**Table 1.** List of North Carolina cold water preference taxa. Distribution and abundance information is also included. Sum_Individuals=the total number of individuals from that taxon in the North Carolina database; Pct_Abund=percent of total individuals in the database comprised of that taxon; Num_Stations=number of stations in the database that the taxon occurred at; Pct_Stations=percent of stations in the database at which the taxon occurred.

| Type | Order | Family | FinalID | Sum_Individs | Pct_Abund | Num_Stations | Pct_Stations |
|---|---|---|---|---|---|---|---|
| cold | Ephemeroptera | Baetidae | Acentrella | 2745 | 0.33 | 427 | 15.19 |
| cold | Trichoptera | Glossosomatidae | Agapetus | 247 | 0.03 | 53 | 1.89 |
| cold | Plecoptera | Nemouridae | Amphinemura | 1210 | 0.14 | 281 | 10 |
| cold | Diptera | Tipulidae | Antocha | 5103 | 0.61 | 711 | 25.29 |
| cold | Trichoptera | Apataniidae | Apatania | 339 | 0.04 | 47 | 1.67 |
| cold | Trichoptera | Hydropsychidae | Arctopsyche | 222 | 0.03 | 40 | 1.42 |
| cold | Diptera | Athericidae | Atherix | 1236 | 0.15 | 240 | 8.54 |
| cold | Diptera | Chironomidae | Cardiocladius | 2300 | 0.27 | 376 | 13.38 |
| cold | Ephemeroptera | Heptagenidae | Cinygmula | 247 | 0.03 | 40 | 1.42 |
| cold | Plecoptera | Perlodidae | Clioperla | 574 | 0.07 | 155 | 5.51 |
| cold | Plecoptera | PERLODIDAE | Cultus | 296 | 0.04 | 70 | 2.49 |
| cold | Diptera | Chironomidae | Diamesa | 734 | 0.09 | 185 | 6.58 |
| cold | Diptera | Tipulidae | Dicranota | 1384 | 0.16 | 284 | 10.1 |
| cold | Plecoptera | Perlodidae | Diploperla | 393 | 0.05 | 122 | 4.34 |
| cold | Trichoptera | Philopotamidae | Dolophilodes | 2905 | 0.35 | 316 | 11.24 |
| cold | Ephemeroptera | EPHEMERELLIDAE | Drunella | 2846 | 0.34 | 218 | 7.76 |
| cold | Ephemeroptera | Heptageniidae | Epeorus | 5226 | 0.62 | 403 | 14.34 |
| cold | Diptera | CHIRONOMIDAE | Eukiefferiella | 2974 | 0.35 | 533 | 18.96 |
| cold | Trichoptera | Glossosomatidae | Glossosoma | 1755 | 0.21 | 309 | 10.99 |
| cold | Diptera | Chironomidae | Heleniella | 95 | 0.01 | 50 | 1.78 |
| cold | Plecoptera | PERLODIDAE | Isoperla | 4556 | 0.54 | 498 | 17.72 |
| cold | Odonata | Gomphidae | Lanthus | 1174 | 0.14 | 300 | 10.67 |
| cold | Plecoptera | Perlodidae | Malirekus | 753 | 0.09 | 132 | 4.7 |
| cold | Ephemeroptera | HEPTAGENIIDAE | Nixe | 64 | 0.01 | 16 | 0.57 |
| cold | Diptera | Chironomidae | Pagastia | 751 | 0.09 | 157 | 5.59 |

**Table 1**. continued…

| Type | Order | Family | FinalID | Sum_Individs | Pct_Abund | Num_Stations | Pct_Stations |
|---|---|---|---|---|---|---|---|
| cold | Trichoptera | Hydropsychidae | Parapsyche | 280 | 0.03 | 52 | 1.85 |
| cold | Diptera | CHIRONOMIDAE | Potthastia | 757 | 0.09 | 292 | 10.39 |
| cold | Coleoptera | Elmidae | Promoresia | 3020 | 0.36 | 332 | 11.81 |
| cold | Diptera | Chironomidae | Rheopelopia | 135 | 0.02 | 64 | 2.28 |
| cold | Ephemeroptera | Heptageniidae | Rhithrogena | 725 | 0.09 | 152 | 5.41 |
| cold | Plecoptera | Peltoperlidae | Tallaperla | 3337 | 0.4 | 377 | 13.41 |
| cold | Plecoptera | NEMOURIDAE | Zapada | 3 | 0 | 3 | 0.11 |

