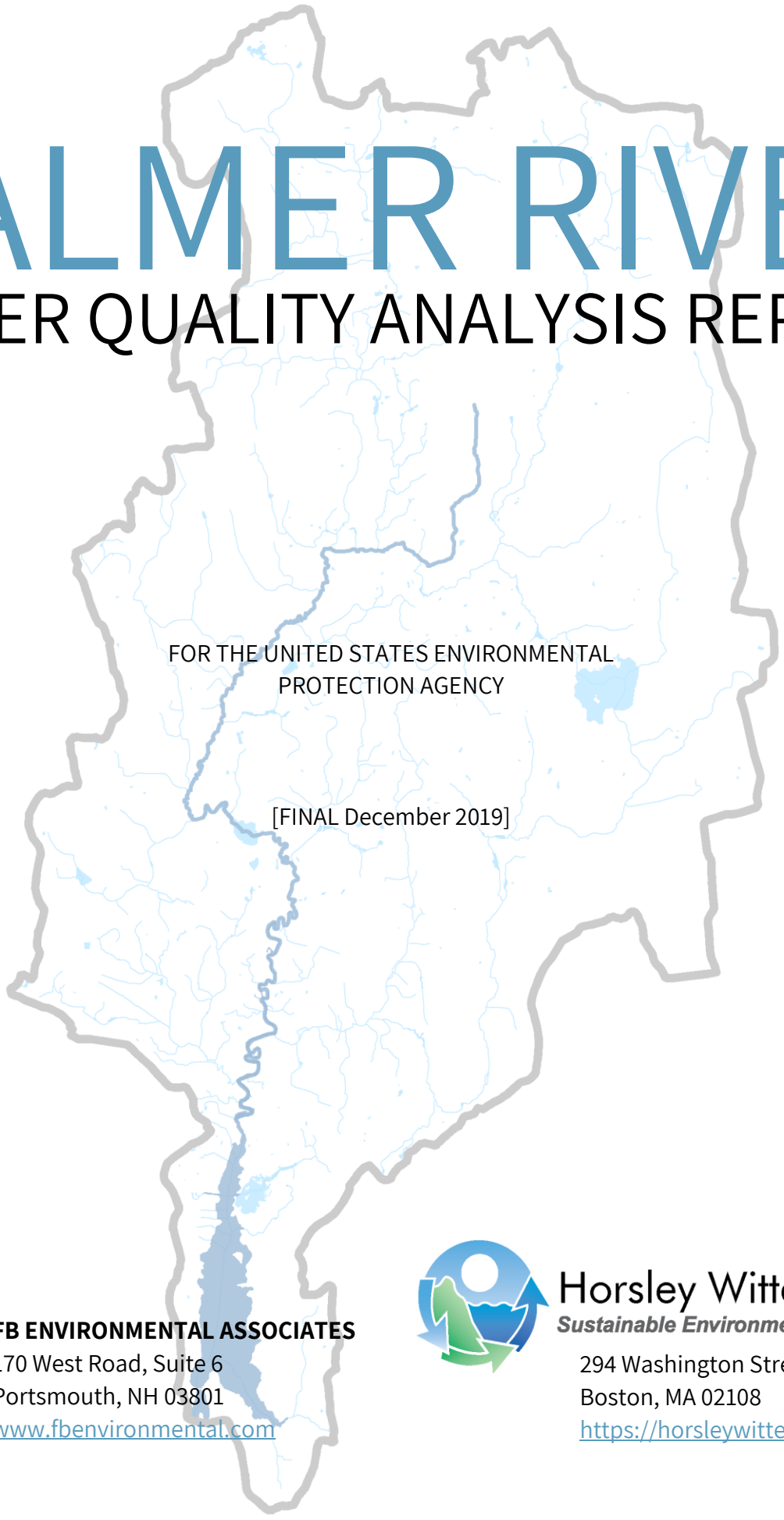
PALMER RIVER

WATER QUALITY ANALYSIS REPORT



FOR THE UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

[FINAL December 2019]

FB ENVIRONMENTAL ASSOCIATES
170 West Road, Suite 6
Portsmouth, NH 03801
www.fbenvironmental.com

Horsley Witten Group
Sustainable Environmental Solutions
294 Washington Street, Suite 801
Boston, MA 02108
https://horsleywitten.com

TABLE OF CONTENTS

The views, thoughts, and opinions expressed in this document may not necessarily represent EPA policies and positions. Mention of trade names or commercial products, services, and enterprises in this presentation do not constitute EPA endorsement or recommendation for use.

EXECUTIVE SUMMARY

As part of Palmer River Source Tracking, Water Quality Trends Summary, and Watershed Plan project to address pathogen pollution sources to the Palmer River, this report evaluates the water quality status of the Palmer River, the efficacy of installed agricultural best management practices (BMPs), the dominant fecal source types using PhyloChip® DNA microarray analysis, and the impact of land use change on water quality. A summary of our findings is presented as follows:

- The Palmer River and its tributaries showed consistently elevated *E. coli* and enterococci counts, along with nutrient concentrations, that exceeded state criteria or were above natural background levels for the coastal ecoregion.
- Poor water quality status for the Palmer River and its tributaries is linked to land use, which is dominated by urban development and agricultural land (both pasture and cropland).
- Increased development in the watershed from 1995 to 2018 increased annual pollutant loads to the river. Pollutant loads to the river will continue to increase if development proceeds in a similar "conventional" manner. Refer to the Land Use & Regulatory Analysis Report (HWG & FBE, 2019b) for an assessment of land use regulations and recommendations for revising land use regulations to reduce the impacts of development on water quality in the Palmer River watershed.
- From these land use types, human, bird, and cow waste were identified by PhyloChip® as the dominate sources of fecal pollution to the Palmer River, and several pathogenic bacteria strains associated with mammalian and bird intestinal tracts were present at eight of the twelve sites selected for analysis.
- Human waste was detected at all eight sites selected for analysis, indicating that septic systems are a significant contributor of fecal contamination to the Palmer River.
- Monitoring sites CR03, TC07, and RR22 were identified as having poor water quality. These sites had elevated nutrients, strong human and/or cow bacterial source signals, and presence of several pathogenic bacteria strains based on PhyloChip® analysis results. We recommend that CR03, TC07, and RR22 be investigated further for septic system failures and that TC07 be targeted for more agricultural BMP installations.
- Significant work to remediate these fecal sources and their associated pathogens has already been accomplished in many of the sub-basins to the twelve monitored sites. From 2015 to 2018, 28 agricultural BMPs were successfully installed in the Palmer River watershed, 11 on cropland and 17 on pasture. Our analyses revealed that despite increased pollutant loads from changes in land use in that time period, installation of agricultural BMPs has had a measurable positive effect on water quality by reducing pollutant loads below what would have been measured without BMPs.
- Continuing to implement agricultural BMPs, along with incorporating low impact development practices on new and existing development, will be necessary to achieve a measurable and sustainable improvement in water quality in the Palmer River.

BACKGROUND

The Palmer River, which flows across the Massachusetts (MA) and Rhode Island (RI) state border, is a major tributary in the Narragansett Bay watershed. The upper freshwater reaches of the Palmer River begin in Rehoboth, MA with the east and west branches of the river extending into Seekonk and Swansea, MA. Head of tide for the Palmer River is at the outlet of the Shad Factory Pond Dam, downstream of which the Palmer River joins with the Barrington River at Tyler Point in RI to form the Warren River before emptying into the Narragansett Bay. While still dominated by forest, the Palmer River watershed contains significant agriculture and development and faces increasing development pressure. In 1992, the Rhode Island Department of Environmental Management (RIDEM) listed its portion of the Palmer River as impaired for primary contact recreation and shellfish consumption due to elevated levels of fecal coliform and as impaired for fish and wildlife habitat due to low dissolved oxygen levels, with a total nitrogen impairment listing added in 1998. The Massachusetts Department of Environmental Protection (Mass DEP) listed its portion of the Palmer River as impaired due to elevated levels of fecal coliform beginning in 2002 (segment 53-03 from the Route 6 bridge to the state line was first listed in 1998), along with nutrient and flow alteration impairment listings. In 2002 and 2004, a total maximum daily load (TMDL) was approved for the Palmer River watershed in RI (fecal coliform) and MA (*E. coli*), respectively (RIDEM, 2002; ESS Group Inc, 2004). The 2004 MA TMDL study found that 33 out of 88 sampling stations along the Palmer River violated state criteria for fecal coliform and/or *E. coli*. The Palmer River also exhibited elevated levels of total suspended solids (TSS) and nutrients (nitrogen and phosphorus). A watershed management plan for the Barrington-Palmer-Warren Rivers was developed by RIDEM and FB Environmental Associates (FBE) for the US Environmental Protection Agency (US EPA) Region 1 in 2012 (FBE, 2012).

In 2012, the Palmer River watershed was included in the National Water Quality Initiative (NWQI) to abate fecal contamination through the installation of agricultural conservation practices or best management practices (BMPs). In the same year, Mass DEP, RIDEM, and US EPA Region 1 began a joint project to further investigate water pollution sources to the Palmer River. By 2015, agricultural BMPs were being installed throughout the southern portion of the watershed and have continued to be installed up to present day. Beginning in 2016, MassDEP, RIDEM, and US EPA Region 1 have collected monthly water quality samples at twelve fixed stations or "core" sites within the lower Palmer River watershed to determine the effectiveness of remediation efforts with agricultural BMP installations. The "core" sites included six saline and six freshwater stations, with three stations on Clear Run sampled for *E. coli*, three stations on the main stem sampled for Enterococci, and the remaining six stations sampled for both parameters. All sites were also sampled for TSS and nutrients. Beginning in 2017, samples were collected for ribonucleic acid (RNA) microarray analysis using PhyloChip®[1].

This report examined several objectives of the Palmer River Source Tracking, Water Quality Trends Summary, and Watershed Plan project (outlined in HWG & FBE, 2019a), including 1) determining the water quality status of the Palmer River using existing water quality data, geospatial information, and summary papers, 2) determining the efficacy of installed agricultural BMPs in the Palmer River watershed, 3) analyzing PhyloChip® results in the context of current water quality and expected pollutant sources, and 4) assessing the impact of changing land use on water quality in the Palmer River watershed. These findings will be used by US EPA Region 1, state agencies, and local municipalities to better inform future effectiveness and placement of agricultural BMP work in the watershed and modify future monitoring of critical sites in the watershed.

DATABASE DEVELOPMENT

All water quality data for the Palmer River watershed were compiled in an MS Excel 2016 database that included metadata, raw data, and site locations. We identified 67 parameters sampled at 158 unique sites from 1960-2018 for a total of 10,111 records. Data came from a variety of federal, state, and local sources and were reviewed and validated for meeting data quality objectives outlined in HWG & FBE (2019a). Refer to the Palmer River Water Quality Database metadata for more details. Validated data were imported to R x64 3.5.1 / RStudio, an open source statistical program, for analysis. Refer to the following sections for analysis methodology.

[1] The PhyloChip is a rapid, high throughput, DNA microarray based on probing environmental samples for the 16S rRNA gene. The main benefits of using the PhyloChip over traditional culturing techniques are its speed, accuracy, and inclusivity of organisms that cannot survive culturing.

WATER QUALITY STATUS

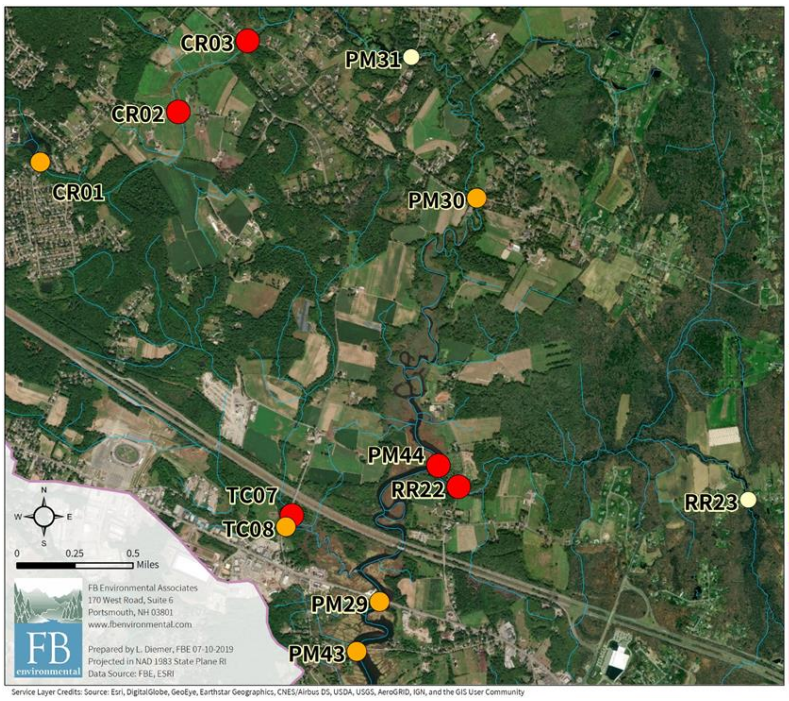
Due to data availability, the analysis was limited to the twelve "core" sampling sites in Massachusetts that Mass DEP, RIDEM, and US EPA Region 1 monitored from 2016-2018 (Appendix 1). Key parameters (i.e., *E. coli*, enterococci, nutrients, and TSS) were summarized (by geomean for log-normalization of fecal indicator bacteria, median for all others) by day, month, and year.

Sites and parameters with ten or more years of annual data were assessed for long-term trends using the Mann-Kendall trend test ($\alpha < 0.05$). Only six sites (CR01, CR02, CR03, PM31, PM30, and RR23) had 9-10 years of water quality data (and only for *E. coli*). A Mann-Kendall trend test[2] using the *rkt* package in R statistical programming was performed on the summarized data and no statistically significant trends were found ($\alpha < 0.05$).

Daily data for all twelve sites were summarized (median, average, minimum, and maximum) by site for application to state water quality criteria or natural background conditions (see Appendices 2 and 3). All sites exceeded state criteria for both *E. coli* and enterococci for either geomean or single-sample or both. Most sites also had elevated nutrient levels compared to natural background levels for the coastal ecoregion (USEPA, 2000).

To rank the twelve "core" sampling sites from relatively better to worse water quality, we summed the magnitude increase (i.e., if greater than 1) above the state criteria or natural background conditions for the average, minimum, and maximum values of seven parameters for each site (refer to Appendix 4 for an example score calculation). *E. coli* was not included for sites with possible salt inundation (PM31, PM30, PM44, RR22, TC07, TC08, PM29, and PM43), as *E. coli* has been shown to result in false positives in marine waters (Pisciotta et al., 2002). Enterococci (if available) was not included for the remaining sites (CR01, CR02, CR03, and RR23) to match the number of summed parameter values for each site (and not artificially increase the score).

The highest scores representing the worst water quality were found at Clear Run (CR03, CR02) followed by Rocky Run (RR22), the mainstem (PM44, which is proximal to the mouth of Rocky Run at RR22), and Torrey Creek (TC07), likely due to the dominance of agriculture in these sub-basins (Figure 1, see further discussion in Land Use Change Impact section). The mainstem (PM31, PM30, PM29, and PM43) scored low to moderate likely due to dilution and/or tidal effects. Clear Run (CR01) and Torrey Creek (TC08) scored moderate; both drain small sub-basins that are impacted by mixed residential, commercial, and industrial development (with minimal agriculture). Rocky Run (RR23) scored low, suggesting that most pollutant sources are likely entering Rocky Run between RR23 and RR22.



Site	Score
PM31	25
RR23	29
PM30	55
PM29	60
CR01	61
TC08	62
PM43	78
TC07	90
PM44	103
RR22	105
CR02	171
CR03	217

Figure 1. Map of the twelve "core" sites with symbols representing the ranking of each site's relative water quality condition from better (yellow, low values) to worse (red, high values). Note that all sites scored above one, indicating one or multiple parameters were above the state criteria or natural background conditions.

[2] Mann-Kendall trend test is a useful non-parametric, statistical test for monotonic trends in time series of environmental data.

AGRICULTURAL BMPS

Agricultural BMP Documentation & Load Reduction Calculations

As noted previously, the Palmer River watershed was included in the NWQI to abate fecal and nutrient contamination through the installation of agricultural conservation practices or BMPs. Through the NWQI, several successful agricultural BMPs have been installed in the Palmer River watershed since 2015 and more are ongoing or soon-to-be installed in the coming years. FBE determined the number, type, and pollutant reduction potential of agricultural BMPs installed in each sub-basin using the Spreadsheet Tool for Estimating Pollutant Load (STEPL) following the latest model and documentation (STEPL 4.4, updated 3/15/18, STEPL 4.4 User's Guide). For data inputs, refer to Appendix A in the *Secondary Data Quality Assurance Project Plan (QAPP) for the Palmer River Source Tracking, Water Quality Trends Summary, and Watershed Plan*, dated February 4, 2019 (HWG & FBE, 2019a). We generated a separate STEPL model spreadsheet for each year of agricultural BMP installation (2015, 2016, 2017, 2018). We used 2015 land use data for the 2015 and 2016 models and 2018 land use data for the 2017 and 2018 models. See HWG & FBE (2019b) for details on land use analysis for model years. STEPL models the total load and total load reduction from installed BMPs for total nitrogen, total phosphorus, and total sediment; load estimates for *E. coli* will be included in the next version update. In the meantime, the range of estimated load reductions for nutrients and sediment can serve as a proxy for *E. coli*, especially sediment since *E. coli* can bind and be transported with sediment and can act more conservatively (i.e., not as readily taken up or transformed) in the environment compared to nutrients that are more readily taken up or transformed through biochemical pathways.

