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METAL CONCENTRATIONS IN BENTHIC INVERTEBRATES IN PEATLANDS

by

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Thesis submitted to the
School of Graduate Studies and Research
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in partial fulfilment of the requirements for the
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ABSTRACT

In order to assess the influence of various abiotic and biotic factors on metal accumulation in peatlands, insects were collected from bogs, mineral poor fens and circumneutral fens located in Central Ontario. The peatlands represented a gradient in alkalinity from 0 (acid bogs) to 200 $\mu eq \cdot L^{-1}$ (circumneutral fens). Further, the peatlands had important hydrological differences with no obvious inflows in the bogs and inflow/outflow streams in the fens. Given these contrasting environments, it was hypothesized that there would also be differences in metal accumulation in the associated biota.

Metal concentrations in Chironomidae (Order: Diptera) larvae were compared among peatlands by One-way Nested ANOVA. Mn and Fe concentrations were greatest in larvae from the fens. Calcium, Al, Zn and Cu did not show clear trends, however concentrations of these elements were consistently higher in larvae from peatlands with low organic matter. Significant negative correlations were observed between organic matter in the sediment and larval Zn and Mn concentrations. Metal concentrations were also examined in *Libellula* nymphs (Order: Odonata). Concentrations were generally highest in the mineral poor fen, Arrowhead, where peat organic matter content was the lowest. Nymphal Zn and Mn concentrations did not differ among peatlands.

In order to assess the potential transfer of metals to the terrestrial food chain, metal concentrations were determined in adult chironomids. Mn, Ca and Zn were readily transferred to the adult stage. In contrast, Al and Fe were greater in larvae, indicating that these metals are not transferred. The trend with Cu was split, with greater transfer to the adult life stage occurring in the bogs. From the adult concentrations, export values (# larvae$\cdot m^{-2}$ * metal concentration) were calculated for a standardized area in order to determine where the greatest potential export of metals could occur. The bog, Bor, showed the highest export of all metals except Mn. This bog had intermediate metal levels and high chironomid productivity. In addition, Bioconcentration factors (BCF) were calculated to assess biomagnification in the predacious *Libellula* nymph. BCFs showed no evidence of biomagnification for any of the metals.

The results of this study indicate that the acidification of peatlands, whether natural or anthropogenic, will not lead to greater availability of metals such as Zn, Cu, Al, and Mn. High organic matter levels may serve to mitigate the effects of acidification on metal availability in such peatlands.

CHAPTER 1.0

INTRODUCTION

1.1 General Overview

The value of wetlands as resources and as environments for biological production has recently been recognised (reviewed in Canadian Committee on Ecological Land Classification, 1988). Still, there is a growing need for further knowledge of wetland dynamics and ecological relationships. Wetlands are prime breeding and nesting grounds for various fish, furbearing mammals and migratory and non-migratory birds (Gorham et al., 1984). The National Wetlands Working Group of Canada recently reported that over 45 species of waterfowl, 155 species of birds and 50 types of mammals depend on wetland systems (Canadian Committee on Ecological Land Classification, 1988). Wetlands also serve a number of other important functions, such as water storage, surface and ground water recharge, water purification and protection against erosion (Environment Canada, 1986). In spite of their ecological importance, millions of hectares of wetlands in Canada have been drained or filled for agriculture, highways, housing and industrial uses (Environment Canada, 1986).

Additional stresses to wetlands in geologically sensitive areas, such as metal contamination and acidic deposition, may further reduce the area suitable for wildlife use. The main industrial sources of atmospheric metal pollution are the mining, smelting and refining of metals, the burning of fossil fuels, and the production and use of metallic commercial products (Nriagu, 1990). Acidic deposition exacerbates the hazards associated with trace metal pollution. This occurs by increasing dissolved metal concentrations in aquatic systems through recruitment from lake sediments and by mobilizing metals from the watershed (National Research Council of Canada, 1988).

Wetlands have traditionally been perceived as sinks for metals because the peat is rich in organic matter. Indeed, artificial wetlands are constructed to deal specifically with acid mine drainage (Dunbabin and Bowmer, 1992). Metal pollution tends to accumulate primarily on the surface layers of the sediment in lakes (Nriagu, 1990). Similarly, metal pollution will also accumulate on peat surfaces in peatlands. It is important to determine what will happen to these metals when increased acidity begins to uncouple complexed metals (Dunbabin and Bowmer, 1992).

Wetlands are generally classified according to their hydrogeological characteristics (for example, presence of inflows and outflows, ground water influence, etc.). When peat accumulations exceed 40 cm they are referred to as peatlands. Bogs and fens are the most common peatland habitats in the boreal zone (Rosenberg et al., 1988), with the former usually having greater peat accumulation as well as a number of other distinguishing chemical differences. Bogs tend to be strongly acidic ($pH < 4.5$), whereas fens are commonly circumneutral ranging from moderately acidic (pH rarely < 4.5) to strongly alkaline (Gorham et al., 1984). Bogs have no significant inflow or outflow streams and receive their mineral supplies mainly from the atmosphere (Mitsch and Gosselink, 1986). In bog pools hydrogen ions are the most abundant cations followed by Ca, Mg, Na and Al. Iron, K and Mn are also present, but usually represent less than 5 percent of the cations (Blancher and McNicol, 1987).

Fens receive their mineral supply from atmospheric sources and from water that has percolated through mineral soil (Gorham et al., 1987). They can be classified as either minerotrophic (high alkalinity, high Ca, and K) or mineral poor (low alkalinity, low Ca). In fen pools Ca ions are the dominant base cation comprising over 50 percent of all cations followed by Mg, Na and Al. Hydrogen ions represent only a small proportion of the cations in these systems (Blancher and McNicol, 1987). In addition, bogs and fens differ in vegetation. Bogs are dominated by *Sphagnum* and *Chamaedaphne* whereas fens are dominated by *Carex* and *Typha* (Table 1).

The following study focuses on three types of peatlands typical of temperate and boreal regions: acidic (ombrotrophic) bogs, mineral poor fens and circumneutral (minerotrophic) fens. The mineral poor systems are included because they are intermediate in water chemistry and vegetation and can be regarded as transitional systems (Clausen and Brooks, 1983).

Gorham et al., (1987) believe that these mineral poor peatlands are the most susceptible to acidification.

