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Chironomidae (diptera) Community Structure in Lakes of Contrasting Morphometry, Landscape Position, and Water Chemistry

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CHIRONOMIDAE (DIPTERA) COMMUNITY STRUCTURE IN LAKES OF CONTRASTING MORPHOMETRY, LANDSCAPE POSITION, AND WATER CHEMISTRY

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ABSTRACT

Chironomidae (Diptera) surface-floating pupal exuviae were collected monthly during the ice-free season in 2010-2011 from six lakes in the North Temperate Lakes Long-Term Ecological Research site in northern Wisconsin. The goal of this study was to determine whether chironomid community structure reflected differences in lake morphometry (i.e. depth, area, shoreline development, etc.), water chemistry and/or landscape position (i.e., elevation). Forty-six genera were identified from four subfamilies: Chironominae (57%), Orthocladiinae (28%), Tanypodinae (11%), and Diamesinae (4%). *Tanytarsus*, *Ablabesmyia*, and *Psectrocladius* were found in all six study lakes; whereas certain genera, such as *Omisus, Protanypus,* and *Epoicocladius* were each observed in only one lake. An analysis of abiotic variables revealed that pH, total phosphorus, and Secchi depth were the most important factors structuring chironomid communities in the NTL-LTER site clearwater study lakes, but a more complete suite of habitat variables that includes biotic factors (i.e., algal growth and availability and macrophyte characteristics) should be sampled in future studies to determine the importance of these variables. Elucidating the factors that influence chironomid community structure can lead to an improved understanding of chironomid ecology and distribution patterns in north temperate lakes and will be a valuable asset in examining the interplay of landscape position and biological communities in lakes.

INTRODUCTION

The dipteran family Chironomidae (non-biting midges) is an ecologically important group of aquatic insects that is widely distributed across aquatic ecosystems worldwide, and is frequently the most abundant insect group in freshwater systems (Pennak 1978; Armitage et al. 1995; Ferrington et al. 2008). Chironomid species richness in most aquatic ecosystems can exceed 80 taxa with some systems having over 100 species (Ferrington et al. 2003, 2008; Ferrington 2008). In many aquatic ecosystems chironomids can account for at least 50% of total macroinvertebrate species richness. The number of species present is the result of complex physical, chemical, biological, and landscape conditions. Chironomids are key biotic components of lakes, providing an important food resource for not only aquatic predators (Epler 2001; Ferrington et al. 2008), but also terrestrial invertebrates and vertebrates (Epler 2001). Chironomids live in pristine to heavily polluted freshwaters and depending on species can tolerate a wide range of gradients, such as temperature, pH, and dissolved oxygen concentration (Ferrington et al. 2008). Not only have chironomids exploited aquatic habitats with strikingly different water quality, but they also live in extreme environments from the depths of Lake Baikal, Russia (~1740m) (Linevich 1971) to the heights of the Himalayan mountain range (~5182m) (Koshima 1984), and are the southernmost (Antarctica) “free-living” holometabolous insect (Usher and Edwards 1984; Edwards and Usher 1985). Aquatic biologists have used chironomids to characterize ecological conditions of lentic systems since the early 1900s (Thienemann 1910) and continue to do so (Brundin 1956; Saether 1975, 1979; Bitusik et al. 2006; Ruse 2010); the first lake typology system, which eventually gave rise to the oligotrophic/eutrophic system of waterbody classification, was based on midges.

Chironomids are holometabolous (complete metamorphosis) having four life stages: egg, larva, pupa, and adult (imago); the larval stage may last from less than two weeks to several years (Armitage et al. 1995; Ferrington et al. 2008). Midge larvae are typically encountered in any study of benthic invertebrates in streams or lakes, and can occur in virtually any aquatic or semi-aquatic habitat (Pennak 1978; Ferrington et al. 2008). Chironomid taxonomic composition in aquatic systems has been widely used as an indicator of water quality and in environmental assessment, however the wide range of larval habitats that must be sampled to characterize a waterbody is costly and time-intensive. Although chironomids can be the most numerous insects in aquatic studies, the collecting and processing of larvae can be time consuming due to their small size and high densities (>50,000 larvae per m^2 is not uncommon) (Ferrington et al. 2008). Identification of chironomid larvae to genus level is exhaustive because of the need to slide mount the severed larval head capsule and body. In addition, identification is hampered by the small percentage of described species, the inability to distinguish larvae of several groups, the lack of taxonomic keys for any instar other than the final larval instar, and the sometimes-subtle differences in certain diagnostic features (Ferrington et al. 2008). The pupa, represents a stage in which major morphological changes occur as the larva transitions into the imago. When the pupa is fully developed, it swims to the water's surface and the adult emerges. The pupal exuviae, or cast skin, remains floating for a short period (i.e., approximately 48-72 hours in the Midwest region) after adult emergence and can be readily collected (Ferrington et al. 1991; Ferrington et al. 2003).

Chironomid surface-floating pupal exuviae (SFPE), or chironomid pupal exuviae technique (CPET) in Europe, have become widely used to address a variety of ecological and environmental questions (Kavanaugh 1988; Rossaro 1991; Bitusik et al. 2006; Ferrington et al. 2008; Ruse 2010). Sampling chironomid SFPE is a simple and effective way of assessing taxonomic composition of midges in freshwater systems, particularly in lakes where chironomid taxa may be heterogeneously distributed across lake regions and depths (Coffman 1973; Wilson and Bright 1973; Saether 1975; Soponis and Russell 1984; Ferrington 1991; Blackwood et al 1995; Ruse et al. 2000; Sealock and Ferrington 2008; Ruse 2010). The method has been effectively used in studies of chironomid ecology and community composition in North America (Kavanaugh 1988; Blackwood and Ferrington 1995; Ruse et al. 2000). SFPE are passively collected at the leeward (downwind) shore of lakes in nearby debris, vegetation, and/or foam that can include chironomids from different locations throughout the lake, including areas from the opposite shore from where the collection is made (Ruse 2010). Large numbers of SFPE can be collected over a short period-of-time and despite differences in larval microhabitat, most emerging species are collected, which allows for an accurate characterization of community composition of the study site (Ferrington et al. 1991, 2008). In addition to the advantages, chironomid exuviae samples take less time to process than larval samples, and allow for better taxonomic resolution (Ferrington et al. 1991; Sealock and Ferrington 2008). The SFPE collecting technique has many advantages over the collection of larvae and is well-suited for use in this study.

Research Objectives and Questions

In this study, chironomid SFPE were collected from lakes within the North Temperate Lakes Long-Term Ecological Research (NTL-LTER) site near Boulder Junction, Wisconsin with contrasting morphometrics (i.e., depth, area, shoreline development, etc.), water chemistry, and landscape position (i.e., elevation), which are all characteristics that may play a role in shaping chironomid community structure (Kratz et al. 1997; Verschuren et al. 2000; Larocque et al. 2006; Rossaro et al. 2007; Free et al. 2009). The primary research objective was to determine the relative importance of lake morphometrics, water chemistry, and landscape position in influencing chironomid communities in north temperate lakes. Lake morphometrics have been found to be important factors in influencing chironomid communities of lakes in northern Sweden (Larocque et al. 2001) and in the Alps (Boggero et al. 2006). The relationship between shoreline development and chironomid community structure has not been studied extensively, but has been suggested as a possible factor shaping chironomid communities (Free et al. 2009); whereas water chemistry, in various studies, has been shown to be an important factor influencing chironomid community structure (Verschuren et al. 2000; Woodward and Schulmeister 2006; Rossaro et al. 2007; Bilton et al. 2009; Free et al. 2009; Ruse 2010). Previous research on lakes in the NTL-LTER site has examined various aspects of water chemistry, plankton, fish, and to a lesser extent, benthic macroinvertebrates. Kratz et al. (1997) found that lake area and landscape position played a role in fish species richness in lakes of the NTL-LTER; large lakes were species-rich and low in the landscape, whereas smaller lakes had fewer species and tended to be high in the landscape. Little work has been conducted examining chironomid community structure in the NTL-LTER area. The specific questions this study addressed were:

1. Is there a relationship between chironomid community structure and lake morphometric parameters?
2. Is there a relationship between lake water chemistry and chironomid community structure?
3. Is chironomid community structure influenced by landscape position (i.e., elevation)?

MATERIALS AND METHODS

Study Location

The North Temperate Lakes Long-Term Ecological Research (NTL-LTER) site (Trout Lake Station) is located within the Northern Highland Lake District (Vilas County) in northern Wisconsin near the town of Boulder Junction (Fig. 1). The NTL-LTER research program, supported by the National Science Foundation (NSF) and administered by the Center for Limnology at the University of Wisconsin-Madison, was established in 1981, and includes seven primary lakes under long-term study and numerous other lakes studied less intensively (Kratz et al. 1997; Schmude et al. 1998; Dodson et al. 2009). Lakes near the LTER site are positioned within a similar geologic setting linked through a common groundwater and surface water flow system, and experience similar weather conditions (Kratz et al. 1997). Although the lakes within the NTL-LTER site are close to each other, they do range from high to low elevation in forested and residential (i.e., lake cottages and campgrounds) landscapes, and differ both in physicochemical characteristics and morphometry (Kratz et al. 1997).

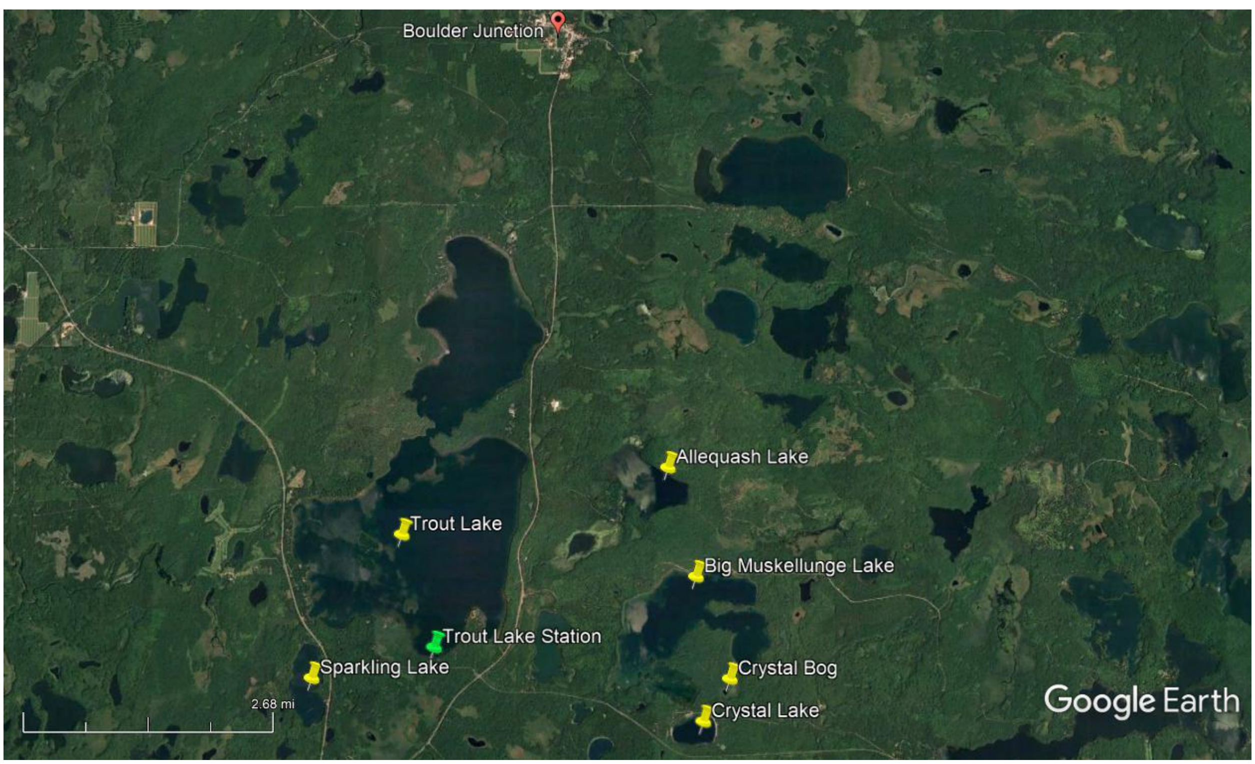
Site Selection

Six lakes, all NTL-LTER primary lakes, were chosen for this study and included Trout Lake (TL), Allequash Lake (AL), Sparkling Lake (SL), Big Muskellunge Lake (BML), Crystal Lake (CL), and Crystal Bog (CB) (Fig. 1). The study lakes differed in morphometry, water chemistry, and landscape position (elevation); three of the study lakes (TL, AL, and SL) were low elevation (~491-494m), and the remaining three study lakes (BML, CL, and CB) were high

Figure 1. A) Map of Wisconsin showing the location of Boulder Junction, WI; B) Location of six study lakes within the North Temperate Lakes Long-Term Ecological Research site in relation to Boulder Junction.

A.

B.



elevation (~499-501m) (Kratz et al. 1997). Although the difference in elevation between the groups is small (10m at the most), it plays an important role in the region's hydrologic gradient (Kratz et al. 1997). High elevation lakes receive a higher percentage of total water input in the form of precipitation, whereas low elevation lakes receive more of their total water input from groundwater, which affects ionic concentration (Kratz et al. 1997). Five of the six study lakes (TL, AL, SL, BML, and CL) were characterized as clearwater lakes, i.e., intermediate to deep Secchi depths (3-6m), and one lake (CB) was characterized as a "stained water" lake, i.e., shallow Secchi depth (1m) and water is brown in color due to decayed vegetation and/or organic matter (Table 1).

Sample Collection and Processing

Chironomid surface-floating pupal exuviae (SFPE) were collected monthly during the open water period in 2010 (June through October) and once in 2011 (May). Surface-floating pupal exuviae were sampled at the leeward (downwind) shore of each study lake using an enamel pan to collect foam samples and to dip the water adjacent to large floating woody debris and vegetation where pupal exuviae accumulate (Ferrington et al. 1991). The shoreline was inaccessible by foot at two study lakes, AL and CB, so collections were made from a kayak or small boat. Contents of the enamel pan were passed through a 125-micron sieve and retained material was transferred to a labeled sample jar and preserved with 70% ethanol. Samples were sorted under a dissecting microscope (Leica MZ75) using 10X magnification and SFPE were removed and mounted in Euparal on microscope slides for later identification using a compound microscope (Leica DM2500). Pupal exuviae were identified to the genus level using Merritt et al. (2008) and Wiederholm (1986). Chironomid taxa were categorized as either "present" or "absent" from a study lake rather than quantifying species-specific densities because the primary objective of this study was to determine the relative importance of lake characteristics in determining chironomid community structure.

Abiotic Variables

Physicochemical parameters from all study lakes are routinely measured and entered into a database maintained by NTL-LTER site staff. The NTL-LTER site has an extensive long-term dataset (since 1981) of physicochemical parameters from the study lakes and are available through the NTL-LTER website (https://lter.limnology.wisc.edu/). Data for the six study lakes, from June 2010 through October 2010 and May 2011, were downloaded, formatted, and used in analyses examining the relationship between chironomid community structure and abiotic variables (Table 1). An additional parameter, shoreline development (SLD), was not available through the database and was calculated as in Lind (1985). Shoreline development is an index of the regularity of a shoreline, which relates to total productivity based on the littoral zone area; a lake that is a perfect circle has a SLD value of 1 (less littoral area for total productivity), whereas an increased shoreline irregularity results in SLD values >1 (more littoral area for total productivity) (Lind 1985).

Data Analysis

Chironomid community structure among lakes and the relationship between chironomid community structure and biotic and abiotic variables were analyzed with non-metric multidimensional scaling (nMDS) and the BIO-ENV procedures using the Primer 5 statistical package (Primer v5, Primer-E Ltd.). Non-metric multidimensional scaling is a multivariate ordination technique that produces a two-dimensional plot showing the relationship among samples. The nMDS procedure creates an among-sample similarity matrix which, when plotted, may be interpreted in terms of relative similarity of samples to each other (Clarke and Warwick

2001). Plots produced by the nMDS are straightforward to interpret; points close together represent samples more similar to each other than those farther apart (Clarke and Warwick 2001). All nMDS ordinations were performed using presence/absence chironomid data, and the similarity matrix used to create the chironomid community nMDS plot was generated using Bray-Curtis similarities. Abiotic variables were transformed by a simple root or logarithmic transformation (Clarke and Gorley 2001; Clarke and Warwick 2001) to account for differences in scale, and the similarity matrix used to create the nMDS plot of abiotic parameters was generated using normalized Euclidean distance. Bray-Curtis similarity (or dissimilarity) is most appropriate for generating similarity matrices of biotic data, whereas Euclidean distance is preferred for abiotic and environmental similarity matrices (Clarke and Gorley 2001). Stress values are given for each plot as an indication of how strongly the relationships are represented by the plot. A stress value ≤0.05 presents an excellent representation with no prospect of misinterpretation, values >0.05 and ≤0.1 correspond to a good ordination with no real prospect of a misleading interpretation, whereas values between >0.1 and ≤0.2 give a potentially useful representation, and stress values >0.2 indicate that the points are randomly placed (Clarke and Warwick 2001).

The BIO-ENV procedure in the Primer 5 statistical package was used to match biotic data to environmental patterns (Clarke and Gorley 2001; Clarke and Warwick 2001). This procedure was used to compare chironomid community composition to abiotic variables and analyze the extent to which the chironomid community in each lake is explained by abiotic variables. Prior to performing BIO-ENV, draftsman plot analysis (i.e., all possible pairwise scatter plots) was performed to detect whether any abiotic variables were highly correlated (pairwise correlations averaging ≥0.95). It is important to remove one of the highly-correlated variables before running the BIO-ENV procedure because including both variables is redundant and may obscure relevant results (Clarke and Ainsworth 1993; Clarke and Warwick 2001). The presence/absence similarity matrix that was used to generate chironomid community nMDS plots was compared to the remaining abiotic variables. BIO-ENV generates a matrix of the best combinations of variables by creating increasingly complex groupings of variables. Spearman rank correlation was used to represent the extent to which chironomid community structure may be explained by abiotic variables (Clarke and Warwick 2001). BIO-ENV is only an exploratory tool and does not demonstrate causality and no statistical significance is implied (Clarke and Gorley 2001). The real causal variables may not have been measured, but they may be strongly correlated with one or more of the variables that were measured (Clarke and Gorley 2001).

Tolerance Values and Water Quality

The evaluation of chironomid community structure can be used to monitor and assess the quality of both lotic and lentic waters. One way to assess water quality is to use chironomid tolerance values. Tolerance values range from 0 to 10, 0 representing the tolerance value of an extremely sensitive taxon and 10 for a tolerant taxon (Barbour et al. 1999); low tolerance values thus imply high quality water, whereas high tolerance values imply low quality water. The tolerance values used for Chironomidae in this study were from the Upper Midwest. If values for this region were not available, tolerance values from other regions were used (Ferrington et al. 2008). The use of tolerance values from other regions occurred in the following order of preference: Midwest > Mid Atlantic > Northwest (Bouchard and Ferrington 2011). Tolerance values are not available for some taxa (i.e., *Epoicocladius*, *Djalmabatista*, *Omisus*, *Sergentia*, *Zavreliella*) due to lack of information and those taxa were not included in analyses using tolerance values. Mean chironomid community tolerance values (MCCTV) for each lake were calculated to assess water quality of the study lakes.

