Winter-Emerging Chironomidae (Insecta: Diptera) in Minnesota Trout Streams

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Dedication

This dissertation is dedicated to the memory of my Grandma, Hazel Flieder.

For always encouraging me to work hard to pursue my dreams;

But more importantly, to take the time to slow down and enjoy life

and the people that make it meaningful.

Abstract

The Chironomidae (Diptera) is among the few aquatic insect families with species that are reliably able to grow, develop, and emerge as fully functional adults throughout the cold winter months that characterize the midwestern United States. However, few studies document their role in trout streams during this time of year. The intent of this dissertation is to enhance the field of Chironomidae research by focusing on the winter dynamics of these insects, including their response to severe flood events, their incorporation in the winter diet of trout, and the morphological and molecular description of three new cold-adapted Chironomidae species. The resistance and resilience of the winter chironomid community was assessed following a severe flood that impacted many streams in southeast Minnesota during the late summer of 2007. Methodology included collections of chironomid surface-floating pupal exuviae from 18 southeast Minnesota streams; streams were selected to include representation of localities that incurred moderate to extreme levels of flooding disturbance. These data were compared to data from the same localities during prior winters. Significantly more taxa emerged during the winter after late-summer flooding as compared to historic collections, and the number of species emerging in winter was positively correlated with rainfall severity, indicating the winter-active Chironomidae are resistant to late summer spates. This indicates that chironomids are an available winter food resource to stream trout recovering from devastating floods. In attempt to examine the importance of Chironomidae and other winter-active aquatic insects to the winter diet of brown trout (*Salmo trutta* L.), stomach contents were collected from trout inhabiting three southeast Minnesota streams; these data were compared to organisms collected in the stream drift and benthos. Results indicated that trout diets differ by stream and by fish size, with larger trout feeding heavily on Trichoptera and *Physella,* and smaller fish relying more on *Gammarus* and Chironomidae larvae. Stomach contents were more similar to the benthos than the drift, indicating a greater reliance on benthic feeding during winter. Trout in all streams selected Trichoptera and Chironomidae over other prey, and appeared to maintain size-selective predation throughout winter. Winter-emerging Chironomidae appear to enhance the trout diet in all streams, and chironomid larvae were particularly dominant in the diet of one trout population. Three new chironomid species within the genus *Micropsectra,* a genus commonly found in the winter trout diet, were discovered from a study of five Minnesota streams. These species, *Micropsectra neoappendica,* n. sp., *Micropsectra penicillata*, n. sp., and *Micropsectra subletteorum,* n. sp., were described using morphological and molecular methods, along with one additional species, *Micropsectra xantha* Roback*,* which was redescribed. Two of the new species initially appeared identical to species known from the Palearctic, however molecular data indicated they are genetically distinct. Subsequently, reexamination of morphological characters revealed slight, but consistent diagnostic differences. These results emphasize the importance of using molecular tools in conjunction with traditional morphological techniques when studying Chironomidae diversity, especially when relying on diagnoses from other regions. The culmination of these studies sheds light on the dynamics of winter-emerging Chironomidae in southeastern Minnesota trout streams, their distribution and abundance, and the larger role they play in stream communities.

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Disclaimer

The species names provided in the following text are provisional and are not yet published within the meaning of the International Code of Zoological Nomenclature (ICZN 1999; Article 9). The names and nomenclatureal acts established in this dissertation, therefore, are not available within the meaning of the Code (ICZN 1999, Article 10).

Prologue

The harsh winter season that characterizes the northern temperate zone imposes a suite of challenges for animals, with critically low temperatures among the most severe of the seasonal trials. Insects have developed various morphological, ecological, behavioral, and biochemical strategies to either cope with or minimize temperature stress (Danks 1991, Lencioni 2004, Danks 2006, Eggermont and Heiri 2012). These strategies include dormancy, relocating to a new habitat or building ornate hibernacula (Danks 1991, Lencioni 2004), or exhibiting brachyptery or aptery (Lencioni 2004). Additionally, many insects are able to enhance their cold hardiness by making internal biochemical adjustments that allow them to either tolerate freezing, or alternatively, enhance their resistance to freezing by lowering the temperature at which their body fluids spontaneously freeze, otherwise known as their supercooling capacity (Lee 1991, Lencioni 2004, Danks 2006).

However, while much work focuses on terrestrial insects in cold environments, only a relatively small base of information focuses on aquatic species (Danks 2007). Cold climates seasonally restrict the activity and development of most aquatic insect species (Danks 2007) and consequently, this has led many people to ignore the winter-active aquatic insect community and believe that this winter community has relatively little ecological impact (Bouchard 2007). However, insects living in habitats that do not freeze during the winter, such as the many groundwater-dominated trout streams that characterize southeastern Minnesota, often show significant activity throughout the coldest months of the year. This lack of ice cover, due to the temperature-moderating influence of groundwater springs, readily permits the growth, development, and winter-emergence of cold-adapted aquatic insects.

The dipteran family Chironomidae, commonly known as non-biting midges, is among the few aquatic insect families with representatives that are able to continue to reliably grow, develop, and emerge as fully-developed and fecund adults throughout the winter months. Many chironomid species and genera from different subfamilies are recognized as dependable indicators of cold environments. Of the eleven chironomid subfamilies, Diamesinae, Prodiamesinae, Podonominae, and Orthocladiinae are recognized as the most cold adapted, however many species within the Chironominae tribe Tanytarsini are often also common in colder waters (Eggermont and Heiri 2012).

In lotic systems of the north temperate ecoregions, Orthocladiinae, Diamesinae, and Tanytarsini are typically the primary groups with representative species that consistently emerge during winter and early spring (Eggermont and Heiri 2012). Adult chironomid activity can be witnessed on stream banks at air temperatures well below 0°C. Perhaps the first documented recording of these winter-active flies was made by Young (1969), who noted flight and breeding activity of *Diamesa arctica* (Boheman) near Smithfield, Utah, USA. Hågvar and Østbye (1973) later recorded that adults of four chironomid species were flying and mating on the snow surface surrounding various Norwegian brooks throughout winter. This early study also showed that adults of *Diamesa permacer* (Walker) are capable of surviving temperatures at least as low as -13.5°C, and can re-gain active movement at a temperature of -4.5°C (Hågvar and Østbye 1973).

More recently, research on another Diamesinae species, *Diamesa mendotae* Muttkowski, has shown that larvae of *D. mendotae* are able to survive freezing, with a lower-lethal temperature with 50% mortality (LLT$_{50}$) of -17.4°C (Bouchard et al. 2006b). Interestingly, this species switches strategies in later life stages, and is freeze-resistant as an adult. As adults, these flies have evolved to survive sub-zero temperatures by enhancing their capacity to supercool, or the temperature at which their body fluids spontaneously freeze, thereby enabling adults of *D. mendotae* to survive air temperatures below -20°C, with a LLT$_{50}$ of -21.5°C (Bouchard et al. 2006a). Adults of *D. mendotae* are commonly observed walking on the banks of Minnesota's groundwater-dominated trout streams at temperatures as low as -7°C, and large emergences often occur on winter days with maximum temperatures around 0°C (Bouchard and Ferrington 2009, personal observation). Other recent winter field work has demonstrated that over 50 chironomid species are capable of emerging from streams in Kansas, USA (Ferrington 2000, 2007, Anderson et al. 2011), and in Chapter 1, I show that nearly 40 species of Chironomidae emerge from trout streams in southeast Minnesota during winter.

While it is likely that these cold-tolerant, winter-active chironomids play critical ecological roles in Minnesota's trout streams, and perhaps act as a key winter food resource for stream trout, little research documents their roles during cold seasons in these systems. The primary aim of my dissertation research is to focus on the winter dynamics of these insects and to further explore and document the intricacies and diversity of the winter chironomid community and their importance in Minnesota's trout stream ecosystems.

The research I present in the following chapters focuses on three distinct aspects of winter-emerging chironomids. The first aspect, covered in Chapter 1, examines the effects of severe summer floods on the diversity of winter-emerging Chironomidae. Briefly, a severe flood occurred in southeast Minnesota during the summer of 2007. I examined 18 streams that received moderate (0-10 cm), heavy (10-20 cm) or extreme (20+ cm) amounts of rainfall. Each stream was sampled bi-weekly during the winter following the flood to determine the type, number, and diversity of chironomid species emerging. Results were then compared to historical collections from the same streams in winters prior to the flood in order to determine how resistant or resilient winter-emerging Chironomidae are to severe flooding. These results are particularly important when considering current climate change predictions, which indicate that Central North America can expect to undergo an increase in both frequency (Christensen et al. 2007) and intensity (Trenberth et al. 2007) of precipitation events. Any changes in the chironomid community due to flooding disturbance may, then, extend to the rest of the stream community, and specifically, may have consequences on the fish community residing in streams that are recovering from devastating summer floods.

This leads into the second area of my research (Chapter 2), which examines the influence of winter-active aquatic insects on the diet of brown trout (*Salmo trutta* Linnaeus), a sport fish that inhabits 124 of southeastern Minnesota’s groundwater-fed streams (Thorn and Ebbers 1997). Importantly, these streams are of high value for anglers and entrepreneurs, accounting for 75% of total stream fishing in Minnesota and generating much of the state's total revenue from stream fishing expenditure ($47 million in sales, $27 million in income, along with providing 820 jobs) (Gartner et al. 2002).

While much research documents the role of aquatic insects in trout diets during spring, summer, and fall, few studies focus on the winter diet and how prey density and composition affect trout during this season. Therefore, the primary objective of my research was to advance the knowledge of the winter diets of brown trout in a subset of Minnesota's groundwater-dominated streams. Specific goals include assessments of: 1.) how the winter diet and fish condition differs between streams; 2.) whether the winter diet is more closely allied with invertebrates found in the stream drift or benthos; 3.) if and how the winter diet varies with fish size, and whether trout demonstrate size-selective predation during winter; and 4.) the importance and potential preference for winter-emerging chironomids and other prey items in the winter diet of trout.

Importantly, enhanced fish growth rates and high condition are among the most desired outcomes for fisheries managers. If a particular winter diet pattern is associated with enhanced winter condition, or if fish show special preference or reliance on certain aquatic insects, this may have significant management implications for Minnesota's streams. For example, if winter-emerging insects are found as an important component of the winter diet, these results may be particularly important considering that populations of winter-emerging insects may be negatively impacted by climate warming. Specific management actions that could result as an outcome of these results include the possibility of reintroducing native forage species or modifying stream habitat to favor production of aquatic insect species that enhance trout growth, thereby increasing the value of the fishery.

The final chapter of my dissertation shifts from the primarily ecologically-based studies described in Chapters 1 and 2, to research focused on taxonomy and systematics of a single genus of winter-emerging Chironomidae. Assessment of chironomid diversity in groundwater streams and their role in trout diets requires accurate and reliable tools to aid in species identification. Current morphology, or appearance-based, identification approaches are often time-consuming and inaccurate, especially if dealing with immature stages of aquatic insects. Molecular-based identification of aquatic insects is a comparatively new, effective method of species identification that can eliminate problems associated with morphological identification (e.g. Sinclair and Gresens 2008, Carew et al. 2011). One specific tool, DNA barcoding, relies on a reference library of short, standardized DNA sequences that can quickly be generated and serve as unique species identifiers, allowing users to easily differentiate species (Kress and Erickson 2008). A clear advantage to this tool is that it allows recognition of all life stages of an organism; this is crucial in groups such as the Chironomidae, where immature stages are difficult to differentiate morphologically. While some studies have used these techniques for chironomid research and identifying chironomid species (e.g. Carew et al. 2003, Ekrem and Willassen 2004, Stur and Ekrem 2006, Carew et al. 2007, Stur and Ekrem 2011b), effort is still needed to develop a comprehensive DNA barcode library for this species-rich insect family (Ekrem et al. 2007).

The research described in Chapter 3 focuses on species elucidation of the genus *Micropsectra* Kieffer, which I have found to be among the most abundant specimens of Chironomidae in the winter trout diet in certain streams in southeastern Minnesota (Chapter 2). Considering the high species richness (over 140 described species are currently known to the Holarctic region) and habitat specificity of the genus, monitoring changes in the abundance and diversity of *Micropsectra* can be useful in biological monitoring studies, particularly when examined at the species level (Ekrem et al. 2010b). Notably, taxonomic research of Nearctic *Micropsectra* is much less advanced than that of Palearctic species; this is problematic considering that many species are morphologically difficult to differentiate. Thus, the goals for this chapter are: 1.) to use a combination of genetic and morphological tools to describe new species of *Micropsectra* found in Minnesota trout streams; 2.) to enhance the reliability of species identification for Nearctic *Micropsectra*; and 3.) to re-examine the taxonomic framework for the genus as a whole.

With recent and forth-coming advances in molecular taxonomy, it is quite likely that taxonomic work that combines molecular and morphological methodologies will rapidly advance our understanding of phylogenetic relationships in species-rich genera such as *Micropsectra*, emphasizing the need for taxonomists to actively use all data available and to be well-versed in both molecular and morphological techniques. Work in this chapter strongly emphasizes the importance of using molecular tools such as DNA barcoding in conjunction with traditional morphological techniques when studying

Chironomidae diversity, particularly when relying on keys and diagnoses from other regions.

The conservation and preservation of biological communities is an ever-growing concern, particularly in light of current climate change predictions. My dissertation takes a unique approach to addressing this issue via a multi-faceted analysis of winter-active Chironomidae. Not only does my research provide basic insights about an important but little-studied community of organisms, but also: 1.) examines the potential impact of precipitation increases caused by climate change on the winter-active Chironomidae community; 2.) explores the inter-relationship of winter-active aquatic insects and stream trout with the intent of providing insights of how streams might be effectively managed to provide optimal winter growth rates for trout; and 3.) identifies and describes three new species of cold-tolerant chironomids within the genus *Micropsectra*, providing identification keys for adults and pupae of the Nearctic members of this genus and expanding the DNA barcode database for the Chironomidae as a whole. The culmination of these areas sheds light on the dynamics of winter-emerging Chironomidae in southeastern Minnesota trout streams, their distribution and abundance, and the larger role they play in stream communities.

All chapters were written in the form of manuscripts to be submitted to peer-reviewed journals for publication. Chapter 1 has been submitted to Hydrobiologia and has recently been accepted for publication, with Dr. Len Ferrington of the University of Minnesota as a co-author. Chapter 2 is being prepared for submission; Dr. Len Ferrington will also be a co-author of this chapter and will be joined by Erin Mittag and Bridget

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Chapter 1

Resistance and Resilience of Winter-Emerging Chironomidae (Diptera): Implications for Minnesota Trout Streams

Chironomidae is one of few insect families that can emerge as active adults during winter. However, little is known about the response of winter-emerging species to extreme flooding events. In August of 2007, a severe flood occurred in southeastern Minnesota, imparting stress on aquatic communities inhabiting the groundwater-dominatedstreams that characterize the region. Collections of chironomid pupal exuviae were obtained biweekly from 18 southeast Minnesota streams during the winter of 2007-2008 to assess resistance and resilience of winter-active chironomids. Streams were selected to include equal representation of moderate (2.5–10cm), heavy (10-20cm), or extreme (20+cm) rainfall categories; rainfall amounts in each category represent total precipitation during the 3-day storm. These samples were compared to samples obtained from the same localities during prior winters. Significantly more taxa emerged during winter after late-summer flooding as compared to historic collections, and the number of species emerging in winter was positively correlated with rainfall severity, indicating that 15 species responded opportunistically to disturbance. Our findings contradict studies of responses by Chironomidae to severe flooding during warmer-water conditions and show that winter-emerging Chironomidae are resistant to stress imposed by summer spates. This indicates that winter-active Chironomidae will be resistant to increased severity of summer spates associated with climate change predictions.

Introduction

In environments that are constantly in a state of flux, such as stream systems, disturbances are best defined as events whose frequency and intensity falls outside of an otherwise predictable range (Resh et al. 1988). Disturbances caused by severe rainfall are common in streams and can have severe short-term consequences on the organisms living within these systems. The resulting impact on stream inhabitants is determined by the frequency, intensity, extent, and predictability of a particular disturbance event (Sousa 1984, Resh et al. 1988, Grimm and Fisher 1989, Townsend et al. 1997).

Global climate warming is accelerating evaporation rates worldwide; this extra water vapor that is added to the atmosphere is among the key drivers necessary to fuel intense storm events (Madsen and Willcox 2012). Across the contiguous United States, extreme precipitation events that are among the largest experienced at a specific location are occurring 30% more often now as compared to 1948 (Madsen and Willcox 2012). Other recent climate change predictions suggest that Central North America will undergo a 3% increase in mean annual precipitation (Christensen et al. 2007) and, perhaps more importantly, an increase in the intensity of individual precipitation events (Trenberth et al. 2007, Madsen and Willcox 2012) , Madsen & Wilcox, 2012). Specifically, over the last 65 years, precipitation intensity has increased by 10% nationwide (Madsen & Wilcox, 2012). Consequently, we can expect more precipitation-related disturbances to impact stream communities. This type of disturbance directly modifies flow regimes of rivers and streams and can cause increased amounts of sedimentation and streambed scouring, with many studies indicating significant changes in species density, richness, and composition (e.g. Fisher et al. 1982, Miller and Golladay 1996, Matthaei et al. 2000, McCabe and Gotelli 2000, Suren and Jowett 2006). Furthermore, disturbances experienced during one season may impact the recovery, diversity, and abundance of organisms active in subsequent seasons.

The consequences of disturbance to streams are often measured by assessing the resistance and resilience of stream biota, which are interpreted in the context of the ecological stability of the disturbed system (Grimm and Fisher 1989, Boulton et al. 1992). Organisms that are resistant to a particular disturbance have the ability to absorb or deflect the effects of the disturbance, with very minimal changes in density (Fig. 1-1a). Resilient organisms, by contrast, initially exhibit dramatic declines in density, however are able to quickly recover to pre-disturbance levels (Fig. 1-1b). Some species may have both low resistance and resilience (Fig. 1-1c) and are either slow or unable to recover. In addition to resistant or resilient response trajectories, we propose that other organisms, such as some species of Chironomidae (Insecta: Diptera), represent an opportunistic response to high levels of disturbance associated with flooding. By our definition, opportunistic taxa quickly become abundant following a disturbance due to the new environmental conditions (Fig. 1-1d), thereby influencing the short-term seasonal trajectory of recovery. These taxa may later exhibit declines after competitively superior, but less resilient, taxa are able to recolonize.

Several studies have examined the resistance and resilience of the aquatic community to severe floods during spring, summer, and fall (e.g. Fisher et al. 1982, Mackay 1992, Miller and Golladay 1996, Hax and Golladay 1998, Gendron and Laville

2000, Melo et al. 2003, Dodds et al. 2004, Fritz and Dodds 2004). While initially imparting severe reductions in taxa abundance and richness following devastating floods, stream communities typically exhibit high levels of resilience, with populations often rebounding within days or weeks of the disturbance (Reice 1985). For example, a study examining the effects of flooding on prairie streams found low resistance (90% decreases in total density) of the benthic community, but a high degree of resilience, especially by Chironomidae, over subsequent seasons (Miller and Golladay 1996). This short recovery period may be facilitated by the high fecundity exhibited by most macroinvertebrates (Reice, 1985), rapid recolonization by species living in suboptimal areas such as the hyperheos (Reice, 1985; Gendron & Laville, 2000), or recolonization by individuals that were able to take advantage of sheltered refuges such as backwaters (Reice 1985), downstream drift (Fisher et al. 1982, Gendron and Laville 2000), or aerial dispersal (Fisher et al. 1982). While this strong research base documents effects of disturbance on the macroinvertebrate community during warmer seasons, there is little documentation of the effects of seasonal disturbances during spring or summer on winter active aquatic invertebrate populations which characterize higher latitudes, such as certain species of Chironomidae.

The Chironomidae play a particularly important role in stream dynamics. They typically comprise over 50% of aquatic insects within a stream system (Coffman and Ferrington 1996), are among the most species-rich of aquatic insect families (Ferrington 2008), and are a major protein source and thereby promote the growth of stream fish (Armitage 1995b). Chironomidae are also unique in that they are among the few aquatic insect families in which several species emerge from streams and are active as adults during the cold winter months that characterize the midwestern United States (e.g. Ferrington 2000, 2007, Bouchard and Ferrington 2009, Anderson et al. 2011). Species within the subfamilies Orthocladiinae and Diamesinae, as well as a limited number of species in the Chironominae tribe Tanytarsini, are the primary constituents of the winter chironomid community in temperate streams and rivers (Ferrington 2000, 2007, Bouchard and Ferrington 2008, Eggermont and Heiri 2012). Some species show very specialized adaptations to cold, such as adults of *Diamesa mendotae* (Muttkowski), which have the ability to survive air temperature less than -20˚C via freezing point depression (Carrillo et al. 2004, Bouchard et al. 2006a). Over 50 Chironomidae species have been shown to emerge within different water temperature ranges from Kansas streams during winter months (Ferrington 2000) and in this study we show that 39 species emerge from groundwater-dominated trout streams in southeastern Minnesota during winter (Table 1-1) when ambient water temperatures do not exceed 8° C.

In August of 2007, a large area of southeastern Minnesota experienced extreme flooding (Fig. 1-2), with some areas receiving over 38 cm of rainfall in a 24-hour period, far surpassing the state's previous 24-hour record rainfall total of 27 cm (Minnesota State Climatology Office 2008). This late-summer spate imposed varying degrees of severe stress (e.g. stream bed scouring, siltation) on aquatic communities inhabiting the numerous economically important trout streams characterizing the area. High-quality historical data, dating back to 2002, of the winter-emerging Chironomidae in southeast Minnesota trout streams provided us with the opportunity to evaluate the effects of this late summer flood on the resistance, resilience and response trajectory of winter-emerging Chironomidae. Specifically, we test the null hypothesis that there is no difference in chironomid species density pre- and post-flood, nor any difference in chironomid richness between streams impacted at different levels.

Materials and Methods

Study Area

The Driftless Region of southeast Minnesota, USA is surrounded, but virtually untouched, by the effects of the last glaciation (Waters 1977). This lack of glacial activity accounts for the unique landscape that distinguishes the area. The region is characterized by steep limestone bluffs, sandstone valleys, and an array of 181 spring-fed trout streams that comprise a total of 1,268 stream kilometers (Dieterman and Merten 2003). Winter water temperatures typically range from 2 - 8°C, with most streams exhibiting a temperature around 4°C.

Eighteen trout streams in southeastern Minnesota with long-term chironomid winter-emergence data were selected for study. Streams were chosen to include multiple representatives of three rainfall categories: extreme (20+ cm) (N = 6 streams), heavy (10-20 cm) (N = 5 streams), and moderate (2.5-10 cm) (N = 7 streams) amounts of rainfall (Fig. 1-2); each rainfall category represents total precipitation received during the 3-day storm (18-20 August, 2007). Rainfall data were obtained from the Minnesota State Climatology Office.

Field and Lab Methodology

Samples of chironomid pupal exuviae, the surface-floating cast-skins left behind by recently-emerged adults, were collected biweekly from each stream from December 7, 2007 – February 23, 2008, following methods of Ferrington et al. (1991), yielding a total of 108 samples from the 18 streams over the 3-month period. Briefly, pupal exuviae were collected from multiple areas of known accumulation (e.g., areas of the stream containing foam or debris buildup) by scooping exuviae, debris, and water into a white enamel pan and pouring all contents through a 125µm sieve. This procedure was repeated for 10 minutes, starting at the downstream end of each sample site and working upstream for a total distance of approximately 100 m. All scoops from an individual stream and sample date were pooled into a single sample. An important benefit of using pupal exuviae is that this technique provides estimates of individuals that were able to successfully survive all immature stages and emerge as adults. Additionally, because collections are made from “natural” areas of accumulation within a stream reach, this method allows evaluation of individuals originating from a wide variety of larval habitats that might be missed with other sampling methods.

All pupal exuviae were preserved in the field using 70% ethanol and later sorted in the lab under 12X magnification to ensure accurate quantification of small to large species. When available, three representatives of every taxon represented in each sample were slide mounted in Euparal® for species-level identification. Genus-level identification was achieved using Wiederholm (1986) or Ferrington et al. (2008) and species-level identification was accomplished with reference to the most recent revision available for each genus. Slide mounted voucher specimens are located in the chironomid collection at the University of Minnesota Insect Collection in St. Paul, MN (UMSP).

Historical Collections

Between one and six collections of pupal exuviae were made by Dr. Len Ferrington during winter in each of the eighteen study streams between 2002 and 2007, with a cumulative total of 40 samples collected historically across the eighteen streams. All sampling techniques and sampling localities were identical to those of the post-flood collections. Species identification information from the historical collections is restricted to presence-absence data, as abundance information is not available for all historical samples.

Data Analysis

A variety of analyses were used to assess the response of winter-emerging Chironomidae to this severe flood event. In our analyses, we test the null hypothesis that there is no difference in chironomid species density pre- and post-flood, nor any difference in chironomid richness between streams impacted at different levels. Statistical programs used were Number Cruncher Statistical Software (NCSS) (Hintze 2001), JMP Pro 9.0.2, and R (version 2.14.2). First, considering that many of the sites in each rainfall category are clustered in space (Fig. 1-2), we tested for any possible effects of geographical location for the historical samples. Sample localities were lumped into four categories based on latitude and analyzed using one-way analysis of variance (ANOVA), with the total number of chironomid taxa as the response variable. One-way ANOVA was also used to verify that historical samples were not significantly different from each other when grouping samples by rainfall category, again, using total number of taxa as the response. In order to evaluate whether post-flood taxonomic sample density of winter-emerging Chironomidae differed from historical sample density values for streams in each of the three rainfall categories, we chose to analyze the data with a 2 x 3 level two-way ANOVA ($\alpha = 0.05$); sample density for each historic and post-flood sample was assessed and included in this analysis. The number of taxa emerging per sample date were not normally distributed, but closely approximated a Poisson distribution. Thus, data were transformed by adding 0.5 to the count and subsequently taking the square root. Means were then back-calculated to yield values reported here. Analysis of Variance was followed with a Tukey-Kramer Multiple Comparison Test.

Linear regression was used to analyze the relationship between actual rainfall amount incurred during the 3-day flood and the average number of taxa collected per stream in historical and post-flood samples. Hierarchical cluster analyses, using Ward's minimum variance method, were performed to assess similarity between streams when considering the average number of taxa detected in each stream on 40 temporally comparable sample dates for historic and post-flood collections. Degree-day data were not available for comparison of pre- and post-flood sample sites, consequently we chose to match our samples by the nearest calendar date for the temporal comparisons; while we recognize that there are limitations with this method, we believe it is the best option given the data available.

Non-metric multidimensional scaling (NMDS) was used to assess similarity among sites based on pre- and post-flood data for temporally comparable sample dates. Post-flood patterns were also evaluated separately based on rainfall intensity and included data for all 108 post-flood samples. For both analyses, the NCSS NMDS software was set to create similarities based on presence/absence data. Initial configuration was set to use a random starting configuration, with 50 iterations and the default values for minimum stress, minimum stress change, and minimum gradient sum. Considering that we achieved stress values less than 0.10, which corresponds to a good ordination with little risk of drawing false interpretations (Clarke 1993) we constrained our analysis to two dimensions. NMDS maps were saved as figures.

Individual-based rarefaction curves were generated using the vegan package (Oksanen et al. 2012) in R version 2.14.2 to assess differences in expected generic richness for a given number of randomly drawn individuals from the Moderate, Heavy, and Extreme rainfall categories. Since abundance data were not available for historical collections, only post-flood data are presented here. Data from all samples and streams within a particular rainfall category were pooled to develop seasonal richness estimates specific for each category. Considering that only a subset of all species collected in a sample could be consistently and accurately identified to species level, we chose to constrain the resolution of our abundance data to the generic level for this richness assessment; all other analyses were conducted with species level data.

Results

A total of 20 species in 14 genera were collected in the 40 historical samples, whereas 29 taxa in 14 genera were collected in the 40 post-flood collections that were temporally closest to the day and month of each historic sample. A cumulative total of 39 taxa within 20 genera were collected across all 108 post-flood samples (Table 1-1). Of the 39 taxa collected post-flood, 26 were within the subfamily Orthocladiinae (Table 1-1). Only two taxa, *Diplocladius cultriger* and *Tvetenia* sp. 2, were unique to historic collections; conversely, 15 taxa were unique to post-flood collections. The most common and abundant taxon in both historic and post-flood collections was *Diamesa* sp. Species within the genera *Orthocladius* and *Micropsectra* were also common.

One-way ANOVA indicated no significant difference in the number of taxa detected when comparing historical samples grouped into either latitudinal ($F_{3,39} = 2.38$, $p = 0.086$) or rainfall categories ($F_{2,39} = 2.16$, $p = 0.129$). Two-way ANOVA found a significant effect when examining the number of taxa collected in historical versus post-flood collections, with historical samples (N = 40) significantly different than that in post-flood collections (N = 108) ($F_{1, 147} = 371.92$, $p = 0.0023$). Post-flood collections exhibited an average of 2.05 more taxa per sample than historic collections. Similarly, this test found a significant effect when examining differences between rainfall categories ($F_{2, 147} = 9.66$, $p = 0.0027$). A Tukey-Kramer Multiple Comparison Test indicated that all rainfall categories were significantly different from one another, with the Extreme rainfall category having 2.6 more taxa than the Moderate category, and 0.9 more taxa than the Heavy category, and the Heavy rainfall category having 1.6 more taxa than the Moderate rainfall category. No interaction was detected between the collection period and rainfall category ($F_{2,147} = 0.01$, $p = 0.985$).

Linear regression models indicated that in post-flood collections, the average number of taxa collected per sample per stream as a function of total rainfall amount indicated a trend of increasing number of taxa with increasing rainfall ($R^2 = 0.259$, $p = 0.031$). Historic collections, conversely, indicated no significant differences in taxa collected among the same streams during non-flood years ($R^2 = 0.040$, $p = 0.425$) (Fig. 1-3). The trend of increasing taxa with increasing rainfall in post-flood collections is also quite apparent in Table 1-1 when examining the comprehensive list of taxa collected in each rainfall category, with 20 taxa found in streams receiving moderate rainfall, 25 taxa in those receiving heavy rainfall, and a total of 31 taxa collected in streams receiving extreme amounts of rainfall (Table 1-1). Table 1-2 shows a similar trend when comparing average taxa collected per sample post-flood as compared to pre-flood.

No discernible clustering patterns were present when examining data from historical collections (Fig. 1-4a), indicating little difference in taxanomic composition between streams prior to the flood. When examining the cluster analysis from the post-flood collections that corresponded closest temporally to historic collections, however, some trends in the data were apparent (Fig. 1-4b). With few exceptions, streams in the Moderate rainfall category tended to cluster together, while streams in the Heavy and Extreme categories tended to form separate clusters. These results correspond well with those described earlier, depicting the trend of higher numbers of taxa in those streams more heavily impacted by the flood.

Differences among streams based on historical data were primarily reflected on the Dimension 1 axis of the NMDS (Figure 5a, stress = 0.075), where we plot samples in species space, reflecting the date-to-date differences in emergence of the smaller number of taxa pre-flood. By contrast, post-flood samples exhibited not only the date-to-date separation on Dimension 1, but also exhibited greater separation of streams along Dimension 2, with the streams that had a higher incidence of opportunistic taxa (e.g. *Brillia retifinis, Corynoneura* sp., *Eukiefferiella* spp., *Dicrotendipes fumidus, Polypedilum* sp., *Micropsectra* spp., *Rheotanytarsus distinctissimus, Tanytarsus nearcticus, Zavrelimyia sinuosa*) being much more strongly differentiated in this dimension. The influence of opportunistic taxa is also illustrated in Figure 1-5b, (stress = 0.086), where we plot streams in species, coded by differing rainfall categories. Most streams with moderate amounts of rainfall occur near the middle of the NMDS plot and streams with heavy and extreme rainfall more strongly separated by both axes.

Rarefaction curves of chironomid generic richness also indicated clear separation between rainfall categories, with the Extreme category exhibiting the highest taxa richness estimates with increasing sample size. Streams within the Heavy category exhibited intermediate richness values, while generic richness was lowest in the Moderate rainfall category (Fig. 1-6). For example, based on a subsample of 1,000 individuals, sites within the Extreme category have an expected generic richness of 14.0 (SE = 1.2), while those in the Heavy or Moderate categories have expected values of 11.0 (SE = .98) and 9.6 (SE = 1.1), respectively.

Discussion

Our results indicate that winter-emerging Chironomidae exhibited high resistance to late summer flooding and were not negatively impacted by severe flooding during the preceding summer season, as demonstrated by the higher number of taxa in post-flood samples. As mentioned, abundance data were not available for historical samples, which restricted the use of taxa richness estimates to post-flood data. This constrained all comparisons made between historic and post-flood data to comparisons of species density. We recognize that there are shortcomings with this type of comparison, especially considering the tendency for more abundant samples to have a higher density of species (McCabe and Gotelli 2000, Gotelli and Colwell 2011). However, we believe that the consistency found in the results of all analyses used to compare historic and post-flood sites, combined with the support of richness results in the post-flood dataset, provide a strong case in support of the conclusion that we have detected a true increase in the number of winter-emerging taxa in the post-flood samples that were most severely impacted by the flood event.

This post-flood increase in Chironomidae taxa is likely triggered by the interplay of a suite of factors. Substrate characteristics, for example, may play a major role in the reduction or persistence of aquatic insect species following severe disturbance. Studies following a single habitat sampling protocol typically sample only in erosional zones because invertebrate density and diversity are generally highest in these areas (Barbour et al. 1999). Research has shown, however, that these habitats, characterized by high amounts of loose cobble and gravel, typically have greatly reduced density and richness following spates as compared to habitats characterized by stable, embedded substrate (Matthaei et al. 2000).

Some aquatic invertebrates may be adapted to seek out areas of refugia during periods of disturbance (Sedell et al. 1990, Dole-Olivier et al. 1997). Matthaei et al. (2000), for example, found that ‘stable stone’ substrates are used as refugia by certain aquatic insects, including Chironomidae. Analogous to the results of our study, chironomid density and richness were found to be significantly higher in post-disturbance samples as compared to pre-disturbance, suggesting that these species may identify and seek these ‘stable’ sites as refugia during periods of high flow (Matthaei et al. 2000).

Several of the winter-emerging species found in our research do not emerge during summer months when water temperatures are warmer, and larvae of *Diamesa* and five *Orthocladius* species from this study are not found actively growing on surface substrates during summer. Although further research is needed to quantify the summer habitat and life stages of several of these winter-emerging Chironomidae species, we presume they are diapausing deep within the hyporheos, using this region as a stable refugium during the summer months before shifting to the stream bed in late autumn and winter. Benthic organisms (Füreder 1999, Malard et al. 2002, Dole-Olivier 2011), including several species of chironomids (Ferrington 1984, 1987, Dole-Olivier et al. 1997, Donley et al. 1998), are known to use the hyporheic zone as a habitat or a refuge from environmental disturbance including floods and warm summer temperatures. For example, chironomid larvae have been found invading the hyporheos at depths of up to 2 m following high discharge events (Dole-Olivier et al. 1997). Additionally, Dole-Olivier

(2011) emphasizes the importance of the hyporheoic zone as a protected 'nursery' for eggs, early instar larva, and quiescent stages of benthic invertebrates. It is quite plausible that winter-emerging taxa found in our study followed a similar strategy by aestivating in the hyporheos as eggs or early instar larvae, thus avoiding mortality associated with stream bed destruction due to severe flooding, and helping to explain the persistence and resistance of these taxa.

Studies of rainfall disturbance often emphasize the devastating impacts of floods on the aquatic invertebrate community, highlighting low resistance of the community (e.g. Fisher et al. 1982, Miller & Golladay 1996, Gendron & Laville 2000). For example, density reductions in excess of 90% are not uncommon following spates (e.g. Fisher et al. 1982, Miller & Golladay 1996). Contrastingly, we predict that some species of Chironomidae actually benefit from extreme flood events. Of the 39 winter-emerging species collected after the late summer spate, 15 were unique to the post-flood collections (Tables 1-1 & 1-3), and 10 of the 15 were restricted to streams receiving either heavy or extreme amounts of rainfall (Table 1-1). We believe that these taxa exhibited an opportunistic response, and thus were able to flourish in the altered stream conditions by quickly taking over void niches left by late-summer developing taxa that are not resistant to severe flooding, thus resulting in reduced competition levels. A study of chironomids in the River Aude also concluded that chironomid diversity was higher following flooding disturbance, and included the appearance of cold stenothermic species (Gendron & Laville, 2000). Conversely, though, while overall species density was higher the year following the flood, Gendron & Laville (2000) also reported a number of species seemed to be expelled by flooding, and conclude that overall, chironomid resistance to flooding was low, with many populations not rebounding for several months.

In an experimental spate disturbance study, McCabe and Gotelli (2000) also reported higher invertebrate species richness following disturbance. They concluded that their results followed predictions of Huston's (1979) 'dynamic equilibrium model' for explaining ecological disturbance (McCabe and Gotelli 2000). Under Huston's model (Huston 1979), which has been deemed applicable to stream systems (e.g. Resh et al. 1988), if the recurrence interval of a disturbance is less than the time required for competitive exclusion to occur, then species that are not strong competitors can thrive in the system, thereby increasing species richness. Additionally, and perhaps more importantly for this research, competitive exclusion of species can be counteracted by population reductions caused by disturbance (Huston 1979). In the case of our study, we propose that since the flood occurred at the end of the summer season and that returning to a state of semi-equilibrium within the most severely affected streams likely did not occur until the onset of cooler weather, it is possible that some competitively superior aquatic invertebrates did not have time, or were otherwise unable to securely re-establishing their position before winter. It is also plausible that some species went into an early seasonal diapause to avoid consequences of the flood; behavioral responses such as suggested here are not uncommon in response to disturbance (Resh et al. 1988).

Thus, without the predominance of these 'superior' species, competition within the impacted streams may have been reduced, allowing certain chironomids to take advantage of the new conditions and emerge earlier than expected by more typical phenology patterns. If, as proposed earlier, certain chironomids are able to take advantage of refugia, such as the deep hyporheos, this could certainly allow them to quickly take advantage of vacant niches. Thus, these species show an alternate, opportunistic type of behavioral response by shortening the time required for development and perhaps also altering patterns of development and voltinism. Future research testing these ideas, as well as incorporating abundance of other groups of aquatic insects along with chironomids, is crucial for evaluating these hypotheses.

Another mechanism that might stimulate these opportunistic species is related to change in the quality and quantity of food available to the aquatic insect community following the disturbance. Increased flow regimes are likely to remove leaves and other types of coarse particulate organic matter (CPOM), causing decreases in this type of food resource (Sweeney et al. 1992). This may be especially critical in headwaters of low order streams, such as those examined in this study, shifting the dominant feeding group from shredders, who rely heavily on CPOM, to collectors, which are able to filter the fine particulate organic matter (FPOM) ingested with sediments (e.g. Lenat 1984, Genito et al. 2002). Importantly, many chironomids, particularly some of the dominant winter-emerging species in our study, are collectors. Twelve of the unique species that were only found in post-flood collections (Table 1-1), are classified as collectors, thus, it is quite plausible that these species are able to opportunistically use these additional food resources, such as increased FPOM resources, resulting in higher population densities or faster growth rates.