From 2015-2018, 28 agricultural BMPs were successfully installed in the Palmer River watershed, 11 on cropland and 17 on pasture (Table 1). Several sites had multiple BMPs installed. Refer to Appendix 5 for a description of the agricultural BMP types based on general STEPL BMP types. Most of the agricultural BMP implementation work in the Palmer River watershed was completed in 2015-2016 with some additional work in 2017-2018 (Table 1). Implementation work completed in 2018 compared to prior implementation work in the sub-basins to PM31, PM44, RR22, and TC07 generated only modest additional reductions in estimated pollutant loads. The pollutant reductions estimated for the sub-basin to CR02 more than doubled with the addition of 2017 BMPs. Additional agricultural BMPs are planned to be implemented in the direct sub-basins to the following sites: CR02, CR03, PM31, PM44, TC07, and PM29.

These BMPs resulted in a total reduction of 528 lbs./yr in nitrogen, 149 lbs./yr in phosphorus, and 25 tons/yr in sediment (Table 1)[3]. The largest percent reduction of total load was for sediment in the TC07 sub-basin (13%); otherwise, most percent load reductions ranged from <1% to 3%. Agricultural BMPs installed in the TC07 sub-basin included three Conservation Tillage 2 BMPs on cropland in a series, as well as Prescribed Grazing on pastureland with a Critical Area Planting. Conservation Tillage 2 has among the highest phosphorus and sediment reduction efficiencies compared to the other BMPs (see Appendix 5).

A Minimum Detectable Change Analysis completed by Tetra Tech, Inc. (2014a) estimated that fecal coliform reductions to the Palmer River would need to be significant (32%) to achieve a measurable improvement in water quality, assuming that improvements were not masked by changes in land use or other source inputs. Since fecal coliform data were not included in this analysis, we are limited in our application of the estimated minimum detectable reduction needed to achieve water quality improvement in the Palmer River. Fecal coliform, *E. coli*, and enterococci are not directly comparable (though *E. coli* is a subset of fecal coliform) and even less so to other parameters of interest such as nutrients and sediment. Fecal indicator bacteria such as fecal coliform, *E. coli*, and enterococci can be notoriously variable both in the environment and in the laboratory compared to other parameters like nitrogen and phosphorus for which the minimum detectable change may be significantly lower than the 32% estimated for fecal coliform. There is also the consideration of lag time for system flushing of some parameters like nutrients and sediment before improvements are measurable, compared to *E. coli* which do not persist for long in the environment (Meals & Dressing, 2008).

With these limitations in mind, applying the fecal coliform minimum detectable change of 32% to the modeled water quality parameters suggests that more reduction efforts may be needed in the Palmer River watershed to achieve a measurable improvement in water quality.

[3] It is important to also note that many of the sub-basins are nested, and any agricultural BMPs installed in the direct sub-basin draining to a given site has cumulative downstream effects on water quality; thus, we identified general BMP types installed in the direct sub-basin to each site but show the total loads and cumulative load reductions for the total drainage area to each site. For example, general BMP types are described for the direct sub-basins draining to CR01 and CR02, but the total loads and load reductions for CR02 combine the loads from the sub-basins draining to both CR01 and CR02 (Table 1).

Table 1. Agricultural BMP types by sub-basin (non-cumulative) and total pollutant loads without BMPs and pollutant load reductions with BMPs by sub-basin (cumulative). Based on 2018 land use. N=nitrogen. P=phosphorus. Sed=sediment. Red=reduction. Dates in brackets [ ] indicate the years in which the BMPs were installed. Refer to Appendix 5 for agricultural BMP descriptions.

Sub-basin	Agricultural BMP Types [implementation years]	N Load (lbs./ yr)	P Load (lbs./ yr)	Sed Load (tons /yr)	N Red (lbs. /yr)	P Red (lbs. /yr)	Sed Red (tons /yr)	N Red (%)	P Red (%)	Sed Red (%)
CR01	No BMPs	3,116	984	25	0	0	0	0%	0%	0%
CR02	Litter Storage and Management + Livestock Exclusion Fencing + Heavy Use Area Protection [2016, 2017]	7,028	1,866	68	46	4	0	1%	0%	1%
CR03	Litter Storage and Management [2016]	8,402	2,208	92	50	5	0	1%	0%	0%
PM31	Diverted Drainage + Grass Swale + Critical Area Planting + Litter Storage and Management [2016, 2018]	48,790	13,556	593	97	16	3	0%	0%	0%
PM30	Litter Storage and Management + Use Exclusion + Heavy Use Area Protection + Grass Swale [2017]	51,317	14,249	619	102	17	3	0%	0%	0%
PM44	Terrace + Conservation Tillage 2 + Prescribed Grazing [2015, 2016, 2018]	54,052	14,820	675	334	98	13	1%	1%	2%
RR23	Litter Storage and Management [2016]	11,662	3,374	134	1	0	0	0%	0%	0%
RR22	Livestock Exclusion Fencing + Grass Buffer + Prescribed Grazing + Use Exclusion [2016, 2018]	18,972	5,207	240	44	7	1	0%	0%	1%
TC07	Conservation Tillage 2 x3 + Prescribed Grazing x2 + Critical Area Planting [2015, 2016, 2018]	4,776	1,252	78	114	42	10	2%	3%	13%
TC08	No BMPs	309	40	6	0	0	0	0%	0%	0%
PM29	Conservation Tillage 2 + Prescribed Grazing [2016]	79,009	21,462	1,012	528	149	25	1%	1%	3%
PM43	No BMPs	79,391	21,561	1,016	528	149	25	1%	1%	2%

Agricultural BMP Efficacy Analysis

METHODOLOGY

We performed a series of calculations and analyses to help determine the efficacy of installed agricultural BMPs in the Palmer River watershed. We hypothesized that 1) observed annual loads would underestimate modeled annual loads for total nitrogen, total phosphorus, and total sediment because sampling generally occurred during low flow summer conditions and that 2) observed annual loads for years and sites with installed agricultural BMPs would be less than modeled annual loads (that do not account for estimated reductions), assuming that there was measurable improvement of water quality as a result of the BMPs. We determined that comparing observed and modeled annual loads would be the best approach because the modeled annual loads helped control for the confounding influence of changing land use that may mask any measurable water quality improvement as a result of remediation efforts (Figure 2; see Land Use Change Impact).

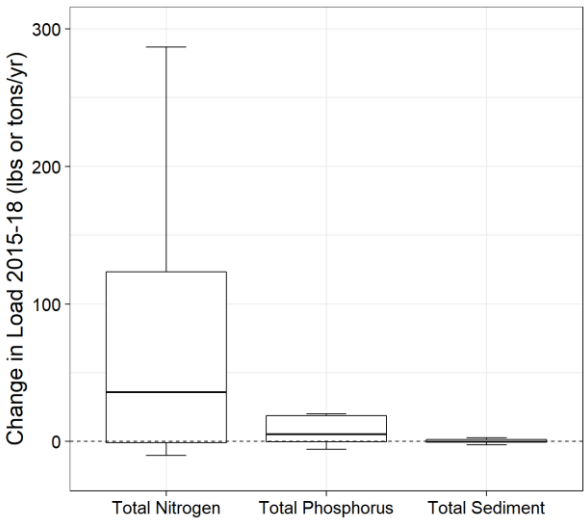


Figure 2. Changes in total nitrogen (lbs./yr), total phosphorus (lbs./yr), and total sediment (tons/yr) load as a result of changes in land use in the twelve sub-basins in the Palmer River watershed from 2015-2018.

The first step in our BMP efficacy analysis was to attach weather data to each data entry; weather data were taken from NOAA NCEI Providence RI US (Station #USW00014765) and summarized for precipitation (on sample day and 1, 2, 3, 4, and 7 days prior), air temperature (average, minimum, and maximum on sample day and 7 days prior), wet versus dry weather distinction (using 0.5" within 72 hours, not including the sample day, as the threshold), and days since last measurable rain event (using 0.25" as the threshold). Next, we attached flow data, along with the ratio of sub-basin drainage area to stream gage drainage area and the flow exceedance probability, to each data entry; flow data were taken from USGS 01109403 Ten Mile River, Pawtucket Ave at East Providence, RI (53.1 square mile drainage area). The flow exceedance probability was determined by ranking average daily flow from

lowest to highest and dividing the ranked position value by the number of records (8,930) plus one. We estimated the average daily flow for each date in the dataset by multiplying the average daily flow taken from the USGS gage and the ratio of sub-basin drainage area to stream gage drainage area (hereafter referred to as the areal-weighted flow); this served as a rough approximation of site-specific daily flow that did not account for localized precipitation events or unique drainage characteristics that would increase or decrease flow. Note: the drainage-area ratio method is commonly used when regional statistics and precipitation-runoff modeling are not readily available (Emerson et al., 2005).

To calculate the observed annual load, we multiplied the areal-weighted flow (cubic feet per second) by concentration (mg/L)[4], along with conversion factors to achieve lbs. per day or tons per day and the flow exceedance probability[5] before averaging by month then year and multiplying by 365 to achieve lbs. per year or tons per year. We plotted observed annual load versus modeled annual load for total nitrogen, total phosphorus, and total sediment and determined the residuals of each linear regression. Residuals were then compared and tested for significant difference for years and sites with and without installed agricultural BMPs as well as for wet and dry years. Using the NOAA NCEI Providence RI US (Station #USW00014765) precipitation data from 1996-2018, we determined "wet" and "dry" years as those years with total annual precipitation greater and less than the median of 1996-2018 total annual precipitation, respectively. We included a wet and dry year comparison because the modeled annual loads did not explicitly account for interannual variation in weather but instead account for annual precipitation normal as a constant input across modeled years; since agricultural BMP installation efforts were concentrated in recent years, there was a risk of annual weather variation driving possible observed changes in water quality. It is important to note that using the nearest USGS stream gage to calculate observed annual loads accounts for antecedent weather patterns on a daily timescale (extrapolated up to an annual timescale with several limitations), as accounted for by higher or lower flow volume. These calculated data failed the Shapiro test for normality, so we used the Kruskal-Wallis test as a non-parametric alternative to analysis of variance (ANOVA).

RESULTS & DISCUSSION

Observed annual loads for total nitrogen, total phosphorus, and total sediment fell below the 1:1 line and underestimated modeled annual loads, which supported our first hypothesis that observed annual loads would underestimate modeled annual loads because sampling generally occurred during low flow summer conditions (Figure 3; Appendix 6). The slopes of the linear regressions of observed versus modeled annual loads were similar for total phosphorus and total sediment but not for total nitrogen, which was closer to the 1:1 line, suggesting that sampling better captured total nitrogen annual loads as compared to total phosphorus and sediment annual loads, which were grossly underestimated. Total phosphorus can attach to sediment particles and be transported to surface waters in stormwater runoff; in contrast, total nitrogen can be captured and transformed by biota from atmospheric sources. Thus, it is reasonable that total phosphorus and sediment would follow similar patterns as compared to total nitrogen. The range of observed annual loads became greater at higher modeled annual loads because of the variability in sample number and distribution for each collection year; for instance, some years had only one sample collected during a low flow period (generating lower observed annual loads compared to modeled annual loads), while other years had many samples collected during a range of flow conditions (generating higher observed annual loads compared to modeled annual loads). To address this and as explained previously, the observed annual loads were calculated from daily average loads that were weighted by the flow exceedance probability (so the influence of higher flow periods were reduced to better approximate annual loads).

Residuals of the linear regressions of observed versus modeled annual loads for total nitrogen, total phosphorus, and total sediment were statistically significantly different for sites and years with and without installed agricultural BMPs, which supported our second hypothesis and showed that there was measurable improvement of water quality likely as a result of the BMPs[6] (Figure 3). To further support this finding, we also found that the residuals of the linear regressions of observed versus modeled annual loads for total nitrogen, total phosphorus, and total sediment were not statistically significantly different for wet and dry years, suggesting that interannual variation in weather was likely not driving observed changes in water quality (i.e., not driving the lower-than-expected observed annual loads for sites and years with installed agricultural BMPs).

[4] Based on generic formula for calculating a mass load. Discharge (Q) x Concentration (C) = Load.

[5] Similar methods described in Tetra Tech, Inc. (2014b).

[6] A significant illicit discharge from a septic system to Rocky Run was discovered and remediated in 2013-2014, resulting in an improvement in water quality to nearby and downstream stations along Rocky Run (based on pre and post remediation testing completed by MassDEP). To ensure that those data were not skewing results of the residual analysis, we re-ran the residuals analysis without RR22 and found similar results, supporting the finding that observed water quality improvements were likely driven by changes in loading as a result of installed agricultural BMPs.

We assessed using two-way ANOVA and Tukey HSD tests how fecal indicator bacteria (*E. coli* and enterococci), nutrients, and sediment varied for sites and years with and without installed agricultural BMPs and for wet and dry weather antecedent conditions ("wet" was defined as >0.5" of precipitation in the prior 3 days not including the day of sampling); the data were approximately normally distributed, so it was reasonable to proceed with a parametric statistical test (Figure 4). We found that *E. coli* was significantly lower during wet weather only; Enterococci, total Kjeldahl nitrogen, and total nitrogen were significantly lower during dry weather only; orthophosphate was significantly higher during wet weather only; and nitrate-nitrate, total phosphorus, and total suspended solids were unchanged from sites and years with installed agricultural BMPs compared to those without BMPs. It is important to note that the method of distinction for wet and dry weather conditions does not include the day of sampling and therefore may be overestimating dry weather conditions. There were several limitations to analyzing the data in this way due to differences in the number of samples across years, seasons, and flow conditions and in various confounding factors such as land use change. Generally, however, the analysis seems to show that there was possible improvement in water quality as a result of installed agricultural BMPs. Due to the variability in *E. coli* and enterococci, it will be important to continue to monitor parameters for nutrients and sediment to assess the efficacy of existing and future installed agricultural BMPs in the Palmer River watershed.

Due to significant data gaps, it was difficult to assess trends in observed data over time or residuals at the individual site level and thus we could not make determinations about which sites and which installed agricultural BMPs had the greatest benefit to water quality. In addition, most sites had multiple types of BMPs of varying size and treatment level that were installed over several years. Even if there were enough data to determine which sites had the greatest benefit to water quality, it would be difficult to ascertain the specific BMP(s) that contributed the most to water quality improvements, as well as the effects of upstream water quality improvements. We can, however, generally conclude that the STEPL reduction estimates for the BMPs can serve as a reasonable reference for selecting the most effective BMP types, depending on the size of the anticipated installation (refer to Appendix 5).

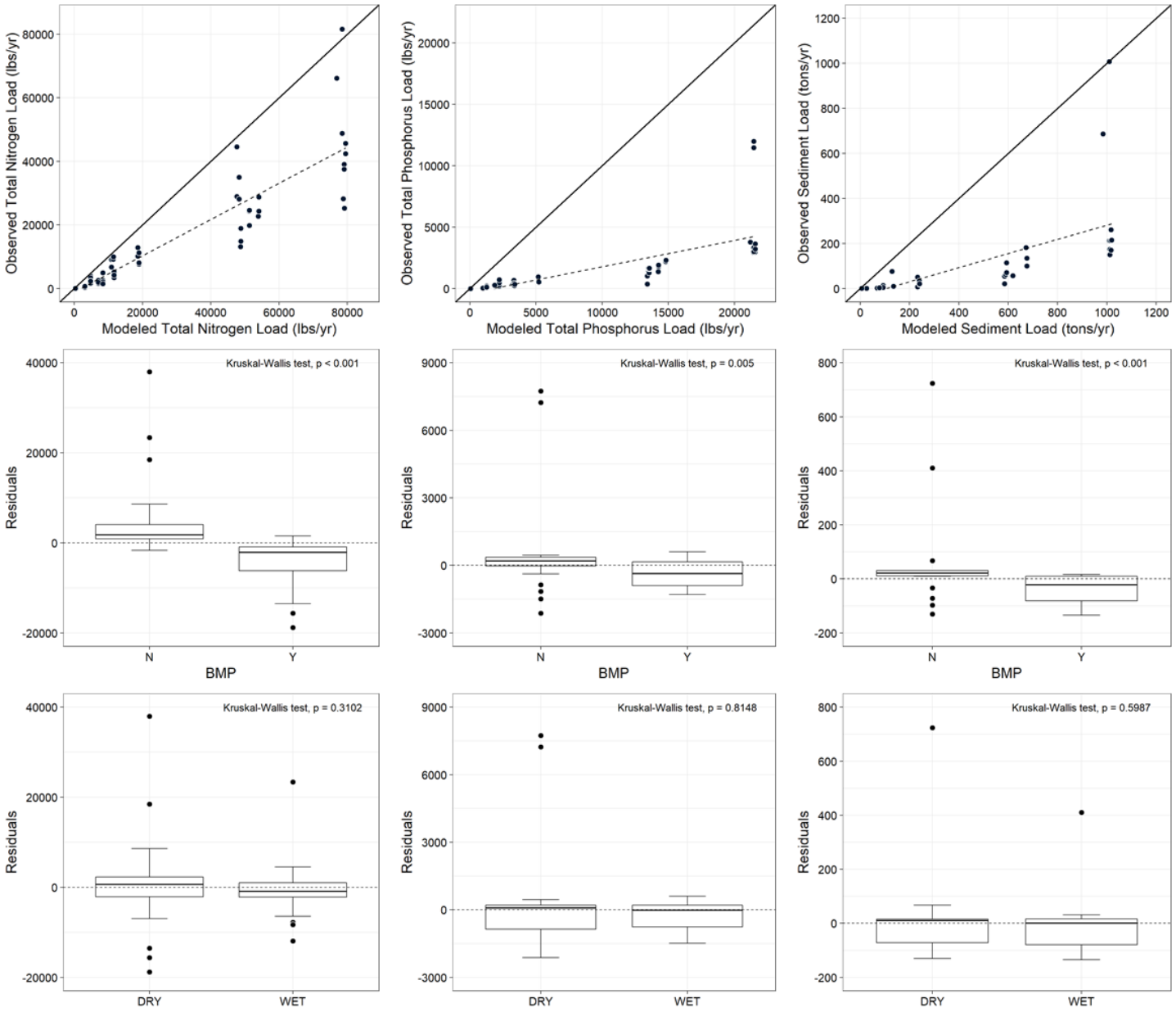


Figure 3. [TOP] Observed annual loads compared to modeled annual loads for total nitrogen (left), total phosphorus (middle), and total sediment (right). Observed annual loads were calculated from average daily measured concentration, as well as areal-weighted flow and flow exceedance probability from USGS 01109403 Ten Mile River, Pawtucket Ave at East Providence, RI. Modeled annual loads were determined using the Spreadsheet Tool for Estimating Pollutant Load (STEPL) and accounted for changes in land use over specific time periods (1995, 2001, 2005, 2011, 2015, and 2018). The dotted line represents the linear regression. The solid line represents the 1:1 ratio as a reference for observed versus modeled comparability. [MIDDLE] Residuals of the linear regressions of observed versus modeled annual loads for total nitrogen (left), total phosphorus (middle), and total sediment (right) binned by sites and years with and without installed agricultural BMPs. [BOTTOM] Residuals of the linear regressions of observed versus modeled annual loads for total nitrogen (left), total phosphorus (middle), and total sediment (right) binned by wet and dry years (determined as greater or less than median annual precipitation from 1996-2018 for the NOAA NCEI Providence RI US (Station #USW00014765), respectively). Significance results ($\alpha < 0.05$) of the non-parametric Kruskal-Wallis test are shown in the plots.