RESULTS

Chironomid Community Structure

A total of 37,071 chironomid surface-floating pupal exuviae (SFPE) were collected during this study, representing 46 genera in four subfamilies (Tables 2 and 3). Thirty-nine of the genera (84.5%) were in two subfamilies, Chironominae (26) and Orthocladiinae (13); the remaining seven genera were in the subfamilies Tanypodinae (5) and Diamesinae (2) (Table 2). Genera in the subfamily Chironominae were represented by all three tribes, Chironomini (19), Tanytarsini (6), and Pseudochironomini (1). Chironomid taxa richness ranged from 15 genera in Crystal Bog (CB) to 26 in Sparkling Lake (SL) (Table 2, Fig. 2). Typical habitats of taxa identified within this study are provided in Appendix A. Most taxa collected were in the collector functional feeding group (FFG) and were present in all lakes followed by predators and shredders (Table 4). Predators were collected from all lakes, whereas shredders were found in four of the six study lakes (Table 4). The highest number of chironomid SFPE was collected in June and the lowest number in October (Appendix B), and monthly taxa richness was highest in August and lowest in October (Fig. 3).

Eight genera of chironomids occurred in all five clearwater lakes and represented three subfamilies: Tanypodinae (*Ablabesmyia*, *Procladius*), Orthocladiinae (*Corynoneura*, *Orthocladius*, *Psectrocladius*), and Chironominae (*Cladotanytarsus*, *Micropsectra*, *Tanytarsus*) (Table 2). Seventeen taxa occurred in only one of the clearwater lakes. These taxa represented four subfamilies: Tanypodinae (*Djalmabatista*, *Larsia*, *Tanypus*), Diamesinae (*Potthastia*, *Protanypus*), Orthocladiinae (*Brillia*, *Epoicocladius*), and Chironominae (*Cladopelma*, *Endochironomus, Glyptotendipes*, *Microtendipes*, *Paratendipes*, *Sergentia*, *Stempellina*, *Stenochironomus*, *Xenochironomus*, *Zavreliella*) (Table 2). Four genera were collected from all six study lakes and represented three subfamilies: Tanypodinae (*Ablabesmyia*, *Procladius*), Orthocladiinae (*Corynoneura*, *Psectrocladius*), and Chironominae (*Tanytarsus*). One taxon occurred exclusively in Crystal Bog from the subfamily Chironominae (*Omisus*) (Table 2).

Among the clearwater lakes, chironomid community structure based on presence/absence was most similar in Sparkling Lake (SL) and Trout Lake (TL) (Fig. 4) with 20 chironomid genera in common resulting in 80% similarity. Six genera were collected in SL and not in TL (Table 2) (*Larsia*, *Cricotopus*, *Chironomus*, *Dicrotendipes, Polypedilum,* and *Stenochironomus*), whereas four genera were collected in TL and not in SL (*Epoicocladius*, *Einfeldia*, *Paratanytarus*, and *Stempellina*). Big Muskellunge Lake (BML) had 17 chironomid genera in common with SL and 16 in common with TL (Table 2, Fig. 4) resulting in 69% and 68% similarity, respectively. Allequash Lake (AL) and Crystal Lake (CL) had 54% similarity, and averaged 57% similarity of genera with SL, TL, and BML (Table 2, Fig. 4). Among all study lakes, Crystal Bog (CB) was most similar to AL with 12 chironomid genera in common resulting in 62% similarity (Table 2, Fig. 5). BML and CB had 53% similarity with 10 chironomid genera in common (Table 2). CB averaged 42% similarity of genera with CL, TL, and SL resulting in less than 10 chironomid genera in common (Table 2, Fig. 5). The only “stained water” lake in the study, CB, was anomalous and omitted from multivariate analyses because of noticeable differences with the five clearwater lakes (e.g., pH, dissolved oxygen, water clarity, substrate, mean depth) (Table 5, Fig. 6).

Table 1. Physicochemical and morphological characteristics (used in multivariate analyses) of study lakes in the North Temperate Lakes Long-Term Ecological Research site[1]. Draftsman Plot Analysis detected 31 highly correlated abiotic variable pairs that resulted in the inclusion of 15 variables (**bold**) and the exclusion of 12 variables in further analyses (BIO-ENV) of Chironomidae community structure.

	Lakes					
Characteristic[2]	**Trout Lake (TL)**	**Allequash Lake (AL)**	**Sparkling Lake (SL)**	**Big Muskellunge Lake (BML)**	**Crystal Lake (CL)**	**Crystal Bog[3] (CB)**
Area (ha)	1050.50	114.60	64.00	396.30	36.70	0.50
Mean Depth (m)	14.60	2.90	10.90	7.50	10.40	1.70
Secchi Depth (m)	5.94	3.36	5.01	5.78	5.43	1.00
Shoreline Development	1.78	2.06	1.51	2.38	1.07	1.13
Elevation (m)	491.88	494.22	494.35	499.35	500.65	501.23
Gravel (%)	40	30	20	20	10	0
Rock (%)	20	10	20	15	0	0
Sand (%)	30	60	60	60	90	1
Muck (%)	10	0	0	5	0	99
pH	7.49	7.40	7.20	7.13	6.25	5.97
Alkalinity (µeq/L)	949.91	983.35	753.21	524.58	54.26	60.25
Specific Conductivity (µS/cm)	106.33	105.18	110.20	58.50	13.20	13.75
Water Temperature (C)	9.10	11.65	10.62	11.43	10.80	12.10
Dissolved Oxygen (mg/L)	9.47	8.47	8.84	8.49	9.62	5.02
Oxygen Saturation (%)	86.09	81.58	84.09	81.60	91.67	50.08
Nitrate (µg/L)	26.08	24.83	6.68	10.07	11.26	7.64
Ammonium (µg/L)	63.54	110.73	119.37	137.84	72.48	158.42
Total Nitrogen (µg/L)	240.88	425.96	336.29	512.25	203.02	824.79
Total Phosphorus (µg/L)	11.29	37.37	23.69	22.93	12.24	27.15
Dissolved Reactive Silica (µg/L)	3083.11	6559.35	3809.95	143.48	32.14	524.15
Total Particulate Matter (mg/L)	0.75	1.61	1.46	1.46	1.27	5.88
Sulfate (mg/L)	3.00	3.68	2.46	3.04	2.14	0.49
Iron (mg/L)	0.03	0.21	0.28	0.08	0.03	0.37
Total Inorganic Carbon (mg/L)	11.34	12.32	9.80	7.36	1.56	3.41
Total Organic Carbon (mg/L)	4.57	4.70	4.13	4.01	2.04	12.41
Calcium (mg/L)	13.98	13.73	12.21	7.22	1.13	0.77
Potassium (mg/L)	0.82	0.77	0.76	0.47	0.35	0.35

[1]Data provided by the North Temperate Lakes Long Term Ecological Research program

[2]Physicochemical characteristics are annual mean values

[3]CB (Crystal Bog) was not included in the BIO-ENV analysis; present in table for comparison to clearwater lakes

Table 2. Chironomidae genera based on surface floating pupal exuviae (SFPE) collections from study lakes (lake abbreviations are presented in Table 1) of the North Temperate Lakes Long Term Ecological Research site, Vilas County, Wisconsin (June 2010 – October 2010, May 2011).

	Genera					
Subfamily	**Trout Lake**	**Allequash Lake**	**Sparkling Lake**	**Big Muskellunge Lake**	**Crystal Lake**	**Crystal Bog**
Tanypodinae	*Ablabesmyia*	*Ablabesmyia*	*Ablabesmyia*	*Ablabesmyia*	*Ablabesmyia*	*Ablabesmyia*
				Djalmabatista		
			Larsia			
	Procladius	*Procladius*	*Procladius*	*Procladius*	*Procladius*	*Procladius*
		Tanypus				
Diamesinae				*Potthastia*		
					Protanypus	
Orthocladiinae					*Brillia*	
	Corynoneura	*Corynoneura*	*Corynoneura*	*Corynoneura*	*Corynoneura*	*Corynoneura*
		Cricotopus	*Cricotopus*			
	Epoicocladius					
	Heterotrissocladius		*Heterotrissocladius*	*Heterotrissocladius*	*Heterotrissocladius*	
	Limnophyes		*Limnophyes*	*Limnophyes*	*Limnophyes*	
	Nanocladius	*Nanocladius*	*Nanocladius*		*Nanocladius*	
	Orthocladius	*Orthocladius*	*Orthocladius*	*Orthocladius*	*Orthocladius*	
	Parakiefferiella	*Parakiefferiella*	*Parakiefferiella*	*Parakiefferiella*		
	Psectrocladius	*Psectrocladius*	*Psectrocladius*	*Psectrocladius*	*Psectrocladius*	*Psectrocladius*
	Synorthocladius		*Synorthocladius*	*Synorthocladius*		*Synorthocladius*
	Thienemanniella		*Thienemanniella*	*Thienemanniella*		
	Zalutschia		*Zalutschia*		*Zalutschia*	
Chironominae		*Chironomus*	*Chironomus*	*Chironomus*		*Chironomus*
					Cladopelma	
	Cladotanytarsus	*Cladotanytarsus*	*Cladotanytarsus*	*Cladotanytarsus*	*Cladotanytarsus*	
	Cryptochironomus	*Cryptochironomus*	*Cryptochironomus*	*Cryptochironomus*		
	Cryptotendipes		*Cryptotendipes*			
		Dicrotendipes	*Dicrotendipes*	*Dicrotendipes*	*Dicrotendipes*	*Dicrotendipes*
	Einfeldia			*Einfeldia*		*Einfeldia*
		Endochironomus				*Endochironomus*
		Glyptotendipes				*Glyptotendipes*
	Micropsectra	*Micropsectra*	*Micropsectra*	*Micropsectra*	*Micropsectra*	
		Microtendipes				
						Omisus
		Parachironomus		*Parachironomus*		*Parachironomus*
	Paratanytarsus	*Paratanytarsus*			*Paratanytarsus*	*Paratanytarsus*
				Paratendipes		
		Polypedilum	*Polypedilum*			*Polypedilum*
	Pseudochironomus		*Pseudochironomus*	*Pseudochironomus*		
				Saetheria	*Saetheria*	
					Sergentia	
	Stempellina					
	Stempellinella		*Stempellinella*			
			Stenochironomus			
	Stictochironomus	*Stictochironomus*	*Stictochironomus*		*Stictochironomus*	
	Tanytarsus	*Tanytarsus*	*Tanytarsus*	*Tanytarsus*	*Tanytarsus*	*Tanytarsus*
		Xenochironomus				
		Zavreliella				
Number of SFPE Collected	25,067	5,661	1,477	2,579	1,726	561

Table 3. Chironomidae taxa richness of pupal exuviae collected from study lakes (lake abbreviations are presented in Table 1) of the North Temperate Lakes Long-Term Ecological Research site, Vilas County, Wisconsin (June 2010 – October 2010, May 2011).

	TL	AL	SL	BML	CL	CB
Subfamily						
Chironominae	11	15	12	11	9	10
Tribe						
Chironomini	4	11	7	7	5	8
Tanytarsini	6	4	4	3	4	2
Pseudochironomini	1	0	1	1	0	0
Subfamily						
Orthocladiinae	11	6	11	8	8	3
Tribe						
Corynoneurini	2	1	2	2	1	1
Orthocladiini	9	5	9	6	7	2
Subfamily						
Tanypodinae	2	3	3	3	2	2
Tribe						
Procladiini	1	1	1	2	1	1
Pentaneurini	1	1	2	1	1	1
Tanypodini	0	1	0	0	0	0
Subfamily						
Diamesinae	0	0	0	1	1	0
Tribe						
Diamesini	0	0	0	1	0	0
Protanypini	0	0	0	0	1	0
Total Number of Taxa	24	24	26	23	20	15

Table 4. Functional feeding groups (FFGs) of chironomids (Diptera: Chironomidae) based on pupal exuviae collected from study lakes (lake abbreviations are presented in Table 1) in the North Temperate Long-Term Ecological Research site, Vilas County, Wisconsin (June 2010 – October 2010, May 2011).

				Lakes					
FFG	**Subfamily**	**Tribe**	**Genus**	**TL**	**AL**	**SL**	**BML**	**CL**	**CB**
Collector[1]	Diamesinae	Diamesini	*Potthastia*				X		
			Protanypus					X	
	Orthocladiinae	Corynoneurini	*Corynoneura*	X	X	X	X	X	X
			Thienemanniella	X		X	X		
		Orthocladiini	*Epoicocladius*	X					
			Heterotrissocladius	X		X	X	X	
			Limnophyes	X		X	X	X	
			Nanocladius	X	X	X		X	
			Orthocladius	X	X	X	X	X	
			Parakiefferiella	X	X	X	X		
			Psectrocladius	X	X	X	X	X	X
			Synorthocladius	X		X	X		X
			Zalutschia	X		X		X	
	Chironominae	Chironomini	*Chironomus*		X	X	X		X
			Cladopelma					X	
			Cryptotendipes	X		X			
			Dicrotendipes		X	X	X	X	X
			Einfeldia	X			X		X
			Microtendipes		X				
			Omisus						X
			Saetheria				X	X	
			Sergentia					X	
			Stenochironomus			X			
			Stictochironomus	X	X	X		X	
			Zavreliella		X				
		Pseudochironomini	*Pseudochironomus*	X		X	X		
		Tanytarsini	*Cladotanytarsus*	X	X	X	X	X	
			Micropsectra	X	X	X	X	X	
			Paratanytarsus	X	X			X	X
			Stempellina	X					
			Stempellinella	X					
			Tanytarsus	X	X	X	X	X	X
Predator	Tanypodinae	Procladiini	*Djalmabatista*				X		
			Procladius	X	X	X	X	X	X
		Pentaneurini	*Ablabesmyia*	X	X	X	X	X	X
			Larsia			X			
		Tanypodini	*Tanypus*		X				
	Chironominae	Chironomini	*Cryptochironomus*	X	X	X	X		
			Parachironomus		X		X		X
			Xenochironomus		X				
Shredder	Orthocladiinae	Orthocladiini	*Brillia*					X	
			Cricotopus		X	X			
	Chironominae	Chironomini	*Endochironomus*		X				X
			Glyptotendipes		X				X
			Polypedilum		X	X			X

[1]Collector represents both collector-gatherers and collector-filterers

Figure 2. Taxa richness of Chironomidae pupal exuviae collections from study lakes of the North Temperate Lakes Long-Term Ecological Research site, Vilas County, Wisconsin (June 2010 – October 2010, May 2011). Lake abbreviations can be found in Table 1.

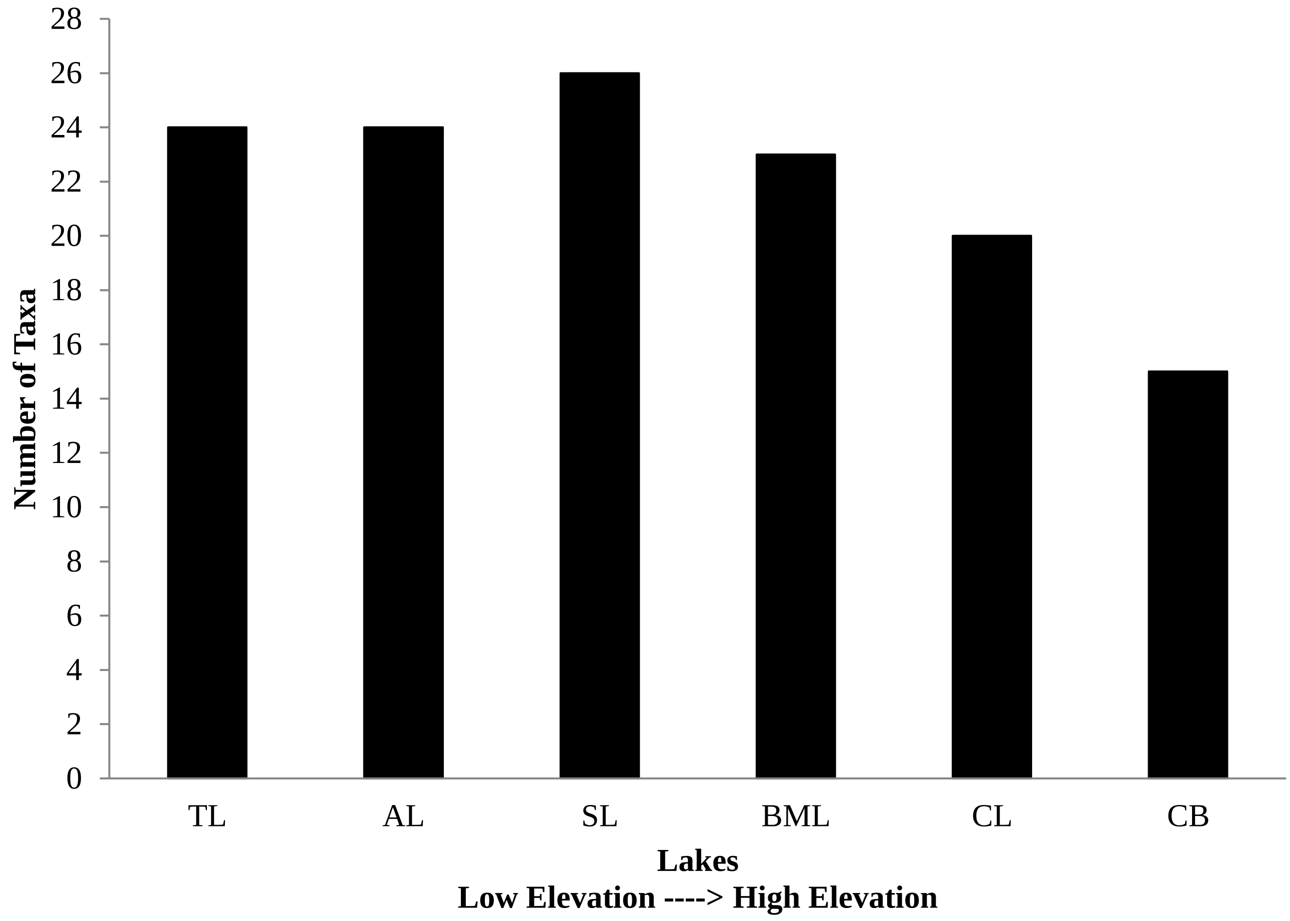


Figure 3. Mean monthly taxa richness of Chironomidae pupal exuviae collections from study lakes of the North Temperate Lakes Long-Term Ecological Research site, Vilas County, Wisconsin (June 2010 – October 2010, May 2011). Error bars represent standard deviation.