1.2 Wetlands and Acidification

Research on the effects of acidification has primarily focused on lakes and streams. Peatlands have been largely overlooked. Although peatlands may be slightly acidic because of naturally derived organic acids (produced from the partial decomposition of plant material) (Gorham et al., 1987), it is important to examine the effects of further acidification of these systems.

Peatlands may be viewed as storage reservoirs for acidity. For years, or perhaps decades, these systems may accumulate sulphate and nitrate; in doing so they delay the acidification of receiving waters (Wood and Rubec, 1989). In the short term, many peatlands appear to neutralize acidic inputs through biological uptake of nitrate and through microbial sulphate reduction (Gorham et al., 1985)). According to Gorham et al., (1987), nutrient poor fens are more sensitive to damage from acidification than other types of peatlands because base saturation in these systems is low. As storage capacity is eventually exceeded (base saturation decreases as organic acids increase), pH decreases, and changes in peat geochemistry may occur. The storage effect delays the recovery of receiving waters (once reductions in deposition have occurred) by releasing historically stored sulphur (Wood, 1989b). Although most lakes may be capable of neutralizing natural acid input from bog drainage, lake waters, such as those on the Precambrian shield, may be especially vulnerable (Gorham et al., 1985).

Peatlands undergo a natural acidification process that transforms circumneutral fens to acidic bogs (Gorham et al., 1987). This transition may be quite rapid, in the order of two to three decades, once a fen has reached critically low concentrations of Ca (Gorham et al., 1987). When the transformation occurs, pH, Ca and alkalinity decline. The surface of the peatland eventually rises because of increased peat accumulation. The system becomes more isolated from ground water sources and therefore from nutrient supplies (Gorham et al., 1987). These raised bogs are referred to as ombrotrophic (Wood and Rubec, 1989).

The transformation from fen to acidic bog is relatively rapid and may explain the bimodal pH frequency distribution observed in peatlands in northern Minnesota (Wood, 1989b) and northern Sweden (Gorham et al., 1984). A greater number of peatlands have been observed with pH 4 and 6, and a relatively low frequency of peatlands have been observed with intermediate pH values (Wood, 1989b).

Anthropogenic acidification of fens may produce similar changes in water chemistry and accelerate natural acidification. Weakly acidic fens of low alkalinity are of particular concern because they may be further acidified by acid deposition (Gorham et al., 1984, Anderson 1986). Increased mineral acidity favours the invasion of acidophilic *Sphagnum*. This invasion represents a critical stage; *Sphagnum* further acidifies its environment by the uptake of cations from solution and the release of H+ (Gorham et al., 1985). Its invasion acts as a positive feedback mechanism since it reduces alkalinity and encourages the development of oligotrophic conditions (Gorham et al., 1987). These changes ultimately result in an acidic bog. Accompanying the above changes in water chemistry will undoubtedly be changes in metal geochemistry. This in turn could influence metal availability to biota. It is therefore important to consider the behaviour of metals in acidifying peatlands.

Metals in aquatic systems

Through both natural and anthropogenic events, metals often accumulate to high levels in sediments (National Research Council of Canada, 1988). In lakes, metals are partitioned among three main sediment components: Fe oxyhydroxides, Mn oxides and organic matter (Luoma, 1983). Relative amounts of the three sediment components have been found to result in differences in metal accumulation by benthic invertebrates (Bendell-Young and Harvey, 1991a). This may also be true for benthic invertebrates in peatlands where differences in peat geochemistry (organic matter and Mn and Fe oxide content) may also affect metal bioavailability. However, one feature that distinguishes peatlands from lakes is the presence of greater concentrations of organic acids in peatlands (see comparison in discussion section).

The role of these organic acids in peatlands is not fully understood, and it is difficult to predict the effects of humic substances on the toxicity and bioaccumulation of metals (Winner, 1984). Kerndorff and Schnitzer (1980) characterized the sorption strength of various metals on humic acids in soils. In pH 4.7 and 5.8 solutions the sorption order was the following: Fe=Cu=Pb=Al>Cd>Zn>Mn. According to Saar and Weber (1982) dissolved organic matter can release metal ions that have been adsorbed on sediments (by Fe and Mn oxides) as well as sequester metal ions in solution (Wood, 1989a). Nutrients may also be sequestered by organic acids. The potential effect of organic material on the availability of metals to biota may play an important role in influencing metal concentrations in invertebrates.

1.3 Metal Concentrations in Benthic Invertebrates and Implications for Food Chain Transfer

In addition to their influence on metal availability, sediment and peat are an important habitat for a number of aquatic organisms. Chironomidae (Order: Diptera) is a relatively abundant family of invertebrates in bogs and fens (Rosenberg and Danks, 1987). The larvae generally live in the upper 10 cm of the sediment (Oliver, 1971). Because they are closely associated with the sediment and may ingest sediment with their food, the larvae bioconcentrate contaminants that have accumulated in the sediment (National Research Council of Canada, 1988). Trace metals concentrated in larval tissues may then be removed from the peat upon insect emergence. The significance of this removal in decreasing concentrations of contaminants in aquatic systems and the potential effects on terrestrial organisms are not fully understood. Recent studies examining the transfer by Chironomidae have been conducted by Bendell-Young and Harvey (1988), Krantzberg and Stokes (1988) and Timmermans and Walker (1989).

Chironomidae are holometabolous insects. Their biology is well known with many species widely distributed (Pinder, 1986). Most species are uni- or bivoltine (Pinder, 1986). The largest part of the life cycle is spent in the larval stage, and all the Chironominae, except a few predacious species, build larval cases on or within the substrate (Oliver, 1971). According to Leuchs and Neumann (1990) there are two characteristic feeding behaviours for chironomid larvae: deposit feeding on the surrounding peat at the tube entrance and filter feeding by pumping water with food particles into the tube. This activity is followed by grazing on the inner tube wall. In this manner, the larvae are exposed to metals in solution and to metals through ingestion of enriched peat (Luoma, 1983). The pupal stage is very brief, from a few hours to a few days. Upon maturation the pupa moves to the surface of the water and adult eclosion occurs. Most adults do not feed and live only long enough to reproduce (a few days to a few weeks)(Oliver, 1971).

Chironomids are suitable in studies of the effects of acidification and metal contamination because several species are extremely tolerant to low pH (Okland and Okland, 1986, Harvey and McArdle, 1986) as well as to moderate heavy metal pollution (Rossaro et al., 1986). Winner et al., (1980) noted that a large number of chironomid species from lakes could tolerate long-term exposure to multiple metal stresses that eliminated most other insect families.

