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Preliminary descriptions of saxicolous lichen communities in North Carolina Piedmont rocky river ecosystems

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Abstract. The lichen biota of eastern North America is fairly well-documented with most taxa reported from terrestrial ecosystems. While some taxa are described as living near water bodies potentially subjected to inundation, no amphibious lichen communities have been described. To address this gap in our understanding of the region's lichen ecology, thirteen rocky river sites in two river basins of central North Carolina, U.S.A. were explored for amphibious and riparian lichen biotas during periods of low water level, restricted to saxicolous species subjected to inundation, however infrequent. Specimens of encountered taxa were collected and their heights above water level were measured during field visits. Three communities were discerned from field observations in increasing height from low water level, here termed: *Mesic Fluvial, Xeric Fluvial* and *Riparian*. These communities are described in terms of species number and composition, height above water, functional traits and taxonomic class composition, as well as characteristic species both in open riverscour and shaded rocky riverbank habitats. From measured heights, two trimlines are described separating the three communities: *Fluvial Trimline*, often co-occurring with a band of deposited silt, and *Riparian Trimline*. With the use of nearby stream gage height data, percent inundation for the 2023 water year was estimated for the three communities as 3–10 months for Mesic Fluvial, 0.5–4 months per year for Xeric Fluvial, and $<$ 1–3 months for Riparian lichens. Environmental variables were explored for relationships with the three communities, finding the most significant positive relationships between Mesic Fluvial species richness and several stream physical and water chemistry variables. Recommendations for future stream lichen surveys are offered.

Keywords. Freshwater lichens, community ecology, eastern North America, functional trait analysis, zonation.

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Streams and rivers are dynamic ecosystems that are shaped by and adapted to flowing water and its variation in flow intensity and height. Fluvial biological communities are shaped by these hydrological processes plus other abiotic factors such as substrate geology, geomorphology, and water chemistry (Allan & Castillo 2007). Species in fluvial habitats are adapted to these environmental factors, including varying amounts of inundation and/or desiccation, along a vertical continuum from fully submerged (i.e., benthic and lotic communities represented by aquatic invertebrate and fish communities) to riparian (e.g., mesic forests on floodplains and along riverbanks). Primary producers in fluvial ecosystems along this gradient include benthic algae, phytoplankton, and macrophytes, the lattermost comprised of flowering plants, bryophytes, macroalgae and lichens (Allan & Castillo 2007).

Vegetation communities are often observed forming a series of distinct horizontal bands or zones of algae, lichens, bryophytes and vascular plants from the low water level or below upwards along creeks, rivers, lakes and coastal shorelines. In fluvial systems, vertical community zonation is most distinct on exposed channel rocks and rocky banks, wherein the communities are dominated by lichens. While such communities occur worldwide, fluvial lichen zoned communities have been most thoroughly described and studied in

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Europe, broadly describing three zones along a height continuum from the low flow water level upwards that corresponds with decreasing amount of water contact or immersion, calling the zones and communities therein by various names [e.g., Coste (2009) in France; James et al. (1977) in Great Britain; Khodosovtsev & Kuzemko (2023) in Poland; Krzewicka et al. (2017) in Ukraine; and Thüs & Schultz (2009) in Middle Europe]. By contrast, reports of zoned lichen communities in freshwater habitats in North America are scarce in the literature [Rosentreter (1984) in Idaho, U.S.A., and Timoney & Marsh (2004) in Alberta, Canada]. No such reports have been found in a literature search for southeastern North America, suggesting this region has not been studied. An opportunity to study fluvial lichen communities and their zonation thus presents itself in central North Carolina, U.S.A., where rocky rivers are numerous.

In France, Costes (2009) and Costes et al. (2023) have quantified submergence durations of zoned lichen communities as > 9 months per year for the lowermost zone, 3–9 months per year for the intermediate zone, and < 3 months per year for the uppermost zone, which they have termed as hyper-hydrophilic, meso-hydrophilic and sub-hydrophilic, respectively. If similar zones are present in rocky rivers of North Carolina, can their submergence too be quantified? The United States Geological Survey (USGS) operates a network of over 11,340 stream gages across the country, recording water levels and/or streamflow for at least part of the year (Normand 2021). Can stream gages be used to measure the frequency and duration of inundation that amphibious lichens are exposed to and thus quantify their ecological inundation tolerances? Similarly, can such measures be used to describe zoned lichen communities?

The lichen biota of North Carolina is fairly well understood with a total of over 1550 taxa recorded (Perlmutter et al. 2024). While floristic reports focus on lichen biodiversity of a given area that include waterways (e.g., Lendemer et al. 2016; Perlmutter 2022), none have focused solely on fluvial habitats.

A recent treatment of riverscour habitats (Estes et al. 2023) define this ecosystem type as "open riparian habitats of rocky, stable-substrate (bedrock, boulder, cobble) zones, often along high-gradient streams, where periodic high-energy flows (water, ice, debris) and edaphic factors inhibit woody vegetation and promote persistent grassland-shrubland-open woodland-outcrop communities rich in conservative heliophytes." In the Piedmont of central North Carolina, riverscour habitats, classified as Rocky Bar and Shore (Mixed Bar Subtype) natural communities by Schafale (2024), are distinct from the surrounding forests, where most lichen surveys have been conducted (e.g., LaGreca et al. 2018; Lendemer et al. 2017; Perlmutter 2008). Rocky riverine and stream habitats also include shaded to semi-shaded banks, wherein species are adapted to both periodic inundation and lower sun exposure.

Objectives of the present study are to document the lichen biodiversity of rocky river (i.e., riverscour and rocky riverbank) habitats in North Carolina; describe their lichen communities by zones of relative inundation with inundation frequencies and durations estimated using hydrological measurements of nearby stream gages; and explore relationships with environmental variables.

Materials and Methods

Study area. Lichens of riverscour and riverbank habitats were explored in 13 sites in two adjacent river basins within the Piedmont Level III Ecoregion (Griffith et al. 2002) in central North Carolina, U.S.A. in southeastern North America (**Fig. 1**). The Piedmont is a moderately dissected peneplain sloping from west to east and consisting of xeric upland ridges, mesic slopes and hydric flat bottomlands, all of which are dissected by streams and rivers. Streams and rivers sampled in the study area lie within two adjacent river basins: the Cape Fear River Basin and the Neuse River Basin, both of which drain from northwest to southeast to empty into the Atlantic Ocean in eastern North Carolina.

The area experiences a four-season climate with mild winters and hot, humid summers. Thirty-year (1981–2010) climatic normals from the Chapel Hill Williams Airport, located approximately in the center of the study area, include a temperature range from –0.1°C (31.9°F) in January to 32.2°C (89.9°F) in July and a total annual precipitation of 1129 mm (44.6 in.), with an average monthly total of 94 mm (range: 79–114 mm) [3.70 in. (range: 3.11–4.49 in.)] without a marked wet or dry season (NOAA 2023).