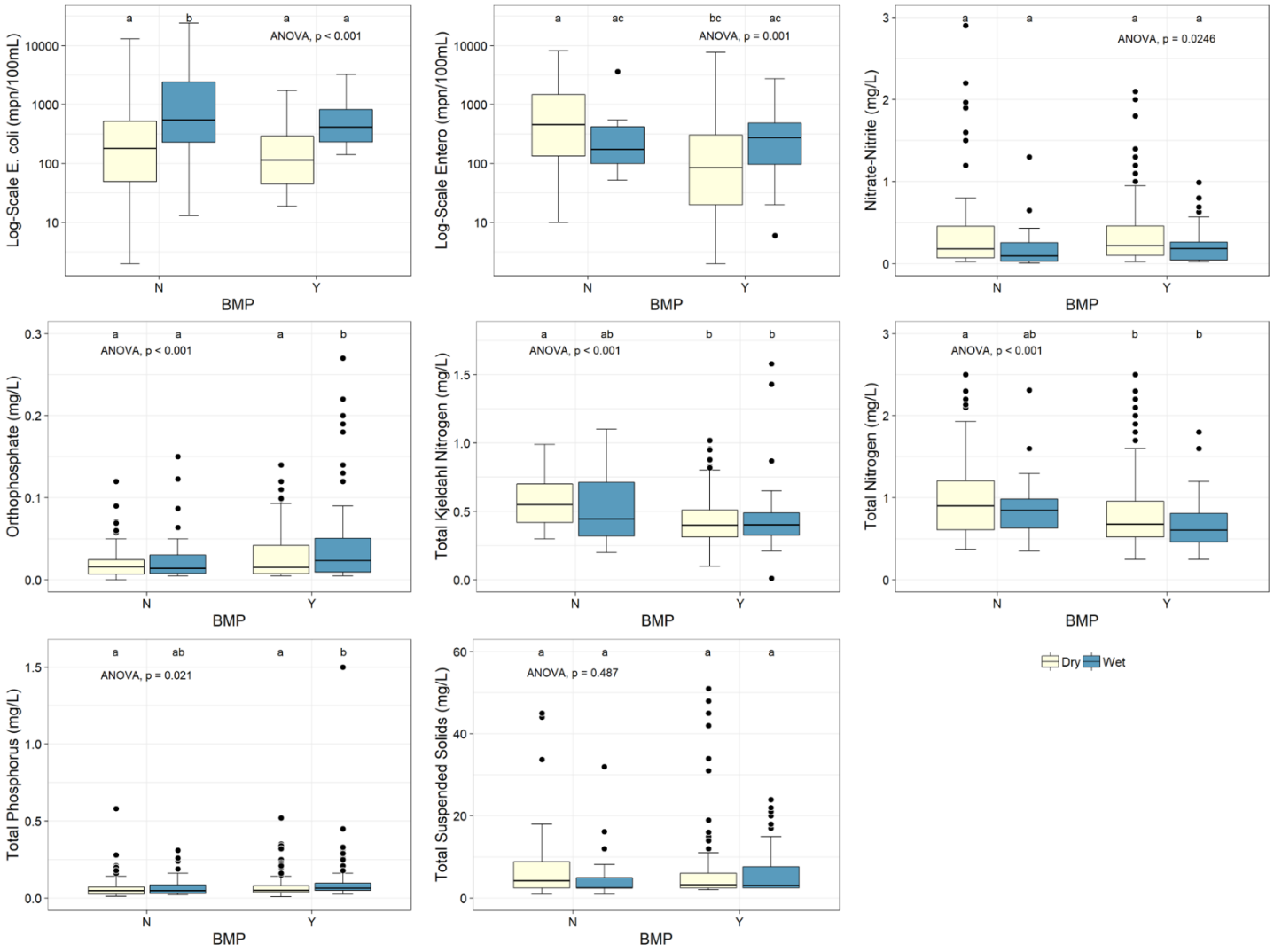


Figure 4. Boxplots of key water quality parameters binned by sites and years with and without installed agricultural BMPs and wet and dry weather antecedent conditions ("wet" was defined as >0.5" of precipitation in the prior 3 days not including the day of sampling). Key parameters analyzed were *E. coli*, enterococci, nitrate-nitrite, orthophosphate, total Kjeldahl nitrogen, total nitrogen, total phosphorus, and total suspended solids. Sites were limited to freshwater (CR01, CR02, CR03, and RR23, did not include PM31 and PM30) for *E. coli*; all sites with available data were included for all other parameters. Significance results ($\alpha < 0.05$) of the ANOVA and Tukey HSD tests are shown in the plots (data were reasonably normally distributed). Dry samples are shown in yellow and wet samples are shown in blue.

PHYLOCHIP® ANALYSIS

Beginning in 2017, samples were collected at the twelve "core" sites for ribonucleic acid (RNA) microarray analysis using the PhyloChip® to identify specific sources of fecal contamination in the Palmer River watershed. Out of the 96 collected samples, a subset of 50 were selected for PhyloChip® analysis. We assessed available information and provided recommendations for prioritizing for analysis (1) sites that met decision matrix metrics identified in the QAPP for factors including water quality trends, presence of agricultural BMPs, magnitude of land use change, and potential pollutant sources and (2) sample dates that met decision matrix metrics for seasonal and antecedent weather conditions (refer to HWG & FBE, 2019c for full discussion of rationale). Our recommended sites (limited to 8 of 12) and dates for the 50 samples were then sent to the Lawrence Berkeley National Laboratory for analysis. The remaining samples will be held with the US EPA Region 1 until additional funding for analysis becomes available.

Limitations to Traditional Source Tracking Methods

As mandated by the USEPA and the US Food and Drug Administration (FDA), state water quality standards use fecal indicator bacteria (primarily *E. coli* in freshwater and enterococci in brackish water) as an estimate of the likelihood that harmful pathogens from fecal source types in the watershed are present in concentrations that make surface waters unsafe for drinking water, shellfish consumption, and/or recreational use. Yet, fecal indicator bacteria are limited in their use as indicators of pathogen contamination which is a primary health risk in surface waters.

For example, previous studies of beaches impacted by point sources of sewage discharge found a significant correlation between fecal indicator bacteria and the probability of gastrointestinal (GI) illness in swimmers caused by bacterial or viral pathogens in the water (Wade et. al., 2003, 2010). However, subsequent studies of surface waters impacted by nonpoint sources of pollution found weaker or no correlation between fecal indicator bacteria and swimmer illness (Colford et. al., 2007; Young et. al., 2016). Studies have concluded that:

- Fecal indicator bacteria come not just from fecal sources but also non-fecal sources such as soils, sediment, algal wrack, decaying vegetation, and beach sands (Badgley et. al., 2010; Byappanahalli et. al., 2003; Hardina & Fujioka, 1991; Imamura et. al., 2011; Ishii et. al., 2006; Park et. al., 2017; Whitman et. al., 2014; Wu et. al., 2017; Yamahara et. al., 2007).
- Fecal indicator bacteria are highly variable and can proliferate or degrade in the environment depending on conditions such as temperature, sunlight, flow, salinity, among other factors (Boehm et. al., 2009; Boehm, 2007; Byappanahalli et. al., 2012; Nelson et. al., 2018; Pisciotta et. al., 2002). Bacterial and viral pathogens have been shown to react differently in the environment, so that external factors may influence the concentration of fecal indicator bacteria but not the viral pathogens of interest for protecting public health. This suggests that the magnitude of fecal indicator bacteria may not reflect a similar level of public health risk.
- Measuring fecal indicator bacteria in the laboratory can be challenging (and potentially confounding) as well due to variability in the ability of cultured specimens in each sample to grow. Because of this, laboratory and field duplicates can vary up to 200% or more, particularly at lower concentrations.

Because fecal indicator bacteria may come from non-fecal sources and/or proliferate/degrade in the environment, fecal indicator bacteria in waters impacted by nonpoint source pollution may not serve as the best proxy for fecal pathogen sources of interest to public health. This research highlights the need for further study into better indicators for pathogen contamination as it relates to public health risk.

For the Palmer River dataset using the PhyloChip® results, we found that false negatives and positives were just as likely as true negatives and positives when comparing culturable fecal indicator bacteria (*E. coli* and enterococci) with source signal strength determined by PhyloChip®, suggesting that fecal indicator bacteria were not a good indicator to determine the likely presence of human, bird, dog, horse, pig, or cow waste (HWG & FBE, 2019c).

PhyloChip® Application in Source Tracking

PhyloChip® is a microbial source identification method that can determine the likelihood of an individual fecal source type such as human or cow being present. It is effective over other similar methods or traditional methods because it uses a series of many diagnostic probes that represent groups of bacteria known to be associated with an individual fecal source type to determine whether a source is present or not. Refer to HWG & FBE (2019c) for additional information on background, uses, and limitations of PhyloChip®.

To perform PhyloChip® analysis, collected water samples are vacuum filtered and centrifuged for DNA extraction. The 16S rRNA gene is amplified using polymerase chain recreation (PCR) for 30 cycles. Microarrays are prepared, stained, and scanned as fluorescent images. Pixel intensities are background-corrected for a hybridization score, along with presence/absence determinations, for individual operational taxonomic units (OTUs)[7] to create a microbial community profile for use in subsequent statistical analyses. A subset of data from DNA probes that target fecal bacteria is run through the "SourceTracker program" to determine the probability (unlikely = no signal, likely = marginal signal, very likely = strong signal) that a source type (human, bird, dog, horse, pig, or cow) is present based on comparison to reference samples from each source type. Full method details are described in Hazen et. al. (2010), Dubinsky et al. (2012), and Dubinsky et al. (2016).

The large dataset generated by the PhyloChip® for a batch of samples can be used to answer several questions related to microbial source tracking, depending on the project's primary objectives. The fecal source signal data can be translated to binary data (presence/absence) or used as a continuous variable to determine significant spatial and temporal differences in source types, as well as possible environmental factors (e.g., water quality, watershed characteristics, etc.) driving the strength of individual sources present across space and time. Similar analyses can be conducted on the microbial community profile, which defines both community composition (richness, binary data) and structure (relative abundance, fold-change in hybridization intensity). We can look at the entire community or individual taxa (such as pathogenic taxa) or co-occurrence among several taxa across space and time or related to environmental factors.

To support the goal of understanding sources of fecal waste and relative health risk within the Palmer River watershed, we focused our analysis on summarizing spatial differences in source types and microbial community composition or richness (both for the entire profile and individual pathogenic taxa). We also analyzed possible connections between environmental factors and source types, the results of which are presented in HWG & FBE (2019c). Due to the limited dataset (only one year and after most agricultural BMP installations were completed), we were unable to make any conclusions about shifts in source types and microbial communities due to seasons or land cover change or water quality improvements from agricultural BMPs.

Detected Source Signals

PhyloChip® analysis results showed 5 of 8 selected sites had strong source signals for human and/or cow. The 8 sites all had at least one source type with a marginal source signal. PhyloChip® results compared to expected sources are summarized in Table 2. The full results table is provided in Appendix 7.

Human fecal sources were strong at three sites (CR03, PM30, and TC07) and marginal at four sites (CR01, PM44, RR22, TC08, and PM43) on at least one occasion (Table 2, Appendix 7). Human fecal sources were detected at the 8 sites selected for analysis, indicating that septic systems are a significant contributor of fecal contamination to surface waters in the Palmer River watershed. In particular, CR03 showed strong human signal on 4 out of 6 samples (from July to November). A prior study using a different fecal source identification method (ribotyping, ESS Group Inc., 2003) found human isolates at RR22 (which had an illicit septic system discharge remediated in 2015). A weak human Bacteroidetes marker (from another prior study under the 2010-2015 Surface Water Monitoring & Assessment MassDEP Division of Watershed Management-Watershed Planning Program) was also detected at TC08. Despite the significant amount of agricultural land contributing to these sites, only one site (TC07) showed a prominent livestock signal for cow (along with a marginal pig and horse signal); these results matched the ribotyping study that found cow and pig isolates at TC07. A marginal livestock signal for cow was found at CR03, PM30, RR22, and TC08. A portion of TC08 contains a hayfield that may be manured. Bird waste was identified at the 8 selected sites and was prominent at CR01 and PM43. The land area immediately adjacent to PM43 was identified as a major goose congregating area according to MassDEP notes. Dog waste was also detected at TC07. Dog and horse waste were not identified as likely fecal sources for any other sites except TC07. The ribotyping study also found pig isolates at CR03, as well as horse and dog isolates at RR22 that were not identified as a prominent or marginal source by the PhyloChip® analysis.

While there were some observable differences in source signal strength across seasons, the limited data do not support any conclusive trend. We can generally note, however, that the snowmelt period in April showed the lowest fecal indicator bacteria counts with marginal signals for human and bird only (except for TC07 which showed marginal signals for dog, horse, and cow). June experienced elevated fecal indicator bacteria counts and marginal signals for human and bird only. Strong signals for human, bird,

[7] OTUs are groups of closely related bacteria with similar DNA sequences. OTUs may not necessarily follow classic taxonomic classification. For instance, a species (lowest taxonomic classification) could have many OTUs.

and cow occurred in mid to late summer under both dry and wet conditions. *E. coli* and enterococci counts were also generally higher in this period, with the linear correlation to human source strength being statistically significant ($p < 0.05$) but weak ($r^2 = 0.143$ for *E. coli* and $r^2 = 0.104$ for enterococci). November experienced a decline in fecal indicator bacteria with marginal signals for human, bird, and cow (except for a strong human signal at CR03). Cow was most prevalent in November (4 out of 8 sites), possibly due to fall manuring of fields.

Table 2. Identified sources of pathogen pollutants by site based on PhyloChip® analysis results, historic Microbial Source Tracking-DNA (MST-DNA) results, and anecdotal information. *NA* signifies that no samples for a site were analyzed using PhyloChip®. The ribotyping study data came from ESS Group Inc. (2003) and the Bacteroidetes data came from a prior study under the 2010-2015 Surface Water Monitoring & Assessment MassDEP Division of Watershed Management-Watershed Planning Program.

Site ID	PhyloChip® Strong Source	PhyloChip® Marginal Source	MST-DNA Results	Other Notes
CR01	Bird	Human		
CR02	*NA*	*NA*		
CR03	Human	Bird, Cow	Cow, pig isolates from ribotyping study	
PM31	*NA*	*NA*		
PM30	Human	Bird, Cow		
PM44		Human, Bird		
RR23	*NA*	*NA*		
RR22		Human, Bird, Cow	Cow, pig, horse, human, deer, rabbit, dog isolates from ribotyping study	Historic septic system failure at RR02 (upstream); remediated by 2015
TC07	Human, Cow	Bird, Pig, Dog, Horse	Cow, pig isolates from ribotyping study	Waterfowl identified in 2004 MA TMDL
TC08		Human, Bird, Cow	Weak human Bacteroidetes marker	
PM29	*NA*	*NA*		Major geese congregation
PM43	Bird	Human		Major geese congregation

Microbial Community Composition

The kingdom of bacteria is a large and complex taxonomic group with a wide range of habitats and functions. Identifying the specific bacteria taxa present can help to distinguish fecal source types or track changes in the occurrence or co-occurrence of taxa across space and time or as related to environmental conditions. Certain taxa are associated with mammalian gut communities, while others occur naturally in soils, while still others are pathogenic and harmful to humans.

ENRICHED TAXA DIFFERENCES AMONG SOURCE TYPES

We determined the top 10% most enriched genera for human, bird, and cow source types using Similarity Percentage (SIMPER) analysis (Appendix 8). Results were similar to those found in other studies that used PhyloChip® (Dubinsky et. al., 2012, 2014, 2016). Samples with human signal were dominated by the Firmicutes phylum (including Bacilli and Clostridia classes) and Bacteroidetes phylum (Bacteroidia class). Bacteroidia and Clostridia comprise most of the bacteria in human waste. Samples with cow signal were similarly dominated by Bacilli and Clostridia classes.

