Note on Chlorophyll Analysis in Homosassa Water Quality Appendix

Section 3.3 of this report describes an analysis of the effects of flow reductions on chlorophyll concentration in which measurements of chlorophyll concentration are compared to a threshold value and calculates risk of individual samples exceeding that value. To perform this analysis a threshold must be selected, and that threshold should be relevant to the system being studied. District staff identified a value of 7.7 µg/L as the most relevant threshold to use. Note, it is critical to distinguish between our use of this value as a threshold for analysis and its prescribed use as a criterion for determining impairment within the Estuary-Specific Numeric Interpretations of the Narrative Nutrient Criterion (NNC). This value, taken as the NNC, is applicable only within WBID 1345F and as an annual geometric mean value according to Rule 62-302.532 F.A.C. Contributing to our decision to use this 7.7 µg/L value, associated with the downstream 1345F WBID, is that the upstream WBID (1345) does not have a chlorophyll NNC value. We used this same value of 7.7 µg/L, but for a different purpose than determination of impairment of the NNC. Thus, an instance of a single exceedance of this threshold, or an increased risk of this exceedance across several repeated samples both inside and outside the WBID boundary cannot and should not be interpreted in the context of impairment of the NNC.

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EXPLORATORY EVALUATION OF WATER QUALITY AND FLOW RELATIONSHIPS FOR THE HOMOSASSA RIVER IN SUPPORT OF MINIMUM FLOWS REEVALUATION

TECHNICAL REPORT IN FULLFILLMENT OF WORK ASSIGNMENT 18TW0001116:

PREPARED FOR:

THE SOUTHWEST FLORIDA WATER MANAGEMENT DISTRICT, BROOKSVILLE, FL

PREPARED BY:

JANICKI ENVIRONMENTAL, INC AND WSP, INC.

FINAL REPORT

DECEMBER 7, 2018

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EXECUTIVE SUMMARY

The Homosassa River is a spring-fed tidal river located along the Springs Coast in Citrus County and within the Southwest Florida Water Management District. This report details efforts to quantify relationships between spring flows from the Homosassa River head springs and water quality throughout the system. Water quality is one of 10 “environmental values” defined in the State Water Resource Implementation Rule to be considered when establishing minimum flows. Salinity is a water quality constituent that represents a direct, physical driver for many estuarine processes. However, other water quality constituents can also affect biological resources in the river. This work effort focused on providing an exploratory examination of the relationships between flows and water quality constituents using the most up to date datasets available for the Homosassa River System. The analysis focused on identifying water quality response endpoints that, under certain conditions, could result in adverse effects to a “resource of concern” within the river as a function of reduced flows. Resources of concern are those attributes of the system that relate to one or more of the 10 environmental values identified in the Water Resource Implementation Rule and have potential quantifiable responses to flow. This updated analysis on relationships between spring flows and water quality uses an expanded list of water quality constituents and additional data collected since the District’s original minimum flow report (Leeper et al 2012) was prepared.

For this analysis, spatial attributes of the river including the headsprings (“Springs”), the “Mainstem” of the river, and the nearshore “Estuary” (outside the river mouth) were identified as potential resources of concern. The specific tasks associated with this work effort consisted of data gathering, exploratory data analysis, stochastic predictive modeling and synthesizing information to supplement existing knowledge on the effects of flows on water quality in the system. Initial tasks included the compilation of available water quality and water quantity data for the Homosassa River and the creation of a Microsoft Access database and database inventory. Additionally, descriptive statistics and plots were generated for each metric of interest to describe both the univariate characteristics and the seasonal and inter-annual distributions. Screening methods were used to identify and qualify potential anomalous data evident in the datasets in the Access database and linear regression was used to explore bivariate relationships between the water quality constituent of interest and flow. Subsequent to initial data compilation and exploration, a statistical analysis plan was developed which outlined potential analytical methods used to approach each of the various data types that exist in the master database. Application of the statistical analysis plan led to the analytical results describing the effects of flows on water quality within the system. Previously developed acceptance criteria for using linear regression relationships in support of minimum flows were applied prior to reporting significant results for linear regression analysis.

For the Springs sites, several water quality constituents were significantly related to flows including alkalinity, calcium, chloride, magnesium, potassium, sodium, and sulfate. This was not a surprising result as it is well known that water that has been in contact with limestone for a relatively short length of time should have low concentrations of calcium and bicarbonate ions; water with a longer period of residency within the flow system should typically have higher concentrations. Total Dissolved Solids is a measure of chemical constituents dissolved in the groundwater and in west-central Florida, TDS is mostly influenced by the concentrations of the major ions: calcium, bicarbonate, magnesium, sodium, sulfate and chloride. TDS can be used to estimate the relative residence time of ground water in the aquifer and typically increases as the length of groundwater flow paths increase (SWFWMD 2001).

Nitrogen enrichment in the Homosassa Springs Group is an ongoing concern due to the presence of algal mats (filamentous and epiphytic algae) which were linked to excessive nutrient concentrations. We reevaluated relationships between flow and all forms of available nitrogen for completeness and found that while some statistically significant relationships with flow were established, the results were inconsistent and not directly useful for supporting reevaluation of minimum flows for the Homosassa River System. Significant nitrogen relationships were found in the Southeast Fork for nitrate-nitrate (total) and total nitrogen both of which were inversely related to flow. The relationship between total nitrogen and flows in Halls River was significant and positive, as was the relationship between nitrite (total) and flow in Hidden River. However, the number of samples was generally less than 40, the R square was less than 50 percent and the results were conflicting with respect to the response as a function of flow. These findings support those of Upchurch et al. (2008) as described in the original minimum flows report (Leeper et al. 2012). In an analysis of the relationships of nitrate and flows for springs in the Suwannee River Water Management District, Upchurch et al (2008) concluded that that minimum flows could not effectively be utilized to control nitrate discharging from the springs by promoting high discharge. The analysis in this evaluation of water quality in the Homosassa River therefore supports the findings of Upchurch et al. (2008) and Heyl (2012) that the current evidence does not support the conclusion that there is a consistent relationship between these forms of nitrogen and flows.

A similar analysis was conducted for the Estuary data (defined as those sample locations outside the mouth of the river) in the nearshore estuarine environment. Analyses of the Estuary data led to similar conclusions as for the Springs data. There were several significant relationships between spring flows and salinity for stations outside of the mouth of the river; however, given the distance from headsprings, it is more likely that salinity in the estuary is driven by a combination of spring discharge, coastal runoff, wetland storage, direct rainfall, and freshwater discharges from other nearby coastal areas that are all seasonally dependent and to some extent correlated with one another. No significant relationships between flows and other water quality constituents that could be used to support the reevaluation of minimum flows established for the Homosassa River System were identified for the Estuary data.

Analysis of the Mainstem of the river did reveal evidence that chlorophyll a distributions, a proxy for phytoplankton abundance, were found to be significantly related to flows under certain conditions. While healthy phytoplankton populations are essential for a healthy estuary, an excess in phytoplankton abundance can have negative impacts on ecosystem health, and, while chlorophyll concentrations are generally low in the Homosassa River System, there is evidence that the system can be susceptible to high phytoplankton biomass with several chlorophyll concentrations observed above 50 ug/l.

For regulatory purposes, the Florida Department of Environmental Protection (DEP) has split the river into Waterbody Identifiers (WBIDs) and the DEP has adopted a Total Maximum Daily Load (TMDL) for nitrate in the springs (Bridger, et al. 2014) including the main spring complex (WBID 1345G), as well as Bluebird Springs (1348A) and Hidden River Springs (1348E). A river kilometer system was developed beginning at the mouth (Rkm 0) to Rkm 12.6 (the headsprings). The mainstem of the river includes WBID 1345F (from Rkm 0 to Rkm 9.2), and WBID 1345 (from 9.2 and 12.6). The upstream WBID is governed by regional standards while the downstream WBID has established site-specific standards known as numeric nutrient criteria, which include total nitrogen, total phosphorus and chlorophyll.

To evaluate the effects of flows on chlorophyll distributions in the mainstem of the river, a mixed-effects logistic regression model was used to predict the probability of a chlorophyll a sample exceeding the NNC for WBID 1345F of 7.7 ug/l. Given that the upstream WBID does not have a site-specific standard for chlorophyll, and that the headsprings are impaired and have a TMDL, while the downstream WBID is currently meeting its designated use, the downstream criterion value for chlorophyll was applied to the entire system. The model results suggested that the probability of a sample exceeding the NNC threshold value was significantly related to flows, especially in the upper portion of the system and when flows approached their seasonal minima. The model results suggested that reduced flows increased the probability of a sample exceeding the site-specific chlorophyll value.

A principal objective of this study was to find water quality relationships to flow that could be used to supplement existing information available for the reevaluation of minimum flows in the Homosassa River. To use the chlorophyll model described above to evaluate the effects of flow reductions on chlorophyll distributions, a “Baseline” condition reflecting flows unimpacted by withdrawals and 1% to 15% flow reduction scenarios (in 1% flow-change increments) were developed. The period of record for evaluating the flow reduction scenarios was 1998-2017 to correspond with the period of measured flows within the system. In addition, because the response of chlorophyll to flow was primarily constrained to the portion of the river above river kilometer 7.1, this area was used for the evaluation. The results of the flow reduction evaluation suggested that a 9% reduction in flows would increase the individual sample exceedance frequency over the Baseline condition by 15% for this section of the river. The 15% change threshold is a common prescriptive standard used to identify “significant harm” for minimum flows evaluation.

While the chlorophyll-flow modeling effort utilized the site-specific chlorophyll threshold value to evaluate response to changes in flow, the results were not intended to be used as a direct assessment of whether or not changes in flow would result in compromises to the river’s “Designated Use” as defined in State statute. The chlorophyll concentrations tend to peak at the WBID boundary, and then decrease both towards the river mouth and upstream of the boundary. This complicates interpretation of the effects of flows on the regulatory criteria. In addition, it would be beneficial to validate the model with additional data prior to application in a regulatory setting. Instead, the analysis illustrates the utility of this type of modeling to assess the sensitivity of chlorophyll a distribution (a proxy for phytoplankton abundance) in the upper 5 kilometers of the river to changes in flow, and suggests the need for more research in this upstream portion of the river that has displayed evidence of sensitivity to changes in flows. Transect data collected at sites throughout the river segment were valuable in this regard because they provided spatially-intensive water quality data. Because of the spatial distribution of the chlorophyll peak within the mainstem of the river, future data collection efforts should consider spatially-intensive sampling in this portion of the system to test hypotheses developed from this work that chlorophyll a distributions are sensitive to changes in flows in the upper reach of the river. Otherwise, this reevaluation has confirmed many of the findings of the District's original minimum flows report.

1.0 INTRODUCTION

1.1 BACKGROUND

Florida law (Chapter 373.042 F.S.) requires Florida's Water Management Districts or the Department of Environmental Protection (FDEP) to establish minimum flows for rivers, streams, estuaries and springs to identify the limit at which further withdrawals would cause significant harm to water resources or ecology of the area. Minimum flows are reviewed periodically and revised as necessary. A minimum flow rule for the Homosassa River System was adopted in 2013 (Rule 40D-8.041, Florida Administrative Code or F.A.C.), with a directive to reevaluate the minimum flow within six years of its adoption (40D-8.041 F.A.C.). The Homosassa River/Homosassa Spring are included on the Southwest Florida Water Management District's 2017 Minimum Flows and Levels Priority List for reevaluation (and its draft 2018 Priority List), with finalization due in 2019. The District is thus currently reevaluating the minimum flows for the Homosassa River System.

As part of the District's efforts to reevaluate the Homosassa River System minimum flow, a work effort was contracted with Janicki Environmental, Inc. in July 2018 (Task Work Assignment [TWA] No: 18TW0001116) to conduct exploratory data analysis through investigation of relationships between springs flows and system water quality. The specific tasks within this TWA consisted of data compilation, exploratory data analysis, stochastic predictive modeling and synthesizing information in support of the minimum flow revaluation. Initial tasks included the compilation of available water quality and water quantity data for the Homosassa River and the creation of a Microsoft Access database and database inventory. Additionally, descriptive statistics and plots were generated for each metric of interest to describe both the univariate characteristics and the seasonal and inter-annual distributions. Screening methods were used to identify and qualify potential anomalous data evident in the datasets in the Access database. The Access database, summary statistics, and tabular/graphical output from the described analyses are provided as deliverables associated with this project. Methods used to compile data, and complete the initial data exploration are described in Section 2.1. Subsequent to initial data compilation and exploration, a statistical analysis plan was developed which outlined the analytical methods used to approach each of the various data types that exist in the master database. The statistical analysis methodology is detailed in Section 2.2.

The results section (Section 3.0) of this report details the application of the statistical analyses plan. The results section is organized by "Resources of Concern" which were identified as part of the exploratory data analysis process. Within each sub-section of the results section, the application of the analytical approach is described including results of exploratory data analysis, formulation of the conceptual model and, where appropriate, results of application of the approach as they pertain to supporting the reevaluation of the minimum flows for the Homosassa River. The assumptions and limitations are described along with recommendation for how to further the research on the relationship between flows and water quality in the final sections of the report.

1.2 REPORT OBJECTIVE

The specific objective of the report was to provide documentation, exploratory analysis, and statistical inference regarding the relationships between flows and water quality constituents in the Homosassa River System to support the assessment of the water quality environmental value as part of the reevaluation of minimum flows for the Homosassa River.

2.0 DATA AND METHODS

This section describes the data sources, exploratory data analysis, and the statistical methods used to evaluate relationships between flows and water quality.

2.1 DATA COMPILATION

Figure 2-1 provides an organizational overview of the types of data compiled for this Task. Multiple datasets, including all water quality, groundwater discharge, and various ancillary datasets were provided by the District to the project team. Water quality data provided by the District include data from ongoing continuous recorder programs as well as from multiple fixed station sampling programs consisting of monthly/quarterly sampling. These programs are described in the sub-sections below. Additionally, period of record river discharge, rainfall and tide data were downloaded as specified below. Individual datasets (whether provided by the District, or downloaded as described above) were read into the Statistical Analysis Systems (SAS Institute, Inc. 2016) software package for summarization and analysis.

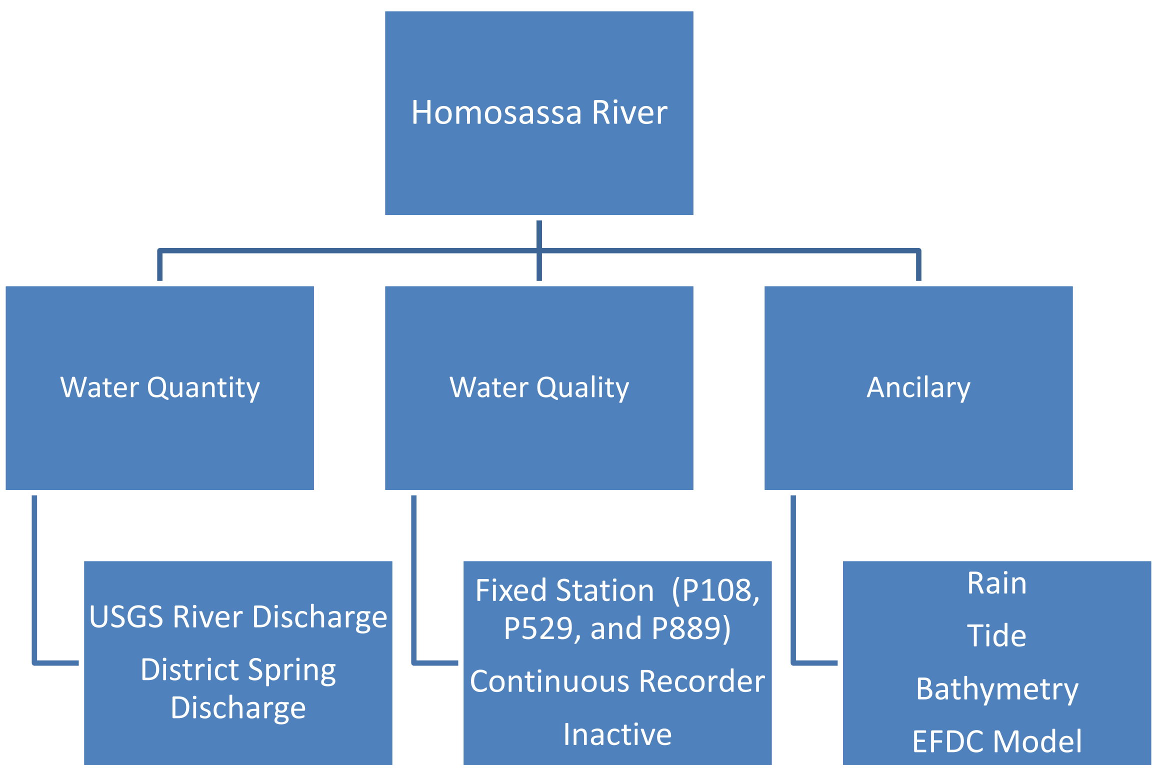


Figure 2-1. Organizational chart for data compiled for this report.

2.1.1 Active Water Quality Sampling

Ongoing, active water quality sampling programs include District Project P108 (Coastal Rivers, Figure 2-2), P529 (Project COAST, Figure 2-3), P889 (Springs, Figure 2-4), and continuous recorders (Figure 2-5). The monitoring period of record for these programs is dependent on which constituent was measured but the general structure of the each network is describe in Table 2-1.

Table 2-1. Monitoring network structure for active monitoring networks.

Monitoring Network	Period of Record	Annual Sampling Frequency	Number of Sampling Events
P108	2005 - 2017	Bi-monthly /quarterly after 2011	65
P529	1996 - 2017	Monthly/quarterly after 2013	140
P889	1993 - 2017	Quarterly	120

Surface-water stations sampled as part of the Districts P108 (Coastal Rivers) sampling program are displayed in Figure 2-2. Sampling began in late 2005. These stations are sampled bimonthly initially and approximatly quarterly after 2011 for a full suite of field and laboratory parameters (Table 2-2).

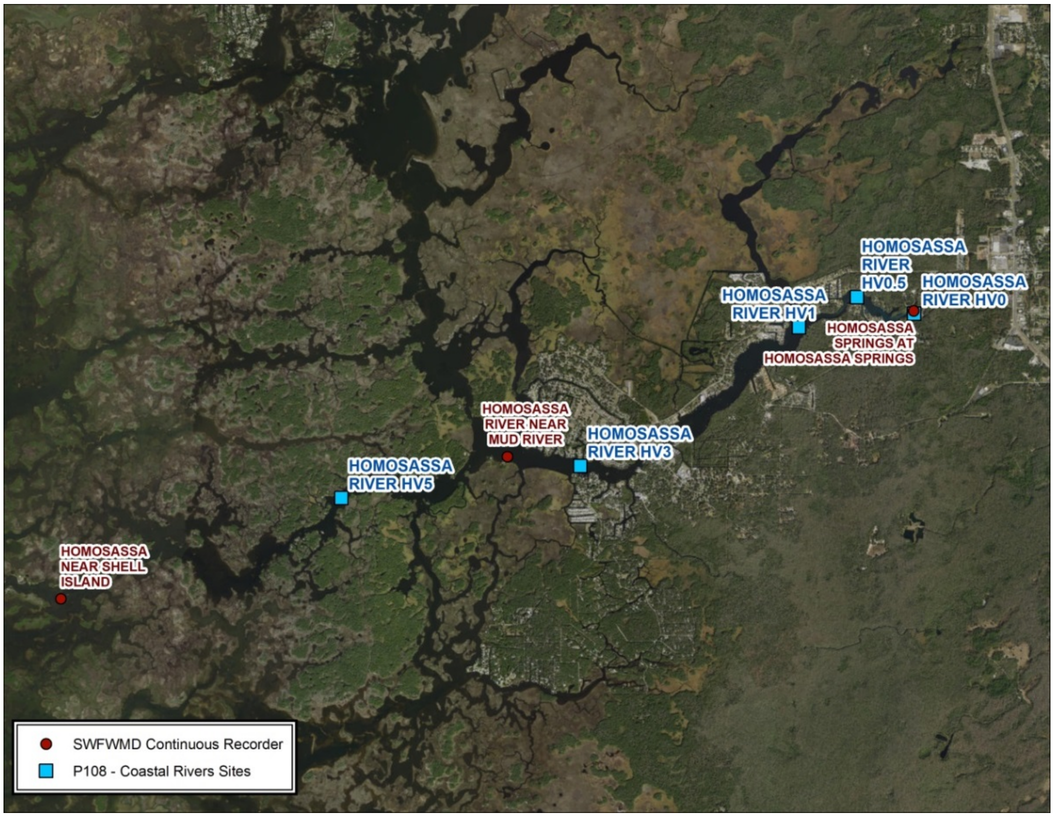


Figure 2-2. Active surface-water sampling conducted for project P108 (Coastal Rivers).

Table 2-2. Standard District surface water quality parameters.	
Ammonia (N) (Total)	pH (Total)*
Calcium (Dissolved)	Pheophytin (Total)
Chlorophyll a (Total)	Phosphorus- Total (Total)
Color (Dissolved)	Potassium (Dissolved)
Depth (Total)*	Residues- Nonfilterable (TSS) (Total)
Depth, bottom (Total)*	Residues- Volatile (Total)
Dissolved Oxygen (Total)*	Salinity (Total)*
Iron (Dissolved)	Secchi-horizontal (Total)*
Magnesium (Dissolved)	Secchi-vertical (Total)*
Nitrite+Nitrate (N) (Total)	Sodium (Dissolved)
Nitrite (N) (Total)	Specific Conductance (Total)*
Nitrogen- Total (Total)	Temperature (Total)*
Orthophosphate (P) (Dissolved)	Turbidity (Total)

*indicates field parameters

Figure 2-3 displays the stations associated with Project P529 (Project COAST). These stations were sampled for a limited suite of field and laboratory parameters by the University of Florida between 1997 and 2010 and subsequently sampled for the full suite of surface water parameters by the District (Table 2-2). Stations 5, 9 , and 10 are not currently sampled.

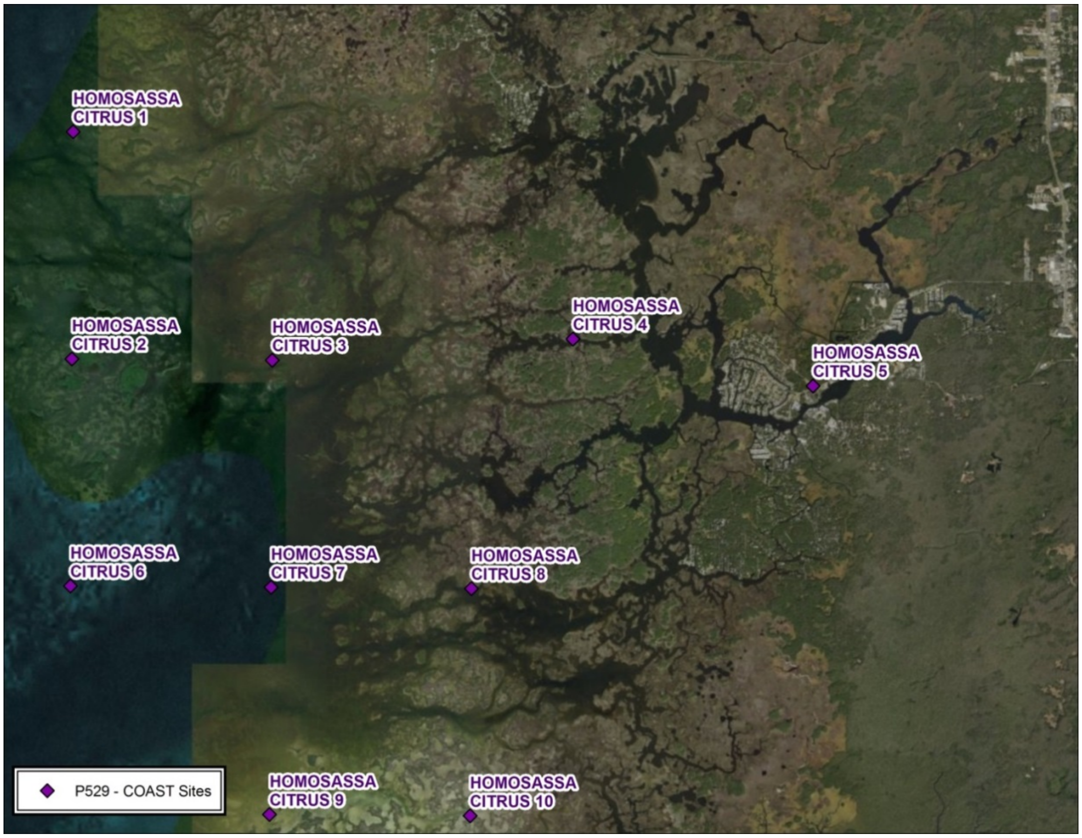


Figure 2-3. Surface water stations associated with P529 (Project COAST).

The principal spring vents of the Homosassa River have been monitored by the District (P889) since 1993. Data were provided by the District for multiple springs. Data for the springs shown in Figure 2-4 were compiled for this task as they are the springs directly on the Homosassa River. Standard District water quality parameters for spring sampling are provided in Table 2-3. Selected springs during older sampling events also include non-standard parameters such as pesticides.

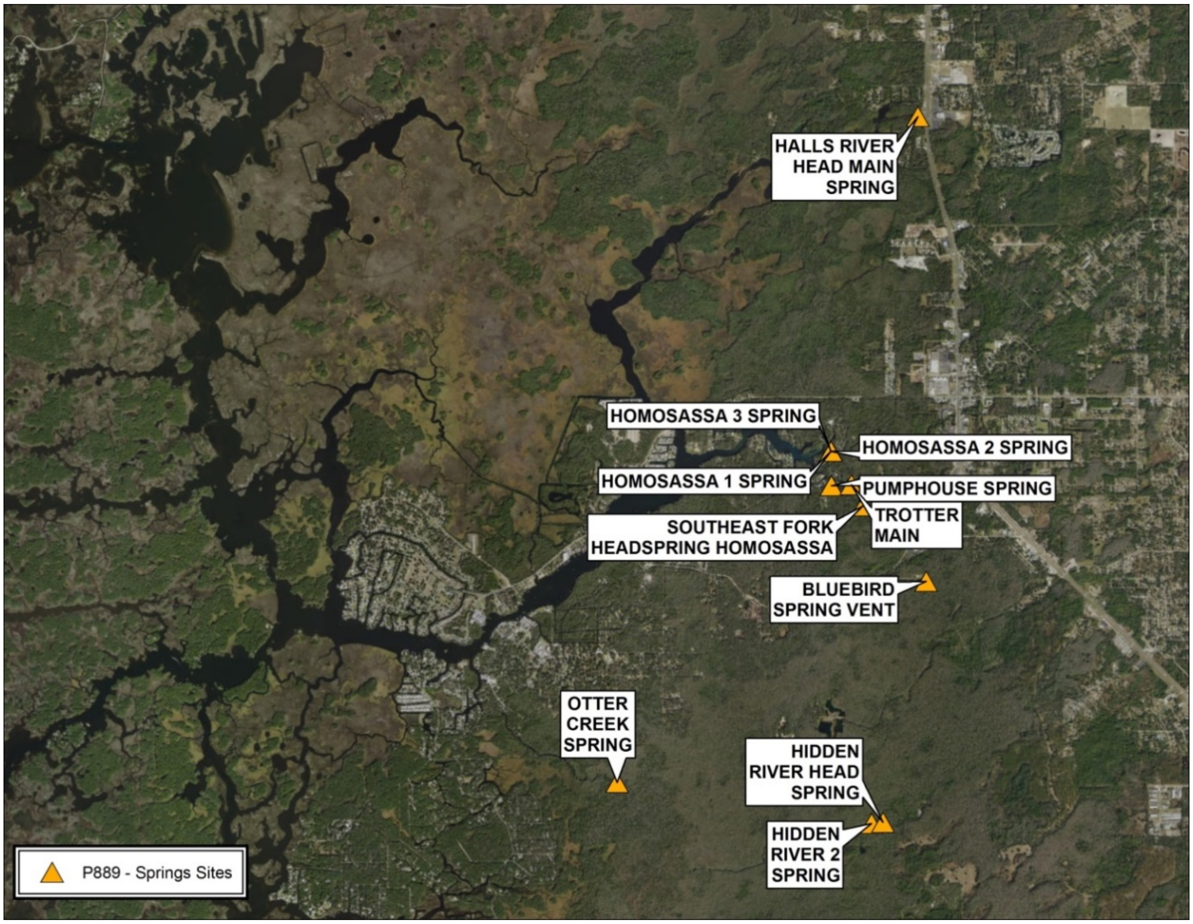


Figure 2-4. Active spring sampling locations on the Homosassa River (project P889).

Table 2-3. Standard District groundwater (spring) parameters.

Alkalinity (Total)	Nitrogen- Total (Total)
Aluminum (Dissolved)	Orthophosphate (P) (Dissolved)
Ammonia (N) (Total)	pH (Total)*
Boron (Dissolved)	Phosphorus- Total (Total)
Calcium (Dissolved)	Potassium (Dissolved)
Carbon- Total Organic (Total)	Residues- Filterable (TDS) (Dissolved)
Chloride (Dissolved)	Silica – Dissolved (Dissolved)
Color (Dissolved)	Sodium (Dissolved)
Dissolved Oxygen (Total)*	Specific Conductance (Total)*

Table 2-3. Standard District groundwater (spring) parameters.	
Fluoride (Dissolved)	Strontium (Dissolved)
Iron (Dissolved)	Sulfate (Dissolved)
Magnesium (Dissolved)	Temperature (Total)*
Manganese (Dissolved)	Turbidity (Total)
Nitrite (N) (Total)	

*indicates field parameters

The District provided data for three water quality continuous recorders on the Homosassa River (Figure 2-5). The continuous recorder monitoring has a short period of record, beginning in 2017. The Homosassa Springs at Homosassa Springs site is monitored continuously for temperature, pH, conductivity, salinity, dissolved oxygen and depth. The other two sites are monitored for a broader suite of parameters listed in Table 2-4.

