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Quantifying flow–ecology relationships across flow regime class and ecoregions in South Carolina

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HIGHLIGHTS

- Quantifying flow-ecology relationships allow for the development of flow standards.
- All flow regime components affected fish and benthic macroinvertebrate assemblages.
- Aquatic organisms' response to flow varied across flow classes and ecoregions.
- Single metric flow standards would underestimate the impacts of any flow alteration.

GRAPHICAL ABSTRACT
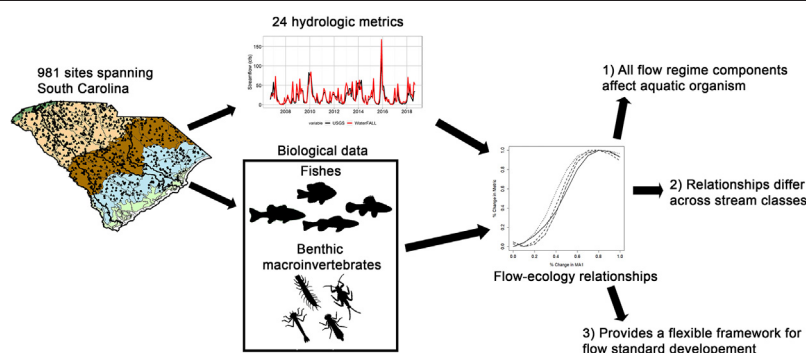
ABSTRACT

The natural flow regime (i.e. magnitude, frequency, duration, timing and rate of change of flow events) is crucial for maintaining freshwater biodiversity and ecosystem services. Protecting instream flow from anthropogenic alterations first requires an understanding of the relationship between aquatic organisms and the flow regime. In this study, we used a unique framework based on random forest modeling to quantify effects of natural flow regime metrics on fish and macroinvertebrate assemblages across ecoregions and flow regime types in the state of South Carolina, USA. We found that all components of the natural flow regime affected both fish and benthic macroinvertebrate assemblages, suggesting that maintaining natural aspects of all flow regime components is critical for protecting freshwater diversity. We identified hydrologic metrics and flow regime components such as magnitude, frequency, and duration of flow events, that were associated with the greatest ecological responses for individual stream classes to help managers prioritize hydrologic and biological metrics of interest during environmental flow standard development. The response of aquatic organisms to hydrologic metrics varied across stream classifications and ecoregions, highlighting the importance of accounting for differences in flow regime and ecoregion when designing environmental flow standards. We provide a flexible framework based on statistical flow-ecology relationships that can be used to inform instream flow management and assess effects of flow alteration on riverine assemblages.

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1. Introduction

Instream flow, deemed the "master variable", shapes the physical processes, water quality, biological components, and energy transfer that drive the ecological characteristics of freshwater systems (Hayes

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et al., 2018; Monk et al., 2006; Poff, 2018; Power et al., 1995). Natural flow regime, defined as the timing, magnitude, frequency, duration, and rate of change of flow events, is crucial for maintaining freshwater biodiversity and ecosystem health (Bunn and Arthington, 2002; Poff et al., 1997). Each flow regime component helps define a unique physical template that acts as an ecological filter and a selective force to differentially shape the ecological and evolutionary characteristics of freshwater systems (Bunn and Arthington, 2002; Naiman et al., 2008; Olden and Kennard, 2010; Poff et al., 1997). Altering the natural flow regime can cause adverse changes in biotic and abiotic characteristics of freshwater ecosystems, presenting a serious conservation concern (Arthington et al., 2018; Poff and Zimmerman, 2010; Webb et al., 2013). Traditional streamflow management of focusing solely on minimum flow may be inadequate in protecting riverine ecosystems because no single flow regime component affects riverine ecosystems alone (Bunn and Arthington, 2002; Poff et al., 2010). Key flow regime components are often neglected during the development of streamflow policy that balance human and ecosystem needs (Postel and Richter, 2003). This neglect is due in part to our limited understanding of aquatic organism response to the various aspects of the flow regime, with studies finding inconsistent responses across time and space (Lynch et al., 2018; Mcmanamay et al., 2013a; Praskievicz and Luo, 2020; Webb et al., 2013). These inconsistences highlight the need for regional studies to determine which flow regime components are most important and how organisms respond to these different components across space and streams with different flow regimes.

Flow regime and hydrology of a given stream is strongly dependent on climate, position in the river network, geomorphology, topology, and landcover characteristics (Winter, 2001). Accordingly, flow regime and stream hydrology vary across landscapes with a mosaic of climate and catchment characteristics, leading to streams with differing habitat templates and assemblage compositions (Poff and Ward, 1989). A consequence of this variation is that the ecological responses to flow regime properties (i.e. flow-ecology relationships) can vary across flow regimes, even within the same drainage division (Arthington et al., 2006; Kennard et al., 2010; Poff et al., 2010). On the other hand, studies suggest flow-ecology relationships should be similar for rivers within similar flow regimes (Arthington et al., 2006; Poff et al., 2010). At a regional scale, collecting biological and hydrologic data for every stream is infeasible. Hydrologic classification of streams into similar flow regimes is, therefore, an important step prior to modeling flow-ecology relationships at large spatial scales, allowing managers to generalize flow-ecology relationships within similar flow regimes (Olden et al., 2012). This step is particularly important for regions with a high diversity of habitat types and flow regimes, such as South Carolina. However, our understanding of the general transferability of flow-ecology relationships across flow regimes is still lacking. Determining extent to which and how flow-ecology relationships change across flow regimes has important implications for the development of management plans.

Modeling regional flow-ecology relationships requires data that adequately capture the complexity of flow regimes and biological characteristics across space and time (Arthington et al., 2018; Fox and Magoulick, 2019; Gwinn et al., 2016; Poff and Zimmerman, 2010). Yet the absence of paired biological observations and hydrologic data for large space and temporal scales is a common problem for regional flow-ecology studies (Patrick and Yuan, 2017; Poff et al., 2010; Wheeler et al., 2018). The space-for-time approach can overcome this issue by using contemporary data over a large spatial scale to understand and model temporal patterns that would be otherwise undetectable (Blois et al., 2013). By using data that spans a full range of hydrologic and biological characteristics across large spatial gradients, this approach captures natural variation in the data that represent future or past scenarios (Blois et al., 2013; Poff and Zimmerman, 2010). Biomonitoring programs for water quality provide baseline data well suited for evaluating the biological

characteristics of assemblages and of the response of assemblages to disturbance over large spatial scales, but this data often lacks concordant hydrologic data (Monk et al., 2006). Addressing the lack of matching hydrologic and biological data in our study area, hydrologic metrics that represent all components of the flow regime were simulated for every stream segment in South Carolina (SC), providing the data needed to quantify flow-ecology relationships at a regional scale using the space-for-time approach. The complexity of relationships between ecological and hydrologic characteristics as well as the development and use of hundreds of flow metrics lends to the inability of finding "general, transferable relationships" (Mcmanamay et al., 2013b; Poff and Zimmerman, 2010). Due to the inability to collect biological data at a river-by-river basis, studies widely advocate for quantifying flow-ecology relationships at regional scale to facilitate the identification of broadly applicable flow-ecology relationships (Arthington et al., 2006; Poff et al., 2010).

Globally, human appropriation of water resources via dam construction, groundwater withdrawals, water diversions/abstractions, and other hydromorphological alterations severely modify the natural flow regime, leading to pervasive declines in aquatic ecosystem integrity (Bunn and Arthington, 2002; Carlisle et al., 2011; Vörösmarty et al., 2010). In the United States, it is estimated that the majority of streams are severely affected by flow alteration (Carlisle et al., 2011), and the growing water demand, increasing droughts, and climate change are likely to exacerbate this problem (Acreman et al., 2014). Over the last few decades, this threat promoted the study of environmental flows and the establishment of environmental flow standards to protect and restore the biodiversity of freshwater systems. Yet, developing management plans that protect flow regimes from anthropogenic alterations is challenging and complex, requiring balancing human water use needs with the natural streamflow required to maintain essential ecosystem goods and services of riverine systems.

A major goal of environmental flow studies is to provide quantitative estimates of relationships between flow regime properties and ecological responses to create targeted management plans for riverine systems and environmental flow standards (Arthington et al., 2006; Bunn and Arthington, 2002; Poff et al., 2010), yet no such study has been completed for SC. Accordingly, the goal of this study is to quantify relationships between key hydrologic and biological response metrics to inform the process of developing recommendations for environmental flow standards in the state of SC, USA. Using a unique statistical framework based on random forest and robust datasets of fish and benthic macroinvertebrate assemblage data, we specifically sought to understand how much variation in biological metrics can be explained by hydrologic metrics, which metrics are most important, and how those relationships varied among ecoregions and types of hydrologic regimes. We also aimed to use model-predictions across various hydrologic gradients describing the natural flow regime to visualize flow-ecology relationships to estimate potential biodiversity loss.

