Stream RMN Protocol Document
Mid-Atlantic Macroinvertebrates (12/29/2022)

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Acknowledgements:
The document was written by Tetra Tech (Jen.Stamp@tetratech.com), with funding from EPA ORD CPHEA (EPA lead: Britta Bierwagen - Bierwagen.Britta@epa.gov) and was developed through a collaborative process with Regional Monitoring Network (RMN) partners.

Why Assess Macroinvertebrates?

Macroinvertebrates are the most commonly-used assemblage in stream bioassessments. They make good indicators for a number of reasons: they respond to a wide range of stressors; they are easy to collect; many (not all) taxa are easily and consistently identified; taxa have limited mobility, relatively long-life cycles (~1 year), and are highly diverse; they play a vital role in the food web; and traits can be linked to environmental conditions.

1 Level of Effort

The RMN framework allows for different levels of effort to maximize participation. Table 1 contains a 'menu' of options for collecting and processing macroinvertebrates, divided into three levels of participation: 'minimum', 'target' and 'better'. If resources permit, participants are encouraged to collect data at the 'target' or 'better' levels, since these levels of effort improve the likelihood of detecting trends over shorter time periods (see text box). Table 1 is intended to provide a basic framework; there may be participation levels that fall in-between those suggested in this table.

When reading Table 1, keep in mind that properly preserved macroinvertebrate samples can last for several years. If you have resources to collect a sample during a given year but lack the resources to process it that year, please go ahead and collect it. The sample can be processed at a later date as resources become available.

Table 1. Stream macroinvertebrate protocols divided into three levels of participation: 'minimum', 'target' and 'better'. Participants are encouraged to collect data at the 'target' or 'better' levels. Higher levels of effort increase the likelihood of detecting trends over shorter time periods and increase the number of ways in which the data can be used. Note that there may be participation levels that fall in-between those suggested in this table.

Data type	Level of participation		
	Minimum	Target	Better
Frequency & timing of collection	1X every 2 years; during spring (March-April)	1X/year; during spring (March–April)	2X/year; during spring (March–April) and summer (July–August)
Target number of organisms	≥ 100 and < 300 organisms	Minimum of 300 organisms	500 organisms
Taxonomic resolution*	Subfamily-level Chironomidae, genus-level for everything else	Lowest practical level; if you lack the resources to do this for all taxa, prioritize EPT taxa first, followed by the list in Attachment 1	Lowest practical level for all taxa

*to the extent allowed by specimen condition and maturity and availability of workable taxonomic keys

Using power analysis to inform design
In 2012, during the design phase of the Northeast stream RMN, US EPA performed a power analysis to explore the influence of collection and processing methods and basic survey design options on trend detection times for macroinvertebrate metrics. Sampling frequency (1 vs. 2 vs. 5-year) had a significant effect on trend detection times, with annual sampling having the shortest trend detection time (this was particularly evident when trends were subtle). Results from the analyses also showed that mean macroinvertebrate richness metric values were inversely related to the number of years needed to detect a trend with 80% power. Put more simply, richness metrics that had higher mean values had shorter trend detection times.

Because richness values increase with subsampling effort, it is important to identify an adequate number of organisms, particularly for metrics with low representation (e.g., cold-water taxa). This is why we are recommending a subsample of 300 or more organisms at RMN sites. While this may increase sample processing costs in the short-term, the resulting decrease in detection times may actually reduce the overall costs of the long-term monitoring effort. Moreover, the earlier detection may lead to management actions that could alleviate additional, costlier impacts. More information on the power analyses can be found in USEPA 2016 (Appendix A).

2 Protocols

The use of consistent and comparable methods is very important for the RMNs, as different methodologies may introduce analytical constraints and contribute to variability, which reduces the sensitivity of indicators and increases trend detection times.

2.1 Time period

To minimize variability associated with seasonal or weather-related changes in composition and abundance of macroinvertebrates, timing of macroinvertebrate sampling should be as consistent as possible from year to year at a given RMN site. Spring (March−April) is the primary sampling period for macroinvertebrates at Mid-Atlantic RMN sites. If resources permit, samples are also being collected during summer (July-August) to explore seasonal differences. Spring was selected as the primary index period because preliminary data suggest that on average, assemblages are comprised of slightly higher proportions of sensitive and cold water taxa during spring.