All fluvial sites visited are in third to fifth order, unaltered perennial streams classified hydro-ecologically

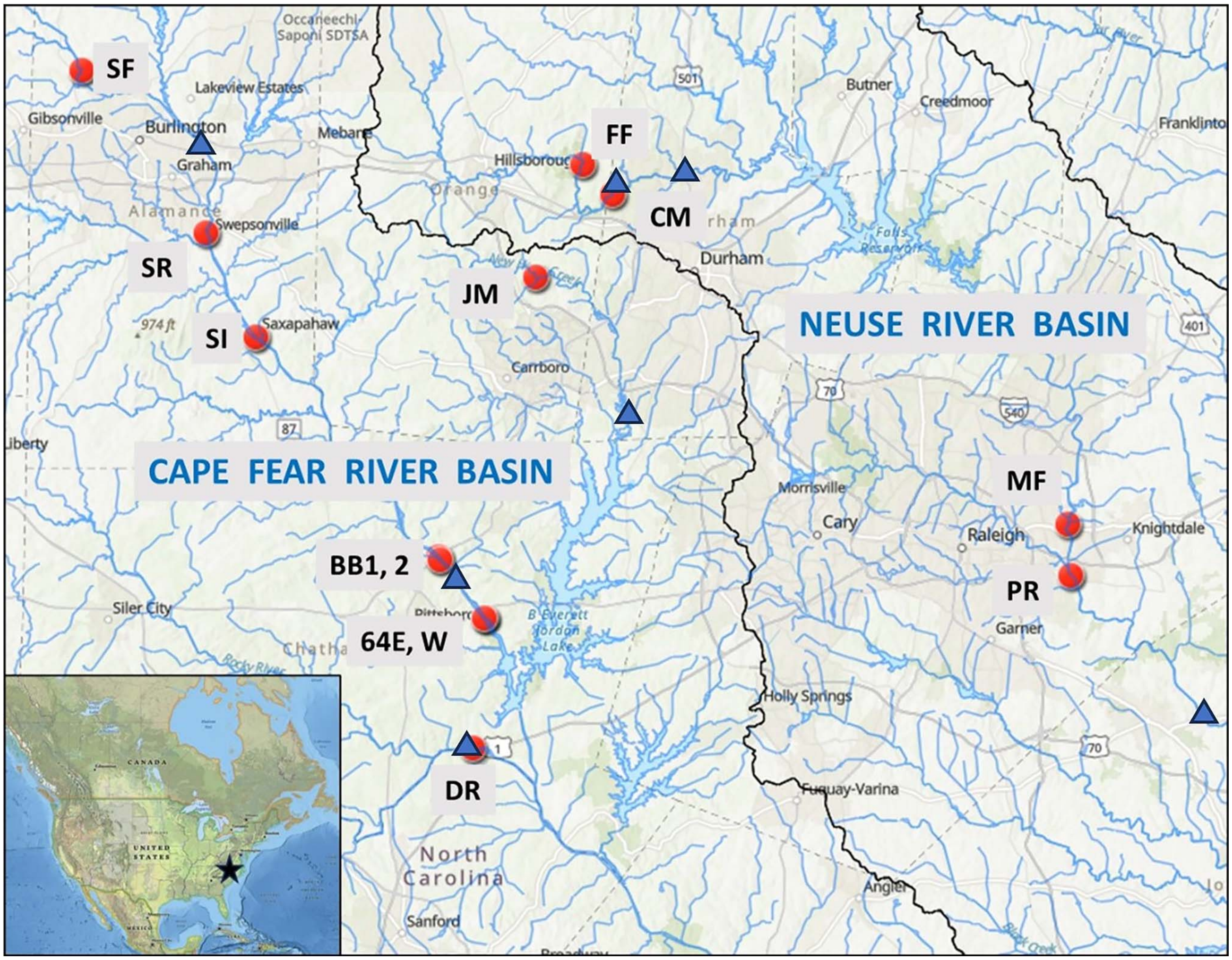

Figure 1. Map of the study area with stream/river lichen site locations (orange circles) with nearby USGS stream gage stations (blue triangles). See Supplementary Table S1 for site code definitions.

as Small Flashy Streams at their nearest USGS streangage stations (**Figs. 2** and **3**). Small Flashy Streams are characterized by: a relativley low median daily flow of 49 cfs (1.4 m^3/s); a relatively low flow predictability of 51% (i.e., flows fluctuate with greater magnitude and frequency and hence considered “flashy”); a low baseflow index of 6% (i.e., a low minimum flow compared to the mean annual flow, which reflects a low flow stability or low groundwater influence); and with the highest number of high flows occuring in December (Henricksen & Heasley 2010). Stream gage data from USGS stations nearest to lichen sites (**Fig. 1**), compiled over several decades, also indicate site flashiness via streamflow statistics of relatively low average flows compared to low (7 day in 10 year minimum) flows and proportionally high (100-yr flood) flows (**Supplementary Table S1**).

Site stream widths ranged 26–272 m (85–892 ft.) with 100-yr floodplain widths ranging 44.5–306 m (146–1005 ft.); annual average streamflows ranged 0.6–39.2 m^3/s (22–1386 cfs) (**Supplementary Table S1**). Stream water quality was assessed by the NC Division of Water Resources (NC DWR) in 2022 as meeting state and federal regulatory standards for a variety of physical, chemical and biological parameters (e.g., Dissolved Oxygen > 5 mg/L, Fecal Coliform < 200 cfu/100 mL, pH between 6.0–9.0 SU, summer maximum Temperature < 32°C, Turbidity < 50 NTU) in stream segments representing all but one site (NC DWR 2022). Substrate geology at all sites was acidic bedrock, including gabbro, mafic to felsic metavolcanic, and granitic (NCGS 1985).

Field surveys and specimen processing. Study sites were visited in the 2023 water year (WY2023:

Figure 2. Neuse River above Poole Road canoe access (Site PR) as an example of a Small Flashy Stream. **A.** Site at high water level in March 2022, with rocks fully submerged. **B.** Site at low water level in October 2022 with rocks exposed. Images by Google Earth. Scale bar = 50 m.

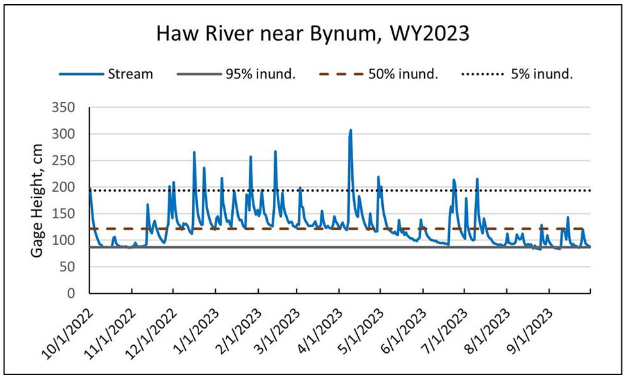

Figure 3. Hydrograph of USGS Station 02096960 Haw River near Bynum data for the 2023 water year as an example of a Small Flashy Stream showing a series of peak gage heights following rain events along with low (95% inundated), median (50% inundated) and high (5% inundated) height overlays.

Oct 2022–Sep 2023) during periods of low water level to sample lichen diversity through collection and observation of taxa encountered. At each site, an effort was made to document all saxicolous, riverine lichen taxa encountered through specimen collection and determination, including taxon associates within a given specimen. Lichens were documented from both shaded riverbank and exposed riverscour rocks, but restricted to habitats that could be submerged or exposed to splashing during flood events.

During site visits, the vertical distance from the water level of representative species was measured in cm including their lowermost and uppermost heights. In addition, lichen trimlines, or lines of abrupt change in lichen community zones (or, rather, transition zones of overlapping community member species), were measured in cm above the water level. Lichen zone communities were described in terms of species composition away from the trimlines and are here termed from the water level upward as *Mesic Fluvial*, *Xeric Fluvial* and *Riparian*, modified from the naming convention of Gilbert (1996) and Gilbert & Giavarini (1997) for stratified lichen communities along streams of England. It should be noted that in some sites not all three communities were observed, either due to rocks being too low to support a riparian community (e.g., PR, **Fig. 2**), or water levels were too high during a visit to reveal the mesic fluvial community.

Collected specimens were studied at NCU and DUKE via examination of morphological features, microscopic examination of reproductive structures, and chemical spot testing following Brodo et al. (2001). When additional help was needed for identification, experts were consulted, and in some cases, specimens were sent for further study and determination.

For two morphologically similar *Dermtocarpon* species encountered, we obtained molecular data from one voucher each of *D. arenosaxi* (*Perlmutter 4503*, NCU) and *D. luridum* (*LaGreca 2900*, DUKE) to verify their identifications. DNA was extracted with a phenol:chloroform protocol for microlichens following the procedure of Hughes et al. (2020). The ITS locus was amplified with primers ITS1F (Stiller & Hall 1997) and ITS4A (Matheny et al. 2002). Sanger sequencing was performed by Eurofins Genomics (Louisville, KY) using PCR primers. Forward and reverse chromatograms were assembled in Geneious Prime 2022 and the assembled sequences were compared to the NCBI nucleotide database and to each other using BLAST (Zhang et al. 2000).

