An Investigation of Disturbance, Sampling Methods, the River Continuum Concept, and Hydroseres in Hardwood Creek, a Ditched Stream in Minnesota, USA

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Abstract

Hardwood Creek is located northeast of the Twin Cities Metropolitan Area near Hugo, Minnesota. Upper portions of the stream have a long history of ditching that has substantially modified the flow regime and physical structure of the stream channel. Ditching has occurred at different intervals along segments of the stream, resulting in a mosaic of segments at varied stages of regression back to more normal channel structure in terms of sinuosity, habitat heterogeneity, and profile.

Ditching is a physical disturbance to streams and is expected to modify stream habitats. Sites which have never been ditched, or have not been ditched recently, are expected to have greater habitat heterogeneity when compared to recently ditched sites. As a result, traditional sampling methods may not provide an accurate estimate of taxa richness. Dipnet (DN) and chironomid surface-floating pupal exuviae (SFPE) methods were employed to capture both chironomid and non-chironomid macroinvertebrates.

The SFPE method detected more taxa over the entire project and on a site-to-site basis for both chironomid and non-chironomid taxa. For chironomids collected in June, the DN method was most effective across all sites, but at disturbed sites there was no difference between methods. In addition, the SFPE method exclusively collected 1.2 to 8.8 times as many non-chironomid macroinvertebrates, on a site-to site basis, as the DN method. A separate analysis found the SFPE method exclusively collected twice as many chironomid genera as the DN method. To obtain a comprehensive estimate of taxa richness in ditches, all macroinvertebrates collected in SFPE samples need to be evaluated in conjunction with DN samples.

The *a priori* test of the IDH to account for the longitudinal patterns of macroinvertebrate biodiversity, as a function of ditching-related disturbance, was also examined. The IDH was tested at the community, order, and family level to determine

whether different levels of taxonomic scale would reveal differing levels of congruence with the IDH. I found the model does not account for the patterns of species richness and diversity, with the exception of one order and three families, the IDH was rejected.

As a consequence of the IDH being rejected, *a posteriori* models were employed in an attempt to explain community composition in Hardwood Creek. The River Continuum Concept (RCC) was tested by analyzing the macroinvertebrate community using three similarity indices to test eight hypotheses. P-values ranged from 0.488 to 0.957 leading to the acceptance of all eight null hypotheses. The similarities of communities of adjacent sites did not conform to the prediction of the RCC that sites adjacent to each other should have the most similar community compositions.

The failure to explain community composition patterns in Hardwood Creek led to the testing of more *a posteriori* models, hydroseres and management categories. Eight taxa were associated with a single hydrosere. Similarity indices generally supported the validity of the hydrosere classification based upon time since the last dredging event. However, the hydrosere models failed to explain patterns of taxa richness for most taxa. An alternative classification based on 303d listing considerations and Total Maximum Daily Load (TMDL) management strategies was also investigated. Based on this classification, the number of taxa at upstream and downstream sites was not significantly different. However, similarity indices generally supported the upstream and downstream management reaches.

Poor water quality, substrate stability, and differences in aquatic vegetation among sample sites are probably the most important factors confounded with ditching in Hardwood Creek. The confounding factors likely played a role in the limited success of IDH, RCC, hydrosere, and management models to explain patterns of macroinvertebrate community composition in Hardwood Creek.

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Chapter 1

Introduction, Literature Review, and Organization of Thesis

Introduction

Hardwood Creek, originates from the outflow of Rice Lake and flows north and west before entering Peltier Lake north of Centerville, Minnesota (Figure 1). Upper portions of the stream have a long history of ditching that substantially modified the flow regime and physical structure of the stream channel. Ditching has occurred at different intervals along segments of the stream, resulting in a mosaic of segments at varied stages of regression back to more normal channel structure in terms of sinuosity, habitat heterogeneity, and profile. In 2003, the Rice Creek Water Management Organization (RCWMO) decided to dredge two stretches of the stream during winter 2003-2004. The stretches are separated by a segment of stream that was ditched greater than 30 years ago. Both state and local groups voiced concern about downstream effects of ditching and a decision was made to investigate potential inadvertent changes in water quality, downstream transport of nutrients, organic matter, eroded inorganic substrates, and patterns of macroinvertebrate composition and biodiversity.

Concurrent with planning for ditching activities, Hardwood Creek was evaluated for potential 303D listing for nutrients and dissolved oxygen by the Minnesota Pollution Control Agency (MPCA). As part of this process, the RCWMO established eight monitoring stations to document ambient conditions. In addition, limited information on fish and macroinvertebrate community composition was generated. However, no comprehensive data on the longitudinal patterns of macroinvertebrates was systematically collected, and filling this data gap was identified as valuable in the decision-making process for potential listing.

Partial funding for this thesis research project was provided to document the patterns of macroinvertebrate composition and biodiversity as a function of ditching practices, and develop a data base of the longitudinal patterns of macroinvertebrates that could be used in the decision-making process by MPCA. Sampling sites closely corresponded to sites established by RCWMO. Consequently, sites include: two sites located near downstream edges of stretches ditched during 2003-2004, five sites within stretches that were ditched at different times in the past, and two downstream sites that had no history of repeated ditching. This placement of sites provided an opportunity to investigate the predictions of a controversial model, the Intermediate Disturbance Hypothesis (IDH), in Hardwood Creek as a function of ditching-related disturbances.

Literature Review: An Overview of Disturbance Ecology With an Emphasis on Lotic Ecosystems

The following is a discussion designed to highlight important aspects of disturbance ecology specifically terminology, paradigms, and history. The literature on disturbance in lotic ecosystems is emphasized to give the reader appropriate background information for the chapters that follow. First the definition, description, response, recovery, and scale as related to disturbance are presented. Community structure, the IDH, and the utility of disturbance follow. A discussion of the hydrological justification for stream disturbance via ditching is also presented.

There exists a debate among ecologists as to the proper definition of disturbance (e.g., Sousa 1984, Pickett and White 1985, Resh et al. 1988, Wallace 1990, Poff 1992, Lake 2000). Researchers note the lack of consensus in its terminology (Yount and Niemi 1990, Glasby and Underwood 1996). Throughout this thesis the definition of disturbance by White and Pickett (1985) is used: “A disturbance is any relatively discrete event in

time that disrupts ecosystem, community, or population structure and changes resources, substrate availability, or the physical environment." This is a general definition of disturbance embraced by others (Risser 1987, Petraitis et al. 1989, Yount and Niemi 1990, Poff 1992, Death and Winterbourn 1994, Reice 1994, Smith and Smith 2001).

Throughout the ecological literature, the terms disturbance and perturbation are used synonymously. In everyday usage the two terms are synonyms. However within the lexicon of disturbance ecology, it is important to differentiate between the terms when distinguishing between the cause of a disturbance and its effect(s). Rykiel (1985) stated that a disturbance is a cause, whereas perturbation is the effect or response. Bender et al. (1984) combined cause and effect into the concept of perturbation.

Lake (2000) noted that disturbance studies at a variety of scales confound the response with the disturbance event. Confusion occurs when a discernible event is both a cause and an effect (Rykiel 1985, Yount and Niemi 1990). For example, eutrophication occurs when nutrient loading leads to a decrease in dissolved oxygen. The disturbance (cause) is the low concentration of dissolved oxygen; the perturbation (effect) is the death of aquatic life (Reice et al. 1990).

Examples of disturbance include excavation, fire, flooding, logging, and wind. Disturbance events are innately biotic or abiotic. In the above examples, fire, flooding, and wind are all abiotic disturbances. Diseases, invasive species, excavation, and logging via man or rodent are biotic disturbance events. Predation is considered a biological disturbance by some (Connell 1978, Sousa 1984, Denslow 1985, Karr and Freemark 1985, Petraitis et al. 1989) but not others (Menge and Sutherland 1987, Lake 1990, Reice 1994). Biotic and abiotic disturbances can act in synergy. For example, a dam is built across a stream (biotic) and its failure results in catastrophic flooding (abiotic).

Disturbance can be described with respect to type, intensity, severity, frequency, and predictability. Bender et al. (1984) introduced two types of disturbance, pulse and press. Lake (2000) described a ramp as a third type of disturbance. Lake (2000) described the three disturbance types in reference to aquatic ecosystems on a temporal and magnitude scale. Floods are generally short lived and are examples of a pulse disturbance (Lake 2000). Press disturbances often occur quickly but persist for a long period of time; examples include fire damage and channelization (Lake 2000). Ramp disturbances slowly increase in magnitude over time; examples include the spread of invasive species and droughts (Lake 2000).

Disturbance intensity and severity determine the magnitude of a disturbance event (Sousa 1984). Disturbance intensity is used to gauge the severity of disturbance. Resh et al. (1988) stated that disturbance intensity is the force exerted by the disturbance per unit time, and provided the example of flood discharge. Wind speed, fire temperature, and wave velocity are also examples of disturbance intensity (Sousa 1984).

Disturbance severity is the total amount of damage produced (Sousa 1984). The severity of a disturbance event can be estimated if the duration and average intensity, expressed as a rate, is known. For example if the average intensity of a forest fire is 45 acres per hour, for 5 hours, then the severity is 225 acres.

Disturbance frequency is a measure of how often a disturbance event occurs over time. Examples include the 1.5-year recurrence interval flood or mechanical disturbance of a stream riffle every 2 weeks for 3 years. Stream substrates such as clay, sand, and boulders have different, yet predictable, frequencies of disturbance because the critical velocities at which they are transported differ (Resh et al. 1988, Reice et al. 1990, Reice 1994). For a given hydrologic disturbance the biota inhabiting one patch

may experience disturbance, whereas the biota inhabiting another patch may not. The discrepancy in the disturbance frequency of lentic and lotic ecosystems is likely a key factor resulting in higher species richness of the later (Resh et al. 1988, Reice et al. 1990, Reice 1994).

Stream ecologists have debated the biological significance of frequent, and therefore predictable, disturbance events. Resh et al. (1988) stated that unpredictable disturbance events usually disturb the biota because organisms are adapted to predictable events. Poff (1992) argued strongly against the ideas of Resh et al. (1988). Reice et al. (1990) lent some support to Resh et al. (1988), in their assertion that the most predictably and frequently disturbed macroinvertebrate communities might be the best adapted to coping with disturbance. However, the lack of predictable disturbance events associated with seasonal changes, can have a greater impact on the biota than the presence of a predictable event (Reice 1994).

When the biota experience a disturbance event their response can be characterized with respect to their resistance, resilience, and persistence (Lake and Barmuta 1986, Lake 2000). Response has also been characterized as pulse, press (Glasby and Underwood 1996), and ramp (Lake 2000). The pulse, press, and ramp characterizations are a conceptually equivalent way to estimate the resistance, resilience, and persistence of the biotic community. The concepts of resistance, resilience, and persistence fit into the broader ecology paradigm of ecosystem stability.

Connell and Sousa (1983) state: "For a system to be considered stable, there must exist one or more equilibrium points or limit cycles (1) at which the system remains when faced with a disturbing force or (2) to which it returns if perturbed by the force." Resistance is a better term than persistence for the first point; elasticity and amplitude

are suggested to describe the concept of adjustment for the second point (Connell and Sousa 1983).

A general definition of ecosystem resistance is the ability of the system to resist an applied force (Connell and Sousa 1983). In lotic ecology, discussion of disturbance resistance is common (e.g., Lake and Barmuta 1986, Fisher 1990, Sedell et al. 1990, Townsend et al. 1997a). It appears that elasticity is rarely discussed or investigated in lotic ecosystems. Instead the term resilience is used. Holling (1973) defined resilience as the ability of an ecosystem to absorb disturbance and other changes, without changing the relationships between populations in the system.

Harrison (1979) found the relationship between resilience and resistance depends upon how sensitive population growth and the density-dependent negative feedback mechanism is to the environment. In lotic ecology, discussion of resilience following disturbance is common (e.g., Niemi et al. 1990, Reice et al. 1990, Sedell et al. 1990, Death 1996, Wetzel 2001).

Connell and Sousa (1983) defined persistence as the ability of a species or population to persist or recolonize following disturbance, within the time required for replacement of all individuals, in the location of interest. Little is known about the persistence of lotic macroinvertebrate communities because few long-term studies have been published (Lake and Barmuta 1986). Fish community persistence in streams has received more attention (e.g., Moyle and Vondracek 1985).

Ecosystem perturbations are two events: the disturbance and the biotic response (Lake 2000). How the biological community responds to disturbance will determine whether and when it will recover. After reviewing the literature on the recovery of aquatic ecosystems, Niemi et al. (1990) concluded that most recovery times were less than three years. However, recovery may take centuries or may not occur (Death 1996).

Fisher (1983) stated that streams recover stochastically, with recovery influenced by colonizers and the severity and timing of disturbance.

Recolonization has three mechanisms: regrowth, migration, and recruitment (Reice 1994). There are good examples of these mechanisms occurring after disturbance in lotic ecosystems. For example, regrowth of survivors and their immature progeny occurs for hydrophytes, macroinvertebrates, and algae. Migration in streams occurs when mobile organisms travel short distances to recolonize denuded substrates. Recruitment is similar to migration, but includes only those individuals that recolonize from distant habitats (Reice 1994). Examples of recruitment in lotic ecosystems include aerial colonization and drift.

Wallace (1990) considered lotic macroinvertebrate community structure within a given habitat recovered when it achieves its historically natural range in variability throughout a year. Recovery of an ecosystem or a community cannot be discussed without reference to the ecological paradigm of succession. Smith and Smith (2001) defined succession as the temporal evolution of communities within an ecosystem. This process usually progresses toward a terminal community, which is thought to be stable (Smith and Smith 2001).

Distinct stages or seres are communities that are identifiable and predictable throughout succession. Pickett and White (1985) demonstrated the linkage between disturbance and succession: "The most obvious role that disturbance plays in ecosystems is in the deflection of a community from some otherwise predictable successional path." How successfully the ecosystem will recover will be influenced by resistance and resilience, both of which encompass succession and are important components of ecosystem recovery (Fisher 1990). Streams offer unique opportunities to study succession, yet stream succession has been almost ignored by researchers

(Fisher 1983). There is a small amount of evidence that succession is not significant in all streams in all regions (Fisher 1990).

Spatial heterogeneity may help lotic ecosystems recover from disturbance (Fisher 1990). Disturbance is a major contributing factor to spatial and/or temporal heterogeneity (Sousa 1984, White and Pickett 1985, Reice et al. 1990, Reice 1994, Ward 1998). The ecological paradigm of spatial heterogeneity is called patch dynamics. White and Pickett (1985) characterized the "patch" concept in patch dynamics as implying patches have a spatial relationship to each other; a spatial pattern is present but internal homogeneity, size, and discreteness do not have limits in any one patch. White and Pickett (1985) emphasized the dynamics part of patch dynamics implies patch evolution.

Patchy or spatially heterogeneous areas provide habitat and refugia for biota. Refugia provide resilience and/or resistance, in space and time, to communities affected by disturbance and is vital for recovery (Sedell et al. 1990). The frequency and magnitude of disturbance experienced by organisms is a product of stream patchiness (Townsend 1989, Reice et al. 1990, Reice 1994). In frequently disturbed streams, the maintenance of high species richness is dependent upon the spatial and temporal heterogeneity of resources (Brooks and Boulton 1991).

Disturbance depends upon the spatial and temporal scales at which it operates; it is predictable at large scales and random at small scales (Reice 1994). Rykiel (1985) stated that a disturbance at one level within a system might not propagate and cause perturbations among all levels. Thus, disturbance at a particular temporal or spatial scale may or may not be exclusive to that scale.

A forest fire is a large disturbance on a scale of several years, but becomes absorbed at larger time scales (Rykiel 1985). Spatially, children playing in a stream can

greatly disturb a small area, but if scale is increased to include the watershed, the children do not cause a disturbance.

The temporal component of disturbance encompasses the fact that disturbance is dynamic in time and relative to the system of interest. Some disturbance events, such as flooding, have a regular recurrence interval. On average most streams experience a flood every 1.5 years (Brooks et al. 2003). Disturbance can be ephemeral or chronic on a temporal scale and should be scaled to the organism of interest. A 15-year disturbance is a pulse for organisms with a life span of centuries, but for organisms living two years this disturbance is a press (Glasby and Underwood 1996). The time between disturbance events must be longer than the life span of the organism(s) of interest, for community change to occur at detectable levels (Fisher 1983).

Community structure is an important component of disturbance ecology and a focus of this thesis. A community is a group of species that interact, with each species belonging to a population at a given location. Community structure is composed of two components, species richness and species evenness. Species richness is the number of species in the community. Species evenness is a measurement of how equitably individuals are distributed among each species.

There are two theories of community structure which can be divided into two camps: equilibrium and non-equilibrium. The debate about which theory best explains the factor(s) responsible for maintaining and producing community structure has raged for decades (McIntosh 1985, 1987).

The term equilibrium suggests balance over time with no net change. Equilibrium models are based upon the idea that community structure is regulated by biotic interactions (Andrewartha and Birch 1954, Connell 1978, McIntosh 1985, Resh et al.1988, Reice et al. 1990, Reice 1994). Examples of biotic interaction include:

mutualism, trophic interaction via predation or parasitism, and competition. Factors that increase in influence as population growth increases have long been labeled as "density-dependent factors" (Nicholson 1958). Andrewartha and Birch (1954) stated that competition and density-dependent factors are used synonymously.

Equilibrium models adopt the premise that when competitively superior species A, competes with competitively inferior species B, the results is competitive exclusion of species B. Equilibrium models trivialize the influence of abiotic parameters and assume a constant environment (Andrewartha and Birch 1954, Resh et al. 1988, Reice et al. 1990, Reice 1994). If the community is disturbed by environmental factors such as fire or flooding it will return to equilibrium relatively quickly. Equilibrium theories adopt the premise that communities contain coevolved populations that are organized, efficient, and stable; the life history strategies of species are optimized (Connell 1978). In equilibrium models, high species diversity is the product of a spatially heterogeneous environment and/or a predator-mediated coexistence of species (Resh et al. 1988, Reice et al. 1990, Reice 1994).

The ideas of equilibrium or balance in natural populations were strongly engrained in early ecological (McIntosh 1985) and western thought back to antiquity (Egerton 1973, DeAngelis 1987). The earliest contributor to the idea of equilibrium theory is likely Möbius (1877), who described a "biocœnotic equilibrium" among an oyster bed (McIntosh 1985). For investigations on community structure the null hypothesis has historically been an equilibrium model, which assumed the environment was constant (Resh et al. 1988, Reice et al. 1990). Equilibrium models are rooted in mathematical models such as the Lotka-Volterra equations that demonstrate competitive exclusion (McIntosh 1985, Smith and Smith 2001).

Influential works that challenged the belief in equilibrium models of community structure include: Andrewartha and Birch (1954), Connell (1978), Pickett and White (1985). Nonequilibrium models are based upon the idea that community structure is regulated by abiotic interactions such as disturbance (Connell 1978, McIntosh 1985, Resh et al.1988, Reice et al. 1990, Reice 1994) or weather (Andrewartha and Birch 1954). Factors that do not increase in influence as population growth increases have long been labeled as "density-independent factors" (Nicholson 1958).

Key differences between the models exist in their assumptions about the environment and the importance of biotic interactions. Nonequilibrium models do not assume environmental factors have negligible effects upon the biota (Andrewartha and Birch 1954, Connell 1978, Pickett and White 1985, Turner 1987). Environmental factors are a source of disturbance, prevent the attainment of equilibrium, and are believed to be primarily responsible for regulating community structure. The attainment of equilibrium within the community is probably a rare event (Connell 1978, Reice 1994). Thus, biotic interactions are seen to be less important than a non-constant environment in determining community structure.

Nonequilibrium models adopt the premise that communities are not coevolved systems with a stabilized species composition, in which efficient associations and optimal life history strategies are present (Connell 1978). High species diversity in nonequilibrium models is the product of two processes, recruitment and distrubance (Reice 1994). The validity of nonequilibrium models in lotic ecosystems was explored conceptually by Hart (1983), Stanford and Ward (1983), Resh et al. (1988), and Reice (1994). Quantitative testing of nonequilibrium mechanisms in lotic ecosystem has been performed by many authors (e.g., Minshall et al. 1983, 1985, Hemphill and Cooper 1983, Ward and Stanford 1983, Reice 1985, Robinson and Minshall 1986, McCabe and Gotelli

2000). Depending upon the season and location, different streams within a watershed can have invertebrate communities that characterize both equilibrium and nonequilibrium conditions (Minshall et al. 1985).

The IDH is a nonequilibrium model that has received much attention from ecologists and is the crux of this thesis. Connell (1978) published the IDH to explain the diversity of coral reefs and tropical rain forests. There is some debate among ecologist as to its origins. Townsend et al. (1997c) stated that the genesis of the hypothesis can be linked to Hutchinson (1953) and Horn (1975). In reference to the hypothesis, Wootton (1998) cited: Paine and Vadas (1969), Dayton (1971), Horn (1975), Lubchenco (1978), and Sousa (1979) in addition to Connell (1978).

The IDH is a nonequilibrium model that predicts the factors responsible for generating and sustaining high species richness. The hypothesis states that species richness will be highest in areas that experience disturbances intermediate in frequency, intensity, and size. Maximum species richness is generated and sustained by disturbance events that interrupt the process of competitive exclusion and prevent the community from achieving equilibrium.

Connell (1978) did not explicitly state that the size of disturbance is a subset of the intensity and frequency parameters. However, this must be inferred from the text: "Diversity is higher when disturbances are intermediate on the scales of frequency and intensity (the 'intermediate distrubance' hypothesis)." Yet, in the first figure and in the text, three disturbance parameters (frequency, intensity, and size) are presented and discussed (Connell 1978). The importance of disturbance frequency and intensity is often discussed (e.g., Townsend et al. 1997a, b, c). It is imperative that investigators of the hypothesis not overlook the importance of the size parameter within the context of disturbance intensity and frequency.

Given the above, it is important to recognize the relationship between these spatio-temoral parameters. An intense, spatially-large disturbance by necessity would affect a large area. Given the low probably of spatially-large disturbances, the frequency of these large events is low. Examples of spatially-large disturbances with a low probability of occurrence include volcanic eruptions, large earthquakes, and massive floods. On the other extreme, spatially-small disturbances occur frequently and effect small areas.

If disturbance frequency, intensity, and size are not intermediate in scale then maximum species richness will not be obtained. If there are few disturbance events over time (low disturbance frequency) then those species that are poor competitors will be competitively displaced. A high disturbance frequency will produce a community composed of a few tolerant species; new colonizers will be displaced and richness will be low. Connell (1978) stated that regeneration of survivors and recruitment after a disturbance of intermediate intensity would result in higher species richness than a very intense disturbance, in which most species are from recruitment. Connell (1978) stated that disturbed areas intermediate in size can be recolonized by migration and recruitment resulting in high species richness.

The utility of disturbance ecology can be demonstrated through its influence on lotic ecology, general ecology, and natural resources policy. Resh et al. (1988) stated: "In fact, to some of us, disturbance is not only the most important feature of streams to be studied, it is the dominant organizing factor in stream ecology." Fisher (1990) proclaimed that everything we observe on earth is controlled by disturbance. Reice (1994) advocated that policy proposals impeding disturbance, heterogeneity, and recolonization should be rejected because they are integral in maintaining biodiversity.

The IDH has become so accepted that it influences natural resources management, including the fire policy of Yellowstone National Park (Wootton 1998).

My study investigated the IDH in Hardwood Creek near the Twin Cites Metropolitan Area of Minnesota. Portions of Hardwood Creek have been dredged repeatedly. Dredging is a physical disturbance to the stream and its biological communities. Yet, the practice of dredging is not uncommon in the Midwestern United States. In the Twin Cities Metropolitan Area alone, hundreds of stream miles have been dredged, straightened, channelized, or modified (Metropolitan Council 2003).

Streams are typically dredged to lower the water table and increase the efficiency at which water is removed from the surrounding landscape. Lowering the water table allows crops to grow closer to the stream and increase the amount of land dedicated to agricultural production. However, dredging eliminates meanders, pools, and riffles that are natural morphological features of a stream. After ditching, a stream is transformed into long straight sections that lack habitat heterogeneity.

The hydrological justification for dredging can be found in the Manning and discharge equations. The Manning equation (Brooks et al. 2003) is defined as: $V = (1.49/n)R_h^{2/3}s^{1/2}$ where, V is the average cross sectional stream velocity (ft/sec), and n is the roughness coefficient. The variable R_h is the hydraulic radius (ft), and $R_h = A/WP$ where, A is stream flow cross-sectional area (ft^2), WP is wetted perimeter (ft), and s is slope at the water surface (ft/ft). The discharge equation (Brooks et al. 2003) is defined as: $Q = VA$ where, Q is stream discharge (m^3/sec), V is the same as above except in (m/sec), and A is the same as above except in (m^2).

Dredging modifies (n), (R_h), and (s) producing higher stream velocities in the following ways. Dredging decreases (n) by removing rough substrates, hydrophytes, and woody debris. Dredging increases (R_h) by decreasing the wetted perimeter and

increases (s) by removing stream meanders. The increase in stream velocity must be considered in the context of the discharge equation. Although the dimensions of the stream change with dredging (A) should remain relatively unchanged. Thus, the increase in stream velocity results from an increase in stream discharge, or the volume of water transported per unit time. As a result, the water table near the stream will be lowered allowing for agricultural cultivation near the steam.

Organization of thesis

As indicated in the Introduction to this chapter, the original intent of this thesis was a test of the IDH to evaluate the longitudinal patterns of macroinvertebrate biodiversity, as a function of ditching-related disturbance. After developing several questions and doing a reconnaissance of sample sites in spring 2004 (after ditching), the extent and magnitude of physical disturbance associated with ditching became apparent. As a consequence, it became clear that standard d-net sampling procedures promoted by Barbour et al. (1999), and used by MPCA to generate data for decision-making activities, would not work well in recently dredged sections. Consequently, a second field sampling strategy using collections of surface-floating pupal exuviae (SFPE) of Chironomidae was incorporated into the field sampling design to complement the DN sampling. This design allowed for a direct comparison of the efficiency of the two methods for detecting macroinvertebrate biodiversity across the gradient of disturbances in Hardwood Creek, which forms the topic of Chapter 2.

Chapter 3 summarizes the *a priori* intention to test the applicability of the IDH to evaluate the longitudinal patterns of macroinvertebrate biodiversity as a function of ditching-related disturbance. Comparisons were performed at the community level, and for orders and families of macroinvertebrates to determine whether different levels of

taxonomic scale would reveal different levels of congruence with predictions of the IDH. I found the IDH model does not account for the patterns of species richness and diversity across the gradient of ditching-related disturbance; with only a few exceptions of families with very low species totals. Thus, the IDH was rejected as an appropriate model.

Two other models, the River Continuum Hypothesis (RCH) of Vannote et al. (1980) and Hydroseres, were tested in an *a posteriori* manner to determine their potential to serve as a model for community structure across the disturbance gradient in Hardwood Creek. The RCH is a very encompassing model that includes several predictable concepts. However, only the prediction relative to similarities of community structure among adjacent sites was tested. Results of this test are provided in Chapter 4, where I conclude that RCH also does not account for observed patterns of community structure in Hardwood Creek.

Two alternative classifications to determine the most appropriate system for aggregating sites into hydroseres are addressed in Chapter 5. In the first classification system, sites are assigned to three categories based on (1) lack of ditching and (2) time since last ditching. The alternative classification tested in this chapter results from recent decisions by MPCA to divide the stream into two segments for 303D listing, and to develop independent management goals and TMDL strategies for each segment. This alternative classification divides the stream into an upstream section (sites 1.5U downstream through site New) and a downstream section (sites 1.0 through 2.0, Figure 1). Community similarity indices were used to determine which classification is most strongly supported.

Chapter 6 provides a review of biological assessment studies that have employed collections of SFPE for study of Chironomidae.

Chapter 7 is a manuscript of a paper presented at the XVI International Symposium on Chironomidae held during July 2006 in Madeira, Portugal. This manuscript was submitted for publication in the Symposium proceedings and is undergoing peer review at this time.

Appendix A consists of an illustrated guide to the Chironomidae taxa collected as SFPE in this project. This guide was prepared as part of a larger-scale project to provide species-level identification guides to Chironomidae for a range of aquatic habitats in Minnesota. No comprehensive regional guides to Chironomidae are presently available. This guide complements a similar guide developed by Rufer (2007) for taxa encountered in lakes of the Twin Cities Metropolitan Area. A similar guide to Chironomidae of trout streams and warmer-water streams in east-central Minnesota is also being developed by Mr. R. Will Bouchard. Care has been taken to utilize the same designations for species that occur in Mr. R. Will Bouchard's study, but cannot be identified formally to species-level at this time.

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Figure

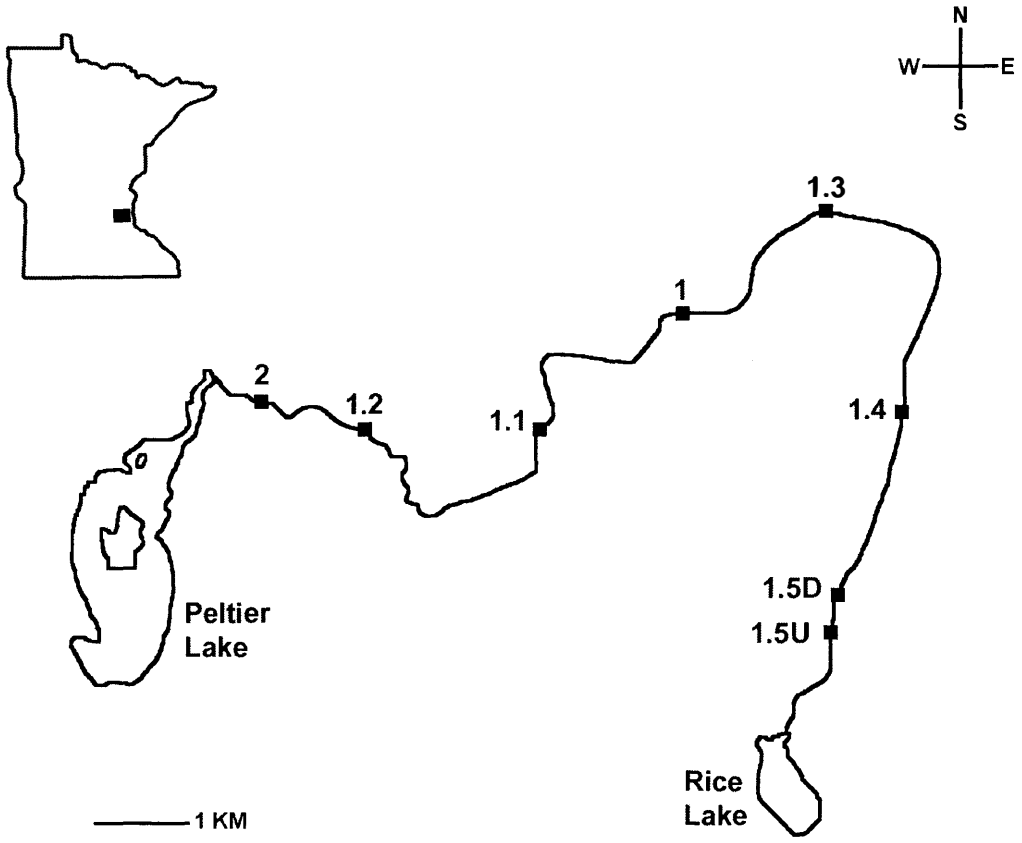

Figure 1. A map of Hardwood Creek and the 9 sample sites. Hardwood Creek flows from Rice Lake into Peltier Lake, north of Centerville, MN.

Chapter 2

Evaluation of Two Sampling Methods in Hardwood Creek, a Ditched Stream in Minnesota, USA

Will be submitted to: Journal of the North American Benthological Society

Abstract

Ditching is a physical disturbance to streams and is expected to modify stream habitats. Sites that have never been ditched, or have not been ditched recently, are expected to have greater habitat heterogeneity when compared to recently ditched sites. As a result, traditional sampling methods employed to sample ditches may not provide an accurate estimate of taxa richness. Dipnet (DN) and chironomid surface-floating pupal exuviae (SFPE) methods were employed to capture both chironomid and non-chironomid macroinvertebrates, for an investigation of the Intermediate Disturbance Hypothesis (IDH) in a ditched stream. A total of 271 macroinvertebrate taxa were detected. The SFPE method detected more taxa over the entire project and on a site-to-site basis, for both chironomid and non-chironomid taxa. In addition, the SFPE method exclusively collected from 1.2 to 8.8 times as many non-chironomid macroinvertebrates, on a site-to site basis, as the DN method. To obtain a comprehensive estimate of taxa richness in ditches, non-chironomid macroinvertebrates collected in SFPE samples should be evaluated in conjunction with DN samples. Dipnet and SFPE collections were highly correlated in this study. Future method comparisons need to incorporate equitable spatial and temporal effort for each method evaluated.

Introduction

Ditching is expected to modify stream habitats. Traditional sampling methods employed to sample ditches may not provide an accurate estimate of taxa richness. Accurate sampling of macroinvertebrate ditch communities is difficult because little is known about the dispersion of macroinvertebrates within ditches (Beltman and Rietveld 1981). Both dipnet (DN) and Chironomidae surface-floating pupal exuviae (SFPE) methods were employed to capture both chironomid and non-chironomid macroinvertebrates. The procedures used to evaluate both methods produced the SFPE, DN, and chironomid larvae data sets; data set(s) will be used instead of method(s) where appropriate. Justification for 2 collection techniques came from the need to obtain an accurate estimate of macroinvertebrate taxa richness in a ditched stream for an investigation of the Intermediate Disturbance Hypothesis (IDH).

A variety of methods have been used to investigate the IDH in streams. Many methods have involved *in situ* experimental manipulation of native or artificial substrates on relatively small spatial and/or temporal scales (Reice 1984,1985, Robinson and Minshall 1986, Malmqvist and Otto 1987, Doeg et al. 1989, Lake et al. 1989, Degani et al. 1992, McCabe and Gotelli 2000, Podraza 2002). Other workers exploited a historic disturbance gradient, in which the cause of disturbance was anthropogenic (Frest and Johannes 2002, Piscart et al. 2005), hydrologic (Rae 1990, Death and Winterbourn 1995, Townsend et al. 1997a, b, Death 2002), or hydrologic and thermal (Death 1995, Fruget et al. 2001). Several studies examined the hypothesis empirically by analyzing results of studies conducted by others (Ward and Stanford 1983, Statzner and Resh 1993).

Despite the prevalence of ditch systems and research on the IDH, no study to date has tested the IDH within a ditched stream. The present study focused on the

differences in sampling methods used to collect macroinvertebrates to evaluate the IDH. In the present study, the methods used to investigate the IDH were novel. Both SFPE and DN methods were employed to collect chironomid and non-chironomid macroinvertebrates. No study to date has evaluated the non-chironomid macroinvertebrates collected in SFPE samples. In addition, there has been no investigation of how efficient DN and SFPE methods are at collecting non-chironomid macroinvertebrates in a ditch.

Sealock and Ferrington (in-press) evaluated the sampling efficiency of DN and SFPE methods for detecting chironomid genera only. Others have examined the efficiency of various collection methods within ditches (Whitaker et al. 1979, Beltman and Rietveld 1981, Clare and Edwards 1983, Higler and Verdonschot 1989). The objective of this study was to evaluate DN, SFPE, and chironomid larvae data sets to compare both DN and SFPE methods using taxa richness, 3 similarity indices, and correlation coefficients.

Methods

Study site

Hardwood Creek is located northeast of the Twin Cities Metropolitan Area near Hugo, Minnesota (Figure 1). Hardwood Creek is a major stream in the Rice Creek Watershed District (RCWD). The district occupies parts of Anoka, Hennepin, Ramsey, and Washington Counties. Hardwood Creek is 1 of 2 major tributaries of Rice Creek, which is approximately 45 kilometers in length before flowing into the Mississippi River. The drainage area of Hardwood Creek is 114 km^2 and occupies the northeastern region of the RCWD.

The water quality in Hardwood Creek is important because the city of Saint Paul obtains part of its water supply from Peltier Lake (Figure 1). Hardwood Creek has been dredged repeatedly, both spatially and temporally, and the upper part of the creek is synonymous with Judicial Ditch 2 of Washington County. Judicial Ditch 2 was established by statute in 1914 (Anonymous 1975).

The topography and underlying geologic composition of the watershed was greatly influenced by the Grantsburg Sublobe glacier which retreated about 12,500 years ago (Anonymous 1975). The Prairie du Chien group composes most of the bedrock found throughout the watershed (Anonymous 1975).

Hardwood Creek flows through the Lino-Isanti and Nessel-Dundas soil associations. The Lino-Isanti association occurs from Rice Lake until just west of U.S. Highway 61. This association can be described as poorly drained, approximately level to gentle slope, and possessing a high water table which limits its development potential (Anonymous 1975, 1990). The Nessel-Dundas association meets the Lino-Isanti and continues until the confluence with Rice Creek. This association can be described as poorly to moderately well drained, approximately level to gentle slope, and can be farmed if the water table is not too high (Anonymous 1975, 1990). More detailed information on each study site can be found in Chapter 3.

Dipnet sampling protocol

Ditching is a physical disturbance to streams and is expected to modify stream habitats. Woody debris, vegetation, and substrates are altered or removed during ditching. Sites that had never been ditched, or were not ditched recently, were expected to have greater habitat heterogeneity when compared to recently ditched sites. Because a disturbance gradient, from recently to never ditched sites exists in Hardwood Creek,

we deployed a sampling approach sensitive to habitat modifications caused by ditching. Based upon *a priori* observations bottom, bank, wood, and riffle habitats were defined and delineated as sample stratification units. When present at each site, 3 DN samples were collected at each stratification unit.

Samples were taken with a d-framed DN with a mesh of 500 µm in June 2004. Samples were collected at all 9 sample locations in Hardwood Creek (Table 1). However, some samples were lost or habitat stratification units were incorrectly sampled. At site 1.2 there were 4 bottom samples, 5 bottom samples at site 1, and 2 bank samples were collected at site 2 (Table 1).

Three consecutive samples were taken at each habitat stratification unit. Great care was taken to minimize site disturbance during sampling. The sampling order of stratification units imparting the smallest amount of disturbance to neighboring units was selected. Samples were deposited individually into 500-mL-polypropylene bottles, and labeled and preserved with 70% isopropyl or ethyl alcohol.

Samples were transferred from the DN into the sample bottle by hand and/or a funnel. Samples at sites with peaty substrates often required the sample to be forced into the sample bottle. Large rocks were thoroughly washed in the DN and discarded. Large pieces of wood were broken into smaller pieces and placed into the bottle.

Material clinging to the sides of the net was condensed by sweeping the net 1 to 3 times through the first several inches of stream water. After the contents were removed from the net it was examined for residual organisms. Before the next sample was obtained, the net was again washed by sweeping it 1 to 3 times through the water.

Sampling began when wood and/or riffle habitats were present, since bottom and bank habitats were present at all sites. When a sample location was selected, an

arbitrary longitudinal boundary, approximately 15 to 25 meters long was established. An effort was made to obtain all samples within this area.

Sites 1.5U, 1.5D, 1.4, and 1.3 were sampled in a kayak because of the boggy and unstable nature of stream substrates at these sites. After DN samples were obtained, information on riparian and stream vegetation was recorded.

Bank samples were taken at every site (Table 1). For bank habitats 2 samples were taken from 1 bank; the remaining sample was retrieved from the opposite bank. Each sample was retrieved from a location below the surface of the water and more lateral in its orientation, when compared to bottom samples. The DN was jabbed into the bank 1 to 3 times and/or swept across the bank for approximately a half-meter, depending upon the bank morphology and composition at each site.

Bottom samples were taken at every site (Table 1), in or near the middle of the channel. Depending upon the type of substrates present, the DN was jabbed into the bottom 1 to 3 times and/or swept across the bottom for approximately a half-meter. Care was taken to avoid wood and riffle stratification units while sampling the bottom and bank stratification units.

Wood samples were taken at 6 sample sites (Table 1). Only woody substrates contributed from riparian, not anthropogenic sources were sampled. Each sample was taken as close as possible to the woody debris of interest, given its size and orientation. Wood size determined the number of times it could be sampled. Small pieces of wood were sampled once, larger pieces were sampled multiple times. As with bank and bottom samples, 1 to 3 jabs and/or approximately a half-meter DN sweep composed a wood sample. Wood toward the middle of the channel was sampled as frequently as possible.

Riffle samples were taken at site 1.2 only (Table 1). All samples were taken by facing the net opening upstream and disturbing the substrate with 5 boot jabs upstream of the net.

Dipnet processing protocol

All preserved DN samples were refrigerated until processed. Sample processing began by removing a sample from the refrigerator and pouring it into a 125 µm sieve positioned within a large funnel. Next, the sample was washed with water. Samples containing a large percentage of peat or sand were repeatedly washed to reduce their size. In most instances the sample identification label was not read until the entire sample was picked, to avoid processing bias.

If the sample occupied approximately 90 to 100% of the sample bottle subsampling occurred. A subsample was obtained in the following way: the sample was mixed and evenly distributed in a sieve, the sieve was rotated several times, and a coin was flipped to determine which half of the sample was kept.

After a sample was washed with water it was partly submerged in a tray 30 cm long, 25.5 cm wide, and 5 cm deep containing water. Next a white-plastic pan 20.5 cm long, 15.5 cm wide, and 3.7 cm deep was filled with approximately 1 to 3 cm of water. The bottom of the pan contained a grid pattern to aid in sample sorting. The sample was transferred to the pan by forceps or a spoon. Large pieces of vegetation were inspected for organisms and then discarded. All macroinvertebrates, except ostracods and zooplankton, were deposited into 1, 2, or 7 dram vials containing alcohol. Fish were also picked and deposited into vials.

When all organisms of interest were removed, the pan was stirred and thoroughly inspected. If no organisms were found, this procedure was repeated. If no organisms

were found after two consecutive inspections, the remaining material was discarded into a 125 µm sieve. If another organism was found it was picked and the procedure begun anew. After the entire sample was picked the remaining residues were placed in alcohol and refrigerated. A sample identification label was added to each vial after picking was completed. To differentiate each sample, within each stratification unit, the letter A, B, or C was assigned to each sample.

Most organisms were identified to genus or species (Appendix C Tables 1-9) using an Olympus 10X to 63X dissecting microscope. Specimens from each sample were placed into 1, 2, or 7 dram vials containing alcohol and labels with locality and identification information. Each taxon and the number of specimens present was recorded. For specimens to be assigned to a taxon, we had to be at least 50% confident that their identification was correct. If there was too much uncertainty, or further resolution was not possible, specimens were classified as undetermined at the next highest taxonomic level.

A parsimonious approach was applied to immature and other hard-to-identify organisms, to avoid over-inflating the estimates of species richness. We assumed that immature and other damaged and hard-to-identify specimens were most likely in a taxon previously identified from the site of interest. This approach established a burden of proof for unique specimens to prevent an inflated estimate of taxonomic richness. Identifications were determined with the help of the following sources: Bouchard (2004), Clarke (1981), Hilsenhoff (1995), Jokinen (1992), Klemm (1991), Merritt and Cummins (1996), Pennak (1989).

All Chironomidae larvae from DN samples were slide mounted using a 15X to 45X Fisher-Scientific dissecting microscope and identified at 200X to 400X using a Leica compound microscope. Larvae were mounted on glass slides 7.5 cm long and 2.5 cm

wide, with locality and determination labels. All larvae with head capsules were dehydrated in 95% ethyl alcohol. Next, Euparal mounting medium was applied to the center of a slide. One or several larvae were removed from the alcohol and placed into Euparal. Head capsules of larvae were oriented ventral side up to expose the mentum. A glass cover slip was placed over the larva(e).

Pressure was applied to the cover slip with forceps to compress the larva(e) and distribute the Euparal. Round cover slips were used for voucher specimens; square cover slips were used when multiple larvae were mounted on a single slide. After specimens were slide mounted, they were identified using Coffman and Ferrington (1996). A voucher collection of larvae will be deposited in the insect museum at the University of Minnesota.

Dipnet quality assurance/quality control

The quality assurance/quality control evaluation was performed by repicking at least 10% of all DN samples. After about 10 to 30 samples were processed 10% were randomly chosen by placing them into numbered locations. The number corresponding to each location was written on a small piece of paper, crumpled, placed into a container, and drawn randomly. The samples chosen were repicked following the above protocol. If the number of organisms found during the repicking procedure exceeded 10% of the organisms found during the initial picking, a repicking procedure by stratification unit ensued. If the number found was less than 10%, the quality assurance/quality control protocol was satisfied.

Repicking procedure for stratification units

For each stratification unit at each site, the average number of organisms found during the initial picking was calculated. Next, 1 sample from each stratification unit was repicked. If the number of organisms found was at least 10% of the average, the rest of the samples within the stratification unit, at that site, were repicked. If the number was less than 10% no further repicking was required. Organisms obtained by repicking were not included in the data analysis. However, the presence of small chironomid and amphipod specimens was noted in repicked samples and greater care was taken during the picking process.

