

Identifying algal indicators for streamflow duration assessment methods in forested headwater streams

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ABSTRACT

Streamflow duration assessment methods (SDAMs) are rapid tools for reach-scale streamflow duration classification. SDAMs use single-visit indicator data as surrogates because direct measurement of long-term flow duration is resource intensive. Algae (diatoms and soft-bodied algae) are potentially strong indicators of streamflow-duration class (SDC) because of geographical ubiquity and biodiversity across moisture gradients. Algal cover and algal assemblage data (presence/absence, density, and biovolume) at species- and genus-levels were analyzed from 508 samples across 22 ephemeral, 37 intermittent, and 51 perennial reaches distributed along 31 forested headwater streams within 4 ecoregions in the contiguous United States. Random forest models using species- and genus-level datasets to predict SDCs had classification accuracy ranging from 69.5% – 88.4%, with accuracy being highest for density, intermediate for presence/absence, lowest for biovolume data. Species-level models had 0.6% – 5.9% higher accuracy for density and presence/absence datasets than genus-level models. Based on their median ranks of minimum node depth among random forest models, season (wet vs dry) was a more important factor than habitat sampled (erosional vs depositional) when distinguishing ephemeral, intermittent and perennial but habitat was more important when distinguishing ephemeral from non-ephemeral reaches. Algal cover index (categorical visual-tactile assessment) was the most important indicator of SDC. Except for the red alga, *Audouinella*, the identified indicator taxa were diatoms. Our findings support epilithic algal cover as an SDAM indicator for forested headwater streams. Until taxonomic tools are available for field identifications, algal cover index and abundance of soft-bodied algae and live diatoms may be candidate indicators for regional SDAMs.

1. Introduction

Streamflow duration describes the length of time and timing of surface flow in stream channels and is controlled by natural and anthropogenic factors (Shanfield et al., 2021; Datry et al., 2023). Streamflow is a fundamental process that controls the transport of resources, pollutants, and services across landscapes, and is also paramount to the habitat template in which aquatic organisms have evolved (Hart and Finelli, 1999; Lytle and Poff, 2004). Therefore, zero flow periods or complete drying, especially if extended, strongly affect the distribution, abundance, and biodiversity of aquatic organisms (Jaeger

et al., 2014; Dalibard et al., 2021).

Different terms have been used to classify streamflow duration, but perennial, intermittent, and ephemeral are the most widely used (Busch et al., 2020). Perennial reaches have continuous surface flow and do not experience recurrent drying except during severe drought. Intermittent reaches have surface flow for part of the year, typically when the streambed is below the water table and/or when snowmelt from surrounding uplands provides sustained flow. Lastly, ephemeral reaches flow only in direct response to precipitation and the streambed elevation is always above the water table. Headwater streams can have perennial reaches, but many reaches are ephemeral or intermittent and contribute

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significantly to the length and discharge in river networks (Fesenmeyer et al., 2021; Brinkerhoff et al., 2024). Because of their abundance and position on the landscape, headwater streams represent a significant interface between uplands and downstream water through the transport, storage, and transformation of materials, and by providing habitat, migratory corridor, and refuge for diverse biota.

Although headwater streams are widespread and abundant, they are rarely gauged (Krabbenhoft et al., 2022), and often poorly mapped (Benstead and Leigh, 2012; Stanislawski et al., 2015). While there have been successful efforts to use remote sensing to map surface water extent in large channels in open landscapes (e.g., Dralle et al., 2023; Cavallo et al., 2025), image resolution, cloud cover, and density of tree canopies can limit the use of remote sensing for classifying the streamflow of headwater streams over extensive areas in many regions (Christensen et al., 2022). Without continuous streamflow data and maps, there is a need to provide accurate SDCs to rapidly inform management decisions for headwater streams. Streamflow duration classes (SDCs), like perennial, intermittent, and ephemeral, are used to support management decisions related to water-quality standards, riparian buffer widths, mitigation, and jurisdictional determinations (Fritz et al., 2017; Messenger et al., 2024; USEPA, 2025). Streamflow duration assessment methods (SDAMs) are rapid field assessments that use indicators to classify streamflow duration at the reach scale (Fritz et al., 2020). Indicators can be physical or biological, and they reflect SDCs because they respond to or control the duration of streamflow.

Epilithic algae (primary producers lacking true tissues, including diatoms, cyanobacteria, green algae, and other soft-bodied forms that live on coarse streambed substrates) are sensitive to environmental changes, support freshwater food webs, and are easy to collect in rocky streams. For these reasons epilithic algae have been extensively used as bioassessment indicators in streams (Stevenson, 2014; Smucker et al., 2024). The distribution of most algal groups spans terrestrial to permanent aquatic habitats, with species being associated with differences in moisture preferences and adaptations (Potts, 1999; Holzinger and Karsten, 2013). Water and its flow are vital to stream algae in several ways. Algae use water for photosynthesis, physical support, reproduction, and nutrient acquisition so desiccation halts processes and can increase exposure to some stressors, such as UV light. Flow can enhance the rate of nutrient exchange, downstream dispersal, and protection from invertebrate grazers (Hart and Finelli, 1999), so periods without flow can be limiting for some stream algae (e.g., Colls et al., 2019; Terada et al., 2025). Other algae have physiological and morphological adaptations to periods with no flow or water (e.g., Timoner et al., 2014; Jiménez et al., 2023). Compared to other biological indicators, like invertebrates and fish, algae are not well characterized in intermittent and ephemeral streams (Novais and Morais, 2024) particularly in North America (Taurozzi et al., 2024; but see Pfister et al., 2015; Busch et al., 2024).

Our study had 3 objectives. The first objective was to identify algal taxa and composite indicators (e.g., total algal cover, total density, total biovolume) that best distinguish among ephemeral, intermittent, and perennial reaches of forested headwater streams in different ecoregions. Our second objective was to determine if different taxonomic resolutions (diatom genus and species) and numeric resolutions (presence, density, and biovolume) affect classification accuracy. Our third objective was to assess the relative influence of season (wet vs dry) and habitat (depositional vs erosional) on classification accuracy.

Based on previous studies comparing algal communities between perennial and intermittent reaches, we expect stronger difference between ephemeral reaches and intermittent and perennial reaches than between intermittent and perennial reaches (e.g., Colls et al., 2019; Tornés et al., 2021; Busch et al., 2024). We also expect season to influence the degree of algal compositional differences across SDCs, in part because hydrological differences would be greater during the dry season (when intermittent and ephemeral reaches are more likely to be dry or have isolated pools; Falasco et al., 2016; Burgazzi et al., 2020) than

during the wet season (when intermittent and ephemeral reaches are more likely to have longer periods of surface flow). We expect habitat (depositional vs erosional) to influence algal compositional differences among SDCs because loss of surface flow is likely to have more impact on taxa of erosional than depositional habitats (Peterson, 1987) and depositional habitats are expected to retain surface water longer than erosion habitats, but we expect habitat to have less influence on compositional differences among SDCs than season (dry vs wet). Lastly, we expect the degree of algal compositional differences among SDCs would vary across regions because previous studies have shown algal assemblages changes predictably based on geographic location (Potapova and Charles, 2002; Soininen, 2007), supporting the need for regional stratification for accurate classification of headwater streams.

2. Methods

2.1. Study area

To address our study objectives, a field study was conducted that compared the taxonomic composition of algal assemblages at 22 ephemeral reaches, 37 intermittent reaches, and 51 perennial reaches in 31 forested headwater streams distributed across 10 mesic forests. The 10 study forests spanned 4 Level II ecoregions (Omernik, 1995) and 9 different U.S. states (Table 1, Figure S-1). Ecoregions are areas containing similar ecosystems (physical and biotic characteristics), including similar type, quality, and quantity of environmental resources (climate, geology, hydrology, landforms, soils, vegetation, wildlife, and human factors). Ecoregion classification is hierarchical, such that they can be used for assessment at continental (Level I), national (Level II), and regional/local (Level III) scales. Algae were sampled during the wet (April and May) and dry (August and September) seasons. Four forests (17 streams in ecoregions 8.3 and 8.4) were sampled during 2003 and 2004, whereas 6 forests (14 streams in ecoregions 5.3, 6.2, 8.3, and 8.4) were sampled in April and May 2005 and August and September 2004 or 2005 (1 y per forest). Study catchments had >90% forest cover and drainage areas <300 ha (see Fritz et al., 2008 for more details). No existing hydrologic data were available for the study reaches, so we identified reaches based on longitudinal position (or drainage area) along the streams to capture varying streamflow duration. Streamflow duration was expected to increase with increasing storage capacity and recharge potential that increases with drainage area in gaining streams. We identified 2 to 4 study reaches (30-m length) along each study stream. Different number of reaches were identified because stream length differed among study streams. Consecutive reaches were typically positioned upstream and downstream from tributary confluences, so that drainage area at the study reaches increased nonlinearly with longitudinal position. The mean channel length between study reaches was 383.8 m (± 42.6 , ± 1 standard error).

2.2. Field sampling

Flow status (continuous surface flow, interstitial flow, isolated pools, or dry) was recorded on each visit. The combination of flow status from wet and dry seasons were used to categorize reaches into SDCs. Perennial reaches had continuous surface flow or interstitial flow on every visit. Intermittent reaches had continuous surface flow or interstitial flow on at least 1 visit. Ephemeral reaches did not have flow on any visit. A subset of reaches (71/110) had continuous data (ranging from 71 to 351 d) from electrical resistance data loggers that recorded streamflow duration in the channel thalweg of erosional habitat (Table S-1, Fritz et al., 2006). Based on these data, we classified reaches that were flowing >90% of the time as perennial, flowing 10 to 90% as intermittent, and <10% of the time as ephemeral. Classification based on flow statuses during site visits and data logger agreed in 75% of study reaches (90.6% agreement for perennial, 58.3% for intermittent, and 66.7% for ephemeral). The SDCs used in analyses were based on the combination

Table 1

Study locations across the United States. Shown are study years, number of streams per forest, number of perennial, intermittent, and ephemeral reaches (R), corresponding number of composite samples (S), and Level II ecoregion names and codes (Omernik, 1995).

Study location	Year	Streams	Perennial		Intermittent		Ephemeral		Level II ecoregion	
			R	S	R	S	R	S	Name	Code
Illinois (IL)	2004–2005	4	1	4	9	36	4	11	Southeastern USA Plains	8.3
Indiana (IN)	2003–2004	4	0	0	11	71	4	24	Southeastern USA Plains	8.3
Kentucky (KY)	2003–2004	5	6	43	8	48	4	24	Ozark, Ouachita-Appalachian	8.4
South-central Ohio (SCOH)	2003–2004	4	9	49	0	0	4	16	Ozark, Ouachita-Appalachian	8.4
Southeastern Ohio (SEOH)	2003–2004	4	8	46	5	19	2	6	Ozark, Ouachita-Appalachian	8.4
New Hampshire (NH)	2005	1	2	4	2	4	0	0	Atlantic Highlands	5.3
New York (NY)	2004–2005	2	6	10	0	0	0	0	Atlantic Highlands	5.3
Vermont (VT)	2005	1	2	8	1	4	1	4	Atlantic Highlands	5.3
Washington (WA)	2004–2005	2	5	20	1	4	0	0	Western Cordillera	6.2
West Virginia (WV)	2004–2005	4	12	46	0	0	3	7	Ozark, Ouachita-Appalachian	8.4
Total		31	51	230	37	186	22	92		

of flow status during study visits. Following Fritz et al. (2006), we measured the following environmental variables: water depth, wetted width, current velocity, modal bed particle size (β -axis), macroinvertebrate grazer density, macroinvertebrate grazer biomass, macroinvertebrate predator density, macroinvertebrate predator biomass, percent canopy cover, discharge, water temperature, dissolved oxygen concentration, pH, specific conductivity, proportion of erosional habitat, drainage area, catchment percent forest cover, depth to bedrock, channel sinuosity, reach slope, and entrenchment ratio.

Six individual stones were selected from the thalweg of each habitat type (depositional and erosional) across multiple habitat units. The habitat units from which stones were selected had the dominant hydrologic condition (flowing, standing water, or dry) for the habitat type within the reach. Many reaches had riffle-pool sequences, but some high gradient reaches had step-pool sequences. Erosional and depositional habitats from which stones were collected were designated based on streambed topography, substrate size, and distribution of organic matter. A 12-cm² area was scraped from the upper surface of each stone and composited into separate erosional and depositional samples and preserved with 10% formalin (see Fritz et al., 2006 for a detailed sampling description).

A visual and tactile Algal Cover Index (ACI) was scored each visit to characterize the algal cover within the study reaches (Table 2; Feminella and Hawkins, 2000; Fritz et al., 2006). The ACI score for at least 25 stones in the thalweg were measured equidistantly along the entire reach and the modal score across stones within the reach was recorded. Higher ACI scores reflect increasing thickness of epilithic algae and were expected to increase with longer streamflow duration (i.e., perennial > intermittent > ephemeral). Although the assessor did not know the SDC of the reach at the time ACI measurements, the hydrological status (flowing, dry, isolated pools) was known, so as for any of the algal indicators we measured, there was potential for observer bias.

2.3. Laboratory analyses

Samples were concentrated to 20 ml, vortexed for 60 s, and split into 2 equal subsamples: “soft-bodied algae” (i.e., Chlorophyta,

Cyanobacteria, Rhodophyta, Pyrrophyta) and a diatom identification subsample. The soft-bodied algae subsample was preserved with formalin (5%), mixed for 20 s, and pipetted (0.1 mL) into a Palmer-Maloney chamber, after which at least 300 living algal counting units (colony, filament, or diatom cell containing protoplast material) were counted using a Zeiss Axioskop compound microscope at 400x magnification (minimum of 1 complete, randomly selected transect). The individual soft-bodied algae filaments were counted if they could be discerned at 400x magnification. However, if individual filaments could not be discerned then each 10- μ m length of filament in the field was counted. Diatoms were counted as living or dead and all other algae were enumerated and identified to lowest possible taxonomic level, typically genus in the soft-bodied algae subsample. A separate diatom subsample was cleaned with concentrated sulfuric acid and rinsed with deionized water to remove organic matter in the diatoms and reveal morphological characteristics used for identification (Hasle and Fryxell, 1970). Then permanent diatom slides were prepared using Naphrax mounting medium (Northern Biological Supplies Limited, Ipswich, England). Using a Zeiss Axioskop compound microscope, at least 500 diatom valves were counted and identified (typically to species) at 1000x magnification (minimum of 1 complete, randomly selected transect). The primary reference for soft-bodied algae taxonomy was Wehr and Sheath (2003) and the primary references for diatom taxonomy were Patrick and Reimer (1966, 1975) and Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b). All taxonomic identifications and counts were made by a single taxonomist (SJD). Taxonomy was updated (see Table S-2) by consulting online resources Diatoms of North America (Spaulding et al., 2021), and Algaebase (Guiry and Guiry, 2021).