In the acidic bogs studied by Walker et al., (1985) 70 percent of the benthic fauna were Chironomidae, specifically *Psectrocladius*, *Monopsectrocladius*, *Chironomus* and *Procladius sp.* Following the experimental acidification of a peatland in the Experimental Lakes Area, Bayley et al., (1988) noted that chironomid larvae represented nearly 50 percent of the standing stock in wet sediment cores. Wiederholm and Eriksson (1977) also found chironomids to dominate the benthic fauna (at all depths) in an acidified lake. Mollusca and other shell-bearing organisms, which are sensitive to acidification, were not found. More recently, Schell and Kerekes (1989) noted that several macroinvertebrate groups, such as Pelecypoda, Hirudinea and Gastropoda, were absent from aquatic systems with $pH < 5.0$. The absence of these invertebrate orders may result directly from H^+ stress or indirectly from pH-associated chemical and biotic factors (Schell and Kerekes, 1989).

Allard and Moreau (1987) noted that following the experimental acidification of a stream channel the only genus unaffected, of an entire lotic macroinvertebrate community, was *Microtendipes*, a member of the tribe Chironomini (of the subfamily Chironominae). This particular tribe is well known for its tolerance to various environmental perturbations. There is some evidence that their resistance to acidity is linked to the presence of a red haemoglobin pigment (Palawski et al., 1989). Wrubleski (1987) suggests that the presence of this pigment in larvae buffers acidity.

Chironomids are also important diet items for a variety of waterfowl and passerines, e.g. the tree swallow (*Tachycineta bicolor* (St. Louis et al., 1990). The availability of chironomids at certain times of the year (principally early spring) may also have considerable influence on the distribution and population size of some bird species (Wrubleski and Ross, 1989). This may be especially true during emergence periods when chironomids are easy prey for young waterfowl (Wrubleski and Ross, 1989).

In systems where metals are readily available, toxic metal accumulation may occur in insectivorous birds (Rossaro et al., 1986). In addition, because insects are 'processors' of large quantities of plant and animal tissues, Walton (1989) claims that they may influence the fate of contaminants in systems even when high body residues are lacking.

A second group of invertebrates, Anisoptera nymphs (Order:Odonata) are also abundant in peatlands. Odonata are hemimetabolous, the nymph having 10 to 15 instars (Westfall, Jr, 1978). The nymphal stage forms an important link in the aquatic food chain for fish and other aquatic invertebrates. A generalized carnivore, the nymph feeds on any aquatic organism of an appropriate size (including chironomids). The nymph may in turn be predated upon by a number of aquatic bird and fish species. Although little research has focused on pollution tolerance in Odonata, Herrmann (1991) noted that Anisoptera nymphs appear to be less affected or even favoured in acid conditions compared to most Crustacea, Mollusca and Ephemeroptera nymphs.

Chironomids and Odonates are important study organisms because of their abundance in aquatic systems and because of their importance as prey items for both aquatic and terrestrial predators. In addition, by examining trends in metal accumulation in an invertebrate predator, one can also determine whether metals biomagnify or whether they are reduced in successive trophic levels.

It is important to also measure the alkali earth metal Ca because of its significance in the diet of higher trophic level organisms (which consume chironomids) and because of its possible interaction with Al.

1.4 Specific Objectives

This research focuses on metal concentrations of larval Chironomidae, adult Chironomidae and Odonata nymphs from a range of peatlands of varying water chemistry. The specific objectives are listed below:

1) Determine whether concentrations of Zn, Cu, Al, Mn, Fe and the alkali earth Ca in larval Chironomidae and Odonata vary among peatlands and whether these differences are related to water chemistry parameters (pH, alkalinity, dissolved organic matter) and peat organic matter;
2) determine which of these metals are transferred from larval to adult Chironomidae and to what extent;
3) quantify the transfer of metals from Chironomidae to Odonata larvae in order to assess biomagnification; and
4) calculate export values for each metal for a standardized area of each peatland.

CHAPTER 2.0

MATERIALS AND METHODS

2.1.0 Field Methods

2.1.1 Study Area

This study follows from a research project conducted by L. Bendell-Young and F. R. Pick in 1990-1992. These authors selected 15 peatlands representing a range of water chemistry parameters and vegetation types in order to determine associated differences in metal biogeochemistry.

The nine peatlands are located in the Muskoka-Haliburton region of Ontario. This area of central Ontario receives moderate levels of acidic deposition with 62 $meq \cdot m^{-2}$ of sulphate deposition per year and an equivalent deposition of hydrogen ions (Lazerte and Burling, 1989). Latitude and longitude measurements, selected water chemistry parameters and dominant vegetation for the nine peatlands are presented in Table 2.1. Additional water chemistry parameters are found in Appendix 7.1.

The peatlands where initially chosen to represent a continuum from bog to fen and represented three main types of peatlands: bogs, mineral poor fens and circumneutral fens. The bogs typically had low pH (< 5.8) and low alkalinity (< 17.0 $\mu eq \cdot L^{-1}$). The circumneutral fens had pH > 6.0 and alkalinities that ranged from 52 - 203 $\mu eq \cdot L^{-1}$. In the mineral poor, or transitional fens, pH ranged from 5.75 to 6.50 and alkalinities from 21.5 to 68 $\mu eq \cdot L^{-1}$ (Fig. 2.1). Dominant vegetation differed among the peatland types with *Carex*, *Typha* and *Nuphar* dominating in fens and *Chamaedaphne* and *Sphagnum* dominating in bogs. The mineral poor peatlands had intermediate water chemistry with two peatlands dominated by sedges and one by *Sphagnum*.

2.1.2 Study Organisms

Table 2.2 represents the benthos collected with the Ekman grab. Two insect taxa were found in all the peatlands: Chironomidae (Order:Diptera) and Anisoptera (Order:Odonata). As it was necessary to obtain study organisms which were both abundant and also present across a range of conditions, these two taxa were selected for further analysis.

2.1.3 Invertebrate Collection

All glassware and plastic equipment used in the field and laboratory procedures were acid-washed i.e. kept in 6 percent HNO_3 for 24 hours and rinsed in distilled deionized water seven times.

