Charlie Creek Water Quality Assessment

Hardee & Polk Counties, Florida

For

Southwest Florida Water Management District

June 2, 2021

By

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Cover art is a derivative, abstract artistic rendering of an aerial photograph of Charlie Creek at County Road 634 in County.

The SWFWMD tasked Applied Technology & Management, Inc. (ATM) with this assessment under Task Work Assignment 20TW0002927. Janicki Environmental, Inc. assisted ATM with the assessment.

Suggested reference:

Applied Technology & Management, Inc. and Janicki Environmental, Inc., 2021. Charlie Creek water quality assessment: Southwest Florida Water Management District task work assignment 20TW0002927 report, Brooksville, Florida, 29 pp.

List of Abbreviations and Acronyms

ATM	Applied Technology & Management, Inc.
BMAP	basin management action plan
C	degrees Celsius
cfs	cubic feet per second
color	colored dissolved organic matter
COD	chemical oxygen demand
DEP	Florida Department of Environmental Protection
DO	dissolved oxygen (without regard to specific measurement)
DO_C	dissolved oxygen analyzed as concentration
DO_{SAT}	dissolved oxygen analyzed as percent saturation
F^-	fluoride
F.A.C.	Florida Administrative Code
Fe^{2+}	iron
HARD	hardness
IWR	Impaired Waters Rule
MFLs	minimum flows and levels
mg/L	milligram per liter
mL	milliliter
MK	Mann Kendall
NAVD88	North American Vertical Datum of 1988
NH_4^+	ammonium
No.	number
NO_3^-	nitrate
NO_2^-	nitrite
$NO_3^-+NO_2^-$	sum of nitrate and nitrite
NTU	Nephelometric turbidity unit
p	probability value
pCi/L	picocurie per liter
PCU	platinum-cobalt unit
pH	quantitative measure of the acidity or basicity of a solution based on the concentration of the hydrogen ion
PO_4^{3-}	orthophosphate
R^2	coefficient of determination
SMK	Seasonal Mann Kendall
SRP	soluble reactive phosphorus
SFWMD	South Florida Water Management District
SJRWMD	St. Johns River Water Management District
SRWMD	Suwannee River Water Management District
SWFWMD	Southwest Florida Water Management District
SU	standard units
TEMP	temperature
TDS	total dissolved solids
TKN	total Kjeldahl nitrogen
TMDL	total maximum daily load
TOC	total organic carbon
TN	total nitrogen
TP	total phosphorus
TURB	turbidity
TSS	total suspended solids
USGS	U.S. Geological Survey
µg/l	microgram per liter
µmho/cm	micromho per centimeter
WBID	waterbody identification
>	threshold exceedance

Executive Summary

The Southwest Florida Water Management District assessed the quality of water in Charlie Creek, from the confluence of Charlie Creek with the Peace River to a prairie southwest of Avon Park Cutoff Road. The assessment was based on regression and time series trend analyses. Southwest Florida Water Management District contracted Applied Technology & Management to conduct this water quality assessment. Applied Technology & Management collaborated with Janicki Environmental on the assessment.

Assessment results support the Southwest Florida Water Management District's consideration of relationships between water quality constituents and flow as an environmental value.

Statistical analyses were used to develop relationships between river flow and the following primary classes of water quality constituents: nutrients and water clarity. (Other constituent classes were also assessed but not described in this executive summary.)

Sixty percent of **nutrient** concentration relationships with flow were positively correlated, such that increases in flow correlated with increases in concentration. Ten percent of these concentration relationships with flow were negatively correlated, and thirty percent did not exhibit a statistically significant correlation. One third of chlorophyll *a* concentration relationships with flow were negatively correlated, such that increases in flow correlated with decreases in chlorophyll *a* concentration. Two thirds of flow-chlorophyll *a* concentration relationships in were seasonal, based on regression of chlorophyll *a* concentration on monthly flows.

Seventy-one percent of water **clarity** relationships with flow were positive, such that increases in flow correlated with increase in water clarity. Eighty-six percent of flow-water clarity correlations were statistically significant.

1.0 Introduction

The Southwest Florida Water Management District (SWFWMD) assessed water quality trends in Charlie Creek, in the Peace River watershed (Figure 1).

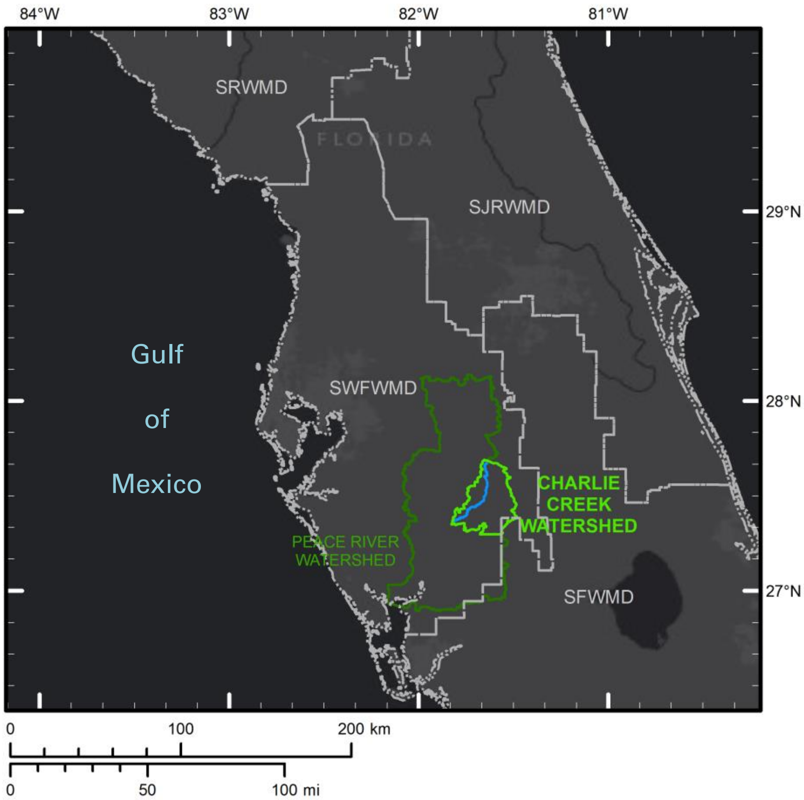

Figure 1. *General, regional location map of Charlie Creek (blue polyline) and Charlie Creek watershed (bright green polygon) in the Peace River watershed (dark green polygon) of the Southwest Florida Water Management District (SWFWMD). Also shown: South Florida Water Management District (SFWMD), St. Johns River Water Management District (SJRWMD), and Suwannee River Water Management District (SRWMD).*

Water quality is an environmental value when determining Minimum Flows and Levels (MFLs) [Water Resource Implementation Rule, Chapter 62–40.473, Florida Administrative Code (F.A.C.)]. This report describes relationships between water quality constituent concentrations and flows in Charlie Creek. The purpose of this report is to present a water quality assessment for Charlie Creek, in which trends in flow and constituent measurements were considered.

In this introduction, the assessed reach is defined (Section 1.1) and assessment objectives are presented (Section 1.2). The organization of the remainder of this report is described in Section 1.3, including a brief description of subsequent sections.

1.1 Assessed Reach

Charlie Creek flows 67 kilometers (42 miles) from a prairie southwest of Avon Park Cutoff Road in Polk County to the confluence with the Peace River north of River Road in Hardee County (Figure 2). Charlie Creek flows through Polk and Hardee Counties, and drains parts of Polk, Hardee, Highlands, and DeSoto Counties. Most of the Charlie Creek watershed is in the SWFWMD; a small southeastern part of the Charlie Creek watershed is in the South Florida Water Management District (SFWMD) (Figure 1). Charlie Creek flows under Hardee County Road 664, Florida State Road 64, Hardee County Road 636, Florida State Road 66 (Florida Cracker Trail), Hardee County Road 634, U.S. Highway 17 (Florida State Road 35) (Figure 3).

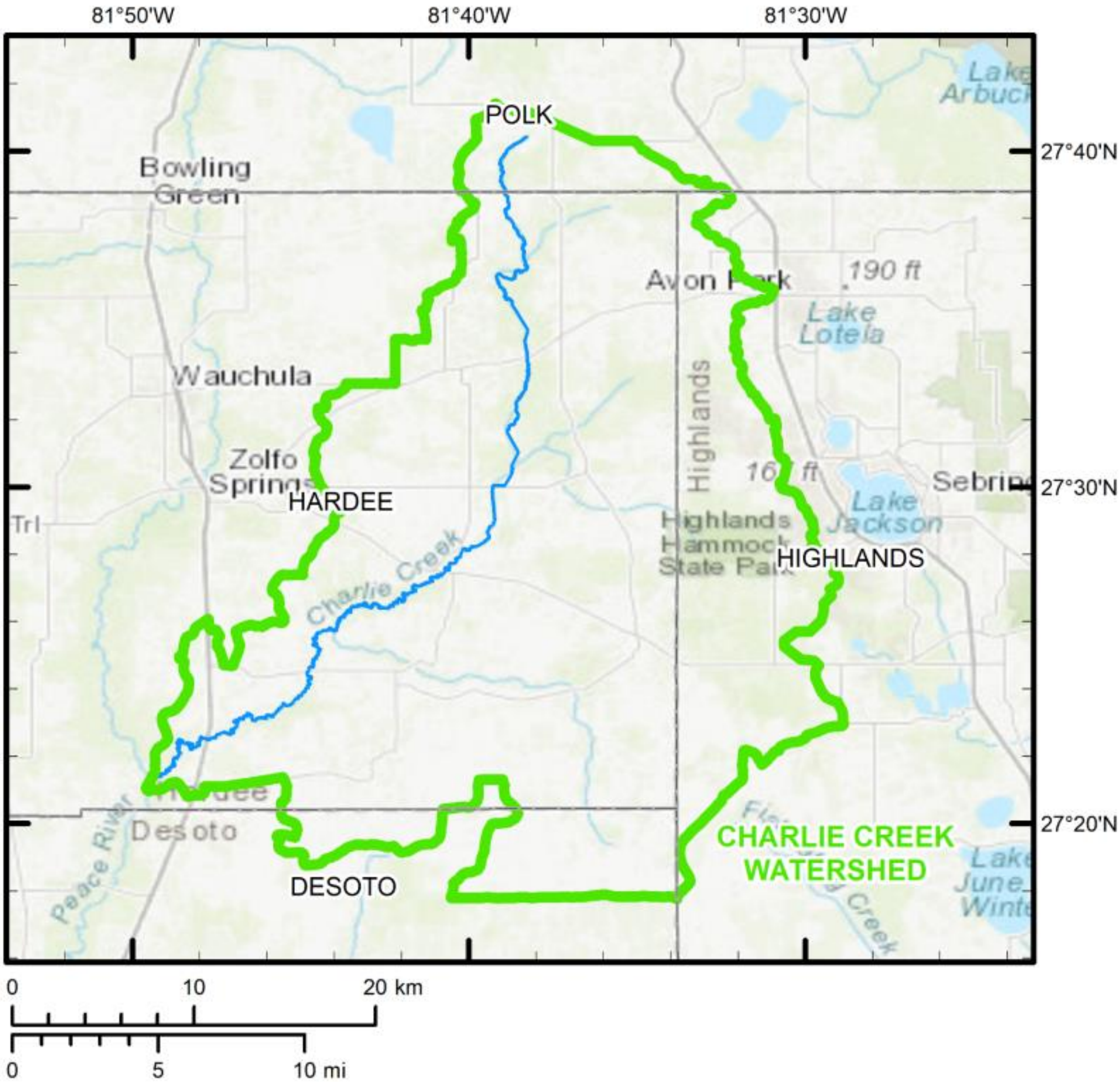

Figure 2*. Charlie Creek watershed in Polk, Hardee, Highlands, and DeSoto Counties.*