When considered at the community level, the opportunistic species introduce more heterogeneity in emergence across all of the study streams during winter. Both the historical data and the results for streams with only moderate rainfall, indicate that a small number of taxa form the nucleus of winter emergence (Table 1). The more intense disturbance increased the stream-to-stream variability during the first winter following the flood. We are not sure how long this effect could persist. However, based on our interpretation that these opportunistic taxa are not efficient competitors, we expect that during one or more subsequent winters without intensive flooding these taxa will disappear or emerge later into the spring and emergence during winter will shift back to a community pattern that is more homogeneous and dominated by the nucleus of winter-emerging taxa that predominated in the streams with moderate rainfall.

It should be noted that Table 1-1 may appear to imply that emergence of two winter-emerging species (i.e., *Orthocladius (Euorthocladius) rivulorum* and *Orthocladius (Euorthocladius) rivicola*) diminished in the Heavy and Extreme categories. This, however, is not the case. While there was less frequent emergence of these species in Heavy and Extreme categories as compared to the Moderate category, overall, the emergence patterns in all categories increased from those in historic collections. For example, 50% of Extreme streams exhibited emergence of *Orthocladius* (*Euorthocladius*) *rivulorum.* This species was only represented in 16.7% of streams corresponding to the Extreme category in historic collections. Similarly, *Orthocladius* (*Euorthocladius*) *rivicola* was only present in 14.3% of Moderate and 20.0% of Heavy streams in historical collections. No collections of this species were made in streams of the Extreme category prior to the flood. Thus, we hypothesize that some mechanisms, other than those described here, account for the flourishing of these species during the post-flood collections.

Work by Bouchard (2007) includes the most comprehensive phenological reports for Chironomidae emerging from groundwater and surface-water dominated streams in Minnesota and provides a strong basis for additional comparisons of our post-disturbance winter-emergence data. Bouchard (2007) compiled an extensive database with estimates of the mean and ranges of water temperatures for emergence of 69 Chironomidae taxa in groundwater-dominated streams and 117 taxa in surface-water dominated streams. We used this database for groundwater-dominated streams when comparing mean emergence temperature of the chironomids collected in this study (Table 1-3) and also to compare dates of first emergence for the more warm-water adapted species, defined as species with a mean emergence temperature greater than 13°C. Our data show that 9 warm-water and one colder-water adapted species are emerging earlier in our post-flood collections than recorded by Bouchard (2007). For example, the earliest emergence date for *Dicrotendipes fumidus* recorded by Bouchard (2007) was 21-April. Similarly, the earliest emergence for *Zavrelimyia sinuosa* was 10-April (Bouchard 2007). In our collections, we found *D. fumidus* as early as 23-February and *Z. sinuosa* as early as 6-January. *Zavrelimyia sinuosa* was again found on 23-February. While we acknowledge that these results could be due to differences in sampling intensity or the individual conducting the actual sampling event, we believe this is highly unlikely since identical field collection protocols were used in our study, the historical collection, as well as in the work done by

Bouchard (2007). We believe that it is quite plausible that changes of in-stream condition associated with the flooding are the primary drivers accounting for earlier detection of these species.

This idea of earlier emergence is further strengthened by examining results for several additional Orthocladiinae taxa. Species within the subfamily Orthocladiinae are recognized as containing very cold-adapted taxa to less cold-adapted species, but with emergence recorded at lower temperatures than taxa within the subfamilies or tribes Tanypodinae, Chironomini, and Tanytarsini (Oliver 1971, Coffman 1973, Berg and Hellenthal 1992, Ferrington et al. 1993, Armitage 1995a, Bouchard and Ferrington 2008). As reported in the phenology patterns of Bouchard (2007), very few Orthocladiinae emerged during the early-winter season; the only taxa that predictably emerged throughout the early-winter was the cold-hardy *Diamesa* sp. Most Orthocladiinae were detected in later-winter collections (Bouchard 2007) or predominantly emerged in spring. Conversely, our results showed that many Orthocladiinae species were predictably found throughout the early and late winter season, and as with the warm-adapted species, these species with varying degrees of cold-adaptedness were much more prevalent in the Heavy and Extreme rainfall categories. Interestingly, the most cold-adapted species found in our collections, *Diamesa* sp. were not found more predictably during early versus late season collections or in streams incurring more extreme rainfall. This seems to further suggest that trends of earlier emergence post-flooding are most important for warm-adapted species and moderately cold-tolerant Orthocladiinae.

Considering current climate change predictions, our results are quite significant, indicating that a strong base of winter-developing Chironomidae species will be present during the winter months as a food resource for stream fish and other higher vertebrates to feed upon. This may be particularly important for fish residing in streams recovering from devastating spring or summer floods when many alternative species of aquatic insects are temporarily reduced or displaced. Mechanisms used by the winter-emerging species described in our study consist of high resistance especially among the most cold-adapted species, life cycle flexibility in moderately cold-adapted species, and earlier emergence combined with potential increases in voltinism in species that are less cold-adapted and constrained to emergence at higher temperatures in streams with low levels of disturbance.

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Tables

Table 1-1. List of the 39 chironomid taxa emerging in southeast Minnesota groundwater-dominated streams and percent of streams in which they occurred by rainfall category post-flood event. Taxa not reliably identifiable to species were compared to and made synonymous with those in Bouchard (2007).

	Percent of streams in each rainfall category with taxon		
Taxa	**Moderate**	**Heavy**	**Extreme**
Orthocladiinae			
Orthocladius (Orthocladius) frigidus	86	80	100
Orthocladius (Orthocladius) obumbratus	100	60	100
Orthocladius (Euorthocladius) rivulorum	100	80	50
Cricotopus triannulatus	71	60	50
Tvetenia sp. 1	57	60	50
Parakiefferiella sp. 3	29	40	67
Orthocladius (Orthocladius) manitobensis	14	40	50
Orthocladius (Euorthocladius) rivicola	43	20	17
Orthocladius (Orthocladius) mallochi	14	40	17
Eukiefferiella claripennis	14	20	33
Eukiefferiella ilkleyensis [a]	0	40	17
Parametriocnemus sp.1	0	20	33
Cricotopus tremulus	0	20	17
Heterotrissocladius marcidus gr.[a]	0	20	17
Orthocladius(Orthocladius) vaillanti	0	20	17
Chaetocladius piger gr., sp. 2	0	0	33
Orthocladius sp. 1	14	0	17
Cricotopus annulator	0	20	0
Cricotopus sp. 4	0	20	0
Brillia retifinis[a]	0	0	17
Chaetocladius dentiforceps gr.	0	0	17
Cricotopus near *tremulus*	0	0	17
Eukiefferiella potthasti [a]	0	0	17
Thienemanniella sp. 1	0	0	17
Corynoneura sp. 1[a]	14	0	0
Cricotopus trifascia	14	0	0

Table 1-1. Continued.

	Percent of streams in each rainfall category with taxon		
Taxa	**Moderate**	**Heavy**	**Extreme**
Prodiamesinae			
Odontomesa fulva	57	60	83
Prodiamesa olivacea [a]	14	0	17
Diamesinae			
Diamesa sp.	100	100	100
Chironomini			
Dicrotendipes fumidus [a]	0	0	17
Polypedilum trigonus ca.[a]	14	0	0
Tanytarsini			
Micropsectra nigripila [a]	14	80	83
Micropsectra subletteorum n. sp.	100	40	17
Micropsectra polita [a]	14	20	50
Micropsectra apposita [a]	0	20	17
Rheotanytarsus distinctissimus [a]	0	20	0
Tanytarsus nearcticus [a]	0	20	0
Micropsectra attenuata [a]	0	0	17
Tanypodinae			
Zavrelimyia sinuosa[a]	0	0	17
Common taxa (present in at least 30% of streams in category)	**9**	**12**	**14**
Unique taxa (present only within specific category)	**3**	**4**	**8**
Total taxa	**20**	**25**	**31**

[a] Taxa unique to samples collected post-flood

Table 1-2. Number of taxa collected per stream and (average per sample) for historic and post-flood collections across rainfall categories.

Category/Stream	Historic	Post-Flood
Moderate		
Gilbertson Stream	3 (3.0)	2 (1.5)
Trout Brook	6 (6.0)	2 (2.0)
Brown's Creek	4 (0.9)	4 (1.8)
Mill Stream	6 (1.5)	5 (2.2)
Hay Creek	7 (5.3)	7 (4.0)
Valley Creek	6 (1.5)	8 (3.6)
Vermillion River	2 (2.0)	11(3.2)
Mean	**4.9 (2.9)**	**5.6 (2.6)**
Heavy		
Duschee Creek	2 (2.0)	2 (1.5)
Trout Valley Creek	1 (1.0)	3 (3.0)
Winnebago Creek	3 (3.0)	4 (4.0)
S. Branch Root River	4 (4.0)	8 (8.0)
Gribben Creek	2 (2.0)	11 (11.0)
Mean	**2.4 (2.4)**	**5.6 (5.5)**
Extreme		
Beaver Creek	1 (1.0)	4 (4.0)
Garvin Brook	4 (4.0)	6 (3.5)
Swede Bottom	4 (4.0)	6 (6.0)
N. Branch Whitewater River	3 (3.0)	7 (7.0)
Badger Creek	6 (6.0)	8 (8.0)
Crooked Creek	2 (2.0)	10 (10.0)
Mean	**3.3 (3.3)**	**6.8 (6.4)**

Table 1-3. List of chironomid taxa emerging in southeast Minnesota groundwater-dominated streams in order of mean emergence temperature (ET50). Adapted from Bouchard (2007).

Taxon	**ET50 (°C)**
Diplocladius sp.[a]	1.6
Orthocladius (E.) rivulorum	5.9
Diamesa sp.	7.1
Chaetocladius piger gr. sp. 2	7.4
Chaetocladius dentiforceps gr. sp. 3	8.0
Orthocladius (O.) frigidus	9.4
Orthocladius (O.) obumbratus	10.1
Heterotrissocladius marcidus gr [b]	10.4
Tvetenia sp. 2 [a]	10.6
Orthocladius (O.) manitobensis [c]	10.8
Orthocladius (O.) vaillanti [c]	10.8
Corynoneura sp. 1 [b]	10.9
Orthocladius (E.) rivicola	10.9
Eukiefferiella claripennis	11.0
Parakiefferiella sp. 3	11.1
Orthocladius (O.) mallochi	11.1
Zavrelimyia sp.[d]	11.2
Odontomesa fulva	11.3
Prodiamesa olivacea [b]	11.3
Micropsectra polita [b]	11.4
Orthocladius (O.) sp. 3	11.4
Eukiefferiella ilkleyensis [b]	11.5
Parametriocnemus sp. 1	11.6
Dicrotendipes fumidus [b]	11.7
Micropsectra nigripila	11.8
Tvetenia sp. 1	11.9
Eukiefferiella potthasti [d]	12.0
Cricotopus (C.) sp. 4	12.6
Thienemanniella sp. 1	12.7
Brillia retifinis[d]	13.3
Rheotanytarsus distinctissimus [b]	13.8

Table 1-3. Continued.

Taxon	**ET50 (°C)**
Cricotopus (C.) annulator [c]	14.3
Cricotopus (C.) tremulus [c]	14.3
Cricotopus (C.) triannulatus [c]	14.3
Cricotopus (C.) near *tremulus* [c]	14.3
Polypedilum trigonus [d]	14.8
Cricotopus (C.) trifascia	14.9
Tanytarsus nearcticus [d]	16.4

[a] Not collected after flood

[b] Only collected after flood

[c] Genus Average for ET50

[d] Only collected after flood and genus average for ET50

Figures

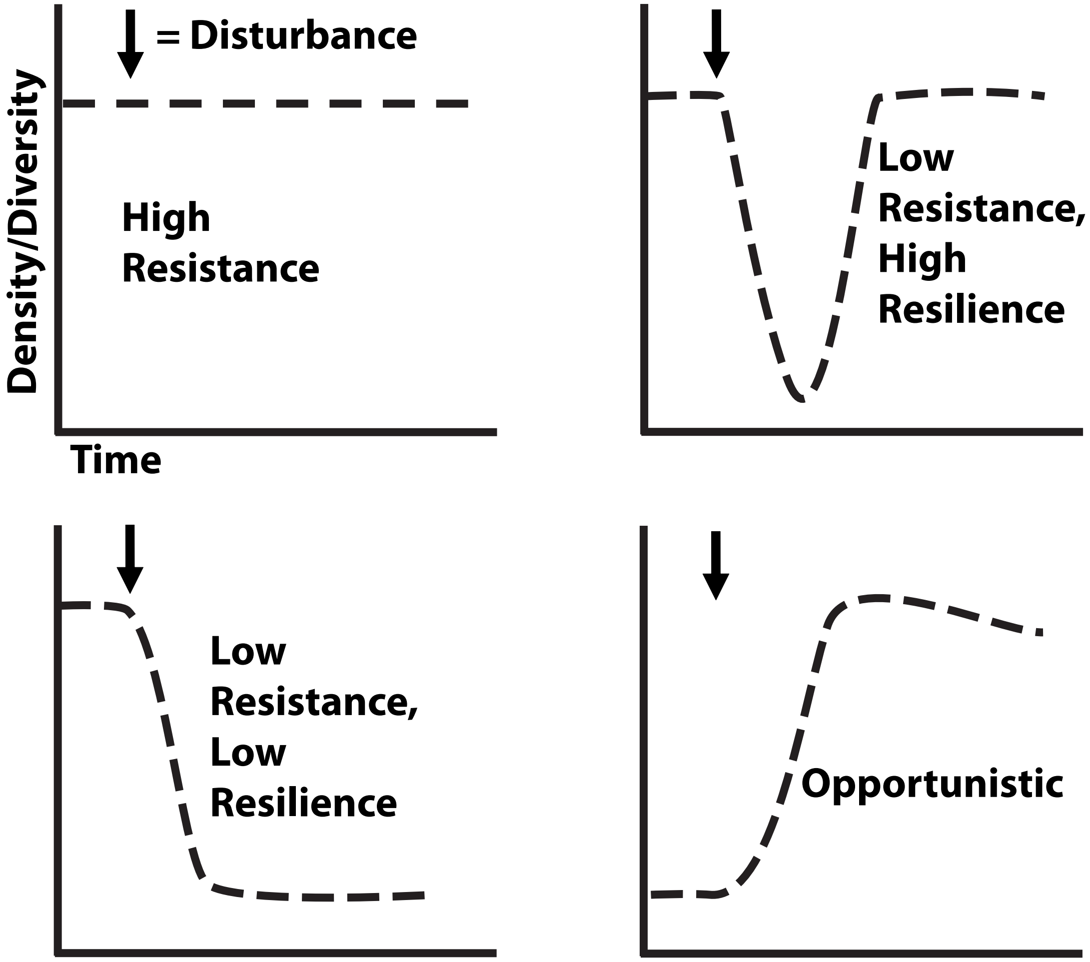

Figure 1-1. Potential community responses to disturbance over time. (a.) Community exhibiting high resistance following a disturbance. (b.) Community exhibiting low resistance and high resilience. (c.) Community exhibiting low resistance and low resilience. (d.) Opportunistic community that is able to become abundant following disturbance.

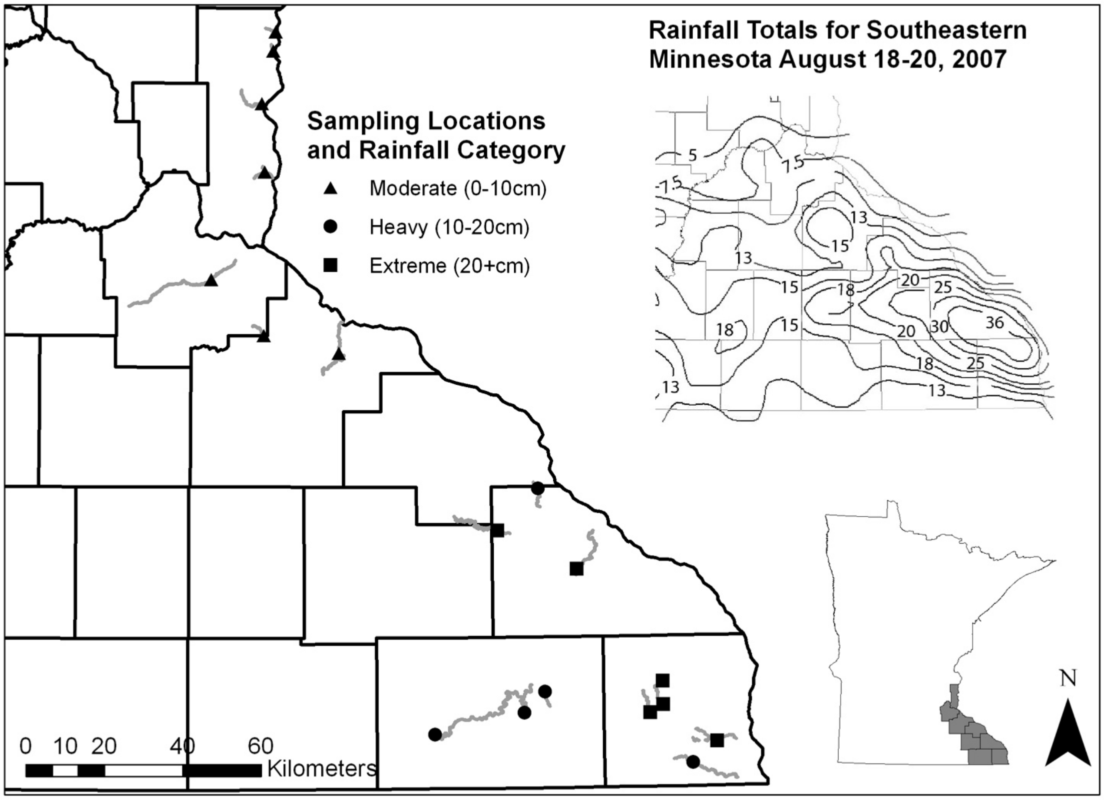

Figure 1-2. Southeastern Minnesota Trout streams sampled for Chironomidae. Streams sampled were equally divided into three rainfall categories. Actual rainfall totals for the area are depicted in the map inset on the top right (Rainfall totals inset modified from Minnesota State Climatology Office, 2008).

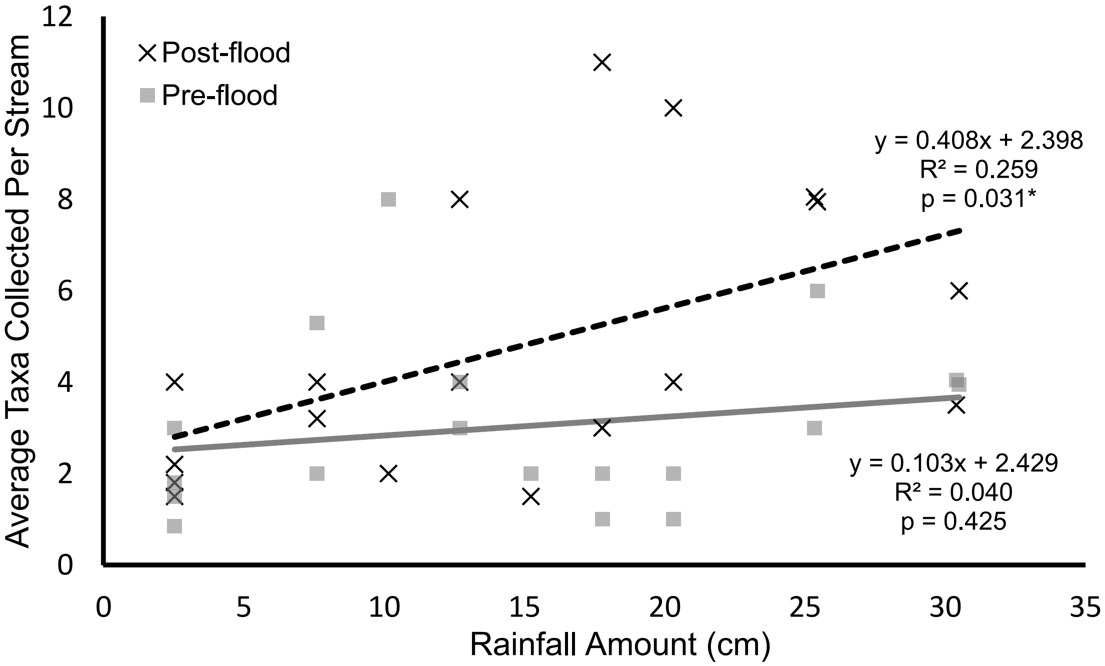

Figure 1-3. Linear regression of average number of taxa collected per stream by rainfall amount. Points were jittered horizontally and vertically to overcome complete overlap. Linear regression analysis indicated a significant response in post-flood collections, indicating that the average number of taxa collected/stream/sample is positively correlated with rainfall (R2 = 0.259; p = 0.031). No trend was apparent in historic collections (R2 = 0.040; p = 0.425).

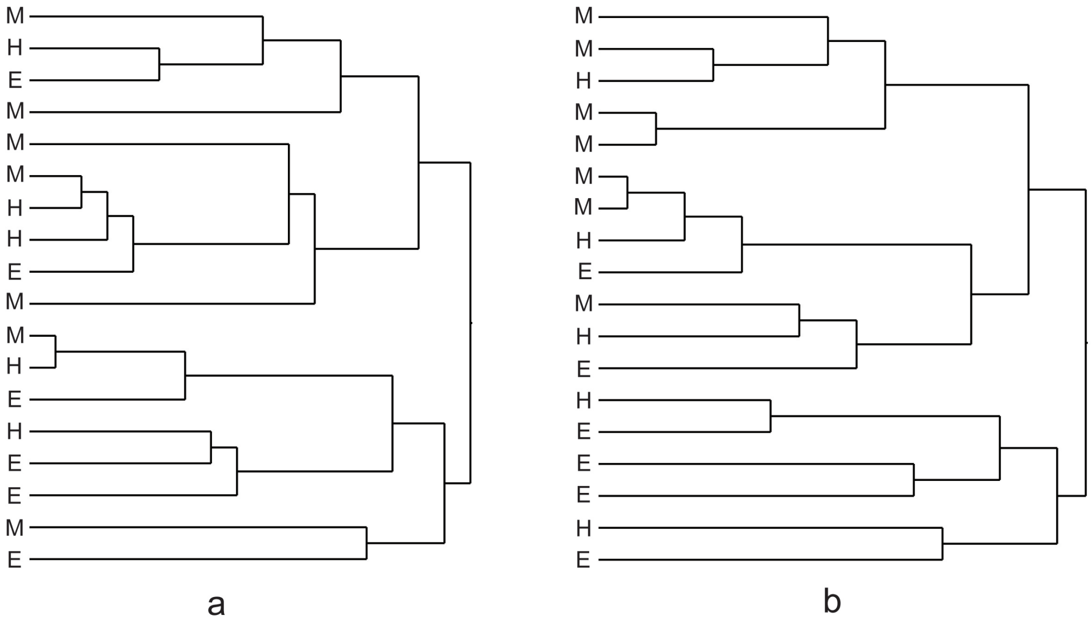

Figure 1-4. Hierarchical cluster analyses of relationships between all streams when considering the average number of taxa per stream for moderate, heavy, and extreme rainfall categories (abbreviated M, H, and E, respectively). Each terminal branch represents an individual stream. (a.) Historical collections; (b.) Post-flood collections.

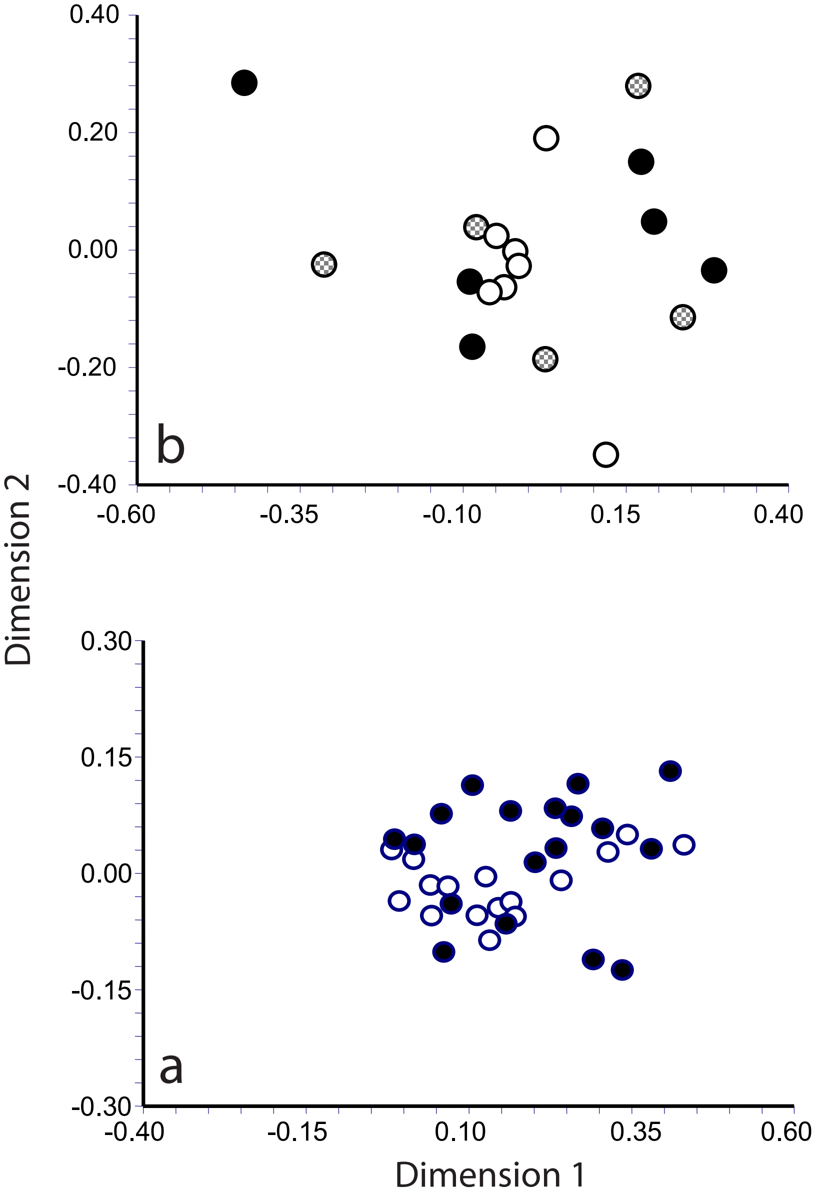

Figure 1-5. Non-metric multidimensional scaling maps of similarity among historical and post-flood data. (a.) Comparison of historical (open circles) and post-flood (filled circles) data (stress = 0.075); (b.) Comparison of rainfall categories using data from all post-flood samples. Open circles represent streams from the 'moderate' category, gray circles represent the 'heavy' category, and black circles represent streams from the 'extreme' rainfall category (stress = 0.086).

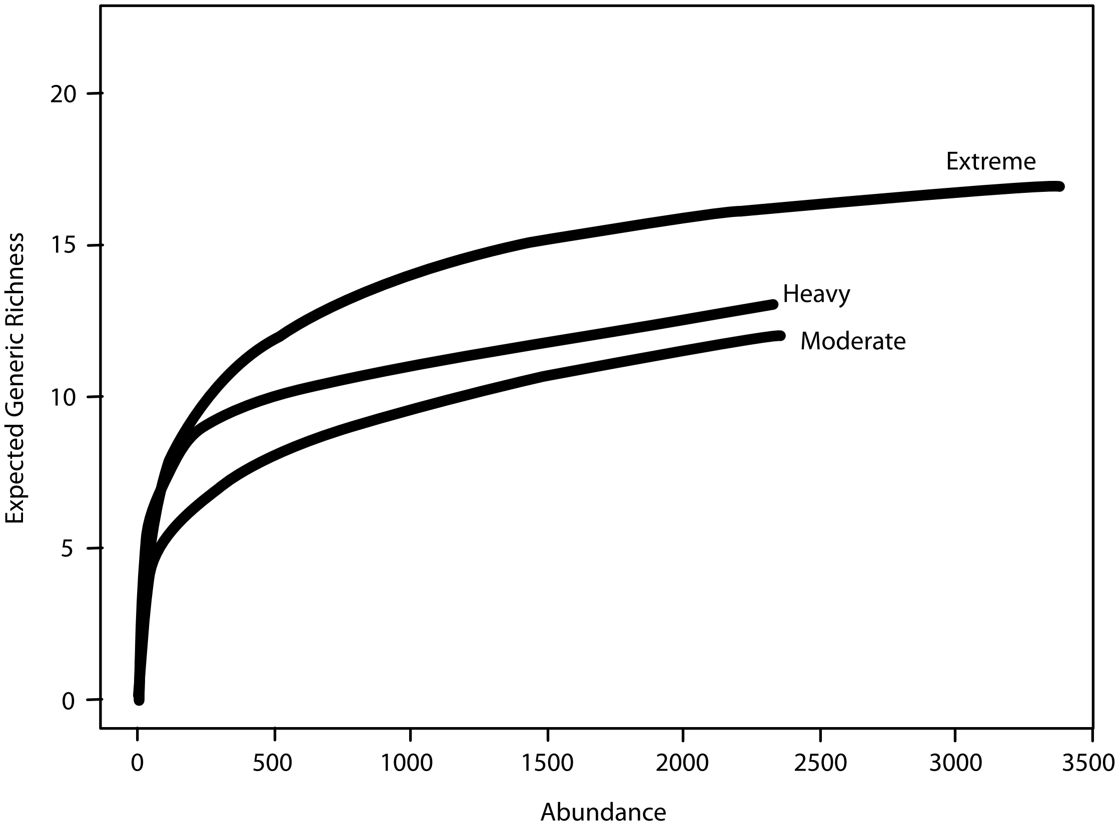

Figure 1-6. Rarefaction curves of the pooled samples within ‘moderate,’ heavy,’ and ‘extreme’ rainfall categories. Each curve shows expected Chironomidae generic richness for a given number of randomly sampled individuals.

Chapter 2

Winter Diets of Brown Trout (*Salmo trutta*) Populations in Southeast Minnesota and the Importance of Winter-active Invertebrates

Winter is a critical period for stream trout, and the need for extending fisheries investigations into this period has long been recognized. Regardless, only a limited number of investigations have focused on winter dynamics and how the winter-active macroinvertebrate community affects trout during this season. The intent of this research was to advance the knowledge of the winter diet of brown trout (*Salmo trutta*) in three groundwater-dominated streams in southeast Minnesota. Specific goals included assessing differences in winter diet between fish of different size classes and whether fish exhibit size-selective predation, whether trout exhibit drift or benthic feeding tendencies, and the importance of winter-emerging insects, such as Chironomidae, in the winter diet. Stomach contents from thirty brown trout were collected from three streams on six occasions during the winter of 2010 and compared to organisms collected in the stream drift and benthos. We found that trout in each stream exhibited distinct diets, and that diets of larger fish differ from younger size classes, with larger trout feeding heavily on Trichoptera and *Physella,* and smaller fish relying more on *Gammarus* and Chironomidae larvae. Stomach contents were more similar to the benthos than drift, indicating a greater reliance on benthic feeding in winter. Trout in all streams preferentially selected Trichoptera and Chironomidae over other prey, and maintained size-selective predation throughout winter, selecting larger *Gammarus* and Chironomidae larvae than found in the environment. Winter-emerging Chironomidae appear to enhance diets in all three streams, and chironomid larvae were particularly dominant in the diet of one trout population. Reductions in these abundant winter-active insects due to climate change may negatively impact winter condition of brown trout, particularly younger size-classes, which may have important implications for fisheries management.

Introduction

Winter is recognized as an energy bottleneck for organisms living at high latitudes, and is an evolutionary challenge for all species faced with surviving the trials of this season (e.g. Halfpenny and Ozanne 1989). Organisms living in freshwater environments, such as stream salmonids, are not immune to the adversities of this critical period (Reimers 1963, Lindroth 1965, Hunt 1969), nor are their prey. Specifically, trout may experience metabolic deficits (Cunjak 1988) and reduced condition (Hunt 1969, Cunjak et al. 1987, Cunjak and Power 1987, Simpkins and Hubert 2000) during winter months.

The need for extending fisheries field investigations into winter was recognized in the early 1900s by Hubbs and Trautman (1935). However, relatively few researchers have chosen to extend their work into winter. While many studies focus on the diet of trout during spring, summer, and fall, only a limited number of investigations have focused on winter dynamics and how macroinvertebrate community composition affect trout during this season (e.g. Lord 1933, Maciolek and Needham 1952, Cunjak et al. 1987, Cunjak and Power 1987, Heggenes et al. 1993, Fochetti et al. 2003, Utz and Hartman 2007, White and Harvey 2007, Johansen et al. 2010). Furthermore, we have not found any studies that compare winter diets among trout of different sizes, and many studies are either restricted to trout over a certain size (e.g. Fochetti et al. 2003, Utz and Hartman 2007), fish residing in a single stream (e.g. Lord 1933, Maciolek and Needham 1952, Cunjak and Power 1987, Johansen et al. 2010) or in mild-winter systems (White and Harvey 2007). Additionally, available studies examine only the prey community associated with the drift (e.g. Heggenes et al. 1993, Simpkins and Hubert 2000) or benthos (e.g. Fochetti et al. 2003), or simply examine those prey found in the diet while ignoring environmental availability (Cunjak et al. 1987, Cunjak and Power 1987). Finally, no winter studies have been conducted in streams that are strongly groundwater-dominated, which have limited, if any, ice cover during the winter season. These 'winter-warm' streams are unique in that they allow unrestricted emergence of aquatic insects that complete their life cycles and develop into active adults during the winter season (Bouchard 2007, Bouchard and Ferrington 2009, Bouchard et al. 2009).

Certain species of Chironomidae are among the few aquatic insects capable of emerging from streams during winter. Adult activity can be witnessed on the banks of groundwater-dominated streams at air temperatures well below 0°C (Bouchard and Ferrington 2009, personal observation). Over 50 chironomid species emerge from streams in Kansas during winter (Ferrington 2000, 2007, Anderson et al. 2011), and nearly 40 species are known to emerge from groundwater-dominated streams in southeast Minnesota (Chapter I). Work by Brown et al. (1980) and Armitage (1995b) indicate that pupae and emerging adults are the primary chironomid life stages fed upon by most trout. Considering that trout are visual predators (Ringler 1979, Klemetsen et al. 2003a, Klemetsen et al. 2003b) that feed extensively on emerging aquatic insects that are found in the stream drift (Elliott 1973), they should be able to effectively exploit winter-emerging chironomids as a food source. Additionally, the large size and distinctive coloration of pupae of some winter-active species, such as *Diamesa mendotae* Muttkowski, may act as a further attraction mechanism. Finally, chironomids are an important source of protein and are contributors to the growth and development of many fish species (Armitage 1995b). Although it is very likely that winter-emerging species play critical ecological roles in groundwater-dominated streams and act as a key food resource for trout, little research documents their roles in these streams.

Studies that have examined the components of the winter drift community in northern regions demonstrate that drift is at a minimum during winter (Clifford 1972, Brittain and Eikeland 1988, Martin et al. 2000) due to either reduced flow rates (Waringer 1992), low invertebrate activity levels (Martin et al. 2000) or perhaps a combination of these factors. Considering that stream trout are often characterized as drift feeding fish (Elliott 1973, Ringler 1979, Bachman 1984, Watz and Piccolo 2011) this may pose a problem for fish that are already experiencing metabolic stress and other problems associated with cold winter temperatures. Watz and Piccolo (2011) emphasize an additional problem that is perhaps exacerbated when considering the potentially low levels of winter drift. They found that prey capture maneuvers of brown trout take significantly longer at temperatures below 8°C, with a 30% decline in capture ability from 10°C to 5.7°C (Watz and Piccolo 2011). The energetic consequences associated with the enhanced risk of missing a drifting prey item during an attack may be more costly when water temperatures are low. Although it is recognized that some trout populations take at least a portion of their food from the stream benthos (Tippets and Moyle 1978, Johansen et al. 2010), as alluded to by Ringler and Brodowski (1983), much less emphasis has been placed on assessing the extent to which trout rely on the stream benthos when foraging.

The intent of this research is to advance the knowledge of the winter diets of brown trout (*Salmo trutta* Linnaeus) residing in three groundwater-dominated streams in southeast Minnesota, USA and to begin clarifying the questions outlined above. Specifically, our goals include evaluation of: 1.) any differences in diet and/or fish condition in the streams studied; 2.) whether winter diet of brown trout corresponds more closely with organisms associated with the stream drift or benthos; 3.) whether the winter diet of trout changes with fish size and if trout exhibit size-selective predation during winter; and 4.) the importance and potential electivity of winter-emerging Chironomidae as well as other possible prey items in the winter diet.

Materials and Methods

Study Area

The study was conducted between January and March 2010 in three groundwater-dominated streams in the Driftless Region of southeast Minnesota, USA. This region was surrounded, but left virtually untouched, by the last glaciation (Waters 1977). The lack of glacial activity accounts for the landscape that distinguishes the area, characterized by steep limestone bluffs, sandstone valleys, and an array of 181 spring-fed trout streams that comprise a total of 1,268 stream kilometers (Dieterman and Merten 2003). Of these streams, 124 are home to brown trout (Thorn and Ebbers 1997), which were first introduced to the region in 1888 (Thorn et al. 1997), and have since become the most abundant trout species in southeast Minnesota (Thorn 1990).

Study sites were located on Beaver Creek, the Middle Branch of the Whitewater River, and Cold Spring Brook (hereafter Beaver, Whitewater, and Cold Spring); Beaver and Whitewater lie in the Mississippi River – Winona watershed, and Cold Spring is within the Zumbro River watershed (Fig. 2-1). Habitat in all three streams has been completely or partially improved in recent years. More information on improvement projects and habitat conditions can be obtained from the Minnesota Department of Natural Resources (e.g. Thorn 1988a, b, Thorn and Anderson 2001, Dieterman and Merten 2003, MNDNR 2003). Other abundant fish species in the streams include brook trout (*Salvelinus fontinalis* (Mitchell)) (Coldspring), rainbow trout (*Oncorhynchus mykiss* (Walbaum)) (Whitewater), and slimy sculpin (*Cottus cognatus* Richardson) (Beaver, Coldspring, Whitewater).

The study reach in each stream was approximately 150 m in length. Two temperature loggers were deployed in each stream at the beginning of winter and left until the conclusion of sampling. Average water temperature for the three-month period was 3.1°C (min = 1.8°C; max = 5.6°C) in Beaver, 4.4°C (min = 0.6°C; max = 8.6°C) in Whitewater, and 7.1°C (min = 5.9°C; max = 8.5°C) in Cold Spring. The stream reach at Cold Spring is in a wooded area and is heavily influenced by a groundwater spring upwelling, resulting in a warmer and more constant winter water temperature, as well as a higher abundance of aquatic vegetation (primarily *Nasturtium* sp.). The Whitewater sampling reach is relatively open, surrounded primarily by pastureland, and exhibited the most significant diurnal fluctuation in water temperature (up to 6°C). The Beaver reach runs through a wooded Wildlife Management Area and exhibited fairly stable diurnal temperature.

Sampling Regime

Three sampling periods occurred in each of the three streams, the first in early January, the second in late January, and the third in late February. Each sampling period consisted of two fish sampling events, spaced three to four days apart, and one invertebrate sampling event; invertebrate sampling coincided with the first fish sampling event in each of the periods. This resulted in a cumulative total of six fish and three invertebrate sampling events per stream. All sampling events occurred between 0800 and 1600-h.

Considering the extended time for brown trout to completely evacuate stomach contents in cold waters (e.g. complete gastric evacuation of *Gammarus, Baetis,* and Chironomidae at 4°C is achieved after 56 hours, whereas complete evacuation of these organisms at 18°C is achieved after only about 12 hours) (Elliott 1972), it was presumed that stomach contents of fish collected during the second event of the sampling period would closely resemble the organisms found in the drift and benthic samples at the beginning of the sampling period. Additionally, this time frame provided an opportunity for fish captured in the first sampling event to have ample opportunity to resume feeding and potentially obtain maximum fullness, eliminating statistically confounding effects of paired data.