Sampling protocol for Chironomidae pupal exuviae

Chironomidae SFPE were sampled approximately monthly, from April through November of 2004 at all sites except New (Table 2). Sampling followed the protocol of Ferrington et al. (1991). A plastic pan 20.5 cm long, 15.5 cm wide, and 3.7 cm high was used in unison with a sieve with a 125 µm mesh. Slow moving water near stream banks or obstacles, where *Lemna* spp. and other floating debris collected, were the primary areas sampled. A sample was obtained by dipping the pan into the water and pouring its contents into the sieve. This process was repeated for approximately 10 minutes. After sampling was completed the contents of the sieve were washed into a 60-ml-glass jar or 500-mL-polypropylene bottle, containing a locality label and 70% isopropyl or ethyl alcohol.

Sites 1.5U, 1.5D, 1.4 and 1.3 were typically sampled from a kayak. In the later months of the study 1.5D and 1.3 could be sampled without a kayak. If 2 individuals were able to travel to the field, sampling would occur concurrently at upstream and downstream sites. One investigator would kayak and sample the 4 upstream sites

starting at 1.5U and ending at 1.3 (Figure 1). The other investigator would sample the 4 downstream sites, starting at site 2 and ending at site 1 (Figure 1).

Laboratory protocol for Chironomidae pupal exuviae

Preserved SFPE samples were refrigerated until processed. Sample processing began by pouring a sample into a 125 µm sieve positioned within a large funnel. The sample was washed with water and partly submerged in a tray 30 cm long, 25.5 cm wide, and 5 cm deep to prevent desiccation. A petri dish with a diameter of 8.6 cm and a depth of 1 cm was filled 50 to 80% with water. Forceps were used to transfer the sample from the sieve to the petri dish. An Olympus 10X to 63X dissecting microscope was used to sort all samples. Pupal exuviae were picked into 1 or 2 dram vials containing an identification label and alcohol. Many exuviae fragments were also picked; however, only whole exuviae were counted.

Adult male chironomids and other macroinvertebrates, including zooplankton and ostracods, were qualitatively subsampled and preserved the same way. Adult males still attached to their pupal exuviae were kept in a separate vial and were not slide mounted or counted. After a thorough inspection, all remaining material in the dish was discarded into a 125 µm sieve. After the entire sample was picked, the remaining material was refrigerated after being placed into a container with an identification label and alcohol.

All non-Chironomidae macroinvertebrates from SFPE samples were identified to the lowest practical taxon, usually genus or species (Appendix C Tables 10-17) using the same equipment, techniques, and literature specified for DN samples. Each taxon and the number of specimens present in the qualitative subsample was recorded.

Pupal exuviae were initially identified and sorted to genus under an Olympus 10X to 63X dissecting microscope by Dr. Len Ferrington (Appendix C Tables 18-25). Exuviae

identified to genus were subsampled from several sampling dates for identification to species (Appendix C Tables 26-33). Within each genus, specimens were sorted to morphospecies under a 10X to 45X Fisher-Scientific dissecting microscope. Five specimens from each morphospecies were slide mounted. If fewer than 5 specimens were present all were mounted.

After SFPE were removed from the 95% ethyl alcohol and placed into Euparal, the cephalothorax was removed from the abdomen. Next the ecdysial suture was completely opened. The cephalothorax was positioned so that all external surfaces would be facing up and in contact with the cover slip. Only 1 exuvium was mounted per slide. After specimens were slide mounted, they were identified using a 200X to 400X Leica compound microscope, Wiederholm (1986), and other literature sources specified in Appendix A. Slide-mounted voucher material will be deposited in the insect museum at the University of Minnesota.

Pupal exuviae quality assurance/quality control

The quality assurance/quality control evaluation was performed by repicking at least 10% of all samples. The evaluation was performed as described for DN samples.

Data analysis

The following taxa were collected in at least 1 sample, but were excluded from all analyses: Collembola, terresterial Curculionidae, Ptiliidae, Staphylinidae, Sciaridae, undetermined Gastropoda, Hydracarina, Cladocera, Ostracoda, Copepoda, and Osteichthyes. However, they are recorded in the raw data (Appendix C Tables 1-17). Macroinvertebrates from SFPE samples were entered as present or absent and

summed across all sample dates on a site-to-site basis to obtain the number of samples in which each taxon was present (Appendix C Tables 10-17).

Whole chironomid pupae were not entered into the raw data even if they were present in DN samples, however they are recorded in SFPE macroinvertebrate samples (Appendix C Tables 10-17). Oligochaetes were counted as present (1) regardless of their abundance in DN and SFPE samples (Appendix C Tables 1-17). Nematoda were counted in SFPE samples, but recorded as present (1) in DN samples (Appendix C Tables 1-17).

In general, unidentifiable specimens were deleted and/or merged into another taxon when genus or species level data were available at the site. For example, immature Corixidae were not analyzed when an adult Corixidae from any genus was found at a site. For the co-occurrence of taxa such as *Hygrotus*/*Hydroporus* sp. and *Hygrotus* sp. at a single site, 2 taxa were considered to be present (*Hygrotus*/*Hydroporus* sp. and *Hygrotus* sp.).

The IDH was tested by arranging each site according to its position within the disturbance gradient, and plotting richness or diversity values for each site. Three data sets were produced to analyze the DN and SFPE methods. The DN data set consisted of all macroinvertebrates collected in DN samples. Chironomid larvae collected in DN samples were used to construct the chironomid larvae data set. The SFPE data set was composed of: SFPE identified under a dissection microscope, and subsampled and slide mounted SFPE identified under a compound microscope. It should be noted that SFPE macroinvertebrates were not included in the SFPE data set. Analyses were conducted using Microsoft® Excel.

Accurate estimates of taxa richness were obtained by deleting the taxa appearing in more than 1 data set, for each method of interest, on a site-by-site basis.

Non-Chironomidae macroinvertebrate taxa present in the SFPE data set, which did not increase taxa richness when compared to the DN data set, were deleted. All chironomid taxa identified from slide mounted SFPE subsamples, SFPE identified under a dissecting microscope, and larvae collected in DN samples were analyzed together. First, the 2 sets of SFPE data were compared. Taxa identified with the dissecting scope that were not unique to the slide mounted data set were deleted. Taxa in the larvae data set were compared to those found in the SFPE data sets and were deleted if previously found. Taxon richness for each site was calculated by adding the number of non-Chironomidae macroinvertebrates and total Chironomidae.

Whittaker's Percent Similarity (WPS), Simple Matching Coefficient (SMC), and Jaccard's Similarity (JAC) similarity indices were calculated for chironomid larvae, SFPE, and DN data sets using ECOMEAS software (version 2.3/release 1986, Kansas Biological Survey, Lawrence, KS). The WPS index (Whittaker 1952, Whittaker and Fairbanks 1958) is defined as: $WPS = \Sigma \min(a, b)$ where, a and b are the frequencies of a given species in samples A and B. The WPS index cannot be calculated for the qualitative SFPE data.

Simple Matching Coefficient and JAC indices were both calculated for all data sets. The SMC index (Heltshe 1988) is defined as: $SMC = (a + d)/S$ where, a is the number of taxa shared by both sites, d is the number of taxa detected in the project but absent at both sites, and S is the total number of taxa found at both sites. The JAC index (Heltshe 1988, Southwood and Henderson 2000) is defined as: $Cj = (a)/(a+b+c)$ where, a is the number of taxa shared by sites B and C, b is the number of taxa at site B only, c is the number of taxa at site C only.

Indices and data sets were used to analyze, (1) the mean similarities of all site combinations, (2) the mean similarities within and across class groupings, and (3) correlation coefficients between data sets.

Results

There were 80 DN and 51 SFPE samples processed for a total of 131 samples processed during the study (Tables 1-2). A total of 271 macroinvertebrate taxa were identified. Dipnets collected 73 non-chironomid macroinvertebrates and 51 chironomid taxa for a total of 124 taxa (Table 3). By contrast, SFPE samples collected 124 non-chironomid macroinvertebrates and 129 chironomid taxa for a total of 253 taxa (Table 3).

For the entire project, the number of non-chironomid macroinvertebrates collected exclusively with DN and SFPE methods was 9 and 60 respectively (Figure 2). The number of chironomid taxa collected exclusively with DN and SFPE methods was 9 and 87 respectively, over the entire project (Figure 2).

On a site-to-site basis the SFPE method exclusively collected many more taxa when compared to the DN method (Table 4). The number of non-chironomid taxa collected exclusively with SFPE samples ranged from 1.2 to 8.8 times that collected in DN samples at sites 1 and 1.5U respectively (Table 4). Over the entire project the SFPE method collected just over twice as many taxa (Table 3). The number of Chironomidae collected with the SFPE method (129 taxa) was greater than the total number of macroinvertebrates collected by the DN method (124 taxa, Table 3). Our ability to identify chironomid exuviae captured with the SFPE method to species or morphospecies contributed to this discrepancy between data sets.

Similarity indices

For the analysis of the mean similarity indices across all possible site combinations for each data set, the SMC index had the highest mean, minimum, and maximum values (Figure 3 and Appendix B Table 1). The DN data set had the highest average similarity value for 2 of the 3 indices; JAC index was more similar for chironomid larvae by 0.01 or 1% more than the DN data set (Figure 3 and Appendix B Table 1). The SFPE data set had the lowest average similarity values for both SMC and JAC indices (Figure 3 and Appendix B Table 1). However, when the June SFPE data set was analyzed, the SMC index was the second highest and the JAC index was the lowest.

In general the correlation coefficients between all 3 data sets and indices were high, the lowest coefficient was 0.532 and the highest was 0.812 (Figure 4 and Appendix B Table 2).

Discussion

Sampling methods

Across all sample sites there was a large difference between the number of taxa collected with DN and SFPE methods. Although both methods detected taxa not collected by the other method, the SFPE method generally detected many more taxa on a site-to-site basis, including both chironomid and non-chironomid taxa.

This study complements and builds on the investigation of SFPE and DN methods in a ditch started by Sealock and Ferrington (in-press). The SFPE method can be expected to exclusively collect about twice as many chironomid taxa as the DN method (Sealock and Ferrington in-press). In addition, we found that the SFPE method exclusively collected from 1.2 to 8.8 times as many non-chironomid macroinvertebrates, on a site-to-site basis, as the DN method.

The results of this study are similar to Whitaker et al. (1979) who used Ekman dredge, DN, and vegetation/debris methods to collect macroinvertebrates in ditches. Whitaker et al. (1979) sampled across a number of sites ranging from 1 to 30 plus years since dredging and found the efficiency of benthic samples to capture taxa varied from site-to-site.

This study supports Higler and Verdonschot (1989) who concluded that different sampling methods should be used for different ditch habitats. Although the DN method was employed to sample each habitat stratification unit, the SFPE method also detected species associated with the water column and aquatic plant communities.

Very different estimates of taxa richness would have resulted if only the DN method was used to collect non-chironomid macroinvertebrates. This study supports the findings of Sealock and Ferrington (in-press), that both DN and SFPE methods should be used in unison to evaluate macroinvertebrate taxa richness in ditches. Non-chironomid macroinvertebrates collected in SFPE samples need be evaluated to obtain a comprehensive estimate of taxa richness.

The findings of this study contrast with Beltman and Rietveld (1981) who found a standard net to be the best method to sample macroinvertebrates in ditches. Clearly, if a single method for sampling Hardwood Creek must be chosen, the SFPE method should be selected.

It was difficult to compare the findings of the present study with Clare and Edwards (1983) because different methods were used in both studies. Clare and Edwards (1983) used 10-meter sweeps with a DN to sample plants and the water column, whereas cores 5 cm in diameter were used to sample the benthos. In the present study, quick jabs were made with a DN and SFPE were collected.

In the present study, DN samples performed poorly when compared to SFPE samples. Clare and Edwards (1983) found DN samples collected about 10 times as many taxa when compared to cores. These findings are not surprising given the spatial scale at which the methods were employed. The method sampling the greatest area would be expected to collect more taxa, all else being equal. In both the present study and Clare and Edwards (1983), those methods which sampled the benthos were employed at a small spatial scale and collected few taxa. The methods employed to sample the water column and vegetation were employed on a larger spatial scale and collected the most taxa. Both studies confounded the scale of the sampling methods with the habitats sampled, which makes it difficult to equitably compare which habitats and/or methods yielded more taxa.

Similarity indices

As expected, the SMC index consistently had the highest index values given that it takes into account joint co-absences as contributing to sample similarity. Differences between JAC and WPS indices can be explained by the fact WPS uses the frequencies of a given species in the samples of interest. The JAC index uses only presence/absence data and ignores abundance data.

In general, DN samples produced the highest average similarity index values, when all possible sites and their combinations were examined. Chironomid larvae had the second highest index values, while SFPE had the lowest. These finding cannot be used to validate one method over another for sampling Hardwood Creek or ditch systems in general. The fact that DN samples had the highest similarity values was not surprising given the diverse fauna and sites sampled in Hardwood Creek. Only a method

capable of collecting the vast majority of the fauna, not just chironomids, would be expected to have the highest similarity indices.

Larvae and SFPE data sets, were focused on the Chironomidae only. Given the relatively diverse sites in Hardwood Creek, we would expect the chironomid taxa to have relatively low similarity values across all site comparisons as different species are able to exploit different niches at different sites. Larvae collected via DN allowed for the collection of taxa that would emerge throughout the year verses the SFPE samples which collected only those taxa that just emerged; this contributed to the discrepancy between larvae and SFPE data sets. The taxonomic resolution made possible by collecting SFPE also contributed to the lower index values of this method.

In addition, the temporal scale at which the methods were employed also contributed to the differences in their similarity indices. The DN method was employed only in June, while the SFPE method was employed 6 or 7 times, from April through November. The method employed to sample the fauna repeatedly over 8 months would be expected to have the lowest similarity values when compared to a method employed only once, all else being equal. This is especially true given the diversity of sample sites and chironomid phonologies. Employing the DN method 6 or 7 times at each site would be a more equitable comparison. However, the data sets for the month of June can be compared. The June SFPE data set showed little deviation from the data set including all sampling dates; the SMC value was the second highest and the JAC index value was the lowest.

The high correlation coefficients for the similarity indices between all data sets is evidence that a relatively strong relationship exists between the number of taxa collected by any two methods. As the number of taxa that can potentially be collected at any site increases, so too will the number of taxa collected by any two methods. However, some

data sets are more strongly correlated with each other. The data suggest that DN and SFPE data sets were the most strongly correlated.

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Tables

Site	Bottom	Bank	Wood	Riffle	Total
1.5U	3	3	0	0	6
1.4	3	3	0	0	6
New	3	3	3	0	9
1.3	3	3	3	0	9
1.5D	3	3	3	0	9
1	5	3	3	0	11
1.1	3	3	3	0	9
1.2	4	3	3	3	13
2	3	2	3	0	8
Total	30	26	21	3	80

Table 1. The number of DN samples collected from each habitat stratification unit at each site in Hardwood Creek.

Site	4/28	5/26	6/23	7/28	9/1	10/6	11/13	Total
1.5U	Yes	Yes	Yes	Yes	Yes	Yes	Yes	7
1.4	*	Yes	Yes	Yes	Yes	Yes	Yes	6
New	*	*	*	*	*	*	*	0
1.3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	7
1.5D	Yes	Yes	Yes	Yes	Yes	Yes	Yes	7
1	Yes	Yes	Yes	Yes	Yes	Yes	*	6
1.1	Yes	Yes	Yes	Yes	Yes	Yes	*	6
1.2	Yes	Yes	Yes	Yes	Yes	Yes	*	6
2	Yes	Yes	Yes	Yes	Yes	Yes	*	6
Total	7	8	8	8	8	8	4	51

Table 2. Sampling dates in 2004 for collection of SFPE samples at each site. An asterisk (*) indicates that a sample was not taken or processed. Downstream sites were frozen on 11/13/2004.

Taxon	DN	SFPE	Total
Ephemeroptera	4	6	6
Odonata	2	4	4
Plecoptera	1	2	3
Hemiptera	6	12	12
Megaloptera	0	1	1
Trichoptera	5	9	10
Lepidoptera	1	1	1
Coleoptera	14	29	29
Diptera	62	159	171
Chironomini and Pseudochironomini (tribe)	20	47	49
Orthocladiinae (subfamily)	16	40	43
Prodiamesinae (subfamily)	1	0	1
Tanypodinae (subfamily)	8	15	18
Tanytarsini (tribe)	6	27	27
Nematoda (phylum)	1	1	1
Veneroida	3	2	3
Basommatophora	13	14	14
Heterostropha	1	1	1
Neotaenioglossa	1	1	1
Arhynchobdellida	1	2	2
Rhynchobdellida	5	5	7
Oligochaeta (subclass)	1	1	1
Isopoda	1	1	1
Amphipoda	1	2	2
Decapoda	1	0	1
Summary			
Non-chironomids	73	124	133
Chironomids	51	129	138
Total	124	253	271

Table 3. The number of taxa collected with DN or SFPE methods within each order (unless labeled other wise).

Non-chironomids				Chironomids			
Site	DN	SFPE	Both	PESM	PEDS	Larvae	Totals
1.5U	6	53	14	48	6	3	130
1.4	6	39	14	39	6	1	105
New	33	*	*	*	*	29	62
1.3	12	25	27	33	6	9	112
1.5D	6	40	25	38	4	2	115
1	18	22	15	47	10	8	120
1.1	16	30	16	42	11	5	120
1.2	9	34	15	38	10	7	113
2	13	30	14	39	6	11	113

Table 4. The totals of non-chironomid taxa collected exclusively with DN and SFPE samples, and those collected with both methods. The right portion of the table summarizes the number of unique chironomid taxa identified from pupal exuviae that were slide mounted (PESM), pupal exuviae examined under a dissection scope (PEDS), and larvae. An asterisk (*) indicates that sampling did not occur.

Figures

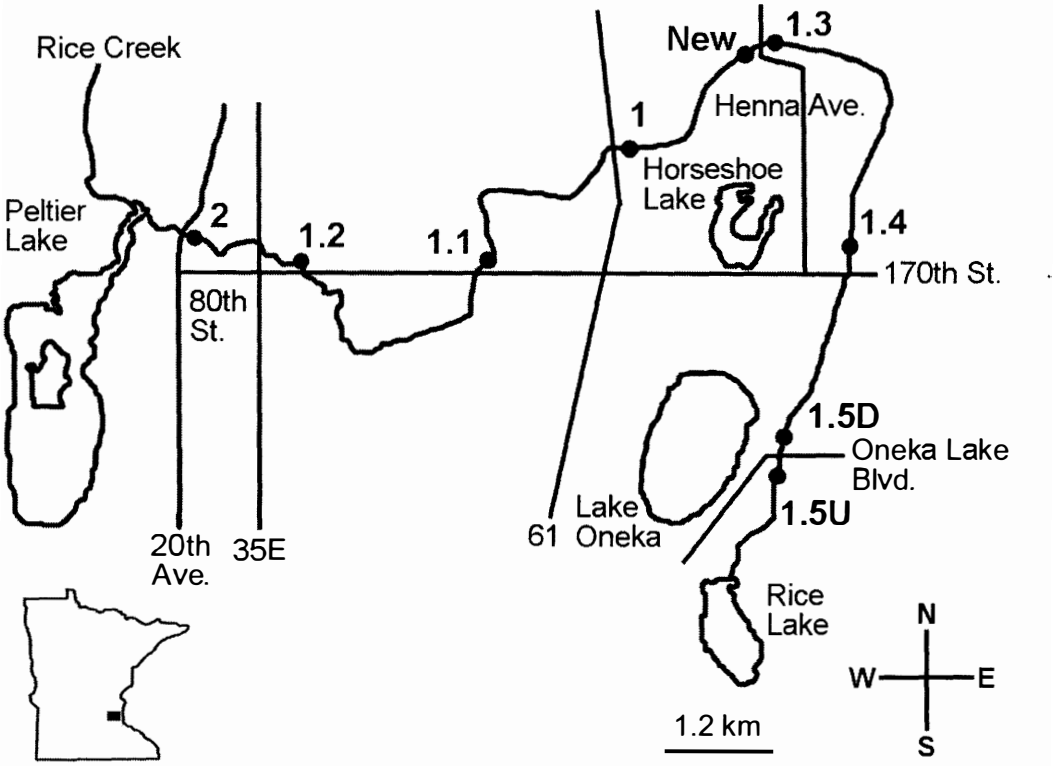

Figure 1. A map of Hardwood Creek and the 9 sample sites. Hardwood Creek flows from Rice Lake into Peltier Lake.

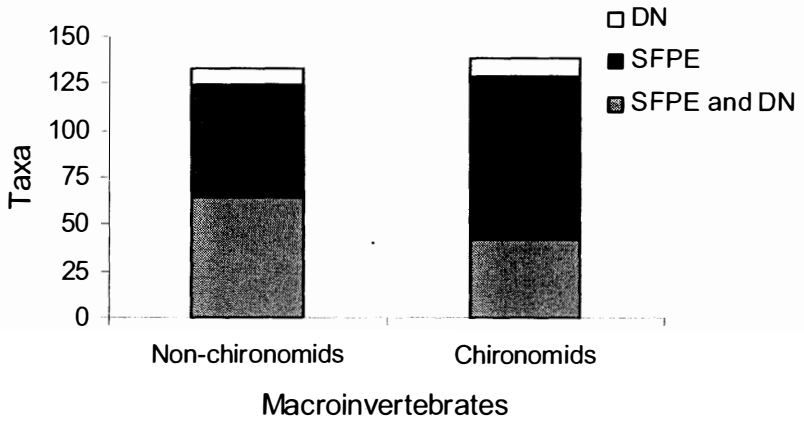

Figure 2. The totals of non-chironomid and chironomid taxa collected exclusively in DN, exclusively in SFPE, and with both methods.

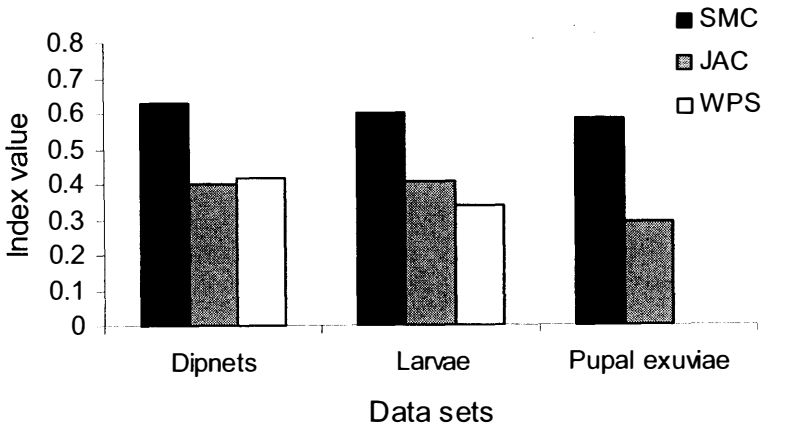

Figure 3. Similarity means for each index across all possible site combinations for taxa from DN, chironomid larvae, and SFPE data sets.

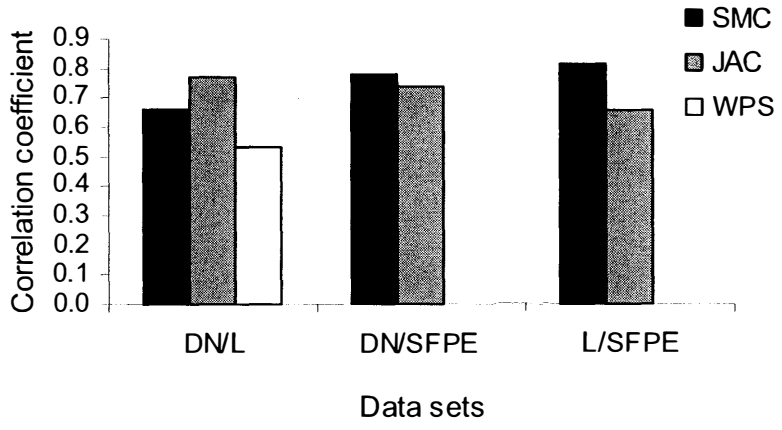

Figure 4. The correlation coefficients of similarity indices for the DN and larvae (DN/L), DN/SFPE, and larvae and SFPE (L/SFPE) comparisons for SMC, JAC, and WPS indices where applicable. Site New has been removed for these comparisons.

Chapter 3

An Investigation of the Intermediate Disturbance Hypothesis in Hardwood Creek, a Ditched Stream in Minnesota, USA

Will be submitted to: Journal of the North American Benthological Society

Abstract

Despite the prevalence of ditch systems and research on stream disturbance, no study to date has tested the Intermediate Disturbance Hypothesis (IDH) within a ditched stream. Four hypotheses were tested at the community, order, and family level for conformity to the IDH in Hardwood Creek. Both dipnet (DN) and chironomid surface-floating pupal exuviae (SFPE) methods were employed to obtain an estimate of taxa richness. A total of 271 macroinvertebrate taxa in 18 orders and 61 families were collected and analyzed. This study failed to confirm the IDH as an explanation for patterns of taxa richness and sample diversity at the community level. However, if the most disturbed and taxa rich site is eliminated from the data set, the pattern of taxa richness more closely conforms to the IDH. A site that was predicted by IDH to have high richness and diversity, had conspicuously lower taxa richness and diversity than many of the sites in Hardwood Creek. Potentially, increased turbidity caused by dredging in the winter of 2004 led to the low diversity at this site. By modifying the taxonomic scale from community, to family or order, valuable insight into the applicability of the IDH to lotic macroinvertebrates was gained. Seventeen orders and 58 families did not conform to predictions of the IDH. However, 1 order (Rhynchobdellida) and 3 families (Dytiscidae, Planorbidae, Glossiphoniidae) conformed to the IDH.

Introduction

The ecology of stream disturbance has received much attention in the last several decades (e.g., Hemphill and Cooper 1983, Resh et al. 1988, Reice et al. 1990, Lake 2000, Piscart et al. 2005). In the United States stream ecologists have largely neglected to investigate disturbance in ditched or channelized systems despite their prevalence. Ditching has been used to drain wetlands and convert land-use to agriculture and pasture. Consequently, many areas have extensive ditch systems that need to be managed to reduce impacts on adjoining streams and lakes. For instance, in the state of Minnesota alone, there are approximately 32,000 to 43,000 kilometers of public drainage ditches (Anonymous 2006).

Investigations of macroinvertebrates in ditches in the United States include biological assessments (e.g., Needham 1949, Hubert and Krull 1973, Maxted et al. 2000, Stone et al. 2005) and effects of channelization (e.g., Duvel et al. 1976, Whitaker 1979, Edwards et al. 1984). European researchers have been very proactive at investigating the dynamics of macroinvertebrates of ditches (Caspers and Heckman 1981, 1982, Clare and Edwards 1983, Scheffer et al. 1984, Higler and Verdonschot 1989, Verdonschot and Higler 1989, Eyre et al. 1990, Verdonschot 1992, Williams et al. 2003), including the role of ditches in species conservation (Foster et al. 1990, Painter 1999, Armitage et al. 2003). The effects of ditch management on macroinvertebrate communities has also received much attention (Clare and Edwards 1983, Beltman 1984, Kaenel et al. 1998, Painter 1998, Twisk 2000, Armitage et al. 2001).

Ditches are relatively good ecosystems in which to test lotic disturbance hypotheses. In addition to being locally ubiquitous in rural landscapes, ditches are often maintained with methods that continuously disturb the stream and its communities. The disturbance imparted by maintenance activities can be chemical, when vegetation is

removed by herbicides, or physical when stream reaches are dredged. Lotic ecologists can take advantage of these planned disturbances by quantifying the effects on the benthos. However, ditches in agricultural regions are also prone to disturbances from livestock, pesticides, and nutrient inputs that can confound the disturbance event(s) of interest. Despite the prevalence of ditch systems and research on stream disturbance, no study to date has tested the Intermediate Disturbance Hypothesis (IDH) within a stream in which sections have different ditching regimes and, consequently, different disturbance histories.

Since Connell (1978) proposed the IDH it has attracted much attention from stream ecologists (e.g., Ward and Stanford 1983, Reice 1985, Townsend et al. 1997a, b, Death 2002). The IDH is a nonequilibrium model of community structure that predicts species richness will be highest in areas that experience disturbances that are intermediate in frequency, intensity, and size. Maximum species richness is generated and sustained by disturbance events interrupting the process of competitive exclusion, thus preventing the community from achieving equilibrium.

Hypotheses tested

In this study, 4 hypotheses tested the conformity of macroinvertebrate taxa richness and diversity to the IDH at the community, order, and family level.

Null hypothesis 1: the IDH does not explain macroinvertebrate taxa richness (the total number of taxa) in Hardwood Creek. Alternative hypothesis 1 is that macroinvertebrate taxa richness in Hardwood Creek conforms to predictions of the IDH.

Null hypothesis 2: the IDH does not explain macroinvertebrate diversity (estimated by Brillouin's diversity index) in Hardwood Creek. Alternative hypothesis 2 is that macroinvertebrate diversity in Hardwood Creek conforms to predictions of the IDH.

Null hypothesis 3: taxonomic richness of individual orders of macroinvertebrate does not conform to predictions of the IDH. Alternative hypothesis 3 is that taxonomic richness of at least some macroinvertebrate taxonomic orders conforms to predictions of the IDH.

Null hypothesis 4: taxonomic richness of individual families of macroinvertebrate do not conform to predictions of the IDH. Alternative hypothesis 4 is that taxonomic richness of at least some macroinvertebrate taxonomic families conform to predictions of the IDH.

Methods

Field methods and laboratory protocols are described in detail in Chapter 2 and are not repeated here. General information about Hardwood Creek and its watershed is also in Chapter 2, however, detailed site descriptions are presented below. When appropriate, polynomial trend lines were applied using Microsoft® Excel to help analyze taxa richness trends. Only sixth order polynomial trend lines or those having the highest R^2 value were used.

Construction of the disturbance gradient

The historic ditching of Hardwood Creek made it possible to construct a disturbance gradient based upon past ditching activities. Historical information on past ditching activities was obtained from 2 individuals familiar with Hardwood Creek (C. Johnson, Rice Creek Watershed District and T. Larson, Minnesota Pollution Control Agency, personal communication). The gradient was constructed by arranging sites from, 1) frequently to infrequently ditched, 2) time since last ditching, and 3) large to small ditching disturbance according to the theoretical framework in Connell (1978).

Description of sample sites

Nine sample sites were established throughout the length of Hardwood Creek (Figure 1). All sites except New were chosen because they were easy to access and near an existing water quality monitoring station operated by the RCWD (Figure 1). Site New was added because its riparian area was dominated by large deciduous trees. The site descriptions that follow are based upon personal observations and notes concerning the location, substrates, vegetation, ditching history (C. Johnson, Rice Creek Watershed District and T. Larson Minnesota Pollution Control Agency, personal communication), and sinuosity of each site. Site descriptions are arranged from the most to least disturbed sites.

Site 1.5U

Site 1.5U is located upstream from the intersection of Hardwood Creek and 157th Street North at: 45° 12.5' north and 92° 57.4' west. Sampling occurred approximately 5 to 175 meters upstream of the road. Only organic-peaty, unconsolidated substrates were observed. The aquatic plant *Lemna* spp. was observed in large quantities throughout 2004. Of all the sample sites, 1.5U was the most disturbed. Dredging occurred in the mid 1980's and in the winter of 2003-2004. The riparian area was dominated by tall grass and was devoid of trees, sinuosity was absent.

Site 1.4

Site 1.4 is north and east of Hugo, downstream of the first intersection of 170th Street North with Hardwood Creek at: 45° 12.3' north and 92° 56.7' west. Sampling occurred approximately 400 to 600 meters downstream from the road, around a conspicuous stream bend. Only organic-peaty, unconsolidated substrates were

observed. Submerged aquatic vegetation was prevalent in June. This site was dredged in the winter of 2003-2004. The riparian area was dominated by tall grass and was devoid of trees. Except for 1 conspicuous stream bend, sinuosity was absent.

Site New

Samples were collected approximately 500 to 600 meters downstream from Harrow Avenue North. Sand, gravel, and pebble substrates were dominant. Submerged vegetation was present at low density in June. This site is disturbed because Washington County Judicial Ditch 7 and parts of Hardwood Creek near New, were dredged in the summer of 2001. Judicial Ditch 7 flows into Hardwood Creek approximately halfway between Harrow Avenue and site New. The understory riparian vegetation was composed of several plant species. When compared to other sites, the deciduous trees in the overstory were larger, however sinuosity was low.

Site 1.3

Site 1.3 is upstream of Harrow Avenue North at: 45° 12.2' north and 92° 57.4' west. Samples were obtained approximately 10 to 350 meters from the road. Both soft peaty substrates and firmer substrates containing pebbles were observed. In June, submerged vegetation was dense and in some locations formed floating mats. Dredging occurred in the mid 1970's and beaver dams have required sporadic small-scale dredging since then. Tall grass was the dominant form of riparian vegetation and little sinuosity was observed.

Site 1.5D

Site 1.5D is downstream of the intersection of Hardwood Creek and 157th Street North at: 45° 11.0' north and 92° 57.3' west. Samples were obtained approximately 50 to 400 meters from the road. Organic-peaty substrates were dominant; however, they appeared to be more consolidated when compared to 1.5U. Late in the summer of 2004, tall grass dominated the riparian area, and in conjunction with *Lemna* spp., choked the stream channel. In June, *Lemna* spp. was less dominant and other aquatic plants were observed. This site was completely ditched in the mid 1970's and it was considered to be the fifth most disturbed in Hardwood Creek. Since then small sections have been ditched occasionally to remove beaver dams. Of all upstream sites, 1.5D was the most sinuous.

Site 1

Site 1 is located just upstream from the intersection of Hardwood Creek and Highway 61 at: 45° 12.5' north and 92° 58.7' west. Sampling occurred approximately 20 to 150 meters from Highway 61. Sand and gravel were the primary substrates observed. In June, aquatic vegetation was present along the margins of the stream, but was largely absent from most of the channel. Dredging occurred several times in the 1950's and 1960's. The riparian vegetation consisted of an understory of grass and other plants with tall trees forming the overstory. Sinuosity was virtually absent at this site.

Site 1.1

When traveling from upstream to downstream, site 1.1 is upstream of the second intersection of Hardwood Creek and 170th Street North at: 45° 11.8' north and 92° and 59.9' west. Samples were collected approximately 20 to 150 meters upstream of the

road. Sand was the primary substrate observed; however, some gravel and pebble substrates were present. A small amount of aquatic vegetation was present in June. Dredging occurred several times in the 1950's and 1960's. Tall grass and low densities of deciduous trees were the dominant forms of riparian vegetation. Some sinuosity was observed at this site.

Site 1.2

Site 1.2 is located west of the Anoka and Washington County border, downstream of where Hardwood Creek intersects 80th Street East at: 45° 11.8' north and 93° 1.5' west. Sample collection occurred approximately 100 to 200 meters downstream of the road. Sand, gravel, and pebble substrates were observed throughout, and riffles were also present. Very little aquatic vegetation was observed in June. There are no records or physical evidence to suggest ditching has ever occurred at site 1.2. The understory riparian vegetation was dominated by grasses and other plants with the overstory dominated by trees. Site 1.2 appeared to have the most sinuosity of all sample sites.

Site 2

Site 2 is the downstream-most site, located just upstream from the intersection of Hardwood Creek and 20th Avenue North at: 45° 12.0' north and 93° 2.4' west. Samples were collected approximately 20 to 70 meters upstream of the road. The dominant substrate was well-consolidated sand. A very small amount of *Lemna* spp. was present at this site in June. There are no records or physical evidence to suggest ditching has ever occurred at site 2. The understory riparian vegetation at this site was composed

primarily of grass, with deciduous trees making up the overstory; a small amount of sinuosity was observed.

Hypothesis 1

Estimates of total taxa richness per sample site from both surface-floating pupal exuviae (SFPE) of Chironomidae and dipnet (DN) methods were used to test hypothesis 1.

Hypothesis 2

Site-specific Brillouin's diversity index values, using natural log as the base resulting in nat units, were calculated from DN samples using 2 methods. A Jackknife approach was used to estimate diversity based on all samples at each site using the equation in Magurran (2004): $\Phi=n(st)-[(n-1)(st-n)]$ where, Φ is a pseudovalue calculated for each sample; n is the number of samples at each site; st is Brillouin's diversity index calculated for all samples at each site; st-n is Brillouin's diversity index calculated for each sample by subtracting the total in the sample of interest from the site total. A 95% confidence interval was constructed for the jackknifed estimate of diversity at each site using Microsoft® Excel. Brillouin's diversity index was also estimated by systematically pooling samples at each site into a successively larger sample, similar to the procedure recommended by Pielou (1975). Samples were systematically added based upon their stratification unit and estimates of diversity were sequentially re-calculated. This was repeated for all samples collected at a given site. This method made it possible to examine whether the estimate of diversity increased, remained constant, or decreased after the last sample was collected.

Pielou (1975) recommended the use of Brillouin's index for estimating the diversity of a collection and the Shannon index for communities of infinite size. Magurran (1988) and Southwood and Henderson (2000) strongly rejected the use of the Shannon index. Consequently, it was determined that Brillouin's index was most appropriate in this study given that non-random dipnet samples were collected (Magurran 1988).

Hypotheses 3 and 4

To test hypotheses 3 and 4 the data from both SFPE and DN methods were used to estimate taxa richness at order and family levels.

Results

A total of 271 macroinvertebrate taxa in 18 orders and 61 families were collected and analyzed for their conformity to the IDH (Tables 1-3). The 3 orders with the most taxa were Diptera, Coleoptera, and Basommatophora with 171, 29, and 14 taxa respectively (Table 1). Taxa richness within many families was low. There was only 1 taxon collected for 36 of the 61 families (Tables 2-3). Non-Diptera taxa with the highest richness were Hydrophilidae, Dytiscidae, and Glossiphoniidae with 10, 9, and 7 taxa respectively (Table 2). The Chironomidae had the highest richness with 138 taxa (Table 3).

Hypothesis 1

Taxa richness patterns of invertebrate communities across sites in Hardwood Creek did not conform to predictions of the IDH, so null hypothesis 1 was not rejected (Figure 2). Taxa richness was both the highest and lowest at the 2 most disturbed sites when compared to all other sites (Figure 2). Taxa richness was highest (130 taxa) at site

1.5U, the most disturbed site in Hardwood Creek (Figure 2). Site 1.4, the second most disturbed site, had the lowest richness with 105 taxa detected (Figure 2). At sites other than these 2, taxa richness steadily increased across the disturbance gradient from 105 taxa at site 1.4 to 120 taxa at site 1 (Figure 2). However, from the fifth to the sixth most disturbed, sites 1 to 1.1 respectively, richness remained at 120 taxa (Figure 2). Taxa richness decreased to 113 taxa at sites 1.2 and 2, the least disturbed sites (Figure 2).

Hypothesis 2

Diversity patterns across sites in Hardwood Creek did not conform to predictions of the IDH, so null hypothesis 2 was not rejected (Figure 3). Diversity estimated from the composite sample was 1.89 nats at site 1.5U, the most disturbed site (Figure 3). Diversity increased from 1.61 nats to 3.02 nats from sites 1.4 to 1.3 respectively (Figure 3). The second lowest diversity value was found at site 1.5D, where the level of disturbance was intermediate, and diversity was estimated to be 1.65 nats (Figure 3). The 4 least disturbed sites had index values that ranged between 2.22 nats to 2.90 nats (Figure 3). Brillouin's index calculated for the composite sample at each site was within the 95% confidence interval of the jackknife estimate of the true mean at all sites (Figure 3).

Hypothesis 3

Patterns of taxa richness across sites at the order level did not conform to predictions of the IDH for 16 of the18 taxonomic orders detected, so null hypothesis 3 was rejected (Appendix B Figures 1-18). The 2 orders that loosely conformed to the IDH were Neotaenioglossa and Rhynchobdellida (Figures 4-5).

Hypothesis 4

Patterns of taxa richness across sites at the family level did not conform to predictions of the IDH for 57 of the 61 taxonomic families detected, so null hypothesis 4 was rejected (Appendix B Figures 19-69). The 4 families that loosely conformed to the IDH were Dytiscidae, Planorbidae, Hydrobiidae, and Glossiphoniidae (Figures 6-9).

Site-to-site diversity trends

Estimates of Brillouin's diversity index generally increased for sites 1.5U, 1.4, New, 1.5D, and 1.3 as samples were sequentially analyzed (Figures 10-14). At sites 1.2, 1.0, and 2.0 the estimate of Brillouin's diversity index was generally stable, with no marked increase or decrease in diversity as samples were sequentially analyzed (Figures 15-17). At site 1.1 there was a decrease in the estimate of diversity as successive samples were analyzed (Figure 18).

Discussion

Conflicting conclusions regarding the validity of the IDH to predict taxa richness and diversity in streams occur in recent literature. Several empirical studies supported the IDH for lotic macroinvertebrates (Ward and Stanford 1983, Townsend et al. 1997b, Fruget et al. 2001, Piscart et al. 2005). Other studies do not confirm the model (Reice 1984), and still others have provided only weak support (Malmqvist and Otto 1987, Townsend et al. 1997a). However, the vast majority of studies do not accepted the IDH for lotic macroinvertebrate communities (Reice 1985, Robinson and Minshall 1986, Doeg et al. 1989, Lake et al. 1989, Rae 1990, Degani et al. 1992, Statzner and Resh 1993, Death 1995, Death and Winterbourn 1995, McCabe and Gotelli 2000, Death 2002, Frest and Johannes 2002, Podraza 2002).

Others who have discussed the validity or reviewed research of the IDH for macroinvertebrates in lotic systems have also produced mixed results. Stanford and Ward (1983), Minshall (1988), Resh et al. (1988), Lake (1990), Lake (2000), and Lepori and Hjerdt (2006) voice at least a little support for the IDH for macroinvertebrates in lotic systems while others (Vinson and Hawkins 1998) questioned its applicability, particularly for mobile invertebrates (Crandall et al. 2003).

It is apparent that the majority of studies, empirical or otherwise, do not support the applicability of the IDH to explain lotic macroinvertebrate richness patterns. This study provides little support for the IDH to explain macroinvertebrate taxa richness patterns at the community, order, and family level across a gradient of disturbance caused by ditching practices along a stream in Minnesota.

Hypothesis 1

The present study failed to confirm the IDH as an explanation for taxa richness patterns in Hardwood Creek. However if site 1.5U, the most disturbed and taxa rich site, was eliminated from the data set the pattern of taxa richness more closely conforms to the IDH in Hardwood Creek (Figure 2).

McCabe and Gotelli (2000) found a similar paradox in that species richness increased as disturbance area and intensity increased. To explain their results they drew upon predictions made by the diversity models of Huston (1994) and Wootton (1998). In addition, McCabe and Gotelli (2000) questioned the use of species richness as a valid measurement of diversity because species density was found to be inverse of species richness. Dai et al. (2003) also found that species richness was enhanced by stream dredging.

In Hardwood Creek, it is possible to resolve this paradox of high taxa richness at one of the most highly disturbed sites through recolonization dynamics. Site 1.5U is approximately 1 km downstream of Rice Lake. Rice Lake may be a source of lentic taxa that increased the number of taxa present at 1.5U. In addition, site 1.5U is close to a second source of lentic taxa from Lake Oneka which is only about 0.25 km west. Although dredging of site 1.5U in the winter of 2004 greatly disturbed this site, the removal of sediments and changes in channel form may have allowed for colonization of taxa via stream drift and aerial colonization from Rice Lake, and possibly via aerial colonization from Lake Oneka.

The genera *Psectrocladius* sp., *Berosus* sp., and *Tropisternus* sp., all found at site1.5U, have well-developed dispersal capabilities and are known to live in both lotic and lentic habitats. The idea that physical disturbance opens up space for colonizing taxa, as hypothesized by others (e.g., Connell 1978, Sousa 1984, Minshall 1988, Townsend 1989, Reice 1994) appears to be supported in this study. This explanation is commensurate with the findings of others who have documented rapid recolonization of macroinvertebrates after dredging of streams for gold (Griffith and Andrews 1981, Thomas 1985, Harvey 1986) or management (Pearson and Jones 1975). Recolonization can be rapid, with increases in species richness detectable after approximately 1 month (Pearson and Jones 1975, Griffith and Andrews 1981, Thomas 1985, Harvey 1986). Sampling in Hardwood Creek began approximately 4 months after ditching and continued until about 11 months post ditching.

If recolonization is a valid explanation of the richness at site 1.5U, we would expect to find many taxa considered to be lentic at the site. Examination of the insect taxa collected at 1.5U for their habitat preference based on Merritt and Cummings (1996), found that 30.7% of the taxa detected at site 1.5U were lentic and 60.2% were

both lentic and lotic (Table 4). This lends support to the explanation that high taxa richness at site 1.5U was from lentic colonizers.

Site 1.4 was also dredged in the winter of 2004 but contained the fewest number of taxa (105) when compared to all other sites. Proximity to lentic colonization sources can be evoked as an explanation for the richness discrepancy between 1.5U and 1.4. Horseshoe Lake is approximately 1 km from site 1.4. The greater distance for lentic colonizers to fly to site 1.4, and its lack of connectivity to a lake, may explain why taxa richness was lower at this site.

Examination of the insect taxa collected at 1.4 based on Merritt and Cummings (1996), found that 29.2% of the taxa were lentic and 64.6% were both lentic and lotic (Table 4). These percentages are almost equal to 1.5U, but 1.5U had 23 more insect taxa when compared to site 1.4 (Table 4). The distance to the nearest lentic colonization source may have limited the number of taxa able to recolonize site 1.4 after ditching.