Peatlands were sampled in the spring of 1991 from April to June. At each peatland benthic invertebrates were collected from three open water sites by Ekman grab. The Ekman sampled an area of 240.25 cm^2. At each site, three containers were filled with peat representing two to five grabs. After sieving the samples were placed in acid-washed jars with adequate peat to minimize mortality prior to laboratory sorting. Re-sampling was done in some peatlands due to low biomass (for example, Deer, Arrowhead and Simp). Fourth instar Chironomidae (Order:Diptera) were then identified to subfamily with greater than 90 percent belonging to the tribe Chironomini. Pooled larval samples (representing between 10 to 50 individuals) were frozen until metal analysis. Collected Odonata nymphs were frozen individually in vials until identification. Prior to metal analysis the nymphs were identified to generic level after the methods of Hutchison (Personal Communication, 1992).

Chironomid adults were collected using submerged transparent funnel traps (after the design of Davies, 1984) which sampled an area of 1029.22 cm^2. The traps, made of cellulose acetate, were weighted and consisted of an inverted funnel with a glass jar at the top where an air pocket simulated the air-water interface. Pupae moved into the funnel and emerged in the air pocket. Three traps were placed at three sites (where larvae were collected) in each peatland. Every two to five days the jars were emptied and adults were stored in polybags until laboratory sorting. Sampling effort was determined by adult abundance. In order to obtain the necessary biomass, many peatlands were resampled. Pooled samples were placed in vials and frozen until metal analysis. Triplicate samples of larvae and adults were analyzed for metals at each site for each peatland.

Identification

Identification to the subfamily level for chironomids was deemed sufficient to meet the study's objectives. The concept of 'taxonomic sufficiency' balances the level of identification against the need for information (Ferraro and Cole, 1992). In this study, further identification would have involved considerable manipulation of the organisms and may have introduced contamination. Furthermore, larval density did not permit duplicate sampling for more detailed taxonomic identification. Larvae collected were all members of the Chironominae and Tanypodinae subfamilies. Bendell-Young and Harvey, 1991a) noted no significant differences in metal concentrations for Zn, Cu, Al, Mn and Fe between larvae from the subfamilies Chironominae and Tanypodinae collected in lakes.

Odonata were identified to the generic level as this taxonomic level could be reached without great manipulation of the nymph. Seven genera were found in the peatlands with the most common genus being *Libellula* (Table 2.3). To determine possible size effects on metal concentrations, total nymph length and head capsule width were recorded.

2.2.0 Metal Analysis

Chironomidae

Pooled samples of Chironomidae were dried at 60° C for 24 hours in small beakers each covered with a watch glass. The beakers were then placed in a desiccator for one hour to obtain a constant dry weight. Samples were digested in 5 mL 75 percent HNO_3 (Mallinckrodt AR Select) for 24 hours. The samples were subsequently boiled to near dryness (approximately 5 hours) and diluted to 5 mL with 6 percent HNO_3. Samples were analyzed for Cd, Zn, Cu, Al, Fe, Mn and Ca by Inductively Coupled Plasma Emission Spectrophotometry (ICP) at the University of Ottawa, Geology Department. Detection limits are shown in Appendix 7.2.

Odonata

Individual samples were dried and weighed according to the methods outlined above. Dried samples were digested in 3.25 mL 75 percent HNO_3 for 4 hours followed by 1.25 mL 70 percent perchloric (BDH-ARISTAR) acid for 24 hours. The samples were boiled until near dryness. The protocol then resumed as above with Chironomidae.

To control the data quality, standard reference material (National Bureau of Standards, Bovine Liver Tissue) were run in parallel with the samples (Appendix 7.3). Blank distilled deionized water samples were routinely run to check for contaminants and to obtain background metal levels. Metal concentrations in $\mu g \cdot g^{-1}$ dry weight were calculated as: (metal concentration in $\mu g \cdot ml^{-1}$ - background) * sample volume in millilitres / sample dry weight in grams.

2.2.1 Metal Concentrations: internal and external

Metal concentrations measured in whole larvae or nymphs are the sum of the metals internally incorporated into the tissues (absorbed), the metals adsorbed to the insect exoskeleton, and the metals contained in the insect gut. Prior to pupation the chironomids purge their gut contents (Wood, M. Personal Communication, 1992). Some studies (Elwood et al., 1976, Chapman, 1985) claim that true metal concentrations are not obtained by including the latter. However, because this study was interested in quantifying metals bioavailable to other organisms, the guts were not purged. The gut provides an additional means for metals to enter the food chain.

2.2.2 Water Chemistry Analysis

At each site within each peatland, 50 mL of water was collected for metal analysis (n=3). Surface water samples were also collected for spring pH and alkalinity measurements. pH was measured with a portable ORION pH meter. Alkalinity was determined using a standard titration technique. And finally, loss on ignition (LOI) of the surface peat and DOC (dissolved organic matter in the surface water) were determined in 1991 as described in Bendell-Young et al., (1992).

2.3.0 Statistical Analysis

2.3.1 Metal Concentrations in Larval Chironomidae

All data were log transformed in order to satisfy the assumptions of normality and equality of residuals. One-way ANOVA (Nested) was applied to determine if larval metal concentrations differed among peatland types and peatlands. This test was also applied to determine if there were differences among peatlands and among sites within peatlands. Where differences among peatlands were detected ($p < 0.05$), Tukey's Studentized Range (HSD) Test (hereafter referred to as Tukey's) was applied to determine where the significant differences occurred. Due to unequal variances (Levene's for Cu larvae $W=6.32$, $p< 0.0001$, and Zn larvae $W=8.63$, $p< 0.0001$), data for Cu and Zn were ranked and Scheffe's test was done. Pearson Product Correlation Coefficients (hereafter referred to as PPC) were determined to examine correlations between larval metal concentrations and abiotic factors such as pH, alkalinity, DOC and percent LOI.

2.3.2 Metal Concentrations in Odonata Nymphs

Libellula were found in adequate numbers in five peatlands to permit statistical analysis. Prior to an analysis of metal concentrations among peatlands, the importance of nymphal size on metal concentration was assessed. This was of particular concern with the nymphs as they were of varying sizes (e.g. 0.90 - 2.71 mm in one peatland). Regressions were done for each metal in a peatland where sample size permitted (Appendix 7.4). Length was not found to be important in its influence upon metal concentration. Further, the mean length of the nymphs did not vary greatly among peatlands with most nymphs approximately 1.5 mm. As size was not important, differences in nymphal metal concentrations among the five peatlands were examined by a one-way ANOVA. Where differences among peatlands were detected ($p < 0.05$), Tukey's Test was applied to determine where the differences occurred. PPC coefficients were calculated, as with larvae, to examine correlations between four abiotic variables and nymph metal concentrations.