Fish Sampling

A Wisconsin™ Abp-3 pulsed DC backpack electrofishing unit and dip nets were used to capture a sample of 30 fish from each stream during every event; attempts were made to collect fish from a wide assortment of size ranges to allow dietary comparisons among size classes. Each fish was anesthetized in an 80mg/L solution of tricaine methanosulfate (MS-222) for 2-3 minutes (Summerfelt and Smith 1990), weighed, and measured for maximum total length to the nearest mm. Gastric lavage (Light et al. 1983, Hartleb and Moring 1995) was used to evacuate stomach contents from each fish. Briefly, a hand-pump with a plastic Pasteur pipette attached to the nozzle was used to deliver a stream of water into the foregut until all items were removed; a coffee straw was inserted into the tip of the pipette to allow small trout (<110 mm) to be sampled. This technique has proven very effective, with studies indicating removal efficiencies of 98% in salmonids, while maintaining 100% survival rates (Light et al. 1983). Stomach contents from each fish were retained on a 125µm sieve and transferred into individual, labeled containers and preserved with 70% ethanol. Following gastric lavage, fish were allowed to recover in stream water for 20-30 minutes before being returned to the stream. All required approvals from the Institutional Animal Care and Use Committee and the Minnesota Department of Natural Resources Division of Fish and Wildlife were obtained before commencing fish sampling procedures.

Invertebrate Sampling

Invertebrates were collected from the stream bed and drift during each of the three sampling periods. To assess available prey in stream drift, three drift nets (28 x 15 x 86 cm; 125µm mesh) were equally spaced along a stream cross section and left in place for 24-hours. Nets were placed at the downstream end of a riffle located at the uppermost portion of the sampling reach. The contents of the nets were passed through a 125µm sieve and preserved in 70% ethanol.

Benthic macroinvertebrate samples were collected with a Waters-Knapp modification of a Hess stream bottom sampler ($0.11m^2$). Five samples were taken in proportion to the erosional and depositional habitat in each reach; sampling locations within each habitat were selected randomly. Samples were then combined to form a single composite sample. As with drift samples, contents were passed through a 125µm sieve and preserved in 70% ethanol.

Laboratory Procedures

Drift samples were sorted and subsampled to 100 individuals per net for a maximum combined sample of 300 invertebrates in a drift sampling session; however, nets often contained less than 100 organisms. Benthic samples were subsampled to 300 individuals. These subsampled invertebrates, along with all invertebrate prey collected from stomach samples, were identified under a dissecting microscope to the lowest possible taxonomic level, usually genus, and were measured for total body length to the nearest 0.5 mm.

Given the considerably slower digestion and stomach evacuation rate due to low winter water temperatures (Elliott 1972), most organisms in the stomach content samples were whole and readily identifiable and measurable. However, in occasions where only parts of an organism were present, the organism was counted by a constant body part, usually the head. When feasible, length was estimated based on comparison with other measurable specimens in the sample. In cases where an accurate length estimate was not feasible, the average length for that specific prey item was used. Average length was estimated as follows: if over five other prey of a given genus with accurate length measurements was consumed by an individual fish, the average length was determined from those prey items. If less than five prey of that genus were consumed by the fish, the average length of the genus was derived from the entire population of prey specimens of the genus found in the trout diet for that stream and sampling period.

Biomass estimates of organisms in trout stomach contents, drift, and benthos were derived using length-dry mass equations. Most estimates were based on Benke et al. (1999), however other resources were utilized for adult insects and non-insect invertebrates (Eckblad 1971, Ganihar 1997, Sabo et al. 2002, Baumgärtner and Rothhaupt 2003). No literature for length-mass regression equations was available for the pupal stages. Thus, in instances where biomass estimates for the pupal stages were required (primarily in the Chironomidae), regression equations for the corresponding larval lifestage were used. While this might be a slight over-estimation of weight, considering that pupae are a non-feeding lifestage and this period is a time when the insect is utilizing stored energy for lifecycle conversion, we believe it is the most reliable estimate currently available. We also chose to use larval biomass estimates for adult chironomids present in our samples rather than conversions available for terrestrial dipteran adults because we believe the larval chironomid lifestage is a closer estimate of adult weight than values that combine various families of Diptera in order to obtain a length-mass relationship.

Data Analysis

Condition Factor

Body condition of each fish was calculated using the Relative Weight (W_r) index (Wege and Anderson 1978). This measure is defined as:

$$W_r = \frac{W}{W_s} \times 100$$

where W is the weight of an individual, and W_s is the standard weight predicted by a length-weight regression for the species, calculated using the equation:

$$\log_{10}(W_s) = a' + b \times \log_{10}(L)$$

where L is the maximum total length of the fish, and a' and b represent the intercept value and slope of $\log_{10}$(weight)-$\log_{10}$(length) regression equation. Standard values for a' and b, developed by Milewski and Brown (1994) and reported in Anderson and Neumann (1996) were used to derive W_s in this study. As is common for most proposed standard weight equations (Blackwell et al. 2000), the equation for brown trout by Milewski and Brown (1994) has a minimum applicable length of 140 mm. Considering differences between juvenile and adult fish and consequences this may have on W_r (Blackwell et al. 2000), we have chosen to include only those fish with total lengths >140 mm. While this eliminates a large subset of our data, we believe this may yield a more accurate assessment of condition than including smaller fish that may skew condition results because of inaccuracies due to differences in juvenile form. To test for differences in body condition (W_r) between streams and sampling periods, a two-way analysis of variance (ANOVA) was carried out, following the General Linear Model procedure (α = 0.05) in Stata/IC 12.1., 2012.

Size Class Delineation

Diets of trout shift with increasing body size (Elliott 1967a, Ozvarol et al. 2011b), however it is not well documented if this trend holds throughout the winter season. In order to assess differences in winter resources among trout of different sizes, we divided the fish sampled in each of our streams into ‘large’ and ‘small’ size classes. Since age data were not available for our fish, we created individual value plots of fish length for each of the three streams and used natural breaks in each of these plots to divide each of the three trout populations into ‘large’ and ‘small’ size classes (Fig. 2-2).

Diet Description and Analysis

The diets of trout sampled in each of the three streams over the course of all sampling periods was quantitatively summarized by calculating the frequency of occurrence, percent composition by number, and percent composition by weight (data available in Appendix A-C).

Size-selective Predation

To determine whether brown trout exhibit size-selective predation throughout winter, we chose to analyze two specific prey types that were common in both the diet and the environment throughout the entire sampling period, Chironomidae larvae and *Gammarus.* Data for all streams and sampling periods was pooled, and data were analyzed non-parametrically because normality conditions were not met. A Kruskal-Wallis test was used to determine whether there were significant differences in median size of these prey items in the trout diet, stream drift, and benthos. Histograms were developed for each taxon, depicting the percent of each prey taxon in varying size categories in both the diet and environment (Figs. 2-3 & 2-4).

Prey Electivity

Manly's alpha for constant prey populations (Manly et al. 1972, Chesson 1978, 1983) was used to estimate prey selection across streams and to determine whether trout of different size classes consumed prey in proportion to that numerically available in the environment. Manly's alpha (α) is defined as:

$$\alpha_i = \frac{r_i}{n_i}\left(\frac{1}{\sum \frac{r_i}{n_i}}\right)$$

where α_i is Manly's alpha for prey type i, r_i is the proportion of prey taxon i in the diet, and n_i is the proportion of each prey taxon i available in the environment. Considering that trout are opportunistic generalist feeders (Cunjak et al. 1987, Cunjak and Power 1987, Bridcut and Giller 1995, Ozvarol et al. 2011a), we chose to combine the prey found in drift and benthic samples to create a depiction of all prey available to trout in the environment; this composited value was used for the n_i parameter when calculating Manly's alpha. We analyzed electivity by use of numerical proportions of organisms available and in the diet rather than by weight because we believe this method better reflects prey encounter rates.

All taxa present in either the trout diet, drift, or benthos with relative abundances greater than 1.5% of the total composition were included as 'major prey' and treated as a separate prey type, i, when calculating Manly's alpha. Taxa with relative abundances less than this amount were lumped together as a combined 'other prey' category when computing prey electivity. In the event that a 'major' taxon was present in the diet (i.e.

percent composition of the taxon was >1.5% of the diet) but was not detected in the drift or benthos, or vice-versa, these groups were included in the 'other prey' category for purposes of this analysis; attention will be given to these cases in the discussion of the results. Values of $\alpha > 1/m$, where m is the total number of prey types available, suggest selective feeding for that taxon, values where $\alpha = 1/m$ indicate neutral selection, and values $< 1/m$ suggest that prey item is avoided.

Niche Breadth

Levins's measure of food niche breadth (Levins 1968) was used to determine whether small and large brown trout in each of the streams exhibit comparable prey specialization levels. Levins's measure (*B)* is calculated as:

$$B = \frac{1}{\sum p_j^2}$$

where p_j is the percentage of items in the diet that are of food category *j*. Levins's measure was standardized (B_A) on a 0 to 1 scale using the following equation suggested by Hurlbert (1978):

$$B_A = \frac{B - 1}{n - 1}$$

where *n* is the number of possible resource states. A B_A value of zero indicates complete specialization on a single prey source, whereas a value of one indicates equal use of all prey items.

Niche Overlap

In order to assess the similarity or niche overlap of winter diets between brown trout of large and small size categories in each of the streams, Schoener's index of percentage overlap (P_{jk}) was used (Renkonen 1938, Schoener 1970). Average prey weight percentages were used to calculate the percent overlap index. This measure, calculated as a percentage, is derived as follows:

$$P_{jk} = \left[\sum_{i=1}^{n} (\text{minimum } p_{ij}, p_{ik}) \right] 100$$

where p_{ij} is the proportion resource i is of the total resources used by species j, p_{ik} is the proportion resource i is of the total resources used by species k, and n is the total number of resource states. Schoener's percentage overlap index was also used to evaluate overlap between the composition of the trout diet, drift, and benthos to determine whether trout diets more closely reflect prey available in the drift or benthos. Niche overlap values greater than 0.60 are considered to be biologically significant (Wallace 1981).

Results

A total of 604 fish were collected, measured, and subjected to gastric lavage across all streams and sample dates, with 207, 202, and 195 from Beaver, Whitewater, and Cold Spring, respectively (Table 2-1). While most fish in our study had food in their stomachs, two of the streams, Beaver and Whitewater, had a larger percent of fish with empty stomachs (14% and 15% of fish sampled, respectively) than the values of 8% or less reported by various other studies documenting winter feeding in salmonids (Maciolek and Needham 1952, Reimers 1957, Cunjak et al. 1987, Cunjak and Power 1987, Simpkins and Hubert 2000). Cold Spring exhibited far fewer instances of trout with empty stomachs (3.6% of fish sampled) as compared to Beaver and Whitewater.

A wide variety of food items were consumed, with totals of 59, 39, and 30 taxa consumed in Beaver, Whitewater, and Cold Spring, respectively. A detailed account of all taxa occurring in trout diets for each stream, including information on the frequency of occurrence, percentage by number, and percentage by weight, along with respective percentages for organisms found in the stream drift and benthos is in Appendices A-E. Each stream had a specific base of taxa that typified the diet; there were also specific taxa in each stream that were more characteristic for large or small trout (Tables 2-2a-c, Appendices A-C). When comparing the numerical composition of the trout diet, in all streams, smaller trout were much more reliant on a base of 'large' chironomid larvae (>3.5mm) (45.7%, 49.5%, and 11.3% in Beaver, Whitewater, and Coldspring, respectively), where this dependence showed a marked decrease in larger trout (33.4%, 17.0%, and 0.77%, respectively), with a corresponding shift in the large trout diet to larger prey such as brachycentrid (Beaver, Whitewater) or limnephilid (all streams) caddisflies. Another example of size-associated diet shift was apparent in Whitewater and Coldspring, where much of the diet of small trout was composed of *Gammarus* (21.0 % and 76.5%, respectively), whereas larger trout did not show as heavy of a reliance (15.6% and 51.2%, respectively). Furthermore, in Cold Spring, *Physella* was virtually absent from the diet of small trout (0.87%), however, was a primary numerical component of the diet of large trout (23.3%).

These same trends are apparent when examining diets by prey weight. In Cold Spring, *Gammarus* dominated the diet of small trout (81.5% of diet by weight), with Limnephilidae (*Limnephilus, Hesperophylax*) adding most of the remaining diet component. The importance of limnephilids in the diets of large trout, on the other hand, increased substantially (49.3% by weight), whereas *Gammarus* was much less important (39.8%). *Physella* accounted for 8.8% of the diet of large trout, whereas this taxon had a much smaller role in small trout diets (0.59%). In Whitewater, *Gammarus* was a primary component of the diet of small trout (53.7% by weight), with *Brachycentrus* and 'large' chironomid larvae (>3.5mm) adding a significant component (24.9% and 12.6%, respectively). Large trout showed a drop in reliance on *Gammarus* (15.9%) and large chironomid larvae (2.07%), and a substantial increase in reliance on *Brachycentrus* (63.0%) and Limnephilidae (10.5%).

Diets trends in Beaver Creek (analyzed by weight) showed the highest diversity of the three streams, but again there were shifts in feeding patterns from small to large trout. The diet of small trout consisted of a greater percentage of *Ephemerella* (6.0%), *Isoperla*

(6.1%), ‘large’ Chironomidae larvae (13.7%), and *Gammarus* (19.4%), than large trout (2.9%, 2.3%, 6.3%, and 15.4%, respectively). Comparatively, large trout showed a much higher reliance on *Brachycentrus* (24.3%, compared to 5.4% for small trout). Both small and large trout fed heavily on Limnephilidae, however, small trout fed primarily on *Limnephilus* (17.4% in small trout, vs. 7.0% in large trout), whereas *Pycnopsyche* and *Hesperophylax* accounted for a larger portion of the large trout diet (9.1% and 3.6%, respectively for large trout, and 2.6% and 0.0% for small trout).

In all streams, predation on other fish was rare; only 10 trout (6 from Beaver) consumed other fish (*Cottus cognatus*, in all identifiable instances). Most trout that fed on *C. cognatus* were over 200 mm, and all were over 120 mm. Three fish, all from Cold Spring, were found to have fed on trout eggs.

Condition Factor

Overall, fish were in good condition in all streams throughout the winter. Two-way ANOVA indicated no significant difference between sampling dates ($F_{2, 325} = 2.87$, $p = 0.058$), indicating no significant declines in condition as the winter season progressed. There were, however, significant differences between streams ($F_{2, 325} = 43.24$, $p < 0.0001$). Tukey’s multiple comparison method indicated that W_r was significantly different in all three streams, with mean condition values of 94.3, 88.5, and 85.5 for Cold Spring, Whitewater, and Beaver, respectively (Table 2-1).

Size-Selective Predation

Kruskal-Wallis tests indicated that trout exhibit strong size selective predation for ‘large’ Chironomidae larvae and *Gammarus* during winter. When analyzing differences between chironomid larval size in the trout diet and the environment, we found significantly different median values for larval length in the trout diet, drift, and benthos ($p < 0.0000$), with median lengths of 6.0 mm, 3.0 mm, and 3.5 mm, respectively (Fig. 2-3). Mann-Whitney tests were then performed to assess pair-wise differences, indicating highly significant differences when comparing median larval length in the diet and drift and diet and benthos ($p = < 0.0000$ for each comparison), also indicating that chironomid size in the benthos was significantly larger than in the drift (p = 0.0026). Similarly, Kruskal-Wallis analysis indicated differences between median *Gammarus* size in the trout diet and the stream drift and benthos ($p < 0.0000$). Median size values for *Gammarus* in the trout diet, drift, and benthos was 7.5 mm, 5.0 mm, and 4.0 mm respectively (Fig. 2-4). Subsequent Mann-Whitney tests indicated that the size of *Gammarus* in the drift and benthos were not significantly different from each other ($p = 0.0632$), whereas size of *Gammarus* in the diet was significantly larger than both drift and benthos ($p < 0.0000$ for both comparisons).

Considering that trout were found to selectively feed primarily on larger Chironomidae larvae than what would be expected if feeding in proportion to what is available in the environment, we chose to divide Chironomidae larvae into ‘large’ and ‘small’ categories. Very few Chironomidae larvae (< 5%) under 3.5 mm were found in the trout diet, however, at least half of the chironomid larvae in the drift and benthos were

3.5 mm or less. Thus, we considered 3.5 mm as a cutoff value, with all chironomid larvae over 3.5 mm considered as ‘large’ Chironomidae and those under 3.5mm as ‘small’ Chironomidae for purposes of the following analyses. While trout feed on larger *Gammarus*, we chose not to subdivide *Gammarus* into large and small categories considering that cutoff values for these categories were not as clear.

Prey Electivity

We analyzed prey electivity based on both numerical and biomass proportions of organisms in the diet and environment, however here we report only results found by use of numerical proportions of organisms rather than by weight because we believe this method better reflects prey encounter rates. Results from prey electivity based on biomass of prey were very similar to those reported here, and results from this analysis can be found in Appendix F-H.

In Cold Spring Brook, *Gammarus* dominated the environmental samples by number (62.9%), with ‘small’ chironomid larvae as the second most abundant taxon at 18.2%. ‘Large’ Chironomidae larvae and baetid mayflies both accounted for over 6.5% of environmental invertebrate numerical abundance. Diets of small brown trout were composed primarily *Gammarus* (76.54%), ‘large’ chironomid larvae (11.3%) and limnephilid caddisflies (6.3%). Comparatively, *Gammarus* and ‘large’ chironomids accounted for a lower percentage of the large brown trout diet (51.2% and 0.77% respectively), whereas limnephilids and *Physella* had greater numerical importance (22.6% and 23.3% respectively) (Table 2-2a; Appendix A). Despite the abundance of

Gammarus in diets of both small and large trout, Manly's alpha did not indicate preference for this taxon. Large trout stongly preferred Limnephilidae and *Physella,* whereas small trout preferred Limnephilidae and 'large' chironomid larvae (Table 2-2a).

Environmental samples in the Middle Branch of the Whitewater River were dominated numerically by 'large' and 'small' Chironomidae (28.5% and 18.4% respectively), along with *Baetis* (10.8%) and *Podura* (Collembola) (10.7%); notably, no collembolans were found in any trout diets. Diets of small trout consisted primarily of 'large' chironomid larvae and pupae (49.5% and 8.8%, respectively), *Gammarus* (21.0%), and *Brachycentrus* (8.5%). When examining diets of large brown trout, *Brachycentrus* dominated diets at 46.8%. 'Large' chironomid larvae, *Gammarus,* and chironomid pupa were not as abundant as in the diet of small trout, with numerically abundances of 16.7%, 15.6%, and 3.3%, respectively. *Physella*, however, comprised a greater proportion of the large trout diet (5.1%). Manly's alpha indicated preference for chironomid pupa, *Brachycentrus,* and *Gammarus*, in both large and small trout; notably, small trout indicated a much greater preference for chironomid pupa, while large trout strongly preferred *Brachycentrus.* Small trout also preferred 'large' chironomid larvae (Table 2-2b; Appendix B). Limnephilidae were found in the diet of large fish, representing 3.1% of the diet numerically (Appendix B), however our sampling methods did not detect this taxon in the environment.

'Large' and 'small' chironomid larvae dominated the environment of Beaver Creek numerically (30.3% and 24.0% respectively). *Podura* (12.8%), baetid and ephemerellid mayflies (4.7% and 5.3%), and the blackfly *Simuliium* (4.0%) were also abundant. ‘Large’ chironomid larvae were numerically abundant in diets of both small and large trout (45.8% and 33.9%, respectively), as were *Gammarus* (4.0% and 5.1%) and *Lepidostoma* (15.3% and 9.4%, respectively); sampling methodology only detected a rare occurrence of *Lepidostoma* in the environment (0.78%). *Ephemerella* (5.1%), ‘small’ chironomid larva (4.0%), and *Allocapnia* (3.4%) were also common in the diet of small brown trout. Other organisms numerically common in the diet of large trout included chironomid pupae and adults (4.8% and 8.5%, respectively), and *Brachycentrus* (8.4%) (Table 2-2c; Appendix C). Two prey taxa common to the diet of large trout but not detected in the environment included Limnephilidae and Uenoidae (Appendix C). Manly’s alpha values indicated preference for Chironomidae pupae and adults, as well *Lepidostoma* and *Allocapnia* in both small and large trout. Alpha values also indicated preference for *Allocapnia*, *Micrasema*, and *Physella* in small brown trout (Table 2-2c).

Niche Breadth

Levins’s measure of niche breadth indicated that small brown trout in Cold Spring exhibited high specialization, while large trout exhibited a more diverse diet. The reverse was true for Whitewater and Beaver, however the differences between niche breadth in large and small trout was were not as pronounced. Overall, trout in Beaver exhibited the greatest degree of niche breadth (Table 2-3).

Niche Overlap

When comparing niche overlap between diets of small and large brown trout based on prey mass, only fish in Beaver exhibited biologically significant overlap between the two size classes (74.9%), whereas less overlap between the size classes was found in Whitewater (49.2%) and Cold Spring (57.3%) (Table 2-3).

Schoener's percent overlap index also indicated that, overall, large and small trout diets showed more overlap with prey found in the benthos than the drift (Table 2-4). With only one exception, all fish in both small and large size categories exhibited biologically significant overlap with prey in the benthos; only large trout in Cold Spring Brook exhibited significant overlap with prey found in the drift. None of the three streams exhibited biologically significant overlap when comparing prey found in the drift and benthos (Table 2-4).

Discussion

A reliable and abundant food supply is one of the primary factors influencing the stability of trout populations; therefore the constancy of a stream's aquatic invertebrate community has a major impact on the diet and growth of trout. Recent studies suggest that populations of brown trout in Minnesota are significantly influenced by differences in forage composition, which often relates directly to water temperature (Dieterman et al. 2004). Furthermore, an 'optimal thermal regime' exists for aquatic insects living within a given geographic area (Vannote and Sweeney 1980, Rossaro 1991). Temperatures that fall outside of this range will often yield smaller and less fecund adults (Vannote and Sweeney 1980), and may even lead to a change in distribution or local extinction of macroinvertebrates found within the stream (Nyman et al. 2005, Durance and Ormerod 2007), which can have corresponding negative impacts on trout that rely on these invertebrates as food.

Trout in Beaver and Whitewater had higher occurrence of empty stomachs (14 and 15%, respectively) than those in Cold Spring (3.6%). This is perhaps an artifact of the higher winter water temperature in Cold Spring (7°C, average), which corresponds to faster rates of gastric evacuation (Elliott 1972) and a greater need for food to sustain or surpass maintenance metabolic rates (Elliott 1982, 1994). While the number of fish in Beaver and Whitewater with empty stomachs may seem comparatively high, this may not be cause for concern, considering that Reimers (1957) has reported that trout are capable of surviving (mortality rate below 10%) without food for at least six months at temperatures below 6°C. Additionally, feeding activity of rainbow trout increases at temperatures in excess of 4.5°C (Reimers 1963) and ingestion and corresponding growth rates are strongly temperature dependent (Elliott 1976, 1982, 1994). These patterns help explain the difference in empty stomachs between our streams, particularly when considering the difference in their winter water temperature regime.

The results we present demonstrate that brown trout have distinct diets that are dependent on fish size and variable by stream. Specifically, prey items such as *Gammarus* and chironomid larvae are often quite dominant in the diet of smaller trout, whereas larger organisms, such as limnephilid and brachycentrid caddisflies, become much more prominent in the diet of larger trout. We also found that brown trout appear to maintain size-selective predation throughout the winter months, selecting for the largest available chironomid larvae and *Gammarus*; this supports the findings of Newman and Waters (1984) who found that trout exhibit size-selective predation on *Gammarus* throughout the year in a similar Minnesota groundwater-dominated stream. They found that the mean size of *Gammarus* preyed upon was approximately 8 mm, whereas the mean size in the environment was 3 mm in January (Newman and Waters 1984); this corresponds almost exactly to the results we found in our study (Fig. 2-4). Passing opportunities to feed on smaller prey can be an important energy-saving mechanism, since smaller prey likely would not compensate for the energy required to capture them (Bachman 1984). These types of energy-conservation behaviors are likely especially critical during the stressful winter months.

Caddisflies and chironomids were the only major taxonomic groups for which trout consistently exhibited positive selection in each of the three streams we studied. The prominence of benthic organisms such as case-building caddisflies has been reported in most other known salmonid winter diet studies (Lord 1933, Maciolek and Needham 1952, Cunjak et al. 1987, Cunjak and Power 1987, Heggenes et al. 1993, Fochetti et al. 2003, Utz and Hartman 2007, Johansen et al. 2010). We found that abundances of Trichoptera in the environment were relatively low, but comprised a substantial part of the winter diet, particularly for large brown trout, which is similar to the winter diet of juvenile Atlantic salmon (Johansen et al. 2010). Limnephilidae, for example, accounted for 10.5% and 21.5% of the prey weight consumed by large brown trout in Whitewater and Beaver, respectively, and 20.0% of the prey weight of small brown trout in Beaver, however this taxon was not detected in drift or benthic samples for either stream. Although the low environmental abundances are likely an artifact of our sampling methodology, especially considering that many limnephilids often prefer slower, deeper pool areas as compared to riffles, our results also indicate that trout are very capable of locating and preferentially selecting for these insects. Research also indicates that larger trout often inhabit areas of deeper water in winter (Cunjak and Power 1986, Thorn 1988a, Heggenes et al. 1993), also corresponding to observations made in this study, thus are more likely to locate limnephilids occupying these areas of the stream.

Although not common or selected for in the diet of large brown trout, small trout appeared to selectively prey on 'large' chironomid larvae in two (Whitewater and Cold Spring) of the three streams studied; while our electivity index did not indicate that 'large' chironomids were selected for in Beaver Creek, these prey clearly dominated the diet of both large and small brown trout in Beaver Creek, numerically comprising 33.9%

of the diet of large trout and 45.8% of the diet of small trout and a large part of the diet by mass (6.3% and 13.5%, respectively), substantially more than the other two streams. Overall, *Diamesa* spp. (Beaver and Whitewater) and *Micropsectra* spp. (Cold Spring) were among the most common chironomid larvae in the trout diet. Although Heggenes et al. (1993) also found high abundances of chironomid larvae in the winter diet of brown trout, this contrasts other salmonid winter diet studies (e.g. Fochetti et al. 2003, Johansen et al. 2010) that found very few chironomids in the trout diet despite high abundances in the stream; importantly though, of these studies, only Fochetti et al. (2003) focused on brown trout, and neither study differentiated diet by fish size. Furthermore, winter-emerging chironomid pupae were positively selected for by both large and small brown trout in two of our three streams, and both large and small trout in one of the streams selected for adult chironomids.

The chironomid species *Diamesa mendotae* is well-adapted to life at low temperatures (e.g. Carrillo et al. 2004, Bouchard et al. 2006a, Bouchard et al. 2006b, Bouchard and Ferrington 2009) and is the most abundant and commonly found winter-emerging chironomid in most groundwater-dominated streams in southeast Minnesota (e.g. Chapter 1). Larvae of this species are among the largest chironomid larvae available to trout during the winter, with a mean larval size of 8mm and a maximum of 12mm. Large emergences of this insect have been observed when daily air temperature maxima are near 0°C, and adults have been observed walking on the snow when daily air temperatures are as low as -7°C (Bouchard 2007, Bouchard and Ferrington 2009). Bouchard and Ferrington (2009) demonstrated that larval growth of *D. mendotae* is limited to water temperatures below 10°C. Thus, if winter stream temperatures increase as a result of predicted climate warming, the abundance of winter-emerging chironomid species such as *D. mendotae* could be reduced. These potential changes in species abundance may have negative consequences for fish communities that rely on an abundant supply of *D. mendotae* and other winter-emerging species throughout the winter months. Trout in Beaver Creek exhibited the greatest reliance on *Diamesa* larvae as a food source and could be negatively impacted if populations of *Diamesa* are reduced. Furthermore, our electivity indices indicated that fish in both Beaver and Whitewater selected for chironomid pupae and adults, the majority of which were *Diamesa.* Although not often the most abundant taxon in the diet, the extra caloric value provided to trout by *Diamesa* and other winter-emerging chironomids may benefit the trout population by providing extra energy during a critical time of year, and the marginal contribution could significantly influence growth rates.

Schoener's percent overlap index indicated that brown trout diets in the three streams were aligned closer with prey associated with the benthos as compared to those in the drift. Trout are opportunistic generalists throughout the year (Lord 1933, Maciolek and Needham 1952, Reimers 1957, Cunjak and Power 1987, Ozvarol et al. 2011a) and many studies emphasize the strong importance of drifting prey to the trout diet. For example, a widely cited paper emphasizes that brown trout of all ages fed primarily on the stream drift, taking less than 15% of their prey from the benthos (Bachman 1984). Winter observations were included in Bachman's research, but these data were not included in the analysis because very few trout were available for observation from

December through March. Bachman (1984) cites numerous other studies (Müller 1954, Nilsson 1957, Elliott 1967b, Waters 1969, Griffith 1974) that suggest that the majority of salmonid prey are drifting organisms. Other important works reference the overarching importance of stream drift to the trout diet (Elliott 1967a, 1970, 1973), and indicate that in swift flowing waters it is perhaps more efficient than benthic feeding (Frost and Brown 1967, Waters 1972). Although this certainly may hold true for many trout populations during warmer weather months, our research indicates that drifting prey may not be as important to brown trout as benthic prey during the winter months. This finding is supported by Johansen et al. (2010), who examined winter stomach contents of juvenile Atlantic salmon in the subarctic River Alta (Norway) and found that prey consumed by the salmon was more similar to the benthos than the drift. The lack of drift feeding was attributed to the reduced light levels caused by ice cover, which thereby impacts the efficiency of drift feeding for visual predators such as salmonids. Tippets and Moyle (1978) came to a similar conclusion for a population of rainbow trout that exhibited benthic feeding in a California river, partially attributing this feeding behavior to the reduced light availability caused by high turbidity.

River dwelling brown trout switched their feeding strategies during the winter months to feed almost exclusively at night; very limited feeding was documented during the day when drift densities were highest (Heggenes et al. 1993). Instead, trout activity during the day was restricted primarily to sheltering within the streambed or vegetation. Conversely, trout were active throughout the night, and tended to hold positions on or above the substrate (Heggenes et al. 1993). This type of seasonal behavioral adaptation is perhaps an important survival strategy to help minimize energy loss during a period of adverse or rapidly changing environmental conditions (Heggenes et al. 1993). Winter feeding observations of brook and brown trout by Cunjak and Power (1986) also indicate reduced levels of feeding during the day, and substantially more time spent holding positions beneath cover.

Combining the findings of Heggenes et al. (1993) and Cunjak and Power (1986) with those of studies that indicate salmonids switch their feeding behavior from drift feeding in the daylight to benthic feeding during periods of darkness (Tippets and Moyle 1978, Jørgensen and Jobling 1992, Fraser and Metcalfe 1997, Johansen et al. 2010), seem to provide a strong basis to help explain the winter-time benthic feeding patterns we describe. Additionally, drift densities are much reduced in the winter (e.g. Clifford 1972, Waters 1972, Brittain and Eikeland 1988). We found that it was not uncommon to find fewer than 100 organisms in a net that had been left out for 24-hours. Furthermore, the main components of the drift, numerically, were ‘small’ chironomid larvae, baetid mayflies, and collembolans, none of which were found to be selected for or abundant in the trout diet. Research by Bachman (1984) indicates that it should not be any more energetically expensive for brown trout to intercept prey in the benthos as compared to the drift, as both of these strategies take an equal amount of time. All of these factors combined seem to lead to the conclusion that benthic feeding is probably the most efficient and relied upon feeding strategy for brown trout during winter.

Although this study sheds light on the winter feeding activity of brown trout in Minnesota’s groundwater-dominated streams and the importance of certain relied-upon aquatic invertebrates, it must be remembered that this is just a snapshot from three streams during a single winter. We hope that this work provides a solid framework for future researchers to base continued winter investigations of the winter diet and behavior of trout and the importance and availability of their invertebrate prey resources.

It will be increasingly important to monitor and note any changes in feeding activity and prey abundances, particularly when considering current climate change predictions and the potential impacts these changes will have on trout streams. For example, Central North America is projected to exhibit a 4°C increase in mean annual temperature due to climate warming (Christensen et al. 2007). An increase in temperature of this extent would require a 30-40% increase in food consumption for trout to achieve current growth levels (Ries and Perry 1995). Furthermore, in a study of nine groundwater-dominated streams in southeast Minnesota, average winter (December – March) water temperatures ranged from 3.0 – 8.5°C (Anderson, unpublished data); the three streams we study here exhibited a similar temperature range. Under the projected temperature increase of 4°C, four of these nine streams would surpass an average winter temperature of 10°C, which would severely reduce and perhaps eliminate populations of winter-emerging chironomids, such as *Diamesa mentodotae* (Bouchard and Ferrington 2009), which we have shown to be an important and often selected-for constituent of the winter trout diet. Furthermore, if the winter season is shortened due to climate warming effects, this may have implications on the voltinism of winter-emerging species. Work by Bouchard and Ferrington (2009) indicate that *D. mendotae* likely produces two or more generations per winter season at temperatures below 10°C; thus, if the winter season is shortened due to climate warming effects, such that there are substantially more days above 10°C at either the beginning or end of winter, this may have implications on the voltinism of cold-reliant species like *D. mendotae.* At minimum, these effects could induce a greater potential for mortality in early or late-season generations, resulting in less chironomid biomass available for fish during early and late winter. Therefore, especially in streams that rely strongly on chironomids as a base component of their winter diet, such as Beaver Creek, and also in streams with fish that enhance their diet with these organisms (Whitewater and Cold Spring), reductions of these abundant winter-emerging insects may negatively impact the overall winter condition of brown trout, imparting potentially important implications for fisheries management.

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Tables

Table 2-1. Total number of brown trout sampled in each stream with breakdown of small and large fish, mean total length (TL) of all fish sampled by stream, and mean relative weight (Wr) for fish over 140mm. Partition cutoffs for large and small fish in Beaver, Whitewater, and Coldspring were 151mm, 180mm, and 188mm, respectively.

		Stream	
Parameter	**Beaver**	**Whitewater**	**Coldspring**
Total # of fish	**207**	**202**	**195**
# of small fish	117	115	148
# of large fish	90	87	47
TL+/-SE	**159**	**177.3**	**164.8**
Range	79-295	90-279	83-295
SE	4.0	4.5	3.7
W_r +- SE	**85.5**	**88.5**	**94.3**
# fish >140mm	94	114	122
Range	66.6-100.9	69.0-107.9	72.3-111.8
SE	0.7	0.7	0.6

Table 2-2a. Percent numerical composition of prey items in the stream environment (drift + benthic) and the diets of small and large brown trout in Cold Spring Brook, with Manly's alpha electivity index scores.

Cold Spring Brook - Percent by Number

Taxon	% Composition in Environment (Drift +Benthic)	% Composition in Diet (Small Trout)	% Composition in Diet (Large Trout)	Manly's alpha* (small trout)	Manly's alpha* (large trout)
Limnephilidae	1.2	6.3	22.6	**0.51**	**0.44**
Physella	1.1	0.9	23.3	0.08	**0.52**
Large chironomid larvae	6.7	11.3	0.8	**0.17**	0.00
Gammarus	62.8	76.5	51.2	0.12	0.02
Other Prey	3.1	2.5	1.9	0.08	0.01
Baetis	6.9	1.4	0.2	0.02	0.00
Small chironomid larvae	18.2	1.1	0.1	0.01	0.00

*Scores > 1/m (0.14) indicate preference. Values indicating preference are bolded.

Table 2-2b. Percent numerical composition of prey items in the stream environment (drift + benthic) and the diets of small and large brown trout in the Middle Branch of the Whitewater River, with Manly's alpha electivity index scores.

Middle Branch of the Whitewater River - Percent by Number

Taxon	% Composition in Environment (Drift +Benthic)	% Composition in Diet (Small Trout)	% Composition in Diet (Large Trout)	Manly's alpha* (small trout)	Manly's alpha* (large trout)
Brachycentrus	2.7	8.5	46.8	**0.15**	**0.63**
Chironomid pupa	1.0	8.8	3.3	**0.42**	**0.12**
Gammarus	4.6	21.0	15.6	**0.22**	**0.12**
Large chironomid larvae	18.4	49.4	17.0	**0.13**	0.03
Physella	5.1	2.9	5.1	0.03	0.04
Simuliium	4.2	1.6	1.9	0.02	0.02
Baetis	10.8	2.7	2.7	0.01	0.01
Chironomid adult	1.9	0.0	1.3	0.00	0.02
Other Prey	16.1	2.3	5.9	0.01	0.01
Ephemerella	3.8	0.3	0.0	0.00	0.00
Small chironomid larvae	28.4	2.3	0.2	0.00	0.00
Acari	3.0	0.1	0.2	0.00	0.00

*Scores > 1/m (0.08) indicate preference. Values indicating preference are bolded.

Table 2-2c. Percent numerical composition of prey items in the stream environment (drift + benthic) and the diets of small and large brown trout in Beaver Creek, with Manly's alpha electivity index scores.

Beaver Creek- Percent by Number

Taxon	% Composition in Environment (Drift +Benthic)	% Composition in Diet (Small Trout)	% Composition in Diet (Large Trout)	Manly's alpha* (small trout)	Manly's alpha* (large trout)
Chironomid pupa	0.1	2.2	4.8	**0.21**	**0.28**
Chironomid adult	0.2	1.7	8.5	**0.11**	**0.34**
Lepidostoma	0.8	15.3	9.4	**0.22**	**0.08**
Micrasema	0.2	2.4	1.5	**0.15**	**0.06**
Allocapnia	0.4	3.4	2.9	**0.11**	**0.06**
Physella	0.4	2.4	2.8	**0.06**	0.05
Glossosoma	0.7	1.8	2.5	0.03	0.03
Other Prey	2	4.9	10.1	0.03	0.03
Gammarus	2	4	5.1	0.02	0.02
Large chironomid larvae	30.3	45.8	33.9	0.02	0.01
Brachycentrus	1.9	0.8	8.4	0.00	0.03
Ephemerella	5.3	5.1	2.9	0.01	0.00
Isoperla	1.7	1.8	1.2	0.01	0.00
Simuliium	4	1.9	2.2	0.01	0.00
Tipulidae	1.9	0.9	0.8	0.01	0.00
Baetis	4.7	1.1	1.6	0.00	0.00
Optioservus larva	3.6	0	0.1	0.00	0.00
Small chironomid larvae	24	4	1	0.00	0.00
Podura	12.8	0	0.1	0.00	0.00
Acari	3.3	0.5	0.3	0.00	0.00

*Scores > 1/m (0.05) indicate preference. Values indicating preference are bolded.

Table 2-3. Niche overlap and niche breadth. Comparison of niche overlap (P_{jk}) and niche breadth (B_A) among small and large brown trout and across streams.

	Niche Measure		
Stream	P_{jk}	$B_{A(\text{small})}$	$B_{A(\text{large})}$
Cold Spring	57.3%	0.08	0.24
Whitewater	49.2%	0.16	0.11
Beaver	74.9%	0.28	0.24

Table 2-4. Comparison of niche overlap (P_{jk}) between percentage prey weight consumed by small or large trout and that found in the drift or benthos. Overlap values >0.60 are biologically significant.