2. Methods

2.1. Overview

We examined relationships among ecologically relevant hydrologic metrics and biological response metrics for fish and macroinvertebrates in wadeable streams of South Carolina, USA (Fig. 1). First, we used the Watershed Flow Allocation model (WaterFALL®; (Eddy et al., 2017) to calculate 171 flow metrics for each stream segment in the state. We then used principal components analysis to reduce this set to 24 metrics representing the key elements of the natural flow regime, stratifying all segments by ecoregions and hydrological classes. We used a powerful machine learning tool—random forest models—to relate hydrologic metrics to a large dataset of stream fishes and benthic
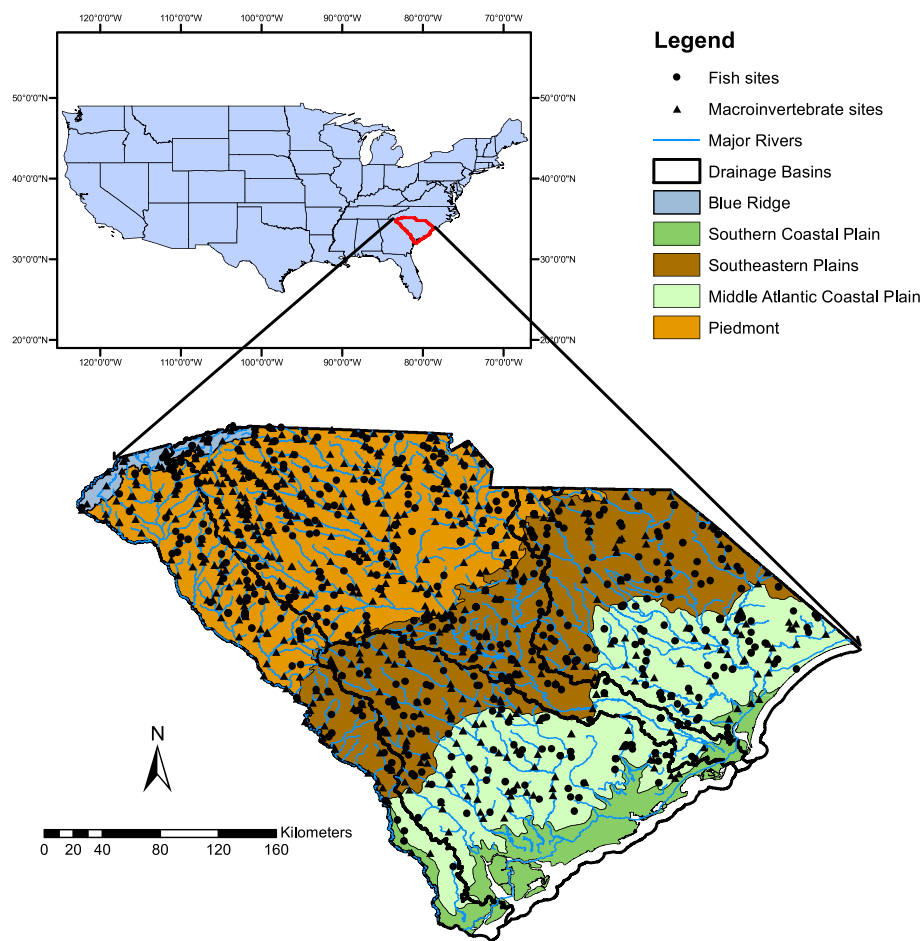
Fig. 1. A graphic depiction of the method work flow.

macroinvertebrates collected by state management agencies. This approach allowed us to (1) quantify variation in biological metrics explainable by hydrologic metrics, (2) identify the most important hydrologic metrics affecting biota in each ecoregion and flow regime type, and (3) develop scaled prediction curves that can be easily interpreted to provide guidance toward flow-related management decisions (Fig. 2).

2.2. Study area

South Carolina (SC) is an ideal area to explore flow-ecology relationships, containing a diversity of stream habitats and flow regimes. Spanning five level III ecoregions, streams in SC range from clear, high gradient mountain streams in the Blue Ridge to low gradient, black water streams in the Middle Atlantic Coastal Plain (Fig. 1; Omernik, 1987). In addition, SC has four major drainage basins, with high aquatic biodiversity and many endemic species within these basins. Roughly 146 freshwater and diadromous fish and 1092 macroinvertebrate taxa occur throughout SC, and these assemblages tend to strongly differ in taxonomic and functional composition from upland to lowland streams and among ecoregions (Denison et al., 2021; Glover et al., 2008; Marion et al., 2015; McManamay et al., 2015a). The streams in our study vary in fluvial habitat type, disturbance level, flow regime, and stream gradient, providing the hydrologic variation needed for a space-for time-approach (Fig. 1). The total upstream cumulative drainage area of our stream segments ranged from 0.185–4369 km^2 (mean = 134 km^2), encompassing headwater streams to small rivers. Our study analyzed a total of 981 stream segments (a catchment inlet and outlet), covering 2% of all stream segments in SC. These stream segments were randomly

selected using a geographic information system-based selection program and stratified by drainage basin, ecoregion, stream size to provide robust coverage of the ecoregions and drainage basins within SC (Scott, 2008).

2.3. Flow metrics and hydrological classification

In a preceding study (Eddy et al., in review), WaterFALL was used to simulate daily streamflows over a 30-year period for each stream segment in SC as defined by the medium resolution National Hydrography Dataset, with the exception of tidally influenced streams, which allowed for the calculation of 171 hydrologic metrics for each simulated stream reach. WaterFALL is a distributed rainfall-runoff model that simulates daily stream flow volumes within each enhanced National Hydrography Dataset (NHDPlus) catchment defining a watershed of interest. The model accounts for withdrawals, discharges, and onstream reservoirs within the network and using the NHDPlus catchments as the hydrologic unit and network. WaterFALL is calibrated and validated against streamflow measurements from US Geological Survey (USGS) gages, using a weight-of-evidence approach across both quantitative performance measures and qualitative graphical representations of model performance (i.e., flow duration curves). Eddy et al. (2017) describes an application of earlier version of WaterFALL to North Carolina streams and rivers to provide hydrologic metrics for subsequent ecological flow analyses (Phelan et al., 2017). The performance of the model in simulating hydrologic metrics within that body of work and in an additional comparison study of WaterFALL with other hydrologic models for a single southeastern US basin (Caldwell et al., 2015) provide support for the use of WaterFALL in pursuing the investigations outlined in this study.
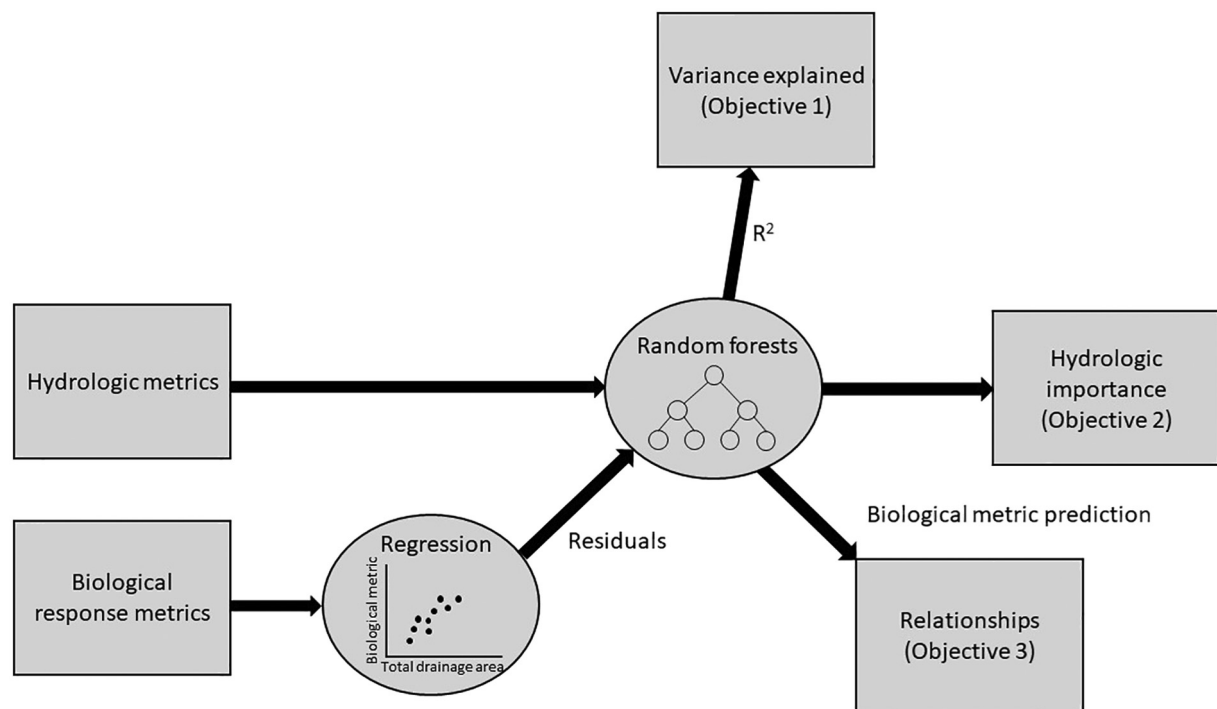
Fig. 2. Maps of sampling sites for macroinvertebrates and fish assemblages, HUC8, major drainage basins, ecoregions, and hydrologic classes.

For the hydrologic simulations supporting this study (Eddy et al., *in review*), WaterFALL was parameterized using the following geospatial data: land-use from the 2016 National Land Cover Database at a 30 m resolution (Jin et al., 2019), climate data (daily temperature and precipitation) at a 4-km resolution from 1987 to 2018 (Daly et al., 2008), subsurface parameters from the Sacramento Soil Moisture Accounting Model (Zhang et al., 2016), and geomorphology from Soil Survey Geographic Database (USDA-NRCS, 2014) at the NHDPlus catchment scale. Additional tabular data were used to parameterize human alterations to the natural flowing system (i.e., reservoir regulation, withdrawals, discharges, small barriers, and channelized areas of retention). Descriptions and sources of these alterations are described in full in Eddy et al. (in review). The simulated flow values from the WaterFALL model were calibrated against observed flow values from 59 USGS gages within the study region, including both reference and non-reference/human-impacted watershed conditions (Fig. S1). These gages span a range of drainage areas from 11 to 1996 km^2 with an average drainage area of 257 km^2. A 12-year calibration period (2006–2018; water years beginning October 1 of the prior year and ending September 30 of the listed year) followed by an 8-year validation period (water years 1998–2005) were used to set and validate model parameters, where each run considered a one-year spin up period to establish initial conditions. These time spans were chosen to include at least one major drought and several wet periods within each period.

