Appendix A

Crosswalk of MassDEP vs SNEP major macroinvertebrate habitat types

Table A1. Crosswalk of MassDEP and SNEP major macroinvertebrate habitat types

MassDEP 2013-2019	SNEP
leaf pack	wood jabs*
snags	wood jabs
other (coarse substrates)	hard bottom jabs
other (non-riffle kick)	hard bottom jabs
other (runs with cobble)	hard bottom jabs
other, bottom kicks	hard bottom jabs
other, coarse substrates	hard bottom jabs
other, cobble kicks	hard bottom jabs
other, run	hard bottom jabs
riffle cobbles	hard bottom jabs
riffles	hard bottom jabs
runs	hard bottom jabs
root mats and submerged macrophytes	undercut banks/overhanging vegetation
overhanging vegetation/stream bank	undercut banks/overhanging vegetation
stream banks	undercut banks/overhanging vegetation
submerged macrophytes	submerged vegetation
submerged macrophytes and root mats	submerged vegetation

*leaf packs are typically associated with snags/wood

Appendix B

Taxa tolerance analyses

B1 Background

Taxon tolerance analyses allow for visualization of the shape of the taxon-stressor relationship across a continuous numerical scale, and can be used to identify optima (the point at which the taxon has the highest probability of occurrence) as well as tolerance limits (the range of conditions in which the taxon can persist) (Yuan 2006). To help inform macroinvertebrate tolerance value assignments related to sensitivity to stressors in low gradient streams in Massachusetts (MA) and Rhode Island (RI), we ran taxa tolerance analyses on four variables that capture anthropogenic disturbance: the Indices of Watershed and Catchment Integrity (IWI & ICI, respectively) (Thornbrugh et al. 2018, Johnson et al. 2019), percent urban and percent agricultural land use. We also ran analyses to better understand the relationship between taxon occurrence and drainage area, flowline slope, elevation and modeled summer stream temperature. The tolerance analyses were run on a regional dataset that included low gradient data from Massachusetts (MA), Rhode Island (RI), Connecticut (CT), Vermont (VT), and New York (NY). The regional scale allowed for a larger sample size than just the MA/RI dataset alone, which improved the robustness of the analyses and allowed tolerance assignments to be generated for more taxa. Biologists from MassDEP reviewed results from the analyses and assigned taxa to three tolerance categories: intolerant, intermediate, and highly tolerant. In this document, we describe the dataset, methods and results and conclude with recommendations on potential future analyses that could further improve our understanding of taxon-stressor relationships in low gradient streams.

B2 Data compilation

B2.1 Macroinvertebrates

The regional dataset was comprised of macroinvertebrate samples from low gradient, freshwater, wadeable, perennial streams in MA, RI, CT, VT and NY that were collected with each state's low gradient collection method (Table B1). Data from 541 sites that spanned nine Level 3 ecoregions were included in the analysis (Table B2, Figure B1).

Table B1. Summary of the regional macroinvertebrate collection methods being used in MA, RI, CT, VT and NY.

Method	Habitat	Effort	Gear	Reach length	Index period	Target # organisms	Taxonomic resolution
MassDEP RBP multihabitat	Snags and root wads, leaf packs, aquatic macrophytes, undercut banks and overhanging vegetation, hard bottom (riffle/cobble/boulder)	Any combination of 10 kicks, sweeps, and/or jabs, which are then combined into a single composite sample. Sampling is proportional to the relative makeup of the reach by the major habitat types	Kick-net with 500-µm mesh, 46-cm wide opening. Brushes are used on woody debris	100-m	July 1 – September 30	300	Lowest practical level
Southern New England Program (SNEP) multihabitat (used in RI and at some MA sites)	Submerged wood (including leaf packs wedged in the wood), submerged vegetation, undercut banks/overhanging vegetation, hard bottom/rocky substrates	Composite of 10 jabs, sweeps, or kicks; each jab/sweep/kick lasted for a minimum of 30 seconds and a maximum of 45 seconds. The goal is to dislodge and capture as many organisms as possible in that area. The habitats will be sampled in rough proportion to their occurrence within the reach*	Kick-net with 500-µm mesh and ~28-cm wide opening; brushes are *not* used on woody debris	100-m	July 1 – September 30	300	Lowest practical level
CT DEEP Standard Semi-Quantitative Low Gradient	Multiple habitat approach that focuses primarily on the most productive habitats (vegetation, woody debris, undercut banks/roots) but also includes, at minimized effort, the less productive fine sediment habitat (sand/silt)	20 jabs/sweeps (1 meter in length, followed by 2-3 sweeps through the suspended material. fixed number of two jabs/sweeps from fine sediments; the other eighteen are based on the percentage of most productive habitats present in sampling reach	Long handled, 500-micron mesh, D-frame net	100 meters		200	Lowest practical level

Table B1. continued...

Method	Habitat	Effort	Gear	Reach length	Index period	Target # organisms	Taxonomic resolution
NYSDEC Low gradient	Four habitats: bank, center channel substrate, woody debris/snags and macrophyte bed	Composite of two jab samples for each of the four habitats (8 samples in total). Consistent effort at each habitat for ~30 seconds (total of ~4 minutes for all samples and habitats). Alternating jabbing and sweeping is performed to catch dislodged macroinvertebrates.	Rectangular kick net (23 cm × 46 cm) with 800–900-µm mesh.	20 times wetted width at sample site	June-September	200	Lowest practical level
VT DEC Low gradient (Sweep Bottom Kick Net Sampling)	Debris dams, vegetation, or root wads. Used in wadeable low gradient streams with substrates dominated by silt or sand and velocities, where velocity is less than 0.2 fps and the depth is less than 1 meter	Four-point composite sample. A jab is performed by jabbing the net into debris dams, vegetation, or root wads, pulling back rapidly to dislodge animals, then sweeping forward again into the same area to scoop up dislodged animals. This jabbing and sweeping motion should be repeated several times at the same point and considered one of four jabs. All four jabs (from different points in reach) are then combined into a single composite sample	Mesh size 500 microns, 18" wide x 9" high		September–mid-October	300	Lowest practical (species whenever possible)

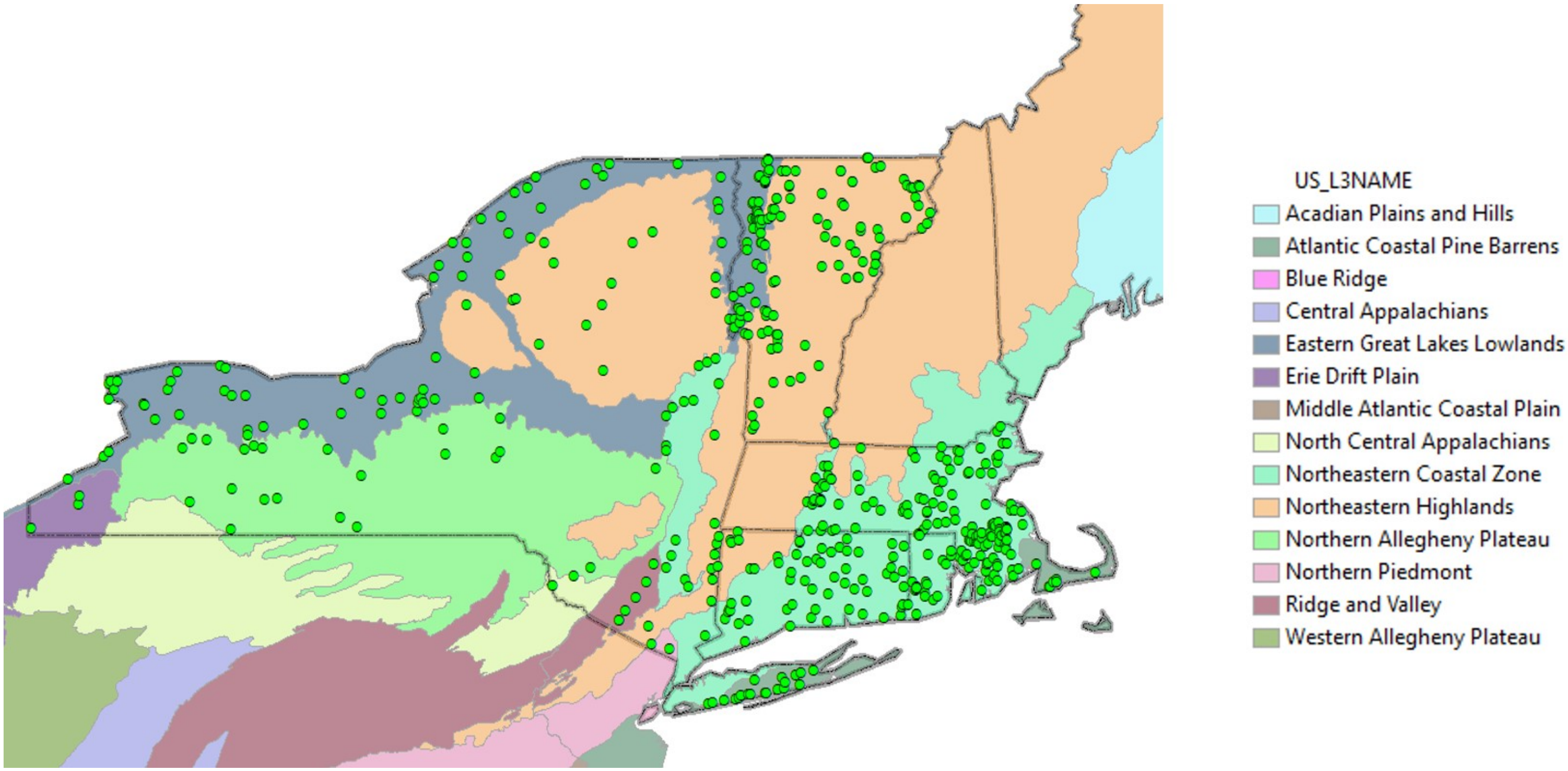


Figure B1. Locations of low gradient sites that were used in the regional tolerance analysis, against a Level 3 ecoregion backdrop.

Table B2. Number of sites in each Level 3 ecoregion.

US_L3NAME	CT	MA	NY	RI	VT	Total
Atlantic Coastal Pine Barrens		7	17			***24***
Eastern Great Lakes Lowlands			66		53	***119***
Erie Drift Plain			3			***3***
North Central Appalachians			2			***2***
Northeastern Coastal Zone	57	152	15	23	1	***248***
Northeastern Highlands	5	2	24		84	***115***
Northern Allegheny Plateau			22			***22***
Northern Piedmont			2			***2***
Ridge and Valley			6			***6***
Total	***62***	***161***	***157***	***23***	***138***	***541***

B2.2 Disturbance variables

We performed the tolerance analysis on four anthropogenic disturbance variables: ICI, IWI, percent urban and percent agricultural land use (Table B3). The data came from the USEPA Stream-Catchment (StreamCat) dataset[1] (Hill et al. 2016), which is associated with the National Hydrography Dataset (NHD) Plus Version 2 (NHDPlusV2) geospatial layer (McKay et al. 2012) via the unique identifiers for the stream segments (COMID) and local catchments (FEATUREID). First we used Geographic Information System software (ArcGIS 10.7.1) to spatially join the biological sampling sites with the NHDPlusV2 dataset. Then we joined the sites with StreamCat data via the NHDPlusV2 identifiers. This was done in a MS Access relational database.

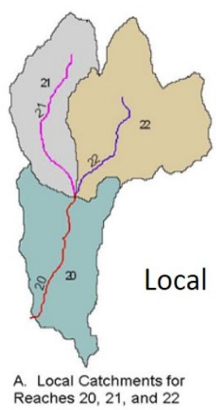
We did several cursory quality control (QC) checks to evaluate whether the biological sampling sites were associated with the correct NHDPlusV2 flowlines. If NHDPlusV2 stream segments had waterbody names (referred to as 'GNIS_Names'), we checked those against the waterbody names of the sites and flagged mismatches for further evaluation. If exact drainage areas were available for the sites, we calculated differences between those and the estimated drainage areas from the StreamCat dataset[2] and flagged sites where differences seemed excessively large (based on our best professional judgment). Next we visually checked the flagged sites to try and determine whether they were associated with the incorrect flowline. One of the most common errors occurred when sites were located on small tributaries that were not captured in the 1:100K NHDPlusV2 dataset and the nearest flowline was a large mainstem. In the end, we excluded 46 sites from the analysis because they were clearly associated with the incorrect flowline.

StreamCat data are available at two spatial scales: local catchment (Cat) (which is defined as the landscape area draining to a single stream segment, excluding upstream contributions) and total watershed (Ws) (which includes the local catchment plus the accumulated area of all upstream catchments) (Figure B2). Three of the disturbance variables (ICI, percent urban and percent agricultural)

[1] https://www.epa.gov/national-aquatic-resource-surveys/streamcat-dataset-0
[2] StreamCat data are not based on exact watershed delineations except in instances where the site happens to be located at the downstream end of the NHDPlusV2 local catchment; instead, a site is characterized based on the attributes that are associated with the catchment in which the site is located.

were at the local catchment scale, while the IWI was at the watershed scale. Because the StreamCat data are not based on exact watershed delineations (except in instances where the site happens to be located at the downstream end of the NHDPlusV2 local catchment), there may be occasional inaccuracies in the attribute data. For example, if a site is located upstream of urban land cover, but the urban land cover is located within the local catchment, the urban land cover data will be (wrongly) associated with the site.

The dataset captured a wide range of disturbance. IWI and ICI scores, which are scaled from 0 (worst) to 1 (best), ranged from 0.16 to 0.92, with most sites falling in the middle of that range (0.4 to 0.7) (Table B4, Figure B3). Urban and agricultural land cover at most sites was < 10% (Figure B3), with median values of 4 and 6%, respectively (Table B4, Figure B3). Figure B4 shows the sites overlaid on the NLCD 2016 land cover geospatial layer.



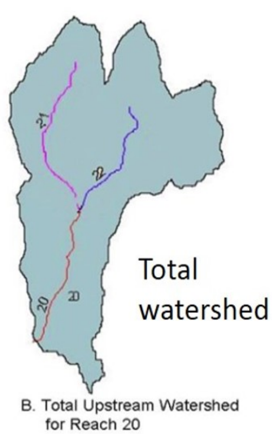
Local catchment

Definition: the landscape area draining to a single stream segment, excluding upstream contributions.

In this example, there are three local catchments (associated with unique flowline segments) –

- # 20 (green)
- # 21 (gray)
- # 22 (brown)

Each local catchment has a unique identifier (COMID or FEATUREID).



Watershed-level

Definition: the local catchment plus the accumulated area of all upstream catchments

In this example there is one total watershed, comprised of the three local catchments (#20 + #21 + #22).

Figure B2. USEPA’s StreamCat metrics (Hill et al. 2016) cover two spatial scales: local catchment and total watershed.

Table B3. Disturbance variables that were included in the taxa tolerance analyses.

Metric (Abbrev)	Scoring scale	Description	Source
Index of Watershed Integrity version 2.1 (IWI_21)	0 (worst) to 1 (best)	Overall watershed condition at the total watershed scale. Scored based on six components: hydrologic regulation, regulation of water chemistry, sediment regulation, hydrologic connectivity, temperature regulation, and habitat provision	EPA StreamCat (Thornbrugh et al. 2018, Johnson et al. 2019)
Index of Catchment Integrity version 2.1 (ICI_21)	0 (worst) to 1 (best)	Overall watershed condition at the local catchment scale. Scored based on the six components listed above	EPA StreamCat (Thornbrugh et al. 2018, Johnson et al. 2019)
% Urban land use - local catchment scale, based on NLCD 2016 (pcUrb_local)	0 to 100%	% of catchment area classified as developed, low-intensity land use (NLCD 2011 class 22) + medium-intensity land use (NLCD 2011 class 23) + high-intensity land use (NLCD 2011 class 24)	EPA StreamCat (NLCD 2016 - Dewitz 2019)
% Agricultural land use - local catchment scale, based on NLCD 2016 (pcAg_local)	0 to 100%	% of catchment area classified as hay land use (NLCD 2011 class 81) + crop land use (NLCD 2011 class 82)	EPA StreamCat (NLCD 2016 - NLCD 2016 - Dewitz 2019)

Table B4. Summary statistics for the anthropogenic disturbance variables.

Variable	Valid N	Minimum	10th percentile	25th percentile	50th percentile	Mean	75th percentile	90th percentile	Maximum	Std.Dev.
Index of Catchment Integrity version 2.1 (ICI_21)	541	0.16	0.35	0.45	0.56	0.57	0.69	0.79	0.92	0.16
Index of Watershed Integrity version 2.1 (IWI_21)	541	0.16	0.36	0.46	0.57	0.57	0.69	0.79	0.92	0.16
% Urban land use - local catchment scale, based on NLCD 2016 (pcUrb_local)	541	0.00	0.35	1.25	4.44	15.69	19.65	51.35	98.91	22.71
% Agricultural land use - local catchment scale, based on NLCD 2016 (pcAg_local)	541	0.00	0.00	0.61	6.12	14.62	21.54	48.17	84.18	18.84

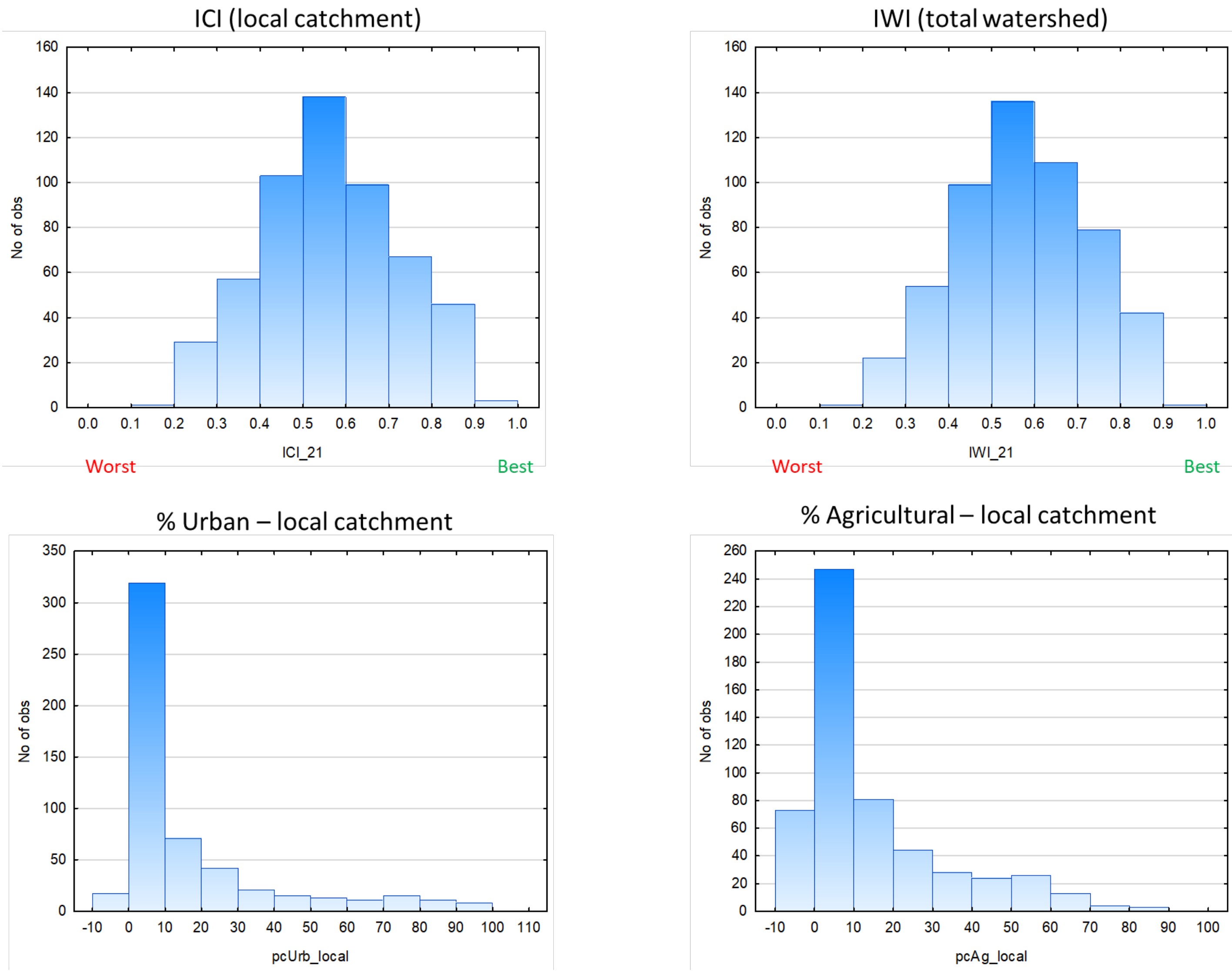


Figure B3. Histograms showing the distribution of sites across the disturbance gradient for each variable (broken into incremental ‘bins’).

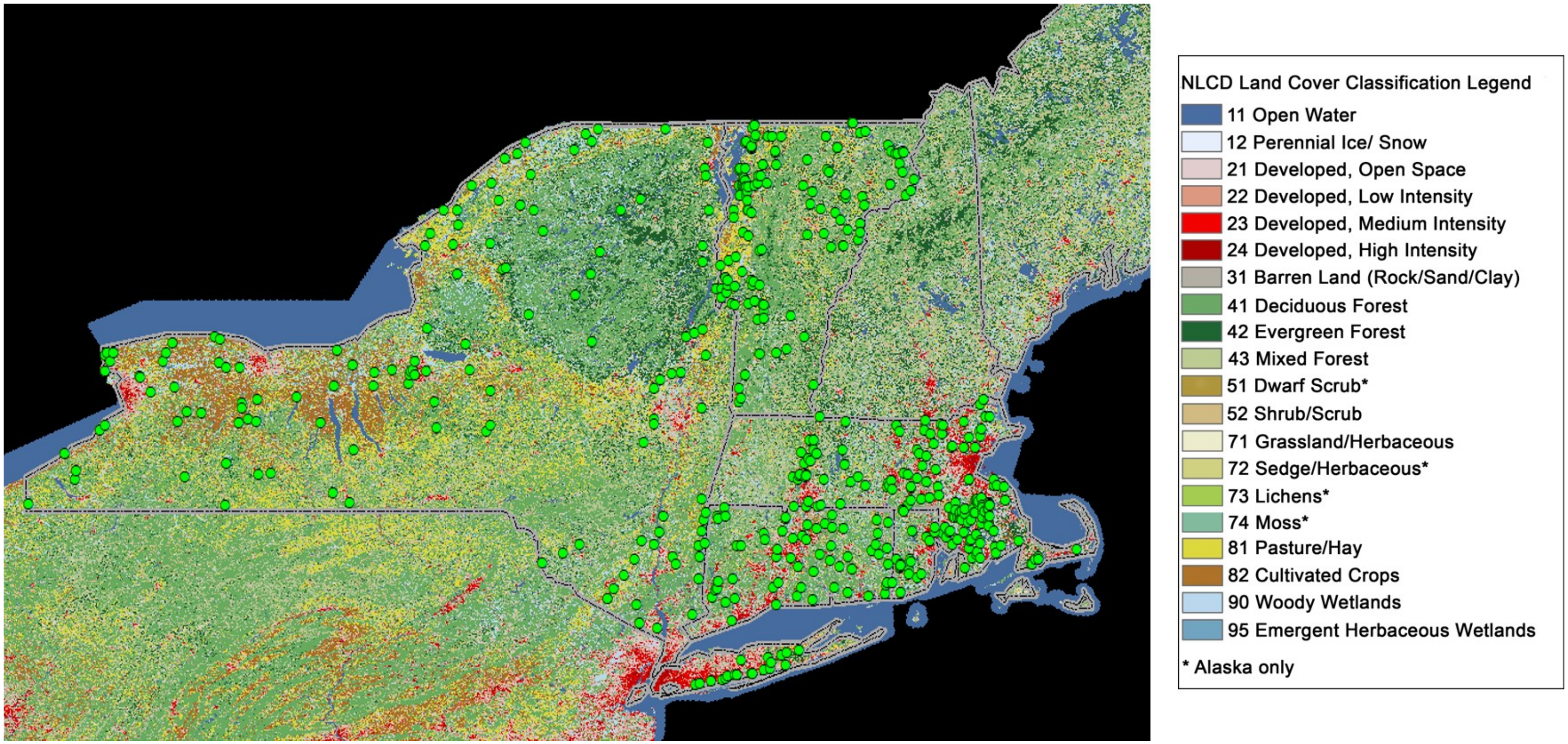


Figure B4. Sites overlaid on the NLCD 2016 land cover geospatial layer.

B2.3 Natural variables

A secondary analysis was performed on four natural variables: drainage area, flowline slope, elevation and modeled summer stream temperature (Table B5). Flowline slope was derived from the NHDPlusV2 attribute data. The source of the other variables was the USEPA Stream-Catchment (StreamCat) dataset (Hill et al. 2016). All four variables are known to influence distributions of macroinvertebrates along a longitudinal gradient (from headwaters to mouth) (Vannote et al. 1980). Most sites had drainage areas less than 100 km^2 (median = 21) and flowline slopes of less than 1% (median = 0.3) (Table B6, Figure B5). Elevation ranged from 7 to 609 meters (median = 111 meters). Most sites had summer stream temperatures in the transitional cool-warm range (18-21°C) (Table B6, Figure B5).

Table B5. Natural variables that were included in the taxa tolerance analyses.

Metric, units (Abbrev)	Description	Source
Drainage area, km2 (DrArea_km2)	Watershed area based on exact delineations where available; where not available, based on EPA StreamCat (estimate from NHDPlusV2 stream segment outlet, i.e., at the most downstream location of the vector line segment	exact delineation or EPA StreamCat estimate
Elevation - local catchment scale, meters (ElevCat)	Mean catchment elevation (m). Obtained from the NHDPlusV2 snapshot of the National Elevation Datasets (NED). Data are distributed through NHDPlusV2 website by HydroRegion.	EPA StreamCat
Flowline slope, % (pcSLOPE)	Slope of flowline (meters/meters) based on smoothed elevations; a value of -9998 means that no slope value is available. See NHDPlusV2 user guide for information about slope computation. Multiplied by 100 to convert to a percentage	NHDPlusV2 (McKay et al. 2012) \NHDPlusAttributes\ElevSlope
Summer stream temperature, °C (MSST_avg)	Modeled mean values for July-August; based on average of 2008, 2009, 2013 and 2014 values in the EPA StreamCat Dataset (which correspond with years of the National Rivers and Streams Assessment (NRSA))	EPA StreamCat (Hill et al. 2013)

Table B6. Summary statistics for the natural variables.

Variable	Valid N	Minimum	10th percentile	25th percentile	50th percentile	Mean	75th percentile	90th percentile	Maximum	Std.Dev.
Drainage area (km2) (DrArea_km2)	541	0.22	4.88	9.75	21.16	45.93	48.72	99.95	1235.12	91.97
Percent Flowline slope (pcSLOPE)	540	0.00	0.03	0.13	0.30	0.64	0.78	1.40	10.30	0.95
Elevation - local catchment scale (m) (ElevCat)	541	7.18	21.34	42.16	111.28	153.75	213.78	376.56	608.97	138.07
Summer stream temperature, degree Celsius (MSST_avg)	536	14.50	16.70	18.00	19.16	19.00	20.18	20.88	22.76	1.58

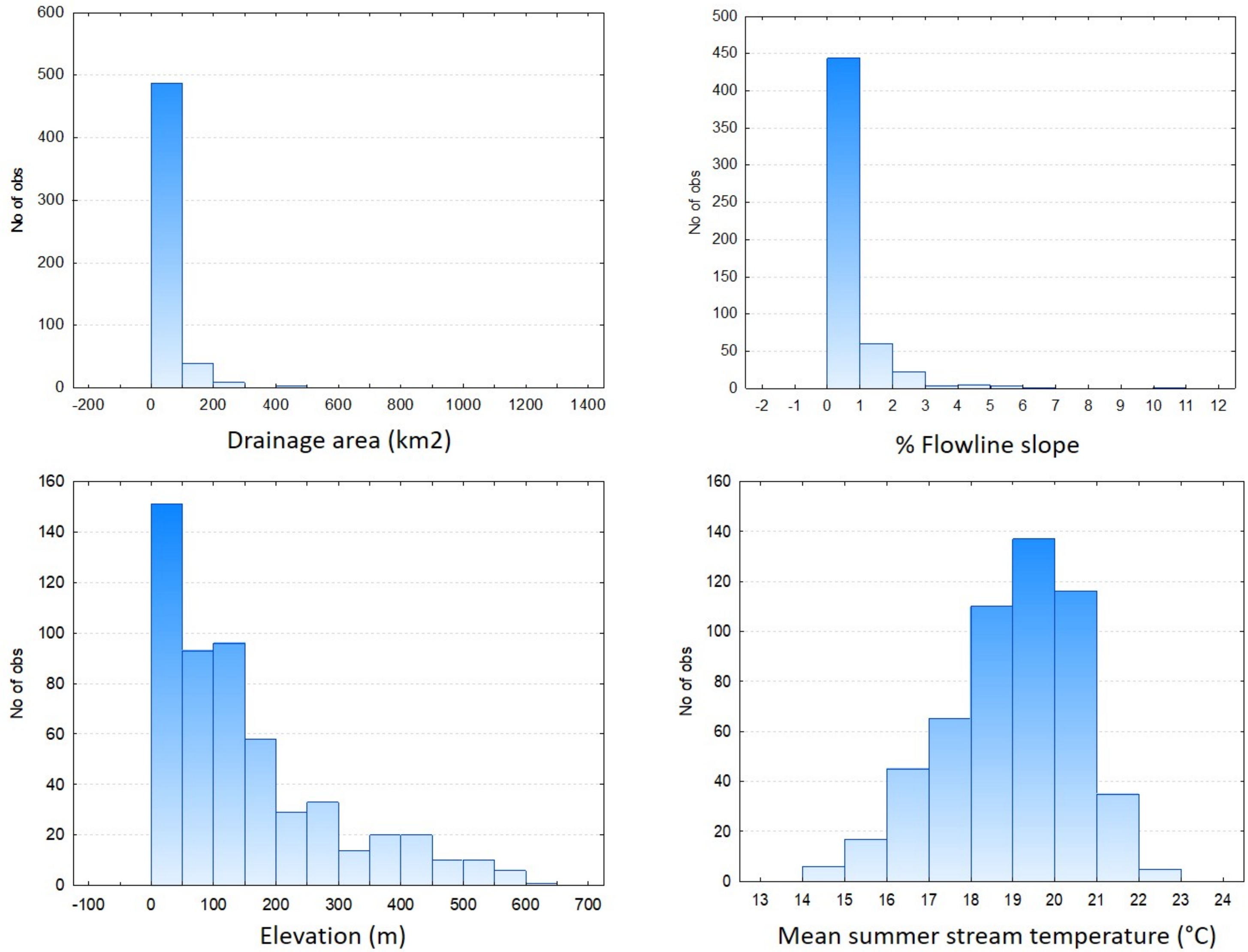


Figure B5. Histograms showing the distribution of sites across gradients for each variable (broken into incremental ‘bins’).