**Table 2**. List of North Carolina warm water preference taxa. Distribution and abundance information is also included. Sum_Individuals=the total number of individuals from that taxon in the North Carolina database; Pct_Abund=percent of total individuals in the database comprised of that taxon; Num_Stations=number of stations in the database that the taxon occurred at; Pct_Stations=percent of stations in the database at which the taxon occurred.

| Type | Order | Family | FinalID | Sum_Individs | Pct_Abund | Num_Stations | Pct_Stations |
|---|---|---|---|---|---|---|---|
| warm | Hemiptera | Belostomatidae | Belostoma | 173 | 0.02 | 99 | 3.52 |
| warm | Coleoptera | Hydrophilidae | Berosus | 1843 | 0.22 | 277 | 9.85 |
| warm | Isopoda | ASELLIDAE | Caecidotea | 3203 | 0.38 | 544 | 19.35 |
| warm | Trichoptera | Philopotamidae | Chimarra | 5178 | 0.62 | 554 | 19.71 |
| warm | Unionoida | UNIONIDAE | Elliptio | 1556 | 0.18 | 189 | 6.72 |
| warm | Odonata | Corduliidae | Epicordulia | 178 | 0.02 | 78 | 2.77 |
| warm | Arhynchobdellida | ERPOBDELLIDAE | ERPOBDELLA/ MOOREOBDELLA | 760 | 0.09 | 210 | 7.47 |
| warm | Rhynchobdellida | Glossiphoniidae | Helobdella | 835 | 0.1 | 225 | 8 |
| warm | Odonata | Corduliidae | Helocordulia | 188 | 0.02 | 95 | 3.38 |
| warm | Odonata | Calopterygidae | Hetaerina | 854 | 0.1 | 153 | 5.44 |
| warm | Odonata | Coenagrionidae | Ischnura | 318 | 0.04 | 101 | 3.59 |
| warm | Coleoptera | Dytiscidae | Lioporeus | 182 | 0.02 | 83 | 2.95 |

**Table 2**. continued…

| Type | Order | Family | FinalID | Sum_Individs | Pct_Abund | Num_Stations | Pct_Stations |
|---|---|---|---|---|---|---|---|
| warm | Odonata | Corduliidae | Macromia | 5064 | 0.6 | 813 | 28.92 |
| warm | Trichoptera | Hydropsychidae | Macrostemum | 1753 | 0.21 | 134 | 4.77 |
| warm | Trichoptera | Polycentropodidae | Neureclipsis | 2092 | 0.25 | 241 | 8.57 |
| warm | Odonata | Corduliidae | Neurocordulia | 1511 | 0.18 | 278 | 9.89 |
| warm | Diptera | Chironomidae | Nilothauma | 180 | 0.02 | 124 | 4.41 |
| warm | Decapoda | Palaemonidae | Palaemonetes | 2262 | 0.27 | 271 | 9.64 |
| warm | Diptera | Chironomidae | Parachironomus | 395 | 0.05 | 128 | 4.55 |
| warm | Diptera | Chironomidae | Pentaneura | 771 | 0.09 | 154 | 5.48 |
| warm | Trichoptera | Dipseudopsidae | Phylocentropus | 576 | 0.07 | 201 | 7.15 |
| warm | Basommatophora | Physidae | Physella | 6677 | 0.79 | 853 | 30.35 |
| warm | Rhynchobdellida | Glossiphoniidae | Placobdella | 677 | 0.08 | 339 | 12.06 |
| warm | Diptera | Chironomidae | Procladius | 3460 | 0.41 | 706 | 25.12 |
| warm | Diptera | Chironomidae | Stenochironomus | 3419 | 0.41 | 750 | 26.68 |
| warm | Odonata | CORDULIIDAE | Tetragoneuria | 687 | 0.08 | 202 | 7.19 |
| warm | Ephemeroptera | Leptohyphidae | Tricorythodes | 4939 | 0.59 | 363 | 12.91 |