First, a combination of classic hydrologic goodness of fit statistics and qualitative evaluation were used to guide the calibration process. The goodness of fit statistics include percent bias (PBIAS), coefficient of determination (R^2), the Nash-Sutcliffe Efficiency (NSE) (Nash and Sutcliffe, 1970), and rNSE, a modified version of the NSE to account for extreme high-flow events that may skew calculations of monthly NSE and gives more weight to low-flow periods. For these quantitative measures, the thresholds set by Moriasi et al. (2007) were used to classify model calibrations into very good, good, and satisfactory performance. Given the range in stream classification assignments, streamflow ranges and regimes, and ratings of observations on which comparisons are made (Table 3), we chose the slightly less restrictive performance

criteria described by Moriasi et al. (2007). Bias in mean streamflow within ±10%, ±15%, and ±25% indicated very good, good, and satisfactory model performance, respectively. Model performance is classified in terms of NSE values as very good (NSE > 0.75), good (0.75 ≥ NSE > 0.65), and satisfactory (0.65 ≥ NSE > 0.50) (Moriasi et al., 2007). Neither analysis in Moriasi et al. (2007) or (2015) provides criteria specifically for rNSE beyond calling for its use. We therefore apply the NSE criteria to the rNSE measure for evaluation. To examine model performance across the tails or the more extreme flow conditions not always well highlighted by single quantitative metrics, flow duration curves were used. In some cases, the calibrations with the best performing quantitative metrics were passed over in favor of calibrations with slightly lower performance measures but better representation of extreme low or high flow conditions. Goodness of fit statistics, PBIAS, NSE, and rNSE, demonstrated an expected range of performance for stream gauges across the study area with full details on the performance described in Eddy et al. (in review). Second, model performance in terms of simulating the 171 hydrologic metric was evaluated using both the simulated and observed streamflows at 31 of the 59 calibration gages across the full 30-year simulation period, where the remaining 28 gages were removed from the evaluation due to limitations in the observed data. Following most studies evaluating simulated hydrologic metrics, we applied the acceptance criteria of ±30% to the PBIAS evaluation for hydrologic metrics (Caldwell et al., 2015; Murphy et al., 2013; Vigiak et al., 2018).

Once calculated, modeled hydrologic metrics were average over a 30-year period of daily streamflows at each catchment from water years 1989–2018. From modeled hydrologic metrics, we selected a subset of metrics corresponding to Indicators of Hydrologic Alteration (IHA) that are minimally redundant and ecologically relevant components of the flow regime (Olden and Poff, 2003). In addition to the IHA metrics, we also used Colwell's index of constancy as a measure of flow stability (Colwell, 1974). To better capture high flow conditions, we also calculated median and specific annual maximum flows, resulting in 24 metrics (Table 1). Metric selection followed the recommendations and methods given in Olden and Poff, 2003. We

Table 1
List of hydrologic metrics, their associated flow regime component, and description based on Henriksen et al. (2006).

Code	Flow regime	Description
DL16	Duration	Low flow pulse duration. The average pulse for flow events below a threshold equal to the 25th percentile value for the entire flow record.
DL17	Duration	Coefficient of vitiation in DL16
DL18	Duration	Number of zero-flow days
DH15	Duration	High flow pulse duration. The average duration for flow events with flows above a threshold equal to the 75th percentile value for each year in the flow record.
DH16	Duration	Coefficient of vitiation in DH15
FL1	Frequency	Low flow pulse count. Average number of flow events with flows below a threshold equal to the 25th percentile value for the entire flow record
FL2	Frequency	Coefficient of vitiation in FL1
FH1	Frequency	High flow pulse count. Average pulse duration for each year for flow events below a threshold equal to the 25th percentile value for the entire flow record.
FH2	Frequency	Coefficient of vitiation in FH1
MA1	Magnitude	Mean daily flow (cfs)
MA3	Magnitude	Mean of the coefficient of vitiation (standard deviation/mean) for each year of daily flows
MA41	Magnitude	Annual runoff computed as the mean of the annual means divided by the
MA42	Magnitude	Coefficient of vitiation of MA41
ML17	Magnitude	Base flow index. The minimum of a 7-day moving average flow divided by the mean annual flow for each year.
ML18	Magnitude	Coefficient of vitiation in ML17
ML22	Magnitude	Specific mean annual minimum flow. Annual minimum flows divided by the drainage area
MH14	Magnitude	Median of annual maximum flows. The ratio of annual maximum flow to median annual flow for each year
MH20	Magnitude	Specific mean annual maximum flow. The annual maximum flows divided by the drainage area
RA8	Rate	Number of reversals. Number of days in each year when the change in flow from one day to the next changes direction
TA1	Timing	Constancy or stability of flow regime computed via the formulation of Colwell (see example in Colwell, 1974).
TL1	Timing	Julian date of annual minimum
TL2	Timing	Coefficient of vitiation in TL1
TH1	Timing	Julian date of annual maximum starting at day 100
TH2	Timing	Coefficient of vitiation in TH1

investigated metric redundancy using the commonly accepted approach of correlation-based principal components analysis (PCA) (Olden and Poff, 2003). We retained PC axes with eigenvalues greater than 1, and only selected hydrologic metrics with loading values greater 0.5 and less than −0.5 on at least one of the retained axes (Mackay et al., 2014; Olden and Poff, 2003). From this subset of metrics, we calculated the Pearson correlation values among all metrics, avoiding metrics with correlations higher than 0.7. The Pearson correlation values among all metrics are presented in Table S1.

Simulation uncertainty varied among hydrologic metrics, with some metric being more confidently simulated than others (Eddy et al., in review). We therefore avoided metrics with larger uncertainty (Fig. S2) and focus on those that confidently estimate observed stream flows and simulate hydrologic metrics (Fig. S2). Timing metrics generally preformed the best out the hydrologic metrics, and average condition magnitude hydrologic metrics consistently performed better than rate, frequency, and duration metrics based on the goodness of fit statistics (Fig. S1). The low flow magnitude metrics had a mixture of both over- (ML18, ML21) and under-prediction (ML4, ML17, ML22) by the model, but unitless baseflow ratio (ML17) was consistent simulated (Fig. S2). High flow magnitude metrics were either well predicted (MH8, MH16, MH20) or slightly over-predicted (MH10, MH14). Metrics indexing low and high flow variability (FL2 and FH2) performed best out of the frequency metrics across all sites (Fig. S2). Low flow pulse count (FL1) and high flood pulse count (FH3) also performed acceptably for most streams and high flow pulse count (FH1) was less stable in its

calculations across a wide range of conditions. High flow duration metrics fell more within the acceptance range of bias than the low flow metrics (Fig. S2), with DL16 and DL17 tending to be overestimated at some sites. Number of reversals (RA8) and to a slightly lesser extent the number of daily rises (RA5) show more acceptable simulation results (Fig. S2). Although a limited number of gages were available in three of the four monitored ecoregions, gages tended to perform better performance at higher elevation (Blue Ridge and Piedmont) sites (Fig. S2).

Hydrologic classification of streams is a critical component of environmental flow assessments because flow-ecology relationships can differ among flow regimes (Poff et al., 2010). This step is particularly crucial for SC, given the diversity of fluvial habitats among ecoregions (Fig. 1). Accordingly, we used a hydrologic classification for rivers of the conterminous US provided by (McManamay and DeRolph, 2019). This classification consists of eight natural stream classes based on reference-quality gauges. Of those eight classes, only four natural hydrologic regimes occurred in SC: perennial runoff streams, characterized by moderately stable flow and distinct seasonal extremes (Class 1, 615 stream segments); stable baseflow streams, characterized by high precipitation, sustained high baseflows, and moderately high run-off (Class 3, 183 stream segments); perennial flashy; characterized by moderately stable flow with high flow variability (coefficient of variation in daily flows) (Class 4, 138 stream segments); and intermittent streams, classified by intermittent periods of no flow punctuated by flooding events (Class 5, 45 stream segments). This approach resulted in five different stream groupings: Piedmont (stream classes 1 and 4), Middle Atlantic Coastal Plains (stream class 1), and Southeastern Plains (stream classes 1 and 3). Limited biological data was available for the Blue Ridge ecoregion and no hydrologic data was calculated for the Southeastern Coast Plains due to the tidal influence in this area (Eddy et al., in review). Therefore, these ecoregions were not induced in our study. Stream classes were analyzed separately in this study to account for the major ecological and evolutionary patterns that occur across the ecoregions (Abell et al., 2008).

2.4. Biological metrics

Fish and benthic macroinvertebrate assemblage composition data were provided by SCDNR (fish) and SCDHEC (benthic macroinvertebrates). All surveys were collected and identified by professional biologists with knowledge of the species and systems. These surveys were intended to quantify species abundance and diversity of the sampling sites. Both the fish and macroinvertebrate stream segments span all major drainage basins and level III ecoregions in SC, except the Southern Coastal Plains, capturing hydrologic variation across SC (Fig. 1). Fish assemblages were surveyed at a total of 492 stream segments between April and October from 2006 through 2011 using a backpack electro-fishing unit following a standardized stream assessment protocol by the SC Department of Natural Resources (Scott et al., 2009). For the Blue Ridge and Piedmont streams, fish were collected using a single pass of 30 times the wetted width of the reach whereas collections in the Southeastern Plains and Middle Atlantic Coastal Plain ecoregions were made using three passes 20 times the wetted width. The differences in sampling methods between the upland and lowland regions were to account for lower capture efficiency observed in lowland streams, presumably due to more complex habitats and heavier cover (Scott, 2008). We calculated a suite of biological response metrics for fish and macroinvertebrate assemblages at each site (Table 2). Taxa richness and Shannon diversity index were calculated for both taxa groups. We used proportional representation of individuals in the genus *Lepomis* because most of these species are ecologically tolerant and habitat generalists (Lynch et al., 2018). Because life history traits of fish are strongly influenced by the hydrologic regime (Mims and Olden, 2012), we used proportional representation of individuals belonging to a breeding strategy in each site. Breeding strategy categories included open substrate spawning, brood hiding, and nest spawning

Table 2
Abbreviation, description, and association with type of biological metrics.

