Savannah River NUCLEAR SOLUTIONS℠

2025 Periodic Report on the Bioassessment of Savannah River Site Streams

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EXECUTIVE SUMMARY

The Savannah River Site (SRS) has employed an Integrator Operable Unit (IOU) program that uses bioassessments to understand the conditions of stream ecosystems that drain different watersheds on the SRS. Bioassessments use information about stream organisms and habitat quality to determine whether a stream can maintain biological communities and stream ecology on par with undisturbed streams. The IOU program at SRS evaluates fish and aquatic macroinvertebrate assemblage bioassessments and stream habitat quality to determine if historically and currently disturbed streams on the SRS are supporting stream ecosystem processes and have biological integrity equivalent to that of undisturbed SRS streams. To determine this, three groups of bioassessment indices are used: 1) the Index of Biotic Integrity (IBI) determined from fish assemblage data that are obtained through electrofishing, 2) the Multiple Habitat Sampling Protocol (MHSP) index determined from macroinvertebrates collected from natural stream habitats, and 3) the number of water-quality sensitive Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa and a Biotic Index value both determined from macroinvertebrates collected from Hester-Dendy artificial substrates deployed in streams. Using these indices, stream locations upgradient (upstream of SRS impacts), downgradient (downstream of SRS impacts), and the lowest reaches of streams (far enough downstream for potential buffering from SRS impacts) were compared. Bioassessments at the SRS have been conducted occasionally starting from 1996 with additional fish data that could be used in bioassessments collected in 1990-1995. The objective of this report is to integrate data collected in the most recent bioassessment (2022/2023) with the prior SRS stream bioassessment report: SRNS-STI-2018-00492 to evaluate the biotic integrity of SRS streams. Lower Three Runs is not included in this report.

The biotic integrity of many downgradient streams at the SRS indicates that they now ~~represent~~host fish and macroinvertebrate communities ~~levels~~ of similar quality to those found in streams upgradient of SRS impacts. In agreement with the findings from 2017, these streams with biotic integrity similar to upgradient stream locations include Indian Grave Branch, the lower portion of Steel Creek, and the lower portion of Pen Branch. Fourmile Branch indicates improvement based on fish IBI metrics and Hester-Dendy artificial substrate EPT data, but the other macroinvertebrate metrics indicate downgradient locations on Fourmile Branch continue to

fall below upgradient levels. These streams and their floodplains were previously damaged by extremely hot reactor cooling water from reactor operations which ceased in 1988 at the latest. Based on this report analysis, which has few upgradient sites to establish the threshold for biotic integrity (one to three sites in 2022), these streams generally have improved to support biotic integrity and ecological processes.

Streams of continued concern may have lower biotic integrity due to habitat quality or organism access. Tims Branch has low biotic integrity and continues to show little improvement based on the fish and macroinvertebrate bioassessment metrics. As the prior report indicated, the low biotic integrity cause has not been confirmed but may be due to formation of extensive iron flocculants associated with ground water upwelling in the study reach and insufficient habitat quality. Crouch Branch also indicates lower biotic integrity possibly due to its low habitat quality of the stream channel due to excessive stormwater flows as bioassessments based on natural habitats (fish IBI and macroinvertebrate MHSP) did not yield scores within the upgradient range, while the macroinvertebrate bioassessment using artificial substrates (Hester-Dendy samplers) did occur in the upgradient range. Upper Steel Creek also continued a trend of poor fish IBI scores that contrasted with macroinvertebrate scores equivalent to upgradient sites. Because the location of L Lake which formerly served as a reactor cooling reservoir isolates upper Steel Creek, fishes may not be able to recolonize that stream reach even though macroinvertebrate metrics suggest it could support more natural fish communities. Previous fish tissue studies did not indicate that metal contaminants would be responsible for the low IBI scores in upper Steel Creek.

The biotic integrity of several streams was challenging to interpret because they had few sampling data points or inconsistent locations. These streams included Meyers Branch, McQueen Branch, and Crouch Branch. The small sample size of upgradient locations also reduced the confidence in robustly identifying an upgradient target threshold.

Recommendations are for continued monitoring and assessment of SRS IOUs to determine conditions and long-term trends. For future efforts, specific recommendations include increasing the consistency of sampling locations, ensuring a standard time of season for sampling, evaluating a transition to habitat-based rather than time-based macroinvertebrate assemblage sampling, and

incorporating ecological principles to the evaluation of the fishes IBI metrics. An additional recommendation is for a walk-down of Meyers Branch (Steel Creek (SC) IOU) to assess stormwater impacts.

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LIST OF ACRONYMS AND ABBREVIATIONS

ABS	aquatic biology section assessment
ac	acres
BI	biotic index
BRA	baseline risk assessment
CB	Crouch Branch
c~~C~~m	centimeters
CMP	Chemicals, Metals, and Pesticides
CI	Confidence Interval
CSM	Conceptual Site Model
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
D	downgradient
EPT	Ephemeroptera, Plecoptera, Trichoptera
FFA	Federal Facility Agreement
FMB	Fourmile Branch
G	G-Rank (NatureServe Global)
ha	hectare
IBI	index of biotic integrity
in	inch
IGB	Indian Grave Branch
IOU	integrator operable unit
km / km^2	kilometer / square kilometer
L	lower reaches
LLC	Limited Liability Company
LTR	Lower Three Runs
m/m^2	meters/square meters
MC	Mill Creek
MHSP	multiple habitat sampling protocol
mi / mi^2	miles / square miles
MQ	McQueen Branch
NA	not applicable
NBN	no building number
NPDES	National Pollutant Discharge Elimination System
OU	operable unit
PB	Pen Branch
PR	Periodic Reports
RCRA	Resource Conservation and Recovery Act
RI	remedial investigation
ROD	Record of Decision
RSCGN	species of greatest conservation need within the southeast region
S	S-Rank (State)
SC	Steel Creek

LIST OF ACRONYMS AND ABBREVIATIONS *(continued/end)*

SCDES[1]	South Carolina Department of Environmental Services
SCDHEC	South Carolina Department of Health and Environmental Control
SGCN	species of greatest conservation need within the state
SNR	unranked; conservation status not yet assessed by SCDNR
SREL	Savannah River Ecology Lab
SRS	Savannah River Site
SWAP	South Carolina's State Wildlife Action Plan
TB	Tims Branch
TC	Tinker Creek
U	Upgradient
USDOE	United States Department of Energy
USEPA	United States Environmental Protection Agency
UTR	Upper Three Runs
WSRC	Westinghouse Savannah River Company
yd / yd^2	yard / square yards

[1] South Carolina Department of Environmental Services (SCDES) was known as South Carolina Department of Health and Environmental Control prior to July 1, 2024.

1.0 INTRODUCTION

The Savannah River Site (SRS) was constructed during the 1950s to produce material used in the fabrication of nuclear weapons, primarily tritium and plutonium, in support of our nation's defense programs. SRS has provided nuclear materials for the space program, as well as for medical, industrial, and research efforts up to the present. Chemical and radioactive wastes are by-products of nuclear material production processes. These wastes have been treated, stored, and in some cases, disposed of at SRS. Past disposal practices (e.g. seepage basins, pits and piles, landfills, untreated release, etc.) have resulted in soil and groundwater contamination.

SRS occupies approximately 802.9 square kilometers (km^2) (310 square miles [mi^2]) of land adjacent to the Savannah River, principally in Aiken and Barnwell counties of South Carolina. SRS is located approximate 40 kilometers (km) (25 miles [mi]) southeast of Augusta, Georgia and 32 km (20 mi) south of Aiken, South Carolina. Approximately 90 percent of SRS land consists of natural and managed forests. The locations at SRS where nuclear materials were produced, stored, and disposed are clustered into distinct industrial areas that are separated by large areas of forest. Waste units and operable units (OUs) are generally contained within, or adjacent to, these industrial areas.

The Integrator Operable Unit (IOU) program was initiated to understand how contaminants from SRS locations, including contaminated streams, waste units, OUs, and SRS industrial activities could influence the ecology of local environments. Since OUs are located within the watershed of SRS water bodies and contaminants from wastewater can be transported into water bodies by overland and subsurface water flow, the water bodies become an "integrator" of disturbances arising from land use within the watersheds and as such are listed as IOUs (Figure 1).

To understand the transport and fate of these contaminants within water bodies on Site, and potentially off Site, chemical and biological approaches can be used. For a chemical approach, extensive suites of chemicals can be monitored within water bodies of each IOU, but the conclusions this approach can be strongly dependent on the timing of sample collection (for example during a precipitation event that remobilizes contaminants or during high flow that dilutes contaminants) and on the suite of chemical compounds measured.

Measuring biological stream communities provides another source of information about longer term ecological effects of contaminants in waterbodies. Bioindicators are valuable tools that can be used to directly evaluate the effects of stream disturbance to aquatic organisms at the individual, population and ecosystem levels (Parmar et al. 2016). Biota exposed to contaminants can integrate the effects of that exposure over time and taxa differ in their sensitivity to pollution and other environmental disturbances (USEPA 1989). The structure of biological communities also serves as an indicator of stream ecological health because it shows the potential for different trophic levels such as primary and secondary consumers to persist in an environment. Thus, using bioassessments one can evaluate the "ability of a stream to support self-sustaining biological communities and ecological processes typical of undisturbed, natural conditions" (Paller and Blas 2018). Typically, assemblages of multiple taxa, such as fish, macroinvertebrates, and periphyton are examined to improve accuracy (e.g. Barbour et al. 1997). Stream bioassessments are a commonly used and validated tool to understand the presence of degraded environmental conditions from poor water quality. Frequently, assessments of biotic community taxa are paired with habitat assessments to understand the potential for habitat availability and quality to influence the biological community results.

The consequences of SRS waste sites for local stream ecological health have been evaluated employing bioassessments for the IOU program using fish assemblages since 1990 and macroinvertebrate communities since 1997. Bioassessment of fish has been based on the Index of Biotic Integrity (IBI) method that uses fish assemblage data from backpack electrofishing. The IBI considers multiple organismal, population, and community level metrics and assesses the ability of a stream to maintain a self-supporting fish community and associated ecological processes (Karr et al. 1986). On the SRS, the IBI method has been demonstrated to accurately differentiate between disturbed and undisturbed sites (Paller et al. 1996). Benthic macroinvertebrates have long been recognized as critical to the functional integrity of aquatic ecosystems and have, thus, been effectively used to monitor factors such as physical habitat alterations and/or pollution affecting stream health (Pallottini et al. 2017, Paller et al. 2020). On the Savannah River Site, the IOU program has effectively used macroinvertebrate bioassessment from South Carolina's Multiple Habitat Sampling Protocol method (MHSP) and deployment of Hester-Dendy artificial substrates to provide critical data on the biotic integrity of streams.

The objectives of this report are to 1) present results from bioassessments conducted in 2022/2023, 2) compare trajectories of stream biological recovery since the previous report, which covered the time period from 1990-2017 (Paller and Blas 2018), 3) compare biotic integrity among SRS streams, 4) update stream reach designation categories to reflect ongoing SRS activities, and 5) recommend modifications to the bioassessment monitoring to improve implementation of the bioassessments. Other reports contain contaminant data summaries and contaminant exposure modeling information (e.g., Paller et al. 2008).

2.0 BACKGROUND

The SRS is divided into six watersheds that align with the stream systems that transverse the SRS including a portion of the Savannah River adjacent to the SRS (Figure 1). The SRS stream systems were added to the Federal Facility Agreement (FFA) in 1997 (FFA 1993). The SRS manages and monitors six IOUs: Upper Three Runs (UTR), Fourmile Branch (FMB), Pen Branch (PB), Steel Creek (SC), Lower Three Runs (LTR), and the Savannah River and Floodplain Swamp (SRFS) that correspond to the surface water bodies (e.g., streams and pond/reservoirs) and associated wetlands within each watershed. These IOUs include the surface water, sediment/soil, and related biota associated with the major stream systems on the site. The term "Integrator Operable Unit" is used because these surface water bodies and associated wetlands could receive and integrate contamination from multiple sources including Resource Conservation and Recovery Act (RCRA)/Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) OUs, Site Evaluation Areas, National Pollutant Discharge Elimination System (NPDES) outfalls, stormwater, and operational facilities. SRS monitors the IOUs because they represent a possible pathway for the release of contamination from SRS activities to human health and ecological receptors.

The SRS IOU program is implemented in three phases to support a final CERCLA Record of Decision (ROD) for each IOU as follows:

- Phase I of the IOU program consisted of an assessment of all existing data to determine if early actions were necessary and to define additional data needs to assess potential impacts to the

IOUs from inactive waste units. Remedial Investigation (RI) Work Plans have been approved for all six IOUs, and Phase I of the IOU program is complete.

- Phase II of the IOU program consists of ongoing sampling and assessment of the IOUs and refinement of the conceptual site model (CSM) continuing in parallel with the OU investigations within each watershed. Figure 2 is a generalized CSM for the IOUs. Prior to the 2019 optimization of the IOU program, the status of each IOU during Phase II was documented through submittal of Periodic Reports (PRs) to the United States Environmental Protection Agency (USEPA) and South Carolina Department of Environmental Services (SCDES) for review and approval. Information in the PRs included a full assessment of the IOU including unreported data, wildlife surveys, OU sampling as it impacts the IOUs, newly identified OUs, an early action evaluation, refined CSM, a data needs evaluation, and a screening level benchmark evaluation to assess contaminant threats to human health and ecological receptors. Phase II concludes when the OU investigations specific to each IOU are complete. Phase II is complete for the LTR IOU and ongoing for the remaining five IOUs.

- In Phase III, each IOU is addressed holistically for a final cleanup decision. Phase III includes an assessment of available data, development of the RI/Baseline Risk Assessment (BRA), and completion of the RI/Feasibility Study process through the ROD, and remedial actions, if warranted. Phase III is complete for the LTR IOU and ongoing for the remaining five IOUs.

Most of the data compiled and reported by the IOU program in recent Phase II implementation is collected by other data stewards and reported under other SRS programs. Scoping of Phase III for the LTR IOU revealed that a significant amount of Phase II historical data from other data stewards and screening level data were not appropriate for use in the quantitative development of the RI/BRA, and extensive characterization was required to support Phase III. For this reason, a scoping meeting was held with the Core Team on March 21, 2019, to discuss optimization of Phase II of the IOU program with respect to future data collection and reporting requirements for the five remaining IOUs in Phase II (SRNS 2019). The Core Team agreed to submit a single Bioassessment Report based on biological data every seven years in place of individual IOU Phase II PRs to continue monitoring the potential impact of waste units and operating facilities on the IOUs. Agreement was reached that the Core Team would be advised should a major change to site

conditions occur as indicated by ongoing SRS environmental monitoring or the SCDES monitoring program to allow for discussion of future IOU data needs and reporting

3.0 INTEGRATOR OPERABLE UNITS

The IOUs addressed in this report include UTR IOU, FMB IOU, PB IOU, and SC IOU (Figure 1). The LTR IOU is excluded from bioassessment monitoring since the Phase III final remedial action for the LTR IOU has been achieved. A final ROD was issued for the LTR IOU in 2021 (SRNS 2021). The SRFSis an SRS IOU but was not included in this bioassessment because the Savannah River is not a wadable stream and the assessment protocols established for small streams would not be applicable. The furthest downstream sampling locations on each of the major SRS streams included in this report are located on fluvial terraces of the Savannah River that lie between the upland area and the active Savannah River floodplain that includes the swamp. These sites indicate the stream biotic integrity at those locations just upstream of the Savannah River swamp/floodplain. While the same biotic integrity may persist downstream of these lowest sampling locations it is not confirmed by this report. As these streams enter the Savannah River floodplain, there is potential for biological and physical remobilization of contaminants stored in sediments of the swamp/floodplain which could reduce biotic integrity in these streams before they enter Savannah River. However, the potential of contaminants reaching the Savannah River and its biota is included in the Annual SRS Environmental Report.

Because the IOUs are large stream systems, they are partitioned into subunits that correspond to portions of a stream that may differ in exposure to contamination or distinctive SRS activities within the watershed. These partitions can reveal consequences from more specific current or historical activity locations on SRS as well as the potential for downstream recovery through dilution or distance. A few subunits of the PB IOU and UTR IOU have no waste sites or industrial facilities within their watershed boundaries and are relatively undisturbed by SRS operations. These relatively undisturbed subunits were designated as “reference sites” prior to the 2025 Bioassessment Report. In the 2025 Bioassessment Report, many of these “reference” sites are now designated as “upgradient” of SRS operations unless the potential for contamination from SRS activities has been identified. Sites downstream of SRS industrial areas but residing onsite and thus potentially impacted by SRS activities were designated as “downgradient” sites. Stream

reaches located far enough downstream of SRS facilities to be potentially buffered from continuous disturbances from SRS industrial facilities were designated "lowest reaches." Bioassessment locations and subunit designations are provided in Figure 3.

3.1 Upper Three Runs Integrator Operable Unit

The UTR IOU encompasses a large area that includes portions of Aiken and Barnwell counties located outside of the SRS (Figure 4). Approximately 250 km^2 (96.5 mi^2) of the UTR watershed is within the SRS boundary. Tributaries of UTR located within the SRS include Tinker Creek, Tims Branch, Crouch Branch, and McQueen Branch. Mill Creek and Reedy Branch are also located within the UTR IOU, but these streams discharge into UTR's main tributary, Tinker Creek. Much of UTR IOU is upstream from SRS industrial areas. Similarly, Tinker Creek and Mill Creek are largely undisturbed by SRS operations. Steed Pond (which received historical uranium and nickel discharges from M Area) lies within Tims Branch.

Upper Three Runs has never received thermal or radiological discharges from SRS reactors. Above its confluence with Tinker Creek, Upper Three Runs is relatively unimpacted by SRS activities. Due to the ecological uniqueness of Upper Three Runs, portions of the stream have been protected from site discharges, and it is currently managed and protected by a United States Department of Energy (USDOE) Stream Management Policy.

3.2 Fourmile Branch Integrator Operable Unit

The FMB IOU originates near the center of SRS and follows a southwesterly direction for approximately 24 km (14.9 mi) (Figure 5). At its headwaters, Fourmile Branch is a small blackwater stream unimpacted by historical SRS operations. The watershed drains about 57 km^2 (22 mi^2) and includes several SRS facility areas including C Area (C Reactor), N Area (Central Shops), and F, H, and E Areas (General Separations Area). Except for the extreme headwaters, most of FMB is potentially influenced by SRS discharges and industrial operations. FMB also received large volumes of heated cooling water from C Reactor in the past, causing extensive habitat destruction. Recovery and recolonization of FMB began in 1985 with the shutdown of C Reactor, and secondary succession is now well underway in the FMB stream channel and riparian zone.

3.3 Pen Branch Integrator Operable Unit

The PB IOU includes Pen Branch and its tributary, Indian Grave Branch, located entirely within the SRS boundary (Figure 5). Pen Branch terminates in the swamp and does not have a clearly defined channel through the swamp to the Savannah River. Except for its extreme headwaters, nearly all Indian Grave Branch has been affected by operation of K Reactor. Heated cooling water from K Reactor entered Indian Grave Branch and flowed into Pen Branch causing the same type of thermal habitat destruction in lower Pen Branch as in Fourmile Branch. Recovery began in 1988 with the cessation of K Reactor operations. Upper and middle Pen Branch are largely undisturbed by SRS operations.

3.4 Steel Creek Integrator Operable Unit

The SC IOU includes Steel Creek and its major tributary, Meyers Branch (Figure 6). Steel Creek originates near P Reactor and flows approximately 3 km (1.9 mi) before entering L Lake, a 7 km (4.3 mi) long, 400 hectare (ha) (988 acre [ac]) cooling reservoir constructed in 1985. Water discharged from L Lake dam enters the lower reach of Steel Creek, flows approximately 5 km (3 mi) to the Savannah River swamp, and then flows about another 2 km (1 mi) through the SRFS to the Savannah River. All of Steel Creek is potentially affected by SRS discharges, waste sites, and/or industrial operations. Steel Creek suffered extensive habitat degradation from the discharge of high temperature reactor cooling water during 1954 to 1968. Recovery began in 1986 subsequent to construction of L Lake. Meyers Branch, which intersects Steel Creek just downstream of L Lake, is largely unaffected by SRS operations.

3.5 Savannah River and Floodplain Swamp Integrator Operable Unit

The Savannah River provides SRS its western boundary for a 56-km (35-mi) stretch from the upstream boundary of the site near Jackson, South Carolina to the southern boundary of the Lower Three Runs corridor. The five major SRS streams feed directly into the river or through the Savannah River Swamp (Upper Three Runs, Fourmile Branch, Pen Branch, Steel Creek, and Lower Three Runs). SRS is approximately 258 river-km (160 river-mi) from the Atlantic Ocean. The SRFS IOU (Figure 1) includes an approximate 72 km (45 mi) stretch along the Savannah

River from the northern boundary of SRS near Jackson, South Carolina southward to the U.S. Highway 301 bridge.

The SRFS IOU is not included in the site-wide bioassessment monitoring at this time because the bioassessment monitoring program assesses the health of wadable stream systems and is not applicable to the Savannah River or Savannah River Swamp. Additionally, the lower portion of the SRS stream systems that ultimately discharge to the Savannah River are considered high integrity/healthy streams. Bioassessment monitoring continues to be collected from the SRFS IOU during Phase II of the IOU program through the Annual Savannah River Site Environmental Report monitoring. The data collected consists of freshwater fish samples that are analyzed for metals (antimony, arsenic, cadmium, chromium, copper, lead, manganese, mercury, nickel, and zinc) and radionuclides (gross alpha, gross beta, cesium-137 and cobalt-60, strontium-90, technetium-99, and iodine-129). Sampling locations include upgradient of the SRS at the New Savannah Bluff Lock and Dam, at the mouth of UTR, FMB, SC, LTR, and the US Highway 301 Bridge. Results of the fish sampling compare current levels to historical levels.

4.0 METHODS AND MATERIALS

4.1 Study Area

The SRS covers parts of Aiken, Allendale, and Barnwell counties in South Carolina and is within the Sandhills ecoregion. The Sandhills ecoregion is a narrow physiographic region in the Upper Coastal Plain that borders the Piedmont along the fall line and covers ~26,000 km^2 (10,039 mi^2) of the southeastern United States (Markewich and Markewich 1994; Schmidt 2013). Historically, the region was dominated by the longleaf pine ecosystem characterized by longleaf pines (*Pinus palustris*), turkey oaks (*Quercus cerris*), and an extensive herbaceous layer. Regular, low-intensity fire maintained a more open savannah-like ecosystem (Markewich and Markewich 1994; Schmidt 2013). On the SRS, and in the Sandhills more broadly, many of the larger streams are considered "blackwater." Blackwater rivers and streams tend to be low-gradient with slow flow and are darkly colored due to the high amounts of tannins from decaying vegetation (Sabater et al. 1993). Upper Three Runs and its tributaries can be classified as blackwater streams. However, not all SRS streams have these characteristics. Streams on the SRS tend to have debris dams from accumulated

woody material that provide aquatic habitat and alter detrital dynamics and geomorphology (Benke and Meyer 1988). Previous research has demonstrated that many features of the streams (e.g., size, instream structure, channel morphometry, water chemistry) affect the fish and macroinvertebrate communities (Sheldon 1968; Paller 1994; Paller et al. 2016). Overall, SRS streams have high biodiversity relative to other streams within the Sandhills ecoregion (Paller 2018). This report includes historical and current sampling of fish and macroinvertebrates across 43 locations within eleven SRS streams (Table 1).

4.2 Upgradient, Downgradient, and Lowest Reaches sites designations

Classification of stream sample collection locations was changed for this report compared with those in the 2018 report (Paller and Blas 2018). Rather than attempting to identify reference sites, we classify "upgradient sites" (U) as those located upstream of potential SRS-sourced impacts. Correspondingly, any sampled stream reach occurring downstream of SRS developed areas and, thus, susceptible to SRS impacts, are now labeled as "downgradient" sites (D). CSMs were developed to aid in these classifications (Figures 7 through 14). Stream reaches located far enough distances downstream of SRS facilities to be potentially buffered from continued disturbance from SRS activities, and which consequently may be recovering from previous disturbance, are now designated "lowest reaches" (L). The lowest reaches sites all occur on fluvial terraces of the Savannah River and are located over 1 km (0.62 mi) downstream from where road SC Highway 125 crosses the stream. Sites near the SC Highway 125 crossings, specifically FMB6 that is only 2.5 km (1.55 mi) below potential impacts from Castor Creek and site SC5 that is less than 2 km (1.24 mi) below L Lake, are both listed as downgradient.

The upgradient site ranges were determined as the upper 95% of upgradient site scores (i.e., the lowest 5% of upgradient site values for all years combined were excluded). IBI and MHSP required only one upgradient data range due to stream size correction of those datasets. For Hester-Dendy sample data, the upper 95% of upgradient site scores were determined separately for small (stream width < 5 m) and large (stream width > 5 m) stream sizes. Excluding the lowest 5% of the upgradient site range was done to avoid inclusion of atypical index values that may arise due to factors such as sampling error. Meyers Branch above Rd 6.2 (MB0) was excluded due to apparent recent habitat degradation which site coordinates show in an ephemeral ditch and Fourmile Branch

at Road F (FMB1) was excluded because it was determined to be in a reach of intermittent stream. Periodic natural drying would make this site incomparable to all of the other perennial stream reach sites.

4.3 Evaluated Integrator Operable Units

4.3.1 Upper Three Runs IOU

The UTR IOU encompasses a large area that includes portions of Aiken and Barnwell counties located outside of the SRS (Figure 4). Approximately 250 km^2 (~97 mi^2) of the UTR watershed is within the SRS. Tributaries of Upper Three Runs located entirely within the SRS include Tims Branch, Crouch Branch and numerous unnamed tributaries. While a large portion of the Tinker Creek drainage lies off of the SRS, some of its tributaries including McQueen Branch, Mill Creek, and Reedy Branch are located entirely within the SRS boundaries. CSMs of the UTR and its tributaries are shown in Figures 7 through 11. Additionally, numerous unnamed perennial tributaries drain directly into Upper Three Runs and its tributaries. There are several SRS operational facilities and OUs within the UTR IOU (WSRC 1998d) (Figure 7), but much of Upper Three Runs is upstream of the SRS and its industrial areas (Figure 4). Tims Branch, Crouch Branch, and McQueen Branch and various unnamed tributaries receive varying amounts of runoff and/or effluents from SRS operational facilities. To a lesser extent, Mill Creek, and potentially Reedy Branch, and several unnamed tributaries may receive discharges from various OUs within their respective drainage basins. The effect of the runoff/effluents correspondingly varies greatly among streams. The Tinker Creek drainage, upstream of Reedy Branch, is largely undisturbed by SRS operations and, thus, designated as upgradient (Figure 4, Figure 8). Occurrence of SRS facilities in the headwaters of Crouch Branch, McQueen Branch, Mill Creek, and Tims Branch results in the entire drainage for each of these small drainages being classified as downgradient (Figures 9 through 11). Reedy Branch would only be a downgradient tributary downstream to the ATTA range. The headwaters/upper portion of the watershed is located outside of the SRS boundary. Several chemical constituents of potential concern from human health or ecological perspectives have been found in water and/or sediments within portions of the UTR IOU (Paller et al. 2008).

4.3.2 Fourmile Branch IOU

Fourmile Branch is a 24 km (~15 mi) long stream that lies entirely within the SRS (Figure 5). The 57 km^2 (22 mi^2) FMB watershed includes several SRS facilities, waste sites, and discharges (WSRC 1998b) (Figure 12). Except for the extreme headwaters that consist of primarily intermittent stream, most of the main stem of Fourmile Branch is potentially influenced by SRS discharges and industrial operations. Several of its tributaries such as Castor Creek are also entirely classified as downgradient. Both radioactive and nonradioactive constituents of potential concern have been detected in environmental media collected from Fourmile Branch (Paller et al. 2008). Via Castor Creek, Fourmile Branch received large volumes of heated cooling water from C Reactor in the past, causing extensive habitat destruction and the elimination of most aquatic biota. Recovery and recolonization of Fourmile Branch began in 1985 with the shutdown of C-Reactor.

4.3.3 Pen Branch IOU

The PB IOU includes Indian Grave Branch and several unnamed tributaries all of which are entirely within the SRS (Figure 5). Pen Branch terminates in the Savannah River swamp and, unlike the other major streams on the SRS, does not have a clearly defined channel through the swamp to the Savannah River. Upper Pen Branch is largely undisturbed by SRS operations upstream of the Chemicals, Metals, and Pesticides (CMP) Pits OU, and the middle segment receives runoff from N, L, and K Areas (Figure 13). Except for its extreme headwaters, nearly all of Indian Grave Branch was affected by the past operation of K Reactor resulting in the type of habitat degradation described for Fourmile Branch. Heated cooling water from K Reactor entered Indian Grave Branch and flowed into Pen Branch where it caused further habitat destruction. Recovery of this stream section began in 1988 with the cessation of K reactor operations. Three additional tributaries are entirely downgradient of N, L, and K Areas.

4.3.4 Steel Creek IOU

The SC IOU includes Steel Creek and its major tributary, Meyers Branch (Figure 6). Steel Creek originates near P-Reactor and flows approximately 3 km (1.9 mi) before entering the headwaters of L Lake, a 7 km (4.3 mi) long, 400 hectares (ha) (988 ac) cooling reservoir constructed in 1985. Water discharged from the L Lake dam enters the middle reach of Steel Creek, flows approximately 5 km (3.1 mi) to the Savannah River swamp, and then flows about another 2 km

(1.2 mi) through the SRFS to the Savannah River. All of the main stem of Steel Creek is potentially affected by SRS discharges, waste sites, and/or industrial operations (Figure 14). The middle and lower portions of Steel Creek suffered extensive habitat degradation from the discharge of high temperature reactor cooling water during 1954 to 1968 and was affected to a lesser degree by discharge from L Lake following its construction. Industrial facilities and waste sites in the SC IOU, as well as potential contaminants of concern, are summarized in WSRC (1998c). The entire main stem of Meyers Branch is classified as downgradient due to runoff from P Area and the railroad yard flowing into its headwaters. However, several of its tributaries that were not sampled in this project are considered upgradient.