Another explanation for the discrepancy may be the fact that the April SFPE sample was not processed for site 1.4. Twenty-seven taxa (16 chironomid and 11 non-chironomid) not collected in DN samples were collected in April SFPE samples at site 1.5U. Given that there were 25 fewer taxa collected at site 1.4, it is possible that the lack of an April SFPE sample explains the discrepancy in richness between sites 1.4 and 1.5U.

It is quite remarkable that the 4 least disturbed sites had very similar richness values given differences in disturbance regimes, habitats, and substrates. All these factors can be expected to greatly influence taxa richness on a site-to-site basis. Sites 1 and 1.1 had 120 taxa, whereas sites 1.2 and 2 each had 113 taxa. This is especially remarkable considering the fact that site 1.2 was the only site containing riffle habitat, which increased habitat complexity, and should reflect higher taxa richness.

Hypothesis 2

Brillouin's diversity index was calculated for the DN data set as an alternative way to measure diversity. Many investigators of lotic ecology have used the number of species or taxa to test the validity of the IDH (Ward and Stanford 1983, Rae 1990, Statzner and Resh 1993, Townsend et al. 1997a, McCabe and Gotelli 2000, and Fruget et al. 2001). The Shannon index has often been used to measure diversity in investigations of the IDH in lotic systems and is often used in concert with species richness (Reice 1984, Reice 1985, Robinson and Minshall 1986, Malmqvist and Otto 1987, Doeg et al. 1989, Lake et al. 1989, Degani et al. 1992, Townsend et al. 1997b, Podraza 2002, Piscart et al. 2005). Death and Winterbourn (1995) and Death (2002) used species richness and Simpson's index. However, little consensus exists among ecologists as to the best measure(s) of species diversity (Magurran 1988, Death and Winterbourn 1995).

The null hypothesis was not rejected for Brillouin's diversity index. No statistical tests for difference are needed for Brillouin's index because index values have no variance (Magurran 2004). Thus 1.5D, the intermediately disturbed site, had a diversity index that was significantly lower than all but 1 site in Hardwood Creek. One explanation for this result is that site 1.5D is immediately downstream of 1.5U, the most disturbed site. It is likely that the increased turbidity caused by dredging in the winter of 2004 led to the low diversity at this site. Because diversity was calculated for DN samples only, the pattern was much different from that observed for taxa richness based on both methods (Figures 2-3).

The acceptance of the null hypothesis was based upon good estimates of Brillouin's diversity index. At each site the estimate of Brillouin's index was within the 95% confidence interval of the jackknife estimate of the true mean (Figure 3). This

finding, in concert with the fact that all 95% confidence intervals did not contain zero, is strong evidence that Brillouin's index is numerically meaningful for each site.

Hypothesis 3

The vast majority of orders detected in this study did not show taxa richness patterns that conformed to predictions of the IDH. Only Neotaenioglossa and Rhynchobdellida conformed to the IDH, so alternative hypothesis three was not rejected. The conformity of Neotaenioglossa richness to the IDH should be approached cautiously because it is based upon data for 1 taxon, *Amnicola walkeri*. Rhynchobdellida richness conforms better to the IDH because richness for 7 taxa followed the richness pattern predicted by the IDH.

Hypothesis 4

An overwhelming majority of families detected in this study did not show taxa richness patterns that conformed to predictions of the IDH. As a result alternative hypothesis four was not rejected. The IDH was accepted as an explanation for the richness patterns found in 4 families. The conformity of Hydrobiidae richness to the IDH should be approached cautiously because it is based upon data for 1 taxon, *Amnicola walkeri*. The families Dytiscidae, Planorbidae, and Glossiphoniidae all had taxa richness curves that were unimodal and commensurate with the IDH. Glossiphoniidae was the only family found within the order Rhynchobdellida, which also conformed to the IDH. All 3 families had the highest taxa richness at site 1.5D.

Site-to-site diversity trends

Given that DN samples did not collect all taxa present at a site, we would theoretically expect Brillouin's index to be generally increasing after the last DN sample was analyzed (Table 5). Yet, only 5 of the 9 sites showed some indications of this trend. By contrast 3 sites had index values that generally did not increase or decrease. Regardless of the changes in diversity trends observed, a numerically meaningful estimate of diversity was obtained for each site because 95% confidence intervals did not contain zero.

The importance of taxonomic scale

Many researchers have noted the importance of both temporal and spatial scales to disturbance (e.g., Minshall 1988, Reice 1994, Downes et al. 1998). However, the present study appears to be the first to examine the applicability of the IDH at different taxonomic scales for macroinvertebrates in lotic systems. At the community level, the IDH did not explain taxa richness and diversity of macroinvertebrates in Hardwood Creek, a stream with a mosaic of ditching patterns. When taxonomic scale was modified to investigate richness within orders and families, the IDH again did not explain taxa richness patterns in Hardwood Creek. Although most orders and families did not conform, the IDH was accepted for a few groups. By modifying taxonomic scale from community, to family or order, valuable insight into the applicability of the IDH to lotic macroinvertebrates was gained.

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Tables

Taxon	Number of taxa
Ephemeroptera	6
Odonata	4
Plecoptera	3
Hemiptera	12
Megaloptera	1
Trichoptera	10
Lepidoptera	1
Coleoptera	29
Diptera	171
Nematoda (phylum)	1
Veneroida	3
Basommatophora	14
Heterostropha	1
Neotaenioglossa	1
Arhynchobdellida	2
Rhynchobdellida	7
Oligochaeta (subclass)	1
Isopoda	1
Amphipoda	2
Decapoda	1
Total	271

Table 1. The number of taxa collected for each order, unless otherwise labeled, in DN and SFPE samples.

Non-Diptera families	Taxa	Non-Diptera families	Taxa
Baetidae	3	Leptoceridae	4
Caenidae	1	Limnephilidae	1
Heptageniidae	1	Pyralidae	1
Leptophlebiidae	1	Curculionidae	3
Calopterygidae	1	Dytiscidae	9
Coenagrionidae	2	Elmidae	3
Libellulidae	1	Haliplidae	2
Capniidae	1	Hydraenidae	1
Nemouridae	1	Hydrophilidae	10
Perlidae	1	Scirtidae	1
Belostomatidae	1	Pisidiidae	3
Corixidae	2	Ancylidae	1
Gerridae	2	Lymnaeidae	4
Hebridae	1	Physidae	3
Hydrometridae	1	Planorbidae	6
Mesoveliidae	1	Valvatidae	1
Nepidae	1	Hydrobiidae	1
Notonectidae	1	Erpobdellidae	2
Pleidae	1	Glossiphoniidae	7
Veliidae	1	Asellidae	1
Corydalidae	1	Gammaridae	1
Glossosomatidae	1	Hyalellidae	1
Hydropsychidae	1	Cambaridae	1
Hydroptilidae	3	Total	98

Table 2. The non-Diptera families and the number of taxa collected for each in DN and SFPE samples.

Diptera	Number of taxa
Ceratopogonidae	6
Chironomidae	138
Chironomini and Pseudochironomini (tribe)	49
Orthocladiinae (subfamily)	43
Prodiamesinae (subfamily)	1
Tanypodinae (subfamily)	18
Tanytarsini (tribe)	27
Culicidae	6
Dixidae	1
Dolichopodidae	1
Empididae	2
Ephydridae	3
Psychodidae	2
Sciomyzidae	2
Simuliidae	1
Stratiomyidae	4
Syrphidae	1
Tabanidae	1
Tipulidae	3
Total	171

Table 3. The families of Diptera, Chironomidae subfamilies and tribes, and the number of taxa collected in both DN and SFPE samples.

Taxa	Count 1.5U	Count 1.4	Percent 1.5U	Percent 1.4
Lentic	27	19	30.7	29.2
Lotic	8	4	9.1	6.2
Lentic/lotic	53	42	60.2	64.6
Total	88	65	100	100

Table 4. Insect taxa by lentic, lotic, or both lentic/lotic habitat preference for sites 1.5U and 1.4 based on Merritt and Cummings (1996). The number of insect taxa analyzed at each site (count) and the percentage of total (percent) is also shown.

Non chironomids				Chironomids			
Site	DN	SFPE	Both	PESM	PEDS	Larvae	Totals
1.5U	6	53	14	48	6	3	130
1.4	6	39	14	39	6	1	105
New	33	*	*	*	*	29	62
1.3	12	25	27	33	6	9	112
1.5D	6	40	25	38	4	2	115
1	18	22	15	47	10	8	120
1.1	16	30	16	42	11	5	120
1.2	9	34	15	38	10	7	113
2	13	30	14	39	6	11	113

Table 5. The totals for non-chironomid taxa collected exclusively in DN and SFPE samples, and those collect with both methods. The number of unique chironomid taxa identified via pupal exuviae that were slide mounted (PESM), pupal exuviae examined under a dissection scope (PEDS), and larvae. An asterisk (*) indicates that sampling and identification did not occur.

Figures

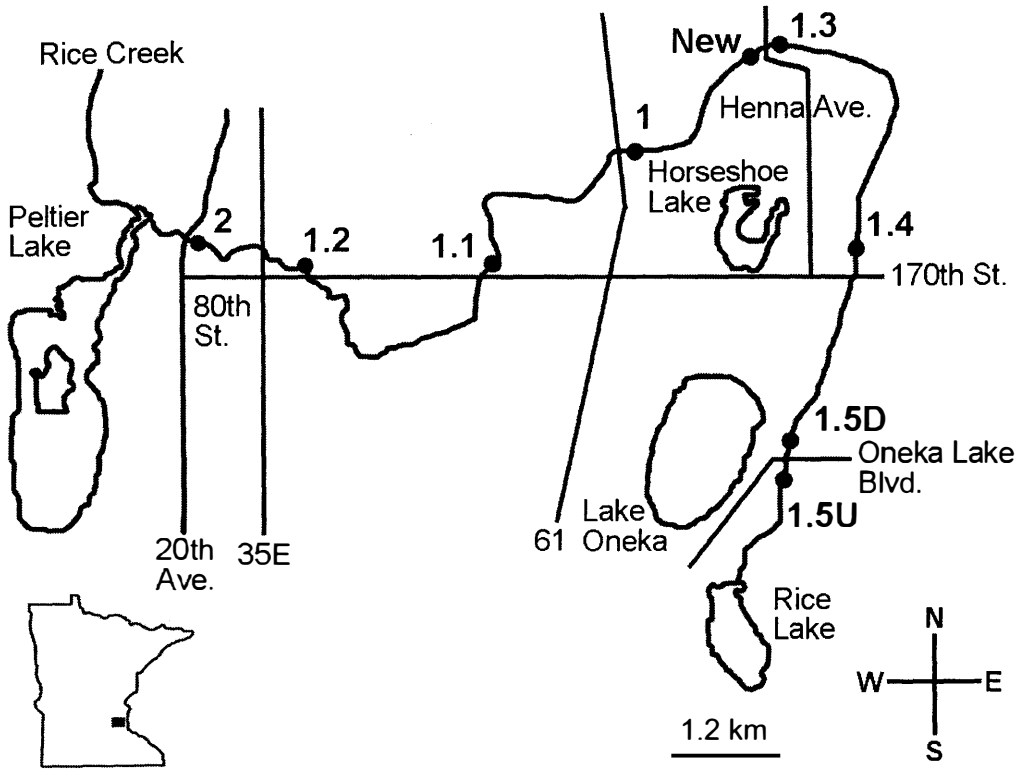

Figure 1. A map of Hardwood Creek and the 9 sample sites. Hardwood Creek flows from Rice Lake into Peltier Lake.

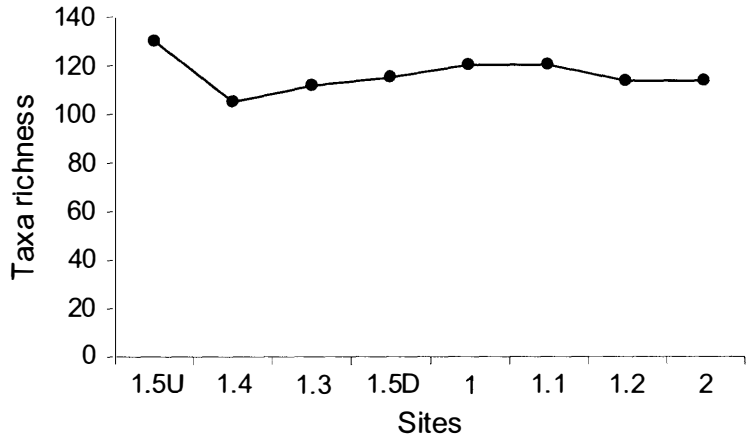

Figure 2. Taxa richness for all taxa from DN and SFPE samples for hypothesis one. Site are arranged from frequently to infrequently ditched, time since last ditching, and large to small ditching disturbance according to the theoretical framework in Connell (1978).

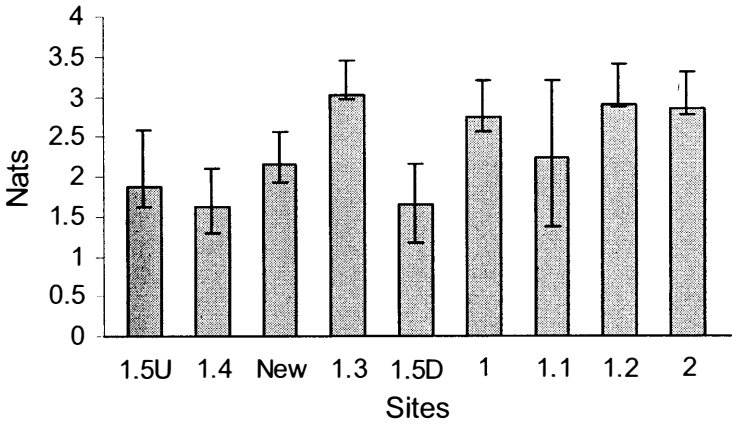

Figure 3. The estimate, not the mean, of Brillouin's index in nats base upon all DN samples at each site. The error bars represent the 95% confidence interval estimate of the true mean based on the jackknife estimate for each site. Site are arranged from frequently to infrequently ditched, time since last ditching, and large to small ditching disturbance according to the theoretical framework in Connell (1978).

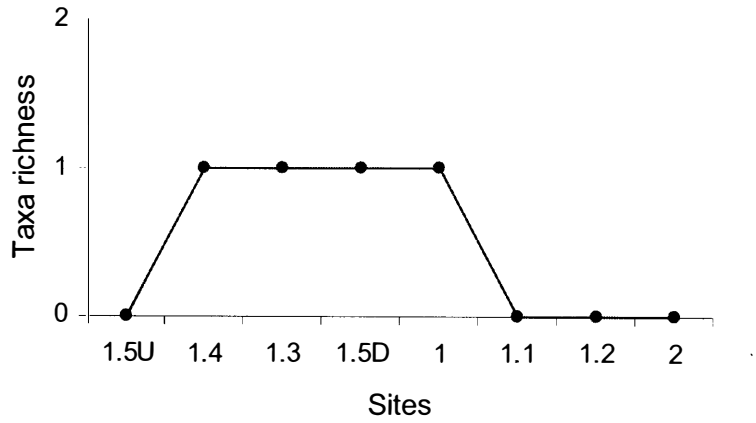

Figure 4. Neotaenioglossa richness for DN and SFPE samples for hypothesis three. Taxa richness equal to 1 is present and 0 is absent at each site. Site are arranged from frequently to infrequently ditched, time since last ditching, and large to small ditching disturbance according to the theoretical framework in Connell (1978)

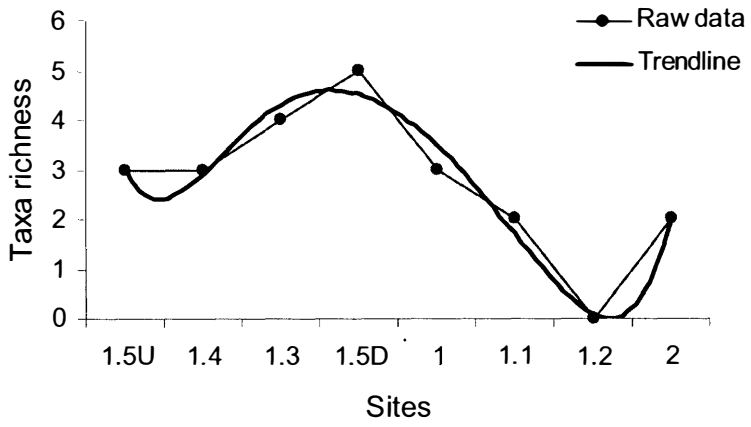

Figure 5. Rhynchobdellida richness for DN and SFPE samples for hypothesis three. Taxa richness equal to 1 is present and 0 is absent at each site. The trendline was fit by selecting the polynomial function with the greatest R^2 value. Site are arranged from frequently to infrequently ditched, time since last ditching, and large to small ditching disturbance according to the theoretical framework in Connell (1978).

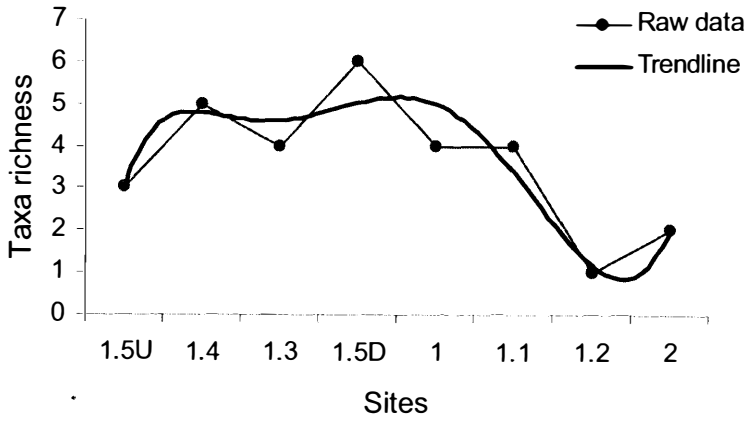

Figure 6. Dytiscidae richness in DN and SFPE samples for hypothesis four. Taxa richness equal to 1 is present and 0 is absent at each site. The trendline was fit by selecting the polynomial function with the greatest R^2 value. Site are arranged from frequently to infrequently ditched, time since last ditching, and large to small ditching disturbance according to the theoretical framework in Connell (1978).

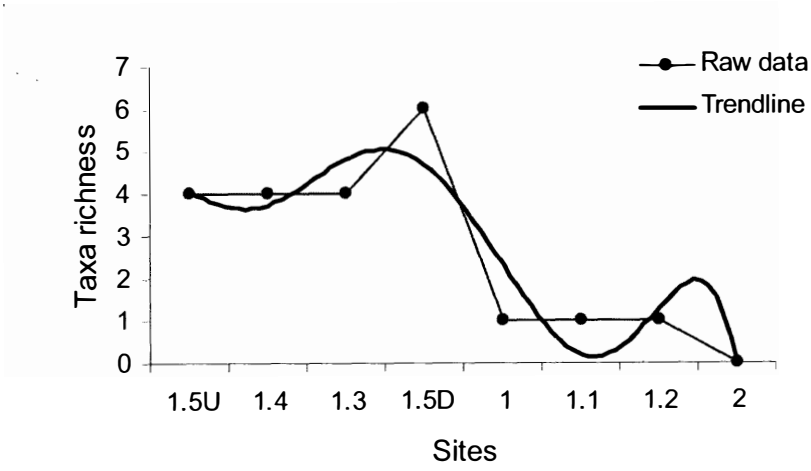

Figure 7. Planorbidae richness in DN and SFPE samples for hypothesis four. Taxa richness equal to 1 is present and 0 is absent at each site. The trendline was fit by selecting the polynomial function with the greatest R^2 value. Site are arranged from frequently to infrequently ditched, time since last ditching, and large to small ditching disturbance according to the theoretical framework in Connell (1978).

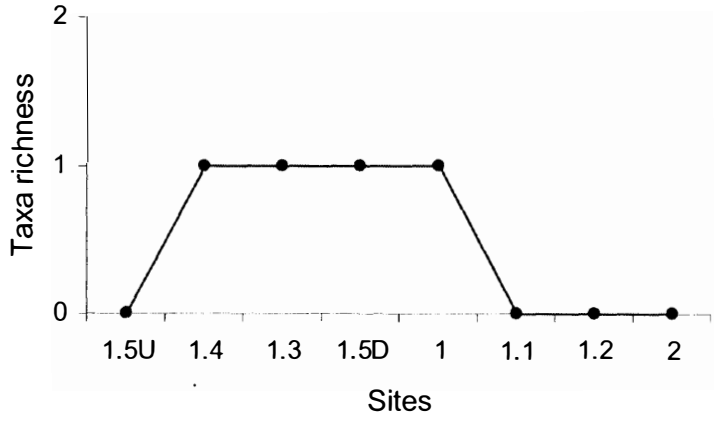

Figure 8. Hydrobiidae richness in DN and SFPE samples for hypothesis four. Taxa richness equal to 1 is present and 0 is absent at each site. Site are arranged from frequently to infrequently ditched, time since last ditching, and large to small ditching disturbance according to the theoretical framework in Connell (1978).

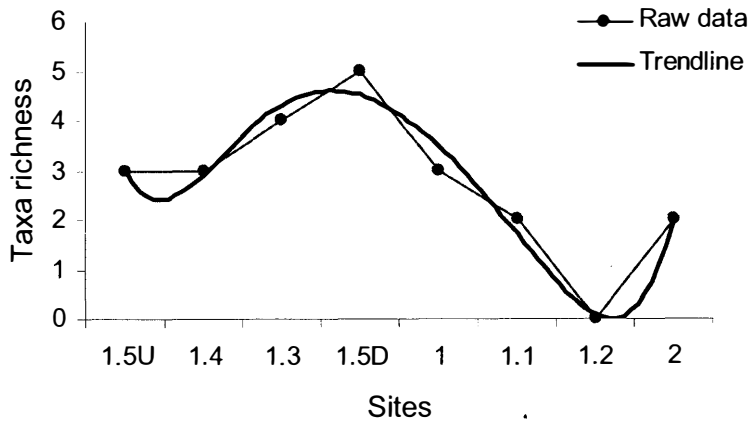

Figure 9. Glossiphoniidae richness in DN and SFPE samples for hypothesis four. Taxa richness equal to 1 is present and 0 is absent at each site. The trendline was fit by selecting the polynomial function with the greatest R^2 value. Site are arranged from frequently to infrequently ditched, time since last ditching, and large to small ditching disturbance according to the theoretical framework in Connell (1978).

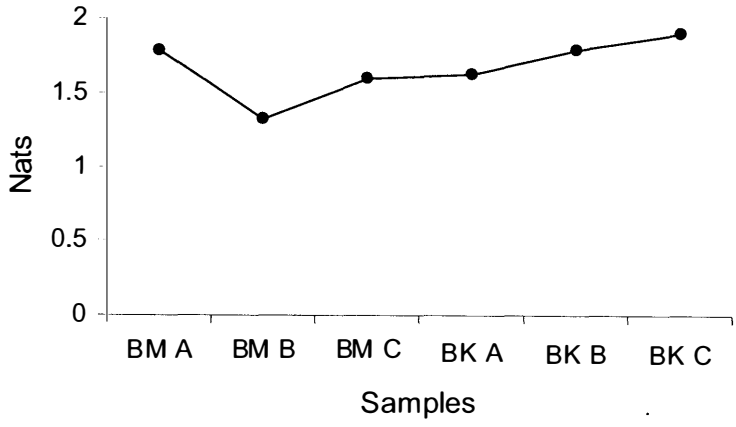

Figure 10. Brillouin's index for site 1.5U calculated by systematically adding each sample and calculating Brillouin's index, such that the last sample is the sum or composite of all samples. Each bottom (BM) and bank (BK) sample was assigned a letter (A, B, or C) to differentiate it from the other samples in each habitat stratification unit.

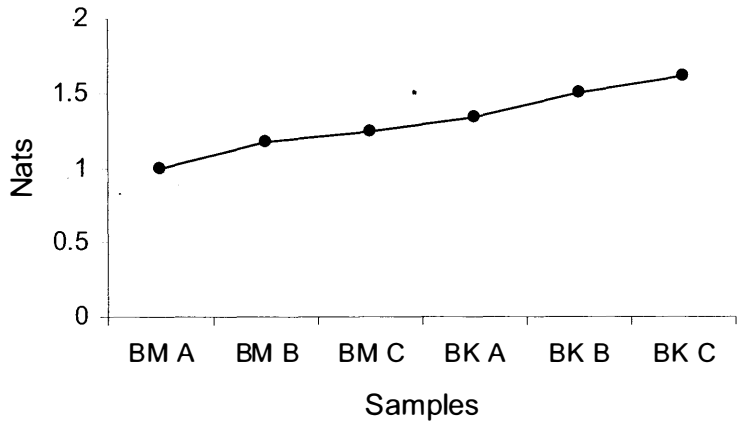

Figure 11. Brillouin's index for site 1.4 calculated by systematically adding each sample and calculating Brillouin's index, such that the last sample is the sum or composite of all samples. Each bottom (BM) and bank (BK) sample was assigned a letter (A, B, or C) to differentiate it from the other samples in each habitat stratification unit.

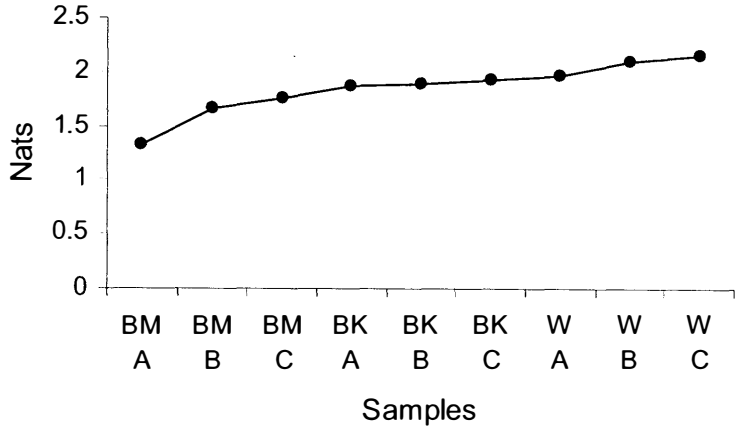

Figure 12. Brillouin's index for site New calculated by systematically adding each sample and calculating Brillouin's index, such that the last sample is the sum or composite of all samples. Each bottom (BM), bank (BK), and wood (W) sample was assigned a letter (A, B, or C) to differentiate it from the other samples in each habitat stratification unit.

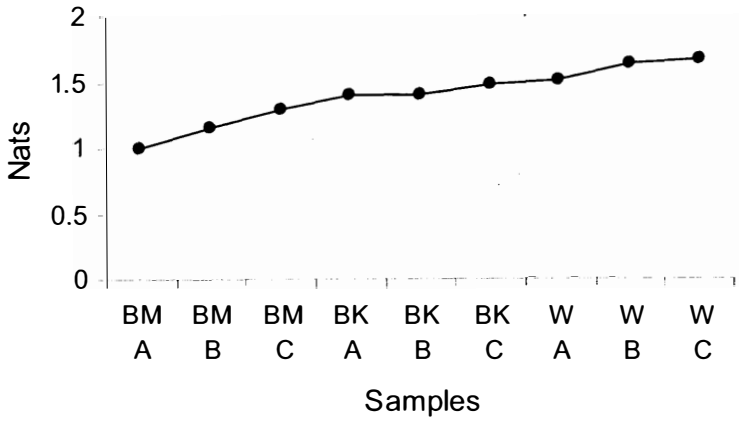

Figure 13. Brillouin's index for site 1.5D calculated by systematically adding each sample and calculating Brillouin's index, such that the last sample is the sum or composite of all samples. Each bottom (BM), bank (BK), and wood (W) sample was assigned a letter (A, B, or C) to differentiate it from the other samples in each habitat stratification unit.

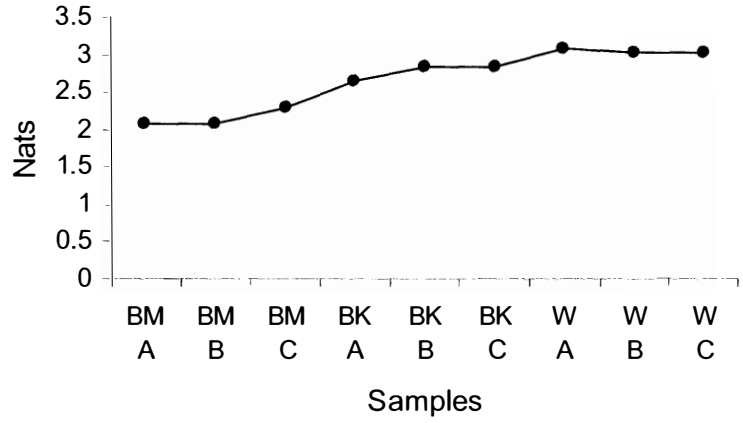

Figure 14. Brillouin's index for site 1.3 calculated by systematically adding each sample and calculating Brillouin's index, such that the last sample is the sum or composite of all samples. Each bottom (BM), bank (BK), and wood (W) sample was assigned a letter (A, B, or C) to differentiate it from the other samples in each habitat stratification unit.

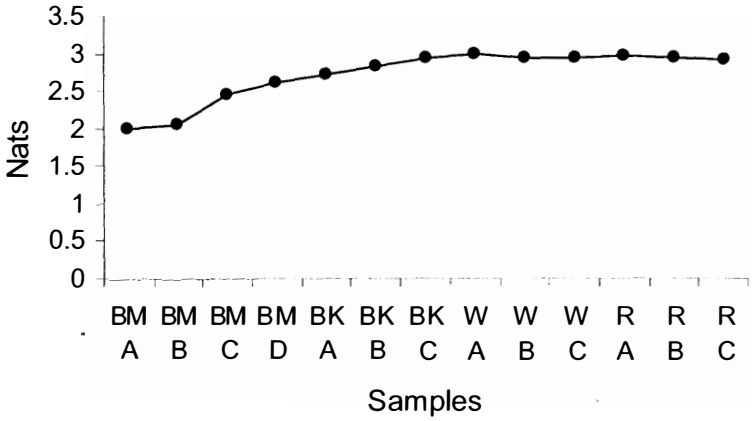

Figure 15. Brillouin's index for site 1.2 calculated by systematically adding each sample and calculating Brillouin's index, such that the last sample is the sum or composite of all samples. Each bottom (BM), bank (BK), wood (W), and riffle (R) sample was assigned a letter (A, B, C, or D) to differentiate it from the other samples in each habitat stratification unit. Bottom D represents an extra bottom sample.

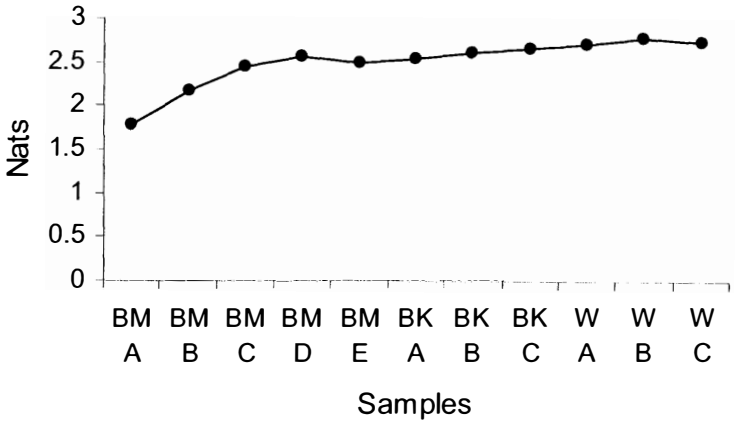

Figure 16. Brillouin's index for site 1 calculated by systematically adding each sample and calculating Brillouin's index, such that the last sample is the sum or composite of all samples. Each bottom (BM), bank (BK), and wood (W) sample was assigned a letter (A, B, C, D, or E) to differentiate it from the other samples in each habitat stratification unit. Bottom D and E represent 2 extra bottom samples.

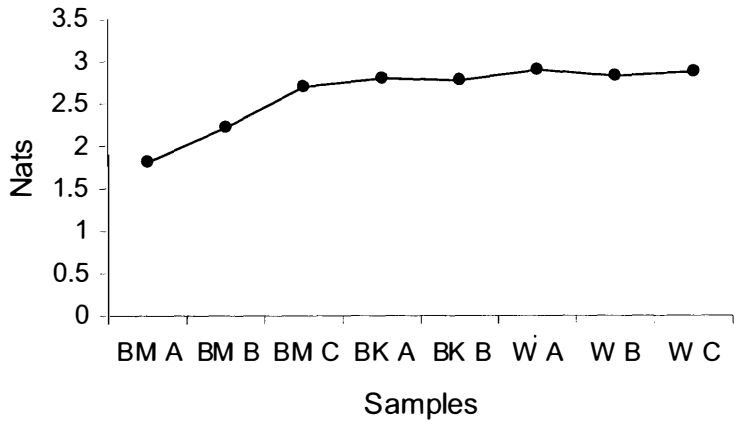

Figure 17. Brillouin's index at site 2, calculated by systematically adding each sample and calculating Brillouin's index, such that the last sample is the sum or composite of all samples. Each bottom (BM), bank (BK), and wood (W) sample was assigned a letter (A, B, or C) to differentiate it from the other samples in each habitat stratification unit. The bank C sample is missing.

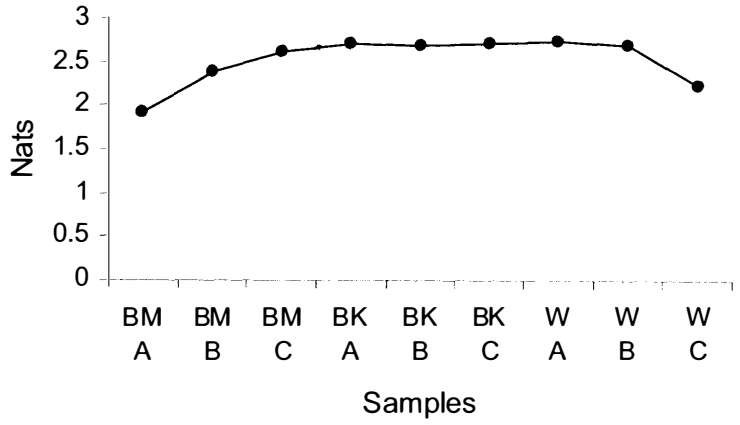

Figure 18. Brillouin's index for site 1.1 calculated by systematically adding each sample and calculating Brillouin's index, such that the last sample is the sum or composite of all samples. Each bottom (BM), bank (BK), and wood (W) sample was assigned a letter (A, B, or C) to differentiate it from the other samples in each habitat stratification unit.

Chapter 4

A Test of the River Continuum Concept in Hardwood Creek, a Ditched Stream in Minnesota, USA

Will be submitted to: Journal of the North American Benthological Society

Abstract

One fundamental prediction of the River Continuum Concept (RCC) is that community composition will change gradually as a function of gradual changes in habitats and resources along natural gradients in streams. Accordingly, community similarity of benthic invertebrates should be most similar among sites that are adjacent and in close proximity. In this study, 3 similarity indices were used to test 8 hypotheses across an upstream to downstream continuum in Hardwood Creek. Whittaker's percent similarity (WPS), simple matching coefficient (SMC), and Jaccard's (JAC) similarity indices were calculated for dipnet (DN), chironomid larvae, and chironomid surface-floating pupal exuviae (SFPE) data sets. Sites were arranged from upstream to downstream in a matrix and similarity values were ranked from highest (1) to lowest (36). Exact Wilcoxon rank sum tests were employed to test all hypotheses. P-values ranged from 0.488 to 0.957, indicating that the similarities of invertebrate communities of adjacent sites do not conform to the prediction of the RCC that sites adjacent to each other should have the most similar community compositions. Several possibilities may account for the acceptance of all the null hypotheses including the effects of habitat modification from ditching, small sample sizes, and the possibility that the RCC is incompatible with Midwestern and/or disturbed streams.

Introduction

The River Continuum Concept (RCC) of Vannote et al. (1980) is a seminal concept in lotic ecology. Resh and Kobzina (2003) found Vannote et al. (1980) was the most-cited article in benthological science between 1995 and 2000. The RCC was developed for stream ecosystems originating from forest ecosystems. The basic premise of the RCC is that the physical structure of river systems changes gradually from small headwater streams to larger streams and rivers downstream. The gradual changes approximately correspond to increasing stream size, or order, and have predictable consequences for the stream biota. As stream order, metabolism, and temperature regimes change, the biological community along the river continuum gradually changes (Vannote et al. 1980). The RCC may be applicable to streams that have experienced anthropogenic disturbances, such as riparian vegetation harvest and reduced water quality (Vannote et al. 1980).

Given the large number of anthropogenically disturbed streams and the influence of the RCC in lotic ecology, many researchers have tested the validity of the concept in disturbed systems. Investigations have focused on streams impacted by agriculture (Wiley et al. 1990, Delong and Brusven 1998, Harding 1999), agriculture and domestic inputs (Dudgeon 1984), and industrial, nuclear, and construction activities (Paller et al. 2006). To date there appears to be no investigation of the applicability of the RCC to predict community composition of benthic invertebrates within a stream that has recently been ditched. Wiley et al. (1990) investigated the RCC within 2 Illinois watersheds channelized 60 years prior to study.

Ditching imposes many confounding factors (e.g., disturbance, turbidity, and resource removal) on an investigation of the RCC. Investigations of the RCC within ditches are necessary given their prevalence throughout the world (e.g., Beltman 1984,

Anonymous 2006), and the need to understand and manage these systems. In addition, some research has questioned how applicable the RCC is to low gradient streams in the Midwestern United States (e.g., Wiley et al. 1990). Further justification for this study comes from the conclusions of Chapter 3 that found the Intermediate Disturbance Hypothesis failed to explain community-level patterns of taxa richness and diversity in Hardwood Creek of Minnesota. This study evaluated whether patterns of community composition at adjacent sites were consistent with the predictions of the RCC.

Based on the RCC, community similarity of benthic invertebrates should be most similar among sites that are adjacent and in close proximity. As distance between sites increases, community structure should become increasingly dissimilar as sites are located further apart across natural gradients of habitats and resources.

To test this prediction of the RCC, we constructed a series of null and alternative hypotheses to test for patterns of similarity among sites using non-parametric rank sums tests. In all null hypotheses, the ranks of similarities of all site comparisons are assumed to be random. The alternative hypotheses are that comparisons of adjacent sites will be most similar and, when ranked by similarity, will have the lowest sum of ranks that can be obtained for a given number of sites investigated in a particular field design.

Hypotheses to be tested

As constructed, the tests for conformity to predictions of the RCC assume that changes in habitats and resources along Hardwood Creek are gradual and represent a continuum across sample sites. Recent ditching during winter of 2004; however, substantially modified the physical nature of habitats and resources at sites 1.5U and 1.4. In addition, sites 1.3, New, 1, and 1.4 had different histories of ditching, and physical conditions at these sites reflect past ditching activities. Consequently, we expected that

ditching has modified sites to the extent that continuous gradients of habitat and resources do not conform to RCC. We expected the null hypotheses to be accepted, and that adjacent sites would not indicate similar community structures.

In this study, 3 similarity indices were used to test 8 hypotheses across an upstream to downstream continuum of sites in Hardwood Creek (Table 1). The indices used to test the 8 hypotheses were Simple Matching coefficient (SMC), Jaccard's coefficient (JAC), and Whittaker's percent similarity (WPS). The indices measure similarity differently and can provide potentially contrasting views of patterns of similarity (refer to Chapter 2 for descriptions of how coefficients were calculated). Consequently, all 3 indices were used to test for conformity to predictions of the RCC.

Nine sample sites were investigated in this study (Figure 1). However, the Chironomid surface-floating pupal exuviae (SFPE) data set contains only 8 sites, all other data sets have 9 sites. The number of site-by-site comparisons is calculated (N (N-1)) / 2 where N is 8 or 9. Of the 36 site-by-site comparisons, only 8 are comparisons of sites located adjacent to each other (e.g., 1.5U x 1.5D, 1.5D x 1.4, 1.4 x 1.3, 1.3 x NEW, NEW x 1.0, 1.0 x 1.1, 1.1 x 1.2, 1.2 x 2.0; Figure 1). The rank sum test was based on the sum of ranks of the similarities of these 8 or 9 adjacent sites, and the null hypothesis assumed that the similarities can vary randomly from most similar to least similar.

Consequently, the corresponding rank of similarity could vary from 1 (most similar) to 36 (least similar). The sum of ranks of the 8 adjacent sites could theoretically vary, assuming no ties in ranks, from 36 if the 8 comparisons were most similar (i.e., ranks vary from 1 to 8), to 260 if the adjacent sites were most dissimilar (i.e., ranks range from 29 to 36). If ties occur, the theoretical minimum sum of ranks can be as low as 8. The rank sum test tests for statistically significant departures from intermediate

values of sums of ranks, when alpha is 0.05 the cut-off value is 20, when tied values occur for at least 1 sample pair.

Null hypothesis 1: the observed sum of ranked SMC values for dipnet (DN) samples is equal to or greater than 21. Alternative hypothesis 1 is that the observed sum of ranked SMC values for DN samples is less than 21.

Null hypothesis 2: the observed sum of ranked SMC values for chironomid larvae across the continuum in Hardwood Creek is equal to or greater than 21. Alternative hypothesis 2 is that the observed sum of ranked SMC values for chironomid larvae across the continuum in Hardwood Creek is less than 21.

Null hypothesis 3: the observed sum of ranked SMC values for SFPE is equal to or greater than 15. Alternative hypothesis 3 is that the observed sum of ranked SMC values for SFPE is less than 15.

Null hypothesis 4: the observed sum of ranked JAC values for DN samples is equal to or greater 21. Alternative hypothesis 4 is that the observed sum of ranked JAC values for DN samples is less than 21.

Null hypothesis 5: the observed sum of ranked JAC values for chironomid larvae is equal to or greater than 21. Alternative hypothesis 5 is that the observed sum of ranked JAC values for chironomid larvae is less than 21.

Null hypothesis 6: the observed sum of ranked JAC values for SFPE is equal to or greater than 15. Alternative hypothesis 6 is that the observed sum of ranked JAC values for SFPE is less than 15.

Null hypothesis 7: the observed sum of ranked WPS values for DN samples is equal to or greater than 21. Alternative hypothesis 7 is that the observed sum of ranked WPS index values for DN samples is less than 21.

Null hypothesis 8: the observed sum of ranked WPS values for chironomid larvae is equal to or greater than 21. Alternative hypothesis 8 is that the observed sum of ranked WPS values for chironomid larvae is less than 21.

Methods

All methods and sample sites used to test the RCC are described in Chapters 2 and 3 respectively. Exceptions include the following: SMC, JAC, and WPS similarity indices were calculated from DN, chironomid larvae, and SFPE data sets (see Chapter 2 for more detail). Whittaker's percent similarity was not calculated for the SFPE data set because quantitative or relative abundance data were not collected with this method. Site New was not included in SFPE similarity calculations because SFPE collections were not made at this site.

Sites were arranged from upstream to downstream in a matrix and similarity values were ranked from highest (1) to lowest (36) to test predictions of the RCC. Similarity values for the SFPE data set were ranked from highest (1) to lowest (28).

Ranked similarity values for the adjacent sites in each matrix (Tables 2-9; Figure 1) were tested against the theoretical rank sum predicted by the RCC (Table 10) to test the hypotheses. Exact Wilcoxon rank sum tests (package version 0.8-15) were employed to test all hypotheses using R statistical software (version 2.4.1/release 2006, R Foundation for Statistical Computing, Vienna, Austria). Each matrix contained at least 1 set of tied similarity ranks; however, the exact Wilcoxon rank sum test accounts for ties.

Results

All exact Wilcoxon rank sum tests were not significant. Analysis of the different measures of community similarity showed the SMC index had the lowest p-values (Table 11). Except for the chironomid larvae data set, the JAC index had the second lowest p-values; the WPS index generally had the highest p-values (Table 11).

P-values ranged from 0.488 to 0.957 leading to the acceptance of all 8 null hypotheses (Table 11). The similarities of invertebrate communities of adjacent sites do not conform to the prediction of the RCC that sites adjacent to each other should have the most similar community compositions.

Discussion

In this study, 3 similarity indices were used to test 8 hypotheses across an upstream to downstream continuum of sites in Hardwood Creek . P-values were all insignificant and led to the acceptance of all 8 null hypotheses. The physical proximity of sample sites did not influence site similarity based on macroinvertebrate community structure.

Similarity indices

The difference in p-values for the 3 similarity indices has implications for finding significant results when testing ranked data using a rank sum test. If an index consistently produces lower similarity values for the sites of interest, when compared to other site combinations in the matrix, it will be harder to find significant results with this index. Index values that are low will result in a higher rank, which increases the rank sum, and can lead to insignificant results when the null hypothesis is an upper-tailed test. In the present study WPS index values were generally lower when the 4 upstream

sites were compared. This resulted in larger ranks and larger rank sums which produced large p-values. Given the discrepancy in p-values observed in this study, it appears that the probability of finding significant results is lower when the WPS index is used, all else being equal.

