

**Evaluating the role of flow and specific conductivity on stream metabolism across a mining-induced salinity gradient in the Appalachian Coalfield**

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## **Scientific Abstract**

Freshwater salinization is a notable concern for headwater streams in Appalachia with the history of resource extraction in the region. Mountaintop removal/valley fill mining techniques in Appalachia result in the burial of headwater streams and mobilization of ions, specifically  $\text{SO}_4^{2-}$ , Ca, and Mg into waters, raising specific conductivity (SpC). Organismal responses to salinization have been well documented in freshwater ecosystems, but there are few measurements assessing how salinity effects on organisms influence whole-ecosystem processes, specifically stream metabolism. Understanding how gross primary production (GPP) and ecosystem respiration (ER) respond to salinity, stream flow, and their association is needed to characterize the consequences of salinization on stream processes. To assess the role of salinization and discharge on metabolism, we recorded high-frequency SpC, discharge, and dissolved oxygen data in three headwater streams in the Appalachian (U.S.) coalfields, where mining has resulted in widespread headwater stream salinization. Sites included a reference stream (REF) with SpC ranging from 0.2 - 57.69  $\mu\text{S}/\text{cm}$ , a mid-salinity site (MID; 2.03- 594.7  $\mu\text{S}/\text{cm}$ ), and a high-salinity site (HI; 84.6-1920  $\mu\text{S}/\text{cm}$ ) with similar flow regimes, helping to characterize these covarying and potentially interacting drivers of metabolism. Across all sites, SpC decreased with increased discharge and with significant breakpoints. This study did not find a clear relationship between SpC and ecosystem metabolism. All streams, regardless of SpC levels, were heterotrophic ( $|\text{ER}| > \text{GPP}$ ). Our high salinity site had the highest GPP and ER, suggesting salt may subsidize ecosystem metabolism, though differences in canopy cover

complicate this. While we saw no consistent effect of SpC on metabolism, deviation in patterns at mining-impacted sites from expected discharge-metabolism patterns at our reference sites suggests some impact of salinity. This study reinforces the need for research across diverse ecosystems and salinization sources to characterize the role of freshwater salinization on ecosystem metabolism, as mediated by discharge.

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## **General Audience Abstract**

Surface coal mining operations in the Appalachian Coalfield region have resulted in decreased water quality and increased salt levels (measured as specific conductance; SpC) in small, headwater streams. Declines in aquatic biodiversity have been documented with increases in SpC, but little work has been done to address impacts on broader stream processes such as ecosystem metabolism, which represents organic carbon fixation and breakdown by gross primary production (GPP) and ecosystem respiration (ER), respectively. High stream flows are a known driver of both ecosystem metabolism and SpC concentrations in headwater streams; however, minimal work has been conducted to connect the roles of these interacting drivers (flow and SpC) on metabolism. This study measured metabolism across three headwater streams in the Appalachian Coalfield of varying salinity and flow regimes from August 2024 to August 2025. We found no relationship between ecosystem metabolism and SpC across our sites. Increases in GPP and ER at our high SpC site suggest a subsidy effect, although small sample sizes and the potential for increased light availability complicate this interpretation. While we found no consistent effect of SpC on metabolism across our sites, variation in flow-metabolism relationships from reference conditions at salinized sites suggests salts exhibit a potential effect on ecosystem processes. We encourage continued work on connecting ecosystem metabolism and freshwater salinization across flow regimes in headwater streams.

## **Dedication**

I would like to dedicate this thesis to:

My parents, John and Laura Meehan, for supporting me throughout my now 23-year education (bet you didn't plan for that). Thank you for showing me I'm capable of doing hard things, even when I believed I couldn't.

My siblings, Madelyn and Aidan. Thank you for keeping me grounded during this stage of my life. Thank you for keeping me entertained and letting me rant from miles away. Sorry, I couldn't help you with Python during this journey, as you can see in this paper, I'm an R girl.

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My roommates, Joe and Shannon, for always being there for me. Thank you for welcoming me into your home and always making me feel like I belong.

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## **Chapter 1: Mining-induced freshwater salinization in Appalachian headwater streams**

Salinization has become a prevalent topic in freshwater research, particularly with the global rise in urbanization and other anthropogenic impacts to ecosystems (Kaushal et al. 2023). Increases in road salts, resource extraction, and wastewater release have led to the steady salinization of freshwater streams over time (Falkenmark 2003, Berger et al. 2017). Freshwater salinization has been documented to impact ecosystem health by causing declines in organismal populations but less is understood about its effects on ecosystem processes. Understanding how salinization influences ecosystem function is essential in aiding ecosystem monitoring, management, and restoration efforts in changing environments.

Freshwater salinization is a notable concern in the Appalachian Coalfield, a region in the Southeastern United States that has had a large portion of its land cover altered by surface mining. Resource extraction has been documented to increase freshwater salinity in Appalachia through the direct release of mine drainage into waterways and through the deposition of mine material in or adjacent to headwater streams, making it a prevalent stressor to water quality in the region (Skousen and Zipper 2021). Increased salinity from mining operations in the Appalachian Coalfield has been a documented stressor to numerous aquatic species, making it a notable environmental concern in the region (Pond et al 2008).

The Appalachian Coalfield was the United States' largest supplier of coal from the early 1800s to the 1970s (Zipper et al. 2021). Over 45 billion tons of coal have been removed from the region to date (Zipper 2020). Historically, coal mining in the area was limited to mining coal seam outcrops (highwall mining) until the advent of mountaintop removal and valley fill (MTR/VF) in the late 1950s (Fritz et al. 2010). The rise of MTR/VF resulted in a dramatic increase in Appalachian coal production, making it the most common resource extraction

technique in the region (Fox 1999, Duffy 2003, Slonecker and Bengert 2009). Appalachian coal production averaged 428 million tons/year in the 1990s, during the peak of MTR/VF mining, and declined to reach production levels of <200 million tons/year by 2016-2019 (Skousen and Zipper 2021). As of 2018, approximately 5900 km<sup>2</sup>, or 7.1%, of central Appalachian land area had been influenced by surface coal mining (Pericak et al. 2018).

The MTR/VF technique removes soil and bedrock near the summit of mountains to access underlying coal; it can remove material to access depths of 100 meters or more (Zipper et al 2021). Material removed from mountaintops to access coal is deposited into surrounding valleys, creating “valley fills”. Valley fills are often situated on top of headwater streams originating in mining-impacted valleys. They are constructed with internal drains, so water entering the structure passes through the fill material. This is done to avoid saturating spoil material, which could lead to instability and potential landslides in the surrounding area. As a result, valley fills strongly influence surface water quality (Cormier et al. 2013a and b, Skousen and Zipper 2021).

The movement of water through valley fill material leads to the weathering of parent material and, in turn, causes the release of soluble elements to headwater streams (e.g., Agouridis et al. 2012, Griffith et al. 2012, Burke et al. 2014). The input of dissolved solids increases in-stream salt concentrations, often measured as specific conductivity (SpC; the ability of water to pass an electric current), impairing once-dilute waterways in the region (Skousen and Zipper 2021). The predominant ions that make up SpC in Appalachian streams influenced by surface coal mining are SO<sub>4</sub><sup>2-</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, and HCO<sub>3</sub><sup>-</sup>, though other trace elements are sometimes present (Pond et al. 2008, Lindberg et al. 2011). Dissolved ion concentrations caused by the weathering of valley-fill mining material into streams accumulate over time, raising

background/reference SpC levels in streams from  $>100 \mu\text{S}/\text{cm}$  to ca.  $1500 \mu\text{S}/\text{cm}$  in heavily impacted streams. (Skousen and Zipper 2021). This increase from background conductivity levels has negative consequences for freshwater organisms and ecosystem processes.

My collaborators at Virginia Tech have been monitoring headwater streams impacted by valley-fill related salinization since 2010. This long-term project includes 23 streams of varying SpC ranges (5 reference and 18 mining-impacted) throughout Southwest Virginia and West Virginia. Selected sites have been monitored for over 10 years to ensure streams meet water and habitat quality standards (with the exception of high SpC levels and ions associated with mining activity) to isolate mining-induced increases in SpC as a stressor that varied among sites (Timpano et al. 2015). These streams have been monitored for changes in biotic composition and ecosystem processes through long-term macroinvertebrate surveys, decomposition studies, and food resource collection and analysis (Timpano et al. 2015, Vander Vorste et al. 2019, Cianciolo et al. 2020). Importantly, minimal water quality recovery has been documented at sites with associated valley fills since monitoring began, indicating mining-induced salinization will be a prevalent, long-term stressor in Appalachia (Cianciolo et al. 2020). Significant declines in the scraper functional-feeding group, Ephemeroptera, have been documented in streams with associated valley fills. An 8-year analysis of macroinvertebrate trends found little evidence for community recovery, suggesting that, due to predicted SpC persistence, the biological effects of mining-induced salinization will persist in the region for years to come (Cianciolo et al. 2020). Salinization has been a documented stressor to macroinvertebrates in the Appalachian Coalfield and is likely to continue given the maintained elevated SpC levels.

While biodiversity declines have been documented in headwaters impacted by MTR/VF techniques, little is known regarding potential shifts in ecosystem processes. One study found

that while increased SpC impacted macroinvertebrate assemblages, it did not significantly alter leaf decomposition, indicating the role of salts on ecosystem processes is complex (Vander Vorste et al. 2019). The role of salinity on in-stream processes, discussed in more detail in Chapter 2, has been understudied in MTR/VF impacted streams and in small headwaters more generally. Expanding our understanding of how high SpC conditions impact ecosystem processes will be essential to assess how mining-induced salinization is changing headwater streams.

Water chemistry, such as variable salinity levels, is not the only dynamic stressor for headwater streams in the Appalachian Coalfield. Flow is highly variable in this region, with small headwaters experiencing both drought and flooding within a single calendar year (Scaife and Band 2017). Appalachian headwater streams can experience near-complete desiccation but are also subject to large storm events, including record flooding during Hurricane Helene in fall 2024. We observed this pattern while conducting field work in a small subset of salinized Coalfield headwaters. A mid-salinity stream, Frying Pan Creek (**Fig. 1.1**), selected for this study, experienced complete drying during August 2024, followed by extremely high flow levels in February 2025, resulting in the scouring of secured sensors following a winter storm. Variable flow patterns present in these mountain streams are an established driver of many ecosystem processes in headwaters (McTammany et al. 2003). Drying, or desiccation, can greatly decrease oxygen levels, having negative implications for stream biota (Sabater et al. 2016). On the other hand, high flows, or large storm events, can scour benthic and surface water populations and increase turbidity through sediment resuspension and terrestrial inputs, decreasing stream production and respiration (Uehlinger 2006, Blaszcak et al. 2018). While high flows can behave as a stressor to freshwater systems, they can also behave as a subsidy in some cases. Low to intermediate flows can bring nutrients and organic carbon into waters, stimulating production

and respiration in stream ecosystems (O'Donnell and Hotchkiss 2019). In addition to influencing ecosystem processes, flow also exhibits control over the physical chemistry of these headwater streams. Indeed, the SpC levels in these streams are dictated by flow levels, with high flow events significantly diluting concentrations. As a result, we must consider flow variability when looking to address the role of SpC on stream ecosystems.



Figure 1: Left: Rickey Branch Creek, a high-salinity/impacted site located near Wise, Virginia, in the Appalachian Coalfield. Photo taken in February 2024. Top right: Frying Pan Creek, a mid-salinity/impacted site located near Dante, Virginia, in the Appalachian Coalfield. Photo taken in August 2024. Bottom right: Eastland Creek, a reference site located near Wise, Virginia, in the

Appalachian Coalfield. Photo taken in October 2023.

The effects of valley fill-induced salinization on ecosystem processes remain unknown. This study investigated the impact of mining-induced salinization in conjunction with variable flow regimes on ecosystem metabolism. Ecosystem metabolism is the fixation and breakdown of carbon in an ecosystem (Odum 1956). This environmental metric incorporates the fundamental processes gross primary production (GPP; carbon fixation by aquatic autotrophs) and ecosystem respiration (ER, the respiration of organic carbon). An environment's net ecosystem production (NEP,  $GPP - |ER|$ ) reflects the degree to which respiration is sustained by in-stream production or subsidized by allochthonous (external) organic matter. Autotrophy,  $GPP > |ER|$  or positive NEP, indicates autochthonous (internal) carbon produced by GPP can sustain respiration and is building autotrophic biomass. Heterotrophy,  $|ER| > GPP$  or negative NEP, indicates allochthonous carbon is subsidizing respiration in excess of GPP. Large shifts in ecosystem metabolism can indicate changes in freshwater health, as it captures changes in autotroph and/or heterotroph populations (Young et al. 2008, Marcarelli et al. 2011). Furthermore, changes in ecosystem metabolism signal a shift in resource allocation that can impact the health and structure of communities downstream. Understanding how ecosystem metabolism changes in response to mining-induced salinization across flow regimes will advance our understanding of how these environmental drivers impact freshwater ecosystems.

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## **Chapter 2: Evaluating the role of discharge and specific conductivity on stream metabolism across a mining-induced salinity gradient**

### **Introduction:**

Freshwater ecosystem health is shifting globally due to land-use changes and increasingly rapid climate change (Carpenter et al. 1992, Nijssen et al. 2021, Maloney and Weller 2011). Freshwater salinization is becoming a prevalent concern for aquatic ecosystems due to an increase in anthropogenic land cover changes and salt use globally (Kaushal et al. 2023b). At the same time, hydrologic variability (e.g., streamflow) is expected to increase with climate change (Sivakumar 2011), which can have direct consequences for aquatic systems (Poff and Alan 1995, Meyer et al 1999) but also interact with ion sources and further lead to variability in salinity levels (Knapp et al. 2020). Dissolved ions from sources such as road salts and brine, mine waste (e.g., drainage, brines from hydrofracking, valley-fills), agricultural runoff, and wastewater enter waterways, increasing the ionic concentration in ecosystems (e.g., Raymond et al. 2008, Vidic et al. 2013, Dugan et al. 2017). Additionally, the threat of climate change and rising sea levels is leading to increased saltwater intrusion in coastal freshwater environments (e.g., Jeppesen et al. 2015, Cañedo-Argüelles et al. 2017, Kaushal et al. 2023a). Between documented salt increases from human development and anticipated increases from climate change, freshwater salinization is predicted to increase globally (Olson 2018, Fanelli et al. 2024). With predicted increases in salinization and hydrologic variability across freshwater ecosystems, it's critical to advance our understanding of these potentially interacting stressors on biota and ecosystem processes.

Freshwater salinization is a documented stressor to aquatic organisms, though responses of individual species vary with salt levels. High salt levels will ultimately kill non-saline organisms, but moderate increases have been documented to subsidize certain populations (Gruntz et al. 2022, Clay et al. 2023). Large increases in ionic concentrations can alter community structure and decrease species richness by inducing osmotic stress and reducing food quality (e.g., Hart et al. 1991, Nielsen et al. 2003, Venâncio et al. 2018). High levels of salt [ $\sim 1500 \mu\text{S}/\text{cm}$ ] can result in the decline of non-salt-tolerant heterotrophs, including certain macroinvertebrate taxa in headwater streams and zooplankton in limnetic systems, which may have implications for vertebrate predator populations (Castillo et al. 2018, Hills et al. 2018, Huber et al. 2023). As such, with increased salinity, biodiversity and associated ecosystem functions often decline in salinized ecosystems (Berger 2018). However, salt can also serve as a subsidy to certain species. For example, increased concentration of some ions (e.g., calcium and magnesium) can act as micronutrients for both plant and animal communities, potentially stimulating population growth (Duan and Kaushal 2015, Cañedo-Argüelles et al. 2020). Additionally, certain microbial communities have exhibited tolerance to, or even increased growth in, high salinity conditions [ $\sim 1400 \mu\text{S}/\text{cm}$ ] (Shu et al. 2023, DeVilbiss et al. 2024). Given such variable organismal responses, it is important to understand emergent effects of salinization on different organisms and ecosystems (Kefford et al. 2016).

While salinity impacts on individual organisms and populations have been documented, less is known regarding how ecosystem processes respond, notably in ecosystems exposed to a large range of salt concentrations. Ecosystem processes, such as nutrient cycling and carbon metabolism, emerge from trophic interactions (Mulholland et al. 2001, Berger 2018, Battin et al. 2023) and may thus vary in response to increased salinity. Further, ecosystem processes are often

more sensitive to finer-scale changes in environmental conditions over time compared to cumulative impacts evaluated on organisms or populations (Segatto et al. 2023). Indeed, ion concentrations often exhibit fine-scale changes with streamflow, either diluting or increasing at higher flows (Johnson & Likens 1969, Clow & Drever 1996). Yet, few studies have tested how salinity effects on organisms are reflected in whole-ecosystem processes and less is known regarding ecosystem sensitivity to time-varying salinity conditions. Here, we focus on one ecosystem process, stream metabolism (carbon fixation by gross primary production (GPP) and the mineralization of organic carbon through ecosystem respiration (ER)), and its response to fine-scale changes in both streamflow and salinity.

Ecosystem metabolism represents ecosystem-scale energetics and can be used to assess the role of environmental drivers, like salinity, on overall stream condition (Marcarelli et al. 2011, Jankowski et al. 2021, Rüegg et al. 2021). Stream net ecosystem production ( $NEP = GPP - |ER|$ ) can be used to predict changes in downstream resource quality and determine whether an ecosystem is a carbon sink or source. Autotrophic streams, where GPP is higher than ER, can sustain ER with internal GPP, where excess autochthonous carbon can either be accumulated in the reach or transported downstream. Heterotrophic waterways ( $GPP < |ER|$ ) rely on allochthonous (i.e., terrestrial) inputs to sustain ER in excess of GPP. Forested headwater streams are usually heterotrophic due to higher allochthonous inputs and lower light relative to larger rivers (Battin et al. 2023); consequently, streams represent hotspots of ER and CO<sub>2</sub> emissions (Raymond et al. 2013, Lui et al. 2022). Furthermore, the amount of organic carbon that is processed in upstream reaches will govern downstream metabolic opportunities and the relative balance between GPP and ER. Thus, changes in stream metabolism will influence stream resource allocation and carbon budgets (Hotchkiss et al 2015, Gounand et al. 2018).

Stream metabolism is sensitive to changes in environmental conditions. GPP and ER can be subsidized or stressed by changing environmental factors depending on stressor type and severity. A known stressor of stream metabolism is increased flow, measured as discharge, where high water velocity can scour benthic autotroph communities and increase turbidity, decreasing GPP (Uehlinger & Naegeli 1998, Blaszcak et al. 2018). This decline in GPP along with the scouring of heterotroph communities can, in turn, decrease stream ER after initial flow increases (O'Donnell and Hotchkiss 2019). In contrast, moderate flows, characteristic of smaller precipitation events, can provide allochthonous carbon and nutrients into the system and stimulate stream metabolism (McLaughlin & Kaplan 2013, Demars 2018). As a result, metabolism may exhibit dynamic changes (i.e. subsidy-stress response (Odum et al. 1979, O'Donnell [DM1] and Hotchkiss 2019) to increases in flow, with GPP and ER being stimulated by allochthonous input from low to intermediate flows until high flows exert severe physical stress.