In concert with the soft-bodied algae and diatom counts and identifications, algal unit dimensions were measured for biovolume determination. Dimensions were measured with an ocular micrometer from 5 algal counting units for each taxon occurring in $\geq 5\%$ of the total abundance within a sample. Dimensions measured and associated biovolume equations were based on Hillebrand et al. (1999) and Sun and Liu (2003). Where a dimension value (depth for diatoms) could not be measured, the associated taxon-specific modal values were used from the Academy of Natural Sciences of Drexel University's Phycology Section database for U.S. Geological Survey's National Water-Quality Assessment Program (Charles et al., 2002).

2.4. Data analysis

We calculated total algal cell density, total algal biovolume, percent soft-bodied algae, percent live diatoms, and percent aerial diatom taxa as composite algal indicators. Except for percent aerial diatom taxa, we expected that the composite algal indicators would be highest in perennial, intermediate in intermittent, and lowest in ephemeral reaches. Aerial diatoms are those taxa with moisture indicator values of 4 or

Table 2

Algal Cover Index (ACI) scores and associated characteristics.

ACI score	Visual and tactile characteristics
1	Substrate is rough with no apparent growth
1.5	Substrate is slimy, but biofilm not visible (i.e., fingernail scratch leaves no visible track)
2	Thin layer visible (0.5–1 mm thick, i.e., fingernail scratch visible)
3	Algal mat thickness 1–5 mm thick and filamentous algae rare
4	Algal mat thickness 5–20 mm thick and filamentous algae common
5	Algal mat thickness >2 cm or filamentous algae dominates

5 in Mertens et al. (2025), where 4 indicates “mainly occurring on wet and moist or temporarily dry places” and 5 indicates “nearly exclusively occurring outside water bodies.” The percent of aerial diatom taxa was expected to be higher in ephemeral than in non-ephemeral reaches. Mertens et al. (2025) included moisture indicator values for 228 of 298 diatom species identified in our dataset (Table S-2). Of the 70 diatom species in our dataset that did not have moisture indicator values in Mertens et al. (2025), 57 species occurred in <10% of the study samples.

We used four-way mixed effects ANOVA in R (*lme4* package, version 4.4.1; R Project for Statistical Computing, Vienna, Austria) to compare composite algae measures (total density, total biovolume, percent abundance of soft-bodied algae, percent abundance of live diatoms, and percent of aerial diatom taxa) among 4 fixed factors: habitat (erosional or depositional), SDC (ephemeral, intermittent, or perennial), season (wet or dry), and Level II ecoregions (5.3, 6.2, 8.3, or 8.4). Habitat was not included as a factor in a three-way mixed effects ANOVA for ACI because ACI was measured for the entire reach (across erosional and depositional habitats) during each visit. A fully nested, random effects structure resulted in overfitting, so the model used did not include the 4-way interaction, and to account for the hierarchical nesting of the sampling design, we used the interaction of forest, stream, and reach as a single random intercept factor to control for non-independence of localized sampling units without destabilizing the variance matrix. In addition, because the dataset lacked ephemeral reaches in Ecoregion 6.2 (Table 1), any detected SDC fixed effect or interaction reflecting differences related to ephemeral reaches would be attributed to the other three ecoregions. Residual plots of fitted vs estimated values and normal Q-Q plots were checked for normality of residual distributions. Total algal density and ACI were log₁₀-transformed; percent soft-bodied algae was square root-transformed; and percent live diatoms was arcsin-transformed prior to analysis. Non-metric multi-dimensional scaling (NMDS) was used to visualize algal compositional patterns based on Bray-Curtis distance of root-root transformed densities of taxa that represented at least 2% in at least 1 sample (156 of 359 taxa across 508 samples). Relationships between NMDS axes scores and environmental variables were assessed using Spearman rank correlation. A permutational multivariate analysis of variance (PERMANOVA) with a mixed effects design was used to test for differences in algal taxonomic composition between habitat (depositional and erosional), SDC (ephemeral, intermittent, and perennial), seasons (wet and dry), and Level II ecoregions (4 ecoregions) based on Bray-Curtis distance of root-root-transformed taxa densities. The fixed factors in the design were habitat, SDC, season, and Level II ecoregion. Unique combinations of stream (31 streams) and forest (10 forests) (stream+forest) was treated as a random factor nested within Level II ecoregion, and all associated interaction terms were also tested. The PERMANOVA was based on 9999 permutations and was done using Primer 7 PERMANOVA+ (Anderson et al., 2008).

We used random forest (RF) classification (Cutler et al., 2007) to identify species- and genus-level taxa and composite measures (ACI, soft-bodied algae, diatoms) as indicators for 3 SDCs (perennial, intermittent and ephemeral reaches) and to distinguish between 2 SDCs (ephemeral from non-ephemeral reaches). Models were developed to distinguish 2 SDCs because distinguishing between ephemeral and non-ephemeral reaches was a high priority use of the SDAMs to inform some management decisions. RF was also used to 1) assess if numeric (presence/absence, density, biovolume) or taxonomic (diatom species or genus) resolution influenced streamflow-duration classification accuracy and 2) assess the relative influence of sample habitat type (erosional and depositional), season (wet or dry), or composite measures (ACI, soft-bodied algae, diatoms) on the classification accuracy. We included habitat type and season in RF to better understand their relative importance for the predictive ability of algal taxa rather than to interpret these factors directly as indicators of SDCs. Our analyses included 12 RF models that used algal indicators to predict SDCs (Table S-6). We used the ranger (version 0.14.1; Wright et al., 2022) and

randomForestExplainer (version 0.10.1; Paluszynska et al., 2020) packages in R (version 4.0.2) to fit models having 500 decision trees. The tuneRanger package was used to optimize settings for mtry (1700) and min.node.size (2). We used the default out-of-bag settings (100% of data with replacement) to estimate classification accuracy for each model.

Imbalanced data can influence RF models because RF fitting is biased toward the classification accuracy of the dominant class over the minority class (Chen et al., 2004; More and Rana, 2017). Our dataset was imbalanced with respect to SDC (perennial – 45.2%, intermittent – 36.6%, and ephemeral – 18.1%, Tables 1 and S-3), so we balanced the decision tree by subsampling across classes during model fitting with evenly weighted random sampling with replacement to prevent bias associated with the imbalanced dataset. Samples were also unevenly distributed across ecoregions (Eco 5.3 – 6.7%, Eco 6.2 – 4.7%, Eco 8.3 – 28.7%, and Eco 8.4 – 59.8%; Table 1), but sample sizes for some combinations of SDC and ecoregion prevented concurrent balancing. We present the indicator results by ecoregion to evaluate potential limitations.

The results across sets of RF models were integrated to address our objectives. Indicators for each of the 3 numeric resolutions (presence/absence, density, biovolume) were identified by ranking the taxa according to how well they predicted SDC in a random forest model. A decision tree identifies predictors that are best able to split a dataset into distinct groups. The first split in a decision tree (attributed to a specific predictor) has a node depth = 0. A lower node depth usually is associated with greater classification ability because predictors identified earlier in a decision tree can classify data more accurately (Paluszynska et al., 2020). Across the 500 decision trees in each RF model, we calculated mean minimal node depth for each predictor (based on only that predictor's first appearance if it appeared multiple times in a decision tree). Because the units of mean node depths are not meaningful for comparison across RF models, we compared RF models by assigning a rank of 1 to the taxon within each RF model with the lowest mean minimal node depth, a rank of 2 to the next lowest, and so on. We calculated Spearman correlation coefficients comparing modeled sets of ranks to assess consistency among the 3 numeric resolutions. Then, for each taxon, we determined median rank across all 3 numeric resolutions. We assessed the relative importance of predictors (habitat, season, and composite measures) on the classification by comparing their median rank of mean node depth across RFs for 3 numeric resolutions. Algal taxonomic indicators were identified as those predictors having >10% difference in percent presence in samples among SDCs. This approach was also applied to compare diatom species + soft-bodied algae genus and diatom genus + soft-bodied algae genus taxonomic resolutions.

3. Results

A total of 359 taxa (29 soft-bodied algae and 330 diatoms), including 13 Chlorophyta genera, 9 Cyanobacteriota genera, 1 Pyrrophyta genus, 1 Rhodophyta genus, and 297 diatom species (79 genera) were identified. The remaining 38 taxa represented undetermined specimens that could not be identified to genus for soft-bodied algae (5) or to a described species for diatoms (33), and except for unverified specimens originally identified as *Diploneis parva* Cleve, did not have mean relative abundance or mean relative biovolume of >1%. Excluding undetermined specimens, the mean diatom richness was 18 species per sample and the mean soft-bodied algae richness was 1 genus per sample. Across all samples, 194 taxa were identified from ephemeral reaches, 256 taxa from intermittent reaches, and 304 taxa from perennial reaches. Diatoms (living and dead), on average, had a relative abundance of 88.1% in all samples with 65.1% being living diatoms. The diatom *Achnanthes minutissimum* was the most abundant species in each SDC, representing >30% of algae on average in the samples and occurring in >78% of samples collected in each SDC. *Eunotia exigua* (8% to 22%) and *Gomphonema parvulum* (4% to 13%) were the next most abundant

diatoms. These 3 diatom species also averaged >10% of the total algal biovolume measured. The most abundant soft-bodied algae collected were the red alga, *Audouinella* (0.6% to 8% abundance) and the cyanobacteria, *Oscillatoria* (2% to 5%) and these were also highest in biovolume among the soft-bodied algae collected (0.7% to 12.8% and 1.1%

to 1.9%, respectively). Chlorophytes were sporadic and typically rare (<1% abundance) in each SDC. There were 43 diatom and 2 soft-bodied algae taxa (*Audouinella* and *Oscillatoria*) that occurred in samples from all Level II ecoregions and only 16 diatom and no soft-bodied algae taxa that occurred in samples from all 10 study forests.

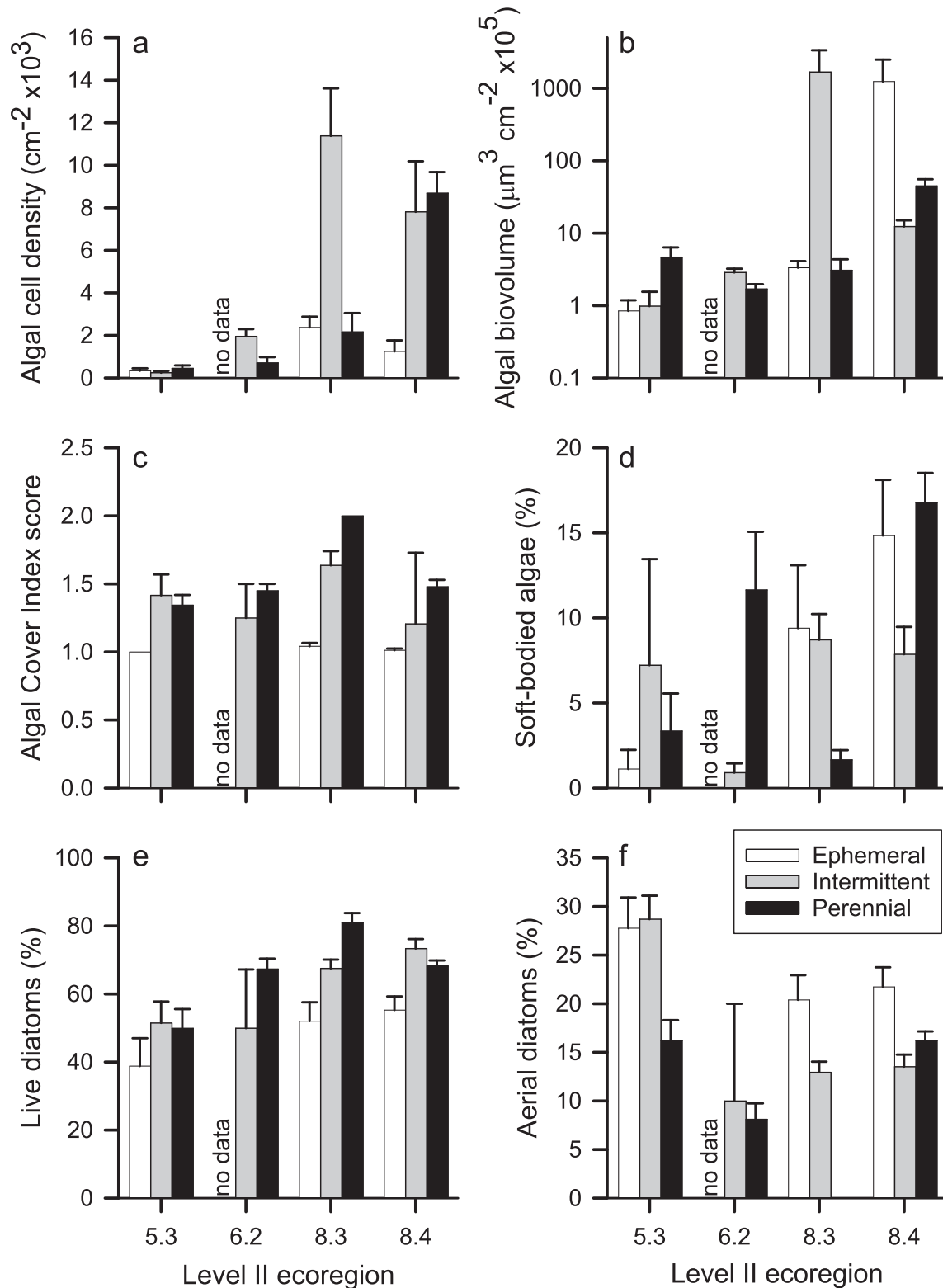


Figure 1. Mean (± 1 standard error, SE) (a) algal density, (b) algal biovolume, (c) Algal Cover Index score, (d) percent soft-bodied algae by density, (e) percent live diatoms by density, and (f) percent aerial diatom taxa by flow duration class displaying variation across Level II Ecoregions (codes labeled on x-axis). No ephemeral reaches were sampled in Ecoregion 6.2.