	Niche Overlap Comparison				
Stream	$P_{small\ trout\ vs\ drift}$	$P_{small\ trout\ vs\ benthos}$	$P_{large\ trout\ vs\ drift}$	$P_{large\ trout\ vs\ benthos}$	$P_{driftt\ vs\ benthos}$
Cold Spring	54.3%	86.3%	88.7%	44.5%	43.2%
Whitewater	30.4%	69.9%	41.5%	70.9%	47.1%
Beaver	27.2%	79.8%	21.3%	82.7%	26.7%

Figures

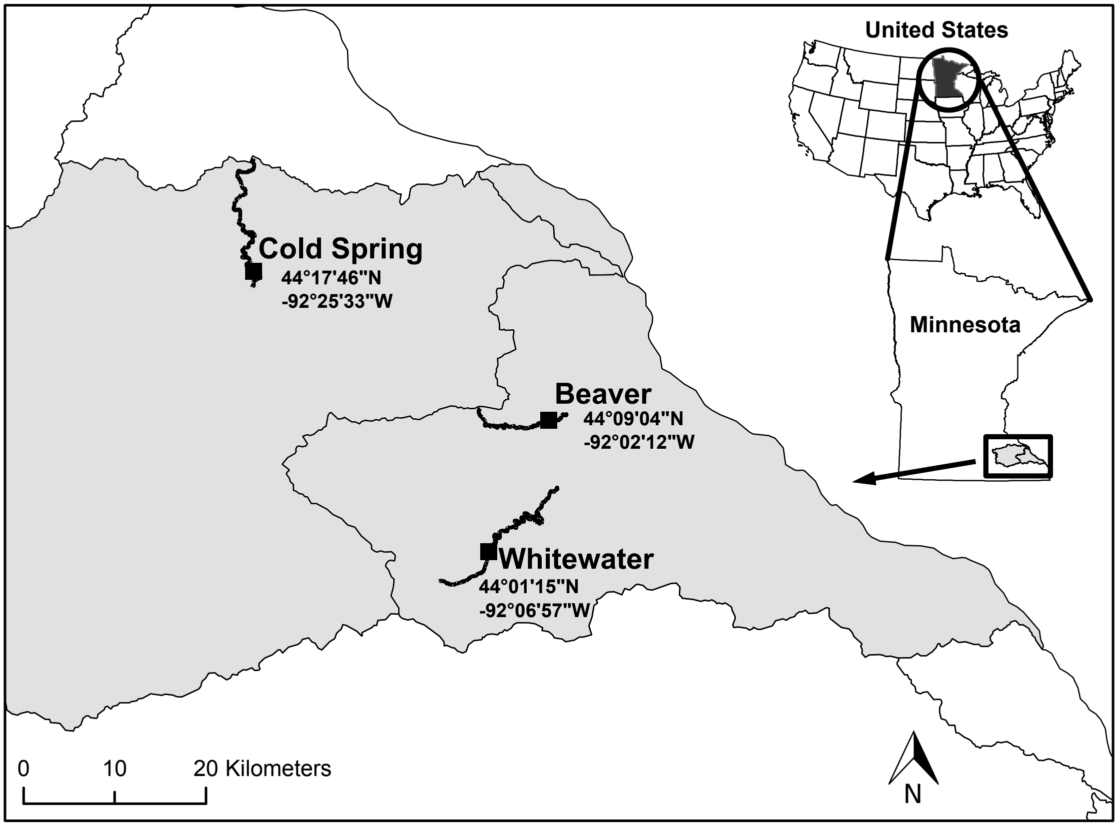

Figure 2-1. Map of trout streams sampled in this study. The gray shaded regions in both the primary map and Minnesota state inset represent the watersheds that contain each stream.

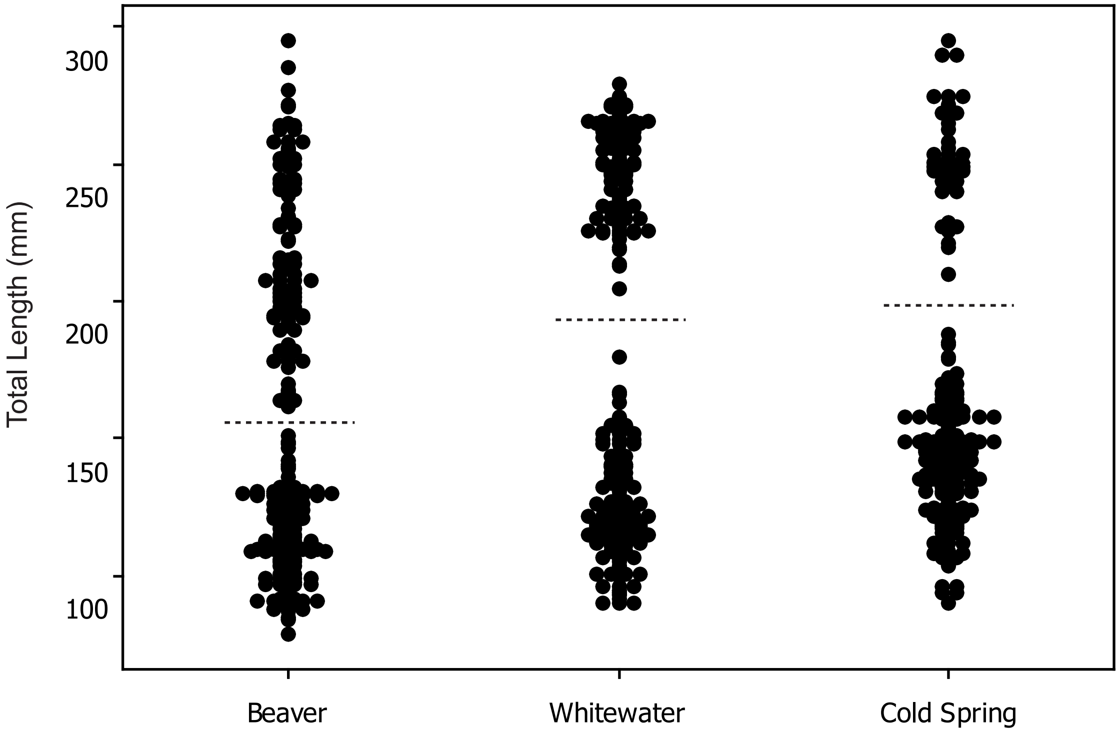

Figure 2-2. Total length for all fish sampled in Beaver, Whitewater, and Cold Spring. Horizontal dashed lines indicate natural cutoff values for small and large fish in each stream.

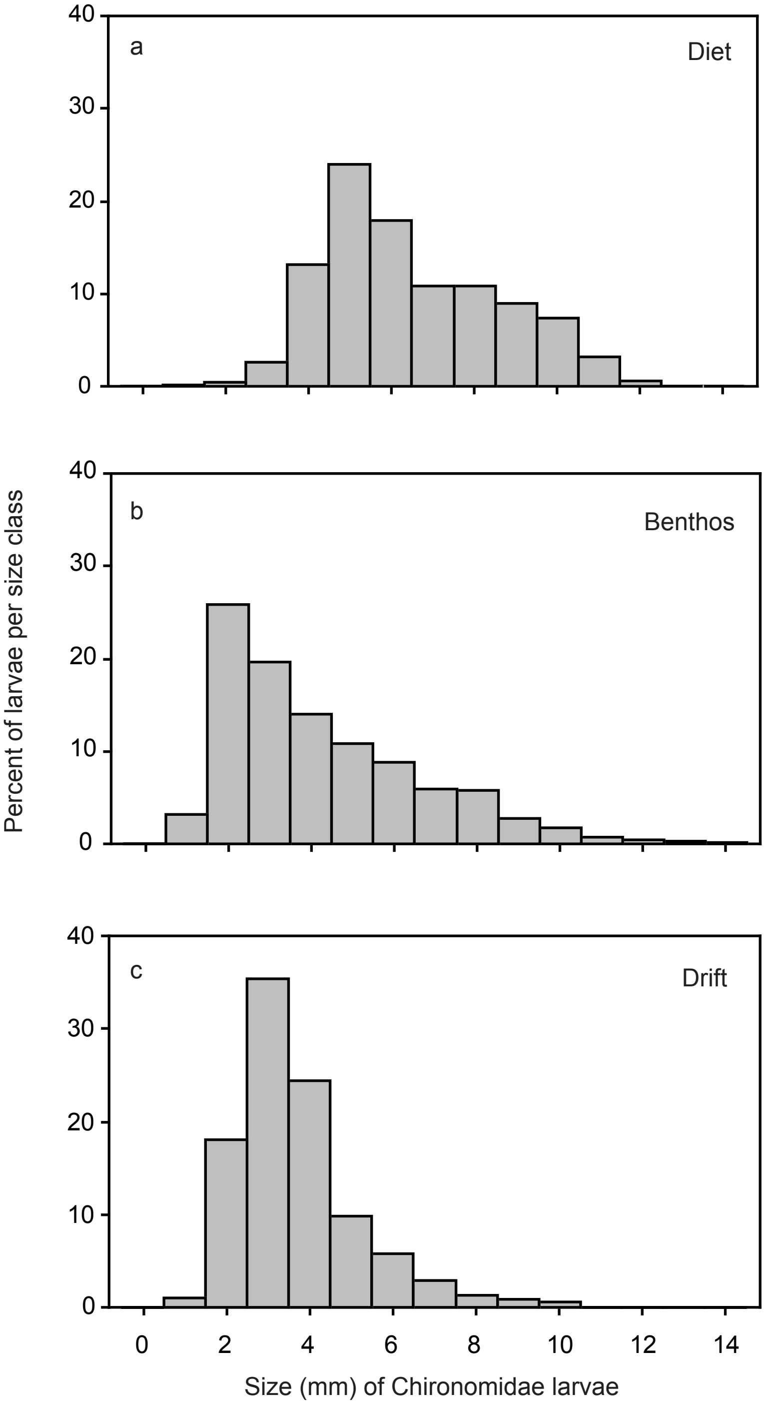

Figure 2-3. Histograms depicting percent of Chironomidae larvae in varying size (mm) categories in the: (a.) diet of brown trout; (b.) stream benthos; and (c.) stream drift.

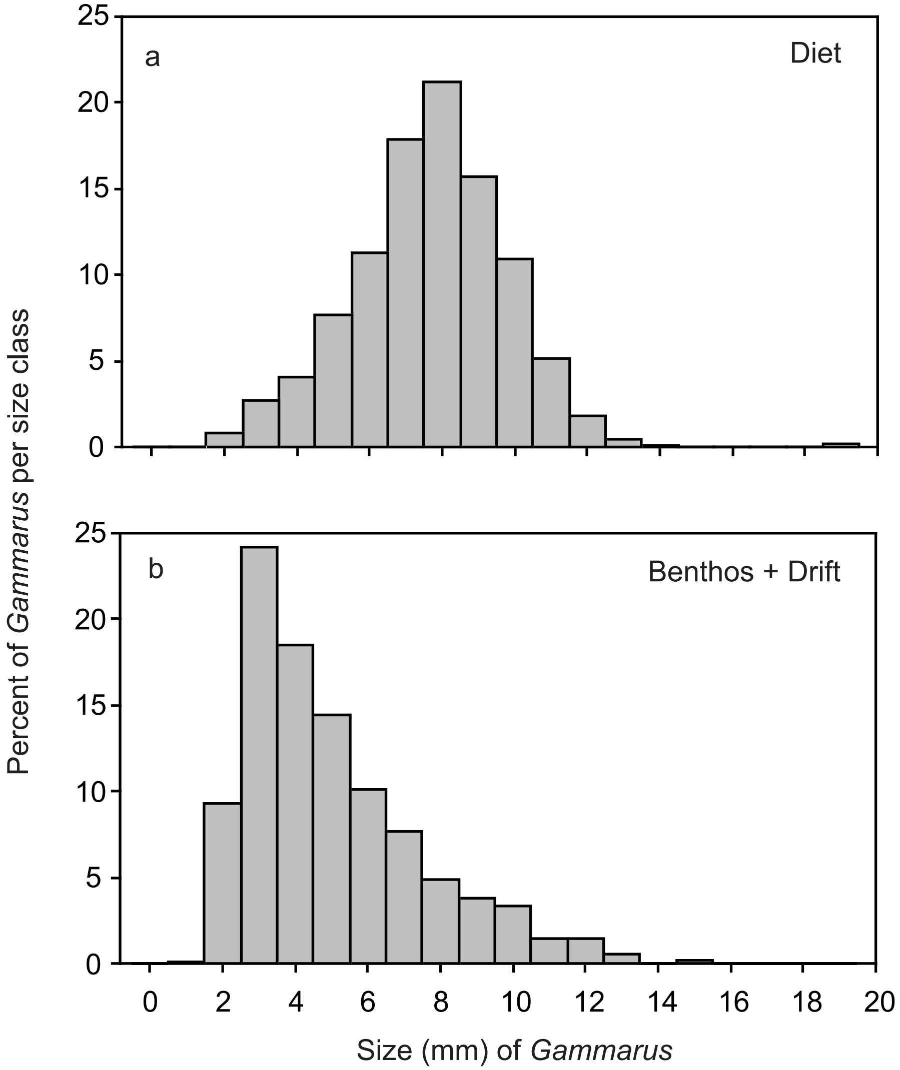

Figure 2-4. Histograms depicting percent of Gammarus in varying size (mm) categories in the: (a.) diet of brown trout; (b.) stream benthos and drift. Considering that statistical analysis indicated no significant difference between Gammarus size in the drift and benthos, these data were combined for presentation purposes.

Chapter 3

Strength of Combined Forces:
Molecular and Morphological Methods Reveal Cryptic Diversity and Three New Species of Nearctic *Micropsectra* (Diptera: Chironomidae)

Micropsectra is a particularly species-rich genus within the Chironomidae, with representatives found in a variety of freshwater habitats throughout the Nearctic and Palaearctic regions. Taxonomic research on Nearctic *Micropsectra*, however, is less advanced than that of Palearctic species, often making species-level identification difficult. Furthermore, while some *Micropsectra* species are thought to be Holarctic in distribution, close examination of Palearctic and Nearctic populations can reveal genetic and morphological differences large enough to be regarded as diagnostic at the species-level. Thus, the intent of this study is to use genetic and morphological characters to help resolve these taxonomic issues and provide a framework for better understanding of North American *Micropsectra.* Larval specimens of *Micropsectra* were collected from five streams throughout Minnesota, USA during late winter/early spring of 2010 and reared to adulthood. A subset of 48 specimens, with several representatives of each morphological species, was selected for DNA extraction, PCR amplification, and sequencing of mitochondrial cytochrome c oxidase subunit I (COI) and nuclear carbamylphosphate synthetase (CAD) genes; sequences were then compared to previously published *Micropsectra* sequences. Our results indicate that we have found three previously undescribed species: *Micropsectra neoappendica*, n. sp., *Micropsectra penicillata*, n. sp., and *Micropsectra subletteorum*, n. sp., and one additional species new to the north-central United States, *Micropsectra xantha* (Roback, 1955). *Micropsectra neoappendica* n. sp. and *Micropsectra subletteorum* n. sp. initially appeared to be morphologically identical to species known from the Palearctic, however molecular data indicated that they are genetically distinct. Reexamination of adult and pupal morphology revealed slight but consistent diagnostic differences. These results emphasize the importance of using molecular tools in conjunction with traditional morphological techniques when studying Chironomidae diversity, especially when relying on diagnoses from other regions.

Introduction

Chironomids are one of the most diverse and widespread families of aquatic insects, with a worldwide total of over 4,000 described species (Ferrington 2008) and species estimates ranging up to 20,000 (Ferrington et al. 2008). Of those species currently described, over 1,200 are known to occur in the Nearctic region, however recent estimates indicate that as many as 2,000 species may occur, many of which are new to science (Ferrington et al. 2008). Chironomids are effective biological indicators for aquatic ecosystems (Wiederholm 1984), thus accurate assessments of chironomid diversity and richness can be particularly important for biological monitoring studies (King and Richardson 2002, Hayford and Ferrington 2005, Calle-Martinez and Casas 2006). Considering the great species richness of the family, there is considerable variation among species in their response to environmental change, often even within a single genus. Thus, it is critical to resolve taxa to the species level to obtain the most biologically informative data (Bailey et al. 2001, Lenat and Resh 2001, King and Richardson 2002). Unfortunately, this can prove quite difficult due to extreme similarity among many chironomid species and lack of appropriate keys, particularly in the Nearctic region.

Chironomidae taxonomy and systematics for many genera is arguably more advanced in the Palearctic region as compared to the Nearctic. Considering this, keys and species descriptions used for many Nearctic Chironomidae are often drawn from Palearctic literature. As is the case for many aquatic invertebrates, it is not uncommon to use an identification key and end with a name that fits the specimen in question; however when making comparisons with species descriptions, slight discrepancies often occur, which may or may not indicate that the specimens are heterospecific. While many trans-Atlantic species do bear a strong resemblance to one another, it is often unknown whether Nearctic and Palearctic counter-parts are truly conspecific (Epler 2001).

The fact that many species descriptions are based primarily on morphology of the adult male, often without incorporating other lifestages, adds to this problem, especially considering that closely related species can exhibit strong similarities in this life stage. For example, Townes (1945) originally synonymized the Nearctic *Polypedilum (Polypedilum) flavum* (Johannsen, 1905) with the Palearctic species *Polypedilum (Polypedilum) convictum* (Walker, 1856) because of morphological similarity in the adult males. It was later discovered that the immature stages were quite different (Epler 2001). This emphasizes the importance of including multiple lifestages in a description whenever possible. In the case of truly cryptic species, however, all lifestages may be quite similar.

Molecular techniques such as DNA barcoding have become much more prominent in recent years and may relieve the problems outlined above by providing an additional mechanism for differentiating species; these techniques have proven especially useful for separating morphologically cryptic species (Sinclair and Gresens 2008, Pauls et al. 2010, Carew et al. 2011, Takano et al. 2011) and associating multiple lifestages (Carew et al. 2005, Willassen 2005, Ekrem et al. 2007, Zhou et al. 2007, Ekrem et al. 2010a, Stur and Ekrem 2011a). As indicated by Sinclair and Gresens (2008), DNA barcoding is an invaluable tool that aids in determining species identity and evaluating whether morphologically similar trans-Atlantic species are truly conspecific.

One of the most species-rich genera within Chironomidae is *Micropsectra* Kieffer, 1909, with at least 140 named species known from the Holarctic region and a few species known from areas outside the Holarctic (see Stur and Ekrem 2006). While some *Micropsectra* are found in oligotrophic or mesotrophic lakes, most species within the genus occur in lentic areas of springs and streams (Säwedal 1982); for example, a study by Stur and Wiedenbrug (2006) found high abundance and diversity of *Micropsectra* species in two groundwater spring communities within Berchtesgaden National Park, Germany. Notably, the new species described below come from similar spring-fed stream habitats. Considering these specific habitat preferences, the genus can be quite important in biological monitoring studies (Stur and Ekrem 2006).

Presently, species within *Micropsectra* are divided into four species groups, including *atrofasciata-*, *attenuata-*, *notescens-*, and *recurvata-* groups, however these groups are based primarily on European fauna and have to be reconsidered as more species from other parts of the world are described. Additionally, all recent revisions of the genus *Micropsectra* are primarily confined to major species groups recognized from the Palearctic (Reiss 1969b, Säwedal 1976, 1981, 1982, Stur and Ekrem 2006, 2008, Ekrem et al. 2010b). While some *Micropsectra* species are thought to be Holarctic in distribution, close examination of some Palearctic and Nearctic populations can reveal genetic and morphological differences large enough to be regarded as diagnostic at the species level.

Here, we use a combination of morphological and molecular approaches to help resolve these taxonomic issues and to provide a framework for a better understanding of North American *Micropsectra* and, subsequently, the genus as a whole. We utilize sequences from mitochondrial cytochrome oxidase I (COI) and nuclear carbamylphosphate synthetase (CAD) genes to determine whether morphologically similar specimens from Nearctic and Palearctic populations are, indeed, conspecifics with a transatlantic distribution or whether there is sufficient genetic variation to classify these specimens as distinct species.

Materials and Methods

Sampling and Data Collection

Larval *Micropsectra* specimens were obtained from the benthos of five streams in Minnesota, USA during late winter/early spring of 2010. All specimens were held in constant-temperature incubators at 5°C to approximate stream temperatures and allowed to develop through to adulthood in mass rearings, resulting in a cumulative total of 99 adult *Micropsectra* with associated pupal exuviae. Rearing chambers were typically checked twice per day to ensure that emerging adults were associated with the appropriate exuviae. Upon emergence, adults and associated exuviae were stored in individual vials and preserved in 95% ethanol; unfortunately in nearly all cases, larval exuviae could not be retrieved. The antennae, head, wings, legs and hypopygium were dissected. The head and hypopygium were cleared in 10% KOH and slide mounted in Euparal®, along with the pupal exuviae, legs, wings, and antennae, for species identification. The thorax and abdomen were slide mounted following DNA extraction, (described below). On the few occasions where more than one fly of the same sex emerged between observations, the adults and exuviae were stored in the same vial until dissection; adults were then mounted on individual slides as described above, however both exuviae were mounted on a single, separate slide. All specimens were identified and separated into four morphotypes. A subset of 44 specimens, chosen as representatives of each morphotype, was selected for molecular analyses (Table 3-1).

DNA Extraction, Amplification and Sequencing

A GeneMole® instrument (Mole Genetics AS, Lysaker, Norway) was used to extract DNA from most specimens, following standard protocol for the MoleStrips DNA Tissue kit; DNA was extracted from remaining specimens using protocol for the Qiagen DNeasy tissue extraction kit, with the exception of using less elution buffer (150µm) than recommended due to small specimen sizes. PCR amplification and sequencing was carried out on partial COI and CAD genes using the primer pairs and PCR programs described in Table 1 of Ekrem et al. (2010b). We used HotStar Taq from Qiagen when amplifying COI and TaKaRa HS Ex*Taq* for amplification of CAD. PCR products were purified using ExoSAP-IT® and sent to an external sequencing service (Eurofins MWG Operon, Ebersberg, Germany). All gene products were sequenced bidirectionally, and forward and reverse sequences were aligned and manually edited using Sequencher 4.8 (Gene Codes Corp., Ann Arbor, Michigan). Conflicting or ambiguous base pairs were initially given the appropriate International Union of Biochemistry ambiguity symbol; initial ambiguities were checked after alignment and corrected, if possible, with reference to trace-files. Consensus sequences were assembled and aligned in BioEdit 7.0.5.3 (Hall 1999) by their amino acid sequences. Introns in CAD sequences were recognized by the GT-AG rule (Rogers and Wall 1980) and eliminated from the alignment considering that these areas exhibit extreme variation among species. All CAD sequences had intron placement at position 725 and there was no indication of paralogous gene copies.

Phylogenetic Analyses

Phylogenetic analysis of COI data was inferred with Neighbor-Joining methods in *MEGA* version 5 (Tamura et al. 2011; available from: www.megasoftware.net), using the Kimura 2-parameter (K2P) substitution model to infer evolutionary distances. After initial analysis of COI data, we further explored phylogenetic relationships using CAD because of its greater ability to reconstruct lower-level phylogenies in Diptera (Winterton et al. 2007, Winkler et al. 2009, Ekrem et al. 2010b). The best-fit model of nucleotide substitution was determined using a maximum likelihood criterion in *MEGA5*; based on the Bayesian (BIC) and Akaike Information Criterion (AIC), we found the Tamura 3-parameter model with a discrete Gamma distribution and invariant sites (T92+G+I) to be the most appropriate model for our data. Multiple tree-building methods were used to determine whether phylogenetic results were robust to different analytical assumptions. Maximum likelihood (ML) analysis with 500 bootstrap replicates was performed with *MEGA5* and Bayesian analysis (BA) was performed using Mr.Bayes 3.1.2 (Huelsenbeck and Ronquist 2001). For BA, we specified flat prior probabilities and ran two parallel analyses, each with four chains, for 3 million generations, sampling every 1000th generation and using a 10% burn-in. We examined the trace files with Tracer 1.5 (Rambaut and Drummond 2008; http://tree.bio.ed.ac.uk/software/tracer/) to verify that MCMC convergence had occurred. Trees created by BA were imported and modified for presentation in *MEGA5*.

The COI dataset consisted of 275 individuals from 39 species. Of these, 4 species (43 individuals) were *Micropsectra* collected in Minnesota (Table 3-1). These taxa included *Micropsectra neoappendica* n. sp., *Micropsectra penicillata* n. sp., *Micropsectra subletteorum* n. sp., and *Micropsectra xantha* (Roback, 1955). The remainder of sequences were acquired from GenBank, and included two different taxa of *Tanytarsus* which served as an outgroup. The CAD dataset consisted of 82 individuals from 38 species; twelve individuals were from the above named *Micropsectra* taxa (Table 3-1); the remaining sequences were acquired from GenBank, including 2 *Paratanytarsus* and 2 *Tanytarsus* species that served as outgroups. GenBank accessions for specimens collected from Minnesota for this study are given in Table 3-1.Voucher and sequence information of all specimens collected are also accessible in BOLD project "Nearctic *Micropsectra* (NAMIC)" (http://boldsystems.org). Remaining DNA extracts are stored at the Museum of Natural History and Archaeology, Norwegian University of Science and Technology (VM) in Trondheim, Norway.

Species Descriptions and Specimen Deposition

The morphological terminology and abbreviations used here follow Sæther (1980). 'Taeniae,' introduced by Langton (1994), is used to describe the flattened setae found on the pupal exuviae and the term 'setiger' (Spies 1998) describes the setae-bearing area of the superior volsella on the male hypopygium. The following abbreviations are also used: P♂ = associated pupal exuviae and adult male; P♀ = associated pupal exuviae and adult female; L_{hc}P♂ = associated larval head capsule, pupal exuviae, and adult male. Measurement techniques follow those outlined by Soponis (1977), with the exception of the gonocoxite length, which was measured along the limb's longest access, as described by Stur and Spies (2011). Lengths of the genital volsellae were measured along their median margin, and the anal point was measured from the anterior ends of the anal crests to the tip of the anal point. Mensural data are presented as ranges, followed by the mean and number of observed specimens.

Holotypes and most paratypes are deposited in the University of Minnesota Insect Collection in St. Paul, Minnesota, USA (UMSP). Additional paratypes are deposited at VM. We also examined type material and other reference material of closely related taxa from the following collections (abbreviations correspond to those used in text):

BMNH	The Natural History Museum, London, England
CNC	Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Ontario, Canada
PHL	Private collection of Peter H. Langton, Londonderry, Northern Ireland
UMSP	University of Minnesota Insect Collection, St. Paul, Minnesota, USA
VM	Museum of Natural History and Archaeology, Norwegian University of Science and Technology in Trondheim, Norway
ZMBN	Natural History Collections, Bergen Museum, University of Bergen, Norway
ZSM	Zoologische Staatssammlung München, Munich, Germany

Results

All Nearctic specimens collected for this study formed distinct monophyletic species groups for both initial COI and subsequent CAD analyses, clearly separating the four Nearctic *Micropsectra* from morphologically similar Palearctic counterparts (Fig. 3-1). This is particularly notable for two of the Nearctic species, *M. neoappendica* n. sp. and *M. subletteorum* n. sp., which are very similar morphologically to species known from the Palearctic. *M. subletteorum* n. sp. is closely allied morphologically with many species within the *notescens*- species group. Males of *M. neoappendica* n. sp. fit the species description of the Palearctic *M. appendica* Stur and Ekrem, 2006 almost perfectly. Only after divergence patterns were revealed with molecular analyses of COI and CAD markers, did subsequent examination reveal subtle but corroborating morphological characters that can be used to differentiate *M. neoappendica* and *M. appendica* (see discussion in species description section below).

CAD phylogenies from ML and BA yielded similar species group patterns, with no notable differences besides the support values for certain clades (Fig. 3-1). One such difference is found in the support for the *M. kurobemaculata* - *M.lindrothi* clade. Bayesian analysis indicated strong support for this clade, placing these two species together (posterior probability = 99%) and as sister to the remaining species within the *notescens*- group (posterior probability = 100%), whereas ML analysis indicated much weaker support for these placements (bootstrap support values < 50%). We choose to leave these two species as members of the *notescens*- group at this point, but recognize that as more species are included in analyses, there may be a more appropriate placement for *M. kurobemaculata* (Sasa and Okazawa, 1992) and *M. lindrothi* Goetghebuer, 1931.

Analyses yielded strongly supported *atrofasciata*- and *notescens*- species groups and a paraphyletic *recurvata*- group, corresponding well with conclusions of Ekrem et al. (2010b) (Fig. 3-1). Note that *M. lacustris* Säwedal, 1975, here designated as a member of the newly re-erected *lacustris*- group was previously considered a member of the *recurvata*- group. We could not confirm the finding of a paraphyletic *attenuata*- group as found in Ekrem et al. (2010b) because CAD-sequences are not currently available for *M. pharetrophora* Fittkau and Reiss, 1999, the specimen accounting for paraphyly in this group. Results for the species described in this study show *M. neoappendica* n. sp. as sister to *M. appendica* in the *atrofasciata*- group, *M. penicillata* n. sp. as sister to *M. lacustris* in the *lacustris*- group, *M. subletteorum* n. sp. as sister to *M. insignilobus* Kieffer, 1924 in the *notescens*- group, and *M. xantha* as sister to *M. recurvata* Goetghebuer, 1928 in the *recurvata*- group (Fig. 3-1).

Description of Species

Micropsectra neoappendica n. sp.

(Figs. 3-2, 3-3a)

Type material.

Holotype. −P♂. **USA, Minnesota**: Cook County, Fiddle Creek, Forest Rd 152/Lima Grade, stream access c. 100m NW of South Brule River bridge near edge of road, 47°56'35"N; 90°26'17"W, 505m, larva collected 11.iv.2010, A.M. Anderson (UMSP, slide mounted specimen; VM, DNA extract; voucher ID: ALY29; GenBank accessions: JQ667045 (COI); JQ672506 (CAD)).

Paratypes.− 8 P♂, 11 P♀. All data as for holotype (7 P♂, 10 P♀ UMSP, 1 P♂, 1 P♀ VM), voucher ID and GenBank accession codes of sequenced specimens in Table 1.

Diagnostic characters. –The following combination of characters separates this species from others currently in the *atrofasciata*-group: Adult male. AR c. 1.27, LR_1 c. 1.52. Anal point long with high, slightly curved crests, with long, rounded point. Elevated hump anterior to base of anal point; hump typically with small rounded point at tip. Superior volsella roundish. Digitus short, thin, and triangular. Median volsella club-shaped with numerous spoon-shaped lamellae on distal 1/3; length of median volsellae c. 123µm. Pupa. Total length c. 4.6mm. Thoracic horn relatively short, c. 306µm with chaetae on distal 2/3. Distance between anterior and posterior dorsocentral setae pairs approximately equal. Nose of wing sheath moderate to strong. Patches of TIV consisting of long longitudinal spines extending nearly to the posterior end of tergite. Patches of TV consisting of spinules and points in a similar pattern to TIV. Anal fringe with c. 30 setae.

Etymology. – The species epithet '*neoappendica'* refers to the striking similarity that this Nearctic species has with its putative sister species in the Palearctic, *Micropsectra appendica.*

Description

Adult male. Measurements and ratios in Table 3-2.

Coloration. Thorax with light green to light brown ground color, dark brown scutal stripes, preepisternum, postnotum, and median anepisternum; scutellum and halteres greenish to light brown; abdomen green to light brown; Head with dark brown antennal pedicel, black eyes, and light brown palps; legs light brown

Head. Antenna with 13 flagellomeres. Frontal tubercles present (8-11, 9µm), temporal setae in 1 row; palpomere 3 with 2 sensilla clavata in subapical pit.

Wing. Subcosta and media bare, brachiolum with 2-4 setae, squama bare.

Legs. Pulvilli present, nearly half as long as claws. Fore tibia with spur (17-25, 22µm); mid and hind tibial combs c. 15µm long; mid ta_1 with 4-5 sensilla chaetica.

Hypopygium (Fig. 3-2). Anal tergite with separate, posteriorly directed anal tergite bands; 4-5, 4.3 median tergite setae situated on an elevated hump anterior to anal point base; 9-11, 9.5 ventral apical setae. Anal point long with high, slightly curved anal crests, apex long and rounded; large knob present between crests; microtrichia-free area at base, except lateral of anal point. Superior volsella round with 6-8, 7 dorsal setae and 3-4 median setae on setiger, 1 strong setae on stem; microtrichia fields located dorsally on stem and ventrally on setiger. Digitus small, thin, triangular, not extending past margin of superior volsella. Median volsella club-shaped, comparatively long and thick, with setiform and spoon-shaped lamellae; spoon-shaped lamellae on distal 1/3; stem nearly reaching apex of inferior volsella. Inferior volsella without dorsoapical or dorsomedian swellings, numerous setae at tip. Inner margin of gonocoxite with 3-4 strong setae.

Pupa. Measurements given in Table 3-3.

Coloration: Pupal exuviae brownish with dark brown apodemes; cephalothorax, TVIII and anal lobe darker brown.

Cephalothorax. Cephalic tubercles present, small, conical; pedicel sheath tubercle weakly developed. Thoracic horn relatively short with numerous long chaetae on distal 2/3; precorneals arranged in triangular pattern, the two anteriormost setae situated closer to each other than to the third; one median and 2 lateral antepronotals (1 seta, 1 sensillum basiconicum); 2 pairs of dorsocentrals, anterior and posterior pair approximately equidistant from each other. Course granulation dorsally on anterior half of thorax. Prealar tubercle absent; nose of wing sheath moderate to strong.

Abdomen (Fig. 3-3a). TII almost covered by shagreen except for two mediolateral and one posteromedian oval patches; pedes spurii B on segment II obvious; hook row width approximately half as long as segment width. Spines of TIII in large, laterally curved patches in posterior half of tergite, shagreen extensively distributed lateral and anterior to spine patches as well as in between. Patches on TIV consisting of spinules in anterior, oval patches with spines heavily distributed in longitudinal, lateral extensions; shagreen present lateral to the anterior patches. Patches on TV similar in shape to those in TIV, however distinctly shorter and consisting only of spinules and points. Patches on TVI similar in shape to those of TIV and TV, however somewhat shorter than patches of TV and consisting only of shagreen. Tergite TVII bare or with weak anterolateral shagreen patches. TVIII with weak anterior shagreen patches. Segment I with 3 D, 1 V and 2 L setae; segment II with 3 D, 4 V, and 3 L; segment III with 5 D, 4 V, and 3 L; segment IV with 5 D, 4 V, 1 L, 2 lateral taeniae; segment V with 5 D, 4 V, 3 lateral taeniae; segment VI with 5 D, 4 V, 4 lateral taeniae; segment VII with 5 D, 4 V, 4 lateral taeniae; segment VIII with 1 dorsal taenia, 1 ventral taenia, 5 lateral taeniae; anal tergite with 1 dorsal taenia. Two pairs of small sensorial setae medially on TII-VII; 1 pair of O-setae present anteriorly on segments II-VII. Anal lobe with 27-33, 30 long taeniae in 1 row. Posterolateral comb of segment VIII narrow with 3-5 apical teeth, median tooth often longer than the rest.

Taxonomic Remarks

Adult males of *Micropsectra neoappendica* are quite similar morphologically to *M. appendica*, thus fit well in the *atrofasciata*-group. Two characters that help to differentiate these two species include the comparatively shorter median volsella of *M. neoappendica* (120-125, 122.5µm as compared to 130-144, 137µm reported in Stur and Ekrem (2006)) and the shape of the elevated hump anterior to the anal point base (Fig. 3-2). In the specimens we have examined, this hump appears less curved in *M.*

neoappendica and typically exhibits a small rounded point or hump at the tip of the elevated region. Setae extending from the gonocoxite and the seta on the stem of the superior volsella appear to be somewhat stronger than those on *M. appendica* (Fig. 3-2). The two species are best differentiated in the pupal stage; pupal exuviae of *M. neoappendica* can be separated by the placement of the dorsocentrals, where the distance between anterior and posterior pairs is approximately equal, as opposed to having a much greater separation of the anterior pair (approximately 4x greater) than the posterior pair as seen in *M. appendica*. Additionally, the thickness of Dc_3 and Dc_4 is similar for *M. neoappendica*, whereas one thick and one thin setae is noted for *M. appendica.* The nose of the wing sheath of *M. neoappendica* is typically quite strong, whereas it is generally weak in *M. appendica*. Spine patches of TIV and TV further differentiate the species, with long longitudinal spines on TIV of *M. neoappendica* extending nearly to the posterior end of the tergite and spinule/point patches of TV distinctly shorter than spine patch of TIV, with patch tapering off about two-thirds the length of the tergite (Fig. 3-3a). Conversely, longitudinal spines of TIV of *M. appendica* typically taper off approximately at the mid-point of the tergite, with much shorter spines and spinules extending to the posterior end of the tergite; the spine/spinule patch of TV is subequal in size and pattern to TIV patches (Fig. 3-3b). Finally, pupae of *M. neoappendica* seem to exhibit fewer anal lobe taeniae than *M. appendica* (27-33, 30 as compared to 35-42, 38 as described in Stur and Ekrem (2006)). Males of *M. neoappendica* are also quite similar to *M. tusimalemea* Sasa and Suzuki, 1999, but can be separated by the differentiating characters for *M. appendica* described in Stur and Ekrem (2006).

Ecology and Distribution. Micropsectra neoappendica is known from a shallow stretch of a small coldwater trout stream in northeastern Minnesota, USA.

Micropsectra penicillata, n. sp.

(Fig. 3-4)

Type material.

Holotype. −P♂, **USA, Minnesota:** Cook County, South Brule River, Forest Rd 152/Lima Grade, 15m upstream of bridge, 47°56'35”N; 90°26'17"W, 499m, larvae collected 11.iv.2010, A.M. Anderson, (UMSP, slide mounted specimen; VM, DNA extract; voucher ID: ALY71; GenBank accessions: JQ667047 (COI); JQ672508 (CAD)).

Paratypes. −11 P♂, 7P♀. All data as for holotype, voucher ID and GenBank accession codes for sequenced specimens in Table 1; 1♂, **USA, Colorado:** Lake Co., East Fork Arkansas River, 3041m, 9-30.viii.1984, J.S. Herrmann, originally determined by J. Sublette as *M. recurvata,* (UMSP); 3♂, **USA, Michigan:** Alcona Co., Mikado, Huron National Forest, White Cender Swamp, Pine River Campground, 8.v.1983, P. Hudson, (UMSP); 1P♂, **USA, South Carolina:** Oconee Co., Horse Pasture River, 17.iii.1979, P.L. Hudson, (UMSP); 1♂, **USA, South Carolina:** Oconee Co., small stream near Devils Fork Landing, Jacassee Reservoir, 19.ii.1982, P.L. Hudson, (UMSP); 1P♂, **USA, Tennessee:** Sevier Co., Gatlinburg, Leconte Creek, 4.v.1980, P.L. Hudson (UMSP); 1♂, **Canada, Alberta:** Hotchkiss, F.L. Kessel-light,16.viii.1961; 2♂, **Canada, Manitoba**: Churchill, Ramsey Creek, 58°43'50”N; 93°46'48”W, 13m; adult collected with malaise trap over brook, 23-24.viii.2006,T. Ekrem & E. Stur, (VM, voucher IDs CHIR-CH9, CHIR-CH119, GenBank Accessions in Table 1); 4♂, **Canada, Ontario:** Caledon Hills, Woodrow Farm Stream, Trap 4, 29.viii-5.ix.1980, (UMSP).