5.0 SAMPLING

For the 2025 Bioassessment Report, fish data were collected following the IBI monitoring previously used and tailored to SRS coastal plain stream systems that were initiated in 1990. Macroinvertebrate community bioassessments were conducted using a modified SCDES MHSP and Hester-Dendy multiplate samplers. The 2025 Bioassessment Report includes historical and current sampling of fish and macroinvertebrates across 43 locations within 11 streams as presented in Table 1. Table 2 provides the results of sampling as described below. The bioassessment sampling locations across all IOUs are shown in Figure 3; individual sampling locations for each IOU are provided in Figures 4-6. Specific data collection methods include:

5.1 Fish Sampling

At each of 20 locations the fish community was sampled by backpack electrofishing a total of 200 meters (m) (219 yards [yd]) per site. The 200 m (219 yd) was broken up into four 50 m (54.7 yd) stream segments. Three passes were made at each site while moving upstream and ensuring all microhabitats (e.g., riffles, runs, pools, etc.) were sampled. While one person moved upstream with the electroshocker, two to three others assisted with netting fish and transferring them to buckets. In streams with larger average widths (>10 m [11 yd]), two electroshockers were used simultaneously to ensure full coverage of the streambed. All sampled fish were kept in 5-gallon buckets with stream water and with oxygen bubblers and kept separate by reach. At the completion of each pass, all buckets were moved to the downstream end of the reach where all fish were

identified, counted, and placed in a mesh holding pen in the stream until all sampling was completed. After completing a sampling site, all fish were released from the pen back into the stream. All sampling occurred between June 27 and September 01, 2022. Fish community surveys in 2022 added 20 additional samples to the previous 124 collected between 1990 and 2017 (Table 2).

5.2 Macroinvertebrate Sampling

To collect macroinvertebrate samples, two approaches were used. First, a method created by South Carolina Department of Health and Environmental Control (SCDHEC) (now SCDES) called the MHSP was used following the same protocol as for previous sampling efforts (SCDHEC 1998). This is a timed, qualitative method with the objective of collecting as many different species as possible in the three person-hours duration. It uses a range of sampling equipment to target different stream habitats, but ultimately with the observational approach, samples all available habitats. For a total of 1.5 person-hours several tools are used to collect macroinvertebrates from different specified habitats or targeting specific macroinvertebrate groups. A fine mesh sampler is used to collect Chironomidae and small macroinvertebrates (15 minutes); a D-frame dip net is used to sample root banks and aquatic vegetation (30 minutes); a fine mesh kick net is used to sample gravel riffles, snags, and leaf packs (30 minutes); and hand sieves are used for all habitat types to collect sediment-dwelling taxa (15 minutes). The other 1.5 hours of sampling is used for visual collections in micro-habitats and is completed by the team using forceps and sieves. Macroinvertebrate samples are photographed and released (unionid mussels) or preserved in ethanol. Samples for this report were measured for radiological activity and sent to ETT Environmental, Inc. for identification. Samples were collected between November 02, 2022, to January 20, 2023, and were not collected in the same season as fish sampling to allow for sufficient recolonization of habitats after disturbance from fish sampling. MHSP sampling in 2022/2023 added 23 additional macroinvertebrate samples to the 91 previous MHSP samples collected from 37 SRS stream sites across 1997, 2000, 2003, 2007, and 2017 for a total of 114 samples (Table 2).

Second, to remove stream-specific effects of habitat on macroinvertebrate community composition, samples were also collected from artificial substrates (Hester-Dendy multiplate samplers) that were deployed in quadruplicate in each stream reach for 28 days before collection.

Each sampler had a total surface area of 0.179 square meters (m^2) (0.214 square yards [yd^2]) derived from 14 square plates (7.6 centimeters [cm] [3 inches (in)] width) spaced 0.3 – 1.0 cm (0.1 – 0.4 in) apart. To prevent the sampler from contacting the sediment, each sampler was hung from a rope or log that was stretched across the stream. Hester-Dendy samplers were retrieved between May 08, 2023, to May 15, 2023 (referred to as the 2022 sampling year). From each stream site, macroinvertebrates from two of the Hester-Dendy samplers were preserved in ethanol, measured for radiological activity, and sent to ETT Environmental, Inc. for identification. Hester-Dendy sampling in 2022 added duplicate samples from each of the 23 macroinvertebrate sites (46 samples) to the 75 site-years collected previously from 35 SRS stream sites across 1997, 2000, 2007, and 2017 (Table 2). Two samplers were analyzed in 2017 and 2022. All other years analyzed five samplers. In total, there were 382 samplers analyzed across sites and years.

5.3 Habitat and Physicochemical Variables

Habitat assessments were conducted in alignment with MHSP sampling and occurred between ~~October 26~~November 2, 2022, to January 20, 2023, using two methods: the USEPA method for low-gradient coastal plain ecoregion streams adopted from the USEPA *Revisions to Rapid Bioassessment Protocols for Use in Streams and Rivers* and the SC Aquatic Biology Section assessment (ABS). These methods were both included in the MHSP Standard Operating and Quality Control Procedures for Macroinvertebrate Sampling (1998). The USEPA approach ascribes a rating from 1 to 20 for each of several variables with 1 as poor and 20 as excellent. The scores are then added for an overall habitat quality score. The low gradient habitat assessment variables include epifaunal (bottom) substrate and available cover, pool substrate, pool variability, sediment deposition, channel flow status, channel alteration, channel sinuosity, bank stability, vegetative protection, riparian zone. The ABS approach focuses on instream macroinvertebrate habitat and uses a rating from zero to five for nonexistent to excellent for five habitat categories. Habitat categories were root banks, logs/sticks/snags, rock/gravel riffle, mature leaf pack, and aquatic vegetation. Additionally, a score for channel braiding, the amount of pine needles in the stream, flow velocity, and sedimentation were recorded, as well as the percentage of pine needle stream detritus. Previous habitat assessments were conducted in 2003, 2007, and 2017 in conjunction with macroinvertebrate sampling (November to December for most sites and years).

Physicochemical parameters of the stream are physical and chemical variables indicating a snapshot of water conditions. These data are collected as part of the SC ABS Macroinvertebrate Habitat Assessment and were recorded using a multi-parameter handheld device (YSI ProQuatro, Pro Series Meter, Fondriest Environmental Inc, Fairborn, OH, US). Parameters included water temperature, pH, dissolved oxygen, and conductivity. Total organic carbon samples were not collected.

6.0 DATA ANALYSIS

In previous reports some samples (those collected from 2000 and earlier in Fourmile Branch, lower Steel Creek, lower Pen Branch, and Indian Grave Branch) were excluded from analysis due to ongoing impacts at the time. Given that other types of ongoing impacts may also affect bioassessments, we have chosen to include all samples with potential ongoing or historical impacts in our analyses. For macroinvertebrate samples, Fourmile Branch at Road F and Meyers Branch at Road 6.2 were excluded in this analysis due to intermittent stream flow and poor habitat location (an ephemeral ditch).

Fish communities were analyzed using the IBI method established by Paller et al. (1996) specifically for the upper coastal plain of South Carolina and for the SRS. From the fish community data, ten individual metrics were calculated that relate to measures of species richness, species composition, trophic composition, indicator species, and abundance (Table 3). Each parameter has an absolute value that is then binned into one of three scoring criteria: 1 (poor), 3 (medium), and 5 (best). The ten metrics are summed without any weighting and thus yield a maximum possible score of 50. In 2022, the metric for fish condition was not directly measured. However, while fish were being identified and counted, no abnormal or obviously diseased fish were noticed. Thus, the top score of 5 was assigned to each site for fish condition. Several metrics involve an equation establishing the “expected number” of fish in particular categories based on estimated width and surface area of the stream being sampled. These equations are provided in Appendix A.

To examine temporal trends of biotic integrity within SRS streams over time the long-term fish data collected since 1990 were evaluated using linear models. Only sites with at least three data points were used. Linear models using year as the explanatory variable were conducted with a least

squares approach. Changes in IBI over time in each stream were visualized by plotting each site as a separate line. Scores were not averaged across sites within the same stream.

To compare biotic integrity among categories of sites (upgradient [U], downgradient [D], and lowest reaches [L]) a subset of the fish data containing the most recent two sampling periods (2017 and 2022) was used. The data are not normally distributed, so the non-parametric Wilcoxon/Kruskal-Wallis tests were used. For the fish component of the study this results in only four data points for upgradient sites: Pen Branch PB1 and Tinker Creek TC1 across 2017 and 2022. Thus, there is little power to compare categories of streams but separate Wilcoxon (when only two categories; U and D) or Kruskal-Wallis (when three categories U, D, and L) tests were run for each stream and the ChiSquare test statistic used to assess significance.

Macroinvertebrate communities were analyzed through data from the two different collection protocols: MHSP and Hester-Dendy samplers. From the MHSP, data were used to calculate two metrics which were then averaged to provide an overall MHSP score. This averaged score relates to the following bioclassifications: 4.5 – 5.0 is excellent, 3.5 – 4.4 is good, 2.5 – 3.4 is good-fair, 1.5 – 2.4 is fair, and 1.0 – 1.5 is poor. The two metrics that are averaged to produce the MHSP score are the Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxonomic richness (number of unique EPT taxa), corrected for stream width, and a Biotic Index value for the average pollution tolerance that is based on taxonomic identification and weighted by categorical abundance groups as described in the MHSP protocol (SCDHEC 1998; Lenat 1993). EPT are generally known to be sensitive to poor water quality and habitat, and the Biotic Index accounts for the sensitivity or tolerance across a wide range of taxa. These metrics receive a score from 1 (poor) to 5 (best), which are then averaged to yield an MHSP score for stream bioclassification. For the Biotic Index, higher values signify greater pollution tolerance, thus, the classification is inverted such that the Biotic Index for MHSP samples has the following categories: $< 5.42 = 5$, $< 6 = 4$, $< 6.67 = 3$, $< 7.68 = 2$, $\geq 7.68 = 1$.

Prior to averaging the MHSP EPT score, it is adjusted for stream width. This adjustment is made because earlier studies of SRS streams (Paller et al. 2007) revealed a positive linear relationship between stream width and the MHSP EPT metric (Figure 15), thus, the EPT metric is adjusted to remove the effects of stream size. This stream width correction removes the bias that would

otherwise classify smaller streams as having worse water quality than large streams regardless of their actual pollution load or habitat degradation. The steps for this adjustment were as follows: (1) using upgradient and known high-quality sites, regress EPT richness on stream width values to obtain a regression equation (Figure 15), (2) subtract the expected EPT richness that was calculated from the regression from the measured EPT richness, (3) plot the residuals excluding the upper 5% (positive residuals indicate greater EPT richness than expected in a stream of that size), and (4) partition the plot into five bioclassification categories with 1 being poor and 5 being excellent (Figure 16). These categories are then used to classify all MHSP macroinvertebrate samples for EPT richness with the following categories: $\geq 5.6 = 5$, $\geq 2.1 = 4$, $\geq -1.1 = 3$, $\geq -4.5 = 2$, $< -4.5 = 1$.

Hester-Dendy samplers were analyzed for EPT richness (number of unique EPT taxa) and Biotic Index. These metrics were evaluated independently and not combined, thus, they were not scored from one to five as were the MHSP data. Data from the duplicate samplers deployed at the same stream site were averaged. Hester-Dendy EPT data were not adjusted for stream width and instead were evaluated with respect to the upgradient sites occurring within each stream size category: small (< 5 m stream width) or large (>5 m stream width). Pen Branch upper (PB1) was the only upgradient small stream location which reduced confidence in statistical analysis comparison among small streams for 2022. Small size streams included Crouch Branch, Mill Creek, Tims Branch, McQueen Branch, Meyers Branch, Indian Grave Branch, and sites SC1 and SC2 in Steel Creek and PB1 and PB2 in Pen Branch. Large size streams included Upper Three Runs, Tinker Creek, Fourmile Branch, and sites SC4, SC5, and SC6 in Steel Creek and PB3 and PB4 in Pen Branch.

Temporal trends of biotic integrity within SRS streams over time using the long-term data collected since 1997 were evaluated using linear models using sites having at least three data points. Linear models using year as the explanatory variable were conducted using the *lm* function in R with either MHSP scores, Hester-Dendy EPT richness, or Hester-Dendy Biotic Index values as the response variable. Linear models were followed by creating Q-Q plots to visualize the residuals. Trends were visualized by plotting the bioassessment score for each year with sites graphed as individual lines for each stream. Scores were presented for each sampling location and not averaged across sites within the same stream.

Categorical comparisons with the long-term data were made between each downgradient or lowest reaches stream site and the pooled upgradient sites combining the prior two years of data collection (2022 and 2017). For Hester-Dendy data, these comparisons were conducted within stream size categories. Given the nonparametric shape of the data, Kruskal-Wallis tests were used to compare differences in category medians using the *kruskal.test* function in the base R package (R, Core Team). Significant Kruskal-Wallis test results were followed by pair-wise comparisons using Dunn's many-to-one comparison with holm adjustments using the *kwManyOneDunnTest* function in R package 'PMCMRplus' (version 1.9.12, Pohlert 2024).

Habitat assessment scores were compared between years using Kruskal-Wallis test and significant results were followed by pair-wise comparisons using Dunn's test for pair-wise. The relationship between habitat assessment scores and fish and macroinvertebrate bioassessment scores was evaluated using Spearman's rank correlations. Relationships were examined for all years combined in addition to using 2022 data alone.

Analyses for fish IBI were run in JMP Student Edition 18. Graphing for fish IBI, and analyses and graphing for macroinvertebrate metrics and habitat assessments were conducted with R software (fish: version 4.1.1, macroinvertebrates: version 4.4.1, R Core Team) in R Studio (version 2024.04.2, Posit Team). Trends and comparisons were considered to be significant when $p < 0.05$. Means are presented ± the Standard Error from the Mean.

7.0 RESULTS

7.1 Temporal trends

7.1.1 Index of Biotic Integrity

Fish community analyses were successfully completed at 20 sites across ten streams and four IOUs. As with the 2017 study, no fish sampling was conducted within the UTR stream due to the unsuitability for backpack shocking (large/deep stream conditions). Across all the historical data there were 26 sampling locations with more than three years of data, allowing for statistical comparison of IBI over time using linear regression. The 2022 sites represent 16 of those locations.

Below, the temporal trends by site type (upgradient, downgradient, and lowest reaches) are further discussed and are illustrated in Figures 17 and 18.

Only two upgradient locations were surveyed in 2022: Tinker Creek (TC1) and Pen Branch (PB1). The Tinker Creek upgradient IBI fell below the lowest limit (36) of the upgradient range. This is the only time this site has fallen below that range, but from 2007, 2017, and 2022 the IBI has declined from 48 to 42 to 34. The regression over time is not significant (estimate = -0.25, statistic = -1.6, p = 0.170) as the IBI increased from 1992 to 2007 before declining. The IBI at the other upgradient site, Pen Branch, has significantly decreased over time (estimate = -0.45, statistic = 0.198, p = 0.004). In 2022 the IBI at PB1 was 36, which matches the lowest value of the upgradient range. The IBI at PB1 reached the maximum of 50 in both 1997 and 2000 but has declined since 2000. This is a very small headwater stream with road crossing culverts creating a historical fish movement impediment downstream of the site.

Among the downgradient sites, significant negative change over time was observed in Tims Branch (TIB1; estimate = -0.90, statistic = -5.25, p = 0.003) and Steel Creek (SC2; estimate = -0.29, statistic = 0.09, p = 0.026). Significant increases in IBI over time were observed in McQueen Branch (MQ1; estimate = 1.17, statistic = 0.12, p = 0.01), though it was not surveyed in 2022. The IBIs in Tims Branch and SC2 have been below the upgradient range since 2003 and 1990, respectively, and have continued to decline. There have been no surveys in MQ1 since 2009, but the IBI did increase consistently from 2000 to 2009, from below the upgradient range to within the 95% confidence interval.

Among the other 13 downgradient sites surveyed in 2022 nine had lower IBIs than in their previous sample period, two stayed the same, and two (MB3 and MQ2) increased. However, 10 downgradient sites fall within the range for the upgradient sites, with 8 of these having IBIs within the 95% confidence interval. The sites that fall below the upgradient range are within Crouch Branch (CB2), Mill Creek (MC1), Tims Branch (TIB1), and Steel Creek (SC2 and SC5). Three of these sites (SC2, MC1, TIB1) have consistently had IBIs below the lowest limits of the upgradient range. For SC5, this represents the first IBI outside of this range since 1990. The IBI in CB2 had increased from 24 in 1995 to 38 in 2017 to fall within the upgradient range but has now declined to 32.

The lowest reaches within Fourmile Branch (FMB7 and FMB8) and Steel Creek (SC6) had IBIs within the lower portion of the upgradient range, while the IBI for the lowest reaches of Pen Branch (PB4) fell within the upgradient 95% confidence interval. The Steel Creek site had not been sampled since 1990 and showed a marked increase in IBI from 24 to 36 during this time. The Pen Branch site has declined for three survey periods in a row and the Fourmile Branch location increased from its 2017 IBI but is still much lower than in 2009 when it had the maximum score.

7.1.2 Multiple Habitat Sampling Protocol

Macroinvertebrate communities were collected from 23 sites in 11 streams within four IOUs using the MHSP which, when combined with historical data, yielded 25 sites with three or more years of data collection. Of the five upgradient stream sites sampled historically (UTR1, UTR2, TC1, TC4, and PB1), the three sampled in 2022 (UTR2, TC1, and PB1) were at or above the 95% confidence interval for upgradient sites (3.94 – 4.59) (Table 2, Figures 19 and 20). Prior to 2017, some samples from upper Pen Branch (PB1) fell below the lowest limits of the upgradient range (3). Indian Grave Branch was the only site with a significant linear trend and indicated an increase in MHSP score over time (estimate = 0.1, statistic = 8.25, $p = 0.001$). Samples collected in 2017 and 2022 at Indian Grave Branch were at or above the 95% confidence interval for the upgradient range. The most downstream downgradient site for Pen Branch (PB3) had a marginally significant increase in MHSP score over time (estimate = 0.5, statistic = 2.48, $p = 0.09$). The only sites with MHSP scores consistently near the bottom limit or below the upgradient range were upper Fourmile Branch (sites FMB3, FMB4, and FMB5), Tims Branch, and CB1 on Crouch Branch which has not been sampled since 2007. Caution should be used to interpret trends in upper Fourmile Branch (sites FMB3, FMB5, and FMB7) in 2022 as collections from these sites yielded fewer than 100 individuals per site which is below that typically expected for the Biotic Index calculation. Sites FMB3 and FMB5 each had 70 individuals while site FMB7 had 83 individuals. The lowest reaches in Upper Three Runs, Pen Branch, and Fourmile Branch FMB8 were at or above the 95% confidence interval for the upgradient MHSP score range while FMB7 and the lowest reach in Steel Creek (SC6) were in the lower portion of the upgradient range.

7.1.3 Hester-Dendy Artificial Substrates

Macroinvertebrates were collected from Hester-Dendy artificial substrates from the same 23 stream sites as the MHSP samples. When these new Hester-Dendy data were combined with historical data it yielded 24 sites with three or more years of data collection. EPT richness from Hester-Dendy samplers collected in 2022 in upgradient sites in large streams (UTR2 and TC1, > 5 m width) were within or above the 95% confidence interval (4.15 – 7.54) for their given stream size (Table 2, Figures 21 and 22). For small streams (< 5 m width), upper Pen Branch (PB1) was the only upgradient stream in the dataset. Compared with 2007 and 2017, PB1 had a higher EPT richness in 2022, but did not return to the even higher EPT richness values found there in 1997 and 2000. Tims Branch was the only site with a significant linear trend and indicated a decrease in EPT richness score over time, dropping from 0.8 to 0 average EPT richness (estimate = -0.037, statistic = -7.23, $p = 0.006$). Although not a significant negative trend, one of the uppermost Steel Creek sites (SC2, small stream) has over time moved from EPT richness exceeding the upgradient 95 % confidence interval (1.25 – 4.50) in 2000 and 2007 before declining back to within the confidence interval in 2017 and 2022. The potential to detect linear trends at Crouch Branch, Mill Creek, and McQueen Branch would benefit from additional data collection—both more years and more sites. Sites at the lowest reaches (UTR8, FMB8, SC6, and PB4) had EPT richness at or above the 95% confidence interval of the upgradient range except for FMB7, which fell just below that.

Biotic Index scores represent pollution tolerance such that higher values indicate lower biotic integrity. For Biotic Index scores from 2022 in the two large stream upgradient sites, Tinker Creek (TC1) was the only one that occurred at or within the 95% confidence interval (4.28 – 5.42), although upper Upper Three Runs (UTR2) was close to the upgradient range (Table 2, Figures 23 and 24). In the 2022 data collection, the small stream upgradient Pen Branch site (PB1) continued a trajectory of improvement after a dip to its poorest score in 2007. One site at Mill Creek (MC2) which was not sampled beyond 2007 was the only site with a significant linear trend and indicated an increase in the Biotic Index score over time signifying a decline in quality (estimate = 0.33, statistic = 16.1, $p = 0.04$). The Biotic Index score for Tims Branch was within the upgradient 95% confidence interval (5.17 – 6.68) for the first time in 2022, but again this confidence interval was based only on one upgradient site (PB1). Upper Fourmile Branch (FMB3) occurred within the upgradient 95% confidence interval for the first time, and the middle Fourmile Branch

downgradient site (FMB5) remained near the upgradient 95% confidence interval. Similar to the Hester-Dendy EPT richness, sites at the lowest reaches had Biotic Index scores near or better than the 95% confidence interval of the upgradient range (Upper Three Runs, Pen Branch, Steel Creek, and Fourmile Branch) with the poorest score in FMB7.

7.2 Comparisons Among Streams

7.2.1 Index of Biotic Integrity

Among the 2022 sites, the IBIs for nine fell within the 95% confidence interval (41.6 – 46.6) for the upgradient sites over time, five fell below the 95% ~~CI~~ confidence interval but within the upgradient range minus the lowest 5% (36 – 50), and the remainder fall below that level (Figure 25). Eleven of the 20 sample locations from 2022 were also sampled in 2017. Within these IBI decreased over time at 10 sites and across all sites but the overall mean IBI (± SE) was not significantly different (Chi-square = 2.72, p = 0.099) in 2022 (34.36 ± 2.90) than in 2017 (39.81 ± 2.90). Combining the 2017 and 2022 data, there were no significant differences in the pooled IBIs of downgradient and lowest reaches of any one stream compared to the pooled upgradient sites. With the multiple comparisons the *p*-value would need to be adjusted to 0.006, but none approached significance even at the 0.05 level. There is very limited power to make these comparisons with a total of four upgradient data points. Further, as mentioned above, the IBI in the upgradient sites has declined. Within Tinker Creek (TC1), the IBI in 2022 was 34, and now falls below the upgradient range while at PB1 it was 36, just at the lowest part of the upgradient range. Within Pen Branch the downgradient (PB2, PB3) and lowest reaches (PB4) had higher IBI scores than the upgradient site. Across other streams downgradient sites in Fourmile Branch (FMB3, FMB4), Indian Grave Branch (IGB1), Meyers Branch (MB2, MB3), Mill Creek (MC3), and McQueen Branch (MQ2) all had higher IBI scores than both upgradient sites. Only locations within Tims Branch (TIB1) and Steel Creek (SC2) had IBI scores lower than both upgradient sites.

7.2.2 Multiple Habitat Sampling Protocol

On average, the MHSP Scores in 2022 occurred above the lowest threshold of the upgradient site range (MHSP score ≥3) except for Tims Branch, Crouch Branch (CB2), and two sites on Fourmile Branch (FMB3 and FMB5) (Figure 26). When MHSP scores from 2017 and 2022 were combined,

some downgradient sites had MHSP scores that were significantly lower than the upgradient sites (upgradient sites n = 7; Chi-square = 27.7, df = 14, p = 0.016). These included Fourmile Branch sites at FMB3, FMB4, and FMB5 (Dunn's test, n = 4, z value = -3.230, p = 0.017). Again, some of these sites had fewer than 100 individuals collected and require caution for interpretation of the Biotic Index which is averaged into the MHSP score. Site FMB3 was the only location to receive a bioclassification rating of "poor" in the 2022 dataset. Tims Branch, which contained only one location, was also lower than the upgradient MHSP scores from 2017 and 2022 combined (Dunn's test, n = 2, z value = -2.962, p = 0.040). Tims Branch was the only site to receive a bioclassification rating of "fair" for the 2022 data. The remaining locations had bioclassification scores of "good-fair", "good", or "excellent" based on the stream-width adjusted MHSP scores. MHSP sampling identified macroinvertebrate taxa of special interest in South Carolina that occurred primarily in large streams irrespective of their contamination history (Tables 6 and 7).

7.2.3 Hester-Dendy Artificial Substrates

Hester-Dendy EPT richness in 2022 for small streams was above the lowest range value of the upgradient small stream sites (1.6), except for Tims Branch which had zero EPT (Figure 27). Crouch Branch had the next lowest average EPT for small streams richness at two taxa. For large streams, the upgradient lowest range value (3.5) was equal to the 2022 sample from a Fourmile Branch site (FMB7), but otherwise the downgradient and lowest reaches sites exceeded this threshold. No downgradient or lowest reaches stream sites had EPT richness from 2017 and 2022 combined data that was significantly different from the upgradient streams in the respective stream size category (small streams: Chi-square = 12.5, df = 8, p = 0.129; large streams: Chi-square = 8.9, df = 8, p = 0.351).

Hester-Dendy Biotic Index values in 2022 for small streams were better (lower) than the threshold of the worst 5% of upgradient sites for small streams (6.6), but for large streams, a Fourmile Branch site (FMB5) and lower Pen Branch site (PB3) were not better than this threshold (5.7) (Figure 28). All of the lowest reaches occurred in large streams and had Biotic Index values in 2022 that met or were better than the lowest range of upgradient large streams. No downgradient or lowest reaches stream sites had Biotic Index values from 2017 and 2022 combined that were significantly

different from the upgradient streams in the respective stream size category (small streams: Chi-square = 13.4, df = 8, $p = 0.10$; large streams: Chi-square = 8.34, df = 8, $p = 0.401$).

7.2.4 Habitat Assessments

USEPA low gradient stream habitat assessment scores differed by year of assessment (Chi-square = 18.4, df = 3, $p < 0.001$, Figure 29). The strongest difference was between 2022 and 2017 (Dunn's test, z value = 4.27, $p < 0.001$) and showed a sharp decline in scores from 2017 to 2022, but assessment scores from all pairwise comparisons except for 2003 vs 2007 were marginally significantly different (all $p < 0.054$). The highest average score was for 2017 (164 ± 7.5) and the lowest average score was for 2022 (130 ± 3.4) Average scores for 2003 and 2007 were 145 (± 5.4) and 144 (± 5.0), respectively. Stream depth ranged from 5.5 – 74.1 cm (2.2 – 29.2 in) and width ranged from 1.4 – 7.4 m (1.5 – 8.1 yd) (Table 8).

Habitat assessment scores from all years of data were positively correlated with only one macroinvertebrate metric: the MHSP scores had a very weak positive correlation with habitat assessment scores (S = 55,602, r = 0.30, $p = 0.008$, Figure 30). When habitat assessments from only 2022 were used, there were no significant correlations with macroinvertebrate metrics or the fish IBI.

8.0 DISCUSSION

Building on the previous bioassessment for SRS streams conducted in 2017 (Paller and Blas 2018), the results from fish and macroinvertebrate communities collected in 2022 generally align with the 2017 assessment and indicate that the majority of stream reaches are not significantly impaired compared with upgradient stream locations. The previous report indicated that ecological recovery was occurring in Pen Branch, Indian Grave Branch, Fourmile Branch, and middle/lower Steel Creek which historically received cooling water discharge from L, K, and C Reactors that killed riparian vegetation and seed banks, scoured the stream channels, destroyed or removed habitat structure, and killed or displaced stream biota due to the extreme hot temperatures and elevated discharge. Reactors were shut down in 1988 aside from a temporary reuse of K Reactor in 1992. Sources of current and historical contamination and runoff on SRS may affect other stream reaches not impacted by reactor cooling water due to groundwater discharge, stormwater runoff or

effluents from industrial areas or waste sites within the watersheds including Tims Branch, McQueen Branch, Crouch Branch, Mill Creek, Meyers Branch, and Upper Three Runs and its direct tributaries. Additionally, streams previously impacted by thermal discharges also have industrial areas and waste units located upstream of the thermal water discharge outfalls including Fourmile Branch, Pen Branch, and Steel Creek. Many of the industrial areas being located in the headwaters of the largest tributaries results in most of the study sites being classified as downgradient of potential SRS stressors. Tims Branch and upper Fourmile Branch based on macroinvertebrates, upper Steel Creek based on fish, and Crouch Branch based on both fish and macroinvertebrates all show signs of community impairment. However, other study sites indicate biological recovery despite habitat assessment scores below those of upgradient sites.

Tims Branch was sampled downstream of Road 2 and Steed Pond which fluctuates between gaining and losing ground water (Parker et al. 2022; Santschi et al. 2024). At this location, Tims Branch continues to consistently indicate poor and worsening biological conditions. Previous explanations have included periodic release of anoxic water from upstream beaver ponds, poor habitat quality, and to a lesser extent elevated metal levels (WSRC 1998a; Paller et al. 2011). The geochemistry of Tims Branch has been extensively studied. Gaining reaches of stream can be identified by the presence of orange iron-rich flocculants that form when anoxic ground water transports iron to oxygenated surface waters where the iron is oxidized (Pérez 2023; Santschi et al, 2024). In addition to metal exposure, the extensive coverage of the stream bottom by these flocculants as we observed in the study reach can harm invertebrate communities by smothering and habitat degradation (Vuori 1995; Reich et al. 2019). However, Tims Branch also has poor habitat with the stream channel, which is eroded down to the hard clay layer, and its bioassessment scores fall below those of the upgradient sites. Future evaluations should include additional sampling locations in Tims Branch to compare differences between the losing stream and gaining stream segments as well as variability in dissolved oxygen concentrations throughout the drainage.