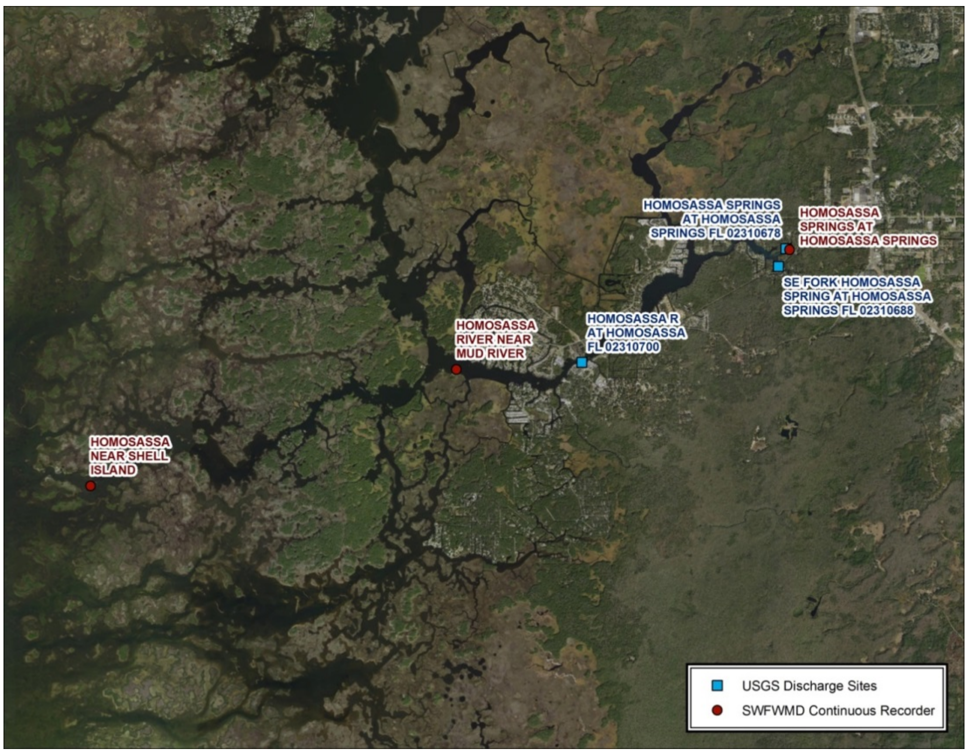


Figure 2-5. Water quality continuous recorders (red circles) on the Homosassa River. Blue squares indicate the locations of USGS river discharge gages.

Table 2-4. Parameters measured at the continuous recorders at Homosassa River Near Mud River and Homosassa Near Shell Island stations.	
Temperature	fDOM
Depth	Chlorophyll
Conductivity	Turbidity
pH	Salinity
Dissolved Oxygen (mg/L and %)	Nitrate
Light Spectrum	Dark Spectrum

2.1.2 Inactive Water Quality Sampling

In addition to data for the active, ongoing water quality monitoring network described in Section 2.1.1, the District provided data for a variety of water quality stations previously sampled in the Homosassa River. These stations are listed in Table 2-5 for stations where the number of observations for at least one constituent exceeded 20 observations. These stations include a spatially intensive water quality and biological monitoring study conducted by the University of Florida (UF 5 Rivers Study: Figure 2-6) between August 1998 and November 2011 (with a gap between 2001 and 2003). For this study, 20 transects were conducted along the length of the river, with three sampling points per station for the 15 upstream stations and a single sample for the 5 most downstream sites. Both field and laboratory parameters were sampled with a total of approximately 138 samples per transect over the study period. The site locations for the other monitoring stations are provided in Figure 2-7.

Table 2-5. Inactive Water Quality Sampling Stations on the Homosassa River/Spring System.

Site Name	Site ID	Surface Water/Spring	Period of Record*	Type of Sampling	Number of Samples*
UF 5 RIVERS STUDY		Surface Water	8/1998-11/2011	Field/Laboratory	138 per transect
HALLS RIVER AB HOMOSASSA	20017	Surface Water	12/1992-9/1998	Field/Laboratory	24
HOMOSASSA RIVER AB GULF	20024	Surface Water	3/1998-9/1998	Field/Laboratory	28
HOMOSASSA RIVER AB HALLS RIVER	20026	Surface Water	3/1992-9/1998	Field/Laboratory	40
HALLS RIVER BRIDGE	21019	Surface Water	10/2002-8/2005	Field/Laboratory	20

*Note: period of record and number of observations vary by parameter. Entries in this table are values for more commonly sampled parameters such as specific conductance or nutrients and should be considered approximations. For some parameters the number of observations will be much less.

Most inactive stations had few sampled over short or intermittent duration and were not useful in support of reevaluation of water quality in support of minimum flows. The U.F. transect data, however, were amenable and used extensively as described in the results section below and included data on alkalinity (mg/l), chlorophyll a (ug/l), color (pcu), dissolved oxygen (mg/l), ammonium (ug/l),nitrate (ug/l), soluble reactive phosphorus (ug/l), salinity (psu), temperature , total nitrogen (ug/l), and total phosphorus (ug/l).

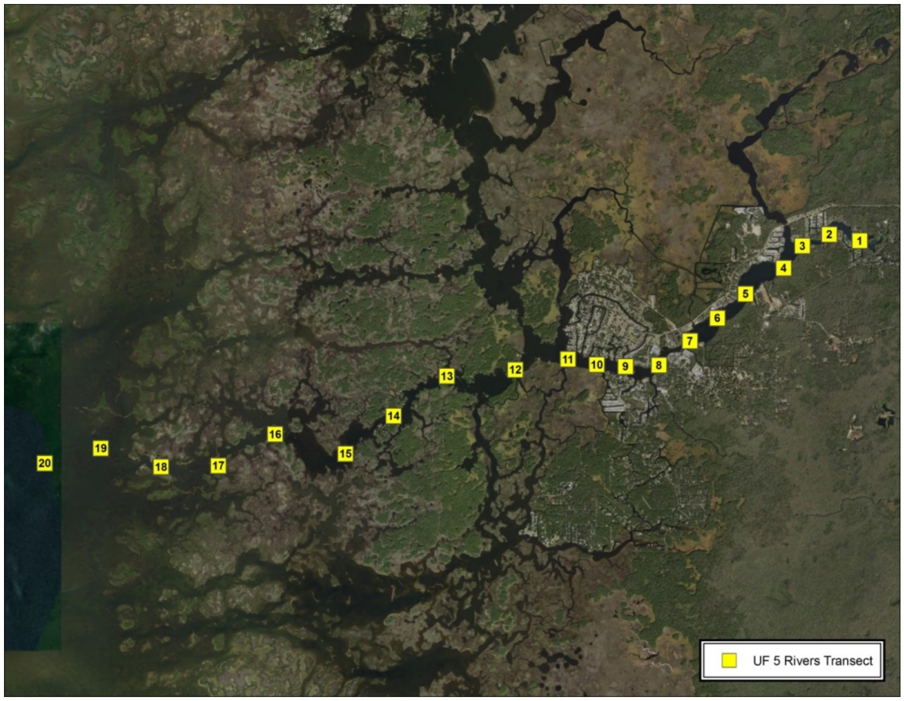


Figure 2-6. **University of Florida 5 Rivers Study transect locations on the Homosassa River.**

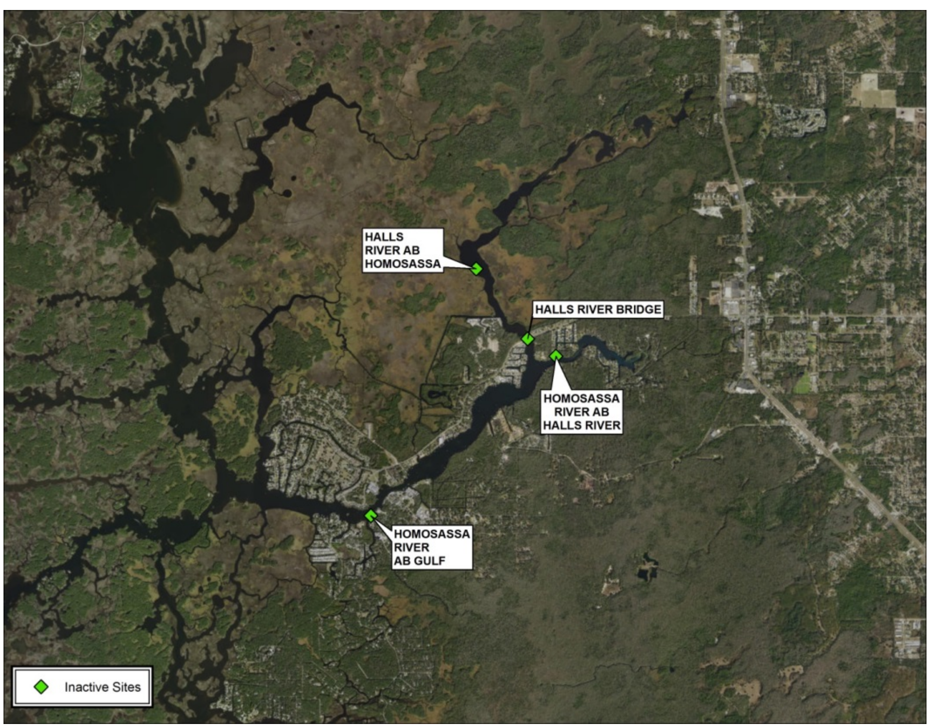


Figure 2-7. **Inactive water quality monitoring stations on the Homosassa River for which the District provided data. Only stations with >20 observations for at least one parameter are included in Table 2-5.**

2.1.3 Hydrologic Data

USGS discharge and/or stage data for the Homosassa River (gages 2310678 Homosassa Springs at Homosassa Springs FL, 2310688 SE Fork Homosassa Springs at Homosassa Springs FL, and 2310700 Homosassa River at Homosassa FL) were downloaded for the available period of record from the National Water Information System (NWIS; https://waterdata.usgs.gov/nwis). These gages are shown above in Figure 2-5. USGS discharge data include a data qualifier indicating whether each data record has been "Accepted" (A) or remains "Provisional" (P). Much of the discharge data from late 2017 into 2018 are flagged as "Provisional" and such data should be used with discretion.

Rainfall data (station name = Inverness3SE, station id = USC00084289) were downloaded for the period of record from the National Climate Data Center (NCDC; https://www.ncdc.noaa.gov/cdo-web/). Tide data (Station 872750 Cedar Key, FL; datum = MLLW) were downloaded for the period of record from the National Oceanic and Atmospheric Administration (NOAA) Center for Operational oceanographic Products and Services Tides and Currents webpage (https://tidesandcurrents.noaa.gov/). These sites are shown in Figure 2-8.

Figure 2-8. Locations of NCDC rainfall station and NOAA tidal gage.

2.1.4 Data Screening Methods

The Task 2 report was accompanied by a master database of all available water quantity, water quality, and available ancillary datasets compiled for the Homosassa River. Quality control procedures were used to identify potential anomalous values. Many of the raw datasets downloaded, or received from the District, contain at least one column indicating the quality of each data record. Water quality grab sample data include a designated qualifier column containing FDEP data qualifier codes as applicable. A list of these qualifier codes is provided in Appendix A. Certain qualifier codes indicate the data should not be utilized in analyses. The following qualifiers were not used in the analysis:

> "Y = flag for improperly preserved sample;
>
> "Q = flag for out of hold time;
>
> "T"= not to be used for analysis; and
>
> "?" = data rejected and should not be used.

It is important to note that a data qualifier including “U” indicates that a compound was analyzed for but not detected. The value associated with the qualifier is the laboratory method detection limit (MDL) though the actual MDL values were not always reported. These values were retained as reported for analytical purposes.

Two data screening methods were used to identify potential anomalous values in each examined dataset. The purpose of the screening methods was simply to identify data points for further investigation; no data were eliminated from the database based on this analysis. Two screening methods were used: an extreme value screening method (e.g. +- 3 standard deviations from the mean) and a functional screening method that evaluates deviations from an expected value based on a timeseries of data using robust regression (SAS Institute, Inc. 2016). Columns were added to the database to identify whether or not each value met the criteria to qualify as a specific data point worthy of further investigation as an anomalous value and these columns were later used in the statistical analysis. A complete list of descriptive statistics and descriptive plots for all constituents evaluated and delivered in the master database is provided in Appendix B.

2.2 Statistical Analysis Methods

A statistical analysis plan was developed as Task 3 of this work effort. The Task 3 document outlined a conceptual analytical pathway to guide the analysis. The analytical pathway is outlined in Figure 2-9.

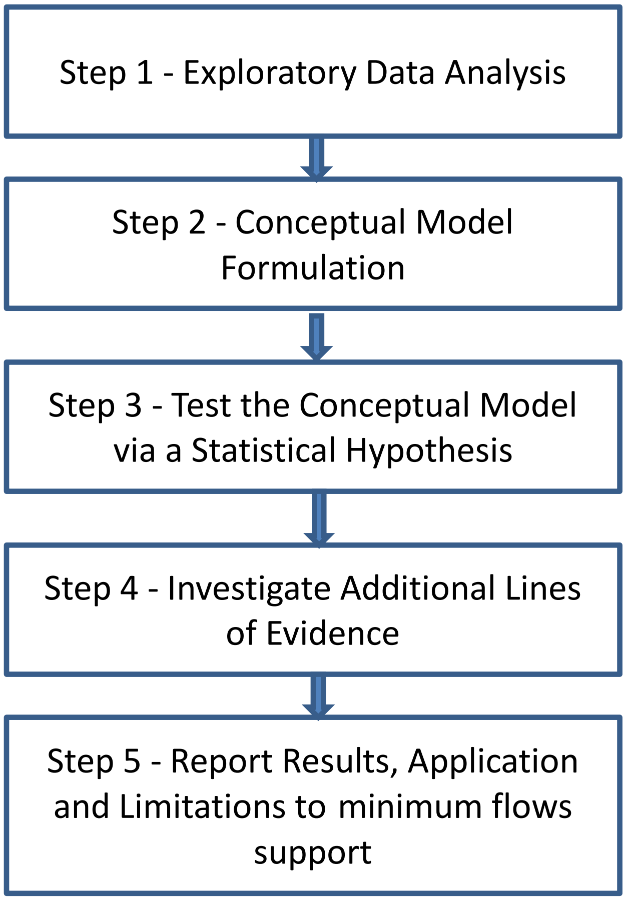


Figure 2-9. Analytical flow path in support of reevaluation of the Homosassa River minimum flows.

The statistical tools applied to this project include ordinary least squares linear regression, general and generalized linear models with inclusion of random effects, timeseries trend analysis, and robust regression techniques. In addition, other methods such as classification trees and timeseries models were considered. Methods are described within, but only reported if applicable to support the establishment of minimum flows for the system. Each of these tools is described in a sub-section below.

2.2.1 Ordinary Least Squares Regression

Ordinary least squares (OLS) regression was used primarily as an exploratory data analysis tool to investigate linear bivariate relationships between flow and a particular analyte of interest. OLS regression maps a response variable such as salinity to a potential explanatory (predictor) variable (e.g. spring discharge). This is accomplished by defining an intercept and a slope for the predictor that defines a straight line minimizing the sum of squared deviations from the line (Zar 1984). Application of OLS regression also provides a reference line through the bivariate distribution that can aid in the selection and elimination of those analytes that are not affected by flow by highlighting relationships that deviate from a straight line. Given the number of analytes evaluated in the exploratory data analyses, linear regression was used as a screening method to refine hypotheses related to the overarching project goal.

A linear regression equation is expressed mathematically as:

$$Y_i = \alpha + \beta X_i + e_i$$

Where :

Y is the response, X is the predictor, i is the indididual observation,

e is the error, α is the intercept, β is the slope of the regression line

The regression coefficient of determination (r^2) is one measure of the variance in the dependent variable that is explained by the model. In linear regression, it is assumed that the data are independent samples from the population. Another important assumption of linear regression is that the error term of the model is normally distributed, with constant variance. However, at times water quality data do not conform to these assumptions. Data transformations such as natural log transforms can help to satisfy the assumption of normality and heteroscedasticity but will not correct for dependencies in the data structure. Therefore, more sophisticated regression modeling techniques were used where there was a potential to generate inferences that could inform the reevaluation of minimum flows for the Homosassa River. These more sophisticated techniques are described in the next sub-section.

2.2.2 General and Generalized Linear Models

General and generalized linear models are extensions (generalizations) of OLS regression models that allow for more flexibility in accounting for artifacts of the data that may affect the underlying assumptions of OLS regression. General linear models are applied when the response variable is continuous and generalized linear models are applied when the response variable is binary or count data. Both classification and continuous predictor variables are allowed and can be expressed as either fixed or random effects representing the deterministic component or the variance component of the model, respectively (Littell et al. 1996).

An example of using a general mixed effects model is provided by the equation below that regresses chlorophyll concentrations on spring discharge. The deterministic component produces a parameter estimate of the intercept and slope and tests that they are different from zero, while the random component of the model allows for each station to have a separate intercept and for the correlation among samples collected at different stations to be accounted for in the error variance. The likelihood ratio test can be used to compare the mixed effects parameterization relative to the null model and Akaike Information Criteria (AIC) can be used to evaluate the model improvement for nested models of the same family. We used a modification of AIC that includes a penalty for including additional model parameters (AICC) in the model evaluation (SAS Institute, Inc. 2016). Residual diagnostics are also helpful to assess model fit and assumptions associated with the regression.

$$Y_{ij} = \beta_0 + \beta_{0j} + \beta_1 * X_{ij} + e_{ij} + e_j$$

Where:

Y_{ij} = chlorophyll concentraion for each sample (i), and station (j)

X_{ij} = spring discharge for each date (i), and station (j)

β_0 = overall intercept

β_{0j} = random intercept for station

β_1 = deterministic effect of spring discharge on chlorophyll

e_{ij} = residual ($N_{(0)\ iid}$)

ej = residual covariance among samples taken at the same station

Generalized linear models are similar to general linear models except the response variable can be from alternative distributions. Logistic regression is an example of a generalized linear model. The formulation is nearly identical to those models above in that they are all linear (i.e. additive) models but generalized linear models use a link function to map the response variable to a known distribution, generally of the exponential family. Logistic regression, in particular, is useful if there are important critical threshold values for an analyte of interest, above which results in some adverse effect. For example, if it were known that a chlorophyll concentration above some threshold value resulted in an adverse effect to the ecology of the system. The general equation for a mixed effect logistic regression model is provided below. Notice that there is no error term as the variance is expressed as a function of the mean value resulting in population level expected values. The formulation for the random effects takes place within the link function.

$$g(E[Y \mid \gamma]) = \log\left(\frac{p_{(y=1)}}{1 - p_{(y=1)}}\right) = X'\beta + Z'\gamma$$

Where:

$X'\beta$ = Fixed effects

$Z'\gamma$ = Random effects

$\log\left(\frac{p_{(y=1)}}{1 - p_{(y=1)}}\right)$ = link function mapping presence absence to independent terms

$g(E[Y \mid \gamma])$ = response conditional on random effects

γ = Random effect $N(0, \sigma^2\gamma)$

We used this model formulation to evaluate the effects of changes in flows on the water column phytoplankton distribution throughout the mainstem of the river.

2.2.3 Robust Regression

Robust regression is a method to account for highly influential data points that may affect statistical inference (Chen, C. 2002). The method relies on iteratively reweighted least squares which successively down weights observations with large residuals until reaching convergence criteria established to evaluate the change in the parameters' estimates. By iteratively reweighting the values, the resulting parameter estimates are "robust" to the influence of extreme values in the dataset and, therefore, the procedure is robust to deviations from assumptions about the data distribution, heterogeneity of variance, and other assumptions of traditional OLS regression approaches. Robust residuals are calculated along with robust standard errors and these estimates can be used to evaluate the robust regression fit to the data. The principal use of robust regression for this project was to detect outliers in an objective way. This was performed as part of the quality control checks where outliers were identified in the dataset for further examination. To illustrate, Figure 2-10 provides an example of a timeseries of total nitrogen measurements for a water quality station in the Homosassa River. The robust regression identified an outlier in the timeseries (denoted by red open triangle) and adjusted the intercept and slope of the regression line to down-weight the influence of the outlier.

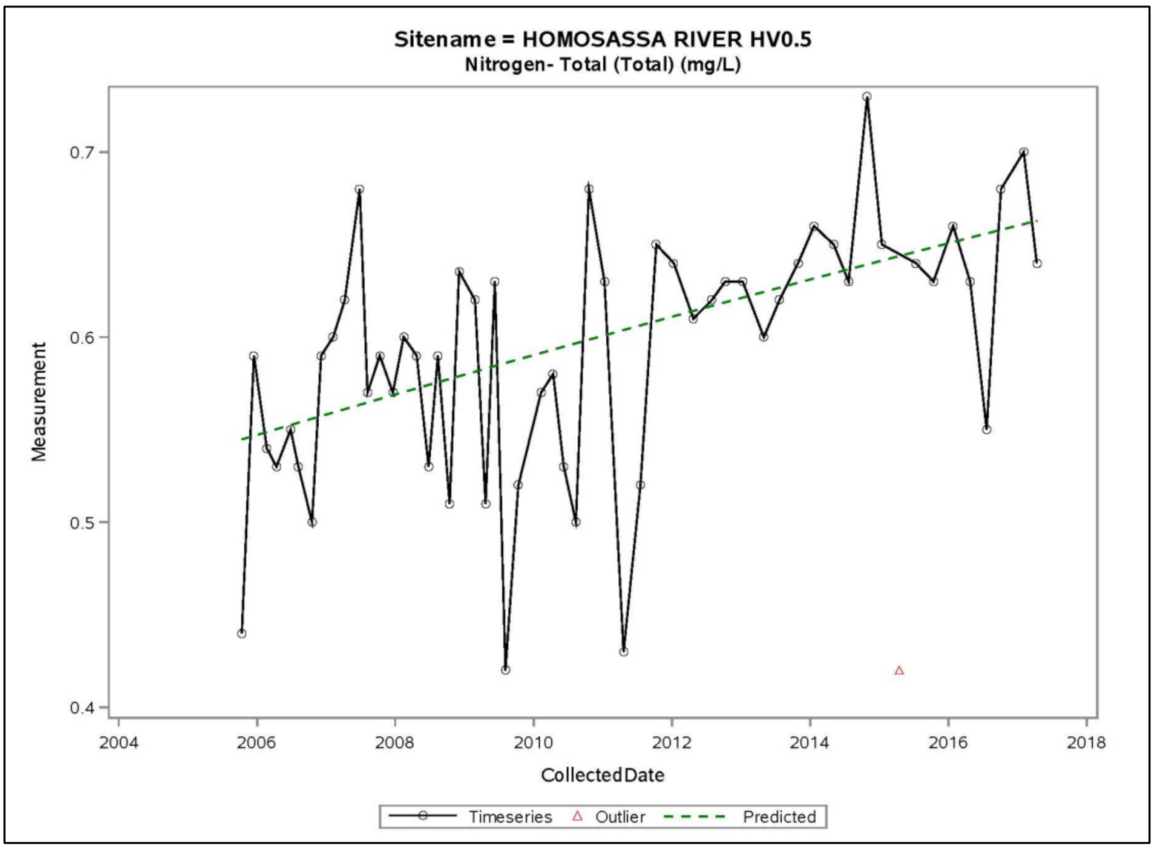


Figure 2-10. Example of application of robust regression to a total nitrogen timeseries for a station in the Homosassa River.

2.2.4 Time Series Trend Tests

Evaluation of long-term trends was performed using the seasonal Mann-Kendall (SMK) test for trend (Hirsch et al., 1982; Hirsch and Slack, 1984) which was developed by the USGS in the 1980s to analyze trends in surface-water quality throughout the United States. The SMK test was modified from the Mann-Kendall trend test (MK, a measure of rank correlation to measure the association between measured quantities), in that the MK test is first performed for individual seasons (months or quarters), and the individual results are combined into an overall test for whether the dependent variable changes in a consistent direction (monotonic trend) over time (Helsel et al., 2006). General time series trend tests were conducted for the long term spring discharge records as well as specific water quality constituents that may be affected by anthropogenic influences over time as well as changes in spring discharge.

2.2.5 Conditional Inference Trees

Conditional inference trees are a class of permutation based methods also known as “Decision Trees” or “Regression and Classification Trees”. This class of methods is applicable to all kinds of regression problems, including nominal, ordinal, and continuous data. A conditional inference tree methodology (Hothorn et al., 2006) was used as an exploratory line of evidence for evaluating water quality stressor-response relationships. The approach is based on recursive partitioning. The partitioning process iteratively searches for a point in the stressors variable which maximizes the difference in the response values between two groups of response data. No *a priori* threshold is specified. The regression tree approach defines the breakpoint as that which maximizes the difference between groups by minimizing the p value associated with some statistical test. The point in the stressor variable at which the p value is minimized, after adjustment for multiple comparisons, is assigned as the breakpoint defining the split of the of the response variable into 2 groups. Once the first split is made, the process continues to test for subsequent splits that are conditional on the first split; hence, the term “conditional inference” or “conditional probability analysis”. Multiple explanatory covariates can be included in the analysis to identify multiple drivers of response dependent on the range of values and can indicate the presence of synergistic relationships among potential explanatory analytes including discharge.

2.2.6 Statistical Analysis of High Frequency Water Quality Data

High frequency (aka continuous) water quality data collection can be a useful tool to identify the different periods of time over which cyclical variability is observed, and to relate these period scales to physical (for example, tides, both diurnal and lunar) and biological (for example, primary production, typically daily) drivers (Downing et al. 2017). One goal of these data collection programs is often to identify important periods of variation (frequencies) in the continuous monitoring data. For example the relative variability of within- and between-day variation is important to put grab sample data in the context of within-day variability. In some instances, frequency analysis such as wavelet or spectral analysis can be used to assess different temporal signatures in the underlying data if those periodicities can predict return intervals. Descriptive plots were constructed to put the timeseries data into context of within- and between-date variability. Base functions in the R computing language (R Core Development

Team 2016), as well as the timeseries regression and seasonal decomposition functions in the forecast package (Hyndman 2018), were used as necessary to decompose the timeseries of water quality data collected by the continuous recorders and identify frequencies relevant for further evaluation. Given the limited period of record for the high frequency data collected in the Homosassa River it was unlikely that analysis of these data would directly inform criteria useful to the reevaluation of the minimum flows. However, analysis of these data provided information on the dominant forms of variability on these data relative to high-frequency periodicities such as fluctuations in tidal amplitudes associated with moon phase and low frequency periodicities that represent more long term seasonal or possibly flow related signals.

3.0 PRESENTATION OF RESULTS

The water quality monitoring networks described in Section 2.0 were established to evaluate different aspects of water quality in the Homosassa River. For example, the “Springs” sampling events were established to characterize the water quality discharging from the spring vents, while the UF transects were designed to characterize the spatial distribution in water quality for the mainstem of the river using a spatially intensive water quality monitoring design. In this way, the monitoring networks represent not only the data sources, but also critical resources of concern within the system. Therefore, the following sections describe the results of data analysis for each of these resources of concern including: the Spring Vents, the Mainstem of the River, and the Estuary. For each of these resources of concern we evaluated the relationship between water quality constituents of interest and flows as directed by the scope of work, and investigated the potential for the constituent to result in an adverse effect that could, under sufficient magnitude and duration, result in significant harm to the integrity of the resource. Therefore, a summary of the flows used for this analysis are also provided as a results section in this report.

The organization of the results section follows the description and application of the analytical pathway discussed in Section 2.0 by first describing the results of exploratory data analysis, then defining a conceptual model that related flows to the resource of concern and then evaluating whether or not a relationship exists that can be useful to support the development of minimum flows for the system. The continuous water quality recorder data represent a special case to evaluate fine scale temporal changes in water quality both within a day and between dates within weeks, months, and seasons. These data have been collected for approximately one year at specific locations in the mainstem of the river and therefore are more representative of high frequency variability at a particular location in the river rather than a long term representation of the expected condition for the system as a whole. The continuous recorder data were evaluated in this context and results also provided as a separate sub-section in the report.

3.1 FLOW GAGE OF RECORD

The USGS began estimating daily discharge at the Homosassa Springs gage (02310678) in October 1995 using a regression with the Weeki Wachee Well (Knochenmus and Yobbi 2001). There are no index velocity or tidally filtered data for discharge at 02310678. The USGS SE Fork gage (02310688) was first reported in October 2000 using a similar regression with groundwater and stage. Therefore, spring flows in the Homosassa Springs Complex were assumed to be directly correlated with the Weeki Wachee well and generally inversely correlated with surface water stage (Knochenmus and Yobbi 2001). The USGS began measuring spring flows for the SE Fork in 2012 using the index velocity method which resulted in tidally filtered daily values. To hindcast flows prior to in situ measurements, Leeper et al. (2012) used a regression equation method between Weeki Wachee well for both the Main Springs and the SE Fork. Once the long term flow record was generated for both the Main Springs and the Southeast Fork, the two stations were summed to represent a long term flow record for the headwaters of the Homosassa River for reevalution of minimum flows. The Halls River is a tributary to the Homosassa River that flows into the mainstem of the river approximately 1.5 kilometers downstream of the Springs complex (Figure 3-1). The Hall's River has only been gaged since 2012. The District has estimated flows using regression based on the SE Fork flows to create a long term flow record for the Hall's River flows to the system, but this record is not used as part of the long term flow for evaluating the minimum flows except as part of a separate hydrodynamic modeling effort conducted in support of reevaluation.