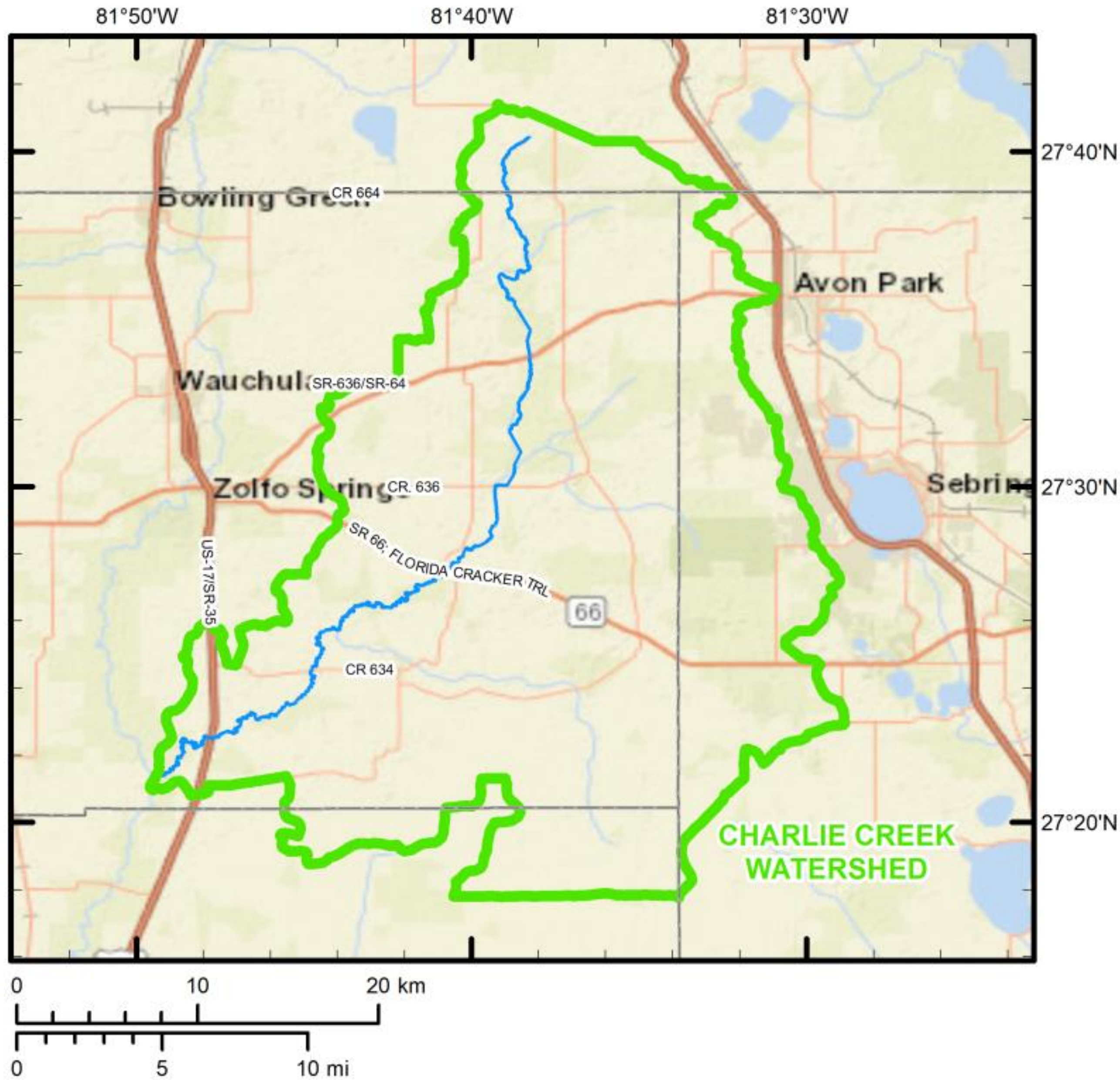

Figure 3. *Charlie Creek road crossings.*

Several major tributaries drain to Charlie Creek (Figure 4). Fish Branch, Oak Creek, Jackson Branch, Buckhorn Creek, Bee Branch, and Old Town Creek drain to Charlie Creek in Hardee County. Little Charlie Bowlegs Creek drains to Bee Branch. Haw Branch drains to a prairie in Hardee County; the prairie eventually drains to Little Charlie Bowlegs Creek.

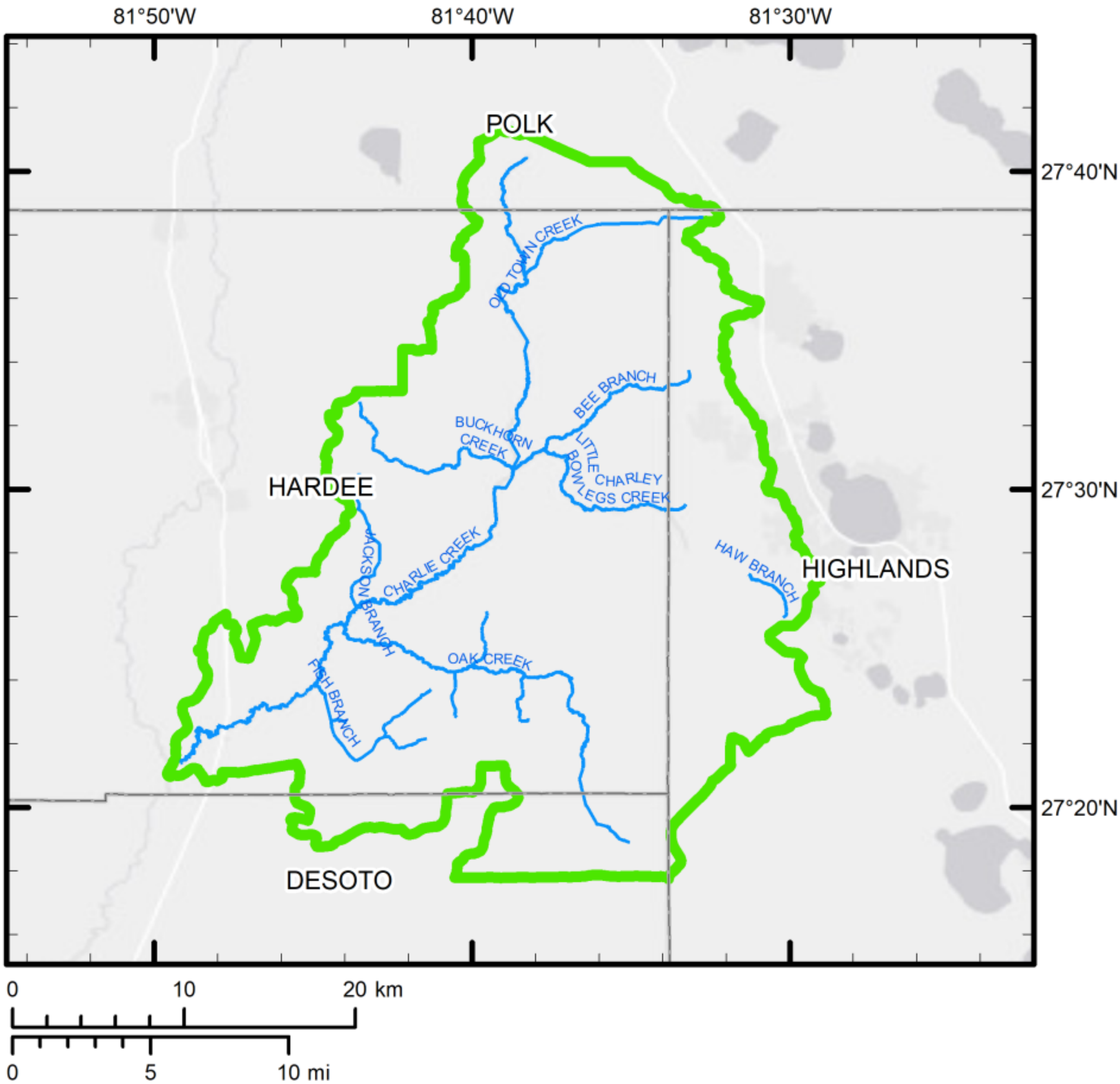

Figure 4. *Selected, major Charlie Creek tributaries.*

The DEP has issued four National Pollution Discharge Elimination System discharge permits in the Charlie Creek watershed (Figure 5).

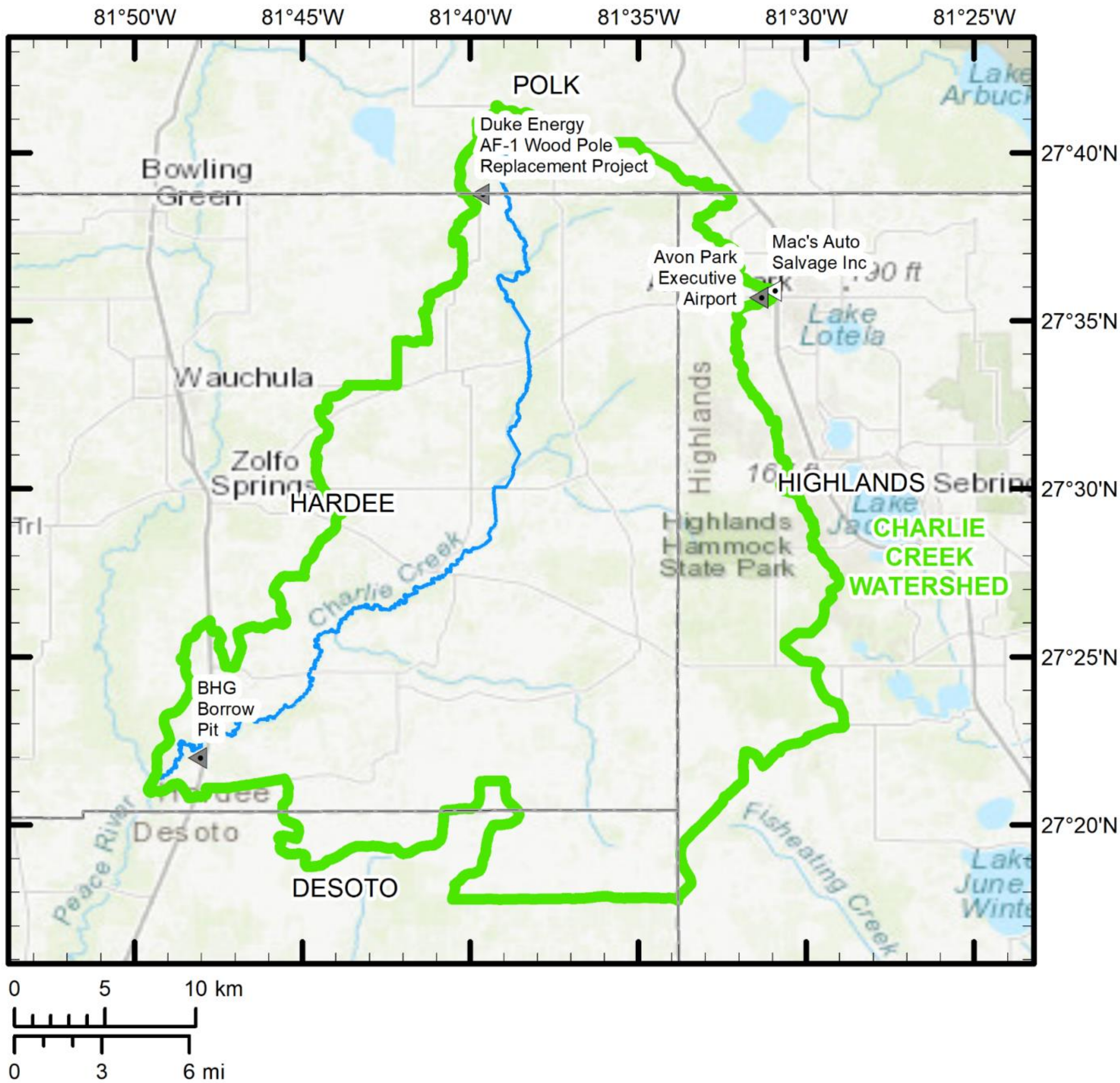

Figure 5. Permitted National Pollution Discharge Elimination System outfalls (triangular arrowhead) in the Charlie Creek watershed.

1.2 Objectives

The objective of this assessment is to analyze water quality in Charlie Creek. To achieve this objective, the following tasks were performed:

- Reviewed and summarized publications related to water quality in Charlie Creek.
- Tabulated regulatory water quality criteria and impairment determinations, and ongoing restoration activities in Charlie Creek and tributaries that drain to Charlie Creek.
- Identified water quality constituents most important to Charlie Creek health.
- Built a database of measured water quality constituents and calculated flows for Charlie Creek.

- Described regression methods to relate water quality measurements to calculated flow.
- Developed and described water quality relationships with flow.
- Identified and described other important relationships to predict changes in water quality in assessed reaches.

1.3 Assessment Report Organization

Section 2 describes present water quality conditions in Charlie Creek based on Florida Department of Environmental Protection (DEP) assessments. Section 3 describes data sources. Section 4 presents methods. Section 5 presents results. Section 6 summarizes findings.

The following statistical tools and supplemental material are presented in a separate appendix: descriptive plots and statistics by data source (appendix A), bivariate plots of flow and water quality (appendix B), covariate linear (log-log) regression summary table and detailed statistical output (appendix C), univariate linear regression summary table and detailed statistical output (appendix D), logistic regression summary table and detailed statistical output (appendix E), and a literature summary (appendix F).

2.0 Water Quality Criteria

This section includes detailed discussion of Charlie Creek water quality criteria, historic and contemporary water quality conditions, and adopted water quality restoration initiatives. Water quality constituents that are most important to the health of Charlie Creek are presented in Section 2.1. Impairment determinations from the DEP are summarized in Section 2.2. This assessment does not determine whether a waterbody or WBID is impaired or not impaired. This assessment also does not evaluate the accuracy or validity of DEP determinations of impairment. Ongoing restoration programs in Charlie Creek are reviewed in Section 2.3.

2.1 Water Quality Parameters Most Important to System Health

Nutrients are the most significant class of water quality constituents because nutrients influence the ecological health of the system. Waterbodies can become eutrophic when loaded with excess nutrients, which can cause algae to bloom, decrease dissolved oxygen (DO) concentrations, and degrade the ecosystem. Chlorophyll *a* concentration is a key indicator of eutrophication. Where nutrient concentrations exceed associated water quality criteria, chlorophyll *a* concentration may be greater than the associated water quality standard, and DO concentrations may be less than associated standard.

2.2 Summary of Designated Use, Criteria, and Florida Department of Environmental Protection Impairment Determinations

The Clean Water Act requires that States classify surface waters according to designated use. Florida (62–302.400, F.A.C.) classifies surface water into one of the following six classes associated with designated use:

- Class I—Potable water supplies: Includes impoundments and associated tributaries, certain lakes, rivers, or portions of rivers used as a source of potable water.
- Class II—Shellfish propagation or harvesting: Generally coastal waters where shellfish harvesting occurs.
- Class III—Fish consumption, recreation, propagation, and maintenance of a healthy, well-balanced population of fish and wildlife: The surface waters of the state are Class III unless described in rule 62–302.400, F.A.C.
- Class III-Limited—Fish consumption; recreation or limited recreation; and/or propagation and maintenance of a limited population of fish and wildlife: This classification is restricted to waters with human-induced physical or habitat conditions that, because of those conditions, have limited aquatic life support and habitat that prevent attainment of Class III uses.
- Class IV—Agricultural water supplies: generally located in agriculture areas around Lake Okeechobee.