Significant differences among SDCs were detected for percent abundance of live diatoms, percent abundance of aerial diatoms, and ACI scores (Table S-4); however, significant interactions between SDC and Level II ecoregion (Figure 1) or season (Figure 2) indicate that any differences among SDCs for these measures were inconsistent. The average percent abundance of live diatoms was higher in the wet season than dry season at ephemeral and intermittent reaches but was comparable between seasons at perennial reaches (Figure 2b). Total algal biovolume (Figure 1b) and percent of soft-bodied algae (Figure 1d) were not detectably different among SDC or for any other factor (Table S-4). Total algal density was higher in depositional habitats ($8213 \text{ cells cm}^{-2} \pm 1224.3$, mean $\pm$ 1SE) than in erosional habitats (5761 ± 674.9 ; $F_{1, 367} = 5.3$, $p = 0.02$) across seasons, SDCs, and ecoregions. Percent of aerial diatom taxa was higher in the dry season ($20.5\% \pm 0.8$) than in the wet season ($11.6\% \pm 0.7$; $F_{1, 386} = 13.1$, $p = 0.0003$) for all SDCs. Dry season ACI scores (1.24 ± 0.03) were lower than wet season ACI scores (1.50 ± 0.06 ; $F_{1, 411} = 10.33$, $p = 0.001$). Most (83.2%) ACI scores reflected no detectable epilithic growth (ACI = 1) or only tactilely detectable growth (ACI = 1.5). Visible growth (ACI > 1.5) was never scored at ephemeral reaches.

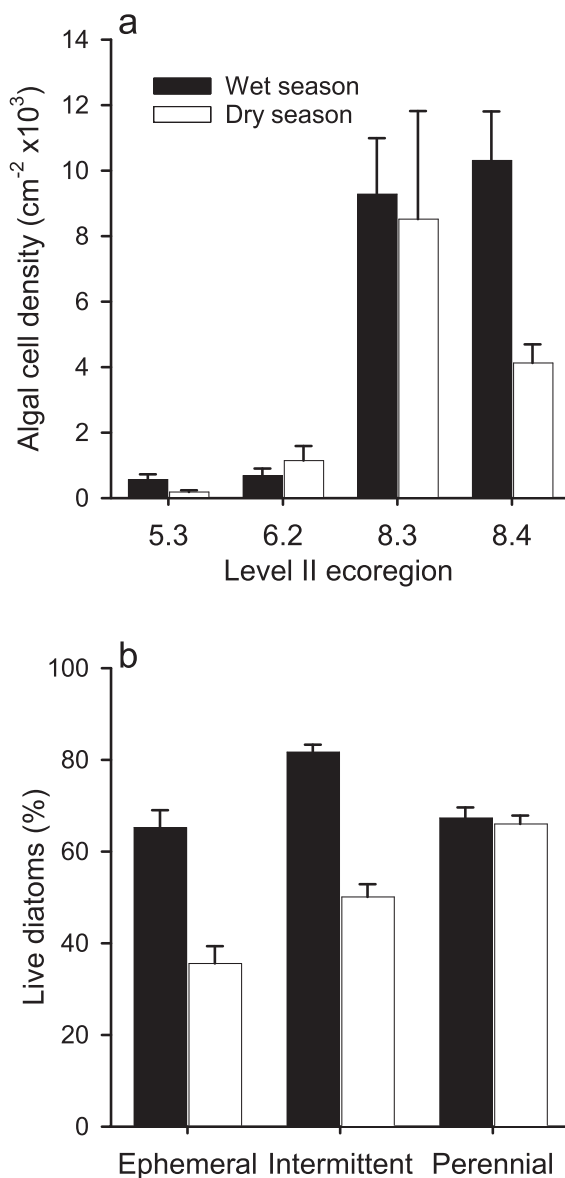


Figure 2. Mean ($\pm$ 1 standard error, SE) (a) algal density by season, and (b) percent live diatoms by density across streamflow duration classes.

The NMDS analysis (2-dimensional, stress = 0.2) did not show clear separation between algal assemblages from erosional and depositional habitats (Figure 3a). Assemblage composition was most clearly organized by forest and Level II ecoregion (Figure 3d) with separation between seasons (Figure 3b) and SDC (Figure 3c) less clear and nested within forests. The environmental variables most strongly correlated to axis 1 were pH ($r_s = -0.52$), specific conductivity ($r_s = -0.48$), and ACI ($r_s = -0.37$) (Figure 3d). There were no detectable differences in taxonomic composition among habitats and SDCs, but there were significant differences detected among seasons and ecoregions as well as interactions among SDCs, seasons, and streams + forests (ecoregions) in the PERMANOVA analysis (Table S-5), confirming NMDS patterns.

The overall classification accuracy ranged from 69.5 to 88.4% across the 12 RF models (Table 3). Overall classification accuracy of RF models varied by numeric resolution more so for the 3 SDCs (ephemeral, intermittent, and perennial) models than 2 SDCs (ephemeral and non-ephemeral) models. Overall classification accuracy of RF models for 3 SDCs based on algal presence/absence and density was 6.1% to 11% higher than models using algal biovolume for species- and genus-level taxonomic resolutions (Table 3). The overall classification accuracy of RF models for 2 SDCs based on biovolume was more comparable (only 1.9% to 2.2% lower) to those based on presence/absence and density. The taxonomic resolution for diatom identification had less effect than numeric resolution. RF models for 3 SDCs using diatom species-level identifications had 1% to 5.9% higher overall accuracy than models using diatom genus-level identifications. The increased accuracy using diatom species-level over genus-level identification was only 0.6% to 1.2% for the RF models developed for 2 SDCs. All RF models for 3 SDCs had higher classification accuracy for perennial reaches than for intermittent reaches, but not always when compared to ephemeral reaches (Table 3), and this was also reflected in the 6.1% to 15.1% higher overall classification accuracies of the RF models for distinguishing ephemeral from non-ephemeral.

The classification accuracy using different taxonomic and numeric resolutions varied among Level II ecoregions to a greater extent for RF models for 3 SDCs than models for 2 SDCs (Table 3). The RF models for 3 and 2 SDCs using presence/absence and density with species- or genus-level diatom taxonomy had higher classification accuracy than the accuracy of the models using biovolume for ecoregions 8.3 and 8.4. The RF model for 3 SDCs using biovolume and genus taxonomy had the highest classification accuracy for ecoregion 5.3. The RF models for 3 SDCs using presence/absence and density with species taxonomy had the highest accuracy for ecoregion 6.2, and accuracy was comparable across numeric resolutions for the RF models for 2 SDCs for this region that did not have any ephemeral study reaches. Species-level instead of genus-level taxonomy for diatoms improved classification of presence/absence-based models for 3 SDCs in 3 of 4 ecoregions, with the highest improvement being 20.6% in ecoregion 5.3. Classification accuracy of the presence/absence-based model for 3 SDCs was higher using species-level than genus-level taxonomy in ecoregion 5.3 but accuracies of density- and biovolume-based models were higher for genus-level taxonomy (Table 3).

Median ranks of minimum node depth among RF models were more strongly correlated between presence/absence and density ($r_s > 0.89$) than with biovolume ($r_s < 0.69$) for species- and genus-level taxonomy and RF models for 3 and 2 SDCs (Table S-7). The ACI score had the top median rank of minimum node depth for all RF models (Tables 4 and 5). Based on their median ranks of minimum node depth among RF, season was a more important factor than habitat in RF models for 3 SDCs (Table 4), and habitat was a more important factor in RF models for 2 SDCs (Table 5). Based on the percentiles associated with the ranks of minimum node depth, season and habitat were less important in models using cell density (17.9–63.9%) than in models using presence/absence and biovolume (5.4–27.9%). Season and habitat were relatively more important in models using species taxonomic resolution for diatoms than models using genus taxonomic resolution.

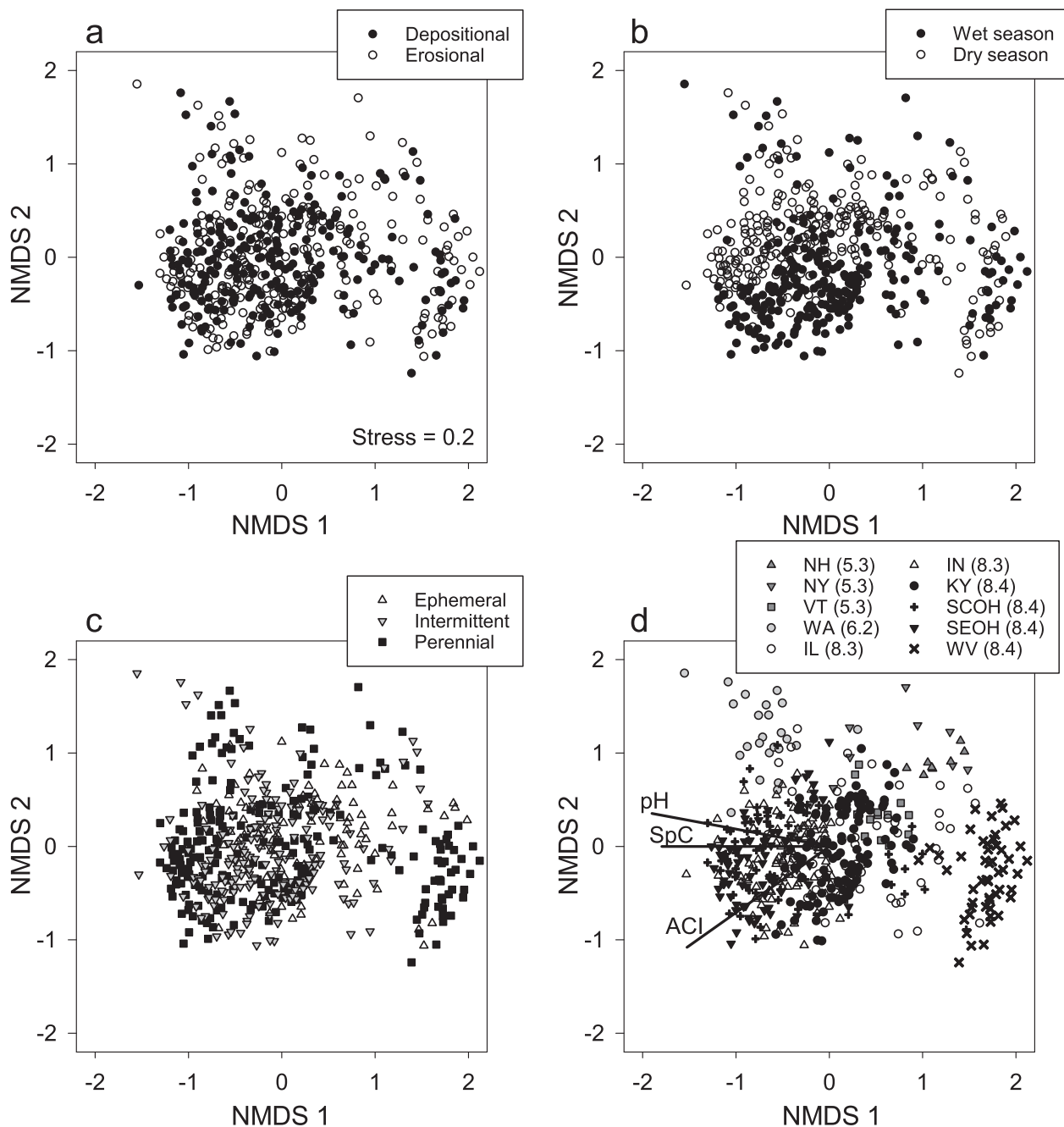


Figure 3. Two-dimensional non-metric multi-dimensional scaling (NMDS) ordination of algal assemblages showing samples by (a) habitat, (b) season, (c) streamflow duration class, and (d) forest (ecoregion) with vectors representing significant environmental variables ($p < 0.05$) and strength of the relationship (Spearman R) corresponds to vector length. SpC – specific conductivity; ACI – Algal Cover Index score.

A total of 21 diatom species and 1 red alga (*Audouinella*) were identified as algal taxonomic indicators with $>10\%$ difference of percent presence in samples among the 3 SDCs (Table 4). These 22 taxa were within the top 23% of median node ranks from the RF models for 3 SDCs (Table S-8). *Audouinella* and 11 diatom species were identified as perennial reach indicators, whereas 3 diatom species were identified as intermittent reach indicators and 7 diatom species were identified as ephemeral reach indicators (Table 4). A total of 22 diatom species, *Audouinella*, live diatoms, and soft-bodied algae were identified as algal taxonomic indicators to distinguish between ephemeral and non-ephemeral reaches. Of these 24 indicators, 23 were within the top 27% of median ranks from the RF models for 2 SDCs (Table S-8). Eleven diatom taxa, including the 8 taxa from the 3 SDC group, were identified

as ephemeral reach indicators. Indicators of non-ephemeral reaches include live diatoms, soft-bodied algae, *Audouinella*, and 10 diatom species, including 7 species from the 3 SDCs perennial and intermittent indicators.

The relative discriminatory ability of the algal taxonomic indicators is shown by plotting the associated probabilities of reach SDC based on the indicators' presence in study samples (Figure 4). The presence of perennial indicators had higher probabilities of occurring in perennial reaches and lower probabilities of occurring in other SDCs compared to intermittent and ephemeral indicators (Figure 4a). Similarly, the probabilities of the non-ephemeral indicators occurring in non-ephemeral reaches were higher than the probabilities of ephemeral indicators not occurring in non-ephemeral reaches (Figure 4b). Seventeen indicator

Table 3

Overall percent of samples (reach visits) classified correctly by random forest analyses majority vote across 500 decision trees for 3 streamflow-duration classes (SDCs; perennial, intermittent, and ephemeral) and 2 SDCs (non-ephemeral and ephemeral) and classification accuracy by SDC and Level II ecoregion (Eco) for the 12 streamflow-duration class balanced random forest models representing different taxonomic and numeric resolutions.

