SPRUCE CREEK WATERSHED

2016 WATER QUALITY MONITORING REPORT

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2016 REPORT SUMMARY

Spruce Creek in Kittery, Maine is listed as impaired due to elevated fecal indicator bacteria. The cause of this impairment is largely attributed to sources of nonpoint source pollution in the watershed (e.g., stormwater runoff, malfunctioning septic systems, leaky sewer lines, etc.). Since 2008, the Town of Kittery, Spruce Creek Association, FB Environmental Associates (FBE), and other partners have worked to address this impairment and to improve the water quality of this natural resource.

FBE Scientist, Lauren Bizzari, deploying a sonde in Spruce Creek at Picott Road. Photo: FBE.

In 2016, FBE continued to monitor water quality in the Spruce Creek estuary. Data sondes were deployed from August through September at two locations to continuously collect data on water temperature, dissolved oxygen (DO), relative depth (i.e., tidal stage), and specific conductivity. Grab samples for bacteria and a suite of nutrient and other parameters were also collected. This monitoring effort yielded several key observations:

- The upper estuary site showed evidence of nutrient and organic enrichment as a persistent source of nonpoint source pollution, possibly from agricultural, residential, or wetland areas upstream.
- Both the upper and middle estuary sites showed evidence of pollutant loading following a significant rain event when suspended sediment and/or fecal coliform bacteria were elevated. Moderate to large rain events mobilize sources of fecal waste and other pollutants from the landscape or groundwater to the Creek.
- Trend analysis indicated that, since monitoring began in 2012, *E. coli* concentrations have increased at the middle estuary site. However, these analyses should be interpreted with caution as *E. coli* is not the preferred bacterial indicator for estuarine environments, and minimal data are available to assess trends.

NEXT STEPS

- Continue to monitor Spruce Creek water quality using continuous data sondes and grab samples during low flow periods (August-September). Continuation of monitoring is critical to determine long-term changes in Spruce Creek water quality because of pollution reduction efforts, land use changes, and climate change.
- Revisit recommendations made in the 2015 Water Quality Monitoring Report, including 1) assess potential sources of fecal waste to the upper estuary site (e.g., bovine DNA analysis and upstream bracket sampling); 2) collect bacteria samples at major tributary sites with high bacteria and/or with future land use changes; and 3) consider eliminating *E. coli* from the testing protocol and replacing it with Enterococci at estuarine sites, along with co-indicators to fecal contamination (e.g., optical brighteners, nutrients, etc.).

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View of the upper estuary of Spruce Creek, just downstream from its crossing with Picott Road. Photo: FBE.

BACKGROUND

The estuarine portion of Spruce Creek is listed in the Maine Department of Environmental Protection (Maine DEP)'s 2014 Integrated Water Quality Monitoring and Assessment Report (Maine DEP, 2014) as impaired under Category 5-B-1: Estuarine and Marine Waters Impaired for Bacteria Only (fecal pollutants) – TMDL Required. Spruce Creek is also identified by Maine DEP on the Threatened Stream and Marine Watersheds Priority List due to negative water quality indicators and its status as an MS4 priority water. Shellfish beds (Department of Marine Resources (DMR) Closed Area 1-B) within Spruce Creek have been closed since July 2005.

In 2009, the Town of Kittery, Spruce Creek Association (SCA), and FB Environmental Associates (FBE) began conducting studies of stormwater outfalls and tributaries to the Creek to identify fecal contamination "hotspots". Results from these studies helped project partners refine the focus of future investigations and implementation efforts. The Spruce Creek Watershed Improvement Project (SCWIP) in currently in Phase IV of funding from Section 319 of the Clean Water Act (from USEPA via Maine DEP). Since the project began in 2008, more than 60 best management practices (BMPs) have been implemented throughout the watershed. As these remediation efforts are now underway, monitoring data becomes essential to assess the trajectory of any changes in **fecal indicator bacteria (FIB)** counts in the estuary.

Fecal indicator bacteria (FIB) are used to detect fecal contamination and the pathogens associated with fecal matter in surface waters. However, there are some limitations to using FIB to track harmful pathogens in fecal matter. Bacterial and viral pathogens react differently in the natural environment, so that external factors (temperature, sunlight, proliferation, etc.) may influence the concentration of FIB, but not the viral pathogens of interest for protecting public health. In addition, laboratory analysis of FIB can be highly variable at lower concentrations. As such, FIB must be interpreted with some caution when determining its actual threat to public health.

Data sondes were first deployed in Spruce Creek in 2012. The sondes collect water quality data at set intervals (typically 15 minutes), providing a picture of in-estuary conditions over the course of multiple days and tidal cycles. Sondes were deployed again in 2016 in the upper and middle estuaries along the main stem of Spruce Creek. Surface grab samples were also collected for chemical analyses.

FECAL INDICATOR BACTERIA

	Fecal Coliform	Enterococci	*E. coli*
USAGE	• Maine DMR • Assess shellfishing safety	• Maine Healthy Beaches (MHB), Maine DEP • Marine/estuarine waters (primary contact recreation, beaches)	• Maine DEP, MS4 • Freshwaters (stormwater discharges, nonpoint sources)

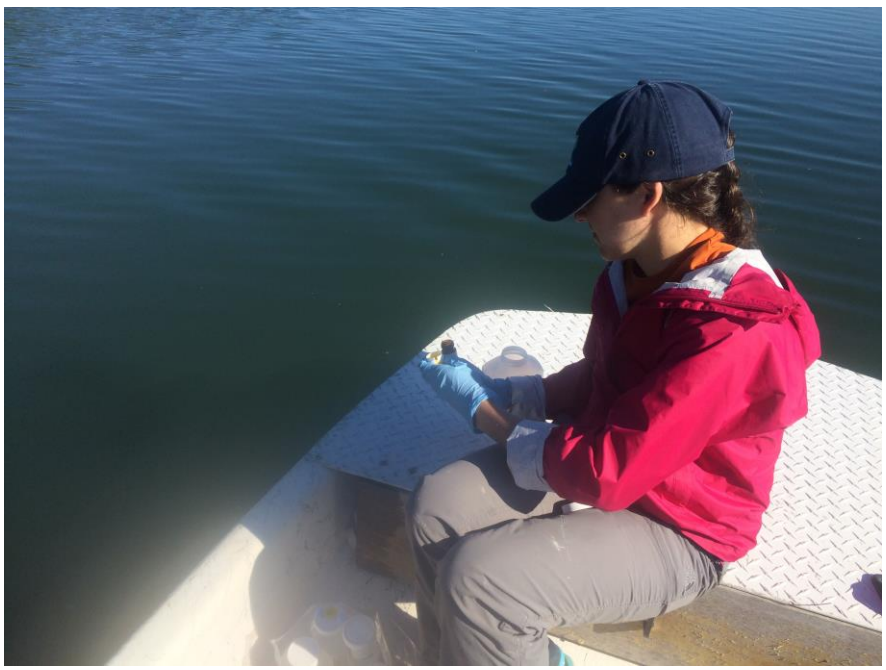
2016 WATER QUALITY RESULTS

Two data sondes (YSI 6-Series) were deployed in Spruce Creek at the middle (MIDEST) and upper estuary (PICOTT) sites for 64 days from 8/8/16 to 10/10/16 (see Figure B1, Appendix B for map). The sondes monitored for dissolved oxygen (saturation and mg/L), temperature, relative water depth, and specific conductivity at 15 minute intervals. Surface grab samples were also collected at both sites on three dates during the deployment period. Additional details on deployments, sampling, and statistical methodology can be found in Appendix A.

CONTINUOUS WATER QUALITY MONITORING (DATA SONDES)

Like previous years, **the upper estuary of Spruce Creek experienced larger swings in daily DO (both mg/L and percent saturation) compared to the middle estuary.** Percent DO saturation fell below the State criterion of 85% for 74% of all readings at the upper estuary site (Figure 1). Percent DO saturation fell below the State criterion of 85% for 19% of all readings at the middle estuary site (Figure 2). Low DO may indicate high concentrations of nutrients and/or organic matter entering the Creek. Excess nutrients can stimulate rapid growth of algae and other aquatic plants; excess organic matter has a high potential biological oxygen demand as decomposition can deplete oxygen in the water column, causing stressful conditions for aquatic organisms, such as fish and shellfish.