While research has assessed how stream metabolism responds to flow variation, less is known regarding metabolic responses to salinization, especially at the whole-ecosystem scale. Salts can behave as both a nutrient or stressor to organisms depending on concentration and ionic makeup, with variable organismal responses. We know high salinity levels can induce osmotic stress in plants, leading to potential GPP declines (Boyer 1982). At the same time, salinization has been shown to mobilize nitrogen and phosphorus from sediment beds (Groffman et al. 2005, Kaushal et al. 2022), which can stimulate autotroph growth and increase in-stream production. There have been documented declines in microbial diversity with increases in salinity (Vander Vorste et al. 2019), which may lead to changes in ER. While in-stream leaf decomposition was not influenced by salinity levels (Vander Vorste et al. 2019), bacterial respiration in mesocosms

increased with increasing salinity (DeVilbiss et al. 2024). Given such variable responses, the effect of salinity on stream metabolism is difficult to predict. Further, headwater streams exhibit variable flow regimes, which may both directly affect metabolism rates while also mediating time-varying salinity levels. Thus, understanding how high-frequency changes in salinity and flow influence GPP and ER is essential to characterize the consequences of salinization on headwater stream processes.

To assess how stream metabolism responds to changes in salinity and flow, we estimated daily GPP, ER, flow, and salinity (via specific conductivity; SpC) in three headwater streams with different SpC ranges in the Appalachian Coalfields in the southeastern United States. We predicted both GPP and ER would exhibit subsidy-stress responses with SpC, with increases in SpC and associated nutrients stimulating these processes until SpC reaches a level at which osmotic stress decreases GPP and ER. We predicted that SpC would have a larger subsidy effect and higher stress threshold on ER compared to GPP. We anticipated metabolism at all sites, regardless of SpC ranges, would be more sensitive to changes in flow than SpC, but follow similar subsidy-stress patterns.

## **Methods:**

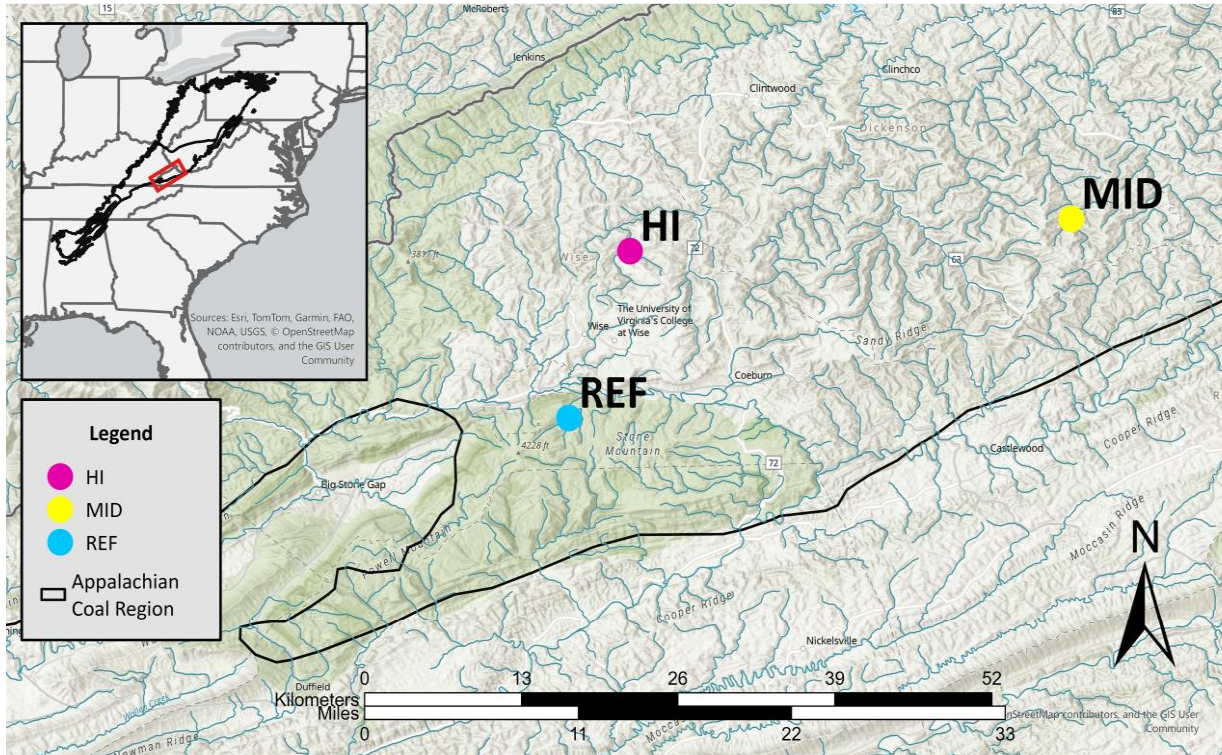
### ***Site Information***

Our study region is the Appalachian coalfields in southwest Virginia and West Virginia, U.S.A., where salinization is a prevalent threat to stream health and water quality due to the large presence of surface mining operations. As of 2018, around 5900 km<sup>2</sup> of central Appalachian land area had been influenced by surface coal mining (Pericak et al. 2018). Bedrock is removed to

reach buried coal seams and deposited into Appalachian valleys, burying headwater streams and creating ‘valley fills’ (Fox 1999, EPA 2011). The deposition of mining products and waste rock allows increased geologic weathering, leaching ions into headwater streams (Hasselmann 2002, Griffith et al. 2012). Sulfuric acid is generated locally, weathering surrounding rocks and leaching additional trace elements into headwater streams (Bernhardt and Palmer 2011). Carbonates in the valley-fill material neutralize sulfuric acid but cause increases in  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{HCO}_3^-$  ions, leading to increased SpC in receiving streams (Beer 2000, Hartman 2005). Increases in SpC in the central Appalachian Coalfield region have led to declines in water quality and aquatic biodiversity, notably macroinvertebrates belonging to scraper feeding groups (Pond et al. 2008).

To assess the impact of freshwater salinization on carbon cycling, we selected three headwater streams, representing low, medium, and high SpC across a mining-induced salinity gradient. These three study sites, as part of a larger set of streams, have been monitored for over 10 years for post-mining recovery assessments (Cianciolo et al. 2020). Sites were previously selected to ensure streams met water and habitat quality standards (excluding SpC levels and ions associated with mining activity) to isolate salinity as a stressor (Timpano et al. 2015). For this study, we selected Eastland Creek (hereafter ‘REF’) (annual mean SpC: 21  $\mu\text{s}/\text{cm}$ ) as a reference stream, Frying Pan Creek (‘MID’) (annual mean: 430  $\mu\text{s}/\text{cm}$ ) as a mid SpC stream, and Rickey Branch Creek (‘HI’) (annual mean: 1427  $\mu\text{s}/\text{cm}$ ) as a high SpC stream (**Table 1, Fig. 2.1**). REF’s watershed has no history of mining activity while MID and HI’s watershed had mining activity cease in 2007 and 2005, respectively (Cianciolo et al. 2020). HI had slightly lower canopy coverage and potentially higher light availability compared to other selected sites

but met similar water and habitat quality standards as REF and MID while having high SpC concentrations.



**Figure 2.1:** Study sites within the Appalachian Coalfield of the eastern United States. The Appalachian Coalfield (black border) is shown in the regional inset, along with our specific study region (red box). Made using ArcGIS Pro version 3.6. (ESRI 2025).

**Table 1:** Specific conductivity (SpC) ( $\mu\text{S}/\text{cm}$ ) ranges and means from our study period (Fall 2024 - Summer 2025) and ion, isotope, and nutrient (mg/L) means of biannual water chemistry sampling using methods from Ciancolo et al. 2020 from Fall 2020 - Summer 2025.

Site	SpC μS/cm	Na <sup>+</sup> mg/L	Mg <sup>2+</sup> mg/L	Ca <sup>2+</sup> mg/L	Cl <sup>-</sup> mg/L	SO <sub>4</sub> <sup>2-</sup> mg /L	TN mg/L	TP mg/L	NO <sub>3</sub> <sup>-</sup> - N mg/L	NH <sub>3</sub> <sup>-</sup> - N mg/L
REF	21, 0.2-57.7	0.7	0.6	2.6	0.3	2.5	0.2	0.009	0.2	0.02
MID	430, 2.0- 594.7	18.4	17.6	39.1	5.9	128.7	0.3	0.01	0.2	0.03
HI	1427, 84.6- 1920.0	12.4	136.4	130.6	9.0	670.3	0.5	0.008	0.3	0.01

### ***Data Collection***

We collected data from dissolved oxygen (DO) logging sensors (miniDOT, Precision Measurement Engineering, Vista, California) at sites from mid-August 2024 through August 2025 to continuously record DO concentration and water temperature at 10-minute intervals. We calibrated sensors before and after deployment by conducting Winkler titrations in oxygenated and anoxic waters to correct any sensor-specific offsets from measured calibration concentrations (Hall and Hotchkiss 2017, Meehan 2026). We deployed sensors downstream in study reaches at a representative location, above any culverts or confluences and avoiding locations with major pools or riffles upstream. We downloaded DO data and cleaned sensors every three months. Final calibration measurements were taken after sensors were removed from

the stream to account for changes in calibration during deployment and assumed linear drift during the course of deployment (Meehan 2026). The REF sensor was replaced with another calibration-checked sensor in October 2024 due to sensor failure.

Pressure transducers and conductivity logging sensors (Conductivity Data Logger, Onset, Bourne, Maine; Water Level Data Logger, Onset, Bourne, Maine) were deployed at all sites to record total pressure and SpC every 30 minutes. We converted pressure measurements to stage (water level relative to streambed), correcting for barometric pressure using additional pressure transducers deployed in upland locations. We manually recorded stage at each water pressure sensor preceding download to correct for sensor drift. We calculated SpC from measured electrical conductivity data using temperature and a linear temperature coefficient (0.02) (Barron and Ashton n.d.). For SpC data, we manually removed periods of burial, represented by prolonged time at 0  $\mu\text{S}/\text{cm}$  or abnormal data patterns, as they were not representative of surface water SpC patterns. MID SpC data from December 2024 to early March 2025 were lost due to sensor washout during a winter storm in February 2025. Small gaps in the SpC data were present in REF and MID due to sensor storage capacity limits.

We created discharge-stage rating curves using periodic low-concentration salt pulse additions throughout the year to calculate daily discharge at each site. We released a known mass of dissolved NaCl 100 meters upstream of a SpC sensor with logging intervals set to 1 second. The area under the SpC spike, distance from sensor, and elapsed time for the salt to pass through the reach were used to calculate one-time discharge and velocity values (Moore 2005). We estimated daily mean stream channel depth using calculated discharge, velocity, and an average wetted width from 10 measurements per site (taken 6 times annually). We created site-specific discharge-stage and depth-stage rating curves with power-law functions using recorded stage to

generate daily estimates of flow and mean channel depth required for metabolism and flow-related assessments described below. We produced 5-6 quality-checked points for each sites rating curve (**Fig. A2–A7**). The range of stage values during the tracer additions used to create the rating curves represented 70-80% of recorded ranges in our sites (72.06% for REF, 78.24% for MID, and 75% for HI), as we were unable to access our sites safely to conduct tracer additions during higher flow periods.

### ***Ecosystem Metabolism Analysis***

We estimated GPP and ER at our sites using the *StreamMetabolizer* package in R version 4.3.3 (R Core Team 2024, Appling et al. 2018). This multilevel Bayesian model solves for the combination of GPP, ER, and air-water gas exchange of oxygen ( $K_o$ ,  $d^{-1}$ ) that best matches modeled and measured DO (Castillo et al. 2018, Appling et al. 2018, **Equation 1**). To model metabolism, we calculated oxygen saturation from calibrated DO (mg/L), temperature, and local barometric pressure. We then modeled light in *StreamMetabolizer* using latitude, longitude, and solar time (Appling et al. 2018). We used single-station Bayesian inverse models to estimate GPP and ER and  $K_o$  in *StreamMetabolizer*, using 4 Monte-Carlo Markov chains (mcmc), 1000 burn-in steps, and 3000 saved steps for each site. To reduce equifinality issues associated with solving for  $K_o$  in addition to GPP and ER, we included a model-generated relationship between Q and  $K_o$  across six Q bins to inform daily model estimates of  $K_o$  (Appling et al. 2018, O'Donnell and Hotchkiss 2019). We constrained model estimates of GPP and ER to be positive and negative, respectively. We used model fit diagnostics in the *rstan* package (Stan Package Development Team, 2018) to remove GPP, ER, and  $K_o$  values that had an Rhat value above 1.1 and  $N_{eff}$  above 20,000 (O'Donnell and Hotchkiss 2019). Of 355 day sensor deployments, 202

daily metabolism estimates for REF, 64 daily metabolism estimates for MID, and 170 daily metabolism estimates for HI passed quality checks. The most common reasons for removing days with poor metabolism estimates were sensor burial and stream drying.

$$mO_t = mO_{\{t-\Delta t\}} + \frac{(GPP \cdot PAR_{\{t-\Delta t\}})}{(Z \cdot \Sigma PAR)} + \left(\frac{ER}{Z}\right)\Delta t + K_O(O_{\{sat(t-\Delta t)\}} - mO_{\{t-\Delta t\}})\Delta t$$

**Equation 1:** Single-station metabolism model (parameters defined in **Table A1**)

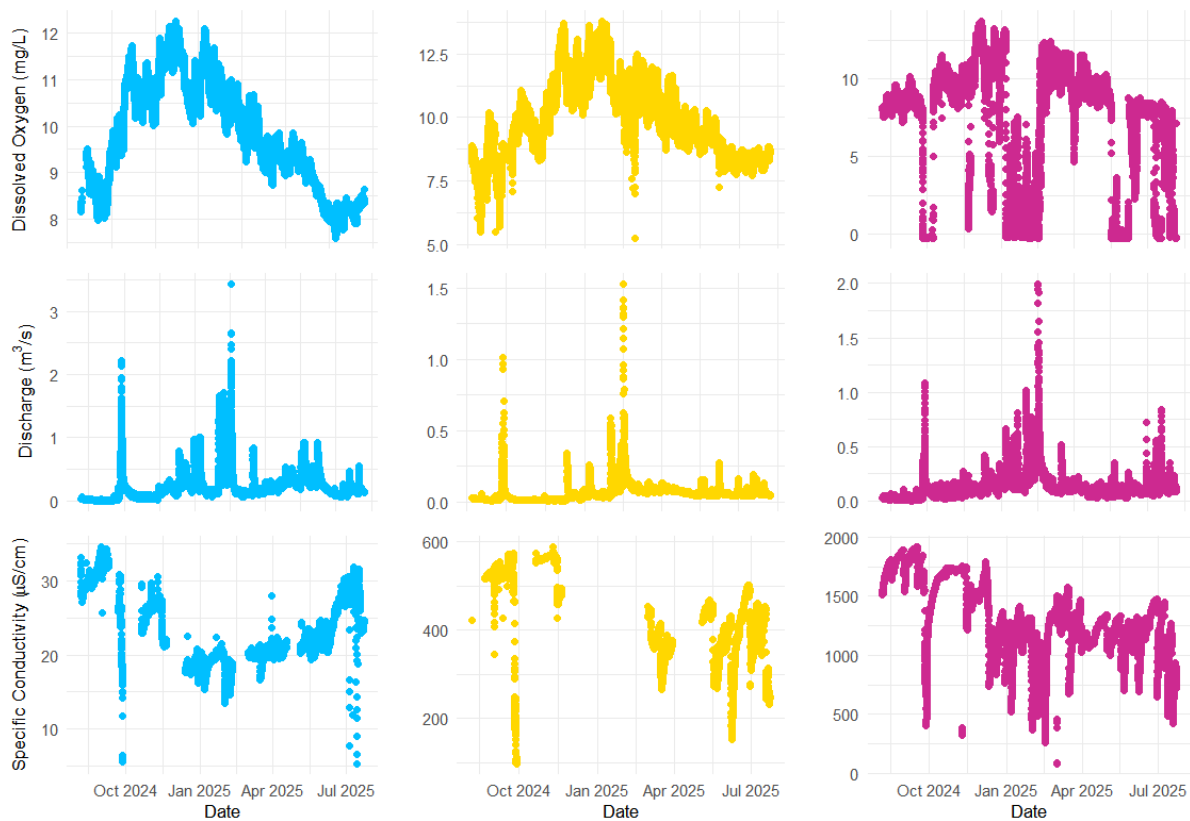
### ***Data Analysis***

For each stream, we conducted regression and breakpoint analyses to characterize how: (1) SpC changes with flow and (2) metabolism values respond to both SpC and flow variation. To do so, we used log-transformed, daily values for median SpC, median flow, GPP, and |ER|. For each analysis, we used the Davies test in the *segmented* R package (Muggeo 2008) to assess if slopes exhibited a model-estimated breakpoint and changed significantly (Meybeck and Moatar 2012, O'Donnell and Hotchkiss 2019). If there were no significant breakpoints, we applied linear regression fits to the full dataset, whereas separate regressions were fit before and following significant breakpoints. We overlaid significant SpC-flow breakpoints on metabolism-flow relationships at mining-impacted sites to assess how flow thresholds for SpC influenced metabolism variation.

### **Results:**

#### ***Site characterization***

Dissolved oxygen was high among all streams and varied seasonally, peaking in winter at all sites (**Fig. 2.2**). The DO time series for HI was variable due to periods of oxygen sensor burial. Estimated discharge varied seasonally with peaks near 3 m<sup>3</sup>/s for REF, 1.5 m<sup>3</sup>/s for MID, and 2 m<sup>3</sup>/s for HI during a winter storm in February 2025. Specific conductivity ranged from 0.2-57.7 μS/cm at our reference site (REF), 2.0-594.7 μS/cm at our mid SpC site (MID), and 84.6-1920.0 μS/cm at our high SpC site (HI). Water temperature varied seasonally with REF ranging from 1.0-20.8 °C, MID ranging from -1.1 to 21.9 °C, and HI ranging from 0.01 to 22.2 °C.