Using macroinvertebrates as biomonitors, Fourmile Branch (upstream of FMB8) did not support full biological recovery as it had significantly lower indices compared to upgradient sites and upper Steel Creek (SC2) showed signs of worsening quality to support EPT taxa. However, based on biotic integrity of fish communities, Fourmile Branch and middle Steel Creek have consistently

fallen within the range of upgradient sites. In 2022 this continued for Fourmile, but there was a decline in IBI in middle Steel Creek to below the range for upgradient sites. L Lake isolates the upper reaches of Steel Creek from the rest of the free-flowing stream, acting as a barrier to stream fish migration, preventing recolonization of the previously disturbed upper Steel Creek by stream fishes. The lower IBI likely results from this isolation and repopulation by lentic species rather than metal contamination (Paller and Blas 2013). Because macroinvertebrate communities in upper Steel Creek still occurred within the upgradient range, habitat quality may be sufficient to support the reintroduction of stream fish communities to upper Steel Creek. Even with the decline of IBI in middle Steel Creek, it is still much higher (34) than in upper Steel Creek (20) further indicating that reintroduction of fish could be successful. While both upper Fourmile Branch and middle Steel Creek (see Table 1 for specific site codes in these IOU subunits) indicated slow ecological recovery from degraded biological conditions, the lowest reaches within those streams supported biological communities on par with those in upgradient streams. In fact, despite being severely impacted by past reactor operations, all four of the lowest reaches downstream of highway SC Highway 125 indicated minimal continued detrimental impacts since the start of their assessments in 2007 or later suggesting that recovery has been faster at longer distances that buffer the stream from current upstream stressors. Although, two of the streams, Fourmile Branch and Pen Branch, received comparatively poor habitat assessment scores which may reflect their history of water scalding and scouring the streams and floodplains.

As noted in the prior report, bioassessments of McQueen Branch have yielded varied results. In 2022, one location on McQueen Branch was sampled and all macroinvertebrate metrics from there occurred within the 95% confidence interval for upgradient streams, suggesting that these communities have recovered to an extent that is similar to upgradient streams. Similarly, the fish metric was well within the lower part of the upgradient range. However, with the prior variability over-time and the assessment of only one sampling location in 2022, additional years and sites of bioassessments would help determine the strength of this conclusion. Crouch Branch has also exhibited varied results for macroinvertebrate and fish community assessments. CB2 sampled in 2022 has a severely scoured and unstable stream channel, consequently it scored at the bottom of the upgradient stream range for two macroinvertebrate metrics and fell out of the range for the fish metric. In fact, the fish metric has steadily declined since 2009. However, sufficient pollution

sensitive macroinvertebrate taxa and quantities were found on Hester-Dendy samplers to move that Biotic Index metric well within the upgradient range for this metric indicating that the community impairments may be due to severe stream instability rather than contaminant exposure. In addition to point sources of copper and other metal contaminants from H and S Areas, Crouch Branch also receives stormwater from multiple industrial areas and consequently is very incised and experiences flashy storm flows which destabilizes stream bottoms. This led to difficulty in keeping the Hester-Dendy samplers submerged in the shifting stream channels.

Meyers Branch was classified as a downgradient site category due to ditches connecting P Area and the Railroad Yard to the headwaters of Meyers Branch. The area, including the P-Area ash basin, was remediated in 2011. Stormwater flows appear to have been increased post remediation. Stormwater from P Area flows across a coal combustion waste plume footprint and into a Carolina Bay before spilling into a ditch and the Meyers Branch headwaters. Meyers Branch has recently been documented to contain elevated contaminants such as iron, aluminum, and arsenic (Dean Fletcher, ongoing research) that may be derived from coal combustion waste. Despite this, fish and macroinvertebrate communities bioassessment scores have remained within the upgradient range over the past eleven years. Meyers Branch may be susceptible to future declines in biological quality.

Mill Creek was classified as a downgradient site due to a history of runoff from R Area. However, within the past two macroinvertebrate bioassessments in 2017 and 2022, Mill Creek has had excellent bioclassification ratings and scored better than the upgradient site range suggesting that it has full ecological recovery for macroinvertebrates. The fish metric for upper Mill Creek falls just below the upgradient range, but downstream it falls within the 95% confidence interval suggesting similar recovery for fish communities.

The quality of habitats varied by year with no significant improvement over time and did not have a strong relationship with stream condition as evaluated from macroinvertebrate and fish scores. Only the MHSP scores were significantly correlated with habitat assessment scores, but this was a very weak positive correlation. This suggests that the macroinvertebrate communities do not respond solely to habitat criteria. Habitat assessment grading can vary based on the team completing the assessment, thus the decrease in average habitat quality from prior assessments

may not indicate a significant change as a new team conducted the survey in 2022. Seasonality also may introduce variability depending on when macroinvertebrates are collected.

Several challenges of the SRS stream biotic assessments include that 1) few upgradient streams are presently included in the study site list, 2) presently included upgradient streams are typically smaller streams relative to many of the included downgradient stream reaches, 3) bioassessments historically were not always conducted in the same season or using the same procedures, and 4) the macroinvertebrate MHSP uses a qualitative approach that limits temporal and spatial comparison. First, stream networks have longitudinal patterns of ecological processes (Vannote et al. 1980) which can make it inappropriate to compare smaller upgradient stream reaches with larger reaches that are downgradient in the same stream network. The inclusion of lowest reaches sections that demonstrate biological recovery relative to the downgradient stream reaches upstream reduces that concern. Second, macroinvertebrate lifecycles vary seasonally leading to potential differences and increased variability in results from bioassessments conducted in spring/summer vs fall/winter. This may explain some of the variability in bioassessment scores. Using the most recently collected data, only Indian Grave Branch, based on macroinvertebrates, had a significant linear trend in biological recovery across stream sites that were sampled at a similar frequency. Another source of variability for temporal trends is likely due to changes in the location of sites sampled in a stream network which is notable in Crouch Branch. Uncertainty is incorporated into the historical fish data because numbers need to be corrected for different study site lengths and different numbers of electrofisher passes made. Lastly, variability can arise from the qualitative nature of sample collection. The MHSP uses timed sampling with an emphasis on capturing high biodiversity, but without standardizing the aerial extent of habitat types sampled. Additionally, the free sampling period will be highly individual-dependent. Thus, collections rely on a consistent speed of sample collection and extensive training to standardize equal sampling of macroinvertebrate microhabitats across collection teams and minimize variation in how collection tools are used by each team. This would also require communication among teams over time. Additionally, because the MHSP samples are field-picked within the allotted time segment, differences in the depth of the water that collectors wade through to collect and then process the sample or the availability of a suitable space on the stream bank to engage in field picking introduces variability. With deep streams such as Upper Three Runs, this limited sampling

efficiency has been noted in prior reports. Because the Biotic Index is weighted by abundance classes, consistent sampling efficiency across sites and teams is critical for the MHSP yet difficult to achieve.

In addition to the challenges listed above, there are some concerns with the fish IBI. The IBI used for this report was developed to detect differences in fish communities between "reference" sites and "potentially impacted" sites that experienced impacts from site operations (Paller 1996). However, the sites that were deemed reference sites have experienced declines in their fish IBIs potentially due to environmental changes such as disrupted flows and land use changes in the watershed area. This essentially means that it is now potentially easier for the downgradient sites to show "recovery" when only comparing to upgradient sites. Further, the selected metrics of the IBI may or may not be the best ones for differentiating sites experiencing these types of impacts. Another potential weakness of the current IBI is that within categories of fish it only counts species richness and not species composition. Thus, it does not take into account the ecological importance of some species. For example, all sunfish species are considered the same and an increased number of species ~~is bad~~decreases the IBI metric. While presence of Bluegill (*Lepomis macrochirus*) that escaped from an upstream impoundment would indicate an impairment to the community, a stream should not be penalized for having some less common habitat specialists such as the Mud sunfish (*Acantharchus pomotis*) or the three species of *Enneacanthus* found on the SRS. Even among the *Lepomis*, habitat heterogeneity will increase the potential spawning locations and thus could increase the numbers of species found in the reach. The result could be habitat heterogeneity that is generally considered good yet reduces the IBI score. In contrast, a highly scoured stream channel will have few spawning sites; consequently, the disturbed stream may be erroneously rewarded for having fewer sunfish species. Similar patterns could arise among minnow species. Moreover, measurements of rarity and specific considerations for the ecology of certain indicator species have become increasingly common in the development of IBIs across the Southeastern United States, including the Coastal Plain (Brumm et al., 2021). Multiple Species of Greatest Conservation Need (SGCNs) listed under South Carolina's State Wildlife Action Plan (SWAP) have been sampled on the SRS as part of surveys for IBIs (Tables 4 and 5), yet their potential significance in determining the health of SRS streams may be masked by the current metrics of the IBI. The Blackbanded Sunfish (*Enneacanthus chaetodon*) inhabits shallow, low-velocity backwaters of lakes and streams

and occurs infrequently on the SRS. It is considered sensitive to poor water quality and was designated as a species of High Conservation Concern under South Carolina's SWAP. However as described above, the occurrence of Blackbanded Sunfish could actually decrease the IBI of an SRS stream by increasing number of sunfish species captured. Conversely, the IBI may also overvalue fish with little support for their significance as indicators of habitat quality. The Blackbanded Darter (*Percina nigrofasciata*) is a species known to inhabit an array of habitats and can even persist in areas of habitat degradation/alteration. Its value as a "Benthic Species" contributes positively towards higher scores in the IBI, yet the presence of this fish may not act as a reliable indicator of stream quality. Incorporation of metrics that explicitly consider the distribution, ecology, and conservation value of species may provide a clearer picture of the drivers of community change of SRS fishes.

In summary, many SRS streams have improved in biotic quality since the early 1990's yet some require further attention to enhance their recovery so that it aligns with upgradient locations. Specifically, Tims Branch, upper Steel Creek, Crouch Branch, and Fourmile Branch indicated impairment based on fish indices, macroinvertebrate indices, or both. Tims Branch indicated the worst quality in most metrics and poor habitat quality may be a primary contributor to biotic integrity impairment there. In upper Steel Creek, reintroductions of fish to overcome the L Lake barrier may improve this biotic condition. Crouch Branch may improve with habitat stabilization. Fourmile Branch shows improvement for some macroinvertebrates metrics and may continue this trend as the surrounding ecosystem recovers. However, none of the impairment has been attributed to contaminant loading into the streams from SRS OUs, but more likely the stronger influences are the physical impacts and stream conditions previously discussed. The interpretation of some streams' biotic integrity, specifically Meyers Branch, McQueen Branch, and Crouch Branch would be improved with additional and consistent samples. Additional upgradient sample locations would also strengthen the confidence of assessing biotic recovery targets.

9.0 RECOMMENDATIONS

Based on the results from the 2025 periodic bioassessment, IOU streams should continue to be monitored to assess conditions and long-term trends.

This bioassessment evaluation does not attribute any declines in stream ecological health to SRS OU contaminant impacts. The SRS recommends a walk-down of potential stormwater impacts to Meyers Branch (SC IOU) to assess erosion and/or the potential for ash redeposition from P-Area ash-related OUs, particularly stormwater flow from around P-Area Ash Basin (188-P) and the Wetland Area at Dunbarton Bay (NBN) that may potentially reach Meyers Branch.

Implementation of the current SRS bioassessment approach revealed some areas of possible improvement. Recommended modifications to the monitoring program, if appropriate, based on this approach include the following:

- Collect additional samples at consistent locations to improve the interpretation of Meyers Branch, McQueen Branch, and Crouch Branch biotic integrity. Identify and establish additional upgradient sample locations.
- Conduct sampling on a standardized seasonal timeframe to reduce seasonal related variability and use the same sampling locations unless impracticable.
- Tailor the macroinvertebrate MHSP sampling procedure to address a full characterization of represented habitats rather than rely on a specified time commitment to conduct the sampling. Also, inclusion and consideration of rare species should be considered in calculating the BI based on macroinvertebrate assemblages or by further adjusting the “bioassessment water quality rating” as also indicated below.
- Incorporate metrics or an adjustment to the bioassessment water quality rating (IBI) that explicitly consider the biogeography, ecology, and conservation value of a species to provide a clearer picture of the drivers of community change (Hilburn et al. 2025) of SRS fishes. Particularly, include endemism as increases or decreases in endemic species may be a more reliable indicator of stream health (Scott and Helfman 2001; Walters et al. 2003; Hilburn et al. 2023).

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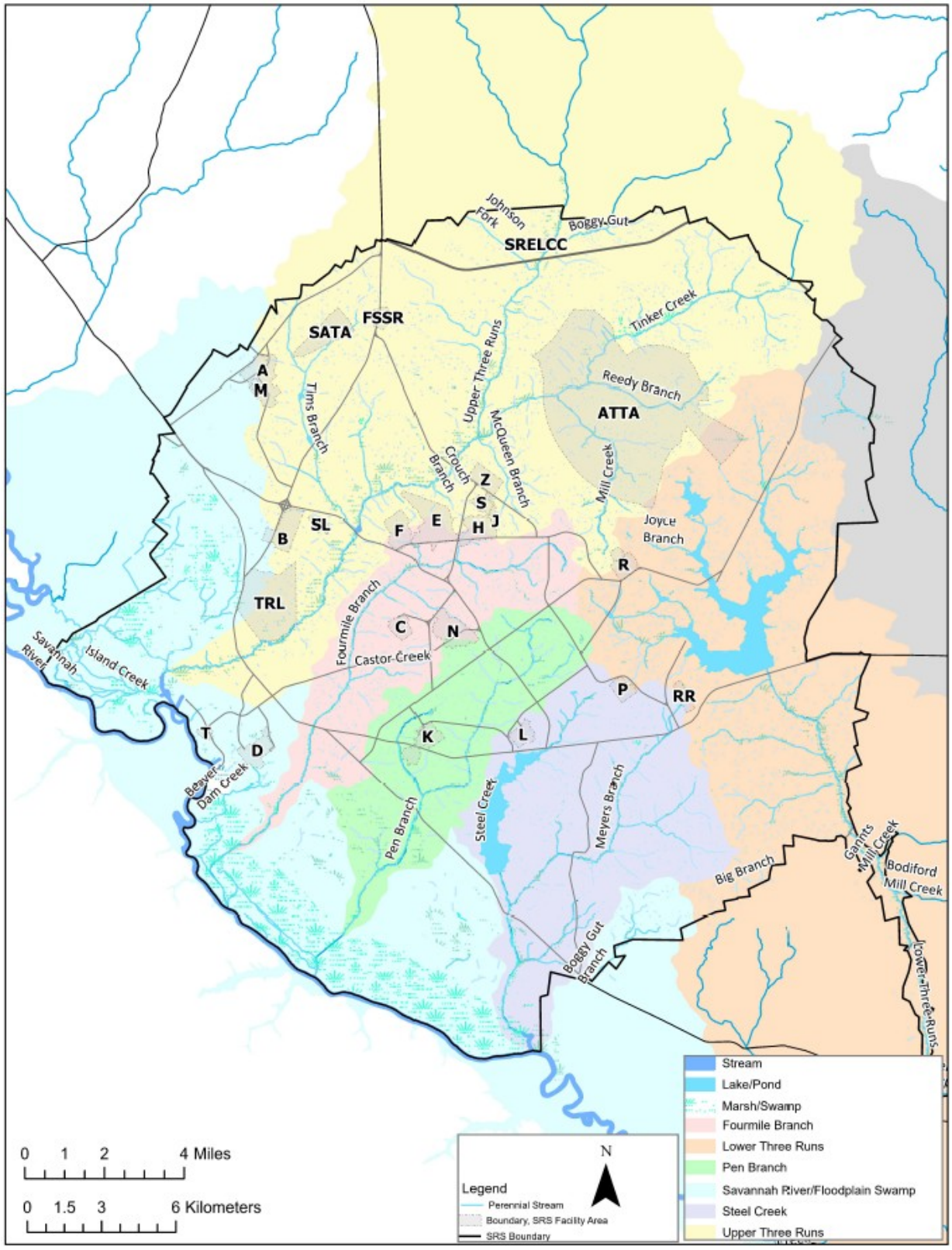


Figure 1. Savannah River Site Integrator Operable Units

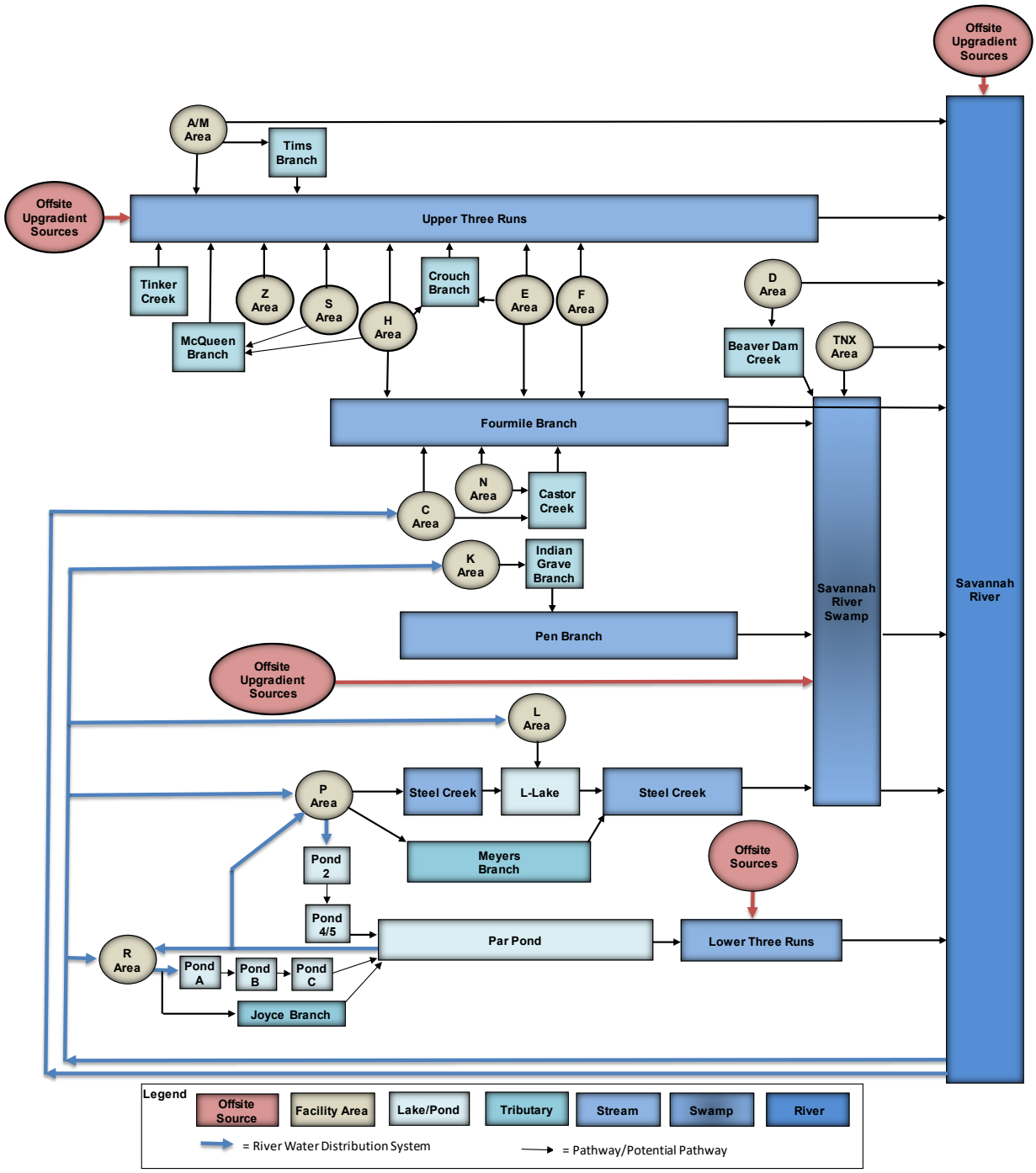


Figure 2. Overall Conceptual Site Model for the SRS IOUs



Figure 3. Bioassessment Sampling Locations

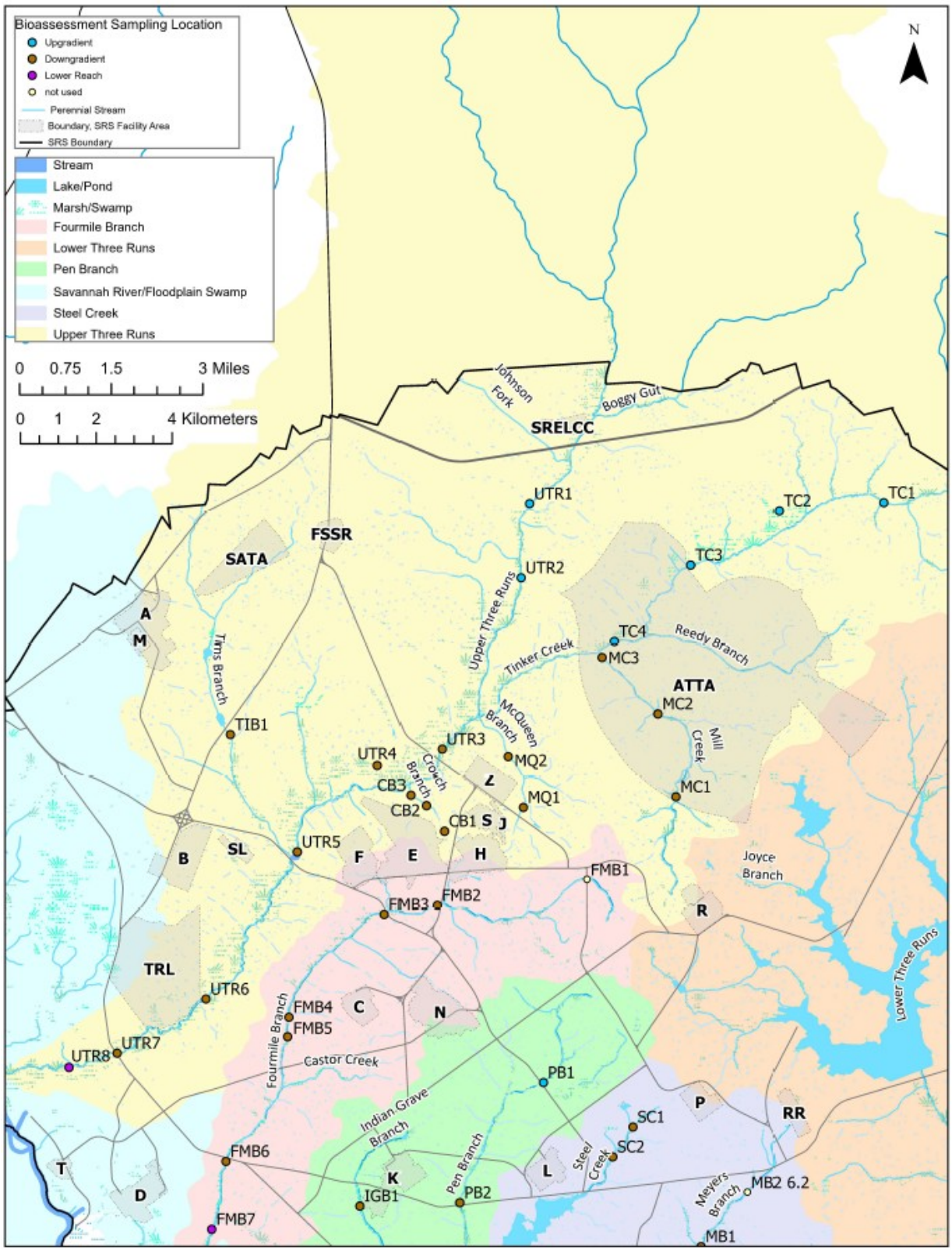


Figure 4. Map of the Upper Three Runs IOU

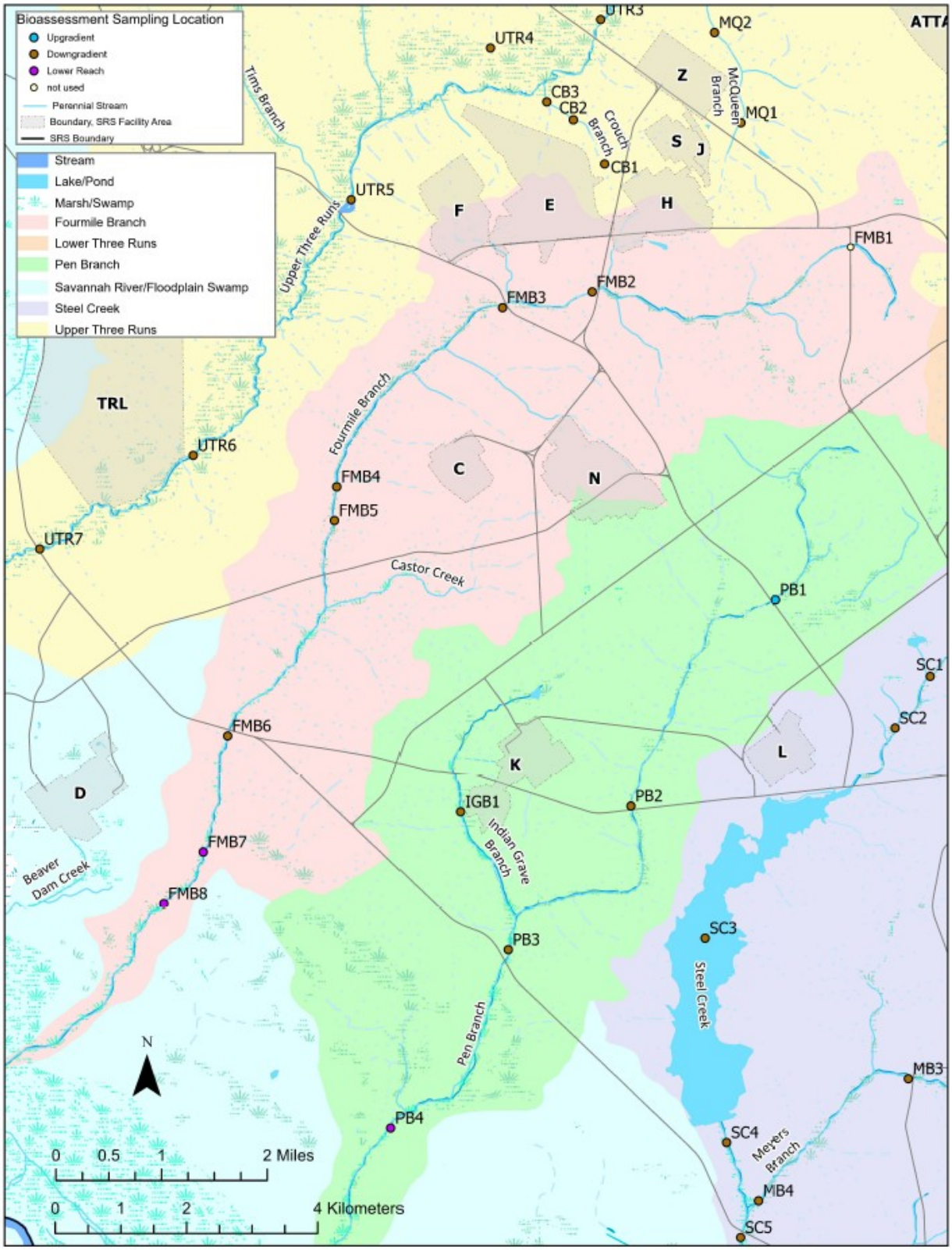


Figure 5. Map of the Fourmile Branch and Pen Branch IOUs

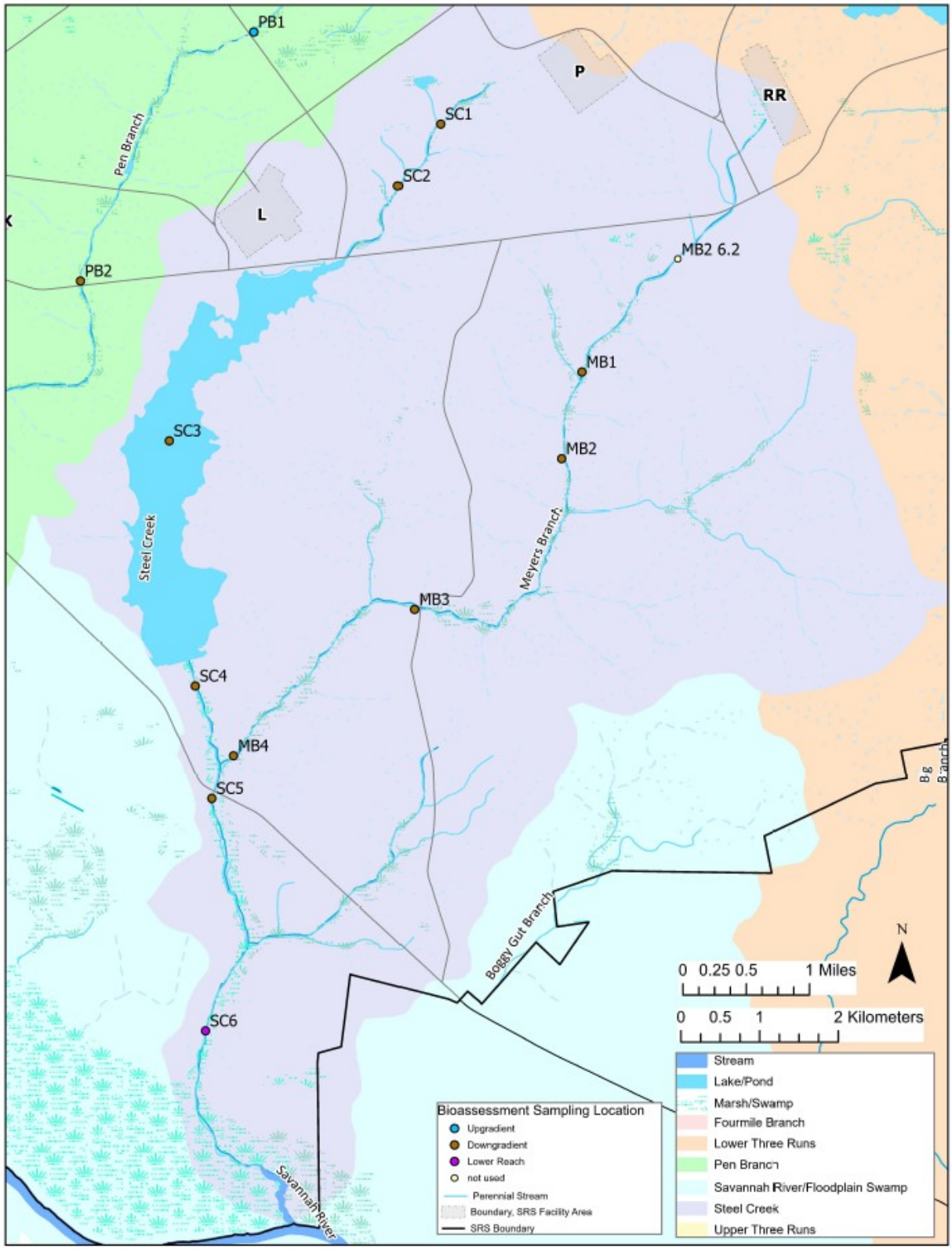


Figure 6. Map of the Steel Creek IOU

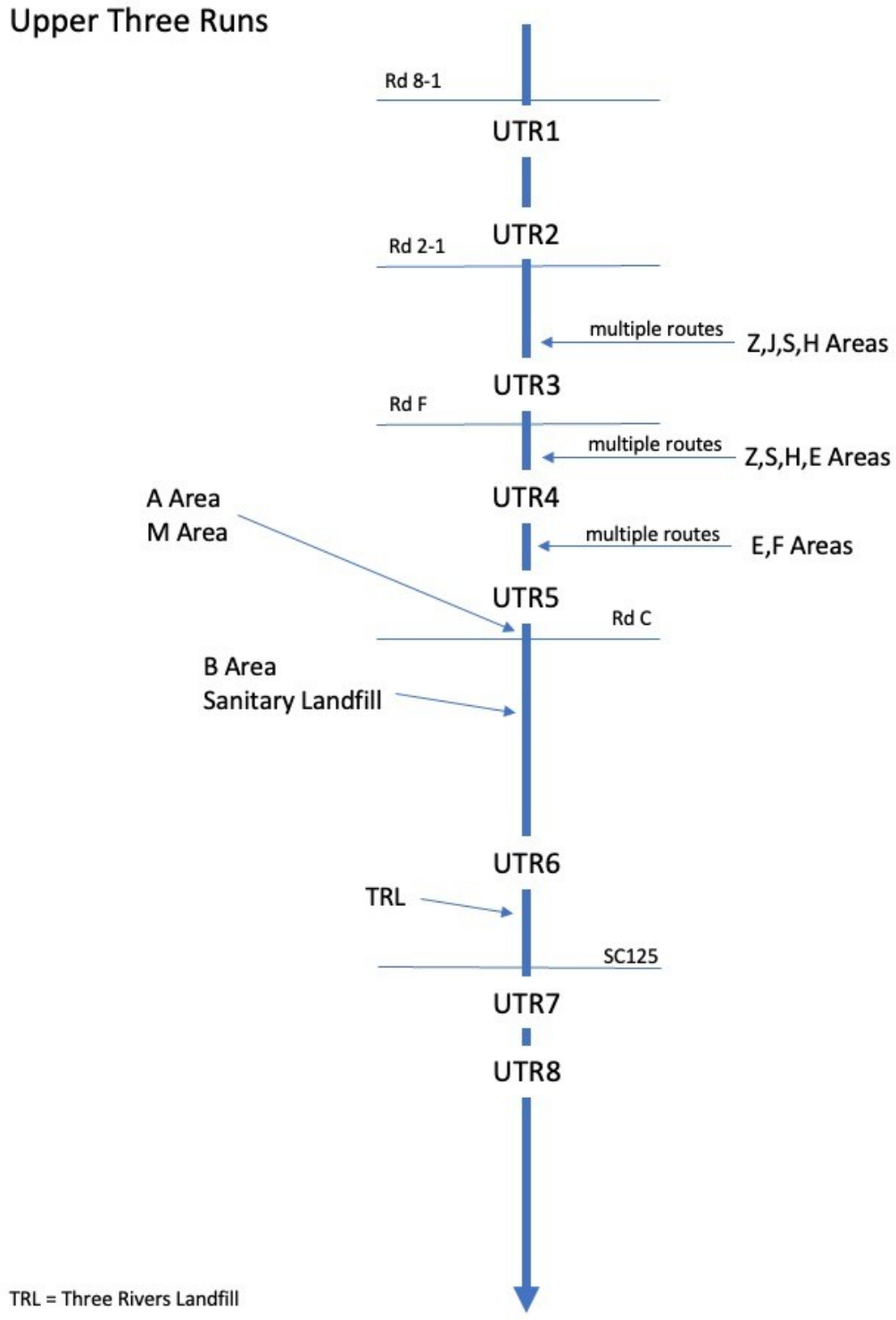


Figure 7. Conceptual Site Model of the Upper Three Runs Potential Impacts Relative to Sampling Location