Specimens collected by GBP were deposited in NCU; those collected by SALG were deposited in DUKE and NCU. Records of our collections were entered into the Consortium of Lichen herbaria (CLH; www.lichenportal.org), which were then compiled into a checklist of North Carolina Piedmont rocky river lichens. Previous specimen records were searched for in the authors' collections as well as records in CLH for inclusion in the checklist. We did not borrow and examine any specimens outside of DUKE or NCU.

Overall diversity, functional trait and taxonomic class analyses. Lichen diversity was measured and analyzed via several metrics, including the overall species richness and species richness of each site, as well as species composition by functional traits and taxonomy. An analysis of similarity (one-way ANOSIM with a Bray-Curtis similarity index) was used to test for differences of lichen biotas between the two river basins. For functional trait and taxonomic analyses, taxa recorded as single occurrences (i.e., from a single site) as well as lichenicolous fungi were excluded, yielding a dataset of 36 taxa. Each species in the dataset was then considered for the following traits: growth form (crustose, squamulose, or foliose); primary reproductive strategy (sexual via ascomata, or vegetative via isidia or soredia); and photobiont (chlorophyte or cyanobacteria). For analysis, squamulose includes thalli also described as either umbilicate (as in *Dermatocarpon*) or peltate (as in *Peltula euploca*); regarding primary reproductive strategy, this means the mode of reproduction that is most often observed (e.g., *Peltula euploca* was found to be predominantly sexual, with weakly sorediate forms found in two of eight specimens while six bore apothecia). For taxonomic analysis, the level of Class was selected as this can be broken down to relatively few types like those within the above functional trait categories (**Supplementary Table S2**). Percent composition of the above functional trait and taxonomic categories was compiled for the overall lichen biota and for each site. Average trait compositions were tested among sites for significance using ANOVA and Student's t-test, the latter assuming unequal variance.

Diversity analysis of zone communities. A nonmetric Multidimensional Scaling (nMDS) analysis was performed using Bray-Curtis distance of similarity on species presence/absence of each site-zone to look for differences in species composition among the three zone communities using the truncated set of 36 taxa and their observed placements across sites. Species composition among zone communities was tested for differences using ANOSIM. For functional trait and taxonomic analyses, each of the 36 taxa was assigned one of the three zone communities it was found most prevalent in, based on field observations, height measurements and collected specimens including associated taxa (**Supplementary Table S2**). The functional trait and taxonomic analyses described above for the biota as a whole were performed on taxa within each zoned community.

Lichen zone inundation estimates. Frequency and duration of inundation that a given lichen species or zone community experiences was estimated using USGS stream gage data along with field-measured lichen and trimline heights for selected sites. For each USGS gage station closest to a given lichen site (some stations represent multiple sites based on proximity), daily gage height and/or flow data were downloaded from the StreamStats website (https://streamstats.usgs.gov/ss/) for WY2023. Since stream gage flow and height data are often highly correlated ($r > 0.90$), the yield equation used for determining a site's annual average flow was applied for estimating daily site river heights as follows:

$$\begin{aligned} \mathit{Site\ Height} &= \mathit{Site\ Drainage\ Area} \\ &\quad \times \left(\mathit{Gage\ Height} / \mathit{Gage\ Drainage\ Area}\right) \end{aligned}$$

Drainage areas were also obtained from SteamStats through delineation (for the lichen site) and the linked gage station webpage. Estimated site data were then tared by subtracting the dataset minimum from each height datum to represent the river heights at each site.

The WY2023 inundation frequency for each field-measured lichen marker (species position or observed trimline) height from the waterline was estimated by first calculating the tared lichen marker height as follows:

$$\begin{gathered} \mathit{Field\text{-}measured\ height} + \mathit{Gage\ height\ on\ day\ measured} \\ -\mathit{Year\ minimum\ height} \end{gathered}$$

The number of days at which estimated site river heights exceeded a given tared lichen height was counted, then divided by 30 to estimate the number of months per year inundated. This metric was selected for comparison against published inundation rates (e.g., Coste 2009; Coste et al. 2023).

For a measurement of scale of extreme floods, the relative height of each site's floodplain was measured via the elevation profile tool in ArcGIS and subtracting the elevation of the streambed (i.e., the lowest point in the profile) from the elevation at the 100-yr floodplain edge.

Multivariate analysis with environmental variables. Lichen zoned communities and lichen species of the surveyed sites were explored for relationships with various environmental variables via nMDS analysis. Environmental variables representing survey sites include stream dimensions (width and floodplain width), annual average flow, drainage area, elevation, plus the following stream water quality parameters: average Temperature, pH, Specific Conductivity, Dissolved Oxygen (DO), Turbidity, and nutrient parameters (Ammonia, Nitrite + Nitrate (NO_2+NO_3), Total Kjeldahl Nitrogen (TKN), and phosphorus; NO_2+NO_3 and TKN were combined to represent Total Nitrogen). Monthly data from ambient water quality monitoring stations nearest to each lichen site were obtained from the Water Quality Portal (https://www.waterqualitydata.us/) spanning January 2017–December 2022 or June 2023 (**Supplementary Table S3**); data from the station nearest to the JM site were not included in the analysis as the station was in a swamp water, distinct from the rocky stream of the lichen site and thus station data were not representative of the lichen site. Mean values were incorporated to the input matrix for nMDS. Apparent relationships between lichen species and environmental variables observed in the nMDS plot were tested by correlation; those correlations of $\geq |0.5|$ were tested for significance by regression.

Level of significance for statistical tests was set *a priori* at 0.05. Analytical tests were run using PAST 4.13 (Hammer et al. 2001) and Microsoft Excel software packages.

RESULTS

Overall lichen diversity. From 164 collections a total of 53 lichen taxa plus two lichenicolous fungi are reported from 13 sites in two river basins in central North Carolina. For the full list, see the accompanying checklist on CLH (https://lichenportal.org/portal/checklists/checklist.php?clid=36859&pid=0); a recent publication (Perlmutter & LaGreca 2024) reports a number of noteworthy taxa we found, including some new to North America as well as some potentially new, as-yet undescribed species. Species richness per site ranged 3–16 with a mean of 10.2 (± 4.3 SD). Site biotas were not found to differ by river basin (ANOSIM; $R = 0.168$; $p = 0.12$, ns). Broken down by habit (i.e., growth form), the lichen flora overall was 61% crustose, 22% squamulose 17% foliose with similar proportions among sites (**Fig. 4**). Averages of the three habit proportions were significantly different among sites (ANOVA; $F = 20.48$; $p < 0.0001$). Reproductive strategies of the lichen biota included 78% sexual (via ascomata) and 22% vegetative (via diaspores: isidia or soredia). The overall lichen biota also comprised 69% chlorolichens and 31% cyanolichens. Both categories were found in significantly different proportions among sites on average (Reproductive strategy: $t = 6.33$; $p < 0.0001$. Photobiont type: $t = 3.29$; $p < 0.005$.). Taxonomically, lichen biota was comprised of four classes: *Eurotiomycetes* (13.9%), *Lecanoromycetes* (63.9%), *Lichinomycetes* (19.4%), and *Dothideomycetes* (2.8%), the lattermost represented by one species. Average proportions across sites differed among the four classes (ANOVA: $F = 15.47$; $p < 0.0001$).

BLAST of our two *Dermatocarpon* ITS sequences against the NCBI nucleotide database confirmed our morphological identifications: *Perlmutter 4503* (GenBank no. PP967966) shared 99%–100% sequence identity with other *D. arenosaxi* sequences in GenBank; and *LaGreca 2900* (GenBank no. PP967967) shared 97%–100% sequence identity with other North American *D. luridum* var. *luridum* sequences in GenBank.