SDCs	Numeric resolution	Diatom genus & soft-bodied algae genus models			Diatom species & soft-bodied algae genus models		
		Presence/absence	Density	Biovolume	Presence/absence	Density	Biovolume
Three	Overall (n = 508)	75.6	75.8	69.5	81.5	79.1	70.5
	Perennial (n = 230)	79.1	80.4	75.6	86.1	80.9	73.5
	Intermittent (n = 186)	69.4	69.9	65.0	78.0	76.3	65.6
	Ephemeral (n = 92)	79.4	76.1	63.0	77.2	80.4	72.8
	Eco 5.3 (n = 34)	52.9	55.9	76.5	73.5	50.0	58.8
	Eco 6.2 (n = 24)	95.8	91.7	87.5	95.8	95.8	87.5
	Eco 8.3 (n = 146)	76.0	71.9	62.3	78.8	77.4	64.4
	Eco 8.4 (n = 304)	76.3	78.6	70.7	82.6	81.9	73.4
Two	Overall (n = 508)	87.0	87.8	84.4	87.6	88.4	85.6
	Non-ephemeral (n = 416)	90.1	90.4	88.2	90.4	90.6	88.2
	Ephemeral (n = 92)	72.8	76.1	67.4	97.5	78.3	73.9
	Eco 5.3 (n = 34)	88.2	88.2	94.1	94.1	85.8	88.2
	Eco 6.2 (n = 24)	100	100	100	95.8	100	100
	Eco 8.3 (n = 146)	84.2	78.1	76.7	84.2	78.8	74.7
	Eco 8.4 (n = 304)	87.2	91.4	85.9	87.8	92.4	89.5

Table 4

Species-level diatom and genus-level soft-bodied algae indicators identified for 3 streamflow-duration classes (perennial (P), intermittent (I), and ephemeral (E)) and 2 streamflow-duration classes (ephemeral (E) and not ephemeral (NE)) of reaches having percent difference >10% in mean percent presence between flow classes. Acronyms for indicators are shown in parentheses. Indicators are ordered from highest to lowest median minimum node rank from the three-class and two-class random forest models. Median rank of minimum node depth for algal cover index, season (wet and dry), and habitat type (erosional and depositional) is also included. Ecoregion codes are from [Omernik \(1995\)](#). - = not applicable

Indicator	3-class		2-Class		Mean % presence by ecoregion			
	Rank	Class	Rank	Class	5.3	6.2	8.3	8.4
Algal cover index (ACI)	1.0	-	1.0	-	-	-	-	-
<i>Gomphonema parvulum</i> (BAGOPAR)	4.0	I	27.3	-	82.4	79.2	91.8	78.9
<i>Eunotia incisa</i> (BAEUINC)	5.0	P	3.0	NE	32.4	4.2	2.7	24.0
<i>Planorhynchium apiculatum</i> (BAPLAPI)	7.5	-	8.0	NE	52.9	100	71.9	51.6
Live diatoms	8.0	-	9.5	NE	94.1	100	91.1	96.1
<i>Audouinella</i> (RHAU)	9.0	P	24.0	NE	5.9	29.2	6.2	28.6
<i>Cocconeis placentula</i> (BACCPLA)	11.0	P	54.0	NE	2.9	83.3	0.7	14.8
<i>Reimeria sinuata</i> (BARESIN)	12.0	P	56.0	NE	17.6	29.2	1.4	17.8
<i>Amphipleura pellucida</i> (BAALPEL)	13.5	P	169.0	NE	0.0	0.0	0.0	18.4
<i>Eunotia exigua</i> (BAEUEXI)	14.0	E	15.0	E	85.3	12.5	62.3	67.4
<i>Luticola mutica</i> (BALUMUT)	18.0	E	21.5	E	17.6	4.2	11.6	11.2
<i>Meridion circulare</i> var. <i>constrictum</i> (BAMECIRCO)	19.0	I	31.0	-	79.4	4.2	27.4	24.3
Season	23.0	-	63.0	-	-	-	-	-
<i>Pinnularia subcapitata</i> (BAPISUB)	24.0	E	21.0	E	64.7	4.2	24.7	25.3
<i>Encyonema silesiacum</i> (BAENSIL)	26.0	I	56.0	-	55.9	29.2	61.6	50.7
Soft-bodied algae	26.0	-	9.0	NE	23.5	66.7	56.8	59.5
<i>Nitzschia dissipata</i> (BANIDIS)	28.0	P	39.0	NE	5.9	8.3	20.5	42.1
<i>Luticola cohnii</i> (BALUCRP)	30.0	P	21.0	NE	38.2	16.7	27.4	42.8
<i>Eunotia rhomboidea</i> (BAEURHO)	31.0	P	19.0	-	70.6	0.0	0.7	11.5
<i>Gomphonema minutum</i> (BAGOMIN)	36.0	P	41.0	-	8.8	70.8	2.7	10.9
<i>Halimophora montana</i> (BAHAMON)	37.0	P	22.0	NE	0.0	0.0	8.2	22.7
<i>Stauroneis thermicola</i> (BASATHE)	41.0	-	36.5	E	32.4	0	21.2	21.1
Habitat	43.0	-	47.0	-	-	-	-	-
<i>Ulnaria ulna</i> (BAULULN)	44.0	-	52.0	NE	32.4	0	17.1	30.3
<i>Eunotia bilunaris</i> (BAEUBIL)	45.0	E	22.0	E	8.8	0.0	11.6	8.6
<i>Eunotia perpusilla</i> (BAEUPER)	47.0	-	40.0	E	5.9	0	19.9	29.9
<i>Fragilaria amphicephaloides</i> (BAFRAMP)	47.5	-	60.5	NE	67.6	20.8	19.2	10.9
<i>Stauroneis smithii</i> (BASASMI)	53.0	-	49.0	E	2.9	0	28.8	29.9
<i>Pinnularia appendiculata</i> (BAPAPP)	56.0	E	70.0	E	17.6	4.2	8.9	22.0
<i>Caloneis bacillum</i> (BACABAC)	56.0	E	81.0	E	0.0	16.7	35.6	33.2
<i>Achnanthes exiguum</i> (BAAHEXI)	62.0	P	120.0	-	2.9	37.5	0.7	6.9
<i>Navicula schroeteri</i> (BANASHR)	79.0	P	139.0	-	0.0	0.0	0.0	18.1
<i>Pinnularia obscura</i> (BAPIOBS)	81.5	E	81.5	E	41.2	4.2	6.2	3.3

taxa were collected from each of the 4 ecoregions (e.g., *Audouinella*, *Encyonema silesiacum*, *G. parvulum*, *Luticola cohnii*), and only *Amphipleura pellucida* did not occur or rarely occurred (<1% of samples) in more than 1 ecoregion (Table 4).

A total of 12 diatom genera and *Audouinella* were identified as algal taxonomic indicators with >10% difference of percent presence in samples among the 3 SDCs (Table 5). These 13 taxa were within the top

30% of median node ranks from the RF models for 3 SDCs (Table S-9). *Audouinella* and 7 diatom genera were identified as perennial reach indicators, whereas 3 and 2 diatom genera were identified as intermittent reach and ephemeral reach indicators, respectively (Table 5). A total of 11 diatom genera, *Audouinella*, live diatoms, and soft-bodied algae were identified as algal taxonomic indicators to distinguish between ephemeral and non-ephemeral reaches. Of these 14 algal taxonomic indicators,

Table 5
Genus-level algal indicators identified for 3 streamflow-duration classes (perennial (P), intermittent (I), and ephemeral (E)) and 2 streamflow-duration classes (ephemeral (E) and not ephemeral (NE)) of reaches having percent difference >10% in mean percent presence between flow classes. Indicators are ordered from highest to lowest median minimum node rank from the three-class random forest models. Median rank of minimum node depth for algal cover index, season (wet and dry), and habitat type (erosional and depositional) is also included. Ecoregion codes are from [Omernik \(1995\)](#). - = not applicable

Indicator	3-class			2-class		Mean % presence by ecoregion			
	Rank	Class	Rank	Class		5.3	6.2	8.3	8.4
Algal cover index	1.0	-	1.0	-		-	-	-	-
<i>Gomphonema</i>	4.0	I	8.0	-		91.2	83.3	95.2	82.2
<i>Diploneis</i>	6.0	-	4.0	NE		5.9	20.8	17.8	19.4
<i>Planothidium</i>	8.0	-	12.0	NE		61.8	100	73.3	51.6
<i>Audouinella</i>	9.0	P	22.0	NE		5.9	29.2	6.2	28.6
Live diatoms	11.5	-	7.5	NE		94.1	100	91.1	96.0
<i>Cocconeis</i>	12.0	P	45.0	NE		2.9	83.3	0.7	16.1
<i>Pinnularia</i>	13.0	E	11.0	E		76.5	41.7	33.6	52.3
<i>Meridion</i>	14.0	I	15.0	-		100	4.2	70.6	54.9
Season	14.0	-	29.0	-		-	-	-	-
<i>Amphipleura</i>	16.5	P	75.5	NE		0.0	0.0	0.0	18.4
<i>Reimeria</i>	17.0	P	33.0	NE		17.6	29.2	1.4	17.8
<i>Fragilaria</i>	17.0	P	26.0	NE		79.4	29.2	1.4	17.8
<i>Navicula</i>	19.0	P	18.0	-		41.2	50.0	34.9	51.0
<i>Halamphora</i>	23.0	P	18.0	NE		0.0	0.0	9.6	26.3
<i>Encyonema</i>	23.0	I	24.0	NE		64.7	29.2	63.7	53.6
Soft-bodied algae	25.0	-	16.0	NE		23.5	66.7	56.8	59.5
<i>Stauroneis</i>	28.0	E	21.0	E		61.8	4.2	41.8	37.5
<i>Ulnaria</i>	31.0	-	37.0	NE		32.4	0.0	17.1	30.3
Habitat	31.0	-	28.0	-		-	-	-	-
<i>Amphora</i>	32.0	P	27.0	-		14.7	41.7	12.3	20.4

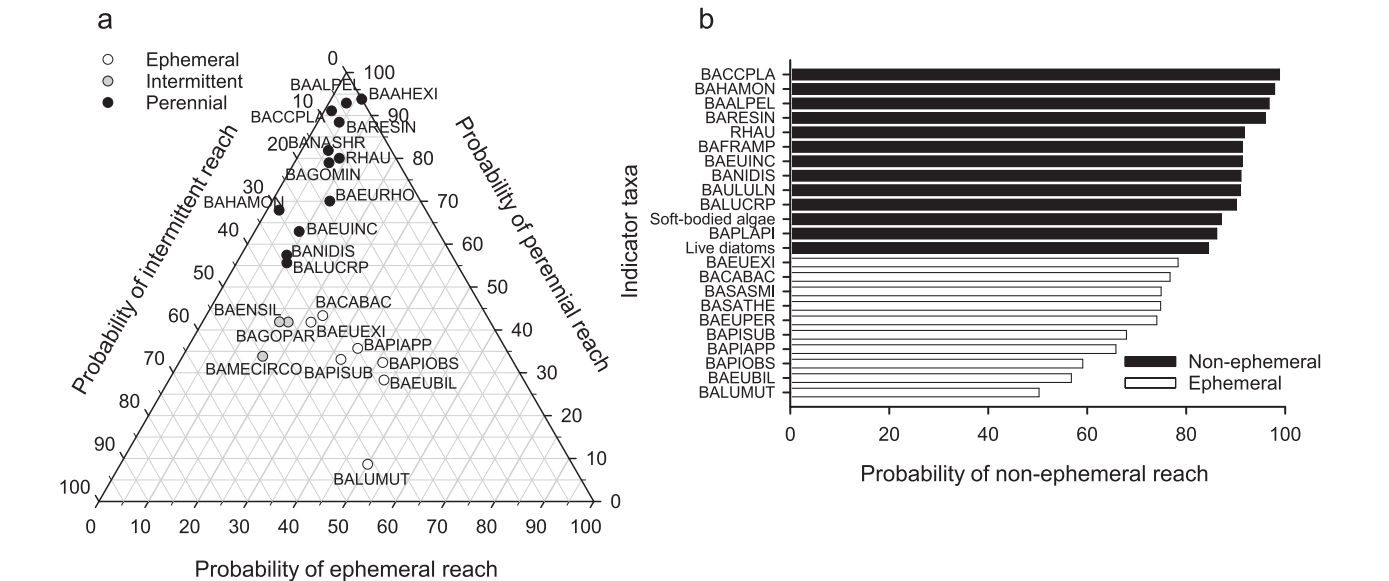


Figure 4. Probabilities of (a) perennial-intermittent-ephemeral classification and (b) non-ephemeral-ephemeral classification if diatom species and soft-bodied algae genera are present. Indicator taxa are those with >10% difference in mean percent presence among streamflow duration classes. Indicator taxa acronyms are defined in [Table 4](#).

13 were within the top 35% of median ranks from the RF models for 2 SDCs (Table S-9). *Pinnularia* and *Stauroneis* were identified as ephemeral indicators. Indicators of non-ephemeral reaches include live diatoms, soft-bodied algae, *Audouinella*, and 9 diatom genera, including 6 genera from the 3 SDCs perennial and intermittent indicators.

The presence of *Amphipleura*, *Cocconeis*, *Reimeria*, and *Audouinella* had the highest probabilities of occurring in perennial reaches and lowest probabilities occurring in other SDCs compared to other algal indicators (Figure 5a). Based on distributions among study samples, the probabilities of the non-ephemeral indicators occurring in non-ephemeral reaches were higher than the probabilities of ephemeral indicators not occurring in non-ephemeral reaches (Figure 5b). Many of the indicator genera, like *Encyonema*, *Gomphonema*, *Meridion*, *Navicula*,

Pinnularia, and *Stauroneis* were well represented across 3 or 4 of the study ecoregions (Table 5). Although *Amphipleura* and *Cocconeis* had among the highest probabilities associated with their presence, *Amphipleura* was only collected in ecoregion 8.4 and *Cocconeis* was more frequently collected in ecoregions 6.2 and 8.4.