The upper estuary also showed larger daily swings in specific conductivity, ranging from 21 to 50 mS/cm, compared to the middle estuary, ranging from 43 to 50 mS/cm. The upper estuary site is located further upstream where the Creek narrows considerably above Picott Rd and becomes more directly connected to freshwater influences from the landscape (e.g., surface runoff and groundwater). The middle estuary site is located where the Creek is much wider and more influenced by marine waters. As measured in previous years, large precipitation events cause a drop in specific conductivity as freshwater runoff from the watershed flows to the Creek.



TOP LEFT: Marine creatures attempt to infiltrate eel crate that housed the sonde at the middle estuary site (MIDEST). Photo: FBE.
RIGHT: FBE Scientist, Carly Ellis, collects grab samples during sonde deployment at the middle estuary site (MIDEST). Photo: FBE.

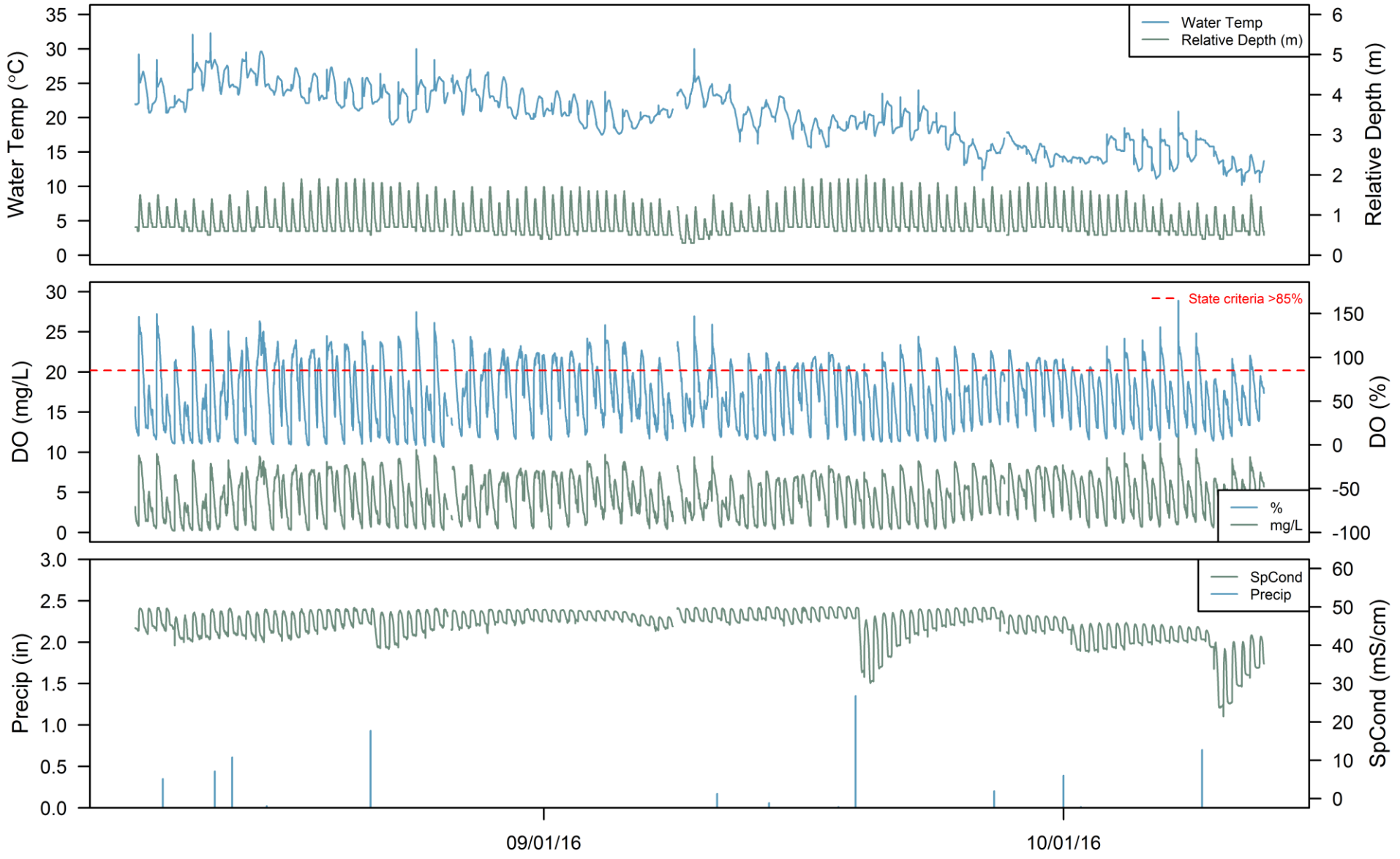


FIGURE 1. Continuous sonde data collected in the upper estuary of Spruce Creek at Picott Road.

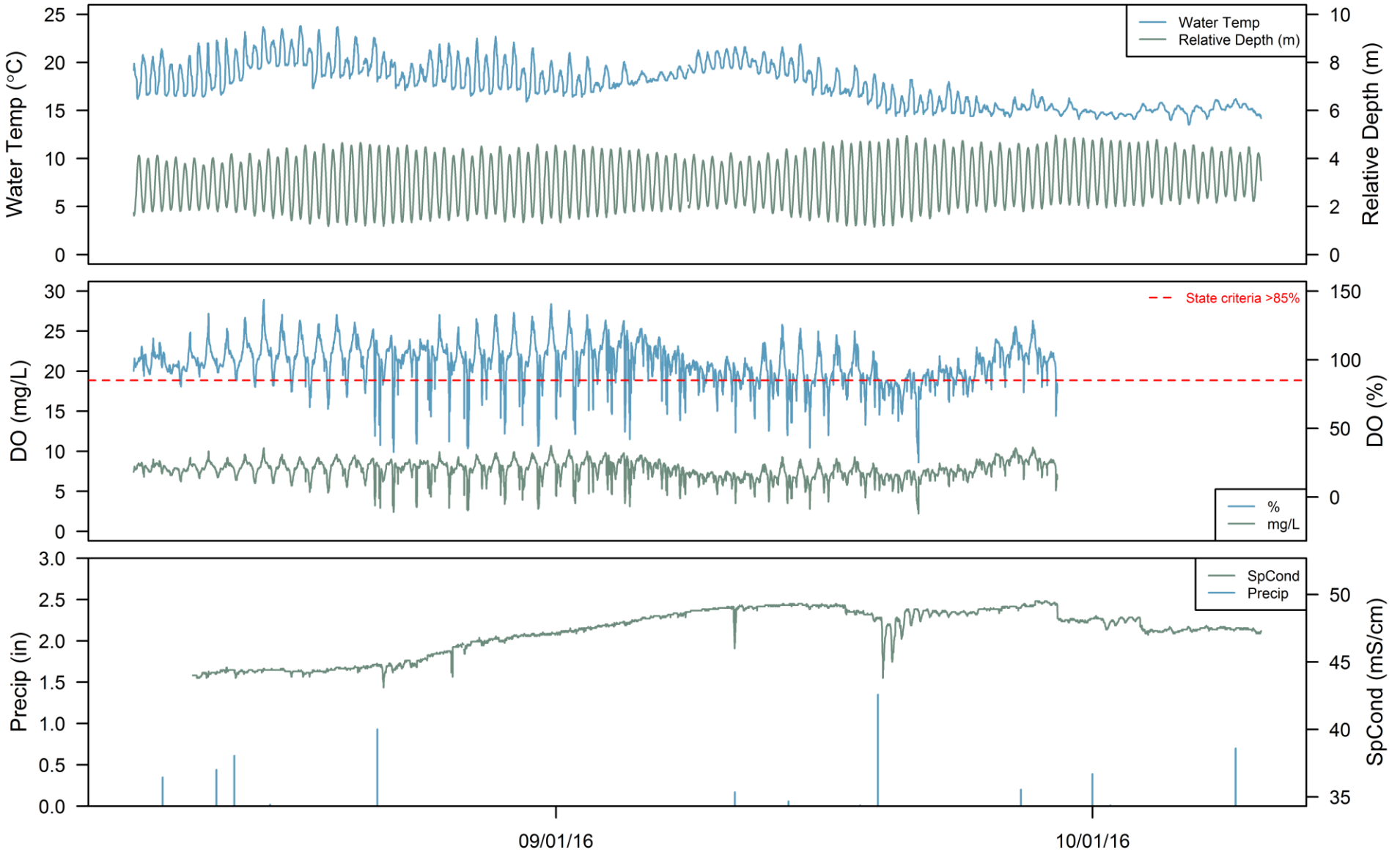


FIGURE 2. Continuous sonde data collected in the middle estuary of Spruce Creek.