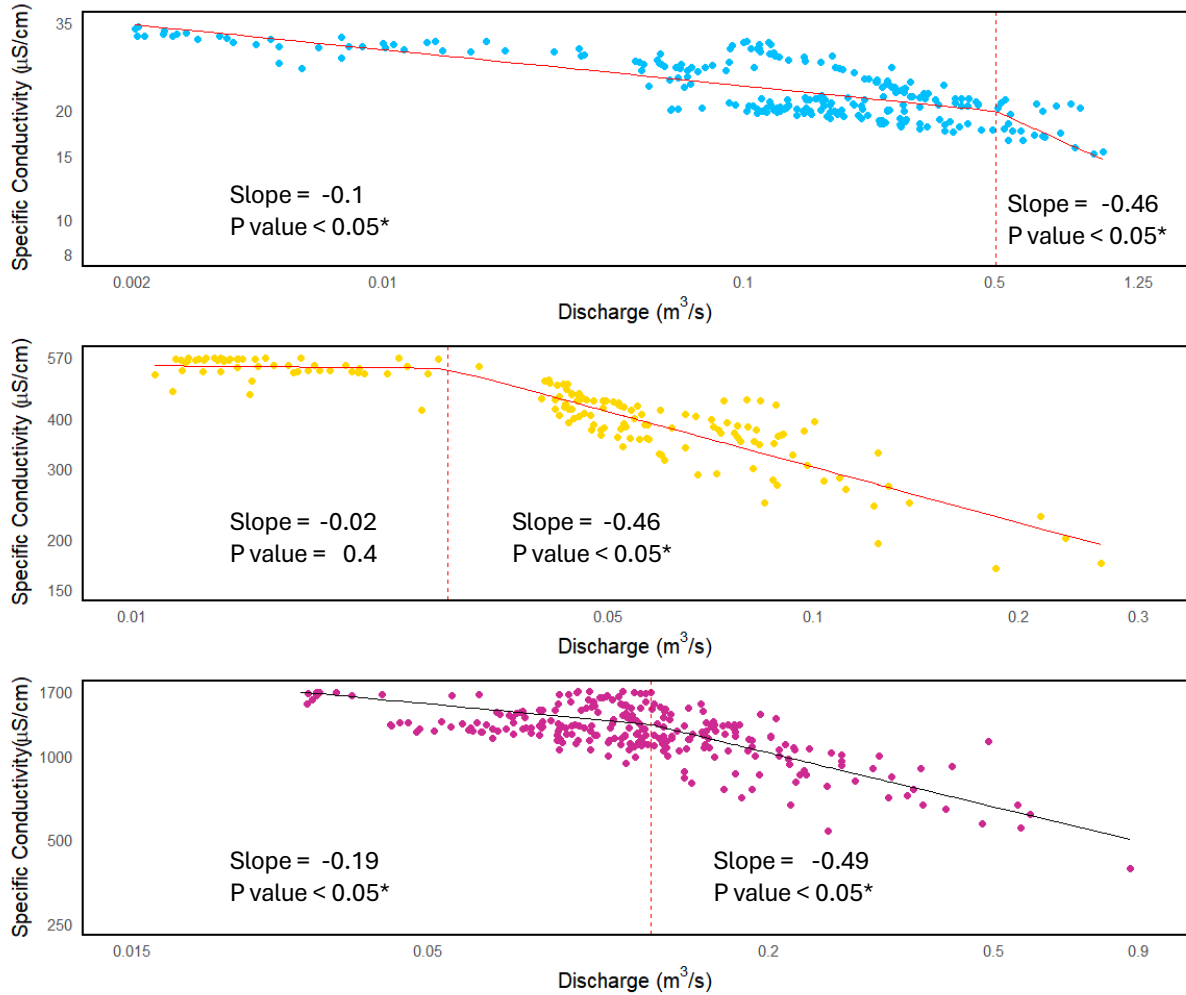


**Figure 2.2:** Dissolved oxygen (top row), discharge (middle row), and specific conductivity (bottom row) time series for REF (blue), MID (yellow), and HI (pink) from October 2024 - August 2025. Note: y-axis ranges differ for each variable.

Ion and nutrient concentrations varied across sites.  $\text{SO}_4^{2-}$ , Mg, and Ca concentrations were the highest at sites impacted by valley-fills (MID and HI). Nutrient levels were low across sites with  $\text{NH}_3^-$  and total phosphorus (TP) levels often below detection. Total Nitrogen (TN) and  $\text{NO}_2^- \text{NO}_3^-$  varied across sites, with highest levels at HI during sampling periods (**Table 1**).

### ***Flow and Conductivity***

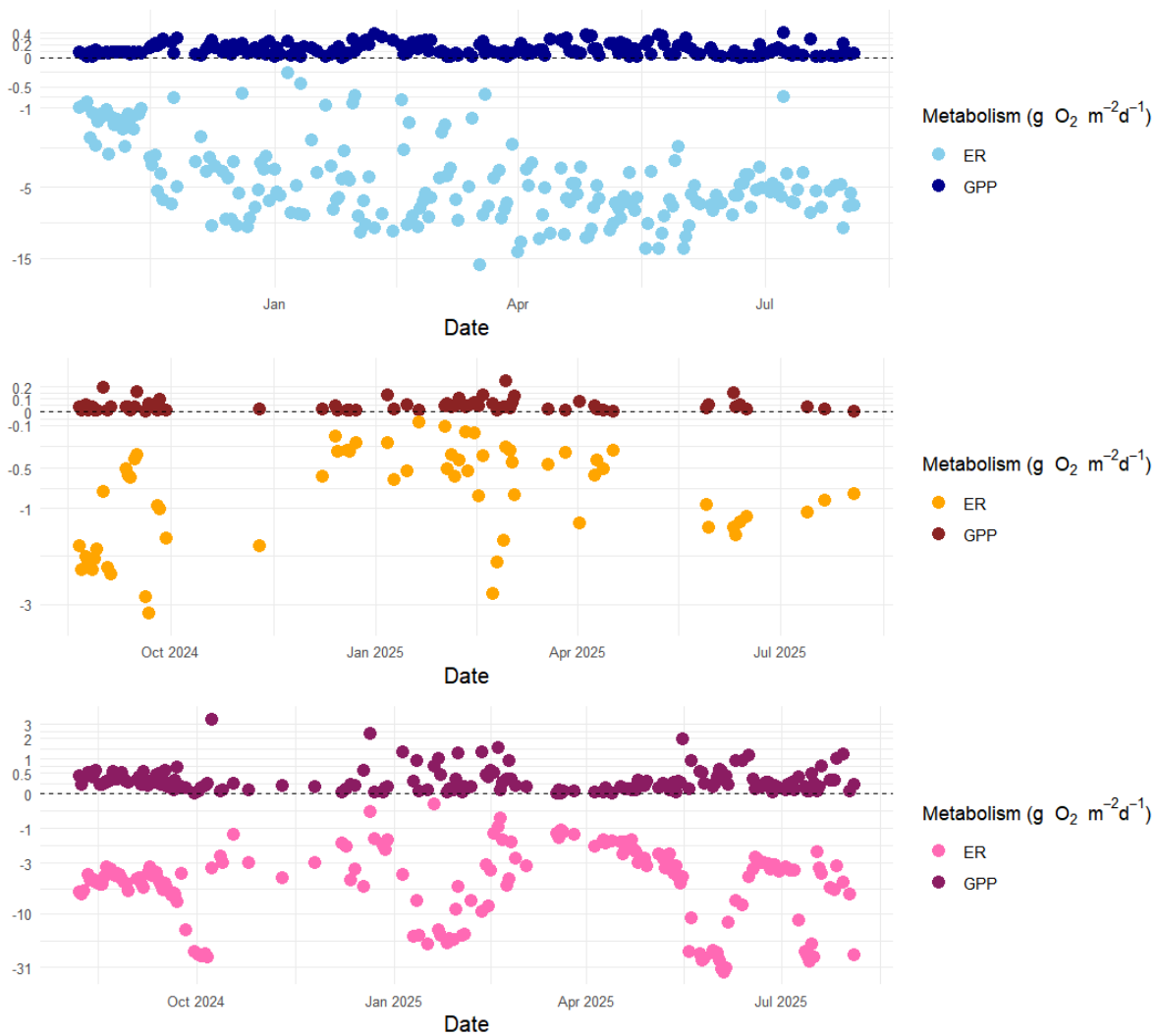
SpC decreased with increased flow in all three streams. Furthermore, SpC-flow relationships had significant breakpoints in all streams, with flow thresholds for REF at  $0.5 \text{ m}^3/\text{s}$  ( $p < 0.001$ ), MID at  $0.03 \text{ m}^3/\text{s}$  ( $p < 0.001$ ), and HI at  $0.12 \text{ m}^3/\text{s}$  ( $p < 0.001$ ) (**Fig. 2.3**). Following model-selected breakpoints, SpC decreased more rapidly with increasing flow in all streams.



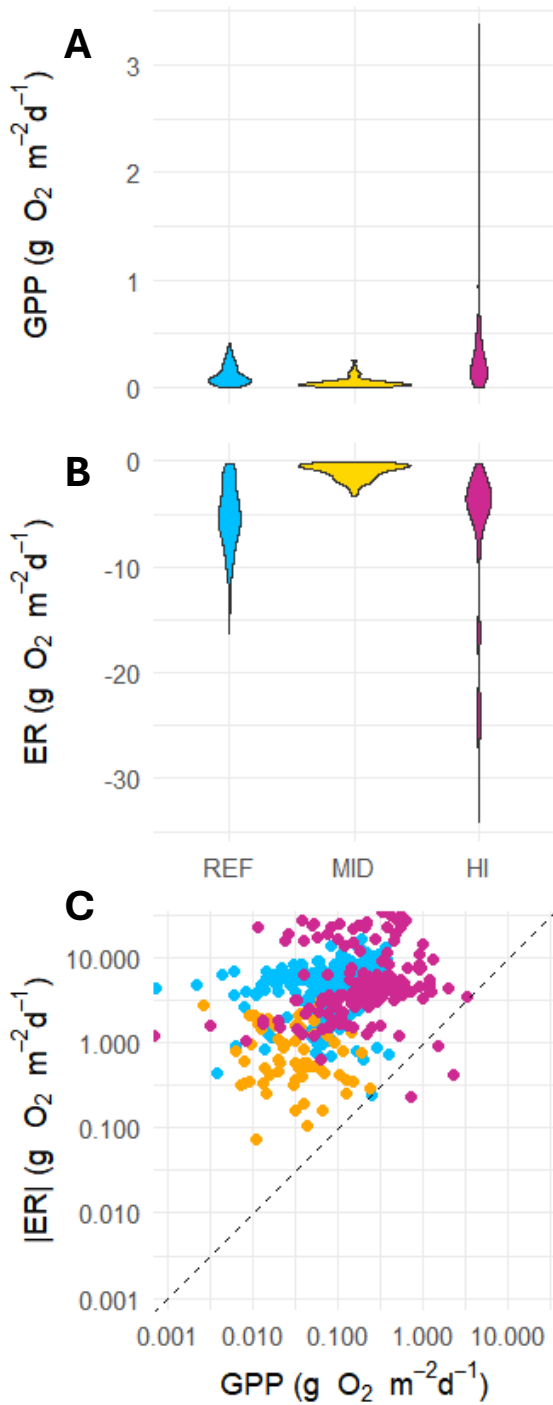
**Figure 2.3** Linear regressions and breakpoints for daily median specific conductivity versus estimated discharge for REF (top, blue), MID (middle, yellow), and HI (bottom, pink) from October 2024 - August 2025. Breakpoints are denoted as dashed red vertical lines, linear relationships are denoted by solid lines. Axis ranges for both specific conductivity and discharge vary across sites.

*Ecosystem Metabolism across Sites*

All sites were consistently heterotrophic ( $|ER| > GPP$ ), regardless of their SpC and discharge ranges (**Figs. 2.4, 2.5**). Across sites, the majority of GPP values were within 0-0.3 g O<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup> and  $|ER|$  values were within 0 to -10 g O<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup>. HI had the largest ranges in metabolism values, with GPP estimates of 0.01 to 3.5 g O<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup> and ER ranging from -0.4 to -32.0 g O<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup>. MID had the lowest metabolism ranges and values, with GPP of 0.003 to 0.2 g O<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup> and ER ranging from -0.07 to -3.3 g O<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup>. Metabolism ranges at MID are constrained by fewer data points for this site following model quality checks. REF had intermediate ranges of both GPP and ER. Autotrophic days ( $GPP > |ER|$ ) were uncommon, occurring on only three days across all sites (1 at REF and 2 at HI).



**Figure 2.4:** Estimates of gross primary production (GPP; rates  $>0$ ) and ecosystem respiration (ER; rates  $<0$ ) in three Appalachian forested streams with varying specific conductivity: REF (top, blue), MID (middle, yellow), and HI (bottom, pink) from October 2024 - August 2025. Axes are log-transformed but axis labels display real, non-log-transformed values.

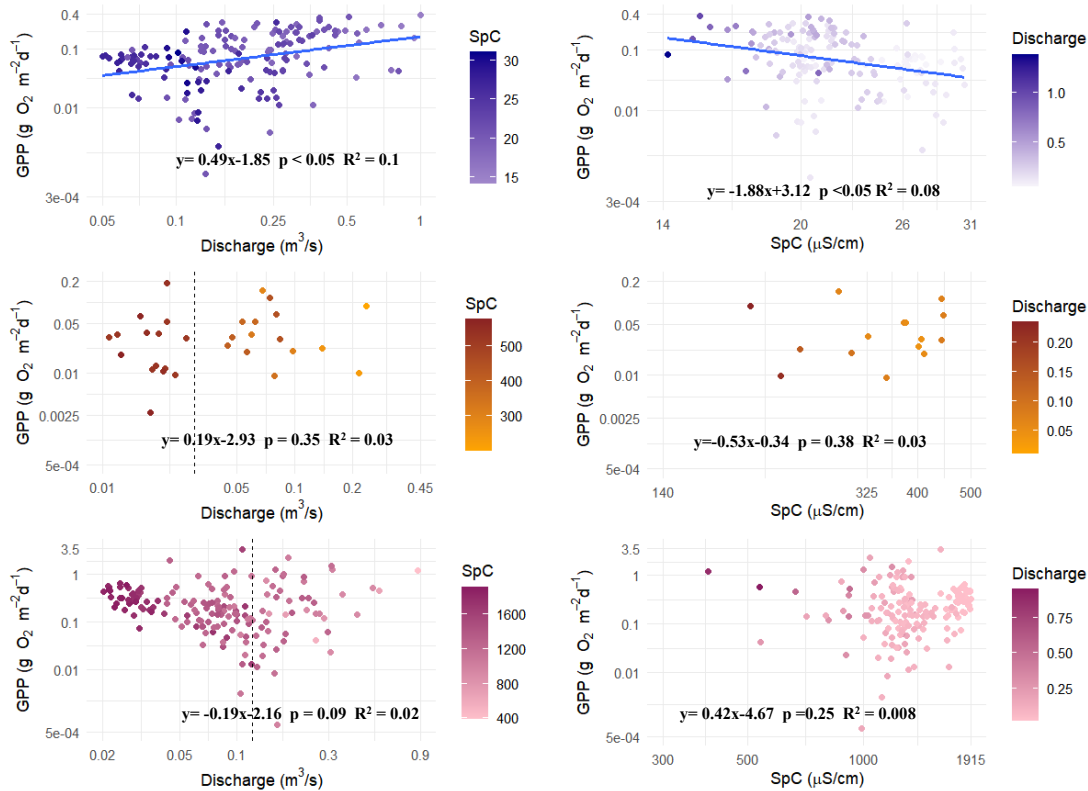


**Figure 2.5:** A -- The range and distribution of gross primary production (GPP) for HI (pink), MID (yellow), and REF (blue). B -- The range and distribution of ecosystem respiration (ER) for

HI (pink), MID (yellow), and REF (blue). C --  $|ER|$  vs. GPP at all sites (blue: REF, yellow: MID, pink: HI). Data are log-transformed, axis values reflect true values. Days above the 1:1 line are heterotrophic ( $GPP < |ER|$ ,  $NEP < 0$ ). Days below the 1:1 line are autotrophic ( $GPP > |ER|$ ,  $NEP > 0$ ).

### ***The Role of Flow and Specific Conductivity on Metabolism***

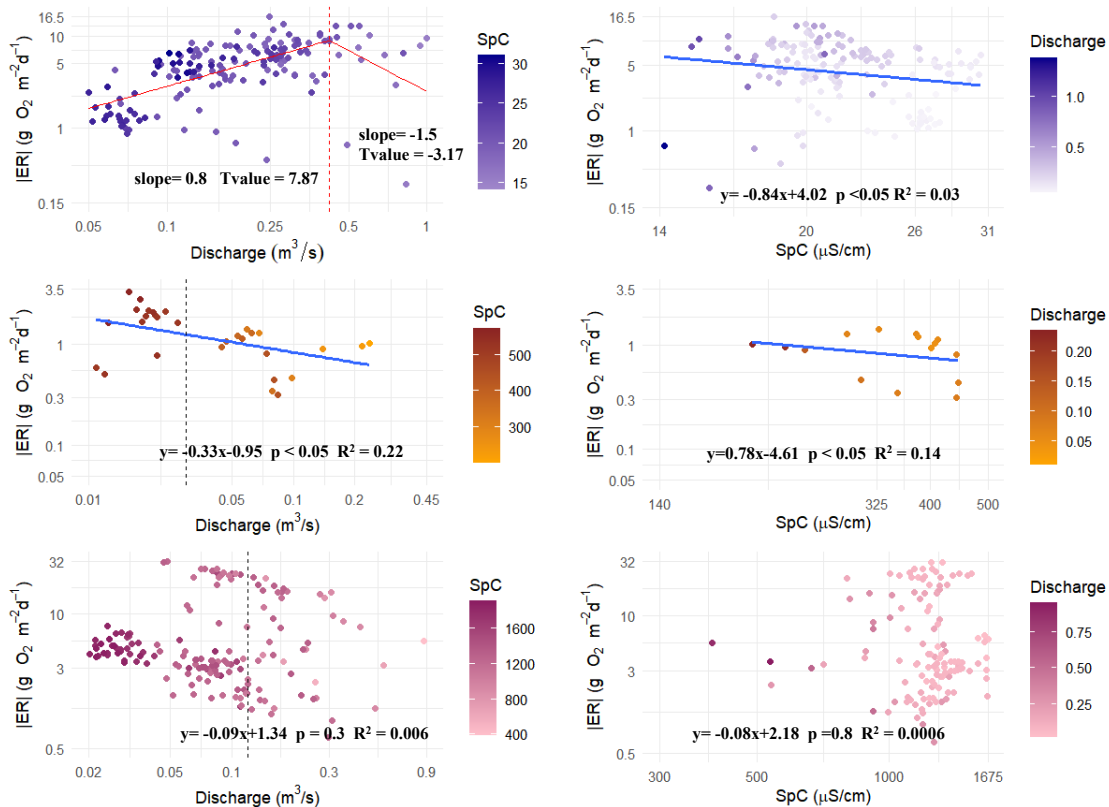
To better characterize the role of flow and SpC on GPP, we assessed site-specific relationships between log-transformed GPP and median daily discharge and SpC (**Fig. 2.6**). GPP had a positive relationship with discharge at REF and no relationship with discharge at MID and HI, with no significant breakpoints across sites. GPP had a negative relationship with SpC in REF, while MID and HI had no significant relationship between GPP and SpC. No distinct patterns emerged between flow and GPP before/after previously calculated SpC-flow breakpoints at MID and HI.



**Figure 2.6:** Log-transformed gross primary production (GPP) plotted against logged discharge (color-ramped by specific conductivity (SpC); left) and logged SpC (color-ramped by discharge; right) for REF (top, blue), MID (middle, yellow), and HI (bottom, pink) from October 2024 - August 2025. Linear relationships are plotted as solid lines if significant. Displayed equations for linear relationships are calculated using log-transformed values, but axes display actual values. Black vertical dashed lines at MID and HI flow graphs represent significant breakpoints shown in **Fig. 2.3**. Axis ranges differ for GPP and associated stressors across sites.

We also assessed how ER responded to flow and SpC variation (**Fig. 2.7**). REF had a significant discharge breakpoint, where  $|ER|$  increased with discharge until 0.42 m<sup>3</sup>/s ( $p < 0.001$ ), at which point the relationship between  $|ER|$  and discharge became negative. There was a

negative relationship between  $|ER|$  and discharge at MID. HI had no significant relationship between  $|ER|$  and discharge. We found no significant breakpoints for  $|ER|$  and flow relationships at MID and HI. There was a negative relationship between  $|ER|$  and SpC at REF, a positive relationship at MID, and no significant relationship at HI. Despite this lack of relationship between  $|ER|$  and SpC, HI displayed large variation in  $|ER|$  (between  $-0.05$  g and  $-32.0$  g  $O_2$   $m^{-2}$   $d^{-1}$ ) until around 1500  $\mu S/cm$ , after which variability decreased (between  $-3.7$  and  $-5.5$  g  $O_2$   $m^{-2}$   $d^{-1}$ ). No distinct patterns emerged between ER and flow before/after previously calculated SpC-flow breakpoints at MID and HI.