Human, bird, and cow signals were also determined by the Gammaproteobacteria class, which is a highly diverse group of bacteria that represent mammalian gut communities and harmful pathogens but also naturally occurring plant and soil communities (along with Deltaproteobacteria). Alphaproteobacteria and Actinobacteria classes are naturally occurring and widespread in the environment but have been found enriched in bird feces, likely from a predominantly grass diet (Dubinsky et. al., 2016). Fusobacteria were also enriched and are associated with bird feces.

Several bacteria taxa that are widespread in marine environments were also enriched in these samples, such as the Nitrospirae, Elusimicrobia, and Planctomycetes phyla. Numerous genera from the Cyanobacteria phylum were prevalent. Enrichment of the naturally occurring and widespread Sphingobacteria class (Bacteroidetes phylum) and Betaproteobacteria class (Proteobacteria phylum), along with Cyanobacteria, suggest that the Palmer River experiences high nutrient and organic matter loading (Dubinsky et. al., 2014).

ENRICHED TAXA DIFFERENCES AMONG SITES

We also analyzed the most enriched taxa across the 8 sites (CR01, CR03, PM30, PM44, RR22, TC07, TC08, and PM43) (Appendix 9). Enriched taxa common in aquatic environments included Betaproteobacteria class (Aquabacteriaceae, Burkholderiaceae, Comamonadaceae, and Oxalobacteraceae families), Actinobacteria class (Corynebacteriaceae family), and non-fecal Bacteroidetes phylum (Flavobacteria family). The most downstream tidally influenced site (PM43) was most enriched in Pelagibacteraceae, which are common marine bacteria.

Mammalian gut taxa represented in these sites were dominated by Bacillaceae (Bacilli class), Clostridiaceae, Lachnospiraceae, and Ruminococcaceae (Clostridia class). These families were most enriched at sites CR03 and TC07, where Bacteroidia (Rikenellaceaell family) was most enriched as well. CR03 and TC07 are impacted by both urban development and agricultural land. Taxa were also consistently enriched across sites in the Enterobacteriaceae and Pseudomonadaceae families (Gammaproteobacteria class), which represent a range of bacteria types that have been associated with mammalian gut sources and human pathogens (Dubinsky et. al., 2014).

PATHOGENIC TAXA

Notably two pathogenic bacteria strains (Streptococcaceae and Staphylococcaceae families from the Firmicutes phylum) were enriched across most sites, especially CR03, RR22, TC07, TC08, and PM43 (Appendix 9; Table 3). *Serratia marcescens*, which occurs naturally in the environment but can cause serious infections, was present at similar counts for all sites but was highest at RR22. *Proteus mirabilis* was highest at PM30 and TC08. *Salmonella enterica* was enriched at PM43. Other pathogenic bacteria associated with sewage contamination were present but to a lesser (though still significant) degree: *Helicobacter* spp. (CR03, PM30, RR22, TC07, PM43), *Campylobacter subantarcticus* (CR01, CR03, PM44, RR22), *Legionella pneumophila* (RR22, TC07), and *Vibrio cholerae* (only RR22). Most of these pathogenic strains can be found in the intestinal systems of mammalian (both human and cow) and bird species. CR03, RR22, and TC07 had the highest total counts of pathogenic bacteria – all three sites have significant urban development and agricultural land and showed strong signals for human and/or cow source types. TC08 had the second highest count of pathogenic bacteria but showed only marginal signals for human, bird, and cow source types. Because of the strong human signal and/or high numbers of pathogenic bacteria at CR03, RR22, and TC07, these sites should be investigated further for septic system failures. TC07 also showed a strong signal for cow source type; thus, we recommend that TC07 be targeted for more agricultural BMP installations. It is likely that the pathogenic bacteria at PM43 were derived largely from geese.

Table 3. Taxonomic richness of pathogenic bacteria in the Palmer River. Values represent the number of detected OTUs summed across all samples for each site. Shading indicates the following: no shading (< 10 OTUs), light yellow (10-50 OTUs), yellow (51-100 OTUs), orange (101-150 OTUs), red (151-300 OTUs), and dark red (>300 OTUs). Taxa (rows) are ordered from greatest to least total counts for all sites. Sites (columns) are ordered from upstream to downstream.

[Class] Genus Species	CR01	CR03	PM30	PM44	RR22	TC07	TC08	PM43
[Bacilli] Staphylococcus spp.	68	265	104	87	260	178	189	82
[Bacilli] Streptococcus spp.	50	125	83	42	126	106	150	175
[Gammaproteobacteria] Serratia marcescens	20	46	43	29	51	43	43	32
[Gammaproteobacteria] Proteus mirabilis	9	12	22	17	16	20	26	18
[Gammaproteobacteria] Salmonella enterica	11	10	20	12	13	16	17	31
[Epsilonproteobacteria] Helicobacter spp.	0	4	1	0	1	1	0	1
[Epsilonproteobacteria] Campylobacter subantarcticus	1	1	0	1	1	0	0	0
[Gammaproteobacteria] Legionella pneumophila	0	0	0	0	1	1	0	0
[Gammaproteobacteria] Vibrio cholerae	0	0	0	0	2	0	0	0

LAND USE CHANGE IMPACT

FBE completed a land use change analysis using the 2003-2004 RIGIS [Land_Use_and_Land_Cover_20032004] and 2005 MassGIS [LANDUSE2005_POLY] layers as a baseline from which to compare change in land use for the years of 1995, 2001, 2011, 2015, and 2018. We applied the land use data to the nearest few years before and after each available year. For example, 2011 land use data were applied to 2008-2013. We assumed that land use changes at a slow pace and large scale, so it was appropriate to replicate the land use data for multiple years.

Overall between 1995-2018, the PM43 sub-basin (which includes all sub-basins) experienced a decrease in forest (555 acres) and agriculture (139 acres) land use types and an increase in water/wetland (11 acres) and urban (683 acres) land use types (Figure 5). More specifically, residential development largely replaced cropland and mixed forest. Increases in water/wetland areas were due to the installation of large stormwater retention ponds or the addition of farm ponds. Refer to the Land Use & Regulatory Analysis Report (HWG & FBE, 2019b) for further discussion on land use change in the Palmer River watershed.

We regressed the modeled and observed annual loads for total nitrogen, total phosphorus, and total sediment with the total area of pasture, crop, urban, and forest land use types in the drainage area to each of the twelve "core" sites to show the impact of changing land use types on water quality; the slopes of the linear regressions for each land use type quantify the rate of modeled and observed annual load increase for every area unit of land use type increase (Figure 6). Increases in pasture and cropland area resulted in greater increases in modeled and observed annual loads (potentially resulting in degrading water quality) compared to increases in urban area. Increases in forest area resulted in only modest increases in modeled and observed annual loads. Thus, a 1:1 conversion of agricultural land to urban land may drive observed improvements in water quality; however, the rate of change in agricultural and urban lands were not equal. On average from 1995-2018, the sub-basin area to PM43 (the most downstream site of the twelve "core" sites) gained about 2 acres of pasture and 30 acres of urban land and lost 10 acres of cropland and 23 acres of forest each year. Thus, for every acre of agricultural land lost, roughly 3 acres of urban land were gained, which increased pollutant loads over time. As a result of increased development in the Palmer River watershed from 1995-2018, total nitrogen, total phosphorus, and total sediment increased by an estimated 2,187 lbs./yr, 261 lbs./yr, and 27 tons/yr, respectively (Figure 7).

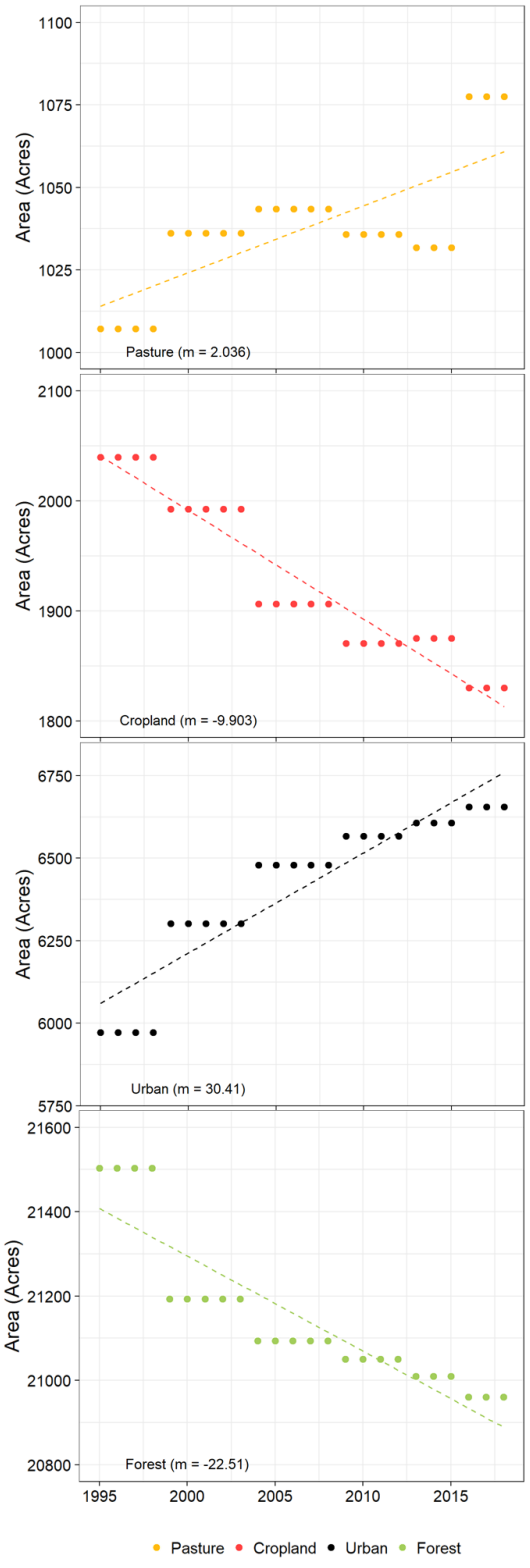


Figure 5. Change in land use types (pasture, cropland, urban, and forest) in the PM43 sub-basin (which includes all sub-basins) from 1995 to 2018. The slopes of the linear regressions for each land use type are shown.

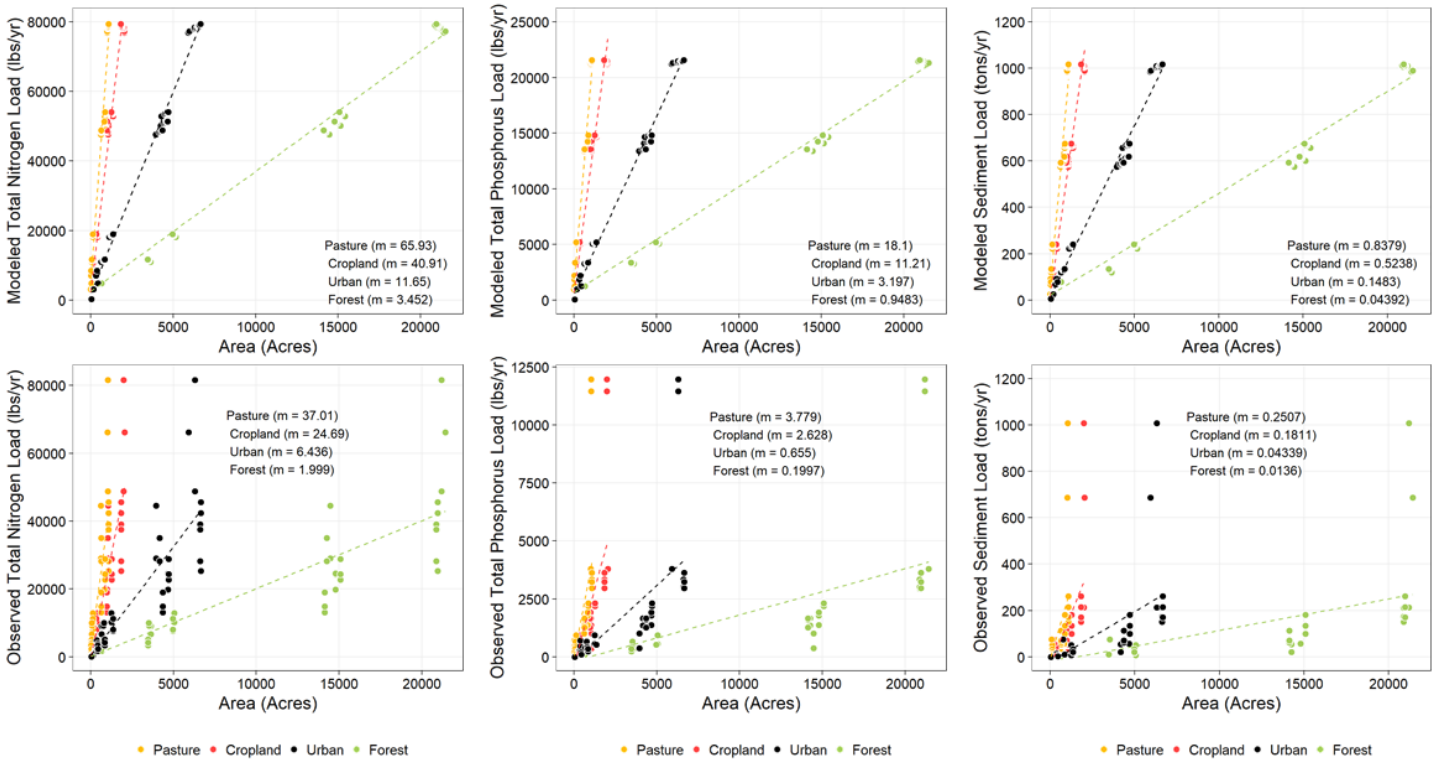


Figure 6. Relationship between the area of land use (pasture, cropland, urban, and forest) in the drainage area to each of the twelve "core" sites and modeled (top) and observed (bottom) annual loads for total nitrogen (left), total phosphorus (middle), and sediment (right). The slopes of the linear regressions for each land use type are shown on each plot.

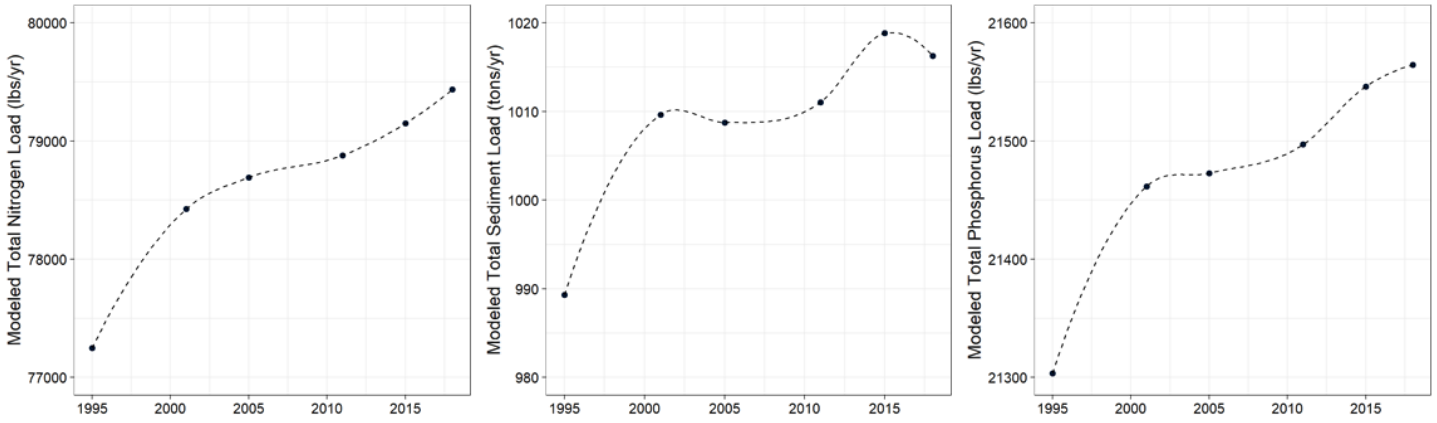


Figure 7. Modeled annual loads for total nitrogen, total phosphorus, and total sediment increased from 1995-2018 as a result of increased development in the Palmer River watershed. Dotted lines represent locally weighted scatterplot smoother (LOESS).

CONCLUSION

This report addressed several objectives of the Palmer River Source Tracking, Water Quality Trends Summary, and Watershed Plan project (outlined in HWG & FBE, 2019a). We analyzed water quality trends to determine the water quality status of the Palmer River using existing water quality data, geospatial information, and summary papers, determined the efficacy of installed agricultural BMPs in the Palmer River watershed, analyzed PhyloChip® results in the context of current water quality and expected pollutant sources, and assessed the impact of changing land use on water quality in the Palmer River watershed.