DISCRETE WATER QUALITY MONITORING (GRAB SAMPLES)

Refer to Tables 1 and 2 for 2016 grab sample results. The following observations were made based on these results:

- **Fecal coliform bacteria levels were highest at the upper estuary site following a significant precipitation event** (0.70" within 24 hours prior to 10/10/16 sampling). Moderate to large rain events mobilize sources of fecal waste from the landscape or groundwater to the Creek. The Maine Department of Marine Resources (DMR) tests for this group of fecal indicator bacteria to monitor shellfish growing areas. *E. coli* results were consistently high at the upper estuary site and much higher than *E.coli* results at the middle estuary site. However, results for *E. coli* should be interpreted with caution as they are not the preferred indicator bacteria for marine or estuarine waters[1].

- **In general, both nitrogen and phosphorus concentrations were higher at the upper estuary site than at the middle estuary site.** Similar to results from 2015, the sum of total kjeldahl nitrogen (TKN) and nitrate-nitrite in the upper estuary exceeded the NH Department of Environmental Services (NHDES) nutrient guidelines during all three sampling events (<0.45 mg TN/L; established for the Great Bay to protect aquatic life). Nitrate-nitrite fell below laboratory's Practical Quantitation Limit (PQL) of 0.1 mg/L during almost all sampling events, indicating that organic nitrogen is the primary pollutant form (supported also by higher total organic carbon (TOC) at the upper estuary site). Proximity of agricultural fields or wetlands to the upper estuary site may be a contributing factor to organic nutrient enrichment. The issue of organic pollutants was further confirmed by elevated biological oxygen demand (BOD) at both sites where BOD was or likely was above 2.0 mg/L[2], which may indicate pollution from industrial, residential, or agricultural organic wastes that enhance oxygen-consuming decomposition processes.

- **Total suspended solids (TSS) were elevated at both sites following a significant precipitation event** (0.70" within 24 hours prior to 10/10/16 sampling). While there are no established guidelines or criteria for TSS, high amounts of suspended solids following wet weather indicate that stormwater runoff carried sediments and other suspended particles to the Creek. Sediment can pick up and carry a variety of other pollutants, including phosphorus.

[1] *E. coli* are a subset of the fecal coliform bacteria group, and thus *E. coli* concentrations should be less than fecal coliform concentrations; this was not the case with 2016 results. The processing laboratory documented this discrepancy in their results report, and noted that the methods used to enumerate *E. coli* and fecal coliforms differ. These methods are each approved for use in marine waters, though the *E. coli* method has been shown to result in false positives in some marine waters (Pisciotta et al., 2002). As such, *E. coli* results should be interpreted with caution.

[2] Per Alpha Analytical, samples were set at the correct dilution for BOD analysis according to prep screening; however, not enough depletion occurred. Therefore, sample results were reported as "non-detect" at an elevated detection limit.

TABLE 1. 2016 Spruce Creek upper estuary (at Picott Road) water quality results from surface grab sampling (laboratory analyses) and in-field measurements using a YSI ProODO. Red bolded values are above established thresholds.

PARAMETER	8/8/16	9/8/16	10/10/16	RL[a]	Threshold
Precip (Prior 24 hrs) (in)	0.00	0.00	0.70	--	--
Precip (Prior 48 hrs) (in)	0.13	0.00	0.70	--	--
Precip (Prior 96 hrs) (in)	0.13	0.00	0.70	--	--
LABORATORY ANALYSES					
E.coli (col/100mL)	**>2,420**	**>2,420**	**2,400**	1	236
Fecal Coliform (CFU/100mL)	80	--	1,600	2	NA[b]
Nitrate-N + Nitrite-N (mg/L)	<0.10	<0.10	0.18	0.10	--
TKN (mg/L)	0.749	0.789	0.808	0.300	--
TN (mg/L)[d]	**0.799**	**0.839**	**0.988**	--	0.450
Total Phosphorus (mg/L)	0.145	0.097	0.084	0.010	--
BOD (mg/L)	**<10**	**3**	**<20**	Variable[c]	2.0
TOC (mg/L)	<1.00	0.70	1.59	Variable[c]	--
TSS (mg/L)	13.0	12.0	21.0	5.0	--
IN FIELD MEASUREMENTS					
Water Temp (°C)	22.0	22.1	13.2	--	--
DO (%)	**25.3**	**73.7**	**64.4**	--	85
DO (mg/L)	2.2	6.4	6.8	--	--
Salinity (ppt)	26	26	13	--	--
Sp. Conductivity (mS/cm)	42.61	44.71	22.17	--	--

[a] RL = Reporting Limit for Alpha Analytical Services; the lowest concentration that the laboratory method can accurately estimate; greater than the method detection limit (MDL).
[b] Minimum of 6 sampling events required to calculate geometric mean or 90th percentile
[c] Variable RL for BOD-5 day and TOC due to dilution factor used.
[d] Calculated as Nitrate-N + Nitrite-N + TKN. If less than RL, 0.5x RL was used for calculation.

TABLE 2. 2016 Spruce Creek middle estuary water quality results from surface grab sampling (laboratory analyses) and in-field measurements using a YSI ProODO. Red bolded values are above established thresholds.

PARAMETER	8/8/16	9/8/16	10/10/16	RL[a]	Threshold
Precip (Prior 24 hrs) (in)	0.00	0.00	0.70	--	--
Precip (Prior 48 hrs) (in)	0.13	0.00	0.70	--	--
Precip (Prior 96 hrs) (in)	0.13	0.00	0.70	--	--
LABORATORY ANALYSES					
E.coli (col/100mL)	**1,300**	**770**	110	1	236
Fecal Coliform (CFU/100mL)	2	--	70	2	NA[b]
Nitrate-N + Nitrite-N (mg/L)	<0.10	<0.10	<0.10	0.10	--
TKN (mg/L)	<0.300	0.345	<0.300	0.300	--
TN (mg/L)[d]	0.200	0.394	0.200	--	0.450
Total Phosphorus (mg/L)	0.114	0.040	0.023	0.010	--
BOD (mg/L)	**<20**	2	**<10**	Variable[c]	2.0
TOC (mg/L)	<0.5	<0.5	<0.5	0.5	--
TSS (mg/L)	12.0	8.6	17.0	5.0	--
IN FIELD MEASUREMENTS					
Water Temp (°C)	19.5	19.8	14.6	--	--
DO (%)	93.8	**80.6**	86.0	--	85
DO (mg/L)	8.64	7.37	8.81	--	--
Salinity (ppt)	32	35	31	--	--
Sp. Conductivity (mS/cm)	42.94	48.14	47.23	--	--

[a] RL = Reporting Limit for Alpha Analytical Services; the lowest concentration that the laboratory method can accurately estimate; greater than the method detection limit (MDL).
[b] Minimum of 6 sampling events required to calculate geometric mean or 90th percentile
[c] Variable RL for BOD-5 day due to dilution factor used.
[d] Calculated as Nitrate-N + Nitrite-N + TKN. If less than RL, 0.5x RL was used for calculation.