Abbreviation	Description	Type
Fish metrics		
Richness	Taxa richness	Ecological integrity
Shannon	Shannon's diversity index	Ecological integrity
Lepomis	Proportional representation of individuals in the genus *Lepomis*	Tolerance
Brood Hider	Proportional representation of individuals in the brood hiding breeding strategy (Balon, 1975).	Ecological integrity
Nest Spawner	Proportional representation of individuals in the nest spawning breeding strategy (Balon, 1975).	Ecological integrity
Open substrate	Proportional representation of individuals an open substrate spawning breeding strategy (Balon, 1975).	Ecological integrity
Lotic	Proportional representation of individuals that prefer lotic environments	Ecological integrity
Tolerance	Proportional representation of tolerant individuals	Tolerance
Benthic Macroinvertebrate metrics		
Richness	Taxa richness	Ecological integrity
Shannon	Shannon's diversity index	Ecological integrity
EPT	Proportional representation of individuals in	Ecological integrity
Chronomidae	Proportional representation of individuals in Chrionomidae family	Tolerance
M-O index	Average of an index indicative of Odonata and Megaloptera taxa preference for lotic or lentic conditions	Ecological integrity
Tolerance	Average tolerance index for macroinvertebrate taxa	Tolerance

species (Balon, 1975; Frimpong and Angermeier, 2009). We also used the proportional representation of tolerant individuals where the number of tolerant individuals should increase in streams with greater hydrological alteration. Tolerance rankings for fish were taken from North Carolina Department of Environmental Quality stream fish community assessment program. In addition, we calculated the proportional representation of individuals that prefer lotic habitats. Lotic fishes have life history strategies and traits adapted to natural flow regimes and would respond to alteration of the flow regime (Poff et al., 1997; Bunn and Arthington, 2002; Frimpong and Angermeier, 2009).

Macroinvertebrates were collected from 530 stream segments between 1995 and 2018, following a timed-qualitative, multiple habitat sampling protocol by SC Department of Health and Environmental Control (see SCDHEC, 2017 for details). This protocol is widely used by many regulatory and non-regulatory agencies and ensures an adequate representation of the macroinvertebrate assemblage. Samples were collected using kick nets, D-frame dip nets, fine mesh samplers, and sieves for a three person-hour sampling effort. Sampling teams independently use one of the sampling methods previously listed to sample the appropriate habitat. Macroinvertebrates were identified to the lowest possible taxonomic resolution within the class Hexapoda. This resulted in 91% of organisms identified to the genus level and 9% to the family level. We calculated the percent contribution of individuals belonging to orders Ephemeroptera, Plecoptera, and Trichoptera (EPT) and the percent contribution of individuals in the family Chironomidae. Species within the EPT orders are generally associated with high water quality and less disturbed habitats. Species in the Chironomidae family are generally considered as tolerant, increasing in abundance within disturbed habitats (Barbour et al., 1999; Karr, 1991). Benthic macroinvertebrate tolerance index was gathered from the literature (Barbour et al., 1999; Bressler et al., 2006; MDEQ, 2003). If no tolerance index was given at the genus level, the tolerance index for that family was used. Finally, we also calculated a Megaloptera-Odonata index. Taxa within the Megaloptera and Odonata orders are long lived and have differing preferences for lotic or lentic conditions. Therefore, changes in this index should reflect dry stream conditions in the past. Based on an index created by SCDHEC for SC, taxa were assigned values ranging from 1 to 10

indicative of their preference for lotic or lentic conditions (personal communication with David Eargle, SCDHEC).

2.5. Statistics

Flow-ecology relationships can be highly complex and nonlinear, particularly in large scale studies across multiple flow regimes (Arthington et al., 2006; Knight et al., 2014; Rosenfeld, 2017). Accordingly, we used random forest models–a powerful machine learning algorithm that combines hundreds of decision trees and their predictions into a single model, improving predictive accuracy and providing a robust predictive tool for quantifying complex and nonlinear flow-ecology relationships (Breiman, 2001; Cutler et al., 2007). Within each stream class, random forest models allowed us to (1) quantify variation in biological metrics explainable by flow metrics (R^2), (2) identify the most important flow metrics affecting biota in each ecoregion and flow classification (i.e. variable importance), and (3) calculate model-predicted values to provide guidance toward flow-related management decisions. Prior to analyses, we removed the pervasive effect of stream size on biological metrics by regressing metrics against the total upstream cumulative drainage area, then extracted the residuals for further analysis. These values represent patterns in biological metrics that cannot be explained by stream size, enabling a clearer signal of flow-ecology relationships to be detected. Random forest models were carried out using the "cforest" function (Hothorn et al., 2015). Model training and performance was evaluated using the "caret" package in R version 4.0.0 (Kuhn et al., 2020; R Core Team, 2020).

Each decision tree in random forest is created using a subset of variables and a bootstrapped sample of the dataset. The remaining data, the out-of-bag (OOB) sample, is used to estimate the predictive performance of the model and variable importance (Breiman, 2001; Cutler et al., 2007). We used pseudo R^2 based on OOB data as a measure of model performance (Ellis et al., 2012). To evaluate the ability of hydrologic metrics to predict the biological response metrics, we randomly split the data into training (70% of data) and test data sets (30% of data), using the models built with the training data to predict the test data. Next, we used linear models to quantify how well these predicted values match the observed values (test data). Selecting random subsets of the data will inevitably cause the predictive ability of models to vary. Therefore, we repeated these steps five times to obtain the average R^2 and quantified the uncertainty of variance explained using standard deviation. Only models with moderate or greater predictive ability (R^2 values greater than 0.5) were considered for further analyses.

The second important inference from random forest, variable importance, can be defined as the mean decrease in model accuracy that would be caused by omitting that variable from the model. Variable importance shows which predictor variables have the greatest influence on the response variable. This step is useful in applied settings because identifying important metrics within stream classes will help managers set priorities during flow standard development. We used 'conditional' variable importance, which is a more robust estimate than traditional important metrics when variables are correlated (Ellis et al., 2012; Strobl et al., 2008). Although we selected minimally redundant hydrologic metrics, some metrics were correlated to some extent (Table S1). Some explanatory variables can have a negative importance, so that inclusion of these variables can produce increased error rates (Pyne et al., 2017). Therefore, variables with negative importance values were sequentially removed through the iterative modeling process, eliminating the variable with the largest negative value and rerunning the analysis. This process was repeated until no negative importance values remain.

Finally, we used the final random forest models to predict biological metrics as a function of changes in hydrologic metrics. To do so, we first selected important predictor variables and model-predicted values to range from zero to one, allowing for clear visualization of potential relationships in like terms. Then, for each hydrologic metric of interest, the maximum value was reduced in 10% increments until the minimum

metric value, keeping all other hydrologic metrics in the model constant at their mean. These results were plotted to examine the flow ecology relationships and identify potential critical values along important flow gradients indicating thresholds of change in assemblage composition and biological responses.

3. Results

A total of 107 fish and 455 macroinvertebrate taxa were collected in SC, with highest taxa richness found in perennial runoff streams within the Piedmont (Table 3). Fish species richness per site fish ranged from 3 to 31 (mean = 14.3). For macroinvertebrates, taxon richness per site ranged from 7 to 83 (mean = 39.3). We retained five axes from the PCA of the hydrologic metric dataset that explained a cumulative 76% of the variation in taxon richness (Fig. S3, Table S2). We retained 24 hydrologic metrics for analysis, each having loadings greater than 0.5 or less than -0.5 on at least one component. We found that redundancy among hydrologic metrics varied (Table S1). Only three pairs of variables were correlated more than $|r \geq 0.70|$ (Table S1). FL1 showed high correlation with FH1 (0.71), DL16 (-0.74), and RA8 (0.82). In addition, ML22 was strongly correlated to MA41 and ML17.

3.1. Variance explained

Random forest models indicated that biological response metrics were poorly to strongly predicted by the 24 selected hydrology metrics across all stream and eco-region groupings (average R^2 = 0.19–0.75; Table 4), with higher average R^2 being found for macroinvertebrates (R^2 = 0.53, SD = 0.12) in comparison to fishes (R^2 = 0.51, SD = 0.12). Fish species richness was the best predicted biological response metric, followed by percentage of nest spawning species (Table 4). In contrast, the percentage of open substrate spawning fish species was

Table 3
The number of stream segments, total number of taxa, average number of taxa, and number of HUC8s in each stream class for fish and macroinvertebrate assemblages.

Fish assemblages	Perennial runoff	Stable baseflow	Perennial flashy
Southeastern Plains			
No. stream segments	92	66	NA
No. taxa present	72	65	NA
Mean No. taxa per site	15	15.5	NA
No. of HUC8	10	11	NA
Piedmont			
No. stream segments	130	NA	69
No. taxa present	77	NA	62
Mean No. taxa per site	14.1	NA	13.9
No. of HUC8	12	NA	9
Middle Atlantic Coastal Plains			
No. stream segments	77	NA	NA
No. taxa present	53	NA	NA
Mean No. taxa per site	13.1	NA	NA
No. of HUC8	10	NA	NA

Macroinvertebrate assemblages	Perennial runoff	Stable baseflow	Perennial flashy
Southeastern Plains			
No. stream segments	67	50	NA
No. taxa present	203	159	NA
Mean No. taxa per site	38.5	43.96	NA
No. of HUC8	14	9	NA
Piedmont			
No. stream segments	225	NA	67
No. taxa present	246	NA	197
Mean No. taxa per site	40.1	NA	42.5
No. of HUC8	15	NA	11
Middle Atlantic Coastal Plains			
No. stream segments	50	NA	NA
No. taxa present	156	NA	NA
Mean No. taxa per site	26.5	NA	NA
No. of HUC8	9	NA	NA

Table 4
The average R^2 values from linear models testing correlation between the observed and predicted biological response metric values from the fish random forest models for each stream class. The standard deviation is shown in the brackets.