Zone community lichen diversities. The nMDS plot showed a clear separation of zone communities between the riparian and combined fluvial communities across sites, and some overlap between the *Mesic Fluvial* and *Xeric Fluvial* communities (**Fig. 5a**). Groupings of zoned communities are aligned with Axis 1 from right to left: *Mesic Fluvial*, *Xeric Fluvial*, and *Riparian*. Most species were positioned within or near the bounds of their respective zone groupings, verifying their affinity for their respective zoned communities. Differences in species composition among the three communities was found significant (ANOSIM; $R = 0.730$, $p = 0.0001$).

Across sites, the three community zones tested significantly different in terms of species number,

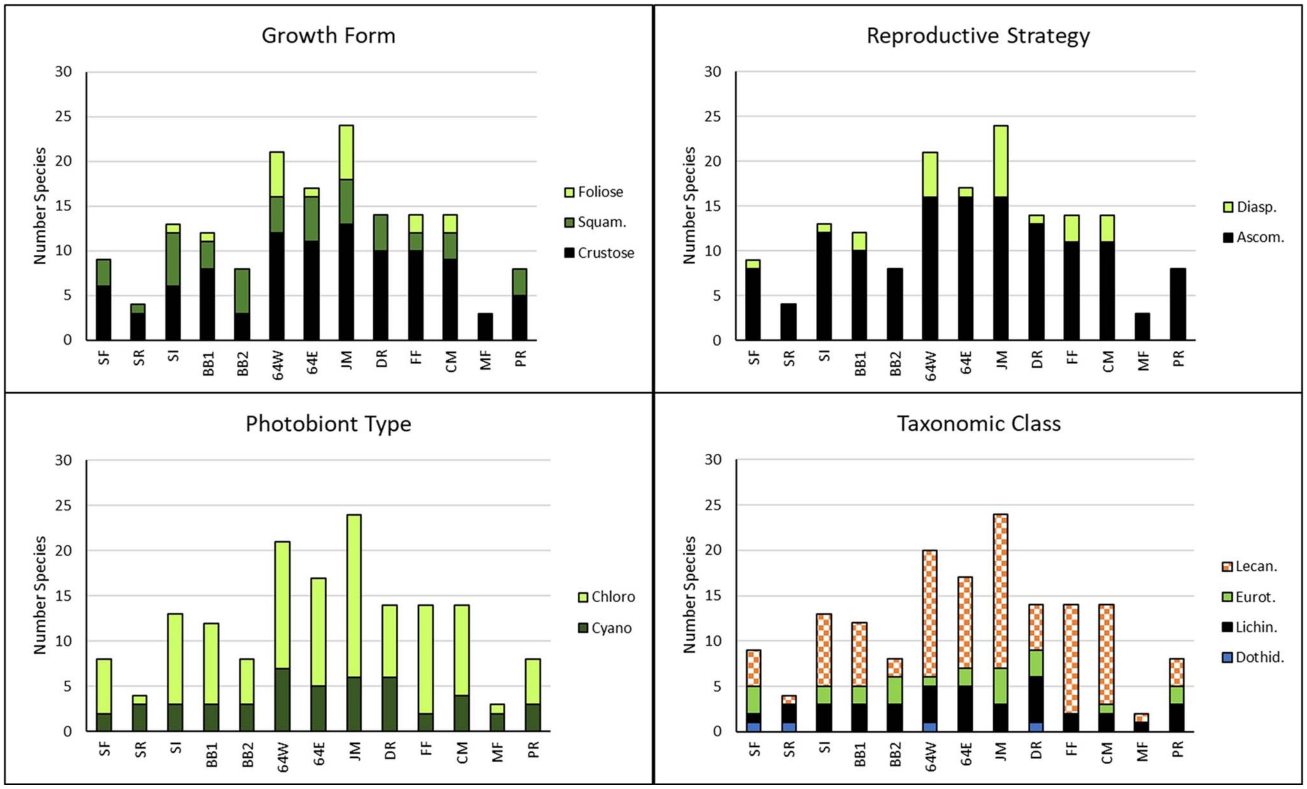

Figure 4. Overall site lichen biotic composition by functional traits and higher taxonomy in 13 Piedmont rocky river sites in central North Carolina, U.S.A. See Supplementary Table S1 for site code definitions.

with the Mesic Fluvial community bearing fewer species on average than either the Xeric Fluvial or Riparian community (ANOVA: $F = 4.0$; $p < 0.05$). Overall community composition percentages are in **Tables 1** and **2** for functional traits and higher taxonomy, respectively.

Mesic Fluvial communities.—A total of eight Mesic Fluvial species were recorded, of which seven were analyzed for functional trait and higher taxonomic composition. Mesic Fluvial communities were 1.6 (± 1.3) crustose and 0.5 (± 0.5) squamulose species across sites with significantly more crustose species found (Student's $t = 4.05$; $p < 0.0005$). Mesic Fluvial lichens were all sexual and were predominantly cyanolichens (1.8 ± 1.3) versus (0.3 ± 0.5) chlorolichens, which were significantly fewer among sites (Student's $t = -5.10$; $p < 0.0005$). Taxonomically, the Mesic Fluvial community was comprised of *Dothideomycetes* (0.3 ± 0.5 species), *Eurotiomycetes* (0.3 ± 0.5 species), and *Lichinomycetes* (1.5 ± 1.1 species), with the lattermost Class significantly more numerous (ANOVA: $F = 12.59$, $p < 0.0001$) than the former two classes, which are represented by one species each (compared to five species representing *Lichinomycetes*). The most common species include a seemingly undescribed areolate-squamulose *Pterygiopsis* (*Forssellia*) species (in seven sites) and the crustose-areolate "*Pterygiopsis*" *neglecta* (in six sites).

A band of dried silt with little to no lichen growth is often observed between the Mesic Fluvial and Xeric Fluvial communities (**Fig. 6**). Specimens collected in both fluvial communities often have caked silt surrounding, or on, thalli.

Xeric Fluvial communities.—A total of 11 Xeric Fluvial species were recorded, of which 10 were analyzed for functional trait and higher taxonomic composition. Xeric Fluvial communities were 1.9 (± 1.0) crustose, 2.3 (± 1.4) squamulose and 0.2 (± 0.4) foliose species across sites, with crustose and squamulose species significantly more numerous than foliose species, of which only *Collema subflaccidum* was recorded (ANOVA: $F = 15.87$; $p < 0.0001$). Xeric Fluvial lichens were primarily sexual in reproductive mode with a mean of 3.8 (± 1.7) species versus a mean of 0.6 (± 0.8) species reproducing via vegetative diaspores, which is significantly fewer (Student's $t = 6.17$;