PPC coefficients were also calculated to determine if significant correlations existed between metal levels in chironomid larvae and *Libellula* nymphs.

2.3.3 Metal Transfer from Larval to Adult Chironomidae

Ratios of metal levels in larvae and adults were calculated as: metal concentration ($\mu g \cdot g^{-1}$) in adults / metal concentration ($\mu g \cdot g^{-1}$) in larvae for each replicate in order to assess the transfer of metals from the larval to the adult form.

2.3.4 Metal Export Calculations

It is important to calculate the estimated removal of metal for a standardized area for each peatland in order to interpret where the greatest metal export could be. These calculations reflect not only the peatlands where metal levels were high, but also where chironomid productivity was great. If a given peatland contained chironomids with high metal levels this would only be of toxicological importance if there was also high productivity.

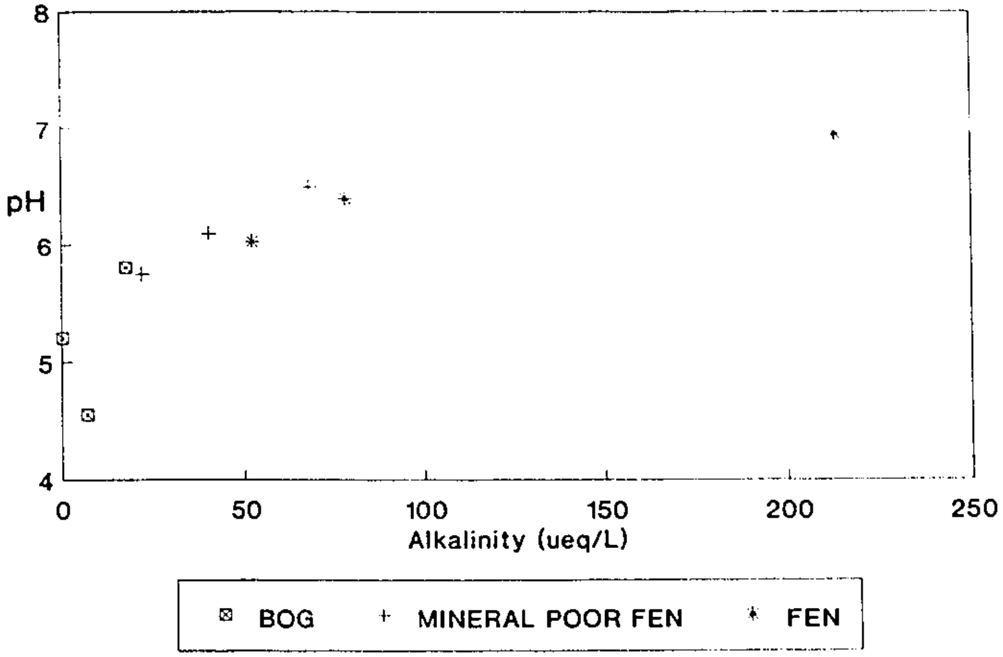
It was necessary to use density values for larvae for the export calculation. As adults emerge over the entire ice-free period, simply using density measurements calculated for a 24 hour period is not sufficient. However, although the adult densities are not complete, it is necessary to first establish that larvae and adult density numbers are correlated. Number of larvae and adults m^{-2} (standardized as number of adults collected per 24 hours) were compared by correlation (Figure 3.7). Because larval and adult density data were correlated, export values were calculated as mean number of larvae$\cdot m^{-2}$ for each site * mean adult dry weight (mg) per site * mean adult metal concentration ($\mu g \cdot g^{-1}$) for each site (n=3 for each peatland except Arrowhead where n=2).

2.3.5 Metals in Larval Chironomidae and Odonata Nymphs

Metals in the two trophic levels were examined by the calculation of bioconcentration factors: the mean concentration of metals in *Libellula* nymphs was divided by the mean concentration of metals in chironomid larvae for each peatland. Values near 1 indicate that metal concentrations were similar in the two trophic levels and that biomagnification of metals did not occur. Values greater than 1 indicate that the metals were biomagnified (greater concentrations found in the higher trophic level) in the *Libellula* nymphs. Finally, values less than 1 indicate that biominification (lower concentrations found in the higher trophic level) occurred and that metals were lost from one trophic level to the next.

All statistical analysis was done using SAS computer software (SAS Institute Inc., 1988). Power analysis was done for ANOVA when the null hypotheses were accepted (as with Zn and Mn concentrations in *Libellula* nymphs) after the methods of Zar (1984).

FIGURE 2.1 Spring alkalinity (ueq.L-1) and pH of the nine peatlands selected for study by peatland type.



Values represent mean of three measurements.

TABLE 2.1. Relevant water chemistry parameters for the nine peatlands located in Central Ontario. Spring sampling conditions, April - June, 1991.

PEATLAND CODE	pH	ALKALINITY ($\mu eq \cdot L^{-1}$)	DOMINANT VEGETATION	LOCATION LAT ° '	LOCATION LONG ° '
BOGS					
BA	4.55	6.5	*Chamaedaphne*	45 35	78 31
BO	5.8	17.0	*Sphagnum*	45 11	78 45
UD	5.2	0	*Sphagnum*	45 30	78 50
MINERAL POOR FENS					
AH	5.75	21.5	Sedges	45 6	78 50
IF	6.10	40	*Sphagnum*	45 5	78 59
HY	6.50	68	Sedges	45 7	78 50
CIRCUMNEUTRAL FENS					
DE	6.03	52	Sedges	45 7	78 51
BU	6.40	78	*Typha*	45 5	78 46
SI	6.95	203	*Nuphar*	45 6	78 41

Water samples collected and analyzed 1990. Dominant vegetation determined by quadrat analysis. Data represent means where n = 3 for pH and alkalinity.

Codes: BA=Bat, BO=Bor, UD=Upper Dawson, AH=Arrowhead, IF=Ideal, HY=Hydro, DE=Deer, BU=Buck and SI=Simp.

TABLE 2.2. Invertebrate presence or absence with an estimate of abundance in peat collected by Ekman grab in nine peatlands located in Central Ontario. Results of spring sampling, 1991.