Once the initial sampling date for a site has been established, the site should be sampled within two-weeks of that date from that point onward, with consideration given to factors such as flow conditions (base flow is preferred, but not too low – the flow must be adequate to carry insects into the net). If you're wondering whether flow conditions are suitable, ask yourself: do conditions allow the collection protocol to be safely and effectively implemented as it normally would? If so, go ahead and sample. If not, wait until conditions allow for normal protocols to be used.

We realize it may not always be possible to sample during the 2-week window (due to various reasons - flow conditions, logistical challenges, etc.). In these situations, sample as close to the normal window as

possible. In your field notes and metadata, explain the reason for the divergence. It is better to collect a sample if safely possible, than to skip sampling.

2.2 Extreme weather events

Capturing impacts from extreme events isn't the primary objective of the RMN macroinvertebrate sampling, but having these types of data are very valuable, especially given how extreme weather events are projected to occur with greater intensity, and in some cases, greater frequency in the future. The more we can learn about what 'signals' to look for in the data, and why assemblages at some sites recover more quickly (or are less impacted to begin with) than others, the better (and faster) we'll be at coming up with strategies and recommendations on how to best improve resiliency of stream biota to future extreme events.

Recommendations are not straightforward for these situations because there are so many variables (each weather event is different, each site is different, impacts will vary depending on timing and antecedent conditions, etc.). If an extreme event impacts a RMN site during your normal sampling period, wait at least two weeks after the extreme conditions subside before sampling (the intent is to give the macroinvertebrate assemblage time to recover). Flow conditions remain a consideration as well. Conditions should allow for safe and effective implementation of normal protocols. Take photographs to document effects of the extreme event on stream habitat. Ideally temperature and hydrologic data are being collected as well; if so, these will provide information on the magnitude and duration of the event.

If you are able to collect additional macroinvertebrate samples (beyond what is described above) to capture response and recovery to the event, please do so! At this time, we don't have recommendations on when exactly to collect those samples, but some RMN partners have collected samples immediately after an event (once conditions were safe enough to access the site) and then another sample one to three months after that. We will update the extreme event protocols as we gain more knowledge and experience.

2.3 Steps for MidAtlantic RMN Method

1. Establish the sampling reach. The sampling reach is 100-m and contains abundant riffle habitat. With a GPS, record the coordinates of the downstream and upstream end. Once established, the same reach should be sampled each year.

2. Sample macroinvertebrates. Start at the downstream end and proceed upstream. If you have an EPA-approved SOP or QAPP, collect the samples in accordance with your existing methods or in such a way that the data can be rendered comparable to historical methods (the goal is to keep the long-term records at RMN sites intact). If you do not have existing protocols, contact Kelly Krock (Krock.Kelly@epa.gov) for guidance. Gear should consist of either a square frame kick net or D-frame net, with mesh size ranging from 450–600 µm. Sample a minimum of 1 m^2 (e.g., four 0.25 m^2 or eleven-0.09 m^2) in riffle habitats throughout the 100-m reach. Composite into a 450–600 µm sieve bucket or put directly into the sample container(s) to get one sample.

3. Preserve and label the sample. Once sampling is complete, the sample material should be preserved as quickly as possible. Transfer the sample material to the sample containers. Sample containers should

contain no more than 30% of their volume as wet weight. Fill sample containers with 95% reagent alcohol to a level that ensures a final alcohol concentration of at least 70%. Be sure to thoroughly clean the bucket and sampling nets of all invertebrates. The use of forceps might be necessary to dislodge some of the smaller organisms. Fill out internal and external sample labels for each sample container. Be sure to use water and alcohol proof writing medium. We recommend that you take a few minutes to "elutriate" samples in the field (remove the mineral material such as stones/sand). This can greatly reduce sample volume (and in turn, amount of preservative and number of containers) and reduces risk of damage to specimens during transport. Consult with experts (e.g., taxonomy labs) to find out the latest guidance on the best preservation techniques. If done correctly, macroinvertebrate samples can last for several years[1]. So even if you don't have the resources to process a sample in a given year, please collect one if you can.

4. In the laboratory, randomly subsample to the target number of organisms. Entities use a variety of techniques to subsample. For the RMNs, each entity can use its own subsampling approach as long as it is random and is performed in accordance with an approved QAPP or SOP. If you do not have existing protocols, contact Kelly Krock (Krock.Kelly@epa.gov) for guidance. For larger picks, computer subsampling is permissible to reach target organism count.

> Optional: after the pick, remove any large and/or rare organisms from the remaining sample material on the tray. This 'large and rare' sample should be kept separate from the other sample.