	Perennial runoff	Stable baseflow	Perennial flashy
Piedmont			
Richness	0.70 (0.03)	NA	0.61 (0.03)
Shannon	0.56 (0.02)	NA	0.59 (0.03)
% Open substrate	0.64 (0.03)	NA	0.39 (0.05)
% Nest spawners	0.63 (0.03)	NA	0.63 (0.03)
% Brood hiders	0.68 (0.04)	NA	0.53 (0.03)
% Lotic	0.50 (0.02)	NA	0.48 (0.02)
% Tolerance	0.51 (0.08)	NA	0.41 (0.04)
% Lepomis	0.48 (0.03)	NA	0.54 (0.05)
Southeastern Plains			
Richness	0.61 (0.02)	0.60 (0.03)	NA
Shannon	0.56 (0.07)	0.19 (0.07)	NA
% Open substrate	0.42 (0.06)	0.48 (0.03)	NA
% Nest spawners	0.49 (0.03)	0.53 (0.02)	NA
% Brood hiders	0.50 (0.02)	0.48 (0.03)	NA
% Lotic	0.71 (0.02)	0.53 (0.02)	NA
% Tolerance	0.61 (0.06)	0.26 (0.07)	NA
% Lepomis	0.59 (0.03)	0.53 (0.03)	NA
Middle Atlantic Coastal Plains			
Richness	0.37 (0.06)	NA	NA
Shannon	0.39 (0.03)	NA	NA
% Open substrate	0.43 (0.08)	NA	NA
% Nest spawners	0.55 (0.03)	NA	NA
% Brood hiders	0.40 (0.07)	NA	NA
% Lotic	0.45 (0.03)	NA	NA
% Tolerance	0.52 (0.08)	NA	NA
% Lepomis	0.40 (0.07)	NA	NA

poorly predicted by the hydrologic metrics (Table 4). For macroinvertebrates, the percentage of EPT taxa and mean tolerance were best predicted by the hydrologic metrics, whereas percentage of Chironomidae species had the lowest average R^2 value (Table 5). We found the highest average R^2 in stream class 1 in the Southeastern Plains and Piedmont ecoregions for both taxa groups (Tables 3 and 4).

### 3.2. Hydrologic metric importance

Conditional importance from the random forest models was used to identify hydrologic metrics that had the greatest influence on the biological response metrics within each stream class. All components of

Table 5
The average R^2 values from linear models testing correlation between the observed and predicted biological response metric values from the macroinvertebrate random forest models for each stream class. The standard deviation is shown in the brackets. Megaloptera-Odonata index is given as M-O index.

	Perennial runoff	Stable baseflow	Perennial flashy
Piedmont			
Richness	0.56 (0.07)	NA	0.54 (0.05)
Shannon	0.54 (0.04)	NA	0.53 (0.03)
EPT	0.51 (0.05)	NA	0.53 (0.05)
Chironomidae	0.60 (0.06)	NA	0.34 (0.05)
M-O index	0.51 (0.03)	NA	0.58 (0.05)
Tolerance index	0.64 (0.02)	NA	0.56 (0.05)
Southeastern Plains			
Richness	0.63 (0.06)	0.23 (0.04)	NA
Shannon	0.66 (0.03)	0.36 (0.07)	NA
EPT	0.56 (0.05)	0.53 (0.06)	NA
Chironomidae	0.50 (0.05)	0.51 (0.06)	NA
M-O index	0.51 (0.08)	0.57 (0.02)	NA
Tolerance index	0.67 (0.03)	0.45 (0.09)	NA
Middle Atlantic Coastal Plains			
Richness	0.75 (0.06)	NA	NA
Shannon	0.67 (0.05)	NA	NA
EPT	0.66 (0.06)	NA	NA
Chironomidae	0.26 (0.04)	NA	NA
M-O index	0.34 (0.04)	NA	NA
Tolerance index	0.51 (0.05)	NA	NA
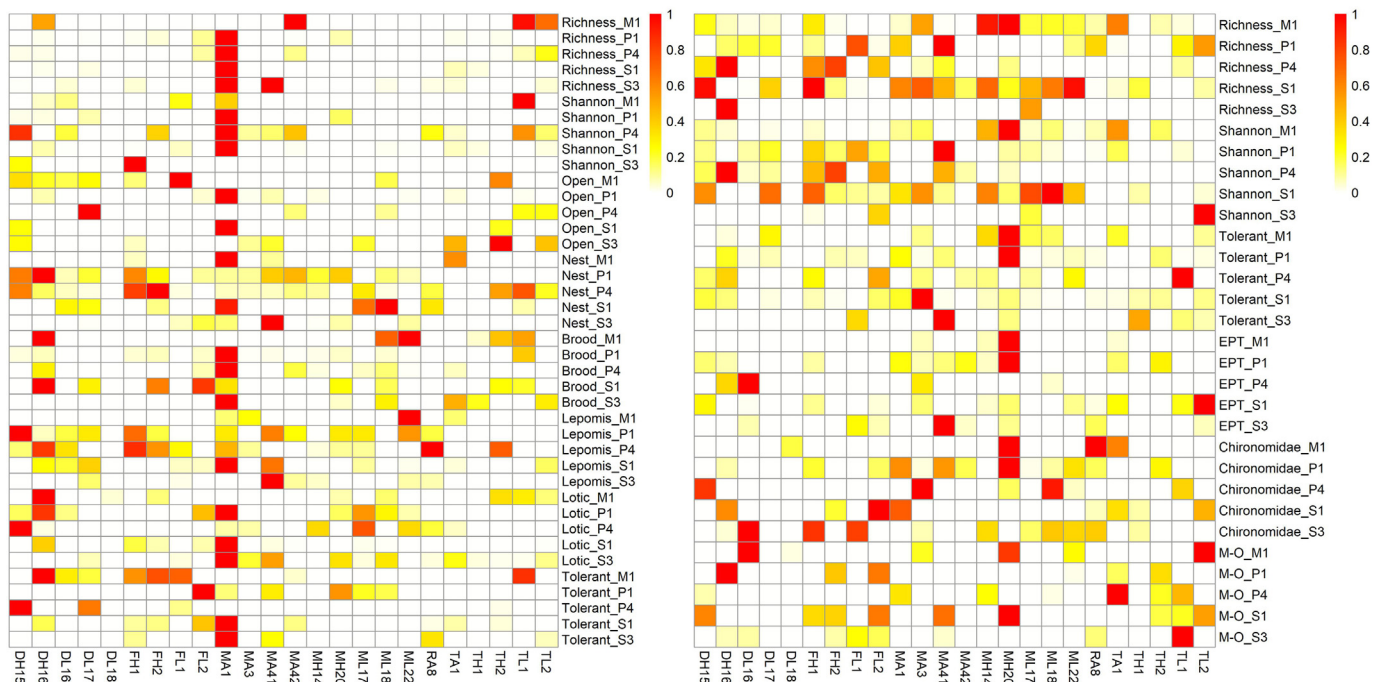
Fig. 3. Heatmaps of conditional importance values from the random forest model for fish (Left graph) and macroinvertebrates (Right graph). Conditional importance values for each biological metric were scaled from 0 (no influence on model) to 1 (largest influence on model). The stream classes are indicated after biological metric name as follows: Middle Atlantic Coastal Plains perennial runoff (M1), Piedmont perennial runoff (P1), Piedmont perennial flashy (P4), Southeastern Plains perennial runoff (S1), and Southeastern Plains stable baseflow (S3). Megaloptera-Odonata index is given as M-O.

the flow regime were important predictors of the biological response metrics in at least one stream grouping except for percentage of open substrate spawning fish (Fig. 3), where the rate at which flow conditions change showed no ability to predict this biological response metric (Fig. 3). In general, magnitude, frequency, and duration of flow events were often associated with high values of conditional importance for fish and macroinvertebrates. The conditional importance value and identity of hydrologic metrics associated with the biologic response metrics varied among stream classes, but no pattern within stream classes was detected. Hydrologic metrics associated with high flow events and MA1 frequently had the highest conditional importance values for fish biological response metrics. For macroinvertebrates, metrics associated with low and high flow events and metrics measuring magnitude commonly had high conditional importance value. In addition, the coefficient of variation for hydrologic metrics also had high conditional importance values for both fish and macroinvertebrates (Figs. 3). For macroinvertebrates in the Middle Atlantic Coastal Plains, MH20 often had the highest conditional importance value across all biological metrics (Fig. 3).

### 3.3. Flow-ecology relationships: biological metrics

Hydrologic metrics indexing all five flow regime components relate to biological response for fish and macroinvertebrates. We detected general trends in the flow ecology relationships for fish and macroinvertebrates. For example, higher values of metrics that characterize the duration and magnitude of both high and low flow events, such as MH14, MH20, DH15, and DL16, tended to decrease diversity metrics and increased those associated with tolerant species or measures, with a few exceptions (Figs. 4). Higher mean daily flow, base flow, and flow constancy had the opposite effect on the biological metrics (Figs. 4). In addition, metrics describing flow variability generally had the opposite trend to those metrics indexing stability (Figs. 4). The shape of the flow-ecology relationships often differed across stream classes (Fig. 5; Figs. S2-8).