4. Discussion

Among the composite algal indicators compared across SDCs, there were detectable differences among SDCs for ACI scores, relative abundance of live diatoms, and percent of aerial diatom taxa. The ACI score was consistently identified as the most important indicator across all RF models for predicting SDC. Similar indicators to the ACI score that

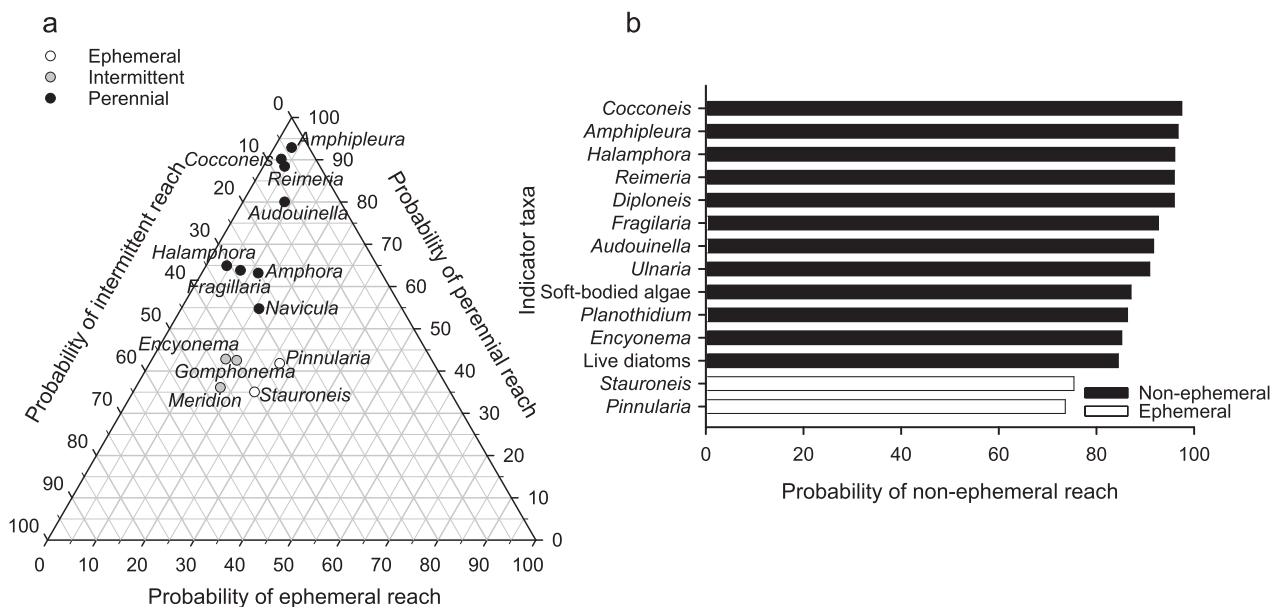


Figure 5. Probabilities of (a) perennial-intermittent-ephemeral classification and (b) non-ephemeral-ephemeral classification if algae genera are present. Indicator taxa are those with >10% difference in mean percent presence among streamflow duration classes.

represent the ordinal scoring of the visual extent of algal cover on the streambed are used in SDAMs developed for North Carolina (NCDWQ, 2010), New Mexico (NMED, 2020), and the Arid West of the United States (Mazor et al., 2024). The ACI score may be considered a surrogate measure of periphyton biomass because it does not distinguish photosynthetic (i.e., chlorophyll) from non-photosynthetic (i.e., heterotrophic, extracellular polymeric substances) periphyton components. Ash-free dry weight (AFDW) of epilithic periphyton was higher in perennial streams compared to non-perennial streams in the northeast Iberian Peninsula and decreased exponentially with the total dry duration over the previous 150 d (Colls et al., 2019). In an experimental mesocosm study that included treatments that manipulated the water level, periphyton on tiles exposed to drying had lower AFDW relative to controls but not where lowered water levels still submerged tiles (Lynch and Magoulick, 2016). Most ACI scores in the present study reflect relatively low periphyton biomass and were higher during the wet season than during the dry season. The low ACI scores and predominance of diatoms are likely related to limited irradiance because of the small channel sizes (median bankfull width range 0.5 – 11.8 m) and the dense canopy cover ($89.3 \pm 0.97\%$, mean $\pm$ 1SE). The higher ACI scores in the wet season compared to the dry season may be in part related to stream drying; however, we did not detect an interaction between SDC and season. Rather, the seasonality of water temperature and irradiance associated with predominantly deciduous riparian vegetation are the most plausible causes that have been previously documented (e.g., Francoeur et al., 1999; Hill et al., 2011). Because flow duration in ephemeral reaches is brief, we expect ACI scoring should still distinguish ephemeral from intermittent and perennial reaches where channels are unshaded. Algae recovery after flow resumed at headwater intermittent reaches in prairie and arid streams required only 10–14 days (Dodds et al., 1996; Peterson and Boulton, 1999). Algal cover was identified as SDAM indicators in the classification model for the Arid West (USA, Mazor et al., 2024) but not in models for the Pacific Northwest, Great Plains, Northeast or Southeast regions (Nadeau, 2015; James et al., 2025a,b) where many intermittent and ephemeral reaches are typically in smaller and more heavily shaded streams.

Although most indicator taxa were diatoms and the percent of soft-bodied algae did not differ among SDCs, *Audouinella* presence and total soft-bodied algae presence were among the more important 3- and 2-class indicators identified for perennial and non-ephemeral reaches,

respectively. Because many soft-bodied algae in streams can become more prevalent with increasing irradiance (e.g., Lowe et al., 1986), we might expect higher soft-bodied algae prevalence in reaches with more open canopy cover than in our heavily shaded study reaches. The soft-bodied algal taxa *Spirogyra*, *Oedogonium*, *Phormidium*, *Oscillatoria*, and *Lyngbya* were identified as becoming dominant in isolated pools of Coastal Plain streams of North Carolina during late summer (Whitford and Schumacher, 1963). The soft-bodied algae assemblage composition from 20 stream reaches with a Mediterranean climate in Southern California was strongly correlated with how long sites had surface water prior to sample collection (Busch et al., 2024). Dried biofilm composed of cyanobacteria and chlorophytes were an important source for recolonization in 2 Australian intermittent streams “with riparian vegetation of open eucalypt forest” (Robson, 2000). After just 1 week following channel rewetting, cyanobacteria and chlorophyte biomass recovered beyond pre-desiccation levels in experimental (recirculating) channels with low shade but remained below pre-desiccation levels in channels with high shade (Zlatanović et al., 2018). Although most studies on algal indicators of SDCs have focused on diatoms (but see Timoner et al., 2020), further investigation for soft-bodied algae indicator taxa is warranted because with some training, many soft-bodied algae taxa can be characterized in the field for rapid assessments (e.g., Kelly et al., 2016; Huynh and Sereidiak, 2006). Our sampling was limited to epilithic algae in the channel thalweg, soft-bodied algae could be more prevalent in other microhabitats that were not sampled.

As expected, our study shows that ephemeral reaches tended to have relatively more aerial taxa than perennial reaches, and aerial diatoms were relatively more abundant during the dry season than wet season in epilithic samples from the thalweg. Aerial diatoms are species which either always or mainly occur in terrestrial habitats. The percent of aerial diatom taxa was based on a list of species and associated moisture classifications for the habitats in which they occur (Mertens et al., 2025). The relative abundance of aerial diatoms has been used to represent hydrologic tracers to characterize the hydrologic connectivity across the hillslope-riparian-stream continuum at the catchment scale (Klaus et al., 2015). Three diatom species (*Pinnularia obscura*, *P. subcapitata*, and *Stauroneis thermicola*) identified as ephemeral indicators in our study were also identified as aerial diatoms in Mertens et al. (2025). Three other diatom species (*Eunotia bilunaris*, *E. exigua*, and *S. smithii*) identified as ephemeral taxa in the present study were classified as “mainly

occurring in water bodies, also rather regularly on wet and moist places, ” and no moisture classifications were provided for *Caloneis bacillum*, *E. perpusilla*, *Luticola mutica*, and *P. appendiculata* (Mertens et al., 2025). Across soil diatom datasets, *E. exigua* has been identified to have low soil moisture optima for growth (Foets et al., 2021), and other references have identified *C. bacillum* and *L. mutica* as aerial diatoms (Johansen, 2010; Van Dam et al., 1994).

The red alga, *Audouinella*, is one of the strongest perennial indicator taxa and was identified in each of our study ecoregions. *Audouinella* was also noted as being more common in a Mediterranean perennial stream than an intermittent stream (Jiménez et al., 2023). However, the association of *Audouinella* with perennial reaches does not apply to all freshwater red algae. The red alga, *Riverina rivularis* (syn. *Hildenbrandia rivularis*), has been reported to be abundant in shaded streams, and its crust-forming thalli are resistant to extended periods of drying (Timoner et al., 2014). The filaments of another red algae, *Paralemanea grandis*, from a North Carolina stream were not resistant to desiccation lasting more than 12 h (Everitt and Burkholder, 1991) but this species produces spores which enable the species to persist during longer dry periods (Mullahy, 1952). In laboratory desiccation experiments, the photosynthetic response of the red algae, *Thorea okadae*, from Sendai-Gawa River in Japan did not recover following up to 1 d of rewetting when desiccation caused the relative water content to drop below 50% (Terada et al., 2025). Similar studies for *Audouinella* are needed to characterize its tolerance and possible adaptations to desiccation.

Given that the other algal indicator taxa identified were diatoms that can be readily hydrologically transported, the longitudinal ordering of our study reaches (upstream ephemeral and intermittent reaches to downstream perennial reaches) may explain why perennial indicators outnumbered and were more diagnostic than ephemeral and intermittent indicators. There could also be more indicators because longer duration of flow at perennial reaches supported more taxa than reaches with shorter flow duration (Falasco et al., 2021), but some studies report no differences in taxa richness with varying flow duration (Tornés et al., 2021; Busch et al., 2024). The diatom *Cocconeis placentula* was among the strongest perennial indicators, a result that supports findings from some European studies comparing diatom assemblages from different SDCs or responses to drought in perennial streams (Barthés et al., 2015; Calapez et al., 2014; Falasco et al., 2018; Jiménez et al., 2023; Tornés et al., 2021). However, *C. placentula* has also been identified as an indicator species for intermittent reaches in other European streams (Novais et al., 2014, 2020). Though not among the most abundant or diagnostic indicators, the diatoms *Halamphora montana* and *Luticola cohnii* were unexpectedly identified as indicators of perennial reaches in our study. *H. montana* has a low moisture optimum for growth (Foets et al., 2021). Both taxa have been classified as terrestrial or aerophilic taxa (Tornés et al., 2021; Mertens et al., 2025) but have also been classified as aquatic elsewhere (Stepanek and Kociolek, 2011; Kociolek et al., 2025).

Classification accuracy was similar between RF models based on algal presence/absence and density and comparable between models for diatom species- and genus-level data. Genus-level presence/absence data requires less time and training to produce than species-level identification and biovolume measurements, making such algal indicators more feasible to use in SDAMS. Phenotypic plasticity in the form of heterovalvy (i.e., Janus cells) can contribute to species misidentification and has been documented in *Gomphonema* taxa in association with periodic drying in swales (Andrejić et al., 2019). Comparable findings across taxonomic and numerical resolutions have been reported in other studies to support simplified bioassessment (Hill et al., 2001; Carneiro et al., 2010). Regional and national multimetric indices developed using genus-level diatom identifications distinguished least- and most-disturbed river and stream reaches and had comparable responsiveness to condition indices using macroinvertebrates and fish assemblages (Riatio et al., 2022). Diatom species identification is conventionally based on morphological characteristics that requires acid digestion to

examine microscopically. This identification method prevents distinguishing living from dead cells for individual species without assuming equal proportion of living and dead individuals across the entire assemblage (Potapova and Charles, 2005). Because diatoms can be transported across the terrestrial-aquatic interface (Pfister et al., 2015) or across reaches of varying SDC, where diatoms are collected may not necessarily reflect their suitable or optimal habitat. Being able to taxonomically identify living cells would provide more definitive evidence of diatoms as SDC indicators (Round, 1998) and is feasible at the genus-level for many diatoms.

Our results indicate that the season in which samples were collected (wet vs dry) was relatively more important for classifying among ephemeral, intermittent and perennial SDCs whereas the habitat sampled (depositional vs erosional) was relatively more important for classifying between ephemeral and non-ephemeral SDCs. This would suggest for epilithic algal assemblages, sampling season was relatively more useful than sample habitat for distinguishing between intermittent and perennial reaches. Sampling season was also more important than sample habitat for classifying intermittent and perennial reaches using invertebrate assemblages (Fritz et al., 2023). Hydrologic condition at intermittent reaches shifted to a greater extent between seasons than at perennial or ephemeral reaches. The upper surface of stones sampled for epilithic algae was submerged in 96% of perennial reach samples but only 73% of intermittent reach samples during the dry season compared to 100% and 99%, respectively, during the wet season. The upper surface of stones sampled from ephemeral reaches was dry in 100% and 86% during the dry and wet seasons, respectively. Despite sample habitat not being an influential factor in overall algal assemblage structure in our study, there may be differences in local conditions that affect distribution of epilithic algae between depositional and erosional habitats in ephemeral reaches. Moisture, temperature, substrates, pH, and nutrient availability are factors documented to control aerial diatoms (Johansen, 2010). Factors driving microhabitat variation in algal composition in ephemeral reaches is ripe for experimental study where factors like moisture, leaf litter, sediment, and bryophyte cover can be readily manipulated.

