

**Congruence between nutrient water quality parameters and Chironomidae
(Diptera) in delineating ecological classifications of Missouri streams
at different environmental scales.**

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by

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SUMMARY

1. Large scale classifications such as nutrient ecoregions have been established in the United States to help regulate surface water resources. These classifications and their constituent classes are hypotheses which may be tested by establishing the strength of the classes to differentiate within class and between class variation. The purpose of our study was to test the strength of three different classification schemes at three different scales: landscape (nutrient ecoregion), watershed (hydrologic unit), and microhabitat (substrate) using Chironomidae community assemblage and metric data and nutrient water quality data. Furthermore, we tested for congruence of classes using nutrient water quality and chironomid community data for the three classification schemes in an attempt to link the biota to nutrient concentrations.
2. Historical data on chironomid communities were compiled from the 20 stream sites in Missouri over multiple years and seasons. Nutrient water quality data were also collected at these sites but under a different study. An analysis of variance was used to determine whether chironomid metrics and nutrient water quality parameters varied significantly between classes for each of the classification schemes. Chironomid community assemblages and metrics and nutrient water quality parameters were used to test the strength of each classification. A mantel test for congruence was used to determine how congruent the classes were for each classification scheme based on three data matrices: chironomid community assemblages; chironomid metrics; and nutrient water quality parameters.
3. The three classification schemes were robust enough to show significant variation between nutrient water quality concentrations, chironomid community assemblages, and chironomid metrics. The classifications based on the nutrient data were strong and the classifications based on the chironomid assemblage and metric data were weak, although comparable to classification strengths based on entire macroinvertebrate communities and fish communities for regional, watershed/basin, and substrate classes.
4. Despite relatively weak classification based on chironomid data, all data were able to separate out the different nutrient ecoregions. Variation from classes of watershed and basin that transcended nutrient ecoregions and from substrates at a very fine environmental filter were not strong enough to break up the grouping of nutrient ecoregions, and therefore were not responsible for the relatively weak classification strengths observed. Finally, the chironomid metric and assemblage data were significantly congruent with the nutrient data but were not strongly correlated indicating that nutrient and biological data can be combined under the nutrient ecoregion classification, but with some caution.

Keywords: Nutrient ecoregions, classification strength, Chironomidae, mantel test, substrate, hydrologic units

Introduction

Classifications are useful tools in aquatic ecology and biological assessment because they allow researchers to examine communities of aquatic organisms within naturally occurring regions at varying scales. Regional classification schemes have been useful in delineating communities of fish (Rohm *et al.*, 1987; Whittier *et al.*, 1988; Van Sickle and Hughes, 2000) and macroinvertebrates (Whittier *et al.*, 1988; Rabeni and Doisy, 2000; Sandin and Johnson, 2000; Johnson, 2000; Mykra *et al.*, 2004). However, tests of the strength of *a priori* classifications such as ecoregions have shown that some classes are weak, particularly between relatively similar ecoregions (Hawkins and Vinson, 2000; Gerritsen *et al.*, 2000), but classes increased in strength when combining similar ecoregions and then comparing them to markedly different ecoregions (Gerritsen *et al.*, 2000). It is likely that some classification schemes will be very useful for water resource managers to identify natural variation between classes; whereas other classifications will be weak due to within class stream heterogeneity (Hawkins and Vinson, 2000; Mykra *et al.*, 2004).

Nutrient ecoregions are a regional scale classification for use in managing U.S. surface waters for nutrient criteria (Gibson *et al.*, 2000). Classes of nutrient regions were formed from aggregates of level III ecoregions (Omernik 1987) and were based on natural and anthropogenic features that affect nutrient loads (Gibson *et al.*, 2000). Although formed for use in creating benchmarks for concentrations of total nitrogen, total phosphorus, chlorophyll *a* and turbidity, they may also prove useful in creating benchmarks for biocriteria; thus, establishing congruence between water quality parameters and community assemblage and biological metric data may provide supporting evidence for a causal link between variation in nutrient criteria and biological communities within the regulatory framework of nutrient ecoregions and nutrient benchmarks.

Classifications are based on three hypotheses: one, that the class is a naturally occurring class rather than a convenience class (e.g. Wiley, 1981); two, that researchers accurately delineate the naturally occurring class (Omernik and Bailey, 1997); and three, that environmental filters working at the scale of the classification select for the communities present within the classification (Chessman and Royal, 2004). Environmental filters are factors that determine the distribution and abundance of species and consequently, the composition of biological communities. Environmental filters can be either physical (e.g. substrate) or chemical (e.g. nutrient concentrations). Assuming that the first hypothesis is correct, we can test the second and third hypotheses for nutrient ecoregions by looking for significant variation in Chironomidae (Diptera) and select nutrient water quality constituent values between regions and by testing the strength of the classes of nutrient ecoregions and of classes at different scales of environmental filters such as substrate and watershed.

Chironomidae are the aquatic insect of focus in this study because they are an abundant, widespread, and heterogeneous group of flies whose larvae inhabit nearly every type of aquatic habitat (Oliver, 1971; Armitage *et al.*, 1995) and they often are numerically dominant in aquatic ecosystems, for example, comprising upwards of 50% of the abundance in streams (Coffman and Ferrington, 1996). Chironomidae may also be numerically dominant in non-stressed to pristine streams or are co-dominant with taxa typically thought to be indicative of high quality habitat such as Ephemeroptera (e.g. Pringle and Ramirez, 1998; Burgherr *et al.*, 2002; Ruegg and Robinson, 2004). Chironomidae are particularly important to study in the Midwestern United States with over 325 species listed for the Great Plains (Hayford and Bouchard, unpublished data). Finally, chironomids show differential preferences for different substrates (Francis and Kane, 1995; Hayford and Ferrington, 2006), which make them useful in testing the robustness of stream classifications based on substrate.

The objective of our study is to determine the efficacy of nutrient ecoregions in Missouri in finding natural differences in nutrient water quality concentrations and biota, specifically Chironomidae, and to search for sources of variation within these large scale classifications by also

looking at one very fine scale classification, substrate, and one classification, hydrologic units, that crosses nutrient ecoregional boundaries. The goals of this study are to:

1. Test the strength of nutrient ecoregion classification for Missouri with chironomid community and selected water quality parameter data.
2. Search for sources of variation within the Missouri nutrient ecoregion classes by testing the strength of a classification at a very fine scale, substrate type.
3. Test the strength of a classification using HUC designations, a classification scheme unrelated to nutrient ecoregions.
4. Test for congruence of classes using water quality and chironomid community data for the three classification schemes in an attempt to link the biota to nutrient concentrations.