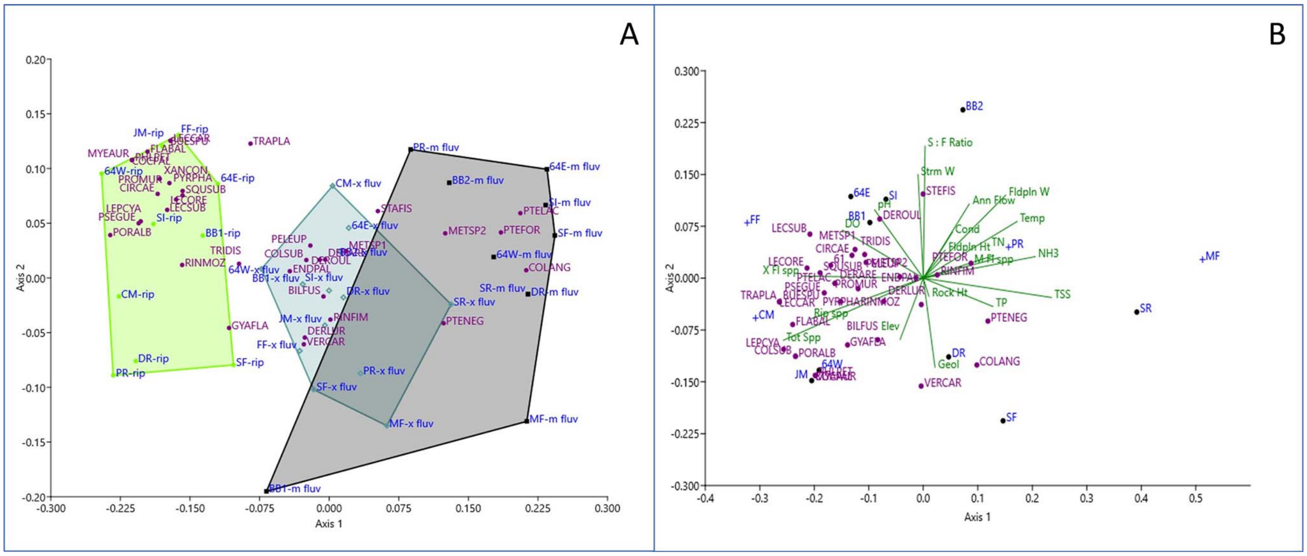

Figure 5. Non-metric multidimensional scaling (nMDS) plots based on Bray-Curtis similarities of Piedmont rocky river lichen species presence/absence data. **A.** Site data split by zone community. The sample labels correspond to sites and symbols refer to hydrological zones (● Mesic Fluvial in black, ◇ Xeric Fluvial in blue-gray, ■ Riparian in chartreuse); species are depicted as purple dots with six-letter species codes. Superimposed convex polygons show sites from different hydrological zones (black outline = Mesic Fluvial, blue-gray = Xeric Fluvial, chartreuse = Riparian). **B.** Site data not split, and including environmental parameters. See Supplementary Table S1 for site code definitions and Supplementary Table S2 for species code definitions.

$p < 0.0001$). Xeric Fluvial lichens were also predominantly chlorolichens (3.1 ± 1.7) versus (1.4 ± 0.0) cyanolichens, which were significantly fewer among sites (Student's $t = 3.19$; $p < 0.005$). Taxonomically, the Xeric Fluvial community was comprised of three Classes, *Eurotiomycetes* (1.5 ± 1.3 species), *Lecanoromycetes* (1.8 ± 0.7 species), and *Lichinomycetes* (1.2 ± 0.8 species), none of which tested different in number among sites (ANOVA: $F = 1.72$, ns). Most common species include *Dermatocarpon arenosaxi* (in 6 sites), *Peltula euploca* (in 10 sites) and *Rinodina fimbriata* (in 12 sites).

Riparian communities.—A total of 28 riparian species were recorded, of which 20 were analyzed for functional trait and higher taxonomic composition (**Supplementary Table S2**) Categories are expressed in mean (±SD) per type. Riparian communities were 3.9 (± 3.1) crustose, 0.5 (± 0.5) squamulose and 1.1 (± 1.7) foliose species across sites, with crustose species significantly more numerous than the other two growth forms (ANOVA: $F = 10.25$; $p < 0.005$). Riparian lichens were primarily sexual in reproductive mode, with a mean of 4.3 (± 3.3) species versus a mean of 1.2 (± 1.9) species reproducing via vegetative diaspores, which is significantly fewer (Student's $t = 2.90$; $p < 0.005$). Riparian lichens were also predominantly chlorolichens (5.7 ± 4.3) versus (0.5 ± 0.9) cyanolichens, which were significantly fewer among sites (Student's $t = 3.77$; $p < 0.005$). Taxonomically, the riparian community was comprised of two Classes, predominantly *Lecanoromycetes* (5.4 ± 4.8) versus a mean of 0.2 ± 0.4 *Lichinomycetes*, the latter represented by one species, *Pyrenopsis phaeococca*. Most common species (i.e., found in > 5 sites) include *Gyalolechia flavovirescens* (in 6 sites), *Rinodina moziana* (in 7 sites) and *Squamulea subsoluta* (in 9 sites).

Table 1. Functional trait composition of saxicolous lichen communities in Piedmont rocky river habitats in North Carolina.

		Growth Form			Reproductive Mode		Photobiont	
Zone	No. spp.	Crustose	Squamulose	Foliose	Sexual	Vegetative	Chlorophyte	Cyanobacteria
Riparian	20	70.0%	5.0%	25.0%	70.0%	30.0%	85.0%	15.0%
Xeric Fluvial	10	40.0%	50.0%	10.0%	80.0%	20.0%	70.0%	30.0%
Mesic Fluvial	7	85.7%	14.3%	0.0%	100%	0%	14.3%	85.7%

Table 2. Taxonomic class composition of saxicolous lichen communities in Piedmont rocky river habitats in North Carolina.

Zone	No. spp.	Dothideomycetes	Eurotiomycetes	Lecanoromycetes	Lichinomycetes
Riparian	20	0.0%	0.0%	95.0%	5.0%
Xeric Fluvial	10	0.0%	40.0%	40.0%	20.0%
Mesic Fluvial	7	14.3%	14.3%	0.0%	71.4%

Lichen zone inundation estimates. Field-measured heights of Mesic Fluvial zone marker taxa (e.g., *Pterygiopsis* sp.) ranged 10–55 cm among sites; those of Xeric Fluvial zone marker taxa (e.g., *Dermatocarpon luridum*) ranged 35–120 cm; and Riparian zone marker taxa (e.g., *Xanthoparmelia conspersa*) ranged from 65–120+ cm above the water line on the day of collection. These heights translated to inundation estimates of 3–10 months per year for Mesic Fluvial species, 0.5–4 months per year for Xeric Fluvial species, and < 1–3 months for Riparian lichens (**Table 3**). Zone marker heights and their relative inundation estimates varied considerably among sites, resulting in the above overlapping of ranges.

Relationships with environmental variables. The species nMDS analysis resulted in an ordination with two dimensions with a stress value of 0.209 and a Shepard plot with R^2 values of 0.732 (Axis 1) and 0.0152 (Axis 2). The ordination yielded a plot with most species occupying the left half of the multivariate space along with Axis 1 with most Riparian species the furthest left, while the Mesic Fluvial taxa *Collemopsidium angermannicum, Pterygiopsis* (*Forssellia*) sp. and *"Pterygiopsis" neglecta* lay the rightmost of the space; species and environmental variables were broadly distributed along Axis 2 (**Fig. 5B**). Most water quality variables also were along the right half of the space.

No apparent relationships (i.e., $r > |0.52|$) were found between any of the lichen species richness variables (total or any of the three zone communities) and any of the following environmental parameters: elevation, floodplain height, geology, substrate rock type, water temperature, dissolved oxygen, pH, or ammonia among sites. The strongest relationships were found between Mesic Fluvial species number and the following parameters in descending order: mean stream flow, width, floodplain width, water conductivity, total nitrogen, and phosphorous, all of which were positive correlations with significant linear regressions (**Table 4**).

DISCUSSION

Three zoned lichen communities were discerned among 13 Piedmont rocky river sites and found to differ in species richness, species composition, functional traits, higher taxonomy, and estimated amounts of inundation from fluctuating watercourse levels. Stratified lichen communities are often characteristic of rocky habitats exposed to periodic inundation and spray adjacent to or within waterbodies (Krzewicka et al. 2017; Thüs et al. 2014; Thüs & Schultz 2009). A review of the literature found descriptions of stream and river rock lichen communities primarily from Europe (Coste 2009; Khodosovtsev & Kuzemko 2023; Krzewicka et al. 2017; Orange 2017; Thüs et al. 2014; Thüs & Schultz 2009) with few studies from North America (Rosentreter 1984; Timoney & Marsh 2004). The zones described from those studies generally follow a similar pattern to those discerned in this study: 1) a lower band occurring near the low water level that is frequently inundated and comprised of semi-aquatic species, 2) a middle band subjected to less frequent inundation and splash, comprised of semi-aquatic species that are less tolerant of prolonged inundation, and 3) an upper band of inundation-sensitive species.

