Table 2. Summary of the protocol elements for the Massachusetts Department of Environmental Protection (MassDEP) and Southeast New England Program (SNEP) low gradient macroinvertebrate methods.

Method	Habitat	Effort	Gear	Reach length	Index period	Target # organisms	Taxonomic resolution
MassDEP 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 listed 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
SNEP multihabitat	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				

*For example, if the habitat is 50% submerged wood, 30% submerged vegetation and 20% vegetated margins/banks, then 5 jabs will be taken from submerged wood, 3 from submerged vegetation, and 2 from vegetated margins/banks. A comparison of habitat types defined by each agency is in Appendix A.

2.1.3 Taxa attributes

We compiled the MassDEP and Tetra Tech macroinvertebrate data into an MS Access relational database. For trait assignments, we used the attribute table that had been created during the calibration of the MassDEP riffle habitat IBI as a starting point (Jessup and Stamp 2020). The table included five sets of traits: functional feeding group (FFG), tolerance value, habit, life cycle/voltinism, and thermal preferences (Table 3). Based on guidance from Cole Ecological, Inc., we updated some of the phylogeny and taxa names to reflect the most current nomenclature and keys and re-checked the attribute assignment based on the sources listed in Table 3.

To help inform tolerance value assignments (which could differ in low vs. higher gradient streams), we ran taxa tolerance analyses on the regional low gradient dataset to explore the distribution of taxa across four generalized disturbance measures: the Indices of Catchment and Watershed Integrity (ICI and IWI, respectively), percent urban and percent agricultural land cover (Thornbrugh et al. 2018, Johnson et al. 2018). Taxa that occurred at fewer than 10 sites were excluded from the analysis because low numbers of occurrences gave unreliable results. 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 increase the sample size and improve the robustness of the analysis, the analyses were also run on a larger regional dataset that included low gradient data from outside of the SNEP region in Massachusetts, Connecticut, Vermont, and New York. Biologists from MassDEP reviewed results from the analyses and assigned taxa to three tolerance categories: intolerant, intermediate, and highly tolerant (Table 3). More detailed information on the tolerance analyses can be found in Appendix B.

The taxa attribute table is provided in Attachment B. Table 3 shows what percentage of the 542 taxa in the SNEP IBI calibration dataset had attribute assignments for each trait group. FFG was the most complete (97%) while voltinism had the lowest number of assignments (46%). Metrics were calculated with the BioMonTools R package (Leppo et al. 2021). Appendix C contains the list of metrics that were calculated and considered as candidates for inclusion in the IBIs. When developing the list of candidate metrics, we researched metrics being used in other existing low gradient IBIs. Results of that exercise are provided in Appendix C. When making metric calculations, non-target taxa (e.g., Hemiptera, crayfish) were excluded from all metrics and redundant/non-distinct taxa were excluded from the richness metrics (for more information, see Appendix C).