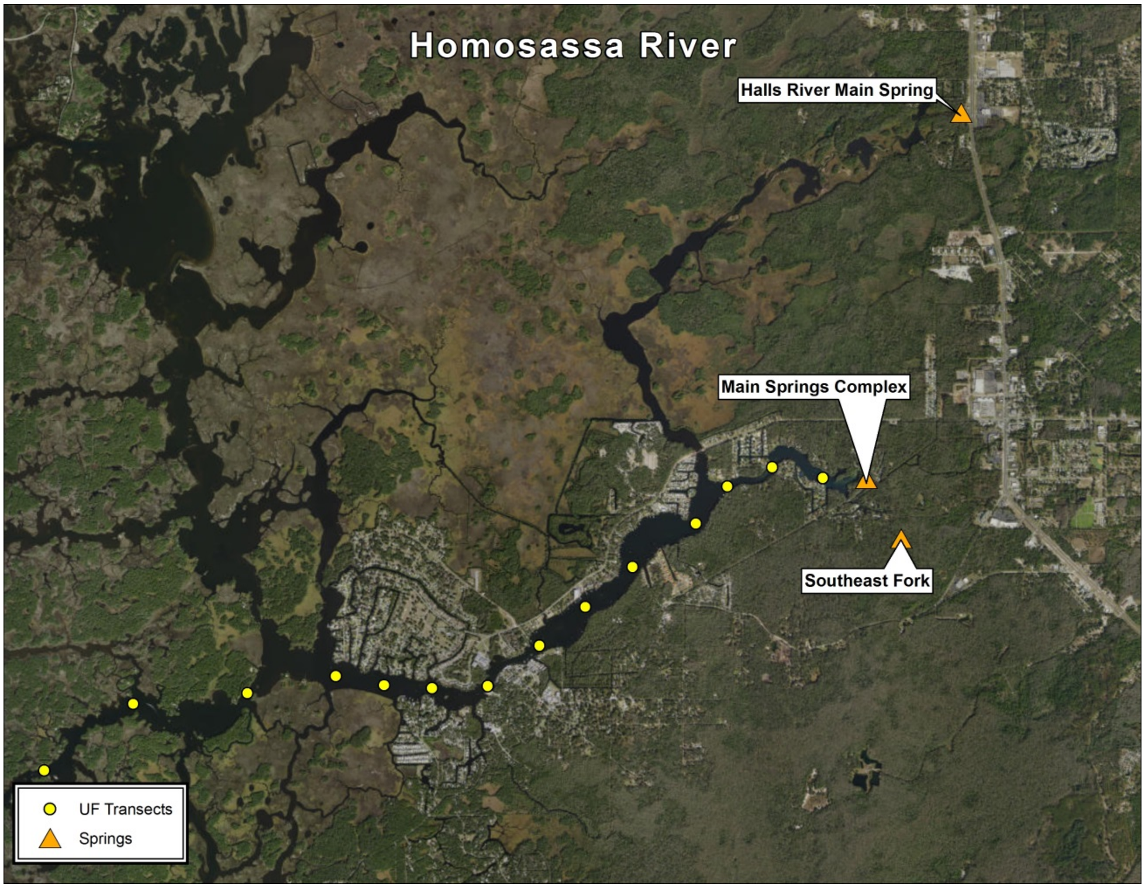


Figure 3-1. Homosassa River with the three principal sources of spring flow to the upper river. The UF water quality transects are shown as filled circles.

The long term timeseries for the Homosassa are presented in Figure 3-2 using the daily flow values (represented with a solid blue line) and the 21 day lag average flow (represented by the solid red line). Clearly, prior to 1995 there was much less variability in the flow estimates as those flows were based solely on well levels.

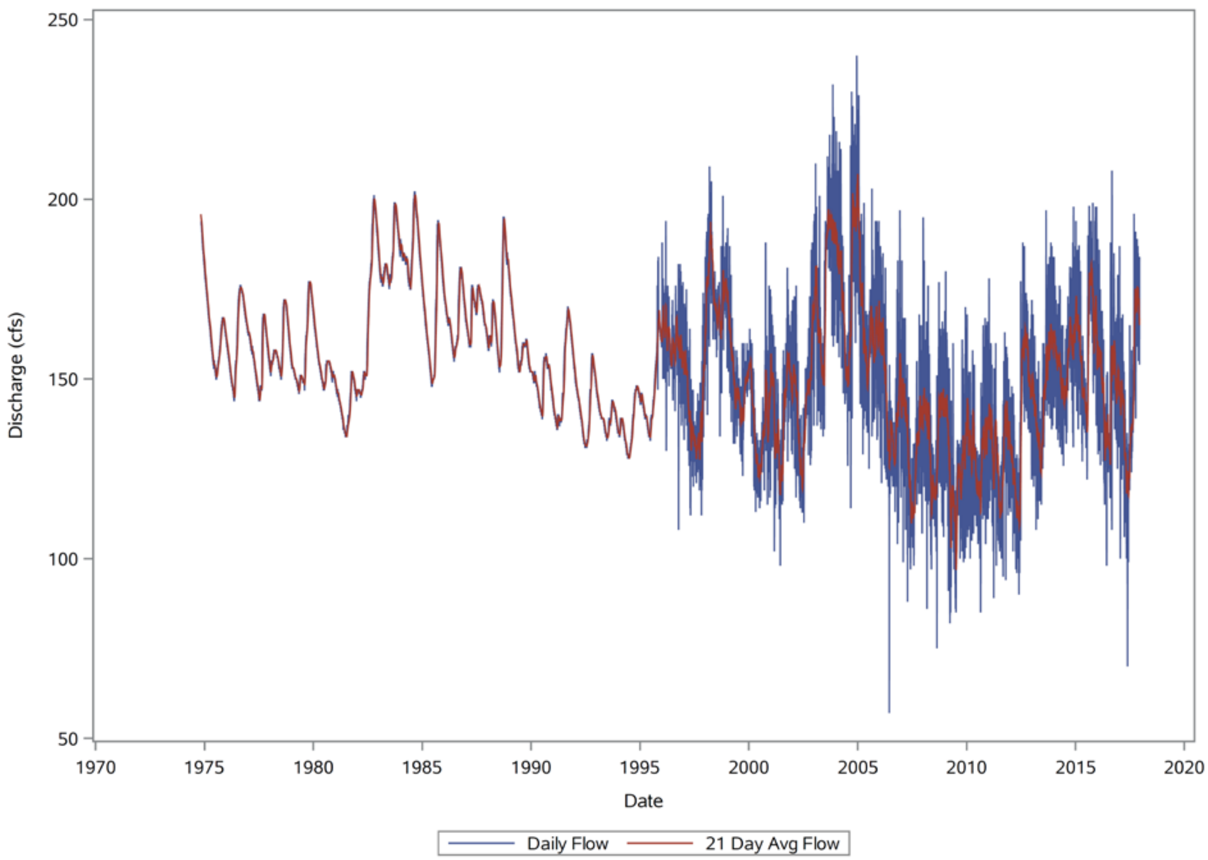


Figure 3-2. Long term flow record for Homosassa including the daily flows (blue) and the 21 day average flows (red).

Summary statistics and a histogram for the Homosassa flows are provided in (Figure 3-3). The mean (153 cfs) and median (152 cfs) values are very similar, within 1 cfs of each other. The range between the 5th (P5) and 95th (P95) percentiles is around 67 cfs, which is less than 50% of the median flow. Quantile plots against the normal distribution (Figure 3-4) suggest very slight deviations from the normal distribution

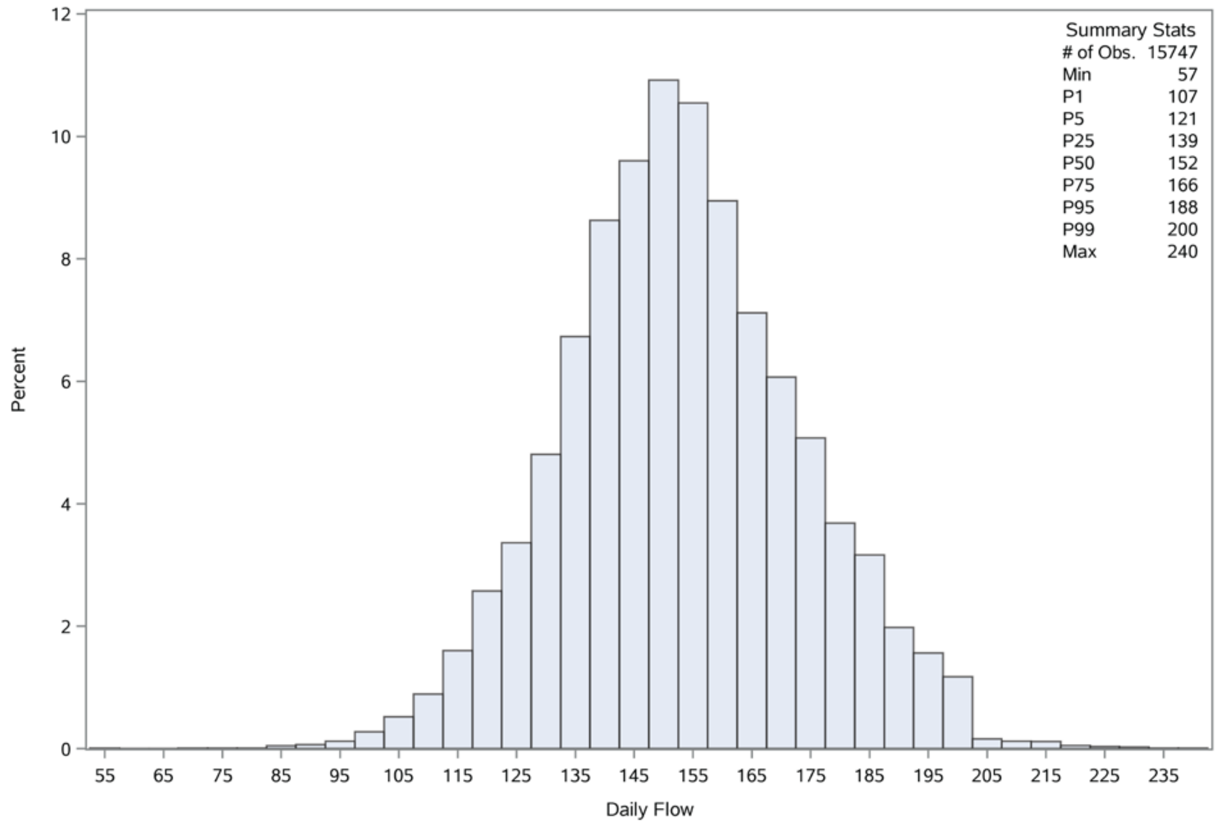


Figure 3-3. Summary statistics and histogram for the combined Homosassa flows estimated daily flow timeseries.

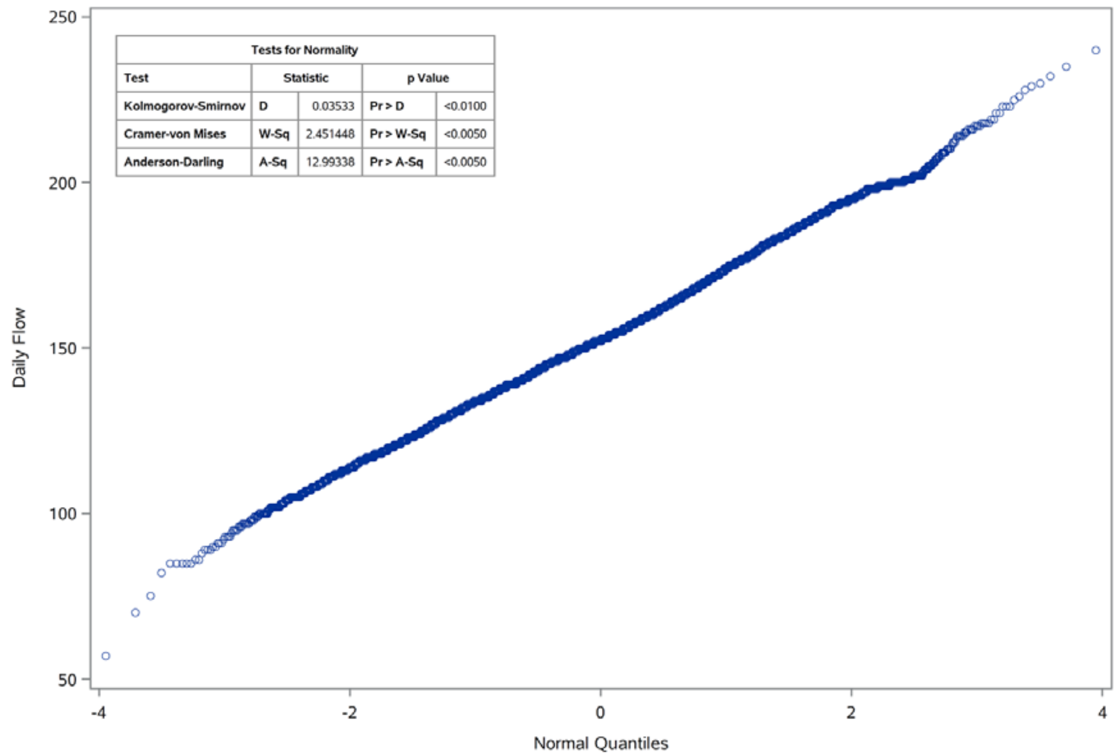


Figure 3-4. Quantile plot of combined Homosassa flows against the normal distribution.

Lag averages of the flows were highly correlated out to a 30 day average (Table 3-1) indicating the general consistency among spring flows up to 30 days and, therefore, any of these estimates would provide similar value to serve as deterministic components to assess the effects of flows on water quality constituents of interest (e.g. salinities, chlorophyll, etc.).

Table 3-1. Spearman rank correlation among various lag average flows between 1 and 30 days.

Flow Statistic	Daily Flows	2d mean	3d mean	5d mean	7d mean	8d mean	14d mean	21d mean	30d mean
Daily Flows	1.00	0.98	0.96	0.94	0.93	0.93	0.92	0.92	0.91
2d mean	0.98	1.00	0.99	0.97	0.96	0.95	0.94	0.94	0.93
3d mean	0.96	0.99	1.00	0.99	0.97	0.97	0.96	0.95	0.94
5d mean	0.94	0.97	0.99	1.00	0.99	0.99	0.97	0.97	0.96
7d mean	0.93	0.96	0.97	0.99	1.00	1.00	0.98	0.98	0.97
8d mean	0.93	0.95	0.97	0.99	1.00	1.00	0.99	0.98	0.97
14d mean	0.92	0.94	0.96	0.97	0.98	0.99	1.00	0.99	0.99
21d mean	0.92	0.94	0.95	0.97	0.98	0.98	0.99	1.00	1.00
30d mean	0.91	0.93	0.94	0.96	0.97	0.97	0.99	1.00	1.00

Monthly median flows were calculated for the period of record and the Seasonal Mann-Kendal (SMK) test for trend was used to evaluate the trend over time. The seasonality in the monthly median flows is portrayed in the box and whisker plots of Figure 3-5. The SMK tests the slope of the time series trend for each month and combines the results to report a statistic representing the significance of the combined results. The results of the SMK test suggest a significant declining trend in the monthly median discharge values over time ($p<0.001$:Figure 3-6). Since the flows are based on regression with Weeki Wachee Well levels (Leeper et al. 2012), the declining trend can be attributed to trends in Weeki Wachee Well levels (Figure 3-7).

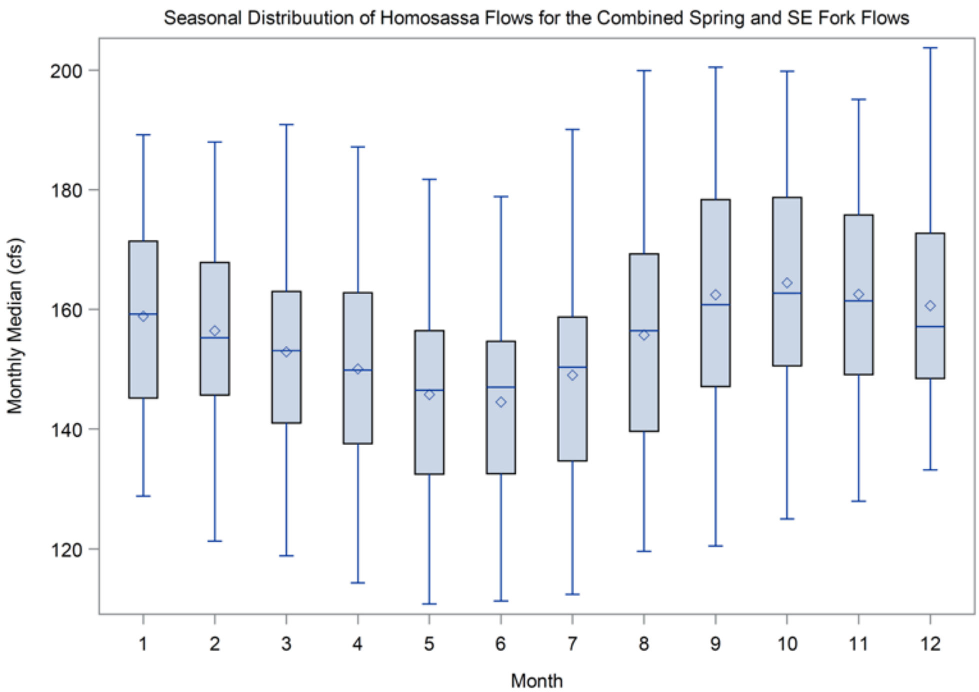


Figure 3-5. **Seasonal (monthly) distribution of flows for the entire period of record.**

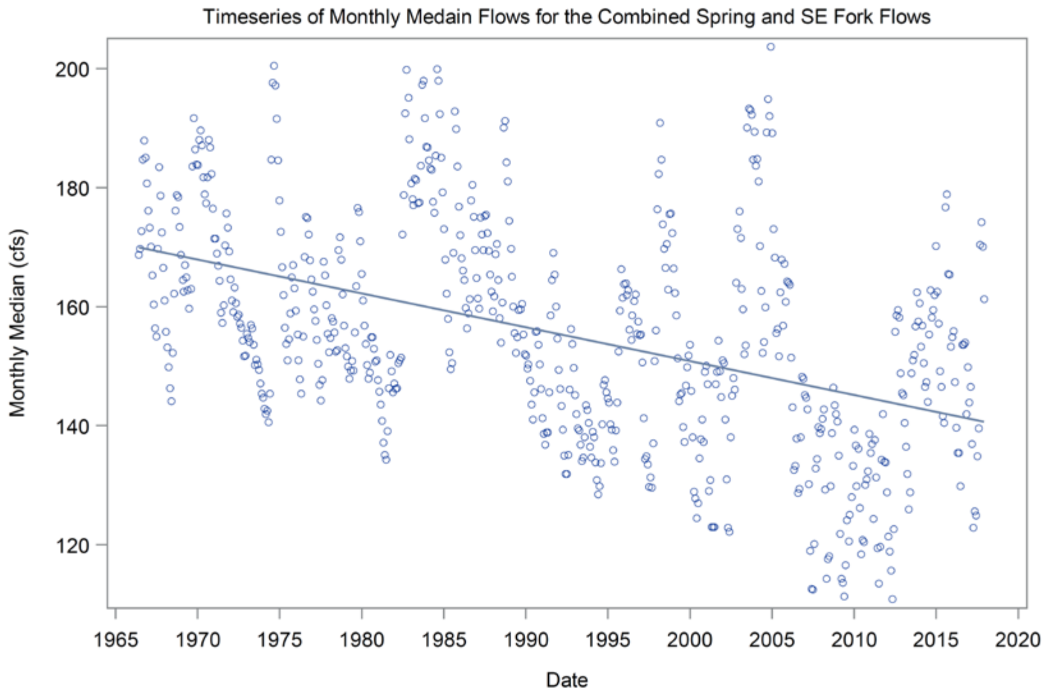


Figure 3-6. **Timeseries of monthly median flows with trend line depicting the decreasing trend in flows over time.**

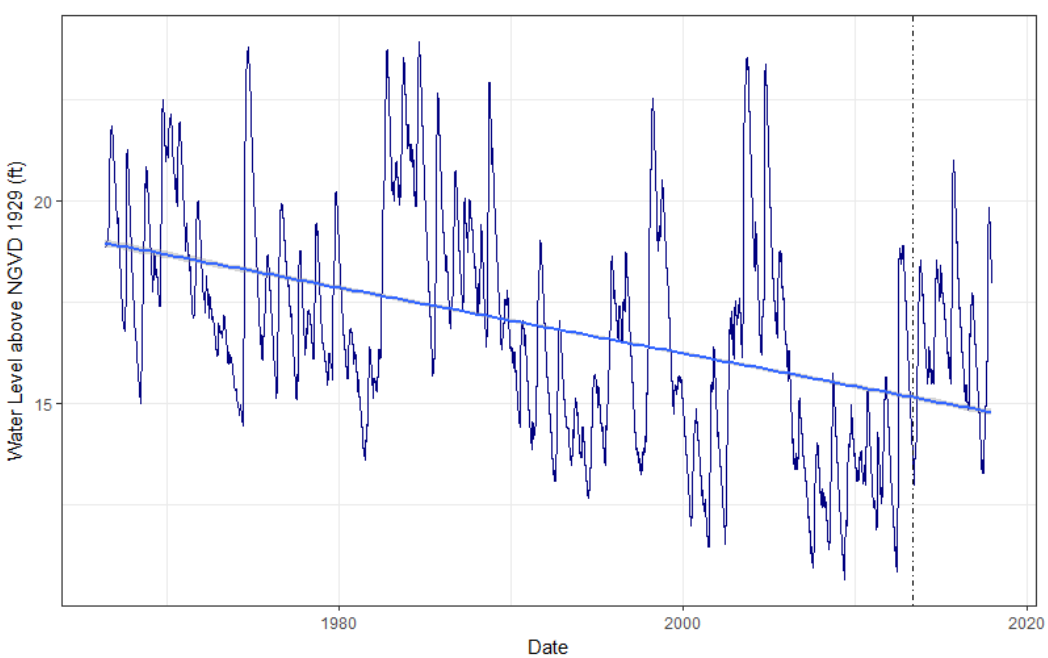


Figure 3-7. **Water levels in Weeki Wachee Well from 16,268 daily values. Dashed vertical line is at start of new well location on 2013-04-30, which has been adjusted by adding 0.3 feet to match with old well location following regression adjustment by USGS.**

3.2 SPRING VENTS

The Springs data included quarterly sampling events generally taken at or near low tide beginning in the early to mid-1990's with the exception of Bluebird, Otter Creek and Southeast Fork Spring vents which have been intermittently sampled between 2010 and 2017 (Figure 3-8).

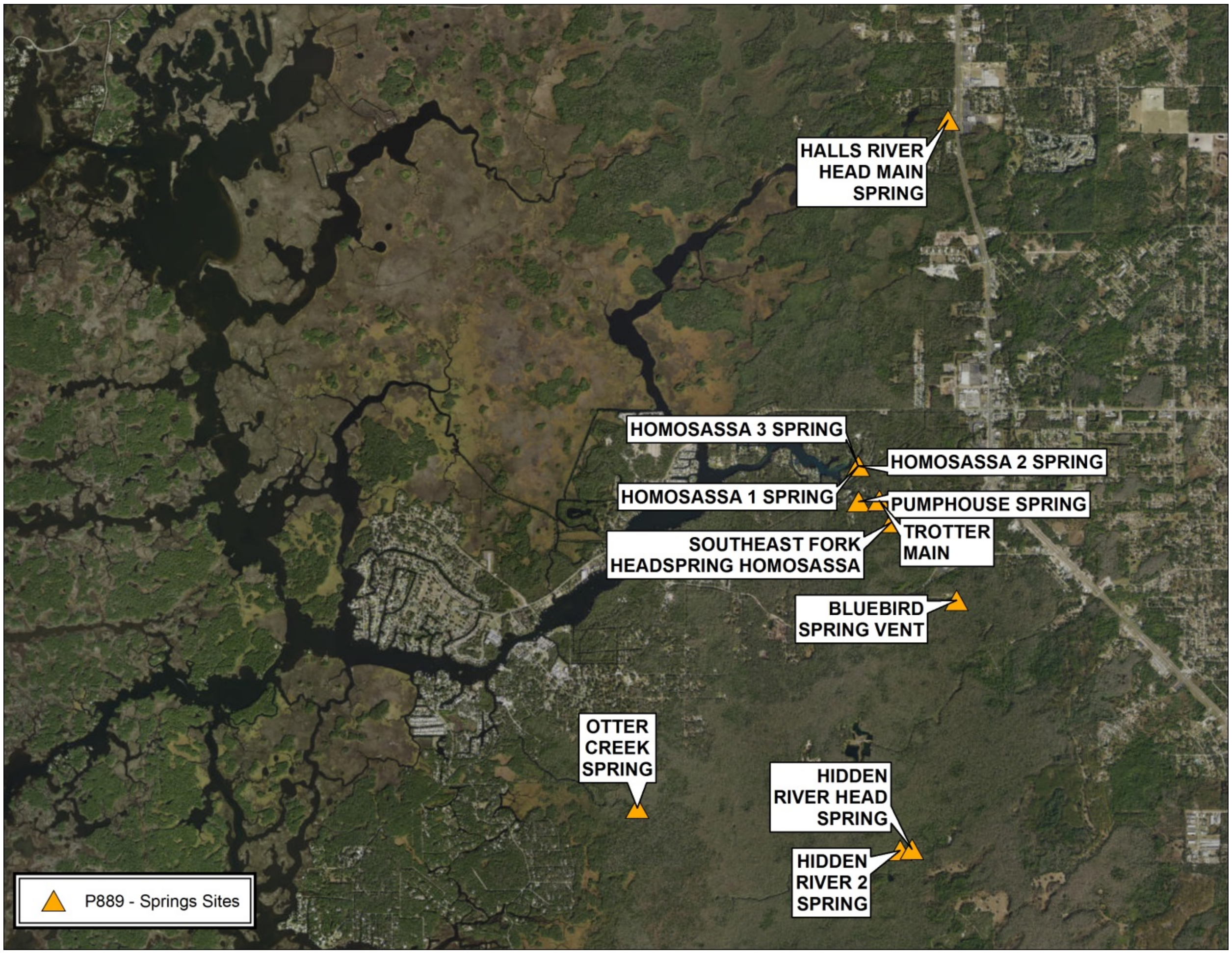


Figure 3-8. Location of sampling sites for the Springs Vent sampling program (District ID P889).

3.2.1 Exploratory Data Analysis

To evaluate the utility of these data to support the reevaluation of the minimum flows analysis, OLS linear regression analysis was conducted to test the hypothesis that concentrations of water quality constituents emanating from the spring vents were related to spring discharge. Because spring discharge is estimated as a function of Weeki Wachee well for the majority of the timeseries, and because these spring vents are located in several areas without long term discharge records, the long term flow record developed to reevaluate minimum flows were used as the estimate of spring discharge for all spring sites. The water quality constituents evaluated are listed in Table 3-2. The District has developed acceptance criteria for using linear regression analysis in support of minimum flows evaluations (Heyl et al. 2012). The acceptance criteria suggest that regressions should include: a) a minimum 10 observations per variable, b) a plausible trend in the response as a function of flow, c) no significant serial correlation and d) an adjusted coefficient of determination (R^2) of at least 0.3.

Table 3-2. List of water quality constituents evaluated for linear relationships with flow.

Alkalinity (Dissolved)	Depth (Total)	Nitrogen- Total Kjeldahl (Dissolved)	Stage (Total)
Alkalinity (Total)	Depth, bottom (Total)	Nitrogen- Total Kjeldahl (Total)	Strontium (Dissolved)
Ammonia (N) (Dissolved)	Dissolved Oxygen (Total)	Nitrogen15/Nitrogen14 Isotope Ratio	Strontium (Total)
Ammonia (N) (Total)	Eh, Field (hydrogen electrode)	Orthophosphate (P) (Dissolved)	Sulfate (Dissolved)
Bicarbonate (Total)	Fluoride (Dissolved)	Orthophosphate (P) (Total)	Sulfate (Total)
Biological Oxygen Demand (Total)	Fluoride (Total)	Pheophytin (Total)	Temperature (Total)
Boron (Dissolved)	Hardness (Total)	Phosphorus (Dissolved)	Total depth at monitored location
Boron (Total)	Iron (Dissolved)	Phosphorus- (Total)	Transparency (Total)
Cadmium (Total)	Iron (Total)	Phosphorus – Soluble Reactive	Turbidity (Total)
Calcium (Dissolved)	Lead (Total)	Potassium (Dissolved)	Zinc (Dissolved)
Calcium (Total)	Light, Attenuation Coefficient	Potassium (Total)	Zinc (Total)
Carbon- Total Organic (Total)	Magnesium (Dissolved)	Purge Volume (Total)	pH (Total)
Chloride (Dissolved)	Magnesium (Total)	Residues- Filterable (TDS) (Dissolved)	
Chloride (Total)	Manganese (Dissolved)	Residues- Nonfilterable (TSS) (Total)	
Chlorophyll (Total)	Manganese (Total)	Residues- Volatile (Total)	
Chlorophyll a (Total)	Molybdenum (Dissolved)	Salinity (Total)	
Chlorophyll b (Total)	Nitrate (N) (Dissolved)	Secchi-horizontal (Total)	
Chlorophyll c (Total)	Nitrate (N) (Total)	Secchi-vertical (Total)	
Cobalt (Dissolved)	Nitrite+Nitrate (N) (Dissolved)	Selenium (Dissolved)	
Coliform Fecal (Total)	Nitrite+Nitrate (N) (Total)	Selenium (Total)	

Those regressions that met the acceptance criteria are described in the paragraphs below. An example of the results for the Homosassa 1 Spring site is provided in Figure 3-9 where several major ions were significantly related to daily flows based on the gage of record. All these constituents displayed inverse relationships with flow (i.e. constituent concentrations decrease with increasing flows). Similar relationships were observed at Homosassa 3 Spring (Figure 3-10; Table 3-3), and Pumphouse Spring (Figure 3-11). This was not a surprising result as it is well known that water that has been in contact with limestone for a relatively short length of time should have low concentrations of calcium and bicarbonate ions; water with a longer period of residency within the flow system should typically have higher concentrations. Total Dissolved Solids is a measure of chemical constituents dissolved in the groundwater and in west-central Florida, TDS is mostly influenced by the concentrations of the major ions: calcium, bicarbonate, magnesium, sodium, sulfate and chloride. TDS can be used to estimate the relative residence time of ground water in the aquifer and typically increases as the length of groundwater flow paths increase (SWFWMD 2001).