**Figure 2.7:** Log-transformed gross primary production (ER) plotted against logged discharge (color-ramped by specific conductivity (SpC); left) and logged SpC (color-ramped by discharge;

right) for REF (top, blue), MID (middle, yellow), and HI (bottom, pink) from October 2024 - August 2025. Linear models are plotted as solid lines when significant ( $p < 0.05$ ) Significant breakpoints are denoted with red, dashed, vertical lines. Displayed equations for linear relationships are calculated using log-transformed values, axes display actual values. Black vertical dashed lines at MID and HI flow graphs represent the previously calculated breakpoints shown in **Fig. 2.3**. Axis ranges differ for GPP and associated stressors across sites.

### **Discussion:**

In this study, we monitored headwater streams across a mining-induced conductivity gradient to test the impact of flow and SpC on headwater stream metabolism. Across our three study sites, GPP exhibited inconsistent relationships with both flow and SpC. Similarly, we found no consistent effect of either flow or SpC on ER across our study sites while SpC always declined with increasing discharge. However, differences in metabolism-flow patterns between reference and salinized sites suggest an indirect effect of salinization on ecosystem processes. Despite inconsistent results, our study provides a unique approach to assess the role of covarying and potentially interacting environmental drivers for future studies in other salinized ecosystems and across larger SpC ranges. With expected salinization and increased hydrologic variability, such continued work is needed to evaluate how these environmental drivers interact to influence ecosystem processes.

### ***Flow and Conductivity***

SpC decreased with increasing flows at all of our sites, regardless of SpC or flow ranges. Further, all sites had discharge thresholds after which SpC exhibited larger declines (**Fig. 2.3**). The degree of dilution prior to breakpoint flow values differed across sites ranging from near chemostatic (slope near zero) at MID and HI to consistent dilution (slopes around  $-0.2$   $(\mu\text{S}/\text{cm})(\text{m}^3/\text{s})^{-1}$ ) at REF, suggesting higher flows are needed for dilution to occur in salinized sites. Slopes beyond flow thresholds were notably steeper at all sites (all around  $-0.5$   $(\mu\text{S}/\text{cm})(\text{m}^3/\text{s})^{-1}$ ). The consistent dilution patterns across sites reaffirm that SpC concentrations in these sites are a result of the long-term upstream weathering of ions from mountaintop removal/valley-fills instead of solutes from terrestrial input/runoff (Fritz 2011, Diamond & Cohen 2018). Dilution of solute concentration with increased flow is characteristic of geogenic sources, while enrichment with increased flow is characteristic of sites that receive high levels of solutes from terrestrial runoff (Johnson & Likens 1969, Clow & Drever 1996, Basu et al. 2011). As such, how the interaction between SpC and flow mediates salinity effects on stream processes is likely different in our streams compared to other salinized ecosystems that receive runoff-derived salts (e.g., via road salts, fertilizers).

### ***Ecosystem Metabolism across Sites***

All stream reaches were heterotrophic for the majority of the study period, which we expect in light-limited, headwater forested streams (**Fig. 2.4**). Increases in GPP, seen in the few days of autotrophy in REF and HI, are common in deciduous forest headwater streams during the spring due to increased light before canopy cover closes after leafout (Loreau et al. 2003, Roberts et al. 2007, Segatto et al. 2023). Despite autotrophic days, NEP was the most negative at our high-SpC site, suggesting increased heterotrophy with higher SpC (**Fig. A17**).

While metabolism values varied among our sites, there was no clear pattern across the site-SpC gradient. Our high-salinity site had the largest range in metabolism values, whereas the MID site had the lowest with REF exhibiting intermediate values (**Fig. 2.5**). HI was the only site with GPP values above  $1 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$  (**Fig. 2.4**). This higher production may be a result of lower canopy cover at HI due to Hemlock die-off and thus increased light, stimulating aquatic primary production. However, maximum water temperatures were similar among sites, which suggests light intensity was not exceptionally different among sites. High GPP with higher SpC may also indicate salts are serving as micronutrients for autotrophs, stimulating overall production levels (Duan and Kaushal 2015, Cañedo-Argüelles et al. 2020). Further, in addition to serving as nutrients themselves, excess salts may mobilize nitrogen from sediments to stream water (e.g. Groffman et al. 2005, Kaushal et al. 2018, Haq et al. 2018). HI notably had the highest total nitrogen and nitrate concentrations in this study, which may be the result of salt-associated nutrient mobilization (**Table 1**). Yet, we note again that our MID site had the lowest GPP values but intermediate nitrogen levels when compared to REF and HI, limiting inferences regarding salinity and nutrient subsidies in our systems. While low GPP values at MID complicate what we can infer from high production at our high-SpC site, MID had poor metabolism model performance in comparison to the rest of our study sites and fewer GPP estimates certainly limiting our understanding of SpC-production dynamics at this site. In addition to having the highest GPP values, HI also had the largest ER values and was the most heterotrophic stream in this study, whereas, similar to GPP, MID exhibited the lowest ER values. The increase in variability seen at HI, characterized by its large range in GPP and ER values, supports previous studies that have documented increases in temporal variability in metabolism with land-use change (Clapcot et al. 2016). While headwater streams are typically heterotrophic and rely, in

part, on allochthonous inputs, a large portion of ER relies on in-stream GPP, meaning the low documented GPP at MID did, in part, lead to its constrained ER (Battin et al. 2023). We also observed dry segments at MID during summer months, which can impact whole-stream production (Sabater et al. 2016) and skew quality-checked annual metabolism ranges. As such, assessments of salinity effects across sites, particularly in only three streams, were potentially confounded by site differences in canopy cover, nutrients, and other environmental drivers in spite of our attempts to keep non-SpC conditions as similar as possible among our three selected sites.

### ***The Role of Flow and Specific Conductivity on Metabolism***

There were inconsistent relationships between GPP and flow across our study sites. GPP was positively, albeit weakly, related to discharge at our reference site, suggesting that flow may be acting as a subsidy to autotrophs in this stream. REF had low nutrient concentrations, and higher flows can potentially increase nutrient inputs and stimulate production (Wurtsbaugh et al. 2019). As such, the metabolism patterns at REF likely represent a typical relationship between GPP and flow for nutrient-poor, low-SpC headwater streams in our study region. In contrast, we saw no relationship between GPP and flow at impacted sites (**Fig. 2.6**). Subsidy effects of flow can decline with increasing discharge as a result of autotrophic communities being scoured and transported downstream by increasing water velocity (Young & Huryn 1996, Uehlinger 2006). Additionally, increased flows can raise turbidity, preventing light from reaching primary producers, maintaining low production levels often seen in headwaters (Roberts & Mulholland 2007, Blaszcak et al. 2018). We note, however, that flow ranges were similar at REF and HI,

complicating interpretation of competing flow effects (nutrient inputs vs. scour and turbidity) on GPP between these two streams.

GPP also had no consistent relationship with SpC across study sites, with only the reference site exhibiting a significant (negative) relationship. While osmotic stress may be causing this decline in GPP, SpC at REF was very low and at levels unlikely to affect GPP (e.g., Allakhverdiev et al. 2000, Krenz et al. 2018, Martínez et al. 2020). Rather, it is likely this negative relationship reflects the positive relationship between GPP and flow at this site, given the strong negative covariance between SpC and flow. MID and HI had elevated SpC concentrations, and we expected to see metabolic shifts from changes in SpC. Yet, we found no significant relationship between GPP and SpC at these mining-impacted sites (**Fig. 2.6**). While we saw no clear relationships between GPP and SpC, elevated GPP levels at our high-SpC site may suggest potential SpC influence, yet we again note the more open canopy at this site.

Separating the role of flow versus SpC in influencing GPP is difficult given their covariance. We used breakpoint values in SpC-flow relationships to help isolate a potential threshold at which stressor dominance changes. That is, at flows above breakpoints, SpC is substantially diluted and flow is likely the dominant driver. Conversely, at flows lower than breakpoints, SpC remains elevated and is likely the dominant potential stressor. With this approach, we expected GPP-flow relationships following breakpoints (0.03 m<sup>3</sup>/s at MID and 0.12 m<sup>3</sup>/s at HI, **Fig. 2.3**) to be increasingly negative and steep due to scour and increased turbidity (Uehlinger & Naegeli 1998, Roberts & Mulholland 2007, Blaszcak et al. 2018). While these expected patterns did not emerge, differences in GPP-flow relationships between our reference and impacted sites suggest some salinity effect. Since flow is assumed to be the dominant driver of GPP at our reference site, SpC may be indirectly interacting with flow in

mining-impacted sites to shift its role in GPP from reference conditions. As flow variability increases in the region with climate change the interactive nature of these two drivers may make metabolic responses difficult to pin down (Gaetner et al. 2020).

Similar to GPP, ER and flow did not have a consistent relationship among our sites. Consistent with our expectation, discharge behaved as both a subsidy and a stressor for ER at our reference site, where  $|ER|$  increased with flows until  $0.42 \text{ m}^3/\text{s}$ , whereafter it declined. Lower levels of precipitation, characteristic of low to intermediate flows, can wash terrestrial organic matter into streams and subsidize heterotrophic respiration. This enrichment can continue until flow levels reach a threshold and begin to induce mechanical stress on heterotrophic populations (e.g. Uehlinger & Naegeli 1998, Roley et al. 2014, Demars 2018). Since REF had lower and constrained SpC, we expect the subsidy-stress response of ER to flow variation is representative of low-SpC waters in the region. In contrast, ER had a significant negative relationship with flow at our mid-salinity site, indicating that flow was acting as a stressor in this site. If heterotrophic communities are constrained or sensitive in this stream, any form of physical stress may reduce ER. We did not see a clear relationship between ER and discharge at HI, suggesting ER may be less influenced by flow changes in high-SpC sites (**Fig. 2.7**).

We also saw no clear relationship between ER and SpC across sites. There was a negative relationship between ER and SpC at REF, yet similar to the GPP-SpC relationship at this site, this is likely not due to changes in SpC given REF's narrow range of SpC ( $0.2 - 57.69 \text{ }\mu\text{S}/\text{cm}$ ) (**Table 1**). SpC and ER had a significant, but slight, positive relationship at our mid-SpC site (**Fig. 2.7**), suggesting increased SpC may be subsidizing heterotroph populations. Salts can alter the reactivity of dissolved organic matter, making it more readily available for heterotroph consumption (e.g. Amon & Benner 1996, Yamashita et al. 2008, DeVilbiss et al. 2024). Our

high-salinity site had the largest  $|\text{ER}|$  values at over  $30 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ , doubling maximum  $|\text{ER}|$  in our reference stream, but with no significant relationship with SpC (**Fig. 2.7**). High ER rates at our highest SpC site along with the significant, positive relationship between  $|\text{ER}|$  and SpC at our mid-SpC site suggests that increased ions may be acting as a subsidy for ER.

Similarly to GPP, we can potentially use SpC-flow relationships to help separate the role of flow versus SpC on ER. If SpC is acting as subsidy to ER, as suggested above, we would expect ER values to remain high at flows below breakpoint values, above which increased flow (and lower SpC values) would scour and stress heterotrophic populations (Błaszczak et al. 2018). While such patterns were not observed, ER at our reference site displayed the expected subsidy-stress response to flow but our mining-impacted sites did not. As flow is believed to be the dominant environmental driver at REF, this may suggest that SpC interacts with flow and exhibits indirect effects on ER.

### ***Whole Ecosystem Implications***

While metabolism patterns exhibited inconsistent responses to SpC, extensive work in our study systems and many others document clear salinity effects to aquatic organisms. Ecosystem metabolism is a measure of total carbon fixation and breakdown in a stream's reach; it cannot measure how specific populations and their metabolism may change. Population declines have been documented in heterotrophic communities, notably aquatic macroinvertebrates belonging to scraper feeding groups, in streams impacted by mining-induced salinization (Pond et al. 2008, Entekin et al. 2018). At the same time, salt-tolerant populations have seen growth under salinized conditions (Shu et al. 2023). This variation in species response to salinization can influence whole-ecosystem metabolism measurements, making population changes difficult to

isolate. Previous studies addressing the role of mining-induced salinization at our study sites found changes in both macroinvertebrate (Pond et al. 2008) and microbial community structure with increased SpC, but not in leaf decomposition rates (Vander Vorste et al. 2019), indicating that heterotroph community structure is changing but not reflected in measured ecosystem processes. Further, given that the majority of stream ER is from microbial respiration, resistance, redundancy and/or replacement in microbial populations may limit the detection of salinity-induced changes in respiration at the whole-reach scale (Kefford et al. 2016, Voss & Bernhardt 2017), overshadowing changes in macroinvertebrate or vertebrate respiration levels (Shu et al. 2023, DeVilbiss et al. 2024). We encourage the inclusion of community metrics in conjunction with ecosystem metabolism to address these conflicting patterns. Ecosystem processes such as metabolism are rarely measured concomitantly with community metrics, limiting opportunities to learn from integrated measurements at the community and ecosystem scale. Understanding trophic dynamics in conjunction with whole-ecosystem carbon fixation and breakdown can give us a more nuanced view into stream health that these metrics, when isolated, cannot (Marcarelli 2011, Rüegg 2021).

While ecosystem metabolism may not capture species-specific changes in ecosystems, assessing reach-scale processes in headwater streams is essential for understanding the state of waterways and their associated watersheds (Hope et al. 1994, Wetzel 2021). Headwater streams play a major role in regulating ecosystem processes throughout river networks; the terrestrial inputs they receive shape the nutrient makeup of the stream network (Bormann et al. 1969, French et al. 2020). Our study sites, regardless of SpC levels, remained heterotrophic with minimal accounts of autotrophy throughout the year, which we anticipate seeing in nutrient-limited headwater streams. While our high-SpC site saw the highest accounts of daily GPP (3.4 g

$\text{O}_2 \text{ m}^{-2} \text{ d}^{-1}$ , a near 3x increase from reference production), we also observed an increase in daily ER, resulting in the lowest NEP values ( $-34.0 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$  at HI versus  $-16.2 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$  at REF). More negative NEP rates at HI indicate sustained and potentially increased heterotrophy with SpC, implying that little excess fixed energy will be transported downstream and reliance on terrestrial organic carbon subsidies will continue (Ruegg et al. 2021, Battin et al. 2023). Increases in ER in relation to GPP with SpC will result in the increased consumption of allochthonous material that would otherwise be transported downstream, potentially altering trophic dynamics (Vannote et al. 1980, Creed et al. 2018). In addition to downstream resource quality, this sustained heterotrophy has implications for gas emissions. We saw few accounts of autotrophy across our SpC gradient, indicating that streams will continue to serve as net  $\text{CO}_2$  sources with increasing SpC (Battin et al. 2023). Results in this study point towards sustained or increased heterotrophy in salinized freshwater systems, and in turn increased  $\text{CO}_2$  emissions from impacted streams. These results support previous studies that have documented increased  $\text{CO}_2$  emissions from urban streams associated with increased pollutant runoff containing high nutrient and salt loads (Xu et al 2024, Zhao et al. 2026).

### ***Conclusions and Future Work***

Characterizing the consequences of freshwater salinization for ecosystem functions is critical given increasing salt concentrations in waterways from urbanization, resource extraction, and other anthropogenic influences. We found no clear relationship between mining-induced SpC increases and ecosystem metabolism, though deviation in flow-metabolism patterns in mining-impacted sites from reference conditions suggest indirect SpC impacts on ecosystem processes. As hydrologic variability and salinization increase in the region (Gaetner et al. 2020)

it will be increasingly to see clear metabolic responses to either environmental driver due to their interactive natures. While our findings were inconclusive, this study reinforces the need for continued research on freshwater salinization and potentially interacting drivers like flow. Continued work addressing how metabolism varies in response to salt level and type in variable flow regimes will assist us in understanding the impact freshwater salinization has on changing aquatic ecosystems.

This study saw no clear changes in ecosystem metabolism across a mining-induced SpC gradient. This lack of relationship between metabolism and SpC is partially a result of our small sample size of three headwater streams. Our mid-SpC site had the lowest number of quality-checked metabolism estimates in comparison to our reference and high-SpC site, further complicating what we can assess across our SpC gradient. Additionally, we may not see strong relationships between SpC and metabolism due to the range of conductivity we were able to capture. While a mean of 1500  $\mu\text{S}/\text{cm}$  at our high salinity site is large in comparison to non-mined streams in the Appalachian Coalfield, these sites remain far from forms of pulse salinization documented to impair biological activity in urban streams, which have been estimated to reach up to 14,000  $\mu\text{S}/\text{cm}$  following road salt application (Berger 2018, Haq et al. 2018, Kaushal et al. 2018). It's possible that high SpC concentrations, beyond the range of conductivity in our study [ $>2000 \mu\text{S}/\text{cm}$ ], may affect production and respiration rates. Further, our flow measurements were limited to 70-80% of the recorded water level potentially leading to inaccuracies to flow estimation and thus metabolism-flow relationships during high flow events. We encourage studies addressing the role of SpC and flow on metabolism to continue with these limitations in mind, expanding the number of sites and capturing larger SpC ranges in future work. Stream metabolism will likely shift with enough salt, but the threshold at which salinity

becomes a stressor to aggregate ecosystem processes may be higher than anticipated when compared to organismal responses (Kaushal et al. 2023a).

We encourage the use of concentration-discharge relationships to assess stressor interactions in future salinization work. For example, identifying flow breakpoints in concentration-discharge and process-discharge analyses may help isolate the role of interactive drivers on ecosystem processes, where metabolism-flow patterns may change with substantial shifts in SpC values. This framework thus couples process (e.g., metabolism)-discharge and concentration-discharge analyses and may be used to examine the role of other solute stressors to ecosystem processes (e.g., nitrogen cycling) in hydrologically dynamic waterways.

We chose to address the role of valley fills and resource extraction-induced salinization on ecosystem processes; however, other methods of salinization may differ in their effects on metabolism. Differences in timing, type, and severity of salinization (e.g., variable salt pulses in urban environments versus slower, long-term salinization from the weathering of valley fills) may be reflected in metabolic changes. Additionally, the ions associated with the salinization methods (e.g., sodium chloride ions for road salts and saltwater intrusion versus sulfate and magnesium ions for resource extraction) may have varying effects on metabolism depending on how recently the ecosystem has experienced salinization as well as the salt tolerance of organisms in the ecosystem (Dailey et al. 2014, Jeppesen et al. 2015, Kaushal et al. 2018). How do these different methods of salinization influence ecosystem metabolism? Is ecosystem metabolism more driven by conductivity levels, specific ions, or timing of salinization? How do pulse and press salinization disturbances differ in their consequences to ecosystem processes and freshwater biota? Expanding studies on salinization and metabolism to address the various forms of freshwater salinization will help answer these unresolved questions and provide a holistic

understanding of the influence of salts on ecosystem processes, which is critical for understanding how our global waterways are changing with increasing salinization.

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### **Chapter 3: Addressing the role of salinization on headwater stream health**

To better understand how freshwater salinization influences ecosystem processes, we documented the role of specific conductivity (SpC) on ecosystem metabolism in headwater streams impacted by mountaintop removal/valley-fill mining techniques. This study found no clear relationship between ecosystem metabolism and SpC in three Appalachian headwater streams spanning a range of SpC. Increased GPP and ER estimates at our high-SpC site may suggest a subsidy effect with increased SpC, but lower sample size, as well as decreased canopy cover and the potential for increased light attenuation at our high-SpC site, complicate these results. While this study showed no statistical relationship between SpC and ecosystem metabolism, it provided a unique assessment of and approach to addressing the role of multiple, dynamic drivers on ecosystem processes for future studies.