5. Taxonomic resolution. When possible, all taxa should be identified to the lowest practical taxonomic level (ideally species level). If this is only feasible for some taxonomic groups, we recommend that (all) Ephemeroptera, Plecoptera and Trichoptera taxa be the highest priority, followed by taxa listed in Attachment 1. Chironomidae require a slide mount and a compound microscope to identify to the species-level, as do oligochaete worms being identified beyond family.

Archiving specimens (using best practices – e.g., glass vials with archival caps) is encouraged.

IMPORTANT UPDATE: In 2023-2024, Tetra Tech will be working with MidAtlantic RMN partners on regional macroinvertebrate tolerance analyses (starting with modeled stream temperature data). Efforts will be made to harmonize the multiple state datasets, update names of taxa that were affected by recent changes in taxonomic nomenclature, correct misspellings, and standardize naming schemes for macroinvertebrate data. The end goal is to refine lists of climate indicator taxa and regional macroinvertebrate attributes that will be used to calculate macroinvertebrate metrics for regional analyses. These analyses will help inform recommendations on levels of taxonomic resolution to improve sensitivity of indicator taxa (and allow us to improve Attachment A – Table A1).

2.4 Quality assurance/quality control (QA/QC)

QA/QC is a critical component of monitoring, as it ensures data quality objectives are being met and ensures that data remain consistent and comparable over time. If you already have an EPA-approved

[1]Samples will likely require some maintenance, such as draining and recharging with fresh alcohol one or more times a year; making sure the bottles are sealed well (threads need to be clean); if storing in plastic (HDPE), bottles can get brittle and crack over time (so check regularly for odors and other indications of liquid loss). This is not an issue with voucher/reference specimens kept in glass vials with archival caps.

SOP or QAPP, continue following your approved QA/QC protocols. If not, below is a list of recommended protocols for RMN sites. Oversight and compliance are left up to participants.

- **Field**
    - Conduct periodic audits to ensure that field crews are adhering to the collection protocols
    - Collect replicate samples (same site, same day) at 10% of sites. Compare results with the primary samples to quantify variability associated with sample collection.
    - To reduce potential variability, have the same person collect the sample each year. If that is not possible, make sure the people collecting the samples are trained and checked for consistency
- **Laboratory**
    - **Picking efficiency**
        - Have a different person check a certain percentage (e.g., 10%) of each sample that is picked. If more than 10% of organisms previously picked are found, the sample is reprocessed. Optional: consider having stricter requirements for new, inexperienced staff.
        - Optional: for 10% of the samples, do a randomized repicking; compare results to quantify variability associated with the subsampling/picking procedure
    - **Taxonomic identifications**
        - Have another taxonomist check a certain percentage (e.g., 5%) of all samples identified; if there are discrepancies, they are resolved by an independent taxonomist
        - Maintain a "voucher" collection that contains three specimens (when possible) of every taxon identified at your RMN sites so they may be examined later to confirm the accuracy of the IDs or to resolve any taxonomic issues. Each vial in the voucher collection should be labeled with the taxon name, sample ID, sample date, taxonomist and any other relevant sample information.
        - Optional: keep a reference collection as well (that contains specimens with IDs that were validated by an authority on the taxonomic group)

High-quality taxonomy is very important. Analyses have shown that the magnitude of taxonomic error varies among taxa, laboratories and taxonomists, and that the variability can affect interpretations of macroinvertebrate data (Stribling et al. 2008). If you encounter unusual taxa or taxa that you are uncertain about, we recommend sending the specimen to one or more outside experts for verification.

What if RMN protocols differ from your normal monitoring procedures?
If possible, collect two sets of samples – one using your normal protocols (that are consistent with your long-term data record; we want your long-term record to stay intact) and one using the RMN protocols. Retain both sets of results for comparison.

Disclaimer: we expect participating entities to follow their own approved safety protocols and thus do not provide any here.

3 Equipment

Table 2 and Figure 1 contain information on basic equipment needs, considerations and estimated costs. This assumes access to waders, safety gear, and a GPS unit for locating the sampling location. These are only examples (and not endorsements of specific brands). This is intended to give you a sense of the range of costs you might encounter when utilizing RMN protocols.

Figure 1. Basic equipment includes a kick net, sieve bucket and waders.