- Class V—Navigation, utility, and industrial use.

All waterbodies in the Charlie Creek watershed are class III.

The DEP uses water quality data from several sources to assess Florida's waterbodies and determine whether waterbodies meet water quality criteria. The DEP must promulgate surface water quality criteria for water quality constituents per Rule 62–302, F.A.C. The DEP organizes Florida's waters with waterbody identification (WBID) numbers. Impaired waterbodies are characterized as "listed" because these waterbodies are on an adopted impairment list. An impaired waterbody is a waterbody with at least one water quality or biological constituent that does not meet associated criteria.

The DEP most recently adopted assessment lists for the Charlie Creek watershed on October 21, 2016 (Cycle 3). Cycle 1 and Cycle 2 lists were adopted in June 2005 and on January 15, 2010, respectively. Waterbodies in the Charlie Creek watershed are in assessment group 3, which will be re-assessed in summer 2021. The DEP is transitioning to a biennial assessment goal, in which all waterbodies in the State of Florida are assessed once every two years (DEP, 2021).

The DEP identified Charlie Creek as impaired upstream of the Peace River (WBID 1763A) and upstream of Old Town Creek (WBID 1763D) (Table 1, Figure 6). Charlie Creek is impaired for fecal coliform bacteria upstream of Peace River (WBID 1763A) and upstream of Old Town Creek (WBID 1763D) due to more than 400 bacteria counts per 100 milliliters of sample water. In Charlie Creek upstream of Peace River (WBID 1763A), 14 of 83 measurements violated the criteria; in Charlie Creek upstream of Old Town Creek (WBID 1763D), 5 of 6 measurements violated the criteria. These reaches of Charlie Creek were verified impaired for fecal coliform in Cycle 3 using data from January 1, 2008 through June 30, 2015. Charlie Creek is impaired for nutrients from the confluence of Charlie Creek with the Peace River to a location 22 kilometers (14 miles) upstream of Peace River at the confluence with Oak Creek (WBID 1763A). This reach of Charlie Creek is impaired for macrophytes due to linear vegetation surveys. This reach of Charlie Creek is also impaired for total phosphorus due to annual geometric mean concentration measurements greater than or equal to 0.49 milligrams per liter.

Table 1. *Impaired waterbody in the Charlie Creek watershed.*

Waterbody WBID	Parameter Assessed Using IWR	Assessment Status	Date Measured	Assessment Data	Criterion Concentration or Threshold Not Met
Charlie Creek above Old Town Creek WBID 1763D	Fecal Coliform Bacteria	Impaired		5 of 6 > 400 counts / 100 mL	≤ 400 counts / 100 mL
Charlie Creek above Peace River WBID 1763A	Fecal Coliform Bacteria	Impaired		14 of 83 > 400 counts / 100 mL	≤ 400 counts / 100 mL
Charlie Creek above Peace River WBID 1763A	Nutrients (Macrophytes)	Impaired		LVS C of C ≥ 2.5 and LVS FLEPPC ≤ 25%	
Charlie Creek above Peace River WBID 1763A	Nutrients (Total Phosphorus)	Impaired	 2008 2009 2010 2011 2012 2013 2014	Annual Geometric Means 0.61 mg/L 0.63 mg/L 0.48 mg/L 0.47 mg/L 0.53 mg/L 0.53 mg/L 0.54 mg/L	annual geometric mean ≥ 0.49 mg/L

Notes: WBID is waterbody identification
IWR is the Impaired Waters Rule
≤ is less than or equal to
mL is milliliter
LVS C of C is linear vegetation survey Coefficient of Conservatism
LVS FLEPPC is linear vegetation survey Florida Exotic Pest Plant Council
mg/L is milligram per liter

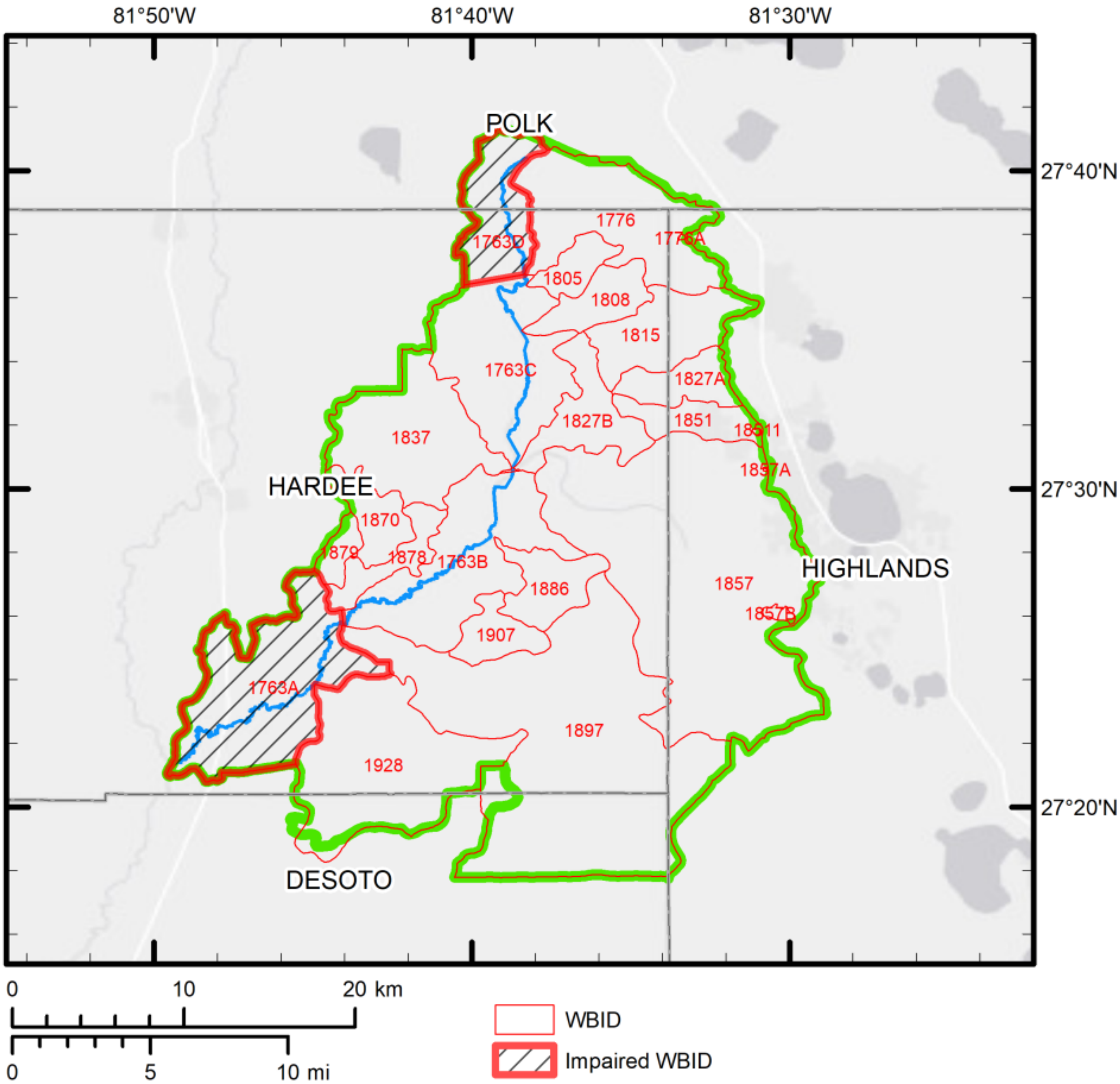

Figure 6*. Unimpaired and impaired waterbodies in the Charlie Creek watershed (green polygon), and Florida Department of Environmental Protection Waterbody Identification (WBID) numbers.*

2.3 Actions to Restore Impaired Waterbodies

The DEP has not established total maximum daily loads (TMDLs) or basin management action plans (BMAPs) for any waterbodies in the Charlie Creek watershed.

The DEP and Florida Department of Health manage bacteria impairment; consequently, the SWFWMD does not manage bacteria impairment. The DEP does not currently emphasize TMDL development for bacteria impairments, preferring to focus resources on nutrient TMDLs. The likelihood of DEP developing a TMDL for Charlie Creek bacteria impairment above Old Town Creek is low. The DEP does not plan to develop a TMDL for nutrients in Charlie Creek, from the confluence of Charlie Creek with the Peace River 22 kilometers upstream to the confluence of Oak Creek with Charlie Creek.

The DEP performed a statewide mercury TMDL because atmospheric deposition is generally considered the mercury source. As mercury is not generally associated with changes in flows, mercury was not assessed, and WBIDs impaired for mercury are not described in this overview. The DEP and Florida Department of Health manage mercury impairment; consequently, the SWFWMD does not manage mercury impairment.

3.0 Data

Data were obtained for this assessment from county, state, and federal sources. This section describes water quantity parameters and data (Section 3.1) and water quality constituents and data (Section 3.2).

Water quantity data were obtained from the U.S. Geological Survey (USGS). Water quality data were obtained from the SWFWMD, the DEP, and the USGS. Applicable counties did not possess water quantity data or water quality data that are not also in State of Florida databases.

3.1 Water Quantity Parameters and Data

3.1.1 Water Quantity Parameters

Four primary constituents are used to describe the quantity of water: water surface elevation, water depth, gage height, and flow. The following discusses each constituent. Regression of flow on water quality in this assessment exclusively use flow data; water quality data were not regressed on water surface elevation, water depth or gage height.

3.1.1.1 Water Surface Elevation

Water surface elevation is the height of the air-water interface in a waterbody above or below a datum. Typically, elevation in the United States is referenced to NAVD88. Water surface elevation is measured in linear units, such as meters or feet.

3.1.1.2 Water Depth

Water depth is the distance from the bed or bottom of a waterbody to the air-water interface of a waterbody. Water depth is measured in linear units, such as meters or feet.

3.1.1.3 Gage Height

Gage height is the vertical distance of the air-water interface in a waterbody above or below a datum. Gage height is also referred to as gauge height, stage, water level, or level. Typically, gage height is measured above an arbitrary point in a river; gage height is not always referenced to NAVD88. Gage height is measured in linear units, such as meters or feet.

3.1.1.4 Flow

Flow in a river is the volume of water moving through a cross section of the river, over a duration of time. Flow is measured in volume per time, such as cubic meters per second, cubic feet per second, or million gallons per day. Flow is typically calculated from water level, using a relationship between flow and water level called a "rating curve." The rating curve is built from coincident measurements of flow and water level. Water level is typically measured at a frequency of once every 5 to 60 minutes. Instantaneous flow is calculated using the rating curve from measured water level. These instantaneous calculations are then averaged over longer periods, such as daily, monthly, or yearly, prior to publication. Peak flow typically is also calculated and published. Peak flow is sometimes measured directly, during a peak-flow event. The type of flow data used in an analysis is a function of the use of the data; for example, an analysis of seasonality may rely on monthly average flows, while an analysis of floods may rely on peak flows. Daily average flows were used in building regression relationships while monthly median flows were used for testing monthly trends in flows over time.

3.1.2 Water Quantity Parameter Measurements

Two USGS water quantity monitoring stations monitor discharge in Charlie Creek (Table 2). A time series plot of the discharge record for these gages is provided in Figure 7. The location of these stations is provided along with the location of the water quality stations in Figure 8 below. The upstream USGS Charlie Creek gage near

Crewsville (No. 02296260) has been monitored since 2004; the USGS estimates flow at this gage with a rating-curve relationship between water-surface elevation and flow. The downstream USGS Charlie Creek gage near Gardner (No. 02296500) has been monitored since 1950; the USGS estimates flow at this gage with a rating-curve relationship between water-surface elevation and flow.