Methods and materials

Data set

Historical data on chironomid communities and nutrient water quality parameters were compiled from the same stream sites in Missouri, but under two different studies. Reference stream nutrient and chironomid data were used to test the classifications of nutrient ecoregions, hydrologic units, and substrate. The Central Plains Center for BioAssessment gathered biological assessment data from state environmental agencies in EPA Region 7 into an historical database (1984-2002) of site macroinvertebrate data from possible reference sites. Only data from one state, Missouri, and one agency, the Missouri Department of Natural Resources were used in this study to reduce variability in sampling and laboratory methodology. Specimens were collected using a 500 μm x 500 μm mesh kick net with approximately one square meter substrate disturbed immediately upstream of the net. Six samples representing various depths, velocities and substrate types were collected and pooled from each site. Data from Missouri were chosen due to the high taxonomic resolution of chironomid identifications and the quality assurance procedures used for these identifications. Data were further confined to those sites that targeted the same types of habitat, riffle/runs or glide/pools, and had been collected for 3 or more sampling events. All sites used in this study had been collected during both spring and fall at least over one year, and most had multi-year sampling. Thus, the data set was comprised of 123 samples representing multi-season, multi-year data from 20 sites (Table 1). Data from all samples at a single site were pooled and chironomid community matrices were constructed, and mean abundances were calculated for each taxon. Absolute abundances were not used in the analysis given the unequal number of sampling events between sites. Mean abundance values, were then used to calculate chironomid metrics and indices. Nutrient ecoregion, HUC, and substrate type were recorded for each site (Table 1).

Water quality data were collected from these same sites from 1999-2002 by the Central Plains Center for BioAssessment under a different study. Nutrients were analyzed from the water quality samples as part of the EPA Region 7 nutrient criteria study following standard EPA protocols and included total phosphorus (TP in $\mu\text{g/L}$), total nitrogen (TN in mg/L), chlorophyll *a* (CHLA in $\mu\text{g/L}$), and turbidity (TURB in NTU). Ratios of TN:TP, CHLA:TP, and CHLA:TN were calculated based on the raw data. Median values from two or more collection events per site were used in this analysis. The assumption on which this analysis is based is that although the biotic data and nutrient data do not completely co-occur in time, chemical conditions in the streams remain relatively unchanged over long periods of time. This assumption underlies the use of nutrient ecoregions as a management tool for setting nutrient water quality standards.

Table 1. Site locations, site codes, and classifications. See text for meaning of codes.

Site	# of samples	County	Latitude	Longitude	Nutrient Ecoregion	HUC	Substrate
1554	5	Dekalb	39.87531	-94.24314	9	1028	4
1568	12	Vernon	37.809	-94.3918	9	1029	6
1569	3	Clark	40.469	-91.7864	9	714	4
1575	8	Putnam	40.487	-93.1309	9	1028	4
1582	9	Davies	39.85903	-93.95003	9	1028	4
1635	7	Adair	40.25286	-92.75708	9	1028	4
1647	4	Sullivan	40.0802	-93.2245	9	1028	1.5
1634	5	Marion	39.7482	-91.537	9	714	2
1580	3	Dunklin	36.32417	-90.00555	10	802	4
1581	3	Mississippi	36.7608	-89.4279	10	802	4
1520	4	Franklin	38.5342	-91.3081	11	1029	3
1528	5	Madison	37.55803	-90.15256	11	714	2.3
1560	7	Crawford	37.82123	-91.17998	11	714	2
1566	4	Ripley	36.7078	-90.6905	11	1107	2
1571	11	Hickory	37.988	-93.1005	11	1029	3
1572	7	Phelps	37.8108	-91.8408	11	1029	2
1586	9	McDonald	36.6263	-94.1817	11	1107	2.3
1586	6	McDonald	36.6215	-94.1801	11	1107	2.3
1588	7	Phelps	37.87205	-91.92923	11	1029	2
1622	4	Ste. Genevieve	37.7966	-90.0526	11	714	2.3

Study Area and Classification Schemes

Missouri is composed of three level III nutrient ecoregions (Gibson *et al.*, 2000), four level one Hydrologic Unit (HUC) watersheds (Seaber *et al.*, 1987). We developed six classes of substrate by modifying substrate size designations outlined in the EPA Rapid Bioassessment Protocol (Barbour *et al.*, 1999).

Nutrient ecoregions were designated to assist in creating nutrient criteria for lakes and streams (Gibson *et al.*, 2000). Missouri contains three nutrient ecoregions. The Southeastern Temperate Forested Plains and Hills nutrient ecoregion (9) has a mixture of forests, row crop, and pastureland. Agricultural land use, in particular poultry and pig operations result in increased nutrient runoff in the region's streams. The Texas-Louisiana Coastal and Mississippi Alluvial Plains nutrient ecoregion (10) is a rich alluvial plains region currently supporting extensive row crop agriculture. Agriculture and localized areas of urban development have resulted in nutrient runoff into the region's streams. Central and Eastern Forest Uplands nutrient ecoregion (11) has forested hills and valleys, fast flowing streams, and usually less agriculture relative to surrounding nutrient ecoregions. However, poultry, aquaculture, and pig operations create point sources of nutrient inflow into the region's streams (Gibson *et al.*, 2000).

The four HUCs used in this study include the upper Mississippi watershed (Region 07), the lower Mississippi watershed (Region 08), the Missouri watershed (Region 10) and the Arkansas-White-Red watershed (hereafter referred to as the Arkansas) (Region 11) (Seaber *et al.*, 1987). Study sites were located in two upper Mississippi watershed level two basins, 711 and 714; one lower Mississippi watershed level two basin, 802; three Missouri watershed level two basins, 1028, 1029, 1030; and, two Arkansas watershed level two basins: 1101 and 1107.

Substrate data were collected for each site. We created a code to represent dominant substrate at each site such that Bedrock/Hardclay =1; Cobble =2; Gravel =3; Sand =4; Mud =5; and Silt=6. In many instances the dominant substrate was mixed and thus would receive a decimal code. For example, if the dominant substrate was cobble at 50% followed by gravel at 40% then the substrate code for that site would be 2.3 with the first number representing the substrate present in the highest percentage and the second being the next highest percentage. If a substrate at a site was clearly dominant, then it did not receive a decimal score.