The ecosystem process measured for this study, ecosystem metabolism, is an integrated measure of all metabolic processes across multiple trophic levels (Marcarelli et al. 2011, Jankowski et al. 2021, Rüegg et al. 2021). We recognize that while metabolism aims to capture trophic dynamics through energy transfer, specific biotic changes in streams may not always be captured by our metabolism estimates. Likely, many populations are negatively impacted by raised SpC levels, as has been documented in organismal studies in the region, but the metabolism of resilient populations can overshadow declines in more sensitive taxa at the whole-ecosystem scale (Vander Vorste et al. 2019). Microbial communities conduct the bulk of respiration in freshwater systems as they make up a large portion of the organismal biomass in streams. Because communities are so diverse, they tend to exhibit resilience to stressors as a result of functional redundancy. If diverse microbial communities are resilient to changes in

SpC, whole-ecosystem respiration may not change (e.g., Shu et al. 2023, DeVilbiss et al. 2024). Similarly, if large autotrophic (algal or macrophyte) populations are resilient to environmental drivers, like SpC, GPP may not change. When looking at broad-ecosystem impacts, ecosystem metabolism is a useful tool as it captures the general condition of all populations in a stream. If we want to assess how individual populations are changing with salinization, ecosystem metabolism may fall short as an assessment tool when used in isolation from other measurements.

Future work will incorporate additional ecosystem metrics with our metabolism results to give us more insight into the role of salinization on stream ecosystem health. This study is a part of a larger, collaborative effort to assess the role of freshwater stressors on different aspects of stream health. In addition to the daily metabolism estimates and water chemistry measurements provided here, researchers are assessing a variety of community dynamics, including invertebrate counts, secondary production, algal biomass, and fine and coarse benthic organic matter measurements. Understanding changes in trophic dynamics in conjunction with whole-ecosystem metrics, like metabolism, can help us see the nuance often lost when looking at just one metric. Results from this study will be used with these additional community metrics, hopefully expanding what we can learn from typically isolated metabolism and trophic metrics.

Finding the threshold at which salt becomes a stressor to whole-ecosystem processes will be necessary to maintain freshwater health in waters subject to freshwater salinization. If ecosystem metabolism rates or variability dramatically shift with increases in SpC, we can assume freshwaters are being significantly altered by salts (Battin et al. 2023). While this study saw no direct change in metabolism with salinization, literature shows that high salt levels will eventually kill organisms adapted to freshwater environments (Pond 2008, Kaushal et al. 2023).

Certain organisms, likely microbial populations, in our headwaters are potentially resilient to the range of SpC measured in this study, following years of salinization, making changes to ecosystem health difficult to measure. In addition to the potential for salt-tolerant populations in our sites, it's possible that the threshold at which SpC significantly alters ecosystem processes is above our maximum measurements of  $\sim 1900 \mu\text{S}/\text{cm}$ . Assessing the role of increased salinization [ $>2000 \mu\text{S}/\text{cm}$ ] on streams subject to long-term salinization or focusing on the role of naive salinization may be more effective when looking to capture distinct changes in ecosystem processes. Further, ecosystem metabolism thresholds to salinity may differ depending on the method of salinization, region, stream size, and length of salt input (long-term vs. pulse salinization) (Dailey et al. 2014, Jeppesen et al. 2015, Kaushal et al. 2018). Isolating the SpC ranges and composition that will cause ecosystem stress, characterized by declines in ecosystem metabolism and other stream functions, across different ecosystems undergoing different methods of salinization will assist in aiding environmental monitoring. If we know when salt loads will significantly impact freshwater ecosystems, we can better monitor the impacts of high salt and ideally reduce salt inputs before significant damage occurs.

Though no clear relationships between mining-induced salinization and ecosystem metabolism were found in this study, GPP was the highest at our high-SpC site. We recognize that high levels of GPP may be a result of the decreased canopy from Hemlock die-off at this site, allowing for increased light penetration, but it may also be a result of increased nitrogen and phosphorus levels at this site. Our high-salinity site had the highest total nitrogen and nitrate/nitrite concentrations in this study (**Table 2**). These raised nutrient concentrations can stimulate algal growth and subsidize production in the system. Salinization has been documented to influence nutrient cycling in freshwater systems. High SpC concentrations can mobilize

nutrients from the streambed into the water column, potentially leading to the high nutrient concentrations seen in our high-SpC site (Groffman et al. 2005, Haq et al. 2018, Kaushal et al. 2018). Further, salts themselves can act as nutrients in low concentrations (Clay et al. 2023). While we may anticipate high salt levels to be a stressor to stream production, the complex role of salts on organisms complicates this. It will be important to monitor metabolism in high salinity streams to further aid in understanding the role of salinization on nitrogen release, and in turn, primary production to advance our understanding of salt subsidy behavior (Clay et al. 2023).

Changes in salt levels and associated nutrient concentrations will have implications for autotroph population growth and, in turn, oxygen and organic carbon cycling in waterways. Nutrient increases from raised SpC levels, combined with nutrient loading from runoff in urban and agricultural regions, can lead to water quality decline and eutrophication (Wurtsbaugh 2019). It will be essential to directly assess how salts influence nutrient cycling as we expand research on freshwater salinization. As mentioned earlier, ecosystem metabolism may not be able to capture all of the changes in these specific biogeochemical pathways. Shifts in biogeochemical cycling will have implications for freshwater health; as a result, more studies addressing the role of increased conductivity on nutrient cycling should be conducted in freshwater streams. Studies on nitrogen cycling and salinization have been conducted in coastal lakes and urban waters, but these ecosystem processes are frequently overlooked in forested headwater streams (e.g., Kinsman-Costello et al. 2023, Sun et al. 2023, Selak et al. 2025). In addition to assessing the role of salinization on nutrient cycling, addressing its influence on carbon spiraling and other forms of carbon transport will further advance our understanding of the role of salinization on production, resource quality, and carbon fate (Newbold et al. 1982). The sustained heterotrophy

( $ER > GPP$ ) we observed in this study helps us understand how salinization influences carbon fate, but studies directly looking at downstream carbon transport and metabolism will be critical in advancing this understanding.

The Appalachian headwater streams studied in this project are subject to salinization from mine drainage and stream burial by valley fills, a persistent stressor in the region (Skousen and Zipper 2021). The restoration of headwater streams impacted by valley fills is complicated; fill material cannot easily be removed from mountain valleys and will continue to weather material into waterways over time (Skousen and Zipper 2021). Projected timelines for recovery are upwards of 25 years for stream reaches with associated valley-fills (Ciancolo et al. 2020). As a result of this slow recovery, the biotic effects of salinization in the Appalachian Coalfield are likely to persist for decades to come. Ecosystem metabolism should continue to be monitored at sites impacted by valley fills with these timelines in mind. Long-term salinization may have compounding effects on aquatic organisms and processes that can shift ecosystem metabolism in the future. Understanding a “baseline” for metabolism in Appalachian streams will also assist in identifying these shifts, which can be an indicator of ecosystem health decline. We hope the metabolism values presented in this study can be used as a baseline for future work on ecosystem processes in the Appalachian Coalfield.

Increased urbanization, resource extraction, and hydrologic manipulation are leading to the steady salinization of freshwater globally. Given the rate of anthropogenic change and extended recovery time predicted for salinized streams, it is likely that salinization will be a prevalent stressor to freshwater systems for years to come (Olson 2018, Fanelli et al. 2024). It will be important to document how different forms of anthropogenic salinization are influencing ecosystem processes. Ion concentration in salinized waterways differs depending on the method

of salinization. Our streams had high concentrations of sulfate, magnesium, and calcium ions, reflecting the geologic material in the valley fills associated with impacted streams (Timpano et al. 2015). Yet, freshwater salinization is not characterized by these ions alone (e.g., Kaushal et al. 2018, Cañedo-Argüelles et al. 2017). Differences in stream bed material, groundwater input, and levels of surrounding urbanization lead to variation in dissolved ion concentrations in waterways. Salinization from the runoff of road salts from impervious surfaces, characteristic of urban environments, or from saltwater intrusion in coastal environments will have higher concentrations of  $\text{Na}^+$  and  $\text{Cl}^-$  compared to streams impacted by valley fills (Dailey et al. 2014, Jeppesen et al. 2015, Dugan et al. 2017). It will be necessary to highlight differences in ion concentrations across methods of salinization to gain a broader understanding of the impacts of freshwater salinization. In addition to variation in SpC levels, differences in ion concentrations may have variable effects on aquatic ecosystems. Ecosystem metabolism should be measured in waterways subject to varying methods of salinization. We encourage a synthesis of the ecosystem effects of different methods of salinization. This can help us understand which forms of salinization most dramatically alter ecosystem health. If specific salts/methods of salinization are more damaging to aquatic ecosystems, we can use this information to help guide mitigation and restoration efforts.

Understanding how salinization and flow influence ecosystem processes will become increasingly relevant as hydrologic variability increases with climate change. Increased temperatures globally are exacerbating drought conditions, leading to the drying of waterways globally (Mukherjee et al. 2018). Desiccation is a method of salinization in itself; drying will lead to increased SpC concentration in waterways, regardless of background levels (Jones and van Villet 2018). Regions predicted to experience increased drought conditions will be subject to

increases in concentrations of total dissolved solids and, in turn, salinity (Perera et al. 2024). This, compounded with secondary salinization (runoff, wastewater, etc.), can lead to dramatic increases in SpC, expediting freshwater salinization in ecosystems. In addition to increased temperatures, flashier hydrology is becoming common with climate change (Xiong and Yang 2024). Long periods of drought followed by large precipitation events, characteristic of flashy hydrology, lead to flood conditions in headwater streams. Large flow events lead to the temporary dilution of salts in Appalachian streams, potentially relieving osmotic stress, but will, in turn, act as a major mechanical stressor to the ecosystem (Uehlinger 2006, Blaszcak et al. 2018). We aimed to understand how these variable drivers interacted and documented that metabolism generally displayed a subsidy-stress response to increases in flow for dilute headwaters but strayed from reference in our salinized streams (Chapter 2, **Fig. 2.6, 2.7**) (O'Donnell and Hotchkiss 2019). Indeed, high conductivity ranges may influence expected flow-metabolism relationships, thus continued work on both drivers should be conducted to best assess the role of the covarying environmental drivers. Understanding how flow variability and increasing salinization interact to drive ecosystem processes will be essential as these conditions are predicted to become more common with climate change.

This study emphasizes the importance of continued work on connecting freshwater salinization and ecosystem metabolism. We did not observe a clear effect of increased SpC on metabolism, but literature supports its role as a stressor to freshwater biota. We encourage future studies on freshwater salinization to cover broader ranges of SpC and account for differences in ionic makeup across methods of salinization. The Appalachian Coalfield is likely to see continued high SpC levels over time, suggesting salinization will be a persistent stressor in the region for decades to come, shifting ecosystem health/structure as a result (Ciancolo et al 2020).

We hope this study can be used as a baseline to document changes in ecosystem metabolism with prolonged salinization. Ecosystem health will shift as salinity increases over time; understanding how and when this environmental driver impacts ecosystem processes should remain a priority for freshwater research.

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## Appendix

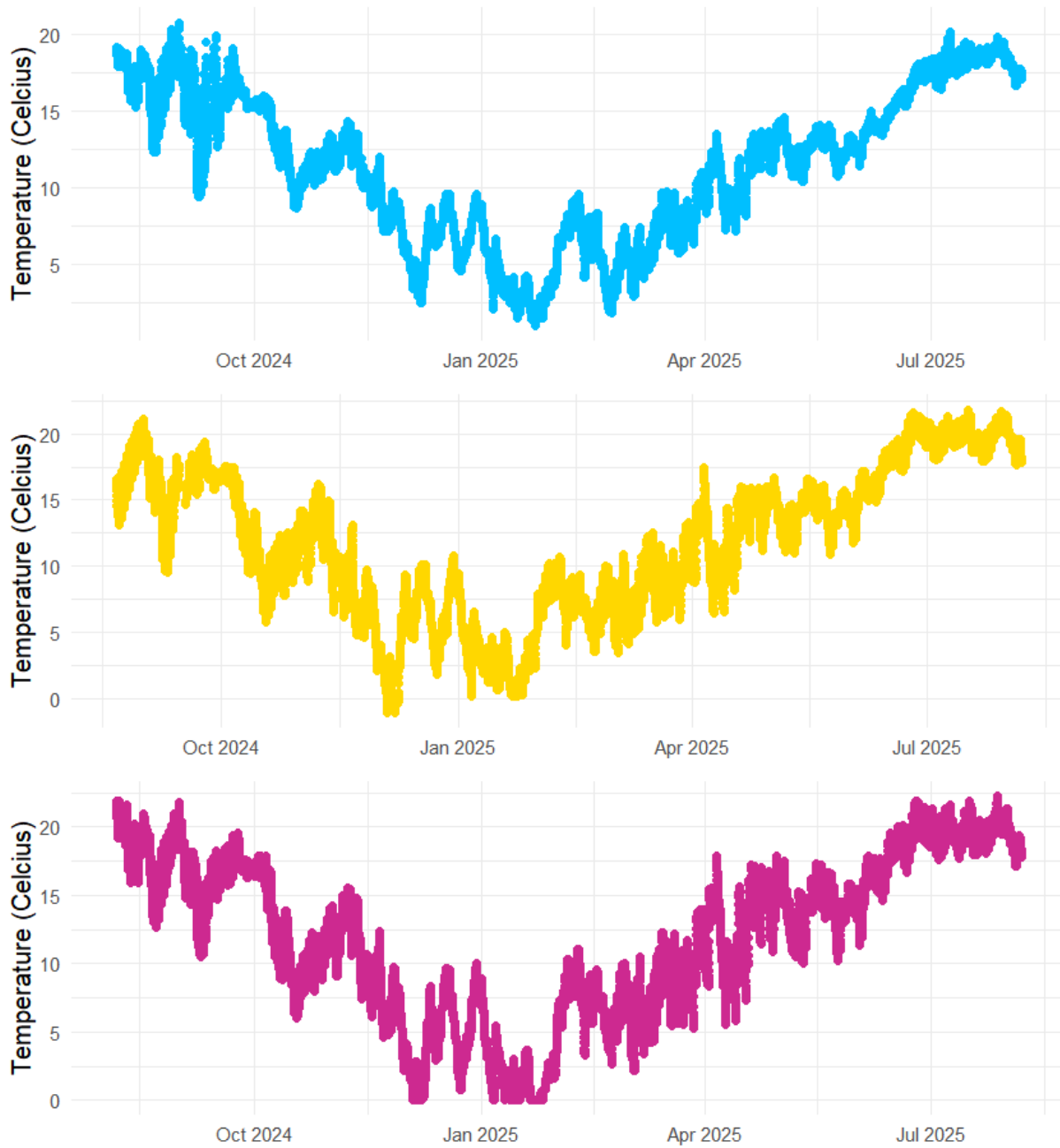


Figure A1: Temperature (°C) from August 2024 - August 2025 at REF (top, blue), MID (middle, gold), and HI (bottom, maroon).

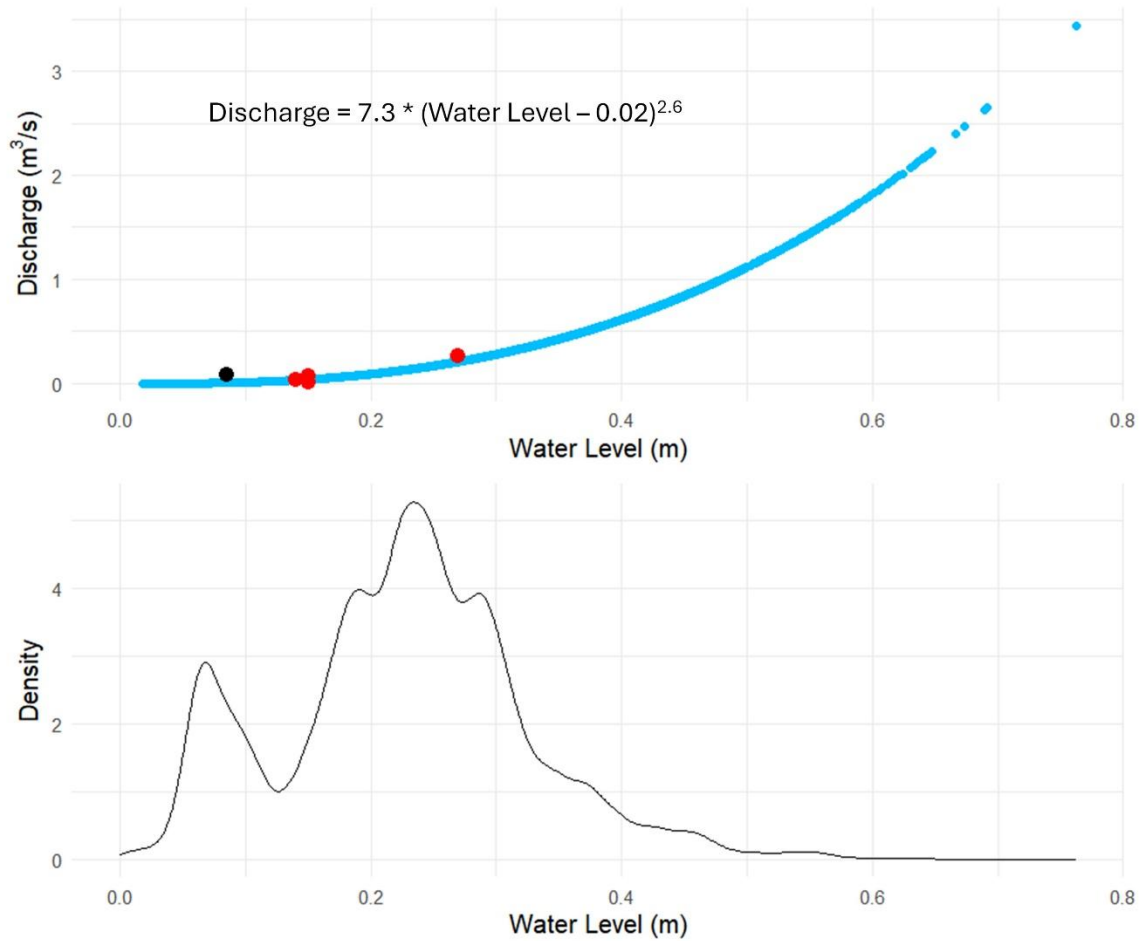


Figure A2: Top: Discharge (m<sup>3</sup>/s) vs. water level (m) at REF from August 2024 to August 2025. Red dots represent one-time solute tracer discharge values and associated recorded discharge used to create the site discharge-stage rating curve; the black dot represents a one-time solute tracer discharge value and manually measured water level from June 2024 due to water level sensor failure on this date. Bottom: A density plot of water level measurements at REF from August 2024 to August 2025.