**Development of the North Carolina cold and warm water preference lists**

**Sources**. The North Carolina cold and warm water lists were developed using several different sources: 1. maximum likelihood calculations based on a subset of the North Carolina biomonitoring database (done by Lei Zheng of Tetra Tech using full-scale collection method data); 2. the thermal preference trait from the Poff et al. 2006 traits matrix; 3. the thermal preference trait from the USGS traits database (Vieira et al. 2006); 4. the thermal preference trait from the compilation of EPA Environmental Requirements and Pollution Tolerance series from the late 1970's (Beck et al. 1977, Harris et al. 1978, Hubbard et al. 1978, Surdick et al. 1978); 5. best professional judgment of the Southeast Climate Change traits feedback group.

**Cold water designation**. Taxa were placed on the North Carolina cold water list if they met the following criteria: 1. They received a rank temperature optima value of 1 or 2 or 3 (the rank optima value is based on percentiles of the dataset; for these taxa, the weighted average optima value was less than the 0.4 percentile value of the dataset it was derived from); or 2. the thermal preference in the Poff et al. 2006 traits matrix was 'cold_cool'; or 3. The thermal preference in the USGS traits database (Vieira et al. 2006) was 'cold stenothermal' or 'cold-cool eurythermal' (temperature preference of less than 15°C); or 4. The thermal preference in the EPA Environmental Requirements and Pollution Tolerance series (which were interpreted by Jen Stamp) was 'oligothermal' or 'stenothermal' or 'metathermal' (temperature preference of less than 15°C); or 5. If anyone in the Southeast Climate Change feedback group felt a taxa should be added to this list.

**Warm water designation**. Taxa were placed on the North Carolina warm water list if they met the following criteria: 1. They received a rank temperature optima value of 5 or 6 or 7 (the rank optima value is based on percentiles of the dataset; for these taxa, the weighted average optima value was greater than the 0.6 percentile value of the dataset it was derived from); or 2. the thermal preference in the Poff et al. 2006 traits matrix was 'warm'; or 3. The thermal preference in the USGS traits database (Vieira et al. 2006) was 'hot euthermal' or 'warm eurythermal' (temperature preference of greater than 15°C); or 4. The thermal preference in the EPA Environmental Requirements and Pollution Tolerance series (which were interpreted by Jen Stamp) was 'euthermal' or 'eurythermal' or 'mesothermal' (temperature preference of greater than 15°C); or 5. If anyone in the Southeast Climate Change feedback group felt a taxa should be added to this list.

**Limitations**. These lists were developed using the best information available, but it should be noted that the available information is limited. The maximum likelihood calculations are based on instantaneous water temperature measurements that were taken at the time of the sampling event. Ideally continuous water temperature data could have been used, since this would provide more information about the thermal regime, especially during times of greatest thermal stress (i.e. summer baseflow conditions). Another limitation of the North Carolina dataset is that the abundance data is categorical (1=rare (1-2 specimens), 3=common (3-9 species) and 10=abundant (10 or more species). The calculations themselves also have limitations. One of the main concerns is that the analysis does not take into account the confounding factors ('noise') that are not related to temperature. However, with a sufficient amount of data, the noise

essentially cancels itself out. Another limitation is that the operational taxonomic unit that was most appropriate for this analysis is at the genus-level (in some instances, family-level was most appropriate). Within certain genera in particular, the thermal preference among species varies, so the assigned thermal preference may not be appropriate for all species within a genera. Attempts were made to note these genera (see 'species-variation' column in the worksheets).