Data analysis

Metrics and indices were selected based on their common usage in biological assessment, to explore new metrics for their utility in differentiation between classes in the proposed classification schemes or based on their habitat or substrate specificity and what this means in terms of both classifications and biological assessments (Table 2). For example, both *Stempelinella* and *Stempellina* are common inhabitants of springs or spring fed streams (Pinder and Reiss, 1983) and it was hypothesized that they would be useful in differentiating between streams in nutrient ecoregion 11 which contained spring fed streams in the Ozark Highlands, and nutrient ecoregions 10 and 9, which are more likely to have surface water dominated streams. Also, many genera within the *Harnischia* complex (Chironominae: Chironomini) are thought to be facultative or obligate psammonophiles (Pinder and Reiss 1983, Barton and Smith 1984) and thus should be good indicators of streams with sandy substrate. Metrics were calculated based on abundance data averaged over the number of sampling events for a site. Most metrics were based on relative percentages of a taxonomic group and/or on their richness values, which did not incorporate abundances. Metrics were examined for redundancy by examining Spearman rank correlations. The less informative of two metrics that were correlated at 0.80 or higher was removed from further analysis. This resulted in the loss of only two metrics: Orthocladiinae richness and taxonomic richness. Correlations were performed using Number Crunching Statistical Software (Hintze, 2000).

Table 2 Candidate metrics examined for significant variation between the classes in the three different classification schemes.

Tanypodinae Richness	Percent <i>Harnischia</i> of Chironomini
Percent Tanypodinae	Percent <i>Harnischia</i> of Chironomini Richness
Percent Tanypodinae Taxa	Percent <i>Harnischia</i> of total richness
Diamesinae Richness	Tanytarsini Richness
Percent Diamesinae	Percent Tanytarsini
Percent Diamesinae Taxa	Percent Tanytarsini Taxa
Orthocladiinae Richness	Percent <i>Stempelinella</i>
Percent Orthocladiinae	Chironomid Richness
Percent Orthocladiinae taxa	Percent Chironomid Richness of Total Richness
Percent <i>Cricotopus</i> of Orthocladiinae	KBI Organic
Chironomini Richness	Shannon Wiener
Percent Chironomini	Evenness
Percent Chironomini Taxa	Taxa Richness
Percent Pseudochironomini	

Three tests were used to determine whether variables were normally distributed: Shapiro-Wilk W, Anderson-Darling, Martinez-Iglewicz. In order to run analysis of variance (ANOVA), a class (= treatment) had to contain data from at least three sites. Nutrient ecoregion 10, and three substrate categories, 1, 5, 3, and 6 had less than three sites so were not used in ANOVAs. There were not enough sites in the HUC level 2 units (e.g. 3 or more sites) to run ANOVAs on that level, rather than

loose too much data, HUC categories were combined for ANOVA such that level 1 hydrologic units remained. However, this still resulted in the removal of HUC 802 which had no other level 2 units in the study and which only had two sites. The remaining HUC sites were combined in the following way: 711 and 714 sites were combined into 714, 1030 was converted to 1028, and 1101 was converted to 1107. Thus, the HUC analysis was run mostly on level 1 hydrologic units, with the exception of the Missouri hydrologic unit (10), which had two level 2 regions.

The General Linear Model ANOVA (GLM ANOVA) was used to test for statistically significant variation between classes in nutrient parameters and chironomid metrics and indices. The GLM ANOVA allows for unequal sample size per treatment and for comparison of multiple treatments. The *a posteriori* Bonferroni Test was used to detect significant differences between all possible paired treatments. Significance was determined at $P \leq 0.05$. The GLM ANOVA was performed using NCSS (Hintze, 2000).

Bray-Curtis index, a quantitative measure of similarity, was used to examine classifications based on median values of nutrients, metric and index values, and dominant taxa. Bray-Curtis similarity index was used to facilitate comparison with other classifications based on macroinvertebrates and fish. Similarity indices were calculated using Poptools (Hood, 2000). Classification strengths of the various classification schemes were calculated following the algorithm proposed by Van Sickle (1997) and Van Sickle and Hughes (2000) in which the average between site similarities, \bar{B} , should be less than the sum of the weighted within site similarities \bar{W} . \bar{W} is calculated as:

$$\bar{W} = \sum_i (n_i / N) W_i$$

Where n_i is the number of sites in class i , and N is the total number in all classes. The classification strength or CS is then calculated either as $CS = \bar{W} - \bar{B}$ or as $M = \bar{B} / \bar{W}$. Classification strengths were calculated in MS. Excel. Some classes removed for the GLM ANOVA were used for the analysis of classification strengths (CS) of the three classifications. However, a class still needed to have at least two sites within it to be used in the CS analyses.

Water quality and chironomid data were collected from the same sites but on different dates, thus data could not be combined. These data were used separately to determine how well *a posteriori* clustering of the data matched the *a priori* classifications used in this study. Clusters were based on Unweighted Paired Group Averages (UPGMA) using Euclidian distances. The Cophenetic Correlation Coefficient was used to determine the degree of fit between the data and the resulting dendrogram. The resulting dissimilarity matrices used in the cluster analyses were then compared via a congruence test. Hierarchical clustering and dissimilarity matrices were run and created using NCSS (Hintz, 2000).

Congruence tests can determine how similar different datasets are at creating classifications. Congruence of matrices for the same classifications but based on different datasets was calculated using a Mantel test which also tests for significance of the correlation relative to correlations values based on a pre-specified number of correlations from random mixes of the data. Mantel tests were run in Poptools (Hood, 2000) with 999 iterations of randomly mixed data. Data did not have a normal distribution so non-parametric Quantile single t-test was run to determine whether the correlation between two datasets was significantly different from that which would occur by chance.

Results

Water quality, metric, and chironomid assemblage data

Five of the seven water quality constituents studied had normal distributions, of which only TN, TP, and CHLA varied significantly between classes within at least one of the classifications schemes (Table 3). All three varied significantly between the two classes of nutrient ecoregions, TP and CHLA varied significantly between classes of substrate, and only CHLA varied significantly

between HUC classes (Table 3). These three parameters were used as the basis for examination of the relative strengths of classifications based on median nutrient concentrations.

Table 3 GLM ANOVA of nutrient parameters by classification scheme. Numbers are p-values with numbers in bold significant at $P \leq 0.05$.