Sample sites

First through third order streams are predicted to be very similar with respect to their physical habitat template and carbon cycle dynamics, which determine the macroinvertebrate community (Vannote et al. 1980). The prediction that community similarity indices at adjacent sites, will be more similar when compared to distant sites, is a logical extension of the RCC.

Analysis of the similarity indices confirms that disturbance from ditching has disrupted the physical habitat template enough that communities do not conform to the RCC. Changes at ditched sample sites include reduction in habitat heterogeneity caused by removal of woody debris and perennial rooted aquatic vegetation, loss of sinuosity and variations in stream flow in highly-sinuous channels, and possible downstream effects related to transport of sediments and changes in flow dynamics. Most of these changes would have differential effects on species with trophic strategies that require solid surfaces and higher flows for filter-feeding. The modifications could also favor burrowing species.

Hypotheses

There exists some debate as to whether the RCC is applicable to prairie and/or disturbed streams. Wiley et al. (1990) found that prairie streams were inverted with respect to production to respiration ratios, riparian vegetation distributions, and

temperatures relative to the example of the RCC illustrated in Vannote et al (1980). Others investigating the RCC have found evidence both for (Paller 2006) and against (Dudgeon 1984, Delong and Brusven 1998, Harding et al. 1999) the applicability of the concept in disturbed streams.

The lack of significant statistical results could be related to the small number of sample sites. The design consisted of only 8 or 9 sites, depending on the sampling method, which made it unlikely that significant differences would be found. For example, to achieve significant results, similarity values would have to be very high among adjacent sites, summing to less than 15 and 21 for comparisons with 8 and 9 sites respectively. Thus, with a small number sample sites, the occurrence of just one large rank, representing random but substantial changes among any pair of adjacent sites, would change the results from being statistically significant to statistically insignificant.

In conclusion, p-values for tests using similarity measures that measured different aspects of community composition were all statistically insignificant, and led to the acceptance of all 8 null hypotheses. The physical proximity of sample sites did not influence site similarity in a predictable manner as a function of distance among sites in Hardwood Creek. The fact that all p-values were not significant may have resulted from several possibilities including the direct effects of habitat modification from ditching, small sample sizes, and the possibility that the RCC is incompatible with Midwestern and/or disturbed streams.

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Tables

Hypothesis	Data set	Index	Null hypothesis
1	DN	SMC	Similarities of adjacent pairs of sites have random rank
2	L	SMC	Similarities of adjacent pairs of sites have random rank
3	SFPE	SMC	Similarities of adjacent pairs of sites have random rank
4	DN	JAC	Similarities of adjacent pairs of sites have random rank
5	L	JAC	Similarities of adjacent pairs of sites have random rank
6	SFPE	JAC	Similarities of adjacent pairs of sites have random rank
7	DN	WPS	Similarities of adjacent pairs of sites have random rank
8	L	WPS	Similarities of adjacent pairs of sites have random rank

Table 1. The 8 RCC hypotheses tested in Hardwood Creek. Different data sets are indicated as DN, chironomid larvae (L), and SFPE. Similarity indices are SMC, JAC, and WPS.

Site	1.5U	1.5D	1.4	1.3	New	1	1.1	1.2	2
1.5U	–	0.683	0.706	0.563	0.563	0.54	0.54	0.556	0.484
1.5D	7	–	0.77	0.675	0.675	0.587	0.635	0.54	0.563
1.4	4	1	–	0.619	0.635	0.563	0.611	0.563	0.587
1.3	18	8	14	–	0.698	0.643	0.595	0.563	0.587
New	18	8	13	5	–	0.675	0.69	0.659	0.667
1	20	17	18	12	8	–	0.73	0.651	0.675
1.1	20	13	15	16	6	2	–	0.73	0.77
1.2	19	20	18	18	10	11	2	–	0.722
2	21	18	17	17	9	8	1	3	–

Table 2. The ranks of site similarities (lower section) based upon the SMC index for DN samples and the index values (upper section) used to assign ranks for each site pair for hypothesis 1. Where each dash (–) represents each site paired with its self and divides the matrix into upper (values to the right and above dashes) and lower (values to the left and below dashes) sections. Ranks are from highest to lowest, with 1 assigned to the sites with the highest index value. Sites are arranged from upstream to downstream.

Site	1.5U	1.5D	1.4	1.3	New	1	1.1	1.2	2
1.5U	–	0.588	0.686	0.529	0.451	0.373	0.451	0.529	0.373
1.5D	7	–	0.549	0.588	0.471	0.51	0.431	0.431	0.353
1.4	4	9	–	0.647	0.608	0.451	0.608	0.569	0.529
1.3	10	7	5	–	0.765	0.725	0.765	0.647	0.686
New	13	12	6	2	–	0.725	0.804	0.765	0.725
1	15	11	13	3	3	–	0.725	0.647	0.686
1.1	13	14	6	2	1	3	–	0.725	0.765
1.2	10	14	8	5	2	5	3	–	0.686
2	15	16	10	4	3	4	2	4	–

Table 3. The ranks of site similarities (lower section) based upon the SMC index for chironomid larvae and the index values (upper section) used to assign ranks for each site pair for hypothesis 2. Where each dash (–) represents each site paired with its self and divides the matrix into upper (values to the right and above dashes) and lower (values to the left and below dashes) sections. Ranks are from highest to lowest, with 1 assigned to the sites with the highest index value. Sites are arranged from upstream to downstream

Site	1.5U	1.5D	1.4	1.3	1	1.1	1.2	2
1.5U	–	0.721	0.682	0.558	0.388	0.403	0.395	0.434
1.5D	4	–	0.651	0.62	0.45	0.481	0.55	0.496
1.4	6	8	–	0.659	0.457	0.488	0.512	0.55
1.3	13	11	7	–	0.612	0.643	0.682	0.659
1	24	20	19	12	–	0.783	0.636	0.659
1.1	22	18	17	9	1	–	0.698	0.767
1.2	23	14	15	6	10	5	–	0.744
2	21	16	14	7	7	2	3	–

Table 4. The ranks of site similarities (lower section) based upon the SMC index for SFPE and the index values (upper section) used to assign ranks for each site pair for hypothesis 3. Where each dash (–) represents each site paired with its self and divides the matrix into upper (values to the right and above dashes) and lower (values to the left and below dashes) sections. Ranks are from highest to lowest, with 1 assigned to the sites with the highest index value. Sites are arranged from upstream to downstream; site New is excluded in this analysis.

Site	1.5U	1.5D	1.4	1.3	New	1	1.1	1.2	2
1.5U	–	0.365	0.275	0.295	0.267	0.284	0.237	0.2	0.177
1.5D	21	–	0.491	0.494	0.474	0.409	0.425	0.284	0.345
1.4	30	11	–	0.351	0.343	0.304	0.31	0.203	0.278
1.3	27	10	22	–	0.548	0.511	0.433	0.368	0.422
New	31	13	24	3	–	0.529	0.519	0.449	0.488
1	28	19	26	9	5	–	0.59	0.47	0.523
1.1	32	17	25	16	8	2	–	0.534	0.613
1.2	34	28	33	20	15	14	4	–	0.521
2	35	23	29	18	12	6	1	7	–

Table 5. The ranks of site similarities (lower section) based upon the JAC index for DN samples and the index values (upper section) used to assign ranks for each site pair for hypothesis 4. Where each dash (–) represents each site paired with its self and divides the matrix into upper (values to the right and above dashes) and lower (values to the left and below dashes) sections. Ranks are from highest to lowest, with 1 assigned to the sites with the highest index value. Sites are arranged from upstream to downstream.

Site	1.5U	1.5D	1.4	1.3	New	1	1.1	1.2	2
1.5U	–	0.192	0.2	0.25	0.2	0.22	0.2	0.25	0.179
1.5D	26	–	0.115	0.364	0.27	0.375	0.237	0.216	0.214
1.4	25	28	–	0.357	0.333	0.263	0.333	0.267	0.294
1.3	20	13	14	–	0.647	0.641	0.647	0.5	0.579
New	25	17	15	4	–	0.65	0.706	0.647	0.632
1	22	12	19	5	3	–	0.65	0.561	0.628
1.1	25	21	15	4	1	3	–	0.6	0.676
1.2	20	23	18	11	4	10	8	–	0.579
2	27	24	16	9	6	7	2	9	–

Table 6. The ranks of site similarities (lower section) based upon the JAC index for chironomid larvae and the index values (upper section) used to assign ranks for each site pair for hypothesis 5. Where each dash (–) represents each site paired with its self and divides the matrix into upper (values to the right and above dashes) and lower (values to the left and below dashes) sections. Ranks are from highest to lowest, with 1 assigned to the sites with the highest index value. Sites are arranged from upstream to downstream.

Site	1.5U	1.5D	1.4	1.3	1	1.1	1.2	2
1.5U	–	0.455	0.414	0.24	0.168	0.163	0.133	0.151
1.5D	4	–	0.318	0.246	0.165	0.173	0.216	0.145
1.4	6	11	–	0.313	0.186	0.195	0.192	0.216
1.3	15	14	13	–	0.315	0.333	0.359	0.313
1	21	22	19	12	–	0.594	0.382	0.397
1.1	23	20	17	10	1	–	0.443	0.531
1.2	26	16	18	9	8	5	–	0.476
2	24	25	16	13	7	2	3	–

Table 7. The ranks of site similarities (lower section) based upon the JAC index for SFPE and the index values (upper section) used to assign ranks for each site pair for hypothesis 6. Where each dash (–) represents each site paired with its self and divides the matrix into upper (values to the right and above dashes) and lower (values to the left and below dashes) sections. Ranks are from highest to lowest, with 1 assigned to the sites with the highest index value. Sites are arranged from upstream to downstream; site New is excluded in this analysis.

Site	1.5U	1.5D	1.4	1.3	New	1	1.1	1.2	2
1.5U	–	0.307	0.653	0.303	0.182	0.209	0.163	0.178	0.22
1.5D	27	–	0.533	0.487	0.432	0.363	0.603	0.211	0.337
1.4	2	6	–	0.492	0.402	0.372	0.422	0.245	0.348
1.3	28	13	12	–	0.475	0.522	0.504	0.386	0.476
New	33	17	20	15	–	0.694	0.525	0.326	0.415
1	32	23	22	9	1	–	0.549	0.46	0.497
1.1	35	4	18	10	7	5	–	0.523	0.611
1.2	34	31	29	21	26	16	8	–	0.611
2	30	25	24	14	19	11	3	3	–

Table 8. The ranks of site similarities (lower section) based upon the WPS index for DN samples and the index values (upper section) used to assign ranks for each site pair for hypothesis 7. Where each dash (–) represents each site paired with its self and divides the matrix into upper (values to the right and above dashes) and lower (values to the left and below dashes) sections. Ranks are from highest to lowest, with 1 assigned to the sites with the highest index value. Sites are arranged from upstream to downstream.

Site	1.5U	1.5D	1.4	1.3	New	1	1.1	1.2	2
1.5U	–	0.127	0.252	0.214	0.218	0.218	0.156	0.196	0.307
1.5D	30	–	0.071	0.156	0.087	0.156	0.115	0.143	0.194
1.4	20	33	–	0.399	0.251	0.268	0.242	0.233	0.34
1.3	25	28	15	–	0.4	0.489	0.501	0.395	0.474
New	24	32	21	14	–	0.599	0.621	0.433	0.435
1	24	28	19	10	4	–	0.717	0.491	0.552
1.1	28	31	22	8	2	1	–	0.519	0.54
1.2	26	29	23	16	13	9	7	–	0.603
2	18	27	17	11	12	5	6	3	–

Table 9. The ranks of site similarities (lower section) based upon the WPS index for chironomid larvae and the index values (upper section) used to assign ranks for each site pair for hypothesis 8. Where each dash (–) represents each site paired with its self and divides the matrix into upper (values to the right and above dashes) and lower (values to the left and below dashes) sections. Ranks are from highest to lowest, with 1 assigned to the sites with the highest index value. Sites are arranged from upstream to downstream.

Site	1.5U	1.5D	1.4	1.3	New	1	1.1	1.2	2
1.5U	–								
1.5D	1 C	–							
1.4	9	2 C	–						
1.3	16	10	3 C	–					
New	22	17	11	4 C	–				
1	27	23	18	12	5 C	–			
1.1	31	28	24	19	13	6 C	–		
1.2	34	32	29	25	20	14	7 C	–	
2	36	35	33	30	26	21	15	8 C	–

Table 10. A hypothetical matrix of site-by-site comparisons in which ranks of similarities conform to the RCC. Sites in the matrix are arranged from upstream to downstream down each column and across all rows and similarity values are ranked from the highest (1) to lowest (36) values. Sites closest to each other from upstream to downstream are adjacent to the diagonal of the matrix and are indicated by (C).

Index and data set		P-value
SMC		
	DN	0.531
	L	0.488
	SFPE	0.822
JAC		
	DN	0.869
	L	0.957
	SFPE	0.837
WPS		
	DN	0.910
	L	0.916
	SFPE	*

Table 11. P-values from exact Wilcoxon rank sum tests of the RCC in Hardwood Creek for SMC, JAC, and WPS similarity indices, calculated from DN, chironomid larvae (L) and SFPE data sets. An asterisk (*) indicates that a p-value could not be calculated.

Figure

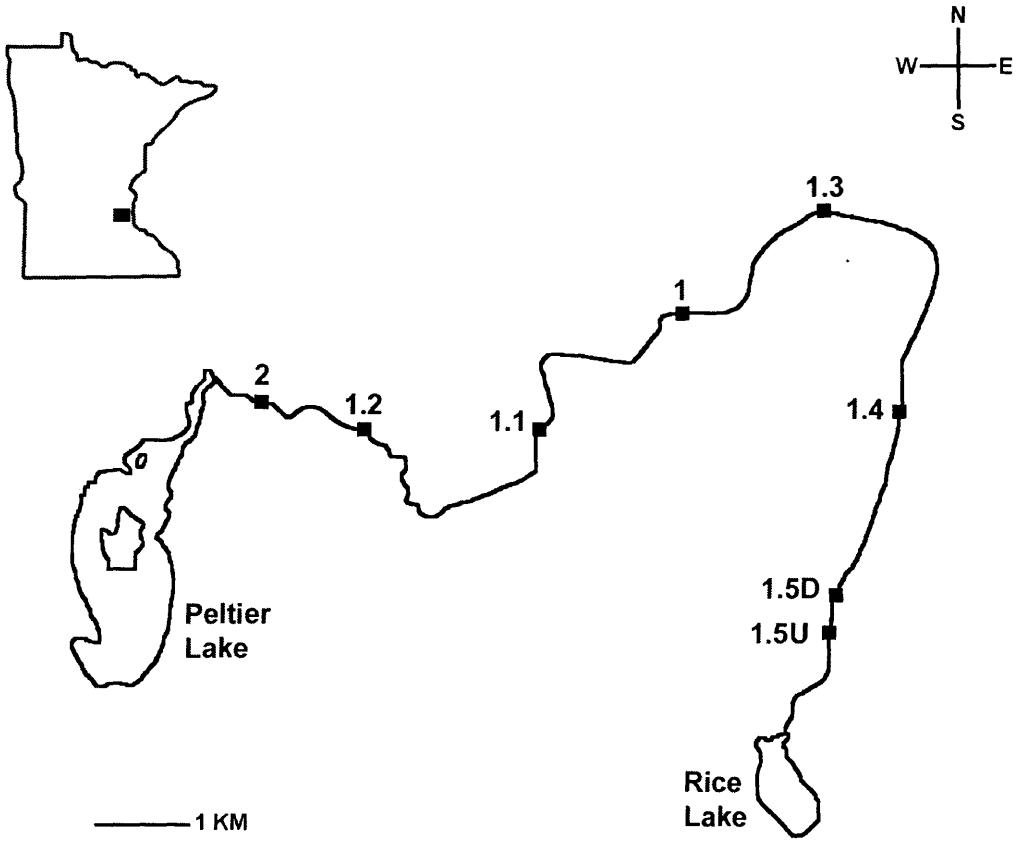

Figure 1. A map of Hardwood Creek and the 9 sample sites. Hardwood Creek flows from Rice Lake into Peltier Lake.

Chapter 5

Hydroseres and Management Categories as Classifications for Understanding Community Composition of Macroinvertebrates in Hardwood Creek, a Ditched Stream in Minnesota, USA

Will be submitted to: Journal of the North American Benthological Society

Abstract

Distinct stages or hydroseres are aquatic communities that are identifiable and predictably occur throughout succession in aquatic ecosystems. Both the Intermediate Disturbance Hypothesis (IDH) and the River Continuum Concept (RCC), Chapters 3 and 4 respectively, failed to explain patterns of species richness, diversity, and community structure in Hardwood Creek. Consequently, 2 alternative classifications, hydroseres and management categories, were tested. Poor water quality, substrate stability, and differences in aquatic vegetation among sample sites are probably the most important factors confounded with ditching in Hardwood Creek, and likely played a role in the rejection of both IDH and RCC models. Time since the last major ditching event was used to structure the hydroseres. The taxa *Ranatra* sp., *Stenelmis* sp., *Sperchopsis* sp., *Pseudosmittia* sp. 1, *Cladopelma* sp. 1, *Dicrotendipes* sp. 4, *Einfeldia* sp. gr. D, and *Stictochironomus* sp. were associated with a single hydrosere. Thus, the hydrosere models failed to explain patterns of taxa richness for a large percentage of taxa in Hardwood Creek. Similarity indices generally supported the validity of the *a priori* hydrosere classification based upon time since the last dredging event. An alternative classification based on a Total Maximum Daily Load (TMDL) management strategy, divided the stream into 2 management reaches, upstream of site 1 and from site 1 downstream to site 2. Based on this classification, the number of taxa at upstream and

downstream sites was not significantly different. However, an analysis of similarity indices generally supported the upstream and downstream management reaches.

Introduction

Succession is defined as community change at a site following a disturbance event (Fisher 1983). Distinct stages or hydroseres are aquatic communities that are identifiable and predictably occur throughout succession in aquatic ecosystems. However, some lotic ecologists question the existence of stream succession (Fisher 1990). Successional processes can be studied successfully within smaller order stream systems that have been ditched. Hydrosere succession in ditches is recognized by many (e.g., Painter 1989, 1999, Strien et al. 1991, Armitage 2003). Others have described the physical and/or biological components associated with each hydrosere (Caspers and Heckman 1981, 1982, Clare and Edwards 1983, Verdonschot 1992).

Justification for an investigation of hydroseres and upstream and downstream site classifications in Hardwood Creek comes from the fact that both the Intermediate Disturbance Hypothesis (IDH) and the River Continuum Concept (RCC), Chapters 3 and 4 respectively, failed to explain patterns of species richness, species diversity, and community composition. The concept of hydroseres, by contrast, allows for temporal comparisons and provides an alternative mechanism to examine community composition patterns across gradients of disturbance. Similarly, a classification of upstream and downstream sites, for development of a Total Maximum Daily Load (TMDL) strategy, can be used to examine community composition patterns too. The objective of this study was to evaluate hydrosere and upstream and downstream site classifications using an t-test, taxa presence and absence, and similarity indices.

Methods

All methods and sample sites used in this chapter are described in Chapters 2 and 3 respectively. Additional methodological procedures and information is described below.

Historical conditions across study sites

Hardwood Creek is one of the major tributaries of the Rice Creek Watershed District (RCWD) and has a long history of poor water quality. In 1973, some of the highest concentrations of Coliform bacteria in the RCWD were found in Hardwood Creek (Anonymous 1975). High concentrations of nitrate, available phosphorus, and solids have also been found (Anonymous 1975). In 2002 and 2004, investigations of the creek were made to determine whether it should be listed on the state of Minnesota's 303(d) List for a low Fish Index of Biological Integrity and low concentrations of dissolved oxygen, respectively. The stream was subsequently listed and was legally divided into 2 sections that were analyzed for development of a TMDL, that could result in potentially different management strategies from the status quo.

Alternative site classifications

Time since the last major ditching event was used to define 3 hydroseres in Hardwood Creek. The recently disturbed hydrosere contains the most disturbed sites 1.5U and 1.4; the intermediately disturbed hydrosere contains sites 1.3, 1.5D, 1, and 1.1; the not disturbed hydrosere contains the least disturbed sites 1.2 and 2.

In addition to hydroseres, Hardwood Creek was divided into upstream and downstream reaches. Sites 1.5U, 1.5D, 1.4, and 1.3 are the upstream sites while 1, 1.1, 1.2, and 2 are the downstream sites. Site New was excluded because SFPE samples

were not collected at this site. The classification largely conforms to that proposed by Emmons and Oliver Resources, Inc. et al. (2005) based upon differences in groundwater, surface water, and biological data. The only difference between the classifications of Emmons and Oliver Resources, Inc. et al. (2005) and this study is site 1 is considered a downstream site.

Analysis

To test whether upstream and downstream sites were significantly different in their taxa richness, an F-test for unequal variance and an t-test (unpooled) was conducted using JMP IN® software (version 5.1.2, SAS Institute Inc., Cary, NC). To determine taxa association with each hydrosere the presence and absence of each taxon was evaluated on a site-by-site basis. In addition, Simple Matching coefficient (SMC), Whittaker's percent similarity (WPS), and Jaccard's (JAC) similarity indices were used to evaluate dipnet (DN), chironomid larvae, and chironomid surface-floating pupal exuviae (SFPE) data sets for each hydrosere.

Results

The hydrosere models

The taxa *Ranatra* sp., *Stenelmis* sp., *Sperchopsis* sp., *Pseudosmittia* sp. 1, *Cladopelma* sp. 1, *Dicrotendipes* sp. 4, *Einfeldia* sp. gr. D, and *Stictochironomus* sp. were associated with a single hydrosere (Figures 1-8). No taxa were exclusively associated with the intermediately disturbed hydrosere, only the recently and not disturbed hydroseres had associated taxa (Figures 1-8 and Appendix B Table 70-172).

Hydrosere similarities

In all but 1 comparison, the sites that were not disturbed had the highest similarity for all indices and all data sets (Figure 9). The SMC index consistently produced the highest index values across all comparisons (Figures 9-10).

The biggest discrepancy was for the WPS index, in which there was a 0.401 or 40% difference in the similarity among the sites for DN taxa and chironomid larvae in the recently disturbed hydrosere (Figure 9). Similarity indices were generally lower at recently and intermediately disturbed hydroseres, especially for JAC and WPS indices (Figure 9).

The SMC index consistently produced the highest index values in comparisons across hydroseres (Figure 10). For comparisons made across hydroseres using the same data set, the intermediate and not disturbed hydroseres had the highest similarity, regardless of the index examined (Figure 10). In 6 out of 8 comparisons, the recently and intermediate disturbed hydroseres and the recently and not disturbed hydroseres had the second and third highest index values respectively, when the same index and data set was compared (Figure 10). The 2 comparisons not conforming to this pattern were observed in DN larvae samples for both JAC and WPS indices (Figure 10).

Upstream/downstream classification

There are several patterns in the similarity indices calculated for DN, chironomid larvae, and SFPE data sets for upstream and downstream, and across site comparisons. First, SMC consistently produced the highest index values for all comparisons. (Figure 11). The downstream sites had the highest index values for all method comparisons (Figure 11).

In comparisons between upstream and across sites, the average similarity values were highest for upstream sites for SMC and JAC indices but not for WPS index. The average index for each sampling method at upstream sites was 0.632, 0.338, and 0.322 for SMC, JAC, and WPS indices respectively (Figure 11). The average index for each sampling method for across site comparisons was 0.559, 0.325, 0.351 for SMC, JAC, and WPS indices respectively (Figure 11).

Taxa richness between upstream and downstream sites did not differ significantly (Table 1; p-value = 0.868). The F-test for equal variance showed that variance between upstream and downstream sites was not equal (p-value = 0.865), so an unpooled t-test was used to evaluate the sites (Table 1, p-value = 0.868).

Discussion

The hydrosere models

Six taxa were associated with the recently disturbed hydrosere and 2 taxa associated with the not disturbed hydrosere. No taxa were exclusively associated with the intermediately disturbed hydrosere. The 6 taxa associated with the recently disturbed hydrosere were *Ranatra* sp., *Pseudosmittia* sp. 1, *Cladopelma* sp. 1, *Dicrotendipes* sp. 4, *Einfeldia* sp. gr. D, and *Stictochironomus* sp. The 2 taxa associated with the not disturbed hydrosere were *Stenelmis* sp. and *Sperchopsis* sp. Thus, the hydrosere models failed to explain patterns of taxa richness for the vast majority of taxa in Hardwood Creek.

In a probabilistic sense, it is not very surprising that 0 taxa were found to be associated with the intermediately disturbed hydrosere. The probability that taxa would be associated with any 2 sites is higher than the probability of being associated with any 4 sites, all else being equal.

The fact that 5 of the 6 taxa associated with the recently disturbed hydrosere were chironomids is not surprising. Many studies have demonstrated chironomids quickly colonize disturbed and/or denuded substrates (Pearson and Jones 1975, Williams and Hynes 1976, Williams 1977, Gray and Fisher 1981, Fisher et al. 1982, Reice 1985, Cushing and Gaines 1989, Doeg et al. 1989, Hare 1995).

The results of this study agree with Gray and Fisher (1981) and Fisher et al. (1982) who found *Dicrotendipes* sp. to be an early colonizing taxa following a flood disturbance in a desert stream. Results also agree with Hare (1995) who found *Cladopelma* sp. and *Stictochironomus* sp. to be early colonizers of lake sediments. The limited number of studies on recolonization, and the poor chironomid taxonomic resolution employed in many studies makes it difficult to compare the results of this study with others.

It is difficult to compare the results of the not disturbed hydrosere with previous studies, because little to no information exists with regards to the presence of *Stenelmis* sp. and *Sperchopsis* sp. in higher order hydroseres. Hydroseres were based upon the last dredging event, thus the proposed hydrosere classification contains sites much different than those proposed by Caspers and Heckman (1981) and Clare and Edwards (1983) for their most mature hydrosere. Sites 1.2 and 2 are the most downstream sites, and the least disturbed, and do not resemble traditional ditches; that further complicates our hydrosere analysis. These findings support the conclusions of Emmons and Oliver Resources, Inc. et al. (2005) who proposed that Hardwood Creek be divided into upstream and downstream reaches.

Hydrosere classification

Eleven site groups or cenotypes, representing a successional gradient were developed by Verdonschot (1992). Given that each site group is based upon detailed information such as velocity, site dimensions, drought duration, acidity, and the combination of several to many species, comparisons with the proposed Hardwood Creek hydroseres with those of Verdonschot (1992) were not possible. Comparisons between Hardwood Creek ditch vegetation and the plant communities presented in Caspers and Heckman (1982) could also not be made. However, the seres defined in Caspers and Heckman (1981) can be compared with Hardwood Creek.

The following comparisons are made between Hardwood Creek and the hydroseres of Caspers and Heckman (1981). Sere 1b fits site 1.5U fairly well in that it was ditched in 2004 and *Lemna* spp. was prevalent at the site. Sere 2 fits the remainder of the sites to varying degrees. Sere 4 describes site 1.5D in the late summer when *Lemna* spp. became dominant throughout much of the channel, and terrestrial and littoral plant communities invaded the channel.

The hydroseres in Hardwood Creek can also be compared with those site group hydroseres discussed in Clare and Edwards (1983). Sites 1.5U and 1.4 fit into group 1 because they were dredged in 2004. The second group best describes parts of sites 1.4 and 1.3 that were dominated by submerged vegetation. Group 4 describes site 1.5D in the late summer when *Lemna* spp. and terrestrial plant communities invaded the channel. The remainder of the site do not fit into any of the hydrosere groups described by Clare and Edwards (1983), largely because submerged, floating, or emergent vegetation was not dominant.

Three hydroseres were developed for testing the hydrosere model in Hardwood Creek. As demonstrated in the above discussion, the hydroseres in Hardwood Creek

differed from those proposed by Caspers and Heckman (1981) and Clare and Edwards (1983). One substantial difference is that sites 1.5U and 1.5D are at the more disturbed end of the continuum, and represent part of the recently and intermediately disturbed hydroseres respectively. This classification is valid given their disturbance history.

However, sites 1.5U and 1.5D would likely be the first to reach a terrestrial climax community, which is theoretically classified as the last hydrosere. This is particularly true for site 1.5D, as demonstrated with the above classification comparisons with Caspers and Heckman (1981) and Clare and Edwards (1983). Regardless of these discrepancies, the time since the last ditching event was used to determine the hydroseres in Hardwood Creek.

The validity of hydrosere classifications based on similarity indices

When hydroseres were compared, it was clear that the sites in the not disturbed hydrosere were more similar to each other than sites within the recently and intermediately disturbed hydroseres. This helps to validate the not disturbed hydrosere as a valid classification. The low JAC and WPS index values for recently and intermediately disturbed hydroseres is evidence that these classifications might not be valid. It is also likely that habitat stability was an overriding factor in determining similarity among sites. Habitat stability has been shown to have a large influence on community structure (e.g., Death 1995, Death and Winterbourn 1995). In addition, habitat stability can be expected to be lowest at recently and intermediately disturbed sites.

Comparisons across hydroseres showed that the intermediate and not disturbed hydroseres were the most similar. The recently and intermediately disturbed hydroseres were generally more similar to each other when compared to the recently and not

disturbed hydroseres. These findings support the validity of the *a priori* hydrosere classifications based upon time since the last dredging event.

Upstream and downstream site similarities and taxa richness

Downstream sites were more similar when compared to upstream and across site comparisons. This is not surprising given that downstream sites were relatively undisturbed when compared to upstream sites. Upstream sites were generally more similar than across site comparisons. These findings support the conclusions of Emmons and Oliver Resources, Inc. et al. (2005) that upstream and downstream reaches in Hardwood Creek are biologically different.

The total number of taxa at upstream and downstream sites were not significantly different (p-value = 0.868). Given the differences in disturbance regimes and habitat types at both upstream and downstream sites, we made the *a priori* assumption that differences would likely be found. Although the temporal and spatial scales of studies investigating the effects of dredging have varied, many studies have concluded that macroinvertebrate species richness, diversity, or abundance is affected by stream dredging. Researchers have found that control reaches had significantly greater macroinvertebrate richness, diversity, or abundance than dredged (Pearson and Jones 1975, Beltman 1984, Edwards et al. 1984, Thomas 1985) or dredged or bulldozed sites (Wydoski and Helm 1980).

However, not all studies have similar results with regards to the effects of stream dredging on the biota. Somer and Hassler (1992) found there was no difference in the number of macroinvertebrates or diversity indices between artificial substrates placed upstream and downstream of areas that were dredged with a suction gold dredge. Dai et al. (2003) found that taxa richness actually increased following stream dredging. Duvel

et al. (1976) found little difference in taxa richness and diversity between channelized and natural sites.

There is much research suggesting that macroinvertebrates are highly resilient and recover quickly following disturbance in streams (e.g., Pearson and Jones 1975, Fisher et al. 1982, Reice 1984,1985, Lake et al. 1989, Matthaei et al. 1996, Dole-Olivier et al. 1997) and ditches (Whitaker 1979).

To date, the only published study conducted in Minnesota similar to the present study is Marsh and Waters (1980), who investigated the downstream effects of drainages ditches and channelization on macroinvertebrates in southwestern Minnesota. Marsh and Waters (1980) found species diversity among all sites to be similar, and concluded that drainage development in upstream regions had little to no impact on the macroinvertebrte fauna in natural downstream reaches.

Disturbance and other factors influencing species richness in Hardwood Creek

Many lotic studies have shown that disturbance results in an initial decrease in macroinvertebrate species richness and/or diversity, but the effects of disturbance are relatively ephemeral and richness and/or diversity recovers relatively quickly (Pearson and Jones 1975, Reice 1984, 1985, Boulton et al. 1988, Doeg et al. 1989, Lake et al. 1989, Matthaei et al. 1996). Others have shown that following disturbance macroinvertebrate diversity (Wydoski and Helm 1980, Degani et al. 1992, Somer and Hassler 1992) and species richness (McCabe and Gotelli 2000, Dai et al. 2003) can increase.

It is difficult to compare the present study with those above because sampling did not occur before dredging. However, there were many confounding factors within the present study that likely influenced our results. Given that this was an observational

study, we could draw upon a myriad of factors that were confound with disturbance via ditching. However, poor water quality, substrate stability, and differences in vegetation among sample sites are probably the most important confounding factors in Hardwood Creek.

Hardwood Creek has a history of poor water quality, back to at least the 1970's (Anonymous 1975). Recently, Hardwood Creek was listed on the state of Minnesota's 303(d) List for a low Fish Index of Biological Integrity in 2002 and low concentrations of dissolved oxygen in 2004. Poor water quality, especially low levels of dissolved oxygen undoubtedly have an effect on macroinvertebrate species richness. Claire and Edwards (1983) found evidence that dissolved oxygen levels were a major factor determining the distribution of macroinvertebrates in ditches.

Others support the idea that general water quality greatly influences the development of macroinvertebrate communities in ditches (Armitage et al. 2003). Turbidity caused by bank erosion, particularly at and downstream of 1.5U is another water quality parameter that is not only a disturbance to the biota, but is a confounding factor with disturbance induced by ditching. Throughout the study peaty organic matter was observed in the water column at many of the sample sites.

Substrate stability is another potential confounding factor within the present study because many of the upstream sites have less stable substrates compared to downstream sites. Benthic substrates at 1.5U, 1.5D, and 1.4 were largely dominated by organic peaty substrates. The benthic substrates at downstream sites were largely dominated by sand, with some sites containing gravel and pebble substrates. Researchers have found evidence that substrate stability is an important parameter affecting the response of macroinvertebrate communities to disturbance (Wydoski and

Helm 1980, Gurtz and Wallace 1984). Substrate stability has been shown to influence community structure (e.g., Death and Winterbourn 1995, Death 2002).

Ditch vegetation was largely ignored in this study, however qualitative differences in vegetation among sample sites were observed (Chapter 3). Vegetation has been shown to have an important influence on macroinvertebrate communities in ditches (Ćlare and Edwards 1983, Beltman 1984, Higler and Verdonschot 1989, Painter 1998, 1999, Armitage et al. 2003). Taxa richness at any site can potential be expected to be a function of vegetative diversity. Both Beltman (1984) and Armitage et al. (2003) found that macroinvertebrate taxa richness increased as ditch vegetation diversity increased.

In conclusion, only 8 taxa were exclusively associated with the recently or not disturbed hydroseres. Sites in the not disturbed hydrosere were most similar to each other; recently and intermediately disturbed hydroseres had some low JAC and WSP similarity index values, which calls into question the validity of these classifications. However, comparisons across hydrosere classes supported the validity of all hydrosere classifications. Upstream and downstream sites were generally more similar than across site comparisons. However, there was no difference in the total number of taxa at upstream and downstream sites. Similarity indices calculated for hydrosere and upstream and downstream sites generally supported the findings of Emmons and Oliver Resources, Inc. et al. (2005), that upstream and downstream sites are biologically different. Poor water quality, substrate stability, and differences in vegetation among sample sites were probably the most important confounding factors in Hardwood Creek.

Acknowledgements

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Table

Site	Location	Richness	F-test	t-test
1.5U	Upstream	130	0.865	0.868
1.5D	Upstream	115		
1.4	Upstream	105		
1.3	Upstream	112		
1	Downstream	120		
1.1	Downstream	120		
1.2	Downstream	113		
2	Downstream	113		

Table 1. The test for significance between upstream and downstream sites. Richness is cumulative taxa richness for all organisms found in DN and SFPE samples and the t-test is unpooled.

Figures

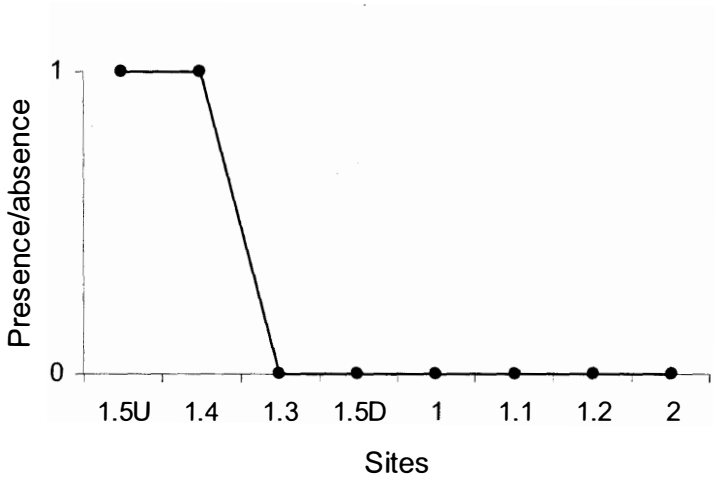

Figure 1. *Ranatra* sp. presence (1) and absence (0). The recently disturbed hydrosere contains the most disturbed sites 1.5U and 1.4; the intermediately disturbed hydrosere contains sites 1.3, 1.5D, 1, and 1.1; the not disturbed hydrosere contains the least disturbed sites 1.2 and 2.

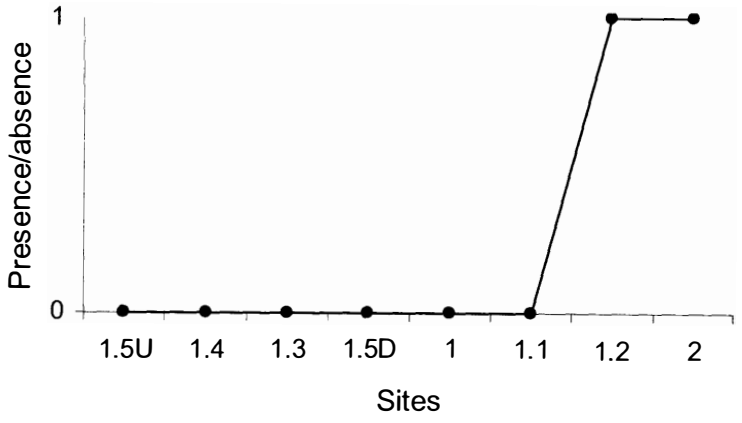

Figure 2. *Stenelmis* sp. presence (1) and absence (0). The recently disturbed hydrosere contains the most disturbed sites 1.5U and 1.4; the intermediately disturbed hydrosere contains sites 1.3, 1.5D, 1, and 1.1; the not disturbed hydrosere contains the least disturbed sites 1.2 and 2.

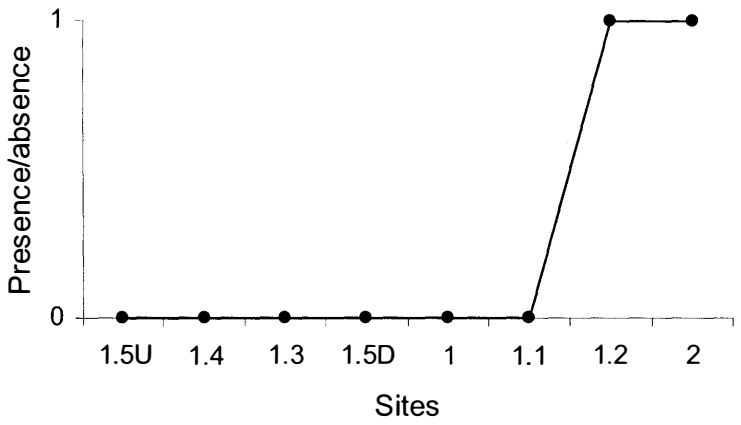

Figure 3. *Sperchopsis* sp. presence (1) and absence (0). The recently disturbed hydrosere contains the most disturbed sites 1.5U and 1.4; the intermediately disturbed hydrosere contains sites 1.3, 1.5D, 1, and 1.1; the not disturbed hydrosere contains the least disturbed sites 1.2 and 2.

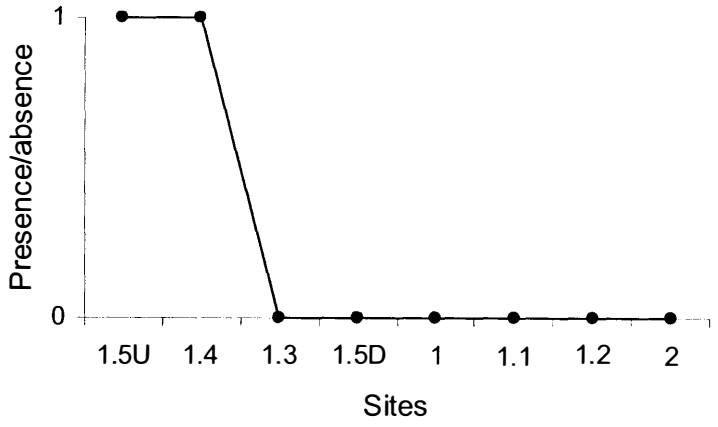

Figure 4. *Pseudosmittia* sp. 1 presence (1) and absence (0). The recently disturbed hydrosere contains the most disturbed sites 1.5U and 1.4; the intermediately disturbed hydrosere contains sites 1.3, 1.5D, 1, and 1.1; the not disturbed hydrosere contains the least disturbed sites 1.2 and 2.

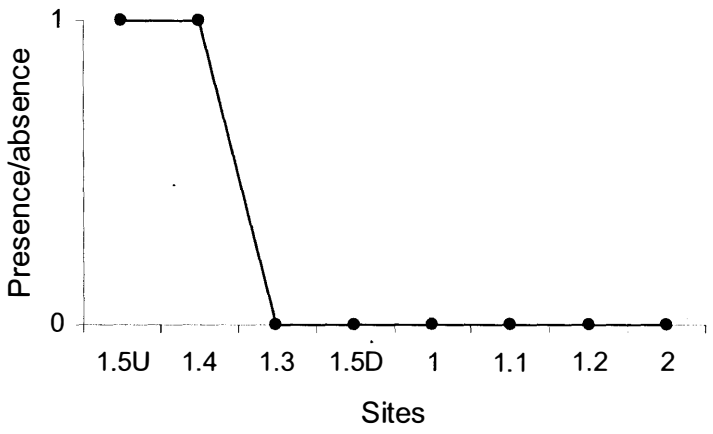

Figure 5. *Cladopelma* sp. 1 presence (1) and absence (0). The recently disturbed hydrosere contains the most disturbed sites 1.5U and 1.4; the intermediately disturbed hydrosere contains sites 1.3, 1.5D, 1, and 1.1; the not disturbed hydrosere contains the least disturbed sites 1.2 and 2.

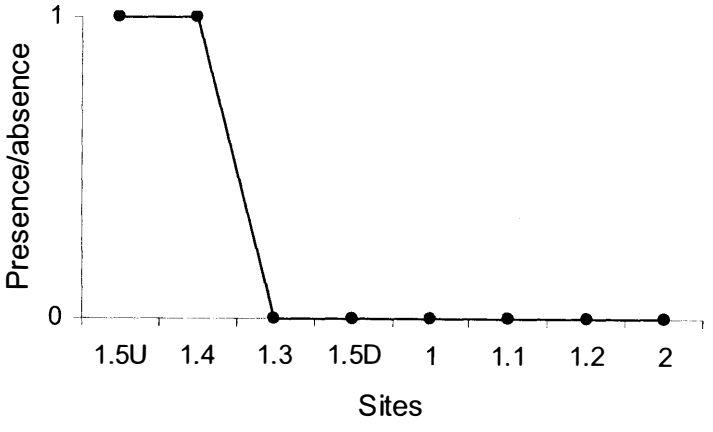

Figure 6. *Dicrotendipes* sp. 4 presence (1) and absence (0). The recently disturbed hydrosere contains the most disturbed sites 1.5U and 1.4; the intermediately disturbed hydrosere contains sites 1.3, 1.5D, 1, and 1.1; the not disturbed hydrosere contains the least disturbed sites 1.2 and 2.

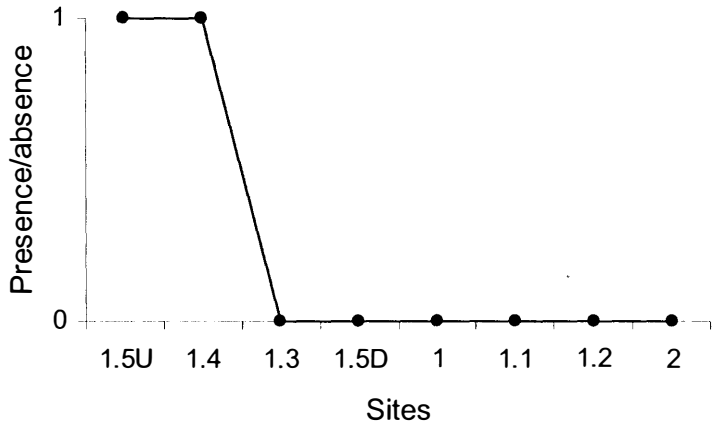

Figure 7. *Einfeldia* sp. gr. D presence(1) and absence(0). The recently disturbed hydrosere contains the most disturbed sites 1.5U and 1.4; the intermediately disturbed hydrosere contains sites 1.3, 1.5D, 1, and 1.1; the not disturbed hydrosere contains the least disturbed sites 1.2 and 2.