B3 Methods

B3.1 Data preparation

Data from 541 sites were included in the analysis. To prevent unequal weighting, only one sample per site (the one from the most recent sampling date) was included. To prepare the data, unique taxa names from each entity's dataset were composited into a single 'master' taxa list. We assigned a 'FinalID' after reconciling differences across entites stemming from misspellings and naming schemes (for example, some entities use 'grp' and others use 'group' – e.g., "Eukiefferiella devonica grp" vs. "Eukiefferiella devonica group"; for the FinalID, we changed all to 'group'). We did not delve into possible differences due to use of different taxonomic keys. For each taxon in each sample, we calculated relative abundance, which was used in the tolerance analysis (vs. straight abundance data).

We generated results for five levels of taxonomic resolution: species, genus, tribe, subfamily and family. Analyses were limited to taxa that occurred in at least 10 samples. Table B7 shows an example of how data for seven species of Polypedilum were collapsed to coarser levels of resolution for the genus, tribe, subfamily and family-level analyses. Because all seven species occurred at 10 or more sites, results were generated for each species. For the genus-level run (Polypedilum), the seven species were collapsed to genus-level (otherwise their counts would have been excluded from the coarser-level analyses). The species and genus-level identifications were further collapsed for the tribe, subfamily and family-level analyses (and combined with data for other Chironomini, Chironominae and Chironomidae taxa, as appropriate). Table B8 shows how many taxa within each major taxonomic group were assessed and at what level of taxonomic resolution.

Table B7. Example of how species-level data (in this case, for the midge Polypedilum) were collapsed to coarser levels of resolution for the genus, tribe, subfamily and family-level analyses.

TaxaID	Total # sites	Species	Genus	Tribe	Subfamily	Family
Polypedilum	438	Exclude	Polypedilum	Chironomini	Chironominae	Chironomidae
Polypedilum aviceps	107	Polypedilum aviceps	Polypedilum	Chironomini	Chironominae	Chironomidae
Polypedilum fallax group	104	Polypedilum fallax group	Polypedilum	Chironomini	Chironominae	Chironomidae
Polypedilum flavum	124	Polypedilum flavum	Polypedilum	Chironomini	Chironominae	Chironomidae
Polypedilum halterale group	50	Polypedilum halterale group	Polypedilum	Chironomini	Chironominae	Chironomidae
Polypedilum illinoense group	284	Polypedilum illinoense group	Polypedilum	Chironomini	Chironominae	Chironomidae
Polypedilum scalaenum group	92	Polypedilum scalaenum group	Polypedilum	Chironomini	Chironominae	Chironomidae
Polypedilum tritum	67	Polypedilum tritum	Polypedilum	Chironomini	Chironominae	Chironomidae

Table B8. Number of taxa within each major taxonomic group that were assessed, along with the level of taxonomic resolution.

Taxonomic Group	Family	Subfamily	Tribe	Genus	Species	Total
Amphipods & Isopods	4			5	5	***14***
Bivalvia	2			4	1	***7***
Chironomidae	1	5	10	73	37	***126***
Coleoptera	10	1	1	23	17	***52***
Decapoda	1			1	2	***4***
Diptera without Chironomidae	11	4		19	3	***37***
Ephemeroptera	10			24	15	***49***
Gastropoda	10	1		11	11	***33***
Megaloptera	2			3	1	***6***
Odonata	7			11	5	***23***
Plecoptera	9			9	2	***20***
Trichoptera	16	1		30	13	***60***
Water mites (Trombidiformes)	8			9		***17***
Worms and Leeches	6	2		11	9	***26***
Total	***97***	***14***	***11***	***233***	***121***	***476***

B3.2 Outputs

We used customized R code to generate weighted average optima (WAopt) and tolerance (WAtol) values for each taxon. The WAopt is a commonly used measure for estimating the central tendency of a taxon along an environmental gradient. The WA is calculated by multiplying taxon relative abundance (=the weighting factor) by the variable of interest (e.g., IWI) for each sample, summing the resulting numbers and dividing that by the sum of all the weights. The width of the bell shape is often called ‘tolerance’ which can also be used to characterize the environmental niche for species along the environmental gradient.

In addition to the WAopt and WAtol values, we generated histograms (Figure B6), relative abundance scatterplots (Figure B7) and cumulative distribution functions (CDFs) (Figure B8) to visualize the relationship between each taxon’s occurrence and the environmental variables. The results provide information on where the taxa occur along stressor gradients and whether they increase or decrease in relative abundance with increasing or decreasing stress. Each output also included taxon distribution maps, with data points sized by relative abundance (such that locations with higher relative abundances had larger dots). Separate sets of output files were generated for each taxonomic group, and disturbance and natural variables were analyzed separately.

The WA optima and tolerance values for each taxon/variable were compiled into a MS Excel worksheet. The worksheet also included sample size. Taxa that occurred in fewer than 30 samples were flagged for low abundance[3] and their outputs were interpreted with caution. In addition to the numeric WAopt

[3] More specifically, those that occurred in 10 to 19 samples were flagged as ‘very low’ and those that occurred in 20-29 samples were flagged as ‘low’.

values for each disturbance variable, the worksheet contained columns with categorical, relative rankings for each variable (five levels, ranging from worst to best, based on the criteria in Table B9).

Table B9. Five narrative rankings were assigned to each taxon for each disturbance variable, using the criteria below. Thresholds were based on statistics (the distributions of WAopt values in the dataset) and best professional judgment.

Category	ICI	IWI	PctUrb	PctAg
Worst	<0.50	<0.50	<5	<5
Worse	0.50-0.54	0.50-0.54	5-9.9	5-9.9
Intermediate	0.55-0.65	0.55-0.65	10-19.9	10-19.9
Better	0.66-0.79	0.66-0.79	20-29.9	20-24.9
Best	≥0.80	≥0.80	≥30	≥25

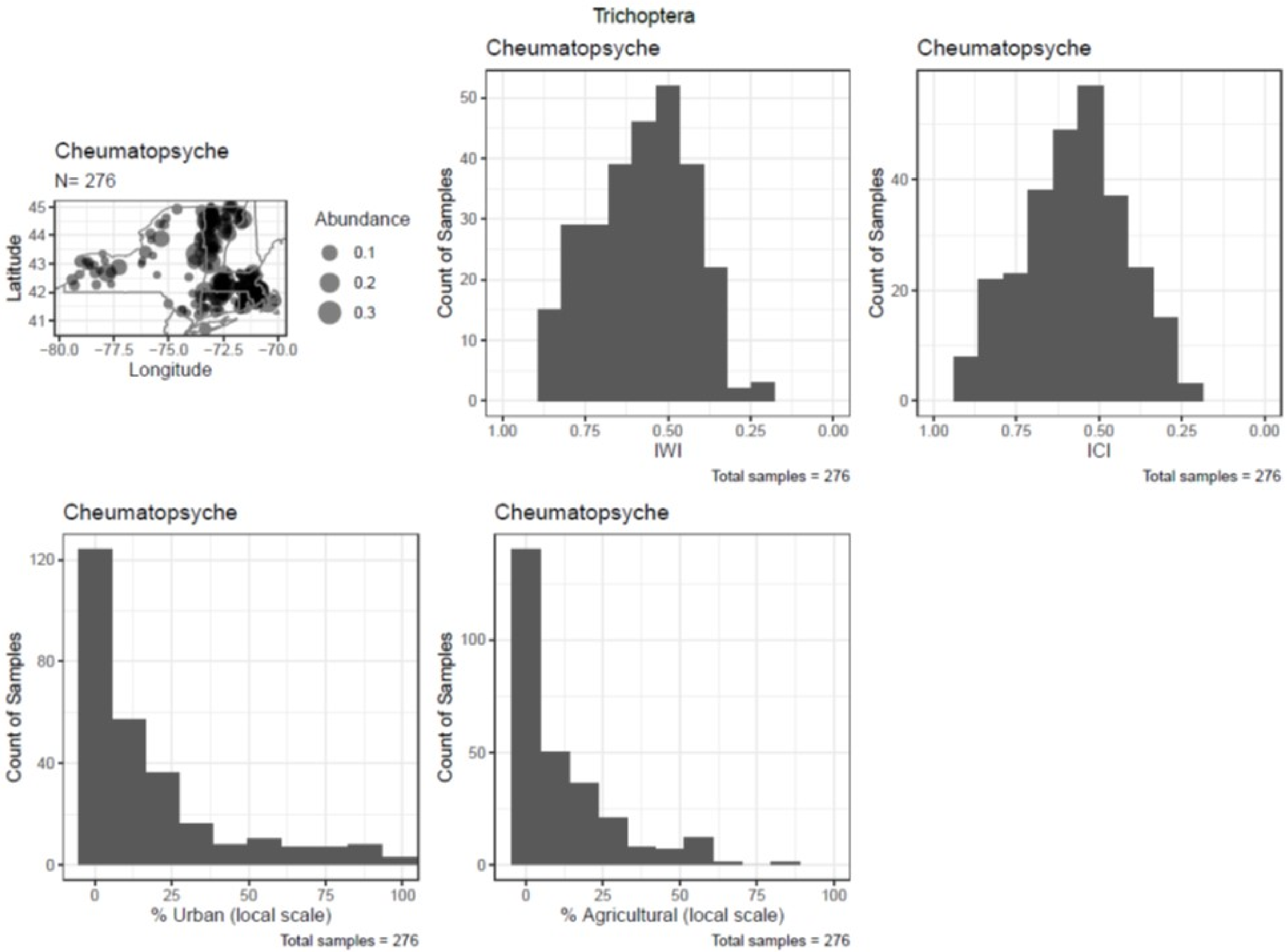


Figure B6. Example of a histogram plot.

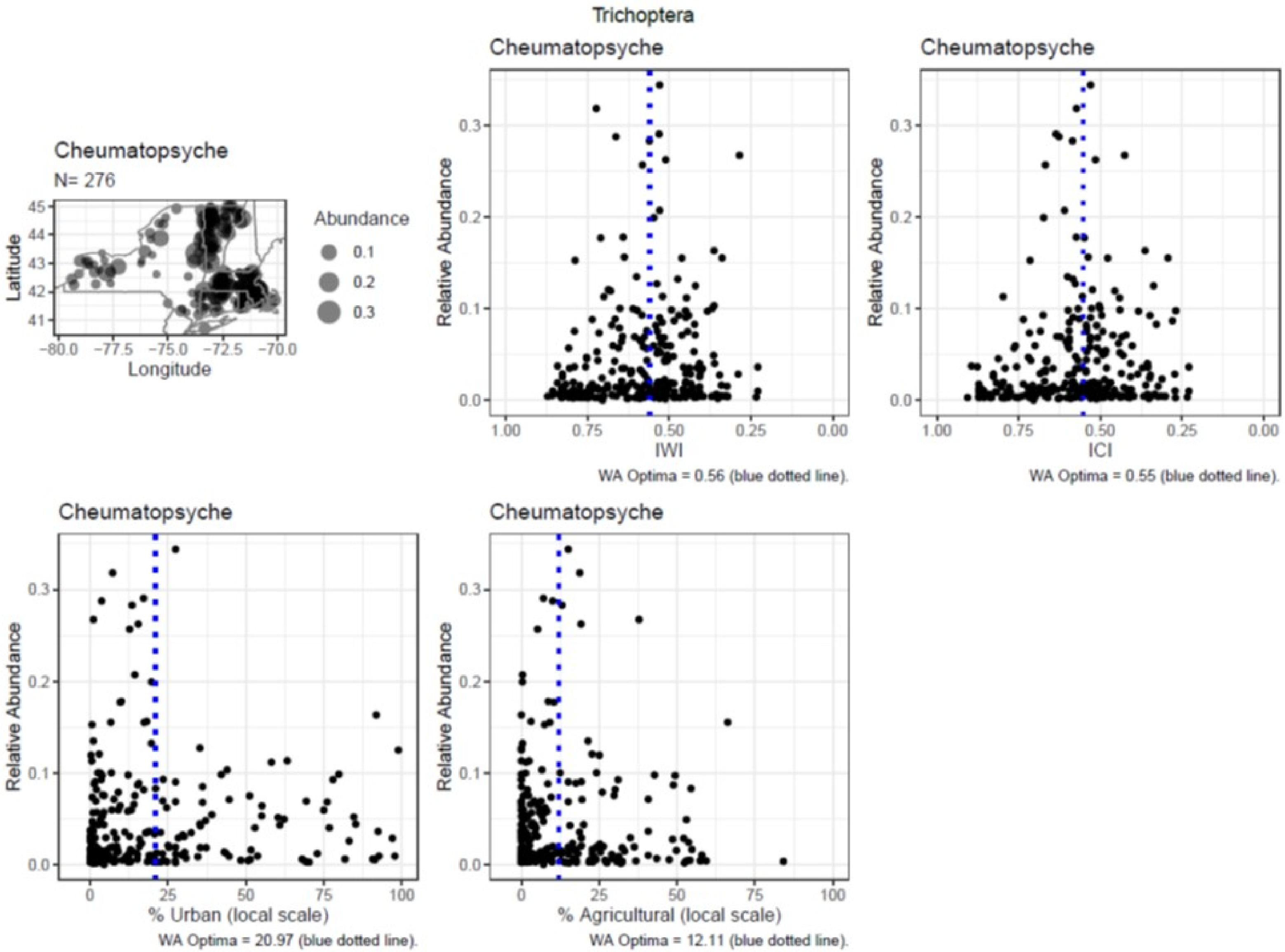


Figure B7. Example of a relative abundance scatterplot. The blue vertical dashed line equals the weighted average optima value.

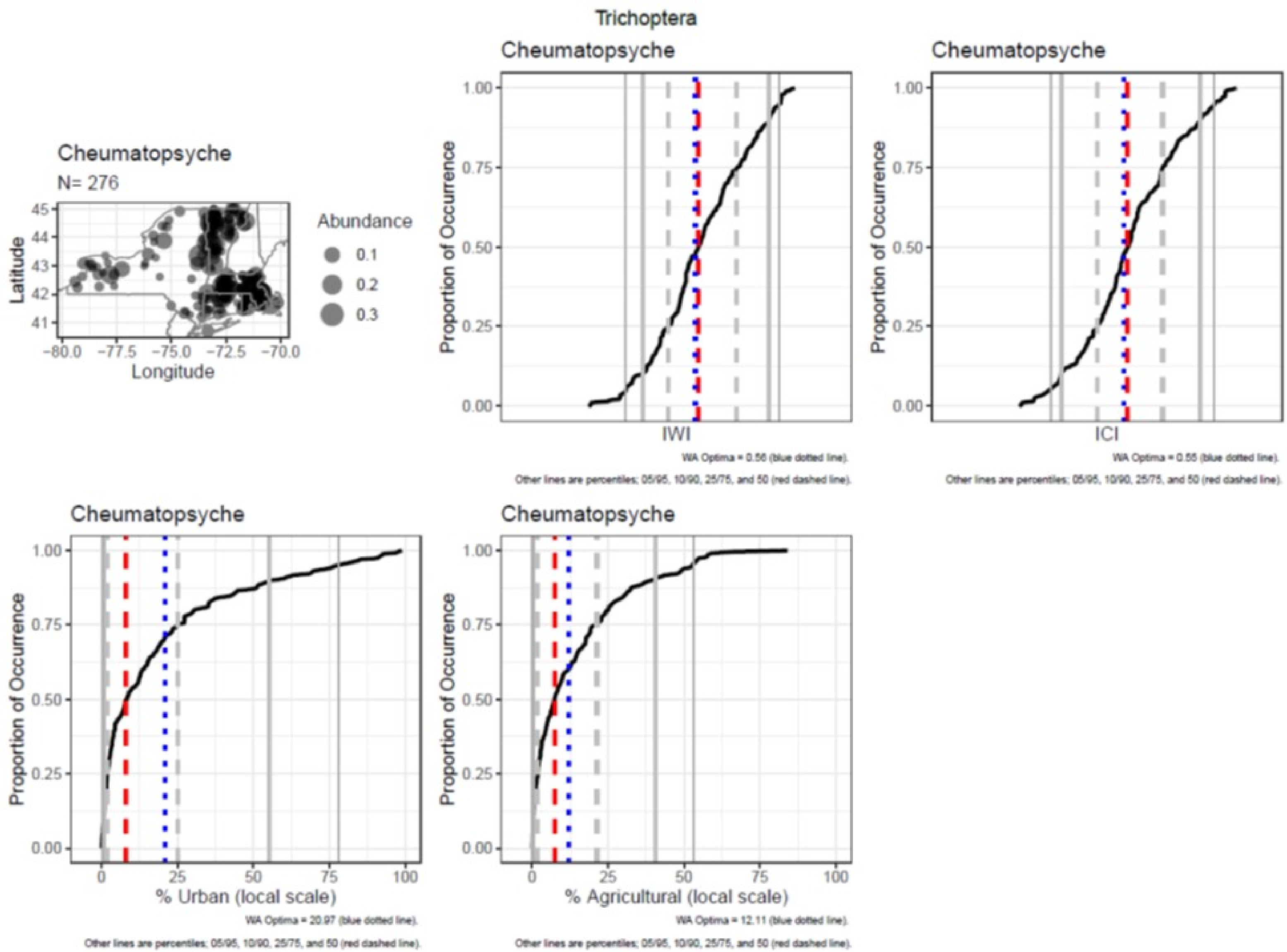


Figure B8. Example of a cumulative distribution function (CDF) plot. The blue vertical dashed line equals the weighted average optima value.

B3.3 Intepretation of results

Biologists from MassDEP who were experienced at assessing macroinvertebrate assemblages reviewed the Excel worksheet and assigned taxa to three tolerance categories: intolerant (numeric value = 2), intermediate (numeric value = 5) and tolerant (numeric value = 8). The worksheet was limited to taxa that occurred in the MA and RI dataset. The review process focused on the disturbance variables, not the natural variables.

The biologists considered multiple lines of evidence when making taxa tolerance assignments, including: 1) WAopt and WAtol values and rankings; 2) distribution across the stressor gradients as shown by the scatterplots, CDFs and histograms; 3) sample size (the more samples the taxon occurred in, the more confident we were in the results); and 4) personal experience and best professional judgment (BPJ). When assigning taxa to the three tolerance categories, the reviewers looked for patterns like those shown in Figure B9. Intolerant taxa occurred mostly (and in higher relative abundance) at sites with the lowest levels of disturbance. Intermediate taxa were generally ubiquitous and most prevalent in the middle of the disturbance gradient. Tolerant taxa tended to occur throughout the stressor gradient and generally increased in relative abundance as stress levels increased. Some taxa showed differing sensitivities to the four disturbance variables. In these situations, the reviewers generally made their assignments based on the 'worst' results (for example, if a taxon was found to be tolerant to stressors associated with urban land cover but not to agricultural land cover, the taxon was generally assigned to the 'tolerant' category).

When interpreting results, it was important for the reviewers to consider both the plots and the WAopt values since WAopt values were sometimes influenced by outliers (see example in Figure B10). The outliers could be either legitimate or incorrect. Potential reasons for erroneous outliers include: the disturbance variable was incorrect (perhaps because the StreamCat data were not based on exact watershed delineations), or the taxon was misidentified. Reasons for the outliers were not investigated. When interpreting results, the reviewers took note of outliers but focused more on the dispersal of data points across the rest of the gradient.

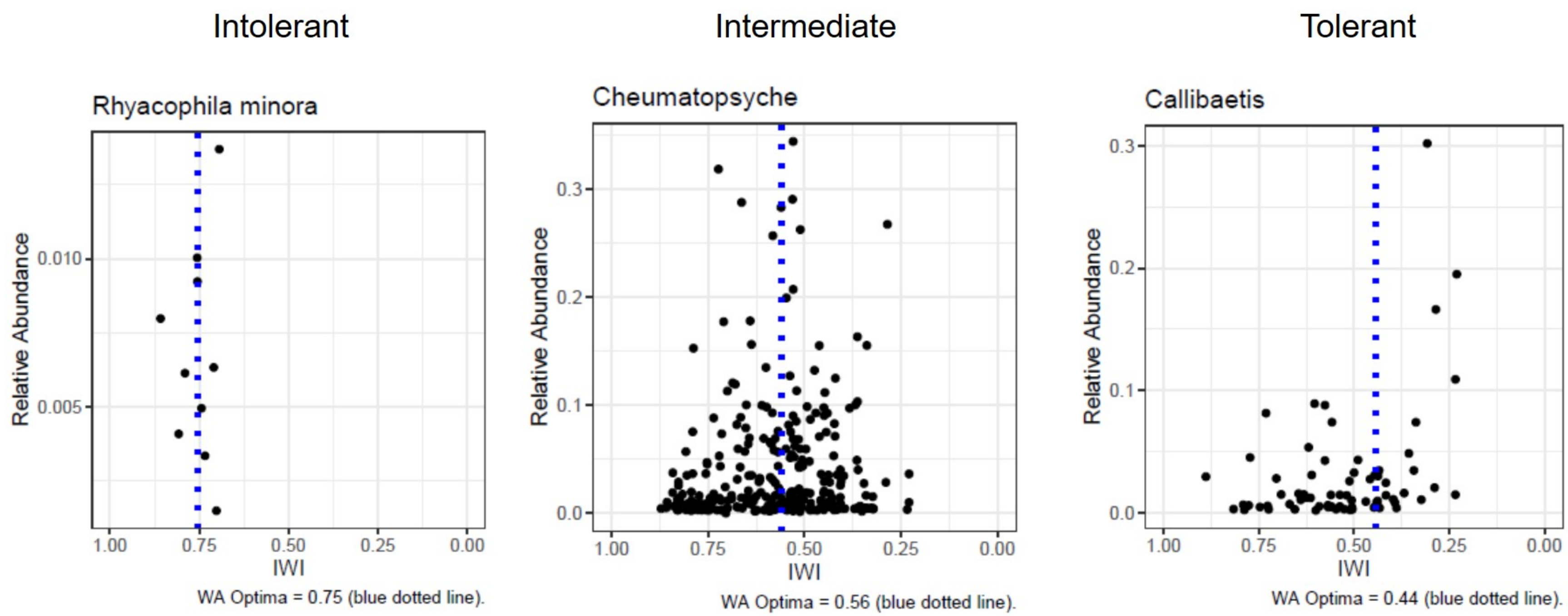


Figure B9. Examples of taxon-response patterns for taxa that were categorized as intolerant, intermediate tolerant and tolerant. The IWI scoring scale ranges from 0 (worst) to 1 (best).

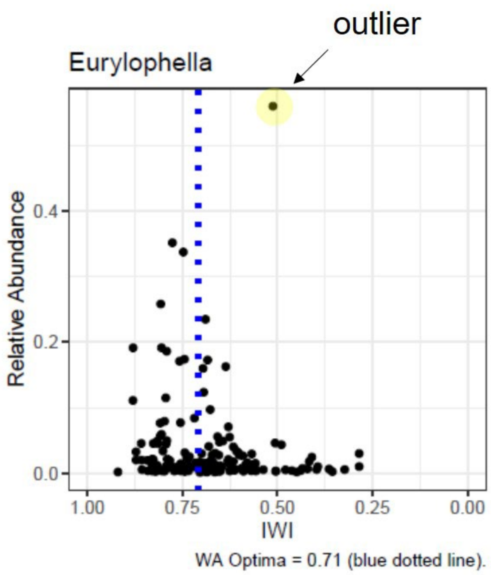


Figure B10. Example of a situation where a taxon's WAopt value was influenced by an outlier.

B4 Results

Table B10 shows the number of taxa in each tolerance category, by taxonomic group. Most taxa were placed in the intermediate group (257 of the 331 taxa that were assessed). The worms/leeches and Chironomidae had the most taxa in the tolerant group, while Plecoptera had the most intolerant taxa, followed by Ephemeroptera and Chironomidae (Table B10). The full set of results (including the plots and worksheet that the reviewers used) are available upon request (contact Jen.Stamp@tetratech.com).

Table B10. Distribution of taxa across tolerance categories, broken into taxonomic groups.

Taxonomic Group	# Intolerant	# Intermediate	# Tolerant	*Total #*
Amphipods & Isopods	0	2	5	***7***
Bivalvia	0	3	2	***5***
Chironomidae	6	80	15	***101***
Coleoptera	1	26	0	***27***
Diptera without Chironomidae	1	21	2	***24***
Ephemeroptera	6	25	1	***32***
Gastropoda	0	14	5	***19***
Megaloptera	0	5	0	***5***
Odonata	2	16	1	***19***
Plecoptera	7	8	0	***15***
Trichoptera	3	42	0	***45***
Water mites (Trombidiformes)	0	6	3	***9***
Worms and Leeches	0	9	14	***23***
Total	***26***	***257***	***48***	***331***

B5 Conclusions

We used low gradient stream macroinvertebrate data provided by regional partners and the StreamCat dataset to examine relationships between taxa occurrence and anthropogenic disturbance. Results helped inform macroinvertebrate tolerance value assignments related to sensitivity to stressors in low gradient streams. The tolerance values were then used to calculate tolerance-based metrics, one of which is included in MassDEP's low gradient Index of Biological Integrity (IBI) (% Tolerant taxa).

While the taxa tolerance analysis described here was an important step forward, more work remains to be done. If resources permit, recommendations for possible future work include:

- Running a similar analysis on data collected from riffle habitats in higher gradient, rocky bottom streams, and then comparing results with the low gradient outputs. This will help biologists better understand differences in the structure and function of macroinvertebrate assemblages in low vs. higher gradient streams, which in turn will improve the ability of biomonitoring programs to identify degradation in biological integrity and water quality.
- Rerunning the low gradient analyses with:
    - New data that MA, RI, CT, NY and VT have collected since the time of the analysis
    - (Possibly) data from low gradient streams in Maine and New Hampshire (caveat: first we'd need to evaluate the suitability of rock basket data for this type of analysis)
    - Environmental data based on exact watershed delineations. Doing exact watershed delineations with the USGS StreatStats stream layer may allow for inclusion of the 46 sites that had to be excluded because they did not match with the NHDPlusV2 flowlines
    - Running an additional set of plots based on Generalized Additive Models (GAM) (see examples in Yuan 2006)
- Working with a group of regional biologists on reviewing results, and through that process, developing better guidance on how to interpret results.
- Developing a regional Biological Condition Gradient (BCG) model for low gradient streams, to go along with the existing New England high gradient streams BCG model (Stamp and Gerritsen 2009)

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Appendix C

C1 Candidate Metrics

List of candidate macroinvertebrate metrics that were calculated with the BioMonTools R package (https://github.com/leppott/BioMonTools).