Nutrient	Normal	NR	HUC	Substrate
TN	Y	0.0400	0.5581	0.9700
TP	Y	0.0002	0.1367	0.0010
CHLA	Y	0.0000	0.0085	0.0128
Chloro-a:TN	Y	0.6930	0.7474	0.3343
Chloro-a:TP	Y	0.4869	0.1924	0.2054

All chironomid metrics met assumptions of having a normal distribution. Eleven of the twenty-five metrics varied significantly between classes in at least one of the three classifications schemes (Table 4). Only metrics based on the percent of the genera *Harnischia* and *Stempelinella* varied significantly between HUC classes, whereas 9 metrics varied significantly between classes of nutrient ecoregions and 8 metrics varied significantly between classes of substrate (Table 4). Some metrics commonly used in biological assessment did not vary significantly, such as Chironomid Richness, KBI organic, Shannon-Wiener diversity, and Evenness (Table 4).

Table 4. GLM ANOVA of chironomid community metrics by classification. Numbers are P -values and those in bold denote significance of $P < 0.05$. Metrics in bold denote those used in analysis of classification strengths.

Metric	NR	HUC	Substrate
Tanypodinae Richness	0.54	0.32	0.52
Percent Tanypodinae	0.28	0.53	0.18
Percent Tanypodinae Taxa	0.30	0.18	0.95
Diamesinae Richness	0.10	0.66	0.06
Percent Diamesinae	0.10	0.62	0.00
Percent Diamesinae Taxa	0.04	0.25	0.01
Percent Orthocladiinae	0.86	0.87	0.76
Percent Orthocladiinae taxa	0.04	0.14	0.02
Percent Cricotopus of Orthocladiinae	0.09	0.08	0.07
Chironomini Richness	0.00	0.06	0.19
Percent Chironomini	0.07	0.04	0.07
Percent Chironomini Taxa	0.00	0.08	0.00
Percent Pseudochironomini	0.88	0.20	0.73
Percent Harnischia of Chironomini	0.51	0.60	0.31
Percent Harnischia of Chironomini Richness	0.15	0.15	0.08
Percent Harnischia of total richness	0.01	0.04	0.01
Tanytarsini Richness	0.05	0.55	0.05
Percent Tanytarsini	0.60	0.70	0.54
Percent Tanytarsini Taxa	0.02	0.09	0.00
Percent Stempelinella like	0.01	0.02	0.01
Chironomid Richness	0.92	0.58	0.64
Percent Chironomid Richness of Total Richness	0.02	0.15	0.06
KBI Organic	0.11	0.13	0.27
Shannon Wiener	0.43	0.35	0.37
Evenness	0.43	0.67	0.70

A total of 90 chironomid taxa, identified to the genus or species group and representing five subfamilies, were used in this analysis (Table 5). Twenty-three taxa were considered dominant in the study with percent abundances of 1 % or higher. Dominant taxa were used as community assemblage data to examine the classification strengths of the three classification schemes.

Table 5. Taxonomic Diversity of Chironomidae from Missouri. Richness by subfamily or tribe is in parentheses beside that subfamily or tribe. Dominant taxa are denoted in bold.

TANYPODINAE (12)	ORTHOCLADIINAE (30)	CHIRONOMINAE (44)	CHIRONOMINAE cont'd
<i>Ablabesmyia</i>	<i>Brillia</i>	Chironomini (35)	<i>Saetheria</i>
<i>Clinotanytus</i>	<i>Cardiocladius</i>	<i>Axarus</i>	<i>Stelechomyia</i>
<i>Krenopelopia</i>	<i>Corynoneura</i>	<i>Chironomus</i>	<i>Stenochironomus</i>
<i>Labrundinia</i>	<i>Cricotopus bicinctus</i>	<i>Cladopelma</i>	<i>Stictochironomus</i>
<i>Larsia</i>	<i>Diplocladius</i>	<i>Cryptochironomus</i>	<i>Tribelos</i>
<i>Nilotanytus</i>	<i>Epoicocladius</i>	<i>Cryptotendipes</i>	<i>Xenochironomus</i>
<i>Paramerina</i>	<i>Eukiefferiella</i>	<i>Demicryptochironomus</i>	Pseudochironomini (1)
<i>Pentaneura</i>	<i>Eukiefferiella brevicar group</i>	<i>Dicrotendipes</i>	<i>Pseudochironomus</i>
<i>Procladius</i>	<i>Eukiefferiella discoloripes group</i>	<i>Endochironomus</i>	Tanytarsini (8)
<i>Tanytus</i>	<i>Eukiefferiella gracei group</i>	<i>Endotribelos</i>	<i>Cladotanytarsus</i>
<i>Thienemannimyia group</i>	<i>Eukiefferiella pseudomontana group</i>	<i>Glyptotendipes</i>	<i>Micropsectra</i>
<i>Zavrelimyia</i>	<i>Heterotrissocladius</i>	<i>Goeldichironomus</i>	<i>Paratanytarsus</i>
DIAMESINAE (3)	<i>Hydrobaenus</i>	<i>Harnischia</i>	<i>Rheotanytarsus</i>
<i>Diamesa</i>	<i>Mesocricotopus</i>	<i>Kiefferulus</i>	<i>Stempellina</i>
<i>Potthastia</i>	<i>Mesosmittia</i>	<i>Lauterborniella</i>	<i>Stempellinella</i>
<i>Sympotthastia</i>	<i>Nanocladius</i>	<i>Lipinella</i>	<i>Sublettea</i>
PRODIAMENSINAE (1)	<i>Orthocladius (Euorthocladius)</i>	<i>Microtendipes</i>	<i>Tanytarsus</i>
<i>Monodiamesa</i>	<i>Orthocladius (Symposiocladius)</i>	<i>Nilothauma</i>	
	<i>Parakiefferiella</i>	<i>Omisus</i>	
	<i>Parametriocnemus</i>	<i>Parachironomus</i>	
	<i>Paraphaenocladius</i>	<i>Paracladopelma</i>	
	<i>Psectrocladius</i>	<i>Paralauterborniella</i>	
	<i>Pseudosmittia</i>	<i>Paratendipes</i>	
	<i>Rheocricotopus</i>	<i>Phaenopsectra</i>	
	<i>Smittia</i>	<i>Polypedilum</i>	
	<i>Synorthocladius</i>	<i>Polypedilum convictum group</i>	
	<i>Thienemanniella</i>	<i>Polypedilum fallax group</i>	
	<i>Tvetenia</i>	<i>Polypedilum halterale group</i>	
	<i>Tvetenia bavarica group</i>	<i>Polypedilum illinoense group</i>	
	<i>Xylotopus</i>	<i>Polypedilum scalaenum group</i>	

Classification Strengths

In general, the HUC classification scheme had the lowest classification strength, CS, ranging from 0.04 for the classification based on chironomid assemblages to 0.08 for the classification based on nutrient concentrations. The nutrient ecoregions had the highest CS, ranging from 0.08 for classification based on both chironomid metric and assemblage data to 0.31 for classification based on nutrient concentrations (Table 6). The classification strengths of the substrate classification scheme were similar to those for the HUC classification, ranging from a CS of 0.04 based on chironomid metric data to a CS of 0.14 based on nutrient concentration data. Similarities based on nutrient data produced the strongest classifications for each of the classification schemes (Table 6).