*Diagnostic characters. –*The following combination of characters separates this species from all other *Micropsectra* species: Adult male. AR c. 1.3. LR_1 c. 1.5. Anal point slender with pointed tip. Superior volsella finger-shaped. Digitus absent. Stem of median volsella long and s-shaped, with numerous narrow, leaf-like lamellae, often held tightly together as in a fine-tipped painting brush. Inferior volsella tapered towards apex and laterally curved, with c. 6-8 distal setae (Fig. 3-4a). Pupa. Total length c. 4.33mm. Thoracic horn relatively short, c. 336µm with numerous chaetae on distal 2/3 (Fig. 3-4b). Anterior pair of dorsocentrals placed further from each other than posterior pair (Fig. 3-4f). Long, strong, longitudinal spines on TIV; spinules and points on TV, similar in shape to TIV (Fig. 3-4b). 23-31, 28 anal fringe setae (Fig. 3-4h).

Etymology. – The species epithet '*penicillata'*- brush like*,* refers to the median volsella in the male hypopygium, which has the appearance of a painter's brush or a small tail.

Description

Adult male. Measurements and ratios in Table 3-2.

Coloration. Thorax with light green to light brown ground color, dark brown scutal stripes, preepisternum, postnotum, and median anepisternum; scutellum and halteres greenish to light brown; abdomen green to light brown; Head with dark brown antennal pedicel, black eyes, and light brown palps; legs light brown.

Head. Antenna with 13 flagellomeres. Frontal tubercles present, 5-12, 8µm; temporal setae in one row; palpomere 3 with 2 sensilla clavata in subapical pit.

Wing. Subcosta and media bare, brachiolum with 3 setae, squama bare.

Legs. Pulvilli present, reaching half length of claws. Fore tibia with spur 20-35, 28µm long, middle and hind tibial combs 12-22, 18µm and 12-22, 19µm respectively. Middle tarsomere Ta_1 with 2-4, 3 sensilla chaeticae.

Hypopygium (Fig. 3-4a). Anal tergite with tergal bands separate, posteriorly directed, not reaching crests of anal point; 3 median tergite setae anterior to anal point base; 7-8 ventral apical setae. Anal point with long, curved anal crests; apex of anal point long, slender with tip pointed; small knob between crests present; superior volsella finger-shaped; setiger with rounded apex and bent in median direction, 4-6, 5 dorsal and 2 median setae on setiger, 1 strong seta on stem; stem of superior volsella lacking field of microtrichia; digitus absent; median volsella long, S-shaped, with numerous narrow, leaf-like lamellae extending from distal end, often held tightly together; tip reaching far beyond apex of superior volsella. Inferior volsella narrowing toward distal end, with c. 6-8 distal setae, tip seemingly bifed and curved medially. Inner margin of gonocoxite with 3-4 strong setae.

Pupa. Measurements in Table 3.

Coloration. Pupal exuviae brownish with dark brown apodemes; cephalothorax, TVIII and anal lobe darker; large pigment-free area dorsally in posterior half of thorax.

Cephalothorax. Cephalic tubercles present, comparatively small, cone-shaped (Fig. 3-4d); pedicel sheath tubercle weakly developed; thoracic horn fairly short with numerous chaetae on distal 3/4, chaetae c.1/3 – 1/2 length of thoracic horn (Fig. 3-4g); precorneals arranged nearly in a line, with one setae offset, the two anteriormost setae situated closer to each other than the third (Fig. 3-4g); 1 median antepronotal, 2 lateral antepronotals (1 sensillum basiconicum); 2 pairs of dorsocentrals, anterior pair placed further from each other than posterior pair and with thin setae as compared to the thicker setae of posterior pair, Dc_1 longer than Dc_2, Dc_3 and Dc_4 typically nearly equal in length (Fig. 3-4f). Some granulation present along median suture line of thorax. Prealar tubercle absent; nose of wing sheath weakly developed.

Abdomen. TI bare. TII almost covered by shagreen except for two lateral round patches and one posteromedian oval patch; pedes spurii B on TII obvious; hook row approximately half as long as segment width. Spines of TIII in large, laterally curved patches in posterior half of tergite; shagreen extensively distributed lateral and anterior to spine patches with numerous points between patches. Patches of TIV consisting of spinules in anterior, oval, slightly diagonal patches with long spines in longitudinal, lateral extensions, with a slight lateral, posterior curve; few points present laterally of longitudinal spines. Patches of TV similar in shape to TIV, somewhat shorter, and consisting only of spinules and points. Patches of TVI consisting only of shagreen, similar to patches TIV and TV in shape, but often much shorter (Fig. 3-4b). Tergite TVII and TVIII with small, anterior, oval shagreen patches, with patches of TVIII somewhat larger and more distinct than TVII in some specimens. Segment I with 3 D and 1 V setae; segment II with 3 D, 5 V, 3 L; segment III with 5 D, 4 V, 3 L setae; segment IV with 5 D, 4 V, 1 L, 2 lateral taeniae; segment V with 5 D, 4 V, 3 lateral taeniae; segment VI with 5

D, 4 V, 4 lateral taeniae; segment VII with 5 D, 4 V, 4 lateral taeniae; segment VIII with 1 dorsal taenia, 1 ventral taenia, 5 lateral taeniae; anal tergite with 1 dorsal taeniae (Fig. 3-4b, h). Two pairs of small sensorial setae medially on TII-VII; 1 pair of O-setae present anteriorly on sternites II-VII. Anal lobe with evenly convex lateral margins, fringe with long taeniae in one row (Fig. 3-4h). Posterolateral comb of segment VIII with 3-6, 4 apical teeth longer than rest (Fig. 3-4e).

Taxonomic Remarks

Micropsectra penicillata exhibits morphological characteristics that tentatively place this species as a member of the Palearctic *recurvata*-group (sensu Säwedal 1981), with the exception of the lack of a digitus, an atypical character for the current *recurvata*-group. Specimens from this species have been collected from various localities throughout North America but have never been formally described. The Sublette collection at UMSP contains adult male specimens of this species from various localities including one adult male from Colorado, USA that was previously identified as *M. recurvata* due to morphological similarities; several others from the collection were left undetermined. Adult males can be differentiated from *M. recurvata* by the absence of the digitus in *M. penicillata,* and appearance of the median volsella lamellae, which lack the spoon-shaped appearance of many *Micropsectra* species, including *M. recurvata*, and instead appear individually as thin leaves that are often held tightly together in a cluster, giving an appearance similar to a thin, pointed, round paintbrush or pointed tail (Fig. 3a), showing most morphological similarity to *M. lacustris* (see discussion below). Pupae of *M.*

penicillata differ from *M. recurvata* by the presence of long longitudinal spines on tergites IV (Fig. 3-4b) as opposed to a short spine patch in *M. recurvata*. Males of *M. penicillata* also share morphological similarities with *M. lacustris*, and phylogenetic analyses based on CAD nuclear gene sequences indicate a sister relationship between these two species (Fig. 1). *M. penicillata* can be differentiated from *M. lacustris* based on a distally tapered and laterally curved inferior volsella, the absence of a digitus, and a comparatively longer median volsella (102-137, 122µm compared to 74-79µm reported by Säwedal (1975) for *M. lacustris*). The median volsella lamellar setae of *M. penicillata* are often held together more tightly than those of *M. lacustris,* which tend to appear more fanned. Pupae of the two species are differentiated based on the pattern of tergite armament; specifically, *M. penicillata* does not exhibit long longitudinal spines on TV (Fig. 3-4b) as seen in *M. lacustris* (see Fig. 6, Säwedal 1975). Further, Säwedal indicates that *M. lacustris* has 2 L and 2 LS setae on TIV, whereas *M. penicillata* has 1 L and 2 LS setae.

Ecology and Distribution. M. penicillata is currently known from stream localities from Colorado eastward in the United States and from Manitoba and Ontario, Canada.

Micropsectra subletteorum, n. sp.

(Fig. 3-5)

Type material.

Holotype. –P♂, **USA, Minnesota**: Washington Co., Valley Creek, Belwin Conservancy, 44°55'09"N; 92°47'54"W, 218m, larva collected 3.iii.2010, A.M. Anderson, (UMSP, slide mounted specimen; VM, DNA extract; voucher ID: ALY95; GenBank accession: JQ667061).

Paratypes. −10 P♂, 4 P♀. All data as for holotype, voucher ID and GenBank accession codes for sequenced specimens in Table 1 (9 P♂ UMSP, 1 P♂ VM); 6 ♂, **USA, Minnesota:** Goodhue Co., Trout Brook, 44°32'97”N; 92°48'07”W, 229m, adults collected 30.iii.2008, R.W. Bouchard. (UMSP, slide mounted specimens, VM, DNA extracts, voucher ID and GenBank accession codes for sequenced specimens in Table 1); 6 P♀, Cook Co., South Brule River, Forest Rd 152/Lima Grade, 15m upstream of bridge, 47°56'35”N; 90°26'17", 499m, larvae collected 11.iv.2010, A.M. Anderson, (UMSP, slide mounted specimens, VM, DNA extracts, voucher ID and GenBank accession codes for sequenced specimens in Table 1); 1 P♀, Cook Co., Fiddle Creek, Forest Rd 152/Lima Grade, stream access c. 100m NW of South Brule River bridge near edge of road, 47°56'35”N; 90°26'17”W, 505m, larva collected 11.iv.2010, A.M. Anderson (VM; voucher ID and GenBank accession code in Table 1); 1♂, **USA, Ohio:** Ottawa Co., Lake Erie, Put-in-Bay, Gibralter Island, 17.vi.1980, P.L. Hudson (UMSP). 1♂, **USA, Wisconsin:** Burnett Co., Spring Brook, 11mi E., 4 mi. S. of Siren, WI; 45°43’N;

92°09’W, 17.iv.1966, Dean Hansen, (UMSP #LRII-64); 1♂ Burnett Co., Spring Brook, 11mi E., 4 mi. S. of Siren, WI, 45°43’N; 92°09’W, 16.iv.1966, Dean Hansen (UMSP, #S73-183); 1♂, **Canada, Manitoba:** Churchill, Ramsey Creek, 58°43'50”N; 93°46'48”W, 13m, adult collected with malaise trap, 23-24.viii.2006, T. Ekrem & E. Stur (VM, voucher ID: CHIR-CH219, GenBank accession code in Table 1; 1♂, **Canada, Ontario:** Renfrew Co., White Lake, 23.vi.1968, R.D. Macdonald (CNC).

Diagnostic characters. –The following combination of characters separates this species from others *Micropsectra* species: Adult male. AR c. 1.6, LR_1 c.1.53. Anal point long and slender, with pointed tip. Superior volsella finger-shaped, field of microtrichia on stem absent. Digitus slender with slight curve, often extending past median margin of superior volsella. Median volsella long, parallel-sided with medially directed spoon-shaped lamellae. Inferior volsella with dorsoapical swelling. Gonostylus relatively narrow and tapering distally. Pupa. Total length c. 5.4mm. Thoracic horn relatively short, c. 450µm long with numerous long chaetae on distal 5/6; chaetae length c. 1/3 – 1/2 length of horn. Abdominal segment IV with 1 L and 2 lateral taeniae. Patches of TIV and TV consisting of spinules in anterior, oval patches, with shagreen lateral and posterior. Anal lobe with c. 44 setae.

Etymology. – This species is named in honor of Jim and Mary Sublette, in recognition of their contribution to Chironomidae taxonomy and systematics and for the large number of Nearctic *Micropsectra* specimens they collected and prepared over the years. Many of these specimens provided a wealth of information for this research and are referred to throughout the study.

Description

Adult male. Measurements and ratios in Table 3-2.

Coloration. Thorax dark brown with yellowish to light brown halteres; abdomen dark brown; head with dark brown antennal pedicel, black eyes, and light brown palps; legs dark brown.

Head. Antennae with 13 flagellomeres. Frontal tubercles small 5-10µm; temporal setae in 1 row; palpomere three with 2-3 sensilla clavata in subapical pit.

Wing. Macrotrichia on entire wing except for most basal part and fields between c and $r_{4+5.}$ Subcosta and media bare, distal 1/3 of cubitus and distal ½ of post cubitus bare; brachiolum with 3 setae, squama bare.

Legs. Pulvilli present, about one-third as long as claws. Fore tibia with 22-38, 30 µm spur, mid and hind tibial combs, respectively 17-25, 21 and 17-25, 22 µm long; mid ta_1 with 3-5, 4 sensilla chaetica.

Hypopygium (Fig. 3-5a). Anal tergite with tergal bands separate, posteriorly directed, reaching crests of anal point; 3-7, 5 median tergite setae anterior to anal point base; anal tergite without hump in front of anal point crests; 8-12, 10 ventral apical setae. Anal point with long, slightly curved anal crests; apex long, slender, and pointed; knob between crests absent; microtrichia between crests absent; microtrichia surrounding base of anal point. Setiger of superior volsella with 4-7, 5 dorsal and 2 median setae, 1 strong seta on stem; dorsal microtrichia on stem absent, field of microtrichia present ventrally on setiger, anterior to base of digitus. Digitus finger-like and slender with slight curve, often reaching past median margin of superior volsella. Median volsella long, parallel sided, with caudally directed setiform and spoon-shaped lamellae, on distal 1/3 - 1/4; tip generally not extending beyond superior volsella. Inferior volsella with moderately developed median, dorsoapical swelling and bearing numerous distal setae. Inner margin of gonocoxite with 3 strong setae.

Pupa. Measurements in Table 3-3.

Coloration. Pupal exuviae light brownish with dark brown apodemes; cephalothorax, TVIII and anal lobe darker; large pigment-free area dorsally in posterior half of thorax.

Cephalothorax. Cephalic tubercles present, cone-shaped, 30-51, 38µm; pedicel sheath tubercle absent (Fig. 3-4d). Thoracic horn 350-580, 450µm long with numerous comparatively long chaetae on distal 5/6; precorneals arranged in triangular pattern, the 2 anteriormost setae situated closer to each other than the third, posterior precorneals longer than the other two (Fig. 3-5e); 1 median and two lateral antepronotals (1 sensillum basiconicum); 2 pairs of dorsocentrals, anterior pair shorter and weaker than posterior pair, Dc_1 longer than Dc_2, Dc_3 and Dc_4 of similar length. Some granulation present along median suture line. Prealar tubercle present, roundish; Nose of wing sheath weak to absent.

Abdomen (Fig. 3-5b, g). TI bare. TII covered by shagreen except for one posteromedian oval patch and two small mediolateral patches; pedes spurii B on TII obvious; hook row over half as long as segment width. Spines of TIII in large, laterally curved patches in posterior half to two-thirds of tergite, shagreen extensively distributed lateral and anterior to spine patches, also covering much of the region between patches. Patches of TIV consisting of anterior, ovoid patches; shagreen present lateral and posterior to spine patches. Spine patches and shagreenation of TV similar to TIV. TVI consisting only of shagreen, similar to shagreenation in TIV and TV, except shagreen does not extend laterally in the posterior region as in TIV and TV. TVII with weak shagreenation in a pattern resembling that of TVI. TVIII with lateral oval shagreen paches. Segment I with 3 D and 1 V setae; segment II with 3 D, 4 V, and 3 L; segment III with 5 D, 4 V, and 3 L setae; segment IV with 5 D, 4 V, 1 L, and 2 lateral taeniae; segment V with 5 D, 4 V, and 3 lateral taeniae; segment VI with 5 D, 4 V, and 4 lateral taeniae; segment VII with 5 D, 4 V, and 4 lateral taeniae; segment VIII with 1 dorsal taenia, 1 ventral taenia, and 5 lateral taeniae; anal tergite with 1 dorsal taenia. One pair of O-setae present anteriolaterally on sternites II-VII. Anal lobe with long taeniae in one row on lateral margins (Fig. 3-5g). Posterolateral comb of segment VIII with 5-6 marginal teeth (Fig. 3-5c).

Taxonomic Remarks

Micropsectra subletteorum is closely allied with many species in the *notescens*-group, particularly *M. apposita* (Walker, 1856), *M. brundini* Säwedal, 1979, *M. contracta* Reiss, 1965, *M. insignilobus, M. junci* (Meigen, 1818), *M. lindebergi* Säwedal, 1976, *M. nigripila* (Johannsen, 1905) and *M. notescens* (Walker, 1856). The primary characteristic distinguishing adult males of *M. subletteorum* from the aforementioned species is that *M. subletteorum* lacks a field of microtrichia on the basal part of the superior volsella as well as lateral teeth on the anal tergite (Fig. 3-5a). The following characteristics are also useful for distinguishing males of *M. subletteorum* from *M. brundini, M. insignilobus* and *M. lindebergi*: a comparatively higher $LR_{1,}$ and stronger anal point crests (see Tables 1-3 in Säwedal 1979). The gonostylus of *M. subletteorum* also is gradually narrowing towards the distal end (Fig. 3-5a) and the AR is lower than seen in the three aforementioned species. Säwedal (1979) indicates that the low number of sensilla chaetica of *M. brundini* (0-2) may also help differentiate *M. brundini* from other *notescens*- group species. However, we have observed specimens of *M. brundini* from the PHL collection that have up to four sensilla chaetica, indicating this may not be a reliable character. *M. subletteorum* can be further differentiated from *M. insignilobus* by having a comparatively longer median volsella (68-85µm compared to 40-59µm). *M. subletteorum* differs from *M. junci* and *M. nigripila* by having a comparatively longer digitus that often extends to or past the margin of the superior volsella (Fig. 3-5a). The digitus of *M. subletteorum* also exhibits more curvature than that of *M. notescens, M. contracta*, and *M. apposita. M. subletteorum* is further differentiated from *M. notescens* by lacking the ball-shaped swelling at the tip of the inferior volsella (see Fig. 10, Säwedal 1976) and having a longer median volsella, 67-85, 74 compared to 38-64µm (Säwedal 1976). The short, blunt-tipped anal point of *M. nigripila* also differentiates males of the two species, and the rounded tip of the superior volsella (Fig. 3-5a) sets *M. subletteorum* apart from the more triangular superior volsella of *M. contracta* and *M. apposita.*

Pupae of *M. subletteorum* can be easily differentiated from *M. contracta* and *M. apposita* by lacking semi-long longitudinal spines posterior to the oval spinule patch on

TIV (Fig. 3-5b). The species differs from *M. insignilobus* and *M. lindebergi* by exhibiting 2 LS and 1L setae on TIV rather than 3 LS setae. The thoracic horn of *M. subletteorum* is also shorter than these two species, together with *M. nigripila* and the parthenogenic *M. silvesterae* Langton, 1994, (c. 450µm as compared to 680µm for *M. insigniliobus* and *M. lindebergi*, 624-947, 825µm for *M. nigripila* (Oliver and Dillon 1994) and 440-835, 672µm for *M. silvesterae* (Säwedal 1976; Oliver and Dillon 1994; Langton 1998)). Further, thoracic horn setae start basally for both outer and inner lateral margins, and filament length is 1/3 – 1/2 the length of the thoracic horn as compared to 1/5 – 1/6 for *M. insignilobus* and *M. lindebergi* (see Säwedal 1976); the particularly long thoracic horn with short chaetae of both *M. nigripila* and *M. silvesterae* also serves to differentiate *M. subletteorum* from these species (compare to Oliver and Dillon 1994, Fig. 10 and Langton 1998, Fig. 2c). *M. sublettorum* can also be distinguished from *M. silvesterae* by the presence of only one median antepronotal seta as compared to two in *M. silvesterae* (Langton 1998). *M. subletteorum* differs from pupae of *M. junci* by lacking the finger-like prealar tuburcle in front of the wing sheath. *M. subletteorum* can be further differentiated from *M. insignilobus* and *M. junci* in that it has 40-47, 44 anal lobe filaments, as compared to 53-60 in *M. insignilobus* and 62-73 in *M. junci.* Pupae of *M. subletteorum* are perhaps most similar to *M. notescens*, and few reliable characters have been found to separate the two. The thoracic horn length and setae arrangement is perhaps the best distinguishing character at present, with the thoracic horn of *M. subletteorum* typically shorter than *M. notescens*. Säwedal (1976) reports the average length of the thoracic horn of *M. notescens* as 660µm, however we have seen specimens with thoracic horn length as low as 525µm, which does fall within the range we report for *M. subletteorum*. Thoracic horn setae appear to begin slightly lower on the inner lateral margin for *M. subletteorum*, covering 4/5 to 5/6 as compared to 2/3 for *M. notescens.*

Ecology and Distribution. This species is common in small groundwater-dominated trout streams throughout Minnesota and has been found in other lotic and lentic localities in the eastern half of the United States and Canada.

Micropsectra xantha (Roback, 1955)

(Figs. 3-6, 3-7a, b)

Calopsectra (*Micropsectra*) *xantha* Roback, 1955: 4

Type material.

Neotype (here designated). –P♂, **USA, Minnesota:** Goodhue Co., Cold Spring Brook, spring pool off of County Rd. 68, c. 1km N of Hwy 60, 44°17'46"N; 92°25'35"W, 256m, larvae collected 25.iv.2010, A.M. Anderson (UMSP, slide mounted specimen; VM, DNA extract; voucher ID: ALY52; GenBank accession: JQ667073).

Additional material examined. −13 P♂, 17 P♀, 1L_{hc}P♀. All data as for neotype, voucher ID and GenBank accession codes for sequenced specimens in Table 1 (12 P♂, 1 P♀ UMSP, 13 P♂, 1 P♀ VM); 3♂, 1P♂, **USA, New York:** Cattaraugus Co., Allegany State Park, adult collected with light trap 28.v – 3.vi.1963, W.W. Wirth, (UMSP, 573-676). 1♂, **USA, Virginia:** Fairfax Co., Falls Church, Holmes Run, adult collected with light trap 31.v.1961, W.W. Wirth, (UMSP); 7♂, **USA, Wisconsin:** Burnett Co., Spring Brook, 11mi E., 4 mi. S. of Siren, WI, 45°43'N; 92°09'W, adults collected with sweep net 13.x.1967, Dean Hansen (UMSP); 3♂, Burnett Co., Spring Brook, 11mi E., 4 mi. S. of Siren, WI, 45°43'N; 92°09'W, adults collected with light trap 7.vi.1968, Dean Hansen (UMSP); 1♂, **Canada, Quebec:** Lewis Co., 31.v.1929, (UMSP).

Diagnostic characters. –The following combination of characters separates this species from others species of *Micropsectra*: Adult male. AR c. 1.3, LR_1 c.1.6. Anal point long and slender, with pointed apex. Superior volsella finger-shaped. Digitus long and narrow, extending beyond median margin of superior volsella. Median volsella moderately long and s-shaped, with numerous long, medially-directed spoon-shaped lamellae. Inferior volsella swollen at tip, with c. 10 strong setae (Fig. 3-6a). Pupae. Total length c. 5.3mm. Thoracic horn relatively long, c. 507µm with numerous chaetae covering distal 5/6; thoracic horn chaetae c. 1/3 length of horn (Fig. 3-6f). Abdominal segment IV with 3 lateral taeniae. Patches of TIV and TV consisting of spinules in anterior, oval patches, with shagreen lateral and posterior (Fig. 3-6b). Anal fringe with c. 43 setae (Fig. 3-6g).

Etymology. Roback (1955) does not include information regarding the name choice for this species in his original description , however, the Greek term 'xanthos' means yellow, and was likely given in reference to the coloration of this species.

Description.

Adult male. Measurements and ratios in Table 3-2.

Coloration. Ground color of head, thorax, abdomen, and legs light yellow or green; antennal pedicel, scutal stripes, preepisternum, postnotum, and median anepisternum darker yellow; eyes black.

Head. Antenna with 13 flagellomeres. Frontal tubercles present as small dots (3-5, 4µm); temporal setae in 1 row; palpomere three with 2-3 sensilla clavata in subapical pit.

Wing. Subcosta and media bare, brachiolum with 2 setae, squama bare.

Legs. Pulvilli present, nearly half the length of claws. Fore tibia with 28-38, 33µm spur; middle and hind tibial combs 15-24 and 15-27µm respectively; middle tarsomere Ta_1 with 3-4 sensilla chaeticae.

Hypopygium (Fig. 3-6a). Anal tergite with tergite bands separate, posteriorly directed, not reaching crests of anal point; 2-4, 3 median tergite setae on slightly elevated ridge; 8-10, 9 ventral apical setae. Anal point with long, pointed apex; anal crests slightly curved; knob between anal crests absent; large microtrichia free area around base. Setiger of superior volsella with 5-7, 6 dorsal setae and 2 median setae; 1 setae on stem; a small field of dorsal microtrichia on stem of superior volsella, and a large field ventrally on setiger. Digitus long and narrow, reaching well beyond the median margin of the superior volsella. Median volsella long and s-shaped, long, medially-directed setiform and spoon-shaped lamellae, c. 10-15 numerous spoon-shaped lamellae extending from tip. Inferior volsella greatly swollen at tip, appearing somewhat ball-shaped and bearing c. 10 strong setae. Inner margin of gonocoxite with 2-3 strong setae.

Pupa. Measurements given in Table 3-3.

Coloration. Pupal exuviae light brownish with darker brown apodemes; cephalothorax, TVIII and anal lobe darker brown.

Cephalothorax. Cephalic tubercles present, small, conical; pedicel sheath tubercle well developed (Fig. 3-6d). Thoracic horn fairly long with numerous chaetae covering distal 5/6; thoracic horn chaetae approximately 1/3 length of thoracic horn (Fig. 3-6f); precorneals arranged nearly in a line, with one setae somewhat offset, and the 2 anteriormost setae situated closer to each other than the third, anterior precorneal typically shorter than the other two; 1 median antepronotal, 2 lateral antepronotals (1 sensillum basiconicum); 2 pairs of dorsocentrals, anterior pair often shorter than posterior pair. Some granulation present along median suture line of thorax. Prealar tubercle present, roundish; nose of wing sheath weak to moderate.

Abdomen (Figs. 3-6 b, g). TI bare. TII almost covered by shagreen except for one posteromedian oval patch; pedis spurii B on TII obvious; hook row over half as long as segment width. Spines of TIII in laterally curved patches in posterior half of tergite, shagreen extensively distributed lateral and anterior to spine patches, and covering majority of the area between patches. Patches of TIV consisting of spinules in anterior, oval patches; shagreen lateral and posterior to patches. TV patches and shagreenation similar to TIV. Patches of TVI consisting only of shagreen; shagreen in anterior region of tergite somewhat larger than those in mid and posterior region, resembling shape of spinule patches in TIV and TV. TVII with shagreen in anterior region. TVIII with small anterolateral shagreen patches. Segment I with 3 D, 1 V setae; segment II with 3 D, 4 V, 3 L setae; segment III with 5 D, 4 V, 3 L; segment IV with 5 D, 4 V, 3 lateral taeniae; segment V with 5 D, 4 V, 3 lateral taeniae; segment VI with 5 D, 4 V and 4 lateral taeniae; segment VII with 5 D, 4 V, and 4 lateral taeniae; segment VIII with 1 dorsal taenia, 1 ventral taenia, and 5 lateral taeniae; anal tergite with 1 dorsal taenia. One pair of O-setae anteriolaterally on sternites II-VII. Anal lobe with fringe of laterally curved taeniae in 1 row. Posterolateral comb of segment VIII with 4-6 teeth longer than the rest.

Taxonomic remarks. The holotype could not be located at the Academy of Natural Sciences Natural History Museum in Philadelphia, USA (ANSP) (J. Weintraub, personal communication). However, as noted by Säwedal (1981), the hypopygium of the holotype was poorly preserved. Additionally, as discussed below, males of *Micropsectra xantha* are very similar morphologically to *M. connexa* (Kieffer, 1906). There are no known paratypes of *M. xantha* and to stabilize future taxonomy of the species we therefore find it necessary to designate a neotype for *M. xantha*.

As noted by Säwedal (1982), the adult male of *M. xantha* is very similar to *M. connexa* (see additional nomenclature notes on this species in Stur and Ekrem 2006) and warrants placement in the Palearctic *recurvata*-group. We have examined the male holotype of *M. connexa*, located in BMNH, and confirm that the most reliable difference between *M. xantha* and *M. connexa* is in the anal point. The anal point of *M. xantha* is slender and parallel-sided towards the tip, whereas in *M. connexa*, the anal point is broader and cone-shaped towards the tip (Figs. 3-7b, d). Additionally, the anal point crest is stronger in *M. xantha* than *M. connexa.* We have also noted differences in the superior volsella (Figs. 3-7a, c); *M. xantha* appears to have a broader superior volsella with a long, narrow digitus that is generally less than one-third the width of the superior volsella. The superior volsella of *M. connexa* is comparatively narrower with a wider digitus that is one-half to one-third the width of the superior volsella. Finally, the seta on the stem of the superior volsella appears more basal on *M. connexa* than *M. xantha.*

Stur and Ekrem (2006) place *M. connexa* with the *attenuata*-group. Here, we refute this placement, and instead find a more appropriate placement in the *recurvata*-

group, together with *M. xantha,* based on the combination of a long and s-shaped median volsella, long anal point (Fig. 3-7d), and a digitus that extends past the margin of the finger-shaped superior volsella (Fig. 3-7c). We attempted to amplify both standard and short (130 bp) COI-fragments from specimens of *M. connexa* found in the Sublette collection (UMSP; all originally labeled as *M. xantha*) to provide additional evidence that *M. xantha* and *M. connexa* are truly separate species. However, we were not successful in obtaining a usable sequence, probably due to the age (>40 years) of the specimens available to us. *M. xantha* is also quite similar to *M. digitata* Reiss, 1971, however the digitus in *M. digitata* is missing and the anal point is wider and more rounded at the tip.

Ecology and Distribution. The original holotype was collected from a small, cold spring west of Concord, Massachusetts, USA (Roback 1955). In the original description, Roback mentions the water temperature of the spring varies from 7-10°C, with a mean temperature of 9°C. The neotype is known from a southeastern Minnesota spring with very similar thermal characteristics. Specimens are also known from various other localities in the eastern half of the United States and Canada.

Discussion

This small survey of only five Minnesota streams, each visited on only one sample occasion, yielded three new species of a single chironomid genus, a clear indication that there are many Nearctic chironomids left to discover. These new specimens more than doubles the number of *Micropsectra* previously known to Minnesota with 24 now known to the Nearctic. Notably, without the aid of molecular techniques or examination of multiple life-stages, two of the species described in this work, *M. neoappendica* and *M. subletteorum,* likely would have gone unnoticed if identified using existing Palearctic keys and species descriptions.

As pointed out by Stur and Spies (2011), there are clear advantages to including species-specific DNA sequences as a standard component of new species descriptions. These advantages include reduced inconsistency in appropriate use of species names (this paper, Stur and Spies 2011), association of multiple or unknown life stages (Zhou et al. 2007, Ekrem et al. 2010a, Pauls et al. 2010, Stur and Ekrem 2011b), and another set of characters that can be used to identify species.

Recent literature has questioned the appropriateness and accuracy of the use of the European-derived species-groups for the *Micropsectra* (Gilka 2001, Gilka and Paasivirta 2008, Ekrem et al. 2010b). While present molecular and morphological evidence currently shows a well-supported *atrofasciata*-group (e.g., Ekrem et al. 2010b, Stur and Ekrem 2006), lines of evidence supporting monophyletic *attenuata, recurvata,* and *notescens* groups are less distinct (e.g., Gilka 2001, Gilka and Abramczuk 2006, Gilka and Paasivirta 2008, Ekrem et al. 2010b), particularly considering the recent synonymy of *Krenopsectra* and *Parapsectra* with the *Micropsectra* (Ekrem et al. 2010b), and are perhaps further blurred when attempting to place Nearctic species in the Palearctic-derived classification system.

As we suggest here, and as Reiss (1995) proposed in an earlier study, most Nearctic *Micropsectra* have not yet been described. Further, many species awaiting description, along with some that have been described, do not conform to the current species-group classification, based primarily on the adult male (e.g., *M. spinigera* Reiss, 1995, *M. borealis* Kieffer, 1922, *M. radialis* Goetghebuer, 1939, and the parthenogenic species *M. sedna* Oliver, 1976, and *M. silvesterae*), highlighting the need for a major revision of the genus and expanded species-group system.

In this research, as well as in the study by Ekrem et al. (2010b), molecular evidence indicates a paraphyletic *recurvata*-group, with former *recurvata*-group members *M. recurvata* and *M. lacustris* falling into genetically distinct clades (Fig. 3-1; Figs. 3 and 4 in Ekrem et al. 2010b). Based on this observation, if chironomid taxonomists choose to retain current species-groups, perhaps Säwedal's original classification of a separate *lacustris*-group (Säwedal 1975) was more appropriate than his later placement of *M. lacustris* in the *recurvata*-group (Säwedal 1981). Molecular evidence from our CAD dataset suggests the placement of *M. penicillata* as sister species to *M. lacustris.* Although *M. penicillata* shares similarities to the *recurvata*-group, as pointed out by preliminary classification of the undescribed species by J. Sublette in his personal collection (now part of UMSP), there are also distinct differences, which we point out in the taxonomic remarks for the species outlined above. These morphological differences, combined with molecular evidence (Fig. 3-1), indicate an alternative grouping for the species.

A further drawback of the existing species-group classification is that it is based primarily on the morphological characteristics of the adult male, without including other life stages, and it was primarily founded before molecular data were commonly used in systematics. As noted by Pinder and Reiss (1986), the current species group classification does not extend to immature stages. Although most chironomid descriptions have historically been based on the adult male, descriptions of immature lifestages are becoming increasingly important to include, particularly for biological monitoring and species diversity studies (e.g., Ekrem et al. 2010a, Zhou et al. 2010).

Chironomid pupal exuviae have been shown to be a simple and effective method for assessing species diversity (e.g., Ferrington et al. 1991, Calle-Martinez and Casas 2006, Raunio et al. 2007, Ruse 2011), particularly as descriptions of pupal exuviae have become more common and readily available. We believe that this life stage may provide equally important features that, combined with characters of the adult male and molecular data, may help resolve future species group concepts. For example, when examining Figure 3-1 (this study) and Figure 3 from Ekrem et al. 2010b, all members of the *atrofasciata*-group that have described exuviae have long longitudinal spine patches on abdominal tergite four. Conversely, exuviae known for the *notescens*-group, with the exception of *M. contracta* and *M. apposita*, have short anterior patches consisting of spinules and points. *Micropsectra contracta* and *M. apposita* have semi-long spines (none long enough to be synonymous with spines from the *atrofasciata*-group) extending partway down TIV. Members of a reduced *recurvata*-group, including *M. xantha,* also fit the exuviae generalization of the *notescens*-group, forming a *recurvata-notescens* clade. Adult male morphology clearly separates the *recurvata*- group members from the *notescens*- group, however we have not yet identified pupal characters that can be used to separate the two groups. Furthermore, if the *lacustris*-group is re-established, with *M. penicillata* as a member, this group fits better with a combined *lacustris-atrofasciata* clade (Fig. 3-1). As with the *recurvata-notescens* clade, members of the *lacustris-atrofasciata* clade have similar patterns on the exuviae, with known species in this clade exhibiting long longitudinal spine patches on TIV. Adult males separate the *lacustris*-group from the *atrofasciata*- group, but no pupal characters distinguish the groups at this point.

To expand this idea further, and express the need for a revision and expansion of current species-groups, *M. radialis* and *M. borealis* form a separate group in our phylogeny (Fig. 3-1) and that of Ekrem et al. (2010b). Additionally, morphology of the adult male and pupa is markedly different from other *Micropsectra* species mentioned here, making it impossible for these species to conform to any of the current species groups or the proposed system described above. Säwedal (1981) mentions the construction of a *borealis*-group, based on redescription of *M. borealis* (Säwedal and Willassen 1980); however, there was apparently no subsequent treatment of the group. Furthermore, Säwedal and Willassen (1980) suggest that *M. borealis* and *M. radialis* form a monophyletic clade with the *atrofasciata*- group based largely on similarities of the superior volsella. We refute placement of these species within the *atrofasciata*- group.

Instead, we suggest erection of the *borealis*-group, currently including *M. borealis* and *M. radialis* as members based on genetic similarity and morphological similarities of the male genitalia, and reasons outlined by Stur and Ekrem (2006).

As mentioned, the former *Parapsectra* and *Krenopsectra* taxa were recently synonymized with the *Micropsectra* (Ekrem et al. 2010b). When considering the former *Parapsectra,* all taxa less one, form a monophyletic group; only *M. mendli* (Reiss, 1983) falls outside of this group, forming its own clade (Fig. 3-1). We therefore suggest formation of a *nana*-group that currently includes *M. nana* (Meigen, 1818)*, M. bumasta* (Giłka & Jazdzewska, 2010), *M. chionophila* (Edwards, 1933)*,* and *M. uliginosa* (Reiss, 1969).

At this point we are hesitant to firmly place *M. acuta* Goetghebuer, 1934 within the *attenuata*- group (Fig. 3-1). The adult male of *M. acuta* exhibits similarities to other species within the *attenuata*- group and molecular results clearly indicate a linkage, however, pupal morphology is somewhat different in that *M. acuta* lacks a laterally curved patch of longitudinal spines on TIII as seen in the other *attenuata*-group species. Additionally, *M. acuta* does not have lateral taeniae on any of the tergites, only lateral setae, a trait unusual for *Micropsectra,* and TIII only has four setae as compared to the usual five. This leads us to believe that as more *Micropsectra* species are described, *M. acuta* could form a new species group within the *Micropsectra*.

The grouping systems proposed here, based on the combination of adult and pupal morphology, along with current genetics clearly points out faults in the current system, and suggests the benefits of creating an expanded species-group system based on a combination of morphological characters for life stages with the most reliable distinguishing characters, such as pupal exuviae and the adult male. Importantly, further proposed morphological groupings and relationships should be tested with appropriate molecular markers.

As *Micropsectra* species continue to be described, revealing new morphological character combinations, and as the DNA barcode database for the genus is expanded, particularly with regard to Nearctic species and those in other less-studied localities, there will be a need to re-think the taxonomic framework for *Micropsectra.* This will likely be a trend seen throughout other major genera of Chironomidae and, perhaps, other large families of aquatic insects. We anticipate that there will be further shifts and alterations in the current species-group classification system and a continued need for chironomid taxonomists to revisit the appropriateness of these groups. With recent and forth-coming advances in molecular taxonomy, taxonomic work that combines forces of molecular and morphological methodologies will rapidly advance our understanding of phylogenetic relationships in species-rich genera such as *Micropsectra*, emphasizing the need for taxonomists to actively use all data available and to be well-versed in both molecular and morphological techniques.

Key to Known Nearctic *Micropsectra* Adult Males

This key adopts several couplets given by Säwedal (1976) or Stur and Ekrem (2006); however, several species and diagnostic characters have been added. Males of the following species are unknown and therefore not included in the key: *Micropsectra sedna* and *M. silvesterae*; both of these species are known to be parthenogenic. We have elected not to arrange the key by species-group at this time considering that many of the species included in the key either are not known in multiple life stages or are not available for inclusion in molecular analyses. Note that it was necessary to format the last couplet in this key differently than previous couplets due to extreme similarity in these three *notescens*- group species.