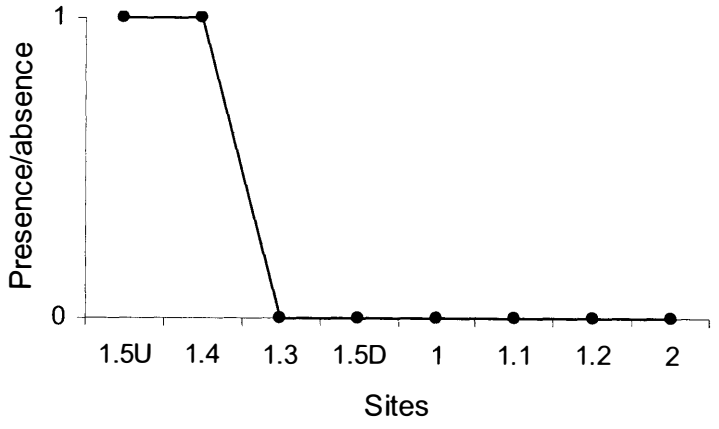

Figure 8. *Stictochironomus* sp. presence (1) and absence (0). The recently disturbed hydrosere contains the most disturbed sites 1.5U and 1.4; the intermediately disturbed hydrosere contains sites 1.3, 1.5D, 1, and 1.1; the not disturbed hydrosere contains the least disturbed sites 1.2 and 2.

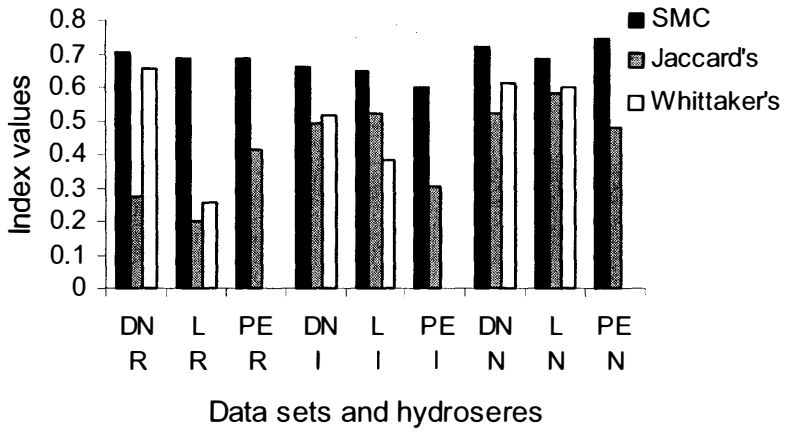

Figure 9. Similarity indices for recently (R), intermediate (I), and not (N) disturbed hydroseres using DN, DN larvae, and SFPE data sets.

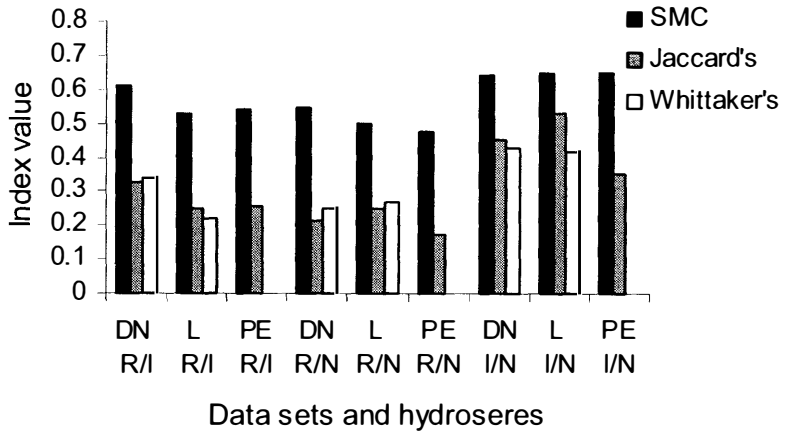

Figure 10. Similarity indices across recently (R), intermediate (I), and not (N) disturbed hydroseres using DN, DN larvae, and SFPE data sets.

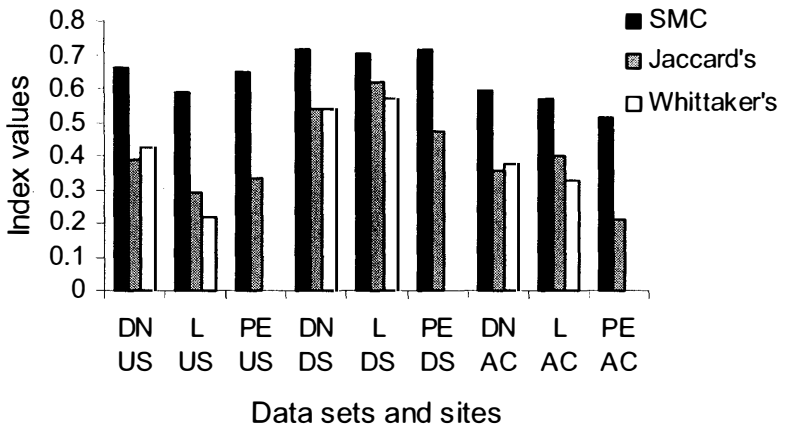

Figure 11. Similarity indices for upstream (US), downstream (DS), and across (AC) sites using DN, DN larvae, and SFPE data sets.

Chapter 6

A Literature Review of Chironomidae (Diptera) Pupal Exuviae Use in Ecological Assessment Studies of Lotic Ecosystems

Introduction

Aquatic flies known as non-biting midges or chironomids are members of the family Chironomidae. Members of this family are usually the most abundant insects in lotic and lentic habitats (Coffman 1973, Pinder 1986, Cranston 1995, Coffman and Ferrington 1996). Given their prominence in aquatic ecosystems, it is critical for sampling methods to efficiently capture the extant chironomid community. Many sampling methods have been employed to capture specimens in the larval or pupal stage of the chironomid life cycle.

The goal of this chapter is a literature review of Chironomidae pupal exuviae use in ecological assessments of lotic ecosystems. Influential studies that have used pupal exuviae, but not for lotic ecological assessment, are also included. This review includes: a discussion of problems associated with methods designed to collect larvae and pupal exuviae, the advantages of using collections of exuviae, and the spatial and ecological information assessed in studies where exuviae were collected. Finally, the methods employed to capture exuviae and a brief history of the method are discussed.

Discussion

Many investigators have noted problems associated with studying Chironomidae larvae. For example, larvae are difficult to identify (Humphries 1938, Wartinbee and Coffman 1976, Wilson and McGill 1977, McGill et al. 1979, Wilson 1980, Ruse and Wilson 1984, Ferrington and Crisp 1989, Coffman and Ferrington 1996, Cranston et al. 1997, Raunio and Muotka 2005, Calle-Martínez and Casas 2006). Larvae can also crawl

out of and actively avoid a net (Wilson and Bright 1973); and larval chironomid samples take longer to process when compared to pupal exuviae samples (Ferrington and Crisp 1989, Ferrington et al. 1991). In addition, early instar larvae can be missed during sampling if the mesh size of the net is too large. Finally, chironomid larvae are generally not identifiable in most keys (Coffman and Ferrington 1996). Regardless of the instar collected, species differentiation can be problematic if mouthparts are substantially worn down.

However, there are also limitations with the collection and identification of pupal exuviae. The exuviae method focuses only on the Chironomidae (Wilson and McGill 1977, Ruse and Wilson 1984) and the specific microhabitat from which specimens are obtained is unknown (Wilson and McGill 1977, Wilson 1980, Ruse and Wilson 1984). The method is also affected by seasonal (Wilson and McGill 1977, Wilson 1980, Hayes and Murray 1988, Raunio and Muotka 2005) and diel (Hayes and Murray 1988) variation in emergence. Pupal exuviae collections are often criticized as being qualitative. However, exuviae can be collected quantitatively (Coffman, 1973, Wilson and Bright 1973, Coffman1974, Wartinbee and Coffman 1976, Wartinbee 1979, Wilson 1980, Chutter 1984, Coffman and Ferrington 1996). In addition, the gaps in taxonomy of chironomid pupae can hamper some studies (Humphries 1938, Wartinbee 1979).

There are several advantages related to collection of surface-floating pupal exuviae instead of larvae. Pupal exuviae samples capture organisms from all habitats (McGill et al. 1979, Wilson 1980, Ruse and Wilson 1984, Coffman and Ferrington 1996, Raunio and Muotka 2005). Identification to genus is easier (Coffman and Ferrington 1996, Raunio and Muotka 2005) and in many cases identification to species can be made (Wilson and McGill 1977, Wilson 1980, Ruse and Wilson 1984, Ferrington et al. 1991, Barton et al. 1995, Coffman and Ferrington 1996).

The pupal exuviae method is also easier and likely safer in larger-deep rivers (Wilson and McGill 1977, McGill et al. 1979, Wilson and Wilson 1983, Ruse and Wilson 1984, Ruse and Davison 2000). In springs, collections of exuviae are efficient, quick, and impart only a small amount of disturbance (Blackwood et al. 1995). Investigators have also noted the smaller amount of time and/or cost associated with pupal exuviae samples (Wilson and Wilson 1983, Ruse and Wilson 1984, Fend and Carter 1995, Ferrington et al. 1991, Wright et al. 1996, Calle-Martínez and Casas 2006).

The pupal exuviae method has been used in a variety of locations to investigate a variety of ecological questions throughout the world. Within the United Kingdom, exuviae have been collected to monitor the impacts of organic enrichment, primarily sewage, in streams (Wilson and Bright 1973, Wilson 1977, Wilson and McGill 1977, McGill et al. 1979, Wilson 1992) and a canal (Wilson 1994). They have also been used in general water quality monitoring (Wilson 1980, Ruse and Wilson 1984, Wilson 1987, Ruse and Wilson 1995). Wilson (1988) applied the method to investigate several streams with zinc pollution. Ruse and Davison (2000) evaluated the exuviae method to determine its effectiveness in detecting ecological changes caused by river flow regulation. In Ireland, exuviae were collected on 3 rivers to assess the temporal variability in Chironomidae emergence and its implications for biological monitoring (Hayes and Murray 1988).

The exuviae method has been used throughout Europe, including the Rhine River, for general surface water quality monitoring (Wilson and Wilson 1983, 1984). In Belgium and the Netherlands on the Meuse River, exuviae were used to determine water quality changes (Frantzen 1993). Exuviae were used in the Kymi River in Finland to detect organic enrichment (Raunio and Muotka 2005), and in south-eastern Finland to study various aspects of the exuviae technique for biomonitoring (Raunio et al. 2007). In

an investigation of the entire chironomid community of the Fulda River in Germany, Lehmann (1971) collected exuviae to augment larval and adult collections.

Calle-Martínez and Casas (2006) used the method to assess the ability of Chironomids to classify streams and monitor water quality in southern Spain. In South Africa exuviae were collected to examine the effects of treated sewage on the Msunduze River (Chutter 1984). In Guinea, Ivory Coast, Sierra Leone, Togo, and Costa Rica, Coffman et al. (1993) used exuviae collections to evaluate the diversity of lotic chironomid communities. Cranston et al. (1997) used exuviae to investigate the effects of acidic waters and heavy metals on the chironomid community in northern Australia.

In North America studies utilizing pupal exuviae have been more limited. Exuviae have been employed in Linesville Creek in Pennsylvania to assess taxonomic composition and phenology (Coffman 1973), quantify emergence densities (Wartinbee and Coffman 1976), and determine diel emergence patterns (Coffman 1974, Wartinbee 1979). Pupal exuviae were used in Kansas to evaluate general water quality (Ferrington et al. 1991), nutrient (Ferrington and Crisp 1989) and organic enrichment (Coler 1984), and various parameters of community ecology (Kavanaugh 1988, Ferrington et al. 1995).

Blackwood et al. (1995) collected exuviae in Kansas and other parts of the High Plains Region to assess emergence and taxa similarity among springs. Exuviae from Missouri streams were used to evaluate the impacts of the pesticide chlordane (Wright et al. 1996) and heavy metals (Hayford and Ferrington 2005). Ruse et al. (2000) used exuviae to evaluate chironomid distributions in relation to heavy metal pollution in the Arkansas River. Exuviae in the Yakima River Basin were employed to evaluate the relationships of chironomid assemblages to habitat variables (Fend and Carter 1995). In

Canada, exuviae have been used to assess the impacts of agriculture on water quality in Ontario streams (Barton et al. 1995).

The field method(s) employed to capture pupal exuviae can differ from country to country. Throughout Europe a handnet is a very popular method for collecting pupal exuviae (Wilson and McGill 1977, McGill et al. 1979, Wilson 1992, Frantzen 1993, Wilson, 1994, Raunio and Muotka 2005, Raunio et al. 2007). However, other methods have been employed including: driftnets (Wilson and Bright 1973, Wilson 1977, Hayes and Murray 1988), a wooden float which collects material later sampled via handnet (Ruse 1995a, b), surface drift net or handnet (Wilson and Wilson 1983, 1984), and both surface drift net and handnet (Wilson 1988, Calle-Martínez and Casas 2006). Humphries (1938) used a row boat and a silk net to collect lentic exuviae.

In North America the methods used are just as diverse and include: a 4-5 liter sample later sieved in the laboratory (Coffman 1973), enclosed channels (Wartinbee and Coffman 1976, Wartinbee 1979), and upstream and downstream surface barriers (Coffman 1974). Pan and sieve (Coler 1984, Ferrington 1987, Kavanaugh 1988, Ferrington and Crisp 1989, Ferrington et al. 1991, Blackwood et al. 1995, Fend and Carter 1995, Ferrington et al. 1995, Wright et al. 1996, Hayford and Ferrington 2005), hand strainer (Barton et al. 1995), and handnet (Ruse et al. 2000) have also been employed.

In South Africa a paddle-wheel sampler was used to collected quantitative samples of pupal exuviae (Chutter 1984). In Guinea, Ivory Coast, Sierra Leone, and Togo, Coffman et al. (1993) used a drift net at night to obtain several samples; however, most exuviae were skimmed from the surface of streams in the above locations and in Costa Rica. Brundin (1966) used a drift net and manually skimmed exuviae in Afrotropic,

Australasia, and Neotropic streams. Cranston et al. (1997) used a driftnet trap in northern Australia to capture exuviae.

The origin of the pupal exuviae method can be traced back to the early 1900's. Numerious authors (Ferrington et al. 1991, Wilson and Wilson 1984, Raunio and Muotka 2005) have attributed the initial idea of using surface-floating pupal exuviae to study chironomid communities to Thienemann (1910). August Thienemann, influenced Humphries (1938) to conduct a novel investigation of the emergence period, and relative density of the chironomid community by collecting pupal exuviae in a eutrophic lake.

In the 1960's pupal exuviae were employed in Afrotropic, Australasia, and Neotropic streams (Brundin 1966) and Lake Constance (Reiss 1968). In 1973 chironomid pupal exuviae were investigated as water quality indicators in streams for the first time (Wilson and Bright 1973). Since then numerous publications have endorsed the pupal exuviae method to assess the ecological integrity of lotic ecosystems (Wilson 1977, Wilson and McGill 1977, McGill et al. 1979, Wilson and Wilson 1983, Chutter 1984, Coler 1984, Ruse and Wilson 1984, Ferrington 1987, Wilson 1987, Ferrington and Crisp 1989, Ferrington et al. 1991, Wilson 1992, Wilson 1994, Barton et al. 1995, Fend and Carter 1995, Ruse and Wilson 1995, Coffman and Ferrington 1996, Wright et al. 1996, Cranston et al. 1997, Ruse and Davison 2000, Raunio and Muotka 2005, Raunio et al. 2007).

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Chapter 7

Sampling Efficiency of Chironomidae (Diptera) Across Disturbance Gradients

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Abstract

We compared detection efficiency and number of exclusively collected genera for surface-floating pupal exuviae and dipnet methods across two disturbance gradients. The most efficient method was collecting exuviae monthly. When compared for June only, the dipnet method was most effective across all sites, but at disturbed sites there were no statistically significant differences between methods. The exuviae method exclusively collected twice as many genera as the dipnet method.

Introduction

Members of the family Chironomidae are usually the most abundant insects in lotic and lentic habitats (COFFMAN, 1973; PINDER, 1986). Given their prominence in aquatic ecosystems, sampling methods must efficiently detect the extant chironomid community. Chironomid sampling methods can be generally classified as larval or pupal exuviae depending upon the life stage that is targeted. The dipnet method is used to collect larvae. Various pan and sieve methods are used to capture the surface-floating pupal exuviae (SFPE) left behind after eclosion of the adult. In this paper, these two sampling approaches are classified as larval and SFPE methods, respectively.

Relatively few investigators have used both larval and SFPE methods in a single study. Exceptions include CHUTTER (1984), RUSE & WILSON (1984), FERRINGTON et al.

(1991), BARTON et al. (1995), and RUSE (1995a,b) in lotic habitats, and KETELAARS et al. (1992) and KUIJPERS et al. (1992) in lentic habitats. Although BARTON et al. (1995) used both larval and SFPE methods, only FERRINGTON et al. (1991) examined the collection efficiencies of the methods when used concurrently in lotic systems. However, other studies report sufficient data to qualitatively compare the collection of efficiencies of both methods (e.g. KUIJPERS et al., 1992; BARTON et al., 1995; RUSE, 1995b).

The aim of this paper is to compare the collection efficiencies of SFPE versus dipnet methods across two disturbance gradients consisting of (1) all sites and (2) a subset of the four most disturbed sites in a ditched stream. Genera collected exclusively with the SFPE method (ESFPE) and dipnet method (EDN) are also evaluated. A comparison of larval and SFPE sampling methods, with regards to efficiencies and collection of exclusive taxa, is needed because no study has focused on the efficiencies of both collection methods across disturbance gradients, particularly within a ditched stream in the United States. BARTON et al. (1995) compared the methods in a ditched stream in Canada, but the authors did not specify if a disturbance gradient was present among sites sampled.

A comparison of methods has implications for biological monitoring. When designing a monitoring project in disturbed streams it is critically important to understand the sampling efficiencies of collection methods. It is also important to determine which taxa are exclusively collected by each method, so the potential limitations of each method can be considered.

In this paper we test eight hypotheses for detection efficiencies of the two methods across two disturbance gradients arising from dredging. The hypotheses were tested for SFPE samples taken approximately monthly versus dipnet samples taken only in June, across all sites and across the four most disturbed sites. The ESFPE and EDN taxa were also evaluated.

Materials and Methods

Eight sample sites were investigated in Hardwood Creek, located in a rapidly urbanizing watershed 34 kilometres northeast of Minneapolis and St. Paul, Minnesota, USA. Hardwood Creek flows from Rice Lake into Peltier Lake (Fig. 1) and drains a catchment of 71 square kilometres within the Rice Creek watershed. The four upstream sites (1.5U, 1.5D, 1.4 and 1.3) were most disturbed due to dredging. Sites 1.5U and 1.4 were dredged in winter of 2004, while 1.5D and 1.3 were last dredged in the 1970's. The four downstream sites (1, 1.1, 1.2 and 2) were less disturbed due to dredging. Sites 1 and 1.1 were dredged in the 1950's and 1960's, while there is no record of ditching at 1.2 and 2. The substrate of the disturbed upstream sites largely consisted of peat while that of the downstream sites was primarily sand. The riparian vegetation at the upstream sites was composed predominantly of tall grasses while riparian zones of downstream sites contained deciduous trees.

In June 2004, benthic samples were taken using a d-framed dipnet with a mesh of 500 μm. Bank, bottom, wood, and riffle habitats were sampled independently, when present (Table 1). At each habitat three consecutive samples were taken except at sites 1, 2, and 1.2 where bottom, bank, and bottom habitats were sampled five, two, and four times respectively. A sample from bank, bottom, and wood habitats consisted of one to three

jabs and/or a half-meter sweep. In riffles a sample consisted of five boot kicks upstream of the net. From April through November 2004, SFPE were sampled approximately monthly following the protocol of FERRINGTON et al. (1991).

Dipnet samples larger than approximately 90% of a 500 ml sample bottle were subsampled. For each subsample, half of the sample was randomly selected, picked, and preserved. Chironomidae larvae were mounted in Euparal® under a dissecting microscope and identified under a compound microscope. SFPE samples were not subsampled. Exuviae were identified to lowest practical level with a dissecting microscope.

Eight paired t-tests were performed using JMP IN 5.1.2 software (SAS Institute) to compare collection efficiencies of both methods. Percent of the chironomid community and number of chironomid genera were used as metrics to evaluate collection efficiencies. Both metrics were calculated on a site-to-site basis, and were analyzed across all sites and the four most disturbed sites for larvae and exuviae collected in June, and exuviae collected approximately monthly. Identifications of larvae and exuviae were initially made to the lowest possible taxon or species group, but were re-classified to genus to provide consistency for data analysis. The ESFPE and EDN genera were summed across all sites and collection dates.

Results

Hypotheses one, three, five, and seven confirmed the SFPE method was significantly more effective detecting genera when applied approximately monthly, across both disturbance gradients (Table 2). Seventy-one genera and 129 species were detected as

pupal exuviae across all sample sites. Twenty genera were collected ESFPE (Table 3). Of these, only Eukiefferiella sp. and Orthocladius sp. were collected in large numbers, with 545 and 171 specimens collected, respectively. ESFPE genera collected only in June were Demeijeria sp., Gillotia/Parachironomus sp., and Guttipelopia sp.

Hypotheses two and four confirmed the dipnet method was significantly more effective for detecting genera in June across all sites (Table 2). For hypotheses six and eight, there was no statistical difference between either method during June at the four most disturbed sites (Table 2). Fifty-one genera were detected as larvae across all sample sites. Ten genera were collected EDN (Table 3). Of these only Paralauterborniella sp. was collected in large numbers, with 39 specimens collected. There were no genera collected EDN in June.

Discussion

The purpose of this study was to compare the detection efficiencies of the SFPE and dipnet methods, across two disturbance gradients consisting of all sites and a subset of the four most disturbed, and to determine genera collected ESFPE and EDN. The SFPE method, when applied approximately monthly across both disturbance gradients, was significantly more efficient detecting genera than the dipnet method applied only in June (Table 2). We would expect samples taken across a greater temporal scale would be more efficient at detecting different species, especially among chironomids, given their diverse phenologies. Conversely, finding that the dipnet method was significantly more efficient, for both metrics at all sites (Table 2), when only June samples of SFPE were compared is not surprising. Although SFPE detected 71 genera, the mean number of genera detected per sample date ranged from 12.5 to 30.9, which clearly demonstrates

that not every genus emerged concurrently. As a result, dipnet samples for developing larvae could be expected to yield more chironomid genera than would be detected on a single collection date for SFPE.

At the four most disturbed sites in June there was not a significant difference between methods (Table 2). This result was likely due to low power of the test related to only three degrees of freedom in the statistical analysis. This explanation was supported by the June comparisons across all sites, which had 7 degrees of freedom and confirmed statistically significant differences.

The SFPE method collected twenty genera exclusively, whereas the dipnet methods collected ten general exclusively (Table 3) when summed for all sites and sample dates. BARTON et al. (1995) also found that approximately twice as many taxa were exclusively collected by their exuvial method when compared to the dipnet method. More taxa were probably detected by BARTON et al. (1995) in exuviae samples because species level identifications could be achieved, as also occurred in this study. However, BARTON et al. (1995) found exuviae at only 35% of their sites, so the number of genera found as exuviae would be expected to increase with more extensive sampling. The number of exclusively collected taxa can be calculated from RUSE (1995b), where it appears that approximately four times as many unique taxa were collected by the exuviae method. However, RUSE (1995b) also noted that many of the larvae could not be identified to species.

Only Limnophyes sp. was collected exclusively using the exuviae method in both this study and by RUSE (1995b), (Table 3). The genera Chaetocladius sp., Larsia sp., and

Zavrelimyia sp. were exclusively collected via a larval method by RUSE (1995b), but we collected these genera ESFPE (Table 3). The genera collected exclusively likely reflect similarities and differences of sampling methods and/or phenologies of the species within the genera preferentially collected. No other studies using both larval and exuviae methods concurrently list exclusively collected taxa, so further comparisons cannot be made.

This study supports the conclusion of KUIJPERS et al. (1992), that exuviae and benthic methods supplement each other. This is particularly important if a comprehensive assessment of the biodiversity of the chironomid fauna is an objective of the research.

Conclusions

The best single strategy for detecting species of chironomids was collecting SFPE approximately monthly, verses collecting dipnet samples in June, as may often be done in monitoring programs. This conclusion is applicable across a gradient containing non-ditched to recently-ditched sites, and across a gradient of intensively disturbed sites containing recently and less-recently ditched sites in Hardwood Creek. However, if June is the only month sampling can occur it is better to use the dipnet method in Hardwood Creek. Across highly disturbed sites in June there was no significant difference between methods, so use of either method would be appropriate. Using the SFPE method approximately monthly in Hardwood Creek will collect twice as many exclusive genera as June dipnet samples. Consequently, both SFPE and dipnet methods should be used if a comprehensive assessment of the biodiversity of the chironomid fauna is needed.

Acknowledgements

We thank the Rice Creek Watershed District for their assistance with this project. We also thank other members of the Chironomidae Research Group at the University of Minnesota, Bruce Vondracek, and Raymond Newman for their valuable critiques of this paper. We gratefully acknowledge a Dayton-Wilkie Fellowship granted to the primary author by the Bell Museum of Natural History at the University of Minnesota.

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Table 1. Conditions at sample sites. Yes indicates the habitat was present and sampled with dipnet in June 2004. No indicates the habitat was not present.

Sample Site	History of Ditching	Habitats Present			
		Bank	Bottom	Wood	Riffle
1.5 U*	Winter 2004	YES	YES	no	no
1.5 D*	1970's	YES	YES	YES	no
1.4*	Winter 2004	YES	YES	no	no
1.3*	1970's	YES	YES	YES	no
1	1950/1960's	YES	YES	YES	no
1.1	1950/1960's	YES	YES	YES	no
1.2	Never	YES	YES	YES	YES
2	Never	YES	YES	YES	no

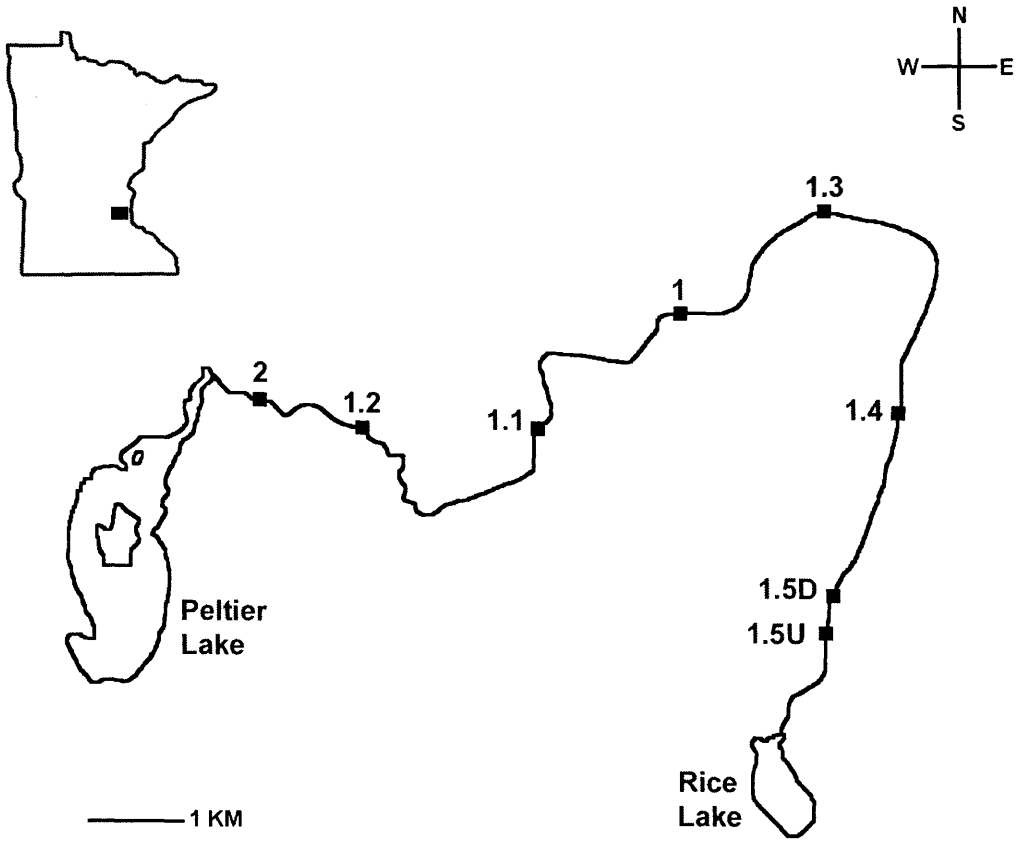
Table 2. The hypotheses (H), metrics tested, site comparisons, collection dates, means, and p-values used to evaluate eight hypotheses of SFPE and dipnet method detection efficiencies.

H	Metric Tested	Sites Included	SFPE Collections	DN Collections	SFPE Mean	DN Mean	P-value
1	Percent of Community	All	Aproximately Monthly	June	82.3	61.8	0.0125
2	Percent of Community	All	June	June	48.0	83.8	0.0060
3	Number of Genera	All	Approximately Monthly	June	30.9	23.9	0.0068
4	Number of Genera	All	June	June	12.8	23.9	0.0070
5	Percent of Community	Four Most Disturbed	Approximately Monthly	June	85.9	55.6	0.0376
6	Percent of Community	Four Most Disturbed	June	June	56.7	74.5	0.2658
7	Number of Genera	Four Most Disturbed	Approximately Monthly	June	26.5	17.3	0.0390
8	Number of Genera	Four Most Disturbed	June	June	12.5	17.3	0.2845

Table 3. The number of specimens collected exclusively with the surface-floating pupal exuviae method (ESFPE) and exclusively with the dipnet method (EDN). An asterisk (*) indicates taxa only collected in June.

Genera	ESFPE	EDN
Orthocladiinae		
Chaetocladius sp.	3	---
Diplocladius sp.	1	---
Eukiefferiella sp.	545	---
Hydrobaenus sp.	1	---
Limnophyes sp.	21	---
Orthocladius sp.	171	---
Paraphaenocladius sp.	6	---
Pseudosmittia sp.	8	---
Stilocladius sp.	---	1
Chironomini		
Demeijeria sp. *	1*	---
Einfeldia sp.	---	2
Endotribelos sp.	---	2
Harnischia sp.	13	---
Microchironomus sp.	---	18
Microtendipes sp.	3	---
Paralauterborniella sp.	---	39
Stictochironomus sp.	2	---
Gillotia/Parachironomus sp. *	6*	---
Tanypodinae		
Clinotanypus sp.	---	1
Conchapelopia sp.	28	---
Guttipelopia sp. *	2*	---
Labrundinia sp.	25	---
Larsia sp.	13	---
Monopelopia sp.	---	1
Natarsia sp.	3	---
Paramerina sp.	7	---
Pentaneura sp.	---	3
Thienemannimyia gr.	---	13
Zavrelimyia sp.	3	---
Prodiamesinae		
Odontomesa sp.	---	2
Total by Collection Method	20	10

Figure 1. Locations of sample sites on Hardwood Creek in Minnesota, USA.



Appendix A

Keys and Descriptions of the Chironomidae Pupal Exuviae Collected in Hardwood Creek

To be submitted: as a PDF and accessible online via the Chironmid Research Group web page: http://www.entomology.umn.edu/midge/

Introduction

The following keys were produced as a guide for future research, and to document the diversity of the chironomid fauna in Hardwood Creek and the state of Minnesota.The methods used to obtain the chironomid pupal exuviae featured in this key are described in previous chapters of this thesis. Many references were used to produce this document, however Wiederholm (1986) and the morphospecies descriptions of R. W. Bouchard Jr. were particurally influential and deserve special recognition here. The majority of the morphospecies described here were synonymized with those created by R. W. Bochard Jr. for his Ph.D. dissertation research. It is possible that several morphospecies were amended by Mr. Bouchard after the production of this document, however the majority of our morphospecies are identical.

The following keys and descriptions are arranged by subfamily and tribe. When using this document the reader should first key out each specimen using the subfamily and tribe key, and then proceed to the appropriate key were a genus level identification can be obtained. Once a genus is known, the reader can proceed to the taxa descriptions section at the end of each subfamily and tribe section to determine the species or morphospecies of each specimen. To increase your success while using the keys refer to the morphological characters and terms in figures 1-4. All of the drawings were produced by the author using the Microsoft® Paint program. The photographs taken by the author were all taken with a Hewlett-Packard 735 HP Photosmart digital camera. Photos were downloaded and modified in HP Photo and Imaging Gallery version 1.1©.

The 17 photographs not produced by the author were taken by Moriya Rufer and are listed in the acknowledgements section at the end of this document.

Morphological Characters and Terms Used:

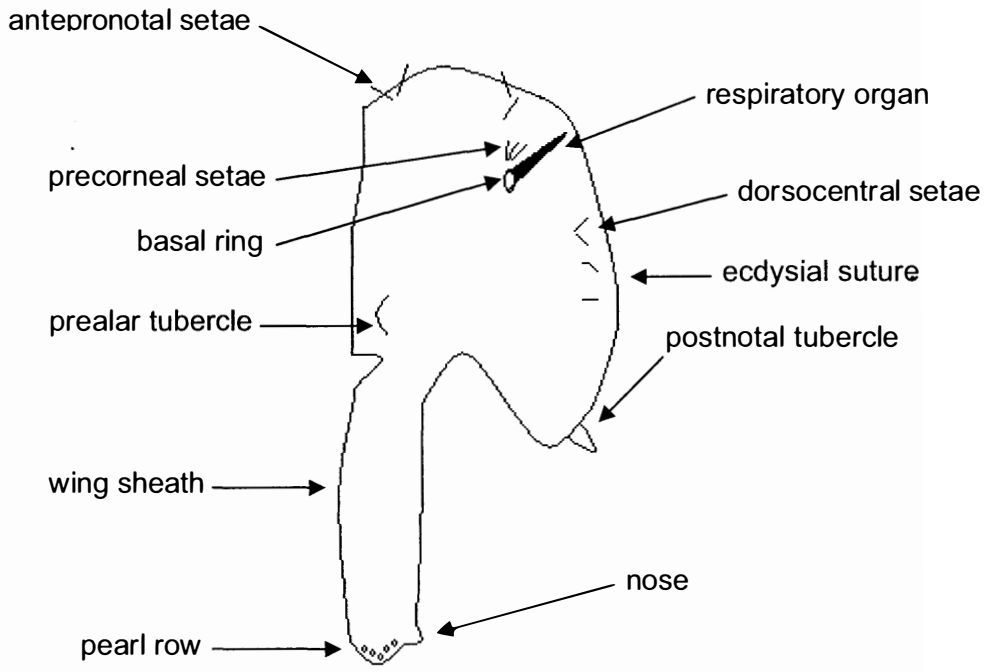


Figure 1. A generalized cephalothorax of a chironomid pupa and its associated characters.

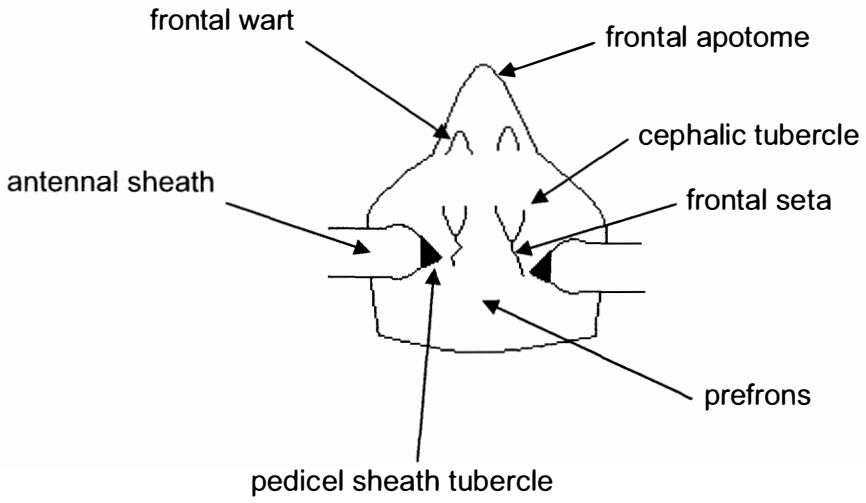


Figure 2. A generalized frontal apotome of a chironomid pupa and its associated characters.

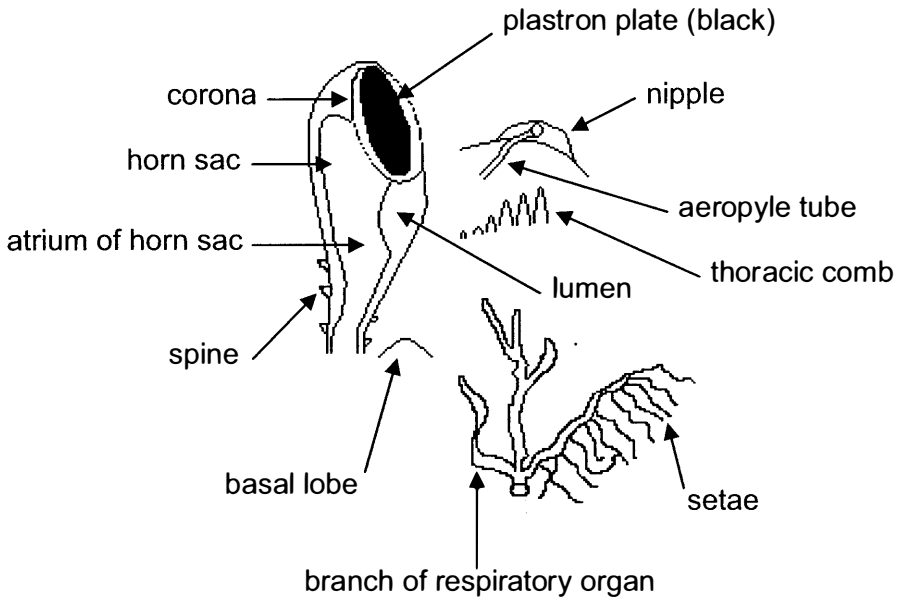


Figure 3. Generalized respiratory organs of chironomid pupae and their associated characters.

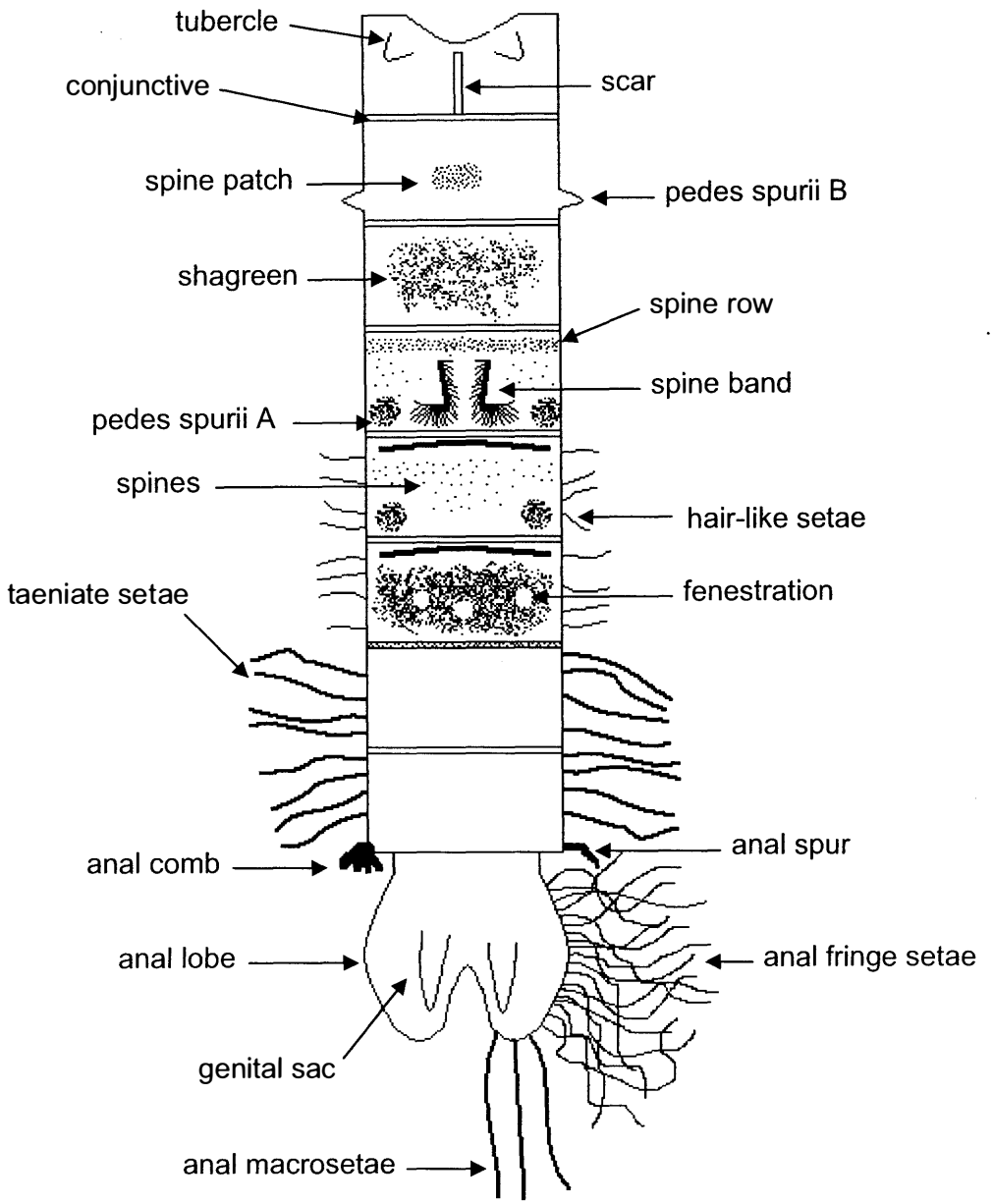


Figure 4. A generalized abdomen of a chironomid pupa and its associated characters.