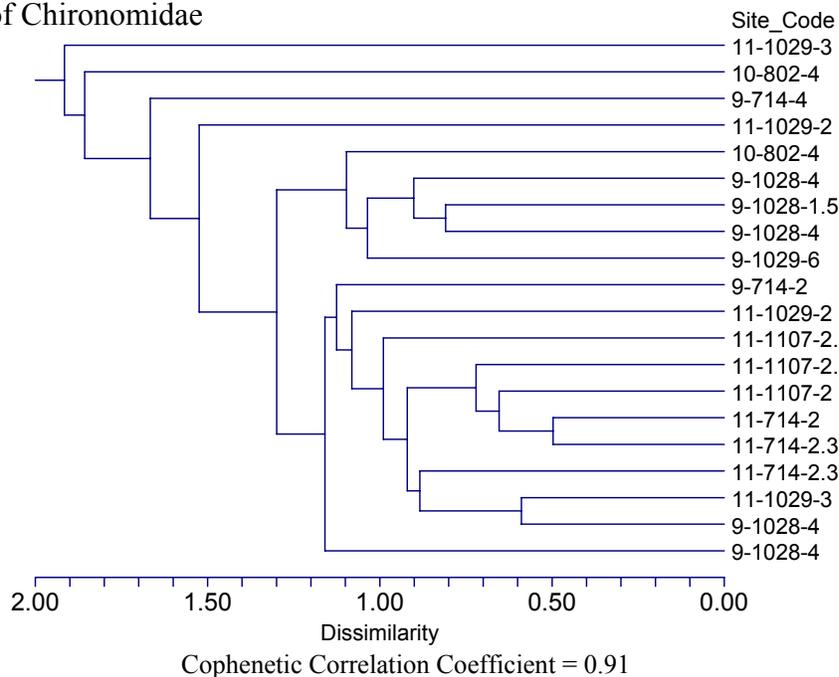
Table 6. Classification strengths of the nutrient region (NR), watershed hydrologic unit (HUC), and substrate classification schemes based on chironomid community metrics, assemblages, and nutrient water quality parameters (NWQ). All calculations are based on Bray-Curtis similarities.

NR	\bar{B}	\bar{W}	$\bar{W} - \bar{B}$ (CS)	\bar{B} / \bar{W}
Metrics	0.79	0.87	0.08	0.91
Assemblages	0.79	0.87	0.08	0.91
NWQ	0.41	0.72	0.31	0.57
HUC	\bar{B}	\bar{W}	$\bar{W} - \bar{B}$ (CS)	\bar{B} / \bar{W}
Metrics	0.82	0.87	0.05	0.94
Assemblages	0.81	0.85	0.04	0.95
NWQ	0.49	0.57	0.08	0.86
Substrate	\bar{B}	\bar{W}	$\bar{W} - \bar{B}$ (CS)	\bar{B} / \bar{W}
Metrics	0.82	0.86	0.04	0.95
Assemblages	0.81	0.87	0.06	0.93
NWQ	0.46	0.6	0.14	0.77

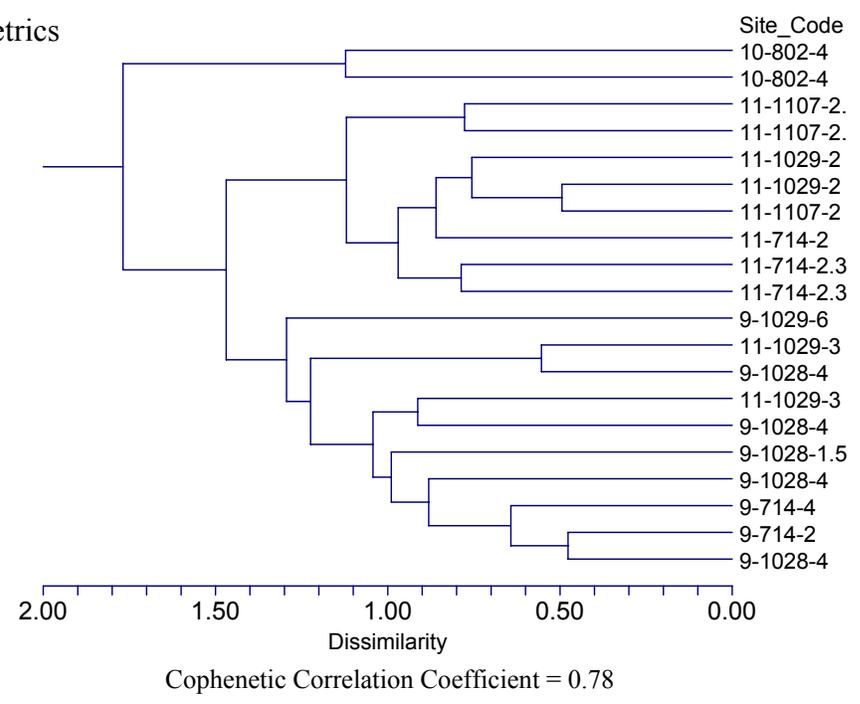
Cluster Analysis

The cluster analysis of sites based on chironomid assemblage data had the greatest fit with a cophenetic correlations coefficient of 0.91; whereas the clusters based on both the chironomid metric data and nutrient concentration data had adequate, but far lower fits with cophenetic correlation coefficients of 0.78 and 0.77 respectively (Figure 1). All three analyses resulted in fairly strong clusters of nutrient ecoregions, whereas the sites from the same HUC classes could be found in different clusters. For example, HUC 1029, a level II HUC from the Missouri watershed located in nutrient ecoregion 11 was found on different clusters in all three cluster analyses (Figure 1). Substrate seemed to vary the most with sites having the same substrate class found on different clusters. For example, substrate class 4, sandy substrate, was found on multiple clusters in each analysis (Figure 1).