***Table 2**. Charlie Creek water quantity measurement sites with associated summary statistic information including daily minimum, maximum, and interquartile statistics.*

				Daily Flow				
Site	Location	Period	Length	Min	25th	50th	75th	Max
			[years]	[cfs]	[cfs]	[cfs]	[cfs]	[cfs]
02296260	Near Crewsville FL	2004-2020	17	0	3.36	28.7	137	6670
02296500	Near Gardner FL	1950-2020	71	0.06	14.3	61	269	9160

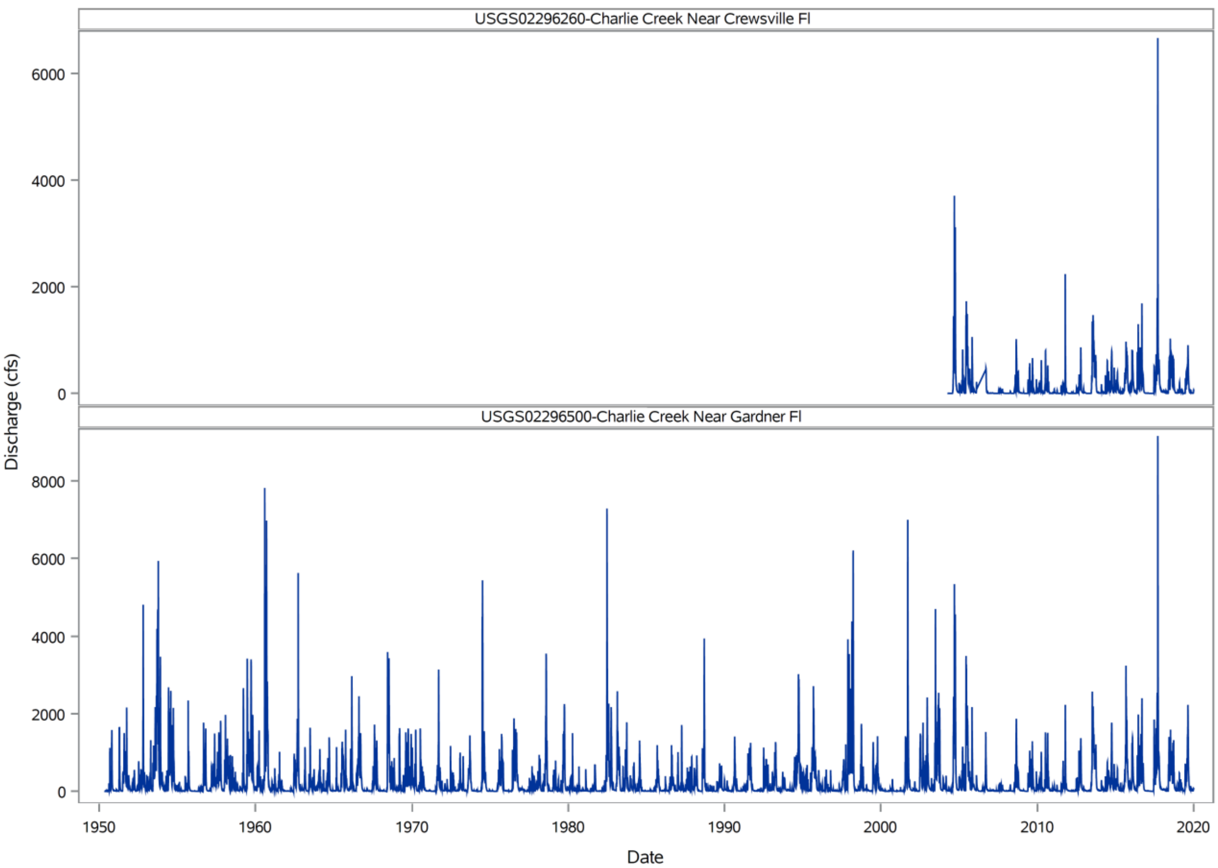

***Figure 7**. Timeseries plots for discharge (flow) in cubic feet per second (cfs) for the two USGS sites in Charlie Creek. Note Y axis scale difference between top (USGS Charlie Creek gage near Crewsville, No. 02296260) and bottom (USGS Charlie Creek gage near Gardner, No. 02296500) plots.*

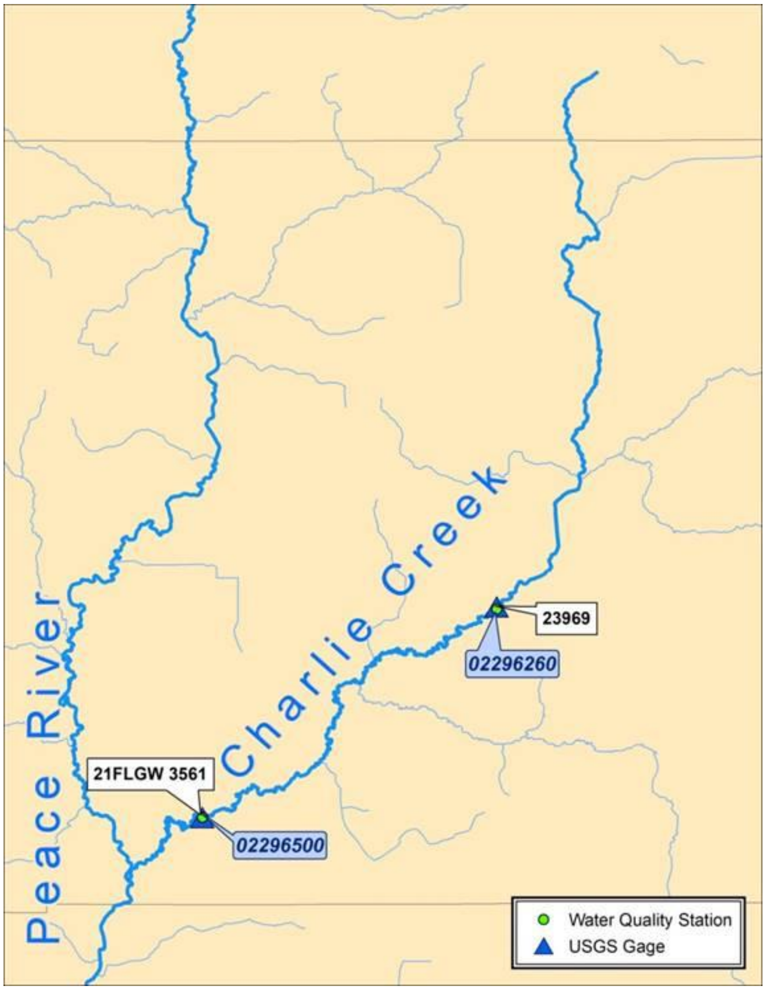

Figure 8. *Water quality stations in Charlie Creek along with associated USGS discharge gage locations (labeled in blue) Note USGS gage 02296500 also reported water quality data which were analyzed for this project.*

Across stations, minimum daily flow ranged from zero to 6670 at the USGS Charlie Creek gage near Crewsville (No. 02296260) and zero to 9160 cfs at the USGS Charlie Creek gage near Gardner (No. 02296500).

3.1.3 Gaps and Outliers

Data gaps were identified in the compiled data that require investigation or elimination before being used in statistical analyses. A data gap exists in the USGS Charlie Creek gage near Crewsville (No. 02296260) between February and September of 2006. No gap filling was conducted as there were sufficient observations for regression analysis without estimating flows for missing periods of record.

No data outliers were identified in the compiled data that require investigation or elimination before being used in statistical analyses.

3.2 Water Quality Constituents and Data

3.2.1 Water Quality Constituents

Water quality constituents of interest were identified in consultation with District staff and grouped for analysis based on their relevance to each other and their impact on water quality (Table 3). The constituent groups included Nitrogen, Phosphorus, Chlorophyll *a*, Physio-Chemical, and Minerals and Metals. Constituents of these groups are summarized in the sub-sections below.

3.2.1.1 Nitrogen

Nitrogen is an essential nutrient for all plants including algae. However, an overabundance of nitrogen can harm aquatic ecosystems by causing an imbalance of algae and other nuisance plants that can disrupt ecosystem function .Total nitrogen (TN) is the sum of inorganic and organic nitrogen. Nitrogen species include nitrate (NO_3^-), nitrite (NO_2^-), Total Kjeldahl nitrogen (TKN), and ammonium (NH_4^+). NO_3^- and NO_2^- are assessed as a sum (NO_3^-+NO_2^-). TKN is the sum of organic nitrogen and NH_4^+. Ammonium NH_4^+ typically occurs in very low concentrations, and is often below the laboratory detection limits. When the method detection limit concentration is reported, the actual NH_4^+ concentration is less than the minimum NH_4^+ concentration that the instrument is capable of measuring. When NH_4^+ data are available, organic nitrogen is calculated as the difference between TKN and NH_4^+: organic nitrogen = TKN - NH_4^+.

3.2.1.2 Phosphorus

Total phosphorus in water exists in two forms, dissolved (soluble) and particulate (attached to particles). Dissolved phosphorus can be further subdivided into soluble reactive phosphorus (SRP) and soluble non-reactive phosphorus. Orthophosphorus (PO_4^{3-}) is a component of soluble reactive phosphorus (SRP) and is the most common dissolved form. Orthophosphorus is readily available to aquatic vegetation including algae and in elevated concentrations can contribute to algae blooms. Like nitrogen, phosphorus is an essential nutrient for all plants including algae. However, an overabundance of phosphorus can harm aquatic ecosystems by causing an imbalance of algae and other nuisance plants that can disrupt ecosystem function.

3.2.1.3 Chlorophyll a

Chlorophyll *a* is a light receptor and primary electron donor during photosynthesis. Chlorophyll *a* concentration is used as a proxy for algal abundance. Pheophytin is a natural chlorophyll *a* degradation product with a light absorption peak in the same spectrum as chlorophyll *a*. Chlorophyll reported as “uncorrected” includes pheophytin while “corrected” chlorophyll *a* measures chlorophyll concentration after removing the pheophytin. The DEP has archived corrected chlorophyll *a* concentration since 2001. Prior to 2001, uncorrected chlorophyll *a* concentration was more common. Data archived for this project included uncorrected chlorophyll *a* concentration and data provided by HCSP which was simply reported as chlorophyll *a*.

3.2.1.4 Physio-Chemical Constituents

The physio-chemical constituent group includes dissolved oxygen, pH, temperature, and conductivity. Dissolved oxygen (DO) is expressed in terms of concentration and as percent saturation. (In this assessment, DO is a generic acronym that references dissolved oxygen, DO_C is a specific acronym that references dissolved oxygen concentration, and DO_{SAT} is a specific acronym that references percent dissolved oxygen saturation.) The percent saturation calculation attempts to account for water’s ability to hold oxygen molecules as a function of temperature (and salinity in estuarine systems). Dissolved oxygen can be added to water through physical processes, interactions with the atmosphere, and photosynthesis. Dissolved oxygen is removed from water by aquatic animals and decomposition. Dissolved oxygen is an important indicator of aquatic ecosystem vitality because it is necessary in many biological processes. The constituent pH is a scaled representation of hydrogen ion concentration. It is a measure of alkalinity or acidity in water. The range for pH is from 0 to 14, where pH 7 is neutral, pH < 7 is acidic, and pH > 7 is alkaline. At different pH levels, different chemical species become soluble and bioavailable, which can benefit or harm aquatic plants and animals. Conductivity is a measure of the ability of water to pass an electrical current and measured in micromhos per centimeter (μmhos/cm) or microsiemens per centimeter (μs/cm). Water temperature is a physical property expressing the average thermal energy of a substance.

3.2.1.5 Minerals and Metals

Minerals and metals include major anions and cations calcium, chloride, fluoride, iron, magnesium, and sulfate. These charged particles affect the conductivity of the water and the presence of these constituents is typically reflective of the geology of the area through which a stream or river flows, or the bedrock through which groundwater flows. Hardness, and total dissolved solids also included in this group. Hardness is the concentration of dissolved minerals, particularly calcium and magnesium, in water.

3.2.1.6 Indicators of Water Clarity

The following constituents were included as indicators of water clarity: color, turbidity, total (and dissolved) suspended solids and total organic carbon. Color is a measure of water clarity which, in southwest Florida, is particularly affected by the presence of tannins from decomposition of organic material. Color is typically measured using a spectrophotometer and reported in platinum cobalt units (pcu). True color is measured after filtering suspended substances (*e.g.*, those contributing to turbidity) while apparent color represents the color one sees with the naked eye and therefore includes the effects of suspended particles. Turbidity is an estimate of the amount of suspended material in the water column. At elevated levels turbidity can cause water to appear cloudy or hazy. Water can be made turbid by sediments, inorganic and organic matter, algae, colored dissolved organic matter, plankton, and other microscopic organisms. Turbidity can increase during rain events due to runoff from surrounding lands, as well as from high flows when greater flow velocities and volumes suspend material from the stream bed. Similarly, suspended solids are particles that are larger than 2 microns found in the water column. Most suspended solids are made up of inorganic materials though bacteria and algae can also contribute to the total solids concentration. Total suspended solids affect water clarity; the more solids present in the water, the less clear the water will be. Some suspended solids can settle to the bottom of a water body particularly during periods of low or no water flow, potentially smothering benthic organisms. Higher concentrations of suspended solids can also serve as carriers of toxics, which cling to suspended particles. Total solids measurements can be useful as an indicator of the effects of runoff and concentrations often increase during rainfall while total dissolved solids can be an indicator of groundwater contributions to streamflow which tend to increase water clarity. Total Organic Carbon (TOC) is a measure of the total amount of carbon in organic compounds found in the water sample. While not specifically a measure of water clarity, it can represent contribution of both anthropogenic and natural sources of carbon inputs into the streambed that affect water clarity.

Table 3. *Water quality constituents measured in field or analyzed in lab along with associated measurement units. Constituent groups were assigned for presentation of results.*

Constituent Group	Field Constituent	Units
Physio-chemical	Conductivity	Micromho per centimeter
Physio-chemical	Dissolved Oxygen	Milligram per liter and Percent Saturation
Physio-chemical	pH	Standard Unit
Physio-chemical	Temperature	Degree Celsius
Constituent Group	**Lab Constituent**	**Units**
Nitrogen	Ammonia	Milligram per liter
Nitrogen	Ammonium	Milligram per liter
Physio-chemical	Biological Oxygen Demand	Milligram per liter
Physio-chemical	Chemical Oxygen Demand	Milligram per liter
Minerals and Metals	Calcium	Milligram per liter
Minerals and Metals	Chloride	Milligram per liter
Chlorophyll	Chlorophyll *a*	Microgram per liter
Water Clarity	Color	Platinum-cobalt unit
Phosphorus	Dissolved Orthophosphate	Milligram per liter
Minerals and Metals	Fluoride	Milligram per liter
Physio-chemical	Hardness	Milligram per liter
Minerals and Metals	Iron	Microgram per liter
Minerals and Metals	Magnesium	Milligram per liter
Nitrogen	Nitrate	Milligram per liter
Nitrogen	Nitrate-Nitrite	Milligram per liter
Nitrogen	Nitrite	Milligram per liter
Nitrogen	Organic Nitrogen	Milligram per liter
Phosphorus	Ortho Phosphate as PO_4^{3-}	Milligram per liter
Phosphorus	Phosphorus in Total Orthophosphate as P	Milligram per liter
Minerals and Metals	Radium 226	Picocurie per liter
Minerals and Metals	Radium 228	Picocurie per liter
Minerals and Metals	Radium Total	Picocurie per liter
Minerals and Metals	Sulfate	Milligram per liter
Water Clarity	Total Dissolved Solids	Milligram per liter
Nitrogen	Total Kjeldahl Nitrogen	Milligram per liter
Nitrogen	Total Nitrogen	Milligram per liter
Water Clarity	Total Organic Carbon	Milligram per liter
Phosphorus	Total Phosphorus	Milligram per liter
Water Clarity	Total Suspended Solids	Milligram per liter
Water Clarity	Turbidity	Milligram per liter
Nitrogen	Unionized Ammonium	Milligram per liter

3.2.2 Water Quality Constituent Measurements

Water quality data were obtained from the SWFWMD, the DEP, and the USGS. For data obtained from the DEP, this assessment used IWR run 59 (dated July 10, 2020). This database contains data collected by the DEP, the SWFWMD, and USGS. Where data are replicated in both IWR run 59 and original data source, the original source data were analyzed.