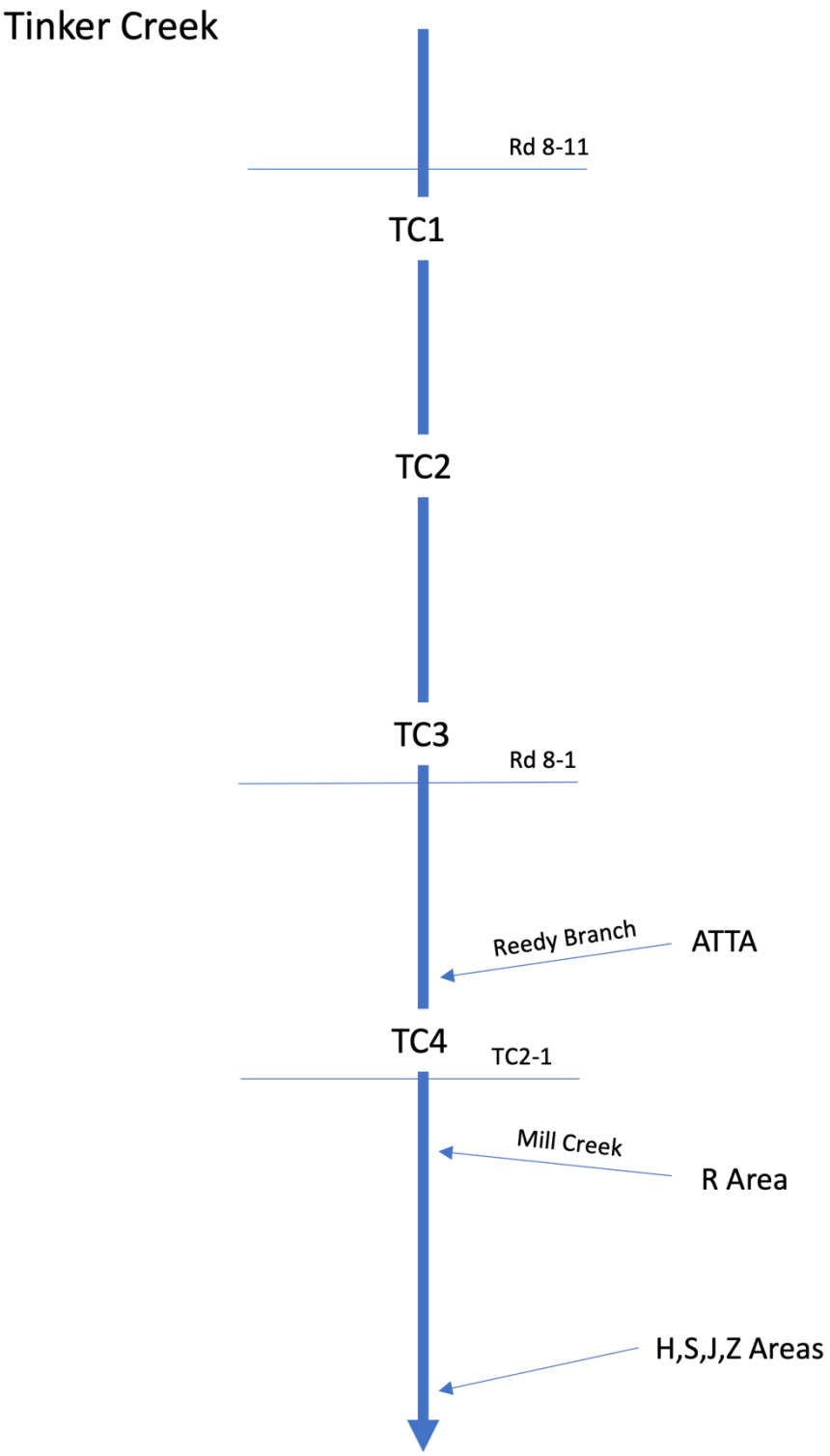


Figure 8. Conceptual Site Model of the Tinker Creek Potential Impacts Relative to Sampling Location

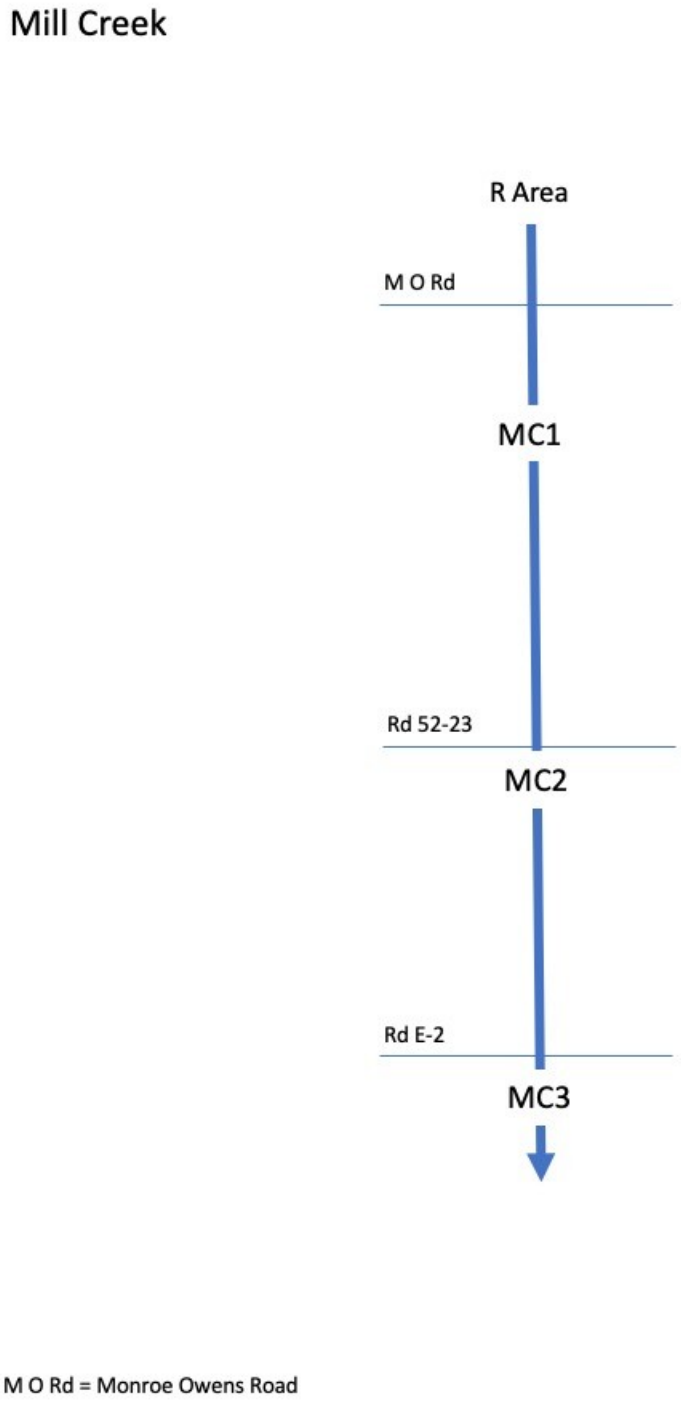


Figure 9. Conceptual Site Model of the Mill Creek Potential Impacts Relative to Sampling Location

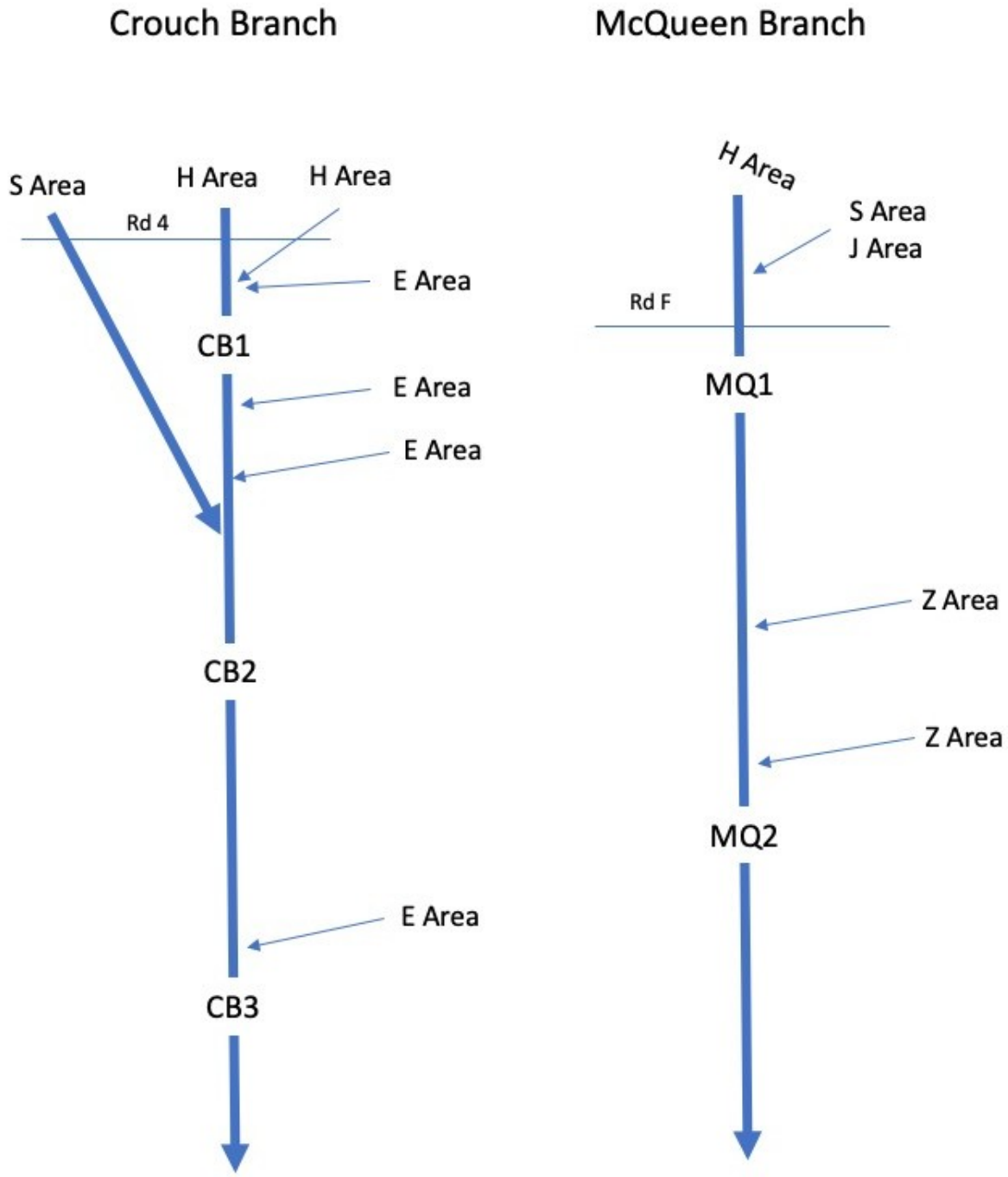


Figure 10. Conceptual Site Model of the Crouch Branch and McQueen Branch Potential Impacts Relative to Sampling Location

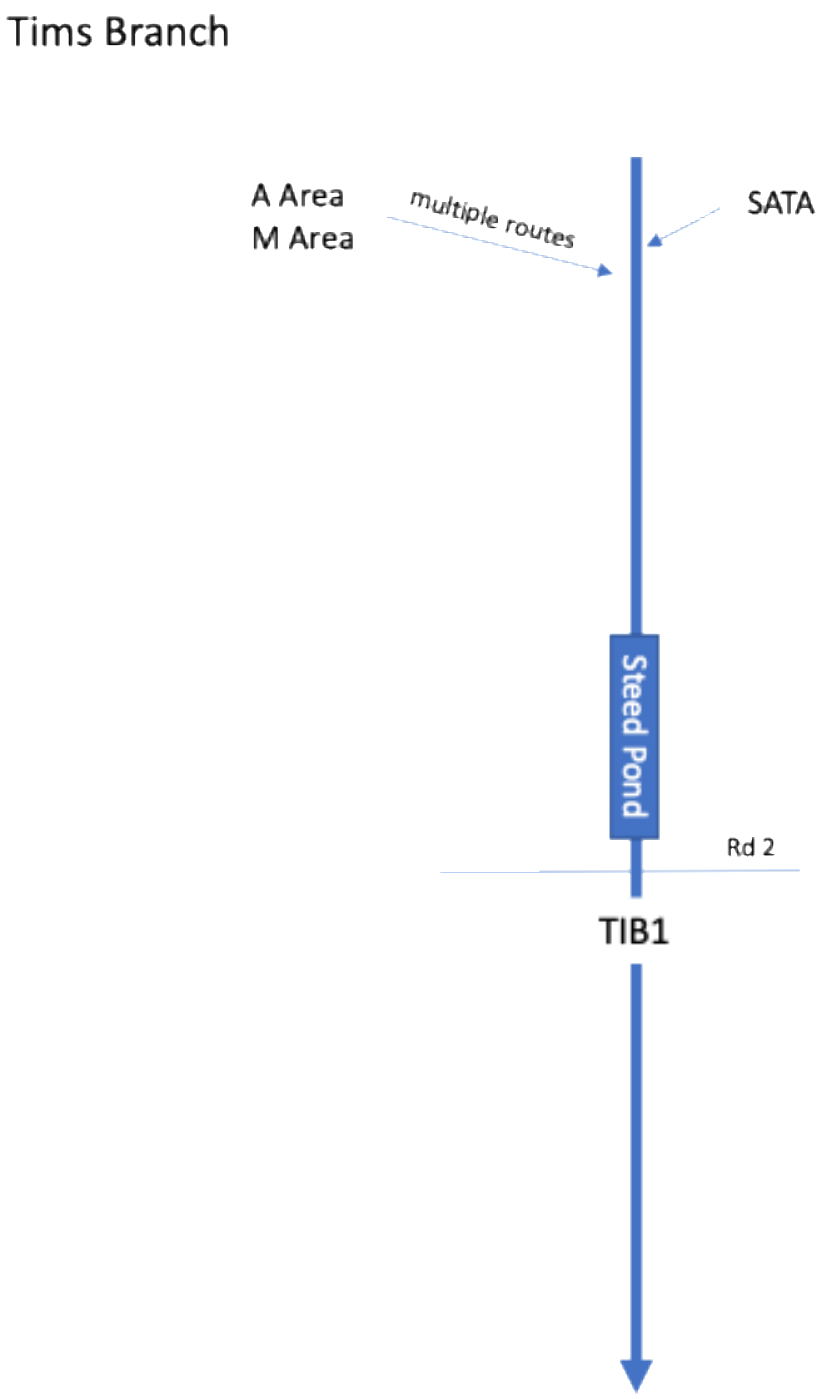


Figure 11. Conceptual Site Model of the Tims Branch Potential Impacts Relative to Sampling Location

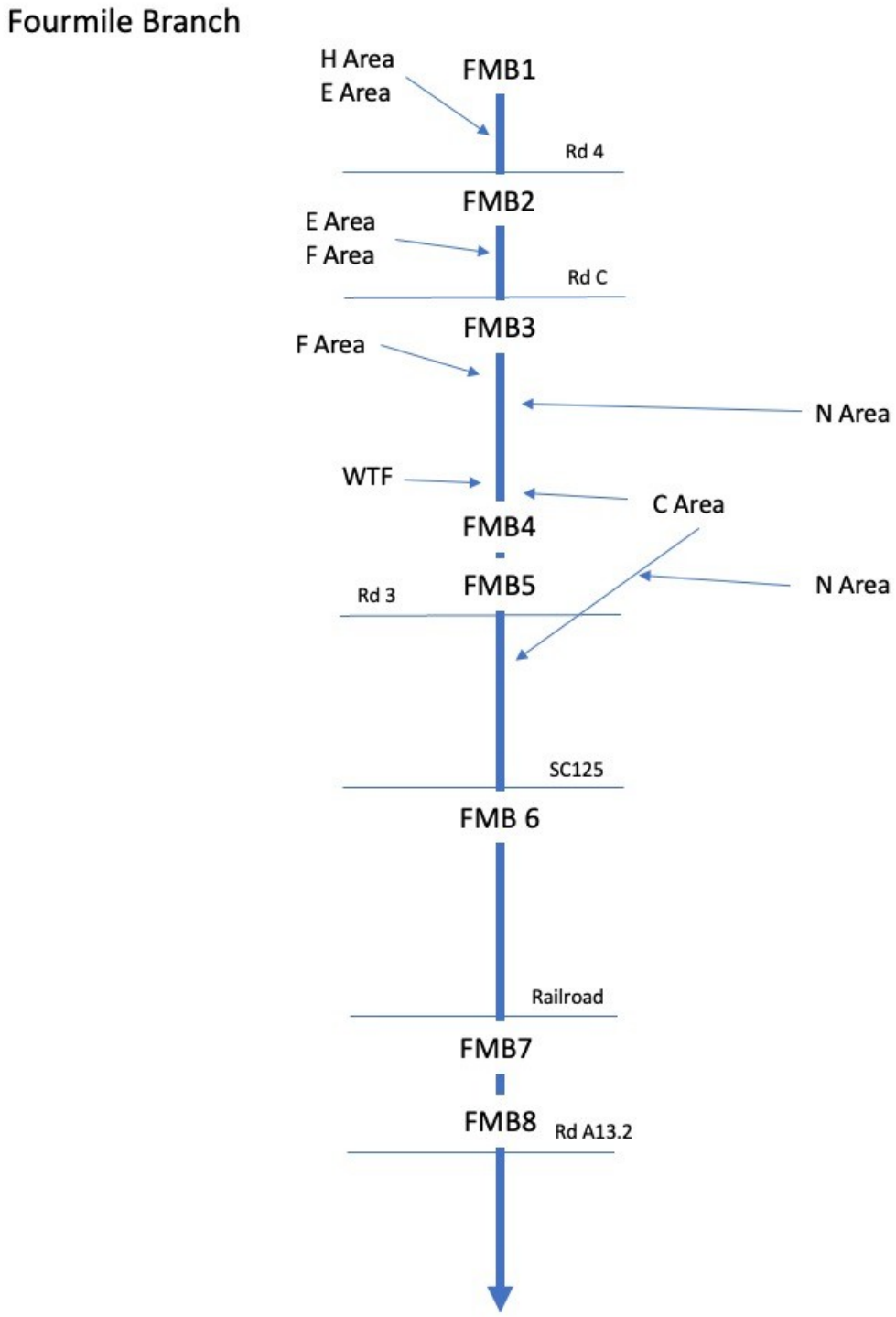
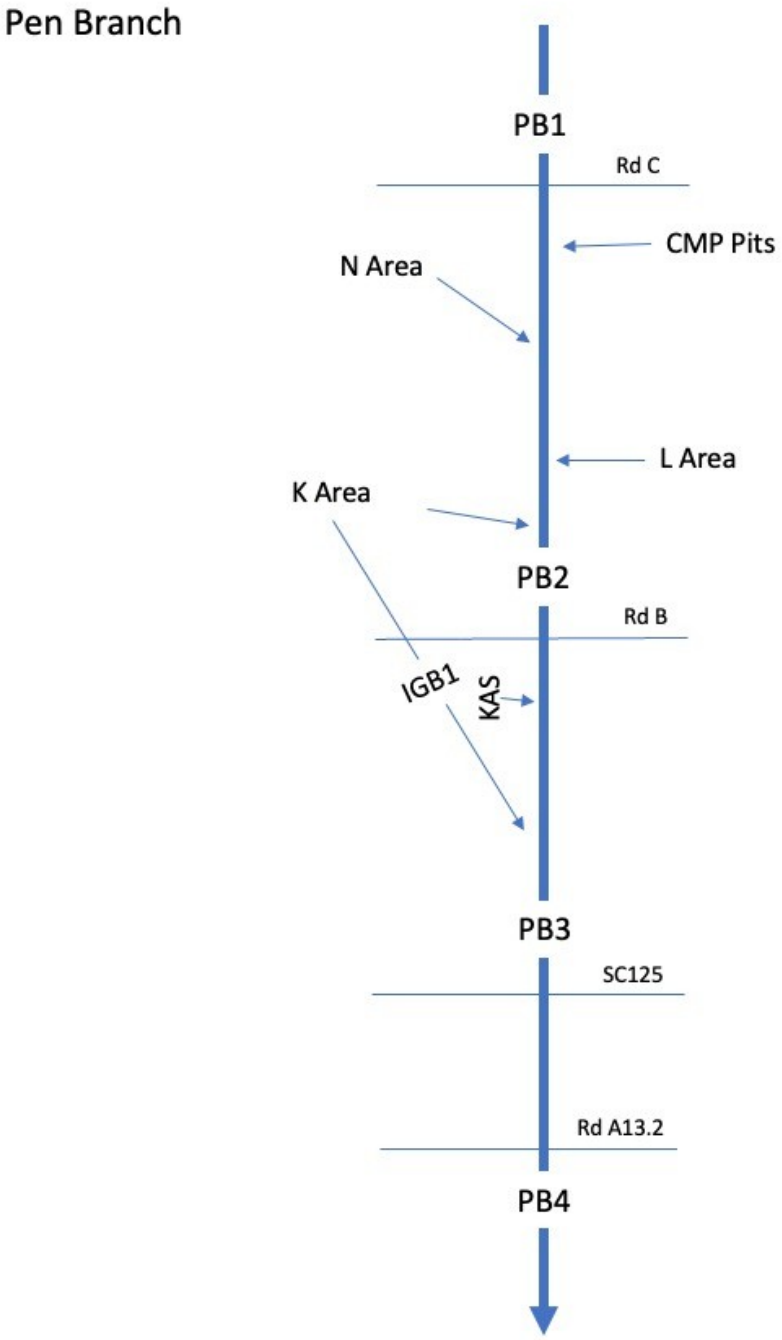


Figure 12. Conceptual Site Model of the Fourmile Branch Potential Impacts Relative to Sampling Location



KAS = K Area sludge application site.

Figure 13. Conceptual Site Model of the Pen Branch and Indian Grave Branch Potential Impacts Relative to Sampling Location

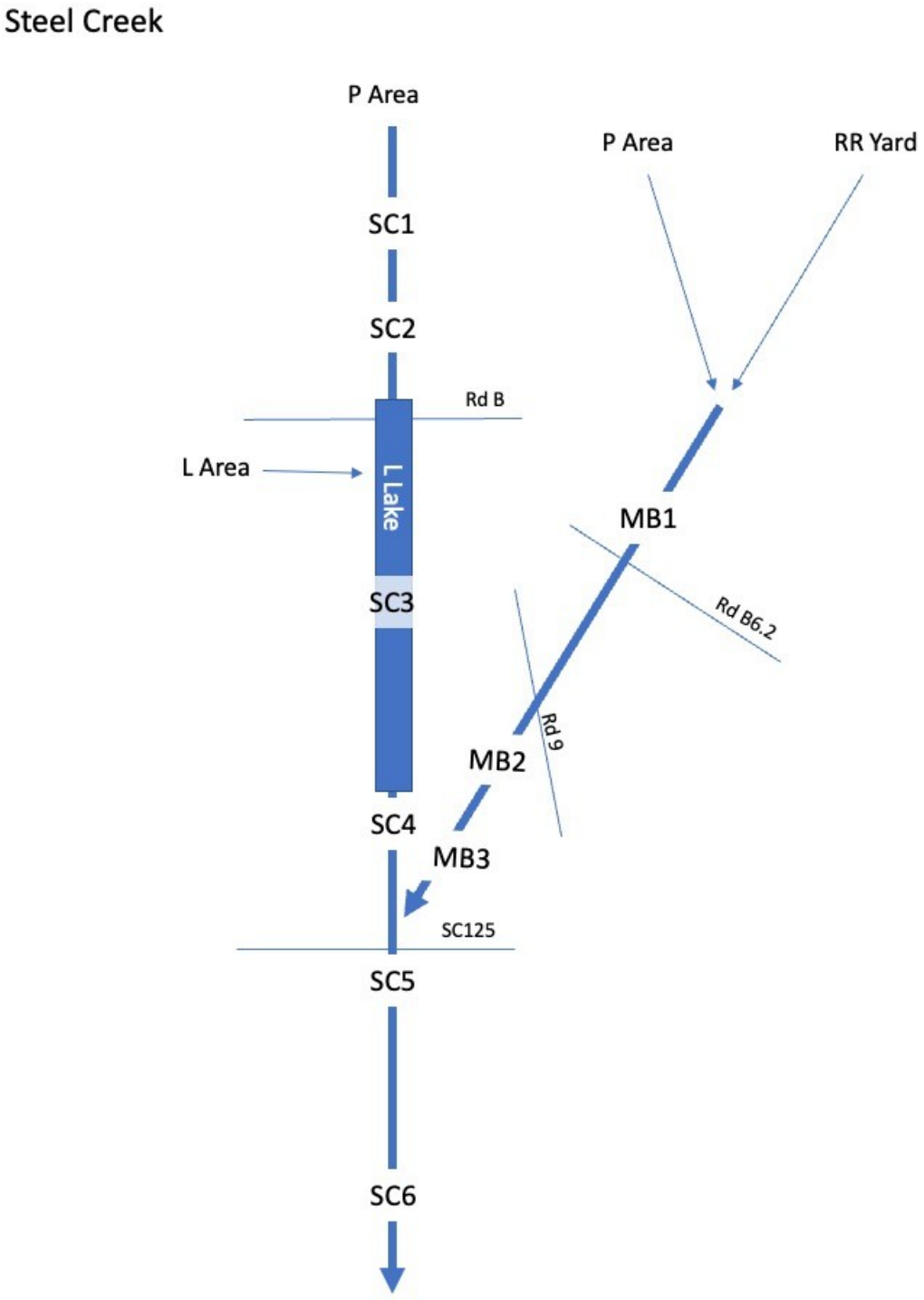


Figure 14. Conceptual Site Model of the Steel Creek and Meyers Branch Potential Impacts Relative to Sampling Location

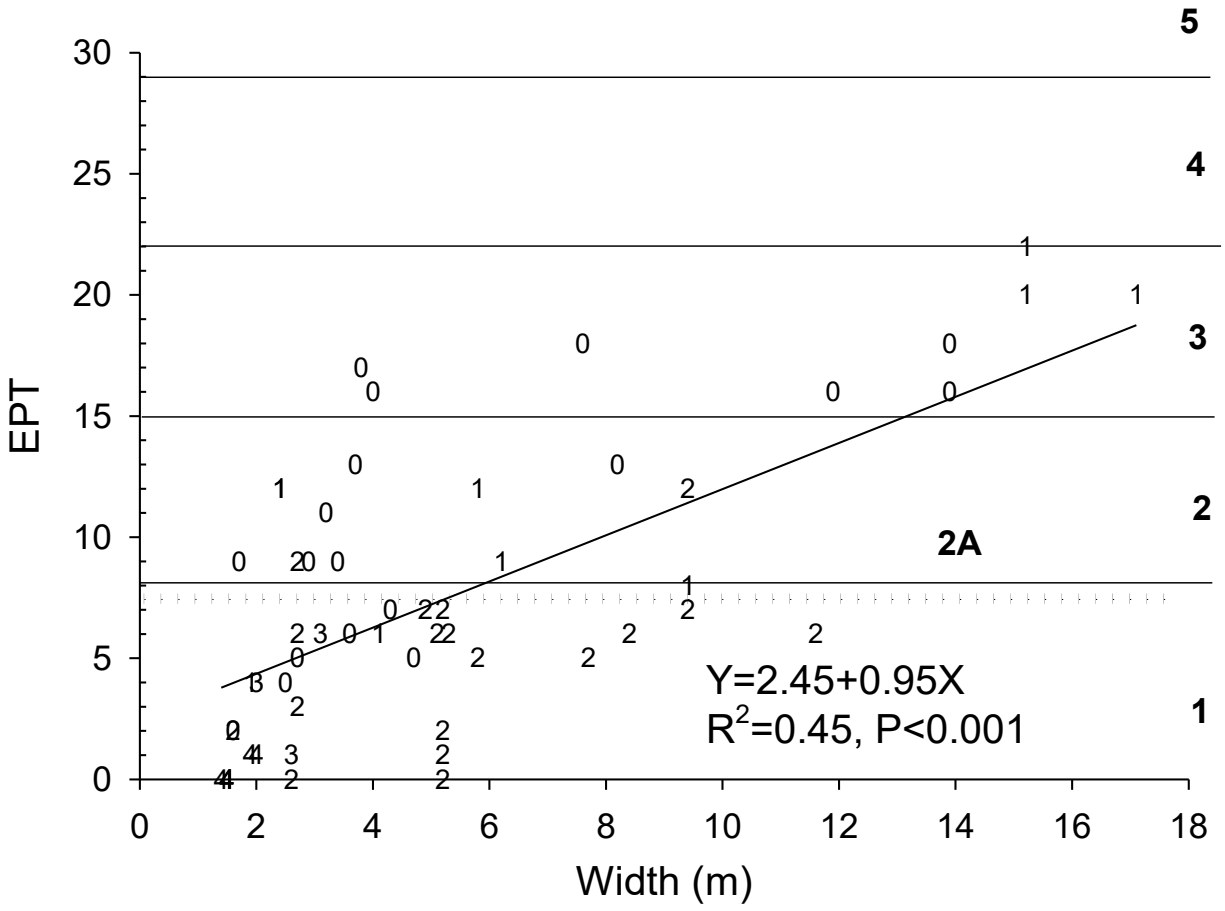


Figure 15. Regression of EPT on Stream Width
Sample site symbols indicate level of disturbance (0=undisturbed to 4=highly disturbed). Numbers 1-5 on the left indicate unadjusted MHSP scoring intervals for EPT taken from SCDHEC (1998). Dotted line represents the average EPT for all sites. Taken from Paller et al. (2007).