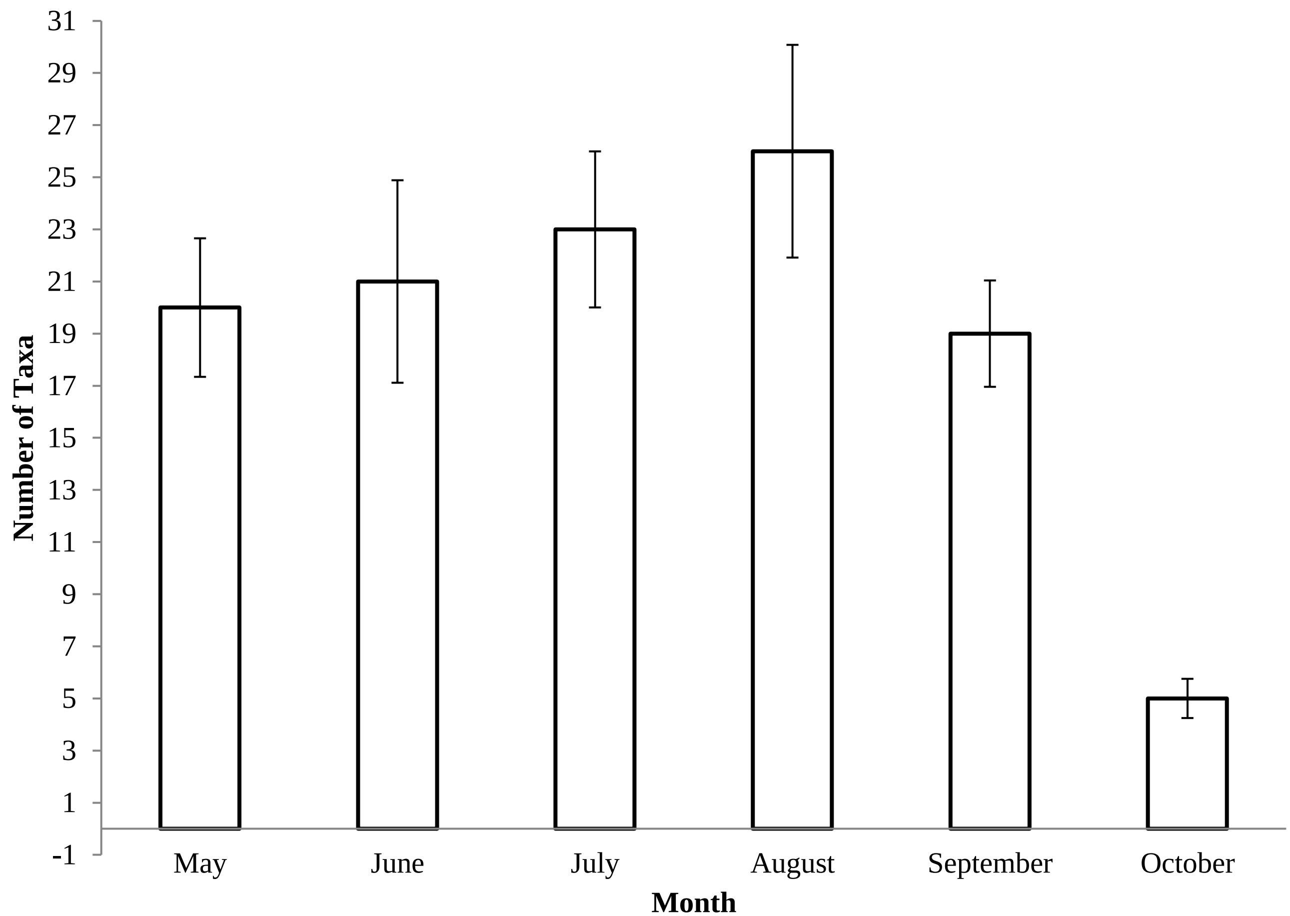


Figure 4. Non-metric multidimensional scaling ordination plot of Chironomidae community structure similarity (presence/absence) in the clearwater study lakes (lake abbreviations are presented in Table 1).

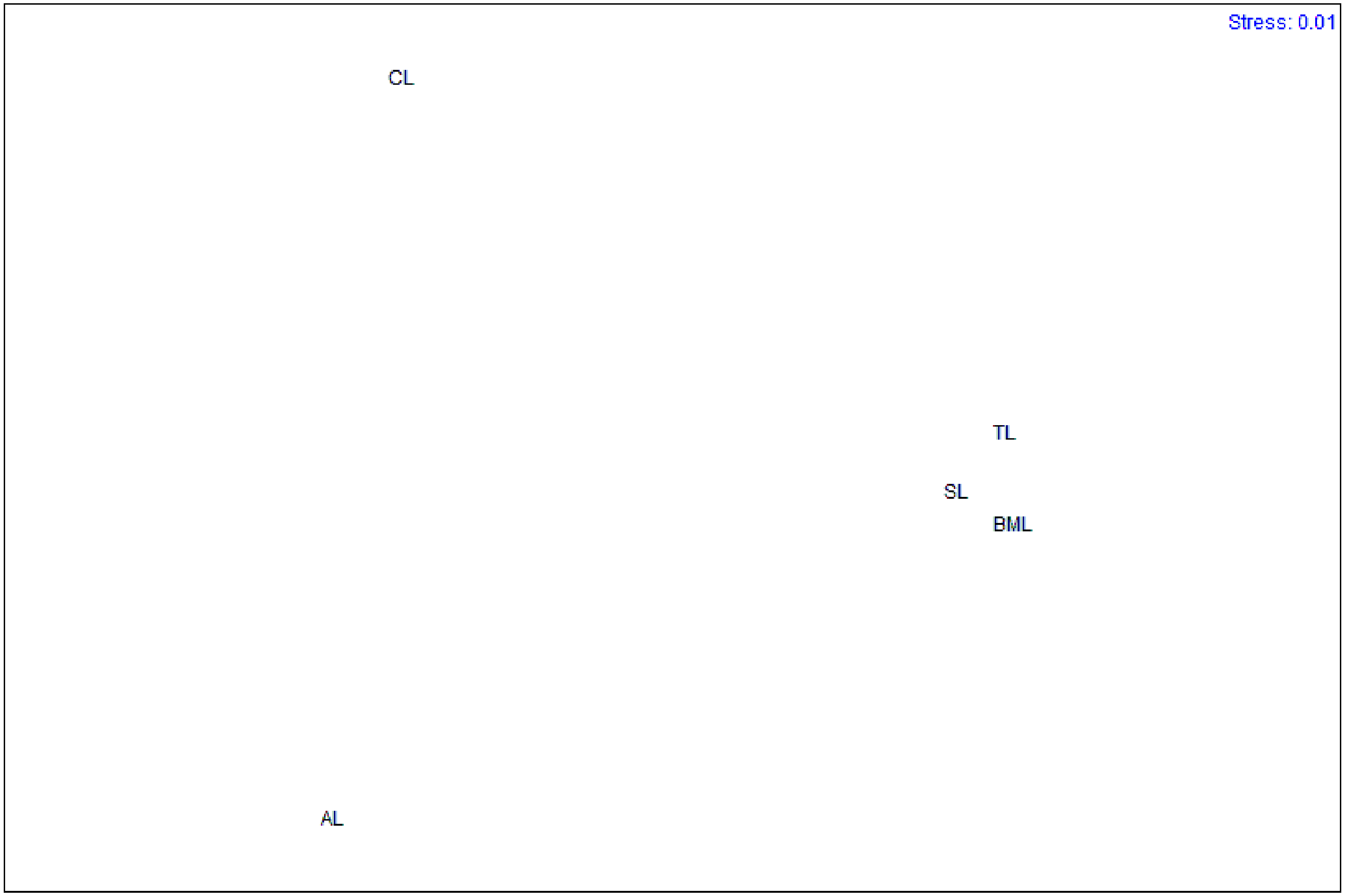


Figure 5. Non-metric multidimensional scaling ordination plot of Chironomidae community structure similarity (presence/absence) in all study lakes (lake abbreviations are presented in Table 1).

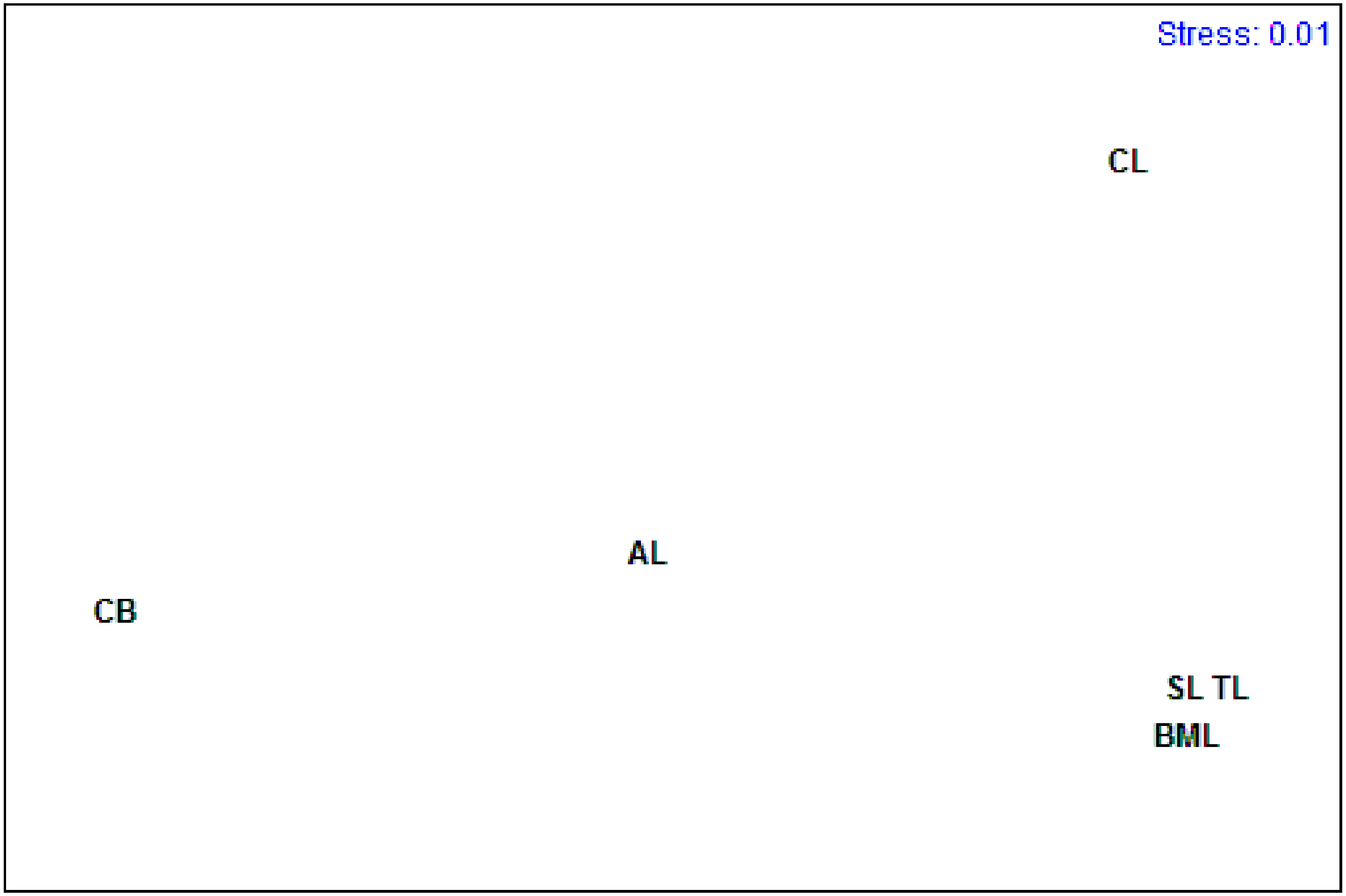
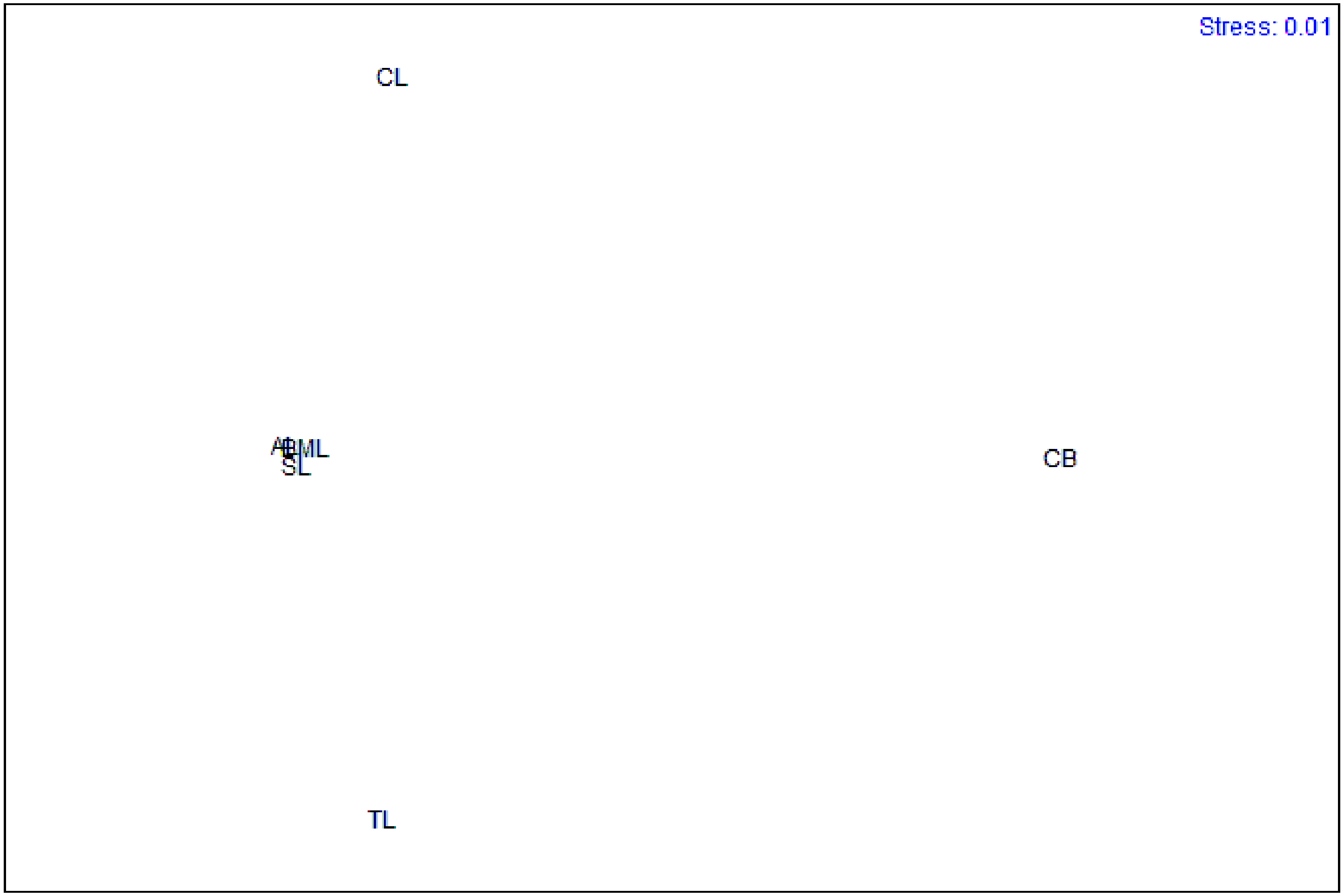


Figure 6. Non-metric multidimensional scaling ordination plot of study site similarity based on abiotic data in all study lakes (lake abbreviations are presented in Table 1).



Influence of Abiotic Variables

Analysis of abiotic variables (Fig. 7) yielded a different ordination of clearwater lakes compared to the chironomid community structure ordination (Fig. 4). SL and BML were most similar based on abiotic data, however, the three remaining lakes (TL, CL, and AL) differed from each other and from SL and BML (Table 4). Both SL and BML had comparable pH, total phosphorus, and Secchi depth and they also had similar substrate composition consisting primarily of sand with areas of gravel and rock. Dissolved reactive silica (DRS) concentration, however, was much higher in SL than in BML. SL is a lower elevation system than BML and therefore receives more groundwater input, which is high in silica (Hurley et al. 1985; Kratz et al. 1997). In contrast, BML receives most of its water input from precipitation with minimal input from groundwater (Kratz et al. 1997; Baines et al. 2000). The primary source of water for lakes in the North Temperate Lakes-Long Term Ecological Research site (NTL-LTER) is either groundwater or precipitation and accounts for chemical similarities and differences (Hurley et al. 1985; Kratz et al. 1997). The three remaining study lakes, TL, CL, and AL, were each grouped separately and were distinct from SL and BML in the NMDS analysis based on abiotic variables. Lake area, DRS, pH, and elevation were the primary variables accounting for the separate ordinations of TL, CL, and AL. For example, TL was the largest lake in surface area, CL was highest in elevation and had the lowest pH, and AL had the highest DRS value among clearwater lakes. In addition, these three lakes differ in substrate composition and primary source of water input; both TL and AL are primarily groundwater-fed, whereas CL is surface water-fed and is highly influenced by precipitation (Kratz et al. 1997). The combined differences in abiotic variables among these three clearwater lakes explains their positioning on the NMDS plot.

Draftsman plot analysis (i.e., all possible pairwise scatter plots) revealed 31 highly correlated pairs of abiotic variables resulting in the removal of 12 variables, thus leaving 15 variables to be included in BIO-ENV (Table 4). The analysis revealed ten multiple variable combinations highly correlated with chironomid community structure (Table 5). Correlation strength (i.e., Spearman correlation coefficient) ranged from 0.903 to 0.939; each of the combinations included variables relating to light and nutrients. Five of the ten combinations incorporated shoreline development, four combinations incorporated pH, and two combinations incorporated mean depth. The combination of variables with the lowest correlation coefficient (0.903) were pH, TP, and Secchi depth. Secchi depth was included in all ten combinations as different variables were added and correlation strength changed. The inclusion of Secchi depth in each combination might indicate it plays an important role in shaping chironomid community structure in the study lakes (Table 5).

Figure 7. Non-metric multidimensional scaling ordination plot of study site similarity based on abiotic data in the clearwater study lakes (lake abbreviations are presented in Table 1).