Table 2. Basic equipment needs and estimated costs (12/29/2022)

Equipment or task	Specifications	Estimated Cost
Kick net	Either a square frame kick net or D-frame net, with mesh size ranging from 450–600 µm	$225[1]
Sieve bucket (also referred to as a 'wash bucket') (optional)	450–600-micron sieve[2]	$150[1]
Jars or bottles in which macroinvertebrate sample is to be preserved	• Preferably non-breakable synthetic (like High-Density Polyethylene - HDPE) • 1-liter capacity (or larger, if preferred) • Wide-mouth • Leak-proof lids	Varies (bulk discounts may be available) Package of 6 - $40 (~$6/bottle) Case of 50 - $125 (~$2.50/bottle) [3] You typically need one 1-liter bottle per site but this may vary[4]
Ethanol[5]	95% reagent alcohol, 4-L poly bottle (this amount generally covers several sites)	Varies (bulk discounts may be available) One 4-L poly bottle - $70[3]
Macroinvertebrate subsampling/sorting	Perform in-house, then send to outside lab for identifications	Highly variable – depends on the target number of organisms and the type of sample. 300-count samples generally take a few hours. If the sample contains lots of debris or sand, it will take longer to process.
	Send to outside lab	Varies; generally less than $100/sample
Macroinvertebrate identifications	300-count, sent to outside lab	Varies depending on the lab and number of samples (bulk discounts may be available). Costs generally range from $250 to $350/sample

[1]Based on quotes for WILDCO products; this is just an example, not an endorsement

[2]If you can only find buckets with 541-micron sieves, those are acceptable

[3]Online quotes are highly variable. It appears alcohol has gotten much more expensive!

[4] If you "elutriate" samples in the field (remove the mineral material such as stones/sand), this can greatly reduce sample volume (and in turn, amount of preservative and number of containers)

[5]Ethanol is flammable; special handling instructions apply

4 Field forms

At a minimum, RMN stream macroinvertebrate field forms should contain the following information:

- Date
- Time
- Person collecting the sample
- Latitude and longitude of upstream and downstream ends of the reach
- Flow conditions – very high/high/moderate/baseflow/very low
- Collection method
- Habitat(s) sampled (in the Northeast, this should be riffles only)
- Notes about anything that might affect the quality of the sample (e.g., difficulty accessing reach due to high flows)

5 Data management

RMN partners will be custodians and owners of their data. The goal is to upload the macroinvertebrate data to the Water Quality Portal/Water Quality Exchange (WQX), where it can be accessed by other regional partners.

There are three options for uploading discrete data into WQX:

- Option 1: Standard web-based application (WQX Web) that uses Microsoft Excel spreadsheets.
- Option 2: Create a custom submission application using WQX XML schema through Exchange Network Nodes or Node Clients
- Option 3: via a third-party system such as The Ambient Water Quality Monitoring System (AWQMS).

Instructions are available online: https://www.epa.gov/waterdata/water-quality-data-wqx

Sometimes new taxa need to be added to WQX before the data can be uploaded; in these situations, send your request to the appropriate EPA WQX contact for assistance.

In addition to uploading data to WQX, entities should also make sure that the original raw biological data are properly archived and stored so that additional uses may be possible in the future. Well-documented metadata are very important as well, as this will allow users to select data that meet their needs (e.g., maybe they only want to use data collected using certain methods and at certain levels of rigor).

There are many possibilities for analyzing the data. People seeking guidance on data analysis can utilize resources developed (or being developed) for the RMNs, which include -

- **RMN data analysis plan**: this plan has applicability across waterbody types (streams, lakes, wetlands). It covers analysis of biological data and continuous temperature and hydrologic data,

both alone and in combination, over short and long-term time periods. There are two sections – one on data preparation steps (QA/QC, documentation of metadata and metric calculations) and one on approaches for analyzing the data. This report is available upon request (Jen.Stamp@tetratech.com)

- **Stream RMN report** (USEPA 2016): has sections describing techniques for summarizing and using stream RMN data.
- **BioMonTools**: this is a free open-source tool for helping people prepare and analyze biological data in a standardized way for regional analyses. It is available as either a website/Shiny app (which does not require use of R software; https://tetratech-wtr-wne.shinyapps.io/BioMonTools/) or a R package (https://github.com/leppott/BioMonTools). For more information, contact Jen.Stamp@tetratech.com

6 Literature Cited

Stribling, JB, Pavlik, KL, Holdsworth, SM, Leppo, EW. 2008. Data quality, performance, and uncertainty in taxonomic identification for biological assessments. J N Am Benthol Soc 27(4):906−919.