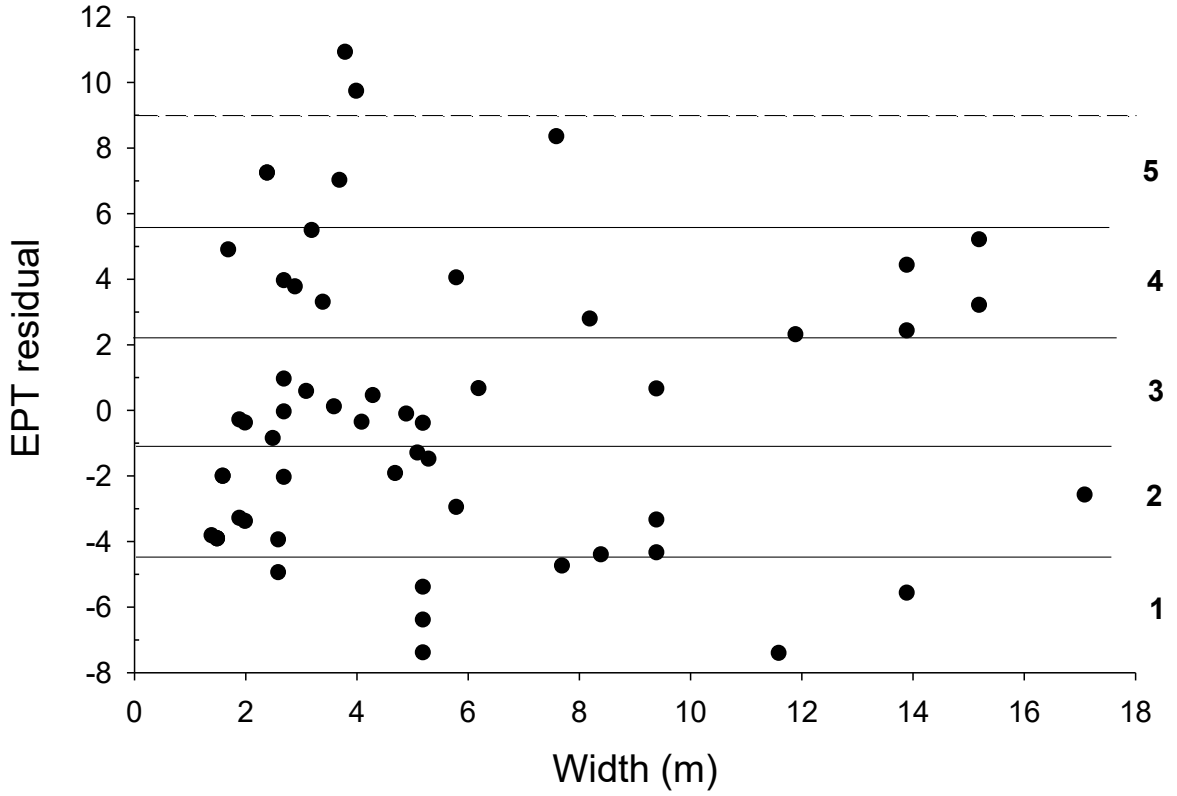


Figure 16. Distribution of EPT Residuals Divided into Five Scoring Intervals after Excluding the Upper 5%

Distribution of EPT residuals were derived from the regression of EPT on stream width. Scoring intervals are shown by the dashed line. Higher EPT residuals indicate greater than expected EPT and receive higher scores. Taken from Paller et al. 2007.

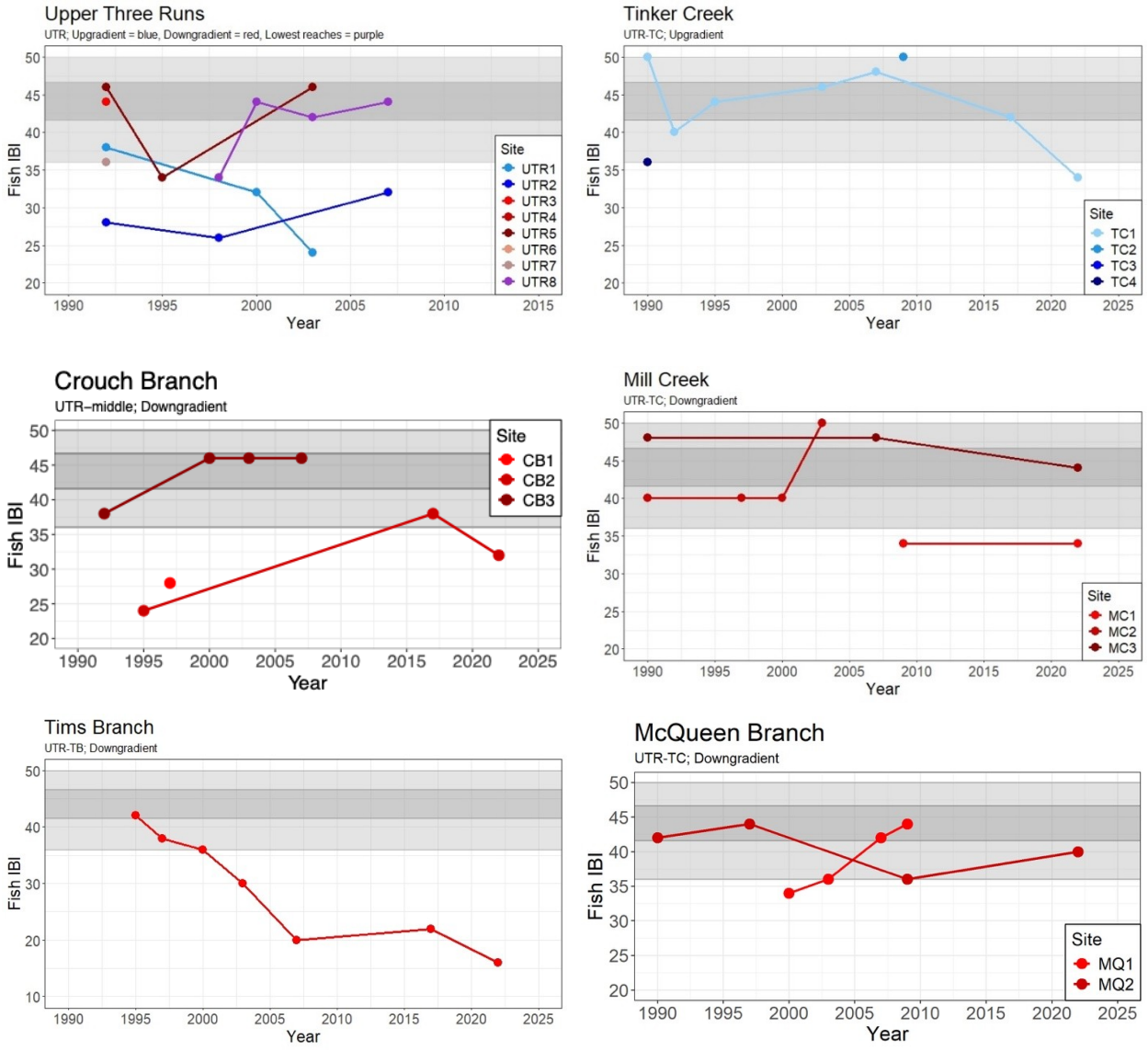


Figure 17. IBI Trends in Upper Three Runs and Tributaries Over Time

Sample Sites are shown as upgradient (blue) or downgradient (red/brown) of potential impacts or were at the lowest stream reaches (purple). The light grey box indicates the range of upgradient IBIs minus the lowest 5% (36-50) and the dark grey is the 95% upper confidence interval of the upgradient range (41.6 – 46.7). Note: No sites in UTR were sampled in 2022.

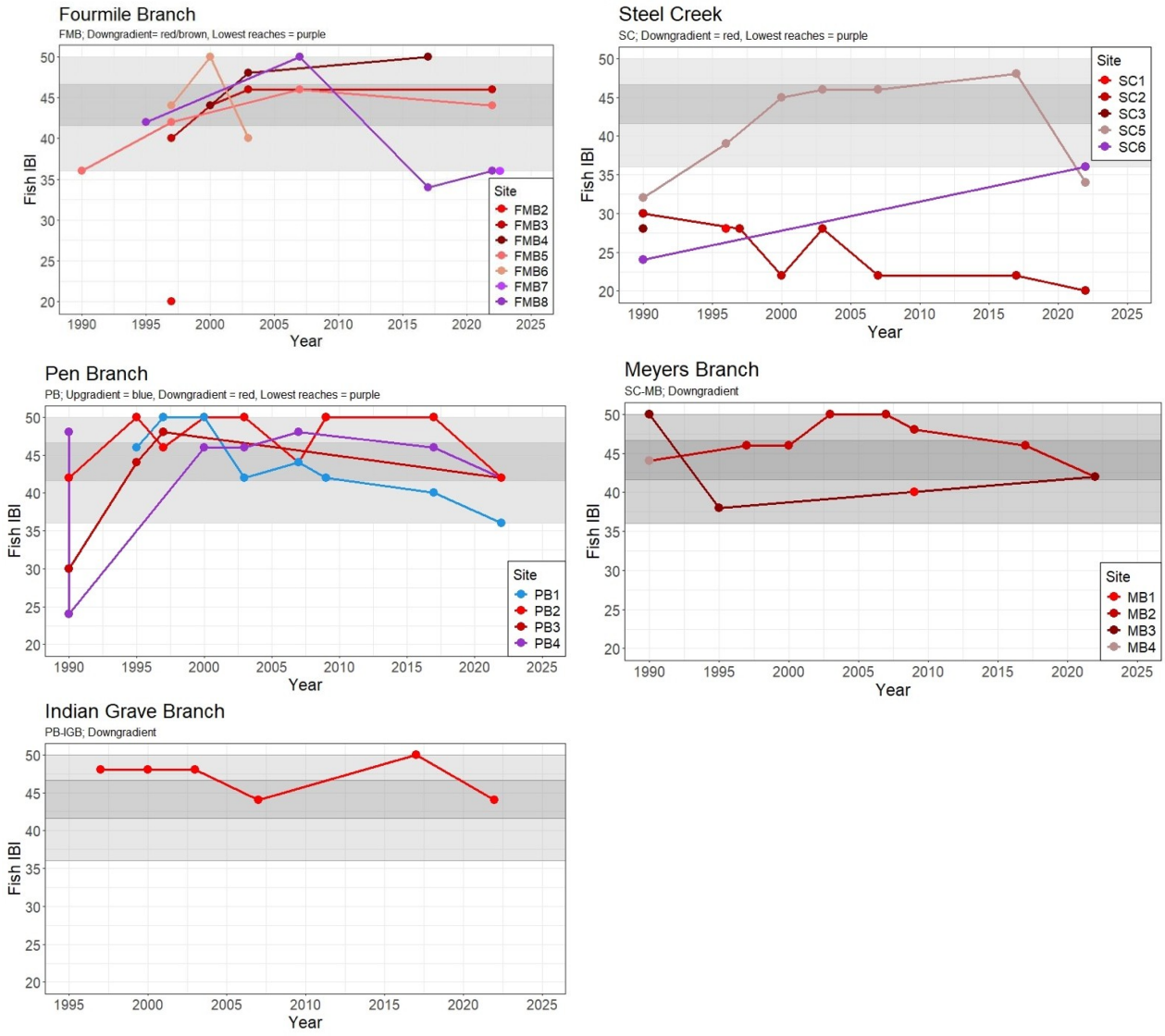


Figure 18. IBI Trends in Fourmile Branch, Pen Branch, and Steel Creek, and Tributaries Over Time

Sample Sites are shown as upgradient (blue) or downgradient (red/brown) of potential impacts or were at the lowest stream reaches (purple). The light grey box indicates the range of upgradient IBIs minus the lowest 5% (36-50) and the dark grey is the 95% confidence interval of the upgradient range (41.6 – 46.7).

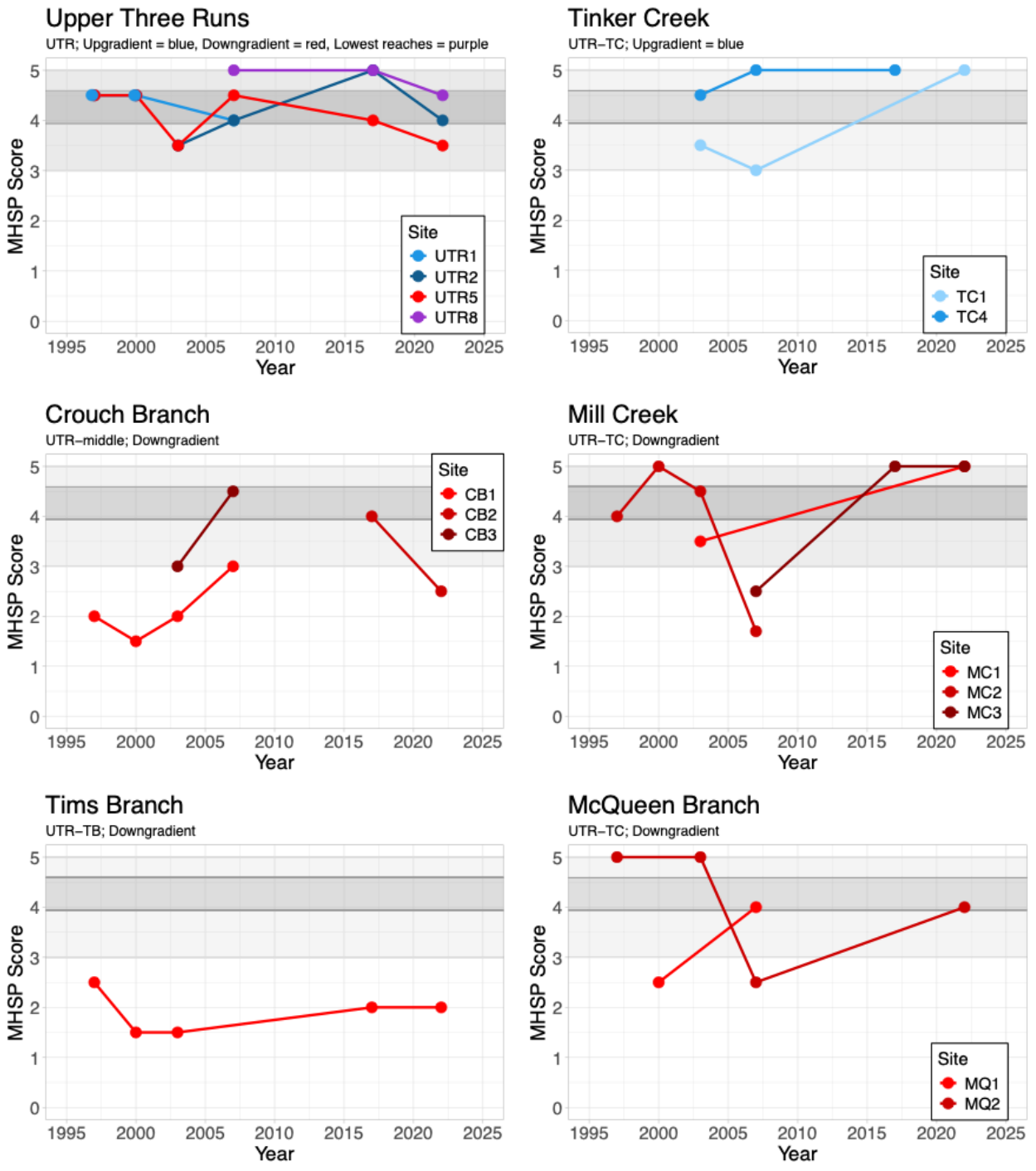


Figure 19. MHSP Trends in Upper Three Runs and Tributaries

Sample sites are denoted as upgradient (blue), downgradient of potential impacts (red/brown), or at the lowest stream reaches (purple). Light grey box indicates the upgradient range minus the lowest 5% (3 – 5) and dark grey box indicates the 95% confidence interval of the upgradient range (3.94 – 4.59). Sites ordered from upstream to downstream.

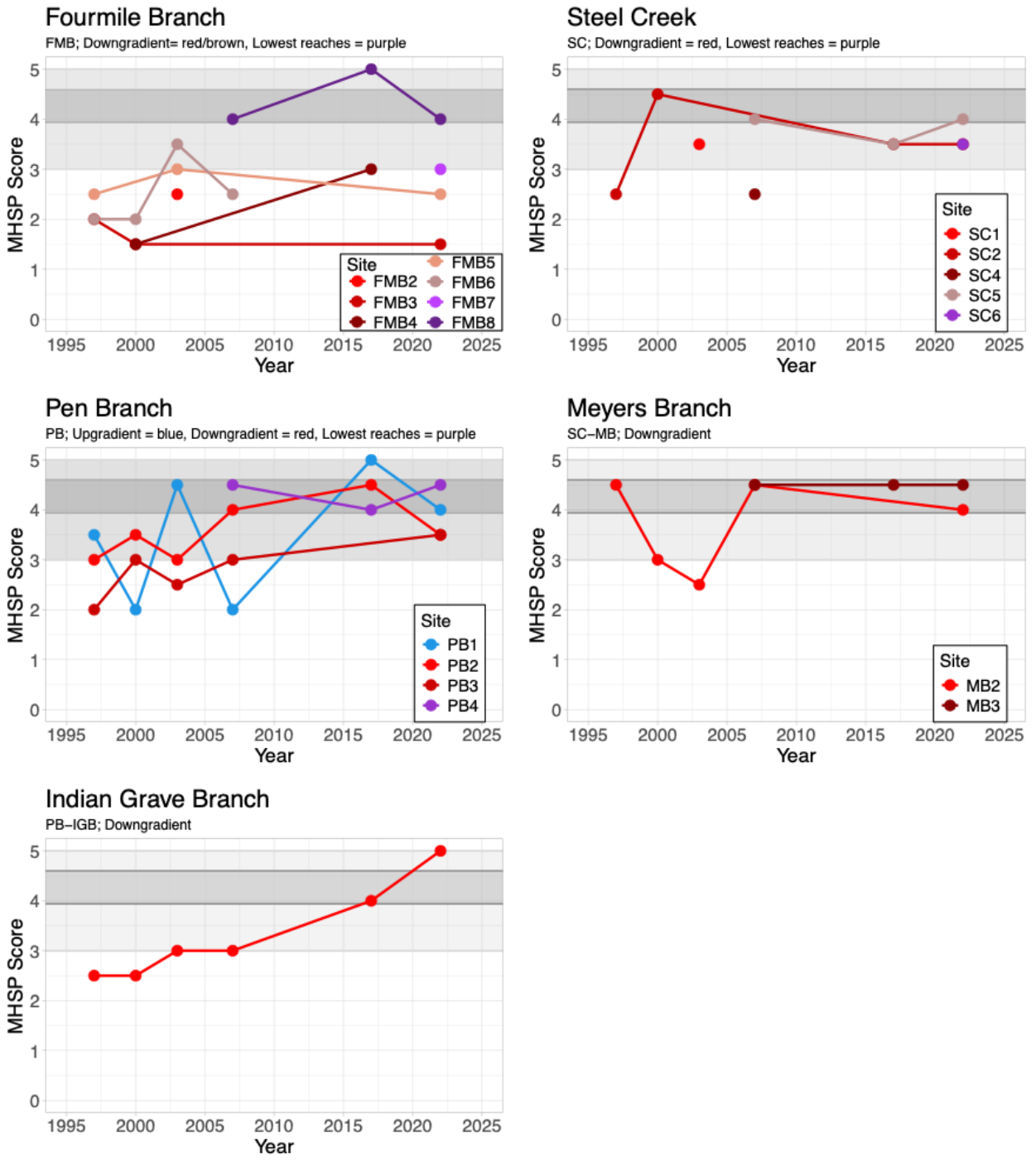


Figure 20. MHSP Trends in Fourmile Branch, Steel Creek, Pen Branch, and Tributaries
Sample sites are denoted as upgradient (blue), downgradient of potential impacts (red/brown), or at the lowest stream reaches (purple). Light grey box indicates the upgradient range minus the lowest 5% (3 – 5) and dark grey box indicates the 95% confidence interval of the upgradient range (3.94 – 4.59). Sites ordered from upstream to downstream.

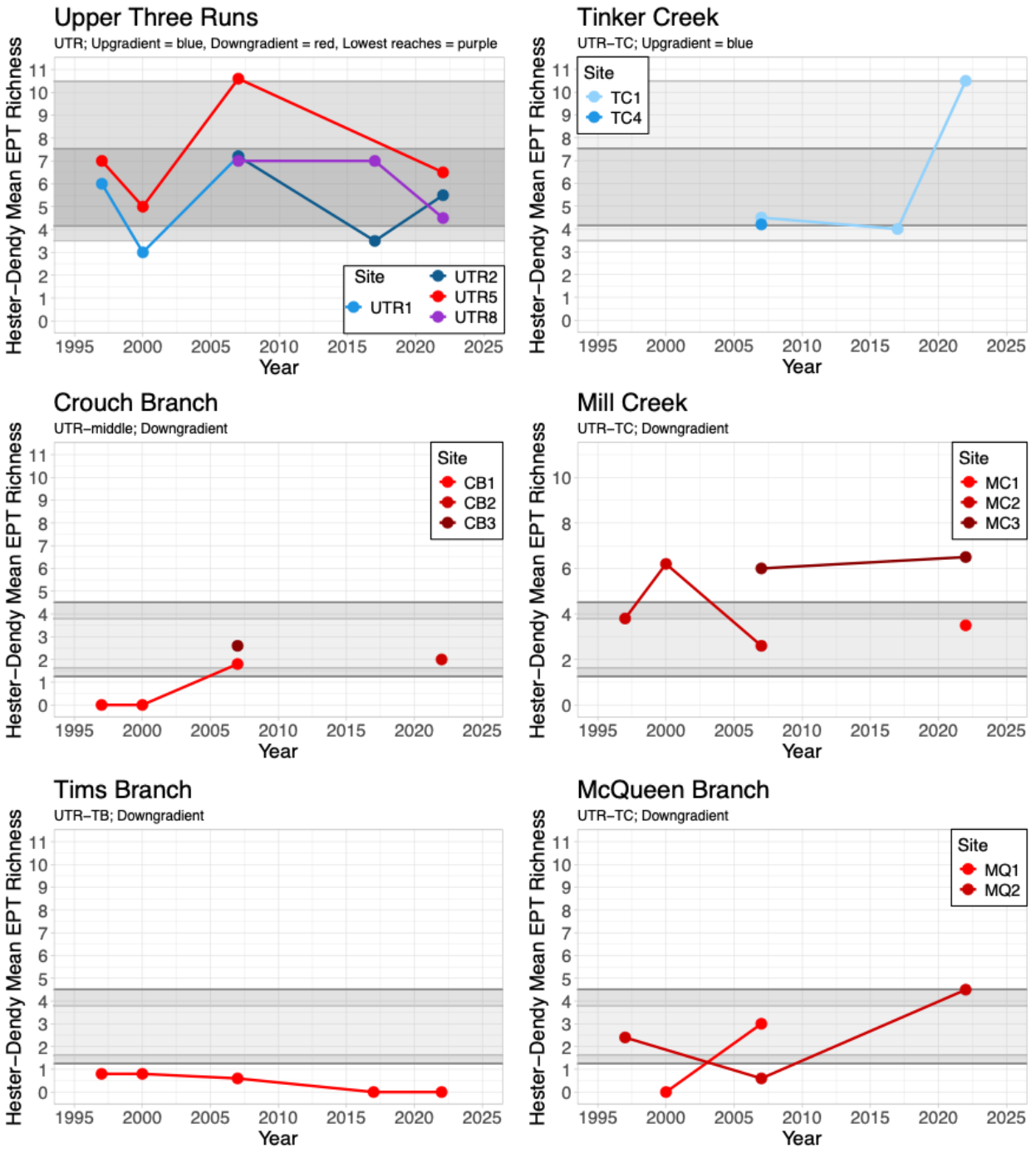


Figure 21. Hester-Dendy EPT Trends in Upper Three Runs and Tributaries

Sample sites are denoted as upgradient (blue), downgradient of potential impacts (red/brown), or at the lowest stream reaches (purple). Light grey box indicates the upgradient range minus the lowest 5% (small streams: 1.6 – 3.8; large streams: 3.5 – 10.5) and dark grey box indicates the 95% confidence interval (CI) of the upgradient range (small streams: 1.25 – 4.50; large streams: 4.15 – 7.54). When both small and large streams are on same graph, yellow indicates small stream range and CI and grey indicates large stream range and CI.