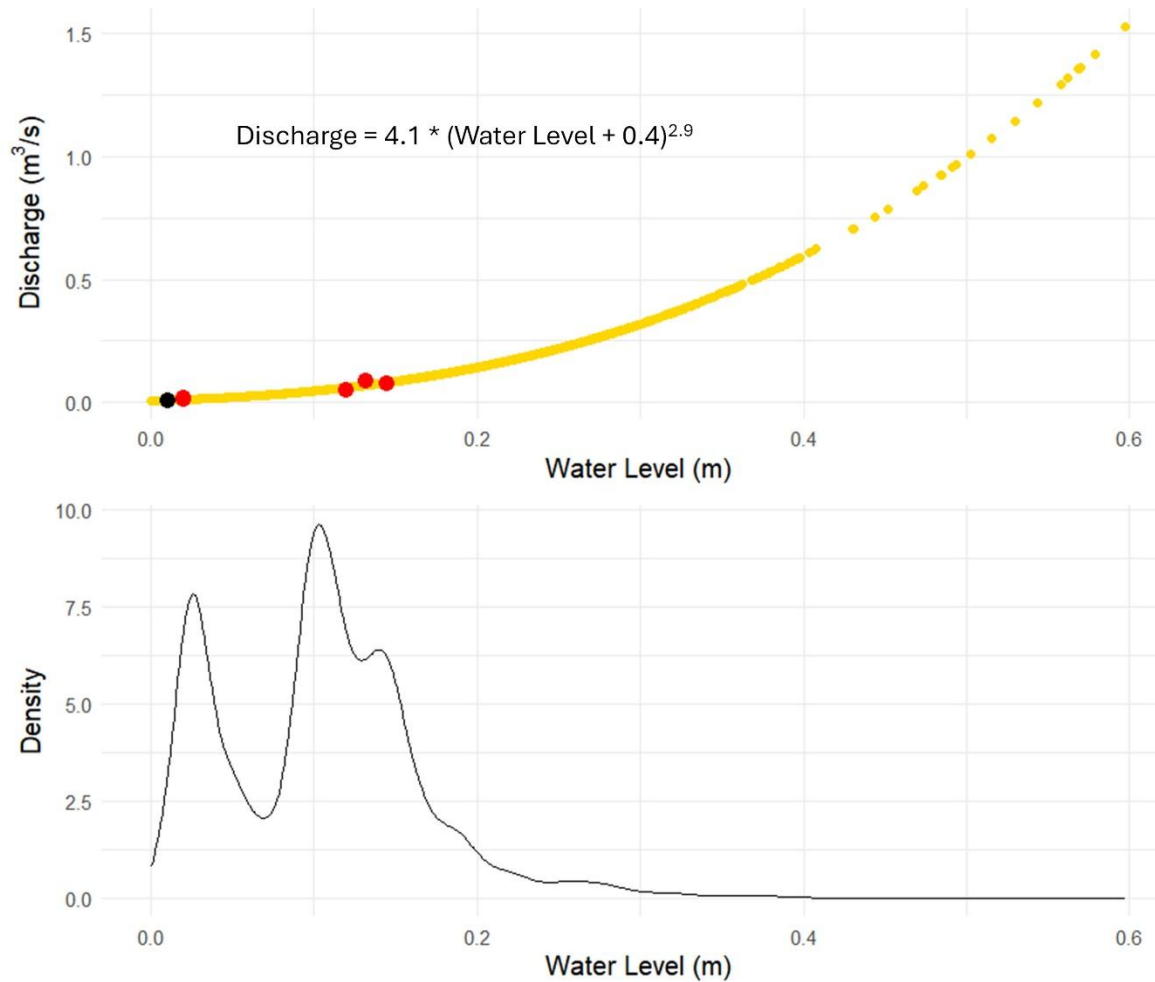


Figure A3: Top: Discharge (m<sup>3</sup>/s) vs. Water Level (m) at MID from August 2024 to August 2025. Red dots represent one-time solute tracer discharge values and associated recorded discharge used to create the site discharge-stage rating curve; the black dot represents a one-time solute tracer discharge value and manually measured water level from June 2024 due to water level sensor failure on this date. Bottom: A density plot of water level measurements at MID from August 2024 to August 2025.

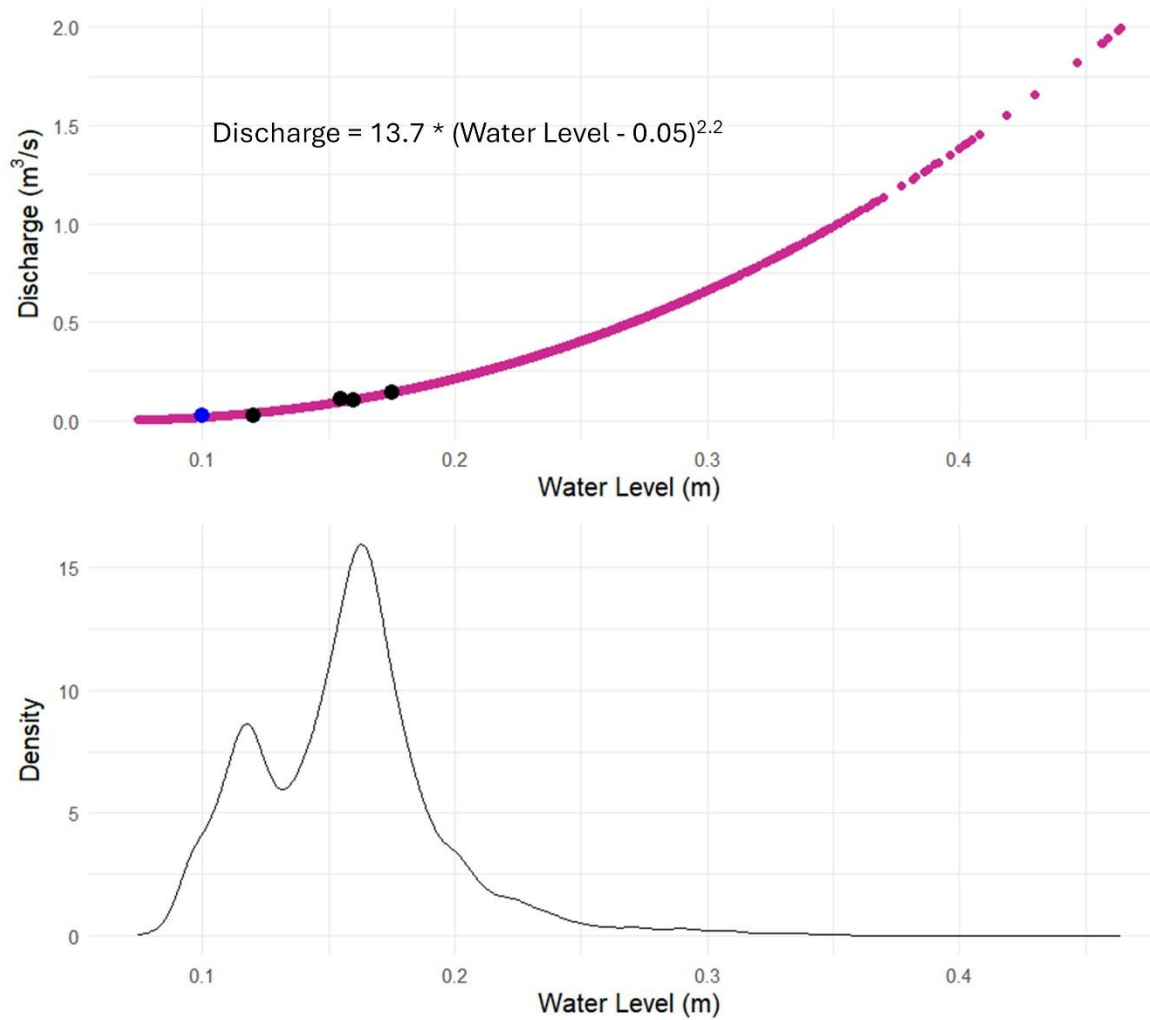


Figure A4: Top: Discharge (m<sup>3</sup>/s) vs. Water Level (m) at REF from August 2024 to August 2025. Red dots represent one-time solute tracer discharge values and associated recorded discharge used to create the site discharge-stage rating curve; the black dot represents a one-time solute tracer discharge value and manually measured water level from June 2024 due to water level sensor failure on this date. Bottom: A density plot of water level measurements at REF from August 2024 to August 2025.

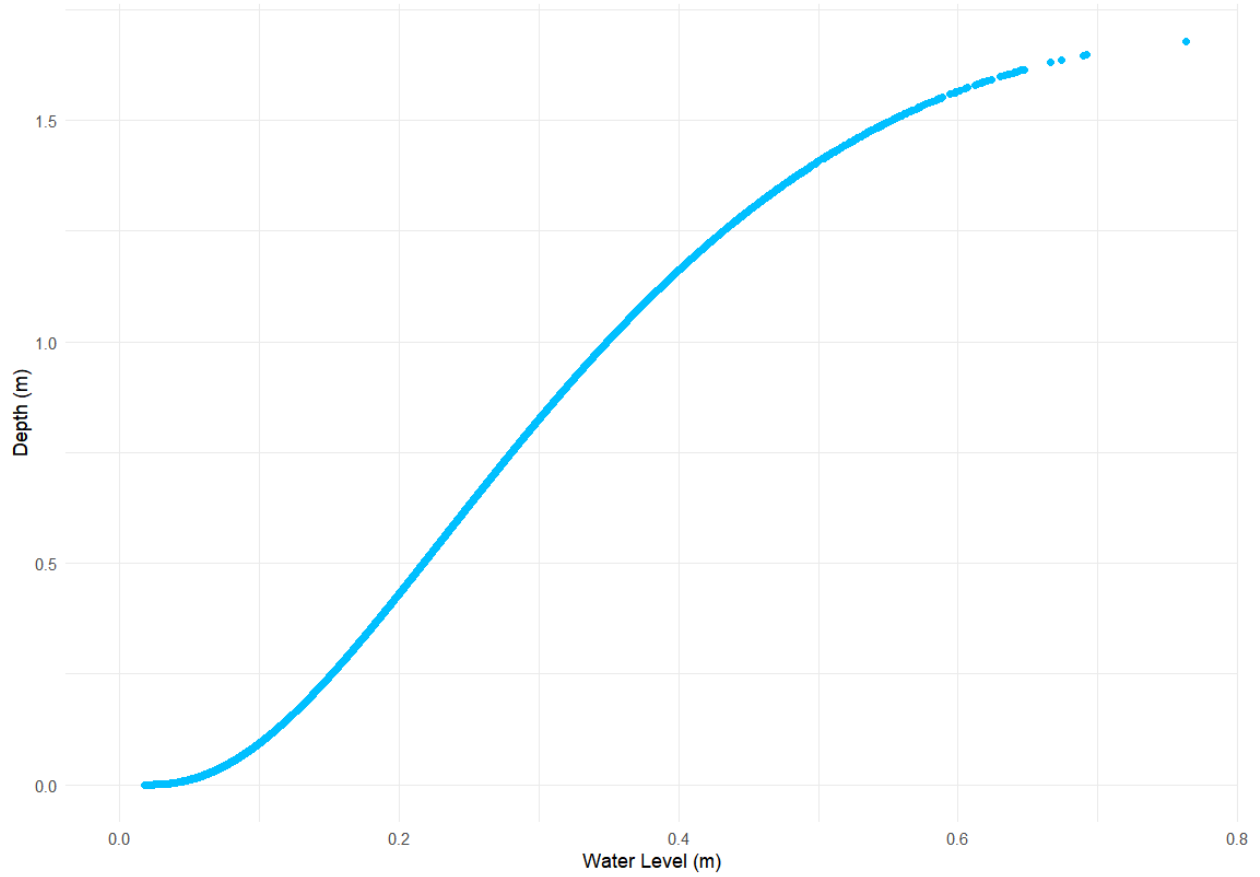


Figure A5: Calculated depth (m) vs. recorded water level (m) at REF from August 2024 to August 2025.

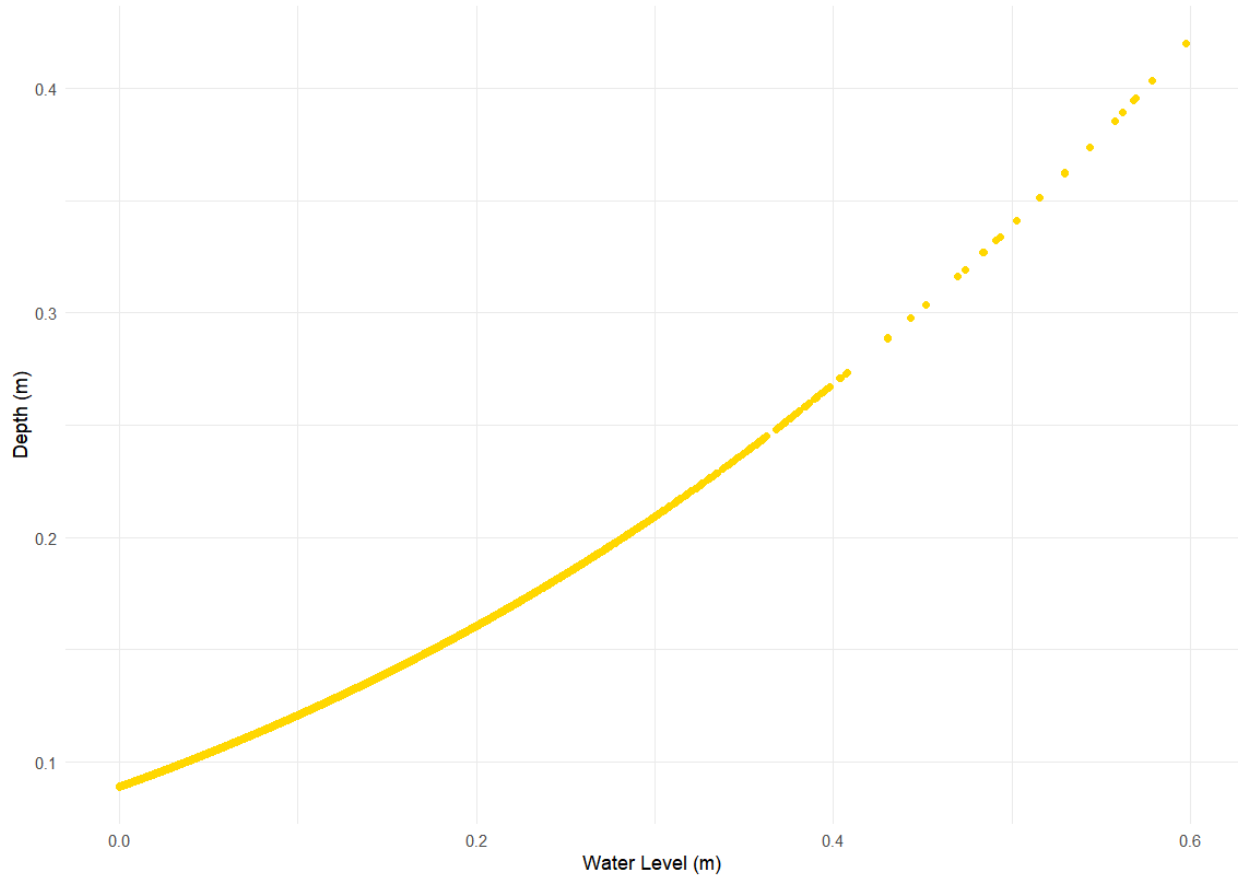


Figure A6: Calculated depth (m) vs. recorded water level (m) at MID from August 2024 to August 2025.

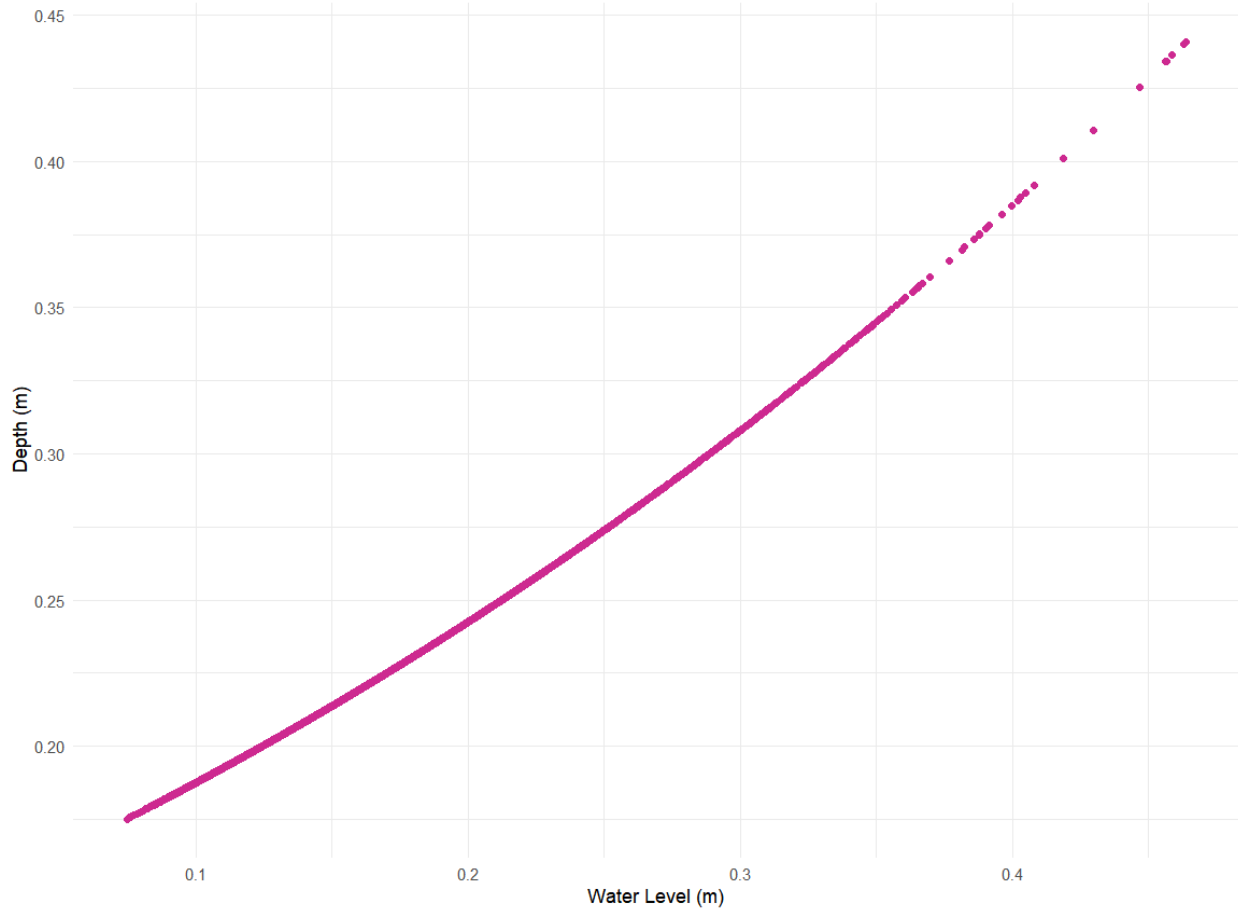


Figure A7: Calculated depth (m) vs. recorded water level (m) at HI from August 2024 to August 2025.