Once again, we want to iterate that when we developed these lists, we did the best we could with the data that was available. These lists should be viewed as a first step, not a final product. It would be very helpful if future research included a combination of short and long-term field and experimental studies designed to better evaluate climate effects on freshwater ecosystems.

**Initial Results**. Initially there were 126 taxa on the cold water list and 112 taxa on the warm water list. These lists were based on maximum likelihood calculations and literature. These lists were further refined through the evaluation of additional evidence. This evidence included analyses of other datasets, case studies, and best professional judgment. Taxa with the greatest amount of evidence were assigned cold or warm water designations. More detailed information about the steps that were used to develop the lists is summarized below:

**Considerations**

**A.** Results from weighted average or maximum likelihood thermal optima and tolerance calculations were a major consideration. Results from the following eight analyses were used:

- California - Herbst and Silldorff (2007)
- Idaho - Brandt (2001)
- Maine – EPA GCRP Maine (2010) (based on site average temperature values (July-September) from 616 sites in the Maine biomonitoring database)
- North Carolina - EPA GCRP North Carolina (2010) (based on maximum likelihood calculations for the North Carolina biomonitoring database, full-scale collection method only)
- Ohio – Rankin and Yoder (2009)
- Oregon - Oregon DEQ (2008)
- Utah - EPA GCRP Utah (2010) (based on 572 fall samples from the Utah biomonitoring database)
- Western EMAP - Yuan (2006) (Estimation and Application of Macroinvertebrate Tolerance Values. Report No. EPA/600/P-04/116F)

A scoring system was developed to summarize results from the eight different analyses. It takes into account thermal preference, thermal tolerance and sample size. Scores were assigned (for each of the eight analyses) as follows:

**COLD WATER TAXA**

- 2=cold stenotherm (rank optima of 1 or 2 or 3 and rank tolerance of 1 or 2 or 3), adequate sample size (20 or more counts)
- 1=cold preference (rank optima of 1 or 2 or 3), adequate sample size (20 or more counts)
- 1=cold stenotherm (rank optima of 1 or 2 or 3 and rank tolerance of 1 or 2 or 3), low sample size (less than 20 counts)
- 0.5=cold preference (rank optima of 1 or 2 or 3), low sample size (less than 20 counts)

**WARM WATER TAXA**

- 2=warm eurythermal (rank optima of 5 or 6 or 7 and rank tolerance of 5 or 6 or 7), adequate sample size (20 or more counts)
- 1=warm preference (rank optima of 5 or 6 or 7), adequate sample size (20 or more counts)
- 1= warm eurythermal (rank optima of 5 or 6 or 7 and rank tolerance of 5 or 6 or 7), low sample size (less than 20 counts)
- 0.5=warm preference (rank optima of 5 or 6 or 7), low sample size (less than 20 counts)

In addition to the weighted average and maximum likelihood results, information on thermal preferences was also derived from literature. The taxon received a score of 1 if it was cited as a cold or warm water taxon in at least one of the following sources: Poff et al. 2006 traits matrix; or USGS traits database (Vieira et al. 2006); or EPA Environmental Requirements and Pollution Tolerance series from the late 1970's (Beck et al. 1977, Harris et al. 1978, Hubbard et al. 1978, Surdick et al. 1978). If the weighted average or maximum likelihood results showed the taxon to have a preference for cold or warm water but the literature showed conflicting results (i.e. based on the weighted average results, the taxon was a cold water taxa, but the literature showed it to be a warm water taxa), then the taxon was not included on the list.

After scores were assigned as described above, they were summed so that each taxon received a total score. The higher the total score, the more evidence there was in the eight analyses and the literature that supported the designation of the cold or warm water taxa.