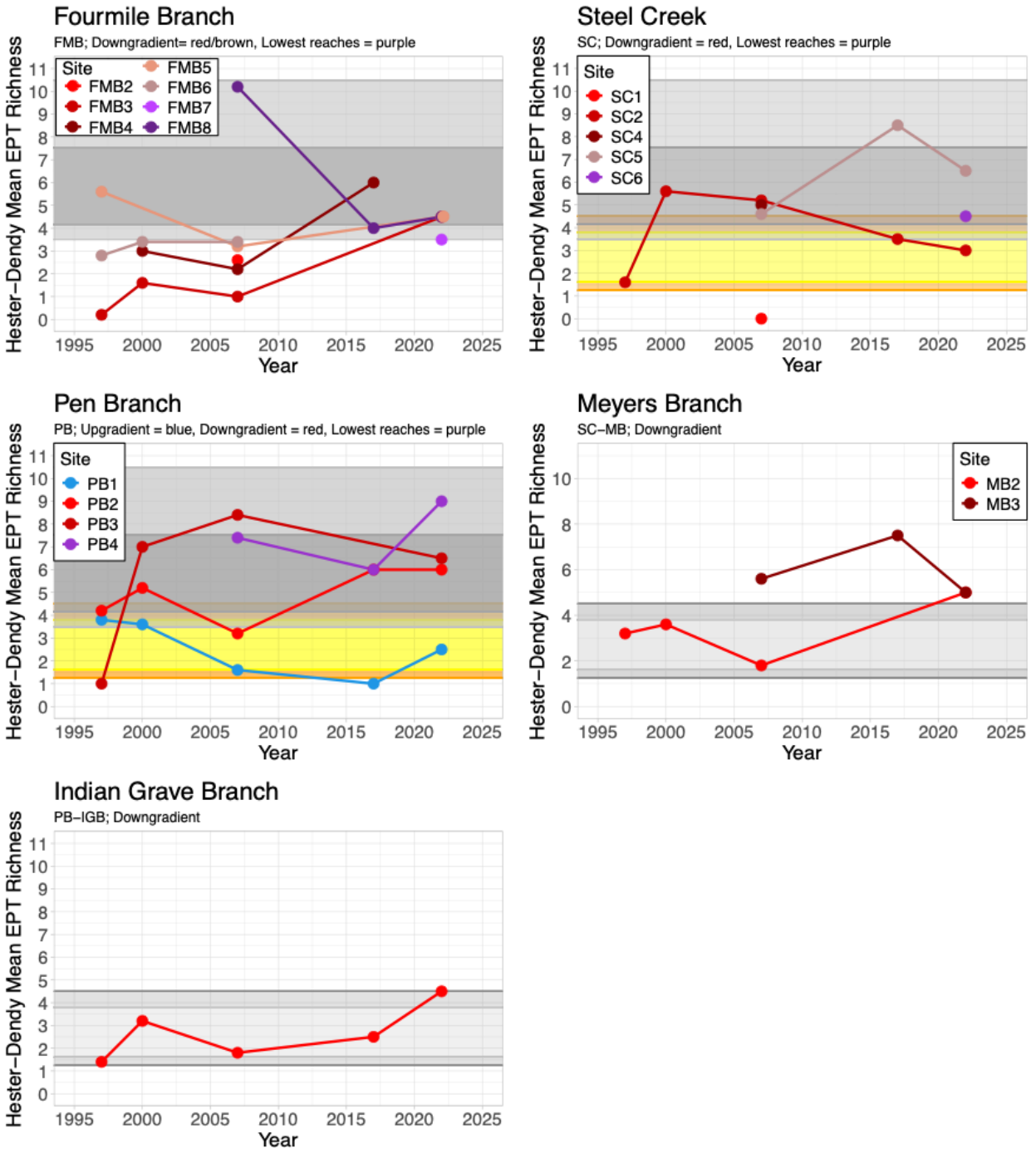


Figure 22. Hester-Dendy EPT Trends in Fourmile Branch, Steel Creek, Pen Branch and Tributaries

Sample sites are denoted as upgradient (blue), downgradient of potential impacts (red/brown), or at the lowest stream reaches (purple). Light grey box indicates the upgradient range minus the lowest 5% (small streams: 1.6 – 3.8; large streams: 3.5 – 10.5) and dark grey box indicates the 95% confidence interval (CI) of the upgradient range (small streams: 1.25 – 4.50; large streams: 4.15 – 7.54). When both small and large streams are on same graph, yellow indicates small stream range and CI and grey indicates large stream range and CI.

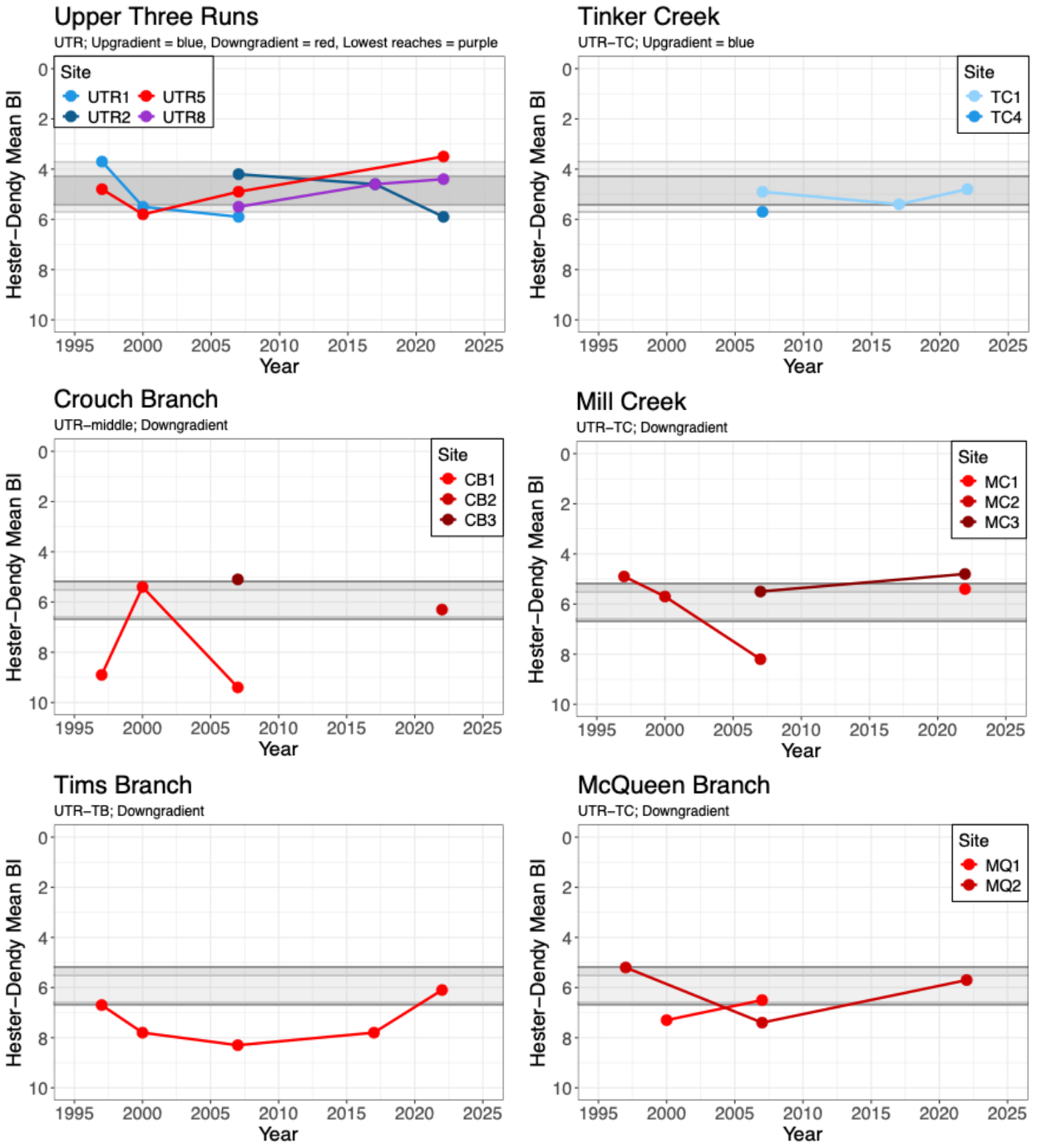


Figure 23. Hester-Dendy BI Trends in Upper Three Runs and Tributaries

Sample sites are denoted as upgradient (blue), downgradient of potential impacts (red/brown), or at the lowest stream reaches (purple). Light grey box indicates the upgradient range minus the lowest 5% (small streams: 5.5 – 6.6; large streams: 3.7 – 5.7) and dark grey box indicates the 95% confidence interval (CI) of the upgradient range (small streams: 5.17 – 6.68; large streams: 4.28 – 5.42). When both small and large streams are on the same graph, yellow indicates small stream range and CI and grey indicates large stream range and CI. Sites ordered from upstream to downstream. Y-axis ordered in reverse to show best values at the top of the graph (lower scores are better).

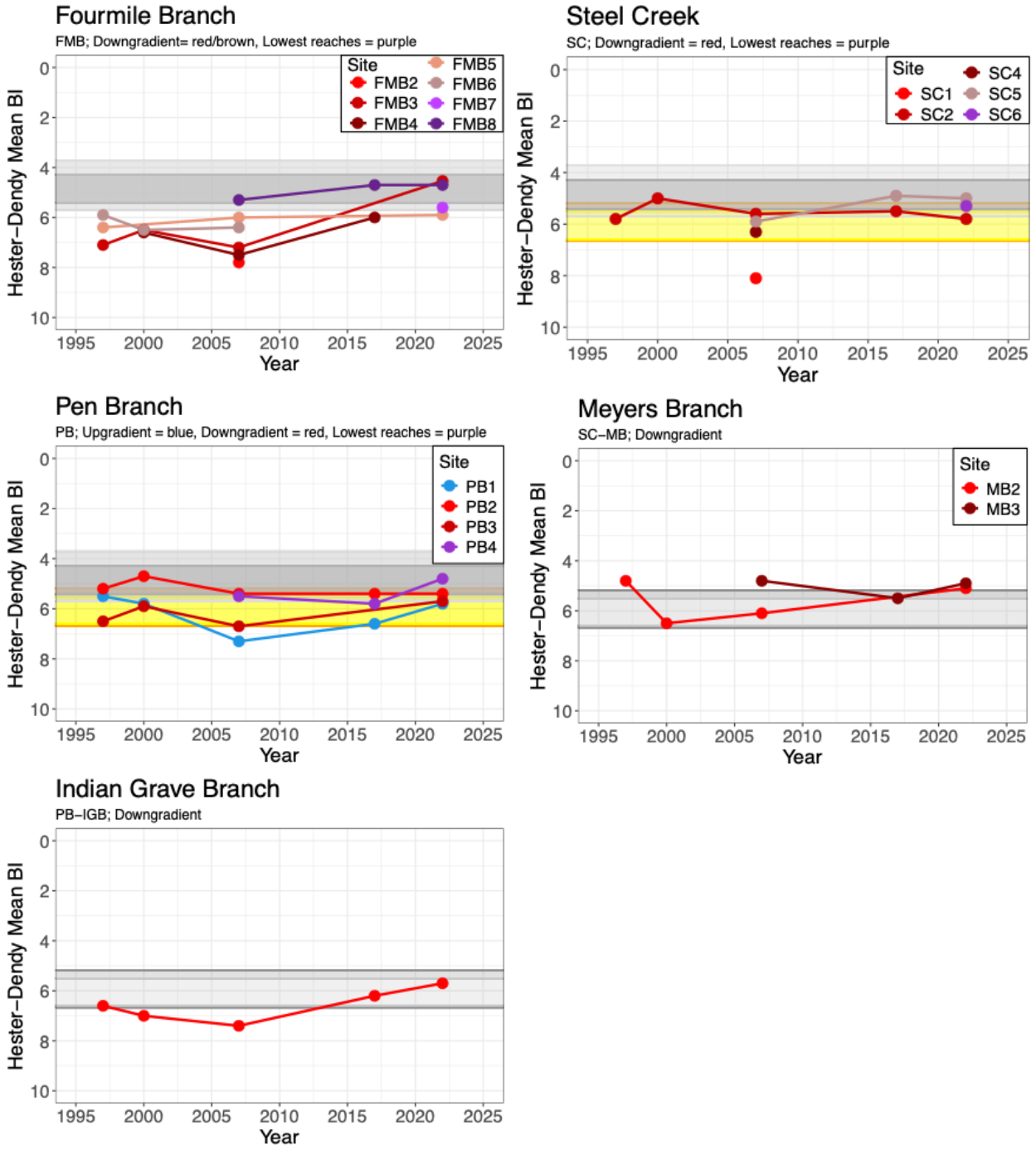


Figure 24. Hester-Dendy BI Trends in Fourmile Branch, Pen Branch, Steel Creek, and Tributaries

Sample sites are denoted as upgradient (blue), downgradient of potential impacts (red/brown), or at the lowest stream reaches (purple). Light grey box indicates the upgradient range minus the lowest 5% (small streams: 5.5 – 6.6; large streams: 3.7 – 5.7) and dark grey box indicates the 95% confidence interval (CI) of the upgradient range (small streams: 5.17 – 6.68; large streams: 4.28 – 5.42). When both small and large streams are on the same graph, yellow indicates small stream range and CI and grey indicates large stream range and CI. Sites ordered from upstream to downstream. Y-axis ordered in reverse to show best values at the top of the graph (lower scores are better).

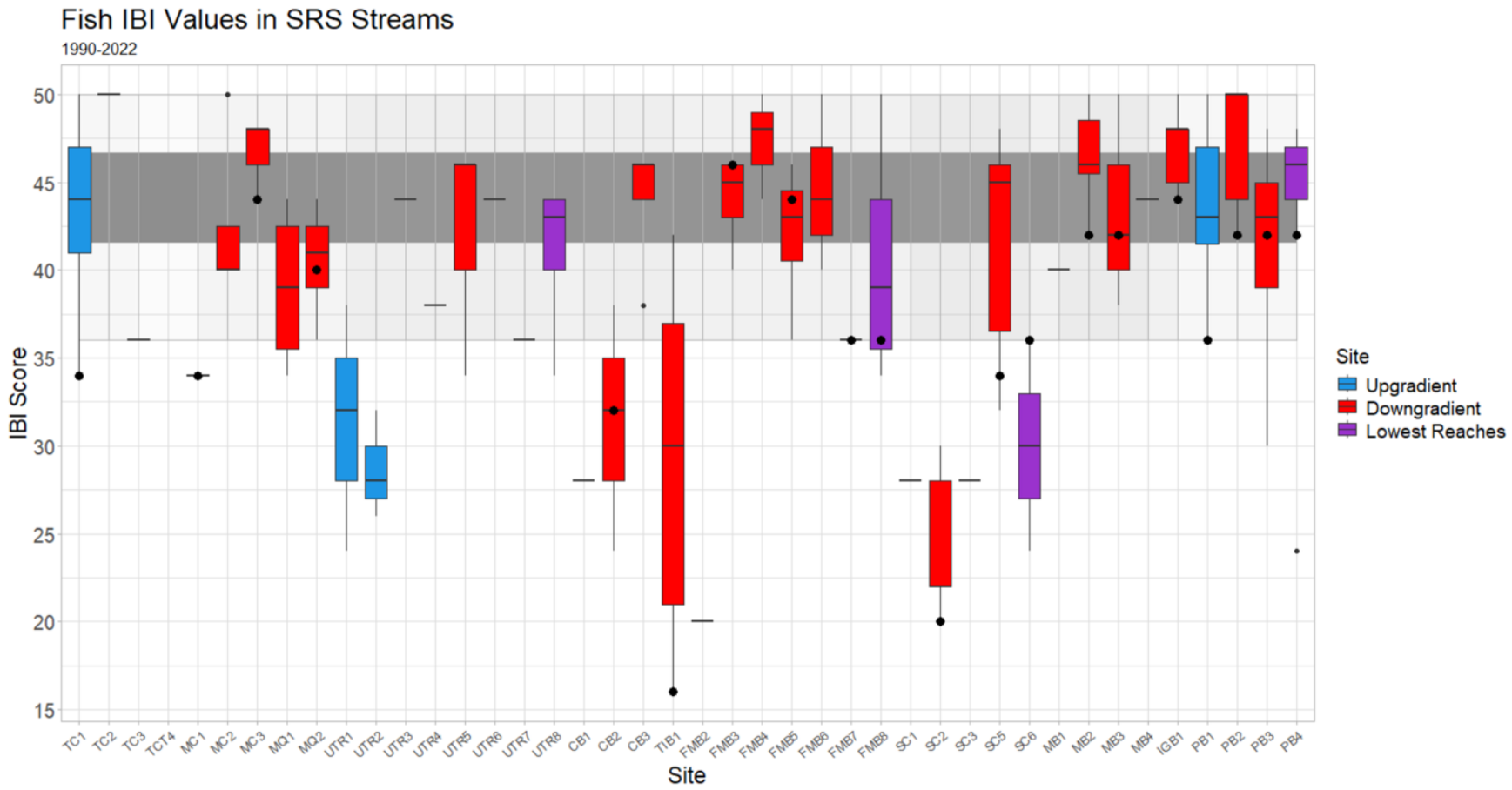


Figure 25. Boxplots of Fish IBI Values from 1900 to 2022

Sample sites are denoted as upgradient (blue), downgradient of potential impacts (red/brown), or at the lowest stream reaches (purple) in SRS Streams. Solid black circle symbol indicates the 2022 IBI. The dashed line is the cut-off values for upgradient sites corresponding to the lower 5% of the upgradient site range. Light grey box indicates the upgradient range minus the lowest 5%, and dark grey box indicates the 95% confidence interval of the upgradient range. Sites ordered by IOU subunits from upstream to downstream within the subunits.

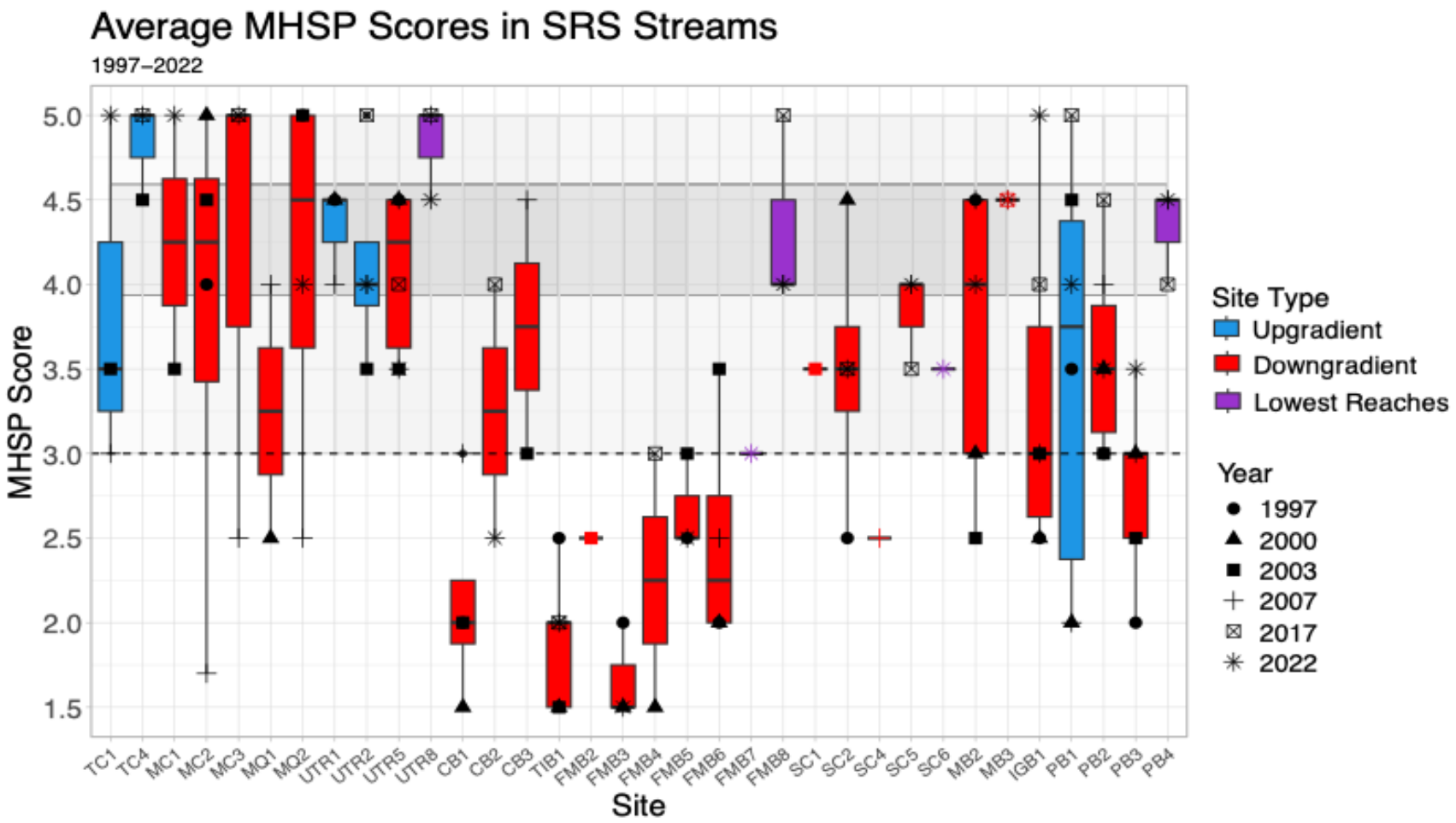


Figure 26. Boxplots of MHSP Values (1997 to 2022)
Sample sites are denoted as upgradient (blue), downgradient of potential impacts (red/brown), or at the lowest stream reaches (purple) in SRS Streams. Symbols indicate specific values for each year sampled. The dashed line is the cut-off values for upgradient sites corresponding to the lower 5% of the upgradient site range. Light grey box indicates the upgradient range minus the lowest 5%, and dark grey box indicates the 95% confidence interval of the upgradient range. Sites ordered by IOU subunits from upstream to downstream within the subunits.

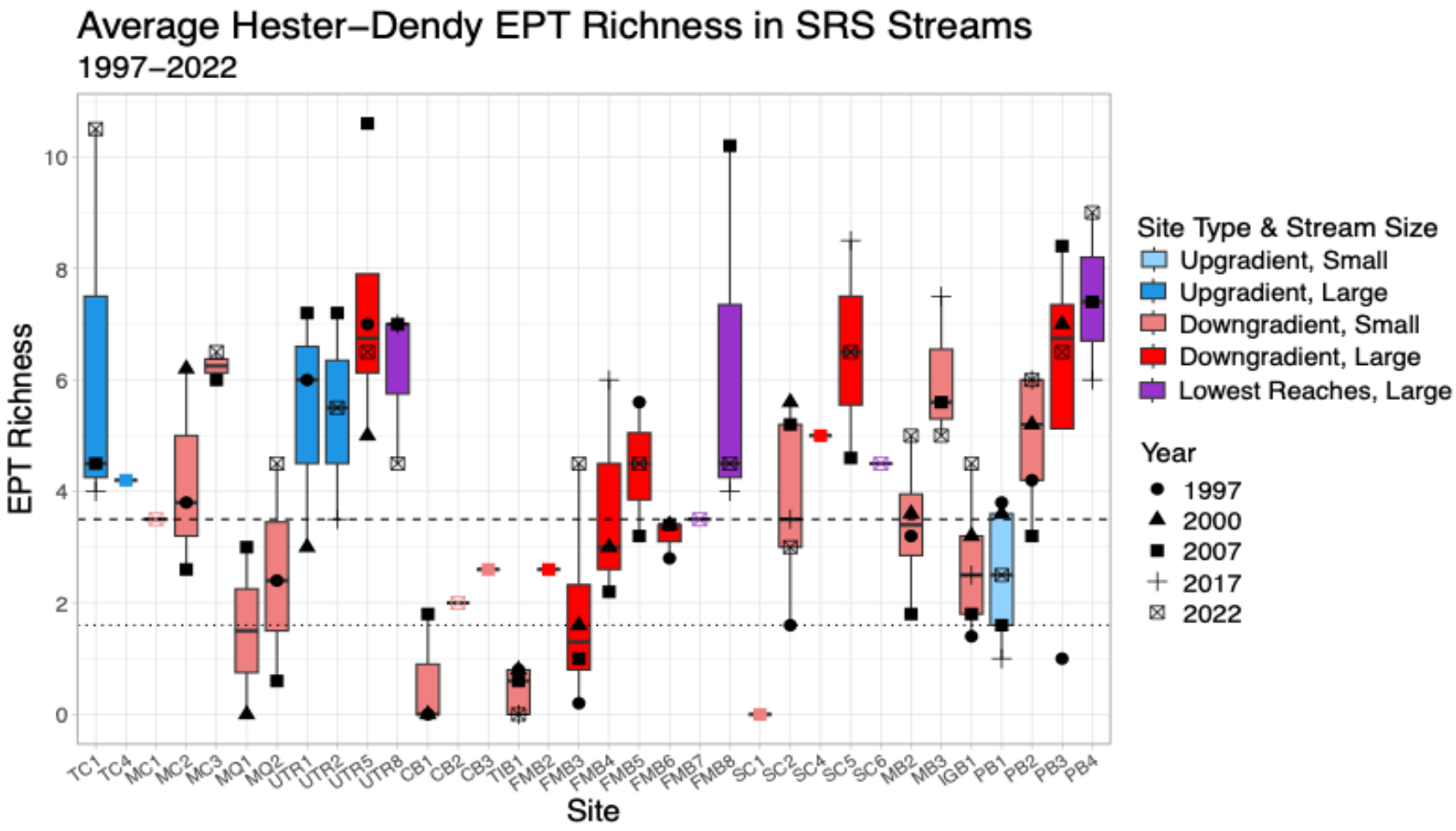


Figure 27. Average Hester-Dendy EPT Richness in SRS Stream (1997 – 2022)

Average number of EPT from Hester-Dendy artificial substrates in samples upgradient (blue), downgradient of potential impacts (red/brown), or at the lowest stream reaches (purple) in SRS Streams. Lighter shades of colors indicate small streams (<5 m wide). Horizontal lines are the cut-off value for upgradient sites corresponding to the lower 5% of the upgradient site ranges for small streams (<5 m wide) (dotted line) and large streams (≥5 m wide) (dashed line). Sites ordered by IOU subunits from upstream to downstream within the subunits.

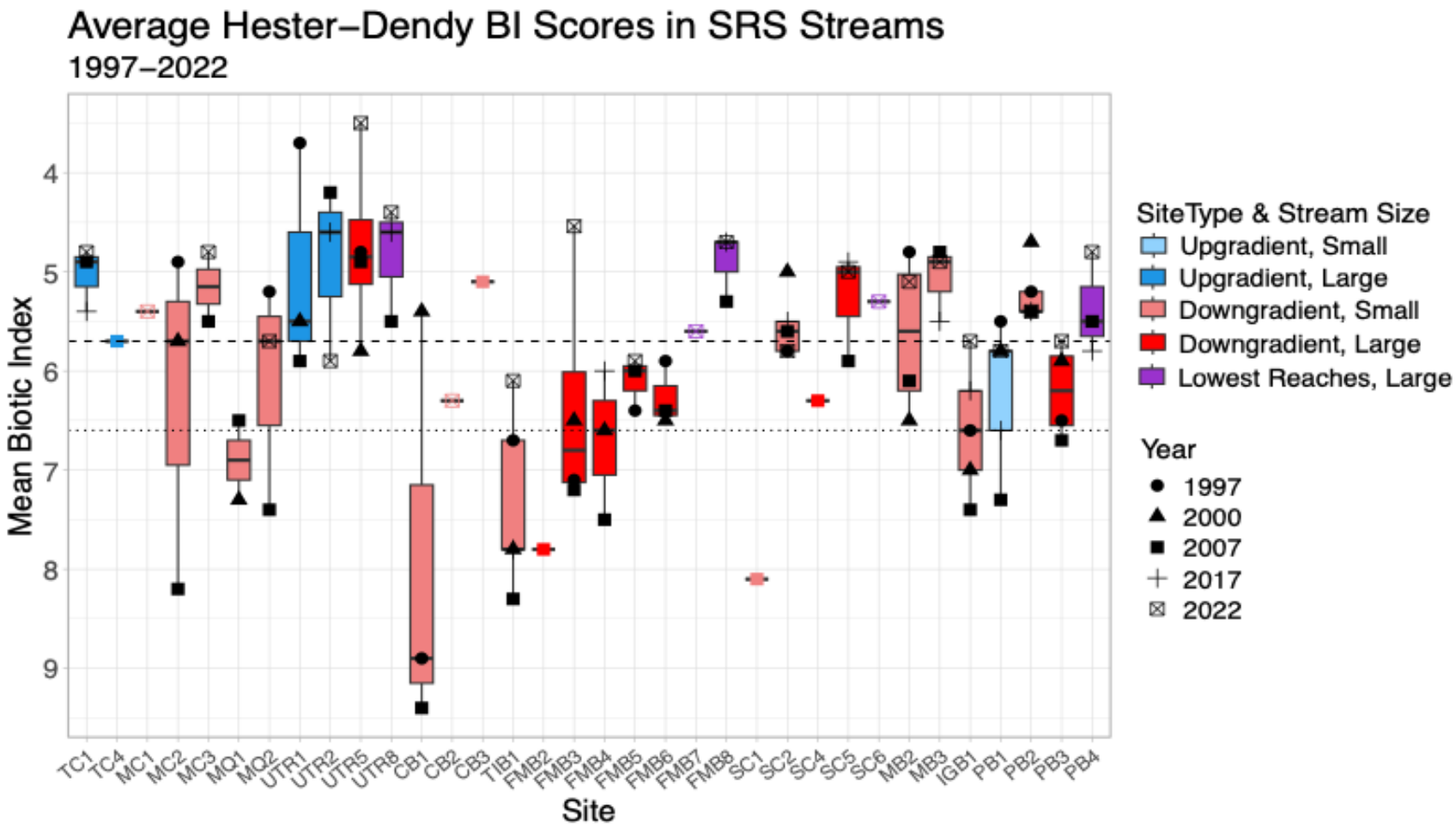


Figure 28. Average Hester-Dendy BI Scores in SRS Stream (1997 – 2022)

Average Hester-Dendy BI scores in upgradient sample sites in samples upgradient (blue), downgradient of potential impacts (red/brown), or at the lowest stream reaches (purple) in SRS Streams. Lighter shades of colors indicate small streams (<5 m wide). Horizontal lines are the cut-off value for upgradient sites corresponding to the lower 5% of the upgradient site ranges for small streams (<5 m wide) (dotted line) and large streams (≥5 m wide) (dashed line). Sites ordered by IOU subunits from upstream to downstream within the subunits