A body of studies have investigated the extent to which environmental factors act as filters on algal assemblages by comparing changes or differences in trait and guild composition (reviewed by Stenger-Kovács and B-Béres, 2024). Algal guilds were developed to test hypotheses that algal growth form reflects characteristic responses to resource use and disturbance stress. In general, low-profile and motile taxa are expected to be more prevalent than high-profile and planktic taxa under increasing disturbance stress (Passy, 2007; Rimet and Bouchez, 2012; Lange et al., 2016). Low-profile taxa were more prevalent at intermittent reaches and high-profile taxa more prevalent at perennial reaches in some streams (Várbíró et al., 2020; Falasco et al., 2021), but this pattern has not always been supported (Novais et al., 2014; Falasco et al., 2016). The 12 indicator taxa for perennial reaches identified in the present study were divided among high profile (4), low profile (4), and motile (3) guilds. The 4 indicator taxa for intermittent reaches were also divided evenly between high profile (2) and low profile (2) guilds. In contrast, the 8 indicator taxa for ephemeral reaches belonged to the motile (6) and high profile (2) guilds. This separation carried over when comparing guilds between indicators of non-ephemeral and ephemeral reaches, where 8 of the 11 indicator taxa for ephemeral reaches and only 2 of 12 indicator taxa for non-ephemeral reaches belong to the motile guild. Some studies report the motile guild to be invariant to drying (Várbíró et al., 2020) or characterize diatom assemblages in perennial reaches (B-Béres et al., 2019), whereas others report increased motile guild prevalence with drying (Novais et al., 2020; Peszek et al., 2021).

Cell size class is another characteristic commonly evaluated with stream drying, with large taxa more prevalent in perennial reaches and smaller taxa more prevalent in intermittent reaches (B-Béres et al., 2019; Várbíró et al., 2020; Lukács et al., 2021; Nicolosi Gelis et al., 2025).

Perennial indicators identified in the present study included 2 diatom species (*Amphipleura pellucida* and *Cocconeis placentula*) in $>1500\ \mu\text{m}^3$ -size class (based on Rimet and Bouchez, 2012) whereas no intermittent or ephemeral indicator taxa belonged to the $>1500\ \mu\text{m}^3$ -size class. All diatom species identified as intermittent indicators and 7 diatom species identified as perennial indicators taxa belong to $300\text{--}599\ \mu\text{m}^3$ - or $600\text{--}1499\ \mu\text{m}^3$ -size classes, whereas 4 diatom species (*Caloneis bacillum*, *Eunotia bilunaris*, *Pinnularia subcapita*, and *Stauroneis smithii*) identified as ephemeral indicator taxa belong to one of these groups. The perennial indicator taxon, *Audouinella*, also belongs to the $300\text{--}599\ \mu\text{m}^3$ -size class (Lange et al., 2016). Four diatom species identified as ephemeral indicator taxa (*Eunotia exigua*, *Luticola mutica*, *Pinnularia appendiculata*, and *P. obscura*), but only 1 identified as a perennial indicator taxon (*Navicula schroeteri* Meister) belong to the smallest cell size classes ($0\text{--}99$ and $100\text{--}299\ \mu\text{m}^3$). One diatom species (*Eunotia rhomboidea*) identified as perennial indicator is not classified in Rimet and Bouchez (2012) for cell size but has been included in a $101\text{--}1000\ \mu\text{m}^3$ -size class elsewhere (Furey, 2011). Overall, our results provide modest support for divergence of the cell size trait among diatom assemblages in reaches with varying SDC.

Although there were some taxa (e.g., *A. minutissimum*, *G. parvulum*, *Planorhynchium apiculatum*, and *Sellaphora atomoides*) that exhibited distributions across all study ecoregions, many taxa had restricted geographical distributions. Our findings support those of previous studies (Potapova and Charles, 2002; Soininen et al., 2004) reporting that lotic algal assemblage composition is spatially structured across broad scales and primarily related to water chemistry and climate. The biogeographical distributions contributed to algal assemblages from reaches of the same SDC across ecoregions to be as compositionally similar to one another as those from reaches of different SDC in the same ecoregion. This finding also supports the use of regional stratification to capture geographic variability of indicators to improve SDAM classification accuracy (Fritz et al., 2020). Incorporating sufficient study reaches for each SDC would better ensure appropriate indicators for regional SDAM development. Although the taxonomic indicators identified may be absent or rare in some study regions, they can be combined into more generalized metrics for evaluation across larger areas. These could include the number of indicator taxa or the relative abundance of indicator taxa.

4.1. Future SDAM indicator use

Some possible obstacles for using algae as SDAM indicators include additional time and expertise required for their identification compared to other indicators. Traditional algal taxonomy requires additional cost and level of effort associated with microscopic identification compared to most field-based measures used in SDAMs. Genus-based identification of algae would require less training, be more consistent, and may be more feasible for automated field identification but may be less sensitive than species-level identifications (Chong et al., 2023; Gifford et al., 2024). Field-based identifications and measurements of cover based on visual assessments or field fluorometers offer more rapid alternatives to traditional laboratory-based methods. Composite measures such as ACI can be rapidly measured. The use of in situ fluorometric (e.g., Piano et al., 2017), chemotaxonomic (Iles et al., 2022), and metagenomic data (Burfeid-Castellanos et al., 2024) can reduce the level of effort needed for measuring algal indicators, like the relative abundance of soft-bodied algae and live diatoms which were identified as strong indicators in RF models for distinguishing ephemeral from intermittent and perennial reaches. However, new technology for these applications will need investment in equipment, taxonomic libraries, and testing methods. There may also be cases where more lines of evidence, beyond a rapid field determination, are needed to support an SDC. Aquatic invertebrate or algal taxonomic assessments for more detailed indicator identification may fill this need and require less time than continuous stream gauging.

Several best practices for using algal indicators in SDAMs are

supported in our study. First, the ACI is a strong candidate indicator for SDAMs, especially for distinguishing ephemeral from non-ephemeral reaches. Not only was the ACI identified as the most important indicator across all RF models explored in this study and was largely invariant among ephemeral reaches, the ACI requires minimal training, no instrumentation, and can be collected in the field (no laboratory measurement) so it was the most rapid of the algal indicators we evaluated. Second, using of genus-level taxonomic resolution and presence/absence numeric resolution may lower cost and effort with minimal sacrifice on discriminating performance among SDCs of forested headwater streams. Third, although season (wet vs dry) and habitat (erosional vs depositional) fell within the top 25% of indicators of SDCs in the RF models, these factors appeared to have less influence on algal composition and most composite indicators than geographic location. Based on our results compositing algal samples across habitats rather than processing them separately could be effective. Percent abundance of live diatoms had higher performance in the dry than wet season. Lastly, our results support, where possible, evaluating indicator taxa for SDAMs at the regional level rather than applying indicator lists derived over continental scales. Algal indicators of SDCs are one of multiple lines of physical and biological evidence that could be used to inform the long-term characterization of hydrologic regimes in streams.

CRedit authorship contribution statement

Ken M. Fritz: Writing – review & editing, Writing – original draft, Visualization, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Roxolana O. Kashuba:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Gregory J. Pond:** Writing – review & editing, Writing – original draft, Investigation. **Jay R. Christensen:** Writing – review & editing, Writing – original draft, Investigation, Visualization. **Susanna J. DeCelles:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Brent R. Johnson:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **David M. Walters:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Conceptualization.

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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Author contributions: KMF, DMW, and BRJ conceived the study and collected field data. KMF coordinated the manuscript, curated data, created figures, and led the writing. ROK, and KMF conducted data analysis. JRC created the study map. SJD conducted taxonomic analyses. All authors contributed to investigation, writing, and reviewing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecolind.2026.115080>.

Data availability

The attached supplemental material file contains the research data.

References

- Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E, Plymouth, UK, p. 214.
- Andrejčić, J.Z., Spaulding, S.A., Manoylov, K.M., Edlund, M.B., 2019. Phenotypic plasticity in diatoms: Janus cells in four *Gomphonema* taxa. *Diatom Res.* 33, 453–470. <https://doi.org/10.1080/0269249X.2019.1572652>.
- Barthés, A., Leflaive, J., Coulon, S., Peres, F., Rols, J.-L., Ten-Hage, L., 2015. Impact of drought on diatom communities and consequences for the use of diatom index values in the River Maureillas (Pyrénées-Orientales, France). *River Res. Appl.* 31, 993–1002. <https://doi.org/10.1002/rra.2793>.
- V., B-Béres, Tóthmérész, B., Bácsi, I., Borics, G., Abonyi, A., Tapolczai, K., Rimet, F., Bouchez, A., Várbró, G., Török, P., 2019. Autumn drought drives functional diversity of benthic diatom assemblages of continental streams. *Adv. Water Resour.* 126, 129–136. <https://doi.org/10.1016/j.advwatres.2019.02.010>.
- Benstead, J.P., Leigh, D.S., 2012. An expanded role for river networks. *Nat. Geosci.* 5, 678–679. <https://doi.org/10.1038/ngeo1593>.
- Brinkerhoff, C.B., Gleason, C.J., Kotchen, M.J., Kysar, D., Raymond, P.A., 2024. Ephemeral stream water contributions to United States drainage networks. *Science* 384, 1476–1482. <https://doi.org/10.1126/science.adg9430>.
- Burfeid-Castellanos, A.M., Mones, P., Dani, M., Beszteri, B., 2024. Non-perennial rivers and streams in extreme hydrological conditions – comparing the effectiveness of amplicon sequencing and digital microscopy for diatom biodiversity appraisal. *Front. Ecol. Evol.* 12, 1355018. <https://doi.org/10.3389/fevo.2024.1355018>.
- Burgazzi, G., Bolpagni, R., Laini, A., Racchetti, E., Viaroli, P., 2020. Algal biomass and macroinvertebrate dynamics in intermittent braided rivers: new perspectives from instream pools. *River Res. Appl.* 36, 1682–1689. <https://doi.org/10.1002/rra.3675>.
- Busch, M.H., Costigan, K.H., Fritz, K.M., Datry, T., Krabbenhoft, C.A., Hammond, J.C., Zimmer, M., Olden, J.D., Burrows, R.M., Dodds, W.K., Boersma, K.S., Shanafield, M., Kampf, S.K., Mims, M.C., Bogan, M.T., Ward, A.S., Perez Rocha, M., Godsey, S., Allen, G.H., Blaszcak, J.R., Jones, C.N., Allen, D.C., 2020. What's in a name? Patterns, trends, and suggestions for defining non-perennial rivers and streams. *Water* 12, 1980. <https://doi.org/10.3390/w12071980>.
- Busch, M.H., Boersma, K.S., Cook, S.C., Jones, C.N., Loffen, C., Mazor, R.D., Stancheva, R., Price, A.N., Stubbington, R., Zimmer, M.A., Allen, D.C., 2024. Macroinvertebrate, algal and diatom assemblages respond differently to both drying and wetting transitions in non-perennial streams. *Freshw. Biol.* 69, 1568–1582. <https://doi.org/10.1111/fwb.14327>.
- Calapez, A.R., Elias, C.L., Almeida, S.F.P., Feio, M.J., 2014. Extreme drought effects and recovery patterns in the benthic communities of temperate streams. *Limnetica* 33, 281–296. <https://doi.org/10.23818/limn.33.22>.
- Carneiro, F.M., Bini, L.M., Rodrigues, L.C., 2010. Influence of taxonomic and numerical resolution on the analysis of temporal changes in phytoplankton communities. *Ecol. Indic.* 10, 249–255. <https://doi.org/10.1016/j.ecolind.2009.05.004>.
- Cavallo, C., Sarno, L., Papa, M.N., Negro, G., Vezza, P., Ruello, G., Gargiulo, M., 2025. Estimating dry bed periods in non-perennial rivers using Sentinel-2 satellite data. *J. Hydrol.* 660, 133416. <https://doi.org/10.1016/j.jhydrol.2025.133416>.
- Charles, D.F., Knowles, C., Davis, R.S. (Eds.), 2002. Protocols for the analysis of algal samples collected a part of the U.S. Geological Survey National Water-Quality Assessment program. Report No. 02-06. The Academy of Natural Sciences, Philadelphia, PA. <https://water.usgs.gov/nawqa/protocols/algprotocol/algprotocol.pdf>.
- Chen, C., Liaw, A., Breiman, L., 2004. Using random forest to learn imbalanced data. Technical report. Department of Statistics, University of California, Berkeley, California. <https://statistics.berkeley.edu/sites/default/files/tech-reports/666.pdf> (accessed on August 28, 2025).
- Chong, J.W.R., Khoo, K.S., Chew, K.W., Vo, D.-V.N., Balakrishnan, D., Banat, F., Munawaroh, H.S.H., Iwamoto, K., Show, P.L., 2023. Microalgae identification: future of image processing and digital algorithm. *Bioresour. Technol.* 369, 128418. <https://doi.org/10.1016/j.biortech.2022.128418>.
- Christensen, J.R., Golden, H.E., Alexander, L.C., Pickard, B.R., Fritz, K.M., Lane, C.R., Weber, M.H., Kwok, R.M., Keefer, M.N., 2022. Headwater streams and inland wetlands: status and advancements of geospatial datasets and maps across the United States. *Earth Sci. Rev.* 235, 104230. <https://doi.org/10.1016/j.earscirev.2022.104230>.
- Colls, M., Timoner, X., Font, C., Sabater, S., Acuña, V., 2019. Effects of duration, frequency, and severity of the non-flow period on stream biofilm metabolism. *Ecosystems* 22, 1393–1405. <https://doi.org/10.1007/s10021-019-00345-1>.
- Cutler, D.R., Edwards Jr., T.C., Beard, K.H., Cutler, A., Hess, K.T., Gibson, J., Lawler, J.J., 2007. Random forests for classification in ecology. *Ecology* 88, 2783–2792. <https://doi.org/10.1890/07-0539.1>.
- Dalibard, M., Laffaille, P., Sánchez-Pérez, J.-M., Sauvage, S., Buisson, L., 2021. Accounting for flow intermittence in freshwater distribution modelling. *Ecohydrology* 14, e2346. <https://doi.org/10.1002/eco.2346>.
- Datry, T., Truchy, A., Olden, J.D., Busch, M.H., Stubbington, R., Dodds, W.K., Zipper, S., Yu, S., Messenger, M.L., Tonkin, J.D., Kaiser, K.E., 2023. Causes, responses, and implications of anthropogenic versus natural flow intermittence in river networks. *BioScience* 73, 9–22. <https://doi.org/10.1093/biosci/biac098>.
- Dodds, W.K., Hutson, R.E., Eiche, A.C., Evans, M.A., Gudder, D.A., Fritz, K.M., Gray, L., 1996. The relationship of floods, drying, flow and light to primary production and producer biomass in a prairie stream. *Hydrobiologia* 333, 151–159. <https://doi.org/10.1007/BF00013429>.
- Dralle, D.N., Lapiques, D.A., Rempe, D.M., Hahn, W.J., 2023. Mapping surface water presence and hyporheic flow properties of headwater stream networks with multispectral satellite imagery. *Water Resour. Res.* 59, e2022WR034169. <https://doi.org/10.1029/2022WR034169>.
- Everitt, D.T., Burkholder, J.A.M., 1991. Phenology and desiccation response of *Lemanea australis* from a fast-flowing stream in North Carolina, USA. *J. Phycol.* 27S, 100. <https://doi.org/10.1111/j.0022-3646.1991.00004.x>.
- Falasco, E., Piano, E., Bona, F., 2016. Diatom flora in Mediterranean streams: flow intermittency threatens endangered species. *Biodivers. Conserv.* 25, 2965–2986. <https://doi.org/10.1007/s10531-016-1213-8>.
- Falasco, E., Piano, E., Doretto, A., Fenoglio, S., Bona, F., 2018. Lenticification in Alpine rivers: patterns of diatom assemblages and functional traits. *Aquat. Sci.* 80, 36. <https://doi.org/10.1007/s00027-018-0587-y>.
- Falasco, E., Bona, F., Rizzo, A.M., Piano, E., 2021. Hydrological intermittency drives diversity decline and functional homogenization in benthic diatom communities. *Sci. Total Environ.* 762, 143090. <https://doi.org/10.1016/j.scitotenv.2020.143090>.
- Feminella, J.W., Hawkins, C.P., 2000. Eyeing the aufwuchs and groping the growth: a rapid method for estimating epilithic periphyton abundance in streams. In: *Bull. North Am. Benthol. Soc. Annual meeting*. Keystone, Colorado, p. 180.
- Fesenmeyer, K.A., Wenger, S.J., Leigh, D.S., Neville, H.M., 2021. Large portion of USA streams lose protection with new interpretation of Clean Water Act. *Freshw. Sci.* 40, 252–258. <https://doi.org/10.1086/713084>.
- Foets, J., Stanek-Tarkowska, J., Teuling, A.J., Van de Vijver, B., Wetzel, C.E., Pfister, L., 2021. Autecology of terrestrial diatoms under anthropic disturbance and across climate zones. *Ecol. Indic.* 122, 107248. <https://doi.org/10.1016/j.ecolind.2020.107248>.
- Francoeur, S.N., Biggs, B.J.F., Smith, R.A., Lowe, R.L., 1999. Nutrient limitation of algal biomass accrual in streams: seasonal patterns and a comparison of methods. *J. North Am. Benthol. Soc.* 8, 242–260. <https://doi.org/10.2307/1468463>.
- Fritz, K.M., Johnson, B.R., Walters, D.M., 2006. Field operations manual for assessing the hydrologic permanence and ecological condition of headwater streams. In: EPA 600/R-06/126. United States Environmental Protection Agency, Office of Research and Development, Washington, DC, p. 134. https://www.epa.gov/sites/production/files/2015-11/documents/manual_for_assessing_hydrologic_permanence_-_headwater_streams.pdf (accessed August 28, 2025).
- Fritz, K.M., Johnson, B.R., Walters, D.M., 2008. Physical indicators of hydrologic permanence in forested headwater streams. *J. North Am. Benthol. Soc.* 27, 690–704. <https://doi.org/10.1899/07-117.1>.
- Fritz, K.M., Cid, N., Autrey, B., 2017. Governance, legislation, and protection of intermittent rivers and ephemeral streams. In: Datry, T., Bonada, N., Boulton, A. (Eds.), *Intermittent Rivers and Ephemeral Streams – Ecology and Management*. Academic Press, London, United Kingdom, pp. 477–507. <https://doi.org/10.1016/B978-0-12-803835-2.00019-X>.
- Fritz, K.M., Nadeau, T.-L., Kelso, J.E., Beck, W.S., Mazor, R.D., Harrington, R.A., Topping, B.J., 2020. Classifying streamflow duration: the scientific basis and an operational framework for method development. *Water* 12, 2545. <https://doi.org/10.3390/w12092545>.
- Fritz, K.M., Kashuba, R.O., Pond, G.J., Christensen, J.R., Alexander, L.C., Washington, B. J., Johnson, B.R., Walters, D.M., Thoeny, W.T., Weaver, P.C., 2023. Identifying invertebrate indicators for streamflow duration assessments in forested headwater streams. *Freshw. Sci.* 42, 247–267. <https://doi.org/10.1086/726081>.
- Furey, P., 2011. Eunotia rhomboidea. In *Diatoms of North America*. https://diatoms.org/species/47898/eunotia_rhomboidea (accessed on June 23, 2025).
- Gifford, S.R., St. Amand, A., Graham, J.L., Foster, G.M., Sauve, C., Clark, D., Schroeder-Larkins, H., 2024. Comparison of imaging flow cytometry and microscopy for freshwater algal bloom detection. *Lake Reserv. Manag.* 40, 221–235. <https://doi.org/10.1080/10402381.2024.2370828>.
- Guiry, M.D., Guiry, G.M., 2021. AlgaeBase. <https://www.algaebase.org/> (accessed August 28, 2025).
- Hart, D.D., Finelli, C.M., 1999. Physical-biological coupling in streams: the pervasive effects of flow on benthic organisms. *Annu. Rev. Ecol. Syst.* 30, 363–395. <https://doi.org/10.1146/annurev.ecolsys.30.1.363>.
- Hasle, G.R., Fryxell, G.A., 1970. Diatoms: cleaning and mounting for light and electron microscopy. *Trans. Am. Microsc. Soc.* 89, 469–474. <https://doi.org/10.2307/3224555>.
- Hillebrand, H., Dürselen, C.-D., Kirschtel, D., Pollinger, U., Zohary, T., 1999. Biovolume calculation for pelagic and benthic microalgae. *J. Phycol.* 35, 403–424. <https://doi.org/10.1046/j.1529-8817.1999.3520403.x>.
- Hill, B.H., Stevenson, R.J., Pan, Y., Herlihy, A.T., Kaufmann, P.R., Johnson, C.B., 2001. Comparison of correlations between environmental characteristics and stream diatom assemblages characterized at genus and species levels. *J. North Am. Benthol. Soc.* 20, 299–310. <https://doi.org/10.2307/1468324>.
- Hill, W.R., Roberts, B.J., Francoeur, S.N., Fanta, S.E., 2011. Resource synergy in stream periphyton communities. *J. Ecol.* 99, 454–463. <https://doi.org/10.1111/j.1365-2745.2010.01785.x>.