1\. Superior volsella deeply divided; anal point long and toothed (Reiss 1995: Fig. 1) …………………………..……………………………….……………...….. *M. spinigera*

1'. Superior volsella undivided; anal point not toothed.………………..………………. 2

2\. Setiger of superior volsella more or less circular (Fig. 3-2), sometimes with straight median margin …...…………………………….………………………………………. 3

2'. Setiger of superior volsella triangular or finger-like (Fig. 3-3a), never with straight median margin ……………………………………………………………………….. 6

3. Mid and hind tibiae each with one obvious spur; digitus digitiform, extending well beyond median margin of superior volsella (Säwedal and Willassen 1980: Fig. 2) ………………………………………………………………………..…..… *M. borealis*

3’. Mid and hind tibiae without spurs; digitus digitiform or triangular, occasionally pointed, sometimes extending slightly beyond median margin of superior volsella (Fig. 3-2; Stur and Ekrem 2006: Figs. 12a, b) …………………………………………………..… 4

4. Median volsella large, thick, club-shaped; knob between crests of anal point present; digitus short, never reaching margin of median volsella (Fig. 3-2) …...…………………………………...……………………..… ***M. neoappendica* n. sp.**

4’. Median volsella small, thin, never club-shaped; knob between crests of anal point absent; digitus extending to or beyond margin of median volsella ...…………………… 5

5. Apex of median volsella not extending to or beyond that of inferior volsella (Stur and Ekrem 2006: Fig. 12a) ……………………………….…….. *M. logani* (Johannsen, 1928)

5’. Apex of median volsella reaches to or beyond that of inferior volsella (Oliver and Dillon 1994: Fig. 7) ……………………….…………………………… *M. polita* Malloch

6. Median volsella with leaf-like lamellae, sometimes held tightly together (Fig. 4a; Säwedal 1976: Fig. 8) or large and spatulate (Reiss 1969b: Fig. 3) ……………...…...… 7

6’. Median volsella with spoon-shaped lamellae (Figs. 3-5a, 3-6a, c)…………….……... 9

7. Lamellae of median volsella large and spatulate; superior volsella triangular; digitus reduced, never reaching margin of median volsella (Reiss 1969b: Fig. 3) …………………………………………………………..…...….. *M. attenuata* Reiss 1969

7'. Lamellae of median volsella leaf-like; superior volsella finger-shaped; digitus either absent or extending to or past margin of superior volsella ……………………………… 8

8. Lamellae of median volsella often held tightly together, appearing brush or tail-like; digitus absent; median volsella long (103-138µm) (Fig. 3-4a) …….. ***M. penicillata* n. sp.**

8'. Lamellae of median volsella not as above; digitus long, often extending to or past margin of superior volsella; median volsella short (23-31µm) (Säwedal 1976: Fig. 8) ………………………………………………………………………………. *M. lindrothi*

9. Median volsella long and sinuous or s-shaped (Fig. 3-4a, 3-6a) ...…….……………… 10

9'. Median volsella of varying length, more or less straight and parallel sided (Fig. 3-5a) ……………………………………………………………………………………...… 15

10. Median volsella nearly as wide as inferior volsella; inferior volsella strongly curved medially, gradually narrowing to tip (Oliver and Dillon 1994: Fig. 1) …………………………………………………………...…… *M. dives* (Johannsen, 1905)

10'. Median volsella never as wide as inferior volsella; inferior volsella may or may not curve medially and/or narrow to tip ……………………………...………………….. 11

11. Inferior volsella either very slender or swollen at tip, appearing somewhat ball-shaped ……………………………………………………………………………………….. 12

11’. Inferior volsella of normal width …………………….…………………………… 14

12. Inferior volsella very slender, distal part bent medially (Reiss 1974: Fig. 3) ……………………………………………………………………….…. *M. recurvata*

12’. Inferior volsella swollen at tip, appearing somewhat ball-shaped (Fig. 3-6a) ……. 13

13. Anal point slender and parallel-sided towards tip (Fig. 3-7b); superior volsella broad with long, narrow digitus; digitus less than one-third width of superior volsella (Fig. 3-7a) ……………………………………………………..………………….. *M. xantha*

13’. Anal point relatively broad and cone-shaped towards tip (Fig. 3-7d); superior volsella comparatively narrow; digitus greater than one-third width of superior volsella (Fig. 3-7c) ……………………………..…………………………….……..…. *M. connexa*

14. Superior volsella finger-like (Säwedal 1981: Fig. 1); LR_1 = 1.38-1.40; AR = 1.57-1.73 (Säwedal 1981) …………....………………………………… *M. tori* Säwedal, 1981

14’. Superior volsella comparatively triangular (Oliver and Dillon 1994: Fig. 2); LR_1 = 1.50-1.69; AR =0.73-1.46 ……………………...… *M. geminata* Oliver and Dillon, 1994

15. Digitus short, rarely reaching margin of superior volsella (Reiss 1969a: Fig. 11; Säwedal 1976: Figs. 22, 24); if digitus reaches close to margin of superior volsella,

gonostylus is thick and stout and anal tergite with large lateral tooth or inferior volsella is enlarged at tip …………………………………………………………………..…. 16

15’. Digitus long, extending to margin of superior volsella or slightly beyond …….…. 17

16. Anal point short, triangular with blunt tip; inferior volsella enlarged at tip; digitus not extending beyond margin of superior volsella (Oliver and Dillon 1994: Fig. 7) ……………………………………….……………………………......…. *M. nigripila*

16’. Anal point long and/or acute; inferior volsella not enlarged at tip ……….…….….. 17

17. Median volsella of medium length (57-78µm); 1.04-1.49; gonostylus thick and stout, narrowing towards tip (Säwedal 1976: Fig. 24); anal tergite with lateral tooth …. *M. junci*

17’. Median volsella short (28-36µm); AR = 0.50-0.73; gonostylus not thick and stout, but may narrow towards tip; anal tergite without lateral tooth ……………….….…. *M. nana*

18. Superior volsella triangular (Säwedal 1976: Fig. 17); anal tergite with small lateral tooth; mid ta_1 with 5-7 sensilla chaetica ……………………………….…… *M. apposita*

18’. Superior volsella finger-shaped (Fig. 3-4a; Säwedal 1976: Fig. 2), more or less parallel at base; anal tergite with or without lateral tooth; mid ta_1 with 5 or less sensilla chaetica …………………………………….…………………………………..…. 19

19a. Gonostylus narrowing toward tip (Fig. 3-5a); LR = 1.52-1.54; anal tergite without lateral tooth; superior volsella lacking field of microtrichia on basal part; AR = 1.47-1.70; digitus often extending to or past margin of superior volsella; median volsella 68-85 µm ………………………………..…………………………………... ***M. subletteorum*** **n. sp.**

19b. Gonostylus not narrowing toward tip (Säwedal 1976: Fig. 2); LR = 1.34-1.51; anal tergite with small lateral tooth; few microtrichia on basal part of superior volsella; AR = 1.47-1.83; digitus extending to or past margin of superior volsella; median volsella = 40-59µm ……………………………………………………………….…… *M. insignilobus*

19c. Gonostylus not narrowing toward tip (Säwedal 1976: Fig. 2); LR = 1.37-1.41; anal tergite with small to medium lateral tooth; large group of microtrichia on basal part of superior volsella; AR = 1.75-2.14; digitus extending to or past margin of superior volsella; median volsella = 63-78µm ………………………………………… *M. brundini*

Key to Known Nearctic *Micropsectra* Pupae and Pupal Exuviae

This key adopts several couplets given by Säwedal (1976), Langton (1991), or Stur and Ekrem (2006); however several species and diagnostic characters have been added. Pupae of the following species are unknown and therefore not included in the key: *Micropsectra connexa, M. spinigera,* and *M. tori.* We have elected not to arrange the key by species-group at this time considering that many of the species included in the key either are not known in multiple life stages or are not available for inclusion in molecular analyses.

1. Abdominal segments II – V each with 4-7 lateral setae + taeniae (Säwedal and Willassen 1980: Fig. 3) ……………………..…………………………… *M. borealis*

1'. Abdominal segments II-V each with 3 lateral setae + taeniae (Fig. 3-4b) …….……. 2

2. Tergite II with shagreen in two triangular patches that are not connected anteriorly ……………………………………………………………………………... *M. sedna*

2.' Tergite II extensively covered with shagreen, leaving only a median and usually lateral rounded patches free of shagreen (Fig. 3-4b) …………………….………………… 3

3. Tergites III-VI with similarly paired, anterior point patches (Wiederholm 1986: Fig. 10.54e) …………………...…………….…………………………………………… *M. nana*

3'. Tergite III with posterior spine patches (Fig. 3-3a, b) ……………………...…….. 4

4. TIV with long longitudinal spine patches extending from anterior spinule patches (Fig. 3-3a,b) ………………………………………..……………..…..……… 5

4'. TIV with only short anterior patches consisting of spinules and points (Fig. 3-5b) ... 9

5. Longitudinal spine patches of TIV extend about half the length of the tergite; a row of fine shagreen extends posteriorly from each spine patch (Säwedal 1976: Fig. 20) .... *M. apposita*

5'. Longitudinal spine patches of TIV extend well over half-way down length of tergite; shagreenation not extending posteriorly from spine patches ……………………..……... 6

6. TIII with 1 lateral setae and 2 lateral taeniae ……..…………………….……… *M. logani*

6'. TIII with 3 lateral setae; no taeniae ..…………………………………...................... 7

7. Longitudinal patches of TV consisting of shagreen, points, spinules, and often spines; spines, if present, similar in size to those of TIV; total patch length subequal to that of TIV (Oliver and Dillon 1994: Fig. 11) ………………………..…… *M. polita* Malloch

7'. Longitudinal patches of TV consisting only of spinules and points; length of patches less than those of TIV (Fig. 3-3a, 3-4b)……………………...……………….………… 8

8. Distance between anterior and posterior pairs of dorsocentrals is approximately equal; nose of wing sheath moderate to strong ………………………… ***M. neoappendica* n. sp.**

8'. Distance between anterior pair of dorsocentrals 2-3x greater than that of posterior pair and anterior pair thinner than posterior pair (Fig. 3-4f); nose of wing sheath weak to moderate ………………...……………………..………………... ***M. penicillata* n. sp.**

9. Prealar tubercle with finger or nose-like projection (Reiss 1969: Fig. 5; Säwedal 1976: Fig. 25) …………………………………………………………………………... 10

9'. Prealar tubercle without finger-like projection .……………………………………. 11

10. Anal lobe with 23-31 lateral taeniae; tergites IV and V almost entirely covered with shagreen and spinules; the spinules of the anterior transverse patches similar in size, but more dense and darker than elsewhere on tergites (Reiss 1969: Fig. 7) ……………………………………………………………………..…. *M. attenuata* Reiss

10'. Anal lobe with 46-52 (Langton 1991) or 62-73 (Säwedal 1976) lateral taeniae; tergites IV and V with distinct patches of spines or spinules which are stronger than surrounding shagreen (Säwedal 1976: Fig. 16) ………………………..……… *M. junci*

11. Tergite VI with a single, crescent-shaped anterior point patch; pleura without shagreen (Oliver and Dillon 1994: Fig. 5) ………………………...…………. *M. dives*

11'. Tergite VI with paired anterior point patches or only with paired longitudinal shagreenation; pleura with or without shagreen …………………...…….……….. 12

12. Tergite IV with 3 lateral taeniae ..…………………………………………….. 13

12'. Tergite IV with 1 or 2 lateral taeniae ………..……………………………………. 16

13. Shagreen absent between spine patches of TIII (Langton 1991: Fig. 146j) …………………………………………………………………...….. *M. recurvata*

13’. Shagreen present between spine patches of TIII (Fig. 3-5b) …..…………………. 14

14. Tergite III with spinules (<40µm) in paired, comparatively longitudinal patches, approximately equal in size to those on TIV and TV, the greater part of the spinule patch anterior to seta D5 (Langton 1991: Fig. 148c); pedicel sheath tubercle weakly developed …………….. *M. insignilobus* and *M. brundini*

14’. Tergite III with spines (>40µm) or spinules in paired, patches that curve in lateral direction posteriorly, if spinules (<40 µm present, they are noticeably larger than those on TIV and TV, the greater part of spine patch posterior to seta D5 or approximately equally distributed above and below seta D5 (Fig. 5b; Langton 1991: Fig. 148i); pedicel sheath tubercle usually well developed …………………..…………………………………… 15

15. Anal lobe with >43 lateral taeniae (48-70, Langton 1991; 43-56, Säwedal 1976); thoracic horn >450 µm (495-710µm Langton 1991; 450µm, Säwedal 1976) ……………………………………………………………………………... *M. lindrothi*

15’. Anal lobe with <48 taeniae; thoracic horn 378-553µm; moderate nose on wing sheath ..………………………………………………………………...….. *M. xantha*

16. Thoracic horn setae covering apical two-thirds and diminishing in length towards tip; setae short, less than one-fifth of horn length (Oliver and Dillon 1994: Fig. 10; Langton 1998: Fig. 2c) …………………………………………………………………………. 17

16’. Thoracic horn setae covering apical five-sixths and not diminishing significantly in length towards tip; setae long, approximately one-third of horn length (Fig. 3-5f; Oliver and Dillon 1994: Fig 4) ……………………………………………………...………. 18

17. Cephalothorax with two median antepronotal setae; wing sheath without nose; anal lobe with 48-66 taeniae …………………………………………...…….. *M. silvesterae*

17’. Cephalothorax with one median antepronotal seta; wing sheath with small nose; anal lobe with 22-52 taeniae …………………………………………………..….. *M. nigripila*

18. Tergite VI with paired anterior point patches (Oliver and Dillon 1994: 3); anal lobe with 31-36 taeniae ……………………………………...……………. *M. geminata*

18’. Tergite VI consisting only of shagreen (Fig. 3-5b); anal lobe with 40-47 taeniae .... ***M. subletteorum* n. sp.**

Taxonomic note: *Micropsectra brundini* was described from oligotrophic lakes in Greenland (Säwedal 1979) and is morphologically similar to *M. lindebergi* and *M. insignilobus* in both the adult male and pupal stages; females from this species are not yet known. The type material of this species could not be located at ZSBS (M. Spies, personal communication). We unsuccessfully attempted to locate type material of this specimen at various other museums. We have, however, examined material identified as *M. brundini* in the collections of Peter Langton, Claus Lindegaard, and the Sublette material at UMSP and confirm that this species is morphologically very similar to *M. lindebergi* and *M. insignilobus*. Values for the AR seem to be the most consistent and obvious differences used to separate *M. brundini* from *M. lindebergi* (1.75-2.14 and 1.31-1.58, respectively) and a longer median volsella aids in differentiation from *M. insignilobus* (63-78µm as compared to 40-59µm). Säwedal (1979) lists other characters that may aid in differentiation; however there is considerable overlap between these species, particularly *M. brundini* and *M. insignilobus*. It is beyond the scope of this paper to resolve these taxonomic issues; however, we believe sampling of localities known to produce *M. brundini* and subsequent molecular analyses of fresh specimens could help assess relationships between these species.

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Tables

Table 3-1. List of specimens sequenced in this study. Pex = associated with pupal exuviae; Lhc = with associated larval head capsule.

Specimen Voucher ID	Accession No. COI; CAD	Species & Lifestage	Collection Locality	Coordinates	Elevation	Collection Date	Gene(s) Sequenced	Collector
***Micropsectra neoappendica* new species**								
Aly10	JQ667044	Male adult, Pex	USA, Minnesota, Cook Co., Brule River	47°56'35"; -90°26'17"	499	11.iv.2010	COI	A.M. Anderson
Aly29	JQ667045; JQ672506	Male adult, Pex	USA, Minnesota, Cook Co., Fiddle Creek	47°56'05"; -90°26'12"	505	11.iv.2010	COI; CAD	A.M. Anderson
Aly31	JQ667037; JQ672504	Male adult, Pex	USA, Minnesota, Cook Co., Fiddle Creek	47°56'05"; -90°26'12"	505	11.iv.2010	COI; CAD	A.M. Anderson
Aly34	JQ667038	Male adult, Pex	USA, Minnesota, Cook Co., Fiddle Creek	47°56'05"; -90°26'12"	505	11.iv.2010	COI	A.M. Anderson
Aly42	JQ667039	Male adult, Pex	USA, Minnesota, Cook Co., Fiddle Creek	47°56'05"; -90°26'12"	505	11.iv.2010	COI	A.M. Anderson
Aly43	JQ667040	Male adult, Pex	USA, Minnesota, Cook Co., Fiddle Creek	47°56'05"; -90°26'12"	505	11.iv.2010	COI	A.M. Anderson
Aly28	JQ667041	Female adult, Pex	USA, Minnesota, Cook Co., Fiddle Creek	47°56'05"; -90°26'12"	505	11.iv.2010	COI	A.M. Anderson
Aly32	JQ667042; JQ672505	Female adult, Pex	USA, Minnesota, Cook Co., Fiddle Creek	47°56'05"; -90°26'12"	505	11.iv.2010	COI; CAD	A.M. Anderson
Aly36	JQ667043	Female adult, Pex	USA, Minnesota, Cook Co., Fiddle Creek	47°56'05"; -90°26'12"	505	11.iv.2010	COI	A.M. Anderson
***Micropsectra penicillata* new species**								
Aly05	JQ667051	Male adult, Pex	USA, Minnesota, Cook Co., Brule River	47°56'35"; -90°26'17"	499	11.iv.2010	COI	A.M. Anderson

Table 3-1. Continued.

Specimen Voucher ID	Accession No. COI; CAD	Species & Lifestage	Collection Locality	Coordinates	Elevation	Collection Date	Gene(s) Sequenced	Collector
Aly17	JQ667050; JQ672509	Male adult, Pex	USA, Minnesota, Cook Co., Brule River	47°56'35"; -90°26'17"	499	11.iv.2010	COI; CAD	A.M. Anderson
Aly18	JQ667049	Male adult, Pex	USA, Minnesota, Cook Co., Brule River	47°56'35"; -90°26'17"	499	11.iv.2010	COI	A.M. Anderson
Aly22	JQ667048	Male adult, Pex	USA, Minnesota, Cook Co., Brule River	47°56'35"; -90°26'17"	499	11.iv.2010	COI	A.M. Anderson
Aly71	JQ667047; JQ672508	Male adult, Pex	USA, Minnesota, Cook Co., Brule River	47°56'35"; -90°26'17"	499	11.iv.2010	COI; CAD	A.M. Anderson
Aly23	JQ667046; JQ672507	Female adult, Pex	USA, Minnesota, Cook Co., Brule River	47°56'35"; -90°26'17"	499	11.iv.2010	COI; CAD	A.M. Anderson
CHIR_CH9	JQ667595	Male adult	Canada, Manitoba, Churchill, Ramsey Creek	58°43'50”; --93°46'48”	13	15.viii.2006	COI	T. Ekrem, E. Stur
CHIR_CH119	JQ667594	Male adult	Canada, Manitoba, Churchill, Ramsey Creek	58°43'50”; --93°46'48”	13	23.viii.2006	COI	T. Ekrem, E. Stur
***Micropsectra subletteorum* new species**								
Aly11	JQ667055	Female adult, Pex	USA, Minnesota, Cook Co., Brule River	47°56'35"; -90°26'17"	499	11.iv.2010	COI	A.M. Anderson
Aly12	JQ667057	Female adult, Pex	USA, Minnesota, Cook Co., Brule River	47°56'35"; -90°26'17"	499	11.iv.2010	COI	A.M. Anderson
Aly74	JQ667056; JQ672510	Female adult, Pex	USA, Minnesota, Cook Co., Brule River	47°56'35"; -90°26'17"	499	11.iv.2010	COI; CAD	A.M. Anderson

Table 3-1. Continued.

Specimen Voucher ID	Accession No. COI; CAD	Species & Lifestage	Collection Locality	Coordinates	Elevation	Collection Date	Gene(s) Sequenced	Collector
Aly75	JQ667058	Female adult, Pex	USA, Minnesota, Cook Co., Brule River	47°56'35"; -90°26'17"	499	11.iv.2010	COI	A.M. Anderson
Aly38	JQ667054	Female adult, Pex	USA, Minnesota, Cook Co., Fiddle Creek	47°56'05"; -90°26'12"	505	11.iv.2010	COI	A.M. Anderson
Aly99	JQ667053	Male adult, Pex	USA, Minnesota, Goodhue Co., Trout Brook	44°32'97"; -92°48'07"	229	30.iii.2008	COI	R.W. Bouchard
Aly100	JQ667052	Male adult, Pex	USA, Minnesota, Goodhue Co., Trout Brook	44°32'97"; -92°48'07"	229	30.iii.2008	COI	R.W. Bouchard
Aly101	JQ667070	Male adult, Pex	USA, Minnesota, Goodhue Co., Trout Brook	44°32'97"; -92°48'07"	229	30.iii.2008	COI	R.W. Bouchard
Aly102	JQ667069; JQ672512	Male adult, Pex	USA, Minnesota, Goodhue Co., Trout Brook	44°32'97"; -92°48'07"	229	30.iii.2008	COI; CAD	R.W. Bouchard
Aly103	JQ667068	Male adult, Pex	USA, Minnesota, Goodhue Co., Trout Brook	44°32'97"; -92°48'07"	229	30.iii.2008	COI	R.W. Bouchard
Aly104	JQ667067	Male adult, Pex	USA, Minnesota, Goodhue Co., Trout Brook	44°32'97"; -92°48'07"	229	30.iii.2008	COI	R.W. Bouchard
Aly98	JQ667066	Female adult, Pex	USA, Minnesota, Goodhue Co., Trout Brook	44°32'97"; -92°48'07"	229	1.iv.2010	COI	A.M. Anderson
Aly89	JQ667065	Male adult, Pex	USA, Minnesota, Washington Co., Valley Creek	44°55'09"; -92°47'54"	218	3.iii.2010	COI	A.M. Anderson
Aly90	JQ667064	Male adult, Pex	USA, Minnesota, Washington Co., Valley Creek	44°55'09"; -92°47'54"	218	3.iii.2010	COI	A.M. Anderson

Table 3-1. Continued.

Specimen Voucher ID	Accession No. COI; CAD	Species & Lifestage	Collection Locality	Coordinates	Elevation	Collection Date	Gene(s) Sequenced	Collector
Aly92	JQ667063	Male adult, Pex	USA, Minnesota, Washington Co., Valley Creek	44°55'09"; -92°47'54"	218	3.iii.2010	COI	A.M. Anderson
Aly93	JQ667062; JQ672511	Male adult, Pex	USA, Minnesota, Washington Co., Valley Creek	44°55'09"; -92°47'54"	218	3.iii.2010	COI; CAD	A.M. Anderson
Aly95	JQ667061	Male adult, Pex, Lhc	USA, Minnesota, Washington Co., Valley Creek	44°55'09"; -92°47'54"	218	3.iii.2010	COI	A.M. Anderson
Aly97	JQ667060	Male adult, Pex	USA, Minnesota, Washington Co., Valley Creek	44°55'09"; -92°47'54"	218	3.iii.2010	COI	A.M. Anderson
Aly86	JQ667059	Female adult, Pex	USA, Minnesota, Washington Co., Valley Creek	44°55'09"; -92°47'54"	218	3.iii.2010	COI	A.M. Anderson
CHIR_CH219	JQ667596	Male adult	Canada, Manitoba, Churchill, Ramsey Creek	58°43'50"; --93°46'48"	13	15.viii.2006	COI	T. Ekrem, E. Stur
***Micropsectra xantha* Roback, 1955**								
Aly47	JQ667071	Male adult, Pex	USA, Minnesota, Goodhue Co., Cold Spring Brook	44°17'46"; -92°25'35"	256	25.iv.2010	COI	A.M. Anderson
Aly50	JQ667072	Male adult, Pex	USA, Minnesota, Goodhue Co., Cold Spring Brook	44°17'46"; -92°25'35"	256	25.iv.2010	COI	A.M. Anderson
Aly52	JQ667073	Male adult, Pex	USA, Minnesota, Goodhue Co., Cold Spring Brook	44°17'46"; -92°25'35"	256	25.iv.2010	COI	A.M. Anderson
Aly59	JQ667074; JQ672513	Male adult, Pex	USA, Minnesota, Goodhue Co., Cold Spring Brook	44°17'46"; -92°25'35"	256	25.iv.2010	COI; CAD	A.M. Anderson

Table 3-1. Continued.

Specimen Voucher ID	Accession No. COI; CAD	Species & Lifestage	Collection Locality	Coordinates	Elevation	Collection Date	Gene(s) Sequenced	Collector
Aly62	JQ667075; JQ672514	Male adult, Pex	USA, Minnesota, Goodhue Co., Cold Spring Brook	44°17'46"; -92°25'35"	256	25.iv.2010	COI; CAD	A.M. Anderson
Aly76	JQ667076	Male adult, Pex	USA, Minnesota, Goodhue Co., Cold Spring Brook	44°17'46"; -92°25'35"	256	25.iv.2010	COI	A.M. Anderson
Aly51	JQ667077	Female adult, Pex	USA, Minnesota, Goodhue Co., Cold Spring Brook	44°17'46"; -92°25'35"	256	25.iv.2010	COI	A.M. Anderson
Aly65	JQ667078; JQ672515	Female adult, Pex	USA, Minnesota, Goodhue Co., Cold Spring Brook	44°17'46"; -92°25'35"	256	25.iv.2010	COI; CAD	A.M. Anderson
Aly81	JQ667079	Female adult, Pex, Lhc	USA, Minnesota, Goodhue Co., Cold Spring Brook	44°17'46"; -92°25'35"	256	25.iv.2010	COI	A.M. Anderson

Table 3-2. Measurements and ratios for adult males of *Micropsectra* species. Lengths in µm unless indicated. Data are presented as ranges, followed by the mean with the number of specimens observed in parentheses.

Parameter	*M. neoappendica*	*M. penicillata*	*M. xantha*	*M. sublettorum*
Wing				
Wing length (mm)	2.39-2.56, 2.46 (6)	2.14-2.44, 2.27 (8)	2.21-2.98, 2.61 (7)	2.77-3.27, 3.03 (8)
Wing width (mm)	0.59-0.62, 0.61 (4)	0.53-0.65, 0.59 (8)	0.57-0.76, 0.65 (6)	0.70-0.85, 0.77 (7)
VR	1.09-1.13, 1.11 (4)	1.05-1.20, 1.10 (8)	1.03-1.13, 1.08 (6)	1.03-1.07, 1.05 (8)
Head				
Terminal flagellomere length	650-705, 681 (5)	590-720, 649 (4)	640-820, 753 (5)	805-930, 858 (6)
AR	1.20-1.37, 1.27 (5)	1.13-1.38, 1.29 (4)	1.16-1.37, 1.29 (5)	1.47-1.70, 1.58 (6)
Head width	450-490, 466 (4)	405-510, 443 (6)	440-540, 492 (7)	500-675, 592 (7)
Distance between eyes	195-240, 226 (5)	150-200, 171 (7)	160-230, 199 (5)	210-260, 234 (7)
Palpomere lengths	45-58, 50 (6); 45-55, 49 (6); 145-158, 153 (6); 130-150, 141 (6); 233-255, 246 (4)	30-50, 42 (7); 40-50, 44 (7); 133-170, 142 (6); 110-150, 127 (6); 170-290, 231 (5)	46-75, 55 (7); 51-75, 63 (6); 153-213, 186 (6); 123-185, 160 (6); 295-320, 304 (4)	36-78, 54 (7); 43-68, 57 (7); 168-213, 195 (7); 168-190, 178 (7); 270-345, 293 (6)
Cephalic tubercle length	8-11, 9 (6)	5-13, 8 (6)	3-5, 4 (3)	5-10, 7 (7)
No. inner verticals	5-6, 5 (4)	4-5, 5 (7)	4-5, 5 (6)	7-9, 7 (7)
No. outer verticals	3-4, 3 (4)	3-4, 4 (7)	4-5, 4 (6)	4-5, 5 (6)
No. post orbitals	1-1, 1 (5)	1-2, 2 (7)	2-3, 3 (4)	2-2, 2 (6)
Legs				
LR_1	1.50-1.55, 1.52 (6)	1.42-1.55, 1.49 (7)	1.56-1.74, 1.66 (6)	1.52-1.54, 1.53 (5)
BR_1	3-5, 4 (5)	3-5, 4 (7)	4-4, 4 (5)	3-5, 4 (5)
BV_1	1.85-1.89, 1.87 (4)	1.89-2.04, 1.98 (7)	1.81-1.88, 1.84 (6)	1.75-1.87, 1.80 (5)
SV_1	1.54-1.60, 1.57 (6)	1.53-1.71, 1.61 (7)	1.43-1.52, 1.47 (6)	1.53-1.59, 1.56 (5)
LR_2	0.55-0.59, 0.57 (6)	0.53-0.57, 0.55 (7)	0.58-0.70, 0.62 (5)	0.56-0.61, 0.59 (5)
BR_2	5-7, 5 (6)	3-6, 5 (7)	3-5, 4 (3)	4-7, 5 (5)
LR_3	0.68-0.75, 0.71 (5)	0.65-0.68, 0.67 (4)	0.68-0.71, 0.70 (6)	0.68-0.71, 0.70 (5)
BR_3	5-7, 6 (5)	5-6, 5 (4)	4-6, 4 (6)	5-7, 6 (5)
Thorax				
No. dorsocentrals	7-11, 10 (6)	9-14, 11 (6)	10-12, 11 (6)	9-14, 11 (6)
No. acrosticals	14-18, 16 (6)	11-13, 12 (6)	11-17, 15 (5)	7-14, 12 (4)

Table 3-2. Continued.

Parameter	*M. neoappendica*	*M. penicillata*	*M. xantha*	*M. sublettorum*
No. prealars	2-3, 3 (6)	2-3, 3 (6)	2-3, 3 (5)	2-4, 3 (5)
No. scutellars	6-8, 7 (6)	7-8, 8 (5)	6-8, 7 (6)	8-11, 10 (6)
No. halterals	6-7, 7 (6)	4-5, 5 (6)	5-11, 7 (5)	7-12, 9 (5)
Hypopygium				
Tergite IX length	163-205, 183 (6)	145-173, 159 (6)	195-248, 228 (6)	205-229, 218 (7)
Gonostylus length	150-170, 163 (6)	143-175, 157 (8)	163-203, 184 (7)	193-223, 210 (7)
Gonocoxite length	165-185, 174 (6)	146-170, 159 (8)	180-200, 193 (6)	180-220, 203 (7)
HR	1.01-1.14, 1.07 (6)	0.93-1.08, 1.01 (8)	0.99-1.10, 1.03 (6)	0.90-1.02, 0.97 (7)
Anal point length	63-74, 68 (6)	53-60, 58 (5)	65-75, 70 (6)	61-74, 68 (7)
No. median setae	4-5, 4 (6)	3-3, 3 (5)	2-4, 3 (7)	3-7, 5 (8)
No. apical setae	9-11, 10 (6)	7-8, 7 (5)	8-10, 9 (6)	8-12, 10 (7)
Median volsella length	120-125, 123 (6)	103-138, 122 (8)	83-114, 96 (6)	68-85, 74 (7)
Median volsella lammellae length	10-16, 13 (6)	20-38, 26 (8)	26-43, 34 (7)	18-31, 24 (7)
Inferior volsella length	125-148, 134 (6)	105-135, 121 (8)	124-148, 135 (7)	135-165, 149 (7)
No. superior volsella dorsal setae	6-8, 7 (6)	4-6, 5 (7)	5-7, 6 (6)	4-7, 6 (8)
No. superior volsella median setae	3-4, 4 (6)	2-2, 2 (8)	2-2, 2 (7)	2-3, 2 (8)

Table 3-3. Measurements of pupal exuviae and pupal structures of *Micropsectra* species. Lengths in µm unless indicated. Data are presented as ranges, followed by the mean with the number of specimens observed in parenthesis.

Parameter	*M. neoappendica*	*M. penicillata*	*M. xantha*	*M. sublettorum*
Total length (mm)	4.34-4.81, 4.63 (6)	3.89-4.68, 4.31 (6)	4.77-5.91, 5.28 (6)	5.05-5.65, 5.39 (6)
Abdomen length (mm)	3.20-3.67, 3.47 (7)	2.87-3.52, 3.19 (6)	3.58-4.42, 3.99 (4)	3.70-4.31, 4.08 (6)
Cephalothorax				
Frontal setae length:	50-123, 80 (6)	95-130, 111 (5)	130-198, 161 (3)	78-180, 132 (6)
Cephalic tubercle length	25-30, 27 (6)	20-35, 26 (6)	25-33, 28 (3)	30-51, 38 (6)
Thoracic horn length	265-395, 306 (6)	303-375, 336 (6)	378-553, 507 (3)	350-580, 450 (6)
Thoracic horn width	20-40, 28 (6)	23-48, 38 (6)	28-58, 44 (3)	48-65, 57 (5)
Thoracic horn chaetae length	148-173, 157 (6)	118-158, 135 (6)	160-233, 189 (3)	175-223, 198 (5)
Anterior precorneal length	73-115, 85 (6)	55-85, 71 (5)	78-125, 99 (3)	80-150, 113 (6)
Median precorneal length	80-133, 100 (6)	55-115, 74 (6)	110-146, 122 (3)	80-148, 110 (6)
Posterior precorneal length	130-173, 146 (6)	128-190, 153 (6)	130-203, 164 (4)	148-243, 194 (6)
Median antepronotal length	80-95, 87 (5)	85-118, 98 (6)	78-130, 109 (3)	95-153, 115 (4)
Lateral antepronotal length	68-80, 73 (6)	78-105, 93 (5)	68-130, 90 (4)	58-83, 68 (5)
DC_1	55-78, 70 (6)	70-105, 85 (5)	53-85, 70 (4)	63-115, 96 (5)
DC_2	38-45, 42 (4)	48-73, 56 (5)	43-75, 50 (2)	45-60, 53 (6)
DC_3	55-100, 85 (6)	85-118, 99 (5)	70-108, 86 (4)	80-145, 112 (6)
DC_4	65-85, 73 (6)	78-113, 95 (5)	70-100, 80 (4)	75-140, 106 (6)
Abdomen				
Hook row width	240-285, 254 (6)	258-315, 278 (5)	270-410, 350 (4)	375-425, 401 (6)
Spine/spinule patch TIII	200-235, 218 (7)	148-190, 165 (6)	170-270, 220 (4)	200-255, 223 (6)

Table 3-3. Continued.

Parameter	*M. neoappendica*	*M. penicillata*	*M. xantha*	*M. sublettorum*
Spine/spinule patch TIV	283-338, 313 (7)	285-325, 308 (6)	300-383, 350 (4)	320-420, 355 (6)
Spine/spinule patch TV	195-268, 236 (7)	190-280, 225 (6)	295-380, 339 (4)	310-410, 348 (6)
Spinepatch VI	193-230, 205 (5)	145-175, 164 (4)	255-350, 308 (4)	310-385, 339 (6)
Spine Length TIII	73-85, 78 (5)	48-60, 56 (3)	50-53, 51 (2)	50-50, 50 (1)
Spine Length TIV	65-85, 76 (4)	53-55, 54 (3)	15-16, 16 (2)	20-20, 20 (2)
Spine Length TV	9-13, 11 (5)	13-18, 15 (3)	13-15, 14 (2)	15-19, 17 (2)
Spine Length TVI	4-5, 4 (4)	4-4, 4 (3)	3-5, 4 (2)	4-6, 5 (2)
No. apical teeth on comb	3-5, 5 (6)	3-6, 4 (5)	4-6, 5 (4)	5-6, 6 (6)
No. taeniae in anal fringe	27-33, 30 (7)	23-31, 28 (6)	35-48, 43 (4)	40-47, 44 (6)

Figures

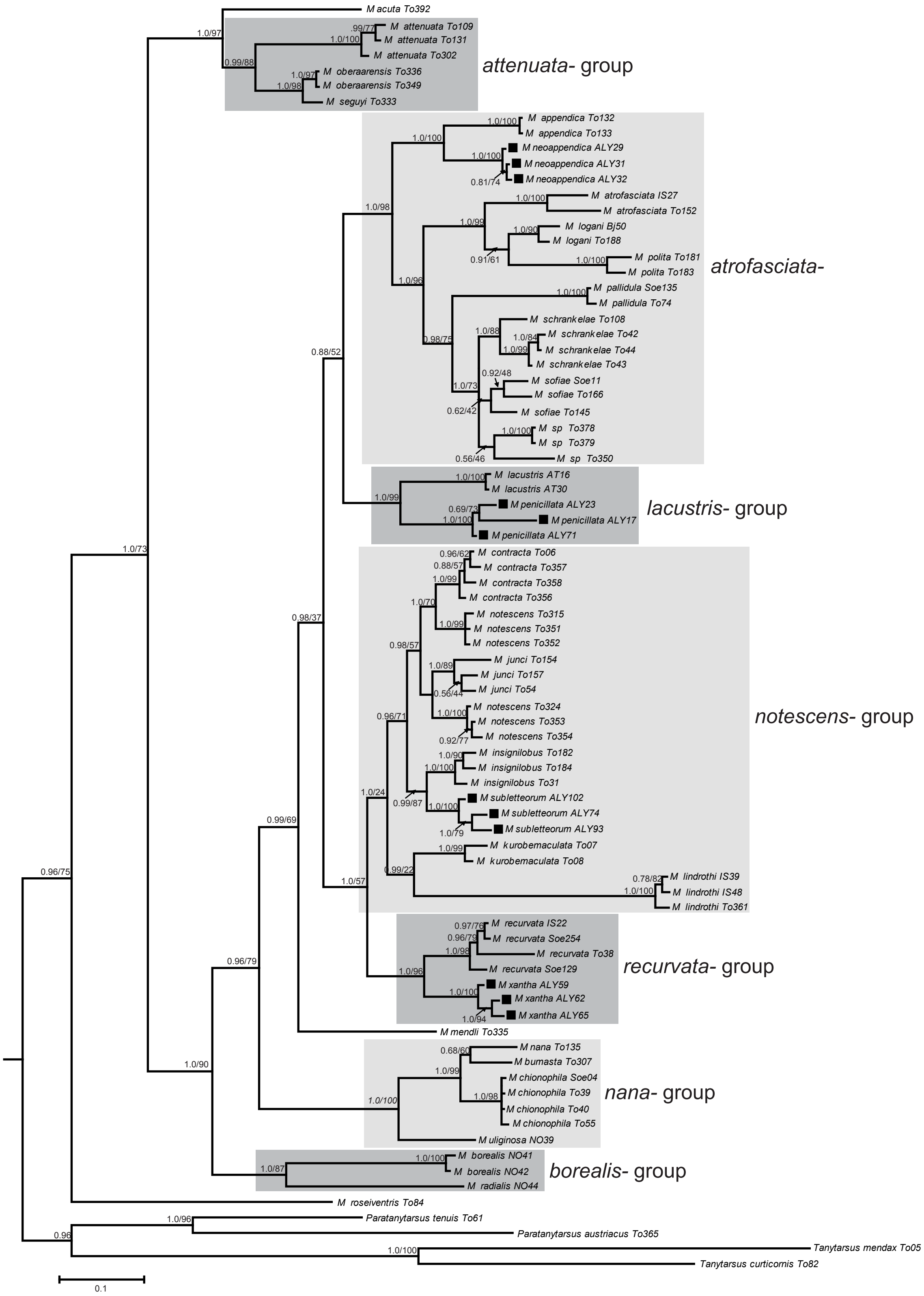

Figure 3-1. See next page for caption.

Figure 3-1. CAD phylogeny of 82 individuals from 38 species. The twelve taxa denoted with a '■' were collected in Minnesota and are described in this study. Shaded boxes enclose current and/or proposed species groups. Values on branches indicate Bayesian posterior probabilities and ML bootstrap support values, respectively. The scale bar indicates branch lengths as number of substitutions per site corrected by the T92+G+I model.

