January 2026

A Hydrobiological Assessment
of the Minimum Flows for the Lower Hillsborough River for the Third Five-Year Assessment Period 2018–2023

Southwest Florida Water Management District

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ESA

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ABBREVIATIONS AND ACRONYMS

AIC	Akaike Information Criteria
ANOVA	Analysis of Variance
BIC	Bayesian Information Criteria
cfs	cubic feet per second
CR	Continuous Recorder
District	Southwest Florida Water Management District
DO	Dissolved Oxygen
EPA	US Environmental Protection Agency
EPC	Environmental Protection Commission of Hillsborough County
FAC	Florida Administrative Code
FDEP	Florida Department of Environmental Protection
FIM	Fisheries-Independent Monitoring
FS	Florida Statutes
FWE	Freshwater Equivalent
FWC	Florida Fish and Wildlife Conservation Commission
GAM	Generalized Additive Modeling
GLM	General Linear Models
HBMP	Hydrobiological Monitoring Program
HD	Hester-Dendy
HIMP	Hillsborough Independent Monitoring Program
JMT	Johnson, Mirmiran, and Thompson
LAMFE	Laterally Averaged Model For Estuaries
LHR	Lower Hillsborough River
mgd	Million Gallons Per Day
MFL	Minimum Flows and Levels
mi^2	Square Miles
mi^3	Cubic Meter
NGVD	National Geodetic Vertical Datum
NMDS	Nonmetric Multidimensional Scaling
NTBWUCA	Northern Tampa Bay Water Use Caution Area
OLS	Ordinary Least Squares
PCU	Platinum-Cobalt Units
POR	Period of Record
ppt	Parts per Thousand
PSU	Practical Salinity Units
REML	Restricted Maximum Likelihood
Rkm	River Kilometer
SAV	Submerged Aquatic Vegetation
SBIO	Statewide Biological Database
SCI	Stream Condition Index
Std	Standard Deviation

TBC	Tampa Bypass Canal
TBW	Tampa Bay Water
USGS	US Geological Survey
WAP	Wetland Assessment Procedure
WAR	Water & Air Research, Inc.
WE	Wetland Evaluation
WIN	Water Information Network
WUP	Water Use Permit

KEY TERMS AND DEFINITIONS

Above the Dam
Spatial zone from the Hillsborough River Dam to the USGS Fowler Gage No. 02304000 to S161.

Adjusted Minimum Flow
Minimum flow required after accounting for adjustments needed based on the USGS Zephyrhills Gage No. 02303000 and time specific minimum flow required (base minimum flow), but does not include freshwater equivalents adjustments.

All Days: This includes all available data from January 1, 1996, through December 31, 2023. This was only used to describe the hydrology to the lower river and calculate statistics regarding the frequency and proportion of days where minimum flow implementation occurred.

Analysis Days: This is a subset of "All Days" that includes days where minimum flow implementation would be required based on the current, adopted rule regardless of year. (includes seasonal adjustments, USGS Zephyrhills Gage No. 02303000 adjustments, and FWE). This was done to include more data that would have met the condition for implementation in the historical record, particularly for biology but was also applied to the water quality analysis for consistency.

Applied Minimum Flow
Minimum flow required after accounting for adjustments needed based on the USGS Zephyrhills Gage No. 02303000, time-specific base minimum flow, and freshwater equivalents factor of 3 cfs (1.9 mgd) applied.

Base of Dam
Area just downstream of the Hillsborough River Dam where water is delivered to the LHR.

Base Minimum Flow
Time-dependent minimum flow requirement with seasonal adjustment. Base minimum flow = 20 cubic feet per second (cfs) (12.9 mgd). Seasonal adjustment for April through June to 24 cfs (15.5 mgd). Between January 1, 2000, and September 30, 2007, base minimum flow is 10 cfs (6.5 mgd). Prior to January of 2000, the minimum flow was 0 cfs.

Continuous Recorder (CR): Device deployed to measure physical chemistry parameters of the water including temperature, specific conductance, and occasionally dissolved oxygen (DO) at high frequency (e.g., hourly) intervals.

Downstream Zone
Spatial zone from Sligh Avenue to the mouth of the LHR (approximately Platt Street).

Freshwater Equivalence
Defined in 40D-8.041(1)(b), FAC, freshwater equivalent means water that has a salinity concentration of 0.0 ppt for modeling purposes. It equates to an additional 3 cfs (1.9 mgd) required for the LHR minimum flow, intended to offset the use of Sulphur Springs brackish water as a freshwater recovery source.

LHR Dam
Structure creating the Hillsborough River Reservoir and defining the upstream extent of the Lower Hillsborough River.

Minimum Flow Implementation
A period where flows were sent to the base of dam from the recovery sources to achieve the required minimum flow.

Minimum Flows
Defined in Section 373.042(1) of the Florida Statutes as "the limit at which further withdrawals would be significantly harmful to the water resources or ecology of the area."

Minimum Flows Met
Periods where implementation of minimum flows were sufficient to achieve the established minimum flow.

Implementation Days: This is a subset of "All Days" that only includes days where minimum flow implementation was required based on the effective rule for that day. Minimum flow implementation is required when the calculated LHR total flow is less than the applied minimum flow (the applied minimum flow includes all temporal, gage, and freshwater equivalents adjustments).

Target Zone
Spatial zone from the base of the Hillsborough River Dam to Sligh Avenue.

EXECUTIVE SUMMARY

As required by Section 373.0421 of the Florida Statutes, if the actual flow of a water course is below an adopted minimum flow or is projected to fall below a minimum flow over the next 20 years, a recovery or prevention strategy must be developed. In 2007, rule amendments incorporated revised minimum flows and a revised recovery strategy for the Lower Hillsborough River (LHR) into Rules 40D-8.041 and 40D-80.073, Florida Administrative Code (FAC), respectively.

The currently adopted minimum flows for the LHR are 20-cubic-feet-per-second (cfs) freshwater equivalent flow from July 1 through March 31 and 24 cfs freshwater equivalent flow from April 1 through June 30 at the base of the Hillsborough River Dam. adjusted based on a proportionate amount that flows when flow at the US Geological Survey (USGS) Hillsborough River Near Zephyrhills, FL (Gage No. 02303000) is below 58 cfs. For purposes of the minimum flows rule, freshwater equivalent means water that has a salinity concentration of 0.0 ppt for modeling purposes.

The recovery strategy requires that in 2013 and for each 5-year period through 2023, the District shall evaluate the strategy regarding its effects on the hydrology, dissolved oxygen (DO), salinity, temperature, pH, and biological characteristics of the LHR that have been achieved from minimum flow implementation, i.e. use of recovery source water when flow over the Hillsborough River Dam was insufficient to meet LHR minimum flow requirements. The first and second 5-year recovery strategy assessment reports documented improvements in salinity, other water quality parameters, and ecological conditions in the river below the dam.

This report represents the third of three consecutive 5-year assessments of the Hillsborough River recovery strategy. The report evaluates the hydrologic, water quality and biological data in the river and the recovery sources utilized to provide the required minimum flows.

The recovery strategy outlines six potential projects and a timeline for their implementation:

1. Sulphur Springs Project (Lower Weir Modifications and Sulphur Springs Pool Upper Weir and Pump Station Modifications).
2. Blue Sink Analysis and Project.
3. Transmission Pipeline Evaluation and Project.
4. Investigation of Storage or Additional Supply Options.
5. Tampa Bypass Canal (TBC) and Hillsborough River Reservoir Diversions.
6. Morris Bridge Sink Project.

These projects are intended to provide a sufficient flow of freshwater and low-salinity water below the Hillsborough River Dam to restore low-salinity habitat within the LHR and achieve an oligohaline zone (salinity < 5 ppt) from the Hillsborough River Dam toward Sulphur Springs. All activities and projects proposed in the adopted recovery strategy are completed or have been deemed not viable or actionable.

Operation of minimum flow implementation for the LHR has been increasingly successful over time; the LHR and Sulphur Springs minimum flows were met for all days beginning in 2023 (a drier than average year). From 2018 through 2023, Sulphur Springs consistently served as the primary recovery source (72% average). The TBC provided the second-largest contribution, on average supplying 15% of the water used for diversions to meet the minimum flow for LHR. Blue Sink contribution is roughly 10% since it came online in 2018. The flows over the dam contribute roughly 3% on average. Morris Bridge Sink has been permitted but not yet used as a recovery source.

Analysis on water levels above the dam indicate minimum flow implementation has not affected water levels upstream of the dam. Sulphur Springs Pool salinity and temperature have increased over time. The City of Tampa has an ongoing feasibility study to further explore this issue; however, the results of that study will not be completed in time to incorporate into this assessment.

Both the water quality assessments in this report and an independent study by the District using an updated Laterally Averaged Model for Estuaries (LAMFE) model indicate minimum flow implementation has extended the low-salinity habitat at the base of the dam toward Sulphur Springs and has had little impact on water temperature in the LHR. Further, statistical modeling suggested that the area of bottom DO greater than 2.5 mg/L increased with successful implementation of the minimum flow, providing more acceptable habitat for biota in the river. Statistical analysis of other water quality parameters (water temperature and pH) indicated little effect of implementation.

The findings of analysis of biological data provide convincing evidence that the current LHR minimum flow is functioning to provide oligohaline (salinity < 5 ppt) habitat conditions for zooplankton, benthic macroinvertebrates, and nekton.

The implementation of the Hillsborough River recovery strategy has successfully:

- Extended the low-salinity habitat from the base of the dam toward Sulphur Springs.
- Improved LHR water quality conditions, particularly salinity and DO levels.
- Enhanced habitat for freshwater and low-salinity adapted organisms.
- Supported diverse and abundant biological communities.

The assessment confirms that the phased implementation approach of the recovery strategy has been appropriate, with each additional water recovery source contributing to the overall success of the program. However, the long-term sustainability of Sulphur Springs as a recovery source remains a concern.

1 INTRODUCTION

As required by Section 373.0421 of the Florida Statutes, if the actual flow of a water course is below an adopted minimum flow or is projected to fall below a minimum flow over the next 20 years, a recovery or prevention strategy must be developed. In 2007, rule amendments incorporated revised minimum flows and a revised recovery strategy for the Lower Hillsborough River (LHR) into Rules 40D-8.041 and 40D-80.80.073, Florida Administrative Code (FAC), respectively.

The recovery strategy requires that in 2013 and for each 5-year period through 2023, the District shall evaluate the strategy regarding its effects on the hydrology, dissolved oxygen (DO), salinity, temperature, pH, and biological characteristics of the LHR that have been achieved from minimum flow implementation, i.e. use of recovery source water when flow over the Hillsborough River Dam was insufficient to meet LHR minimum flow requirements.

This report represents the third of three consecutive 5-year assessments of the Hillsborough River recovery strategy. The report evaluates the hydrologic, water quality and biological data in the river, and the recovery sources used to provide the required minimum flows.

1.1 WATERSHED OVERVIEW

The Hillsborough River watershed, which covers approximately 675 square miles (mi^2) in Hillsborough and Pasco Counties, comprises the largest river drainage basin that flows to Tampa Bay (SWFWMD and Atkins 2015). The river originates in the Green Swamp and flows approximately 55 miles in a generally southwest direction through the Cities of Temple Terrace and Tampa to the mouth of the river in downtown Tampa (Figure 1.1-1).

Major tributaries to the river upstream of the Hillsborough River Dam include Crystal Springs, Blackwater Creek, Trout Creek, and Cypress Creek. During the dry season, the majority of baseflow in the river comes from Crystal Springs, a second-order spring near the headwater region of the river in Pasco County. Blackwater Creek, which is the river's largest tributary, flows to the river between the Zephyrhills and Morris Bridge gages (Nos. 02303000 and 02303330). As part of the Florida Department of Environmental Protection (FDEP) *Lake Bioassessment/Regionalization Initiative*, Griffith et al. (1997) note that few lakes occur within the lake region containing the middle river. Among area lakes that may drain to the river, Lake Thonotosassa, an 819-acre lake in Hillsborough County, is the largest (Leeper 2009).

The portion of the river above Fletcher Avenue is considered the Upper Hillsborough River. The reach of the river from the Hillsborough River Dam to Fletcher Avenue is referred to as the Middle Hillsborough River. The reach of the river below the Hillsborough River Dam is referred to as the LHR.

The US Geological Survey (USGS) Hillsborough River at State Park near Zephyrhills FL Gage (No. 02303000) is approximately 40 miles upstream of the mouth of the river in Hillsborough River State Park. The USGS Hillsborough River at Morris Bridge near Thonotosassa FL Gage (No. 02303330) is approximately 29 miles upstream of the river mouth (Figure 1.1-2). Downstream of Morris Bridge, the two main tributaries to the river are Cypress Creek and Trout Creek. The USGS operates streamflow gages on both tributaries (Nos. 02303800 and 02303350). The combined drainage area covered by these two gages plus the USGS Hillsborough River at Morris Bridge Near Thonotosassa FL Gage (No. 02303330) totals 558 square miles, or approximately 86% of the drainage area to the Hillsborough River above the dam (SWFWMD and Atkins 2015). The river is impounded at the Hillsborough River Dam, about 10 miles (16.1 kilometers) upstream of the river mouth in the City of Tampa. The river discharges into Hillsborough Bay, which is the most northeast lobe of Tampa Bay.

Figure 1.1-1: Hillsborough River watershed

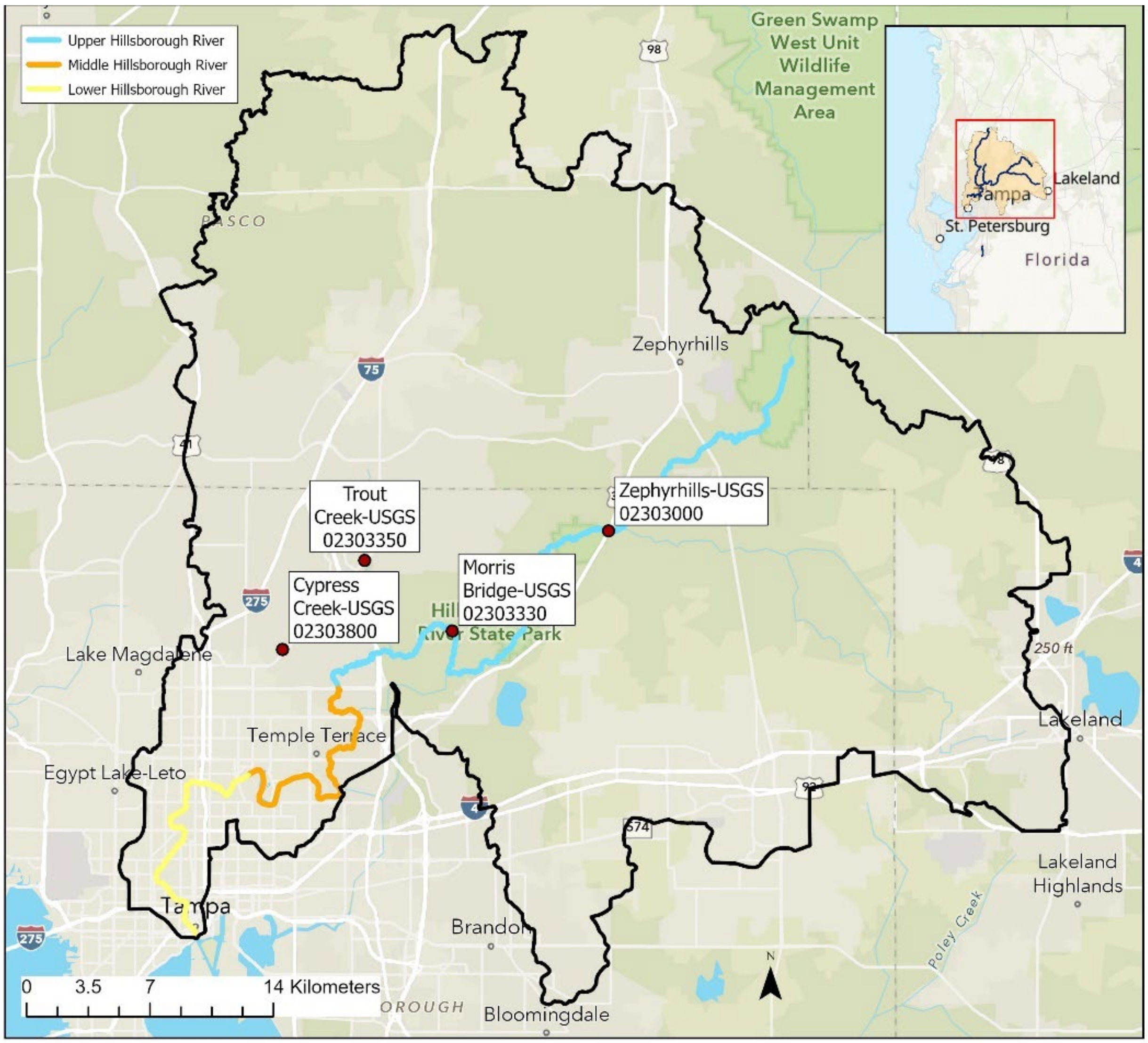
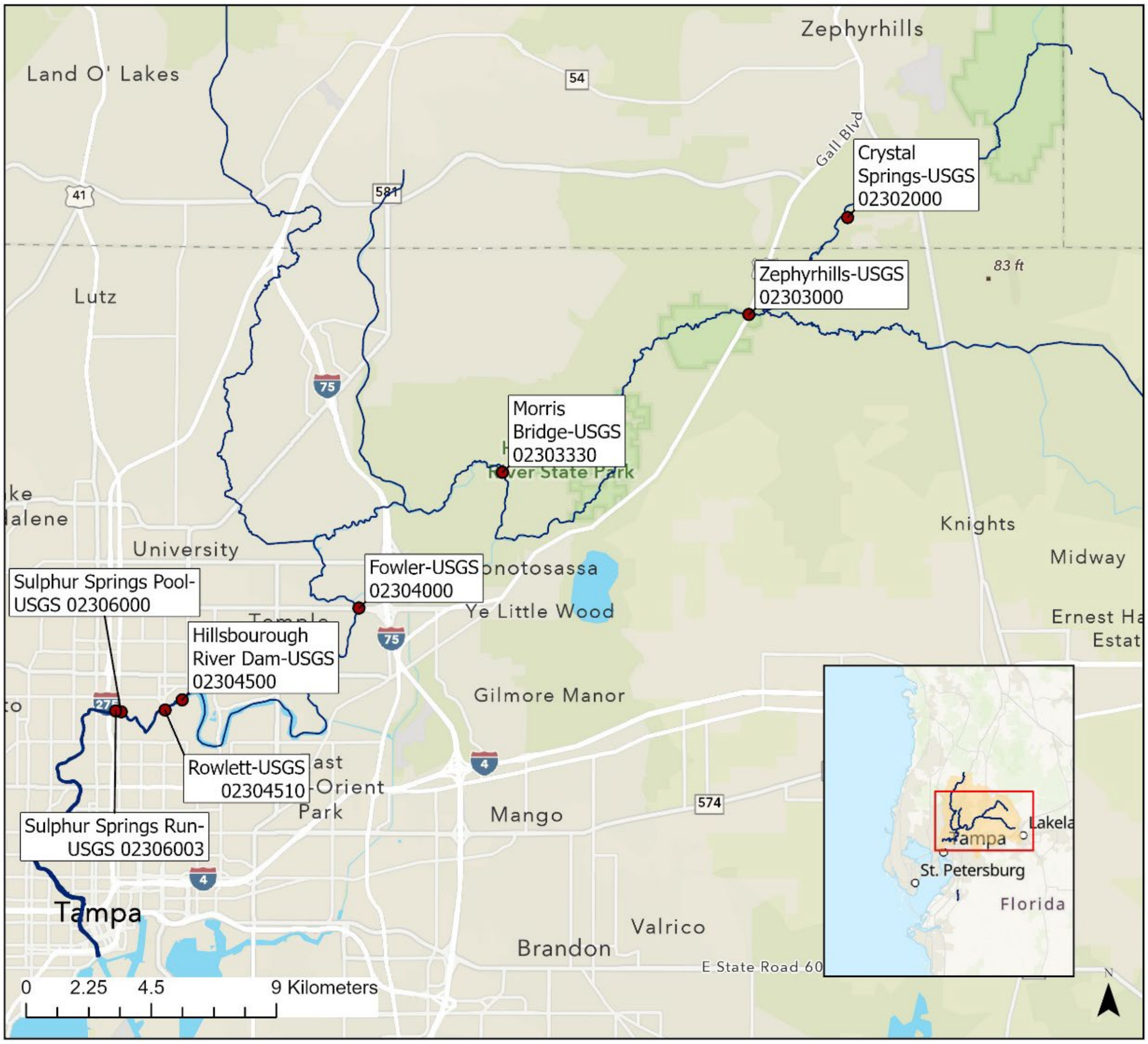


Figure 1.1-2: USGS continuous recorder discharge gages of the Hillsborough River



1.2 Water Supply

Withdrawals from the Hillsborough River Reservoir by the City of Tampa are regulated under a Water Use Permit (WUP, Permit No. 20002062.006), which was renewed by the District in 2004. The permit requires that the annual average withdrawal quantity cannot exceed 82 million gallons per day (mgd) and a maximum daily withdrawal rate of 120 mgd. The 2004 permit renewal increased the maximum daily quantity from 104 to 120 mgd to allow for the increase of aquifer storage and recovery facilities by the City to store water in the Upper Floridan aquifer in the wet season for subsequent withdrawal and use in the dry season. The City of Tampa has submitted a renewal application for the WUP with no increases in quantities proposed.

An important modification of the City of Tampa's water use from the reservoir occurred in the 1980s, when augmentation of the reservoir with water pumped from the Tampa Bypass Canal (TBC) first began. The District acknowledged that constructing the TBC greatly increased groundwater discharge to the canal and concluded that pumpage from the TBC could be used to augment the water supplies available from the reservoir (SWFWMD and Atkins 2015). Pumpage from the TBC to the reservoir began in 1985 using a temporary pumping facility that pumped water from the Harney Canal around Structure S-161 into the reservoir. This temporary pump was replaced in 1992 by the current pumping facility at Structure S-161.

Diversions from the TBC to the reservoir are regulated under a District Water Use Permit (WUP,Permit No. 20006675.006), which requires that the average annual pumpage rate cannot exceed 20 mgd (31 cfs) and a peak monthly quantity of 40 mgd (62 cfs). Although this water use permit is held by Tampa Bay Water (TBW), the Tampa Water Department determines the timing and rate of the daily pumping rates from the canal to the reservoir. Augmentation may commence from the TBC when the water levels at the Hillsborough River Dam (measured at Gage No. 02304500) recede to less than the crest gate elevation of 22.50 feet National Geodetic Vertical Datum (NGVD) 29, the crest gate and the two tainter gates are closed, and the flow in the Hillsborough River measured at Morris Bridge Road (USGS flow Gage No. 02303330) is less than the draft from the City of Tampa Hillsborough River Reservoir to the City of Tampa D.L. Tippin Water Treatment Facility (measured at District ID No. 11 associated with WUP No. 20002062.006). However, withdrawals from the Harney Canal must not cause water levels in the middle pool of the canal to fall below regulatory levels, which are based on maintaining acceptable head differences between the middle pool and the reservoir.

The City of Tampa is also permitted to augment water supplies in the reservoir with diversions from Sulphur Springs (WUP No. 2002062 District ID No. 10), which discharges to the channel of the LHR about 2.2 miles downstream of the Hillsborough River Dam. A pipe to transmit flow from Sulphur Springs to the reservoir has been in place since the mid-1960s. As part of their permit for reservoir withdrawals, the City can withdraw an annual average of 5 mgd from the spring and a maximum daily quantity of 20 mgd, which frequently equals the total flow of the spring in the dry season. However, the discharge from Sulphur Springs is highly mineralized and exceeds Class I potable water quality standards for certain constituents (SWFWMD 2004). Therefore, the City of Tampa has diverted water from Sulphur Springs for potable supply only during times of water shortage, relying on the blending of the spring water with water in the reservoir to not exceed potable water supply standards in their withdrawals from the reservoir. Sulphur Springs has not been used for potable water supply since 2009. The adoption of a minimum flow for Sulphur Springs has further reduced diversions from the spring to the reservoir.

In 1999, a WUP was issued to TBW (Permit No. 20011796.000) to also withdraw water from the TBC for potable water supply. Withdrawals from the middle or lower pool of the TBC are sent to a water treatment plant near the east shore of the lower pool that was constructed in 2002. The current permit (WUP No. 20011796.002) for TBW is structured so that water can be obtained directly from the TBC, or during times of relatively high river flow (greater than 100 cfs), water can be diverted from the Hillsborough River Reservoir through the Harney Canal to the TBC where TBW can withdraw the diverted river flows. A graduated diversion schedule applies in which up to 40% of flow from the river can be diverted based on the rate of flow at the dam. The current permitted quantities for total withdrawals by TBW from the river and the TBC are an average annual rate of 85 mgd (132 cfs) and maximum daily rate of 258 mgd (399 cfs).

1.3 Minimum Flows

The requirement to establish Minimum Flows and Levels (minimum flows or MFL) in Florida was first introduced in the Water Resources Act of 1972. This act mandated the state's water management districts to identify and set minimum flows to prevent significant harm to water resources and ecosystems due to water withdrawals. Minimum flows were required to be adopted by the State of Florida under subsection 373.042(3) of the Florida Statutes (FS). The District is directed by the Florida Legislature to establish minimum flows for flowing watercourses within its boundary. Minimum flows are defined in Section 373.042(1), FS, as "the limit at which further withdrawals would be significantly harmful to the water resources or ecology of the area."

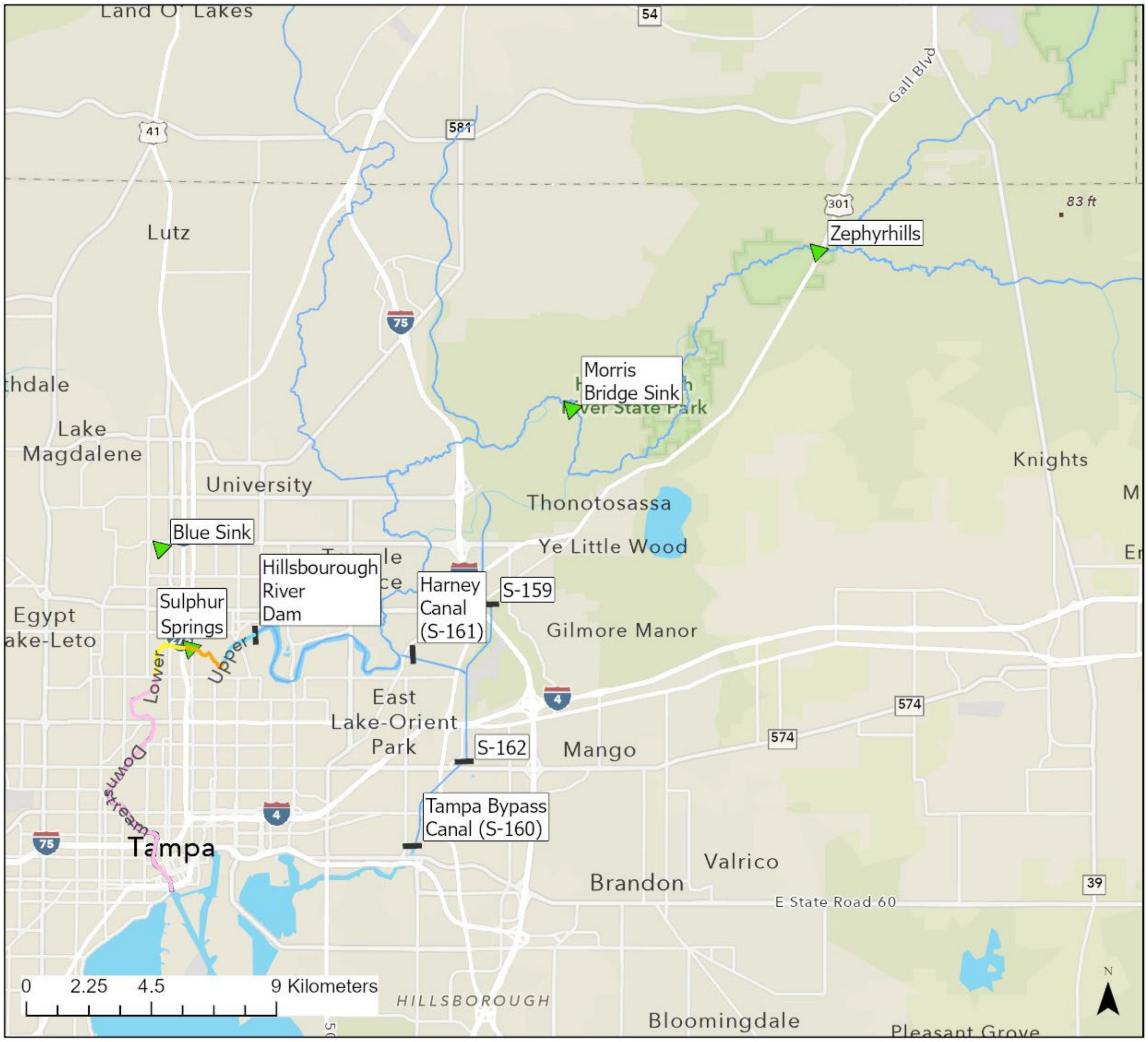
The initial minimum flow for the LHR, which was adopted in 2000, was established at 10 cfs (6.5 mgd) at the base of the dam. This flow was based on a 1999 analysis conducted by the District titled *An Analysis of Hydrologic and Ecological Factors Related to the Establishment of Minimum Flows for the Hillsborough River* (SWFWMD 1999). The LHR was found to need recovery to meet the established minimum flow, and a recovery strategy was developed to meet this goal. Subsequently, the minimum flow rule was revised, and the recovery strategy was revised to meet the revised minimum flow adopted in 2007. Appendix A provides the current minimum flow rules for the LHR.

1.4 Geographic Range of the Study Area for this Report

1.4.1 Recovery Sources

Recovery sources include Sulphur Springs, Blue Sink, The Tampa Bypass Canal, and Morris Bridge Sink (Figure 1.4-1).

Figure 1.4-1: Recovery sources to meet the Lower Hillsborough River recovery strategy



1.4.1.1 Sulphur Springs

Sulphur Springs is within a heavily urbanized area of North Tampa. The spring lies just east of the intersection of Nebraska Avenue and Bird Street in the City of Tampa. Sulphur Springs discharges to the Hillsborough River at a point 3.5 km (~2.2 miles) downstream of the dam and is influenced by runoff from the surrounding urban watershed within the Hillsborough River Basin. The spring and its associated features are part of the Sulphur Springs system, which includes the spring pool, vent, and adjacent spring run (SWFWMD, 2004). Groundwater flow of the spring can be influenced by runoff to nearby sinks, which are hydraulically connected. However, evidence indicates that previous groundwater connections of these sinks to Sulphur Springs have become clogged with debris. (Schreuder 1999, 2001, 2004).

1.4.1.2 Blue Sink

Blue Sink is within an urbanized area of North Tampa. The sink is northwest of the intersection of 115th Avenue and Florida Avenue in the City of Tampa. Blue Sink receives runoff from the Curiosity Creek watershed within the Hillsborough River Basin. The creek drains a 3.5-square-mile closed basin area (SWFWMD, 2009). The sink is part of the Curiosity Creek/Blue Sink Complex, which consists of a series of sinkholes east of the creek (Schreuder, 2001).

1.4.1.3 Tampa Bypass Canal

The TBC is a 22.5 km (~14 miles) waterway designed and constructed by the US Army Corps of Engineers to divert floodwaters around Temple Terrace and Downtown Tampa. It serves as one component of the Four River Basins drainage project that commenced following widespread flooding in the wake of Hurricane Donna in 1960 (SWFWMD, 2015). The TBC connects the Lower Hillsborough Wilderness Preserve/Lower Hillsborough Flood Detention Area with McKay Bay.

Based on these structures and their operation, the TBC is divided into lower, middle, and upper pools:

- Upper Pool: The upper pool is the portion upstream of Structure S-159 and receives water from the Lower Hillsborough Flood Detention Area and Hillsborough River.
- Middle Pool: The middle pool extends from Structure S-159 downstream to Structure S-162. It connects to the Hillsborough River Reservoir by the Harney Canal about 9.7 km (6 miles) upstream of the dam.
- Lower Pool: The lower pool extends from Structure S-162 downstream to tructure S-160.

1.4.1.4 Morris Bridge Sink

Morris Bridge Sink is on District-owned property referred to as the Lower Hillsborough Wilderness Preserve. It is approximately 1.2 km (~0.75 mile) northeast of the TBC and approximately 0.6 mile southeast of the Hillsborough River. It is approximately 135 feet in diameter and 200 feet deep (Basso, 2010).

1.4.2 Hillsborough River

1.4.2.1 Upper Hillsborough River

The Upper Hillsborough River originates in the Green Swamp, a large hydrologically significant wetland area spanning portions of Pasco and Polk Counties. From its headwaters, the river flows southwest through a mosaic of conservation lands, forested floodplains, and urbanizing areas within the Hillsborough River Basin. For this report, the downstream extent of the Upper Hillsborough River is defined as the USGS stream gage at Fowler Avenue near Temple Terrace, Florida (No. 02304000).

1.4.2.2 Hillsborough River Reservoir

The Hillsborough River Reservoir is also considered the Middle Hillsborough River. For this report, the upstream boundary of the reservoir is the USGS Hillsborough River at Fowler Avenue Near Temple Terrace FL Gage (No. 02304000). The downstream boundary is the Hillsborough River Dam at the USGS Hillsborough River Near Tampa FL Gage (No. 02304500). The Harney Canal connects the TBC to the reservoir at District Structure S-161.

1.4.2.3 Lower Hillsborough River

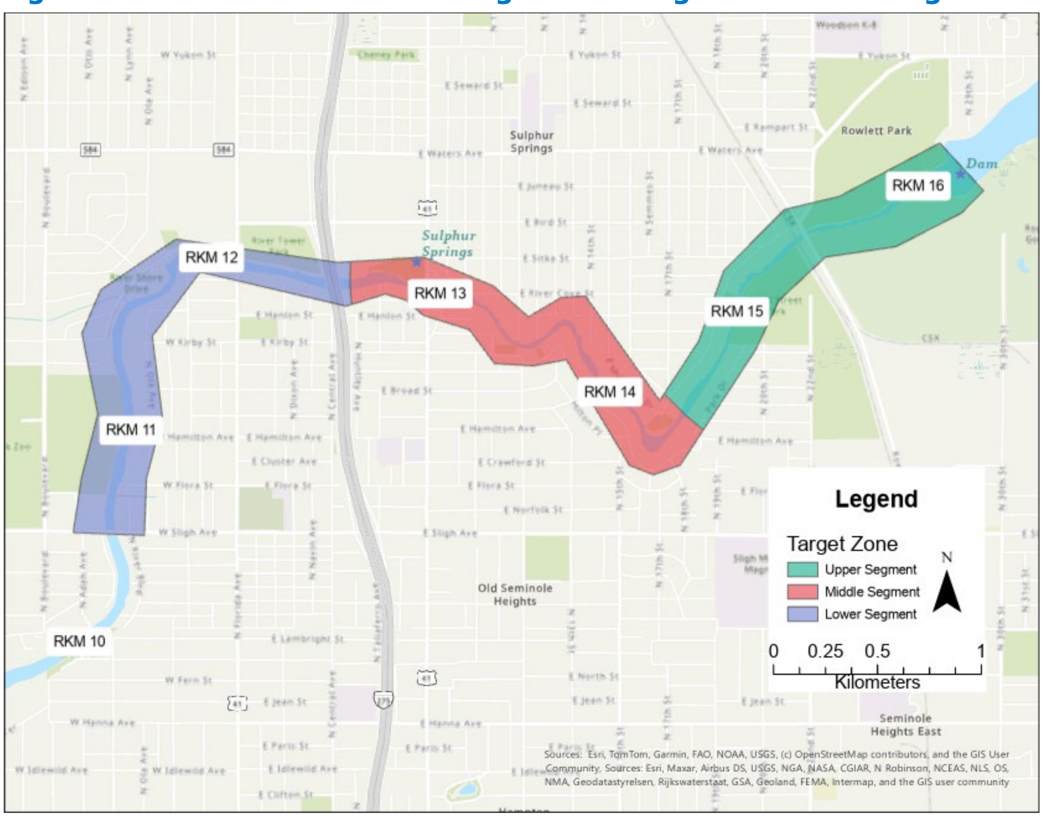
For this report, the LHR begins at the Hillsborough River Dam, a low-head lift-gate structure in the City of Tampa at the USGS stream gage Hillsborough River Near Tampa, Florida (No. 02304500). From the dam, the river flows approximately 10 miles (~16.1 Rkms) southwest through a densely urbanized corridor that includes residential neighborhoods, public parks, and commercial development within the Hillsborough River Basin. For this report, the downstream extent of the Lower Hillsborough River is defined as the USGS stream gage at Platt Street in downtown Tampa (No. 02306028), where the river discharges into Hillsborough Bay.

LHR Target Zone

To align with the spatial extent referenced in the LHR minimum flow rule (toward Sulphur Springs), the target zone was defined as the reach from the Hillsborough River Dam to Sligh Avenue. Within this target zone, three segments were delineated by river kilometer (Rkm) (Figure 1.4-2):

- Upper Segment: Rkm 16.2 to Rkm 14.5 (Dam to Hannah's Whirl)
- Middle Segment: Rkm 14.5 to Rkm 12.6 (Hannah's Whirl to Sulphur Springs)
- Lower Segment: Rkm 12.6 to Rkm 10.6 (Sulphur Springs to Sligh Avenue)

Figure 1.4-2: Lower Hillsborough River target zone river segments



LHR Downstream Zone

The LHR downstream zone begins at Sligh Avenue and extends to the mouth of the river at Platt Street.

1.5 OBJECTIVES OF THE ASSESSMENT

The purpose of this report is to meet the intent of 40D-80.073(8) FAC. The minimum flow for the river, which was adopted in Rule 40D-8.041. FAC, has been adjusted and implemented over time, and the recovery strategy requires the District to assess the river response to minimum flow implementation every 5 years starting in 2013 and running through 2023.

The requirements for the 5-year assessments are listed in Section 40D-80.073(8), FAC:

> *(8) In 2013, and for each five-year period through 2023, the District shall evaluate the hydrology, dissolved oxygen, salinity, temperature, pH and biologic results achieved from implementation of the recovery strategy for the prior five years, including the duration, frequency and impacts of the adjusted minimum flow as described in paragraph 40D-8.041(1)(b), F.A.C. As part of the evaluation, the District will assess the recording systems used to monitor these parameters. The District shall also monitor and evaluate the effect the Recovery Strategy is having on water levels in the Hillsborough River above the City's dam to at least Fletcher Avenue. The District will evaluate all projects described in this Recovery Strategy relative to their potential to cause unacceptable adverse impacts prior to their implementation.*

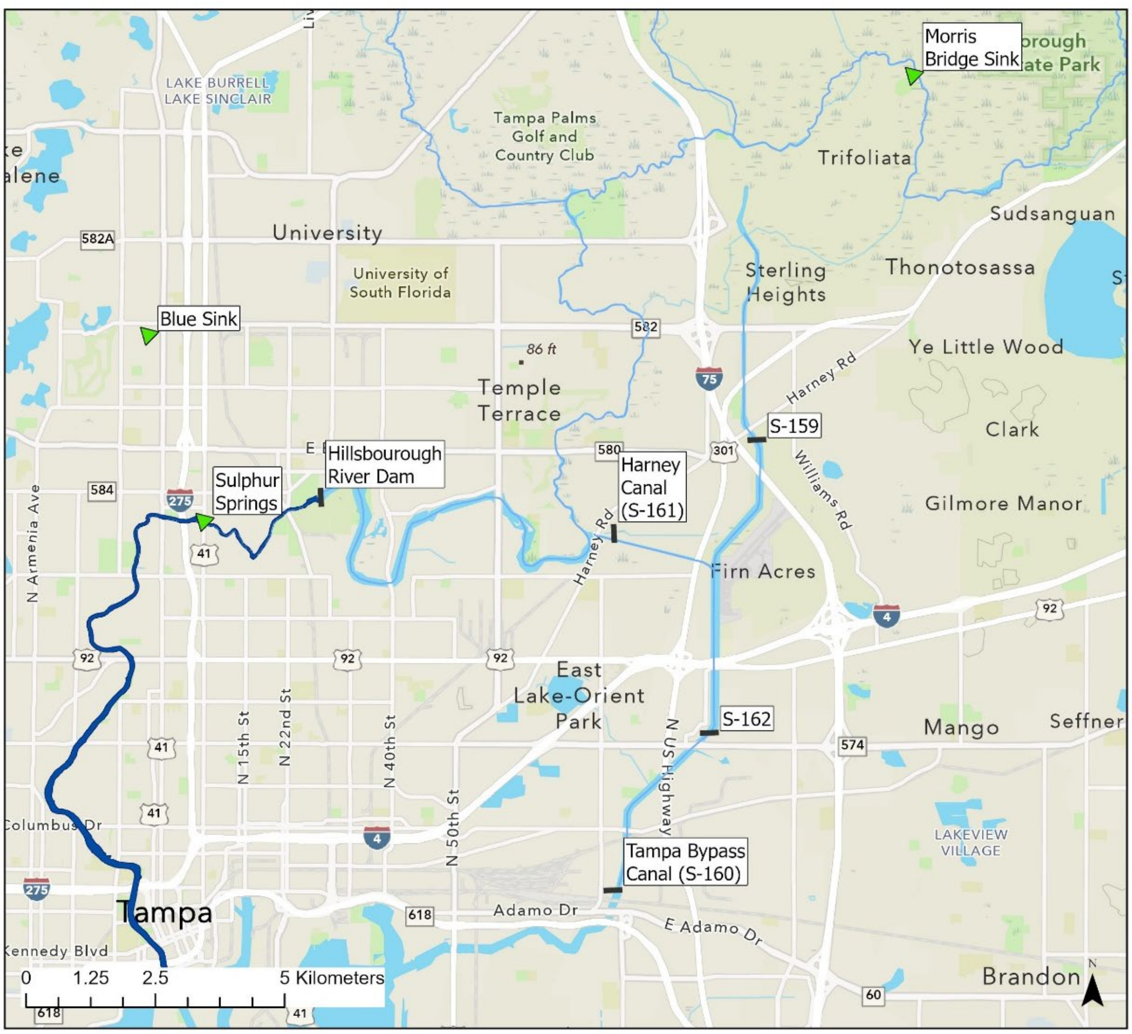
The objectives of this report are to:

- Meet the requirements listed in Rule 40D-80.073(8), FAC.
- Describe the period of record (POR) data collected between 1996 and 2023.
- Evaluate the effects of minimum flow implementation on the water quality and the biological communities of the LHR.
- Summarize results to evaluate if the recovery strategy, as implemented to date, has increased low salinity habitat in the area from the base of the Hillsborough River Dam toward Sulphur Springs, as specified in the LHR adopted minimum flow (40D-8.041 FAC).

2 THE HILLSBOROUGH RIVER RECOVERY STRATEGY

The Hillsborough River has a long history of impoundment for use in agriculture, energy production, and water supply, and its current configuration is the result of the creation of the Hillsborough River Dam in 1960 after major flooding destroyed the functionality of the previous structure (Leeper 2009). Continued flooding issues in Tampa after the dam's construction instigated the creation of the TBC, which was completed in 1981 to divert flood waters from the river around the cities of Tampa and Temple Terrace (Figure 2-1). The uppermost reaches of the TBC are connected to the river upstream of Fletcher Avenue where Structure S-155 can be used to route flow from the river through the TBC. The TBC is also connected to the Hillsborough River Reservoir via the Harney Canal, which is a lateral canal that extends from the TBC to the reservoir about 6 miles upstream of the dam. Flows in the river can be diverted from the reservoir to the TBC via the Harney Canal through Structure S-161 (SWFWMD and Atkins 2015). The diversion of moderately high flows through the Harney Canal is fairly frequent, occurring during the wet season of most years. In contrast, the diversion of flood flows from the river via the closing of Structure S-155 is infrequent.

Figure 2-1: Map of water sources to the Hillsborough River Reservoir and the LHR



2.1 ESTABLISHED MINIMUM FLOWS FOR THE LOWER HILLSBOROUGH RIVER

The initial minimum flow for the LHR (SWFWMD 1999), which was adopted in 2000, established a minimum flow of 10 cfs (6.5 mgd) at the base of the dam and noted that the minimum flow should provide immediate improvements to the ecological characteristics of the river below the dam, but also acknowledged that the data during low flow conditions were limited (SWFWMD 1999). To address this limitation, the District reevaluated the minimum flow once it was implemented.

The results of that reevaluation, which were documented in the report *Lower Hillsborough River Low Flow Study Results and Minimum Flow Recommendations* (SWFWMD 2006), were the basis for increasing the minimum flow in 2007. The 2007 rule, which is still in effect, establishes a minimum flow at the base of the dam of 20 cfs (12.9 mgd) freshwater equivalent (FWE) from July 1 through March 31 and 24 cfs (15.5 mgd) FWE from April 1 through June 30 (FAC 40D-8.041, Appendix A).

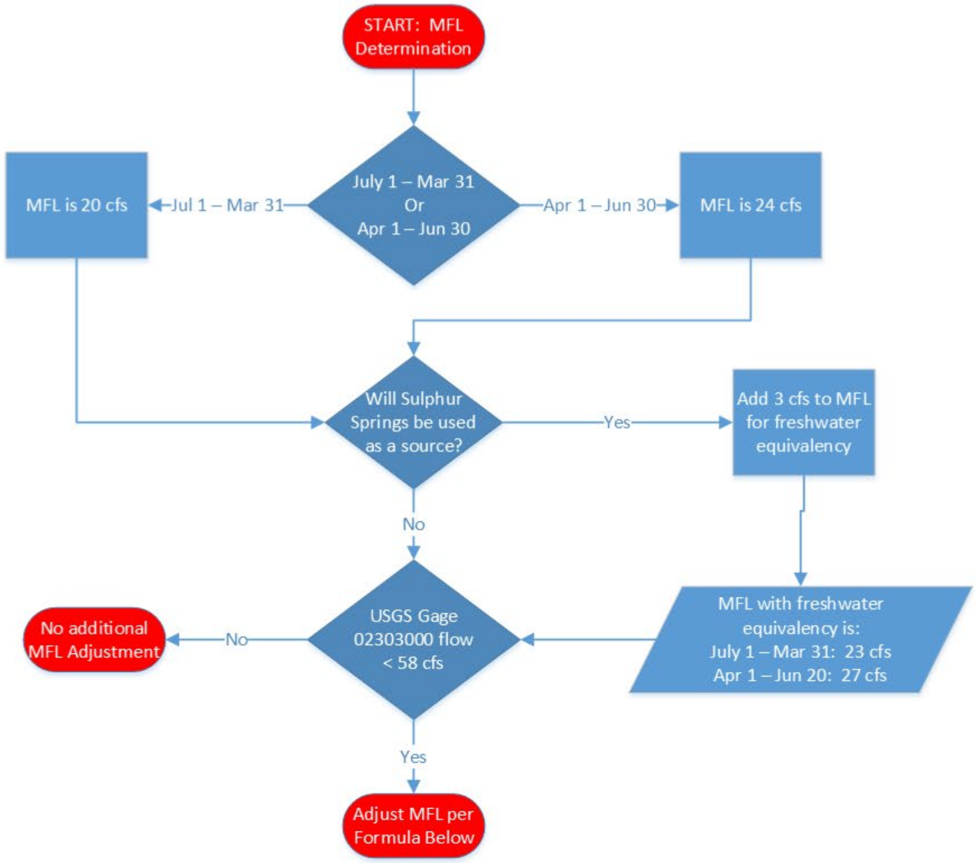
The District's 2006 report proposed a year-round minimum flow of 20 cfs FWE for the LHR. The report further indicated that this flow could be achieved through a combination of 15 cfs (9.7 mgd) from Sulphur Springs and 8 cfs (5.2 mgd) of freshwater. Sulphur Springs flow contains salinity values higher than freshwater limits (0.0 ppt for modeling purposes), reducing its effectiveness in lowering estuarine salinity. Therefore, to meet the intended freshwater equivalent of 20 cfs, an additional 3 cfs adjustment is needed to account for the reduced freshwater quality of Sulphur Springs discharge.

Following publication of the 2006 SWFWMD report, the peer review panel recommended establishing a 24 cfs freshwater equivalent minimum flow for April through June, citing the importance of this period for fish nursery habitat utilization. In response, the District incorporated this recommendation in the adopted rule, which specifies a 24 cfs FWE minimum flow April, May, and June—an update not reflected in the original 2006 report. The same 3 cfs freshwater equivalent adjustment was applied to the 24 cfs minimum flow for consistency with the approach used for the 20 cfs FWE target, creating an operational minimum of 27 cfs in April, May, and June.

2.2 MINIMUM FLOW RULE 40D-8.041(1) FAC

The current adopted minimum flow is defined in 40D-8.041(1) FAC (Appendix A). The rule provides a series of criteria that determine what the LHR minimum flow should be (Figure 2.2-1); it is not one static number. The base minimum flow without adjustments is quantified as flows at the base of the dam of 20 cfs (12.9 mgd) FWE from July 1 through March 31 and 24 cfs (15.5 mgd) FWE from April 1 through June 30. It takes into account low-flow conditions in the watershed under drought conditions, and the minimum flow is adjusted based on the flow at the upstream USGS Hillsborough River at State Park Near Zephyrhills FL Gage (No. 02303000). The July 1 through March 31 minimum flow is reduced by 0.35 cfs for every cfs that the flow at the gage is below 58 cfs (37.5 mgd), and the April 1 through June 30 minimum flow is reduced by 0.40 cfs for every cfs the flow at the gage is below 58 cfs (37.5 mgd).

Figure 2.2-1: Minimum flow rule criteria for the LHR (40D-8.041(1) FAC)



LHR MFL Calculations				
If USGS Gage 02303000 flow is < 58 cfs, adjust MFL with this formula: *Adjusted MFL value = Unadjusted MFL - (MFL adjustment factor * (58 - actual USGS flow)) **Adjusted MFL value with freshwater equivalency = (Unadjusted MFL + 3) - (MFL adjustment factor * (58 - actual USGS flow))				
Season	**Jul 1 - Mar 31**		**Apr 1 - Jun 30**	
Unadjusted MFL	20		24	
MFL Adjustment Factor	0.35		0.4	
Flow (cfs) @ USGS Gage 0203000 (Zephyrhills)	Adjusted MFL value (cfs)*	Adjusted MFL value with freshwater equivalency (cfs)**	Adjusted MFL value (cfs)*	Adjusted MFL value with freshwater equivalency (cfs)**
58	20.00	23.00	24.00	27.00
57	19.65	22.65	23.60	26.60
56	19.30	22.30	23.20	26.20
55	18.95	21.95	22.80	25.80
54	18.60	21.60	22.40	25.40
53	18.25	21.25	22.00	25.00
52	17.90	20.90	21.60	24.60
51	17.55	20.55	21.20	24.20
50	17.20	20.20	20.80	23.80

40D-8.041(1)(b) FAC

2.3 RECOVERY STRATEGY 40D-80.073 FAC

The District initially adopted the Hillsborough River Recovery Strategy in 2000 as part of the Northern Tampa Bay Water Use Caution Area (NTBWUCA) Recovery Strategy because the minimum flow adopted for the LHR was not being met. The District revised the recovery strategy in 2007 concurrently with the adoption of revised minimum flows for the river that also were not being met. The NTBWUCA Recovery Strategy sunset on December 31, 2020, and was deleted from Rule 40D-80.073, FAC. The Hillsborough River Recovery Strategy, which had successfully been implemented for recovery of other area waterbodies, remains in effect.

The recovery strategy in 40D-80.073 FAC (Appendix B) identified four potential water sources that can be used as recovery flows in the LHR:

- Sulphur Springs
- Blue Sink
- Tampa Bypass Canal
- Morris Bridge Sink

Using these recovery sources required feasibility assessments and projects involving installing or constructing pumps and piping to convey water from each recovery source to the base of the dam. In addition to these four recovery sources, transmission pipe evaluation and the investigation of water storage or additional water supply options were also listed in the recovery strategy as described in the subsections below.

2.3.1 SULPHUR SPRINGS

Diversions of water from Sulphur Springs to meet the initial minimum flow for the LHR began in spring 2002. The Hillsborough River Recovery Strategy required the City to provide 10 cfs (6.5 mgd) from Sulphur Springs to the base of the dam if needed to achieve the LHR minimum flow by November 25, 2007. This flow rate was the maximum flow that the temporary pumping facilities could supply. To provide additional flows for recovery, the City was required to complete weir and pump station modifications needed for Sulphur Springs to provide up to 18 cfs (11.6 mgd) water from the Sulphur Springs Pool to the LHR by October 1, 2012. These modifications allowed variable pump rates to the LHR and Sulphur Springs run in addition to the greater capacity. Sulphur Springs has an adopted minimum flow (40D-8.041(3) FAC) that must be met before diversions can be made to support the LHR minimum flow. In October 2008, the City and District entered into a cooperative agreement to perform modifications to the weir and pump station. The Lower Weir project, which was completed in October 2011, involved installing an operable weir at the mouth of the spring run to:

- Prevent incursions of higher-salinity water from the river during low-flow periods.
- Allow access to the run by manatees and other organisms during higher-flow periods when incursions of saline water were less of a concern.
- Enhance management flexibility for the City regarding use of spring water to meet minimum flow requirements for the LHR and Sulphur Springs Run.

Modification of the pump station was completed in March 2012. The pumping facility has separate pumps to divert water from the spring pool to the base of the Hillsborough River

Dam or to Sulphur Springs Run. Pump station modifications, combined with the previously completed weir modifications, increased the reliability of flow diversions to the LHR by lowering water levels in the spring pool and inducing greater flow from the spring vent. Modifications also provided for variable pumping rates, allowed control of flows between Sulphur Springs Pool and Sulphur Springs Run based on the temperature and salinity of adjacent monitoring stations, and allowed for pumping at a greater range of anticipated water levels in Sulphur Springs Pool by modification/replacement of the pump station intake.

Although the increased flows available for diversion were clearly beneficial to the LHR, the increased diversions in 2012 initially resulted in lower elevations of Sulphur Springs Pool (from just above 7 feet before diversions to 3–5 feet NGVD). These lower water levels increased the salinity of the spring discharge from an average of 1.6 practical salinity units (PSU) from March 2002 through February 2012 to 2.7 PSU between March 2012 and May 2013 when pool levels were lowered to induce greater spring discharge (SWFWMD 2015).

Increased diversions from Sulphur Springs may have also contributed to increases in filamentous algae in the spring run by reducing current velocities in the spring run (SWFWMD, 2004). University of Florida researchers found that coverage of filamentous algae in Sulphur Springs was less than 6% in 2003 (SWFWMD 2004), and algae have become more widespread after diversion rates increased in spring 2012 (SWFWMD and Atkins 2015).

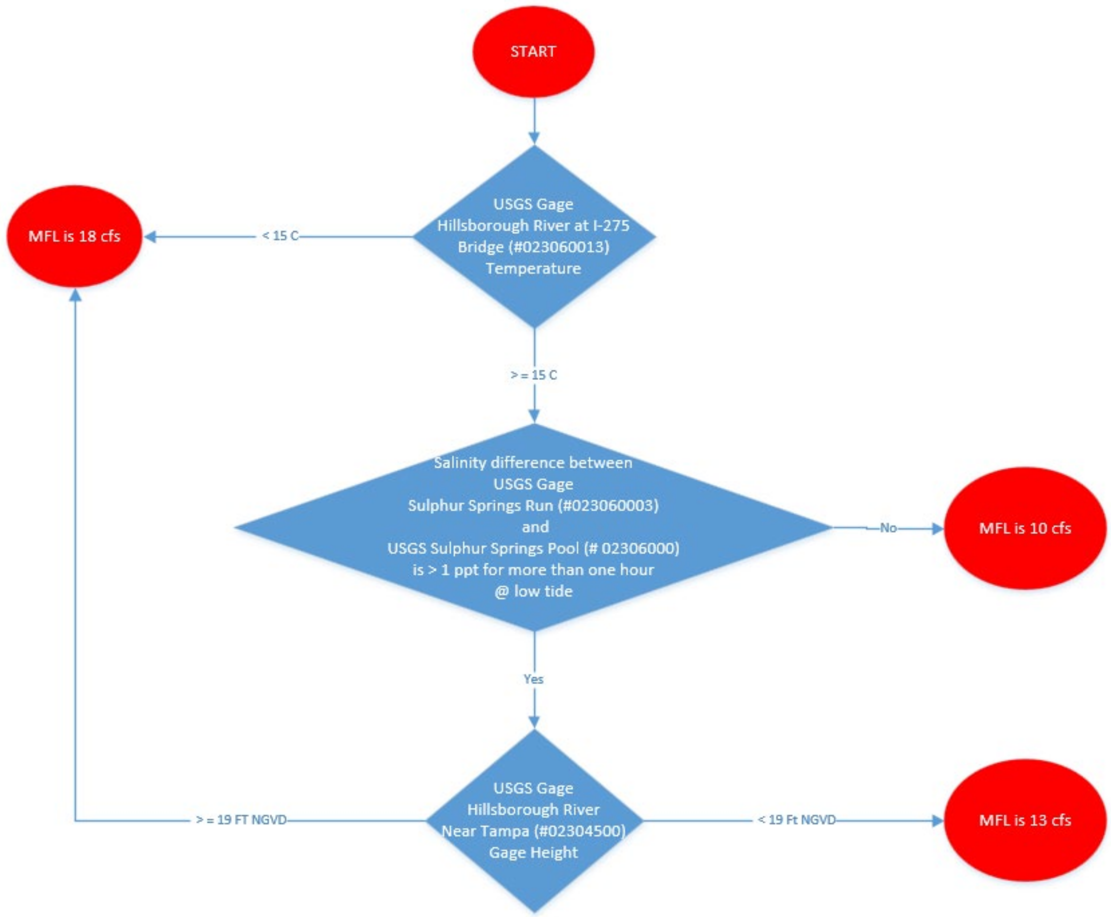
The Sulphur Springs minimum flow was designed to minimize salinity incursions in the spring run and to moderate temperatures within the manatee protection zone of the LHR. As of October 1, 2012, the City is required to maintain minimum flows for Sulphur Springs, as shown in Figure 2.3-1, as follows:

1. 18 cfs, as measured at USGS Sulphur Springs at Sulphur Springs FL Gage (No. 02306000).
2. 13 cfs when water levels in the reservoir fall below 19 feet NGVD.
3. 10 cfs during low tide stages in the LHR, provided that salinity levels measured in the spring run are not more than 1 ppt greater than the value measured at the spring pool for more than 1 hour.

The minimum flows listed in Items 2 and 3 are superseded if the temperature of the LHR near the spring run outlet is below 15°C, in which case the minimum flow is 18 cfs.

The adopted minimum flow for Sulphur Spring under 40D-8.041(3), FAC, incorporates a 15°C temperature trigger to protect manatee thermal refuge (Figure 2.3-1). However, US Army Corps of Engineers Permit SAJ-2010-01672(LP-LDD) and Hillsborough County Environmental Protection Commission Permit 09-047 require the City of Tampa to apply a 20°C trigger in place of the 15°C criterion in Rule 40D-8.041(3). The higher threshold reflects a more conservative protection measure to ensure that manatees continue to have access to suitable warm-water habitat during colder conditions.

Figure 2.3-1: Minimum flow rule criteria for Sulphur Springs (40D-8.041(3) FAC)



2.3.2 Blue Sink

The District initiated studies of the feasibility of using Blue Sink as a recovery source for flows to the LHR in 2007 by conducting a site evaluation of historical water surface elevations in Blue Sink and the hydrologically connected Ewanowski Spring (SWFWMD 2007). As part of this evaluation, District staff reviewed three hydrogeological investigations conducted by Schreuder, Inc., which had previously concluded:

- The underground conduit connecting Blue Sink to the downgradient Sulphur Springs was effectively blocked between 1974 and 1985 by trash and sediment deposition and that the water levels within the system observed over the previous 2 to 3 decades were approximately 6–8 feet higher than before the blockage (Schreuder 1999).
- The area directly north of Sulphur Springs, including Poinsettia Sink, still has a rapid and direct connection to Sulphur Springs (Schreuder 2001).
- It was not feasible to reconnect the underground hydrologic connection between Blue Sink and Sulphur Springs (Schreuder 2004).

To address the increased water levels and associated flooding in the Blue Sink system caused by the blockage of outflow from Blue Sink, the City installed a 65 cfs (42 mgd) pumping station at a stormwater retention pond (F-100c) to pump the excess water to the Hillsborough River (Schreuder 2004). Although this helped improve stormwater management, it also highlighted that excess flows are available from Blue Sink. The District then conducted two 30-day pumping tests of Blue Sink in May–June 2008 and March–April 2009 to evaluate the potential yield of Blue Sink. Based on the 2009 pumping test results, the District concluded that Blue Sink could provide up to 2 mgd (3.1 cfs) as a minimum flow recovery source for the LHR. The District noted that impacts to nearby lakes were not significant and that domestic wells in the area should not be impacted by the resultant 0.5 to 2.5 feet of drawdown of the unconfined aquifer (SWFWMD 2009).

The District's proposed safe pumping rate was largely consistent with the withdrawals conducted previously by the City during an April 1996 pumping test of Blue Sink and an emergency pumping project conducted from April to August 2000 in which water was pumped from Blue Sink into Poinsettia Sink. Based on the City's 1996 test, the City initially concluded that an average pumping rate of 4.3 mgd (6.6 cfs) would result in a drawdown of 2.7 feet. However, during the emergency pumping 4 years later, the City initially pumped at 4 mgd (6.1 cfs) but decreased the pumping rate to approximately 1.6 mgd (2.5 cfs) after one week in response to decreases in aquifer elevation (SWFWMD 2022).

Following the District's 2009 30-day pumping tests (Appendix C), the City and the District entered into a cooperative funding agreement in October 2010 to fund construction of a pump station at Blue Sink and a pipeline to connect water pumped from the sink to the existing pipeline used to transfer water from Sulphur Springs to the LHR.

Withdrawal authorization was permitted under WUP No. 20020382 for Blue Sink. The permit was issued to the City by the District in December 2013. The permit allows a peak monthly withdrawal of up to 2 mgd (~3.1 cfs) and an annual average withdrawal rate of approximately 1.7 mgd (~2.6 cfs). Because the City also needed to obtain applicable permits to construct project elements using various municipal rights-of-way, the City requested that the completion date for the project be changed to December 31, 2015. Accordingly, a new cooperative agreement with the City with an expiration date of October 1, 2017, was developed in July 2014 to accommodate the revised timeline. The agreement was subsequently amended in February 2016 to include a construction completion date of January 14, 2017.

The City completed design for the pipeline and pump station project elements in March 2015. Construction activities for the Blue Sink pipeline were completed in May 2016. Initial pump station testing began in March 2017, and pumping evaluations identified a leak in the transmission line used to deliver pumped water to the LHR. Once this issue was corrected and additional testing by the City was conducted, the project construction was completed in September 2017. In March 2018, the City began operation of the Blue Sink pumping facility for minimum flow implementation at the LHR. Pumping from Blue Sink is not expected to impact Sulphur Springs flow because, as noted previously, the hydrologic connection between Blue Sink and Sulphur Springs was blocked by trash and sediment deposition.

The District has been monitoring the stage of both Blue Sink and the Ewanowski Spring Pool. However, in April 2022 the District no longer had access to the spring for pool stage measurements. This should not limit the ability to evaluate the impacts of diversions on

Ewanowski Spring based on an evaluation of the water levels in Blue Sink and Ewanowski Spring conducted by the District; Blue Sink stage observations can serve as a surrogate for the Ewanowski Spring Pool stage. The sink and spring are hydraulically connected by a short conveyance ditch, and the stages are very highly correlated with an R^2 value of 0.98 (SWFWMD 2022, Appendix D). Further, the USGS continues to measure the flow from the Ewanowski Spring Outlet.

2.3.3 Tampa Bypass Canal

The TBC has been used to augment the Hillsborough River Reservoir since 1985 in support of withdrawals from the reservoir by the City. Flow from the TBC to the base of the Hillsborough River Dam was added in 2008 as part of the Hillsborough River Recovery Strategy, which required the District, by January 1, 2008, to divert up to 7.1 mgd (11 cfs) of water from the middle pool of the TBC to the Hillsborough River Reservoir at Structure S-161. The recovery strategy also established a priority for sources of diversions from the TBC to the Hillsborough River as follows:

- Priority Source One are diversions from the TBC middle pool when the middle pool is above 12 feet NGVD and the flow over Structure S-162 is at least 11 cfs (~7.1 mgd). This scenario included several constraints:
    - Seventy-five percent of the water diverted to the Hillsborough River from TBC must be delivered to the base of the Hillsborough River Dam (allows for the 25% assumed loss at the time of adoption).
    - The flow diverted is limited to the amount needed to achieve the minimum flow and must not exceed 7.1 mgd (11 cfs) on any day.
    - The diversions must cease if the difference in elevation between the TBC middle and lower pool exceeds 7 feet.
    - When the minimum flow for the LHR is met, any water in the TBC middle and lower pools above elevations 12 and 9 feet NGVD, respectively, is available for water supply.
- Priority Source Two are diversions from the TBC middle pool when the middle pool is above 12 feet NGVD but the flow over Structure S-162 is less than 11 cfs (~7.1 mgd). Under this scenario, additional provisions require the District to divert flow from the TBC lower pool to the TBC middle pool equivalent to the flow diverted from the middle pool to the Hillsborough River Reservoir. Operations must cease diversions if the TBC lower pool elevation reaches 6 feet as measured at Structure S-160. Further, the District is only allowed to reinitiate withdrawals from the TBC lower pool when the elevation reaches 9 feet for at least 20 consecutive days.
- Priority Source Three are diversions from the TBC middle pool when middle pool elevations are between 10 and 12 feet NGVD. Under this scenario, diversions are only allowed when the TBC lower pool elevation is 6 feet NGVD or greater and are limited to the quantity needed to achieve the LHR minimum flow after using other available sources. Diversions must not exceed 7.1 mgd (~11 cfs) on any day. Diversions must cease if the difference in elevation between the Hillsborough River Reservoir and the TBC middle pool exceeds 9.5 feet, the difference in elevation between the TBC middle and lower pools exceeds 7 feet, or the stage in the middle pool goes below 10 feet

NGVD. The District is only allowed to reinitiate withdrawals from the TBC middle pool when the elevation reaches 12 feet NGVD for at least 20 consecutive days.

As part of the recovery strategy, water has been supplied from the TBC to the LHR as needed since December 31, 2007. WUP No. 20020575 for these diversions was issued by FDEP to the District on December 17, 2015.

The District's temporary pumping facilities at Structure S-161 and at the dam were transferred to the City in November 2017. WUP No. 20020802 for diversion of water from the TBC to the reservoir was issued to the City by the District on April 23, 2019. The WUP previously issued by FDEP to the District for these diversions was cancelled. An agreement between the City and District for the Dam Control Gate Facilities (Project N492) to replace temporary pumping facilities at the dam was finalized in October 2017. Construction and operational tests for the gate were completed on July 20, 2018, and the City began using it for minimum flow implementation on April 1, 2019.

During the transition of responsibility from the District and City, some initial miscommunications about operational responsibility resulted in gaps in the flow records. The District stopped recording these diversions on January 1, 2018, and the City did not start recording until March 15, 2018. This delay left two data gaps during periods of minimum flow implementation (January 1, 2018, to January 23, 2018, and March 2, 2018, to March 18, 2018).

Pumping activities for the LHR minimum flow implementation have been conducted mainly by the City since 2018. In accordance with the recovery strategy, however, the District has continued to own and operate the facilities necessary to transfer water from the lower pool to the middle pool of the TBC at Structure S-162.

2.3.4 Morris Bridge Sink

The Hillsborough River Recovery Strategy specifies that by October 1, 2012, or earlier and upon completion of the Morris Bridge Sink Project, the District will divert up to 3.9 mgd (6.0 cfs) of water on any day from Morris Bridge Sink to the TBC middle pool for the City to transfer to the reservoir and release to the base of the Hillsborough River Dam to help achieve minimum flows in the LHR. This flow rate was based on a 30-day pumping test conducted by District in 2009, which concluded that the sink could sustain a withdrawal rate of 6 cfs (3.9 mgd) during extremely dry conditions (SWFWMD 2010, Appendix E). However, the diversions from the sink were limited to those needed to meet the LHR minimum flow after using other sources (Sulphur Springs and Blue Sink) and after the District diverts up to 7.1 mgd (11 cfs) from the TBC lower pool to the TBC middle pool (depending on water levels).

On January 15, 2016, FDEP issued WUP No. 20020574 to the District for withdrawals from Morris Bridge Sink. This permit allows for a peak monthly and maximum daily pumping rate of 3.9 mgd (6 cfs) and an annual average withdrawal rate of approximately 2 mgd (3 cfs) from Morris Bridge Sink. Rule 40D-2.302(2), FAC established the reservation of water in Morris Bridge Sink. The WUP requires the reservation to be repealed before the District can use the recovery source. In February 2016, the District initiated a project for consultant services for the design of a pump station at Morris Bridge Sink to divert water from the sink to the upper pool of the TBC, a pipeline, and a second pump station at Structure S-159 to divert water from the upper to the middle pool of the TBC. Project design and permitting have been completed. To date the pumping station has not been constructed; however, portable temporary pumps and pipes could be installed to commence withdrawals of Morris Bridge Sink when needed to meet the LHR minimum flow requirements once the reservation under 40D-2.302(2) is repealed.

2.3.5 Transmission Pipeline Evaluation and Project

The recovery strategy required 75% of the permitted 11 cfs (~7.1 mgd) to be transmitted from the TBC to the LHR. This allowed for an assumed 25% loss of water via the transmission of that water. As part of the Hillsborough River Recovery Strategy, the construction of a pipeline from the TBC middle pool at Structure S-161 to the base of the Hillsborough River Dam was considered to address potential water savings. A peer review of this project was conducted and submitted to the District and City in September 2008 (Davis et al. 2008, Appendix F). The peer review concluded "…the projected water saving by transporting the augmentation water in a pipeline rather than through the reservoir is relatively small." District and City staff concluded that the transmission pipeline project is no longer a viable project for recovery of the LHR (SWFWMD 2008). The Recovery Strategy required the City to provide 1.9 mgd from another permittable source if the pipeline was not constructed. In 2022, the City provided a letter to the District (Appendix G) identifying that the 25% assumed loss would be used as the other permittable source and has been releasing that water to the base of the dam since 2022.

2.3.6 Investigation of Storage or Additional Supply Options

Consistent with the recovery strategy, the City and District entered into a joint funding agreement in July 2010 to investigate other storage and supply options to meet recovery strategy objectives for the LHR. A review of other available sources (MHW Americas, Inc. 2011) indicated that the identified sources of water in the recovery strategy may be sufficient for achieving minimum flow requirements in the LHR. A project completion report (Weber 2018, Appendix H) submitted to the District by the City in October 2018 also suggested that the City is positioned and committed to implementing and investigating projects that will ensure that the LHR minimum flows are met.

2.3.7 Wetland Restoration

Paragraph 40D-80.073(9) FAC discusses a wetland restoration project concept adjacent to McKay Bay and Palm River estuary. It describes the commitment from the City of Tampa to provide up to 7.1 mgd (~ 11 cfs) reclaimed water for the wetland project. However, before spending public funds on a feasibility study or on project design, District staff desired a greater understanding of the potential for obtaining necessary permits from FDEP. District and FDEP staff met on November 2, 2010, and concluded that obtaining a permit for the discharge of reclaimed water from the City's Howard F. Curren Advanced Wastewater Treatment Plant into McKay Bay would not be feasible unless the project resulted in a net reduction in nutrients.

2.3.8 Recovery Project Status Summary

The Hillsborough River Recovery Strategy outlines six potential projects and a timeline for their implementation. Four projects are identified for joint funding by the District and the City of Tampa, and two are to be implemented by the District. Implementation of specific projects is subject to applicable diagnostic/feasibility studies and contingent on obtaining any required permits. Projects to be jointly funded by the District and the City are:

- Sulphur Springs Project (Lower Weir Modifications and Sulphur Springs Pool Upper Weir and Pump Station Modifications).
- Blue Sink Analysis and Project.
- Transmission Pipeline Evaluation and Project.
- Investigation of Storage of Additional Supply Options.

Projects to be implemented by the District:

- Tampa Bypass Canal (TBC) and Hillsborough River Reservoir Diversions.
- Morris Bridge Sink Project.

The projects are intended to provide a sufficient flow of freshwater and low-salinity water below the Hillsborough River Dam to restore low-salinity habitat within the LHR and achieve an oligohaline zone (salinity < 5 ppt) from the dam toward Sulphur Springs. All projects proposed in the adopted recovery strategy are either completed or have been deemed not viable or actionable.

2.4 Assessment of the Recovery Strategy

2.4.1 Schedule

The Hillsborough River Recovery Strategy provides a detailed schedule for phased implementation of the recovery flows in 40D-8.041(1), FAC, by the District and City. The strategy identifies specific recovery sources, the order of priority for using each recovery source, the expected volumes from each source, and the specific water elevations and flows governing withdrawal volumes from individual sources. The schedule also incorporates by reference a joint funding agreement between the District and City for implementing the recovery projects needed to meet the minimum flow.

Although the schedule provided flexibility in how the minimum flow was ultimately attained (including evaluating using aquifer storage and recovery and additional source options), it required that the minimum flow be met by October 1, 2017, or earlier. As noted later in this document, since 2018 flows have come close to meeting the goals of the recovery strategy and by 2023, the minimum flows have been regularly met.

2.4.2 Five-Year Assessments

2.4.2.1 First (2015)

The first 5-year report was finalized in April 2015 and examined the changes in hydrobiological conditions of the LHR in response to implementation of minimum flows between 2002 and 2013 (SWFWMD and Atkins 2015). The report's findings support the minimum flows that were adopted in 2007. However, the report also noted that minimum flows in the range of 23 cfs (14.9 mgd) to 26 cfs (16.8 mgd) produced more consistent, beneficial results and that achieving a volume of flow corresponding to total minimum flow rates in this range should be a primary management goal. Minimum flow rates between 23 cfs (14.9 mgd) to 26 cfs (16.8 mgd) produced significantly lower and less variable salinity conditions compared to 20 cfs (12.9 mgd) (which is the minimum flow that applies from July 1 through March 31). However, the report found that the benefits of the increased minimum flows were largely limited to shallow waters beginning about 2 km below the dam due to the vertical stratification in the area. Minimum flow rates between 22 cfs (14.2 mgd) and 25 cfs (16.2 mgd) are effective at preventing hypoxic conditions upstream of Hannah's Whirl, but as was seen for salinity the improved DO levels from Hannah's Whirl to Sulphur Springs were limited to shallow waters less than 2 meters given the vertical salinity stratification. Implementing the minimum flows moderated temperature and color levels below the dam when using diversions from Sulphur Springs but had little effect on pH. No negative side effects were expected from these results. Implementing the minimum flows did not adversely affect abundance or taxonomic richness of zooplankton but did reduce diversity slightly due to a shift to freshwater species.

Implementing the minimum flows resulted in improved conditions for freshwater fish during the dry season, and a number of benthic macroinvertebrate species characteristic of freshwater and low salinity waters increased. Increased diversions from Sulphur Springs benefit the lower river, but diversion of greater quantities from Sulphur Springs to the base of the dam has contributed to the growth of filamentous algae in the spring run. Many of the analyses in this report focused on selected dates where flows over the spillway at the dam were less than 1 cfs (0.65 mgd), and statistical tests were conducted on the differences in analyte among various minimum flow periods where the recovery source contributions changed.

2.4.2.2 Second (2020)

The second 5-year report assessed the effects of the recovery strategy on water quality and quantity above and below the Hillsborough River Dam from October 1, 2012, through May 31, 2018 (WAR, 2020) and compared the results to three previous periods with varying levels of minimum flows:

- Period 1 with no established minimum flow from October 1, 1979, to February 28, 2002.
- Period 2 with a minimum flow of 10 cfs (6.5 mgd) from March 1, 2002, to December 31, 2007.
- Period 3 with the current minimum flow from January 1, 2008, to September 30, 2012.
- Period 4 with minimum flows requiring 20 or 24 cfs (12.9 – 15.5 mgd) or freshwater equivalents, adjusted for flow at the USGS Hillsborough River at State Park near Zephyrhills FL Gage (No. 02303000), from October 1, 2012, to May 31, 2018.

The assessment found significant differences in water quality between Period 1 (no minimum flow implementation) and Periods 2, 3, and 4. Most notably, the report concluded that recovery strategy implementation had successfully extended the low salinity (< 5-ppt) zone from the base of the dam toward Sulphur Springs. There were also significant differences in water quality between Period 2 and Periods 3 and 4 with recovery source utilization, which generally had similar water quality and have the same minimum flow requirements. Like the previous report, Water & Air Research, Inc. (WAR) assessed water quality when flow over the dam was < 1 cfs rather than all flow conditions during each period; however, they used a different methodology for evaluating the effects of the implementation by using monthly average flows in some cases. Conclusions about the biological community health included:

- Nekton habitats improved following recovery strategy implementation but catch per unit effort decreased with lower salinity.
- Benthic macroinvertebrate richness and diversity increased with minimum flow implementation.
- Zooplankton taxa richness increased with minimum flow implementation.
- Benthic macroinvertebrates and zooplankton communities showed evidence of a shift toward more freshwater community.

2.4.2.3 Third (2025)

Given the difference in methodologies applied in the first and second assessment reports, the goal of this report is to provide a unified assessment method applied to the data over the span of the minimum flow development and implementation process (i.e., 1996 through 2023). The overall goals of the assessment were to *evaluate the effects of minimum flow implementation on the Lower Hillsborough River* including:

- Descriptive and quantitative evaluation of flows and other hydrologic data identified for inclusion in the 5-year assessment.
- Descriptive and quantitative evaluation of changes in salinity and other water quality parameters as a function of minimum flows implementation.
- Descriptive and quantitative evaluation of changes in biology (benthos, zooplankton, and nekton) as a function of minimum flows implementation.
- Descriptive evaluation of changes in water levels above the Hillsborough River Dam as a function of minimum flows implementation.

3 METHODS

This section describes the data sources and analytical methods used in assessing the recovery strategy.

3.1 DATA SOURCES

Principal data sources included the USGS, Environmental Protection Commission of Hillsborough County (EPC), City of Tampa, the District, TBW, and data collected by other entities under the auspices of these entities. The raw data were compiled and described in memorandums included in the report appendices (Appendices I1–H6*)*. The POR data were compiled for includes January 1, 1996, through December 31, 2023.

3.1.1 HYDROLOGIC

Hydrologic data includes flow and water levels. The hydrologic data that were compiled for this report is documented in Appendix I1. Data were obtained from USGS, City of Tampa, and the District and include:

- USGS Hillsborough River Near Tampa FL Gage (No. 02304500)
- USGS Hillsborough RV at State Park NR Zephyrhills, FL Gage (No. 02303000)
- USGS Sulphur Springs at Sulphur Springs FL (Sulphur Springs Pool) Gage (No. 02306000)
- City of Tampa Blue Sink Flow Contribution
- HRR to Base of Dam Flow Contribution
- City of Tampa Sulphur Springs Pumped to Base of Dam Flow Contribution
- City of Tampa Sulphur Springs Pumped to Run Flow
- TBC Middle Pool to HR Reservoir Diversion Flow (S161)
- WMD TBC Lower Pool to Middle Pool Flow Diversions (S162)

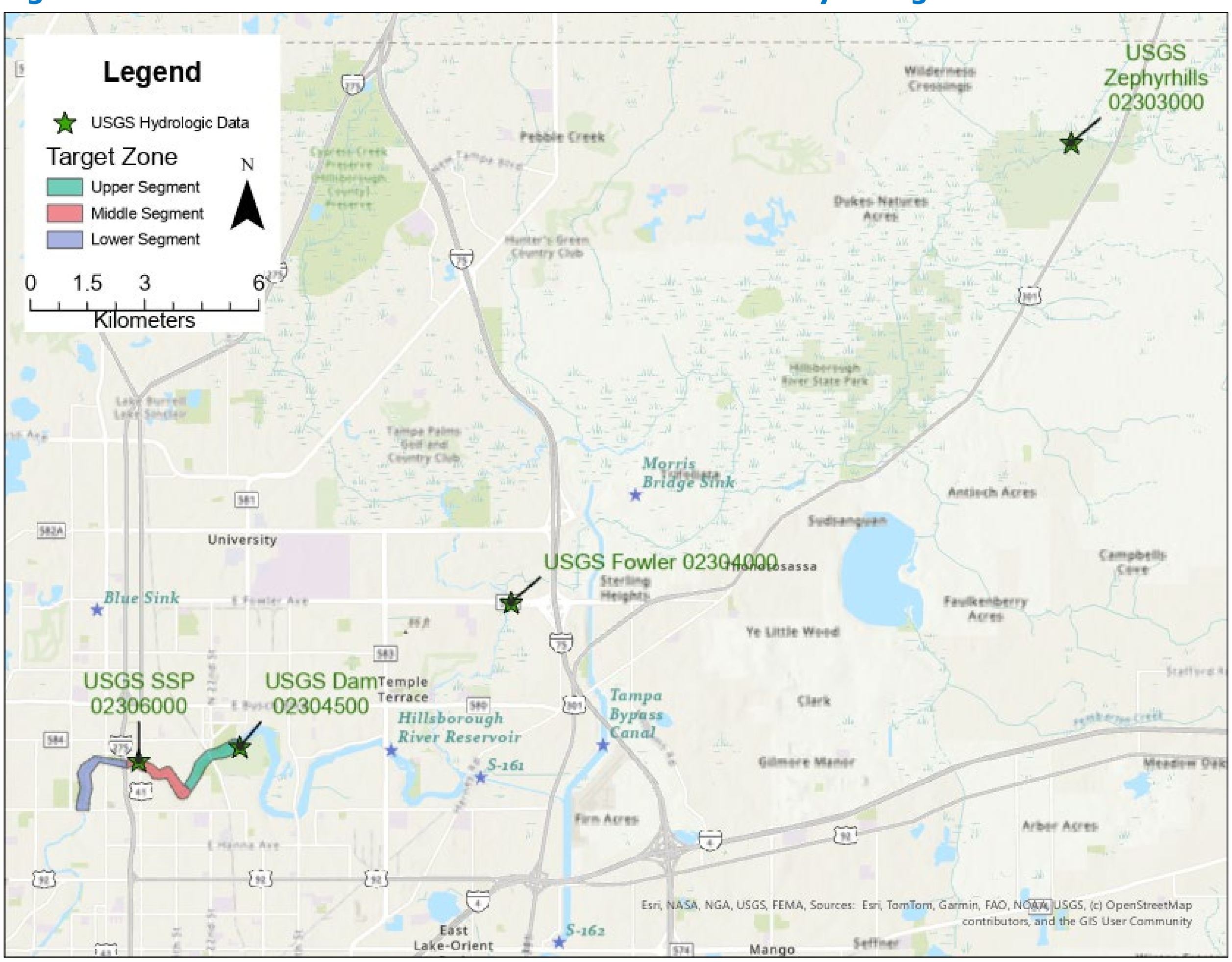
3.1.1.1 USGS

Continuous record (CR) data (high frequency, at 15-minute intervals) are collected via automated sensors installed at fixed monitoring locations. The USGS CR stations considered in this report are listed in Table 3.1-1, and the locations are displayed in Figure 3.1-1.

Table 3.1-1: USGS continuous recorders – hydrologic data

Gage Name	Parameter	Date Range Data is Available
USGS Hillsborough RV at State Park Nr Zephyrhills, FL (Zephyrhills) Gage No. 02303000	Discharge	03/15/1987–12/31/2023
USGS Hillsborough R at Fowler Av Near Temple Terrace, FL (Fowler) Gage No. 02304000	Water Level	05/31/2008–12/31/2023
USGS Hillsborough River Near Tampa FL (Dam) Gage No. 02304500	Discharge Water Level	10/01/2007–12/31/2023 10/01/2007–12/31/2023
USGS Sulphur Springs at Sulphur Springs FL (SSP) Gage No. 02306000	Discharge Water Level	10/09/1986–12/31/2023 10/01/2007–12/31/2023

Figure 3.1-1: USGS continuous recorder locations – hydrologic data



3.1.1.2 City of Tampa

City of Tampa hydrologic data were provided by the District, as reported to them by the City. Continuous data (high frequency, at 1-hour intervals) were collected via automated sensors installed at fixed monitoring locations. Data were provided as both hourly data and daily averages. Table 3.1-2 summarizes the daily average data availability.

Table 3.1-2: City of Tampa continuous recorders – hydrologic data

Gage Name	Parameter	Date Range Data is Available
COT Blue Sink to Base of Dam	Discharge	12/17/2017–12/31/2023
COT Sulphur Springs to Base of Dam	Discharge	01/01/2002–12/31/2023
COT Sulphur Springs Pool Pumped to Run	Discharge	02/22/2012–12/31/2023
COT S161 - TBC Middle Pool to Hillsborough River Reservoir	Discharge	01/01/2018–12/31/2023
COT Pumped to Base of Dam	Discharge	01/01/2018–12/31/2023

3.1.1.3 District

District hydrologic CR data are collected via automated sensors installed at fixed monitoring locations at a high rate of frequency (15-minute intervals). Table 3.1-3 summarizes the daily average water level and flow data availability.

Table 3.1-3: District continuous recorders – hydrologic data

Gage Name	Parameter	Date Range Data is Available
WMD S161 - TBC Middle Pool to Hillsborough River Reservoir	Water Level Discharge	12/31/2007–12/31/2017
WMD S162 - TBC Lower Pool to Middle Pool	Water Level Discharge	12/31/2007–12/31/2023
WMD Pumped to Base of Dam	Discharge	12/31/2007–12/31/2017

3.1.2 Water Quality

Water quality data for this project included data collected in the lower river and in the recovery sources including Sulphur Springs, Blue Sink, Morris Bridge Sink, the Hillsborough River Reservoir, Tampa Bypass Canal, and long-term water quality sites above the dam.

Collecting agencies included the USGS, FDEP, EPC, the District, and Tampa Bay Water (TBW) for the Hydrobiological Monitoring Program (HBMP), as well as entities reporting data to Florida's Water Information Network (WIN) database, which was queried for this project.

Physical measurements and water quality data collected during biological sampling events were included in the water quality data set for analysis. The water quality data that were compiled for this report include CR data documented in Appendices I2 (A and B) and for synoptic sampling in Appendix I3. All CR data were converted to daily averages for analysis in this report.

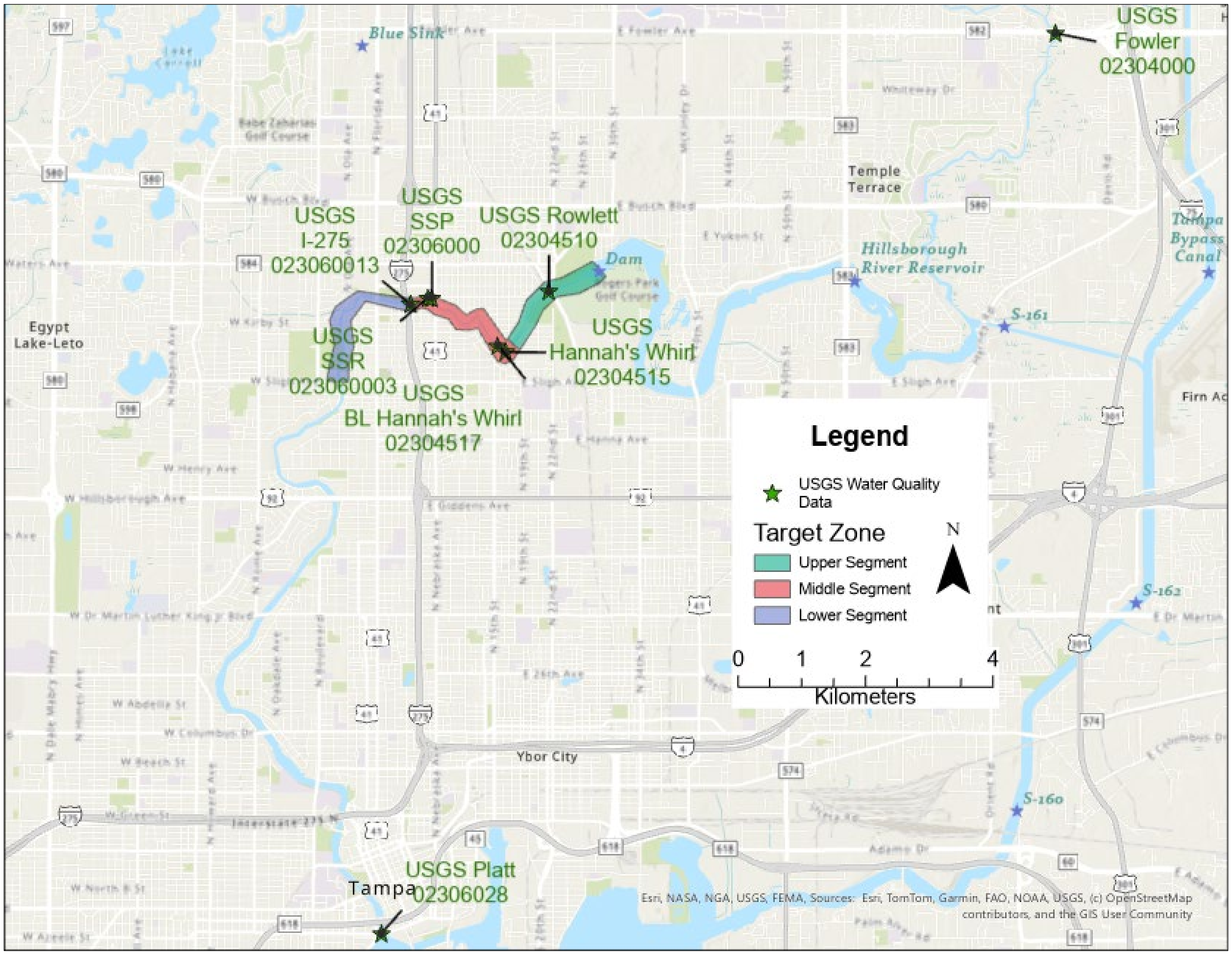
3.1.2.1 USGS

Water quality data was collected by USGS via automated sensors at a number of fixed-location continuously recording sites (15-minute intervals). The water quality data available is summarized in Table 3.1-4, and the locations are displayed in Figure 3.1-2.

Table 3.1-4: USGS continuous recorders – water quality fixed stations

Gage Name	Parameter	Date Range Data is Available
USGS Hillsborough River at Rowlett PK DR Near Tampa FL (Rowlett) Gage No. 02304510	Specific Conductance Water Temperature	12/02/1996–12/31/2023 12/02/1996–12/31/2023
USGS Hillsborough River at Hanna's Whirl at Tampa FL Gage No. 02304515	Specific Conductance Water Temperature Dissolved Oxygen	06/15/2001–10/11/2005 06/15/2001–10/11/2005 06/15/2001–06/30/2002
USGS Hillsborough R BL Hannahs Whirl NR Sulphur Spgs FL (BL Hannahs) Gage No. 02304517	Specific Conductance Water Temperature Dissolved Oxygen	10/25/2017–12/31/2023 10/25/2017–12/31/2023 10/25/2017–12/31/2023
USGS Hillsborough R. at I-275 Bridge at Sulphur Spgs FL (I-275) Gage No. 023060013	Specific Conductance Water Temperature	10/19/2012–12/31/2023 06/30/2011–12/31/2023
UGSG Hillsborough River at Platt Street at Tampa FL (Platt) Gage No. 02306028	Specific Conductance Water Temperature	01/09/1997–12/31/2023 01/09/1997–12/31/2023
USGS Sulphur Springs at Sulphur Springs FL (SSP) Gage No. 02306000	Specific Conductance Water Temperature	05/02/1999–12/31/2023 05/02/1999–12/31/2023
USGS Sulphur Springs Run at Sulphur Springs FL (SSR) Gage No. 023060003	Specific Conductance Water Temperature	05/25/1999–12/31/2023 05/25/1999–12/31/2023

Figure 3.1-2: USGS continuous recorders – water quality fixed stations locations



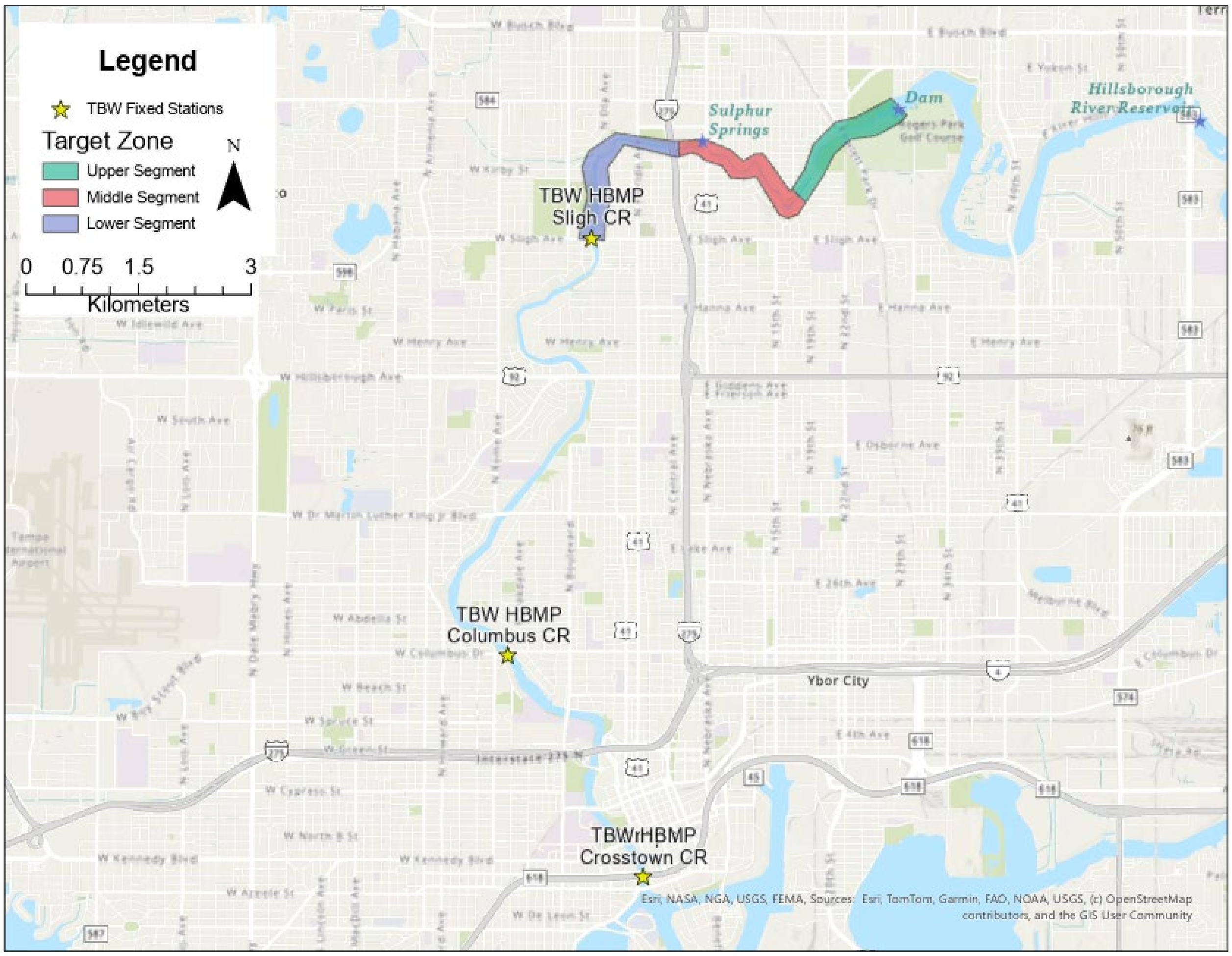
3.1.2.2 Tampa Bay Water HBMP

TBW implements an HBMP in accordance with special conditions in its WUP. The LHR HBMP reporting unit extends from the mouth of the river at Platt Street to the Hillsborough River Dam. This spatial reporting unit was divided into six strata, five of equal length (2.55 km) below Sulphur Springs and one of 3.61 km in length from Sulphur Springs upstream to the dam. The HBMP sampling program on the LHR originally included three continuous conductivity, temperature, salinity recorders (15-minute intervals), monthly water column profiles using a multi-parameter sonde at stratified random locations, and monthly water quality grab samples at the same locations. In addition, water column profiles were taken in association with benthic, plankton, and fish surveys, each conducted monthly. All elements but the CRs were discontinued in the LHR after WY2012. The CR water quality data availability is summarized in Table 3.1-5, and the locations are displayed in Figure 3.1-3.

Table 3.1-5: TBW continuous recorders – water quality fixed stations

Gage Name	Parameter	Date Range Data is Available
TBW HBMP Columbus	Salinity	02/15/2001–12/31/2023
	Water Temperature	02/15/2001–12/31/2023
TBW HBMP Sligh	Salinity	02/15/2001–12/31/2023
	Water Temperature	02/15/2001–12/31/2023
TBW HBMP Crosstown	Salinity	02/15/2001–12/31/2023
	Water Temperature	02/15/2001–12/31/2023

Figure 3.1-3: TBW continuous recorders – water quality fixed stations locations



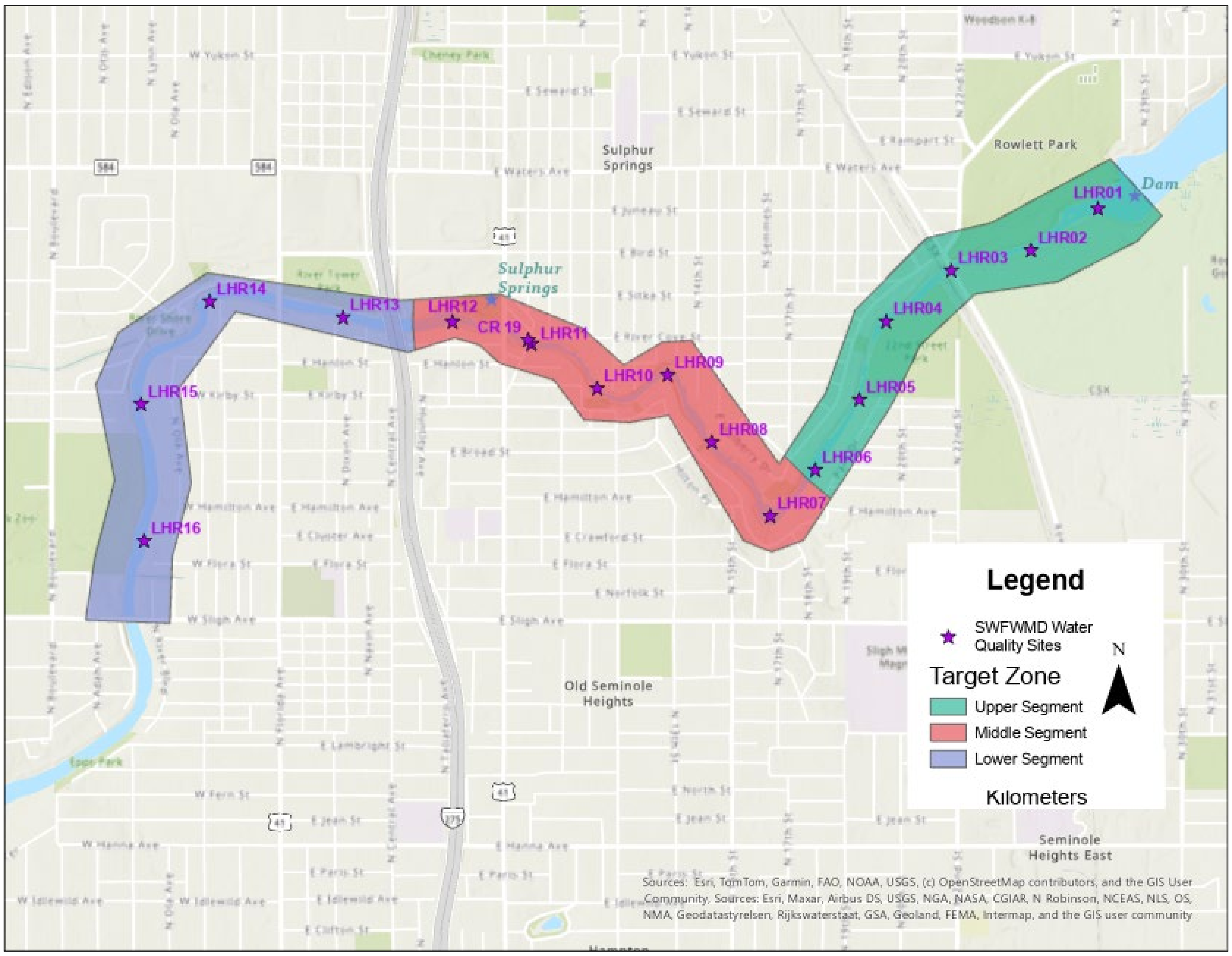
3.1.2.3 District

Water quality data collected by the District included grab samples, CR data, and a transect study to characterize the longitudinal variability in water quality within the target zone of the river. The District used an Rkm system to identify stations. This Rkm system was adopted from a hydrodynamic model grid and ordered from the mouth (Rkm 0) to the Dam (Rkm 16.2). This river kilometer system was also used to assign all water quality and biology data to an Rkm using GIS. The CR water quality data availability is summarized in Table 3.1-6, and the District fixed location stations are displayed in Figure 3.1-4.

Table 3.1-6: District – water quality fixed station

Gage Name	Parameter	Date Range Data is Available
WMD Station Number 19206	Salinity	04/15/2020–12/31/2023
	Water Temperature	04/15/2020–12/31/2023
	Dissolved Oxygen	04/15/2020–12/31/2023
	pH	04/15/2020–12/31/2023

Figure 3.1-4: District – water quality fixed station locations within the target zone



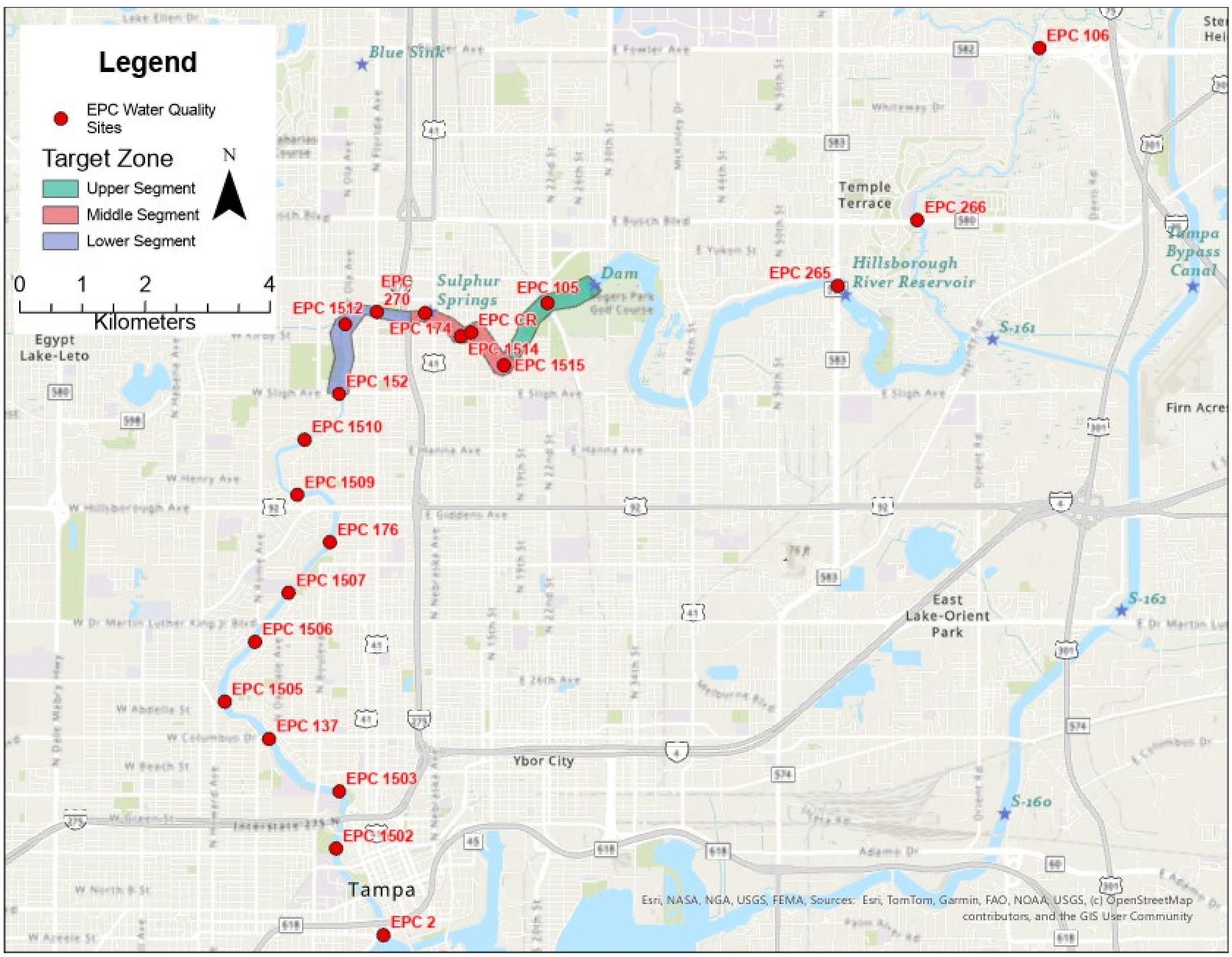
3.1.2.4 Hillsborough County EPC

The EPC established a County-wide surface water quality monitoring program in 1972. Originally, 53 fixed sampling stations were established throughout Tampa Bay; in 1973 sampling in the tributaries was added. In 1975, the program began collecting water column profiles to collect hydrographic data at the surface, mid, and bottom depths. The monitoring program was expanded in 2000 in response to the construction of several potable water projects including withdrawal structures on the Alafia River and TBC and a desalination plant at Apollo Beach. This supplemental monitoring, known then as the Hillsborough Independent Monitoring Program (HIMP), included fixed continuous monitoring stations, specialized diurnal studies, and monthly hydrographic surveys on the Hillsborough River, Palm River/McKay Bay system, Alafia River, Little Manatee River and in the vicinity of the Apollo Beach desalinization plant. The HIMP ended in 2006; however, fixed continuous monitoring on the Hillsborough and Alafia Rivers and the monthly hydrographic surveys have been incorporated into the monitoring program. One CR fixed station has been discontinued, located at Rkm 13.7 (Nebraska Avenue). The CR water quality data availability is summarized in Table 3.1-7, and all EPC fixed location stations are displayed in Figure 3.1-5.

Table 3.1-7: EPC – water quality fixed stations

Gage Name	Parameter	Date Range Data is Available
EPC CR Nebraska (Rkm 13.7)	Salinity	05/14/2002–05/15/2015
	Water Temperature	05/14/2002–05/15/2015
	Dissolved Oxygen	05/14/2002–05/15/2015

Figure 3.1-5: EPC – water quality fixed station locations



3.1.2.5 Florida Department of Environmental Protection (FDEP)

FDEP runs a comprehensive Water Quality Assessment Program. This program is designed to monitor and assess the quality of Florida's surface water resources including implementing statewide status and trend monitoring networks, developing monitoring plans and coordinating with regional operation centers to collect data for assessing water quality, or executing strategic monitoring plans and using collected data to identify impaired waters and determine restoration actions. These data are housed in the statewide WIN repository, which was queried for this project. All data were collected within the Hillsborough River Reservoir.

3.1.3 Biological Data

Biological monitoring for zooplankton, benthic invertebrates, and nekton was conducted over time by several entities. The qualitative analysis was limited to Analysis Days within the target zone and downstream. Quantitative analysis was limited to Analysis Days within the target zone.

3.1.3.1 Zooplankton

The zooplankton data compiled for this report are documented in Appendix I4. Sampling locations within the LHR target zone (base of dam to Sligh Avenue) are shown in Figure 3.1-6, and locations within the LHR downstream area (Sligh Avenue to Platt Street) are shown in Figure 3.1-7. Data sources are described below.

Figure 3.1-6: Zooplankton station locations within the LHR target zone

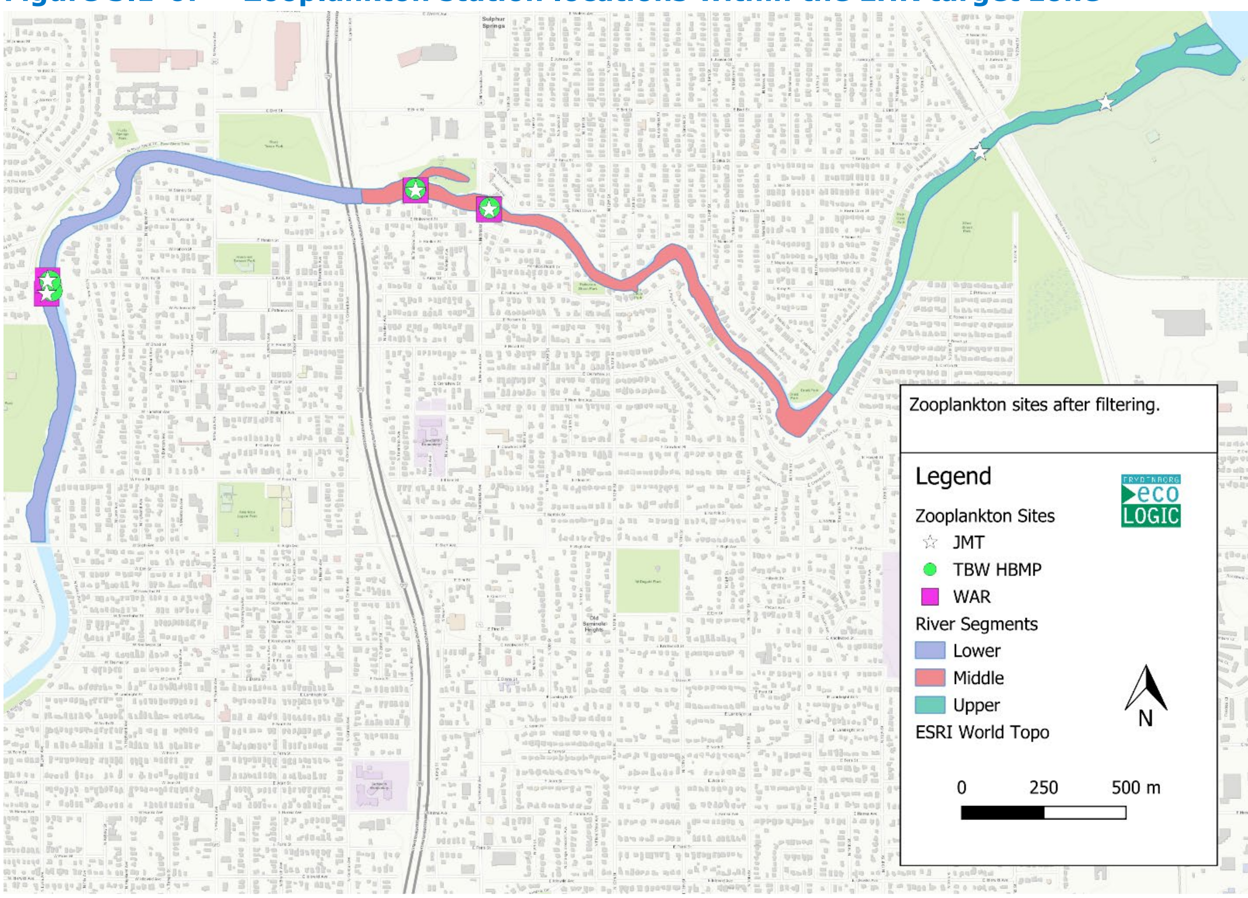
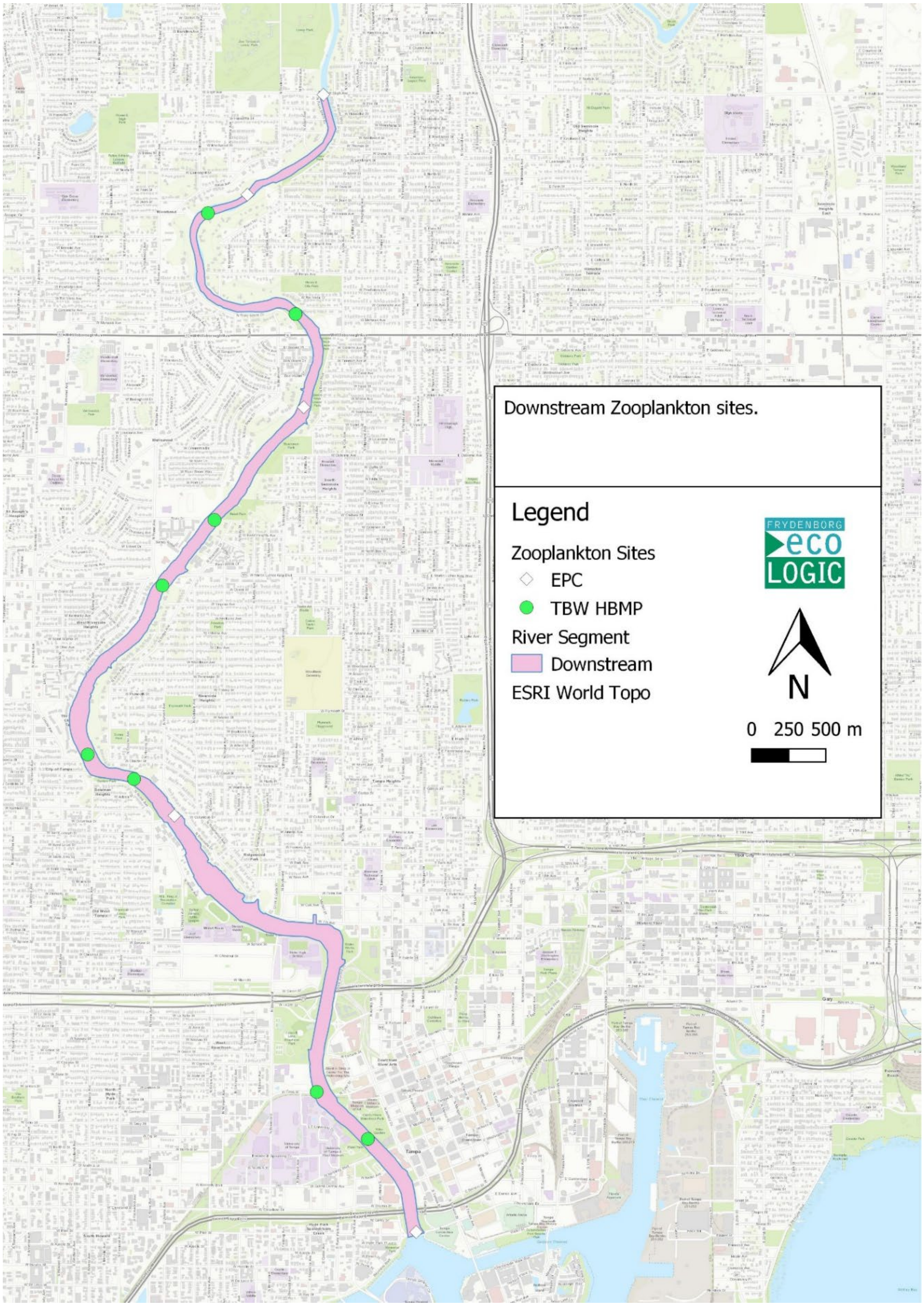


Figure 3.1-7: Zooplankton station locations within the LHR downstream



Tampa Bay Water HBMP

From April 2000 through September 2012, the TBW HBMP performed monthly sampling at fixed stations, originally selected using a stratified-random approach (Atkins & JEI 2015). Sampling locations were split into several river strata, two of which overlapped with the middle and lower river segments of the study area used for this evaluation, with two sampling tows per strata. Sampling occurred during flood tides, beginning approximately 2 hours after sunset, to maximize zooplankton catch.

Zooplankton tows were completed using a 500-µm Nitex mesh conical net with a 0.5-m mouth diameter and a 3:1 length to mouth ratio. Deployments consisted of oblique tows divided between the bottom, mid-depth, and surface waters, pulled for a duration of 5 minutes in the center of the river channel at a boat speed of 1.0 to 1.5 m/s. This resulted in sampling of approximately 70 to 80 cubic meters of water (SWFWMD & Atkins 2015). Zooplankton taxa were identified and enumerated in the laboratory by Dr. Ernst Peebles from the University of South Florida.

District

Water & Air Research

Water & Air Research (WAR) sampled four of the TBW HBMP fixed stations in April and May 2018 in the middle and lower river segments within the target zone. Sampling methods and parameters mimicked those employed by the TBW HBMP. Samples were rinsed from the net into a cod-end jar, and the final volume in the jar was reduced to 500–800 mL prior to the addition of 50 mL of formaldehyde in the field.

In the laboratory, samples were separated using stacked sieves with mesh openings of 4 mm and 250 µm. Fish and macroinvertebrates in the 4-mm fraction were typically identified and enumerated without magnification. The > 250 µm fraction of organisms was identified and enumerated under magnification (as high as 90X). The smaller fraction was processed in two steps. First the entire sample, divided into 10–15 mL aliquots, was scanned using a gridded Petri dish. Uncommon taxa (n < 50) were enumerated during this step. For the second step, the aliquots from the first step were recombined, and the total volume was recorded, well-mixed, and a single 30–60-mL aliquot was examined. This subset was identified and enumerated by Dr. Ernst Peebles.

Johnson, Mirmiran, and Thompson

Zooplankton were collected by Johnson, Mirmiran, and Thompson (JMT) for the District during seven events: May 2020, November 2020, May 2021, March 2022, April 2023, June 2023, and December 2023(JMT 2024). The methods for zooplankton collection, preservation, and sample processing replicated those used by TBW HBMP and WAR, as described above. Sampling occurred at the same locations in the lower and middle segments of the river target zone as previously sampled by WAR and the HBMP, and two additional fixed sampling locations were established in the upper segment of the target zone. Zooplankton taxa were identified and enumerated in the laboratory by Dr. Ernst Peebles from the University of South Florida.

3.1.3.2 Benthic Macroinvertebrates

The benthic macroinvertebrate data compiled for this report are documented in Appendix I5. Figure 3.1-8 shows sampling locations within the LHR target zone (base of dam to Sligh Avenue), Figure 3.1-9 shows sampling locations within the LHR downstream area (Sligh Avenue to Platt Street). Data sources are described below.

Figure 3.1-8: Benthic macroinvertebrate station locations within the LHR target zone

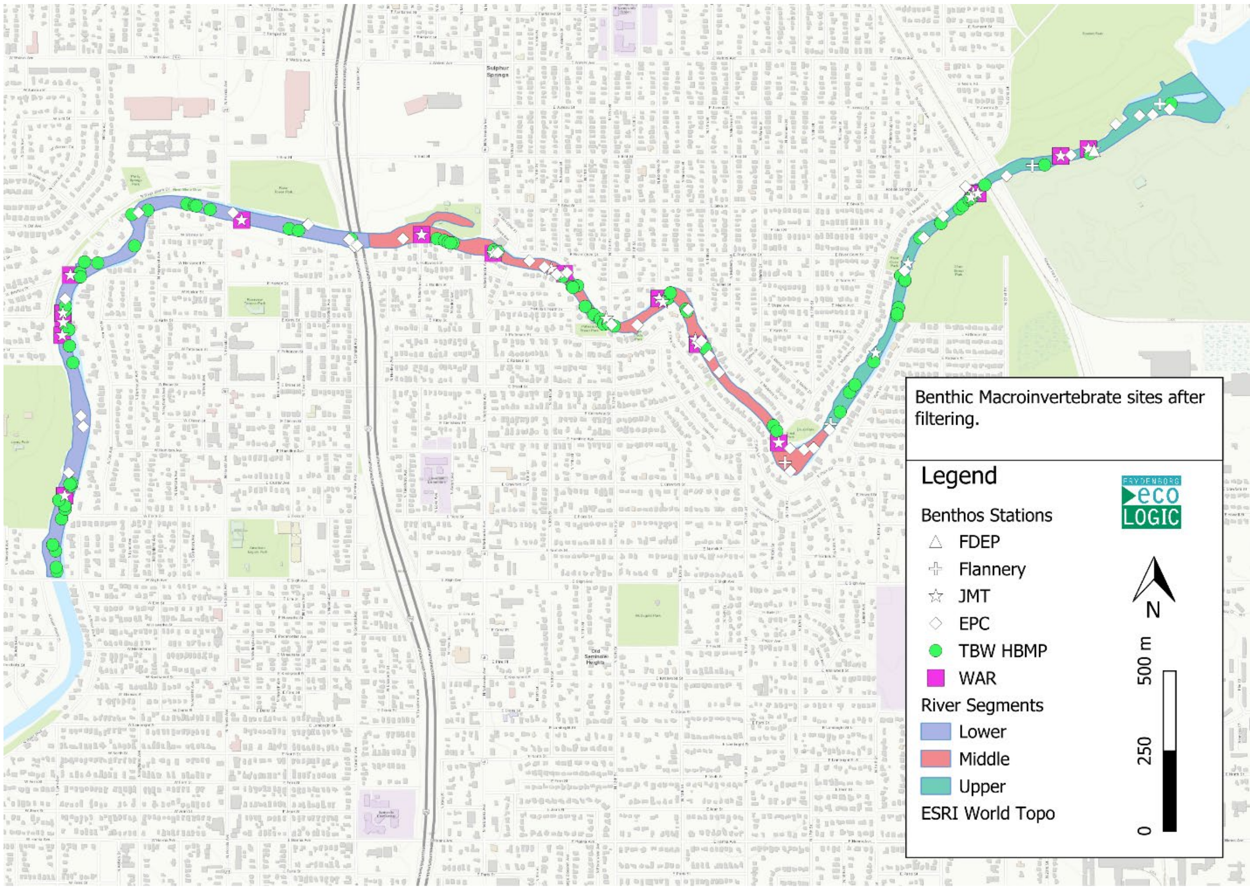
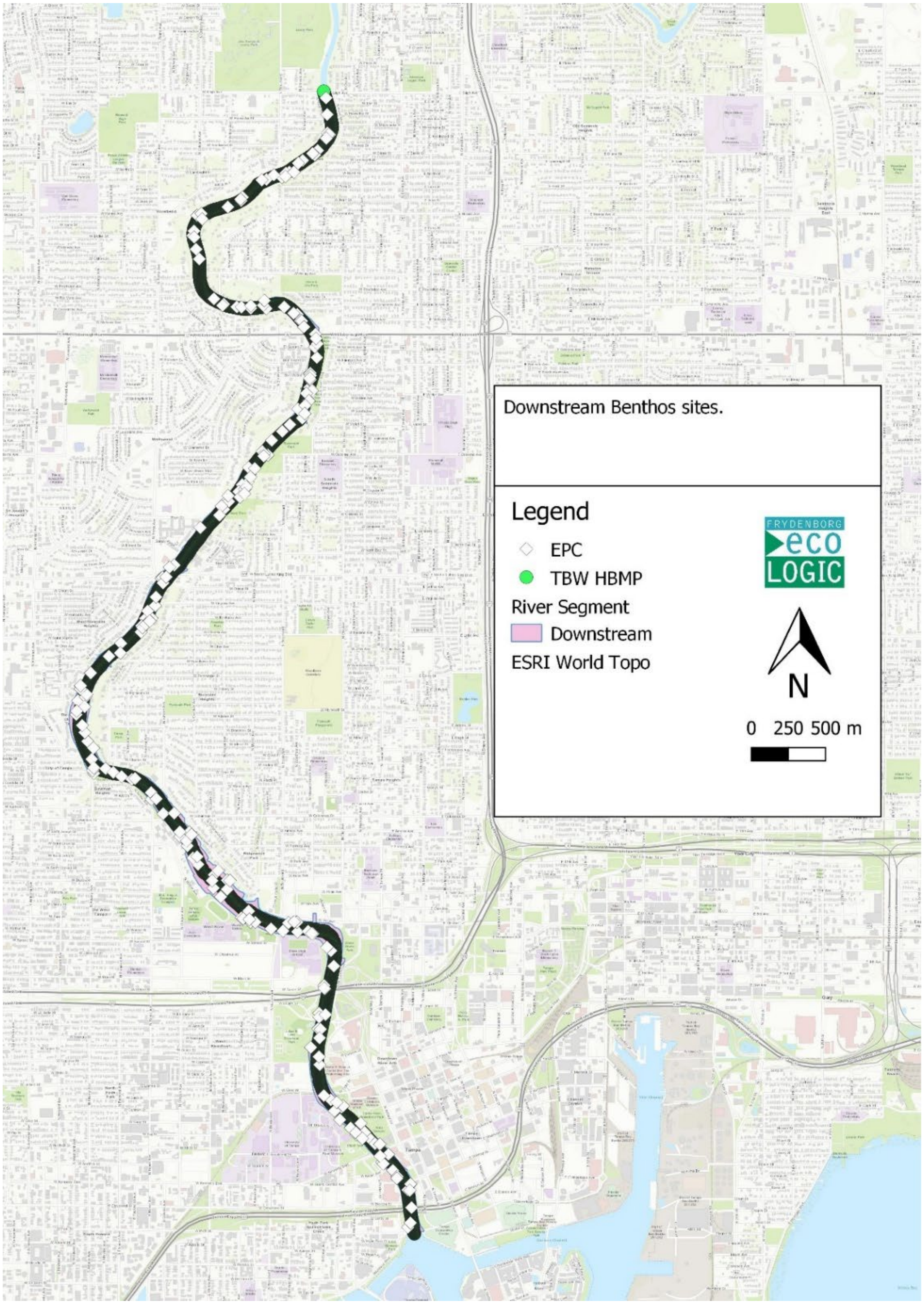


Figure 3.1-9: Benthic station locations within the LHR downstream



TBW HBMP

The TBW HBMP conducted monthly benthic macroinvertebrate sampling from April 2000 through September 2010 using a probabilistic design that randomized sampling locations within defined river strata. Because strata defined by the TBW HBMP did not perfectly match those river segments defined in this assessment, the number of samples sampled per month within the lower, middle, and upper segments varied depending on where the randomized samples occurred.

Within each TBW HBMP-defined stratum, a Young-modified Van Veen grab sampler was used to collect two samples. Each grab sampled a 0.04 m^2 area of sediment. Taxa were identified and enumerated in the laboratory; however, the processing laboratory changed throughout the project. Consistent taxonomic identification at the same laboratory began in August 2005. Only samples from January to March and July to September were fully processed (WAR 2020).

District

WAR

In April and May 2018, WAR completed duplicate sampling of six TBW HBMP stations, selecting two stations from the lower, middle, and upper reaches of the target zone. Samples were collected with a petite Ponar grab (0.023 m^2 sediment surface area) and were fixed with 10% formalin and stained with rose Bengal dye in the field (WAR 2020).

In the laboratory, samples were rinsed with tap water in a 500-μm sieve. Macroinvertebrates were identified to the lowest practical taxonomic level under dissecting or compound microscopes, as appropriate. Five percent of the organisms were re-identified by a different technician for quality assurance.

JMT

Benthic macroinvertebrate samples were collected by JMT at fixed sampling locations in May 2020, November 2020, May 2021, March 2022, April 2023, June 2023, and December 2023 (JMT 2024). Six of the sampling locations replicated those used by WAR in 2018. Beginning in March 2022, two additional grab samples were taken in the middle and upper segments of the target zone, from fixed locations selected based upon the Consultant’s professional judgement. The methods for sample collection, preservation, and processing replicated those employed by TBW HBMP, as described above.

Hillsborough County EPC

The EPC collected samples by Van Veen dredge (0.04 m^2) between 1995 and 2009. These data only have years (not month or day) associated with the collection events. An estimated date of June 1 was assigned.

FDEP

FDEP has collected biological data from the Hillsborough River, accessible in their Statewide Biological Database (SBIO). This includes Rapid Periphyton Surveys, Ponar dredges, Stream Condition Index (SCI), Hester-Dendy (HD) deployments, and Lake Vegetation Surveys.

These data were evaluated spatially to determine if sampling locations intersected the study area. One SCI event and one HD sample occurred in the upper river segment, on the same date. All other samples occurred further upstream.

Non-Agency Data

Sid Flannery, Peggy Morgan, and Judy Ashton retired environmental scientists from the District and FDEP, respectively, collected macroinvertebrates at four stations on five dates between Hannah's Whirl and the dam using the FDEP BioRecon method (BRN 1000; Flannery et al. 2025). This method uses four 0.5-meter sweeps per site with a D-frame dip net (mesh size of 600 microns) to assess the most productive habitats for freshwater organisms, including rocks, tree roots, snags, and vegetation. Samples were collected between December 8, 2021, and May 24, 2023, at low tide. Sampling was designed to occur after prolonged wet periods or after minimum flows had established fresh conditions, as confirmed by continuous specific conductance recorders. Data collected by Flannery et al. (2025) were considered to be qualitative and were not used for quantitative analyses.

Using this method, nineteen taxa were collected that were not observed in benthic grab samples. The species composition of samples varied between upstream and downstream sites and also at each individual station after prolonged flow versus periods of minimum flow.

3.1.3.3 Nekton

The nekton data compiled for this report are documented in Appendix I6. Figure 3.1-10 shows sampling locations within the LHR target zone (base of dam to Sligh Avenue), and Figure 3.1-11 shows sampling locations within the LHR downstream area (Sligh Avenue to Platt Street). Data sources are described below.

Figure 3.1-10: Nekton station locations within the LHR target zone

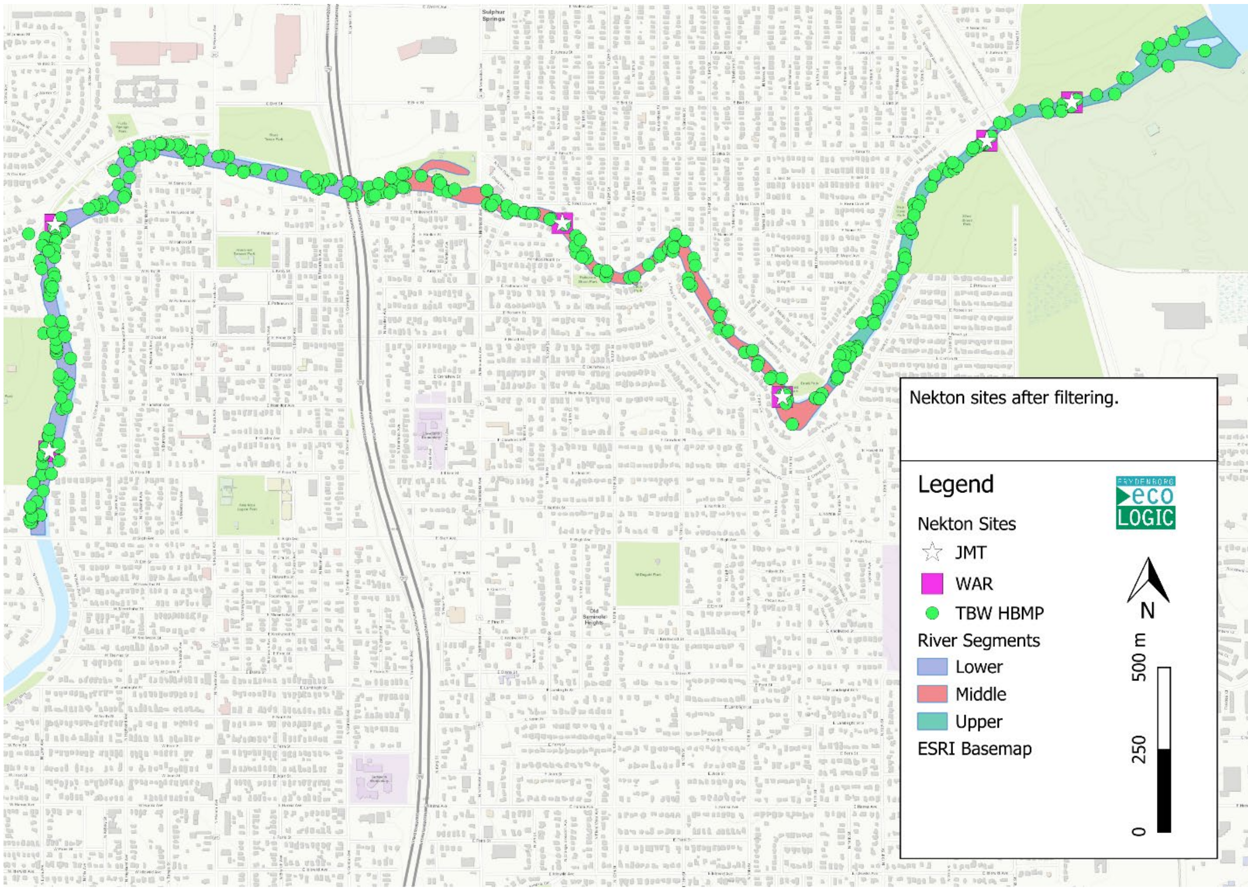
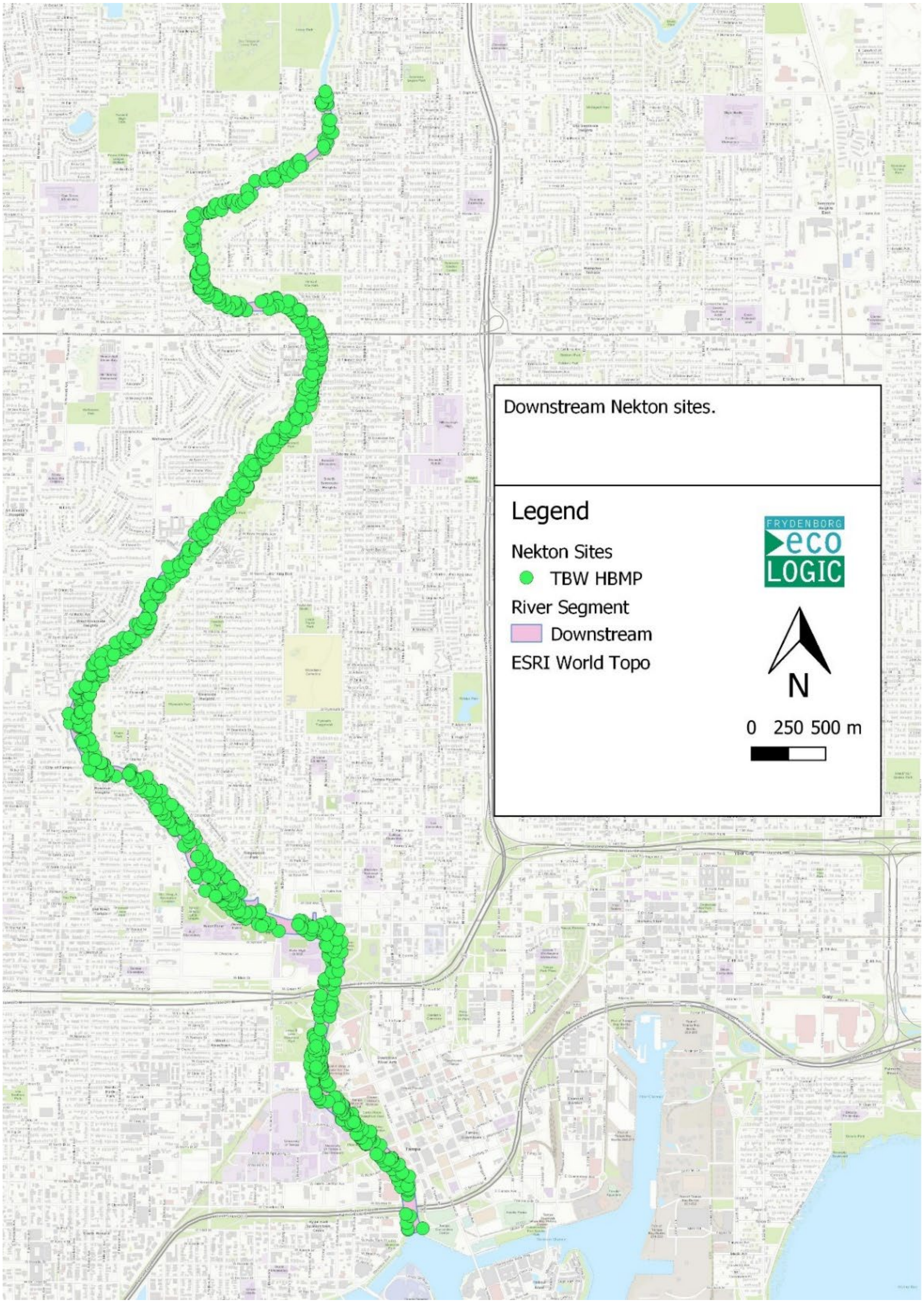


Figure 3.1-11: Nekton station locations within the LHR downstream



TBW HBMP

The TBW HBMP program performed monthly sampling at random locations within each of their defined river strata from May 2000 through June 2012. Within each strata, two 21.3-m seine hauls and one 6.1-m otter trawl were collected. Nekton were identified and enumerated in the field and released after documentation (Atkins & JEI 2015). Sample collection, processing, and effort calculations followed procedures outlined in the Florida Fish and Wildlife Conservation Commission (FWC) state-wide Fisheries-Independent Monitoring (FIM) sampling handbook (FWC 2019).

District

WAR

In April and May 2018, WAR collected two seine samples within the lower, middle, and upper segments of the target zone, using the same sampling locations each month, WAR 2020). Sites were randomly chosen from those sampled by TBW HBMP, and modified as needed for appropriate seine operation. Methods for sample collection and processing replicated those performed by the TBW HBMP and were in compliance with the FIM procedure manual.

JMT

Nekton data were collected within the target zone by JMT during May 2020, November 2020, May 2021, March 2022, April 2023, June 2023, and December 2023 at the same fixed locations that were sampled by WAR in 2018. Methods for sample collection and processing replicated those performed by TBW HBMP and WAR and were in compliance with the FIM procedure manual.

3.2 Flow Calculations

Total flows for the LHR and Sulphur Springs cannot be measured at a single gage and must be calculated from multiple data inputs. The data included in the calculation and the equations are detailed in Appendix I1.

3.2.1 Lower Hillsborough River Flows

Several sources of data and calculations are required to generate total flows provided to the base of the dam for the LHR. Table 3.2-1 provides the necessary data and contributing agencies.

Table 3.2-1: LHR total flow calculation

Source Agency	Flow Contribution Description
USGS (1996–2023)	+ Hillsborough River Dam Cresting Flow (cfs) measured at USGS 02304500
WMD (2008–2017) COT (2018–2023)	+ TBC Dam Flow (cfs) water pumped or released through the dam sluice gate for minimum flow implementation
COT (2002–2023)	+ Sulphur Springs Pumped to Base of Dam Flow (cfs)
COT (2018–2023)	+ Blue Sink Flow (cfs)
Calculation (1996–2023)	= Total Flow to LHR (cfs)

The total flow to the LHR is calculated as the sum of the USGS dam flow, City of Tampa flows pumped from Blue Sink and Sulphur Springs to the base of the dam, and diversions from Hillsborough River Dam Pump Station/Sluice Gate (sourced from TBC).

Provisional data from USGS gages were accepted along with all reported values. Fifteen days with missing values for USGS Dam flow were filled using linear interpolation, which results in the total flow over the dam always being zero or greater once the minimum flow was established.

The base minimum flow is a time-dependent criterion value that provided a foundation when establishing the required minimum flows for the LHR. Base minimum flow does not include any adjustments subsequently defined in the final minimum flow required by FAC for the LHR. The first minimum flow adopted for LHR became effective in 2000. The base minimum flow for 1999 and prior was set to zero since a minimum flow had not been adopted yet. The minimum flow adopted in 2000 for LHR was 10 cfs (6.5 mgd), and remained at 10 cfs (6.5 mgd) until the revised minimum flow was adopted in August of 2007 to its current form. The base minimum flow is set to 10 cfs (6.5 mgd) for January 1, 2000, through September 30, 2007. After September 30, 2007, the base minimum flow is set to 24 cfs (15.5 mgd) April through June and 20 cfs (12.9 mgd) July through March.

Beginning on October 1, 2007, an adjustment to the required minimum flow for the LHR was implemented. When flows at the USGS Hillsborough River at State Park Near Zephyrhills, FL Gage (No. 02303000) are below 58 cfs (37.5 mgd), an adjustment to the minimum flows is made based on the difference between Zephyrhills flow and 58 cfs (37.5 mgd). This difference is multiplied by a seasonal adjustment factor, which is then subtracted from the base minimum flow criteria to derive an adjusted criterion value ("Adjusted Minimum Flow"). Seasonal adjustment factors used if the USGS Hillsborough River at State Park Near Zephyrhills, FL Gage (No. 02303000) is less than 58 cfs (37.5 mgd) and the date is October 1, 2007, or later:

- 0.35 (July 1 through March 31)
- 0.40 (April 1 through June 30)

The applied minimum flow includes a 3 cfs (1.9 mgd) FWE beginning on October 1, 2007, where 3 cfs (1.9 mgd) is added on to the adjusted criterion value to derive the final minimum flow requirement for the LHR.

The applied minimum flow can be visualized in Figure 3.2-1 and represents the final rule required minimum flow, accounting for all temporal, USGS Hillsborough River at State Park Near Zephyrhills, FL Gage (No. 02303000), and FWEs adjustments.

3.2.2 Hillsborough River Reservoir to Base of Dam Flows

Freshwater is delivered to the base of the dam from the Hillsborough River Reservoir. This total freshwater input is calculated using USGS gage data and City of Tampa pumpage information (Table 3.2-2).

Table 3.2-2: Hillsborough River Reservoir to base of dam flow calculation

Source Agency	Flow Contribution Description
USGS (1996–2023)	+ Flow @ USGS Hillsborough River Near Tampa FL Gage (No. 02304500)
COT (2008–2023)	+ Hillsborough River Reservoir Pumped to Base of Dam
Calculation (1996–2023)	= Hillsborough River Reservoir to Base of Dam Flow

3.2.3 Sulphur Springs Flows

Two separate flow calculations pertain to Sulphur Springs—Sulphur Springs Pool corrected for withdrawals and Sulphur Springs Run. Both calculations are important in evaluating ecological relationships with the provided flows. The data to complete the calculations are provided by USGS and City of Tampa.

Total flows for Sulphur Springs Pool corrected for withdrawals require the data shown in Table 3.2-3.

Table 3.2-3: Sulphur Springs Pool flow corrected for withdrawals calculation

Source Agency	Flow Contribution Description
USGS (1996–2023)	+ Sulphur Springs Pool flow @ USGS Sulphur Springs at Sulphur Springs FL Gage (No. 02306000)
COT (2002–2023)	+ Sulphur Springs Pumped to Base of Dam
COT (2012–2023)	+ Sulphur Springs Pumped to Run
Calculation (1996–2023)	= Total Sulphur Springs Flow adjusted for Withdrawals

Total flows for Sulphur Springs run can be calculated using data provided by USGS and COT. The necessary data and contributing agencies are shown in Table 3.2-4.

Table 3.2-4: Sulphur Springs Run flow calculation

Source Agency	Flow Contribution Description
USGS (1996–2023)	+ Sulphur Springs Pool flow @ USGS Sulphur Springs at Sulphur Springs FL Gage (No. 02306000)
COT (2012–2023)	+ Sulphur Springs Pumped to Run
Calculation (1996–2023)	= Total Sulphur Springs Run Flows

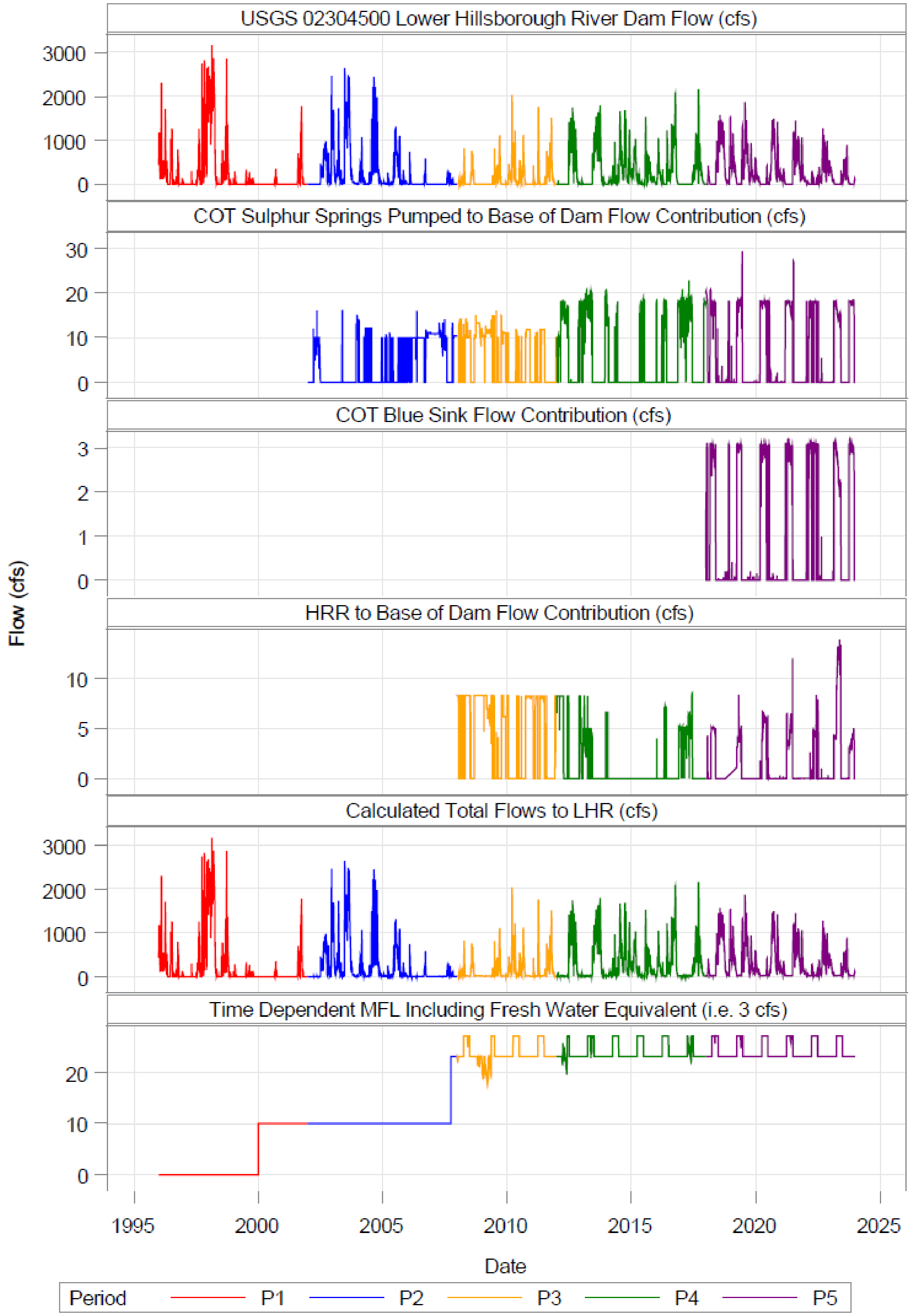
3.2.4 Hillsborough River Reservoir to Base of Dam Flows

Freshwater is delivered to the base of the dam from the Hillsborough River Reservoir. This total freshwater input is calculated using USGS gage data and City of Tampa pumpage information (Table 3.2-5).

Table 3.2-5: Hillsborough River Reservoir to base of dam flow calculation

Source Agency	Flow Contribution Description
USGS (1996–2023)	+ Flow @ USGS Hillsborough River Near Tampa FL Gage (No. 02304500)
COT (2008–2023)	+ Hillsborough River Reservoir Pumped to Base of Dam
Calculation (1996–2023)	= Hillsborough River Reservoir to Base of Dam Flow

Figure 3.2-1: Time series plot of flow contributions and contributing terms to the calculation of the total flow to the LHR and selection criteria used for analysis POR 1996–2023 (All Days)



The POR for the third 5-year assessment is January 1, 1996c through December 31, 2023. The defined periods for long-term comparisons were as follows:

Period 1: 2001 and prior	No minimum flow pumping
Period 2: 2002–2007	Sulphur Springs pumps up to 10 cfs (6.5 mgd) for minimum flow
Period 3: 2008–2011	TBC pumps up to 11 cfs (7.1 mgd) for minimum flow (8.25 cfs (5.3 mgd) to base of dam)
Period 4: 2012–2017	Sulphur Springs pumps up to 18 cfs (11.6 mgd) for minimum flow
Period 5: 2018–2023	Blue Sink pumps up to 3.1 cfs (2.0 mgd) for minimum flow

3.3 Analytical Methods

Qualitative and quantitative analysis were used to characterize the effect of minimum flow implementation on water quality and biology of the LHR. The lower river consists of the "target zone" from the dam to Sligh Avenue and the "downstream" section from Sligh Avenue to the river mouth just below Platt Street. For this report, the quantitative analysis focuses on the target zone, while the downstream section is principally qualitative since the impacts of implementation are primarily restricted to the target zone. The target zone is further divided into three river segments (Figure 1.4-2):

- Upper Segment - Rkm 16.2 to Rkm 14.5 (Dam to Hannah's Whirl)
- Middle Segment - Rkm 14.5 to Rkm 12.6 (Hannah's Whirl to Sulphur Springs)
- Lower Segment - Rkm 12.6 to Rkm 10.6 (Sulphur Springs to Sligh Avenue)

All Days: This includes all available data from January 1, 1996, through December 31, 2023. This was only used to describe the hydrology to the lower river and calculate statistics regarding the frequency and proportion of days where minimum flow implementation occurred.

Implementation Days: This is a subset of "All Days" that only includes days where minimum flow implementation was required based on the effective rule for that day. Minimum flow implementation is required when the calculated LHR total flow is less than the applied minimum flow (the applied minimum flow includes all temporal, gage, and freshwater equivalents adjustments).

Analysis Days: This is a subset of "All Days" that includes days where minimum flow implementation would be required based on the current, adopted rule regardless of year (includes seasonal adjustments, USGS Hillsborough River at State Park Near Zephyrhills, FL Gage (No. 02303000) adjustments, and FWE). This was done to include more data that would have met the conditions for implementation in the historical record, particularly for biological data but was also applied to the water quality analysis for consistency.

All analyses of water quality and biology data are restricted to Analysis Days only unless otherwise noted. Hydrology is characterized as either All Days, Implementation Days, or Analysis Days and is noted as such in the tables and figures.

3.3.1 WATER QUALITY/FLOW STATISTICAL METHODS

3.3.1.1 Ordinary Least Squares Linear Regression

Ordinary least squares (OLS) linear regression, as well as logistic regression models, were used as a first step to evaluate relationships between flow and water quality constituents (Zar 1998). The assessment was conducted to identify constituents where variations in flow describe a significant proportion of the variation in a water quality constituent of interest. A seasonal term was added to the model to evaluate the improvement to the model by accounting for seasonal differences in water quality in addition to evaluating the relationship with flows. Linear regression was used to explore site-specific relationships with flow, and logistic regression was used to investigate the relationship between flows and the probability of exceeding ecologically relevant water quality thresholds for a subset of water quality constituents (i.e., chlorophyll, DO, salinity). While linear regression estimates the change in a water quality constituent for a unit change in flows, logistic regression estimates the change in the probability of exceeding a threshold value for a water quality constituent as a function of flows. Thereby, logistic regression was a complementary assessment to evaluate if variations in flows resulted in an increased or decreased probability of exceeding ecologically relevant thresholds where those thresholds have been established. Logistic regression analysis required more data for proper inference and was therefore restricted to stations and constituents with 100 or more observations as well as more than 10% of the observations showing exceedances of the thresholds.

3.3.1.2 General Linear Models

General linear models (GLM) are extensions (generalizations) of regression models that allow for more flexibility in accounting for artifacts of the data that may affect the underlying assumptions of OLS regression. They are applied when the response variable is continuous. Both classification and continuous predictor variables are allowed and can be expressed as fixed or random effects representing the deterministic component or the variance component of the model, respectively (Little et al. 2017) (Littell et al. 1996). These models were used in an analysis of variance framework to test the differences among periods while controlling for the effect of changes in the sampling scheme over time. An example of using a general linear mixed effects model is provided below where the deterministic component produces a test for differences among periods while the random component of the model allows for each station to have a separate intercept and residual error correlation component. The GLMs were used to test for differences among periods within target zone strata and across zones and periods using an interaction term.

$$Y_{ijk} = \beta_0 + \beta_{0_j} + \beta_k * X_k + e_{ijk} + e_j$$

Where:

$Y_{ij} =$ dissolved oxygen concentration for each sample (i), and station (j) in period (k)

$X_k =$ categorical value representing period k

$\beta_0 =$ overall intercept

$\beta_{0_j} =$ random intercept for station

$\beta_k =$ deterministic effect of period k on overall mean chlorophyll

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

$ejk =$ residual covariance among samples taken at the same station

3.3.1.3 Generalized Additive Modeling

Generalized Additive Modeling (GAM) was used to estimate non-linear responses between flows and river kilometer as drivers of water quality in the LHR. The GAMs were employed using R software (R Core Development Team 2024) and the mgcv package. The mgcv package provides a flexible framework for fitting GAMs, allowing for the inclusion of both linear and smooth terms. The smooth terms were represented using tensor product smoothing, which is particularly useful for modeling interactions between multiple predictors, especially when the predictors have different units or scales. The model was fitted using the Restricted Maximum Likelihood (REML) method to ensure robust estimation of the smoothing parameters. Diagnostic plots and statistical summaries were examined to assess the model's fit and validate the assumptions of the GAM.

The model selection process involved creating lag average flows up to 30 days, merging with the water quality data of interest, sub-setting the data to "Analysis Days" and applying univariate smoothing GAM models against various lag average flow conditions ranging from 0 to 30 days to identify the best lag average to use in the GAM models. Akaike Information Criteria (AIC) and Bayesian Information Criteria (BIC) were used to select the best univariate lag average flow condition for inclusion, and then a series of five GAM models of increasing complexity was run to select the best model to represent the surface response. The models included:

- Model 1: Linear main effect of same day's flow, smoothed function of river kilometer, interaction of same days flow and river kilometer and a month covariate term.
- Model 2: Smoothed main effect of same day's flow, smoothed function of river kilometer, interaction of same days flow and river kilometer and a month covariate term.
- Model 3: Smoothed main effect of best lag average, smoothed function of river kilometer, interaction of best lag average and river kilometer and a month covariate term.
- Model 4: Smoothed main effect of best lag average, no main effect for river kilometer, interaction of best lag average and river kilometer and a month covariate term.
- Model 5: Smoothed main effect of same day's flow, smoothed function of river kilometer, multiple interaction terms using lag average flows bracketing the best lag average and a month covariate term.

Final selection of the GAM model was completed using AIC and BIC to select the best full model for predicting water quality response throughout the target zone as well as other fit statistics and plots. Model 3 was consistently the best model fit to the data; however, at times Model 5 had slightly smaller AIC and BIC values (smaller is better). Despite Model 5 being slightly better in terms of AIC and BIC in some cases, the Model 5 results showed negligible improvement in model fit as indicated by the R^2 coefficient and complicated the inference associated with the predictions by including three different lag terms. Model 5 was therefore not used in any final model predictions. Once the final model was selected, predictions of the response were generated for all Analysis Days between 2018 and 2023 for the observed ("Existing") condition and for a condition representative of flows if the minimum flow had not been implemented ("No MFL"). This scenario modeling is conceptually analogous to the method used by Chen (2024, Appendix J) to evaluate the effects of minimum flow implementation based on hydrodynamic modeling though the GAM model uses different data and a different methodology for estimating the potential non-linear effects of flows on water quality as a function of location in the river as discussed below.

3.3.1.4 Laterally Averaged Model for Estuaries

The District has developed and refined the LAMFE model to characterize and evaluate the hydrodynamics of the LHR (Chen 2024, Appendix J). The LAMFE model is a laterally averaged two-dimensional model that is particularly suitable for narrow and meandering waterbodies such as the LHR, where current and salinity vary mainly in the longitudinal and vertical directions. Numerical methods used in the LAMFE model have been rigorously peer-reviewed in previous MFL studies and published in reputable journals.

The newly updated LAMFE model for the LHR was developed based on the most recent bathymetry data and LiDAR data. Other improvements over the previous LAMFE model for the LHR included:

- Refined longitudinal resolution (the updated model used 144 longitudinal grids to resolve the LHR main stem, while the previous model used 88 longitudinal grids).
- Inclusion of the Sulphur Springs run in the simulation domain.
- Temperature simulated.
- Consideration of weir flow in the Sulphur Springs run.
- Use of newly collected data by USGS, City of Tampa, and the District.
- Longer calibration and verification periods (the updated LAMFE model for the LHR was calibrated and verified using 5-years of real-time data collected between October 25, 2017, and October 12, 2021.

The calibrated LAMFE model for the LHR was used to simulate hydrodynamics, salinity transport process, and thermal dynamics in the river during 1997 and 2001–2023 for the existing (operations), MFL, and No MFL flow conditions (Chen 2024, Appendix J). Model results of water level, salinity, and temperature were processed to calculate salinity habitats for various isohalines and thermal habitats for temperature ≥ 20^{o}C and 15–20 ^{o}C. The salinity habitat calculation was carried out for the river segment between the dam and the confluence of the Sulphur Springs run, while the thermal habitat calculation was for the refuge for manatees, which was defined as the spring run plus a 50-m box of the LHR at the confluence of the spring run.

The model output from Chen (2024) was used for this report to calculate daily water column average salinity within each two-dimensional (longitudinal and vertical) grid established for the lower river. The model results were then matched spatially to the biological data for analysis. The water column average salinity for each LAMFE cell for a 28-day period prior to each biological sampling event was calculated to take biological recruitment into account (FDEP 2017). These 28-day antecedent salinities, which are directly related to freshwater inflows and minimum flow implementation, were considered to be a primary driver influencing the estuarine biological community composition during each of the zooplankton, benthic, and nekton collections. The main predictors of interest for the biological response variables are the period and river segment. Particularly, the upper river segment of the study area before and after full MFL implementation was of interest, as this is where changes to biological communities as a result of changes to salinity as a result of flow should be noticeable and attributable to management actions. Flow, whether flow was met or not, and salinity are also of interest as predictors of biology, though correlated to period and segment. The biology response variables of interest, including those for the analyses of salinity sensitive species, included abundance, density, richness, and diversity. Details of the analytical methods are provided in the following subsections for water quality and biology, respectively.

3.3.2 Biological Statistical Methods

3.3.2.1 Abundance, Richness, and Shannon Diversity

Abundance refers to the number of individuals captured at a sampling location. Species richness refers to the number of unique taxa observed. The Shannon diversity index (H) is a calculation involving taxa richness and the proportional abundance of organisms in a community (FDEP 2017). It is calculated as follows:

$$H = -\Sigma p_i * ln(p_i)$$

where:

Σ: Sum

***ln*:** Natural log

p_i: The proportion of the entire community made up of species *i*

3.3.2.2 Cluster Analysis and Ordination

Cluster analysis is a statistical technique that groups data objects using an algorithm, ensuring that objects within the same cluster are more similar to each other than those in different clusters (Blashfield and Aldenderfer 1978). Cluster analysis does not test hypotheses but is useful for exploring groupings and patterns in data.

Hierarchical agglomerative clustering was performed using Bray-Curtis distances calculated for taxa with the *vegdist* function in the *vegan* R library. Species considered rare (comprising < 5% of samples) were removed. The square root of this distance matrix was used by the *hclust* function with Ward's method. Ward's minimum variance method aims to minimize the sum of squares error, similar to the Analysis of Variance (ANOVA). Each sample unit begins in its own cluster. Iterations are performed to select a pair of units to be fused with the aim of minimally increasing the sum of squared distance. Ward's method has been recommended regularly in the literature, though other methods are also appropriate (McCune and Grace 2002; Singh et al. 2011). In the resulting dendrogram, sites were color coded by the river segment in which they occurred.