- **Water Quality Status.** All twelve sites exceeded state criteria for fecal indicator bacteria (*E. coli* and/or enterococci) for either geomean or single-sample or both. Most sites also had elevated nutrient levels compared to natural background levels for the coastal ecoregion. After ranking the twelve "core" sampling sites from relatively better to worse water quality, the highest ranked scores representing the worst water quality were found at Clear Run (CR03, CR02) followed by Rocky Run (RR22), the mainstem (PM44, which is proximal to the mouth of Rocky Run at RR22), and Torrey Creek (TC07), likely due to the dominance of agriculture in these sub-basins. The mainstem (PM31, PM30, PM29, and PM43) scored low to moderate likely due to dilution and/or tidal effects. Clear Run (CR01) and Torrey Creek (TC08) scored moderate; both drain small sub-basins that are impacted by mixed residential, commercial, and industrial development (with minimal agriculture). Rocky Run (RR23) scored low, suggesting that most pollutant sources are likely entering Rocky Run between RR23 and RR22.
- **Agricultural BMP Efficacy.** We found that observed annual loads for total nitrogen, total phosphorus, and total sediment were significantly less than expected (compared to modeled annual loads) for sites and years with installed agricultural BMPs compared to sites and years without installed agricultural BMPs, and interannual variation in weather did not explain observed improvements in water quality. Thus, we can preliminarily state that there was measurable improvement of water quality as a result of installed agricultural BMPs in the Palmer River watershed. Due to significant data gaps at the individual site level and the number and varying size of BMPs installed over time, we were unable to determine which installed agricultural BMPs had the greatest benefit to water quality, but we can generally conclude that the STEPL reduction estimates for the BMPs serve as a reasonable reference for selecting the most effective BMP types, depending on the size of the anticipated installation.
- **PhyloChip® Analysis.** PhyloChip® DNA microarray analysis generated a complete microbial community profile for each sample, along with the presence probability of six major source types: human, bird, dog, horse, pig, and cow. Human, bird, and cow were the dominant source types with the strongest signals found in the Palmer River watershed, and several pathogenic bacteria strains associated with mammalian and bird intestinal tracts were present at all sites. Human waste was detected at the 8 sites selected for analysis, indicating that septic systems are a significant contributor of fecal contamination to the Palmer River. Cow source type was prominent at TC07 and marginal at CR03, PM30, RR22, and TC08. A portion of TC08 contains a hayfield that may be manured, but TC08 is also tidally influenced by sources outside the watershed (which may help account for the second highest count of pathogenic bacteria at TC08). CR03, RR22, and TC07 had the highest counts of pathogenic bacteria – all three sites have significant urban development and agricultural land and showed strong signals for human and/or cow source types. Because of the strong human signal and/or high numbers of pathogenic bacteria at CR03, RR22, and TC07, these sites should be investigated further for septic system failures. TC07 also showed a strong signal for cow source type; thus, we recommend that TC07 be targeted for more agricultural BMP installations. Bird waste was also identified at the 8 sites and was prominent at CR01 and PM43. The land area immediately adjacent to PM43 was identified as a major goose congregating area, and thus it is likely that the pathogenic bacteria at PM43 were largely from geese.
- **Land Use Change Impact.** Increases in pasture and cropland area resulted in greater increases in annual loads for total nitrogen, total phosphorus, and total sediment compared to increases in urban area from 1995-2018. Thus, it would be expected that a 1:1 conversion of agricultural land to urban land would improve water quality. However, the rate of change in agricultural and urban lands were not equal. On average from 1995-2018, the sub-basin area to PM43 (the most downstream site of the twelve "core" sites) gained about 2 acres of pasture and 30 acres of urban land and lost 10 acres of cropland and 23 acres of forest each year. Thus, for every acre of agricultural land loss, roughly 3 acres of urban land were gained, which increased pollutant loads over time. As a result of increased development in the Palmer River watershed from 1995-2018, total nitrogen, total phosphorus, and total sediment increased by an estimated 2,187 lbs./yr, 261 lbs./yr, and 27 tons/yr, respectively. In sum, changes in land use over time in the Palmer River watershed have degraded water quality and

will continue to degrade water quality if left unchecked. Refer to the Land Use & Regulatory Analysis Report (HWG & FBE, 2019b) for an assessment of land use regulations and recommendations for revising land use regulations to reduce the impacts of land development on water quality in the Palmer River watershed.

In closing, while substantial progress has been made to date, continuing to implement agricultural BMPs, along with incorporating low impact development practices on new and existing development, will be necessary to achieve a measurable and sustainable improvement in water quality in the Palmer River.

REFERENCES

Badgley, B.D., Thomas, F.I.M., Harwood, V.J. (2010). The effects of submerged aquatic vegetation on the persistence of environmental populations of Enterococcus spp. *Environ. Microbiol.* 12, 1271-1281. http://dx.doi.org/10.1111/j.1462-2920.2010.02169.x

Boehm, A. B. (2007). Enterococci Concentrations in Diverse Coastal Environments Exhibit Extreme Variability. *Environ. Sci. Technol.* 41 (24), 8227−8232.

Boehm, A. B., Yamahara, K. M., Love, D. C., Peterson, B. M., McNeill, K., Nelson, K. L. (2009). Covariation and Photoinactivation of Traditional and Novel Indicator Organisms and Human Viruses at a Sewage-Impacted Marine Beach. *Environ. Sci. Technol.* 43 (21), 8046−8052.

Byappanahalli, M., Fowler, M., Shively, D., Whitman, R. (2003). Ubiquity and persistence of *Escherichia coli* in a midwestern coastal stream. *Appl. Environ. Microbiol.* 69, 4549-4555. http://dx.doi.org/10.1128/AEM.69.8.4549-4555.2003

Byappanahalli, M.N., Nevers, M.B., Korajkic, A., Staley, Z.R., Harwood, V.J. (2012). Enterococci in the environment. *Microbiol. Mol. Biol. Rev.* 76, 685-706. http://dx.doi.org/10.1128/MMBR.00023-12

Colford, J.M., Wade, T.J., Schiff, K.C., Wright, C.C., Griffith, J.F., Sandhu, S.K., Burns, S., Sobsey, M., Lovelace, G., Weisberg, S.B. (2007). Water Quality Indicators and the Risk of Illness at Beaches with Nonpoint Sources of Fecal Contamination. *Epidemiology*, 18: 27–35. DOI: 10.1097/01.ede.0000249425.32990.b9

Dubinsky, E.A., and Andersen, G. (2014). Russian River Human Impact Study PhyloChip Microbial Community Analysis. Final Report: May 1, 2014.

Dubinsky, E.A., Butkus, S.R., Andersen, G.L. (2016). Microbial source tracking in impaired watersheds using PhyloChip and machine-learning classifications. *Water Research* 105: 56-64. http://dx.doi.org/10.1016/j.watres.2016.08.035

Dubinsky, E.A., Esmaili, L., Hulls, J.R., Cao, Y., Griffith, J.F., Andersen, G.L. (2012). Application of Phylogenetic Microarray Analysis to Discriminate Sources of Fecal Pollution. *Environmental Science & Technology*, 46, 4340-4347. DOI: dx.doi.org/10.1021/es2040366

Emerson, D., Vecchia, A., & Dahl, A. (2005). Evaluation of Drainage-Area Ratio Method Used to Estimate Streamflow for the Red River of the North Basin, North Dakota and Minnesota. U.S. Geological Survey, Scientific Investigations Report 2005-2007, Reston, VI. Online at: https://pubs.usgs.gov/sir/2005/5017/pdf/sir20055017.pdf

ESS Group Inc. (2003). Palmer River Microbial Source Tracking (MST) Study. Produced for Massachusetts Department of Environmental Protection. M252-000.11, May 16, 2003.

ESS Group Inc. (2004). Bacteria TMDL for the Palmer River Basin. Massachusetts Department of Environmental Protection. Final Report MA 53 03 2004-2 CN 182.0.

FBE. (2012). Barrington-Palmer-Warren Rivers Watershed Plan. Barrington, Bristol, East Providence, and Warren, RI. Rehoboth, Seekonk, and Swansea, MA. Prepared for the United States Environmental Protection Agency Region 1, November 2012.

Hardina, C.M., Fujioka, R.S. (1991). Soil: the environmental source of *Escherichia coli* and enterococci in Hawaii's streams. *Environ. Toxicol. Water Qual.* 6, 185-195. http://dx.doi.org/10.1002/tox.2530060208

Hazen et. al. (2010). Deep-Sea Oil Plume Enriches Indigenous Oil-Degrading Bacteria. *Science*, 330 (6001), 204-208. DOI: 10.1126/science.1195979

HWG & FBE. (2019a). Secondary Data Quality Assurance Project Plan (QAPP) for the Palmer River Source Tracking, Water Quality Trends Summary, and Watershed Plan. Prepared for the US Environmental Protection Agency by Horsley Witten Group and FB Environmental Associates, February 4, 2019.

HWG & FBE. (2019b). Palmer River Land Use & Regulatory Analysis Report. Prepared for the US Environmental Protection Agency by Horsley Witten Group and FB Environmental Associates, December 2019.

HWG & FBE. (2019c). Technical Memorandum: Task 3: PhyloChip® Prioritization & Results. Prepared for the US Environmental Protection Agency by Horsley Witten Group and FB Environmental Associates, December 2019.

Imamura, G.J., Thompson, R.S., Boehm, A.B., Jay, J.A. (2011). Wrack promotes the persistence of fecal indicator bacteria in marine sands and seawater. *FEMS Microbiol. Ecol.* 77, 40-49. http://dx.doi.org/10.1111/j.1574-6941.2011.01082.x

Ishii, S., Ksoll, W.B., Hicks, R.E., Sadowsky, M.J. (2006). Presence and growth of naturalized *Escherichia coli* in temperate soils from lake superior watersheds. *Appl. Environ. Microbiol.* 72, 612-621. http://dx.doi.org/10.1128/AEM.72.1.612- 621.2006

Meals, D.W., & Dressing, S.A. Lag time in water quality response to land treatment. Tech Notes 4, September 2008. Developed for U.S. Environmental Protection Agency by Tetra Tech, Inc., Fairfax, VA, 16 p. Online at https://www.epa.gov/polluted-runoffnonpoint-source-pollution/nonpoint-source-monitoring-technical-notes.

Nelson, K.L., Boehm, A.B., Davies-Colley, R.J., Dodd, M.C., Kohn, T., Linden, K.G., Liu, Y., Maraccini, P.A., McNeill, K., Mitch, W.A., Nguyen, T.H., Parker, K.M., Rodriguez, R.A., Sassoubre, L.M., Silverman, A.I., Wigginton, K.R., Zepp, R.G. (2018). Sunlight-mediated inactivation of health-relevant microorganisms in water: a review of mechanisms and modeling approaches. Environmental Science: Processes & Impacts, 20, 1089. DOI: 10.1039/c8em00047f

Park, Y., Pachespsky, Y., Hong, E-M., Shelton, D., Coppock, C. (2017). Escherichia coli Release from Streambed to Water Column during Baseflow Periods: A Modeling Study. *Journal of Environmental Quality,* 46, 219-226. doi:10.2134/jeq2016.03.0114

Pisciotta, et al. (2002). Marine bacteria cause false-positive results in the Colilert-18 rapid identification test for *Escherichia coli* in Florida waters. Applied and Environmental Microbiology, February 2002, 68 (2), p. 539-544.

RIDEM. (2002). Fecal Coliform TMDL for the Palmer River, Rhode Island. Rhode Island Department of Environmental Management.

Tetra Tech, Inc. (2014a). Minimum detectable change analysis, Palmer River, MA. April 7, 2014, p. 1-11.

Tetra Tech, Inc. (2014b). Load Estimation from Monitoring Data. US EPA Archive Document, December 17, 2014. Online at: https://archive.epa.gov/region5/agriculture/web/pdf/webinar-20141217.pdf

US EPA. (2000). Ambient Water Quality Criteria Recommendations: Information Supporting Development of State and Tribal Nutrient Criteria: Rivers and Streams in Nutrient Ecoregion XIV. EPA 822-B-00-022, December 2000. Online at: https://www.epa.gov/sites/production/files/documents/rivers14.pdf

US EPA. (2003). Developing Water Quality Criteria for Suspended and Bedded Sediment (SABS): Potential Approaches. DRAFT, August 2003. Online at: https://www.epa.gov/sites/production/files/2015-10/documents/sediment-report.pdf

Wade, T. J., Pai, N., Eisenberg, J. N. S., Colford, J. M. (2003). Do U. S. Environmental Protection Agency Water Quality Guidelines for Recreational Waters Prevent Gastrointestinal Illness? A Systematic Review and Meta-Analysis. *Environ. Health Perspect.* 111 (8), 1102−1109.

Wade, T.J., Sams, E., Brenner, K.P., Haugland, R., Chern, E., Beach, M., Wymer, L., Rankin, C.C., Love, D., Li, Q., Noble, R., Dufour, A.P. (2010). Rapidly measured indicators of recreational water quality and swimming-associated illness at marine beaches: a prospective cohort study. *Environmental Health*, 9 (66), 1-14. doi:10.1186/1476-069X-9-66

Whitman, R.L., Harwood, V.J., Edge, T.A., Nevers, M.B., Byappanahalli, M., Vijayavel, K., Brand~ao, J., Sadowsky, M.J., Alm, E.W., Crowe, A., Ferguson, D., Ge, Z., Halliday, E., Kinzelman, J., Kleinheinz, G., Przybyla-Kelly, K., Staley, C., Staley, Z., Solo-Gabriele, H.M. (2014). Microbes in beach sands: integrating environment, ecology and public health. *Rev. Environ. Sci. Biotechnol.* 13, 329-368. http://dx.doi.org/10.1007/s11157-014-9340-8

Wu, M.Z., O'Carroll, D.M., Vogel, L.J., Robinson, C.E. (2017). Effect of Low Energy Waves on the Accumulation and Transport of Fecal Indicator Bacteria in Sand and Pore Water at Freshwater Beaches. *Environ. Sci. Technol.* 51 (5), 2786-2794. https://doi.org/10.1021/acs.est.6b05985

Yamahara, K. M., Layton, B. A., Santoro, A. E., Boehm, A. B. (2007). Beach Sands along the California Coast Are Diffuse Sources of Fecal Bacteria to Coastal Waters. *Environ. Sci. Technol.* 41 (13), 4515−4521.

Young, N. (2016). The association between marine bathing and infectious diseases – a review. Journal of the Marine Biological Association of the United Kingdom, 96(1), 93-100. doi:10.1017/S0025315415002003

APPENDIX 1

Sub-basin drainage areas to twelve "core" sample site locations in the Palmer River watershed.

APPENDIX 2

Summary statistics (median, average, minimum, maximum, number of samples (after duplicate days averaged), number of years, start year, and end year) by site and parameter for twelve "core" sites monitored in the Palmer River watershed. Values exceeding state criteria or natural background conditions are displayed as bold red or orange, respectively. Refer to the end of the table for a list of applied thresholds and other assumptions. *E. coli* for saline sites were greyed out because *E. coli* has been shown to result in false positives in marine waters (Pisciotta et al., 2002) and thus is not the preferred indicator for saline sites.

Site	Parameter	Median	Average	Min	Max	*n* (samples)	*n* (years)	Start Year	End Year
CR01	E. COLI	55	57	2	**4884**	41	9	2001	2018
CR01	NITRATE + NITRITE	**0.330**	**0.703**	0.023	**2.900**	23	3	2016	2018
CR01	ORTHOPHOSPHATE	0.014	0.020	0.005	**0.087**	20	3	2016	2018
CR01	TOTAL KJELDAHL NITROGEN	**0.425**	**0.474**	0.240	**0.889**	22	3	2016	2018
CR01	TOTAL NITROGEN	**0.890**	**1.118**	0.370	**2.500**	23	3	2016	2018
CR01	TOTAL PHOSPHORUS	**0.071**	**0.092**	0.018	**0.240**	23	3	2016	2018
CR01	TOTAL SUSPENDED SOLIDS	3.4	5.6	2.5	18.0	24	3	2016	2018
CR02	E. COLI	**471**	**414**	18	**24196**	44	10	1999	2018
CR02	NITRATE + NITRITE	**1.200**	**1.206**	0.200	**2.100**	23	3	2016	2018
CR02	ORTHOPHOSPHATE	**0.065**	**0.084**	**0.027**	**0.220**	20	3	2016	2018
CR02	TOTAL KJELDAHL NITROGEN	0.300	**0.354**	0.010	**0.800**	23	3	2016	2018
CR02	TOTAL NITROGEN	**1.600**	**1.560**	**0.640**	**2.500**	23	3	2016	2018
CR02	TOTAL PHOSPHORUS	**0.150**	**0.164**	**0.060**	**0.450**	23	3	2016	2018
CR02	TOTAL SUSPENDED SOLIDS	2.7	6.0	2.5	31.0	24	3	2016	2018
CR03	E. COLI	**315**	**324**	12	**24196**	52	11	1999	2018
CR03	NITRATE + NITRITE	**0.460**	**0.533**	0.023	**1.400**	23	3	2016	2018
CR03	ORTHOPHOSPHATE	**0.100**	**0.116**	0.005	**0.270**	21	5	2001	2018
CR03	TOTAL KJELDAHL NITROGEN	**0.370**	**0.411**	0.210	**1.100**	25	5	2001	2018
CR03	TOTAL NITROGEN	**0.840**	**0.986**	0.450	**2.310**	30	6	2001	2018
CR03	TOTAL PHOSPHORUS	**0.215**	**0.266**	**0.080**	**1.500**	30	6	2001	2018
CR03	TOTAL SUSPENDED SOLIDS	4.1	9.7	2.0	48.0	26	5	2001	2018
PM31	E. COLI	31	33	2	**2420**	35	10	1999	2018
PM31	ENTEROCOCCI	14	23	2	**426**	26	4	2015	2018
PM31	NITRATE + NITRITE	0.145	0.197	0.012	**1.965**	34	5	1996	2018
PM31	ORTHOPHOSPHATE	0.008	0.012	0.001	**0.031**	45	7	1996	2018
PM31	TOTAL KJELDAHL NITROGEN	**0.330**	**0.347**	0.200	**0.840**	27	5	2001	2018
PM31	TOTAL NITROGEN	0.515	**0.594**	0	**2.132**	38	7	1996	2018
PM31	TOTAL PHOSPHORUS	**0.039**	**0.038**	0.011	**0.066**	38	7	1996	2018
PM31	TOTAL SUSPENDED SOLIDS	2.5	4.7	1.0	24.0	28	5	2001	2018
PM30	E. COLI	**136**	**169**	16	**2420**	36	10	1999	2018
PM30	ENTEROCOCCI	**142**	**125**	10	**2910**	27	5	2014	2018
PM30	NITRATE + NITRITE	0.280	**0.333**	0.026	**1.000**	23	3	2016	2018
PM30	ORTHOPHOSPHATE	0.012	0.018	0.005	**0.123**	30	4	2001	2018
PM30	TOTAL KJELDAHL NITROGEN	**0.301**	**0.325**	0.220	**0.700**	23	3	2016	2018
PM30	TOTAL NITROGEN	**0.590**	**0.658**	0.320	**1.700**	23	3	2016	2018
PM30	TOTAL PHOSPHORUS	**0.042**	**0.046**	0.018	**0.099**	23	3	2016	2018
PM30	TOTAL SUSPENDED SOLIDS	2.5	3.3	2.5	9.8	24	3	2016	2018
PM44	*E. COLI*	*1230*	*957*	*95*	*6328*	*5*	*3*	*2013*	*2016*
PM44	ENTEROCOCCI	**426**	**326**	10	**7701**	26	4	2015	2018
PM44	NITRATE + NITRITE	0.210	0.182	0.023	**0.380**	23	3	2016	2018
PM44	ORTHOPHOSPHATE	0.015	0.018	0.005	**0.044**	16	3	2016	2018
PM44	TOTAL KJELDAHL NITROGEN	**0.449**	**0.489**	0.300	**0.879**	23	3	2016	2018
PM44	TOTAL NITROGEN	**0.650**	**0.669**	0.400	**1.100**	23	3	2016	2018
PM44	TOTAL PHOSPHORUS	**0.057**	**0.062**	**0.026**	**0.110**	23	3	2016	2018
PM44	TOTAL SUSPENDED SOLIDS	5.0	8.5	2.5	45.0	24	3	2016	2018
RR23	E. COLI	**154**	126	4	**1099**	42	11	2001	2018
RR23	ENTEROCOCCI	**95**	**90**	10	**776**	25	4	2015	2018
RR23	NITRATE + NITRITE	0.170	0.222	0.005	**0.802**	34	5	1996	2018
RR23	ORTHOPHOSPHATE	0.016	0.021	0.000	**0.120**	41	6	1996	2018
RR23	TOTAL KJELDAHL NITROGEN	**0.382**	**0.437**	0.271	**0.990**	25	4	2001	2018