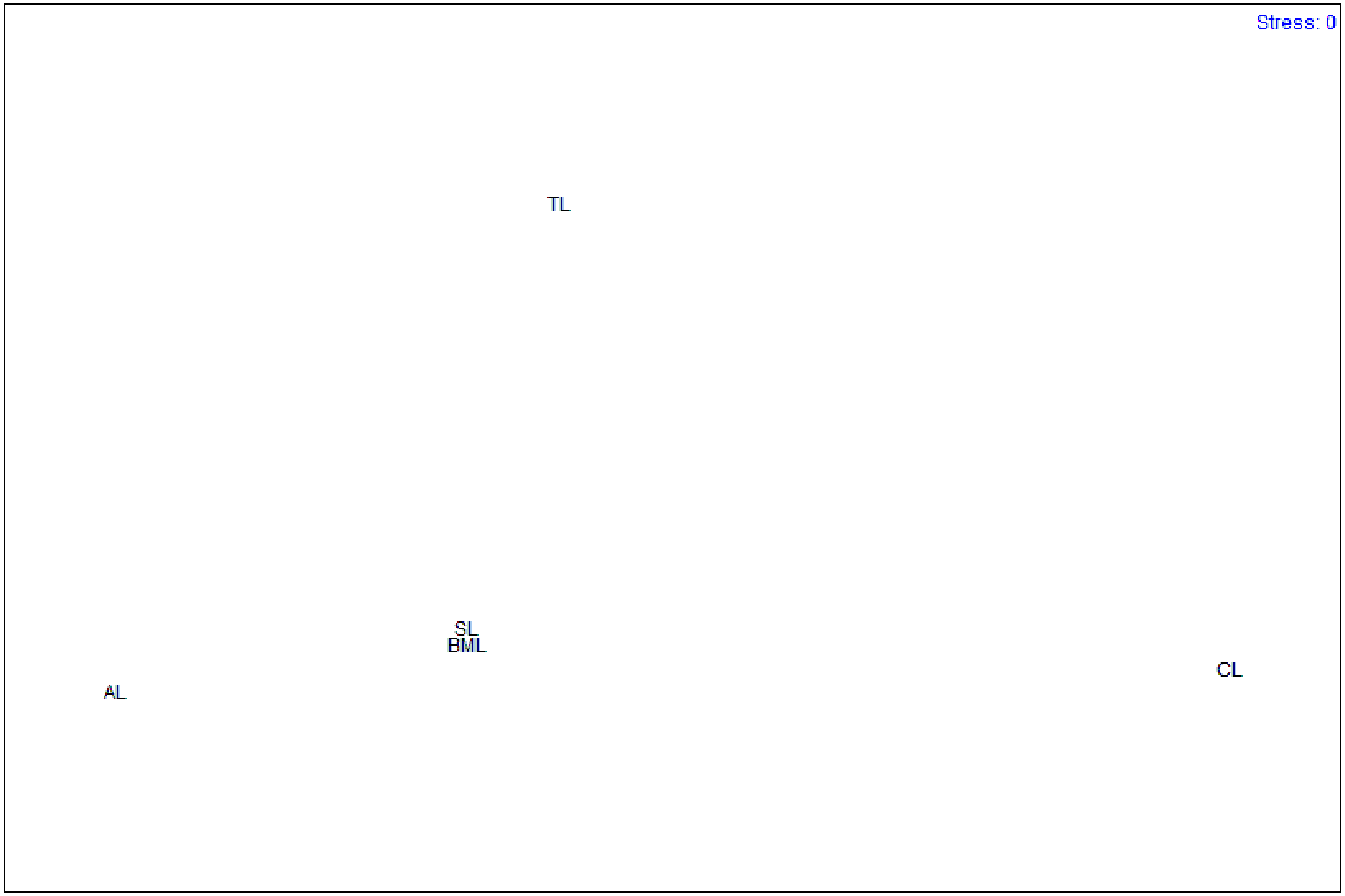


Table 5. Combinations of abiotic factors that best explain Chironomidae community structure in the North Temperate Lakes-Long Term Ecological Research site clearwater study lakes in Vilas County, Wisconsin. Presence/absence Chironomidae data were used to equally weight all taxa. The abiotic variable combinations listed below are the highest correlations that explain Chironomidae community structure according to the BIO-ENV procedure. Correlation coefficients are all significant at $p < 0.05$.

Spearman Correlation Coefficient	**Abiotic Variable Combination**
0.903	pH, Total Phosphorus (µg/L), Secchi Depth (m)
0.915	Dissolved Reactive Silica (µg/L), Iron (mg/L), Mean Depth (m), Secchi Depth (m), Shoreline Development
0.915	Total Phosphorus (µg/L), Dissolved Reactive Silica (µg/L), Sulfate (mg/L), Secchi Depth (m), Shoreline Development
0.927	pH, Total Nitrogen (µg/L), Total Phosphorus (µg/L), Dissolved Reactive Silica (µg/L), Sulfate (mg/L), Mean Depth (m),
0.927	pH, Total Nitrogen (µg/L), Total Phosphorus (µg/L), Secchi Depth (m)
0.927	pH, Total Nitrogen (µg/L), Total Phosphorus (µg/L), Secchi Depth (m)
0.939	Total Nitrogen (µg/L), Total Phosphorus (µg/L), Dissolved Reactive Silica (µg/L), Sulfate (mg/L), Secchi Depth (m)
0.939	Total Phosphorus (µg/L), Dissolved Reactive Silica (µg/L), Secchi Depth (m), Shoreline Development
0.939	Total Nitrogen (µg/L), Dissolved Reactive Silica (µg/L), Sulfate (mg/L), Secchi Depth (m)
0.939	Total Nitrogen (µg/L), Total Phosphorus (µg/L), Dissolved Reactive Silica (µg/L), Secchi Depth (m)

The ten variables that were best correlated with chironomid community structure were individually examined with ordination plots using chironomid presence/absence data. BML, TL, and SL were grouped based on highly similar chironomid community structures, whereas CL and AL were positioned separately from each other and the three-lake group (Fig. 4). An examination of multiple log transformed variables (i.e., total nitrogen, total phosphorus, dissolved reactive silica, and sulfate) represented by bubble size superimposed on the plot of chironomid presence/absence data revealed no clear patterns (Figs. 8-11). However, the plot reveals that BML had the largest total nitrogen concentration of the group with TL and SL (Fig. 8, Table 1). Also, SL and BML total phosphorus concentrations were twice as large as TL (Fig. 9, Table 1). Dissolved reactive silica concentration in SL and TL was 24 times higher than BML, and AL had the highest value, whereas CL had the lowest (Fig. 10, Table 1). Sulfate concentrations were similar across all clearwater study lakes (Fig. 11, Table 1). When log transformed Secchi depth was superimposed on the community structure plot, the BML, TL, and SL group and CL had deep Secchi readings, whereas AL had a shallow Secchi reading (Fig. 12, Table 1). In addition, superimposing shoreline development, BML had the most irregular shoreline, whereas CL had a circular or the most regular shoreline (Fig. 13, Table 1). All clearwater study lakes had similar pH values, which was reflected in bubble size (Fig. 14, Table 1). The mean depth bubble for TL was the largest compared to BML and SL, whereas BML was the smallest in the group (Fig. 15). The plot of square-root percent muck showed that CL, AL, and SL all had no muck substrate, while TL and BML had bubbles representing 10% and 5%, respectively (Fig. 16, Table 1). Iron was present in all lakes, but SL had the largest concentration (0.28 mg/L) and BML, TL, and CL had the lowest (Fig. 17, Table 1).

Tolerance Values and Water Quality

Among all study lakes, CB had the lowest water quality of all study lakes as indicated by the highest chironomid tolerance values (mean chironomid community tolerance value (MCCTV) = 7.4) due to the occurrence of highly tolerant taxa such as *Chironomus*, *Glyptotendipes*, and *Parachironomus* (Table 6). AL had the lowest water quality (MCCTV = 7.0) among clearwater lakes due to the occurrence of the same highly tolerant chironomids as in CB (Table 7). In contrast, TL had the highest water quality (MCCTV = 5.7) among all lakes due to the occurrence of extremely sensitive chironomids such as *Heterotrissocladius*, *Synorthocladius*, and *Stempellina* (Table 8). The mean tolerance values of SL, BML, and CL were intermediate to CB and TL (Tables 9-11).

Figure 8. Non-metric multidimensional scaling ordination of Chironomidae community structure similarity (presence/absence) in the clearwater study lakes. Bubble sizes represent log transformed total nitrogen (µg/L) in each study lake (lake abbreviations are presented in Table 1).

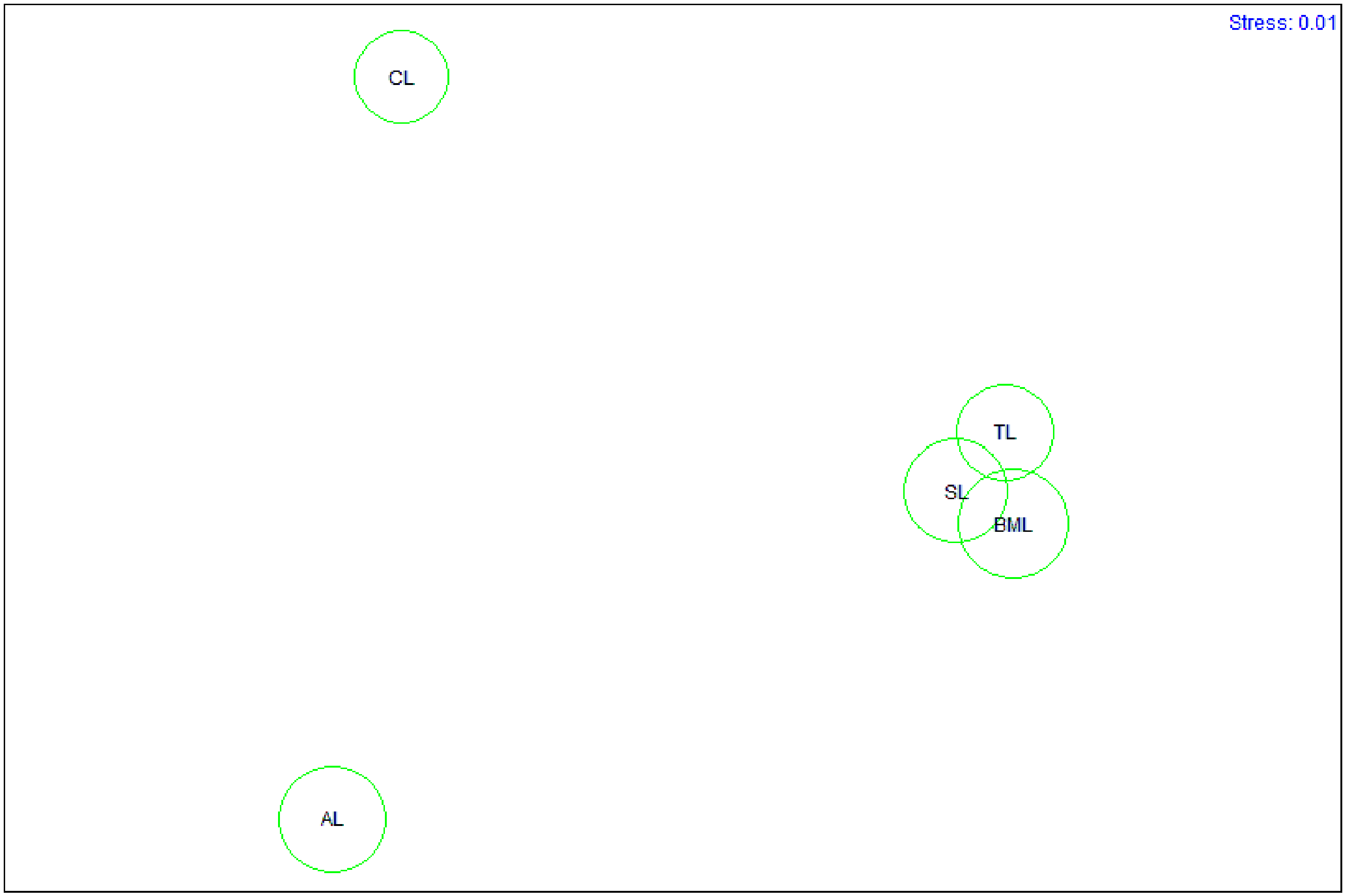


Figure 9. Non-metric multidimensional scaling ordination plot of Chironomidae community structure similarity (presence/absence) in the clearwater study lakes. Bubble sizes represent log transformed total phosphorus (µg/L) at each study lake (lake abbreviations are presented in Table 1).

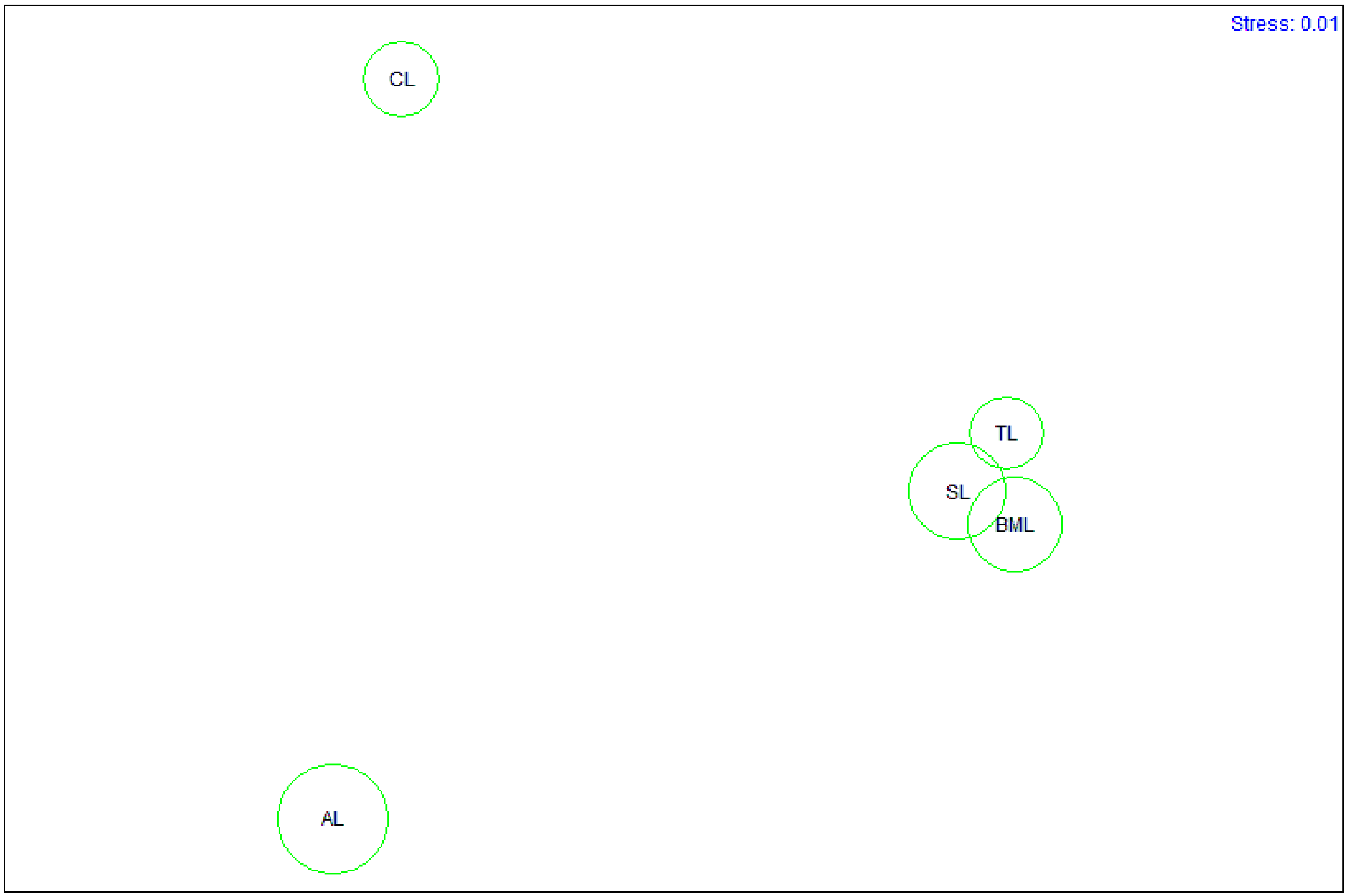


Figure 10. Non-metric multidimensional scaling ordination plot of Chironomidae community structure similarity (presence/absence) in the clearwater study lakes. Bubble sizes represent log transformed dissolved reactive silica (μg/L) at each study lake (lake abbreviations are presented in Table 1).

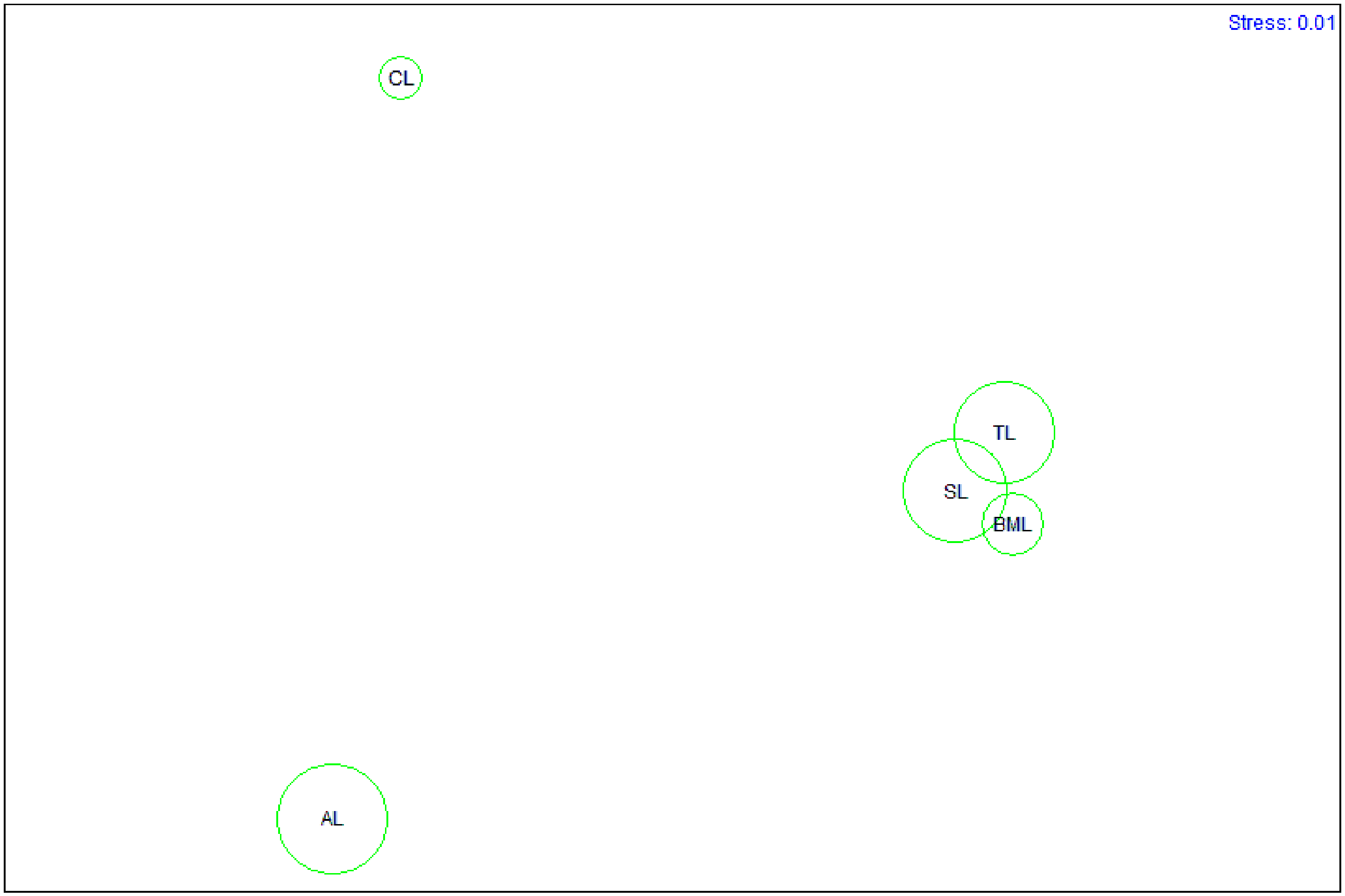


Figure 11. Non-metric multidimensional scaling ordination plot of Chironomidae community structure similarity (presence/absence) in the clearwater study lakes. Bubble sizes represent log transformed sulfate (mg/L) at each study lake (lake abbreviations are presented in Table 1).