Ordination is a statistical technique in which data from a large number of sites or populations are represented as points in a two- or three-dimensional coordinate frame, resulting in a reduction from many dimensions to few. Nonmetric Multidimensional Scaling (NMDS) is a type of ordination in which a small number of axes are explicitly chosen prior to the analysis and the data are fitted to those dimensions; there are no hidden axes of variation (Holland 2008). An NMDS is a numerical technique that iteratively seeks a solution and stops computation when an acceptable solution has been found (Holland 2008).

Using standardized Bray-Curtis distances (appropriate for relative abundance of species), NMDS was conducted on the taxonomic data with the *metaMDS* function in R. Species considered rare (comprising < 5% of samples) were removed. A random initiation process with three axes and 200 iterations found a convergent solution. The fit of the model was evaluated for acceptability using model stress measurement. Values of stress under 0.2 are considered satisfactory for inference, with lower values being preferable (Clarke 1993). A stress plot was also examined for the fit of the data. The NMDS results were plotted and color-coded by segment and/or period.

3.3.2.3 Mixed Models

Mixed effects models are used to predict a single outcome variable using two or more predictor variables (Bakker 2024). In these models, main effects represent the direct influence of independent variables on the outcome, while random effects account for variability that arises from grouping structures or repeated measures in the data. Mixed models are particularly useful for handling unbalanced designs, hierarchical data structures, covariates, grouping factors, and small sample size, all of which are characteristics of this study.

Data filtered for Analysis Days and salinity-sensitive species were fit to generalized linear mixed effect models, incorporating main effects of river segment and minimum flow period, along with random effects of date and site, to analyze the interaction between river segment and period of minimum flow. Different distributions for model fit were examined. In general, abundance data were fit with a Poisson distribution, density data were fit with a Gamma distribution, richness data were fit with a Poisson distribution, and diversity data were fit with a Gaussian distribution.

A significant interaction effect between period and segment, along with subsequent examination of where the effects differ, provides strong inferential power. Ideally, increased freshwater flow during Period 5 should lead to lower salinity habitats in the upper river segment and portions of the middle segments. As a result, salinity-sensitive taxa in these segments are expected to respond to this change in environmental conditions when compared to earlier time periods, particularly Period 1. By using pre-defined periods and river segments, this method allows the integration of data from multiple agencies that used different collection methods, with random effect terms accounting for error.

The *lme, glmer,* and *glmmTMB* functions from the *lme4* and *glmmTMB* R packages were used to generate mixed effect models. The *Anova* function with Type III sum of squares was used to determine p-values. Model assumptions were checked by examining QQ plots and residual plots with the *performance* package.

3.3.2.4 Effect Size Contrasts

Pre-selected comparisons of interest were examined for subsets of the mixed models. These contrasts estimate differences between groups and quantify the associated uncertainty, such as the 95% confidence intervals. This approach allows for useful and informative comparisons within complex models. For example, the difference in the biological community in the upper river segment during Period 5 (at the end of full minimum flow implementation) and Period 1 (at the beginning of implementation) provides insight into the impact of the minimum flow on the biological community. The *emmeans* and *contrast* functions from the *emmeans* package were used to perform these comparisons, with predefined contrasts for river segments, including: Upper Period 5 – Upper Period 1, Upper Period 5 – Lower Period 5, Upper Period 5 – Middle Period 5, Upper Period 5 – Lower Period 5, Lower Period 5 – Lower Period 1, and Middle Period 5 – Middle Period 1.

For zooplankton, which lacked data for Upper Period 1, the predefined contrasts included: Upper Period 5 – Middle Period 1, Upper Period 5 – Middle Period 5, Upper Period 5 – Lower Period 5, Lower Period 5 – Lower Period 1, and Middle Period 5 – Middle Period 1.

3.3.2.5 Salinity Models

A key objective of the LHR minimum flow was to provide essential low salinity habitat conditions downstream of the Hillsborough River Reservoir. Salinity-sensitive benthic macroinvertebrate, zooplankton, and nekton taxa were determined via a literature review specific to the organisms collected in the LHR estuary (Brodie et al. 2013; FDEP 2021; Freshwater Inflows 2023; Froese and Pauly 2023; Greenwood et al. 2006; Holzwart et al. 2023; Janicki Environmental 2003; MacDonald et al. 2006; Ocean Biodiversity Information System 2023; Peebles 2005; Peebles 2008; Smithsonian 2023; SWFWMD 2007; Tolley et al. 2010; US Fish and Wildlife 2023; World Register Marine Species 2023).Taxa were classified as salinity-sensitive if they inhabit oligohaline or tidal freshwater environments, defined as having a salinity of 5 ppt or lower, as part of their salinity range (Odum et al. 1984). This salinity threshold was also recommended for the Hillsborough River estuary by Montagna et al. (2007), who stated that "the oligohaline biotic community, including freshwater benthic macroinvertebrates and juvenile stages of important estuarine-dependent fish, would benefit from salinities < 5 ppt." Taxa consistently found in salinities above 5 ppt or taxa with no documented salinity preference were not classified as "salinity-sensitive." Species-specific salinity preferences were reported as a salinity range or as an explicit salinity central tendency (in ppt).

Each LAMFE model cell was matched to biological sampling sites using the geographic information system program QGIS. Depth-averaged daily salinity values from January 1, 1996, to December 31, 2023, were used to calculate the 28-day prior LAMFE salinity for a cell before each sampling event. The salinity for this full 28-day antecedent period was averaged within that cell for each sample. Mixed effects models with a main effect of salinity and random effects of date and sampling site were used to examine the relationship between model salinity and a biological factor, including abundance, density, richness, and diversity. The models were fit using the *glmer* or *lmer* function from the *lme4* R package, the *glmmTMB* function from the *glmmTMB* package, and the *gam* function from the *gam* package. The *Anova* function from the *car* package or the *anova.gam* function from the *gam* package, with Type III sum of squares, was used to determine p-values. Model assumptions were checked with the performance package, including examination of residuals, dispersion, and influential values (for an example, see Figure 6.2-6). The R^2 value was calculated using the *r2* function from the *performance* package.

3.3.2.6 Individual Species Comparisons

Previous 5-year analyses that used a graphical approach of mean and standard error indicated a potential shift in the abundance of several taxa coinciding with the changes to minimum flow periods. This approach was re-created and improved by incorporating nonparametric Kruskal-Wallis tests followed by a post-hoc Dunn's test (with multiple comparisons corrected via Benjamini-Hochberg) for each period within each river segment (*i.e.*, *Laeonereis culveri* abundance in the upper river segment over each period). Periods with significant differences are denoted via compact letter display (*e.g.*, "a" is significantly different than "b" by Dunn's contrast).

4 DESCRIPTIVE EVALUATION OF THE RECOVERY SOURCE DATA

The recovery strategy identifies Sulphur Springs, Blue Sink, the TBC and Morris Bridge Sink as recovery sources for minimum flow implementation. Each recovery source had descriptive statistics generated for the hydrologic, water quality, and biologic datasets.

This section provides a characterization of the hydrology and water quality of the recovery sources that contribute water to the LHR. Water from upstream of the dam (the Hillsborough River Reservoir and waters upstream of the reservoir) that flows over the dam is also included in this chapter. The descriptions in this chapter pertain only to data collected between January 1, 1996, and December 31, 2023 and, unless otherwise noted, only when minimum flow implementation was required under the FAC-required minimum flow (Implementation Days) or (for water quality) when implementation would have been required if the current minimum flow was implemented for the entire time series of data (Analysis Days).

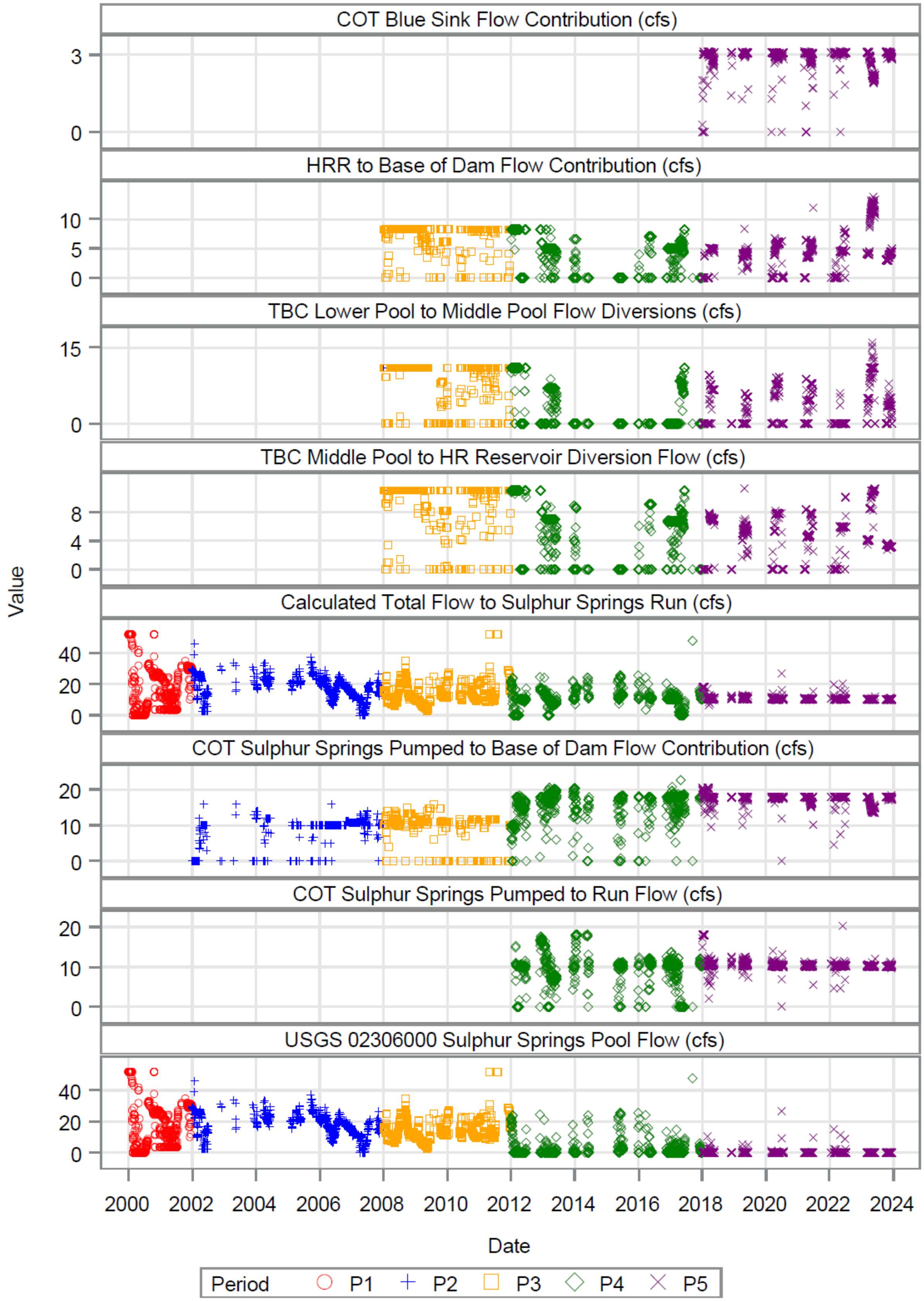
4.1 RECOVERY SOURCE HYDROLOGY

Summary statistics for the various sources of recovery water to the LHR are provided in Table 4.1-1. The statistics are calculated for each period for each identified source for all dates when flows were reported on Implementation Days. These tables include rows solely for those periods where discharge data were available. In addition, a time series plot for each source is provided in Figure 4.1-1. Given that several sources are used as recovery to the total LHR minimum flow, data for several sources are not available prior to the adoption of the recovery strategy. Details for each source are provided in the subsections below. Morris Bridge Sink is not included in these analyses because no pumping to date for minimum flows has occurred from this source.

Table 4.1-1: Summary statistics of daily flows for recovery strategy sources (Implementation Days)

Recovery Water Source	Period	N	Min	Max	Mean	Std
COT Blue Sink Flow Contribution (cfs)	P5	696	0.00	3.13	2.84	0.55
HRR to Base of Dam Flow Contribution (cfs)	P2	1	8.25	8.25	8.25	.
	P3	828	0.00	8.29	7.44	2.01
	P4	786	0.00	8.29	3.94	3.12
	P5	685	0.00	13.86	4.19	3.23
TBC Lower Pool to Middle Pool Flow Diversions (cfs)	P2	1	11.00	11.00	11.00	.
	P3	828	0.00	11.00	8.69	4.25
	P4	786	0.00	11.00	2.97	4.35
	P5	696	0.00	16.00	3.51	3.85
TBC Middle Pool to HR Reservoir Diversion Flow (cfs)	P2	1	11.00	11.00	11.00	.
	P3	828	0.00	11.00	9.76	3.01
	P4	786	0.00	11.00	5.16	4.17
	P5	695	0.00	11.29	4.62	3.32
Sulphur Springs Pool Flow Corrected for Withdrawals (cfs)	P1	605	0.00	52.00	17.15	15.34
	P2	974	0.00	46.00	16.94	7.52
	P3	828	13.94	52.00	23.36	5.11
	P4	786	8.82	47.80	25.62	5.64
	P5	696	21.19	38.54	28.24	2.19
Calculated Total Flow to Sulphur Springs Run (cfs)	P1	605	0.00	0.00	0.00	0.00
	P2	974	0.00	16.00	8.90	3.89
	P3	828	2.43	52.00	13.77	7.00
	P4	786	0.00	47.80	10.01	5.90
	P5	696	6.40	26.81	10.82	1.77
COT Sulphur Springs Pumped to Base of Dam Flow Contribution (cfs)	P1	605	0.00	0.00	0.00	0.00
	P2	974	0.00	0.00	0.00	0.00
	P3	828	0.00	16.00	9.59	4.27
	P4	786	0.00	22.73	15.61	4.34
	P5	696	0.06	20.68	17.42	1.67
COT Sulphur Springs Pumped to Run Flow (cfs)	P1	605	0.00	52.00	17.15	15.34
	P2	974	0.00	46.00	16.94	7.52
	P3	828	0.00	0.00	0.00	0.00
	P4	786	0.00	18.16	7.85	5.18
	P5	696	0.11	20.25	10.61	1.73
USGS 02306000 Sulphur Springs Pool Flow (cfs)	P3	828	2.43	52.00	13.77	7.00
	P4	786	0.00	47.80	2.16	5.28
	P5	696	0.00	26.70	0.22	1.51

Figure 4.1-1: Time series of recovery source daily average flow (1996–2023, Implementation Days Only)



4.1.1 Sulphur Springs Hydrology

The adopted minimum flow for Sulphur Spring under 40D-8.041(3), FAC, incorporates a 15°C temperature trigger to protect manatee thermal refuge (Figure 2.3-1). However, US Army Corps of Engineers Permit SAJ-2010-01672(LP-LDD) and Hillsborough County Environmental Protection Commission Permit 09-047 require the City of Tampa to apply a 20°C trigger in place of the 15°C criterion in Rule 40D-8.041(3). The higher threshold reflects a more conservative protection measure to ensure that manatees continue to have access to suitable warm-water habitat during colder conditions.

Table 4.1-1 and Figure 4.1-1 provide information on flows related to Sulphur Springs by period. Water from Sulphur Springs has been pumped to the base of the dam since Period 2. Average measured Sulphur Springs Run flow during Implementation Days has decreased from the earlier periods from 17.15cfs (11 mgd) prior to 2002 (P1) to near 10 cfs (6.5 mgd) by 2023 as water is both pumped to the base of dam to meet the lower Hillsborough flow and controlled to meet the Sulphur Springs MFL. Pump station modifications, combined with weir modifications, allowed an increase in flows available to be pumped to the base of the dam for LHR minimum flow implementation. This is particularly evident in the increase in maximum volume of water diversion to the base of the dam from Sulphur Springs from Periods 2 and 3 of 16 cfs (10.3 mgd) to more than 20 cfs (ca. 13 mgd) in Periods 4 and 5.

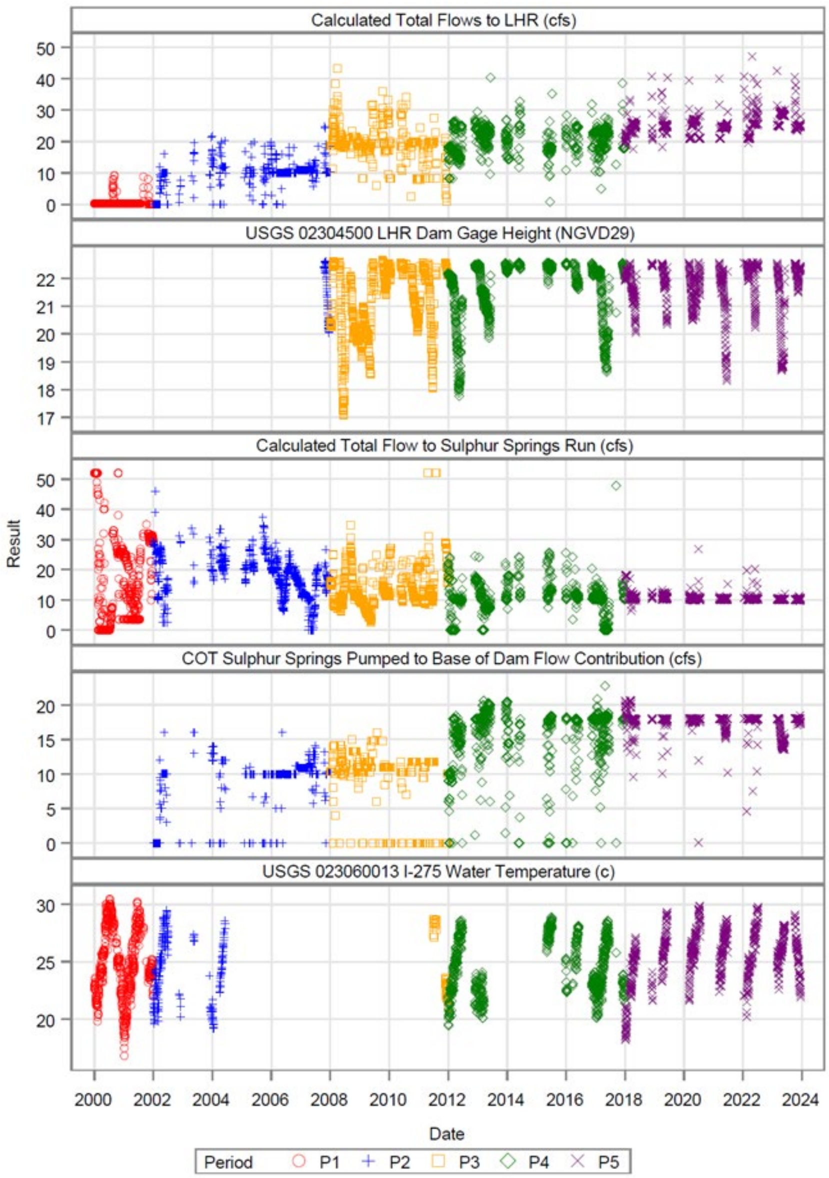
A comparison of the summary statistics for the calculated Sulphur Springs run flows and estimated pool flows corrected for withdrawals is provided in Table 4.1-2; however, given the modifications to the weir and other management interventions, it does not necessarily represent what flows would have gone to the run if those interventions had not occurred.

Table 4.1-2: Summary Statistics of Sulphur Springs flows (Implementation Days)

Source	Period	N	Min	Max	Mean	Std
Calculated Total Flow to Sulphur Springs Run (cfs)	P1	605	0.00	52.00	17.15	15.34
	P2	974	0.00	46.00	16.94	7.52
	P3	828	2.43	52.00	13.77	7.00
	P4	786	0.00	47.80	10.01	5.90
	P5	696	6.40	26.81	10.82	1.77
Sulphur Springs Pool Flow Corrected for Withdrawals (cfs)	P1	605	0.00	52.00	17.15	15.34
	P2	974	2.50	46.00	25.84	6.76
	P3	828	13.94	52.00	23.36	5.11
	P4	786	8.82	47.80	25.62	5.64
	P5	696	21.19	38.54	28.24	2.19

The need to provide water to the base of the dam to meet the LHR minimum flow and to meet the needs of the Sulphur Springs run minimum flow results in a complex and highly managed system. The minimum flow for Sulphur Spring itself is complex and dependent on water levels at the reservoir as well as temperature and salinity in the LHR to determine the required flow. The Hillsborough River Dam water levels appear to rarely drop below 19 feet in elevation during times when minimum flow implementation was required to meet the LHR minimum flow, suggesting that much of the time the Sulphur Springs minimum flow would need to be 18 cfs (11.6 mgd) as seen in Figure 4.1-2. Daily average surface water temperatures at the USGS I-275 gage (No. 023060013) were never below 15 degrees Celsius when minimum flow implementation was required. Sulphur Springs Run flows have been consistent, particularly since 2019, between 10 and 20 cfs (6.45 and 12.9 mgd). Similarly, flows pumped to the base of the dam for LHR minimum flow implementation have been consistent since 2019 at approximately 18 cfs (11.6 mgd) with some episodic variations.

Figure 4.1-2: Time series plots of Sulphur Springs flow components and parameters relevant to its minimum flow (Implementation Days)



To evaluate the potential effects of pumping Sulphur Springs water to the dam to meet the LHR minimum flow, regression plots of the relationship between Sulphur Springs flows to the base of the dam against gage height, water temperature, and total flows to the LHR were plotted (Figure 4.1-3). The results indicate:

- Sulphur Springs flows to the base of the dam are correlated with total flows to the LHR during minimum flow implementation, as expected.
- Gage heights at the dam are somewhat lower when Sulphur Springs flow pumped to the base of the dam are higher.
- As more water is pumped to the base of the dam from Sulphur Springs, there tends to be less water in the run.
- Surface water temperatures at I-275 are always above 15 degrees Celsius and almost exclusively between 20 and 30 degrees Celsius.
- A correlation exists between higher flows pumped to the base of the dam and lower temperatures, likely due to seasonality. The need for minimum flow implementation is reduced as temperatures warm with the onset of the rainy season.

A time series plot of all data sources related to Sulphur Springs Gage height at the pool and at the dam for All Days (to show the full range of gage heights) compared to the flow components over Implementation Days is provided in Figure 4.1-4. This plot suggests a modest decrease in Pool gage (No. 02306000) height over time and a modest increase in Run gage (No. 02306003) height over time as the Sulphur Springs minimum flow was implemented.

Figure 4.1-3: Scatter plot with regression line depicting relationship between flows pumped to the base of the Hillsborough Dam to meet the LHR minimum flow and other parameters related to the Sulphur Springs minimum flow (Implementation Days)

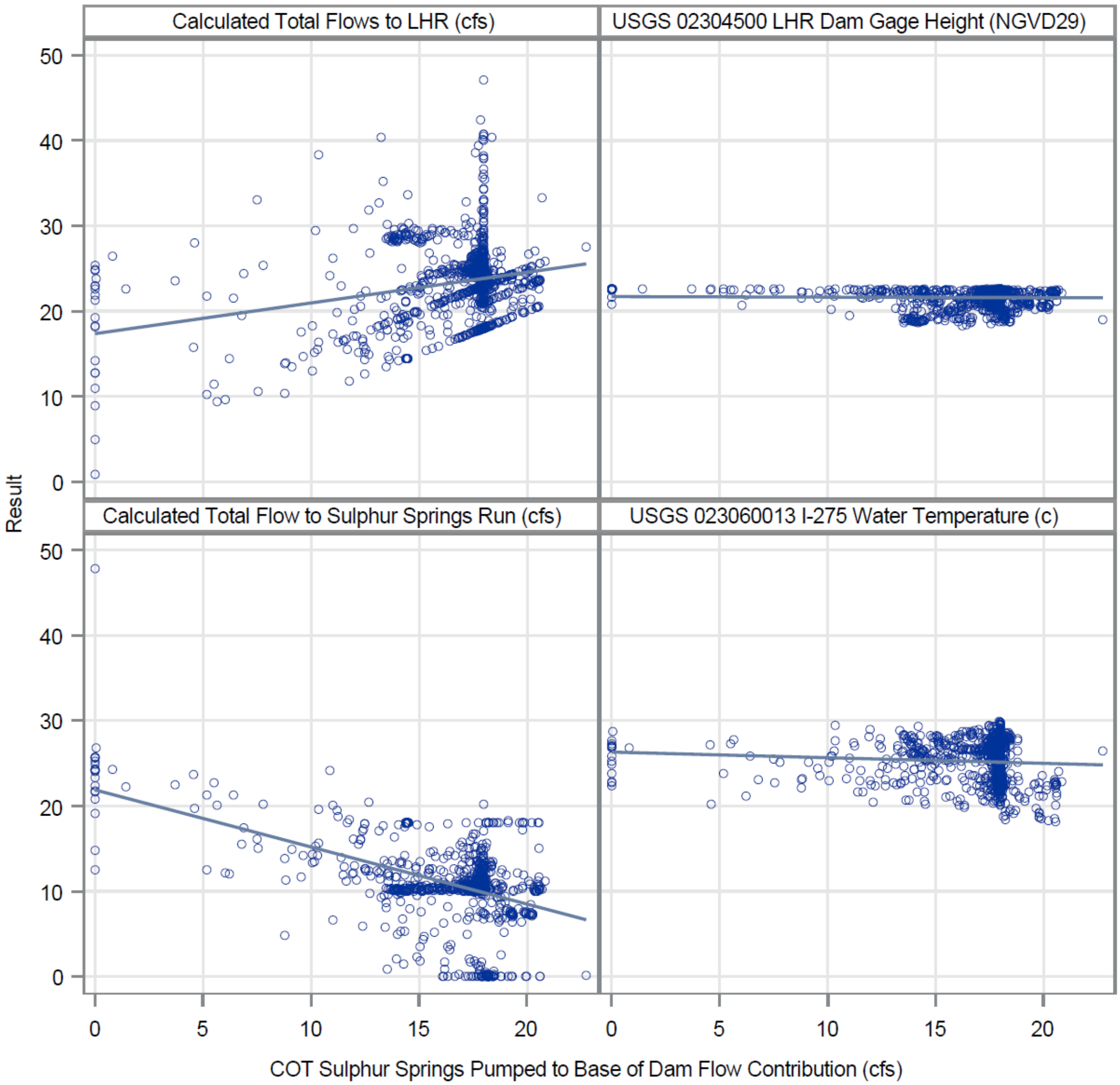
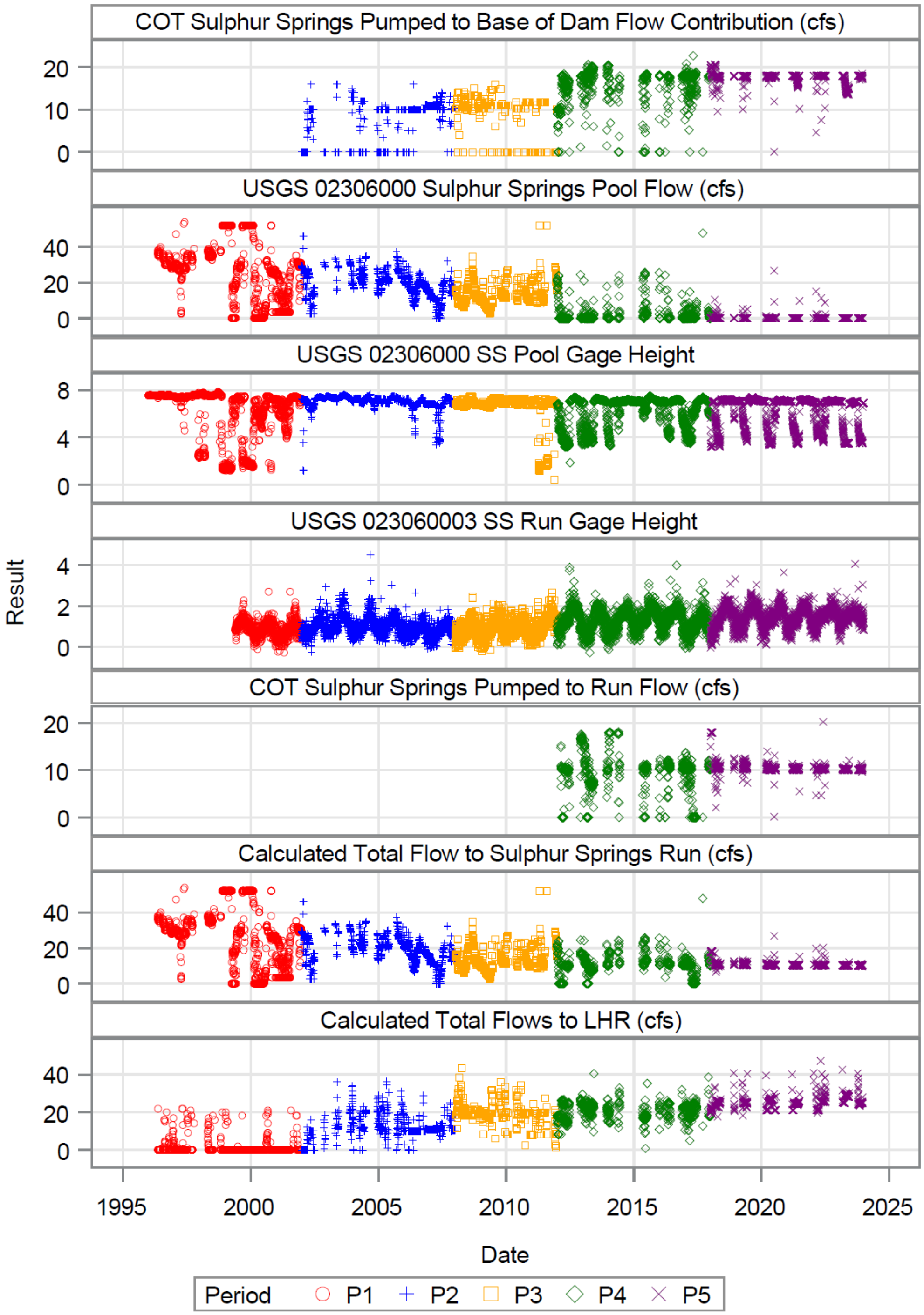


Figure 4.1-4: Time series of source contributions (Implementation Days) and gage height measurements (All Days) related to the use of Sulphur Springs for the LHR minimum flow implementation and to meet its own minimum flow for the Sulphur Springs Run



4.1.2 Blue Sink Hydrology

WUP No. 20020382 issued to the City by the District for pumping from Blue Sink allows a peak monthly withdrawal of up to 2 mgd (~3.1 cfs) and an annual average withdrawal rate of approximately 1.7 mgd (~2.6 cfs). Operationally, water from Blue Sink has been used to supplement LHR minimum flows during Period 5 only. A mean value of 2.84 cfs (1.8 mgd) was used for minimum flow implementation during this timeframe, with little variability over the 5-year period (Table 4.1-1, Figure 4.1-1). The maximum volume pumped for minimum flow implementation 3.1 cfs (2.0 mgd) was within the permitted amount during the timeframe of operation.

4.1.3 TBC/Hillsborough River Reservoir Hydrology

As part of the recovery strategy, water has been supplied from the TBC to the LHR as needed since December 31, 2007. Water can be diverted directly from the TBC middle pool or first from the lower pool to the middle pool depending on water levels and TBC flows. The average daily diversions from the lower pool to the middle pool and from the middle pool to the reservoir on Implementation Days have gone down over time. as demonstrated in Table 4.1-1, Figure 4.1-1. During Periods 3 and 4, the volume of water transferred from the reservoir to the base of the dam for minimum flow implementation has historically corresponded to 75% of the volume transferred from the TBC to the reservoir. Following the discontinuation of the evapotranspiration and leakage adjustment (as a result of the pipeline project cancellation), an equivalent volume of water moved from the TBC to the reservoir has been released from the reservoir to the base of the dam for minimum flow implementation, beginning in 2023.

4.1.4 Morris Bridge Sink Hydrology

WUP No. 20020574 issued to the District by FDEP for pumping from Morris Bridge Sink allows a peak monthly withdrawal of up to 3.9 mgd (~6 cfs) and an annual average withdrawal rate of approximately 1.7 mgd (~2.6 cfs). Water from Morris Bridge Sink has not been pumped for minimum flow implementation to date. The District collects hydrologic data at three sinkholes, 10 wells, and three wetlands. Hydrologic data for these sites are provided to FDEP each year as a requirement of the WUP. The final report submitted for calendar year 2023 can be found in Appendix K.

4.1.5 Effects of the Recovery Strategy on Water Levels above the Dam

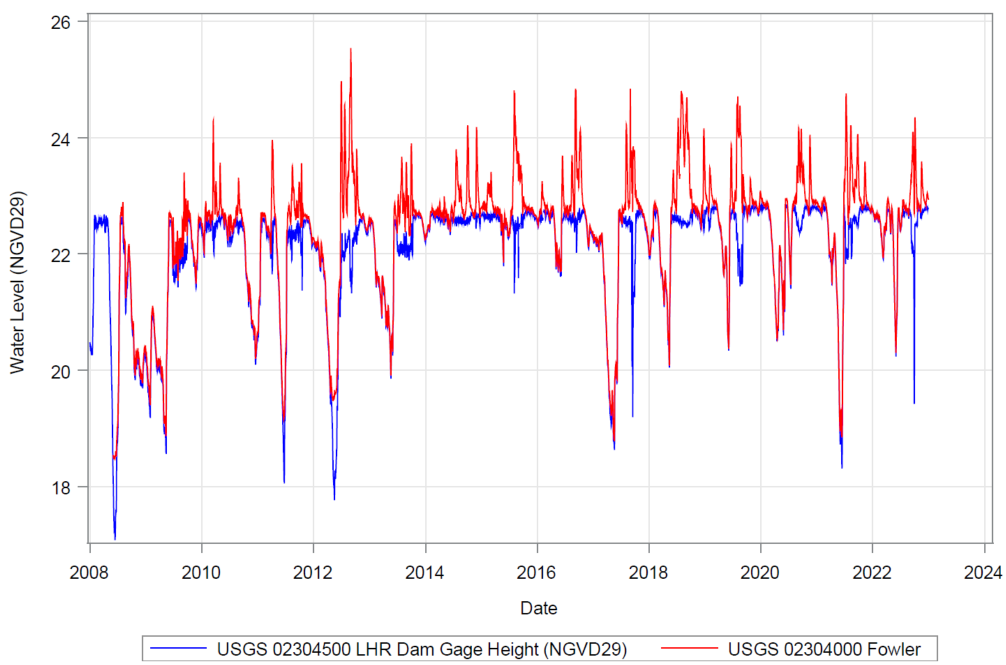
A requirement of the recovery strategy is that the District "*shall also monitor and evaluate the effect the Recovery Strategy is having on water levels in the Hillsborough River above the City's dam to at least Fletcher Avenue.*" – 40D-80.073(8) FAC. In response to concerns about how the implementation of minimum flows for the LHR might affect water levels in the river above the dam, the District completed a study of the Middle Hillsborough River in 2009 (Leeper 2009). In addition, the first 5-year assessment report (SWFWMD and Atkins 2015) produced a figure (12-4) describing the relationship between water levels at the dam and water levels at the USGS Fowler gage (No. 02304000) using data collected between 2008 and 2013 to address the evaluation. The first assessment report (SWFWMD and Atkins 2015) characterized this relationship as follows:

> *There is very close agreement between the two sites over time except during two types of conditions. The first is when water levels at the dam fell to levels*

near 17 to 18 feet, and water levels at Fowler stabilized at higher levels, such as in 2008, 2011, and 2012. When water levels at the dam drop below 18.2 feet, water levels at Fowler Avenue and points upstream tended to maintain higher water levels, due to changes in bathymetry of the bed of the river near Fowler Avenue, which was verified by a bathymetric survey of the middle river conducted in 2007 (Ping and Beck, 2008). The second is when water levels at the dam were near the spillway elevation of 22.5 feet and water levels at Fowler Avenue was considerably higher. This second category corresponds to periods of high flow in the Hillsborough River when water is released from the reservoir using the tainter gates located at the dam. During these high flow events, releases from the reservoir maintains water levels near the dam at 22.5 feet or lower, but constrictions in the reservoir cross section allow water levels at Fowler to rise as water accumulates further upstream. The regulation of high water levels in the middle river is closely managed by the District's Structure Operations Division and is not discussed further in this report, which focuses on periods of minimum flows when there is either zero or very low flow at the dam spillway.

This analysis was repeated with additional data through 2023 (Figure 4.1-5).

Figure 4.1-5: Water levels at the Hillsborough River Dam and USGS 02304000 Fowler (All Days)

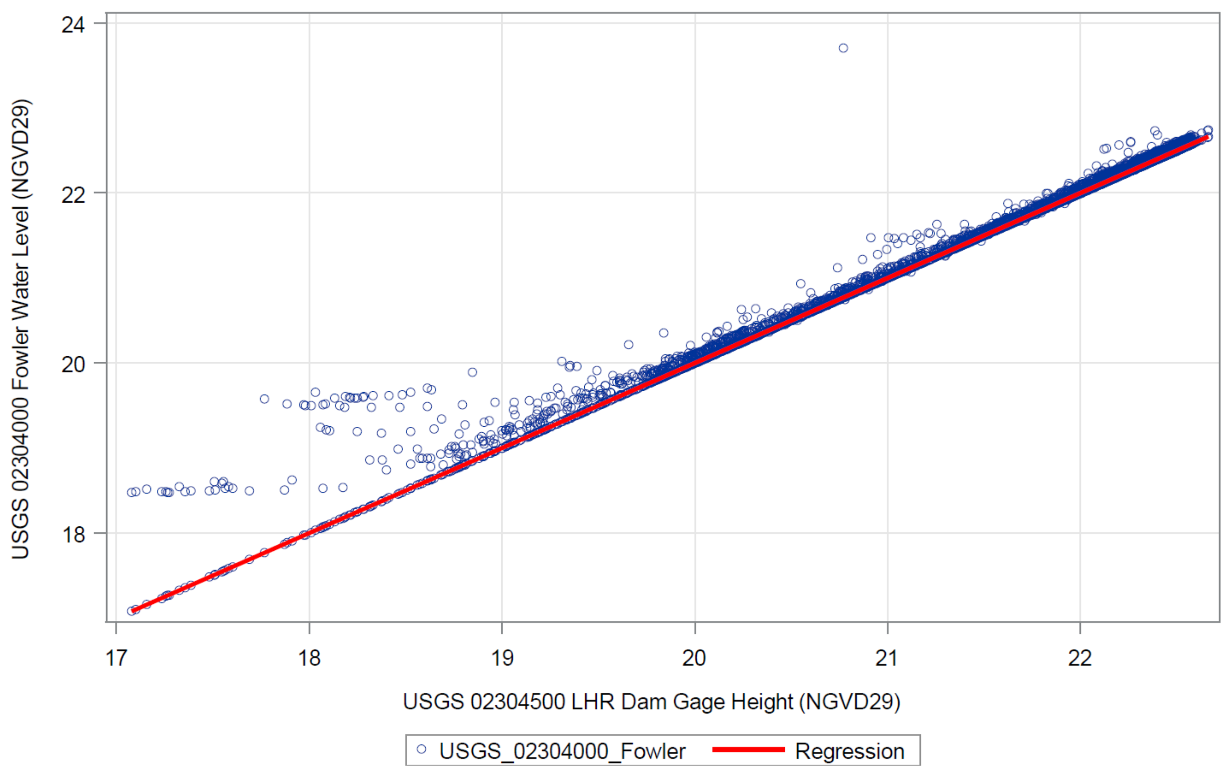


An x y plot of the two gages is presented in Figure 4.1-6. For clarity, the data for this plot are reduced to Implementation Days. This was done to remove a cloud of high flow points obscuring the relationship being examined. The results support the conclusions of the first report regarding the relationship between the water levels at the dam and those at USGS 02304000 Fowler.

Water levels compared at the two USGS gages indicate the following:

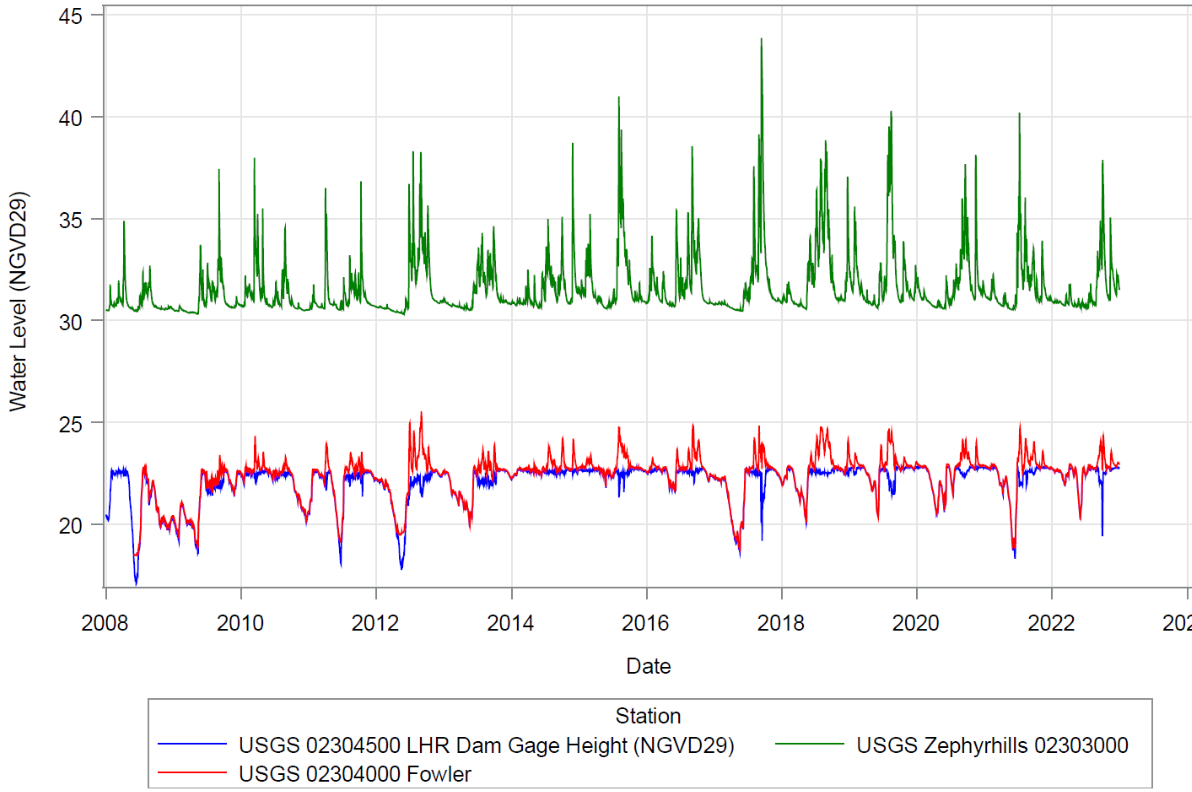
- Close agreement of water levels between USGS Gages Fowler (No. 02304000) and Hillsborough River Dam (No. 02304500) except when:
  - Low Flows – dam < 18.2 feet, Fowler will maintain higher levels than the dam.
  - High Flows – dam > 22.5 feet, Fowler will maintain higher levels than the dam.

Figure 4.1-6: Comparison of water levels at the Hillsborough River Dam and at USGS 02304000 Fowler (Implementation Days)



Finally, the Zephyrhills Gage (USGS No. 02303000) was added to the full time series plot to evaluate the relationships further upstream of the dam as was considered in the Middle Hillsborough Report (Leeper 2009) (Figure 4.1-7). As concluded by Leeper (2009), similarities in the hydrographs of the Middle Hillsborough River, Florida reservoirs, and area lakes indicated that it may be appropriate to consider water level fluctuations in the middle river analogous to those occurring in lakes given the highly managed system and dam operations. As discussed in SWFWMD & Atkins (2015) and Leeper (2009) and supported by the analysis conducted for this report, it can be concluded that implementing the recovery strategy for the LHR has not resulted in a lowering of water levels in the Middle Hillsborough River and should not do so in the future given the management specifications adopted in the recovery strategy.

Figure 4.1-7: Water levels upstream of the Hillsborough River Dam (All Days)



4.2 Recovery Source Water Quality

Data for the water quality analysis were filtered to "Analysis Days." Once filtered, water quality data was limited for many sources (Table 4.2-1), so only those sources with sufficient data and pertinent results are displayed in this section. Time series and descriptive plots and statistics for all available data during Analysis Days are provided in Appendix L.

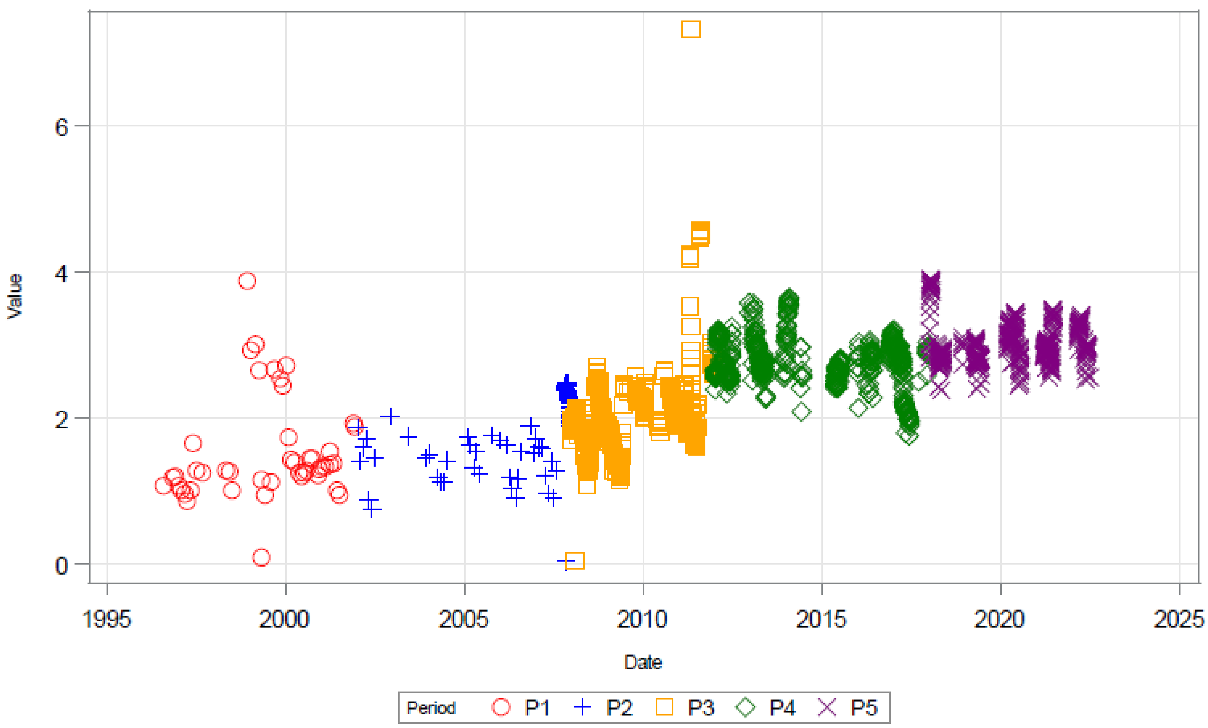
Table 4.2-1: Recovery source water quality data frequency (N) 1996–2023 (Analysis Days)

Recovery Source	Station	CHLA	CHLAC	COLOR	COND	DO	DOSAT	NH3	NH4+	NO3	NO32	OPO4	PH	SALIN	TEMP	TKN	TN	TP
Sulphur Springs Pool	USGS Sulphur Springs Pool 02306000	.	.	.	1,828	.	.	.	.	.	.	.	.	1,828	1,835	.	.	.
	WUP 2062 DID 10 - SS	.	.	63	159	139	.	122	.	129	.	.	139	140	139	116	138	135
Sulphur Springs Run	EPC SN 174	65	65	64	70	67	67	62	.	.	66	68	67	70	72	63	65	65
	USGS Sulphur Springs Run 023060003		.	.	1,796	.	.	.	.	.	.	.	.	1,796	1,824	.	.	.
Blue Sink	SWFWMD Blue Sink SN 670721	.	.	3	3	.	.	.	.	.	2	3	3	3	3	.	2	2
TBC	WUP 6675 DID 49 – TBC	3	.	76	75	76	.	62	.	75	.	.	76	63	75	62	63	62
Hillsborough Reservoir	IWR Run65 All Reservoir	84	84	104	794	787	754	.	117	.	133	.	786	764	794	99	370	357
	WUP 2062 DID11 - HRR	.	.	140	141	139	.	111	.	121	.	.	139	134	140	117	137	136
Morris Bridge Sink	SWFWMD MBS 1	.	.	.	120	120	109	.	.	.	.	.	120	.	120	.	.	.
	SWFWMD MBS 2	.	.	.	120	120	109	.	.	.	.	.	114	.	120	.	.	.
	SWFWMD MBS 3	4	.	3	110	110	99	.	.	4	2	3	110	.	110	2	4	3

4.2.1 Sulphur Springs Water Quality

Although conductivity (and therefore calculated salinity) has remained relatively stable for most of the recovery sources, salinity in the Sulphur Springs system is not only higher than the other recovery sources but has also risen over time (Figure 4.2-1, Figure 4.2-2). As noted in Chapter 2, increased diversions from Sulphur Springs initially resulted in lower elevations of Sulphur Springs Pool, which in turn increased the salinity of the spring discharge. When Pool levels were lowered to induce greater spring discharge, the salinity increased. Additionally, the discharge from Sulphur Springs is highly mineralized and exceeds Class I potable water quality standards for certain constituents (SWFWMD 2004). Therefore, the City of Tampa has diverted water from Sulphur Springs for potable supply only during times of water shortage, relying on the blending of the spring water with water in the reservoir to not exceed potable water supply standards in their withdrawals from the reservoir. Sulphur Springs has not been used for potable water since 2009 (SWFWMD 2015). Data on conductivity, temperature, and calculated salinity for Sulphur Springs Pool was supplied by USGS Sulphur Springs Pool (02306000) and District WUP 2062- DID 10, while data for Sulphur Springs Run was provided by USGS Sulphur Springs Run (023060003) and EPC site 174.

Figure 4.2-1: Sulphur Springs Pool calculated salinity in ppt, 1996–2023 (Analysis Days)



To illustrate this effect further, water levels data and calculated salinity for the Sulphur Springs Pool Gage (USGS No. 02306000) were plotted as a time series using all days when water levels were recorded (All Days) to show the entire distribution of water levels (Figure 4.2-2) and as an x y plot for only Analysis Days (Figure 4.2-3). The plots clearly demonstrate that increased salinity is associated with decreased water levels in Sulphur Springs Pool. The relationship between lowering water levels in Sulphur Springs Pool increasing to salinity of the spring discharge is described in *The Determination of Minimum*

Flows for Sulphur Springs, Tampa Florida report (SWFWMD 2004) and the first five-year assessment for LHR (SWFWMD and Atkins 2015).

Figure 4.2-2: Time series plot of USGS Sulphur Springs Pool (Gage No. 02306000) calculated salinity and observed water levels (All Days)

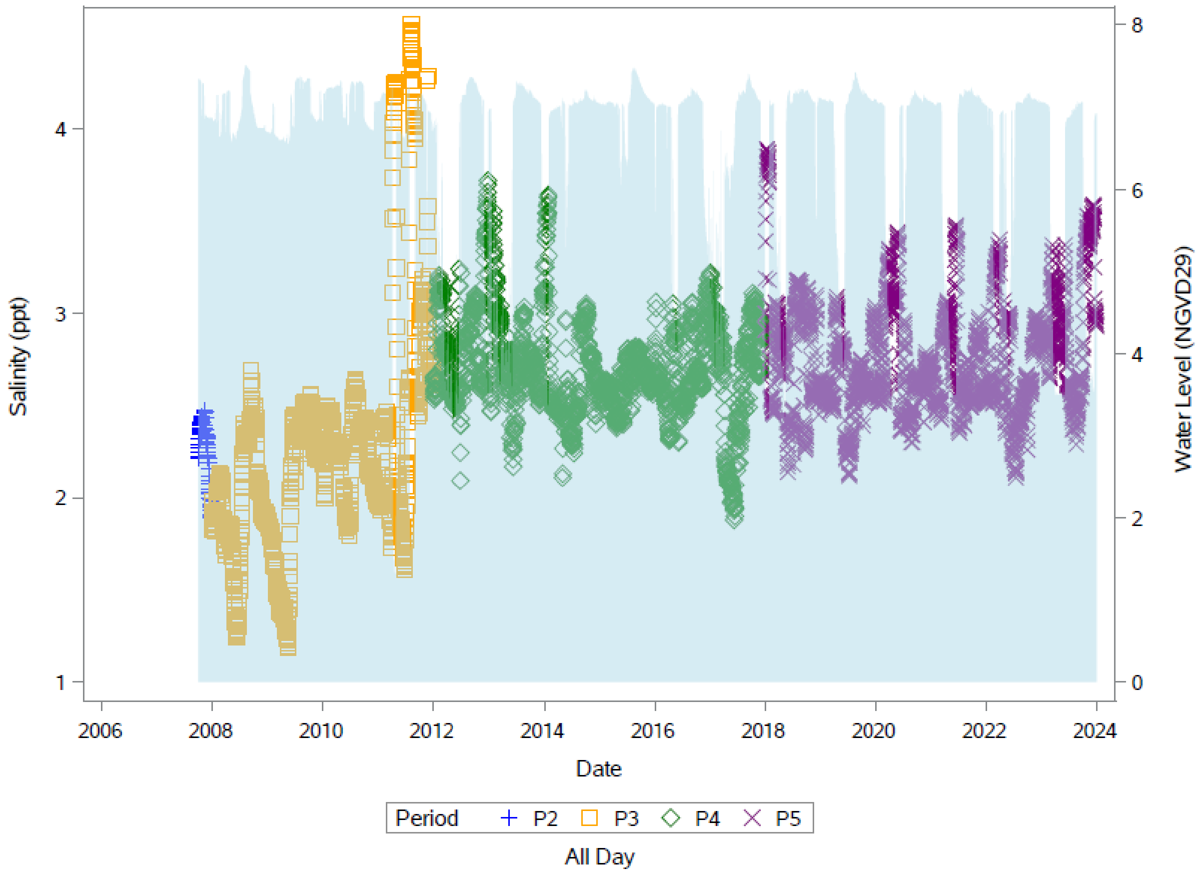
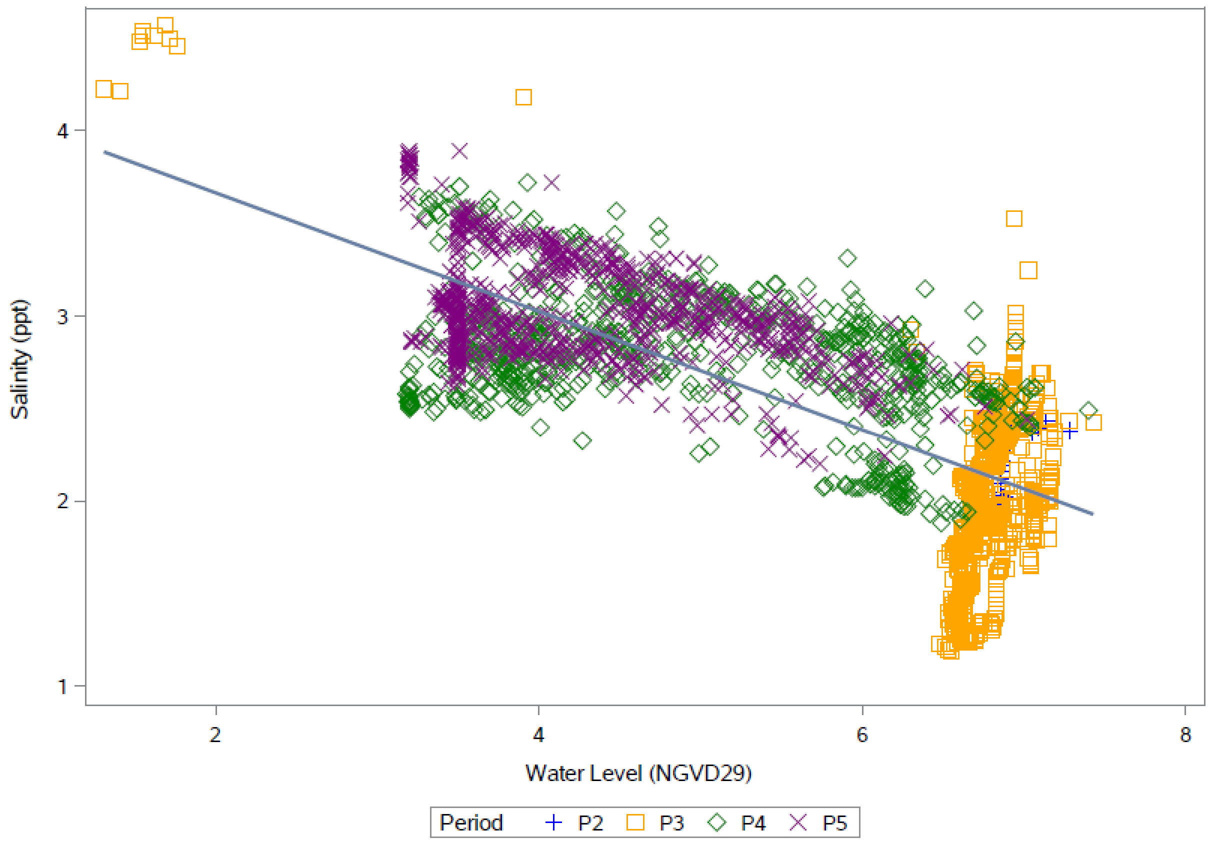
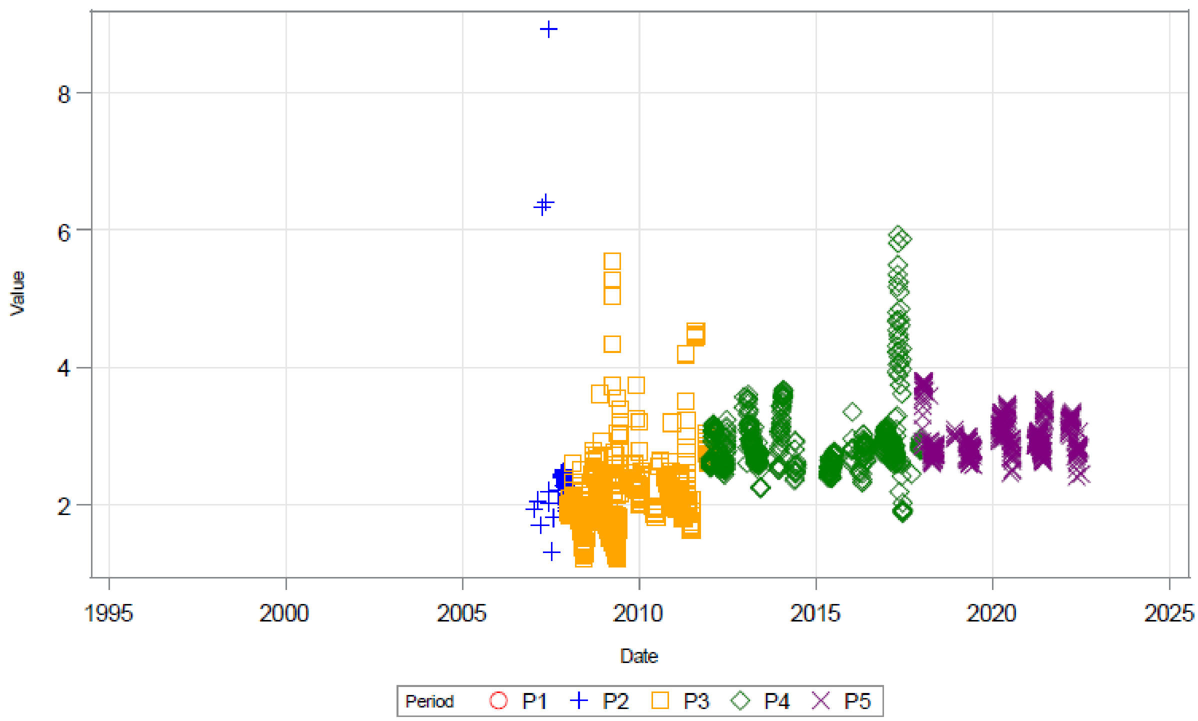


Figure 4.2-3: Scatter plot of USGS Sulphur Springs Pool (Gage No. 02306000) observed water levels vs. calculated salinity (Analysis Days)



Sulphur Springs Run data suggests a similar increase in calculated salinity noted for the Sulphur Springs Pool data between Period 3 and Period 4 (Figure 4.2-4). There also appears to be a smaller step change in salinity between Period 2 and Period 3.

Figure 4.2-4: Sulphur Springs Run calculated salinity in ppt, 2007–2023 (Analysis Days)



Sharping et al. (2018) suggested that salinity at the spring increased during dry-season pumping and following wet-season recharge events, likely due to elevated artesian pressure in the confined saline aquifer units. They hypothesized that salinization of Sulphur Springs may disrupt the cave microbe and stygobite communities and eventually make the spring unsuitable to maintain low-salinity habitat in the LHR. A feasibility study is being conducted by the City of Tampa to consider alternatives to reduce salinity and improve flow to Sulphur Springs. This study is ongoing, and the results will not be completed in time to incorporate into this assessment.

The Sulphur Springs Pool and Run exhibit increases in temperature over time, while the remaining recovery sources have remained relatively similar over the study period (Figure 4.2-5, Figure 4.2-6). This may be due to the installation of an operable weir at the mouth of the spring run that prevents incursions of higher-salinity water from the river during low-flow periods while allowing access to the run by manatees during higher-flow periods when incursions of saline water are less of a concern. Therefore, the spring run temperature variability is more affected by river water incursions.

Figure 4.2-5: Sulphur Springs Pool water temperature values in degrees C, 1996–2023 (Analysis Days)

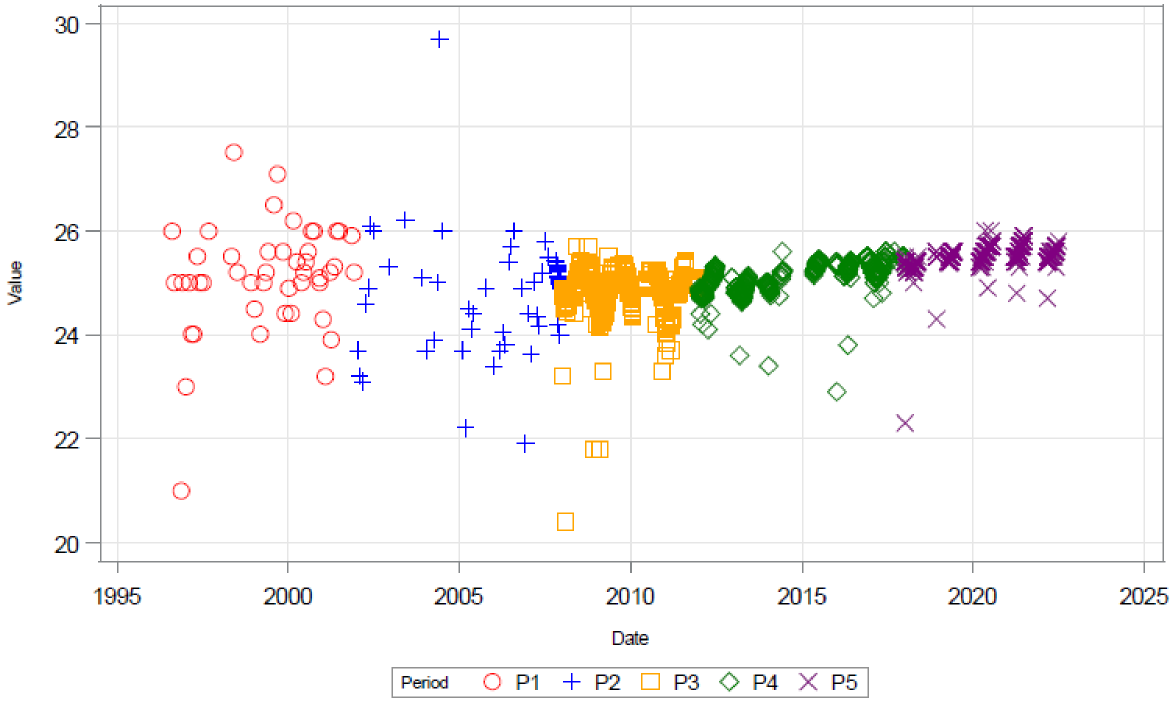
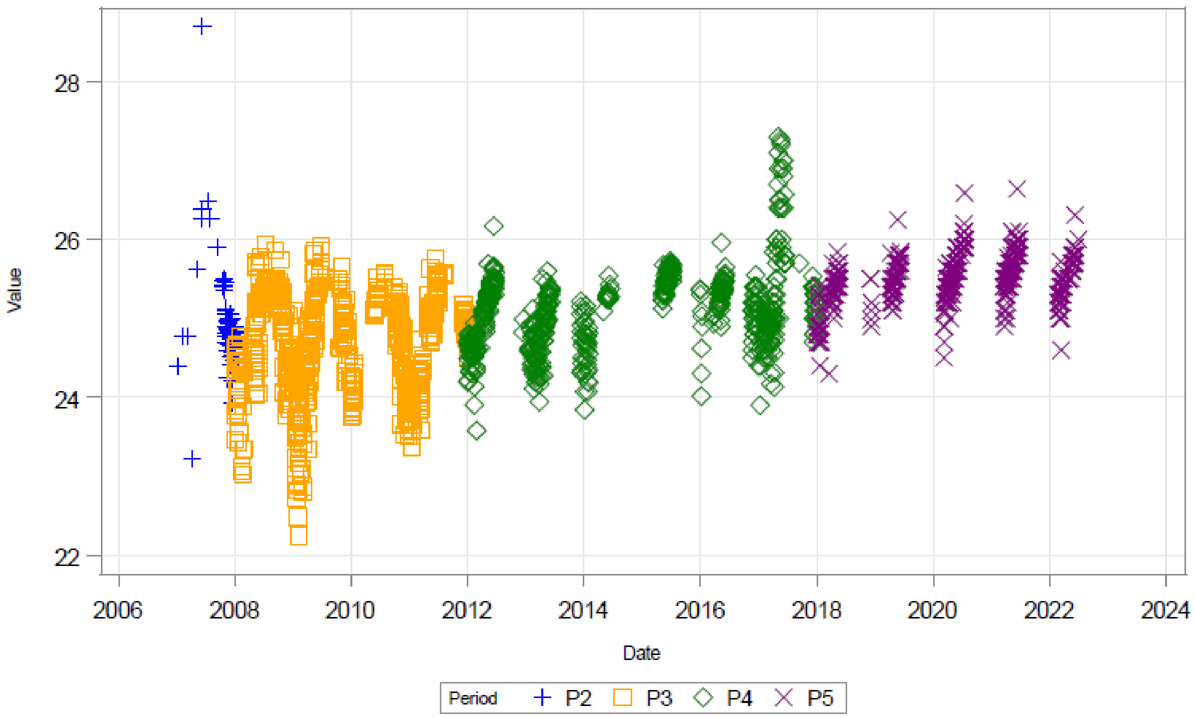
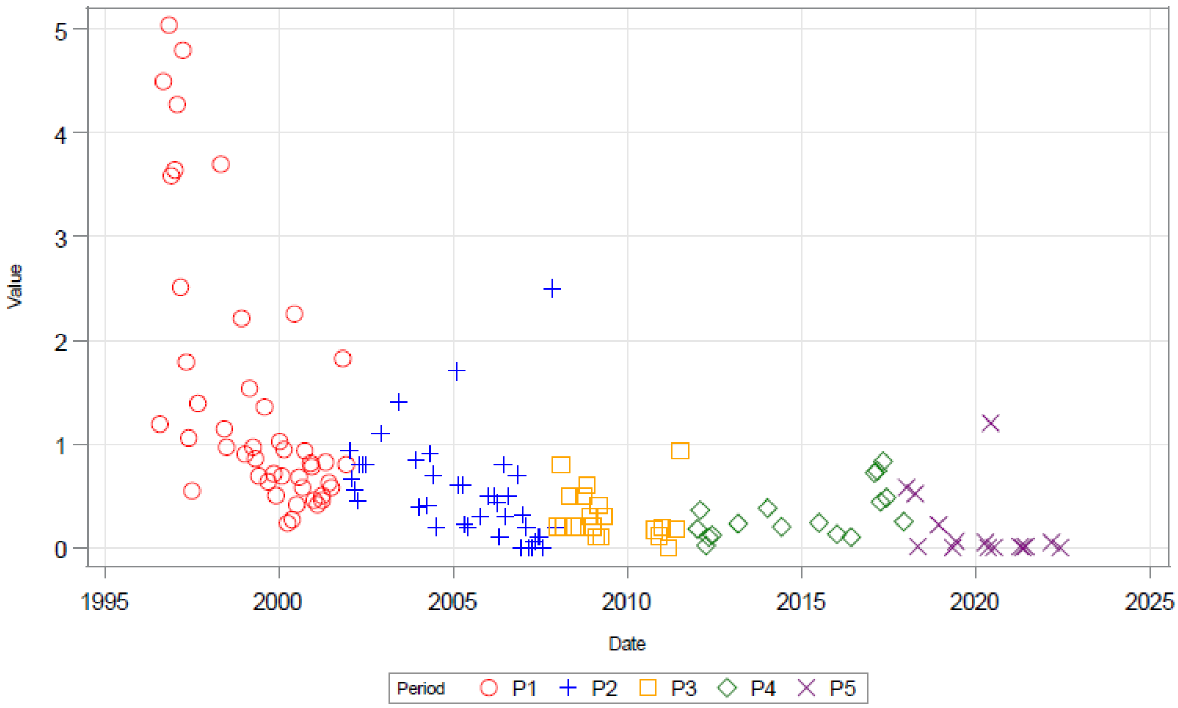


Figure 4.2-6: Sulphur Springs Run water temperature values in degrees C, 2007– 2023 (Analysis Days)



The Sulphur Springs Pool exhibits a decline in total nitrogen concentrations over the study period (Figure 4.2-7). Remaining parameters analyzed did not exhibit a noteworthy pattern. Appendix L includes scatterplots, histograms, boxplots, and descriptive statistics for all parameters analyzed.

Figure 4.2-7: Sulphur Springs Pool total nitrogen concentrations in mg/L, 1996–2023 (Analysis Days)



4.2.2 Blue Sink Water Quality

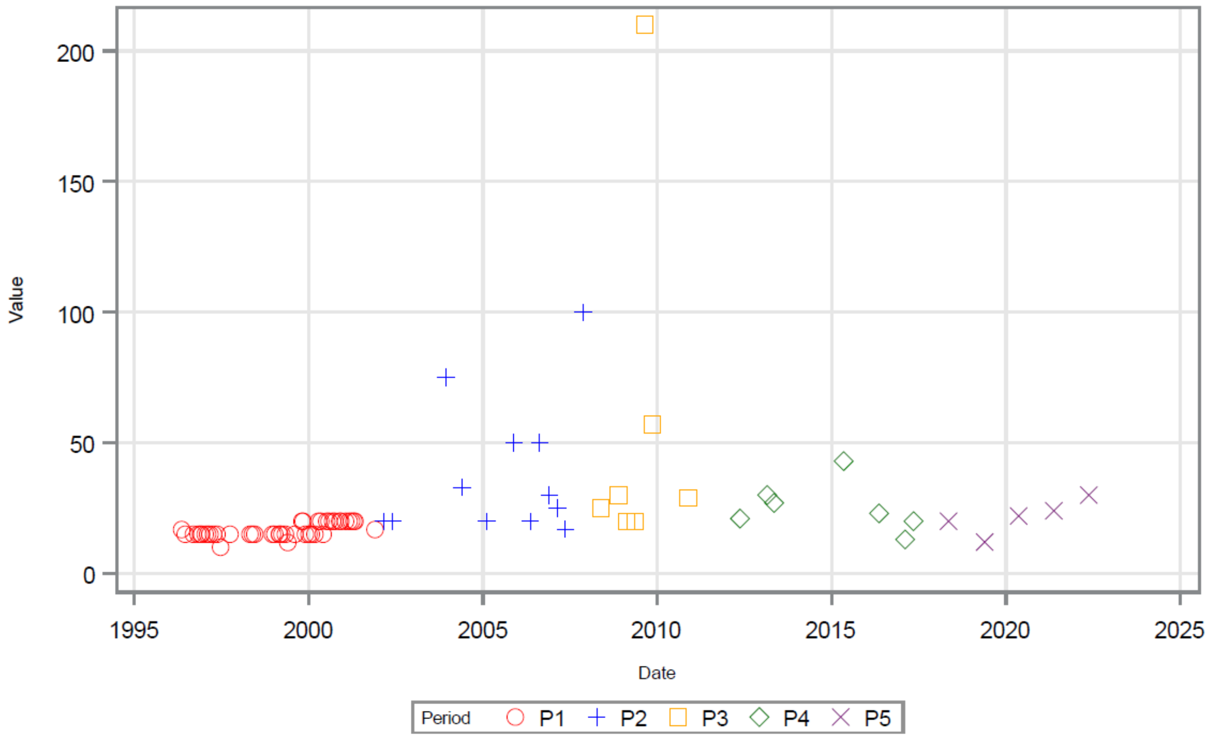
As indicated in Table 4.2-1, only three water quality samples were collected from Blue Sink over the study period. All three sampling events were conducted during Period 3. Parameters include conductivity, salinity, temperature, color, total nitrogen, nitrate+nitrite, total phosphorus, orthophosphate, and uncorrected chlorophyll. Descriptive graphics of these limited data are provided in Appendix L.

4.2.3 TBC AND HILLSBOROUGH RIVER RESERVOIR WATER QUALITY

Although many parameters sampled in the TBC remained largely stable over the study period (all plots available in Appendix L), some parameters with noted patterns included color and nutrients.

Color values in the TBC exhibited a substantial increase over the study period with no recorded values above 20 before 2002, then an increase in values over 50 in Periods 2 and 3. The values consistently remain under 50 in Periods 4 and 5. (Figure 4.2-8).

Figure 4.2-8: Tampa Bypass Canal color in PCU, 1996–2023 (Analysis Days)



Total nitrogen concentrations have increased over the study period according to available data (1996–2010) within the TBC (Figure 4.2-9). However, nitrate+nitrite concentrations declined over the whole study period (Figure 4.2-10) in the TBC. Total phosphorus concentrations declined over time in the TBC (Figure 4.2-11).

Figure 4.2-9: Tampa Bypass Canal total nitrogen concentrations in mg/L, 1996–2010 (Analysis Days)

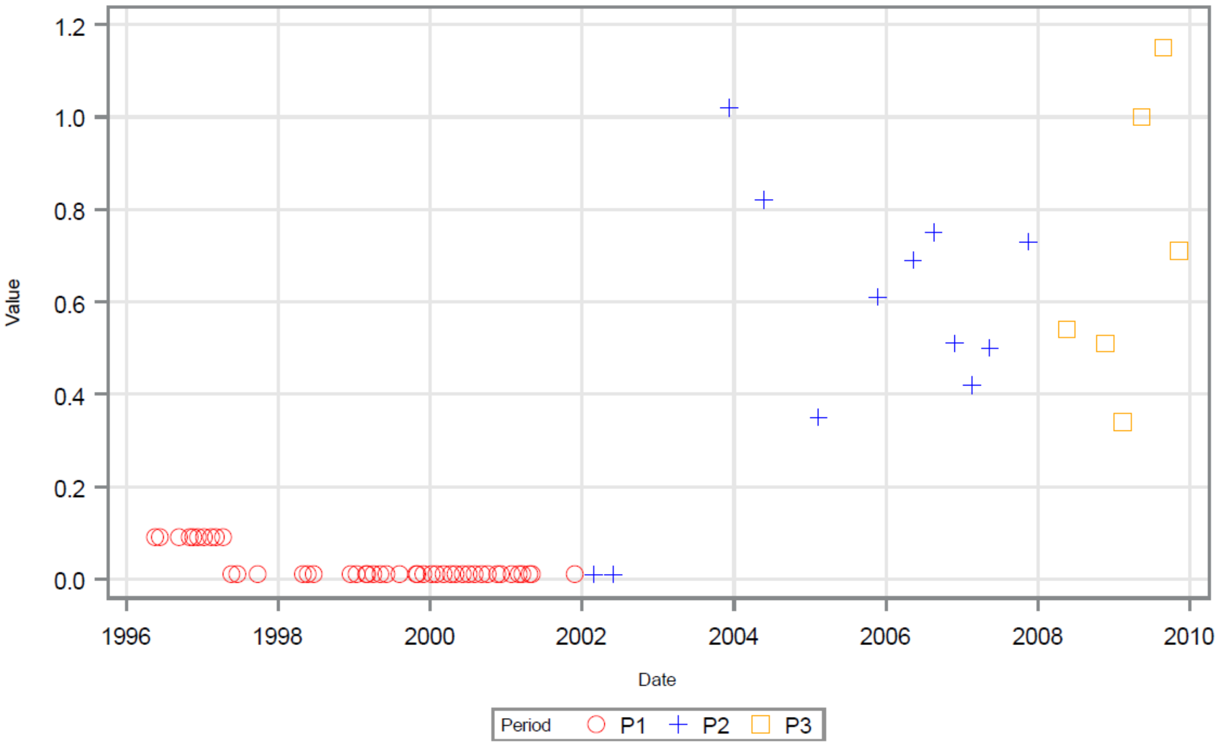


Figure 4.2-10: Tampa Bypass Canal Nitrate-Nitrite concentrations in mg/L, 1996–2010 (Analysis Days)

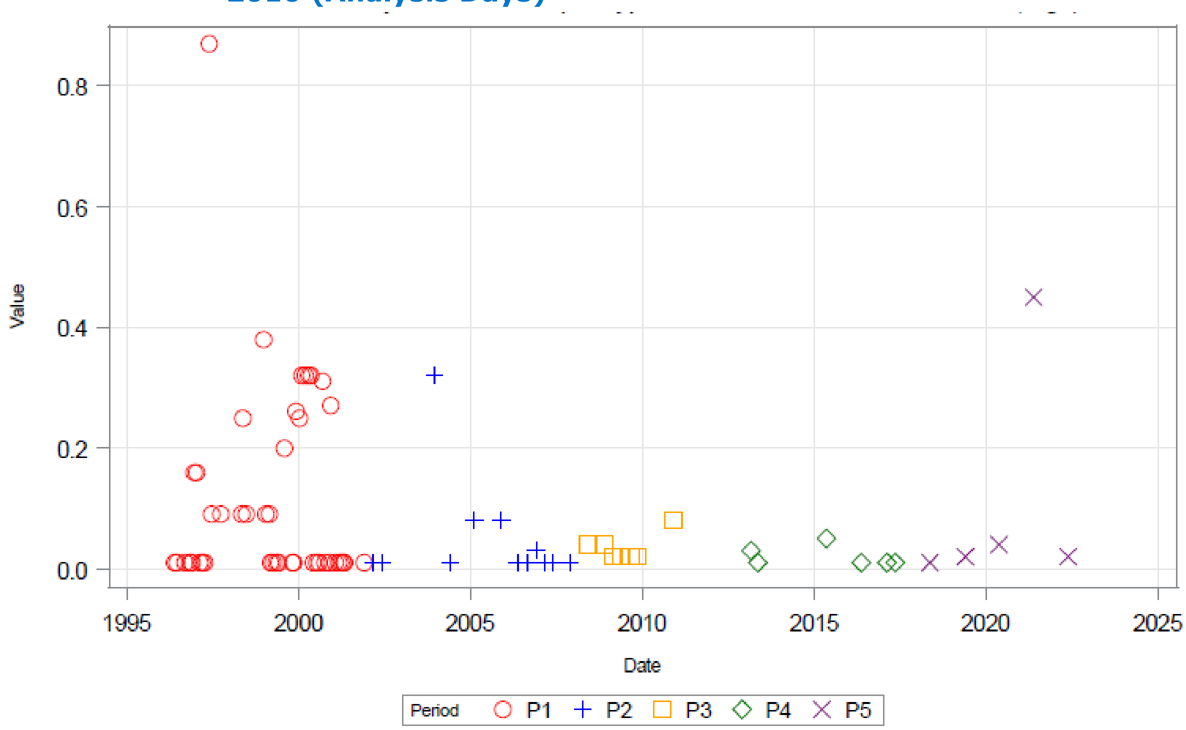
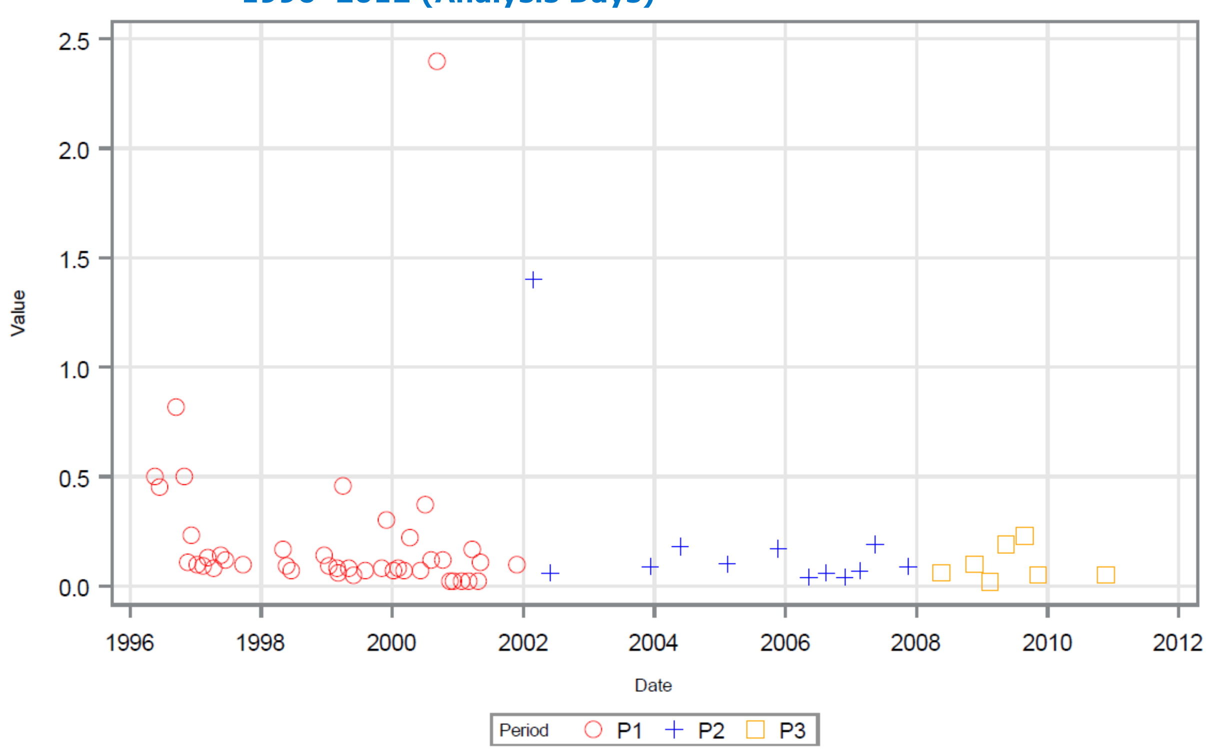
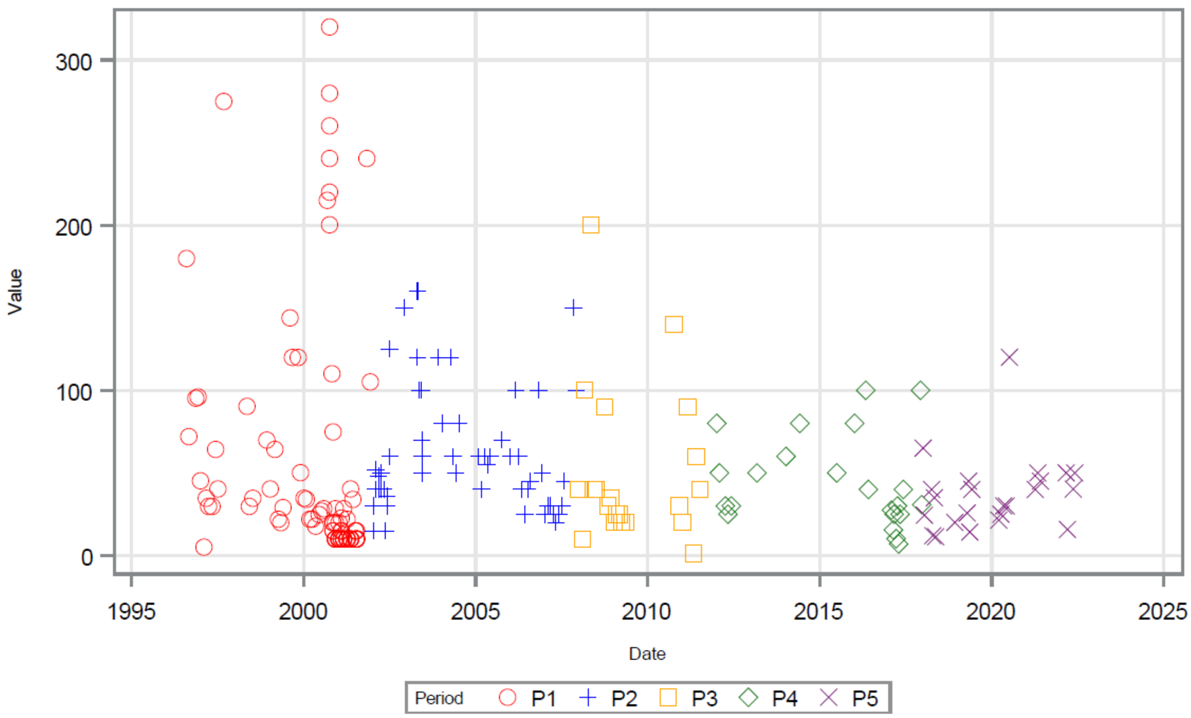


Figure 4.2-11: Tampa Bypass Canal total phosphorus concentrations in mg/L, 1996–2012 (Analysis Days)



Color in the reservoir decreased slightly from the earliest period to Period 3, and then maximum values rose slightly again in the more recent periods (Figure 4.2-12). However, unlike the TBC, values were regularly greater than 50 PCU throughout the study period.

Figure 4.2-12: Hillsborough River Reservoir color in PCU, 1996–2023 (Analysis Days)



In terms of nutrients, both total nitrogen and total phosphorus concentrations have decreased over time in the Reservoir (Figure 4.2-13, Figure 4.2-14). The declines in total nitrogen were also observed in total Kjeldahl nitrogen and nitrate+nitrite concentrations over the study period (Appendix L).

Figure 4.2-13: Hillsborough River Reservoir total nitrogen concentrations in mg/L, 1996–2023 (Analysis Days)

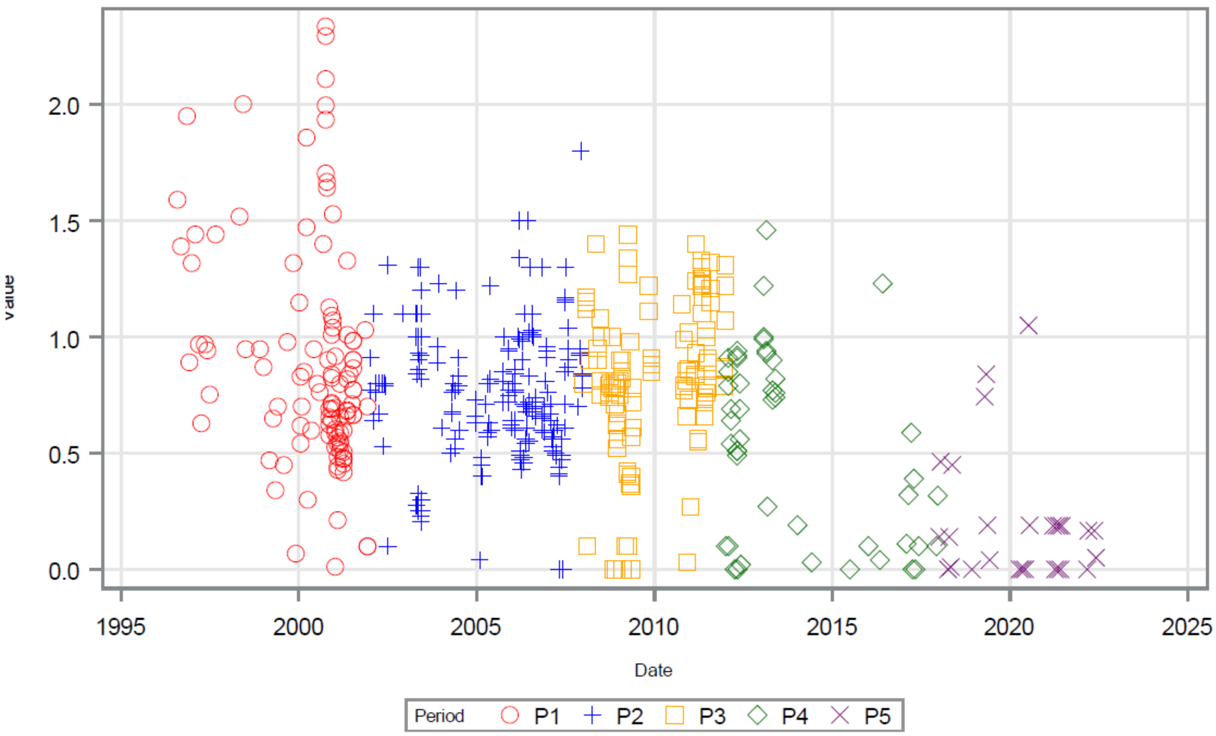
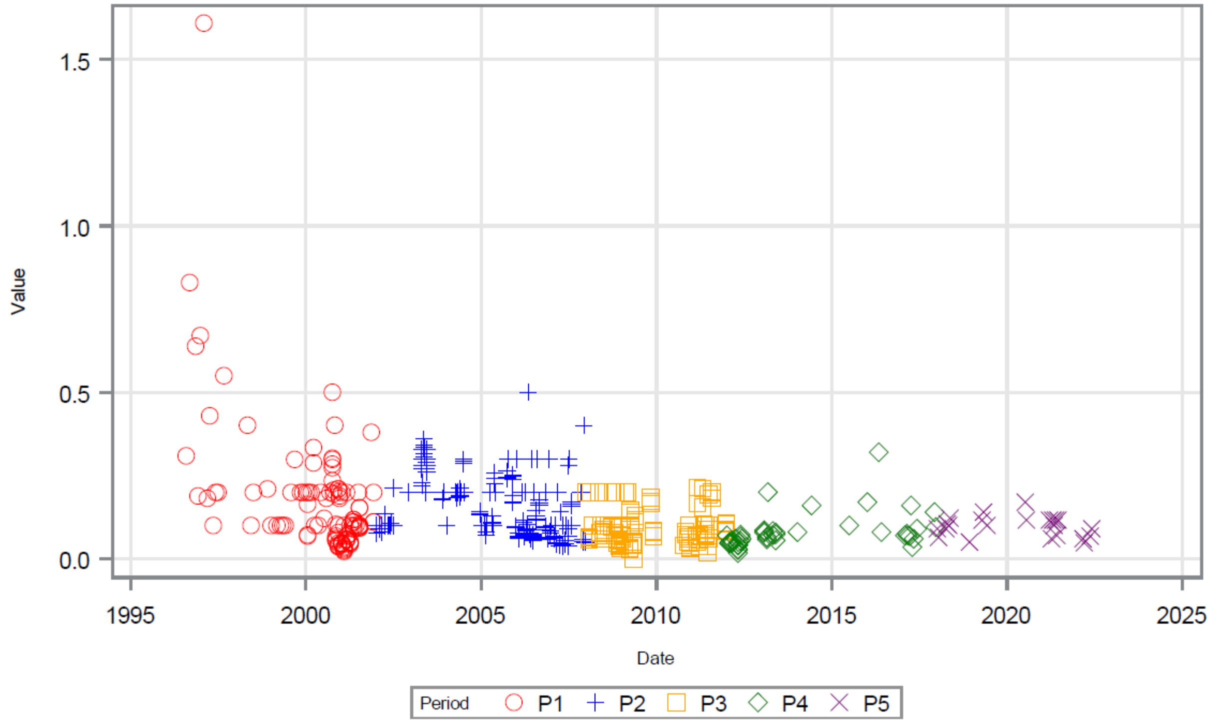


Figure 4.2-14: Hillsborough River Reservoir total phosphorus concentrations in mg/L 1996–2023 (Analysis Days)



4.2.4 MORRIS BRIDGE SINK WATER QUALITY

Water quality data have been collected at least annually in Morris Bridge Sink since 2016. Table 4.2-1 summarizes the parameters sampled. These data are summarized and provided to FDEP annually (Appendix K). All sampling events were conducted during Period 4 and 5. Parameters include conductivity, salinity, temperature, color, total nitrogen, nitrate+nitrite, total phosphorus, orthophosphate, and uncorrected chlorophyll. Scatter plots & boxplot of these limited data are provided in Appendix L.

4.3 RECOVERY SOURCE BIOLOGY

4.3.1 SULPHUR SPRINGS RUN FLORAL COMMUNITY

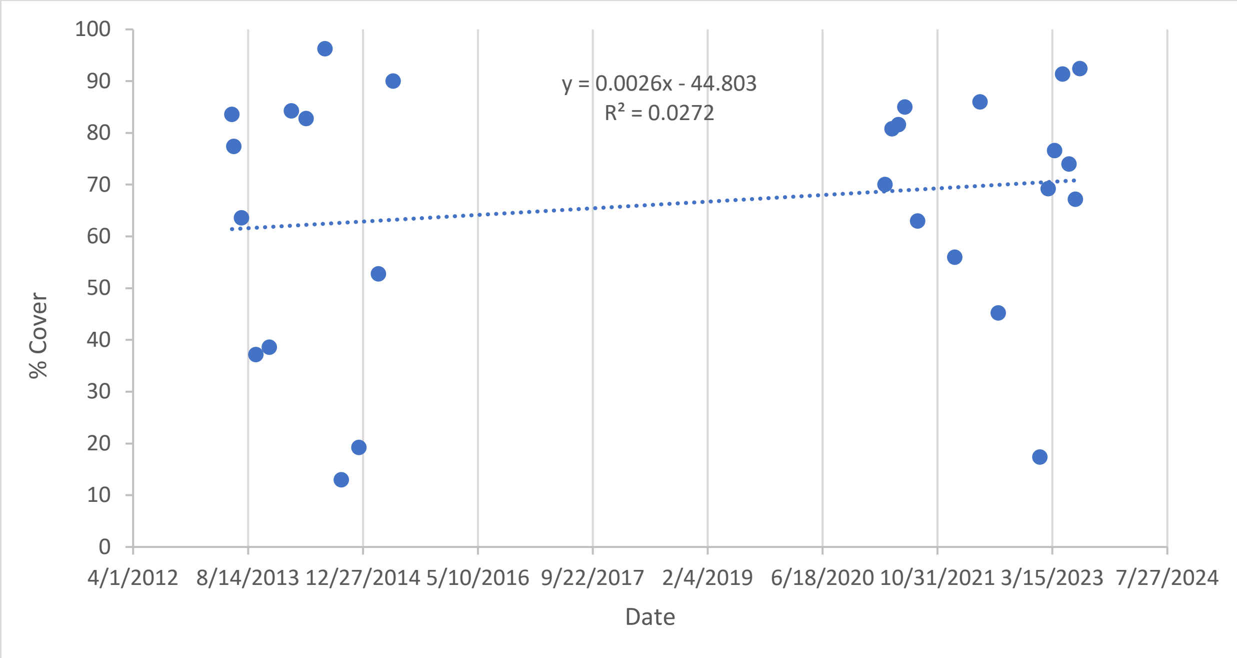
Filamentous algae mats in Sulphur Springs Run were first observed during the 2000–2001 drought when large flow diversions from Sulphur Springs reduced spring run velocities (SWFWMD 2004). These blooms subsided during wetter years (2003–2005) when diversions were infrequent, and algae coverage remained low. However, since minimum flows were implemented and higher diversion rates to the dam began in 2012, large filamentous algae growths have reappeared and become more widespread (SWFWMD 2015).

The Sulphur Springs Run was sampled for macroalgae and submerged aquatic vegetation (SAV) using four to five transects situated across the run. At each transect, three quadrats were employed to determine presence and percent coverage of macroalgae and SAV, which were generally identified to the genus level.

A subset of the Sulphur Springs Pool water quality data described in Section 4.2.1 was used to calculate mean nitrate-nitrite and salinity. These data (spanning the period of the SAV/macroalgal collections between 2013 and 2023) were used to help interpret the biological community data. The mean nitrate-nitrite concentration during this period in the Sulphur Springs Pool was 0.19 mg/L. This level complies with the 0.35 mg/L nitrate-nitrite water quality criterion in Chapter 62-302, FAC, indicating nitrate-nitrite enrichment was not an issue. Based on the chloride concentration collected in the pool between 2013 and 2023, the mean salinity of Sulphur Springs was 2.3 ppt, which could support taxa tolerant of moderate salinities.

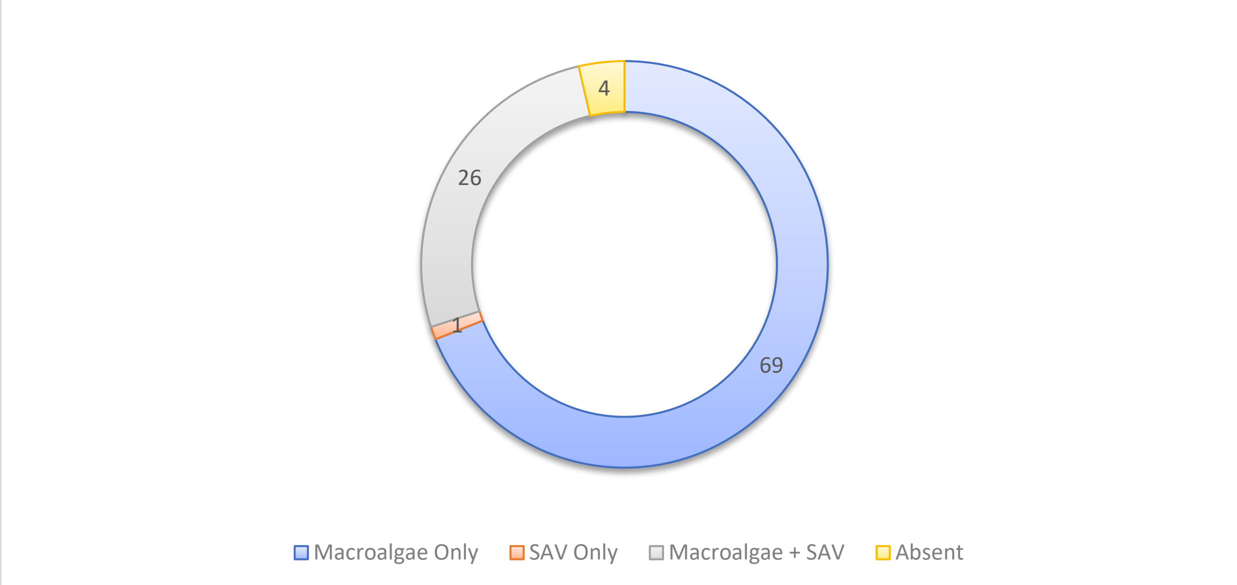
SAV and macroalgae sampling at Sulphur Springs occurred in 2013 and 2014 and then later in 2021–2023. The mean percent coverage of macroalgae and SAV in the spring run ranged from 13% to 97%, with a long-term mean of 66% (Figure 4.3-1). There appeared to be no temporal trend in the percent coverage data between the two clouds of points representing Periods 4 and 5 sampling, evidenced by the low coefficient of determination (r^2= 0.03).

Figure 4.3-1: Sulphur Springs Run mean macroalgae and SAV percent coverage over time



Data collected within each quadrat were amenable to analysis through calculating the number of occurrences of the various taxa identified. Because one to multiple SAV or macroalgae taxa were identified within a quadrat, it was not practical to determine the percent occurrence of each individual taxonomic unit. However, the number of occurrences of macroalgae only, SAV only, a mixture of both, or the absence of macroalgae and SAV within the quadrat could be determined (Figure 4.3-2). The system is clearly dominated by macroalgae, occurring as a single taxon or as multiple algal taxa in 69% of the sampled quadrats. SAV, occurring without macroalgae, were found in only 1% of the samples. A mixture of SAV and macroalgae was found in 26% of the quadrats, while SAV and macroalgae were both absent in 4% of the samples.

Figure 4.3-2: Occurrence-based Sulphur Springs Run quadrat percent cover composition



Common macroalgae collected include *Chaetomorpha* sp., *Cladophora* sp., *Vaucheria* sp., and *Compsopogon* sp. Common SAV included *Hydrilla* sp., *Chara* sp. (a macroscopic algae considered to function as SAV and therefore is grouped with SAV here), *Najas* sp., *Zannichellia* sp., and *Potamogeton* sp. The following provides a brief description of these periphyton and SAV species.

Chaetomorpha sp. is a green alga, often forming dense, tangled mats that float around or snag on other seaweed in the mid to low intertidal zone in summer. It is usually found in protected to semi-exposed habitats, tidepools, and mud flats (SOA 2024).

Cladophora sp. is a green alga found in a variety of marine and fresh waters and provides habitat and food for numerous organisms. It may be the most ubiquitous macroalgae in freshwater worldwide. This filamentous green alga often reaches nuisance levels as a result of cultural eutrophication (Dodds and Gudder 1992).

Vaucheria sp., a yellow-green algae, is found nearly worldwide. Most species occur in fresh water, though some are marine, inhabiting almost any wetland habitat, including mudflats, salt marshes, estuaries, and springs. *Vaucheria* sp. has been identified as a chief nuisance organism in Florida springs (Stevenson et al. 2007).

Compsopogon sp. is a red algae that lives in fresh water, brackish lagoons, and estuaries, tolerating a wide range of conditions (Algaebase 2024).

Hydrilla sp. is an invasive vascular plant species, infesting Florida waters and costing millions of dollars each year to control its spread (UF Center for Aquatic and Invasive Plants 2024).

Chara sp., known as Muskgrass, is a submersed macro-alga generally considered to function as SAV, characterized by a distinctive garlic odor. The plant has no leaves, and tiny spines and calcium deposits make muskgrass rough to the touch (UF Center for Aquatic and Invasive Plants 2024).

Najas sp., known as Southern Naiad, may be found in springs, fresh and brackish lakes, ponds, and canals, sometimes forming mats (UF Center for Aquatic and Invasive Plants 2024).

Zannichellia sp., the Horned Pondweed, is a plant found in fresh to brackish waters in the United States, Europe, Asia, Australia, and South America. It is recognizable by its long, thread-like leaves (Oregon Flora 2024).

Potamogeton pectinatus (likely the species found), or Sago Pondweed, grows in fresh, brackish, and saline waters throughout the state. It is found in stagnant ponds, spring-fed rivers, and slow-flowing marshes (UF Center for Aquatic and Invasive Plants 2024).

4.3.2 Sulphur Springs Run Floral Community Conclusions

A temporal trend was not identified in the percent coverage data in Sulphur Springs Run, evidenced by the low coefficient of determination in Figure 4.3-1 (r^2= 0.03). Macroalgae alone occurred in 69% of the quadrats, whereas a mixture of SAV and macroalgae was found in 26% of the quadrats.

Cladophora sp. and *Vaucheria* sp. were two macroalgae taxa present in the system that have been associated with nuisance growth in some springs, while *Hydrilla* sp., a vascular plant, is known as an invasive species. The other macroalgae and SAV species present are common in spring runs and/or brackish waters.

4.3.3 Morris Bridge Sink Descriptive Biology

Overall, the ecological and environmental data collected at Morris Bridge through 2023 signify a collection of healthy wetland environments. No pumping from Morris Bridge Sink has occurred to date. Annual monitoring has been conducted by the District since 2016 to establish baseline conditions. A description of baseline biological data is provided for reference, but no impacts from minimum flow implementation are expected since no pumping has occurred.

4.3.3.1 Zooplankton

Zooplankton were sampled in Morris Bridge Sink seven times between 2016 and 2023 by consultants hired by the District (Appendix K). Both Amec Foster Wheeler/Wood (through Earth Resources) and Frydenborg Ecologic (through Jones Edmunds) employed similar sampling methodologies. The samples were collected from a boat by vertical tow with an 8-inch diameter plankton net with 80-micron mesh. Samples were taken from three zones in the Sink: the edge (approximately 3 meters from shore), the center (approximately 25 meters from shore), and a midpoint between the edge and center (approximately 10 meters from shore). The depths of each sampling location varied, as depths increase from the edge of the Sink to the center. For collections in 2016, sampling occurred at 2.5 meters depths at the edge, 4.5 meters depth at the midpoint, and 7 meters depth at the center. Later sampling depths were constrained by DO concentrations, preferentially targeting depths where DO concentrations were above 3 mg/L. If DO concentrations did not reach this level, as was frequent away from the edge, the consultants defaulted to the depths used in 2016. Net contents were preserved in the field and taxa were identified and enumerated.

Sid Flannery, a former Chief Environmental Scientist for the District, sampled the sink on four occasions (Table 4.3-1) between 2017 and 2019 using a Wisconsin style sampling net with a diameter of 4.5 inches and 80-micron mesh. The first event consisted of qualitative sampling using vertical and oblique tows of the plankton net at beginning depths of 1-2 meters. Subsequent events occurred using vertical tows beginning at 1-2.5 meters of depth, dependent on the depth of hypoxic conditions (DO < 0.4 mg/L). At least one tow was taken near the center of the Sink during each event. Net contents were preserved, and subsamples were enumerated and identified.

Table 4.3-1: Morris Bridge Sink zooplankton data 2016–2023

Sample Date	Sampling Entity	Taxa	Density (#/m^3)
November 6, 2016	Amec Foster Wheeler	NA	0
November 8, 2017	Amec Foster Wheeler	NA	0
September 17, 2018	Sid Flannery	*Calanoida spp.*	54,200
		Copepod nauplii	60,000
		Cladocera spp.	1,200
February 13, 2019	Sid Flannery	*Calanoida spp.*	1,100
		Copepod nauplii	1,000
		Cladocera spp.	400
May 16, 2019	Wood (formerly Amec Foster Wheeler)	*Cladocera spp.*	1,782
		Calanoida spp.	1,971
		Rotifera spp.	286
June 4, 2019	Sid Flannery	*Calanoida spp.*	80,500
		Copepod nauplii	24,300
		Cladocera spp.	0
March 10, 2020	Frydenborg Ecologic	*Calanoida spp.*	250
April 21, 2021	Frydenborg Ecologic	*Calanoida spp.*	7,046
		Rotifera spp.	5,372
April 12, 2022	Frydenborg Ecologic	*Calanoida spp.*	1,411
April 25, 2023	Frydenborg Ecologic	*Calanoida spp.*	1,990
		Rotifera spp.	16
		Chironomidae spp.	16

4.3.3.2 Benthic Macroinvertebrates

Benthic macroinvertebrates were sampled in Morris Bridge Sink on seven occasions between 2016 and 2023, by consultants hired by the District (Table 4.3-2). Consultants followed the FDEP guidelines for BioRecon sample collection outlined in the FDEP's "BioRecon Field Method BRN1000" document.

Briefly, each event consisted of four sweeps of a D-frame dipnet with a 600-micron mesh within available productive habitats. After each sweep, contents of the dipnets were transferred to jars and samples were preserved with formalin in the field. Samples taken by Earth Resources, Inc. were analyzed by the Amec Foster Wheeler taxonomy lab in Gainesville, Florida, and those sampled by Frydenborg Ecologic, LLC. were analyzed in the field by staff scientists and in the laboratory by Dr. John Epler.

Beginning in 2021, the consultants noted the prevalence of shoreline habitat smothering by periphyton. The 20 most abundant benthic macroinvertebrate taxa observed in the data, with the number of sampling events in which they occurred are provided in Table 4.3-2.

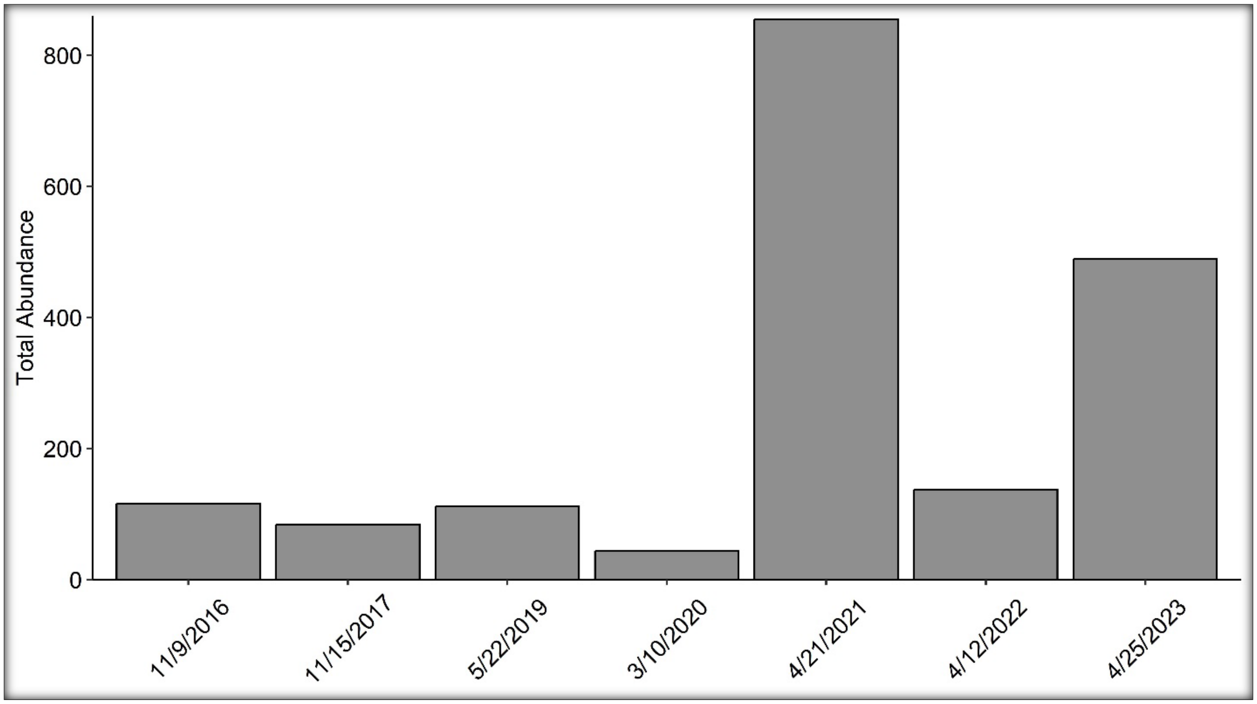
Table 4.3-2: Morris Bridge Sink benthic macroinvertebrate 20 most abundant species

Taxa	Abundance (n)	Frequency of Occurrence (number of sampling events)
Hydrobiidae spp.	1,238	3
Chironomidae spp.	248	3
Hyalella azteca spp. complex	243	6
Oligochaeta spp.	136	3
Planorbella spp.	43	3
Amnicola dalli	30	4
Planorbella trivolvis	27	4
Ancylidae spp.	17	2
Goeldichironomus carus	15	1
Pyrgophorus platyrachis	15	4
Peltodytes spp.	13	4
Coptotomus interrogatus	10	3
Polypedilum beckae	10	3
Glyptotendipes paripes	7	1
Goeldichironomus cf. natans	7	2
Hirudinea spp.	7	1
Atrichopogon spp.	6	1
Caenis diminuta	6	2
Caenis spp.	6	1
Kiefferulus spp.	5	2

4.3.3.3 Nekton

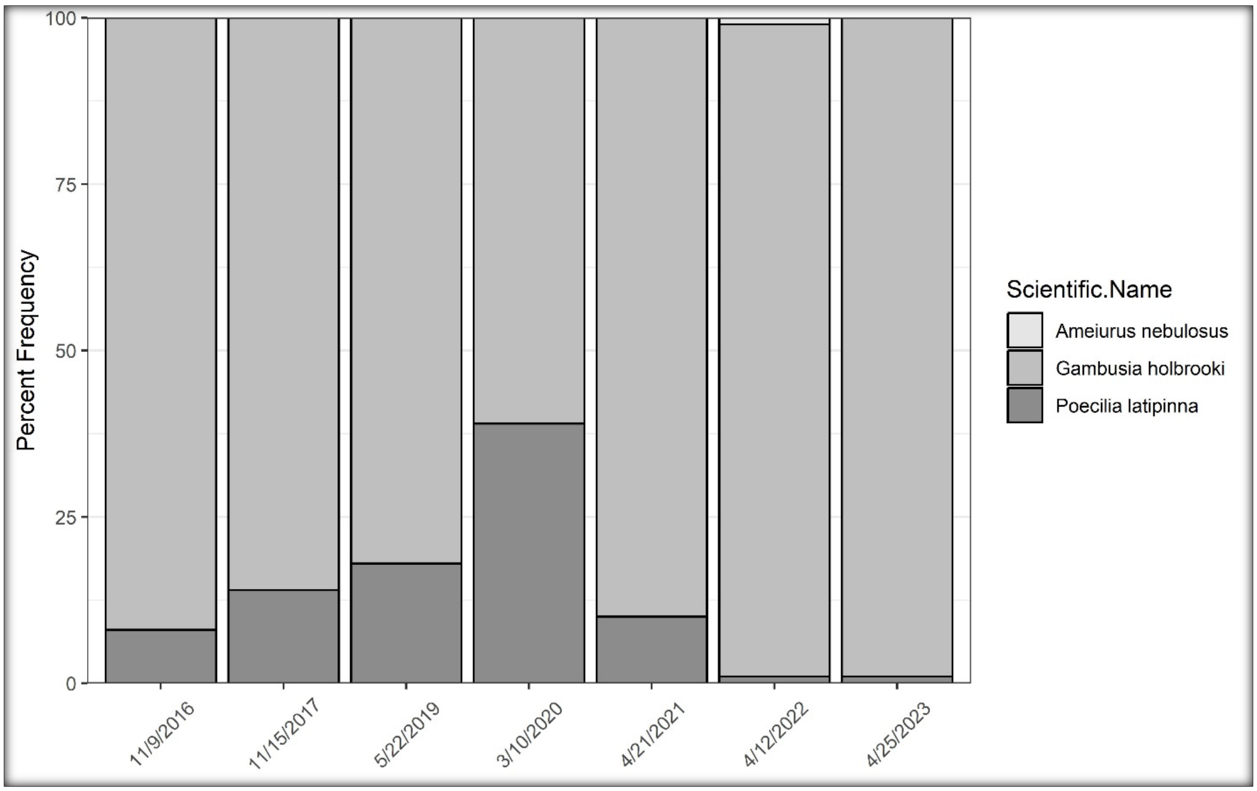
Nekton (fish) were sampled in Morris Bridge Sink on seven occasions between 2016 and 2023, by consultants hired by the District (Figure 4.3-3, Appendix K). Samples were collected by four minnow seine hauls taken at four different locations of the Sink's littoral shelf. Seine mesh size was reduced from 0.25 inch to 0.125 inch in 2021 due to the observation of fish escaping the larger sized mesh. All vertebrates collected by seine were identified, enumerated, and returned to the Sink.

Figure 4.3-3: Morris Bridge Sink total nekton catch 2016–2023



Total abundance by sampling event varied from 44 fish in 2020 to 855 fish in 2022 (Figure 4.3-4). Variability in catch was likely due to different levels of DO, zooplankton availability for food, and periphyton prevalence, which served both as cover and limited seine catch by interfering with net deployment. Three species were observed in the Sink, with *Gambusia holbrooki* (Eastern Mosquitofish) dominating the majority of catch during each sampling event (Figure 4.3-4). The consultants noted the limited number of fish species observed was likely due to the lack of recruitment potential from other water bodies.

Figure 4.3-4: Morris Bridge Sink percent frequency of nekton catch 2016–2023



4.3.3.4 Wetland Vegetation

Wetland vegetation data was collected along three established transects using the Wetland Assessment Procedure (WAP) and the Wetland Evaluation (WE) wetland health methodologies (Figure 4.3-5). Each method uses a scoring system from 5 to 1, with a score of 5 indicating normal wetland plant zonation and hydrology and a score of 1 indicating high numbers of upland plants in the wetland's center and no hydrology. Data collection began in 2009 at Nursery Marsh (Wetland ID 539) and Nursery Cypress Wetland (Wetland ID 540) and began in 2016 at Cypress Marsh (Wetland ID 543). The WAP zonation scores are summarized in Table 4.3-3. Scores have remained consistent over the monitoring period.

Figure 4.3-5: Morris Bridge Sink wetland transects

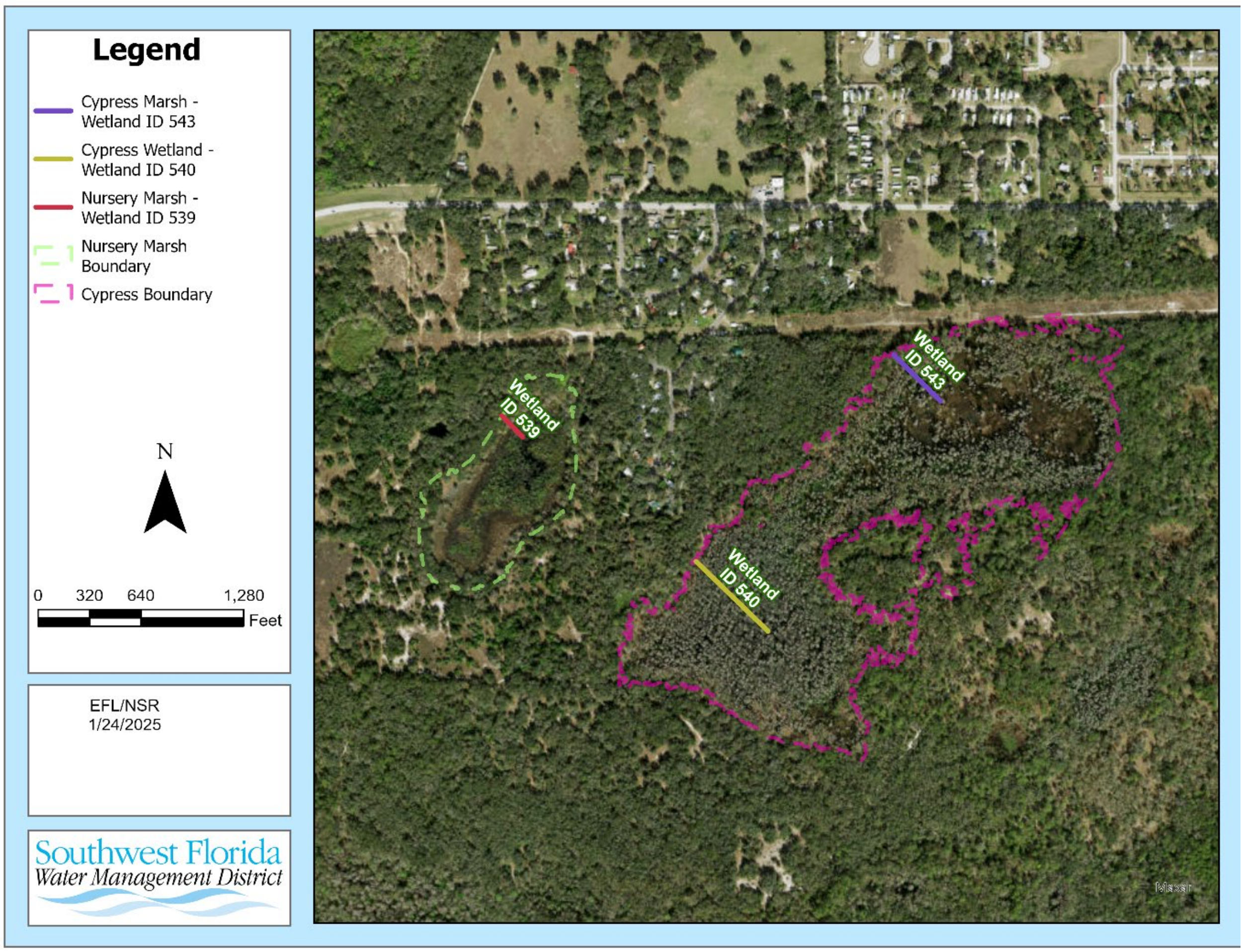


Table 4.3-3: Morris Bridge Sink wetland transect WAP zonation scores 2013–2023

Year	Nursery Marsh Wetland ID 539			Cypress Wetland Wetland ID 540			Cypress Marsh Wetland ID 543		
	G	S	T	G	S	T	G	S	T
2013	4	3	5	5	3	3			
2014	3	3	3	4	3	4			
2015	4	3	3	5	4	3			
2016	4	3	3	NA	4	3	5	4	3
2017	3	3	3	5	3	3	5	2	3
2018	3	3	3	4	4	5	4	3	3
2019	4	3	3	4	4	5	4	3	3
2020	3	3	3	4	5	5	5	5	3
2021	3	3	3	3	4	5	3	5	3
2022	3	3	3	4	3	3	3	5	3
2023	3	3	3	4	4	3	3	4	3

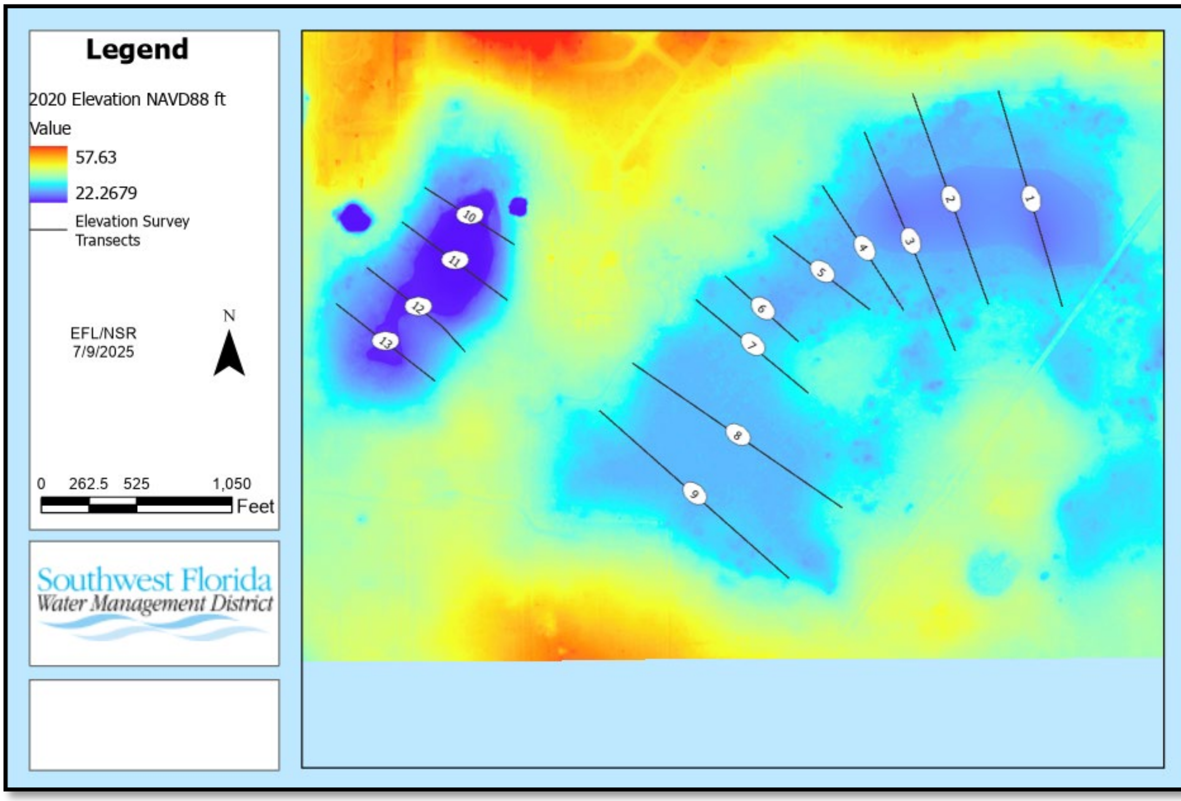
G = Groundcover
S = Shrub/Small Trees
T = Tree
NA= Not enough cover to make evaluation
Gray= Not sampled

4.3.3.5 Wetland Soils

Subsidence of wetland soils can greatly impact wetland health and can eventually lead to tree fall. To monitor soil subsidence from baseline conditions prior to initiation of pumping at Morris Bridge Sink, soil monitoring stations were established along each wetland transect by driving aluminum rods to refusal at 10-m intervals from the wetland's edge to the center (Figure 4.3-5). The elevation of each soil monitoring station was determined, and the ground surface elevation was measured during each monitoring event to determine if soil subsidence had occurred. A hydric soil analysis was completed at each soil monitoring station, and three 5 ft deep soil profiles were also documented. Soil monitoring data was collected in 2016, 2017, 2019, and 2021. No evidence of soil subsidence or recent tree fall has been observed (Jones Edmunds, 2021).

Additional bathymetric data were collected at the monitored wetlands along 13 survey transects in 2016 and 2020 (Figure 4.3-6). Survey data were combined with 2017 LiDAR data to generate a digital elevation model (DEM), which can be used to track inundation area and frequency at the monitored wetlands over time.

Figure 4.3-6: Morris Bridge Sink baseline survey transects



4.3.3.1 Feral Hog

Feral hog surveys were conducted at each transect beginning in 2016 using the US Department of Agriculture's feral pig disturbance ranking (USDA 2009). This methodology ranks feral hog damage from Category 1 – surficial rooting to Category 4 – a wallow or open depression created by rolling activity of hogs. Surveys were conducted by consultants or District staff (Table 4.3-4). There were slight modifications to the sampling protocol in 2020, when the assessment boundary was limited to the same area as the wetland vegetation assessment transect. During all other surveys, the assessment area included both the area of the wetland vegetation assessment transect and the areas in the vicinity of the transect. Hog damage has been documented at every wetland transect during each survey, with substantial damage including wallows identified at each transect (Table 4.3-4).

Table 4.3-4: Morris Bridge Sink feral hog assessment disturbance rankings 2016–2023

Wetland Transect	2016	2017	2018	2019	2020	2021	2022	2023
Cypress Marsh	2	1	1	4	1	1	2	4
Cypress Wetland	1	1	1	4	2	4	2	2
Nursery Marsh	1	2	3	2	2	3	4	2

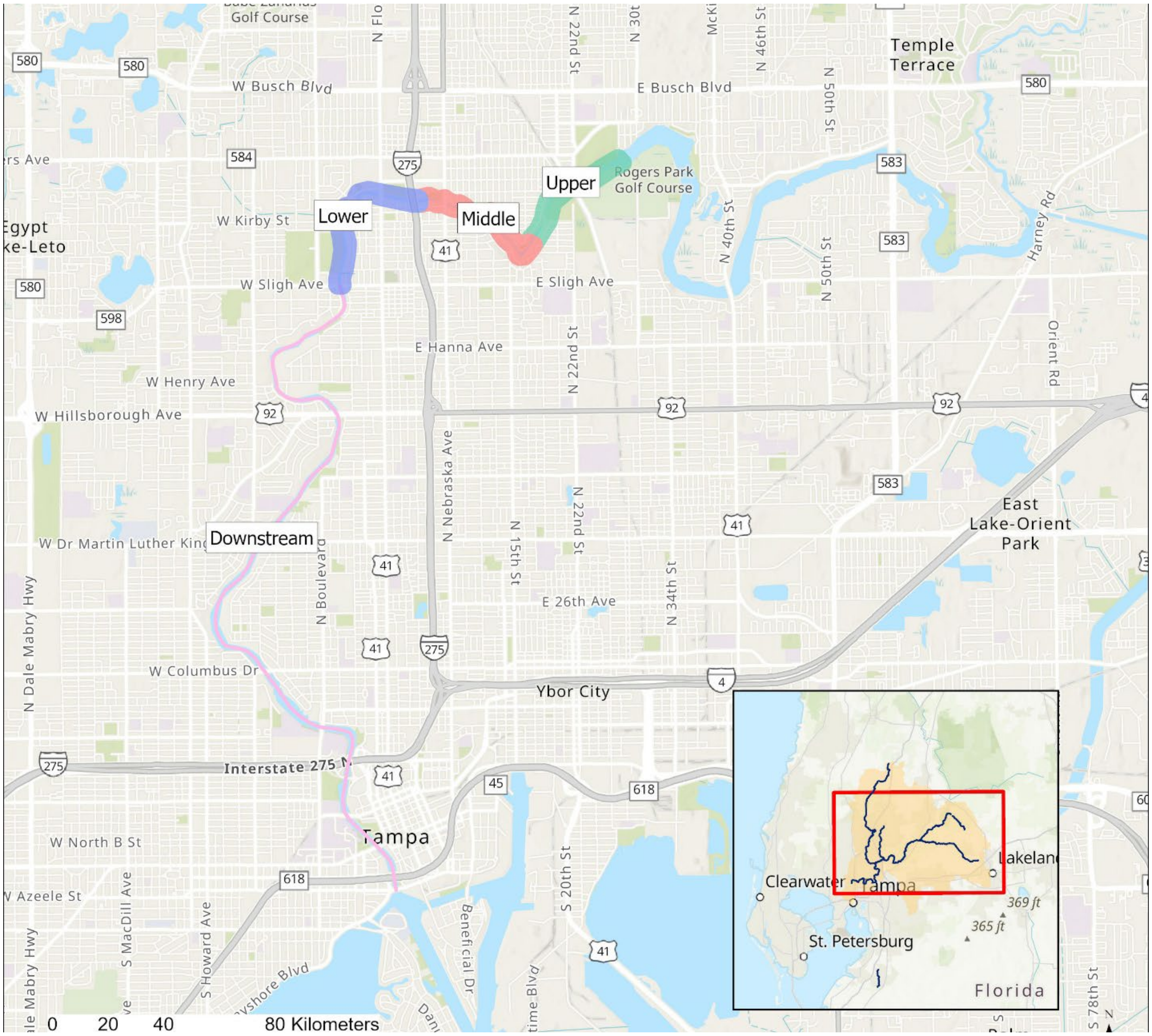
Notes: 1 = Surficial rooting; 2 = Moderate rooting; 3 = Extensive rooting; 4 = Wallow

5 DESCRIPTIVE EVALUATION OF THE LOWER HILLSBOROUGH RIVER DATA

This chapter begins with a general description of hydrology (rainfall and flows) to the LHR in Section 5.1 to characterize flows for all days (All Days) in the POR and then for flows only when implementation was required under the MFL (Implementation Days). Descriptive statistics displaying the percentage of days when implementation was required and how often the MFL was achieved are provided to characterize the evolution of the recovery strategy over time.

The hydrology section is followed by a detailed description of water quality data collected in the LHR (Section 5.2). The data were separated into two spatial segments; downstream and the target zone (Figure 5-1). The downstream zone runs from Platt Street to Sligh Avenue. The target zone begins at Sligh Avenue and ends at the base of the Hillsborough River Dam. Descriptive statistics have been summarized for the water quality data collected in the downstream and targets zone segments of the river for Analysis Days. Restricting the characterization of water quality to Analysis Days allowed for the compatibility with analysis of the biological data, which is detailed in Section 5.3. Analysis Days represent days where implementation would have occurred if the current minimum flow was in place for the entire time series of data since 1996. The descriptive discussion includes analysis on general trends in water quality and biota over the different minimum flow periods within each segment.

Figure 5-1: Downstream and target zone river segments in the LHR



5.1 Lower Hillsborough River Hydrology

5.1.1 Total Calculated Flows to the LHR

Total calculated flows to the LHR represent the sum of flow measured at the dam and flow from recovery sources pumped to the base of the dam; specifically, Sulphur Springs, the TBC/Hillsborough River Reservoir, and Blue Sink. Table 5.1-1 summarizes the total flows for all days in the POR. Table 5.1-2 provides the flow statistics for these stations, limited to Implementation Days only.

Available data reflects an observed decline in upper river flows reported at USGS gage 02303000 (Zephyrhills) during Periods 2 and 3 (Table 5.1-1). Although peak total flow values (highest flow events) in the LHR have not since returned to pre-2002 (Period 2) levels, the average total flows have since increased. A time series of total flows since 1996 is shown in Figure 5.1-1, illustrating the more natural hydrology of the upper river (as indicated in the Zephyrhills time series) in contrast to the more regulated, flattened flow patterns observed in the lower river (i.e., fewer high flow spikes occurring on the lower river because of the reservoir and dam).

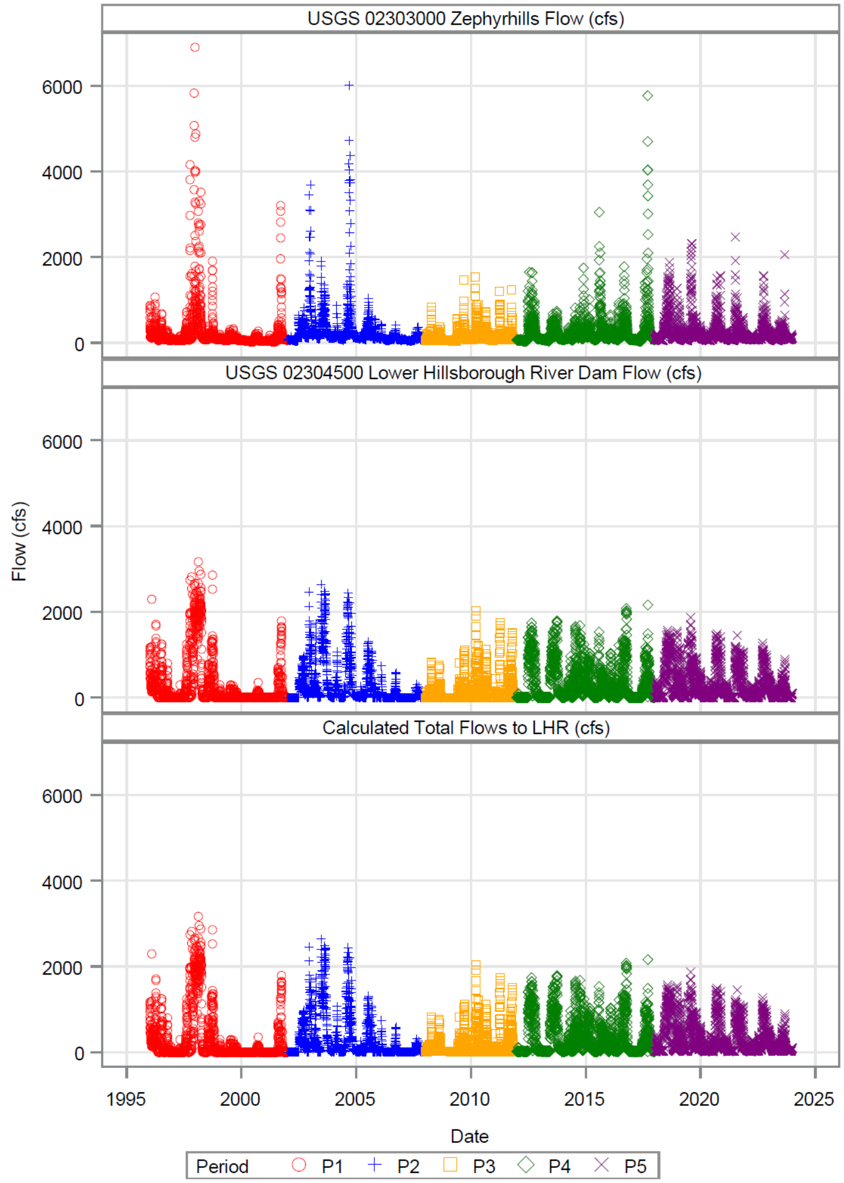
Table 5.1-1: Summary statistics of daily average flows in the LHR by period (All Days)

Source	Period	N	Min	Max	Mean	Std
Calculated Total Flows to LHR (cfs)	P1	2,192	0.00	3,160.00	250.40	543.92
	P2	2,191	0.00	2,640.00	248.49	464.27
	P3	1,461	1.04	2,030.00	148.69	265.28
	P4	2,192	0.82	2,160.00	306.88	411.14
	P5	2,191	17.60	1,870.00	297.01	366.50
USGS 02304500 LHR Dam Flow (cfs)	P1	2,192	0.00	3,160.00	250.40	543.92
	P2	2,191	0.00	2,640.00	243.99	466.51
	P3	1,461	0.00	2,030.00	138.70	270.17
	P4	2,192	0.00	2,160.00	299.75	416.14
	P5	2,191	0.00	1,870.00	288.88	372.21
USGS 02303000 Zephyrhills Flow (cfs)	P1	2,192	27.00	6,900.00	212.33	484.11
	P2	2,191	35.80	6,010.00	236.80	419.33
	P3	1,461	38.60	1,530.00	141.58	146.72
	P4	2,192	39.50	5,770.00	236.42	352.20
	P5	2,191	52.10	2,470.00	241.94	294.96

Table 5.1-2: Summary statistics of daily flows in the LHR by period (Implementation Days)

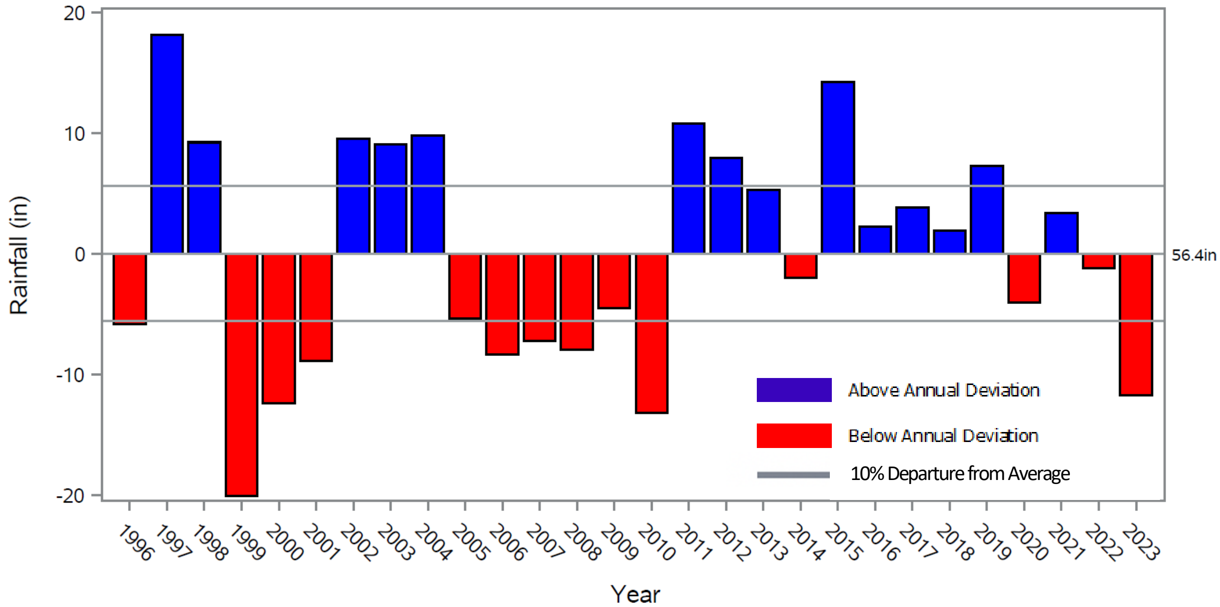
Source	Period	N	Min	Max	Mean	Std
Calculated Total Flows to LHR (cfs)	P1	605	0.00	9.30	0.38	1.09
	P2	974	0.00	24.76	9.57	4.11
	P3	828	1.04	43.15	18.48	5.01
	P4	786	0.82	40.38	20.79	4.26
	P5	696	17.60	47.09	25.37	3.46
USGS 02304500 LHR Dam Flow (cfs)	P1	605	0.00	9.30	0.38	1.09
	P2	974	0.00	18.20	0.67	2.20
	P3	828	0.00	25.60	1.46	4.25
	P4	786	0.00	25.60	1.25	4.17
	P5	696	0.00	26.10	0.99	3.64
USGS 02303000 Zephyrhills Flow (cfs)	P1	605	27.00	266.00	54.36	27.89
	P2	974	35.80	638.00	85.42	55.11
	P3	828	38.60	558.00	78.92	43.45
	P4	786	39.50	5,770.00	81.95	204.19
	P5	696	52.10	656.00	88.25	42.28

Figure 5.1-1: Time series of calculated daily average flow at USGS Zephyrhills (Gage No. 02303000), USGS dam flows (Gage No. 02304500) and total LHR calculated flows (All Days)



These patterns in flow are largely a function of rainfall in the region. Average annual rainfall fell below the POR average of 56.4 inches between 2007 and 2010 when the region was experiencing severe drought (Figure 5.1-2). Rainfall then increased between 2011 and 2019, and subsequently flow also increased and remained relatively steady during this period. Recent years reflect declining rainfall relative to long-term average.

Figure 5.1-2: Deviation of annual rainfall (Hillsborough River at Sulphur Springs station (District 19436), 1996–2023



5.1.2 Implementation of Minimum Flows

The minimum flow for the LHR has evolved over time, beginning in 2000 as a 10 cfs (6.5-mgd) minimum flow applied to the base of the Hillsborough River Dam to a seasonal flow with a freshwater equivalence to account for the salinity of Sulphur Springs water being utilized as a recovery source. Diversions of water from Sulphur Springs to meet minimum flows began in spring 2002, and therefore the evaluation of the flow regime begins in 2002 (the beginning of Period 2).

The number of days that minimum flow implementation was needed (i.e. when dam flows were not sufficient to meet the required minimum flow) in each calendar year from 2000–2023 are shown in Table 5.1-3. Days needing minimum flow implementation per calendar year ranged from a minimum of 22 days in 2003 to a maximum of 331 days in 2000 (Table 5.1-3). While the MFL was established in 2000, implementation did not occur until 2002. During Period 2 (2002–2007) when Sulphur Springs was supplying up to 10 cfs (6.5 mgd) for minimum flow implementation but no TBC inputs had begun, the average number of days requiring implementation per year was 162. This average increased to 207 days for Period 3 and then decreased to 131 and 116 days on average for Periods 4 and 5, respectively (Table 5.1-4).

Table 5.1-3 also provides the number of days the minimum flow was met during times when minimum flow implementation was needed (according to the minimum flow rule in effect for that year. For a time series plot of these sources, see Figure 4.1-1). Between 2008 and 2023, the number of days needing minimum flow implementation decreased and the percentage of days meeting the minimum flow increased as effort became more successful in providing sufficient water to meet the minimum flows through a substantial increase in diversions to the river from Sulphur Springs, Blue Sink, and the TBC. Successful minimum flow implementation is seen in the increasing percentage of days meeting the minimum flow from 8% over Period 3, to 20% for Period 4, and 32% over Period 5 (Table 5.1-4). During

Period 5, the flow deficit was less than the operational freshwater equivalent of 3 cfs. Finally, in 2023 the minimum flow was met 100% of the time despite the region experiencing significantly below average rainfall.

Table 5.1-3: Annual minimum flow implementation statistics between 2000–2023

Year	Days Min Flow Imp Req'd	Percent of Days Min Flow Imp Req'd	Days Min Flow Met	Percent of Days Min Flow Met	Avg Flow Deficit (cfs)	Days Flow <58 cfs at Zephyrhills	Percent of Days Flow <58 cfs at Zephyrhills
2000	331	90	0	0	9.5	235	71
2001	274	75	0	0	9.7	176	64
2002	189	52	59	31	8.5	56	30
2003	22	6	15	68	2.5	0	0
2004	79	22	67	85	7.0	0	0
2005	100	27	29	29	1.0	0	0
2006	305	84	117	38	0.2	29	10
2007	279	76	185	66	8.1	92	33
2008	258	70	28	11	4.1	80	31
2009	249	68	41	16	4.2	120	48
2010	148	41	7	5	7.6	0	0
2011	173	47	2	1	8.8	0	0
2012	203	55	62	31	6.1	77	38
2013	166	45	35	21	3.2	22	13
2014	51	14	9	18	5.6	0	0
2015	63	17	2	3	8.9	0	0
2016	105	29	14	13	3.6	0	0
2017	198	54	62	31	4.1	56	28
2018	120	33	35	29	1.7	7	6
2019	72	20	7	10	2.1	7	10
2020	122	33	16	13	1.3	0	0
2021	106	29	4	4	2.1	13	12
2022	104	28	34	33	1.6	0	0
2023	172	47	172	100	0	0	0

The number of days for which the minimum flow was adjusted based on Zephyrhills Gage (USGS No. 02303000) data ranged from 0 days in several years to a maximum of 120 days in 2009. The average percentage of days the Zephyrhills Gage (USGS No. 02303000) measured flow was less than 58 cfs (37.5 mgd) varied for each period, with averages of 12% for Period 2, 20% for Period 3, 13% for Period 4, and only 5% for Period 5.

Table 5.1-4: Descriptive Statistics for minimum flow implementation over Periods 2 through 5

Period	Days Min Flow Imp Req'd	Percent of Days Min Flow Imp Req'd	Days Min Flow Met	Percent of Days Min Flow Met	Avg Flow Deficit (cfs)	Days Flow < 58 cfs at Zephyrhills	Percent of Days Flow < 58 cfs at Zephyrhills
Average 2002–2023	149	41	46	30	4.2	25	12
Minimum 2002–2023	22	6	2	1	1.5	0	0
Maximum 2002–2023	305	84	185	100	6.2	120	48
P2 Average 2002–2007	162	45	79	53	4.6	30	12
P3 Average 2008–2011	207	57	20	8	6.2	50	20
P4 Average 2012–2017	131	36	31	20	5.2	26	13
P5 Average 2018–2023	116	32	45	32	1.5	5	5

Sulphur Springs has consistently been the largest source of water used for minimum flow implementation, averaging 72% of the flow contribution over the 2002–2023 period when minimum flow implementation was required by the minimum flow rule (Figure 5.1-3, Table 5.1-5). Although these percentages indicate recovery source contribution, it does not guarantee implementation was met during the respective period. The percent contribution from Sulphur Springs was highest between 2002–2007 (86%) before TBC and Blue Sink flows were available (beginning in 2008 and 2018, respectively). The dam flows make up the difference to the total contributions from other recovery sources and generally constituted only 14% to the total LHR calculated total flow when minimum flow implementation was required. Percent contributions from the dam were highest during Period 2 with maximum contribution of 39% in 2003 as other sources were being brought online. Since 2005, the percentage flow from the dam has been less than 10% except in 2014 and 2015 when they were 20% and 26%, respectively (Table 5.1-5).

Figure 5.1-3: Stacked bar plot of recovery source contributions and dam flows (USGS 02304500) to the calculated total LHR flow

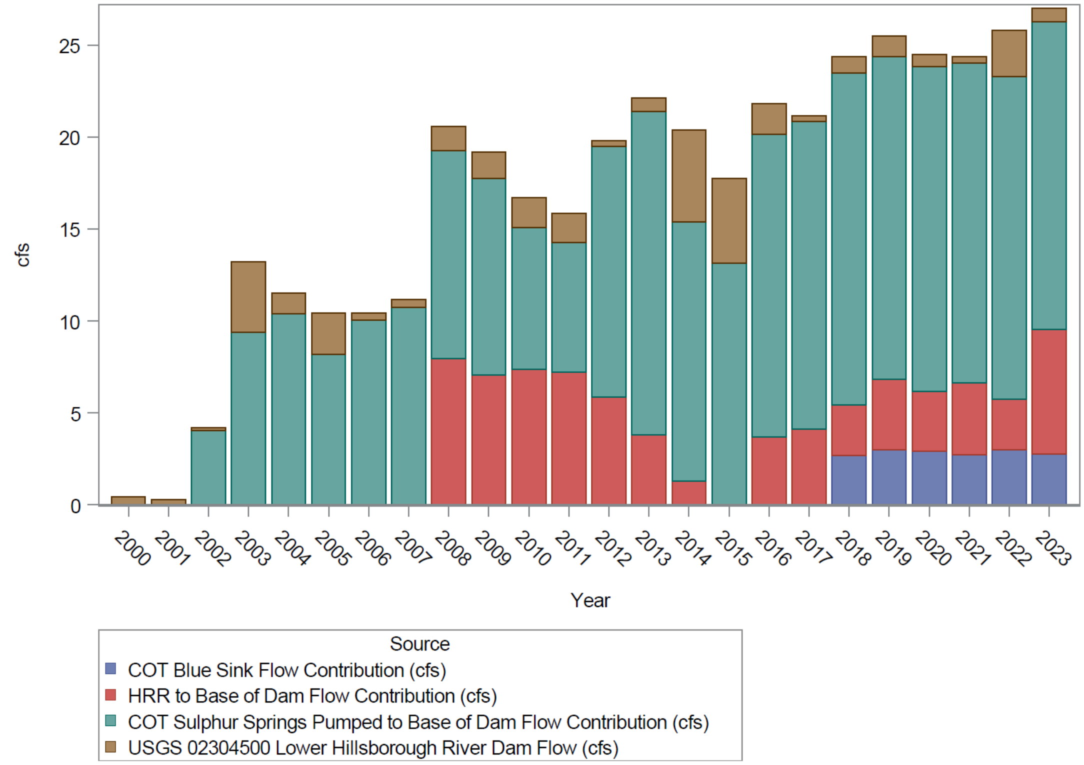


Table 5.1-5: Annual recovery source contributions (percent of total) to LHR minimum flow, 2002–2023(Implementation Days)

Year	LHR Dam	Blue Sink	Sulphur Springs	HRR to BOD
2002	5	0	95	0
2003	39	0	61	0
2004	11	0	89	0
2005	24	0	76	0
2006	3	0	97	0
2007	2	0	98	0
2008	5	0	55	40
2009	6	0	56	39
2010	8	0	39	53
2011	10	0	37	53
2012	1	0	69	30
2013	3	0	80	17
2014	20	0	73	6
2015	26	0	74	0
2016	7	0	76	16
2017	1	0	79	19
2018	3	11	75	11
2019	3	12	69	15
2020	2	12	73	12
2021	1	11	72	16
2022	7	12	70	10
2023	2	10	63	25

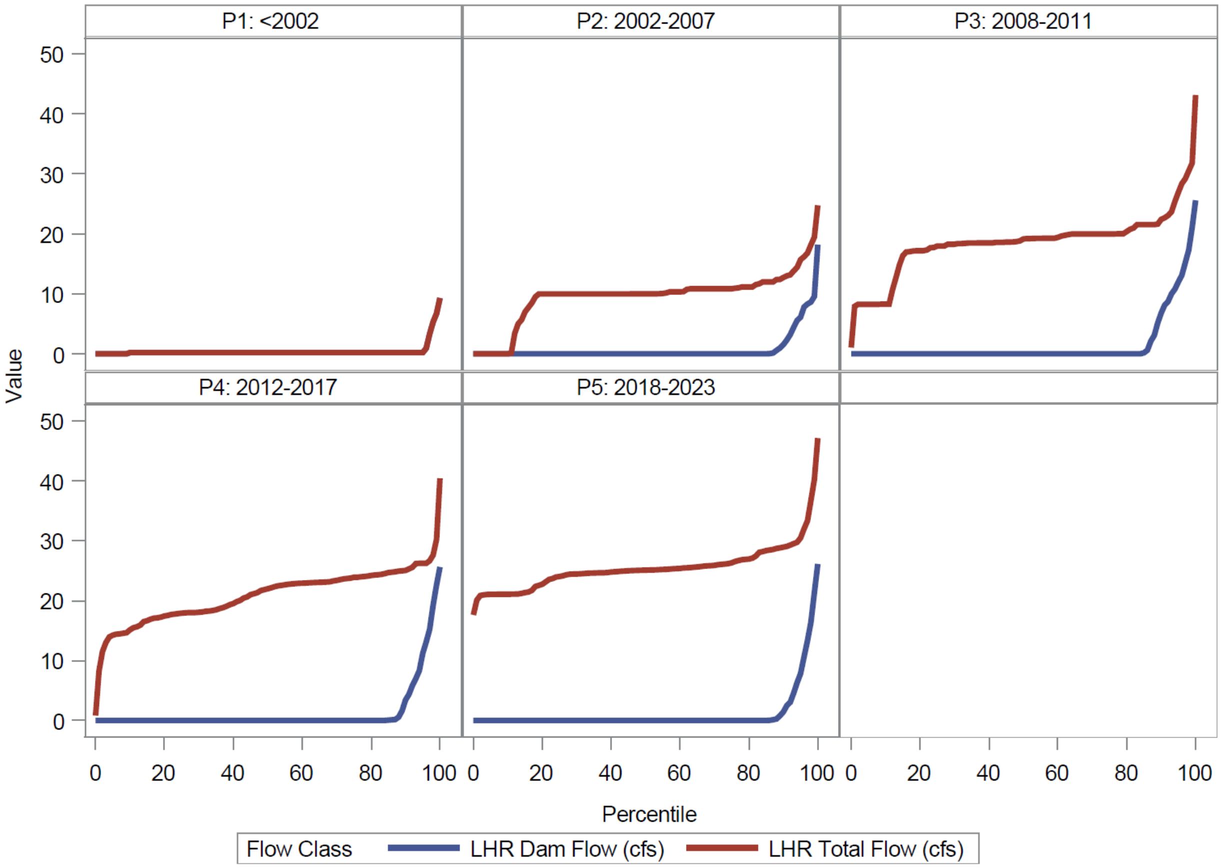
Note: HRR to BOD = Hillsborough River Reservoir to Base of Dam (cfs); LHR Dam = USGS 02304500 (cfs)

Table 5.1-6: Summary statistics for recovery source contributions to LHR minimum flow, 1996–2023(Implementation Days)

	Period	N	Min	Max	Mean	Std
COT Blue Sink Flow Contribution (cfs)	P5	696	0.00	3.13	2.84	0.55
HRR to Base of Dam Flow Contribution (cfs)	P2	1	8.25	8.25	8.25	.
	P3	828	0.00	8.29	7.44	2.01
	P4	786	0.00	8.29	3.94	3.12
	P5	685	0.00	13.86	4.19	3.23
COT Sulphur Springs Pumped to Base of Dam Flow Contribution (cfs)	P2	956	0.00	16.00	9.06	3.72
	P3	828	0.00	16.00	9.59	4.27
	P4	786	0.00	22.73	15.61	4.34
	P5	696	0.06	20.68	17.42	1.67
USGS 02304500 Lower Hillsborough River Dam Flow (cfs)	P1	605	0.00	9.30	0.38	1.09
	P2	974	0.00	18.20	0.67	2.20
	P3	828	0.00	25.60	1.46	4.25
	P4	786	0.00	25.60	1.25	4.17
	P5	696	0.00	26.10	0.99	3.64

The impact of minimum flow implementation is readily seen in cumulative distribution function plots comparing dam flows and total flows when implementation was required for each period (Figure 5.1-4). Before adoption of the minimum flow in 2000, no recovery source pumping was occurring and "total flows" for LHR were the same as dam flow. Sulphur Springs began providing recovery source water in 2002 for the LHR minimum flow implementation. For this pre-minimum flow pumping period (prior to 2002), dam and total flows were 0 cfs over 90% of the time. While dam flows remain low throughout the rest of the assessment period, total calculated flows started to increase in 2002 through 2007, with flows greater than 10 cfs (6.5 mgd) approximately 80% of the time due to flows from Sulphur Springs. Total flows further increased during 2008 through 2011 as Hillsborough River Reservoir flows increased, such that 0 flow conditions at the USGS Dam Gage (No. 02304500) were rare (total calculated flows exceeded 5 cfs (3.2 mgd) over 95% of the time), and total calculated flows were greater than 20 cfs (12.9 mgd) approximately 80% of the time. Zero flow conditions were even less frequent from 2012 to 2017, and the lower end of the curve was lifted, with total calculated flows of 15 cfs (9.7 mgd) occurring over 90% of the time. No zero total calculated flows occurred during Period 5, with total flows almost always above 20 cfs (12.9 mgd).

Figure 5.1-4: Cumulative distribution function plots of USGS Dam (Gage No. 02304500) flows and total calculated LHR flows (Implementation Days)



5.2 Lower Hillsborough River Water Quality

5.2.1 LHR Downstream

Water quality data were available from three CRs in the downstream section of the river (between Sligh Avenue and Platt Street), 12 routine fixed station sampling sites with varying frequency of sampling and different suites of parameters, and probabilistic monitoring associated with TBW HBMP, which ended in 2012. The approximate period of data collection associated with these sites is provided in Table 5.2-1. Results are reported by parameter of interest as separate sub-sections, first reporting overall time series trends and a table of descriptive statistics at each level for each period. These data are followed by boxplots of data distributions differentiated by the periods and then presenting summary statistics and distributions of the measured parameters at routinely collected fixed station sites ordered by their location in the river. A map of the fixed station locations that reported the parameter of interest is provided within each sub-section followed by a spatial distribution boxplot of parameter values for each station available. All plots and statistics are provided for Analysis Days only; that is, those dates where minimum flow implementation would have been required by the current adopted minimum flow. Time series plots, boxplots, and distributional statistics for each individual station using the same criteria are provided in Appendix M.

Table 5.2-1: Water quality stations within the LHR downstream

Sampling Type	Station Name	River Kilometer	Start Date	End Date
CRs	USGS Platt 02306028	0.02	1/10/1997	12/16/2023
	TBW @ Crosstown CR	0.10	5/17/2001	12/16/2023
	TBW @ Columbus CR	3.75	2/15/2001	12/16/2023
Fixed Stations	EPC SN 2	0.00	6/11/1996	12/13/2023
	EPC SN 1502	1.46	1/5/2009	12/13/2023
	EPC SN 1503	2.31	1/5/2009	12/13/2023
	EPC SN 137	3.64	5/14/1996	12/13/2023
	EPC SN 1505	4.52	1/5/2009	12/13/2023
	EPC SN 1506	5.53	1/5/2009	12/13/2023
	EPC SN 1507	6.38	1/5/2009	12/13/2023
	EPC SN 176	7.32	1/5/2009	12/13/2023
	EPC SN 1509	8.44	1/5/2009	12/13/2023
	EPC SN 1510	9.59	1/5/2009	12/13/2023
Probabilistic Sites	TBW HBMP	Varies	4/12/2000	6/14/2012

NOTE: Start and end dates can vary by parameter; those listed encompass the broadest range of the greatest number of parameters.

5.2.1.1 LHR Downstream Salinity

Time series of surface, midwater, and bottom salinity within the downstream section of the LHR, for all stations combined for Analysis Days are illustrated in Figure 5.2-1. All plots in this section are for Analysis Days only, which explains the gaps in the time series. The variation in the time series reflects the general effects of drought or surplus flow conditions with a dense cloud of data points in Period 1 represented by the red circles corresponding to drought conditions and reporting some of the highest observed salinities in the time series. More gaps in the data exist in Period 2, which was mostly above average rainfall in the watershed. The high density of points in Period 3 is associated with a persistent drought that affected the area between 2008 and 2011. Rainfall returned to normal or above normal conditions in 2011 until dry conditions occurred in 2023 when some of the highest salinities in the POR were again observed. This variability is largely attributed to differences in rainfall among periods, resulting in changes in freshwater flow but could also be affected by changes in the location and POR of stations reporting data. Summary statistics for the combined stations in the downstream segment are presented in Table 5.2-2 for each period and each sampling type. The table includes the number of samples collected (n), minimum salinity (min), maximum salinity, (max), the average salinity (mean), and the associated standard deviation (Std). Average salinity at the bottom tended to be about 6 ppt higher than at the surface for each period.

The distribution of salinity concentrations by period is also graphically represented in box and whisker plots in Figure 5.2-2. In these plots, the circle represents the mean, the horizontal line represents the median, the “box” represents the interquartile range, the whiskers represent the 1.75 times the interquartile range, and the open circles outside the whiskers represent “outlying” or unusual observations. The box and whisker plots also clearly illustrate the vertical gradient between surface and bottom salinities in the downstream portion of the river.