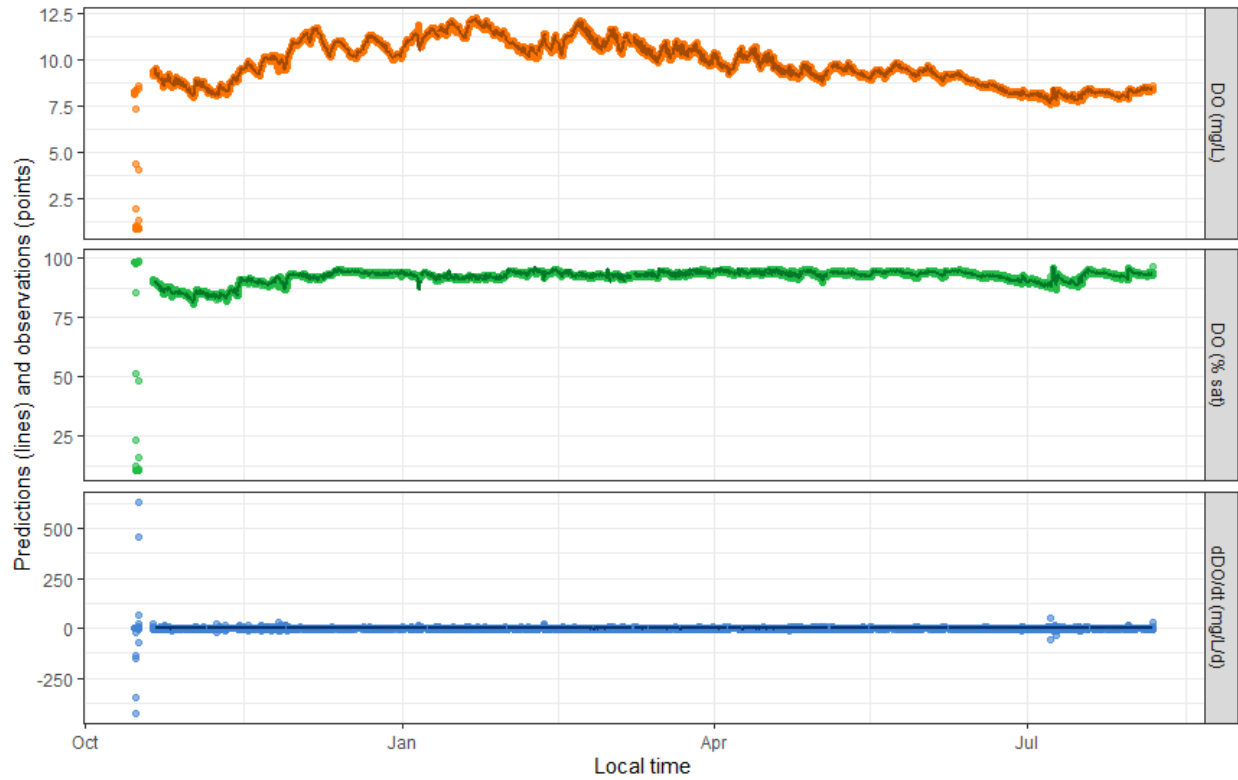


Figure A8: Predicted (modeled) and observed (recorded) dissolved oxygen concentration (mg/L), dissolved oxygen concentrations (%), and change in dissolved oxygen per day (mg/L/d) at REF. Observed data was collected from miniDOT high-frequency oxygen sensors and predicted data was modeled using *StreamMetabolizer* in R.

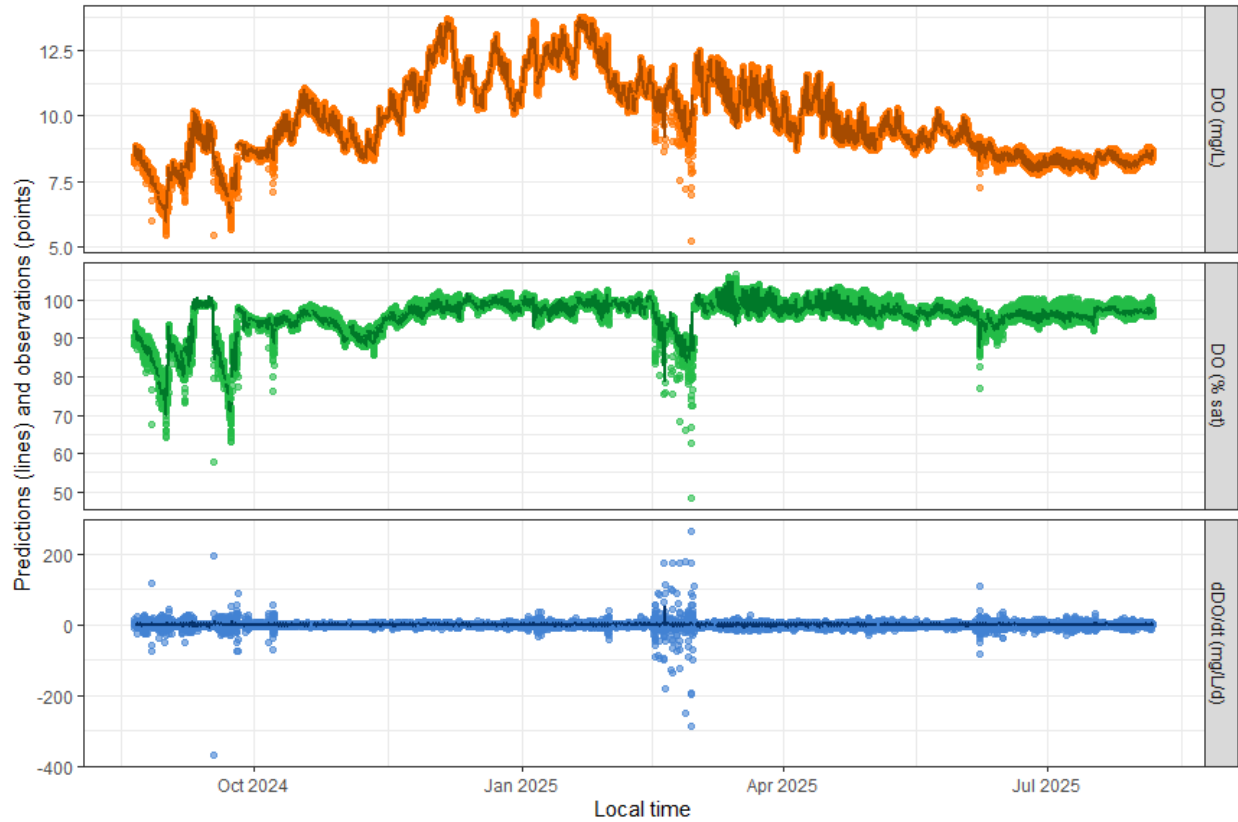


Figure A9: Predicted (modeled) and observed (recorded) dissolved oxygen concentration (mg/L), dissolved oxygen concentrations (%), and change in dissolved oxygen per day (mg/L/d) at MID. Observed data was collected from miniDOT high-frequency oxygen sensors and predicted data was modeled using *StreamMetabolizer* in R.

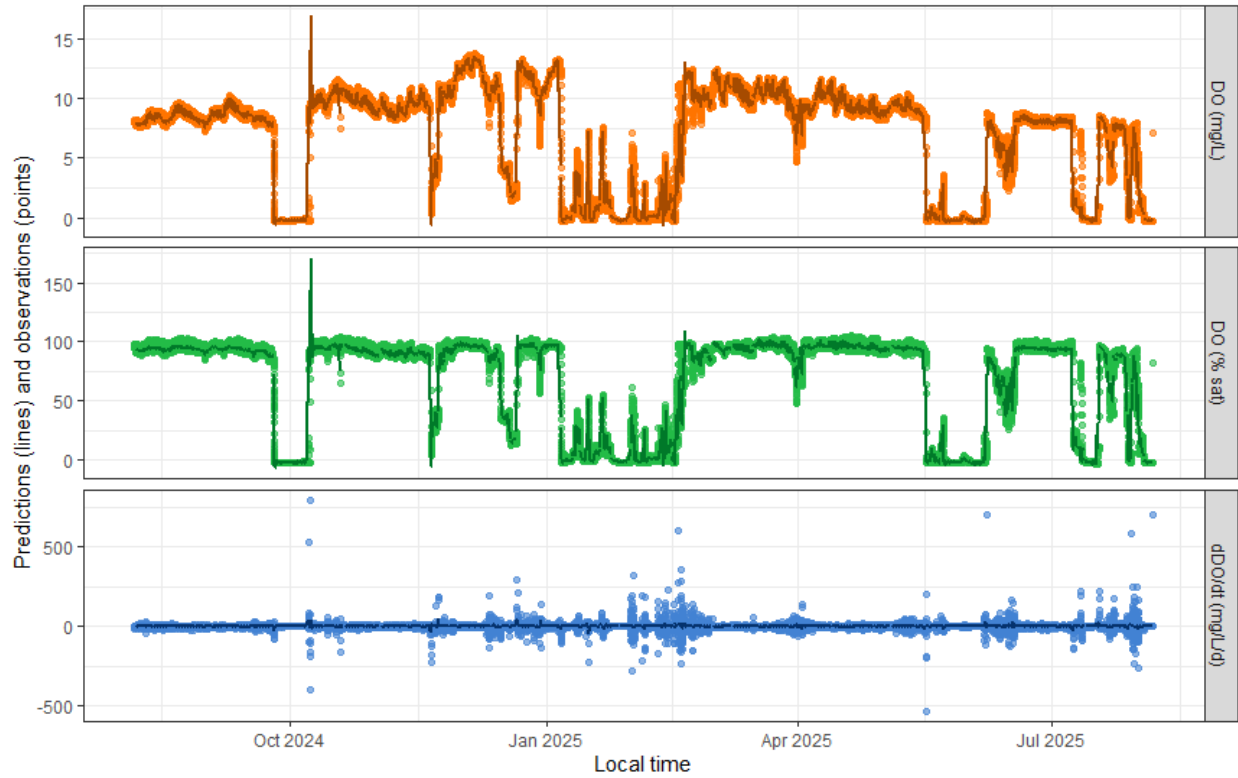


Figure A10: Predicted (modeled) and observed (recorded) dissolved oxygen concentration (mg/L), dissolved oxygen concentrations (%), and change in dissolved oxygen per day (mg/L/d) at HI. Observed data was collected from miniDOT high frequency oxygen sensors and predicted data was modeled using *StreamMetabolizer* in R.

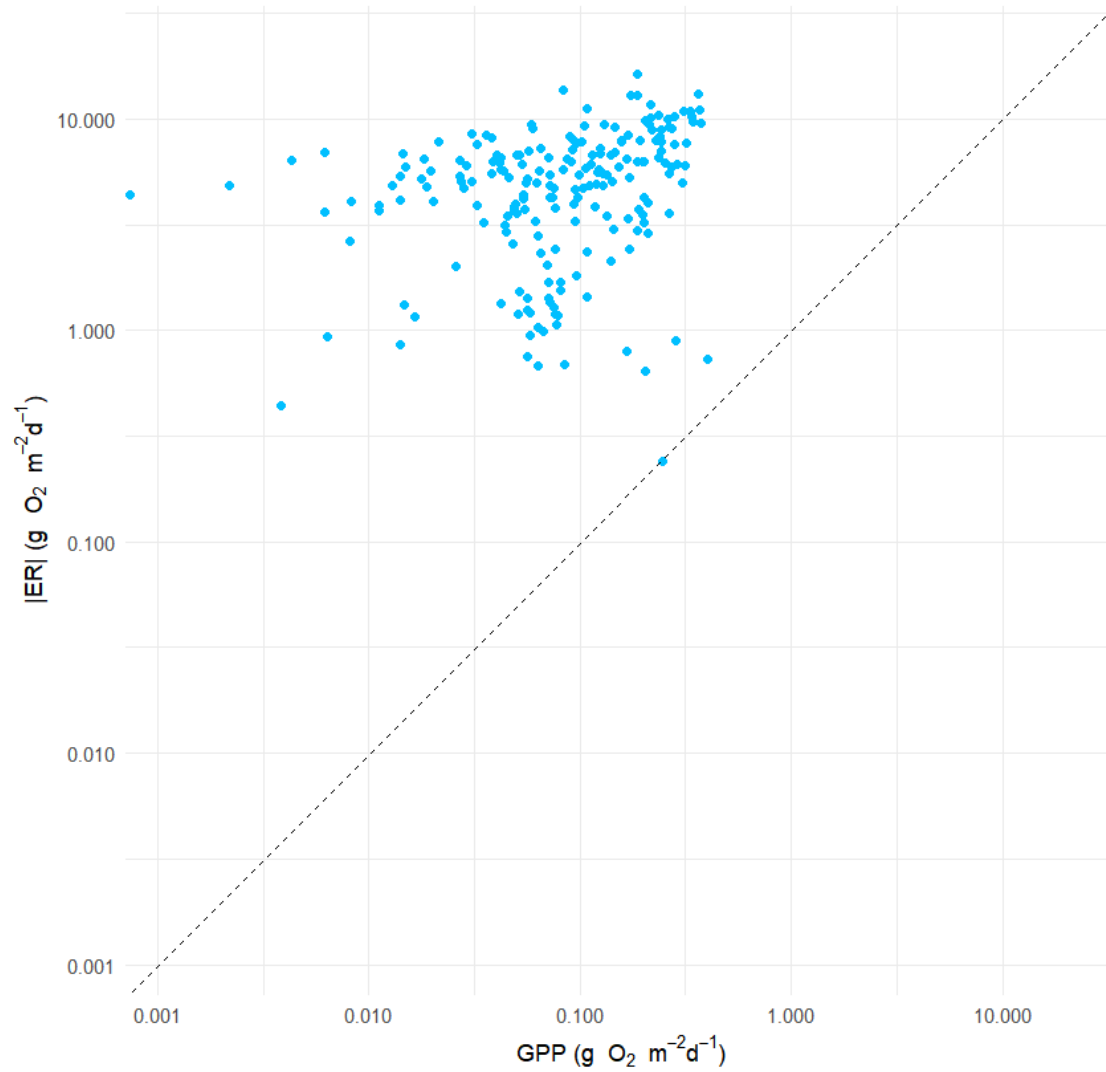


Figure A11:  $|ER|$  ( $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ) vs.  $GPP$  ( $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ) at REF. Data is log-transformed, but axis values reflect true values. Days above the 1:1 line are heterotrophic ( $GPP < |ER|$ ,  $NEP < 0$ ). Days below the 1:1 line are autotrophic ( $GPP > |ER|$ ,  $NEP > 0$ ).

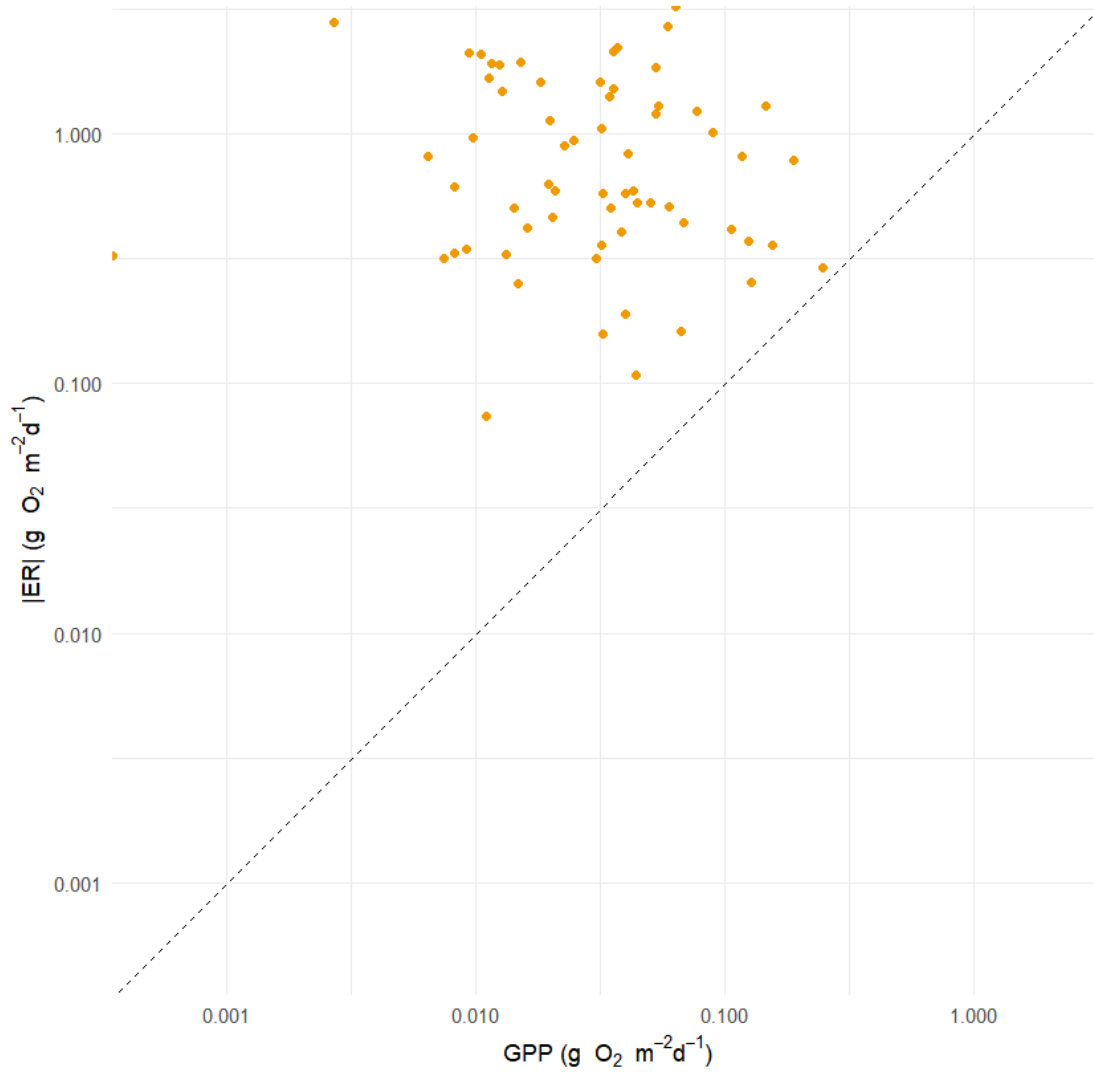


Figure A12:  $|ER|$  ( $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ) vs.  $GPP$  ( $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ) at MID. Data is log-transformed, but axis values reflect true values. Days above the 1:1 line are heterotrophic ( $GPP < |ER|$ ,  $NEP < 0$ ). Days below the 1:1 line are autotrophic ( $GPP > |ER|$ ,  $NEP > 0$ ).



Figure A13:  $|ER|$  ( $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ) vs.  $GPP$  ( $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ) at HI. Data is log-transformed, but axis values reflect true values. Days above the 1:1 line are heterotrophic ( $GPP < |ER|$ ,  $NEP < 0$ ). Days below the 1:1 line are autotrophic ( $GPP > |ER|$ ,  $NEP > 0$ ).