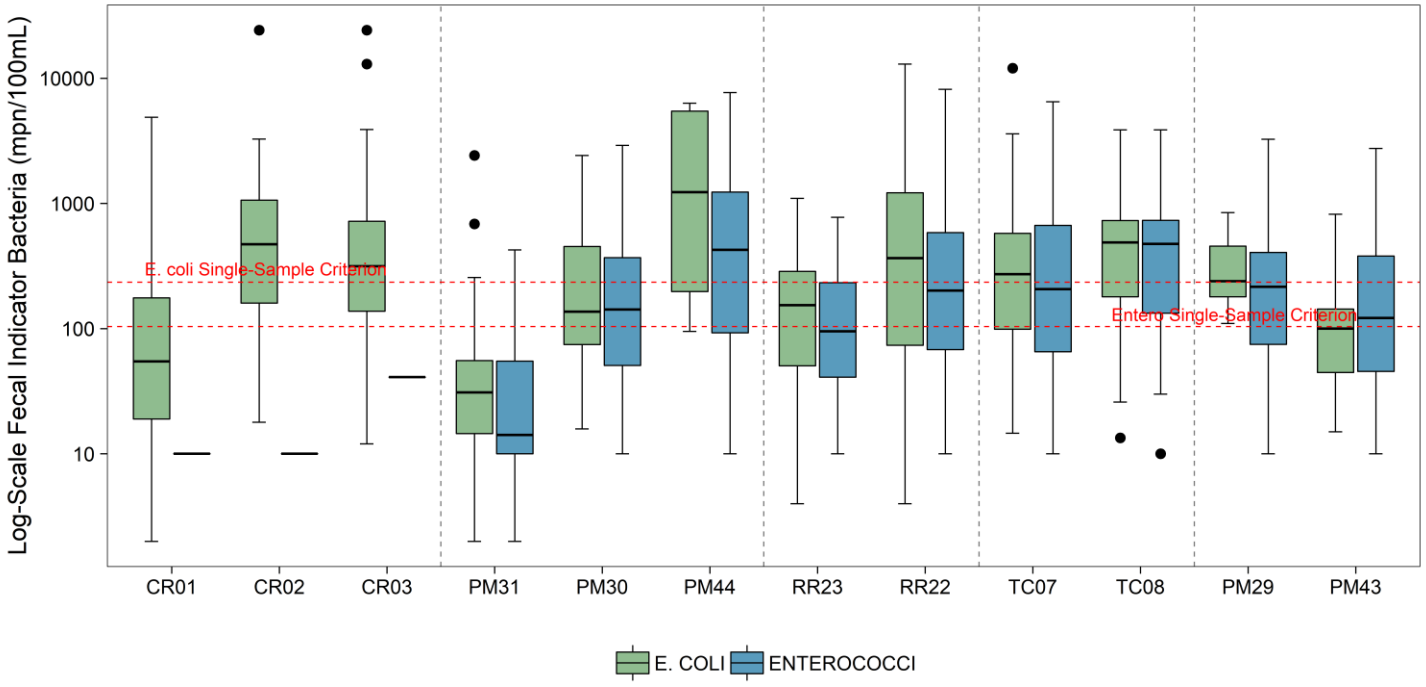
Site	Parameter	Median	Average	Min	Max	*n* (samples)	*n* (years)	Start Year	End Year
RR23	TOTAL NITROGEN	0.655	0.769	0.360	1.424	40	7	1996	2018
RR23	TOTAL PHOSPHORUS	0.043	0.046	0.010	0.140	41	7	1996	2018
RR23	TOTAL SUSPENDED SOLIDS	2.5	4.3	1.0	44.0	26	4	2001	2018
RR22	*E. COLI*	*365*	*336*	*4*	*12997*	*43*	*12*	*1999*	*2018*
RR22	ENTEROCOCCI	201	192	10	8160	29	6	2013	2018
RR22	NITRATE + NITRITE	0.180	0.215	0.023	0.500	23	3	2016	2018
RR22	ORTHOPHOSPHATE	0.014	0.021	0.005	0.050	17	5	2001	2018
RR22	TOTAL KJELDAHL NITROGEN	0.490	0.571	0.300	1.020	27	5	2001	2018
RR22	TOTAL NITROGEN	0.810	0.808	0.410	1.400	27	5	2001	2018
RR22	TOTAL PHOSPHORUS	0.040	0.050	0.018	0.120	27	5	2001	2018
RR22	TOTAL SUSPENDED SOLIDS	2.9	5.8	1.0	51.0	28	5	2001	2018
TC07	*E. COLI*	*272*	*266*	*15*	*12033*	*39*	*9*	*2001*	*2018*
TC07	ENTEROCOCCI	206	211	10	6488	26	5	2013	2018
TC07	NITRATE + NITRITE	0.460	0.503	0.054	1.000	23	3	2016	2018
TC07	ORTHOPHOSPHATE	0.010	0.015	0.005	0.050	24	5	2001	2018
TC07	TOTAL KJELDAHL NITROGEN	0.360	0.414	0.240	0.900	27	5	2001	2018
TC07	TOTAL NITROGEN	0.940	0.993	0.400	1.930	27	5	2001	2018
TC07	TOTAL PHOSPHORUS	0.037	0.041	0.020	0.080	27	5	2001	2018
TC07	TOTAL SUSPENDED SOLIDS	2.9	4.0	2.5	18.0	28	5	2001	2018
TC08	*E. COLI*	*487*	*348*	*13*	*3873*	*37*	*8*	*2002*	*2018*
TC08	ENTEROCOCCI	475	326	10	3873	27	5	2013	2018
TC08	NITRATE + NITRITE	0.081	0.084	0.023	0.260	23	3	2016	2018
TC08	ORTHOPHOSPHATE	0.005	0.008	0.005	0.027	19	3	2016	2018
TC08	TOTAL KJELDAHL NITROGEN	0.559	0.569	0.339	0.919	23	3	2016	2018
TC08	TOTAL NITROGEN	0.610	0.650	0.350	1.000	23	3	2016	2018
TC08	TOTAL PHOSPHORUS	0.026	0.031	0.014	0.096	23	3	2016	2018
TC08	TOTAL SUSPENDED SOLIDS	2.5	5.4	2.5	45.0	24	3	2016	2018
PM29	*E. COLI*	*239*	*281*	*110*	*846*	*6*	*4*	*2012*	*2016*
PM29	ENTEROCOCCI	216	177	10	3255	29	6	2013	2018
PM29	NITRATE + NITRITE	0.120	0.132	0.023	0.300	26	4	1998	2018
PM29	ORTHOPHOSPHATE	0.023	0.025	0.005	0.056	17	4	1998	2018
PM29	TOTAL KJELDAHL NITROGEN	0.543	0.559	0.290	1.580	26	4	1998	2018
PM29	TOTAL NITROGEN	0.630	0.688	0.370	1.800	26	4	1998	2018
PM29	TOTAL PHOSPHORUS	0.054	0.060	0.027	0.130	26	4	1998	2018
PM29	TOTAL SUSPENDED SOLIDS	7.0	8.5	2.5	33.7	27	4	1998	2018
PM43	*E. COLI*	*100*	*91*	*15*	*820*	*8*	*5*	*2001*	*2016*
PM43	ENTEROCOCCI	121	142	10	2755	28	6	2013	2018
PM43	NITRATE + NITRITE	0.079	0.106	0.023	0.240	23	3	2016	2018
PM43	ORTHOPHOSPHATE	0.027	0.037	0.007	0.099	27	5	2001	2018
PM43	TOTAL KJELDAHL NITROGEN	0.554	0.616	0.300	1.430	27	5	2001	2018
PM43	TOTAL NITROGEN	0.730	0.742	0.350	1.600	27	5	2001	2018
PM43	TOTAL PHOSPHORUS	0.060	0.089	0.036	0.580	27	5	2001	2018
PM43	TOTAL SUSPENDED SOLIDS	7.4	8.9	3.0	32.0	28	5	2001	2018

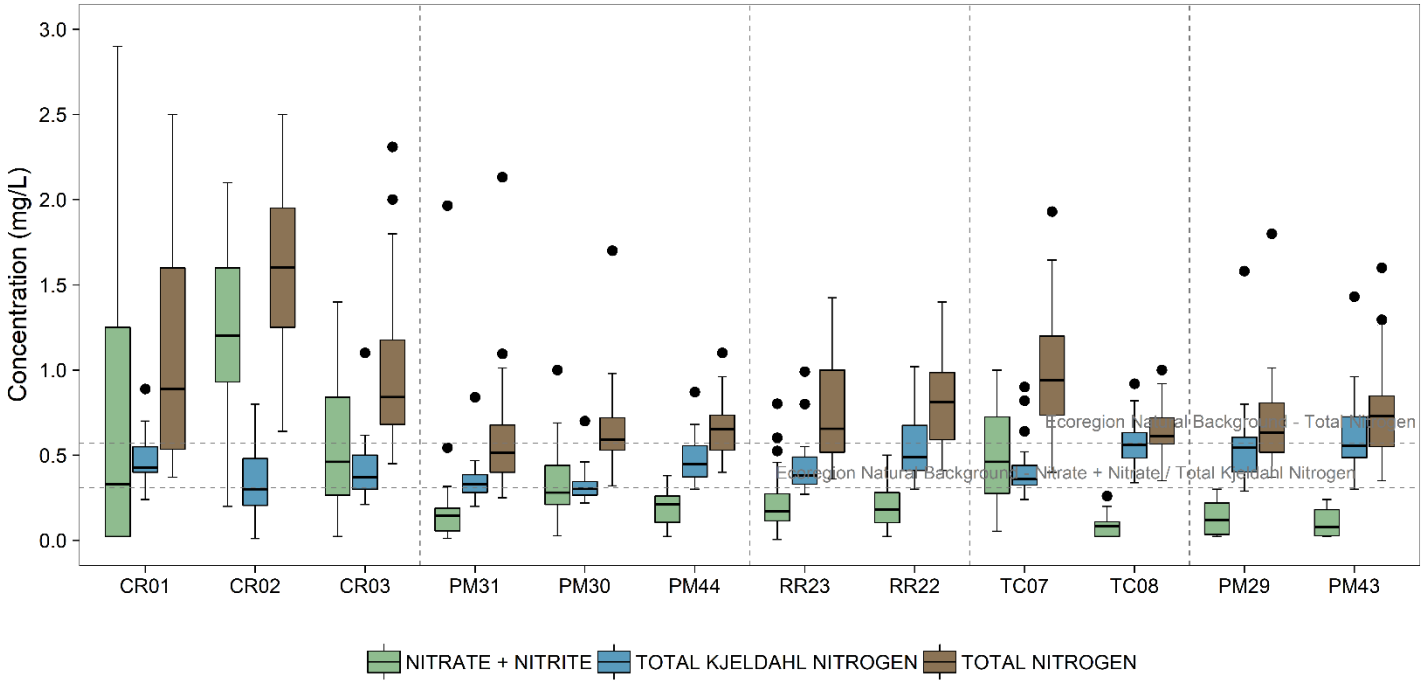
E. coli	*126 mpn/100mL (geomean); 235 mpn/100mL (single)*
Enterococci	*35 mpn/100mL (geomean); 104 mpn/100mL (single)*
Nitrate + Nitrite	*0.31 mg/L*
Total Kjeldahl nitrogen	*0.30 mg/L*
Total Nitrogen	*0.57 mg/L*
Orthophosphate	*0.024 mg/L (used Total Phosphorus Reference Condition)*
Total Phosphorus	*0.024 mg/L*
Total suspended solids	*30 mg/L (30-day average), 58 mg/L (daily max)*

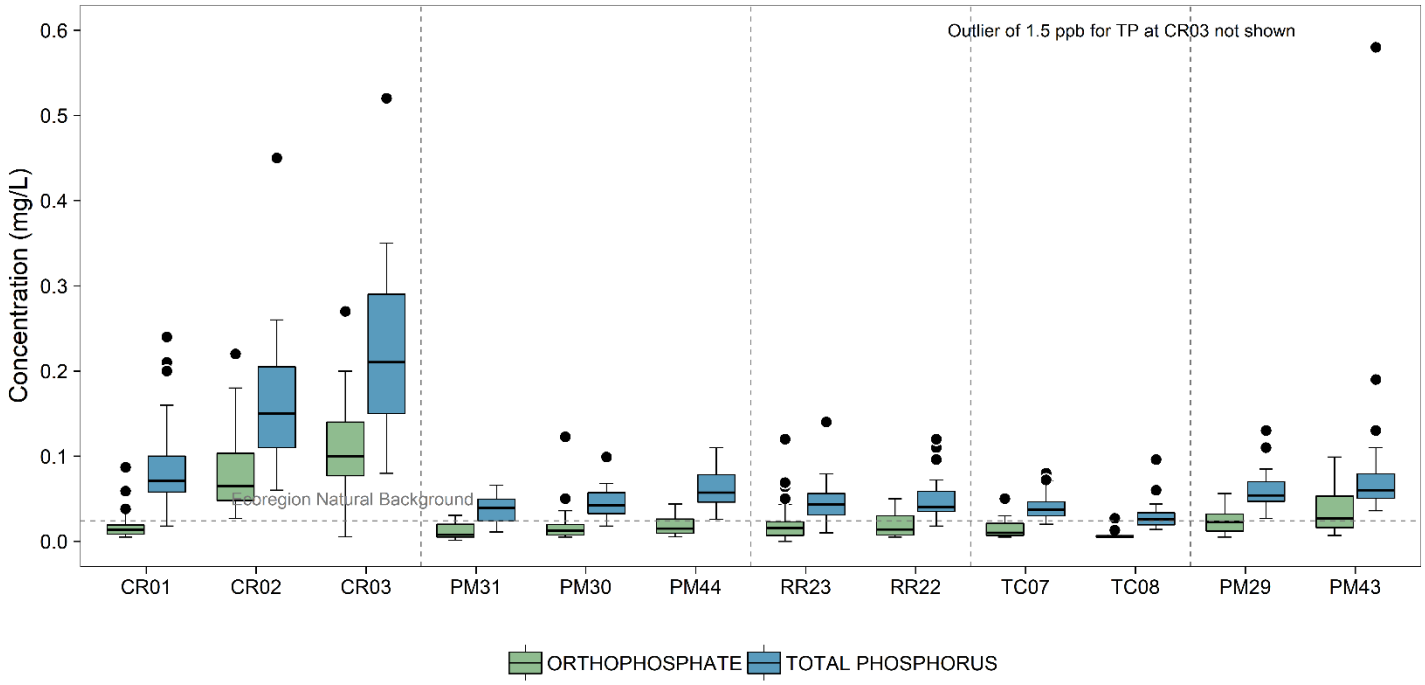
Note: both median and average *E. coli* and enterococci values were log-transformed before summarized (average represents true geomean)