	BOG			MINERAL POOR FEN			FEN		
	BA	UD	BO	AH	IF	HY	DE	BU	SI
Diptera									
Chironomidae	X	X	X	*	*	X	*	X	*
Tabanidae				X		*	X		
Bezzia		*	*			*	*	X	*
Chaoborus			X						
Odonata									
Anisoptera	X	X	X	X	*	*	*	X	*
Zygoptera	*								*
Trichoptera		*		X	*	*	*	*	*
Ephemeroptera				X					
Coleoptera									
Chrysomelidae				X		*	X		
Dytiscidae					*				
Bivalvia		*	X	X	*	*	X	X	X
Gastropoda	*	*		*		*	*	*	*
Oligochaeta	*	X		X		*	X	X	*
Hirudinea				*		*	*	*	*
Crustacea									
Gammarus									*
Asellidae				X					

* = present and X = abundant (n>2)

TABLE 2.3. Presence or absence of seven Odonata genera in Ekman grab samples from peatlands located in Central Ontario. Results of spring sampling, 1991.

	PEATLAND							
	BOGS			MINERAL POOR FENS		FENS		
GENUS								
Libellula	BA	UD	BO	AH	IF	DE	BU	SI
Gomphus		UD		AH	IF	DE		
Leucorrhinia	BA	UD						SI
Cordulegaster				AH		DE		
Anax								SI
Tetragoneuria					IF			
Aeshna		UD						

CHAPTER 3.0

RESULTS

3.1 Metal concentrations in Larval Chironomidae and Nymphal Odonata

3.1.1 Larval Metal Concentrations by Peatland Type

Cadmium concentrations in larval chironomids were below the detection limit of 0.002 $\mu g \cdot g^{-1}$. With the exception of Cu (Nested ANOVA F=1.20, p=0.31, complete ANOVA in Appendix 7.5a), metal concentrations in larvae were found to be different among the three peatland types (Table 3.1 Nested ANOVA, full ANOVA are presented in Appendix 7.5a). Metal concentrations were also significantly different within peatland types, which suggested that pooling the peatlands by type may not be acceptable. The results of a Tukey's should be interpreted with caution given the significance of within peatland type variability. Nevertheless, Zn concentrations were significantly greater in fen larvae than bog larvae. In contrast, larval Al and Ca concentrations were greatest in the mineral poor fens. Larvae from the bogs were found to have the lowest Ca concentrations. Larval Mn and Fe was highest in the circumneutral fens and lowest in the bogs (Tukey's, p=0.05).

3.1.2 Larval Metal Concentrations by Peatland

Statistically significant differences in larval concentrations among peatlands were found for all metals. No significant differences were found among sites within the peatlands (Table 3.2, Nested ANOVA, complete table in Appendix 7.5b). Metal concentrations for the three sites in each peatland were therefore pooled for further analysis.

Differences in larval Zn concentrations were observed between Arrowhead (mineral poor fen) and two bogs (Bor and Bat) and between Deer (circumneutral fen) and the bog Bat (Scheffe, p=0.05). A negative correlation was observed between larval Zn and organic matter in the peat as measured by percent LOI (r=-0.76, p=0.03, Table 3.3). Hydro and Bat, a mineral poor fen and a low pH bog, had greater Cu concentrations than two of the fens Ideal and Deer (Scheffe, p=0.05). Larval Cu was negatively correlated (r=-0.77, p=0.03) with DOC in the surface water. Larval Cu and Zn concentrations in the other peatlands varied, but were not significantly different.

There were significant differences in larval Al concentrations among peatlands, but these differences were not related to pH and alkalinity. Larvae from two peatlands, Deer (a circumneutral fen) and Arrowhead (a mineral poor) fen had significantly greater Al (Tukey's, p=0.05) than a number of other peatlands including two bogs (Bat and Bor) and two fens (Buck and Simp).

Although fen larvae appeared to have greater Ca, this finding was not significant (Tukey's p=0.05). However, larval Ca in one bog (Bor) was significantly lower than larval levels in all other peatlands, except one bog. Larvae from one of the fens (Deer) had significantly higher (at $p=0.05$) Ca than larvae from all other peatlands. These results must be interpreted with caution due to the high degree of variation (25 percent) in the standards from the National Bureau of Standards certified values.

Larval Fe and Mn concentrations were significantly different among peatlands and in general these differences were consistently significant between larvae from bogs and larvae from circumneutral fens. Circumneutral and mineral poor fen larvae were found to have greater concentrations of Mn than larvae from the three bogs (Tukey's $p = 0.05$). Indeed, a significant negative correlation was observed between larval Mn and organic matter in the peat (Table 3.3, $r=-0.71$, $p=0.05$). Larval Fe in two fens, Buck and Deer, were greater ($p = 0.05$) than larval concentrations from the three bogs. Concentrations of these two metals appear to be related to peatland type with the greater concentrations being found in larvae from high pH, high alkalinity peatlands.

3.1.3 Metal Concentrations in Odonata Nymphs

Cadmium concentrations in *Libellula* were below the detection limit of 0.006 $\mu g \cdot g^{-1}$ (Table 3.4, complete ANOVA table in Appendix 7.5b). Nymphal Zn and Mn concentrations did not vary among peatlands ($p>0.05$; POWER for Zn 0.34: therefore a 66 percent chance of having committed a Type II error and POWER for

Mn 0.60). A similar trend was observed for Cu, Fe and Ca concentrations in nymphs. The highest concentration of these metals was observed in Arrowhead mineral poor fen. Nymphal Cu, Fe and Ca concentrations in the other peatlands were relatively similar. This observed with nymphal Mn concentrations as well, however, it was not of statistical significance. Nymphal Mn and organic matter in the peat showed a significant negative correlation ($r=-0.97$, $p=0.04$, Table 3.5).

Correlations were calculated to determine if metal levels observed in chironomid larvae were reflected in metal levels in *Libellula* nymphs (Table 3.6). Ca and Al were positively correlated ($r=0.70$, $p<0.0001$ and $r=0.36$, $p=0.05$, Table 3.6). However, when individual peatland PPC were calculated, no significant correlations were found.