Metric Name	Category	Description
nt_total	RICH	number of taxa - total
nt_Amph	RICH	number of taxa - Order Amphipoda
pi_Amph	COMP	percent individuals - Order Amphipoda
pt_Amph	RICH	percent taxa - Order Amphipoda
nt_Isop	RICH	number of taxa - Order Isopoda
pi_Isop	COMP	percent individuals - Order Isopoda
pt_Isop	RICH	percent taxa - Order Isopoda
pi_AmphIsop	COMP	percent individuals - Orders Amphipoda & Isopoda
pi_Baet	COMP	percent individuals - Family Baetidae
nt_Bival	RICH	number of taxa - Class Bivalvia
pi_Bival	COMP	percent individuals - Class Bivalvia
pt_Bival	RICH	percent taxa - Class Bivalvia
pi_Caen	COMP	percent individuals - Family Caenidae
nt_Chiro	RICH	number of taxa - Family Chironomidae
pi_Chiro	COMP	percent individuals - Family Chironomidae
pt_Chiro	RICH	percent taxa - Family Chironomidae
nt_Coleo	RICH	number of taxa - Order Coleoptera
pi_Coleo	COMP	percent individuals - Order Coleoptera
pt_Coleo	RICH	percent taxa - Order Coleoptera
nt_COET	RICH	number of taxa - Order Coleoptera, Odonata, Ephemeroptera & Trichoptera
pi_COET	COMP	percent individuals - Order Coleoptera, Odonata, Ephemeroptera & Trichoptera
pt_COET	RICH	percent taxa - Order Coleoptera, Odonata, Ephemeroptera & Trichoptera
nt_CruMol	RICH	number of taxa - Crustacea & Mollusca
pi_CruMol	COMP	percent individuals - Crustacea & Mollusca
nt_Dipt	RICH	number of taxa - Order Diptera
pi_Dipt	COMP	percent individuals - Order Diptera
pt_Dipt	RICH	percent taxa - Order Diptera
nt_Ephem	RICH	number of taxa - Order Ephemeroptera
pi_Ephem	COMP	percent individuals - Order Ephemeroptera
pt_Ephem	RICH	percent taxa - Order Ephemeroptera
pi_EphemNoCae	COMP	percent individuals - Order Ephemeroptera, excluding Family Caenidae
pi_EphemNoCaeBae	COMP	percent individuals - Order Ephemeroptera, excluding Families Caenidae & Baetidae

nt_EPT	RICH	number of taxa - Orders Ephemeroptera, Plecoptera & Trichoptera (EPT)
pi_EPT	COMP	percent individuals - Orders Ephemeroptera, Plecoptera & Trichoptera (EPT)
pt_EPT	RICH	percent taxa - Orders Ephemeroptera, Plecoptera & Trichoptera (EPT)
nt_Gast	RICH	number of taxa - Class Gastropoda
pi_Gast	COMP	percent individuals - Class Gastropoda
pt_Gast	RICH	percent taxa - Class Gastropoda
pi_Hydro	COMP	percent individuals - Family Hydropsychidae
nt_Insect	RICH	number of taxa - Class Insecta
pi_Insect	COMP	percent individuals - Class Insecta
pt_Insect	RICH	percent taxa - Class Insecta
nt_Mega	RICH	number of taxa - Order Megaloptera
pi_Mega	COMP	percent individuals - Order Megaloptera
pt_Mega	RICH	percent taxa - Order Megaloptera
nt_NonIns	RICH	number of taxa - Class not Insecta
pi_NonIns	COMP	percent individuals - Class not Insecta
pt_NonIns	RICH	percent taxa - Class not Insecta
nt_Odon	RICH	number of taxa - Order Odonata
pi_Odon	COMP	percent individuals - Order Odonata
pt_Odon	RICH	percent taxa - Order Odonata
nt_OET	RICH	number of taxa - Orders Odonata, Ephemeroptera & Trichoptera (OET)
pi_OET	COMP	percent individuals - Orders Odonata, Ephemeroptera & Trichoptera (OET)
pt_OET	RICH	percent taxa - Orders Odonata, Ephemeroptera & Trichoptera (OET)
nt_Oligo	RICH	number of taxa - Class Oligochaeta
pi_Oligo	COMP	percent individuals - Class Oligochaeta
pt_Oligo	RICH	percent taxa - Class Oligochaeta
nt_Pleco	RICH	number of taxa - Order Plecoptera
pi_Pleco	COMP	percent individuals - Order Plecoptera
pt_Pleco	RICH	percent taxa - Order Plecoptera
nt_POET	RICH	number of taxa - Orders Plecoptera, Odonata, Ephemeroptera & Trichoptera (POET)
pi_POET	COMP	percent individuals -Orders Plecoptera, Odonata, Ephemeroptera & Trichoptera (POET)
pt_POET	RICH	percent taxa - Orders Plecoptera, Odonata, Ephemeroptera & Trichoptera (POET)
nt_Trich	RICH	number of taxa - Order Trichoptera
pi_Trich	COMP	percent individuals - Order Trichoptera
pt_Trich	RICH	percent taxa - Order Trichoptera

pi_TricNoHydro	COMP	percent individuals - Order Trichoptera, excluding Family Hydropsychidae
pi_SimBtri	COMP	percent individuals - Families Simuliidae & Baetis tricaudatus
pi_dom01	RICH	percent individuals - most dominant taxon [max(N_TAXA)]
pi_dom02	RICH	percent individuals - two most dominant taxa
pi_dom03	RICH	percent individuals - three most dominant taxa
pi_dom04	RICH	percent individuals - four most dominant taxa
pi_dom05	RICH	percent individuals - five most dominant taxa
x_Shan_2	RICH	Shannon Wiener Diversity Index (log base 2) - x_Shan_Num/log(2)
x_D	RICH	Simpson's Index
x_Evenness	RICH	Evenness=x_Shan_e/log(nt_total)
x_Becks	TOLER	Becks Biotic Index = 2*[C1Taxa]+[C2Taxa] (see footnote)
x_HBI	TOLER	Hilsenhoff Biotic Index (references the TolVal field)
nt_tv_intol	TOLER	number of taxa - tolerance value - intolerant ≤ 3
pi_tv_intol	TOLER	percent individuals - tolerance value - intolerant ≤ 3
pt_tv_intol	TOLER	percent taxa - tolerance value - intolerant ≤ 3
nt_tv_toler	TOLER	number of taxa - tolerance value -tolerant ≥ 7
pi_tv_toler	TOLER	percent individuals - tolerance value -tolerant ≥ 7
pt_tv_toler	TOLER	percent taxa - tolerance value -tolerant ≥ 7
nt_ffg_col	FFG	number of taxa - Functional Feeding Group (FFG) - collector-gatherer (CG)
pi_ffg_col	FFG	percent individuals - Functional Feeding Group (FFG) - collector-gatherer (CG)
pt_ffg_col	FFG	percent taxa - Functional Feeding Group (FFG) - collector-gatherer (CG)
nt_ffg_filt	FFG	number of taxa - Functional Feeding Group (FFG) - collector-filterer (CF)
pi_ffg_filt	FFG	percent individuals - Functional Feeding Group (FFG) - collector-filterer (CF)
pt_ffg_filt	FFG	percent taxa - Functional Feeding Group (FFG) - collector-filterer (CF)
nt_ffg_pred	FFG	number of taxa - Functional Feeding Group (FFG) - predator (PR)
pi_ffg_pred	FFG	percent individuals - Functional Feeding Group (FFG) - predator (PR)
pt_ffg_pred	FFG	percent taxa - Functional Feeding Group (FFG) - predator (PR)
nt_ffg_scrap	FFG	number of taxa - Functional Feeding Group (FFG) - scraper (SC)
pi_ffg_scrap	FFG	percent individuals - Functional Feeding Group (FFG) - scraper (SC)
pt_ffg_scrap	FFG	percent taxa - Functional Feeding Group (FFG) - scraper (SC)
nt_ffg_shred	FFG	number of taxa - Functional Feeding Group (FFG) - shredder (SH)
pi_ffg_shred	FFG	percent individuals - Functional Feeding Group (FFG) - shredder (SH)
pt_ffg_shred	FFG	percent taxa - Functional Feeding Group (FFG) - shredder (SH)
nt_habit_burrow	HABIT	number of taxa - Habit - burrowers (BU)
pi_habit_burrow	HABIT	percent individuals - Habit - burrowers (BU)

pt_habit_burrow	HABIT	percent taxa - Habit - burrowers (BU)
nt_habit_climb	HABIT	number of taxa - Habit - climbers (CB)
pi_habit_climb	HABIT	percent individuals - Habit - climbers (CB)
pt_habit_climb	HABIT	percent taxa - Habit - climbers (CB)
nt_habit_cling	HABIT	number of taxa - Habit - clingers (CN)
pi_habit_cling	HABIT	percent individuals - Habit - clingers (CN)
pt_habit_cling	HABIT	percent taxa - Habit - clingers (CN)
nt_habit_sprawl	HABIT	number of taxa - Habit - sprawlers (SP)
pi_habit_sprawl	HABIT	percent individuals - Habit - sprawlers (SP)
pt_habit_sprawl	HABIT	percent taxa - Habit - sprawlers (SP)
nt_habit_swim	HABIT	number of taxa - Habit - swimmers (SW)
pi_habit_swim	HABIT	percent individuals - Habit - swimmers (SW)
pt_habit_swim	HABIT	percent taxa - Habit - swimmers (SW)
nt_volt_multi	VOLT	number of taxa - multivoltine (MULTI)
pi_volt_multi	VOLT	percent individuals - multivoltine (MULTI)
pt_volt_multi	VOLT	percent taxa - multivoltine (MULTI)
nt_volt_semi	VOLT	number of taxa - semivoltine (SEMI)
pi_volt_semi	VOLT	percent individuals - semivoltine (SEMI)
pt_volt_semi	VOLT	percent taxa - semivoltine (SEMI)
nt_volt_uni	VOLT	number of taxa - univoltine (UNI)
pi_volt_uni	VOLT	percent individuals - univoltine (UNI)
pt_volt_uni	VOLT	percent taxa - univoltine (UNI)
nt_ti_cc	TEMP	number of taxa - thermal indicator - cold/cool
pi_ti_cc	TEMP	percent individuals - thermal indicator - cold/cool
pt_ti_cc	TEMP	percent taxa - thermal indicator - cold/cool
nt_ti_w	TEMP	number of taxa - thermal indicator - warm
pi_ti_w	TEMP	percent individuals - thermal indicator - warm
pt_ti_w	TEMP	percent taxa - thermal indicator - warm

C2 Summary of input metrics in existing low gradient IBIs

Vermont DEC (in progress; personal communication Aaron Moore)

Hybrid low gradient (HLG)

1. Density
2. EOT Richness
3. BCG intolerant richness
4. BCG intolerant COTE %
5. Modified EOT/EOT+Chiro
6. PMA-O
7. Amphipoda+Isopoda %
8. Biotic Index
9. PPCS-F
10. Shr%/CF+Shr%

Soft/slow low gradient (SLG)

1. Density
2. EOT Richness
3. BCG intolerant richness
4. BCG intolerant COTE %
5. Modified EOT/EOT+Chiro
6. PMA-O
7. Amphipoda+Isopoda %
8. Biotic Index
9. PPCS-F
10. Modified EOT Density

New York State DEC (in progress; personal communication Gavin Lemly)

Provisional IBIs by regions for low-gradient streams for three regions:

<u>Great Lakes</u>
- rich_family: decrease with stress
- pct_dom1_order: increase with stress
- shannon_family: decrease with stress
- rich_scraper: decrease with stress

<u>Adirondacks</u>
- pct_insecta: decrease with stress
- rich_mollusca_amphipoda_fa: increase with stress
- rich_intolerant: decrease with stress
- rich_et_macro_genspecies: decrease with stress

<u>Hudson Valley+Southern Tier</u>:
- pct_rich_cote_family: decrease with stress
- pct_et: decrease with stress
- pct_filterer: decrease with stress
- shannon_genus: decrease with stress

Location	Index Identifier	Metrics	Metric response to stress	Citation	Remarks
Coastal plain region of the 6 states (New Jersey, Delaware, Maryland, Virginia, North Carolina, and South Carolina)	Coastal Plain Macroinvertebrate Index (CPMI)	# of taxa # of EPT taxa % Ephemeroptera Hilsenhoff Biotic Index (HBI) % clingers	**# taxa**: decrease; 45% overall assessment accuracy **# EPT taxa**: decrease; high assessment accuracy (84% overall); correlated with Ephem and Trichop metrics; historic reliability. **% Ephemeroptera**: decrease; 57% overall-assessment accuracy; lower redundancies with HBI & # EPT; high redundancy with % EPT metrics already selected **HBI**: increase; high assessment accuracy (80%overall); strongly correlated with other tolerance metrics; historic reliability. **% clingers**: decrease; not redundant with TT and %E metrics already selected, moderately redundant with the HBI and EPT metrics	Maxted et al. 2000	Oct/Nov sampling period. Accurately identified 86% of impaired sites overall (varied 83-100% across the 3 regions classified). 90% CI for the 5 core metrics were ±6.0 taxa for TT, ±2.5 taxa for EPT, ±8.9% for %E, ±0.28 units for the HBI, ±13.8% for %CL, and ±3.1 units for the CPMI.

Location	Index Identifier	Metrics	Metric response to stress	Citation	Remarks
Florida	Stream Condition Index (SCI)	# total taxa # EPT taxa # Chironomidae taxa Florida Index % dominant taxa % Diptera % gatherers % filterers	**# total taxa**: decrease **# EPT taxa**: decrease **# Chironomidae taxa**: decrease **Florida Index**: decrease **% dominant taxa**: increase **% Diptera**: increase **% gatherers**: variable **% filtere**rs: decrease; "filter feeders are also thought to be sensitive in low-gradient streams (Wallace et al. 1977)."	Barbour et al. 1996	Summer index sampling period (Jul-Sep). 3 classified regions: panhandle, peninsular Florida, & the northeastern portion of Florida Scores (5, 3, or 1) developed for 8 metrics to allow aggregation into an index

Location	Index Identifier	Metrics	Metric response to stress	Citation	Remarks
Choctawhatchee-Pea Rivers watershed, AL	Invertebrate community index (ICI)	# EPT taxa # Trichoptera taxa # Diptera taxa # Crustacea + Mollusca % Dominant taxa % Ephemeroptera % Diptera % Chironominae to chironomids Family Biotic Index (FBI) % Shredders	# EPT taxa: decrease # Trichoptera taxa: decrease # Diptera taxa: decrease # Crustacea + Mollusca: decrease % Dominant taxa: increase % Ephemeroptera: decrease % Diptera: increase % Chironominae to chironomids: decrease Family Biotic Index (FBI): increase % Shredders: decrease	Bennet et al. 2004	Within the coastal plains ecoregion in southeast Alabama; low elevation and loosely compacted, sandy soils. 34 wadeable first through sixth-order streams; plus for validation 7 additional least impacted and 8 impacted streams. 49 sites sampled once during April and May 2001. The 10 selected metrics (of 38 tested) had significant correlations with one or more physiochemical variables. ICI calculated by summing the 10 metric scores from 34 sites; ranged from 18 to 56 out of a possible score of 60. The ICI was not always capable of discriminating between artificially enriched sites and good quality sites

Location	Index Identifier	Metrics	Metric response to stress	Citation	Remarks
Central Valley, CA	Central Valley IBI	collector richness predator richness percent EPT taxa percent clinger taxa Shannon diversity	collector richness: decrease predator richness: decrease percent EPT taxa: decrease percent clinger taxa: decrease Shannon diversity: decrease *Note: these expectations deduced from the scoring ranges presented in Table 2 of paper.*	Rehn et al. 2008	Perennial streams on the valley floor In the Central Valley, minimally disturbed reference sites no longer available. Most streams are highly alteredby human activities such as urbanization, agriculture and water diversions. 80 metrics evaluated; metric criteria: 1) sufficient range for scoring; 2) responsiveness to land use and reach-scale disturbance variables (as data allowed); 3) good discrimination between reference and test sites; 4) lack of correlation with other responsive metrics. Lack of intolerant and shredder taxa in Valley floor streams. Final IBI more strongly related to reach-scale physical habitat variables than to water chemistry or land use variables. The final 5 IBI metrics did not vary between spring and fall samples and did not require seasonal adjustments in scoring.

Location	Index Identifier	Metrics	Metric response to stress	Citation	Remarks
Austria	Multimetric index (for A01 - Mid-sized (low-gradient) streams in the Hungarian Plains)	# of total families # of EP # taxa # of Plecoptera (abundance) [%] EP # individuals [%] EP # taxa Saprobic index # of sensitive taxa [%] Shredder Diversity (Margalef)	# of total families: decrease # of EP # taxa: decrease # of Plecoptera (abundance): [%] EP # individuals: decrease [%] EP # taxa: decrease Saprobic index: increase # of sensitive taxa: decrease [%] Shredder: decrease Diversity (Margalef): decrease	Ofenböck et al. 2004	Stressor – organic pollution

Location	Index Identifier	Metrics	Metric response to stress	Citation	Remarks
Willow Creek, Nebraska	Composite Biotic Index (CBI)	Percent dominance EPT index (i.e. # EPT taxa) EPT abund/EPT + chironomid abundance Scraper abund/filterer abund Taxa richness Hilsenhoff index	Not specified in paper	Whiles et al. 2002	Developed with metrics used previously by the NDEQ during their statewide stream survey (NDEQ 1991). CBI scores actually are based on a “reference condition” for Nebraska rather than the reference stream (site 4) in our basin Corrected metrics for stream size (based on discharge) using relationships generated from a prior investigation; i.e. metrics were scored 1, 3, or 5 based on regression equations generated by the NDEQ (1991) that divided scatter plots of stream size vs metric scores into thirds

Location	Index Identifier	Metrics	Metric response to stress	Citation	Remarks
Netherlands	Multimetric index (for slow-running streams)	See metrics listed in Table 2 copied below from paper.	complex	Vlek et al. 2004	Included metrics that indicated the different classes (from 5 (high quality) to 1 (low quality); final index equation combined these; for slow running streams: $$S = \frac{T_1 * \frac{1}{2} + T_2 * \frac{1}{2} + T_3 * \frac{1}{2} + T_4 \frac{1}{4}}{n_1 * \frac{1}{2} + n_2 * \frac{1}{2} + n_3 * \frac{1}{2} + n_4 * \frac{1}{4}}$$ Where: S, final score; T1, sum of scores for the individual metrics indicating class 1; T2, sum of scores for the individual metrics indicating class 2; etc. And n1, number of indices indicating class 1; etc. Validation showed that 54% of the streams were classified correctly

References – existing low gradient IBIs

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C3 Non-target taxa

The following non-target taxa were excluded from metric calculations:

ORDER	TAXAID	NONTARGET
Hemiptera	Belostoma	TRUE
Hemiptera	Belostomatidae	TRUE
Hemiptera	Corixidae	TRUE
Hemiptera	Gerridae	TRUE
Hemiptera	Gerris	TRUE
Hemiptera	Microvelia	TRUE
Hemiptera	Neoplea striola	TRUE
Hemiptera	Notonecta	TRUE
Sarcoptiformes	Oribatida	TRUE
Hemiptera	Pleidae	TRUE
Hemiptera	Ranatra	TRUE
Hemiptera	Rhagovelia	TRUE
Hemiptera	Veliidae	TRUE

For the purposes of IBI calculations, “macroinvertebrate” is defined to include:

- all aquatic Annelida;
- all aquatic Mollusca;
- aquatic macro Crustacea (except as noted below);
- all aquatic Arachnida except for Oribatid mites (which are not truly aquatic); and
- the aquatic life stages of Insecta except Hemiptera and adult Coleoptera other than Elmidae.

Those macroinvertebrates excluded from the above list are not used for one of three reasons: either there is insufficient ecological information on them to make them useful for biomonitoring, they are surface film dwellers, or they are capable of escaping the aquatic environment at will to avoid temporarily unfavorable conditions. One further exception is crayfish (Class Crustacea, Family Cambaridae), which often are seen evacuating the immediate area as kick-sampling begins, and even swimming out of the kick-net. Crayfish species are noted when present in the sample but are not counted toward total numbers.

C4 Exclusion criteria for redundant taxa

When calculating metrics for benthic macroinvertebrates, there are occasions when certain taxa are not included in taxa richness metrics but the individuals are included for all other metrics. This is done to avoid double counting taxa that may have been identified to a more coarse level when taxa of a finer level are present in the same sample.

These taxa have been referred to by many names – e.g., Excluded Taxa, NonUnique Taxa, or Ambiguous Taxa. This document will use the term Excluded.

We used the 'markExcluded' function in the BioMonTools R package (https://github.com/leppott/BioMonTools) to mark redundant taxa in the low gradient samples prior to metric calculations. Redundant taxa were identified on a sample-by-sample basis and excluded from the richness calculations.

Redundant taxa were identified based on the following steps:

1. Calculate and find all taxa names that appear in a sample at each taxonomic rank more than once (for an example, see Figure 1). These are the potential "parents" to be excluded.

2. Check if any of the potential "parents" equal a final ID in their respective samples.

3. If you get a match these are marked as "Excluded"

All Excluded decisions are sample-specific and the rules should be reapplied if sample contents change. Also, if the level of effort or operational taxonomic units change, the Excluded taxa designations should be recalculated.

TAXA LIST

BCG Attribute	FinalID	Count	FFG	Thermal	Toler_Sed	Redundant	Excluded
4	*Nais*	7	NA	--	NA	FALSE	FALSE
4	*Atractides*	1	PR	--	NA	FALSE	FALSE
4	*Hygrobates*	3	PR	--	NA	FALSE	FALSE
4	*Lebertia*	6	PR	--	NA	FALSE	FALSE
4	*Sperchon*	2	PR	--	NA	FALSE	FALSE
3	*Torrenticola*	1	PR	--	NA	FALSE	FALSE
4	*Dytiscidae*	3	PR	--	NA	TRUE	FALSE
3	*Oreodytes*	1	PR	--	NA	FALSE	FALSE
3	*Heterlimnius corpulentus*	19	GC	--	5	FALSE	FALSE
3	*Narpus concolor*	2	GC	--	5	FALSE	FALSE
3	*Clinocera*	1	PR	--	NA	FALSE	FALSE
4	*Neoplasta*	1	NA	--	NA	FALSE	FALSE
2	*Glutops*	2	PR	--	NA	FALSE	FALSE
x	*Ceratopogoninae*	2	PR	--	NA	FALSE	FALSE
4	*Thienemannimyia group*	9	PR	--	NA	FALSE	FALSE
4	*Micropsectra*	19	GC	--	NA	FALSE	FALSE

... Data_Taxa_Master Data_Metrics Data_Habitat Data_Taxa_Samps Samp0001 Samp0009 Samp0018

Figure 1. Example - Dytiscidae (family-level) is excluded from the richness metrics in this sample because these organisms could be the same taxon as Oreodytes (genus-level). The exclusion rule is applied on a sample by sample basis.

Below is a more detailed description of the process that the markExcluded function follows. Before starting, it is necessary to have a complete and correct master taxa list (all phylogenetic information and ranks).

Terminology

- Target Rank = intended level of taxonomy for identification, e.g., genus. Typically, specified in the project's SOP but can be adjusted during the OTU process.
- Parent or Parent Taxon = a taxon that occurs in the data in addition to other taxa in the same group that are identified to a more specific level. For example, the family Baetidae may occur in the data in addition to genera within the family Baetidae. In this case the name Baetidae is a parent to the other taxa within the family. Parents do not have to be only a single rank above the child taxon. That is, the class and order ranks are parents of any family ranks within them.
- Child or Children Taxa = a taxa or taxon that occurs in the data in addition to individuals identified to a coarser level. For example, the genera Baetis and Procloeon may occur in addition to the family Baetidae (of which the 2 genera listed are a member). In this case Baetis and Procloen are children of Baetidae.

Rule Development

For each sample:

1. Determine "potential" taxa for exclusion based on rank (or level) names appearing more than once in a sample.
    a. This is done for all ranks present; phylum, class, order, family, tribe, genus, species.
2. Check if any "potential" taxa are equal to a final (unique) ID in the same sample.
3. Stage is combined with taxa names if used in the dataset.

Requirements

1. A sample taxa table or data frame.
    a. All non-count and zero individual taxa have been removed.
    b. Unique sample ID code in a single column.
    c. A column with a final identification that is narrative not numeric. That is, Baetidae is ok but the ITIS number is not.
    d. Phylogenetic rank/level columns.
        i. This can be applied from a master taxa table but needs to be included in this table. One column per rank.
        ii. Names need to be consistently spelled.

Procedures

1. Find all potential Parents (those with a rank coarser than the target rank). This is done by creating a list of taxa rank names that appear more than once in a sample. This is done for each taxonomic rank.
2. The above list is compared to the final identifications for each sample.
    a. Special consideration is made for ranks of finer detail than genus. That is, names that are a combination of more than one field.
3. Any matches are marked as "Excluded".

There is still a need for manual review / QC check of the final list of Excluded designations.

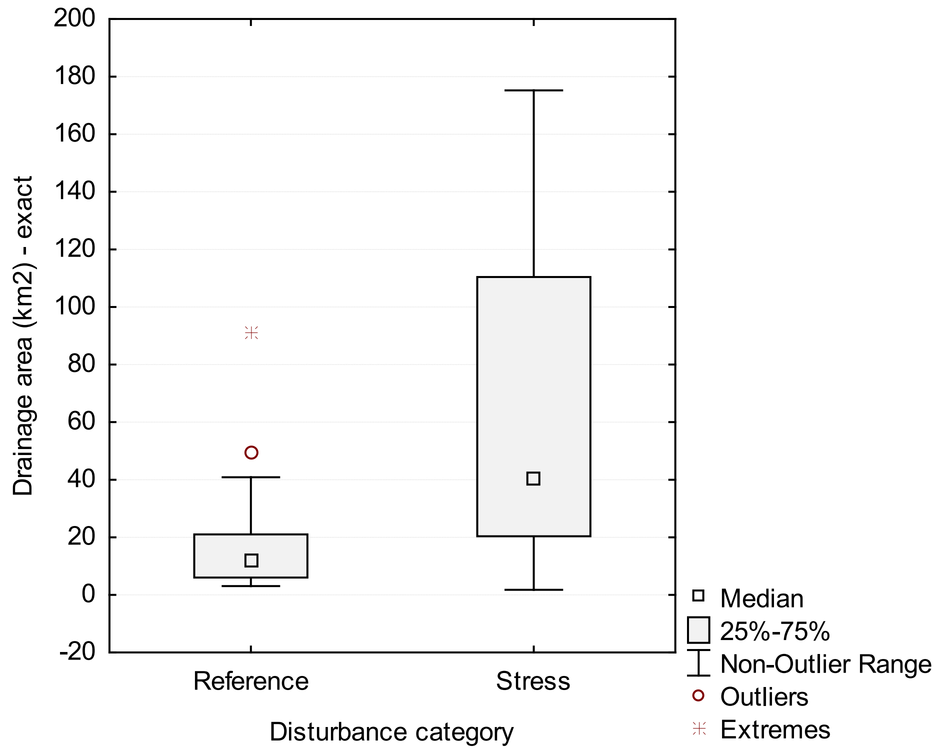
Appendix D

Characterization of reference vs. stressed sites

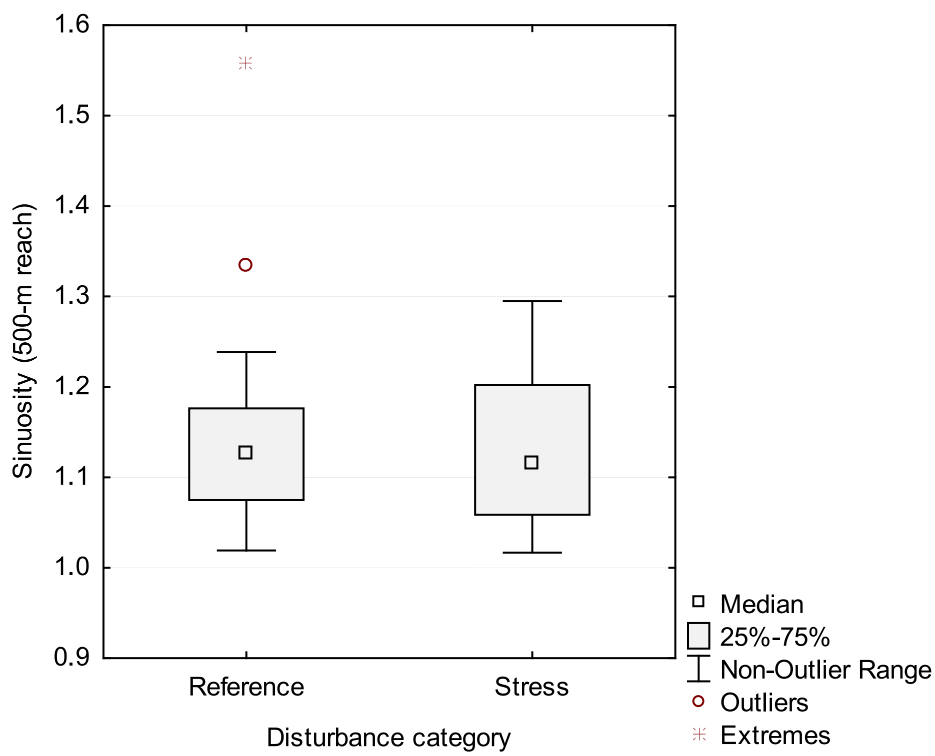
Natural and disturbance variables

Drainage area, sinuosity, slope, elevation, baseflow, temperature, precipitation, ICI, IWI, land cover statistics, RBP habitat assessment score, macroinvertebrate jab allocations, % sediment composition

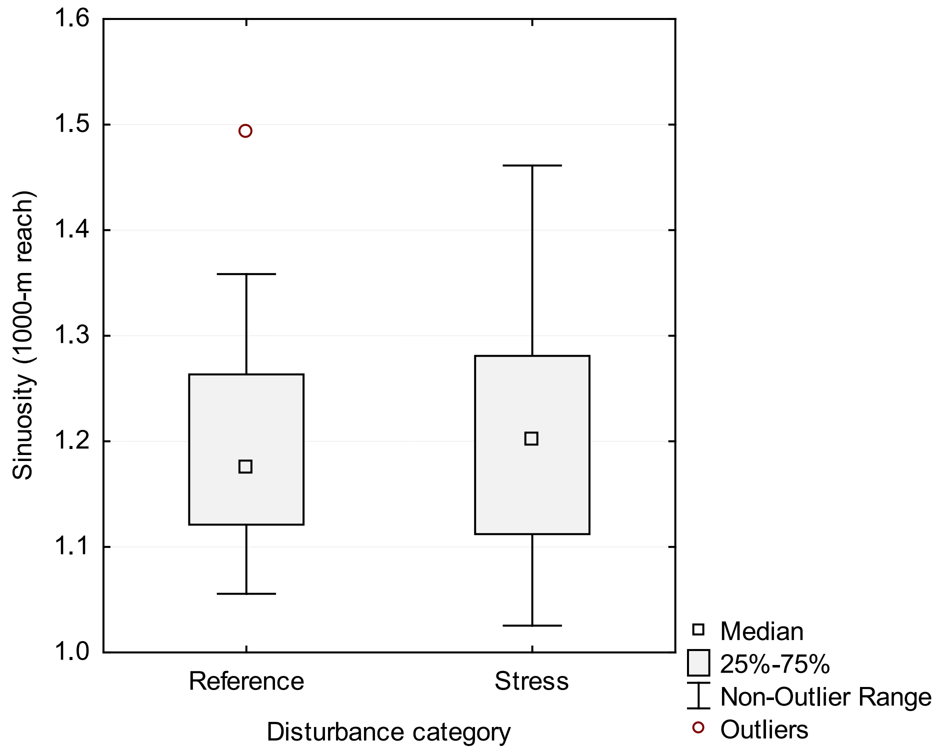
MassDEP & Tetra Tech/SNEP sites
26 reference sites, 23 stressed sites