Figure 5.2-1: Time series for salinity (ppt) within the LHR downstream for specified water column strata (Analysis Days)

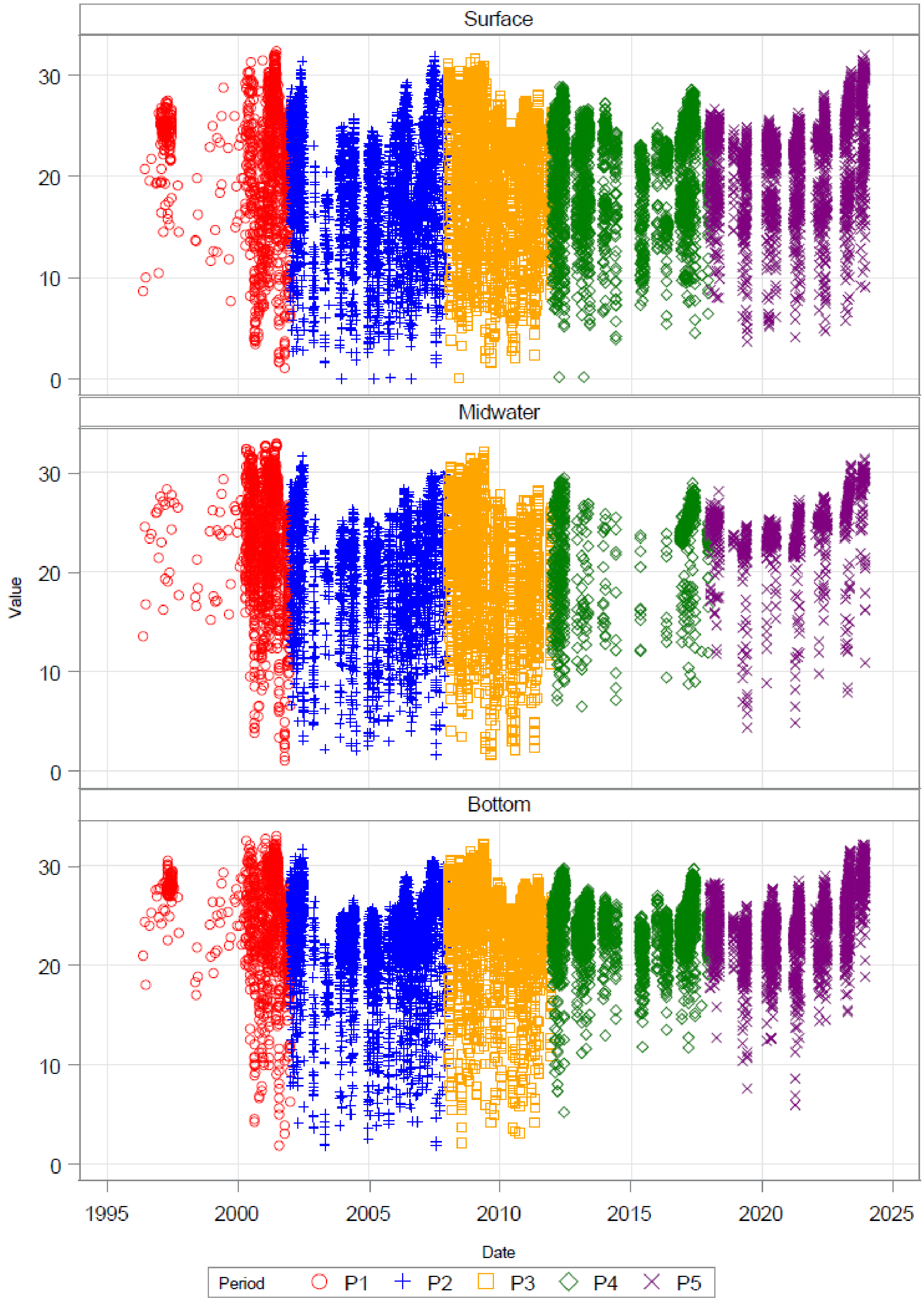
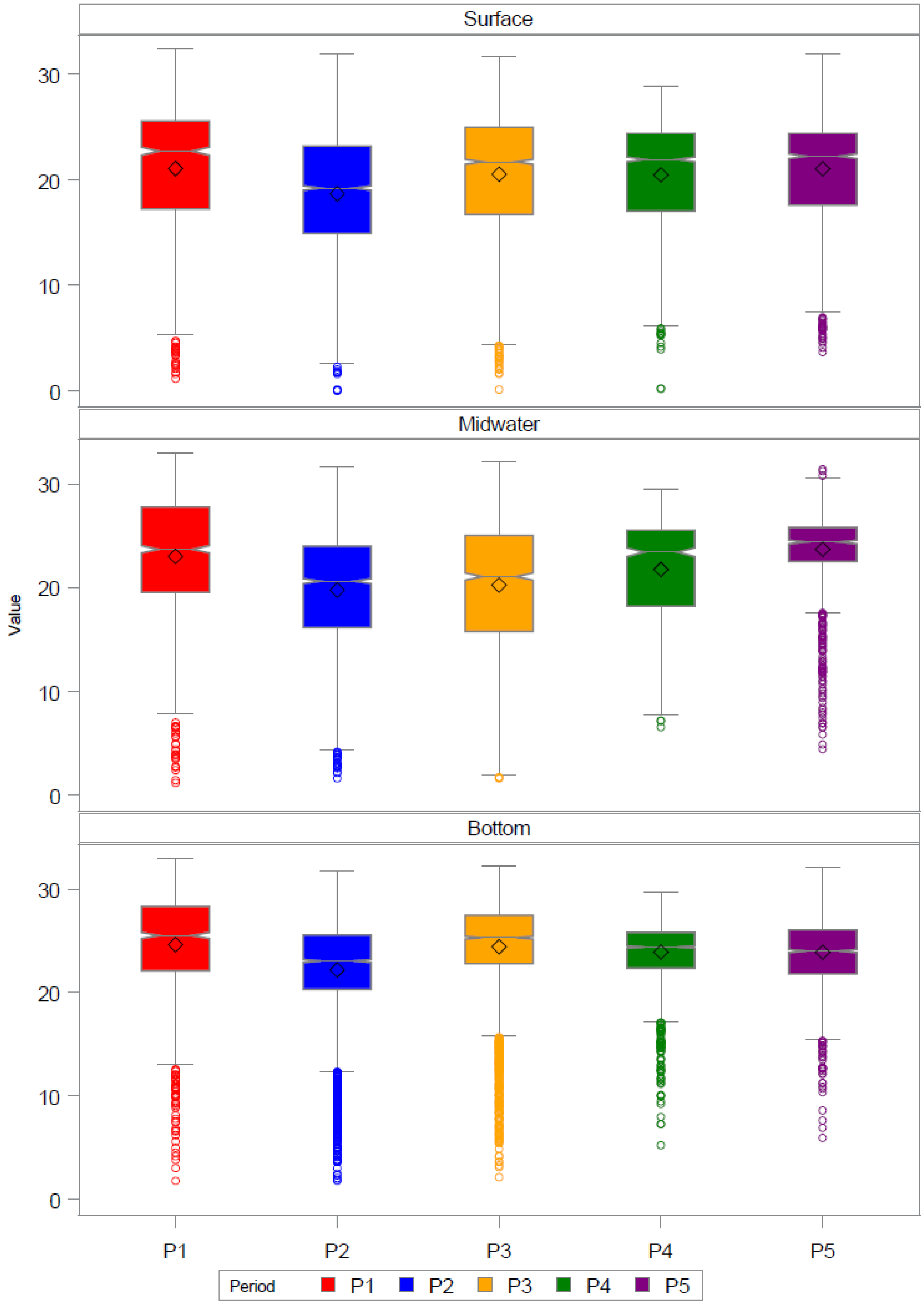


Table 5.2-2: Descriptive statistics for salinity (ppt) within the LHR downstream for specified water column strata (Analysis Days)

Type	Level	Period	N	Min	Max	Mean	Std
CR	Surface	P1	640	3.66	31.90	24.32	3.89
		P2	2.473	4.45	31.90	21.00	4.48
		P3	2.174	2.97	31.70	23.00	4.30
		P4	2.222	5.32	28.86	21.32	4.39
		P5	2.053	5.17	31.99	21.69	4.30
	Midwater	P1	212	19.98	31.01	27.33	2.46
		P2	517	17.26	30.72	23.03	2.87
		P4	244	12.04	28.22	25.03	1.67
		P5	687	19.79	31.40	25.04	2.33
	Bottom	P1	543	17.39	31.85	27.01	2.88
		P2	2,524	13.87	30.90	24.00	2.96
		P3	2,238	13.51	32.22	26.11	2.72
		P4	2,194	11.77	29.70	24.32	2.37
		P5	2,071	12.57	32.14	24.11	3.23
Fixed	Surface	P1	86	7.70	31.80	20.40	5.90
		P2	88	0.01	31.40	17.14	6.16
		P3	188	6.09	30.58	17.87	6.14
		P4	242	0.18	28.84	15.36	6.38
		P5	210	3.67	29.85	14.79	6.14
	Midwater	P1	87	13.60	32.20	24.23	4.67
		P2	88	10.79	31.60	21.05	4.59
		P3	188	11.60	30.73	21.64	4.80
		P4	240	6.52	29.46	19.80	5.36
		P5	210	4.39	30.28	19.24	5.96
	Bottom	P1	86	17.00	32.30	26.15	3.65
		P2	88	13.54	31.70	23.24	3.46
		P3	188	13.83	30.87	23.17	4.20
		P4	240	11.17	29.74	21.75	4.27
		P5	210	5.92	30.63	21.60	4.94
Random	Surface	P1	540	1.10	32.38	17.31	6.78
		P2	1224	0.00	30.52,	14.05	6.06
		P3	833	0.06	31.24	14.64	5.84
		P4	133	5.17	26.80	15.25	5.34
	Midwater	P1	1,048	1.12	32.90	22.02	6.02
		P2	2,046	1.62	30.74	18.85	6.19
		P3	1,701	1.63	32.08	20.06	6.39
		P4	245	7.11	29.30	20.32	5.22
	Bottom	P1	551	1.80	33.00	22.05	5.95
		P2	1,301	1.79	30.77	18.57	6.26
		P3	818	2.09	32.09	20.07	6.38
		P4	139	5.20	29.40	20.98	5.49

Figure 5.2-2: Distribution for salinity (ppt) within the LHR downstream by period for specified water column strata (Analysis Days)



Salinity was measured at 15 fixed stations including three CR stations (Figure 5.2-3) in the downstream portion of the river. This map represents only stations where salinity was recorded. CR data were converted to daily averages for this water quality characterization. The distribution of surface and bottom salinity concentrations observed at each of the fixed location stations over its POR, ordered by location along the longitudinal river transect are presented in Figure 5.2-4. Despite showing only the downstream portion of the LHR and while individual stations may have different periods of data collection, these figures clearly illustrate the salinity gradient and vertical salinity stratification that exists in the river. The CR sites are characterized by a greater number of values outside of the whiskers. This is due to the fact that they represent daily values as opposed to a single measurement in a day while still remaining restricted to only Analysis Days.

Figure 5.2-3: Fixed-location stations within the LHR downstream with salinity data

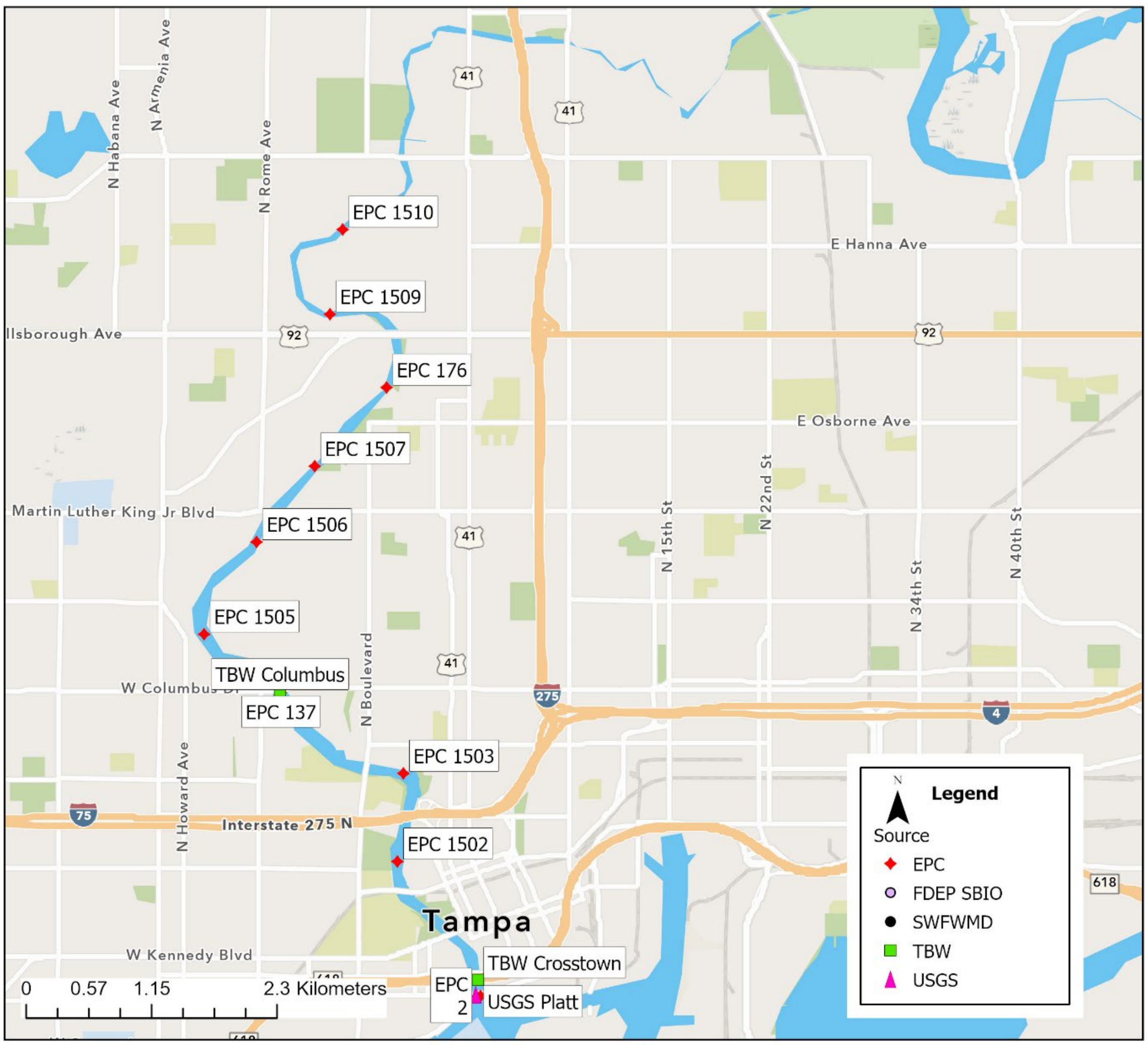
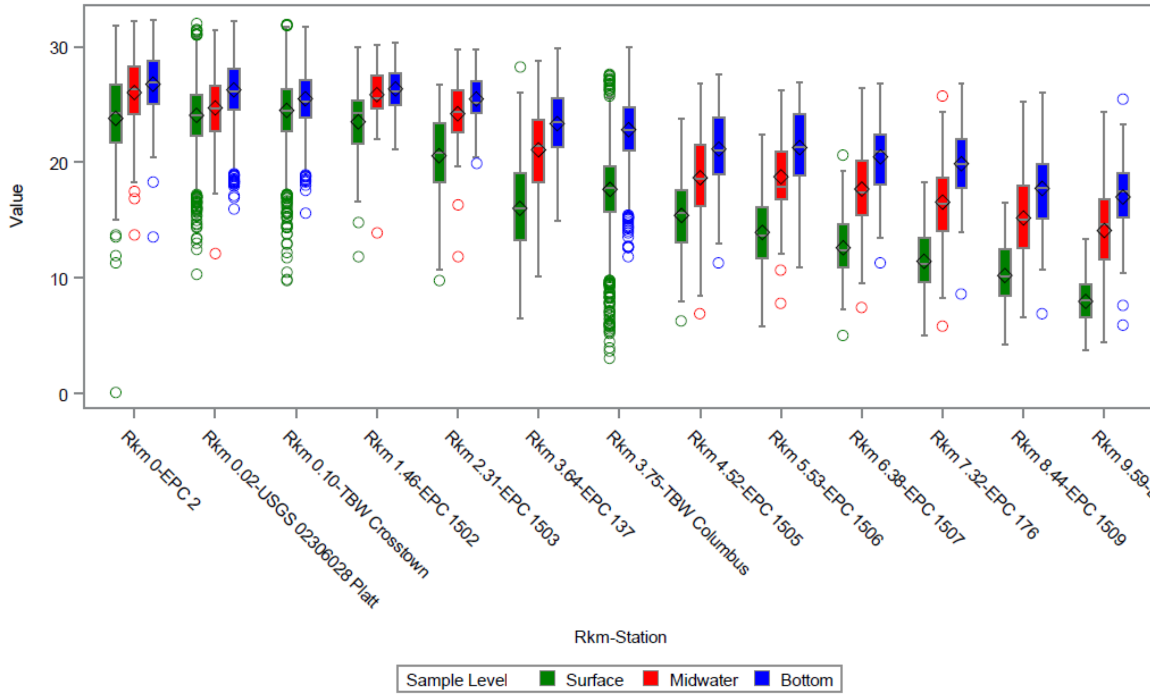


Figure 5.2-4: Distribution for salinity (ppt) within the LHR downstream by fixed-location stations for specified water column strata (Analysis Days)



5.2.1.2 LHR Downstream Dissolved Oxygen

Time series of surface, midwater, and bottom DO concentration and percent saturation within the downstream section of the LHR, for all stations combined over the study period during Analysis Days, are illustrated in Figure 5.2-5 and Figure 5.2-6, respectively. A reduction in the range of values observed during the last two periods is indicated, particularly at the high end of the scale associated with increased implementation of the minimum flow. Summary statistics (Table 5.2-3 and Table 5.2-4) illustrate that maximum values at fixed stations have dropped over time and that mean midwater and bottom values have also slightly declined.

The distribution of DO concentration values is also displayed in the form of box and whisker plots in Figure 5.2-7. Box and whisker plots for DO percent saturation are provided in Figure 5.2-8. Large differences between the periods of analyses were not evident. Variability within the data can be explained at the station level where DO at a particular station can fluctuate within its own local conditions but can differ due to proximity to freshwater input, estuarine influence, and depth or flow dynamics.

Figure 5.2-5: Time series for DO concentration (mg/L) within the LHR downstream for specified water column strata (Analysis Days)

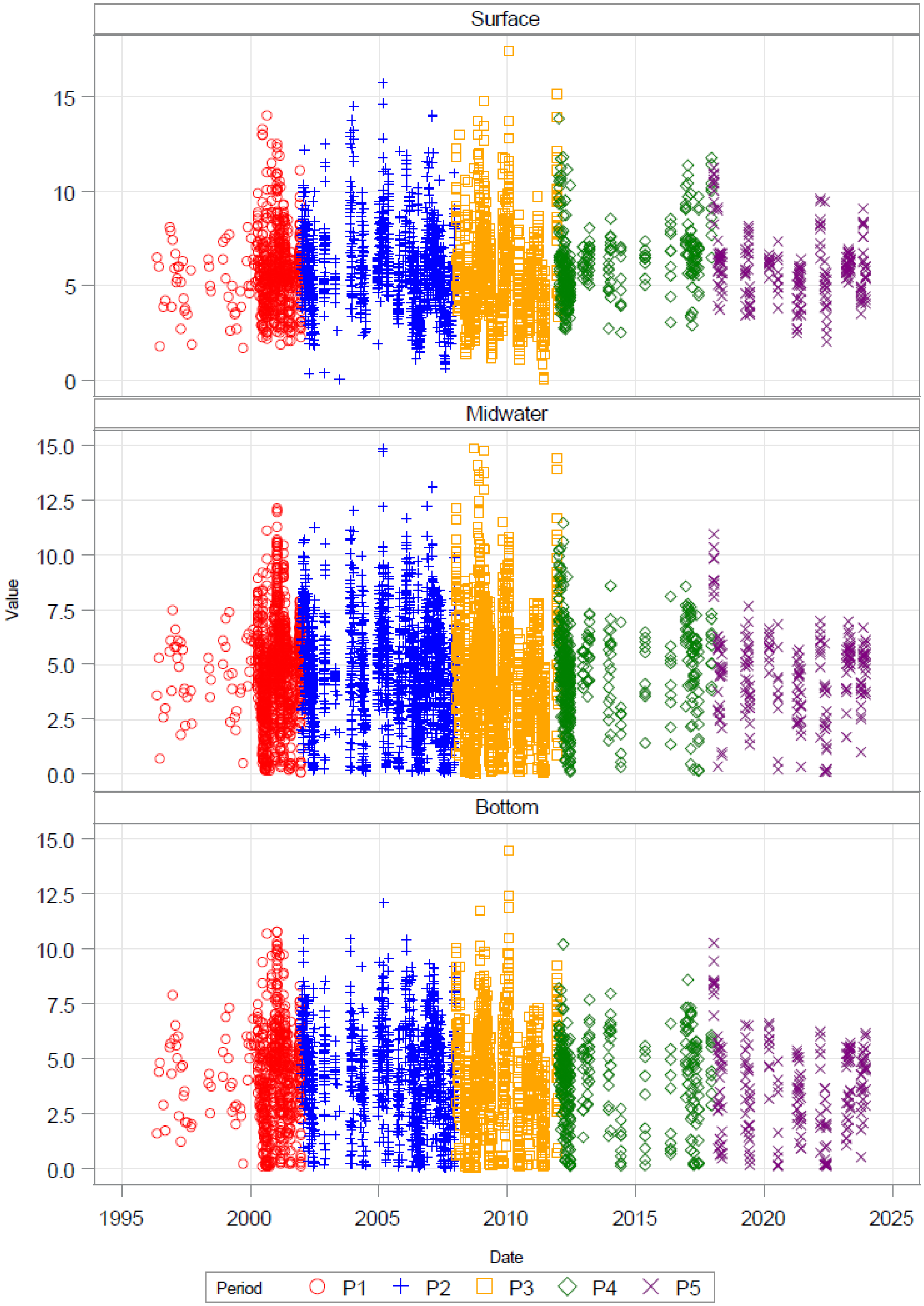


Figure 5.2-6: Time series for DO percent saturation (%) within the LHR downstream for specified water column strata (Analysis Days)

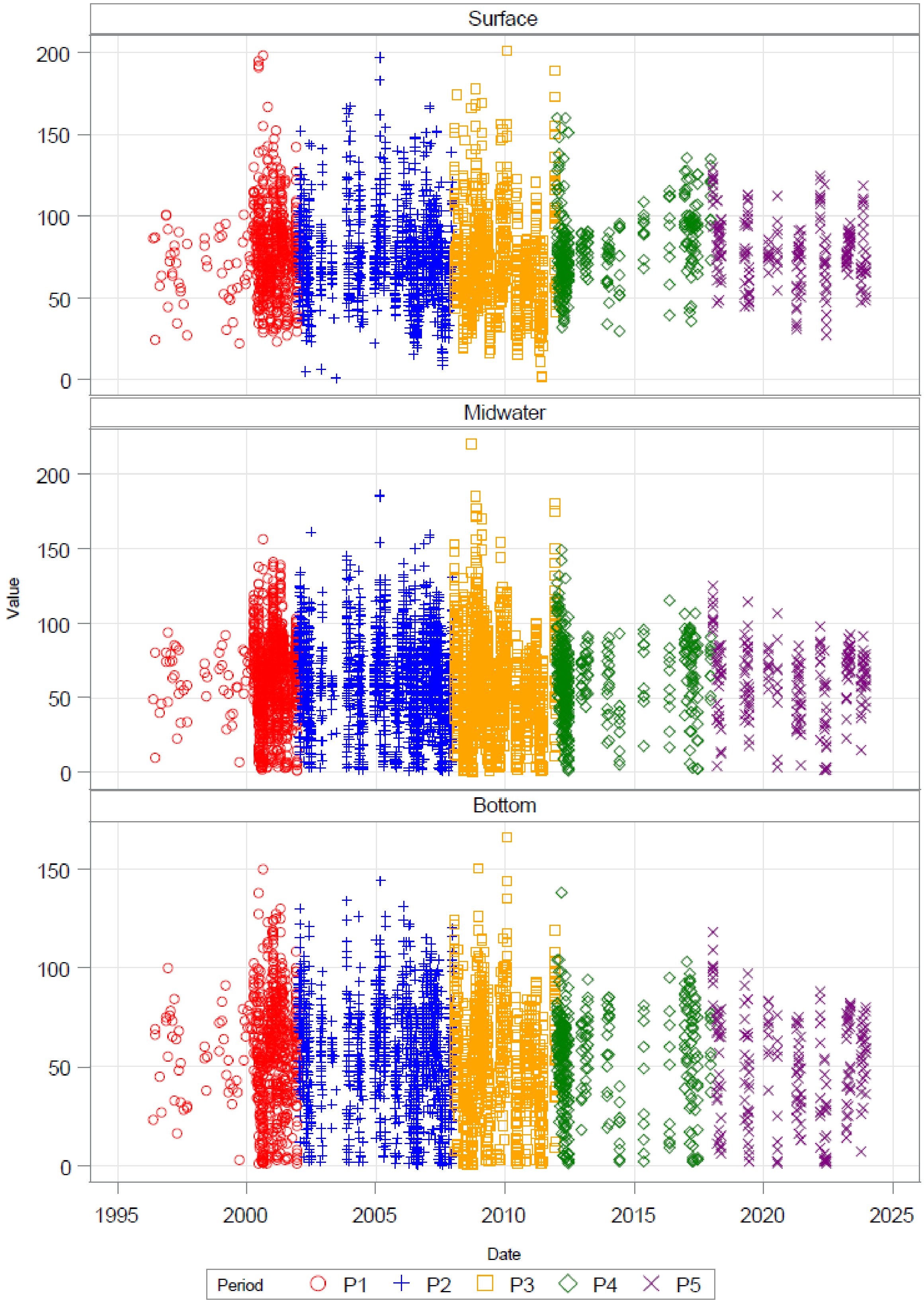


Table 5.2-3: Descriptive statistics for DO Concentration (mg/L) within the LHR downstream by period for specified water column strata (Analysis Days)

Type	Level	Period	N	Min	Max	Mean	Std
Fixed	Surface	P1	85	1.70	12.50	5.16	1.76
		P2	82	0.04	9.28	4.71	1.94
		P3	168	0.81	10.78	5.51	1.59
		P4	212	2.52	11.76	6.42	1.66
		P5	211	2.03	11.23	5.92	1.69
	Midwater	P1	85	0.50	9.30	4.48	1.67
		P2	82	0.51	7.92	4.32	1.79
		P3	168	0.05	8.26	4.31	1.82
		P4	210	0.15	8.59	4.82	1.80
		P5	210	0.11	10.94	4.51	1.98
	Bottom	P1	85	0.20	8.20	4.10	1.77
		P2	82	0.74	7.40	4.32	1.73
		P3	168	0.10	8.60	4.18	1.94
		P4	210	0.11	8.59	4.16	1.96
		P5	210	0.09	10.27	3.57	2.10
Random	Surface	P1	540	1.89	14.00	5.89	2.02
		P2	1,216	0.63	15.72	5.96	2.18
		P3	833	0.05	17.40	5.61	2.45
		P4	133	2.70	13.84	5.77	2.19
	Midwater	P1	1,048	0.10	12.11	4.64	2.27
		P2	2,031	0.04	14.85	4.72	2.40
		P3	1,701	0.02	14.86	4.46	2.50
		P4	245	0.10	11.45	4.60	2.20
	Bottom	P1	551	0.08	10.78	4.19	2.31
		P2	1,293	0.01	12.10	4.28	2.27
		P3	818	0.01	14.50	4.06	2.40
		P4	139	0.10	10.21	3.72	1.91

Table 5.2-4: Descriptive statistics for DO Percent Saturation (%) within the LHR downstream by period for Specified Water Column Strata (Analysis Days)

Type	Level	Period	N	Min	Max	Mean	Std
Fixed	Surface	P1	85	22.00	147.00	68.56	22.06
		P2	82	0.60	117.80	60.38	23.00
		P3	168	10.80	122.60	71.49	18.66
		P4	211	29.40	135.50	82.72	20.55
		P5	211	27.00	129.80	78.83	20.86
	Midwater	P1	85	7.00	110.00	60.81	20.42
		P2	82	7.50	118.70	56.97	21.54
		P3	168	0.60	113.50	56.55	22.00
		P4	210	2.10	115.20	63.15	23.03
		P5	210	1.50	125.00	61.02	25.02
	Bottom	P1	85	3.00	100.00	55.85	21.46
		P2	82	11.00	104.00	57.29	20.42
		P3	168	1.00	115.00	55.21	23.91
		P4	210	2.00	103.00	54.84	25.14
		P5	210	1.00	118.00	48.59	26.99
Random	Surface	P1	540	23.00	198.00	78.86	26.50
		P2	1,216	8.60	197.00	76.72	26.78
		P3	833	1.00	201.00	71.55	29.40
		P4	133	35.00	160.00	75.85	26.33
	Midwater	P1	1,048	1.00	156.00	62.97	28.69
		P2	2,031	1.00	186.00	62.42	30.34
		P3	1,701	0.00	220.00	58.70	31.13
		P4	245	1.00	149.00	61.81	27.55
	Bottom	P1	551	1.00	150.00	56.76	29.63
		P2	1,293	0.00	144.00	55.83	28.30
		P3	818	0.00	166.00	52.40	28.98
		P4	139	1.00	138.00	50.01	25.09

Figure 5.2-7: Distribution for DO concentration (mg/L) within the LHR downstream by period for water column strata (Analysis Days)

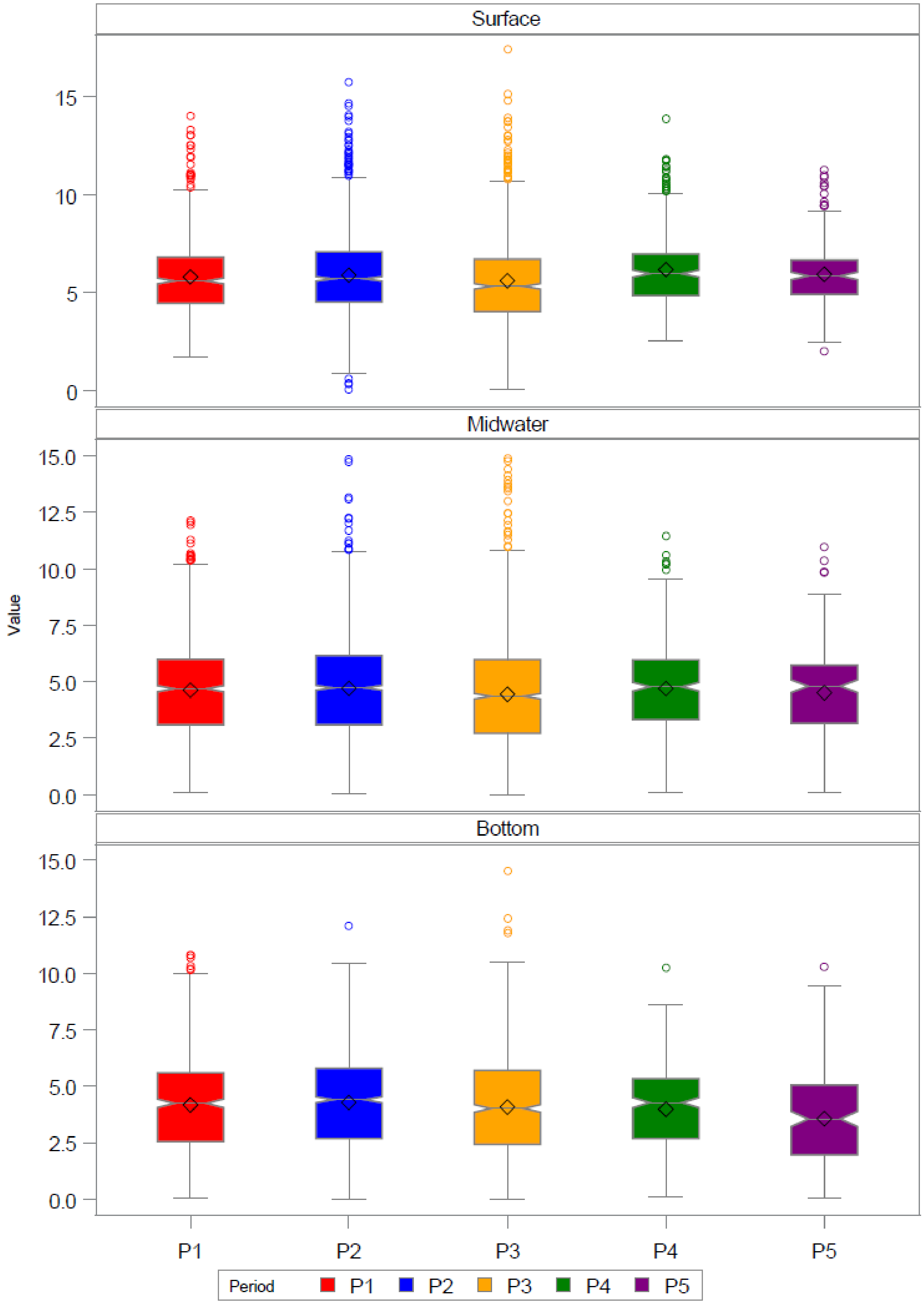
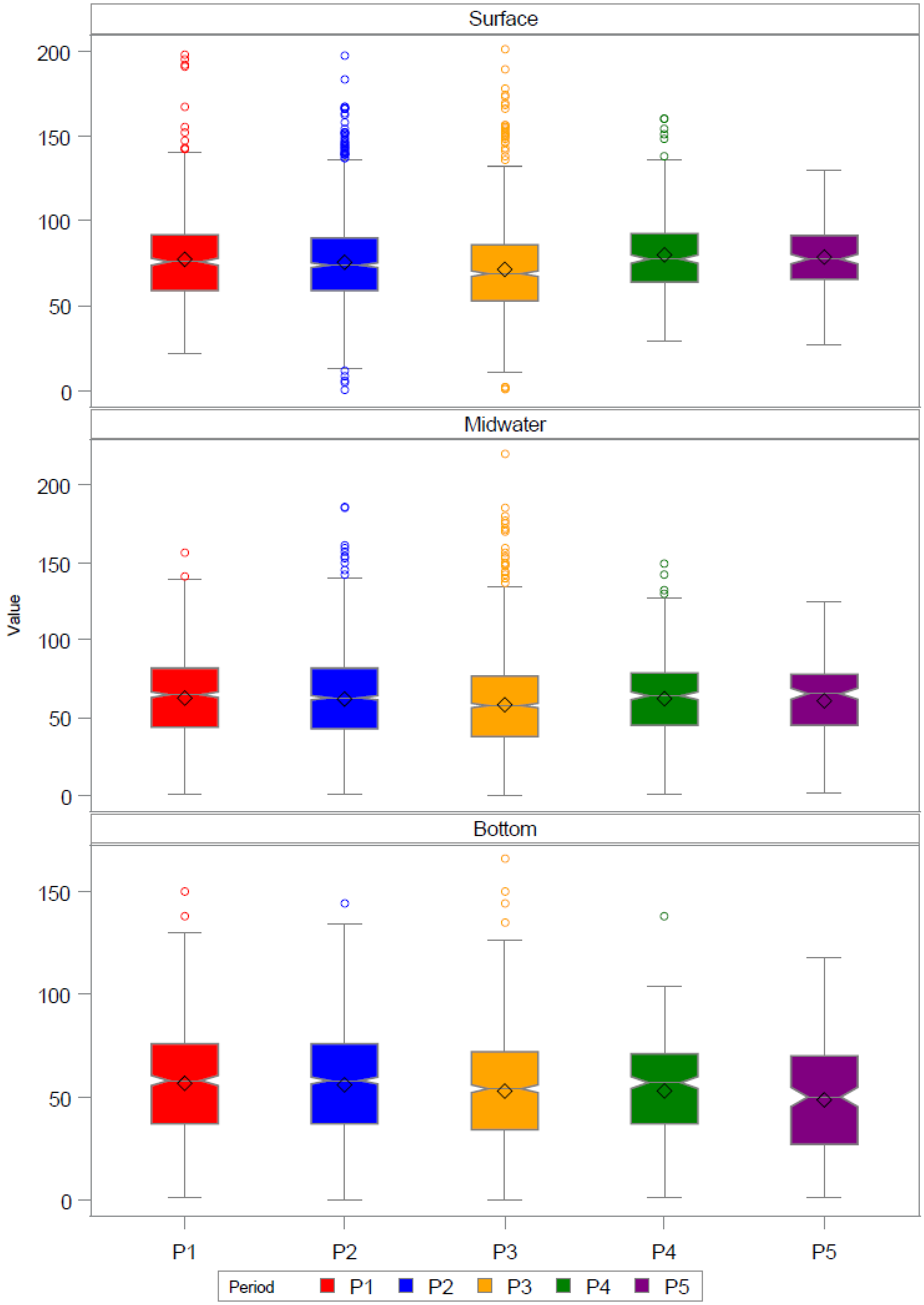


Figure 5.2-8: Distribution of DO percent saturation (%) within LHR downstream by period for specified water column strata (Analysis Days)



DO was measured using both CRs and vertical water column profile measurements (surface, midwater, and bottom levels) taken at fixed locations between 1996 and 2023 (Figure 5.2-9). CR data were converted to daily averages for this water quality characterization. Measurements were also conducted as part of the probabilistic (random) sampling associated with the TBW HBMP between 2000 and 2012.

Figure 5.2-9: Fixed-location stations within the LHR downstream with DO concentration data

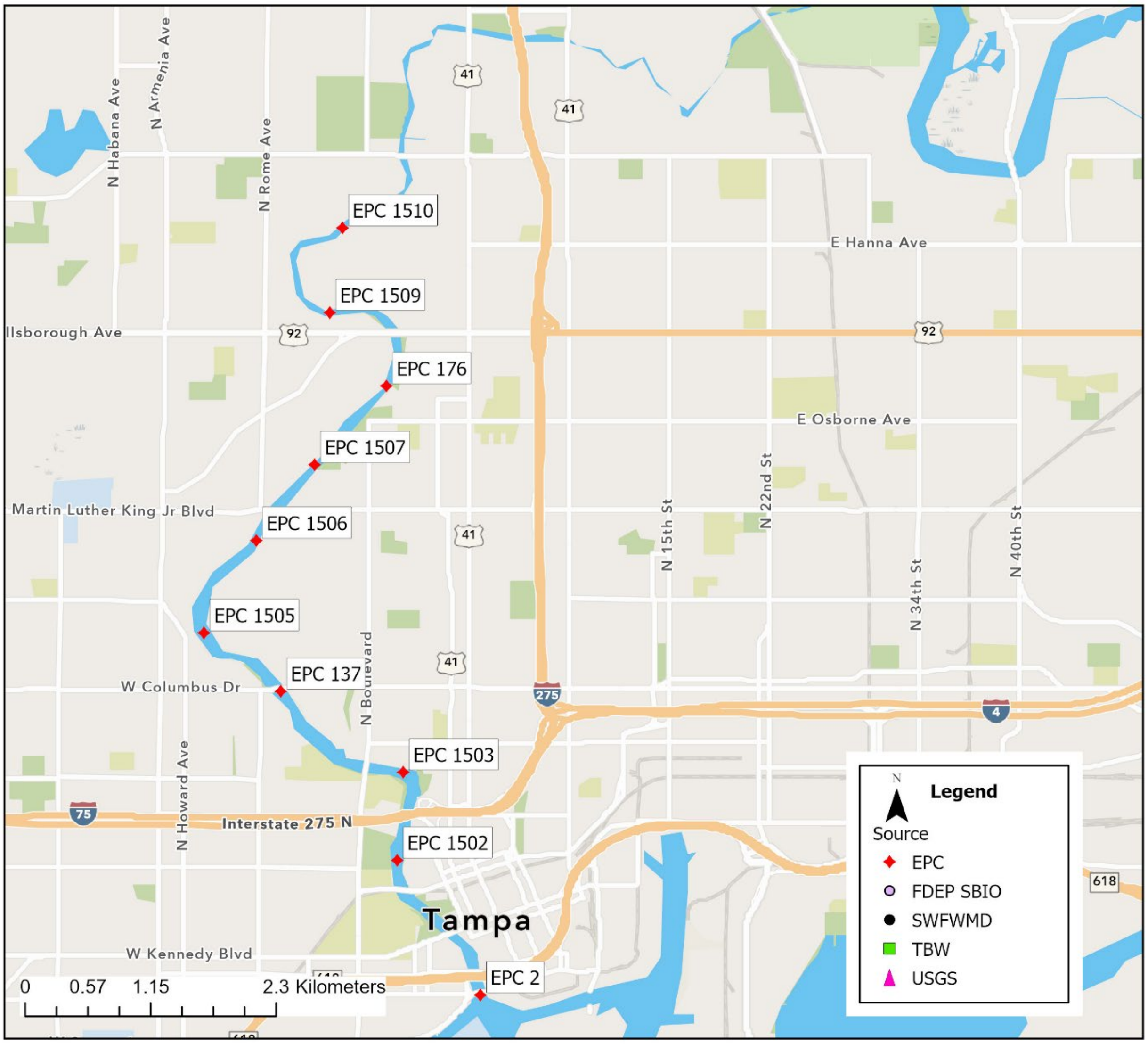


Figure 5.2-10 and Figure 5.2-11 illustrate the range of surface and bottom DO concentration and percent saturation values, respectively, observed at each of the fixed-location stations over its POR, ordered by location along the longitudinal river transect. The vertical gradient is evident in these figures with surface values exceeding bottom values for all stations. The difference between surface and bottom increases with distance up the river, largely due to decreased bottom values.

Figure 5.2-10: Distribution for DO concentration (mg/L) within the LHR downstream by fixed-location stations for specified water column strata (Analysis Days)

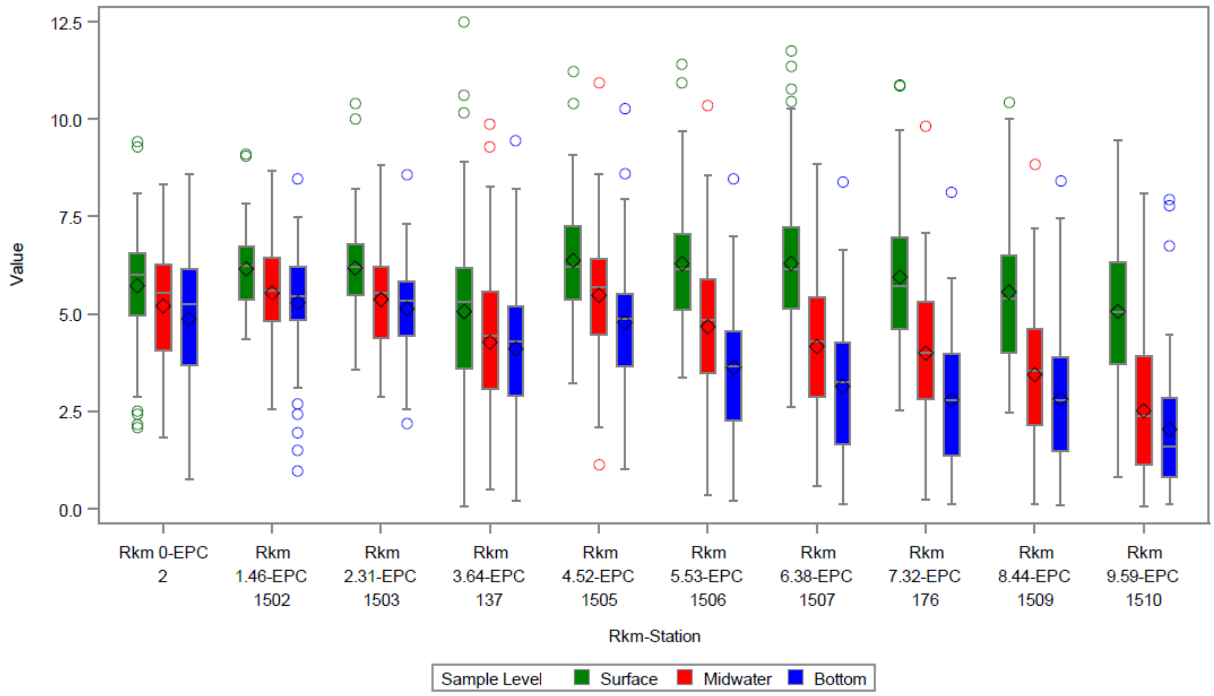
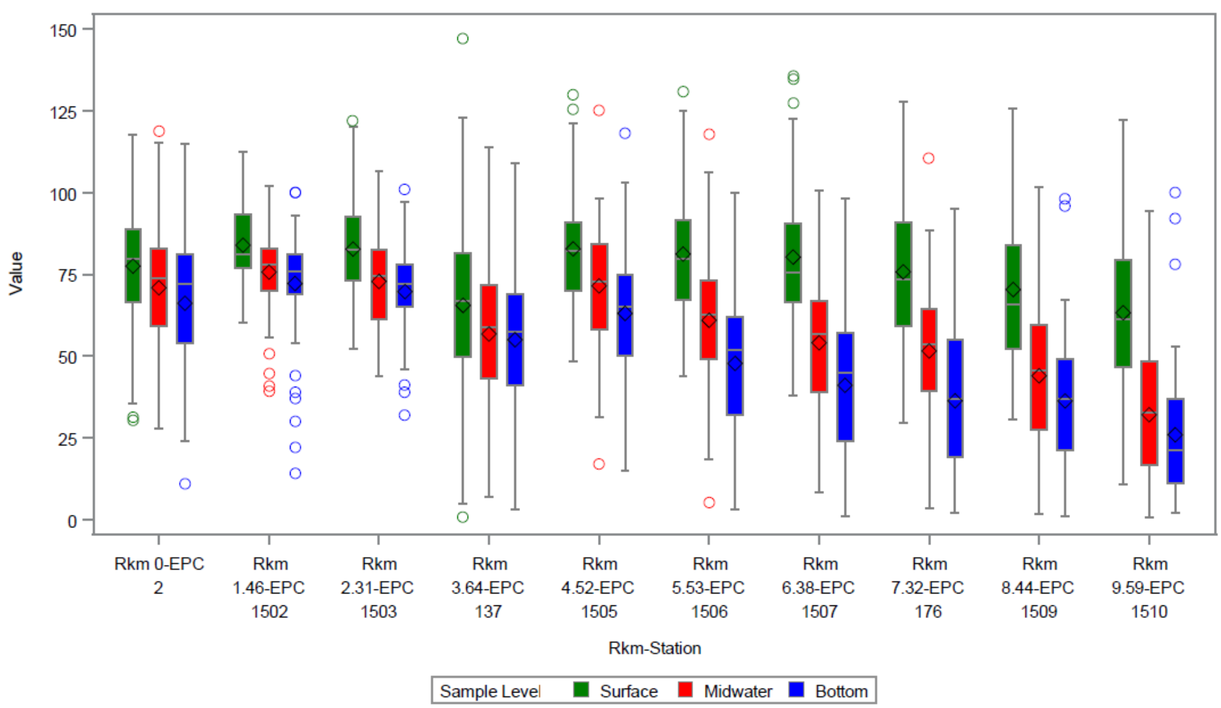


Figure 5.2-11: Distribution for DO percent saturation (%) within LHR downstream by fixed-location stations for specified water column strata (Analysis Days)



5.2.1.3 LHR Downstream Water Temperature

Time series of surface, midwater, and bottom water temperature within the downstream section of the LHR illustrate that, overall, there is little variation in water temperature between depth measurements in this portion of the LHR (Figure 5.2-12). Values below 15^{o}C were infrequently recorded and have not been observed since around 2018. As seen from the CR data available through 2023, low water temperatures likely have increased over time.

Summary statistics are presented in Table 5.2-5. Mean values at fixed station locations (including CRs) indicate that mean values of water temperature on Analysis Days have increased over the study period. Maximum values have also increased over time.

The distribution of water temperatures by period is also graphically represented in Figure 5.2-13. The mean and median water temperature had a slight but non-significant decrease through Periods 1–3 with a slight increase in subsequent periods for each water level. The interquartile range for the most recent period is narrower than the prior implementation periods.

Figure 5.2-12: Time series for water temperature (degrees C) within the LHR downstream for specified water column strata (Analysis Days)

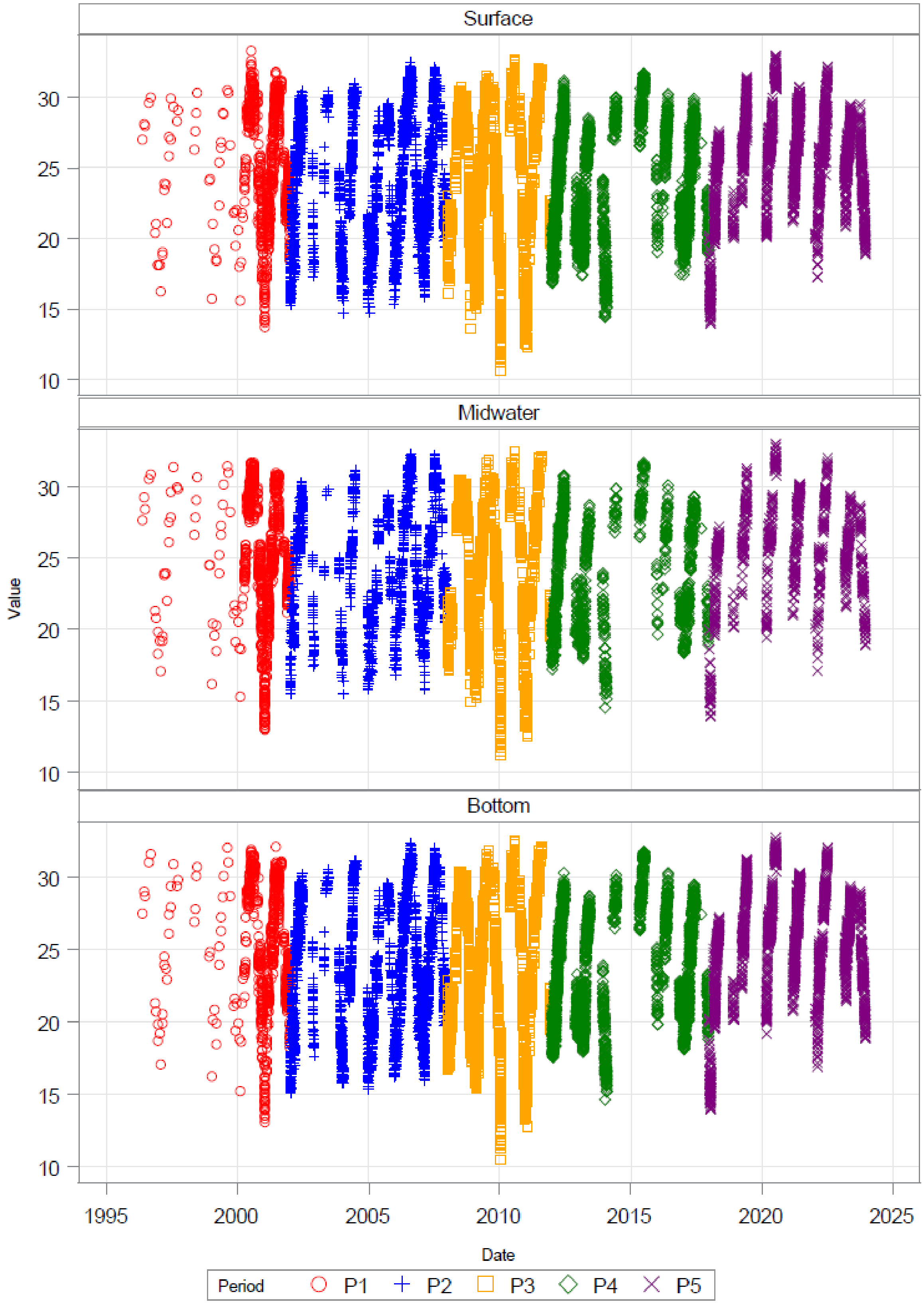
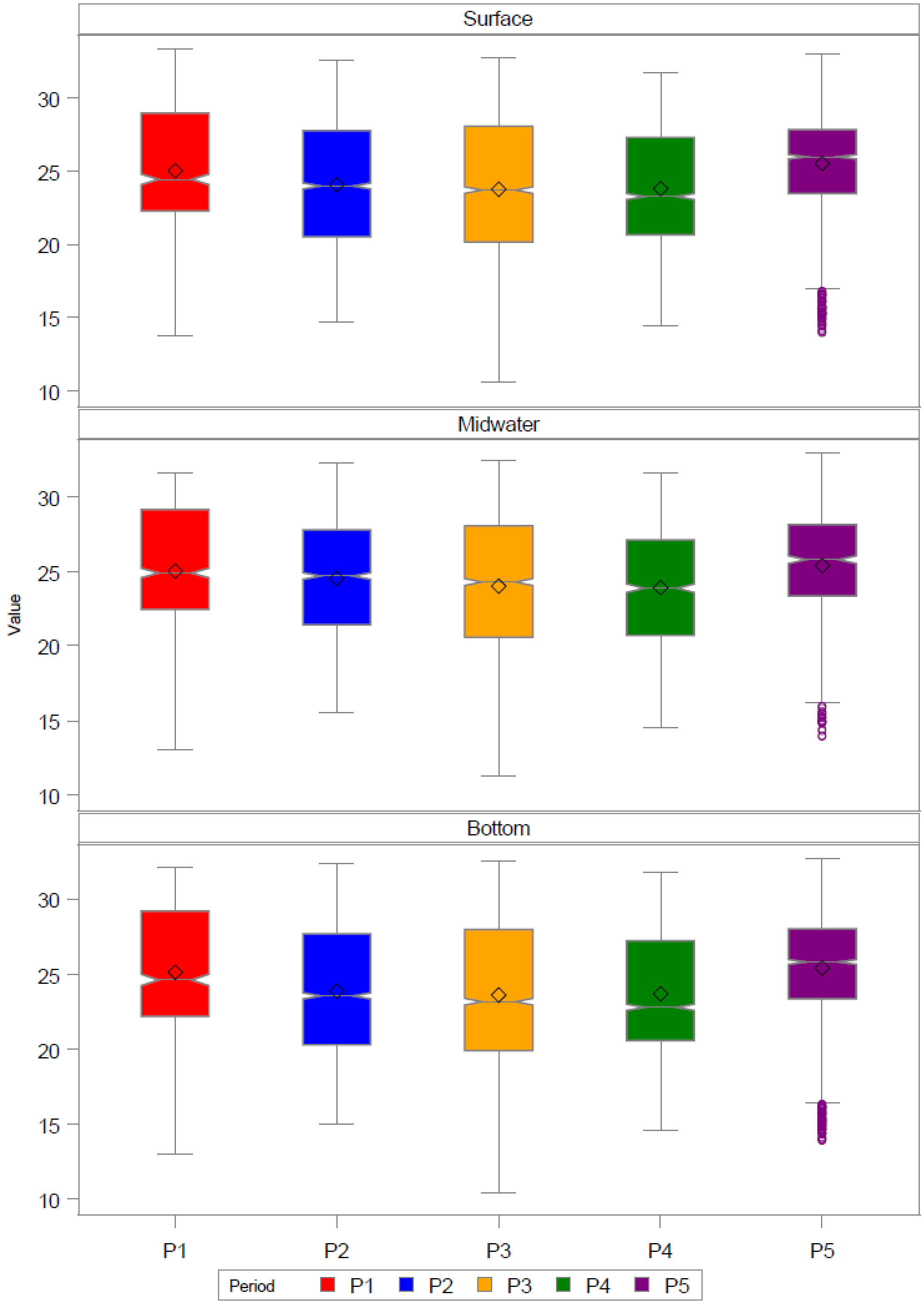


Table 5.2-5: Descriptive statistics water temperature (degrees C) within the LHR downstream by period for specified water column strata (Analysis Days)

Type	Level	Period	N	Min	Max	Mean	Std
CR	Surface	P1	275	18.46	31.17	25.06	3.64
		P2	1,948	15.15	32.51	23.97	4.54
		P3	2,202	11.47	32.68	23.74	5.01
		P4	2,268	14.44	31.69	23.65	3.84
		P5	2,053	13.94	32.74	25.47	3.45
	Midwater	P3	725	11.24	32.43	23.61	5.24
		P4	786	15.14	31.64	23.60	3.90
		P5	694	13.91	31.99	25.38	3.56
	Bottom	P1	234	18.21	31.06	25.33	3.65
		P2	2,006	15.04	32.33	23.61	4.49
		P3	2,306	11.40	32.55	23.58	4.98
		P4	2,286	15.17	31.77	23.61	3.86
		P5	2,078	13.93	32.08	25.39	3.53
Fixed	Surface	P1	88	14.40	30.50	23.92	4.33
		P2	88	14.69	30.54	23.42	4.62
		P3	188	13.15	31.23	23.54	4.79
		P4	242	14.42	31.01	24.26	3.82
		P5	211	15.69	32.96	25.68	3.80
	Midwater	P1	88	13.30	31.40	24.31	4.58
		P2	88	15.49	31.43	23.92	4.64
		P3	188	13.23	30.76	23.25	4.65
		P4	240	14.54	30.25	23.77	3.52
		P5	210	14.88	32.91	25.49	3.78
	Bottom	P1	88	13.30	32.00	24.39	4.61
		P2	88	15.53	31.56	23.92	4.71
		P3	188	13.77	30.76	23.21	4.67
		P4	240	14.60	29.66	23.66	3.46
		P5	210	14.90	32.73	25.53	3.79
Random	Surface	P1	540	13.73	33.29	25.14	4.22
		P2	1,224	15.80	32.08	24.22	3.96
		P3	833	10.60	32.08	23.78	4.43
		P4	133	16.81	31.18	24.96	3.26
	Midwater	P1	1,048	13.03	31.63	25.10	4.49
		P2	2,047	15.77	32.25	24.57	4.08
		P3	1,701	11.50	31.99	24.28	4.36
		P4	245	17.21	30.73	25.13	3.25
	Bottom	P1	551	13.04	32.09	25.14	4.35
		P2	1,302	15.62	32.24	24.23	4.11
		P3	818	10.46	31.82	23.75	4.65
		P4	139	19.00	30.29	24.75	2.97

Figure 5.2-13: Distribution for water temperature (degrees C) within the LHR downstream by period for specified water column strata (Analysis Days)



Water temperature was measured using both continuous and fixed vertical water column profile measurements (surface, midwater, and bottom levels; Figure 5.2-14) at various time intervals between 1996 and 2023. Measurements were also conducted as part of the probabilistic (random) sampling associated with the TBW HBMP between 2000 and 2012. Figure 5.2-15 illustrates the range of water temperatures observed at each of the fixed-location stations ordered by location along the longitudinal river transect. There is little variability along the length of the downstream zone in water temperature at any depth level.

Figure 5.2-14: Fixed-location stations within the LHR downstream with water temperature data

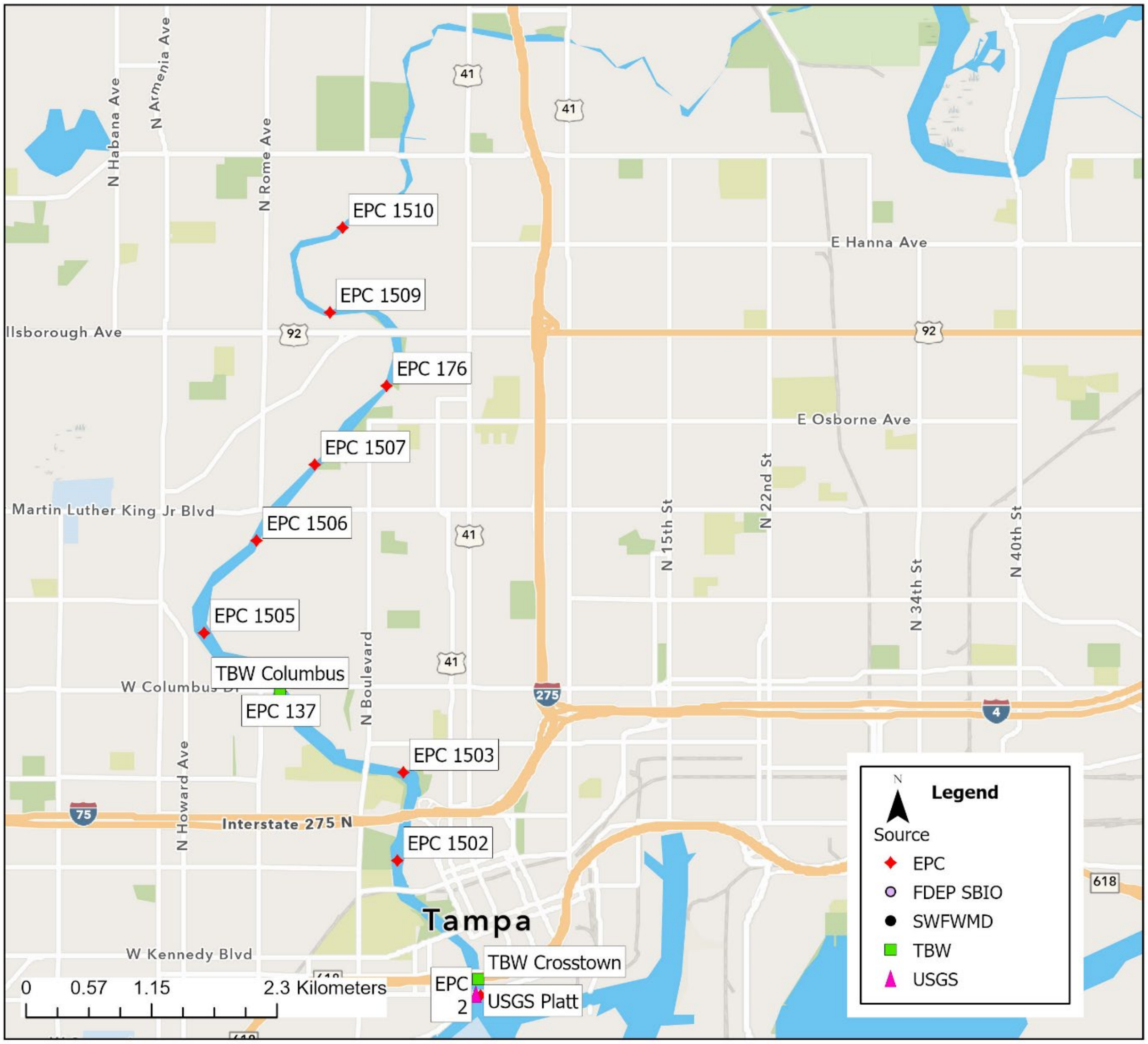
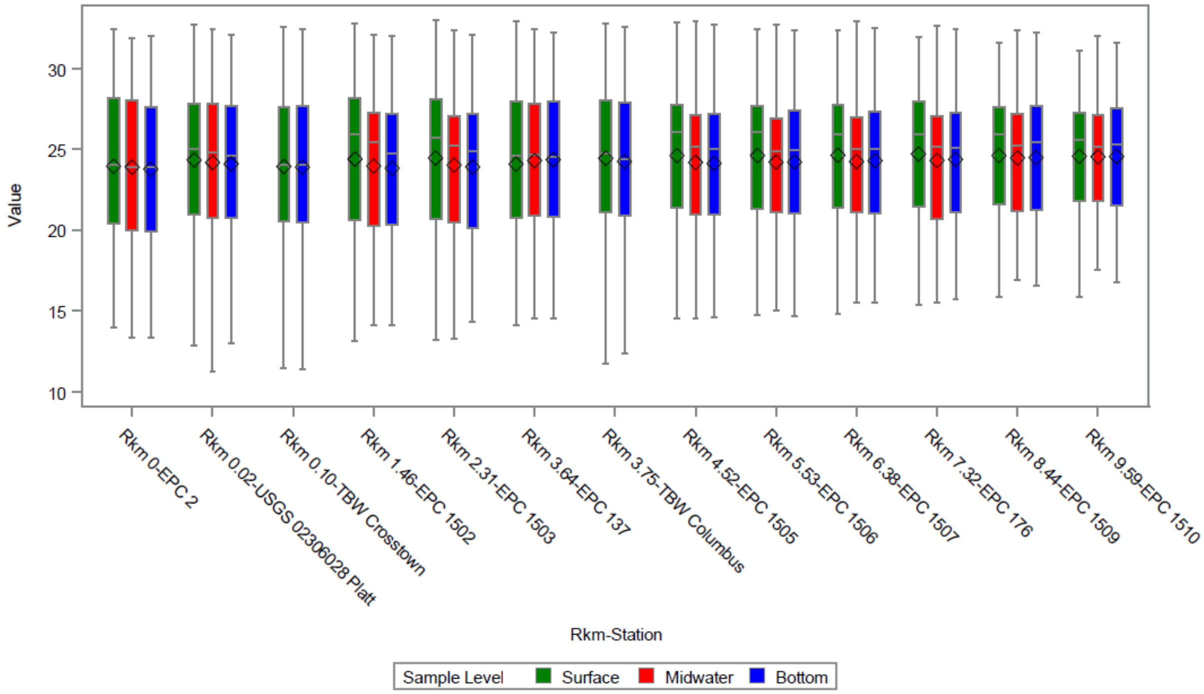


Figure 5.2-15: Distribution of water temperature (degrees C) within the LHR downstream by fixed-location stations for specified water column strata (Analysis Days)



5.2.1.4 LHR Downstream pH

Values for pH within the downstream section of the LHR are typically between seven and eight consistently across the five periods (Figure 5.2-16). A narrowing in range is indicated for the last two periods.

Mean pH by period has varied little, regardless of depth of measurement (Table 5.2-6). Maximum and mean values were somewhat larger for random stations than for fixed station samples. The random sampling ceased in 2012. In addition, by their nature, random sampling changes location every sampling event, and locations at times may have been closer to inputs of higher pH source water.

The distribution of pH values by period is also graphically represented in box and whisker plots in Figure 5.2-17. Mean and median values for pH have stayed consistent across the POR; however, the range of observed values was less during the more recent two periods with fewer extreme values.

Figure 5.2-16: Time series for pH (SU) within the LHR downstream for specified water column strata (Analysis Days)

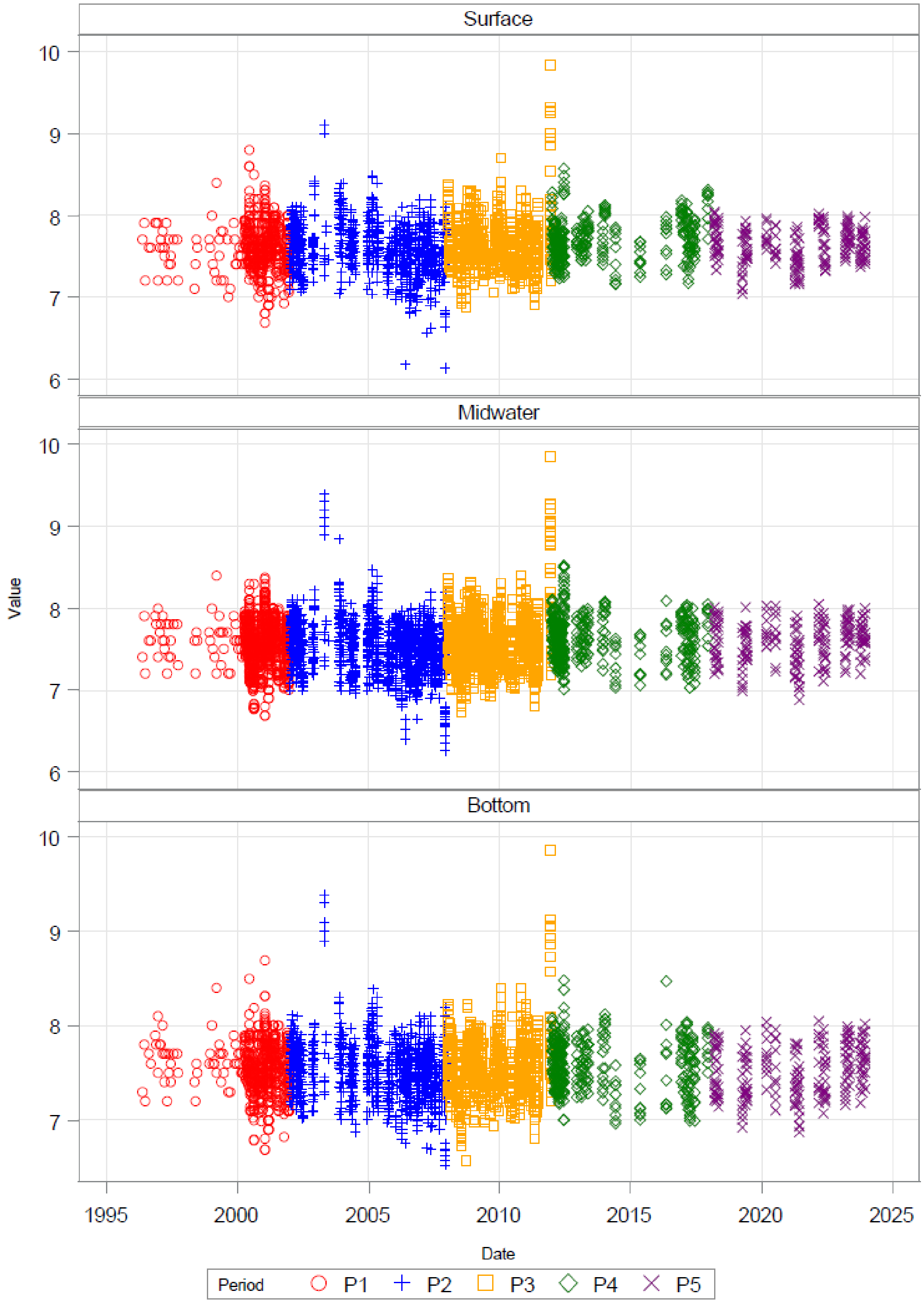
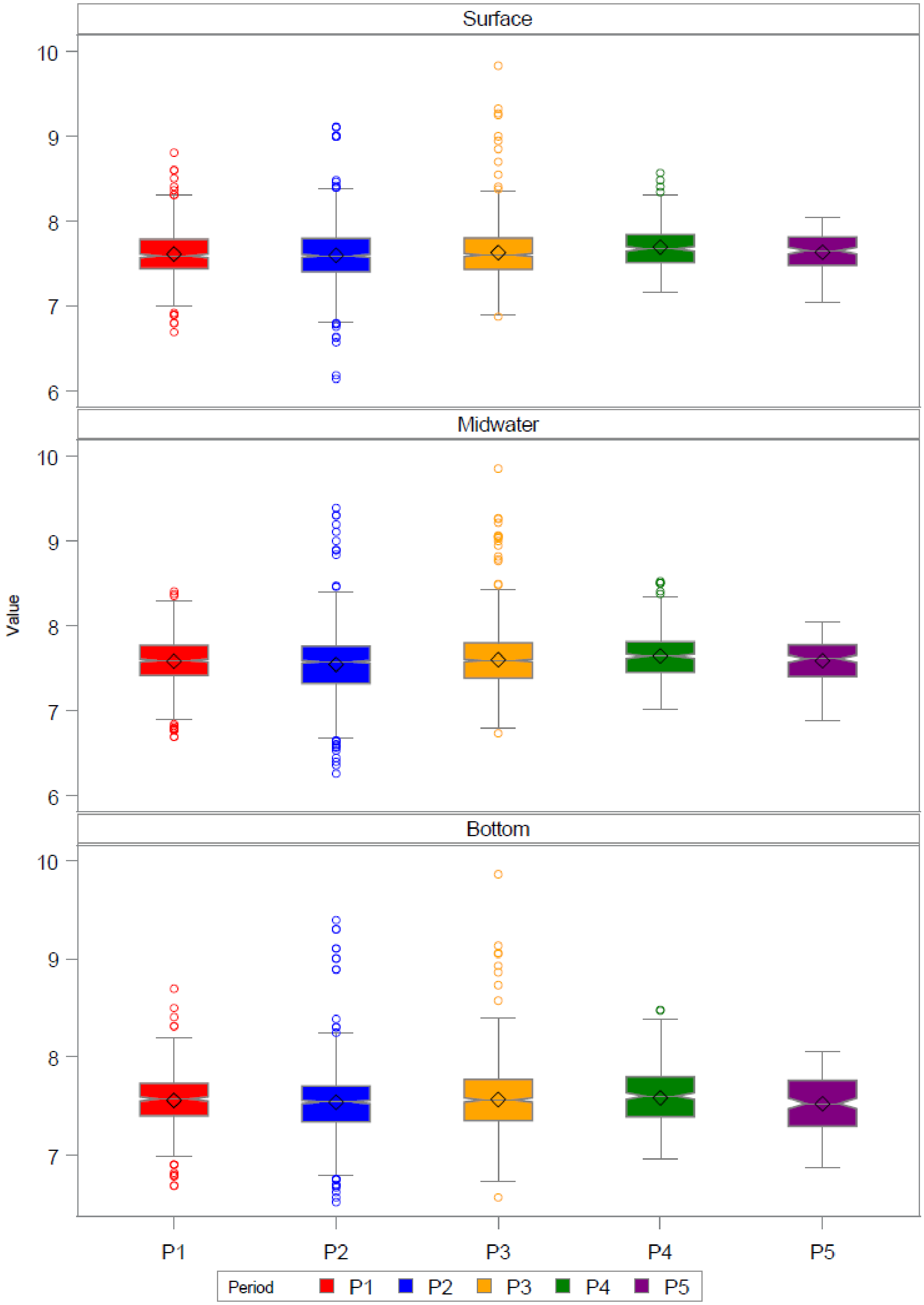


Table 5.2-6: Descriptive statistics of pH (SU) within the LHR downstream by period for specified water column strata (Analysis Days)

Type	Level	Period	N	Min	Max	Mean	Std
Fixed	Surface	P1	88	7.00	8.40	7.57	0.26
		P2	88	6.94	8.22	7.64	0.24
		P3	174	7.17	8.21	7.71	0.22
		P4	230	7.16	8.31	7.71	0.24
		P5	200	7.04	8.04	7.63	0.22
	Midwater	P1	88	7.07	8.40	7.60	0.24
		P2	88	7.06	7.99	7.66	0.21
		P3	174	7.03	8.23	7.63	0.25
		P4	230	7.03	8.09	7.62	0.25
		P5	200	6.88	8.05	7.59	0.26
	Bottom	P1	88	7.10	8.40	7.62	0.22
		P2	88	7.08	8.03	7.68	0.19
		P3	174	7.03	8.23	7.59	0.26
		P4	230	6.96	8.47	7.57	0.28
		P5	200	6.87	8.05	7.52	0.27
Random	Surface	P1	512	6.69	8.80	7.62	0.28
		P2	1,188	6.14	9.10	7.59	0.31
		P3	829	6.87	9.83	7.61	0.31
		P4	133	7.25	8.57	7.66	0.26
	Midwater	P1	1,036	6.69	8.37	7.58	0.27
		P2	2,046	6.26	9.39	7.54	0.32
		P3	1,697	6.73	9.85	7.59	0.32
		P4	245	7.01	8.52	7.67	0.27
	Bottom	P1	523	6.69	8.69	7.55	0.27
		P2	1,266	6.52	9.39	7.53	0.31
		P3	814	6.57	9.86	7.56	0.32
		P4	139	7.00	8.48	7.60	0.26

Figure 5.2-17: Distribution of pH (SU) within the LHR downstream by period for specified water column strata (Analysis Days)



Measurements of pH were obtained from fixed vertical water column profiles at locations shown in Figure 5.2-18. Measurements were also conducted as part of the probabilistic (random) sampling associated with the Tampa Bay Water HBMP between 2000 and 2012. A spatial gradient along the sampling transect in the downstream portion of the river is apparent, particularly for bottom measurements (Figure 5.2-19), with values decreasing with increasing distance upstream.

Figure 5.2-18: Fixed-location stations within the LHR downstream with pH data

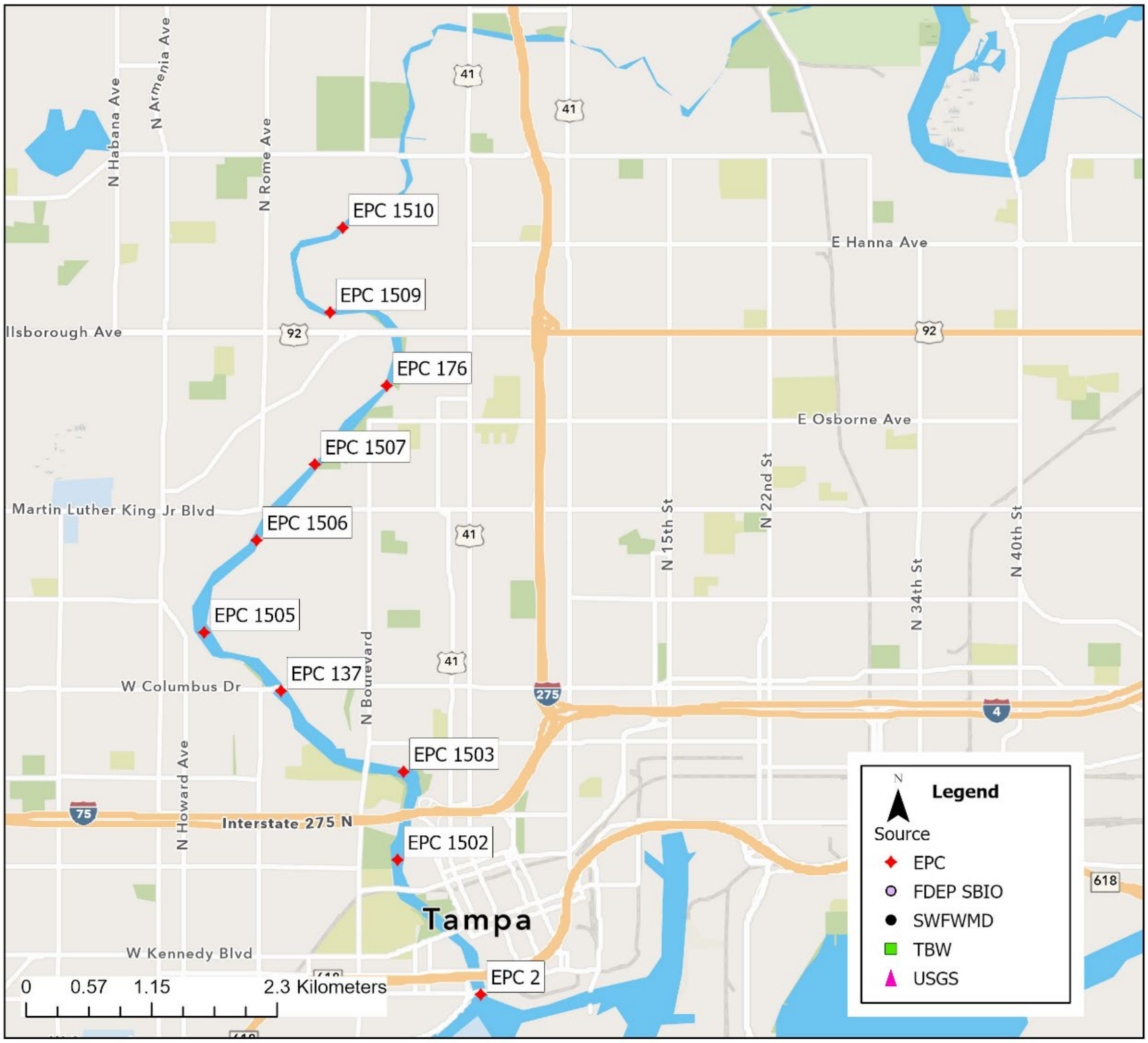
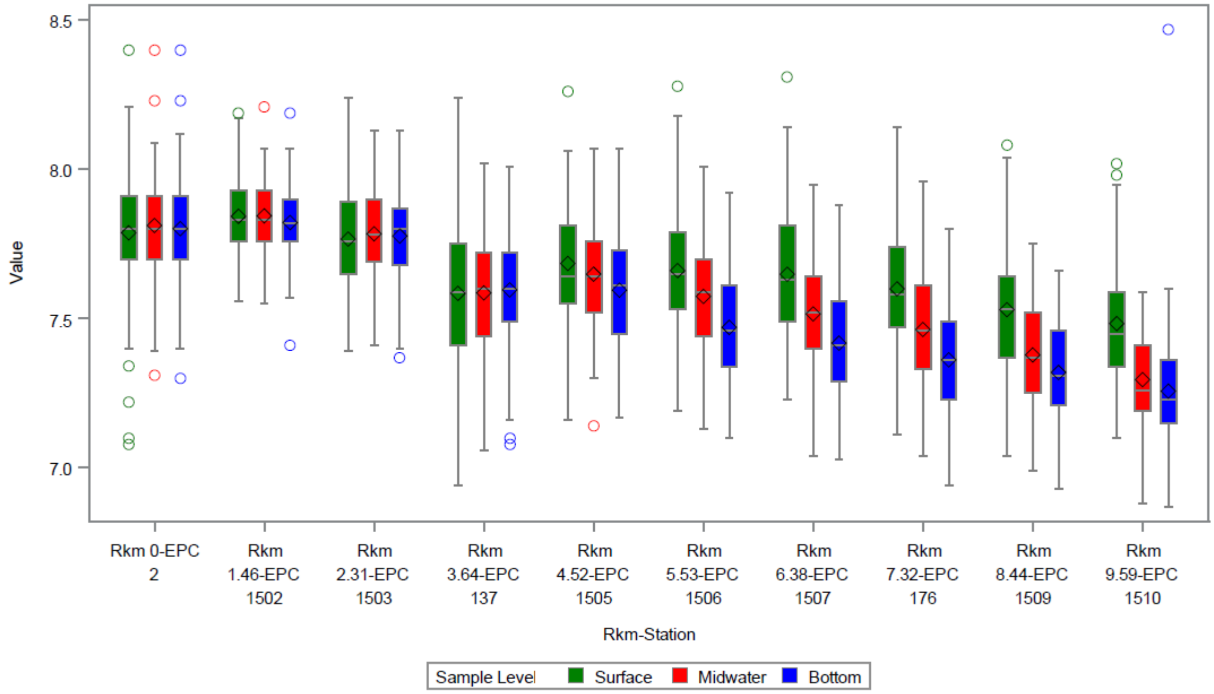


Figure 5.2-19: Distribution for pH (SU) at LHR downstream by fixed-location stations for specified water column strata (Analysis Days)



5.2.1.5 LHR Downstream Color

Color data were principally from two sources – EPC and the District – that measure color using different methods. Even within the EPC data, two methods were used with color being measured using spectrophotometric methods at 345 wavelength prior to 2010 and florescence-based color measured at 345 wavelength after 2010. There was also a gap in the EPC data between 2020 and 2022 in reported color. For the descriptive plots, different color methods were grouped for analysis since they were of similar wavelength but should be interpreted with caution using combined data. Reporting the data separately resulted in sparse data for large segments of time.

A time series plot of the color measurements is provided in Figure 5.2-20. A decline in color values, for at least the first three periods, is observed. Since 2010, color values have remained under 40 PCU and are most frequently under 20 PCU.

Color at random stations was only sampled before 2002, and all summary statistics from this period for random stations are higher than the same period for fixed stations, as well as all other periods for fixed stations (Table 5.2-7). Mean color at fixed stations for all periods were 15 PCU or lower. Figure 5.2-21 supports the decline in color over time, with a slight increase in the most recent period. The method change for EPC could have contributed to this outcome.

Figure 5.2-20: Time series for surface color (PCU) within the LHR downstream (Analysis Days)

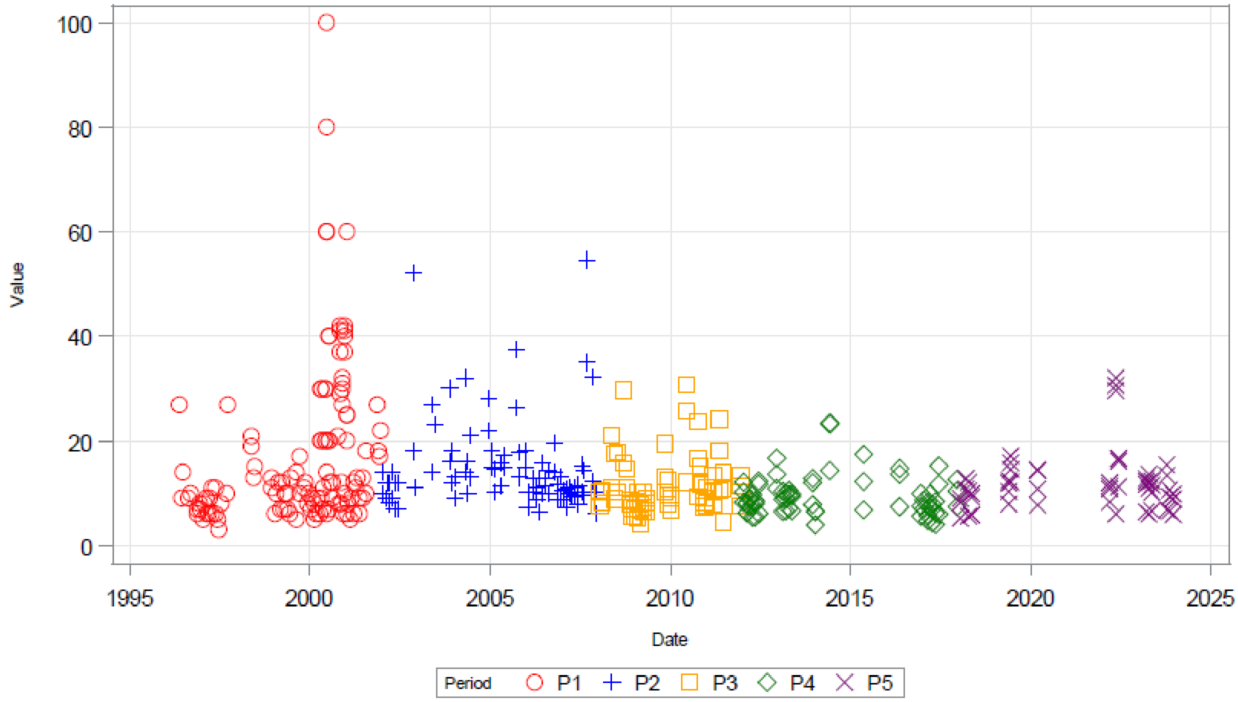
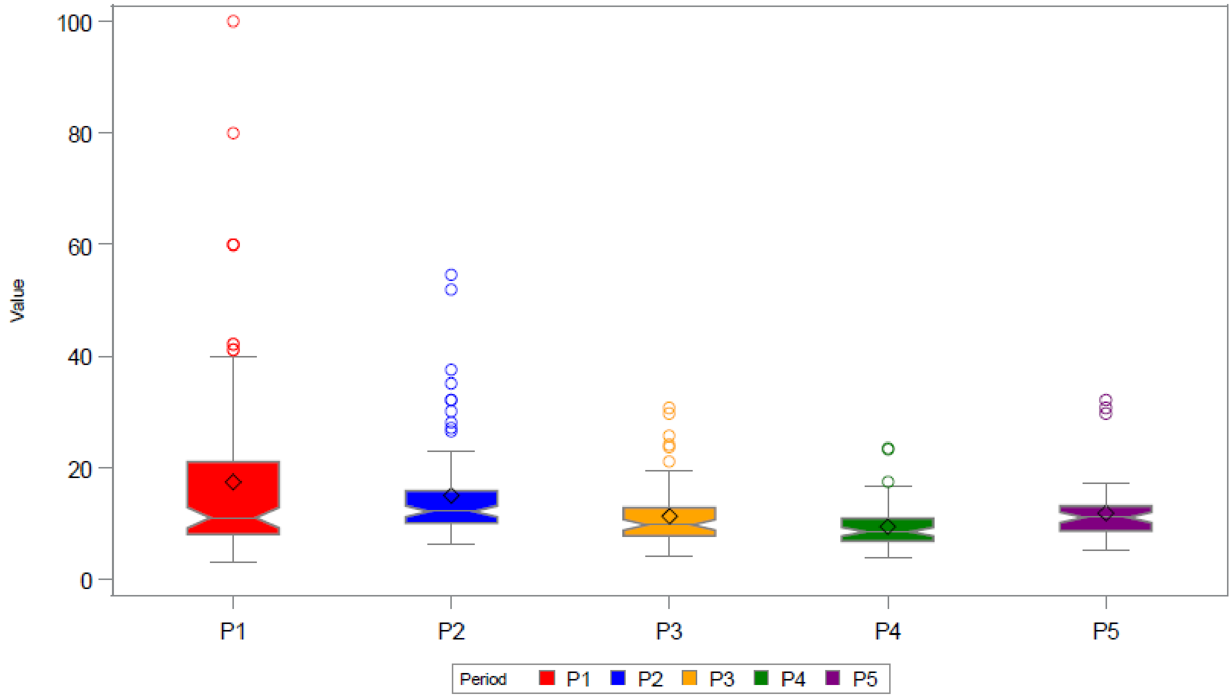


Table 5.2-7: Descriptive statistics for color (PCU) within the LHR downstream by period (Analysis Days)

Type	Level	Period	N	Min	Max	Mean	Std
Fixed	Surface	P1	89	3.00	27.00	10.42	5.01
		P2	88	6.20	54.60	14.97	8.75
		P3	72	4.10	30.70	11.26	5.58
		P4	70	3.90	23.40	9.370	3.83
		P5	56	5.20	32.00	11.83	5.50
Random	Surface	P1	32	20.00	100.00	36.84	18.22

Figure 5.2-21: Distribution for color (PCU) within the LHR downstream by period (Analysis Days)



The EPC measured color at four fixed-location sites shown in Figure 5.2-22**.** Box and whisker plots of these data indicate lower color values near the mouth of the river (Rkm 0) and increasing color values between Rkm 3.64 and Rkm 9.59 (Figure 5.2-23).

Figure 5.2-22: Fixed-location stations within the LHR downstream with color data

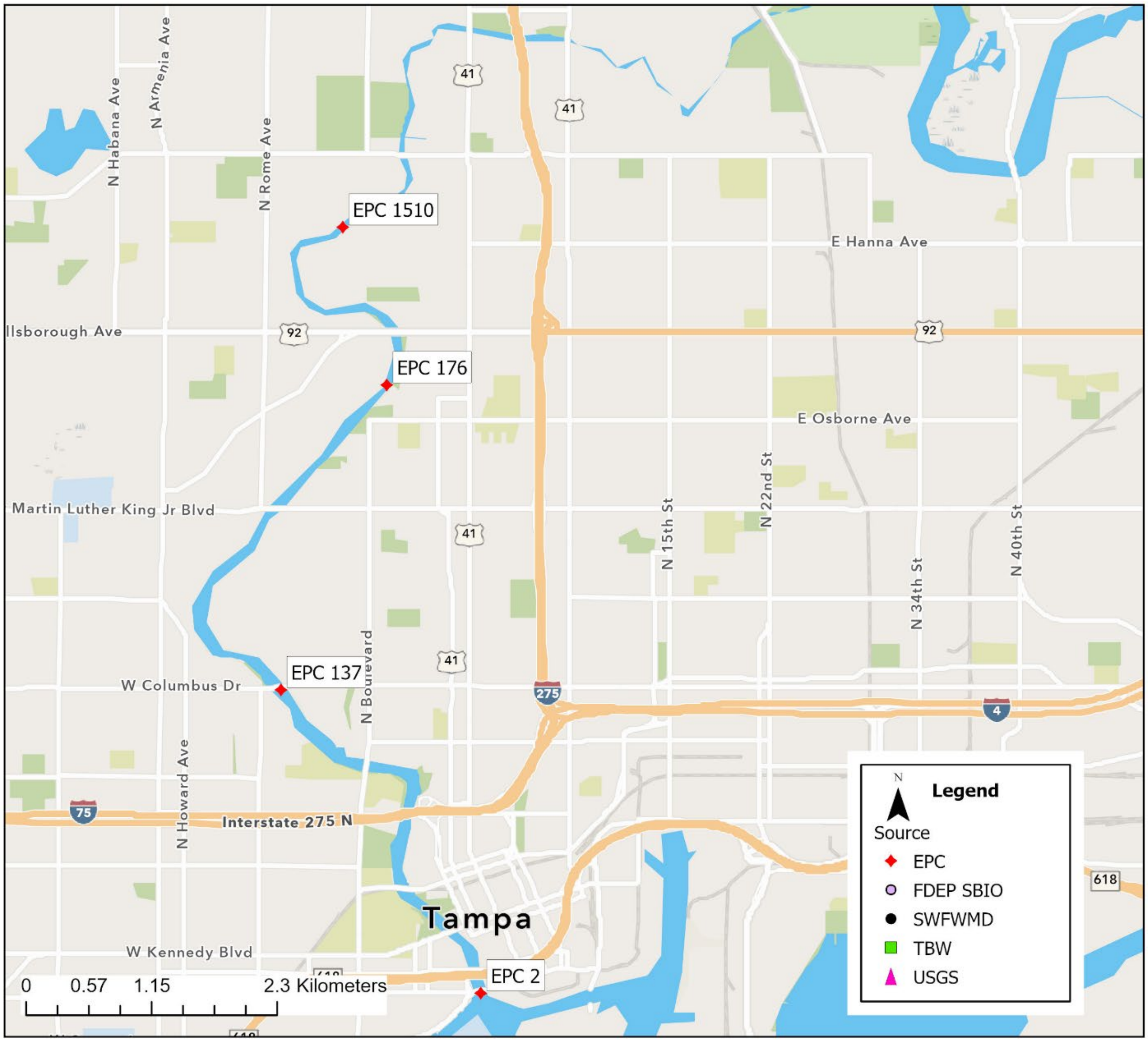
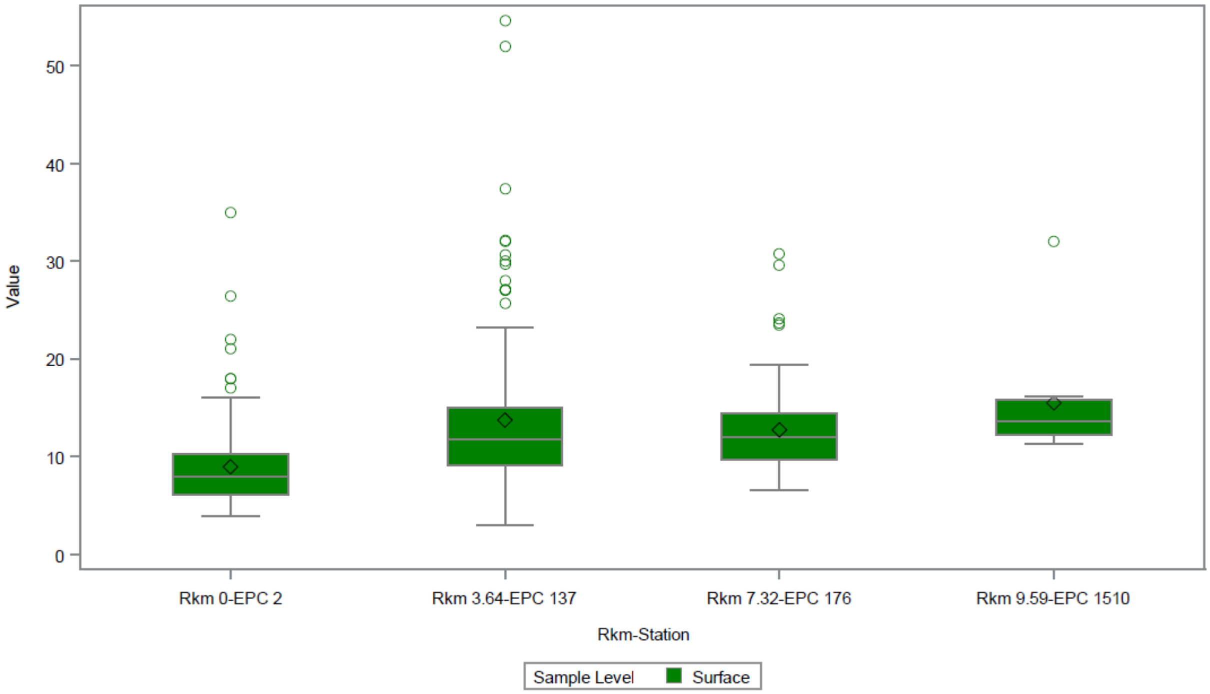


Figure 5.2-23: Distribution of color (PCU) within the LHR downstream by fixed-location stations (Analysis Days)



5.2.1.6 LHR Downstream Total Nitrogen

Total nitrogen values in the downstream portion of the LHR have declined over the study period (Figure 5.2-24). The average value of total nitrogen at fixed stations has been reduced by 50% or more from Period 1 to Period 5 (Table 5.2-8). Maximum values have declined by more than 1 mg/L over that timeframe.

Total nitrogen has predominantly remained below 1.5 mg/L for each period in this analysis and across all stations (Figure 5.2-25). Except for isolated high nitrogen events in Period 3, total nitrogen demonstrates a decrease in concentrations over time with the highest variability between 2008 and 2017.

Figure 5.2-24: Time series for surface total nitrogen (mg/L) within the LHR downstream (Analysis Days)

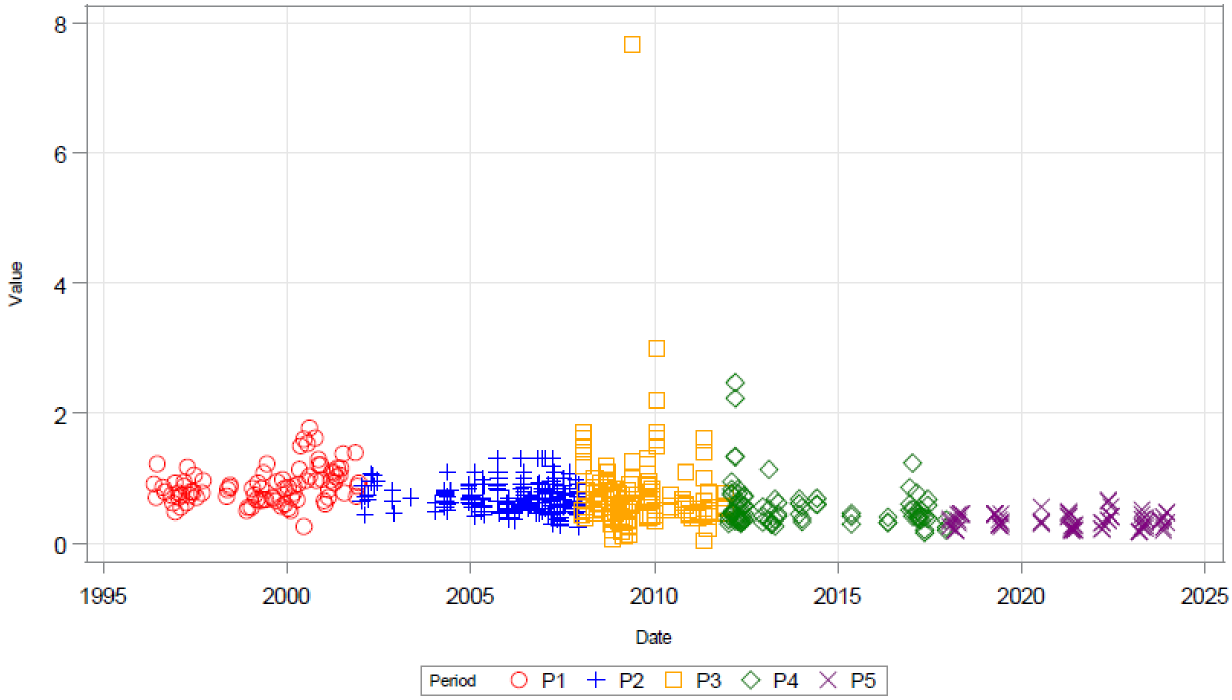


Table 5.2-8: Descriptive statistics for total nitrogen (mg/L) within the LHR downstream by period (Analysis Days)

Type	Level	Period	N	Min	Max	Mean	Std
Fixed	Surface	P1	89	0.27	1.77	0.89	0.28
		P2	80	0.25	1.08	0.64	0.19
		P3	69	0.04	1.18	0.54	0.19
		P4	72	0.17	1.24	0.46	0.19
		P5	73	0.18	0.66	0.35	0.11
Random	Surface	P2	120	0.28	1.30	0.76	0.23
		P3	89	0.07	7.67	0.87	0.86
		P4	20	0.33	2.47	0.88	0.57

Total nitrogen was measured at both fixed stations at the surface between 1996 and 2023 (Figure 5.2-26) and as part of the probabilistic (random) sampling associated with the TBW HBMP between 2000 and 2012. Figure 5.2-27 illustrates the range of surface total nitrogen concentrations observed at each of the fixed stations over its POR ordered by location along the longitudinal river transect. Values were lowest at the upper end of the downstream portion of the LHR. The greatest variability was shown at Rkm 3.64.

Figure 5.2-25: Distribution total nitrogen (mg/L) within the LHR downstream by period (Analysis Days)

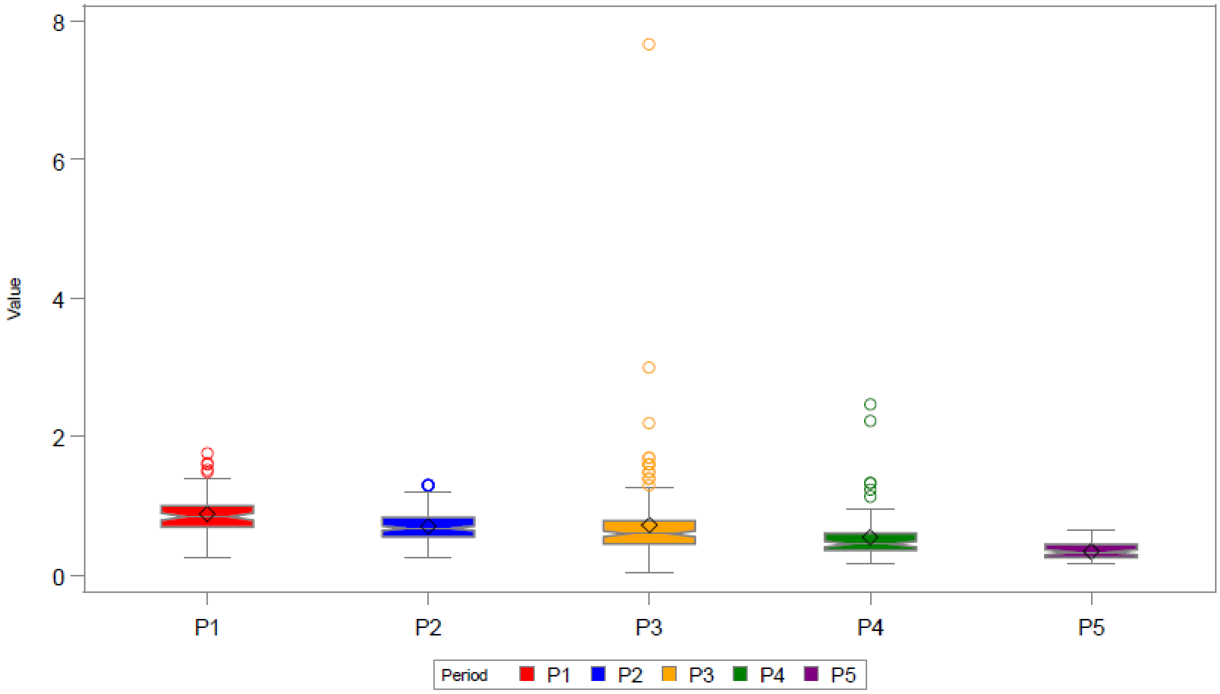


Figure 5.2-26: Fixed-location stations within the LHR downstream with total nitrogen data

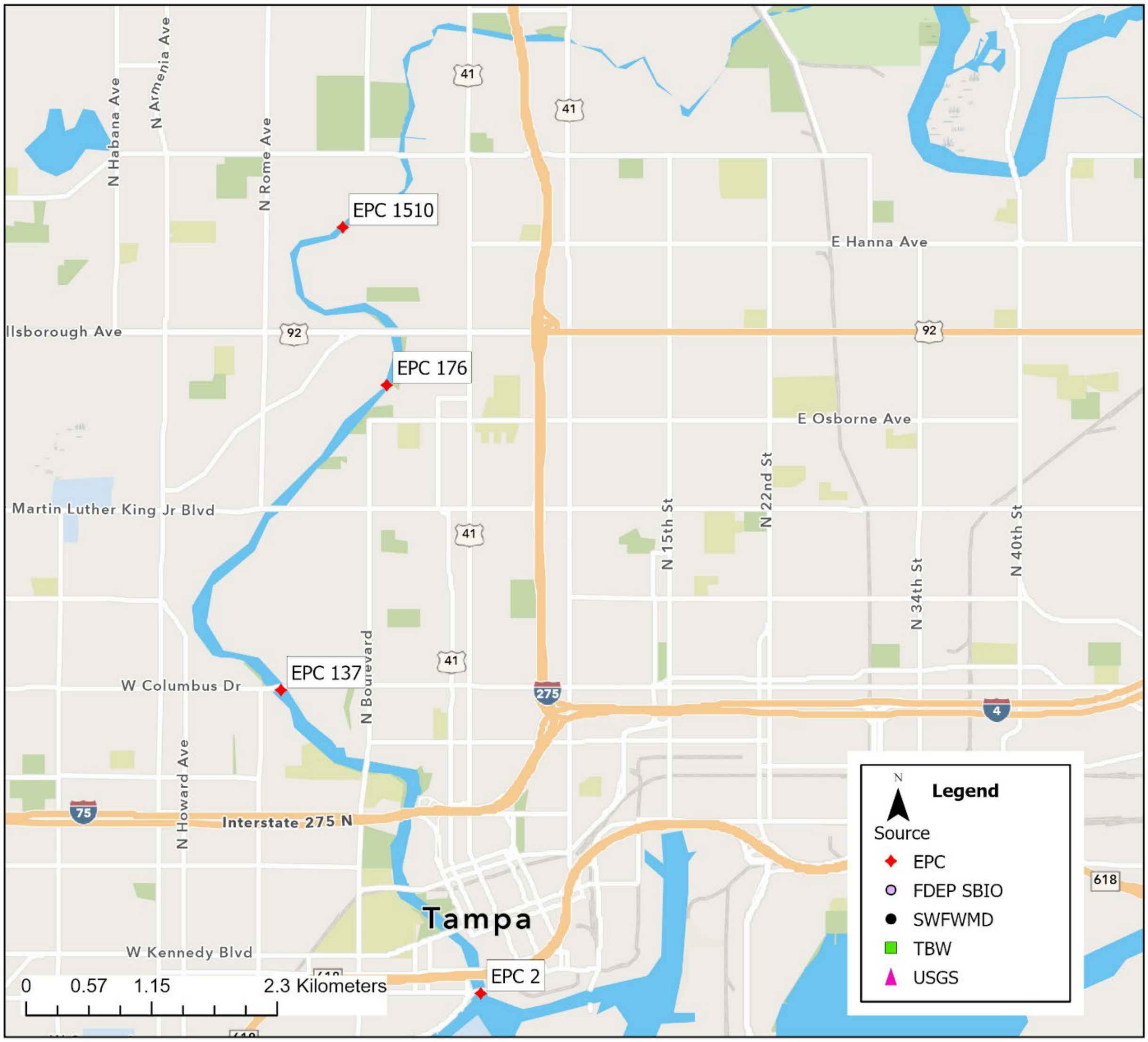
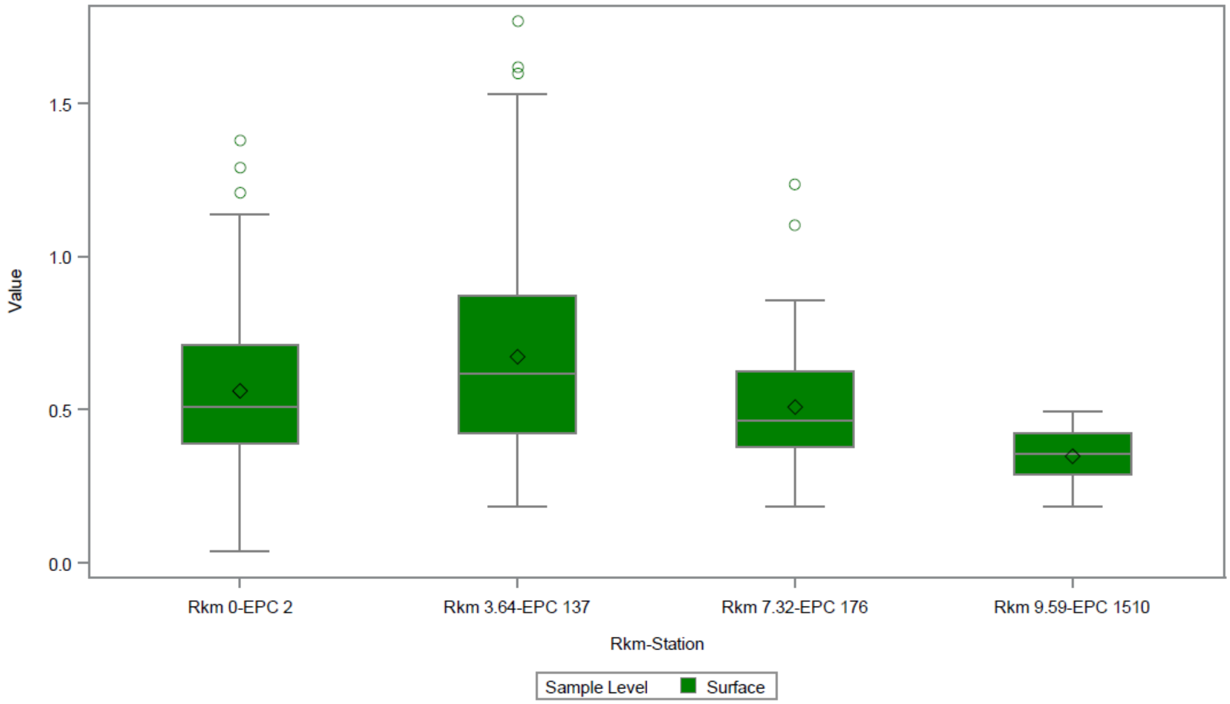


Figure 5.2-27: Distribution for total nitrogen (mg/L) within the LHR downstream by fixed-location stations (Analysis Days)



5.2.1.7 LHR Downstream Nitrate + Nitrite

Higher nitrate + nitrite (NO_3^- + NO_2^-) concentrations occurred between 2002–2011 than the other periods examined (Figure 5.2-28). Mean values ranged from 0.03 to 0.05 mg/L across the implementation periods at fixed stations, but maximum values were lowest in the most recent period (Table 5.2-9). Random sampling for NO_3^- + NO_2^- was conducted under a shorter timeframe, but the results from the 2002–2011 periods were more than twice as high, on average, than those for the fixed stations from the same period.

Similar to observations in total nitrogen, the highest variability exists between 2002–2011 (Figure 5.2-29). Both the range and mean/median values of NO_3^- + NO_2^- declined following Period 3.

Figure 5.2-28: Time series for surface nitrate + nitrite (mg/L) within the LHR downstream (Analysis Days)

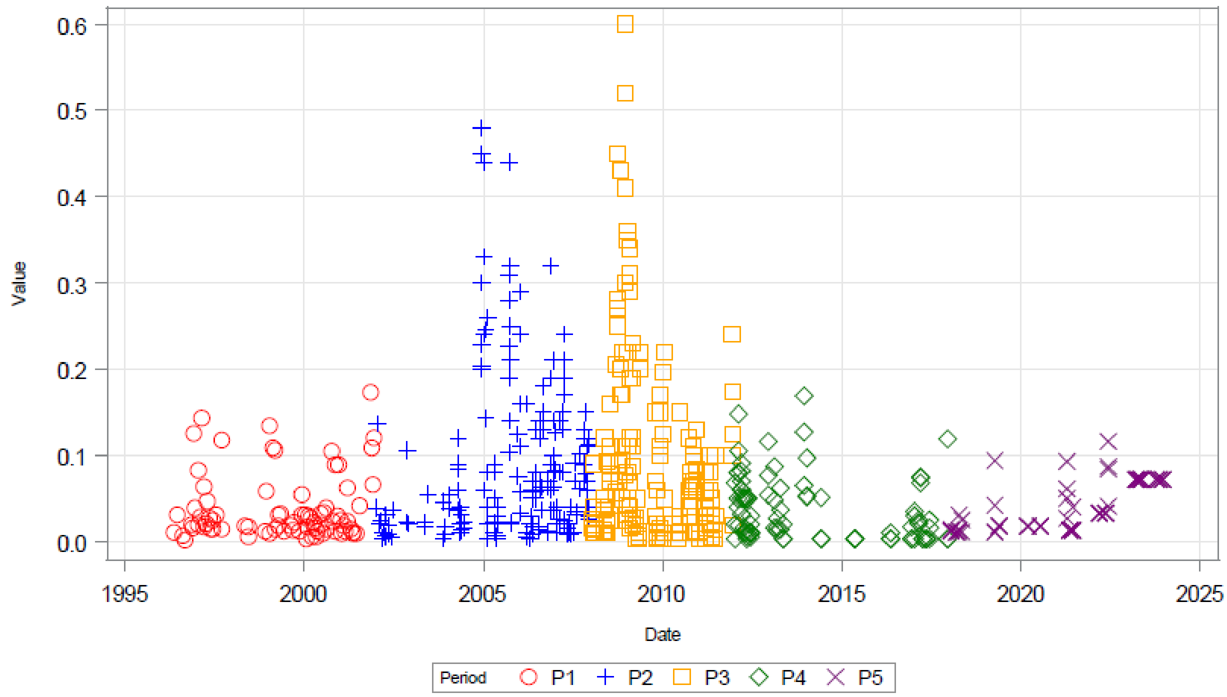
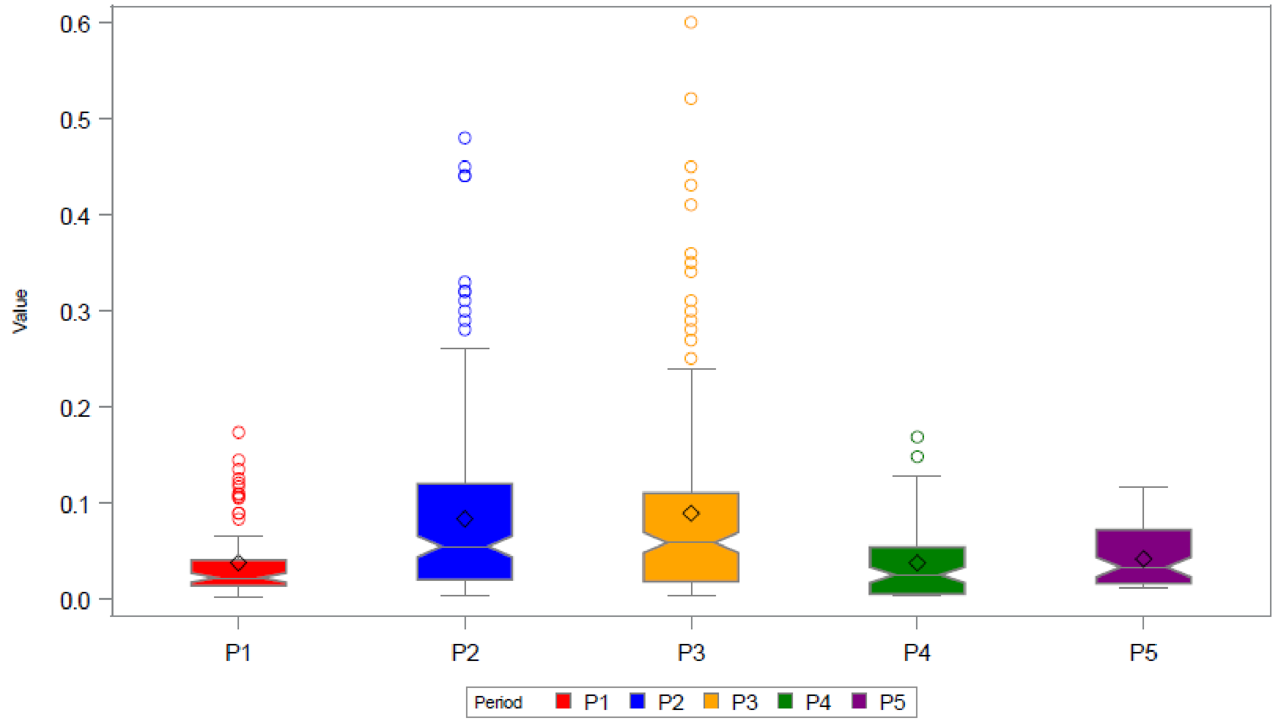


Table 5.2-9: Descriptive statistics for nitrate + nitrite (mg/L) within the LHR downstream by period (Analysis Days)

Type	Level	Period	N	Min	Max	Mean	Std
Fixed	Surface	P1	80	0.00	0.17	0.04	0.04
		P2	86	0.00	0.25	0.05	0.05
		P3	69	0.00	0.21	0.04	0.05
		P4	71	0.00	0.17	0.03	0.04
		P5	72	0.01	0.12	0.04	0.03
Random	Surface	P2	120	0.01	0.48	0.11	0.11
		P3	117	0.01	0.60	0.12	0.12
		P4	20	0.01	0.08	0.05	0.02

Figure 5.2-29: Distribution of surface nitrate + nitrite (mg/L) within the LHR downstream by period



Nitrate + nitrite were measured at both fixed stations at the surface between 1996 and 2023 (Figure 5.2-30) and as part of the probabilistic (random) sampling associated with the TBW HBMP between 2000 and 2012. The concentrations of nitrate + nitrite were similar between stations, with the exception of a lower average and lower range of concentrations observed right near the mouth of the river (EPC 2: Figure 5.2-31).

Figure 5.2-30: Fixed-location stations within the LHR downstream with nitrate + nitrite data

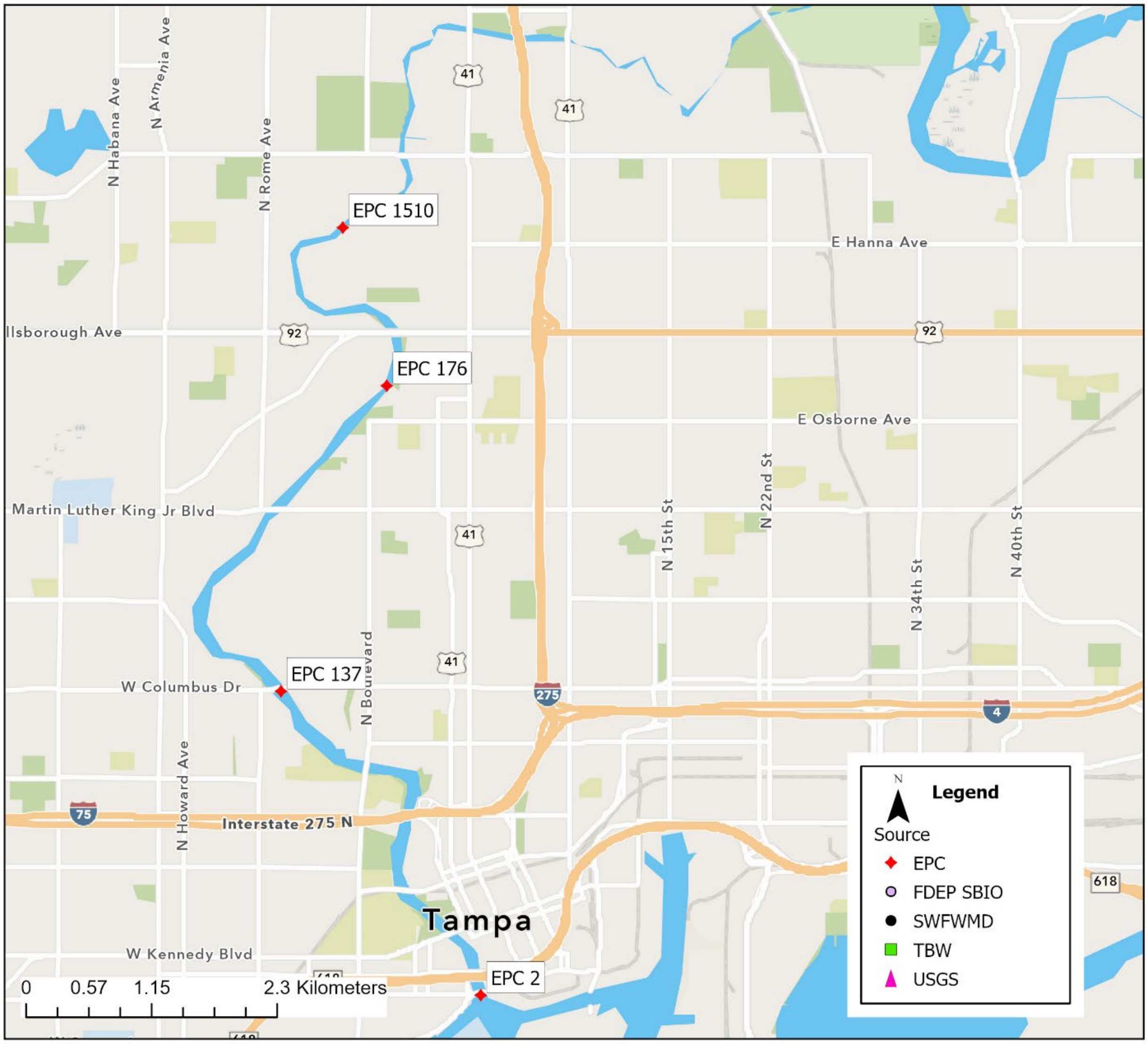
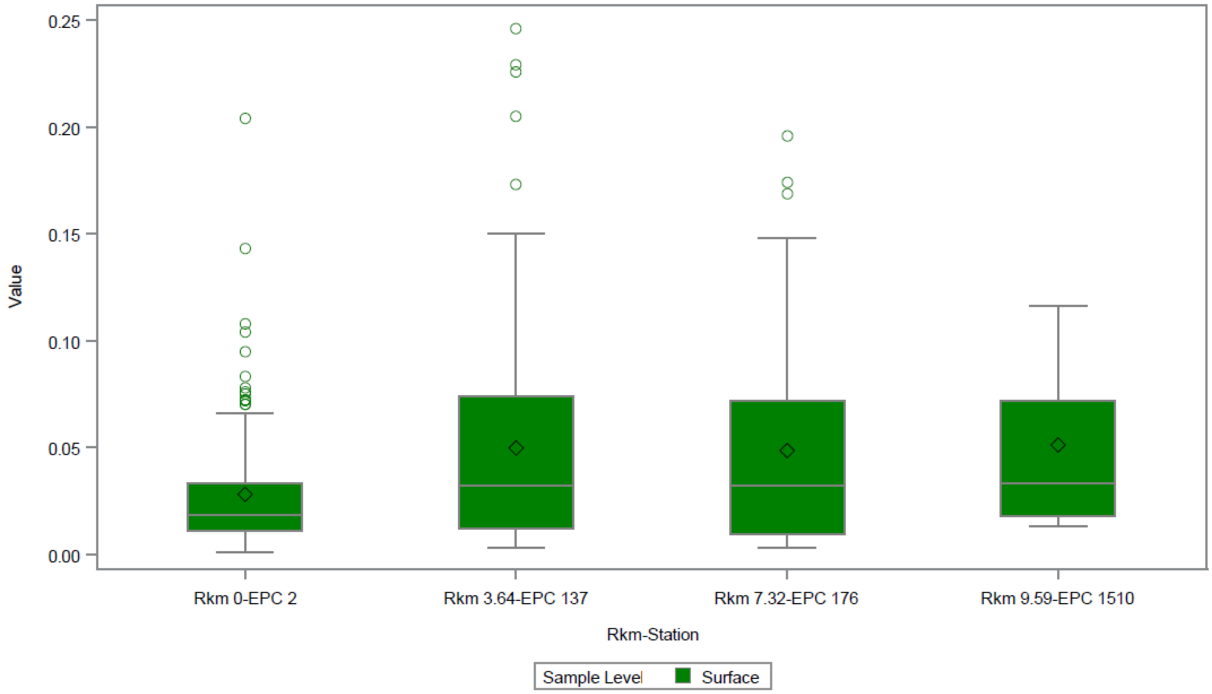


Figure 5.2-31: Distribution of nitrate + nitrite (mg/L) within the LHR downstream by fixed-location stations (Analysis Days)



5.2.1.8 LHR Downstream Total Phosphorus

The time series of total phosphorus concentrations (Figure 5.2-32) suggests a slight decline over time. The greatest values were observed during the end of the 2008–2011 study period and at the beginning of Period 4.

Mean values at fixed stations declined from 0.2 to 0.16 mg/L over the five implementation periods despite a higher maximum value in the last period (Table 5.2-10). With the exception of Period 3, minimum values varied very little.

Combined with the summary statistics in Table 5.2-10, the box and whisker plot (Figure 5.2-33) indicates that mean total phosphorus remained constant across the POR; however, concentrations exhibited an increasing pattern in maximum concentrations over time. The data additionally shows increased variability between 2010 and 2011 where concentration maxima exceeded that of the rest of the POR.

Total phosphorus was measured at four EPC fixed stations (Figure 5.2-34) as well as part of the probabilistic (random) sampling associated with the TBW HBMP between 2000 and 2012. The distribution of concentrations collected at each individual station over its POR is illustrated in Figure 5.2-35. In the downstream portion of the LHR, the distributions of total phosphorus values were larger, with the exception of lower values at the most upstream station at Rkm 9.59.

Figure 5.2-32: Time series for total phosphorus (mg/L) within the LHR downstream (Analysis Days)

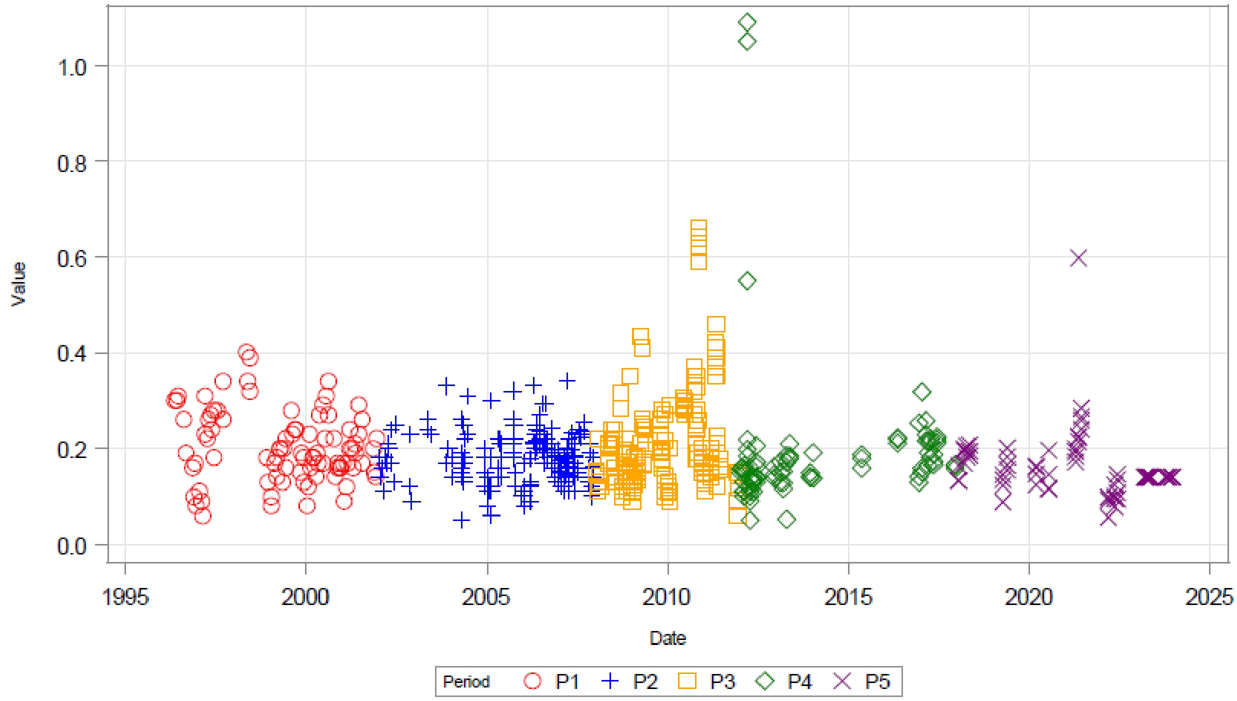


Table 5.2-10: Descriptive statistics for total phosphorus (mg/L) within the LHR downstream by period (Analysis Days)

Type	Level	Period	N	Min	Max	Mean	Std
Fixed	Surface	P1	88	0.06	0.40	0.20	0.07
		P2	88	0.05	0.33	0.19	0.06
		P3	68	0.11	0.43	0.18	0.06
		P4	68	0.05	0.32	0.17	0.04
		P5	73	0.06	0.60	0.16	0.07
Random	Surface	P2	120	0.08	0.34	0.17	0.05
		P3	113	0.06	0.66	0.21	0.12
		P4	17	0.05	1.09	0.25	0.33

Figure 5.2-33: Distribution for total phosphorus (mg/L) within the LHR downstream by period (Analysis Days)

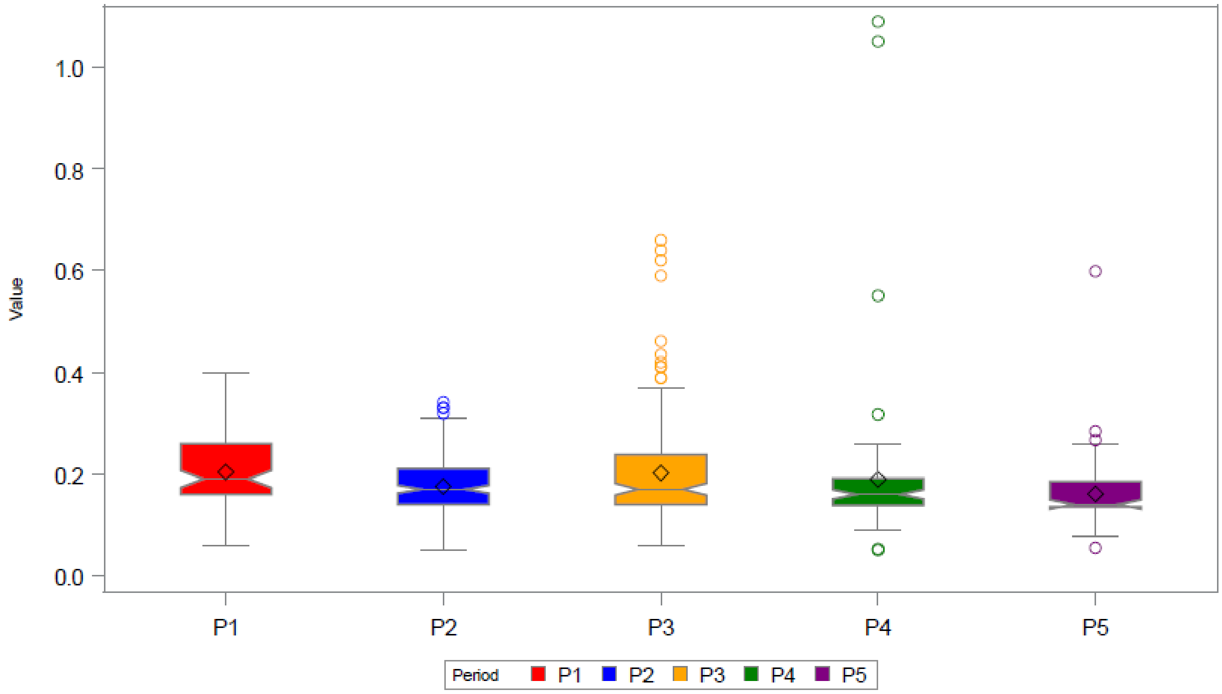


Figure 5.2-34: Fixed-location stations within the LHR downstream with total phosphorus data

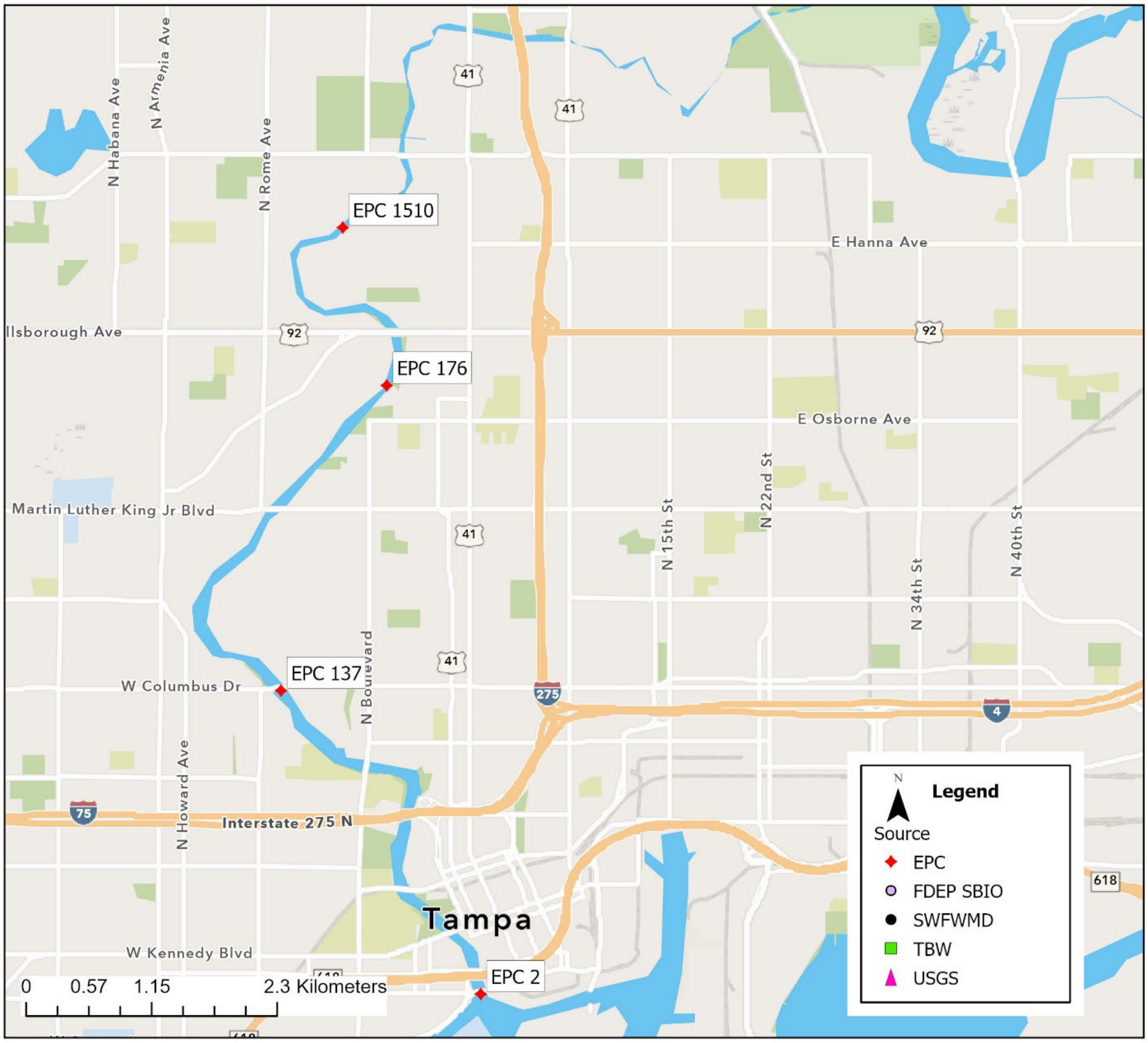
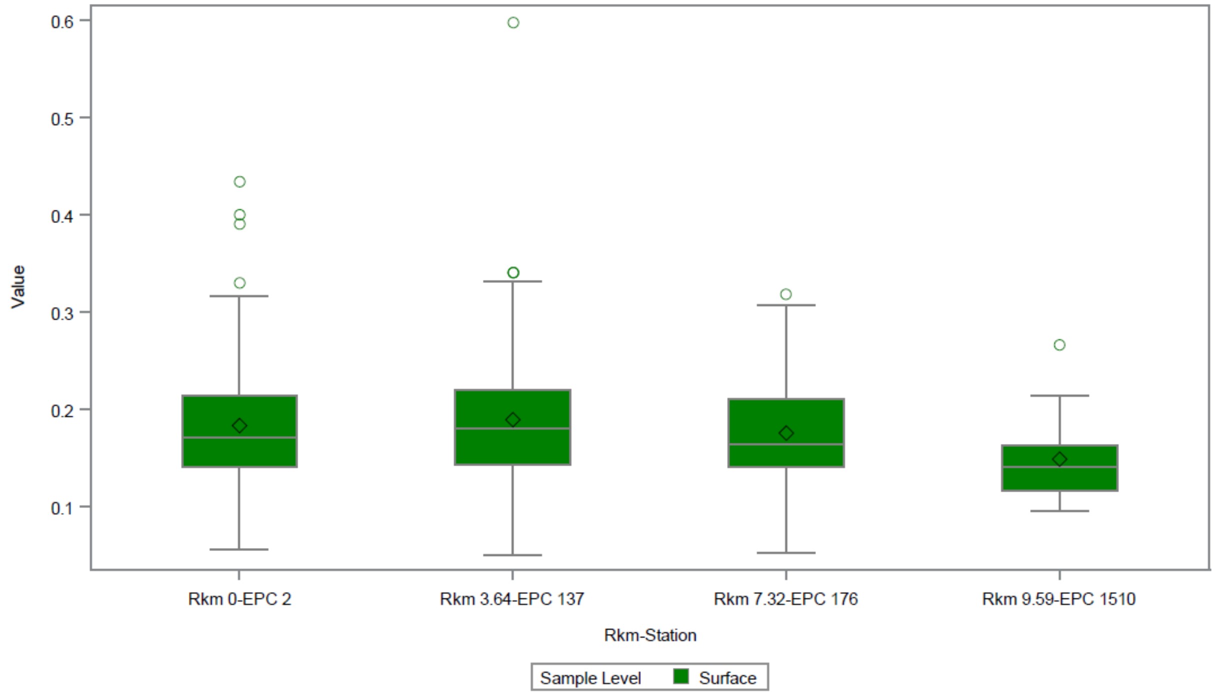


Figure 5.2-35: Distribution of total phosphorus (mg/L) within LHR downstream by fixed-location stations (Analysis Days)



5.2.1.9 LHR Downstream Orthophosphate

Patterns in orthophosphate (Figure 5.2-36) were similar to those for total phosphorus over the time series. A decline is indicated over the study period, and maximum values and variability were observed during the 2008–2011 period.

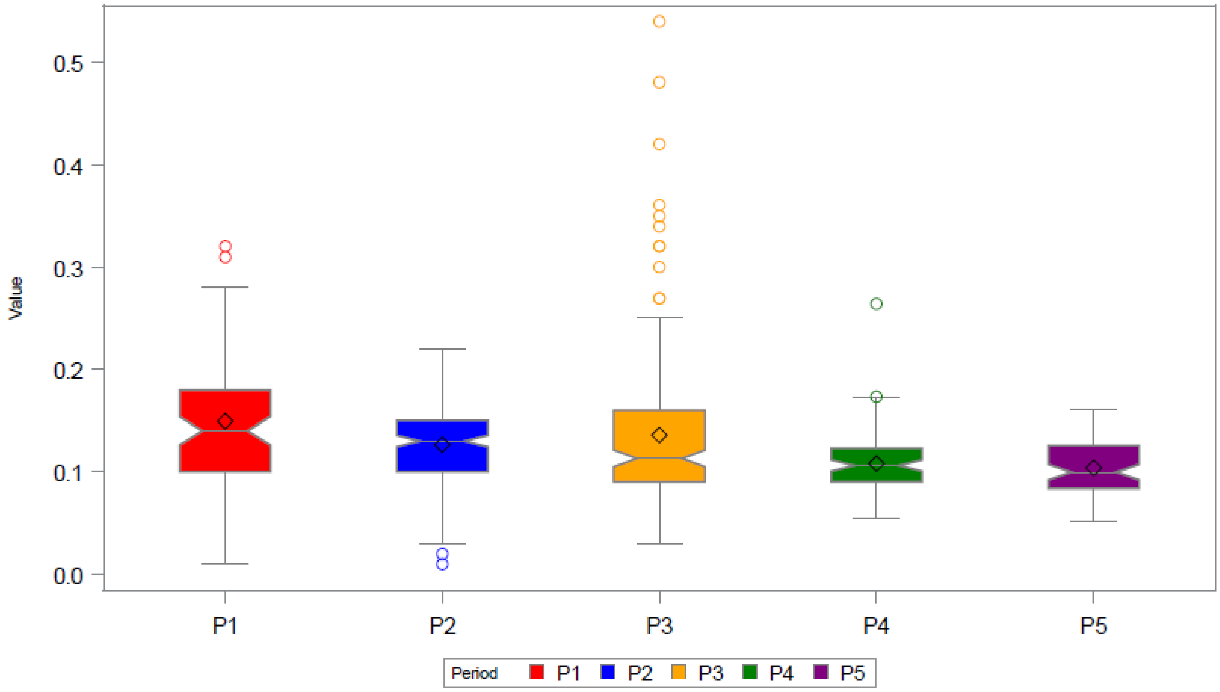
The mean concentration of orthophosphate fell from 0.15 mg/L in the earliest period to 0.10 mg/L in the most recent period (Table 5.2-11). Maximum values also decreased. Box and whisker plots of the orthophosphate concentrations are shown in Figure 5.2-37. Observations of orthophosphate demonstrate a slight decrease in concentrations across the POR with the highest values being noted between 2008 and 2011. Average concentrations range between 0.1 and 0.15 mg/L with only a few outliers falling above 0.3 mg/L during the entire POR.

Figure 5.2-36: Time series for orthophosphate (mg/L) within the LHR downstream (Analysis Days)

Table 5.2-11: Descriptive statistics for orthophosphate (mg/L) within the LHR downstream by period (Analysis Days)

Type	Level	Period	N	Min	Max	Mean	Std
Fixed	Surface	P1	84	0.01	0.32	0.15	0.06
		P2	88	0.06	0.22	0.13	0.04
		P3	69	0.05	0.21	0.11	0.04
		P4	71	0.06	0.26	0.11	0.03
		P5	76	0.05	0.16	0.10	0.03
Random	Surface	P2	112	0.01	0.22	0.13	0.04
		P3	113	0.03	0.54	0.15	0.09
		P4	20	0.08	0.13	0.10	0.01

Figure 5.2-37: Distribution for orthophosphate (mg/L) within the LHR downstream by period (Analysis Days)



Orthophosphate was measured at four EPC fixed stations (Figure 5.2-38), and measurements were conducted as part of the probabilistic (random) sampling associated with the TBW HBMP between 2000 and 2012 only. The spatial pattern of orthophosphate concentrations (Figure 5.2-39) matches that of total phosphorus.

Figure 5.2-38: Fixed-location stations within the LHR downstream with orthophosphate data

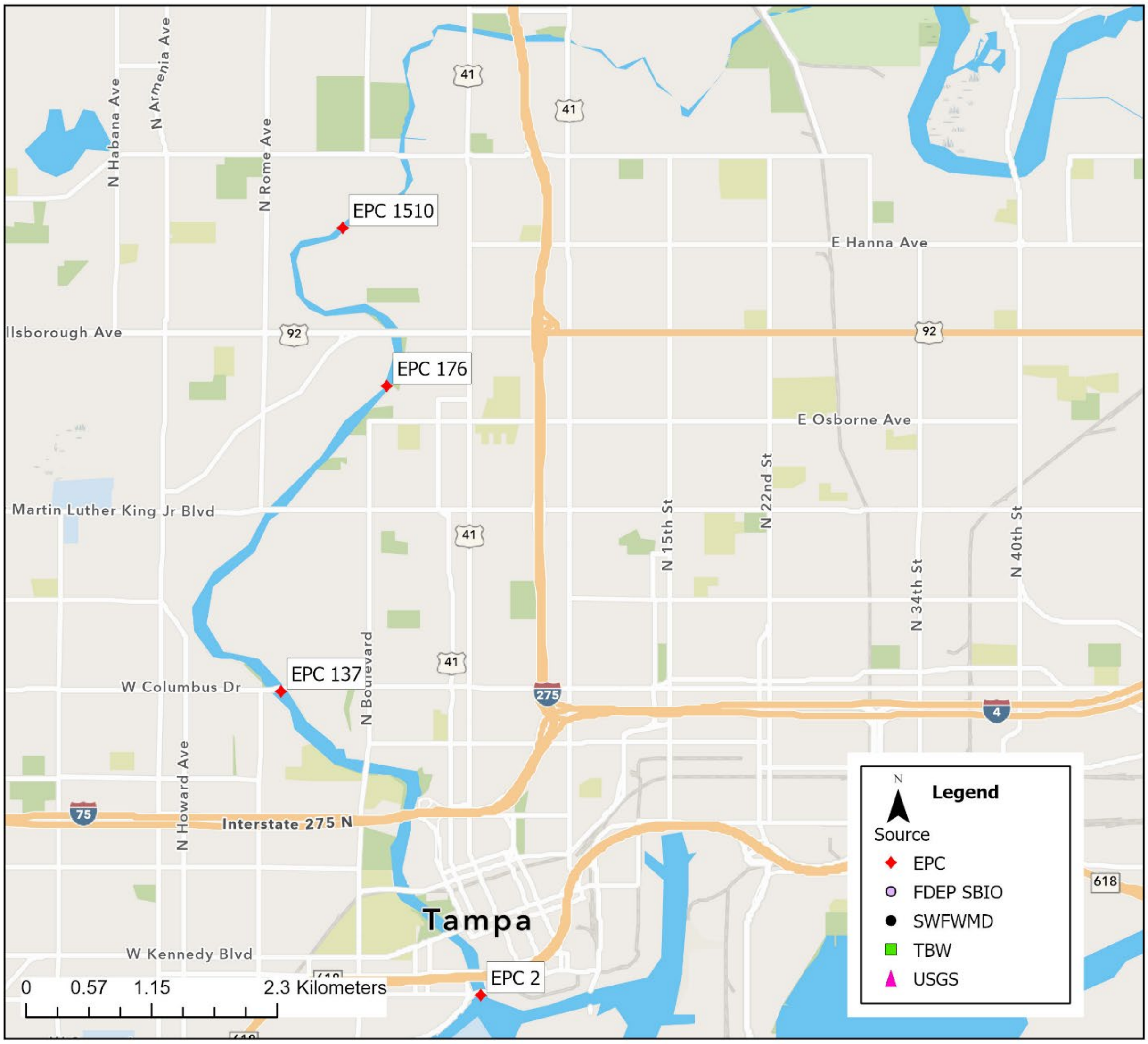
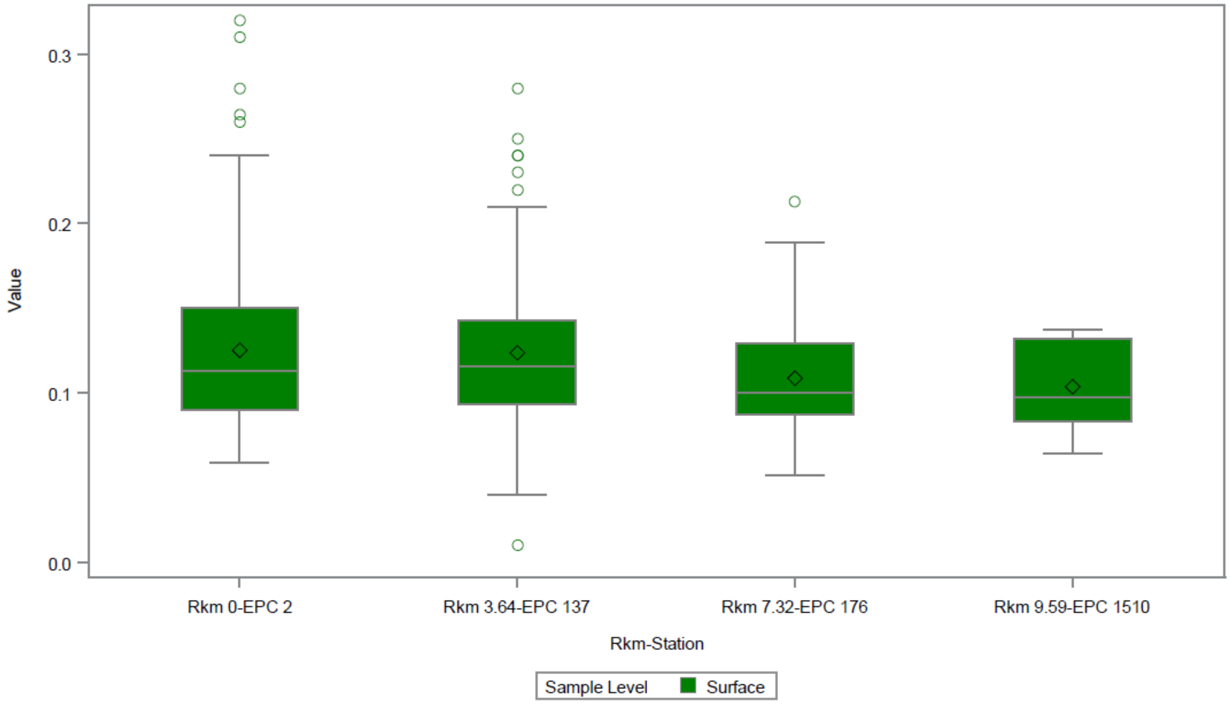


Figure 5.2-39: Distribution for orthophosphate (mg/L) within the LHR downstream by fixed-location stations (Analysis Days)



5.2.1.10 LHR Downstream Chlorophyll

Chlorophyll measurements have been reported in five different ways by the different agencies collecting water quality data in the LHR (Figure 5.2-40). The EPC switched from only reporting uncorrected chlorophyll *a* until 2004 to reporting both uncorrected and corrected chlorophyll *a* since that time. TBW reported corrected chlorophyll *a* for the entire POR (2002 to 2012), and the District reported various forms of chlorophyll including total chlorophyll, chlorophyll *a*, monochromatic, and trichromatic. Previous 5-year assessments (SWFWMD and Atkins 2015; WAR 2020) evaluated uncorrected chlorophyll because it provided the most robust dataset. Therefore, uncorrected chlorophyll is also reported here, and the District data reporting other methods were considered uncorrected chlorophyll for this report. Differences between uncorrected and corrected chlorophyll were generally minimal relative to the variation in magnitudes over time as can be seen in Figure 5.2-40 when both forms were reported. Time series plots of chlorophyll are presented in Figure 5.2-41. The time series suggests some variability over time but no clear pattern in terms of increase or decrease.

There were some higher values in Period 4 that appear to have influenced the mean (18.08 µg/L) being slightly higher than the other periods (Table 5.2-12). Minimum values were largely consistent across the periods.

Figure 5.2-40: Time series for chlorophyll (µg/L) within the LHR downstream (Analysis Days)

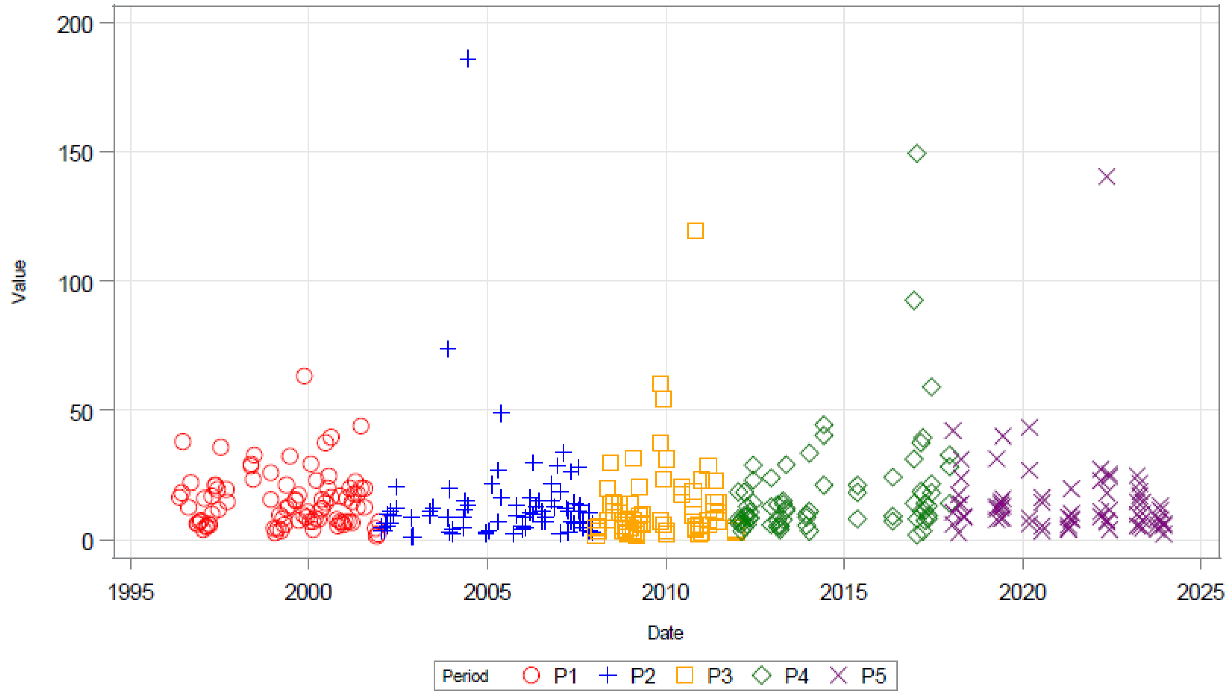
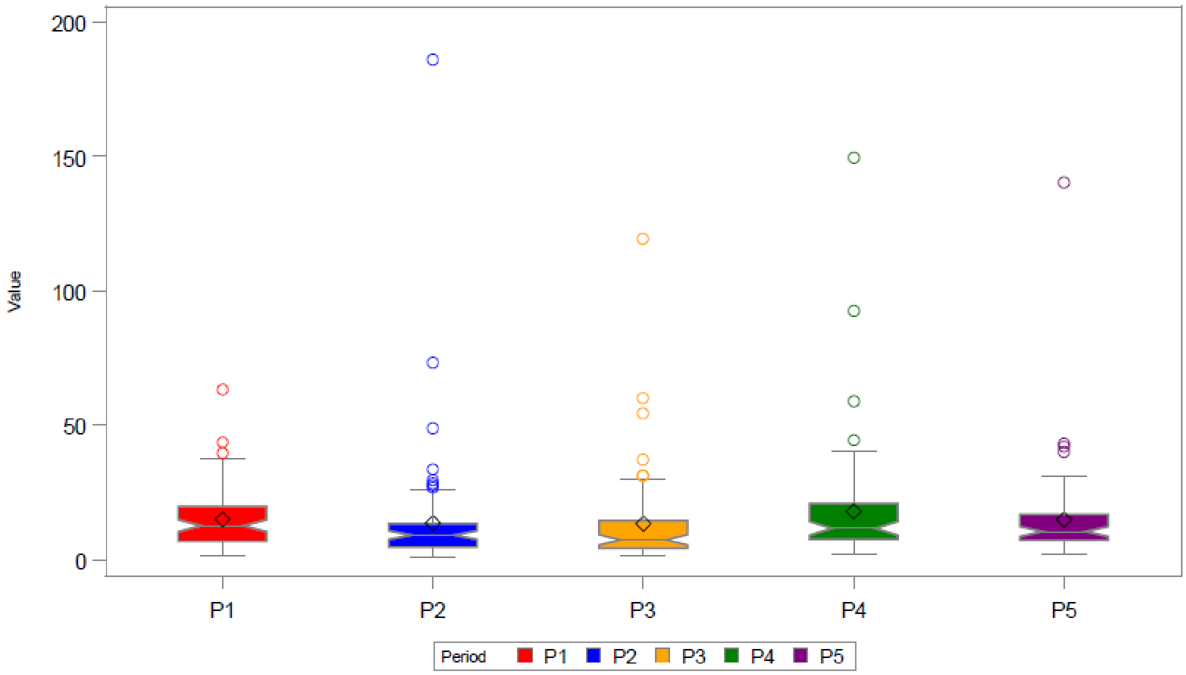


Table 5.2-12: Descriptive statistics of chlorophyll (µg/L) within the LHR downstream by period (Analysis Days)

Type	Level	Period	N	Min	Max	Mean	Std
Fixed	Surface	P1	89	1.53	63.41	15.13	10.82
		P2	81	1.10	185.92	13.74	22.23
		P3	69	1.80	119.40	13.50	17.33
		P4	72	1.90	149.30	18.08	21.29
		P5	73	2.20	140.40	14.99	17.48

Figure 5.2-41: Distribution for chlorophyll (µg/L) within the LHR downstream by period (Analysis Days)



Chlorophyll values were measured at four EPC fixed location stations in the downstream portion of the LHR (Figure 5.2-42). Although results across the stations were relatively similar when combined over the five periods, Figure 5.2-43 indicates a slight longitudinal pattern of increasing concentration with distance upstream from the river mouth.

Figure 5.2-42: Fixed-location stations within the LHR downstream with chlorophyll data

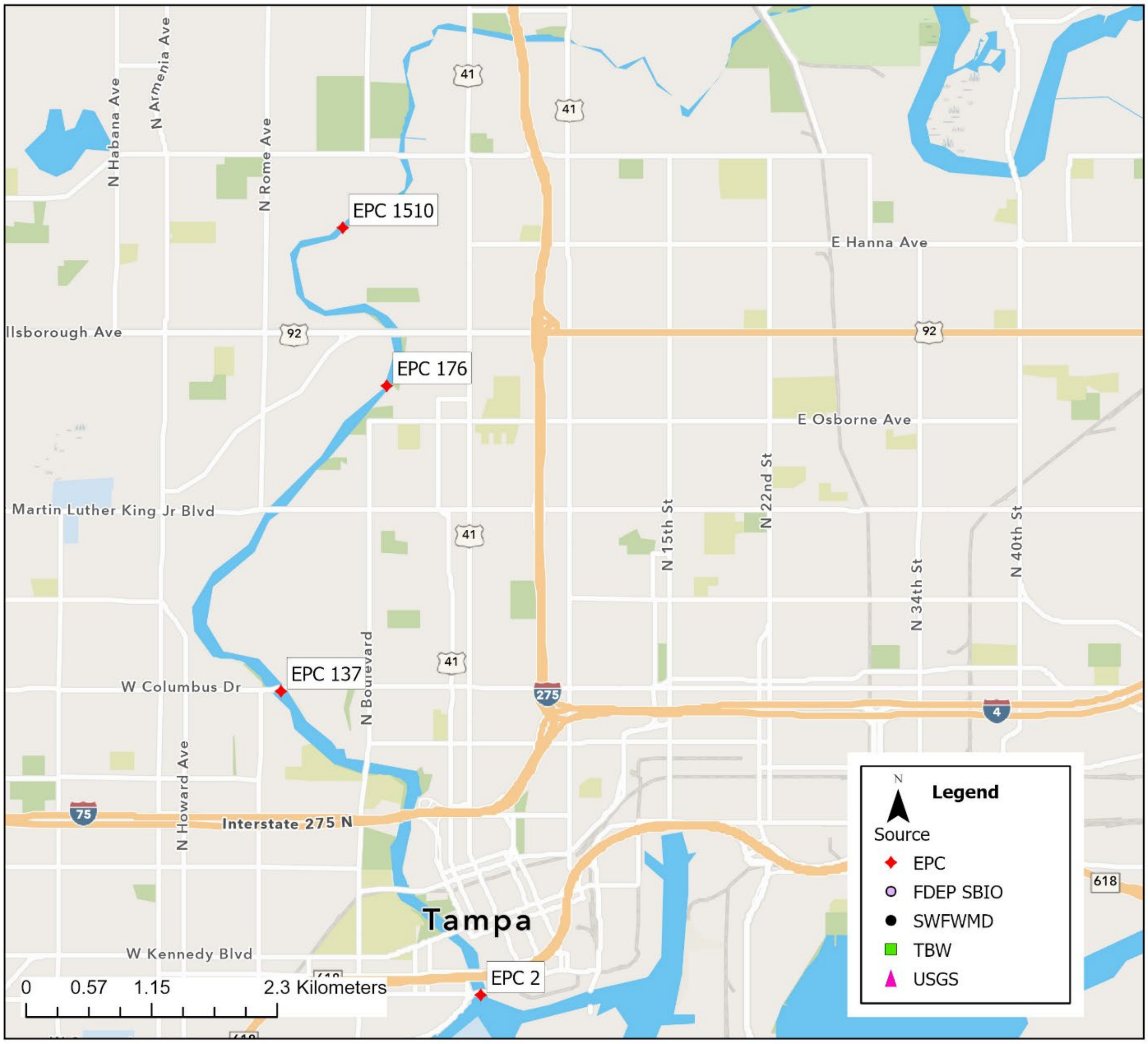
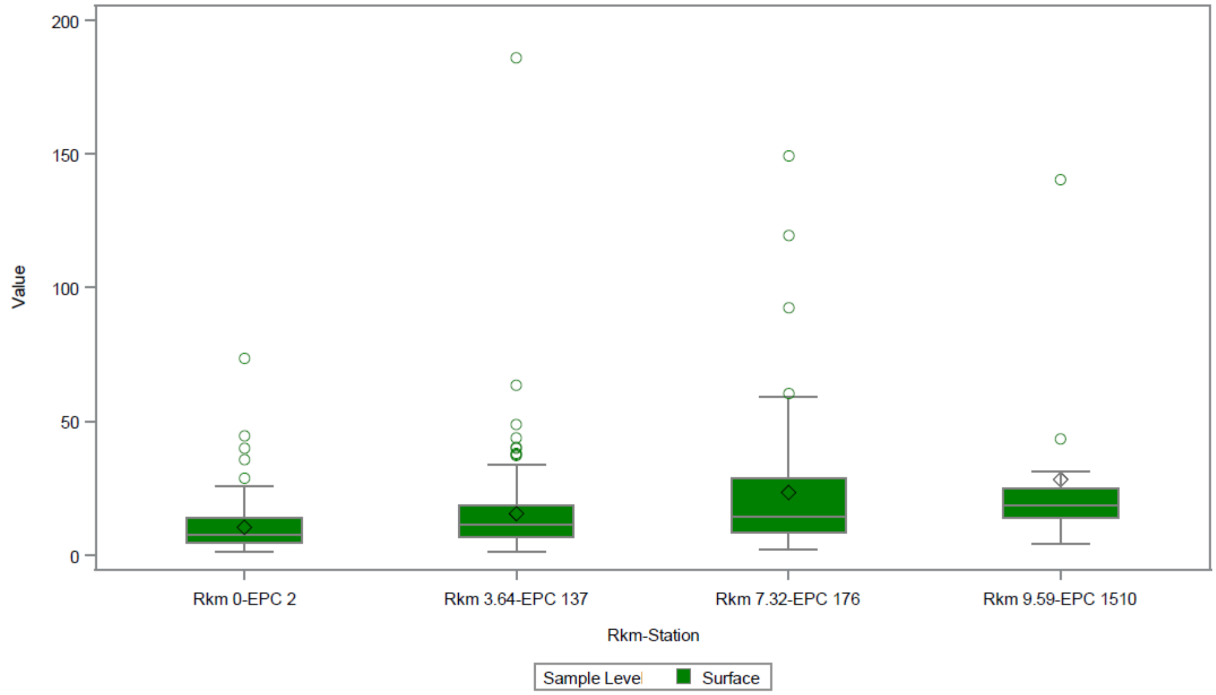


Figure 5.2-43: Distribution for chlorophyll (µg/L) within the LHR downstream by fixed-location stations (Analysis Days)



5.2.2 LHR Target Zone

The target zone of the LHR is the area between the base of the Hillsborough River Dam and Sligh Avenue. Six CRs (continuous recorders) are in the LHR target zone: the District CR 19206, TBW CR at Sligh, and four USGS stations. There were 23 fixed station sampling sites, in addition to the random sampling program of TBW HBMP and some probabilistic sampling by JMT with varying frequency of sampling. Data from all stations were combined for the characterization of the target zone data presented here. The stations are listed in Table 5.2-13. Plots of the data collected at each individual station during Analysis Days are provided in Appendix M. This section contains summary tables and plots that combine the data collected at all stations for each parameter in the target zone of the study area. These plots are further grouped by their vertical location within the water column (surface, middle, bottom) to examine near vertical stratification in water quality within the target zone.