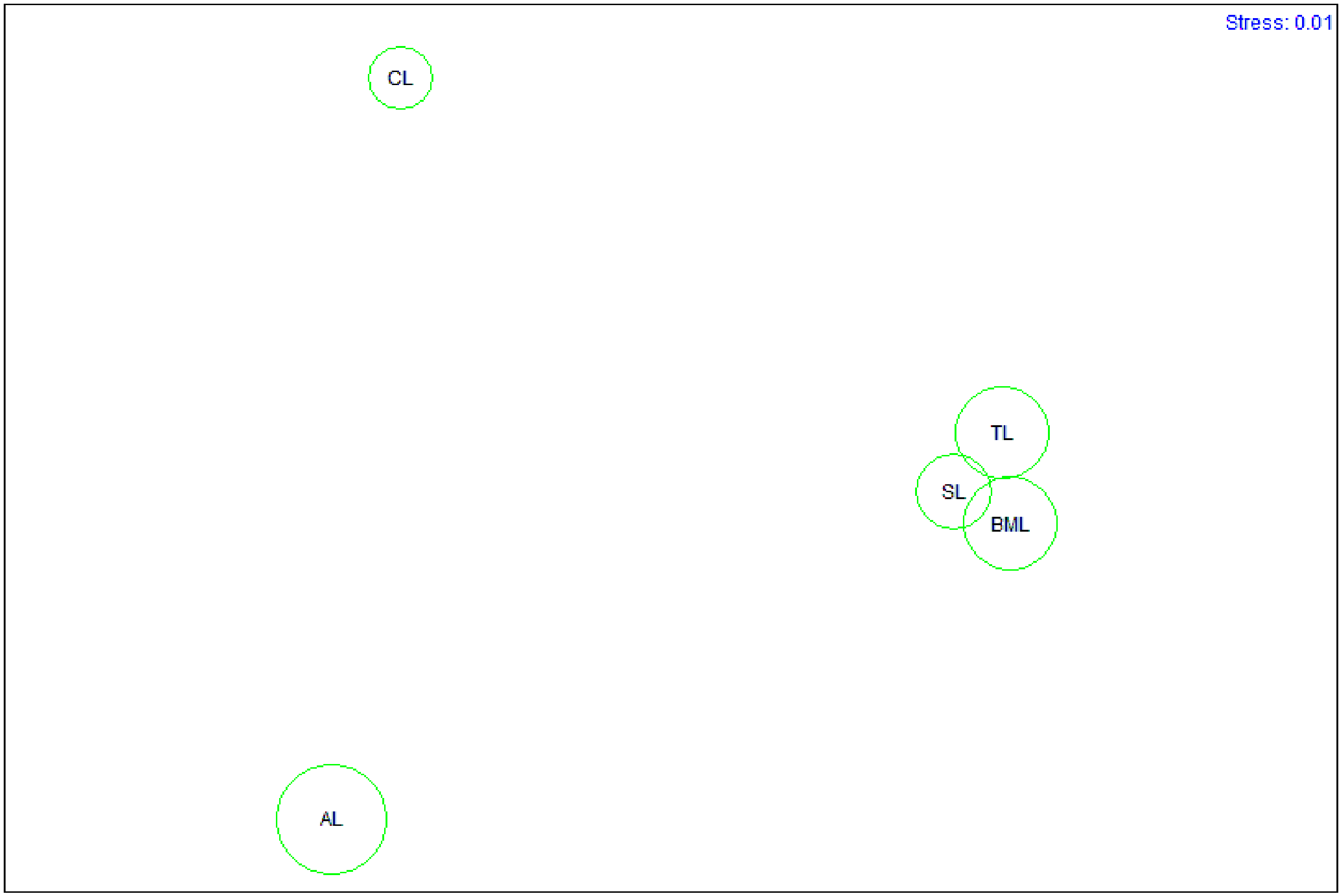


Figure 12. Non-metric multidimensional scaling ordination plot of Chironomidae community structure similarity (presence/absence) in the clearwater study lakes. Bubble sizes represent log transformed Secchi depth (m) at each study lake (lake abbreviations are presented in Table 1).

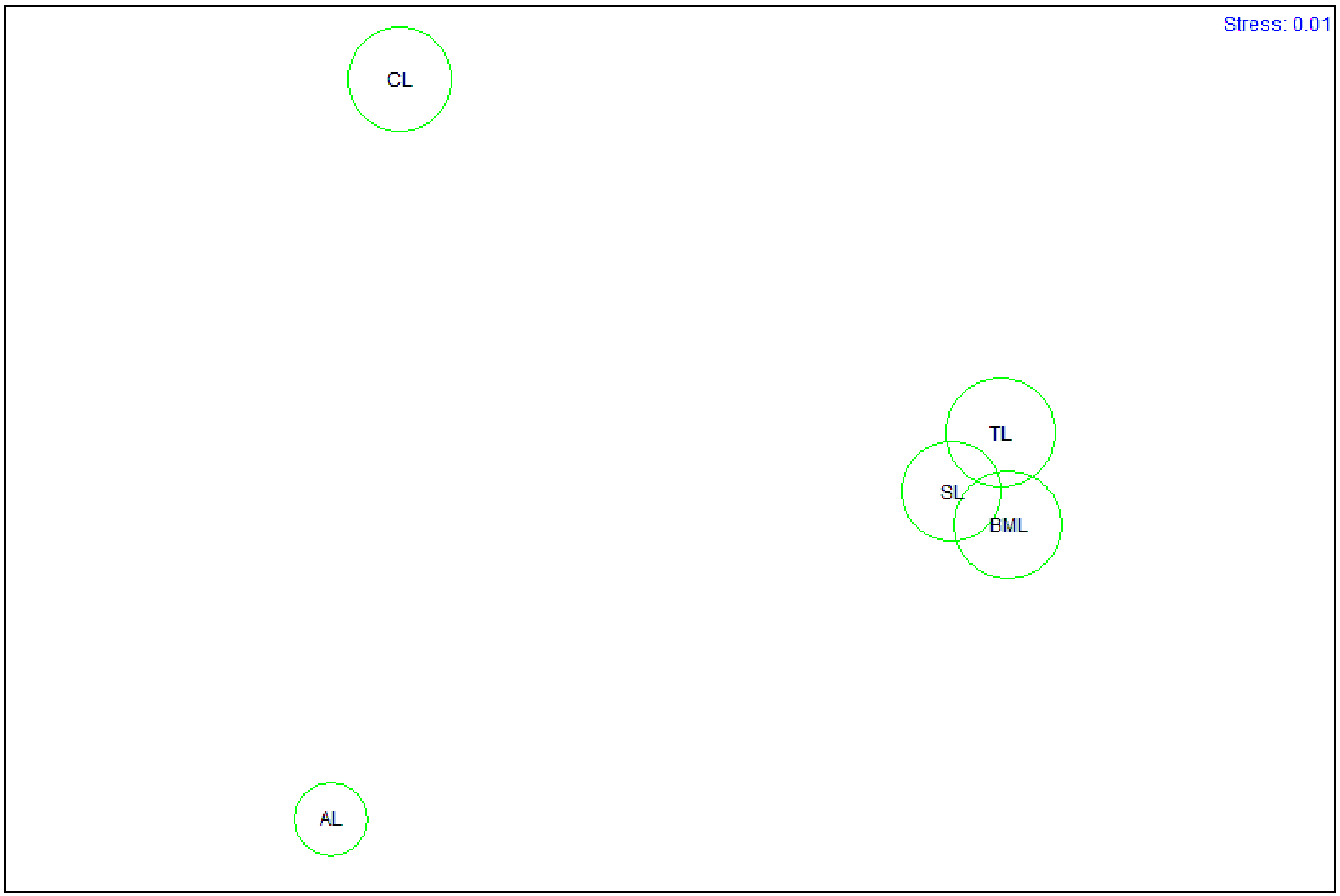


Figure 13. Non-metric multidimensional scaling ordination plot of Chironomidae community structure similarity (presence/absence) in the clearwater study lakes. Bubble sizes represent shoreline development at each study lake (lake abbreviations are presented in Table 1).

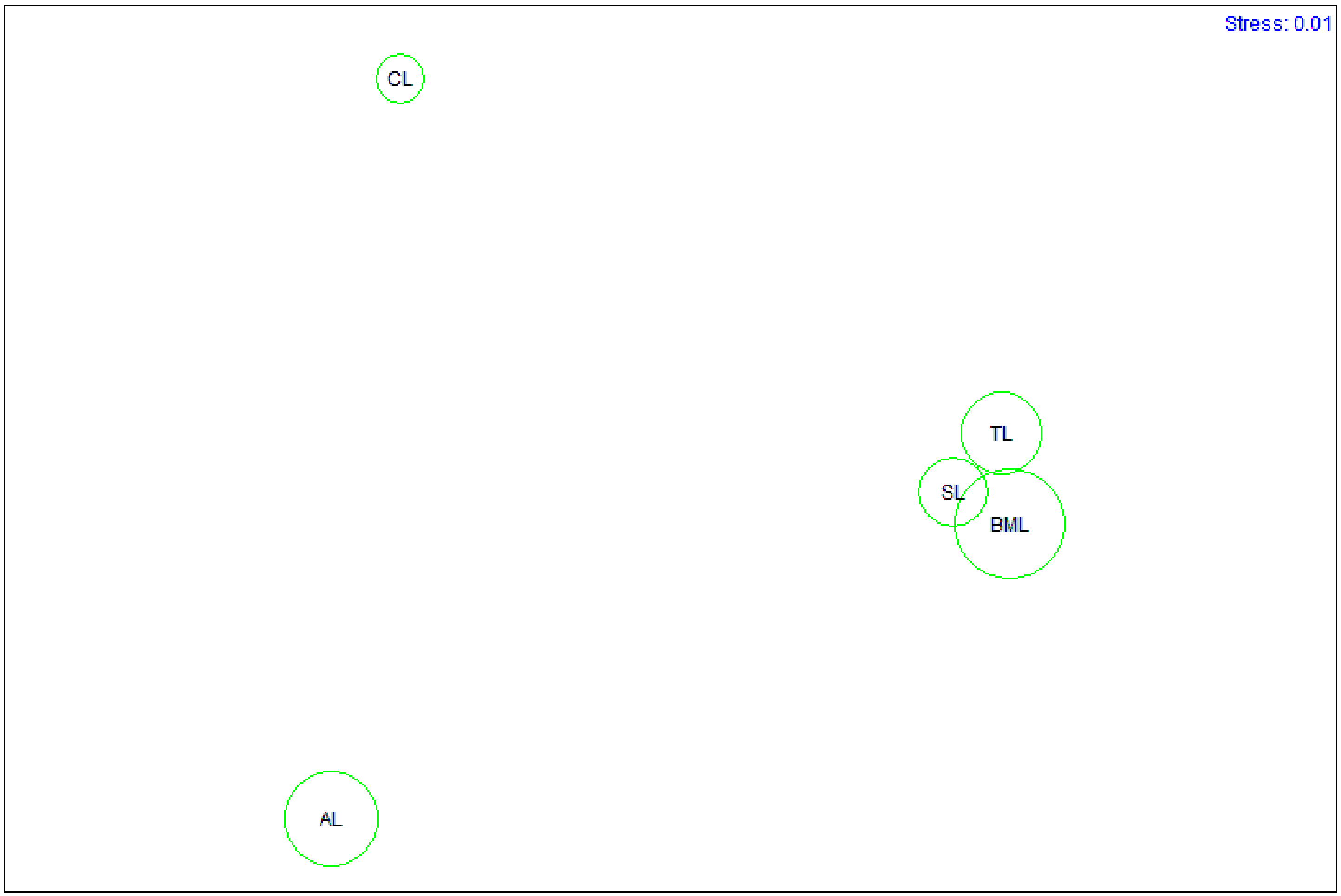


Figure 14. Non-metric multidimensional scaling ordination plot of Chironomidae community structure similarity (presence/absence) in the clearwater study lakes. Bubble sizes represent log transformed pH at each study lake (lake abbreviations are presented in Table 1).

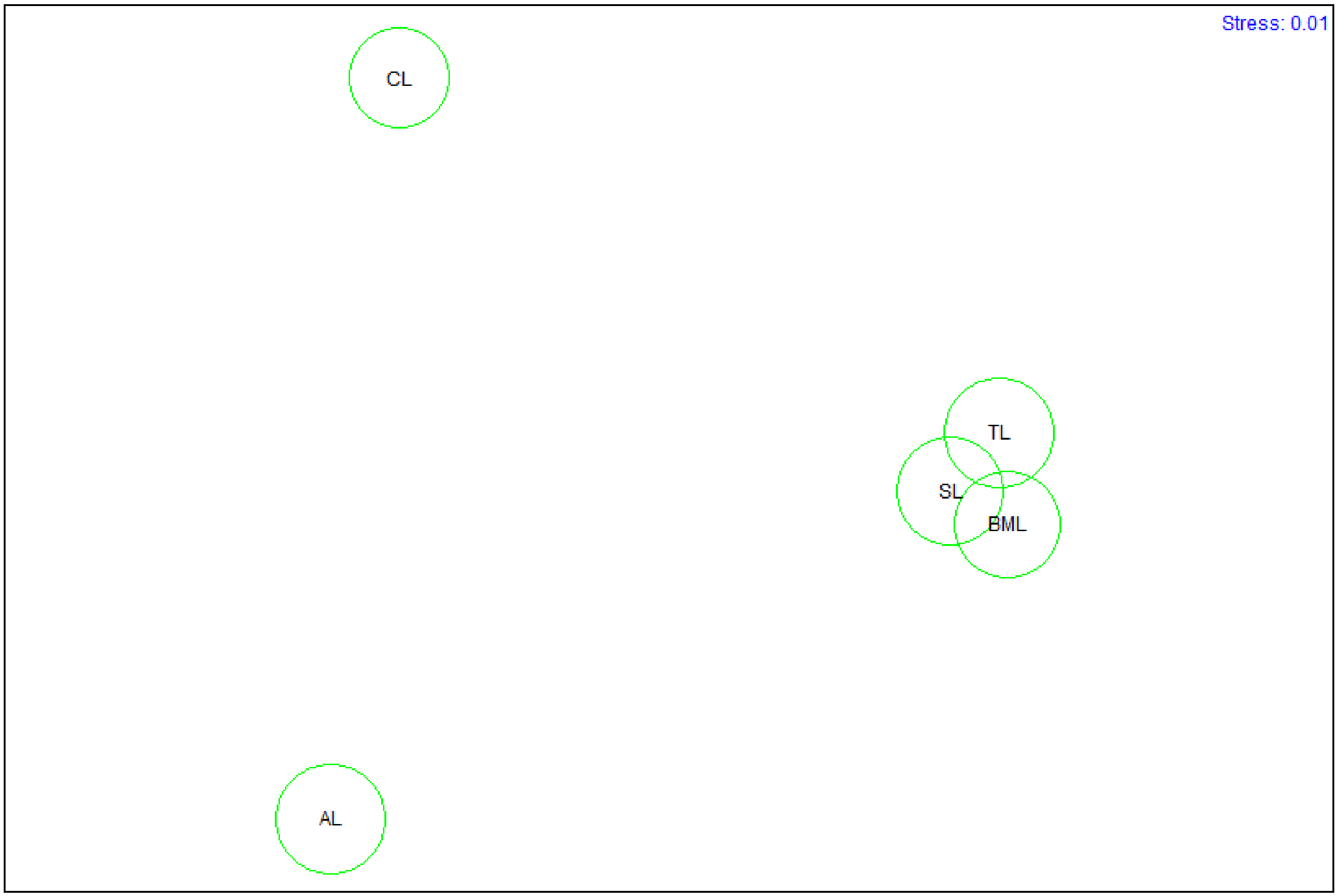


Figure 15. Non-metric multidimensional scaling ordination plot of Chironomidae community structure similarity (presence/absence) in the clearwater study lakes. Bubble sizes represent mean depth (m) at each study lake (lake abbreviations are presented in Table 1).

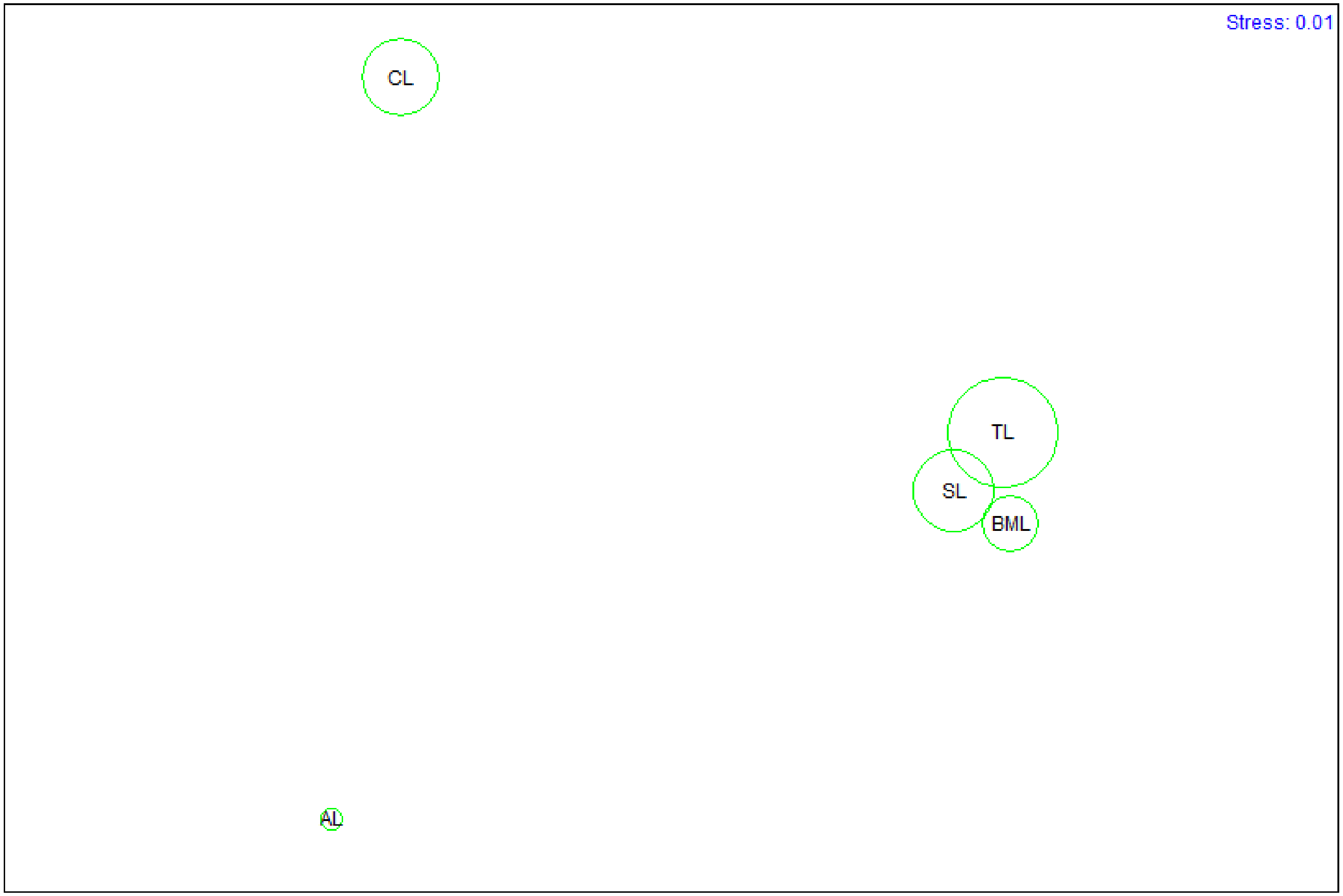


Figure 16. Non-metric multidimensional scaling ordination plot of Chironomidae community structure similarity (presence/absence) in the clearwater study lakes. Bubble sizes represent square root transformed muck (%) at each study lake (lake abbreviations are presented in Table 1).