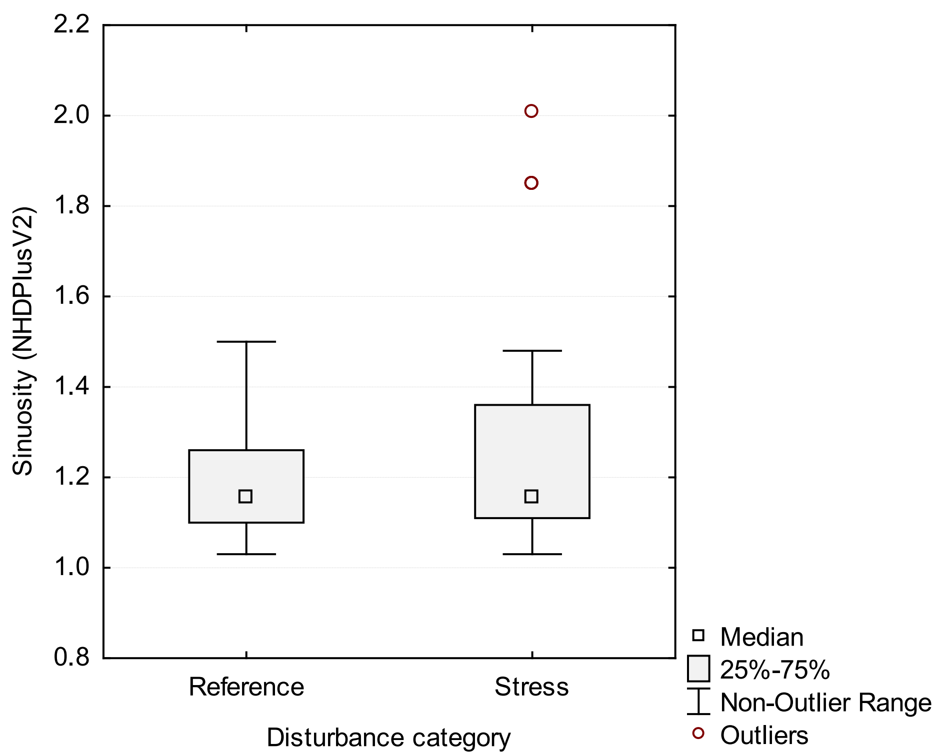
200
180
160
140
120
100
80
60
40
20
0
-20
Drainage area (km2) - exact
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Outliers
Extremes



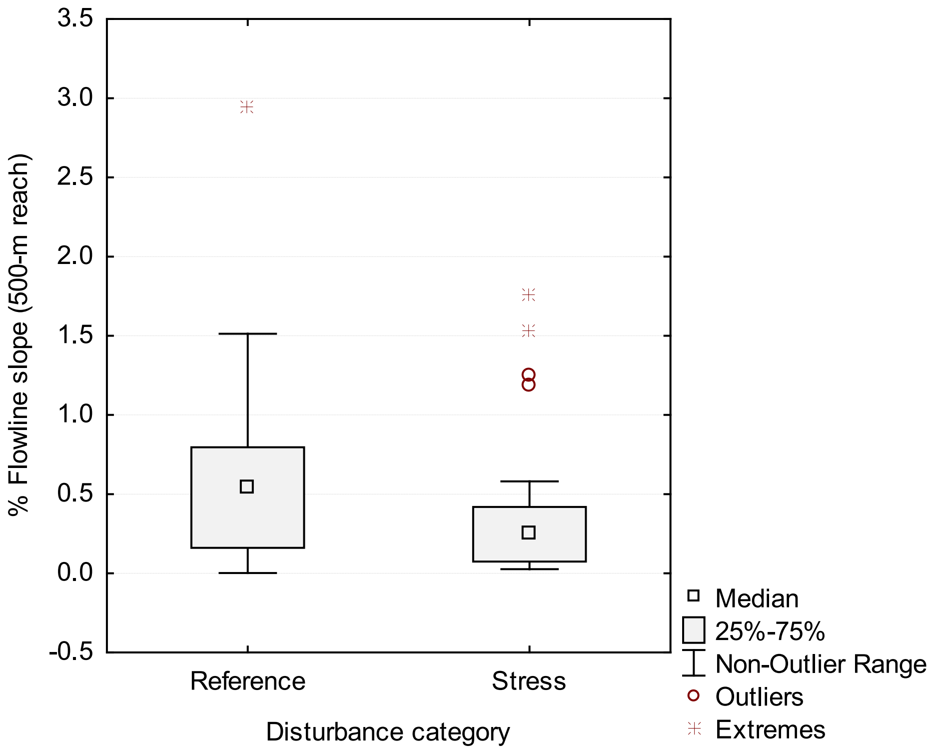
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
Sinuosity (500-m reach)
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Outliers
Extremes

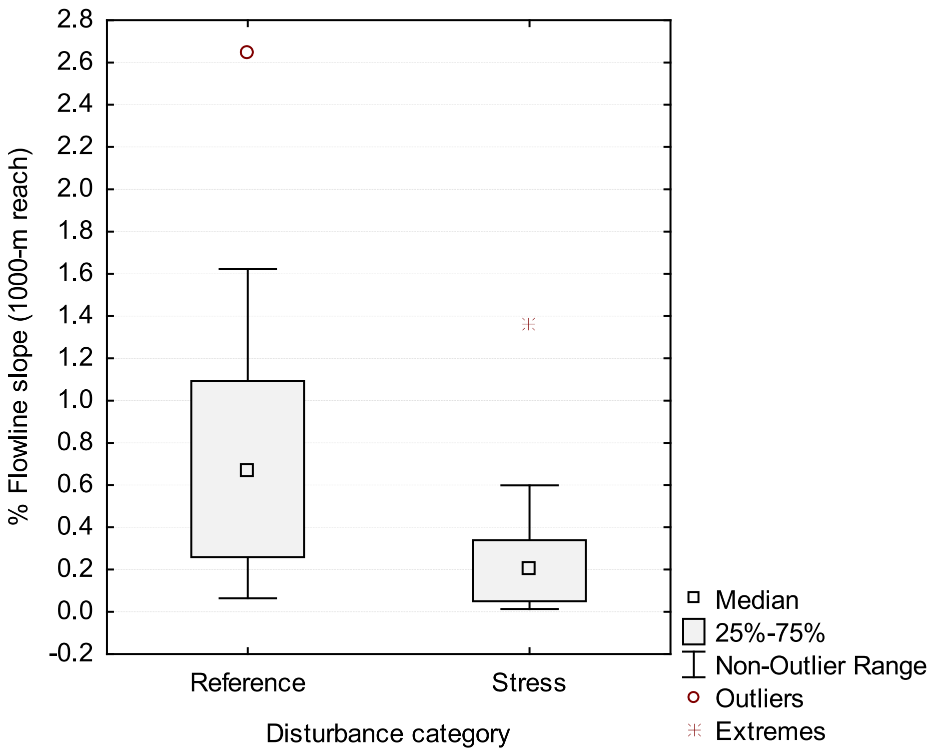


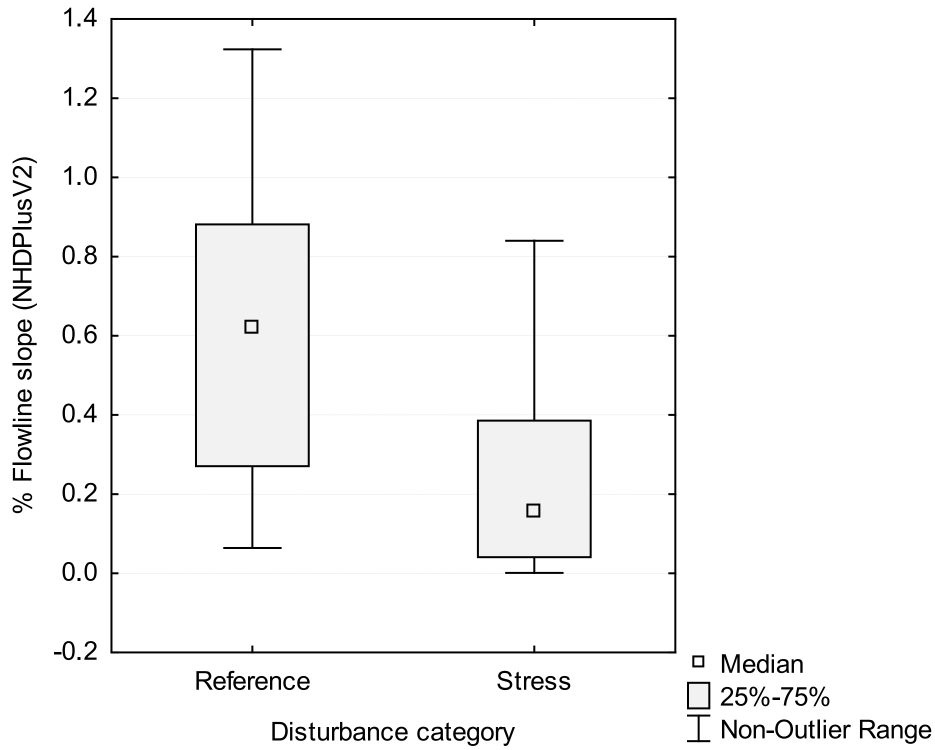
1.6
1.5
1.4
1.3
1.2
1.1
1.0
Sinuosity (1000-m reach)
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Outliers

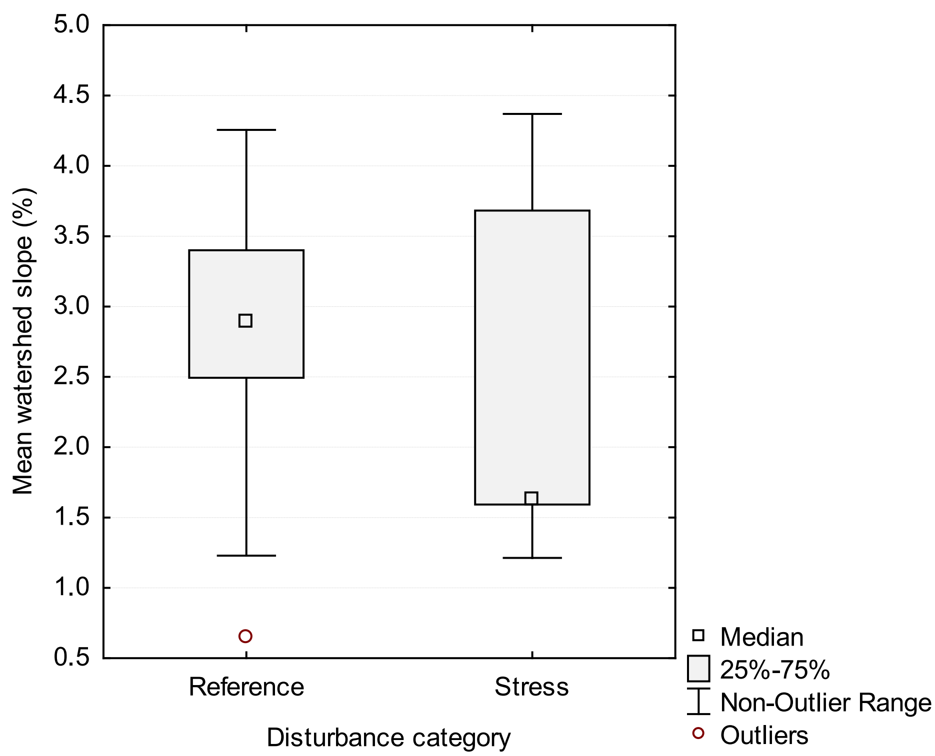


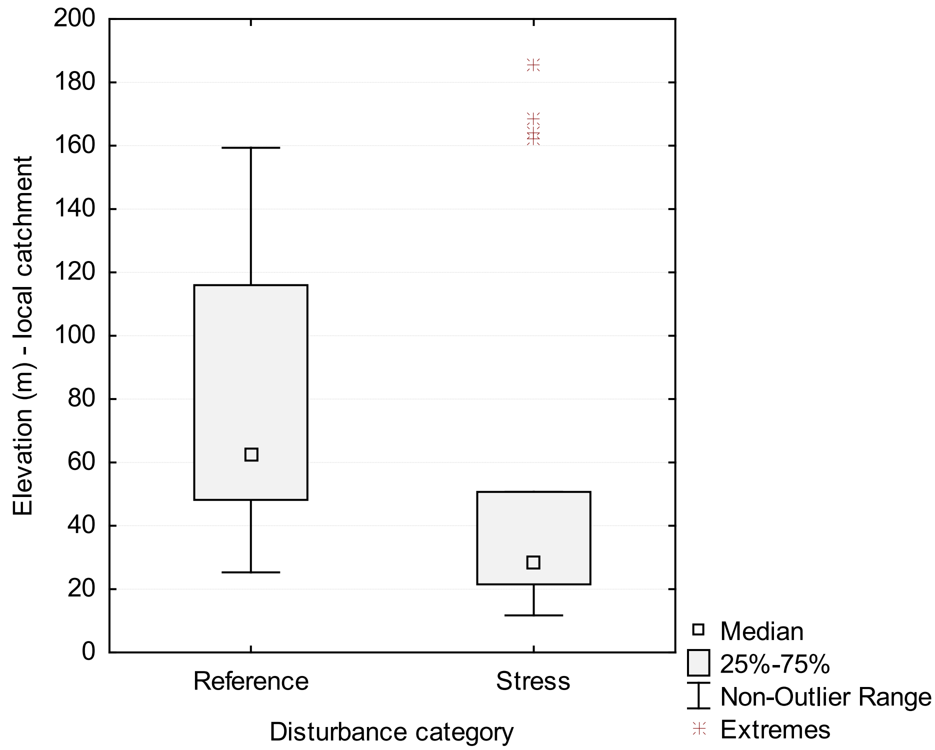
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
Sinuosity (NHDPlusV2)
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Outliers

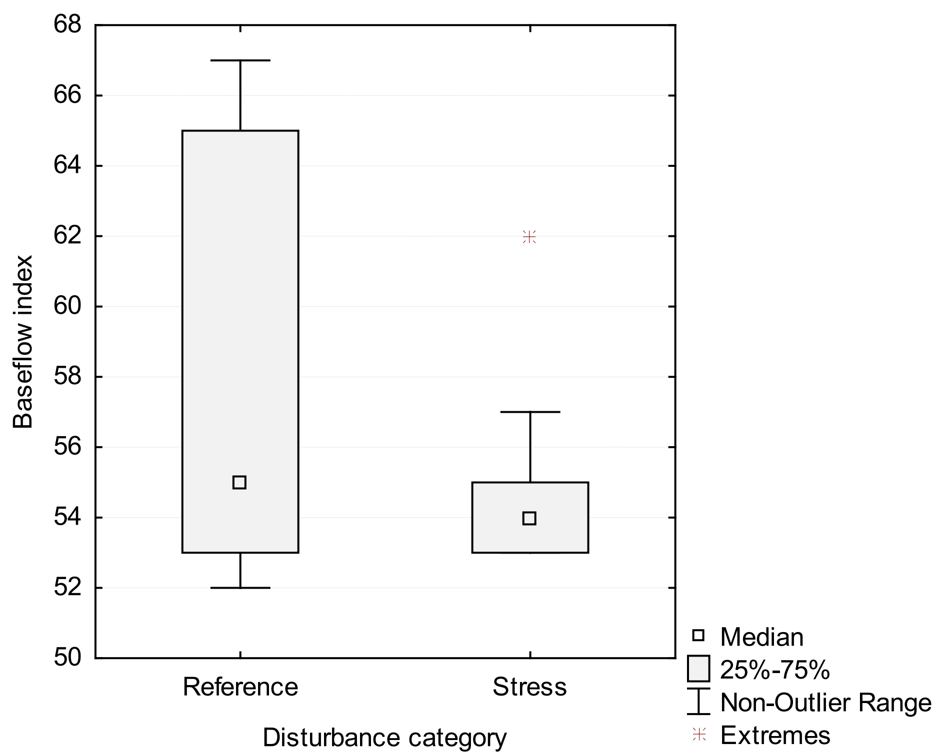

3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
% Flowline slope (500-m reach)
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Outliers
Extremes

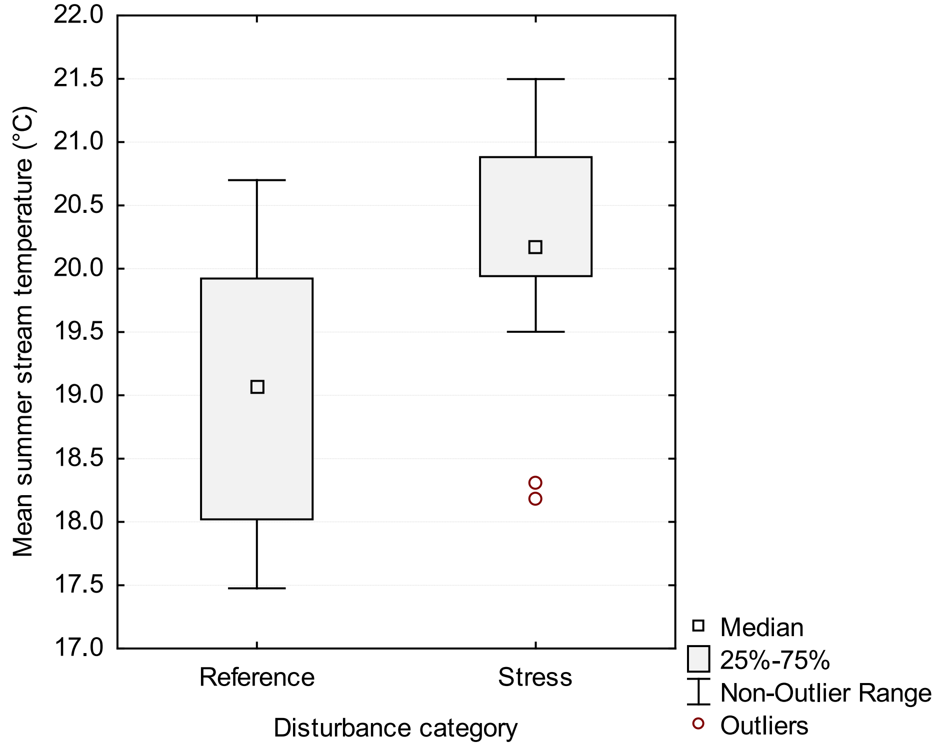

2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
% Flowline slope (1000-m reach)
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Outliers
Extremes

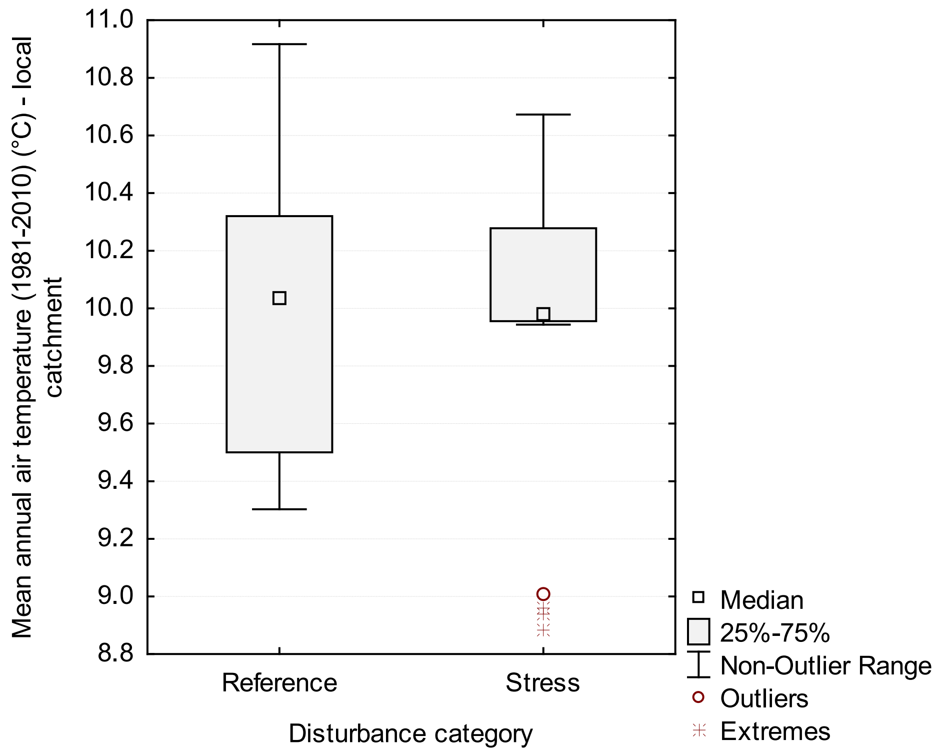

1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
% Flowline slope (NHDPlusV2)
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range

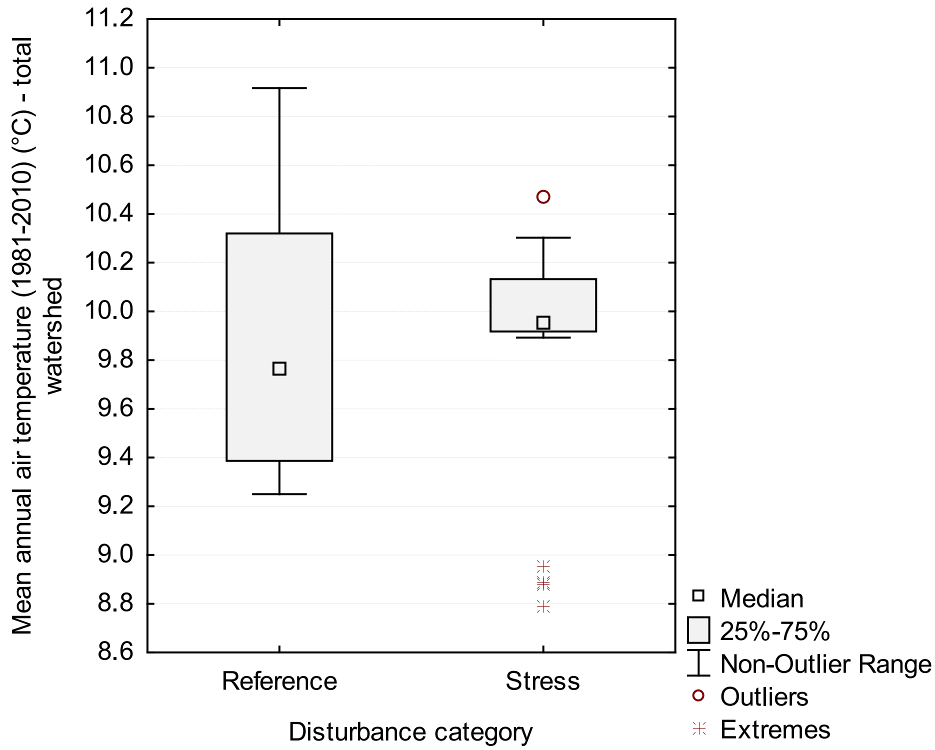

5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Mean watershed slope (%)
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Outliers

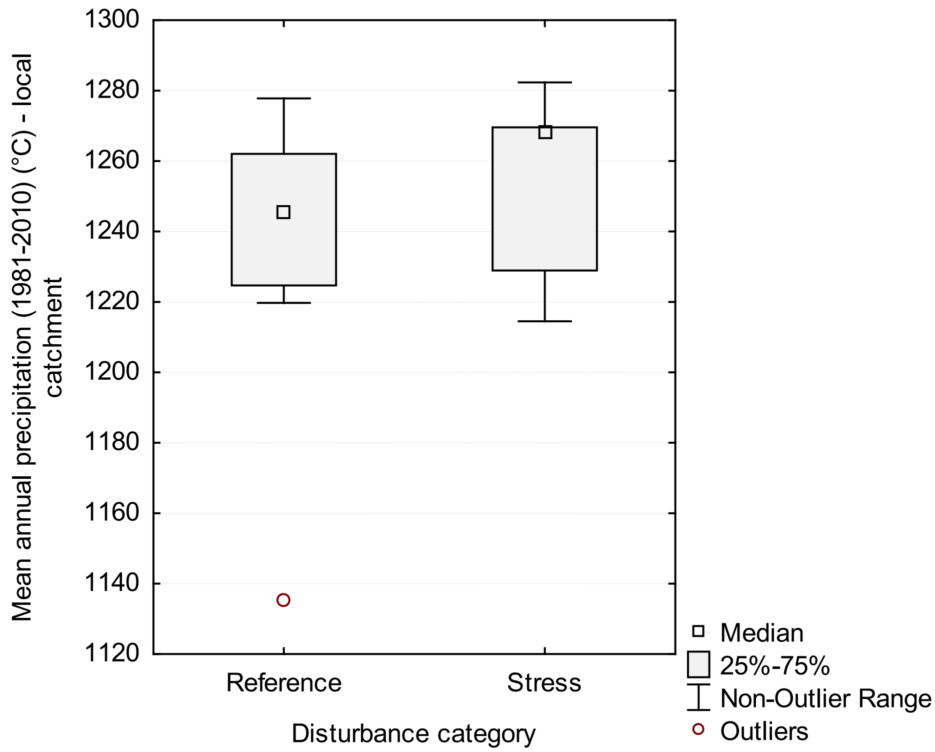

200
180
160
140
120
100
80
60
40
20
0
Elevation (m) - local catchment
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Extremes

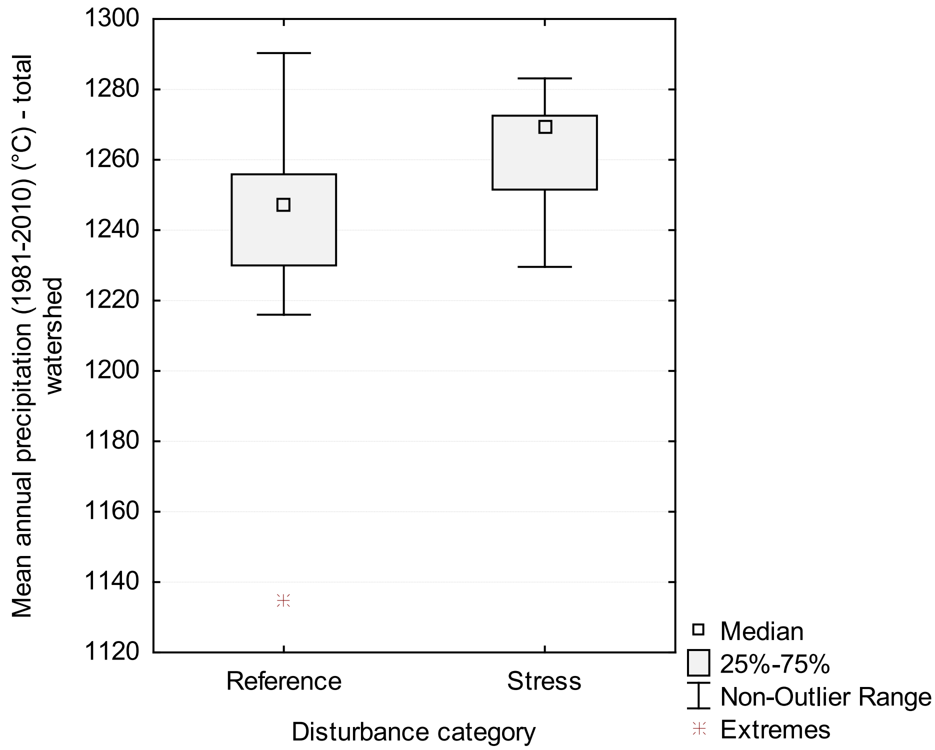

68
66
64
62
60
58
56
54
52
50
Baseflow index
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Extremes