U.S. EPA (Environmental Protection Agency). 2012. Implications of climate change for state bioassessment programs and approaches to account for effects. [EPA/600/R-11/036F]. Washington, DC: Global Change Research Program, National Center for Environmental Assessment. http://cfpub.epa.gov/ncea/global/recordisplay.cfm?deid=239585

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Attachment 1

Level of taxonomic resolution

Level of Taxonomic Resolution

IMPORTANT UPDATE: In 2023-2024, Tetra Tech will be working with MidAtlantic RMN partners on regional macroinvertebrate tolerance analyses (starting with modeled stream temperature data). Efforts will be made to harmonize the datasets from regional partners, update names of taxa that were affected by recent changes in taxonomic nomenclature, correct misspellings, and standardize naming schemes for macroinvertebrate data. The end goal is to refine lists of climate indicator taxa and regional macroinvertebrate attributes that will be used to calculate macroinvertebrate metrics for regional analyses. These analyses will help inform recommendations on levels of taxonomic resolution to improve sensitivity of indicator taxa (and allow us to improve Attachment A – Table A1).

For now, here are our recommendations -
When possible, all taxa should be identified to the lowest practical taxonomic level (ideally species level). If this is only feasible for a limited number of taxonomic groups, we recommend that (all) Ephemeroptera, Plecoptera and Trichoptera taxa be the highest priority, followed by the taxa listed below (taken to the specified level of resolution), where practical. Chironomidae require a slide mount and a compound microscope to identify to the species-level, as do oligochaete worms being identified beyond family.

The taxa in Table A-1 were originally selected based on a Northeast U.S. dataset. The taxa were selected based on differences in thermal tolerances that were evident in analyses (USEPA 2012; unpublished Northeast pilot study) and from best professional judgment. The list in Table A-1 should be regarded as a starting point and should be updated as better data become available in the future.

Literature Cited:

U.S. EPA (Environmental Protection Agency). 2012. Implications of climate change for bioassessment programs and approaches to account for effects. [EPA/600/R-11/036F]. Washington, DC: Global Change Research Program, National Center for Environmental Assessment.
http://cfpub.epa.gov/ncea/global/recordisplay.cfm?deid=239585

Table A1. At RMN sites, when possible, all taxa should be identified to the lowest practical taxonomic level (ideally species level). If this is only possible for a limited number of taxonomic groups, we recommend that (all) EPT taxa be the highest priority, followed by taxa listed below (identified to the specified level of resolution), where practical. Chironomidae require a slide mount and a compound microscope to identify to the species-level, as do oligochaete worms being identified beyond family.

Order	Family	Genus	Level of resolution	Notes
Amphipoda	Gammaridae	Gammarus	species	*G. pseudolimnaeus* is regarded as a cold- or cool-water taxon in Vermont (and is tolerant of nutrients). *Gammarus* (assumed to be *pseudolimnaeus*) is also regarded as a cold-water indicator in Minnesota (Gerritsen and Stamp, 2012).
Amphipoda	Hyalellidae	Hyallela	species	*H. azteca* is regarded as a cold/cool water taxon in Vermont (note: this species had previously been thought to be monotypic in the Northeast but recently it was discovered that there are actually multiple species, which may account for some variability). In Kentucky, Hyallela it is believed to be a completely warm-water genus.
Coleoptera	Elmidae	Optioservus	species	Cool to cold water species: *Optioservus trivittatus*
Coleoptera	Elmidae	Oulimnius	species	*O. latiusculus* is regarded as a cold-water taxon in Vermont, but species-level IDs may not be necessary for the larger region because most of the taxa are *O. latiusculus.*
Coleoptera	Elmidae	Stenelmis	species	Cool to cold water species: *Stenelmis cheryl, mera, sandersoni*
Diptera	Ceratopogonidae		subfamily	General agreement that there is variability in thermal preferences, but the taxonomy for this family needs to be further developed.
Diptera	Chironomidae	Eukiefferiella	species	Potential variability in thermal preferences of *E. brevicalar group, E. brehmi group, and E. tirolensis group* (cold); and *E. claripennis/coerulescens groups* and *E. devonica group* (warm)