Table 5.2-13: Water Quality Data within the LHR Target Zone

Sampling Type	Station Name	River Kilometer	Start Date	End Date
CRs	TBW @ Sligh CR	10.55	12/12/2002	12/29/2023
	USGS I-275 023060013	12.58	9/11/1999	12/16/2023
	District CR 19206	12.75	4/15/2020	12/16/2023
	USGS BL Hannah's Whirl 02304517	14.14	12/1/2017	12/16/2023
	USGS Hannah's Whirl 02304515	14.33	6/15/2001	9/29/2005
	USGS Rowlett 02304510	15.41	12/23/1996	12/16/2023
Fixed Stations	EPC SN 152	10.5	8/17/1999	12/13/2023
	SWFWMD Lower Hillsborough River16 SN 19237	10.8	3/27/2002	11/27/2023
	SWFWMD Lower Hillsborough River15 SN 800055	11.3	3/27/2002	11/27/2023
	EPC SN 1512	11.56	1/5/2009	12/13/2023
	SWFWMD Lower Hillsborough River14 SN 800054	11.8	3/27/2002	11/27/2023
	EPC SN 270	12.12	4/17/2019	5/10/2023
	SWFWMD Lower Hillsborough River13 SN 19235	12.3	3/27/2002	11/27/2023
	SWFWMD Lower Hillsborough River12 SN 800053	12.7	3/27/2002	11/27/2023
	SWFWMD Lower Hillsborough River11 SN 800052	13	3/27/2002	11/27/2023
	SWFWMD Lower Hillsborough River10 SN 800050	13.3	3/27/2002	11/27/2023
	EPC SN 1514	13.41	1/5/2009	12/13/2023
	SWFWMD Lower Hillsborough River09 SN 19209	13.6	3/27/2002	11/27/2023
	SWFWMD Lower Hillsborough River08 SN 800049	13.9	3/27/2002	11/27/2023
	SWFWMD Lower Hillsborough River07 SN 800048	14.3	3/27/2002	11/27/2023
	EPC SN 1515	14.35	1/5/2009	11/8/2023
	SWFWMD Lower Hillsborough River06 SN 800047	14.5	3/27/2002	11/27/2023
	SWFWMD Lower Hillsborough River05 SN 800046	14.8	3/27/2002	11/27/2023
	SWFWMD Lower Hillsborough River04 SN 800045	15.1	3/27/2002	11/27/2023
	SWFWMD Lower Hillsborough River03 SN 19208	15.4	3/27/2002	11/27/2023
	EPC SN 105	15.45	5/14/1996	12/13/2023
	SWFWMD Lower Hillsborough River02 SN 800044	15.7	3/27/2002	11/27/2023
	SWFWMD Lower Hillsborough River01 SN 800043	16	4/10/2002	11/27/2023
	EPC SN 165	16.2	1/18/2005	12/12/2023

Sampling Type	Station Name	River Kilometer	Start Date	End Date
Probabilistic Sites	TBW HBMP	Varies	4/12/2000	6/14/2012
	JMT Lower A	Varies	5/16/2020	12/13/2023
	JMT Lower B	Varies	5/16/2020	12/13/2023
	JMT Middle A	Varies	5/16/2020	12/13/2023
	JMT Middle B	Varies	5/16/2020	12/13/2023
	JMT Middle C	Varies	3/27/2022	12/13/2023

Notes: Start and end dates may vary by parameter, those listed encompass the broadest range of the greatest number of parameters.

5.2.2.1 LHR Target Zone Salinity

Surface salinity in the target zone was principally under 10 ppt with excursions above 10 ppt more frequent during drought conditions (e.g. the 2000 drought: Figure 5.2-44). Salinity generally trended lower over time as implementation flows increased. Of particular note is the salinity spike at the end of these time series (2023) which is discussed further at the end of this section Gaps in the time series represent periods where implementation was not necessary based on the current adopted minimum flow. Midwater and bottom salinities tend to be about 5 ppt higher than the surface salinity based on the summary statistics in Table 5.2-14. Appendix N presents the descriptive statistics breakdowns by target zone river segment, water column strata, and period. The box and whisker plots of Figure 5.2-45 display the data distribution by period as well as separated by the three river segments of the target zone (upper, middle, and lower). Salinity clearly shows a strong gradient within the target zone with the upper segment always lower than the lower segment. As implementation of the minimum flows has clearly increased over time, salinity has been reduced in the upper and middle segments of the target zone and the effects have been less pronounced in the lower segment.

Figure 5.2-44: Time series for salinity (ppt) within the LHR target zone for specified water column strata (Analysis Days)

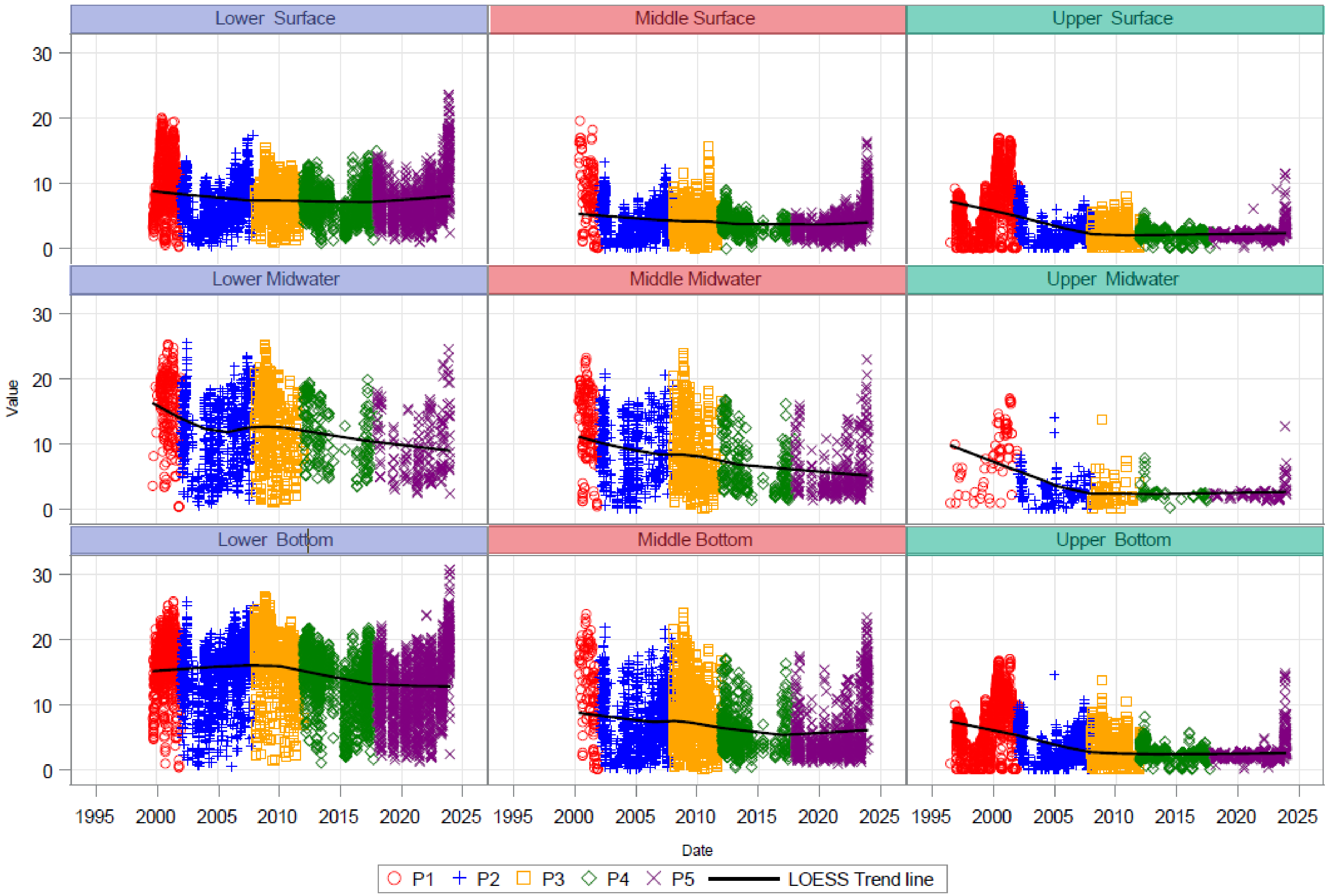
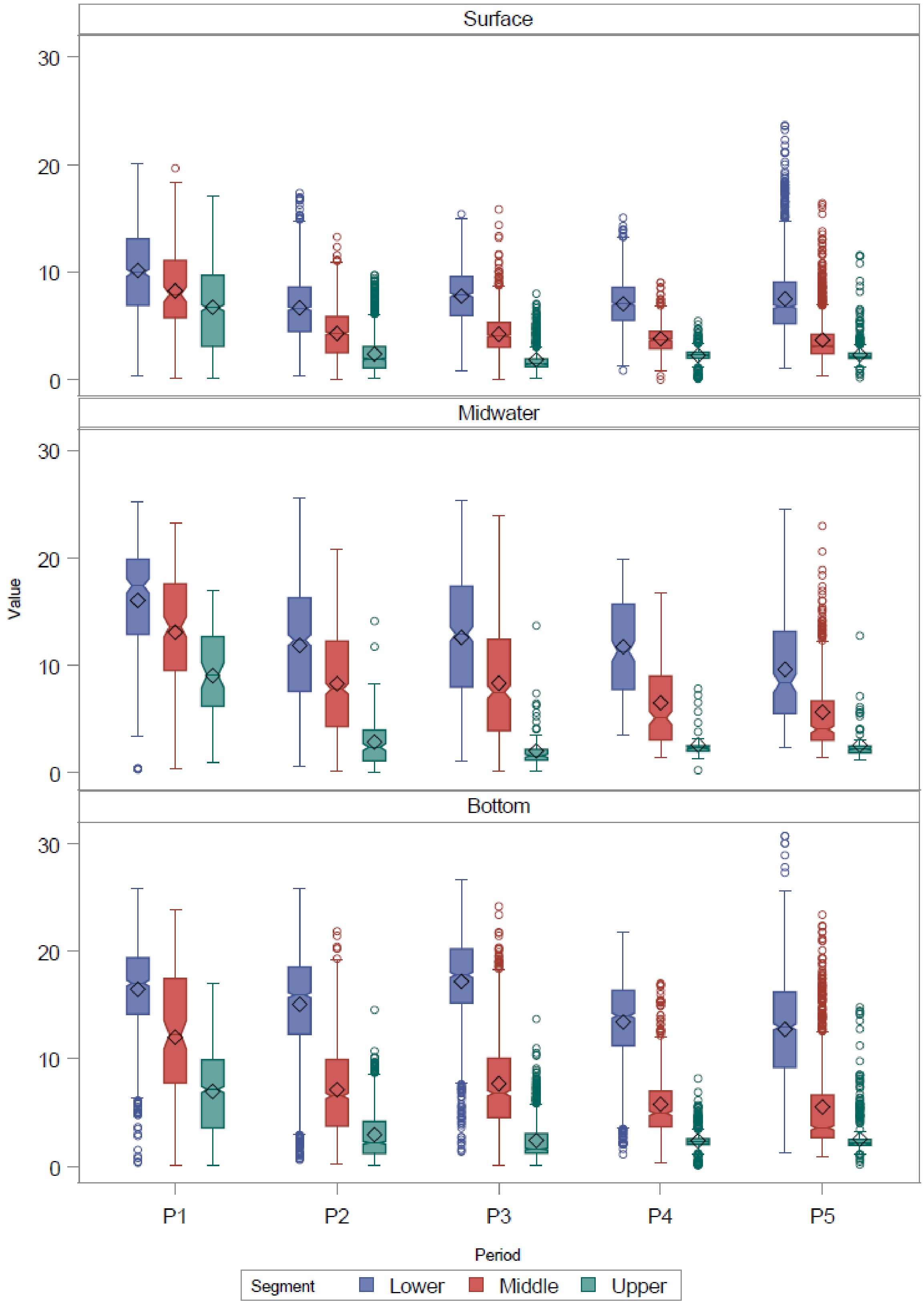


Table 5.2-14: Descriptive statistics for salinity (ppt) within the LHR target zone for specified water column strata (Analysis Days)

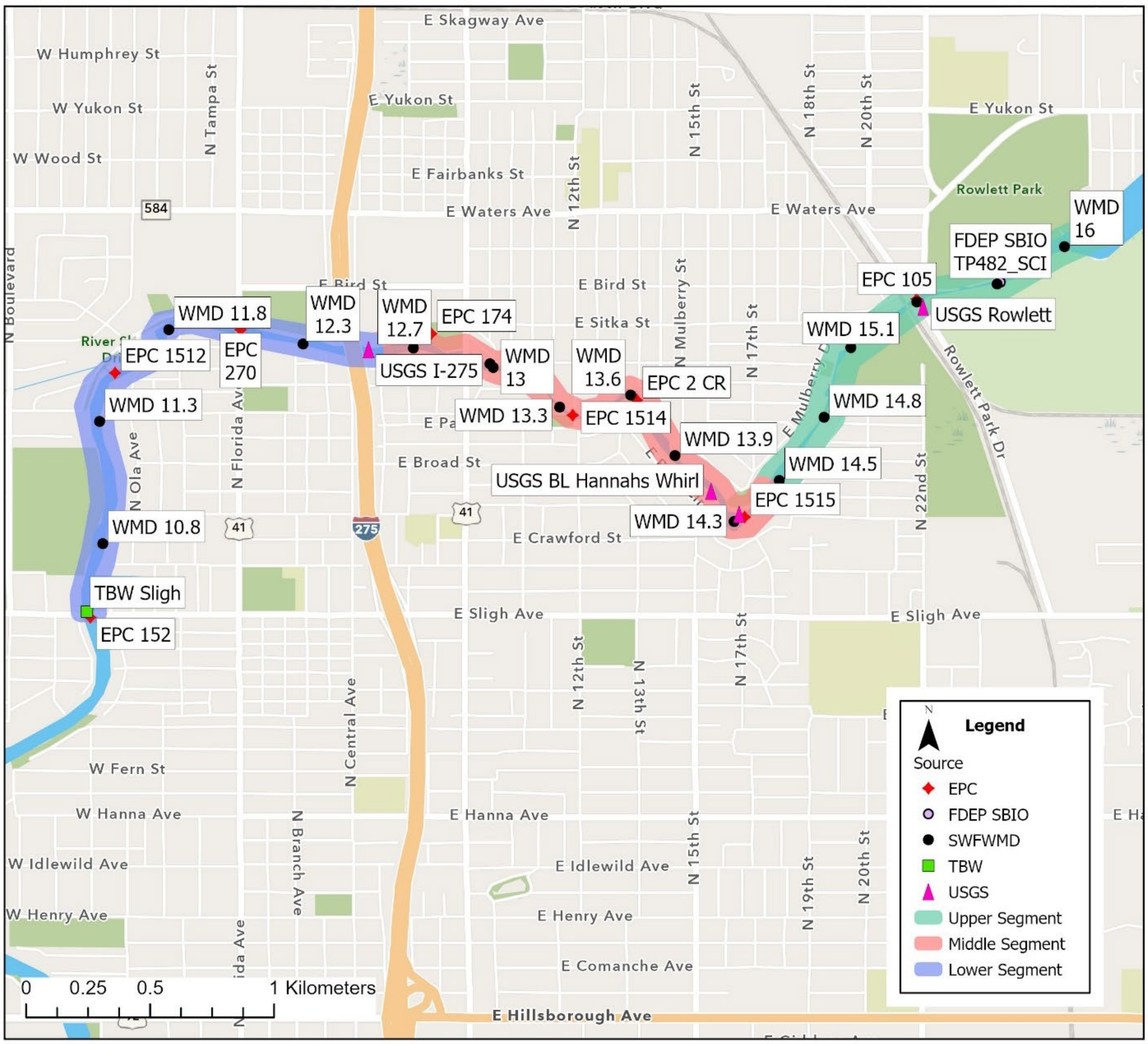
Sampling Type	Level	Period	N	Min	Max	Mean	Std
CRs	Surface	P1	1,986	0.11	20.07	7.89	4.62
		P2	3,171	0.01	16.91	4.69	3.14
		P3	2,332	0.01	15.77	4.77	3.35
		P4	2,370	0.01	15.01	5.00	2.88
		P5	3,154	0.17	23.61	5.06	3.51
	Midwater	P1	.	.	.	.	.
		P2	.	.	.	.	.
		P3	.	.	.	.	.
		P4	.	.	.	.	.
		P5	.	.	.	.	.
	Bottom	P1	1,931	0.10	24.59	10.31	6.10
		P2	3,350	0.11	25.06	8.64	6.58
		P3	2,375	0.12	26.62	8.84	7.20
		P4	2,350	0.13	21.73	8.35	5.98
		P5	2,689	0.18	30.67	8.16	6.21
Fixed Stations	Surface	P1	66	1.00	19.20	7.50	4.50
		P2	401	0.10	11.74	3.54	2.51
		P3	310	0.22	11.09	4.26	2.55
		P4	335	0.25	9.90	3.91	1.92
		P5	451	0.61	9.67	3.59	1.65
	Midwater	P1	66	1.00	23.30	9.90	6.21
		P2	705	0.10	20.20	7.93	5.43
		P3	427	0.22	21.72	9.46	5.72
		P4	372	0.27	19.87	7.23	4.74
		P5	562	1.24	24.53	6.42	4.79
	Bottom	P1	66	1.00	25.60	11.05	6.98
		P2	397	0.10	22.52	8.62	6.21
		P3	305	0.22	22.29	11.21	6.80
		P4	315	0.32	20.47	8.42	5.52
		P5	422	1.27	25.23	7.97	5.84
Random	Surface	P1	237	0.10	19.98	9.65	4.85
		P2	560	0.02	17.37	4.78	2.78
		P3	328	0.14	11.57	4.36	2.28
		P4	52	2.00	10.41	5.34	2.38
		P5	86	1.62	17.78	5.07	3.04
	Midwater	P1	448	0.36	25.23	14.56	5.38
		P2	837	0.10	25.50	10.46	5.82
		P3	640	0.14	25.32	9.58	6.55
		P4	82	2.00	19.39	11.75	5.13
		P5	81	1.52	19.40	5.99	3.78
	Bottom	P1	240	0.20	25.80	14.93	5.94
		P2	583	0.10	25.79	10.18	6.48
		P3	326	0.14	25.42	10.54	6.93
		P4	53	2.00	19.90	10.88	6.23
		P5	86	1.49	30.67	7.73	5.71

Figure 5.2-45: Distribution for salinity (ppt) within the LHR target zone by period and river segment for specified water column strata (Analysis Days)



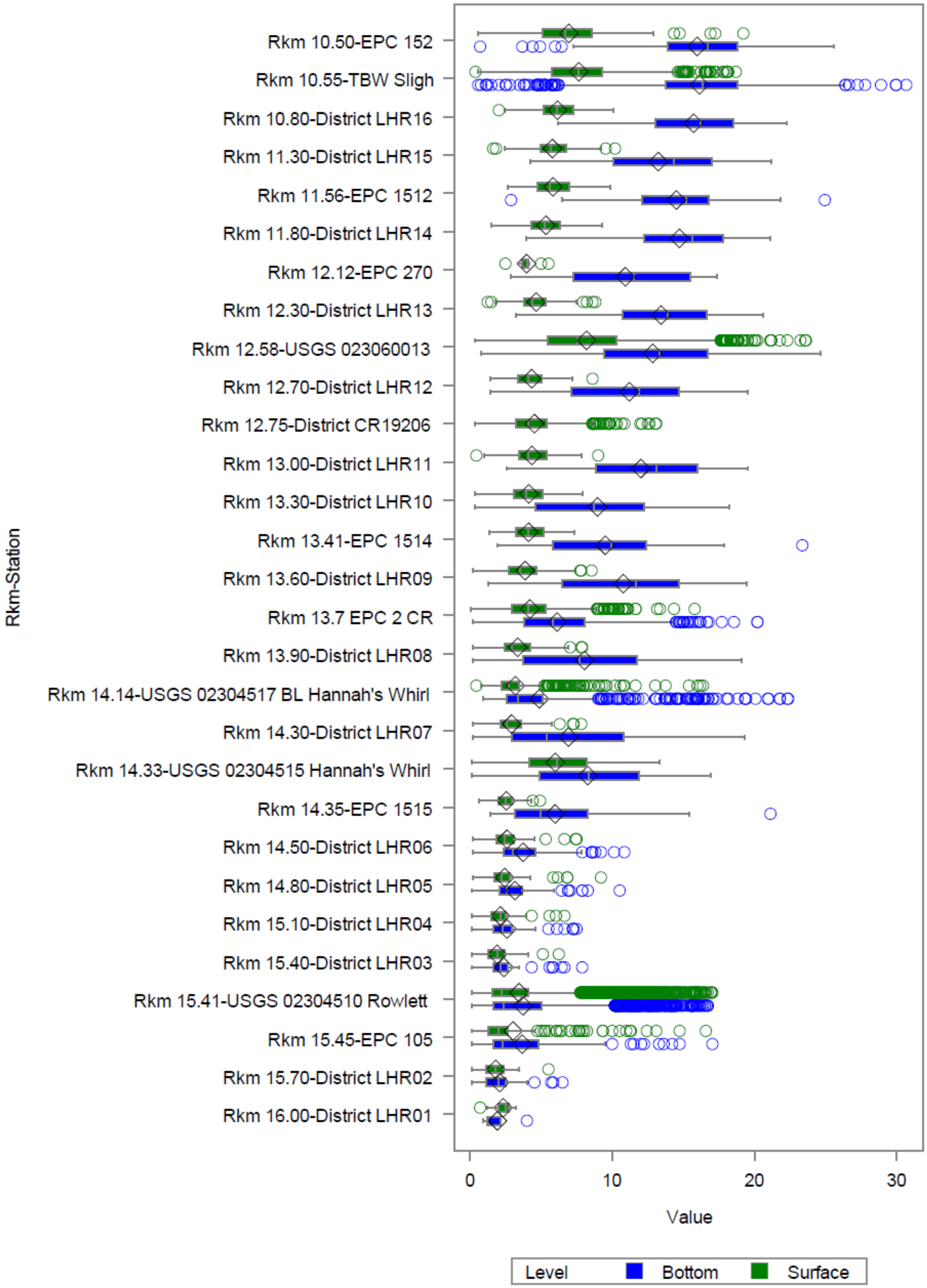
The individual stations that make up the data distribution within the target zone are presented in Figure 5.2-46 and include CRs as well as vertical water column profile measurements. Measurements from probabilistic (random) sampling associated with the TBW HBMP program from 2000 through 2012 and synoptic biological data collections thereafter are not represented as they would obscure the visibility of fixed station locations. These maps represent only those stations where salinity was reported.

Figure 5.2-46: Fixed-location stations within the LHR target zone with salinity data



The data distribution of surface and bottom salinity values for these sites is presented in Figure 5.2-47. In these plots the sites are oriented longitudinally by river kilometer to display the longitudinal gradient in salinity within the target zone. Although individual stations may have different periods of data collection, the longitudinal gradient is still evident in salinity within the target zone. In addition, below Rkm 13.6 the difference between surface and bottom salinity clearly becomes greater, suggesting increased vertical stratification in the water column downstream. The USGS CR typically presents a wider distribution of values, since they represent daily values, but remain restricted to only Analysis Days in Figure 5.2-47.

Figure 5.2-47: Distribution of salinity (ppt) within the LHR target zone by fixed-location stations for specified water column strata (Analysis Days)



To further investigate the observed salinity spikes in 2023, sampling dates with the 30 highest salinity values recorded anywhere in the target zone were identified for the POR during Analysis Days, and all salinity values for those dates were plotted and identified by the year in which the sample occurred (Figure 5.2-48). The 30 highest salinity values in the target zone occurred over 22 dates, all during drought conditions. Ten of the 22 occurred in 2023, one occurred in 2009, nine occurred in 2008, and one occurred in 2002 and 2001. In particular, the TBW recorder at Sligh Avenue, as well as the USGS CRs at I-275 (No. 023060013) and Hannah's Whirl No. (02304515), had the highest observed values in 2023 which was a particularly dry summer, while the single highest values at USGS Cr at Rowlett (No. 02304510) occurred in 2001. The same effect can be seen in the full timeseries plots of all salinity values for these gauges over all "analysis" days (Figure 5.2-49). Interestingly, the 2023 salinity spikes occurred in the Fall and despite total flows over the dam achieving the LHR minimum flow. All dates in 2023 and 2008 occurred during the months of November and December which is an atypical time of year for implementation to be required under the MFL. This is considered an anomalous event due to antecedent regional hydrological conditions and not controllable by MFL implementation.

Figure 5.2-48: Surface and bottom salinity values for dates where the 30 highest salinity values within the LHR target zone (Analysis Days)

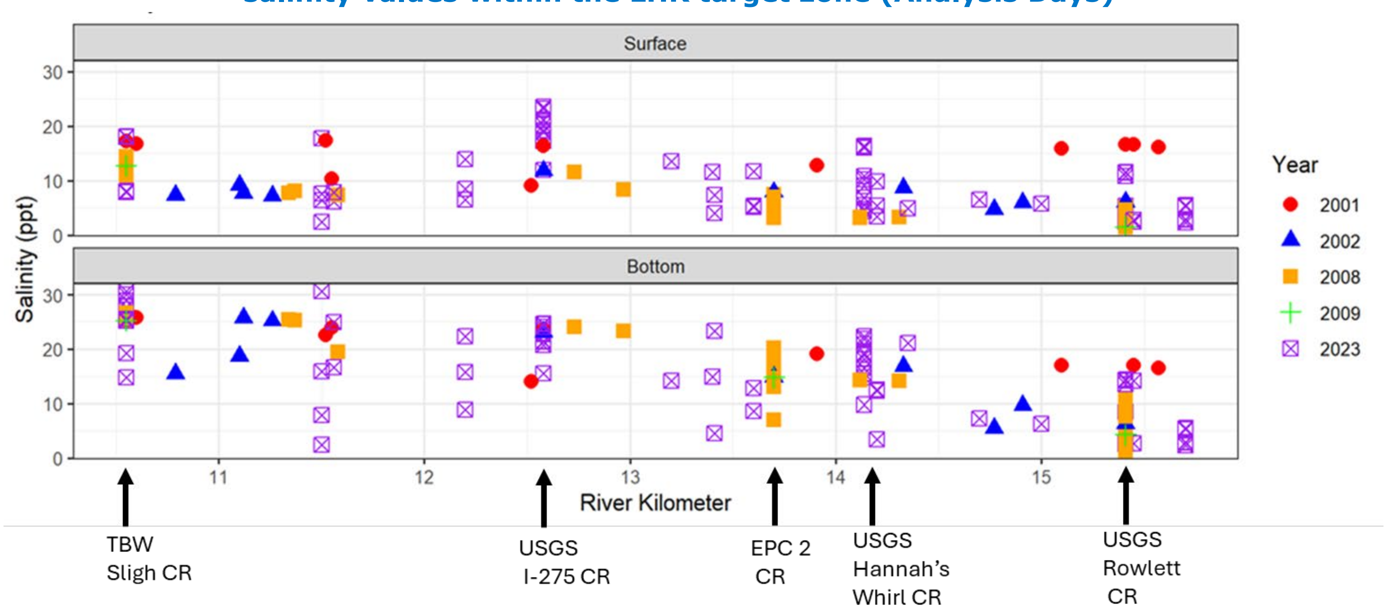
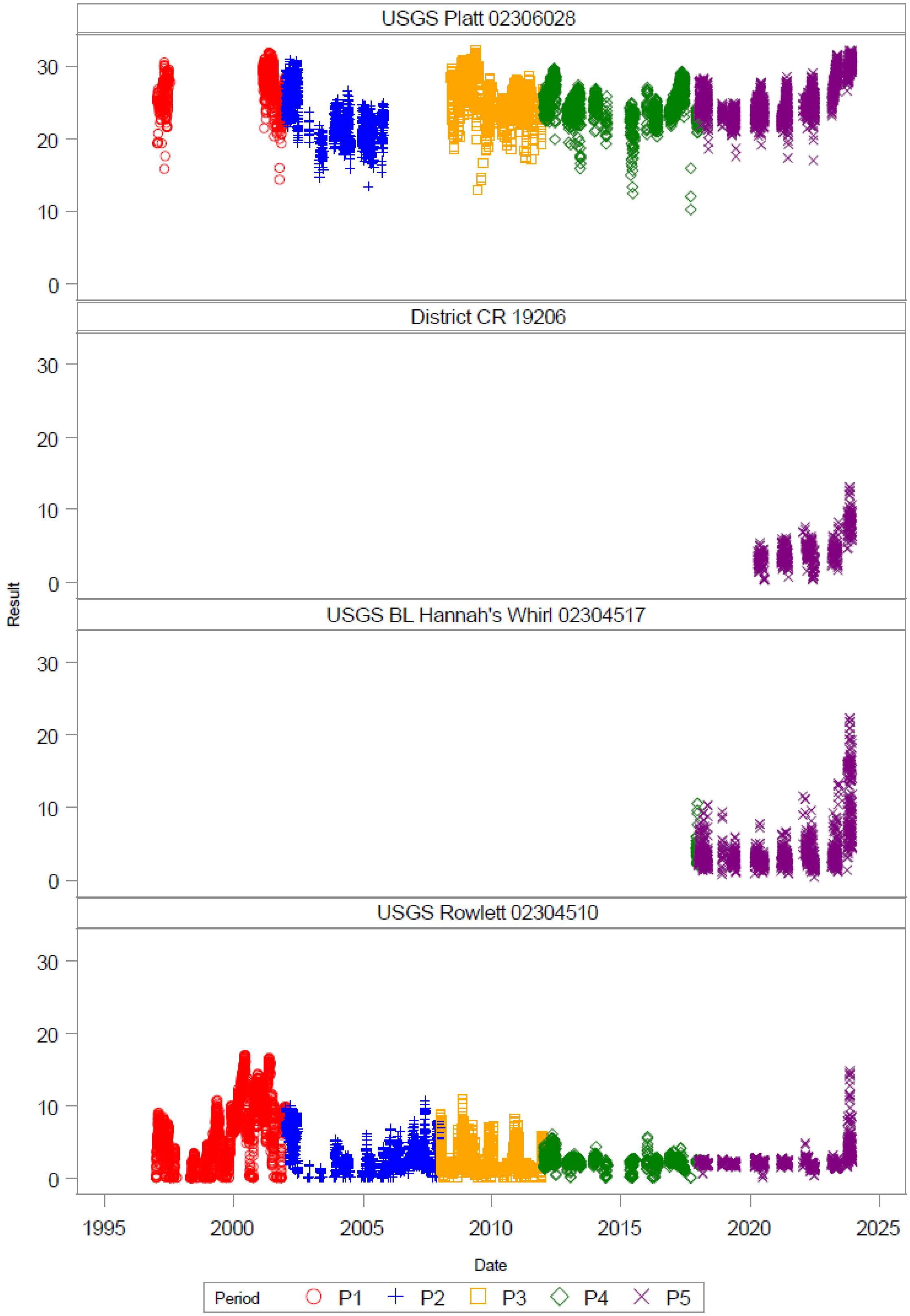


Figure 5.2-49: All salinity values at four principal continuous recorders in the LHR during analysis days



5.2.2.2 LHR Target Zone Dissolved Oxygen

DO measurements are expressed as concentrations as well as percent saturation. The time series of DO concentrations in the target zone is displayed in Figure 5.2-50, and the percent saturation time series is presented in Figure 5.2-51. These figures indicate improving time series trend in the Upper segment of the target zone and significant variability in DO in all target zone segments. Average concentrations were approximately 3 mg/L in the bottom waters and 5 mg/L in surface waters with no trend across periods (Table 5.2-15 and Table 5.2-16) when evaluating the plots across the target zone. Appendix N presents the descriptive statistics breakdowns by target zone river segment, water column stratum, and period The segment-specific boxplots suggest that the Upper segment of the river has seen an increase in DO for all sample levels over the most recent period, and the middle segment has exhibited some more muted improvements as well (Figure 5.2-52, Figure 5.2-53).

Figure 5.2-50: Time series for DO concentration (mg/L) within the LHR target zone for specified water column strata (Analysis Days)

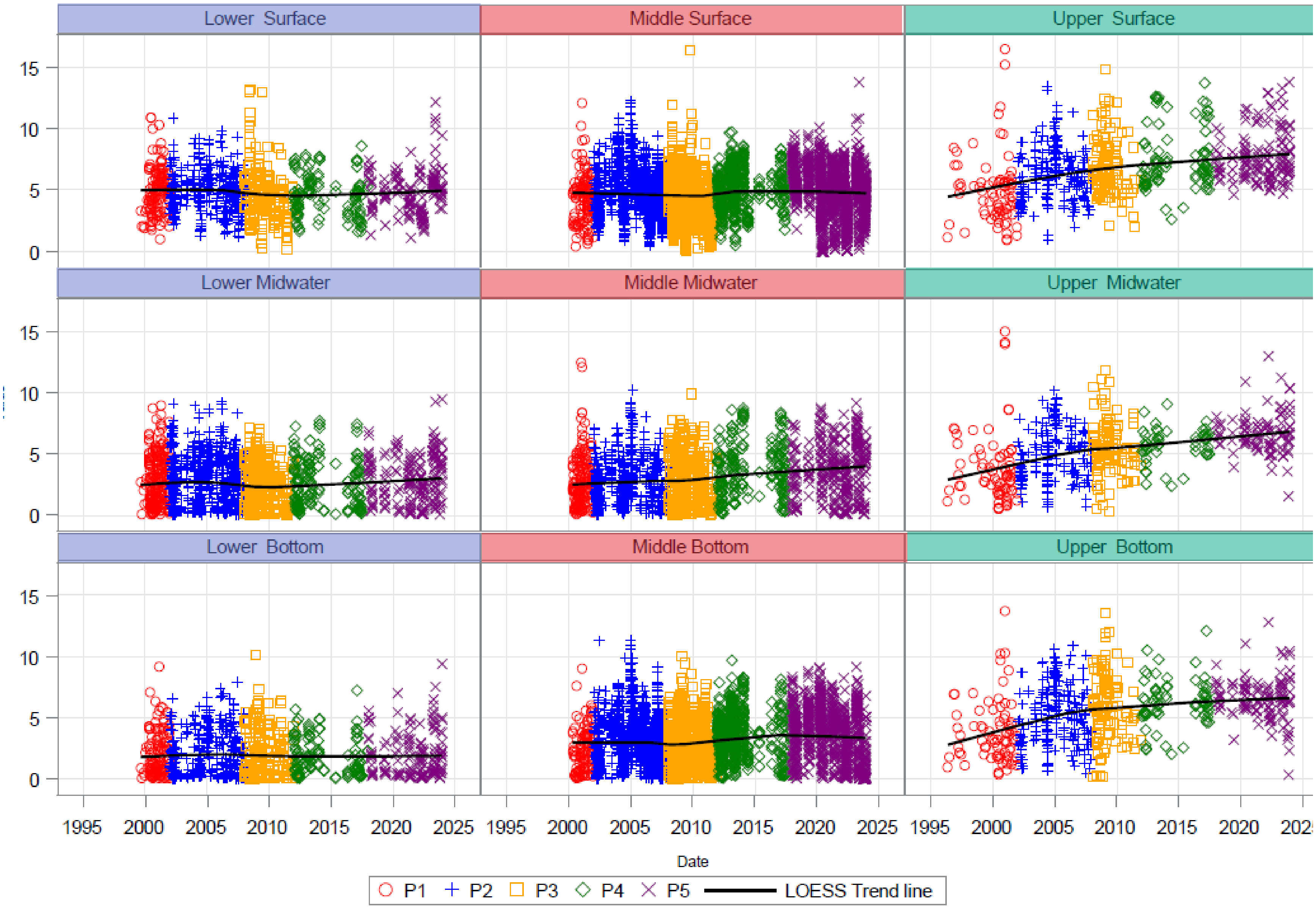


Figure 5.2-51: Time series for DO percent saturation (%) within the LHR target zone for specified water column strata (Analysis Days)

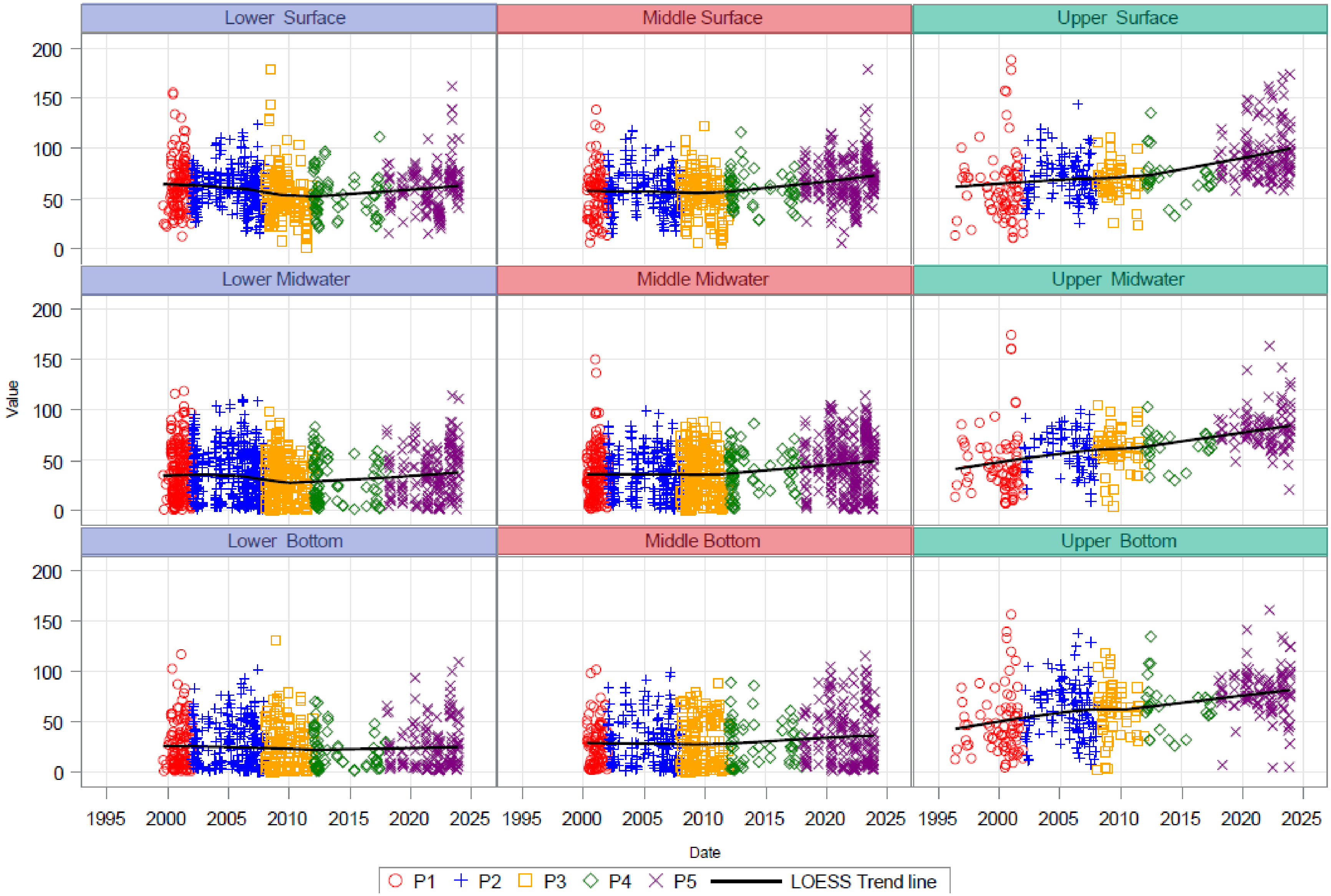


Table 5.2-15: Descriptive statistics for DO concentration (mg/L) within the LHR target zone by period for specified water column strata (Analysis Days)

Type	Level	Period	N	Min	Max	Mean	Std
CR	Surface	P1	37	0.64	6.88	4.03	1.62
		P2	970	0.42	12.25	4.67	1.97
		P3	741	0.08	16.33	3.93	1.80
		P4	377	0.47	8.78	5.03	1.63
		P5	1,157	-0.07	10.10	4.70	2.06
	Bottom	P1	17	2.55	6.02	4.11	1.09
		P2	967	0.26	11.31	3.23	1.88
		P3	767	0.07	7.42	2.66	1.45
		P4	426	0.24	8.26	3.90	1.55
		P5	685	0.00	9.10	3.82	2.18
Fixed	Surface	P1	66	1.10	8.80	4.41	1.73
		P2	396	0.84	13.45	5.21	2.22
		P3	308	0.19	14.79	5.68	2.45
		P4	320	1.58	13.70	5.84	2.23
		P5	451	1.08	13.77	5.89	2.14
	Midwater	P1	65	0.10	7.10	3.00	1.89
		P2	702	0.00	10.19	2.84	2.16
		P3	422	0.06	11.83	2.79	2.38
		P4	357	0.09	9.04	3.69	2.24
		P5	562	0.07	9.32	3.71	2.29
	Bottom	P1	64	0.09	7.00	2.55	1.89
		P2	393	0.00	10.64	3.14	2.57
		P3	301	0.07	13.56	3.15	2.91
		P4	300	0.08	12.08	3.40	2.55
		P5	422	0.08	10.10	3.12	2.59
Random	Surface	P1	237	0.42	16.46	4.90	2.35
		P2	560	1.28	11.30	5.10	1.84
		P3	328	0.09	13.12	4.72	1.90
		P4	52	1.60	10.53	4.93	1.90
		P5	86	2.84	12.88	6.39	2.04
	Midwater	P1	448	0.12	14.99	2.79	2.26
		P2	837	0.04	9.21	2.99	2.08
		P3	640	0.00	9.05	2.71	2.03
		P4	82	0.11	8.41	2.73	2.01
		P5	81	0.08	12.95	5.63	2.49
	Bottom	P1	240	0.11	13.72	2.44	2.19
		P2	583	0.00	10.89	2.73	2.35
		P3	325	0.00	10.11	2.45	2.34
		P4	53	0.09	10.50	2.69	2.59
		P5	86	0.10	12.78	4.95	2.84

Table 5.2-16: Descriptive statistics for DO percent saturation (%) within the LHR target zone by period for specified water column strata (Analysis Days)

Type	Level	Period	N	Min	Max	Mean	Std
Fixed	Surface	P1	66	14.00	112.00	54.56	21.40
		P2	83	15.10	105.50	57.66	18.87
		P3	89	10.00	109.10	52.44	20.10
		P4	105	22.80	116.30	58.80	18.48
		P5	437	5.71	178.70	72.99	27.43
	Midwater	P1	65	1.00	94.00	36.78	22.36
		P2	93	1.00	108.20	50.05	29.32
		P3	113	0.80	156.40	48.82	35.52
		P4	126	1.20	108.10	49.42	27.17
		P5	559	1.10	135.20	48.33	29.68
	Bottom	P1	64	1.00	88.00	31.31	22.57
		P2	83	1.00	94.00	35.13	27.96
		P3	89	1.00	112.00	31.87	25.61
		P4	105	1.00	89.00	33.05	23.60
		P5	408	1.00	130.00	38.22	31.40
Random	Surface	P1	237	6.00	188.00	62.93	30.01
		P2	560	16.00	144.00	62.42	21.60
		P3	328	1.00	179.00	56.83	22.01
		P4	52	21.00	135.50	60.85	22.82
		P5	86	34.70	161.90	80.54	26.21
	Midwater	P1	448	2.00	175.00	36.04	27.97
		P2	837	1.00	111.00	37.41	25.18
		P3	640	0.00	105.00	33.61	24.42
		P4	82	2.00	103.00	34.61	24.68
		P5	81	1.10	163.80	70.63	30.81
	Bottom	P1	240	1.90	157.00	31.88	27.97
		P2	583	0.00	138.00	33.53	27.83
		P3	325	0.00	131.00	29.64	27.28
		P4	53	1.00	134.70	33.34	31.90
		P5	86	1.30	161.30	62.89	35.28

Figure 5.2-52: Distribution of DO concentration (mg/L) within the LHR target zone by period and river segment for specified water column strata (Analysis Days)

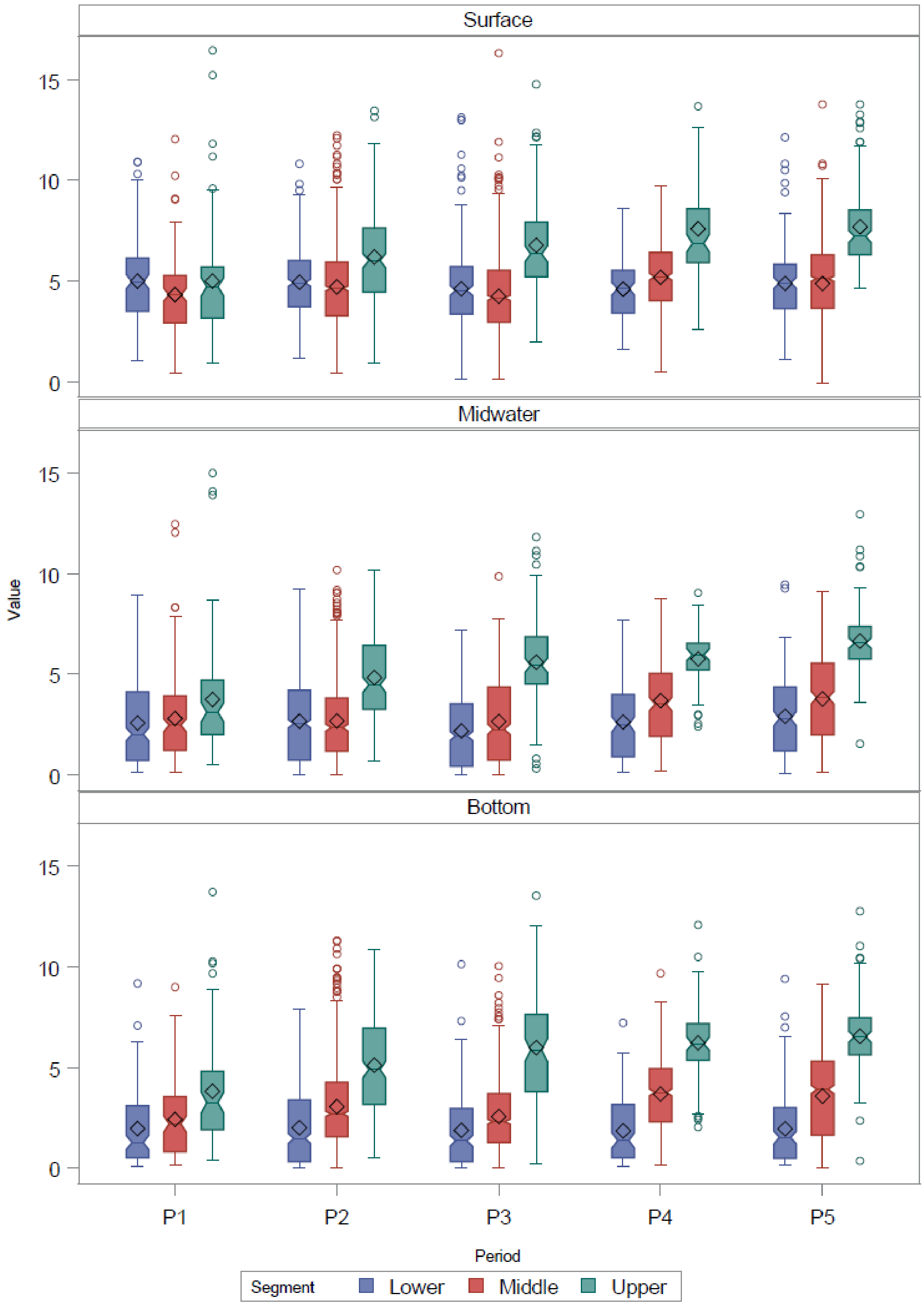
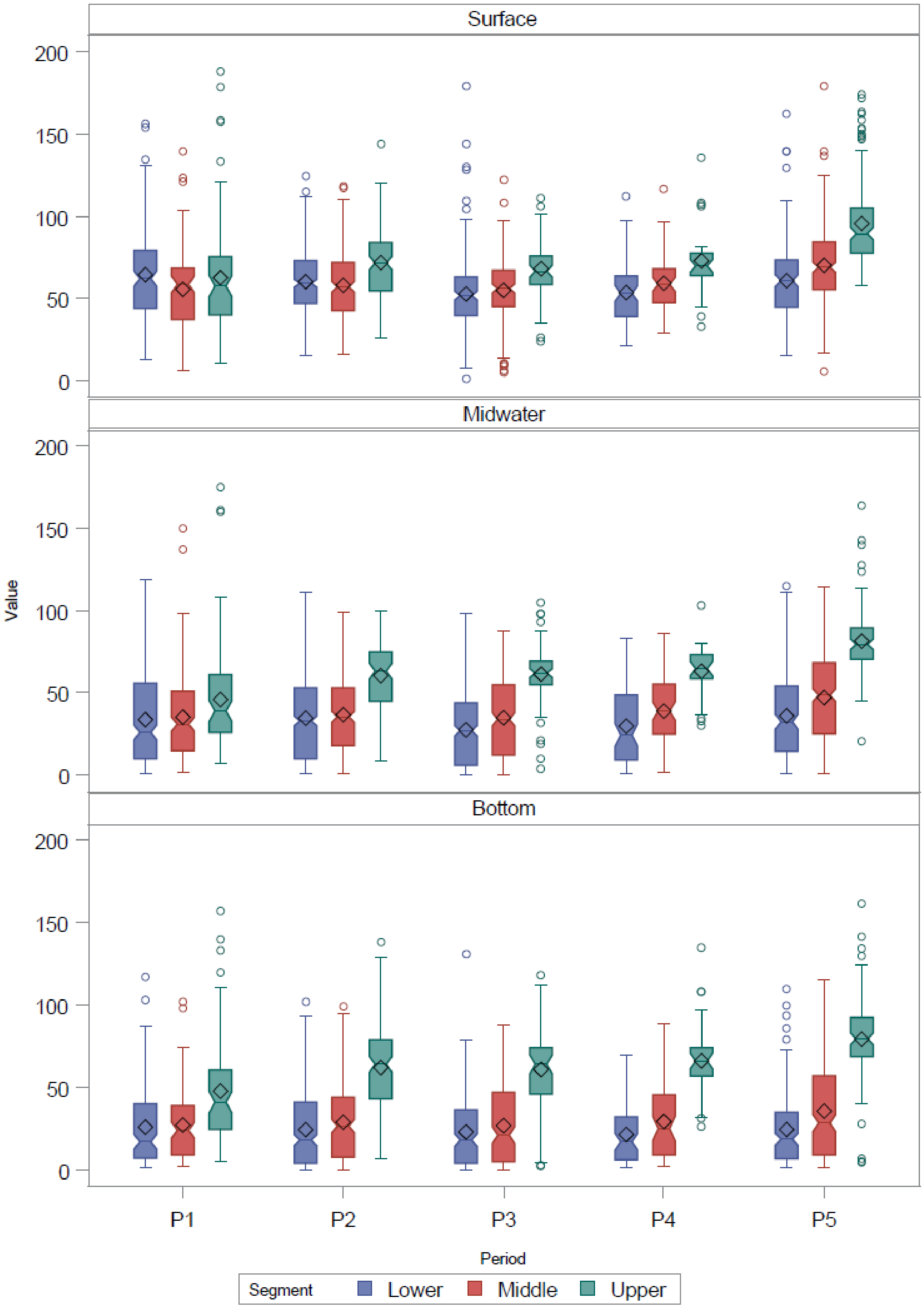


Figure 5.2-53: Distribution of DO saturation (%) within the LHR target zone by period and river segment for specified water column strata (Analysis Days)



The fixed stations that reported DO measurements are shown in Figure 5.2-54 and similar to salinity, a noticeable longitudinal break was observed in the difference between surface and bottom measurements (Figure 5.2-55). Below Rkm 14, the difference between surface and bottom DO concentrations (and DO percent saturation) become more apparent (Figure 5.2-55 and Figure 5.2-56).

Figure 5.2-54: Fixed-location stations within the LHR target zone with DO data

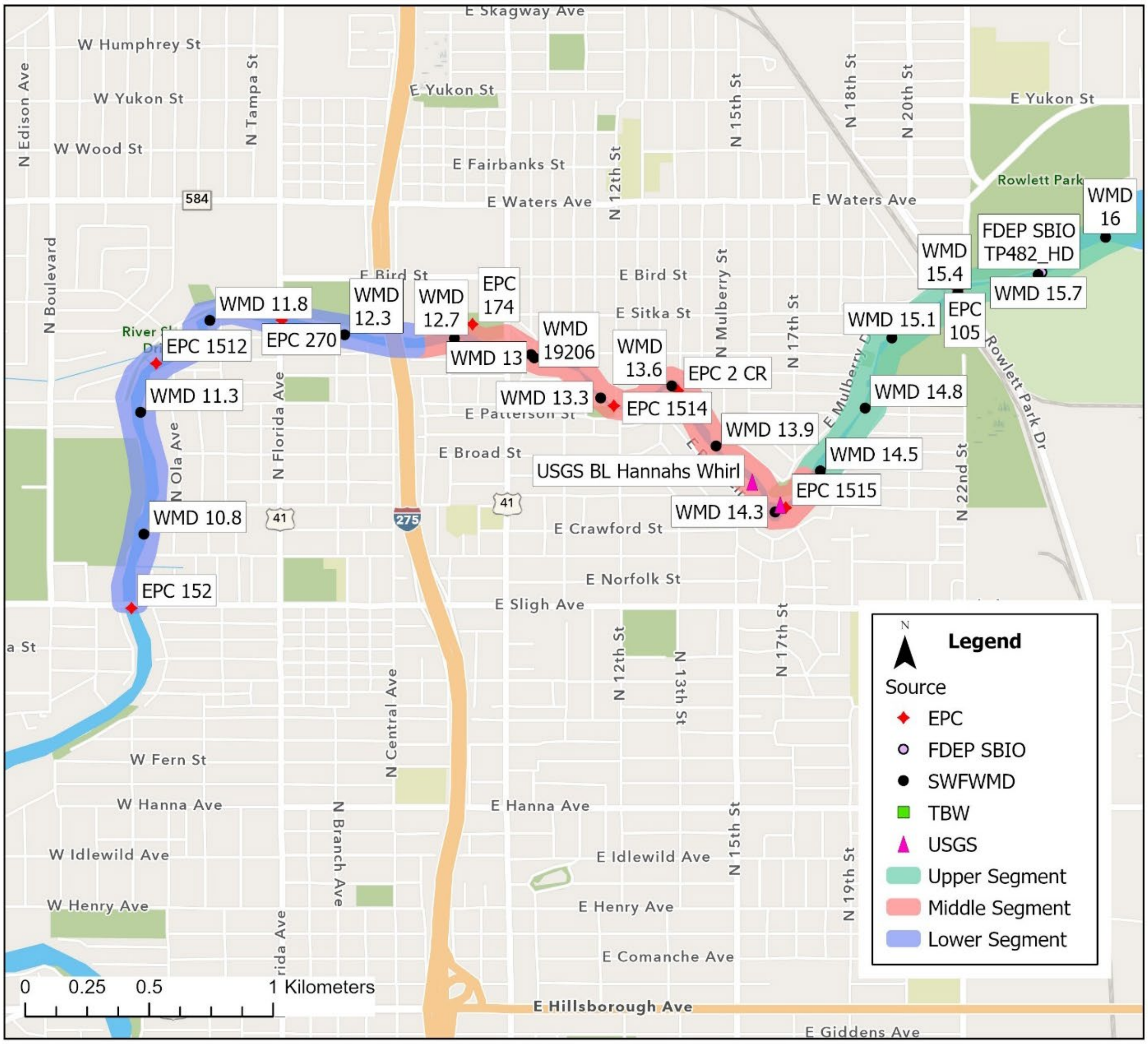


Figure 5.2-55: Distribution for DO concentration (mg/L) within the LHR target zone by fixed-location stations for specified water column strata (Analysis Days)

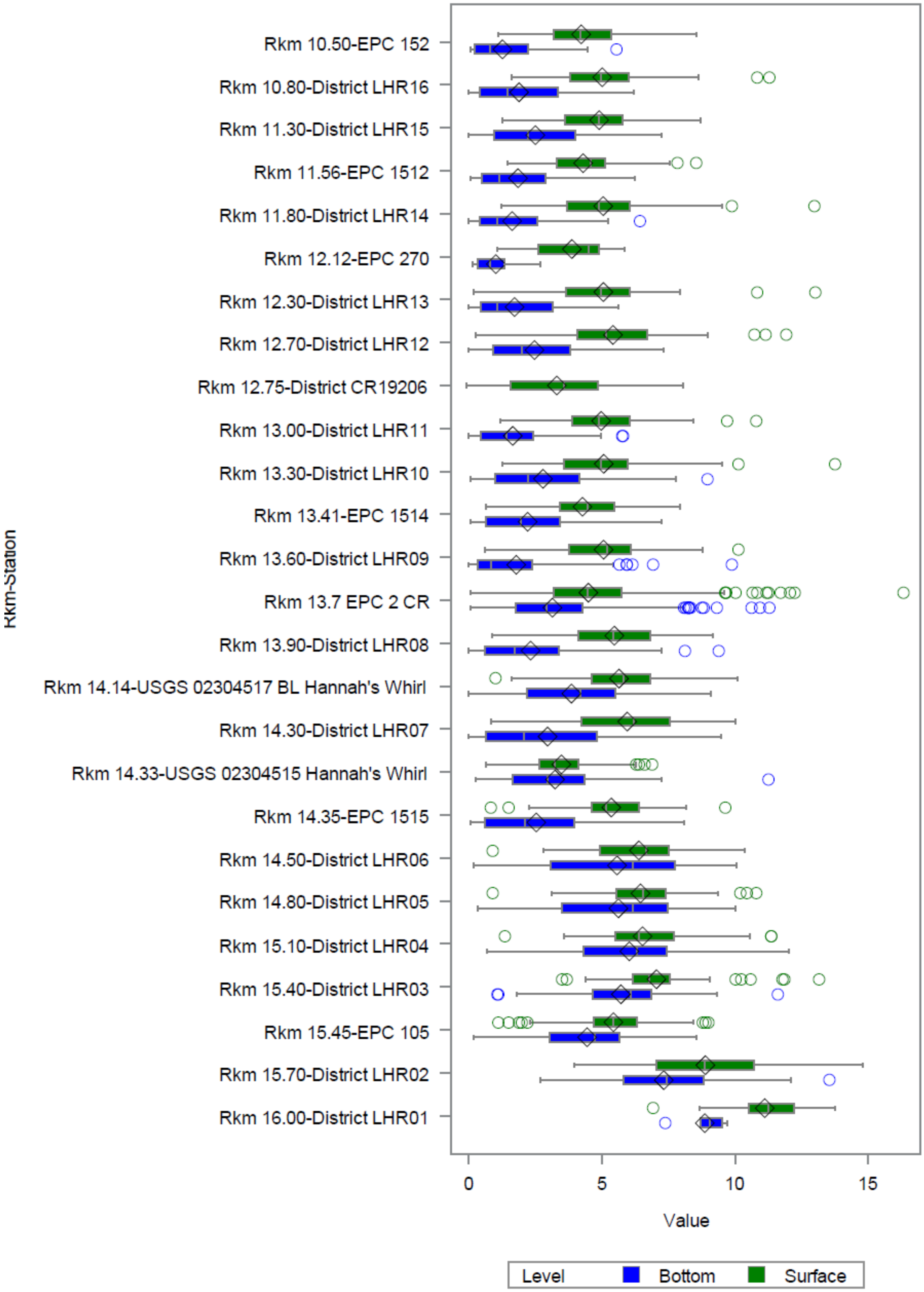
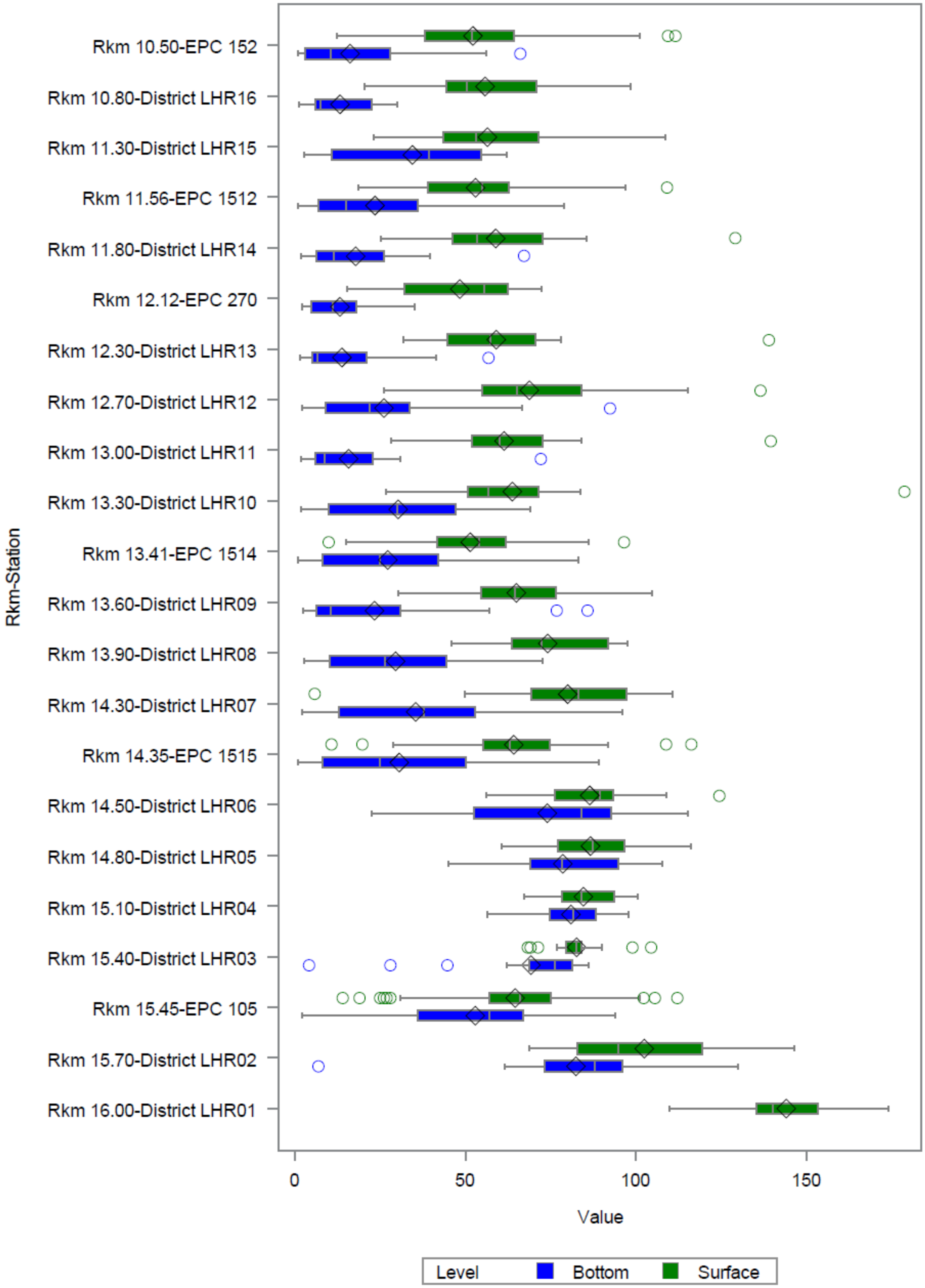


Figure 5.2-56: Distribution of DO percent saturation (%) within the LHR target zone by fixed-location stations for specified water column strata (Analysis Days)



5.2.2.3 LHR Target Zone Water Temperature

The time series of water temperature measurements in the target zone is displayed in Figure 5.2-57. The time series plots indicate that, since 2010, there have been fewer days with water temperatures below 15 degrees Celsius. This could be attributed to natural climate variability or due to additions of Sulphur Spring water making up a higher percentage of the total flow to the LHR. Temperature typically ranged between 15 and 30 degrees Celsius before 2012 and between approximately 13 and 30 degrees Celsius since 2012. Minimum surface values in CR data jumped from between 10–12 degrees to 15–16 degrees after 2011 (Table 5.2-17). Appendix N presents the descriptive statistics breakdowns by target zone river segment, water column stratum, and period . The differences in temperature are more difficult to see in the temperature boxplots (Figure 5.2-58) except for a rather pronounced contraction of the whiskers on the low end of the distribution. This lack of differences among strata may suggest that the observed trend in the minima may be more related to natural variation than a result of implementation of the minimum flows.

Figure 5.2-57: Time series for temperature (degrees C) within the LHR target zone for specified water column strata (Analysis Days)

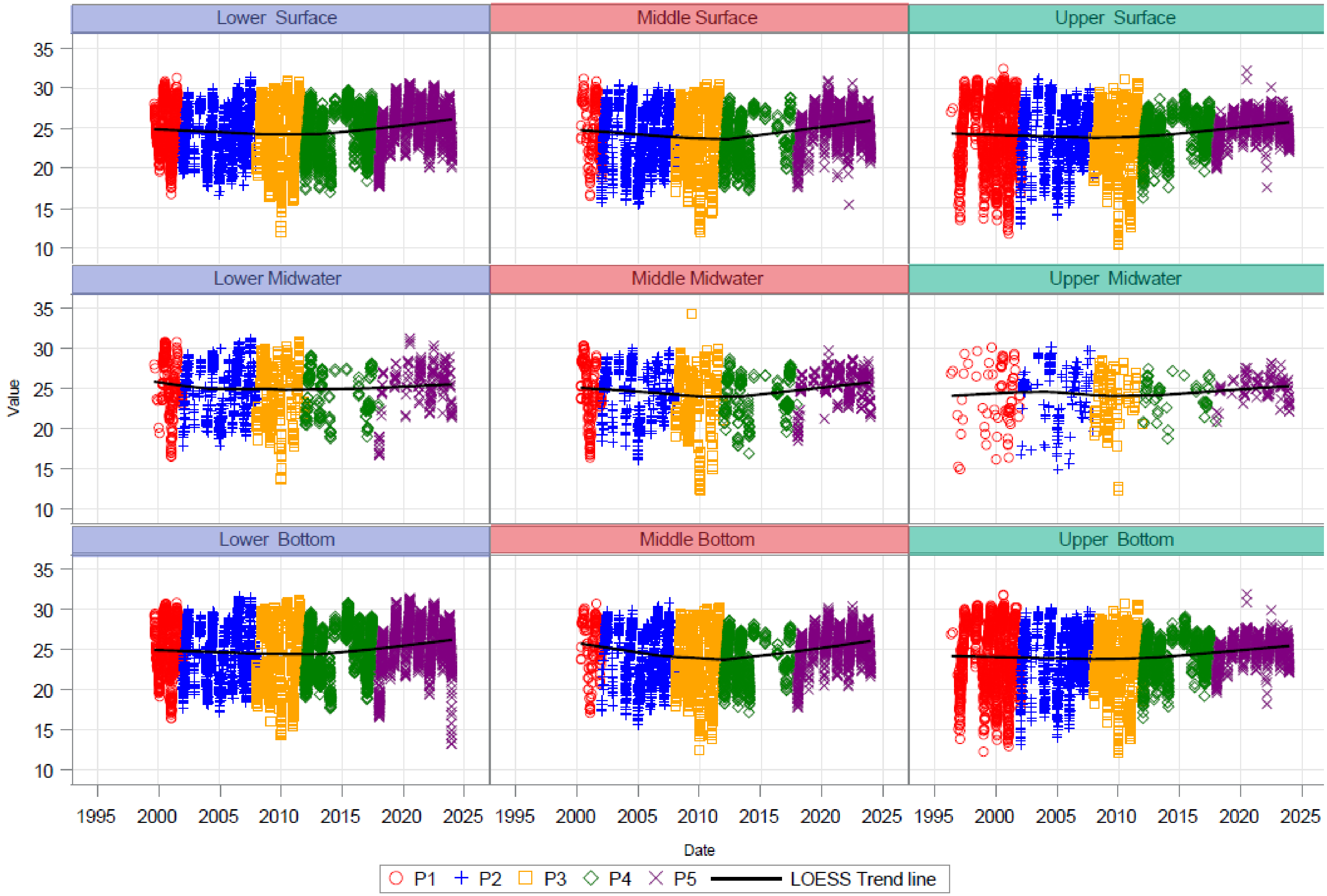
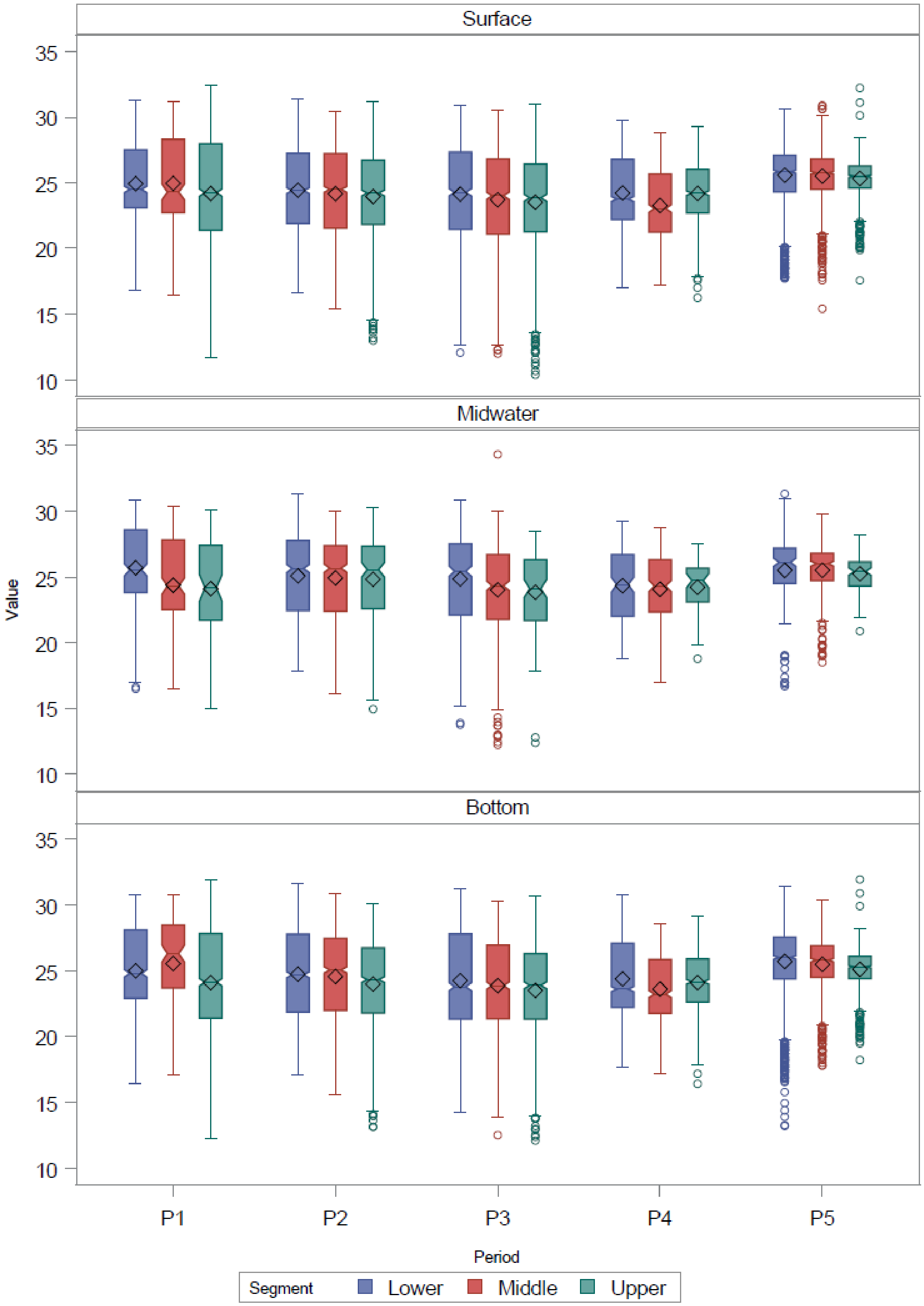


Table 5.2-17: Descriptive statistics for water temperature (degrees C) within the LHR target zone by period for specified water column strata (Analysis Days)

Type	Level	Period	N	Min	Max	Mean	Std
CR	Surface	P1	2,055	11.76	31.39	24.47	3.79
		P2	3,555	12.97	31.38	24.13	3.35
		P3	2,381	10.45	31.04	23.90	3.64
		P4	2,570	16.29	29.75	24.06	2.58
		P5	3,158	15.46	32.20	25.51	2.12
	Bottom	P1	1,988	12.23	30.76	24.43	3.80
		P2	3,348	13.13	31.61	24.41	3.41
		P3	2,426	12.06	31.24	23.92	3.64
		P4	2,593	16.44	30.79	24.17	2.62
		P5	2,717	13.21	31.90	25.48	2.36
Fixed	Surface	P1	68	13.80	30.70	23.94	4.02
		P2	402	15.03	31.19	24.52	3.63
		P3	313	11.34	29.59	22.98	3.84
		P4	335	17.24	29.01	23.80	2.77
		P5	451	19.19	30.55	25.47	1.82
	Midwater	P1	68	15.00	30.80	24.14	3.96
		P2	708	14.93	30.47	25.13	3.37
		P3	428	12.36	34.33	23.52	3.64
		P4	372	16.93	28.73	23.97	2.60
		P5	561	16.70	31.29	25.48	2.17
	Bottom	P1	68	15.10	30.80	24.21	4.02
		P2	399	14.96	30.95	24.54	3.66
		P3	308	12.49	30.11	23.29	3.67
		P4	315	17.15	28.32	23.83	2.60
		P5	422	16.60	31.41	25.58	2.18
Random	Surface	P1	237	16.50	32.40	25.10	3.40
		P2	560	16.69	30.99	24.44	3.10
		P3	328	12.05	29.84	23.86	3.33
		P4	52	17.58	29.03	24.66	2.62
		P5	86	20.10	28.60	25.35	2.31
	Midwater	P1	448	16.47	30.82	25.12	3.52
		P2	837	16.89	31.29	24.84	3.10
		P3	640	12.26	30.86	24.90	3.25
		P4	82	20.53	29.18	25.12	2.53
		P5	81	21.44	28.50	25.28	2.22
	Bottom	P1	240	16.46	31.89	25.24	3.55
		P2	583	17.20	30.94	24.65	3.22
		P3	326	15.43	30.85	24.24	3.50
		P4	53	21.18	28.66	24.78	2.21
		P5	86	21.48	28.28	25.45	2.15

Figure 5.2-58: Distribution for temperature (degrees C) within the LHR target zone by period and river segment for specified water column strata (Analysis Days)



The distribution of stations reporting temperature data in the target zone is provided in Figure 5.2-59. No longitudinal trend nor vertical stratification of temperature was apparent throughout the target zone (Figure 5.2-60).

Figure 5.2-59: Fixed-location stations within the LHR target zone with water temperature data

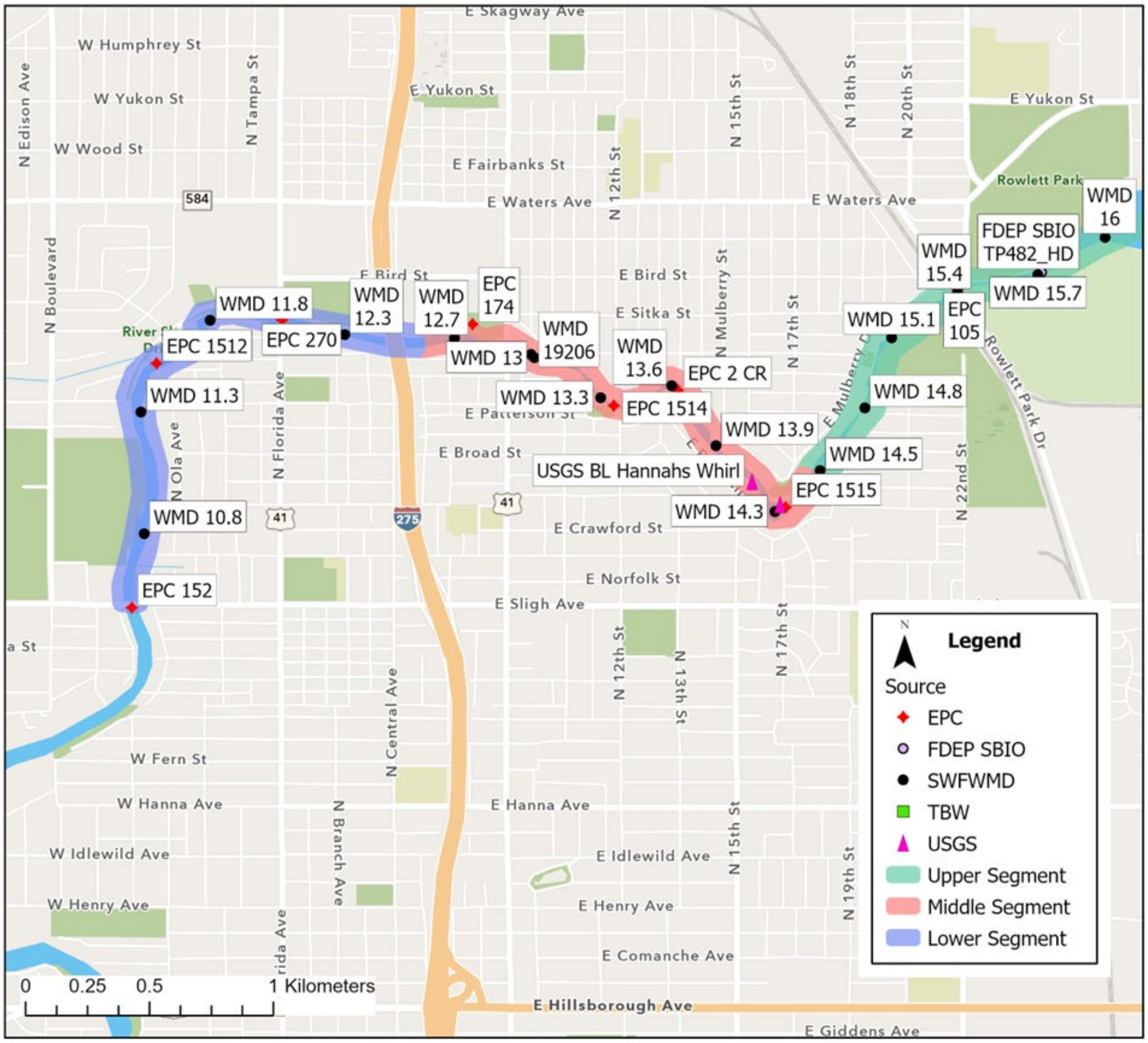
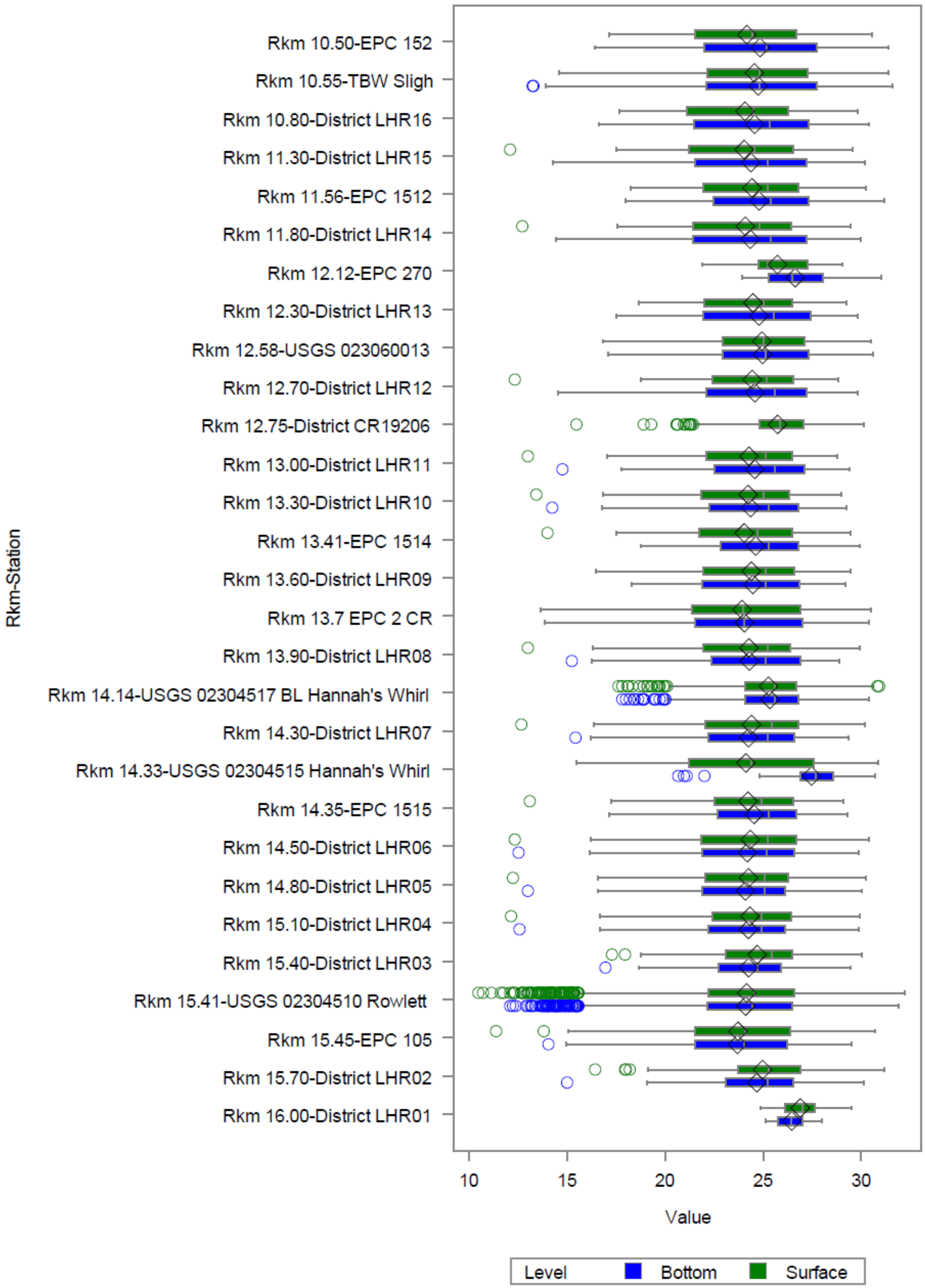


Figure 5.2-60: Distribution for water temperature (degrees C) within the LHR target zone by fixed-location stations for specified water column strata (Analysis Days)



5.2.2.4 LHR Target Zone pH

The time series of pH measurements in the target zone is displayed in Figure 5.2-61. The plots do not show any discernable trend. Summary statistics are presented in Table 5.2-18 for each period and sampling type. Appendix N presents the descriptive statistics breakdowns by target zone river segment, water column stratum, and period. pH values tended to be higher in the upper segment of the target zone relative to the lower and middle strata as seen in the strata boxplots (Figure 5.2-62).

Figure 5.2-61: Time series for pH (SU) within the LHR target zone for specified water column strata (Analysis Days)

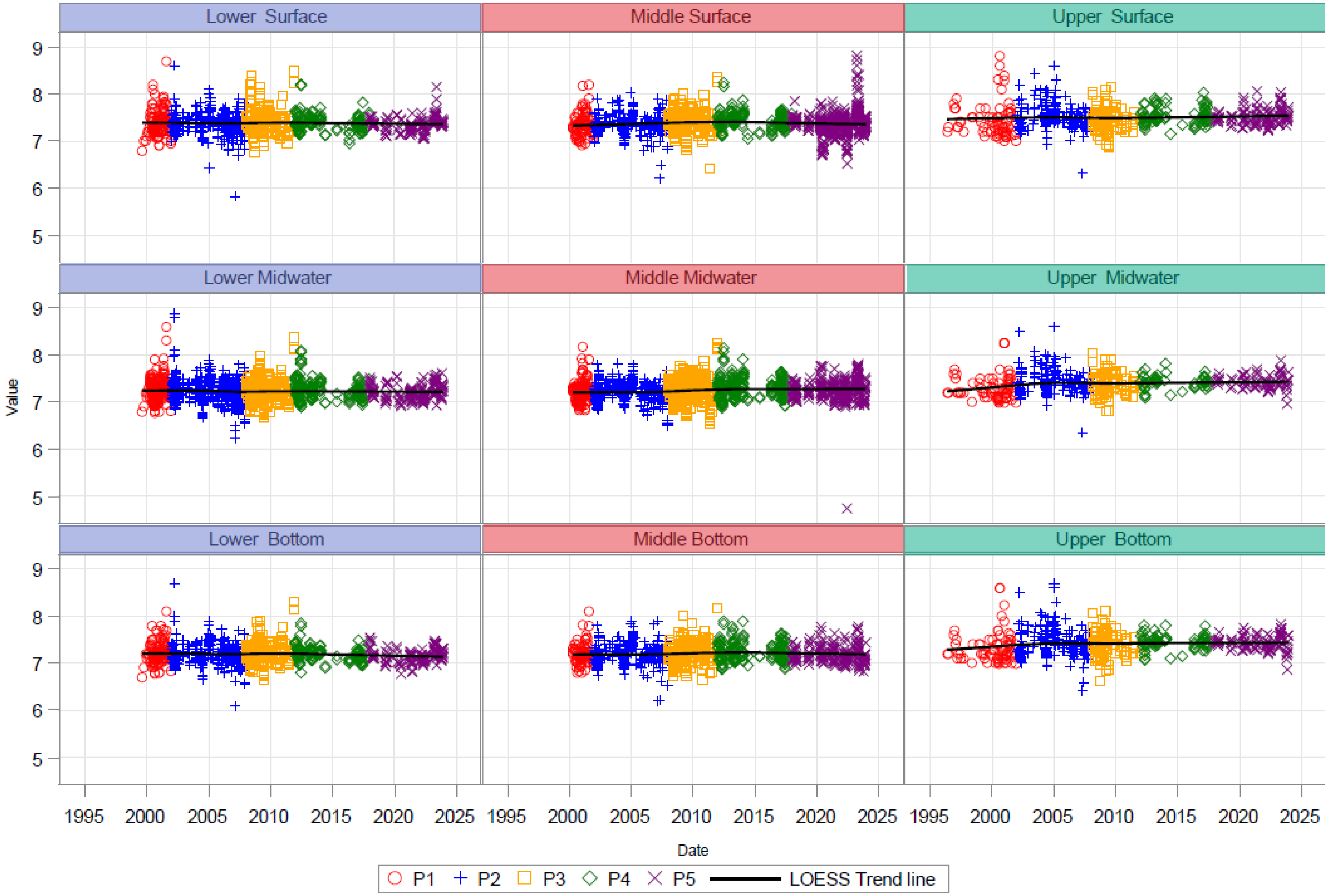
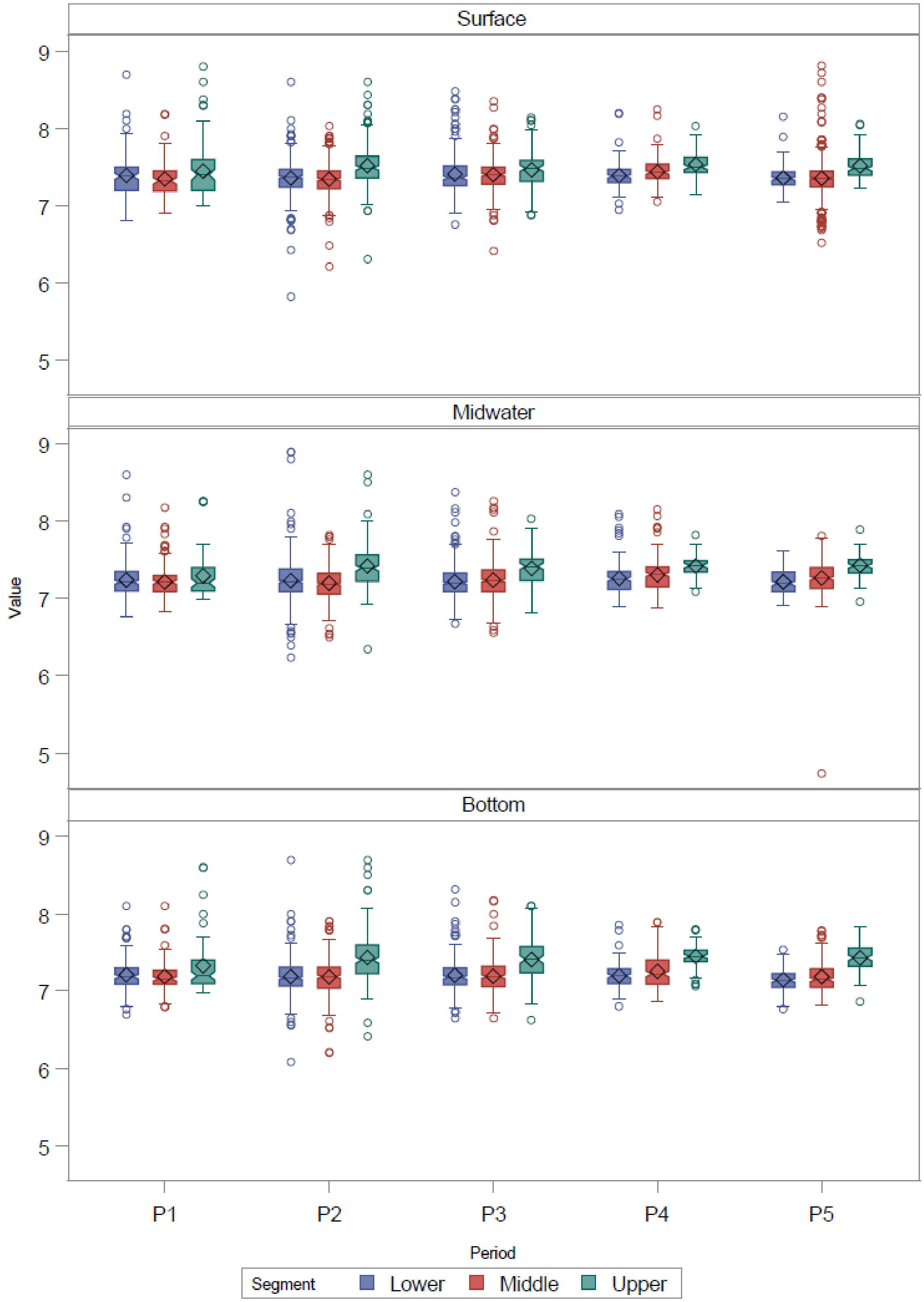


Table 5.2-18: Descriptive statistics for pH (SU) within the LHR target zone by period for specified water column strata (Analysis Days)

Type	Level	Period	N	Min	Max	Mean	Std
CR	Surface	P5	461	6.52	8.81	7.32	0.23
Fixed	Surface	P1	68	6.80	7.90	7.33	0.23
		P2	402	6.43	8.43	7.40	0.22
		P3	294	6.81	8.25	7.39	0.21
		P4	330	6.95	8.03	7.44	0.17
		P5	444	7.02	8.40	7.43	0.18
	Midwater	P1	68	6.80	7.70	7.23	0.20
		P2	708	6.67	8.09	7.25	0.23
		P3	402	6.67	8.03	7.22	0.20
		P4	367	6.88	7.91	7.28	0.18
		P5	554	4.74	7.89	7.27	0.22
	Bottom	P1	68	6.70	7.70	7.18	0.19
		P2	399	6.65	8.02	7.26	0.25
		P3	281	6.65	8.07	7.20	0.23
		P4	310	6.87	7.89	7.27	0.19
		P5	414	6.77	7.83	7.23	0.20
Random	Surface	P1	226	6.90	8.80	7.41	0.30
		P2	523	5.82	8.60	7.38	0.26
		P3	326	6.42	8.48	7.44	0.26
		P4	52	7.20	8.24	7.49	0.25
	Midwater	P1	442	6.77	8.60	7.24	0.23
		P2	837	6.24	8.89	7.23	0.24
		P3	634	6.56	8.37	7.26	0.23
		P4	82	6.90	8.15	7.40	0.28
	Bottom	P1	229	6.77	8.60	7.25	0.27
		P2	546	6.09	8.69	7.23	0.28
		P3	324	6.63	8.31	7.27	0.25
		P4	53	6.80	7.89	7.34	0.23

Figure 5.2-62: Distribution of pH (SU) within the LHR target zone by period and river segment for specified water column strata (Analysis Days)



The distribution of stations reporting pH data is provided in Figure 5.2-63. A noticeable vertical stratification trend was observed downstream of river kilometer 14 where the distribution of pH values was typically lower than surface values Figure 5.2-64.

Figure 5.2-63: Fixed-location stations within the LHR target zone with pH data

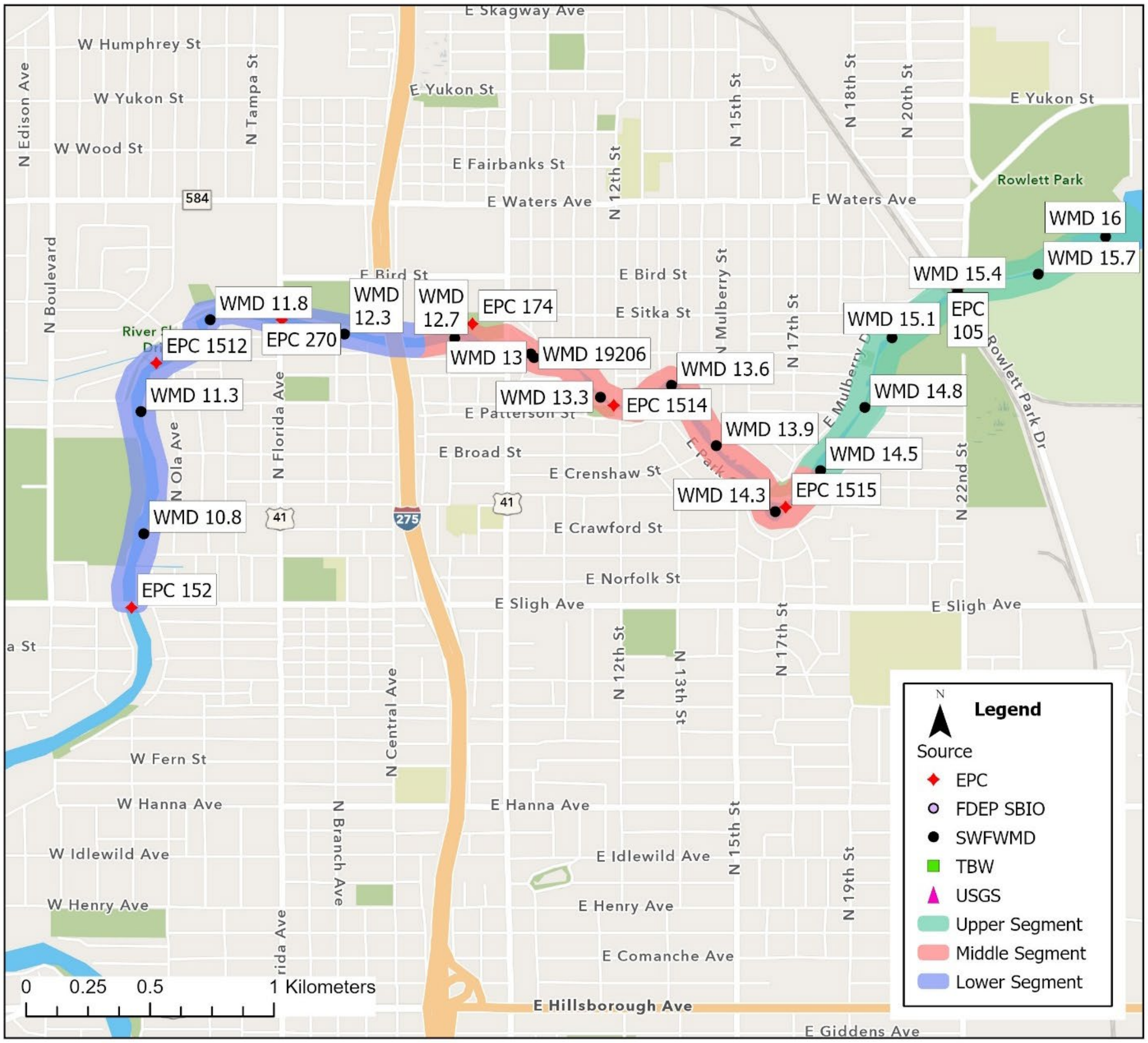
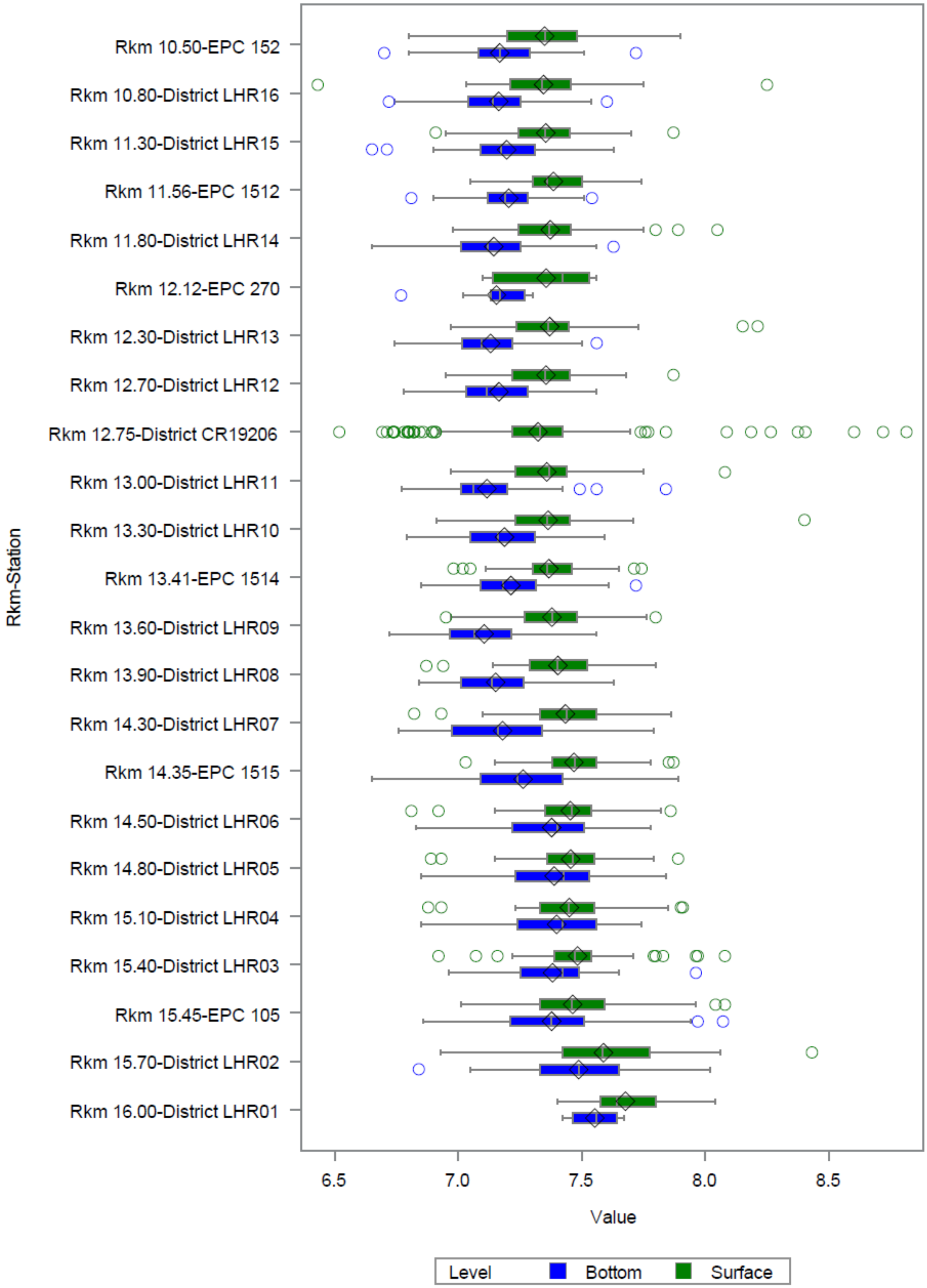


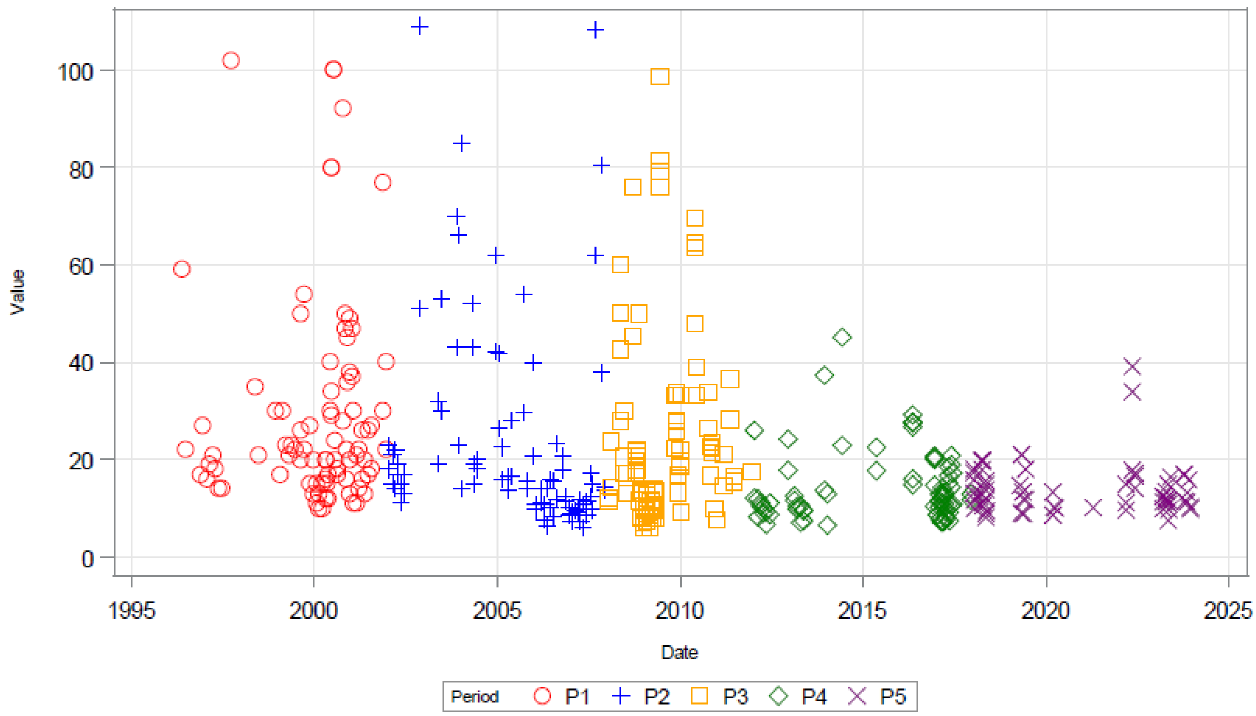
Figure 5.2-64: Distribution for pH (SU) within the LHR target zone by fixed-location stations for specified water column strata (Analysis Days)



5.2.2.5 LHR Target Zone Color

The time series of surface color values in the target zone is displayed in Figure 5.2-65 and suggests much higher values prior to 2011 than since 2011 when larger percentages of the water to the lower river for implementation was from Sulphur Springs.

Figure 5.2-65: Time series of surface color (PCU) within the LHR target zone (Analysis Days)

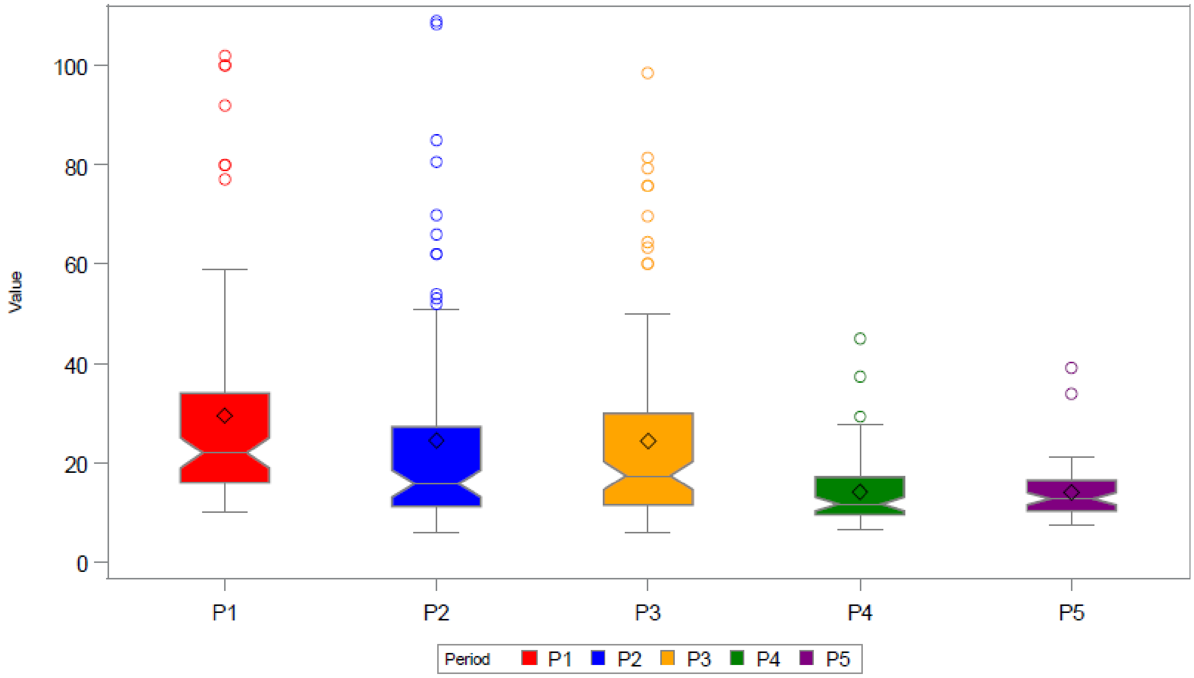


Summary statistics are presented in Table 5.2-19 for each period and sampling type. The first two periods demonstrated relatively elevated color concentrations, whereas more recent data show average color concentrations below 20 PCU (Figure 5.2-66). Random sampling data is limited to the period before 2002 with no current data to help illuminate existing trends in color in the target zone.

Table 5.2-19: Descriptive statistics for color (PCU) within the LHR target zone (Analysis Days)

Type	Level	Period	N	Min	Max	Mean	Std
Fixed	Surface	P1	70	10.00	102.00	24.54	17.05
		P2	99	0.50	109.00	22.72	21.12
		P3	88	6.00	109.00	24.51	21.71
		P4	106	5.90	98.60	24.40	19.15
		P5	79	6.50	45.10	14.11	7.11
Random	Surface	P1	16	20.00	100.00	51.19	25.34

Figure 5.2-66: Distribution for surface color (PCU) within the LHR target zone by period (Analysis Days)



Color was reported primarily for EPC and District sites (Figure 5.2-67), though some random sampling efforts associated with the TBW HBMP reported values between 2000 and 2012 but were sporadic and ended in 2012 and thus are not presented in Figure 5.2-67. The distribution of values collected at each station is provided in Figure 5.2-68 and suggests that the higher values upstream are a combination of water over the dam and measurements taken further back in time.

Figure 5.2-67: Fixed-location stations within the LHR target zone with color data

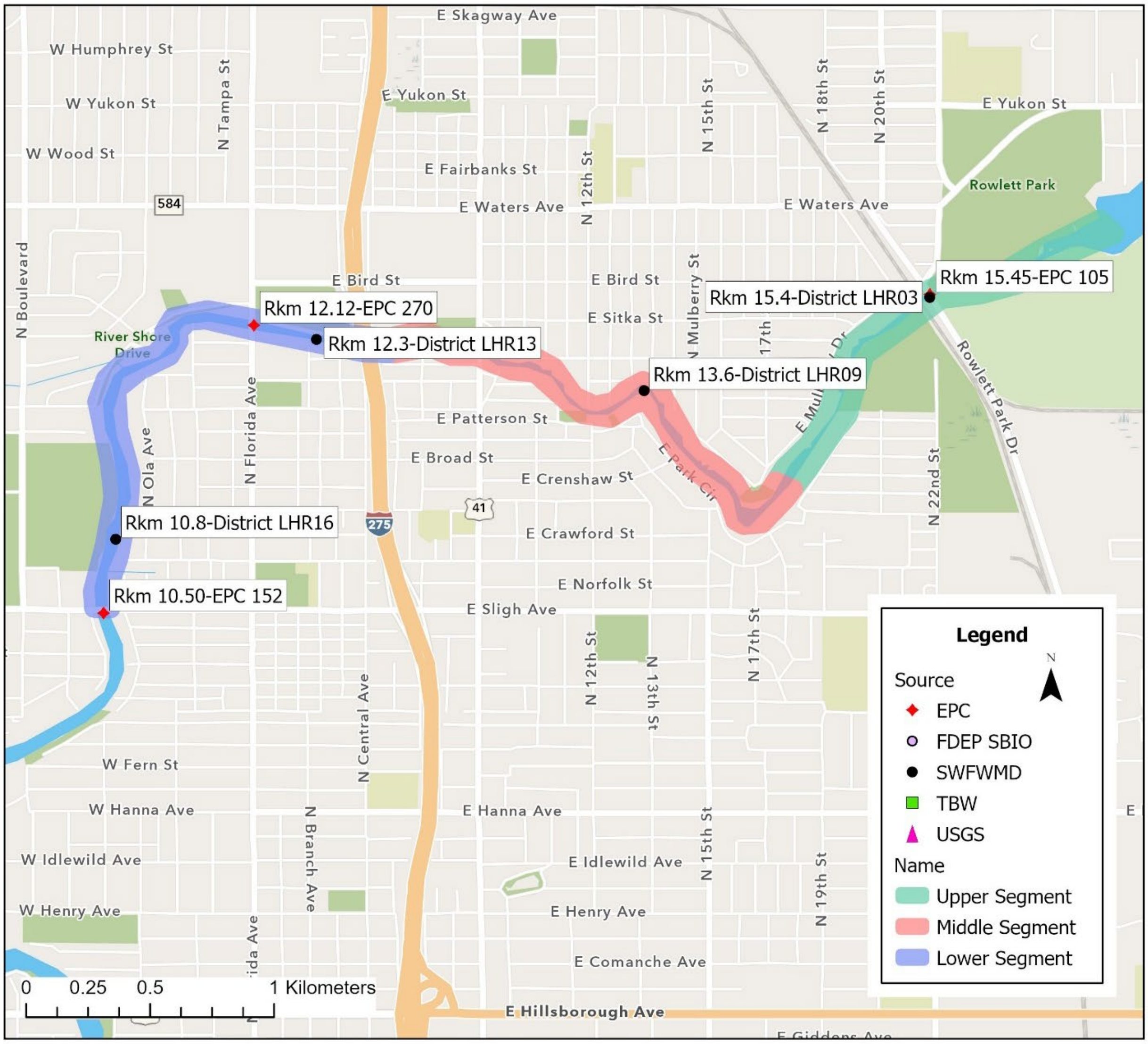
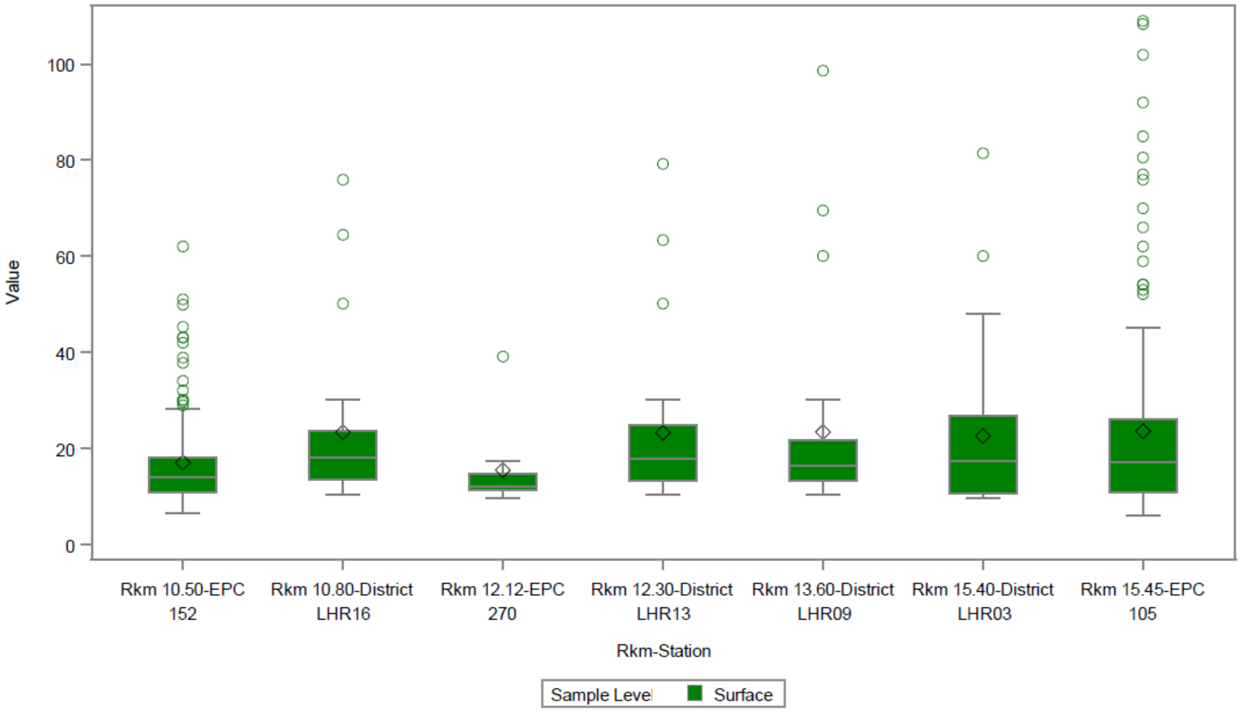


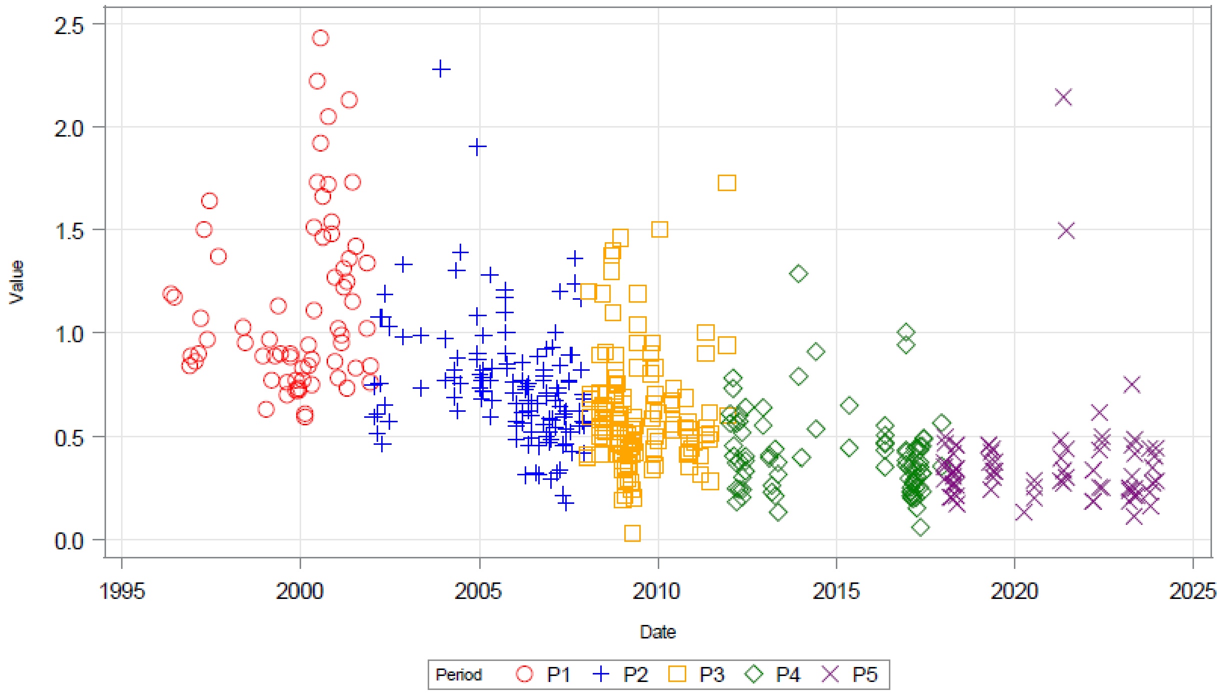
Figure 5.2-68: Distribution for surface color (PCU) within the LHR target zone by fixed-location stations (Analysis Days)



5.2.2.6 LHR Target Zone Total Nitrogen

The time series of surface total nitrogen values in the target zone is displayed in Figure 5.2-69 and suggests a substantial decrease in concentration over time, with a stabilization of values after 2010.

Figure 5.2-69: Time series for surface total nitrogen (mg/L) within the LHR target zone (Analysis Days)

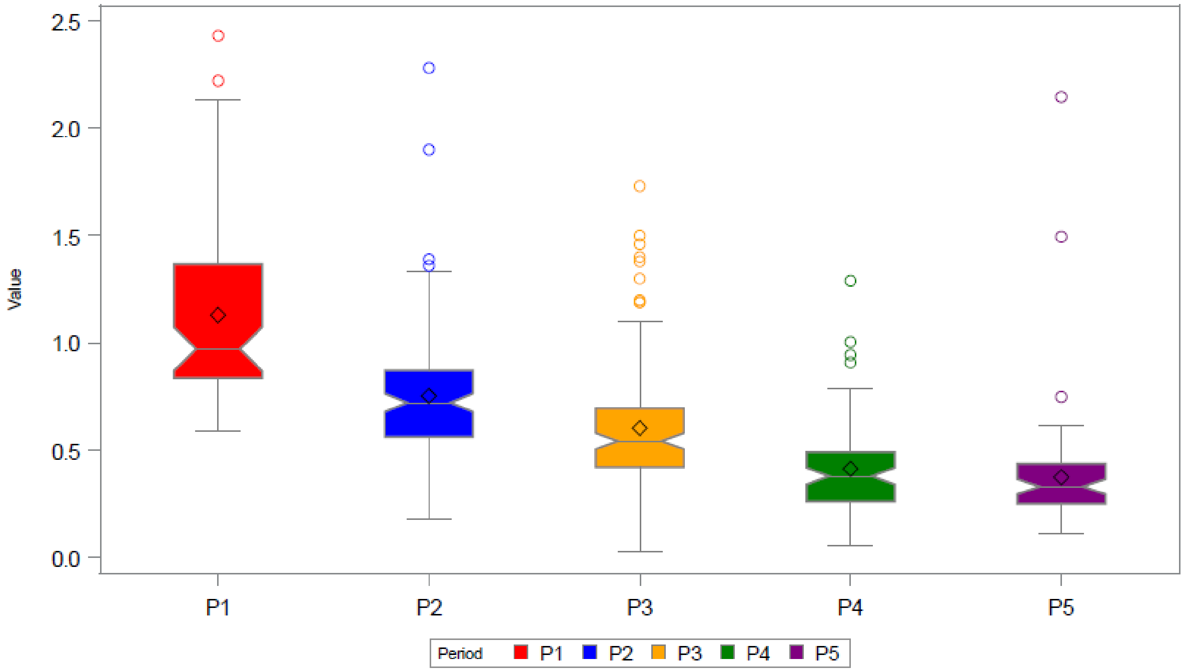


Summary statistics are presented in Table 5.2-20 for each period and sampling type; the data distributions by period are also illustrated in Figure 5.2-70. Total nitrogen decreases from averages before 2002 over 1 mg/L to less than 1 mg/l before stabilizing during the last decade with an average of 0.5 mg/L.

Table 5.2-20: Descriptive statistics for total nitrogen (mg/L) within the LHR target zone by period (Analysis Days)

Type	Level	Period	N	Min	Max	Mean	Std
Fixed	Surface	P1	68	0.59	2.43	1.13	0.42
		P2	92	0.13	2.28	0.71	0.33
		P3	81	0.17	2.28	0.76	0.32
		P4	100	0.19	1.38	0.56	0.22
		P5	77	0.06	1.29	0.40	0.20
Random	Surface	P2	52	0.31	1.90	0.74	0.27
		P3	37	0.03	1.73	0.72	0.39
		P4	10	0.18	0.78	0.50	0.21

Figure 5.2-70: Distribution for surface total nitrogen (mg/L) within the LHR target zone by period (Analysis Days)



The spatial distribution of fixed stations reporting total nitrogen concentrations in the LHR is provided in Figure 5.2-71, though some random sampling efforts associated with the TBW HBMP reported values between 2000 and 2012 (not shown). Spatial distributions of TN data are presented in Figure 5.2-72. Two EPC stations, 142 and 105, which bookend the longitudinal distribution of sampling, tended to have both the highest averages and the highest variability.

Figure 5.2-71: Fixed-location stations within the LHR target zone with total nitrogen data

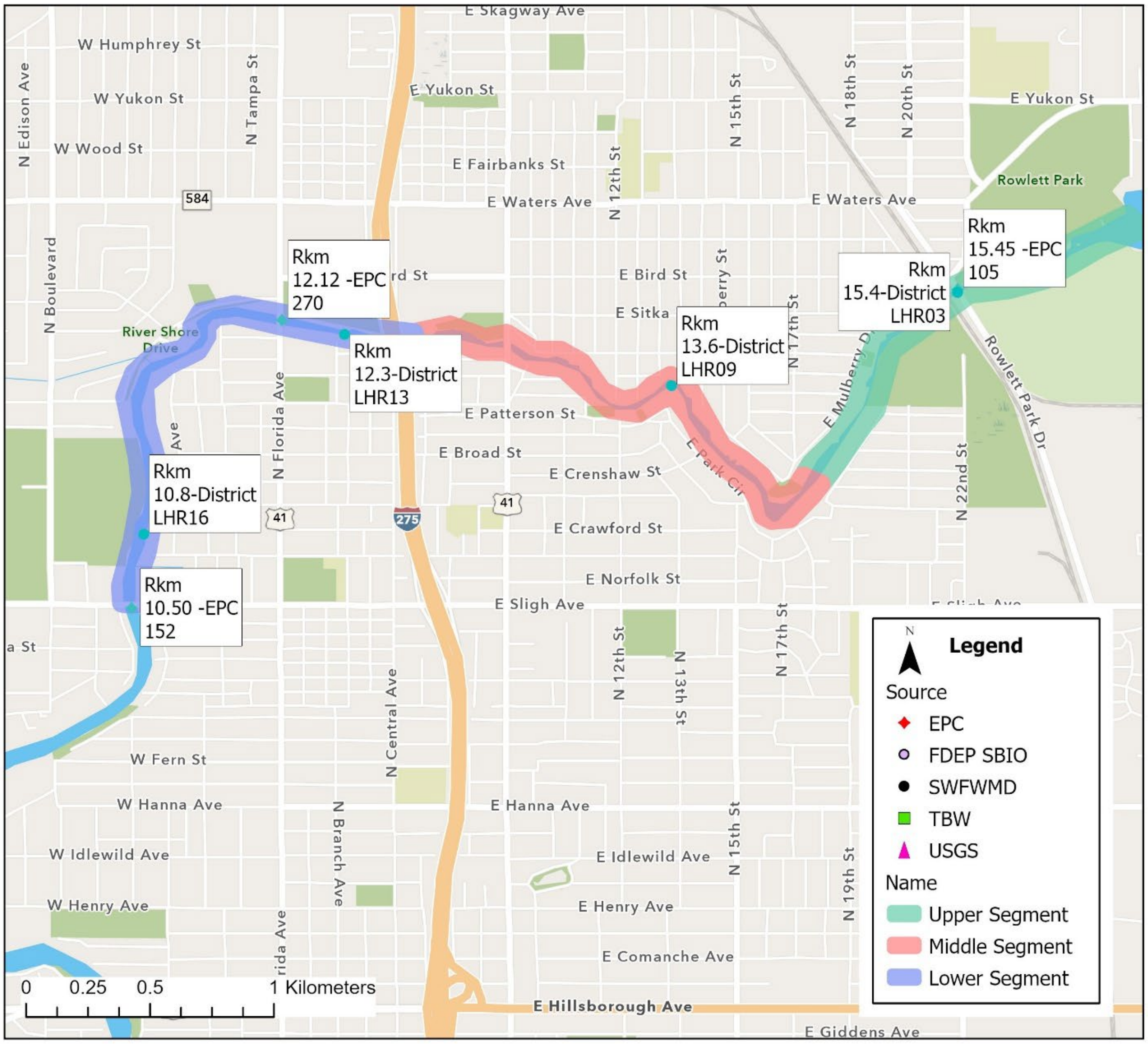
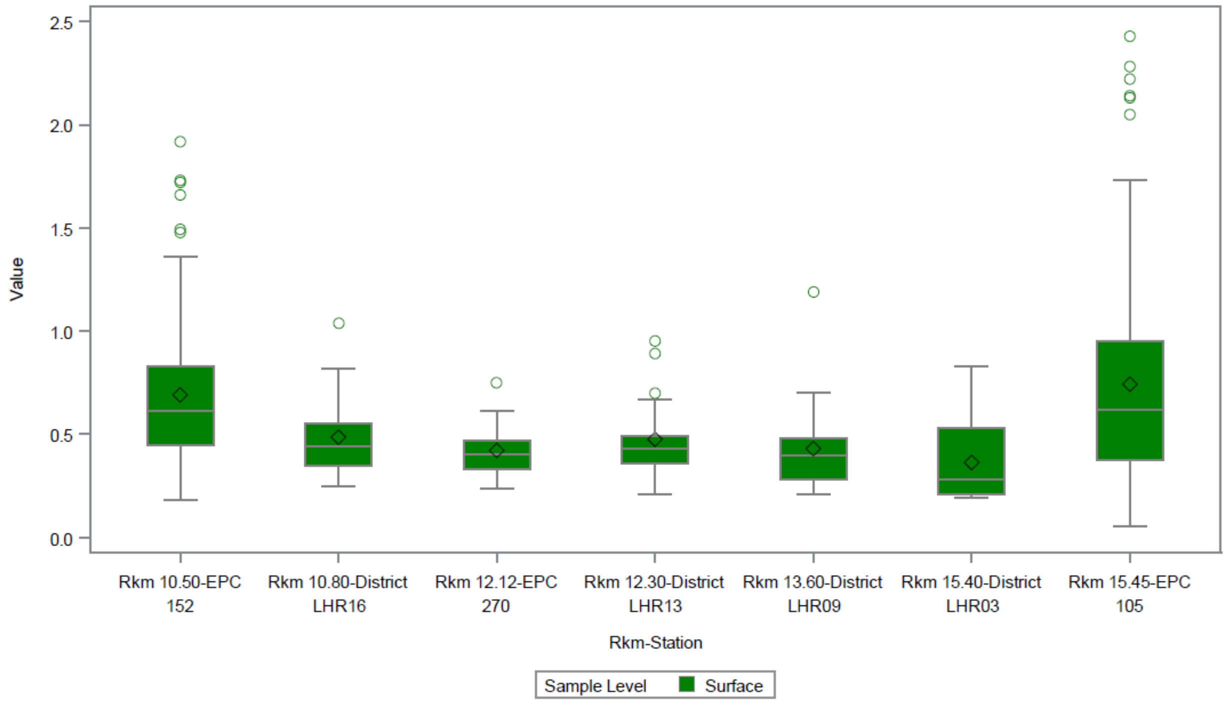


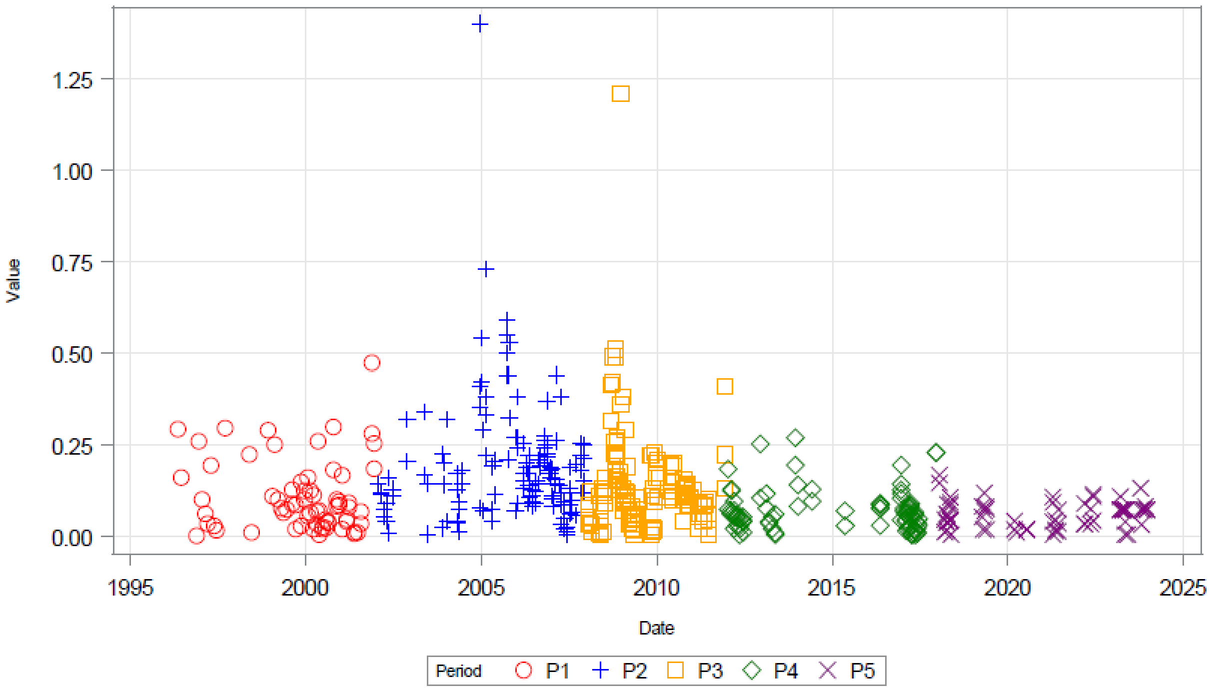
Figure 5.2-72: Distribution for surface total nitrogen (mg/L) within the LHR target zone by fixed-location stations (Analysis Days)



5.2.2.7 LHR Target Zone Nitrate + Nitrite

The time series of nitrate + nitrite concentrations in the target zone is displayed in Figure 5.2-73, which suggests lower nitrate + nitrite concentrations over time, similar to the trend observed for total nitrogen, but with a discernable peak in nitrate + nitrite between 2005 and 2009.

Figure 5.2-73: Time series for surface nitrate + nitrite (mg/L) within the LHR target zone (Analysis Days)

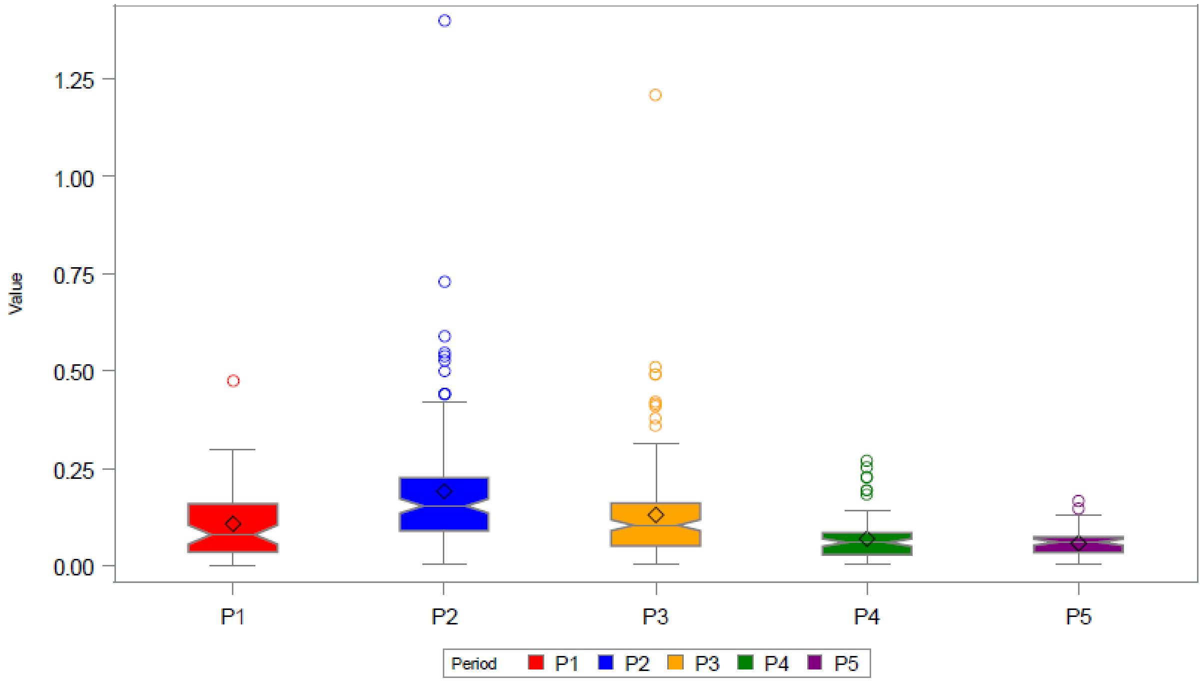


Summary statistics are presented in Table 5.2-21 for each period and sampling type. These statistics, and the box and whisker plot in Figure 5.2-74, also support a reduction in nitrate + nitrite concentrations over the period. Peak concentrations were evident in the period between 2002–2007 before a steady decrease over the remaining POR.

Table 5.2-21: Descriptive statistics for nitrate + nitrite (mg/L) in the LHR target zone by period (Analysis Days)

Type	Level	Period	N	Min	Max	Mean	Std
Fixed	Surface	P1	66	0.00	0.47	0.11	0.10
		P2	96	0.00	0.59	0.16	0.11
		P3	86	0.00	0.59	0.16	0.11
		P4	100	0.00	0.42	0.10	0.07
		P5	79	0.00	0.27	0.07	0.06
Random	Surface	P2	52	0.01	1.40	0.24	0.23
		P3	51	0.01	1.21	0.18	0.20
		P4	10	0.01	0.07	0.04	0.02

Figure 5.2-74: Distribution for surface nitrate + nitrite (mg/L) within the LHR target zone by period (Analysis Days)



Nitrate + nitrite were measured primarily at EPC and District fixed station sites (Figure 5.2-75). The distribution of nitrate + nitrite samples at each fixed-location station is shown in Figure 5.2-76.

Figure 5.2-75: Fixed-location stations within the LHR target zone with nitrate + nitrite data

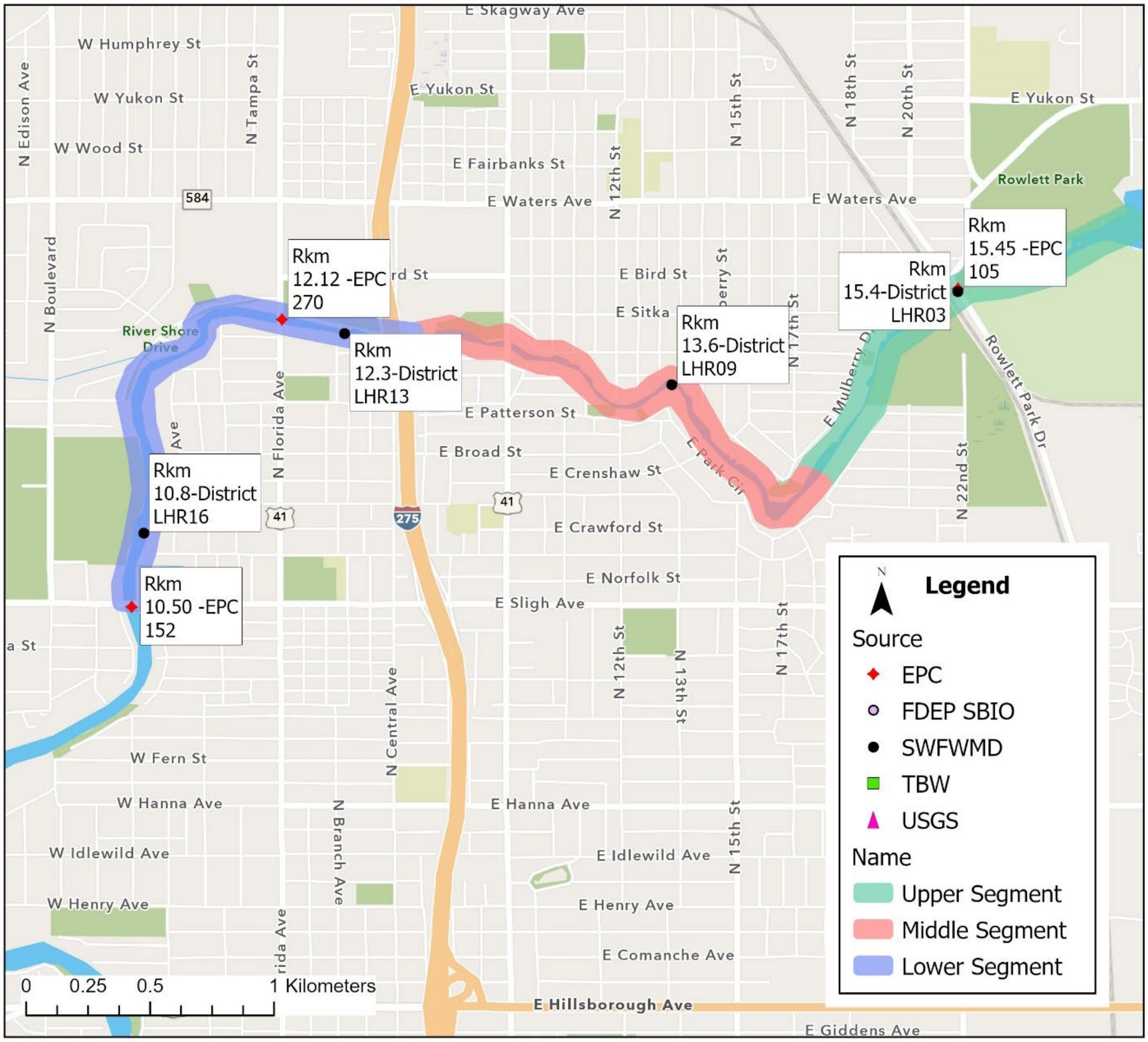
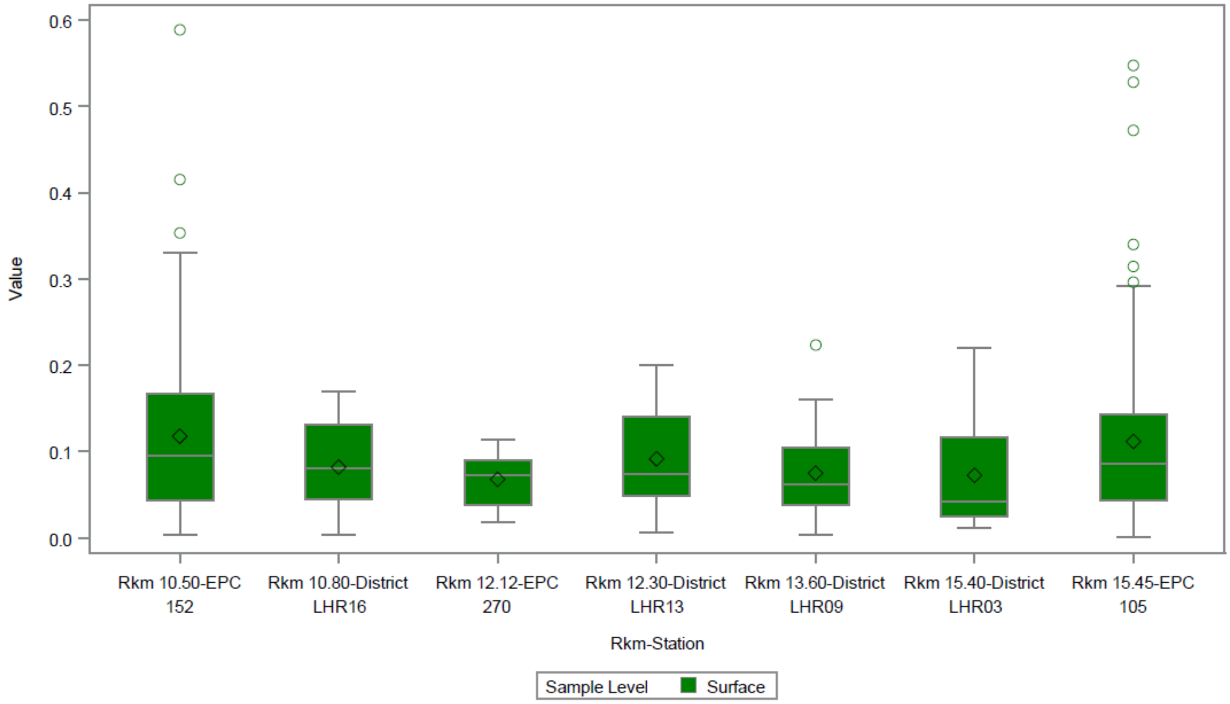


Figure 5.2-76: Distribution for surface nitrate and nitrite (mg/L) within the LHR target zone by fixed-location stations (Analysis Days)



5.2.2.8 LHR Target Zone Total Phosphorus

The time series of total phosphorus concentrations in the target zone is displayed in Figure 5.2-77 and shows a decreasing trend from higher concentrations before 2012 and then concentrations mostly stabilizing under 0.2 mg/L since 2012.

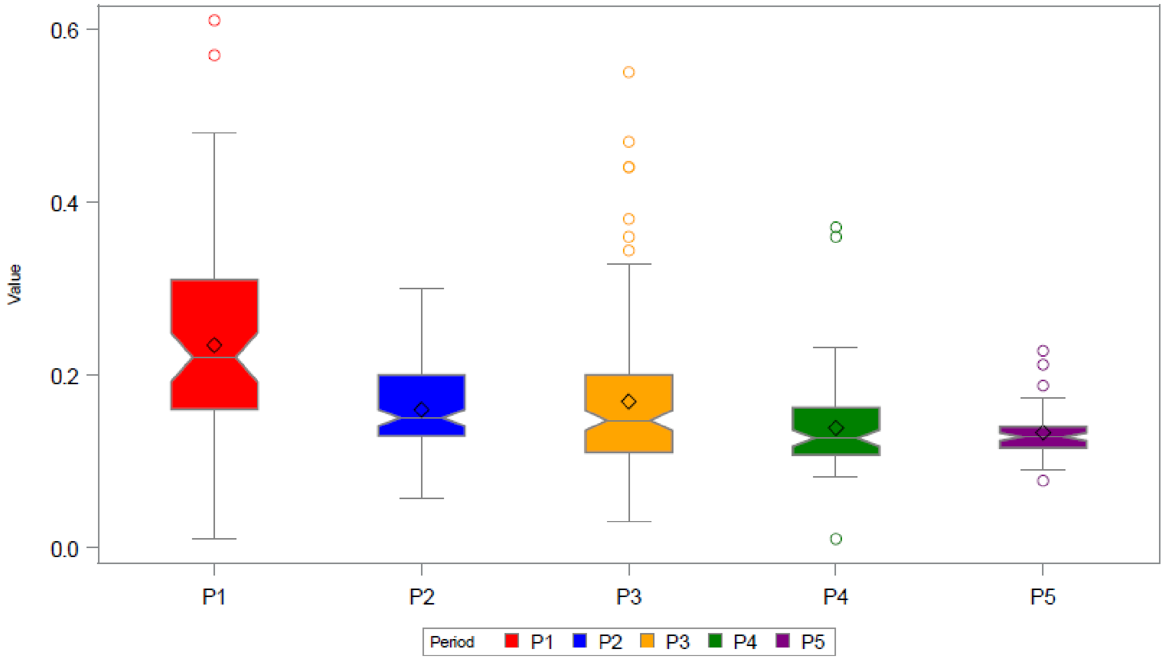
Figure 5.2-77: Time series for surface total phosphorus (mg/L) within the LHR target zone (Analysis Days)

Summary statistics are presented in Table 5.2-22 for each period and sampling type. Total phosphorus decreased across the POR (Figure 5.2-78) culminating in a Period 5 average of 0.14 mg/L.

Table 5.2-22: Descriptive statistics for total phosphorus within the LHR target zone by period (Analysis Days)

Type	Level	Period	N	Min	Max	Mean	Std
Fixed	Surface	P1	69	0.01	0.61	0.23	0.12
		P2	99	0.06	0.30	0.16	0.05
		P3	88	0.06	0.30	0.17	0.05
		P4	100	0.07	0.34	0.16	0.07
		P5	77	0.08	0.23	0.14	0.04
Random	Surface	P2	52	0.08	0.26	0.15	0.04
		P3	49	0.03	0.55	0.19	0.11
		P4	9	0.01	0.37	0.16	0.12

Figure 5.2-78: Distribution for surface total phosphorus (mg/L) within the LHR target zone by period (Analysis Days)



Total phosphorus was measured at fixed stations (Figure 5.2-79) and as measurements conducted as part of the random sampling associated with the TBW HBMP between 2000 and 2012 (not shown). The distribution of total phosphorus collected at each fixed location is provided in Figure 5.2-80. The only discernable longitudinal trend was the higher variability in EPC 105 near the base of the dam relative to the other sites.

Figure 5.2-79: Fixed-location stations within the LHR target zone with total phosphorus data

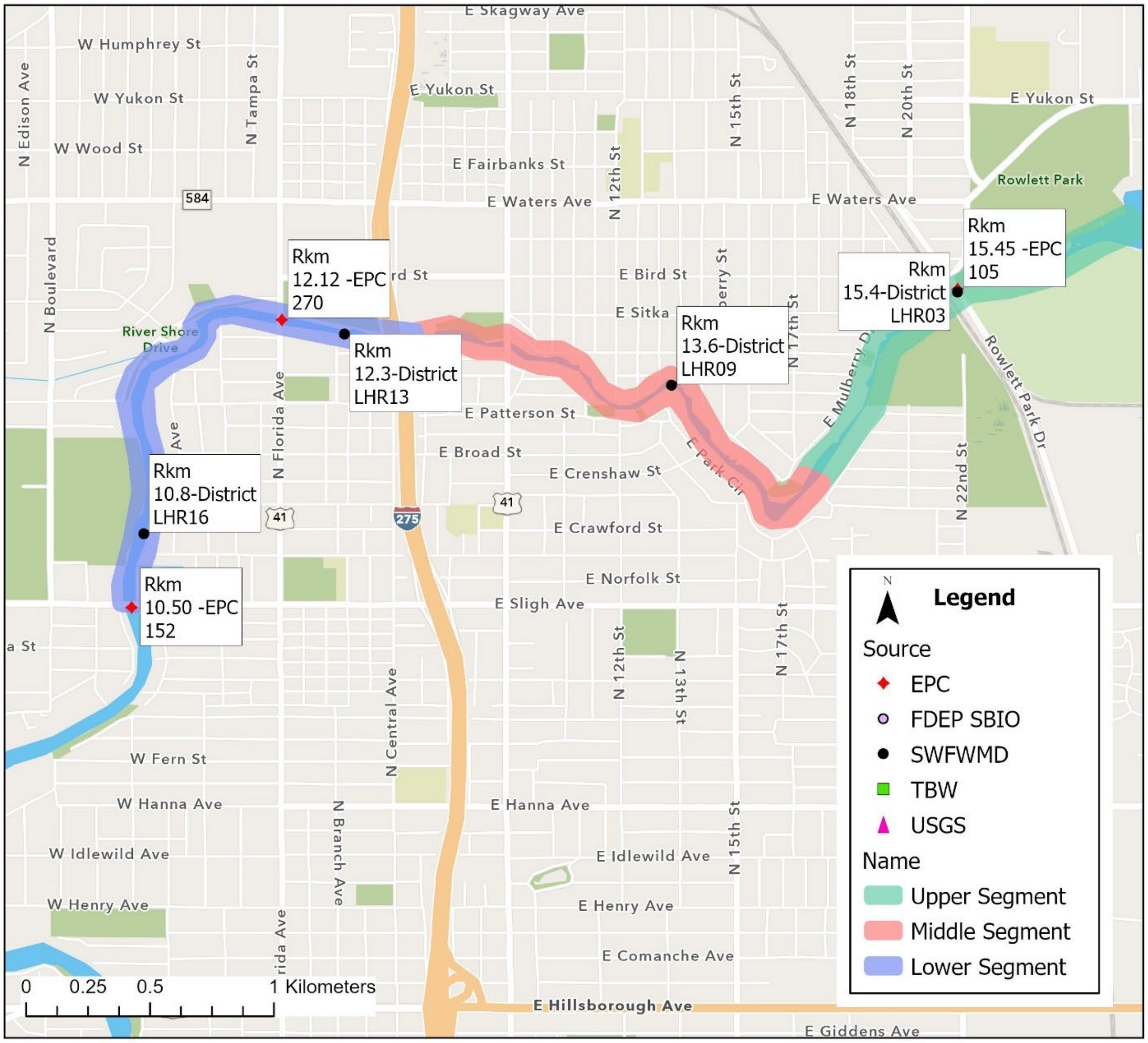
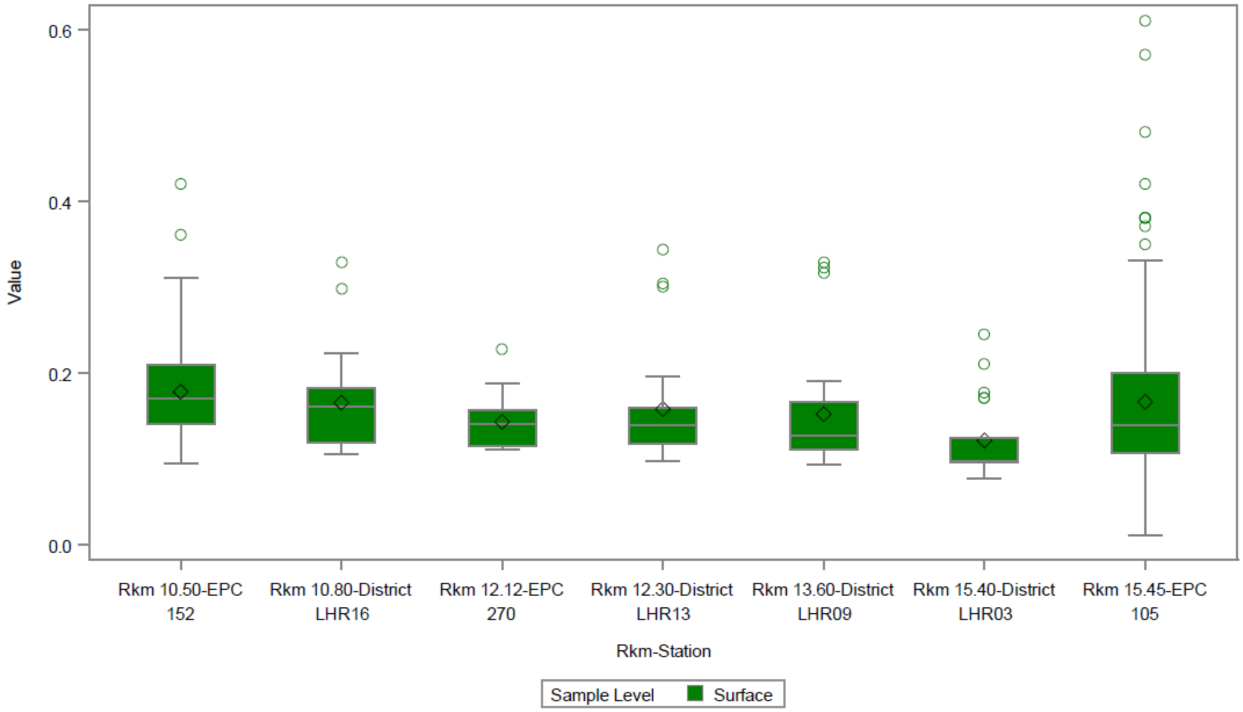


Figure 5.2-80: Distribution for total phosphorus (mg/L) within the LHR target zone by fixed-location stations (Analysis Days)



5.2.2.9 LHR Target Zone Orthophosphate

The time series of orthophosphate concentrations in the target zone is displayed in Figure 5.2-81. This figure suggests a decline in orthophosphate concentrations over time. This is supported by summary statistics presented in Table 5.2-23 for each period and sampling type, and by the distribution of orthophosphate concentrations being higher in the early period boxplots of Figure 5.2-82.

Figure 5.2-81: Time series for surface orthophosphate (mg/L) within the LHR target zone (Analysis Days)

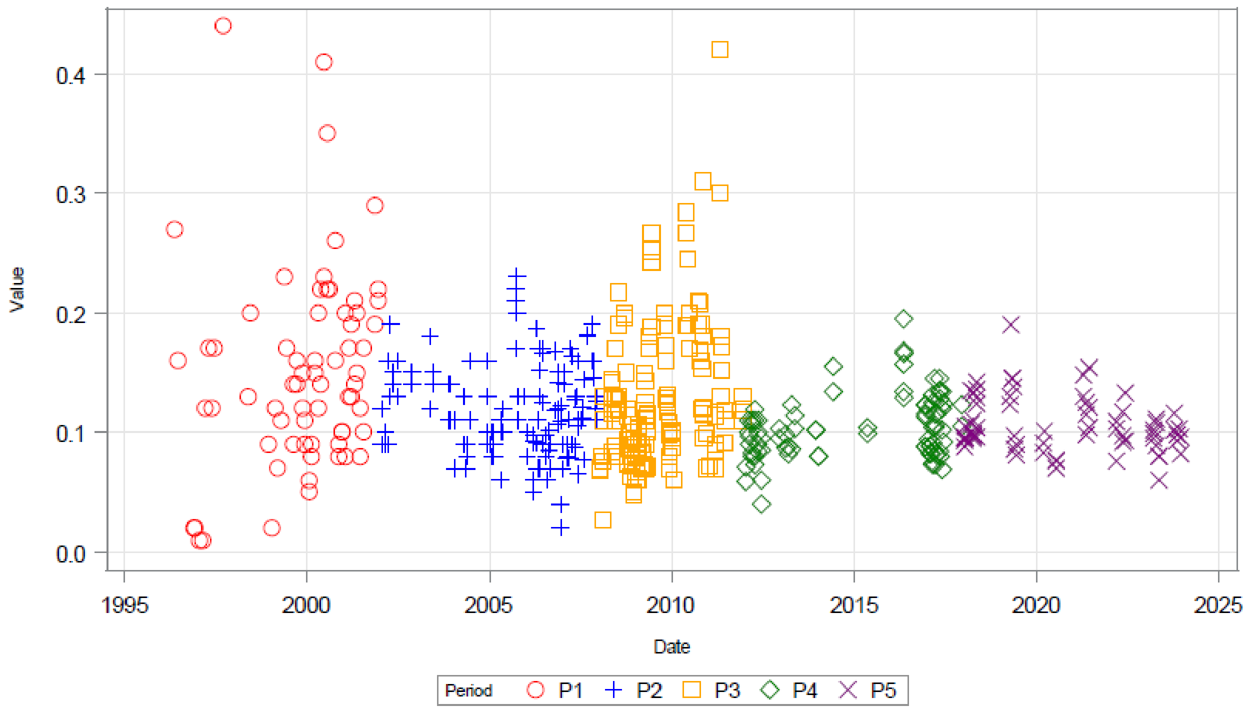
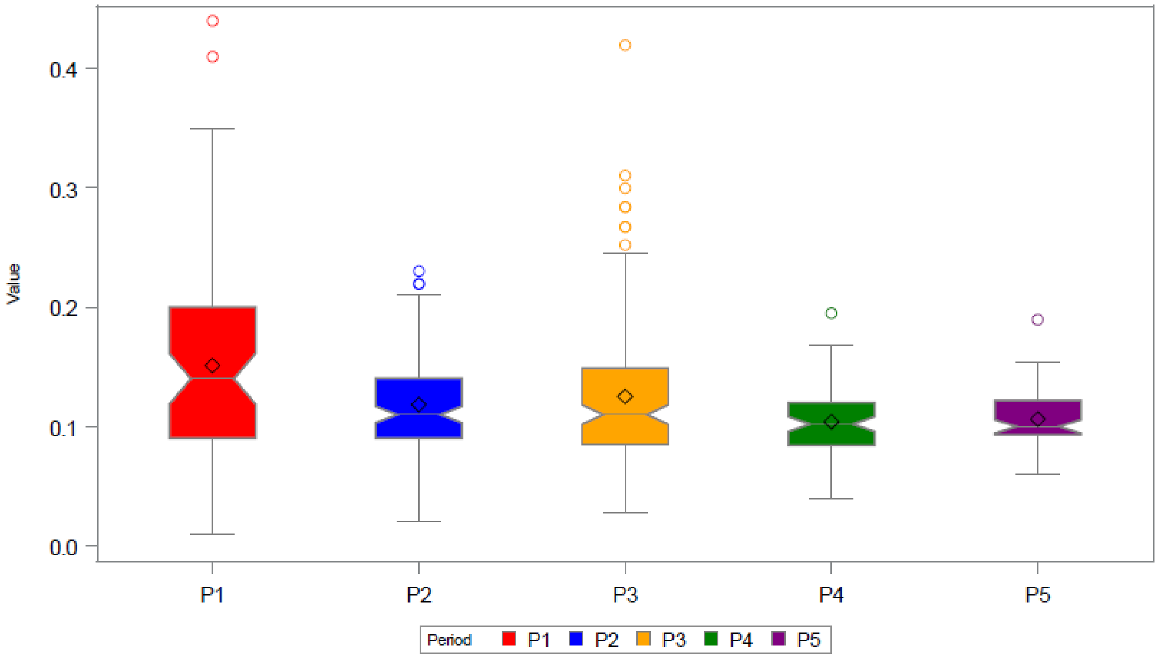


Table 5.2-23: Descriptive statistics for orthophosphate (mg/L) within the LHR target zone by period (Analysis Days)

Type	Level	Period	N	Min	Max	Mean	Std
Fixed	Surface	P1	67	0.01	0.44	0.15	0.08
		P2	99	0.06	0.22	0.12	0.03
		P3	88	0.06	0.22	0.12	0.03
		P4	104	0.03	0.28	0.12	0.05
		P5	76	0.06	0.20	0.11	0.03
Random	Surface	P2	48	0.02	0.23	0.12	0.05
		P3	50	0.05	0.42	0.14	0.07
		P4	10	0.04	0.11	0.09	0.02

Figure 5.2-82: Distribution for surface orthophosphate (mg/L) within the LHR target zone by period (Analysis Days)



Total phosphorus was measured at fixed stations (Figure 5.2-82) and as measurements conducted as part of the random sampling associated with the TBW HBMP between 2000 and 2012 (not shown). Spatial patterns in orthophosphate across stations (Figure 5.2-84) do not indicate a specific pattern in the fixed station data.