Once constituents were selected from the datasets, the following data processing procedures were used as data screening procedures prior to analysis:

1. Selected for analyses, stations meeting minimum sample size requirements of 30 observations, as agreed upon by the SWFWMD. An exception to this rule included analysis of WSI data from an ongoing study that is now approaching the minimum sample size criteria.
2. Removed of observations with one or more of the following fatal DEP qualifier codes: (?, V, N, O, Y, H, J, K, Q)
3. Formatted data into an analytical format

4. Performed outlier analysis to identify potential influential or erroneous observations
5. Output analytical dataset with outliers identified (not removed)
6. Described data with plots and statistics

Descriptive plots and statistics, including results of outlier analysis, are delivered as Appendix B provided as part of the Supplemental Materials document associated with this task assignment. The Appendix contains:

1. A table listing each station meeting inclusion criteria with number of observations and period of record for each constituent as well as the percent of observations identified as potential outliers by each method.
2. A univariate histogram for each constituent at each site including distributional statistics and curves based on normal and log normal distributions.
3. Temporal box plots of the data distribution for each station/constituent combination.
4. Timeseries plots with outliers identified for each station/constituent combination.

The water quality sites in Charlie Creek are shown in Figure 8 and listed in Table 4, along with each station's period of record and sample size for field and lab-measured constituents. TN, nitrogen constituents, TP, phosphorus constituents, chlorophyll *a*, DO, color, specific conductance, pH, temperature, total suspended solids, turbidity, and select mineral and metal constituents were assessed.

Table 4*. Sample locations, data source, period of record and associated sample sizes for water quality data in Charlie Creek.*

Source	Station	Station Name	First Date	Most Recent Date
USGS	02296500	Charlie Creek near Gardner	01/26/1965	09/28/1999
IWR	21FLGW 3561	Charlie Creek near Gardner	10/08/1998	12/05/2017
SWFWMD	23969	Charlie Creek at State Road 66	05/16/2007	03/05/2019

Notes: USGS is United States Geological Survey
IWR is Impaired Waters Rule
SWFWMD is Southwest Florida Water Management District

4.0 Methods

4.1 Flows and Antecedent Flow Conditions

Each water quality station was assigned a discharge record for developing relationships between water quality and flow. Since all water quality stations were collocated with USGS discharge gages, the water quality stations were matched to the corresponding discharge gage (Figure 8). No antecedent flows were considered for analysis.

4.2 Analytical Methods

4.2.1 Trend Tests

Evaluating trends in water quality over time can provide important context to consider in assessing the relationships between water quality and flows. Two iterations of the non-parametric Mann Kendall test for trend (Mann 1945; Kendall 1975) were used for this evaluation. Monthly median flows were evaluated to determine if flows in any month were trending over the entire period of record for each gage. That is, a Mann Kendall (MK) trend test was performed for each month based on the monthly median flow value. Second, the Seasonal Mann Kendall (SMK) with correction for serial dependence (Hirsch and Slack 1984) was used to evaluate if water quality data were consistently trending over time across months to provide general assessments of trends in water quality over the time period of data collection. Inclusion criteria for these tests included having data collected in a recent time period (*i.e.*, since 2017) and, for the SMK test, at least 5 years of data and 60 observations. Results of the SMK were adjusted for multiple comparisons using the False Discovery Rate method (Benjamini and Hochberg 1993). Importantly, these methods describe trends but do not account for the effects of other potential explanatory factors affecting observed trends and therefore do not explain why a trend might be observed. Adjustments for serial correlation and multiple comparisons were included to be conservative in declaring the presence of a trend. Both serial correlation and multiple comparisons can lead to increased false positive rates and these methods attempt to minimize that artifact in the data analysis. Trend test results are reported for each site and constituent meeting criteria defined above and reported with number of observations (n), the p value of the statistical test, a column identifying whether or not serial correlations was present in the timeseries (which can affect the p value associated with the statistical test), the trend direction, and the Theil-Sen slope estimate (*i.e.*, the median of all pairwise slope estimates: Hirsch and Slack 1984).

4.2.2 Linear Regression

Linear regression is a common statistical method relating a predictor variable (*e.g.*, flow) to a response variable (*e.g.*, water quality constituent concentration) under a specific set of assumptions; principally that the relationship is linear. Additional assumptions include that the data are normally distributed, independent, and homoscedastic. These attributes are not typical properties of either flows or most common water quality constituents and to comply with those assumptions, natural log transformations were applied to both the predictor and response variables for useful relationships. To test for seasonal differences in the mean response to flow, a seasonal classification term was added to the model using dummy variables to evaluate how different months changed the average response between flows and water quality constituent concentrations. When seasonality was not detected, the results of univariate regressions with flow are presented. The coefficients of determination describe variance in water quality constituents attributable to both flow and season when both are significant (α= 0.05). Due to limited data availability for most sites, interaction terms were not considered for these models. To evaluate the model fit and potential utility in assessing these water quality relationships, the sign of the slope statistic (*i.e.*, positive or negative), the p value indicating the statistical significance of the slope statistic, and the coefficient of determination (R^2) defining the proportion of variation explained by the model were reported to aid in evaluating the resultant linear regression relationships. The following equation depicts the form of the linear regression that was applied.

$$Ln(Y_{ij}) = \beta_0 + \beta_1 * Ln(flow_i) + \beta_2 * season_j + e_{ij}$$

where:
Y = water quality response
β_0= intercept
β_1= slope estimate for the rate of change in Ln(Y)
per unit change in Ln(flow)
β_2 = effect of seasonal covariate defined by month

and the null hypothesis being evaluated is Ho: $\beta_1 = 0$.

Linear regression models were used to evaluate relationships between flow and water quality constituents to facilitate the SWFWMD's consideration of water quality as an environmental value in Charlie Creek. The assessment was conducted to identify those constituents where variations in flow describe a significant proportion of the variation in a water quality constituent of interest. The modeling effort explicitly acknowledges that the results do not imply causation. Establishing causality is an extremely challenging endeavor and no one study can establish causality, particularly from observational data. Hill (1965) established 10 criteria for establishing causality, which include the strength of association, consistency among multiple studies, and experimental evidence. Therefore, establishing causality is beyond the scope of this effort.

Results of linear regression analyses are reported for "primary sites" which are defined as those with at least 100 observations for the constituent of interest, and "secondary sites" defined as those with fewer than 100 observations for the constituent of interest. The same location could be reported as a primary site for one constituent and a secondary site for another constituent. This separation was performed because many sampling locations had fewer than 100 observations, and it was determined to include these sites in the results but to isolate them from interpretation with those sites with a more robust sample set. Linear regression analyses are presented for those sites with smaller sample sizes but should be interpreted with caution.

4.2.3 Logistic Regression

Logistic regression is an alternative to natural-log transformation of both predictor and response variables (Hosmer and Lemeshow, 2000). Logistic regression relies on fewer assumptions than linear regression to model response relationships of the exponential family and relies on a relatively large sample size for estimation. Logistic regression is based on a binary transformation of the response variable to be either greater than or less than a specified a priori determined threshold value. DEP criteria were used for TN, TP, chlorophyll *a*, and DO (percent saturation) as ecologically relevant threshold values to classify exceedance or non-exceedance with the understanding that these models do not relate to determination of impairment, classification, or listing of a waterbody according to state water quality criteria. The general model structure was defined as:

$$g(y) = \ln\left[\frac{p(y)}{1-p(y)}\right] = \beta_0 + \beta_1 x_1 + \beta_2 x_2$$

where:

$p(y)$ = the probability of exceedance as a function of x;

$g(y)$ = the logit transformation of the odds of exceedance;

x_1 = the flow condition; and

x_2 = the covariate such as month used as a dummy variable; and β_0, β_1, and β_2 are regression coefficients.

Evaluation of the logistic regression model fits included calculating a generalized R^2 and evaluating the predictive capacity of the model with receiver operator curves (SAS Institute Inc., 2018). The computation of R^2 was derived by comparing the maximum likelihood estimate of the intercept-only model to the maximum likelihood estimate for the specified model:

$$R^2 = 1 - [\frac{L(O)}{L(\beta)}]^{2/n}$$

where:

L(O) = the maximum likelihood estimate under the intercept-only model;

L(β) = the maximum likelihood estimate under specified model; and

n = the sample size.

The generalized R^2 statistic was then rescaled to conform to the typical inference regarding R^2, in which the maximum value is 1.

The intent of using logistic regression in addition to linear regression was to investigate the relationship between flows and the probability of exceeding ecologically relevant water quality thresholds for a subset of water quality constituents (*i.e.*, TN, TP, corrected chlorophyll *a*, and dissolved oxygen). While linear regression estimates the change in a water quality constituent for a unit change in flows, logistic regression estimates the change in probability of exceeding a threshold value for a water quality constituent as a function of flows. Thereby, logistic regression was a complimentary assessment to evaluate if variations in flows resulted in an increased or decreased probability of exceeding ecologically relevant thresholds where those thresholds have been established. Threshold values associated with state water quality standards were used. Those threshold values include the maximum TN concentration for freshwater streams (1.65 mg/l), the maximum chlorophyll *a* corrected concentration for freshwater streams (20 µg/l), a maximum TP concentration for freshwater streams (0.49 mg/L), and minimum DO_{SAT} criteria of 38% saturation. The purpose of using these threshold values is that, at some temporal scale, they represent an ecologically relevant threshold value and that if the findings suggest that reductions in flows are associated with an increased probability of exceeding those thresholds, that finding may be worthy of further investigation. However, the state standards were derived as annual statistics and this assessment explicitly warns that the results of this analysis should not in any way be used to suggest that variations in flows would lead to impairments according to state standards. Logistic regression analysis was restricted to those stations and constituents with 100 or more observations as well as more than 10% of the observations showing exceedances of the thresholds.

5.0 Results of Statistical Analyses

This section presents the results of the statistical analyses described above.

5.1 Trend Test Results

5.1.1 Flow Trend Tests

No months were observed to be trending over the long-term period of record at USGS Charlie Creek gage near Gardner (No. 02296500) (Figure 9) and only a single month (June) resulted in a statistically significant trend at USGS Charlie Creek gage near Crewsville (No. 02296260). The June trend at USGS Charlie Creek gage near Crewsville (No. 02296260) has a v-shaped trend based on the non-parametric LOESS smoother. The observation of a v-shaped trend combined with the short period of record for the USGS Charlie Creek gage near Crewsville (No. 02296260) make inference from the statistical result tenuous and should be interpreted with caution.

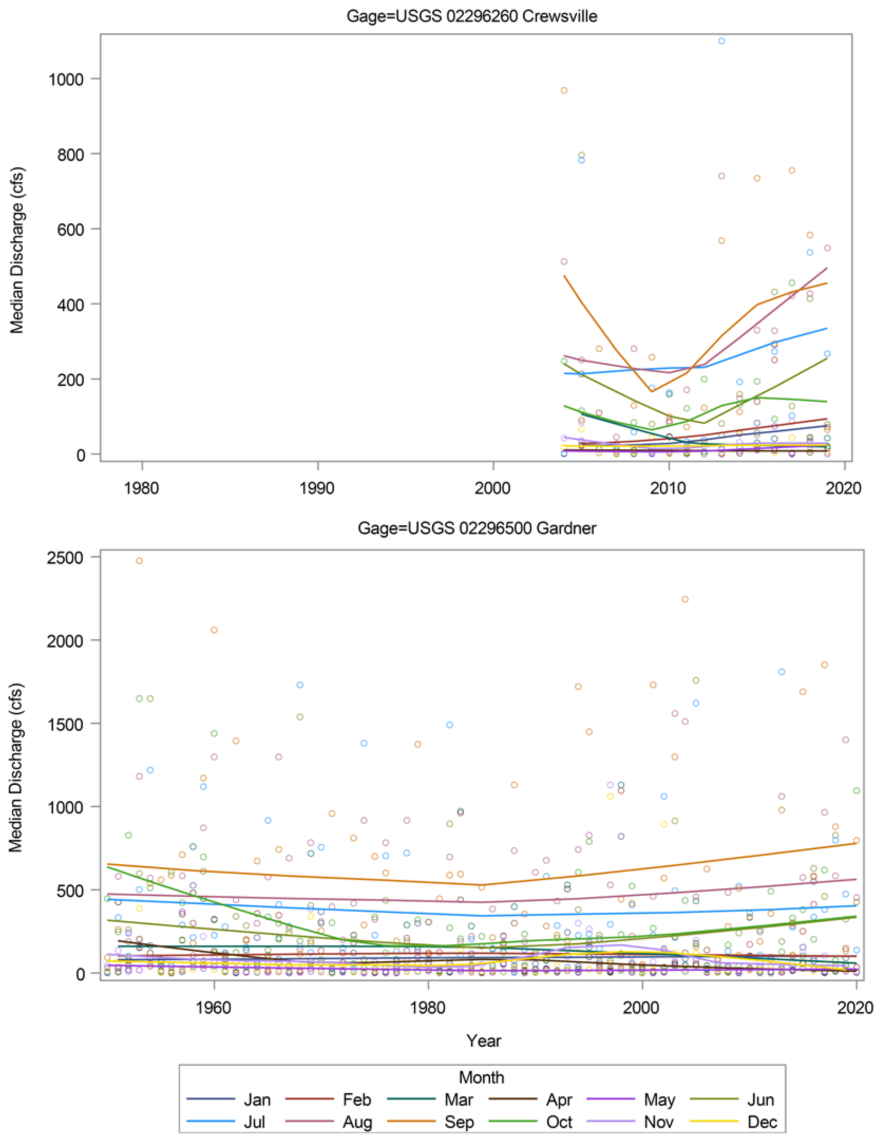

***Figure 9**. Monthly median flow time series (points) and locally-estimated scatterplot smoothing (LOESS) polylines for USGS Charlie Creek gage near Crewsville (No. 02296260) (Top), and USGS Charlie Creek gage near Gardner (No. 02296500) (Bottom).*

5.1.2 Water Quality Trend Tests

This section presents the results of Seasonal Mann Kendall trend tests. Trend tests were conducted on 44 constituent/station combinations. The results for the five statistically significant trends are listed by constituent and station in Table 5. Constituents with significant results were principally physio-chemical constituents from *in situ* measurements. Four of the five significant results were decreasing over time while conductivity increased over the period of record for the USGS station 02296500. This station is more downstream than Station 23969 where conductivity exhibited a decreasing trend. However, the two stations also have different periods of record (Table 4) All other constituents evaluated, including nutrients, chlorophyll *a*, and indicators of water clarity were stable over the respective periods of record.