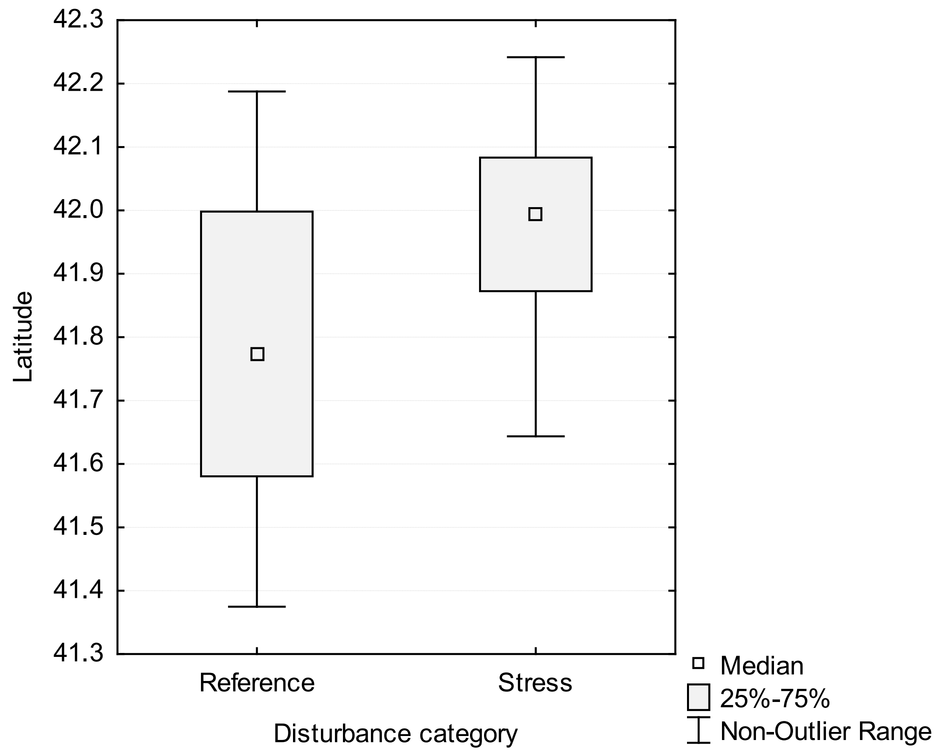

22.0
21.5
21.0
20.5
20.0
19.5
19.0
18.5
18.0
17.5
17.0
Mean summer stream temperature (°C)
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Outliers

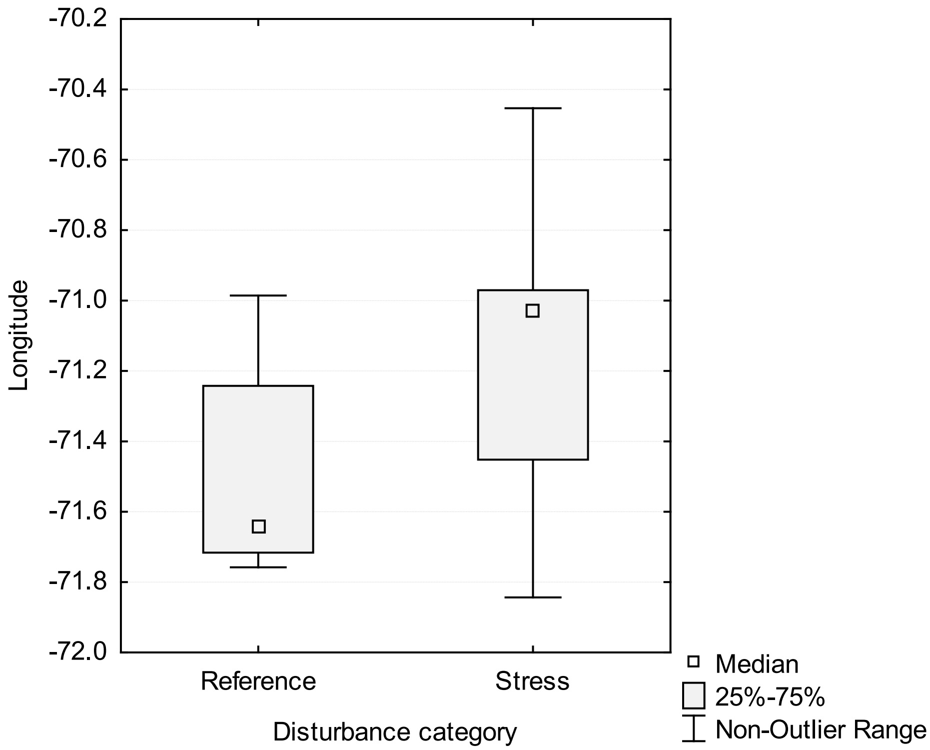

11.0
10.8
10.6
10.4
10.2
10.0
9.8
9.6
9.4
9.2
9.0
8.8
Mean annual air temperature (1981-2010) (°C) - local catchment
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Outliers
Extremes

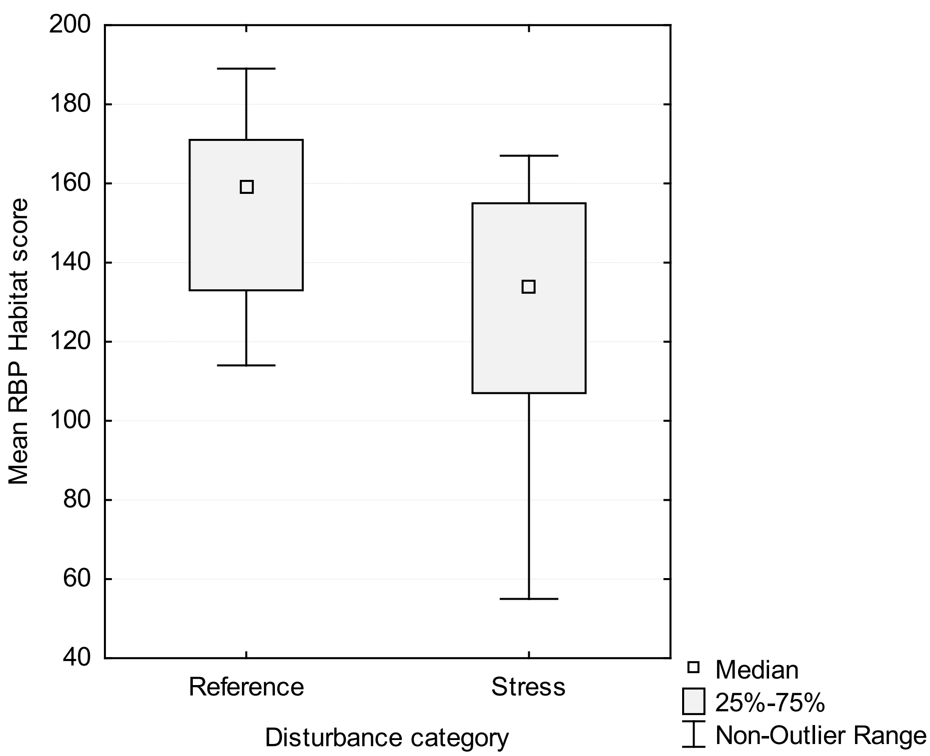

11.2
11.0
10.8
10.6
10.4
10.2
10.0
9.8
9.6
9.4
9.2
9.0
8.8
8.6
Mean annual air temperature (1981-2010) (°C) - total watershed
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Outliers
Extremes

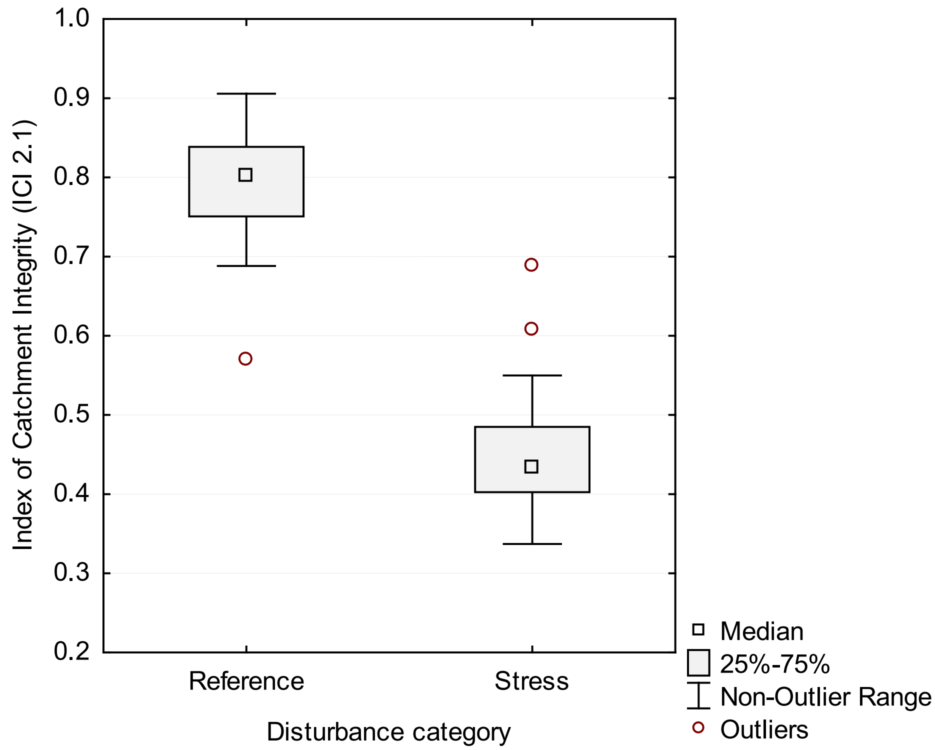

1300
1280
1260
1240
1220
1200
1180
1160
1140
1120
Mean annual precipitation (1981-2010) (°C) - local catchment
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Outliers

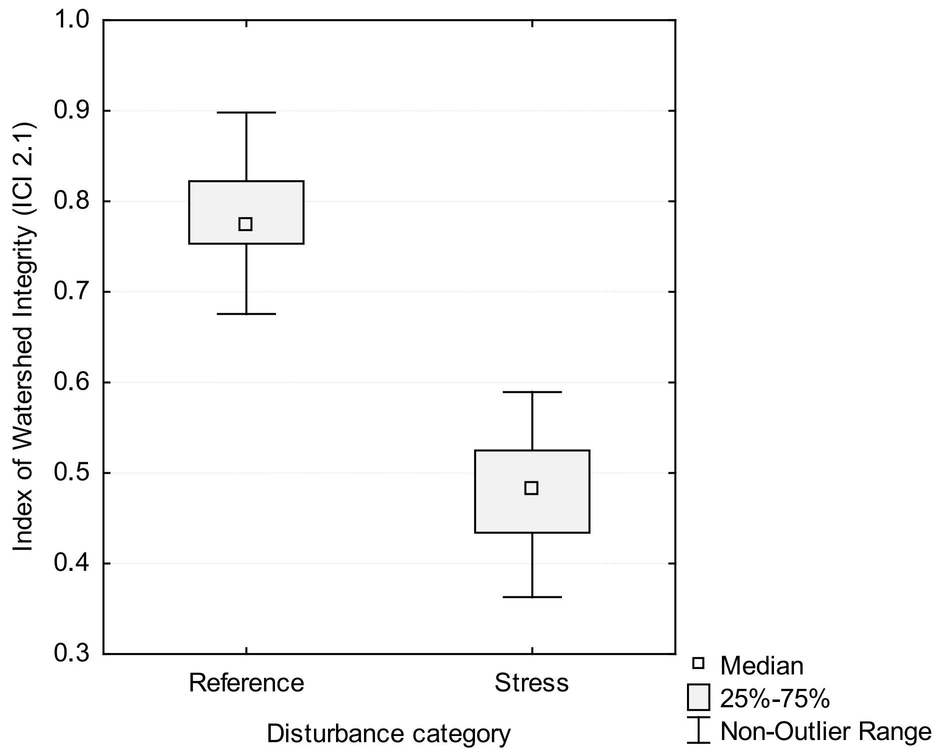

Mean annual precipitation (1981-2010) (°C) - total watershed
1300
1280
1260
1240
1220
1200
1180
1160
1140
1120
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Extremes

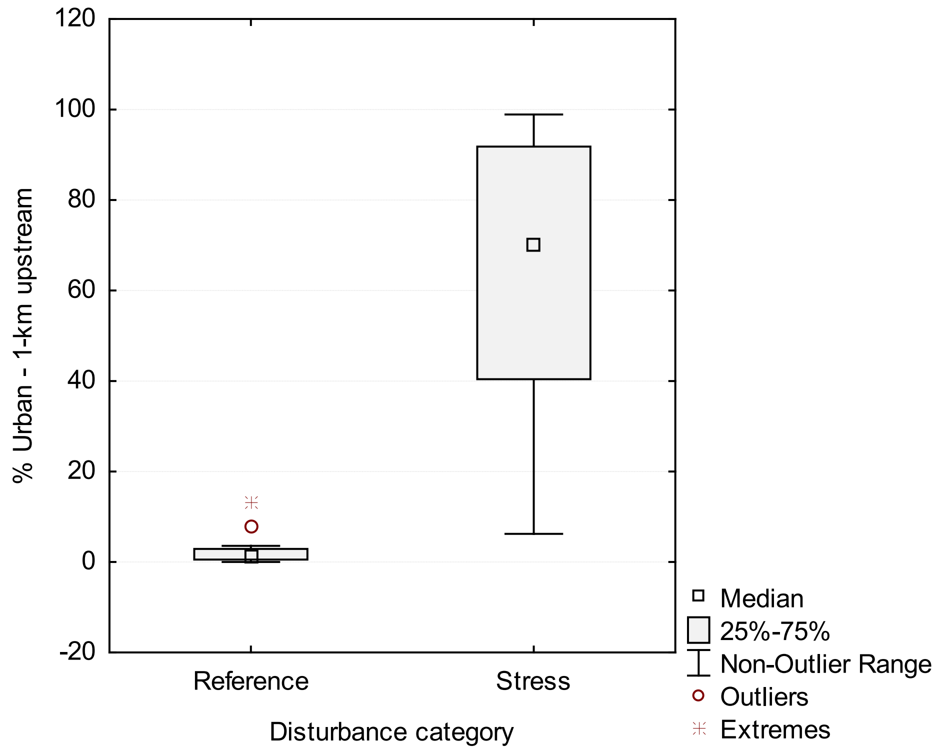

Latitude
42.3
42.2
42.1
42.0
41.9
41.8
41.7
41.6
41.5
41.4
41.3
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range

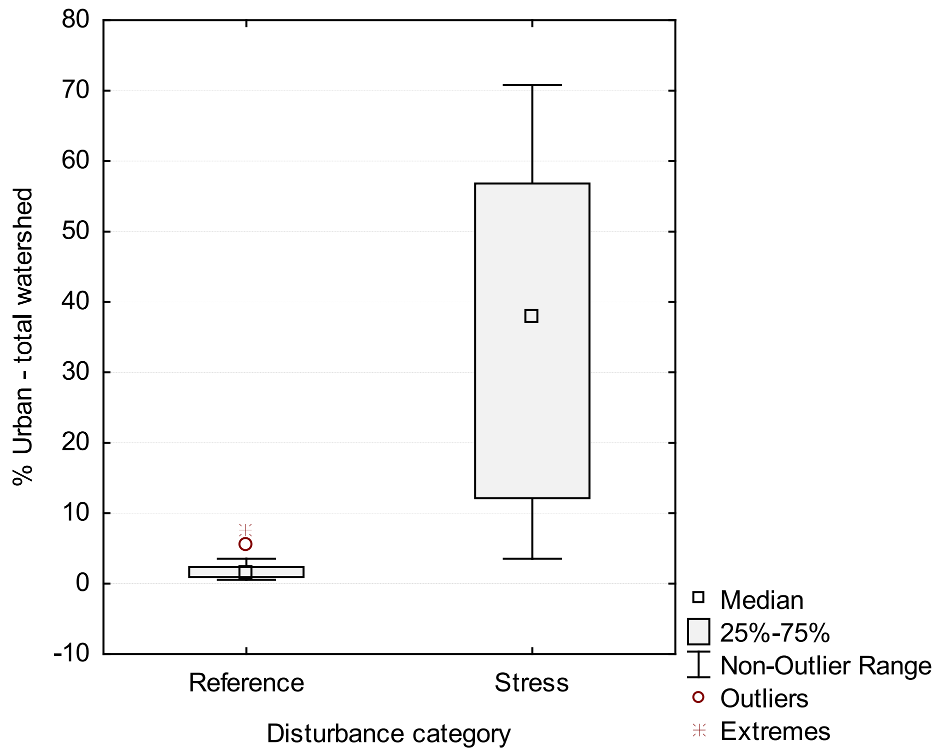

-70.2
-70.4
-70.6
-70.8
-71.0
-71.2
-71.4
-71.6
-71.8
-72.0
Longitude
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range

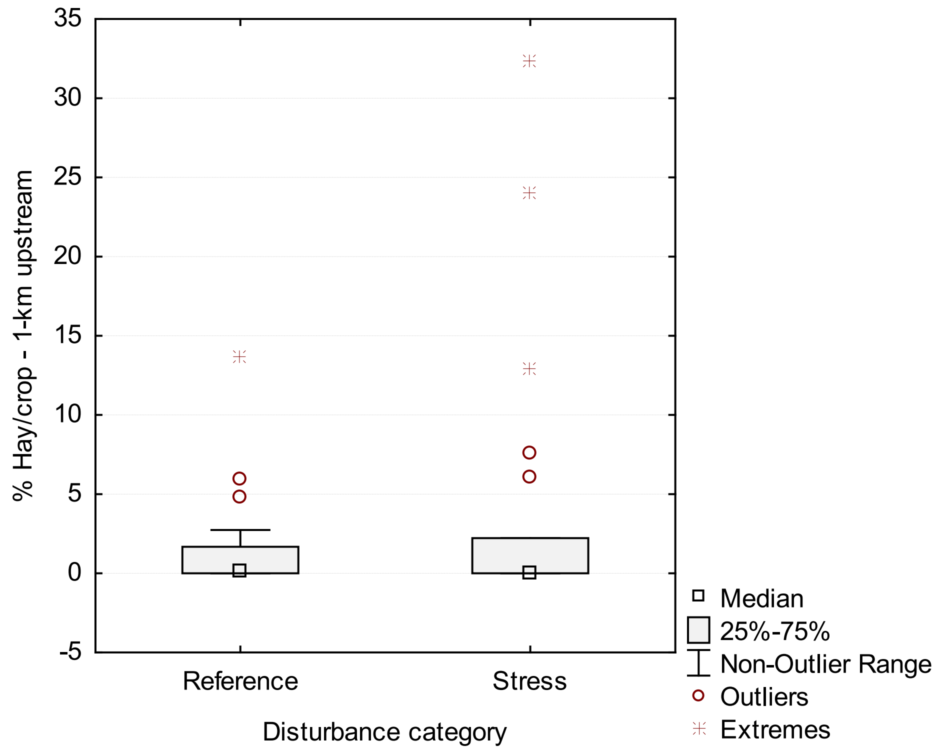

200
180
160
140
120
100
80
60
40
Mean RBP Habitat score
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range


1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
Index of Catchment Integrity (ICI 2.1)
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Outliers

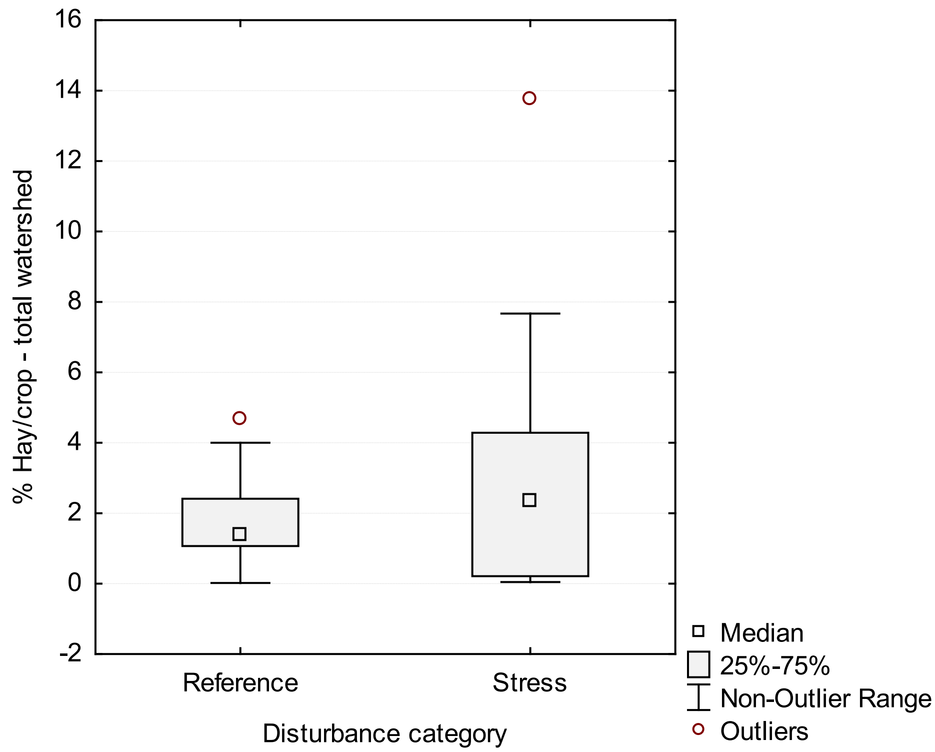

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
Index of Watershed Integrity (ICI 2.1)
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range


120
100
80
60
40
20
0
-20
% Urban - 1-km upstream
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Outliers
Extremes

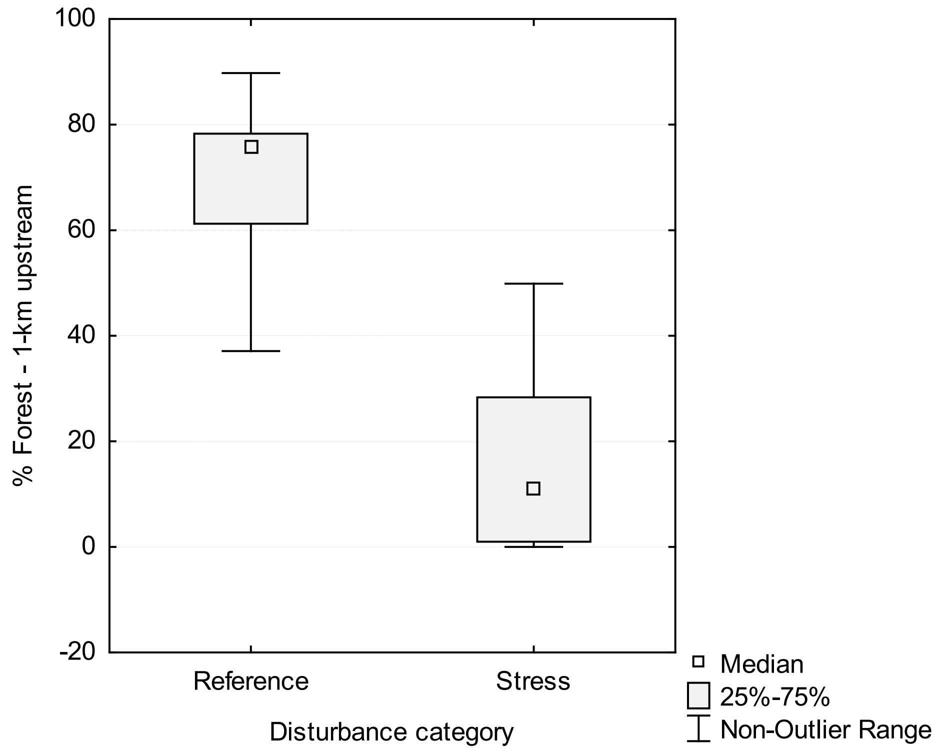

80
70
60
50
40
30
20
10
0
-10
% Urban - total watershed
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Outliers
Extremes



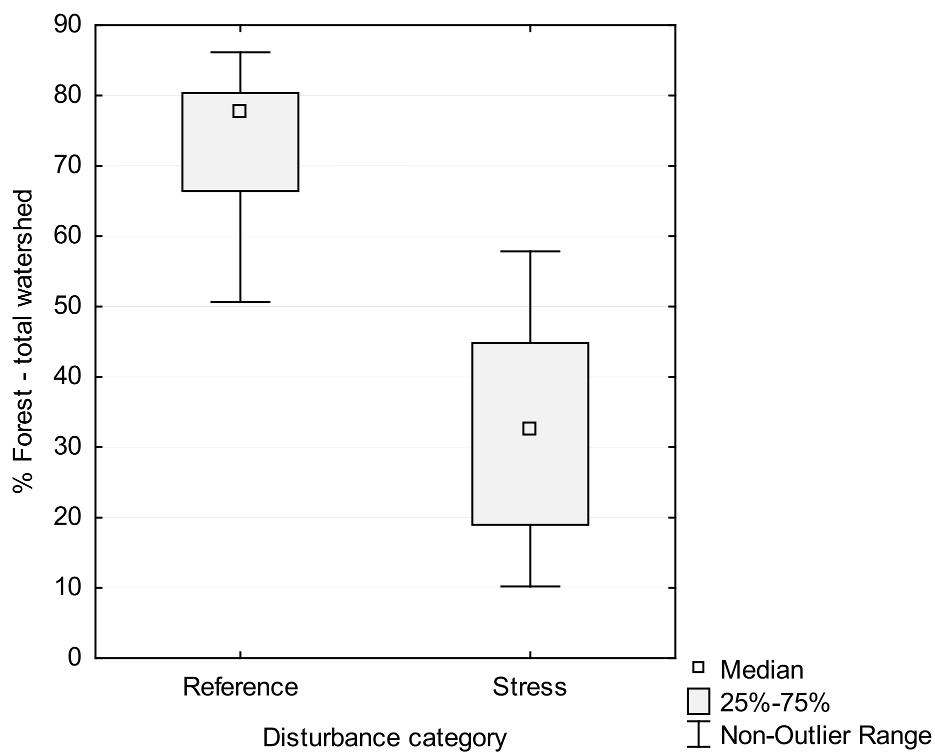
35
30
25
20
15
10
5
0
-5
% Hay/crop - 1-km upstream
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Outliers
Extremes



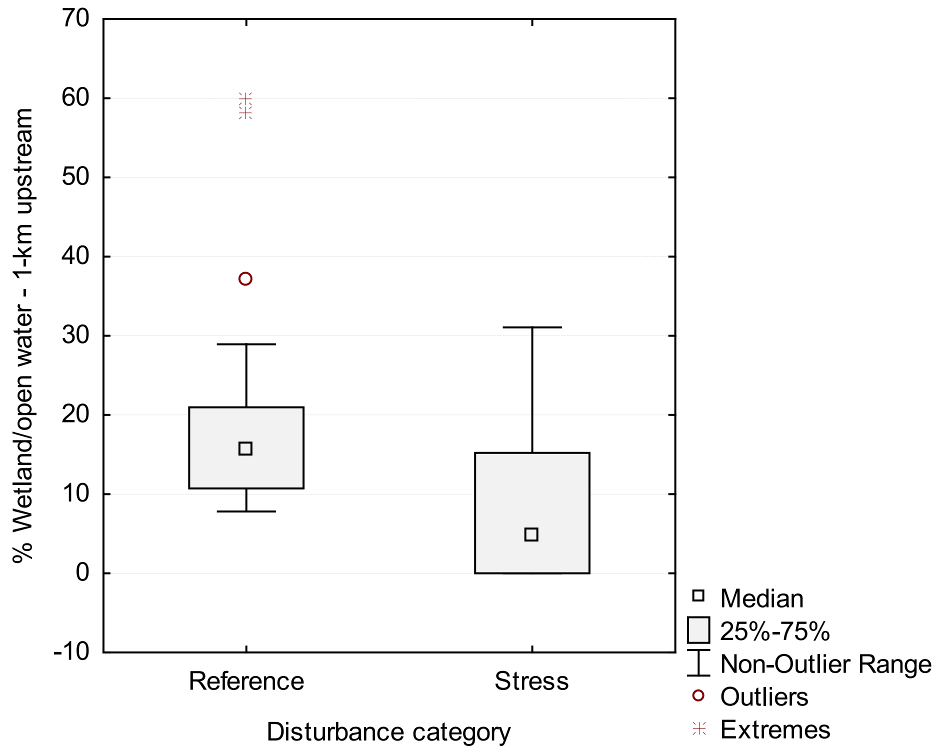
16
14
12
10
8
6
4
2
0
-2
% Hay/crop - total watershed
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Outliers