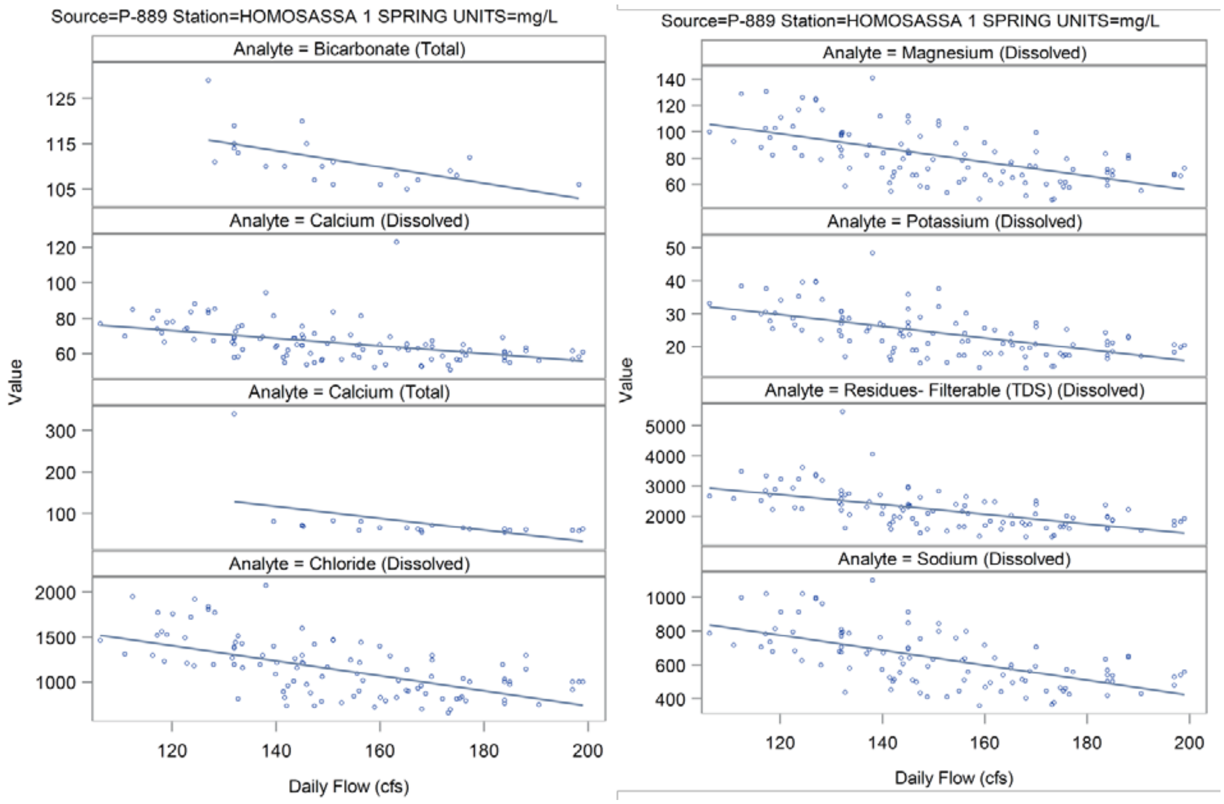


Figure 3-9. Regression relationships between a select group of water quality constituents of interest and the Homosassa flow gage of record.

While many of the components of TDS (i.e. bicarbonate, magnesium, and sodium) illustrated a decrease in concentration with increasing flows, there was no evidence that these trends would result in significant harm to the system. The fact that there were statistically significant relationships does not imply that there was an ecologically meaningful interpretation of this result that could aid in reevaluation of the minimum flows. TDS concentrations vary greatly across the spring group and chloride concentrations also widely range, indicating that water quality at the spring group is strongly influenced by the coastal transition zone, even at low tide (SWFWMD 2001).

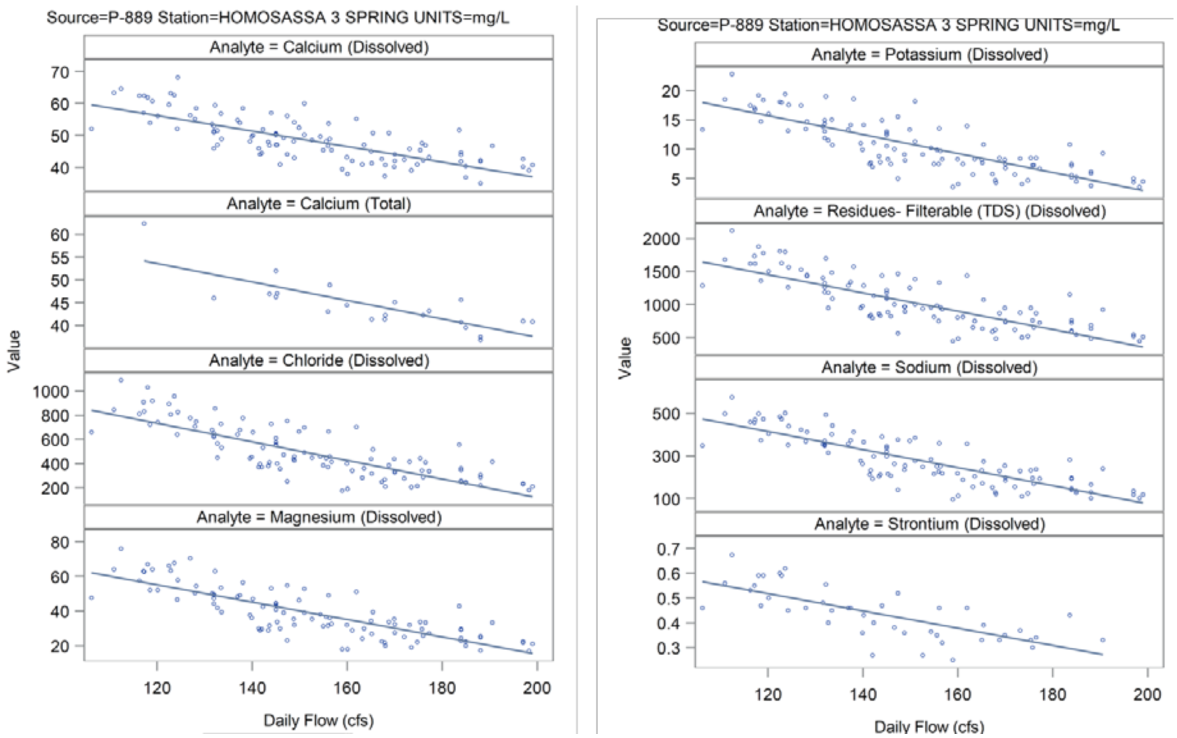


Figure 3-10. **Regression relationships between the Homosassa flow gage of record and concentrations of water quality constituents of interest at Homosassa 3 Spring.**

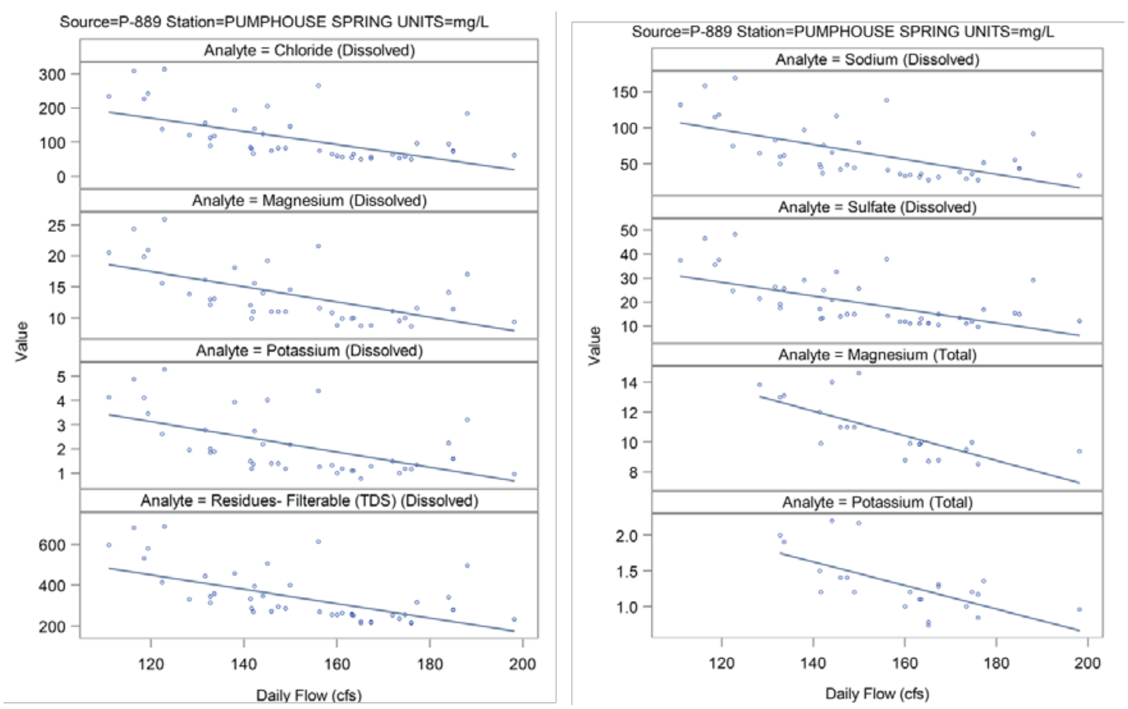


Figure 3-11. **Regression relationships between the Homosassa flow gage of record and concentrations of water quality constituents of interest at Pumphouse Spring.**

Interestingly, no forms of nitrogen were significantly related to flows for Homosassa 1through 3 or Pumphouse spring (Table 3-3). Nitrogen is of particular interest with respect to water quality issues within the Springs coast systems. The Homosassa-Trotter-Pumphouse Springs Group has exceeded the state standard for nitrates and a regulatory action known as a total maximum daily load (TMDL) has been developed to establish allowable level of nutrient loadings that would restore the waterbodies so that they meet applicable water quality criterion for nutrients (Bridger et al. 2014).

Table 3-3. Significant regression results for Homosassa 1, 2 and 3 Springs data.

Spring Number	Parameter	Units	Intercept	Slope	DF	R Square	P Value
1	Turbidity (Total)	FTU	11.2377	-0.0550	12	0.48	0.0058
1	Bicarbonate (Total)	mg/L	130.4976	-0.1306	19	0.33	0.0063
1	Calcium (Dissolved)	mg/L	100.8252	-0.2363	114	0.35	0.0000
1	Calcium (Total)	mg/L	116.7772	-0.3032	20	0.45	0.0006
1	Chloride (Dissolved)	mg/L	2333.0610	-7.9620	116	0.35	0.0000
1	Magnesium (Dissolved)	mg/L	159.8680	-0.5197	101	0.36	0.0000
1	Potassium (Dissolved)	mg/L	49.7150	-0.1700	101	0.37	0.0000
1	Residues- Filterable (TDS) (Dissolved)	mg/L	4380.6287	-14.6869	115	0.39	0.0000
1	Sodium (Dissolved)	mg/L	1307.1923	-4.4381	102	0.36	0.0000
1	Strontium (Dissolved)	mg/L	1.4098	-0.0049	43	0.48	0.0000
1	Sulfate (Dissolved)	mg/L	351.5412	-1.2241	117	0.35	0.0000
1	Specific Conductance (Total)	uS/cm	7970.1242	-25.3900	180	0.40	0.0000
2	Turbidity (Total)	NTU	-2.0838	0.0252	89	0.31	0.0000
2	Color (Dissolved)	PCU	-4.4538	0.0675	43	0.54	0.0000
2	Iron (Dissolved)	ug/L	-96.1410	1.3711	110	0.32	0.0000
2	Manganese (Dissolved)	ug/L	2.3415	0.0369	22	0.40	0.0009
3	Calcium (Dissolved)	mg/L	84.1959	-0.2361	115	0.56	0.0000
3	Calcium (Total)	mg/L	67.9628	-0.1458	19	0.56	0.0001
3	Chloride (Dissolved)	mg/L	1655.2600	-7.6765	118	0.63	0.0000
3	Magnesium (Dissolved)	mg/L	115.1335	-0.5002	101	0.65	0.0000
3	Magnesium (Total)	mg/L	78.4187	-0.3067	34	0.46	0.0000
3	Potassium (Dissolved)	mg/L	35.1156	-0.1616	101	0.65	0.0000
3	Potassium (Total)	mg/L	25.9411	-0.1144	37	0.59	0.0000
3	Residues- Filterable (TDS) (Dissolved)	mg/L	3097.7020	-13.7901	116	0.64	0.0000
3	Sodium (Dissolved)	mg/L	922.5090	-4.2363	101	0.69	0.0000
3	Sodium (Total)	mg/L	792.0325	-3.6174	38	0.65	0.0000
3	Strontium (Dissolved)	mg/L	0.9351	-0.0035	43	0.54	0.0000
3	Sulfate (Dissolved)	mg/L	239.9213	-1.0884	118	0.61	0.0000
3	Specific Conductance (Total)	uS/cm	5998.8118	-26.2340	181	0.67	0.0000
3	Boron (Dissolved)	ug/L	371.0426	-1.5372	22	0.71	0.0000
3	Manganese	ug/L	3.2881	-0.0120	20	0.42	0.0011

Table 3-4. Significant regression results for Pumphouse Springs						
Parameter	Units	Intercept	Slope	DF	R Square	P Value
Chloride (Dissolved)	mg/L	376.5549	-1.7762	56	0.33	0.0000
Fluoride (Total)	mg/L	0.2704	-0.0011	22	0.36	0.0019
Magnesium (Dissolved)	mg/L	32.1935	-0.1227	39	0.37	0.0000
Magnesium (Total)	mg/L	20.8538	-0.0667	17	0.49	0.0009
Potassium (Dissolved)	mg/L	6.8831	-0.0313	39	0.33	0.0001
Potassium (Total)	mg/L	3.9400	-0.0165	22	0.42	0.0006
Residues- Filterable (TDS) (Dissolved)	mg/L	877.0180	-3.5516	56	0.37	0.0000
Sodium (Dissolved)	mg/L	220.8779	-1.0317	39	0.37	0.0000
Sodium (Total)	mg/L	123.3618	-0.5249	21	0.52	0.0001
Sulfate (Dissolved)	mg/L	52.7090	-0.2262	59	0.35	0.0000
Specific Conductance (Total)	uS/cm	1845.5692	-7.6599	68	0.39	0.0000

The observed statistically significant relationships with any form of nitrogen were tenuous with low numbers of observations and less than 50% of the total variability explained by the model. Significant nitrogen relationships are reported for nitrite+nitrate (total) and total nitrogen in the Southeast Fork, both of which were inversely related to flow. The relationship between total nitrogen and flows in Hall's River was significant and positive, as was the relationship between nitrite (total) and flow in Hidden River. The nitrite results are considered especially tenuous since the concentrations tend to be very small and near the detection limits. In addition, the results of the nitrogen regressions were conflicting with respect to the direction of the relationship with positive relationships observed for Hall and Hidden River and negative relationships observed for the SE fork. These findings support those of Upchurch et al. 2008 who evaluated the relationship between nitrates and spring flow in the Suwannee Management District to address the question "can management of spring flows be utilized to mitigate nitrate discharging from the springs?". The results of that analysis concluded that minimum flows cannot be utilized to control nitrate discharging from the springs by promoting high discharge.

Table 3-5. Significant regression results for other Springs in the Homosassa complex.							
Site Name	Parameter	Units	Intercept	Slope	DF	R Square	P Value
SE Fork	Temperature	Deg. C	24.8467	-0.0131	19	0.32	0.0078
SE Fork	Nitrite+Nitrate (N) (Total)	mg/L	0.9283	-0.0017	19	0.31	0.0090
SE Fork	Nitrogen- Total (Total)	mg/L	0.9037	-0.0013	19	0.38	0.0029
Trotter	Sodium (Total)	mg/L	130.2328	-0.6084	20	0.50	0.0002
Halls River	pH (Total)	SU	6.8135	0.0045	35	0.30	0.0004
Halls River	Nitrogen- Total (Total)	mg/L	0.0747	0.0020	23	0.32	0.0033
Hidden River	Nitrite (N) (Total)	mg/L	-0.0048	0.0001	39	0.35	0.0001
Hidden River	Strontium (Total)	ug/L	1933.2599	-10.6594	17	0.50	0.0007

3.2.2 Conceptual Model

Despite the existence of many significant water quality relationships with flow, there was no evidence that a conceptual model could be developed that provided a plausible connection between these relationships and the establishment of a minimum flow for the Homosassa River. The relationship between major ions and flow would only be problematic if they were considered contaminants. Instead, many of these constituents are trace nutrients that are valuable for biological growth. In addition, even if the concentrations decrease with flow, the total mass of the constituent may be increasing and that total mass may be a more important driver of response of biota in the receiving water bodies. In summary, there was no evidence that the relationship of any of these constituents with flow would result in significant harm to the receiving waters of the Homosassa River. Therefore, the investigation of relationship between these water quality constituents and flow for the Springs dataset was not pursued further. Plots for all relationships examined for the Springs data are provided in Appendix C.

3.3 River Mainstem

The river mainstem includes water quality samples collected from various monitoring networks from just below the headsprings to the mouth of the river. The monitoring programs in the mainstem of the Homosassa River were described in Section 2.1.1. The following sections describe the exploratory analysis and implementation of the statistical analysis plan for those data.

3.3.1 Exploratory Data Analysis

Linear regression analysis was conducted on data collected in the mainstem of the river in a similar manner to that described above for the Springs resource. The Project Coast monitoring network data (P-529) is the most data rich of the sampling programs. After application of the District acceptance criteria, the only significant constituent related to flow measured by the Project Coast monitoring network was salinity, which was significantly inversely related to flow for all 10 stations in the network. The Coastal Rivers network (P-108) has a reduced sampling frequency, but also recorded significant relationships between flows and either salinity or specific conductivity. In addition, major ions including calcium, magnesium, and potassium were significantly related to flows. Again, all these constituents were inversely related to flows as described for the Springs data. The same inference described for these results in the Springs data can be applied to the mainstem of the river. That is, there is no evidence that these constituents (other than salinity) would result in significant harm to the system. The hydrodynamic model developed for the Homosassa is considered the best available tool for evaluating the effects of flows on salinity and was applied to the reevaluation as described in a separate report.

The U.F. transect regression results suggested that, in addition to salinity and specific conductivity throughout the system, nutrients including ammonium, and soluble reactive phosphorus were positively related to flows in the upper portion of the system. Chlorophyll was also significantly related to flow at three stations in the upper portion of the river (transect 1, 4 and 6) and the regressions suggested an inverse relationship with flow at all these locations. Interestingly, no forms of nitrogen were significantly related to flows based on the linear regression analysis of the UF data. Appendix D details the results of this exploratory analysis, with plots for all significant relationships, and details of the statistical output

The findings with respect to chlorophyll were of particular interest since it appeared that the effects were somewhat nonlinear at the lowest flows for some of the transects in the upper portion of the river. To further explore this relationship, the UF data were deemed the best representation of the river from which to evaluate the effects of flows on water quality in the mainstem of the river. The sampling design is spatially intensive with 20 transect locations within 14 kilometers of the river (Figure 3-12). At each of the first 15 transect locations, three samples were collected along a lateral cross-section of the river while at the 5 most downstream locations, a single water quality sample was collected (Frazer et al. 2001).

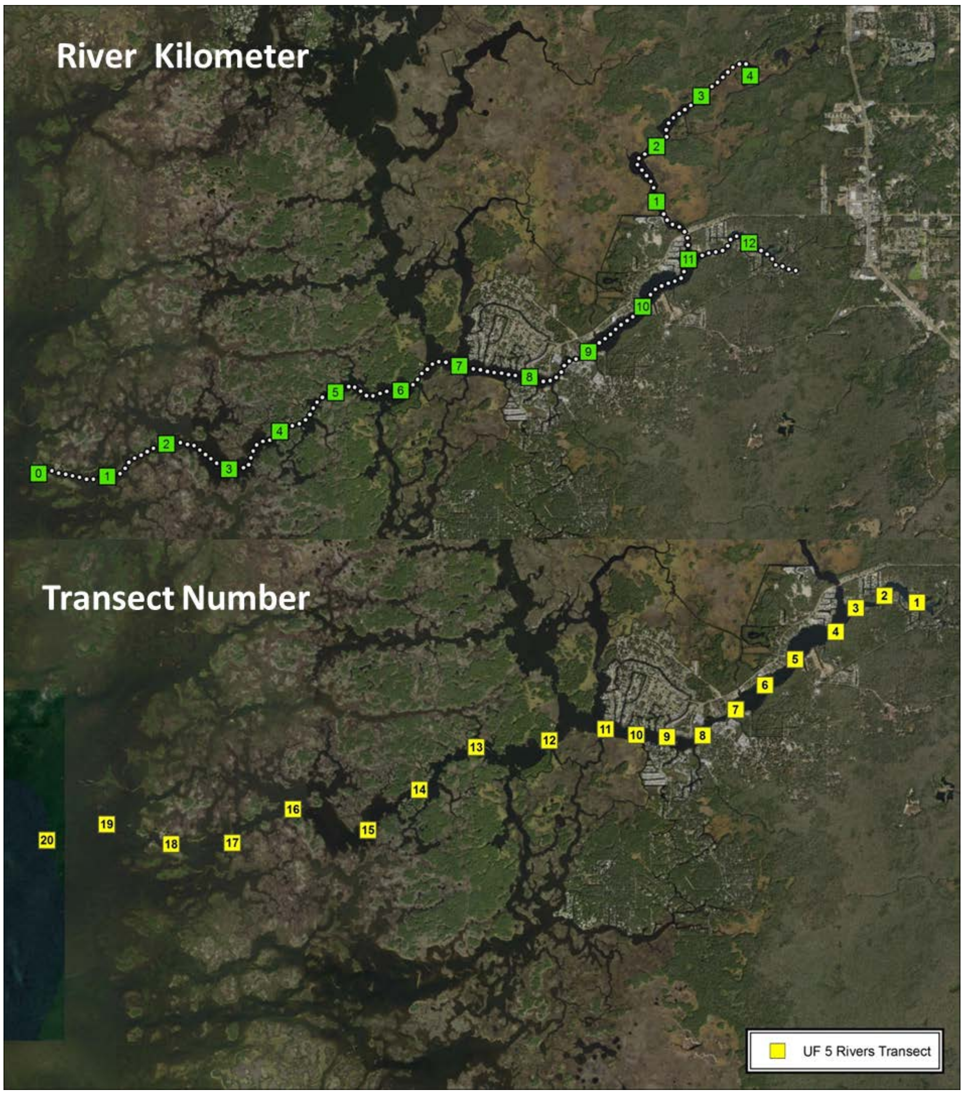


Figure 3-12. River kilometer and transect numbering system for the Homosassa River.

Details of the sampling design for the UF transect study can be found in Frazer et al. (2001). Briefly, the 20 sites were located at approximately 0.5 km intervals along the mainstem of the river. These sites were sampled quarterly between 1998 and 2011 with a data gap between 2001 and 2003. At each site, three water quality samples were collected. These samples are not exactly replicates in that they were collected at different lateral positions across the river at the site location to correspond with measurements of macrophyte and other biological measurements across the river. While the concentrations of chlorophyll a are low in general, exploratory data analysis suggested that the mainstem of the river was susceptible to chlorophyll a concentrations that were in many cases more than twice the annual geometric mean. This was evident in the spatial (Figure 3-13) and temporal (Figure 3-14) plots of the UF transect data as well and confirmed by other data sources in the mainstem of the river (Figure 3-15). Note the UF data have a longer timeseries of chlorophyll a uncorrected for pheophytin and therefore these data were used for the following analysis.

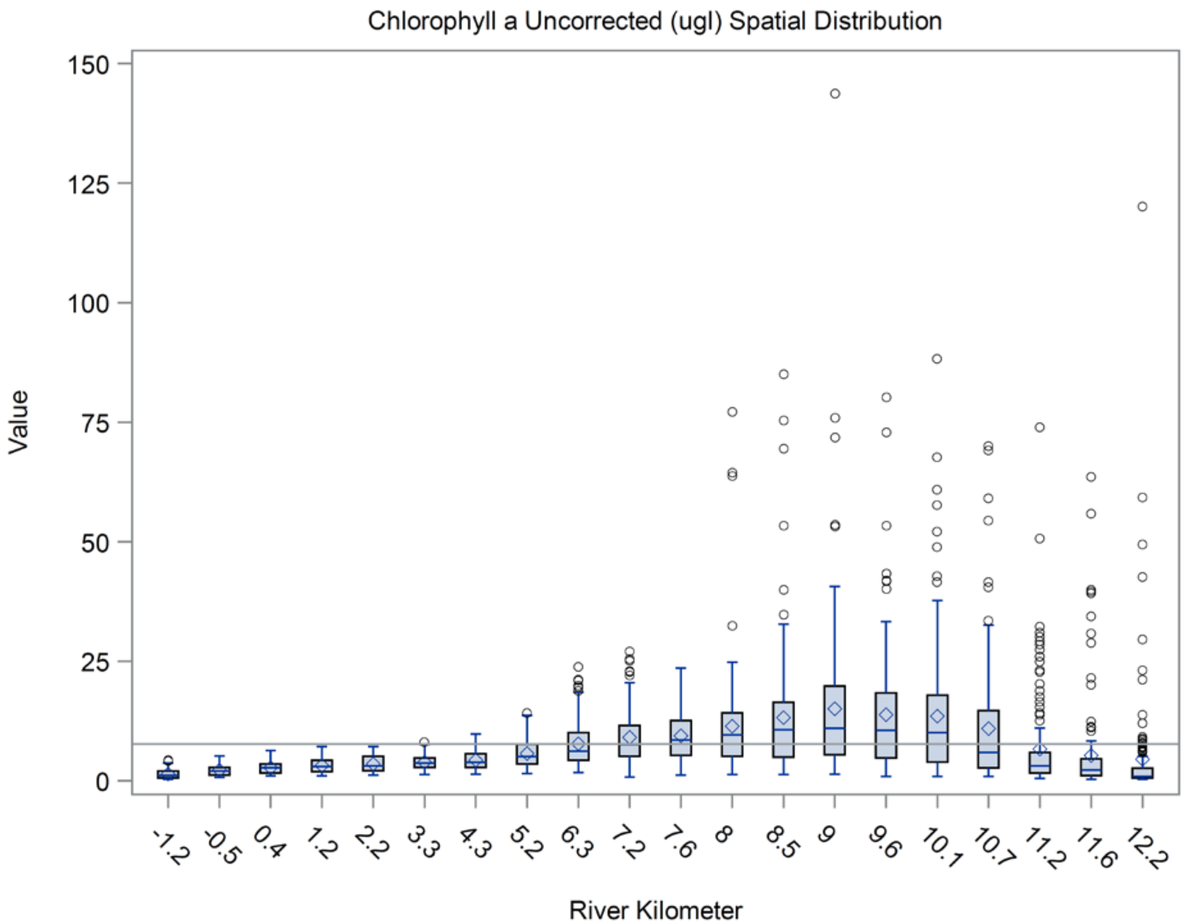


Figure 3-13. **Distribtuon of uncorrected chlorophyll a concentrations from the University of Florida transect data collection effort between 1998 and 2011.Horizontal reference line represents 10 ug/l for reference only.**

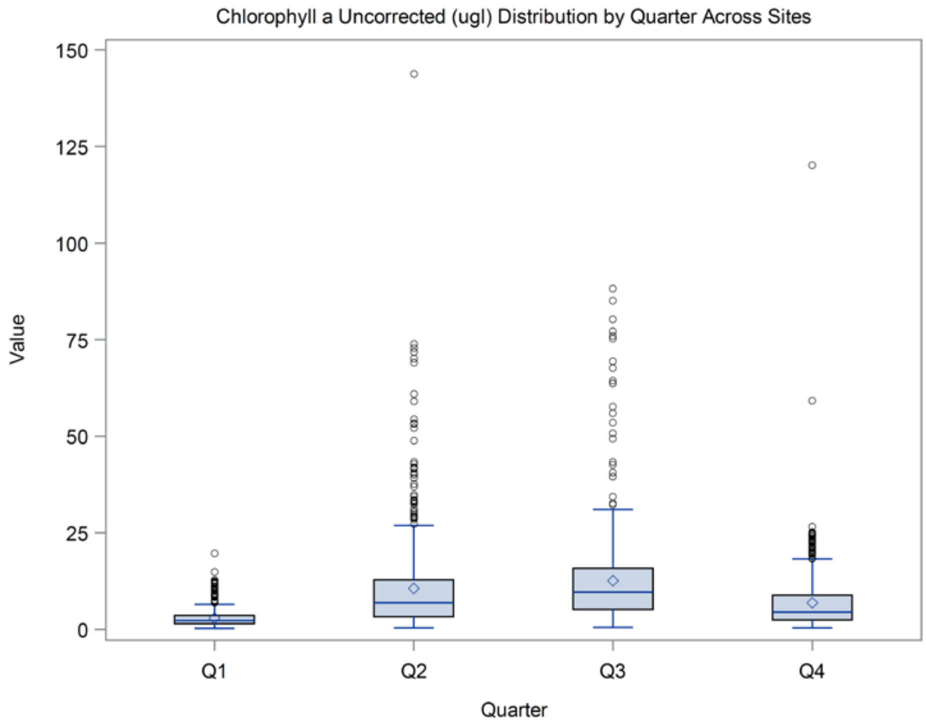


Figure 3-14. Temporal distribution of uncorrected chlorophyll a concentrations based on quarterly sampling from University of Florida transect study in the Homosassa River.

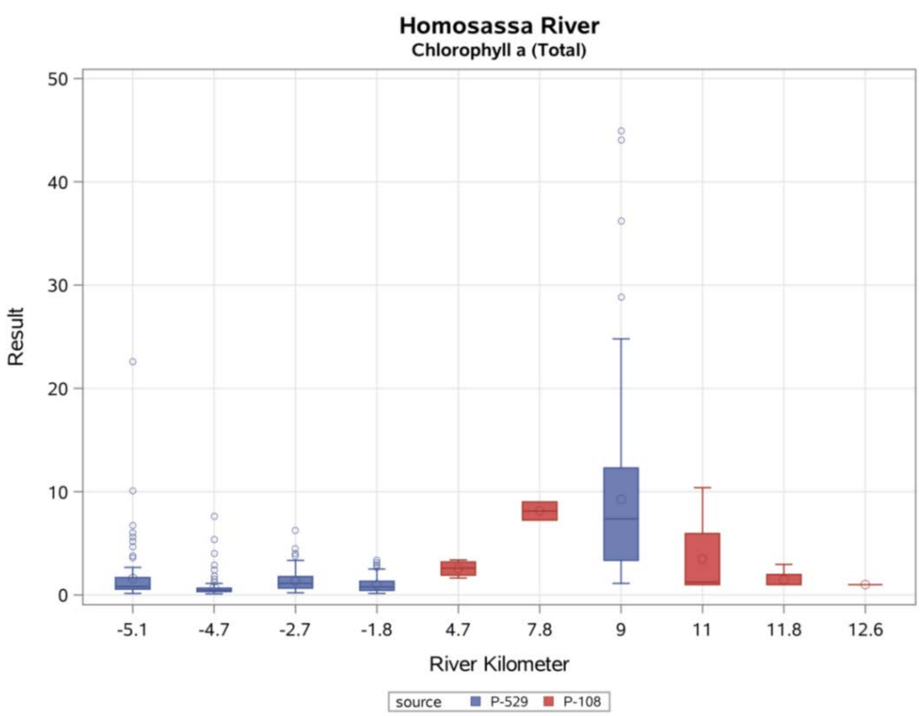


Figure 3-15. Corrected chlorophyll (ug/l) distribution at fixed locations in the Homosassa River from the active sampling programs in the Homosassa River.