In the proceeding paragraphs, we characterize relationships as either positive (+) or negative (−). For fish assemblages, we found associations between the percentage of brood hiding species and FH1 (−), FL1 (−), MA1 (−), MH20 (−), and ML18 (−) when analyzing perennial runoff and perennial flashy streams in the Piedmont perennial runoff and stable bases flow streams (Fig. 4; Figs. S4–5). We found relationships among the percentage of nest spawning species and DH15 (−), DH16 (+), DL16 (−), FH1 (+), FH2 (−), FL2 (−), MA1 (+), MA3 (−), MA41 (+), MA42 (−), ML17 (−), ML18 (+), ML17 (− and +), MH14 (−), MH20 (+), RA8 (−), TA1 (+), and TL2 (+) for all ecoregions (Fig. 4; Figs. S4-S6). Annual minimums between 240 and 280 Julian days were associated with higher percentages of nest spawning species in Piedmont streams (Fig. S7). The percentage of open-substrate spawning species was related to DH16 (−) and MA1 (+) in Piedmont perennial runoff streams (Fig. 4; Figs. S2–S3). We detected relationships among the percentage of tolerant fish species and DH16 (−), DL16 (−), DL17 (−), FH1 (+), FH2 (−), FL1 (+), FL2 (−), MA1 (−), MA42 (−) and TA (−) for perennial runoff in the Southeastern Plains, Middle Atlantic Coastal Plains, and Piedmont (Fig. 4; Figs. S4–S6). In addition, we found higher values of the percentage of tolerant fish species when annual minimum flows occurred between 200 and 230 Julian days in the Middle Atlantic Coastal Plains (Fig. S5). We found associations among percentage of *Lepomis* species and DH15 (−), DH16 (−), DL16 (−), FH1 (+), FH2 (−), MA1 (+), MA41 (+), ML17 (−), RA8 (+), TA1 (+), and TH2 (+) for perennial runoff streams in the Southeastern Plains as well as perennial runoff and perennial flashy streams in the Piedmont (Fig. 4; Figs. S2–S4). We found relationships among the percentage of lotic species and DH16 (+), FH1 (−), FH2 (+), FL2 (+), MA1 (+), MH20 (−), ML17 (+), ML18 (−), and TA1 (+) (Fig. 4; Fig. S4–S6). Shannon diversity index was associated with DH15 (−), DL16 (−), MA1 (+), MA42 (−), MH20 (−) and TL2 (−), for perennial flashy and perennial runoff streams in the Piedmont (Fig. 4; Fig. S4–S6). Higher values of Shannon diversity index were associated with annual minimum flows between 220 and 280 Julian days for perennial flashy streams in the Piedmont (Fig. S5). We found relationships among species richness
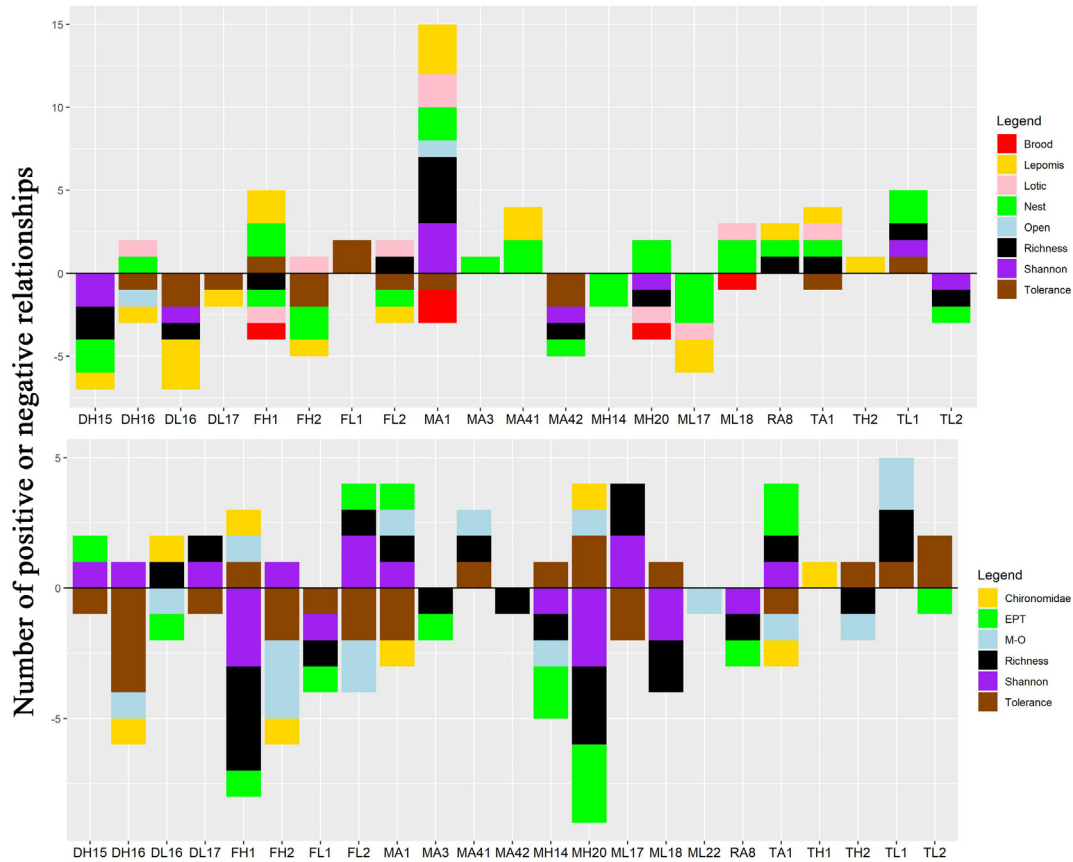
Fig. 4. Summarizing the relationships found by the random forest models given in Figs. S2–S9, stacked bar plots depicting the number of positive or negative relationships among biological and hydrologic metrics for fish (A) and macroinvertebrates (B). Hydrologic-biological metric relationships were assigned a value based on whether they were negative (−1) or positive (1). The sum of these values is shown in the stacked bar plot, showing which biological metric was associated with each hydrologic metric, number of times this relationship occurred across stream classes, and relationship direction.
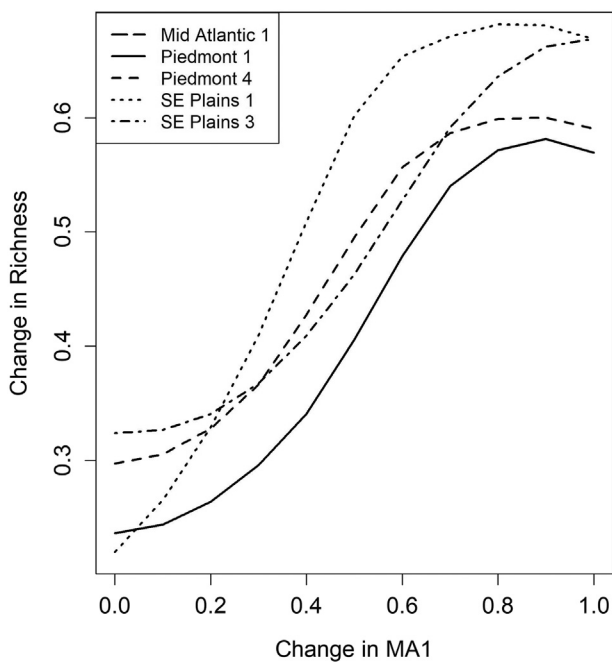
Fig. 5. The predicted relationships among fish richness and MA for stream classes Middle Atlantic Coastal Plains perennial runoff (Mid Atlantic 1), Piedmont perennial runoff (Piedmont 1), Piedmont perennial flashy (Piedmont 4), Southeastern Plains perennial runoff (SE Plains 1), Southeastern Plains stable baseflow (SE Plains 3).

and DH15 (−), DL16 (−), FH1 (−), FL1 (−), FL2 (+), MA1 (+), MA42 (−), MH20 (−), RA8 (+), TA1 (+), and TL2 (−) for perennial runoff and perennial flashy streams in the Piedmont as well as perennial runoff and stable baseflow streams in Southeastern Plains (Fig. 4; Fig. S4–S6). Additionally, higher values of species richness were associated with low flow events around 280 Julian days for these regions (Fig. S7).

For macroinvertebrates, we found associations among the percentage of EPT species and DH15 (+), DL16 (−), FH1 (−), FL1 (−), FL2 (+), MA1 (+), MA3 (−), MH14 (−), MH20 (−), ML17 (+), RA8 (−), TA1 (+), and TL2 (−) (Fig. 4; Figs. S8–S10). We showed relationships among percentage of Chironomidae and DL16 (+), DH16 (−), FH1 (+), FH2 (−), FL2 (+), MA1 (−), MH20 (+), and TA1 (−) for perennial runoff streams in the Piedmont and Southeastern Plains (Fig. 4; Figs. S8–S10). The percentage of Chironomidae species was highest when annual maximum flow events occurred around 250 Julian days for perennial runoff streams in the Southeastern Plains (Figs. S11). We showed relationships among macroinvertebrate tolerance index and DH15 (−), DH16 (−), DL17 (−), FH1 (+), FH2 (−), FL1 (+), FL2 (−), MA1 (−), MA41 (+), ML17 (−), ML18 (+), MH14 (+), MH20 (+), TA1 (−), TL2 (+), and TH2 (+) for all ecoregions and stream classes except stable baseflow (Fig. 4; Figs. S8–S10). Higher tolerance values were seen during annual minimum flows between 240 and 260 Julian days for perennial flashy streams in the Piedmont (Fig. S11). We found associations among Megaloptera-Odonata index and DH16 (−), DL16 (−), FH1 (+), FH2 (−), FL2 (−), MA1 (+), MA41 (+), MH20 (+), MH14 (−), ML22 (−), TA1 (+), and TH2 (−) for all ecoregions except the Middle Atlantic Coastal Plains (Fig. 4; Figs. S8–S10). We saw higher values of Megaloptera-Odonata index at roughly 220 and 250 Julian

days for perennial runoff streams in the Southeastern Plains and perennial flashy streams in the Piedmont, respectively (Figs. S11). Shannon diversity index increased along gradients of DH15, DH16, DL17, FH2, FL2, MA1, ML17, and TA1 and decreased along gradients of FL1, FH1, MH14, MH20, ML18, and RA8 for all stream classes except stable baseflow (Fig. 4; Figs. S8–S10). We found relationships among species richness and DL16 (+), DL17 (+), FH1 (−), FL1 (−), FL2 (+), MA1 (+), MA3 (−), MA42 (+), MA41 (+), MH14 (−), MH20 (−), ML17 (+), ML18 (−), RA8 (−), TA1 (+), and TH2 (−) for all stream classes except stable baseflow (Fig. 4; Figs. S8–S10). In the Middle Atlantic Coastal Plains, we saw the highest richness values during annual minimum flows around 300 Julian days for perennial runoff streams (Fig. S11).