(a) Dominant taxa of Chironomidae



(b) Chironomidae metrics



(c) Nutrient water quality parameters

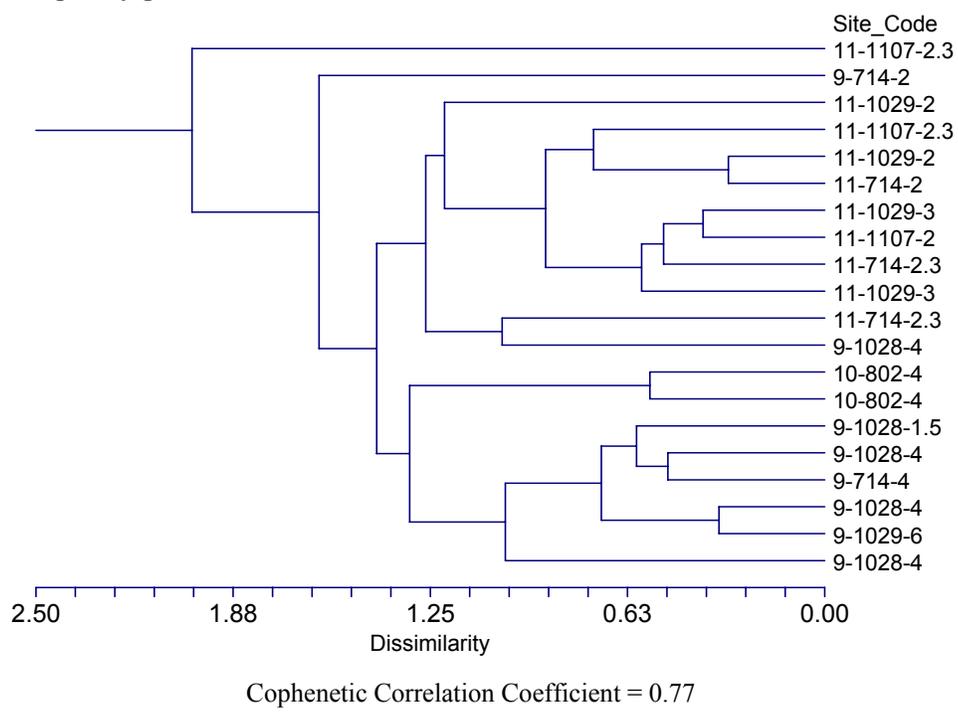


Figure 1a-c. Dendrograms resulting from hierarchical cluster analyses. Site codes consist of first the nutrient ecoregion code followed by the hydrologic unit code and then the substrate code. Fig. 2(a) is a dendrogram of sites based on the dominant taxa chironomid community assemblage. Fig. 2(b) is a dendrogram of sites based on chironomid community metrics. Fig. 2(c) is a dendrogram of sites based on nutrient water quality parameters.

Congruence Analysis

Congruence between classifications based on different datasets (e.g. metrics versus nutrients) ranged from correlation values of 0.26 to 0.57 and all pairwise comparisons passed the mantel test for having actual correlations higher than those computed from randomizations of the data (Table 7). Similarity matrices of all three classifications were most congruent when comparing those based on metric data and macroinvertebrate assemblage data. Correlations of similarity matrices for the classifications were lower when comparing those based on either metric data or assemblage data with the nutrient data (Table 7). Similarity matrices for the substrate classification scheme had both the highest congruence at 0.57 between classification based on metric and assemblage data and the lowest congruence at 0.26 between classification based on assemblage and nutrient data (Table 7).

Table 7. Results from the Mantel test of congruence using a Quantile T-test. Congruence was tested for between the chironomid community metric (MET) data and assemblage (ASMBLG) data matrices and each of these between the nutrient water quality (NWQ) data matrix for each of the classification schemes. The Number Lower and Number Higher values indicate the number of 998 random mixes of the original data which resulted in lower or higher correlation values than the pairwise comparisons between the original data matrices.

Classification Scheme	Comparison	Correlation	Number Lower	Number Higher	Pass/Fail
NR	MET vs ASMBLG	0.55	998	0	Pass
HUC	MET vs ASMBLG	0.55	998	0	Pass
Substrate	MET vs ASMBLG	0.57	998	0	Pass
NR	MET vs NWQ	0.36	998	0	Pass
HUC	MET vs NWQ	0.36	998	0	Pass
Substrate	MET vs NWQ	0.35	998	0	Pass
NR	ASMBLG vs NWQ	0.33	998	0	Pass
HUC	ASMBLG vs NWQ	0.33	997	1	Pass
Substrate	ASMBLG vs NWQ	0.26	978	20	Pass

Discussion

Water quality, metric, and chironomid assemblage data

A significant implication of the results is that three of the four water quality parameters analyzed for nutrient criteria benchmarks varied significantly between the two nutrient ecoregions, 9 and 11. Turbidity is the fourth major water quality parameter measured for nutrient criteria, but it was excluded from use in ANOVAs because it did not meet the assumption of having a normal distribution of data. Nutrient ecoregion 9 corresponds to the Central Irregular Plains of Missouri and nutrient ecoregion 11 corresponds to the Ozark Highlands, which have vastly different ecophysiological characters and land uses (Gibson *et al.*, 2000). Our data support the discrete nature and validity of these two classes of nutrient ecoregions in Missouri.

Total Phosphorus and CHLA varied significantly between classes of substrate, but this may be explained by the fact that substrate classes did not overlap too much between nutrient ecoregion 9 and 11. Nutrient ecoregion 9 was characterized by sandy substrate, silt, and some hard clay; while nutrient ecoregion 11 was characterized by a mixture of gravel and cobble substrate (Table 1). On the other hand, HUC classes did not fall into discrete groups within nutrient ecoregion 9 and 11, but overlapped between the two regions (Table 1). Thus, significant variation of CHLA between the HUC watershed basins may represent the effect of an environmental filter at the basin scale driving changes in the concentrations of chlorophyll *a*, which may, in turn, have a confounding impact on interpretation of variation of concentrations of CHLA at a higher scale such as nutrient ecoregions.