**B**. Several 'case studies' were performed to see whether the cold or warm water taxa occurred at sites in North Carolina that had the warmest or coldest summer water temperatures. The following case studies were performed:

a. **Cold Water Case Study #1**. Taxa lists from two Blue Ridge reference sites(NC1560-BEAR CR and NC1561-HAZEL CR) that have full-scale collection method data, have <5% urban and <10% agricultural land use within a 1 km buffer, and have the coldest recorded summer water temperatures (13-14° C in July). Note: there were a number of sites with temperature readings of 0°C; these readings seemed questionable so they were not used.

b. **Cold Water Case Study #2**. The taxa list from the Piedmont site (NC0634-TOWN FORK CR) that has full-scale collection method data and has the coldest recorded Piedmont summer water temperature (9° C in August). This site has 4.3% urban and 11% agricultural land use within a 1 km buffer.

c. **Cold Water Case Study #3**. Taxa lists from three Piedmont reference sites (NC0248-BARNES CR, NC0713-CATAWBA R, NC1607-MARLOWE CR) that have full-scale collection method data, have <5% urban and <10% agricultural land use within a 1 km buffer, and have the coldest recorded summer water temperatures (16-17° C in August and September).

d. **Warm Water Case Study #1**. Taxa lists from the two warmest reference sites in the state (NC1466-CAPE FEAR R and NC1467-CAPE FEAR R) that have full-scale collection method data, have <5% urban and <10% agricultural land use within a 1 km buffer, and have the warmest recorded summer water temperatures (30-32° C in July).

e. **Warm Water Case Study #2**. Taxa lists from the two Piedmont reference sites (NC0219-TAR R and NC0573-DEEP R) that have full-scale collection method data, have <5% urban and <10% agricultural land use within a 1 km buffer, and have the warmest recorded summer water temperatures (28-29° C in July).

f. **Warm Water Case Study #3**. Taxa list from the warmest Blue Ridge reference site(NC1285-CROOKED CR) that has full-scale collection method data, has <5% urban and <10% agricultural land use within a 1 km buffer, and has the warmest recorded summer water temperature (24° C in July).

**C.** In addition to the case studies, best professional judgment from the Southeast Climate Change group[1] was taken into account.

[1] Southeast Climate Change group: Trish MacPherson (formerly NC DNR), Jim Glover (South Carolina DHEC), Debbie Arnwine (Tennessee)

**Development of the Cold Water Taxa List.** Taxa were placed on the cold water list if the following criteria were met:

1. The taxon received a 'yes' per best professional judgment AND has been recorded at one or more of the cold water case study sites AND has NOT been recorded at either of the two warm water case study sites.

2. The taxon received a 'yes' per best professional judgment AND received a Total Score of 5 or more.

3. The taxon received a 'no comment' per best professional judgment AND has been recorded at two or more of the cold water case study sites AND no species variation was noted.

**Development of the Warm Water Taxa List.** Taxa were placed on the warm water list if the following criteria were met:

1. The taxon received a 'yes' per best professional judgment AND has been recorded at one or more of the warm water case study sites AND has NOT been recorded at either of the two cold water case study sites.

2. The taxon received a 'yes' per best professional judgment AND received a Total Score of 5 or more.

3. The taxon received a 'no comment' per best professional judgment AND has been recorded at two or more of the warm water case study sites AND no species variation was noted.

4. The taxon received a 'no comment' per best professional judgment AND received a Total Score of 5 or more AND has been recorded at one or more warm water case study sites AND NOT at any of the cold water case study sites AND no species variation was noted.

**Cold and Warm Water Lists.** The cold water taxa list was comprised of 32 taxa and the warm water taxa list was comprised of 27 taxa. Lists can be found in Tables 1 & 2.

**Important Notes – variation within genera**. Some noteworthy genera were left off the North Carolina cold water taxa list. These included Ephemerella, Neophylax, Rhyacophila, Goera, Eurylophella and Paragnetina. The reason they were not included is because there is variation in temperature preferences among species within these genera, and this was noted by the Southeast Climate Change feedback group. Genera that were left off the warm water list due to species variation included Hydropsyche, Oecetis and Polypedilum.

**Abundance and Distribution**. In addition to dispersal ability, abundance and distribution are also important considerations. Those taxa that are widespread and common are likely to have greater genetic diversity and greater chance of adapting than rare taxa that only occur in isolated, localized populations (Sweeney et al. 1992). Moreover, the more abundant taxa are more likely to affect the state biomonitoring assessments. Abundance and distribution information for the cold and warm water taxa can be found in Tables 1 & 2. It should be noted once again that the abundance data in the North Carolina dataset is categorical (1=rare (1-2 specimens), 3=common (3-9 species) and 10=abundant (10 or more species).