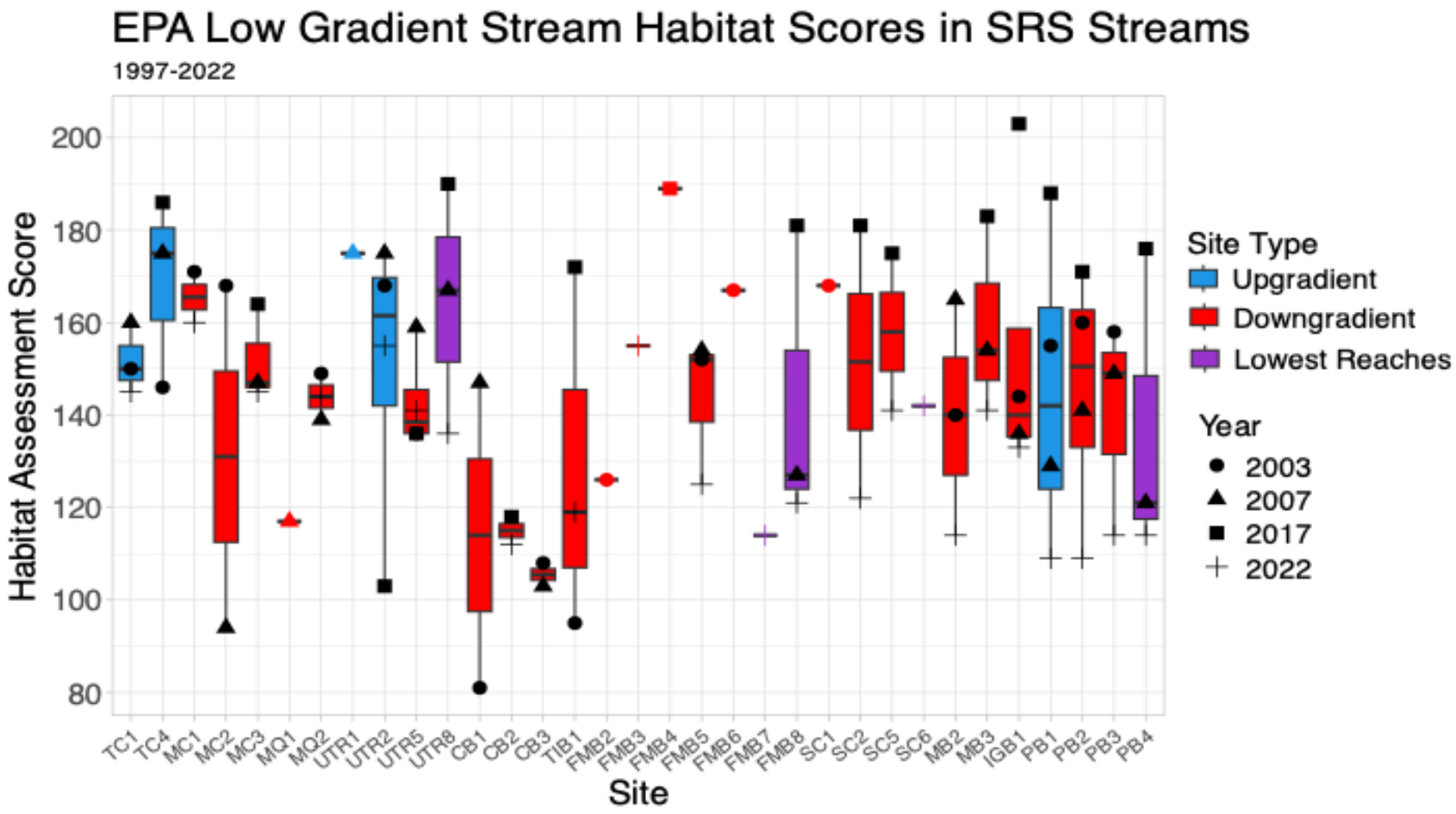


Figure 29. Boxplots of EPA Low-Gradient Stream Habitat Assessment Scores

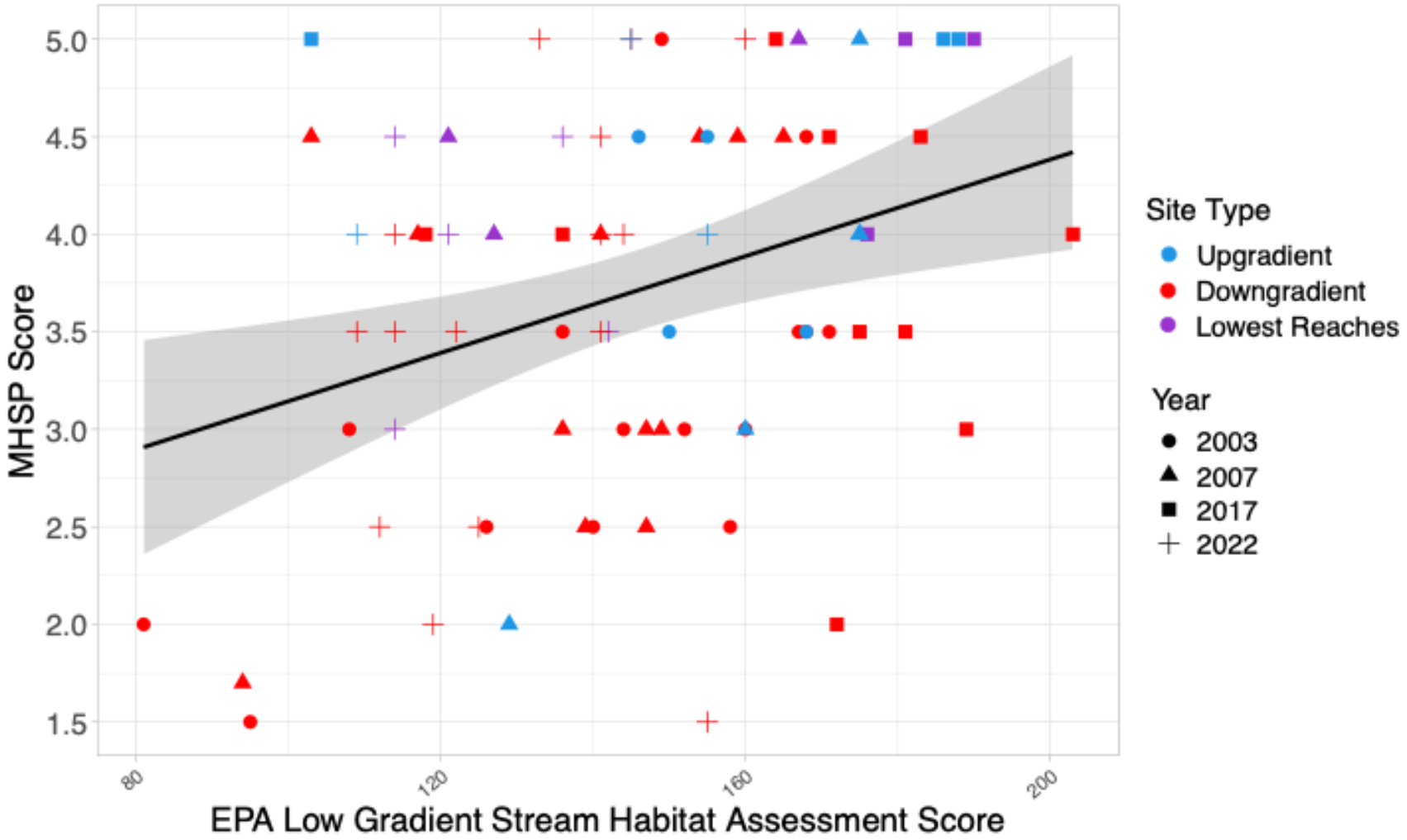


Figure 30. Scatterplot of EPA Low-Gradient Stream Habitat Assessment Scores and MHSP Scores for SRS Streams (2003 – 2022).

Grey band represents 95% confidence interval (Spearman's Rank Correlation: rho = 0.297, p = 0.008).

Table 1. Information for All Sampling Locations for Fish and Macroinvertebrate Surveys over Time

IOU	Stream	Stream abbrev.	IOU subunit	Type	Location	Latitude	Longitude	Site Code	Longitudinal Order
Fourmile Branch	Fourmile Branch	FMB	FMB-upper	*	Rd F	33.2809843	-81.6118631	FMB1	1
			FMB-middle	D	Rd 4	33.2746087	-81.6541117	FMB2	2
				D	Rd C	33.2723646	-81.6687777	FMB3	3
				D	RD A-6	33.2478241	-81.6954815	FMB4	4
				D	RD A-7	33.2431886	-81.6958227	FMB5	5
			FMB-lower	D	Rd A	33.2135086	-81.7130537	FMB6	6
				L	Rd A Railroad	33.1973771	-81.7169048	FMB7	7
				L	Rd A13.2	33.1902566	-81.7232807	FMB8	8
Pen Branch	Indian Grave Branch	IGB	PB-IGB	D	Near cooling tower site	33.2031390	-81.6751047	IGB1	1
	Pen Branch	PB	PB-upper	U	Rd C	33.2326956	-81.6238207	PB1	1
			PB-middle	D	Rd B	33.2040806	-81.6472527	PB2	2
			PB-lower	D	Rd A	33.1842586	-81.667164	PB3	3
				L	Rd A13.2	33.1596746	-81.6859847	PB4	4
Steel Creek	Meyers Branch	MB	SC-MB	D	Headwaters	33.194	-81.579	MB1	1
			SC-MB	D	Old Dunbarton Rd	33.1842206	-81.5816878	MB2	2
			SC-MB	*	Rd 6.2 (upper)	33.207	-81.566	MB0	3
			SC-MB	D	Rd 9	33.1668686	-81.6016128	MB3	3
			SC-MB	D	Boardwalk	33.1507282	-81.6264100	MB4	4
	Steel Creek	SC	SC-upper	D	P area	33.2222904	-81.5984301	SC1	1
				D	Rd C	33.2151826	-81.6041088	SC2	2
				D	Rd B-5	33.186	-81.635	SC3	3
			SC-middle	D	below L Lake dam	33.1579691	-81.6312759	SC4	4
			SC-lower	D	Rd A	33.1449526	-81.6289057	SC5	5
				L	Cypress Bridge below	33.1183386	-81.6295828	SC6	6

Table 1. Information for All Sampling Locations for Fish and Macroinvertebrate Surveys over Time *(continued/end)*

IOU	Stream	Stream abbrev.	IOU subunit	Type	Location	Latitude	Longitude	Site Code	Longitudinal Order
Upper Three Runs	Upper Three Runs	UTR	UTR-upper	U	Rd 8-1	33.3703505	-81.6286933	UTR1	1
				U	Tyler Bridge Rd	33.3527690	-81.630973	UTR2	2
			UTR-lower	D	Rd F-5	33.312	-81.653	UTR3	3
				D	Rd F-4	33.3017288	-81.6740103	UTR4	4
				D	Rd C	33.2870491	-81.6934380	UTR5	5
				D	Cato Rd	33.252	-81.719	UTR6	6
				D	Rd A	33.239	-81.744	UTR7	7
				L	Rd A.2	33.2355342	-81.7575827	UTR8	8
	Crouch Branch	CB	UTR-middle	D	near Rd 4	33.2921811	-81.6522046	CB1	1
				D	middle CB	33.2982246	-81.6573697	CB2	2
				D	lower CB, near UTR	33.3006533	-81.6617484	CB3	3
	McQueen Branch	MQ	UTR-TC	D	Rd F	33.2979599	-81.6299475	MQ1	1
			UTR-TC	D	Rd Z	33.3103086	-81.6343507	MQ2	2
	Mill Creek	MC	UTR-TC	D	Monroe Owens Rd	33.3006666	-81.5868028	MC1	1
			UTR-TC	D	Telephone cable Rd	33.3206415	-81.5920508	MC2	2
			UTR-TC	D	Rd E-2	33.3339586	-81.6079347	MC3	3
	Tims Branch	TB	UTR-TB	D	Rd 2	33.3151076	-81.7126127	TIB1	1
	Tinker Creek	TC	UTR-TC	U	Kennedys Pond Rd	33.3710726	-81.5284068	TC1	1
				U	Btwn Ken Pd Rd and Rd 8-1	33.3650355	-81.5545287	TC2	2
				U	Rd 8-1	33.356	-81.583	TC3	3
				U	Tyler Bridge Rd	33.3378053	-81.6044804	TC4	4

*Excluded from analysis due to poor habitat quality.
IOU = integrator operable unit on the SRS
Station Type:
U = Upgradient, potentially impacted by SRS waste sites or operations
D = Downgradient, or potentially in recovery from SRS impacts
L = Lowest reaches

Table 2. Collated Data Across All Sampling Periods and Sample Types

Stream abbrev.	IOU subunit	Type	Site Code	Year sampled	Fish Data			MHSP Data			Hester-Dendy Samplers			
					Reach length	No. passes	IBI	EPT score	BI score	MHSP	Number samplers	Mean BI	Mean number EPT	Mean number taxa
FMB	FMB-upper	*	FMB1	1997	NA	NA	NA	2	2	2	5	8.1	0	1
				2000	NA	NA	NA	2	1	1.5	5	8.8	0.4	4
	FMB-middle	D	FMB2	1997	150	1	20	NA	NA	NA	NA	NA	NA	NA
				2003	NA	NA	NA	3	2	2.5	NA	NA	NA	NA
				2007	NA	NA	NA	NA	NA	NA	5	7.8	2.6	16.4
			FMB3	1997	150	1	40	1	3	2	5	7.1	0.2	2.8
				2000	150	1	44	1	2	1.5	5	6.5	1.6	9.6
				2003	150	1	46	NA	NA	NA	NA	NA	NA	NA
				2007	NA	NA	NA	NA	NA	NA	5	7.2	1	8.4
				2022	200	3	46	1	2	1.5	2	4.54	4.5	6.5
			FMB4	2000	150	1	44	1	2	1.5	5	6.6	3	10.8
				2003	150	1	48	NA	NA	NA	NA	NA	NA	NA
				2007	NA	NA	NA	NA	NA	NA	5	7.5	2.2	15.6
				2017	200	2	50	3	3	3	2	6	6	16.5
			FMB5	1990	250	7	36	NA	NA	NA	NA	NA	NA	NA
				1997	150	1	42	2	3	2.5	5	6.4	5.6	11
				2003	NA	NA	NA	3	3	3	NA	NA	NA	NA
				2007	150	1	46	NA	NA	NA	5	6	3.2	19
				2022	200	3	46	3	2	2.5	2	5.9	4.5	12.5
	FMB-lower	D	FMB6	1997	150	1	44	2	2	2	5	5.9	2.8	5.8
				2000	150	1	50	2	2	2	5	6.5	3.4	17.2
				2003	150	1	40	4	3	3.5	NA	NA	NA	NA
				2007	NA	NA	NA	3	2	2.5	5	6.4	3.4	19.4
		L	FMB7	2022	200	3	36	3	3	3	2	5.6	3.5	10
			FMB8	1995	173	5	42	NA	NA	NA	NA	NA	NA	NA
				2007	150	1	50	4	4	4	5	5.3	10.2	27.2
				2017	200	2	34	5	5	5	2	4.7	4	11
				2022	200	3	36	4	4	4	2	4.7	4.5	7.5

Table 2. Collated Data Across All Sampling Periods and Sample Types *(continued/~~end~~)*

Stream abbrev.	IOU subunit	Type	Site Code	Year sampled	Fish Data			MHSP Data			Hester-Dendy Samplers			
					Reach length	No. passes	IBI	EPT score	BI score	MHSP	Number samplers	Mean BI	Mean number EPT	Mean number taxa
IGB	PB-IGB	D	IGB1	1997	150	1	48	3	2	2.5	5	6.6	1.4	6.6
				2000	150	1	48	3	2	2.5	5	7	3.2	13.8
				2003	150	1	48	3	3	3	NA	NA	NA	NA
				2007	150	1	44	4	2	3	5	7.4	1.8	12.3
				2017	200	2	50	4	4	4	2	6.2	2.5	11
				2022	200	3	44	5	5	5	2	5.7	4.5	7.5
PB	PB-upper	U	PB1	1995	300	5	46	NA	NA	NA	NA	NA	NA	NA
				1997	150	1	50	4	3	3.5	5	5.5	3.8	5.4
				2000	150	1	50	2	2	2	5	5.8	3.6	10.2
				2003	150	1	42	4	5	4.5	NA	NA	NA	NA
				2007	150	1	44	2	2	2	5	7.3	1.6	7.2
				2009	150	2	42	NA	NA	NA	NA	NA	NA	NA
				2017	200	2	40	5	5	5	2	6.6	1	6
				2022	200	3	36	4	4	4	2	5.8	2.5	11
	PB-middle	D	PB2	1990	200	7	42	NA	NA	NA	NA	NA	NA	NA
				1995	300	5	50	NA	NA	NA	NA	NA	NA	NA
				1997	150	1	46	3	3	3	5	5.2	4.2	8.8
				2000	150	1	50	3	4	3.5	5	4.7	5.2	14.6
				2003	150	1	50	2	4	3	NA	NA	NA	NA
				2007	150	1	44	5	3	4	5	5.4	3.2	10.6
				2009	200	2	50	NA	NA	NA	NA	NA	NA	NA
				2017	200	2	50	4	5	4.5	2	5.4	6	13.5
				2022	200	3	42	3	4	3.5	2	5.4	6	14
	PB-lower	D	PB3	1990	160	7	30	NA	NA	NA	NA	NA	NA	NA
				1995	240	5	44	NA	NA	NA	NA	NA	NA	NA
				1997	150	1	48	2	2	2	5	6.5	1	7.4
				2000	NA	NA	NA	3	3	3	5	5.9	7	19.8
				2003	NA	NA	NA	2	3	2.5	NA	NA	NA	NA

Table 2. Collated Data Across All Sampling Periods and Sample Types *(continued/~~end~~)*

Stream abbrev.	IOU subunit	Type	Site Code	Year sampled	Fish Data			MHSP Data			Hester-Dendy Samplers			
					Reach length	No. passes	IBI	EPT score	BI score	MHSP	Number samplers	Mean BI	Mean number EPT	Mean number taxa
PB	PB-lower	D	PB3	2007	NA	NA	NA	4	2	3	5	6.7	8.4	25.8
				2022	200	3	42	3	4	3.5	2	5.7	6.5	12.5
		L	PB4	1990	100	6	24	NA	NA	NA	NA	NA	NA	NA
				2000	150	1	46	NA	NA	NA	NA	NA	NA	NA
				2003	150	1	46	NA	NA	NA	NA	NA	NA	NA
				2007	150	1	48	5	4	4.5	5	5.5	7.4	19.8
				2017	200	2	46	3	5	4	2	5.8	6	16
				2022	200	3	42	5	4	4.5	2	4.8	9	14
MB	SC-MB	D	MB1	2009	160	2	40	NA	NA	NA	NA	NA	NA	NA
			MB2	1990	400	7	44	NA	NA	NA	NA	NA	NA	NA
				1997	150	1	46	5	4	4.5	5	4.8	3.2	8.2
				2000	150	1	46	4	2	3	5	6.5	3.6	19.2
				2003	150	1	50	3	2	2.5	NA	NA	NA	NA
				2007	150	1	50	5	4	4.5	5	6.1	1.8	7.4
				2009	200	2	48	NA	NA	NA	NA	NA	NA	NA
				2017	200	2	46	NA	NA	NA	NA	NA	NA	NA
				2022	200	3	42	5	3	4	2	5.1	5	11
		*	MB0	2003	NA	NA	NA	2	1	1.5	NA	NA	NA	NA
		D	MB3	1990	140	7	50	NA	NA	NA	NA	NA	NA	NA
				1995	300	4	38	NA	NA	NA	NA	NA	NA	NA
				2007	NA	NA	NA	5	4	4.5	5	4.8	5.6	13.8
				2017	NA	NA	NA	4	5	4.5	2	5.5	7.5	15.5
				2022	200	3	42	5	4	4.5	2	4.9	5	9.5
			MB4	1990	300	7	44	NA	NA	NA	NA	NA	NA	NA
SC	SC-upper	D	SC1	1996	150	1	28	NA	NA	NA	NA	NA	NA	NA
			SC1	2003	NA	NA	NA	3	4	3.5	NA	NA	NA	NA
			SC1	2007	NA	NA	NA	NA	NA	NA	5	8.1	0	8

Table 2. Collated Data Across All Sampling Periods and Sample Types *(continued)*

Stream abbrev.	IOU subunit	Type	Site Code	Year sampled	Fish Data			MHSP Data			Hester-Dendy Samplers			
					Reach length	No. passes	IBI	EPT score	BI score	MHSP	Number samplers	Mean BI	Mean number EPT	Mean number taxa
SC	SC-upper	D	SC2	1990	180	7	30	NA	NA	NA	NA	NA	NA	NA
				1997	150	1	28	3	2	2.5	5	5.8	1.6	4.2
				2000	150	1	22	4	5	4.5	5	5	5.6	9.4
				2003	150	1	28	NA	NA	NA	NA	NA	NA	NA
				2007	150	1	22	NA	NA	NA	5	5.6	5.2	17.4
				2017	200	2	22	4	3	3.5	2	5.5	3.5	10
				2022	200	3	20	3	4	3.5	2	5.8	3	8.5
			SC3	1990	220	5	28	NA	NA	NA	NA	NA	NA	NA
	SC-middle	D	SC4	2007	NA	NA	NA	2	3	2.5	5	6.3	5	22
	SC-lower	D	SC5	1990	180	7	32	NA	NA	NA	NA	NA	NA	NA
				1996	180	7	39	NA	NA	NA	NA	NA	NA	NA
				2000	180	7	45	NA	NA	NA	NA	NA	NA	NA
				2003	150	1	46	NA	NA	NA	NA	NA	NA	NA
				2007	150	1	46	5	3	4	5	5.9	4.6	18.8
				2017	200	2	48	3	4	3.5	2	4.9	8.5	14.5
				2022	200	3	34	4	4	4	2	5	6.5	13
		L	SC6	1990	340	3	24	NA	NA	NA	NA	NA	NA	NA
				2022	200	3	36	3	4	3.5	2	5.3	4.5	10.5
CB	UTR-middle	D	CB1	1997	150	1	28	2	2	2	5	8.9	0	1.2
				2000	NA	NA	NA	2	1	1.5	5	5.4	0	6
				2003	NA	NA	NA	2	2	2	NA	NA	NA	NA
				2007	NA	NA	NA	3	3	3	5	9.4	1.8	6
			CB2	1995	281	5	24	NA	NA	NA	NA	NA	NA	NA
				2017	200	2	38	4	4	4	NA	NA	NA	NA
				2022	200	3	32	2	3	2.5	2	6.3	2	5.5
			CB3	1992	300	5	38	NA	NA	NA	NA	NA	NA	NA
				2000	150	1	46	NA	NA	NA	NA	NA	NA	NA
				2003	150	1	46	3	3	3	NA	NA	NA	NA
				2007	150	1	46	4	5	4.5	5	5.1	2.6	8.2

Table 2. Collated Data Across All Sampling Periods and Sample Types *(continued)*

Stream abbrev.	IOU subunit	Type	Site Code	Year sampled	Fish Data			MHSP Data			Hester-Dendy Samplers			
					Reach length	No. passes	IBI	EPT score	BI score	MHSP	Number samplers	Mean BI	Mean number EPT	Mean number taxa
MC	UTR-TC	D	MC1	2003	NA	NA	NA	3	4	3.5	NA	NA	NA	NA
				2009	150	2	34	NA	NA	NA	NA	NA	NA	NA
				2022	200	3	34	5	5	5	2	5.4	3.5	7
			MC2	1990	280	3	40	NA	NA	NA	NA	NA	NA	NA
				1997	150	1	40	4	4	4	5	4.9	3.8	6.4
				2000	150	1	40	5	5	5	5	5.7	6.2	15.4
				2003	150	1	50	5	4	4.5	NA	NA	NA	NA
				2007	NA	NA	NA	2	1	1.7	5	8.2	2.6	16
			MC3	1990	320	7	48	NA	NA	NA	NA	NA	NA	NA
				2007	150	1	48	3	2	2.5	5	5.5	6	14
				2017	NA	NA	NA	5	5	5	NA	NA	NA	NA
				2022	200	3	44	5	5	5	2	4.8	6.5	13.5
MQ	UTR-TC	D	MQ1	2000	150	1	31	2	3	2.5	5	7.3	0	2.4
				2003	150	1	36	NA	NA	NA	NA	NA	NA	NA
				2007	150	1	42	4	4	4	5	6.5	3	14.8
			MQ2	1990	350	7	42	NA	NA	NA	NA	NA	NA	NA
				1997	150	1	44	5	5	5	5	5.2	2.4	3.8
				2003	NA	NA	NA	5	5	5	NA	NA	NA	NA
				2007	NA	NA	NA	4	1	2.5	5	7.4	0.6	17.2
				2009	150	2	36	NA	NA	NA	NA	NA	NA	NA
				2022	200	3	40	5	3	4	2	5.7	4.5	10
TC	UTR-TC	U	TC1	1990	300	7	50	NA	NA	NA	NA	NA	NA	NA
				1992	300	7	40	NA	NA	NA	NA	NA	NA	NA
				1995	241.5	4	44	NA	NA	NA	NA	NA	NA	NA
				2003	150	1	46	4	3	3.5	NA	NA	NA	NA
				2007	150	1	48	3	3	3	5	4.9	4.5	11.5
				2017	200	2	42	NA	NA	NA	2	5.4	4	12
				2022	200	3	34	5	5	5	2	4.8	10.5	16

Table 2. Collated Data Across All Sampling Periods and Sample Types *(continued)*

Stream abbrev.	IOU subunit	Type	Site Code	Year sampled	Fish Data			MHSP Data			Hester-Dendy Samplers			
					Reach length	No. passes	IBI	EPT score	BI score	MHSP	Number samplers	Mean BI	Mean number EPT	Mean number taxa
TC	UTR-TC	U	TC2	2009	210	2	50	NA	NA	NA	NA	NA	NA	NA
			TC3	1990	240	7	36	NA	NA	NA	NA	NA	NA	NA
			TC4	1990	360	5	36	NA	NA	NA	NA	NA	NA	NA
				2003	NA	NA	NA	5	4	4.5	NA	NA	NA	NA
				2007	NA	NA	NA	5	5	5	5	5.7	4.2	14
				2017	NA	NA	NA	5	5	5	NA	NA	NA	NA
TB	UTR-TB	D	TIB1	1995	294	4	42	NA	NA	NA	NA	NA	NA	NA
				1997	150	1	38	2	3	2.5	5	6.7	0.8	6.5
				2000	150	1	36	1	2	1.5	5	7.8	0.8	4
				2003	150	1	30	2	1	1.5	NA	NA	NA	NA
				2007	150	1	20	NA	NA	NA	5	8.3	0.6	5.4
				2017	200	2	22	2	2	2	2	7.8	0	4.5
				2022	200	3	16	1	3	2	2	6.1	0	3.5
UTR	UTR-upper	U	UTR1	1992	300	4	38	NA	NA	NA	NA	NA	NA	NA
				1997	150	1	32	4	5	4.5	5	3.7	6	9.6
				2000	NA	NA	NA	4	5	4.5	5	5.5	3	13.4
				2003	150	1	24	NA	NA	NA	NA	NA	NA	NA
				2007	NA	NA	NA	4	4	4	5	5.9	7.2	23.4
			UTR2	1992	300	6	28	NA	NA	NA	NA	NA	NA	NA
				1998	150	1	26	NA	NA	NA	NA	NA	NA	NA
				2003	NA	NA	NA	4	3	3.5	NA	NA	NA	NA
				2007	150	1	32	4	4	4	5	4.2	7.2	23
				2017	NA	NA	NA	5	5	5	2	4.6	3.5	11.5
				2022	NA	NA	NA	3	5	4	2	5.9	5.5	16
	UTR-lower	D	UTR3	1992	300	7	44	NA	NA	NA	NA	NA	NA	NA
			UTR4	1992	300	6	38	NA	NA	NA	NA	NA	NA	NA
			UTR5	1992	300	7	46	NA	NA	NA	NA	NA	NA	NA
				1995	200	4	34	NA	NA	NA	NA	NA	NA	NA

Table 2. Collated Data Across All Sampling Periods and Sample Types *(continued/end)*

Stream abbrev.	IOU subunit	Type	Site Code	Year sampled	Fish Data			MHSP Data			Hester-Dendy Samplers			
					Reach length	No. passes	IBI	EPT score	BI score	MHSP	Number samplers	Mean BI	Mean number EPT	Mean number taxa
UTR	UTR-lower	D	UTR5	1997	NA	NA	NA	4	5	4.5	5	4.8	7	14.4
				2000	NA	NA	NA	4	5	4.5	5	5.8	5	14
				2003	150	1	46	2	5	3.5	NA	NA	NA	NA
				2007	NA	NA	NA	4	5	4.5	5	4.9	10.6	25
				2017	NA	NA	NA	3	5	4	NA	NA	NA	NA
				2022	NA	NA	NA	2	5	3.5	2	3.5	6.5	12
			UTR6	1992	300	5	44	NA	NA	NA	NA	NA	NA	NA
			UTR7	1992	300	6	36	NA	NA	NA	NA	NA	NA	NA
		L	UTR8	1998	150	1	34	NA	NA	NA	NA	NA	NA	NA
				2000	150	1	44	NA	NA	NA	NA	NA	NA	NA
				2003	150	1	42	NA	NA	NA	NA	NA	NA	NA
				2007	150	1	44	5	5	5	5	5.5	7	19.2
				2017	NA	NA	NA	5	5	5	2	4.6	7	13.5
				2022	NA	NA	NA	4	5	4.5	2	4.4	4.5	6

U = Upgradient; D = Downgradient (potentially impacted by SRS waste sites or operations); L = Lowest reaches (potentially in recovery from SRS impacts)
IBI = Index of Biotic Integrity
MHSP = Multiple Habitat Sampling Protocol
EPT = Ephemeroptera, Plecoptera, Trichoptera
BI = Biotic Index

Table 3. Metrics and scoring criteria used in the Index of Biotic Integrity (Modified for use in SRS Streams)

Metrics	Scoring Criteria		
	1	3	5
Species Richness			
Percentage of expected number of total species (TSP)[a]	<70	70-90	>90
Percentage of expected number of native minnow species (CSP)[a]	<55	55-80	>80
Percentage of expected number of piscivorous species (PSP)[a]	<65	65-85	>85
Percentage of expected number of madtom and darter species (BSP)[a]	<55	55-80	>80
Species Composition			
Percent native minnows	<20	20-35	>35
Percent sunfish	<5 and >45	25-45	5-24
Trophic Composition			
Percent generalized insectivores	>75	50-75	<50
Local Indicator Species			
Percent tolerant fish	>15	5-15	<5
Fish Abundance (Number/100m^2)			
Stream orders 1-3, ≥4 passes[b]	<25		≥25
Stream orders 1-3, 1 pass	<10		≥10
Stream order 4, ≥4 passes	<5		≥5
Stream order 4, 1 pass	<2		≥2
Fish Condition			
Percent with disease or anomalies	>5	2-5	<2

a - Percentage determined on the basis of sample site surface area and sampling effort (Paller et al. 1996).
b - Passes refer to number of electrofishing passes through the sample reach.