Figure 17. Non-metric multidimensional scaling ordination plot of Chironomidae community structure similarity (presence/absence) in the clearwater study lakes. Bubble sizes represent log transformed iron (mg/L) at each study lake (lake abbreviations are presented in Table 1).

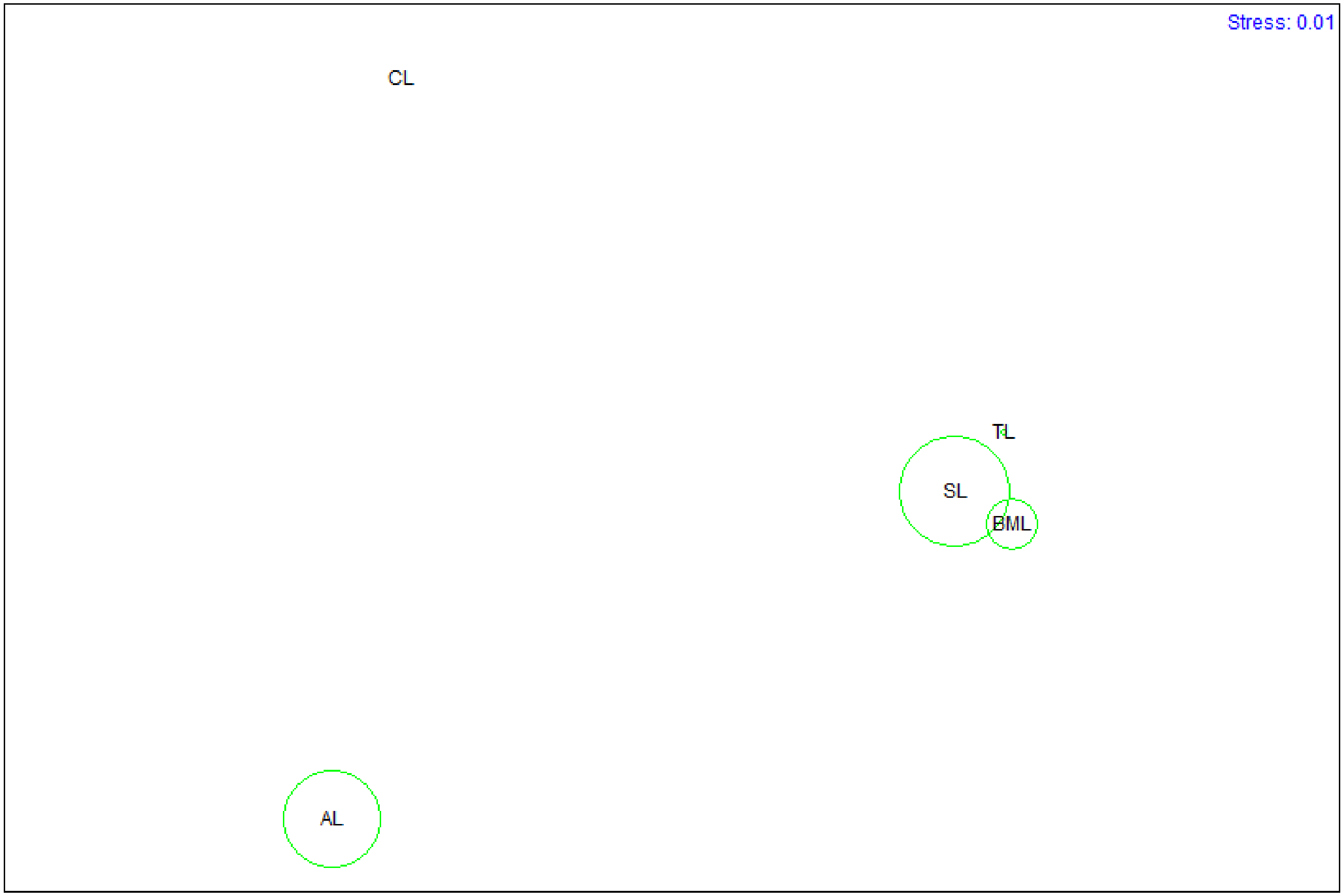


Table 6. Tolerance values (T.V.[1]) of Chironomidae taxa (Ferrington et al. 2008) and average T.V. for Crystal Bog (North Temperate Lakes Long-Term Ecological Research site).

Crystal Bog		
Taxa	**Tolerance Value**	**Region[2]**
TANYPODINAE		
Ablabesmyia	8	Upper Midwest
Procladius	9	Upper Midwest
ORTHOCLADIINAE		
Corynoneura	7	Upper Midwest
Psectrocladius	8	Upper Midwest
Synorthocladius	2	Upper Midwest
CHIRONOMINI		
Chironomus	10	Upper Midwest
Dicrotendipes	5	Upper Midwest
Einfeldia	8	Northwest
Endochironomus	8	Upper Midwest
Glyptotendipes	10	Upper Midwest
Omisus		
Parachironomus	10	Upper Midwest
Polypedilum	6	Mid Atlantic
TANYTARSINI		
Paratanytarsus	6	Upper Midwest
Tanytarsus	6	Upper Midwest
Average T.V.	**7.4**	

[1]T.V. = tolerance value

[2]Region indicates the region from which the tolerance values were derived

Table 7. Tolerance values (T.V.[1]) of Chironomidae taxa (Ferrington et al. 2008) and average T.V. for Allequash Lake (North Temperate Lakes Long-Term Ecological Research site).

Allequash Lake		
Taxa	**Tolerance Value**	**Region[2]**
TANYPODINAE		
Ablabesmyia	8	Upper Midwest
Procladius	9	Upper Midwest
Tanypus	10	Upper Midwest
ORTHOCLADIINAE		
Corynoneura	7	Upper Midwest
Cricotopus	7	Upper Midwest
Nanocladius	3	Upper Midwest
Orthocladius	6	Upper Midwest
Parakiefferiella	4.8	Midwest
Psectrocladius	8	Upper Midwest
CHIRONOMINI		
Chironomus	10	Upper Midwest
Cryptochironomus	8	Upper Midwest
Dicrotendipes	5	Upper Midwest
Endochironomus	8	Upper Midwest
Glyptotendipes	10	Upper Midwest
Microtendipes	7	Upper Midwest
Parachironomus	10	Upper Midwest
Polypedilum	6	Mid Atlantic
Stictochironomus	9	Upper Midwest
Xenochironomus	0	Upper Midwest
Zavreliella		
TANYTARSINI		
Cladotanytarsus	7	Upper Midwest
Micropsectra	7	Upper Midwest
Paratanytarsus	6	Upper Midwest
Tanytarsus	6	Upper Midwest
Average T.V.	**7.0**	

[1]T.V. = tolerance value

[2]Region indicates the region from which the tolerance values were derived

Table 8. Tolerance values (T.V.[1]) of Chironomidae taxa (Ferrington et al. 2008) and average T.V. for Trout Lake (North Temperate Lakes Long-Term Ecological Research site).

Trout Lake		
Taxa	**Tolerance Value**	**Region[2]**
TANYPODINAE		
Ablabesmyia	8	Upper Midwest
Procladius	9	Upper Midwest
ORTHOCLADIINAE		
Corynoneura	7	Upper Midwest
Epoicocladius		
Heterotrissocladius	0	Upper Midwest
Limnophyes	3.1	Midwest
Nanocladius	3	Upper Midwest
Orthocladius	6	Upper Midwest
Parakiefferiella	4.8	Midwest
Psectrocladius	8	Upper Midwest
Synorthocladius	2	Upper Midwest
Thienemanniella	6	Upper Midwest
Zalutschia	7	Mid Atlantic
CHIRONOMINI		
Cryptochironomus	8	Upper Midwest
Cryptotendipes	6	Upper Midwest
Einfeldia	8	Northwest
Stictochironomus	9	Upper Midwest
PSEUDOCHIRONOMINI		
Pseudochironomus	5	Upper Midwest
TANYTARSINI		
Cladotanytarsus	7	Upper Midwest
Micropsectra	7	Upper Midwest
Paratanytarsus	6	Upper Midwest
Stempellina	2	Upper Midwest
Stempellinella	4	Upper Midwest
Tanytarsus	6	Upper Midwest
Average T.V.	**5.7**	

[1]T.V. = tolerance value

[2]Region indicates the region from which the tolerance values were derived

Table 9. Tolerance values (T.V.[1]) of Chironomidae taxa (Ferrington et al. 2008) and average T.V. for Sparkling Lake (North Temperate Lakes Long-Term Ecological Research site).

Sparkling Lake		
Taxa	**Tolerance Value**	**Region[2]**
TANYPODINAE		
Ablabesmyia	8	Upper Midwest
Larsia	6	Upper Midwest
Procladius	9	Upper Midwest
ORTHOCLADIINAE		
Corynoneura	7	Upper Midwest
Cricotopus	7	Upper Midwest
Heterotrissocladius	0	Upper Midwest
Limnophyes	3.1	Midwest
Nanocladius	3	Upper Midwest
Orthocladius	6	Upper Midwest
Parakiefferiella	4.8	Midwest
Psectrocladius	8	Upper Midwest
Synorthocladius	2	Upper Midwest
Thienemanniella	6	Upper Midwest
Zalutschia	7	Mid Atlantic
CHIRONOMINI		
Chironomus	10	Upper Midwest
Cryptochironomus	8	Upper Midwest
Cryptotendipes	6	Upper Midwest
Dicrotendipes	5	Upper Midwest
Polypedilum	6	Mid Atlantic
Stenochironomus	5	Upper Midwest
Stictochironomus	9	Upper Midwest
PSEUDOCHIRONOMINI		
Pseudochironomus	5	Upper Midwest
TANYTARSINI		
Cladotanytarsus	7	Upper Midwest
Micropsectra	7	Upper Midwest
Stempellinella	4	Upper Midwest
Tanytarsus	6	Upper Midwest
Average T.V.	**6.0**	

[1]T.V. = tolerance value

[2]Region indicates the region from which the tolerance values were derived

Table 10. Tolerance values (T.V.[1]) of Chironomidae taxa (Ferrington et al. 2008) and average T.V. for Big Muskellunge Lake (North Temperate Lakes Long-Term Ecological Research site).

Big Muskellunge Lake		
Taxa	**Tolerance Value**	**Region[2]**
TANYPODINAE		
Ablabesmyia	8	Upper Midwest
Djalmabatista		
Procladius	9	Upper Midwest
DIAMESINAE		
Potthastia	4	Northwest
ORTHOCLADIINAE		
Corynoneura	7	Upper Midwest
Heterotrissocladius	0	Upper Midwest
Limnophyes	3.1	Midwest
Orthocladius	6	Upper Midwest
Parakiefferiella	4.8	Midwest
Psectrocladius	8	Upper Midwest
Synorthocladius	2	Upper Midwest
Thienemanniella	6	Upper Midwest
CHIRONOMINI		
Chironomus	10	Upper Midwest
Cryptochironomus	8	Upper Midwest
Dicrotendipes	5	Upper Midwest
Einfeldia	8	Northwest
Parachironomus	10	Upper Midwest
Paratendipes	8	Upper Midwest
Saetheria	4	Upper Midwest
PSEUDOCHIRONOMINI		
Pseudochironomus	5	Upper Midwest
TANYTARSINI		
Cladotanytarsus	7	Upper Midwest
Micropsectra	7	Upper Midwest
Tanytarsus	6	Upper Midwest
Average T.V.	**6.2**	

[1]T.V. = tolerance value

[2]Region indicates the region from which the tolerance values were derived

Table 11. Tolerance values (T.V.[1]) of Chironomidae taxa (Ferrington et al. 2008) and average T.V. for Crystal Lake (North Temperate Lakes Long-Term Ecological Research site).

Crystal Lake		
Taxa	**Tolerance Value**	**Region[2]**
TANYPODINAE		
Ablabesmyia	8	Upper Midwest
Procladius	9	Upper Midwest
DIAMESINAE		
Protanypus		
ORTHOCLADIINAE		
Brillia	5	Upper Midwest
Corynoneura	7	Upper Midwest
Heterotrissocladius	0	Upper Midwest
Limnophyes	3.1	Midwest
Nanocladius	3	Upper Midwest
Orthocladius	6	Upper Midwest
Psectrocladius	8	Upper Midwest
Zalutschia	7	Mid Atlantic
CHIRONOMINI		
Cladopelma	9	Upper Midwest
Dicrotendipes	5	Upper Midwest
Saetheria	4	Upper Midwest
Sergentia		
Stictochironomus	9	Upper Midwest
TANYTARSINI		
Cladotanytarsus	7	Upper Midwest
Micropsectra	7	Upper Midwest
Paratanytarsus	6	Upper Midwest
Tanytarsus	6	Upper Midwest
Average T.V.	**6.1**	

[1]T.V. = tolerance value

[2]Region indicates the region from which the tolerance values were derived

DISCUSSION

Chironomid Community Structure

Most chironomid taxa collected during this study represented-two subfamilies, Chironominae and Orthocladiinae; remaining taxa were in the subfamilies Tanypodinae and Diamesinae. Chironominae and Orthocladiinae taxa are widely distributed and collected in both lotic and lentic systems because of their ability to tolerate a broad range of conditions (Larocque et al. 2001; Boggero 2006; Bitusik et al. 2006; Ruse 2010). The ratio of Chironominae to Orthocladiinae genera was similar across clearwater lakes (1:1), except for Allequash Lake (AL), which had two-fold more Chironominae genera than Orthocladiinae. The littoral zone of AL is a mixture of macrophytes, gravel, and soft sediments, including both sand and silt. Pinder (1980) reported that Chironominae are typically associated with soft sediments, whereas Orthocladiinae are generally associated with coarse substrates (i.e., gravel) and submergent macrophytes. Although AL's littoral zone had a mixture of substrates, sand and silt were dominant and were observed covering submergent macrophytes and gravel. Chironominae taxa are known to use detritus, algae, and small sand grains and silt to build 'feeding' tubes/cases with catchnets (Pennak 1975; Berg 1995), so the predominance of sand and silt in AL is a likely explanation for the 2:1 Chironominae:Orthocladiinae ratio.

Common and Unique Occurring Chironomid Taxa

Taxa that were considered common occurrences in this study, i.e., found in all five clearwater lakes, have been reported to be broadly distributed among a wide range of freshwater habitats and ecological conditions (Lindeman 1942; Walker et al. 1985; Wiederholm 1986; Epler 2001). Two of the common taxa included the predaceous chironomids *Ablabesmyia* (Tanypodinae) and *Procladius* (Tanypodinae), which are known to inhabit lakes, ponds, swamps, bogs, and slow-moving portions of streams and rivers (Lindeman 1942; Wiederholm 1986). Three Orthocladiinae taxa, *Corynoneura*, *Orthocladius*, and *Psectrocladius*, were considered common occurrences in the clearwater study lakes and are frequently collected from a variety of terrestrial, freshwater, coastal estuarine, and littoral marine areas (Epler 2001). The remaining three common taxa in this study, *Cladotanytarsus* (Chironominae), *Micropsectra* (Chironominae), and *Tanytarsus* (Chironominae), are all in the subfamily Chironominae and can be found in fresh, brackish, and salt water. All common occurrence taxa were collected from lakes with different substrates ranging from the sandy bottom of Crystal Lake (CL) to the marsh-type littoral zone of AL.

In addition to common occurrence taxa, some taxa were unique occurrences, i.e., found in only one clearwater lake. Three of these taxa *Protanypus* (Diamesinae), *Sergentia* (Chironominae), and *Cladopelma* (Chironominae), were collected from oligotrophic CL. These results are consistent with reports of *Protanypus* and *Sergentia* occurring in profundal zones of oligotrophic lakes (Saether 1975; Wiederholm 1986; Merritt et al. 2008) and *Cladopelma* most commonly collected from sand substrates in lentic systems (Hudson et al. 1990), the predominant substrate in CL. Another unique chironomid occurrence was *Epoicocladius* collected only from Trout Lake (TL), the largest lake in this study. *Epoicocladius* has been reported to live either commensally or as a parasite on Ephemeroptera (mayflies) nymphs, specifically burrowing mayflies in the family Ephemeridae: *Ephemera* spp. and *Hexagenia* spp.

(Wiederholm 1986; Epler 2001). Exuviae of burrowing mayflies were collected only from TL in June 2010; no burrowing mayflies were collected from the other clearwater study lakes. AL had the most chironomid taxa considered to be unique occurrences, and included *Glyptotendipes* (Chironominae), *Endochironomus* (Chironominae), *Microtendipes* (Chironominae), *Tanypus* (Tanypodinae), *Zavreliella* (Chironominae), and *Xenochironomus* (Chironominae). The presence of these taxa exclusively in AL is likely due to characteristics of the littoral zone that were not present in any of the other clearwater lakes. AL has a marsh-type littoral zone with gravel, sand, silt, and well-developed beds of emergent, submergent, and floating macrophytes such as wild rice (*Zizania palustris*), large-leaf pondweed (*Potamogeton amplifolius*), common pondweed (*Potamogeton natans*), northern water-nymph (*Najas flexilis*), coon's-tail (*Ceratophyllum demersum*), common bladderwort (*Utricularia vulgaris*), and white water-lily (*Nymphaea odorata*) (a complete list can be found at the Wisconsin Department of Natural Resources website http://dnr.wi.gov/topic/lands/naturalareas/). Substrate type strongly influences chironomid communities within lakes (Pinder 1980), particularly the presence of macrophytes that serve as a stable substrate for many chironomid taxa (Tokeshi and Pinder 1985; Brodersen et al. 2001). *Glyptotendipes, Endochironomus*, and *Microtendipes* are reported to be associated with periphyton on macrophytes (Wiederholm 1986; Epler 2001). *Tanypus* is often found in soft sediments of the littoral zone, whereas *Zavreliella* move amongst submerged vegetation in their silken cases (Wiederholm 1986; Epler 2001). Lastly, *Xenochironomus* are obligate miners in freshwater sponges (Wiederholm 1986), which tend to colonize plant stems, pieces of wood, rocks, and other submersed objects (Manconi and Pronzato 2008).