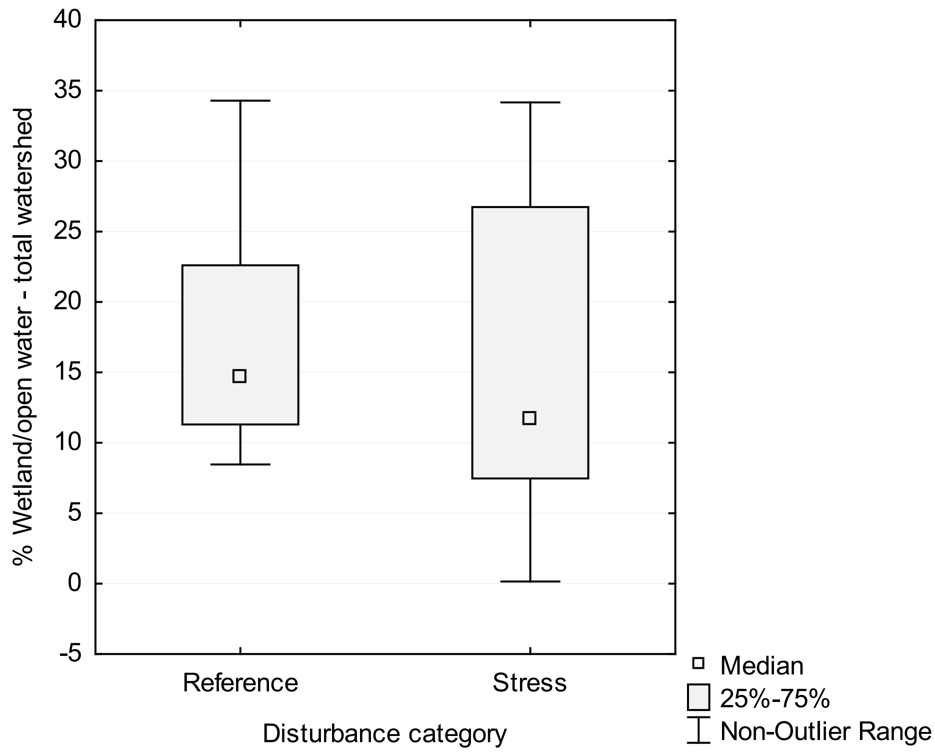


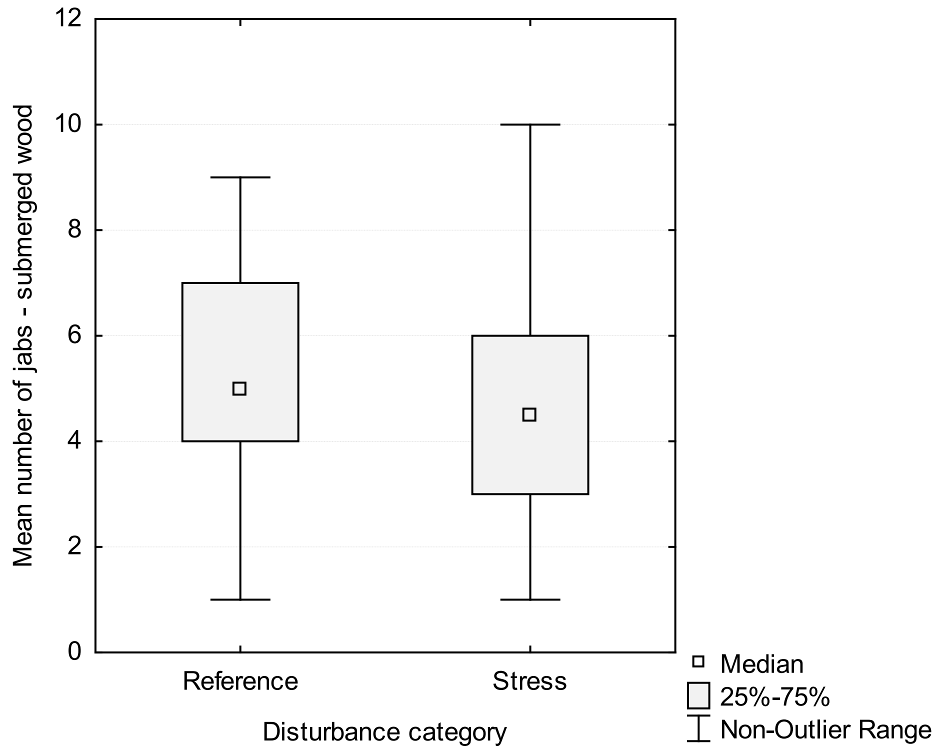
100
80
60
40
20
0
-20
% Forest - 1-km upstream
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range

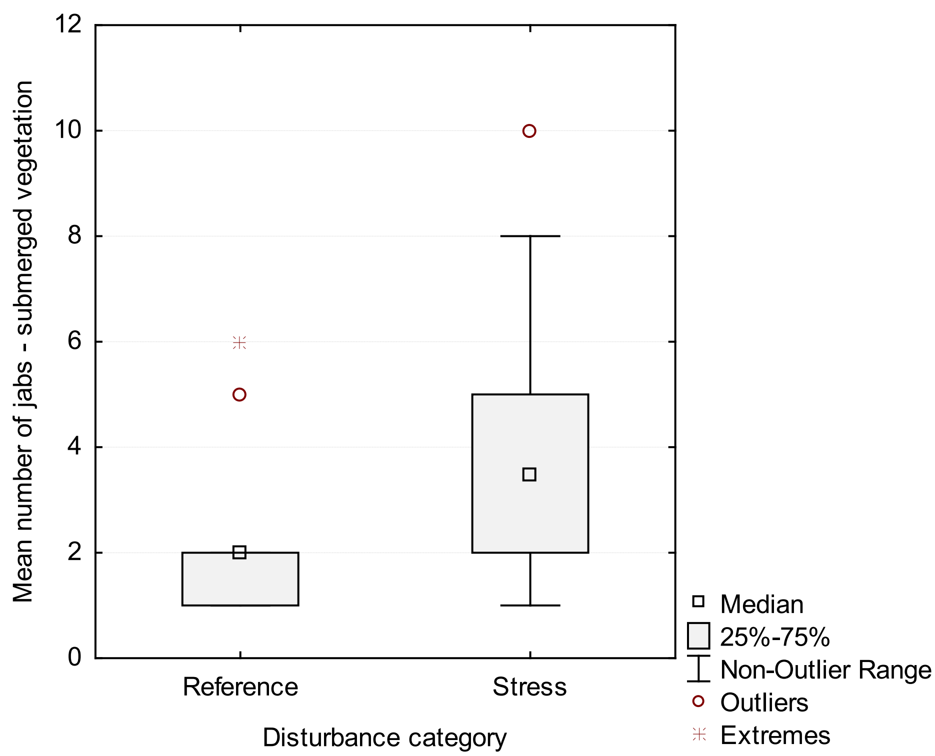


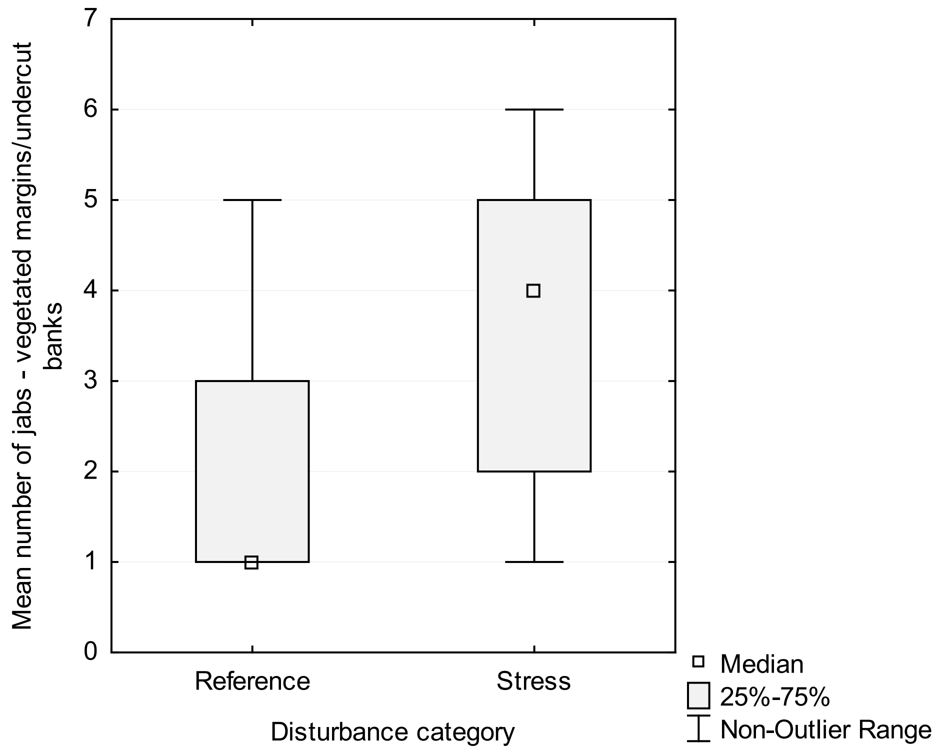
90
80
70
60
50
40
30
20
10
0
% Forest - total watershed
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range

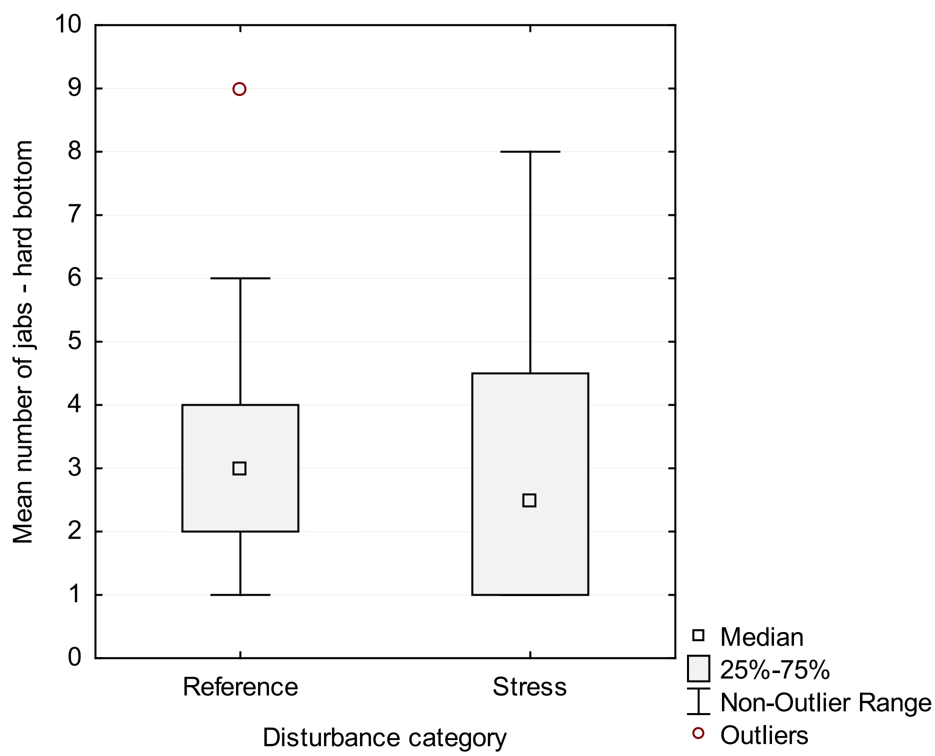

% Wetland/open water - 1-km upstream
70
60
50
40
30
20
10
0
-10
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Outliers
Extremes

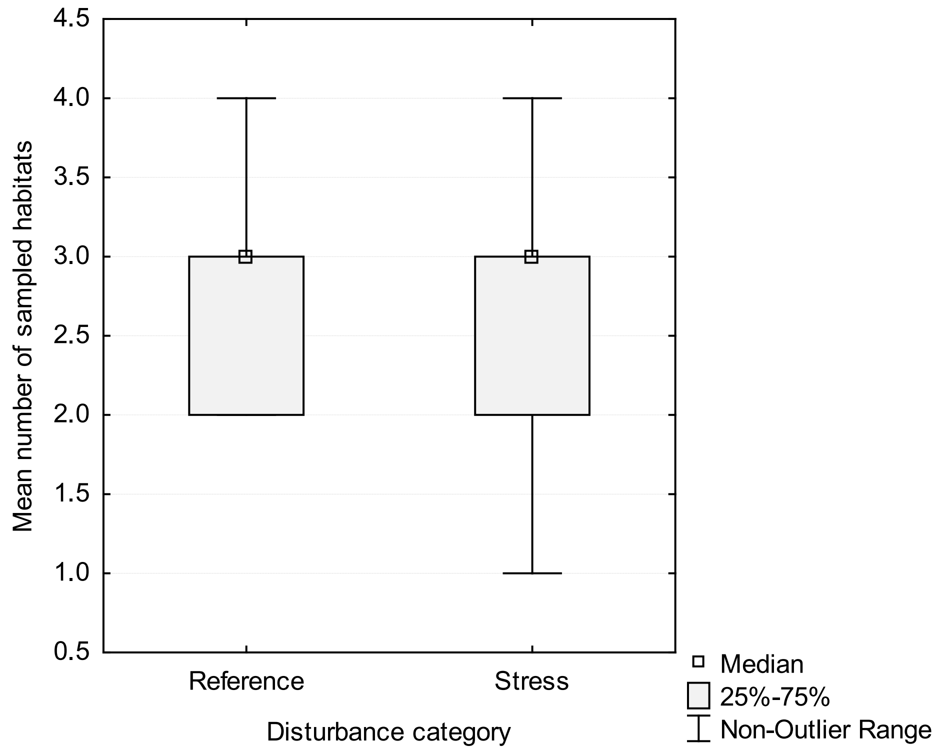

% Wetland/open water - total watershed
40
35
30
25
20
15
10
5
0
-5
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range

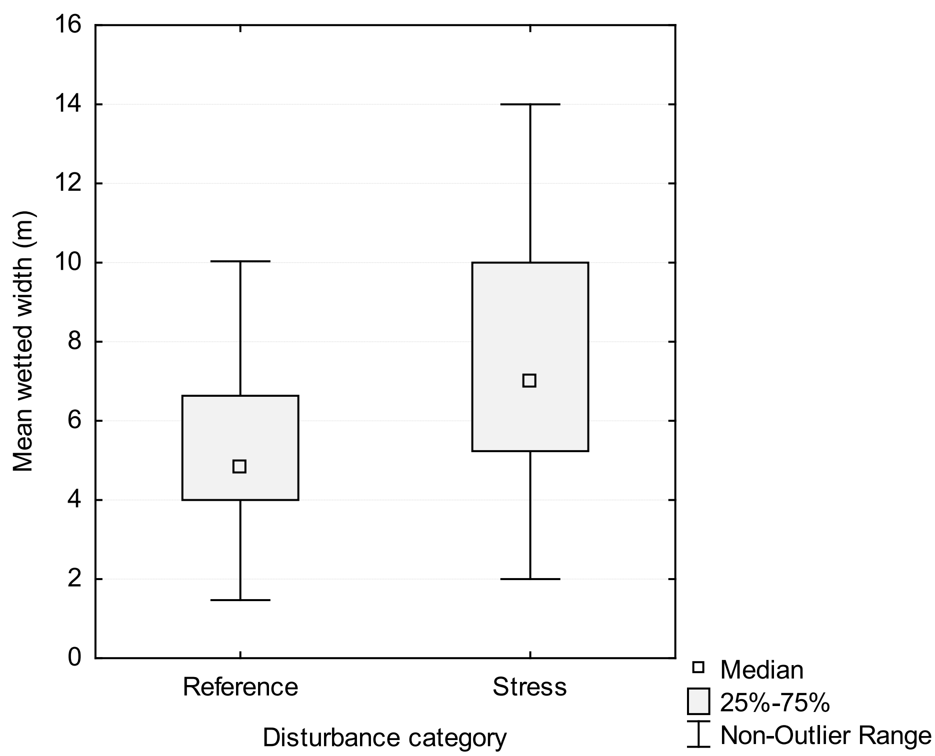

Mean number of jabs - submerged wood
12
10
8
6
4
2
0
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range

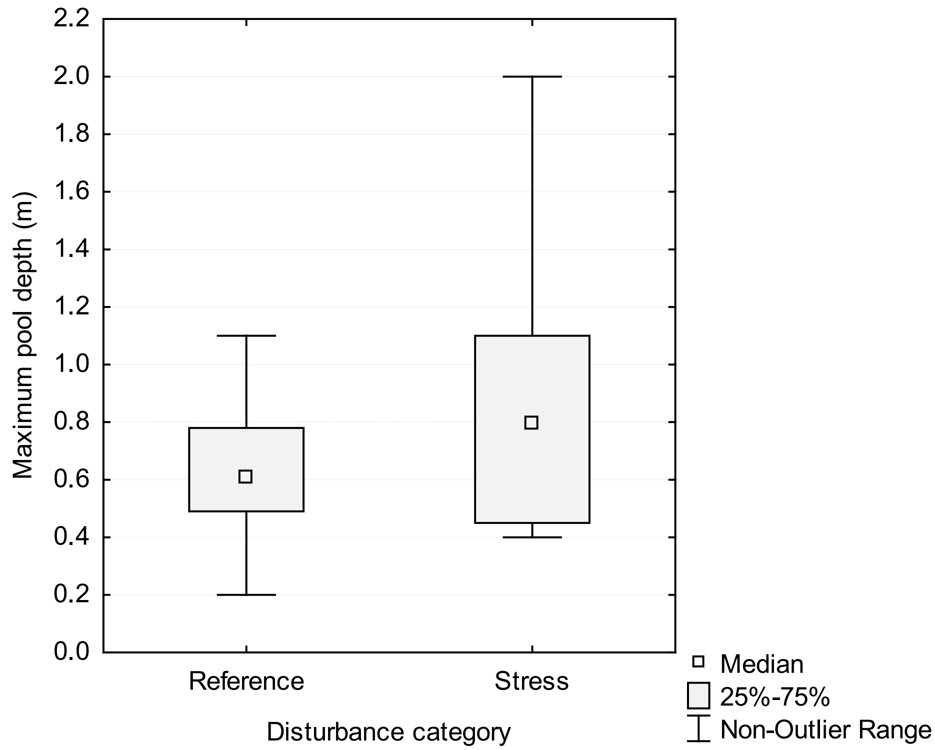

Mean number of jabs - submerged vegetation
12
10
8
6
4
2
0
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Outliers
Extremes

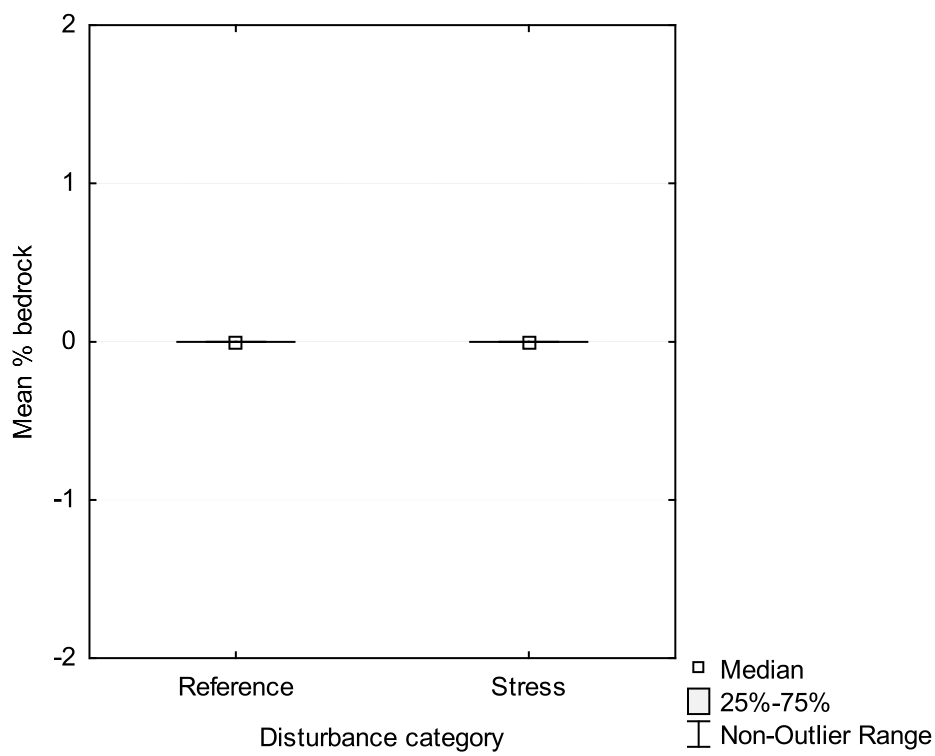

Mean number of jabs - vegetated margins/undercut banks
0
1
2
3
4
5
6
7
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range

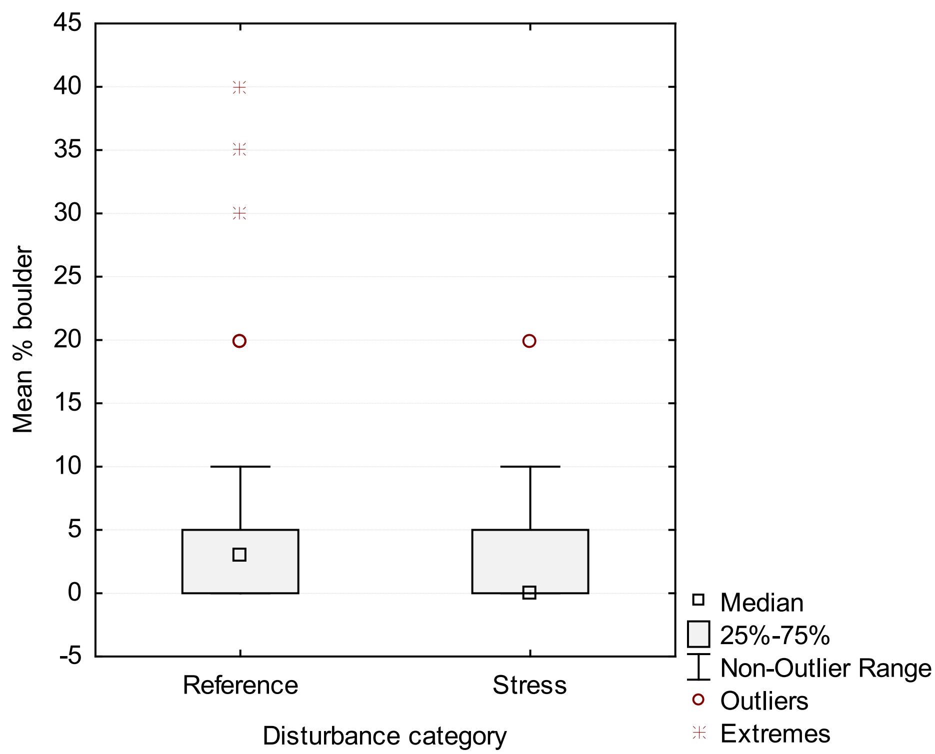

Mean number of jabs - hard bottom
0
1
2
3
4
5
6
7
8
9
10
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Outliers

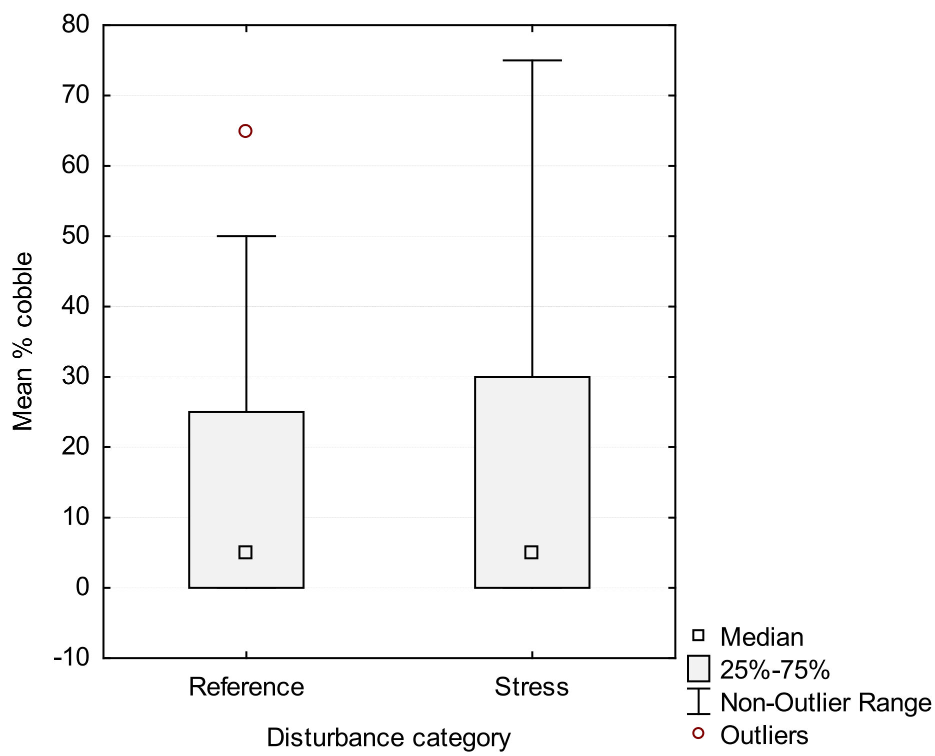

4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Mean number of sampled habitats
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range

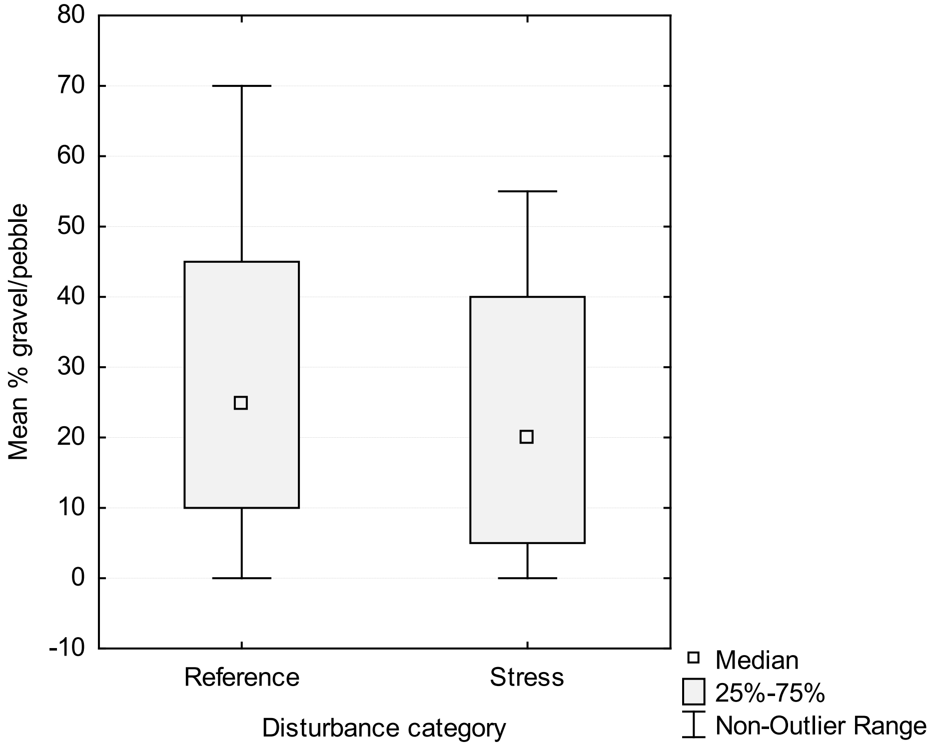

16
14
12
10
8
6
4
2
0
Mean wetted width (m)
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range

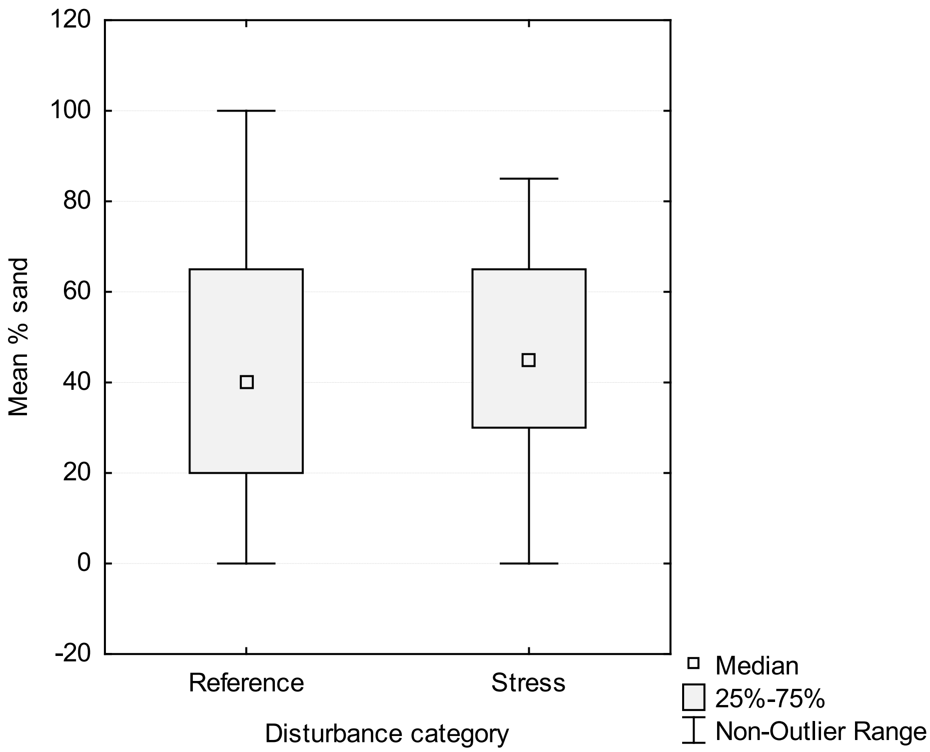

2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Maximum pool depth (m)
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range

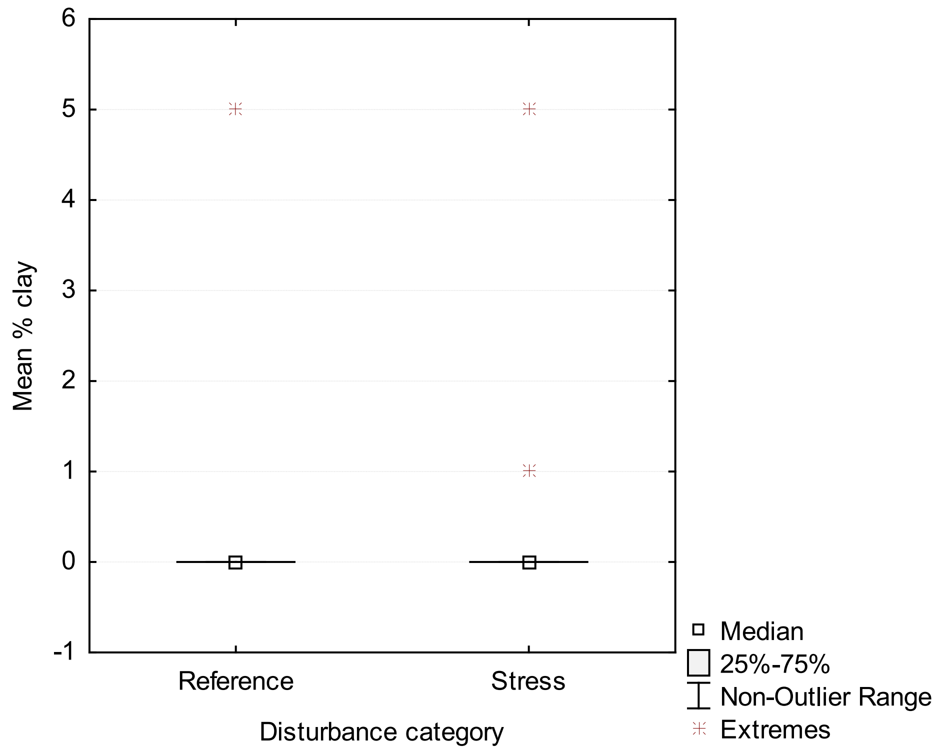

2
1
0
-1
-2
Mean % bedrock
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range