Key to the Subfamilies or Tribes of Chironomidae Pupae Collected in Hardwood Creek:

1. Respiratory organ large, well developed, and not branched. Tips of anal lobes are rounded or pointed with 2 distinct macrosetae projecting from the lateral edge of each lobe (Figs. 158-159, 162-163, 165-168, 170, 172-174). ---------- **Tanypodinae (p.)**
1. Respiratory organ less well developed or absent. If present, organ with no to many branches. Anal lobe with 3 distinct macrosetae at or near the tip and/or with lateral edge of each lobe containing a fringe of several to many setae (Figs. 5-6, 15-18, 21, 25, 27, 31-32, 63-64, 70-73, 76, 79, 85, 120-121, 123, 129). ---------- **2**

2. Anal lobe with 3 distinct macrosetae, however, these can be absent. Respiratory organ is often absent, however, when present the organ will not be branched. No spines or a single spur present on posterolateral corner of segment VIII (Figs. 5-6, 15-21, 25, 27, 31-32). ---------- **Orthocladiinae (p.)**
2. Anal lobe without macrosetae and usually containing a fringe of several to many setae. Respiratory organ present and with or without branches. Spines or a single spur present on posterolateral corner of segment VIII (Figs. 63-64, 70-73, 76, 79, 85-86, 120-121, 123, 129). ---------- **3**

3. Respiratory organ without branches and usually on top of a small rounded base. Tergites III-V often with spine or point patches, paired or unpaired (tergites II and VI often containing these patches too). Wing sheaths with pearl row and/or nose (Figs. 124-130). ---------- **Tanytarsini (p.)**
3. Respiratory organ branched and often containing many branches, organ not on top of a small rounded base. Wing sheaths without pearl row, nose very rare. Sternite I with or without paired tubercles containing spines (Figs. 71-73, 75, 79, 119-120). ---------- **4**

4. Sternite I with paired tubercles containing spines. Respiratory organ with 2 rounded branches. Frontal setae and cephalic tuberacles absent (Figs. 119-120). ---------- **Pseudochironomini (p.)**
4. Sternite I usually without paired tubercles containing spines. Respiratory organ often with many thin and pointed branches. Frontal setae and/or cephalic tuberacles usually present (Figs. 69, 71-73, 79). ---------- **Chironomini (p.)**

<u>Key to the Genera of Orthocladiinae Pupae Collected in Hardwood Creek</u>:

1. Anal lobes with a distinct fringe of setae (Fig. 6). ---------- **2**
1. Anal lobes without a fringe of setae (Fig. 5). ---------- **12**

2. No respiratory organ. ---------- **3**
2. Respiratory organ present (Figs. 15, 17-18, 27). ---------- **4**

3. Small spines prevalent on abdominal segment conjunctives. No pearl rows on wing sheaths (Fig. 7). ---------- ***Thienemanniella* sp. (p.)**
3. Small spines not prevalent or absent on conjunctives. Pearl rows present on wing sheaths (Figs. 8-9). ---------- ***Corynoneura* sp. (p.)**

4. Segment VIII with wide taeniate lateral setae; pedes spurii B present or absent on segment II, if present, not tapering to a point (Fig. 20). ---------- **5**
4. Segment VIII with hair-like lateral setae, if taeniate then setae are not very wide; pedes spurii B on segment II tapering to a point (Fig. 19). -- ***Parametriocnemus* sp. (p.)**

5. Each anal lobe with long setae (Figs. 6, 16). ---------- **6**
5. Each anal lobe with short setae (Fig. 32). ---------- **22**

6. Tergite IV without distinct arrangement of spines in rows or patches. Segment II without pedes spurii B (Fig. 11). ---------- ***Nanocladius* sp. (in part) (p.)**
6. Tergite IV with distinct arrangement of spines in rows or patches. Segment II with or without pedes spurii B (Fig. 12). ---------- **7**

7. Tergite IV possesses the following characters: at least 1 posterior spine row and 1 or more distinct medial spine patch(es) or row(s) are both present (Fig. 12). ---------- **8**
7. Tergite IV with posterior spine row(s), possibly with medial shagreen pattern, but lacking distinct medial spine patch(es) or row(s) (Fig. 11). ---------- **11**

8. Two precorneal setae dominant and distinctly larger than the third. All setae are on 1 tuberacle, however, the 2 dominant setae can be found on different tuberacles (Fig. 13). ---------- **9**
8. If precorneals unequal in size then: 2 dominant setae are not present and setae not on different tuberacles, however, precorneals can all be equal in size (Fig. 14). -------- **10**

9. Respiratory organ digitform, well developed, and possessing many spines. Anal lobe possessing long and robust macrosetae, which are distinctive from the other, more numerous setae on the lobes' margins (Figs.15-16). ---------- ***Doncricotopus* sp. (p.)**
9. Respiratory organ slender with pointed tip or organ is short with base and/or middle one-half of organ being very broad. Anal lobe possessing weak and thin macroseate, which are difficult to distinguish from the other, more numerous setae on the lobes' margins (Figs. 6, 17-18). ---------- ***Nanocladius* sp. (in part) (p.)**

10. One medial spine patch often present on tergites IV-VI, a patch may also be present on tergite VII (Fig. 12). ---------- ***Psectrocladius* sp. (in part) (p.)**
10. One pair of spine patches on tergites IV-VI and sometimes on III (Fig. 12). ---------- ***Psectrocladius* sp. (in part) (p.)**

11. Anal lobe possessing weak and long macroseate, two precorneal setae dominant and distinctly larger than the third. All setae are on 1 tubercle, however, the 2 dominant setae can be found on different tubercles (Figs. 6, 13). --- ***Nanocladius* sp. (in part) (p.)**
11. Anal lobe possessing robust and long macrosetae, precorneal setae can be equal or unequal in size. However, if 2 of 3 setae are large they are not present on tuberacles or the setae are not too much larger than the other (Figs. 14, 16, 20). ---------- ***Rheocricotopus* sp. (in part) (p.)**

12. When excluding any medial setae that may be present on anal lobes, there are a total of 3 marginal or apical macrosetae on each lobe. Some setae may resemble spines and should not be counted as macrosetae (Figs. 5, 21). ---------- **13**
12. When excluding any medial setae that may be present on anal lobes, the number of macrosete present on each lobe's margin or apex is equal to any number but 3. Some setae may resemble spines and should not be counted as macrosetae (Fig. 31). ------ **21**

13. Respiratory organ present, frontal setae present or absent (Figs. 15, 17-18, 27). --**14**
13. No respiratory organ, frontal setae present (Fig. 22). --------- ***Parakiefferiella* sp. (p.)**

14. Pearl rows on wing sheaths; frontal apotome containing frontal setae. Anal lobes with strong and robust median (1) and macro (3) setae (Figs. 9, 21-22). - ***Tvenia* sp. (p.)**
14. No pearl rows on wing sheaths; frontal setae present or absent on frontal apotome. Median setae absent from anal lobes (Figs. 5-6, 16, 25, 31-32). ---------- **15**

15. At least tergites III-IV and sometimes tergite V with 1 hook row, containing several to many well-developed recurved hooks, located on segement conjunctives or the posterior edge of segments (Fig. 23). ---------- ***Eukiefferiella* sp. (p.)**

15. Tergites III-IV or III-V often without 1 hook row, containing several to many well-developed recurved hooks, located on conjunctives or the posterior edge of segments. However, if hooks are present they are arranged in more than 1 row, or hooks are weak and not well developed (Figs. 24, 26, 28-30). ---------- **16**

16. All tergites except I with rows of conspicuous and long spines on posterior margins of tergites; macrosetae on anal lobe not resembling spines (Fig. 24). ---------- ***Limnophyes* sp. (p.)**
16. All tergites except I without rows of conspicuous and long spines on posterior margins of tergites; if spines are present in rows, these spines are short (Figs. 10-12, 23, 26, 28, 33). ---------- **17**

17. Anal lobe very unique, macrosetae inserted apically into an elongated dorsal flap protruding from each lobe. Flaps attached to the medial part of each lobe (Fig. 25). ---------- ***Diplocladius* sp. (p.)**
17. Anal lobe without flaps attached to the medial part of each lobe (Figs. 5-6, 21, 31-32). ---------- **18**

18. Tergite II without hooks on posterior edge, however, small spines that point anteriorly are often present. Anal lobe macroseate or spines not greater than half the length of the organ, with 1 setae or spine locate on the margin of each lobe (Fig. 26). ---------- ***Chaetocladius* sp. (p.)**
18. Hooks present on posterior edge of tergite II (Figs. 29-30). ---------- **19**

19. Respiratory organ relatively long. Tergites with a pair medial spine patches present at least on tergites III-VI (Figs. 27-28). ---------- ***Acricotopus* sp. (p.)**
19. Respiratory organ variable. No paired medial spine patches on tergites (Figs. 11-12, 15, 17-18, 23-24, 26, 33). ---------- **20**

20. Tergite II with 2 posterior hook rows (Fig. 29). ---------- ***Cricotopus* sp. (p.)**
20. Tergite II with 3 or more posterior hook rows, with hook rows being more randomly arranged (Fig. 30). ---------- ***Orthocladius* sp. (p.)**

21. Respiratory organ present and relatively conspicuous; in male exuviae the genital sacs are approximately twice as long as the anal lobe (Fig. 31). ---------- ***Paraphaenocladius* sp. (p.)**
21. No respiratory organ present or if present clear and poorly developed; genital sacs variable in male exuviae (Figs. 6, 16, 21). ---------- **23**

22. Anal lobe with marginal fringe located primarly in the anterior half of the lobe, fringe primarly composed of short setae that are not densely arranged. ---------- ***Hydrobaenus* sp. (in part) (p.)**
22. Anal lobe with fringe throughout the length of the lobes' margins, fringe composed of long or short setae that are densely arranged (Fig. 32). - ***Hydrobaenus* sp. (in part) (p.)**

23. Anal lobe unique and reduced in size, composed of 2 structures that are apically pointed and contain spines (Fig. 58). ---------- ***Rheosmittia* sp. (p.)**
23. Anal lobe not reduced in size (Figs. 5-6, 16, 31-32). ---------- **24**

24. At least 1 segment conjunctive with conspicuous spinule bands conmposed of small spinules (Fig. 33). ---------- ***Pseudosmittia* sp. (in part) (p.)**
24. No segment conjuncitve with conspicuous spinule bands, however, if these are present it is obvoious that these spinules are a part of the shagreen spinules present on each tergite. ---------- **25**

25. There are 0 or not greater than 1 antepronotal seta on the cephalothorax (Fig. 34). -- ---------- ***Smittia* sp. (p.)**
25. There are 2 or more antepronotal setae present (Fig. 35). ---------- ---------- ***Pseudosmittia* sp. (in part) (p.)**

<u>Orthocladiinae Figures</u>:

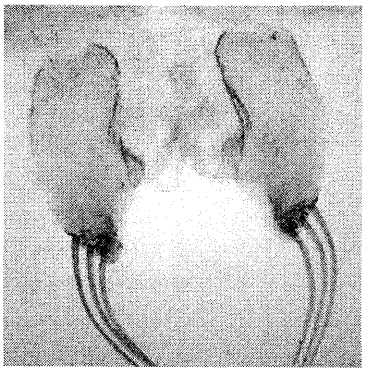


Figure 5. Anal lobe of Orothocladiinae.

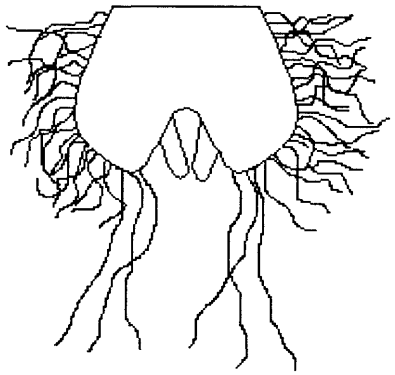


Figure 6. Anal lobe of *Nanocladius* sp.

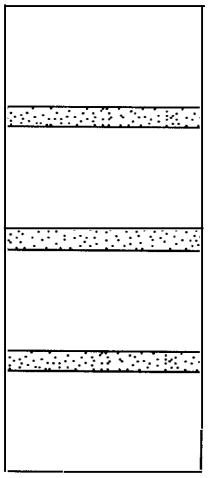


Figure 7. Conjunctives of *Thienemanniella* sp.

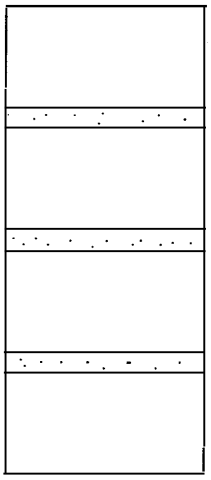


Figure 8. Conjunctives of *Corynoneura* sp.

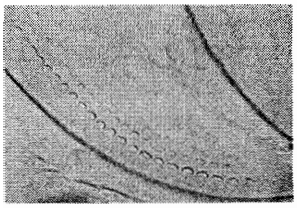


Figure 9. Pearl rows of *Corynoneura* sp.

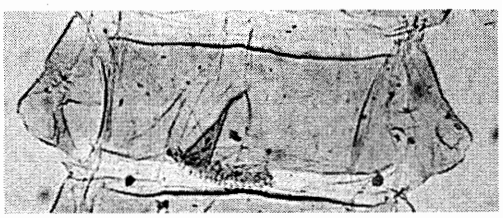


Figure 10. Segment II with pedes spurii B of *Nanocladius rectinervis*.

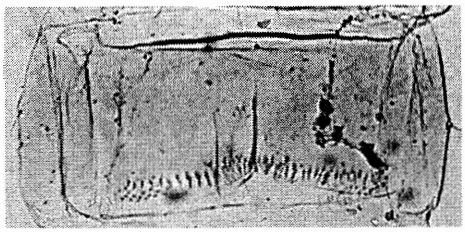


Figure 11. Tergite IV of *Nanocladius rectinervis*.

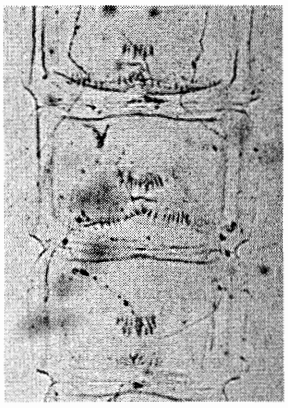


Figure 12. Tergites IV-VI of *Psectrocladius schlienzi* type.

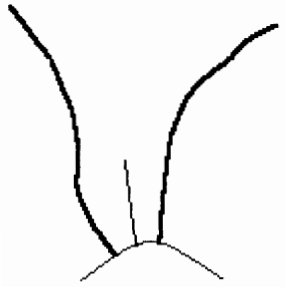


Figure 13. Precorneal setae of *Nanocladius* sp.

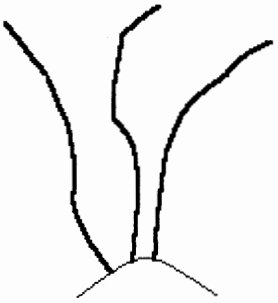


Figure 14. Precorneal setae of some Orthocladiinae.

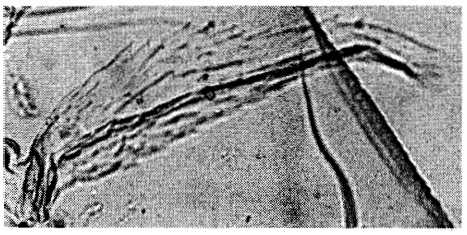


Figure 15. Respiratory organ of *Doncricotopus bicaudatus*.

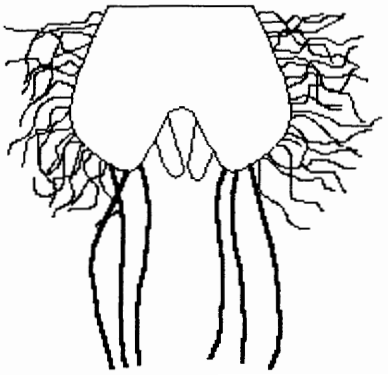


Figure 16. Anal lobe of *Doncricotopus* sp.

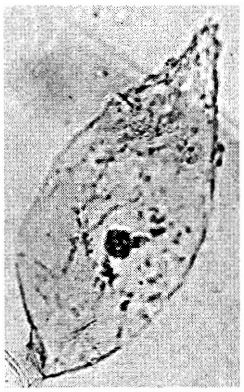


Figure 17. Respiratory organ of *Nanocladius crassicornus*.

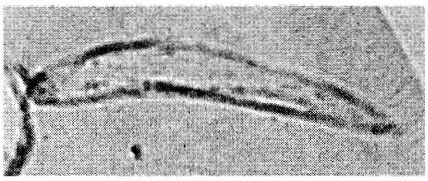


Figure 18. Respiratory organ of *Nanocladius rectinervis*.

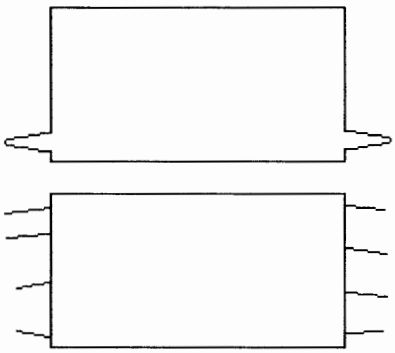


Figure 19. Segment II (top) and VIII (bottom) of *Parametriocnemus* sp.

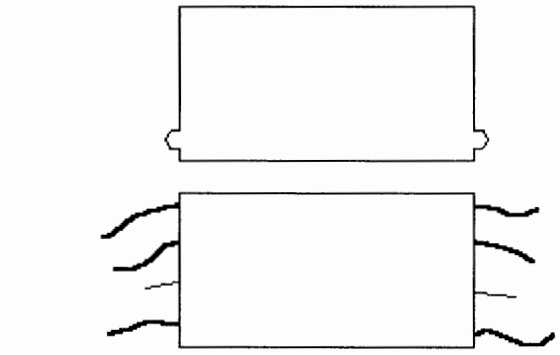


Figure 20. Segment II (top) and VIII (bottom) of *Rheocricotopus* sp.

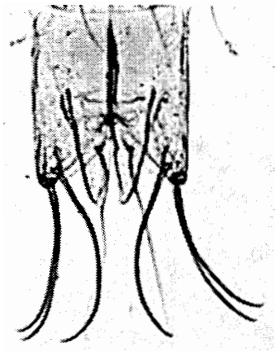


Figure 21. Anal lobe of *Tvetenia* sp. 1.

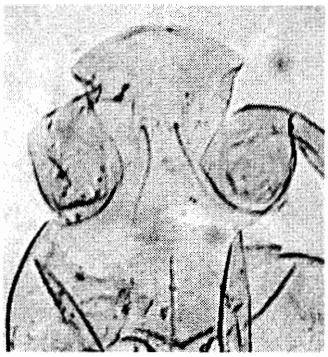


Figure 22. Frontal setae of *Tvetenia* sp. 1.

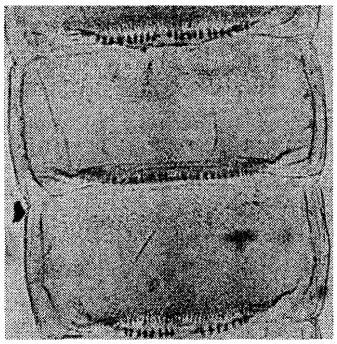


Figure 23. Hook rows on tergites III-V of *Eukiefferiella* sp. 2.

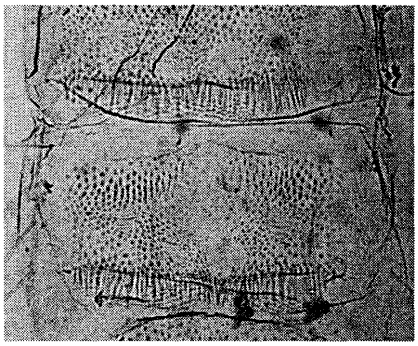


Figure 24. Tergites III-IV of *Limnophyes* sp. 3.

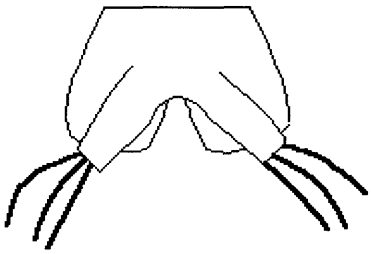


Figure 25. Anal lobe of *Diplocladius* sp.

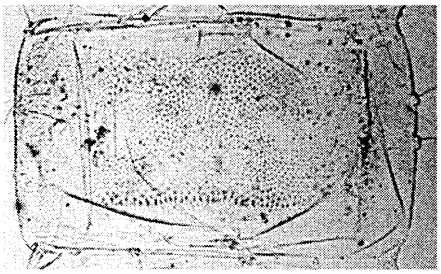


Figure 26. Tergite II of *Chaetocladius* sp.

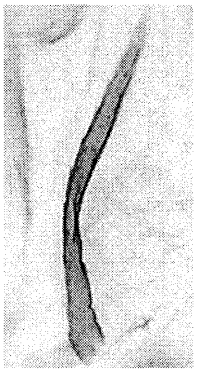


Figure 27. Respiratory organ of *Acricotopus nitidellus*.

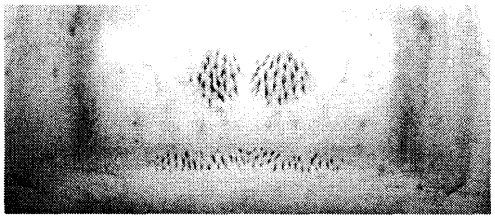


Figure 28. Tergite IV of *Acricotopus nitidellus*.

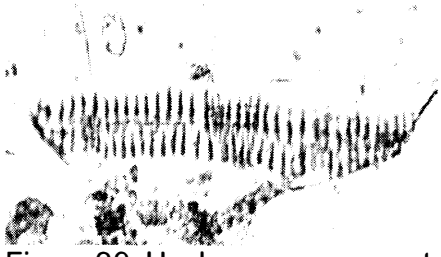


Figure 29. Hook row on segment II of *Cricotopus* sp. 5.

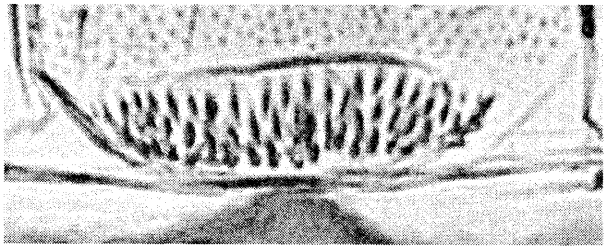


Figure 30. Hook row on segment II of *Orthocladius oliveri*.

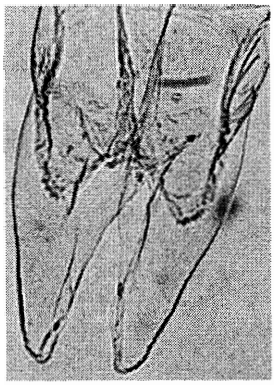


Figure 31. Anal lobe of *Paraphaenocladius exagitans*.

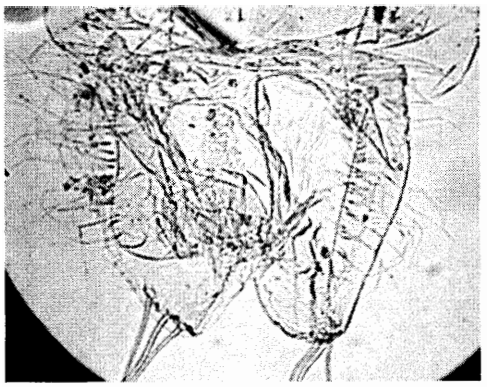


Figure 32. Anal lobe of *Hydrobaenus* sp.

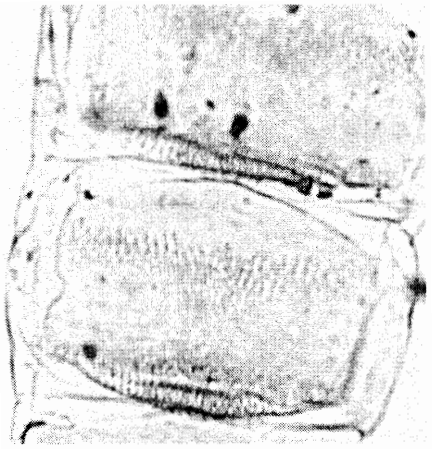


Figure 33. Conjunctives V/VI-VI/VII of *Pseudosmittia* sp. 2.

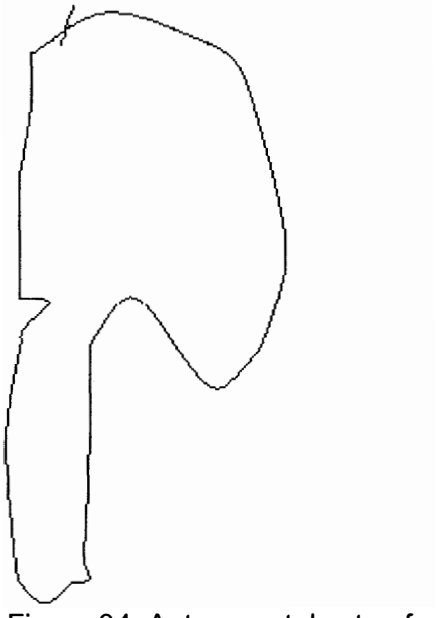


Figure 34. Antepronotal seta of *Smittia* sp.

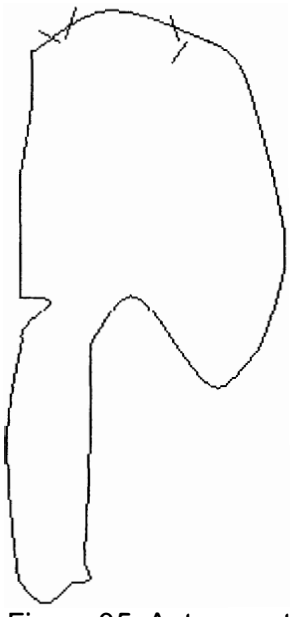


Figure 35. Antepronotal setae of *Pseudosmittia* sp.

Orthocladiinae Taxa Descriptions:

Acricotopus nitidellus
Reference: Oliver et al. 1990
Description: Respiratory organ well developed, thin, and tapered apically with spines particuraly prevelant in the apical 1/3 of the organ. Pedicle sheath tuberacle well developed, frontal setae present on small cephalic tubercles, pedes spurii B on segment II. Tergites II-VI with 1 pair of conspicuous, round, spine patches which become more developed from anterior to posterior tergites. Conjuctives III/IV-V/VI with 3-4 rows of well-developed spines; posterior spine rows present on tergites III-VI with II possessing weak spins in rows (Figs. 27-28).
Notes: Only 1 *Acricotopus* species is known from the lower 48 states of the United States.

Chaetocladius sp.
Reference: Wiederholm 1986
Description: Respiratory organ 4 or more times longer than wide, with its distal most end pointed and usually possessing spines throughout most of it length. Anal lobe macroseta very unique in that they are somewhat tapered from base to apex, smaller than the anal lobe, and usually bent and resempling a thorn or spine but do not contain an apical hook (Figs. 26, 36).
Notes: No specimens of this genus were slide mounted, they were identified via a dissecting microscope only.

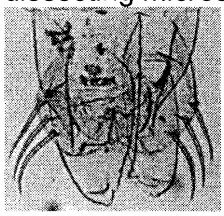


Figure 36. Anal lobe macrosetae of *Chaetocladius* sp.

Corynoneura sp. 1
Description: Recurved hooks present on tergites IV-VII, anal lobe with full fringe of setae, no long spines on sternite II.

Corynoneura sp. 2
Description: Recurved hooks present on tergites IV-VII, hooks often present on tergite III, anal lobe with full fringe of setae, sternite II with long spines (visible at 200X).

Cricotopus bicinctus
Reference: Simpson et al. 1983
Description: Tergites with both anterior and posterior shagreen patches widely spaced from each other. Pedes spurii B on segments II-III, respiratory organ present, the fourth lateral setae on segment VIII approximately equal to other setae. Frontal apotome containing frontal setae, anal lobe with macrosetae approximately the same length (Fig. 37).

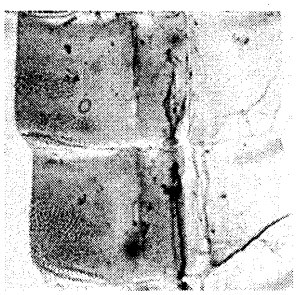


Figure 37. Tergites III-IV of *Cricotopus bicinctus*.

Cricotopus polaris
Reference: Simpson et al. 1983
Description: The same description as *C. tibialis* except dorocentral setae 1 is situated near 2 (Fig. 38).

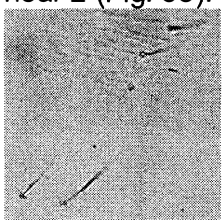


Figure 38. Dorocentral setae of *Cricotopus polaris*.

Cricotopus sylvestris
Reference: Simpson et al. 1983
Description: Pedes spurii B present on segment II, respiratory organ long and relatively colorless. Cephalothorax rough looking due to the presence of many bumps/wort-like projections (Fig. 39).

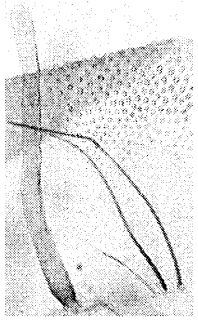


Figure 39. Respiratory organ, precorneal setae, and bumps/wort-like projections of *Cricotopus sylvestris*.

Cricotopus tibialis
Reference: Simpson et al. 1983
Description: Tergites with shagreen pattern separated into 2 discernable fields with some connection between anterior and posterior fields. Respiratory organ with spinnules, prefrons containing frontal setae. Dorocentral seta 2 is situated near 3 and 4, pedicle sheath tubercle absent or very poorly developed (Fig. 40).

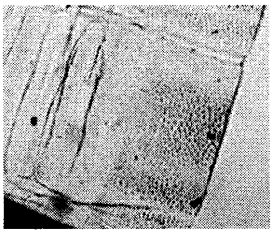


Figure 40. Tergite V of *Cricotopus tibialis*.

Cricotopus (Isocladius) sp. 1
Description: Frontal setae on frontal apotome when present. Respiratory organ, when present, extremely variable in form. Three anal lobe macrosetae approximately similar in length, shagreen on tergites not distinctly separated into anterior and posterior fields. Segment VIII with 4, hair-like, lateral setae (Fig. 41).

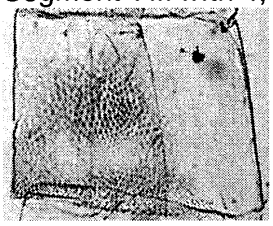


Figure 41. Tergite V of *Cricotopus* (Isocladius) sp. 1.

Cricotopus sp. 5
Description: Respiratory organ small and variable, pedes spurii B present on segments II-III. Exuviae is dark brown in color, frontal setae on prefrons or frontal apotome (Figs. 29, 42).
Notes: Keys to *Cricotopus triannulatus* in Simpson et al. (1983) but pedes spurii B is present on segments II-III, but, sometimes absent on segment II.

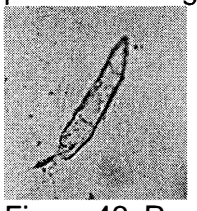


Figure 42. Respirtaory organ of *Cricotopus* sp. 5.

Diplocladius sp.
Reference: Wiederholm 1986
Description: Tergites II-VII containing shagreen, coverage of shagreen on each tergite decreases from anterior to posterior. Segment II with pedes spurii B; pedes spurii A on segments IV-VI. Anal lobe different from other Orthocladiinae, macrosetae inserted apically into an elongated dorsal flap protruding from each lobe. Flaps attached to the medial part of each lobe (Fig. 25).
Notes: Only 1 specimen slide mounted; at present R. W. Bouchard Jr. has not created morphospecies for this genus.

Doncricotopus bicaudatus
Reference: Sæther 1981
Description: Frontal setae well developed on small, finger-like, cephalic tubercles. Respiratory organ large, well developed, and covered with spines. Strong, spine-like,

dorocentral setae that are approximately equally spaced, large pedes spurii B on segment II. Conspicuous tuberacle on tergite II with hooklets, tergites III-VI with posterior spine patches or rows, tergites IV-VI with circular spine patches medially. Anteriorly pointing spines just posterior of posterior spine rows on tergites III-IV, segment VII with 4 and VIII with 5 lateral setae that are more hair like than taeniate (Figs. 15, 43).
Notes: The specimens collected fit pretty well with the description in Sæther (1981). However, there might be some discrepancies with the number of lateral setae, Sæther (1981) examined only 3 specimens for his description.

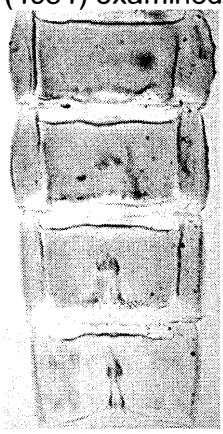


Figure 43. Tergites III-VI of *Doncricotopus bicaudatus*.

Eukiefferiella sp. 2
Description: Respiratory organ tip is longer than the base, anal lobe macrosetae are approximately equal in size, no recurved hooks on sternites VI-VII (Figs. 23, 44).
Notes: The only character that separates this species from *E. claripennis* is the respiratory organ tip is longer than the base. Keys to *E. fuldensis* in Lehmann (1972).

Figure 44. Respiratory organ of *Eukiefferiella* sp. 2.

Hydrobaenus sp.
Reference: Wiederholm 1986
Description: Each anal lobe with fringe of small setae, with the fringe being well or less developed. Segment II with conspicuous sedes spurii B, respiratory organ always with at least a few small spines (Fig. 32).
Notes: No specimens of this genus were slide mounted, they were identified via a dissecting microscope only.

Limnophes sp. 3
Description: Segment VIII with 4, hair-like, lateral setae that are not the same size, with 2 setae being larger and 2 being smaller in size. Dorocentral setae 2 and 3 with contacting scars, 4 is separated from other setae. Respiratory organ without much pigmentation, long, thin, and whip like. Tergites II-VIII with shagreen (Fig. 24).

Nanocladius crassicornus
Reference: Sæther 1977
Description: Pedes spurii B and conspicuous protuberance with hooklets well developed on segment II. Respiratory organ wide and short; less than three times as long as wide. Median spine patches on tergites IV-VII with patches on IV being reduced in size (Fig. 17).

Nanocladius distinctus
Reference: Sæther 1977
Description: Well-developed pedes spurii B on segement II. Respiratory organ tapering with distinct spines at the end of the organ, three times longer than wide; organ only a little bit wider 1/3 up from its base when compared to the base. Segment VI possessing 4, hair-like, setae; VIII with 5 taeniate lateral setae. Anal lobe macrosetae thick, each anal lobe with fringe of approximately 20-44 setae (Fig. 45).

Figure 45. Respiratory organ of *Nanocladius distinctus*.

Nanocladius incomptus
Reference: Sæther 1977
Description: Broad and short respiratory organ, no distinct median spine patches on any tergite. Segment VII with 4, hair-like, lateral setae; VIII has 4, taeniate, lateral setae (Fig. 46).

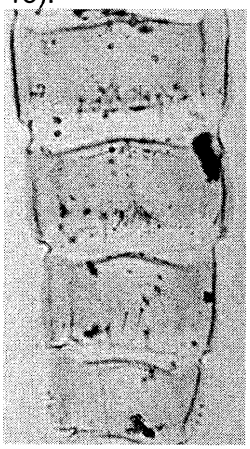


Figure 46. Tergites V-VIII of *Nanocladius incomptus*.

Nanocladius rectinervis
Reference: Sæther 1977
Description: Well-developed pedes spurii B on segement II. Respiratory organ not tapering much with blunt spines at tip, organ three times longer than wide. Segments VI-

VII both with at least 1, taeniate, lateral seta. Tergite VI with long, posterior spines. Anal lobe macrosetae are long and strong (Figs. 10-11, 18).

Nanocladius spiniplenus
Reference: Sæther 1977
Description: Well-developed pedes spurii B on segment II. Respiratory organ tapering much with spines at its end blunt, organ three times longer than wide. Segments VI-VII both with at least 1, taeniate, lateral seta. Thin and weak anal lobe macrosetae; conjunctive between IV/V with spine rows experiencing little or no interruption medially (Fig. 47).

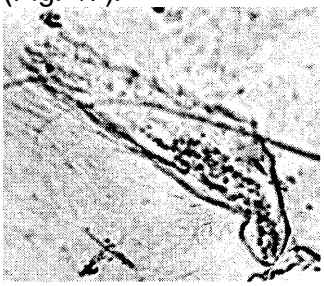


Figure 47. Respiratory organ of *Nanocladius spiniplenus*.

Orthocladius clarkei
Reference: Soponis 1977
Description: No pedes spurii B on segment III, frontal setae present. Spines in posterior patches of segment III and IV (which are pointed anteriorly) are approximately equal to the width of the recurved spines on II. No chitinous rings on tergites I-III. Anal lobe setae longer than 40% of the anal lobe's radius, spurs on anal lobes (Fig. 48).
Notes: This species is a little darker than *O. oliveri*, but this is a miserable character for distinguishing these species.

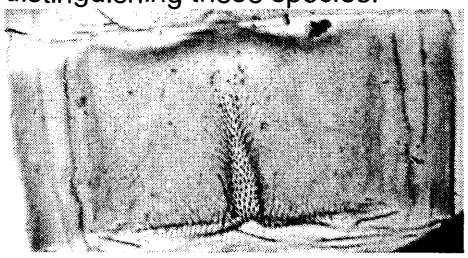


Figure 48. Tergite IV of *Orthocladius clarkei*.

Orthocladius nigritus
Reference: Soponis 1977
Description: Respiratory organ large, anterior half of tergites IV and V with spines/dense shagreen, no spurs on anal lobes (Fig. 49).

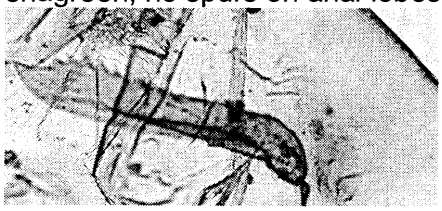


Figure 49. Respiratory organ of *Orthocladius nigritus*.

Orthocladius obumbratus
Reference: Soponis 1977
Description: No pedes spurii B on segment III. Spines in posterior patches of tergites III-IV (which are pointed anteriorly) extend laterally well beyond the width of recurved spines on II. No chitinous rings on tergites I-III. Anal lobe setae longer than 40% of the anal lobe's radius, anal lobe spurs are bifid or trifid. Frontal setae present, cephalic tuberacles not prominent, flat (Figs. 50-51).
Notes: Color is a miserable character to differentiate this species from *O. oliveri*, I used the following: *O. obumbratus* has bifid or tirfid anal lobe spurs and usually scale-like spines on the respiratory organ. Only *O. oliveri* has chitinous threads near the anal lobe macrosetae.

Figure 50. Anal lobe spurs of *Orthocladius obumbratus*.

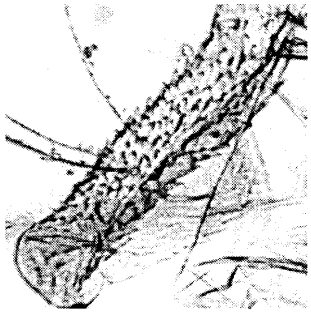


Figure 51. Respiratory organ of *Orthocladius obumbratus*.

Orthocladius oliveri
Reference: Soponis 1977
Description: No pedes spurii B on segment III. The spines in posterior patches of tergites III and IV (which are pointed anteriorly) extend laterally well beyond the width of the recurved sines on II. Respiratory organ with dense cover of moderately sized and pointed spines, organ fairly large, no chitinous rings on tergites I-III. Anal lobe setae longer than 40% of the anal lobe's radius, spurs on anal lobes. Frontal setae present; cephalic tubercle not prominent, flat. Apophyses on tergites are dark (Figs. 30, 52).
Notes: Color is a miserable character to differentiate this species from *O. obumbratus*, I used the following: *O. oliveri* has chitinous threads near the anal lobe macrosetae. Only *O. obumbratus* has bifid or trifid anal lobe spurs and usually scale-like spines on the respiratory organ.

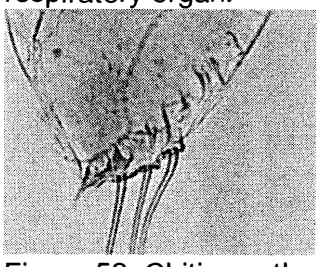


Figure 52. Chitinous threads on the anal lobe of *Orthocladius oliveri*.

Orthocladius trigonolabis
Reference: Soponis 1977
Description: Respiratory organ small, no spurs on anal lobes.

Parakiefferiella sp. 7
Description: Pedes spurii B on segments II and III, these segments also have strong patches of slender spines; 3 macrosetae on each anal lobe. Tergite II also has a posterior circular patch of spines, no respiratory organ, and extensive shagreen on tergites (Fig. 53).
Notes: R. W. Bouchard Jr. did not collect this species.

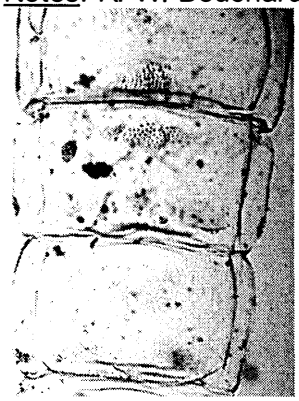


Figure 53. Tergites II-IV of *Parakiefferiella* sp. 7.

Parametriocnemus sp. 1
Description: Pedes spurii B on segement II well developed. No anterior spine patches on tergite II, each anal lobe with fringe of approximately 13 setae. Exuvium with dark brown pigmentation, not clear (Fig. 54).

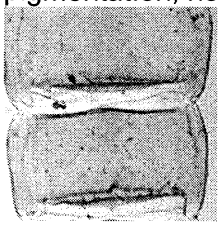


Figure 54. Tergites III-IV of *Parametriocnemus* sp. 1.

Parametriocnemus sp. 4
Description: Tergites III-VIII and sternites III-VIII with well-developed, spine rows that fold over when mounted. No anterior spine patches on tergite II. Anal lobe with very weak fringe and a patch of conspicuous shagreen. Exuvium with dark brown pigmentation, not clear (Fig. 55).

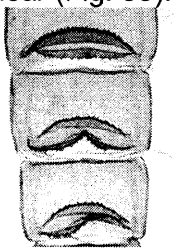


Figure 55. Tergites IV-VI of *Parametriocnemus* sp. 4.

Paraphaenocladius exagitans
Reference: Sæther and Wang 1995
Description: Nose on wing sheath; 1seta at tip of anal lobe (may be difficult to see). Pearl row on wing sheaths and pedes spurii B both poorly developed (Figs. 31, 56).


Figure 56. Nose on wing sheath of *Paraphaenocladius exagitans*.

Psectrocladius schlienzi type
Reference: Wiederholm 1986
Description: Tergites III-VI with a medial spine patch, three strong anal lobe macrosetae on each lobe. Tergites VII-VIII without posterior spine rows or patches (Fig. 12).

Pseudosmittia sp. 1
Description: Uniform shagreen composed of well-developed spinules and covering the majority of tergites II-VIII; the vast majority of spinules are approximately the same size. Anterior region of cephalothorax/ecdysial suture region with shagreen of small spines that fade posteriorly. At least 1 and as many as approximately 5 fenestrations in the shagreen of tergites II-VIII (Fig. 57).
Notes: At present R. W. Bouchard Jr. has not created morphospecies for this genus.


Figure 57. Tergites V-VII of *Pseudosmittia* sp. 1.

Pseudosmittia sp. 2
Description: Shagreen on tergites II-VIII composed of spinules of two different sizes. Longer, more-developed spinnules present on the anterior and posterior parts of each segment. Smaller, less-developed spinnules in the medial region of each tergite. No shagreen patch present in the anterior region of cephalothorax/ecdysial suture. Tergites II-VIII generally without fenestrations in the shagreen pattern, when present usually not conspicuous (Fig. 33).
Notes: At present R. W. Bouchard Jr. has not created morphospecies for this genus.

Rheocricotopus (Psilocricotopus) sp. 1
Reference: Sæther 1985
Description: No pedes spurii B, no median spinule patches on any tergite. Conjunctives III/IV-V/VI with spinules in rows, frontal setae present on the prefrons. Strong, anal lobe macrosetae, each anal lobe with fringe of approximately 21 setae. Lateral setae on segments V-VIII numbering: 3, 3, 4, 4-5 respectively.
Notes: This species keys to *R. chalybeatus* in Sæther (1985), however this species has not been found in the Neartic.

Rheosmittia sp.
Reference: Wiederholm 1986
Description: Anal lobe reduced and forming 2 structures that are apically pointed and contain spines, no respiratory organ or anal lobe macrosetae (Fig. 58).
Notes: Only 1 specimen was slide mounted; at present R. W. Bouchard Jr. has not created morphospecies for this genus.

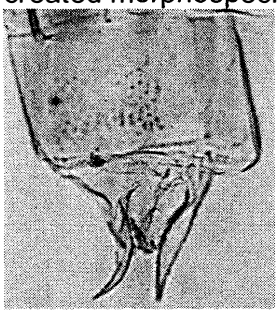


Figure 58. Tergite VIII and anal lobe of *Rheosmittia* sp.

Smittia sp.
Reference: Wiederholm 1986
Description: Anal lobe, respiratory organ, frontal warts, cephalic tubercles, and frontal setae are all absent. Segment conjunctives without hooks or spines (Fig. 34).
Notes: No specimens of this genus were slide mounted, they were identified via a dissecting microscope only.

Thienemanniella boltoni
Reference: Hestenes and Sæther 2000
Description: Tergite I with posterior hook row. Tergite II with shagreen composed of small spines; 2 hair-like and 2, taeniate, lateral setae. Long, spinule bundles absent on sternite II. Tergite VII with approximately 6 hooks on posterior hook row (Fig. 59).
Notes: This can be a difficult species to identify because it is hard to decide if the posterior projections on VII are hooks or spines. In determining this species I decided that the posterior projections must be robust and discernable enough at the lowest magnification to be considered hooks.

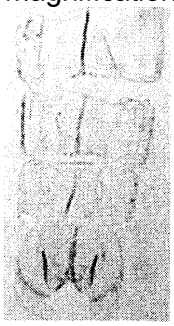


Figure 59. Tergites VI-VIII and anal lobe of *Thienemanniella boltoni*.

Thienemanniella lobapodema
Reference: Hestenes and Sæther 2000
Description: Posterior hooks absent on tergite I. Tergite II with fine shagreen and 3, taeniate, lateral setae. Posterior hook row with relatively-large hooks and long, spinule bundles absent on sternite II.

Thienemanniella similis
Reference: Hestenes and Sæther 2000
Description: Tergite I with posterior hook row. Tergite II with shagreen composed of small spines, and 3, taeniate, lateral setae also present. Three, long, spinule bundles absent on sternite II; tergite VII without posterior hook row.

Thienemanniella sp. 1
Description: Posterior hooks absent on tergite I. Tergite II with: fine shagreen; 2, hair-like, lateral setae; 1, taeniate, lateral seta; small hooks in posterior hook row. Long, spinule bundles absent on sternite II; long spines on posterior segments (Fig. 60).
Notes: Keys to *T. xena* in Hestenes and Sæther (2000) except for the presence of long spines on posterior segments.

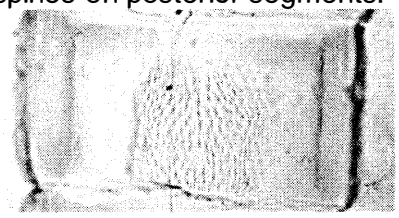


Figure 60. Tergite VII of *Thienemanniella* sp. 1.

Tvetenia sp. 1 (cf. *paucunca*).
Description: Tergites III-IV possessing recurved hooks posteriorly. Sternites IV-VIII with patches of hooks posteriorly. Respiratory organ with oval base and somewhat elongated, base not globular or round, a conspicuously long filament is inserted at the apex of the base (Figs. 21-22, 61).

Figure 61. Respiratory organ of *Tvetenia* sp. 1 (cf. *paucunca*).