3.2.0 Metal Transfer from Chironomidae Larvae to Adult

Figures 3.1 to 3.6 illustrate the ratio of metal concentration ($\mu g \cdot g^{-1}$ dry weight) in the adult:larvae. Three separate trends were observed with the metals. First, both Zn and Mn were greater in adults than larvae (Figures 3.1 and 3.2). As the organism looses a substantial portion of its larval body weight upon emergence (Appendix 7.6), Zn and Mn were being concentrated in adults. A similar trend was observed with Ca (Figure 3.3).

A different trend was observed for Fe and Al (Figures 3.4 and 3.5) where concentrations were generally greater in larvae. This implied that generally Fe and Al were lost upon emergence. Finally, a third trend was observed with Cu as seen in Figure 3.6. In lower pH sites the Cu was lost upon emergence and in the higher pH sites the Cu was retained in the adults.

3.3.0 Metal Transfer from Chironomidae Larvae to Odonata Nymphs

Table 3.7 indicates the calculated bioconcentration factors for the five peatlands. Values near 1, e.g. 1.14, were considered as 1 and that biomagnification had not occurred. Biomagnification did not occur with Zn, Al and Fe. Biomagnification was observed with Cu in nymphs from only one of the peatlands, Arrowhead mineral poor fen (BCF=2.24). Ca levels were greater in nymphs from Buck, Arrowhead, and Upper Dawson. Finally, Mn concentrations were greater in the Upper Dawson nymphs. With the exception of Ca, metals were generally greater in the chironomid larvae.

3.4.0 Metal Export for Each Peatland

A partial correlation test found that the mean number of larvae$\cdot m^{-2}$ was correlated with the mean number of adults$\cdot m^{-2}\cdot 24$ hours^{-1} (r= 0.68, p=0.0001) (Figure 3.7). This correlation permitted the use of the larval density data in the export calculation.

The greatest removal of Zn, Cu, Fe, Al and Ca occurred at Bor, a bog where adult metal levels were intermediate, but where chironomid biomass was high (Table 3.8). Hydro and Buck also had relatively high export values which again was related to high biomass. Iron export was greater in the bogs (Bor and Upper Dawson) than the fens, although the fen Buck had a relatively high export value of 150.77 $\mu g \cdot m^{-2}$. This trend implied that although fens typically had higher levels of Fe, the greater biomass in some bogs resulted in greater transfer. Mn export was highest in the fen Buck, where levels were greater in the larvae. Although the biomass was greater in Bor, this peatland did not have the highest export of this metal.

FIGURE 3.1 Mean ratio of Zn concentration in Chironomid adults:larvae with standard errors.

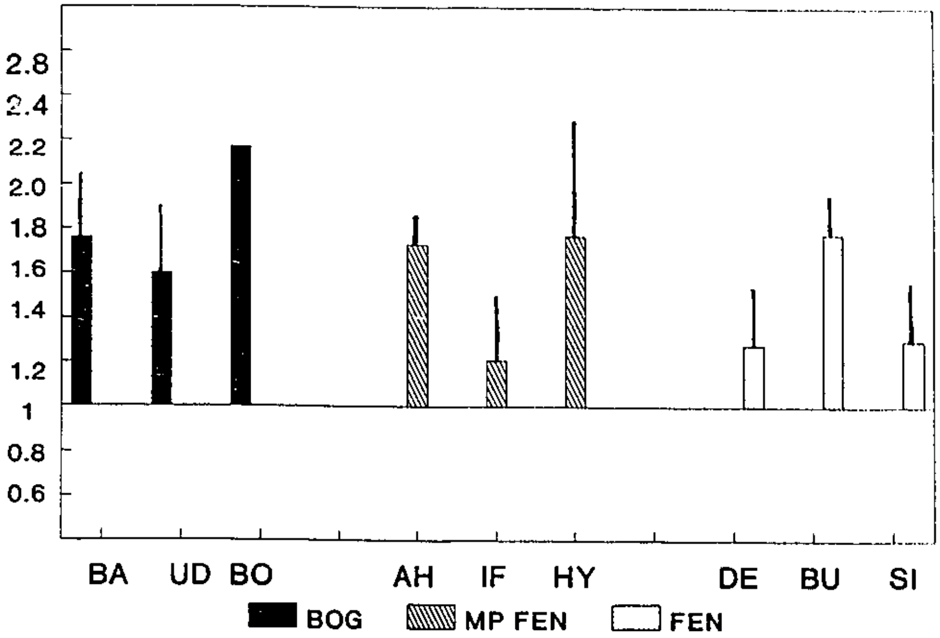


FIGURE 3.2 Mean ratio of Mn concentration in Chironomid adults:larvae with standard errors.

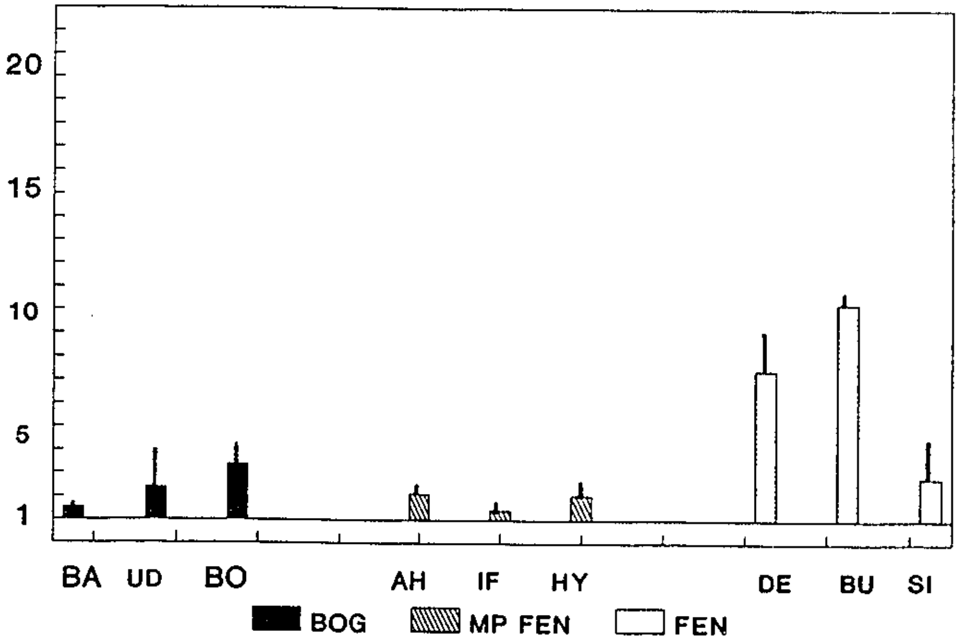


FIGURE 3.3 Mean ratio of Ca concentration in Chironomid adults:larvae with standard errors.