Differences in site lichen biotas were not discerned between the two river basins, which suggests that most lichens are not restricted to one particular river basin or stream. A comparison of two mountain streams in the Polish Carpathians likewise did not discern a difference in lichen communities (Krzewicka et al. 2017). One species that was found in the Cape Fear River basin but not the Neuse River basin is the locally endemic *Dermiscellum oulocheilum*, which appears to be restricted to the Haw River. Historic specimens from the Catawba River are known from both North and South Carolina (Lendemer 2003), but there are no modern collections from that river (CLH 2024). Further exploration of the Catawba River, and similar rocky river habitats in other river basins in North

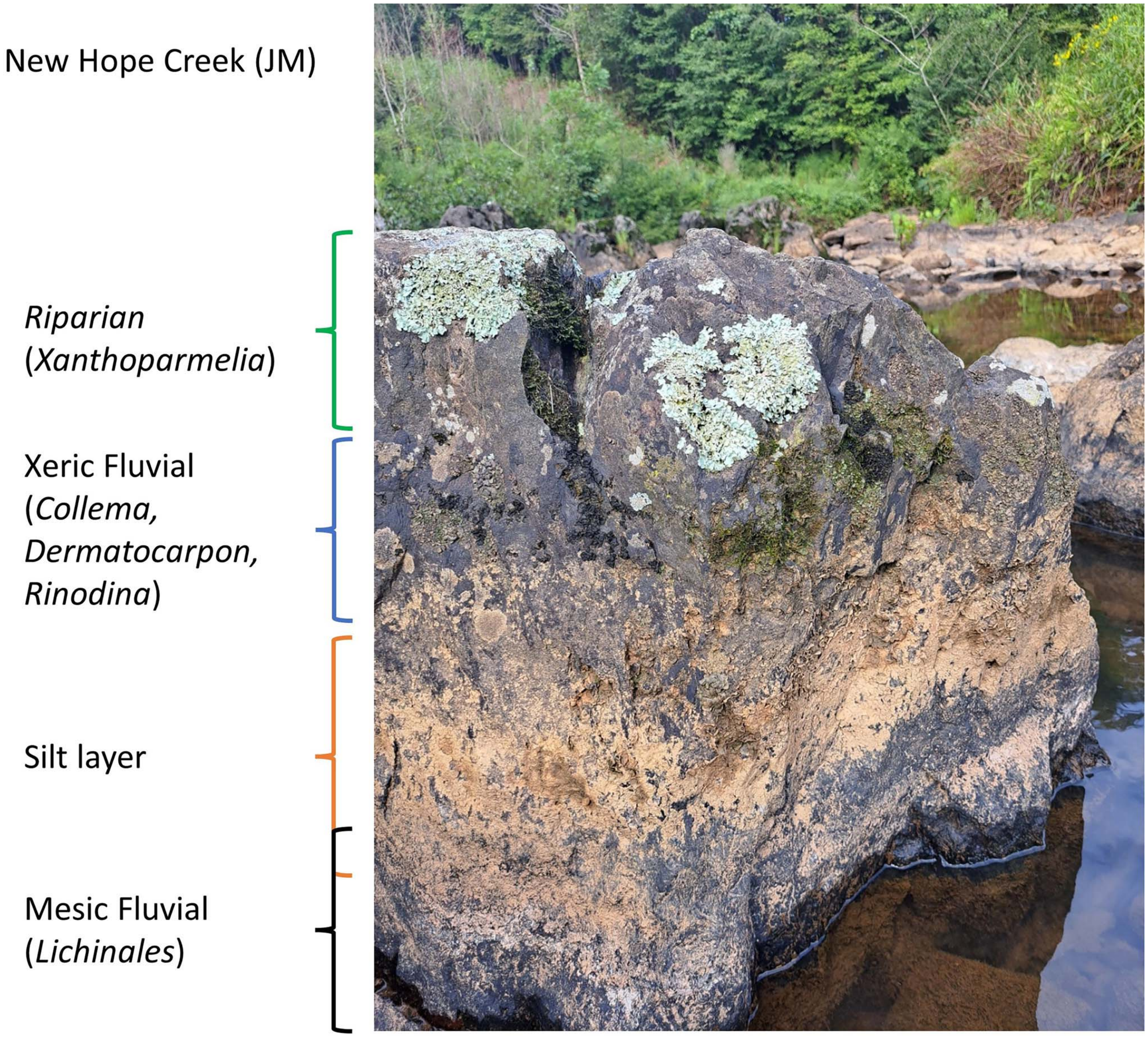

Figure 6. An exposed stream rock showing four lateral zones. New Hope Creek at Johnston Mill Nature Preserve (Site JM), August 2023.

Carolina and adjacent states, may reveal additional populations of *D. oulocheilum*.

The inundation durations of the three zones, i.e., 3–10 months per year for Mesic Fluvial species, 0.5–4 months per year for Xeric Fluvial species, and < 1–3 months for Riparian, roughly approximate those described in streams throughout France that are based on multiple years of observations (i.e., > 10 months for hyper-hydrophilic, 3–10 months for meso-hydrophilic, and < 3 months for sub-hydrophilic zones; Coste et al. 2023), providing a promising method of estimating relative lichen zone inundations using nearby publicly available stream gage data. Field measurements of lichen zone marker taxa in this study (**Table 3**) also approximate those of similar zones in France (hyper-hydrophilic at ±10 cm above/below low water level; meso-hydrophilic at 10–100 cm above low water level; and sub-hydrophilic at 100–200 cm above low water level; Coste et al. 2023). Not all sites were completely measured for their lichen heights, and visits were not made at consistently low stream flows due to the exploratory, preliminary nature of the present study. Recommendations to provide more consistent measurements for more precise and accurate inundation estimates in future surveys are offered at the end of this paper.

Table 3. Inundation estimates of measured lichen marker heights in selected sites in Piedmont rocky river habitats in North Carolina for WY2023.