HISTORICAL ANALYSIS

Trend analyses were completed for continuous monitoring data from 2012-2016 and for discrete grab sample results from 2013-2016 at the middle estuary site[3]. Trend analysis for the upper estuary could not be completed due to fewer years of available data. Weather trends for air temperature and precipitation during the monitoring periods were also assessed. Full details of statistical methods can be found in Appendix A. Please note that this represents the minimum amount of annual data needed for water quality monotonic trend analysis (Lettenmaier et.al., 1982); thus, all results should be interpreted with caution. A longer dataset will provide a better view of changes to Spruce Creek water quality during ongoing restoration efforts in the watershed.

CONTINUOUS WATER QUALITY MONITORING (DATA SONDES)

- No significant trends were detected in monthly median water temperature, specific conductivity, or dissolved oxygen (mg/L or % saturation) at the middle estuary (Table 3).
- The highest monthly median water temperature occurred in August for 2014-16 (2012-13 data was only collected in fall) at the middle estuary (Figure B2, Appendix B).
- The highest monthly median dissolved oxygen (both concentration and % saturation) occurred in August and/or September from 2014-2016 (2012-13 data was only collected in fall) at the middle estuary (Figures B4 and B5, Appendix B).
- Across all years, dissolved oxygen percent saturation fell below the state criterion (85%) more frequently at the upper estuary site than at the middle estuary site (Table 4).

TABLE 3. Results of Mann-Kendall trend analysis of monthly medians for data collected at the middle estuary site in Spruce Creek. S = Kendall's score; Tau = Kendall's tau; p-value = significance level (values <0.05 represent a significant trend in the data).

Parameter	# data points	# data points excluded*	S	Tau	p-value
Water Temperature (°C)	14	4	4	0.200	0.60
Specific Conductivity (mS/cm)	14	4	2	0.100	0.86
Dissolved Oxygen (mg/L)	13	4	2	0.125	0.84
Dissolved Oxygen (%)	13	4	2	0.125	0.84

* Values were excluded if less than 4 data points (years) per block (month). As a result, only September and October were analyzed.

[3] Trend analyses for discrete grab sample results could only be performed on parameters with at least four years of consistent data that were also above laboratory detection limits.

TABLE 4. Exceedances of the dissolved oxygen (DO) percent saturation criterion for Maine (85%).

Middle Estuary (MIDEST)

	August			September			October			November			Grand Total		
Year	**N**	**Y**	**%**	**N**	**Y**	**%**	**N**	**Y**	**%**	**N**	**Y**	**%**	**N**	**Y**	**% Total Exceedance**
2012	--	--	--	239	66	22%	170	159	48%	--	--	--	409	225	35%
2013	--	--	--	1,001	758	43%	1,629	313	16%	--	--	--	2,630	1,071	29%
2014	2,089	591	22%	978	126	11%	904	682	43%	--	--	--	3,971	1,399	26%
2015	2,252	109	5%	2,794	86	3%	1,225	1,738	59%	--	925	100%	6,271	2,858	31%
2016	2,052	211	9%	1,977	710	26%	--	--	--	--	--	--	4,029	921	19%
Grand Total	**6,393**	**911**	**12%**	**6,989**	**1,746**	**20%**	**3,928**	**2,892**	**42%**	**--**	**925**	**100%**	**17,310**	**6,474**	**27%**

* excludes observations removed due to biofouling errors.

Upper Estuary (PICOTT)

	August			September			October			November			Grand Total		
Year	**N**	**Y**	**%**	**N**	**Y**	**%**	**N**	**Y**	**%**	**N**	**Y**	**%**	**N**	**Y**	**% Total Exceedance**
2014	--	--	--	208	584	74%	685	1,177	63%	--	--	--	893	1,761	66%
2015	648	1,709	73%	735	1,974	73%	1,442	1,160	45%	569	359	39%	3,394	5,202	61%
2016	702	1,542	69%	714	2,131	75%	180	932	84%	--	--	--	1,596	4,605	74%
Grand Total	**1,350**	**3,251**	**71%**	**1,657**	**4,689**	**74%**	**2,307**	**3,269**	**59%**	**569**	**359**	**39%**	**5,883**	**11,568**	**66%**

* excludes observations removed due to biofouling errors.

DISCRETE WATER QUALITY MONITORING (GRAB SAMPLES)

- *E. coli* concentrations showed a statistically-significant increase at the middle estuary site from 2013 to 2016 (Table 5; Figure B6, Appendix B). Fecal coliform could not be statistically analyzed for trends due to lack of data, but do not show any trend for available years (2014-2016). This analysis was conducted with only four years of data; generally, a minimum of five years of data is recommended for analysis.
- TOC at the middle estuary site showed a statistically-significant decrease at the middle estuary site from 2013 to 2016 (Table 5; Figure B6, Appendix B). This analysis was conducted with only four years of data; generally, a minimum of five years of data is recommended for analysis.
- Total kjeldahl nitrogen (TKN) and TSS did not show any statistically-significant trends over this period (Table 5).

TABLE 5. Results of Mann-Kendall trend analysis of grab samples for data collected at the middle estuary site in Spruce Creek. S = Kendall's score; Tau = Kendall's tau; p-value = significance level (values <0.05 represent a significant trend in the data).

Parameter	# of data points	# excluded*	S	Tau	p-value
E. coli (col/100mL)	12	4	10	0.833	***0.03***
TOC (mg/L)	11	3	-10	-0.830	***0.03***
TKN (mg/L)	11	3	2	0.167	0.81
TSS (mg/L)	11	3	-7	-0.580	0.14

* Values were excluded if less than 4 data points (years) per block (month). As a result, only September and October were analyzed.

WEATHER TRENDS

- Only minimum monthly air temperature showed a statistically-significant increasing (warming) trend in Kittery from 2013 to 2016 (Table 6; Figure B7, Appendix B).

TABLE 6. Results of Mann-Kendall trend analysis of monthly weather data (August through November only) at Pease Air Force Base (Portsmouth, NH; WBAN:04743) from 2012-2016. S = Kendall's score; Tau = Kendall's tau; p-value = significance level (values <0.05 represent a significant trend in the data).

Parameter	# of data points	# excluded*	S	Tau	p-value
Maximum Temp. (°C)	16	0	2	0.083	0.86
Minimum Temp. (°C)	16	0	15	0.625	***0.01***
Average Temp. (°C)	16	0	10	0.417	0.13
Monthly Total Precip. (inches)	16	0	8	0.200	0.39

* Values were excluded if less than 4 data points (years) per block (month).

CONCLUSIONS AND NEXT STEPS

The water quality monitoring dataset for Spruce Creek becomes more robust for every year that monitoring is continued. As the data record expands, water quality analysis will provide better insights to long-term trends and allow better understanding of interannual changes in water quality that may be related to specific conditions within a given year (e.g., weather patterns, dramatic land use changes, etc.). For instance, New England experienced significant drought during the 2016 monitoring period. Certain parameters reflected this condition (see below), while other parameters seemed unaffected by the drought. Trend analysis of the available data revealed a limited number of significant changes in water quality since 2012. These trends should be interpreted with caution, as they only reflect a very short time frame in the context of changing water quality (which may occur over decades or longer).

Major conclusions from the 2016 monitoring season include the following:

- **The upper estuary site showed evidence of nutrient and organic enrichment.** The upper estuary experienced large swings in daily DO, daily minimum DO that regularly fell below the State criterion of 85% saturation, and elevated nitrogen concentrations and biological oxygen demand. These results are consistent year to year; historical analysis of sonde data collected since 2012 revealed that DO fell below the State criterion of 85% saturation more frequently at the upper estuary than the middle estuary. This indicates a persistent source of nonpoint source pollution from upstream agricultural, residential, or wetland areas is impacting the upper estuary.
- **Both the upper and middle estuary sites showed evidence of pollutant loading following a significant precipitation event** (0.70" in the previous 24 hours on 10/10/16). Total suspended solids (TSS) were elevated at both locations, and fecal coliform bacteria were highest at the upper estuary. Moderate to large rain events mobilize sources of fecal waste and other pollutants from the landscape or groundwater to the Creek.
- Trend analysis indicated that, since monitoring began in 2012, total organic carbon has decreased and *E. coli* concentrations have increased at the middle estuary site. However, these analyses should be interpreted with caution as *E. coli* is not the preferred bacterial indicator for estuarine environments, and only four to five years of data are available to assess trends. Continuation of monitoring is critical to determine long-term changes in Spruce Creek water quality.