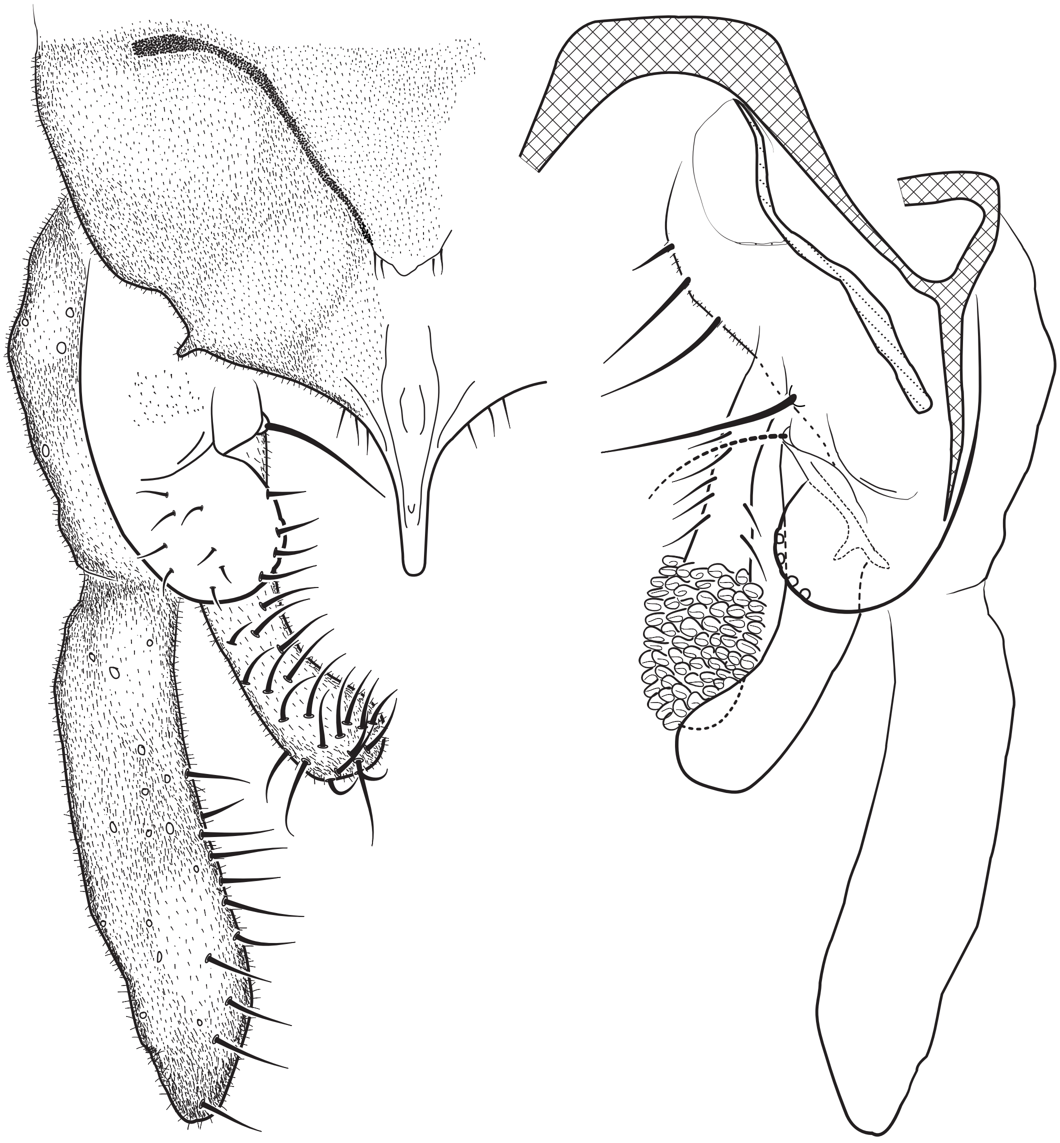

Figure 3-2. *Micropsectra neoappendica* n. sp., male hypopygium.

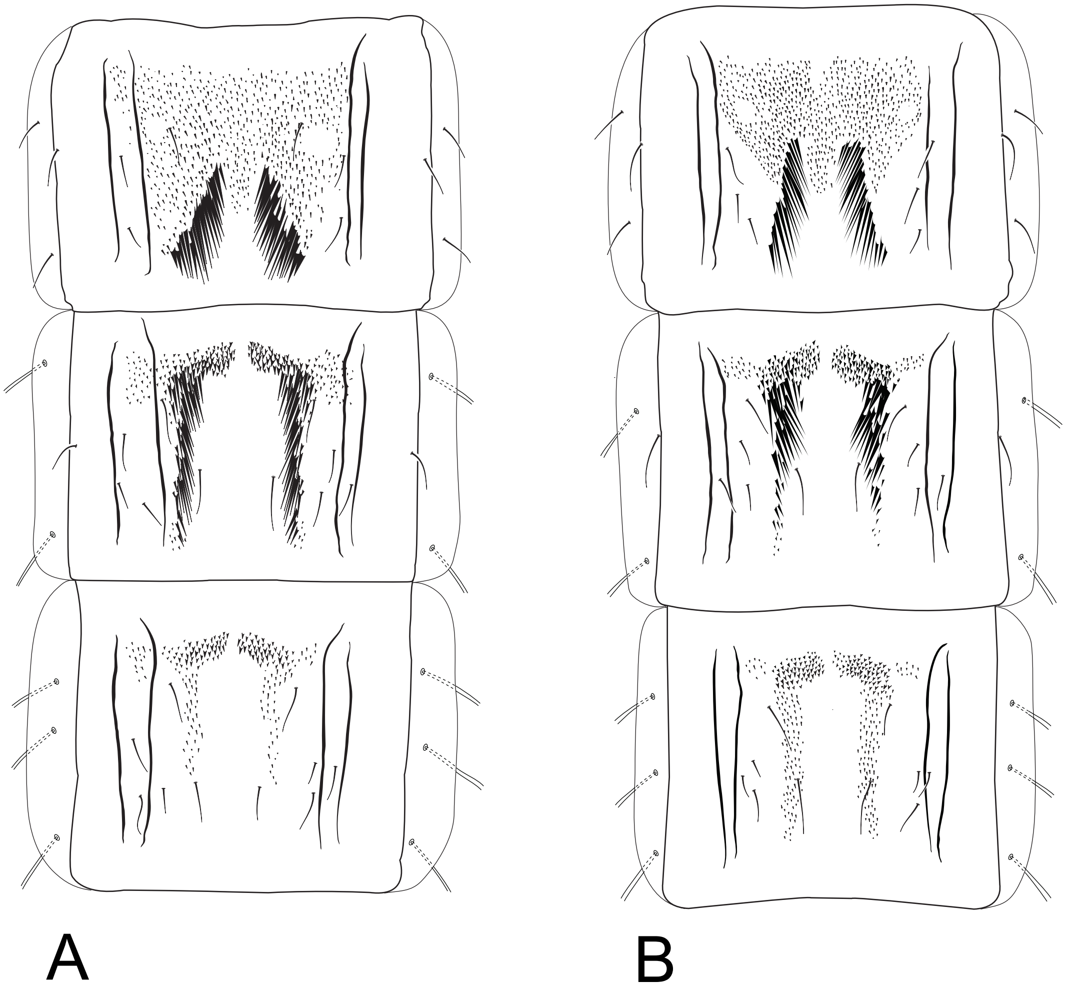

Figure 3-3. Pupal abdominal segments III-V. A. –*Micropsectra neoappendica*, n. sp. B. –*Micropsectra appendica*.

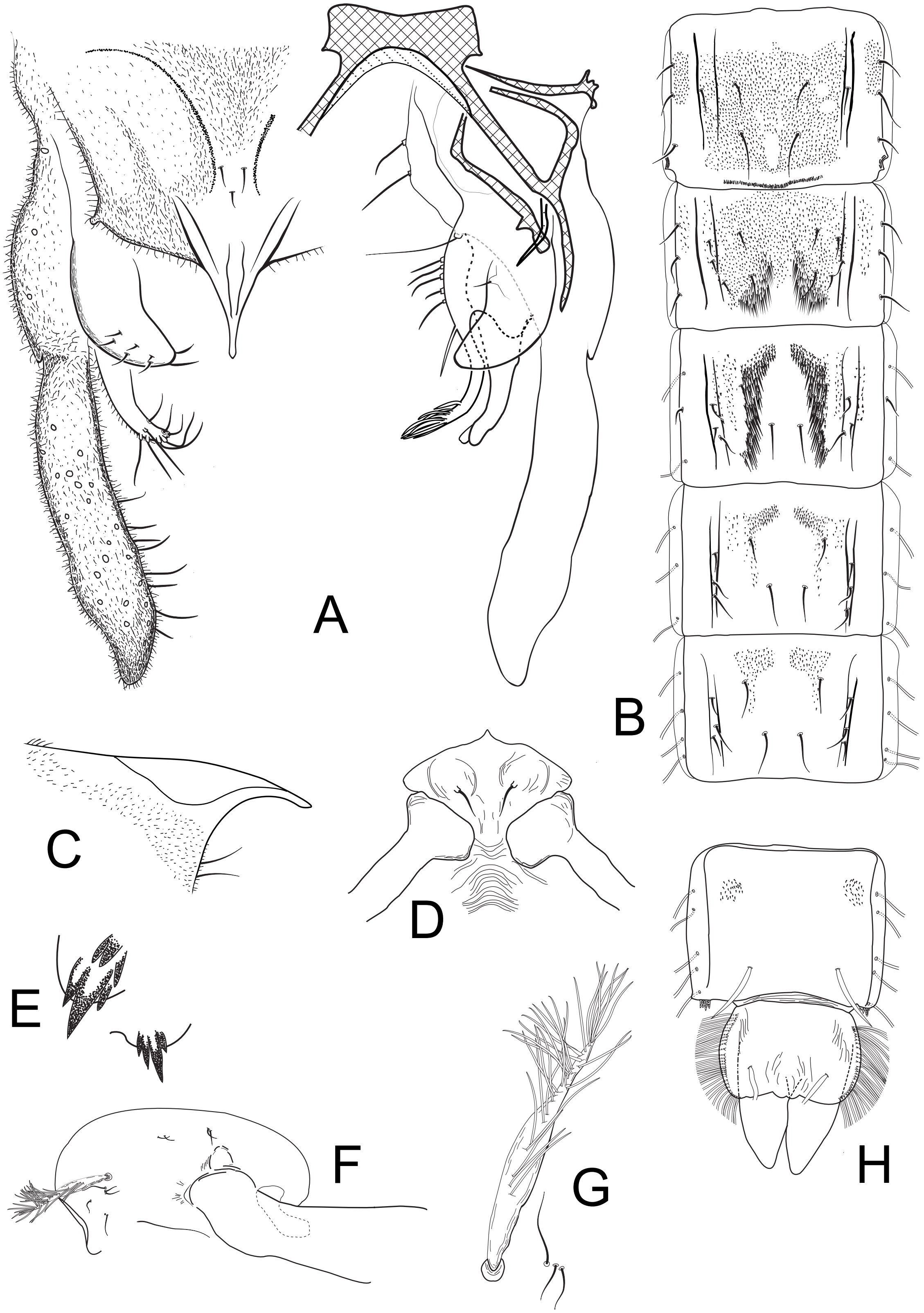

Figure 3-4. See next page for caption.

Figure 3-4. *Micropsectra penicillata* n. sp., male. (a.) Hypopygium; (b.) Pupal abdominal segments II-VI, dorsal; (c.) Anal point, lateral view; (d.) Pupal frontal apotome; (e.) Posterolateral combs of pupal segment VIII; (f.) Pupal thorax; (g.) Pupal thoracic horn; (h.) Pupal abdominal segments VIII-IX.

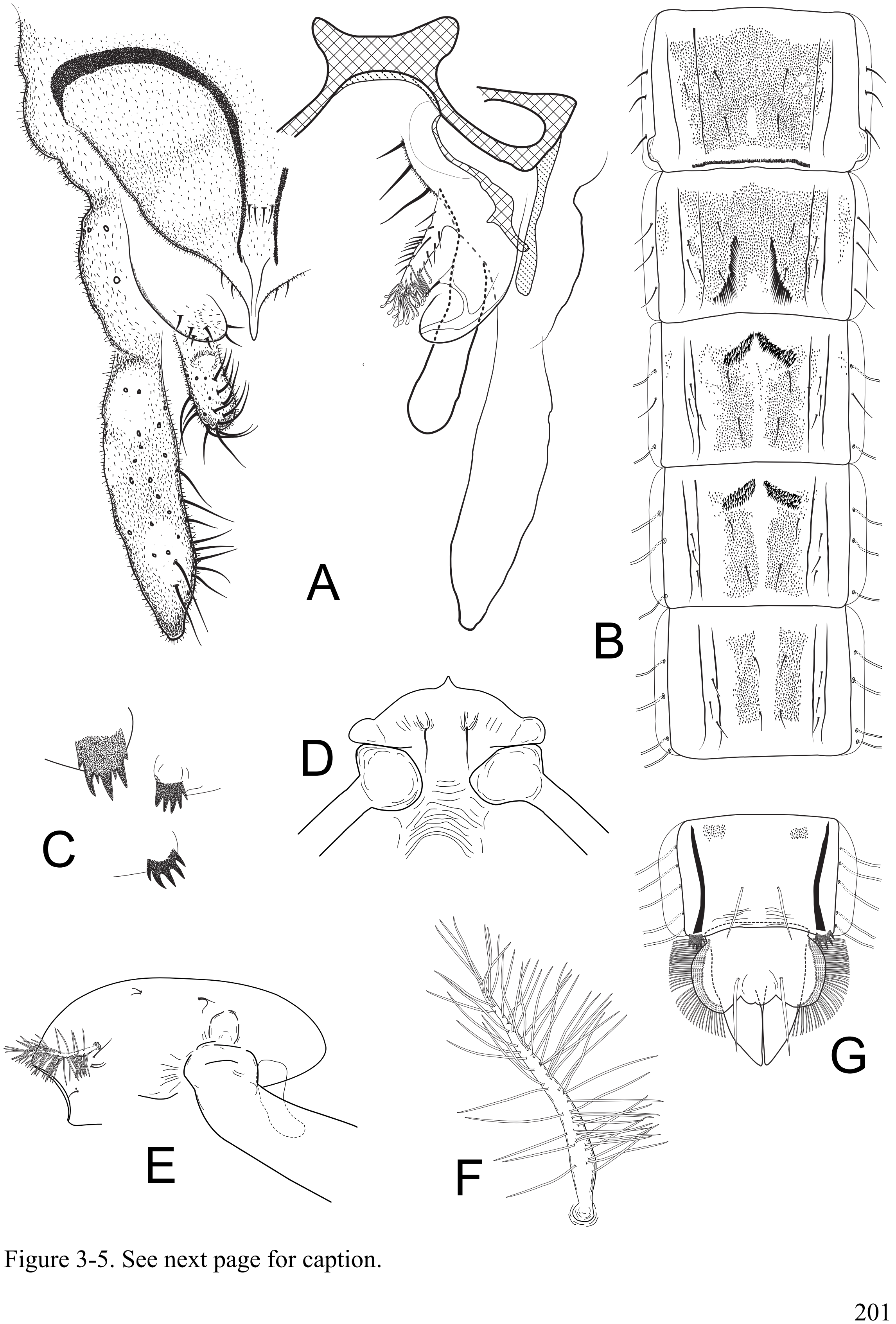

Figure 3-5. See next page for caption.

Figure 3-5. *Micropsectra subletteorum* n. sp., male. (a.) Hypopygium; (b.) Pupal abdominal segments II-VI, dorsal; (c.) Posterolateral combs of pupal segment VIII; (d.) Pupal frontal apotome; (e.) Pupal thorax; (f.) Pupal thoracic horn; (g.) Pupal abdominal segments VIII-IX.

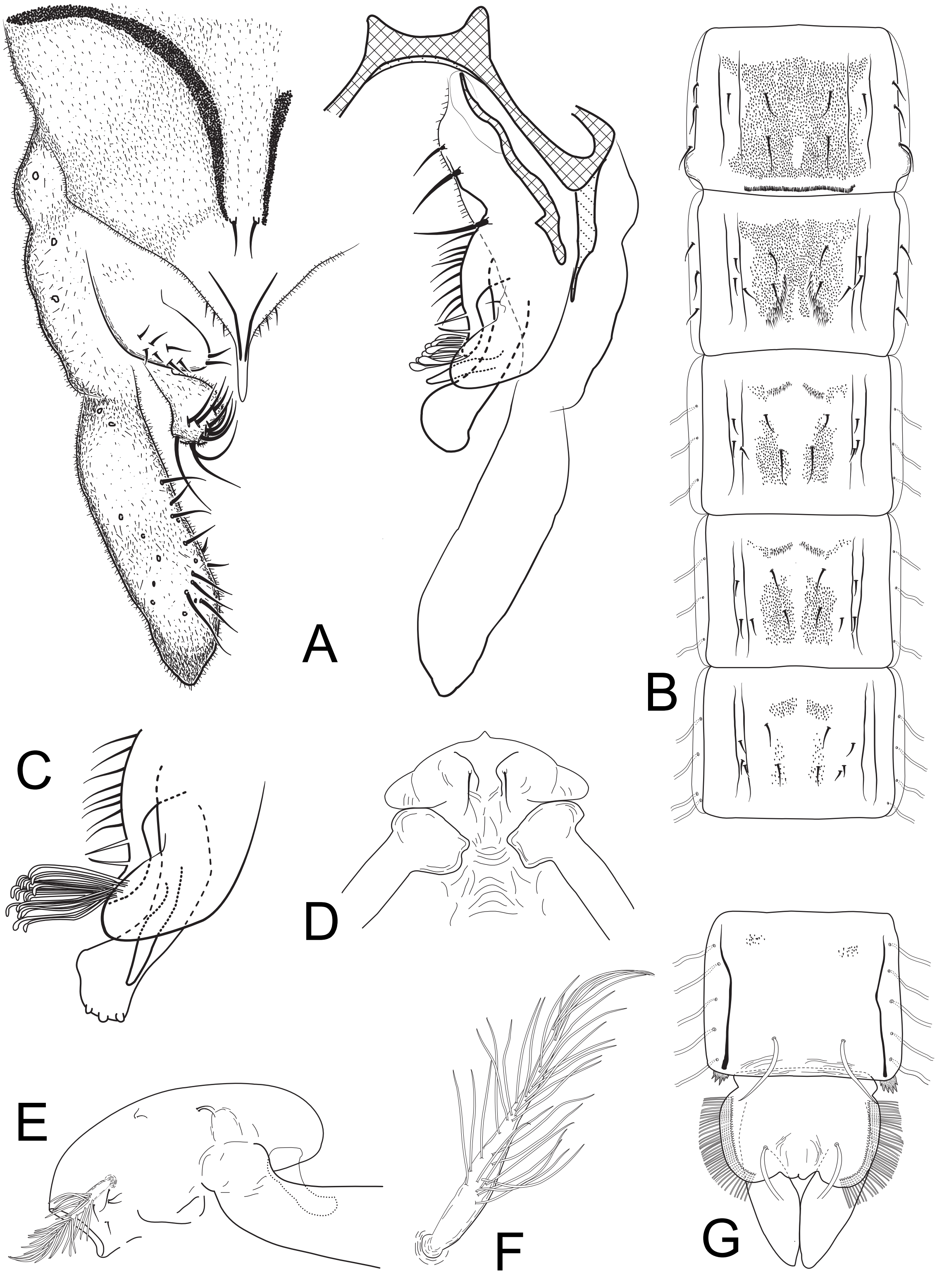

Figure 3-6. See next page for caption.

Figure 3-6. *Micropsectra xantha*, male. (a.) Hypopygium; (b.) Pupal abdominal segments II-VI, dorsal; (c.) Superior, median, and inferiorior volsellae; (d.) Pupal frontal apotome; (e.) Pupal thorax; (f.) Pupal thoracic horn; (g.) Pupal abdominal segments VIII-IX.

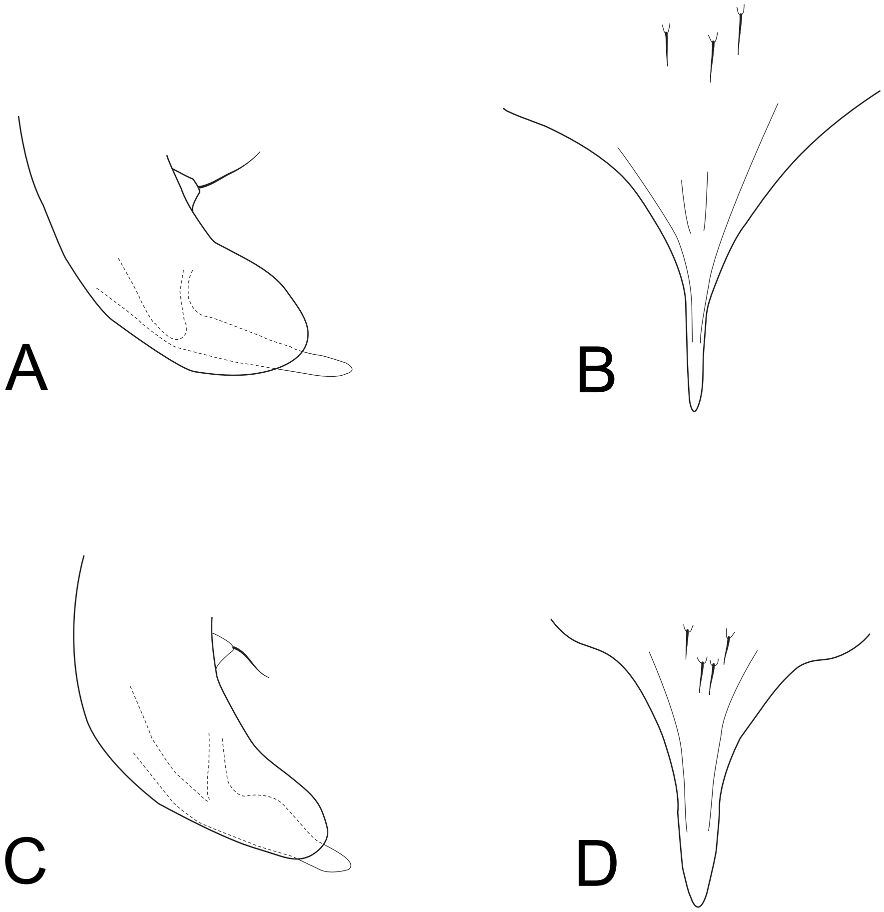

Figure 3-7. (a.) Superior volsella, *M. xantha*; (b.) Anal point, *M. xantha*; (c.) Superior volsella, *M. connexa*; (d.) Anal point, *M. connexa*.

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Appendices

Appendix A. Frequency of occurance, percent by number, and percent by weight of prey found in trout diets in Beaver Creek. Values represent the composite of samples from all sampling periods.

Beaver Creek	**Frequency of Occurance**			**Percent by Number**			**Percent by Weight**		
	All fish sampled n = 207	**Small fish (<151mm) n = 117**	**Large fish (>151mm) n = 90**	**All fish sampled n = 207**	**Small fish (<151mm) n = 117**	**Large fish (>151mm) n = 90**	**All fish sampled n = 207**	**Small fish (<151mm) n = 117**	**Large fish (>151mm) n = 90**
Ephemeroptera	**27.50%**	**18.80%**	**38.89%**	**5.35%**	6.66%	4.69%	4.26%	7.38%	3.52%
Baetidae	12.08%	5.98%	20.00%	1.43%	1.09%	1.60%	0.56%	0.56%	0.56%
Baetis	12.08%	5.98%	20.00%	1.43%	1.09%	1.60%	0.56%	0.56%	0.56%
Ephemerellidae	20.29%	14.53%	27.78%	3.64%	5.08%	2.90%	3.50%	5.97%	2.92%
Ephemerella	20.29%	14.53%	27.78%	3.64%	5.08%	2.90%	3.50%	5.97%	2.92%
Heptageniidae	0.97%	1.71%	0.00%	0.08%	0.24%	0.00%	0.13%	0.66%	0.00%
Stenonema	0.97%	0.85%	0.00%	0.04%	0.12%	0.00%	0.05%	0.28%	0.00%
Leptophlebiidae	1.93%	1.71%	2.22%	0.16%	0.24%	0.12%	0.07%	0.20%	0.04%
Leptophlebia	1.93%	0.85%	1.11%	0.16%	0.24%	0.12%	0.07%	0.20%	0.04%
Siphlonuridae	-	-	-	-	-	-	-	-	-
Odonata	**0.48%**	**0.00%**	**1.11%**	**0.04%**	**0.00%**	0.06%	0.52%	0.00%	0.65%
Aeshnidae	0.48%	0.00%	1.11%	0.04%	0.00%	0.06%	0.52%	0.00%	0.65%
Plecoptera	**23.67%**	**19.66%**	**30.00%**	**4.62%**	5.21%	4.32%	4.74%	8.51%	3.85%
Capniidae	16.91%	12.82%	21.11%	3.19%	3.39%	3.08%	1.67%	2.41%	1.50%
Allocapnia	15.94%	12.82%	20.00%	3.06%	3.39%	2.90%	1.51%	2.41%	1.44%
Allocapnia (a)	0.48%	0.00%	1.11%	0.04%	0.00%	0.06%	0.12%	0.00%	0.00%
Paracapnia	0.97%	0.00%	2.22%	0.08%	0.00%	0.12%	0.04%	0.00%	0.05%
Perlodidae	9.18%	6.84%	11.11%	1.39%	1.82%	1.17%	3.06%	6.10%	2.34%
Isoperla	9.18%	6.84%	12.22%	1.39%	1.82%	1.17%	3.06%	6.10%	2.34%
Taeniopterygidae	-	-	-	-	-	-	-	-	-
Taeniopteryx	-	-	-	-	-	-	-	-	-

Appendix A. Continued.

Beaver (cont.)	**Frequency of Occurance**			**Percent by Number**			**Percent by Weight**		
Megaloptera	**0.48%**	**0.00%**	**1.11%**	**0.04%**	**0.00%**	0.06%	0.12%	0.00%	0.15%
Sialidae	0.48%	0.00%	1.11%	0.04%	0.00%	0.06%	0.12%	0.00%	0.15%
Sialis	0.48%	0.00%	1.11%	0.04%	0.00%	0.06%	0.12%	0.00%	0.15%
Coleoptera	**3.86%**	**2.56%**	**5.56%**	0.37%	0.36%	0.37%	0.16%	0.29%	0.13%
Dryopidae	0.48%	0.00%	1.11%	0.04%	0.00%	0.06%	0.03%	0.00%	0.04%
Helichus	0.48%	0.00%	1.11%	0.04%	0.00%	0.06%	0.03%	0.00%	0.04%
Elmidae	1.45%	0.00%	2.22%	**0.16%**	**0.12%**	0.19%	0.06%	0.12%	0.05%
Optioservus	0.48%	0.00%	1.11%	0.08%	0.00%	0.12%	0.01%	0.00%	0.01%
Optioservus adult	0.48%	0.85%	0.00%	0.04%	0.12%	0.00%	0.02%	0.12%	0.00%
Stenelmis	0.48%	0.00%	1.11%	0.04%	0.00%	0.06%	0.03%	0.00%	0.04%
Dytiscidae	1.93%	1.71%	2.22%	0.16%	0.24%	0.12%	0.06%	0.17%	0.03%
Hygrotus	0.48%	0.85%	0.00%	0.04%	0.12%	0.00%	0.01%	0.07%	0.00%
Laccophilus	-	-	-	-	-	-	-	-	-
Neoporus	0.48%	0.00%	1.11%	0.04%	0.00%	0.06%	0.01%	0.00%	0.02%
Dytiscidae adult	-	-	-	-	-	-	-	-	-
Hydrophilidae	-	-	-	-	-	-	-	-	-
Hydrobius	-	-	-	-	-	-	-	-	-
Diptera	**67.63%**	**58.97%**	**78.89%**	**53.49%**	56.78%	51.82%	14.58%	17.28%	13.95%
Athericidae	-	-	-	-	-	-	-	-	-
Atherix	-	-	-	-	-	-	-	-	-
Ceratopogonidae	-	-	-	-	-	-	-	-	-
Chironomidae	63.29%	55.56%	72.22%	50.02%	53.63%	48.18%	8.65%	14.35%	7.30%
Chironomidae larva	60.87%	52.99%	72.22%	39.89%	49.76%	34.86%	7.70%	13.69%	6.29%
Diamesa	24.64%	14.53%	37.78%	8.79%	9.93%	8.20%	2.60%	5.05%	2.02%
Micropsectra	7.25%	6.84%	7.78%	0.90%	1.57%	0.56%	0.13%	0.37%	0.08%

Appendix A. Continued.

Beaver (cont.)	**Frequency of Occurance**			**Percent by Number**			**Percent by Weight**		
Prodiamesa	-	-	-	-	-	-	-	-	-
Other chironomid larva	49.28%	44.44%	55.56%	30.20%	38.26%	26.10%	4.97%	8.27%	4.19%
Chironomid pupa	15.94%	27.78%	6.84%	3.92%	2.18%	4.75%	0.55%	0.47%	0.57%
Diamesa pupa	14.98%	5.98%	26.67%	3.51%	1.33%	4.63%	0.53%	0.39%	0.57%
Chaetocladius pupa	1.45%	1.71%	2.22%	0.20%	0.36%	0.12%	0.01%	0.04%	0.00%
Cricotopus pupa	0.48%	0.85%	0.00%	0.04%	0.12%	0.00%	0.00%	0.01%	0.00%
Orthocladius pupa	0.97%	1.71%	0.00%	0.12%	0.36%	0.00%	0.01%	0.03%	0.00%
Other chironomid pupa	0.48%	0.00%	0.00%	0.04%	0.00%	0.00%	0.00%	0.00%	0.00%
Chironomid adult	5.80%	3.42%	8.89%	6.21%	1.69%	8.51%	0.39%	0.19%	0.44%
Diamesa adult	7.73%	3.42%	8.89%	6.21%	1.69%	8.51%	0.39%	0.19%	0.44%
Empididae	0.48%	0.85%	0.00%	0.08%	0.24%	0.00%	0.04%	0.23%	0.00%
Ephydridae	-	-	-	-	-	-	-	-	-
Muscidae	-	-	-	-	-	-	-	-	-
Psychodidae	2.42%	0.85%	4.44%	0.20%	0.12%	0.25%	0.06%	0.04%	0.06%
Pericoma	2.42%	0.85%	3.33%	0.16%	0.12%	0.19%	0.06%	0.04%	0.06%
Tipulidae	9.18%	4.27%	15.56%	0.94%	0.85%	0.99%	5.11%	1.88%	5.88%
Antocha	-	-	-	-	-	-	-	-	-
Dicranota	4.35%	3.42%	5.56%	0.45%	0.61%	0.37%	0.51%	0.65%	0.47%
Tipula	4.83%	1.71%	8.89%	0.45%	0.24%	0.56%	4.56%	1.23%	5.35%
Pedicia	-	-	-	-	-	-	-	-	-
Simuliidae	15.46%	11.10%	22.20%	2.08%	1.94%	2.16%	0.68%	0.79%	0.65%
Simuliium	15.46%	11.11%	22.22%	2.08%	1.94%	2.16%	0.68%	0.79%	0.65%
Stratiomyiidae	0.48%	0.00%	1.10%	0.04%	0.00%	0.06%	0.01%	0.00%	0.01%
Caloparyphus	0.48%	0.00%	1.11%	0.04%	0.00%	0.06%	0.01%	0.00%	0.01%

Appendix A. Continued.

Beaver (cont.)	**Frequency of Occurance**			**Percent by Number**			**Percent by Weight**		
Trichoptera	**66.18%**	**62.39%**	**72.22%**	**27.34%**	23.61%	29.24%	57.59%	45.91%	60.36%
Brachycentridae	27.54%	15.38%	44.44%	7.64%	3.27%	9.87%	21.43%	6.93%	24.86%
Brachycentrus	19.81%	6.84%	38.89%	5.84%	0.85%	8.39%	20.70%	5.44%	24.31%
Micrasema	10.63%	10.26%	12.22%	1.80%	2.42%	1.48%	0.74%	1.49%	0.56%
Glossosomatidae	9.66%	4.27%	16.67%	2.33%	1.82%	2.59%	4.02%	4.93%	3.81%
Glossosoma	9.18%	4.27%	15.56%	2.29%	1.82%	2.53%	4.01%	4.93%	3.79%
Glossosoma pupa	0.48%	0.00%	1.10%	0.04%	0.00%	0.06%	0.02%	0.00%	0.02%
Hydropsychidae	2.90%	0.00%	5.56%	0.37%	0.12%	0.49%	0.98%	0.47%	1.10%
Ceratopsyche	1.45%	0.85%	2.22%	0.16%	0.12%	0.19%	0.37%	0.47%	0.34%
Hydropsyche	0.97%	0.00%	2.22%	0.12%	0.00%	0.19%	0.44%	0.00%	0.54%
Lepidostomatidae	45.89%	47.01%	43.33%	11.36%	15.25%	9.38%	4.34%	7.31%	3.64%
Lepidostoma	45.89%	47.01%	44.44%	11.36%	15.25%	9.38%	4.34%	7.31%	3.64%
Limnephilidae	16.91%	5.13%	32.22%	2.17%	0.85%	2.84%	21.24%	19.95%	21.55%
Anabolia	0.48%	0.00%	1.11%	0.04%	0.00%	0.06%	0.35%	0.00%	0.44%
Frenesia	-	-	-	-	-	-	-	-	-
Hesperophylax	2.42%	0.00%	5.56%	0.33%	0.00%	0.49%	2.93%	0.00%	3.62%
Limnephilus	6.76%	4.27%	11.11%	0.69%	0.61%	0.74%	8.94%	17.36%	6.95%
Pycnopsyche	7.25%	1.71%	14.44%	0.94%	0.24%	1.30%	7.81%	2.59%	9.05%
Philopotamidae	-	-	-	-	-	-	-	-	-
Phryganeidae	1.93%	0.00%	3.33%	0.16%	0.12%	0.19%	3.56%	5.25%	3.15%
Ptilostomis	1.93%	0.85%	3.33%	0.16%	0.12%	0.19%	3.56%	5.25%	3.15%
Polycentropodidae	0.48%	0.85%	0.00%	0.04%	0.12%	0.00%	0.00%	0.02%	0.00%
Uenoidae	16.91%	11.11%	24.44%	3.11%	1.94%	3.70%	2.01%	1.06%	2.24%
Neophylax	16.91%	11.11%	25.56%	3.11%	1.94%	3.70%	2.03%	1.06%	2.24%
Hymenoptera	-	-	-	-	-	-	-	-	-

Appendix A. Continued.

Beaver (cont.)	**Frequency of Occurance**			**Percent by Number**			**Percent by Weight**		
Amphipoda	**26.57%**	**18.80%**	**37.78%**	**4.74%**	4.00%	5.12%	16.13%	19.35%	15.36%
Gammaridae	26.57%	17.95%	36.67%	4.74%	4.00%	5.12%	16.13%	19.35%	15.36%
Gammarus	26.57%	17.95%	37.78%	4.74%	4.00%	5.12%	16.13%	19.35%	15.36%
Isopoda	**0.48%**	**0.85%**	**0.00%**	**0.04%**	**0.12%**	0.00%	0.08%	0.41%	0.00%
Collembola	**0.48%**	**0.00%**	**1.11%**	**0.04%**	**0.00%**	0.06%	0.01%	0.00%	0.02%
Poduridae	0.48%	0.00%	1.11%	0.04%	0.00%	0.06%	0.01%	0.00%	0.02%
Podura	0.48%	0.00%	1.11%	0.04%	0.00%	0.06%	0.01%	0.00%	0.02%
Gastropoda	**12.56%**	**4.27%**	**23.33%**	**2.66%**	**2.42%**	**2.78%**	**1.60%**	**0.73%**	**1.80%**
Physidae	12.56%	4.27%	23.33%	2.66%	2.42%	2.78%	1.60%	0.73%	1.80%
Physella	12.56%	4.30%	23.30%	2.66%	2.42%	2.78%	1.60%	0.73%	1.80%
Bivalvia	**0.97%**	**0.00%**	**1.10%**	**0.57%**	**0.12%**	**0.80%**	**0.15%**	**0.02%**	**0.18%**
Acari	**4.35%**	**3.42%**	**5.56%**	**0.37%**	**0.48%**	**0.31%**	**0.05%**	**0.13%**	**0.04%**
Annelida	-	-	-	-	-	-	-	-	-
Cottidae	**2.41%**	**0.00%**	**4.44%**	**0.33%**	**0.24%**	0.37%	na	na	na
Cottus cognatus	2.41%	0.00%	4.40%	0.33%	0.24%	0.37%	na	na	na
Salmonidae eggs	-	-	-	-	-	-	-	-	-
	Total Taxa Consumed			**Total # of Prey Consumed**			**Total Prey Weight Consumed**		
	59	40	48	2405	823	1582	3190.8mg	610.1mg	2580.7mg
	Average # Taxa Consumed/Fish			**Average # of Prey Consumed/Fish**			**Average Prey Weight Consumed/Fish**		
	3.8	2.6	5.4	11.4	7	17.1	15.4	5.1	28.6
Range:	0-14	0-10	0-14	0-89	0-74	0-89	0-165.7	0-64.5	0-165.7
SEM:	0.23	0.2	0.41	1.2	1.2	2.11	1.8	0.84	3.52
SD:	3.3	2.2	3.9	17.1	12.9	20	25.8	9.1	33.4
T-test comparing means for small and large trout	p<0.001; 95%CI: (1.88, 3.68); DF=131; t=6.10)			p<0.001; 95%CI: (5.39, 14.96); DF=143; t=4.20)			p<0.001; 95%CI: (16.24, 30.6) DF=99; t=6.47)		

Appendix B. Frequency of occurance, percent by number, and percent by weight of prey found in trout diets in the Middle Branch of the Whitewater River. Values represent the composite of samples from all sampling periods.

Middle Branch of the Whitewater River	**Frequency of Occurance**			**Percent by Number**			**Percent by Weight**		
	All fish sampled n = 202	**Small fish (<180mm) n = 115**	**Large fish (>180mm) n = 87**	**All fish sampled n = 202**	**Small fish (<180mm) n = 115**	**Large fish (>180mm) n = 87**	**All fish sampled n = 202**	**Small fish (<180mm) n = 115**	**Large fish (>180mm) n = 87**
Ephemeroptera	12.38%	9.57%	16.09%	2.81%	3.01%	2.74%	0.60%	1.28%	0.52%
Baetidae	11.88%	8.70%	16.09%	2.70%	2.74%	2.69%	0.56%	1.13%	0.49%
Baetis	11.88%	8.70%	16.09%	2.70%	2.74%	2.69%	0.56%	1.13%	0.49%
Ephemerellidae	0.99%	1.74%	0.00%	0.07%	0.27%	0.00%	0.02%	0.15%	0.00%
Ephemerella	0.99%	1.74%	0.00%	0.07%	0.27%	0.00%	0.02%	0.15%	0.00%
Heptageniidae	-	-	-	-	-	-	-	-	-
Stenonema	-	-	-	-	-	-	-	-	-
Leptophlebiidae	-	-	-	-	-	-	-	-	-
Leptophlebia	-	-	-	-	-	-	-	-	-
Siphlonuridae	0.50%	0.00%	1.15%	0.04%	0.00%	0.05%	0.02%	0.00%	0.03%
Odonata	-	-	-	-	-	-	-	-	-
Aeshnidae	-	-	-	-	-	-	-	-	-
Plecoptera	-	-	-	-	-	-	-	-	-
Capniidae	-	-	-	-	-	-	-	-	-
Allocapnia	-	-	-	-	-	-	-	-	-
Allocapnia (a)	-	-	-	-	-	-	-	-	-
Paracapnia	-	-	-	-	-	-	-	-	-
Perlodidae	-	-	-	-	-	-	-	-	-
Isoperla	-	-	-	-	-	-	-	-	-
Taeniopterygidae	-	-	-	-	-	-	-	-	-
Taeniopteryx	-	-	-	-	-	-	-	-	-

Appendix B. Continued.