Lichen Marker(s)	Zone	Field Height, cm (ft.)	Frequency Submerged, days (mo.)
Haw River at Shallow Ford (SF)			
Gyalolechia flavovirescens, Rinodina moziana	Rip.	80–95 (2.6–3.1)	20–28 (0.7–0.9)
Dermatocarpon luridum	X. Fluv.	35–60 (1.1–2.0)	42–82 (1.4–2.7)
Collempsidium angermannicum	M. Fluv.	10–20 (0.3–0.7)	137–201 (4.6–6.7)
Haw River above Swepsonville River Park (SR)			
Rinodina fimbriata	X. Fluv.	45–50 (1.5–1.6)	85–92 (2.8–3.1)
Pterygiopsis (Forssellia) sp., "*Pt.*" *neglecta*	M. Fluv.	20–35 (0.7–1.1)	116–182 (3.9–6.1)
Haw River at Saxapahaw Island (SI)			
Xanthoparmelia conspersa	Rip.	100 (3.3)	63 (2.1)
D. luridum	X. Fluv.	55–90 (1.8–3.0)	73–110 (2.4–3.7)
Pt. (Forssellia) sp.	M. Fluv.	10 (0.3)	305 (10.2)
Haw River near Bynum Bridge (BB1, BB2)			
Lecanora oreinoides, X. conspersa	Rip.	75 (2.5)	19 (0.6)
Peltula euploca	X. Fluv.	35–70 (1.1–2.3)	24–61 (0.8–2.0)
Pt. (Forssellia) sp.	M. Fluv.	20 (0.6)	98 (3.3)
Haw River below Hwy 64 (64E, 64W)			
L. oreinoides, X. conspersa	Rip.	≥120 (3.9)	≤12 (0.4)
Peltula euploca	X. Fluv.	55–120 (1.8–3.9)	12–81 (0.4–2.7)
C. angermannicum, Metamelanea, Pterygiopsis	M. Fluv.	10–40 (0.3–1.3)	141–265 (4.7–8.8)
New Hope Creek at Johnston Mill Preserve (JM)			
L. oreinoides, X. conspersa	Rip.	≥75 (2.5)	–
D. luridum, C. subflaccidum	X. Fluv.	20–75 (0.6–2.5)	–
R. fimbriata	X. Fluv.	20 (0.6)	–
Deep River at Deep River State Trail (DR)			
R. moziana	Rip.	65–95 (2.1–3.1)	10–26 (0.3–0.9)
R. fimbriata	X. Fluv.	40–60 (1.3–2.0)	35–70 (1.2–2.3)
Pt. (Forssellia) sp., "*Pt.*" *neglecta*	M. Fluv.	15–35 (0.5–1.1)	86–195 (2.9–6.5)
Eno River at Fews Ford (FF)			
X. conspersa	Rip.	65 (2.1)	≤1 (0.03)
P. euploca	X. Fluv.	50 (1.6)	3 (0.1)
Eno River at Cole Mill canoe launch (CM)			
G. flavovirescens, R. moziana	Rip.	≥75 (2.5)	≤1 (0.03)
Collema subflaccidum	X. Fluv.	45 (1.5)	4 (0.13)
Neuse River at Milburnie Falls (MF)			
R. fimbriata	X. Fluv.	100 (3.3)	43 (1.4)
"*Pt.*" *neglecta*	M. Fluv.	55 (1.8)	114 (3.8)
Neuse River above Poole Road (PR)			
D. luridum, P. euploca, R. fimbriata	X. Fluv.	60–135 (2.0–4.4)	22–79 (0.7–2.6)
Pt. (Forssellia) sp.	M. Fluv.	40 (1.3)	126 (4.2)

Here we present the following descriptions of the three saxicolous lichen communities of rocky river habitats based on results of the present study; a visual depiction of the lichen zones interspersed by a broad silt zone is in **Fig. 6**.

Mesic Fluvial community (Fig. 7). Mesic Fluvial communities here described are species poor yet almost exclusively crustose, sexual cyanolichens in the Class *Lichinomycetes* with one representative of *Dothideomycetes* (*Collemopsidium angermannicum*) and *Eurotiomycetes* (*Staurothele fissa*) each [see Perlmutter & LaGreca (2024) for brief descriptions and images]. Several of these species are as yet undetermined including one such species, "*Pterygiopsis*" sp. that is sufficiently distinct to be identifiable in the field by its black orbicular rosettes (M. Schultz, pers. comm.). Other lichens for which species-level determinations have been made,

Table 4. Lichen site parameter correlation (r) and linear regression (F) statistics with associated environmental parameters. $^*p < 0.05$; $^{**}p < 0.01$.

	Mesic Fluvial		Xeric Fluvial		Riparian	
Parameter comparison	***r***	***F***	***r***	***F***	***r***	***F***
Lichen spp. v Elevation	–0.13	1.18 ns	–0.05	0.03 ns	0.012	0.15 ns
Lichen spp. v Mean flow	0.77	15.66**	–0.10	0.10 ns	–0.32	0.13 ns
Lichen spp. v Stream width	0.64	7.67*	–0.08	0.07 ns	–0.01	0.001 ns
Lichen spp. v Floodplain width	0.74	13.36**	–0.42	2.36 ns	–0.48	3.27 ns
Lichen spp. v Floodplain height	–0.17	0.35 ns	–0.22	0.53 ns	–0.19	0.41 ns
Lichen spp. v Temperature	0.33	1.23 ns	–0.10	0.11 ns	–0.24	0.62 ns
Lichen spp. v pH	0.51	3.60 ns	–0.001	0.00002 ns	0.38	1.72 ns
Lichen spp. v Conductivity	0.67	8.05*	–0.23	0.57 ns	0.08	0.06 ns
Lichen spp. v Dissolved Oxygen	0.25	0.68 ns	0.07	0.05 ns	0.44	2.34 ns
Lichen spp. v Turbidity	0.29	0.90 ns	–0.35	1.42 ns	–0.43	2.34 ns
Lichen spp. v Total Nitrogen	0.70	8.73*	–0.34	1.17 ns	–0.11	0.11 ns
Lichen spp. v Phosphorus	0.66	7.12*	–0.16	0.23 ns	–0.17	0.28 ns

such as *Pterygiopsis lacustris* and *"Pterygiopsis" neglecta*, have been reported from similar low-lying, frequently submerged habitats in Europe (Gilbert & Giavarini 1997; Jørgensen 2007; Orange 2017); however, the taxonomy of the latter species remains unresolved (M. Schultz, pers. comm.). As noted above, *Lichinomycetes* includes *Lichinales*, one of the two orders that contributes most freshwater lichen species (Thüs et al. 2014). Cyanolichens are often dominant in humid to aquatic habitats because cyanobacteria require wetting to be physiologically active (Gauslaa et al. 2012).

Growth form and reproductive traits of Mesic Fluvial lichens appear to be adapted to a semi-aquatic to aquatic lifestyle. Coste et al. (2023) noted that species with crustose habit and perithecial or sunken apothecial ascomata are adaptations to the sheer stresses of running water among hydrophilic lichens in French streams. The smooth crustose habit and the often sunken, punctiform ascomata of *Pterygiopsis* spp., or the perithecial ascomata of *Collemopsidium* and *Staurothele*, observed in this study appear to agree observations of Coste et al.

Figure 7. Mesic Fluvial lichen community, dominated by Lichinales species, including *Metamelanea* spp., *Pterygiopsis lacustris*, *"Pterygiopsis" neglecta* and other incompletely determined species, some undescribed. Pale brown layer is silt deposited during moderate to high water events. Haw River below US Highway 64 (Site 64W), August 2023.

Xeric Fluvial community (Fig. 8). Xeric Fluvial communities described here are moderate in species richness and proportion of cyanolichens and are distinct in having the highest proportion of squamulose species and of *Eurotiomycetes* taxa among the three communities. Lichens in these fluvial communities are considered amphibious. Characteristic species include the well-known, amphibious *Dermatocarpon luridum* and the darker *D. arenosaxi*, both of which often form extensive mats extending from 30–70 cm above the low water level as observed in sites 64E, BB1 and SI. In the upper range of this community, which abuts the riparian trimline, is found the locally endemic *Dermiscellum oulocheilum*. This species is often associated with the similarly sized, peltate-squamulose cyanolichen *Peltula euploca*, which, like *D. luridum*, has a cosmopolitan distribution and is found in a range of shade to sunny exposures (Amtoft et al. 2008; Clewell et al. 2009; Shivarov et al. 2018). We sometimes found *P. euploca* forming extensive mats on vertical rock faces, extending to the riparian trimline as observed at sites 64W and PR. On lower rocks, the light brown, crustose thallus

Figure 8. Xeric Fluvial lichen communities. **A.** *Collema subflaccidum, Dermatocarpon luridum* and *Rinodina fimbriata* in New Hope Creek at Johnston Mill Nature Preserve (Site JM), August 2023. **B.** *Dermiscellum oulocheilum, Peltula euploca* and *Rinodina* spp. in Haw River at Saxapahaw Island (Site SI), November 2022.

of *Rinodina fimbriata* forms extensive patches on horizontal surfaces, with its lower edge forming the fluvial trimline below which the darker species of *Lichinomycetes* occur. Also, on low shaded rocks and shaded rocky banks, is the distinctive, sorediate, green crust *Bilimibia fuscoviridis*, recently reported from eastern North America along waterways (Curtis et al. 2023), often with the dark olive pyrenolichen *Pseudosagedia guentheri*, which is also reported along waterways in Europe and North America (Coste et al. 2023, Gilbert & Giavarini 1997, Krzewicka et al. 2017, Perlmutter 2022). Analogous communities described in Europe are labeled the "splash zone" (e.g., Krzewicka et al. 2017).