4. Discussion

We quantified relationships between biological and hydrologic metrics for fish and aquatic macroinvertebrates within wadeable stream classes. Our random forest models had a reasonable ability to predict the distribution of the biological metrics for all stream classes, with no metric being clearly better predicted than others. We observed many relationships among hydrologic and biological metrics, which is a first step toward developing recommendations for environmental flow standards in South Carolina and potentially in other geologically similar regions (Arthington et al., 2006). All components of the flow regime played a role in shaping ecological response for both taxa groups. Based on conditional importance, the magnitude, frequency, and duration of flow events had the greatest influence on the biological metrics. However, the response of aquatic organisms to hydrologic metrics varied across stream classes, highlighting the importance of accounting for differences in flow regime and ecoregion when designing environmental flow standards.

4.1. Flow-ecology relationships and components of the flow regime

All five components of the flow regime affected fish and invertebrate assemblage metrics, indicating that natural hydrological variability is critical for maintaining essential ecosystem goods and services and anthropogenic alteration to the flow regime would negatively impact aquatic biodiversity in SC (Arthington et al., 2006; Poff and Zimmerman, 2010). With South Carolina's population growing rapidly, increasing 11.3% between 2010 and 2019 (US Census Bureau 2021), human induced alteration of flow regime is expected to become a greater threat to aquatic biodiversity in the future. In addition, droughts, land use, and climate change will continue to exacerbate this problem, negatively impacting society and natural systems. Identifying stream segments that will be negatively impacted in the future would allow policy and managers to prioritize areas of conservation concern. However, this study did not identify specific stream segments with an increased threat of future hydrologic alteration and biological degradation. Predictions of future flow alterations and the associated ecological impacts as human population increases would greatly benefit managers and future water planning in SC.

Many flow-ecology relationships were consistent with prior understanding of the taxon-specific ecology. For example, mean annual flow (MA1) was noticeably the most important predictor of many fish biological metrics in our study, even after removing the pervasive effect of stream size. Other studies also observed a positive relationship between mean flow and biological metrics (Hayes et al., 2018; Mcmanamay et al., 2013a; Poff and Zimmerman, 2010; Rapp and Reilly, 2017). Reductions in mean stream discharge can lead to loss of aquatic habitat connectivity, refugia for aquatic species, changes in geomorphic complexity, alteration of habitat space, rise in water temperature, increased risk of anoxia, and other detrimental outcomes (Graf, 2006; Hayes et al., 2018; Welcomme et al., 2006). Overall, significant reductions in mean annual flow are likely to have negative effects on both fish and benthic macroinvertebrate assemblages.

In general, we found that higher magnitude high-flow events (MH20) reduced key fish and macroinvertebrate biological metrics and increased representation of tolerant macroinvertebrate taxa. This flow metric often had the greatest influence on the macroinvertebrate metrics. Sufficiently high-flow pulses can negatively affect benthic macroinvertebrates and fishes by dislodging benthic macroinvertebrates from the streambed, washing juvenile fishes downstream, reducing prey density, or increasing sediment load (Gido et al., 2013; Mcmanamay et al., 2013a). Extreme flows caused by climatic events such as typhoons or hurricanes, can strongly influence aquatic insect diversity and assemblage structure. Increased runoff due land cover change can cause unnaturally high-flow pulses and is often associated with lower and longer low-flow events and reductions in baseflow (Shuster et al., 2005). However, anthropogenic reduction in the magnitude of high-flow events via flow regulation can also negatively impact aquatic communities (Graf, 2006; Hayes et al., 2018; Webb et al., 2013). Ultimately, ecological responses to high flow events depend on how the timing, duration, and magnitude of high-flow events interact with the traits of the organisms responding to them (Gido et al., 2013; Mims and Olden, 2012). For example, flood-pulses can increase recruitment for broadcast-spawners fish species but reduced recruitment for species with other life history characteristics (King et al., 2003; Rodger et al., 2016; Zeug and Winemiller, 2008).

The frequency and duration of flow events were also particularly important for both macroinvertebrates and fish assemblages in this study, with frequency of high-flow events (FH1) consistently having a larger influence than other frequency and duration metrics. Response of macroinvertebrate and fish assemblages to the frequency and duration of low-flow events (FL1 and DL16) follows ecological expectations with FL1 and DL16 negatively related to biological metrics associated with diversity and positively related to tolerance metrics. Increased frequency and duration of low-flows can result in diminished water quality and aquatic biodiversity (Hayes et al., 2018). Higher frequency (FH1) and duration (DH15) of high flows tended to reduce biological metrics associated with diversity and increased tolerance metrics in our study. Increasing FH1 can cause habitats to become more unstable, higher watershed disturbance, and increased sediment transportation, resulting in the decline of fish and macroinvertebrates biodiversity (Kennen et al., 2008; Knight et al., 2008). However, the response of biological metrics to FH1 varied in our study. For example, frequency of high-flow events was positively associated with proportional representation of Megaloptera and Odonata individuals, but was negatively associated with macroinvertebrate taxa richness. Other studies find differential responses to high-flow frequency as well (Booker et al., 2015; Webb et al., 2013). For example, high-flow frequency reduced species richness and winter spawners but was positively related with periodic strategists (Bruckerhoff et al., 2019; George et al., 2021; Mcmanamay et al., 2013a). Alteration of high-flow frequency and duration can also cause deleterious effects on aquatic organisms by altering habitats characteristics and changing reproductive cues (Hayes et al., 2018; Lynch et al., 2019; Poff and Zimmerman, 2010; Webb et al., 2013). Our results corroborate the findings of other studies suggesting that the duration and frequency of flow events can strongly influence aquatic organisms and alteration of flow duration and frequency would likely affect aquatic organisms (George et al., 2021; Hayes et al., 2018; Poff and Zimmerman, 2010; Webb et al., 2013). Additionally, the magnitude, duration, and frequency of high and low flow events flow components will inevitably impact aquatic organisms to a greater extent as future global climate change further alters the hydrologic cycle (Wilhite et al., 2014; Xenopoulos et al., 2005).

The coefficients of variation in flow events (DH16, DL17, FH2, FL2, MA3, MA42, TL2, and TH2) were positively associated with fish and macroinvertebrate biological response metrics, consistent with findings of other studies (Gido et al., 2013; Iwasaki et al., 2012; Kennen et al., 2008, 2012; Knight et al., 2014; Poff and Allan, 1995). These metrics often better predicted the biological metrics better than the actual

flow metrics they describe. The positive relationships among variability of flow events (coefficients of variation in a flow metric) and biological integrity metrics may result from losses of weak competitors as hydrologic events stabilize (Hayes et al., 2018). However, studies report inverse relationship between flow variability and biological integrity of native species (Gido et al., 2013; Knight et al., 2014) while others suggest that flow variability may not be ecologically useful in areas with wide ranging flow regimes (Clausen and Biggs, 2000). For example, Kennen et al., 2012 observed decreases in annual flow variability diminished taxa richness. Consistent with other studies, we also found constancy of flow events (TA1) was an important predictor of the biological metrics, particularly for macroinvertebrate metrics (Bruckerhoff et al., 2019; George et al., 2021; Knight et al., 2008, 2014). This result suggests that fish and macroinvertebrates are responding positively to less disturbed and more stable conditions. High constancy or lower flashiness is associated with longer spawning season length, more habitat for spawning, less harsh habitats, and increased surface area for macroinvertebrate colonization and reproduction that could result in the positive relationship between biological integrity metric and TA1 seen in our study (Bruckerhoff et al., 2019; Knight et al., 2008; Poff and Allan, 1995). The timing of low-flow events was also an important predictor of the biological metrics in our study suggesting changes in the timing of flow events, such as altering TH1 by releasing high volumes of water, may adversely impact fish and macroinvertebrate assemblages by disruption of life-cycle cues. Similar to other studies, we also found that higher baseflow (ML17) increased macroinvertebrate biological integrity metrics (Zorn et al., 2012), suggesting reductions in baseflow could negatively impact aquatic organisms.

4.2. Inter- and intraregional variability

Although we saw similar trends in the direction of relationships between hydrologic and biological metrics, the shape of the flow-ecology relationships often differed across stream classes. For example, the relationship between fish Shannon diversity and MA1 varied among the stream classes with a linear relationship found for perennial runoff streams in the Piedmont but a logistic curve detected for perennial runoff streams in the Middle Atlantic Plains (Fig. S3). In addition, we often found relationships between hydrologic and biological metrics that were absent in the other stream classes. These results are unsurprising given the high variation in habitat type, geography, flow regime, and assemblage composition across SC. Variation in flow-ecology relationships across stream classes is congruent with other studies (Bruckerhoff et al., 2019; George et al., 2021; Monk et al., 2006; Poff et al., 2010; Praskievicz and Luo, 2020; Rapp and Reilly, 2017). For example, fish richness showed positive correlations with natural flow magnitudes in the coastal plains of the southeastern US, whereas no relationship was apparent in upland areas (Mcmanamay et al., 2013a). This pattern implies that biological assemblages in some flow regimes may be more sensitive to flow alterations than others.