Figure 5.2-83: Fixed-location stations within the LHR target zone with orthophosphate data

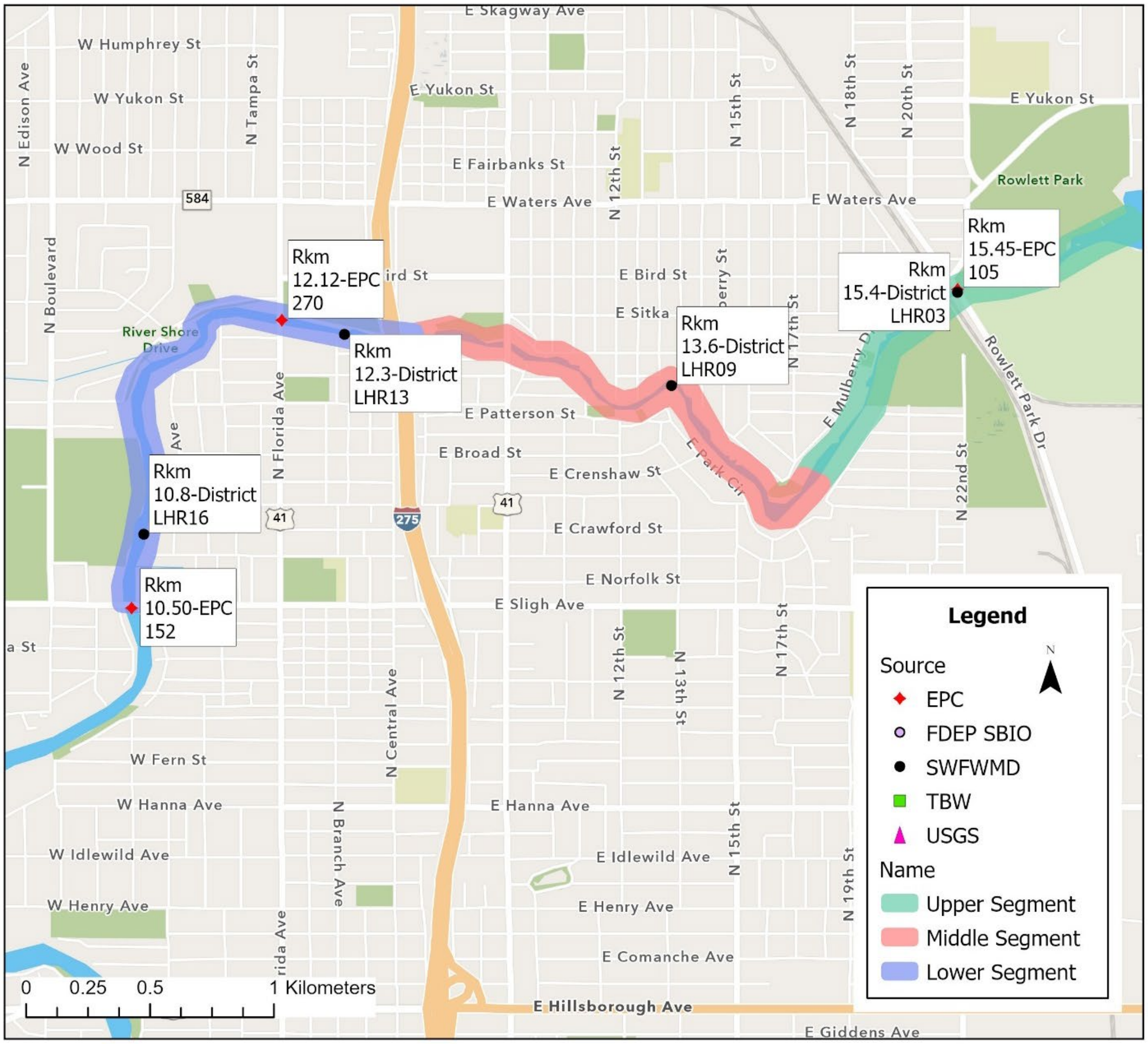
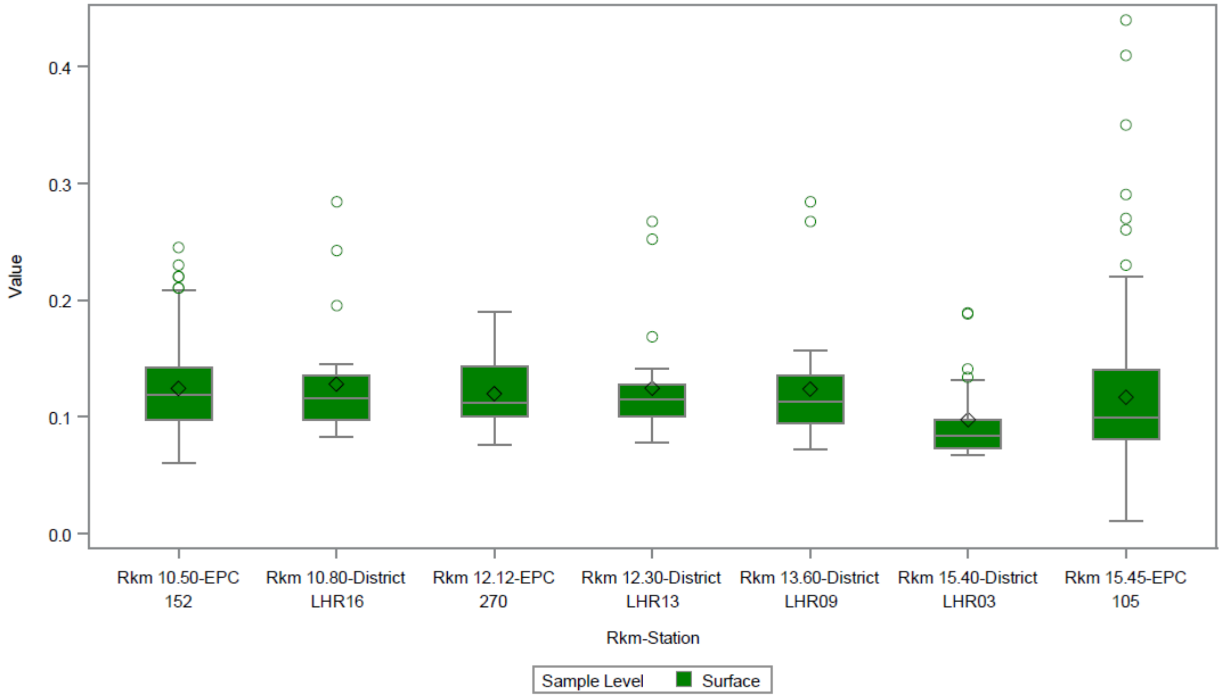


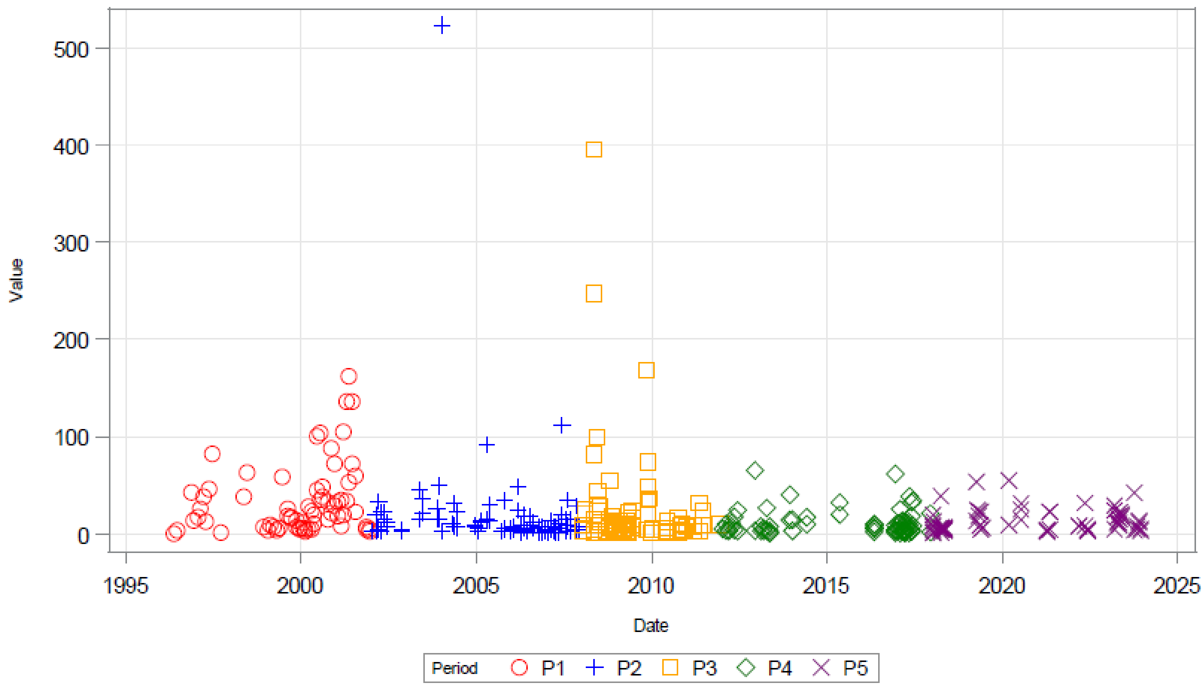
Figure 5.2-84: Distribution for orthophosphate (mg/L) within the LHR target zone by fixed-location stations (Analysis Days)



5.2.2.10 LHR Target Zone Chlorophyll

Figure 5.2-85 displays the time series of chlorophyll concentrations in the target zone. Concentrations of chlorophyll above 50 and 100 µg/L are due to phytoplankton blooms. Some very high chlorophyll values occurred in the time series before 2010, but no samples over 100 µg/L have been observed since, possibly due to increased flushing with minimum flow implementation. Summary statistics are presented in Table 5.2-24 for each period and sampling type. Only 11 samples were taken that were not at the surface; therefore, only surface values are plotted for chlorophyll. Plots for all values are provided in the water quality appendix (Appendix M).

Figure 5.2-85: Time series for surface chlorophyll (µg/L) within the LHR target zone (Analysis Days)

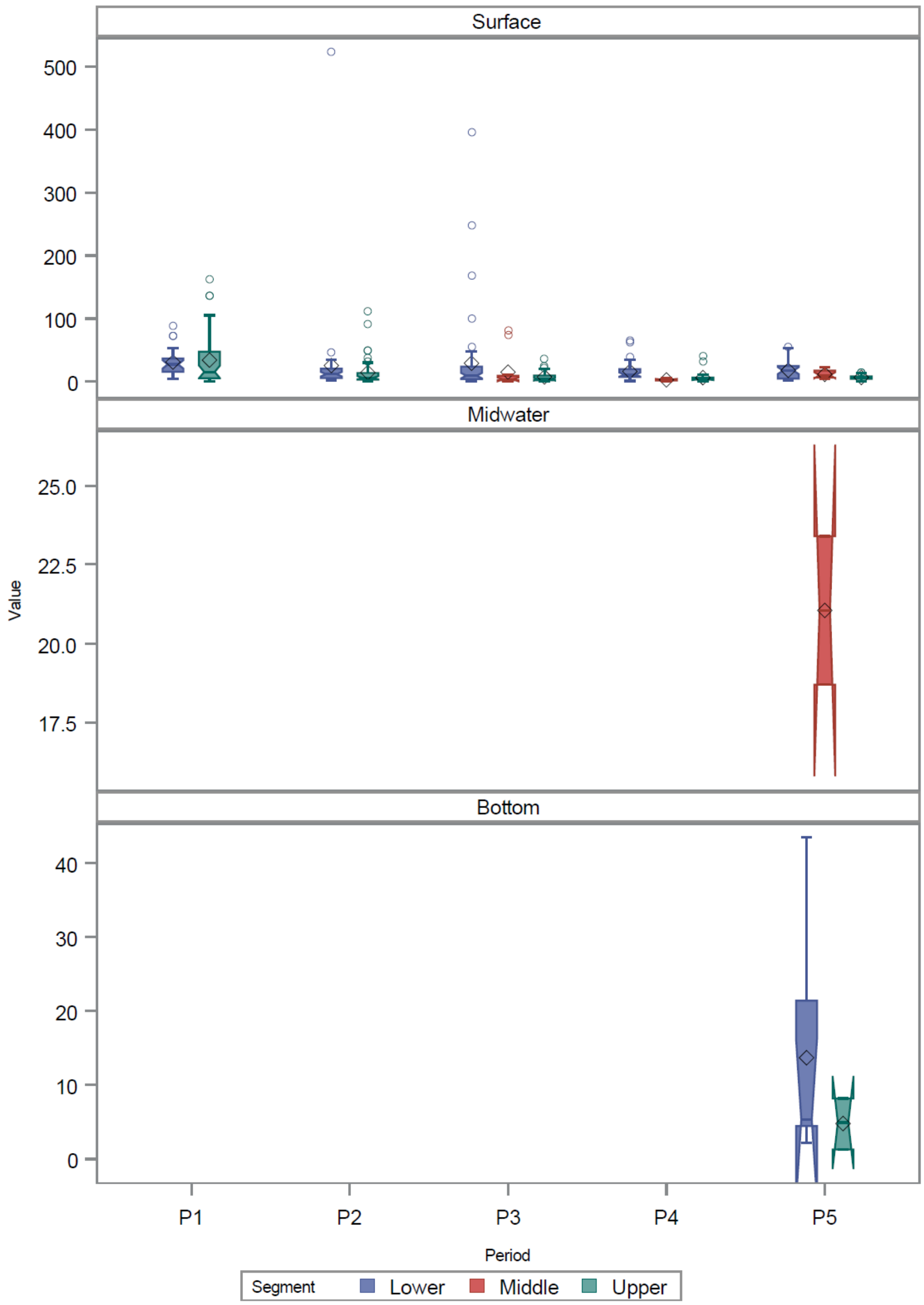


Chlorophyll concentrations demonstrate large variability throughout the POR but with fewer high (> 100 µg/L) concentrations in more recent periods (Figure 5.2-86). Overall concentrations remained consistent throughout the periods with no clear temporal trend in median (Figure 5.2-86).

Table 5.2-24: Descriptive statistics for chlorophyll (µg/L) within the LHR target zone by period for specified water column strata (Analysis Days)

Type	Level	Period	N	Min	Max	Mean	Std
Fixed	Surface	P1	68	0.71	162.15	33.06	36.00
		P2	97	0.80	523.15	18.34	54.54
		P3	88	1.10	523.15	19.96	57.04
		P4	104	1.00	395.10	19.76	49.18
		P5	77	1.00	65.50	10.70	12.69
	Midwater	P5	2	18.70	23.40	21.05	3.32
	Bottom	P5	9	1.23	43.50	10.69	13.66

Figure 5.2-86: Distribution for chlorophyll (µg/L) within the LHR target zone by period and river segment for specified water column strata (Analysis Days)



Chlorophyll was measured at EPC and District fixed stations between 1996 and 2023 (Figure 5.2-87). Figure 5.2-88 illustrates the distribution of chlorophyll concentrations at each of the target zone fixed stations and does not show much of a pattern over time though the scale is difficult to interpret. Chlorophyll concentrations are more closely evaluated in the quantitative chapter on water quality (Chapter 6).

Figure 5.2-87: Fixed-location stations within the LHR target zone with chlorophyll data

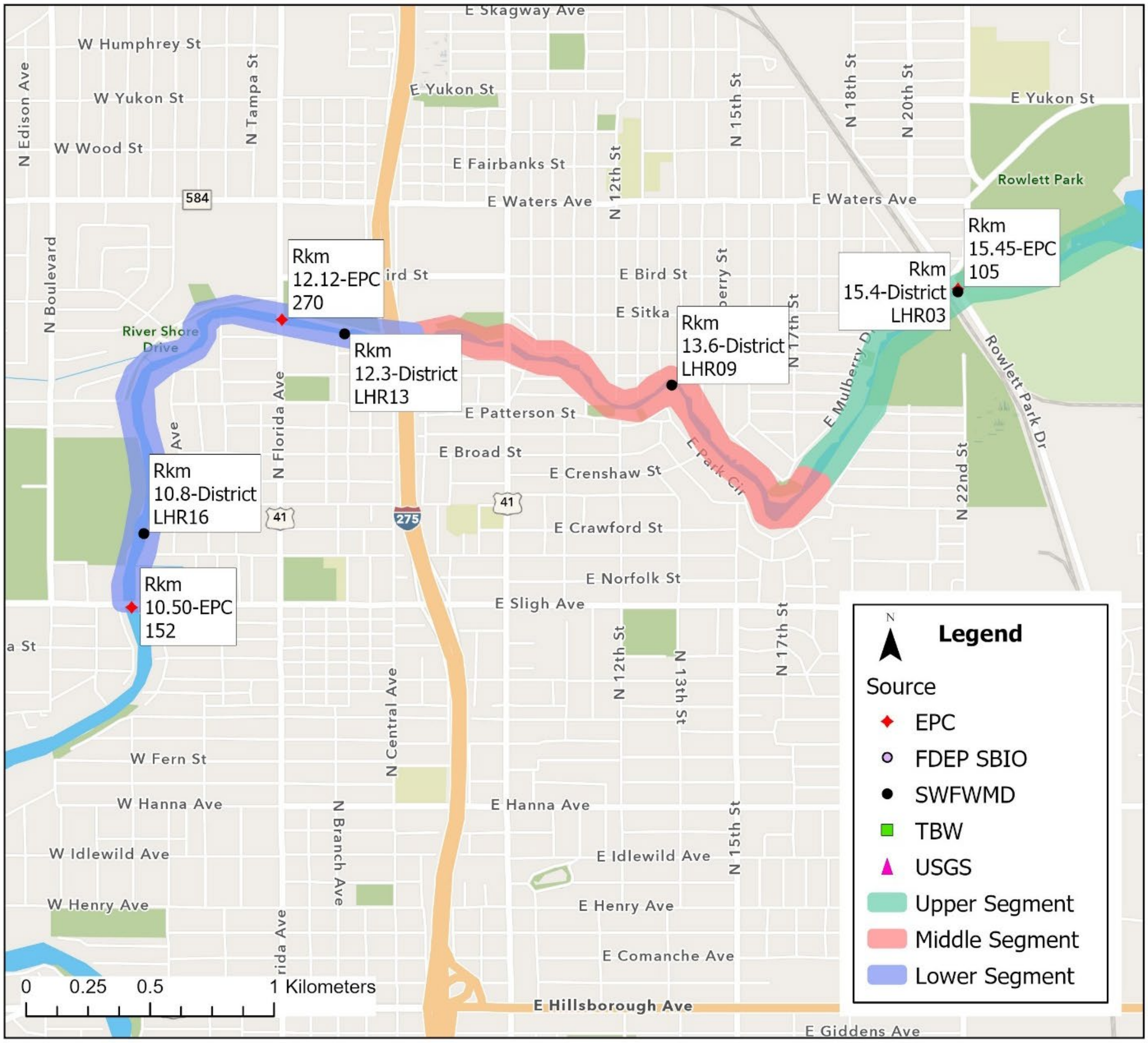
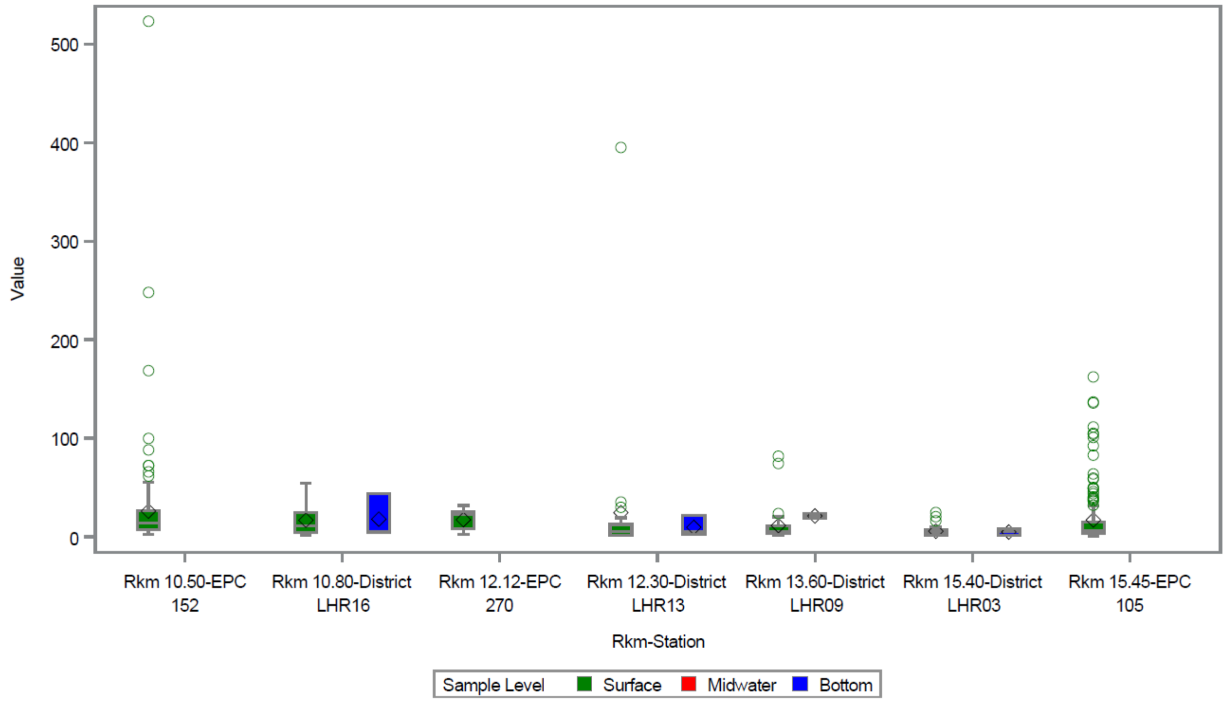


Figure 5.2-88: Distribution for chlorophyll (µg/L) within the LHR target zone by fixed-location stations for specified water column strata (Analysis Days)



5.3 Lower Hillsborough River Biology

Estuaries are characterized by dynamic changes in salinity, which influence the structure of biological communities. These variations are driven by the timing, duration, and magnitude of freshwater inflows into these semi-enclosed coastal systems connected to an ocean or high-salinity bay (FDEP 2011). Estuaries are highly productive areas that serve as spawning, nursery, and feeding grounds. As such, estuaries provide important habitats for many economically important fish and shellfish species (Barnes and Hughes 1993). Estuarine-dependent species constitute more than 95% of the commercial fishery harvests from the Gulf of Mexico, and many important recreational fishery species also depend on estuaries at some stage of their life cycle (Janicki Environmental 2003).

The minimum flow was established to protect biota inhabiting the freshwater/oligohaline conditions below the Hillsborough River impoundment. However, the presence of freshwater/oligohaline taxa is naturally dynamic in the Hillsborough River estuary below the reservoir. As noted by Montagna et al. 2007, "because the entire segment of the LHR below the dam is a tidally affected reach, lunar and wind tides can carry marine waters into the river making it impossible to maintain permanent freshwater (< 0.5 ppt) conditions, even near the dam. The freshwater conditions present during wet periods will naturally give way to brackish conditions in the tidal river segment during prolonged dry periods." However, previous plots of salinity vs. flow and modeling performed using LAMFE indicates that maintaining freshwater discharge at the dam at rates similar to the minimum flow rule (20 and 24 cfs) would sustain a predominantly fresh (<0.5 ppt) to near-fresh (<1 ppt) salinity zone near the dam for a substantial portion of the time that minimum flows are implemented (SWFWMD 1999, SWFWMD 2006).

The primary spatial focus of the biological community analyses was the LHR target zone, the area immediately downstream of the Hillsborough River Reservoir to Sligh Avenue. The biology of the target zone was both qualitatively and quantitatively evaluated. The biology of the lower portion of the system past the LHR target zone, denoted "LHR downstream," was qualitatively described. Full descriptive statistics for data filtered for salinity-sensitive species are available in Appendix O. Taxa were classified as salinity-sensitive if they inhabit oligohaline or tidal freshwater environments, defined as having a salinity of 5 ppt or lower, as part of their salinity range (Odum et al. 1984). Descriptive statistics for data with no filtering by salinity sensitivity are available in Appendix P1. Additional details regarding the twenty most abundant species within each target zone segment by period may be found in Appendix P2.

The number of samples used to analyze the zooplankton, benthic macroinvertebrate, and nekton communities within the LHR are provided in Table 5.3-1. These data were filtered by Analysis Days.

Table 5.3-1: Number of observations (site-date combinations) used for qualitative evaluation of zooplankton, benthic macroinvertebrates, and nekton (Analysis Days)

Data Set	Upper	Middle	Lower	Downstream
Zooplankton	10	160	160	584

Data Set	Upper	Middle	Lower	Downstream
Benthos	60	74	56	388
Nekton	92	103	185	998

5.3.1 LHR Downstream

The cumulative zooplankton, benthic macroinvertebrate, and nekton data from the area downstream of the target zone were analyzed (Sligh Avenue to Platt Street). Table 5.3-2 summarizes taxa richness, total abundance, and the proportion of the 20 most abundant taxa within each sampled biological community. A qualitative description of the 20 most abundant taxa for each community type below provides ecological information for these taxa.

Table 5.3-2: Taxa richness, total abundance, and proportion of the 20 most abundant taxa relative to total abundance for zooplankton, benthic macroinvertebrates, and nekton within the LHR downstream (Analysis Days)

Parameter	Zooplankton	Benthic Macroinvertebrates	Nekton
Total Abundance	7,302,426	130,544	854,376
Total Taxa Richness	280	398	117
20 Most Abundant Taxa Proportion to Total Taxa (%)	98	60	75

5.3.1.1 LHR Downstream Zooplankton

The 20 most abundant zooplankton taxa from the downstream LHR are found in Table 5.3-3.

Table 5.3-3: Twenty most abundant zooplankton taxa within the LHR downstream (Analysis Days)

Top 20 Zooplankton Taxa	Total Abundance	% of Total
Decapod zoeae	4,640,822	63.55
Acartia tonsa	727,242	9.96
Americamysis almyra	318,188	4.36
Clytia sp.	239,725	3.28
Gobiosoma spp. postflexion larvae	132,065	1.81
Amphipods, gammaridean	124,283	1.70
Decapod megalopae	116,888	1.60
Decapod mysis	106,515	1.46
Unidentified *Americamysis* juveniles	105,002	1.44
Appendicularian, *Oikopleura dioica*	100,161	1.37
Chaetognaths, sagittid	79,435	1.09
Lucifer faxoni juveniles and adults	68,933	0.94
Fish eggs, percomorph	63,272	0.87
Anchoa mitchilli juveniles	62,578	0.86

Top 20 Zooplankton Taxa	Total Abundance	% of Total
Polychaeta	58,022	0.79
Gobiid preflexion larvae	55,232	0.76
Labidocera aestiva	41,340	0.57
Unidentified calanoids	31,544	0.43
Cumaceans	30,898	0.42
Gobiid flexion larvae	24,211	0.33

Decapoda is an order of crustacea that includes crabs, shrimp, and lobster. The zoea, megalopae, and mysis life stages of decapod crustaceans comprised 66.6% of the total zooplankton abundance in the downstream Hillsborough estuary. Sub-adult life stages are typically difficult to identify to a lower taxonomic level, but likely included common taxa such as *Americamysis* sp., *Paleomenetes* sp., *Farfantepanaeus* sp., and *Callinectes* sp., which are known to occur in Gulf coast estuaries (FDEP 2013a).

Acartia tonsa is a species of omnivorous calenoid copepod that can tolerate a wide range of salinities and can survive rapid salinity changes. These copepods are a food source for many species including birds, corals, crustaceans, fishes, jellyfishes, polychaete worms, and seahorses (Animal Diversity Web 2024).

Americamysis is a genus of Mysid Shrimp that is native to the Atlantic Ocean and Gulf of Mexico, inhabiting estuarine and shallow shelf waters (FDEP 2013c). These omnivorous shrimp are commonly found on sandy or muddy sediments in bays and may also be associated with *Thalassia* seagrass beds. Mysid Shrimp are vital food sources for many commercially and recreationally important fish such as anchovies, catfish, seatrout and drum (FDEP 2013c). *Americamysis* is known to be sensitive to environmental stressors, and the US Environmental Protection Agency (EPA) promotes the use of *Americamysis bahia* for laboratory testing for acute and chronic toxicity assays (FDEP 2013c).

Clytia is a genus of cnidaria (Jellyfish) with a polyhaline planktonic life stage that feeds on other zooplankton (Smithsonian 2023).

Gobiosoma is a genus of small coastal fish, generally found in salinities between 0.3 ppt to 25 ppt and is frequently associated with oyster beds, grass beds, and marsh pools (Smithsonian 2023).

Gammarid amphipods occur in a variety of estuarine habitats and serve as prey for juvenile and adult fish of several species, and also for large decapods. In terms of secondary productivity, populations of gammarid amphipods are important ecosystem components and as such are considered to be keystone taxa (FDEP 2013b).

Oikopleura dioica is a species of small pelagic bioluminescent tunicate found in the surface waters of most of the world's oceans. It is preyed on by ctenophore, *Mnemiopsis leidyi*, copepods, and fish (Scripps 2024).

Chaetognaths, known as Arrow Worms, are a phylum of predatory marine worms that are a major component of zooplankton worldwide.

Lucifer faxoni is a marine shrimp that feeds on mangrove leaf detritus (World Register Marine Species 2023).

Anchoa mitchilli (Bay Anchovy) is primarily a pelagic (water column) zooplanktivorous species, common in protected waters and tide pools. Bay Anchovies are a major component in the diets of several species of piscivorous fish, including commercially important species such as Weakfish (*Cynoscion regalis*) and Striped Bass (*Morone saxatilis*) and represent a critical component of marine and estuarine food webs, both as a predator and a prey species (FDEP 2013c).

Polychaetes are an extremely diverse and often abundant group of predominantly marine worms. They play essential ecological roles, serving as predators on small invertebrates and as food for fish and large invertebrates (Smithsonian 2023).

Labidocera aestiva is a marine calenoid copepod that is a food source for many species including birds, corals, crustaceans, fishes, jellyfishes, polychaete worms, and seahorses (Animal Diversity Web 2024).

Calanoid copepods are marine or freshwater arthropods commonly found as zooplankton. The order includes around 46 families with about 1,800 species. Calanoid copepods are primarily suspension feeders eating mainly phytoplankton and protozoans (Smithsonian 2023).

Cumaceans are small crustaceans, known as Hooded Shrimp, which occur in marine and brackish waters throughout the world (World Register Marine Species 2023).

5.3.1.2 LHR Downstream Benthic Macroinvertebrates

The 20 most abundant benthos taxa from the downstream zone are found in Table 5.3-4.

Table 5.3-4: Twenty most abundant benthic macroinvertebrate taxa within the LHR downstream (Analysis Days)

Top 20 Benthic Taxa	Total Abundance	% of Total
Grandidierella bonnieroides	21,500	16.47
Stenoninereis martini	11,659	8.93
Naididae	10,997	8.42
Laeonereis culveri	8,141	6.24
Kirkegaardia sp.	5,822	4.46
Streblospio gynobranchiata	5,405	4.14
Streblospio sp.	5,094	3.90
Mytilopsis leucophaeata	4,795	3.67
Ampelisca abdita	3,840	2.94
Melinna maculata	3,653	2.80
Monticellina dorsobranchialis	3,609	2.76
Polypedilum halterale grp.	3,521	2.70
Tubificoides brownae	2,670	2.05
Capitella capitata sp. complex	2,486	1.90
Laeonereis cf. longula	2,198	1.68
Hargeria/Leptochelia sp. complex	2,029	1.55
Pyrgophorus platyrachis	1,957	1.50
Littoridinops palustris	1,930	1.48

Top 20 Benthic Taxa	Total Abundance	% of Total
Melanoides tuberculata	1,317	1.01
Limnodriloides sp.	1,034	0.79

Grandidierella bonnieroides is an amphipod, widely distributed in the Gulf of Mexico, inhabiting oligohaline to lower euryhaline (1 ppt to 40 ppt) waters. It occurs in shallow bays, lagoons, tidal rivers, bayous, and tide pools in marshes and mangrove swamps. *G. bonnieroides* is common in the diet of many estuarine fishes and serves as an intermediate host for an acanthocephalan parasite (*Dollfusentis chandleri*) whose adult stage develops in the Atlantic Croaker, spot, and other estuarine fish (Heard 1979).

Stenoninereis martini is a small polychaete worm that grows up to 8 mm long with 34 body segments. It ranges from North Carolina to the Gulf of Mexico inhabiting brackish waters in salt marsh ponds, tidal creeks and rivers, generally on silt and mud bottoms (Heard 1979).

The Naididae are a family of oligochaete worms recognized as being the most cosmopolitan freshwater oligochaete family, being present in all biogeographic regions, including sub-Antarctic islands (Science Direct 2024).

Laeonereis culveri is a large (70 mm long), carnivorous polychaete worm living on the Atlantic and Gulf coasts. It is a euryhaline species occupying intertidal to shallow subtidal areas in substrates with at least some sand (Heard 1979). The polychaete species *Laeonereis longula* is a more recently described species based on material previously identified *as L. culveri* (Conde-Vela 2021).

Kirkegaardia sp. is a genus of marine, benthic-dwelling polychaete (World Register Marine Species 2023).

Streblospio gynobranchiata is a recently described spionid polychaete. In Florida, the genus *Streblospio* is known to inhabit a wide range of salinities and is typically found in muddy or soft-sediment areas, such as mudflats, seagrass beds, and marshes. *Streblospio* is also adapted to rapid colonization, due to its capacity for small-scale dispersal following larval development (Smithsonian 2023).

Mytilopsis leucophaeata, Dark Falsemussel, is a filter-feeding mussel native to the east coast of the Atlantic and the Gulf of Mexico. The species has wide salinity tolerances but typically inhabits estuarine environments (US Fish and Wildlife 2023).

Ampelisca abdita is a gammarid tube-building amphipod native to Florida and the Gulf of Mexico. It inhabits soft sediment from the mid-intertidal to 60 m depth in estuarine and shallow coastal waters (Smithsonian 2023).

Melinna maculata is a terebellid polychaete common in seagrass beds, soft bottoms, and mangroves throughout the Gulf of Mexico (World Register Marine Species 2023).

Monticellina dorsobranchialis, now classified as *Kirkegaardia dorsobranchialis*, is a sedentary marine polychaete species known for building mud balls on the seafloor (World Register Marine Species 2023). *Monticellina dorsobranchialis* is a bi-tentaculate cirratulid polychaete ranked fourth in dominance in Boca Ciega Bay (Grabe & Karlen 1995).

Polypedilum halterale grp. (a species complex) is a freshwater chironomid midge that is commonly found in streams and rivers. Midges are consumed by demersal fish and predatory benthic invertebrates (Merritt and Cummins 1978).

Tubificoides brownie is a marine oligochaete, resembling terrestrial earthworms, but is usually less than 60-70 mm long. They are hermaphroditic deposit feeders, generally occurring in silty or muddy sediments (Smithsonian 2023).

Capitella capitata sp. complex is a group of sibling species known for inhabiting disturbed marine environments, particularly those with high organic matter or pollutants. They are considered ecological indicators because of their ability to colonize and thrive in such conditions (Smithsonian 2023).

The *Hargeria/Leptochelia* sp. complex consists of tanaidacean crustaceans, which are fairly common in the Gulf of Mexico. Tanaidaceans are an important food source for various marine organisms, including fish, crustaceans, and birds. They are also considered indicator species due to their sensitivity to pollutants and changes in water quality (Ferreire et al. 2015).

Melanoides tuberculata (Red-Rim Melania) and *Pyrgophorus platyrachis* (Crown Snail) are invasive freshwater snails capable of living in estuaries. They graze on microalgae and detritus and in turn are eaten by crabs, fishes, and birds. *M. tuberculata* is host to a number of parasitic species and in some areas is known to outcompete native snails for algal resources (Smithsonian 2023).

Littoridinops palustris, known as the Bantam Hydrobe, is a small aquatic snail found in brackish and tidal freshwater marshes and estuaries, typically feeding on detritus, algae, and plant material. *Littoridinops palustris* is found in the Gulf of Mexico and is known to inhabit both oligohaline and mesohaline environments (Smithsonian 2023).

Limnodriloides is a genus of freshwater and marine oligochaete worms, which inhabits muddy substrates, seagrass beds, and mangrove areas, as well as fine to medium shell/sand (World Register Marine Species 2023).

5.3.1.3 LHR Downstream Nekton

The 20 most abundant nekton taxa in the downstream zone are found in Table 5.3-5.

Table 5.3-5: Twenty most abundant nekton taxa within the LHR downstream (Analysis Days)

Top 20 Nekton Taxa	Total Abundance	% of Total
Anchoa mitchilli	534,862	62.60
Menidia sp.	160,507	18.79
Palaemonetes pugio	39,228	4.59
Mugil cephalus	14,873	1.74
Eucinostomus sp.	13,000	1.52
Leiostomus xanthurus	10,398	1.22
Eucinostomus harengulus	8,779	1.03
Microgobius gulosus	8,692	1.02
Anchoa hepsetus	7,679	0.90
Brevoortia sp.	7,508	0.88
Cyprinodon variegatus	5,345	0.63
Lucania parva	5,342	0.63
Lagodon rhomboides	5,067	0.59
Trinectes maculatus,	3,681	0.43
Opisthonema oglinum	2,613	0.31
Fundulus grandis	2,562	0.30
Harengula jaguana	2,557	0.30
Poecilia latipinna	2,305	0.27
Gobiosoma bosc	1,910	0.22
Fundulus similis	1,873	0.22

Anchoa mitchilli (Bay Anchovy) and *Anchoa hepsetus* (Broad-striped Anchovy) are primarily pelagic (water column), zooplanktivorous species, common in protected waters and tide pools. Anchovies are a major component in the diets of several species of piscivorous fish, including commercially important species such as Weakfish (*Cynoscion regalis*) and Striped Bass (*Morone saxatilis*) and represent a critical component of marine and estuarine food webs, both as a predator and a prey species (FDEP 2013c).

Menidia beryllina, Inland Silverside (a small fish), is often found in shallow water with frequent migrations to open water in search of food. This species feeds primarily on zooplankton and is in turn fed on by larger fish and birds. Due to its sensitivity to environmental stressors, the inland silverside is approved by the EPA as a standard test organism for acute and chronic toxicity testing (FDEP 2013a).

Palaemonetes pugio, the Dagger Blade Shrimp, normally inhabits the areas where freshwater and saltwater combine, with a salinity range of 4.4 ppt to 17 ppt. Their basic habitat is salt marshes and connecting streams (Animal Diversity Web 2024).

Mugil cephalus (Striped Mullet) inhabit estuarine intertidal, freshwater, and coastal marine habitats. Juveniles occupy the high intertidal zone of estuaries where water temperatures and salinity fluctuate greatly. Older mullet inhabit deeper waters with more stable environmental conditions. Given their specialized gizzard-like stomach, they can feed on a wide variety of food substrates, such as detritus and epiphytic material. Mullet are commonly consumed by humans also serve as a food source for valuable game fish such as mahi, snook, and snapper (FDEP 2013d).

Eucinostomus harengulus (Tidewater Mojarra) are primarily found in estuaries on vegetated bottoms and in mangroves with sand and mud bottoms, but they also inhabit freshwater areas including springs. Most species travel in large schools to avoid large predators. Their diet consists of shrimp, plants, and invertebrates (Florida Springs Institute 2024).

Leiostomus xanthurus (Spot) is an estuarine-dependent fish, migrating between shallow tidal creeks that serve as this species' nursery area and deeper waters throughout their development. Because spot are so abundant, they serve as key species in the transfer of biologic energy from nearshore areas to offshore. Therefore, they are considered an important species indicating healthy estuarine systems (FDEP 2013d).

The Clown Goby (*Microgobius gulosus*) and the Naked Goby *(Gobiosoma bosc*) are small estuarine or freshwater fish that inhabit primarily low-energy tidal zones, sometimes vegetated and with sand or mud substrates, including bays, tidal creeks, canals, ditches and coastal rivers (Florida Museum 2024).

Brevoortia sp. (Menhaden) is a filter-feeding fish that is common in brackish and marine waters. Predators of menhaden consist of such aquatic animals as sharks, rays, and bony fish. Menhaden are considered to be a valuable commercial fish and are used in the production of oil, fertilizer, and fishmeal. Menhaden are also marketed for human consumption, either fresh, smoked, salted, or canned (Animal Diversity Web 2024).

Cyprinodon variegatus (Sheepshead Minnow) is tolerant of wide variations in salinity and is found in brackish water in bays, inlets, lagoons, and saltmarshes with little wave action and sandy or muddy bottoms. It is omnivorous, feeding on organic detritus and algae as well as microcrustaceans, and dipteran larvae (USGS 2024).

Rainwater Killifish (*Lucania parva*) is an estuarine species, ranging from euryhaline to tidal fresh waters, but nontidal freshwater populations also occur in highly mineralized waters. Rainwater Killifish is usually associated with dense vegetation including Tapegrass (*Vallisneria americana*) and Pondweed (*Potamogeton* spp*.*). As omnivores, Rainwater Killifish feed mostly on small invertebrates such as annelids, mollusks, and amphipods (Smithsonian 2023).

Lagodon rhomboides (Pinfish) is an estuarine-dependent species inhabiting coastal waters of the Gulf and Atlantic states, preferring habitats such as seagrass beds, rocky bottoms, jetties, and mangrove areas. The primary diet of Pinfish consists of shrimp, mysids, and amphipods; however, they exhibit strict herbivory or carnivory depending on conditions or development stage. *Lagodon rhomboides* is commonly consumed by larger fish, including game species such as spotted sea trout and flounder (FDEP 2013c).

Trinectes maculatus (Hogchoker) is a member of the American sole family. The Hogchoker is an euryhaline species that enters streams and rivers and is often found far inland. Their diet depends on the salinity of the waters they inhabit, consisting of benthic organisms such as aquatic crustaceans and insects, mollusks, and polychaete and oligochaete worms (Florida Museum 2024).

Opisthonema oglinum (Atlantic Thread-herring) are found in shallow waters and harbors along the Atlantic and Gulf of Mexico, preferring higher salinity waters. Atlantic Thread-herring are filter feeders and mostly feed on phytoplankton and zooplankton using their numerous gill rakers (filtering bars on the gills). They also feed on small fishes, crabs, shrimps, copepods, gastropods, bivalves, larval barnacles, plant detritus, fish scales, and sediments (UWI 2024).

Primarily estuarine, *Fundulus grandis* (Gulf Killifish) and *Fundulus similis* (Longnose Killifish*)* are inshore fish associated with a variety of low-salinity habitats, including marshes, seagrass beds, and oyster reefs. Killifish feed throughout the water column, consuming fishes, terrestrial insects on the water surface, benthic algae, and crustaceans (USGS 2024).

Sailfin Mollies (*Poecilia latipinna*) are small live-bearing freshwater fish native to the Gulf Coast that have established populations in estuarine habitats. Sailfin mollies can colonize a wide range of habitats such as ditches, canals, and disturbed marshes. Sailfin mollies have shown aggressive behavior towards other fishes, but many native fishes prey upon them (Smithsonian 2023).

5.3.2 LHR Target Zone

5.3.2.1 LHR Target Zone Zooplankton

Methods for calculations of abundance, richness, and diversity are described in Section 3.3.2.1. The 20 most common zooplankton within the target zone included: decapod crustaceans (e.g., *zoeae, megalopae*, and *mysis* stages; *Palaemonetes* spp*.*), jellyfish (e.g., *Clytia* sp.), mysid shrimp (e.g., *Americamysis almyra*), copepods, polychaetes, amphipods, isopods (e.g., *Cassidinidea ovalis*) and fish (e.g., *Gobiosoma sp., Anchoa mitchilli, Brevoortia* sp*.*) (Table 5.3-6). Taxa richness ranged from 161 in the lower segment to 39 in the upper segment; however, the upper segment was sampled only during Period 5 (Table 5.3-7). Shannon diversity ranged from 0.13 in the lower segment to 1.27 in the middle segment (Table 5.3-8).

Table 5.3-6: Abundance of 20 most common zooplankton within the LHR target zone by segment and period (Analysis Days)

Taxon Name	Total	Period 1 Middle	Period 1 Lower	Period 2 Middle	Period 2 Lower	Period 3 Middle	Period 3 Lower	Period 4 Middle	Period 4 Lower	Period 5 Upper	Period 5 Middle	Period 5 Lower
Decapod zoeae	2,068,763	156,375	146,594	287,476	525,267	157,805	186,296	110,593	257,353	77,016	49,261	114,727
Clytia sp.	241,791	43,557	82,117	44,527	67,489	1,381	2,289	14	1	0	14	402
Americamysis almyra	108,296	11,014	9,718	14,061	67,106	401	3,455	34	600	203	198	1,506
Acartia tonsa	70,039	89	783	11,722	50,827	90	6,428	6	38	0	0	56
Polychaeta	29,846	1909	286	10,563	5,542	527	8,986	377	946	0	275	435
Gobiosoma spp. postflexion larvae	26,760	6,877	7,641	745	9,240	187	719	6	13	13	1,004	315
Unidentified *Americamysis* juveniles	26,377	202	99	4,887	17,562	107	2,783	43	448	17	59	170
Decapod megalopae	23,945	5,793	1,915	3,503	11,988	82	299	23	101	120	8	113
Amphipods, gammaridean	19,558	24	33	2,981	5,087	2,760	3,991	3,361	607	73	438	203
Decapod mysis	17,807	5,203	2,297	6,433	2,569	155	722	19	33	6	89	281
Anchoa mitchilli juveniles	10,966	1,376	5,019	107	2,964	141	663	97	596	0	2	1
Cymothoid sp. a (Lironeca) juveniles	5,362	1,495	1,975	95	675	139	197	290	496	0	0	0
Gastropods, prosobranch	3,312	172	591	592	1,166	325	216	24	34	47	21	124
Chaetognaths, sagittid	2,501	289	1,447	677	84	0	4	0	0	0	0	0
Gobiid flexion larvae	2,432	1,022	385	349	435	7	104	0	3	1	72	54
Palaemonetes spp. post larvae	2,335	520	59	282	1,004	32	382	0	10	0	4	42
Brevoortia spp. metamorphs	2,176	845	1,297	1	1	4	9	0	0	0	14	5
Cassidinidea ovalis	1,981	1	0	161	60	114	46	28	13	1,543	10	5
Microgobius spp. postflexion larvae	1,976	593	233	348	665	2	118	0	0	0	2	15
Gobiid preflexion larvae	16,82	119	74	96	95	24	1,053	20	52	2	80	67

Table 5.3-7: Zooplankton taxa richness of the LHR target zone by segment and period (Analysis Days)

Segment	Period 1	Period 2	Period 3	Period 4	Period 5	Segment Richness
Upper	NA	NA	NA	NA	39	39
Middle	79	93	78	38	53	144
Lower	78	100	101	53	55	161

Table 5.3-8: Zooplankton diversity of the LHR target zone by segment and period (Analysis Days)

River Segment	Period	Shannon Diversity
Upper	5	0.19
Middle	1	1.27
Middle	2	1.09
Middle	3	0.28
Middle	4	0.21
Middle	5	0.32
Lower	1	1.26
Lower	2	1.24
Lower	3	0.78
Lower	4	0.13
Lower	5	0.31

5.3.2.2 LHR Target Zone Benthic Macroinvertebrates

The 20 most common invertebrates collected via grab sampler within the target zone included: polychaetes (e.g., *Stenoninereis martini*, *Laeonereis culveri0*, snails (e.g., *Melanoides tuberculata, Pyrgophorus platyrachis*), oligochaete worms (e.g., *Naididae*), clams (e.g., *Mytilopsis leucophaeata*), isopods (e.g., *Cassidinidea ovalis*), amphipods (e.g., *Grandidierella bonnieroides*), and midges (e.g., Chironomus sp., *Polypedilum halterale* grp.) (Table 5.3-9). Taxa richness ranged from 114 in the lower segment to 184 in the upper segment (Table 5.3-10). Shannon diversity ranged from 1.6 in the middle segment to 3.0 in the upper segment (Table 5.3-11).

BioRecon (dip net) sampling by retired state scientists between the dam and Hannah's Whirl during Period 5 captured 19 taxa not observed in grab samples taken during the same period (Flannery et al. 2025).

Table 5.3-9: Abundance of 20 most common benthic macroinvertebrates within the LHR target zone by segment and period (Analysis Days)

Taxon Name	Total	Period 1 Upper	Period 1 Middle	Period 1 Lower	Period 2 Upper	Period 2 Middle	Period 2 Lower	Period 3 Upper	Period 3 Middle	Period 3 Lower	Period 5 Upper	Period 5 Middle	Period 5 Lower
Stenoninereis martini	6,191	23	169	141	485	1,652	491	11	831	620	184	541	1,043
Melanoides tuberculata	5,183	23	6	4	1,258	1,303	166	1,036	518	270	325	151	123
Pyrgophorus platyrachis	3,371	105	0	7	1,273	573	697	365	158	14	79	21	79
Naididae	3,219	248	197	46	962	962	382	372	50	0	0	0	0
Laeonereis culveri	2,815	53	1	2	104	385	1,313	2	105	283	37	294	236
Hydrobiidae	2,591	3	1	0	687	1,331	17	296	80	25	14	29	108
Tubificidae w/o hair	2,052	1	0	0	74	1	10	0	0	0	889	952	125
Mytilopsis leucophaeata	1,784	57	6	2	133	180	430	131	343	49	28	237	188
Grandidierella bonnieroides	1,601	7	5	0	219	62	284	20	50	358	93	203	300
Corbicula fluminea	1,539	216	72	12	593	290	133	95	11	0	96	20	1
Cassidinidea ovalis	1,312	3	0	0	245	13	6	535	10	1	489	8	2
Streblospio gynobranchiata	1,168	0	1	0	0	0	54	11	23	1,078	0	0	1
Limnodrilus hoffmeisteri	1,113	0	0	0	248	148	23	67	1	0	400	200	26
Chironomus sp.	738	22	3	1	76	160	17	31	143	117	6	95	67
Laeonereis cf. *longula*	730	216	160	135	115	40	5	0	1	58	0	0	0
Hyalella azteca	709	0	0	0	108	2	0	564	1	0	34	0	0
Polypedilum halterale grp.	699	0	0	0	121	47	39	32	6	0	325	123	6
Streblospio sp.	620	0	1	0	1	44	556	0	0	0	0	0	18
Polypedilum scalaenum grp.	281	0	0	0	85	5	24	0	2	0	157	8	0
Dicrotendipes sp.	267	0	0	0	160	5	0	58	34	4	0	5	1

Table 5.3-10: Benthic macroinvertebrate taxa richness of the LHR target zone by segment and period (Analysis Days)

River Segment	Period 1	Period 2	Period 3	Period 5	Segment Richness
Upper	31	97	81	90	184
Middle	16	74	55	55	125
Lower	21	70	36	55	114

Table 5.3-11: Benthic macroinvertebrate diversity of the LHR target zone by river segment and period (Analysis Days)

River Segment	MFL Period	Diversity
Upper	Period 1	2.3
Upper	Period 2	2.8
Upper	Period 3	2.6
Upper	Period 5	3.0
Middle	Period 1	1.6
Middle	Period 2	2.4
Middle	Period 3	2.3
Middle	Period 5	2.5
Lower	Period 1	1.8
Lower	Period 2	2.5
Lower	Period 3	2.0
Lower	Period 5	2.3

5.3.2.3 LHR Target Zone Nekton

Nineteen of the 20 most common nekton taxa within the target zone were fish (e.g., *Menidia* sp., *Anchoa mitchilli*, and *Gambusia holbrooki*). The Daggerblade Shrimp (*Palaemonetes pugio*) was also commonly found (Table 5.3-12). Taxa richness ranged from 59 in the upper segment to 69 in the lower segment (Table 5.3-13). Shannon diversity varied from 0.51 to 2.02, with this range occurring in the lower segment (Table 5.3-14).

Table 5.3-12: Abundance of 20 most common nekton in the LHR target zone by segment and period (Analysis Days)

Taxon Name	Total	Period 1 Upper	Period 1 Middle	Period 1 Lower	Period 2 Upper	Period 2 Middle	Period 2 Lower	Period 3 Upper	Period 3 Middle	Period 3 Lower	Period 4 Upper	Period 4 Middle	Period 4 Lower	Period 5 Upper	Period 5 Middle	Period 5 Lower
Menidia sp.	92,443	1,524	30,681	15,815	3,283	13,689	23,015	539	191	2,445	481	1	779	0	0	0
Menidia beryllina	40,178	0	0	0	0	0	0	0	0	0	0	0	0	3,582	3,242	33,354
Anchoa mitchilli	30,745	431	57	11,383	6	1,806	1,847	14	7,110	6,444	21	807	1	43	206	569
Palaemonetes pugio	29,126	3,205	11,060	7,521	186	1,131	2,897	58	167	2,779	0	13	107	0	0	2
Brevoortia sp.	22,868	162	19,658	1,759	0	410	178	1	49	649	0	0	0	0	1	1
Gambusia holbrooki	15,620	2,683	4,306	351	1,196	19,19	875	712	1,247	1,371	13	2	147	358	269	171
Lucania parva	10,258	1,861	835	726	2,384	377	802	752	666	1,264	19	22	15	108	3	424
Poecilia latipinna	3,993	447	2170	301	130	78	504	9	13	316	0	0	7	1	1	16
Cyprinodon variegatus	2,009	8	9	108	336	306	871	8	38	323	0	0	2	0	0	0
Eucinostomus harengulus	1,604	0	14	23	19	75	63	8	59	274	0	0	21	151	77	820
Trinectes maculatus	1,582	69	106	205	104	150	205	61	56	144	17	16	106	71	220	52
Mugil cephalus	1,241	0	10	80	0	1	440	0	4	271	0	0	2	1	9	423
Microgobius gulosus	1,063	47	45	152	51	189	209	0	7	9	0	20	7	0	19	308
Leiostomus xanthurus	930	2	168	573	7	16	66	0	3	23	0	0	3	0	3	66
Tilapia sp.	880	76	173	46	233	221	78	0	18	35	0	0	0	0	0	0
Eucinostomus sp.	785	0	5	42	0	53	70	3	103	506	0	0	3	0	0	0
Fundulus seminolis	572	0	0	0	133	21	5	86	64	37	3	0	2	115	48	58
Fundulus grandis	485	1	28	180	10	14	155	0	0	48	0	0	3	0	0	46
Gobiosoma bosc	469	31	4	64	16	71	135	3	3	16	0	4	16	11	48	47
Lepomis macrochirus	431	5	1	0	99	18	4	9	35	6	142	0	33	65	14	0

Table 5.3-13: Nekton taxa richness of the LHR target zone by segment and period (Analysis Days)

Segment	Period 1	Period 2	Period 3	Period 4	Period 5	Segment Richness
Upper	25	44	25	15	25	58
Middle	31	44	37	13	32	66
Lower	38	46	46	28	34	68

Table 5.3-14: Nekton diversity of the LHR target zone by segment and period (Analysis Days)

River Segment	Period	Shannon Diversity
Upper	1	1.79
Upper	2	1.86
Upper	3	1.67
Upper	4	1.24
Upper	5	1.08
Middle	1	1.41
Middle	2	1.37
Middle	3	1.20
Middle	4	0.50
Middle	5	1.04
Lower	1	1.57
Lower	2	1.27
Lower	3	2.02
Lower	4	1.67
Lower	5	0.51

5.3.3 LHR Target Zone and Downstream Zone Qualitative Conclusions

Zooplankton, benthic macroinvertebrate, and nekton communities in the LHR target zone and downstream area were generally characterized by high abundance, high taxa richness, and moderate-to-low diversity. The taxa present in this nursery area of transitional salinity are common in Gulf Coast estuaries, representing a variety of functional feeding groups, indicating the presence of a robust food web.

6 QUANTITATIVE EVALUATION OF THE TARGET ZONE

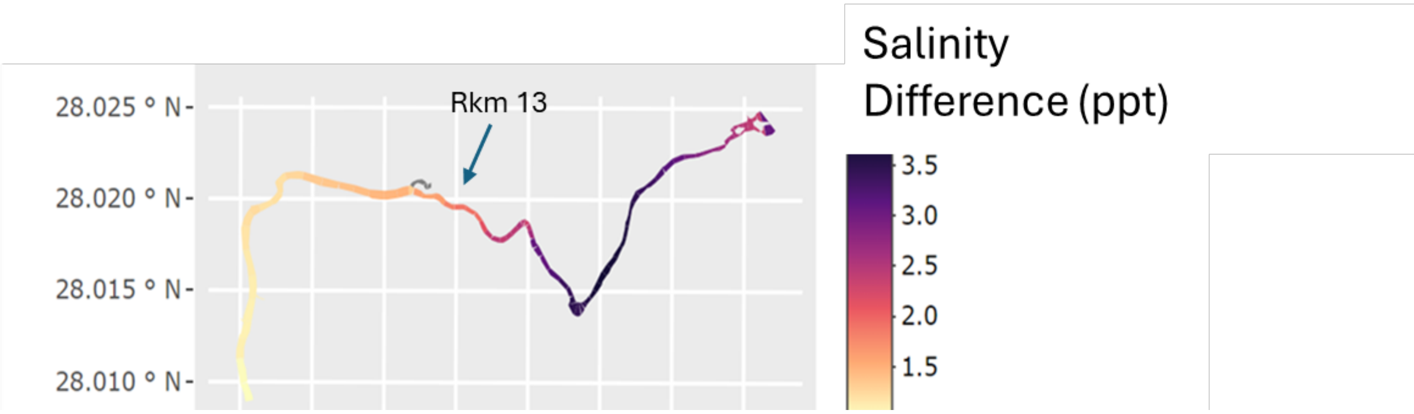
6.1 Target Zone Water Quality

This chapter details the quantitative evaluation of the effects of minimum flows implementation in the LHR to address the specific question: "What were the effects of minimum flow implementation on water quality in the Lower Hillsborough River?" The analysis focuses on the target zone, that area between the dam and the Sligh Avenue overpass, as supported by the cumulative results of qualitative analysis (Chapter 5), suggesting that the effects are generally restricted to the target zone of the river. Similarly, the analysis is restricted to water quality parameters including salinity, DO, water temperature, and pH. The quantitative analysis includes results of a mixed-effects GLM to test for differences among periods and segment within the target zone, site-specific regression analysis (linear and logistic) of the relationship between flow and the parameter of interest to determine if flows during times of minimum flow implementation effected water quality (or water quality threshold values) at a particular site, and GAM models to estimate nonlinear responses of these parameters throughout the target zone as a function of multiple explanatory variables including implementation flows based on all data collected within the target zone. Logistic regressions were only applied to those parameters for which there are known threshold values affecting the biological integrity of the lower river (i.e., DO < 2.5 mg/L and salinity > 5 ppt) and only at sites where at least 100 observations were available for analysis. All quantitative evaluations were based on data collected during Analysis Days only. Modeling results with statistically significant outcomes are summarized within the main body of this report in the tables and figures below, and detailed statistics for all modeling fits are provided in Appendix Q4.

The GAM modeling effort was performed to extend the conceptual framework used by LAMFE to estimate the response of salinity throughout the target zone as a function of flows to the other water quality parameters of interest. GAM modeling uses a different approach for estimation than LAMFE, and LAMFE is considered the gold standard for evaluating the effects of flows on salinity and temperature in the LHR. The LAMFE model uses hydrodynamic equations to mechanistically describe and predict the movement of water and changes to salinity and temperature, making it the most accurate model for evaluating the effects of flows on salinity in the LHR. The GAM models were developed for salinity to build confidence that the GAM method can reasonably capture the effect of flows by comparing the inference from the GAM to that of LAMFE (Chen 2024, Appendix J) and then the GAM framework was extended to model the effects of dissolved oxygen, pH, and water temperature.

Hydrodynamic modeling using LAMFE demonstrated a substantial improvement of low-salinity habitats in the upstream portion of the river. An example plot of that output for the 2018–2023 period is provided in Figure 6.1-1, representing the average water column salinity difference between an existing condition (the implementation of the MFL as observed to date) and the No MFL condition (an estimate of what the flows would have been without the MFL implementation). The LAMFE modeling results suggest that, on average over this period, salinity differences greater than 2 ppt would be restricted to areas above Rkm 13, or approximately the center of the middle segment of the LHR. This result also supports the focus of quantitative analysis of the biological data (Section 6.2) to the target zone of the river since salinity is the principal forcing function for changes in biological communities in estuarine systems.

Figure 6.1-1: Average difference in water column salinity between the existing and No MFL condition predicted by LAMFE model for implementation flows between 2018 and 2023



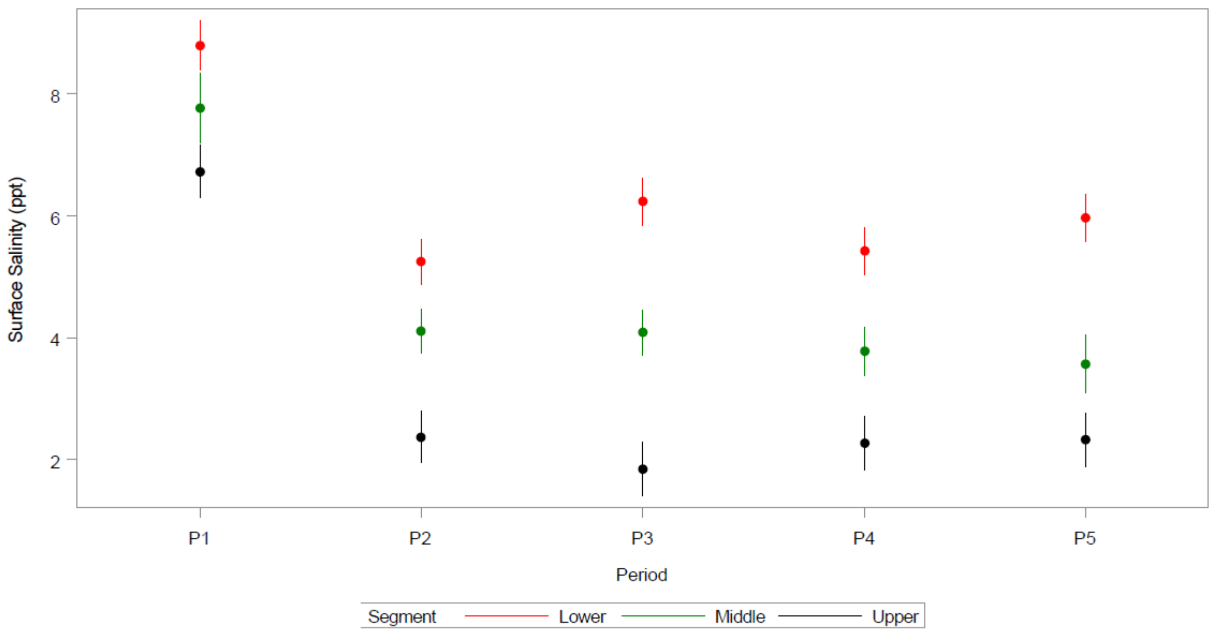
6.1.1 Salinity

6.1.1.1 Surface Level

Comparison of Means with General Linear Models (GLM)

The mixed effects GLM results suggested highly significant effects for period, segment, and the interaction between segment and period for surface salinity. The average salinity concentrations with adjusted 95% confidence intervals resulting from these comparisons are provided in Figure 6.1-2. The effect of initial implementation is seen in all segments with significantly higher surface salinity in Period 1 than in other periods. Although there were clearly differences among segments in later comparisons, differences among periods were segment-dependent with the upper segment salinities being the closest to freshwater and similar among Periods 2 through 5.

Figure 6.1-2: Average surface salinity (ppt) (least squared means) with confidence intervals for surface salinity



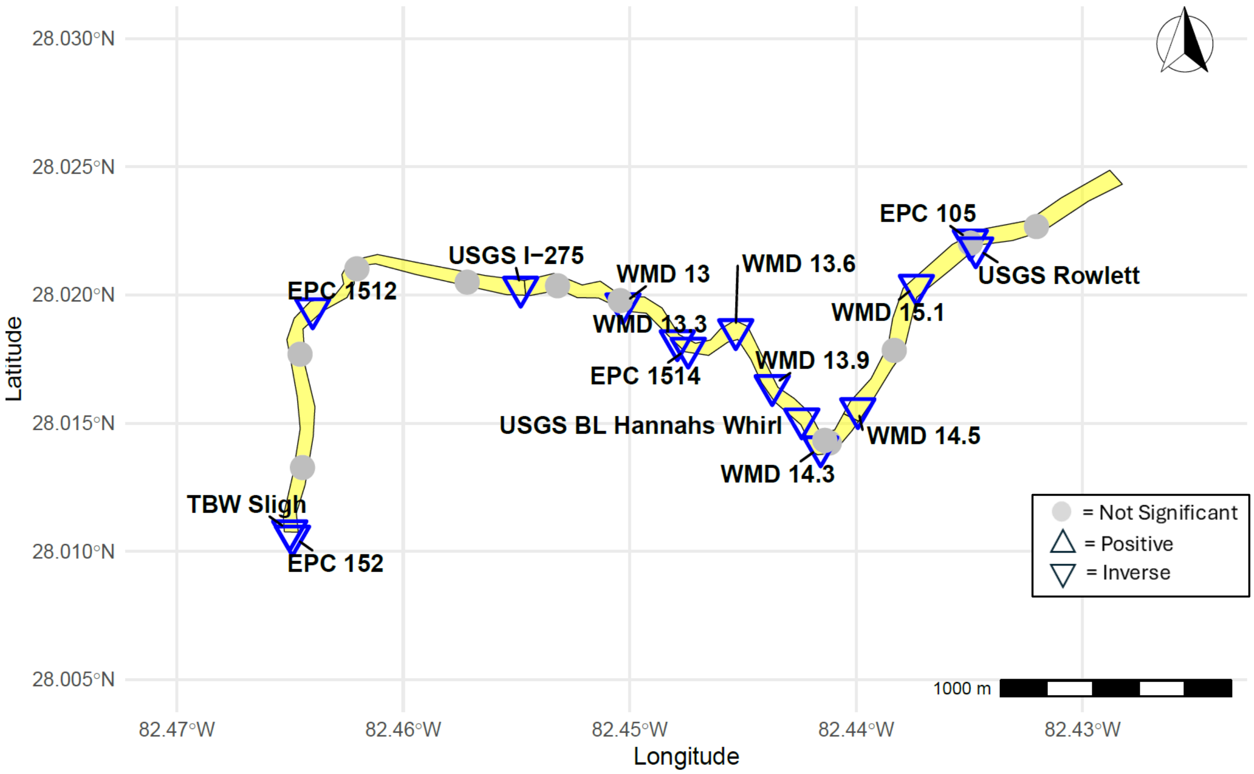
Linear Regression

The results of station-specific surface salinity linear regression relationships with the 7-day lag average flows are provided in Table 6.1-1. In univariate assessments of the most appropriate lag term, the 7-day average was the most consistently significant and the best fit lag average term to use for this analysis of the target zone. All significant relationships were inverse (indicated by a value of negative 1 in the far right column of Table 6.1-1 and by a downward facing triangle in Figure 6.1-3), suggesting that higher flows decrease salinity during implementation conditions. The regressions generally explained less than 50% of the total variation in salinity despite efforts to identify the most appropriate antecedent condition to capture the systematic effects of implementation on surface salinities. The spatial distribution of stations with significant results is provided in Figure 6.1-3 and depicts that the effects of salinity were mostly oriented toward the upper portion of the target zone near the dam, as expected; however, not every site displayed a statistically significant relationship (as denoted by grey filled circle in Figure 6.1-3). All results, including those without statistically significant effects, are provided in Appendix Q1. Although there was potential for autocorrelation in the CR time series to have affected the determination of statistical significance for some stations, the fragmented time series caused by only selecting Analysis Days eliminated the ability to perform true time series analysis for these gages. However, the fact that both fixed grab sample station and CR stations reported similar outcomes supports the observed results and the underlying premise of the recovery strategy that increasing flows to the LHR will reduce salinity in the target zone.

Table 6.1-1: Linear regression results for surface salinity in the target zone of the LHR

Station	N	Month p Value	Flow p Value	Flow Slope	R square	Slope Direction
EPC 105	144	ns	<0.001	-0.183	0.38	-1
EPC 1512	59	ns	0.006	-0.124	0.13	-1
EPC 1514	59	0.002	0.036	-0.069	0.49	-1
EPC 152	131	0.012	<0.001	-0.133	0.30	-1
SWFWMD LHR04_800045_RKm_15.1	68	<0.001	0.003	-0.058	0.44	-1
SWFWMD LHR06_800047_RKm_14.5	71	0.004	<0.001	-0.092	0.43	-1
SWFWMD LHR07_800048_RKm_14.3	70	0.003	<0.001	-0.098	0.43	-1
SWFWMD LHR08_800049_RKm_13.9	70	0.014	<0.001	-0.107	0.40	-1
SWFWMD LHR09_19209_RKm_13.6	66	ns	0.004	-0.095	0.12	-1
SWFWMD LHR10_800050_RKm_13.3	70	ns	0.008	-0.078	0.10	-1
SWFWMD LHR11_800052_RKm_13	70	ns	0.024	-0.068	0.07	-1
TBW Sligh	2,887	<0.001	<0.001	-0.089	0.17	-1
USGS 02304510 Rowlett	4,476	<0.001	<0.001	-0.194	0.42	-1
USGS 02304517 BL Hannah's Whirl	710	<0.001	0.006	-0.031	0.55	-1
USGS 023060013 I-275	2,126	<0.001	<0.001	-0.113	0.18	-1

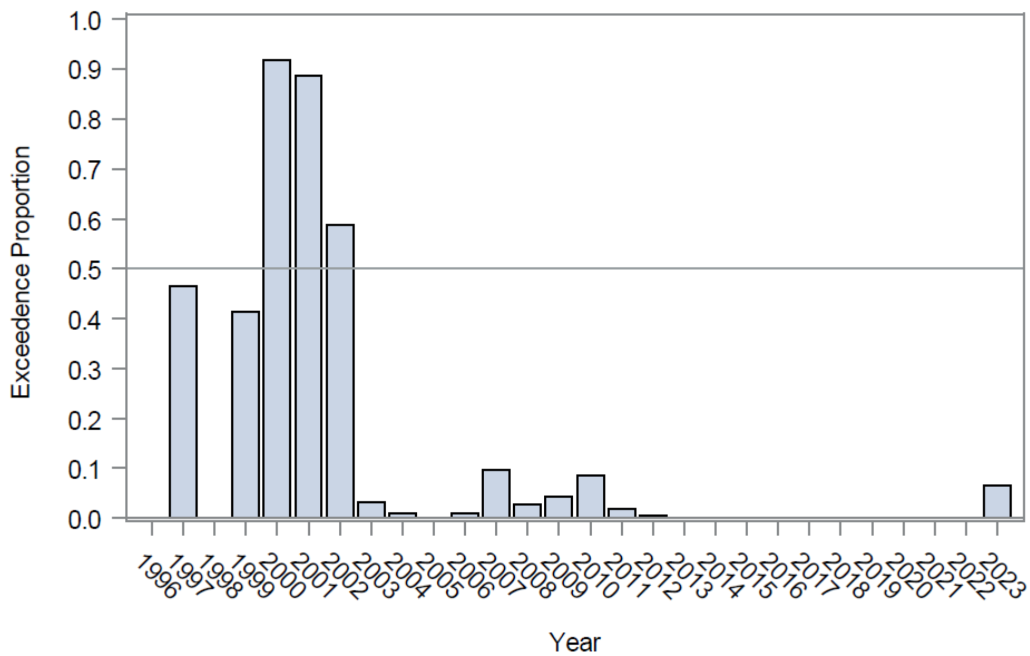
Figure 6.1-3: Surface salinity stations with statistically significant relationships with flow in the target zone (labeled) and direction of the triangle indicating direction of relationship with flow



Logistic Regression (Salinity > 5ppt)

Logistic regression requires more samples for proper inference, and once sites were restricted to only those with at least 100 observations, four stations (USGS 02304510 Rowlett, TBW Sligh, USGS 023060013 I-275, and EPC 152) reported a significant effect of flow on the probability of exceeding a surface salinity of 5 ppt. In all cases the direction of the effect was inverse, indicating that as flows increase the probability of an exceedance decreases, which supports the results of linear regression. Evaluation of the exceedance proportions at USGS 02304510 Rowlett suggests that once implementation of the initial MFL was established, the probability of exceeding 5 ppt surface salinity at this station was dramatically reduced (Figure 6.1-4). The logistic regression version of the R^2 statistic less than 0.50 suggests a relatively weak relationship at all but USGS 02304510 Rowlett, the most upstream station. Details of logistic model results for stations meeting criteria are provided in Appendix Q2.

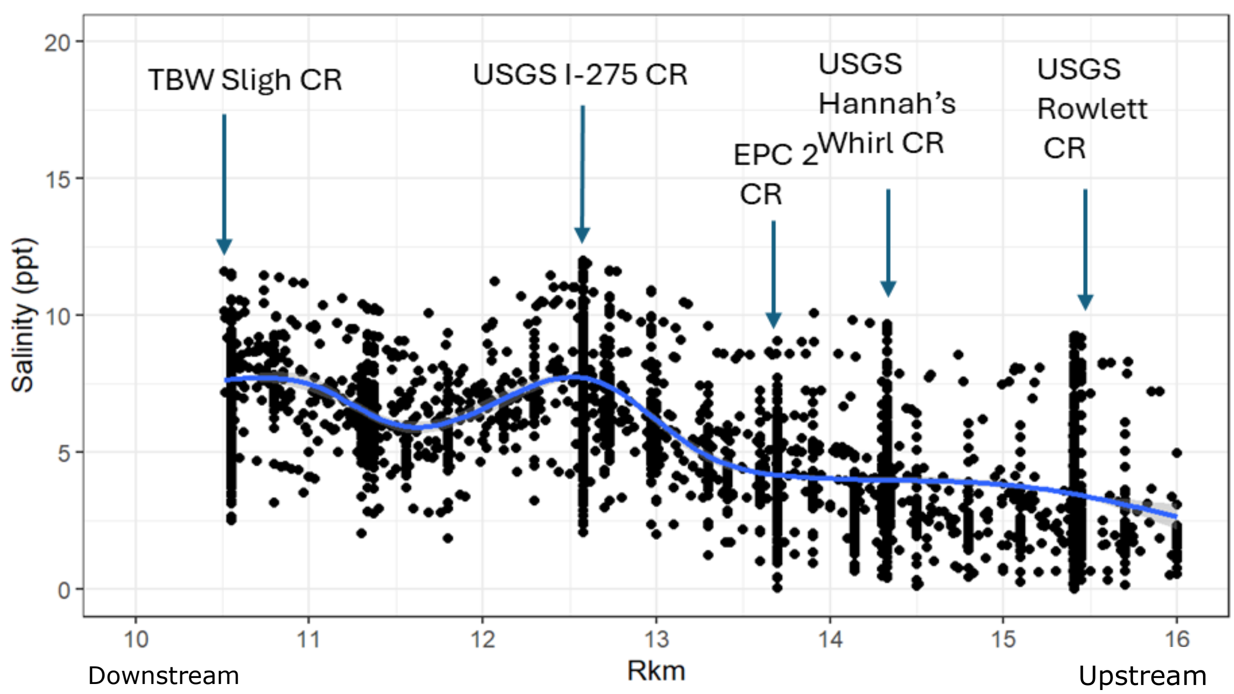
Figure 6.1-4: Annual surface salinity exceedance proportions of 5 ppt at USGS 02304510 Rowlett CR (4476 daily average observations)



Nonlinear Regression (GAM) Modeling

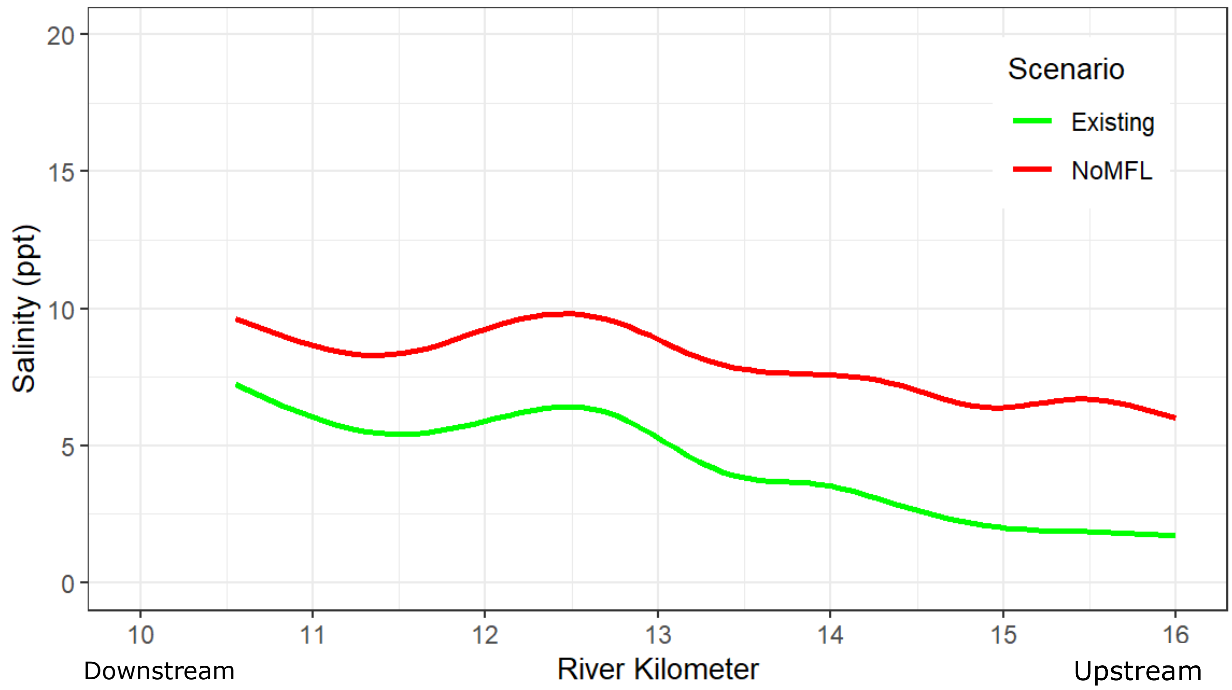
Advantages of generalized additive models (GAM) are that they allow for the generation of predictions throughout the entire target zone for any given day or for a combination of days using a statistic (e.g., the average) and allow for the simulation of the effects of management actions on the response. Based on AIC and BIC criteria, the 14-day lag average flow was best suited for GAM modeling of surface salinity, and the final model selected (Model 3) included smoothed functions for river kilometer, lag average flow, and an interaction term for river kilometer and lag average flow as well as the month covariate. This model explained 56% of the deviance in the observed data and all smoothed functions were highly statistically significant ($p<0.001$). The smoothed average model prediction for the observed data used to develop the model is plotted in Figure 6.1-5 where the blue line is the local average model prediction and the black-filled circles represent observed data. The smoothed average line does not represent date-specific model predictions and therefore may reflect more curvature than model predictions for any specific date. The USGS CR are highlighted in this plot since they contributed such a large portion of the data used to develop the model. The CR data are daily averages rather than instantaneous profiles as with the other data used to develop the model, which may also contribute to the sinusoidal shape of the smoothed curve. GAM modeling with and without the use of the CR data (provided as supplemental content in Appendix Q3), also supports this conclusion that the CR data may be introducing some sinusoidal shape to the curve; however, the intent of this report was to use all available data in the modeling effort. The predicted salinity at the base of the dam was approximately 2.5 ppt, which corresponds well to the average salinity concentration of the Sulphur Springs source water that made up a large percentage of the flow. Predicted salinity increases with distance downstream as expected and reached a predicted average of 5 ppt at approximately kilometer 13.25 and 7.5 ppt near the end of the target zone.

Figure 6.1-5: GAM model predictions for surface salinity (blue line) as a function of river kilometer based on observed data (black-filled circles) used to develop the model



The model was then used to predict surface salinity for all Analysis Days for the most recent period (i.e. 2018–2023) under both the existing condition and a No MFL condition. The results of that comparison suggest that the existing condition provides a low-salinity habitat as defined by the minimum flows rule for the first approximately 3 kilometers of the LHR and that a No MFL condition would result in salinity of at least 5 ppt on average to the base of the dam (Figure 6.1-6). These predictions generally agree with the LAMFE model results in suggesting that an approximately 3-kilometer low-salinity surface habitat in the LHR is expected under existing conditions on average, lending support to the use of GAMs for other water quality parameters. The difference in model predictions diminishes toward the downstream end of the target zone where the difference between the existing condition and the No MFL condition is only approximately 2 ppt, indicating that there were diminishing returns on implementing minimum flows with distance downstream, also supporting the LAMFE results.

Figure 6.1-6: Surface salinity GAM model predictions under Existing and No MFL scenarios for Analysis Days between 2018 and 2023

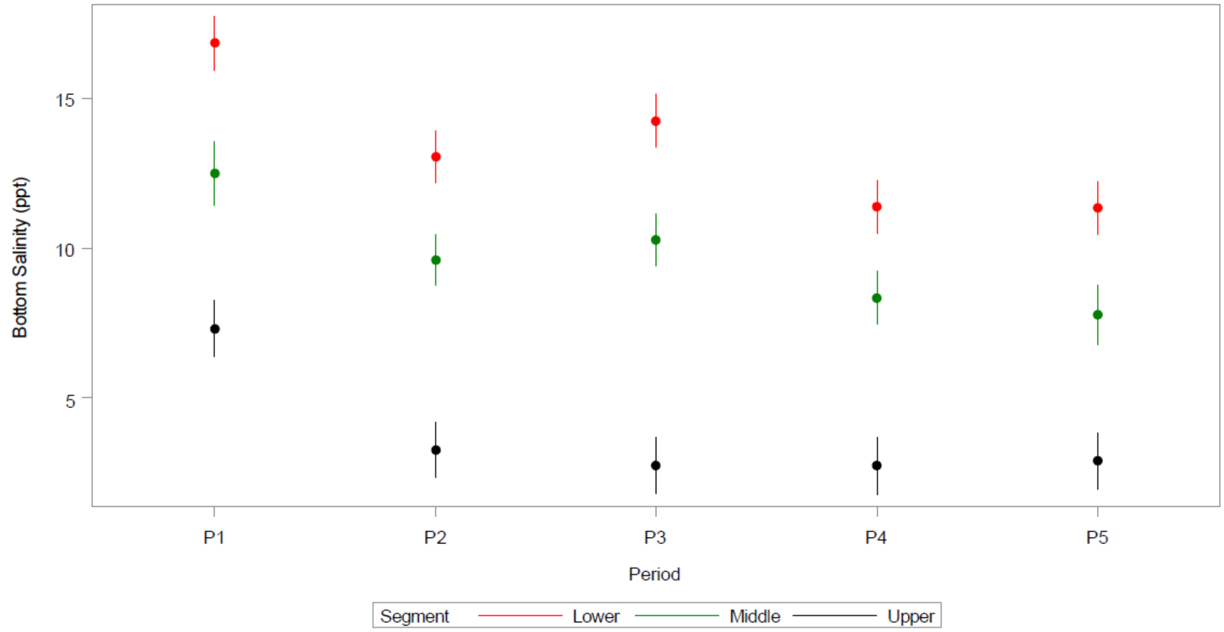


6.1.1.2 Bottom Salinity

Comparison of Means with General Linear Models (GLM)

The mixed effects GLM results suggested highly significant effects for period, segment, and the interaction between segment and period. The least squared means resulting from these comparisons are provided in Figure 6.1-7. The effect of initial implementation is seen in all segments with significantly higher bottom salinity in Period 1 than other periods. The period differences after Period 1 were seen more in the middle and lower segment as implementation flows increased and the bottom salinity in the middle and lower segment decreased. However, the most recent two periods were not different from one another in any segment.

Figure 6.1-7: Least squared means with confidence intervals for bottom salinity (ppt)



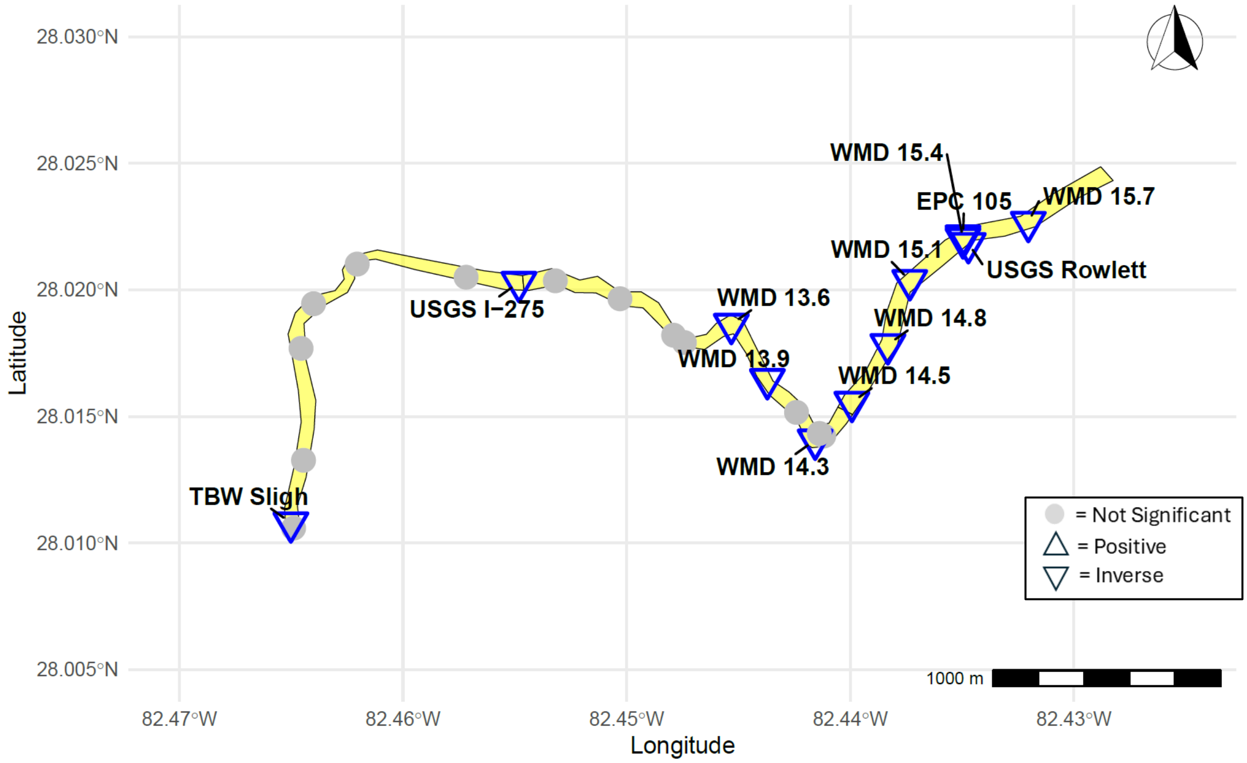
Linear Regression

Linear regression results for bottom salinity were similar to the surface salinity results in that all significant relationships were inverse as presented in Table 6.1-2. Significant results were concentrated in the upper and middle sections of the river, though not every station displayed significant results (Figure 6.1-8).

Table 6.1-2: Linear regression results for bottom salinity in the target zone of the LHR

Station	N	Month p Value	Flow p Value	Flow Slope	R square	Slope Direction
EPC 105	144	0.007	<0.001	-0.207	0.47	-1
SWFWMD LHR02_800044_RKm_15.7	47	0.011	0.046	-0.050	0.46	-1
SWFWMD LHR03_19208_RKm_15.4	62	0.002	0.001	-0.084	0.45	-1
SWFWMD LHR04_800045_RKm_15.1	60	0.002	<0.001	-0.109	0.49	-1
SWFWMD LHR05_800046_RKm_14.8	69	<0.001	<0.001	-0.135	0.56	-1
SWFWMD LHR06_800047_RKm_14.5	69	<0.001	<0.001	-0.144	0.56	-1
SWFWMD LHR07_800048_RKm_14.3	70	0.004	0.005	-0.228	0.36	-1
SWFWMD LHR08_800049_RKm_13.9	70	0.022	0.004	-0.238	0.32	-1
SWFWMD LHR09_19209_RKm_13.6	65	ns	0.002	-0.284	0.14	-1
TBW Sligh	2,997	<0.001	<0.001	-0.119	0.18	-1
USGS 02304510 Rowlett	4,416	<0.001	<0.001	-0.196	0.44	-1
USGS 023060013 I-275	2,121	<0.001	<0.001	-0.202	0.33	-1

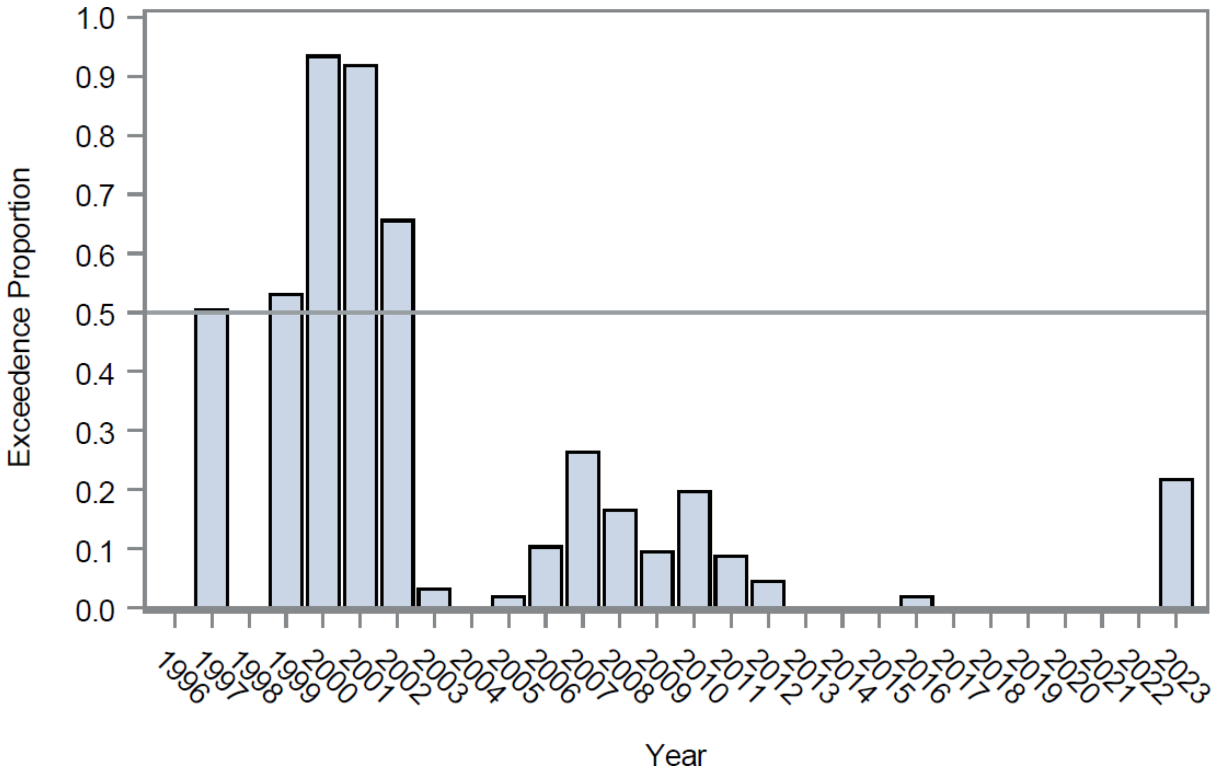
Figure 6.1-8: Bottom salinity stations with statistically significant relationships with flow in the target zone (labeled) and direction of the triangle indicating direction of relationship with flow



Logistic Regression (Salinity > 5ppt)

Logistic results for bottom salinity were similar to surface salinity but restricted to more upstream locations in the target zone (i.e. USGS 02304510 Rowlett, USGS 023060013 I-275, EPC 2 CR, and EPC 105). Similar to surface salinity, all sites were inversely related to flows, indicating that as flows during analysis days increased, the probability of exceeding 5 ppt decreased. The exceedance frequency for USGS 02304510 Rowlett bottom salinity Figure 6.1-9 shows that, similar to surface salinity results, once initial implementation of the MFL was established, the exceedance percentage was dramatically reduced and greater than 10% only during drought years.

Figure 6.1-9: Annual bottom salinity exceedance proportions of 5 ppt at USGS 02304510 Rowlett CR



Nonlinear Regression (GAM) Modeling

Based on AIC and BIC criteria, Model 3 using the 28-day lag average flow was best suited for GAM modeling of bottom salinity, and the final model selected included smoothed functions for river kilometer, lag average flow, and an interaction term for river kilometer and lag average flow, which were all highly statistically significant ($p<0.001$). This model explained 74% of the variance in the observed data. The smoothed average model prediction for the observed data used to develop the model is plotted in Figure 6.1-10 where the blue line is the local average model prediction and the black-filled circles represent observed data. The predicted salinity at the base of the dam over the course of the entire POR was approximately 2.5 ppt, which corresponds well to the average salinity concentration of the Sulphur Springs source water. Salinity increases downstream as expected and reached a predicted average of 5 ppt at approximately Rkm 15, farther upstream than surface salinity (Figure 6.1-11). The inference from this comparison is that minimum flows have, on average, resulted in an approximately 1.5-kilometer increase in available low-salinity bottom habitat defined as a salinity < 5 ppt (Figure 6.1-12).

Figure 6.1-10: GAM model predictions for bottom salinity (blue line) as a function of river kilometer based on observed data (black filled circles) used to develop the model

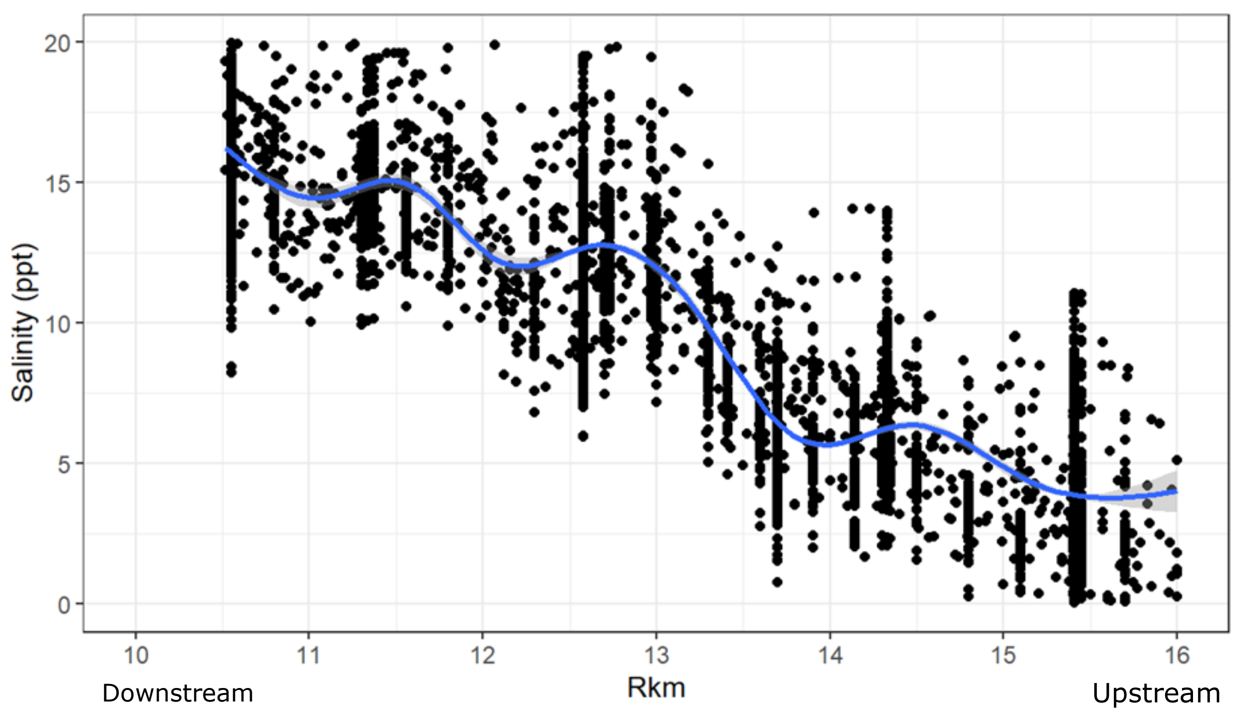


Figure 6.1-11: Bottom salinity GAM model predictions under existing and No MFL scenarios for Analysis Days between 2018 and 2023

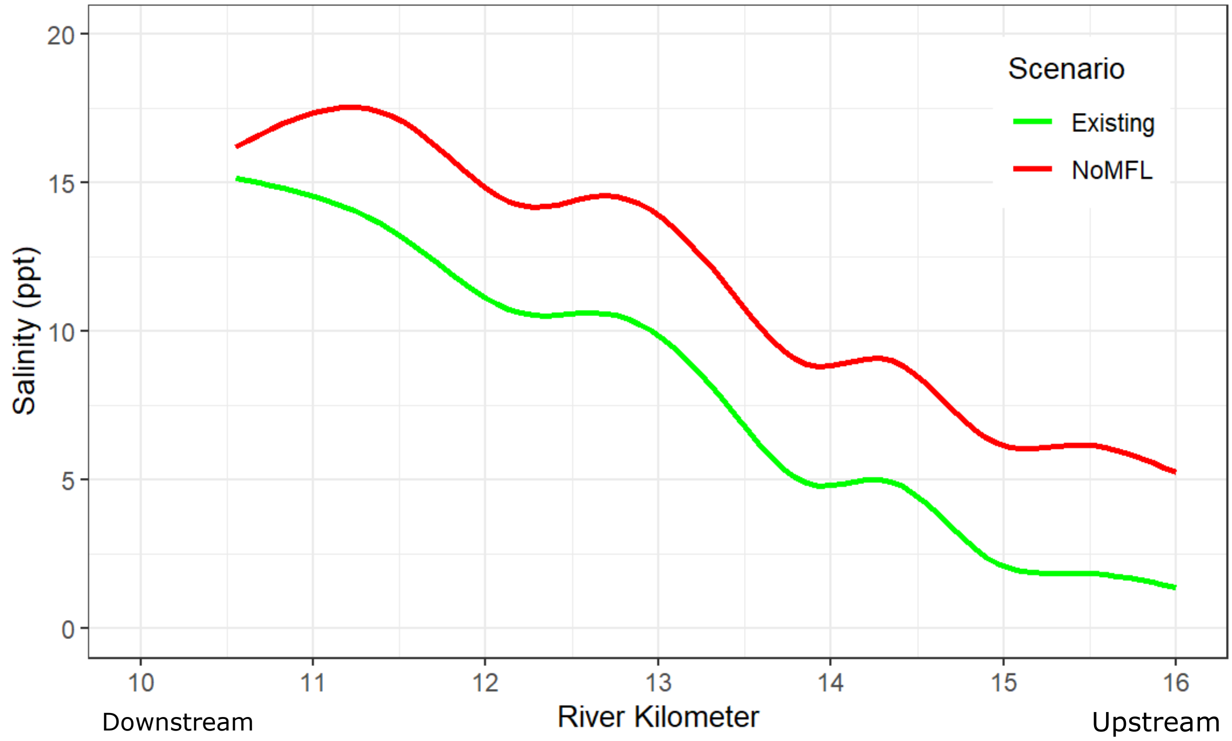
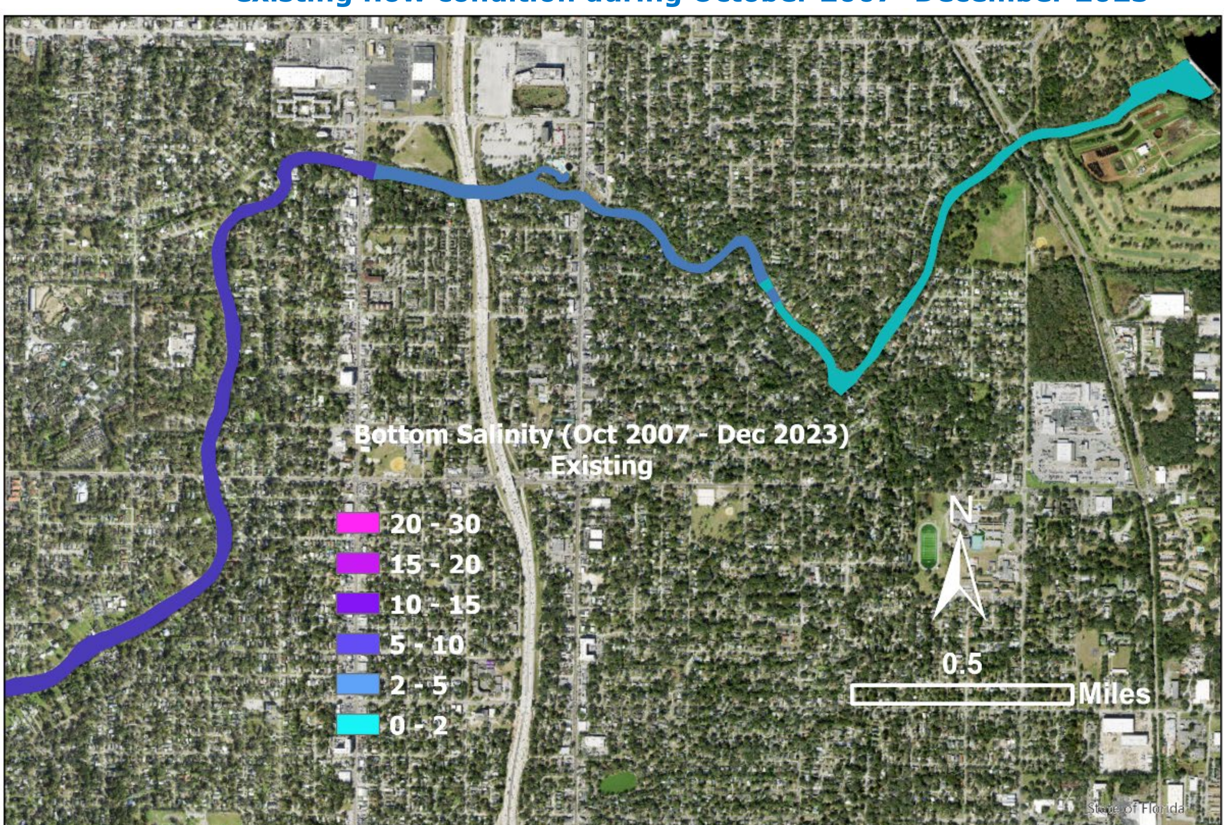


Figure 6.1-12: Simulated mean bottom salinity by the LAMFE model under the existing flow condition during October 2007–December 2023

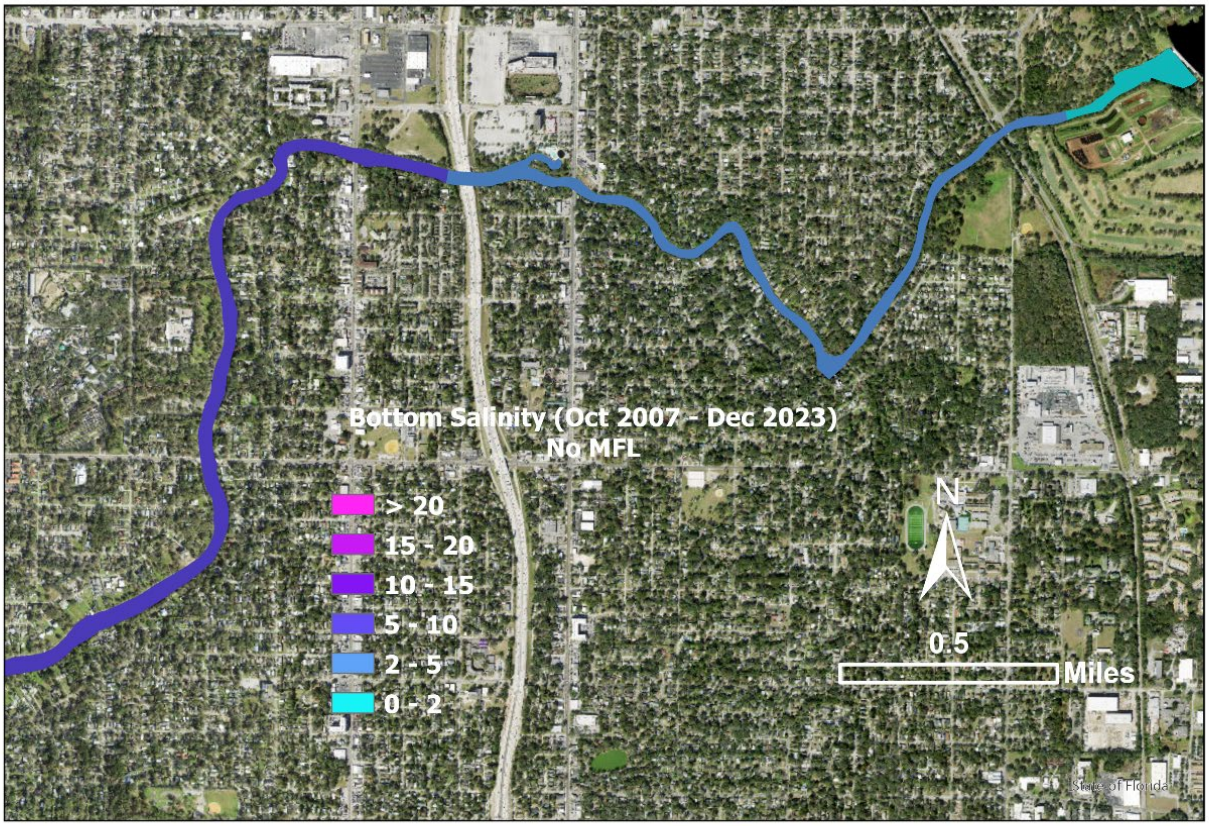


LAMFE Salinity Summary

In 2023, the laterally averaged 2D model LAMFE was used to simulate hydrodynamics, salinity transport processes, and thermal dynamics during October 2007–June 2023, before the simulation period was extended to December 2023 once data driving the model for the second half of 2023 were available in early 2024. Later, the LAMFE model was used to simulate hydrodynamics and salinity transport processes in the LHR for the periods of January 1997–August 1997 and February 2001–December 2023. Three flow scenarios were simulated, including the existing flow condition (actual operations), the full minimum flow implementation condition (the adopted minimum flow at that point in time), and the no minimum flow implementation condition.

Model simulations indicate a significant improvement for low salinity habitats in the upstream portion of the LHR. Figure 6.1-12 shows simulated mean bottom salinity in the upstream portion of the LHR during October 2007–December 2023 under the existing flow condition, while Figure 6.1-13 shows simulated mean bottom salinity during the same period under the no minimum flow condition. From the two figures, one can visually see the differences of the location of the 2-ppt and 5-ppt isohalines. The LAMFE model bottom layer follows the thalweg of the river and thus represents the lowest layer of each river transect. Because the thalweg varies along the river, salinity results shown in Figure 6.1-12 and Figure 6.1-13 are not at the same elevation. In addition, there are some differences between LAMFE bottom salinity and GAM bottom salinity. GAM and LAMFE used different sets of salinity data in model development, and GAM predictions were based on salinity measurements not necessarily taken at the thalweg.

Figure 6.1-13: Simulated mean bottom salinity by the LAMFE model under the no MFL flow conditions during October 2007–December 2023



If only days when MFL implementation were required are considered during October 2007–December 2023, the improvement is even more impressive. Table 6.1-3 lists annual averages of water volumes for salinity ≤ 2 ppt and ≤ 5 ppt between the dam and the Sulphur Springs confluence in the LHR during minimum flow implementation required days under the existing (operations), MFL (current adopted minimum flow rule), and No MFL (no minimum flow pumping) conditions. From the table, one can see that 2015 was a relatively wet year and had the highest values of ≤ 2 ppt and ≤ 5 ppt water volumes under any of the three flow conditions. On the other hand, 2023 was quite dry and had the lowest values of the low salinity habitats. Under the No MFL flow condition, the average ≤ 2 ppt water volume during the MFL-required days in 2023 was only 8,280 m^3, which was only about 6.3% of that in 2015. Nevertheless, 2023 had the biggest improvement of the ≤ 2 ppt water volume during the MFL-required days with a full MFL implementation, which expanded the salinity habitat to 36,090 m^3, representing an increase of 335.9%. The improvement of ≤ 5 ppt water volume during the MFL-required days caused by full MFL implementation was even more significant in 2023, when the salinity habitat increased from 19,720 m^3 to 123,130 m^3, or a 524.4% increase.

Similar improvements for ≤ 2 ppt and ≤ 5 ppt bottom areas and shoreline lengths were also achieved with the implementation of MFL during the 16-year period, especially for the MFL-required days. For example, 2023 had the biggest improvement of ≤ 2 ppt bottom area and shoreline length, with a respective increase of 321.4% and 340.0%. More details can be found in Chen (2024, Appendix J).

Table 6.1-3: Annual average water volumes (in 1000 cubic meters) during MFL-required days for salinity ≤ 2 ppt and ≤ 5 ppt between the dam and the Sulphur Springs confluence in the LHR during January 2018 –December 2023 under the existing, MFL, and No MFL flow conditions

	Salinity ≤ 2 ppt			Salinity ≤ 5 ppt		
Year	Existing	MFL	No MFL	Existing	MFL	No MFL
2008	85.19	95.54	30.26	135.64	145.35	60.44
2009	78.00	87.19	23.75	125.81	134.45	54.26
2010	77.59	103.10	20.10	140.26	160.65	62.82
2011	88.34	118.92	34.67	152.22	174.51	71.80
2012	45.85	70.38	14.21	129.43	145.91	42.86
2013	33.40	54.25	33.94	154.63	160.05	98.69
2014	61.60	78.59	63.93	174.61	183.18	140.66
2015	101.05	155.39	131.07	198.36	202.77	198.23
2016	29.30	51.47	22.35	150.01	158.73	92.32
2017	34.60	45.30	10.29	135.84	143.54	38.74
2018	39.06	51.46	18.85	149.03	154.42	76.78
2019	53.12	79.67	20.85	176.82	182.51	109.50
2020	34.19	41.41	26.48	159.24	164.04	73.95
2021	35.49	58.63	19.59	162.06	168.96	65.01
2022	57.23	66.04	39.49	152.61	156.87	85.15
2023	36.08	36.09	8.28	123.14	123.13	19.72

As the LAMFE model was set to output salinity results every 30 minutes, time series of low salinity habitats can be calculated and evaluated, revealing more details about the effect of the MFL implementation on low salinity habitats in the upstream portion of the LHR. For simplicity, only some key findings of such an evaluation of the time series of low salinity habitats are given in the following bullets. Readers are referred to Chen (2024, Appendix J) for more details.

Key findings from the evaluation of low salinity habitat time series:

- Low-salinity habitats in the most upstream segment of the LHR for the existing flow condition had a significant improvement over the No MFL flow condition.
- Since October 2007, no instances in the LHR where the water volume, bottom area, or shoreline length at salinities ≤ 5 ppt was reduced to zero have occurred.
- If no MFL rules were implemented, there would be numerous times when ≤ 5 ppt salinity habitats became zero during the 16-year period.
- For most part of the simulation period between October 2007 and December 2023, simulated low-salinity habitats for the existing flow condition were very close to those of the MFL flow condition. However, some improvements could be achieved when the MFL rules were fully implemented.

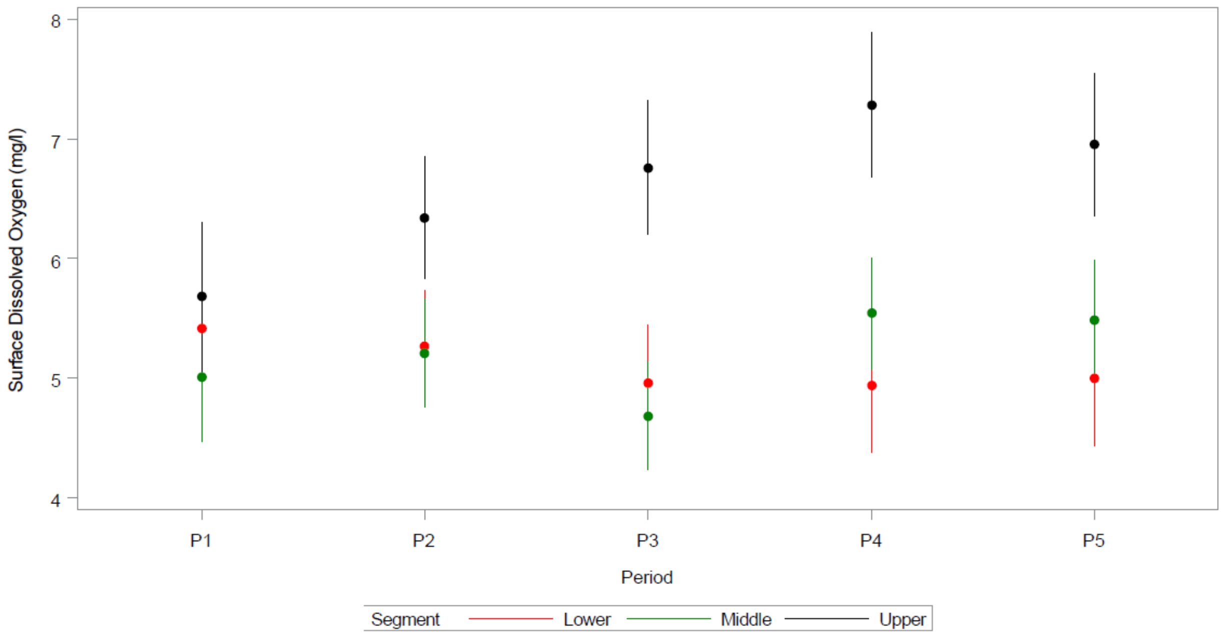
6.1.2 Dissolved Oxygen

6.1.2.1 Surface DO

Comparison of Means with General Linear Models (GLM)

Generalized linear model (GLM) results for surface DO indicated that all main effects and interactions were highly significant. However, post-hoc multiple comparison tests revealed that only the upper segment exhibited significant differences among periods (Figure 6.1-14), with Period 1 showing lower DO concentrations than Periods 3, 4, and 5, which did not differ significantly from one another.

Figure 6.1-14: Least squared means with 95% confidence intervals for surface dissolved oxygen concentrations (mg/L)



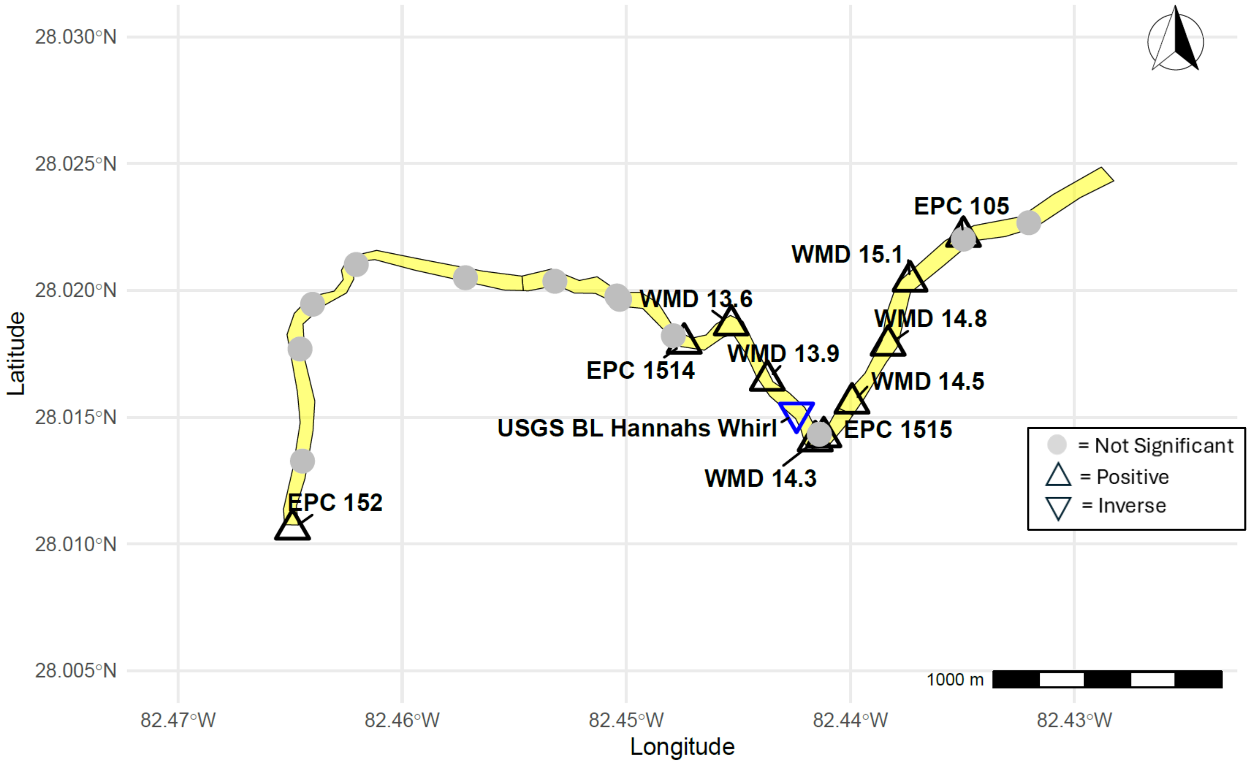
Linear Regression

Linear regression results suggested statistically significant results were mainly positive, meaning increasing implementation flows increased surface DO concentrations with the exception of the USGS site (02304517) below Hannah’s Whirl, which exhibited a negative response to increasing flows (Table 6.1-4). The significant relationships were again concentrated in the upper and portions of the middle segment of the river (Figure 6.1-15).

Table 6.1-4: Linear regression results for surface dissolved oxygen concentrations in the target zone of the LHR

Station	N	Month p Value	Flow p Value	Flow Slope	R square	Slope Direction
EPC 105	139	0.004	0.001	0.036	0.24	1
EPC 1514	55	<0.001	0.004	0.120	0.53	1
EPC 1515	51	<0.001	0.005	0.158	0.50	1
EPC 152	122	ns	0.048	0.028	0.03	1
USGS 02304517 BL Hannah's Whirl	710	<0.001	<0.001	-0.042	0.31	-1
SWFWMD LHR09_19209_RKm_13.6	66	0.001	0.023	0.070	0.40	1
SWFWMD LHR08_800049_RKm_13.9	70	<0.001	<0.001	0.097	0.44	1
SWFWMD LHR07_800048_RKm_14.3	70	<0.001	<0.001	0.144	0.57	1
SWFWMD LHR06_800047_RKm_14.5	71	<0.001	<0.001	0.144	0.48	1
SWFWMD LHR05_800046_RKm_14.8	70	0.003	<0.001	0.118	0.42	1
SWFWMD LHR04_800045_RKm_15.1	68	<0.001	<0.001	0.102	0.46	1
SWFWMD LHR01_800043_RKm_16	28	ns	0.036	0.104	0.16	1

Figure 6.1-15: Surface DO stations with statistically significant relationships with flow in the target zone (labeled) and direction of the triangle indicating direction of relationship with flow



Logistic Regression (DO < 2.5 mg/L)

Surface DO regressions resulted in significant main effects for season and flow at sites EPC 2 CR and WMD 19206 CRs; however, those results suggested that increasing flows increase the probability of a DO exceedance (i.e., lower DO in surface waters). This result does not comport with the general findings of the linear regression analysis or the GAM modeling and was further explored by including an interaction term between season and flow, which resulted in both the flow and the flow/season interaction terms becoming insignificant. Dropping the season main effect also resulted in a nonsignificant flow effect. Other sites evaluated for surface DO also resulted in nonsignificant results. While the results of the surface DO analysis might be affected by site-specific elements, the GAM modeling results and the linear regression modeling results both suggest that increasing flows have beneficial effects on surface DO in the target zone.

Nonlinear Regression (GAM) Modeling

Based on AIC and BIC criteria, Model 3 using the 28-day lag average flow was best suited for GAM modeling of surface dissolved oxygen, and the final model selected included the month covariate and smoothed functions for river kilometer, lag average flow, and an interaction term for river kilometer and lag average flow, which were all highly statistically significant ($p<0.001$). This GAM model resulted in an R squared of 37%. Nonlinear regression modeling (GAM) suggested average surface DO concentrations of approximately 5 mg/L except in the most upstream portion of the river where the DO concentrations may be affected by the recovery flows from Sulphur Springs, which are aerated by the flume discharging at the base of the dam (Figure 6.1-16). The GAM scenario evaluation suggests that the implementation flows improved surface DO concentrations by approximately 1 mg/L on average between 2018 and 2023 and that there were diminishing returns on that response with increased distance downstream (Figure 6.1-17).

Figure 6.1-16: GAM model predictions for surface DO (blue line) as a function of river kilometer based on observed data (black-filled circles) used to develop the model

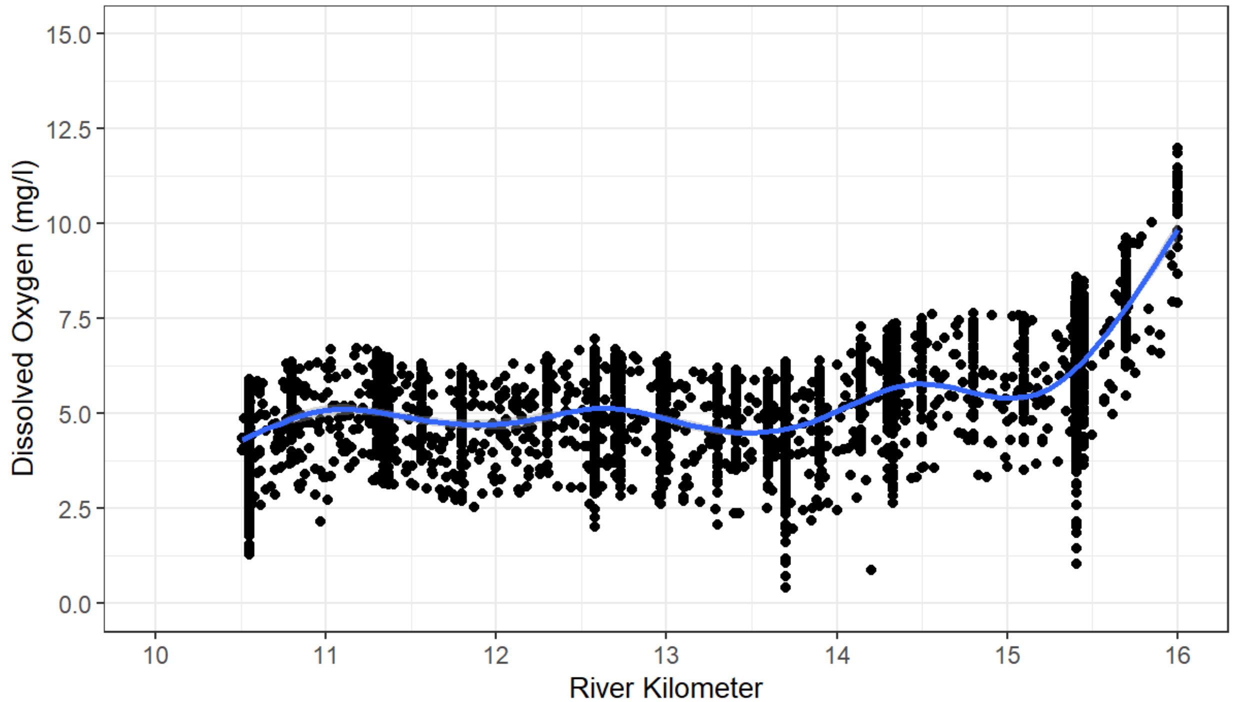
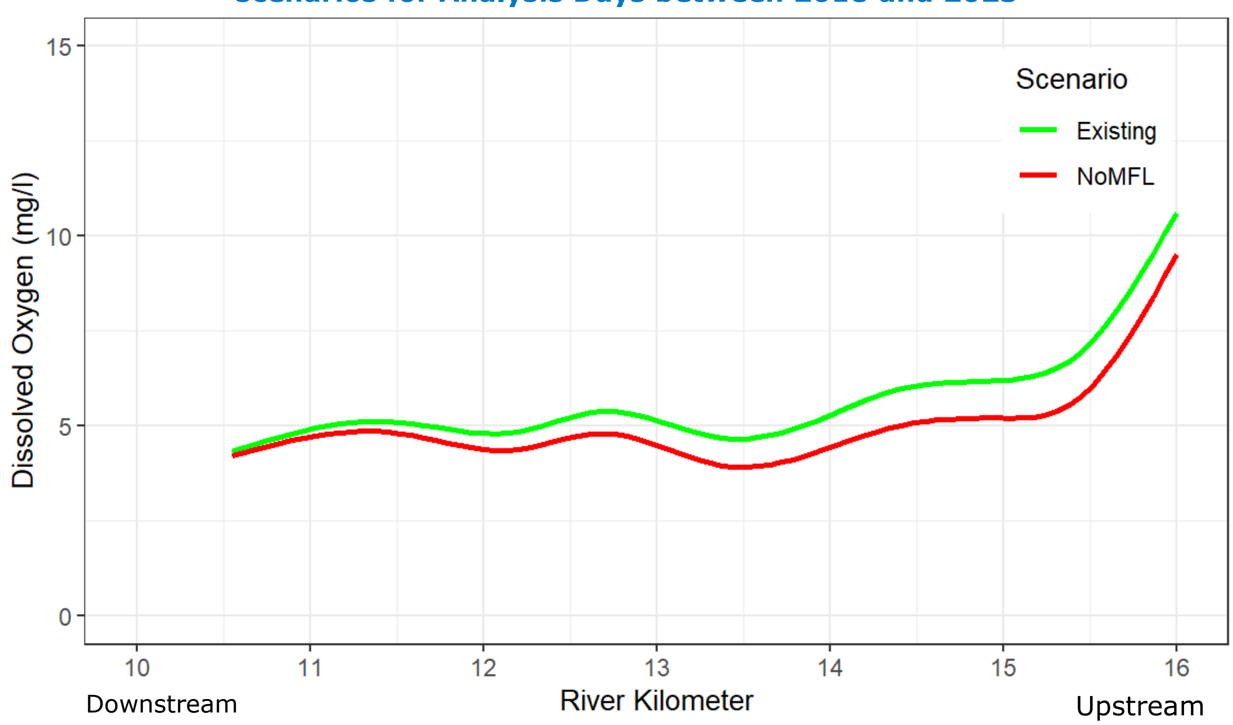


Figure 6.1-17: Surface DO GAM model predictions under existing and No MFL scenarios for Analysis Days between 2018 and 2023

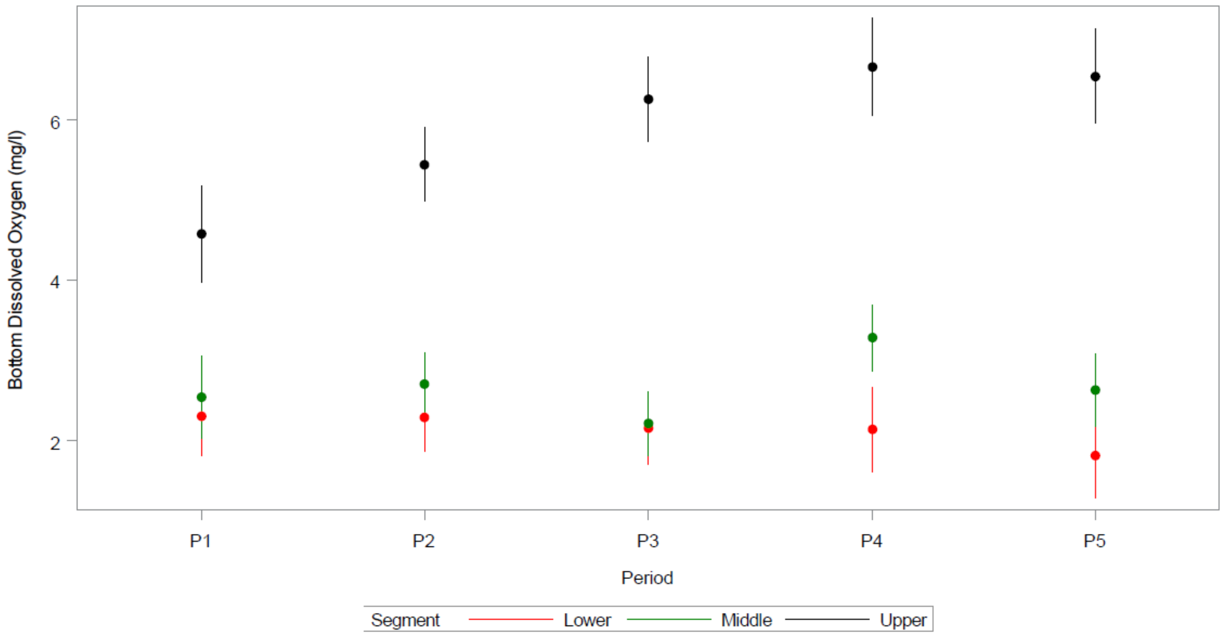


6.1.2.2 Bottom DO

Comparison of Means with General Linear Models (GLM)

GLM test results were similar to surface DO in that all main effects and interactions were highly significant and suggested bottom DO concentrations differences among periods were restricted primarily to the upper segment of the LHR target zone (Figure 6.1-18).

Figure 6.1-18: Least squared means with confidence intervals for bottom DO concentrations (mg/L)



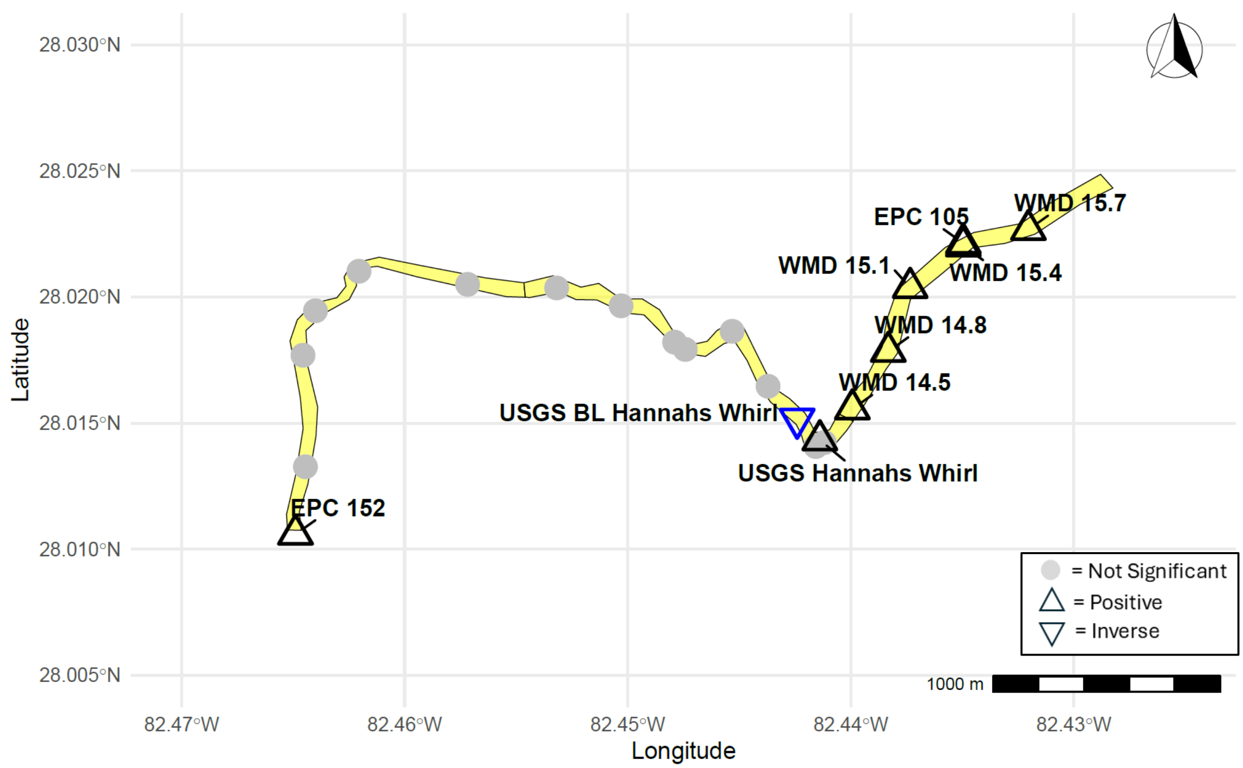
Linear Regression

Linear regression of bottom DO concentrations resulted in similar outcomes as surface DO with mainly significant positive relationship with flow during Analysis Days (Table 6.1-5), restricted to the upper segment down to Hannah’s Whirl and a single negative relationship with flow at the USGS 02304517 BL Hannah's Whirl site (Figure 6.1-9).

Table 6.1-5: Linear regression results for bottom DO concentrations in the target zone of the LHR

Station	N	Month p Value	Flow p Value	Flow Slope	R square	Slope Direction
EPC 105	139	0.021	<0.001	0.075	0.30	1
EPC 152	120	<0.001	0.04	0.021	0.33	1
USGS 02304517 BL Hannah's Whirl	699	<0.001	<0.001	-0.053	0.34	-1
USGS 02304515 Hannah's Whirl	70	0.016	0.045	0.152	0.18	1
SWFWMD LHR06_800047_RKm_14.5	69	<0.001	<0.001	0.155	0.52	1
SWFWMD LHR05_800046_RKm_14.8	70	<0.001	<0.001	0.167	0.57	1
SWFWMD LHR04_800045_RKm_15.1	61	0.002	<0.001	0.171	0.51	1
SWFWMD LHR03_19208_RKm_15.4	62	<0.001	<0.001	0.153	0.60	1
SWFWMD LHR02_800044_RKm_15.7	47	ns	0.042	0.090	0.09	1

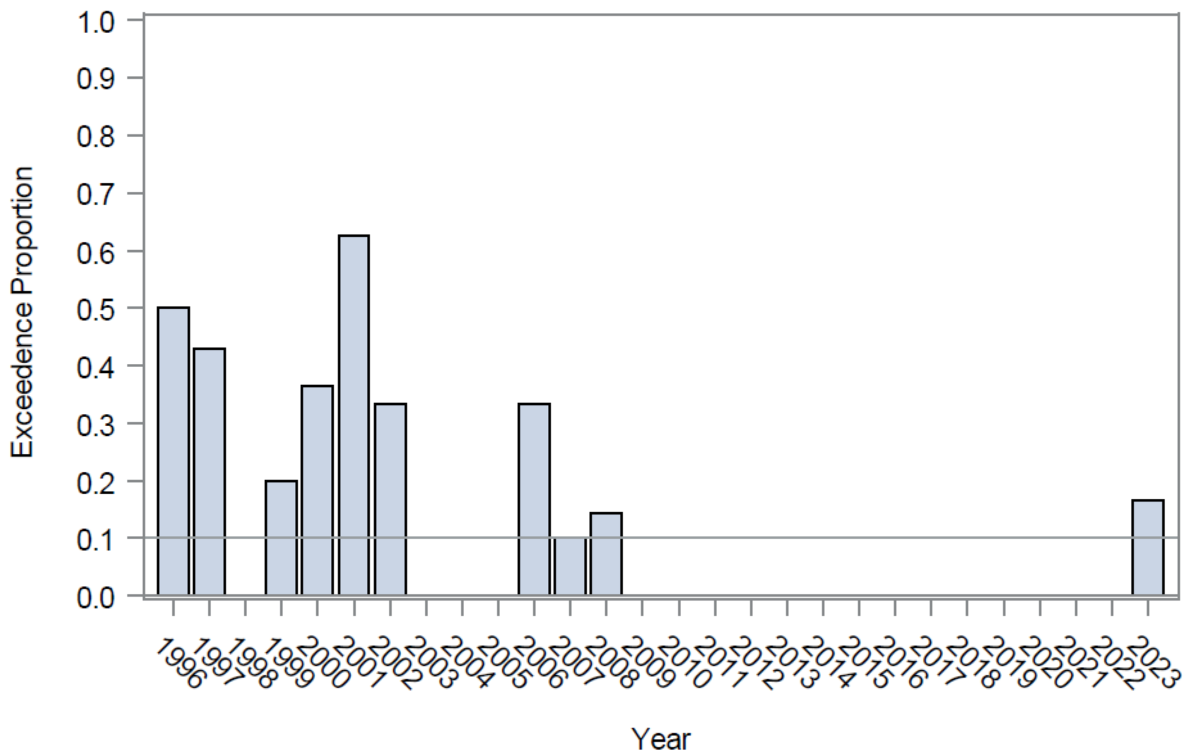
Figure 6.1-19: Bottom DO stations with statistically significant relationships with flow in the target zone (labeled) and direction of the triangle



Logistic Regression (DO < 2.5 mg/L)

The exceedance proportions in target zone bottom DO was statistically related to flows at EPC 105 and EPC 2 CR, where results suggested that as flows increase, the probability of a value less than 2.5 mg/L is reduced. The EPC 2 CR only recorded data from 2002 until 2014. The exceedance proportions (i.e., proportion of values less than 2.5 mg/L) at EPC 105 are provided in Figure 6.1-20 and suggest that since 2010 there have been few values below 2.5 mg/L during Analysis Days at EPC 105. This is not to say that bottom DO concentrations at other sites were not often below 2.5 mg/L; however, only EPC 105 exhibited the proportion of values below 2.5 influenced by implementation flows. For the BL Hannah's Whirl gage (USGS 02304517), the results were similar to linear regression in that as flows increased the probability of observing a value less than 2.5 mg/L increased indicating potential stratification at this site. Full logistic regression summary tables for bottom DO are provided in Appendix Q2.

Figure 6.1-20: Exceedance frequencies for bottom DO values < 2.5 mg/L at EPC 105 in the upper segment of the LHR



Nonlinear Regression (GAM) Modeling

Based on AIC and BIC criteria, Model 3 using the 28-day lag average flow was best suited for GAM modeling of bottom dissolved oxygen, and the final model selected included the month covariate and smoothed functions for river kilometer, lag average flow, and an interaction term for river kilometer and lag average flow which were all highly statistically significant ($p<0.001$).This GAM model resulted in an R squared of 42.3%. The model predicted, on average, bottom DO concentrations above 5 mg/L to river kilometer 15 and then steadily declining to average DO concentrations below 2.5 mg/L at Rkm 13 and below (Figure 6.1-21). The GAM scenario evaluation suggests that the implementation flows improved bottom DO concentrations by approximately 1 mg/L on average between 2018 and 2023 and that there were diminishing returns on that response with increased distance downstream (Figure 6.1-22).

Figure 6.1-21: GAM model predictions for bottom DO (blue line) as a function of river kilometer based on observed data (black-filled circles) used to develop the model

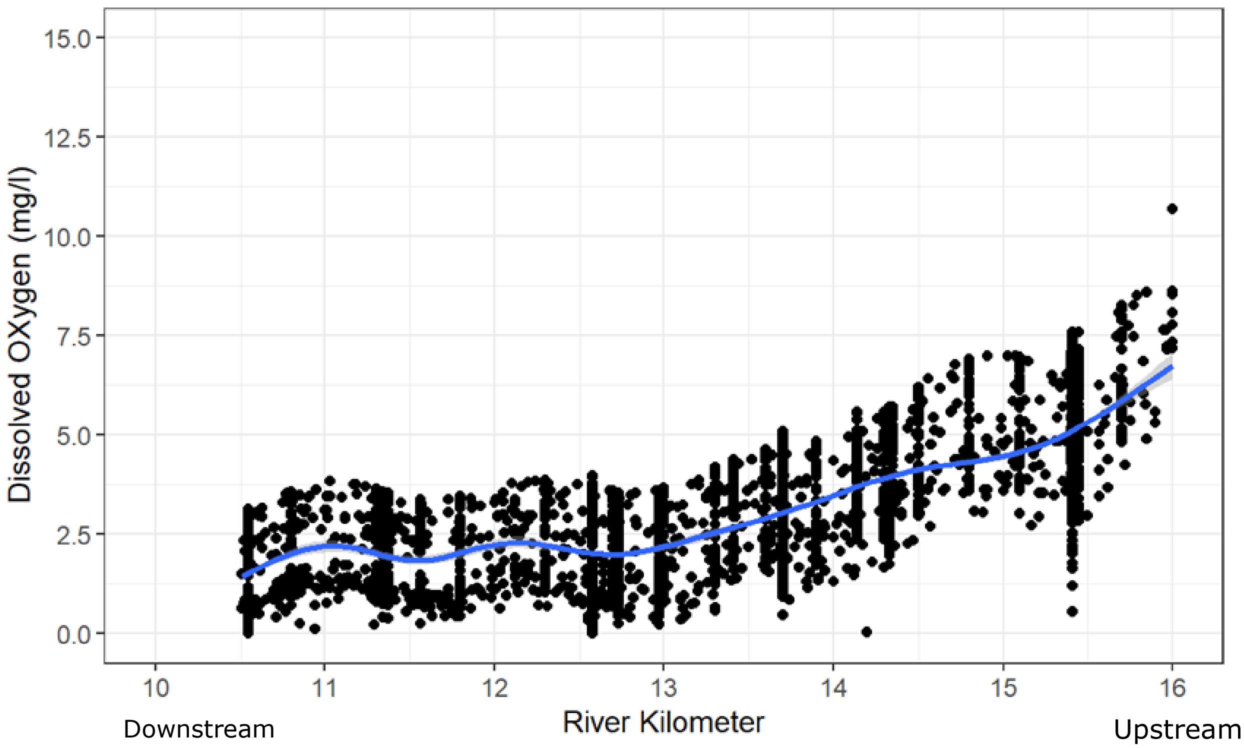
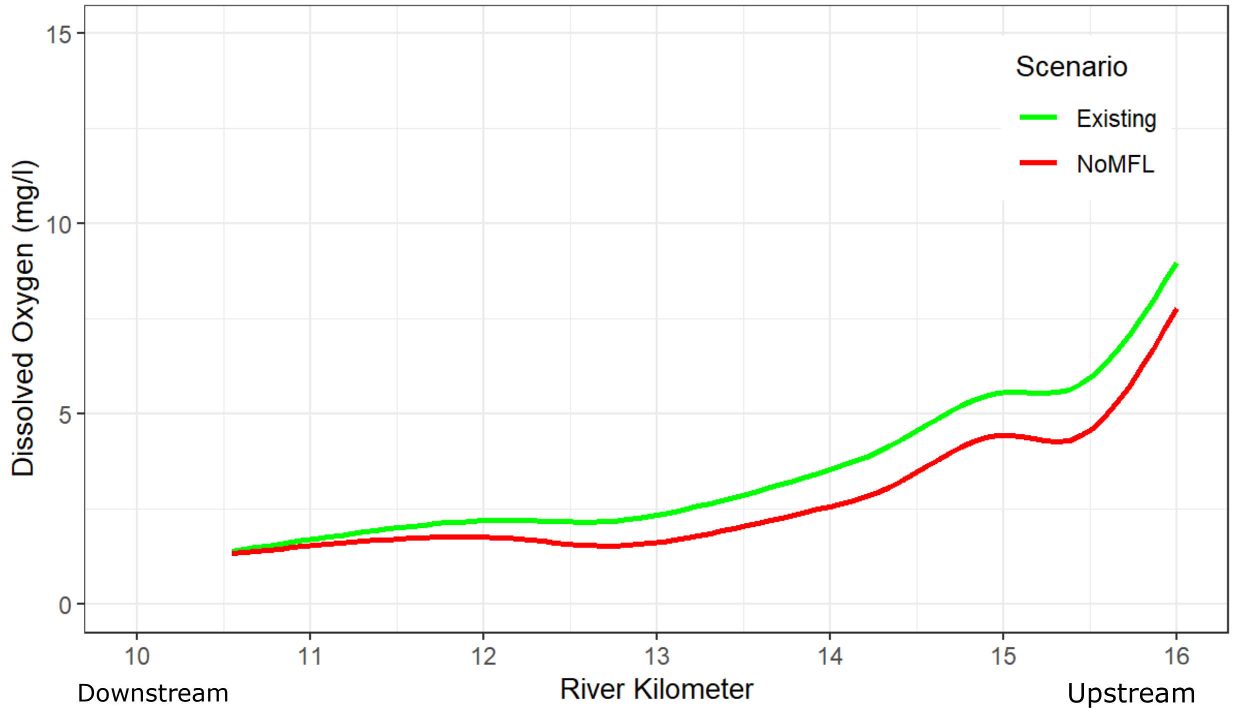


Figure 6.1-22: Bottom DO GAM model predictions under existing and No MFL scenarios for Analysis Days between 2018 and 2023



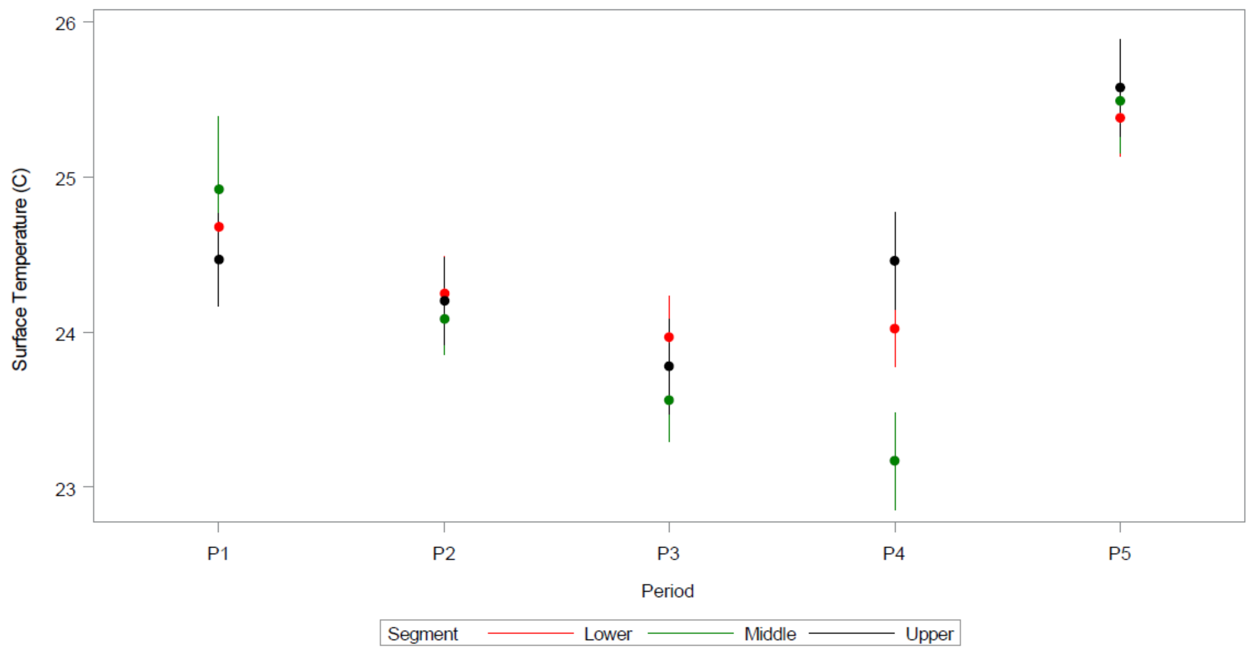
6.1.3 Water Temperature

6.1.3.1 Surface Water Temperature

Comparison of Means with General Linear Models (GLM)

Results of mixed effects GLM tests suggest a significant period effect and significant interaction between period and segment mostly driven by differences in segment temperature in Period 4 (Figure 6.1-23).

Figure 6.1-23: Least squared means with 95% confidence intervals for surface temperature (C)



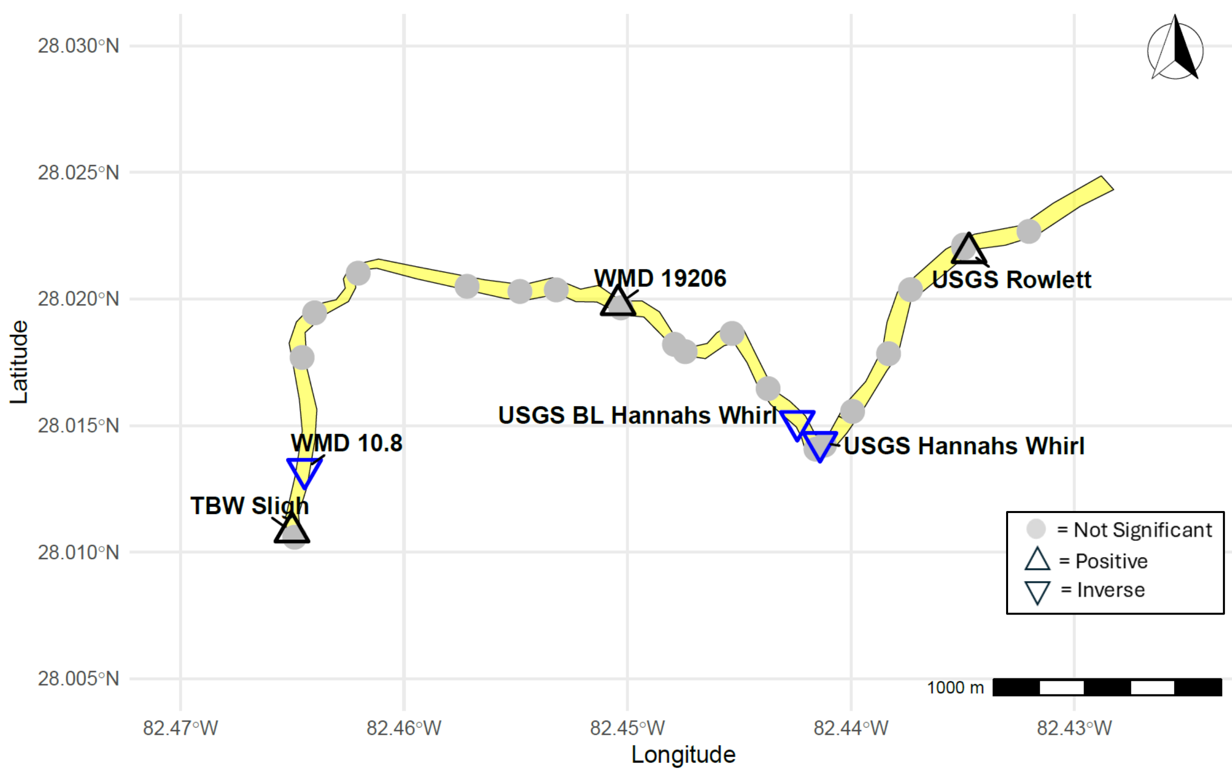
Linear Regression

Results of linear regression analysis were mixed with three positive and three negative relationships with implementation flows (Table 6.1-6). The site-specific temperature results did not indicate any consistent spatial patterns during minimum flow implementation (Figure 6.1-24). Given the strong temporal correlation in temperature, these findings may reflect natural variability rather than flow-related effects. The lack of a temperature response at most sites suggests that minimum flow implementation exerts only a limited influence on temperature conditions across the range of observed values. Additional insight into localized temperature responses will be informed by the LAMFE model results.

Table 6.1-6: Linear regression results for surface temperature in the target zone of the LHR

Station	N	Month p Value	Flow p Value	Flow Slope	R square	Slope Direction
TBW Sligh	2,891	<0.001	<0.001	0.024	0.81	1
USGS 02304517 BL Hannah's Whirl	710	<0.001	0.006	-0.027	0.75	-1
USGS 02304515 Hannah's Whirl	536	<0.001	<0.001	-0.061	0.81	-1
USGS 02304510 Rowlett	4,468	<0.001	<0.001	0.027	0.71	1
SWFWMD LHR16_19237_RKm_10.8	61	<0.001	0.006	-0.094	0.76	-1
SWFWMD WMD_19206	446	<0.001	0.045	0.029	0.66	1

Figure 6.1-24: Surface temperature stations with statistically significant relationships with flow in the target zone (labeled) and direction of the triangle indicating direction of relationship with flow



Nonlinear Regression (GAM) Modeling

Surface response modeling results for surface temperature were highly statistically significant, using the same day flow ($p<0.001$) and explaining 75% of the model variance. Results suggest slightly elevated temperatures near the base of the dam and then consistent temperatures throughout the target zone between 23 and 25ºC (Figure 6.1-25). A seasonal (monthly) effect is clearly seen in these plots as well with predicted values between 20 ºC and 29 ºC (68–84ºF). Although flow was a significant term in the model, the predictions of the No MFL scenario (Figure 6.1-26) suggest that the effects of implementation flows are minimal and restricted to river kilometer above 13.5 where the existing condition was predicted to result in slightly increased temperatures on average. These effects are averaged over all dates and therefore there may be times when implementation increased temperatures and times when implementation decreased temperatures relative to ambient conditions. The LAMFE model may be better suited for more detailed inference regarding the effects of implementation on temperature.

Figure 6.1-25: GAM model predictions for surface temperature (blue line) as a function of river kilometer based on observed data (black-filled circles) used to develop the model

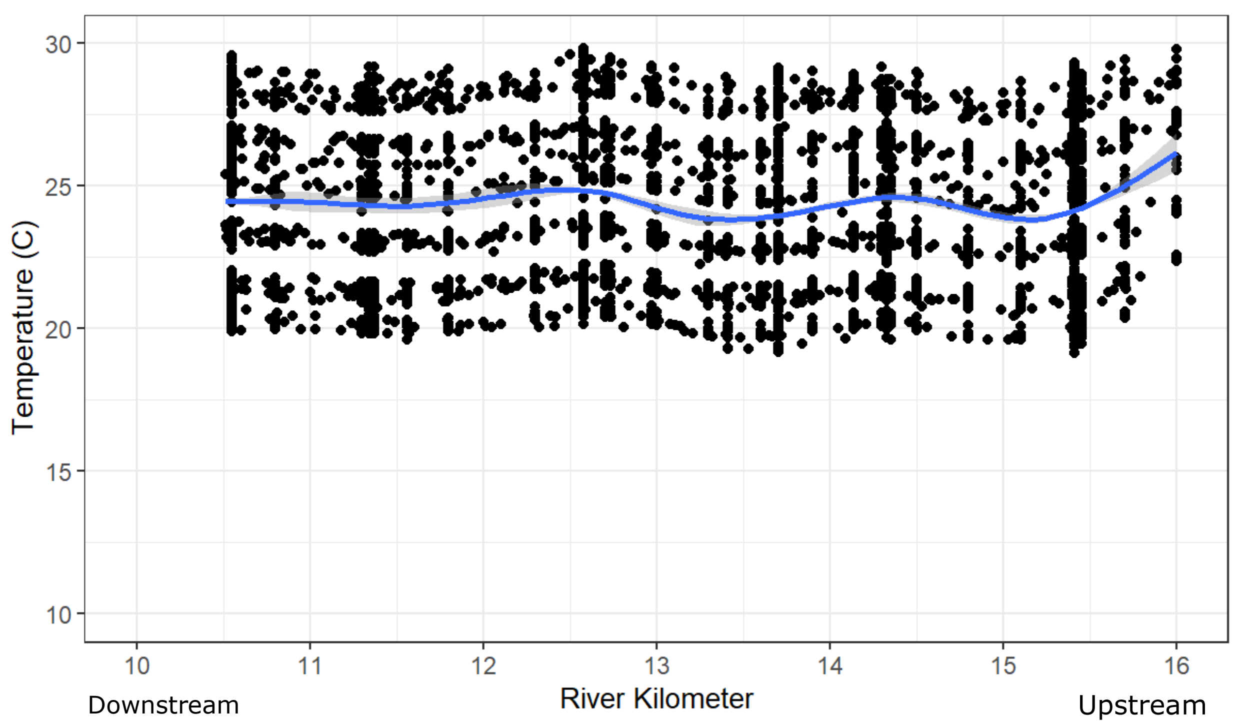
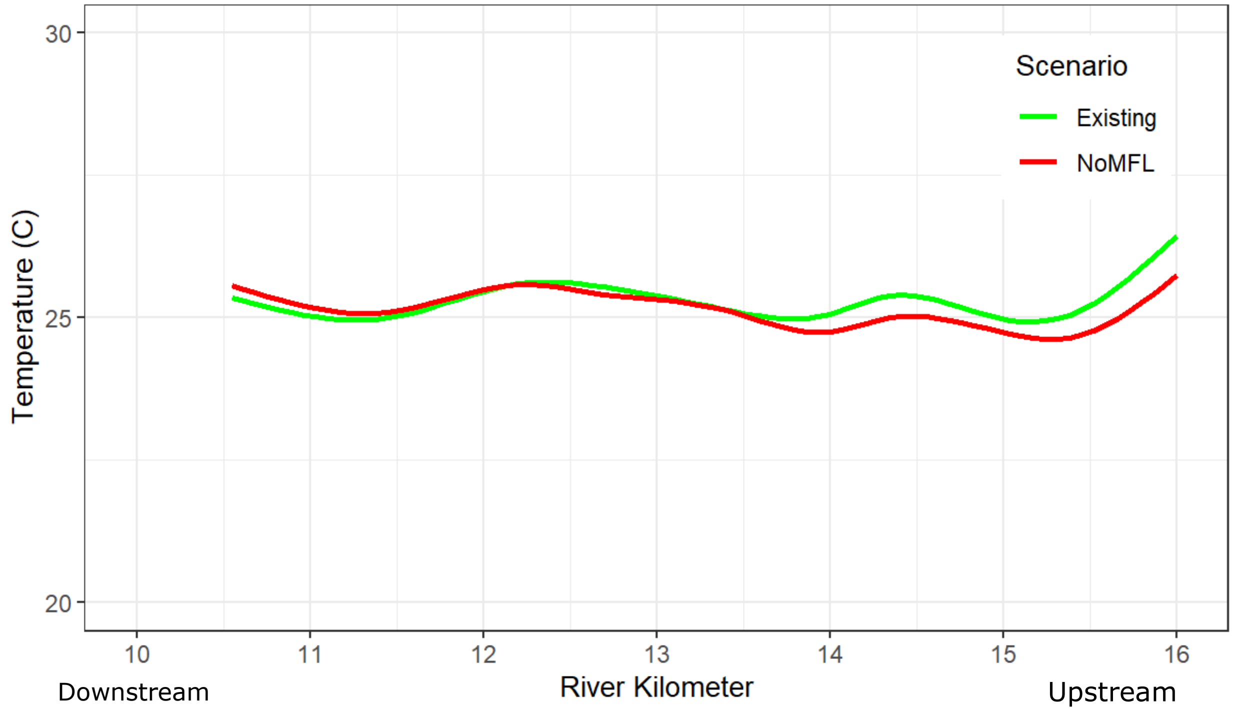


Figure 6.1-26: Surface temperature GAM model predictions under existing and No MFL scenarios for Analysis Days between 2018 and 2023



LAMFE

As mentioned before, thermal dynamics under the three flow scenarios were simulated by the LAMFE model for the simulation period between October 2007 and December 2023. In most of the LHR, heat exchange with the atmosphere at the water surface is a major process determining the surface water temperature. However, for the river segment between the dam and I-275, the longitudinal transport of Sulphur Springs flow, which is routed to the base of the dam, becomes an important factor affecting water temperature during cold days when water temperature in the LHR is much lower than that of Sulphur Springs water or during the summer when the LHR is much warmer than Sulphur Springs water. Unlike how the MFL implementation affects salinity distribution and low-salinity habitat availability in the upstream portion of the LHR, effects of the MFL implementation on temperature distribution and thermal habitats for manatees are more complicated, as the release of Sulphur Springs water to the base of the dam would create a thermal plume at the base of the dam, while the thermal plume near the confluence of the Sulphur Springs run would be altered by the division of the Sulphur Springs flow. During MFL Analysis Days in winter, routing of the Sulphur Springs flow would increase water temperature between the dam and the Sulphur Spring confluence; however, during MFL Analysis Days in summer, it would decrease water temperature in the river segment.