- Holzinger, A., Karsten, U., 2013. Desiccation stress and tolerance in green algae: consequences for ultrastructure, physiological and molecular mechanisms. *Front. Plant Sci.* 4, 327. <https://doi.org/10.3389/fpls.2013.00327>.
- Huynh, M., Serediak, N., 2006. *Algae Identification Field Guide*. Agriculture and Agri-Food Ottawa, Canada (46 pp). https://publications.gc.ca/collections/collection_2011/agr/A125-8-1-2011-eng.pdf (accessed on August 28, 2025).
- Iles, J.A., Pettit, N.E., Cawthray, G., Grierson, P., 2022. Chemotaxonomic responses of autotrophic periphyton communities to nutrient additions in pools of an intermittent stream. *Freshw. Biol.* 67, 2148–2160. <https://doi.org/10.1111/fwb.14002>.
- Jaeger, K.L., Olden, J.D., Pelland, N.A., 2014. Climate change poised to threaten hydrologic connectivity and endemic fishes in dryland streams. *Proc. Natl. Acad. Sci.* 111, 13894–13899. <https://doi.org/10.1073/pnas.1320890111>.
- James, A., Fritz, K.M., Topping, B., Fertik Edgerton, R., Nicholas, K., Mazor, R., Nadeau, T.-L., 2025a. Streamflow Duration Assessment Methods for the Northeast and Southeast of the United States. Version 2.0. Document No. EPA-843-B-25001. https://www.epa.gov/system/files/documents/2025-08/ne-se_sdam_august_2025.pdf (accessed on August 28, 2025).
- James, A., Fritz, K.M., Topping, B., Nadeau, T.-L., Fertik Edgerton, R., Nicholas, K., Mazor, R., 2025b. Streamflow Duration Assessment Methods for the Great Plains of the United States. Version 2.0. Document No. EPA-843-B-24001. https://www.epa.gov/system/files/documents/2024-10/gp-sdam_user_manual_october2024.pdf (accessed on August 28, 2025).
- Jiménez, L., Freixa, A., Besolí, N., Sabater, S., 2023. Resistance but not recovery is related to the role of specialist taxa in river communities submitted to hydric stress. *Sci. Total Environ.* 871, 161952. <https://doi.org/10.1016/j.scitotenv.2023.161952>.
- Johansen, J.R., 2010. Diatoms of aerial habitats. In: Stoermer, E.P., Smol, J.P. (Eds.), *The Diatoms: Applications for the Environmental and Earth Sciences*. Cambridge University Press, Cambridge, UK, pp. 465–472. <https://doi.org/10.1017/CBO9780511763175.025>.
- Kelly, M.G., Krokowski, J., J.P.C., Harding, 2016. RAPPER: a new method for rapid assessment of macroalgae as a complement to diatom-based assessments of ecological status. *Sci. Total Environ.* 568, 536–545. <https://doi.org/10.1016/j.scitotenv.2015.12.068>.
- Klaus, J., Wetzel, C.E., Martínez-Carreras, N., Ector, L., Pfister, L., 2015. A tracer to bridge the scales: on the value of diatoms for tracing fast flow path connectivity from headwaters to meso-scale catchments. *Hydrol. Process.* 29, 5275–5289. <https://doi.org/10.1002/hyp.10628>.
- Kociotek, J.P., Blanco, S., Coste, M., Ector, L., Liu, Y., Karthick, B., Kulikovskiy, M., Lindholm, N., Ludwig, T., Potapova, M., Rimet, F., Sabbe, K., Sala, S., Sar, E., Taylor, J., Van de Vijver, B., Wetzel, C.E., Williams, D.M., Witkowski, A., Witkowski, J., 2025. DiatomBase. *Luticola cohni* (Hilse) D.G.Mann, 1990. <https://diatombase.org/aphia.php?p=taxdetails&id=610484> (accessed on June 20, 2025).
- Krabbenhoft, C.A., Allen, G.H., Lin, P., Godsey, S.E., Allen, D.C., Burrows, R.M., DelVecchia, A.G., Fritz, K.M., Shanafield, M., Burgin, A.J., Zimmer, M., Datry, T., Dodds, W.K., Jones, C.N., Mims, M.C., Franklin, C., Hammond, J.C., Zipper, S.C., Ward, A.S., Costigan, K.H., Beck, H.E., Olden, J.D., 2022. Assessing placement bias of the global river gauge network. *Nat. Sustainability* 5, 586–592. <https://doi.org/10.1038/s41893-022-00873-0>.
- Krammer, K., Lange-Bertalot, H., 1986. Bacillariophyceae, Teil 1. Naviculaceae. In: Ettl, H., Gerloff, J., Heynig, H., Mollenhauer, D. (Eds.), *Süßwasserflora von Mitteleuropa*. VEB Gustav Fischer Verlag, Jena, Germany, pp. 1–876.
- Krammer, K., Lange-Bertalot, H., 1988. Bacillariophyceae, Teil 2. Epithemiaceae, Bacillariaceae, Surirellaceae. In: Ettl, H., Gerloff, J., Heynig, H., Mollenhauer, D. (Eds.), *Süßwasserflora von Mitteleuropa*. VEB Gustav Fischer Verlag, Jena, Germany, pp. 1–612.
- Krammer, K., Lange-Bertalot, H., 1991a. Bacillariophyceae, Teil 3. Centrales, Fragilariaceae, Eunotiaceae, Achnantheaceae. In: Ettl, H., Gerloff, J., Heynig, H., Mollenhauer, D. (Eds.), *Süßwasserflora von Mitteleuropa*. VEB Gustav Fischer Verlag, Stuttgart, Germany, pp. 1–576.
- Krammer, K., Lange-Bertalot, H., 1991b. Bacillariophyceae, Teil 4. Achnantheaceae, Kritische Ergänzungen zu *Navicula* (Lineolatae) und *Gomphonema*. In: Ettl, H., Gartner, G., Gerloff, J., Heynig, H., Mollenhauer, D. (Eds.), *Süßwasserflora von Mitteleuropa*. VEB Gustav Fischer Verlag, Stuttgart, Germany, pp. 1–437.
- Lange, K., Townsend, C.R., Matthaei, C.D., 2016. A trait-based framework for stream algal communities. *Ecol. Evol.* 6, 23–36. <https://doi.org/10.1002/eece3.1822>.
- Lowe, R.L., Golladay, S.W., Webster, J.R., 1986. Periphyton response to nutrient manipulation in streams draining clearcut and forested watersheds. *J. North Am. Benthol. Soc.* 5, 221–229. <https://doi.org/10.2307/1467709>.
- Lukács, Á., Bácsi, I., Nemes-Kókai, Z., Borics, G., Várbíró, G., E., T.-Krasznai, V., B-Béres, 2021. Strong influence of climatic extremes on diversity of benthic algae and cyanobacteria in a lowland intermittent stream. *Ecology* 14, e2286. <https://doi.org/10.1002/eco.2286>.
- Lynch, D.T., Magoulick, D.D., 2016. Effects of pulse and press drying disturbance on benthic stream communities. *Freshw. Sci.* 35, 998–1009. <https://doi.org/10.1086/687843>.
- Lytle, D.A., Poff, N.L., 2004. Adaptation to natural flow regimes. *Trends Ecol. Evol.* 19, 94–100. <https://doi.org/10.1016/j.tree.2003.10.002>.
- Mazor, R., James, A., Fritz, K.M., Topping, B., Nadeau, T.-L., Fertik Edgerton, R., Nicholas, K., 2024. Streamflow Duration Assessment Methods for the Arid West and Western Mountains of the United States. Version 2.0. Document No. EPA-843-B-24002. https://www.epa.gov/system/files/documents/2024-10/aw-wm-sdam_user_manual_october2024.pdf (accessed on August 28, 2025).
- Mertens, A., van der Wal, J., Verweij, G., Pex, B., van Dulmen, A., van Dam, H., 2025. A revised list of diatom ecological indicator values in The Netherlands. *Ecol. Indic.* 172, 113219. <https://doi.org/10.1016/j.ecolind.2025.113219>.
- Messenger, M.L., Pella, H., Datry, T., 2024. Inconsistent regulatory mapping quietly threatens rivers and streams. *Environ. Sci. Technol.* 58, 17201–17214. <https://doi.org/10.1021/acs.est.4c01859>.
- More, A.S., Rana, D.P., 2017. Review of random forest classification techniques to resolve data imbalance. Institute of Electrical and Electronics Engineers, Aurangabad, India. In: First International Conference on Intelligent Systems and Information Management. Institute of Electrical and Electronics Engineers, Aurangabad, India, pp. 72–78. <https://doi.org/10.1109/ICISIM.2017.8122151>.
- Mullahy, J.H., 1952. The morphology and cytology of *Lemanea australis* Atk. *Bull. Torrey Bot. Club* 79, 471–484. <https://doi.org/10.2307/2482261>.
- Nadeau, T.-L., 2015. Streamflow duration assessment method for the Pacific Northwest. In: Document No. EPA-910-K-14-001, U.S. Environmental Protection Agency, Region 10, Seattle, WA, p. 36. https://www.epa.gov/sites/default/files/2016-01/documents/streamflow_duration_assessment_method_pacific_northwest_2015.pdf (accessed on August 28, 2025).
- NCDWQ (North Carolina Division of Water Quality), 2010. Methodology for Identification of Intermittent and Perennial Streams and Their Origins. Version 4.11. In: North Carolina Department of Environment and Natural Resources, Division of Water Quality, Raleigh, North Carolina, p. 41. https://files.nc.gov/ncdeq/Water%20Quality/Surface%20Water%20Protection/401/Policies_Guides_Manuals/StreamID_v_4point11_Final_sept_01_2010.pdf (accessed on August 28, 2025).
- Nicolosi Gelis, M.M., Sathicq, M.B., Paredes del Puerto, J.M., Pazos, R.S., Tarda, A.S., Gómez, N., 2025. Impact of extreme drought on diatom traits and species composition in temperate lowland streams. *Hydrobiologia* 852, 629–644. <https://doi.org/10.1007/s10750-024-05710-3>.
- NMED (New Mexico Environmental Department), 2020. Hydrology Protocol for the Determination of Uses Supported by Ephemeral, Intermittent, and Perennial Waters. Albuquerque, NM, Surface Water Quality Bureau, New Mexico Environment Department, p. 39. <https://www.env.nm.gov/wp-content/uploads/sites/25/2019/11/WQMP-CPP-Appendix-C-Hydrology-Protocol-20201023-APPROVED.pdf> (accessed on August 28, 2025).
- Novais, M.H., Morais, M., 2024. Diatoms in temporary rivers: importance in a global climate change context. In: Maidana, N.I., Licursi, M., Morales, E. (Eds.), *Diatom Ecology: From Molecules to Metacommunities*. Scrivener Publishing, Beverly, MA, pp. 361–391. <https://doi.org/10.1002/9781394174898.ch11>.
- Novais, M.H., Morais, M.M., Rosado, J., Dias, L.S., Hoffmann, L., Ector, L., 2014. Diatoms of temporary and permanent watercourses in southern Europe (Portugal). *River Res. Appl.* 30, 1216–1232. <https://doi.org/10.1002/rra.2818>.
- Novais, M.M., Morales, E.A., Penha, A.M., Potes, M., Bouchez, A., Barthés, A., Costa, M. J., Saigado, R., Santos, J., Morais, M., 2020. Benthic diatom community dynamics in Mediterranean intermittent streams: effects of water availability and their potential as indicators of dry-phase ecological status. *Sci. Total Environ.* 719, 137462. <https://doi.org/10.1016/j.scitotenv.2020.137462>.
- Omernik, J.M., 1995. Ecoregions: A framework for managing ecosystems. *Geo. Wright Forum* 12, 35–50. <https://www.jstor.org/stable/43597408>.
- Paluszynska, A., Biecek, P., Jiang, Y., 2020. randomForest-Explainer: Explaining and visualizing random forests in terms of variable importance. (Available from: <https://cran.r-project.org/web/packages/randomForestExplainer/index.html>).
- Passy, S.I., 2007. Diatom ecological guilds display distinct and predictable behavior along nutrient and disturbance gradients in running waters. *Aquat. Bot.* 86, 171–178. <https://doi.org/10.1016/j.aquabot.2006.09.018>.
- Patrick, R., Reimer, C.W., 1966. The Diatoms of the United States, exclusive of Alaska and Hawaii. *Monogr. Acad. Nat. Sci. Philad.* 1, 1–688.
- Patrick, R., Reimer, C.W., 1975. The Diatoms of the United States, exclusive of Alaska and Hawaii. *Monogr. Acad. Nat. Sci. Philad.* 2 (13), 1–213.
- Peszek, L., Zgrundo, A., Noga, T., Kochman-Kędziora, N., Poradowska, A., Rybak, M., Puchalski, C., Lee, J., 2021. The influence of drought on diatom assemblages in a temperate climate zone: A case study from the Carpathian Mountains. *Poland. Ecol. Indic.* 125, 107579. <https://doi.org/10.1016/j.ecolind.2021.107579>.
- Peterson, C.G., 1987. Influences of flow regime on development and desiccation response of lotic diatom communities. *Ecology* 68, 946–954. <https://doi.org/10.2307/1938366>.
- Peterson, C.G., Boulton, A.J., 1999. Stream permanence influences microalgal food availability to grazing tadpoles in arid-zone springs. *Oecologia* 118, 340–352. <https://doi.org/10.1007/s004420050735>.
- Pfister, L., Wetzel, C.E., Martínez-Carreras, N., Iffy, J.F., Klaus, J., Holko, L., McDonnell, J.J., 2015. Examination of aerial diatoms flushing across watersheds in Luxembourg, Oregon and Slovakia for tracing episodic hydrological connectivity. *J. Hydrol. Hydromech.* 63, 235–245. <https://doi.org/10.1515/johh-2015-0031>.
- Piano, E., Falasco, E., Bona, F., 2017. Mediterranean rivers: consequences of water scarcity on benthic algal chlorophyll a content. *J. Limnol.* 76, 39–48. <https://doi.org/10.4081/jlimnol.2016.1503>.
- Potapova, M.G., Charles, D.F., 2002. Benthic diatoms in USA rivers: distribution along spatial and environmental gradients. *J. Biogeogr.* 29, 167–187. <https://doi.org/10.1046/j.1365-2699.2002.00668.x>.
- Potapova, M.G., Charles, D.F., 2005. Choice of substrate in algae-based water-quality assessment. *J. North Am. Benthol. Soc.* 24, 415–427. <https://doi.org/10.1899/03-111.1>.
- Potts, M., 1999. Mechanisms of desiccation tolerance in cyanobacteria. *Eur. J. Phycol.* 34, 319–328. <https://doi.org/10.1080/09670269910001736382>.
- Riatio, L., Hill, R.A., Herlihy, A.T., Peck, D.V., Kaufmann, P.R., Stoddard, J.L., Paulsen, S. G., 2022. Genus-level, trait-based multimetric diatom indices for assessing the ecological condition of rivers and streams across the conterminous United States. *Ecol. Indic.* 141, 109131. <https://doi.org/10.1016/j.ecolind.2022.109131>.