Data on anthropogenic land uses such as row crop were used to designate classes of nutrient ecoregions (Gibson *et al.*, 2000). Candidate reference streams were used in these analyses, however, and so the results of the chironomid metric ANOVAs allows us to identify metrics that will be useful in supporting the classification scheme. Some of the new candidate metrics were effectively quite different between nutrient ecoregion 9 and 11. For example, our expectation that Percent *Stempelinella* would vary between the two regions due to the dominance of spring fed streams in nutrient ecoregion 11 (Pinder and Reiss, 1986) was supported. However, Percent *Stempelinella* also varied significantly with HUC and with substrate class. We expected the metric Percent *Harnischia* to vary significantly according to substrate because some genera within the *Harnischia* complex are psammonophiles (Barton and Smith, 1984) and they should increase in numbers with increased prevalence of sandy substrate. The percent of *Harnischia* complex did vary significantly with substrate and correspondingly with nutrient ecoregion, but also with classes of HUC (Table 4). Other, more commonly used chironomid metrics varied significantly between classes as well. For example, Percent Chironomini Taxa varied between nutrient ecoregions and substrate classes, while other commonly used chironomid metrics did not vary significantly between any of the classifications (Table 4).

Classification strengths

To meet assumptions required for parametric analysis, some of the data had been removed for the above ANOVAs, but most of the data were replaced to conduct the analyses of classification strengths. For example, the analyses of classification strengths now includes nutrient ecoregion 10 which corresponds to the Mississippi alluvial flood plains (see methods for further explanation).

Classification strengths were weakest for the basin scale (HUC) classification scheme regardless of the type of data used to examine the classification (Table 6), but were comparable to other studies of classification strengths using community assemblage and metric data (Table 8). In general, most HUC or basin scale classifications were weak, even weaker than regional scale nutrient ecoregion (Table 8). The exception was the study by Hawkins and Vinson (2000) which found that HUC classification based on macroinvertebrate assemblage data was slightly stronger than the classification based on ecoregions and the catchment classification was considerably stronger (Table 8). In fact, the strength for the catchment classification in their study was considerably greater than the other regional scale classifications, with only the classification of ecoregions based on fish metric data reported by Van Sickle and Hughes (2000) being close with a CS of 0.11 or 11% (Table 8).

The strength of the nutrient ecoregion classification based on both the chironomid assemblage data and metric data is comparable to a range of other regional scale classifications based on macroinvertebrate assemblage data, fish assemblage data, and fish metric data (Table 8). The chironomid assemblage and metric data produced the same CS; whereas, Van Sickle and Hughes (2000) found that their fish assemblage data produced a stronger ecoregion classification with a CS of 0.11 or 11% relative to the CS of 0.04, or 4 % produced by their fish metric data (Table 8). One reason that the chironomid metrics performed well relative to the assemblage data was that we created metrics that we hypothesized would vary significantly between the nutrient ecoregions, such as Percent *Stempelinella*. If we were correct, then we would expect the *a priori* metric data to perform similarly to the assemblage data as both types of data would behave in a similar manner.

Table 8. A comparison of classification strengths at two different scales between chironomid only assemblage and metric data and classification strengths published in other studies based on macroinvertebrate and fish data. Data are reported in both probabilities and percents to facilitate the discussion in text.

Biological Data	Landscape Scale		Watershed/Catchment Scale	
	Ecoregion	Nutrient Region	HUC/HAU	Catchment Level
Macroinvertebrate Assemblages				
This Study: Chironomid Assemblage	-----	0.08, 8%	0.04, 4%	-----
This Study: Chironomid Metric	-----	0.08, 8%	0.05, 5%	-----
Mid-Atlantic Streams ^a	0.01, 1%	-----	-----	0.01, 1%
Swedish Streams ^b	0.06, 6%	-----	-----	-----
Wyoming Streams ^c	0.09, 9%	-----	-----	-----
North America Streams ^d	0.06, 6%	-----	0.07, 7%	0.14, 14%
Fish Assemblages				
Fish Species Abundance Data ^e	0.11, 11%	-----	0.05, 5%	-----
Fish Metric Data ^e	0.04, 4%	-----	0.03, 3%	-----
Fish Assemblage (reference streams) ^f	-----	-----	-----	0.03, 3%

^a Waite *et al.*, (2000)

^b Sandin & Johnson (2000)

^c Gerritsen *et al.*, (2000)

^d Hawkins & Vinson (2000)

^e Van Sickle & Hughes (2000)

^f McCormick *et al.*, (2000)

We can conclude from our data that chironomid data, taken alone amongst macroinvertebrates, was as useful as general macroinvertebrate and fish data in examining classification strengths and therefore chironomid assemblage and metric data may be effective tools for future use in stream management. We can also conclude, when examining our results and the results from other research (Table 8), that neither regional scale nor basin scale classifications are very strong. Many researchers still find them useful, if modified. For example, Gerritsen *et al.* (2000) found that they increased the strength of their classification of ecoregions for a CS of 0.87, or 9% to 0.13 or 13 % when they combined their two mountain ecoregions into one and compared it to prairie and basin ecoregions in Wyoming. Sandin and Johnson (2000) suggest that a possible approach to stream management would be to follow a stratified approach with local scale variables nested within regional scale classes.

One of the goals of this study was to search for sources of variation within the Missouri nutrient ecoregion classes by testing the strength of a classification at a very fine scale, substrate type. As an environmental filter, substrate should exert a very strong selective pressure on insect populations and thus community assemblages. Substrate provides habitat, food, points of attachment, and refuges from predators (Minshall, 1984). Feio *et al.* (2006) found that substrate quality was an important determinant in macroinvertebrate distribution. It is generally thought that the diversity of aquatic insects increases as a function of increased substrate heterogeneity, but amongst the relatively few studies that exist on the subject, there are conflicting results (Vinson and Hawkins, 1998). Vinson and Hawkins (1998) also found a relationship between substrate type and the number of taxa such that different substrate types supported different insect diversities. Our question is whether different substrate types support different communities entirely. Francis and Kane (1995) found that communities of chironomids differed by substrate type in a study of colonization of substrates in experimental ponds and Mebane (2001) found that the standard macroinvertebrate metrics he examined were significantly correlated to his fine substrate class.