NEXT STEPS

We strongly recommend that the Town of Kittery continue to monitor Spruce Creek water quality. Without continued monitoring, there will be no method of tracking the impact of changes in the watershed on water quality (both pollution reduction efforts and land use changes).

We also recommend that the Town revisit recommendations submitted with the 2015 Spruce Creek Water Quality Monitoring Report. A modified list of those recommendations is presented below.

1. **Assess potential sources of bacteria to PICOTT and the unnamed tributary along Picott Road. This site consistently shows impacts from nonpoint source pollution. The Town should consider agricultural, wildlife, and human sources of bacteria and excess nutrients.**
    a. Use microbial source tracking to further pinpoint the source of bacteria to the unnamed tributary by also testing for bovine (cow) DNA.
    b. Work with local farmers to install agricultural BMPs, including increasing the width of vegetative stream buffer and minimizing the use of manure, if currently used. The Town of Kittery has indicated that one of the farms may become land trust property soon (J. Kellogg, Kittery Shoreland Resource Officer, pers. comm.), which may change the land use in this area.
    c. Investigate locations along Picott Road to install stormwater BMPs that treat road runoff.

2. **Continue to monitor sites for changes in water quality in Spruce Creek. This will provide valuable information and allow the Town of Kittery to track progress toward improving water quality in Spruce Creek. Consistency and continuity of monitoring efforts is essential to detecting long term trends in water quality.**
    a. Redeploy data sondes at the upper and middle estuary sites in 2017 for at least August and September. *However, September and October are the months with the longest record; October should also be considered for monitoring to enhance future trend analyses.*
    b. Continue to collect at least three sets of surface grab samples for key water quality parameters at the upper and middle estuaries.
    c. Re-start collection of bacteria samples at major tributary sites, particularly those sites with consistently-high bacteria counts and/or with future changes to land use. This could be aided by the reactivation and expansion of the Spruce Creek Association Volunteer Monitoring Program.

3. **For estuarine sites, continue to test for fecal coliform bacteria (used by DMR for assessments). Eliminate *E. coli* from testing protocol and replace with *Enterococci* testing at estuarine sites.**
    a. The record of *E. coli* testing is nearly ten years long for freshwater tributary sites in the Spruce Creek watershed. However, *Enterococci* is a better fecal bacteria indicator to use for comparison across freshwater/estuarine boundaries. Recent research also suggests that *E. coli* testing protocols in cold marine waters may be confounded by the presence of other bacteria.

REFERENCES

Lettenmaier, D.P., L.L. Conquest and J.P. Hughes. 1982. Routine Streams and Rivers Water Quality Trend Monitoring Review. C.W. Harris Hydraulics Laboratory, Technical Report No. 75, Seattle, WA.

Marchetto, A. 2015. Package "rkt". http://cran.r-project.org/web/packages/rkt/rkt.pdf

MEDEP. 2014. Integrated Water Quality Monitoring and Assessment Report. 190 p. http://www.maine.gov/dep/water/monitoring/305b/2014/2014report-final.pdf

Pisciotta, J. M., Rath, D. F., Stanek, P. A., Flanery, D. M., & Harwood, V. J. 2002. Marine Bacteria Cause False-Positive Results in the Colilert-18 Rapid Identification Test for Escherichia coli in Florida Waters. Applied and Environmental Microbiology, 68(2), 539–544. http://doi.org/10.1128/AEM.68.2.539-544.2002

APPENDIX A: Methodology

FIELD METHODOLODY

FBE conducted baseline continuous and discrete water quality monitoring of the estuarine portion of Spruce Creek at the upper and middle estuaries in 2016. Two data sondes were deployed in Spruce Creek at the middle (YSI 600 XLM) and upper estuary (YSI 600 OMS-V2) stations for 64 days from 8/8/16 to 10/10/16. The sondes measured dissolved oxygen (saturation and mg/L), temperature, relative water depth, and specific conductivity at 15 minute intervals.

The middle estuary sonde was secured inside a metal eel trap using cable ties. The eel trap was tied with thick rope to a bright orange buoy that floated on the surface of the water for retrieval. Two bricks were placed inside the eel trap to help anchor the trap in place and prevent any significant drift. The upper estuary sonde was secured to a PVC pipe using cable ties and tied with rope to a metal handle that was attached to the inside of the concrete culvert under Picott Road. Rocks were placed around and on top of the crate to secure the sonde and hide it from public view.

At the middle estuary site, the sonde was replaced with a freshly-calibrated sonde on 9/8/16, so that the retrieved sonde could be taken back to the safety of the office for proper post-calibration checks. A small lobster was found stuck in an exterior cavity of the eel trap during final retrieval on 10/10/16. Additionally, moderate accumulation of silt and muck was found surrounding the dissolved oxygen sensor upon final retrieval. Data were truncated beginning 9/29/16 as post-processing indicated this was when fouling began. Additional biofouling was evident during post-processing and was corrected whenever possible.

The sonde at the upper estuary was recalibrated more frequently due to battery life limitations. The sonde was retrieved, recalibrated in the office, and returned to the field on 8/26/16, 9/8/16, 9/27/16, and 10/12/16. Minimal evidence of biofouling was evident on this sonde.

Quality assurance and quality control of the data followed the USGS Guidelines and Standard Procedures for Continuous Water-Quality Monitors (http://pubs.usgs.gov/tm/2006/tm1D3/), as well as the Quality Assurance Project Plan (QAPP) for Spruce Creek Watershed-Scale Bacteria Monitoring, dated September 3, 2015.

Three sets of grab samples were also collected (8/8/16, 9/8/16, 10/10/16) at the middle and upper estuaries and sent to Alpha Analytical in Portsmouth, NH for various analyses (fecal coliform[4] and *E. coli*, nitrate-nitrite, total phosphorus, total suspended solids, biological oxygen demand, total kjeldahl nitrogen, and total organic carbon). All samples were collected within two hours of dead low tide. It is important to note that grab samples are a very limited "snapshot" of the water quality of Spruce Creek.

Daily precipitation data for 2016 figures were retrieved from station KMEKITTE11 on Weather Underground. Daily air temperature maximum, minimum, and average and total monthly precipitation from 2012-2016 were retrieved from NOAA National Climatic Data Center (Pease Air Force Base, Portsmouth, NH; WBAN:04743).

STATISTICAL ANALYSES

All statistical analyses were conducted in R (version 3.0). Sonde data were first aggregated into an hourly median (for years with multiple measurements per hour). Hourly medians were then aggregated into daily and monthly medians. Surface grab samples were also aggregated by monthly median for years in which multiple samples were collected per month. Weather data were aggregated into average, maximum, and minimum monthly temperatures (from daily averages, minima, and maxima).

Parameter trends were assessed using the seasonal Mann-Kendall test, with month as the "seasonal" block to control for variation attributed to month instead of individual years (rkt, R statistical program; Marchetto, 2015).

[4] Fecal coliform samples were not collected on 9/8/2016.