3.3.2 Conceptual Model

Evaluating the effects of flows on water quality required the development of a conceptual model that summarizes the linkages related to the criteria established for deriving a line of evidence to support reevaluation of minimum flows. That is, the analysis must identify an adverse effect of reduced flows that, if of sufficient magnitude and duration, would result in significant harm to the ecology of the system. State water quality standards (62.303.530 F.A.C.) were viewed as one set of criteria that were applicable to this effort. The state water quality standards for the Homosassa River estuary which includes the downstream half of the mainstem of the river (1345F) are listed in Table 3-6.

Table 3-6. Florida water quality standards for WBID 1345F in the Homosassa River.

	Total Phosphorus	Total Nitrogen	Chlorophyll a
Homosassa River Estuary	0.028 mg/L as AGM	0.51 mg/L as AGM	7.7 µg/L as AGM

** AGM = Annual Geometric Mean

The FDEP split the river into Waterbody Identifiers (WBID) depicted in Figure 3-16. A river kilometer (Rkm) system is also established for the river which begins at the mouth (Rkm 0) and ends at the headsprings (Rkm 12.6). WBID 1345F includes the portion of the river from Rkm 0 to Rkm 9.2. Between Rkm 9.2 and 12.6 is WBID 1345, representing the upper river run which has no site-specific standard. The TMDL applies to WBIDs identifying the spring vents including the main spring complex (WBID 1345G), as well as Bluebird Springs (1348A) and Hidden River Springs (1348E).

While the chlorophyll water quality standard in Table 3-6 does not apply in a regulatory sense to the uppermost portion of the mainstem of the river (WBID 1345), we used that value for the entire portion of the river above Rkm 7 to represent an indicator that has relevance to an adverse effect for the following reasons. Excessive phytoplankton concentrations (as measured by chlorophyll a) are known to reduce water clarity and limit sunlight available to submerged aquatic vegetation such as the native macrophytes that are considered an indicator of good water quality conditions in the mainstem of the river. Phytoplankton blooms can increase the production of organic material that, upon deposition, can reduce dissolved oxygen concentrations in the river bottom, and phytoplankton blooms can change the ratio of water column to benthic primary production that is thought to be an important characteristic of these historically oligotrophic tidal springs systems (Burghart et al. 2013). Given that the Homosassa River is listed as an Outstanding Florida Waterbody with portions of the system designated as a Class II waterbody (i.e. shellfish propagation), the more stringent criterion value was selected as an indicator of an adverse condition. It should be noted, therefore, that exceedances of the 7.7 ug/l threshold as applied here are not directly applicable to inference regarding the effects of threshold exceedances on the declaration of the River as “Impaired” according to state laws.

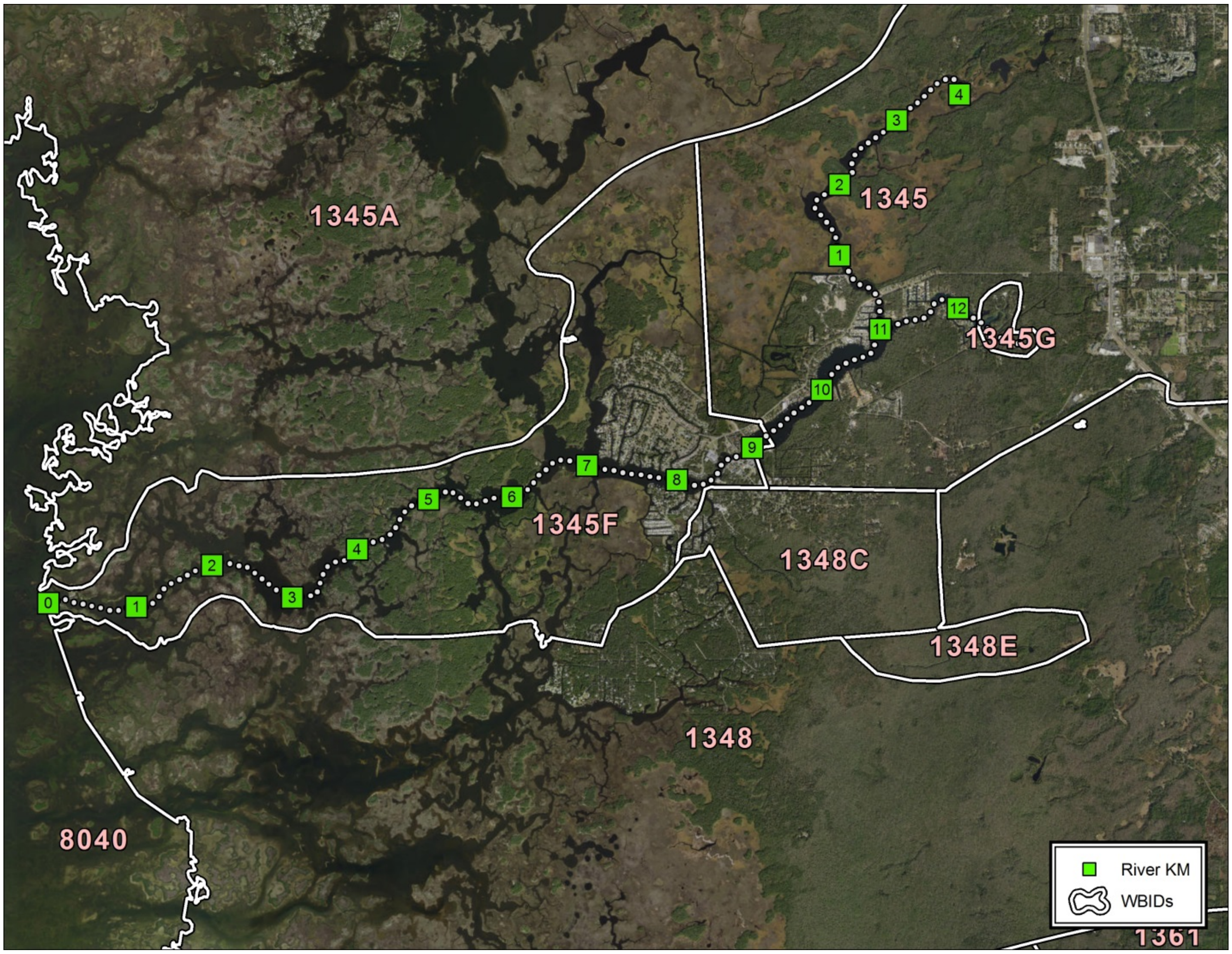


Figure 3-16. Map of Homosassa identifying waterbody identifiers (WBIDs) of relevance within the systems.

A conceptual model was developed to evaluate the effects of flow on the probability of exceeding the site-specific threshold for chlorophyll a. As described in Section 2.0, the pathway includes:

- the development of a conceptual model,
- development of a hypothesis,
- an analytical approach,
- application of the analytical approach, and
- application of the results to evaluate the effects of flow reductions on the response of interest.

The final bullet point is vital with respect to the reevaluation as it requires that the modeling approach be amenable to conducting a series of simulated flow reduction scenarios. Thereby, data used as model inputs must be available for a long term daily timeseries to conduct the simulations.

A conceptual model is presented in Figure 3-17 that illustrates the proposed relationship between spring flows, season and the distribution of chlorophyll a in Homosassa River. The model considers the effects of spring flow along with season as principal drivers of chlorophyll a distribution, and that the effect of flow and season is location dependent. That is, the effect of flow differs depending on the location in the river and season.

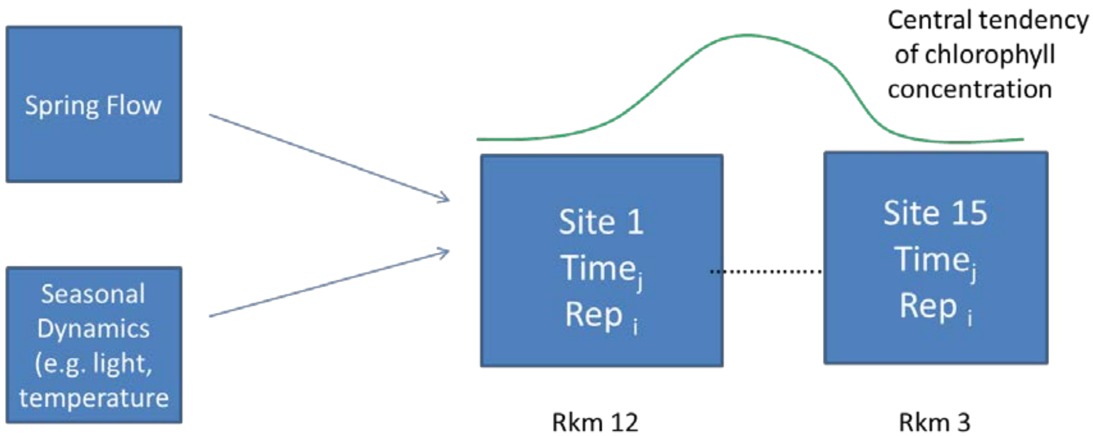


Figure 3-17. Conceptual model of the effects of spring flow and seasonal dynamics on chlorophyll concentrations in the Homosassa River.

A plot of the 3-dimensional locally weighted average observed uncorrected chlorophyll a concentrations as estimated using locally weighted scatter plot smoothing (LOESS) is presented in (Figure 3-18). The curve suggests that the highest chlorophyll a concentrations occur when flows are the lowest and that increasing flows are associated with corresponding decreases in concentration. In addition, the peak of the curve tends to occur between river kilometers 6 and 10 indicating that this section of the river is where the majority of the phytoplankton production tends to occur. There is also some evidence in the plot that when flows are above the median the peak of the chlorophyll distribution is moved downstream.

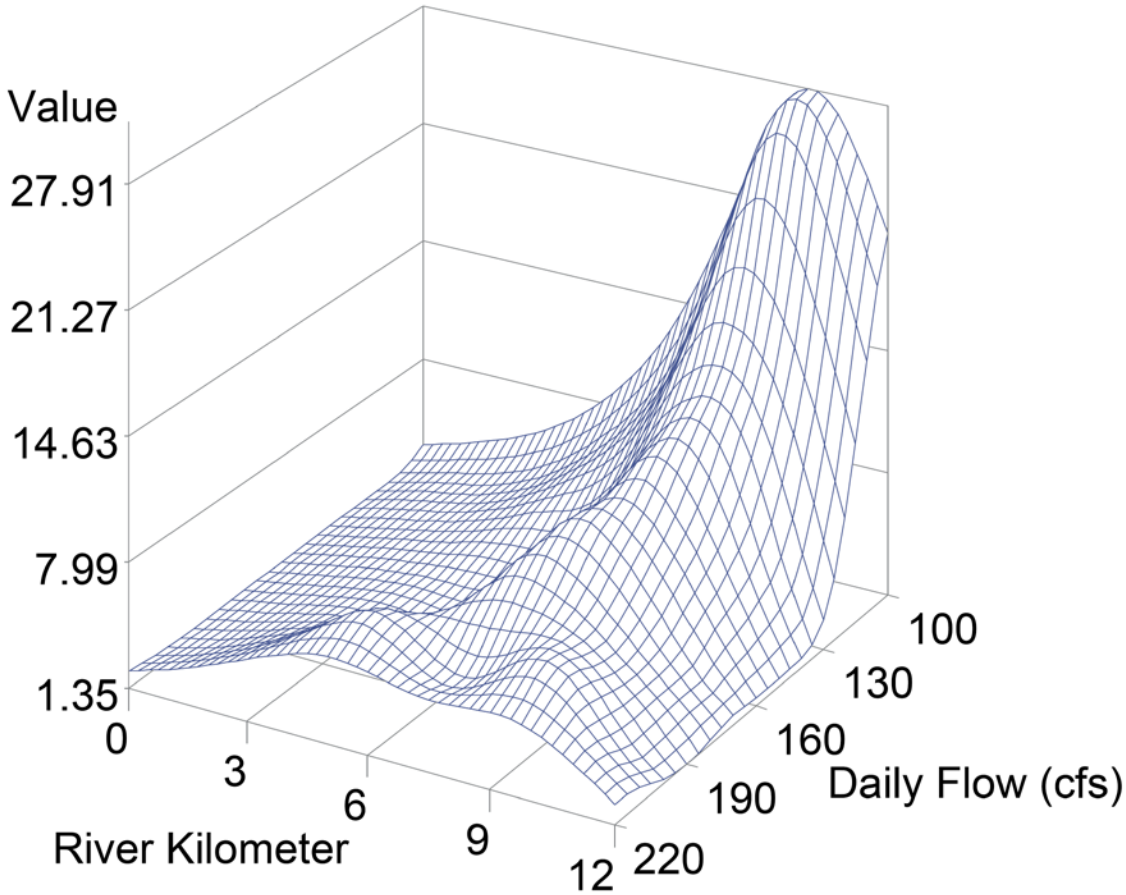


Figure 3-18. **LOESS 3-dimensional smoothed curve of chlorophyll concentrations in the Homosassa River from the UF transect data as a function of location and spring flow using the long term existing condition flow record.**

The curve generally corresponds to the results of an analysis of "water age" by the District using the revised hydrodynamic model. Water age is defined as the estimated time it takes for a particle to move downstream of a particular location in the river. There is a rather dramatic increase in water age in the upper portion of the system as flows decrease. An example using the 3 day average flows (to smooth out the influence of tides) for four flow values (122, 140, 160, and 196 cfs) is provided in Figure 3-19. The increase in water age in the upper portion of the system is especially apparent when flows drop below the median value. For example, the difference between the 160 cfs and 122 cfs curve nearly tripled the water age from ca. 50 hours to ca. 150 hours at Rkm 10.

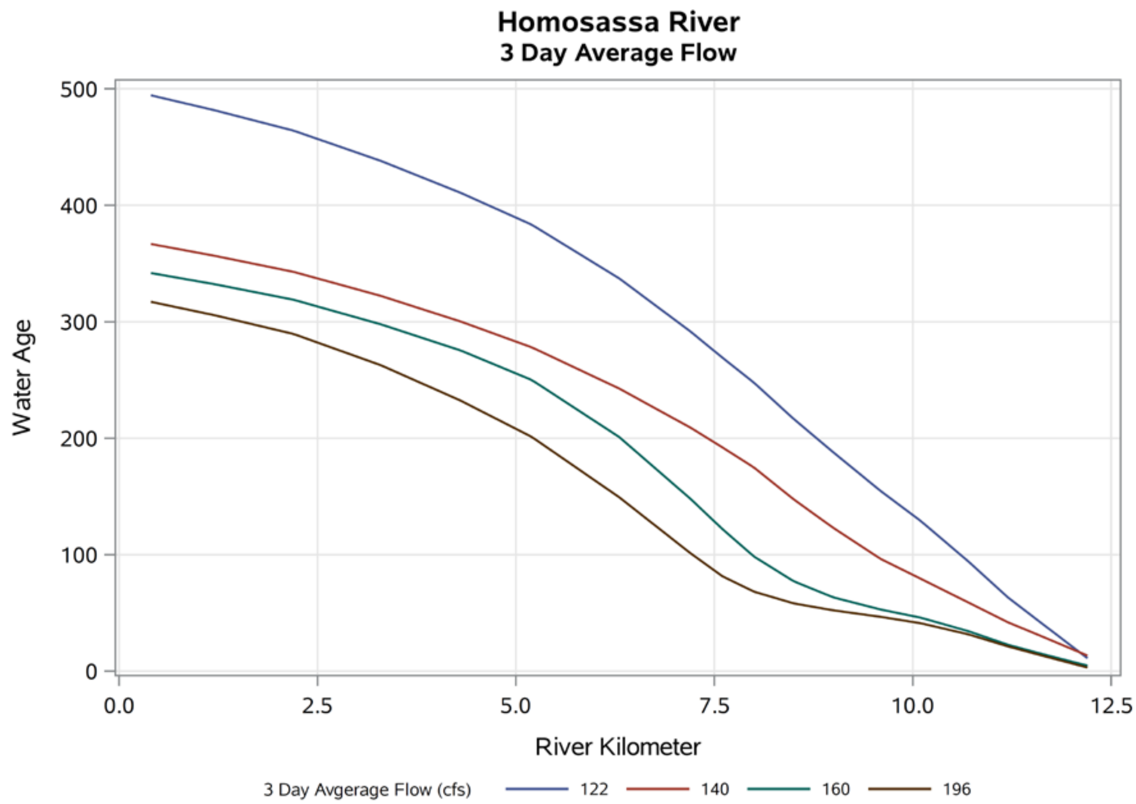


Figure 3-19. Water age (in hours) curves for four different 3 day average flows.

3.3.3 Analytical Approach

The conceptual model was then formulated as a statistical model to test the hypothesis that exceedances of the regulatory chlorophyll threshold were related to spring flow. The general form of the model is expressed by the equation below as a generalized linear mixed effects model predicting the probability of an exceedance of the chlorophyll standard (a binomial response) as a function of flow and season (i.e. quarter) with interaction terms to allow for the effects of flows on chlorophyll to be location and seasonally dependent. The model is similar to a standard logistic regression model in that it is linear (additive) on the logit (log odds) scale but includes random effects components. Flow and river kilometer were treated as continuous variables in the model while quarter is treated as a categorical variable with Quarter 1 (i.e. Winter) being considered as the reference level. Because the UF data were sampled quarterly (February, May, August, and November), quarters were defined as Q1 = Jan-Mar, Q2=Apr-Jun, Q3=Jul-Sep, and Q4=Oct-Dec). A quadratic term for the river kilometer effect was also initially included in an attempt to capture the parabolic curve observed in the empirical data as a function of location in the river. These are the “fixed” effects defining the deterministic component of the model (i.e. the predictive equation).

The "random effects" component of the model allows for specific properties of the sampling design to be incorporated into the analysis in order to appropriately estimate the standard errors associated with the statistical tests used to evaluate significance of the model. This results in what is called "design-based inference" and is important in this analysis to account for the site-specific properties of the sampling locations within the river. Three parameterizations of the random effect component of the statistical model were considered:

Parameterization 1: Random Site Intercepts

Parameterization 2: Random Site and nested Rep within (Site) effect

Parameterization 3: Rep averaged with random site intercepts

The "rep" term refers to the fact that three samples are taken in close proximity to one another at a particular longitudinal location along the river. The equation for the model using Parameterization 1 is given below:

$$E(y) = \log\left(\frac{p_{(y=1)}}{1-p_{(y=1)}}\right) = \beta_0 + \beta_{0s} + \beta_1 * \text{flow} + \beta_2 * \text{rkm}_j + \beta_3 * \text{quarter}_k + \beta_4 * \text{rkm} * \text{flow} + \beta_5 * \text{quarter}_k * \text{flow} + \beta_6 * \text{Rkm}^2$$

Where:

$\text{logit}(p_{ijk})$ = probability of exceedence for each sample

S = Site specific properties of location at rkm $_i$

β_0 = Intercept

β_{0s} = random intercepts for site $N(0, \sigma^2_s)$

β_{1-6} = regression coefficients

The random intercepts for the site term is a "variance component" to allow for the fact that each sampled site in the river has a random but quantifiable difference from the overall effect. The benefit of adding the random effects is that it allows the model to capture a variance component associated with variability in sites when estimating the statistical significance of the fixed effects (Zuur et al 2009) and allows for inference at any location within the modeled portion of the system. The difference between Parameterization 1 and Parameterization 2 is additional term to describe the correlation that exists between the three replicate samples that were taken at the same longitudinal point in the river (although at a different location laterally) on the same date. Parameterization 3 is similar to Parameterization 1 except that the three replicates were first averaged and then the average was used to determine if the value exceeded the site-specific chlorophyll threshold.

The statistical model was implemented using the GLIMMIX procedure in SAS (V9.4: SAS Institute, 2016) using the general principles for model fitting outlined by Zuur et al. (2009) and described as follows. The full fixed effects model was implemented first and the benefit of including the random effects was evaluated using Restricted Maximum Likelihood (REML) and the residual pseudo-likelihood as described in the SAS Stats User's Guide for the GLIMMIX procedure (SAS Institute: STAT User's Guide v14.1: 2016). Once the random effects were established, Maximum Likelihood (ML) methods were used to evaluate the benefit of the fixed effects model terms using the goodness of fit evaluated by changes in likelihood ratio and the Akaike Information Criteria (AICC) statistics. Individual terms were dropped from the model if they did not contribute improvement to the model fit as evaluated based on a reduction in either the log likelihood or AICC. Once the fixed effects were established for the final model, the final model was run and reported using REML.

3.3.4 Analytical Results

The three candidate model parameterizations for the random effects were considered using the full model fixed effects. Because the random effects parameterization can change the fixed effects estimates, the fixed effects parameter estimates were evaluated for all models as well. Parameterization 3 would not converge when the quadratic term was included in the model and so the quadratic term was subsequently dropped from the modeling effort for the Homosassa River. Since interaction terms are present in the models this can alter the results of the significance tests of the main effects if the interaction results in a cross over effect. For example, in the models below, the main effect for flow is highly statistically significant overall (Table 3-7); however, the main effect of flow was not significant for some parameterizations in the solutions table (Table 3-8). This can happen if the interaction term results in no effect at for example, the mean for a continuous variable (e.g. river kilometer). Since the interaction terms were highly significant, the main effects were retained in the model. The inference from the results suggests that the effects of flow change depending on location in the river and season.

The random intercepts term for site significantly improved the model fit over the fixed effect model based on the likelihood ratio test results (LRT under Fit Statistics: Table 3-7). The dispersion statistic is also used as a test of model goodness of fit. An over-dispersed model can lead to improper estimates of the standard errors and inflated type I error while a dispersion parameter below 1 is generally conservative with respect to the statistical significance of the parameter estimates (Burnham, K. P. and D. R. Anderson. 2002).

Table 3-7. Results of Type 1 tests for fixed effects for the three mixed effects models evaluated to predict chlorophyll exceedances in the Homosassa River.

	Parameterization 1	Parameterization 2	Parameterization 3
Fixed Effects	P Value	P Value	P Value
Flow	<.0001	<.0001	0.0098
Rkm	0.0002	0.0004	0.0002
Flow*Rkm	<.0001	0.0001	0.0009
Quarter	<.0001	<.0001	<.0001
Flow*Quarter	<.0001	0.0076	0.0079
Fit Statistics			
Random Effects : LRT	<0.001	<0.001	<0.001
Dispersion (Chisq / DF)	1.05	0.38	0.58

Table 3-8.	**Solutions table for fixed effects for the three mixed effects models evaluated to predict chlorophyll exceedances in the Homosassa River.**					
	Parameterization 1		Parameterization 2		Parameterization 3	
	Estimate	P Value	Estimate	P Value	Estimate	P Value
Fixed Effects						
Intercept	-4.6969	0.2194	-14.4284	0.0001	8.1818	0.4476
Flow	-0.02967	0.2467	0.05498	0.0012	-0.1278	0.1132
River Kilometer	1.5737	<.0001	1.5946	<.0001	1.4822	<.0001
Flow*River Kilometer	-0.00713	<.0001	-0.0071	0.0002	-0.00634	0.0009
Quarter 2	-4.8915	0.1275	7.5349	0.0413	-16.5928	0.107
Quarter 3	-4.7541	0.1349	5.1722	0.0061	-16.9231	0.0994
Quarter 4	-9.2765	0.0034	5.5805	0.0009	-21.7636	0.0341
Quarter 1	0		0		0	
Flow*Quarter 2	0.05718	0.0186	-0.07351	0.0062	0.1453	0.0688
Flow*Quarter 3	0.06683	0.0054	-0.03116	0.0181	0.16	0.0445
Flow*Quarter 4	0.08395	0.0005	-0.02187	0.0475	0.178	0.0253
Flow*Quarter 1	0		0	0.0001	0	

The area under the receiver operator curve (ROC: Hosmer and Lemeshow 2000) is another metric used to evaluate the model goodness of fit. The ROC curves plot the sensitivity (defined as correctly predicting an exceedance when one is observed in the empirical data) against 1-specificity (defined as correctly predicting a non exceedance when a non exceedance is observed in the empirical data). A ROC curve that is high into the upper left hand corner of the plot is most preferred because it has both high sensitivity and high specificity. Parameterization 3 had the largest area under the ROC curve (ROC=0.85: Figure 3-20). Based on the fact that the dispersion statistic is less than 1 and had the highest ROC value, in addition to results of internal discussions with the project team, Parameterization 3, the “rep averaged” model was considered the most appropriate representation of the system under study for evaluating the effects of flows on the probability of a chlorophyll exceedance.

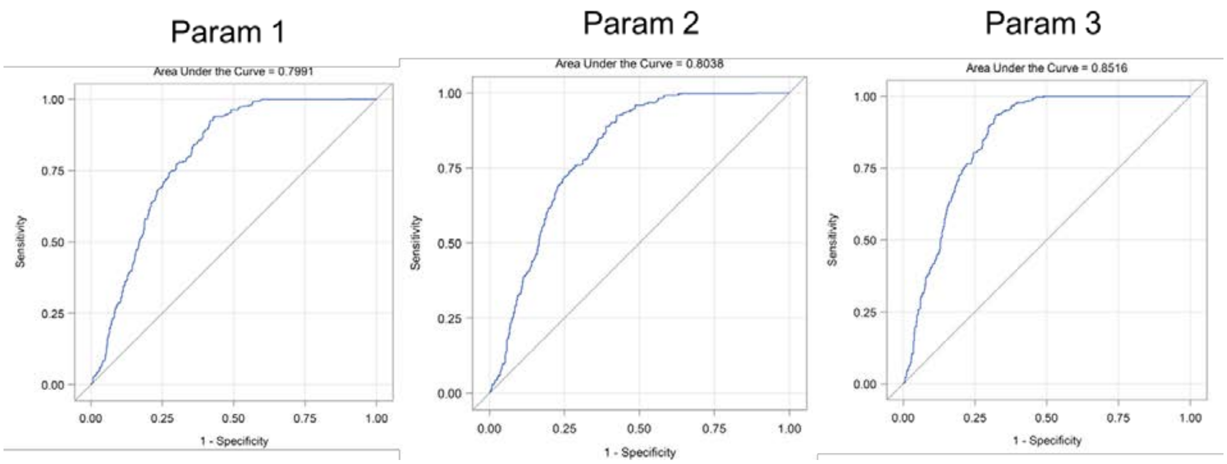


Figure 3-20. Receiver operator curves based on the fixed effects for the three generalized mixed effects model parameterizations considered.

Once the parameterization for the random effects was established, the final fixed effects were evaluated by sequentially eliminating effects from the full model, beginning with the interaction terms, and evaluating the effects on AICC. The full model, which included all main effects and interactions was the best of the candidate models for describing the fixed effects (Table 3-9). Based on the results of AICC suggesting the best fit included all parameters in the model, all terms were kept in the model. To confirm that the main effect of flow should be retained despite being reported as not significant in the solutions table, the AICC were compared for the full model and one without the flow main effect. The results suggest that the full model (AICC = 1612.58) is a better fit than the model without the main flow effect (AICC 1630.63).

Table 3-9. Comparison of Akaike Information Criteria for nested models with various fixed effects. For AICC smaller numbers represent improved model fit. Models fit using maximum likelihood.

Model Parameterization	AICC
Flow rkm quarter	1678.07
Flow rkm Flow*rkm quarter	1641.38
Flow rkm Flow*rkm quarter Flow*quarter	1612.58

Once the final model was selected, diagnostic plots and prediction curves were generated to evaluate the model fit across a range of conditions. A summary plot of the predicted probability of occurrence as an effect of season and river kilometer is provided in Figure 3-21A. The plots present estimates of one effect while holding other effects at a constant value. For example, in Figure 3-21A, flow is held at its mean value. The predictions suggest that In the first quarter, the exceedance probability is low throughout the river while in Q3, higher probabilities of exceedance extend farthest downstream. Quarters 2 and 4 had simlar prediction curves and were not significantly different from one another when holding other parameters at a constant value as presented in the diffogram (Figure 3-21B). A diffogram provides multiple comparison test adjusted differences for the least squares mean estimates for the Quarter effect.

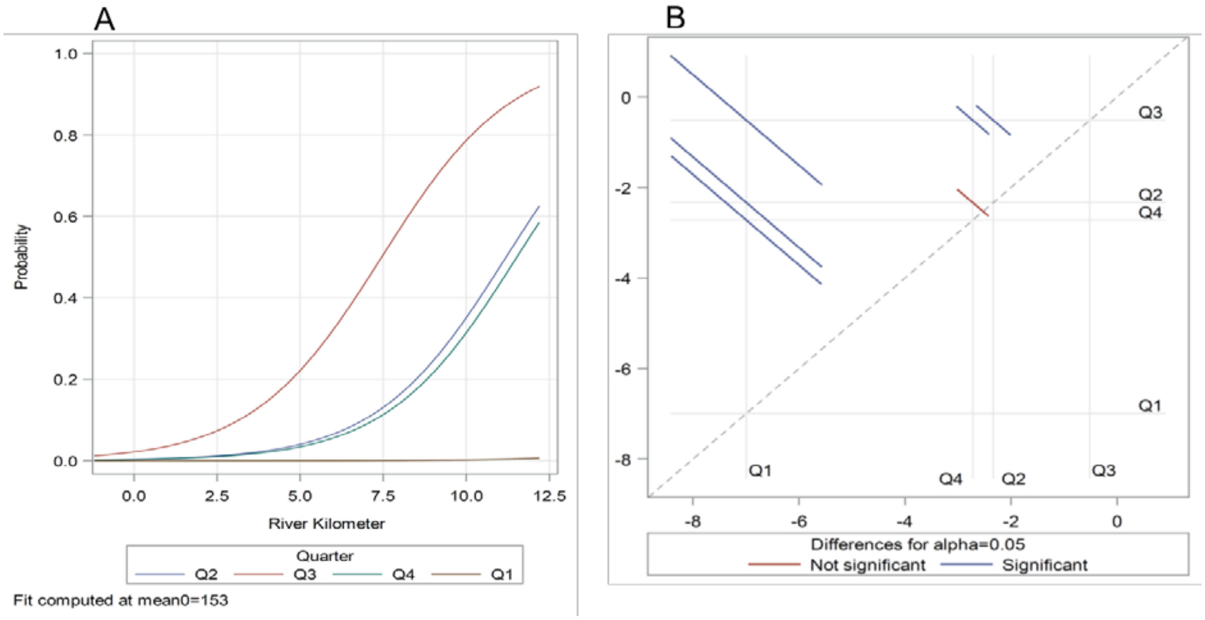


Figure 3-21. Predicted probabilities for each quarter at a fixed daily flow of 153cfs (left) and a diffogram of the multiple comparisons test to assess differences between quarters(right).