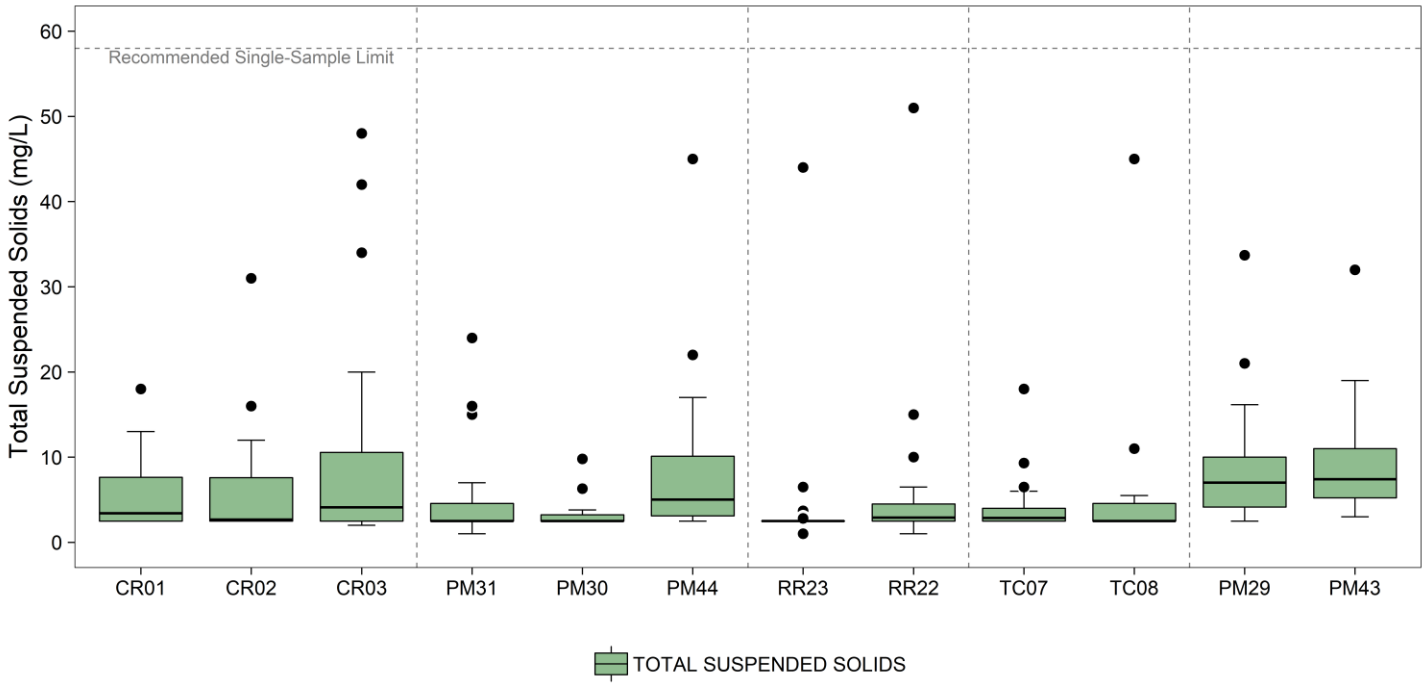
APPENDIX 3

Summary of all data distribution by parameter for sites ordered from upstream to downstream (vertical gray dashed lines represent tributary inputs to the mainstem). The top and bottom of the box area in each boxplot represent the 75th and 25th percentiles of the data, respectively. The solid horizontal line in each box represents the median or 50th percentile of the data. The top and bottom whiskers represent the maximum and minimum non-outliers of the data, respectively. Any points above or below the whiskers are outliers, defined as 1.5 times the interquartile range (or the length of the box). Single horizontal lines represent only a single data point. Applicable criteria or natural background conditions are shown in red or grey horizontal dashed lines, respectively.






Outlier of 1.5 ppb for TP at CR03 not shown
Concentration (mg/L)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Ecoregion Natural Background
CR01
CR02
CR03
PM31
PM30
PM44
RR23
RR22
TC07
TC08
PM29
PM43
ORTHOPHOSPHATE
TOTAL PHOSPHORUS


Recommended Single-Sample Limit
Total Suspended Solids (mg/L)
60
50
40
30
20
10
0
CR01
CR02
CR03
PM31
PM30
PM44
RR23
RR22
TC07
TC08
PM29
PM43
TOTAL SUSPENDED SOLIDS

APPENDIX 4

Example score calculation for CR01 to rank the twelve "core" sampling sites from relatively better to worse water quality. *E. coli* is the only parameter with criteria set by state water quality standards. Natural background conditions for the nutrient parameters were obtained from USEPA (2000). Recommended criteria for total suspended solids were obtained from USEPA (2003). We distinguished between "chronic" and "acute" water quality criteria to account for geomean and single-sample criteria for fecal indicator bacteria (*E. coli)* and 30-day average and daily max recommended criteria for total suspended solids. Sub-scores less than 1 (shown as grey italicized text) indicate no exceedance and were not included in the total score.

Parameter	Avg	Min	Max	Chronic WQ Criteria	Acute WQ Criteria	Avg/Chronic WQ Criteria	Min/Acute WQ Criteria	Max/Acute WQ Criteria	Sum Score
E. COLI (MPN/100ML)	57	2	4884	126	235	*0.45*	*0.01*	20.78	20.78
NITRATE + NITRITE (MG/L)	0.703	0.023	2.900	0.310	0.310	2.27	*0.07*	9.35	11.62
ORTHOPHOSPHATE (MG/L)	0.020	0.005	0.087	0.024	0.024	*0.84*	*0.21*	3.63	3.63
TOTAL KJELDAHL NITROGEN (MG/L)	0.474	0.240	0.889	0.300	0.300	1.58	*0.80*	2.96	4.54
TOTAL NITROGEN (MG/L)	1.118	0.370	2.500	0.570	0.570	1.96	*0.65*	4.39	6.35
TOTAL PHOSPHORUS (MG/L)	0.092	0.018	0.240	0.024	0.024	3.82	*0.75*	10.00	13.82
TOTAL SUSPENDED SOLIDS (MG/L)	5.6	2.5	18.0	30.0	58.0	*0.19*	*0.04*	*0.31*	0.00
								Total Score	**60.74**

APPENDIX 5

Agricultural BMP definitions and pollutant reduction efficiencies were adapted from STEPL 4.4 documentation. TN=Total Nitrogen. TP=Total Phosphorus. Sed=Total Sediment. Red.=Reduction. Eff.=Efficiency. Reduction efficiencies are based on a per unit area, except for grass buffer which is based on a minimum of 35 linear feet.

BMP	Definition	TN Red. Eff.	TP Red. Eff.	Sed. Red. Eff.
Terrace	A terrace is an earth embankment, or a combination ridge and channel, constructed across the field slope to enable water to be stored temporarily to allow sediment deposition and water infiltration. This practice is applied as part of a management system to either reduce erosion and trap sediment or retain runoff for moisture conservation.	0.253	0.308	0.400
Prescribed Grazing	Prescribed grazing is the controlled harvest of vegetation with grazing or browsing animals, managed with the intent to maintain or improve water quality and quantity. For example, on grazed forest, native pasture, or rangeland, grazing is limited so that the grazing animals will consume no more than 50 percent (by weight) of the annual growth of high or medium preferred grazing species.	0.408	0.227	0.333
Critical Area Planting	Critical area planting is the planting of grasses, legumes, or other vegetation to stabilize slopes in small, severely eroding areas. The permanent vegetation stabilizes areas such as gullies, over-grazed hillsides and terraced backslopes. Although the primary goal is erosion control, the vegetation can also provide nesting cover for birds and small animals.	0.175	0.200	0.420
Conservation Tillage 2 (equal or more than 60% residue)	Limiting soil disturbance to manage the amount, orientation and distribution of crop and plant residue on the soil surface year-round. This will reduce sheet, rill and wind erosion and excessive sediment in surface waters; reduce tillage-induced particulate emissions; maintain or increase soil health and organic matter content; increase plant-available moisture; reduce energy use; and provide food and escape cover for wildlife.	0.250	0.687	0.770
Diverted Drainage	Capturing runoff from paved surfaces and diverting the flow away from agricultural fields.	0.450	0.700	0.00
Grass Swale	Grass swales are elongated depressions in the land surface that are at least seasonally wet, usually heavily vegetated, and normally without flowing water. Swales direct stormwater flows into primary drainage channels and allow some of the stormwater to infiltrate to the ground. Swales are vegetated with erosion resistant and flood tolerant grasses. Sometimes check dams are strategically placed in swales to moderate flow and an engineered soil mixture might underlie swales.	0.100	0.250	0.650

BMP	Definition	TN Red. Eff.	TP Red. Eff.	Sed. Red. Eff.
Litter Storage and Management	Can consist of a manure storage facility, bedded pack, manure composting, etc. Any practice which confines animal litter to an area designed to manage litter via confinement, treatment, or removal.	0.140	0.140	0.00
Livestock Exclusion Fencing	Fencing is used to restrict livestock access to streambanks because animal traffic erodes streambanks, increases sediment load, and contributes animal waste in and near the stream, impairing water quality.	0.203	0.304	0.620
Grass Buffer	A newly established area along a waterbody that intercepts overland flow and is used to maintain bank stabilization, reduce the impacts of upland sources of pollution by trapping, filtering, and converting sediments, nutrients, and other chemicals to supply food, cover and thermal protection to fish and other wildlife. To achieve these results, the recommended minimum width is 35 feet and should include native grass(es).	0.868	0.766	0.648
Use Exclusion	Pasteurized land no longer used for pasture. Land use converted away from pasture. All animals are sold, but the land is not necessarily retired from crop production, development, or regular mowing.	0.390	0.040	0.589
Heavy Use Area Protection	Heavy use area protection is used to stabilize ground surface that is frequently and intensively used by people, animals, or vehicles. Heavy use area protection is used to provide a stable, non-eroding surface and to protect or improve water quality.	0.183	0.193	0.333

APPENDIX 6

Observed annual loads compared to modeled annual loads for total nitrogen (N), total phosphorus (P), and total sediment (Sed) by year for twelve "core" sites in the Palmer River watershed. Observed annual loads were calculated from average daily measured concentration, as well as areal-weighted flow and flow exceedance probability from USGS 01109403 Ten Mile River, Pawtucket Ave at East Providence, RI. Modeled annual loads were determined using the Spreadsheet Tool for Estimating Pollutant Load (STEPL) and accounted for changes in land use over specific time periods (1995, 2001, 2005, 2011, 2015, and 2018). BMP Installed indicates whether one or more agricultural BMPs under the NWQI were installed in a given sub-basin to a sampling site. Wet or Dry indicates whether a given year was above (wet) or below (dry) the median total annual precipitation from 1996-2018 for NOAA NCEI Providence RI US (Station #USW00014765).

Year	Site	Observed N Load (lbs./yr)	Observed P Load (lbs./yr)	Observed Sed Load (tons/yr)	Modeled N Load (lbs./yr)	Modeled P Load (lbs./yr)	Modeled Sed Load (tons/yr)	BMP Installed	Wet or Dry
1996	PM31	28,993	1,012	*NA*	47,652	13,407	575	N	WET
1996	RR23	9,094	369	*NA*	10,934	3,262	119	N	WET
1997	PM31	44,583	376	*NA*	47,652	13,407	575	N	DRY
1997	RR23	6,710	372	*NA*	10,934	3,262	119	N	DRY
1998	PM29	66,166	3,789	687	76,866	21,204	985	N	WET
2001	CR03	2,572	186	11	8,425	2,210	92	N	DRY
2001	PM31	28,179	1,360	54	48,294	13,494	585	N	DRY
2001	PM43	48,791	11,460	213	78,424	21,462	1,010	N	DRY
2001	RR22	12,864	941	51	18,625	5,154	232	N	DRY
2001	RR23	9,203	668	75	11,425	3,330	129	N	DRY
2001	TC07	3,897	79	5	4,796	1,266	82	N	DRY
2002	CR03	4,941	556	13	8,425	2,210	92	N	DRY
2002	PM31	35,045	1,659	21	48,294	13,494	585	N	DRY
2002	PM43	81,600	11,972	1,008	78,424	21,462	1,010	N	DRY
2002	RR22	10,169	561	7	18,625	5,154	232	N	DRY
2002	TC07	3,406	194	6	4,796	1,266	82	N	DRY
2009	CR03	2,020	507	*NA*	8,403	2,208	93	N	WET
2009	RR23	9,969	426	*NA*	11,463	3,340	130	N	WET
2016	CR01	358	51	2	3,117	985	25	N	DRY
2016	CR02	1,571	149	5	7,029	1,866	68	Y	DRY
2016	CR03	1,452	271	7	8,403	2,208	93	Y	DRY
2016	PM29	28,225	2,947	212	78,766	21,447	1,014	Y	DRY

Year	Site	Observed N Load (lbs./yr)	Observed P Load (lbs./yr)	Observed Sed Load (tons/yr)	Modeled N Load (lbs./yr)	Modeled P Load (lbs./yr)	Modeled Sed Load (tons/yr)	BMP Installed	Wet or Dry
2016	PM30	24,319	1,379	56	51,193	14,229	616	Y	DRY
2016	PM31	13,154	1,275	61	48,676	13,536	590	Y	DRY
2016	PM43	25,267	2,960	215	79,148	21,546	1,019	Y	DRY
2016	PM44	22,714	2,197	181	53,929	14,800	672	Y	DRY
2016	RR22	7,630	477	33	18,979	5,203	239	Y	DRY
2016	RR23	3,417	237	10	11,662	3,372	135	Y	DRY
2016	TC07	1,696	74	6	4,786	1,257	79	Y	DRY
2016	TC08	69	3	1	238	34	5	N	DRY
2017	CR01	810	48	2	3,116	984	25	N	WET
2017	CR02	2,628	217	5	7,028	1,866	68	Y	WET
2017	CR03	2,147	459	13	8,402	2,208	92	Y	WET
2017	PM29	39,053	3,337	151	79,053	21,465	1,012	Y	WET
2017	PM30	24,608	1,780	59	51,317	14,249	619	Y	WET
2017	PM31	18,956	1,595	71	48,790	13,556	593	Y	WET
2017	PM43	42,414	3,627	261	79,435	21,564	1,016	Y	WET
2017	PM44	28,809	2,339	100	54,052	14,820	675	Y	WET
2017	RR22	11,246	518	19	18,972	5,207	240	Y	WET
2017	RR23	5,178	345	11	11,662	3,374	134	Y	WET
2017	TC07	2,386	101	3	4,776	1,252	78	Y	WET
2017	TC08	77	3	0	309	40	6	N	WET
2018	CR01	712	35	1	3,116	984	25	N	WET
2018	CR02	1,978	263	2	7,028	1,866	68	Y	WET
2018	CR03	1,530	712	4	8,402	2,208	92	Y	WET
2018	PM29	37,528	3,011	174	79,053	21,465	1,012	Y	WET
2018	PM30	19,871	1,919	57	51,317	14,249	619	Y	WET
2018	PM31	14,828	1,663	114	48,790	13,556	593	Y	WET
2018	PM43	45,622	3,228	171	79,435	21,564	1,016	Y	WET
2018	PM44	24,344	2,314	135	54,052	14,820	675	Y	WET
2018	RR22	8,087	520	21	18,972	5,207	240	Y	WET
2018	RR23	4,223	338	10	11,662	3,374	134	Y	WET
2018	TC07	2,327	106	3	4,776	1,252	78	Y	WET
2018	TC08	76	3	0	309	40	6	N	WET

APPENDIX 7

PhyloChip® DNA microarray analysis and select water quality results for 50 sites and dates in the Palmer River watershed. PhyloChip® analysis was performed on 8/23/2019 by the University of California at Lawrence Berkeley National Laboratory, CA. Source signal data represent proportions (ranging from 0 to 1). Signal strength thresholds are project-specific based on calibration samples analyzed with diagnostic probes. Values ≥0.2 (red) indicate a strong source signal (likely source). Values ≥0.1 and <0.2 (yellow) indicate a marginal source signal (possible source, recommend additional testing). Values <0.1 indicate low source signal (unlikely source). Gray shaded and bold text indicate exceedance of state single sample criteria for *E. coli* (235 MPN/100mL) and enterococci (104 MPN/100mL). Gray shaded text indicate exceedance of natural background conditions for the ecoregion for total nitrogen (TN, 0.57 mg/L) and total phosphorus (TP, 0.024 mg/L). "Wet" weather determinations were based on more than 0.5" within 72 hours of sample day.