Taxonomically, the Xeric Fluvial community includes all three Classes in similar proportions, including taxa in the Orders *Lichinales* (*Metamelanea, Peltula*) and *Verrucariales* (*Dermatocarpon, Endocarpon*)—the two orders of lichenized fungi known to comprise the most freshwater taxa (Thüs et al. 2014).

Riparian community (Fig. 9). Riparian lichen communities described here are the richest in species number and include species that are primarily crustose in growth form; contain chlorophyte photobionts; reproduce sexually; and belong in the Class *Lecanoromycetes*. Compared to the other zone communities, the riparian also has the highest proportion of foliose species, as well as the highest proportion species reproducing primarily by vegetative diaspores. Taxonomically, the riparian community is also the most diverse, with species in 19 genera representing 14 families of the analyzed set. Several of these species are also found in terrestrial habitats both forested (Perlmutter 2008; Perlmutter & Lendemer 2008) and open (LaGreca et al. 2018; Perlmutter 2022) within the study region. More specifically, riparian lichens on exposed riverscour rocks (e.g., *Squamulea subsoluta, Xanthoparmelia conspersa*) are also found on granitic flatrocks and other exposed or semi-exposed rocks (LaGreca et al. 2018; Perlmutter

Figure 9. Riparian lichen community, represented by *Protoparmeliopsis muralis* (P), *Rinodina moziana* (R), *Squamulea subsoluta* (S) and *Xanthoparmelia conspersa* (X), in Haw River below US Highway 64 (Site 64W), May 2023.

2013). Further, *X. conspersa* has been reported as a riparian species above the trimline from European studies of fluvial lichen communities (Gilbert & Giavarini 1997; Hawksworth 2000; James et al. 1977; Orange 2017). Riparian communities along shaded riverbanks include species (e.g., *Leptogium cyanescens, Porpidia albocaerulescens, Pseudosagedia cestrensis*) that are also found in the adjacent terrestrial forests and can thus be considered facultative riparian species. This finding is in agreement with other described riparian or terrestrial zones among streamside or riverine communities (Krzewicka et al. 2017; Thüs et al. 2014; Thüs & Schultz 2009).

Riparian communities are subjected to infrequent inundation of short periods during flood events, as shown by the spikes of high-water levels in the example hydrograph (**Fig. 3**). High river levels above the trimline are also evidenced by the observation of logs and other woody debris sitting atop of rocks over 1 m above the low water level at multiple sites during field visits. This community is persistent likely due to the relative short duration of immersion periods, with the maximum duration being five days. Most terrestrial lichens are sensitive to inundation as the thallus will disintegrate when submerged for extended periods of time (Clewell et al. 2009; Thüs et al. 2014). In addition, their photobionts are sensitive to supersaturation, resulting in negative net photosynthesis rates when soaked (Kappen 1973; Nascimbene 2013; Stanton et al. 2023). It should be noted that riparian communities were not observed in three sites (SR, CM and MF), likely due to river rocks not extending above the riparian trimline. Higher trimlines have been reported to be indicative of greater water level fluctuations (Timoney & Marsh 2004).

Overall patterns of zoned communities. A pattern is apparent across lichen communities of varying rock heights above water, with the lowermost comprising species that are specialized for inundation tolerance with the following functional traits: a crustose habit that is often gelatinous when wet; small ascomata with sunken, often punctiform disks; a cyanobacterial photobiont that requires wetting to be physiologically active; and belonging to Classes *Dothideomycetes* and *Lichinomycetes*, the latter of which includes the families *Lichinaceae* and *Peltulaceae*, species of which are characterized by the above traits. Moving up, species number increases with an increasing diversity of functional trait components (i.e. more growth form diversity, reproductive strategies, and photobiont types), as well as an increase in higher taxonomic diversity and a decrease in species specialized for frequent inundation. The Xeric Fluvial community is a transitional community intermediate between the inundation-adapted Mesic Fluvial and the inundation-sensitive Riparian community, yet it is distinct in bearing the highest proportion of squamulose species as well as those in Class *Eurotiomycetes*. The Riparian zone communities on channel rocks sometimes share taxa with upland exposed rocky habitats, whereas those along shaded stream and riverbanks sometimes share taxa with the surrounding forest.

Relationships with environmental variables. Our study found that the lowest fluvial zone community—that experiencing the highest amount of inundation—is affected by stream size and water quality, with greater species richness occurring in larger streams carrying greater concentrations of dissolved solids and nutrients (nitrogen and phosphorus) at higher specific conductance. Since lichens receive their nutrients directly from the environmental medium passively, it appears that the semi-aquatic species found here are benefiting from waters bearing nutrients including nitrogen and phosphorus. Nearly all lichens of the Mesic Fluvial zone community harbor cyanobacteria as their photobiont, and these bionts have been shown to require wetting to be physiologically active (Rikkinen 2002 and sources therein). Experimental treatments of nitrogen and phosphorus on terrestrial cyanolichens have shown increase in growth (Johansson et al., 2011; McCune & Caldwell 2009). Freshwater cyanobacteria are well-known to be responsive to excess nitrogen and phosphorus in aquatic environments, causing harmful algal blooms (Heisler et al. 2008). These patterns appear to be corroborated by the similar positive relationship of Mesic Fluvial lichen species number with specific conductance. Specific conductance, which is a measure of electrical conductivity of water at 25°C, is an approximate measure of total dissolved ions from inorganic dissolved solids and is affected by geology as well as discharges from anthropogenic sources—including wastewater containing nutrients like chloride, phosphate and nitrate (EPA 2012). The site with the highest nutrient concentrations and conductivity is SR, which is situated in the Haw River below the discharges of two major municipal wastewater treatment plants.

Conclusions and Recommendations

The present study was of a preliminary and exploratory nature, yielding results herein reported (i.e., zoned lichen community descriptions with characteristic species and inundation estimates) that can be useful for potential structured stream lichen surveys in North Carolina and beyond. For future surveys, we recommend using either a plot or transect method, and measuring the distance of a given lichen observation from the shore as well as its height above the water line. For greater consistency with low water levels, visits should be made in summer months with at least 3–5 days after the most recent rain event to approximate summer base flow levels. Amount of shading or exposure should also be measured at a given observation via % canopy cover. If a plot method is used, we recommend estimating or measuring percent lichen cover, including total and/or of a given species, to provide a measure of abundance, which the present study lacked. Also, orientation of the rockface (i.e., horizontal to vertical and compass direction) should be noted, as this could affect abundance and composition of lichen species. Maximum rock heights should be measured, as low rocks subject to total inundation were found to lack riparian communities. Sites should be chosen relatively close to an active USGS gage station (i.e., within 5 km) and share similar geomorphology (i.e., stream width, floodplain width and annual mean flow) for accurate inundation estimates for lichen species and communities; ideally, sites should be co-located with an active gage station. Similarly, lichen sites should be chosen close to an ambient monitoring station for representative water quality data. With such a structured study plan developed and implemented, a greater understanding of fluvial lichen ecology can be found and better comparisons can be made across stream locations within and among river basins, as well as larger regions such as across ecoregions (e.g., coastal plain vs. piedmont vs. mountain in North Carolina) and even across continents. Further, such a survey can also supplement measures of stream ecological health classically assessed via benthic invertebrate and fish community samplings (e.g., Tomkiewicz & Dunson 1977).

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Supplementary documents online:

Supplementary Table S1. River lichen site and nearby stream gage metadata, ordered from upstream to downstream.

Supplementary Table S2. Saxicolous lichen taxa of surveyed Piedmont rocky river sites in central North Carolina, U.S.A. with functional traits, taxonomic and hydrological zone affiliations.

Supplementary Table S3. Water Quality parameter mean (range) summary statistics from ambient monitoring stations nearest to lichen sites.