The predicted probability of exceedance by quarter can also be plotted for various other potential flow values. For example, in Figure 3-22, the prediction curves are generated for four flow values between the near minimum and maximum observed flows. Two principal results are observed from these plots. First, in Quarter 1, predicted probabilities are low overall except during the lowest flows when the model predicts the probabilities to increase rather dramatically between 150 and 120 cfs. Second, the separation in curves for Q2 and Q4 becomes more apparent as flows decrease from 150 to 120 cfs.

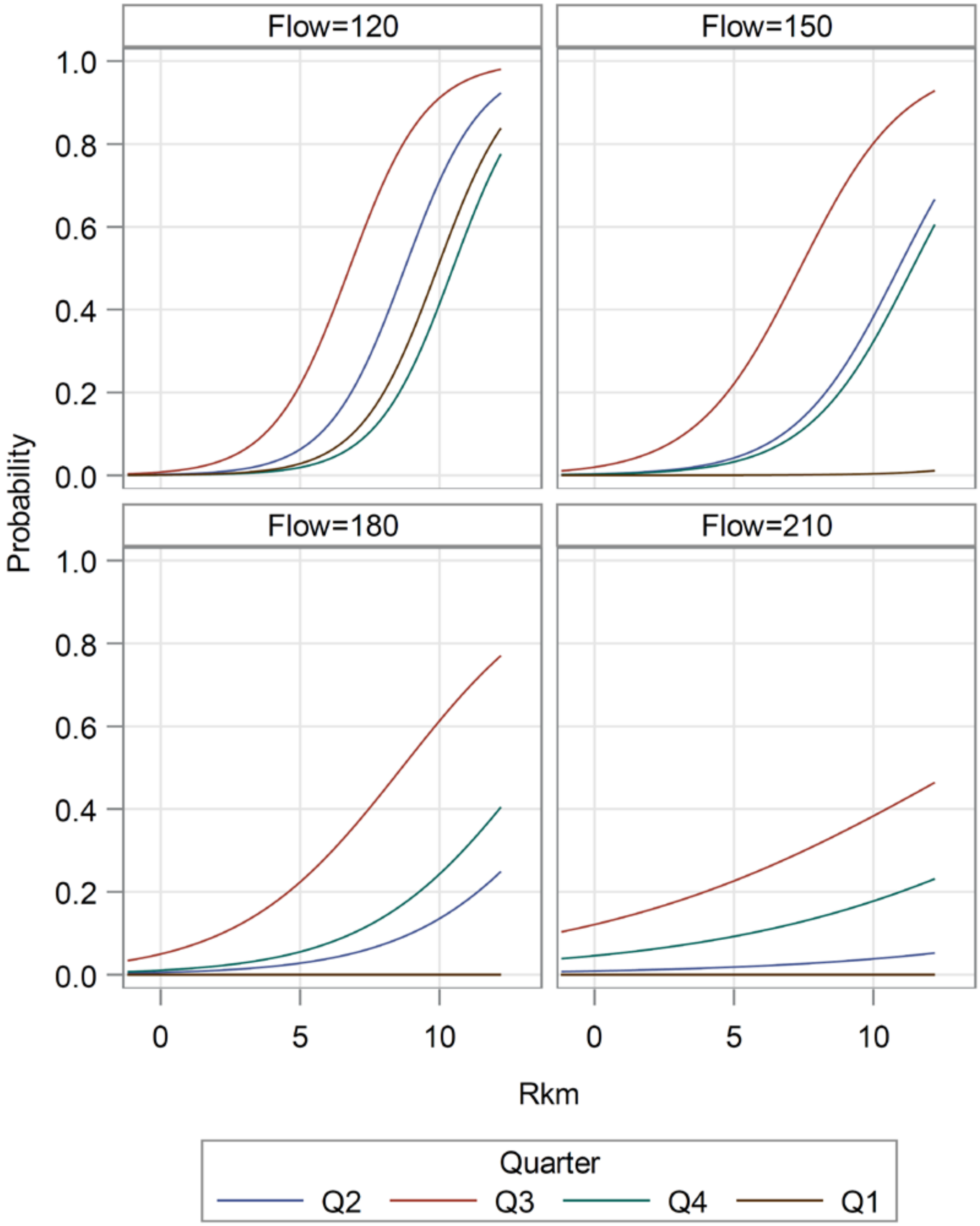


Figure 3-22. Predicted probability of exceedance as a function of river kilometer by quarter for four fixed flow values in the Homosassa River.

To evaluate the effect of the interaction terms, the relationship between flow and the predicted probability of exceedance by quarter is plotted at four different locations in the river (Rkm 0, 3, 6, 9) in Figure 3-23. At the most upstream end of the domain, increasing flows resulted in a decreased probability of occurrence for all quarters, while at the downstream end of the model domain, increased flows may result in additional supply of nutrients to the system and an increased probability of exceedance with increasing flows. This is the manifestation of the interaction term with flow and river kilometer in the model.

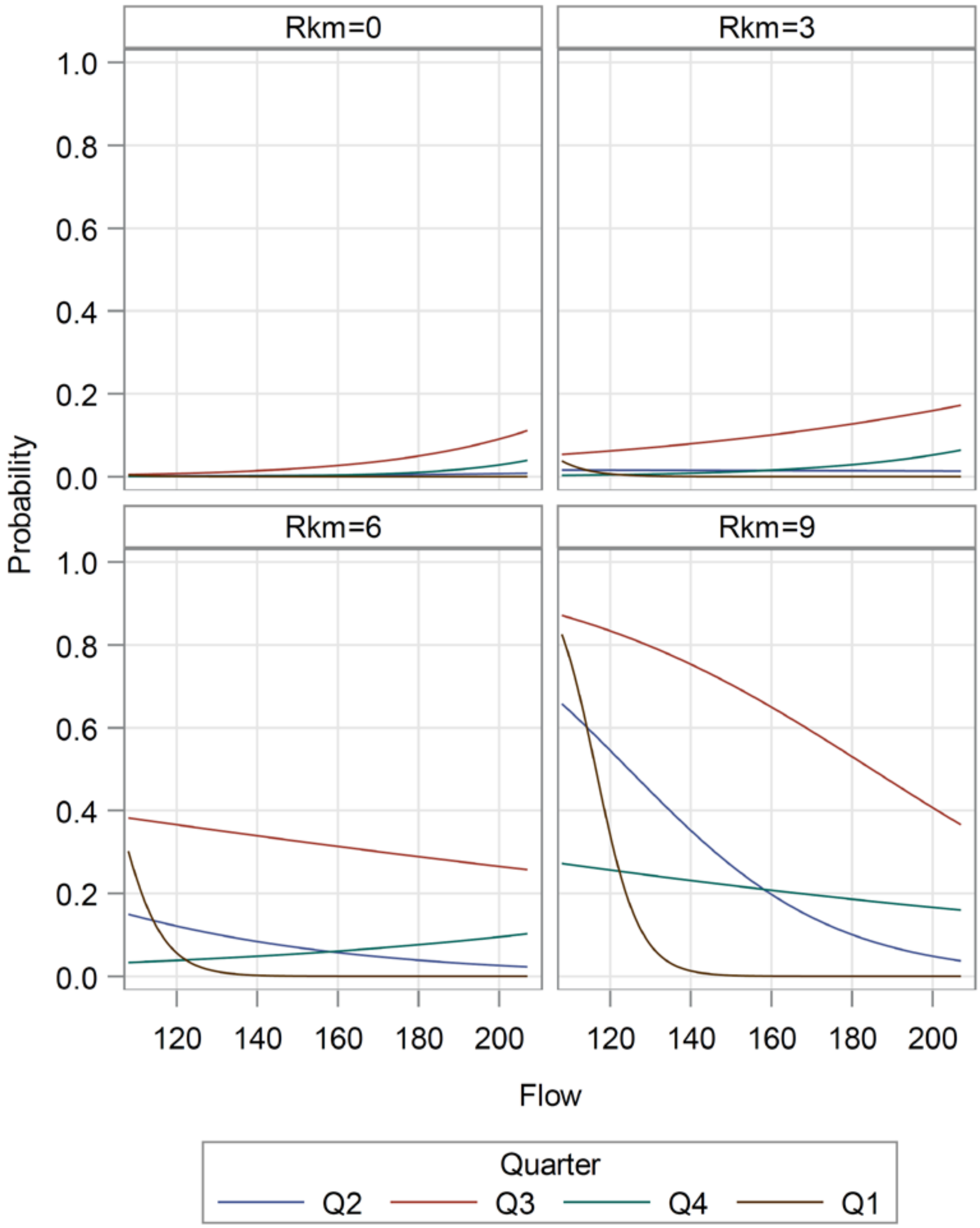


Figure 3-23. Predicted probability curves as a function of flow by quarter at 4 different locations in the system.

This model was subsequently used to evaluate the effects of flow reductions on changes in chlorophyll a exceedance probabilities for the upper portion of the river. The results of that evaluation are described in Chapter 4.

3.4 ESTUARY

The goal of the estuary analysis was to assess relationships between flows and water quality constituents of interest for sites located outside the hydrodynamic model domain. Sites for the mainstem of the river were described by the analysis above. The "Estuary" sites include four

Project COAST (P529) sampling stations, as well as two transects from the previously completed UF 5 Rivers Study which are bounded by the blue rectangle in Figure 3-24. Three Coast sites (1,9 and 10) were considered too far removed from the river mouth to have a direct influence.

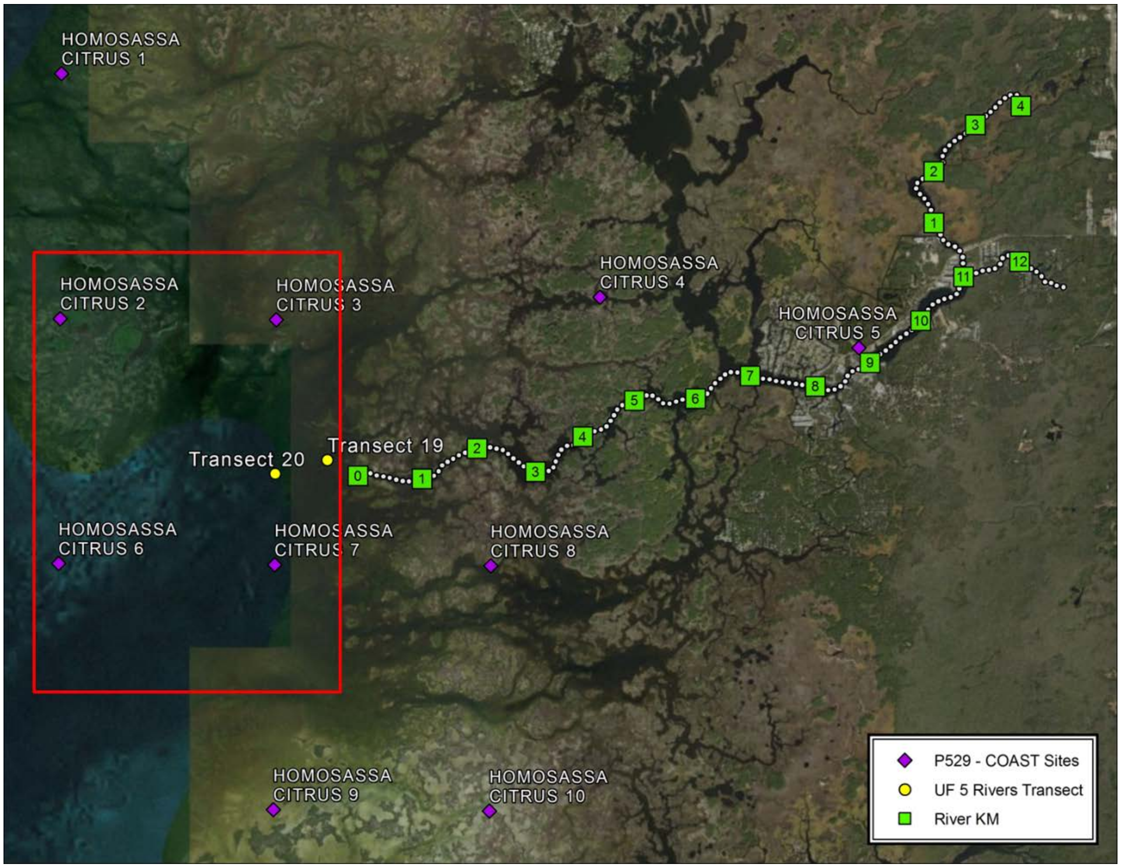


Figure 3-24. Sampling areas in the Homosassa River estuary outside of the hydrodynamic model domain (highlighted by red rectangle).

The same regression process used for the Springs data was applied to the Estuary sites. Outliers indicated by robust regression and data points with qualifiers indicating unreliable data were removed from the analyses. The parameters listed in Table 3-10 were tested for significant relationships with flow.

Table 3-10. List of water quality constituents evaluated for linear relationships with flow.	
Parameter	Sampling Program(s)
Alkalinity (Total)	UF 5 Rivers
Ammonia	UF 5 Rivers
Chlorophyll a (corrected)	UF 5 Rivers
Chlorophyll a (uncorrected)	UF 5 Rivers
Chlorophyll a (Total)	P-529
Chlorophyll (Total)	P-529
Color	UF 5 Rivers, P-529
Dissolved Oxygen	UF 5 Rivers, P-529
Light Attenuation Coefficient	P-529
Nitrogen – Total	UF 5 Rivers, P-529
Nitrate	UF 5 Rivers
pH	UF 5 Rivers, P-529
Phosphorus – Total	UF 5 Rivers, P-529
Salinity	UF 5 Rivers, P-529
Secchi-vertical	P-529
Specific Conductivity	UF 5 Rivers
SRP	UF 5 Rivers
Temperature	UF 5 Rivers, P-529

After application of the linear regression acceptance criteria adopted for this project, salinity was the principal water quality constituent affected by springs flows (Figure 3-25). The two UF transects did not meet the criteria for inclusion (R^2 =0.25) but are presented here to illustrate that the trend is the same as the other station that had more data. The list of constituents that met the criteria is provided in Table 3-11. Other than salinity, dissolved oxygen (water column average) data for the UF transects was the only constituent with a predictive relationship with flows, where increased flows increased DO concentrations; this likely reflects the fact that higher flows during winter are correlated with cooler temperatures which allow water to hold more oxygen. Application of the salinity regressions suggest that for each unit change in the 3 day flow, salinity would change by a range from between 0.11 psu (at Homosassa Citrus 2) to 0.13 psu (at Homosassa Citrus 3). If these regressions were used to evaluate the effects of a prescriptive reduction in flow using the median flow of 152 cfs, a 15 % reduction would result in a 2.6-3.0 psu increase in salinity at the examined estuary sites.

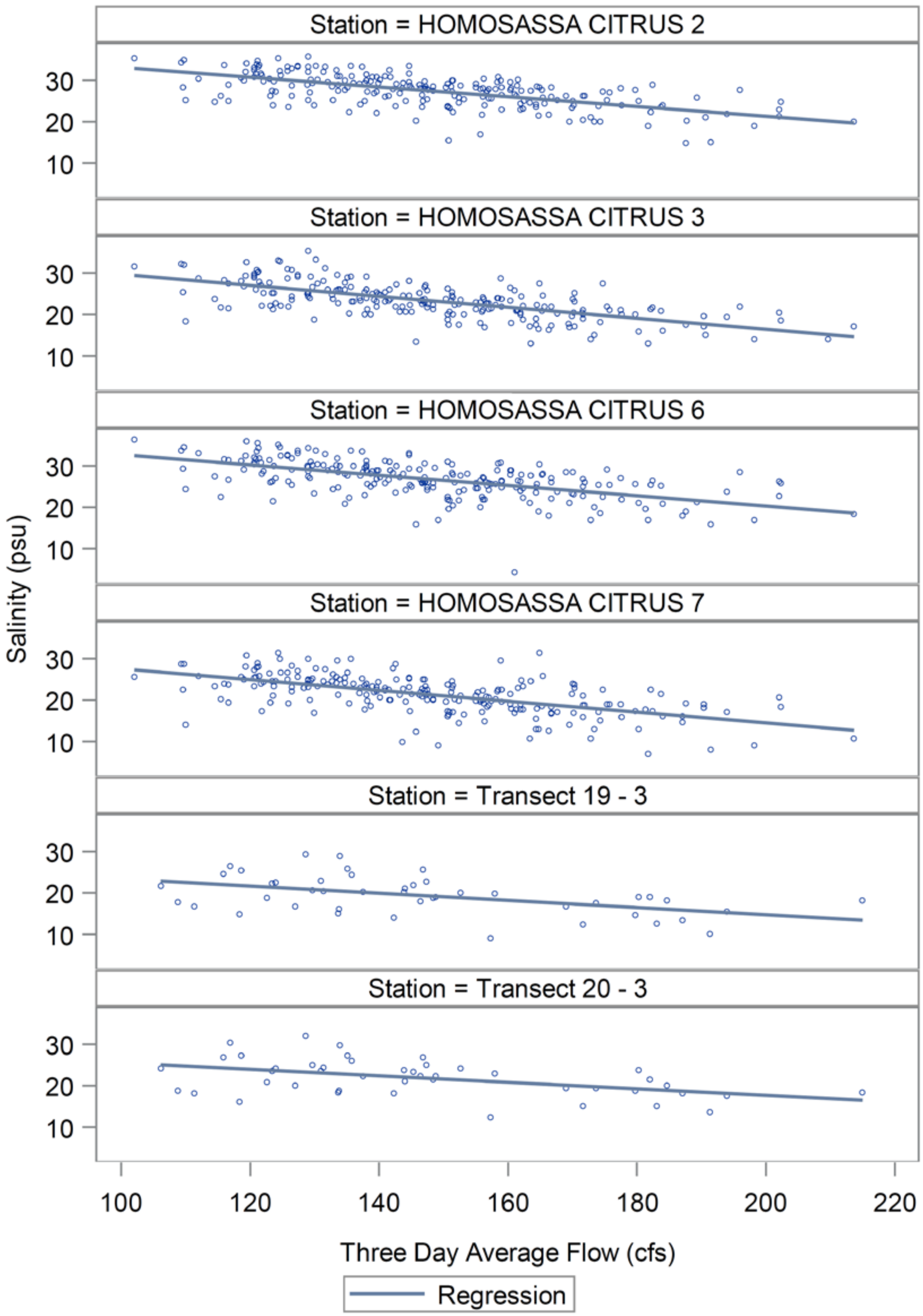


Figure 3-25. **Regression relationships of salinity at the Homosassa River estuary stations and 3-day average flow.**

Table 3-11. Significant regression results for estuary data.						
Site Name	Parameter	Intercept	Slope	DF	R Square	P Value
HOMOSASSA CITRUS 2	Salinity (Total)	44.42432	-0.114	217	0.40	<0.001
HOMOSASSA CITRUS 2	Secchi-vertical (Total)	2.439325	-0.011	18	0.41	0.0061
HOMOSASSA CITRUS 3	Salinity (Total)	42.91336	-0.132	218	0.44	<0.001
HOMOSASSA CITRUS 6	Salinity (Total)	44.78269	-0.121	219	0.37	<0.001
HOMOSASSA CITRUS 7	Salinity (Total)	40.6104	-0.130	220	0.36	<0.001
Transect 19	DO_mgL	-0.81787	0.0507	43	0.47	<0.001
Transect 20	DO_mgL	0.278693	0.0445	43	0.36	<0.001

In a study by Yobbi and Knochenmus (1988), the location of 25-ppt salinity isohaline in the Homosassa River had a range in movement that was more than three times as great as the range in movement of the upstream extent of the zone of saltwater mixing. The 25-ppt was generally found between six miles outside the mouth to about 1 mile upstream of the mouth. The authors report that the 18-ppt salinity isohaline is generally found between 2 miles outside the river mouth to about 4 miles upstream of the mouth. Given that this area is so far removed from the springs flows and is affected by direct rainfall, surface flows from coastal zone runoff, and wetland storage, the regression results presented above offer little direct utility to evaluate the effects of flow reductions on estuarine salinity west of the river but provide evidence that salinity in the estuary is correlated with spring flows from the Homosassa River. Plots for all stations and parameters analyzed for the Estuary resource of concern are provided in Appendix E.

3.5 Continuous Recorders

There are three continuous recorder sites in the Homosassa (Figure 3-26). The most upstream site collected data on salinity, temperature, dissolved oxygen, pH and nitrite+nitrate, while the downstream locations (near Mud River and Shell Island) include several additional fluorescence-based estimates including chlorophyll, fluorescence dissolved organic matter (FDOM), light spectra, nitrite+nitrate (NO23), and turbidity. These latter parameters are of particular interest since the salinity and temperature parameters are modeled by the hydrodynamic model developed separately for reevaluation of the minimum flows.

Exploratory data analysis consisted of evaluating the relative contribution of within- and between-day variability on the distribution of data available within each quarter for the period of record between January 207 and March of 2018. Much of these data are still provisional and therefore the results are meant only for exploratory analysis. The coefficient of variation (i.e. CV= the standard deviation divided by the mean) was used to quantify the variability around the expected value. For the within-day variation, the result was a distribution of CV values. For the between-day variability, the average value for each date was calculated and then the CV of the daily average values was calculated resulting in a single CV value to represent the between-day variation. These results were then overlaid to evaluate the relative difference between the within- and between-day variability.

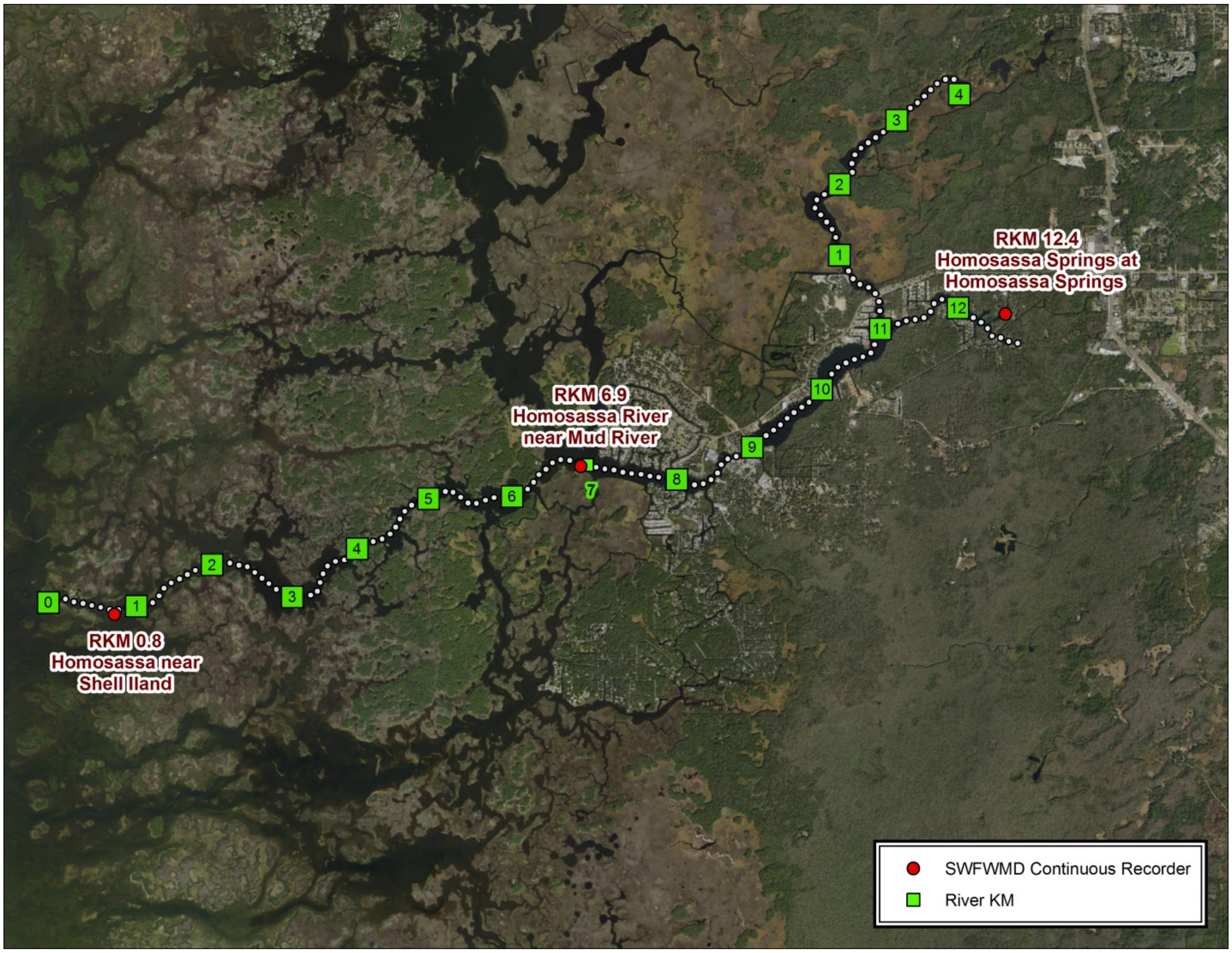
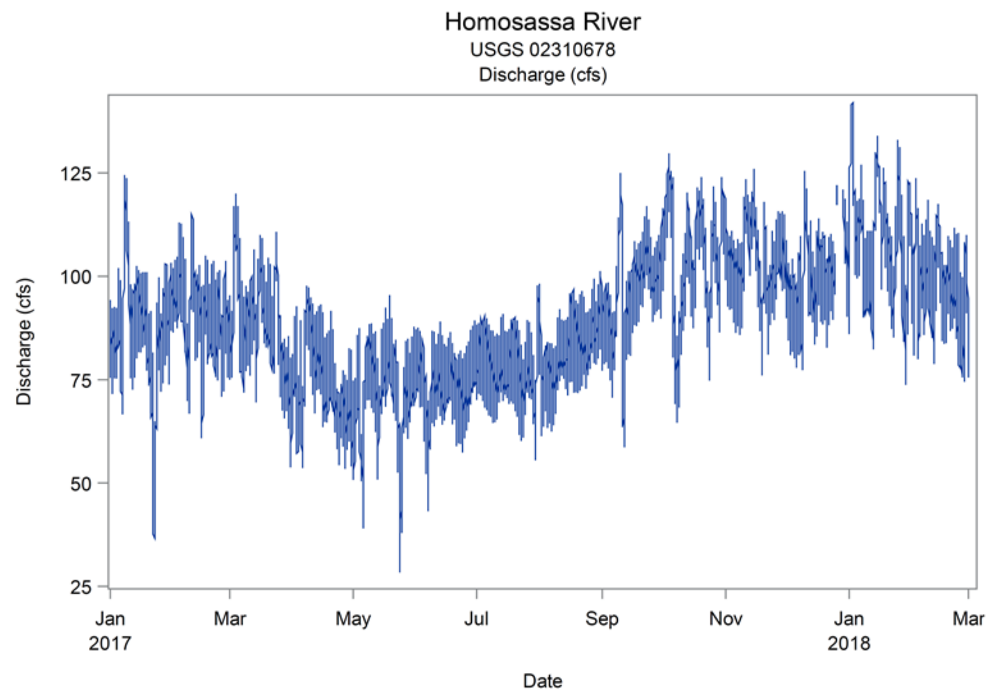


Figure 3-26. Location of continuous recorder gages in the Homosassa River.

An example application of this approach is provided for the USGS gage at Homosassa Springs that includes discharge measurements from the main spring as well as continuous NO23 data. These are 15-minute data that were averaged by hour. A plot of the timeseries for discharge and nitrite+nitrate is provided in Figure 3-27. Missing data are evident in both timeseries but more prevalent in the NO23 data. The CV plots are provided in Figure 3-28 and suggest that between-day variability may be substantial relative to the within-day variability in a quarter for both flow and NO23. The CV of the daily discharge was consistently above the within-day CV in all quarters, suggesting that seasonal influence tends to be a greater source of variability at this site than the effects of tidal action. The NO23 variations are extremely small (i.e. typically standard deviation is less than 3% of the mean) for both within- and between-day measurements. It should be noted that the within-day CV is calculated based on the standard deviation of 24 observations while the between-day variability is calculated from 90 daily observations and, as such, the standard deviation for the within-day CVs may not represent and asymptotic value in all cases.



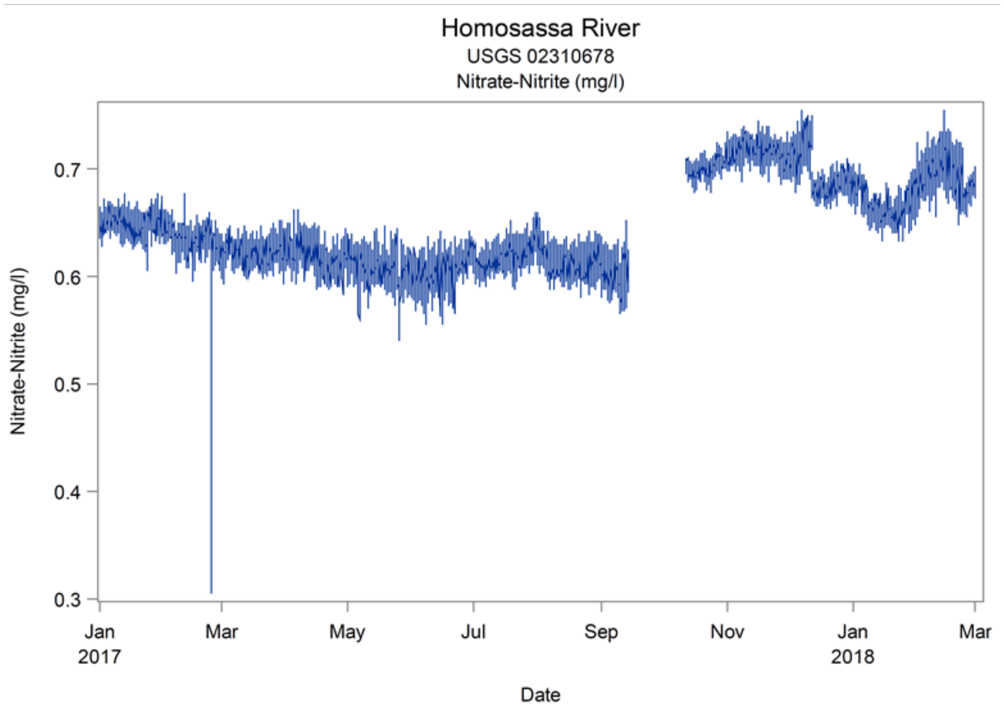


Figure 3-27. Timeseries of Discharge (top) and nitrite+nitrate (bottom) from the continuous recorder gage at Homosassa Springs (USGS 02310678).