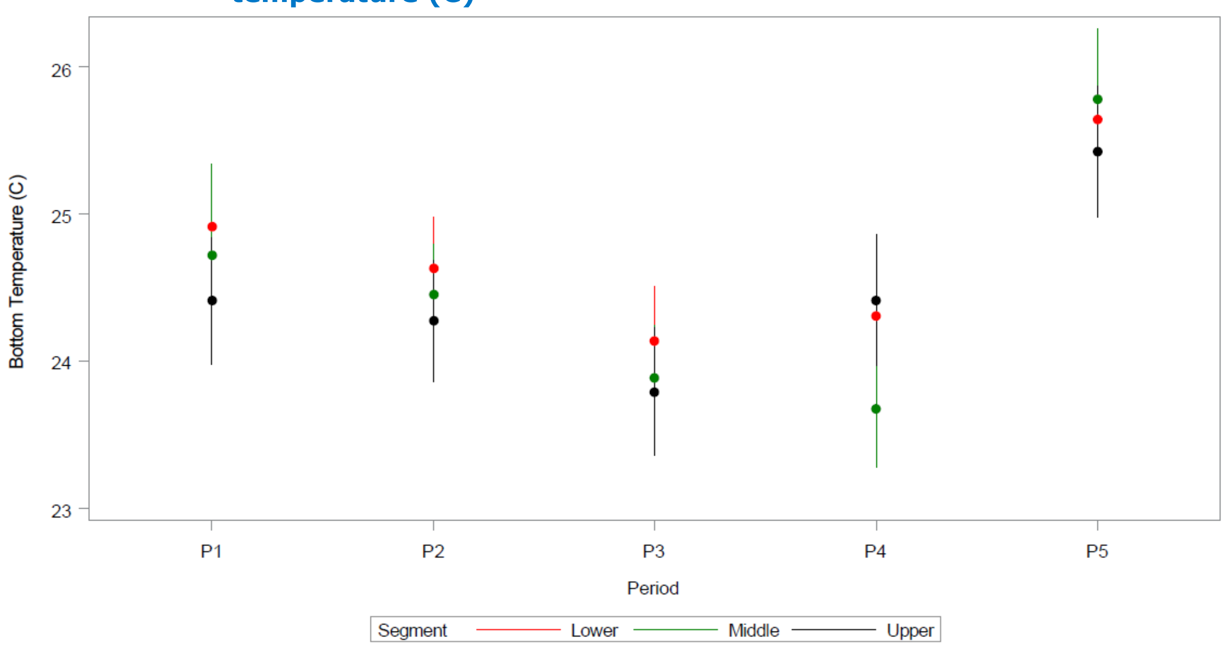
Simulated temperature results by the LAMFE model were processed to calculate thermal habitats in the thermal refuge for manatees, which is defined as the entire SS run and a 50-m box in the LHR that is centered at the Sulphur Springs confluence (SWFWMD, 2004). Comparisons of simulated thermal habitats in the thermal refuge for manatees indicate that effects of the implementation of the MFLs on various thermal habitats for manatees are complicated, not just because of the complexity of the MFL rules for the LHR and the Sulphur Springs run, but also because of the dynamic nature of the thermal plume of the Sulphur Springs flow in the LHR. While the thermal refuge for manatees is a fixed area, the shape and extent of the plume vary constantly, depending on many factors such as tides, Sulphur Springs flow in the run, Sulphur Springs flow routed to the base of the dam, freshwater flow entering the river at the dam, ungauged flows, boundary conditions at the Platt Street, as well as meteorological conditions. Simulated results suggest that the spring run temperature barely drops below 20 °C, which occurs only below the weir structure for the existing and MFL flow conditions. For the no MFL flow condition, no thermal habitats less than 20 °C exist in the entire spring run. In any case, calculated thermal habitats for temperature between 15 °C and 20 °C in the thermal refuge for manatees mainly or entirely exist in the 50-m box in the LHR.

6.1.3.2 Bottom Water Temperature

Comparison of Means with General Linear Models (GLM)

Results of mixed effects GLM tests suggest a significant period effect and significant interaction between period and segment mostly driven by differences in segment temperature in Period 4 (Figure 6.1-27).

Figure 6.1-27: Least squared means with 95% confidence intervals for bottom temperature (C)



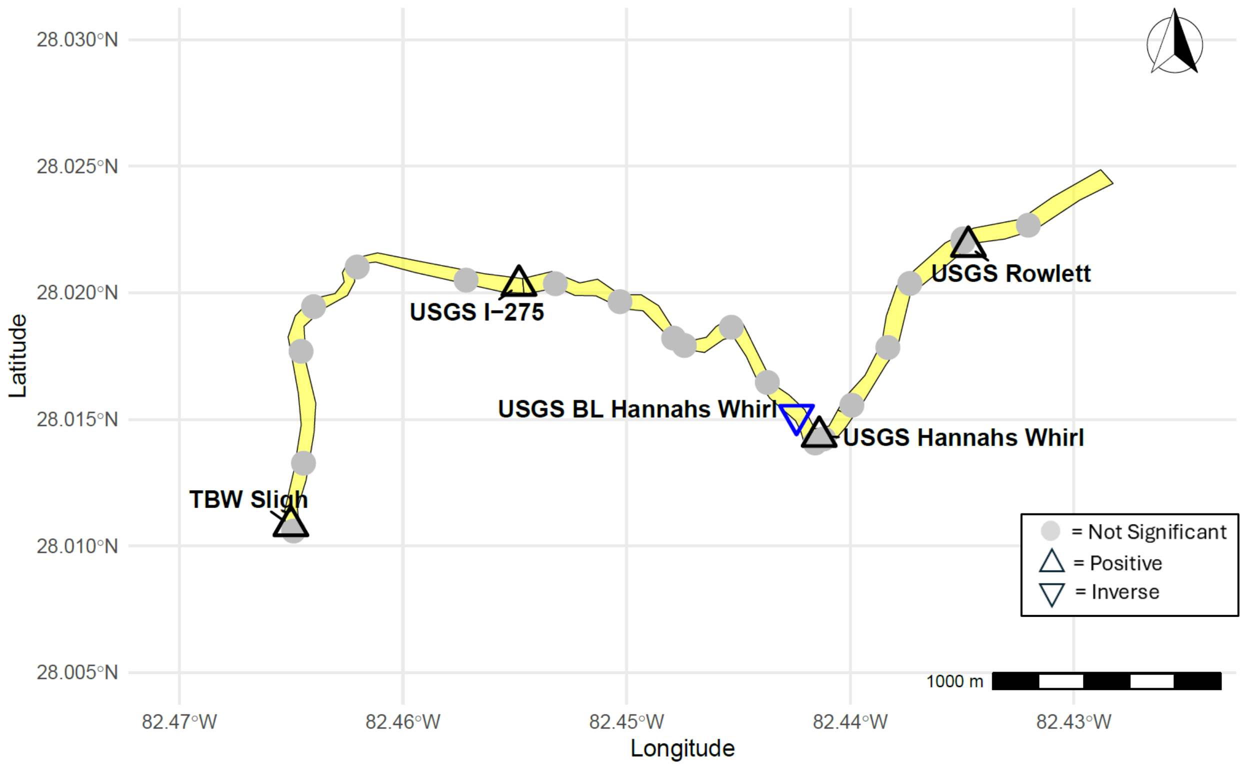
Linear Regression

Results of linear regression analysis were mixed with four positive and one negative relationship with implementation flows (Table 6.1-7). Site-specific temperature results did not indicate any consistent spatial patterns during minimum flow implementation (Figure 6.1-28). Given the strong temporal correlation in temperature, these findings may reflect natural variability rather than flow-related effects. The lack of a temperature response at most sites suggests that minimum flow implementation exerts only a limited influence on temperature conditions across the range of observed values. Additional insight into localized temperature responses will be informed by the LAMFE model results.

Table 6.1-7: Linear regression results for bottom temperature in the target zone of the LHR

Station	N	Month p Value	Flow p Value	Flow Slope	R square	Slope Direction
TBW Sligh	2,997	<0.001	<0.001	0.027	0.85	1
USGS 02304517 BL Hannah's Whirl	710	<0.001	0.024	-0.021	0.77	-1
USGS 02304515 Hannah's Whirl	108	<0.001	<0.001	0.082	0.65	1
USGS 023060013 I-275	2,379	<0.001	0.017	0.005	0.81	1
USGS 02304510 Rowlett	4,535	<0.001	<0.001	0.023	0.72	1

Figure 6.1-28: Bottom temperature stations with statistically significant relationships with flow in the target zone (labeled) and direction of the triangle indicating direction of relationship with flow



Nonlinear Regression (GAM) Modeling

Based on AIC and BIC criteria, Model 3 using the same day flow was best suited for GAM modeling of surface temperature, and the final model selected included the month covariate and smoothed functions for river kilometer, lag average flow, and an interaction term for river kilometer and lag average flow which were all highly statistically significant (p<0.001). The model explained 75% of the total variance. Results suggest slightly elevated temperatures near the base of the dam and then consistent temperatures throughout the target zone between 23 °C and 25 °C (73.4=77 °F) (Figure 6.1-29). A seasonal (monthly) effect is clearly seen in these plots as well and a large portion of the overall effect was explained by seasonality in temperature. The similarity between surface and bottom temperature predictions is notable. Although flow was a significant term in the model, the predictions of the No MFL scenario (Figure 6.1-30) suggest that the effects of implementation flows are minimal and restricted to above Rkm 13.5 where the existing condition was predicted to result in slightly increased temperatures on average. These effects are averaged over all dates and therefore there may be times when implementation increased temperatures and times when implementation decreased temperatures relative to ambient conditions. Again, the LAMFE model may be better suited for more detailed inference regarding the effects of implementation on temperature.

Figure 6.1-29: GAM model predictions for bottom temperature (blue line) as a function of river kilometer based on observed data (black-filled circles) used to develop the model

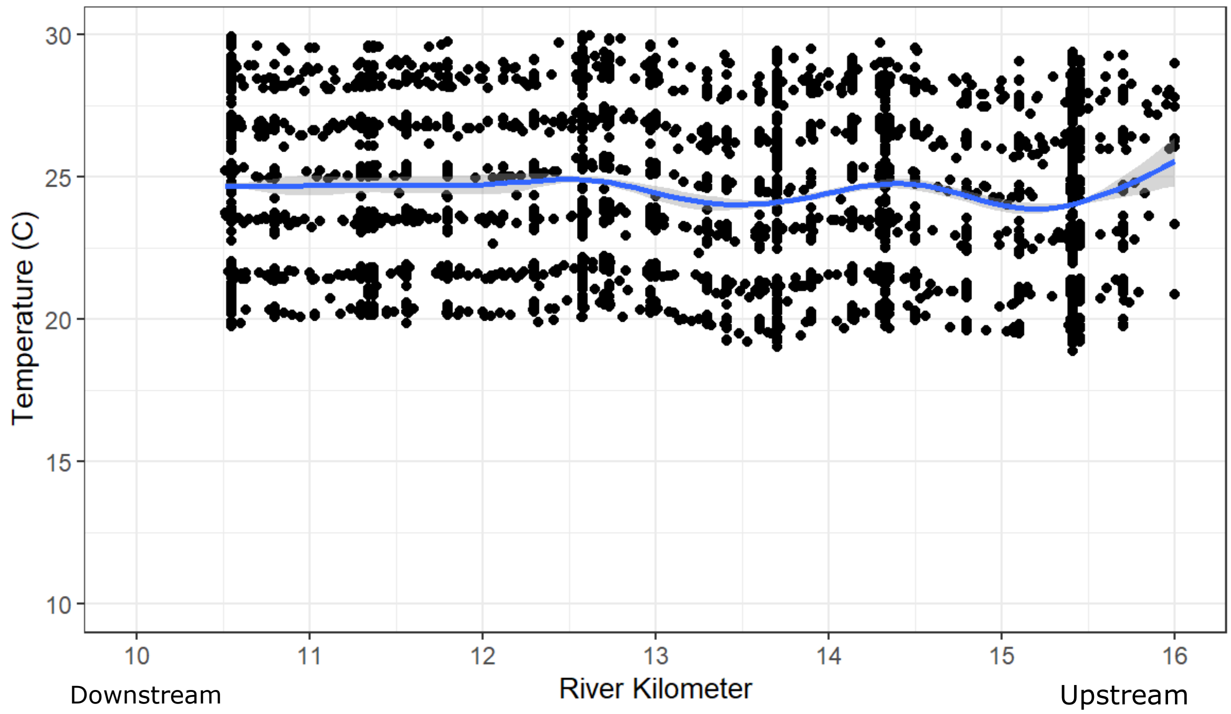
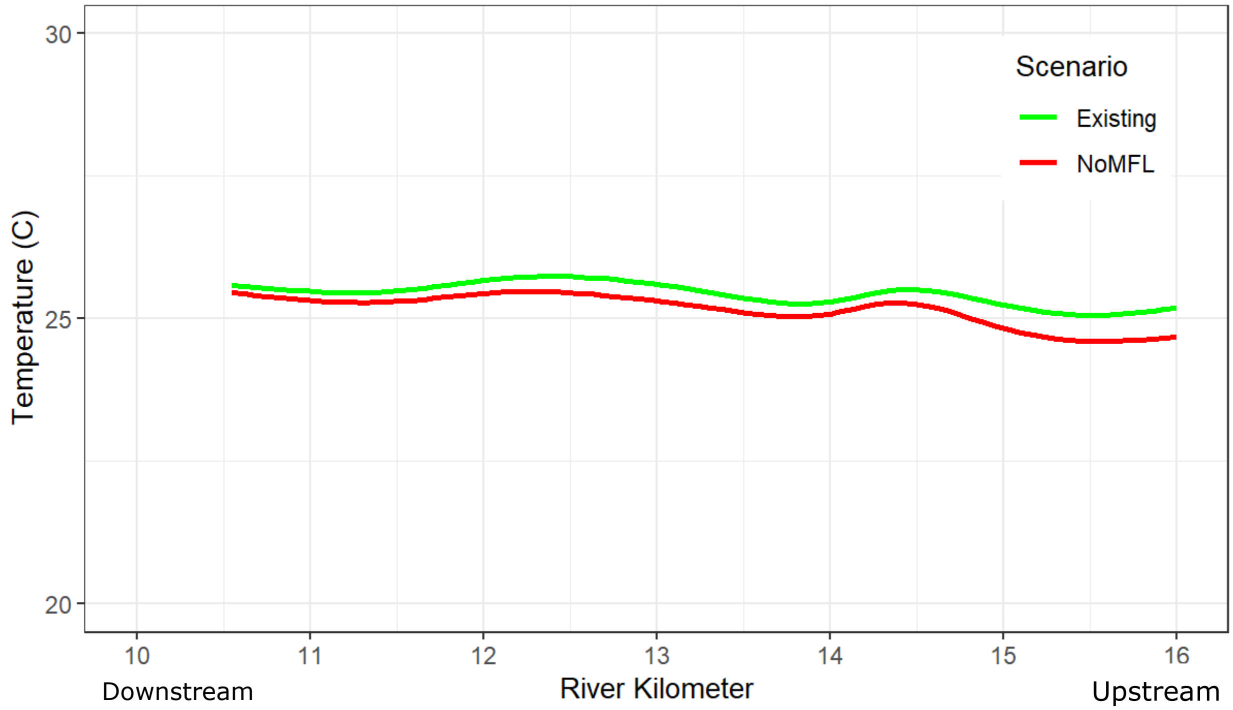


Figure 6.1-30: Bottom temperature GAM model predictions under existing and No MFL scenarios for Analysis Days between 2018 and 2023



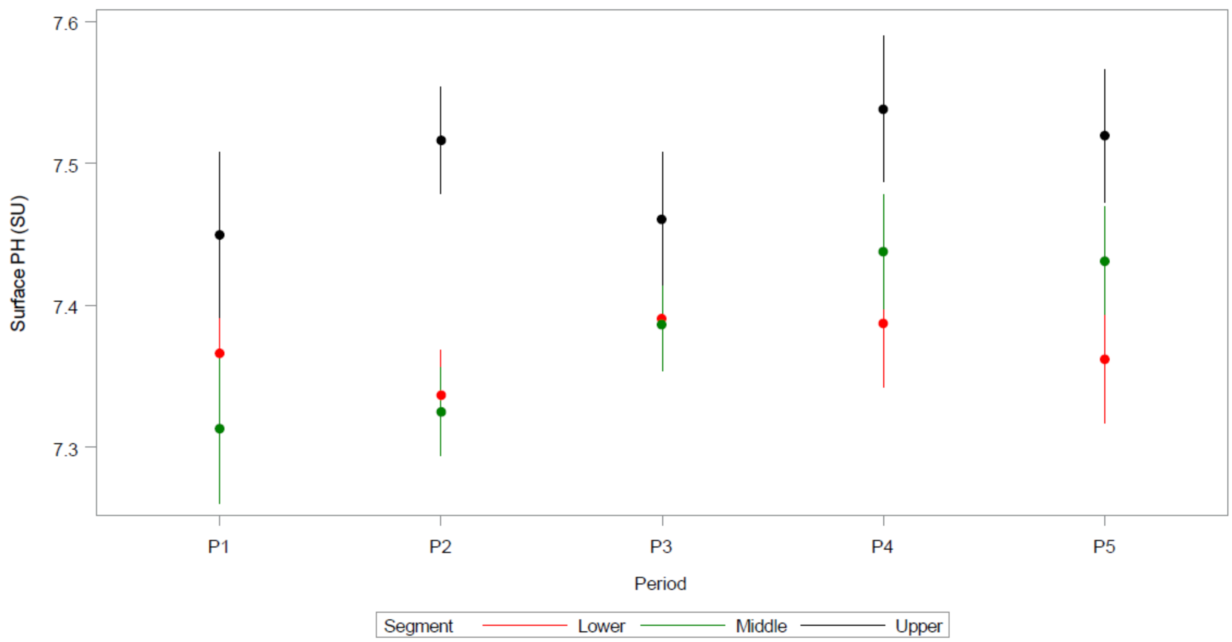
6.1.4 pH

6.1.4.1 Surface pH

Comparison of Means with General Linear Models (GLM)

Results of mixed effects GLM tests suggest significant effects for period, river segment, and interaction between period and river segment (Figure 6.1-31) despite the overall effect size of the differences being small, ranging from 7.3 to 7.55 standard units. The river segment differences were mostly due to the upper river segment being more basic than the other two river segments and the middle river segment becoming more basic over time, presumably as increased contributions from Sulphur Springs impacted the pH of the target zone.

Figure 6.1-31: Comparison of surface pH (su) means with 95% confidence intervals



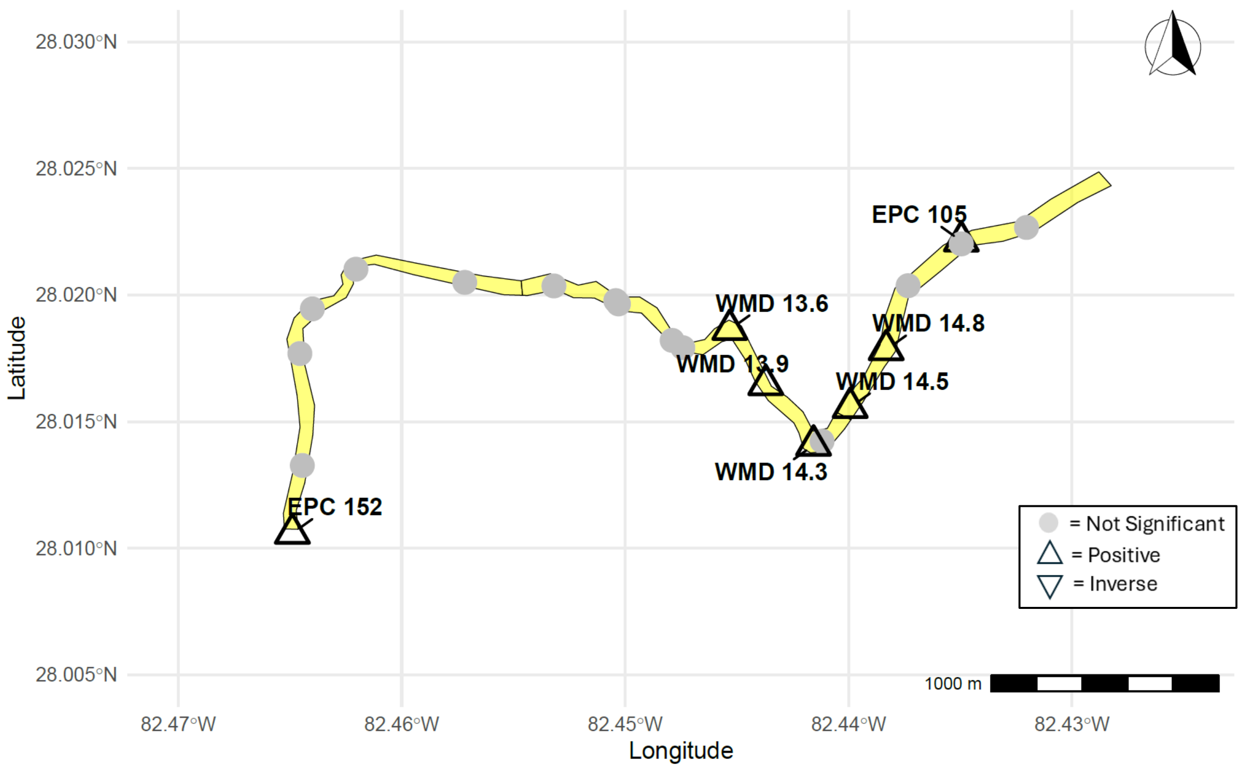
Linear Regression

Seven stations exhibited significant linear relationships with flow, all of which were positive, indicating that as implementation flows increased, the pH of the water increased (Table 6.1-8). Most of the significant relationships were located in the upper and middle segments of the LHR target zone (Figure 6.1-32).

Table 6.1-8: Linear regression results for surface pH in the LHR target zone

Station	N	Month p Value	Flow p Value	Flow Slope	R square	Slope Direction
EPC 105	142	0.005	<0.001	0.006	0.25	1
EPC 152	126	0.038	<0.001	0.007	0.29	1
SWFWMD LHR09_19209_RKm_13.6	66	0.021	0.026	0.007	0.31	1
SWFWMD LHR08_800049_RKm_13.9	69	0.024	0.002	0.009	0.32	1
SWFWMD LHR07_800048_RKm_14.3	69	0.046	<0.001	0.011	0.33	1
SWFWMD LHR06_800047_RKm_14.5	70	ns	0.011	0.008	0.09	1
SWFWMD LHR05_800046_RKm_14.8	69	ns	0.032	0.006	0.07	1

Figure 6.1-32: Surface pH stations with statistically significant relationships with flow in the target zone (labeled) and direction of the triangle indicating direction of relationship with flow



Nonlinear Regression Modeling

Based on AIC and BIC criteria, Model 3 using a 28-day lag average flow was best suited for GAM modeling of surface pH, and the final model selected included the month covariate and smoothed functions for river kilometer, lag average flow, and an interaction term for river kilometer and lag average flow, which were all highly statistically significant ($p<0.001$). However, the model explained little of the overall variation in pH (17%), indicating much of the variability in pH was unexplained by the model. Predicted pH values were stable throughout the target zone with a slight increasing trend toward the base of the dam (Figure 6.1-33). Model predictions comparing an existing condition and No MFL condition resulted in a predicted slight increase in pH in the upper portions of the target zone and diminishing differences below river kilometer 13 (Figure 6.1-34).

Figure 6.1-33: GAM model predictions for surface pH (blue line) as a function of river kilometer based on observed data (black-filled circles) used to develop the model

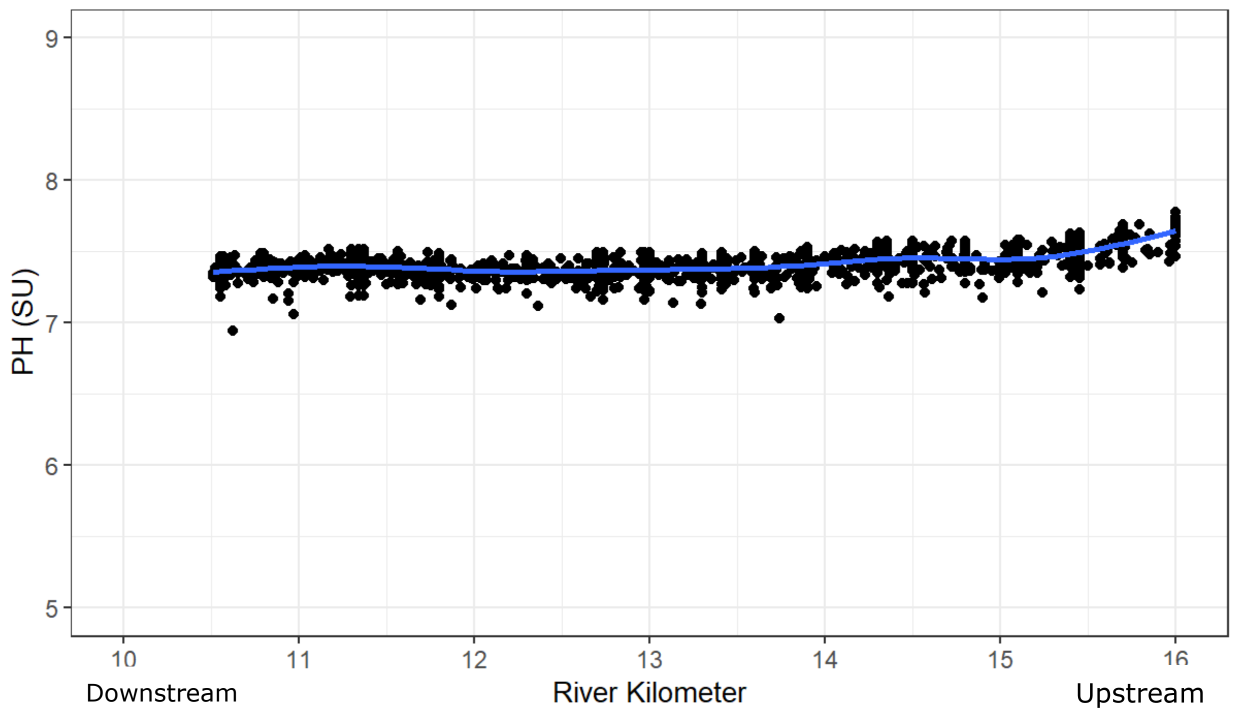
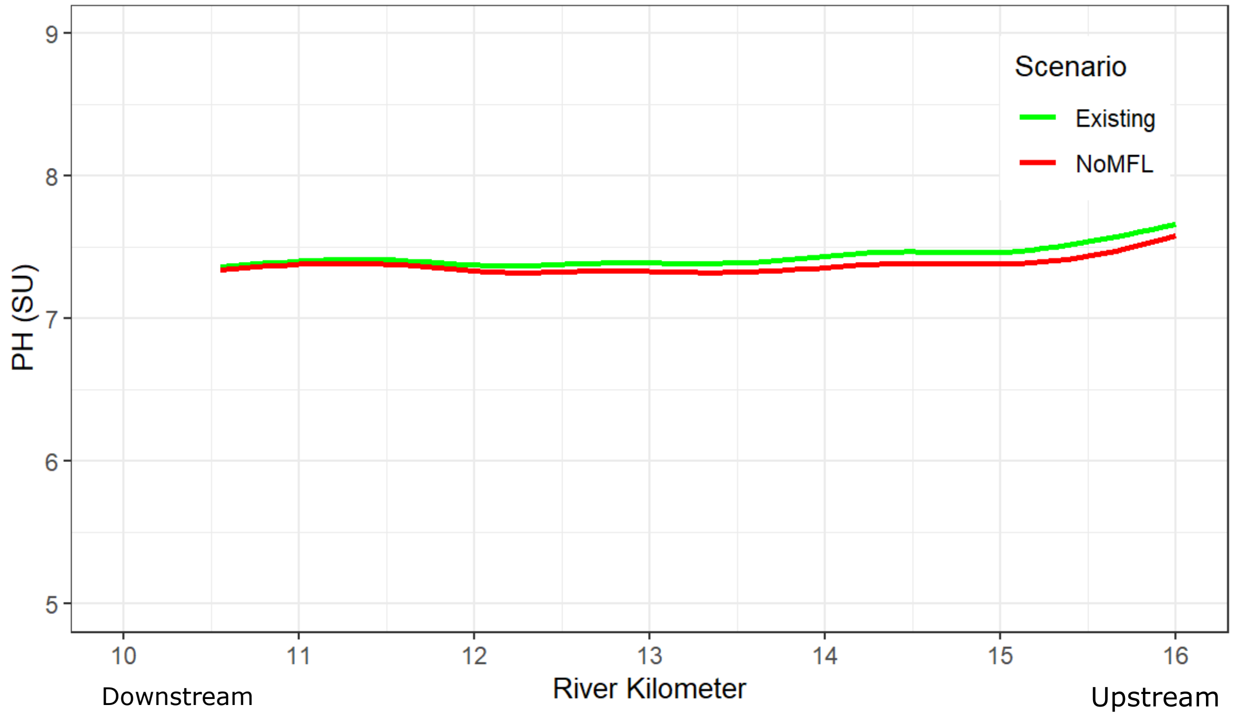


Figure 6.1-34: Surface pH GAM model predictions under existing and No MFL scenarios for Analysis Days between 2018 and 2023

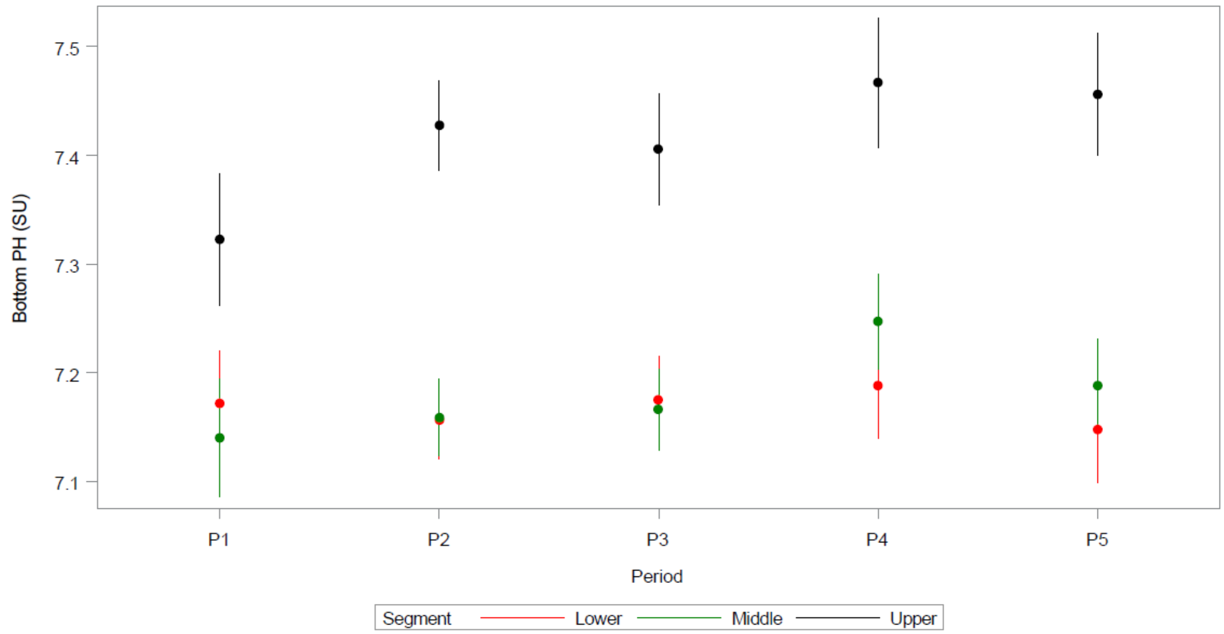


6.1.4.2 Bottom pH

Comparison of Means with General Linear Models (GLM)

Results of mixed effects GLM tests suggested significant effects for period, segment, and interaction, mostly driven by separation of the upper segment from the middle and lower segment (Figure 6.1-35).

Figure 6.1-35: Least squared means with 95% confidence intervals for bottom pH (su)



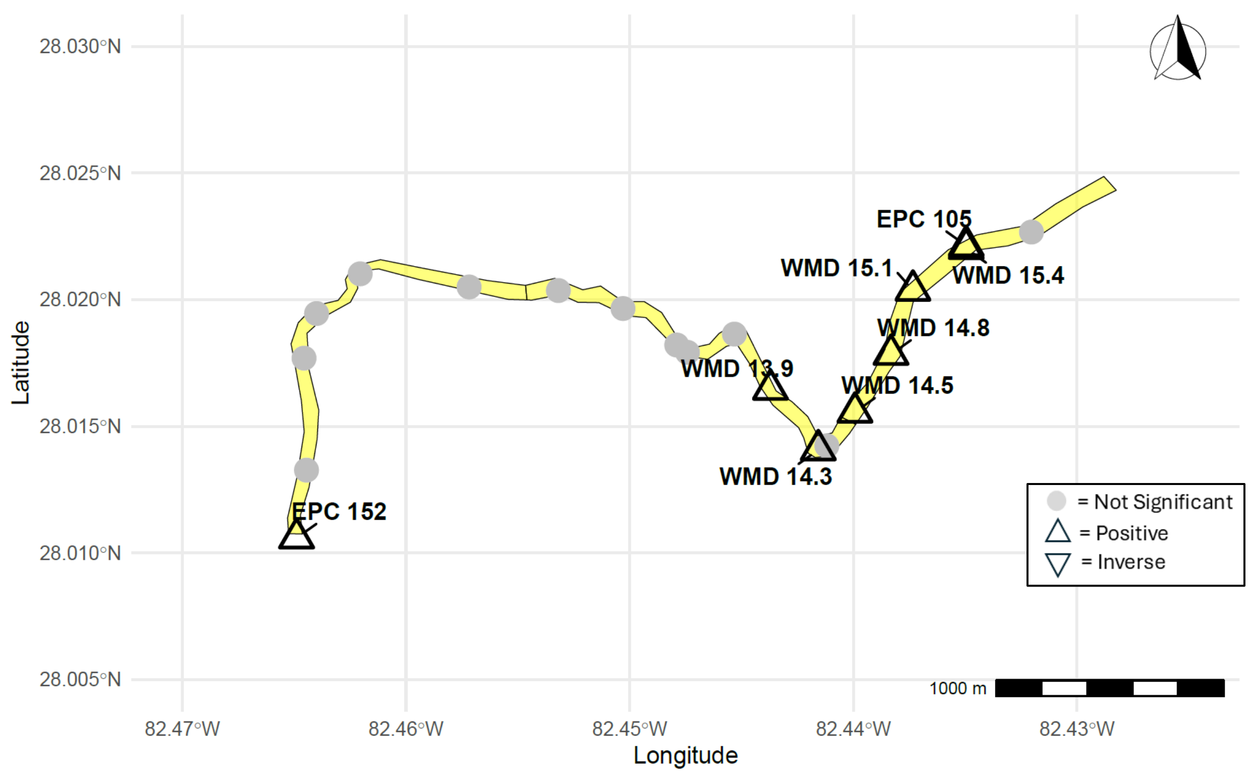
Linear Regression

Results of linear regression analysis suggested eight significant site-specific regressions, all with positive relationships with flow (Table 6.1-9). The significant results were principally restricted to the upper portions of the targets zone, above Rkm 13 (Figure 6.1-36).

Table 6.1-9: Linear regression results for bottom pH in the target zone of the LHR

Station	N	Month p Value	Flow p Value	Flow Slope	R square	Slope Direction
EPC 105	142	0.002	<0.001	0.010	0.40	1
EPC 152	126	<0.001	0.009	0.003	0.32	1
SWFWMD LHR08_800049_RKm_13.9	69	0.002	0.034	0.007	0.36	1
SWFWMD LHR07_800048_RKm_14.3	69	0.018	0.034	0.008	0.30	1
SWFWMD LHR06_800047_RKm_14.5	68	<0.001	<0.001	0.013	0.46	1
SWFWMD LHR05_800046_RKm_14.8	69	0.012	<0.001	0.012	0.37	1
SWFWMD LHR04_800045_RKm_15.1	60	ns	0.006	0.009	0.12	1
SWFWMD LHR03_19208_RKm_15.4	62	0.021	0.006	0.009	0.35	1

Figure 6.1-36: Bottom pH stations with statistically significant relationships with flow in the target zone (labeled) and direction of the triangle indicating direction of relationship with flow



Nonlinear Regression Modeling

Based on AIC and BIC criteria, Model 3 using a 28-day lag average flow was best suited for GAM modeling of bottom pH, and the final model selected included the month covariate and smoothed functions for river kilometer, lag average flow, and an interaction term for river kilometer and lag average flow which were all highly statistically significant ($p<0.001$). The model explained approximately 28% of the variation in observed values. Predicted pH values were stable throughout the target zone with a slight increasing trend towards the base of the dam (Figure 6.1-37). Model predictions comparing an existing condition and No MFL condition resulted in a predicted slight increase in pH in the upper portions of the target zone and diminishing differences below river kilometer 13 (Figure 6.1-38).

Figure 6.1-37: GAM model predictions for bottom pH (blue line) as a function of river kilometer based on observed data (black-filled circles) used to develop the model

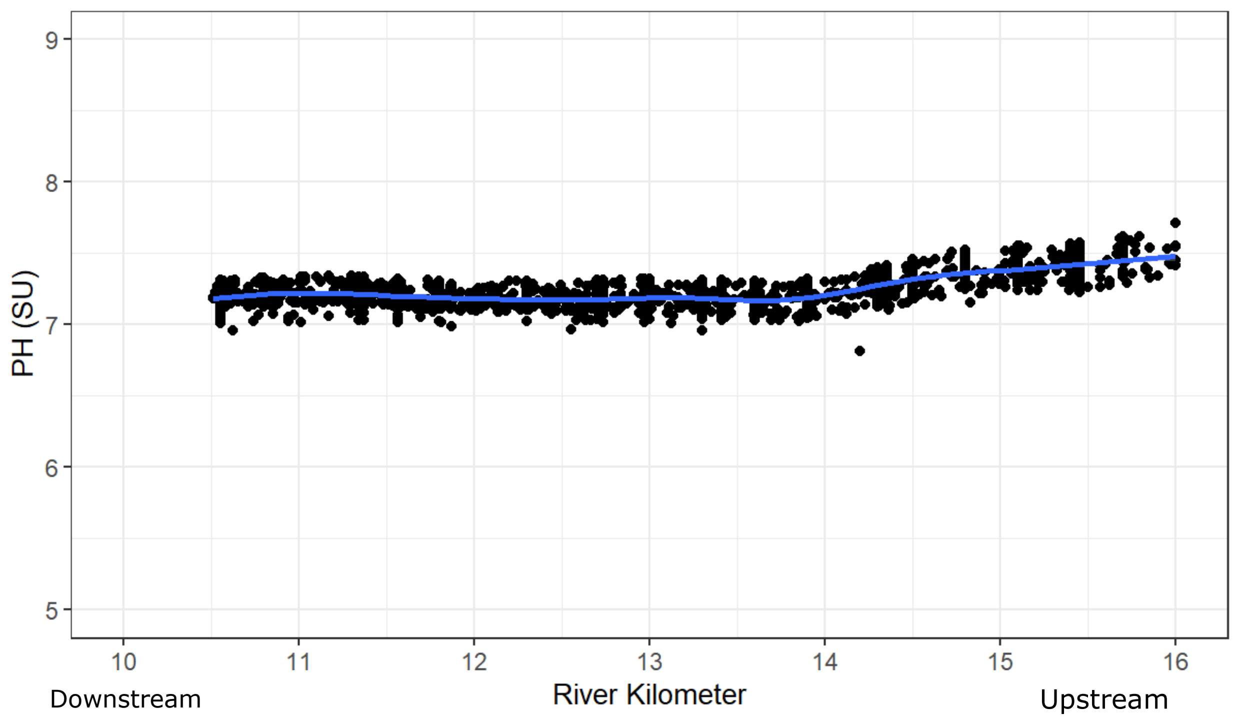
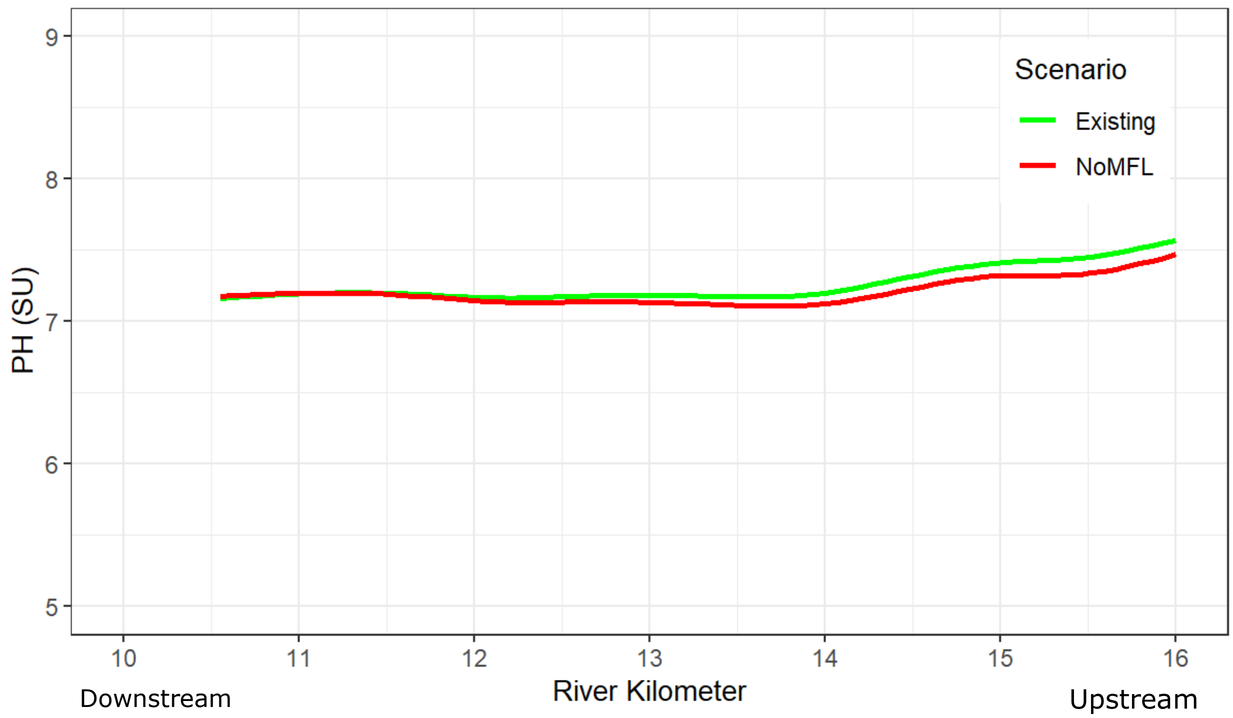


Figure 6.1-38: Bottom pH GAM model predictions under Existing and No MFL scenarios for Analysis Days between 2018 and 2023



6.2 TARGET ZONE BIOLOGICAL DATA

The guiding principles for the quantitative analysis of the LHR target zone biological data were to determine:

- If minimum flow implementation has influenced biological community structure within the target zone, resulting in conditions favorable to species requiring low salinity (<5 ppt) habitat.
- Whether the implementation of the most current minimum flow regime has increased the temporal or spatial extent of the low-salinity habitat or increased the taxa richness, abundance, or diversity of the organisms that are known to be associated with low-salinity conditions.

Abundance refers to the number of individuals captured at a sampling location. Species richness refers to the number of unique taxa observed. The Shannon diversity index (H) is a calculation involving taxa richness and the proportional abundance of organisms in a community (FDEP 2017). It is calculated as follows:

$$H = -\Sigma p_i * \ln(p_i)$$

where:

Σ: Sum
***ln*:** Natural log
p_i: The proportion of the entire community made up of species *i*

The analytical approaches for evaluating zooplankton, benthic macroinvertebrates, and nekton used multivariate techniques, along with mixed models and effect size contrasts based on literature-determined salinity-sensitive taxa (see Section 3.3.1). Evaluation of species previously hypothesized to respond to minimum flow implementation was conducted via Kruskal-Wallis and Dunn's post-hoc tests. Multivariate techniques (cluster analysis and ordination) were used to explore differences between segments and periods. Modeling to assess organism responses to LAMFE-determined salinity was also conducted to determine potential statistically significant effects from minimum flow implementation, focusing on taxa with low salinity (< 5 ppt) preferences. Statistical analyses examined the effects of minimum flow implementation on the abundance, density, taxa richness, and diversity of salinity-sensitive taxa within the three biological communities of interest. Quantitative analyses were performed on data after filtering for "Analysis Days" and salinity-sensitive species (Table 6.2-1).

Data were collected by multiple groups with differing frequency across target zone river segments and periods, which may impact the results. To account for this, standardization efforts included harmonizing taxonomic classification; eliminating rare taxa (<5% of samples) from quantitative analysis; using mixed effects models to account for random variation among sites, dates, and sampling effort; limiting quantitative analysis to days meeting minimum flow implementation to ensure similar hydrologic conditions were experienced by the biological community; and considering salinity-sensitive taxa (with preferences for salinities < 5 ppt). Key modeling and statistical results are presented below; however, observed differences may be influenced by variations in sampling effort between target zone segments and periods.

Table 6.2-1: Number of observations (site-date combinations) used for zooplankton, benthic macroinvertebrate (benthos), and nekton within the LHR target zone (Analysis Days)

Dataset	MFL Period	Upper	Middle	Lower	Total
Benthos	Period 1	6	3	3	12
Benthos	Period 2	18	25	21	64
Benthos	Period 3	7	10	9	26
Benthos	Period 5	26	26	15	67
Nekton	Period 1	16	17	23	56
Nekton	Period 2	29	35	47	111
Nekton	Period 3	19	27	36	82
Nekton	Period 4	8	4	10	22
Nekton	Period 5	16	16	16	48
Zooplankton	Period 1	0	28	23	51
Zooplankton	Period 2	0	55	40	95
Zooplankton	Period 3	0	37	33	70
Zooplankton	Period 4	0	11	11	22
Zooplankton	Period 5	10	11	13	34

6.2.1 Zooplankton

Zooplankton are defined here as marine or aquatic animals that are carried by tides and currents because their swimming ability is insufficient to move against these forces. Many zooplankton exhibit age-related changes in salinity tolerance (e.g., from egg, to larvae, to fry); however, their dependance on distribution by tide and currents complicates the prediction of species presence or absence based upon the antecedent salinity conditions (Tolley et al. 2010).

6.2.1.1 Cluster Analyses

Hierarchical cluster analysis, using standardized Euclidean distance, was conducted using on all zooplankton taxa from the target zone. Three small groups of zooplankton taxa found in the upper segment of the LHR target zone (coded blue) appeared as being distinct from the clusters of taxa inhabiting the middle and lower segments (Figure 6.2-1). Of note, zooplankton were only sampled in the upper segment during Period 5. The results of the cluster analysis prompted further analyses involving ordination and salinity sensitive taxa.

6.2.1.2 Ordination

An NMDS using standardized Bray-Curtis distance was conducted on the zooplankton taxonomic data. A random initiation process was used with three axes, and a convergent solution found with less than 40 starts. The fit of the model was acceptable, with a value of 0.16, which was below the 0.2 threshold for stress considered satisfactory for inference

(Clarke 1993). A stress plot was also examined for the fit of the data (Figure 6.2-2). Results indicated that during Period 5, when data for all three segments were available, zooplankton taxa in the upper segment (blue cloud) differed from the taxa inhabiting the middle and lower segments (Figure 6.2-3). This suggests that the salinity regime in the upper segment could be an environmental driver. Additional analyses were conducted with salinity-sensitive taxa.

Figure 6.2-1: Hierarchical clustering of zooplankton data

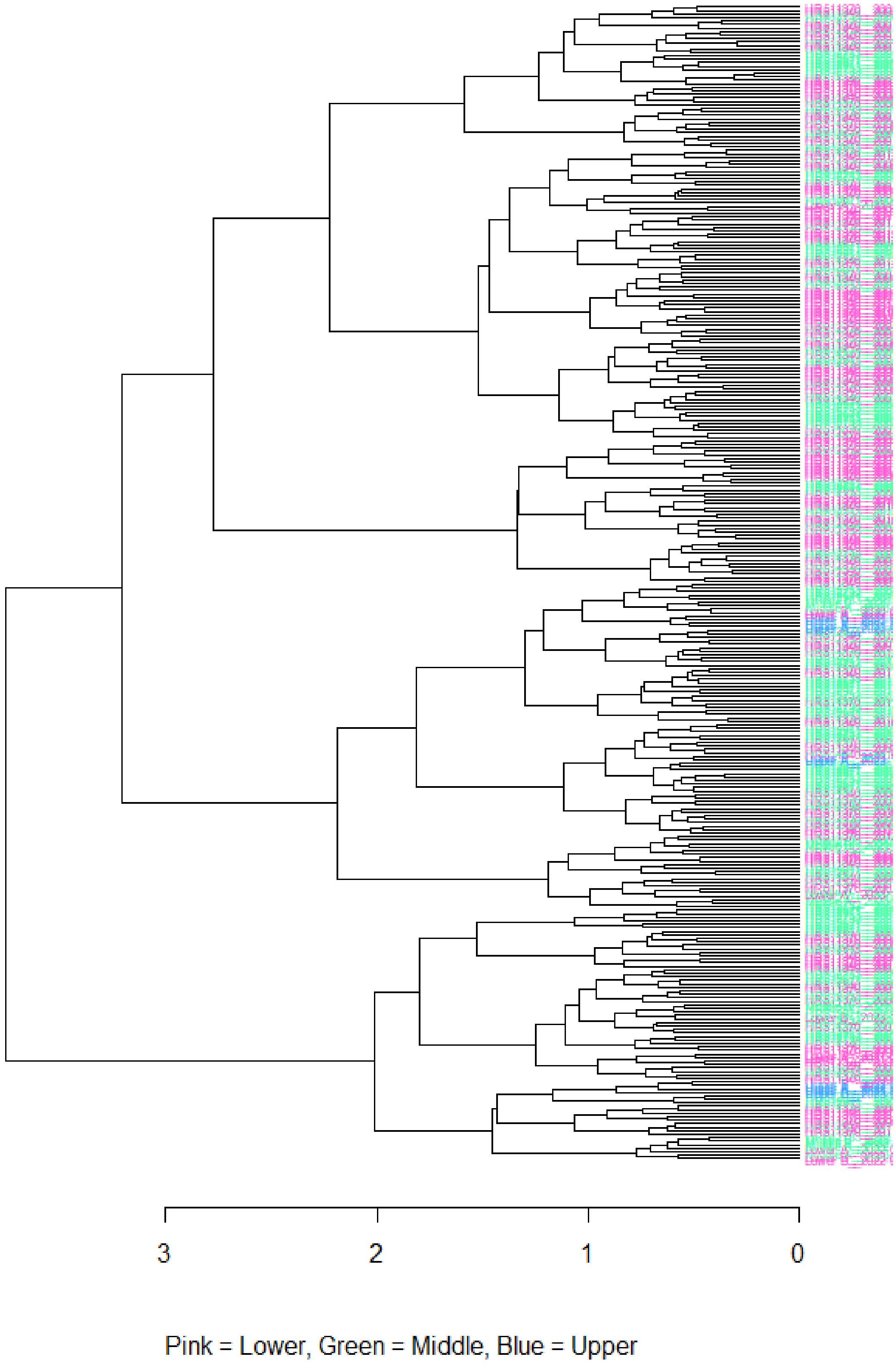


Figure 6.2-2: Stress plot of zooplankton data

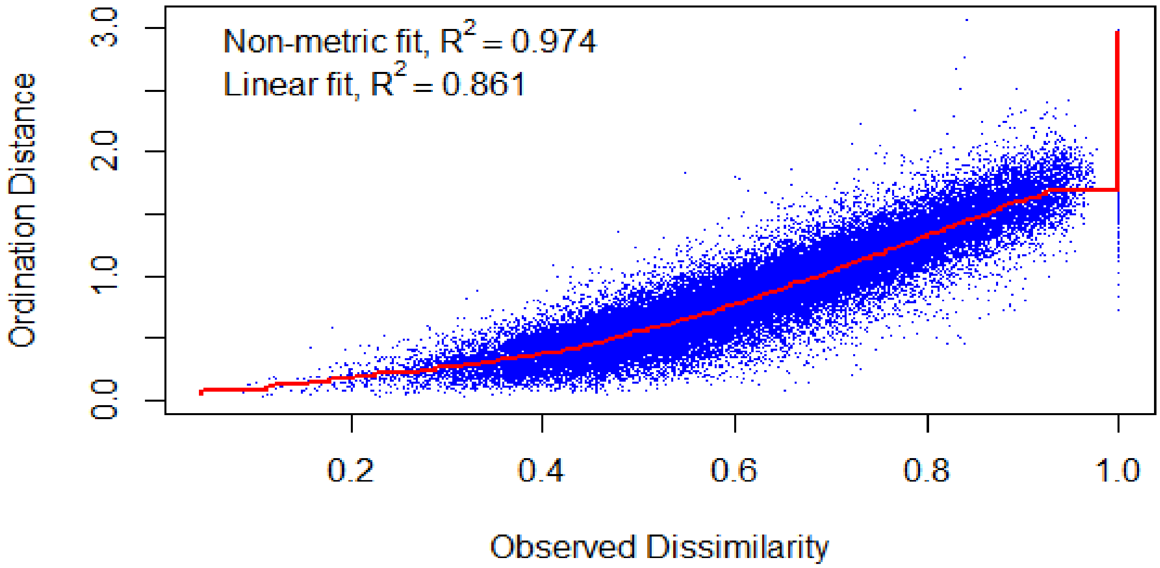
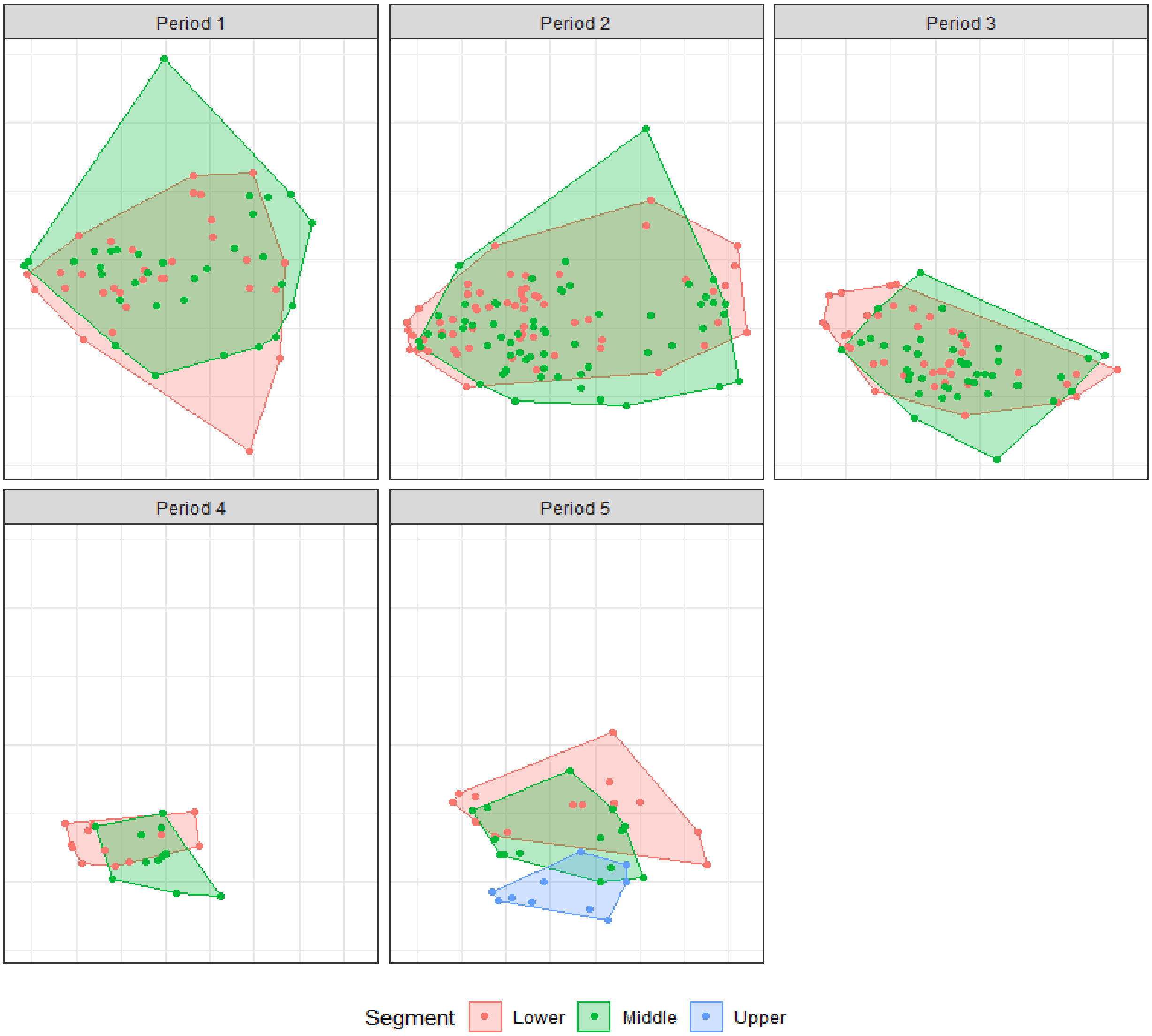


Figure 6.2-3: NMDS ordination of zooplankton by segment



6.2.1.3 Salinity Sensitivity

For this analysis, taxa were classified as salinity-sensitive if they inhabit oligohaline or tidal freshwater environments, defined as having a salinity of 5 ppt or lower, as part of their known salinity range (Odum et al. 1984) (Table 6.2-2). This target was also recommended for the Hillsborough River estuary by Montagna et al. (2007) who stated that "the oligohaline biotic community, including freshwater benthic macroinvertebrates and juvenile stages of important estuarine-dependent fish, would benefit from salinities < 5 ppt." Taxa that were always found in salinities greater than 5 ppt or that had no documented salinity preference were not considered to be "salinity-sensitive." A list of salinity-sensitive zooplankton taxa used for these analyses is found in Table 6.2-2.

Table 6.2-2: List of zooplankton taxa within the LHR target zone inhabiting low salinity (< 5 ppt) habitats (Analysis Days)

Acari	Diaphanosoma brachyurum	Hemipterans, Belostomatid adults	Microgobius gulosus adults
Anopsilana jonesi	*Diaptomus sp.*	*Hemipterans, Corixid adults*	*Microgobius gulosus juveniles*
Anuran larvae	*Dipteran, Chaoborus punctipennis larvae*	*Hemipterans, Gerrid adults*	*Micropterus salmoides juveniles*
Cassidinidea ovalis	*Dipterans, Ceratopogonid larvae*	*Hemipterans, Pleid adults*	*Nematodes*
Cladocerans, Daphnia spp.	*Dipterans, Chironomid larvae*	*Heterandria formosa juveniles*	*Odonates, Zygopteran larvae*
Coleopterans, Curculionid adults	*Dipterans, Ephydrid larvae*	*Ictalurus punctatus juveniles*	*Oligochaetes*
Coleopterans, Dytiscid adults	*Dipterans, Stratiomyid larvae*	*Ilyocryptus sp.*	*Orthocyclops modestus*
Coleopterans, Dytiscid larvae	*Dorosoma spp. preflexion larvae*	*Lepidopterans, Pyralid larvae*	*Poecilia latipinna juveniles*
Coleopterans, Elmid adults	*Ephemeropteran larvae*	*Lepomis macrochirus juveniles*	*Simocephalus vetulus*
Coleopterans, Elmid larvae	*Eurytemora affinis*	*Lepomis punctatus juveniles*	*Taphromysis bowmani*
Coleopterans, Gyrinid larvae	*Fundulus seminolis postflexion larvae*	*Leydigia sp.*	*Tilapia melanotheron juveniles*
Coleopterans, Haliplid larvae	*Gambusia holbrooki adults*	*Lucania parva juveniles*	*Trichopteran larvae*
Coleopterans, Noterid adults	*Gambusia holbrooki juveniles*	*Macrocyclops albidus*	*Unidentified freshwater cyclopoids*
Coleopterans, Noterid larvae	*Gobiid eggs*	*Menidia spp. juveniles*	*Xanthid juveniles*
Coleopterans, Scirtid larvae	*Gobiosoma bosc juveniles*	*Menidia spp. preflexion larvae*	
Cyclops sp.	*Gobiosoma robustum* juveniles	*Mesocyclops edax*	

6.2.1.4 Zooplankton Abundance

Although zooplankton were only collected in the upper river segment during Period 5, the average and cumulative abundance of salinity-sensitive zooplankton taxa appeared highest in this LHR target zone river segment, when compared with the middle and lower segment data (Figure 6.2-4, Figure 6.2-5).

Figure 6.2-4: Salinity-sensitive zooplankton abundance over time within the LHR target zone (Analysis Days)

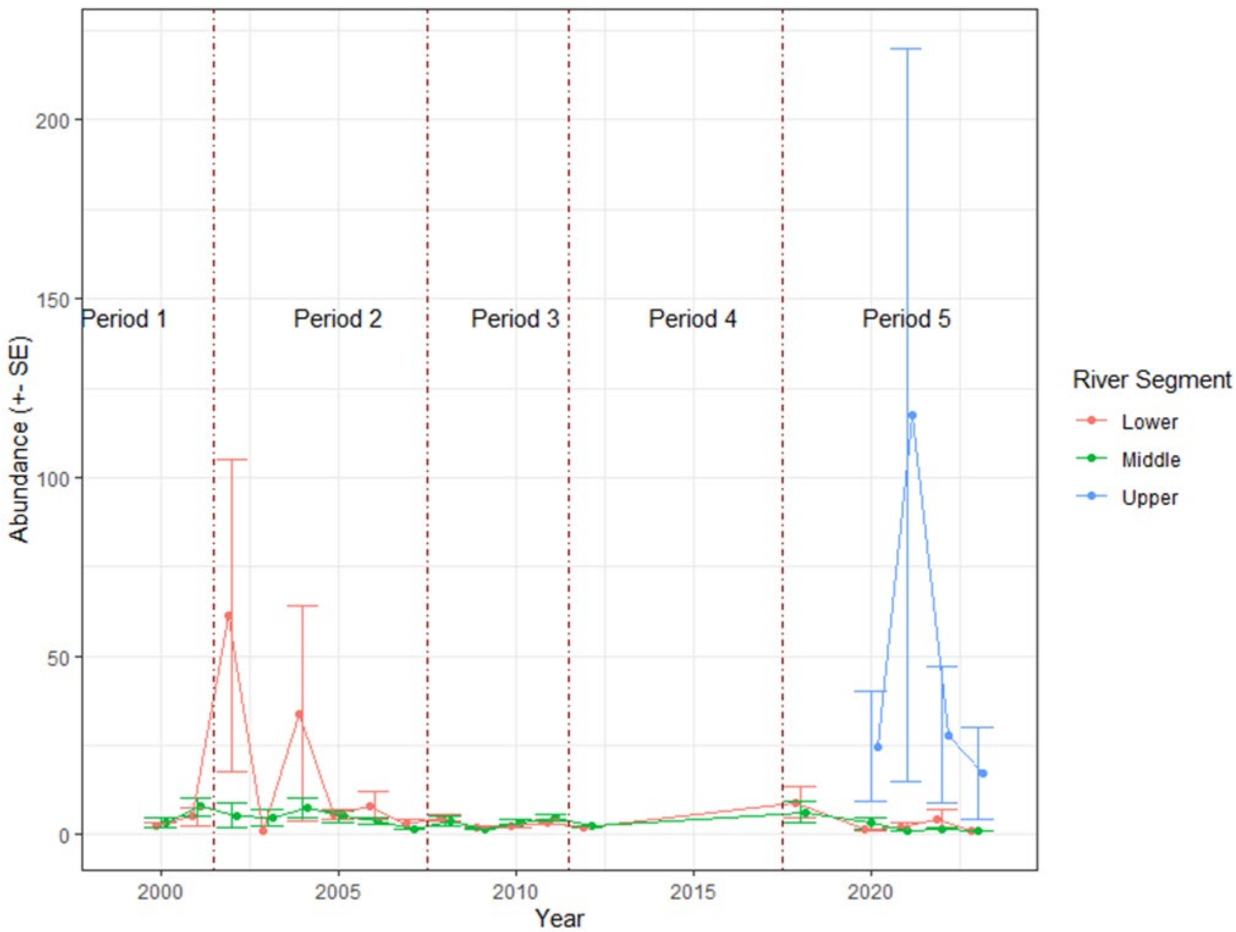
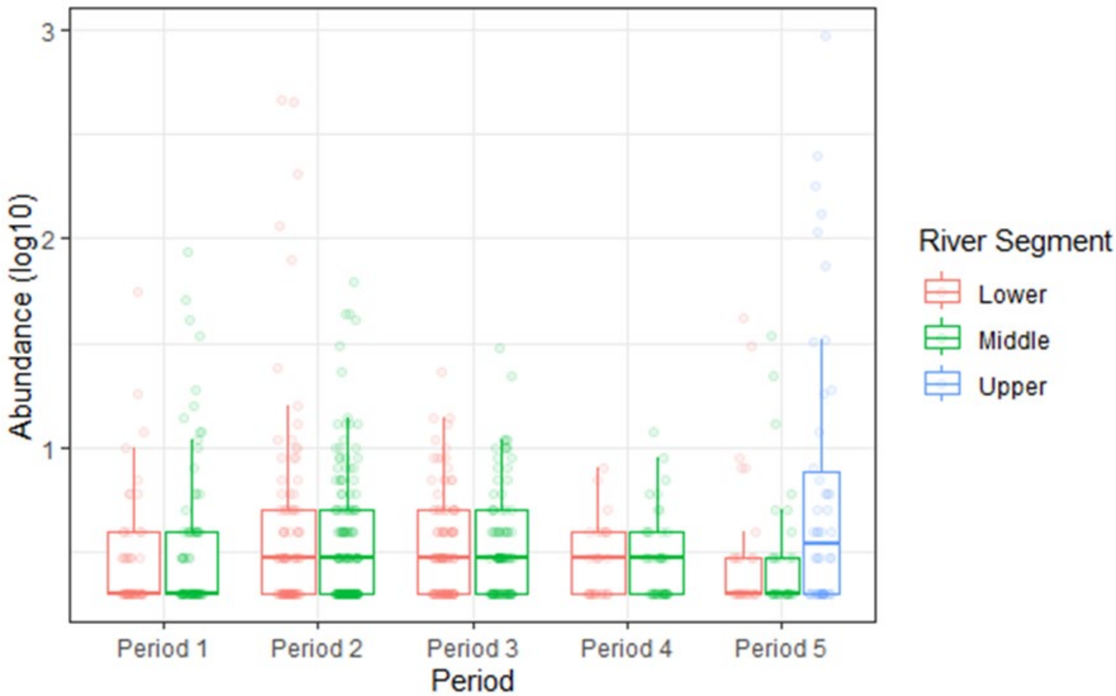


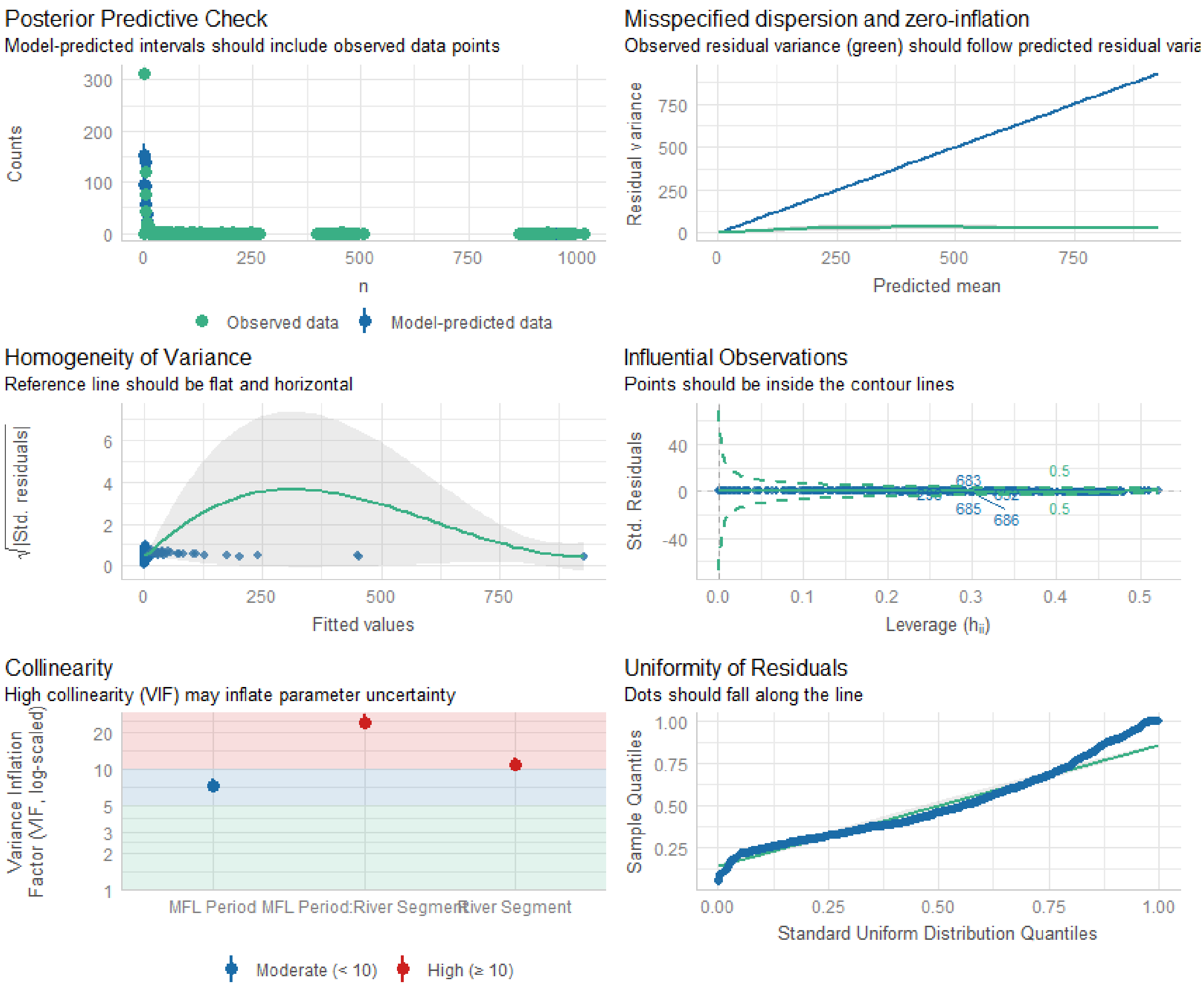
Figure 6.2-5: Salinity-sensitive zooplankton abundance boxplots



Zooplankton Abundance Modeling

Statistical models are used to assess data by testing hypotheses, estimating parameters, or determining the significance of the results. Mixed effects models are used to predict a single variable using two or more other variables (Bakker 2024). A chi-square test is a statistical procedure used to compare observed results with expected results to determine if a difference between observed data and expected data is due to a genuine relationship between the variables or due to random variation. Several potential zooplankton abundance models were evaluated, including linear mixed effects models (with and without log transformation of the data), with a Poisson distribution, and a generalized linear mixed effects model with a negative binomial distribution. Model assumptions were assessed for each approach. For the linear models, the assumption of normality was violated due to right-skewed data, a common occurrence with count data. Equidispersion was violated by the Poisson model. However, a negative binomial model was unable to resolve overdispersion. Additionally, the negative binomial model variances were high and collinearity was violated, though collinearity is an expected violation when examining interaction effect models. An observation level random effect was added to the generalized mixed Poisson model, which accounted for the overdispersion (dispersion ratio = 0.11, $p > 0.05$), and therefore, the mixed Poisson model was considered appropriate. The residuals for this model predominantly exhibited an acceptable fit (Figure 6.2-6).

Figure 6.2-6: Model assumption tests for salinity-sensitive zooplankton abundance



A mixed model for salinity-sensitive zooplankton abundance using the negative binomial distribution indicated no significant interaction effect (p = 0.24) between minimum flow period and segment and a significant effect of river segment (p < 0.05) (Table 6.2-3). As noted above, zooplankton data were only available for the upper segment during Period 5.

Table 6.2-3: Generalized linear mixed model for salinity-sensitive zooplankton abundance using Poisson distribution

	Chi squared	Degrees of Freedom	p value
(Intercept)	3.89	1	< 0.05
Minimum Flow Period	5.39	4	0.25
River Segment	15.50	2	< 0.05
Minimum Flow Period: River Segment	5.49	4	0.24

Zooplankton Abundance Effect Sizes

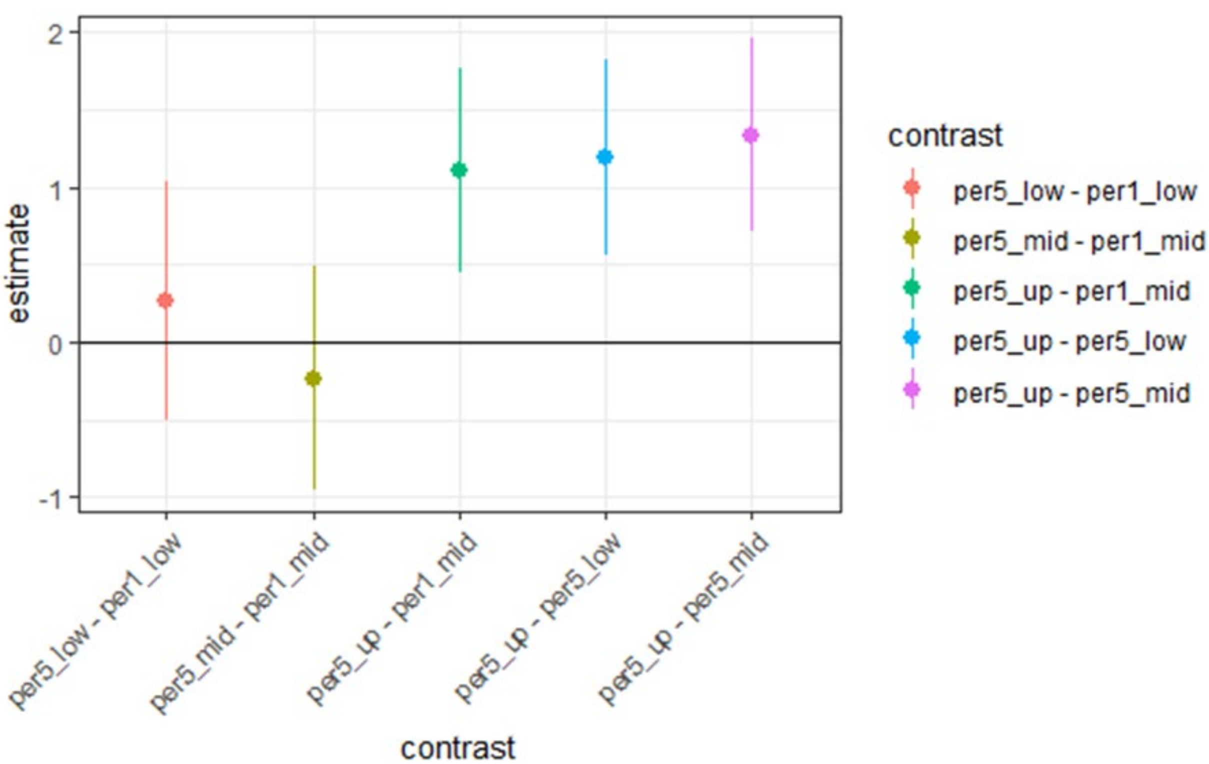
Effect size is a quantification of the difference between two group means. In this analysis, the differences in various measures of salinity-sensitive taxa were quantified by comparing the segment means calculated from various minimum flow implementation periods. The effect size was measured by the standardized difference between the mean of group one (e.g., salinity sensitive taxa after minimum flow implementation) minus the mean of group two (e.g., salinity sensitive taxa prior to minimum flow implementation), divided by the standard deviation.

Selected effect size contrasts indicated that during Period 5 the upper segment had a higher abundance of salinity-sensitive zooplankton than did the middle and lower segments. The upper segment during Period 5 also had a higher abundance of salinity-sensitive zooplankton compared to the middle segment during Period 1, with 95% confidence limits that did not cross zero (Table 6.2-4, Figure 6.2-7). Again, zooplankton data were only available for the upper segment during Period 5.

Table 6.2-4: Selected contrasts for salinity-sensitive zooplankton abundance

Contrast	Estimate	Standard Error	Degrees of freedom	Asymptotic Lower Confidence Limit	Asymptotic Upper Confidence Limit
Period 5 Upper – Period 1 Middle	1.10	0.34	Inf	0.45	1.76
Period 5 Upper – Period 5 Middle	1.34	0.32	Inf	0.72	1.95
Period 5 Upper – Period 5 Lower	1.18	0.32	Inf	0.55	1.81
Period 5 Lower – Period 1 Lower	0.26	0.39	Inf	-0.51	1.04
Period 5 Middle – Period 1 Middle	-0.23	0.37	Inf	-0.95	0.49

Figure 6.2-7: Selected contrasts for salinity-sensitive zooplankton abundance



Salinity and Zooplankton Abundance Models

A generalized linear mixed model was developed using the Poisson distribution for 28-day depth-averaged salinity, as determined via the LAMFE model. Model assumptions were examined and upheld except for overdispersion, which was accounted for with an observation level random effect.

A mixed model predicting salinity-sensitive zooplankton abundance across all river segments was significant ($p = 0.01$), while the mixed model for the upper segment alone was not ($p = 0.67$) (Table 6.2-5, Table 6.2-6). Salinity-sensitive taxa abundance predicted by 28-day depth-averaged salinity (determined via the LAMFE model) showed a higher abundance of salinity-sensitive taxa in salinities < 5 ppt, which occurred primarily during the Period 5 sampling event (Figure 6.2-8). Figure 6.2-9 depicts boxplots of LAMFE predicted 28-day antecedent salinity associated with zooplankton sampling events for all periods and segments.

Table 6.2-5: Linear mixed model with Poisson distribution for 28-day depth-averaged salinity (determined via the LAMFE model) as a predictor for salinity-sensitive zooplankton abundance

	Chi Squared	Degrees of Freedom	p value
(Intercept)	11	1	0.00
salin_depavg_28day	6	1	0.01

Table 6.2-6: Linear mixed model with Poisson distribution for 28-day depth-averaged salinity (determined via the LAMFE model) as a predictor for salinity-sensitive zooplankton abundance within the upper segment of the study area

	Chi Squared	Degrees of Freedom	p value
(Intercept)	0.39	1	0.53
salin_depavg_28day	0.18	1	0.67

Figure 6.2-8: Salinity-sensitive zooplankton taxa abundance predicted by 28-day depth-averaged salinity (in ppt, determined via the LAMFE model)

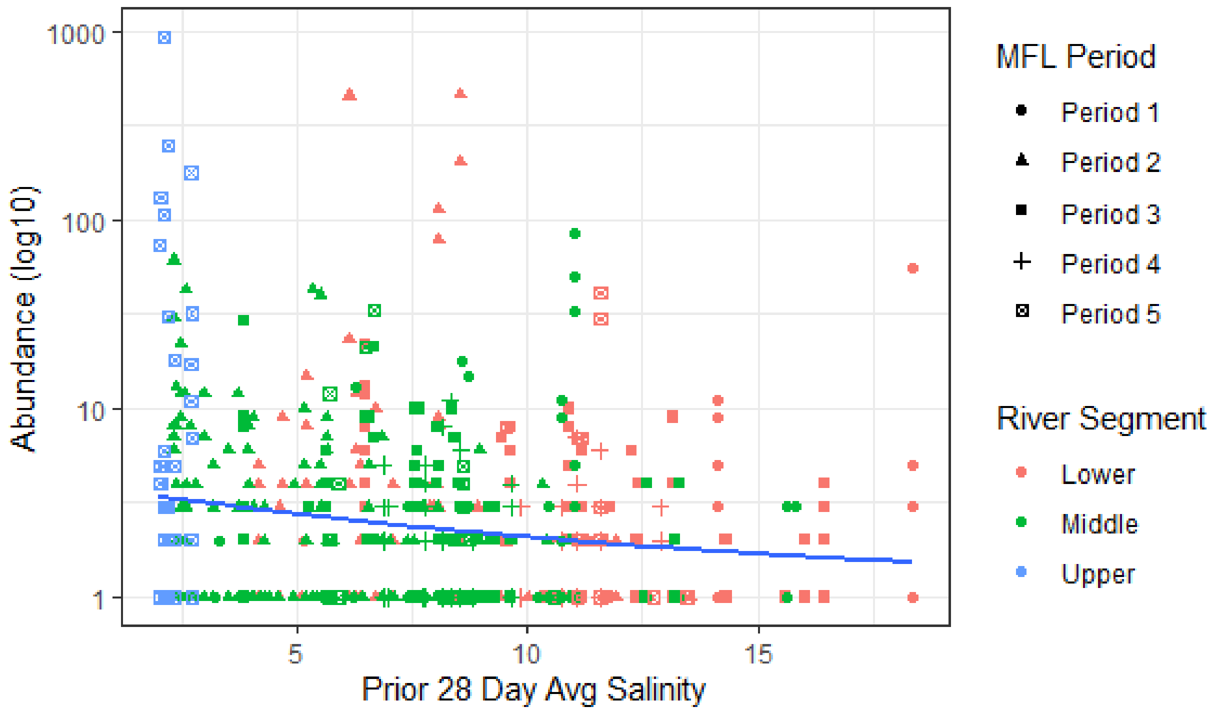
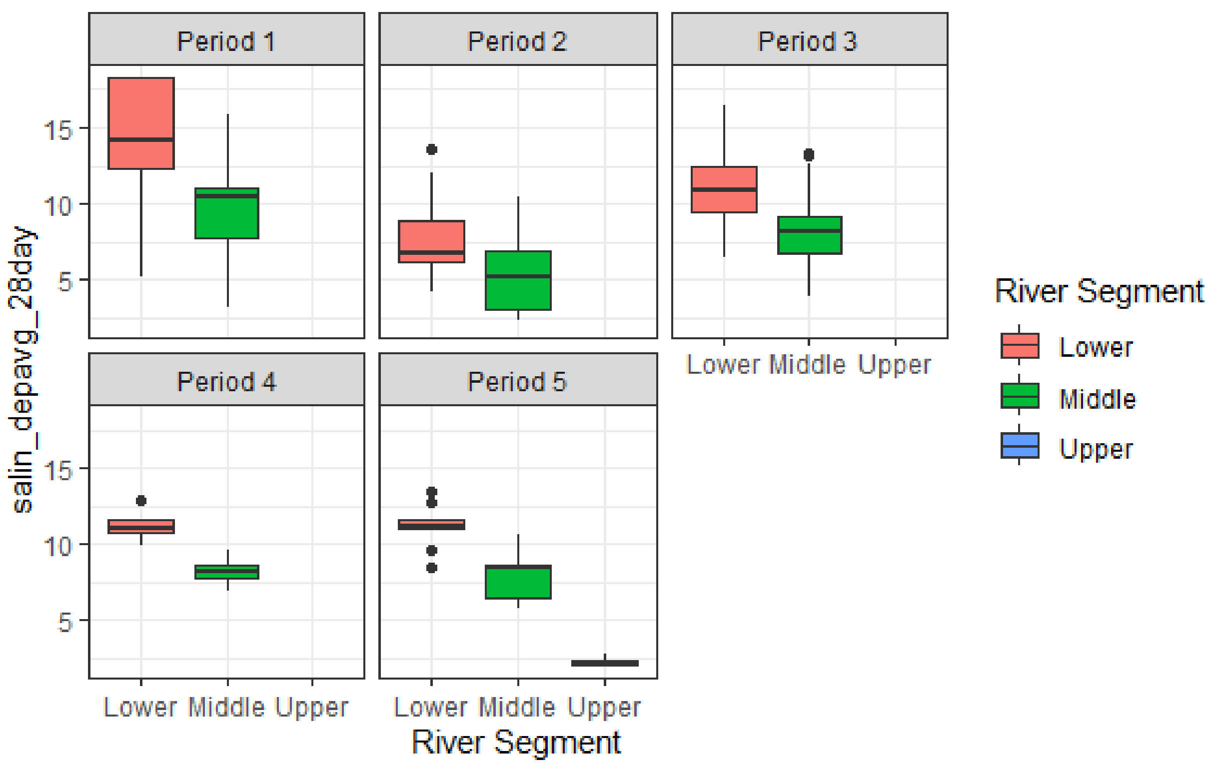


Figure 6.2-9: Antecedent 28-day depth averaged salinity (in ppt, determined via LAMFE model) by river segment and period for zooplankton collections



6.2.1.5 Zooplankton Density

The average density of salinity-sensitive zooplankton taxa was highest in the upper segment during Period 5. Another noticeable peak occurred in the lower segment during Period 2 (Figure 6.2-10, Figure 6.2-11).

Densities above 20 per m^3 were removed for these analyses as outliers. The majority of these observations (22 of 23) occurred in 2018, with many of those densities above 50 per m^3, an order of magnitude or more above all other densities in all other years.

Figure 6.2-10: Density of salinity-sensitive zooplankton over time

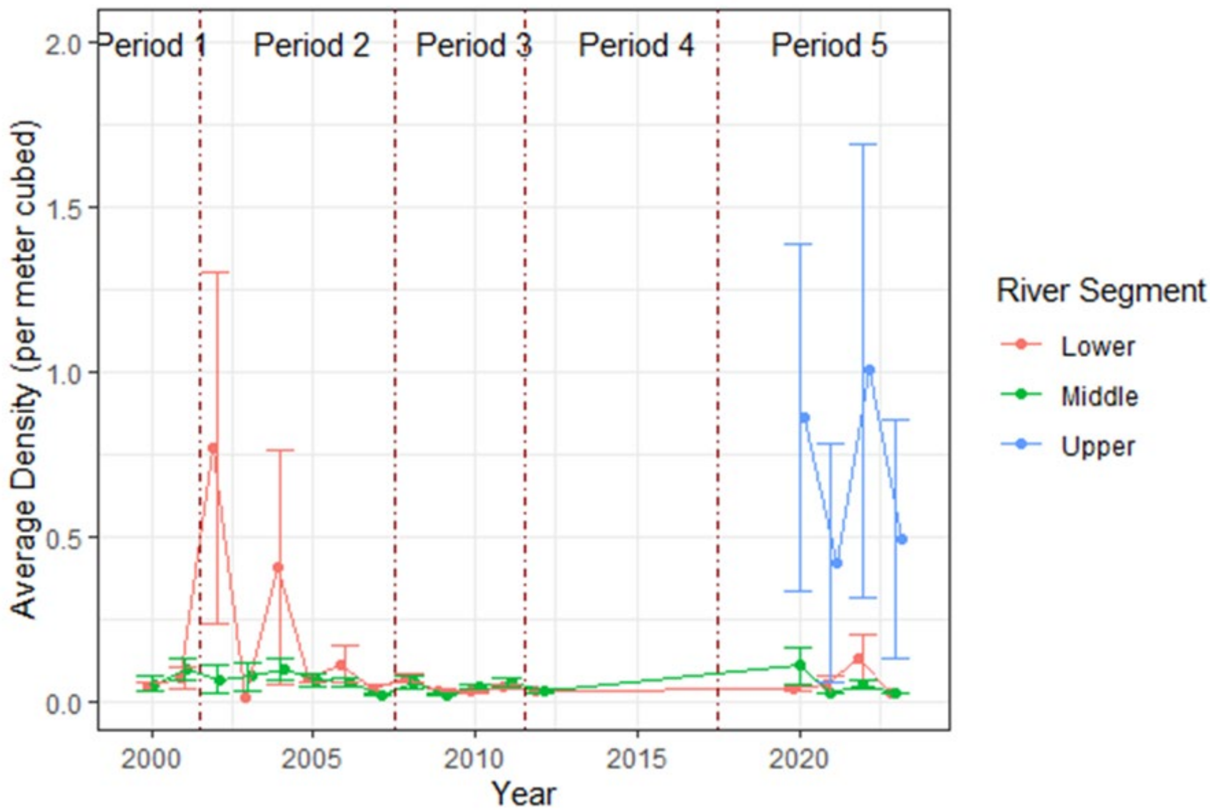
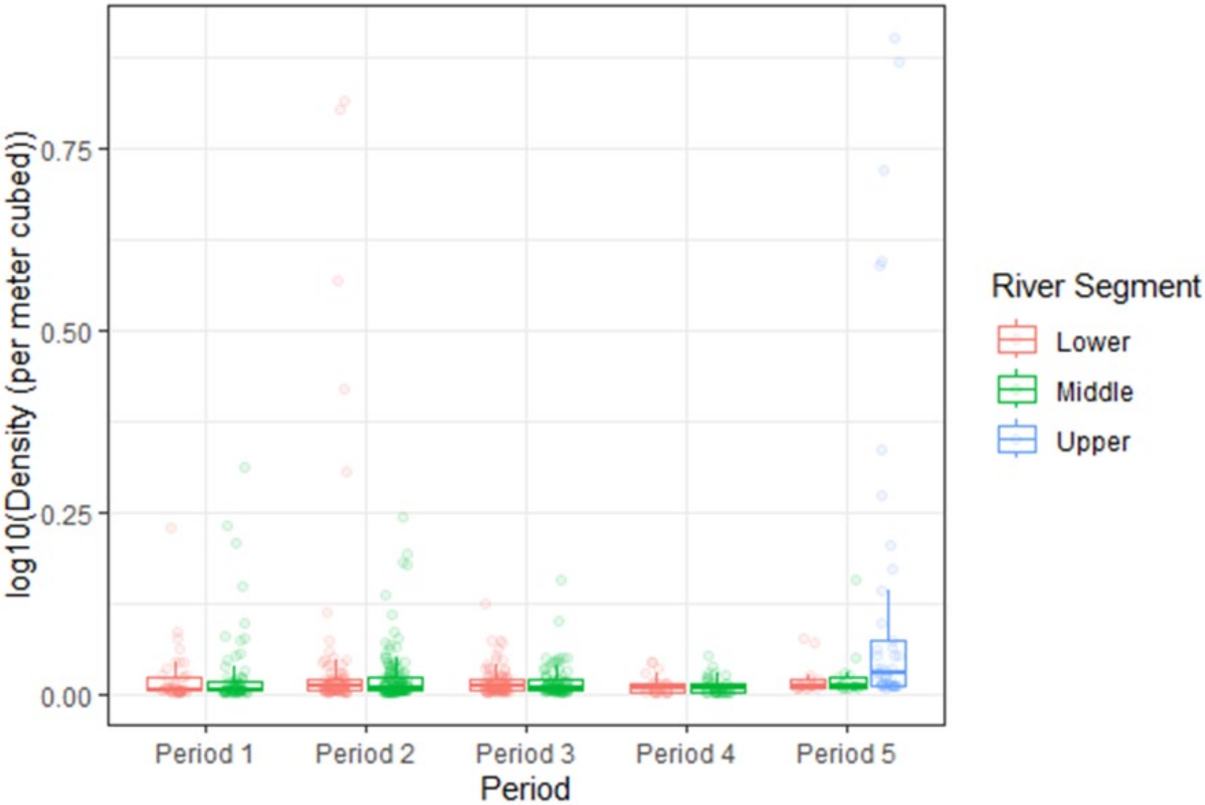


Figure 6.2-11: Density boxplots for salinity-sensitive zooplankton



Zooplankton Density Modeling

Density data present similar challenges to modeling as count data. To address violations of linear model assumptions, a generalized linear model with a gamma distribution was used. Model assumptions (independent observations, a gamma distribution, and equidispersion) were examined and found to be met. (Figure 6.2-12). Although collinearity was elevated, this is common and expected when including interaction effects in a model.

The model for zooplankton density using gamma distribution indicated no significant interaction effect (p = 0.12) between period and segment (Table 6.2-7).

Figure 6.2-12: Model assumption tests for salinity-sensitive zooplankton density

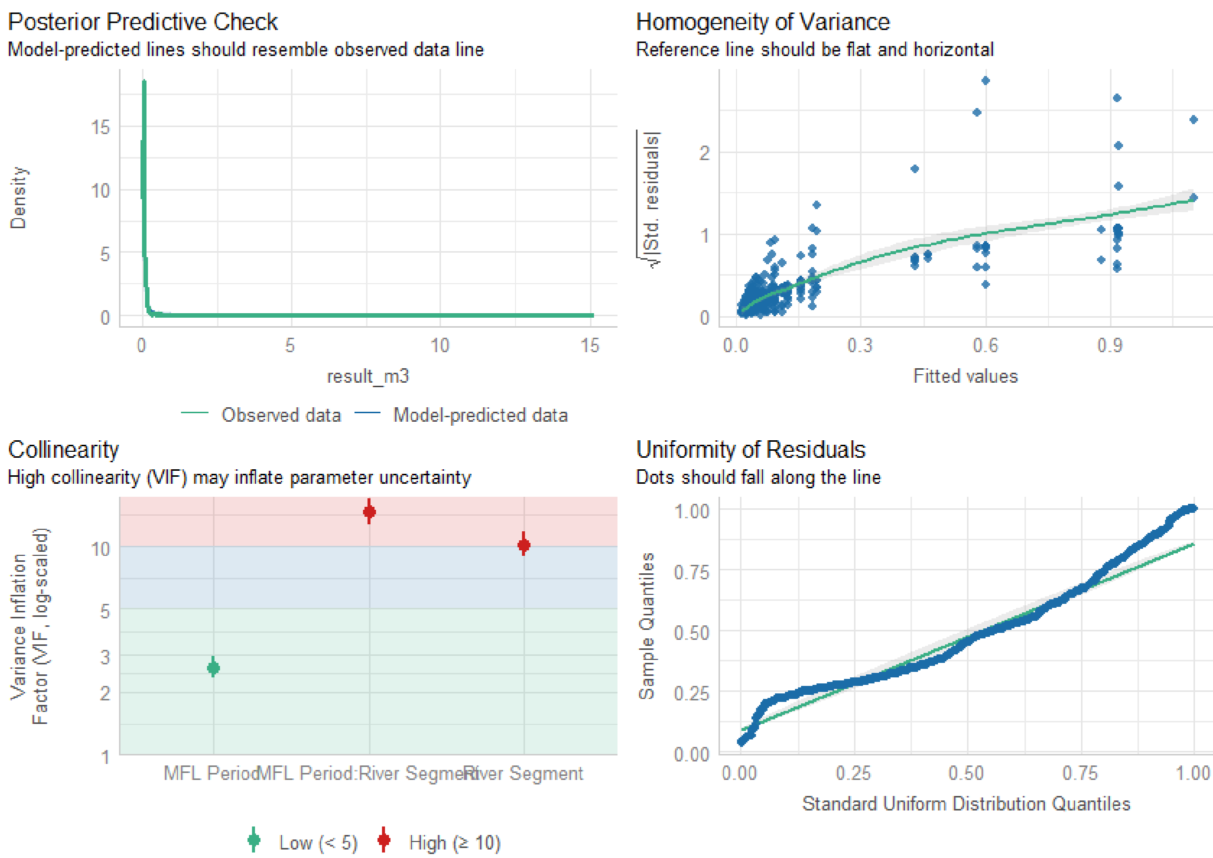


Table 6.2-7: Linear mixed model for the density of salinity-sensitive zooplankton using log transformation

	Chi Squared	Degrees of Freedom	p value
Minimum Flow Period	5.17	4	0.27
River Segment	66.33	2	< 0.05
Minimum Flow Period: River Segment	7.38	4	0.12

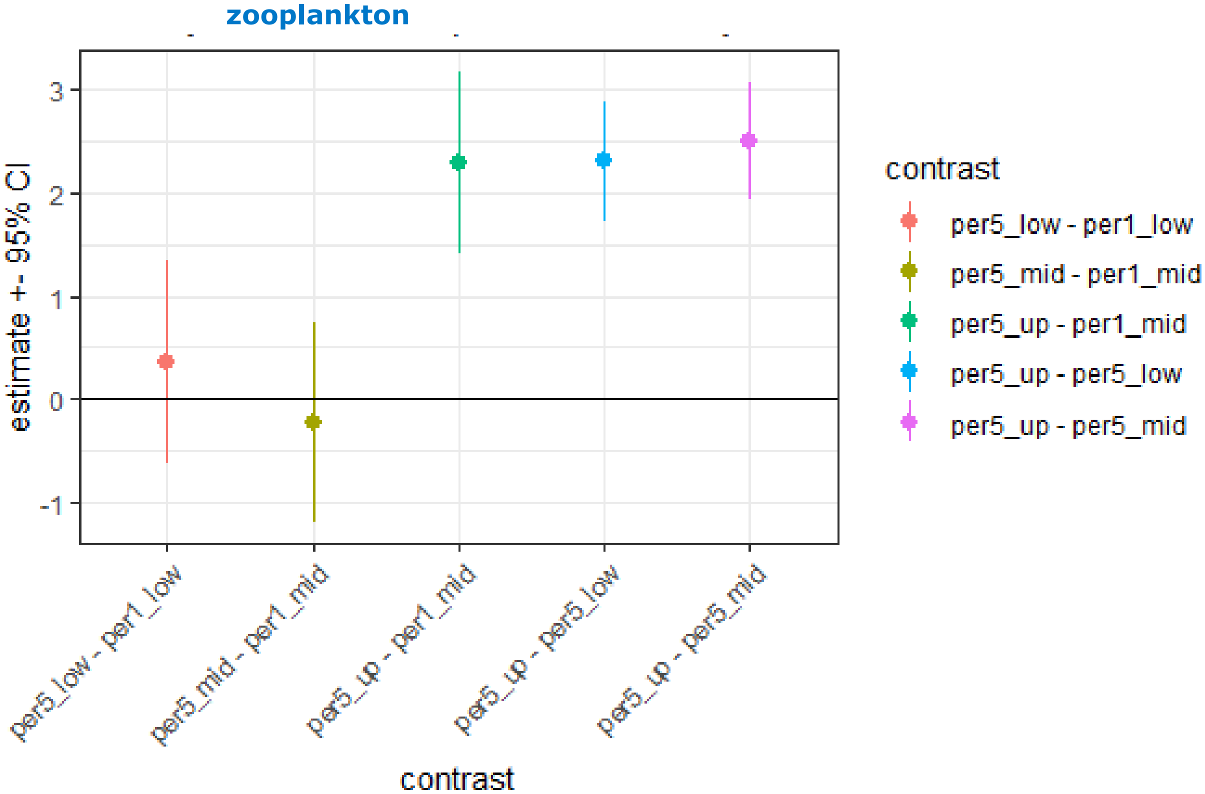
Zooplankton Density Effect Sizes

Selected contrasts for salinity sensitive zooplankton density indicated that the upper segment during Period 5 had a significantly higher density of salinity-sensitive zooplankton than the middle and lower segments during Period 5 (Table 6.2-8, Figure 6.2-13).

Table 6.2-8: Selected contrasts for salinity sensitive zooplankton density

Contrast	Estimate	Standard Error	Degrees of Freedom	Lower Confidence Limit	Upper Confidence Limit
Period 5 Upper – Period 1 Middle	2.28	0.45	Inf	1.41	3.16
Period 5 Upper – Period 5 Middle	2.51	0.29	Inf	1.94	3.07
Period 5 Upper – Period 5 Lower	2.30	0.29	Inf	1.73	2.87
Period 5 Lower – Period 1 Lower	0.37	0.50	Inf	-0.62	1.35
Period 5 Middle – Period 1 Middle	-0.22	0.49	Inf	-1.19	0.75

Figure 6.2-13: River segment contrasts for the density of salinity-sensitive zooplankton



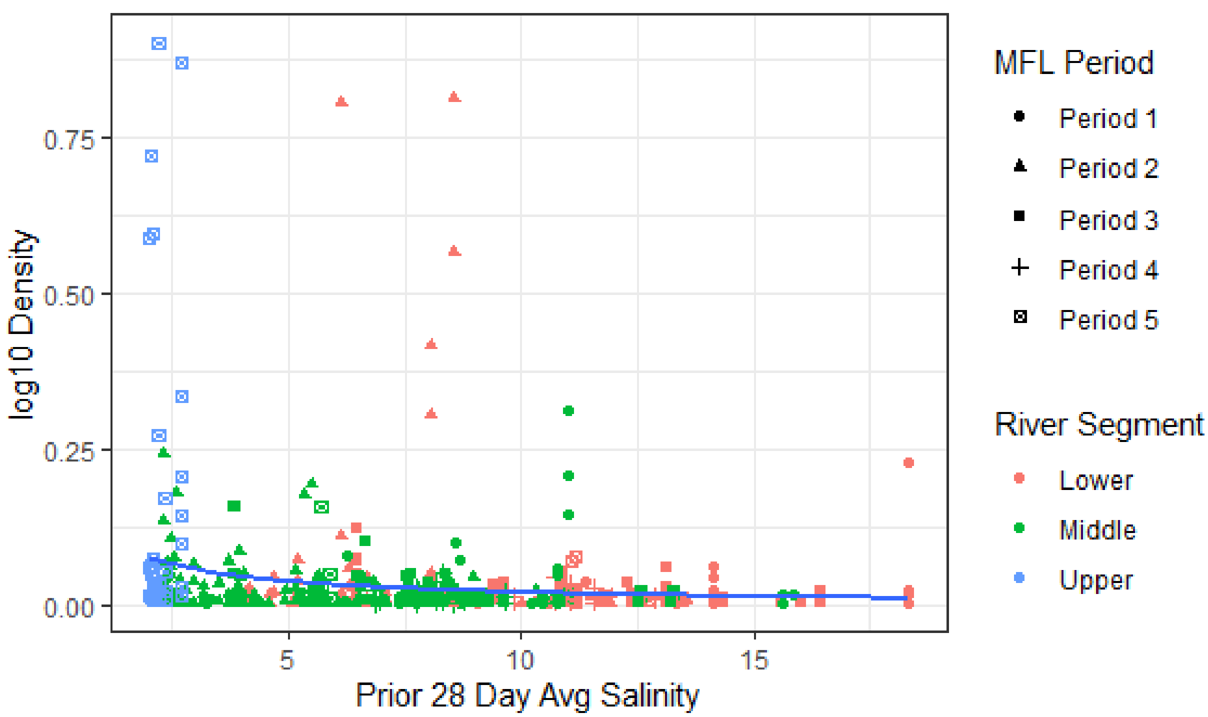
Salinity and Zooplankton Density

A generalized linear mixed model with a gamma distribution was used with the 28-day depth-averaged salinity (as determined via LAMFE model). Model assumptions were evaluated and were met, with a slight violation of homogeneity. This was deemed acceptable, as residual fit was far better than other models examined. The mixed model was significant ($p < 0.01$) as a predictor for salinity-sensitive zooplankton density (Table 6.2-9). The R^2 was 0.2 for the main effect, suggesting the predictor variable explained 20% of the variance in the response variable. The density of salinity-sensitive zooplankton predicted by 28-day depth-averaged salinity (as determined via LAMFE model) appeared higher in salinities < 5 ppt (Figure 6.2-14).

Table 6.2-9: Linear mixed model with log transformation for 28-day depth-averaged salinity (determined via the LAMFE model) as a predictor for salinity sensitive zooplankton density

	Chi Squared	Degrees of Freedom	p value
(Intercept)	9.1	1	< 0.05
salin_depavg_28day	5.7	1	< 0.05

Figure 6.2-14: Density (per m^3) of salinity-sensitive zooplankton as predicted by 28-day depth-averaged salinity (in ppt, determined via the LAMFE model)



6.2.1.6 Zooplankton Taxa Richness

Taxa richness is defined as the number of distinct types of organisms found at a location or date, as identified to the lowest practical taxonomic level (FDEP 2017).

During Period 5, when data were available at all three river segments, the richness of salinity-sensitive zooplankton taxa was highest within the upper segment, as compared to the middle and lower segments (Figure 6.2-15, Figure 6.2-16). This suggests that salinity-sensitive taxa richness is an effective metric for discriminating differences in salinity regimes that may be associated with minimum flow implementation.

Figure 6.2-15: Salinity sensitive zooplankton richness over time

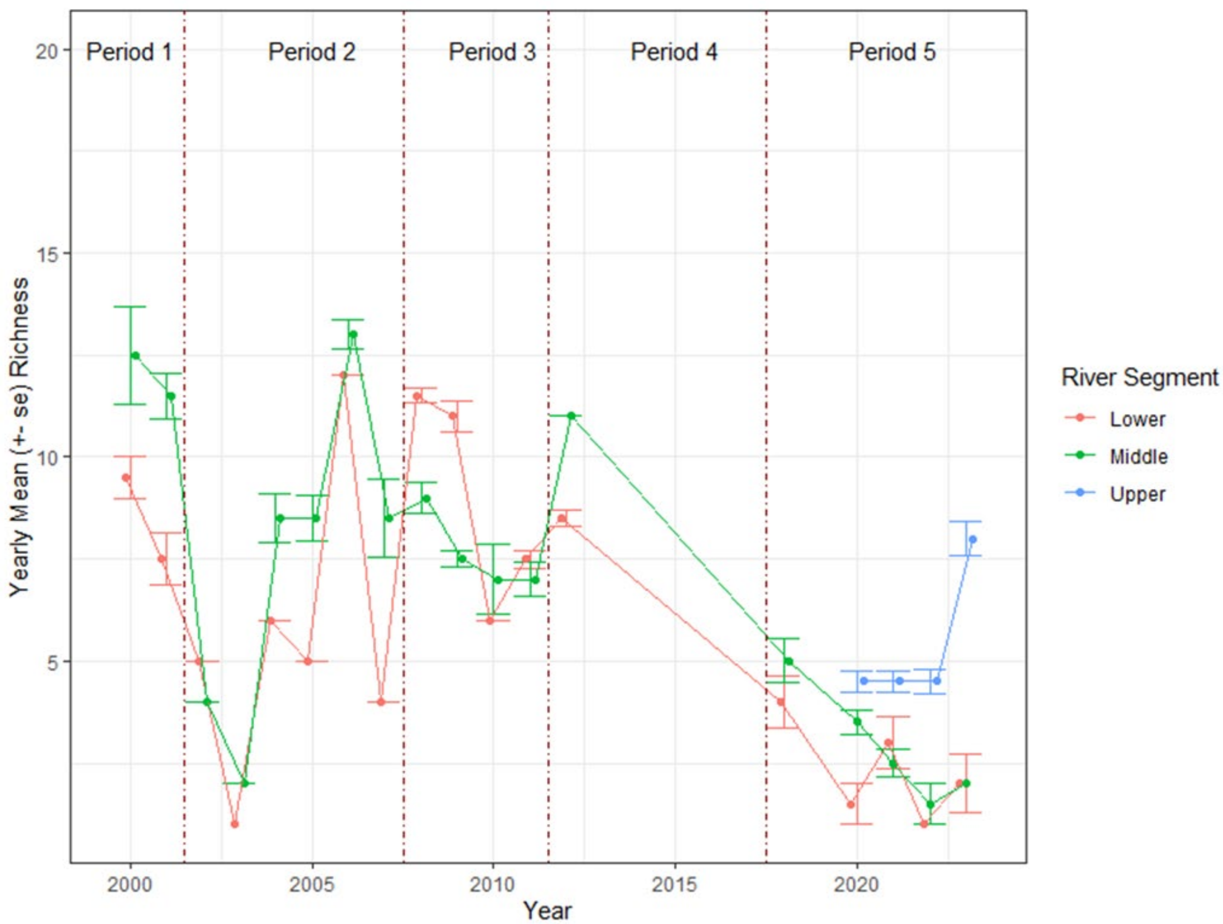
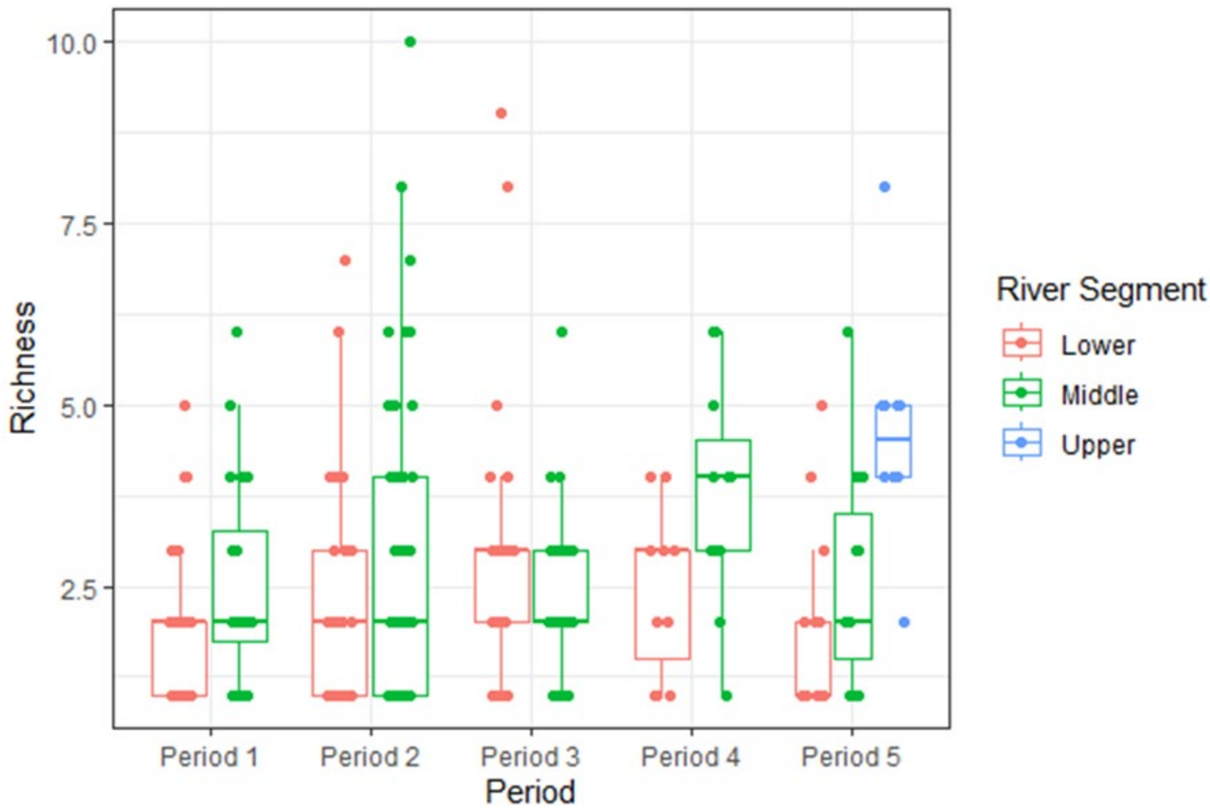


Figure 6.2-16: Boxplots of salinity-sensitive zooplankton richness by period and segment



Zooplankton Taxa Richness Modelling

A linear mixed model with Poisson distribution was determined to provide the best fit of salinity-sensitive zooplankton taxa richness data (Figure 6.2-17). Collinearity was elevated, which is a common and expected occurrence when interaction effects are included in a model.

The model indicated a statistically significant ($p < 0.1$) difference in the richness of salinity-sensitive zooplankton taxa by segment. The interaction effect between period and segment was not significant ($p = 0.20$), indicating that this trend is consistent over time, rather than varying by period, possibly a result of lack of upper segment data across periods (Table 6.2-10).

Figure 6.2-17: Model tests for richness of salinity-sensitive zooplankton taxa

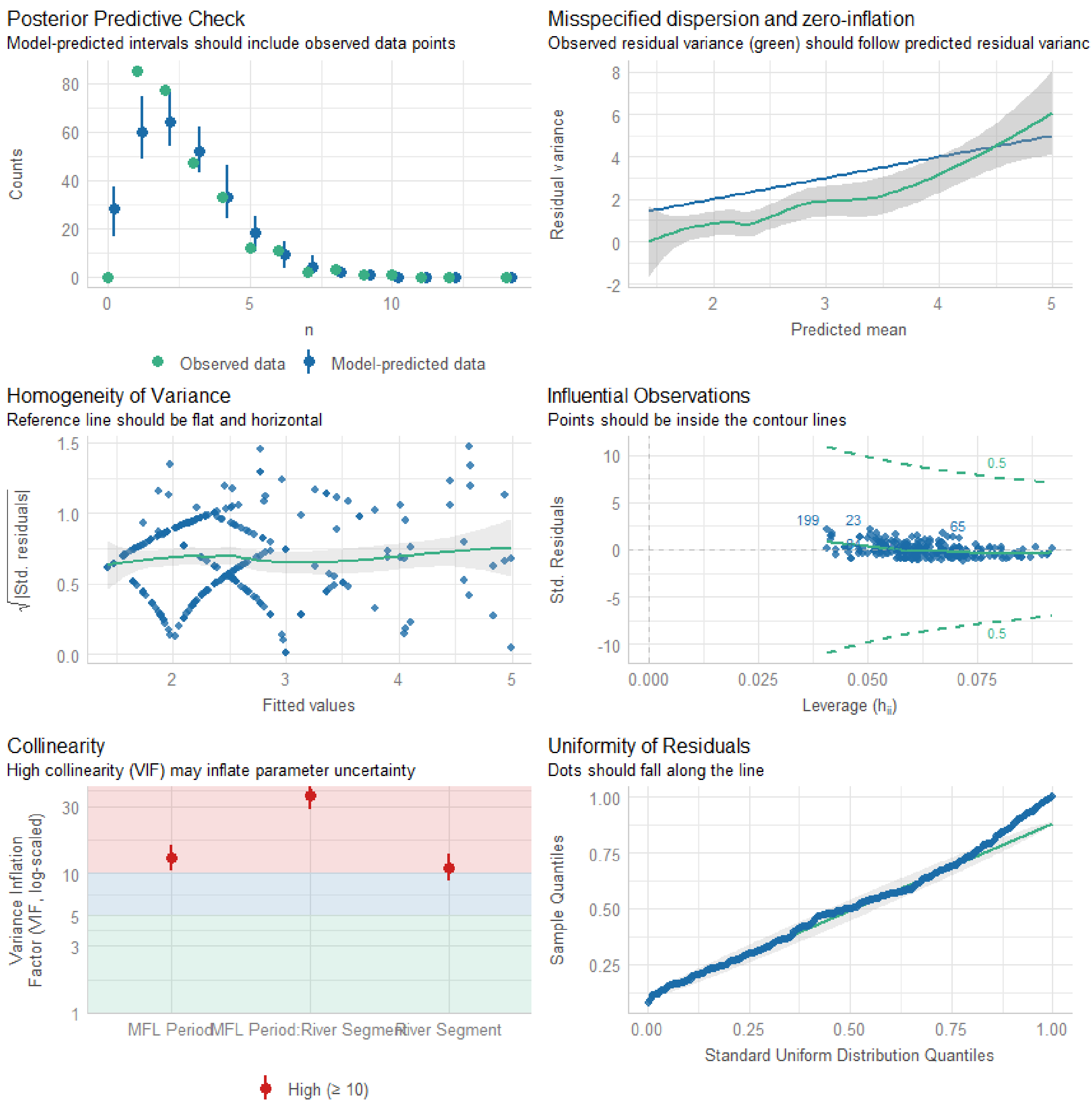


Table 6.2-10: Linear mixed model with a Poisson distribution for salinity-sensitive zooplankton taxa richness

	Chi Squared	Degrees of Freedom	p value
Minimum Flow Period	4.45	4	0.35
River Segment	15.27	2	< 0.05
Minimum Flow Period: River Segment	5.95	4	0.20

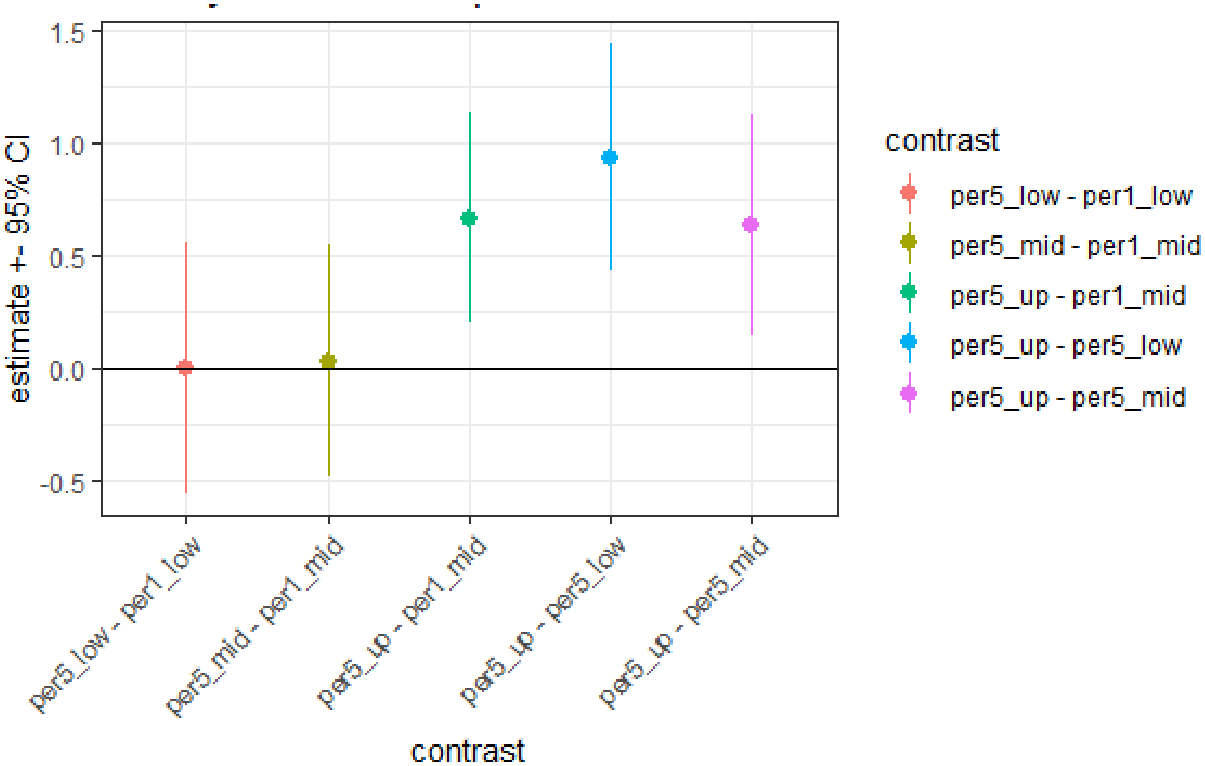
Zooplankton Taxa Richness Effect Sizes

Selected contrasts for salinity sensitive zooplankton taxa richness indicated that the upper segment during Period 5 had more salinity-sensitive taxa than did the middle and lower segments during the same period. The upper segment during Period 5 also had more salinity-sensitive taxa than the middle segment during Period 1, as indicated by 95% confidence limits that did not overlap zero (Table 6.2-11, Figure 6.2-18).

Table 6.2-11: Selected contrasts for salinity-sensitive zooplankton taxa richness

Contrast	Estimate	Standard Error	Degrees of Freedom	Lower Confidence Limit	Upper Confidence Limit
Period 5 Upper – Period 1 Middle	0.67	0.24	Inf	0.20	1.14
Period 5 Upper – Period 5 Middle	0.64	0.25	Inf	0.14	1.13
Period 5 Upper – Period 5 Lower	0.94	0.26	Inf	0.43	1.44
Period 5 Lower – Period 1 Lower	0.00	0.29	Inf	-0.56	0.56
Period 5 Middle – Period 1 Middle	0.03	0.26	Inf	-0.48	0.55

Figure 6.2-18: Selected contrasts for salinity-sensitive zooplankton taxa richness



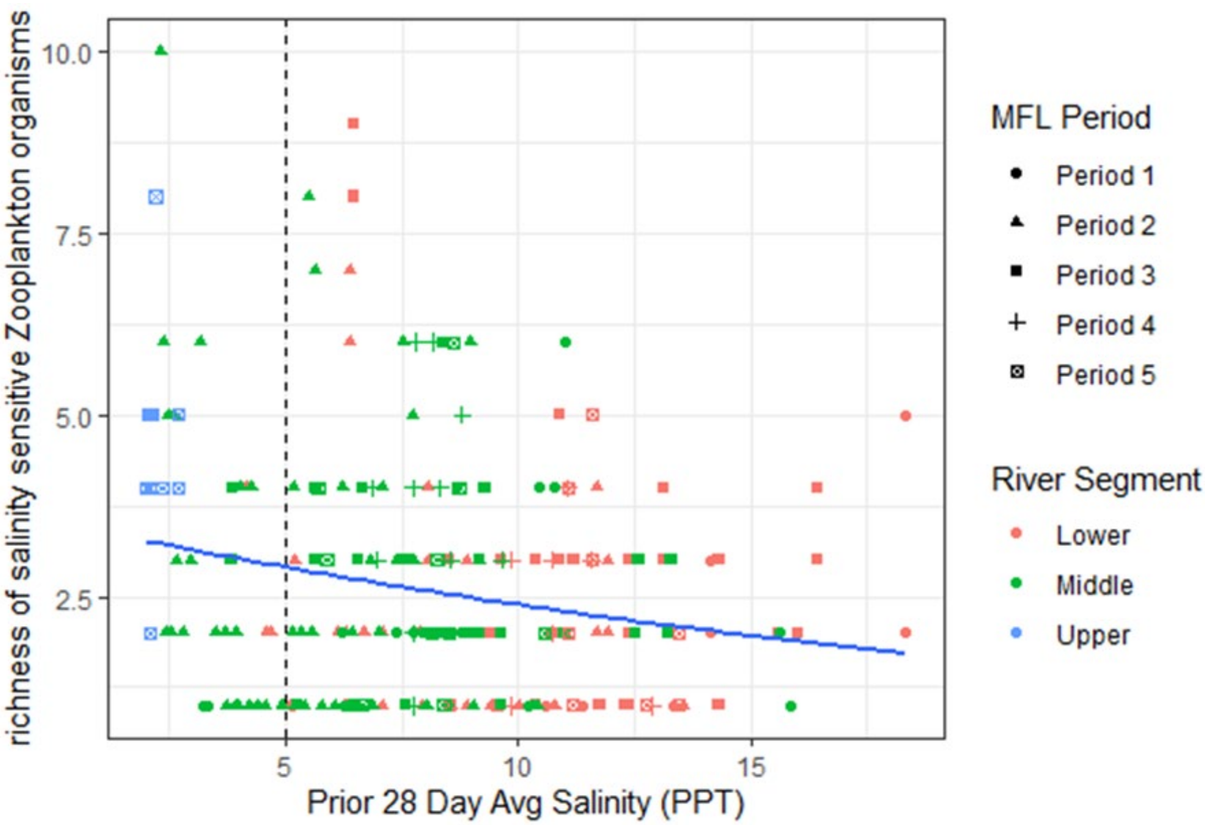
Salinity and Zooplankton Taxa Richness

A linear mixed model using the Poisson distribution for 28-day depth-averaged salinity (as determined via the LAMFE model) as a predictor for salinity-sensitive zooplankton taxa richness was statistically significant ($p < 0.01$) (Table 6.2-12), although the R^2 was low (0.1). The greatest richness was associated with low salinity values, and richness decreased as salinity increased; however, there was variability in the data (Figure 6.2-19).

Table 6.2-12: Generalized linear mixed model for 28-day depth-averaged salinity (in ppt, determined by the LAMFE model) as a predictor for salinity-sensitive zooplankton richness

	Chi Squared	Degrees of Freedom	p value
(Intercept)	113	1	0.00
salin_depavg_28day	17	1	<0.01

Figure 6.2-19: Salinity-sensitive zooplankton taxa richness vs. 28-day depth averaged salinity (in ppt) determined by the LAMFE model



6.2.1.7 Zooplankton Diversity

Although Shannon diversity has been shown to exhibit variability in tidal fresh and oligohaline waters (Weisberg et al. 1997), it was calculated for salinity-sensitive taxa to explore potential effects of minimum flow implementation.

The diversity analysis was complicated by the lack of zooplankton data in the upper segment for Periods 1–4 and by the presence of > 80 samples with a single salinity-sensitive zooplankton taxon, which resulted in diversity values of zero. A zero-inflation term was incorporated into the model to account for zero values.

The diversity of salinity-sensitive taxa was generally higher over time at the middle segment as compared to the lower segment (Figure 6.2-20, Figure 6.2-21). During Period 5, salinity-sensitive taxa diversity in the upper segment was not higher than that of the middle segment.

Figure 6.2-20: Salinity-sensitive zooplankton Shannon diversity over time

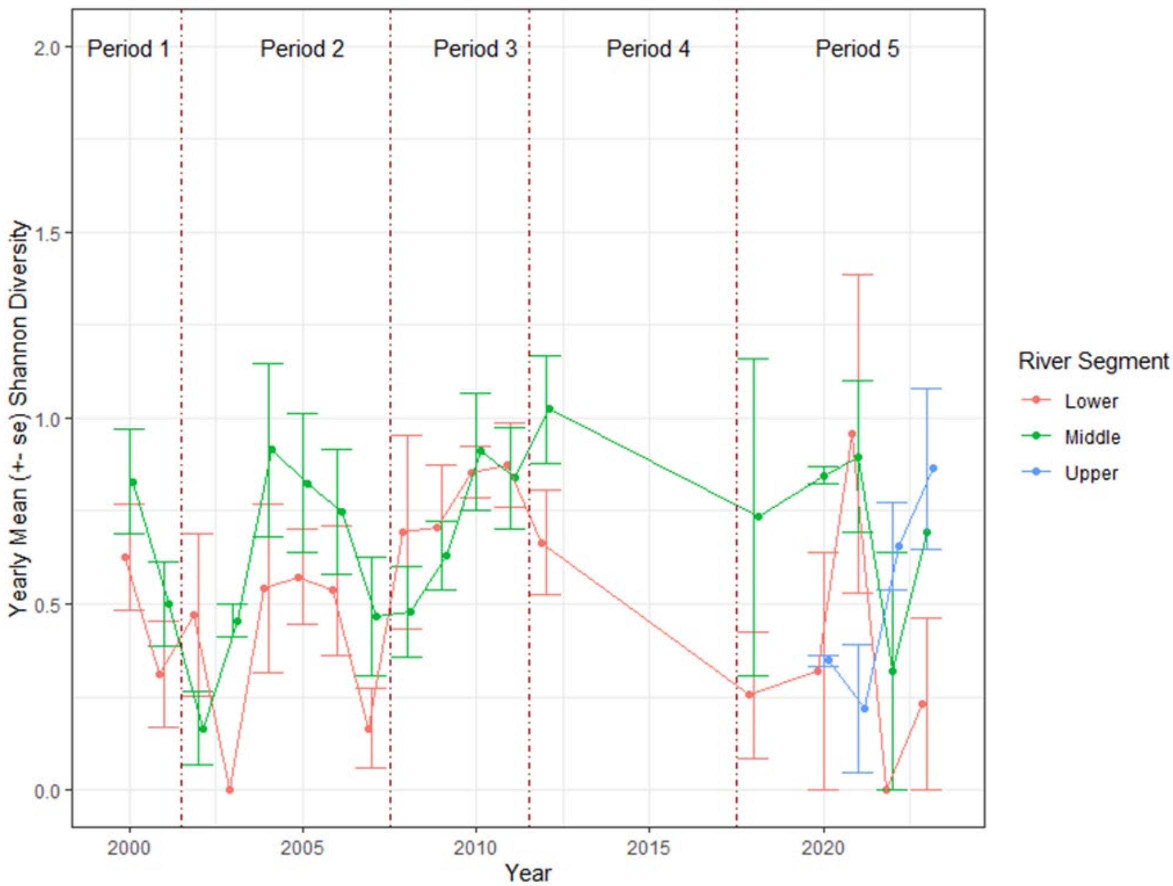
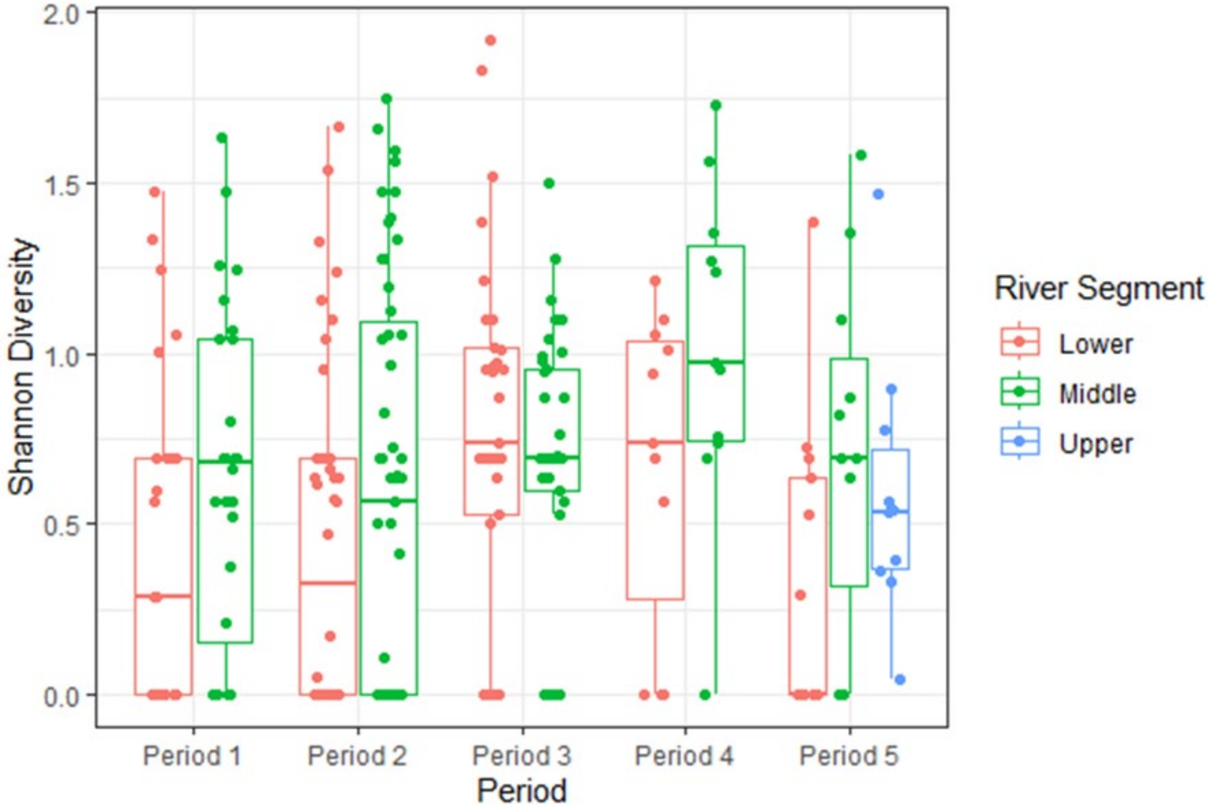


Figure 6.2-21: Boxplots of salinity-sensitive zooplankton diversity by segment



Zooplankton Diversity Modeling

Model tests showed issues due to a large number of zero values (> 80). A zero-inflated generalized linear model was fit with a Gaussian distribution (Figure 6.2-22). This model for salinity-sensitive zooplankton diversity found no significant interaction between period and segment (p =0.08) (Table 6.2-13).

Figure 6.2-22: Model tests for salinity-sensitive zooplankton diversity

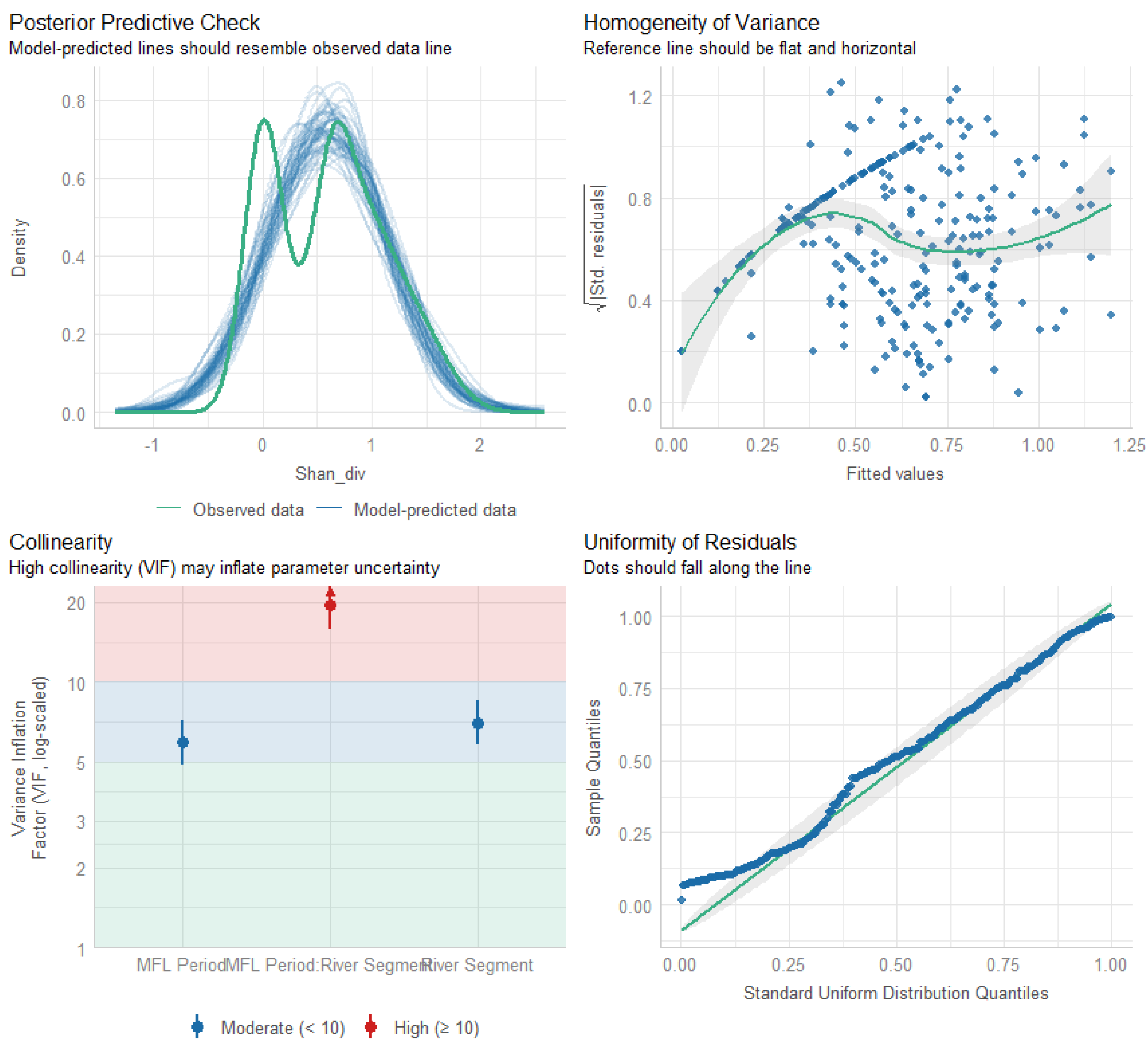


Table 6.2-13: Generalized linear mixed model for salinity-sensitive zooplankton diversity using a zero inflated Gaussian distribution

	Chi Squared	Degrees of Freedom	p value
Minimum Flow Period	9.85	4	<0.05
River Segment	4.34	2	0.11
Minimum Flow Period: River Segment	8.26	4	0.08

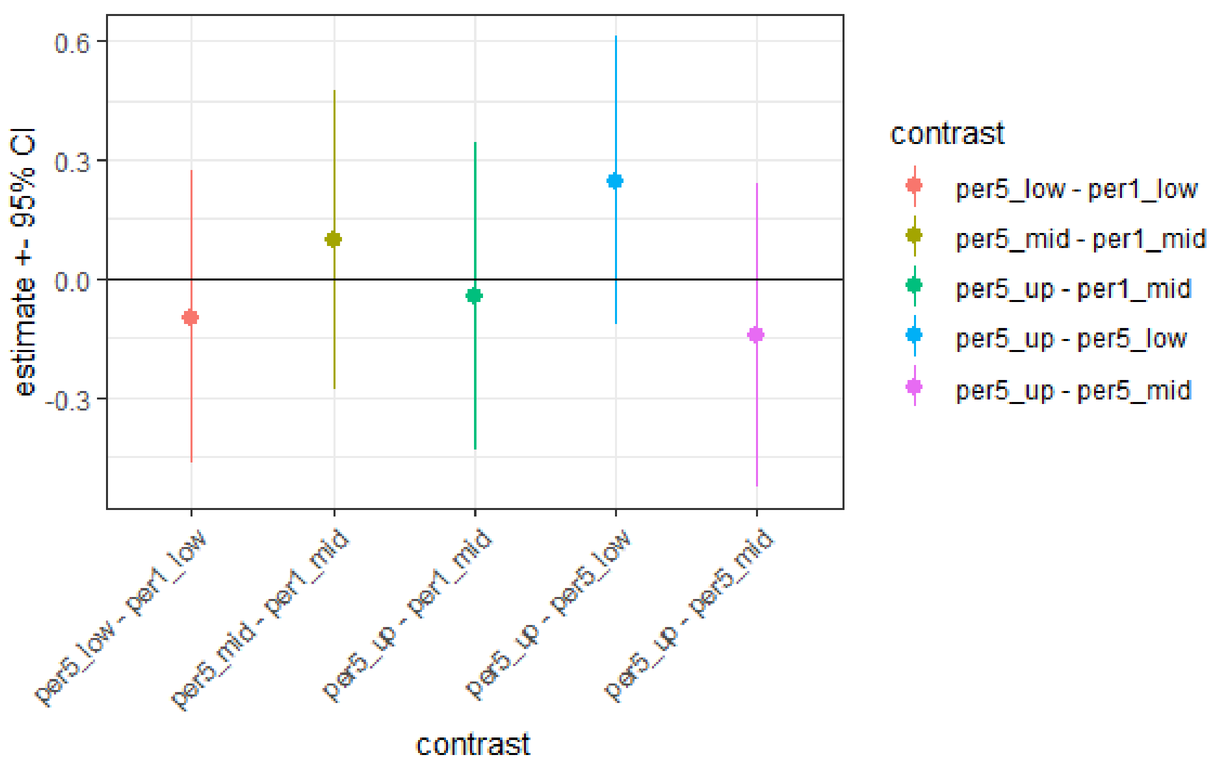
Zooplankton Diversity Effect Sizes

River segment contrasts for salinity-sensitive zooplankton diversity indicated no meaningfully significant differences (Table 6.2-14, Figure 6.2-23).

Table 6.2-14: Selected contrasts for salinity-sensitive zooplankton diversity

Contrast	Estimate	Standard Error	Degrees of Freedom	Lower Confidence Limit	Upper Confidence Limit
Period 5 Upper – Period 1 Middle	-0.04	0.20	247	-0.43	0.35
Period 5 Upper – Period 5 Middle	-0.14	0.19	247	-0.53	0.24
Period 5 Upper – Period 5 Lower	0.25	0.18	247	-0.12	0.61
Period 5 Lower – Period 1 Lower	-0.10	0.19	247	-0.47	0.27
Period 5 Middle – Period 1 Middle	0.10	0.19	247	-0.28	0.48

Figure 6.2-23: Selected contrasts for salinity-sensitive zooplankton diversity



Salinity and Zooplankton Diversity

For 28-day depth-averaged LAMFE salinity as a predictor for salinity-sensitive zooplankton diversity, a generalized additive model (p = 0.41) performed better than a zero-inflated generalized linear mixed model (p = 0.64), though neither were significant (Table 6.2-15 and Table 6.2-16). Salinity-sensitive zooplankton diversity did not exhibit a noticeable trend over the salinity range (Figure 6.2-24).

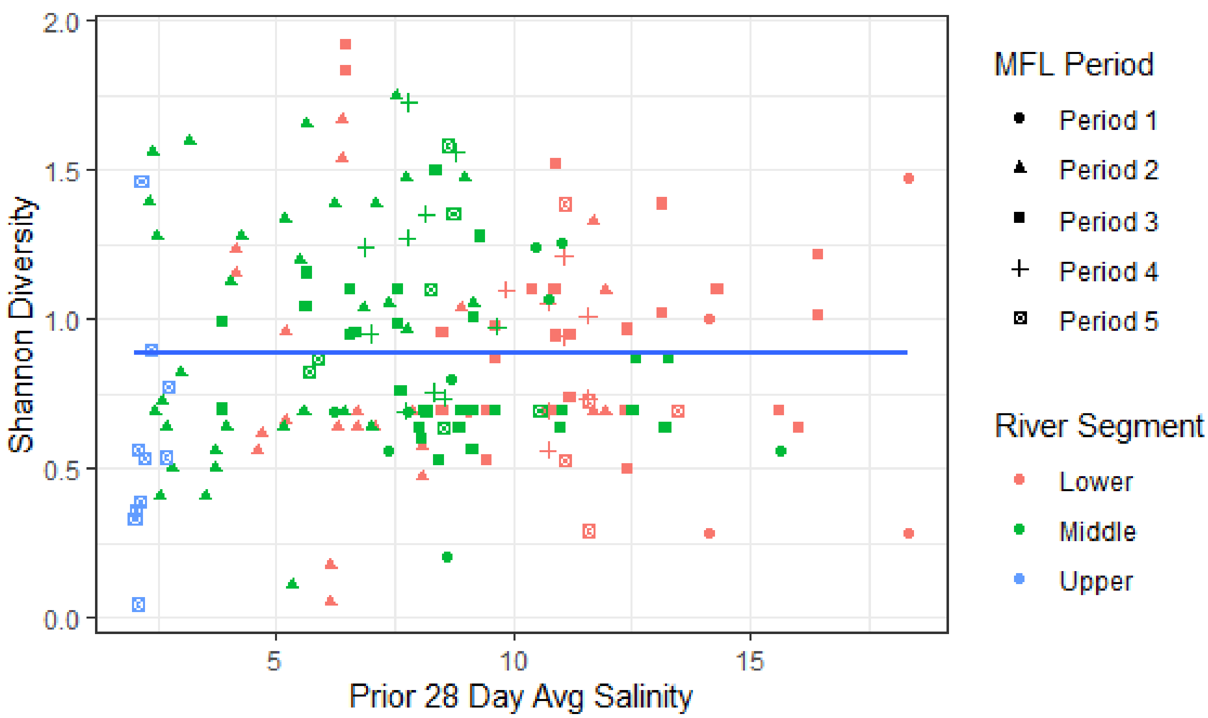
Table 6.2-15: Generalized additive model for 28-day depth-averaged LAMFE salinity as a predictor for salinity-sensitive zooplankton diversity

	Chi Squared	Degrees of Freedom	p value
(Intercept)	36.73	1	0.00
salin_depavg_28day	0.55	1	0.41

Table 6.2-16: Generalized linear model for 28-day depth-averaged LAMFE salinity as a predictor for salinity-sensitive zooplankton diversity

	Chi Squared	Degrees of Freedom	p value
(Intercept)	0.10	1	0.75
salin_depavg_28day	0.55	1	0.64

Figure 6.2-24: Salinity-sensitive zooplankton diversity by segment and period as predicted by 28-day depth-averaged LAMFE salinity (in ppt)



6.2.1.8 Zooplankton Conclusions

Cluster analysis and ordination suggested taxonomic differences when comparing the upper segment with the middle and lower segments, encouraging additional analyses involving salinity-sensitive taxa.

Zooplankton were only collected in the upper river segment during Period 5, complicating the statistical examination. Despite this, analyses of salinity sensitive zooplankton abundance, density, and taxa richness demonstrated increases in the upper segment associated with minimum flow implementation (Table 6.2-17 and Table 6.2-18).

Table 6.2-17: Summary of salinity-sensitive zooplankton mixed model results

Mixed Models	Type	Interaction Effect?	Meaningful contrasts?
Abundance ~ Period * Segment + Date(R) + Site(R)	Quasi-Poisson	No	Yes
Density ~ Period * Segment + Date(R) + Site(R)	Gamma	No	Yes
Richness ~ Period * Segment + Date(R) + Site(R)	Poisson	No	Yes
Diversity ~ Period * Segment + Date(R) + Site(R)	Zero-inflated Gaussian	No	No

Table 6.2-18: Summary of salinity-sensitive zooplankton predictive model results

Predictive Models	Type	Significant?	R^2
Abundance ~ Salinity + Date(R) + Site(R)	Poisson	Yes	0.04
Density ~ Salinity + Date(R) + Site(R)	Gamma	Yes	0.16
Richness ~ Salinity + Date(R) + Site(R)	Poisson	Yes	0.06
Diversity ~ Salinity + Date(R) + Site(R)	Zero-inflated Gaussian, GAM	No	0.01

The average and cumulative abundance of salinity-sensitive zooplankton taxa was highest in the upper segment compared with the middle and lower segment data collected during Periods 1–4, as well as higher than the upper and middle segments during Period 5. Selected effect size contrasts indicated that during Period 5, the upper segment had a higher abundance of salinity-sensitive zooplankton than did the middle and lower segments during this period and higher than the middle segment during Period 1. A model as a predictor for salinity-sensitive zooplankton abundance for all river segments was significant ($p = 0.01$), with a higher abundance of these zooplankton in salinities < 5 ppt, which occurred primarily during the Period 5 sampling event.

Selected contrasts for salinity-sensitive zooplankton density indicated that during Period 5 the upper segment had a significantly higher density of salinity-sensitive zooplankton than did the middle and lower segments. A generalized linear mixed model as a predictor for salinity-sensitive zooplankton density was significant ($p < 0.01$), demonstrating an increase in salinity-sensitive zooplankton taxa density in salinities < 5 ppt.

During Period 5, when data were available at all three river segments, the richness of salinity-sensitive zooplankton taxa was highest within the upper segment, as compared with the middle and lower segments. A linear mixed model for salinity-sensitive zooplankton taxa richness using a Poisson distribution indicated that there was a significant difference by segment ($p < 0.01$).

Selected contrasts for salinity sensitive zooplankton taxa richness indicated that during Period 5 the upper segment had statistically more sensitive taxa than did the middle and lower segments during the same period and also more sensitive taxa than the middle segment during Period 1 (indicated by 95% confidence limits that did not overlap zero). A linear mixed model using the Poisson distribution for 28-day depth-averaged salinity (as determined via the LAMFE model) as a predictor for salinity-sensitive zooplankton taxa richness was statistically significant ($p < 0.01$). Salinity sensitive zooplankton taxa richness was generally higher when salinity was < 5 ppt for the preceding 28-day period.

The zooplankton diversity analysis was complicated by the lack of upper segment zooplankton data during Periods 1-4 and the presence of > 80 samples with a single salinity-sensitive zooplankton taxon, which resulted in diversity values of zero for those cases. A zero-inflation term was incorporated into the model to account for zero values. Potentially related to this, salinity-sensitive zooplankton diversity did not exhibit a noticeable trend over the salinity range.

6.2.2 Benthic Macroinvertebrates

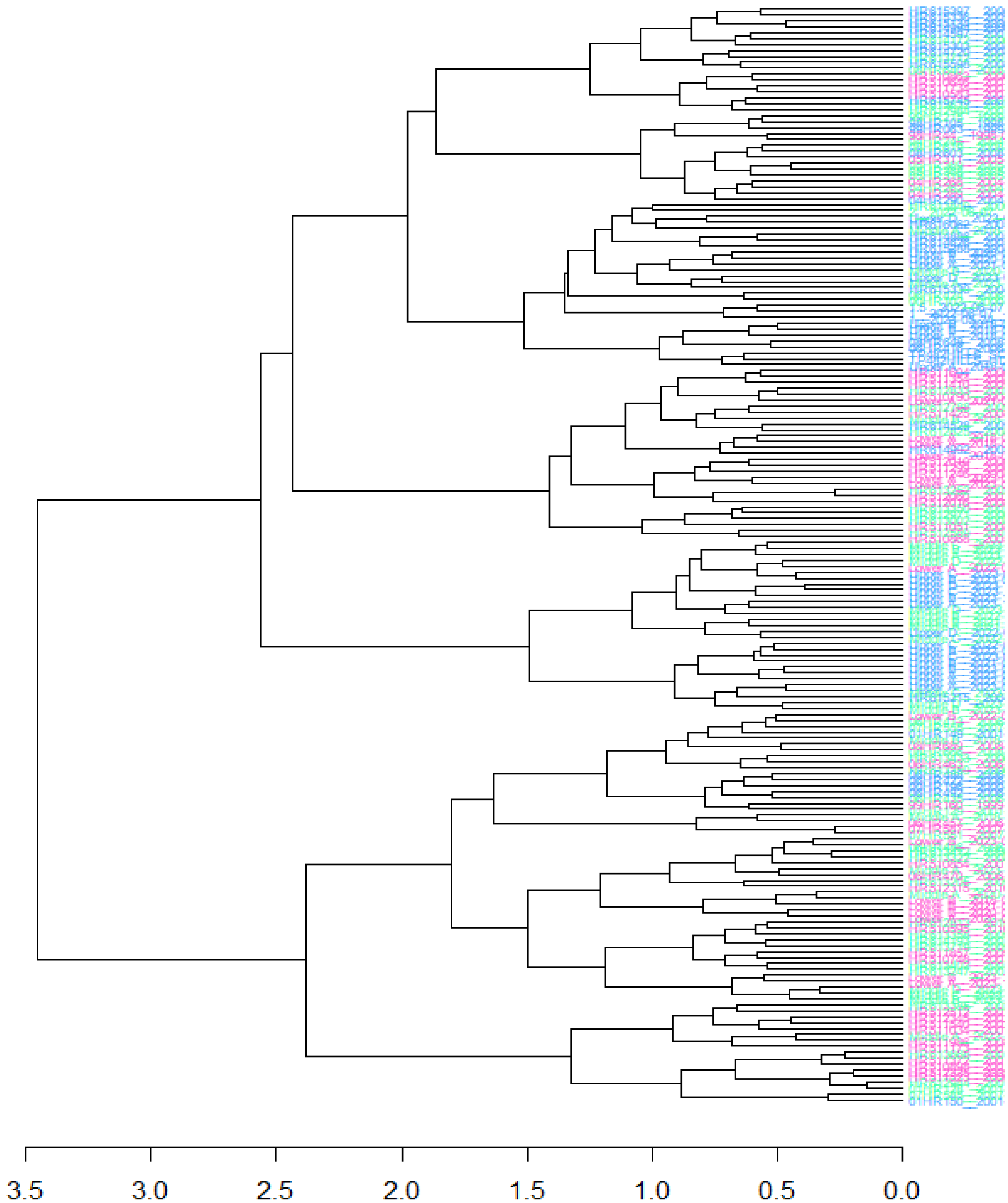
Benthic macroinvertebrates (benthos) are defined as relatively sessile, bottom-dwelling animals without a backbone that are retained by a US Standard 30 mesh sieve (FDEP 2017). A potential benefit of using benthic macroinvertebrates as an assessment tool includes their general lack of mobility, which enables the benthic community to respond directly to preceding water quality conditions (FDEP 2017). This section provides a description and quantitative evaluation of changes in benthos as a function of minimum flow implementation. No benthic macroinvertebrate data were collected during Period 4.

6.2.2.1 Cluster Analyses

Hierarchical cluster analysis, using standardized Euclidean distance, was conducted for all benthic macroinvertebrate taxa from the target zone (Figure 6.2-25). The parameter β was set to -0.5, which has been suggested as a value that balances clustering behavior and minimizes the influence of outliers (Milligan 1989).

Several small clusters of benthic macroinvertebrate taxa found in the upper segment (coded blue) were distinct from the clusters of taxa inhabiting the middle and lower segments (Figure 6.2-25). This suggested potential differences in the types of taxa inhabiting the upper segment as compared to the middle and lower segments, encouraging further analyses involving ordination and salinity-sensitive taxa.

Figure 6.2-25: Hierarchical clustering of benthic macroinvertebrates



Pink = Lower, Green = Middle, Blue = Upper

6.2.2.2 Ordination

An NMDS using standardized Bray-Curtis distances (appropriate for relative abundance of species) was conducted on the benthic macroinvertebrate taxonomic data. A random initiation process was used with three axes, and a convergent solution found in less than 40 starts. The fit of the model was acceptable, as stress was 0.16 and below the 0.2 stress threshold satisfactory for inference (Clarke 1993).

A stress plot was examined for the fit of the data (Figure 6.2-26). Although benthic macroinvertebrate taxa in the upper segment (blue cloud) were different from the taxa inhabiting the middle and lower segments, those differences appeared largest during Period 5. This suggests that full minimum flow implementation may have been associated with community changes over time (Figure 6.2-27). Additional analyses were conducted with salinity-sensitive taxa.

Figure 6.2-26: Stress plot of benthic macroinvertebrate data

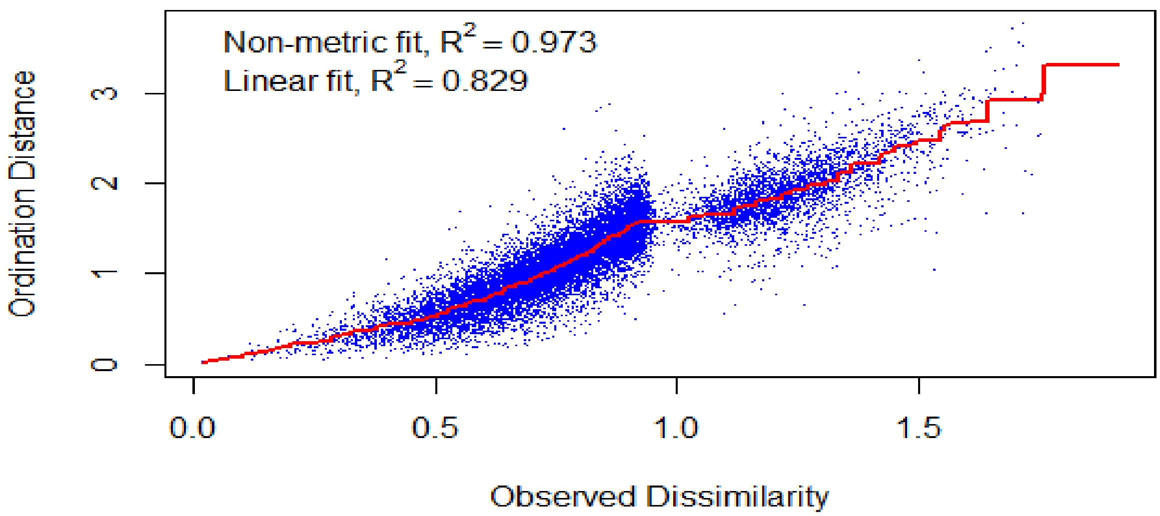


Figure 6.2-27: NMDS ordination of benthic macroinvertebrates by segment

6.2.2.3 Salinity Sensitivity

A key component of the Hillsborough River minimum flow was to provide essential low-salinity habitat conditions downstream of the Hillsborough River Reservoir. A list of salinity-sensitive benthic macroinvertebrate taxa used for these analyses is found in Table 6.2-19.

Table 6.2-19: List of benthic macroinvertebrate taxa inhabiting low salinity (< 5 ppt) habitat within the LHR target zone (Analysis Days)

Ablabesmyia rhamphe grp.	Cladotanytarsus sp.	Djalmabatista sp.	Larsia sp.	Parachironomus frequens	Procladius (Holotanypus) sp.
Ablabesmyia sp.	Cladotanytarsus sp.	Dubiraphia sp.	Leptoceridae	Parachironomus sp.	Procladius bellus var.1 Epler
Ancylidae	Clinotanypus sp.	Dubiraphia vittata	Libellula incesta	Parachironomus tenuicaudatus	Procladius sp.
Apedilum sp.	Coenagrionidae	Enallagma sp.	Libellula sp.	Paralauterborniella nigrohalteralis	Prostoma sp.
Aphylla williamsoni	Collembola	Ephemeroptera	Libellulidae	Paranais litoralis	Pseudochironomus sp.
Arhynchobdellida sp.	Corbicula fluminea	Erpobdella punctata	Limnodriloides sp.	Paranais sp.	Psychodidae
Asheum beckae	Cricotopus bicinctus	Euhirudinea sp.	Limnodrilus hoffmeisteri	Peltodytes sp.	Pyrgophorus platyrachis
Berosus sp.	Cryptochironomus sp.	Ferrissia cf. hendersoni	Melanoides tuberculata	Pisidium punctiferum	Pyrgophorus tuberculatus
Bezzia/Palpomyia grp.	Cryptotendipes sp.	Gloiobdella elongata	Mytilopsis leucophaeata	Pisidium sp.	Rhabditophora sp.
Boccardiella ligerica	Cyrnellus fraternus	Glossiphoniidae sp.	Naididae	Placobdella ornata	Rhithropanopeus harrisii
Branchiobdellidae	Daphnia sp.	Goeldichironomus carus	Naidinae sp. A of EPC	Planorbella scalaris	Sparganophilus sp.
Bratislavia unidentata	Dero nivea	Goeldichironomus sp.	Nais communis sp. complex	Polycladida sp.	Sphaerium sp.
Caenis diminuta	Dero obtusa	Hebetancylus excentricus	Nanocladius sp.	Polymesoda caroliniana	Stenelmis sp.
Caenis sp.	Dero pectinata	Helobdella elongata	Neureclipsis sp.	Polypedilum beckae	Stenochironomus sp.
Callibaetis sp.	Dero sp.	Helobdella papillata	Odonata	Polypedilum halterale grp.	Tanypodinae
Ceratopogonide	Dicrotendipes lobus	Helobdella stagnalis	Oecetis inconspicua	Polypedilum scalaenum grp.	Tanypus sp.
Ceriodaphnia sp.	Dicrotendipes modestus	Hirudinea	Oecetis inconspicua complex	Polypedilum sp.	Tanytarsus sp.
Chaoborus punctipennis	Dicrotendipes neomodestus	Hyalella azteca	Oecetis nocturna	Pristina (Pristina) proboscidea	Tanytarsus sp. G
Chaoborus sp.	Dicrotendipes nervosus	Hyalella sp.	Oecetis sp.	Pristina (Pristinella) cf. osborni	Tanytarsus sp. G of Epler, 2001
Chironomidae	Dicrotendipes simpsoni	Hyalella sp. A	Oecetis sp. A of Epler, 2001	Pristina (Pristinella) sima	Tanytarsus sp. K
Chironomus decorus grp.	Dicrotendipes sp.	Hyalella sp. C	Oribatida	Pristina (Pristinella) sp.	Tarebia granifera
Chironomus sp.	Djalmabatista pulcher	Hydroptila sp.	Parachironomus carinatus	Pristina leidyi	Trichoptera
Cladopelma sp.	Djalmabatista pulcher var.	Hydroptilidae	Parachironomus directus	Pristina sp.	Uromunna reynoldsi
					Zygoptera

6.2.2.4 Benthic Macroinvertebrate Abundance

The average and total abundance of salinity-sensitive benthic macroinvertebrate taxa was inconsistent over time, with the middle and upper segments generally having the most salinity-sensitive individuals (Figure 6.2-28, Figure 6.2-29). The differences in salinity sensitive benthic macroinvertebrate abundance can be illustrated more clearly with boxplots delineated by segment and period (Figure 6.2-30).