Key to the Genera of Chironomini Pupae Collected in Hardwood Creek:

1. Tergites II-V and sometimes VI with 1 pair of conspicuous, longitudinal, spine patches (Fig. 62). ---------- ***Zavreliella* sp. (p.)**
1. Tergites II-VI without conspicuous spine patches (Figs. 65-68, 77-78, 81-82, 87). ---- **2**

2. Fringe on anal lobe with a conspicuous tuft of setae on each lobe (Fig. 63). ----------
---------- ***Endochironomus* sp. (p.)**
2. Tufts of setae not on anal lobes (Figs. 64, 70, 76, 85). ---------- **3**

3. Several tergites with a conspicuous group of anteriomedial, clumped spines, or spines on a mace (Figs. 65-66). ---------- **4**
3. No tergite with a conspicuous group of anteriomedial, clumped spines, or spines on a mace (Figs. 67-68, 77-78, 81-82, 87). ---------- **5**

4. Tergite IV and possible tergites V-VI with group of clumped spines (Fig. 65). ----------
---------- ***Demeijerea* sp. (p.)**

4. Tergites III-VI and possible tergite II with a mace containing spines (Fig. 66). ---------- ---------- ***Glyptotendipes* sp. (p.)**

5. Tergite VI possessing a posteromedial spine mound (Fig. 67). --- ***Cladopelma* sp. (p.)**
5. No posteromedial spine mound on tergite VI (Fig. 87). ---------- **6**

6. Conspicuous black or dark areas on the lateral most part of conjunctives I/II-IV/V. Cephalic tuberacles with apical spinules (Figs. 68-69). ---------- ***Phaenopsectra* sp. (p.)**
6. Conspicuous back or dark areas laterally on anterior conjunctives sometimes present in *Polypedilum* sp. Cephalic tuberacles present or absent, if present, apical spinules are absent. ---------- **7**

7. Anal lobe with conspicuous, posterior projection possessing 2 finger-like lobes (Fig.70). ---------- ***Cryptochironomus* sp. (p.)**
7. No conspicuous, anal lobe projection possessing 2 lobes (Figs. 63-64, 76). ---------- **8**

8. Respiratory organ very long, wide, well developed; apical half of organ with many branches. Organ more than half the length of the entire exuviae; most of tergites II-VI with a pair of projections containing spines posteriorly. Sternites II-VI many also have spiny projections (Fig. 71). ---------- ***Cryptotendipes* sp. (p.)**
8. Respiratory organ less than half the length of the entire exuviae; no projections containing spines on tergites or sternites II-VI (Figs. 68, 72-73, 77-79). ---------- **9**

9. Respiratory organ with no more than 25 branches (Figs. 72, 79). ---------- **10**
9. Respiratory organ with 30 to 100's of branches (Fig. 73). ---------- **13**

10. Frontal warts projected dorsally; frontal setae and cephalic tubercles are not present (Fig. 74). ---------- ***Microtendipes* sp. (p.)**
10. Frontal warts often absent; frontal setae are present with cephalic tubercles present or absent. ---------- **11**

11. Nose on wing sheaths; segments II-IV with 4 lateral setae (Fig. 75). ---------- ---------- ***Paralauterborniella* sp. (p.)**
11. No nose on wing sheaths; segments II-IV with 3 lateral setae. ---------- **12**

12. Tergites II-VI with an anterior row of spines discernable from surrounding shagreen. Tergites VII-VIII with an anterior pair of lightly-shagreened patches (Figs. 76-77). -------- ---------- ***Polypedilum* sp. (p.)**
12. Tergites II-VI without anterior row of spines discernable from shagreen (Fig. 78). ----- ---------- ***Paratendipes* sp. (p.)**

13. The following are not present: pedes spurri B on segment II, pedes spurii A on segment IV, cephalic tubercles, frontal setae. Respiratory organ branched, with 1 branch not further branched and not containing setae, instead spines are present on this branch (Fig. 79). ---------- ***Stenochironomus* sp. (p.)**
13. The following are usually present: pedes spurii B on segment II, pedes spurii A on segment IV. Respiratory organ highly branched and not containing spines (Figs. 73, 80). ---------- **14**

14. Segment VIII without anal comb or spur. Tergites III-VI and sometimes II, with posterior row of spines; hook row on segment II is conspicuously divided medially. Often a small spine patch is just anterior to the hook row division (Fig. 81). ***Harnischia* sp. (p.)**
14. Segment VIII with or without comb or spur; hook row on segment II not conspicuously divided medially. Tergites II-VI with anterior spine band present or absent, if present, anterior row of spines usually present in conjunction with a posterior band which many have a medial interruption (Figs. 68, 78). ---------- **15**

15. Tergites without posterior spine band(s). Tergites II-VI possessing conspicuous anterior spine row, frontal setae conspicuous. ---------- ***Stictochironomus* sp. (p.)**
15 .Tergites II-VI without anterior spine row, however, if present this row it is weakly expressed and is accompanied by a conspicuous posterior spine band, that is often interrupted medially (Fig. 82). ---------- **16**

16. Basal ring with 3, conspicuous, circular, tracheal openings (Fig. 83). ----------
---------- ***Einfeldia* sp. (p.)**
16. Basal ring with less than 3 tracheal openings (Fig. 84). ---------- **17**

17. No pedes spurii B on segment II. ---------- **18**
17. Pedes spurii B present on segment II (Figs. 78, 80). ---------- **19**

18. Segment VIII without anal spur or comb and possessing 4 lateral setae. ----------
---------- ***Saetheria* sp. (p.)**
18. Segment VIII possessing 5 lateral setae, however, if 4 lateral setae are present, an anal comb or spur is present. ---------- ***Paracladopelma* sp. (p.)**

19. Segment conjunctives with small, fine, lateral seta on III/IV and/or IV/V. Broad, conspicuous, and usually pigmented anal spur present; exuviae are characteristerically large in size (Fig. 85). ---------- ***Chironomus* sp. (p.)**
19. Segment conjunctives without a lateral seta. Anal comb is present, or if a spur is present not broad, conspicuous, and pigmented; exuviae smaller in size (Fig. 86). ---- **20**

20. Segment I lacking lateral setae. Distinct anal spur that is "s" shaped, usually tapering to a sharp point; large basal ring with conspicuous medial constriction (Fig. 86). ----------
---------- ***Dicrotendipes* sp. (p.)**
20. Segement I with lateral setae. Anal comb, if present, consisting of spines; when comb is absent, tergite VI has a bulbous medial projection. The projection can be present when the anal comb is present (Fig. 87). ---------- ***Parachironomus* sp. (p.)**

Chironomini Figures:

Figure 62. Tergites II-V of *Zavreliella* sp.

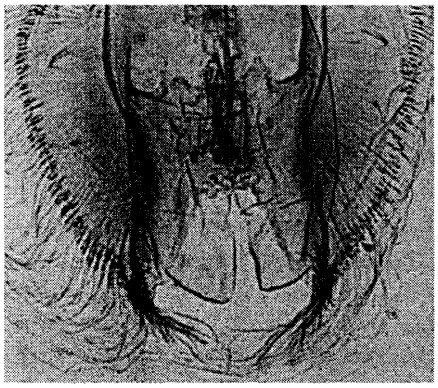


Figure 63. Anal lobe of *Endochironomus nigricans*.

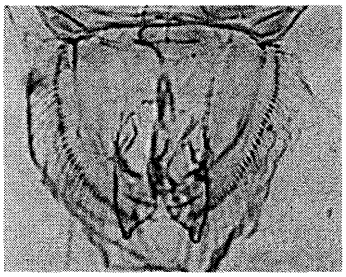


Figure 64. Anal lobe of *Cladopelma* sp. 1.

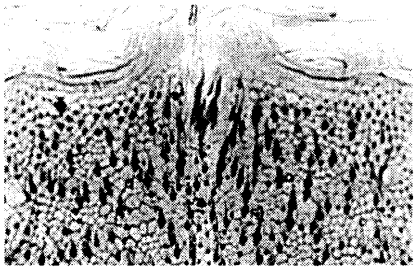


Figure 65. Tergite V of *Demeijerea* sp.

Figure 66. Mace on tergite VI of *Glyptopendipes* sp. 1.

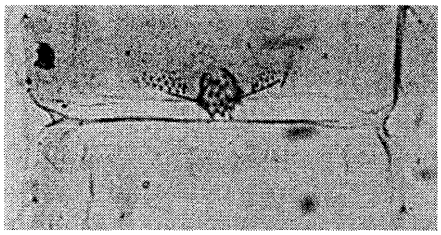


Figure 67. Posterior spine mound on tergite VI of *Cladopelma* sp. 1.

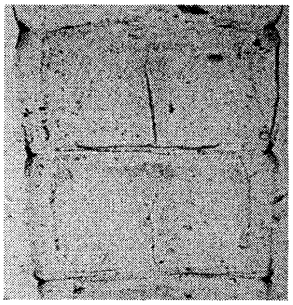


Figure 68. Conjunctives I/II-III/IV of *Phaenopsectra* sp. 1.

Figure 69. Cephalic tubercles of *Phaenopsectra* sp.

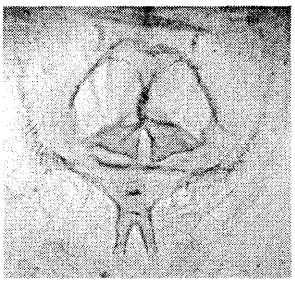


Figure 70. Anal lobe of *Cryptochironomus* sp.

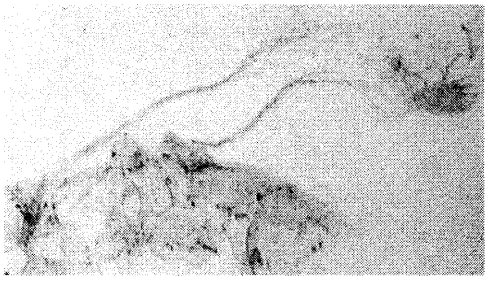


Figure 71. Respiratory organ of *Cryptotendipes* sp.

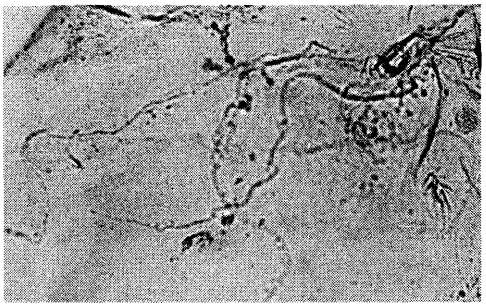


Figure 72. Respiratory organ of *Polypedilum scalaenum* gr. sp. 8.

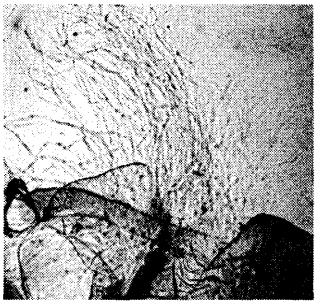


Figure 73. Respiratory organ of *Chironomus* sp. 1.

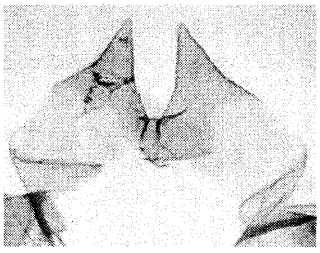


Figure 74. Frontal warts of *Microtendipes* sp.

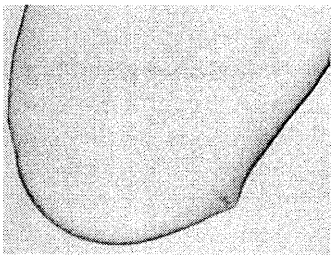


Figure 75. Wing sheath nose of *Paralauterborniella* sp.

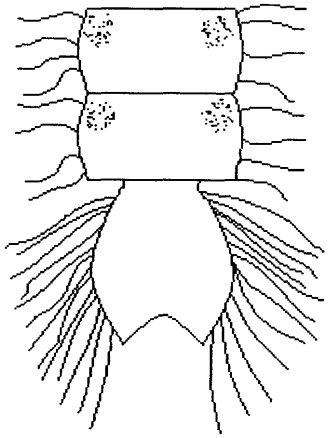


Figure 76. Segments VII-VIII and anal lobe of *Polypedilum* sp.

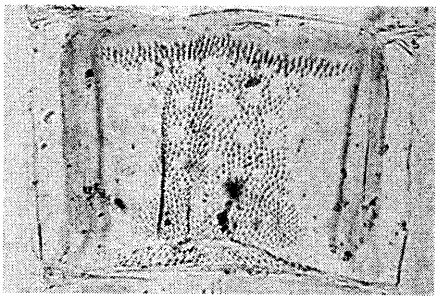


Figure 77. Tergite IV of *Polypedilum obtusum*.

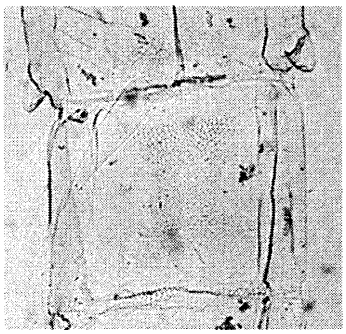


Figure 78. Tergite III and part of II of *Paratendipes* sp. 1.

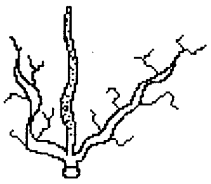


Figure 79. Respiratory organ of *Stenochironomus* sp.

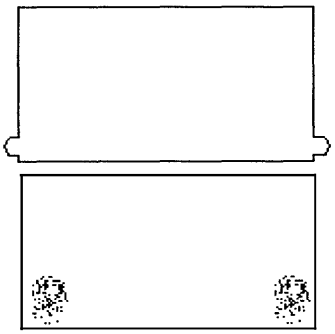


Figure 80. Segment II (top) and segment IV (bottom) of some Chironomini.

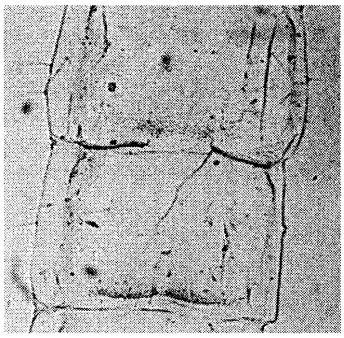


Figure 81. Tergites II-III of *Harnischia* sp.

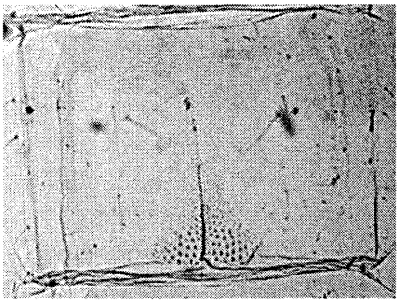


Figure 82. Tergite V of *Parachironomus* sp. gr. C.

Figure 83. Basal ring of *Einfeldia* sp. gr. D.

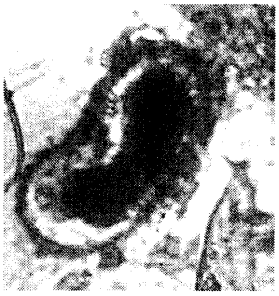


Figure 84. Basal ring of *Chironomus* sp. 1.

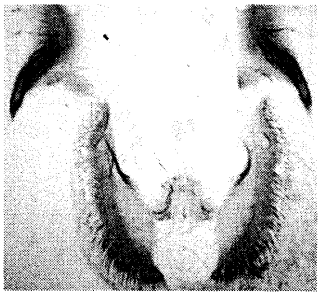


Figure 85. Anal lobe and spurs of *Chironomus* sp.

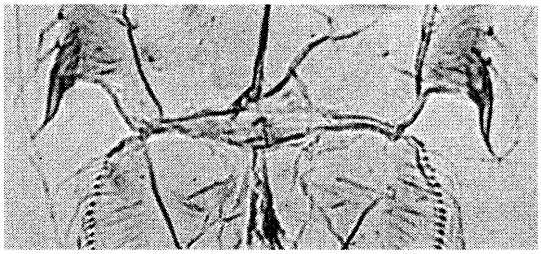


Figure 86. Anal spurs of *Dicrotendipes* sp. 6.

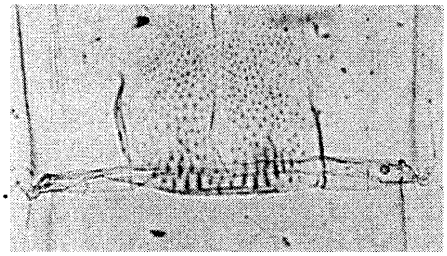


Figure 87. Bulbous projection on tergite VI of *Parachironomus arcuatus* gr.

Chironomini Taxa Descriptions:

Chironomus sp. 1
Description: Only cephalic tubercles present (Figs. 73, 84).
Notes: This morphospecies is very encompassing and can likely be divided into many more species.

Chironomus sp. 2
Description: Cephalic tubercles and frontal warts present (Fig. 88).
Notes: This morphospecies is very encompassing and can likely be divided into many more species.

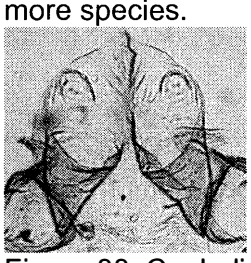


Figure 88. Cephalic tuberacles and frontal warts of *Chironomus* sp. 2.

Cladopelma sp. 1
Description: Segment II with 1 anterior and posterior row of spines. Anterior row with long and well-developed spines. Posterior row with greater than or equal to 33 total spines which are generally larger and better developed than those of species 2 (Figs. 64, 67, 89).
Notes: At present R. W. Bouchard Jr. has not created morphospecies for this genus.

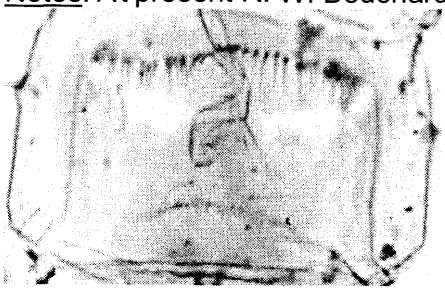


Figure 89. Segment II of *Cladopelma* sp. 1.

Cladopelma sp. 2
Description: Segment II with 1 anterior and usually posterior row of spines, with the posterior row absent in some specimens. Anterior row with long and well-developed

spines. Posterior row with less than or equal to 32 total spines, which are generally weaker and less well developed than those of species 1 (Fig. 90).
Notes: At present R. W. Bouchard Jr. has not created morphospecies for this genus.

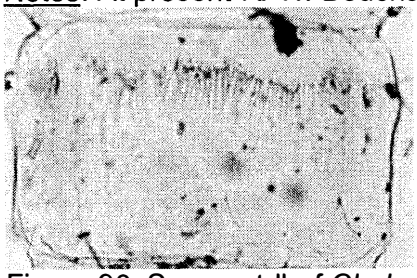


Figure 90. Segment II of *Cladopelma* sp. 2.

Cryptochironomus eminentia
Reference: Mason 1985
Description: Large and conspicuous cephalic tubercles with bulbous base that is divided between the 2 tubercles (Fig. 91).

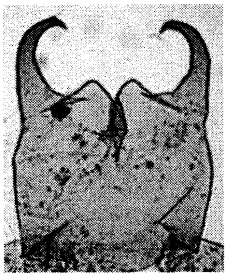


Figure 91. Cephalic tuberacles of *Cryptochironomus eminentia*.

Cryptochironomus ponderosus
Reference: Mason 1985
Description: Large and conspicuous cephalic tubercles with bulbous base that is not divided between the 2 tubercles (Fig. 92).

Figure 92. Cephalic tuberacles of *Cryptochironomus ponderosus*.

Cryptochironomus sp. 1 (nr. *conus*)
References: Mason 1985
Description: Cephalic tuberacles are slender, long, and pointed with subapical seta. Tergite I with 1-2 pairs of anterior tubercles with spines, heavy reticulation only on anterior segments. Shagreen on tergites II-VI covering half to three-quarters of tergites. Tergites and sternites II-VII with posterior spine rows. Tergites VII-VIII with light, anteriolateral, shagreen patches; sternite VIII with spine rows only in male exuviae. Each anal lobe with dense and well-developed fringe of greater than 30 setae (Fig. 93).
Notes: A variable species with regards to light reticulation pattern, especially on posterior segments, and the development of shagreen spinules and cephalic tuberacles.

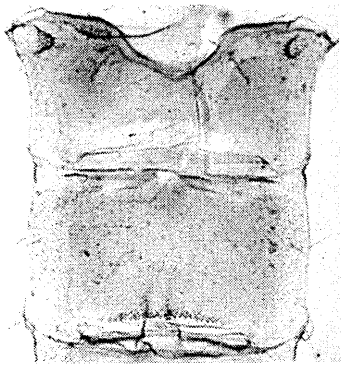


Figure 93. Tergites I-II of *Cryptochironomus* sp. 1 (nr. *conus*).

Cryptochironomus sp. 2
Description: Cephalic tuberacles large and conicle, heavy reticulation pattern approximately resembling snake skin on segments I-V. Tergite VI sometimes with reticulation in anterior-quarter of the segment. Specimens of this species tend to be large in size (Fig. 94).

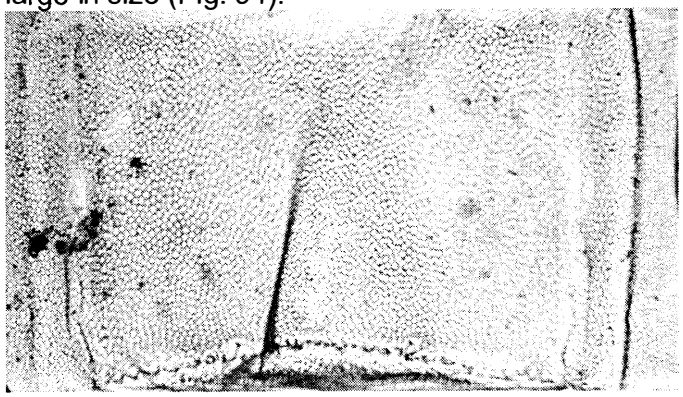


Figure 94. Tergite III of *Cryptochironomus* sp. 2.

Cryptotendipes sp.
Description: Respiratory organ very long, wide, and well developed. Apical half of organ with many branches. Organ more than half the length of the entire exuviae. Cephalic tuberacles small, finger-like projections; tergites II-VI with 1 pair of spine mounds becoming more developed on posterior segments. Thick, brown filaments not present in anal lobe fringe; anal spur single and resembling those found in the genus *Dicrotendipes* sp. Male genital sheath with 2 conspicuous finger-like projections, cephalothorax rough and possessing many wart-like projections (Fig. 71).
Notes: Only 1 specimen slide mounted and described above; at present R. W. Bouchard Jr. has not created morphospecies for this genus.

Demeijerea sp.
Reference: Wiederholm 1986
Description: Tergites IV, IV-V or IV-VI with conspicuous pattern of reticulation and dark spines. Each anal lobe with fringe of approximately 110-190 setae (Fig. 65).
Notes: Only 1 specimen slide mounted; at present R. W. Bouchard Jr. has not created morphospecies for this genus.

Dicrotendipes modestus/neomodestus
Reference: Epler 1988
Description: Sternite II with spine row. Tergite VI with anterior and posterior spinules in the medial shagreen patch more developed than those present in the center of the patch. Tergite VIII with 4, taeniate, lateral setae; anal spur single or closely appressed double. Anal lobe with many setae (Fig. 95).
Notes: A morphologicaly variable species.

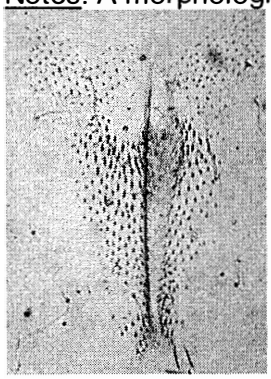


Figure 95. Tergite VI shagreen of *Dicrotendipes modestus/neomodestus*.

Dicrotendipes nervosus gr.
Reference: Epler 1988
Description: Tergites II-V with approximately equal shagreen spinules, no spine row on sternite II. Tergites VI-VIII with moderately to poorly developed reticulate pattern, VI with the longest spinules present in the middle of the shagreen. Segment VIII with 4, taeniate, lateral setae (Fig. 96).

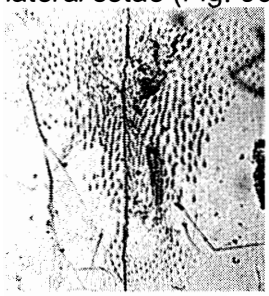


Figure 96. Tergite VI shagreen of *Dicrotendipes nervosus* gr.

Dicrotendipes tritomus
Reference: Epler 1988
Description: Cephalic tuberacles wide and short, sternite II with spines. Segment VIII with 4, taeniate, lateral setae. Anal spur composed of 2 well-separated spurs.

Dicrotendipes sp. 4
Description: Tergites III-VI with relaively extensive shagreen coverage. Keys to *D. modestus/neomodestus* but anterior shagreen on tergites IV-VI is different (Fig. 97).

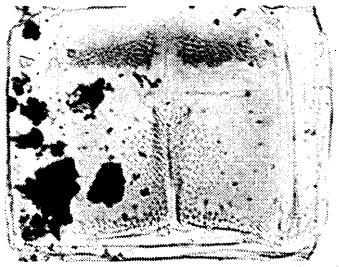


Figure 97. Tergite IV of *Dicrotendipes* sp. 4.

Dicrotendipes sp. 6
Description: Sternites I-II with spines. Tergite VI with anterior spinules in the medial shagreen patch more developed than those in the middle and posterior part of the patch (Figs. 86, 98).
Notes: Keys to *D. californicus* complex in Epler (1988).

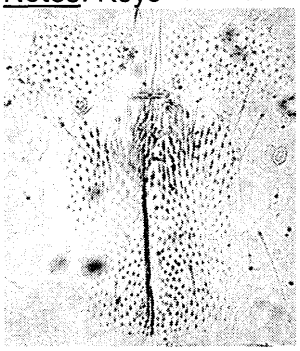


Figure 98. Tergite VI shagreen of *Dicrotendipes* sp. 6.

Einfeldia sp. gr. D
Reference: Wiederholm 1986
Description: Tergites III-VI with shagreen pattern divided into anterior, medial, and posterior fields. Sternite VIII with 1 pair of larval, ventral tubules; cephalic tubercles present. Basal ring of respiratory organ has 3 branches, anal comb with slender spines arranged in a transverse row (Figs. 83, 99).


Figure 99. Tergite III of *Einfeldia* sp. gr. D.

Endochironomus nigricans
Reference: Grodhaus 1987
Description: Pedes spurii A absent on sternite IV; segments V-VI with hair-like, lateral setae. Segment VIII usually with more than 1, well-developed, spine on posteriolateral corner. If cephalic tuberacles are present, they are without an apical circle of small papillae. Exuviae yellow and anal lobe with tuft of setae (Fig. 63).

Notes: *E. subtendens* is approximately colorless.

Glyptotendipes sp. 1
Description: Maces increasing in size with tergite VI having the largest mace. Cephalic tuberacles are moderaltely large and finger like. Segment VIII with several, very-small spines in on its posterolateral corner. Each anal lobe with dense fringe of setae, cephalothorax with brown pigmentation and warts giving it a rough appearance (Fig. 66).

Harnischia sp.
Description: Cephalic turberacles resemble finger-like projections and are relatively-well developed. Exuvium relatively clear, some specimens with a little pigmentation. Large bump present near edysal margin region in anterior half of the cephalothorax (Fig. 81).
Notes: All 6 specimens that were mounted are described above and appear to be the same species.

Microtendipes sp. 3
Description: Frontal warts well developed, long, and slender. Anal comb with approximately 2-4 spines, exuviae yellow to brown in color (Fig. 100).

Figure 100. Anal comb of *Microtendipes* sp. 3.

Parachironomus arcuatus gr.
Reference: Wiederholm 1986
Description: Sternite II with anterior and posterior spine rows, tergite VI with a distinct flap containing spines. Posterolateral region of segment VIII with 1 to several colorless spines; 5, taeniate, lateral setae also present on VIII (Fig. 87).

Parachironomus frequens gr.
Reference: Wiederholm 1986
Description: Tergite VI without a distinct flap containing spines, lateral reticulation on VII-VIII. Posterolateral region of segment VIII with 1 to several colorless spines; 5, taeniate, lateral setae also present on VIII (Fig. 101).
Notes: The lateral reticulation on terites VII-VIII can be a difficult character to use because many species of *Parachironomus* sp. have a reticulate pattern on these segments, however, it is particurarly pronounced in *P. frequens* gr.

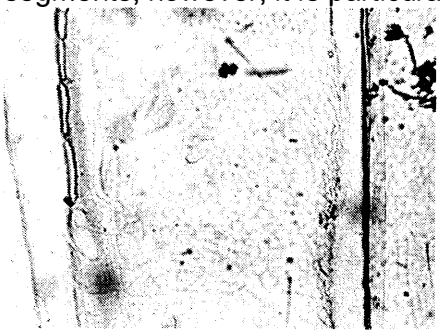


Figure 101. Lateral reticulation on part of tergite VII of *Parachironomus frequens* gr.

Parachironomus varus gr.
Reference: Wiederholm 1986
Description: Segment VIII with 5, taeniate, lateral setae; posterolateral region of VIII without spines.

Parachironomus vitiosus gr.
Reference: Wiederholm 1986
Description: Anterior or posterior spine rows absent on sternite II. Tergite VI with a distinct flap contajning spines. Posterolateral region of segment VIII with 1 to several colorless spines and 5, taeniate, lateral setae also present on VIII (Fig. 102).

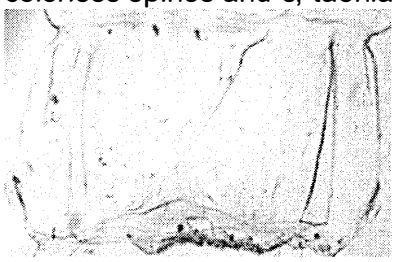


Figure 102. Segment II of *Parachironomus vitiosus* gr.

Parachironomus sp. gr. C
Reference: Wiederholm 1986
Description: Tergite II with continous hook row 4, taeniate, lateral setae on segments V-VIII (Figs. 82, 103).

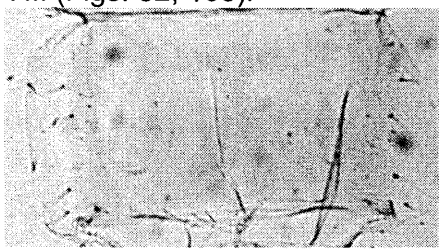


Figure 103. Four, taeniate, lateral setae on segment VIII of *Parachironomus* sp. gr. C.

Paracladopelma nereis
Reference: Jackson 1977
Description: Tergites II-IV with 4, hair-like, lateral setae, with 2 of these setae being present on the caudolateral angles of the segments. Posterior hook row on tergite II with 54-58 hooks, shagreen on tergite VIII, anal spur absent (Fig. 104).

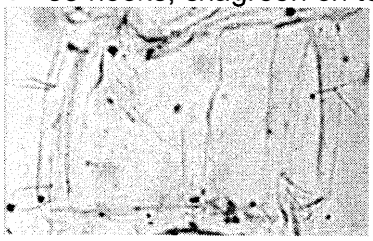


Figure 104. Tergite II of *Paracladopelma nereis*.

Paralauterborniella nigrohalterale
Reference: Wiederholm 1986
Description: No lateral setae on segment I and 4, hair-like, lateral setae on segments II-IV. Segement IV with pedes spurii A not resembling a vortex and 1, taeniate, dorsal setae on the anal lobe.

Paratendipes sp. 1
References: Hayford 1998
Description: Cephalic tuberacles with frontal setae. Tergites II-VI without variable shagreen patterns, shagreen is square or hourglass shaped. Tergites III/IV and IV/V with setae between conjunctives. Respiratory organ with 10-12 branches which are often difficult to see (Figs. 78, 105).
Notes: This morphospecies is probally *P. albimanus*.

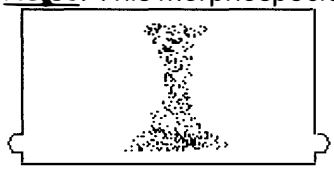


Figure 105. Tergite II of *Paratendipes* sp. 1.

Paratendipes (?) sp. 2
Description: Cephalic tuberacles absent and tergites II-VI have well-developed, shagreen patches covering most of the segment. The lateral setae on segments IV-VIII numbering: 1, 3, 4, 4, 4 respectively. Each anal lobe with fringe of approximately 13 setae (Fig. 106).
Notes: This species is possibly in the genus *Apedilum* sp.

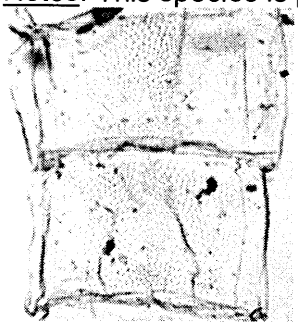


Figure 106. Tergites II-III of *Paratendipes* (?) sp. 2.

Phaenopsectra sp. 1
Description: Anterior shagreen on tergites II-V only slightly stronger than shagreen on the rest of the tergite. Anal comb with 3-4 spines (Figs. 68, 107).

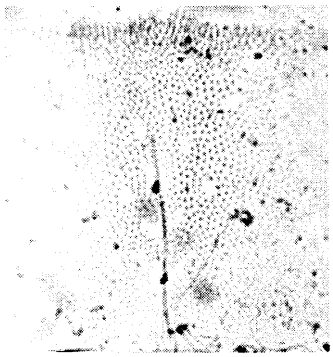


Figure 107. Tergite III shagreen of *Phaenopsectra* sp. 1.

Phaenopsectra sp. 2
Description: Anterior shagreen distinct with many spines being larger than shagreen spines in medial part of segments. Anal comb with approximately 2-3 spines, the largest spine is usually not the most lateral (Fig. 108).
Notes: A very morphologicaly variable species.

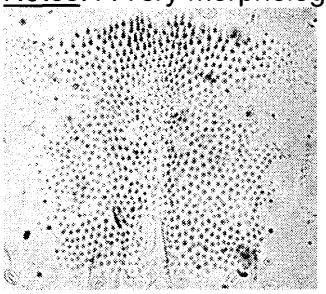


Figure 108. Tergite III shagreen of *Phaenopsectra* sp. 2.

Polypedilum (nr. *falciforme*)
Reference: Maschwitz and Cook 2000
Description: Prealar tubercle and anal spur both present. No cephalic tuberacles, each anal lobe with fringe of less than 40 setae, exuviae greater than 5 mm long.
Notes: Dose not quite have less than 40 setae but specimens were greater than 5 mm.

Polypedilum fallax
Reference: Maschwitz and Cook 2000
Description: Prealar tubercle absent to poorly developed, anal spur present with many small spines at its base. No cephalic tuberacles, many spinules on segment conjunctives III/IV-IV/V. Shagreen fairly extensive on all tergites, especially II-III, brown tinge on posterior tergites (Fig. 109).
Notes: Looks very much like many *Phaenopsectra* sp. based on the dark pigmentation on the lattermost part of anterior conjunctives.

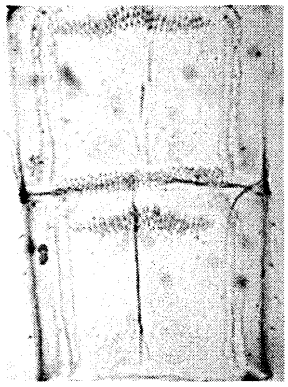


Figure 109. Tergites IV-V of *Polypedilum fallax.*

Polypedilum illinoense/angulum
Reference: Maschwitz and Cook 2000
Description: Prealar tubercle absent to poorly developed, anal spur present without small spines at its base. No cephalic tuberacle, many spinules between segments III-IV. Anterior spine rows on tergites III-VI are larger than conjunctive spinules and hooks in posterior hook row on tergite II. Pedes spurii A present on segment IV (Fig. 110).

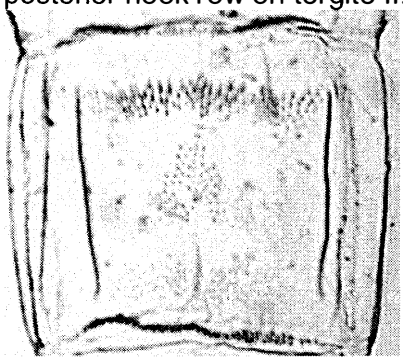


Figure 110. Tergite IV and conjunctive spunules of segments III/IV-IV/V of *Polypedilum illinoense/angulum.*

Polypedilum obtusum
Reference: Maschwitz and Cook 2000
Description: No prealar tubercle, anal spur present, no cephalic tuberacles. Each anal lobe with fringe of greater than 30 setae. Tergite II with anterior spinule row weak to approximately absent. Pedes spurii B on segement II present; shagreen pattern without an anterior to posterior bald midline on tergites III-VI (Fig. 77).

Polypedilum scalaenum gr. sp. 1
References: Maschwitz and Cook 2000
Description: Prealar tubercle absent to poorly developed, anal spur present without small spines at its base. No cephalic tuberacles, many spinules on conjunctive III/IV. Tergites III-VI with anterior row of spinules approximately the size of, or smaller than the hook row on tergite II. No triangle shaped shagreen pattern on tergites III-VI (Fig. 111).


Figure 111. Tergites II-III of *Polypedilum scalaenum* gr. sp. 1.

Polypedilum scalaenum gr. sp. 8
References: Maschwitz and Cook 2000
Description: Prealar tuberacle absent to poorly developed, anal spur with lateral spines (approximately 3-4). No cephalic tuberacles, spinules on conjunctive III/IV, tergites II-V with very-dense shagreen covering most of the tergite. Shagreen spinules almost as large as the anterior spinule row; each anal lobe with fringe of approximately 28 setae (Figs. 72, 112).


Figure 112. Tergite III of *Polypedilum scalaenum* gr. sp. 8.

Polypedilum simulans
Reference: Maschwitz and Cook 2000
Description: Tergites with distinct anterior row of spinules, cephalic tuberacles present.
Notes: The presence of cephalic tuberacles is a key character for separating this species from other *Polypedilum* sp. (Fig. 113).

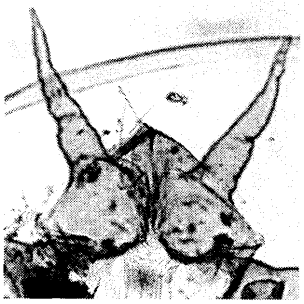


Figure 113. Cephalic tuberacles and frontal warts of *Polypedilum simulans*.

Polypedilum trigonus
Reference: Maschwitz and Cook 2000

Description: No prealar tuberacle, anal spur present, and no cephalic tuberacles. No spinules on conjunctives III/IV, prominent anterior spinule row on tergite II. Tergites IV-V with medial shagreen that connects both anterior and posterior spinule rows.

Polypedilum sp. 4
Description: Cephalic tubercles with many worts; anal spur with 1 prominent spur and 2-3 smaller accessory spurs. Each anal lobe with fringe of approximately 32-35 setae (Fig. 114).

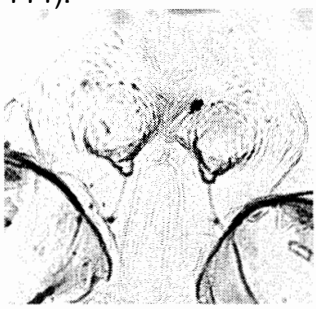


Figure 114. Cephalic tuberacles of *Polypedilum* sp. 4.

Saetheria tylus
Reference: Jackson 1977
Description: Tergites III-V with posterior band of spines expanded to each side of the tergite. Segment II-IV with 2 ,strong, hair-like setae; last quarter of tergite III-IV with posterior band of spines (Fig. 115).

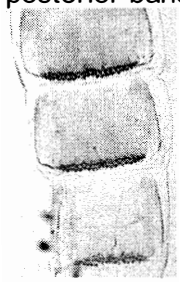


Figure 115. Tergites III-V of *Saetheria tylus*.

Saetheria sp. 1
Reference: Jackson 1977
Description: Tergites III-IV with shagreen in a triangular pattern. The most distinctive spines are also the most posterior, with spines decrease in size anteriorly toward the tip of the triangle. Segments V-VIII with 4, taeniate, lateral setae (Fig. 116).
Notes: This is a morphospecies created by Jackson 1977.


Figure 116. Tergites III-IV of *Saetheria* sp. 1.

Stenochironomus sp. 3
Description: Tergite II with posterior hook row extended laterally near the edge of the segment and not divided. Tergites II-V with large, anterior, spine rows and small shagreen spines medially. Sternites II-III with bilobed "M" pattern that is sometimes present on other segments too. Anal comb with approximately 2 pointed spines medially, and approximately 4 or more rounded and broad spines laterally (Fig. 117).

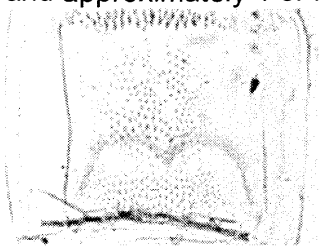


Figure 117. Tergite II of *Stenochironomus* sp. 3.

Stictochironomus sp.
Reference: Wiederholm 1986
Description: Segments II and IV with pedes spurii B and A respecively. Tergites II-V possessing a conspicuous spine row anteriorly and a large, medial, shagreen patch that is separated from the anterior spine row; posterior spine row absent. Frontal setae, anal comb, cephalic tuberacles, and highly branched respiratory organ all well developed (Fig. 118).
Notes: No specimens of this genus were slide mounted, they were identified via a dissecting microscope only.

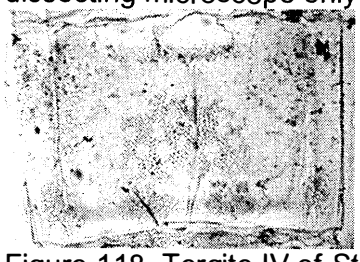


Figure 118. Tergite IV of *Stictochironomus* sp.

Zavreliella sp.
Reference: Wiederholm 1986

Description: Tergites II-V and sometimes VI with 1 pair of conspicuous, longitudinal, spine patches; conjunctives between segements bare. Segment II with posterior hook row not interuped medially; VI with 3, taeniate, lateral setae; respiratory organ with 4 branches (Fig. 62).
Notes: No specimens of this genus were slide mounted, they were identified via a dissecting microscope only.

Key to the Genera of Pseudochironomini Pupae Collected in Hardwood Creek:

1. Sternite I with 2 pairs of tubercles very often containing spines. Respiratory organ consisting of only two plump branches, both of which are rounded apically. No marginal fringe of setae on anal lobes (Figs. 119-120). ---------- **Pseudochironomus (p.)**
1. Sternite I usually without terbercles containing spines. Respiratory organ with or without branches. If branched the organ is not plump and can have 1 to 100's of branches, spines, and setae. Anal lobes usually with relatively, well-developed, marginal fringe (Figs. 63-64, 70-73, 76, 79, 85, 121, 123, 129). ---------- ---------- **Tanytarsini (p.) or Chironomini (p.)**

Pseudochironomini Figures:

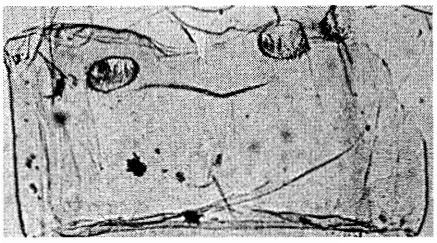


Figure 119. Segment I of *Pseudochironomus richardsoni*.

Figure 120. Respiratory organ of *Pseudochironomus* sp.

Pseudochironomini Taxa Descriptions:

Pseudochironomus richardsoni
Reference: Sæther 1977
Description: Taeniate lateral setae on segments V-VIII numbering: 3, 4, 4, 5 respectively. Each anal lobe with fringe of approximately 15 setae, exuvium darker in color, anal comb composed of 3-7 spines (Fig. 119).
Notes: There were 2 characters that did not quite fit with Sæther (1977). First, I counted approximately 30-35 setae in the anal lobe fringe of my specimens. Second, sternite I had both pairs of tubericles covered in spines.

Key to the Genera of Tanytarsini Pupae Collected in Hardwood Creek:

1. Segement IV with pedes spurii A; segment VIII without an anal comb, instead, an anal spur is present (Figs. 121-122). ---------- **2**
1. Segment IV without pedes spurii A; segment VIII with an anal comb (Fig. 123). ------- **4**

2. Tergites II-IV or II-VI will 1 anterior pair of well-developed, round, spine patches (Fig. 124). ---------- ***Rheotanytarsus* sp. (p.)**
2. Tergites without round spine patches, instead, tergites II-V with shagreen (Fig. 125). **3**

3. The shagreen coverage on tergite II is obvously not equal to that found on tergite III. ---------- ***Stempellinella* sp. (p.)**

3. The shagreen coverage on tergite II is approximately equal to that found on tergite III (Fig. 125). ---------- ***Zavrelia* sp. (p.)**

4. Tergite IV with 1 anteromedial patch of well-developed spines, longitudinal spine patch present or absent on tergite IV (Fig. 126). ---------- ***Paratanytarsus* sp. (p.)**

4. Tergite IV with 1 pair of anteriomedial patches containing of well-developed spines, longitudinal spine patches present or absent on tergite IV (Figs. 127-128, 130). ---------- **5**

5. Tergite III with 1 posterior pair of spine patches that curve and are present in the last half of the tergite. Tergites IV-V possessing paired, anterior, spine patches that are horizontally elongated; respiratry organ with conspicuous fringe of long setae (Fig. 127). ---------- ***Micropsectra* sp. (p.)**

5. Tergite II and/or III with 1 anterior pair of spine patches. Respiratory organ can possess conspicuously long setae, however, the organ is often bare or has spines (Figs. 128-130). ---------- **6**

6. Tergites II-VI with 1 pair of small, anterior, spine patches that are anteriorly to posteriorly elongated, espically on posterior tergites. Segment VIII possessing 5 lateral setae, respiratory organ often with long setae (Figs. 128-129). ---------- ***Cladotanytarsus* sp. (p.)**

6. Tergites III-IV with 1 pair of small, anterior, spine patches that are usually anteriorly to posteriorly elongated, or 1 pair of large longitudinal spine patches may be present on at least 1 of these tergites. Tergite II usually possessing shagreen patches that are pigmented. Segment VIII possessing 5 lateral setae, however, 4 are sometimes present (Fig. 130). ---------- ***Tanytarsus* sp. (p.)**

<u>Tanytarsini Figures</u>:

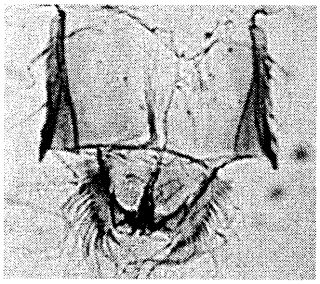


Figure 121. Segment VIII and anal lobe of *Zavrelia* sp.