Table 5. *Results of Seasonal Mann Kendall test for trend for those constituent/ station combinations with statistically significant (p<0.05) results.*

Station	Constituent	Start Date	End Date	N	Serial Correlation	Adjusted p Value	Theil Sen Slope (Change /Year)	Trend Direction
23969	Conductivity (μmho)	05/16/2007	03/05/2019	74	No	0.0174	-6.8333	Decreasing
21FLGW 3561	Hardness (mg/l)	10/08/1998	12/05/2017	68	Yes	0.0033	-5.6250	Decreasing
02296500	pH (SU)	01/26/1965	09/28/1999	98	No	0.0169	-0.0200	Decreasing
02296500	Temperature (C)	01/26/1965	09/28/1999	122	Yes	0.0030	-0.1143	Decreasing
02296500	Conductivity (μmho)	01/26/1965	09/28/1999	132	Yes	0.0011	4.3765	Increasing

Notes: N is sample size
μmho is micromho
SU is standard unit
C is degrees Celsius
$p < 0.05$ is significant $p \geq 0.05$ is not significant
mg/L is milligram per liter

5.2 Linear Regression Results

This section presents the results of linear regression models; the results are presented by constituent. Bivariate (*i.e.*, XY plots) plots of each constituent against flow followed by detailed statistical output for each regression performed are provided in the Appendix B and C of the Supplemental Materials document, respectively. In the regression tables below, regression statistics are reported where significant (p value less than 0.05) and an “ns” is reported where the p value was greater than or equal to 0.05. If the seasonal term was not significant in the model, the regression statistics reported for the intercept and flow terms represent the results from a univariate regression of the constituent against flow. Details for these regressions can be found in Appendix D of the Supplemental Materials document.

5.2.1 Associating Flows and Water Quality

To assess the relationships between water quality and flows, water quality stations were assigned discharge data from the USGS station co-located with the water quality location (Figure 8). Since all water quality stations were co-located with discharge stations, no antecedent conditions were considered.

5.2.2 Nitrogen

Charlie Creek gage near Gardner reported three nitrogen constituents (ammonium, nitrate-nitrite and TKN) with samples sizes greater than or equal to 100 (Table 6). All three constituents yielded significant positive relationships with flow and all three indicated seasonality was a significant factor. An additional 11 regressions were conducted for seven nitrogen constituents (ammonia, nitrite, nitrate-nitrite, organic nitrogen, TKN and TN) at two stations with sample sizes less than 100 (Table 7). All TN regressions yielded significant positive relationships with flow, as did both nitrite regressions and the single organic nitrogen and TKN analyses. The remaining regressions yielded non-significant relationships with flow. Only nitrate-nitrite at Crewsville gage indicated seasonality was a significant factor.

***Table 6**. Linear regression for nitrogen constituents at primary water quality sites where sample size ≥ 100 in Charlie Creek, on flows in cubic feet per second at the USGS Charlie Creek gage near Gardner (No. 02296500).*

Gage	Constituent	Station	N	Month p Value	Flow p Value	Flow Slope	R^2	Slope Direction
Gardner	Ammonium (mg/l)	21FLGW 3561	222	<0.001	<0.001	0.115	0.39	1
Gardner	Nitrate-Nitrite (mg/l)	21FLGW 3561	216	<0.001	<0.001	0.290	0.29	1
Gardner	Total Nitrogen (mg/l)	21FLGW 3561	220	<0.001	<0.001	0.126	0.67	1

Notes: Flow slope is the slope of the flow regression in ln(units)/ln(cfs)
cfs is cubic feet per second
N is sample size
$p < 0.05$ is significant $p \geq 0.05$ is not significant
ns indicates p value is not significant
R^2 is the coefficient of determination
mg/l is milligram per liter

***Table 7**. Linear regression for nitrogen constituents at primary water quality sites where sample size< 100 in Charlie Creek, on flows in cubic feet per second at either the USGS Charlie Creek gage near Gardner (No. 02296500) or the USGS Charlie Creek gage near Crewsville (No. 02296260).*

Gage	Constituent	Station	N	Month p Value	Flow p Value	Flow Slope	R^2	Slope Direction
Crewsville	Ammonia (mg/l)	23969	69	ns	ns	0.069	0.05	0
Gardner	Nitrite (mg/l)	02296500	72	ns	0.006	0.074	0.10	1
Crewsville	Nitrite (mg/l)	23969	70	ns	<0.001	0.078	0.22	1
Gardner	Nitrate (mg/l)	02296500	70	ns	ns	0.005	0.00	0
Crewsville	Nitrate-Nitrite (mg/l)	23969	72	<0.001	ns	0.023	0.40	0
Gardner	Nitrate-Nitrite (mg/l)	02296500	71	ns	ns	0.075	0.01	0
Gardner	Organic Nitrogen (mg/l)	02296500	76	ns	<0.001	0.156	0.39	1
Gardner	Total Kjeldahl Nitrogen (mg/l)	02296500	73	ns	<0.001	0.151	0.39	1
Crewsville	Total Nitrogen (mg/l)	23969	73	ns	<0.001	0.049	0.16	1
Gardner	Total Nitrogen (mg/l)	02296500	73	ns	<0.001	0.127	0.26	1
Gardner	Total Nitrogen (mg/l)	21FLGW 3561	31	ns	<0.001	0.235	0.37	1

Notes: Flow slope is the slope of the flow regression in ln(unit)/ln(cfs)
cfs is cubic feet per second
N is sample size
ns indicates p value is not significant
mg/l is milligram per liter
$p < 0.05$ is significant $p \geq 0.05$ is not significant
R^2 is the coefficient of determination

When sample sizes were combined, 71% of nitrogen constituent regressions yielded positive relationships with flow. The remainder was not significant.

5.2.3 Phosphorus

Charlie Creek gage near Gardner reported two phosphorus constituents (dissolved ortho P, and total phosphorus) with samples sizes greater than or equal to 100 (Table 8). Both of these relationships were significant and had negative slopes. Seasonality was also significant for both constituents. An additional four regressions were conducted on three phosphorus constituents (dissolved Ortho P, orthophosphate as PO4 and TP) at two stations with sample sizes less than 100 (Table 9). The regression for dissolved ortho P and one of the two TP regressions yielded significant positive relationships with flow at Charlie Creek gage near Gardner, while the remaining two regressions were not significant. Seasonality was determined to be a significant factor for all four analyses. When sample sizes were combined, 33% of phosphorus constituent relationships yielded negative relationships with flow, while 33% yielded positive flow relationships.

***Table 8**. Linear regression for phosphorus constituents at primary water quality sites where sample size ≥ 100 in Charlie Creek, on flows in cubic feet per second at the USGS Charlie Creek gage near Gardner (No. 02296500).*

Gage	Constituent	Station	N	Month p Value	Flow p Value	Flow Slope	R^2	Slope Direction
Gardner	Dissolved Ortho P (mg/l)	21FLGW 3561	111	<0.001	0.032	-0.050	0.33	-1
Gardner	Total Phosphorus (mg/l)	21FLGW 3561	217	<0.001	<0.001	-0.047	0.32	-1

Notes: Flow slope is the slope of the flow regression in ln(unit)/ln(cfs)
cfs is cubic feet per second
N is sample size
$p < 0.05$ is significant $p \geq 0.05$ is not significant
ns indicates p value is not significant
R^2 is the coefficient of determination
mg/l is milligram per liter

***Table 9**. Linear regression for phosphorus constituents at primary water quality sites where sample size< 100 in Charlie Creek, on flows in cubic feet per second at either the USGS Charlie Creek gage near Gardner (No. 02296500) or the USGS Charlie Creek gage near Crewsville (No. 02296260).*

Gage	Constituent	Station	N	Month p Value	Flow p Value	Flow Slope	R^2	Slope Direction
Crewsville	Dissolved Ortho P (mg/l)	23969	74	0.021	<0.001	0.070	0.59	1
Gardner	Orthophosphate as PO4 (mg/l)	02296500	74	<0.001	ns	-0.027	0.48	0
Gardner	Total Phosphorus (mg/l)	02296500	73	<0.001	ns	-0.021	0.51	0
Crewsville	Total Phosphorus (mg/l)	23969	71	0.006	0.008	0.051	0.58	1

Notes: Flow slope is the slope of the flow regression in ln(unit)/ln(cfs)
cfs is cubic feet per second
N is sample size
ns indicates p value is not significant
$p < 0.05$ is significant $p \geq 0.05$ is not significant
R^2 is the coefficient of determination
mg/l is milligram per liter

5.2.4 Chlorophyll *a*

One station with at least 100 samples had data for both corrected and uncorrected chlorophyll *a*. The regressions against flow for both of these constituents were not significant (Table 10). However, seasonality was found to be a significant factor for both.

***Table 10**. Linear regression for chlorophyll a constituents at primary water quality sites where sample size ≥ 100 in Charlie Creek, on flows in cubic feet per second at the USGS Charlie Creek gage near Gardner (No. 02296500).*

Gage	Constituent	Station	N	Month p Value	Flow p Value	Flow Slope	R^2	Slope Direction
Gardner	Chlorophyll Uncorrected (µg/l)	21FLGW 3561	119	<0.001	ns	-0.036	0.26	0
Gardner	Chlorophyll Corrected (µg/l)	21FLGW 3561	214	0.001	ns	0.036	0.15	0

Notes: Flow slope is the slope of the flow regression in ln(unit)/ln(cfs)
cfs is cubic feet per second
N is sample size
$p < 0.05$ is significant $p \geq 0.05$ is not significant
ns indicates p value is not significant
R^2 is the coefficient of determination
µg/l is micrograms per liter

In addition, one station with fewer than 100 samples (Table 11) had data for uncorrected chlorophyll *a*. This regression against flow was found to have a significant negative slope; however, seasonality was not found to be a significant factor.

***Table 11**. Linear regression for uncorrected chlorophyll a at primary water quality sites where sample size< 100 in Charlie Creek, on flows in cubic feet per second at the USGS Charlie Creek gage near Crewsville (No. 02296260).*

Gage	Constituent	Station	N	Month p Value	Flow p Value	Flow Slope	R^2	Slope Direction
Crewsville	Chlorophyll Uncorrected (µg/l)	23969	73	ns	<0.001	-0.308	0.42	-1

Notes: Flow slope is the slope of the flow regression in ln(unit)/ln(cfs)
cfs is cubic feet per second
N is sample size
R^2 is the coefficient of determination
$p < 0.05$ is significant $p \geq 0.05$ is not significant
ns indicates p value is not significant
µg/l is micrograms per liter

5.2.5 Physio-Chemical

Eight regressions for in situ constituents (conductivity, dissolved oxygen, pH, and temperature) were conducted for stations with a minimum of 100 samples (Table 12). All eight regressions (two for each constituent) resulted in significant negative relationships with flow. Additionally, with the exception of pH, all indicated seasonality as a significant factor.