Figure 6.2-28: Average abundance of salinity-sensitive benthic macroinvertebrates over time

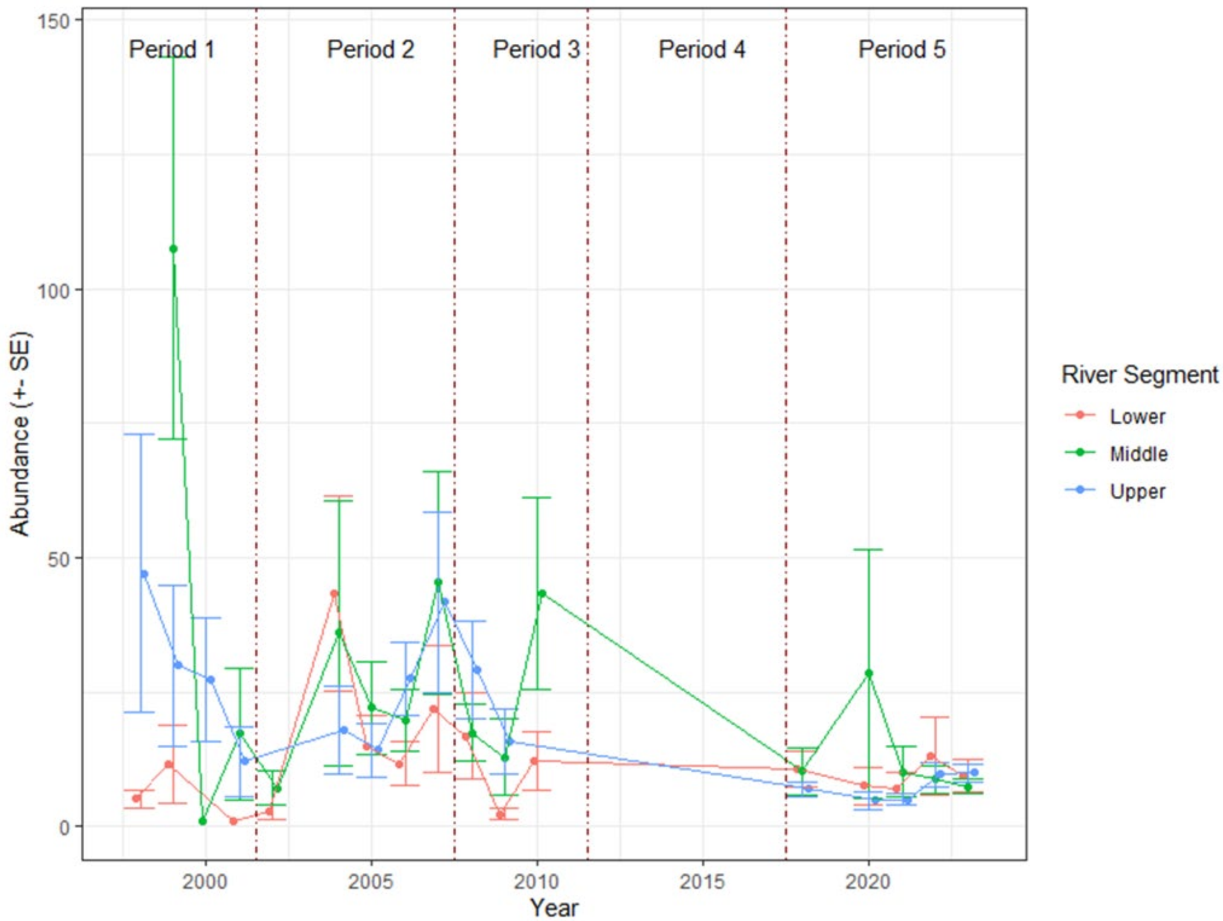


Figure 6.2-29: Total abundance of salinity-sensitive benthic macroinvertebrates over time

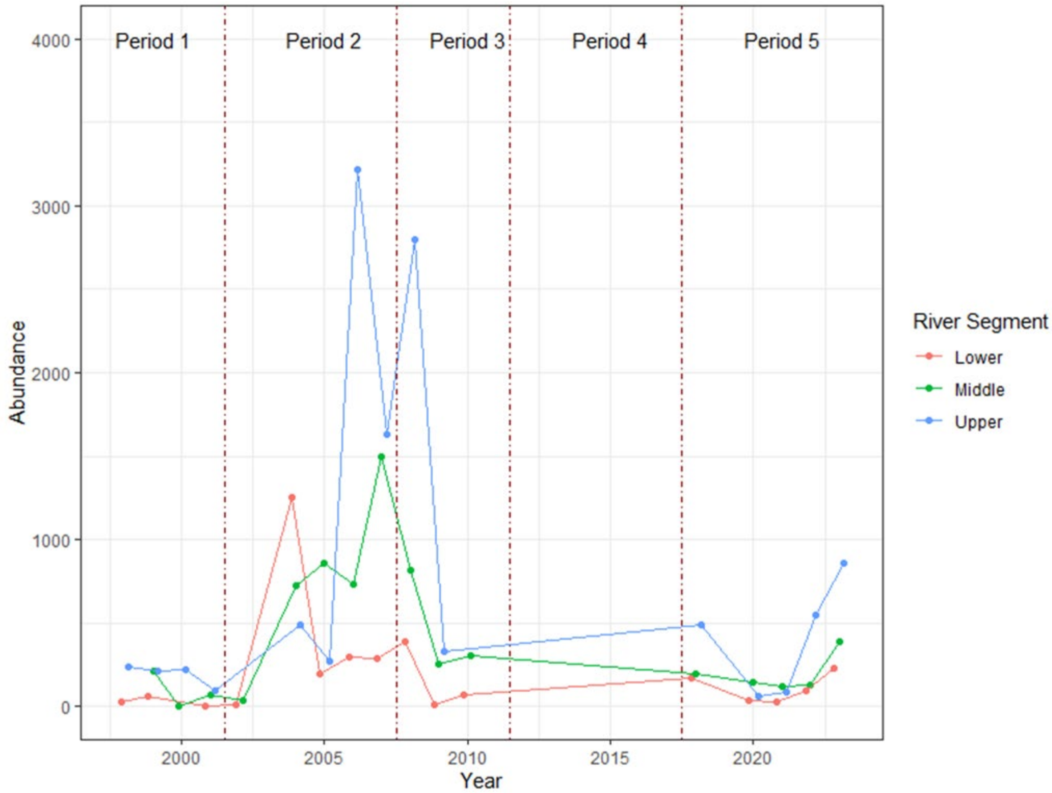
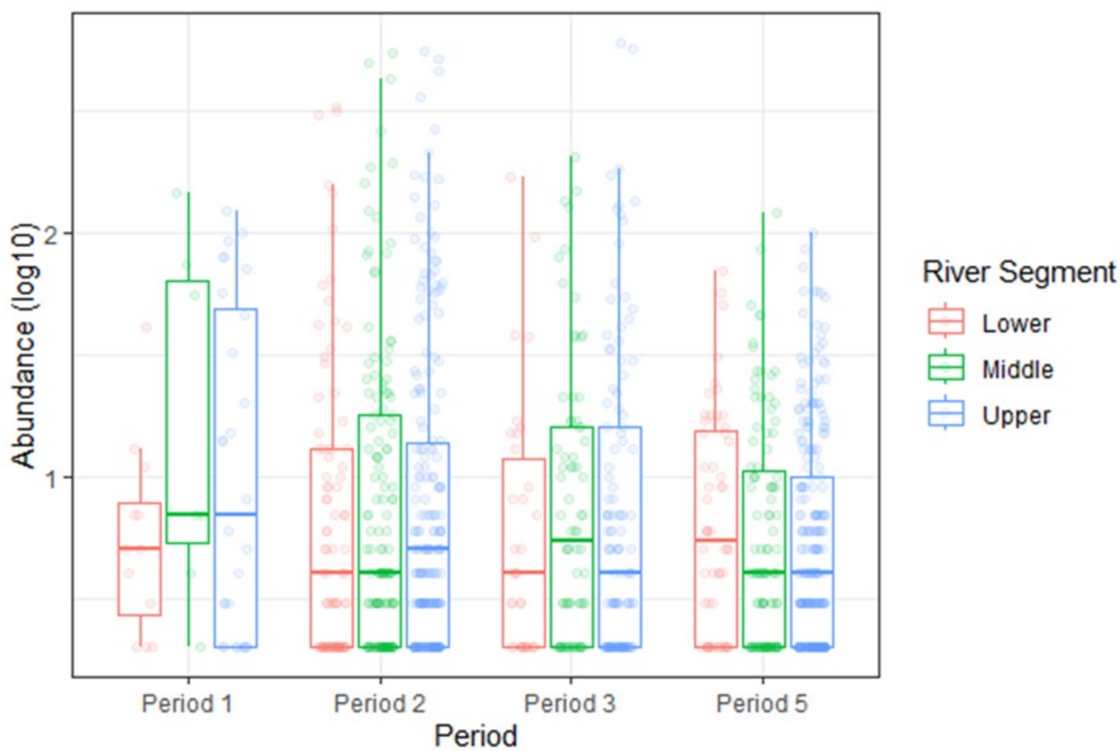


Figure 6.2-30: Boxplots of salinity-sensitive benthic macroinvertebrate abundance



Benthic Macroinvertebrate Abundance Modeling

A generalized linear mixed model for benthic macroinvertebrate abundance using the Poisson distribution indicated that there was no significant interaction effect (p = 0.53) between period and river segment (Table 6.2-20). A negative binomial distribution did not remove the overdispersion and overfit, leading to a singular model with zero variance-covariance and suggesting the estimated parameters were unreliable. A generalized linear mixed model with a Poisson distribution and an observation level random effect was used, which was able to account for overdispersion.

Table 6.2-20: Linear mixed model for benthic macroinvertebrate abundance using Poisson distribution

	Chi Squared	Degrees of Freedom	p value
(Intercept)	9.33	1	< 0.05
Minimum Flow Period	0.66	3	0.88
River Segment	2.72	2	0.26
Minimum Flow Period: River Segment	5.09	6	0.53

Benthic Macroinvertebrate Abundance Effect Sizes

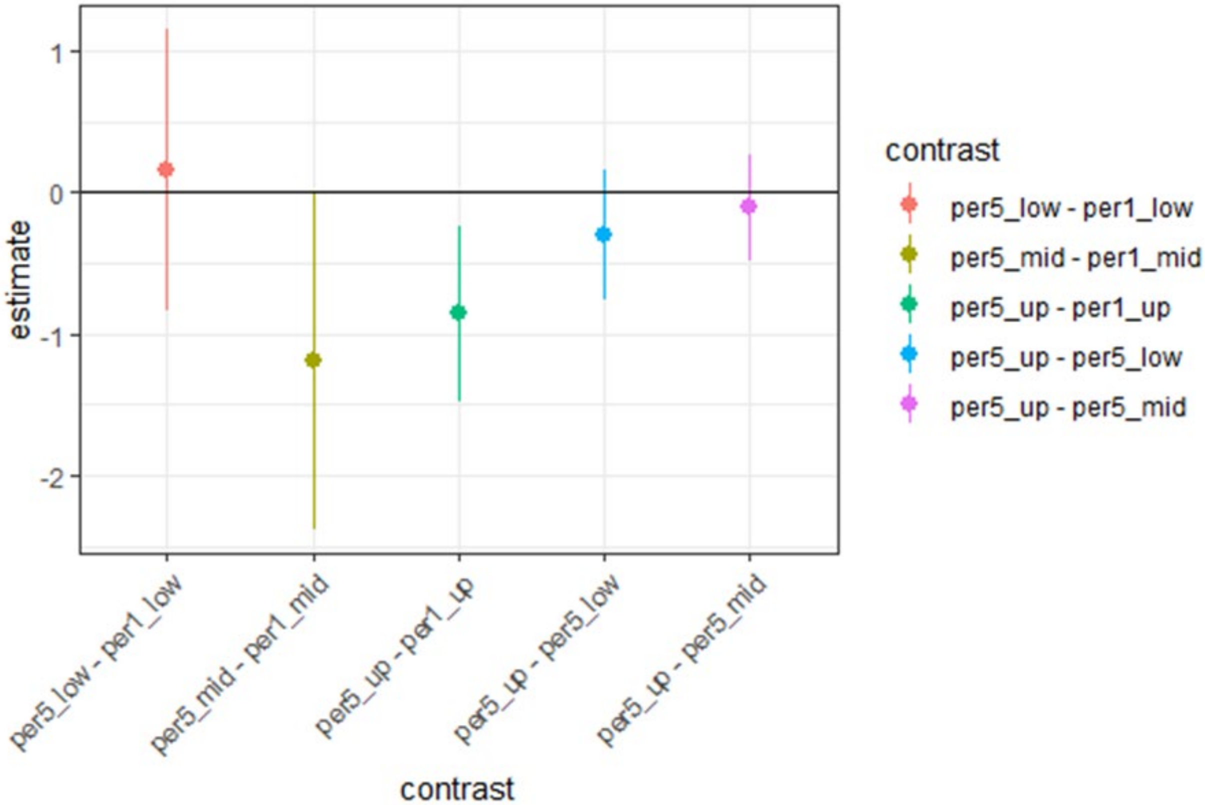
Effect size is a quantification of the difference between two group means. In this analysis, the differences in various measures of salinity sensitive taxa were quantified, comparing segment means calculated from various periods.

Selected contrasts for salinity-sensitive benthic macroinvertebrate abundance indicated that no meaningful segment versus period comparisons evaluated had 95% confidence limits that did not extend beyond zero (Table 6.2-21, Figure 6.2-31).

Table 6.2-21: Selected contrasts for salinity-sensitive benthic macroinvertebrate abundance

Contrast	Estimate	Standard Error	Degrees of Freedom	Lower Confidence Limit	Upper Confidence Limit
Period 5 Upper – Period 1 Upper	-0.86	0.32	Inf	-1.48	-0.24
Period 5 Upper – Period 5 Middle	-0.11	0.19	Inf	-0.48	0.26
Period 5 Upper – Period 5 Lower	-0.30	0.24	Inf	-0.76	0.16
Period 5 Lower – Period 1 Lower	0.15	0.51	Inf	-0.84	1.15
Period 5 Middle – Period 1 Middle	-1.19	0.61	Inf	-2.38	0.00

Figure 6.2-31: Selected contrasts for salinity-sensitive benthic macroinvertebrate abundance



Salinity and Benthic Macroinvertebrate Abundance Models

Generalized linear mixed models were developed using the Poisson distribution for 28-day depth-averaged salinity as determined via the LAMFE model. A mixed model as a predictor for salinity-sensitive benthic macroinvertebrate abundance for all river segments was statistically significant ($p < 0.01$) (Table 6.2-22). However, a mixed model as a predictor for salinity-sensitive abundance for the upper segment of the LHR was not significant ($p = 0.23$) (Table 6.2-23). Boxplots of the LAMFE-predicted 28-day antecedent salinity associated with benthic macroinvertebrate sampling events by period and segment are shown in Figure 6.2-32. The abundance of salinity-sensitive benthic macroinvertebrate taxa predicted by 28-day depth averaged salinity (determined via the LAMFE model) showed no compelling trend with salinity. A wide spread of data was evident (Figure 6.2-33).

Table 6.2-22: Linear mixed model with Poisson distribution for 28-day depth-averaged salinity (determined via the LAMFE model) as a predictor for salinity-sensitive benthic macroinvertebrate abundance

	Chi Squared	Degrees of Freedom	p value
(Intercept)	347	1	0
salin_depavg_28day	60	1	<0.01

Table 6.2-23: Linear mixed model with Poisson distribution for 28-day depth-averaged salinity (determined via the LAMFE model) as a predictor for salinity-sensitive benthic macroinvertebrate abundance for upper segment

	Chi Squared	Degrees of Freedom	p value
(Intercept)	135.4	1	0.00
salin_depavg_28day	1.4	1	0.23

Figure 6.2-32: Antecedent 28-day depth-averaged salinity (in ppt, determined via the LAMFE model) by river segment and minimum flow period for benthic macroinvertebrates

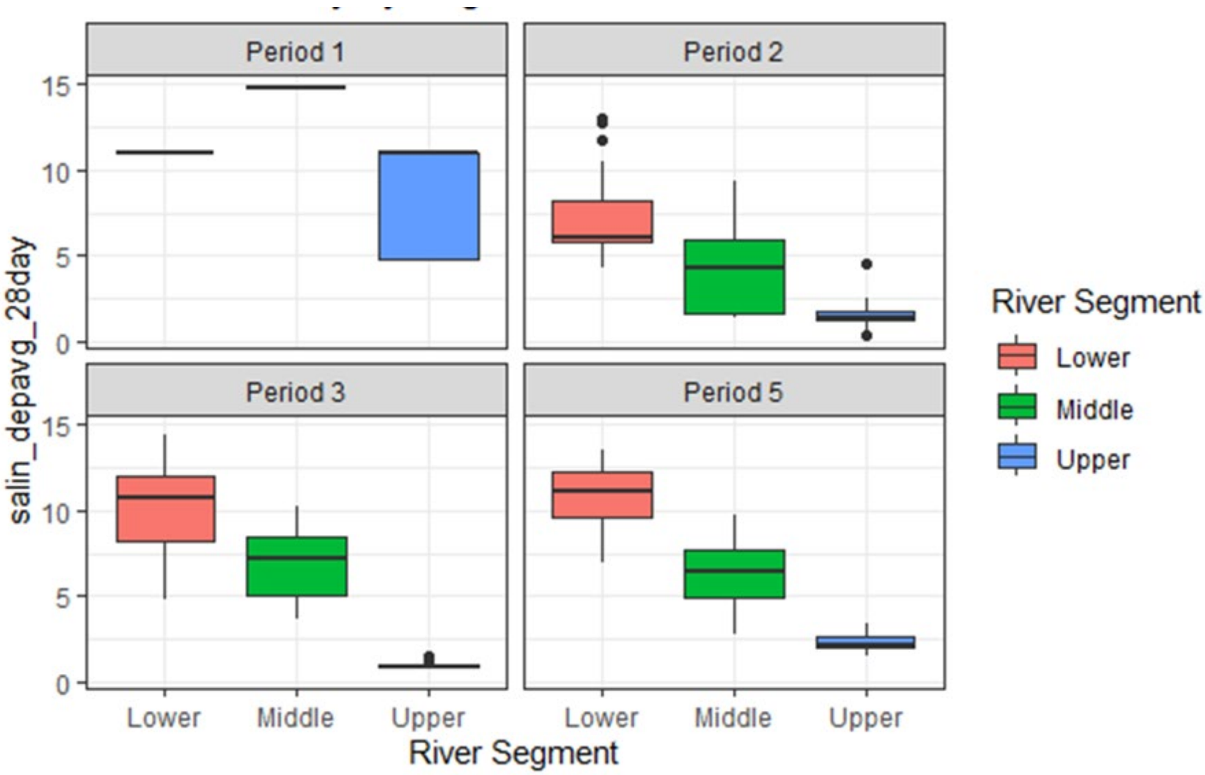
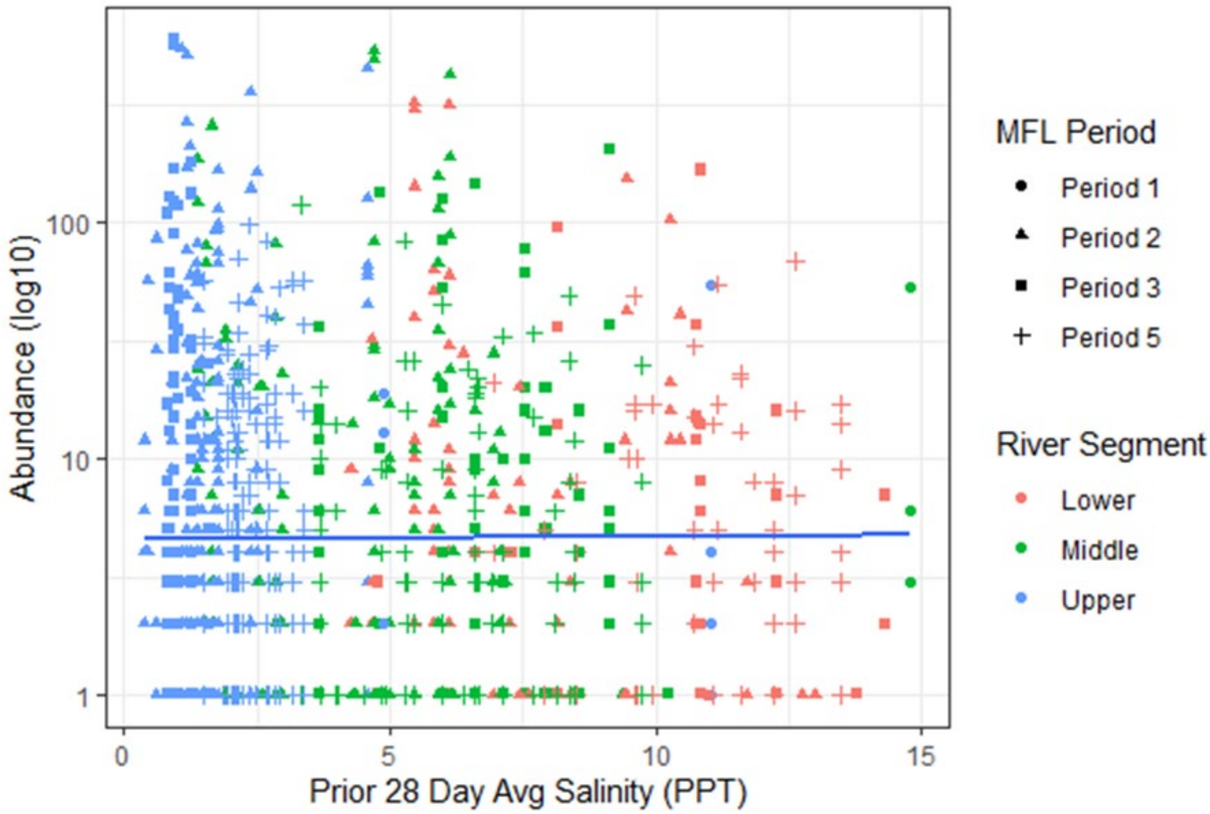


Figure 6.2-33: Salinity-sensitive benthic macroinvertebrate abundance as predicted by 28-day depth-averaged salinity (in ppt, determined via the LAMFE model)



6.2.2.5 Benthic Macroinvertebrate Density

The average density of salinity-sensitive benthic macroinvertebrate taxa was inconsistent over periods, with no clear spatial or temporal trends (Figure 6.2-34). Salinity-sensitive benthic macroinvertebrate abundance is also shown in boxplots by segment and period (Figure 6.2-35). For these data, density does not appear to be useful in discriminating between periods.

Figure 6.2-34: Salinity-sensitive benthic macroinvertebrate density over time

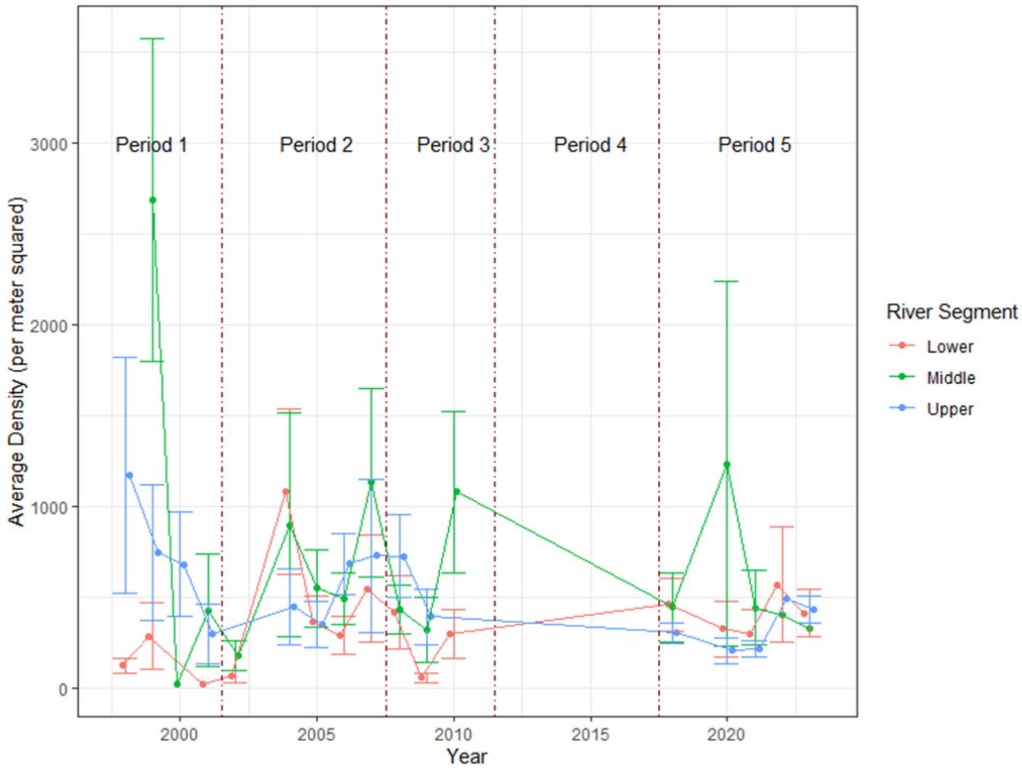
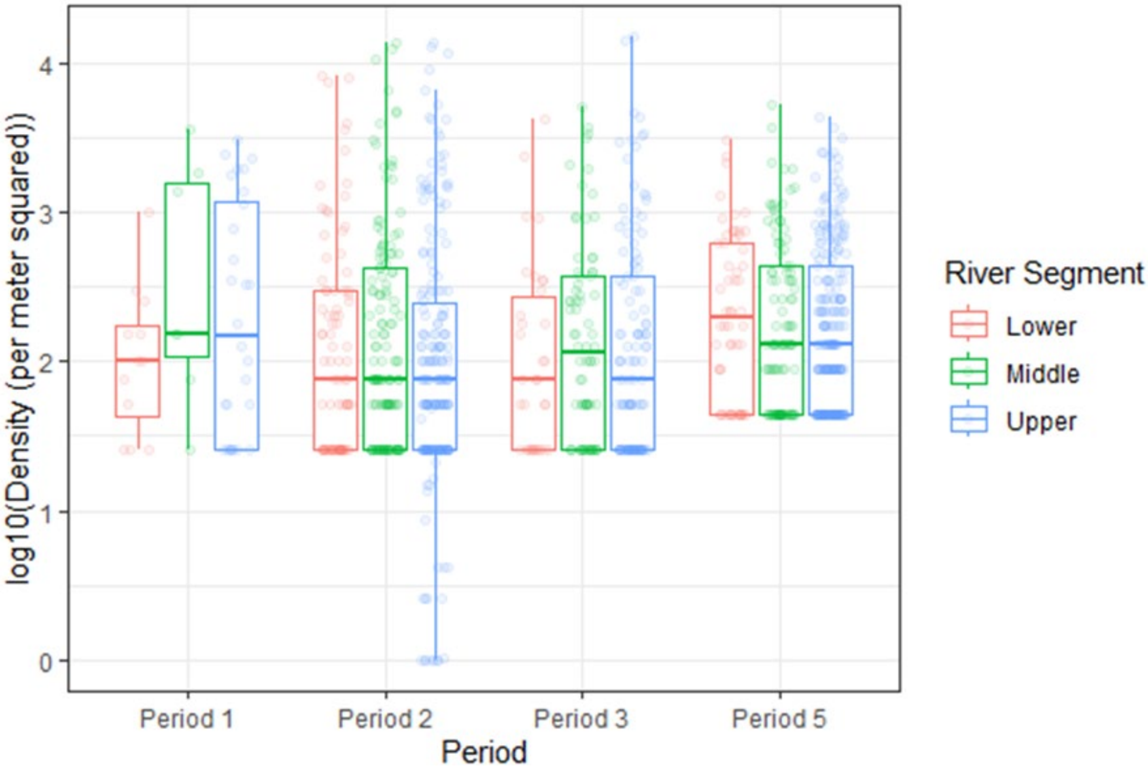


Figure 6.2-35: Boxplots of salinity-sensitive benthic macroinvertebrate density



Benthic Macroinvertebrate Density Modeling

A generalized linear model was fit with a gamma distribution. The gamma distribution is suitable for data that are continuous, positive, and right-skewed and where variance is near-constant on the log-scale. This model assumes independence of observations, equidispersion, and collinearity. Collinearity was elevated, though in this case it was not possible to correct for, as the interaction between segment and time was the main effect of interest. Collinearity does not mean the model is not correct nor should it be confused with correlation between predictors. Rather it is conditional on the other variables of the model. Multicollinearity does not mean predictors are not strongly associated with effect and is inevitable when interaction terms are included (Francoeur 2013, McElreath 2020) (Figure 6.2-36).

A generalized linear mixed model for benthic macroinvertebrate abundance using log linked gamma distribution indicated no significant interaction effect ($p = 0.79$) between period and river segment (Table 6.2-24).

Figure 6.2-36: Model tests for salinity-sensitive benthic macroinvertebrate density

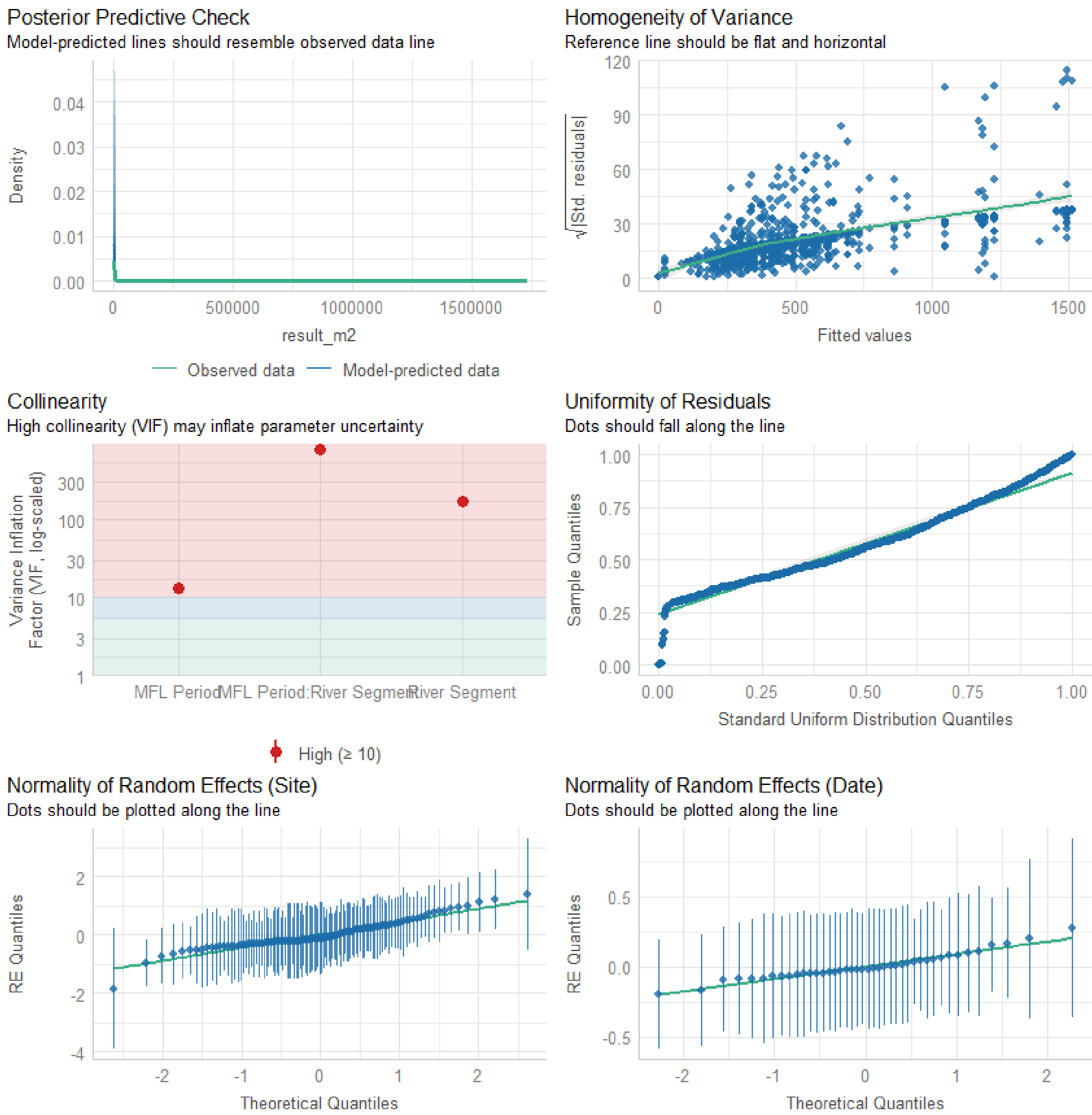


Table 6.2-24: Linear mixed Chi-squared (Chisq) model for benthic macroinvertebrate density using log transformation

	Chi Squared	Degrees of Freedom	p value
(Intercept)	64.3	1	0.00
Minimum Flow Period	1.9	3	0.59
River Segment	2.0	2	0.36
Minimum Flow Period: River Segment	3.1	6	0.79

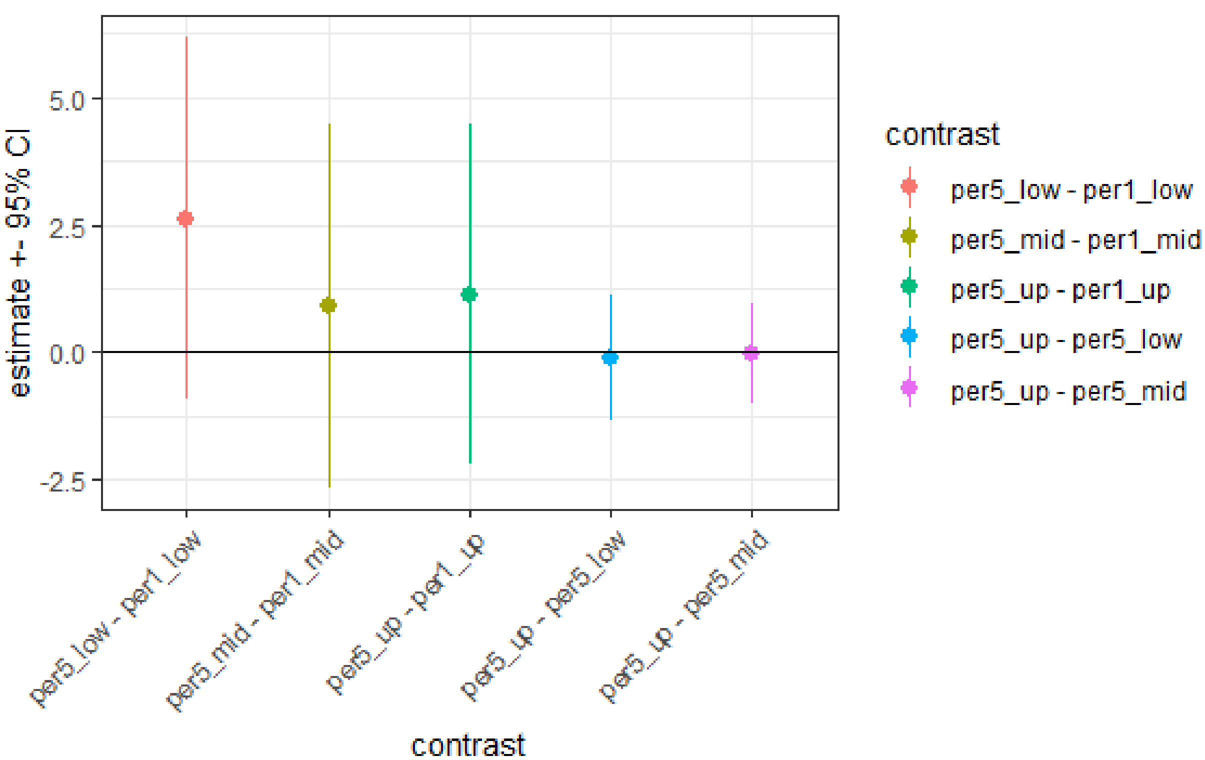
Benthic Macroinvertebrate Density Effect Sizes

Selected contrasts for salinity-sensitive benthic macroinvertebrate density indicated that no segment by period comparisons had 95% confidence limits that did not extend beyond zero (Table 6.2-25, Figure 6.2-37).

Table 6.2-25: Selected contrasts for salinity-sensitive benthic macroinvertebrate density

Contrast	Estimate	Standard Error	Degrees of Freedom	Lower Confidence Limit	Upper Confidence Limit
Period 5 Upper – Period 1 Upper	-0.04	0.22	66	-0.47	0.39
Period 5 Upper – Period 5 Middle	0.01	0.20	37	-0.39	0.41
Period 5 Upper – Period 5 Lower	-0.06	0.24	37	-0.55	0.44
Period 5 Lower - Period 1 Lower	0.32	0.32	78	-0.32	0.96
Period 5 Middle – Period 1 Middle	-0.27	0.33	164	-0.92	0.37

Figure 6.2-37: River segment contrasts for salinity-sensitive benthic macroinvertebrate density



Salinity and Benthic Macroinvertebrate Density

A linear mixed model was developed using the log distribution for 28-day depth-averaged salinity (as determined via the LAMFE model). Date was included as a random effect. The mixed model as a predictor for salinity-sensitive benthic macroinvertebrate density was not significant (p= 0.14) (Table 6.2-26). A generalized linear mixed model with log linked gamma distribution was fit for 28-day depth-averaged salinity (determined via the LAMFE model) as a predictor for salinity-sensitive benthic macroinvertebrate density for the upper segment of the LHR was not significant (p = 0.33) (Table 6.2-27).

Salinity-sensitive benthic macroinvertebrate taxa density, as predicted by 28-day depth-averaged salinity (determined via the LAMFE model), appeared to have no discernible trend (Figure 6.2-38).

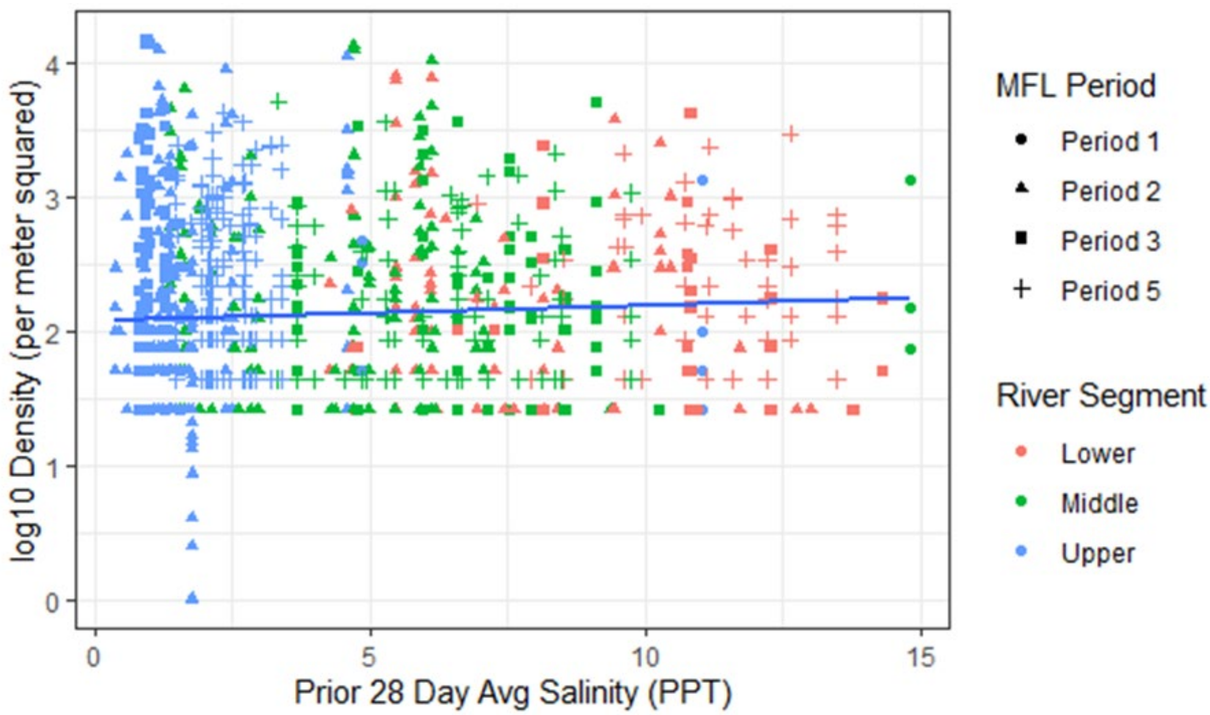
Table 6.2-26: Linear mixed model with gamma distribution and log link for 28-day depth averaged salinity (in ppt, determined via LAMFE model) as a predictor for salinity sensitive benthos density

	Chi Squared	Degrees of Freedom	p value
(Intercept)	1,250.69	1	< 0.05
salin_depavg_28day	0.01	1	0.14

Table 6.2-27: Linear mixed model with gamma distribution and log link for 28-day depth averaged LAMFE salinity (ppt) as a predictor for salinity sensitive benthos diversity for upper segment of river

	Chi Squared	Degrees of Freedom	p value
(Intercept)	80.88	1	< 0.05
salin_depavg_28day	0.95	1	0.33

Figure 6.2-38: Salinity-sensitive benthic macroinvertebrate density predicted by 28-day depth-averaged salinity (determined via the LAMFE model)



6.2.2.6 Benthic Macroinvertebrate Taxa Richness

The richness of salinity-sensitive benthic macroinvertebrate taxa generally increased throughout the study period at the upper segment, with the highest richness occurring during Period 5 and the lowest richness during Period 1 (Figure 6.2-39). The trends in salinity-sensitive richness are more clearly seen in boxplots by segment and period (Figure 6.2-40). Salinity-sensitive benthic macroinvertebrate taxa richness appears to be a strong metric for discriminating differences in periods, potentially associated with minimum flow implementation.

Figure 6.2-39: Salinity-sensitive benthic macroinvertebrate taxa richness over time

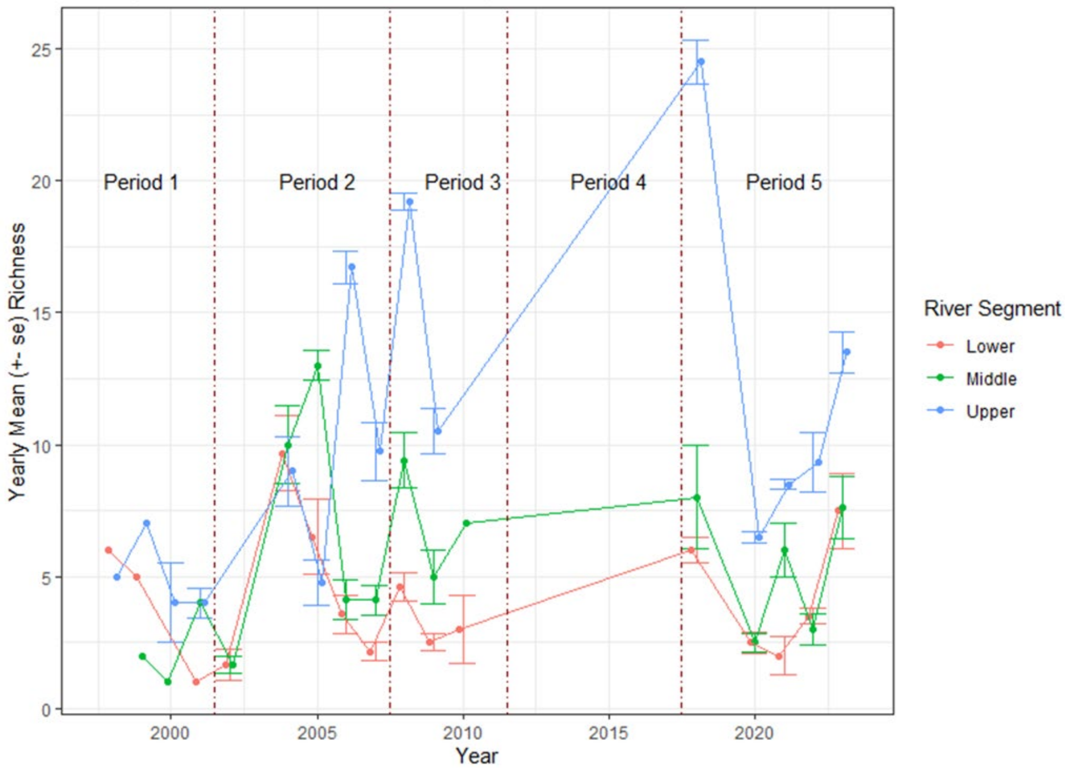
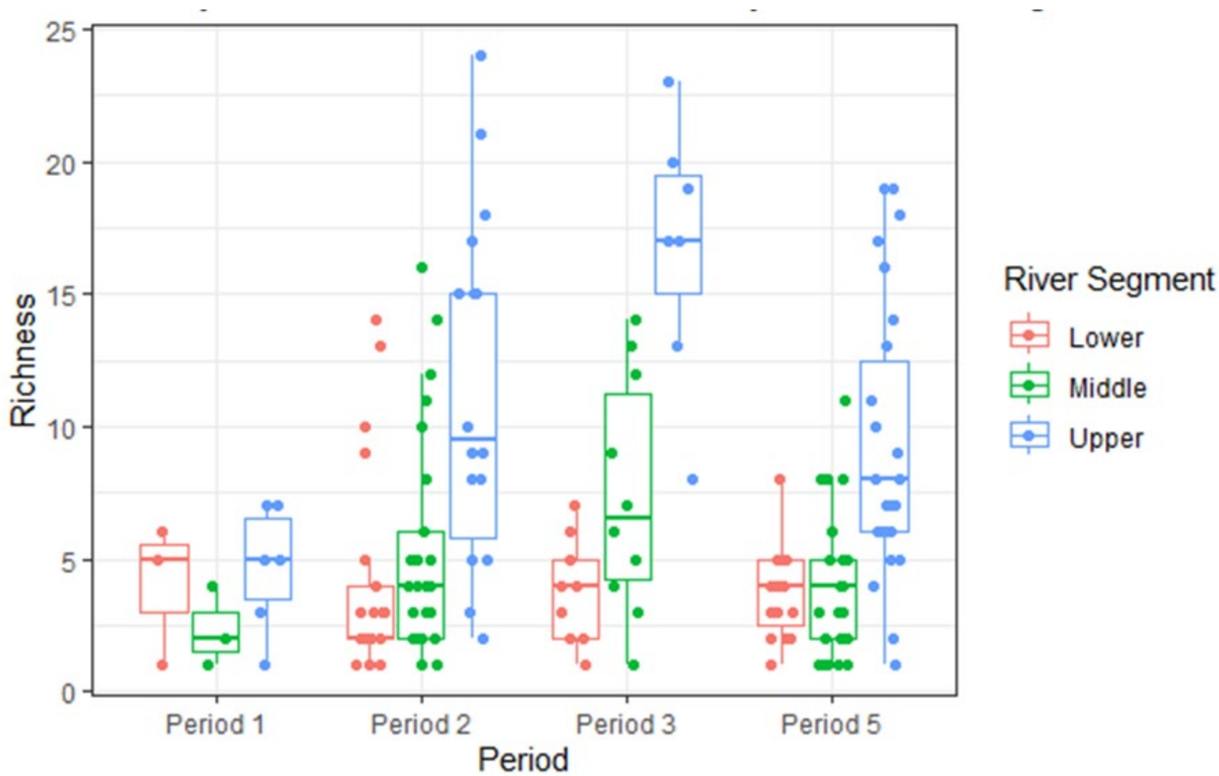


Figure 6.2-40: Boxplots of salinity-sensitive benthic macroinvertebrate taxa richness



Benthic Macroinvertebrate Taxa Richness Modelling

Taxa richness was fit to a generalized linear model using the Poisson distribution and date as a random effect (Figure 6.2-41). There were no concerns with model violation. Collinearity was elevated, but that is expected for examining interaction effects. There was no statistically significant interaction effect ($p = 0.09$) between period and segment (Table 6.2-28). A reduced model based on only Period 1 and 5 was examined for interaction effect and was found to be statistically significant ($p < 0.05$) (Table 6.2-29).

Figure 6.2-41: Model tests for salinity-sensitive benthic macroinvertebrate taxa richness

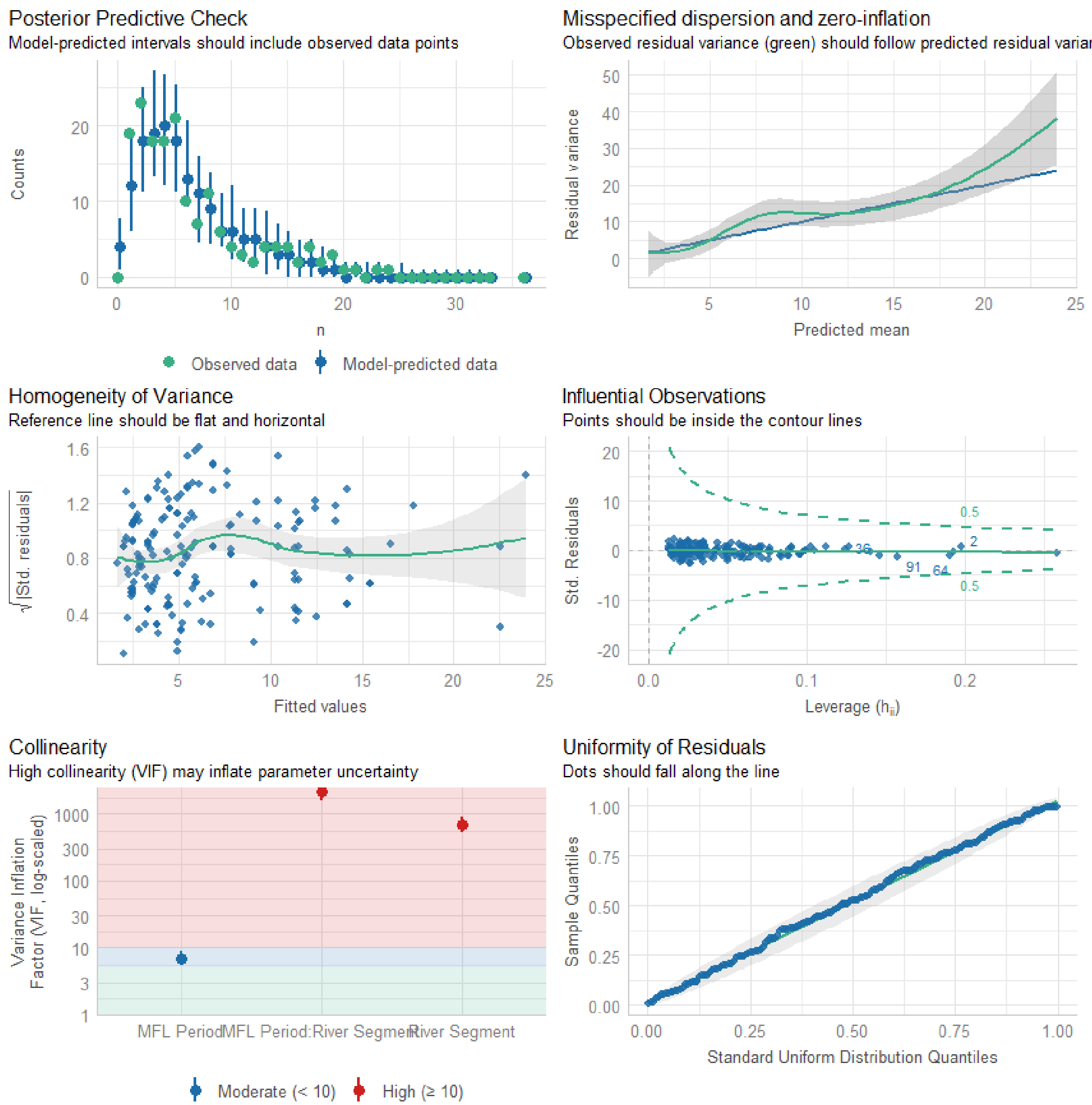


Table 6.2-28: Linear mixed model for salinity-sensitive benthic macroinvertebrate taxa richness using log transformation

	Chi Squared	Degrees of Freedom	p value
Minimum Flow Period	0.25	3	0.98
River Segment	2.24	2	0.28
Minimum Flow Period: River Segment	7.91	6	0.09

Table 6.2-29: Reduced model for salinity-sensitive benthic macroinvertebrate taxa richness

	Chi Squared	Degrees of Freedom	p value
Minimum Flow Period	0.27	3	0.97
River Segment	3.07	2	0.22
Minimum Flow Period: River Segment	13.41	6	< 0.05

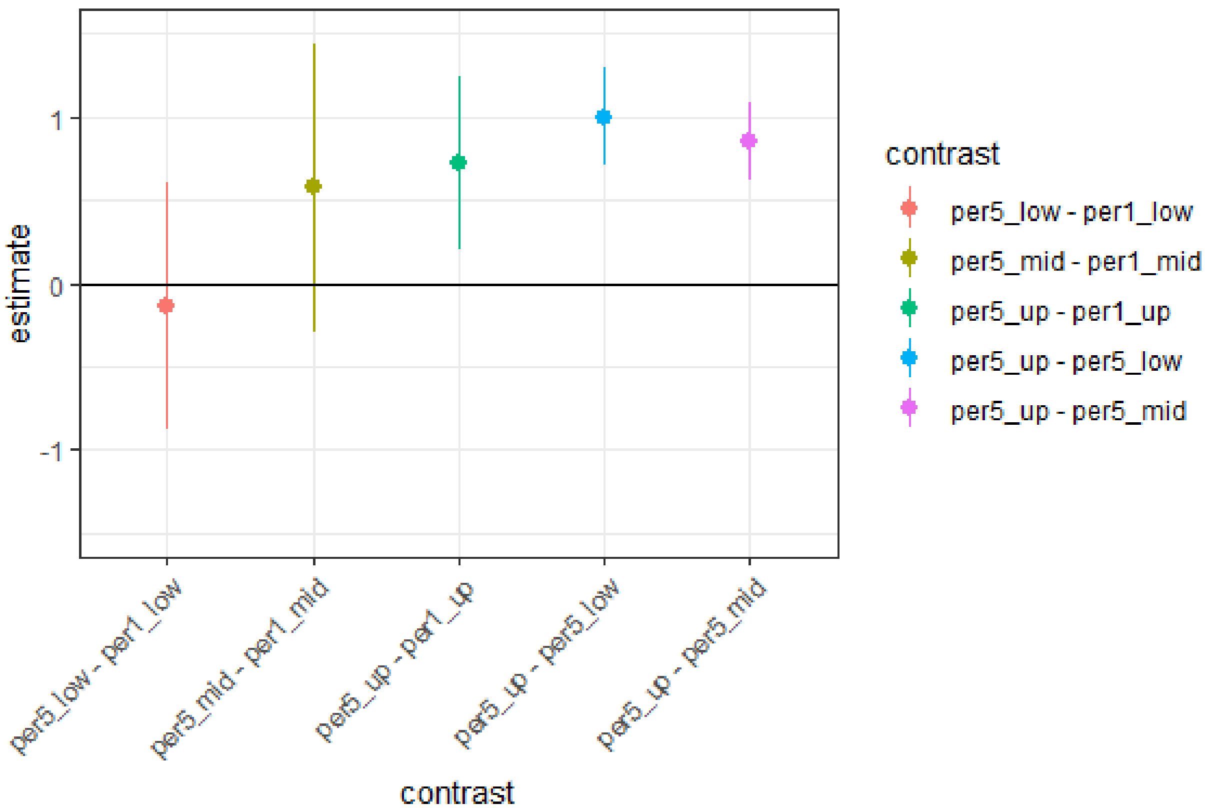
Benthic Macroinvertebrate Taxa Richness Effect Sizes

Contrasts of interest for salinity-sensitive benthic macroinvertebrate taxa richness indicated that the upper segment during Period 5 had more salinity-sensitive taxa than the middle and lower segments during the same period and as compared to the upper segment during Period 1, where 95% confidence limits did not overlap zero (Table 6.2-30, Figure 6.2-42). The estimates were log transformed.

Table 6.2-30: Selected contrasts for salinity-sensitive benthic macroinvertebrate taxa richness

Contrast	Estimate	Standard Error	Degrees of Freedom	Lower Confidence Limit	Upper Confidence Limit
Period 5 Upper – Period 1 Upper	0.72	0.27	Inf	0.20	1.25
Period 5 Upper – Period 5 Lower	1.00	0.15	Inf	0.70	1.29
Period 5 Upper – Period 5 Middle	0.86	0.12	Inf	0.63	1.09
Period 5 Lower – Period 1 Lower	-0.14	0.38	Inf	-0.88	0.61
Period 5 Middle – Period 1 Middle	0.58	0.44	Inf	-0.29	1.44

Figure 6.2-42: Selected contrasts for salinity-sensitive benthic macroinvertebrate taxa richness



Salinity and Benthic Macroinvertebrate Taxa Richness

A generalized linear mixed model using the Poisson distribution for 28-day depth-averaged salinity (as determined via the LAMFE model) as a predictor for salinity-sensitive benthic macroinvertebrate taxa richness was statistically significant ($p < 0.05$) (Table 6.2-31). The R^2 for the model was 0.69. Salinity-sensitive benthic macroinvertebrate taxa richness was higher when salinity was < 5 ppt for the preceding 28-day period as determined via the LAMFE model (Figure 6.2-43). A model for 28-day depth-averaged salinity (determined via the LAMFE model) as a predictor for salinity-sensitive benthic macroinvertebrate taxa richness for the upper segment of the study area also was statistically significant ($p < 0.05$) but with a low R^2 (0.13). Greater taxa richness was associated with lower salinities, particularly salinities < 5 ppt (Table 6.2-32, Figure 6.2-43). This provides evidence that more types of salinity-sensitive benthic macroinvertebrate taxa are in the upper segment when flows are increased, and salinity is decreased.

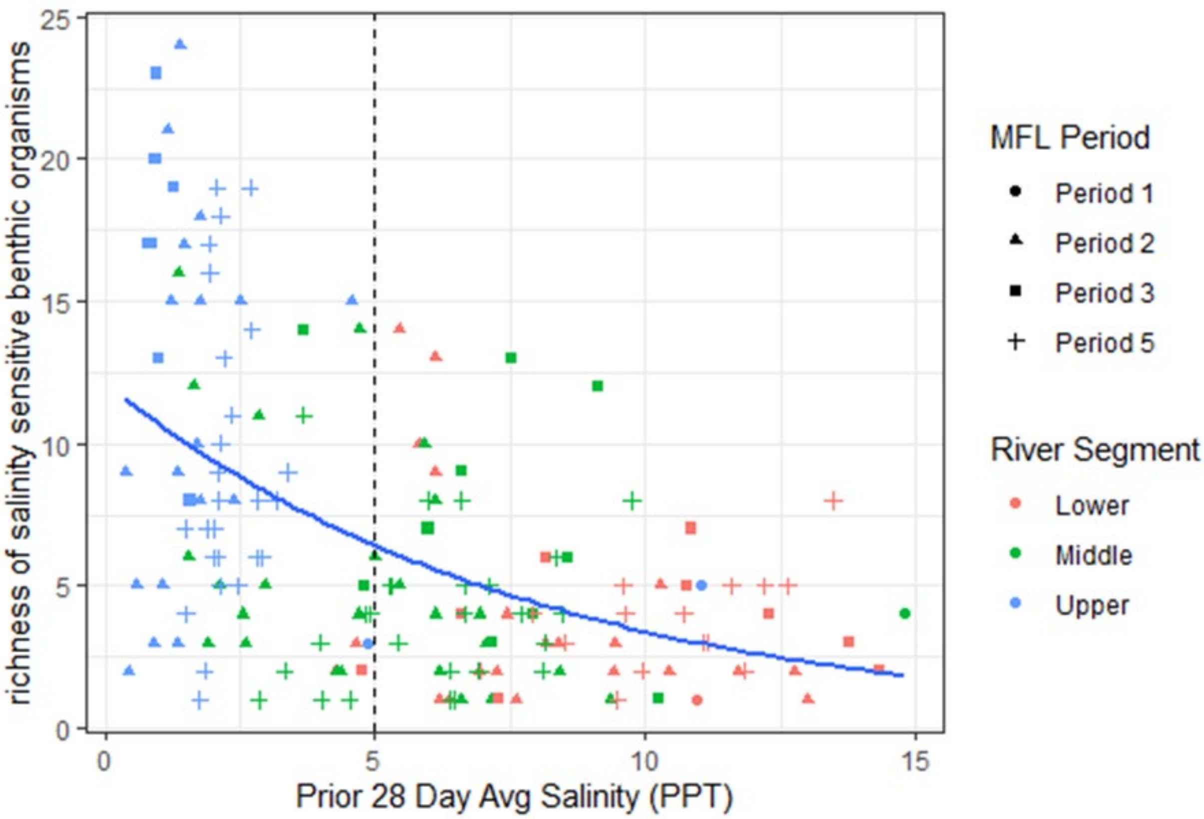
Table 6.2-31: Linear mixed model for 28-day depth-averaged salinity (determined by the LAMFE model) as a predictor for salinity-sensitive benthic macroinvertebrate taxa richness

	Chi Squared	Degrees of Freedom	p value
(Intercept)	77	1	<0.05
salin_depavg_28day	22	1	<0.05

Table 6.2-32: Linear mixed model for 28-day depth-averaged salinity (determined by the LAMFE model) as a predictor for upper segment salinity-sensitive benthic macroinvertebrate taxa richness

	Chi Squared	Degrees of Freedom	p value
(Intercept)	26.3	1	< 0.05
salin_depavg_28day	4.3	1	< 0.05

Figure 6.2-43: Salinity-sensitive benthic macroinvertebrate taxa richness vs. 28-day depth-averaged salinity (in ppt, determined by the LAMFE model)



6.2.2.7 Benthic Macroinvertebrate Diversity

Although variable, the diversity of salinity-sensitive benthic macroinvertebrate taxa was generally highest within the upper segment in Periods 2, 3, and 5 and was highest overall in the upper segment during Period 5 (Figure 6.2-44, Figure 6.2-45), similar to the pattern shown by salinity-sensitive benthic macroinvertebrate taxa richness.

Figure 6.2-44: Salinity-sensitive benthic macroinvertebrate Shannon diversity over time

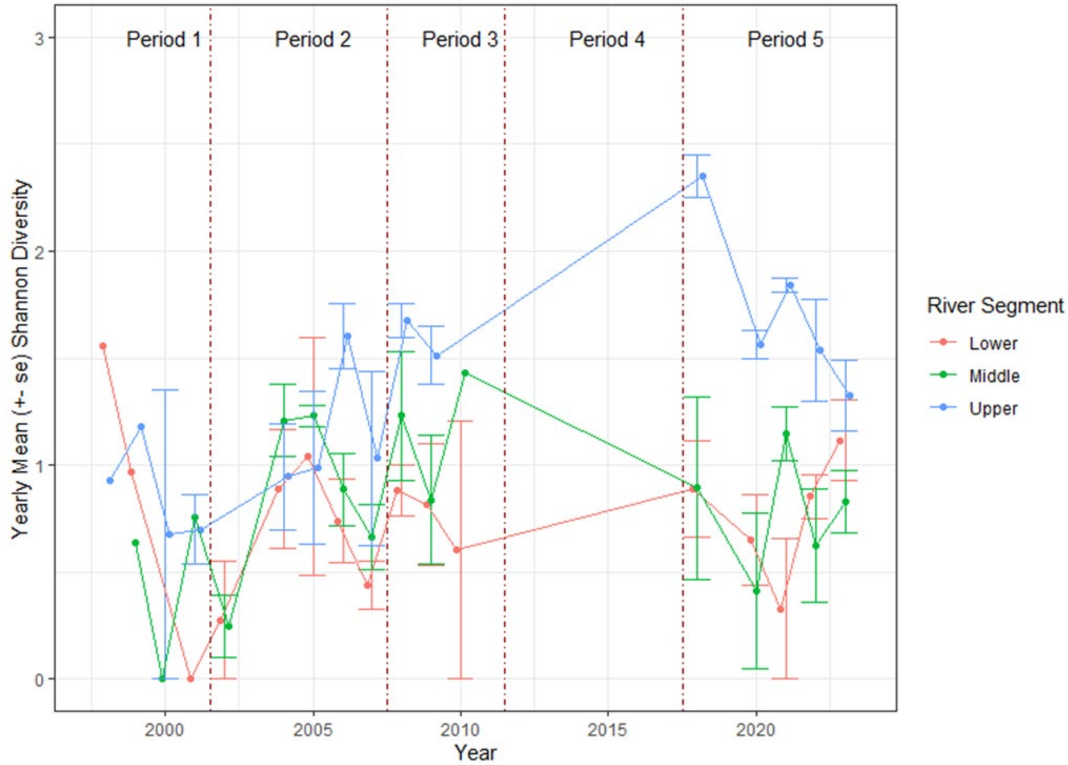
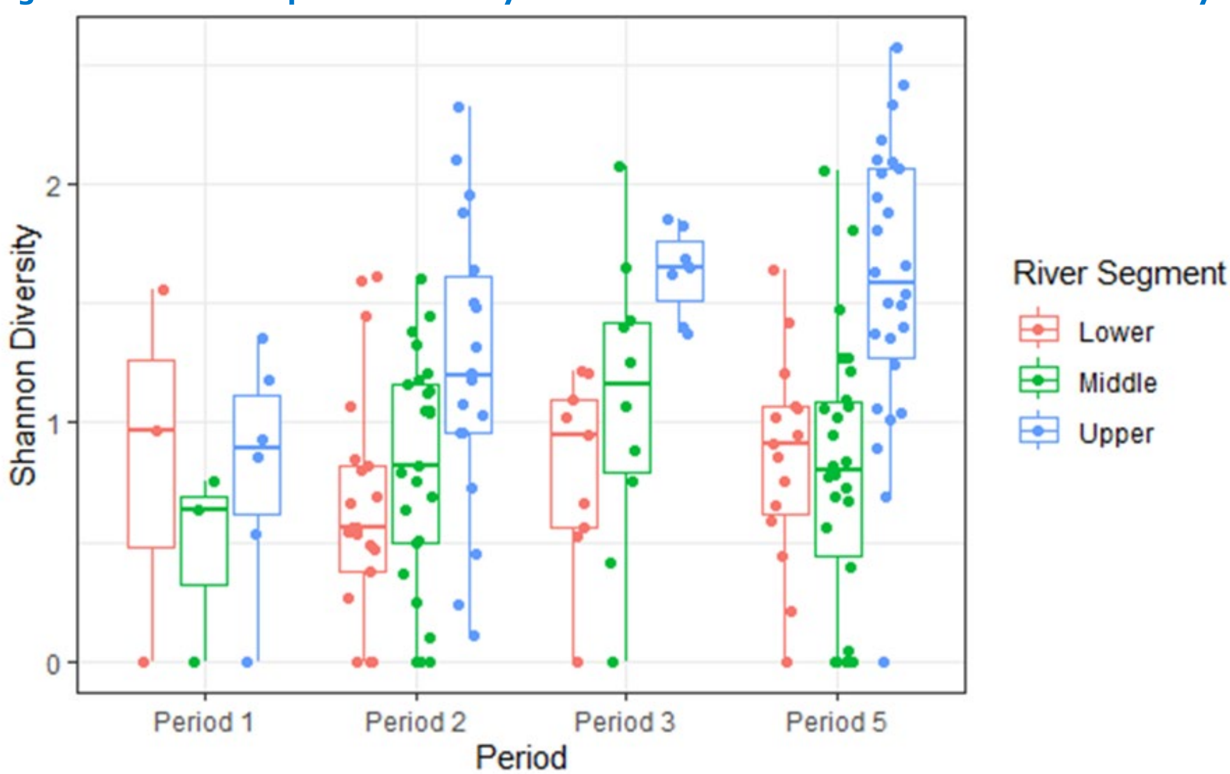


Figure 6.2-45: Boxplots of salinity-sensitive benthic macroinvertebrate diversity



Benthic Macroinvertebrate Diversity Modelling

A mixed effects linear model with Gaussian distribution was used and model assumptions were checked (Figure 6.2-46). Collinearity was observed but was a result of the nature of the interaction effect model. The linear mixed model for benthic macroinvertebrate diversity indicated that the interaction between period and segment was not statistically significant (p=0.49) (Table 6.2-33).

Figure 6.2-46: Model tests for salinity-sensitive benthic macroinvertebrate diversity

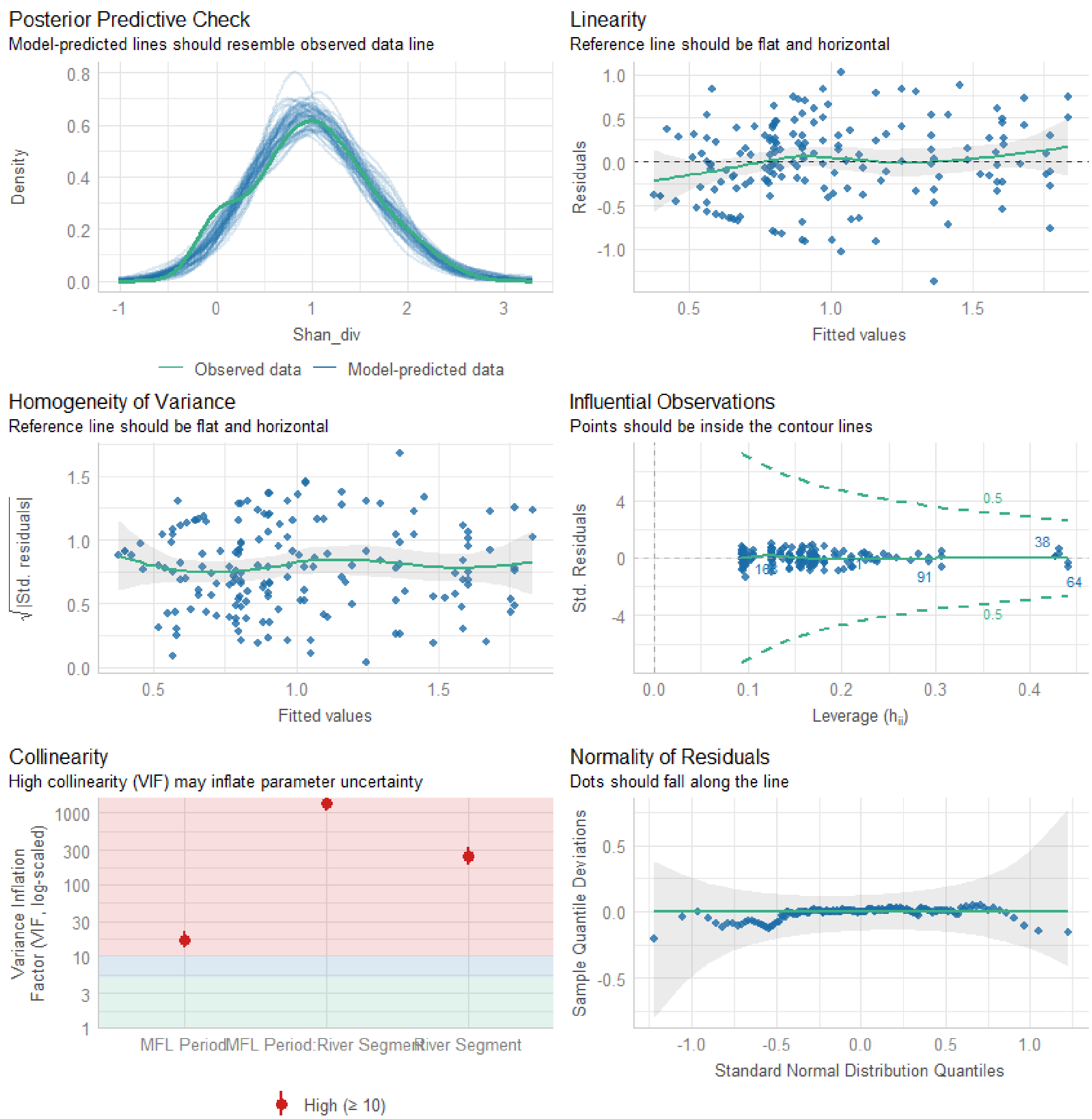


Table 6.2-33: Linear mixed model for benthic macroinvertebrate diversity using Poisson distribution

	Chi Squared	Degrees of Freedom	p value
Minimum Flow Period	1.25	3	0.74
River Segment	0.95	2	0.62
Minimum Flow Period: River Segment	7.75	6	0.26

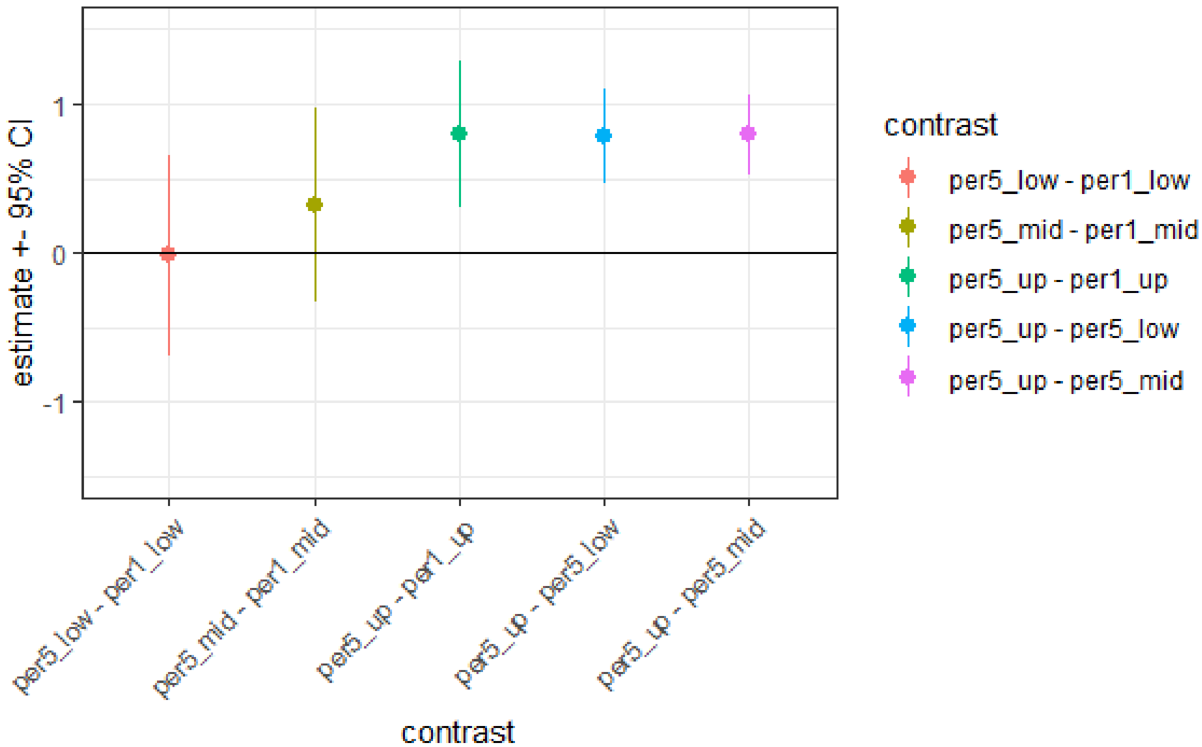
Benthic Macroinvertebrate Diversity Effect Sizes

River segment contrasts indicated the diversity of salinity-sensitive benthic macroinvertebrate taxa was greater within the upper segment during Period 5 as compared to the middle and lower segments during the same periods and the upper segment during Period 1 (Table 6.2-34, Figure 6.2-47). These values were not transformed and can be interpreted as shown (*i.e.*, a mean increase of 0.8 diversity).

Table 6.2-34: Selected contrasts for salinity-sensitive benthic macroinvertebrate diversity

Contrast	Estimate	Standard Error	Degrees of Freedom	Lower Confidence Limit	Upper Confidence Limit
Period 5 Upper – Period 1 Upper	0.80	0.25	116.77	0.31	1.30
Period 5 Upper – Period 5 Lower	0.78	0.16	128.64	0.46	1.10
Period 5 Upper – Period 5 Middle	0.80	0.14	124.53	0.53	1.07
Period 5 Lower - Period 1 Lower	-0.01	0.34	140.65	-0.68	0.66
Period 5 Middle – Period 1 Middle	0.33	0.33	137.03	-0.32	0.98

Figure 6.2-47: Selected contrasts for salinity-sensitive benthic macroinvertebrate diversity



Salinity and Benthic Macroinvertebrate Diversity

Both a linear model and a general additive model were created and compared using the AIC (Figure 6.2-48). Based on the AIC results, the GAM model was chosen. A generalized additive model for 28-day depth-averaged salinity (determined via the LAMFE model) as a predictor for salinity-sensitive benthic macroinvertebrate diversity was statistically significant ($p < 0.05$) (Table 6.2-35). Salinity-sensitive benthic macroinvertebrate diversity was higher when salinity was < 5 ppt and diversity decreased as salinity increased, however, the R^2 was low (0.17) (Figure 6.2-49).

Figure 6.2-48: Model tests for 28-day depth-averaged salinity (as determined by the LAMFE model) and salinity-sensitive benthic macroinvertebrate diversity

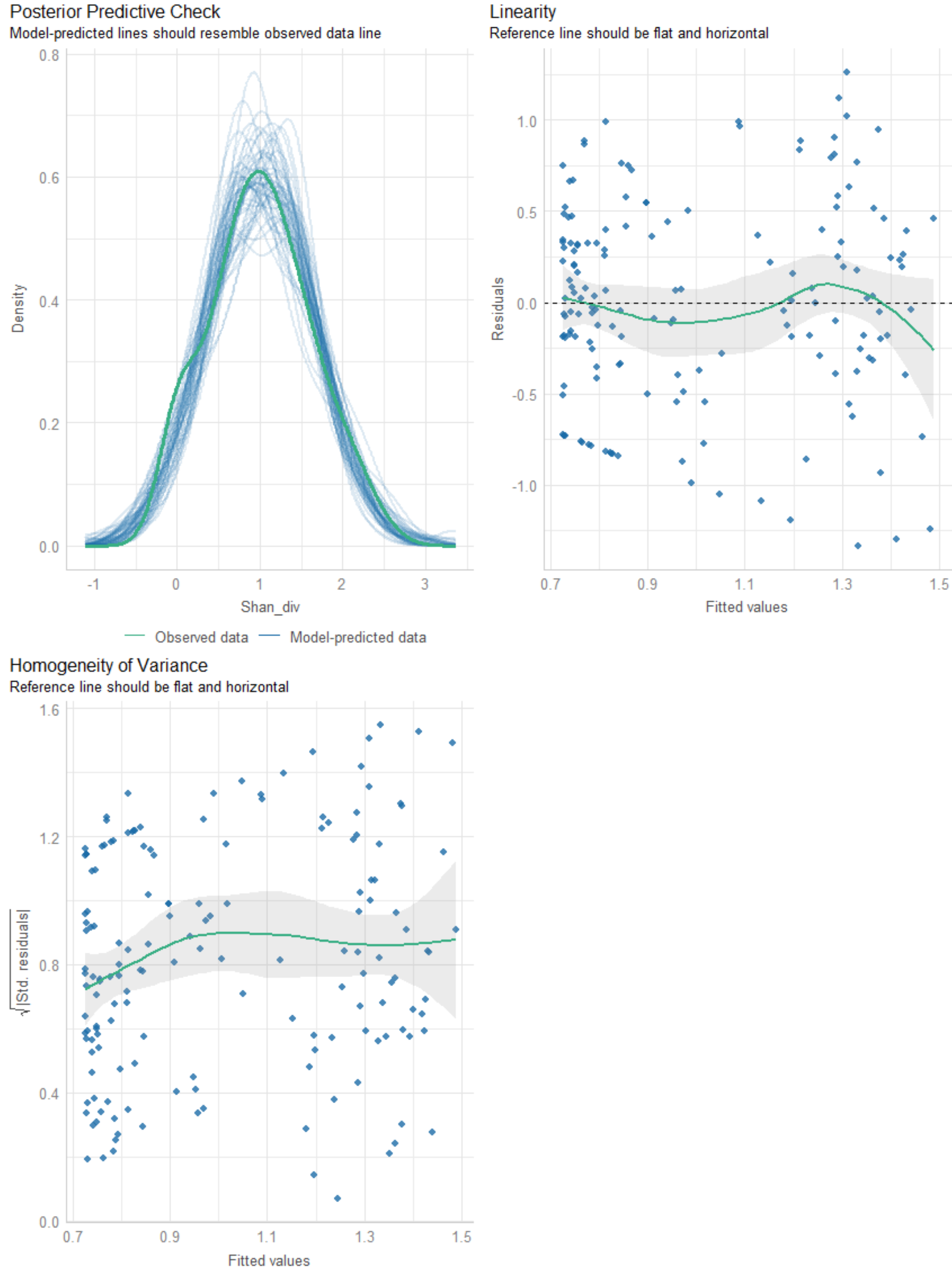
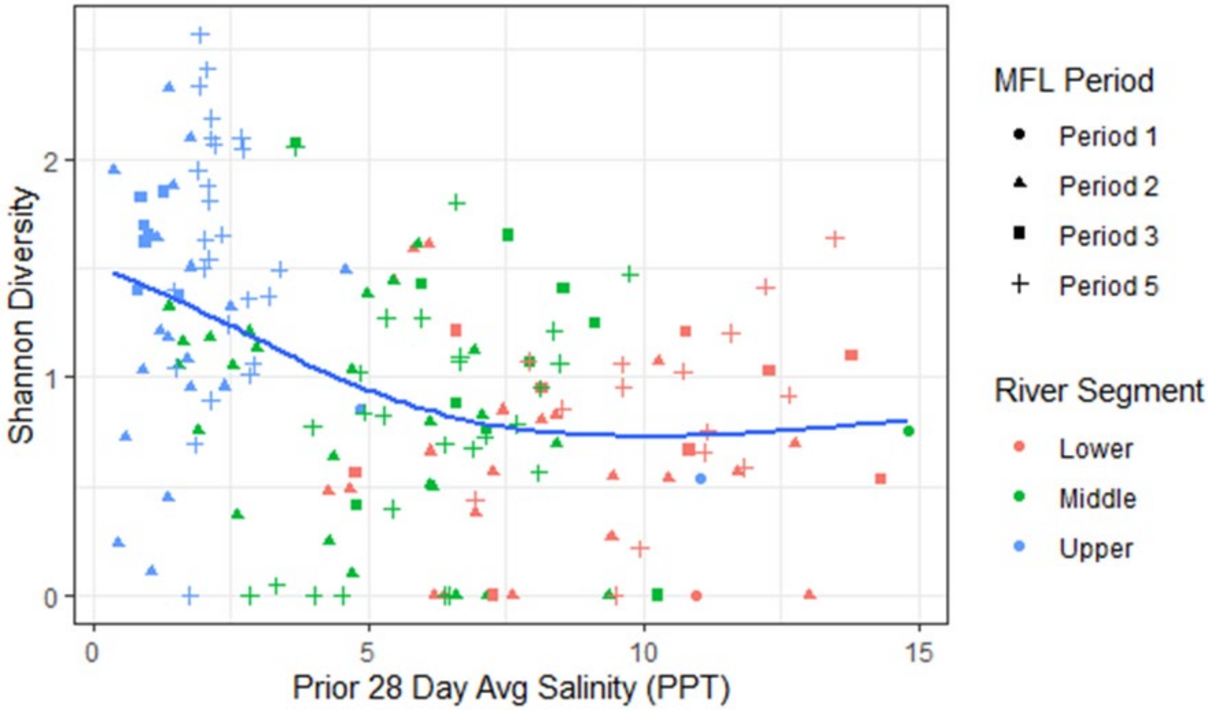


Table 6.2-35: Linear mixed model for 28-day depth-averaged salinity (as determined by the LAMFE model) as a predictor for salinity-sensitive benthic macroinvertebrate diversity

	Chi Squared	Degrees of Freedom	p value
(Intercept)	169	1	< 0.05
salin_depavg_28day	20	1	< 0.05

Figure 6.2-49: Salinity-sensitive benthic macroinvertebrate diversity by segment and period as predicted by 28-day depth-averaged salinity (in ppt, determined by the LAMFE model)



6.2.2.8 Benthic Macroinvertebrate Conclusions

Cluster analysis and ordination suggested taxonomic differences when comparing the upper segment with the middle and lower segments, encouraging additional analyses involving salinity-sensitive taxa.

Benthic density did not appear to be a useful indicator. However, as described below, abundance, taxa richness, and the diversity of salinity-sensitive benthic macroinvertebrate taxa indicated distinct ecological benefits associated with minimum flow implementation (Table 6.2-36, Table 6.2-37, and Table 6.2-38).

Table 6.2-36: Summary of mixed model results

Mixed Model	Type	Interaction Effect?	Meaningful Contrasts?
Abundance ~ Period * Segment + Date(R) + Site(R)	Quasi-Poisson	No	No
Density ~ Period * Segment + Date(R) + Site(R)	Gamma	No	No
Richness ~ Period * Segment + Date(R) + Site(R)	Poisson	Yes	Yes
Diversity ~ Period * Segment + Date(R) + Site(R)	Gaussian	No	Yes

Table 6.2-37: Summary of predictive model results

Predictive Model	Type	Significant?	R^2
Abundance ~ Salinity + Date(R) + Site(R)	Poisson	Yes	0.15
Density ~ Salinity + Date(R) + Site(R)	Gamma	No	0.01
Richness ~ Salinity + Date(R) + Site(R)	Poisson	Yes	0.69
Diversity ~ Salinity + Date(R) + Site(R)	GAM	Yes	0.18

Table 6.2-38: Summary of salinity-sensitive benthic macroinvertebrate taxa richness contrast results

Contrast	Estimate	Conclusion
Period 5 Upper – Period 1 Upper	0.8	On the log scale, 0.8 more salinity-sensitive taxa in upper segment with full minimum flow implementation compared to no implementation
Period 5 Upper – Period 5 Middle	0.9	On the log scale, 0.9 more salinity-sensitive taxa in upper segment with full minimum flow implementation compared to middle segment
Period 5 Upper – Period 5 Lower	1	On the log scale, 1 more salinity-sensitive taxa in upper segment with full minimum flow implementation compared to lower segment

The interaction effect for the richness model was significant and contrasts demonstrated significantly greater benthic macroinvertebrate taxa richness in the upper segment during Period 5 as compared to the middle and lower segments during the same period and as compared to the upper segment during Period 1.

A linear mixed model using the Poisson distribution for 28-day depth-averaged salinity (as determined via the LAMFE model) as a predictor for salinity-sensitive benthic macroinvertebrate taxa richness was statistically significant ($p < 0.05$), with a strong R^2 (0.69). A linear mixed model relating salinity to salinity-sensitive benthic macroinvertebrate taxa richness for the upper segment was also statistically significant ($p < 0.05$), providing additional evidence of a salinity-richness relationship in the upper segment of the study area, with greater richness associated with lower salinities, particularly salinities < 5 ppt. Significantly more types of salinity sensitive benthic macroinvertebrate taxa are in the upper segment of the target zone when flows are increased and salinity decreased. The diversity of salinity-sensitive benthic macroinvertebrate taxa was greater in the upper segment during Period 5 as compared to the middle and lower segment during the same period and as compared to the upper segment during Period 1 (Table 6.2-39). A GAM model for 28-day depth-averaged salinity (as determined by the LAMFE model) as a predictor for salinity-sensitive benthic macroinvertebrate diversity was statistically significant ($p < 0.05$) though the R^2 value indicated fairly low variance. Despite this, the model demonstrated that salinity-sensitive benthic macroinvertebrate diversity increased as salinity decreased and was higher when salinity was < 5 ppt.

Table 6.2-39: Summary of salinity-sensitive benthic macroinvertebrate diversity contrast results

Contrast	Estimate	Conclusion
Period 5 Upper – Period 1 Upper	0.8	~ 0.8 greater diversity of salinity-sensitive taxa in upper segment with full minimum flow implementation compared to no implementation
Period 5 Upper – Period 5 Middle	0.8	~ 0.8 greater diversity of salinity-sensitive taxa in upper segment with full minimum flow implementation compared to middle segment
Period 5 Upper – Period 5 Lower	0.8	~ 0.8 greater diversity of salinity-sensitive taxa in upper segment with full minimum flow implementation compared to lower segment

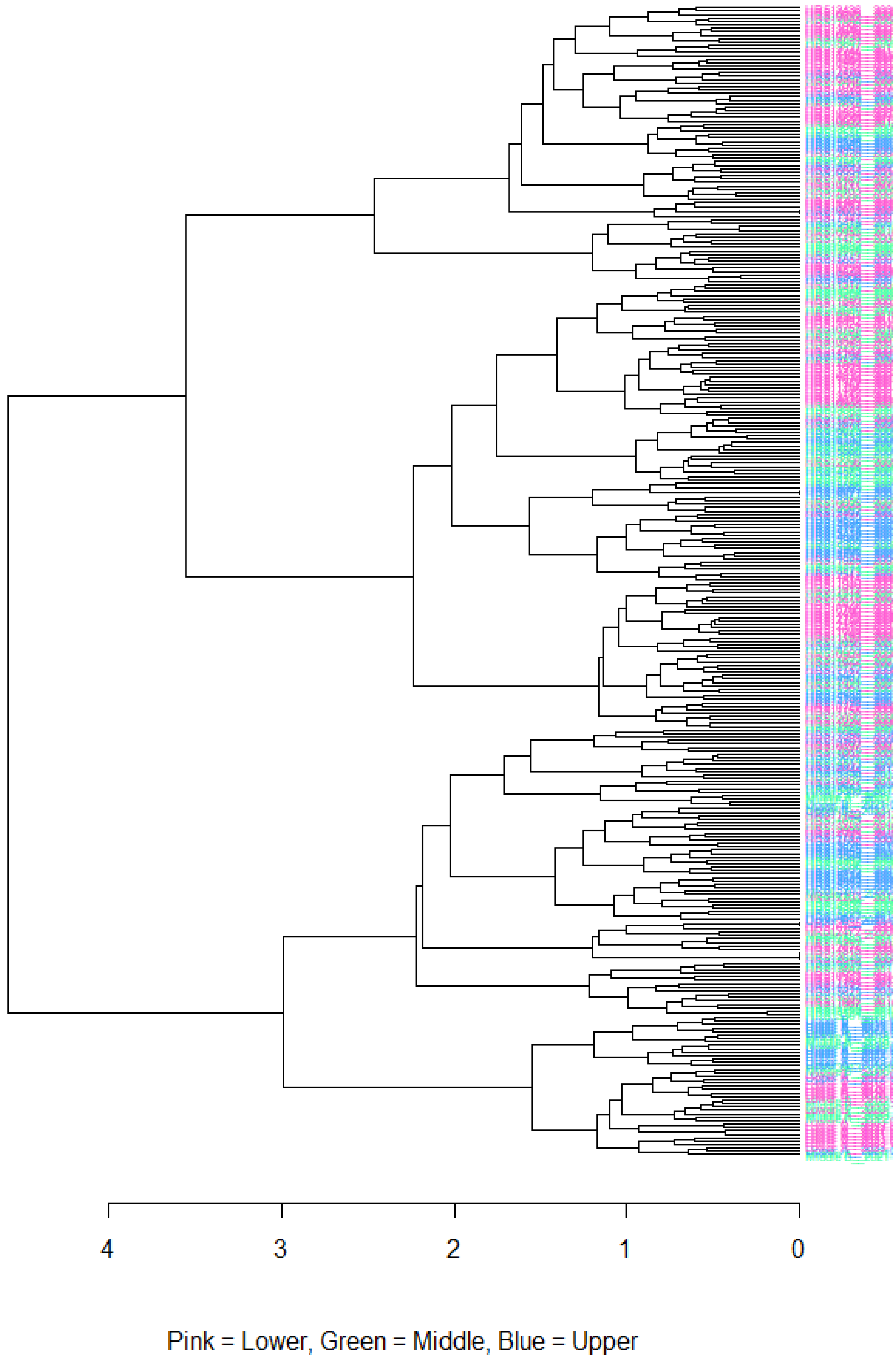
6.2.3 Nekton

Nekton are defined here as marine or aquatic animals with a sufficiently strong swimming ability to move independently of tides and currents. Nekton have the capability to migrate throughout the tidal zone to follow food sources, which, along with salinity, influences species presence or absence (Janicki Environmental 2003). The interaction between static (habitat) and dynamic (salinity) components is thought to be critical in defining nekton community structure (Guenther & MacDonald 2012, Brodie et al. 2013). This section provides a description and quantitative evaluation of changes in nekton as a function of minimum flow implementation.

6.2.3.1 Cluster Analyses

Hierarchical cluster analysis, using standardized Euclidean distance, was conducted using on all nekton taxa from the target zone. A few small clusters of nekton taxa found in the upper segment (coded blue) were potentially distinct from the clusters of taxa inhabiting the middle and lower segments (Figure 6.2-50).

Figure 6.2-50: Hierarchical clustering of nekton



6.2.3.2 Ordination

An NMDS using standardized Bray-Curtis distances (appropriate for relative abundance of species) was conducted on the nekton taxonomic data. A random initiation process was used with three axes, and a convergent solution was found with less than 40 starts (Figure 6.2-51). The fit of the model was acceptable as the stress value of 0.19 was below the threshold considered satisfactory for interference (0.2) (Clarke 1993). A stress plot was also examined for the fit of the data (Figure 6.2-52). The NMDS did not clearly distinguish nekton taxa groups from the three segments over the five periods as being distinct from one another. Additional analyses were conducted with salinity-sensitive taxa.

Figure 6.2-51: NMDS ordination of nekton by segment

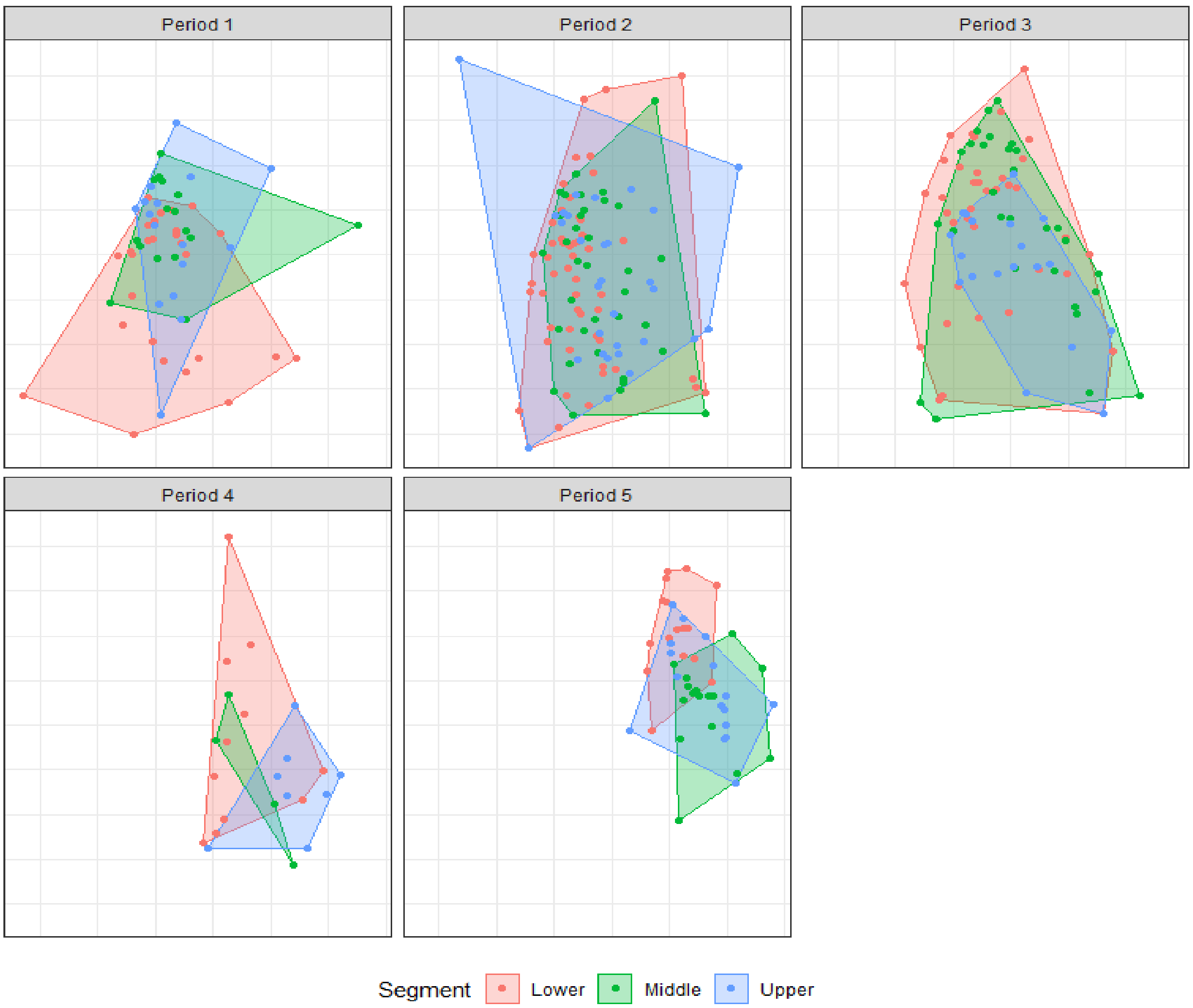
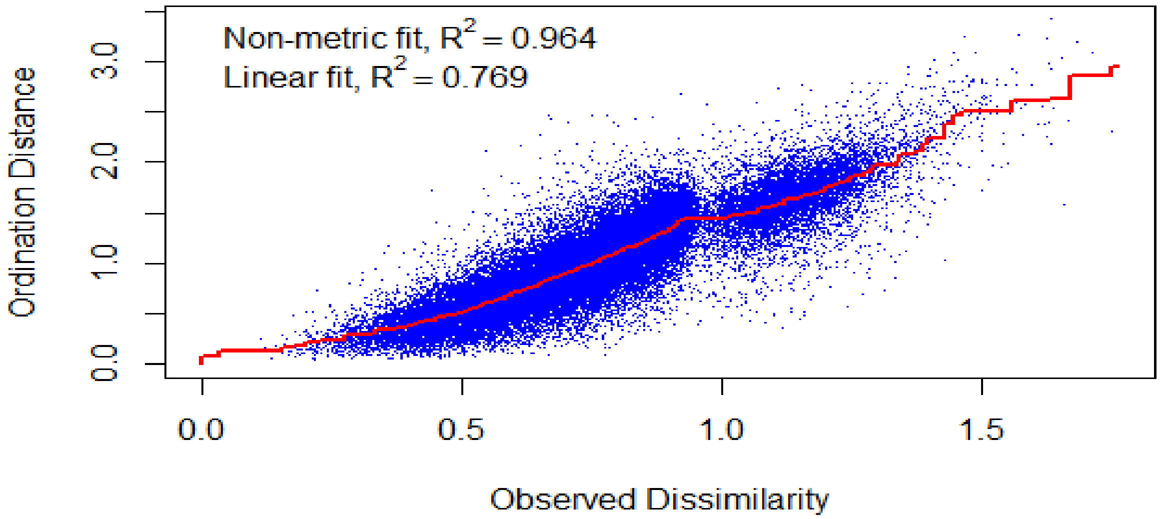


Figure 6.2-52: Stress plot of nekton data



6.2.3.2.1 Salinity Sensitivity

A key component of the LHR minimum flow was to provide essential low salinity habitat conditions downstream of the Hillsborough River Reservoir. A list of salinity-sensitive nekton taxa from the target zone used for these analyses are found in Table 6.2-40.

Table 6.2-40: List of nekton taxa inhabiting low salinity (< 5 ppt) habitat within the LHR target zone (Analysis Days)

Etheostoma fusiforme	*Heterandria formosa*	*Lepomis microlophus*	*Mugil cephalus*	*Palaemonetes pugio*
Eugerres plumieri	*Labidesthes sicculus*	*Lepomis punctatus*	*Notemigonus crysoleucas*	*Pomoxis nigromaculatus*
Fundulus chrysotus	*Lepomis auritus*	*Lepomis sp.*	*Notropis maculatus*	*Trachemys scripta*
Fundulus seminolis	*Lepomis macrochirus*	*Lucania goodei*	*Notropis petersoni*	*Trinectes maculatus*
Gambusia holbrooki	*Lepomis marginatus*	*Micropterus salmoides*	*Oreochromis aureus*	

6.2.3.2.2 Nekton Abundance

The average and total abundance of salinity-sensitive nekton taxa was inconsistent over time, with Period 1 exhibiting the most salinity-sensitive individuals (Figure 6.2-53, Figure 6.2-54). The temporal differences in salinity-sensitive abundance are more clearly seen in boxplots by segment and period (Figure 6.2-55). The abundance of the Daggerblade Shrimp (*Palamonetes pugio),* with a salinity range of 4.4 ppt to 17 ppt, was predominantly responsible for the abundance peaks during Period 1.

Figure 6.2-53: Average salinity-sensitive nekton abundance over time

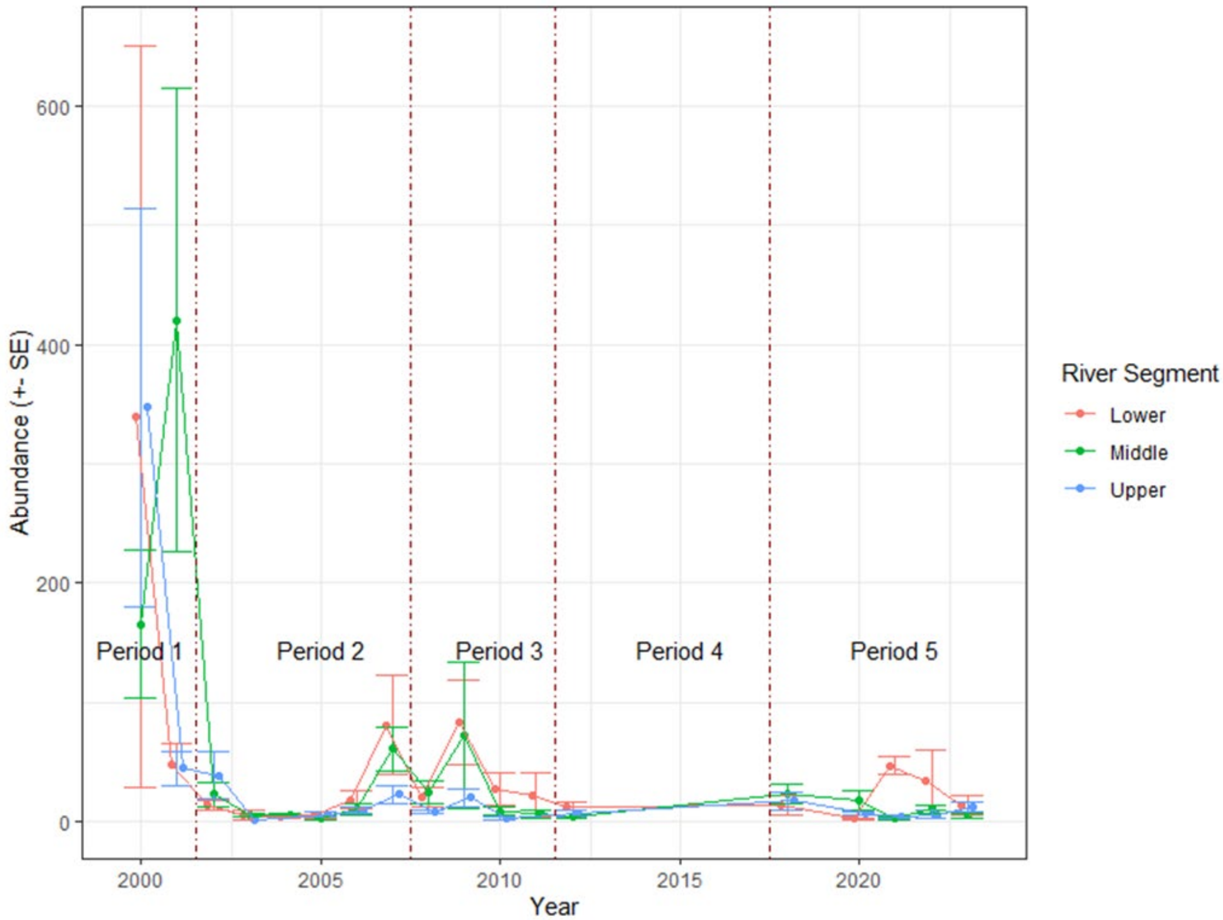


Figure 6.2-54: Total salinity sensitive nekton abundance over time

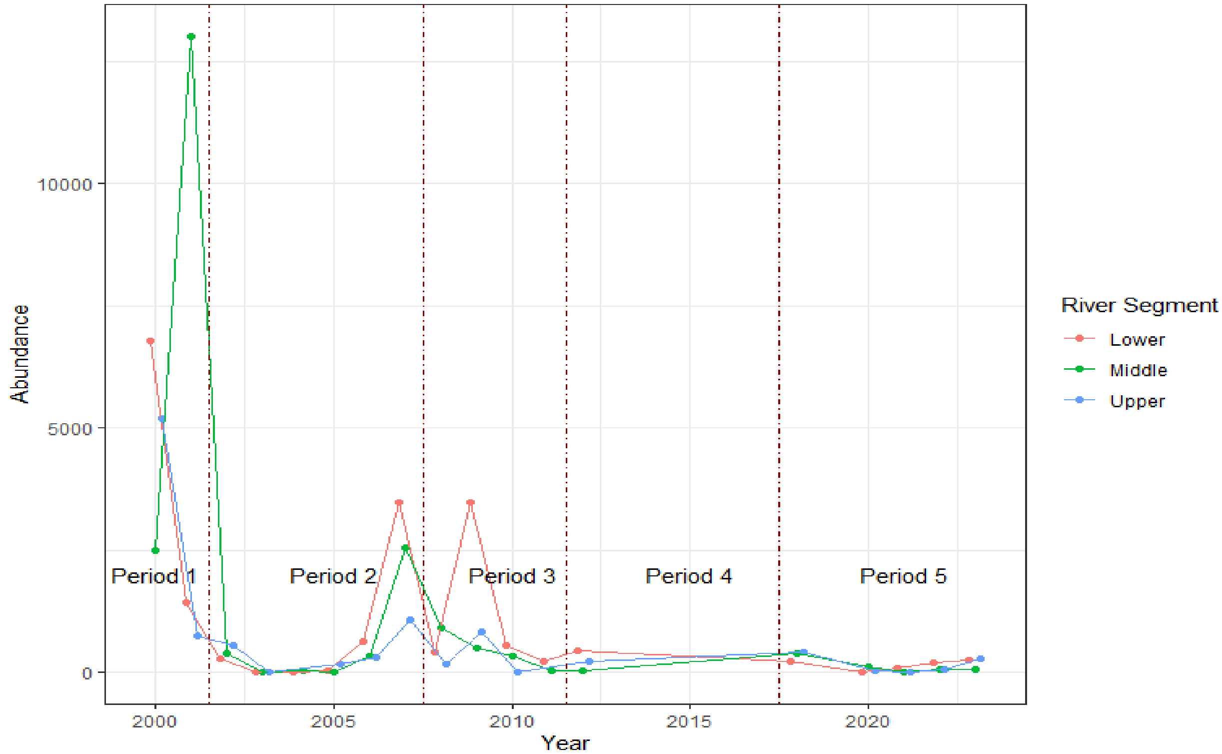
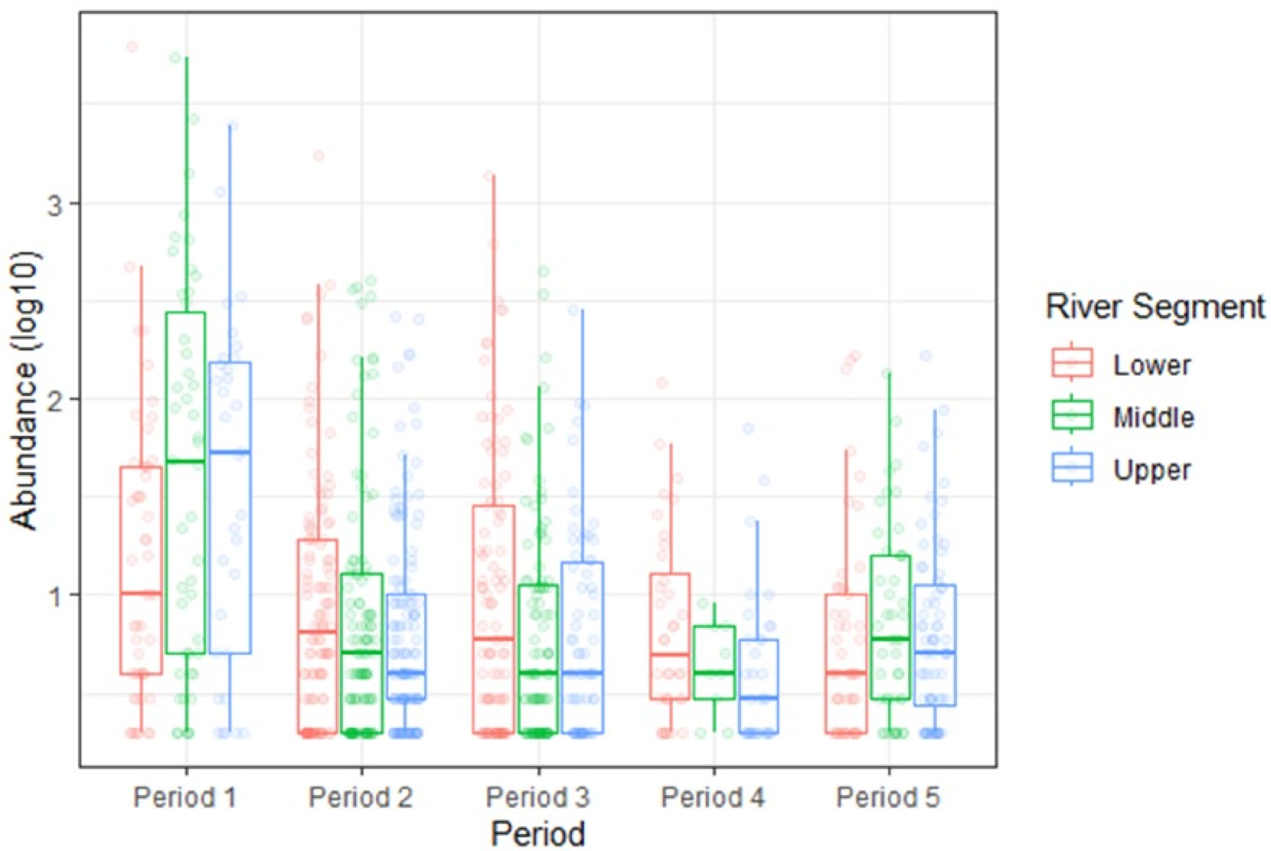


Figure 6.2-55: Boxplots of salinity-sensitive nekton abundance



Nekton Abundance Modelling

The abundance data were modeled using a Poisson distribution with an observation level random effect. A Poisson distribution was considered along with a negative binomial distribution, and although a negative binomial better accounted for the high variance relative to the mean in the nekton abundance data (dispersion ratio and residuals), neither were able to fully account for high overdispersion. A Poisson model with an observation model effect modelled overdispersion and was chosen. Model tests indicated high collinearity, which is common and often unavoidable when including interaction effects (Figure 6.2-56).

A generalized linear mixed model for salinity-sensitive nekton abundance using the Poisson distribution indicated a statistically significant interaction effect ($p < 0.05$) between abundance and segment (Table 6.2-41).

Figure 6.2-56: Model tests for salinity-sensitive nekton abundance

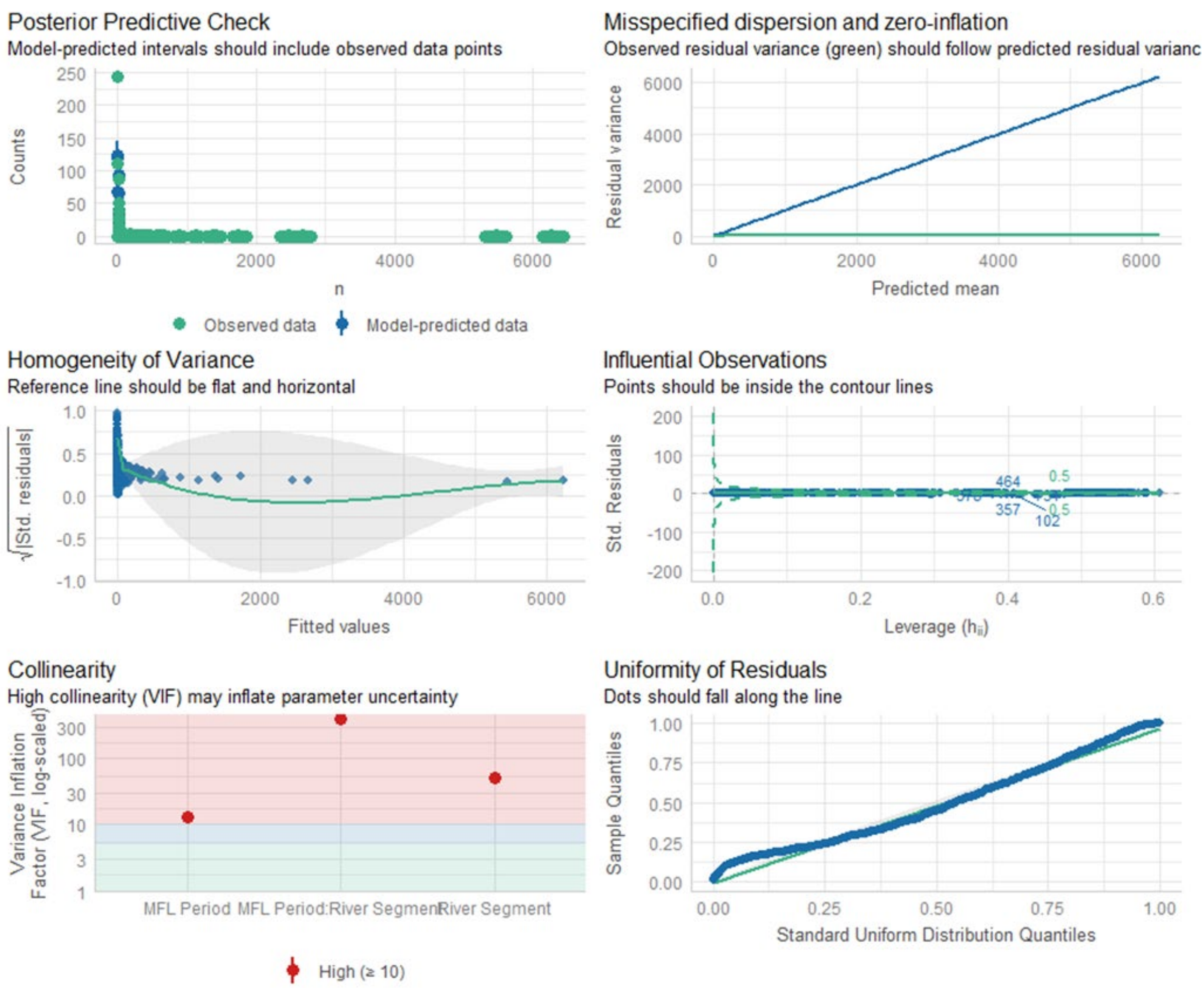


Table 6.2-41: Generalized linear mixed model for salinity-sensitive nekton abundance using the Poisson distribution

	Chi Squared	Degrees of Freedom	p value
(Intercept)	93.19	1.00	< 0.05
Minimum Flow Period	10.23	4.00	< 0.05
River Segment	12.45	2.00	< 0.05
Minimum Flow Period: River Segment	23.26	8.00	< 0.05

Nekton Abundance Effect Sizes

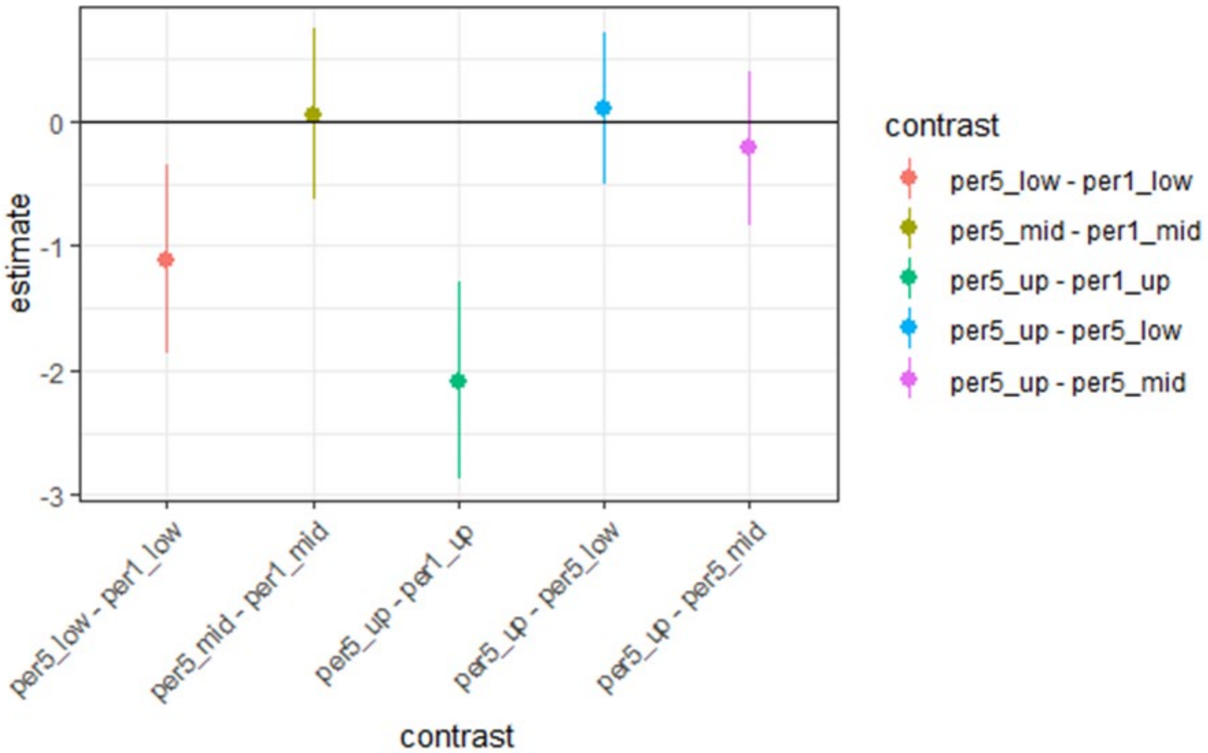
Selected contrasts for salinity-sensitive nekton abundance fit with the Poisson distribution indicated that salinity-sensitive nekton abundance was higher during Period 1 than during Period 5 in the upper segment. During Period 5 there was no difference in salinity-sensitive nekton abundance between upper, middle, and lower segments, indicated by 95% confidence limits that overlap zero (Table 6.2-42, Figure 6.2-57).

The abundance of the Daggerblade Shrimp, which inhabits a known salinity range of 4.4 ppt to 17 ppt, was predominantly responsible for the observed trends. As a result of the apparent flexibility in the salinity range that Daggerblade Shrimp may inhabit, these results were not considered relevant to minimum flow implementation.

Table 6.2-42: Selected contrasts for salinity-sensitive nekton abundance

Contrast	Estimate	Standard Error	Degrees of Freedom	Asymptotic Lower Confidence Limit	Asymptotic Upper Confidence Limit
Period 5 Upper – Period 1 Upper	-2.09	0.40	Inf	-2.88	-1.29
Period 5 Upper – Period 5 Lower	0.10	0.31	Inf	-0.50	0.71
Period 5 Upper – Period 5 Middle	-0.22	0.32	Inf	-0.84	0.41
Period 5 Lower – Period 1 Lower	-1.11	0.39	Inf	-1.86	-0.35
Period 5 Middle – Period 1 Middle	0.06	0.35	Inf	-0.62	0.74

Figure 6.2-57: Selected contrasts for salinity-sensitive nekton abundance



Salinity and Nekton Abundance Models

Generalized linear mixed models were developed using the Poisson distribution for 28-day depth-averaged salinity (determined via the LAMFE model). Mixed models as predictors for salinity-sensitive nekton abundance for all river segments were not significant (Table 6.2-43 and Table 6.2-44). Predicted antecedent 28-day depth-averaged salinity (determined by the LAMFE model) prior to the nekton sampling events is shown in

Figure 6.2-58. Salinity-sensitive taxa abundance predicted by these data showed no clear relationship (Figure 6.2-59).

Table 6.2-43: Generalized linear mixed Chi-squared (Chisq) model with Poisson distribution for 28-day depth-averaged salinity (determined via the LAMFE model) as a predictor for salinity-sensitive nekton abundance

	Chi Squared	Degrees of Freedom	p value
(Intercept)	225.73	1	< 0.05
salin_depavg_28day	0.17	1	0.68

Table 6.2-44: Generalized linear mixed model with Poisson distribution for 28-day depth-averaged salinity (determined via the LAMFE model) as a predictor for salinity-sensitive nekton abundance for upper segment

	Chi Squared	Degrees of Freedom	p value
(Intercept)	47	1	< 0.05
salin_depavg_28day	3	1	0.09

Figure 6.2-58: Antecedent 28-day depth-averaged salinity (in ppt, determined via the LAMFE model) by river segment and minimum flow period for nekton

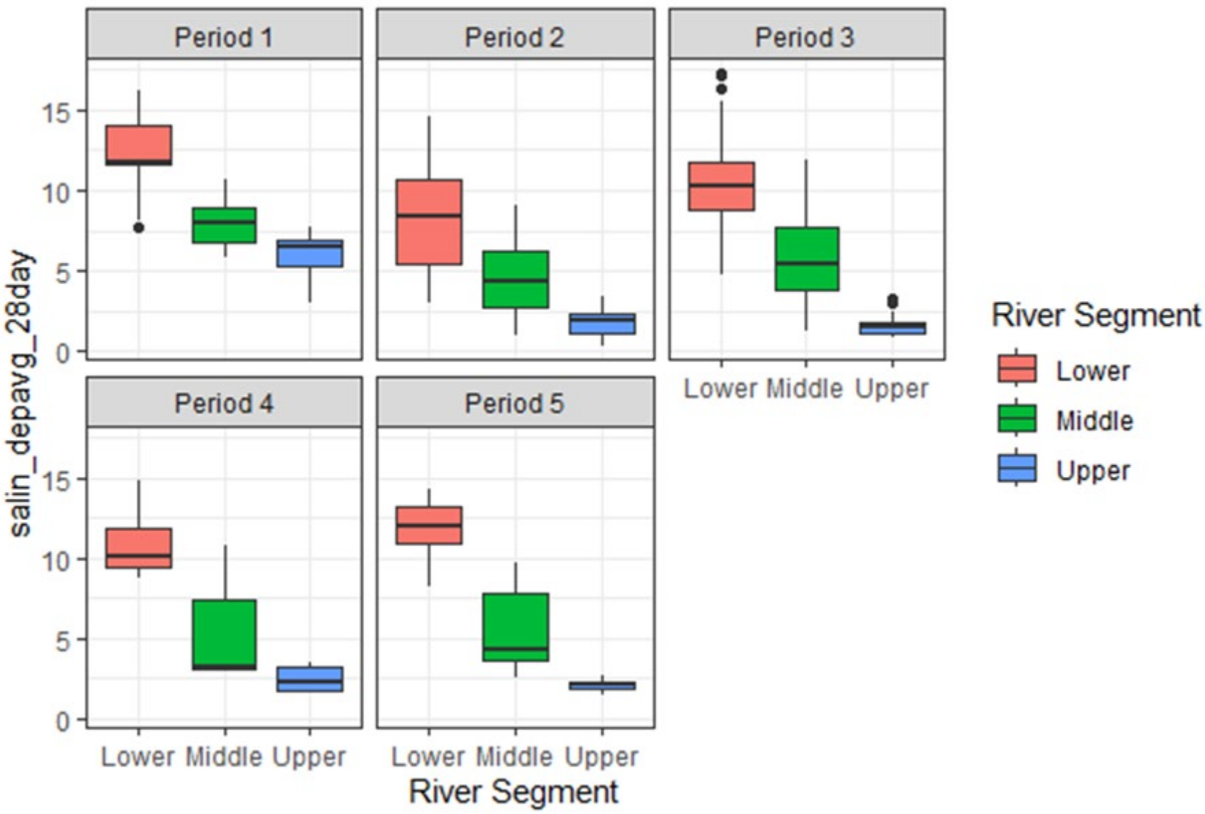
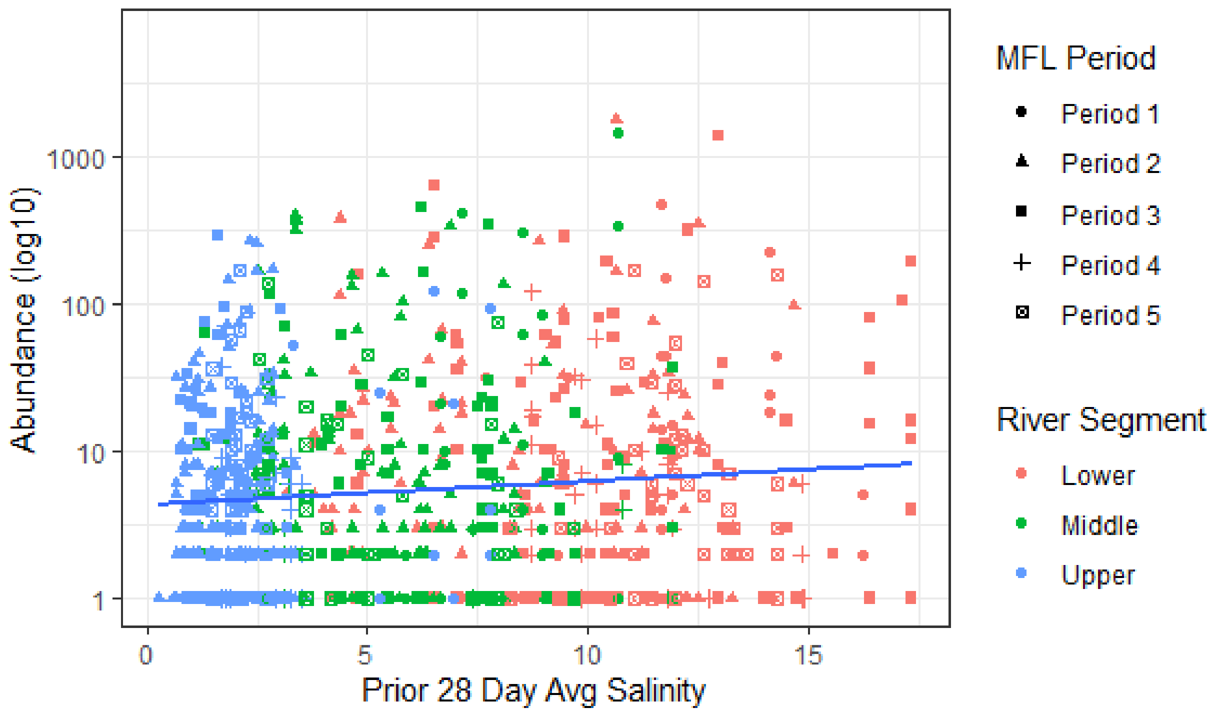


Figure 6.2-59: Salinity-sensitive nekton taxa abundance predicted by 28-day depth-averaged salinity (in ppt, determined via the LAMFE model).



6.2.3.3 Nekton Density

The average and total density of salinity-sensitive nekton taxa was inconsistent over time, with Period 1 exhibiting the highest density of salinity-sensitive nekton (Figure 6.2-60). The temporal differences in salinity-sensitive nekton density are more clearly seen with boxplots by segment and period (Figure 6.2-61). The abundance of the Daggerblade Shrimp, which inhabits a salinity range of 4.4 ppt to 17 ppt, was predominantly responsible for the density peaks in Period 1. These peaks occurred within each river segment during this period.

Figure 6.2-60: Salinity-sensitive nekton density over time

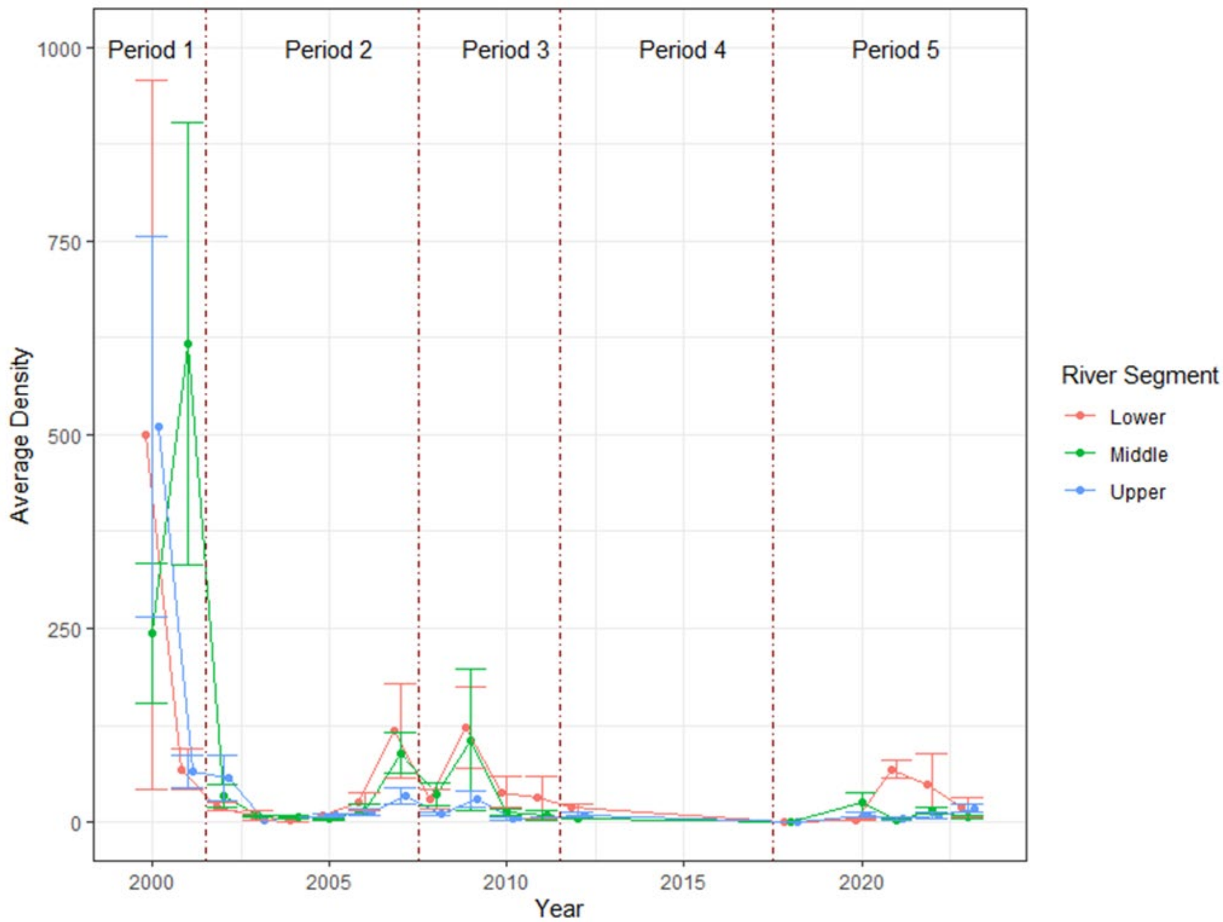
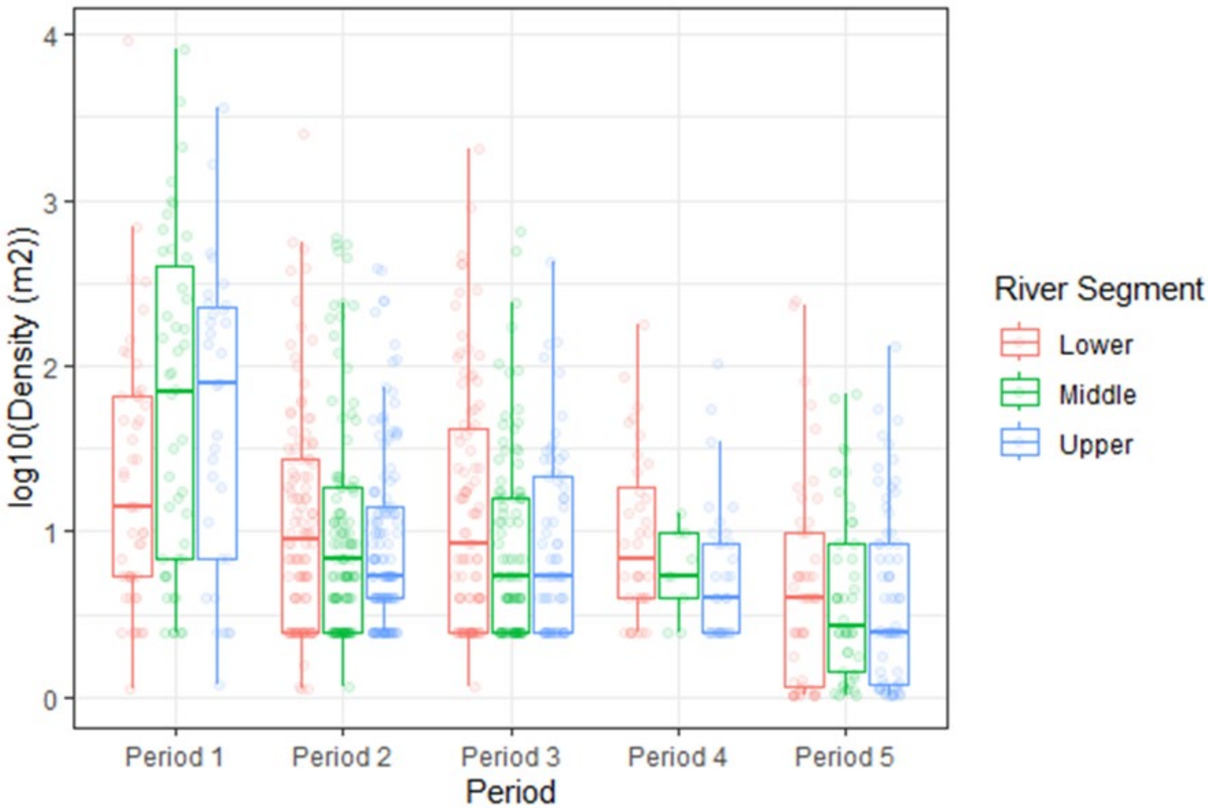


Figure 6.2-61: Boxplots of salinity-sensitive nekton density



Nekton Density Modeling

Density data were fit with a generalized linear mixed effects model with a gamma distribution using a log link. Despite an increased dispersion ratio, the model was not significantly overdispersed. Collinearity was elevated, which is expected in models that include interaction effects (Figure 6.2-62).

The model for salinity-sensitive nekton abundance indicated a statistically significant interaction effect ($p < 0.05$) between period and segment (Table 6.2-45). This appeared to be associated with the high density of Daggerblade Shrimp, which can thrive in both low and moderate salinities, during Period 1.

Figure 6.2-62: Model tests for salinity-sensitive nekton density

Table 6.2-45: Linear mixed Chi-squared (Chisq) model for salinity-sensitive nekton density using log transformation

	Chi Squared	Degrees of Freedom	p value
(Intercept)	115.84	1	<0.05
Minimum Flow Period	11.82	4	0.18
River Segment	11.43	2	<0.05
Minimum Flow Period: River Segment	26.02	8	<0.05

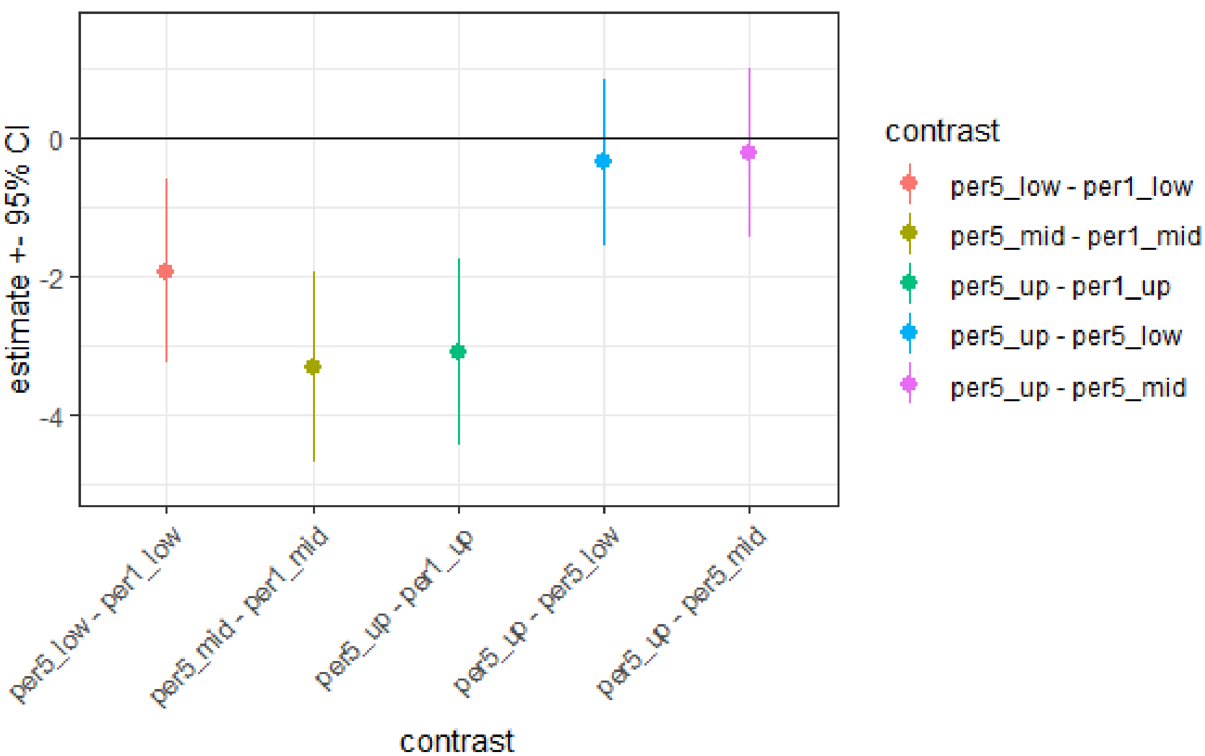
Nekton Density Effect Sizes

River segment contrasts for salinity-sensitive nekton density are shown in Table 6.2-46. Because of high Daggerblade Shrimp density during Period 1, river segment contrasts for salinity-sensitive nekton density were higher for all segments during Period 1 as compared to during Period 5 (Figure 6.2-63). There were no differences between river segments during Period 5. The flexibility of Daggerblade Shrimp to inhabit a range of salinities appeared to produce a result independent of minimum flow implementation.

Table 6.2-46: Selected contrasts for salinity-sensitive nekton density

Contrast	Estimate	Standard Error	Degrees of Freedom	Asymptotic Lower Confidence Limit	Asymptotic Upper Confidence Limit
Period 5 Upper – Period 1 Upper	-3.09	0.69	Inf	-4.44	-1.74
Period 5 Upper – Period 5 Lower	-0.35	0.62	Inf	-1.56	0.87
Period 5 Upper – Period 5 Middle	-0.21	0.63	Inf	-1.44	1.01
Period 5 Lower – Period 5 Middle	-1.92	0.68	Inf	-3.24	-0.60
Period 5 Middle – Period 1 Middle	-3.31	0.70	Inf	-4.68	-1.94

Figure 6.2-63: River segment contrasts for salinity-sensitive nekton density



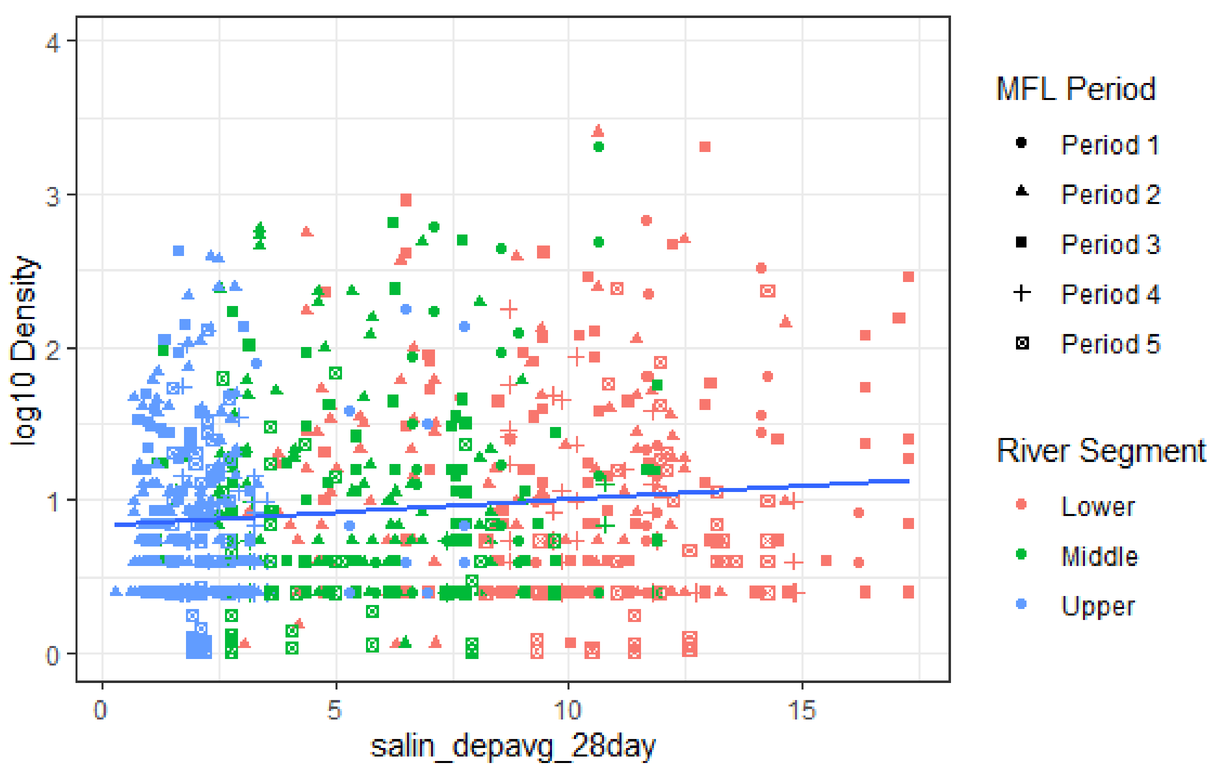
Salinity and Nekton Density

A linear mixed model was developed using the log distribution for 28-day depth-averaged salinity (as determined via the LAMFE model) and model assumptions were met. The mixed model as a predictor for salinity-sensitive nekton density was statistically significant ($p < 0.05$) (Table 6.2-47). However, the R^2 for the main model was low (0.02), with most of the model being explained by random effects ($R^2 = 0.59$). Salinity-sensitive nekton taxa density predicted by the 28-day depth-averaged salinity (determined via the LAMFE model) appeared to slightly increase across the salinity gradient, associated with the Daggerblade Shrimp trends (Figure 6.2-64).

Table 6.2-47: Generalized linear mixed model fit to a gamma distribution for 28-day depth-averaged salinity (in ppt, determined via the LAMFE model) as a predictor for salinity-sensitive nekton density

	Chi Squared	Degrees of Freedom	p value
(Intercept)	356.0	1	< 0.05
salin_depavg_28day	6.6	1	< 0.05

Figure 6.2-64: Salinity-sensitive nekton density predicted by 28-day depth-averaged salinity (in ppt, determined via the LAMFE model) over time by river segment.



6.2.3.4 Nekton Taxa Richness

The richness of salinity-sensitive nekton taxa generally increased throughout the study period within the upper segment, with the highest richness of salinity-sensitive nekton taxa occurring during Period 5 and the lowest richness of salinity-sensitive taxa during Period 1 (Figure 6.2-65). The trends in salinity-sensitive nekton taxa richness are also shown in boxplots by segment and period (Figure 6.2-66). Salinity-sensitive nekton taxa richness appears to discriminate differences in periods, potentially associated with minimum flow implementation.

Figure 6.2-65: Salinity-sensitive nekton taxa richness over time

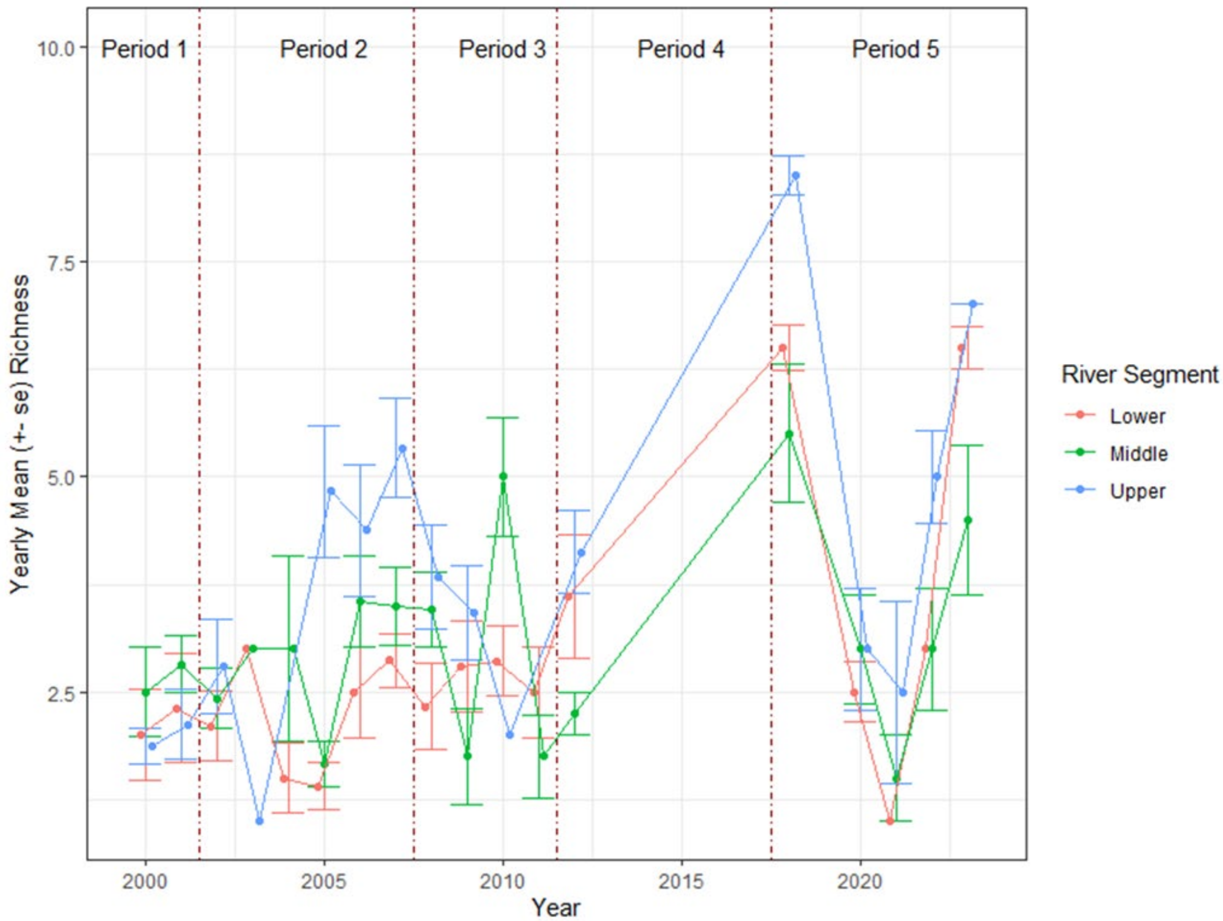
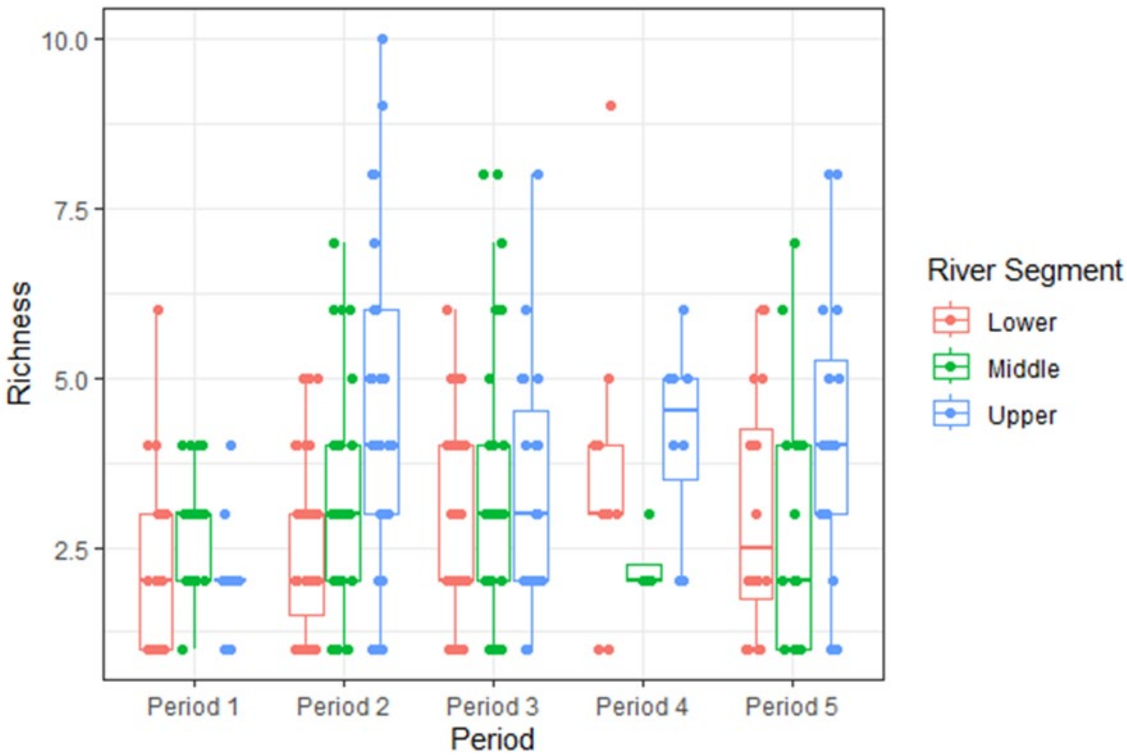


Figure 6.2-66: Salinity-sensitive nekton taxa richness boxplots by period and segment



Nekton Taxa Richness Modeling

A generalized linear mixed model with the Poisson distribution was chosen to fit richness data and model assumptions were met. Collinearity was elevated, but this is expected when fitting an interaction effect (Figure 6.2-67).

The mixed model for salinity-sensitive nekton taxa using the Poisson distribution indicated no statistically significant interaction effect ($p = 0.08$) between period and segment (Table 6.2-48). A reduced model using only Period 1 and Period 5 data had a significant interaction effect ($p < 0.05$) (Table 6.2-49).

Figure 6.2-67: Model tests for salinity-sensitive nekton taxa richness

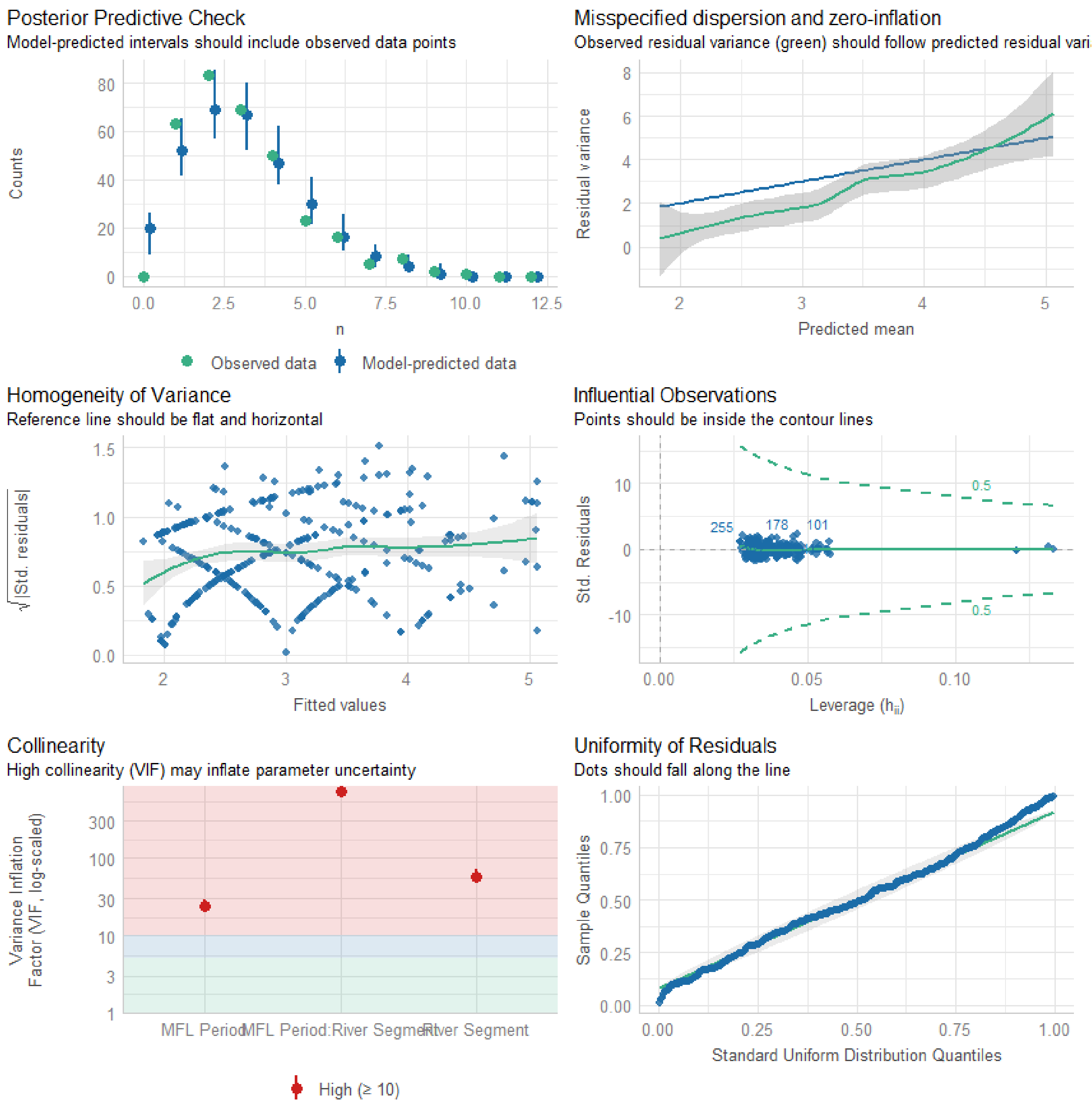


Table 6.2-48: Generalized linear mixed model for salinity-sensitive nekton taxa richness using log transformation

	Chi Squared	Degrees of Freedom	p value
Minimum Flow Period	6.10	4.00	0.19
River Segment	1.26	2.00	0.53
Minimum Flow Period: River Segment	14.03	8.00	0.08

Table 6.2-49: Reduced generalized linear effects model (Period 1 and 5) for salinity-sensitive nekton taxa richness

	Chi Squared	Degrees of Freedom	p value
Minimum Flow Period	2.89	1.00	0.09
River Segment	1.96	2.00	0.37
Minimum Flow Period: River Segment	6.16	2.00	< 0.05

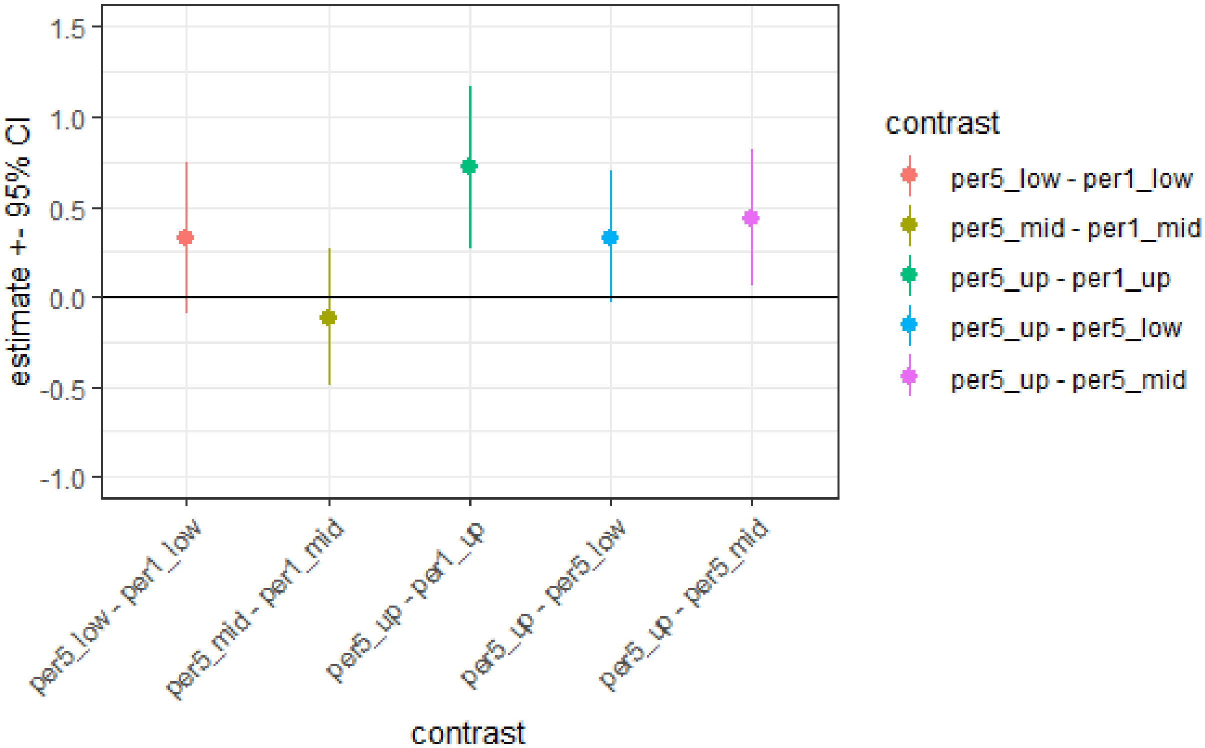
Nekton Taxa Richness Effect Sizes

Selected contrasts indicated that salinity-sensitive nekton taxa richness was higher in the upper segment during Period 5 than the middle segment during the same period, and was higher than taxa richness in the upper segment during Period 1, because 95% confidence limits did not overlap zero (Table 6.2-50 and Figure 6.2-68). Although the Period 5 upper segment taxa richness was higher than Period 5 lower segment taxa richness, the confidence interval overlapped zero. These results indicate an increase in nekton taxa that prefer salinity < 5 ppt associated with the minimum flow implementation within the upper segment, both over time and when compared to downstream segments. However, salinity-sensitive taxa richness increased in the lower segment over time as well, predominantly due to the flexible salinity range of the Daggerblade Shrimp.

Table 6.2-50: Selected contrasts for salinity-sensitive nekton taxa richness

Contrast	Estimate	Standard Error	Degrees of Freedom	Asymptotic Lower Confidence Limit	Asymptotic Upper Confidence Limit
Period 5 Upper – Period 1 Upper	0.72	0.23	Inf	0.27	1.17
Period 5 Upper – Period 5 Lower	0.33	0.19	Inf	-0.04	0.70
Period 5 Upper – Period 5 Middle	0.44	0.19	Inf	0.06	0.81
Period 5 Lower – Period 1 Lower	0.33	0.22	Inf	-0.10	0.75
Period 5 Middle – Period 1 Middle	-0.12	0.19	Inf	-0.50	0.26

Figure 6.2-68: Selected contrasts for salinity-sensitive nekton taxa richness



Salinity and Nekton Taxa Richness

A generalized linear model using the Poisson distribution for 28-day depth-averaged salinity (as determined via the LAMFE model) as a predictor for salinity-sensitive nekton taxa richness was statistically significant ($p < 0.05$), however, the R^2 (0.1) was low (Table 6.2-51). Salinity-sensitive taxa richness tended to increase as salinity decreased, with the highest taxa richness concentrated at < 5 ppt salinity (Figure 6.2-69). A model was also fit for 28-day depth-averaged salinity (determined via the LAMFE model) as a predictor for salinity-sensitive nekton taxa richness exclusively within the upper segment, which was not statistically significant ($p = 0.28$) (Table 6.2-52).