Diptera	Chironomidae	Micropsectra	species	General agreement that there is variability in thermal preferences, but the taxonomy for this genus needs to be further developed.
Diptera	Chironomidae	Microtendipes	species groups	may be differences between species groups (pedellus vs. rydalensis groups)
Diptera	Chironomidae	Polypedilum	species	*P. aviceps* is generally regarded as a cold water taxon.
Diptera	Chironomidae	Thienemannimyia group	genus level	there appear to be differences between genera (Rheopelopia, Trissopelopia, Telopelopia may be cool to cold water inhabitants)
Diptera	Chironomidae	Tvetenia	species group	In the Northeast, *T. vitracies* is warm water oriented and *T. bavarica* is cool water oriented
Diptera	Simuliidae		genus	General agreement that Prosimilium is a cold water indicator but there is potential for variability within this genus (e.g., *P. mixtum* vs. *P. vernale*), and species-level systematics are not well developed at this time.
Ephemeroptera	Baetidae	Baetis	species	Potential variability in thermal preferences (e.g., *B. tricaudatus complex*—cool; *B. intercalaris* and *B. flavistriga complex* —warm).
Ephemeroptera	Ephemerellidae	Drunella	species	Variability in thermal tolerances within this genus was noted in the Utah pilot study, but in the Eastern states, species are believed to be all cold/cool water.
Ephemeroptera	Ephemerellidae	Ephemerella	species (as maturity allows)	Potential variability in thermal preferences (e.g., *E. subvaria*—colder); need mature individuals (early instars are difficult to speciate).
Ephemeroptera	Ephemerellidae	Eurylophella	species	Some variability was noted in a pilot study in North Carolina (U.S. EPA, 2012); could be seasonal phenology vs. thermal preference.
Ephemeroptera	Heptageniidae	Epeorus	species	Some variability was noted in a pilot study in Utah (U.S. EPA, 2012); can be difficult to speciate.

Ephemeroptera	Heptageniidae	Stenacron	species	In the Mid-Atlantic region, some regard *S. interpunctatum* as a warm-water taxon and the others as cooler/some cold. Taxonomy may be tricky.
Isopoda	Asellidae	Caecidotea	species	*C. brevicauda* has been noted as a potential cold-water indicator in the Midwest (Gerritsen and Stamp, 2012).
Neoophora*	Dugesiidae	Cura	species	*C. formanii* is regarded as a cold-water taxon in Vermont. Can be difficult to speciate in speciose regions.
Neoophora*	Planariidae	Dugesia	species	*D. tigrina* is regarded as a warm-water taxon in Vermont, as well as in New Jersey. Can be difficult to speciate in speciose regions.
Oligochaeta			family	Enchytraeidae is regarded as a cold-water family in Vermont. In the Mid-Atlantic region, it is found mostly in small streams. In New Jersey, it is found throughout the state.
Plecoptera	Perlidae	Acroneuria	species	Potential variability in thermal preferences of *A. abnormis* (warmer) and *A. carolinensis* (cooler).
Plecoptera	Perlidae	Isoperla	species	several species are cold water species but speciation is difficult during September/October because most are immature (at least this is the case in VT)
Plecoptera	Perlidae	Paragnetina	species	Potential variability in thermal preferences of *P. immarginata* (cold) and *P. media* and *P. kansanensis.*
Plecoptera	Pteronarcyidae	Pteronarcys	species	*P. dorsata* may be warmer water oriented.
Trichoptera	Brachycentridae	Brachycentrus	species	Potential variability in thermal preferences in the Northeast.
Trichoptera	Goeridae	Goera	species	Some variability was noted in a pilot study in North Carolina (U.S. EPA, 2012). The two species found in Kentucky are associated with cold water. In New Jersey, this genus is found as often in the coastal plain as in northern high gradient streams and is currently not taken to the species level.

Trichoptera	Hydropsychidae	Hydropsyche	species	Some variability was noted in a pilot study in New England (U.S. EPA, 2012, unpublished data); is generally considered to be eurythermal but some species are regarded as cold water taxa. Ceratopsyche has been changed to Hydropsyche.
Trichoptera	Leptoceridae	Oecetis	species	Some variability was noted in a pilot study in North Carolina (U.S. EPA, 2012). The species found in Kentucky are associated with warm water. In New Jersey, this genus is typically found in low gradient coastal plain streams.
Trichoptera	Philopotamidae	Chimarra	species	Some variability was noted in a pilot study in New England (U.S. EPA, 2012, unpublished data) but most species were warm-water oriented. *C. obscura* and *C. atterima* predominate, but tend to co-occur.
Trichoptera	Rhyacophilidae	Rhyacophila	species	Most species are cold water, but some variability has been documented in the Northeast (U.S. EPA, 2012, unpublished data).
Trichoptera	Uenoidae	Neophylax	species	Some variability was noted in a pilot study in North Carolina (U.S. EPA, 2012).

*non-target group in some states (e.g., MassDEP does not include it in its bioassessments)