Community Composition Among All Lakes

Chironomid community structures among the six north temperate study lakes were generally distinct, although Sparkling Lake (SL), TL and Big Muskellunge Lake (BML) formed a loose cluster in the NMDS ordination (Fig. 4). Community composition in SL and TL was most similar and included two taxa, *Cryptotendipes* and *Stempellinella*, only collected from these lakes. This similarity is likely due to the relatively close geographic proximity of the lakes (Fig. 1) and the high vagility of aerial adult chironomids that would result in a similar species pool available for colonization in the two lakes. BML was similar in chironomid community structure to both SL and TL, however, BML is not geographically close to the other two lakes (Fig. 1). The lakes are similar in available substrate and comparable shoreline habitat that could be suitable for the same chironomid taxa. Although shoreline habitat was not specifically examined in this study, similar shorelines characterized by a mixture of vegetation, woody debris, sand, gravel, and trees were observed during sampling at SL, TL, and BML. Additionally, cottages are present along BML and SL, and the NTL-LTER site field station is located on the south end of TL. These structures emit artificial light that could have a strong influence on aerial adult movements, especially in the absence of other lights across vast tracts of land and open water. Ali et al. (1984, 1986) found chironomids to be attracted to light in both laboratory conditions and in the field. In the southeastern United States, homes and business establishments emitting light and located on or near the lakeshore were affected by dense swarms of adult chironomids emerging from adjacent lakes (Ali et al. 1986). In contrast, chironomid community structures and physicochemical and morphological characteristics differed between BML and CL, even though the lakes are in close geographic proximity (Table 1, Fig. 1). For example, the substrate in CL is primarily sand, whereas in BML the substrate is a mixture of sand, gravel, rock, and some muck. This difference in substrate availability might explain the differences in chironomid community structure of these geographically close lakes. CL and AL had the highest number of unique taxa among all clearwater lakes resulting in community structures that were distinct from each other and from communities in other clearwater lakes (Table 2, Fig. 4). Likewise, CL and AL's distinctive habitat, one with a primarily sandy habitat, (CL) and the other with a marsh-type littoral zone (AL), most likely played a role in what chironomids were found in each system. Reuss et al. (2014) found chironomid community structure to be influenced by microhabitats within lakes in southwest Greenland, which included macrophytes, stones, and soft sediment at various depths. Chironomids adjusted to the available substrate that changed with depth from mosses in the upper littoral zone to aquatic plants, algae, and exposed stones mid-depth to patches of bare, soft sediment in the deeper portions of the lakes (Reuss et. 2014).

Crystal Bog (CB) was the only "stained water" lake in this study and is a small, hypoxic, acidic lake with sediment primarily consisting of muck (i.e., peat debris including decayed vegetation or organic matter that is typical of dystrophic lakes) (Butler and Anderson 1990). Chironomid community structure in CB was dominated in taxa richness by the subfamily Chironominae, but also included taxa in the subfamilies Orthocladiinae and Tanypodinae. Chironominae are reported to be more resilient in stressful conditions, such as low pH and oxygen concentrations (Boggero 2006), which may explain why this subfamily was more common in CB. Also, all Chironominae have hemoglobin, which has a high affinity for oxygen, so when a chironomid larva undulates its body within their tubes or burrows, the hemoglobin is saturated with oxygen allowing them to inhabit low oxygen conditions (Cranston 1995). The harsh physicochemical conditions, i.e., low dissolved oxygen and low pH, influenced CB's chironomid community structure. Non-metric multidimensional scaling revealed the distinctiveness of CB's chironomid community structure from communities in the five clearwater lakes. Chironomid taxa considered common in the clearwater lakes, i.e., *Ablabesmyia*, *Procladius*, *Corynoneura*, *Psectrocladius*, *Tanytarsus*, were also collected from CB, which suggests these taxa are tolerant of a wide range of conditions. *Ablabesmyia* and *Psectrocladius* are common in bogs (Walker 1985; Mousavi 2002). The Chironominae *Omisus* was a unique occurrence only collected from CB. *Omisus* are primarily collected from small, dystrophic ponds, shallow peat pools, and in fens and peat bogs (Wiederholm 1986; Berezina 1999; Epler 2001; Heiri et al. 2007). They have also been found in late-glacial lake sediments from the Netherlands and boreal lakes of North America and northern Europe, and in both cases the lakes were shallow with a low pH (Mousavi 2002; Heiri et al. 2007).

Influence of Abiotic Variables on Chironomidae Community Structure

Abiotic variables related to light, nutrients, and water chemistry had the greatest influence on chironomid communities in the study lakes as indicated by the BIO-ENV analysis. Studies have commonly reported that chironomid community structure in lakes is influenced by multiple factors (Aagaard 1986). Boggero et al. (2006) found that altitude, temperature, and pH influenced composition of chironomid assemblages in lakes of the Alps, whereas Bitusik et al. (2006) showed that altitude, pH, ion concentrations, and nutrients played a role in chironomid community structure of Tatra Mountain, Slovakia, lakes. Chironomid distributions in the low elevation lakes of northwestern Quebec, Canada were mainly influenced by water depth, dissolved organic carbon, water temperature, and August air temperature (Larocque et al. 2006), whereas Ruse (2010) concluded that conductivity and alkalinity were the key predictors for chironomid community structure in the low-to-mid elevation lakes of England, Wales, and Scotland. In the present study, the simplest combination of variables distinguishing chironomid communities was pH, total phosphorus, and Secchi depth. The inclusion of other variables in ordination analyses did not provide more insight into chironomid community structure because additional factors were either not important, highly variable, or related to pH, total phosphorus, and Secchi depth in the NTL-LTER site lakes system.

pH

One abiotic factor that had measurable influence on chironomid community structure was pH. Hydrogen ion concentration, pH, is an important environmental factor that is influenced by local geology (i.e. bedrock) and biological processes in the waterbody (i.e., photosynthesis, decomposition of organic matter, etc.) (Berezina 2001). Berezina (2001) found that chironomids were the numerically dominant organisms relative to other macroinvertebrates at all pH levels, however, chironomid communities only consisted of a few taxa in both highly acidic (low pH) and highly alkaline (high pH) water bodies. All clearwater lakes in this study were in the tolerable range of pH for freshwater systems (Ellis 1937; Kalff 2002). TL, AL, SL, and BML had circumneutral pH, whereas CL was slightly acidic. Overall, clearwater lakes had similar taxa richness, however lower pH was associated with reduced taxa richness, i.e., CL. Woodcock et al. (2005) showed that chironomid community structure in wetlands was related to pH, and suggested that competitive dominance of a few tolerant taxa was responsible for lower richness in low pH wetlands, whereas competition was weaker in high pH wetlands, facilitating the coexistence of more chironomid taxa. Similarly, Boggero et al. (2006) showed that the number of chironomid taxa was highest in circumneutral alpine lakes of the Alps, whereas more acidic and alkaline alpine lakes had lower numbers of taxa, which were primarily tolerant. Leuven et al. (1987) noted that in acidified lakes of the Netherlands the dominating submerged macrophytes were replaced over time by more tolerant macrophytes, which played a role in influencing chironomid assemblages. Bitusik et al. (2006) found that acidified subalpine and alpine lakes in the Tatra Mountains were characterized by reduced chironomid taxa richness and increased dominance of tolerant chironomid taxa. Additional NTL-LTER site lakes with varying pH values need to be studied over a longer period of time before a definitive pH trend can be determined, however taxa richness was higher in circumneutral SL, TL, AL, and BML, compared to the slightly-acidic CL.

Total Phosphorus

The second abiotic factor that had measurable influence on chironomid community structure was total phosphorus. AL had the highest phosphorus concentration among the clearwater study lakes, whereas TL had the lowest, and CL, BML, and SL were intermediate. Phosphorus concentrations in the clearwater study lakes were representative of Wisconsin natural lakes, indicating fair (AL) to good water quality (TL, SL, BML, CL) (Lillie and Mason 1983). Baines et al. (2000) reported phosphorus limitation of algal reproduction in the same clearwater lakes as the present study. Wiederholm (1980) noted that phosphorus was a key element for both plant and algal growth, and Saether (1979) noted that chironomid community structure was related to concentrations of phosphorus and chlorophyll-*a*. Aagaard (1986) studied the chironomid fauna of north Norwegian lakes and found a positive correlation between phosphorus, Secchi depth, total nitrogen, chlorophyll-*a*, and other variables that influenced community structure. In this study, no clear pattern was observed between phosphorus concentrations and chironomid community structure in the clearwater lakes, but phosphorus concentrations might influence other factors, such as plant and algal growth, which were not examined in this study.

The physicochemical and landscape variables examined in this study are not all-inclusive, and potentially important variables might have been omitted. The results of this study suggest that biotic variables should also be examined to find out how they relate to chironomid community structure of the study lakes. Algal growth and availability were not specifically examined in this study, but in other studies have been found to be an important variable in influencing chironomid community structure in lakes (e.g. Moore 1981, Aagaard 1986, Brodersen and Lindegaard 1999). Also, macrophyte presence and distribution could lend further insight into chironomid community structure of the clearwater study lakes as AL had a littoral zone with an abundant mixture of macrophyte species not found in the same density, or at all, in the other lakes. Weatherhead and James (2001) concluded that algal and macrophyte biomass and detritus were the most important factors influencing chironomid community structure in New Zealand lakes, which were indirectly affected by Secchi depth. Reuss et al. (2014) found that macrophytes and algae (i.e., habitat and food availability) are key factors in the structuring of chironomid communities. Algal community species composition and biomass and macrophytes should be assessed in future studies of the NTL-LTER study lakes because of their relationship with phosphorus and possible role in influencing chironomid community structure.

Secchi Depth

The final abiotic factor that had measurable influence on chironomid community structure was Secchi depth. TL, BML, CL and SL all had deep Secchi depth readings, whereas AL had a shallower Secchi depth reading. Saether (1975) suggested that light penetration had a strong influence on chironomid distribution in lakes and chironomid communities could be used as indicators of lake typology. Light penetration is affected by suspended particles in the water column such as terrestrial organic matter, phytoplankton, and zooplankton (all potential food items for chironomids) and can affect food availability (Mayer et al. 2001; Karlsson et al. 2009). Secchi depth values of all the clearwater study lakes fell into the ‘very good’ category per Lillie and Mason’s (1983) water clarity scale. Furthermore, the Wisconsin Department of Natural Resources (WDNR) Lakes information site (http://dnr.wi.gov/lakes), which obtains lake characteristic data through various partnerships, such as the Citizen Lake Monitoring Network, is consistent with Lille and Mason’s (1983) water clarity scale and categorized TL, BML, CL, and SL as oligotrophic (clear water) and AL as mesotrophic (moderately clear water). Although this study did not specifically consider chironomid community structure and trophic status or primary productivity, based solely on Secchi depth, the trophic status classification of clearwater study lakes is consistent with previous discussions of taxa and habitats in each lake. TL, BML, CL, and SL have sparse macrophytes and deep Secchi readings, which are the characteristics of oligotrophic systems. Conversely, AL had a shallower Secchi reading and was the only clearwater study lake with a littoral zone containing beds of emergent, floating, and submergent macrophytes, which results in accumulated organic matter and characteristic of a mesotrophic lake.

The relationship between chironomid fauna and productivity has been used in the development of lake classification systems (Saether 1979; Wiederholm 1980). Woodward and Shulmeister (2006) concluded that temperature and lake productivity, measured as chlorophyll-*a* concentration, were the most significant variables that influenced chironomid taxa in New Zealand. Brodersen and Lindegaard (1999) found that Secchi depth and chlorophyll-*a* concentration were best correlated to chironomid faunal data in Danish lakes. Moore (1981) showed algal density and availability stimulated by highly transparent water played an important role in chironomid communities in a northern Canadian lake. Algae are known to be a major food resource for chironomids (Ali et al. 2002); depending on feeding mode, chironomids feed on benthic algae, epiphytic algae, suspended algae and/or benthic macro-algae (Berg 1995). Filter-feeding chironomids ingest suspended algae, i.e., phytoplankton, collector-gatherers feed on deposited algae and detritus, scrapers remove or feed on algae from the surfaces of plants, rocks, wood, etc., and shredders mainly feed on coarse particulate organic matter (CPOM) i.e., submerged wood, leaf litter and living macrophytes, but are known to feed on macro-algae (Berg 1995). Filter-feeders, collector-gatherers, and scrapers were collected from all the clearwater study lakes, whereas shredder chironomids were mainly found in AL. The possible effects of light penetration in the clearwater study lakes should be studied further to determine the relationship with algal quantity, quality, and availability for chironomids and the possible effects on lake community structure.

Tolerance Values and Water Quality

Multiple attributes are typically used to assess water quality of lentic systems (Kratz et al. 1997). These attributes include fish communities, habitat availability, benthic macroinvertebrate community (including Chironomidae), and water chemistry. The use of chironomid communities as water quality indicators is well-established (Saether 1979; Ruse 2010); where mean chironomid community tolerance values (MCCTV) are commonly used in water quality assessments of lentic systems. CB had the highest MCCTV among all study lakes resulting in the lowest water quality. Caution should be used, however, when interpreting the water quality of CB because it is not a deteriorated water body, but rather a dystrophic lake. CB is a small, acidic lake with low dissolved oxygen and is surrounded by a floating peat mat (Butler and Anderson 1990; Riera et al. 1999), which is consistent with its highly tolerant chironomid community. Most chironomids collected from CB had tolerance values ≥8. Three genera, *Chironomus*, *Glyptotendipes*, and *Parachironomus* are considered highly tolerant, i.e., tolerance value 10. The genus *Omisus* also was collected from CB and is known to inhabit mainly bogs or bog-type conditions, however the genus has no tolerance value due to the lack of biological and ecological information. If *Omisus* was assigned a tolerance value, it would most likely be on the higher end of the scale because of its typical habitat. Chironomid community structure in CB consisting of only tolerant taxa is consistent with reports of a single fish species, the Central Mudminnow (*Umbra limi*), occurring in the bog (Kratz et al. 1997; 2018 NTL-LTER website). This species is highly tolerant and often found in waters with low dissolved oxygen concentrations, extensive aquatic vegetation, and with a benthic substrate covered by a thick layer of organic matter (Schilling et al. 2006). The presence of one fish species and mostly tolerant chironomids in CB suggests that water quality is not necessarily low, but only inhabitable by the most tolerant organisms compared to the clearwater study lakes.

Lillie and Mason's (1983) 14-year study of Wisconsin lakes, which summarized information from 1,140 lakes, concluded that water quality of the clearwater study lakes in the NTL-LTER site is 'good' based on circumneutral pH and total phosphorus and dissolved oxygen concentrations. When MCCTVs of only clearwater lakes were compared, AL had the highest value due to the presence of primarily moderately to highly tolerant chironomids. Twenty of the 24 taxa collected from AL had tolerance values ranging from 5 – 10. Tolerant chironomids, by definition, can inhabit a wide range of habitats and ecological conditions (Wiederholm 1986). Although the chironomid community in AL is comprised of mostly tolerant chironomids, it should not be inferred that the lake is of low water quality and/or similar to CB. Most physicochemical and morphological characteristics of AL, except for dissolved reactive silica (DRS) due to high groundwater input, are not substantially different from other clearwater lakes that had lower mean chironomid tolerance values. Although water clarity in AL was considered 'very good' based on Secchi depth, total phosphorus concentration was the highest among all clearwater lakes resulting in a 'fair' water quality rating (Lillie and Mason 1983). Several sensitive chironomid taxa were collected from AL and included *Xenochironomus*, which has a tolerance value of 0, and *Nanocladius* with a tolerance value of 3. The NTL-LTER website (2018) reported 36 fish species collected from AL, with only TL having a higher fish species richness. AL had the same number of chironomid taxa as TL, which was the largest lake in this study and had the best water quality according to MCCTVs.

The MCCTV in TL was the lowest, which implied high water quality, of all study lakes due to the occurrence of mainly sensitive to moderately tolerant chironomids. Six of the 24 taxa collected from TL had a tolerance value ranging from 0 to 4. Three of the six sensitive chironomid taxa, *Heterotrissocladius*, *Synorthocladius*, and *Stempellina*, were considered very sensitive with tolerance values ranging from 0 – 2. The same tolerant chironomids collected in AL were also found in TL, which again, demonstrates their ability to live in lakes across a wide range of water quality. The physicochemical characteristics and fish community of TL was consistent with the MCCTV. For example, TL had the highest water clarity and lowest total phosphorus of all study lakes, classifying it as having good to very good water quality (Lillie and Mason 1983). The NTL-LTER website (2018) reported 42 fish species from TL, which was the highest number of species collected among the study lakes.

The three remaining lakes, Sparkling, Crystal, and Big Muskellunge, had similar MCCTVs. As expected, chironomid communities of each lake consisted mainly of moderately and highly tolerant chironomids with few sensitive taxa. SL, CL, and BML were similar in both physicochemical and morphological characteristics and were consistent with the MCCTVs. Also, the number of fish taxa reported in each lake, i.e., 30 in SL, 24 in CL, and 34 in BML, was intermediate between TL (42 species) and CB (1 species; NTL-LTER website 2018). All three lakes had water clarity and total phosphorus concentrations intermediate to CB and the other study lakes resulting in good water quality (Lillie and Mason 1983).

CONCLUSION

The ongoing focus of the North Temperate Lakes-Long Term Ecological Research (NTL-LTER) project has been the importance of landscape position in structuring biological communities in lakes (Swanson et al. 1988; Kratz et al. 1991, 1997). Even though lakes within the Trout Lake Region are geographically close to each other, positioned within a similar geologic setting, and experience the same weather conditions, the physicochemical and biological properties of the lakes are often quite different from one another (Kratz et al. 1997). This is due to differences in lake morphometry, internal dynamics (i.e., trophic dynamics), and spatial positioning (i.e., lakes that are isolated versus lakes near roads or residential developments) (Kratz et al. 1997). Kratz et al. (1997) found that elevation influenced fish communities in the lakes of the NTL-LTER site, which were the same lakes as in this study. Species-rich lakes were larger in size and located in low elevation, whereas lakes located in higher elevation were smaller in size and had fewer fish species (Kratz et al. 1997). A similar pattern was not found for chironomids in this study as elevation and lake-size were not important factors influencing chironomid community structure. The aerial stage of chironomids enables them to potentially be found at all the study lakes no matter the elevation or distance between them; not only does their ability to fly give them accessibility to the other lakes, but once in the air they can be carried by the wind (Lindeberg 1964; Vaughn 1982). Also, chironomids have been reported to be dispersed between water-bodies through migratory birds (Green and Sanchez

2005) and transferred by traffic, i.e., train and automobile (Hoffrichter 1973), which also gives chironomids accessibility to all the NTL-LTER site study lakes.