APPENDIX B: Additional Maps and Tables

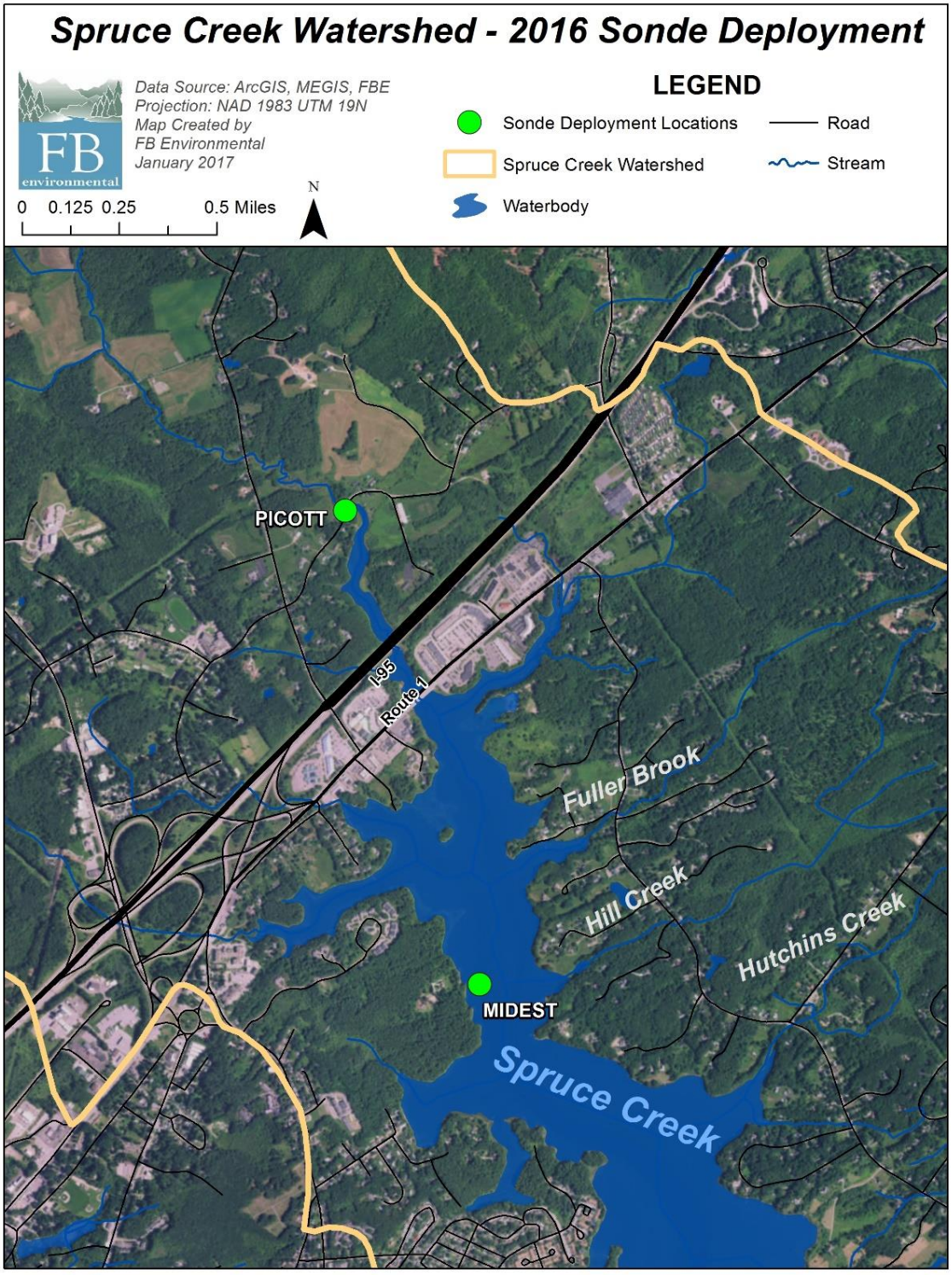


FIGURE B1. Map of 2016 sonde deployment locations. PICOTT = upper estuary, MIDEST = middle estuary.

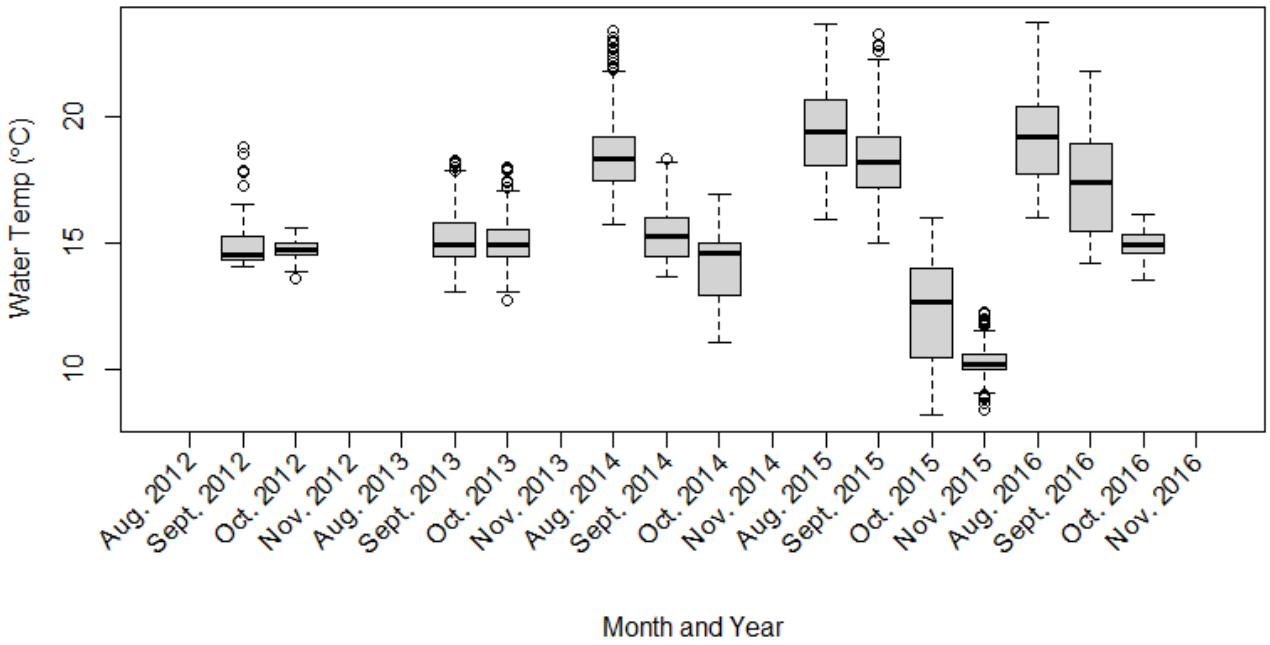


FIGURE B2. Boxplots of hourly median water temperatures (by month and year) at the middle estuary site in Spruce Creek. Boxes represent the 50th percentile or median (thick horizontal line), the 25th quartile (bottom of box), and the 75th quartile (top of box). Upper and lower whiskers represent 1.5x the interquartile range (or maximum/minimum of the data if within the interquartile range). Outliers are single points greater than 1.5x the interquartile range.