Table 4. Abundance of Fish Considered Species of Greatest Conservation Concern by the South Carolina Department of Natural Resources

IOU	Stream	Site Code	Type	Year	*Alburnops chalybaeus*	*Ameiurus platycephalus*	*Anguilla rostrata*	*Chologaster cornuta*	*Cyprinella analostana*	*Cyprinella leedsi*	*Enneacanthus chaetodon*	*Etheostoma fricksium*	*Etheostoma inscriptum*	*Etheostoma serrifer*	*Hybopsis rubrifrons*	*Morone saxatilis*	Total Abundance
Fourmile Branch	Fourmile Branch	FMB3	D	2000	-	-	3		-	-	-	2	-	-	-	-	5
		FMB3	D	2003	-	-	1		-	-	-	24	-	-	-	-	25
		FMB3	**D**	**2022**	**-**	**-**	**2**		**-**	**-**	**-**	**15**	**-**	**-**	**-**	**-**	**17**
		FMB4	D	2003	17	2	1		-	-	-	9	-	-	3	-	32
		FMB5	D	1997	-	3	2		-	-	-	-	-	-	-	-	5
		FMB5	D	2000	-	2	-		-	-	-	-	-	-	-	-	2
		FMB5	D	2007	69	5	7		-	-	-	5	-	-	33	-	119
		FMB5	**D**	**2022**	**-**	**3**	**2**		**-**	**-**	**-**	**9**	**-**	**-**	**-**	**-**	**14**
		FMB6	D	1997	-	3	8		-	-	-	1	-	-	-	-	12
		FMB6	D	2000	-	3	2		1	-	-	-	-	-	-	-	6
		FMB6	D	2003	-	-	1		-	-	-	3	-	-	-	-	4
		FMB7	**D**	**2022**	**-**	**1**	**2**		**-**	**-**	**-**	**4**	**-**	**-**	**-**	**-**	**7**
		FMB8	D	2007	-	1	17		-	-	-	-	-	-	6	-	24
		FMB8	**D**	**2022**	**-**	**8**	**6**		**-**	**-**	**-**	**7**	**-**	**-**	**-**	**-**	**21**
Pen Branch	Indian Grave Branch	IGB1	D	1997	-	31	1		13	-	-	-	-	-	-	-	45
		IGB1	D	2000	-	27	4		11	-	-	-	-	-	-	-	42
		IGB1	D	2003	3	-	-		-	-	-	1	-	-	-	-	4
		IGB1	D	2007	-	1	-		-	-	-	-	-	-	-	-	1
		IGB1	**D**	**2022**	**-**	**-**	**2**		**-**	**-**	**-**	**8**	**-**	**-**	**-**	**-**	**10**

Table 4. Abundance of Fish Considered Species of Greatest Conservation Concern by the South Carolina Department of Natural Resources *(continued)*

IOU	Stream	Site Code	Type	Year	*Alburnops chalybaeus*	*Ameiurus platycephalus*	*Anguilla rostrata*	*Chologaster cornuta*	*Cyprinella analostana*	*Cyprinella leedsi*	*Enneacanthus chaetodon*	*Etheostoma fricksium*	*Etheostoma inscriptum*	*Etheostoma serrifer*	*Hybopsis rubrifrons*	*Morone saxatilis*	Total Abundance
Pen Branch	Pen Branch	PB1	U	1995	-	-	4		-	-	-	6	-	-	-	-	10
		PB1	U	1997	-	-	3		-	-	-	1	-	-	-	-	4
		PB1	U	2000	-	-	2		-	-	-	1	-	-	-	-	3
		PB1	U	2003	4	-	-		-	-	-	-	-	-	-	-	4
		PB1	U	2007	-	-	1		-	-	-	-	-	-	-	-	1
		PB1	**U**	**2022**	**-**	**-**	**-**		**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0**
		PB2	D	1990	-	1	1		-	-	-	-	-	-	-	-	2
		PB2	D	1995	-	-	1		-	-	-	7	-	1	-	-	9
		PB2	D	1997	-	-	-		-	-	-	2	-	-	-	-	2
		PB2	D	2000	-	1	-		-	-	-	1	-	-	-	-	2
		PB2	D	2003	1	-	-		-	-	-	4	-	-	-	-	5
		PB2	D	2007	-	-	-		-	-	-	2	-	-	-	-	2
		PB2	**D**	**2022**	**-**	**1**	**1**		**-**	**-**	**-**	**28**	**-**	**-**	**-**	**-**	**30**
		PB3	D	1990	-	2	5		-	-	-	-	-	-	-	-	7
		PB3	D	1995	-	7	-		-	-	-	1	-	-	-	-	8
		PB3	D	1997	-	1	3		17	-	-	-	-	-	-	-	21
		PB3	**D**	**2022**	**-**	**-**	**2**		**-**	**-**	**-**	**3**	**-**	**-**	**-**	**-**	**5**
		PB4	L	1990	-	2	3		-	-	-	-	-	-	-	-	5
		PB4	L	2000	-	-	1		18	-	-	-	-	-	-	-	19
		PB4	L	2003	14	-	1		-	-	-	-	-	1	6	-	22
		PB4	L	2007	13	2	2		-	-	-	-	-	-	-	-	17
		PB4	**L**	**2022**	**-**	**-**	**-**	**1**	**-**	**-**	**-**	**4**	**-**	**-**	**-**	**-**	**5**

Table 4. Abundance of Fish Considered Species of Greatest Conservation Concern by the South Carolina Department of Natural Resources *(continued)*

IOU	Stream	Site Code	Type	Year	*Alburnops chalybaeus*	*Ameiurus platycephalus*	*Anguilla rostrata*	*Chologaster cornuta*	*Cyprinella analostana*	*Cyprinella leedsi*	*Enneacanthus chaetodon*	*Etheostoma fricksium*	*Etheostoma inscriptum*	*Etheostoma serrifer*	*Hybopsis rubrifrons*	*Morone saxatilis*	Total Abundance
Steel Creek	Meyers Branch	MB1	D	2009	-	-	-		-	-	-	4	-	-	-	-	4
		MB2	D	1997	-	-	1		-	-	-	7	-	-	-	-	8
		MB2	D	2000	-	1	4		-	-	-	-	-	-	-	-	5
		MB2	D	2003	2	-	5		-	-	-	7	-	-	-	-	14
		MB2	D	2007	-	-	1		-	-	-	2	-	-	-	-	3
		MB2	D	2009	-	-	-		-	-	-	1	-	-	-	-	1
		MB2	**D**	**2022**	**-**	**-**	**3**		**-**	**-**	**-**	**12**	**-**	**-**	**-**	**-**	**15**
		MB3	D	1990	-	10	2		-	-	-	-	-	-	-	-	12
		MB3	D	1995	-	-	3		-	-	-	5	-	-	-	-	8
		MB3	**D**	**2022**	**-**	**2**	**3**		**-**	**-**	**-**	**9**	**-**	**-**	**-**	**-**	**14**
		MB4	D	1990	1	18	99		-	-	-	-	-	-	-	-	118
	Steel Creek	SC2	D	1990	-	26	2		7	-	-	-	-	-	-	-	35
		SC2	D	1997	-	22	-		-	-	-	-	-	-	-	-	22
		SC2	D	2000	-	1	-		-	-	-	-	-	-	-	-	1
		SC2	D	2003	-	6	-		-	-	-	-	-	-	-	-	6
		SC2	D	2007	-	5	-		-	-	-	-	-	-	-	-	5
		SC2	**D**	**2022**	**-**	**-**	**-**		**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0**
		SC3	D	1990	-	-	9		-	-	-	-	-	-	-	-	9
		SC5	D	1990	3	1	6		-	-	-	-	-	-	-	-	10
		SC5	D	2003	4	-	4		-	-	-	1	-	-	-	-	9
		SC5	D	2007	-	-	21		-	-	-	-	-	-	-	-	21
		SC5	**D**	**2022**	**-**	**-**	**11**		**-**	**-**	**-**	**4**	**-**	**-**	**-**	**-**	**15**

Table 4. Abundance of Fish Considered Species of Greatest Conservation Concern by the South Carolina Department of Natural Resources *(continued)*

IOU	Stream	Site Code	Type	Year	*Alburnops chalybaeus*	*Ameiurus platycephalus*	*Anguilla rostrata*	*Chologaster cornuta*	*Cyprinella analostana*	*Cyprinella leedsi*	*Enneacanthus chaetodon*	*Etheostoma fricksium*	*Etheostoma inscriptum*	*Etheostoma serrifer*	*Hybopsis rubrifrons*	*Morone saxatilis*	Total Abundance
Steel Creek	Steel Creel	SC6	L	1990	-	1	2		-	-	-	-	-	-	-	-	3
		SC6	**L**	**2022**	**-**	**3**	**45**		**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**48**
Upper Three Runs	Crouch Branch	**CB2**	**D**	**2022**	**-**	**-**	**-**		**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0**
		CB3	D	2000	-	-	4		-	-	-	-	-	-	-	-	4
		CB3	D	2000	-	-	1		-	-	-	-	-	-	-	-	1
		CB3	D	2003	-	-	2		-	-	-	-	-	-	-	-	2
		CB3	D	2007	12	-	1		-	-	-	-	-	-	-	-	13
	Mill Creek	**MC1**	**D**	**2022**	**-**	**-**	**1**		**-**	**-**	**1**	**9**	**-**	**-**	**-**	**-**	**11**
		MC2	D	2000	-	2	-		-	-	-	4	-	-	-	-	6
		MC2	D	2003	-	-	-		-	-	-	2	-	-	-	-	2
		MC3	D	1990	-	2	3		-	-	-	-	-	-	-	-	5
		MC3	D	2007	-	-	2		-	-	-	1	-	-	-	-	3
		MC3	**D**	**2022**	**-**	**8**	**3**		**-**	**-**	**-**	**7**	**-**	**-**	**-**	**-**	**18**
	McQueen Branch	MQ1	D	2007	1	-	-		-	-	-	-	-	-	1	-	2
		MQ2	**D**	**2022**	**4**	**4**	**-**		**-**	**-**	**-**	**1**	**-**	**-**	**-**	**-**	**9**
		MQ2	D	1997	-	-	1		-	-	-	-	-	-	-	-	1
	Tinker Creek	TC1	U	1990	3	2	8		-	-	-	-	-	-	-	-	13
		TC1	U	1992	3	-	1		-	-	-	1	-	-	-	-	5
		TC1	U	1995	-	-	2		-	-	-	4	-	2	-	-	8
		TC1	U	2003	1	-	-		-	-	-	2	-	4	-	-	7
		TC1	U	2007	-	2	1		-	-	-	2	-	-	-	-	5
		TC1	**U**	**2022**	**-**	**6**	**-**		**-**	**-**	**1**	**16**	**-**	**-**	**-**	**-**	**23**

Table 4. Abundance of Fish Considered Species of Greatest Conservation Concern by the South Carolina Department of Natural Resources *(continued/end)*

IOU	Stream	Site Code	Type	Year	*Alburnops chalybaeus*	*Ameiurus platycephalus*	*Anguilla rostrata*	*Chologaster cornuta*	*Cyprinella analostana*	*Cyprinella leedsi*	*Enneacanthus chaetodon*	*Etheostoma fricksium*	*Etheostoma inscriptum*	*Etheostoma serrifer*	*Hybopsis rubrifrons*	*Morone saxatilis*	Total Abundance
Upper Three Runs	Tinker Creek	TC3	U	1990	-	1	2		4	-	-	-	-	-	-	-	7
		TC4	U	1990	1	1	21		8	-	-	-	-	-	-	-	31
	Tims Branch	TIB1	D	1995	-	-	4		-	-	-	-	-	-	-	-	4
		TIB1	D	2003	1	-	-		-	-	-	-	-	-	-	-	1
		TIB1	**D**	**2022**	**-**	**-**	**-**		**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0**
	Upper Three Runs	UTR1	D	1992	1	-	10		-	-	1	-	-	-	-	-	12
		UTR2	D	1992	-	-	8		-	-	-	1	-	-	-	-	9
		UTR2	D	1997	-	-	4		-	-	-	-	-	-	-	-	4
		UTR2	D	2003	-	-	-		-	-	1	-	-	-	-	-	1
		UTR2	D	2007	-	-	4		-	-	-	-	-	-	-	-	4
		UTR3	D	1992	8	-	10		-	-	-	1	-	-	-	-	19
		UTR4	D	1992	5	-	16		2	-	-	-	-	-	-	-	23
		UTR5	D	1992	1	-	4		-	-	-	1	-	-	-	-	6
		UTR5	D	1995	-	-	3		-	-	1	1	-	-	-	-	5
		UTR6	D	1992	-	-	16		-	-	-	-	-	-	-	-	16
		UTR7	D	1992	-	-	12		-	-	-	-	-	-	-	-	12
		UTR8	L	1997	-	-	9		1	-	-	-	-	-	-	5	15
		UTR8	L	2000	-	3	10		-	-	-	-	-	-	-	-	13
		UTR8	L	2003	-	-	11		-	-	-	-	-	-	-	-	11
		UTR8	L	2007	-	1	11		-	3	-	-	1	-	-	-	16
Grand Total					**172**	**235**	**497**	**1**	**82**	**3**	**5**	**253**	**1**	**8**	**49**	**5**	**1311**

For previous years only sites with positive findings are included but all sites from the 2022 sampling are included and are in bold font.
U = Upgradient; D = Downgradient (potentially impacted by SRS waste sites or operations); L = Lowest reaches (potentially in recovery from SRS impacts)

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Table 5. Status of Fish Species Included in Bioassessment.

Scientific name	Common name	G-Rank	S-Rank	Protection Status[1]	SGCN Priority	RSGCN Priority	Other
Alburnops chalybaeus	Ironcolor shiner	G4	S3		High	High	
Ameiurus platycephalus	Flat bullhead	G4	S3S4		Moderate		Cultural significance
Anguilla rostrata	American eel	G5	S2S3	State regulated	Highest	NA	Cultural significance
Chologaster cornuta	Swampfish	G5	S3		Moderate	Moderate	
Cyprinella analostana	Satinfin shiner	G5	S3		Moderate		
Cyprinella leedsi	Bannerfin shiner	G4	S3		Moderate		
Enneacanthus chaetodon	Blackbanded sunfish	G3G4	S2S3		High	High	
Etheostoma fricksium	Savannah darter	G4	S3		High	High	
Etheostoma inscriptum	Turquoise darter	G4	S2S3		High	Moderate	
Etheostoma serrifer	Sawcheek darter	G5	S4		Moderate		
Hybopsis rubrifrons	Rosyface chub	G4	S3		Moderate	Moderate	
Morone saxatilis	Striped bass	G5	S1	State regulated	Highest		Cultural significance

1 – Protection Status refers to state or federal regulations.
G-Rank = NatureServe global
S-Rank = State
G5/S5 = secure
G4/S4 = apparently secure
G3/S3 = vulnerable
G2/S2 = imperiled
G1/S1 = critically imperiled
SGCN = Species of Greatest Conservation Concern within the state
RSGCN = Species of Greatest Conservation Concern within southeast region

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Table 6. Aquatic Macroinvertebrate Abundances of Species Identified for Tracking by South Carolina Natural Heritage Program.

IOU	Stream	Site Code	Type*	Year	Scientific Name												Total Abundance
					Macromia margarita	*Oecetis morsei*	*Agarodes libalis*	*Somatogyrus virginicus*	*Gillia altilis*	*Elliptio fraterna*	*Procambarus raneyi*	*Psilotreta frontalis*	*Taeniopteryx robinae*	*Callinina intertexta*	*Procambarus pubescens*	*Procambarus hirsutus*	
Fourmile Branch	Fourmile Branch	FMB3	D	2007	-	-	-	-	1	-	-	-	-	-	-	-	**1**
		FMB4	D	2017	3	-	-	-	-	-	-	-	-	-	-	-	**3**
		FMB5	D	2022	-	-	-	-	-	-	-	-	-	-	-	1	**1**
		FMB6	D	2007	-	-	-	-	1	-	-	-	-	-	-	-	**1**
		FMB7	D	2022	-	-	-	-	-	-	-	-	-	1	-	-	**1**
		FMB8	L	2007	-	-	-	-	1	-	-	-	-	-	-	-	**1**
		FMB8	L	2017	4	-	-	-	-	-	-	-	-	-	-	-	**4**
Pen Branch	Indian Grave Branch	IGB1	D	2007	-	-	-	9	-	-	-	-	-	-	-	-	**9**
		IGB1	D	2022	-	-	-	-	-	-	-	-	43	-	-	-	**43**
	Pen Branch	PB1	U	2017	-	-	-	-	-	-	-	16	-	-	6	-	**22**
		PB1	U	2022	-	-	-	-	-	-	-	1	-	-	-	-	**1**
		PB2	D	2022	-	-	-	-	-	1	-	-	3	-	-	-	**4**
		PB3	D	2007	-	-	-	4	1	-	-	-	-	-	-	-	**5**
		PB3	D	2022	-	-	-	-	-	-	-	-	3	-	2	-	**5**
Steel Creek	Meyers Branch	MB2	D	2022	-	-	-	-	-	-	-	-	-	-	9	-	**9**
		MB3	D	2022	-	-	-	-	-	-	-	-	-	-	13	-	**13**
	Steel Creek	SC2	D	2007	-	-	-	-	1	-	-	-	-	-	-	-	**1**
		SC2	D	2022	-	-	-	-	-	-	-	-	-	-	11	-	**11**
		SC6	L	2022	-	-	-	-	-	-	-	-	-	-	10	-	**10**
Upper Three Runs	Mill Creek	MC1	D	2022	-	-	-	-	-	-	-	1	-	-	2	-	**3**
		MC2	D	2007	1	-	-	-	-	-	-	-	-	-	-	5	**6**
		MC3	D	2007	2	-	-	-	-	-	-	-	-	-	-	2	**4**
		MC3	D	2017	1	-	-	-	-	-	-	-	-	-	1	-	**2**
		MC3	D	2022	-	-	-	-	-	-	-	-	-	-	11	-	**11**
	McQueen Branch	MQ2	D	2022	-	-	-	-	-	-	-	-	-	-	24	-	**24**

Table 6. Aquatic Macroinvertebrate Abundances of Species Identified for Tracking by South Carolina Natural Heritage Program *(continued/end)*

IOU	Stream	Site Code	Type*	Year	Scientific Name												Total Abundance
					Macromia margarita	*Oecetis morsei*	*Agarodes libalis*	*Somatogyrus virginicus*	*Gillia altilis*	*Elliptio fraterna*	*Procambarus raneyi*	*Psilotreta frontalis*	*Taeniopteryx robinae*	*Callinina intertexta*	*Procambarus pubescens*	*Procambarus hirsutus*	
Upper Three Runs	Tinker Creek	TC1	U	2007	1	-	-	-	-	-	-	-	-	-	-	5	**6**
		TC1	U	2022	-	-	-	-	-	1	-	-	-	-	13	-	**14**
		TC4	U	2007	1	-	-	-	-	-	-	-	-	-	-	6	**7**
		TC4	U	2017	1	-	-	-	-	-	-	-	-	-	-	-	**1**
	Upper Three Runs	UTR1	U	2000	-	-	2	-	-	-	-	-	-	-	-	-	**2**
		UTR1	U	2007	-	-	-	-	-	-	3	-	-	-	-	-	**3**
		UTR2	U	2003	-	3	-	-	-	-	-	-	-	-	-	-	**3**
		UTR2	U	2007	-	-	-	-	-	-	2	-	-	-	-	-	**2**
		UTR2	U	2017	-	-	-	-	-	-	2	1	-	-	3	-	**6**
		UTR2	U	2022	-	-	-	-	-	-	-	7	-	-	-	-	**7**
		UTR5	D	2003	-	-	1	-	-	-	-	-	-	-	-	-	**1**
		UTR5	D	2007	-	-	-	-	-	-	11	-	-	-	-	-	**11**
		UTR5	D	2022	-	-	-	-	-	-	-	-	-	-	8	-	**8**
		UTR8	L	2007	-	-	-	-	1	-	-	-	-	-	-	-	**1**
		UTR8	L	2017	-	-	-	-	-	1	-	-	-	-	-	1	**2**
		UTR8	L	2022	-	-	-	-	-	-	-	-	-	-	3	-	**3**
Total Abundance					**14**	**3**	**3**	**13**	**6**	**3**	**18**	**26**	**49**	**1**	**116**	**20**	**272**

U = Upgradient; D = Downgradient (potentially impacted by SRS waste sites or operations); L = Lowest reaches (potentially in recovery from SRS impacts)

Note: Hester-Dendy samplers picked up the only *Somatogyrus virginicus* and the only *Callinina intertexta* sampled (*Gillia altilis* was the only other species collected with Hester-Dendy samplers and was also found with MHSP sampling).

Table 7. Status of macroinvertebrate taxa in Table 6 tracked by South Carolina Natural Heritage Program

EST ID	Scientific Name	Common Name	Federal Status	State Status	SWAP Priority	G-Rank	S-Rank	Informal Taxonomy
5,053	*Procambarus hirsutus*	Shaggy Crayfish	NA	NA	Moderate	G4	S4	Crayfishes
5,652	*Callinina intertexta*	Rotund Mysterysnail	NA	NA	NA	G4	S2S3	Freshwater Snails
5,542	*Taeniopteryx robinae*	Savannah Willowfly	NA	NA	NA	G1	SNR	Stoneflies
5,055	*Procambarus pubescens*	Brushnose Crayfish	NA	NA	Moderate	G4G5	S3	Crayfishes
5,559	*Psilotreta frontalis*	A Caddisfly	NA	NA	NA	G5	SNR	Caddisflies
5,423	*Macromia margarita*	Mountain River Cruiser	ARS	NA	NA	G3	S1	Dragonflies and Damselflies
5,056	*Procambarus raneyi*	Disjunct Crayfish	NA	NA	NA	G4	S3	Crayfishes
5,617	*Elliptio fraternal*	Brother Spike	NA	SE	Highest	G1G2	S1?	Freshwater Mussels
5,654	*Gillia altilis*	Buffalo Pebblesnail	NA	NA	High	G5	S1S3	Freshwater Snails
7,329	*Somatogyrus virginicus*	Panhandle Pebblesnail	NA	NA	High	G2G3	S2S4	Freshwater Snails
7,597	*Agarodes libalis*	Spring-loving Psiloneuran Caddisfly	NA	NA	NA	G3	SNR	Caddisflies
5,580	*Oecetis morsei*	Morse's Long-horn (Sedge) Caddisfly	NA	NA	NA	G3	SNR	Caddisflies

Note: Status and ID from SC Natural Heritage Program
NA = Not Applicable
ARS = At-Risk Species
SE = State Endangered
G-Rank = NatureServe global
S-Rank = State
SNR = unranked; conservation status not yet assessed by SCDNR
? = The most likely rank is assigned, but the question mark indicates uncertainty

Table 8. Average (Geometric Means) Maximum Stream Depth and Width and Water Quality Variables in 2022 at Sampling Location During Habitat Assessment

Site Code	Average width (m)	Average max depth (cm)	Date water quality sampled	Time of collection	Water temperature (° C)	pH	Dissolved oxygen (mg/L)	Conductivity (µS)	Specific Cond. (S/m)
CB2	2.096	5.5	2022-11-04	09:53	15.1	7.25	9.75	48.7	59.4
FMB3	3.544	27.4	2022-11-02	11:01	15.9	6.49	6.31	50.39	65.8
FMB5	4.751	51	2022-11-02	14:54	17.5	6.75	8.17	95.8	112.2
FMB7	4.958	50.1	2022-11-14	11:48	11.2	6.72	10.11	44.6	60.6
FMB8	5.937	43.3	2022-12-01	10:46	11.3	6.23	10.46	45.3	61.3
IGB1	3.224	23.1	2022-11-16	09:47	14.8	7.05	9.98	63.2	78.6
MB2	2.496	15.8	2022-11-17	13:50	13.4	6.31	9.64	25.9	33.3
MB3	4.395	21	2022-11-17	10:11	11.6	6.65	10.80	37.2	50.0
MC1	1.917	19.1	2022-11-09	09:51	15.3	5.99	8.33	24.3	29.8
MC3	4.357	50.4	2022-11-18	09:34	7.3	7.03	11.15	33.4	50.4
MQ2	2.632	15.8	2022-11-16	12:44	13.6	6.76	9.95	43.5	55.6
PB1	1.424	6.6	2022-11-15	14:43	14.0	6.69	8.40	38.7	49.1
PB2	3.889	18.2	2022-11-21	10:11	8.0	7.10	11.65	43.2	63.8
PB3	5.009	38.2	2022-11-03	09:41	17.0	6.95	8.79	69.9	76.6
PB4	5.03	30.7	2022-12-01	15:06	12.9	7.13	10.05	41.9	54.6
SC2	2.438	18.2	2022-11-21	14:05	13.1	6.65	10.34	31.8	41.3
SC5	6.851	49.2	2022-11-22	11:09	13.1	7.07	10.07	46.5	60.2
SC6	6.607	74.1	2022-11-29	11:09	14.1	7.04	9.54	49.5	62.5
TC1	7.424	44.1	2022-12-05	10:31	13.4	6.84	10.04	23.7	30.5
TIB1	2.403	8.4	2022-11-08	10:23	18.7	5.84	6.64	27.7	31.6
UTR2	NA	NA	2022-12-07	09:34	15.8	5.28	8.82	16.1	19.5
UTR5	NA	NA	2023-01-20	12:52	14.1	6.72	10.2	17.9	22.7
UTR8	NA	NA	2023-01-20	09:38	13.8	6.43	9.52	19.4	24.7

NA = Not Applicable

APPENDIX A

Fish IBI Metric Calculation Macro for use in Excel (from Michael Paller)

```
Function score(ord, area, pass, total, cyp, bi, pisc, pertol, percyp, persun, pergen, den, dis)
If pass >= 5 Then
   f = 1
   cf = 1
ElseIf pass = 4 Then
   f = 0.997
   cf = 1
ElseIf pass = 3 Then
   f = 0.952
   cf = 0.977
ElseIf pass = 2 Then
   f = 0.868
   cf = 0.947
ElseIf pass = 1 Then
   f = 0.765
   cf = 0.932
End If
If ord = 1 Then
   t = (total / ((-12.305 + 9.3409 * (Log(area) / Log(10))) * f)) * 100
      If t > 100 Then t = 100
   c = (cyp / ((-5.5503 + 3.1334 * (Log(area) / Log(10))) * cf)) * 100
      If c > 100 Then c = 100
   p = (pisc / ((-2.1605 + 1.4077 * (Log(area) / Log(10))) * f)) * 100
      If p > 100 Then p = 100
   b = (bi / ((-4.7433 + 2.878 * (Log(area) / Log(10))) * f)) * 100
      If b > 100 Then b = 100
 ElseIf ord = 2 Then
   t = (total / ((-9.3407 + 9.3413 * (Log(area) / Log(10))) * f)) * 100
      If t > 100 Then t = 100
   c = (cyp / ((-5.5503 + 3.1334 * (Log(area) / Log(10))) * cf)) * 100
      If c > 100 Then c = 100
   p = (pisc / ((-1.4189 + 1.4078 * (Log(area) / Log(10))) * f)) * 100
      If p > 100 Then p = 100
   b = (bi / ((-4.251 + 2.878 * (Log(area) / Log(10))) * f)) * 100
      If b > 100 Then b = 100
ElseIf ord = 3 Then
   t = (total / ((-5.6573 + 9.3423 * (Log(area) / Log(10))) * f)) * 100
      If t > 100 Then t = 100
   c = (cyp / ((-5.5503 + 3.1334 * (Log(area) / Log(10))) * cf)) * 100
      If c > 100 Then c = 100
   p = (pisc / ((-0.6681 + 1.4079 * (Log(area) / Log(10))) * f)) * 100
      If p > 100 Then p = 100
   b = (bi / ((-4.0532 + 2.878 * (Log(area) / Log(10))) * f)) * 100
      If b > 100 Then b = 100
ElseIf ord = 4 Then
   t = (total / ((-6.3604 + 9.3423 * (Log(area) / Log(10))) * f)) * 100
      If t > 100 Then t = 100
```

```
   c = (cyp / ((-5.5503 + 3.1334 * (Log(area) / Log(10))) * cf)) * 100
      If c > 100 Then c = 100
   p = (pisc / ((1.8311 + 1.4079 * (Log(area) / Log(10))) * f)) * 100
      If p > 100 Then p = 100
   b = (bi / ((-7.3892 + 2.878 * (Log(area) / Log(10))) * f)) * 100
      If b > 100 Then b = 100
End If
If t < 70 Then
      tsc = 1
   ElseIf (t >= 70) And (t < 90) Then
      tsc = 3
   ElseIf t >= 90 Then
      tsc = 5
   End If
If c < 55 Then
      csc = 1
   ElseIf (c >= 55) And (c < 80) Then
      csc = 3
   ElseIf c >= 80 Then
      csc = 5
   End If
If p < 65 Then
      psc = 1
   ElseIf (p >= 65) And (p < 85) Then
      psc = 3
   ElseIf p >= 85 Then
      psc = 5
   End If
If b < 65 Then
      bsc = 1
   ElseIf (b >= 65) And (b < 85) Then
      bsc = 3
   ElseIf b >= 85 Then
      bsc = 5
   End If
If pertol > 15 Then
      tolsc = 1
   ElseIf (pertol >= 5) And (pertol <= 15) Then
      tolsc = 3
   ElseIf pertol < 5 Then
      tolsc = 5
   End If
If (percyp < 20) Or (percyp > 95) Then
      cypsc = 1
   ElseIf (percyp >= 20) And (percyp <= 35) Then
      cypsc = 3
   ElseIf (percyp > 35) And (percyp <= 95) Then
      cypsc = 5
   End If
If (persun > 45) Or (persun < 5) Then
      sunsc = 1
```

```
   ElseIf (persun >= 25) And (persun <= 45) Then
      sunsc = 3
   ElseIf (persun < 25) And (persun >= 5) Then
      sunsc = 5
   End If
If (pergen > 75) Or (pergen < 5) Then
      gensc = 1
   ElseIf (pergen >= 50) And (pergen <= 75) Then
      gensc = 3
   ElseIf (pergen < 50) And (pergen >= 5) Then
      gensc = 5
   End If
If dis > 3 Then
      dissc = 1
   ElseIf (dis >= 1) And (dis <= 3) Then
      dissc = 3
   ElseIf dis < 1 Then
      dissc = 5
   End If
If (pass = 4) Or (pass = 5) Or (pass = 6) Or (pass = 7) Then
      If den < 25 Then
         denscr = 1
      Else
         denscr = 5
      End If
   ElseIf pass = 3 Then
      If den < 20 Then
         denscr = 1
      Else
         denscr = 5
      End If
   ElseIf pass = 2 Then
      If den < 15 Then
         denscr = 1
      Else
         denscr = 5
      End If
   ElseIf pass = 1 Then
      If den < 10 Then
         denscr = 1
      Else
         denscr = 5
      End If
   End If
 score = tsc + csc + psc + bsc + tolsc + cypsc + sunsc + gensc + denscr + dissc
 End Function
```

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