Table 12*. Linear regression for physio-chemical constituents at primary water quality sites where sample size ≥ 100 in Charlie Creek, on flows in cubic feet per second at the USGS Charlie Creek gage near Gardner (No. 02296500).*

Gage	Constituent	Station	N	Month p Value	Flow p Value	Flow Slope	R^2	Slope Direction
Gardner	Conductivity (µmho)	21FLGW 3561	223	<0.001	<0.001	-0.162	0.74	-1
Gardner	Conductivity (µmho)	02296500	149	0.021	<0.001	-0.166	0.57	-1
Gardner	Dissolved Oxygen (mg/l)	21FLGW 3561	217	<0.001	<0.001	-0.039	0.52	-1
Gardner	Dissolved Oxygen (mg/l)	02296500	133	<0.001	<0.001	-0.038	0.51	-1
Gardner	pH (SU)	21FLGW 3561	226	ns	<0.001	-0.019	0.37	-1
Gardner	pH (SU)	02296500	113	ns	<0.001	-0.028	0.20	-1
Gardner	Temperature (C)	21FLGW 3561	225	<0.001	0.015	-0.013	0.76	-1
Gardner	Temperature (C)	02296500	140	<0.001	0.042	-0.014	0.71	-1

Notes: Flow slope is the slope of the flow regression in ln(unit)/ln(cfs)
cfs is cubic feet per second
N is sample size
R^2 is the coefficient of determination
µmho is a micromho
$p < 0.05$ is significant $p \geq 0.05$ is not significant
ns indicates p value is not significant
mg/l is milligram per liter
SU is standard units
C is degrees Celsius

An additional seven regressions were analyzed at stations with fewer than 100 samples (Table 13) for alkalinity, conductivity, dissolved oxygen, hardness, pH, and temperature. All but two of these (DO_C and temperature at the same station) yielded significant (and negative) relationships. Seasonality was a significant factor for Conductivity, DO_C and DO_{SAT}, and temperature.

Table 13*. Linear regression for physio-chemical constituents at primary water quality sites where sample size< 100 in Charlie Creek, on flows in cubic feet per second at either the USGS Charlie Creek gage near Gardner (No. 02296500) or the USGS Charlie Creek gage near Crewsville (No. 02296260).*

Gage	Constituent	Station	N	Month p Value	Flow p Value	Flow Slope	R^2	Slope Direction
Crewsville	Alkalinity (mg/l)	23969	69	ns	<0.001	-0.171	0.74	-1
Crewsville	Conductivity (µmho)	23969	76	0.001	<0.001	-0.107	0.75	-1
Crewsville	Dissolved Oxygen (mg/l)	23969	75	<0.001	ns	-0.047	0.57	0
Gardner	Dissolved Oxygen (%sat)	02296500	29	0.007	<0.001	-0.083	0.87	-1
Gardner	Hardness (mg/l)	21FLGW 3561	72	ns	<0.001	-0.189	0.79	-1
Crewsville	pH (SU)	23969	76	ns	<0.001	-0.018	0.66	-1
Crewsville	Temperature (C)	23969	76	<0.001	ns	-0.006	0.72	0

Notes: Flow slope is the slope of the flow regression in ln(unit)/ln(cfs)
cfs is cubic feet per second
N is sample size
R^2 is the coefficient of determination
µmho is micromho
$p < 0.05$ is significant $p \geq 0.05$ is not significant
ns indicates p value is not significant
mg/l is milligram per liter
SU is standard units
C is degrees Celsius

For the combined 15 regressions, 87% yielded significant negative relationships with flow, and seasonality was a significant factor for all but three. All conductivity and pH regressions and the sole dissolved oxygen saturation, hardness and alkalinity regressions were characterized by significant negative slopes, while two-thirds of all dissolved oxygen concentration and temperature regressions had significant negative slopes.

5.2.6 Minerals and Metals

Regressions for four constituents (calcium, chloride, fluoride, and sulfate; one regression per constituent) were analyzed from stations with at least 100 samples (Table 14). All four regressions with flow were found to have significant negative slopes. Seasonality was a significant factor for all constituents in this group..

Table 14. Linear regression for specified constituents at primary water quality sites where sample size ≥ 100 in Charlie Creek, on flows in cubic feet per second at the USGS Charlie Creek gage near Gardner (No. 02296500).

Gage	Constituent	Station	N	Month p Value	Flow p Value	Flow Slope	R^2	Slope Direction
Gardner	Calcium Total (mg/l)	21FLGW 3561	226	0.002	<0.001	-0.194	0.77	-1
Gardner	Chloride (mg/l)	21FLGW 3561	227	<0.001	<0.001	-0.104	0.72	-1
Gardner	Fluoride (mg/l)	21FLGW 3561	226	0.004	<0.001	-0.196	0.85	-1
Gardner	Sulfate Total (mg/l)	21FLGW 3561	221	<0.001	<0.001	-0.264	0.69	-1

Notes: Flow slope is the slope of the flow regression in ln(unit)/ln(cfs)
cfs is cubic feet per second
N is sample size
$p < 0.05$ is significant $p \geq 0.05$ is not significant
ns indicates p value is not significant
R^2 is the coefficient of determination
mg/l is milligram per liter

An additional seven regressions (calcium n=1, chloride n=1, fluoride n=2, magnesium n=1, sulfate n=1 each total and dissolved) were analyzed from stations with fewer than 100 samples (Table 15). All but the single chloride regression yielded significant negative slopes; seasonality was significant for fluoride, dissolved sulfate, and dissolved magnesium.

Table 15. Linear regression for specified constituents at primary water quality sites where sample size< 100 in Charlie Creek, on flows in cubic feet per second at either the USGS Charlie Creek near Gardner FL site (No. 02296500) or the USGS Charlie Creek near Crewsville FL site (No. 02296260).

Gage	Constituent	Station	N	Month p Value	Flow p Value	Flow Slope	R^2	Slope Direction
Crewsville	Calcium Dissolved (mg/l)	23969	74	ns	<0.001	-0.142	0.70	-1
Gardner	Chloride (mg/l)	02296500	31	ns	ns	-0.041	0.06	0
Crewsville	Fluoride (mg/l)	23969	63	0.034	<0.001	-0.098	0.70	-1
Gardner	Fluoride (mg/l)	02296500	30	ns	<0.001	-0.145	0.54	-1
Crewsville	Magnesium dissolved(mg/l)	23969	74	0.045	<0.001	-0.133	0.73	-1
Gardner	Sulfate Total (mg/l)	02296500	30	ns	0.005	-0.224	0.25	-1
Crewsville	Sulfate Dissolved (mg/l)	23969	73	0.003	<0.001	-0.172	0.61	-1

Notes: Flow slope is the slope of the flow regression in ln(unit)/ln(cfs)
cfs is cubic feet per second
N is sample size
ns indicates p value is not significant
R^2 is the coefficient of determination
$p < 0.05$ is significant $p \geq 0.05$ is not significant
mg/l is milligram per liter

When sample sizes were combined, 91% of the regressions yielded significant relationships with flow, all characterized by negative slopes..

5.2.7 Indicators of Water Clarity

Four regressions were analyzed for four water clarity constituents (color, TOC, TSS, and turbidity) at stations with at least 100 samples (Table 16). While the regression for TSS resulted in a non-significant relationship, the remaining regressions indicated significant positive relationships with flow. Seasonality was found to be significant for all four.

Table 16. Linear regression for water clarity constituents at primary water quality sites where sample size ≥ 100 in Charlie Creek, on flows in cubic feet per second at the USGS Charlie Creek gage near Gardner (No. 02296500).

Gage	Constituent	Station	N	Month p Value	Flow p Value	Flow Slope	R^2	Slope Direction
Gardner	Color (PCU)	21FLGW 3561	226	<0.001	<0.001	0.261	0.71	1
Gardner	Total Organic Carbon (mg/l)	21FLGW 3561	223	0.007	<0.001	0.164	0.65	1
Gardner	Total Suspended Solids (mg/l)	21FLGW 3561	225	<0.001	ns	0.014	0.20	0
Gardner	Turbidity (NTU)	21FLGW 3561	226	0.02	<0.001	0.125	0.29	1

Notes: Flow slope is the slope of the flow regression in ln(unit)/ln(cfs)
cfs is cubic feet per second
N is sample size
R^2 is the coefficient of determination
$p < 0.05$ is significant $p \geq 0.05$ is not significant
ns indicates p value is not significant
mg/l is milligram per liter
PCU is platinum-cobalt units
NTU is Nephelometric Turbidity Units

An additional three regressions (one each for color, TOC, and turbidity) were conducted at a station with fewer than 100 samples (Table 17). The regression for turbidity was found to be non-significant while the remaining two resulted in significant positive slopes. None of these regressions indicated seasonality as a significant factor. When combined, 71% of the regressions yielded significant positive relationships with flow. The remainder were not significant.

Table 17. *Linear regressions for water clarity constituents at primary water quality sites where sample size< 100 in Charlie Creek, on flows in cubic feet per second at the USGS Charlie Creek gage near Crewsville (No. 02296260).*

Gage	Constituent	Station	N	Month p Value	Flow p Value	Flow Slope	R^2	Slope Direction
Crewsville	Color (PCU)	23969	72	ns	<0.001	0.131	0.51	1
Crewsville	Total Organic Carbon (mg/l)	23969	73	ns	<0.001	0.070	0.29	1
Crewsville	Turbidity (NTU)	23969	74	ns	ns	-0.003	0.00	0

Notes: Flow slope is the slope of the flow regression in ln(unit)/ln(cfs)
cfs is cubic feet per second
N is sample size
ns indicates p value is not significant
R^2 is the coefficient of determination
$p < 0.05$ is significant $p \geq 0.05$ is not significant
mg/l is milligram per liter
PCU is platinum-cobalt units
NTU is Nephelometric Turbidity Units

5.3 Logistic Regression Results

Only TP at a single station qualified for logistic regression (Table 18). The remaining possible station-constituent combinations either had too few observations (<100) or did not have an exceedance rate of at least 10%. For the single qualifying analysis, the relationship between flow and TP was negative.

Table 18. *Results of logistic regression for the sole constituent meeting the requirements of at least 100 observations and a 10% threshold exceedance rate. Threshold = 0.49 mg/l (Total Phosphorus).*

Constituent	Station	N	Below	Above	Month p Value	Flow p Value	Flow Slope	R^2	Slope Direction
Total Phosphorus	21FLGW 3561	217	74	143	<0.001	0.017	-0.264	0.28	-1

Notes: Flow slope is the slope of the flow regression in ln(unit)/ln(cfs)
N is sample size
R^2 is the coefficient of determination
$p < 0.05$ is significant $p \geq 0.05$ is not significant
mg/L is milligram per liter

6.0 Summary of Results

Table 19 and Table 20 provide a summary of regression results for primary and secondary evaluations, respectively. Results are summarized for the study area as a whole in these tables. A total of 23 regressions were performed on 19 constituents from stations with a minimum of 100 samples (Table 19). Sixty one percent (n=14) of these regressions yielded significant negative relationships, while the remainder were either positive significant relationships (n=6) or were not significant (n=3). Conversely, the 33 regressions conducted with data from stations with fewer than 100 samples (but greater than 30) for 27 constituents were divided almost evenly among these three categories (negative n=12, positive n=11, not significant n=10). When sample sizes were combined, 46% of the regressions yielded significant negative relationships while 30% resulted in significant positive relationships; the remainder were not significant.

It is important to note that the results described above are presented without regard to the strength of the association between flow and the constituent, expressed in this analysis by the R^2 statistic which quantifies the amount of variation in the response explained by the model. In some cases, the models were statistically significant but the R^2 values were low, suggesting the models explain little of the observed variability in water quality. To evaluate the results across sites graphically in a similar manner to Table 19 and Table 20, summary plots were constructed to display the R^2 values for all significant regression by constituent. For clarity, regressions results with a negative slope against flow were plotted as negative R^2 values though technically R^2 is always a positive value. Primary and secondary sites were combined for this plot since there were few stations available for analysis. The resulting graphics are instructive to evaluate how strong the association between water quality and flow when significant. For example, the Minerals and Metals group had generally strong negative relationships with flow with R^2 values greater than 0.50 suggesting the majority of variation in these constituents was explained by flow (and season) in these models. Indicators of water clarity including color, TOC and turbidity were positively related to flow with R^2 values ranging between ca. 0.25 and ca. 0.75. Physio-chemical constituents were negatively correlated with flow at most stations with the majority of significant results yielding an R^2 greater than 0.50. Only a single chlorophyll regression had a significant relationship which flow which had a negative slope but an R^2 less than 0.50. Phosphorus regressions yielded conflicting results with both constituents indicating negative slopes at one station and positive results at another while nitrogen constituents yielded all positive results though R^2 were less than 0.50 in all but one case.