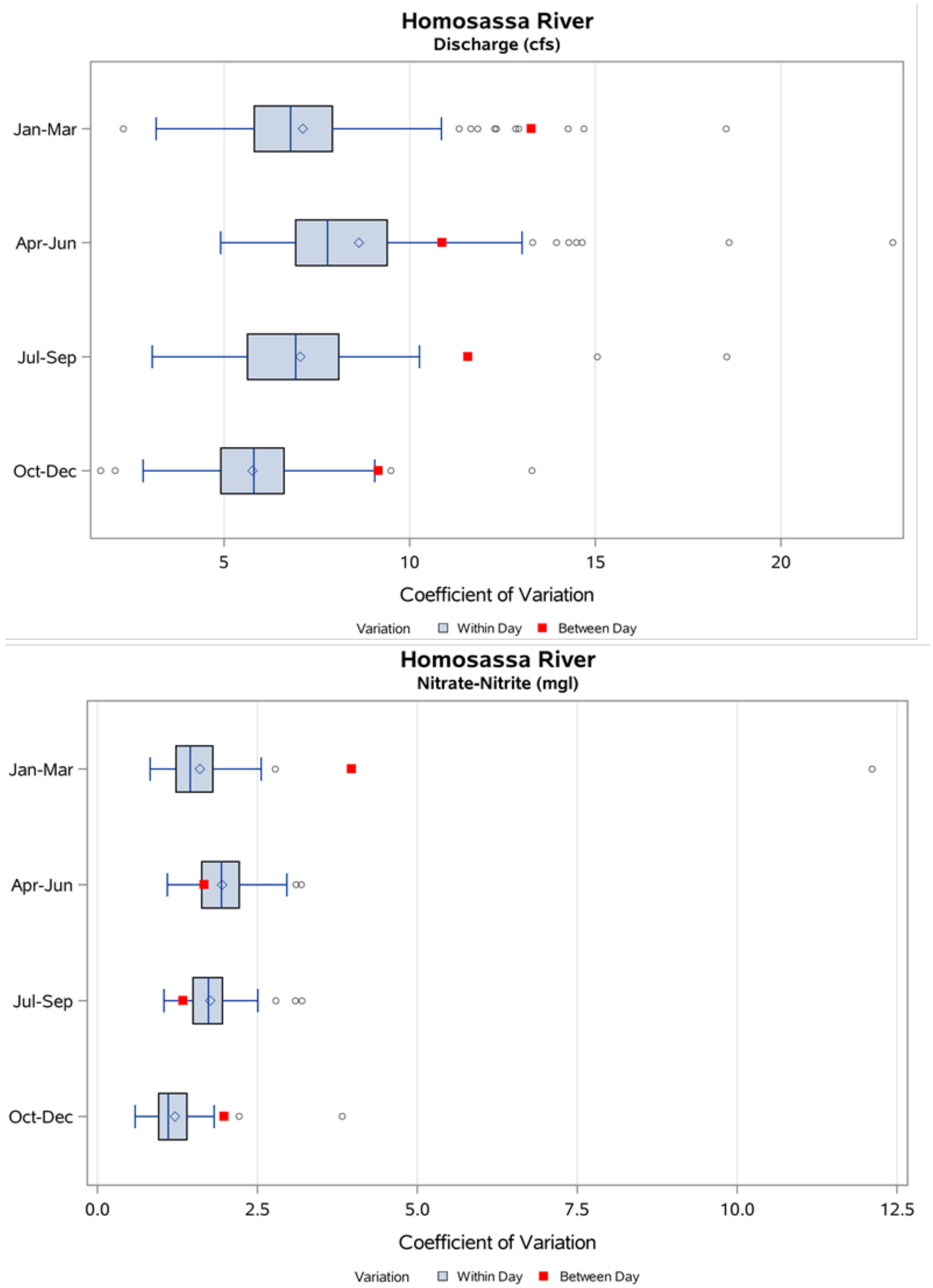


Figure 3-28. Coefficient of variation plots for discharge (top) and nitrite+nitrate (bottom) from the continuous recorder gage at Homosassa Springs (USGS 02310678).

A scatter plot of the relationship between discharge and NO23 for this gage is provided in Figure 3-29 and suggests little direct linear correspondence between flows and NO23 but two distinct clouds of data points with higher flows tending to have higher NO23 concentrations and a mean centered around 0.62 for lower flows and near 0.7 for higher flows.

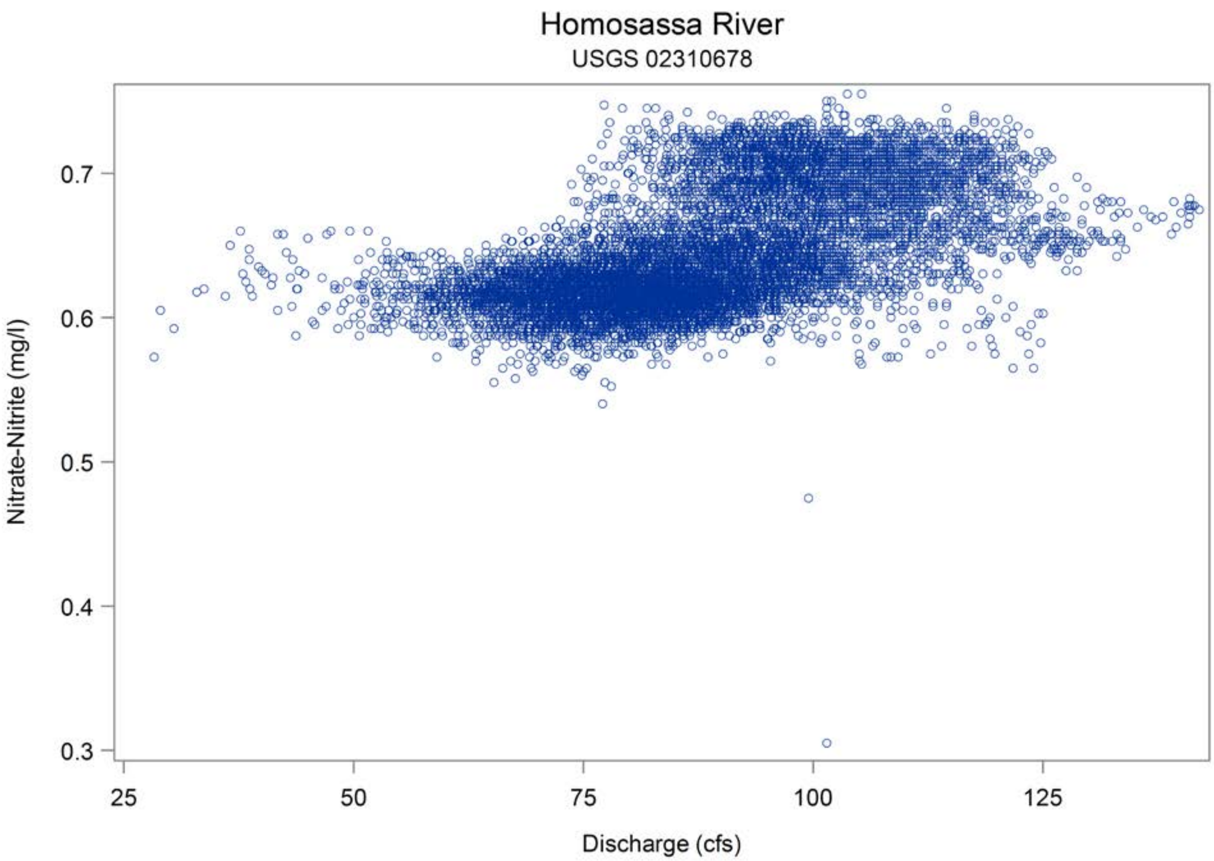


Figure 3-29. Relationship between discharge and NO23 from the continuous recorder gage at Homosassa Springs (USGS 02310678).

The hourly distribution of NO23 is also quite consistent with no tendency for within day fluctuation (Figure 3-30).

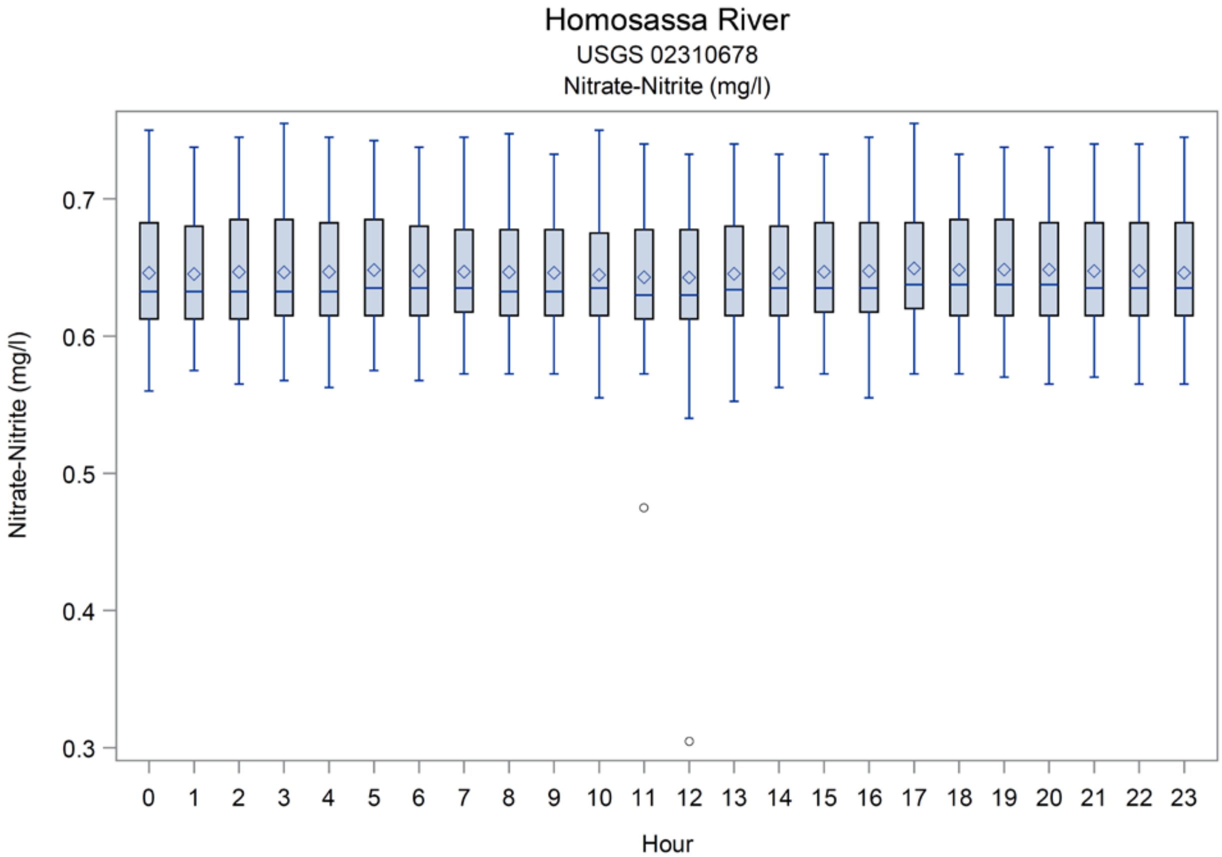


Figure 3-30. **Distribution of hourly nitrite+nitrate concentrations (mg/l) from the continuous recorder gage at Homosassa Springs (USGS 02310678).**

The two downstream locations included more parameters but also more missing data. Timeseries for NO23 at the two downstream continuous recorders is provided in Figure 3-31and suggest considerable seasonal differences when data are available. The CV plots (Figure 3-32) confirm that the between-day CV tends to be larger than the within-day distribution of CVs. Note that the x-axis scales are different in these plots as well.

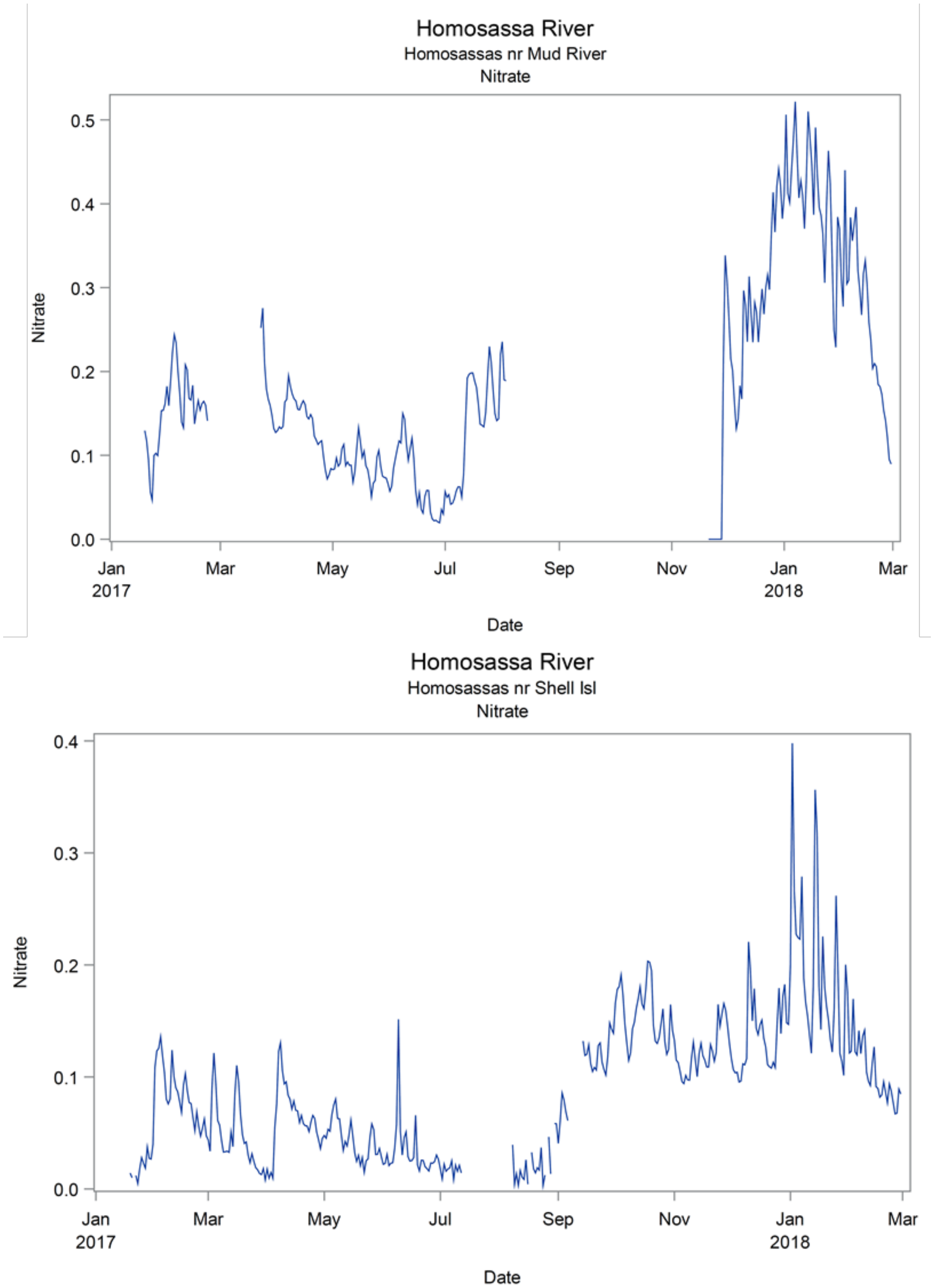
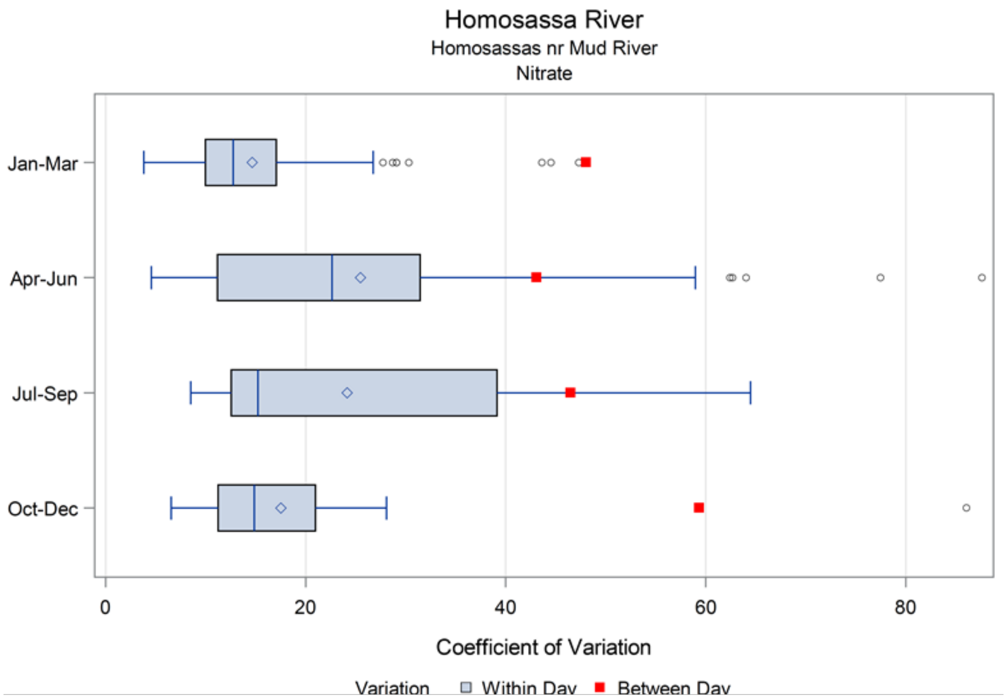


Figure 3-31. **Timeseries plots for nitrite+nitrate at the two downstream continuous recorder sites, near Mud River (Top) and Shell Island (Bottom).**



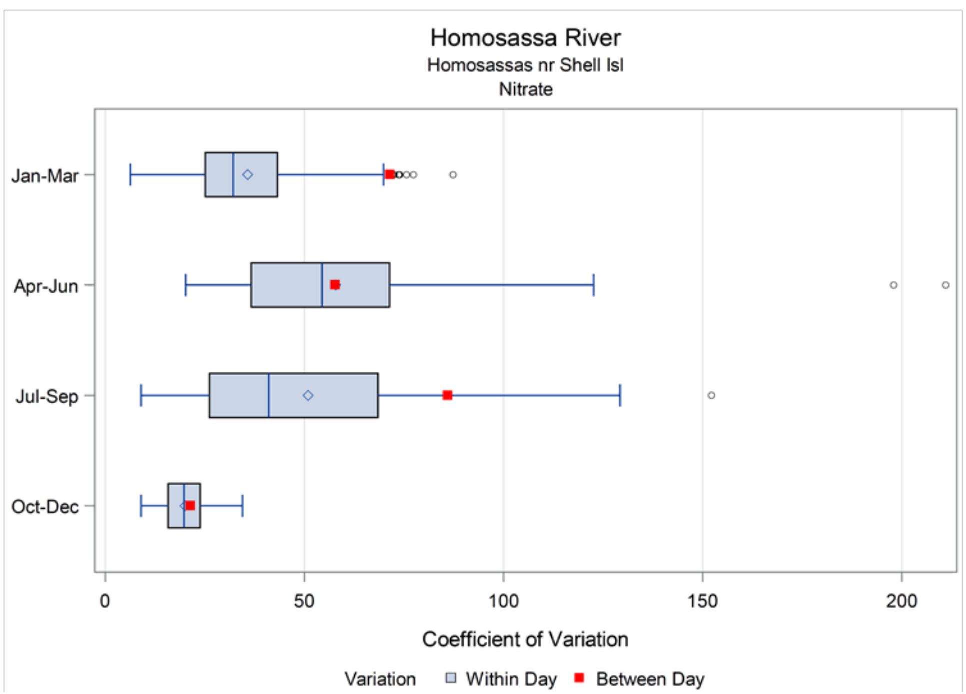


Figure 3-32. Coefficient of variation plots for nitrate-nitrate for the two downstream continuous recorder sites, near Mud River (Top) and Shell Island (Bottom).

Plots for all constituents are provided in Appendix F. Part of the work effort also included spectral decomposition analysis to identify principal dominant frequencies in the continuous recorder data. Spectral decomposition analysis does not allow for missing values which hampers the ability to evaluate the data for dominant frequencies. However, an example of the analysis was performed using short segment of data for dissolved oxygen (DO) at the near Mud River site to investigate the ability of the spectral decomposition approach to detect short term seasonal signals in the continuous recorder data. The data were hourly DO measurements in mg/l. The analysis cannot include missing values so we used the timeseries between January and September of 2017. DO measurements over that period ranged from 1.5 mg/l to 11.1 mg/l. Spectral analysis suggested the first dominant frequency was 24 indicating a diurnal signal (a frequency = the number of observations required to complete a cycle); this is termed “seasonal” irrespective of the frequency so in this case “season” is a day. Defining the dominant frequency for decomposition yields Figure 3-33 that includes the observed data (top); the 24 hour frequency (second); the de-seasonalized trend (third) , and the residuals. The time axis is displayed as the number of the cycles, so in this case, days.

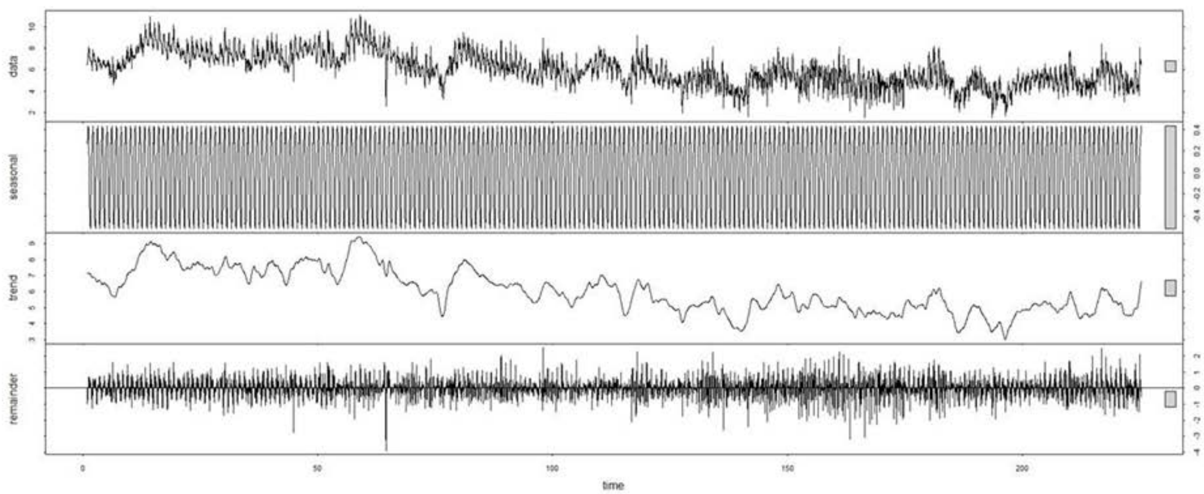


Figure 3-33. Seasonal decomposition of dissolved oxygen timeseries including raw data (top), seasonal cycle identified (second from top), the de-seasonalized timeseries trend, (second from bottom), and the residual (bottom).

To discover an additional frequency in the data we performed spectral analysis on the residuals resulting from the first analysis. This yielded a 12-hour frequency indicating the potential of a tidal signal (plot not shown). These outcomes make sense for dissolved oxygen dynamics in tidal systems including a diurnal component associated with production and respiration and a tidal component associated with mixing of fresh and salt waters. It seemed possible that lunar cycle might also have an effect though no additional dominant frequencies were identified by spectral decomposition. There is a way to specify multiple frequencies into a decomposition method. It is much more complex and has a number of complex embedded functions that perform ARIMA modeling on residuals and box cox transformations, all automatically. We used this method and specified tidal (12-hour), diurnal (24-hour), and lunar (672-hour) frequencies. The results are provided in Figure 3-34. The “observed” plot (top) is simply the raw data. The “level” and “slope” plots below that present the step changes between observations from one time step to another. The three “seasonal” signals from top to bottom are 12, 24, and 672 hour frequencies. The time axis in this plot is the largest cycle, so the first 112 days though the first cycle isn’t plotted.

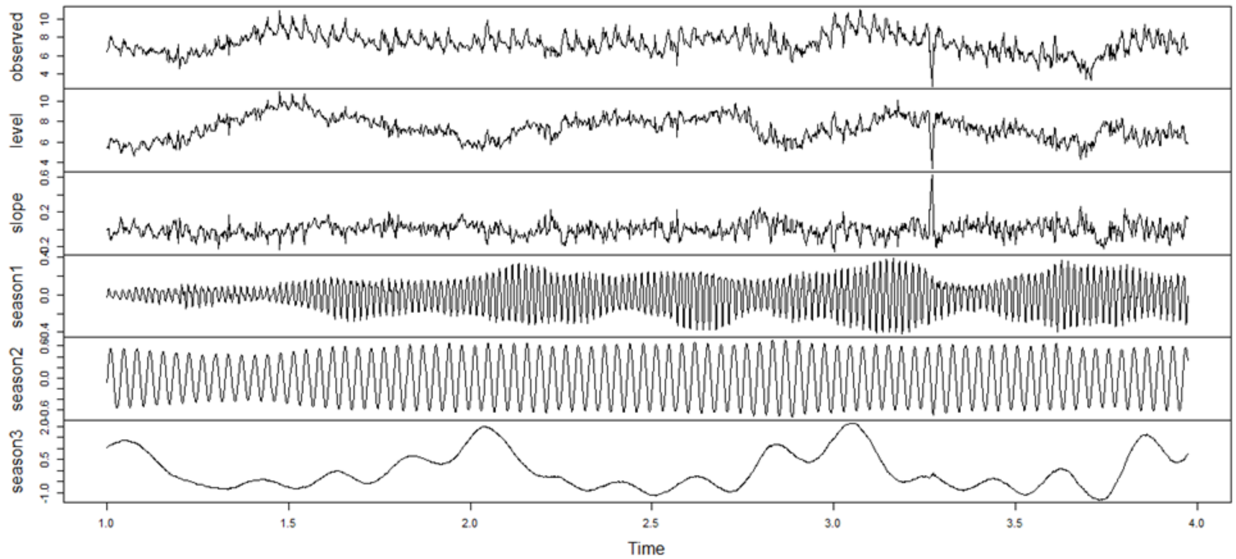


Figure 3-34. Specification of multiple frequencies in a spectral decomposition of the dissolved oxygen timeseries for the near Mud River site.

The results suggest that spectral decomposition may be a valuable approach to identify short term cycles in continuous recorder data, but more data are needed and an approach to handle missing data needs to be developed in order to accurately identify longer term dominant wave forms in the continuous recorder data.

4.0 APPLICATION TO MINIMUM FLOWS ASSESSMENT

This work effort was completed to support the District's consideration of the water quality environmental value in its reevaluation of minimum flows for the Homosassa River System. The tools developed as part of this work effort may be used in future analyses to support various aspects of flow management in these systems. In an effort to evaluate the efficacy of these tools for future assessments of the effects of flows on water quality, a summary of the minimum flow evaluation process is provided, and the chlorophyll model developed for the river mainstem was used to assess the potential for this type of model to be used in future assessments.

The goal of a minimum flows determination is to protect the resource from significant harm due to withdrawals. This goal was broadly defined in the enacting legislation as "the limit at which further withdrawals would be significantly harmful to the water resources or ecology of the area." What constitutes "significant harm" was not defined. In the absence of specific stressor-response threshold values identifying significant harm, a 15% reduction in a beneficial attribute of a resource of concern has been identified as a prescriptive standard by which significant harm has been defined. This 15% threshold has been used and supported in the development of the majority of minimum flows developed for Southwest Florida Water Management District which have been peer reviewed and subsequently adopted into Florida Administrative Code. The identification of the threshold values relies on a "percent of flow" approach in which predictive equations or mechanistic models are used in an iterative fashion to evaluate the effects of daily flow reduction scenarios of various increasing percentages of flow until the response threshold is achieved.

Results of the analysis described in section 3.4 suggested that the model developed to assess the response of chlorophyll distributions to changes in flows had potential to provide supporting evidence to evaluate the water quality environmental value as part of the minimum flows reevaluation for the Homosassa River, though the model would require validation before implementation as a regulatory tool. The sections below detail how the model could be implemented and presents results of that implementation for a hypothetical set of flow reduction evaluations under the assumption that the model is predictive of future conditions.

4.1 FLOW REDUCTION SCENARIOS

To apply results of the chlorophyll a model to evaluate the effects of flow reductions on increases in the exceedance frequency of the chlorophyll threshold, 15 flow reduction scenarios corresponding to 1% to 15% reductions from the baseline flow record for the Homosassa River, in 1% flow-change increments, were developed. The period from 1998 through 2017, generally corresponding to when gauged flows were available for the system, was identified as the period of record for the analyses. Season (i.e. Quarter) was assigned to each date based on month such that January-March was defined as Q1, April-June as "Q2", July-September as "Q3" and October-December as "Q4". In addition, after initial discussion of the model results, the area between sites 1 and 11 was identified as the focus area for analysis since this portion of the system is most likely to be directly influenced by spring flows to the system though the model was developed for the entire system. This spatial area is referred to for the remainder of this document as the “inset stratum”. Below site 11 (i.e. Rkm 7) the morphometrics of the river change dramatically with the potential for influence from additional tributaries and sheet flow from expansive marsh areas in the lower section of the River (Figure 4-1).