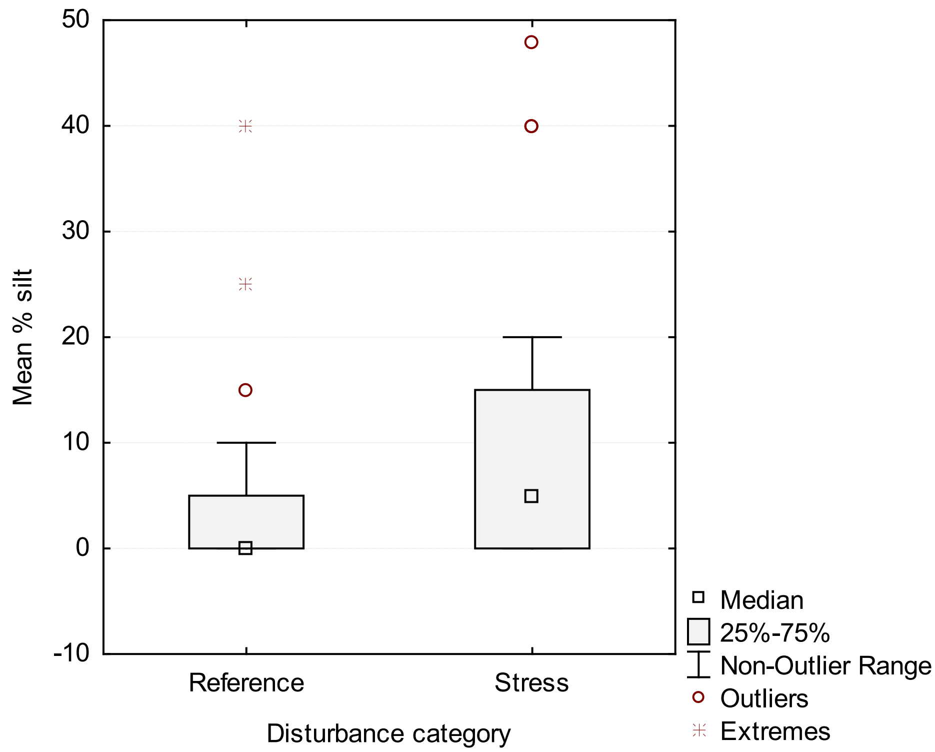

Mean % boulder
45
40
35
30
25
20
15
10
5
0
-5
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Outliers
Extremes

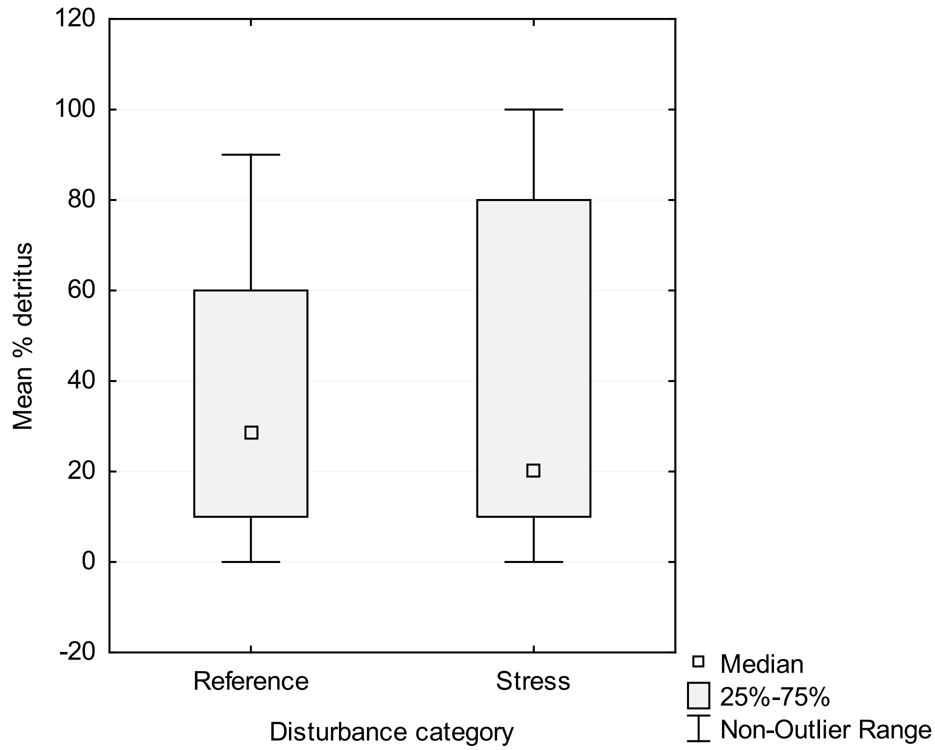

Mean % cobble
80
70
60
50
40
30
20
10
0
-10
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Outliers

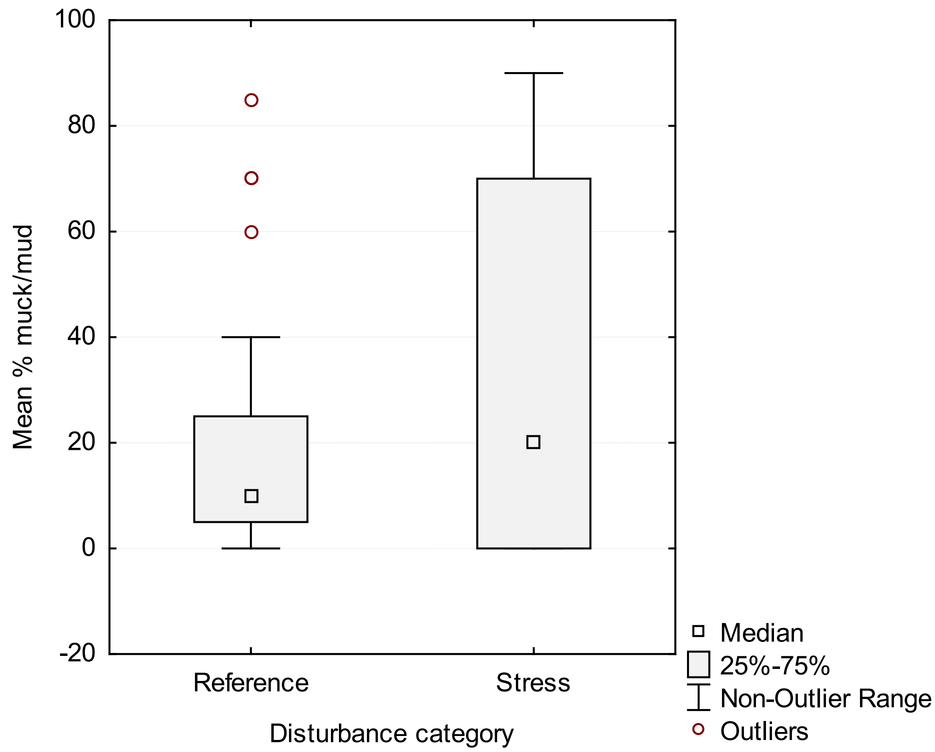

80
70
60
50
40
30
20
10
0
-10
Mean % gravel/pebble
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range

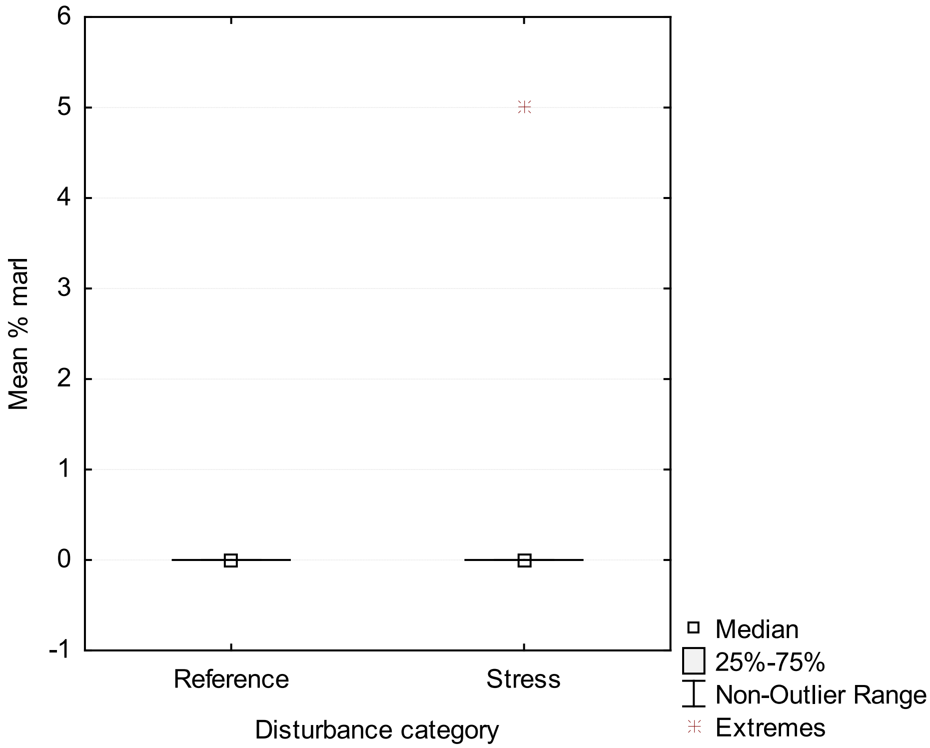

120
100
80
60
40
20
0
-20
Mean % sand
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range


6
5
4
3
2
1
0
-1
Mean % clay
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Extremes


50
40
30
20
10
0
-10
Mean % silt
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Outliers
Extremes


120
100
80
60
40
20
0
-20
Mean % detritus
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range

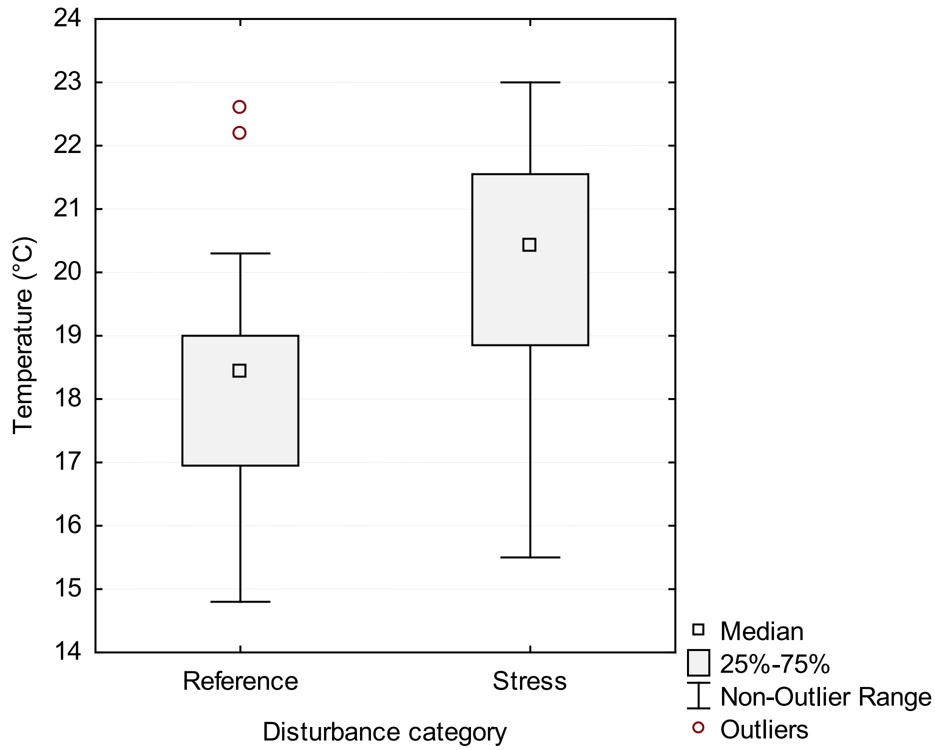

100
80
60
40
20
0
-20
Mean % muck/mud
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Outliers

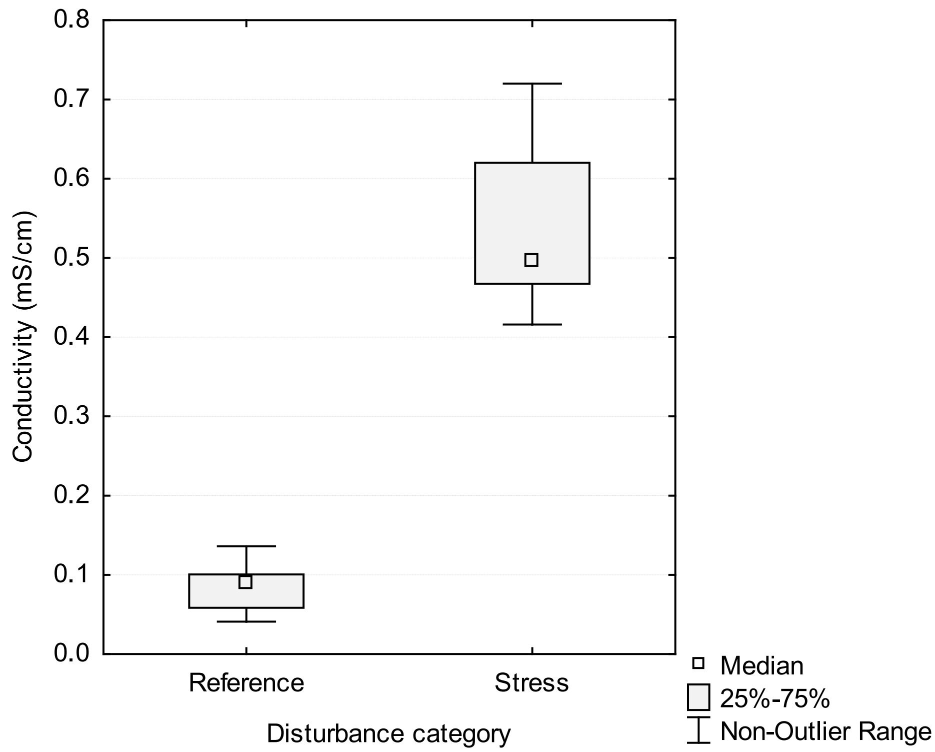


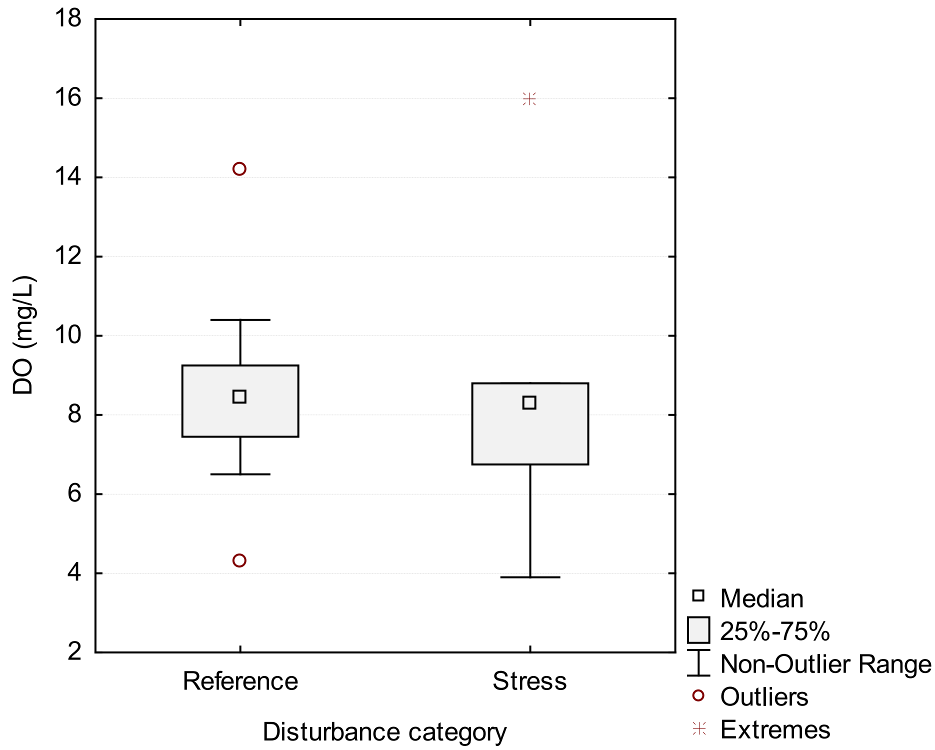
Mean % marl
6
5
4
3
2
1
0
-1
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Extremes

In situ water quality

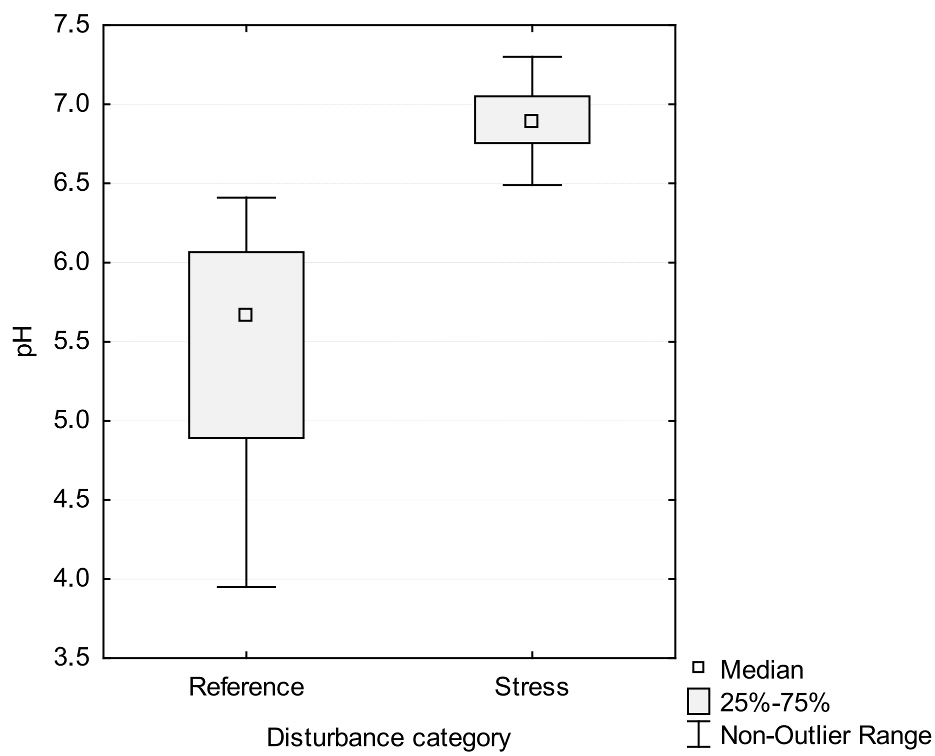
Tetra Tech/SNEP sites only
20 reference sites, 8 stressed sites


24
23
22
21
20
19
18
17
16
15
14
Temperature (°C)
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Outliers


0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Conductivity (mS/cm)
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range



18
16
14
12
10
8
6
4
2
DO (mg/L)
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range
Outliers
Extremes



7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
pH
Reference
Stress
Disturbance category
Median
25%-75%
Non-Outlier Range

Appendix F

SNEP IBI Metric Response Mechanisms

Metrics in the SNEP low gradient IBI were selected for inclusion in the index based on performance statistics (DE and Z-score), response mechanisms, and metric diversity (metrics representative of many metric categories). The recommended IBI consists of metrics representative of relative taxonomic richness, community composition, pollution tolerance, functional feeding groups, and voltinism. The IBI input metrics (Table F1) have comprehensible mechanisms of response to increasing environmental stress, as described below. Interpretable metrics provide easier interpretation of assemblage structure in relation to index scores. Taxa attributes related to the metrics are in Attachment B.

Table F1. Metrics included in the low gradient IBI.

Metric (abbrev)
% Plecoptera, Odonata, Ephemeroptera, and Trichoptera (POET) taxa (pt_POET)
% Predator taxa (pt_ffg_pred)
% Non-insect taxa (pt_NonIns)
% Odonata, Ephemeroptera, and Trichoptera (OET) individuals (pi_OET)
% Tolerant taxa (pt_tv_toler)
% Semivoltine taxa (pt_volt_semi)

% Non-insect taxa (pt_nonIns)

Description: Of all taxa, the percentage of taxa that are non-insects

Taxa richness generally decreases with increasing stress, as the sensitive and specialist taxa emigrate or perish when exposed to intolerable conditions such as pollution, greater sedimentation, or reduced food quality. Non-insects (primarily gastropods, bivalves, crustaceans, and worms) can be tolerant or take advantage of stresses, and therefore, an increase in relative richness indicates the presence of disturbance. Relative richness of non-insects can increase either when non-insect taxa increase or when insect taxa decrease.

Metric Category: Relative Richness

Trend: Expected to increase with stress and increases in the SNEP dataset.

References: Barbour et al. 1999; Yuan and Norton 2003

% POET taxa (Plecoptera, Odonata, Ephemeroptera, and Trichoptera) (pt_POET)

Description: Of all taxa, the percentage of taxa that are in the insect orders Plecoptera (stoneflies), Odonata (dragonflies and damselflies), Ephemeroptera (mayflies), and Trichoptera (caddisflies)

In riffle dominated streams, EPT taxa are generally sensitive to environmental degradation such as reduced dissolved oxygen, unstable substrates, reduced food quality, and contamination due to heavy metals and other pollutants. EPT are also sensitive in low gradient streams and Odonata (dragonflies) can be a fourth sensitive insect order. As environmental conditions become worse, the sensitive and specialist taxa of these insect orders will emigrate or perish.

Metric Category: Relative Richness

Trend: Expected to decrease with stress and decreases in the SNEP dataset.

References: Angradi 1999; Barbour et al. 1999; Yuan and Norton 2003; Hutchens et al. 2009; Steele 2013; Onana et al. 2019; Gomez-Tolosa et al. 2020

% OET individuals (Percent of Odonata, Ephemeroptera, and Trichoptera individuals) (pi_OET)

Description: Of all individuals, the percentage of individuals that are in the insect orders Odonata (dragonflies and damselflies), Ephemeroptera (mayflies), and Trichoptera (caddisflies)

The stressor mechanisms described for % POET taxa also affect the relative abundance of sensitive insect individuals in a stream. Plecoptera (stoneflies) are more meaningful as a presence/absence signal than they are as a relative abundance signal because they are usually not abundant in low gradient streams. Therefore, this metric does not include stoneflies. The sensitive and specialist individuals of the dragonfly, mayfly, and caddisfly insect orders emigrate or perish with increasing stress.

Metric Category: Composition

Trend: Expected to decrease with stress and decreases in the Michigan dataset.

References: Angradi 1999; Barbour et al. 1999; Yuan and Norton 2003; Hutchens et al. 2009; Steele 2013; Onana et al. 2019; Gomez-Tolosa et al. 2020

% Predator taxa (Percent taxa of the predator (PR) Functional Feeding Group) (pt_ffg_pred)

Description: Of all taxa, the percentage of taxa that consume other organisms using different strategies to capture them

Predators employ a diversity of strategies for capturing prey, including modified mouth parts and behavior. Some species of invertebrates are predators in both the larval and adult stages of their life.

Metric Category: Functional Feeding Groups

Trend: Expected to decrease with stress and decreases in the SNEP dataset.

References: Kerans and Karr 1994; Merritt et al. 2008; Hutchens et al. 2009; Xu et al. 2014; Lan Fu et al. 2016;

% Tolerant taxa (Percent tolerant taxa with tolerance value ≥ 7) (pt_tv_toler)

Description: Of all taxa, the percentage of taxa that are relatively tolerant to stressors

Taxa respond differently to environmental stressors, therefore, can be arranged on a continuum from intolerant to tolerant. Intolerant taxa will emigrate or perish as environmental conditions worsen. Conversely, tolerant taxa may not respond negatively to environmental conditions and may actually increase as niches open from extirpated intolerant taxa.

Metric Category: Tolerance

Trend: Expected to increase with stress and increases in the SNEP dataset.

References: Hilsenhoff 1987; Yuan 2006; Megan et al. 2007; USGS 2013

% Semivoltine taxa (Percent Semivoltine taxa) (pt_volt_semi)

Description: Of all taxa, the percentage of taxa that require more than one year in a reproduction cycle

Taxa respond differently to environmental stressors, therefore, can be arranged on a continuum from intolerant to tolerant. Intolerant taxa will emigrate or perish as environmental conditions worsen. Conversely, tolerant taxa may not respond negatively to environmental conditions and may actually increase as niches open from extirpated intolerant taxa.

Metric Category: Voltinism

Trend: Expected to increase with stress and increases in the SNEP dataset.

References: Barbour et al. 1994; Dole´dec et al. 2006; Statzner and Beˆche 2010

References:

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Appendix E

Site Classification Analysis

and

Additional maps –

- Baseflow
- Mean annual air temperature
- Mean summer stream temperature
- Elevation

Site Classification Analysis

Site classification addresses the recognition that even with the least disturbance to streams, there might be different expectations of the sampled benthic assemblage due to natural effects and influences. Natural variation in stream slope, stream size, dominant substrates, temperature, and other factors that are components of ecoregional characteristics that might cause a sample to contain more or less of certain taxa groups, sensitive taxa, or functionally specialized taxa. These types of taxa and some of the metrics derived from their traits are expected to exhibit variation not only with natural variation but also with human disturbance and unnatural stressors. When we use the benthic assemblage to indicate biological conditions relative to disturbance, we attempt to account for different expectations due to the background natural setting.

Accounting for different biological expectations was explored by an investigation of natural variation in samples from the least-disturbed reference sites. If the variation in taxa or metrics can be associated with natural categories or gradients, then those categories or gradients can be used to characterize different reference conditions. Comparisons of metrics between reference sites and those with high disturbance will be more sensitive to stressors if the natural variation is filtered out through classification.

The classification investigation proceeded through the ordination of taxa and metrics in reference sites so that samples could be organized by similar biological characteristics. To increase the sample size for the classification analyses, one ‘borderline reference’ site was included in the reference dataset (n=27; these sites are marked in Attachment C). Non-metric multidimensional scaling (NMS) ordination was used to find sites with similar taxa. Principle components analysis (PCA) was used to organize sites by similar metric values, using 45 selected metrics. In each of these ordinations, the biological gradients were mapped in two dimensions, with each axis describing orthogonal composite aspects of the community. The axes were then associated with continuous natural variables through correlation. Categorical variables (e.g., level 4 ecoregions) were superimposed on the ordination diagrams to visually discern separation of categories. Any strong associations of environmental factors with the axes prompted further investigation of the factors as possible classification variables.

Due to the region and small data set, only a few discrete site classes could possibly be recognized before the separate classes would become too small to robustly represent the reference condition in each class or to allow comparisons between reference and disturbed data within each class. For adjustment of expectations along a continuous gradient, the optimal metric values were defined relative to the strongly correlated environmental variables. Metric scoring was thereby specific to the natural factor in each site.

Non-metric multidimensional scaling (NMS)

The NMS ordinations were run on presence/absence data from the reference sites, with the dataset limited to 105 common taxa. Taxa that occurred in less than three sites were removed to prevent a bias in the sample similarities. The ordination resulted in a 2-dimensional solution with a final stress of 16.6 (< 20 is acceptable). The first two axes explained 84% of the variance in the data and these axes were explored with correlation analysis and visual inspection.

Level 4 ecoregions were fairly distinct for reference SNEP sites using presence/absence ordinations (Figure E-1). The first NMS axis (horizontal) was related to stream sinuosity, land slope, temperature, watershed size, and stream substrate (Table E-1). Larger, steeper streams with larger substrates in the western SNECPAH ecoregion were on the right of the diagram. These samples had higher numbers of clinger taxa and sensitive and Coleoptera, Odonata, Ephemeroptera, and Trichoptera (COET) taxa. On the left of the diagram were samples soft-bottom, sinuous streams from the eastern NBL ecoregion. These had greater percentages of non-insect individuals and more individuals were dominant in the five most common taxa. The second (vertical) axis was related to wide streams with diverse habitats at the top of the diagram. The opposite end of the axis had sites with higher temperatures in the east. The sinuosity measures traced in the GIS exercises did not confirm the relationship suggested by the NHD sinuosity. The top of the second axis had higher non-insect and clinger taxa and the bottom had higher percent Trichoptera individuals.

On the first axis, sinuosity, longitude, land slope, and substrate characteristics, and percent water and wetland cover in the watershed are the major correlated natural variables that might be useful for site classification (Table E-1). Drainage area was also correlated but might not be appropriate for classification. The correlation with drainage area was driven by three large sites (> 30 km^2). In more disturbed non-reference sites, watersheds were up to 700 sq km. If drainage area was used in site classification, the reference condition derived mostly from small sites might represent a natural condition that would not be applicable to large non-reference sites.