The lack of relationships in some stream classes that were present in others may result from several factors. First, some biological metrics were not well represented in certain stream classes. For example, very few brood hiding fish species were collected from perennial runoff streams in the Southeastern Plains ecoregion, limiting our ability to detect relationships for certain metrics and areas if one existed. Second, some stream classes may display a relatively limited range of hydrological conditions and biological variation within a stream class compared to other stream classes. Third, the response of organisms to hydrology and strength of flow-ecology relationships can vary spatially and temporally (Bruckerhoff et al., 2019; Lynch et al., 2019), particularly during extreme hydrologic events such as drought (Lynch et al., 2018). Combining data sampled across different years that spans drought and flood years may diminish our ability to detect flow-ecology relationships since species response to flow may differ across years with

extreme hydrologic events. However, a review of flow-ecology relationships across space and time suggest that flow-ecology relationships are readily transferable across time (Chen and Olden, 2018). Fourth, the ecological response of aquatic organisms to natural changes in flow can differ from the ecological response to anthropogenic-induced flow alterations (Mcmanamay et al., 2013b). For example, fish in the South Atlantic Region of the US generally had positive ecology responses to increases in natural flow magnitudes, whereas no ecological response was observed for anthropogenic increases in flow magnitude (Mcmanamay et al., 2013b). Our study encompassed both altered and "natural" systems, potentially inhibiting the detection of flow-ecology relationships if they change dramatically with anthropogenically-induced flow variation. Fifth, some hydrologic metrics characterizing high and low flow events, are difficult to model with a degree of uncertainty (Eddy et al., in review). This potential source of modeling error may impact our ability to find flow-ecology relationships and should be considered when interpreting the results. The selection of hydrologic metrics for the development of environmental standards requires careful consideration, with model uncertainty being one critical aspect. For this study, we selected metrics with good performance (i.e., low simulation bias), minimal redundancy, and ecological relevance. However, not all metrics performed equally, with metric performance varying among metrics, flow regime components, and watersheds. Managers should also be aware of the variation in model performance and hydrologic estimation across ecoregions, stream classes, and low conditions when developing flow standards. Finally, while hydrology plays a strong role in determining aquatic assemblages, it may work in concert with many other environmental factors such as temperature, water quality, geomorphology, and landcover (Booker et al., 2015; Bruckerhoff et al., 2019; McManamay et al., 2013b, 2015b). Hydrologic factors often indirectly influence aquatic organisms through physical and/or chemical environmental factors, making it hard to untangle the mechanisms responsible for change in aquatic assemblages because flow-ecology relationships may be concealed or complicated by other environmental factors (Bruckerhoff et al., 2019; Monk et al., 2006).

Some flow-ecology relationships in this study were complex and sometimes unanticipated within regions. For example, percentage of brood hiding fishes was negatively related to mean daily flow, in contrast to our expectations. Brood hiders prefer areas with higher annual runoff in stable systems and are associated with high gradient streams (McManamay et al., 2015b; McManamay and Frimpong, 2015). The pattern seen in our study may be due to the strong association of brood hiders with high gradient streams. In our study area, high gradient streams with more brood hiding species, would be smaller with less daily discharge than low gradient streams. Another unexpected result was the positive relationship among nest-spawners and high-flow magnitude and frequency. With high parental care, we would expect nest-spawning to be advantageous in more predictable environments with less frequent flooding events during the spawning season (Winemiller, 2005). In addition, we presume large floods would increase the risk of nests being scoured away. However, nest guarding may be beneficial in unstable environments because nesting sites can be chosen in areas protected from frequent and high floods (Carlisle et al., 2011), and some nest-spawners can spawn multiple times even if nest are destroyed (Lukas and Orth, 1993). In addition, connection among main river channel and lentic habitats, where nest-spawners are abundant, may increase with high-flow magnitudes and frequency, allowing for great exchange nest-spawners among these habitats (Zeug and Winemiller, 2008). Expecting macroinvertebrate richness to decrease with longer low-flow durations, we instead observed a unimodal response. Species richness may increase as drought tolerant species enter the system until the selective pressures of low flow are strong enough to extirpate sensitive species, creating a unimodal response. Other studies noted that both tolerant and intolerant macroinvertebrates were able to persist in streams with reduced high-flows if their traits allow them to adapt to the environment (Brooks et al., 2011;

Carlisle et al., 2009). A similar pattern was observed for fish species in North Carolina, where fish abundance and diversity increase during drought as drought immigrants moved into the system (Grossman and Ratajczak, 1998).

4.3. Toward developing management recommendations

The flow-ecology relationships identified in this study provide a quantitative basis for implementation of environmental flow standards and offer managers a flexible framework to guide discussions on water permitting and management policy. Our approach allows managers to prioritize metrics for a proposed water withdrawal project, with metric selection based on which flow regime components will be altered, the stream class where the proposed project will be placed, and biological metrics of interest. Flow-ecology relationships of prioritized hydrologic and biological metrics can then be used to estimate the biological response to changes of the flow regime components within a given stream class. A major benefit to quantifying flow-ecology relationships at regional scale study is the potential to make predictions for unsampled stream segments within the same stream class (Olden et al., 2012; Poff et al., 2010). For example, a proposed water withdrawal from a stream segment within the Southeastern Plains stable baseflow stream class will reduce MA1 and increase DL16. Given the current flow conditions of the stream, managers can estimate the impact of these streamflow changes could have on prioritized biological metrics, helping managers determine the best management practices for sampled or unsampled streams.

It is important to account for the reliability of flow-ecology relationships because they vary depending on metric, taxonomic group, and stream class. Our hydrologic metrics were not able to predict biological metrics with high reliability across all stream classes, adding to the complexity of setting flow standards. For example, the predictive ability of most fish biological metrics was low in the Middle Atlantic Plains, but many models for benthic macroinvertebrates in the Middle Atlantic Plains performed well in this ecoregion. In this case, managers should use the benthic macroinvertebrates biological metrics rather than fish biological metrics when discussing flow standards in this area. This also occurs across stream classes. For instance, macroinvertebrate richness is well predicted in all stream classes except SE plains stable baseflow streams. Managers will need to prioritize biological metrics based on variance explained and metrics suited to their goals.

Our results reveal important differences in flow-ecology relationships both among and within ecoregions, demonstrating complexities of in flow-ecology relationships among flow regime types (Arthington et al., 2006; Kennard et al., 2010; Poff et al., 2010). In addition, many flow-ecology relationships occur in one stream class but not others. These inter-stream class differences emphasize the importance of tailoring environmental flow standards toward individual stream flow classes and their distinctive ecological communities. This is particularly true in areas with high taxonomic, geographic, and hydrologic diversity such as SC. Furthermore, subdividing streams based on both ecoregion and flow regime offers a means of not only increasing the predictive ability of models but also provides a more precise tool for the management of fluvial systems (Mcmanamay et al., 2013b). Creating a single statewide flow standard would likely over or under estimate the impacts of any given flow alteration. Developing flow standards adapted to each stream class would provide managers with a more accurate tool in which to predict the ecological impact of flow alteration. Additionally, all components of the flow regime (i.e. magnitude, frequency, duration, timing and rate of change of flow events) affected numerous aspects of both fish and benthic macroinvertebrate assemblages in all ecoregions and flow classes, implying the use of a single metric in the development of flow standards would not adequately protect riverine biodiversity (Bunn and Arthington, 2002; Poff et al., 1997). Instead, managing instream flow to protect all aspects of the natural flow regime will likely be a more effective approach.

The goal of many environmental flow standards is to determine a static water withdrawal threshold or "rule of thumb". The idea of straightforward thresholds that are consistent and transparent is attractive. However, thresholds neglect our fundamental understanding of the natural flow regime and the vital role of flow variability plays in maintaining essential riverine ecosystem goods and services and limits the flexibility in creating more comprehensive flow standards (Arthington et al., 2006). In areas with varying and complex flow-ecology relationships such as SC, static flow standards can hinder managers' ability to determine unacceptable level of risk of degradation. Although random forests can detect and fit non-linear relationships, we observed few obvious thresholds that indicated a distinct change in the biological condition of fish and macroinvertebrates along hydrologic gradients. Many of the relationships in our study were linear or wedge-shaped (Knight et al., 2014). These relationships avoid the pitfall of creating a minimum flow target that can be easily applied but may actually cause harm to river ecosystems (Annear et al., 2004; Arthington et al., 2006). Instead, managers can determine acceptable levels of flow alteration and biodiversity degradation within each stream class, using prioritized biological metrics and hydrologic metrics.

Although our data covered some fifth and sixth order streams, we were unable to model flow-ecology relationships for large rivers. Only wadeable streams and smaller rivers, sampled using standardized methods, were included in this study. Sampling of large rivers requires different gear types and sampling methods, and merging data with such different sampling methods and frameworks could bias results. Moreover, the concept of ecosystem health may be defined much differently for large rivers, being potentially less associated with biotic community structure and more associated with other ecosystem services such as water quality or food production (Yates et al., 2019). However, in SC much of the direct surface water withdrawals and large-scale hydrologic alterations from impoundments occur in larger rivers. Accordingly, a logical next step in this will be to analyze large river systems in SC, comparing and contrasting the result obtained in this study. Another data limitation of this study was the lack of sites in the Blue Ridge and Southern Coastal Plains ecoregions. Robust hydrologic models could not be created for the Coastal Plain due to the tidal influence in this area, and few biological sites were available for the Blue Ridge because it covers such a small portion of the state. However, important hydrologic metrics in the Piedmont may be similar to the Blue Ridge (Praskievicz and Luo, 2020).

Ultimately, management policy that minimizes changes to the natural flow regime will help maintain the essential ecosystem goods and services of freshwater streams. However, environmental flows require balancing human water use needs with the needs of riverine ecosystem. Quantifying flow-ecology relationships are an initial step toward improving environmental flow standards. Here, we provide a flexible framework based on statistical flow-ecology relationships using empirical data from which managers can enhance environmental flow standards for SC and assess the impact of stream flow alteration on aquatic assemblages.

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CRediT authorship contribution statement

Luke Bower: Conceptualization, Methodology, Formal analysis, Writing - Original Draft. **Brandon People**: Conceptualization, Methodology, Writing- Original draft preparation. **Michele Eddy**: Conceptualization, Data Curation, Methodology. **Mark Scott**: Conceptualization, Writing - Review & Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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