- Rimet, F., Bouchez, A., 2012. Life-forms, cell-sizes and ecological guilds of diatoms in European rivers. *Knowl. Manag. Aquat. Ecosyst.* 406, 01. <https://doi.org/10.1051/kmae/2012018>.
- Robson, B.J., 2000. Role of residual biofilm in the recolonization of rocky intermittent streams by benthic algae. *Mar. Freshw. Res.* 51, 725–732. <https://doi.org/10.1071/MF00012>.
- Round, F.E., 1998. A problem in algal ecology—contamination of habitats from adjacent communities. *Cryptogam. Algal.* 19, 49–55.
- Shanafield, M., Bourke, S.A., Zimmer, M.A., Costigan, K.H., 2021. An overview of the hydrology of non-perennial rivers and streams. *WIREs Water* 8, e1504. <https://doi.org/10.1002/wat2.1504>.
- Smucker, N.J., Pilgrim, E.M., Nietch, C.T., Gains-Germain, L., Carpenter, C., Darling, J. A., Yuan, L.L., Mitchell, R.M., Pollard, A.I., 2024. Using DNA metabarcoding to characterize national scale diatom-environment relationships and to develop indicators in streams and rivers of the United States. *Sci. Total Environ.* 939, 173502. <https://doi.org/10.1016/j.scitotenv.2024.173502>.
- Soininen, J., 2007. Environmental and spatial control of freshwater diatoms – a review. *Diatom Res.* 22, 473–490. <https://doi.org/10.1080/0269249X.2007.9705724>.
- Soininen, J., Paavola, R., Muotka, T., 2004. Benthic diatom communities in boreal streams: community structure in relation to environmental and spatial gradients. *Ecography* 27, 330–342. <https://doi.org/10.1111/j.0906-7590.2004.03749.x>.
- Spaulding, S.A., Potapova, M.G., Bishop, I.W., Lee, S.S., Gasperak, T.S., Jovanoska, E., Furey, P.C., Edlund, M.B., 2021. Diatoms.org: supporting taxonomists, connecting communities. *Diatom Res.* 36, 291–304. <https://doi.org/10.1080/0269249X.2021.2006790>.
- Stanislawski, L.V., Buittenfield, B.P., Doumbouya, A., 2015. A rapid approach for automated comparison of independently derived stream networks. *Cartogr. Geogr. Inf. Sci.* 42, 435–448. <https://doi.org/10.1080/15230406.2015.1060869>.
- Stenger-Kovács, C., V., B-Béres, 2024. Guilds for diatoms: history and future prospects. In: Maidana, N.I., Licursi, M., Morales, E. (Eds.), *Diatom Ecology: From Molecules to Metacommunities*. Scrivener Publishing, Beverly, MA, pp. 233–279. <https://doi.org/10.1002/9781394174898.ch8>.
- Stepanek, J., Kociolek, P., 2011. *Halamphora montana*. In *Diatoms of North America*. Retrieved June 20, 2025, from: https://diatoms.org/species/45413/halamphora_montana.
- Stevenson, R.J., 2014. Ecological assessments with algae: a review and synthesis. *J. Phycol.* 50, 437–461. <https://doi.org/10.1111/jpy.12189>.
- Sun, J., Liu, D., 2003. Geometric models for calculating cell biovolume and surface area for phytoplankton. *J. Plankton Res.* 25, 1331–1346. <https://doi.org/10.1093/plankt/fbg096>.
- Taurozzi, D., Cesarini, G., Scalici, M., 2024. Diatoms as bioindicators for health assessments of ephemeral freshwater ecosystems: a comprehensive review. *Ecol. Indic.* 166, 112309. <https://doi.org/10.1016/j.ecolind.2024.112309>.
- Terada, R., Makino, K., Nishihara, G.N., 2025. The photosynthetic response of the freshwater red alga *Thorea okadae* to environmental gradients of temperature, irradiance, desiccation, and salinity: adaptations to its stream habitat. *J. Appl. Phycol.* 37, 2753–2768. <https://doi.org/10.1007/s10811-025-03528-7>.
- Timoner, X., Buchaca, T., Acuña, V., Sabater, S., 2014. Photosynthetic pigment changes and adaptations in biofilms in response to flow intermittency. *Aquat. Sci.* 76, 565–578. <https://doi.org/10.1007/s00027-014-0355-6>.
- Timoner, X., Colls, M., Salomón, S.M., Oliva, F., Acuña, V., Sabater, S., 2020. Does biofilm origin matter? Biofilm responses to non-flow period in permanent and temporary streams. *Freshw. Biol.* 65, 514–523. <https://doi.org/10.1111/fwb.13447>.
- Tornés, E., Colls, M., Acuña, V., Sabater, S., 2021. Duration of water flow interruption drives structure and functional diversity of stream benthic diatoms. *Sci. Total Environ.* 770, 144675. <https://doi.org/10.1016/j.scitotenv.2020.144675>.
- United States Environmental Protection Agency (USEPA), 2025. Regional streamflow duration assessment methods. <https://www.epa.gov/streamflow-duration-assessment> (accessed on 28 August 2025).
- Van Dam, H., Mertens, A., Sinkeldam, J., 1994. A coded checklist and ecological indicator values of freshwater diatoms from The Netherlands. *Neth. J. Aquat. Ecol.* 28, 117–131. <https://doi.org/10.1007/BF02334251>.
- Várbíró, G., Borics, G., Novais, M.H., Morais, M.M., Rimet, F., Bouchez, A., Tapolczai, K., Bácsi, I., Usseglio-Polatera, P., V., B-Béres, 2020. Environmental filtering and limiting similarity as main forces driving diatom community structure in Mediterranean and continental temporary and perennial streams. *Sci. Total Environ.* 741, 140459. <https://doi.org/10.1016/j.scitotenv.2020.140459>.
- Wehr, J.D., Sheath, R.G., 2003. *Freshwater Algae of North America: Ecology and Classification*. Academic Press, Boston, MA, p. 900.
- Whitford, L.A., Schumacher, G.J., 1963. Communities of algae in North Carolina streams and their seasonal relations. *Hydrobiologia* 22, 133–196. <https://doi.org/10.1007/BF00039686>.
- Wright, M.N., Wager, S., Probst, P., 2022. ranger: A fast implementation of random forests. (Available from: <https://cran.r-project.org/web/packages/ranger/ranger.pdf>).
- Zlatanović, S., Fabian, J., Premke, K., Mutz, M., 2018. Shading and sediment structure effects on stream metabolism resistance and resilience to infrequent droughts. *Sci. Total Environ.* 621, 1233–1242. <https://doi.org/10.1016/j.scitotenv.2017.10.105>.