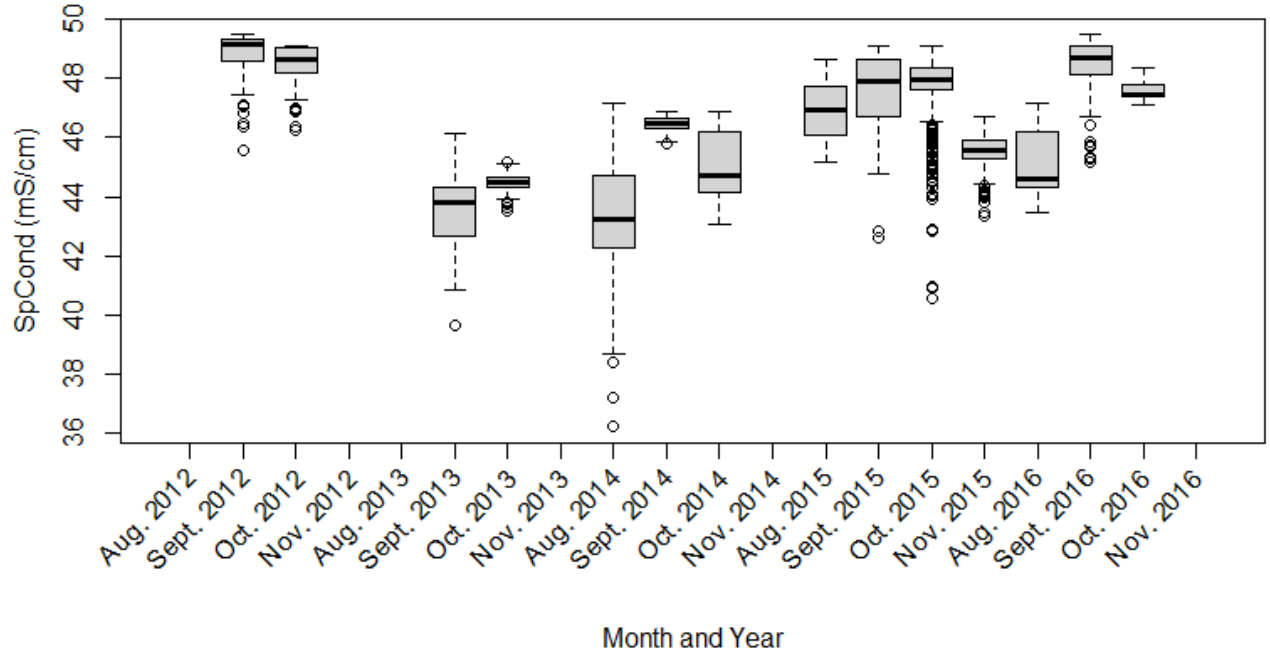


FIGURE B3. Boxplots of hourly median specific conductivity (by month and year) at the middle estuary site in Spruce Creek. Boxes represent the 50th percentile or median (thick horizontal line), the 25th quartile (bottom of box), and the 75th quartile (top of box). Upper and lower whiskers represent 1.5x the interquartile range (or maximum/minimum of the data if within the interquartile range). Outliers are single points greater than 1.5x the interquartile range.

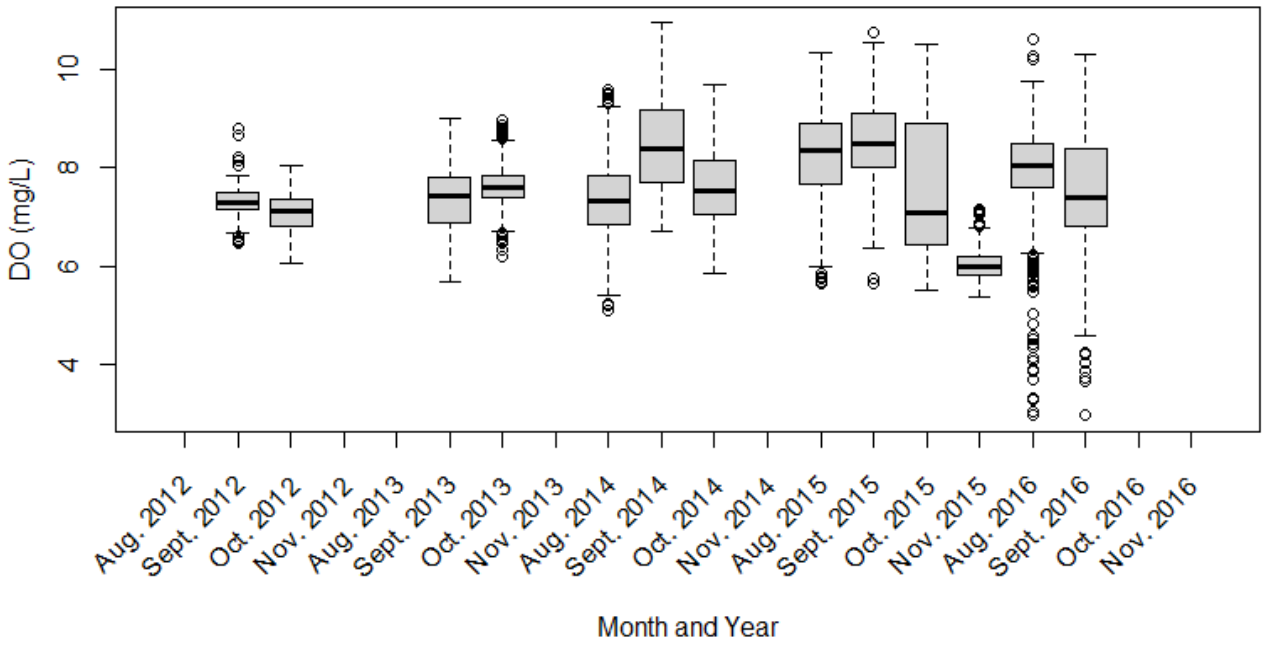


FIGURE B4. Boxplots of hourly median DO concentration (by month and year) at the middle estuary site in Spruce Creek. Boxes represent the 50th percentile or median (thick horizontal line), the 25th quartile (bottom of box), and the 75th quartile (top of box). Upper and lower whiskers represent 1.5x the interquartile range (or maximum/minimum of the data if within the interquartile range). Outliers are single points greater than 1.5x the interquartile range.

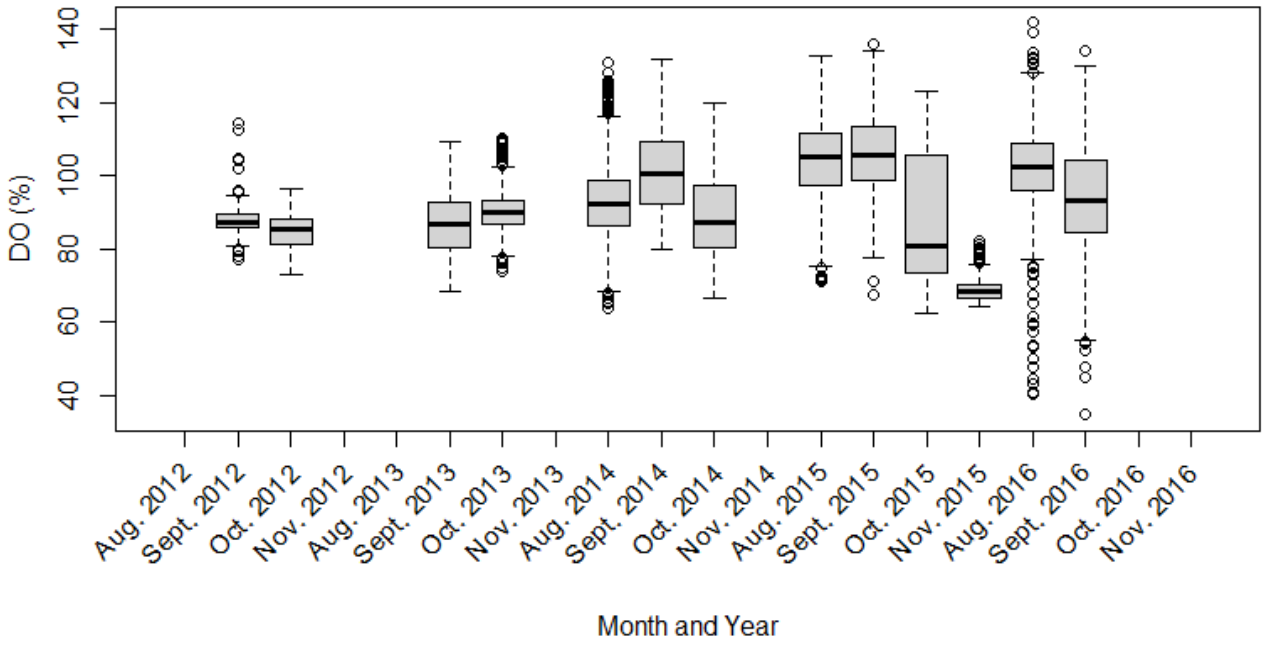


FIGURE B5. Boxplots of hourly median DO % saturation (by month and year) at the middle estuary site in Spruce Creek. Boxes represent the 50th percentile or median (thick horizontal line), the 25th quartile (bottom of box), and the 75th quartile (top of box). Upper and lower whiskers represent 1.5x the interquartile range (or maximum/minimum of the data if within the interquartile range). Outliers are single points greater than 1.5x the interquartile range.