Table 6.2-51: Generalized linear mixed model for 28-day depth-averaged salinity (determined by the LAMFE model) as a predictor for salinity-sensitive nekton taxa richness

	Chi Squared	Degrees of Freedom	p value
(Intercept)	346	1	< 0.05
salin_depavg_28day	20	1	<0.05

Figure 6.2-69: Salinity-sensitive nekton taxa richness vs. 28-day depth-averaged salinity (in ppt, determined by the LAMFE model)

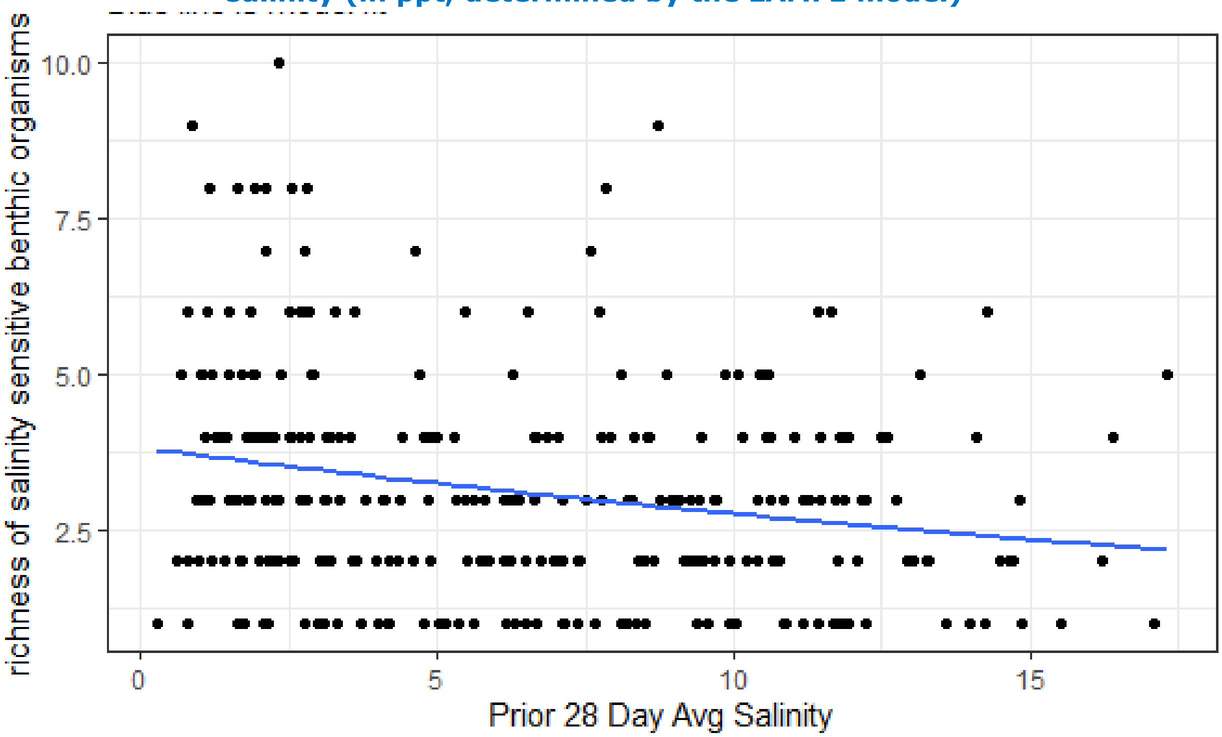


Table 6.2-52: Generalized linear mixed effects model with log transformation for 28-day depth-averaged salinity (determined via the LAMFE model) as a predictor for salinity-sensitive nekton richness for the upper segment

	Chi Squared	Degrees of Freedom	p value
(Intercept)	74.12	1	< 0.05
salin_depavg_28day	0.93	1	0.28

6.2.3.5 Nekton Diversity

Although variable, the diversity of salinity-sensitive nekton taxa was generally highest within the upper segment during Period 5 (Figure 6.2-70, Figure 6.2-71), similar to the pattern shown by salinity-sensitive nekton taxa richness.

Figure 6.2-70: Shannon diversity of salinity-sensitive nekton over time

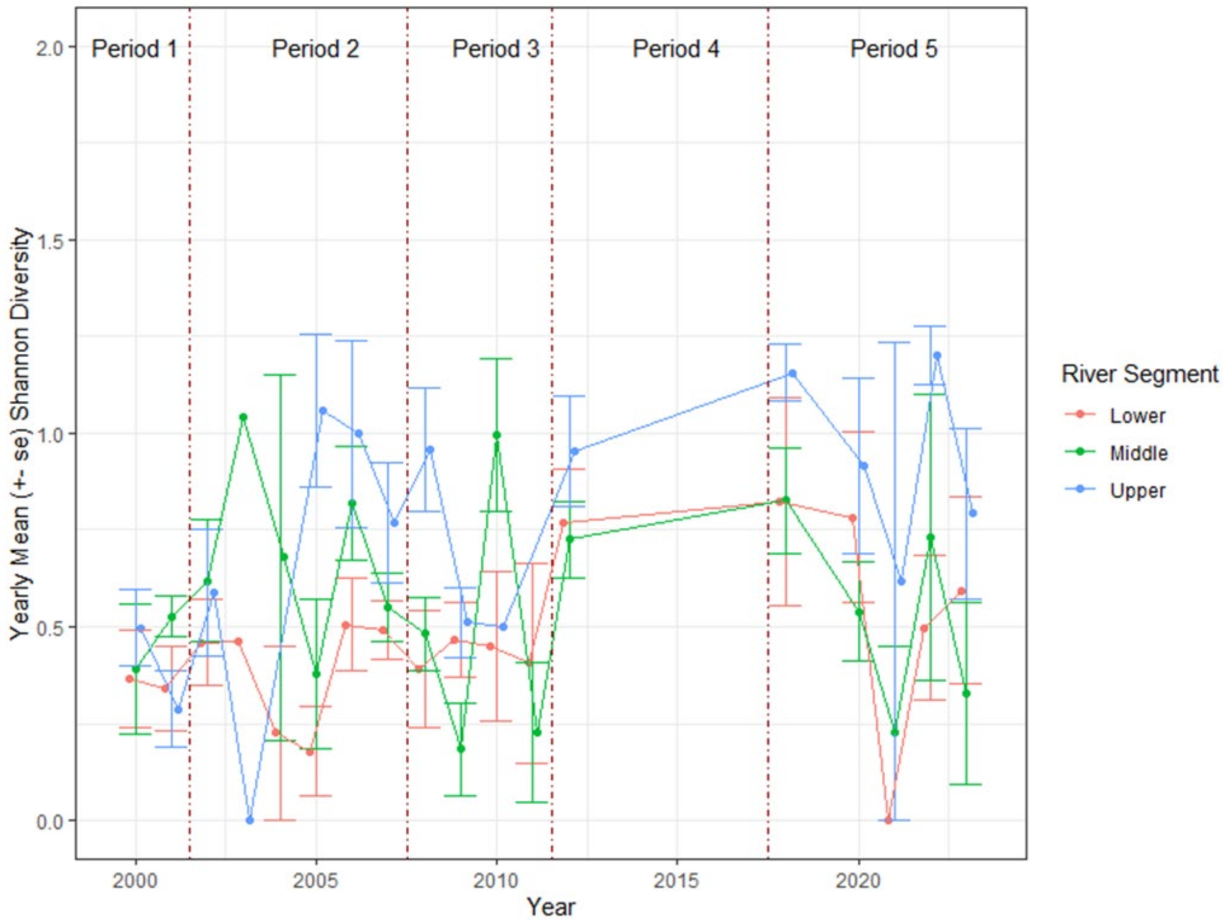
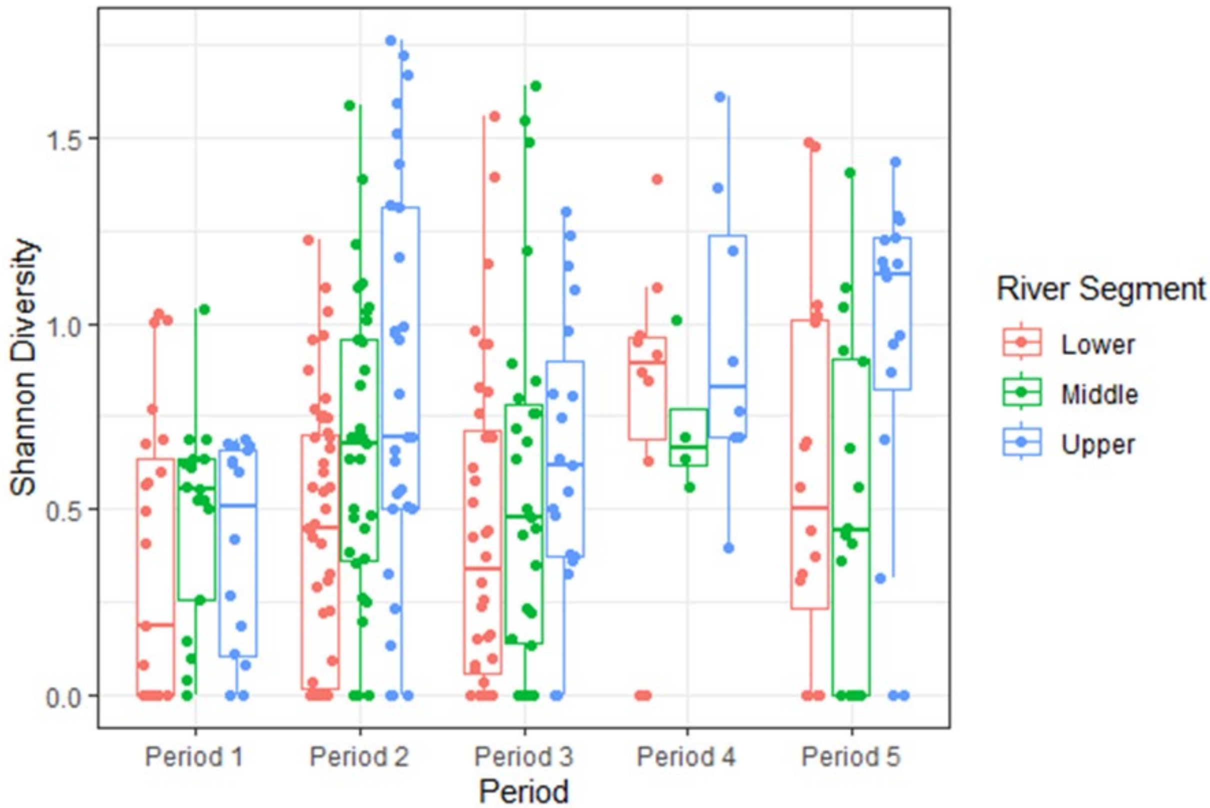


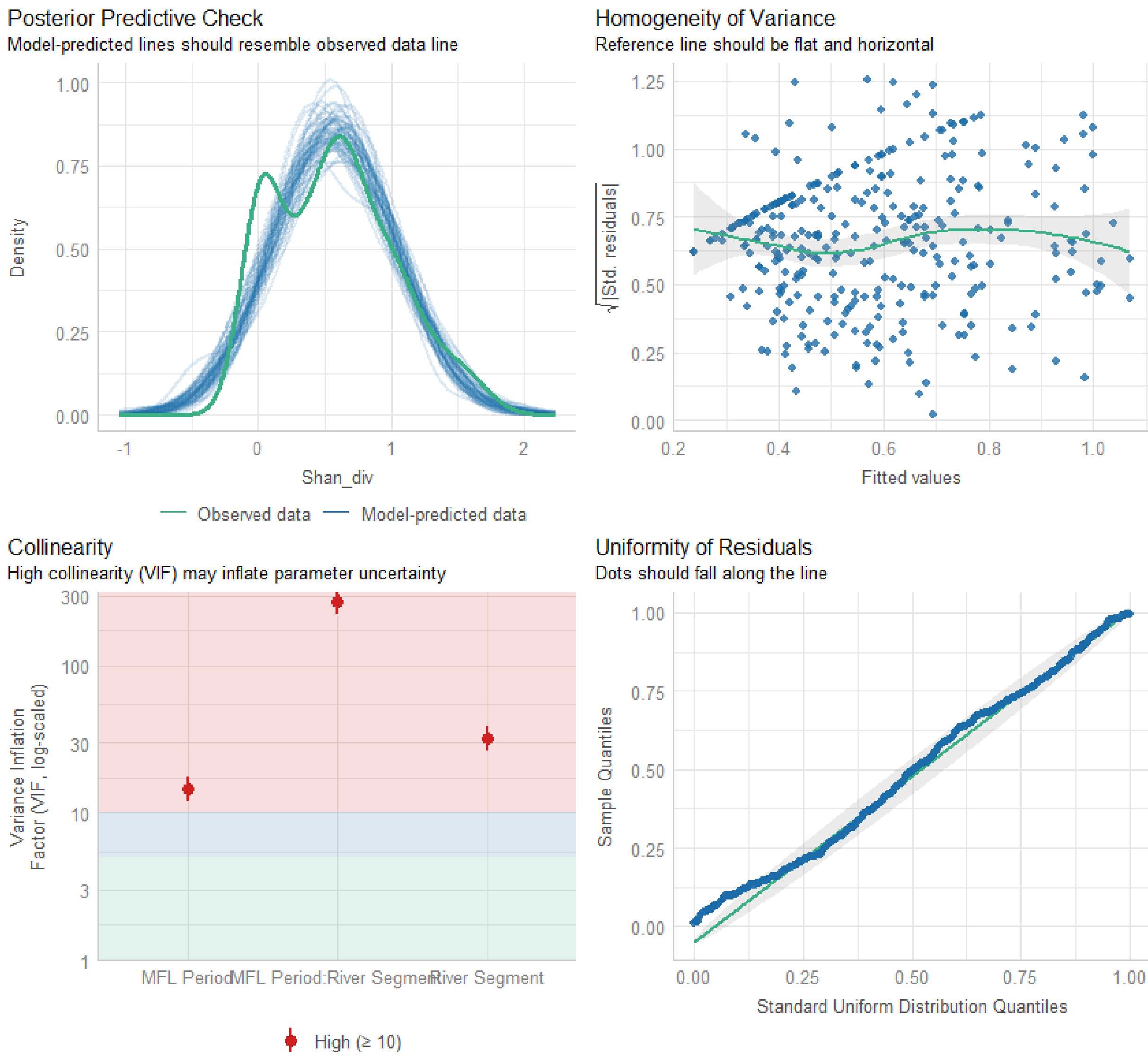
Figure 6.2-71: Boxplot of salinity-sensitive nekton diversity



Nekton Diversity Modelling

The salinity-sensitive nekton diversity data demonstrated zero inflation, because many sites had a single salinity-sensitive species observed. Therefore, a generalized linear mixed model with a zero-inflation term was fit to the data. Model tests indicated elevated collinearity, an expected result of models with interaction terms (Figure 6.2-72).

Figure 6.2-72: Model tests for salinity-sensitive nekton diversity



The linear mixed model for salinity-sensitive nekton diversity indicated that the interaction between minimum flow period and segment was not significant ($p = 0.38$) (Table 6.2-53). A reduced model for salinity-sensitive nekton diversity using Period 1 and Period 5 data was statistically significant ($p < 0.04$) (Table 6.2-54).

Table 6.2-53: Linear mixed Chi-squared (Chisq) model for salinity-sensitive nekton diversity

	Chi Squared	Degrees of Freedom	p value
Minimum Flow Period	7.68	4	0.10
River Segment	0.80	2	0.67
Minimum Flow Period: River Segment	8.62	8	0.38

Table 6.2-54: Linear mixed model for Period 1 and Period 5 salinity-sensitive nekton diversity

	Chi Squared	Degrees of Freedom	p value
Minimum Flow Period	3.04	1	0.08
River Segment	0.95	2	0.62
Minimum Flow Period: River Segment	6.05	2	< 0.05

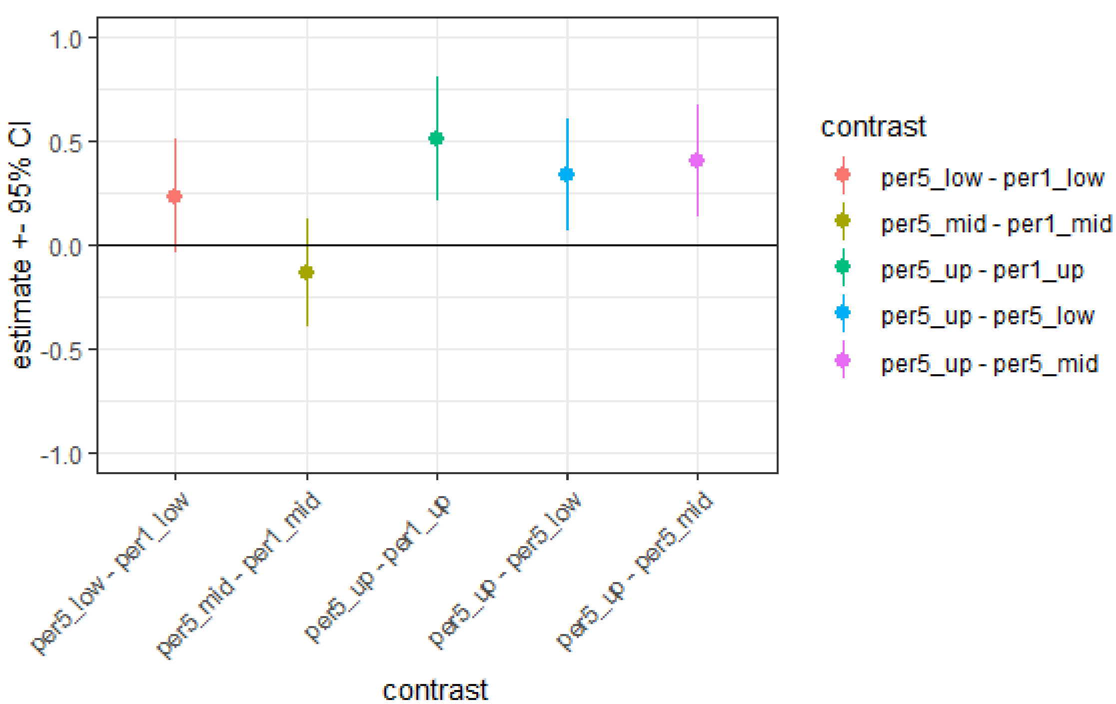
Nekton Diversity Effect Sizes

River segment contrasts for salinity-sensitive nekton diversity indicated that the diversity of salinity-sensitive nekton taxa was higher in the upper segment during Period 5 as compared to the middle and lower segment during Period 5 and as compared to the upper segment during Period 1 (Table 6.2-55, Figure 6.2-73). The largest change in salinity-sensitive nekton diversity occurred within the upper segment between Period 1 and Period 5, when Shannon diversity increased by 0.5.

Table 6.2-55: Selected contrasts for salinity-sensitive nekton diversity

Contrast	Estimate	Standard Error	Degrees of Freedom	Asymptotic Lower Confidence Limit	Asymptotic Upper Confidence Limit
Period 5 Upper – Period 1 Upper	0.51	0.15	286	0.21	0.81
Period 5 Upper – Period 5 Lower	0.34	0.14	286	0.07	0.61
Period 5 Upper – Period 5 Middle	0.41	0.14	286	0.14	0.68
Period 5 Lower – Period 1 Lower	0.24	0.14	286	-0.04	0.51
Period 5 Middle – Period 1 Middle	-0.13	0.13	286	-0.39	0.12

Figure 6.2-73: Selected contrasts for salinity-sensitive nekton diversity



Salinity and Nekton Diversity

A zero-inflated generalized linear mixed model evaluating 28-day depth-averaged salinity (estimated using the LAMFE model) as a predictor for salinity-sensitive nekton diversity indicated a statistically significant relationship ($p < 0.05$) (Table 6.2-56). A GAM of the same relationship was also statistically significant ($p < 0.01$). Model comparisons showed similar AIC values, with the linear model providing slightly greater parsimony. The GAM explained a modestly higher proportion of variability ($R^2 = 0.1$).

A zero-inflated model using only upper segment data was statistically significant ($p < 0.05$) but had a lower R^2 than did the model incorporating all river segments (Table 6.2-57). Salinity-sensitive nekton diversity was higher in < 5 ppt salinity (Figure 6.2-74). Additionally, for the upper segment, Period 1 diversity was clustered in the low diversity and higher salinity quadrant (Figure 6.2-75).

Table 6.2-56: Linear mixed model for 28-day depth-averaged salinity (as determined via the LAMFE model) as a predictor for salinity-sensitive nekton diversity

	Chi Squared	Degrees of Freedom	p value
(Intercept)	213	1	< 0.05
salin_depavg_28day	16	1	< 0.05

Table 6.2-57: Linear mixed Chi-squared (Chisq) model for 28-day depth-averaged salinity (in ppt, as determined via the LAMFE model) as a predictor for salinity-sensitive nekton diversity for upper segment

	Chi Squared	Degrees of Freedom	p value
(Intercept)	53.9	1	< 0.05
salin_depavg_28day	1.7	1	< 0.05

Figure 6.2-74: Salinity-sensitive nekton diversity by segment and period as predicted by 28-day depth-averaged salinity (in ppt, as determined by the LAMFE model) with GAM fit

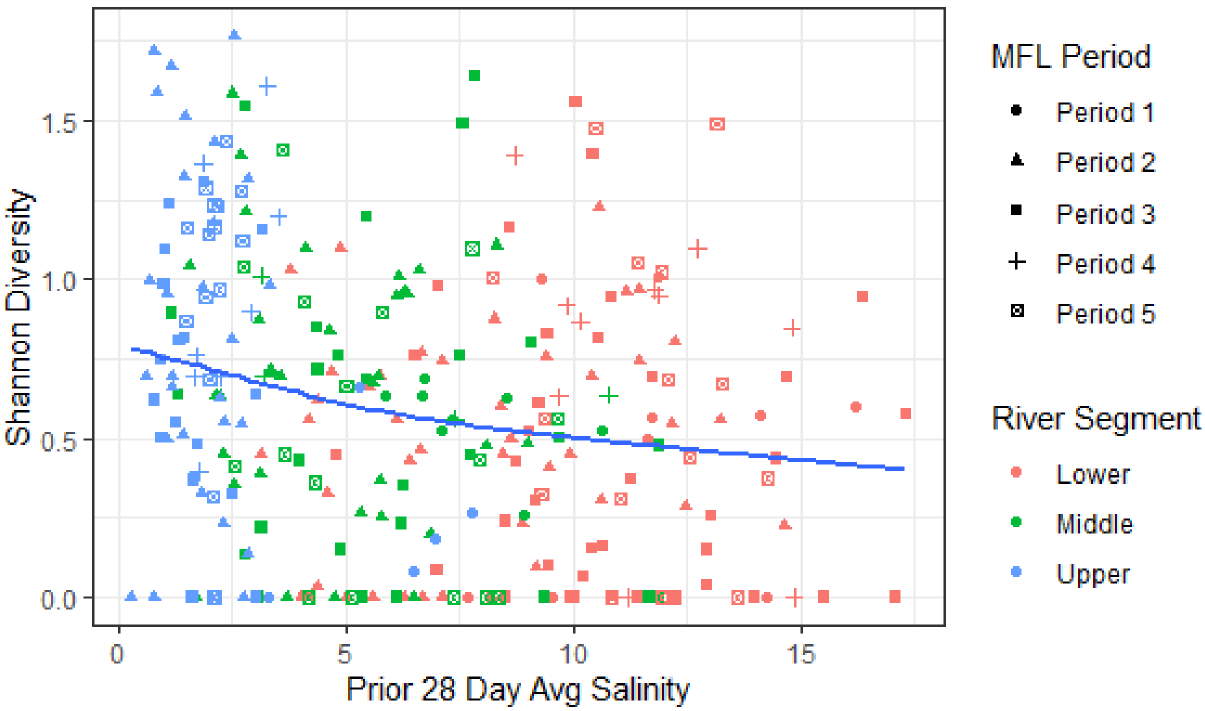
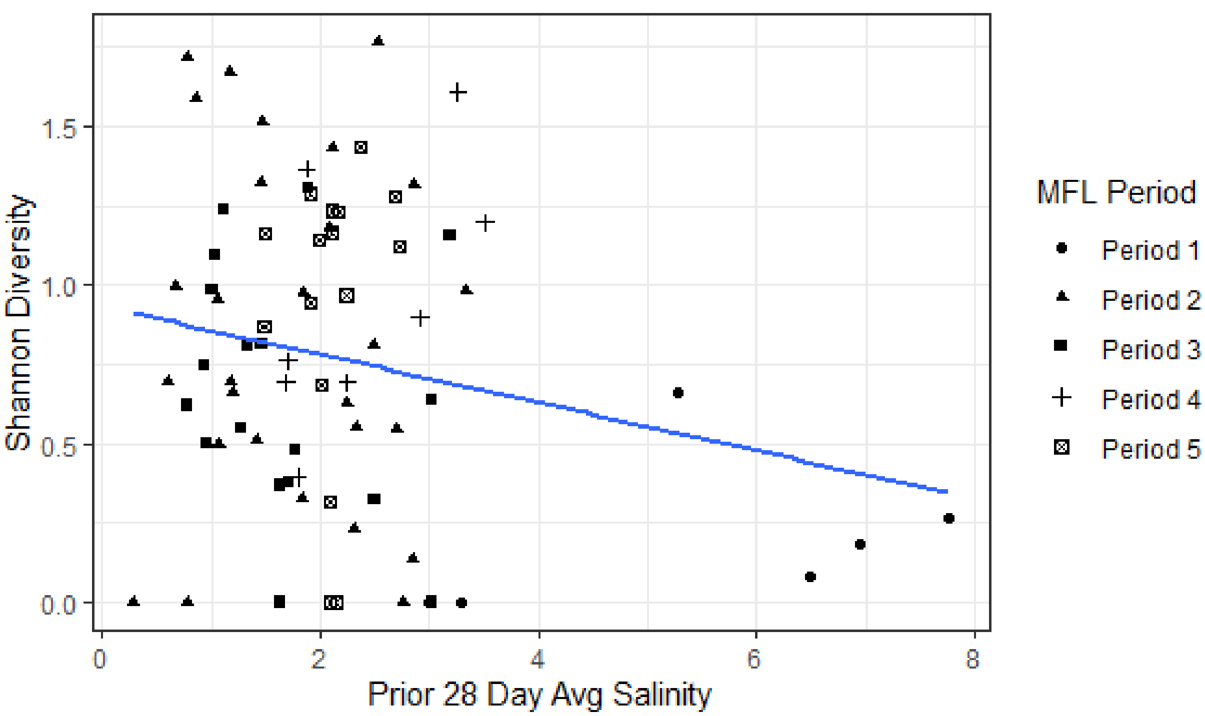


Figure 6.2-75: Salinity-sensitive nekton diversity by segment and period as predicted by log 28-day depth-averaged salinity (in ppt, as determined by the LAMFE model) for the upper segment



6.2.3.6 Nekton Conclusions

Cluster analysis and ordination were inconclusive for distinguishing taxonomic differences between the upper, middle, and lower segments.

Although nekton abundance and density were not useful indicators, salinity-sensitive nekton taxa richness and diversity demonstrated the efficacy of minimum flow implementation (Table 6.2-58, Table 6.2-59).

Table 6.2-58: Summary of salinity-sensitive nekton mixed model results

Interaction Model	Type	Interaction Effect?	Meaningful contrasts?
Abundance ~ Period * Segment + Date(R) + Site(R)	Quasi-Poisson	Yes	No
Density ~ Period * Segment + Date(R) + Site(R)	Gamma	Yes	No
Richness ~ Period * Segment + Date(R) + Site(R)	Poisson	Yes	Yes
Diversity ~ Period * Segment + Date(R) + Site(R)	Zero inflated Gaussian	No	Yes

Table 6.2-59: Summary of salinity-sensitive nekton predictive model results

Predictive Model	Type	Significant?	R^2
Abundance ~ Salinity + Date(R) + Site(R)	Poisson	No	0.00
Density ~ Salinity + Date(R) + Site(R)	Gamma	Yes	0.02
Richness ~ Salinity + Date(R) + Site(R)	Poisson	Yes	0.09
Diversity ~ Salinity + Date(R) + Site(R)	Zero inflated Gaussian, GAM	Yes	0.06

The richness of salinity-sensitive nekton taxa generally increased throughout time within the upper segment, with the highest richness of salinity-sensitive nekton taxa occurring during Period 5 and the lowest richness of salinity-sensitive taxa occurring during Period 1.

A generalized linear mixed model for salinity-sensitive nekton taxa using Poisson distribution for Period 1 and Period 5 data had a significant interaction effect ($p < 0.04$). Selected contrasts for salinity-sensitive nekton taxa richness indicated that the upper segment during Period 5 had statistically more salinity-sensitive nekton taxa than did the middle segment during the same period, as indicated by 95% confidence limits that did not overlap zero (Table 6.2-60). This indicates a statistically significant increase in nekton taxa that prefer salinity < 5 ppt associated with the minimum flow implementation. Note estimate values are on a log scale due to the standard transformation of ecological data with high variability.

A linear mixed model using the Poisson distribution for 28-day depth-averaged salinity (as determined via the LAMFE model) as a predictor for salinity-sensitive nekton taxa richness was statistically significant ($p < 0.01$); however, the R^2 was low (0.09), indicating approximately 9% of the variability in the data was captured by the model (Table 6.2-61). Low R^2 values are common in ecological data, due to high variability. Salinity-sensitive taxa richness tended to increase as salinity decreased, with the highest taxa richness concentrated at salinities < 5 ppt.

Table 6.2-60: Summary of salinity-sensitive nekton taxa richness contrasts

Contrast	Estimate	Conclusion
Period 5 Upper – Period 1 Upper	0.7	On the log scale, 0.7 more salinity-sensitive taxa in upper segment with minimum flow implementation compared to no implementation
Period 5 Upper – Period 5 Middle	0.4	On the log scale, 0.4 more salinity-sensitive taxa in upper segment with full minimum flow implementation compared to middle segment

River segment contrasts for salinity-sensitive nekton diversity indicated that the diversity of salinity-sensitive taxa was statistically higher within the upper segment during Period 5 as compared with middle and lower segments during the same period and as compared to the upper segment during Period 1 (Table 6.2-61). A GAM for 28-day depth-averaged salinity (determined via the LAMFE model) as a predictor for salinity-sensitive nekton diversity was significant ($p < 0.05$, $R^2 = 0.06$). Salinity-sensitive nekton diversity increased as salinity decreased, with higher diversity associated with salinities < 5 ppt.

Table 6.2-61: Summary of salinity-sensitive nekton diversity contrasts

	Estimate	Conclusion
Period 5 Upper – Period 1 Upper	0.51	Greater diversity of salinity-sensitive taxa in upper segment with full minimum flow implementation compared to no implementation
Period 5 Upper – Period 5 Middle	0.41	Greater diversity of salinity-sensitive taxa in upper segment compared to middle segment with full minimum flow implementation
Period 5 Upper – Period 5 Lower	0.34	Greater diversity of salinity-sensitive taxa in upper segment compared to the lower segment with full minimum flow implementation

6.2.4 Responses of Selected Species to Minimum Flow Implementation in the Target Zone Compared with Previous Studies

In their 2020 report, WAR identified taxa that appeared to demonstrate a response associated with minimum flow implementation. In the present study, these taxa were further evaluated for responses to minimum flow implementation based on all cumulative data collected through 2023 (Table 6.2-62). Quantification of the preferred salinity for taxa in Table 6.2-62 is from literature cited earlier. Since salinity tolerances differ, minimum flow implementation may have resulted in increases in some taxa, decreases in other taxa, or potentially no change in taxa adapted to a wide range of salinity conditions. The Kruskal-Wallis test compares independent groups to determine if there are significant differences in their distributions (Bakker 2024). Dunn's test is a post-hoc evaluation for significance based on the data's range distribution. Comparisons were made on a per segment basis. The Dunn's test used the Benjamini-Hochberg adjustment for multiple comparisons.

Visualization of the abundance distribution of a species is represented by boxplots, which show the mean, median, and interquartile range (25^{th} to 75^{th} percentile) with whiskers extending 1.5 times the interquartile range beyond the quartiles. Compact letter display is used to represent the results of the Dunn's post hoc test (*e.g*. "a" and "b" are significantly different from one another).

Table 6.2-62: Results of selected taxa analyzed for abundance changes by segment and period (red = taxa that appear to prefer higher salinities)

Groups	Taxon Identifier	Common Name	Cited Salinity (ppt)	Kruskal-Wallis Significant?	Pattern Consistent with Higher Salinity?	Notes
Zooplankton	*Clytia*	Jellyfish	10.9	No	Yes	Never found in upper segment
Zooplankton	*Chaetognatha*	Arrow worm	23.5	Yes	Yes	Decreased during Periods 3 and 4 in middle and lower, never found in upper
Zooplankton	*Palaemonetes pugio*	Daggerblade shrimp	4.4–16.8	No	Yes	Never found in upper segment in zooplankton samples
Zooplankton	Prosobranchia	Group of snails	6.9	No	No	No significant differences in middle and lower over time, found in upper during Period 5
Benthos	*Laeonereis culveri*	Polychaete	20–35	No	No	Not significantly different in middle and lower over time
Benthos	*Stenonereis martini*	Polychaete	30–35	No	No	No, not significantly different in upper, middle, or lower over time
Benthos	*Hydrobiidae*	Group of snails	Not quantified	No	No	Increased in the lower during Period 5 but not significantly different in the upper and middle over time
Benthos	*Melanoides tuberculata*	Introduced snail	Tends Fresh	No	No	Not significantly different in the upper, middle, or segments over time
Benthos	*Pyrgophorus platyrachis*	Introduced snail	Tends Fresh	No	No	Not significantly different in the upper, middle, or segments over time
Nekton	*Menidia beryllina*	Silverside	5–30	No	No	Not significantly different in the upper or middle over time, but increased in abundance in the lower during Period 5
Nekton	*Palaemonetes pugio*	Daggerblade shrimp	4.4–16.8	Yes	Yes	Not found in the upper during Periods 4 and 5 and not found in the middle during Period 5. Not significantly different in the lower over time
Nekton	*Eucinostomus harengulus*	Tidewater mojarra	16.8	No	No	Not significantly different in the upper and middle over time, increased in lower during Period 5
Nekton	*Brevoortia*	Menhaden	10–30	No	Yes	Not significantly different in the middle and lower over time, but not found in the upper during Periods 4 and 5

Groups	Taxon Identifier	Common Name	Cited Salinity (ppt)	Kruskal-Wallis Significant?	Pattern Consistent with Higher Salinity?	Notes
Nekton	*Anchoa mitchilli*	Anchovy	0.5–40	No	No	Not significantly different in the upper, middle, and lower over time
Nekton	*Gambusia holbrooki*	Mosquitofish	1–20	No	No	Not significantly different in the upper or lower over time, and while there was variability in the middle, Period 5 abundances were similar to those in Period 1
Nekton	*Lucania parva*	Rainwater killifish	10.6	No	No	Not significantly different in the upper, middle, or lower segments over time
Nekton	*Poecilia latipinna*	Sailfin molly	0–9.1	No	No	Not significantly different in the upper, middle, or lower segments over time
Nekton	*Cyprinodon variegatus*	Sheepshead minnow	26.5	No	Yes	Not found in the upper and middle during Periods 4 and 5, although it was fairly common prior to those periods
Nekton	*Mugil cephalus*	Mullet	3.9–30	No	No	A single specimen was found in the upper during Period 5, but *Mugil cephalus* was common with no significant changes over time in the middle and lower segments
Nekton	*Trinectes maculatus*	Hog choker	3.7–30	No	No	Not significantly different in the upper, middle, and lower over time
Nekton	*Microgobius gulosus*	Clown goby	7	No	Yes	Not significantly different in the middle and lower over time, but not found in the upper segment during Periods 3, 4, and 5
Nekton	*Fundulus seminolis*	Seminole killifish	17	No	No	Not found in any segment during Period 1, but was fairly common at the upper, middle, and lower thereafter, with no significant changes over time
Nekton	*Micropterus salmoides*	Largemouth bass	2	No	No	Not found in any segment during Period 1, absent from the middle and lower during Period 4, but fairly common at the upper, middle, and lower during other periods, with no significant changes over time

6.2.4.1 Zooplankton

Clytia is a hydrozoan–group cnidarian (jellyfish) with both a colonial, vegetatively propagating polyp stage and free-living, sexual medusae, which is a component of the zooplankton (MARIMBA 2024). *Clytia* feed on shrimp or other zooplankton with their stinging tentacles (Smithsonian 2023). The WAR (2020) study suggested that higher flows associated with the minimum flow resulted in the displacement of *Clytia* from the upper portion of the study area, improving habitat quality for other species. Analysis of cumulative data, including applying the Kruskal-Wallis test, showed that *Clytia* was never found in the upper segment and that *Clytia* abundance did not change over time in the middle and lower segments (Figure 6.2-76, Table 6.2-63). Note, sampling for zooplankton in the upper segment began in 2020.

Figure 6.2-76: Boxplots of abundance of *Clytia sp*. by period and segment with Dunn's test comparisons represented by compact letter display.

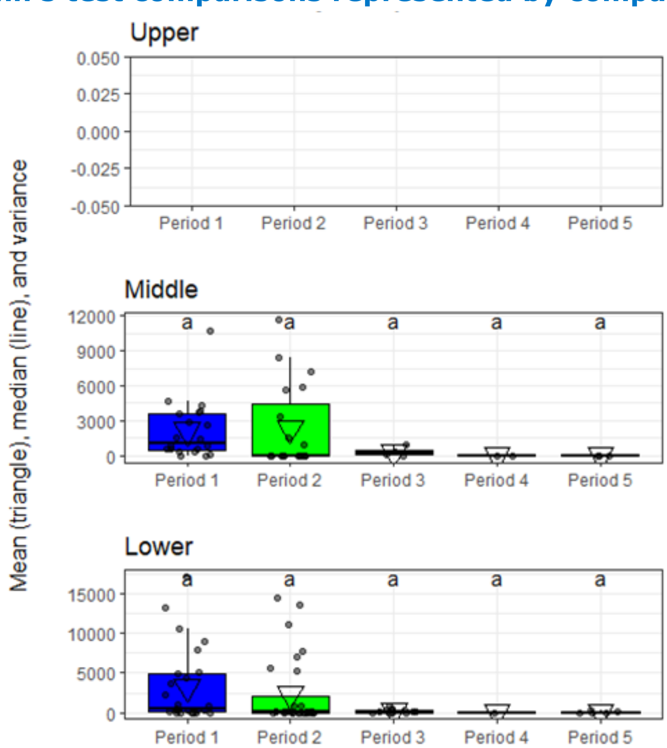


Table 6.2-63: Post-hoc Dunn's test for *Clytia* sp. Multiple comparison adjusted significance is designated by ns (not significant) or by an asterisk (significant at alpha = 0.05)

River Segment	group1	group2	n1	n2	statistic	p	P adj.	P adj. significance
Middle	Period 1	Period 2	20	19	-1.86	0.06	0.21	ns
Middle	Period 1	Period 3	20	4	-1.49	0.14	0.27	ns
Middle	Period 1	Period 4	20	2	-2.21	0.03	0.14	ns
Middle	Period 1	Period 5	20	3	-2.65	0.01	0.08	ns
Middle	Period 2	Period 3	19	4	-0.39	0.69	0.77	ns
Middle	Period 2	Period 4	19	2	-1.40	0.16	0.27	ns
Middle	Period 2	Period 5	19	3	-1.68	0.09	0.23	ns
Middle	Period 3	Period 4	4	2	-0.95	0.34	0.43	ns
Middle	Period 3	Period 5	4	3	-1.08	0.28	0.40	ns
Middle	Period 4	Period 5	2	3	-0.01	0.99	0.99	ns
Lower	Period 1	Period 2	25	28	-2.01	0.04	0.13	ns
Lower	Period 1	Period 3	25	12	-2.26	0.02	0.13	ns
Lower	Period 1	Period 4	25	1	-1.95	0.05	0.13	ns
Lower	Period 1	Period 5	25	5	-2.21	0.03	0.13	ns
Lower	Period 2	Period 3	28	12	-0.70	0.49	0.54	ns
Lower	Period 2	Period 4	28	1	-1.41	0.16	0.32	ns
Lower	Period 2	Period 5	28	5	-1.09	0.28	0.39	ns
Lower	Period 3	Period 4	12	1	-1.14	0.25	0.39	ns
Lower	Period 3	Period 5	12	5	-0.54	0.59	0.59	ns
Lower	Period 4	Period 5	1	5	0.82	0.41	0.51	ns

Chaetognaths are transparent and shaped like a torpedo; commonly called "Arrow Worms" (University of Washington 2024). These worms are predators of copepods, larval fish, crustaceans, and other Chaetognaths (University of Washington 2024). Besides being active predators themselves, Chaetognaths are an important food source for fish and other marine animals. The WAR (2020) study surmised that the reduction in Chaetognath densities after implementation of the minimum flow was likely related to improved freshwater inflow. Analysis of cumulative data, including applying the Kruskal-Wallis test, showed that Chaetognaths were never found in the upper segment and that Chaetognath abundance was reduced during Periods 3, 4, and 5 in the middle and lower segments (Figure 6.2-77, Table 6.2-64). When Chaetognaths were present, the differences in abundance were not significant; however, the absence of Chaetognaths in the upper segment and in the middle and lower segments after minimum flow implementation suggests this taxon prefers higher salinity.

Figure 6.2-77: Boxplots of abundance of Chaetognaths by period and segment with Dunn's test comparisons represented by compact letter display

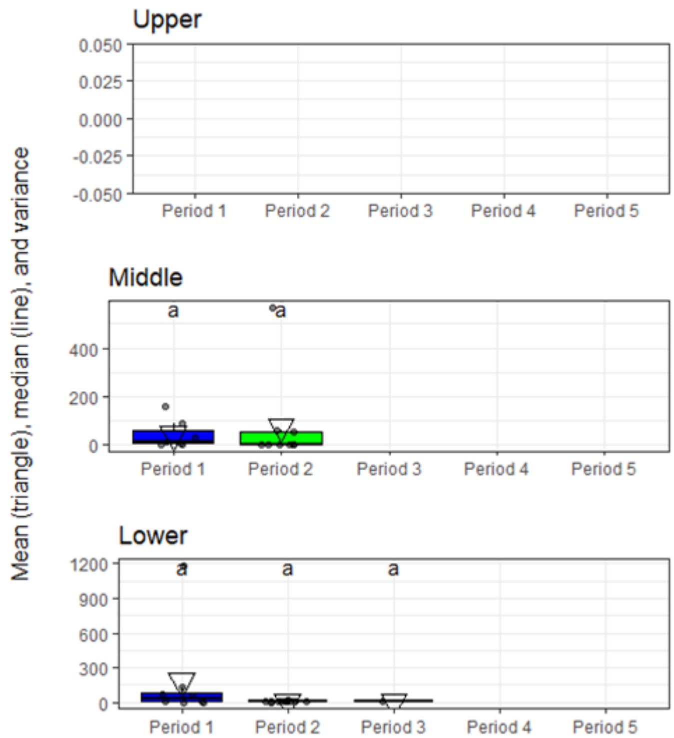


Table 6.2-64: Post-hoc Dunn's test for Chaetognaths. Multiple comparison adjusted significance is designated by ns (not significant) or by an asterisk (significant at alpha = 0.05).

River Segment	group1	group2	n1	n2	statistic	p	P adj.	P adj. significance
Middle	Period 1	Period 2	7	9	-1.27	0.20	0.20	ns
Lower	Period 1	Period 2	8	12	-1.03	0.31	0.55	ns
Lower	Period 1	Period 3	8	1	-0.90	0.37	0.55	ns
Lower	Period 2	Period 3	12	1	-0.47	0.64	0.64	ns

Palaemonetes pugio, commonly known as Daggerblade Shrimp, normally inhabits areas where freshwater and saltwater combine, feeding on oligochaetes, polychaetes, and harpacticoid copepods. They in turn are consumed by many valuable commercial and sport fishes (Animal Diversity Web 2024). The WAR (2020) study suggested that elevated flows associated with minimum flow implementation shifted the *P. pugio* habitat downstream toward the lower tidal river. Analysis of cumulative data, including applying the Kruskal-Wallis test, showed that *P. pugio* was never found in the upper segment and that *P. pugio* abundance was significantly reduced in the middle and lower segments between Periods 1 and 2. *P. pugio* was absent from the middle segment during Period 4 and from the lower segment during Period 5 (Figure 6.2-78, Table 6.2-65). The abundance pattern does not clearly coincide with minimum flow implementation.

Figure 6.2-78: Boxplots of abundance of *Palaemonetes pugio* adults by period and segment with Dunn's test comparisons represented by compact letter display

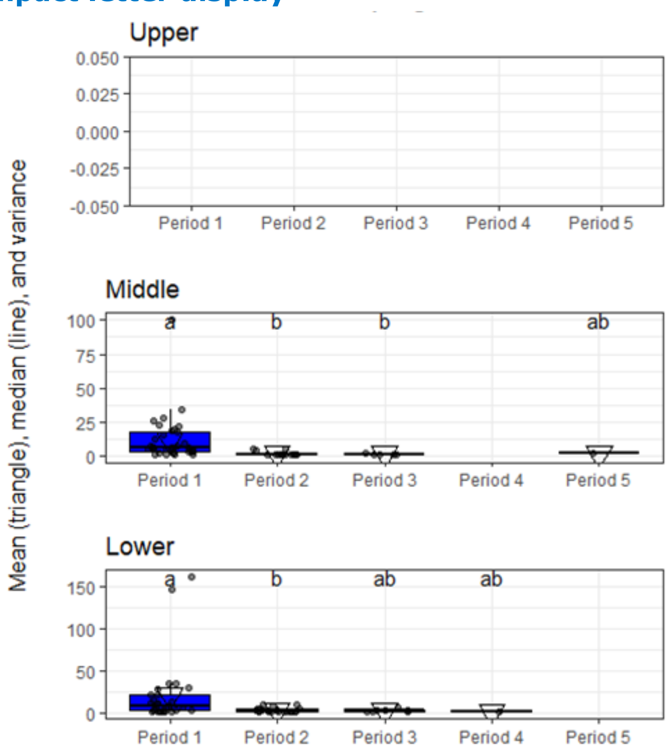


Table 6.2-65: Post-hoc Dunn's test for *Palaemonetes pugio.* Multiple comparison adjusted significance is designated by ns (not significant) or by an asterisk (significant at alpha = 0.05)

River Segment	group1	group2	n1	n2	statistic	p	P adj.	P adj. significance
Middle	Period 1	Period 2	33	13	-4.01	<0.01	<0.01	*
Middle	Period 1	Period 3	33	5	-3.08	<0.01	0.01	*
Middle	Period 1	Period 5	33	1	-0.85	0.40	0.75	ns
Middle	Period 2	Period 3	13	5	-0.32	0.75	0.75	ns
Middle	Period 2	Period 5	13	1	0.44	0.66	0.75	ns
Middle	Period 3	Period 5	5	1	0.57	0.57	0.75	ns
Lower	Period 1	Period 2	27	21	-3.08	<0.01	0.01	*
Lower	Period 1	Period 3	27	7	-2.08	0.04	0.11	ns
Lower	Period 1	Period 4	27	1	-1.64	0.10	0.20	ns
Lower	Period 2	Period 3	21	7	0.03	0.98	0.98	ns
Lower	Period 2	Period 4	21	1	-0.75	0.45	0.56	ns
Lower	Period 3	Period 4	7	1	-0.73	0.46	0.56	ns

Prosobranch gastropods, commonly known as Gilled Snails, are characterized by strong torsion (shell twisting) and inhabit both marine and fresh water habitats (British Geological Society 2024). In WAR (2020) it was postulated that because some of the water used for minimum flow implementation originates from Sulphur Springs, which has low color and higher transparency than river water, the minimum flow resulted in an improved light environment in the upper river, favoring taxa with a scraping trophic strategy. Analysis of cumulative data, including applying the Kruskal-Wallis test, showed that prosobranch gastropods were found in the upper segment only during Period 5 and that prosobranch gastropod abundance was not significantly different in middle and lower segments over time, suggesting a wide salinity tolerance (Figure 6.2-79, Table 6.2-66).

Figure 6.2-79: Boxplots of abundance of prosobranch gastropods by period and segment with Dunn's test comparisons represented by compact letter display

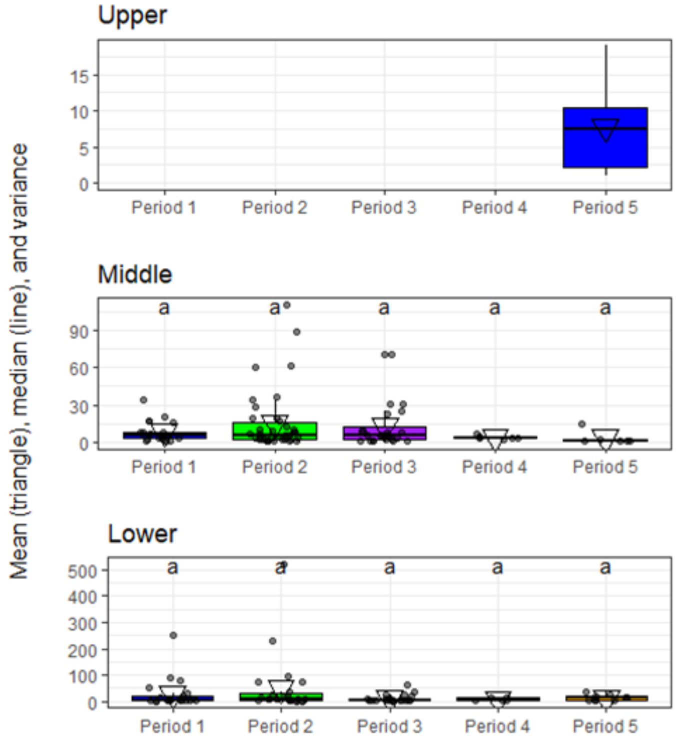


Table 6.2-66: Post-hoc Dunn's test for prosobranch gastropods. Multiple comparison adjusted significance is designated by ns (not significant) or by an asterisk (significant at alpha = 0.05)

River Segment	group1	group2	n1	n2	statistic	p	P adj.	P adj. significance
Middle	Period 1	Period 2	21	38	0.17	0.86	0.97	ns
Middle	Period 1	Period 3	21	24	0.01	0.99	0.99	ns
Middle	Period 1	Period 4	21	6	-0.56	0.57	0.82	ns
Middle	Period 1	Period 5	21	6	-2.05	0.04	0.14	ns
Middle	Period 2	Period 3	38	24	-0.16	0.87	0.97	ns
Middle	Period 2	Period 4	38	6	-0.70	0.48	0.82	ns
Middle	Period 2	Period 5	38	6	-2.26	0.02	0.14	ns
Middle	Period 3	Period 4	24	6	-0.58	0.56	0.82	ns
Middle	Period 3	Period 5	24	6	-2.09	0.04	0.14	ns
Middle	Period 4	Period 5	6	6	-1.19	0.23	0.58	ns
Lower	Period 1	Period 2	21	23	0.93	0.35	0.59	ns
Lower	Period 1	Period 3	21	20	-1.04	0.30	0.59	ns
Lower	Period 1	Period 4	21	5	-0.81	0.42	0.59	ns
Lower	Period 1	Period 5	21	9	0.35	0.73	0.81	ns
Lower	Period 2	Period 3	23	20	-1.99	0.05	0.47	ns
Lower	Period 2	Period 4	23	5	-1.39	0.16	0.59	ns
Lower	Period 2	Period 5	23	9	-0.36	0.72	0.81	ns
Lower	Period 3	Period 4	20	5	-0.16	0.88	0.88	ns
Lower	Period 3	Period 5	20	9	1.16	0.25	0.59	ns
Lower	Period 4	Period 5	5	9	0.98	0.33	0.59	ns

6.2.4.2 Benthic Macroinvertebrates

Laeonereis culveri is a large (70 mm long), carnivorous polychaete worm that lives on the Atlantic and Gulf coasts. It is a euryhaline species that occupies intertidal to shallow subtidal areas in substrates with at least some sand (Heard 1979). Analysis of cumulative data, including applying the Kruskal-Wallis test, showed that *L. culveri* abundance was not significantly different in the upper, middle, and lower segments over time (Figure 6.2-80, Table 6.2-67).

Figure 6.2-80: Boxplots of abundance of *Laeonereis culveri* by period and segment with Dunn's test comparisons represented by compact letter display

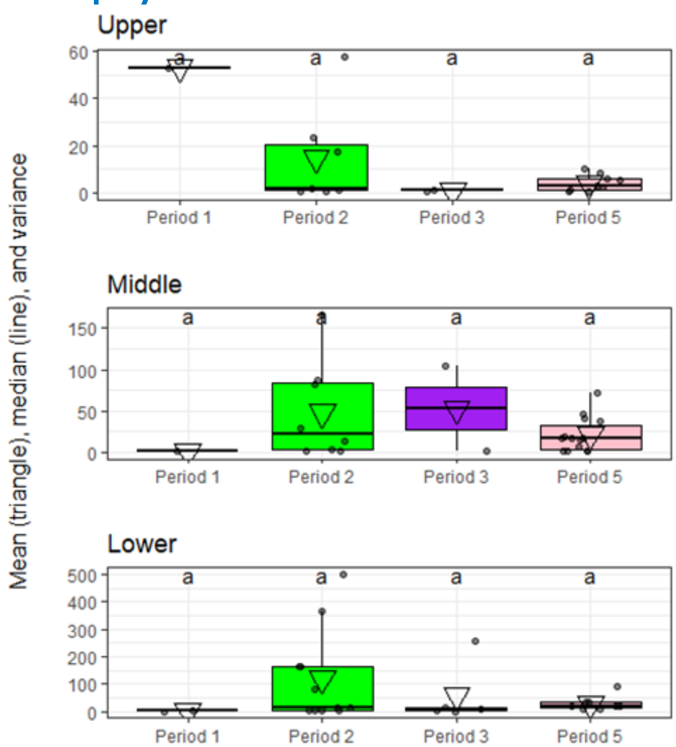


Table 6.2-67: Post-hoc Dunn's test for *Laeonereis culveri*. Multiple comparison adjusted significance is designated by ns (not significant) or by an asterisk (significant at alpha = 0.05).

River Segment	group1	group2	n1	n2	statistic	p	P adj.	P adj. significance
Middle	Period 1	Period 2	1	8	1.45	0.15	0.50	ns
Middle	Period 1	Period 3	1	2	1.15	0.25	0.50	ns
Middle	Period 1	Period 5	1	14	1.20	0.23	0.50	ns
Middle	Period 2	Period 3	8	2	-0.17	0.86	0.86	ns
Middle	Period 2	Period 5	8	14	-0.67	0.50	0.75	ns
Middle	Period 3	Period 5	2	14	-0.21	0.83	0.86	ns
Lower	Period 1	Period 2	2	11	2.13	0.03	0.10	ns
Lower	Period 1	Period 3	2	5	1.36	0.17	0.35	ns
Lower	Period 1	Period 5	2	8	2.19	0.03	0.10	ns
Lower	Period 2	Period 3	11	5	-0.91	0.36	0.43	ns
Lower	Period 2	Period 5	11	8	0.21	0.84	0.84	ns
Lower	Period 3	Period 5	5	8	1.03	0.30	0.43	ns

Stenoninereis martini is a small polychaete worm, that ranges from North Carolina to the Gulf of Mexico, where it inhabits brackish waters in salt marsh ponds, tidal creeks, and rivers, generally on silt and mud bottoms (Heard 1979). Analysis of cumulative data, including applying the Kruskal-Wallis test, showed that *S. martini* abundance was not significantly different in the upper, middle, or lower segments over time (Figure 6.2-81, Table 6.2-68).

Figure 6.2-81: Boxplots of abundance of *Stenoninereis martini* by period and segment with Dunn's test comparisons represented by compact letter display

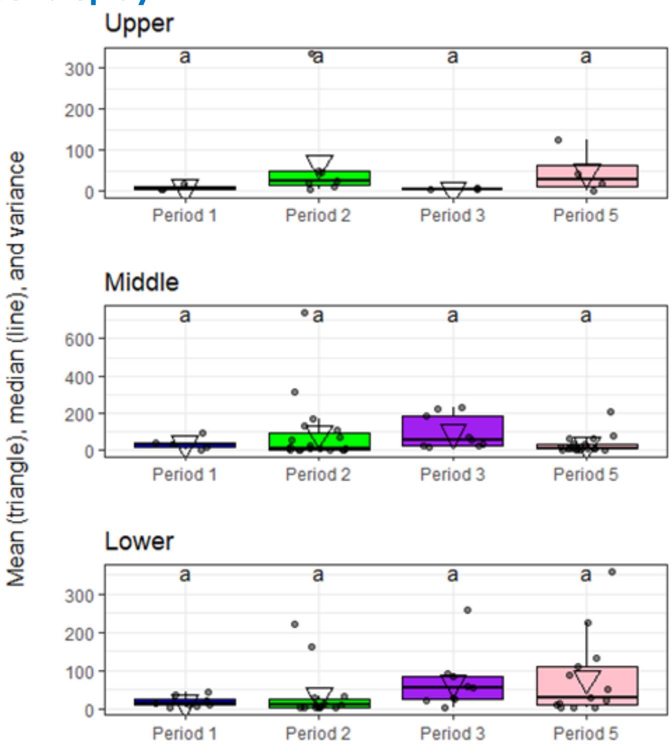


Table 6.2-68: Post-hoc Dunn's test for *Stenoninereis martini*. Multiple comparison adjusted significance is designated by ns (not significant) or by an asterisk (significant at alpha = 0.05)

River Segment	group1	group2	n1	n2	statistic	p	P adj.	P adj. significance
Middle	Period 1	Period 2	5	19	-0.37	0.71	0.71	ns
Middle	Period 1	Period 3	5	9	1.08	0.28	0.56	ns
Middle	Period 1	Period 5	5	19	-0.77	0.44	0.64	ns
Middle	Period 2	Period 3	19	9	1.95	0.05	0.15	ns
Middle	Period 2	Period 5	19	19	-0.63	0.53	0.64	ns
Middle	Period 3	Period 5	9	19	-2.45	0.01	0.09	ns
Lower	Period 1	Period 2	8	14	-0.49	0.63	0.63	ns
Lower	Period 1	Period 3	8	9	1.69	0.09	0.18	ns
Lower	Period 1	Period 5	8	13	1.27	0.20	0.31	ns
Lower	Period 2	Period 3	14	9	2.43	0.02	0.09	ns
Lower	Period 2	Period 5	14	13	2.04	0.04	0.12	ns
Lower	Period 3	Period 5	9	13	-0.58	0.56	0.63	ns

Hydrobiidae is a diverse family of gastropods (snails). Most hydrobiid species in this family live in freshwater (lakes, ponds, rivers, streams), but some are found in brackish water or at the borders between freshwater and brackish water (Heard 1979). Analysis of cumulative data, including applying the Kruskal-Wallis test, showed that Hydrobiidae abundance was not significantly different in the upper and middle segments over time, but Hydrobiidae abundance increased in the lower segment during Period 5 when compared to Period 2 (Figure 6.2-82, Table 6.2-69). This appeared to be independent of MFL implementation.

Figure 6.2-82: Boxplots of abundance of Hydrobiidae by period and segment with Dunn's test comparisons represented by compact letter display

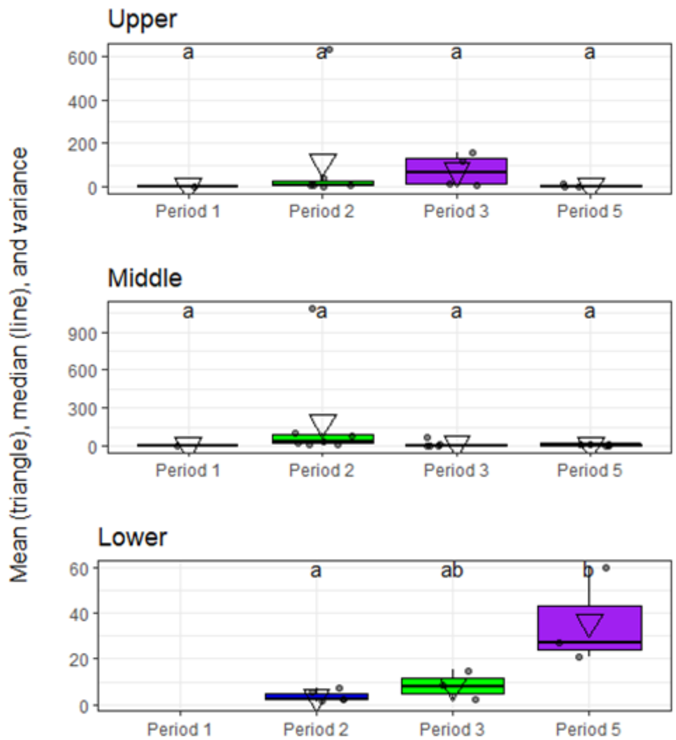


Table 6.2-69: Post-hoc Dunn's test for *Hydrobiidae*. Multiple comparison adjusted significance is designated by ns (not significant) or by an asterisk (significant at alpha = 0.05)

River Segment	group1	group2	n1	n2	statistic	p	P adj.	P adj. significance
Middle	Period 1	Period 2	1	7	2.16	0.03	0.08	ns
Middle	Period 1	Period 3	1	5	0.99	0.32	0.38	ns
Middle	Period 1	Period 5	1	5	0.99	0.32	0.38	ns
Middle	Period 2	Period 3	7	5	-2.08	0.04	0.08	ns
Middle	Period 2	Period 5	7	5	-2.08	0.04	0.08	ns
Middle	Period 3	Period 5	5	5	0.00	1.00	1.00	ns
Lower	Period 2	Period 3	5	3	1.00	0.32	0.32	ns
Lower	Period 2	Period 5	5	3	2.67	0.01	0.02	*
Lower	Period 3	Period 5	3	3	1.49	0.14	0.20	ns

Melanoides tuberculata (Red-Rim Melania) and *Pyrgophorus platyrachis* (Crown Snail) are invasive freshwater snails capable of living in estuaries. They graze on microalgae and detritus and in turn are eaten by crabs, fishes, and birds (Smithsonian 2023). From visual inspection, WAR 2020 suggested that *M. tuberculata* and *P. platyrachis* increased in abundance during Period 2 and then dropped in abundance during subsequent periods. Analysis of cumulative data, including applying the Kruskal-Wallis test, showed that *M. tuberculata* abundance was not significantly different in the upper, middle, or lower segments over time (Figure 6.2-83, Table 6.2-70). Although *P. platyrachis* abundance was different during Period 2 compared with Period 5, Period 5 abundance was not significantly different from Period 1 (Figure 6.2-84, Table 6.2-71).

Figure 6.2-83: Boxplots of abundance of *Melanoides tuberculata* by period and segment with Dunn's test comparisons represented by compact letter display

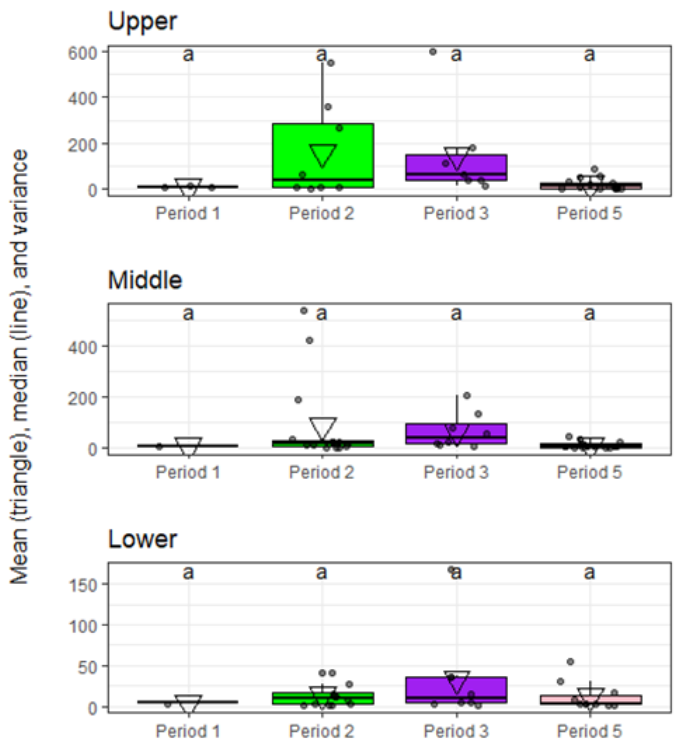


Table 6.2-70: Post-hoc Dunn's test for *Melanoides tuberculata*. Multiple comparison adjusted significance is designated by ns (not significant) or by an asterisk (significant at alpha = 0.05).

River Segment	group1	group2	n1	n2	statistic	p	P adj.	P adj. significance
Middle	Period 1	Period 2	1	15	0.50	0.62	0.74	ns
Middle	Period 1	Period 3	1	8	0.94	0.35	0.52	ns
Middle	Period 1	Period 5	1	15	-0.13	0.90	0.90	ns
Middle	Period 2	Period 3	15	8	1.10	0.27	0.52	ns
Middle	Period 2	Period 5	15	15	-1.76	0.08	0.24	ns
Middle	Period 3	Period 5	8	15	-2.57	0.01	0.06	ns
Lower	Period 1	Period 2	1	12	0.05	0.96	0.96	ns
Lower	Period 1	Period 3	1	8	0.43	0.67	0.96	ns
Lower	Period 1	Period 5	1	10	-0.08	0.93	0.96	ns
Lower	Period 2	Period 3	12	8	0.88	0.38	0.96	ns
Lower	Period 2	Period 5	12	10	-0.34	0.74	0.96	ns
Lower	Period 3	Period 5	8	10	-1.15	0.25	0.96	ns

Figure 6.2-84: Boxplots of abundance of *Pyrgophorus platyrachis* by period and segment with Dunn's test comparisons represented by compact letter display

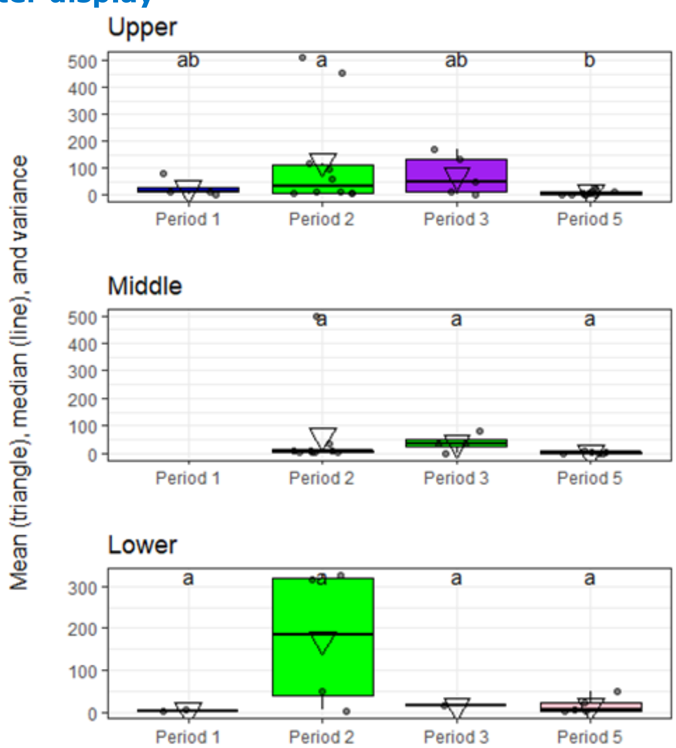


Table 6.2-71: Post-hoc Dunn's test for *Pyrgophorus platyrachis.* Multiple comparison adjusted significance is designated by ns (not significant) or by an asterisk (significant at alpha = 0.05).

River Segment	group1	group2	n1	n2	statistic	p	P adj.	P adj. significance
Middle	Period 2	Period 3	9	4	0.67	0.50	0.50	ns
Middle	Period 2	Period 5	9	6	-1.71	0.09	0.13	ns
Middle	Period 3	Period 5	4	6	-2.02	0.04	0.13	ns
Lower	Period 1	Period 2	2	4	1.81	0.07	0.25	ns
Lower	Period 1	Period 3	2	1	0.74	0.46	0.65	ns
Lower	Period 1	Period 5	2	5	0.48	0.63	0.65	ns
Lower	Period 2	Period 3	4	1	-0.59	0.55	0.65	ns
Lower	Period 2	Period 5	4	5	-1.73	0.08	0.25	ns
Lower	Period 3	Period 5	1	5	-0.46	0.65	0.65	ns

6.2.4.3 Nekton

Menidia beryllina (Inland Silverside Minnow) is an estuarine species that feeds on zooplankton, invertebrates, fish eggs and larvae and diatoms (Froese and Pauly 2023). They are an important food source for fish and other marine animals. Analysis of cumulative data, including applying the Kruskal-Wallis test, showed that *M. beryllina* (grouped with *Menidia* sp.) abundance was not significantly different in the upper segment over time, and that differences in the middle and lower segments appeared unrelated to MFL implementation (Figure 6.2-85, Table 6.2-72.)

Figure 6.2-85: Boxplots of abundance of *Menidia beryllina* by period and segment with Dunn's test comparisons represented by compact letter display

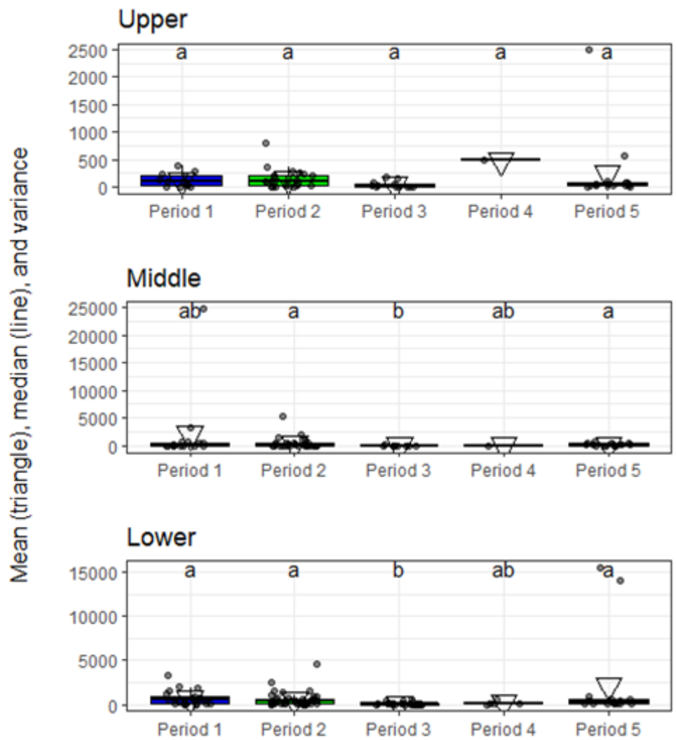


Table 6.2-72: Post-hoc Dunn's test for *Menidia beryllina.* Multiple comparison adjusted significance is designated by ns (not significant) or by an asterisk (significant at alpha = 0.05)

River Segment	group1	group2	n1	n2	statistic	p	P adj.	P adj. significance
Upper	Period 1	Period 2	12	23	0.14	0.89	0.89	ns
Upper	Period 1	Period 3	12	12	-1.86	0.06	0.21	ns
Upper	Period 1	Period 4	12	1	1.31	0.19	0.28	ns
Upper	Period 1	Period 5	12	15	-0.61	0.54	0.60	ns
Upper	Period 2	Period 3	23	12	-2.27	0.02	0.21	ns
Upper	Period 2	Period 4	23	1	1.29	0.2	0.28	ns
Upper	Period 2	Period 5	23	15	-0.87	0.39	0.48	ns
Upper	Period 3	Period 4	12	1	2.04	0.04	0.21	ns
Upper	Period 3	Period 5	12	15	1.35	0.18	0.28	ns
Upper	Period 4	Period 5	1	15	-1.55	0.12	0.28	ns
Middle	Period 1	Period 2	15	29	0.24	0.81	0.81	ns
Middle	Period 1	Period 3	15	9	-2.36	0.02	0.06	ns
Middle	Period 1	Period 4	15	1	-1.64	0.1	0.17	ns
Middle	Period 1	Period 5	15	14	0.66	0.51	0.64	ns
Middle	Period 2	Period 3	29	9	-2.81	0.01	0.03	*
Middle	Period 2	Period 4	29	1	-1.74	0.08	0.16	ns
Middle	Period 2	Period 5	29	14	0.52	0.6	0.67	ns
Middle	Period 3	Period 4	9	1	-0.66	0.51	0.64	ns
Middle	Period 3	Period 5	9	14	2.91	<0.01	0.03	*
Middle	Period 4	Period 5	1	14	1.87	0.06	0.15	ns
Lower	Period 1	Period 2	22	41	-0.72	0.47	0.67	ns
Lower	Period 1	Period 3	22	24	-4.05	<0.01	<0.01	*
Lower	Period 1	Period 4	22	5	-1.9	0.06	0.14	ns
Lower	Period 1	Period 5	22	16	-0.62	0.53	0.67	ns
Lower	Period 2	Period 3	41	24	-3.91	<0.01	<0.01	*
Lower	Period 2	Period 4	41	5	-1.59	0.11	0.23	ns
Lower	Period 2	Period 5	41	16	-0.05	0.96	0.96	ns
Lower	Period 3	Period 4	24	5	0.52	0.61	0.67	ns
Lower	Period 3	Period 5	24	16	3.07	<0.01	0.01	*
Lower	Period 4	Period 5	5	16	1.44	0.15	0.25	ns

Palaemonetes pugio (Daggerblade Shrimp) is also a component of the nekton. The WAR (2020) report suggested that elevated flows associated with minimum flow implementation shifted the *P. pugio* habitat downstream towards the lower tidal river, causing densities in the upper river to decline. Analysis of cumulative data, including applying the Kruskal-Wallis test, showed that *P. pugio* abundance was reduced in the upper segment during Periods 2 and 3 compared to Period 1, and was absent in the upper segment during Periods 4 and 5. *Palaemonetes pugio* was not found in the middle segment during Period 5 and *P. pugio* abundance was not significantly different in the lower segment over time (Figure 6.2-86, Table 6.2-73).

Figure 6.2-86: Boxplots of abundance of *Palaemonetes pugio* by period and segment with Dunn's test comparisons represented by compact letter display

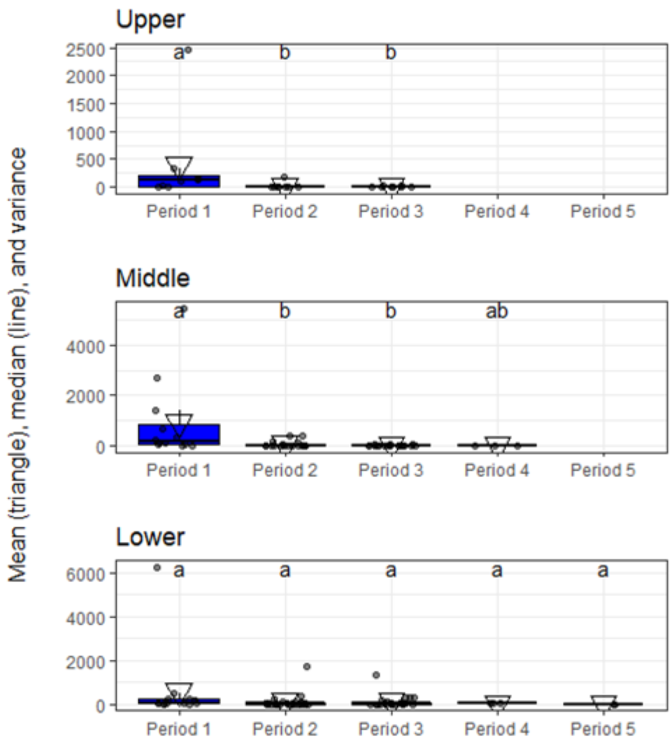


Table 6.2-73: Post-hoc Dunn's test for *Palaemonetes pugio*. Multiple comparison adjusted significance is designated by ns (not significant) or by an asterisk (significant at alpha = 0.05)

River Segment	group1	group2	n1	n2	statistic	p	P adj.	P adj. significance
Upper	Period 1	Period 2	8	8	-2.67	0.01	0.02	*
Upper	Period 1	Period 3	8	8	-2.51	0.01	0.02	*
Upper	Period 2	Period 3	8	8	0.16	0.87	0.87	ns
Middle	Period 1	Period 2	12	19	-2.70	0.01	0.02	*
Middle	Period 1	Period 3	12	18	-3.22	<0.01	0.01	*
Middle	Period 1	Period 4	12	3	-2.47	0.01	0.03	*
Middle	Period 2	Period 3	19	18	-0.62	0.53	0.53	ns
Middle	Period 2	Period 4	19	3	-0.97	0.33	0.50	ns
Middle	Period 3	Period 4	18	3	-0.64	0.52	0.53	ns
Lower	Period 1	Period 2	13	25	-2.10	0.04	0.12	ns
Lower	Period 1	Period 3	13	23	-1.62	0.10	0.17	ns
Lower	Period 1	Period 4	13	3	-0.34	0.73	0.73	ns
Lower	Period 1	Period 5	13	2	-2.67	0.01	0.08	ns
Lower	Period 2	Period 3	25	23	0.54	0.59	0.66	ns
Lower	Period 2	Period 4	25	3	0.82	0.41	0.59	ns
Lower	Period 2	Period 5	25	2	-1.78	0.07	0.15	ns
Lower	Period 3	Period 4	23	3	0.56	0.57	0.66	ns
Lower	Period 3	Period 5	23	2	-1.98	0.05	0.12	ns
Lower	Period 4	Period 5	3	2	-1.98	0.05	0.12	ns

Eucinostomus harengulus (Tidewater Mojarra) live primarily in estuaries on vegetated bottoms, mangroves, and sand and mud bottoms, and will penetrate into freshwater (Smithsonian 2023). The WAR (2020) study found that the mean densities of *Eucinostomus* (Mojarra) individuals increased over time. Analysis of cumulative data, including applying the Kruskal-Wallis test, showed that *E. harengulus* abundance was not significantly different in the upper and middle segments over time. It increased in abundance at the lower segment during Period 5 compared to Periods 1, 2, and 3, but median *E. harengulus* abundance in Period 5 was not significantly different from Period 4 (Figure 6.2-87, Table 6.2-74).

Figure 6.2-87: Boxplots of median abundance of *Eucinostomus harengulus* by period and segment with Dunn's test comparisons represented by compact letter display

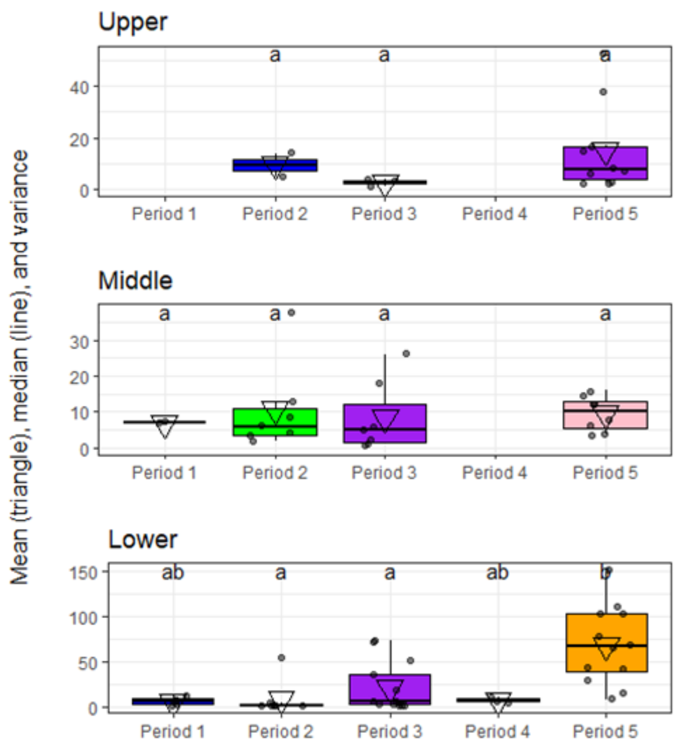


Table 6.2-74: Post-hoc Dunn's test for *Eucinostomus harengulus*. Multiple comparison adjusted significance is designated by ns (not significant) or by an asterisk (significant at alpha = 0.05)

River Segment	group1	group2	n1	n2	statistic	p	P adj.	P adj. significance
Upper	Period 2	Period 3	2	3	-1.27	0.20	0.31	ns
Upper	Period 2	Period 5	2	10	0.01	0.99	0.99	ns
Upper	Period 3	Period 5	3	10	1.78	0.08	0.23	ns
Middle	Period 1	Period 2	2	7	-0.23	0.82	0.86	ns
Middle	Period 1	Period 3	2	7	-0.58	0.56	0.86	ns
Middle	Period 1	Period 5	2	8	0.18	0.86	0.86	ns
Middle	Period 2	Period 3	7	7	-0.53	0.60	0.86	ns
Middle	Period 2	Period 5	7	8	0.63	0.53	0.86	ns
Middle	Period 3	Period 5	7	8	1.17	0.24	0.86	ns
Lower	Period 1	Period 2	4	7	-0.65	0.52	0.69	ns
Lower	Period 1	Period 3	4	13	0.59	0.55	0.69	ns
Lower	Period 1	Period 4	4	3	0.39	0.70	0.77	ns
Lower	Period 1	Period 5	4	12	2.56	0.01	0.03	*
Lower	Period 2	Period 3	7	13	1.59	0.11	0.22	ns
Lower	Period 2	Period 4	7	3	1.02	0.31	0.51	ns
Lower	Period 2	Period 5	7	12	3.97	<0.01	<0.01	*
Lower	Period 3	Period 4	13	3	-0.06	0.95	0.95	ns
Lower	Period 3	Period 5	13	12	2.85	<0.01	0.02	*
Lower	Period 4	Period 5	3	12	1.83	0.07	0.17	ns

Brevoortia sp. (Menhaden) is a commercially valuable filter-feeding fish that is common in brackish and marine waters. Their predators consist of such aquatic animals as sharks, rays, and bony fish (Animal Diversity Web 2024). *Brevoortia* sp. were the third-most abundant taxa in Period 1 but became less common in subsequent periods (WAR 2020). Analysis of cumulative data, including applying the Kruskal-Wallis test, showed that *Brevoortia* sp. abundance was not significantly different within the upper, middle, and lower segments over time, but the upper segment abundances were reduced compared to the middle and lower segments. *Brevoortia* sp. was not collected in the upper segment during Periods 2, 4, and 5 but was found in the middle and lower segments during Period 5, suggesting an affinity for higher salinity habitat (Figure 6.2-88, Table 6.2-75).

Figure 6.2-88: Boxplots of abundance of *Brevoortia sp*. by period and segment with Dunn's test comparisons represented by compact letter display

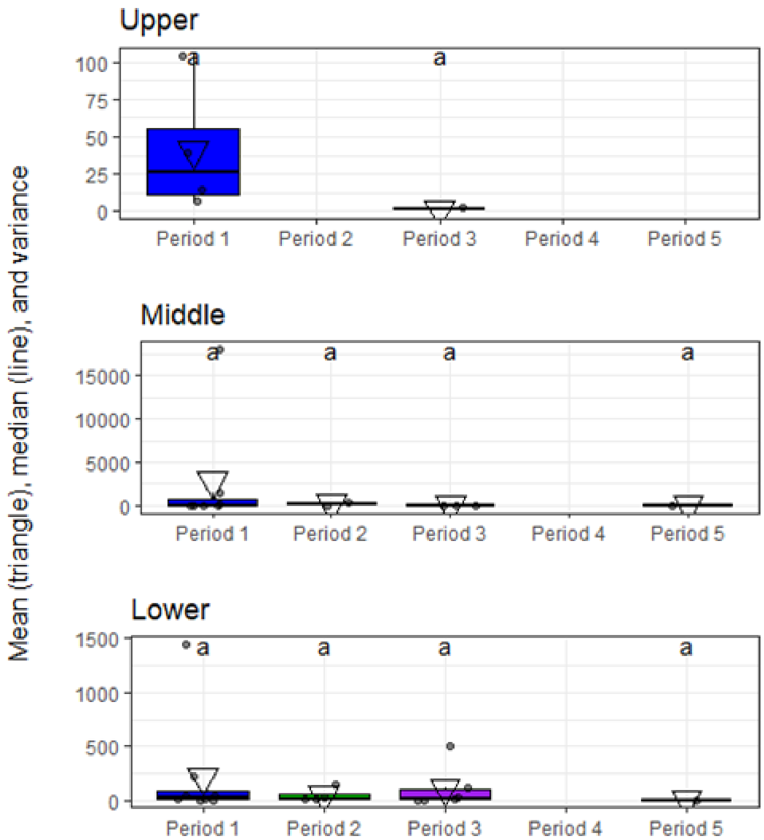


Table 6.2-75: Post-hoc Dunn's test for *Brevoortia sp*. Multiple comparison adjusted significance is designated by ns (not significant) or by an asterisk (significant at alpha = 0.05).

River Segment	group1	group2	n1	n2	statistic	p	P adj.	P adj. significance
Upper	Period 1	Period 3	4	1	-1.41	0.16	0.16	ns
Middle	Period 1	Period 2	7	2	-0.71	0.48	0.60	ns
Middle	Period 1	Period 3	7	3	-1.39	0.16	0.49	ns
Middle	Period 1	Period 5	7	1	-1.62	0.10	0.49	ns
Middle	Period 2	Period 3	2	3	-0.42	0.67	0.67	ns
Middle	Period 2	Period 5	2	1	-0.95	0.34	0.60	ns
Middle	Period 3	Period 5	3	1	-0.67	0.50	0.60	ns
Lower	Period 1	Period 2	8	4	0.00	1.00	1.00	ns
Lower	Period 1	Period 3	8	6	-0.21	0.84	1.00	ns
Lower	Period 1	Period 5	8	1	-1.37	0.17	0.43	ns
Lower	Period 2	Period 3	4	6	-0.17	0.86	1.00	ns
Lower	Period 2	Period 5	4	1	-1.30	0.19	0.43	ns
Lower	Period 3	Period 5	6	1	-1.24	0.21	0.43	ns

Anchoa mitchilli (Bay Anchovy) is a commercially useful, primarily pelagic zooplanktivorous species, common in protected waters and tide pools. *Anchoa mitchilli* represent a critical component of marine and estuarine food webs, both as a predator and a prey species (FDEP 2013c). The WAR (2020) study found high abundances of *A. mitchilli* in minimum flow Periods 1, 2, and 3. Analysis of cumulative data, including applying the Kruskal-Wallis test, showed that *A. mitchilli* abundance was not significantly different in the upper, middle, or lower segments over time, although overall *A. mitchilli* abundance was decreased in the upper segment over time, compared with the middle and lower segments (Figure 6.2-89, Table 6.2-76).

Figure 6.2-89: Boxplots of abundance of *Anchoa mitchilli* by period and segment with Dunn's test comparisons represented by compact letter display

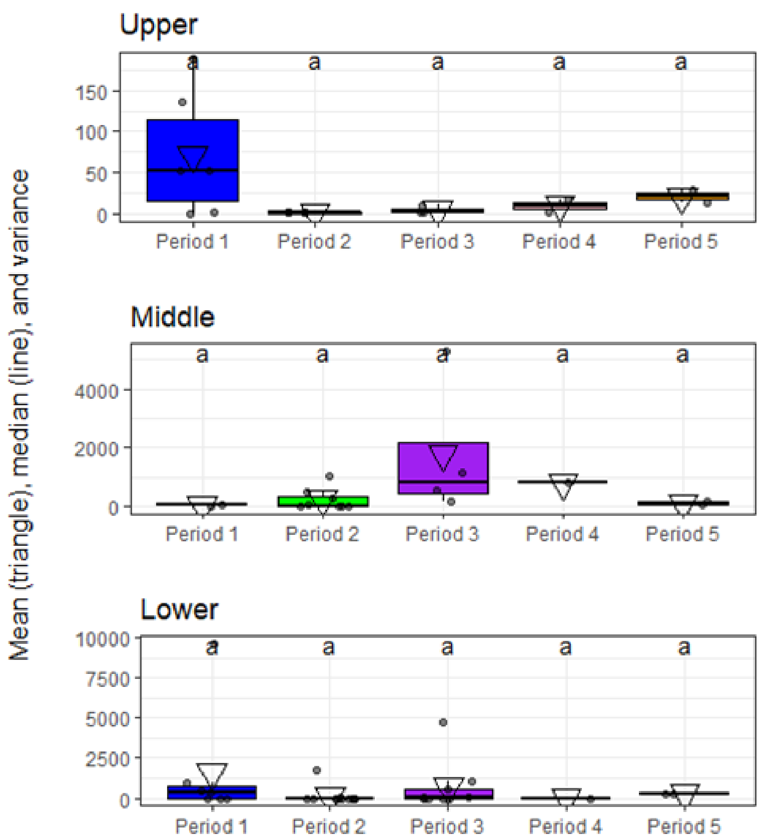


Table 6.2-76: Post-hoc Dunn's test for *Anchoa mitchilli*. Multiple comparison adjusted significance is designated by ns (not significant) or by an asterisk (significant at alpha = 0.05)

River Segment	group1	group2	n1	n2	statistic	p	P adj.	P adj. significance
Upper	Period 1	Period 2	6	4	-2.28	0.02	0.23	ns
Upper	Period 1	Period 3	6	3	-1.56	0.12	0.40	ns
Upper	Period 1	Period 4	6	2	-0.33	0.74	0.85	ns
Upper	Period 1	Period 5	6	2	0.04	0.97	0.97	ns
Upper	Period 2	Period 3	4	3	0.48	0.63	0.85	ns
Upper	Period 2	Period 4	4	2	1.39	0.16	0.41	ns
Upper	Period 2	Period 5	4	2	1.74	0.08	0.40	ns
Upper	Period 3	Period 4	3	2	0.92	0.36	0.60	ns
Upper	Period 3	Period 5	3	2	1.25	0.21	0.43	ns
Upper	Period 4	Period 5	2	2	0.30	0.76	0.85	ns
Middle	Period 1	Period 2	2	8	0.09	0.93	0.96	ns
Middle	Period 1	Period 3	2	4	1.66	0.10	0.45	ns
Middle	Period 1	Period 4	2	1	1.21	0.22	0.45	ns
Middle	Period 1	Period 5	2	2	0.30	0.77	0.96	ns
Middle	Period 2	Period 3	8	4	2.23	0.03	0.26	ns
Middle	Period 2	Period 4	8	1	1.33	0.18	0.45	ns
Middle	Period 2	Period 5	8	2	0.28	0.78	0.96	ns
Middle	Period 3	Period 4	4	1	0.04	0.96	0.96	ns
Middle	Period 3	Period 5	4	2	-1.32	0.19	0.45	ns
Middle	Period 4	Period 5	1	2	-0.97	0.33	0.55	ns
Lower	Period 1	Period 2	7	11	-2.04	0.04	0.29	ns
Lower	Period 1	Period 3	7	9	-0.65	0.51	0.57	ns
Lower	Period 1	Period 4	7	1	-1.66	0.10	0.29	ns
Lower	Period 1	Period 5	7	2	0.22	0.82	0.82	ns
Lower	Period 2	Period 3	11	9	1.46	0.14	0.29	ns
Lower	Period 2	Period 4	11	1	-0.75	0.45	0.57	ns
Lower	Period 2	Period 5	11	2	1.52	0.13	0.29	ns
Lower	Period 3	Period 4	9	1	-1.37	0.17	0.29	ns
Lower	Period 3	Period 5	9	2	0.65	0.51	0.57	ns
Lower	Period 4	Period 5	1	2	1.59	0.11	0.29	ns

Gambusia holbrooki (Mosquitofish) live in fresh or brackish waters, preferring warm, slow flowing or still waters, and water depths of 10 cm or less (US Fish and Wildlife 2016). Adults feed on small terrestrial insects, usually in the drift and amongst aquatic plants, as well as aquatic invertebrates, including hemipterans, beetles, dipteran larvae, zooplankton, filamentous algae and fragments of fruit and other plant tissues (US Fish and Wildlife 2016). The WAR (2020) report found that *G. holbrooki* was abundant in all four periods and in all river segments. Analysis of cumulative data, including applying the Kruskal-Wallis test, showed that *G. holbrooki* abundance was variable in the upper and middle segments over time. However, the abundance during Period 5 at these segments was not different than abundance during Period 1, suggesting this organism has a wide salinity tolerance range (Figure 6.2-90, Table 6.2-77).

Figure 6.2-90: Boxplots of abundance of *Gambusia holbrooki* by period and segment with Dunn's test comparisons represented by compact letter display

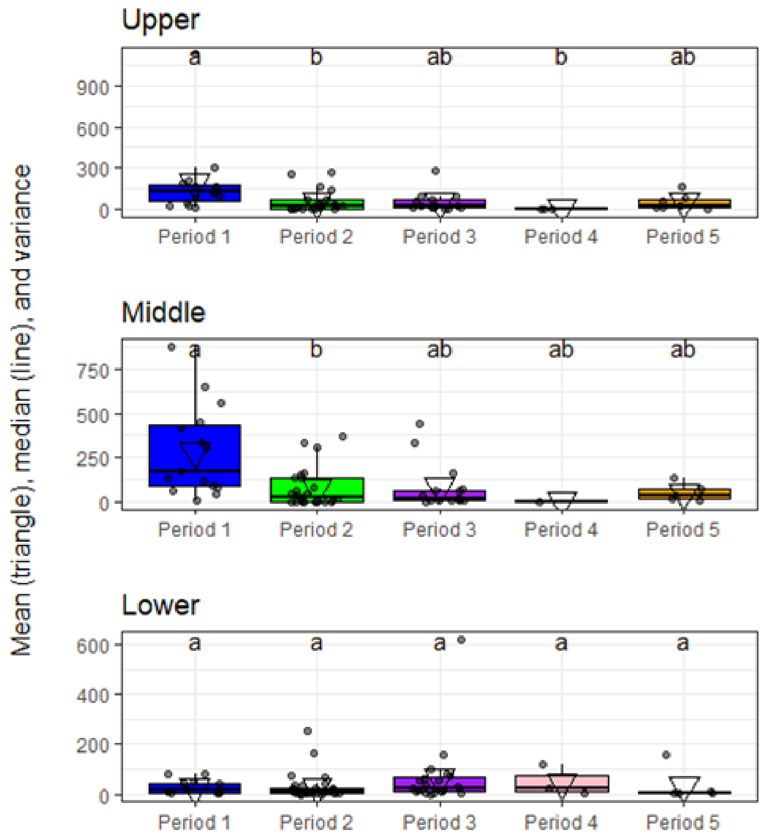


Table 6.2-77: Post-hoc Dunn's test for *Gambusia holbrooki*. Multiple comparison adjusted significance is designated by ns (not significant) or by an asterisk (significant at alpha = 0.05)

River Segment	group1	group2	n1	n2	statistic	p	P adj.	P adj. significance
Upper	Period 1	Period 2	14	21	-2.91	<0.01	0.02	*
Upper	Period 1	Period 3	14	14	-2.58	0.01	0.03	*
Upper	Period 1	Period 4	14	3	-3.09	<0.01	0.02	*
Upper	Period 1	Period 5	14	7	-1.87	0.06	0.15	ns
Upper	Period 2	Period 3	21	14	0.08	0.93	0.93	ns
Upper	Period 2	Period 4	21	3	-1.56	0.12	0.17	ns
Upper	Period 2	Period 5	21	7	0.31	0.75	0.91	ns
Upper	Period 3	Period 4	14	3	-1.56	0.12	0.17	ns
Upper	Period 3	Period 5	14	7	0.23	0.82	0.91	ns
Upper	Period 4	Period 5	3	7	1.60	0.11	0.17	ns
Middle	Period 1	Period 2	15	24	-3.52	<0.01	<0.01	*
Middle	Period 1	Period 3	15	15	-2.75	0.01	0.03	*
Middle	Period 1	Period 4	15	1	-2.21	0.03	0.09	ns
Middle	Period 1	Period 5	15	5	-1.73	0.08	0.21	ns
Middle	Period 2	Period 3	24	15	0.47	0.64	0.71	ns
Middle	Period 2	Period 4	24	1	-1.11	0.27	0.38	ns
Middle	Period 2	Period 5	24	5	0.54	0.59	0.71	ns
Middle	Period 3	Period 4	15	1	-1.24	0.21	0.36	ns
Middle	Period 3	Period 5	15	5	0.21	0.84	0.84	ns
Middle	Period 4	Period 5	1	5	1.27	0.20	0.36	ns
Lower	Period 1	Period 2	12	30	-0.83	0.41	0.62	ns
Lower	Period 1	Period 3	12	20	0.72	0.47	0.62	ns
Lower	Period 1	Period 4	12	3	0.25	0.80	0.87	ns
Lower	Period 1	Period 5	12	5	-1.16	0.25	0.62	ns
Lower	Period 2	Period 3	30	20	1.89	0.06	0.40	ns
Lower	Period 2	Period 4	30	3	0.74	0.46	0.62	ns
Lower	Period 2	Period 5	30	5	-0.69	0.49	0.62	ns
Lower	Period 3	Period 4	20	3	-0.16	0.87	0.87	ns
Lower	Period 3	Period 5	20	5	-1.75	0.08	0.40	ns
Lower	Period 4	Period 5	3	5	-1.06	0.29	0.62	ns

Lucania parva (Rainwater Killifish) is an estuarine species, ranging from euryhaline to tidal fresh waters. As omnivores, they feed mostly on small invertebrates such as annelids, mollusks, and amphipods (Smithsonian 2023). *Lucania parva* was found during all four periods (WAR 2020). During the first three periods, it was found in all three river segments and in the middle and upper segments in 2018 (WAR 2020). Analysis of cumulative data, including applying the Kruskal-Wallis test, showed that *L. parva* abundance was not significantly different in the upper, middle, or lower segments over time (Figure 6.2-91, Table 6.2-78).

Figure 6.2-91: Boxplots of abundance of *Lucania parva* by period and segment with Dunn's test comparisons represented by compact letter display

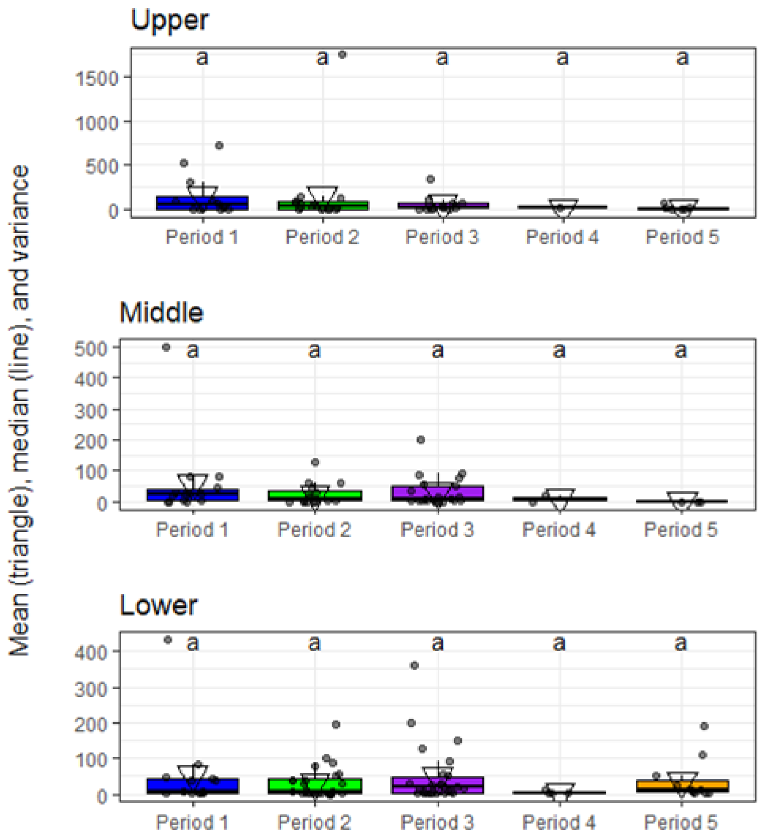


Table 6.2-78: Post-hoc Dunn's test for *Lucania parva*. Multiple comparison adjusted significance is designated by ns (not significant) or by an asterisk (significant at alpha = 0.05)

River Segment	group1	group2	n1	n2	statistic	p	P adj.	P adj. significance
Upper	Period 1	Period 2	12	15	-0.40	0.69	0.97	ns
Upper	Period 1	Period 3	12	13	-0.43	0.66	0.97	ns
Upper	Period 1	Period 4	12	1	-0.20	0.84	0.97	ns
Upper	Period 1	Period 5	12	6	-1.74	0.08	0.53	ns
Upper	Period 2	Period 3	15	13	-0.05	0.96	0.97	ns
Upper	Period 2	Period 4	15	1	-0.06	0.95	0.97	ns
Upper	Period 2	Period 5	15	6	-1.48	0.14	0.53	ns
Upper	Period 3	Period 4	13	1	-0.04	0.97	0.97	ns
Upper	Period 3	Period 5	13	6	-1.41	0.16	0.53	ns
Upper	Period 4	Period 5	1	6	-0.61	0.54	0.97	ns
Middle	Period 1	Period 2	14	15	-0.80	0.43	0.61	ns
Middle	Period 1	Period 3	14	21	-0.46	0.65	0.69	ns
Middle	Period 1	Period 4	14	2	-0.98	0.33	0.61	ns
Middle	Period 1	Period 5	14	3	-2.52	0.01	0.10	ns
Middle	Period 2	Period 3	15	21	0.41	0.69	0.69	ns
Middle	Period 2	Period 4	15	2	-0.59	0.55	0.69	ns
Middle	Period 2	Period 5	15	3	-2.07	0.04	0.13	ns
Middle	Period 3	Period 4	21	2	-0.79	0.43	0.61	ns
Middle	Period 3	Period 5	21	3	-2.35	0.02	0.10	ns
Middle	Period 4	Period 5	2	3	-0.95	0.34	0.61	ns
Lower	Period 1	Period 2	13	25	-0.63	0.53	0.88	ns
Lower	Period 1	Period 3	13	26	0.00	1.00	1.00	ns
Lower	Period 1	Period 4	13	4	-2.08	0.04	0.18	ns
Lower	Period 1	Period 5	13	11	-0.20	0.84	0.93	ns
Lower	Period 2	Period 3	25	26	0.77	0.44	0.88	ns
Lower	Period 2	Period 4	25	4	-1.81	0.07	0.18	ns
Lower	Period 2	Period 5	25	11	0.36	0.72	0.93	ns
Lower	Period 3	Period 4	26	4	-2.21	0.03	0.18	ns
Lower	Period 3	Period 5	26	11	-0.24	0.81	0.93	ns
Lower	Period 4	Period 5	4	11	1.89	0.06	0.18	ns

Poecilia latipinna (Sailfin Molly) is a small, live-bearing freshwater fish native to the Gulf Coast that has established populations in estuarine habitats. It feeds mainly on algae but also consumes animals, including rotifers, small crustaceans (such as copepods and ostracods) and aquatic insects. They in turn are consumed by native fishes (Smithsonian 2023). The WAR (2020) study noted that *P. latipinna* was most abundant during Period 1 and less common in the subsequent periods. Analysis of cumulative data, including applying the Kruskal-Wallis test, showed that *P. latipinna* abundance was not significantly different in the upper or lower segments over time. Although *P. latipinna* abundance was variable in the middle segment, Period 5 abundance was not significantly different from that of Period 1 (Figure 6.2-92, Table 6.2-79).

Figure 6.2-92: Boxplots of abundance of *Poecilia latipinna* by period and segment with Dunn's test comparisons represented by compact letter display

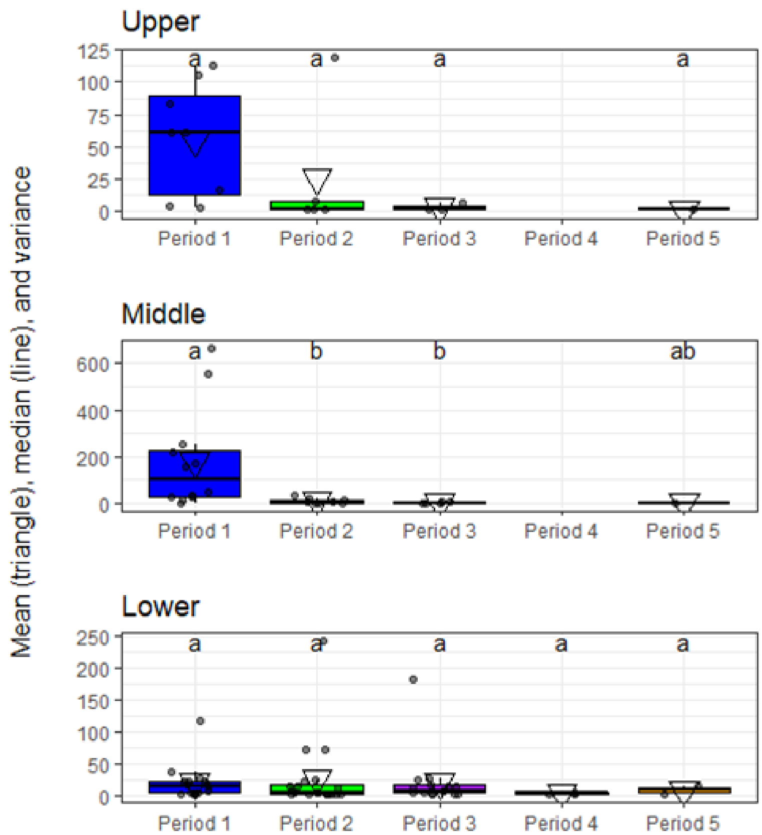


Table 6.2-79: Post-hoc Dunn's test for *Poecilia latipinna*. Multiple comparison adjusted significance is designated by ns (not significant) or by an asterisk (significant at alpha = 0.05)

River Segment	group1	group2	n1	n2	statistic	p	P adj.	P adj. significance
Upper	Period 1	Period 2	8	5	-1.69	0.09	0.18	ns
Upper	Period 1	Period 3	8	3	-1.78	0.08	0.18	ns
Upper	Period 1	Period 5	8	1	-1.70	0.09	0.18	ns
Upper	Period 2	Period 3	5	3	-0.33	0.74	0.74	ns
Upper	Period 2	Period 5	5	1	-0.77	0.44	0.66	ns
Upper	Period 3	Period 5	3	1	-0.52	0.60	0.72	ns
Middle	Period 1	Period 2	12	8	-2.80	0.01	0.02	*
Middle	Period 1	Period 3	12	5	-3.12	<0.01	0.01	*
Middle	Period 1	Period 5	12	1	-2.03	0.04	0.09	ns
Middle	Period 2	Period 3	8	5	-0.67	0.50	0.60	ns
Middle	Period 2	Period 5	8	1	-0.78	0.43	0.60	ns
Middle	Period 3	Period 5	5	1	-0.41	0.68	0.68	ns
Lower	Period 1	Period 2	15	20	-1.18	0.24	0.71	ns
Lower	Period 1	Period 3	15	15	-0.65	0.51	0.71	ns
Lower	Period 1	Period 4	15	3	-1.69	0.09	0.71	ns
Lower	Period 1	Period 5	15	2	-0.85	0.40	0.71	ns
Lower	Period 2	Period 3	20	15	0.48	0.63	0.71	ns
Lower	Period 2	Period 4	20	3	-1.07	0.28	0.71	ns
Lower	Period 2	Period 5	20	2	-0.32	0.75	0.75	ns
Lower	Period 3	Period 4	15	3	-1.31	0.19	0.71	ns
Lower	Period 3	Period 5	15	2	-0.53	0.60	0.71	ns
Lower	Period 4	Period 5	3	2	0.47	0.64	0.71	ns

Cyprinodon variegatus (Sheepshead Minnow) is tolerant of wide variations in salinity and is found in brackish water in bays, inlets, lagoons, and salt marshes with little wave action and sandy or muddy bottoms. It is omnivorous, feeding on organic detritus and algae as well as microcrustaceans, and dipteran larvae (USGS 2024). *Cyprinodon variegatus* was found in approximately half the seine samples during Periods 1-3, but was not observed in 2018 (WAR 2020). Analysis of cumulative data, including applying the Kruskal-Wallis test, showed that *C. variegatus* was not found in the upper and middle segments during Periods 4 and 5 or in the lower segment during Period 5, despite being fairly common prior to those periods (Figure 6.2-93, Table 6.2-80). The recent absence of *C. variegatus* in the upper and middle segments may potentially be attributable to salinity reduction observed during full MFL implementation.

Figure 6.2-93: Boxplots of abundance of *Cyprinodon variegatus* by period and segment with Dunn's test comparisons represented by compact letter display

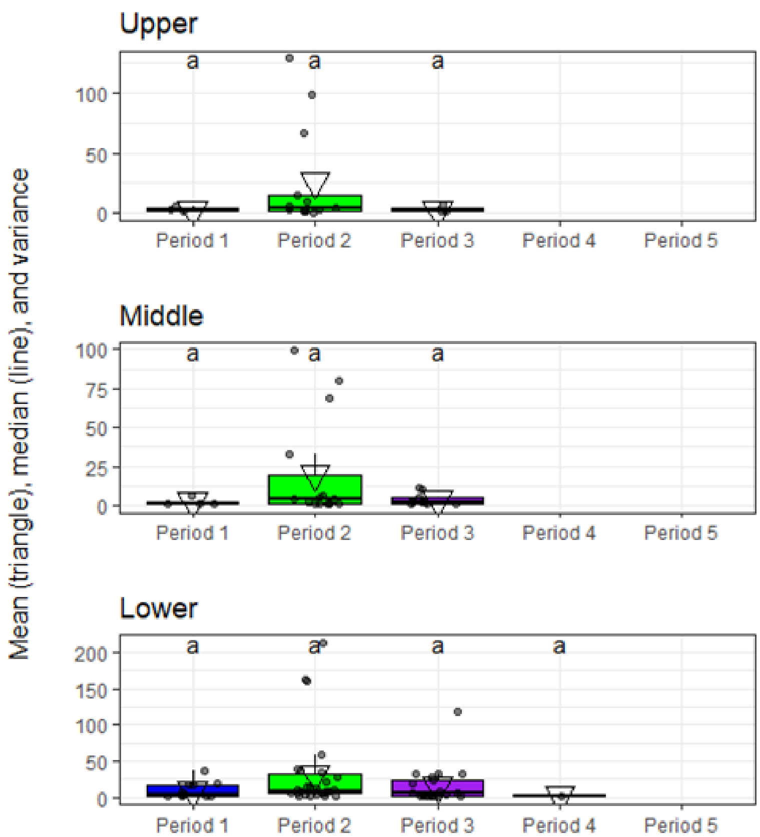


Table 6.2-80: Post-hoc Dunn's test for *Cyprinodon variegatus*. Multiple comparison adjusted significance is designated by ns (not significant) or by an asterisk (significant at alpha = 0.05)

River Segment	group1	group2	n1	n2	statistic	p	P adj.	P adj. significance
Upper	Period 1	Period 2	3	13	0.76	0.45	0.67	ns
Upper	Period 1	Period 3	3	3	-0.22	0.82	0.82	ns
Upper	Period 2	Period 3	13	3	-1.04	0.30	0.67	ns
Middle	Period 1	Period 2	4	15	1.11	0.27	0.54	ns
Middle	Period 1	Period 3	4	10	0.91	0.36	0.54	ns
Middle	Period 2	Period 3	15	10	-0.21	0.84	0.84	ns
Lower	Period 1	Period 2	11	27	1.68	0.09	0.37	ns
Lower	Period 1	Period 3	11	20	0.39	0.70	0.70	ns
Lower	Period 1	Period 4	11	1	-0.60	0.55	0.66	ns
Lower	Period 2	Period 3	27	20	-1.54	0.12	0.37	ns
Lower	Period 2	Period 4	27	1	-1.20	0.23	0.46	ns
Lower	Period 3	Period 4	20	1	-0.75	0.45	0.66	ns

Mugil cephalus (Striped Mullet) inhabit estuarine intertidal, freshwater, and coastal marine habitats. They feed on a wide variety of food substrates, such as detritus and epiphytic material. Mullet are commonly consumed by humans and also serve as a food source for valuable game fish such as mahi, snook, and snapper (FDEP 2013d). *Mugil cephalus*, a schooling fish, was found during all periods and were most abundant in the lower segment (WAR 2020). Analysis of cumulative data, including applying the Kruskal-Wallis test, showed that while a single specimen of *M. cephalus* was found in the upper segment during Period 5, *M. cephalus* was common within the middle and lower segments with no significant changes over time (Figure 6.2-94, Table 6.2-81).

Figure 6.2-94: Boxplots of abundance of *Mugil cephalus* by period and segment with Dunn's test comparisons represented by compact letter display

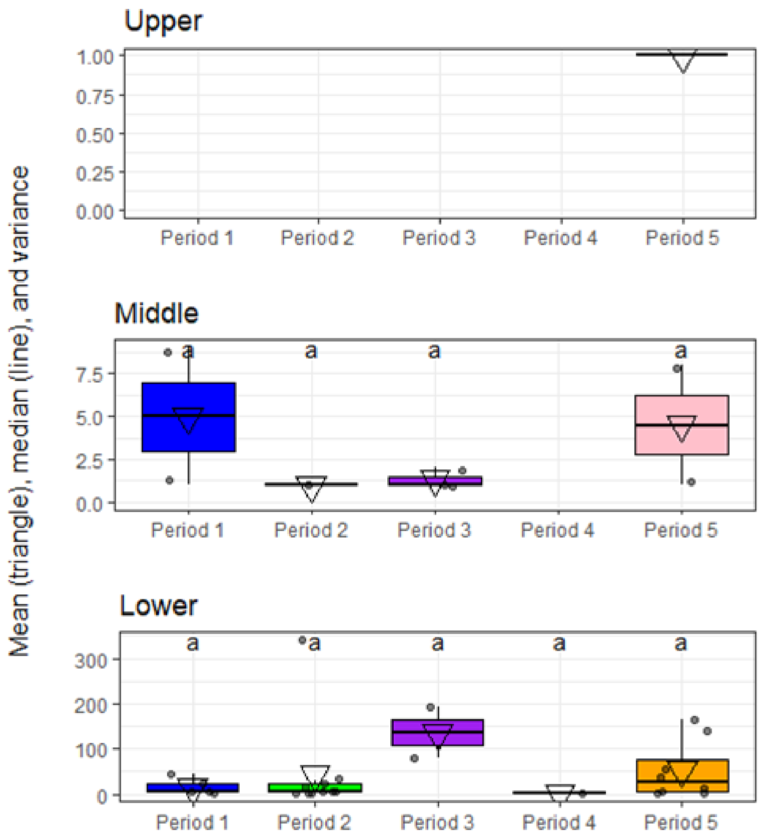


Table 6.2-81: Post-hoc Dunn's test for *Mugil cephalus*. Multiple comparison adjusted significance is designated by ns (not significant) or by an asterisk (significant at alpha = 0.05)

River Segment	group1	group2	n1	n2	statistic	p	P adj.	P adj. significance
Middle	Period 1	Period 2	2	1	-0.95	0.34	0.82	ns
Middle	Period 1	Period 3	2	3	-0.77	0.44	0.82	ns
Middle	Period 1	Period 5	2	2	-0.23	0.82	0.82	ns
Middle	Period 2	Period 3	1	3	0.41	0.69	0.82	ns
Middle	Period 2	Period 5	1	2	0.76	0.45	0.82	ns
Middle	Period 3	Period 5	3	2	0.51	0.61	0.82	ns
Lower	Period 1	Period 2	5	10	0.12	0.90	0.90	ns
Lower	Period 1	Period 3	5	2	1.86	0.06	0.21	ns
Lower	Period 1	Period 4	5	1	-0.79	0.43	0.48	ns
Lower	Period 1	Period 5	5	8	0.78	0.43	0.48	ns
Lower	Period 2	Period 3	10	2	1.93	0.05	0.21	ns
Lower	Period 2	Period 4	10	1	-0.89	0.37	0.48	ns
Lower	Period 2	Period 5	10	8	0.80	0.42	0.48	ns
Lower	Period 3	Period 4	2	1	-1.98	0.05	0.21	ns
Lower	Period 3	Period 5	2	8	-1.41	0.16	0.40	ns
Lower	Period 4	Period 5	1	8	1.24	0.22	0.43	ns

Trinectes maculatus (Hog Choker) is a euryhaline species that enters streams and rivers and is often found far inland. Their diet depends on the salinity of the waters they inhabit and consists of benthic organisms such as aquatic crustaceans and insects, mollusks, and both polychaete and oligochaete worms (Florida Museum 2024). The WAR (2020) report found that *T. maculatus* was found during all minimum flow periods in all river segments, with somewhat higher abundances in the upper segment. Analysis of cumulative data, including applying the Kruskal-Wallis test, showed that *T. maculatus* abundance was not significantly different in the upper, middle, or lower segments over time (Figure 6.2-95, Table 6.2-82).

Figure 6.2-95: Boxplots of abundance of *Trinectes maculatus* by period and segment with Dunn's test comparisons represented by compact letter display

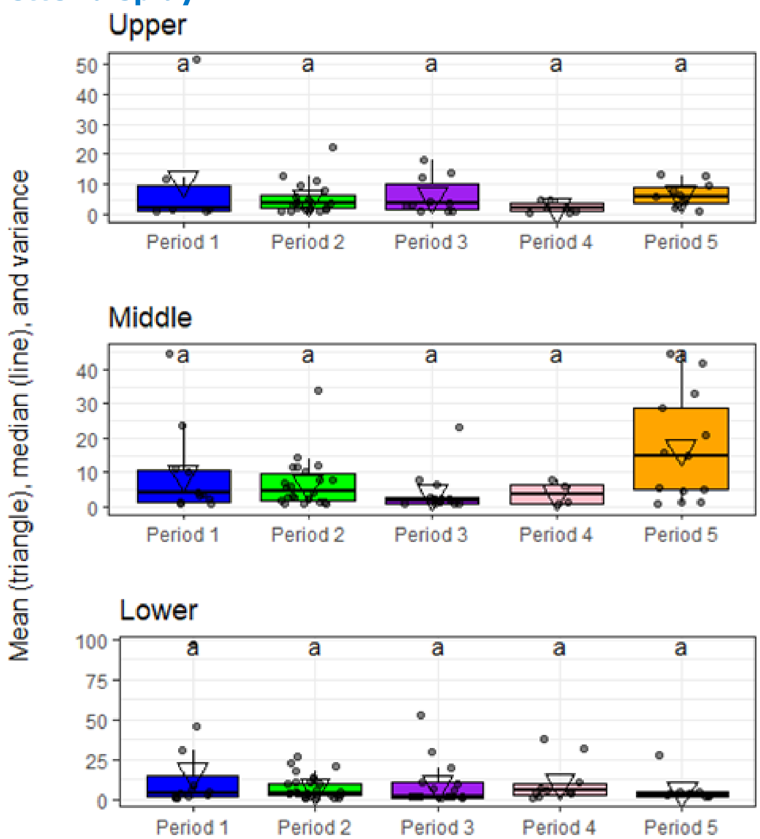


Table 6.2-82: Post-hoc Dunn's test for *Trinectes maculatus*. Multiple comparison adjusted significance is designated by ns (not significant) or by an asterisk (significant at alpha = 0.05)

River Segment	group1	group2	n1	n2	statistic	p	P adj.	P adj. significance
Upper	Period 1	Period 2	6	20	0.27	0.79	0.86	ns
Upper	Period 1	Period 3	6	10	0.37	0.71	0.86	ns
Upper	Period 1	Period 4	6	7	-0.79	0.43	0.61	ns
Upper	Period 1	Period 5	6	11	1.15	0.25	0.50	ns
Upper	Period 2	Period 3	20	10	0.17	0.86	0.86	ns
Upper	Period 2	Period 4	20	7	-1.28	0.20	0.50	ns
Upper	Period 2	Period 5	20	11	1.23	0.22	0.50	ns
Upper	Period 3	Period 4	10	7	-1.28	0.20	0.50	ns
Upper	Period 3	Period 5	10	11	0.90	0.37	0.61	ns
Upper	Period 4	Period 5	7	11	2.12	0.03	0.34	ns
Middle	Period 1	Period 2	11	22	0.03	0.98	0.98	ns
Middle	Period 1	Period 3	11	14	-1.27	0.21	0.37	ns
Middle	Period 1	Period 4	11	4	-0.70	0.49	0.61	ns
Middle	Period 1	Period 5	11	13	1.21	0.22	0.37	ns
Middle	Period 2	Period 3	22	14	-1.52	0.13	0.37	ns
Middle	Period 2	Period 4	22	4	-0.77	0.44	0.61	ns
Middle	Period 2	Period 5	22	13	1.39	0.16	0.37	ns
Middle	Period 3	Period 4	14	4	0.18	0.85	0.95	ns
Middle	Period 3	Period 5	14	13	2.62	0.01	0.09	ns
Middle	Period 4	Period 5	4	13	1.58	0.11	0.37	ns
Lower	Period 1	Period 2	12	28	-0.18	0.86	0.94	ns
Lower	Period 1	Period 3	12	15	-0.71	0.48	0.88	ns
Lower	Period 1	Period 4	12	10	0.25	0.80	0.94	ns
Lower	Period 1	Period 5	12	9	-0.69	0.49	0.88	ns
Lower	Period 2	Period 3	28	15	-0.66	0.51	0.88	ns
Lower	Period 2	Period 4	28	10	0.46	0.65	0.92	ns
Lower	Period 2	Period 5	28	9	-0.63	0.53	0.88	ns
Lower	Period 3	Period 4	15	10	0.93	0.35	0.88	ns
Lower	Period 3	Period 5	15	9	-0.07	0.94	0.94	ns
Lower	Period 4	Period 5	10	9	-0.90	0.37	0.88	ns

Microgobius gulosus (Clown Goby) is a small estuarine fish that inhabit primarily low-energy, sometimes vegetated, tidal zones, with sand or mud substrates, including bays, tidal creeks, canals, ditches and coastal rivers (Florida Museum 2024). The WAR (2020) report found that *M. gulosus* became less abundant after Period 2. Analysis of cumulative data, including applying the Kruskal-Wallis test, showed that *M. gulosus* abundance was not significantly different in the middle segment over time, was variable in the lower segment, and that *M. gulosus* was not found in the upper segment after Period 2 (Figure 6.2-96, Table 6.2-83).

Figure 6.2-96: Boxplots of abundance of *Microgobius gulosus* by period and segment with Dunn's test comparisons represented by compact letter display

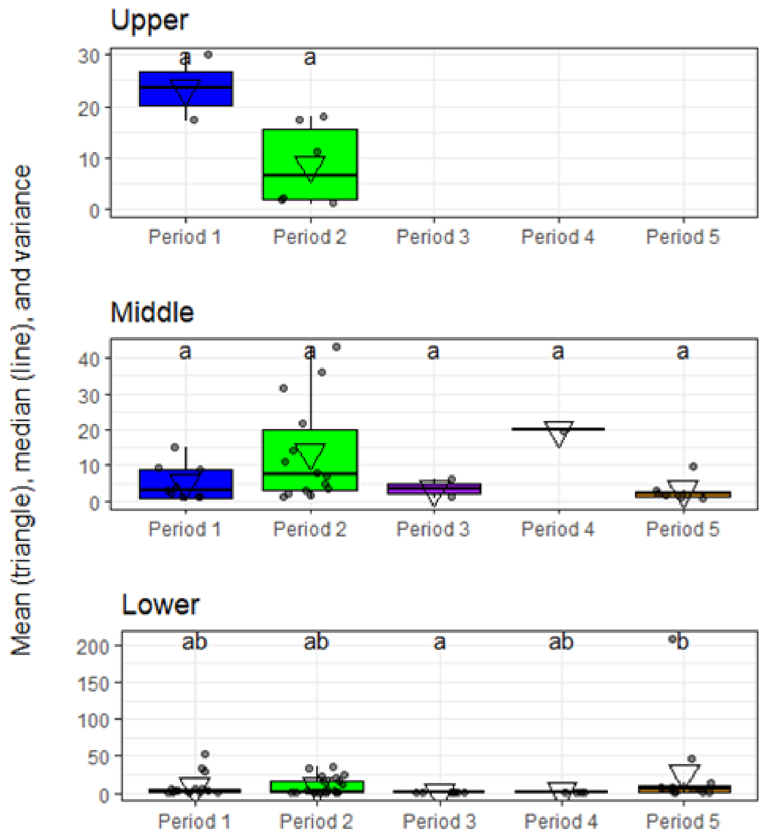


Table 6.2-83: Post-hoc Dunn's test for *Microgobius gulosus*. Multiple comparison adjusted significance is designated by ns (not significant) or by an asterisk (significant at alpha = 0.05)

River Segment	group1	group2	n1	n2	statistic	p	P adj.	P adj. significance
Upper	Period 1	Period 2	2	6	-1.52	0.13	0.13	ns
Middle	Period 1	Period 2	9	14	1.61	0.11	0.30	ns
Middle	Period 1	Period 3	9	2	-0.34	0.74	0.82	ns
Middle	Period 1	Period 4	9	1	1.43	0.15	0.30	ns
Middle	Period 1	Period 5	9	6	-0.58	0.56	0.70	ns
Middle	Period 2	Period 3	14	2	-1.26	0.21	0.35	ns
Middle	Period 2	Period 4	14	1	0.79	0.43	0.61	ns
Middle	Period 2	Period 5	14	6	-2.04	0.04	0.30	ns
Middle	Period 3	Period 4	2	1	1.45	0.15	0.30	ns
Middle	Period 3	Period 5	2	6	-0.05	0.96	0.96	ns
Middle	Period 4	Period 5	1	6	-1.68	0.09	0.30	ns
Lower	Period 1	Period 2	15	23	-0.34	0.74	0.74	ns
Lower	Period 1	Period 3	15	7	-2.30	0.02	0.10	ns
Lower	Period 1	Period 4	15	4	-1.09	0.28	0.46	ns
Lower	Period 1	Period 5	15	12	0.80	0.42	0.53	ns
Lower	Period 2	Period 3	23	7	-2.18	0.03	0.10	ns
Lower	Period 2	Period 4	23	4	-0.92	0.36	0.51	ns
Lower	Period 2	Period 5	23	12	1.18	0.24	0.46	ns
Lower	Period 3	Period 4	7	4	0.70	0.48	0.53	ns
Lower	Period 3	Period 5	7	12	2.86	<0.01	0.04	*
Lower	Period 4	Period 5	4	12	1.59	0.11	0.28	ns

Fundulus seminolis (Seminole Killifish), a topminnow species endemic to Florida, is found in lakes and quiet pools of streams. Small individuals are most often found near vegetation, and adults are more often found in open water (Florida Museum 2024). *Fundulus seminolis* increased in abundance after Period 1 (WAR 2020). Analysis of cumulative data, including applying the Kruskal-Wallis test, showed that *F. seminolis* was not found during Period 1, but was fairly common within the upper, middle, and lower segments thereafter, with no significant changes over time (Figure 6.2-97, Table 6.2-84).

Figure 6.2-97: Boxplots of abundance of *Fundulus seminolis* by period and segment with Dunn's test comparisons represented by compact letter display

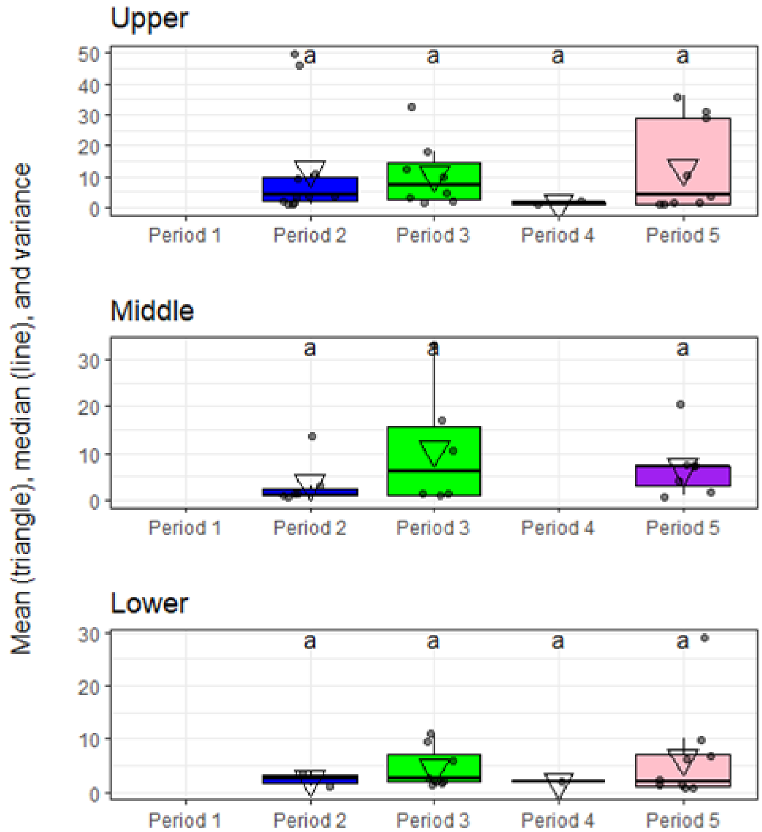


Table 6.2-84: Post-hoc Dunn's test for *Fundulus seminolis*. Multiple comparison adjusted significance is designated by ns (not significant) or by an asterisk (significant at alpha = 0.05)

River Segment	group1	group2	n1	n2	statistic	p	P adj.	P adj. significance
Upper	Period 2	Period 3	11	8	0.62	0.53	0.64	ns
Upper	Period 2	Period 4	11	2	-1.35	0.18	0.42	ns
Upper	Period 2	Period 5	11	9	-0.14	0.89	0.89	ns
Upper	Period 3	Period 4	8	2	-1.68	0.09	0.42	ns
Upper	Period 3	Period 5	8	9	-0.72	0.47	0.64	ns
Upper	Period 4	Period 5	2	9	1.25	0.21	0.42	ns
Middle	Period 2	Period 3	6	6	1.09	0.27	0.41	ns
Middle	Period 2	Period 5	6	7	1.43	0.15	0.41	ns
Middle	Period 3	Period 5	6	7	0.30	0.77	0.77	ns
Lower	Period 2	Period 3	2	8	0.75	0.45	0.92	ns
Lower	Period 2	Period 4	2	1	0.11	0.92	0.92	ns
Lower	Period 2	Period 5	2	9	0.41	0.68	0.92	ns
Lower	Period 3	Period 4	8	1	-0.44	0.66	0.92	ns
Lower	Period 3	Period 5	8	9	-0.56	0.58	0.92	ns
Lower	Period 4	Period 5	1	9	0.18	0.86	0.92	ns

Micropterus salmoides (Largemouth Bass) is a predatory gamefish found in freshwater lakes, ponds, and pools of creeks and small to large rivers (Florida Museum 2024). The WAR (2020) report noted that *M. salmoides* became more abundant after Period 1 and was more prevalent in upper portions of the target zone. Analysis of cumulative data, including applying the Kruskal-Wallis test, showed that *M. salmoides* was not found during Period 1, was absent from the middle and lower segments during Period 4, and was fairly common within the upper, middle, and lower segments during other periods, with no significant change over time (Figure 6.2-98, Table 6.2-85).

Figure 6.2-98: Boxplots of abundance of *Micropterus salmoides* by period and segment with Dunn's test comparisons represented by compact letter display

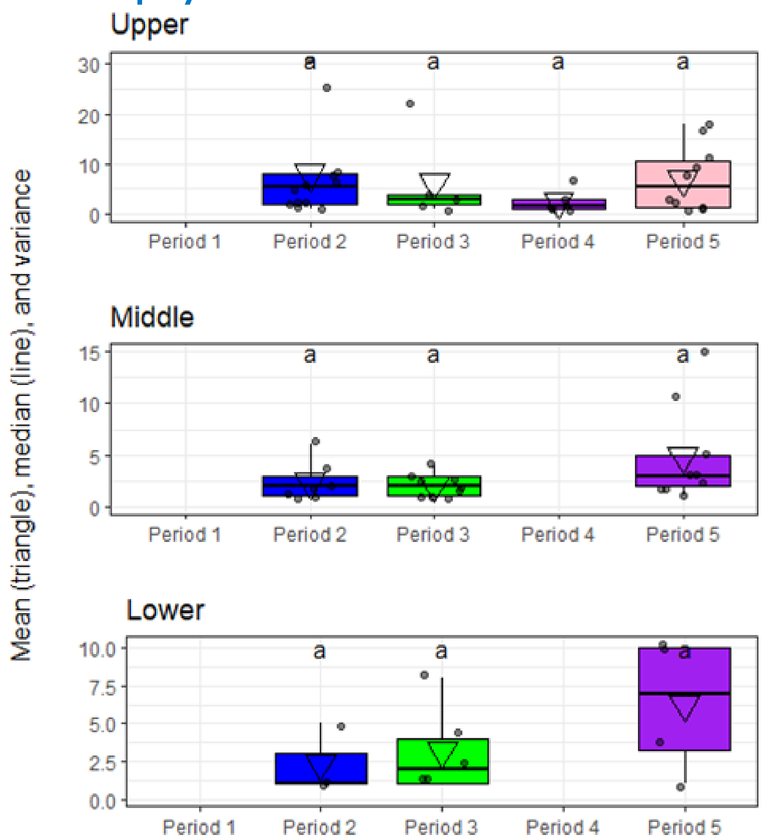


Table 6.2-85: Post-hoc Dunn's test for *Micropterus salmoides*. Multiple comparison adjusted significance is designated by ns (not significant) or by an asterisk (significant at alpha = 0.05)

River Segment	group1	group2	n1	n2	statistic	p	P adj.	P adj. significance
Upper	Period 2	Period 3	12	5	-0.37	0.71	0.91	ns
Upper	Period 2	Period 4	12	6	-1.58	0.11	0.45	ns
Upper	Period 2	Period 5	12	10	-0.11	0.91	0.91	ns
Upper	Period 3	Period 4	5	6	-0.98	0.33	0.66	ns
Upper	Period 3	Period 5	5	10	0.28	0.78	0.91	ns
Upper	Period 4	Period 5	6	10	1.44	0.15	0.45	ns
Middle	Period 2	Period 3	7	9	0.03	0.97	0.97	ns
Middle	Period 2	Period 5	7	9	1.34	0.18	0.27	ns
Middle	Period 3	Period 5	9	9	1.40	0.16	0.27	ns
Lower	Period 2	Period 3	3	5	0.36	0.72	0.72	ns
Lower	Period 2	Period 5	3	4	1.28	0.20	0.43	ns
Lower	Period 3	Period 5	5	4	1.07	0.29	0.43	ns

6.2.4.4 Species Responses Compared with Previous Studies: Conclusions

Previous studies suggested that certain taxa responded to minimum flow implementation in the LHR (Atkins and JEI 2015, WAR 2020). Analyses of cumulative data, including the calculation of individual species' mean and median abundances by segment and period and subjecting the data to the Kruskal-Wallis and Dunn's post-hoc tests, indicated six taxa in the Hillsborough River estuary that were more abundant in higher salinity zones and less abundant in lower salinity zones over time. These taxa included *Clytia sp.* (jellyfish), Chaetognatha (Arrow Worm), *Palaemonetes pugio* (Daggerblade Shrimp), *Brevoortia* (Menhaden), *Cyprinodon variegatus* (Sheepshead Minnow), and *Microgobius gulosus* (Clown Goby).

Analyses of the remaining taxa evaluated, including *Laeonereis culveri* (predatory polychaete), *Menidia beryllina* (Inland Silverside Minnow), Prosobranchia (a group of snails), *Stenonereis martini* (Polychaete), Hydrobiidae (a group of snails), *Melanoides tuberculata* (Red-rim Melania), *Pyrgophorus platyrachis* (Crown Snail), *Eucinostomus harengulus* (Tidewater Mojarra), *Anchoa mitchilli* (Bay Anchovy), *Gambusia holbrooki* (Mosquitofish), *Lucania parva* (Rainwater Killifish), *Poecilia latipinna* (Sailfin Molly), *Mugil cephalus* (Striped Mullet), *Trinectes maculatus* (Hogchoker), *Fundulus seminolis* (Seminole Killifish), and *Micropterus salmoides* (Largemouth Bass), demonstrated no clear pattern or statistically significant responses attributable to salinity changes by segment or period.

7 SYNTHESIS AND CONCLUSIONS

This report represents the third of three consecutive 5-year assessments of the Hillsborough River Recovery Strategy. The assessment evaluates the effectiveness of the recovery strategy in meeting minimum flow requirements between 1996 and 2023.

This assessment used data from multiple sources including the USGS, EPC, City of Tampa, the District, and TBW. The analysis focused on:

- Hydrologic data – Flow measurements from various monitoring stations and water sources.
- Water quality data – Physical and chemical parameters collected at fixed stations.
- Biological data – Zooplankton, benthic macroinvertebrates, and nekton (fish and mobile invertebrates) collected through various sampling methods.

The assessment employed both descriptive and quantitative analytical approaches. For water quality analysis, mixed effects models were used to evaluate period differences and interactions and linear, logistic, and GAM models were used to evaluate water quality relationships with flow. For biological analyses, multivariate techniques (cluster analyses and NMDS) were used to identify community patterns, and statistical tests evaluated differences between implementation periods and river segments. The analysis focused particularly on taxa known to inhabit low-salinity (< 5 ppt) environments, as extending this habitat zone was a primary goal of the minimum flow implementation.

7.1 HYDROLOGY

The recovery strategy has progressively improved flow conditions in the LHR over time. Implementing minimum flows has been increasingly successful, with the most recent period (2018–2023) coming close to meeting the goals of the recovery strategy. By 2023, the minimum flows were completely met.

Recovery sources used to meet the LHR minimum flow requirements have evolved over time:

- Sulphur Springs has been a primary source since 2002.
- The TBC became a significant contributor starting in 2008.
- Blue Sink began contributing in 2018.
- Other Permittable Source (elimination of the 25% loss term) was identified and used consistently beginning in summer 2022
- Morris Bridge Sink has been permitted but not yet used as a recovery source.

The analysis showed that implementing the minimum flow has not adversely affected water levels in the Hillsborough River Reservoir above the dam.

7.2 WATER QUALITY

Implementing minimum flows has improved water quality conditions in the upper portions of the LHR target zone (Sligh Avenue to the dam):

- Salinity decreased in both surface and bottom waters, extending the low salinity (< 5 ppt) zone from the dam toward Sulphur Springs.
- DO has generally increased, particularly in the upper target zone, with bottom DO values >2.5 mg/L occurring more frequently and extending farther downstream, though low DO persists in deeper, middle and lower segments.
- Temperature and pH showed minimal changes and no adverse effects.

GAMs confirmed the positive influence of minimum flows on salinity and DO compared to the "no minimum flow" scenario (absence of recovery water application) and aligned with independent LAMFE salinity model findings, which remains the primary tool for assessing flow-related salinity and temperature responses in the LHR.

There was no evidence that the Upper Hillsborough River or the other recovery sources were negatively affected by implementing the minimum flow. Nitrogen concentrations have generally declined over time in recovery sources as better stormwater and wastewater management practices have been implemented in the watershed. However, increasing temperature and salinity in Sulphur Springs have been observed.

7.3 BIOLOGY

Analyses of biological communities provided compelling evidence that the current LHR minimum flow is functioning to provide oligohaline (< 5 ppt) habitat conditions for aquatic organisms. To reduce the effects of different sampling frequencies across segments and periods, data were standardized (e.g., consistent taxonomy, removal of rare taxa, mixed-effects models, limiting analyses to minimum flow implementation days, and considering salinity-sensitive taxa), though some observed differences may still reflect uneven sampling effort. Key modeling and statistical results are presented below; however, observed differences may be influenced by variations in sampling effort between target zone segments and periods:

- Zooplankton – Salinity-sensitive zooplankton abundance, density, and taxa richness were higher in lower-salinity areas associated with minimum flow implementation. Statistical models showed significant relationships between these metrics and implementation periods.
- Benthic Macroinvertebrates – Salinity-sensitive benthic macroinvertebrate abundance, richness, and diversity increased in lower salinity areas associated with minimum flow implementation. The community composition showed shifts toward more freshwater-adapted species.
- Nekton (Fish and Mobile Invertebrates) – Salinity-sensitive nekton richness and diversity improved in lower salinity areas associated with minimum flow implementation. Several fish species characteristic of freshwater and low-salinity environments showed increased abundance.

Additional analyses of specific taxa that had previously been shown to respond to minimum flow implementation confirmed these patterns, identifying several organisms that became more abundant in low-salinity zones created by the minimum flow implementation.

The biological assessment also evaluated communities downstream of the target zone, finding high taxa richness, high organism abundance, and evidence of a robust, functioning food web throughout the LHR.

7.4 SUMMARY

The findings presented in this report provide compelling evidence that the current LHR minimum flows are functioning as intended to provide oligohaline (<5 ppt) habitat conditions for aquatic organisms. The implementation of the minimum flow and associated recovery strategy has successfully:

- Achieved the adjusted minimum flow for the LHR on all days in 2023, a drought year.
- Extended the low-salinity zone from the base of the dam toward Sulphur Springs.
- Improved water quality conditions in the LHR, particularly salinity and DO levels.
- Enhanced habitat for freshwater and low-salinity adapted organisms.
- Supported diverse and abundant biological communities.

The most recent implementation period (2018–2023) has shown the greatest success in meeting minimum flow requirements, with regular achievement of target flows by 2023. The water quality and biological responses to these improved flow conditions demonstrate that the recovery strategy is effectively protecting the ecological resources of the LHR while balancing water supply needs.

The assessment confirms that the phased implementation approach of the recovery strategy has been appropriate, with each additional water recovery source contributing to the overall success of the program. The continued monitoring and assessment of the LHR will be important to ensure that these ecological benefits are maintained over time; however, the long-term sustainability of Sulphur Springs as a recovery source remains a concern.

8 LITERATURE CITED

Algaebase. 2024. Algaebase: https://www.algaebase.org/

Animal Diversity Web. 2024. https://animaldiversity.org/

Atkins & Janicki Environmental (JEI). 2015. Tampa Bypass Canal/Hillsborough River Water Supply Facility Hydrobiological Monitoring Program Year 15 Interpretive Report. Prepared for Tampa Bay Water, Clearwater, Florida.

Bakker, JD. 2024. Applied Multivariate Statistics in R. University of Washington.

Barnes, RSK; Hughes, RN. 1993. An introduction to marine ecology. Blackwell Science Ltd. Cambridge, MA.

Basso, R. 2010. Results of Morris Bridge Sink Pumping Test. Hillsborough County. Florida. Prepared by and for the Southwest Florida Water Management District. Brookville, Florida.

Blashfield, RK; Aldenderfer, MS. 1978. The Literature On Cluster Analysis. *Multivariate Behav Res* 13:271–295.

British Geological Society. 2024. Gastropods. https://www.bgs.ac.uk/discovering-geology/fossils-and-geological-time/gastropods/

Brodie, RB; MacDonald, TC; McMichael. RH. 2013. Salinity Effects Due to Channel Deepening on Estuarine Dependent Nekton in the Lower St. Johns River Estuary. Prepared for the US Army Corps of Engineers.

Central Michigan University. 2024. Zooplankton of the Great Lakes.

Chen 2024. Use of the Updated LAMFE Model for the Third 5-Year Assessment of the Lower Hillsborough River MFLs. Southwest Florida Water Management District technical report. Brooksville, Florida.

Clarke, KR. 1993. Non-Parametric Multivariate Analyses of Changes in Community Structure. *Austral Ecol.* 18:117–143.

Conde-Vela VM. 2021. Revision of Laeonereis Hartman, 1945 (Annelida: Phyllodocida: Gymnonereidinae), with a review of shaft morphology in nereidids. *J Nat Hist* 55:381–455.

Davis, P.R., Ross, M.A. and Motz, L.H. 2008. Draft peer review panel report - Tampa Pipeline Project. Prepared for the Southwest Florida Water Management District, Brooksville, Florida and the City of Tampa, Florida.

Dodds, WK; Gudder, DA. 1992. The ecology of Cladophora. *J Phycol* 28:415–427.

Ferreire A, Ambrosio E, Capitulo A. 2015. Population ecology of *Sinelobus stanfordi* (Crustacea: Tanaidacea) in a temperate southern microtidal estuary. N Z J Mar Fresh Res 49.

Flannery, S., Morgan, P., and Ashton, J. 2025. BioReconnaissance sampling of macroinvertebrates in the Lower Hillsborough River between the dam and Hannah's Whirl. Technical Memo.

Florida Department of Environmental Protection (FDEP). 2021. Statewide Biological Database: https://floridadep.gov/dear/florida-dep-laboratory/content/statewide-biological-database

Florida Department of Environmental Protection (FDEP). 2017. FDEP SOPs: LT 7000, Biological Communities.

Florida Department of Environmental Protection (FDEP). 2013a. Site-Specific Information in Support of Establishing Numeric Nutrient Criteria for Pensacola Bay.

Florida Department of Environmental Protection (FDEP). 2013b. Site-Specific Information in Support of Establishing Numeric Nutrient Criteria for Perdido Bay.

Florida Department of Environmental Protection (FDEP). 2013c. Site-Specific Information in Support of Establishing Numeric Nutrient Criteria for St. Andrew Bay.

Florida Department of Environmental Protection (FDEP). 2013d. Site-Specific Information in Support of Establishing Numeric Nutrient Criteria in Apalachicola Bay.

Florida Department of Environmental Protection (FDEP). 2013e. Site-Specific Information in Support of Establishing Numeric Nutrient Criteria for Choctawhatchee Bay.

Florida Department of Environmental Protection (FDEP). 2011. Overview of Approaches for Numeric Nutrient Criteria Development in Marine Waters.

Florida Fish and Wildlife Conservation Commission (FWC). 2019. The Fisheries Independent Monitoring Program Procedure Manual. Fisheries Independent Monitoring (FIM). St Peterburg, Florida.

Florida Museum. 2024. Fish database. https://www.floridamuseum.ufl.edu/fish/

Florida Springs Institute. 2024. Springs Field Guide.

Francoeur RB. 2013. Could Sequential Residual Centering Resolve Low Sensitivity in Moderated Regression? Simulations and Cancer Symptom Clusters. *Open J Stat* 03:24–44.

Freshwater Inflows. 2023. Freshwater Inflows: Salinity Effects: https://www.freshwaterinflow.org/salinity-effects/.

Froese, R; Pauly, D. 2023. Fish Base. World Wide Web electronic publication. http://www.fishbase.org.

Grabe, S., and D. Karlen. 1995. Technical Report: Synoptic Survey of Benthic Macroinvertebrates in the Boca Ciega Bay Estuarine System (Pinellas County, Florida). Envoron. Protection Division, Hillsborough Co.

Greenwood, MFD; Peebles, EB; Burghart, SE; MacDonald, TC; Matheson, RE; McMichael, RH. 2006. Freshwater Inflow Effects on Fishes and Invertebrates in the Chassahowitzka River and Estuary.

Griffith, G., Canfield, D., Jr., Horsburgh, C., Omernik, and J. Azevedo, S. 1997. Lake regions of Florida (map). United States Environmental Protection Agency, University of Florida Institute of Food and Agricultural Sciences, Florida Lakewatch, Florida Department of Environmental Protection, and the Florida Lake Management Society. Gainesville and Tallahassee, Florida.

Guenther, CB; MacDonald, TC. 2012. Comparison of Estuarine Salinity Gradients and Associated Nekton Community Change in the Lower St. Johns River Estuary. *Estuaries Coasts* 35:1443–1452.

Heard, R. 1979. Guide to common tidal marsh invertebrates of the northeastern Gulf of Mexico.

Holland, S. 2008. Non-Metric Multidimensional Scaling (MDS).

Holzwart, KR; Ghile, Y; Chen, X; Herrick, G; Deak, K; Miller, J; Basso, R; Leeper, D; Wessel, M; Pribble. R. 2023. Recommended Minimum Flows for the Little Manatee River 2nd Revised Draft Report. *Environmental J.*

Janicki Environmental. 2003. Salinity tolerances for the major biotic components in the Anclote River and anchorage and nearby coastal waters.

Johnson, Mirmiran, & Thompson, Inc. (JMT). 2024. Summary Data Analysis H400 Biological Monitoring for the Lower Hillsborough River 2021-2023. Technical Memo. Prepared for the Southwest Florida Water Management District, Brooksville, Florida.

Jones Edmunds & Associates, Inc. 2024. Morris Bridge Sink Project H404 2023 Annual Report for Water Use. Prepared for SWFWMD.

Leeper, D.A. 2009. Middle Hillsborough River: Water Levels, Water Quality, and Water Management. Report of the Southwest Florida Water Management District, Brooksville Florida.

Littell, R. C., G.A. Milliken, W.W. Stroup and R. D. Wolfinger. 1996. *SAS System for Mixed Models*. Cary, N.C.: SAS Institute Inc.

Little S, Wood PJ, Elliott M. 2017. Quantifying salinity-induced changes on estuarine benthic fauna: The potential implications of climate change. Estuar Coast Shelf Sci 198:610–625.

MacDonald, TC; Peebles, EB; Greenwood, MFD; Matheson, RE; McMichael, RH. 2006. Freshwater Inflow Effects on Fishes and Invertebrates in the Hillsborough River Estuary.

MARIMBA. 2024. Clytia hemisphaerica. Mar Invertebr Model Database. https://voices.uchicago.edu/jocelynmalamylab/clytia-resources/

McCune, B; Grace, JB. 2002. Analysis of Ecological Communities. MjM Software Design, Gleneden Beach, OR.

McElreath, R. 2020. Statistical rethinking: A Bayesian Course with Examples in R and Stan. Chapman and Hall/CRC.

Merritt, RW; Cummins, KW. 1978. Introduction to the Aquatic Insects of North America.

MHW Americas, Inc. 2011. Memorandum to Brian Pickard, PE/City of Tampa dated April 18, 2011. Subject: Lower Hillsborough recovery strategy status and minimum flow evaluation technical memorandum. Tampa, Florida.

Milligan, GW. 1989. A Study of the Beta-Flexible Clustering Method. *Multivar Behav Res* 24:163–176.

Montagna, P; Powell, GL; Boyer, JN. 2007. Scientific Peer Review of the Lower Hillsborough River Low Flow Study Results and Minimum Flow Recommendation.

Ocean Biodiversity Information System. 2023. Taxa Information: https://obis.org/.

Odum, WE; Smith, TJ; Hoover, JK; McIvor, CC. 1984. The Ecology of Tidal Freshwater Marshes of the United States East Coast: a Community Profile.

Oregon Flora. 2024. Oregon Flora: https://oregonflora.org/

Peebles, EB. 2005. An Analysis of Freshwater Inflow Effects on Early Stages of Fish and Their Invertebrate Prey in the Alafia Estuary.

Peebles, EB. 2008. Freshwater Inflow Effects on Fishes and Invertebrates in the Little Manatee River Estuary; an Update of Data Analyses.

R Core Development Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Schielzeth, H; Dingemanse, NJ; Nakagawa, S; Westneat, DF; Allegue, H; Teplitsky, C; Réale, D; Dochtermann, NA; Garamszegi, LZ; Araya-Ajoy, YG. 2020. Robustness of Linear Mixed-Effects Models to Violations of Distributional Assumptions. *Methods Ecol Evol*.

Schreuder, 2004. Schreuder, Inc., *Hydrogeological Investigation of the Blue Sink Complex, Tampa, Florida – Phase 3.* January 2004.

Schreuder, 2001. Schreuder, Inc., *Hydrogeological Investigation of the Blue Sink Complex, Tampa, Florida – Phase 2.* June 2001.

Schreuder, 1999. Schreuder, Inc., *Hydrogeological Investigation of the Blue Sink Complex, Tampa, Florida.* October 1999.

Science Direct. 2024. Naididae: https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/naididae

Scripps. 2024. Zooplankton Guide: http://sio-legacy.ucsd.edu/zooplanktonguide/welcome-zooplankton-san-diego-region

Sharping, R; Garman, M; Henry, R; Eswara, P; Garey, J. 2018. The fate of urban springs: Pumping-induced seawater intrusion in a phreatic cave. https://doi.org/10.1016/j.jhydrol.2018.07.016.

Singh, W; Hjorleifsson, E. Stefansson, G. 2011. Robustness of Fish Assemblages Derived from Three Hierarchical Agglomerative Clustering Algorithms Performed on Icelandic Groundfish Survey Data. *ICES J Mar Sci* 68:189–200.

Smithsonian. 2023. NEMISIS Species Profiles: https://invasions.si.edu/nemesis/all_taxa.

SOA. 2024. Seaweeds of Alaska: https://www.seaweedsofalaska.com/misc_plants.asp.

Southwest Florida Water Management District (SWFWMD), 2022. Southwest Florida Water Management District, Environmental Flows and Levels Section. Technical Memorandum by Ron Basso and Jill Qi. An Evaluation of the need for future Ewanowski Spring Pool Stage Data. April 25, 2022

Southwest Florida Water Management District (SWFWMD) and Water & Air Research, Inc. (WAR). 2020. A hydrobiological assessment of the Minimum Flows for the Lower Hillsborough River for the Second Five-Year Assessment Period - October 2012 to May 2018. Brooksville, Florida.

Southwest Florida Water Management District (SWFWMD) and Atkins, North America, Inc. 2015. A hydrobiological assessment of the phased implementation of minimum flows for the lower Hillsborough River. Brooksville, Florida.

Southwest Florida Water Management District. (SWFWMD) 2015. Lower Hillsborough River recovery strategy implementation - five-year assessment. Submit and file report to the Resource Management Committee. Governing Board meeting agenda and meeting information, March 31, 2015. Brooksville, Florida.

Southwest Florida Water Management District (SWFWMD). January 2010. Results of Morris Bridge Sink Pumping Test, Hillsborough County, FL.

Southwest Florida Water Management District (SWFWMD), 2009. Southwest Florida Water Management District, Hydrologic Evaluation Section. “Results of Blue Sink Pumping Test No. 2, Hillsborough County, Florida.” September 2009.

Southwest Florida Water Management District (SWFWMD). 2008. Lower Hillsborough River minimum flows recovery strategy - implementation. Included in the significant water resource and development projects routine report to the Resource Management Committee. Governing Board meeting agenda and meeting information, November 18, 2008. Brooksville, Florida.

Southwest Florida Water Management District (SWFWMD) 2007. Southwest Florida Water Management District, Ecologic Evaluation Section. Technical Memorandum by Doug Leeper and Richard Grant on Ewanowski Spring and Blue Sink. May 31. 2007.

Southwest Florida Water Management District (SWFWMD). 2007. Proposed Minimum Flows and Levels for Dona Bay/Shakett Creek below Cow Pen Slough Appendices.

Southwest Florida Water Management District (SWFWMD). 2006. Lower Hillsborough River Low Flow Study Results and Minimum Flow Recommendation, Draft – August 31, 2006.

Southwest Florida Water Management District. 2004. The Determination of Minimum Flows for Sulphur Springs, Tampa Florida. Report of the Southwest Florida Water Management District, Brooksville Florida.

Southwest Florida Water Management District (SWFWMD). June 15, 1999. An Analysis of Hydrologic and Ecological Factors Related to the Establishment of Minimum Flows for the Hillsborough River.

Stevenson, JR; Pinowska, A; Albertin, A; Sickman, JO. 2007. Ecological Condition of Algae and Nutrients in Florida Springs: The Synthesis Report.

Tampa Bypass Canal/Alafia River Water Supply Projects. no date.

Tolley, SG; Fugate, D; Parson, M; Burghart, SE; Peebles, EB. 2010. The Responses Of Turbidity, CDOM, Benthic Microalgae, Phytoplankton and Zooplankton to Variation in Seasonal Freshwater Inflow to the Caloosahatchee Estuary.

UF Center for Aquatic and Invasive Plants. 2024. UF Center for Aquatic and Invasive Plants: https://plants.ifas.ufl.edu/.

University of Washington. 2024. Invertebrates in the Plankton: Chaetognatha: https://depts.washington.edu/fhl/zoo432/plankton/plchaetognatha/chaetognatha.html#:~:text=Invertebrates%20in%20the%20Plankton%3A%20Chaetognatha,%2C%20crustaceans%2C%20and%20other%20chaetognaths.

US Department of Agriculture (USDA)/Wildlife Services/National Wildlife Research Center. 2009. Implementation and evaluation of management tools to reduce feral swine damage to sensitive plant sites at Avon Park AFR, Florida. Annual Report, Year 1 to U.S. DoD/Avon Park Air Force Range

US Fish and Wildlife Service (USFWS). 2023. Ecological Risk Screening Summaries: Species Accounts.

US Fish and Wildlife Service (USFWS). 2016. Eastern Mosquitofish (*Gambusia holbrooki*) Ecological Risk Screening Summary.

US Geological Survey (USGS). 2024. Nonindigenous Aquatic Species.

University of the West Indies (UWI). 2024. The Online Guide to the Animals of Trinidad and Tobago: https://sta.uwi.edu/fst/lifesciences/animals-trinidad-tobago.

Water & Air Research (WAR). 2020. A Hydrobiological Evaluation of the Minimum Flows for the Lower Hillsborough River for the Second Five-Year Assessment Period – October 2012 to May 2018.

Weber, C. 2018. Technical Memorandum to Eric DeHaven, PG, Southwest Florida Water Management District, dated October 30, 2018. Subject: Summary report on the Investigation of Additional Water Supply Options (H400). City of Tampa Water Department. Tampa, Florida.

Weisberg, SB; Ranasinghe, A; Dauer, D; Schaffner, L. 1997. An Estuarine Benthic Index of Biotic Integrity (B-IBI) for Chesapeake Bay. Est Coasts 20:149–158.

Wood, S.N. 2017. Generalized Additive Models: An Introduction with R (2nd edition). CRC/Taylor & Francis. https://www.maths.ed.ac.uk/~swood34/

World Register Marine Species 2023. Taxa Details: https://www.marinespecies.org/aphia.php?p=taxdetails&id=131191.

Zar JH 1998. Biostatistical Analyses. Fourth Edition. Prentice Hall, NJ.

Appendix A

Minimum Flow Rule

Appendix B

Recovery and Prevention Strategies for Minimum Flows and Levels

Appendix C

Results of Blue Sink Pumping Test No. 2 (SWFWMD 2009)

Appendix D

An Evaluation of the need for future Ewanowski Spring Pool Stage Data (SWFWMD 2022)

Appendix E

Results of Morris Bridge Sink Pumping Test (SWFWMD 2010)

Appendix F

Draft Peer Review Panel Report – Tampa Pipeline Project (Davis et al. 2008)

Appendix G

City of Tampa 1.9-MGD Source Identification

Appendix H

Summary Report on the Investigation of Additional Water Supply Options (Weber 2018)

Appendix I1

Flow Raw Data Source Description

Appendix I2A

Continuous Recorder Raw Data Source Description

Appendix I2B

Continuous Recorder Raw Data Plots

Appendix I3

Water Quality Raw Data Source Description

Appendix I4

Zooplankton Raw Data Source Description

Appendix I5

Benthics Raw Data Source Description

Appendix I6

Nekton Raw Data Source Description

Appendix J

Use of the Updated LAMFE Model for the Third 5-Year Assessment of the Lower Hillsborough River MFL (Chen 2024)

Appendix K

Morris Bridge Sink Project H404 2023 Annual Report

Appendix L
Recovery Source Water Quality Plots
(Analysis Days)

Appendix M

Water Quality Descriptive Plots and Statistics by Station (Analysis Days)

Appendix N

Descriptive Statistics Breakdowns by Target Zone River Segment, Water Column Strata, and Period

(Analysis Days)

Appendix O

Biological Community Metrics Filtered for Salinity Sensitive Species

Appendix P

Biological Community Metrics No Filtering

Appendix Q1

Water Quality Modeling Results

Linear Regression Summary

Appendix Q2

Water Quality Modeling Results

Logistic Regression Summary

Appendix Q3

Water Quality Modeling Results

GAM Model Subsets

Appendix Q4

Water Quality Modeling Results

GAM Model Chapter 6 Detailed Results