Clinger taxa and percent Plecoptera, Odonata, Ephemeroptera, and Trichoptera (POET) taxa were associated with higher land slopes, larger drainage areas, and gravel and pebble substrates. More non-insect individuals and greater dominance of the five most common taxa per sample were associated with the sinuous, warmer, small sites with fine sediments.

Longitude is related to ecoregion and could be used as a continuous variable for classification whereas ecoregion would be categorical. Eastern sites in the lower left of the diagram are mostly in the Narragansett-Bristol Lowlands (NBL, L4 ecoregion 59e), though there is some overlap with sites of the Southern New England Coastal Plains and Hills (SNECPAH, L4 ecoregion 59c) (Figure E-1). The single Gulf of Maine Coastal Lowland (L4 ecoregion 59f) reference site is intermediate to the other ecoregions. Because of the overlap in longitudes without a distinctive break-point or threshold, the categorical ecoregions would be better classification variables than longitude.

Sinuosity was on the same axis as land slope and drainage area (Figure E-2). These three variables are often related, as large catchments are generally in flatter valleys with low slopes and meandering streams. In this data set, the three variables suggest that the flatter streams were sometimes more sinuous than steeper streams, but that these flatter, sinuous streams were in small catchments. The sinuosity measures traced in the GIS exercises did not confirm the relationship suggested by the NHD sinuosity.

Land slopes were steeper in the upper right of the diagram. Only two NBL sites had slopes similar to those in the SNECPAH sites (Figure E-3). Correlated substrate characteristics included the percent muck-mud in the reach and the percent gravel and cobble (estimated substrate areal percent). Though these had a higher correlation with the axis, there was no apparent threshold that could be used to classify biological types.

The second (vertical) axis was related to temperature, stream width, and longitude. The top of the diagram had warmer, wider, western sites, which were also related to the SNECPAH ecoregion. Temperature could be related to urbanization and clearing of riparian canopy and that could lead to misclassification of new sites. The top of the diagram also had greater richness of non-insect taxa, more clinger taxa, and fewer Trichoptera individuals.

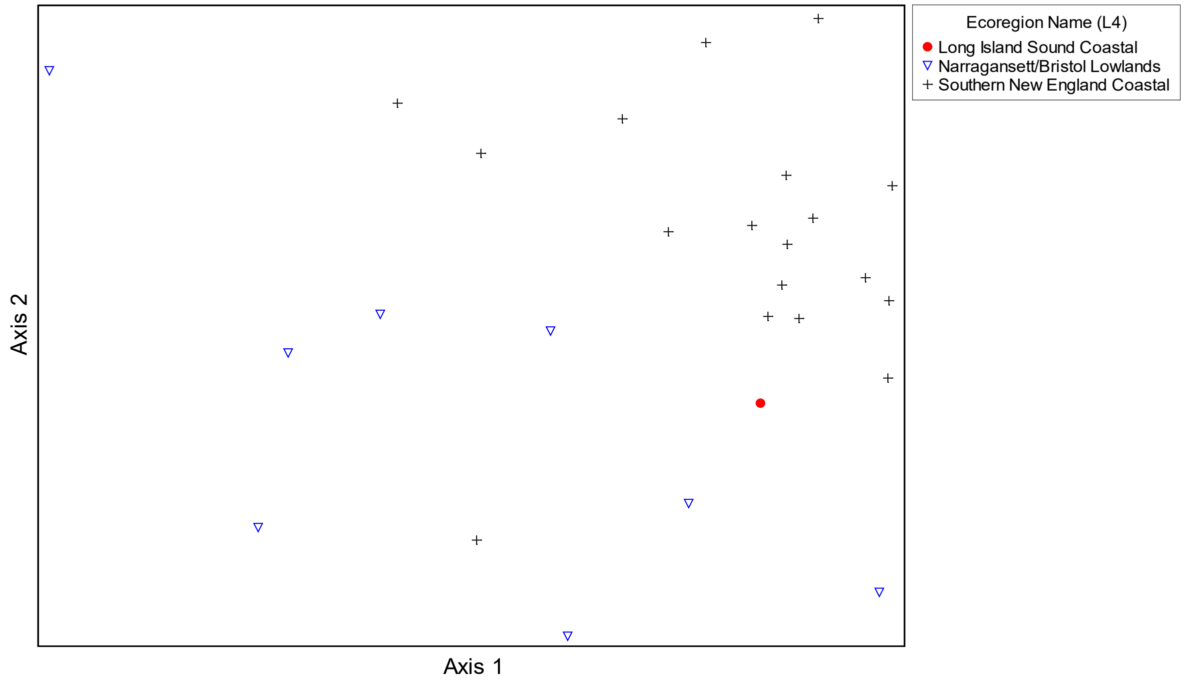


Figure E-1. Non-metric multidimensional scaling (NMS) ordination of taxa presence/absence in SNEP reference sites, with samples coded by Level 4 ecoregion. Samples with similar taxonomic composition are plotted in close proximity.

Table E-1. Correlation coefficients of the major environmental variables and biological metrics related to the non-metric multidimensional scaling (NMS) ordination axes.

Axis	Variables	r	Axis	Variables	r
Axis 1 (60% variance)	**Environmental**		Axis 2 (23% variance)	**Environmental**	
	Sinuosity	-0.66		# Habitat Types	0.66
	Land Slope	0.59		Minimum Temperature	-0.63
	Longitude	-0.54		Stream Width	0.61
	Maximum Temperature	-0.51		Longitude	-0.54
	% Wetland & Water	-0.49		**Biological**	
	% Muck and Mud	-0.44		% Trichoptera Taxa	-0.68
	Drainage Area	0.41		Non-insect Taxa	0.66
	% Gravel & Pebble	0.40		Clinger Taxa	0.62
	Biological				
	Clinger Taxa	0.81			
	% COET Taxa	0.81			
	% Dominant 5 Taxa	-0.76			
	% Non-insect Individuals	-0.75			

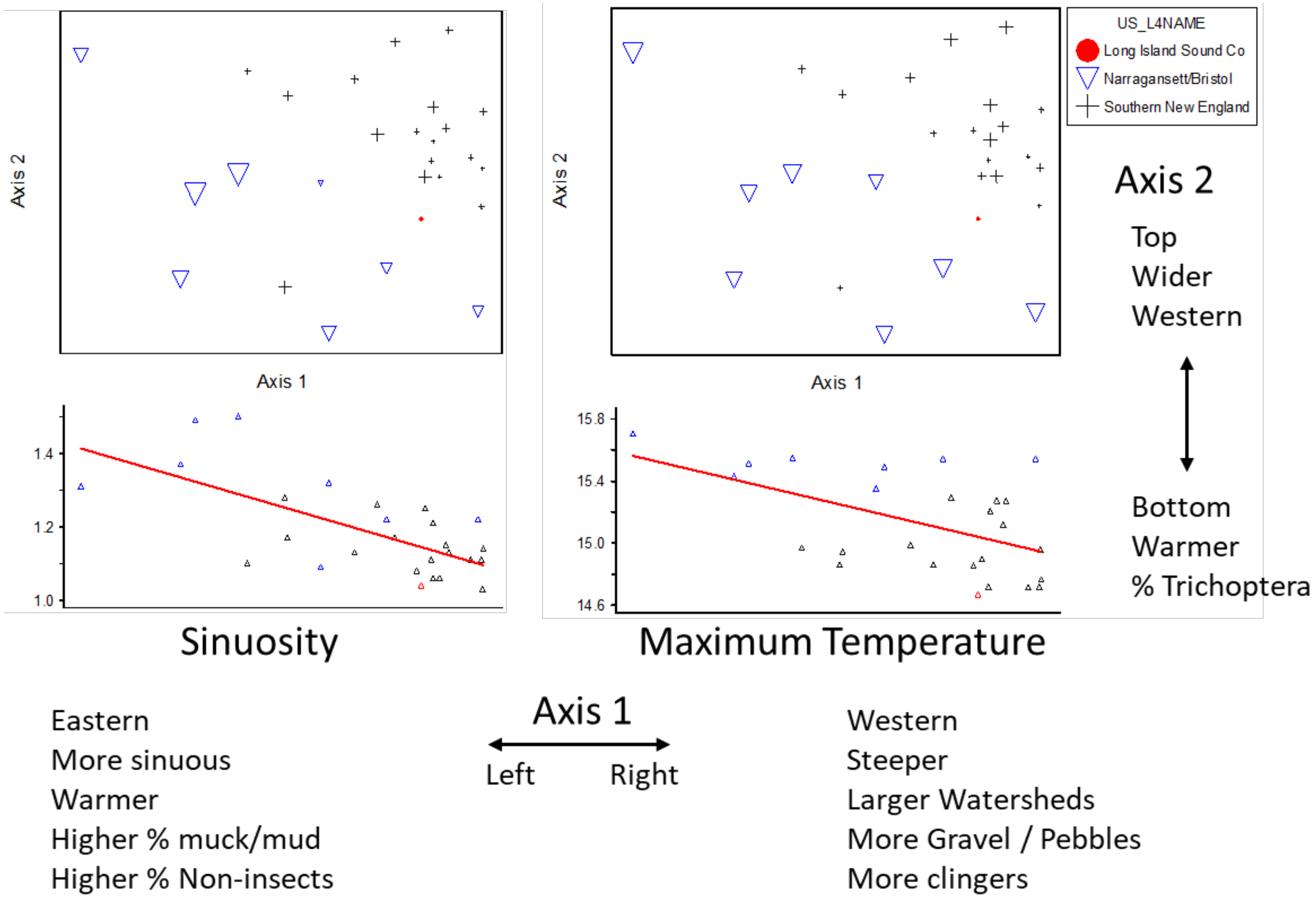


Figure E-2. NMS ordination of taxa presence/absence data in SNEP reference sites with samples coded by Level 4 ecoregion. The lower plots show how sinuosity and maximum water temperature relate to Axis 1.

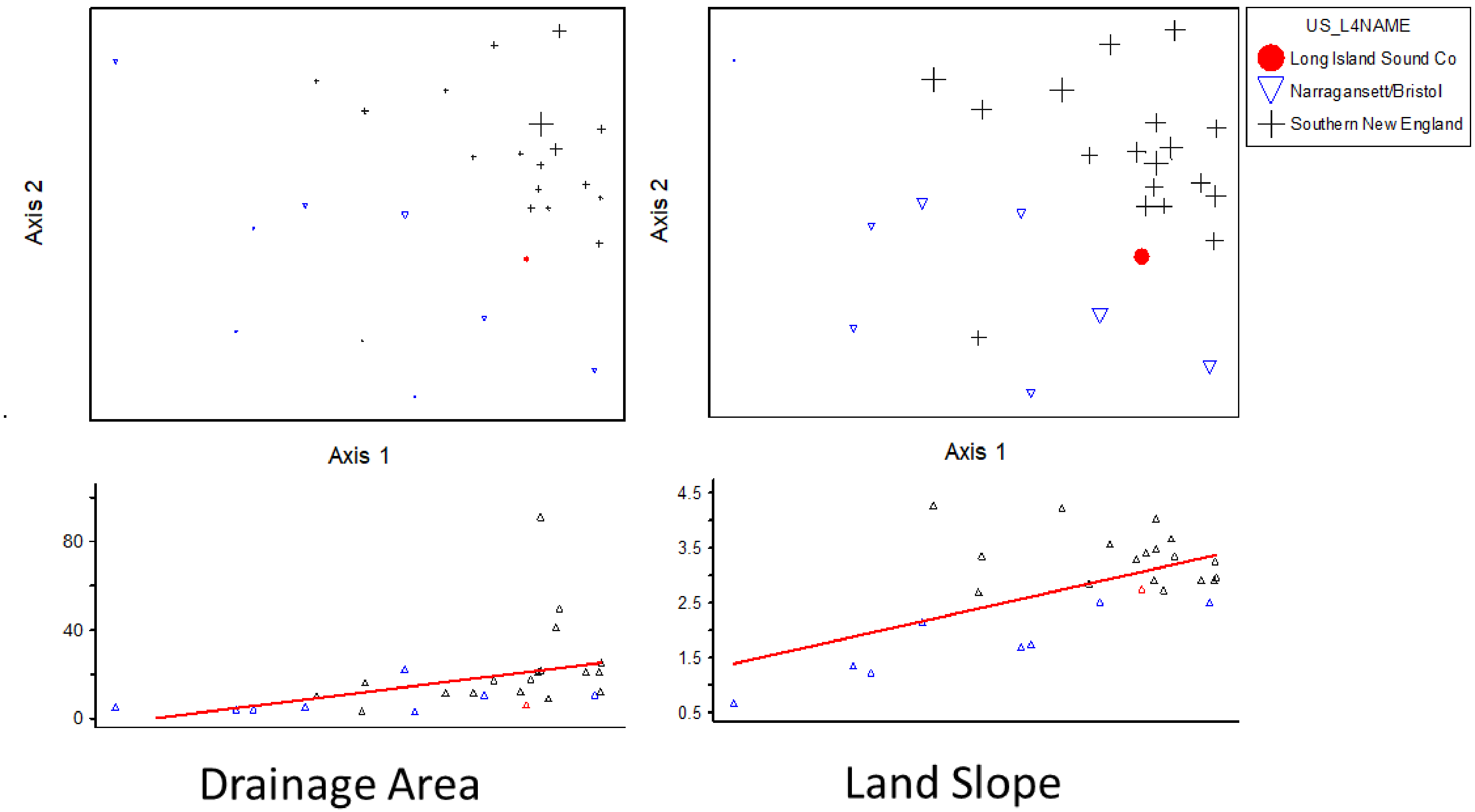


Figure E-3. NMS ordination diagram of taxa presence/absence data in SNEP reference sites with samples coded by Level 4 ecoregion. The lower plots show how drainage area and land slope relate to Axis 1.

Principle components analysis (PCA)

To explore the effects of environmental variables on metric distributions, a PCA was performed with 45 metrics that represented a variety of metric formulations and taxa characteristics. The PCA identified the same variables on the first axis as were identified in the NMS of taxa presence absence, though in a slightly different order of importance. These included sinuosity, land slope, percent water and wetland cover in the watershed, longitude, and drainage area. Substrate characteristics were also correlated, though not as strongly. The first axis explained 39% of variance in the ordination. On the second axis, less variance (13%) was explained by the variables stream width, longitude, and temperature.

Richness metrics were associated strongly with the first axis, including clinger taxa, Coleoptera, Odonata, Ephemeroptera, and Trichoptera (COET) taxa, and insect taxa. These were on the end of the axis with steeper slopes. Patterns of the PCA ordination were similar to those seen in the NMS ordination, though the separation of ecoregions was less distinct (Figure E-4).

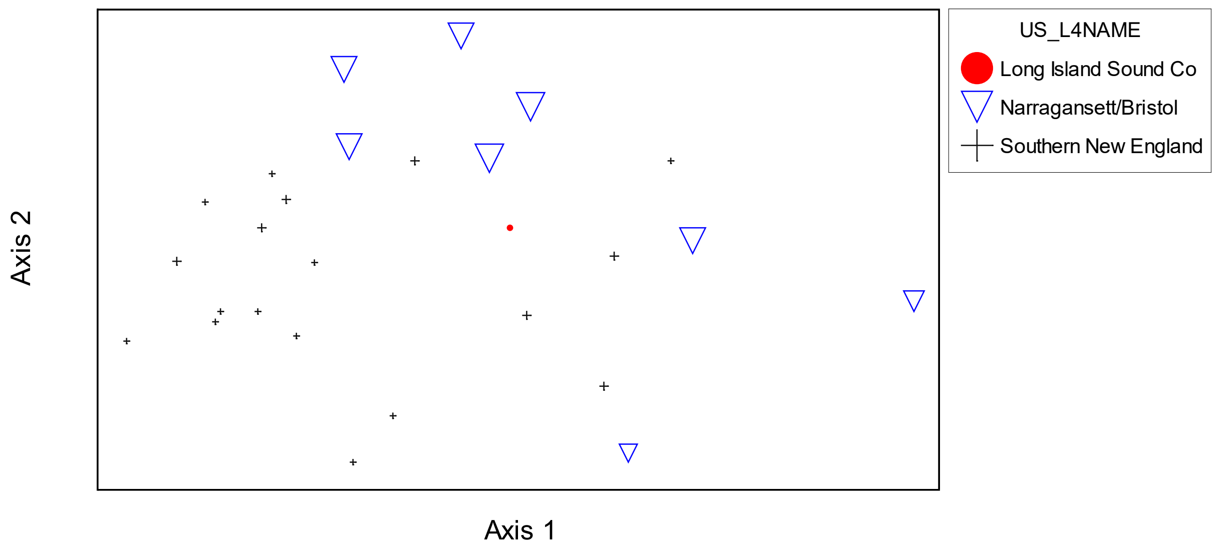


Figure E-4. Principle Components Analysis (PCA) ordination of 45 metrics in reference sites of the SNEP region, with larger marker size indicating eastern longitude of the sites

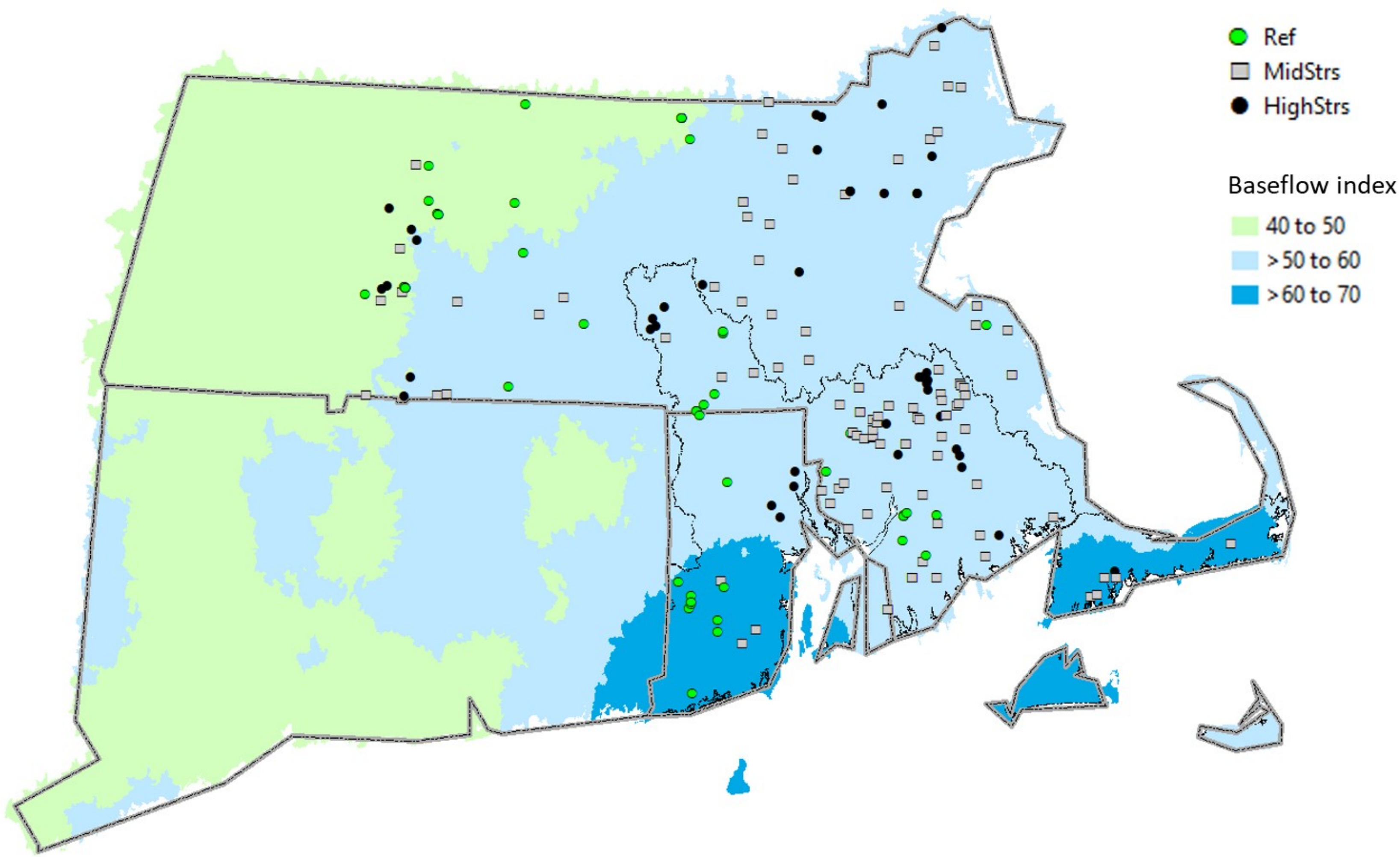
Classification Summary

Classification schemes related to Level 4 ecoregions and drainage area were considered but ruled out based on results from the NMS and PCA analyses. Level 4 ecoregion did not cluster distinctly in the PCA ordination of metrics. Moreover, defining site classes based on Level 4 ecoregions might be untenable because it would result in small sample sizes for index calibration. All the reference sites in the NBL were <15 km2, which is smaller than the bulk of stressed sites, suggesting that a classification scheme based on drainage area or ecoregion would result in insufficient comparable samples for index calibration.

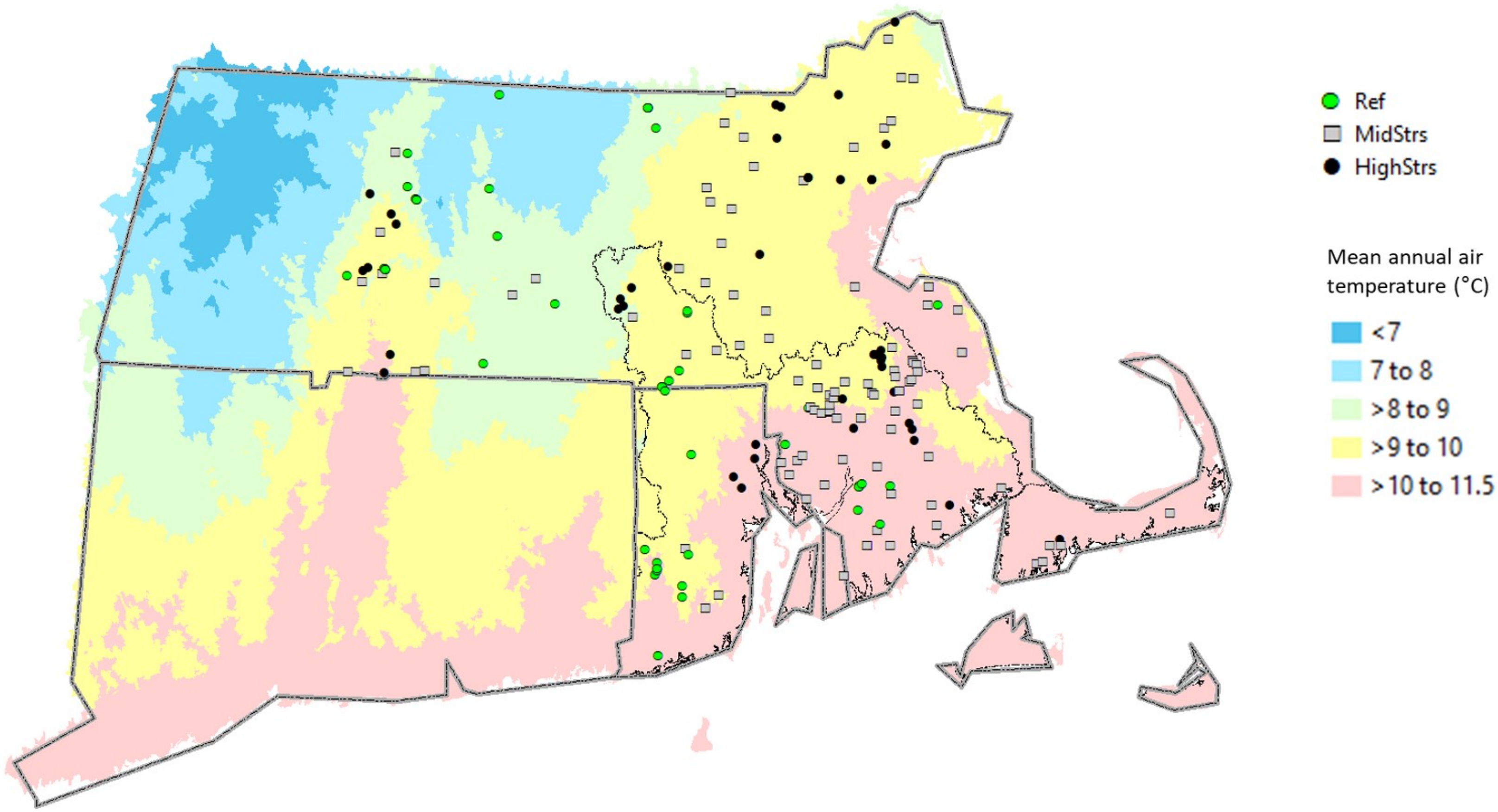
Continuous variables that showed potential for classification included: annual air temperature (PRISM 1981-2010), sinuosity, longitude, land slope, substrate types, and drainage area. Because there are no clear break-points to distinguish classes based on the continuous variables, scores for individual metrics that showed strong correlations with these natural variables were adjusted during index development (see Section 5.1).

Additional Maps

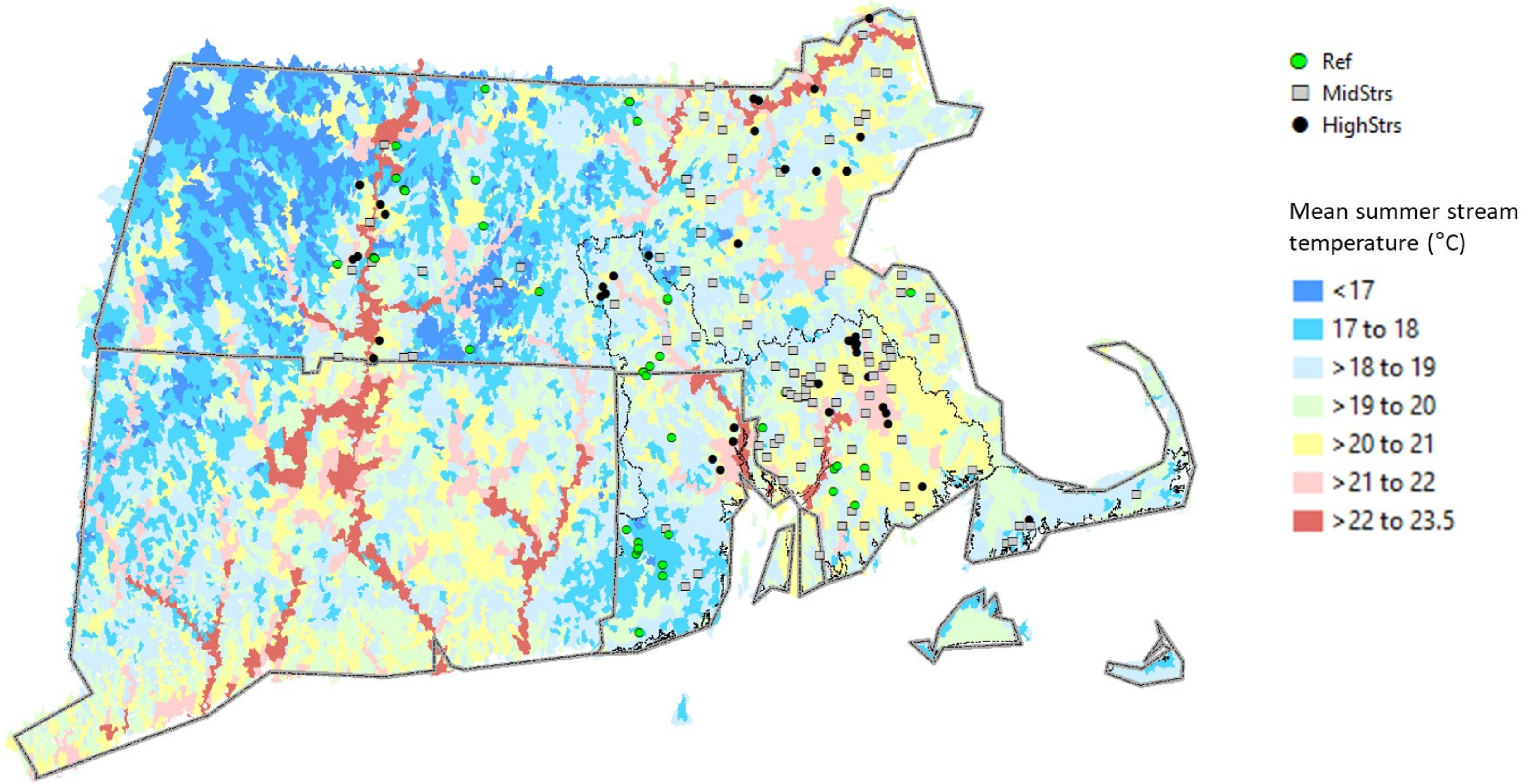
Baseflow index - https://water.usgs.gov/GIS/metadata/usgswrd/XML/bfi48grd.xml



Mean annual air temperature (PRISM 1981-2010) - https://prism.oregonstate.edu/normals/



Mean summer stream temperature (July-August) –
Hill, R.A., C.P. Hawkins, and D.M. Carlisle. 2013. Predicting thermal reference conditions for USA streams and rivers. Freshwater Science 32(1):39-55. doi:10.1899/12-009.1.



Elevation