Over all, our local scale, substrate classification scheme was stronger than our basin scale, HUC classification scheme, but was weaker than our regional scale, nutrient ecoregion classification scheme (Table 6). However, the strength of the substrate classification based on chironomid metrics, with a *CS* of 0.04 or 4 %, was weaker than for that of either the HUC or nutrient ecoregion classifications. The classification strengths for this local-scale classification were comparable to the strengths of the basin scale and regional scale classifications with the exception of Hawkins' and Vinson's (2000) catchment classification (Table 8). They were also comparable to finer scale classifications for fish assemblages based on stream order, with *CS* of 0.05 or 5 % (Van Sickle and Hughes 2000), and a *CS* of 0.07 or 7 % (McCormick *et al.*, 2000). They were slightly higher than the strengths of local scale classifications for macroinvertebrates based on slope with a *CS* of 0.028, or 2.8% and water chemistry with a *CS* of 0.011 or 1.1% (Waite *et al.*, 2000).

The greatest classification strength for substrate was based on nutrient concentrations, with a *CS* of 0.14 or 14 %. In fact, the classification strength of each classification scheme was greatest if based on nutrient concentrations (Table 6). The strength of the nutrient ecoregion classification when based on nutrient concentrations was greatest for the entire study with a *CS* of 0.31 or 31 %, which is higher than most of the classification strengths reviewed for this paper with the exception of the classification of Oregon streams based on cluster analyses of fish and a composite combining fish assemblages and geographic distribution, with *CS*s ranging from 0.30 or 30 % to 0.35 or 35 % (Van Sickle and Hughes, 2000) and from the Mid-Atlantic streams, with a *CS* of 0.27 or 27% (McCormick *et al.*, 2000). The relatively strong classification of nutrient ecoregions based on nutrient data indicates that these may be natural classes which have been accurately delineated and will be useful to watershed resource managers in establishing nutrient criteria.

Cluster Analysis

An *a posteriori* clustering of sites was performed based on chironomid assemblage and metric data and on the nutrient water quality data and compared to the *a priori* classes used in this study (Figure 2). All three cluster analyses were fairly good at clustering sites based on the regional scale nutrient ecoregion classes, but less effective at clustering sites based on the basin scale HUC classes or the local scale substrate classes (Figure 2). The relative ability of the data to differentiate between these three scales of classes in the cluster analyses is similar to the relative strengths of the different classification schemes, with nutrient ecoregions having the strongest classification strengths, followed by substrate and HUC classes (Figure 2, Table 6).

One of the goals of this analysis was to determine whether substrate or watershed and basin as hydrologic unit cause variation of nutrients and chironomid assemblage and metrics within the Missouri nutrient ecoregion classification. It is clear from the cluster analysis that the strongest determiner of chironomid community assemblages is the regional scale nutrient ecoregion, but the question remains whether filters at smaller scales are driving changes in these assemblages. McCormick *et al.* (2000) conclude that weak classification strengths for their ecoregion classification scheme suggest that they do not need to interpret fish assemblage data in an ecoregional framework and that identification of local factors may be necessary in establishing reference criteria for fish Mid Atlantic Highland streams. Waite *et al.* (2000), Sandin and Johnson (2000), and Mykra *et al.* (2004) suggest that stratification of smaller scale variables within regional scale classification schemes should be considered if *a priori* classifications will be used in biological assessments and creation of reference criteria. However, our findings show that basin scale and local scale substrate strata were not strong enough to form their own groups disrupting the regional scale nutrient ecoregions (Figure 2). If, for example, substrate was a strong environmental filter then we would expect it to create groups either within the regional classification or instead of the regional classification. This, in fact does not happen with the minor exception or two, such as site 9-714-2 in the cluster based on chironomid assemblage

data, which separated out from the larger cluster based on nutrient ecoregion 9 to reside in a cluster dominated by substrate category 2 (Figure 2). Our cluster analyses support the *a priori* regional scale nutrient ecoregion classification and do not support stratification within nutrient ecoregions.

Congruence Analysis

Since we used two disparate sets of data for this analysis, chironomid and nutrient data, we had the goal of determining how congruent these separate data sets were in determining the strengths of our three classification schemes. In addition, we calculated the congruence between the chironomid assemblage and metric data to each other to determine how similar or dissimilar these data sets are in a classification scheme. Not surprisingly, congruence was highest between chironomid metric and assemblage matrices used to analyze the three classifications schemes, with the highest congruence, 0.57, for the substrate classification (Table 7). It was surprising that the chironomid assemblage and metric data were not more congruent, since the metrics were based on the original source assemblage data.

On the other hand, the nutrient data matrices were similarly congruent with both chironomid assemblage and metric matrices, but with low correlations ranging from 0.26-0.36 (Table 7). The relative lack of congruence between matrices based on nutrient versus chironomid assemblage and metric data supports the result of the classification strength analysis, with the nutrient data producing strong classifications relative to the metric and assemblage data (Table 6), but differs from the outcome of the cluster analyses (Figure 2). In the cluster analyses, the dendrograms based on chironomid metric and nutrient data appear more congruent to each other than either does to the dendrogram based on the chironomid assemblage data. The discrepancy may be explained by the lower cophenetic correlations coefficients for the metric and nutrient dendrograms (Figure 2). The lower the coefficient value the more likely the difference between the resulting dendrogram and the original data matrix (Hintz, 2000). The congruence analysis compares the original data matrices rather than the final dendrogram matrices, thus the congruence analysis and the CS analysis are more reflective of the relationships between the source data and classification schemes than are the dendrograms, with the exception of the dendrogram based on the chironomid assemblage data, which had a high Cophenetic Correlation Coefficient of 0.91. The significant congruence, but low correlation between the chironomid assemblage and metric data to the nutrient data indicates that the data can be combined within the nutrient ecoregions, but should be done with some caution.

Chironomidae have proven to be an effective group in this study due to their diversity, heterogeneity in habitat preference, and numerical dominance. However, it is these qualities which other researchers have used to exclude chironomids from basic and applied ecological research. Mykra *et al.* (2004) argued to remove the extremely heterogeneous chironomids from their study on boreal headwater streams, rather than using their heterogeneity in response to environmental conditions, gradients, and stressors as an analytical tool. Rabeni and Wang (2001) went so far to argue that chironomids need not be used beyond the family level at all in biological assessments as a way to cut costs and put those costs into sampling more sites. The debate whether to include genus or species level data for Chironomidae is part of a larger discussion on taxonomic resolution (see Lenat and Resh, 2001; Bailey *et al.*, 2001 for thoughtful discussion on the topic), but there is a very real danger that valuable information is being missed when excluding chironomids from analyses, in particular when performing analyses at the landscape scale (Waite *et al.*, 2004).

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