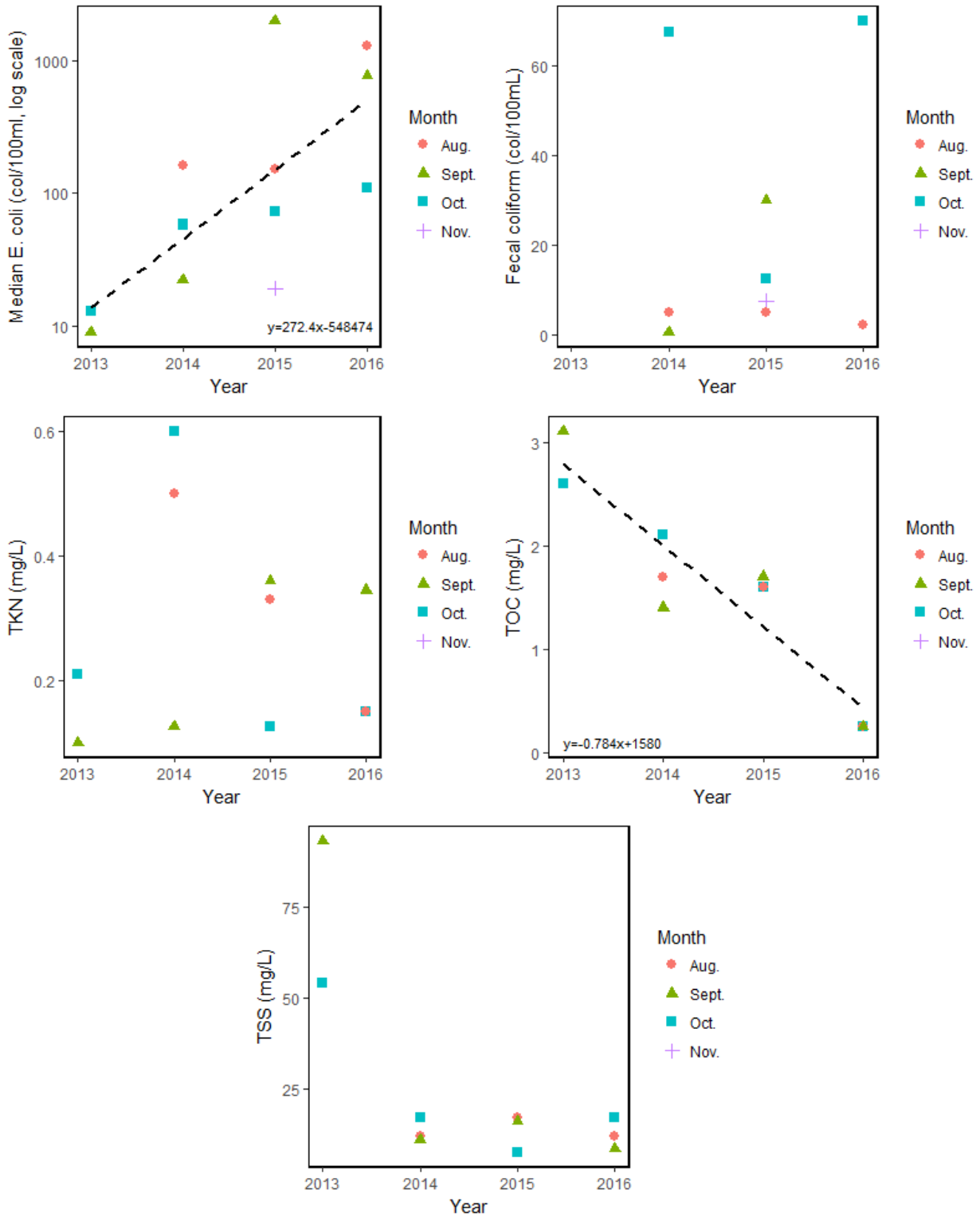


FIGURE B6. Median monthly E. coli, fecal coliform, total kjeldahl nitrogen (TKN), total organic carbon (TOC), and total suspended solids (TSS) for the middle estuary site in Spruce Creek. In some cases, only a single sample was collected per month. Mann-Kendall trend analysis results: TKN, TSS (not significant, $p>0.05$); E. coli, TOC (significant, $p = 0.03$; trend shown by dotted line); fecal coliform (not enough data to assess).

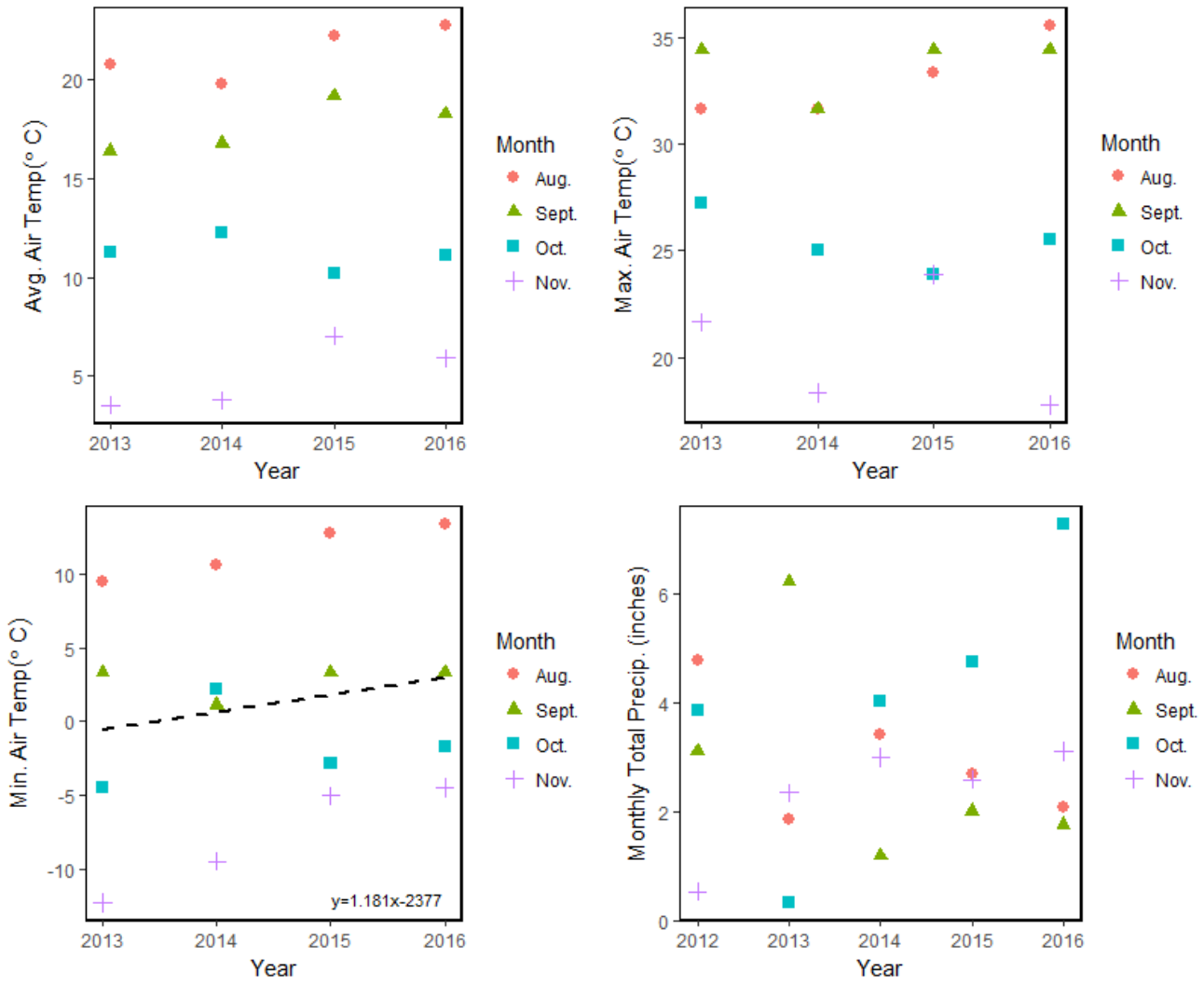


FIGURE B7. Monthly average, minimum, and maximum air temperature and cumulative monthly rainfall at Pease Air Force Base in Portsmouth, NH (approximately 5 miles from Spruce Creek in Kittery, ME). Mann-Kendall trend analysis results: average and maximum monthly temperature, cumulative monthly precipitation (not significant, $p>0.05$); minimum monthly air temperature (significant, $p = 0.01$; trend shown by dotted line).