Figure 122. Segment IV of some Tanytarsini.

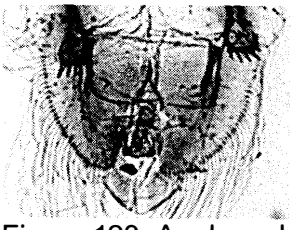


Figure 123. Anal comb and anal lobe of *Tanytarsus confusus*.

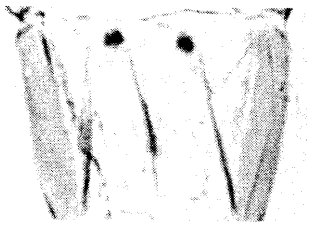


Figure 124. Tergite III of *Rheotanytarsus distinctissimus*.

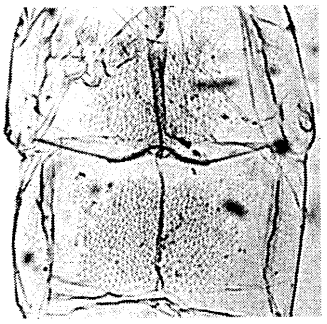


Figure 125. Tergites II-III of *Zavrelia* sp.

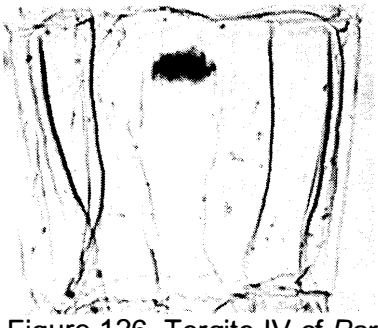


Figure 126. Tergite IV of *Paratanytarsus* sp. 2.

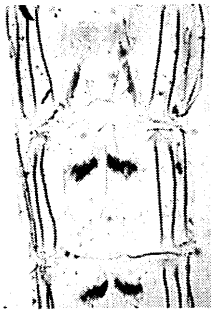


Figure 127. Tergites III-IV and part of V of *Micropsectra nigripila*.

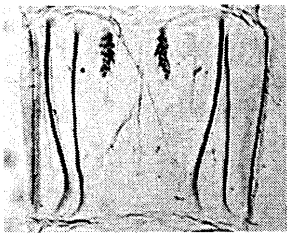


Figure 128. Tergite V of *Cladotanytarsus* sp. 1.

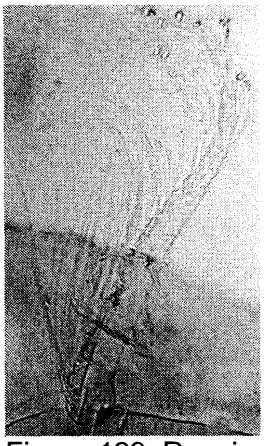


Figure 129. Respiratory organ of *Cladotanytarsus* sp.

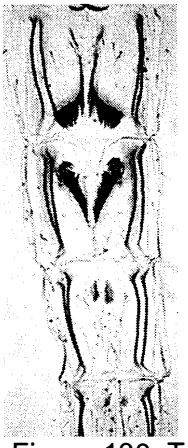


Figure 130. Tergites III-V and part of VI of *Tanytarsus confusus*.

<u>Tanytarsini Taxa Descriptions</u>:

Cladotanytarsus sp. 1
<u>Description</u>: Paired spine patches on segments II-VI going from round to more elongated as you go from anterior to posterior segments. Respiratory organ on a pedicle, well developed, and relatively large compaired to other *Cladotanytarsus* sp. with fringe throughout the tapered length of the organ. Cephalic tuberacles short, finger-like projections, frontal setae present. Spinules not present on the frontal apotome, relatively-large spines near the edysial suture, abodemen and cephalothorax with

pigmentation. Anal comb well developed and variable and each anal lobe with fringe (Figs. 128, 131).

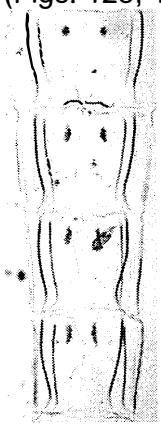


Figure 131. Tergites II-V of *Cladotanytarsus* sp. 1.

Cladotanytarsus sp. 2
Description: Respiratory organ small and bulbous with long setae present apically. Cephalic tuberacles finger shaped and long with frontal setae. No spinnules/shagreen on frontal apotome; spines present near ecdysial suture (Fig. 132).

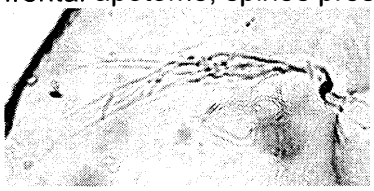


Figure 132. Respiratory organ of *Cladotanytarsus* sp. 2.

Micropsectra nigripila
Reference: Oliver and Dillon 1994
Description: Paired spine patches on tergites III-V. Respiratory organ relatively long with shorter setae; 4, taeniate, lateral setae on segment VIII (Fig. 127).

Micropsectra polita
References: Webb 1981 and Oliver and Dillon 1994
Description: Paired longitudinal spine patches on tergites III-V. Tergites IV-V with horizontally elongated spine patches, with longitudinal patch on V shorter than IV. Respiratory organ relatively short with long setae; 5, taeniate, lateral setae on segment VIII (Fig. 133).

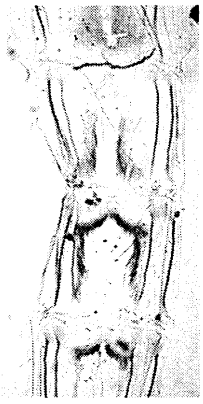


Figure 133. Tergites III-IV and part of tergites II and V of *Micropsectra polita*.

Paratanytarsus inopertus gr. sp. 1
Description: Tergite II with 2, pigmented, triangle shaped areas of continous shagreen. Tergite III with long spinules in posterior half of segment. Tergite IV with an anteriomedial spine patch usually connected with long spinules to lateral spine patches. Tergite V with 2, anteriomedial spine patches; VI blank. Frontal setae well-developed, flattened setae; cephalic tuberacles small bumps. Prealar tuberacle not well developed or absent. Dorsocentral seta 1 taeniate and strong, but not as strong as 2-4. There are some intermediate dorsocentral seta 1 between species 1 and 3 (Fig. 134).
Notes: To be species 1 dorsocentral seta 1 must be a strong and long taeniate seta. The degree of spinulation on tergite IV varies, if any long spinules are present than it was classified as species 1. Some specimens had weak anteriomedial spine patches on tergite VI.


Figure 134. Tergites III-IV and part of tergites II and V of *Paratanytarsus inopertus* gr. sp. 1.

Paratanytarsus inopertus gr. sp. 3
Description: Spine patches on tergites IV-V a bit weaker than those present in species 1. Dorsocentral seta 1 much smaller than 2-4 and much smaller than the seta present in *P. inopertus* gr. sp. 1. Prealar tuberacle is absent (Fig. 135).

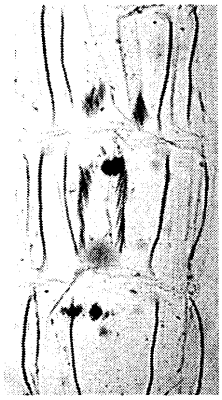


Figure 135. Tergites III-V of *Paratanytarsus inopertus* gr. sp. 3.

Paratanytarsus inopertus gr. sp. 4
Description: Tergite III with long spines; IV with anterior spine patch with short spines and 2 lateral spine patches with elongate spines. Tergite V with paired, anterior, spine patches of short spines and 2 lateral patches with elongate spines; VI with paired spine patches that are weak (Fig. 136).
Notes: R. W. Bouchard Jr. did not collect this species.

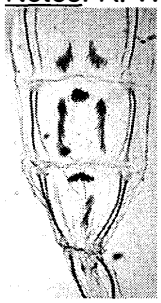


Figure 136. Tergites III-VI of *Paratanytarsus inopertus* gr. sp. 4.

Paratanytarsus (cf. *laccophilus*)
Description: Tergite IV with "U" shaped spine patch that bends very gradually, V with 1 pair of horizontaly elongated spine patches. No spine patch(es) on tergite VI, respiratory organ very small. Dorsocentral seta 2 with setal socket that is larger in diameter and darkened with pigment around its circumfrance. Each anal lobe with fringe of approximately 14 flattened and well-developed setae (Figs. 137-138).

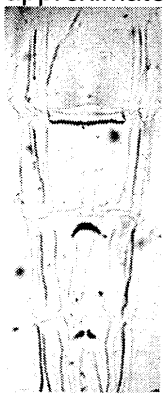


Figure 137. Tergites II-V of *Paratanytarsus* (cf. *laccophilus*).

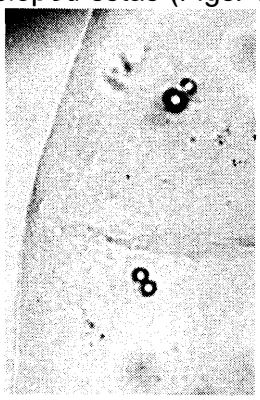


Figure 138. Setal sockets of dorsocentral setae 1-4 of *Paratanytarsus* (cf. *laccophilus*).

Paratanytarsus laccophilus gr. sp. 1
Description: Tergite III with shagreen better developed posteriorly; IV with 1 patch that looks like 2 patches stuck together because of some medial indentation in the spine patch. Tergite V is approximately the same as IV; VI with a poorly-developed spine patch. Dorsocentral setae 2-4 very long and well developed, 1 long and less well developed with 1-2 and 3-4 being close together, but both pairs being separated by a considerable distance. Frontal setae present on poorly-developed cephalic tuberacles. Prealar tuberacle poorly developed, anal comb with approximately 4 marginal spines, each anal lobe with fringe of well-developed setae (Fig. 139).
Notes: R. W. Bouchard Jr. did not collect this species.

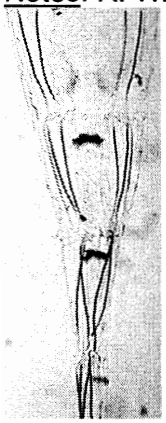


Figure 139. Tergites III-V and part of VI of *Paratanytarsus laccophilus* gr. sp. 1.

Paratanytarsus sp. 2
Description: Tergite III with shagreen and no elongated spines; IV-VI with 1, anteromedial, spine patch that decrease in size from anterior to posterior segments. Frontal setae present but not flattened, anal comb with approximately 5-7 sharp spines. Each anal lobe with fringe of approximately 45 setae (Figs. 126, 140).
Notes: I keyed this species to *austriacus* gr. in Wiederholm (1986).

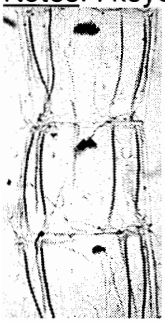


Figure 140. Tergites IV-VI of *Paratanytarsus* sp. 2.

Reotanytarsus distinctissimus
Reference: Lehmann 1970
Description: Tergite II with a distinct pair of medially directed points just anterior of the hook row, frontal apotome bumpy (Figs. 124, 141).


Figure 141. Tergite II of *Reotanytarsus distinctissimus*.

Rheotanytarsus sp. 1
Description: Respiratory organ long with small spines in apical half; apical end resembling a rat tail. Tergite II with small shagreen/spine patch anterior of the hook row. Round spine patches on tergites II-VI decreasing in size from anterior to posterior tergites (Fig. 142).
Notes: Spine patches on tergite VI can be very weak to absent.

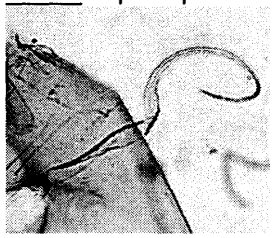


Figure 142. Respiratory organ of *Rheotanytarsus* sp. 1.

Stempellinella sp.
Reference: Wiederholm 1986
Description: Tergite II with 1 posterior-pair of shagreen patches, shagreen density on II is obvously not equal to that found on III. Each anal lobe with fringe of approximately 14-30 setae (Fig. 143).
Notes: No specimens of this genus were slide mounted, they were identified via a dissecting microscope only. The best way to distinguish this species from *Zavrelia* sp. is the shagreen density on tergites II-III, *Zavrelia* sp. has an approximately equally density on both segments.


Figure 143. Shagreen on tergites II-III of *Stempellinella* sp.

Tanytarsus confusus
References: Ekrem et al. 2003
Description: Tergite III with 1 pair of conspicuous, longitiudinal, spine bands that reach almost to the anterior edge of the tergite and migrate laterally in the posterior part of the tergite. Tergite IV with 1 pair of conspicuous, longitiudinal, spine bands that are approximately "C" shaped. Tergites V-VI with 1 pair of round, anterior, spine patches with patches on VI being smaller. Tergites III-VI with middle, lateral seta the strongest.

Respiratory organ long and slender, on a tuberacle, and many small spinules are scattered throughout the organ. Prealar tuberacle large and bulbous, dorsocentral setae in 2 pairs with dorsocentral 4 strong and very long, pedicle sheath tuberacle small. Precorneal setae in a triangle pattern with seta 1 smaller than seta 2, cephalic tuberacle small and broad. Anal comb with approximately 10 marginal spines, each anal lobe with fringe of approximately 50 setae and with shagreen (Figs. 123, 130).

Tanytarsus lobiger
References: Ekrem et al. 2003
Description: Tergite II with "Π" shaped shagreen patch, III with 1 pair of diagonal bands of long spines that are posteriorly directed. Tergite IV-VI with 1 pair of circular spine patches that have spines that are elongated a little more than other *Tanytarsus* sp. Tergite IV sometimes with a spine patch that is elongated, with spine patches decreasing in size from IV-VI. No taeniate, lateral setae on tergite III-V; VII with 2-3 taeniate, lateral setae; VIII with 5, teaniate, lateral setae. Respiratory organ small and slender and on a pestisal with small spines scattered throughout. Prealar tuberacle low or absent, dorsocentral setae in 2 pairs, pedicle sheath tuberacle well developed. Precorneal setae in a triangular pattern with anterior seta longer than others, cephalic tuberacles are low mounds. Anal comb with approximately 5 marginal teeth (Fig. 144).

Figure 144. Tergites II-VI of *Tanytarsus lobiger*.

Tanytarsus neoflavellus
References: Ekrem et al. 2003
Description: Tergite II with 1 pair of large shagreen patches. Tergite III with 1 pair of conspicuous longitiudinal spine bands that migrate laterally in the posterior part of the tergite. Tergite IV with 1 pair of conspicuous, longitiudinal, spine bands that are approximately question mark shaped. Tergites V-VI with spinules in oval, paired patches; VIII with a horizontal stripe. Respiratory organ long and skinny with setae that can be difficult to see. Prealar tuberacle well developed, dorsocentral seta 4 more robust than others, pedicle sheath tuberacle weak. Cephalic tuberacle low and conical. Anal comb with approximately 6 marginal spines, each anal lobe with fringe of approximately 10-14 setae (Figs. 145-146).
Notes: The horizontal stripe on segment VIII, a well-developed prealar tuberacle, and anal lobe fringe with approximately 10-14 setae are very good characters for identifying this species. There were several specimens in which the horizontal stripe was not quite complete, in at least on of these specimens the pedicle sheath tuberacle was strong.

Figure 145. Tergites III-V and part of VI of *Tanytarsus neoflavellus*.

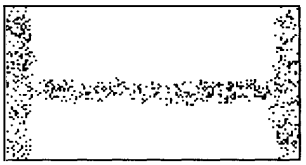


Figure 146. Tergite VIII of *Tanytarsus neoflavellus*.

Tanytarsus sepp
References: Ekrem et al. 2003
Description: Tergite II with 1 pair of large shagreen patches. Tergite III with 1 pair of conspicuous, longitiudinal, spine bands that migrate laterally in the posterior part of the tergite and are restricted to the posterior part of the segment; an anterior shagreen patch is also present. Tergite IV with 1 pair of conspicuous, longitiudinal, spine bands that are approximately "C" shaped. Tergite V-VI with 1 pair of circular spine patches, taeniate lateral setae on segments V-VIII numbering: 1, 1, 3, 5 respectively. Respiratory organ long and possessing spines, prealar tuberacle small, dorsocentral setae in 2 pairs with the anterior pair the longest. Precorneal setae in a triangular pattern with seta 1 the largest, cephalic tuberacles small. Anal comb with 5-6 marginal spines, each anal lobe with fringe of greather than 35 setae. Ecdysial suture region with irregularly-pointed or rounded warts or spines (Fig. 147).
Notes: A key difference between species 16 and *T. sepp* is the number of taeniate setae on each segment. Taeniate setae on segments V-VIII numbering: 0, 0, 2, 5 respectively in species 16.

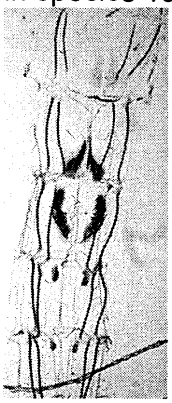


Figure 147. Tergites II-VI of *Tanytarsus sepp*.

Tanytarsus wirthi
References: Ekrem et al. 2003
Description: Tergite with 1 pair of anterior and 1 pair of posterior triangle patches. Posterior patches with darker triangles and becoming more broad posteriorly. Tergites III-VI with 1 pair of elongate, oval, spinule patches decreasing in size anteriorly to posteriorly with patches on tergite IV possibly larger than those on III. Taeniate, lateral

setae on segments VI-VIII numbering: 1, 2-3, 5 respectively. Respiratory organ long, middle half of organ with spines longer than horn's width at this point. Prealar tuberacle low, dorsocentral setae arranged in 2 pairs with seta 4 being robust, pedicle sheath tuberacle pointed and well developed. Precorneal setae in an approximately triangle pattern, with the most anterior seta being the strongest. Anal comb with approximately 5-6 marginal spines (Fig. 148).

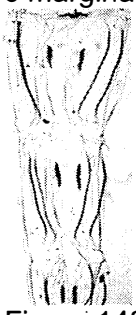


Figure 148. Tergites III-IV and part of V of *Tanytarsus wirthi*.

Tanytarsus sp. 1 (cf. *gregaris*)
Description: Tergite II shagreen with approximately "Π" shaped patch with brown pigment on the posterior part of the patch. Tergite III-IV with 1 pair of conspicuous, longitudinal, spine bands containing long spines; IV with spine bands broader anteriorly and possessing longer spines posteriorly. Tergites V-VI with 1 pair of anterior, oval, spine patches possessing short spines. Tergite VIII with 5, taeniate, lateral setae and shagreen. Anal lobe shagreen more pronounced than shagreen on VIII. Respiratory organ on round pedestal, long, and without setae. Prealar tuberacle large and well developed, dorsocentral setae in 2 widely separated pairs with seta 2 and 4 heavier than others, pedicle sheath tuberacle moderately developed. Precorneal setae in triangle pattern with seta 1 large and thick, cephalic tuberacles present. Anal comb with approximately 8-12 spines, each anal lobe with dense fringe of setae (Fig. 149).

Figure 149. Tergites III-V and part of II and VI of *Tanytarsus* sp. 1 (cf. *gregaris*).

Tanytarsus sp. 10
Description: Tergite II with well-developed shagreen patch containing a bald section in the lower middle part of the tergite. Tergite III with 1 pair of conspicuous, longitudinal, spine bands, with anterior shagreen patch near the bands' anterior ends. Tergite IV with 1 pair of conspicuous, longitudinal, spine bands containing larger spines in the posterior part of each band. Tergites V-VI with 1 pair of spine patches containing smaller spines; taeniate lateral setae on segments V-VI and VIII numbering: 3, 3, 4 respectively. Respiratory organ without spines and on a pedestal. Prealar tuberacle well developed,

dorsocentral seta 4 the strongest. Cephalic tuberacles low mounds, anal comb with approximately 4-5 spines, each anal lobe with fringe of many setae (Fig. 150).

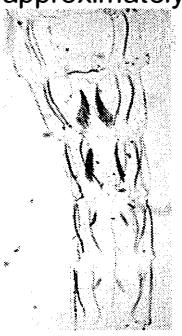


Figure 150. Tergites II-VI of *Tanytarsus* sp. 10.

Tanytarsus sp. 11
Description: Tergite II shagreen pattern composed of an anterior group of spines and 2 dark triangles of spines becoming broader posteriorly, with little to no connectivity between anterior and posterior spine groups. Tergite III with 1 pair of longitudinal, spine bands that are only slightly curved and contain moderately long spines. Tergites IV-V with 1 pair of anteriorly to posteriorly elongated patches containing short spines, with no spine patchs on VI. Respiratory organ broad basially with 1 row of fringe. Prealar tuberacle moderately developed, dorsocentral setae in 2 widely separated pairs with setae 2 and 4 slightly larger, pedicle sheath tuberacle appears to be weak. Cephalic tuberacles long and finger like, anal comb well developed (Fig. 151).
Notes: This species is in the *mendax* species group. The lack of a spines on tergite VI and the basially broad respiratory organ with a single row of fringe are key characters for identifying this species.

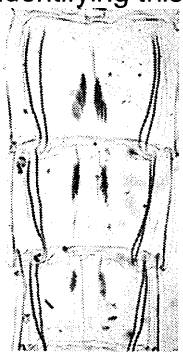


Figure 151. Tergites III-V of *Tanytarsus* sp. 11.

Tanytarsus sp. 12
Description: Tergite II with 1 pair of large shagreen patches. Tergites III-IV with 1 pair of conspicuous, longitudinal, spine bands containing long spines. Tergites V-VI with 1 pair of circular spine patches containing small spines; respiratory organ relatively long, without setae or spines and on a pedestal. Prealar tuberacle poorly developed; dorsocentral setae in 2 widely separated pairs, with seta 4 large and robust; pedicle sheath tuberacle small. Precorneal seta 1 the largest with 2-3 close together, cephalic tuberacle poorly developed or absent. Anal comb with approximately 5 marginal spines, each anal lobe with fringe of approximately 22 setae (Fig. 152).
Notes: Similar to species 10; ecdysial suture region with warts that are sometimes absent or reduced.

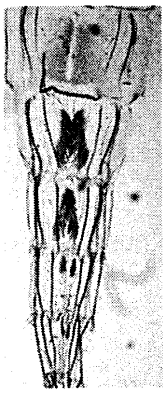


Figure 152. Tergites II-VI of *Tanytarsus* sp. 12.

Tanytarsus sp. 16
Description: Tergite II containing shagreen; III with 1 pair of conspicuous, longitiudinal, spine bands that migrate laterally in the posterior part of the tergite. Tergite IV with 1 pair of conspicuous, longitiudinal, spine bands that are approximately "C" shaped; V-VI with 1 pair of circular spine patches containing small spines. Taeniate lateral setae on segments V-VIII numbering: 0, 0, 2, 5 respectively. Respiratory organ relatively long with small spines scattered over whole length of the organ. Prealar tuberacle poorly developed, pedicle sheath tuberacle moderately developed. Precorneal setae in a triangle pattern with the anterior most seta the largest, cephalic tuberacle low to absent. Anal comb with approximately 7 marginal spines, each anal lobe with fringe of approximately 35-40 setae. Ecdysial suture region with warts (Fig. 153).
Notes: A key difference between species 16 and 21 is the prealar tuberacle, which is well developed in species 21. A key difference between species 16 and *T. sepp* is the number of taeniate lateral setae on each segment. Taeniate lateral setae on segments V-VIII numbering: 1, 1, 3, 5 respectively in *T. sepp*.


Figure 153. Tergites III-VI of *Tanytarsus* sp. 16.

Tanytarsus sp. 19
Description: Tergite II with 1 pair of strong, anterior, spine patches. Tergites III-VI with 1 pair of elongated spine patches. Taeniate lateral setae on segments VI-VIII numbering: 3, 4, 4 respectively. Respiratory organ long and without spines or setae. No prealar tuberacle, dorsocentral seta 4 well developed when compaired to other setae. Cephalic tuberacles are short finger like projections; anal comb with approximately 8 marginal spines (Fig. 154).


Figure 154. Tergites II-IV and part of V of *Tanytarsus* sp. 19.

Tanytarsus sp. 21
Description: Tergite II with "( )" shagreen pattern. Tergite III with 1 pair of conspicuous, longitiudinal, spine bands that migrate laterally in the posterior part of the tergite. Tergite IV with 1 pair of conspicuous, longitiudinal, spine bands that are approximately "C" shaped; V-VI with 1 pair of circular spine patches containing small spines. Taeniate lateral setae on segments V-VIII numbering: 1, 1, 2-3, 5 respectively. Respiratory organ moderately sized with small scattered spines and positioned atop a pedicel. Prealar tuberacle quadrate and well developed, dorsocentral setae all equal in size, pedicle sheath tuberacle moderaltly well developed. Cephalic tuberacles are low mounds. Anal comb with approximately 5-10 marginal spines and each anal lobe with fringe of approximately 50 setae (Fig. 155).
Notes: A key difference between species 16 and 21 is the prealar tuberacle, which is weak in species 16.


Figure 155. Tergites III-V and part of VI of *Tanytarsus* sp. 21.

Tanytarsus sp. 22
Description: Tergites II-VI with 1 pair of round spine patches containing small spines; II with a relatively-weak pair. Taneiate lateral setae on segments V-VIII numbering: 1, 1, 3, 5 respectively. Respiratory organ large, long, and ribbon to whip like. Dorsocentral setae in 2 well seperated pairs; seta 4 strong and conspicuous with setae 1-3 much less well developed. Cephalic tuberacles absent, anal comb well developed, each anal lobe with fringe of approximately 25 setae (Fig. 156).

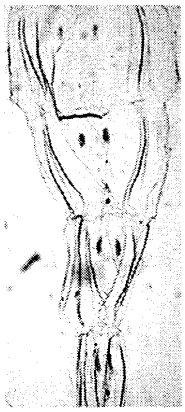


Figure 156. Tergites II-IV and part of V of *Tanytarsus* sp. 22.

Tanytarsus sp. 24
Description: Tergite II shagreen with approximately "Π" shaped patch with brown pigment on the posterior part of the patch. Tergite III with 1 pair of longitiudinal spine bands longer than those bands on IV. Anterior half of tergite IV spine band with small lateral spines much stronger than those small lateral spines present on III. Tergite V with 1 pair of circular spine patches containing small spines, VI without spine patches; segment VIII with 5 ,taeniate, lateral setae. Respiratory organ broad and amorphous with a conspicuous textured area between the ecdysial stuture and the base of the organ. Prealar tuberacle well developed, dorsocentral setae 1 and 3 weak with 2 and 4 robust, pedicle sheath tuberacle well developed. Cephalic tuberacles long and tubular, anal comb with approximately 5 marginal spines (Fig. 157).
Notes: Two key characters for identifying this species are a lack of spine patches on tergite VI and the texture patch near the ecdysial suture. However, other *Tanytarsus* sp. also have a texture patch.


Figure 157. Tergites III-IV and part II and V of *Tanytarsus* sp. 24.

Zavrelia sp.
Reference: Wiederholm 1986
Description: Tergites II-VI with dense shagreen coverage, shagreen density on II is approximately equal to that found on III. Each anal lobe with fringe of approximately 17-20 setae (Figs. 121, 125).
Notes: Only one specimen slide mounted; at present R. W. Bouchard Jr. has not created morphospecies for this genus. The best way to distinguish this species from *Stempellinella* sp. is the shagreen density on tergites II-III, *Stempellinella* sp. has an unqual density of shagreen on II when compaired to III.

Key to the Genera of Tanypodinae Pupae Collected in Hardwood Creek:

1. Anal lobe with conspicuous spines on its outer margins. In male exuviae the genital sacs do not extend beyond half the length of the lobe. Anal lobes possessing rounded inner and/or outer margins, with the lobes touching or approximately touching medially and resembling a "paddle" (Fig. 158). ---------- ***Procladius* sp. (p.)**
1. Anal lobe with or without spines on its outer margins. In male exuviae the genital sacs usually extend beyond half the length of the lobe. Anal lobes smaller or pointed apically and not resembling a "paddle" (Figs. 159, 163, 165-166, 170-171). ---------- **2**

2. Anal lobe less than 2 times long as broad; no medial scar on tergite I (Fig. 159). ---------- ***Tanypus* sp. (p.)**
2. Anal lobe approximately or conspicuously greater than 2 times long as broad; medial scar present or absent on tergite I (Figs. 160, 163, 165-166, 170-171). ---------- **3**

3. All segments but VIII with 1 small and 1 large lateral setae situated close together (Fig. 161). ---------- ***Natarsia* sp. (p.)**
3. Lateral setae not paired and not situated together. ---------- **4**

4. Respiratory organ larger and sometimes very wide, the lumen of the organ is approximately filled by the horn sac. Plastron plate conspicuously reduced, thoracic comb is present (Figs. 162, 167). ---------- **5**
4. Respiratory organ smaller, tube, trumpet, or club shaped. The lumen of the organ not filled by the horn sac, plastron plate and thoracic comb are present or absent (Figs. 167-168, 172-174). ---------- **7**

5. Segment VIII with posterolateral corners posteriorly elongated. Conspicuous cone or thorn shaped basal lobe, postnotal tubercle present (Figs. 163-164). ---------- ***Guttipelopia* sp. (p.)**
5. Segment VIII without elongated corners, poorly developed or rounded basal lobe, no postnotal tubercle (Figs. 162, 167). ---------- **6**

6. Anal lobe less than 3 times long as broad, respiratory organ with a relatively-conspicuous reticulate pattern, lumen 100% filled by the horn sac, basal lobe poorly developed (Figs. 162, 165). ---------- ***Ablabesmyia* sp. (p.)**
6. Anal lobe greater than 3 times long as broad, respiratory organ without or with a faint reticulate pattern, lumen not 100% filled by the horn sac, basal lobe conspicuous and well developed (Figs. 166-167). ---------- ***Labrundinia* sp. (p.)**

7. Tergites with shagreen spinules containing 2 or more branches. Respiratory organ with a large plastron plate that is as wide or wider than the maximum width of the entire organ (Figs. 168-169). ---------- ***Conchapelopia* sp. (p.)**
7. Tergites with shagreen spinules containing 0 branches, or shagreen is absent. Respiratory organ and plastron plate variable. ---------- **8**

8. Anal lobe tapered and approximately pointed at apex, the tapered ends are bent so the apex of each lobe points medially. Segment VII with 1, taeniate, lateral seta; tergites with 1 or more posterior rows of spines or tuberacles. Male exuviae with conspiclusly long and tapering genital sacs (Fig. 170). ---------- ***Nilotanypus* sp. (p.)**

8. Anal lobe with apex not pointed medially. Segment VII with greater than 1, taeniate, lateral seta; tergites without posterior rows of spines or tuberacles. Male exuviae with variable genital sacs (Figs. 163, 165-166, 171). ---------- **9**

9. Small spines present on outer and inner margins of anal lobes (Fig. 171). ---------- **10**
9. Small spines present on outer margins of anal lobes, spines absent on inner margins of lobes. ---------- **11**

10. Corona present, respiratory organ with a thick-walled horn sac, plastron plate not very large (Figs. 171-172). ---------- ***Zavrelimyia* sp. (in part) (p.)**
10. Corona absent, respiratory organ with a thick-walled horn sac, plastron plate not very large (Fig. 171). ---------- ***Zavrelimyia* sp. (in part) (p.)**

11. Respiratory organ without lobed horn sac, slightly or not resembling the morphology of a brain. Tergites with single, small, shagreen spinules not in arched rows (Fig. 173). -- ---------- ***Paramerina* sp. (p.)**
11. Respiratory organ with lobed horn sac, approximately resembling the morphology of a brain. Tergites possessing shagreen spinules in arched rows (Figs. 174-175). ---------- ---------- ***Larsia* sp. (p.**

<u>Tanypodinae Figures</u>:

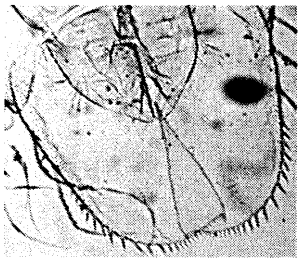


Figure 158. Anal lobe of *Procladius subletteildenticulatus*.

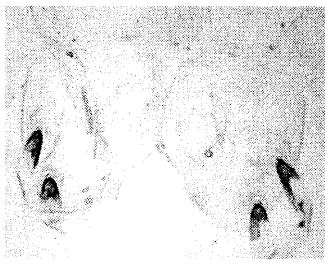


Figure 159. Anal lobe of *Tanypus* sp.

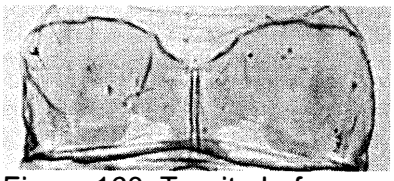


Figure 160. Tergite I of *Ablabesmyia mallochi*.

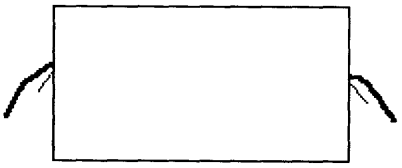


Figure 161. Lateral setae on segments I-VII of *Natarsia* sp.

Figure 162. Respiratory organ, basal lobe, and thoracic comb of *Ablabesmyia mallochi*.

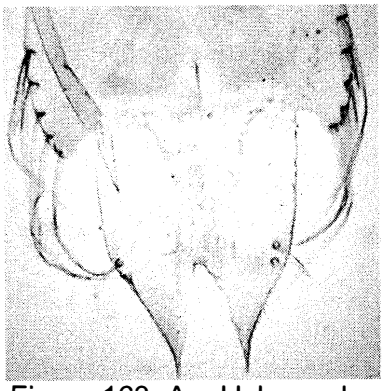


Figure 163. Anal lobe and part of segment VIII of *Guttipelopia* sp.

Figure 164. Basal lobe, postnotal tubercle, and respiratory organ of *Guttipelopia* sp.

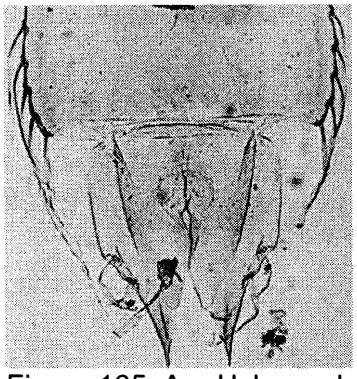


Figure 165. Anal lobe and part of segment VIII of *Ablabesmyia monilis*.

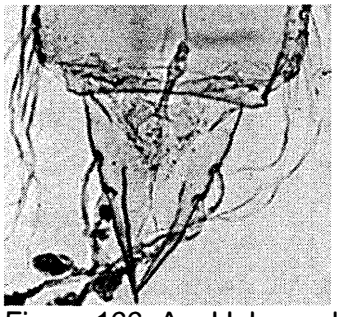


Figure 166. Anal lobe and part of segment VIII of *Labrundinia pilosella*.

Figure 167. Respiratory organ, basal lobe, and thoracic comb of *Labrundinia pilosella*.

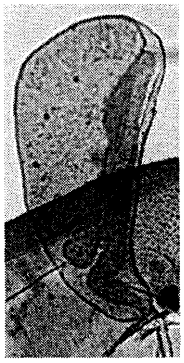


Figure 168. Respiratory organ of *Conchapelopia rurika*.

Figure 169. Shagreen spinules of *Conchapelopia* sp.

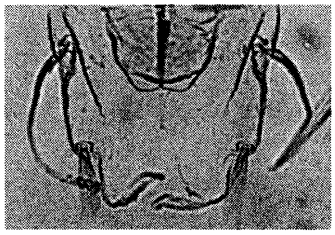


Figure 170. Anal lobe of *Nilotanypus fimbriatus*.

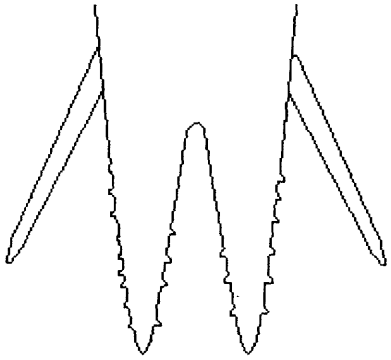


Figure 171. Anal lobe of *Zavrelimyia* sp.

Figure 172. Respiratory organ of *Zavrelimyia* sp.

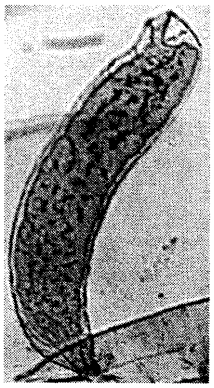


Figure 173. Respiratory oragan of *Paramerina* sp.

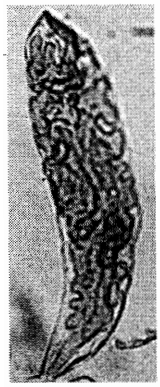


Figure 174. Respiratory organ of *Larsia* sp.

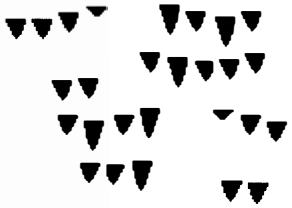


Figure 175. Shagreen spinules of *Larsia* sp.

Tanypodinae Taxa Descriptions:

Ablabesmyia mallochi
Reference: Roback 1985
Description: Brown veins in wing pad not distinct, much brown pigment in wing pads with veins usually not being descernable and having a spotty appearance. Mesal area of tergite IV with 3 white mesal spots, a variable and hard to see character in some specimens (Figs. 160, 162, 176).

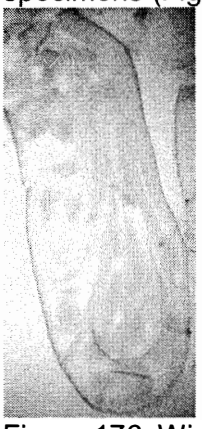


Figure 176. Wing pad of *Ablabesmyia mallochi*.

Ablabesmyia monilis
Reference: Roback 1985
Description: Wing pad with brown veins that are strong and distinct, connecting to the brown margin of the wing pad. Tergite IV without pigmentation on its lateral boarders. Base of M vein in wing pad with base usually absent, respiratory organ nipple short, not elongated; aeropyle tube apex club shaped (Figs. 165, 177).

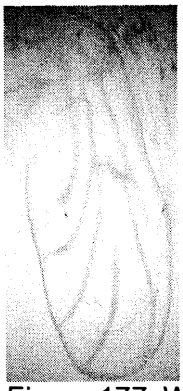


Figure 177. Wing pad of *Ablabesmyia monilis*.

Conchapelopia dusena
Reference: Roback 1981
Description: Corona present on respiratory organ; length of plastron plate less than half the total length of the organ. Taeniate lateral setae present on segments VII-VIII (Fig. 178).

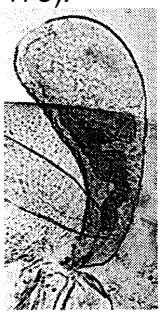


Figure 178. Respiratory organ and basal lobe of *Conchapelopia dusena*.

Conchapelopia rurika
Reference: Roback 1981
Description: Corona present on respiratory organ; length of plastron plate greater than half the total length of the organ. Taeniate lateral setae present on segments V-VIII; tips of anal lobes hooked (Fig.168).

Guttipelopia sp.
Reference: Wiederholm 1986
Description: Respiratory organ large and wide and in some specimens approximately 2 times longer than wide, with a conspicuous cone or thorn shaped basal lobe. Scar present on tergite I; VIII with posterolateral corners posteriorly elongated. Postnotal tubercle present (Figs. 163-164).
Notes: No specimens of this genus were slide mounted, they were identified via a dissecting microscope only.

Labrundinia pilosella
Reference: Roback 1987
Description: Respiratory organ with very distinct preapical groove and organ is approximately club shaped. Aeropyle tube in the preapical groove, tube not along the outer margin of the horn sac. Atrium of respiratory organ not "s" shaped (Figs. 166-167).

Larsia sp.
Reference: Wiederholm 1986
Description: Respiratory organ relatively unique because horn sac approximately resembles the morphology of a brain. Inner margin of anal lobe without teeth, and shagreen not in arched rows (Figs. 174-175).
Notes: Only one specimen slide mounted; at present R. W. Bouchard Jr. has not created morphospecies for this genus.

Natarsia sp.
Reference: Wiederholm 1986
Description: Scar present on tergite I, no thoracic comb. Respiratory organ greater than 3 times as long as wide with the apical end swollen to accomidate the plastron plate; organ on top of a tubercle (Figs. 161, 179).
Notes: No specimens of this genus were slide mounted, they were identified via a dissecting microscope only.

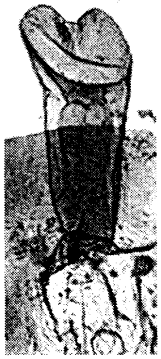


Figure 179. Respiratory organ and tubercle of *Natarsia* sp.

Nilotanypus fimbriatus
Reference: Roback 1986
Description: No spines on tip of respiratory organ, tergites I-VIII clear to dark in color. Tergites with mesal and lateral infuscation (Fig. 170).
Notes: This species and *N. americans* are similar but the known distribution of *N. americans* is the southeastern United States.

Paramerina sp.
Description: Tergites II-V and sometimes VI with 2 pairs of lightened, circular areas lateral of the midline of each tergite; near the center of each circle there is at least 1 seta or a setal scar. The base of each antennal sheath has a very long and well-developed seta (Fig. 173).
Notes: I looked at all my specimens and they all appear to be the same species which is described above. At present R. W. Bouchard Jr. has not created morphospecies for this genus.

Procladius bellus
Reference: Roback 1980
Description: Anal lobe with inner corner that is usually rounded, exuviae clear (Fig. 180).

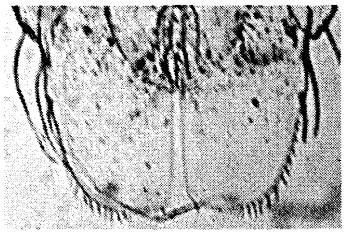


Figure 180. Anal lobe of *Procladius bellus*.

Procladius freemani
Reference: Roback 1980
Description: Atrium of respiratory organ occupying much, but not the entire horn sac; organ with spines that are denser when compared to *P. subletteil denticulatus*. Anal lobe concave toward inner apex, spines on each lobe broad and close together. Abdomen with pigmentation, not clear (Fig. 181).

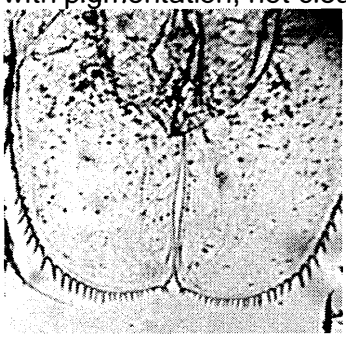


Figure 181. Anal lobe of *Procladius freemani*.

Procladius subletteil denticulatus
Reference: Roback 1980
Description: Respiratory organ with triangular spines that are relatively well spaced, neck of organ visible. Atrium of respiratory organ occupying all to most of the horn sac. Anal lobe straight toward inner apex, spines on lobes widely spaced, abdomen with pigmentation, not clear (Fig. 158).

Tanypus sp.
Reference: Wiederholm 1986
Description: Anal lobe reduced in size. Respiratory organ not symmetrical and with no plastron plate, horn sac filling 100% of the lumen and possessing a reticulated pattern. Segments VII-VIII with posterolateral corners posteriorly elongated (Figs. 159, 182).
Notes: Only one specimen slide mounted; at present R. W. Bouchard Jr. has not created morphospecies for this genus. There is much variation in the characters representative of this genus.


Figure 182. Slightly damaged respiratory organ of *Tanypus* sp.

Zavrelimyia sp.
Reference: Wiederholm 1986
Description: Small spines always present on outer and inner margins of anal lobes. Respiratory organ elongated anteriorly to posteriorly and usually 3 to 6 times longer than wide, basal lobe well developed. Lateral taeniate setae on segments VII-VIII numbering: 4, 5 respectively (Figs. 171-172).
Notes: All 3 specimens that were mounted appeared to be the same species; at present R. W. Bouchard Jr. has not created morphospecies for this genus.

Acknowledgements

I would like to acknowledge the help and taxonomic expertise of my advisor Dr. Len C. Ferrington Jr. and R. W. Bouchard Jr. during the production of this document. Mr. Bouchard was gracious in providing me with his pupal exuviae morphospecies descriptions and vouchers which were invaluable resources throughout this project. I would also like to acknowledge the 17 pictures taken by Moriya Rufer. These pictures are figures: 5, 9, 27-28, 39, 62, 70-71, 74-75, 85, 129, 159, 163, 176-177, and 182.

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