Results from this study suggest that pH, total phosphorus, and Secchi depth, as a group, were the most important factors structuring chironomid communities in the NTL-LTER site long-term clearwater study lakes, and this study provides insights for future work. Algal growth and availability should be examined in greater detail in the NTL-LTER site as it has been known to be closely related to the primary factors (i.e., pH, total phosphorus, and Secchi depth) found in this study influencing chironomid community structure. In addition, macrophyte abundance and species composition of the NTL-LTER site study lakes were not evaluated in detail and should be considered in future studies, as they are known to be associated with certain chironomid taxa and provide habitat, food resources, and substrate for epiphytic algae. Future work should include these biotic variables and further examination may uncover important relationships. The results of this research should be considered as an exploratory and introductory assessment, a snapshot, of chironomid community structure in lakes of the NTL-LTER region. A long-term chironomid sampling program using pupal exuviae would increase the likelihood of collecting any taxa missed and provide a more comprehensive picture of chironomid community structure in the NTL-LTER site study lakes. Also, expanding sample collections to include more seasons will provide additional insight into the factors influencing chironomid community structure.

The NTL-LTER site and surrounding area includes a diversity of lakes that, while different from one another, are also susceptible to similar biotic and abiotic conditions, e.g. invasive species and climate change, that are relevant to lakes throughout the world. Little work has been conducted examining chironomid community structure in the NTL-LTER site lakes and surrounding area lakes. This study provides the necessary background to inform the design and implementation of future studies. For example, a future study may look at the susceptibility of lakes in the NTL-LTER site region to climate change and compare chironomid communities by referencing results from this study. Climatic shifts to drier conditions have been studied in northern Wisconsin, but chironomid community structure was not considered (Webster et al. 1996). Furthermore, chironomids play an important role as food resources for other organisms, such as aerial-feeding and water birds, fish, bats, and spiders, and any changes in chironomid community structure have the potential to cascade to higher trophic levels (Leuven et al. 1987; Armitage 1995; Gratton et al. 2008; Sabo and Power 2008; Jonsson and Wardle 2009; Hoekman et al. 2011; Dreyer et al. 2012). Elucidating the factors that influence chironomid community structure will allow researchers to predict chironomid community structure at other lakes, and can lead to an improved understanding of chironomids in north temperate lakes. Also, recognizing the factors that influence chironomid communities will contribute to our understanding of the importance of landscape position in structuring lake characteristics, and be an asset to future studies examining the interplay of landscape position and biological communities in lakes.

APPENDIX A

TYPICAL HABITATS OF CHIRONOMID TAXA IDENTIFIED IN THE NORTH TEMPERATE LAKES LONG-TERM ECOLOGICAL RESEARCH REGION

TANYPODINAE

Ablabesmyia:
(Pentaneurini)

-Eurytopic - able to tolerate a wide range of habitats and ecological conditions (Wiederholm 1986)
-Found in flowing water, lakes, ponds, and swamps. Roback (1985) noted that *Ablabesmyia* were found over a pH range of <4.1 - >8.1, but were predominantly found in circumneutral range of 6.1-7.0. He observed that they preferred softer, less alkaline water (Epler 2001).
-Littoral (Merritt et al. 2008).

Djalmabatista:
(Procladiini)

-Occur in littoral zone of soft water, low alkaline to weakly acidic lakes and ponds (Wiederholm 1986)
-Found in ponds, lakes, streams, and rivers; prefer soft water, low alkalinity, a slightly acidic to circumneutral pH, and are tolerant of moderate levels of iron (Epler 2001).

Larsia:
(Pentaneurini)

-Found in variety of habitats, such as springs, ditches, bog pools among *Sphagnum,* in the littoral zones of lakes. Many species are polyoxybiontic and cold-stenothermic. (Wiederholm 1986)
-Most often found in marshes, ponds, and the littoral zone of lakes, but can also be found in the slower moving portions of rivers and streams; at least one species has been collected from hot spring in Colorado (Epler 2001).
-Littoral (Merritt et al. 2008).

Procladius:
(Procladiini)

-Live in muddy sediments of lentic or slow-flowing waters. They have been recorded from a variety of habitats, in bog pools, ponds, the littoral and profundal of lakes (Wiederholm 1986).
-Found in the bottom sediments of bogs, ponds, lakes, and the slower moving portions of streams and rivers. Able to tolerate a wide range of habitats and ecological conditions (Epler 2001).
-Profundal (some littoral) (Merritt et al. 2008).

Tanypus:
(Tanypodini)

-Live in sediments in slow-flowing and standing waters and are predominantly found in regions with temperate or warm climates (Wiederholm 1986).
-Usually found in or on soft sediments of marshes, ponds, and lakes but also occur in the slower portions and side pools of streams and rivers. A few species can be common in organically enriched systems. Data in Roback (1977) indicate that *Tanypus* are found in a pH range of <4-8.0, an alkalinity range of <40-160 ppm, a total hardness range of <50-200 ppm, and a specific conductivity of <100-400 µmhos @25°C (Epler 2001).
-Littoral (Merritt et al. 2008).

DIAMESINAE

Potthastia:
(Diamesini)

-Those of the *longimana* group also inhabit lakes (Wiederholm 1986).
-In general, cool-adapted flowing water inhabitants, but some are also found in springs and lakes (Epler 2001).

Protanypus:
(Protanypini)

-Inhabit lakes, usually oligotrophic systems (Wiederholm 1986).
-Profundal (Merritt et al. 2008).

ORTHOCLADIINAE

Brillia: (Orthocladiini)	-Inhabit small to large bodies of flowing water, springs, the littoral zone of lakes and the hygropetric zone. They are usually associated with submerged wood and leaves (Wiederholm 1986). -Are almost always associated with submerged allochthonous wood and leaves, and may be found in springs, streams, rivers, and the littoral margins of lakes (Epler 2001). -Depositional detritus (Merritt et al. 2008).
Corynoneura: (Corynoneurini)	-Found in nearly all types of aquatic habitat and pupae may occur throughout the year. Many species appear to be polyvoltine (Wiederholm 1986). -Found in a wide variety of habitats (Epler 2001). -Littoral (Merritt et al. 2008).
Cricotopus: (Orthocladiini)	-Inhabit all types of freshwater and, to a lesser extent, saline coastal and inland waters. They are frequently associated with aquatic plants, including algae, and some mine living parts of aquatic macrophytes (Wiederholm 1986). -Found in a variety of aquatic habitats, where they are often associated with plants (Epler 2001). -Vascular hydrophytes, algal mats, sediments, and detritus (Merritt et al. 2008).
Epoicocladius: (Orthocladiini)	-Found on the bodies of Ephemeroptera (*Ephemera* spp. and *Hexagenia* spp.) The most frequent site for pupation on the Ephemeropteran is beneath the wing buds and on the dorsal surface where a loose cocoon is spun (Wiederholm 1986). -Live commensally or as parasites on larvae of the mayfly family Ephemeridae (Epler 2001).
Heterotrissocladius: (Orthocladiini)	-Inhabit the littoral and profundal zones of lakes; some species are found in streams, springs, rivers, ponds, or puddles (Wiederholm 1986). -Found in rivers, streams, seeps, pools and lakes (Epler 2001). -Littoral and profundal (Merritt et al. 2008).
Limnophyes: (Orthocladiini)	-Found in aquatic, semi-terrestrial, terrestrial habitats (Wiederholm 1986). -Occurs in rivers, streams, springs, seeps, in moss on rock surfaces, stream margins, and other semi-aquatic habitats, as well as in terrestrial habitats (Epler 2001). -Littoral (macroalgae) (Merritt et al. 2008).
Nanocladius: (Orthocladiini)	-Inhabit streams, rivers, lakes (both littoral and upper profundal zones; oligotrophic to mesotrophic lakes) (Wiederholm 1986). -Associated with lakes, rivers, and streams; some species tolerant of high levels of organic nutrients (Epler 2001). -Littoral (Merritt et al. 2008).

Orthocladius: (Orthocladiini)	-Found in various freshwater habitats, including lakes, ponds, streams, rivers, swamps, temporary *sloughs*, and thermal waters (Wiederholm 1986). -Inhabit a wide variety of habitats (Epler 2001). -Littoral (erosional) and profundal (Merritt et al. 2008).
Parakiefferiella: (Orthocladiini)	-Encountered in both lentic and lotic habitats (Epler 2001; Wiederholm 1986). -Littoral (Merritt et al. 2008).
Psectrocladius: (Orthocladiini)	-Occurs in a wide variety of habitats (Wiederholm 1986) -Found in lentic and lotic habitats (Epler 2001). -Littoral (Merritt et al. 2008).
Synorthocladius: (Orthocladiini)	-Inhabit springs, small to large bodies of flowing water, and small bodies or shallower parts of still water (Wiederholm 1986). -Most often found in running water, but may occur in springs and lentic habitats (Epler 2001).
Thienemanniella: (Orthocladiini)	-Most lotic habitats (Wiederholm 1986). -Found in running and standing water, usually in streams and rivers, and can be encountered in clean or enriched habitats (Epler 2001). -Littoral (Merritt et al. 2008).
Zalutschia: (Orthocladiini)	-Primarily found in lakes (oligotrophic and dystrophic), but are also found in ponds, puddles, ditches, and occasionally streams (Wiederholm 1986). -Inhabit lakes, streams, and rivers (Epler 2001). -Littoral (Merritt et al. 2008).
CHIRONOMINAE	
Chironomus (Chironomini)	-Predominantly found in soft sediments of standing water, more rarely in flowing water; some species prefer humic water (Wiederholm 1986). -Inhabit sediments, can occur in highly polluted conditions or in relatively clean water (Epler 2001). -Littoral and profundal (Merritt et al. 2008).
Cladopelma: (Chironomini)	-Occur in sandy and muddy substrata of lakes and rivers (Wiederholm 1986). -Usually found on or in bottom sediments of lakes and rivers; some species are tolerant of low oxygen conditions (Epler 2001). -Littoral (Merritt et al. 2008).
Cladotanytarsus: (Tanytarsini)	-Occur in a wide range of habitats, including lakes, rivers, thermal springs, and brackish waters (Wiederholm 1986). -Found in many types of water bodies, including brackish water and hot springs (Epler 2001). -Vascular hydrophytes (Merritt et al. 2008).

Cryptochironomus: (Chironomini)	-Occur in a wide variety of freshwater habitats, including streams, large rivers, and lakes (Wiederholm 1986) -Mostly benthic and seem to prefer sandy substrates (Epler 2001). -Littoral and profundal (Merritt et al. 2008).
Cryptotendipes: (Chironomini)	-Inhabit sandy and muddy substrata in both flowing and standing waters (Wiederholm 1986) -Found in lentic and lotic situations; they are usually benthic and appear to tolerate organically enriched habitats (Epler 2001). -Littoral (Merritt et al. 2008).
Dicrotendipes: (Chironomini)	-Inhabit littoral sediments of standing, occasionally flowing, waters; most often found in "Aufwuchs" (vegetation) (Wiederholm 1986). -Found in brackish and fresh waters, in lotic and lentic conditions, in pristine or degraded habitats; occur in sediments but most often encountered on vegetation (Epler 2001). -Littoral (wide range of microhabitats) (Merritt et al. 2008).
Einfeldia: (Chironomini)	-Live in littoral, soft sediments of small lakes, ponds, old river beds, and small water bodies with a tendency towards eutrophic conditions; some species occur in dystrophic, small water bodies. *Einfeldia* is apparently absent from flowing waters (Wiederholm 1986). -Found most often in eutrophic standing water, but can occur in lotic situations (Epler 2001). -Littoral and profundal (Merritt et al. 2008).
Endochironomus: (Chironomini)	-Occur in "Aufwuchs" of living and dead substrata in almost all types of still water or mine in leaves and stems of macrophytes with a tendency of preferential occurrence in small, eutrophic standing waters (Wiederholm 1986). -Often associated with moderate eutrophic conditions and occur in lentic and lotic situations (Epler 2001). -Littoral (algal mats) and profundal (Merritt et al. 2008).
Glyptotendipes: (Chironomini)	-Occur in detritus-rich littoral sediments and in "Aufwuchs" of lakes, ponds, small water bodies, and running water. A considerable proportion of species are "semi-miners" under the bark of submerged branches, in submerged plants or plants with floating leaves (Wiederholm 1986). -Occur in usually eutrophic standing and slow moving water, where they are found in or on sediments and aquatic plants; several species are miners in plants or decaying wood (or they live in burrows in plant material made by other organisms) (Epler 2001). -Littoral and profundal (Merritt et al. 2008).
Micropsectra: (Tanytarsini)	-Found living in a wide range of habitats, including hygropetric situations, hot springs, and temporary pools, but they are most characteristic of soft sediments in streams, small rivers, and lakes (Wiederholm 1986) -Occur in a wide range of lentic and lotic habitats (Epler 2001) -Littoral (Merritt et al. 2008).

Microtendipes: (Chironomini)	-Inhabit littoral and sublittoral sediments of larger bodies of still water; sporadically also in "Aufwuchs"; also in sediments and submerged mosses in running water (Wiederholm 1986). -Occur in streams, rivers, ponds, and lakes (Epler 2001). -Littoral (Merritt 2008 et al.).
Omisus: (Chironomini)	-Occur in small, partly dystrophic lakes and in dystrophic small water bodies (e.g. bogs) (Wiederholm 1986). -Found in seeps, small streams, ponds, marshes and peat bogs (Epler 2001).
Parachironomus: (Chironomini)	-Found in a wide variety of water bodies and habitats. Most species inhabit soft sediments, but some live in association with Bryozoa or are ectoparasitic on other invertebrates and some are leaf and stem-miners in submerged macrophytes (Wiederholm 1986). -Found in lentic and lotic water bodies under a wide range of conditions (Epler 2001). -Littoral (Merritt et al. 2008).
Paratanytarsus: (Tanytarsini)	-Able to tolerate a wide range of habitats (Wiederholm 1986). -Inhabit a variety of aquatic habitats, as well as brackish water (Epler 2001). -Littoral (Merritt et al. 2008).
Paratendipes: (Chironomini)	-Occur in standing waters (lakes, ponds, small water bodies, bogs) and in flowing waters (streams, rivers), in soft sediments and in sandy bottoms (Wiederholm 1986). -Occur in a variety of habitats, preferring sandy bottoms of streams and rivers (Epler 2001). -Littoral (Merritt et al. 2008).
Polypedilum: (Chironomini)	-Occur in virtually all kinds of still and flowing waters; sediments are the preferred substratum. A few species are also found on hard substrata or mining in water plants (Wiederholm 1986). -Found in a wide range of habitats under a variety of environmental conditions; ranging from pristine to heavily degraded (Epler 2001). -Vascular hydrophytes (floating zone) (Merritt et al. 2008).
Pseudochironomus: (Pseudochironomini)	-Found in sandy or gravelly littoral sediments in primarily mesotrophic to oligotrophic lakes or in large, slowly flowing rivers (Wiederholm 1986). -Inhabit sandy substrata of lakes and rivers and may also be found in brackish water (Epler 2001). -Lentic, erosional (in algae) and depositional (Merritt et al. 2008).
Saetheria: (Chironomini)	-Inhabit sandy substrata in lakes and streams (Wiederholm 1986). -Found in sandy substrata, usually running water (Epler 2001). -Sandy bottom (Merritt et al. 2008).

Sergentia: (Chironomini)
-Occur in the sublittoral and profundal of oligotrophic to mesotrophic lakes. They are probably cold-stenothermic (Wiederholm 1986).
-Lentic (Merritt et al. 2008).

Stempellina: (Tanytarsini)
-Have been recorded from a wide variety of freshwater habitats (Wiederholm 1986).
-Live in portable cases and may occur in lotic and lentic situations (Epler 2001).
-Littoral (Merritt et al. 2008).

Stempellinella: (Tanytarsini)
-Occur in springs and small streams as well as in lakes (Wiederholm 1986).
-Found in springs, streams, and rivers; they are also recorded from lakes (Epler 2001).
-Littoral (Merritt et al. 2008).

Stenochironomus: (Chironomini)
-Are obligate miners in living and dead vegetation, including wood (Wiederholm (1986)
-Mine in dead submerged leaves or in submerged dead wood (Epler 2001).
-Vascular hydrophytes (Merritt et al. 2008).

Stictochironomus: (Chironomini)
-Found in profundal soft sediments or littoral sand of oligotrophic to mesotrophic lakes; also, ubiquitous in sandy sediments of streams and slowly flowing rivers (Wiederholm 1986).
-Inhabit sandy sediments of streams, rivers and lakes (Epler 2001).

Tanytarsus: (Tanytarsini)
-Occur in all types of freshwater and a few species are marine (Wiederholm 1986).
-Found in a variety of aquatic habitats, including brackish water (Epler 2001).
-Vascular hydrophytes (floating zone) and profundal (Merritt et al. 2008).

Xenochironomus: (Chironomini)
-Obligate miners in freshwater sponges in both standing and flowing waters (Wiederholm 1986; Epler 2001).
-In sponges (Merritt et al. 2008).

Zavreliella: (Chironomini)
-*Zavreliella* are mobile amongst submerged vegetation in small bodies of standing water (Wiederholm 1986).
-Found in marshes, vegetation-choked, eutrophic ponds and lakes, and the sluggish portions of streams and rivers, where they swim around in their hydroptilid caddisfly-like silken cases (Epler 2001).
-Lentic (Merritt et al. 2008).

APPENDIX B

NUMBER OF CHIRONOMID EXUVIAE COLLECTED BY DATE FROM NORTH TEMPERATE LAKES LONG-TERM ECOLOGICAL RESEARCH SITE STUDY LAKES

	Collection Dates					
Lakes	June 2010	July 2010	August 2010	September 2010	October 2010	May 2011
Trout Lake	24,263	338	345	91	0	30
Allequash Lake	70	118	5,067	309	3	94
Sparkling Lake	1,021	169	141	49	3	94
Big Muskellunge Lake	1,708	409	164	25	3	270
Crystal Lake	1,123	33	22	202	2	344
Crystal Bog	70	341	116	16	0	18
Total Number Per Date	28,255	1,408	5,855	692	11	850
Taxa Richness By Date	21	23	26	19	5	20

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VITA

Conrad S. Zack graduated from the University of New Mexico in 2003 with a Bachelor of Science degree in Biology. As an undergraduate, he had a scientific article published in Wildlife Society Bulletin about the southern oscillation index and encounters between humans and black bears in New Mexico. After graduation, he worked for the New Mexico Department of Game and Fish (NMDGF) in the Conservation Services Division – Endangered Species Program in Santa Fe where he was introduced to and developed an interest in aquatic biology. Following the position with NMDGF, in 2005, Conrad moved to Chicago, IL and found employment with EA Engineering, Science, and Technology Inc., PBC., an environmental consulting firm in Deerfield, IL. During this position, he solidified his love of aquatic biota and refined his aquatic macroinvertebrate taxonomy skills, becoming a Certified Taxonomist in General, EPT, and Chironomidae – eastern taxa in 2013 by the Society for Freshwater Science. He also has advanced his ichthyoplankton taxonomy skills since 2005 working with well-known experts Darrel Snyder, Robert (Bob) Wallus, and Larry K. Kay. He has been with the company for 13 years and currently works as an Aquatic Biologist/Benthic Macroinvertebrate and Larval Fish Taxonomist.