Whitewater (cont.)	**Frequency of Occurance**			**Percent by Number**			**Percent by Weight**		
Megaloptera	-	-	-	-	-	-	-	-	-
Sialidae	-	-	-	-	-	-	-	-	-
Sialis	-	-	-	-	-	-	-	-	-
Coleoptera	2.48%	0.87%	4.60%	0.18%	0.14%	0.19%	0.21%	0.15%	0.22%
Dryopidae	-	-	-	-	-	-	-	-	-
Helichus	-	-	-	-	-	-	-	-	-
Elmidae	-	-	-	-	-	-	-	-	-
Optioservus	-	-	-	-	-	-	-	-	-
Optioservus adult	-	-	-	-	-	-	-	-	-
Stenelmis	-	-	-	-	-	-	-	-	-
Dytiscidae	1.98%	0.87%	3.45%	0.14%	0.14%	0.14%	0.14%	0.15%	0.14%
Hygrotus	-	-	-	-	-	-	-	-	-
Laccophilus	0.50%	0.87%	0.00%	0.04%	0.14%	0.00%	0.02%	0.00%	0.00%
Neoporus	-	-	-	-	-	-	-	-	-
Dytiscidae adult	-	-	-	-	-	-	-	-	-
Hydrophilidae	0.50%	0.00%	1.15%	0.04%	0.00%	0.05%	0.08%	0.00%	0.08%
Hydrobius	0.50%	0.00%	1.15%	0.04%	0.00%	0.05%	0.08%	0.00%	0.08%
Diptera	58.42%	60.00%	56.32%	34.47%	62.60%	24.60%	7.41%	15.18%	6.43%
Athericidae	0.50%	0.00%	1.15%	0.04%	0.00%	0.05%	0.02%	0.00%	0.02%
Atherix	0.50%	0.00%	1.15%	0.04%	0.00%	0.05%	0.02%	0.00%	0.02%
Ceratopogonidae	0.50%	0.87%	0.00%	0.04%	0.14%	0.00%	0.01%	0.06%	0.00%
Chironomidae	55.45%	58.26%	51.72%	31.87%	60.55%	21.82%	3.65%	14.26%	2.30%
Chironomidae larva	53.60%	56.52%	50.57%	26.18%	51.78%	17.20%	3.26%	12.63%	2.07%
Diamesa	24.75%	22.61%	27.59%	14.02%	21.64%	11.34%	2.43%	7.71%	1.75%
Micropsectra	12.87%	12.17%	14.94%	2.70%	4.66%	2.02%	0.17%	0.66%	0.11%

Appendix B. Continued.

Whitewater (cont.)	**Frequency of Occurance**			**Percent by Number**			**Percent by Weight**		
Prodiamesa	0.99%	0.87%	1.15%	0.07%	0.14%	0.05%	0.01%	0.02%	0.01%
Other chironomid larva	37.13%	27.83%	29.89%	9.39%	25.34%	3.80%	0.65%	4.24%	0.20%
Chironomid pupa	13.40%	7.90%	20.70%	4.73%	8.77%	3.32%	0.37%	1.63%	0.21%
Diamesa pupa	10.89%	6.96%	16.09%	4.45%	8.49%	3.03%	0.36%	1.61%	0.20%
Chaetocladius pupa	-	-	-	-	-	-	-	-	-
Cricotopus pupa	-	-	-	-	-	-	-	-	-
Orthocladius pupa	2.48%	0.87%	4.60%	0.28%	0.27%	0.29%	0.01%	0.03%	0.01%
Other chironomid pupa	-	-	-	-	-	-	-	-	-
Chironomid adult	1.00%	0.00%	2.30%	0.96%	0.00%	1.30%	0.02%	0.00%	0.02%
Diamesa adult	1.00%	0.00%	2.30%	0.96%	0.00%	1.30%	0.01%	0.00%	0.01%
Empididae	0.99%	0.00%	2.30%	0.11%	0.00%	0.14%	0.02%	0.00%	0.02%
Ephydridae	-	-	-	-	-	-	-	-	-
Muscidae	-	-	-	-	-	-	-	-	-
Psychodidae	-	-	-	-	-	-	-	-	-
Pericoma	-	-	-	-	-	-	-	-	-
Tipulidae	4.95%	0.87%	10.34%	0.53%	0.14%	0.67%	3.39%	0.33%	3.77%
Antocha	0.50%	0.87%	0.00%	0.04%	0.14%	0.00%	0.04%	0.33%	0.00%
Dicranota	1.98%	0.00%	4.60%	0.14%	0.00%	0.19%	0.10%	0.00%	0.11%
Tipula	2.97%	0.00%	6.90%	0.36%	0.00%	0.48%	3.25%	0.00%	3.66%
Pedicia	-	-	-	-	-	-	-	-	-
Simuliidae	10.40%	7.83%	13.79%	1.85%	1.64%	1.92%	0.33%	0.52%	0.31%
Simuliium	10.40%	7.83%	13.79%	1.85%	1.64%	1.92%	0.33%	0.52%	0.31%
Stratiomyiidae	-	-	-	-	-	-	-	-	-
Caloparyphus	-	-	-	-	-	-	-	-	-

Appendix B. Continued.

Whitewater (cont.)	**Frequency of Occurance**			**Percent by Number**			**Percent by Weight**		
Trichoptera	48.02%	35.65%	64.37%	40.70%	10.14%	51.42%	70.07%	28.84%	75.31%
Brachycentridae	41.09%	27.83%	58.62%	36.86%	8.49%	46.80%	58.68%	24.90%	62.97%
Brachycentrus	41.09%	27.83%	58.62%	36.86%	8.49%	46.80%	58.68%	24.90%	62.97%
Micrasema	-	-	-	-	-	-	-	-	-
Glossosomatidae	-	-	-	-	-	-	-	-	-
Glossosoma	-	-	-	-	-	-	-	-	-
Glossosoma pupa	-	-	-	-	-	-	-	-	-
Hydropsychidae	3.47%	3.48%	3.45%	0.28%	0.55%	0.19%	0.18%	0.50%	0.14%
Ceratopsyche	0.99%	0.00%	2.30%	0.07%	0.00%	0.10%	0.07%	0.00%	0.08%
Hydropsyche	1.98%	3.48%	0.00%	0.14%	0.55%	0.00%	0.06%	0.50%	0.00%
Lepidostomatidae	2.48%	1.74%	3.45%	0.18%	0.27%	0.14%	0.03%	0.08%	0.02%
Lepidostoma	2.48%	1.74%	3.45%	0.18%	0.27%	0.14%	0.03%	0.08%	0.02%
Limnephilidae	8.91%	0.87%	19.54%	2.35%	0.14%	3.12%	9.65%	2.79%	10.52%
Anabolia	-	-	-	-	-	-	-	-	-
Frenesia	-	-	-	-	-	-	-	-	-
Hesperophylax	-	-	-	-	-	-	-	-	-
Limnephilus	7.43%	0.87%	16.09%	1.78%	0.14%	2.35%	7.53%	2.79%	8.13%
Pycnopsyche	1.98%	0.00%	4.60%	0.53%	0.00%	0.72%	1.80%	0.00%	2.03%
Philopotamidae	0.50%	0.87%	0.00%	0.04%	0.14%	0.00%	0.00%	0.02%	0.00%
Phryganeidae	0.99%	0.00%	2.30%	0.07%	0.00%	0.10%	0.77%	0.00%	0.87%
Ptilostomis	0.99%	0.00%	2.30%	0.07%	0.00%	0.10%	0.77%	0.00%	0.87%
Polycentropodidae	-	-	-	-	-	-	-	-	-
Uenoidae	7.92%	1.74%	16.09%	0.78%	0.41%	0.91%	0.76%	0.46%	0.80%
Neophylax	7.92%	1.74%	16.09%	0.78%	0.41%	0.91%	0.76%	0.46%	0.80%
Hymenoptera	-	-	-	-	-	-	-	-	-

Appendix B. Continued.

Whitewater (cont.)	**Frequency of Occurance**			**Percent by Number**			**Percent by Weight**		
Amphipoda	55.45%	59.13%	50.57%	16.97%	20.96%	15.57%	20.13%	53.68%	15.87%
Gammaridae	55.45%	59.13%	50.57%	16.97%	20.96%	15.57%	20.13%	53.68%	15.87%
Gammarus	55.45%	59.13%	50.57%	16.97%	20.96%	15.57%	20.13%	53.68%	15.87%
Isopoda	0.99%	0.00%	2.30%	0.07%	0.00%	0.10%	0.05%	0.00%	0.05%
Collembola	-	-	-	-	-	-	-	-	-
Poduridae	-	-	-	-	-	-	-	-	-
Podura	-	-	-	-	-	-	-	-	-
Gastropoda	**12.38%**	**4.35%**	**22.99%**	**4.52%**	**2.88%**	**5.09%**	**1.42%**	**0.86%**	**1.49%**
Physidae	12.38%	4.35%	22.99%	4.52%	2.88%	5.09%	1.42%	0.86%	1.49%
Physella	16.83%	6.96%	29.89%	4.52%	2.88%	5.09%	1.42%	0.86%	1.49%
Bivalvia	**0.50%**	**0.00%**	**1.15%**	**0.04%**	**0.00%**	**0.05%**	**0.00%**	**0.00%**	**0.00%**
Acari	**2.48%**	**0.87%**	**4.60%**	**0.18%**	**0.14%**	**0.19%**	**0.09%**	**0.01%**	**0.10%**
Annelida	0.50%	0.00%	1.50%	0.04%	0.00%	0.05%	na	na	na
Cottidae	0.50%	0.09%	0.00%	0.04%	0.14%	0.00%	na	na	na
Cottus cognatus	0.50%	0.09%	0.00%	0.04%	0.14%	0.00%	na	na	na
Salmonidae eggs	-	-	-	-	-	-	-	-	-
	Total Taxa Consumed			**Total # Prey Consumed**			**Total Prey Weight Consumed**		
	39	23	32	2811	730	2081	8330.8mg	939.1mg	7391.7mg
	Average # Taxa Consumed/Fish			**Average # of Prey Consumed/Fish**			**Average Prey Weight Consumed/Fish**		
	2.7	2.1	3.4	13.7	6.4	23.6	43	8.4	90.2
Range:	0-11	0-7	0-11	0-188	0-90	0-188	0-722.1	0-47.9	0-722.1
SEM:	0.17	0.14	0.33	1.74	1.12	3.52	6.6	0.89	14.1
SD:	2.4	1.5	3.1	24.9	12.2	33	93.5	9.6	129.5
T-test comparing means for small and large trout	p<0.001; 95%CI: (0.600, 2.01); DF=117; t=3.65)			p<0.001; 95%CI: (9.76, 24.6); DF=103; t=4.60)			p<0.001; 95%CI: (53.8, 109.8); DF=84; t=5.81)		

Appendix C. Frequency of occurance, percent by number, and percent by weight of prey found in trout diets in Cold Spring Brook. Values represent the composite of samples from all sampling periods.

Cold Spring Brook	**Frequency of Occurance**			**Percent by Number**			**Percent by Weight**		
	All fish sampled n = 195	**Small fish (<188mm) n = 148**	**Large fish (>188mm) n = 47**	**All fish sampled n = 195**	**Small fish (<188mm) n = 148**	**Large fish (>188mm) n = 47**	**All fish sampled n = 195**	**Small fish (<188mm) n = 148**	**Large fish (>188mm) n = 47**
Ephemeroptera	5.64%	6.04%	4.35%	0.93%	1.41%	0.17%	0.18%	0.30%	0.07%
Baetidae	5.64%	6.04%	4.35%	0.93%	1.41%	0.17%	0.18%	0.30%	0.07%
Baetis	5.64%	6.04%	4.35%	0.93%	1.41%	0.17%	0.18%	0.30%	0.07%
Ephemerellidae	-	-	-	-	-	-	-	-	-
Ephemerella	-	-	-	-	-	-	-	-	-
Heptageniidae	-	-	-	-	-	-	-	-	-
Stenonema	-	-	-	-	-	-	-	-	-
Leptophlebiidae	-	-	-	-	-	-	-	-	-
Leptophlebia	-	-	-	-	-	-	-	-	-
Siphlonuridae	-	-	-	-	-	-	-	-	-
Odonata	-	-	-	-	-	-	-	-	-
Aeshnidae	-	-	-	-	-	-	-	-	-
Plecoptera	11.30%	10.10%	15.22%	0.83%	0.98%	0.61%	0.90%	1.27%	0.56%
Capniidae	0.51%	0.67%	0.00%	0.03%	0.05%	0.00%	0.01%	0.01%	0.00%
Allocapnia	-	-	-	-	-	-	-	-	-
Allocapnia (a)	-	-	-	-	-	-	-	-	-
Paracapnia	0.51%	0.67%	0.00%	0.03%	0.05%	0.00%	0.01%	0.01%	0.00%
Perlodidae	-	-	-	-	-	-	-	-	-
Isoperla	-	-	-	-	-	-	-	-	-
Taeniopterygidae	10.77%	9.40%	15.22%	0.80%	0.92%	0.61%	0.90%	1.26%	0.56%
Taeniopteryx	10.77%	9.40%	15.22%	0.80%	0.92%	0.61%	0.90%	1.26%	0.56%

Appendix C. Continued.

Cold Spring (cont.)	**Frequency of Occurance**			**Percent by Number**			**Percent by Weight**		
Megaloptera	-	-	-	-	-	-	-	-	-
Sialidae	-	-	-	-	-	-	-	-	-
Sialis	-	-	-	-	-	-	-	-	-
Coleoptera	3.59%	0.67%	13.04%	0.27%	0.05%	0.61%	0.53%	0.04%	1.00%
Dryopidae	-	-	-	-	-	-	-	-	-
Helichus	-	-	-	-	-	-	-	-	-
Elmidae	-	-	-	-	-	-	-	-	-
Optioservus	-	-	-	-	-	-	-	-	-
Optioservus adult	-	-	-	-	-	-	-	-	-
Stenelmis	-	-	-	-	-	-	-	-	-
Dytiscidae	3.08%	0.67%	10.87%	0.20%	0.05%	0.43%	0.08%	0.04%	0.11%
Hygrotus	-	-	-	-	-	-	-	-	-
Laccophilus	1.03%	0.00%	4.35%	0.07%	0.00%	0.17%	0.01%	0.00%	0.03%
Neoporus	0.51%	0.00%	2.17%	0.03%	0.00%	0.09%	0.01%	0.00%	0.01%
Dytiscidae adult	-	-	-	-	-	-	-	-	-
Hydrophilidae	1.03%	0.00%	4.35%	0.07%	0.00%	0.17%	0.46%	0.00%	0.89%
Hydrobius	-	-	-	-	-	-	-	-	-
Diptera	24.62%	28.19%	13.04%	8.67%	13.54%	1.04%	0.53%	0.98%	0.10%
Athericidae	-	-	-	-	-	-	-	-	-
Atherix	-	-	-	-	-	-	-	-	-
Ceratopogonidae	-	-	-	-	-	-	-	-	-
Chironomidae	23.08%	27.52%	10.87%	8.37%	13.05%	0.86%	0.45%	0.85%	0.06%
Chironomidae larva	22.05%	26.17%	10.90%	7.97%	12.41%	0.86%	0.43%	0.82%	0.06%
Diamesa	3.08%	3.36%	2.17%	0.80%	1.25%	0.09%	0.07%	0.13%	0.01%
Micropsectra	8.21%	8.72%	6.52%	3.87%	6.07%	0.35%	0.16%	0.31%	0.01%

Appendix C. Continued.

Cold Spring (cont.)	**Frequency of Occurance**			**Percent by Number**			**Percent by Weight**		
Prodiamesa	0.51%	0.67%	0.00%	0.13%	0.22%	0.00%	0.02%	0.03%	0.00%
Other chironomid larva	3.08%	3.36%	2.17%	3.17%	4.98%	0.43%	0.19%	0.35%	0.04%
Chironomid pupa	1.50%	2.00%	0.00%	0.23%	0.38%	0.00%	0.01%	0.02%	0.00%
Diamesa pupa	-	-	-	-	-	-	-	-	-
Chaetocladius pupa	-	-	-	-	-	-	-	-	-
Cricotopus pupa	-	-	-	-	-	-	-	-	-
Orthocladius pupa	1.03%	1.34%	0.00%	0.20%	0.33%	0.00%	0.01%	0.02%	0.00%
Other chironomid pupa	0.51%	0.67%	0.00%	0.03%	0.05%	0.00%	0.00%	0.00%	0.00%
Chironomid adult	0.51%	0.67%	0.00%	0.17%	0.27%	0.00%	0.01%	0.01%	0.00%
Diamesa adult	-	-	-	-	-	-	-	-	-
Empididae	-	-	-	-	-	-	-	-	-
Ephydridae	0.51%	0.67%	0.00%	0.03%	0.05%	0.00%	0.01%	0.03%	0.00%
Muscidae	0.51%	0.67%	0.00%	0.03%	0.05%	0.00%	0.01%	0.03%	0.00%
Psychodidae	-	-	-	-	-	-	-	-	-
Pericoma	-	-	-	-	-	-	-	-	-
Tipulidae	1.54%	0.67%	4.35%	0.13%	0.11%	0.17%	0.04%	0.04%	0.04%
Antocha	-	-	-	-	-	-	-	-	-
Dicranota	-	-	-	-	-	-	-	-	-
Tipula	-	-	-	-	-	-	-	-	-
Pedicia	1.54%	0.67%	4.35%	0.13%	0.11%	0.17%	0.04%	0.04%	0.04%
Simuliidae	1.54%	2.01%	0.00%	0.10%	0.16%	0.00%	0.02%	0.04%	0.00%
Simuliium	1.54%	2.01%	0.00%	0.10%	0.16%	0.00%	0.02%	0.04%	0.00%
Stratiomyiidae	-	-	-	-	-	-	-	-	-
Caloparyphus	-	-	-	-	-	-	-	-	-

Appendix C. Continued.

Trichoptera	47.18%	36.24%	82.61%	12.83%	6.50%	22.92%	32.99%	15.28%	49.63%
Brachycentridae	0.51%	0.00%	2.17%	0.03%	0.00%	0.09%	0.09%		0.18%
Brachycentrus	0.51%	0.00%	2.17%	0.03%	0.00%	0.09%	0.09%		0.18%
Micrasema	-	-	-	-	-	-	-	-	-
Glossosomatidae	2.05%	1.34%	4.35%	0.13%	0.11%	0.17%	0.15%	0.14%	0.15%
Glossosoma	1.54%	0.67%	4.35%	0.10%	0.05%	0.17%	0.14%	0.12%	0.15%
Glossosoma pupa	0.51%	0.67%	0.00%	0.03%	0.05%	0.00%	0.01%	0.02%	0.00%
Hydropsychidae	-	-	-	-	-	-	-	-	-
Ceratopsyche	-	-	-	-	-	-	-	-	-
Hydropsyche	-	-	-	-	-	-	-	-	-
Lepidostomatidae	-	-	-	-	-	-	-	-	-
Lepidostoma	-	-	-	-	-	-	-	-	-
Limnephilidae	45.13%	34.90%	78.26%	12.57%	6.28%	22.58%	32.74%	15.14%	49.29%
Anabolia	-	-	-	-	-	-	-	-	-
Frenesia	1.03%	1.34%	0.00%	0.07%	0.11%	0.00%	0.04%	0.07%	0.00%
Hesperophylax	30.26%	18.79%	67.39%	7.27%	2.71%	14.53%	15.98%	3.76%	27.47%
Limnephilus	28.72%	18.79%	60.87%	5.03%	3.14%	8.04%	16.46%	10.75%	21.83%
Pycnopsyche	0.51%	0.67%	0.00%	0.03%	0.05%	0.00%	0.02%	0.03%	0.00%
Philopotamidae	-	-	-	-	-	-	-	-	-
Phryganeidae	-	-	-	-	-	-	-	-	-
Ptilostomis	-	-	-	-	-	-	-	-	-
Polycentropodidae	-	-	-	-	-	-	-	-	-
Uenoidae	-	-	-	-	-	-	-	-	-
Neophylax	-	-	-	-	-	-	-	-	-
Hymenoptera	0.51%	0.67%	0.00%	0.03%	0.05%	0.00%	0.00%	0.00%	0.00%

Appendix C. Continued.

Cold Spring (cont.)	**Frequency of Occurance**			**Percent by Number**			**Percent by Weight**		
Amphipoda	95.38%	91.28%	95.65%	66.83%	76.54%	51.21%	60.03%	81.53%	39.81%
Gammaridae	95.38%	91.28%	95.65%	66.83%	76.54%	51.21%	60.03%	81.53%	39.81%
Gammarus	95.38%	91.28%	95.65%	66.83%	76.54%	51.21%	60.03%	81.53%	39.81%
Isopoda	-	-	-	-	-	-	-	-	-
Collembola	-	-	-	-	-	-	-	-	-
Poduridae	-	-	-	-	-	-	-	-	-
Podura	-	-	-	-	-	-	-	-	-
Gastropoda	**19.49%**	**6.04%**	**60.87%**	**9.50%**	**0.87%**	**23.27%**	**4.84%**	**0.59%**	**8.83%**
Physidae	19.49%	6.04%	60.87%	9.50%	0.87%	23.27%	4.84%	0.59%	8.83%
Physella	19.49%	6.04%	60.87%	9.50%	0.87%	23.27%	4.84%	0.59%	8.83%
Bivalvia	-	-	-	-	-	-	-	-	-
Acari	-	-	-	-	-	-	-	-	-
Annelida	-	-	-	-	-	-	-	-	-
Cottidae	1.50%	0.70%	4.30%	0.10%	0.05%	0.17%	na	na	na
Cottus cognatus	1.50%	0.70%	4.30%	0.10%	0.05%	0.17%	na	na	na
Salmonidae eggs	1.50%	2.00%	0.00%	na	na	na	na	na	na
	Total Taxa Consumed			**Total # Prey Consumed**			**Total Prey Weight Consumed**		
	30	26	18	3002	1846	1156	7626.1mg	3695.4mg	3930.7mg
	Average # Taxa Consumed/Fish			**Average # of Prey Consumed/Fish**			**Average Prey Weight Consumed/Fish**		
	2.4	2	3.5	14.9	12.6	22.5	39	24.7	85.4
Range:	0-7	0-6	0-7	0-81	0-81	0-67	0-293.9	0-175.8	0-293.9
SEM:	0.11	0.11	0.24	1.11	1.13	2.62	3.57	2.26	10.9
SD:	1.52	1.3	1.6	15.43	13.79	17.91	50	27.7	73.6
T-test comparing means for small and large trout	p<0.001; 95%CI: (1.00, 2.03); DF=65; t=5.88)			p=0.001; 95%CI: (4.23, 15.6); DF = 64; t = 3.48)			p=0.001; 95%CI: (38.5, 83.0); DF = 48; t = 5.48)		

Appendix D. Percent by number, and percent by weight of invertebrates found in the drift for each stream. Values represent the composite of samples from all sampling periods.

Drift Taxa	**Percent by Number**			**Percent by Weight**		
	Beaver	**Whitewater**	**Cold Spring**	**Beaver**	**Whitewater**	**Cold Spring**
Ephemeroptera	9.87%	11.36%	17.14%	8.49%	6.33%	5.31%
Baetidae	6.84%	10.12%	17.14%	4.04%	5.06%	5.31%
Baetis	6.84%	10.12%	17.14%	4.04%	5.06%	5.31%
Ephemerellidae	3.03%	1.23%	-	4.44%	1.27%	-
Ephemerella	3.03%	1.23%	-	4.44%	1.27%	-
Heptageniidae	-	-	-	-	-	-
Stenonema	-	-	-	-	-	-
Leptophlebiidae	-	-	-	-	-	-
Leptophlebia	-	-	-	-	-	-
Plecoptera	0.92%	-	-	1.10%	-	-
Capniidae	0.53%	-	-	0.69%	-	-
Allocapnia	0.53%	-	-	0.69%	-	-
Perlodidae	0.39%	-	-	0.41%	-	-
Isoperla	0.39%	-	-	0.41%	-	-
Megaloptera	-	-	-	-	-	-
Sialidae	-	-	-	-	-	-
Sialis	-	-	-	-	-	-
Coleoptera	0.92%	0.49%	-	1.92%	2.55%	-
Elmidae	0.92%	0.49%	-	1.92%	2.55%	-
Dubiraphia	0.13%	-	-	0.07%	-	-
Optioservus larva	0.26%	-	-	0.84%	-	-
Optioservus adult	0.53%	-	-	1.01%	-	-
Diptera	51.45%	52.35%	59.08%	20.28%	15.14%	4.77%
Ceratopogonidae	-	-	-	-	-	-
Chironomidae	41.97%	44.07%	57.54%	11.60%	9.59%	4.42%
Chironomidae larva	41.32%	38.64%	53.71%	11.24%	7.23%	3.72%
Diamesa larva	4.21%	3.09%	-	2.92%	2.44%	-
Micropsectra larva	-	0.49%	-	-	0.13%	-
Prodiamesa larva	-	-	-	-	-	-
Other chironomid larva	37.11%	35.06%	53.71%	8.32%	4.67%	3.72%
Chironomid pupa	0.26%	1.36%	-	0.24%	0.76%	-
Diamesa pupa	0.26%	1.23%	-	0.24%	0.76%	-
Orthocladius pupa	-	0.12%	-	-	-	-
Other chironomid pupa	-	-	-	-	-	-

Appendix D. Continued.

Drift Taxa	**Percent by Number**			**Percent by Weight**		
Chironomid adult	0.39%	4.07%	3.84%	0.12%	1.56%	0.71%
Diamesa adult	0.26%	4.07%	-	0.09%	1.56%	-
Orthocladius adult	-	-	3.84%	-	-	0.71%
Other chironomid adult	0.13%	-	-	0.03%	-	-
Empididae	0.13%	-	-	0.21%	-	-
Ephydridae	-	0.12%	-	-	-	-
Psychodidae	0.79%	0.62%	-	0.71%	0.06%	-
Pericoma	0.79%	0.74%	-	0.71%	0.06%	-
Tipulidae	-	0.37%	-	-	1.53%	-
Antocha	-	-	-	-	-	-
Dicranota	-	0.12%	-	-	1.53%	-
Hexatoma	-	-	-	-	-	-
Tipula	-	-	-	-	-	-
Simuliidae	8.16%	5.80%	1.53%	7.75%	3.65%	0.34%
Simuliium	8.16%	5.80%	1.53%	7.75%	3.65%	0.34%
Tabanidae	-	-	-	-	-	-
Chrysops	-	-	-	-	-	-
Trichoptera	1.97%	1.11%	3.07%	5.17%	7.56%	52.20%
Brachycentridae	0.26%	0.25%	-	0.71%	4.53%	-
Brachycentrus	0.13%	0.25%	-	0.66%	4.53%	-
Micrasema	0.13%	-	-	0.04%	-	-
Glossosomatidae	0.66%	-	-	2.52%	-	-
Glossosoma	0.26%	-	-	1.06%	-	-
Glossosoma pupa	0.39%	-	-	1.46%	-	-
Hydropsychidae	0.66%	0.49%	-	1.80%	2.59%	-
Ceratopsyche	-	0.12%	-	-	0.64%	-
Chematopsyche	0.39%	-	-	1.44%		-
Hydropsyche	0.13%	0.37%	-	0.33%	1.95%	-
Lepidostomatidae	0.39%	0.25%	-	0.15%	0.07%	-
Lepidostoma	0.39%	0.25%	-	0.15%	0.07%	-
Limnephilidae	-	-	3.07%	-	-	52.20%
Hesperophylax	-	-	1.28%	-	-	8.53%
Limnephilus	-	-	1.79%	-	-	43.67%
Uenoidae	-	-	-	-	0.36%	-
Hymenoptera	-	0.12%	-	-	0.43%	-

Appendix D. Continued.

Drift Taxa	Percent by Number			Percent by Weight		
Amphipoda	0.26%	0.62%	18.16%	0.57%	10.07%	36.02%
Gammaridae	0.26%	0.62%	18.16%	0.57%	10.07%	36.02%
Gammarus	0.26%	0.62%	18.16%	0.57%	10.07%	36.02%
Collembola	28.16%	22.47%	-	56.74%	42.07%	-
Poduridae	28.16%	22.47%	-	56.74%	42.07%	-
Podura	28.16%	22.47%	-	56.74%	42.07%	-
Gastropoda	0.13%	6.30%	1.02%	1.57%	14.08%	1.36%
Physidae	0.13%	5.93%	1.02%	1.57%	14.07%	1.36%
Physella	0.13%	5.93%	1.02%	1.57%	14.07%	1.36%
Planorbidae	-	0.37%	-	-	0.02%	-
Gyraulus	-	0.12%	-	-	0.00%	-
Bivalvia	-	-	-	-	-	-
Acari	6.32%	5.19%	1.53%	4.16%	1.78%	0.33%
	Total Number of Prey:			**Total Weight of Prey (mg):**		
	760	810	391	244.23	314.99	237.90

Appendix E. Percent by number, and percent by weight of invertebrates found in the benthos for each stream. Values represent the composite of samples from all sampling periods.

Benthic Taxa	**Percent by Number**			**Percent by Weight**		
	Beaver	**Whitewater**	**Cold Spring**	**Beaver**	**Whitewater**	**Cold Spring**
Ephemeroptera	11.03%	17.61%	2.44%	9.72%	7.32%	2.16%
Baetidae	2.84%	11.40%	2.44%	0.63%	3.32%	2.16%
Baetis	2.84%	11.40%	2.44%	0.63%	3.32%	2.16%
Ephemerellidae	7.21%	6.09%	-	5.84%	3.51%	-
Ephemerella	7.21%	6.09%	-	5.84%	3.51%	-
Heptageniidae	0.76%	0.11%	-	3.01%	0.48%	-
Stenonema	0.66%	-	-	2.75%	-	-
Leptophlebiidae	0.22%	-	-	0.24%	-	-
Leptophlebia	0.22%	-	-	0.24%	-	-
Plecoptera	2.95%	-	-	8.53%	-	-
Capniidae	0.22%	-	-	0.25%	-	-
Allocapnia	0.22%	-	-	0.25%	-	-
Perlodidae	2.73%	-	-	8.28%	-	-
Isoperla	2.73%	-	-	6.82%	-	-
Megaloptera	0.11%	-		2.45%	-	-
Sialidae	0.11%	-		2.45%	-	-
Sialis	0.11%	-	-	2.45%	-	-
Coleoptera	6.44%	1.58%	-	12.85%	2.55%	-
Elmidae	6.44%	1.58%	-	12.85%	2.55%	-
Dubiraphia	-	-	-	-	-	-
Optioservus larva	6.44%	1.02%	-	-	-	-
Optioservus adult	-	0.56%	-	-	-	-
Diptera	68.23%	60.05%	13.44%	26.80%	16.04%	3.09%
Ceratopogonidae	0.33%	0.34%	-	0.18%	0.10%	-
Chironomidae	64.96%	54.97%	12.33%	14.57%	9.90%	0.96%
Chironomidae larva	64.96%	54.29%	12.33%	14.57%	9.59%	0.96%
Diamesa larva	4.69%	8.58%	-	4.26%	4.14%	-
Micropsectra larva	1.86%	3.05%	0.89%	0.71%	0.59%	0.21%
Prodiamesa larva	-	-	-	-	-	-
Other chironomid larva	58.41%	42.66%	11.44%	9.60%	4.86%	0.75%
Chironomid pupa	-	0.68%	-	-	0.31%	-
Diamesa pupa	-	0.56%	-	-	0.29%	-
Orthocladius pupa	-	0.11%	-	-	0.02%	-
Other chironomid pupa	-	-	-	-	-	-

Appendix E. Continued.

Benthic Taxa	**Percent by Number**			**Percent by Weight**		
Chironomid adult	-	-	-	-	-	-
Diamesa adult	-	-	-	-	-	-
Orthocladius adult	-	-	-	-	-	-
Other chironomid adult	-	-	-	-	-	-
Empididae	-	-	-	-	-	-
Ephydridae	-	-	-	-	-	-
Psychodidae	0.11%	0.11%	-	0.08%	0.07%	-
Pericoma	0.11%	0.11%	-	0.08%	0.07%	-
Tipulidae	1.86%	1.92%	0.78%	8.27%	4.77%	1.45%
Antocha	-	-	-	-	-	-
Dicranota	1.31%	1.92%	0.44%	2.61%	4.77%	1.18%
Hexatoma	-	-	0.33%	-	-	0.26%
Tipula	0.55%	-	-	5.66%	-	-
Simuliidae	0.55%	2.71%	-	0.35%	1.21%	-
Simuliium	0.55%	2.71%	-	0.35%	1.21%	-
Tabanidae	0.44%	-	0.33%	3.35%	-	0.68%
Chrysops	0.11%	-	0.33%	1.34%	-	0.68%
Trichoptera	6.33%	7.00%	0.67%	25.50%	42.66%	2.02%
Brachycentridae	3.60%	4.85%	-	22.01%	35.60%	-
Brachycentrus	3.38%	4.85%	-	21.65%	35.60%	-
Micrasema	0.22%	-	-	0.36%	-	-
Glossosomatidae	1.09%	0.34%	-	2.19%	0.28%	-
Glossosoma	0.98%	-	-	2.02%	-	-
Glossosoma pupa	0.11%	0.34%	-	0.17%	0.28%	-
Hydropsychidae	0.55%	1.47%	-	0.61%	6.61%	-
Ceratopsyche	-	-	-	-	2.68%	-
Chematopsyche	0.33%	-	-	0.56%	-	-
Hydropsyche	-	0.79%	-	-	3.94%	-
Lepidostomatidae	1.09%	0.34%	-	0.69%	0.16%	-
Lepidostoma	1.09%	0.34%	-	0.69%	0.16%	-
Limnephilidae	-	-	0.44%	-	-	1.49%
Hesperophylax	-	-	0.44%	-	-	1.49%
Limnephilus	-	-	-	-	-	-
Uenoidae	-	-	0.22%	-	-	0.53%
Hymenoptera	-	-	-	-	-	-

Appendix E. Continued.

Benthic Taxa	**Percent by Number**			**Percent by Weight**		
Amphipoda	3.38%	8.24%	82.22%	12.39%	28.20%	91.58%
Gammaridae	3.38%	8.24%	82.22%	12.39%	28.20%	91.58%
Gammarus	3.38%	8.24%	82.22%	12.39%	28.20%	91.58%
Collembola	-	-	-	-	-	-
Poduridae	-	-	-	-	-	-
Podura	-	-	-	-	-	-
Gastropoda	0.66%	4.40%	1.11%	1.57%	3.05%	1.14%
Physidae	0.66%	4.40%	1.11%	1.57%	3.05%	1.14%
Physella	0.66%	4.40%	1.11%	1.57%	3.05%	1.14%
Planorbidae	-	-	-	-	-	-
Gyraulus	-	-		-	-	-
Bivalvia	0.11%	0.11%	-	0.03%	0.01%	-
Acari	0.76%	1.02%	0.11%	0.17%	0.17%	0.02%
	Total Number of Prey:			**Total Weight of Prey (mg):**		
	916	886	902	576.48	695.70	718.42

Appendix F. Percent composition of prey by weight in the stream environment (drift + benthic) and the diet of small and large brown trout in Cold Spring Brook, with Manly's alpha electivity index scores.

Cold Spring- Percent by Weight

Taxon	% Composition in Environment (Drift +Benthic)	% Composition in Diet (Small Trout)	% Composition in Diet (Large Trout)	Manly's alpha* (small trout)	Manly's alpha* (large trout)
Physella	1.20%	0.59%	8.83%	0.12	**0.60**
Limnephilidae	14.11%	15.14%	49.29%	**0.26**	**0.28**
Gammarus	77.76%	81.53%	39.81%	**0.25**	0.04
Other Prey	2.35%	1.62%	1.93%	**0.16**	0.07
Large chironomid larvae	1.06%	0.80%	0.06%	**0.18**	0.00
Baetis	2.94%	0.30%	0.07%	0.02	0.00
Small chironomid larvae	0.59%	0.02%	0.00%	0.01	0.00

*Scores > 1/m (0.14) indicate preference. Values indicating preference are bolded.

Appendix G. Percent composition of prey by weight in the stream environment (drift + benthic) and the diet of small and large brown trout in the Middle Branch of the Whitewater River, with Manly's alpha electivity index scores.

Whitewater- Percent by Weight

Taxon	% Composition in Environment (Drift +Benthic)	% Composition in Diet (Small Trout)	% Composition in Diet (Large Trout)	Manly's alpha* (small trout)	Manly's alpha* (large trout)
Brachycentrus	25.92%	24.90%	62.97%	**0.10**	**0.47**
Chironomid pupa	0.45%	1.63%	0.21%	**0.39**	**0.09**
Gammarus	22.55%	53.68%	15.87%	**0.25**	**0.14**
Large chironomid larvae	8.79%	12.57%	2.07%	**0.15**	0.05
Other Prey	24.99%	4.48%	16.46%	0.02	**0.13**
Simuliium	1.97%	0.52%	0.31%	0.03	0.03
Baetis	3.87%	1.13%	0.49%	0.03	0.02
Physella	6.49%	0.86%	1.49%	0.01	0.04
Acari	0.67%	0.01%	0.10%	0.00	0.03
Ephemerella	2.81%	0.15%	0.00%	0.01	0.00
Small chironomid larvae	1.01%	0.06%	0.00%	0.01	0.00
Chironomid adult	0.49%	0.00%	0.02%	0.00	0.01

*Scores > 1/m (0.08) indicate preference. Values indicating preference are bolded.

Appendix H. Percent composition of prey by weight in the stream environment (drift + benthic) and the diet of small and large brown trout in Beaver Creek, with Manly's alpha electivity index scores.

Beaver Creek- Percent by Weight

Taxon	% Composition in Environment (Drift +Benthic)	% Composition in Diet (Small Trout)	% Composition in Diet (Large Trout)	Manly's alpha* (small trout)	Manly's alpha* (large trout)
Lepidostoma	0.53%	7.31%	3.64%	**0.28**	**0.15**
Chironomid adult	0.04%	0.19%	0.44%	**0.10**	**0.28**
Chironomid pupa	0.07%	0.47%	0.57%	**0.13**	**0.18**
Allocapnia	0.38%	2.41%	1.44%	**0.13**	**0.09**
Micrasema	0.27%	1.49%	0.56%	**0.11**	0.05
Glossosoma	1.74%	4.93%	3.79%	**0.06**	0.05
Gammarus	8.87%	19.35%	15.36%	0.04	0.04
Other Prey	17.69%	28.58%	29.37%	0.03	0.04
Brachycentrus	15.40%	5.44%	24.31%	0.01	0.04
Physella	1.57%	0.73%	1.80%	0.01	0.03
Ephemerella	5.43%	5.97%	2.92%	0.02	0.01
Isoperla	5.94%	6.10%	2.34%	0.02	0.01
Large chironomid larvae	12.34%	13.52%	6.27%	0.02	0.01
Tipulidae	5.81%	1.88%	5.88%	0.01	0.02
Baetis	1.65%	0.56%	0.56%	0.01	0.01
Simuliium	2.55%	0.79%	0.65%	0.01	0.01
Optioservus larva	0.25%	0.00%	0.01%	0.00	0.00
Small chironomid larvae	1.24%	0.17%	0.02%	0.00	0.00
Podura	16.89%	0.00%	0.02%	0.00	0.00
Acari	1.35%	0.13%	0.04%	0.00	0.00

*Scores > 1/m (0.05) indicate preference. Values indicating preference are bolded.