Site	Date	E. coli (MPN/100mL)	Enterococci (MPN/100mL)	TN (mg/L)	TP (mg/L)	Water Temp (°C)	Precip in Prior 7 Days (in)	Wet/Dry	Human	Bird	Dog	Horse	Pig	Cow
TC07	6/20/2017	**355**	**556**	0.94	0.07	19.9	2.30	Dry	0.04	0.14	0.04	0.08	0.03	0.07
TC07	11/14/2017	84	73	1.00	0.03	5.8	0.51	Dry	0.07	0.08	0.05	0.03	0.03	0.09
CR01	4/24/2018	4		2.30	0.05	13.7	0.46	Dry	0.05	0.12	0.01	0.02	0.02	0.03
CR03	4/24/2018	21		1.20	0.13	8.9	0.46	Dry	0.09	0.12	0.03	0.03	0.06	0.04
PM30	4/24/2018	20	10	0.50	0.02	11.6	0.46	Dry	0.06	0.15	0.02	0.03	0.03	0.04
PM43	4/24/2018		10	0.67	0.04	12.8	0.46	Dry	0.11	0.02	0.02	0.05	0.02	0.02
PM44	4/24/2018		10	0.51	0.03	11.9	0.46	Dry	0.05	0.12	0.02	0.03	0.02	0.04
RR22	4/24/2018	31	20	0.53	0.02	9.7	0.46	Dry	0.15	0.04	0.04	0.10	0.04	0.08
TC07	4/24/2018	15	10	1.30	0.02	9.4	0.46	Dry	0.09	0.04	0.13	0.13	0.06	0.11
TC08	4/24/2018	26	10	0.59	0.01	7.8	0.46	Dry	0.15	0.04	0.05	0.06	0.04	0.08
CR01	6/7/2018	13		0.86	0.10	17.7	0.84	Wet	0.14	0.02	0.02	0.02	0.02	0.02
CR03	6/7/2018	**278**		0.65	0.29	14.8	0.84	Wet	0.12	0.11	0.03	0.06	0.06	0.09
PM30	6/7/2018	**548**	**350**	0.52	0.07	15.1	0.84	Wet	0.07	0.13	0.03	0.04	0.03	0.03
PM43	6/7/2018		**515**	0.88	0.08	16.0	0.84	Wet	0.07	0.11	0.00	0.03	0.04	0.03
PM44	6/7/2018		**563**	0.57	0.07	16.2	0.84	Wet	0.07	0.17	0.00	0.05	0.04	0.03
RR22	6/7/2018	**517**	**265**	0.60	0.05	14.5	0.84	Wet	0.03	0.12	0.02	0.04	0.03	0.05
TC07	6/7/2018	**272**	**347**	0.83	0.07	13.9	0.84	Wet	0.10	0.04	0.01	0.08	0.05	0.07
TC08	6/7/2018	**487**	**163**	0.56	0.03	13.5	0.84	Wet	0.11	0.03	0.03	0.08	0.04	0.08
CR01	7/9/2018	4		0.49	0.08	25.3	0.03	Dry	0.08	0.07	0.02	0.02	0.03	0.01
CR03	7/9/2018	198		0.51	0.52	18.1	0.03	Dry	0.26	0.03	0.02	0.04	0.06	0.07
PM30	7/9/2018	133	**177**	0.63	0.06	21.4	0.03	Dry	0.11	0.12	0.02	0.03	0.05	0.03
PM43	7/9/2018		**171**	0.56	0.06	26.2	0.03	Dry	0.04	0.09	0.02	0.02	0.01	0.01
PM44	7/9/2018		**641**	0.68	0.06	24.8	0.03	Dry	0.08	0.08	0.03	0.02	0.01	0.03
RR22	7/9/2018	**1300**	**176**	0.98	0.06	21.8	0.03	Dry	0.10	0.10	0.01	0.03	0.01	0.02
TC07	7/9/2018	**272**	**265**	1.20	0.04	18.3	0.03	Dry	0.23	0.02	0.04	0.07	0.11	0.24
TC08	7/9/2018	**820**	**702**	1.00	0.04	19.5	0.03	Dry	0.16	0.06	0.01	0.02	0.02	0.02
CR01	8/7/2018	**384**		0.42	0.13	28.7	1.63	Wet	0.06	0.08	0.03	0.02	0.02	0.02
CR03	8/7/2018	**1462**		0.60	1.50	22.2	1.63	Wet	0.22	0.04	0.03	0.06	0.07	0.11
PM30	8/7/2018	140	**142**	0.59	0.04	24.7	1.63	Wet	0.14	0.09	0.03	0.04	0.04	0.03
PM43	8/7/2018		**842**	0.57	0.07	29.1	1.63	Wet	0.04	0.11	0.03	0.02	0.01	0.01
PM44	8/7/2018		31	0.65	0.09	28.0	1.63	Wet	0.03	0.14	0.01	0.04	0.01	0.02
RR22	8/7/2018	**1203**	**189**	0.87	0.05	26.4	1.63	Wet	0.06	0.12	0.02	0.03	0.01	0.02

Site	Date	E. coli (MPN/100mL)	Enterococci (MPN/100mL)	TN (mg/L)	TP (mg/L)	Water Temp (°C)	Precip in Prior 7 Days (in)	Wet/Dry	Human	Bird	Dog	Horse	Pig	Cow
TC07	8/7/2018	**345**	**132**	0.67	0.03	23.2	1.63	Wet	0.22	0.05	0.03	0.10	0.07	0.16
TC08	8/7/2018	**1872**	**3654**	0.67	0.02	23.9	1.63	Wet	0.15	0.11	0.03	0.03	0.03	0.04
CR01	9/19/2018	**273**		0.89	0.03	21.7	1.92	Wet	0.05	0.24	0.03	0.01	0.03	0.01
CR03	9/19/2018	**1549**		0.71	0.33	19.2	1.92	Wet	0.22	0.07	0.02	0.04	0.04	0.09
PM30	9/19/2018	**2420**	**2481**	0.61	0.10	20.0	1.92	Wet	0.30	0.09	0.02	0.04	0.06	0.11
PM43	9/19/2018		**2755**	1.60	0.08	22.2	1.92	Wet	0.05	0.21	0.02	0.03	0.03	0.02
PM44	9/19/2018		**2481**	1.10	0.07	20.3	1.92	Wet	0.19	0.12	0.02	0.04	0.06	0.05
RR22	9/19/2018	**1300**	**1014**	0.81	0.06	19.5	1.92	Wet	0.10	0.09	0.01	0.06	0.06	0.06
TC07	9/19/2018	**1120**	**749**	0.80	0.06	19.3	1.92	Wet	0.16	0.07	0.02	0.09	0.07	0.10
TC08	9/19/2018	**1014**	**185**	0.71	0.04	19.1	1.92	Wet	0.08	0.07	0.02	0.05	0.03	0.06
CR01	11/5/2018	**432**		1.60	0.05	10.4	1.63	Wet	0.19	0.11	0.04	0.02	0.01	0.02
CR03	11/5/2018	208		0.84	0.25	8.1	1.63	Wet	0.23	0.08	0.03	0.05	0.06	0.16
PM30	11/5/2018	101	98	0.32	0.05	9.2	1.63	Wet	0.02	0.14	0.01	0.01	0.02	0.02
PM43	11/5/2018		**135**	0.66	0.07	9.7	1.63	Wet	0.09	0.17	0.02	0.06	0.03	0.05
PM44	11/5/2018		**384**	0.40	0.05	9.8	1.63	Wet	0.01	0.07	0.01	0.02	0.02	0.02
RR22	11/5/2018	31	52	0.41	0.04	8.9	1.63	Wet	0.10	0.14	0.03	0.07	0.06	0.13
TC07	11/5/2018	52	31	0.53	0.07	8.5	1.63	Wet	0.16	0.07	0.02	0.09	0.07	0.11
TC08	11/5/2018	201	52	0.35	0.03	8.4	1.63	Wet	0.09	0.09	0.03	0.09	0.05	0.12

APPENDIX 8

Top 10% enriched genera (SIMPER) summed to class for human, bird, and cow source types determined by PhyloChip® DNA microarray analysis. Shading indicates the following: no shading (< 10 OTUs), light yellow (10-50 OTUs), yellow (51-100 OTUs), orange (101-150 OTUs), red (151-300 OTUs), and dark red (>300 OTUs). Classes are ordered from highest to lowest counts for human source type.

P_Phlym c_Class	Human	Bird	Cow
p__Proteobacteria c__Alphaproteobacteria	645	557	305
p__Proteobacteria c__Gammaproteobacteria	631	418	301
p__Firmicutes c__Bacilli	470	362	229
p__Firmicutes c__Clostridia	378	251	216
p__Proteobacteria c__Deltaproteobacteria	397	266	189
p__Actinobacteria c__Actinobacteria	431	391	176
p__Bacteroidetes c__Bacteroidia	102	36	64
p__TM7 c__TM7-3	123	84	64
p__Chloroflexi c__Dehalococcoidetes	101	45	51
p__Planctomycetes c__Planctomycea	112	169	51
p__TG3 c__TG3-2	132	67	49
p__TM6 c__SJA-4	111	83	47
p__WS3 c__PRR-12	77	38	43
p__Acidobacteria c__iii1-8	61	60	35
p__Elusimicrobia c__Elusimicrobia	63	36	34
p__Acidobacteria c__BPC102	46	26	34
p__Nitrospirae c__Nitrospira	50	28	29
p__Proteobacteria c__Betaproteobacteria	65	50	28
p__Chloroflexi c__Ktedonobacteria	51	29	25
p__Bacteroidetes c__Sphingobacteria	38	58	23
p__AC1 c__SHA-114	42	26	23
p__Cyanobacteria c__Chloroplast	58	41	21
p__Acidobacteria c__Chloracidobacteria	40	20	19
p__Chlorobi c__Chlorobia	29	22	16
p__Cyanobacteria c__Nostocophycideae	44	32	13
p__unclassified c__unclassified	23	15	12
p__ZB2 c__unclassified	24	15	12
p__TM7 c__TM7-1	18	12	11
p__Tenericutes c__Erysipelotrichi	21	19	10
p__Chloroflexi c__Anaerolineae	18	8	10
p__Chloroflexi c__Thermomicrobia	20	14	10
p__OP3 c__koll11	22	14	10
p__Deferribacteres c__Deferribacteres	20	10	9
p__Chloroflexi c__TK-SH13	17	12	8
p__Tenericutes c__Mollicutes	12	8	8
p__ABY1_OD1 c__unclassified	18	18	8
p__OP3 c__BD4-9	16	10	8
p__Proteobacteria c__Epsilonproteobacteria	13	7	8
p__Cyanobacteria c__4C0d-2	26	30	7
p__HDBW-WB69 c__unclassified	16	10	7
p__NKB19 c__GN13	13	9	7
p__OP11 c__unclassified	12	4	7
p__Fusobacteria c__Fusobacteria	25	29	6
p__Cyanobacteria c__Oscillatoriophycideae	19	23	6
p__Cyanobacteria c__Synechococcophycideae	19	31	6
p__SAR406 c__AB16	8	10	6
p__Chlamydiae c__Chlamydiae	10	14	5
p__GN02 c__VC12-cl04	11	12	4

APPENDIX 9

Taxonomic richness of bacteria in the Palmer River. Values represent the number of detected OTUs in 50 taxonomic families with the highest summed OTU richness across all samples for each site. Shading indicates the following: no shading (< 10 OTUs), light yellow (10-100 OTUs), yellow (101-500 OTUs), orange (501-1000 OTUs), red (1001-2000 OTUs), and dark red (>2000 OTUs). Taxa are ordered alphabetically.

p_Phylum c_Class o_Order f_Family	CR01	CR03	PM30	PM44	RR22	TC07	TC08	PM43
p__Acidobacteria c__Acidobacteria o__Acidobacteriales f__Acidobacteriaceae	93	344	363	216	413	595	475	265
p__Acidobacteria c__Solibacteres o__Solibacterales f__Solibacteraceae	66	217	229	130	236	378	249	125
p__Actinobacteria c__Actinobacteria o__Actinomycetales f__Corynebacteriaceae	978	1010	1156	1048	1132	1705	1095	1205
p__Actinobacteria c__Actinobacteria o__Actinomycetales f__Microbacteriaceae	191	318	329	238	282	448	240	327
p__Actinobacteria c__Actinobacteria o__Actinomycetales f__Micrococcaceae	114	206	182	150	162	362	179	176
p__Actinobacteria c__Actinobacteria o__Actinomycetales f__Mycobacteriaceae	63	388	306	212	332	486	185	250
p__Actinobacteria c__Actinobacteria o__Actinomycetales f__Streptomycetaceae	76	656	398	189	479	472	208	245
p__Actinobacteria c__Actinobacteria o__MC47 f__unclassified	55	162	163	99	167	254	175	111
p__Bacteroidetes c__Bacteroidia o__Bacteroidales f__RikenellaceaeII	270	557	426	307	364	819	435	362
p__Bacteroidetes c__Flavobacteria o__Flavobacteriales f__Flavobacteriaceae	1344	1661	1466	1375	1239	1819	1208	1680
p__Bacteroidetes c__Sphingobacteria o__Sphingobacteriales f__Chitinophagaceae	241	266	308	215	282	458	250	250
p__Firmicutes c__Bacilli o__Bacillales f__Bacillaceae	942	2484	2070	1389	1577	2471	998	1523
p__Firmicutes c__Bacilli o__Bacillales f__Paenibacillaceae	59	357	220	123	188	335	152	127
p__Firmicutes c__Bacilli o__Bacillales f__Planococcaceae	42	181	165	124	172	211	75	125
p__Firmicutes c__Bacilli o__Bacillales f__Staphylococcaceae	70	272	104	89	260	179	190	83
p__Firmicutes c__Bacilli o__Lactobacillales f__Streptococcaceae	98	187	114	66	129	164	201	202
p__Firmicutes c__Clostridia o__Clostridiales f__Clostridiaceae	576	1047	942	483	655	910	553	451
p__Firmicutes c__Clostridia o__Clostridiales f__Clostridiales Family XI. Incertae Sedis	98	175	149	100	118	222	158	122
p__Firmicutes c__Clostridia o__Clostridiales f__Lachnospiraceae	478	2058	1342	794	1073	2303	1138	881
p__Firmicutes c__Clostridia o__Clostridiales f__Ruminococcaceae	244	777	531	336	513	1289	618	303
p__Firmicutes c__Clostridia o__Clostridiales f__Veillonellaceae	85	184	148	91	138	221	139	98
p__Planctomycetes c__Planctomycea o__Planctomycetales f__Planctomycetaceae	84	163	161	154	215	292	181	236
p__Proteobacteria c__Alphaproteobacteria o__Caulobacterales f__Caulobacteraceae	79	262	276	186	244	433	231	178
p__Proteobacteria c__Alphaproteobacteria o__Rhizobiales f__Bradyrhizobiaceae	82	475	488	294	499	796	528	352
p__Proteobacteria c__Alphaproteobacteria o__Rhizobiales f__Hyphomicrobiaceae	81	346	316	219	319	571	352	286
p__Proteobacteria c__Alphaproteobacteria o__Rhizobiales f__Phyllobacteriaceae	72	182	149	135	184	293	180	199
p__Proteobacteria c__Alphaproteobacteria o__Rhizobiales f__Rhizobiaceae	158	352	301	303	348	561	365	380
p__Proteobacteria c__Alphaproteobacteria o__Rhizobiales f__unclassified	120	233	254	182	248	398	242	241
p__Proteobacteria c__Alphaproteobacteria o__Rhodobacterales f__Rhodobacteraceae	230	307	287	489	409	514	352	800
p__Proteobacteria c__Alphaproteobacteria o__Rhodospirillales f__Rhodospirillaceae	235	487	520	379	524	822	548	474
p__Proteobacteria c__Alphaproteobacteria o__Rickettsiales f__Pelagibacteraceae	25	78	93	391	395	261	335	1022
p__Proteobacteria c__Alphaproteobacteria o__Sphingomonadales f__Erythrobacteraceae	132	144	154	117	176	242	162	184
p__Proteobacteria c__Alphaproteobacteria o__Sphingomonadales f__Sphingomonadaceae	433	563	669	454	604	957	651	524
p__Proteobacteria c__Betaproteobacteria o__Burkholderiales f__Alcaligenaceae	160	302	300	259	256	444	235	282
p__Proteobacteria c__Betaproteobacteria o__Burkholderiales f__Aquabacteriaceae	1330	726	1096	888	1256	1587	1278	1152
p__Proteobacteria c__Betaproteobacteria o__Burkholderiales f__Burkholderiaceae	524	527	619	424	576	1037	652	546
p__Proteobacteria c__Betaproteobacteria o__Burkholderiales f__Comamonadaceae	2171	2380	1834	1583	2294	3448	2617	1968
p__Proteobacteria c__Betaproteobacteria o__Burkholderiales f__Oxalobacteraceae	374	458	540	399	482	761	556	450
p__Proteobacteria c__Betaproteobacteria o__Rhodocyclales f__Rhodocyclaceae	251	245	276	195	304	435	307	230
p__Proteobacteria c__Betaproteobacteria o__Rhodocyclales f__unclassified	65	159	178	101	167	297	191	122
p__Proteobacteria c__Deltaproteobacteria o__Desulfuromonadales f__Geobacteraceae	84	189	174	95	142	266	195	114
p__Proteobacteria c__Epsilonproteobacteria o__Campylobacterales f__Helicobacteraceae	57	219	229	264	288	269	256	353
p__Proteobacteria c__Gammaproteobacteria o__Aeromonadales f__Aeromonadaceae	357	257	292	198	372	302	349	344
p__Proteobacteria c__Gammaproteobacteria o__Chromatiales f__Chromatiaceae	136	285	264	304	296	411	320	434
p__Proteobacteria c__Gammaproteobacteria o__Chromatiales f__Sinobacteraceae	89	165	166	157	200	298	189	251
p__Proteobacteria c__Gammaproteobacteria o__Enterobacteriales f__Enterobacteriaceae	1252	2264	2433	1972	2449	2888	2510	2045
p__Proteobacteria c__Gammaproteobacteria o__Legionellales f__Coxiellaceae	47	163	154	126	182	272	185	132
p__Proteobacteria c__Gammaproteobacteria o__Pseudomonadales f__Moraxellaceae	54	213	251	160	136	209	147	128
p__Proteobacteria c__Gammaproteobacteria o__Pseudomonadales f__Pseudomonadaceae	850	2328	2521	1939	2386	3491	2497	2314
p__Proteobacteria c__Gammaproteobacteria o__Xanthomonadales f__Xanthomonadaceae	232	568	451	388	440	553	368	354