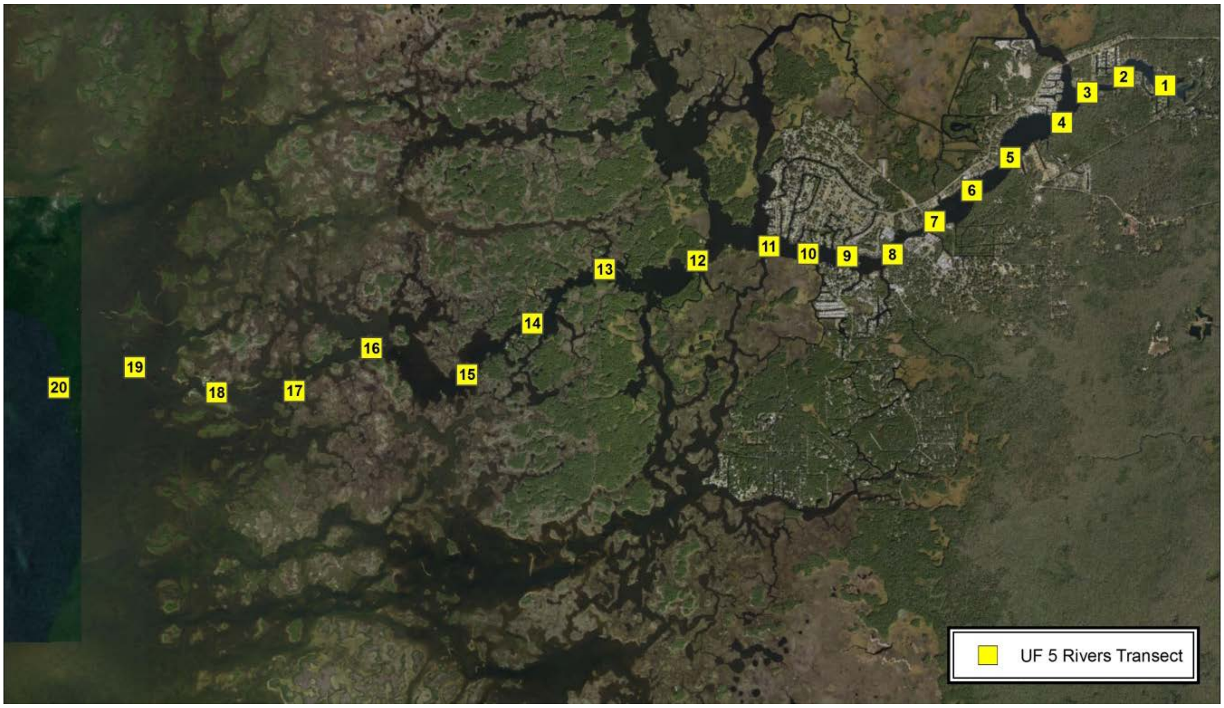


Figure 4-1. Station locations in the Homosassa River displaying the locations relative to morphometric and landscape characteristics of the system.

The GLMM outputs two types of predictions; “marginal” prediction also known as Best Linear Unbiased Estimates (BLUEs), or population level predictions, and “Conditional” estimates (i.e. conditional on the random “site” effects) known as Best Linear Unbiased Predictions (BLUPs). Both predictions are valid and the choice of which to use depends on the question being addressed. For example, if the expectation for the population average at any point in the river is desired, the BLUEs might be chosen to infer the model results to the entire model domain. However, if site-specific characteristics are important to the inference then the conditional estimates (BLUPs) might be chosen to ensure an adequate representation of the response at the sampled locations (Littell et al. 1996). We consider both these estimates as potential outcomes and describe the differences associated with each outcome.

To evaluate the effects of the flow reduction scenarios, a cutpoint had to be defined to identify whether or not a predicted probability of exceedance would be classified as an exceedance of the site-specific value. A cutpoint value of 0.50 was chosen based on its common use as a standard for a logistic curve of predicted probabilities that approach 1 at some point along the gradient. A plot of the effect of potential alternative cutpoint values on the model fit suggested there was not a clear alternative that would improve the model accuracy relative to the empirical data. The final model (Parameterization 3) was used to predict the probability of exceedance for each date in the timeseries at each station location above Rkm 7 and those predicted probabilities were converted to presence/absence identifiers for each scenario using the cutpoint value. The predicted exceedance frequencies were then summed across the entire time period for each flow reduction scenario and summary statistics were generated to evaluate the results. There are several statistics commonly used to evaluate outcomes of logistic regressions that have analogous applications for describing the predicted relative difference in the number of exceedances between the Baseline and a flow reduction scenarios including:

Risk of Exceedance: The percent of the values expected to exceed the standard for a particular scenario.

Risk Difference: Expressed as the difference between the Baseline risk of exceedance and the risk predicted by a flow reduction scenario.

Relative Risk (or Risk Ratio): the risk in the scenario group divided by the risk in the Baseline group

Odds Ratio: the ratio of the odds of an exceedance in the scenario group divided by the odds of exceedance in the baseline group

These statistics were used to evaluate the predicted effects of the flow reduction scenarios on the exceedance frequencies and to identify the flow reduction scenario that resulted in a 15% change from the Baseline exceedance rate. The results for the relative risk calculations are presented for each flow reduction scenario in Figure 4-2 for both the BLUP and BLUE estimates. When considering the total predicted exceedance rate for the segment of the river above Rkm 7 (i.e. The BLUE), the 6% flow reduction scenario resulted in a Relative Risk of 1.15, equivalent to a 15% increase in risk of exceedance relative to the Baseline run (Figure 4-2 left). The overall Risk Difference was 5.5% and the Odds Ratio was 1.25, indicating that the odds of exceedance was 1.25 as likely under the 6% flow reduction scenario compared to the Baseline. However, using the BLUP results which include the site-specific effects, a 9% reduction would be allowed before reaching the 15% threshold (Figure 4-2 right). The difference for the BLUP results would be 7.0% and the odds ratio would be 1.33.

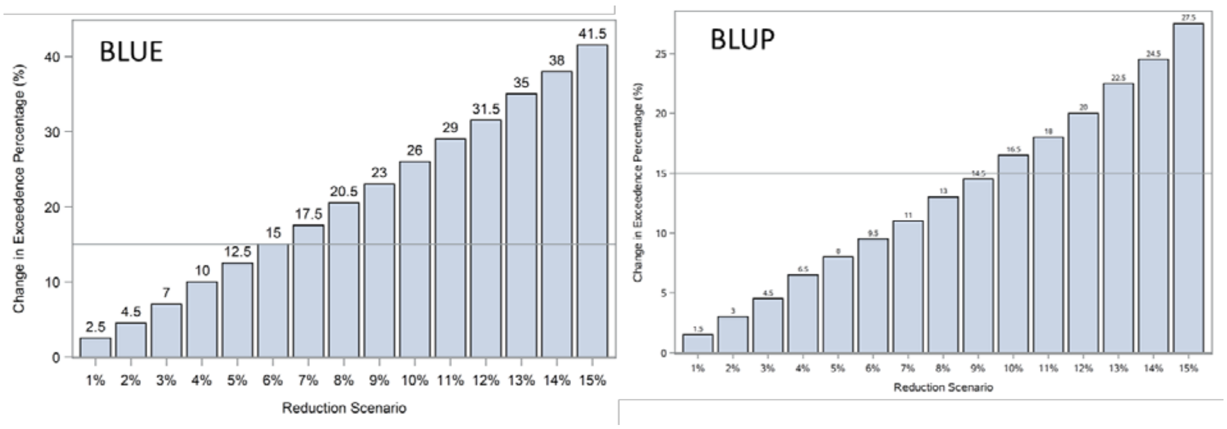


Figure 4-2. **Results of flow reduction scenarios on increase in relative risk of exceeding state water quality standard for chlorophyll a in the inset stratum of the Homosassa River using the BLUE and BLUP estimates. Numbers above bars represent the relative risk compared to Baseline for each scenario.**

To compare the predictions at each sampling site, a panel of paired box plots of the predicted probabilities by quarter under the Baseline and critical reduction scenario are presented for the BLUP and BLUE estimates in Figure 4-3. The effects of the interaction terms is apparent in the plots with the flow reductions resulting in a reduced probability of exceedance in Q1 (Winter) and an increased probability in Q2 (May). In Q3, the Rkm interaction results in flow reductions increasing the probability upstream and decreasing it downstream. The difference between the BLUP and BLUE predictions is also apparent in the site-specific difference for Q2 where the BLUP predictions drop dramatically for both the Baseline and Reduction scenario while the BLUE predictions remain a smooth increasing function of river kilometer which does not match the empirical data. This is problematic and is a function of not being able to appropriately fit a quadratic term for river kilometer to the data as described above. The BLUP predictions on the other hand, still represent the site specific attributes of the data and result in lower overall predictions in the upstream most portion of the river and still predict an increase in the probability of exceedance as flows decrease. For this reason, the BLUP predictions were chosen as the best linear unbiased predictions to use to assess the effects of flow reductions on the probability of exceeding the threshold chlorophyll value of 7.7 ug/l.

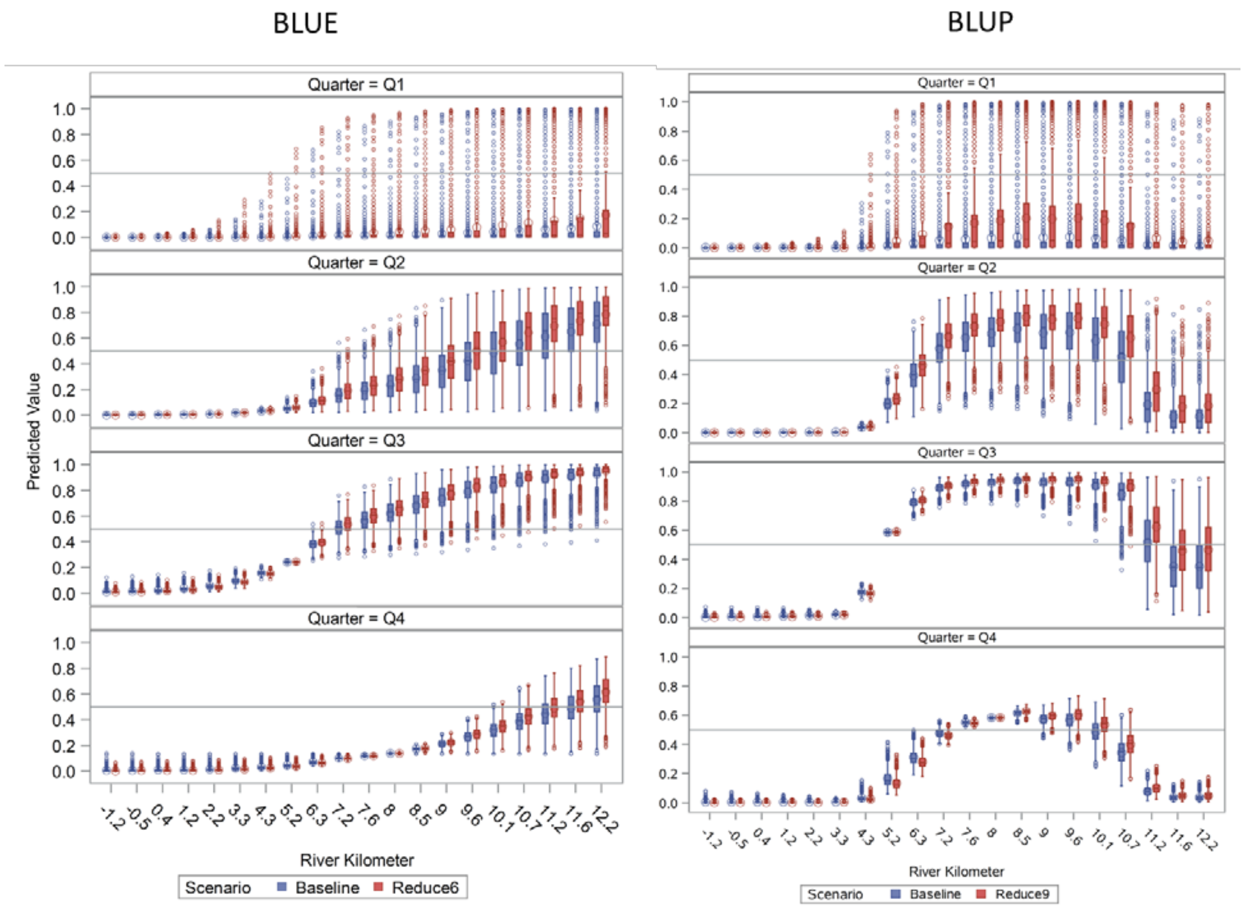


Figure 4-3. **Results of flow reduction scenarios on increase in relative risk of exceeding state water quality standard for chlorophyll a for each site in the inset stratum of the Homosassa River. Numbers above bars represent the relative risk compared to Baseline for each scenario.**

4.2 Monte Carlo Simulation

The analysis described in this investigation for the Homosassa River was designed to assess the increased risk of an exceedance of applicable water quality standards to hypothetical flow reduction scenarios and used a 15% change from the Baseline condition as a prescriptive standard by which to identify significant harm. However, the assessment did not, and was not intended to, directly evaluate whether the flow reduction scenario would result in a violation of the site-specific chlorophyll threshold. The state standard is expressed as an annual geometric average and the evaluation was based on a chlorophyll value exceeding that AGM on a particular date. The AGM is used as a regulatory statistic to minimize the effects of data that can be skewed by high values when calculating summary statistics such as the arithmetic mean. By taking the logarithm of a distribution of data that exhibit tendencies to be positively skewed such as chlorophyll, the transformed data exhibit the bell shaped pattern associated with the normal distribution. This is a common and convenient method used in data analysis to reduce the influence of extreme values on statistical analysis. Since the mean and the median of a normal distribution are nearly equivalent, the AGM generally represents the median of the log normal distribution (Helsel and Hirsch 2002). If one considers the AGM as the median then 50% of the data should lie above the median and 50% should lie below the median. These properties of the distribution were used to simulate the effects additional exceedances of the standard would have on the AGMs as described below.

The overall distribution of chlorophyll a values on the natural scale is provided in Figure 4-4a with a median values of 4.06 ug/l and an arithmetic average of 7.38 ug/l. The distribution of the natural log transformed values is provided in Figure 4-4b and shows how the transformation leads to an approximately bell shaped curve. The mean of the transformed data is 1.4854. Exponentiation of that number provides the overall geometric mean value of 4.41 ug/l, close to but not exactly the median value.

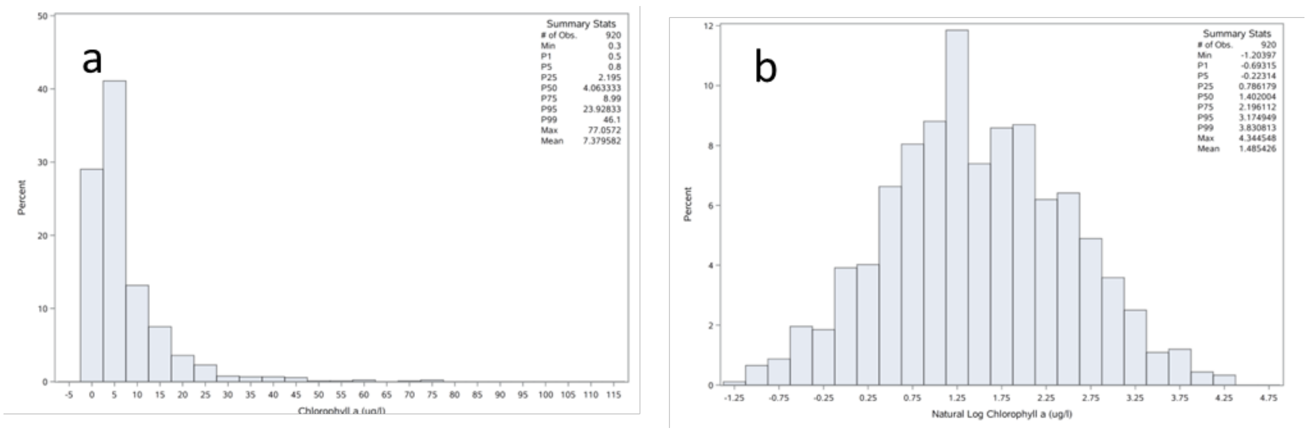


Figure 4-4. Distribution of chlorophyll a on the natural scale (left) and natural log scale (right).

The properties of the empirical distribution can be used to generate an extremely large dataset via monte-carlo simulation and that dataset can be used to evaluate the effects of changes in the exceedance frequency of the annual geometric average. The process involved the following steps:

- Generate the monte-carlo data pool using the properties of the empirical distribution
- Calculate percent exceedance under the existing condition
- Generate a representative sample for a given year by randomly selecting 44 samples (quarterly samples from 11 sites) at the empirical exceedance frequency
- For each replicate, calculate the AGM
- Repeat 1000 times

The simulation pool was constructed by using the distributional statistics for each site to generate independent distributions for each site within the system which were then combined into a single large dataset. A sample size of 44 was chosen to represent an individual year for each replicate sample, and for each replicate an AGM was calculated. The distribution of AGMs was then evaluated to define the increased risk of exceeding the standard under the critical flow management scenario identified above. The simulation was performed for the inset stratum including sites 1 through 11.

The empirical AGMs for the inset stratum are shown in Figure 4-5a while the monthly flow timeseries is provided in Figure 4-5b. The horizontal reference line on the plot of AGMs denotes the site-specific chlorophyll standard of 7.7 ug/l, which was exceeded in several of the more recent years in the timeseries in the inset stratum. This time period corresponded to a period of reduced flows in the Homosassa relative to the long term record (Figure 4-5b).

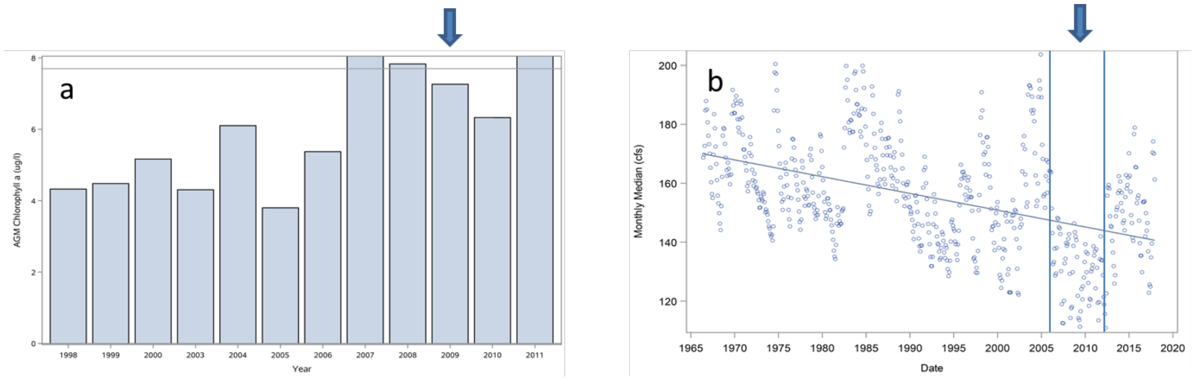


Figure 4-5. **Annual geometric average chlorophyll a concentrations in the Homosassa River above Rkm 7 based on the UF transect data (a) and flow timeseries for the Homosassa River with low flow time period highlighted by vertical bars (b).**

The overall geometric average (i.e. the average of the individual AGMs in Figure 4-5) for the inset stratum was 6.0 ug/l. The distribution of AGMs from the simulation of the existing condition is provided in Figure 4-6. The mean and median of this distribution are nearly identical at 5.99 ug/l, and a very close approximation to the empirical AGMs. The vertical reference line in Figure 4-6 indicates the standard of 7.7 ug/l and the simulated distribution exceeds the standard approximately 2% of the time. This suggests that the simulation is not accounting for the fact that correlation exists among sites. However, adjusting the exceedance frequency to represent the critical flow scenario of 9% results in a substantial increase in the expected exceedance frequency, from 3% to ca. 13% (Figure 4-7).

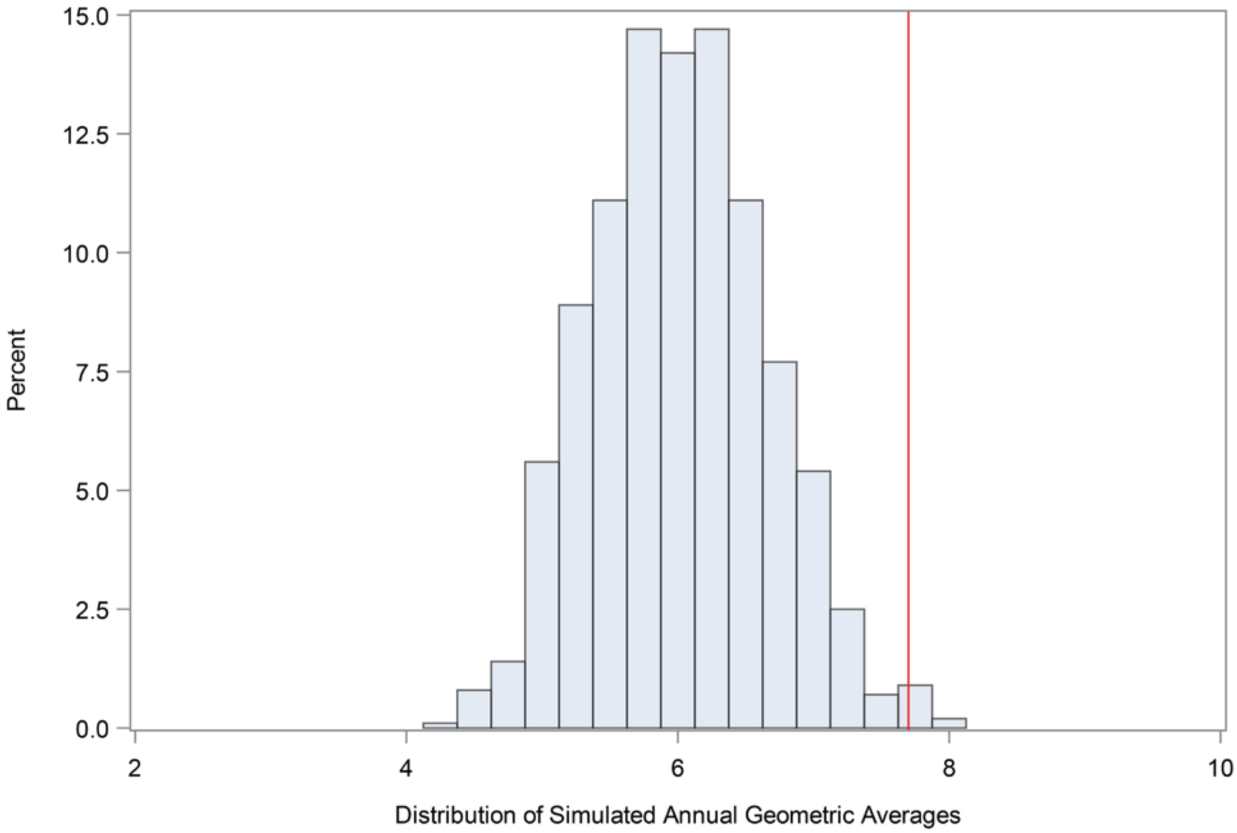


Figure 4-6. **Distribution of simulated annual geometric averages for existing condition above Rkm 7 in the Homosassa River. Vertical reference line is 7.7 ug/l.**

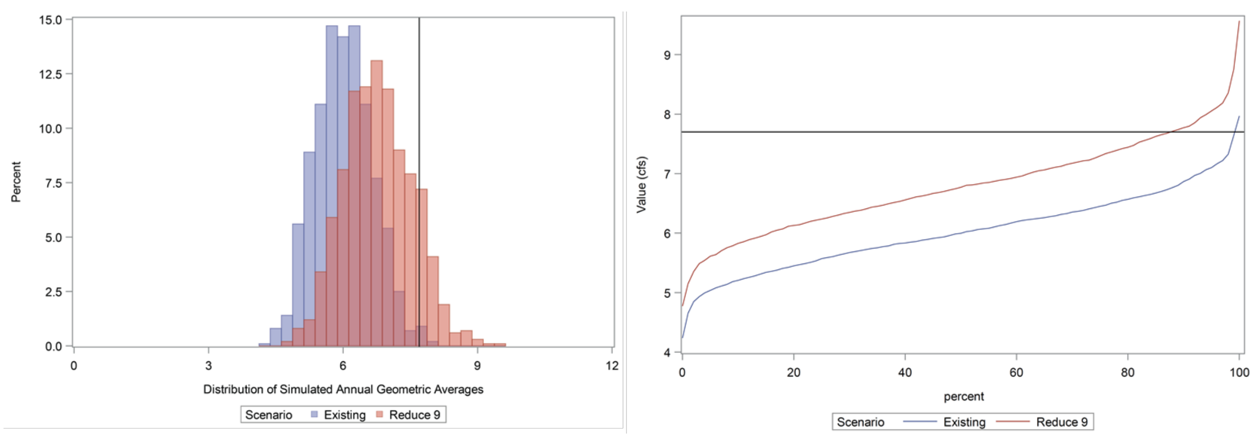


Figure 4-7. **Distributions of simulated annual geometric average chlorophyll a concentrations for the existing and 6% flow reduction condition shown as a frequency histogram (left) and a cumulative distribution plot (right), both with 7.7 ug/l reference lines.**

The results of this monte-carlo simulation illustrate how changes in the exceedance frequency as modeled for the flow reduction scenarios are related to the actual chlorophyll a concentration distributions in the Homosassa River. Increased exceedance frequencies will increase the overall mean value, but without the monte-carlo approach it is difficult to determine the magnitude to which the actual concentrations are changed. The results of this analysis suggest that, on average the AGM is expected to increase from 6.0 ug/l to 6.8 ug/l due to the 9% reduction scenario equating to a percent change in concentration of 13%, however, the results also suggest that an estimate of the correlation that exists among samples, accounted for in the mixed effects model, should be included in the monte carlo simulation to accurately estimate the change in concentrations. This was beyond the scope of this effort.

4.3 Limitations and Future Research

To date, phytoplankton distributions have not been previously used within the District as the principal determinant of minimum flows for a District tidal river. Chlorophyll a concentrations have been used to support the low-flow threshold established for the Lower Alafia River (Flannery et al. 2008) and have recently been used by the South Florida Water Management District in comparison to state water quality standards as one line of supporting evidence in the reevaluation of the minimum flow for the Caloosahatchee River Estuary (SFWMD 2018). While phytoplankton distributions are important indicators of riverine and estuarine condition they are notoriously difficult to model due to complex interactions between nutrient availability, light availability, and residence times. That being said, in recent years, Florida has experienced several high profile algae blooms including a protracted red tide event in 2018 and a blue green algae bloom that has affected Lake Okeechobee and its receiving waterbodies on both the east and west coast. These blooms have attracted much media attention and raised awareness as to the potential negative effects of excessive algal production in both fresh and estuarine systems. In addition, the Homosassa River springs complex has an established TMDL to reduce effects of increased nutrient loads to the system that have resulted in excessive primary production and nuisance algal mats for the Spring vents serving as headwaters for the river. While data collected in the Homosassa River have not indicated consequential negative impacts associated with phytoplankton bloom conditions, elevated concentrations that are indicative of bloom potential have been observed in the data.

While the modeling effort utilized the site-specific chlorophyll a threshold value established as a regulatory tool for a portion of the river, the results were not intended to be used as a direct assessment of whether or not changes in flow would result in compromises to the rivers "Designated Use" as defined in State statute. Rather, the modeling effort was developed to illustrate the utility of this type of modeling to assess the sensitivity of phytoplankton (chlorophyll a) concentrations in the upper 5 kilometers of the river to changes in flows. The model results predict that flow reductions, especially in April through June when flows tend towards their annual minimum, would increase the probability of exceeding a value of 7.7 ug/l. However, the location of the geographic boundary for evaluating water quality against regulatory criteria and the spatial distribution of chlorophyll in the system complicated the interpretation of the results of flow reduction scenarios. The modeling effort represents a novel application in support of environmental flow considerations that support minimum flow development, and more research should be completed before this approach can be more directly used in establishing minimum flows for the Homosassa River System and other District rivers.

The reported differences associated with the flow reduction scenarios are fairly small in terms of overall risk difference and it is unlikely that one could state with certainty that the reported differences represent statistically different conditions. Unfortunately, there is no standard way of evaluating the statistical certainty of the predictions when evaluating management scenarios such as this. However, it is important to consider that uncertainty exists in the predicted probabilities, and this uncertainty is not accounted for when evaluating the results of changes associated with the flow reduction scenarios. Several tributaries, including Hall's River, contribute flow and nutrients to the system which are unaccounted for by the flow records or the chlorophyll modeling efforts. Flow records for these sites are lacking long term records and are presumed to covary with the long term flow record developed for the minimum flows reevaluation. While these limitations are important to note, they do not obviate the need for protective limits to protect the system from degradation of water quality. The modeling effort has focused on the effects of flow and assumes that other factors that may affect the distribution of chlorophyll in the system are at a stable state for the flow reduction evaluations. In this sense, the results provide the best estimate of the effects of flow on the probability of exceeding the site-specific chlorophyll threshold as applied but do not imply that there are no other factors might also affect the distribution of phytoplankton in the system.

Future research should consider the utility of developing nitrate loadings from the head springs. Using nitrate loads as an explanatory variable would eliminate the potentially confounding effects of nutrient dynamics in the downstream portion of the river, and provide a truly independent variable for modeling chlorophyll concentrations. However, considerable additional effort would be required to develop the long term timeseries of daily nitrate loads needed to simulate the effects of flow reductions on the chlorophyll a response. In addition, evaluating the efficacy of using the downstream, site-specific chlorophyll criterion value of 7.7 ug/l established for WBID 1345F as a management threshold for the entire upper portion of the river (above Rkm 7) should be considered. Currently, the WBID boundary bisects the peak of the spatial distribution of chlorophyll. This fact, combined with the fact that the upstream WBID does not have a site-specific chlorophyll threshold, results in a disconnect between the existing criterion and the system dynamics. Evaluating whether or not the current downstream criterion is applicable to upstream portion of the river or developing an alternative criterion value to protect the upstream portion of the river (i.e., above Rkm 7) would result in a more site-specific protective standard for the portion of the river most affected by variation in spring discharge. There are also alternative modeling choices that could be made that consider the actual chlorophyll concentrations as a response variable; however, to apply those models to flow reduction scenarios associated with the development of minimum flows, an appropriate chlorophyll concentration would need to be developed based on an established threshold for significant harm.

5.0 RECOMMENDATIONS

This study has shown that chlorophyll concentrations are related to flows in the Homosassa River System. Chlorophyll concentrations have not been previously used as a criterion for establishing minimum flows and the modeling approach summarized in this report is a novel approach that should be further investigated. Further research is needed to determine if the site-specific numeric nutrient criterion (NNC) for chlorophyll a established for the downstream portion of the river is applicable to the entire upstream portion of the system, and for model validation. In the meantime, the results summarized in this report support consideration of water quality as part of the environmental values assessment associated with reevaluation of the minimum flow established for the Homosassa River System.

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