The most abundant cold water preference taxa are Epeorus (Ephemeropteran), Antocha (Dipteran), Isoperla (Plecopteran) and Tallaperla (Plecopteran). These taxa comprise only 0.4 to 0.6% of the total individuals in the North Carolina database. Seventeen of the cold water taxa have overall abundances of less than 0.1%. Physella (Basommatophora), Chimarra (Trichopteran) and Macromia (Odonata) are the most abundant warm water taxa, with overall abundances ranging from 0.6 to 0.8%. Twelve of the warm water taxa have overall abundances of less than 0.1%. Of the cold water taxa, Antocha occurs at the largest percentage of sites (25%), followed by a Chironomidae, Eukiefferiella, and a Plecopteran, Isoperla, which occur at 18-19% of the sites. Eighteen of the cold water taxa occur at less than 10% of the sites. Among the warm water taxa, Physella occurs at the highest percentage of sites (30%), followed by an Macromia (29%) and Stenochironomus (27%). Nineteen of the warm water taxa occur at less than 10% of the sites.

**Additional information – Cold Water Taxa.** Ten of the cold water taxa are Dipterans, eight are Plecopterans, six are Ephemeropteran and six are Trichopterans (Table 3a). The rest are Coleopterans and Odonates. The families with the most number of taxa on the cold water list are Chironomidae, Perlodidae and Heptageniidae (Table 3b).

**Additional information – Warm Water Taxa.** Seven of the warm water taxa are Odonates, five are Dipterans and four are Trichopterans (Table 4a). The families with the most number of taxa on the warm water list are Chironomidae and Corduliidae (Table 4b).

**Table 3a**. Number of cold water taxa in each order.

| Order | Total |
|---|---|
| Diptera | 10 |
| Plecoptera | 8 |
| Ephemeroptera | 6 |
| Trichoptera | 6 |
| Coleoptera | 1 |
| Odonata | 1 |

**Table 3b**. Number of cold water taxa in each family.

| Family | Total |
|---|---|
| Chironomidae | 7 |
| Perlodidae | 5 |
| Heptageniidae | 4 |
| Glossosomatidae | 2 |
| Hydropsychidae | 2 |
| Nemouridae | 2 |
| Tipulidae | 2 |
| Apataniidae | 1 |
| Athericidae | 1 |
| Baetidae | 1 |
| Elmidae | 1 |
| Ephemerellidae | 1 |
| Gomphidae | 1 |
| Peltoperlidae | 1 |
| Philopotamidae | 1 |

**Table 4a**. Number of warm water taxa in each order.

| Order | Total |
|---|---|
| Odonata | 7 |
| Diptera | 5 |
| Trichoptera | 4 |
| Coleoptera | 2 |
| Rhynchobdellida | 2 |
| Arhynchobdellida | 1 |
| Basommatophora | 1 |
| Decapoda | 1 |
| Ephemeroptera | 1 |
| Hemiptera | 1 |
| Isopoda | 1 |
| Unionoida | 1 |

**Table 4b**. Number of warm water taxa in each family.

| Family | Total |
|---|---|
| Chironomidae | 5 |
| CORDULIIDAE | 5 |
| Glossiphoniidae | 2 |
| Asellidae | 1 |
| Belostomatidae | 1 |
| Calopterygidae | 1 |
| Coenagrionidae | 1 |
| Dipseudopsidae | 1 |
| Dytiscidae | 1 |
| ERPOBDELLIDAE | 1 |
| Hydrophilidae | 1 |
| Hydropsychidae | 1 |
| Leptohyphidae | 1 |
| Palaemonidae | 1 |
| Philopotamidae | 1 |
| Physidae | 1 |
| Polycentropodidae | 1 |
| Unionidae | 1 |