Table 19*. Summary of linear regression results for each constituent analyzed; number of regressions resulting in negative, non-significant, or positive slopes; and the percentage of regressions with negative and positive slopes from all stations with greater than 100 samples.*

	Slope Direction					
Constituent	Negative	Not Significant	Positive	Total	% Negative	% Positive
Calcium Total (mg/l)	1	0	0	1	100.0	0.0
Chlorophyll Uncorrected (µg/l)	0	1	0	1	0.0	0.0
Chlorophyll Corrected (µg/l)	0	1	0	1	0.0	0.0
Chloride (mg/l)	1	0	0	1	100.0	0.0
True Color (PCU)	0	0	1	1	0.0	100.0
Conductivity (µmho)	2	0	0	2	100.0	0.0
Dissolved Oxygen (mg/l)	2	0	0	2	100.0	0.0
Fluoride (mg/l)	1	0	0	1	100.0	0.0
Ammonium (mg/l)	0	0	1	1	0.0	100.0
Nitrate-Nitrite (mg/l)	0	0	1	1	0.0	100.0
PH (SU)	2	0	0	2	100.0	0.0
Dissolved Ortho P (mg/l)	1	0	0	1	100.0	0.0
Sulfate Total (mg/l)	1	0	0	1	100.0	0.0
Temperature (C)	2	0	0	2	100.0	0.0
Total Kjeldahl Nitrogen (mg/l)	0	0	1	1	0.0	100.0
Total Organic Carbon (mg/l)	0	0	1	1	0.0	100.0
Total Phosphorus (mg/l)	1	0	0	1	100.0	0.0
Total Suspended Solids (mg/l)	0	1	0	1	0.0	0.0
Turbidity (NTU)	0	0	1	1	0.0	100.0
All	14	3	6	23	60.9	26.1

Notes: mg/L is milligram per liter
µg/L is microgram per liter
PCU is platinum-cobalt unit
µmho/cm is micromho per centimeter
% sat is percent saturation
pCi/L is picocurie per liter
C is degrees Celsius

***Table 20**. Summary of linear regression results for each constituent analyzed; number of regressions resulting in negative , not significant, or positive slopes; and percentage of regressions with negative and positive slopes from all stations with 30 to 99 observations.*

	Slope Direction					
Constituent	**Negative**	**Not Significant**	**Positive**	**Total**	**% Negative**	**% Positive**
Alkalinity (mg/l)	1	0	0	1	100.0	0.0
Calcium Dissolved (mg/l)	1	0	0	1	100.0	0.0
Chlorophyll Uncorrected (µg/l)	1	0	0	1	100.0	0.0
Chloride (mg/l)	0	1	0	1	0.0	0.0
True Color (PCU)	0	0	1	1	0.0	100.0
Conductivity (µmho)	1	0	0	1	100.0	0.0
Dissolved Oxygen (mg/l)	0	1	0	1	0.0	0.0
Dissolved Oxygen (%sat)	1	0	0	1	100.0	0.0
Fluoride (mg/l)	2	0	0	2	100.0	0.0
Hardness (mg/l)	1	0	0	1	100.0	0.0
Magnesium dissolved(mg/l)	1	0	0	1	100.0	0.0
Ammonia (mg/l)	0	1	0	1	0.0	0.0
Nitrite (mg/l)	0	0	2	2	0.0	100.0
Nitrate (mg/l)	0	1	0	1	0.0	0.0
Nitrate-Nitrite (mg/l)	0	2	0	2	0.0	0.0
Organic Nitrogen (mg/l)	0	0	1	1	0.0	100.0
PH (SU)	1	0	0	1	100.0	0.0
Dissolved Ortho P (mg/l)	0	0	1	1	0.0	100.0
Orthophosphate as PO4 (mg/l)	0	1	0	1	0.0	0.0
Sulfate Total (mg/l)	1	0	0	1	100.0	0.0
Sulfate Dissolved (mg/l)	1	0	0	1	100.0	0.0
Temperature (C)	0	1	0	1	0.0	0.0
Total Kjeldahl Nitrogen (mg/l)	0	0	1	1	0.0	100.0
Total Nitrogen (mg/l)	0	0	3	3	0.0	100.0
Total Organic Carbon (mg/l)	0	0	1	1	0.0	100.0
Total Phosphorus (mg/l)	0	1	1	2	0.0	50.0
Turbidity (NTU)	0	1	0	1	0.0	0.0
All	12	10	11	33	36.4	33.3

Notes: mg/L is milligram per liter
µg/L is microgram per liter
PCU is platinum-cobalt unit
µmho/cm is micromho per centimeter
% sat is percent saturation
pCi/L is picocurie per liter
C is degrees Celsius
NTU is Nephelometric turbidity unit

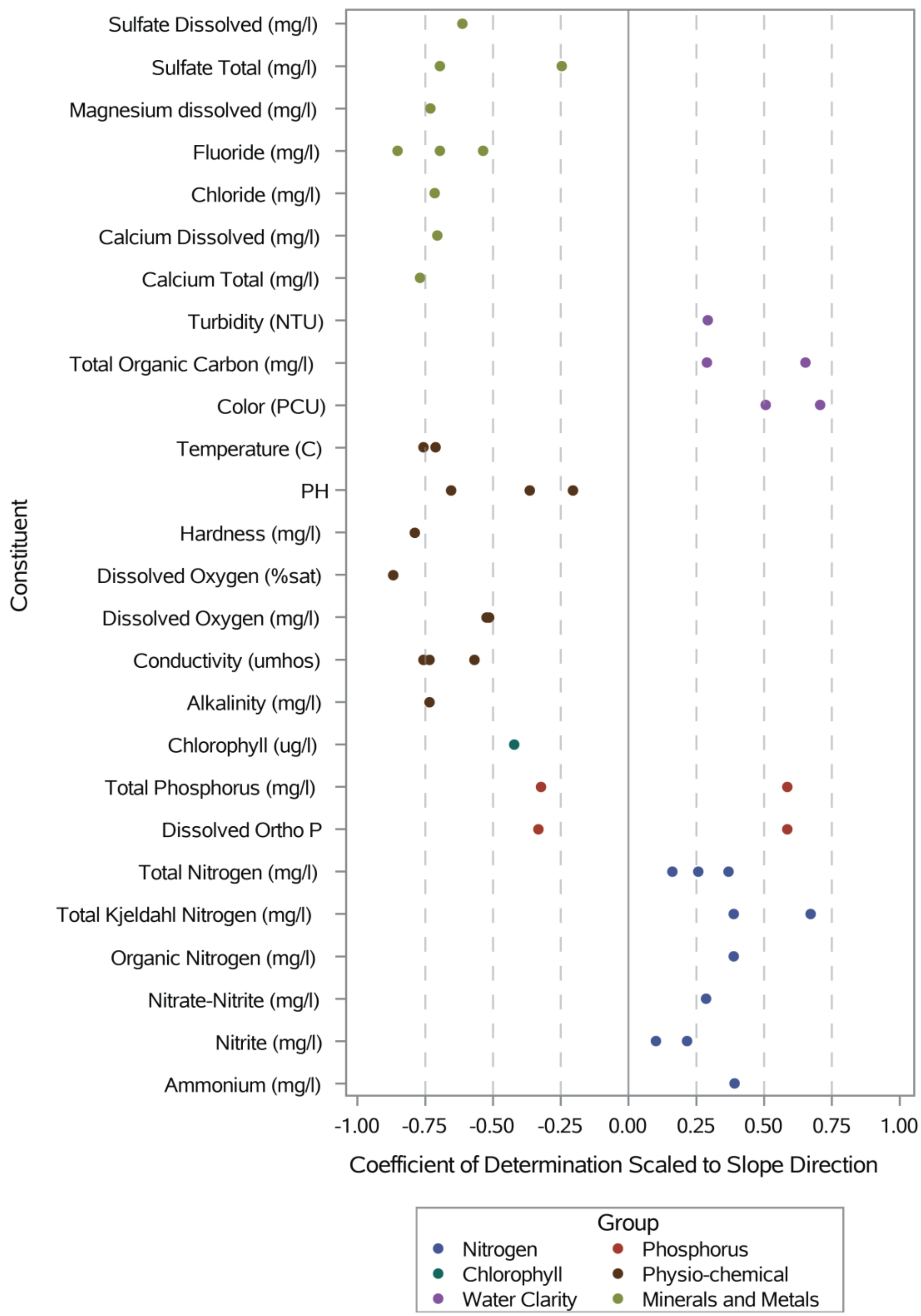

Figure 10. *Coefficient of determination (R^2) results for all significant regressions with at least 100 observations. The R^2 statistic is negative when the relationship with flow was negative. Color of filled circle indicates water quality group.*

6.1 Ecological Patterns and Processes

Time series trend tests on monthly median flow statistics suggest all months were stable over their respective periods of record with the exception of June at USGS Charlie Creek gage near Crewsville (No. 02296260). The June trend at USGS Charlie Creek gage near Crewsville (No. 02296260) is v-shaped based on the non-parametric LOESS smoother. The observation of a v-shaped trend combined with the short period of record for the USGS Charlie Creek gage near Crewsville (No. 02296260) make inference from the statistical result tenuous and the result should be interpreted with caution.

Time series trend tests on water quality constituents suggested that most constituents were stable over the period of record evaluated, corresponding to the results of the flow time series analysis above. Exceptions included one increasing trend and one decreasing trend in conductivity at different locations with differing periods of record, decreasing hardness at one station, and decreasing pH and temperature at one station. Differences in rainfall and flow patterns over the differing periods of record, as well as changes in land use patterns between locations over time, may help explain observed trends in these constituents.

The observed water quality relationships with flow were not unexpected for Florida rivers. For example, total nitrogen increases were correlated with increased flows though R^2 were less than 0.50. These findings are similar to results reported in similar water quality flow assessments conducted for the District in the Upper Withlacoochee River (Applied Technology & Management and Janicki Environmental, 2020) and Lower Withlacoochee River (Applied Technology & Management and Janicki Environmental, 2021) and generally result from increased flushing of organic rich wetland areas where decomposing organic matter and captured stormwater runoff contribute nitrogen to the system during the wet season. Indicators of water clarity suggest increasing flows reduce water clarity which also agrees with findings from other similar District studies. Conductivity, dissolved oxygen, and pH also tended to be inversely correlated with flow and the limited data for minerals also suggested inverse correlation with flow as the contribution of runoff from the lands surface is typically lower in pH and conductivity than groundwater contributions. Physical factors such as residence time and velocity are largely driven by the variation in rainfall and flows. Residence time, or its converse, flushing rate, can significantly affect such water quality constituents as chlorophyll *a* and DO. Longer residence times can result in higher primary production and eventual algal abundance in planktonic and attached forms. In turn, DO concentrations depend upon the relative amounts of primary production and respiration. Elevated primary production can result in supersaturated DO conditions often seen in eutrophic systems. This excessive production can subsequently cause hypoxic or even anoxic conditions once productivity crashes, which influences organisms in higher trophic levels. However, Charlie Creek does not appear to be experiencing these symptoms.

6.2 Limitations, Uncertainties, and Assumptions

This report characterized relationships between flow and a large group of water quality constituents to support the District's consideration of water quality as a water resource value for Charlie Creek. These relationships represent associations between flows and water quality concentrations and are not meant to be interpreted as directly causal relationships. Many factors can confound the relationship between flows and water quality. For example, seasonality in flows and water quality is common. The rainfall in this portion of the state follows the typical central Florida rainfall pattern with peaks in the summer months and particularly dry conditions in April and May. Seasonality in water quality represents the cumulative manifestation of a number of physical and biogeochemical drivers of ecological process in river systems that often co-vary with rainfall and flow temporal patterns. We attempted to account for seasonality in evaluating the relationship between water quality and flow by including a term to account for differences in the monthly intercept of the linear regression relationship. Seasonality was often (33 of 56 regressions) a contributing factor and sometimes (8 of 56 regressions), after accounting for seasonality, there was not a relationship between the constituent and flow. The potential for the relationship between a water quality constituent and flow to vary by season (*i.e.*, month) was not evaluated due to the limited sample sizes from which to assess this affect over the wide range of constituents evaluated. If seasonal blocks are defined in future District efforts, the potential for this effect may be examined for a subset of constituents thought relevant to aid the District in evaluating water quality as an environmental value for its research needs.

In several cases the R^2 values were quite low indicating little of the overall variation in the water quality constituent was explained by the model. Fit statistics such as the R^2 are one indication of the reliability of the model that should be considered but are not a sole means of judging the adequacy of the models. The results presented in this document are intended to provide information the District can use to identify potential relationships and pursue additional analyses as they determine would be beneficial for their research and management needs.

6.3 Recommendations for Future Monitoring

Charlie Creek and tributaries are presently monitored at only two stations (Figure 8). We identify the following additional monitoring that SWFWMD may consider, to improve future analyses:

- Add continuous flow and monthly water quality measurement in Charlie Creek at the southern end of WBID 1763D (Figure 6)
- Add continuous flow and monthly water quality measurement in Oak Creek upstream of the confluence with Charlie Creek (Figure 4)
- Add continuous flow and monthly water quality measurement in Bowlegs Creek upstream of the confluence with Charlie Creek (Figure 4)

SWFWMD may consider measuring a general suite of water quality constituents, on a monthly basis, such as nutrients, chlorophyll *a*, total suspended solids, and turbidity.

The District water quality data collected in Charlie Creek are an important attribute to maintain future data collection within Charlie Creek.

7.0 References

Applied Technology & Management, Inc. (ATM) and Janicki Environmental, Inc., 2020. Upper Withlacoochee River water quality assessment: Southwest Florida Water Management District task work assignment 19TW0002680 report , Brooksville, FL, 60 pp. with 2,871 pp. supplemental materials appendix.

Applied Technology & Management, Inc. (ATM) and Janicki Environmental, Inc., 2021. Water Quality Assessment for the Lower Withlacoochee River, including Lake Rousseau and the Withlacoochee River to Dunnellon: Southwest Florida Water Management District task work assignment 20TW0002925 report, Brooksville, FL, 64 pp. with 5,889 pp. supplemental materials appendix.

Benjamini, Y. and Y. Hochberg. 1995. Controlling the false discovery rate: a powerful approach to multiple testing. Journal of the Royal Statistical Society. Series B 57(1): 289-300

Hirsch, R.M. and Slack, J.R. (1984) A Nonparametric Trend Test for Seasonal Data with Serial Dependence. Water Resources Research, 20, 727-732.
http://dx.doi.org/10.1029/WR020i006p00727

Hosmer, D.W., Lemeshow, S, 2000. Applied Logistic Regression: John Wiley & Sons, Inc., New York, 2nd Edition.

Kendall, M. G., Rank Correlation Methods, 4th ed., Charles Griffin: London, 1975.

Livingston, R.J. & E.A. Fernald. 1991. Introduction pp. 1–15. In: R.J. Livingston (ed.) The Rivers of Florida, Springer-Verlag, New York.

Mann, H.B., 1945, Nonparametric test against trend: Econometrica, v. 13, no. 3, p. 245–259, accessed October 2019 at https://doi.org/10.2307/1907187

SAS Institute, Inc, 2018. SAS/STAT® 14.2 User's Guide: Cary, NC.

Zar, J. H., 1984. Biostatistical Analysis: Prentice Hall, Englewood Cliffs, New Jersey.