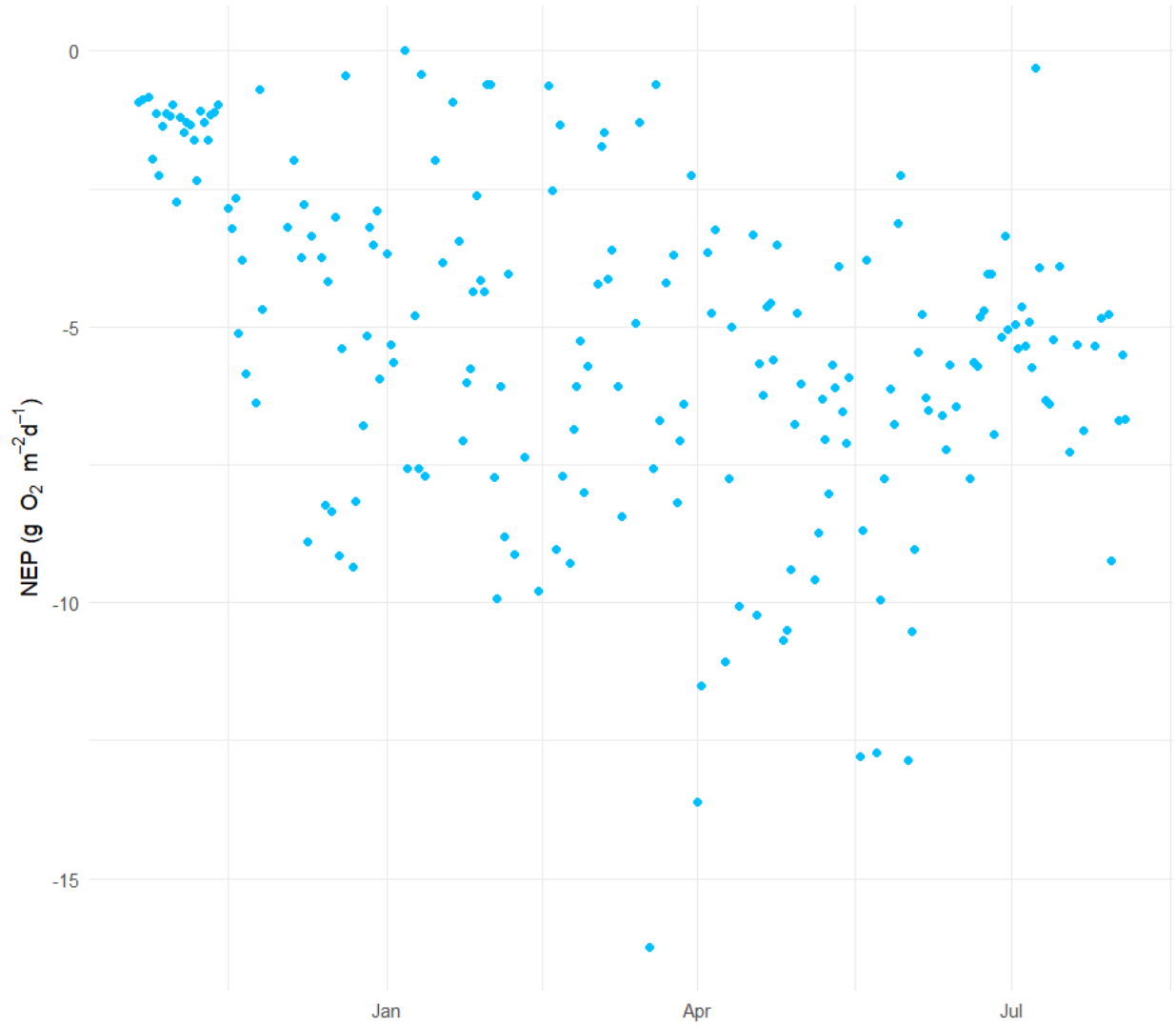


Figure A14: Net Ecosystem Production (NEP) ( $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ) (GPP + ER) time series at REF.

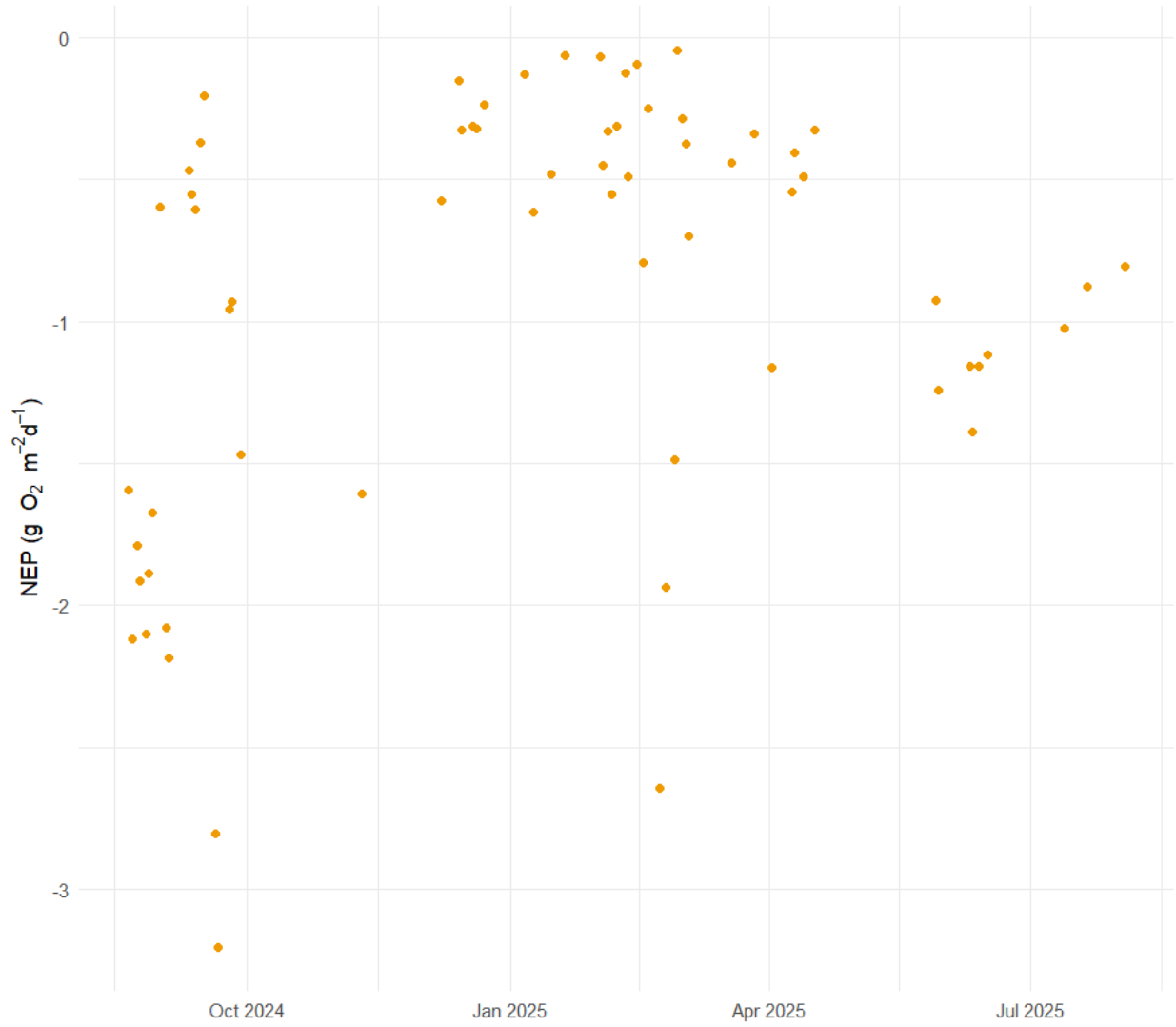


Figure A15: Net Ecosystem Production (NEP) (g O<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup>) (GPP + ER) time series at MID.

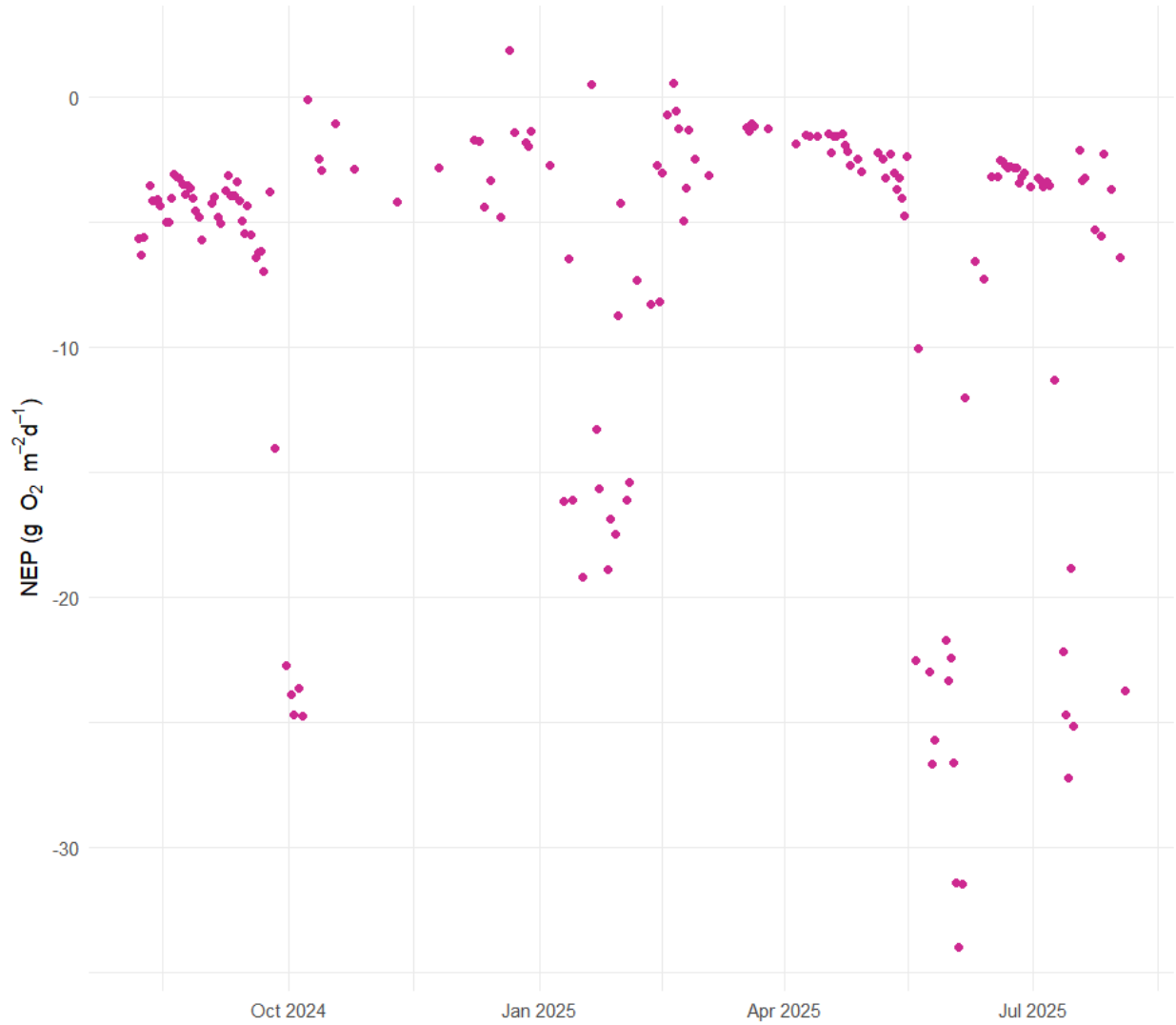


Figure A16: Net Ecosystem Production (NEP) ( $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ) (GPP + ER) time series at HI.

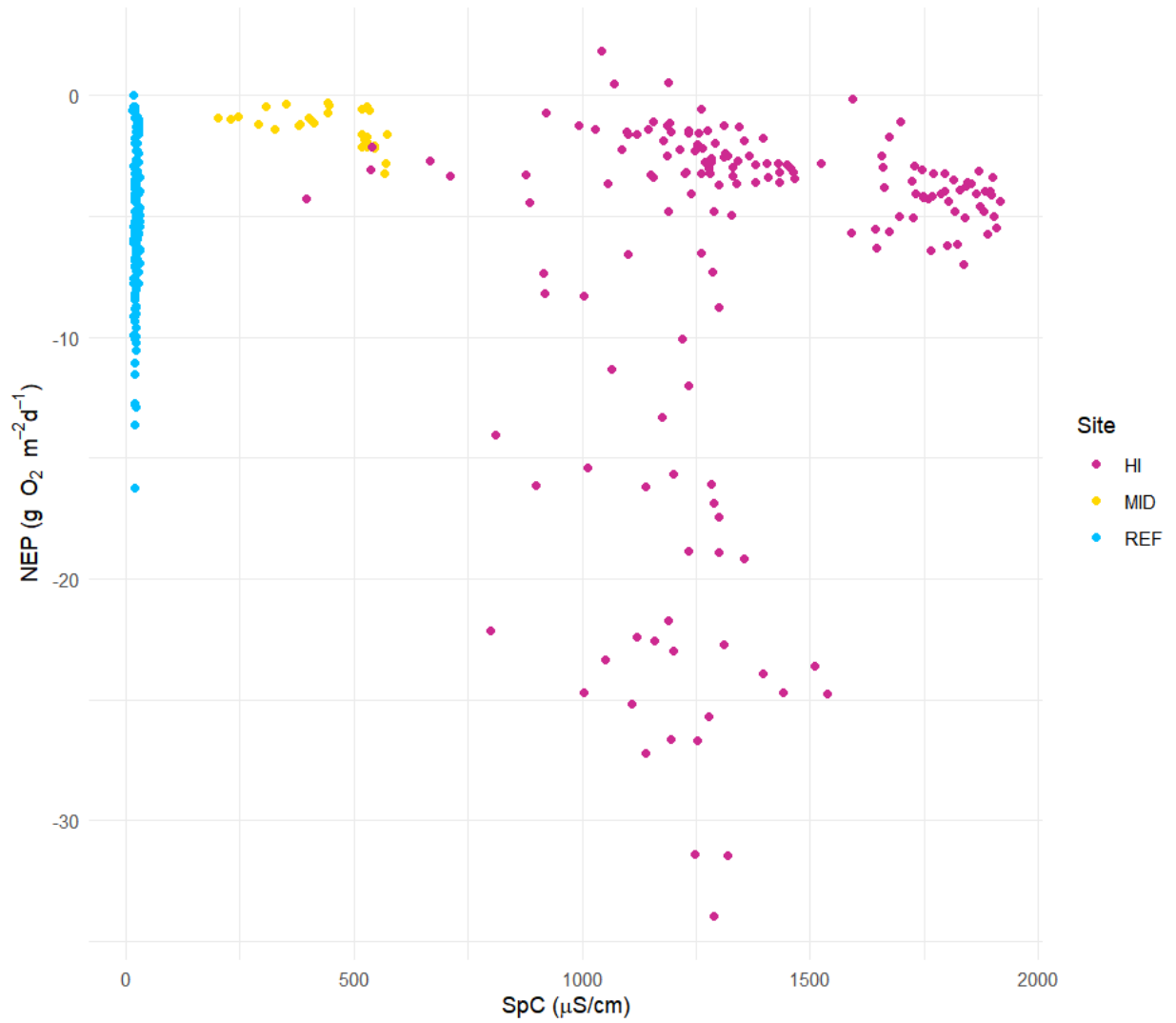


Figure A17: NEP (g O<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup>) (GPP + ER) vs. daily median specific conductivity (μS/cm) across all sites from August 2024 - August 2025.

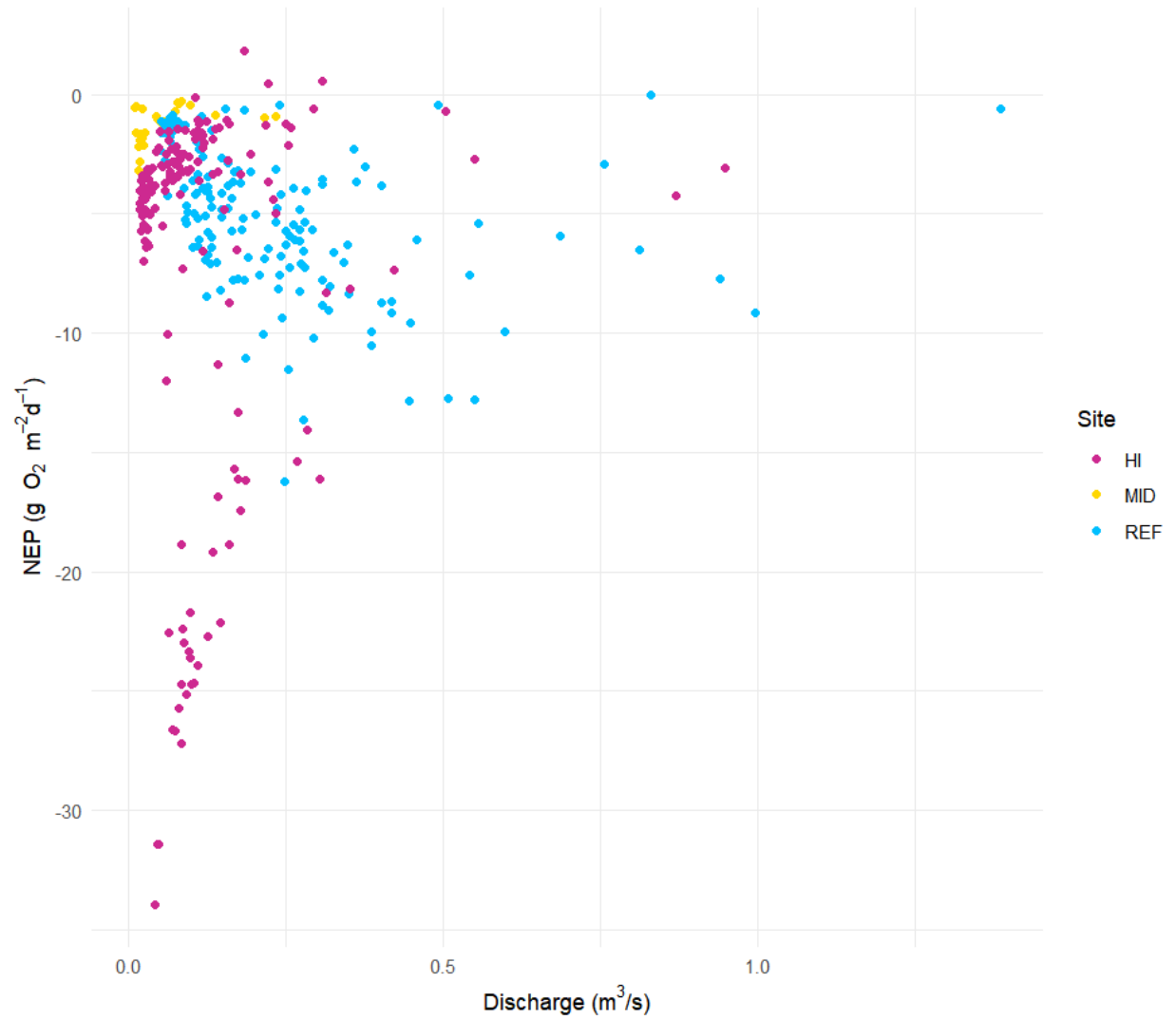


Figure A18: NEP (g O<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup>) (GPP + ER) vs. daily median discharge (m<sup>3</sup>/s) across all sites from August 2024 - August 2025.

## Supplemental Figures

**Table S1:** List of terms used in this paper

<b>Term</b>	<b>Description</b>	<b>Units</b>
mO	Modeled oxygen	$\text{g m}^{-3}$
$\Delta t$	Measurement interval	d
GPP	Gross Primary Production	$\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$
ER	Ecosystem Respiration	$\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$
$z$	Average stream channel depth	m
$K_o$	Air-water gas exchange of $\text{O}_2$	$\text{d}^{-1}$
$O_{\text{sat}}$	Oxygen saturation	$\text{g m}^{-3}$
PAR	Photosynthetically active radiation	$\mu\text{mol m}^{-2} \text{ d}^{-1}$
SpC	Specific conductivity	$\mu\text{S/cm}$
$Q$	Stream discharge	$\text{m}^3/\text{s}$
DO	Dissolved oxygen	mg/L