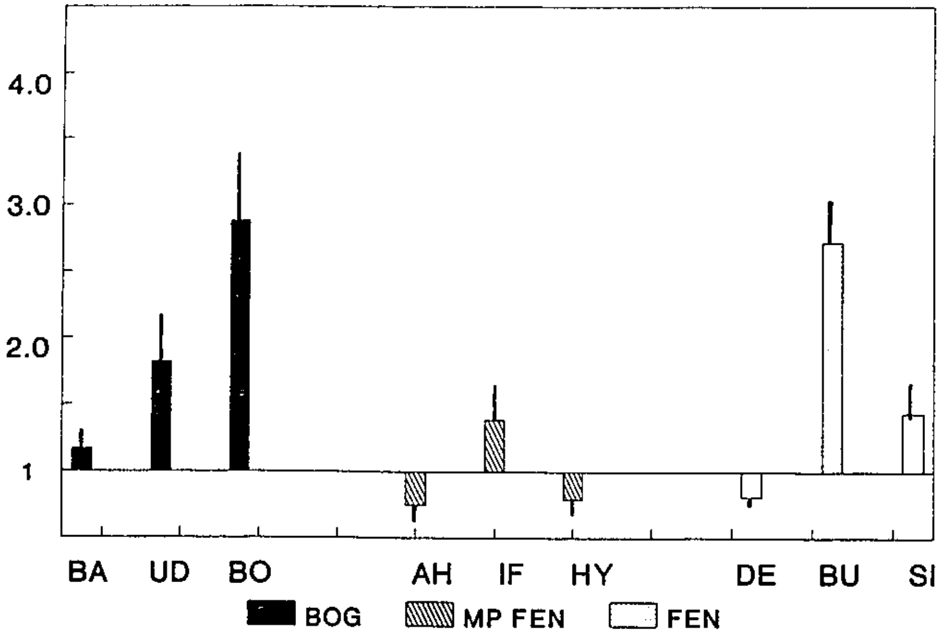


FIGURE 3.4 Mean ratio of Fe concentration in Chironomid adults:larvae with standard errors.

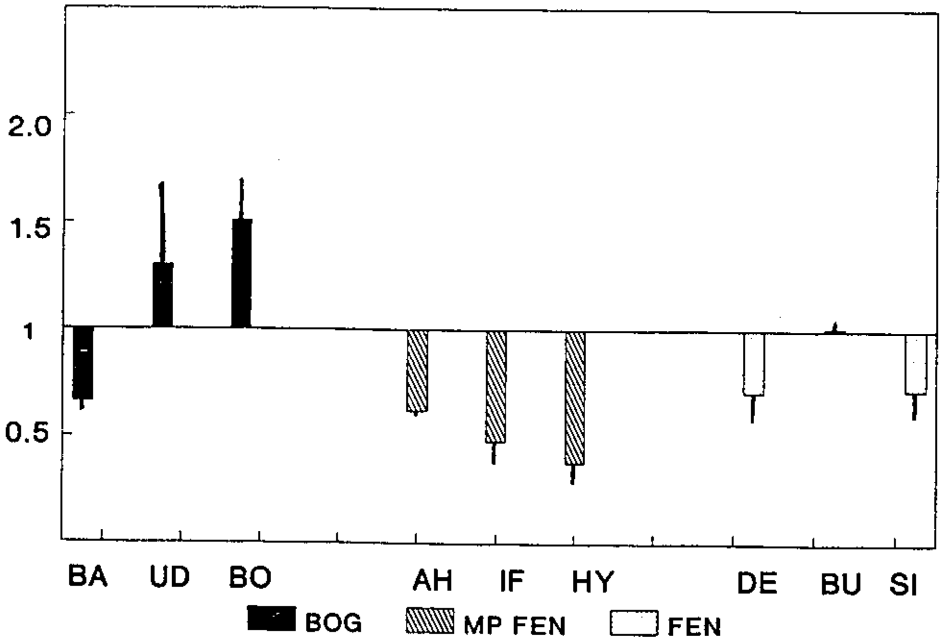


FIGURE 3.5 Mean ratio of Al concentration in Chironomid adults:larvae with standard errors.

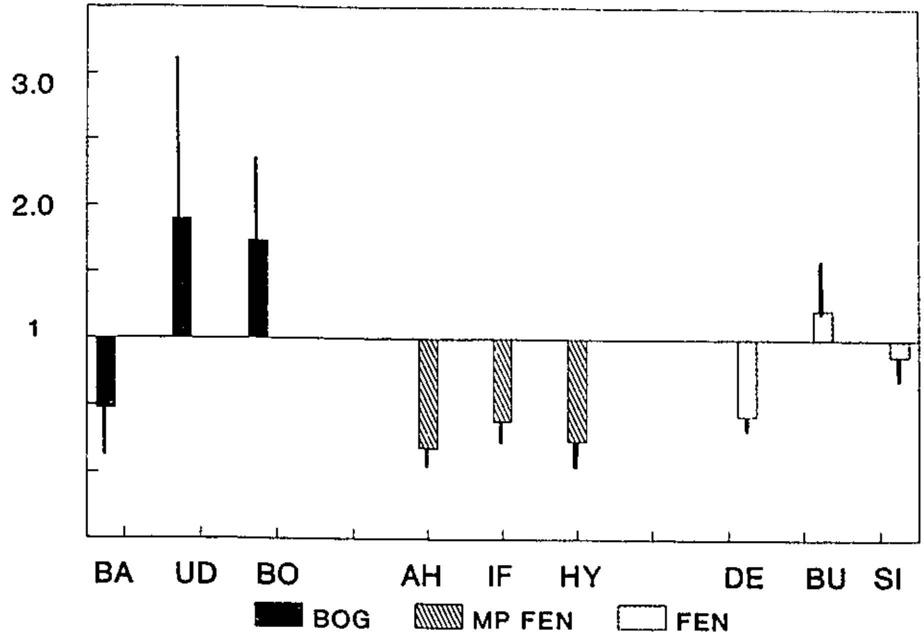


FIGURE 3.6 Mean ratio of Cu concentration in Chironomid adults:larvae with standard errors.

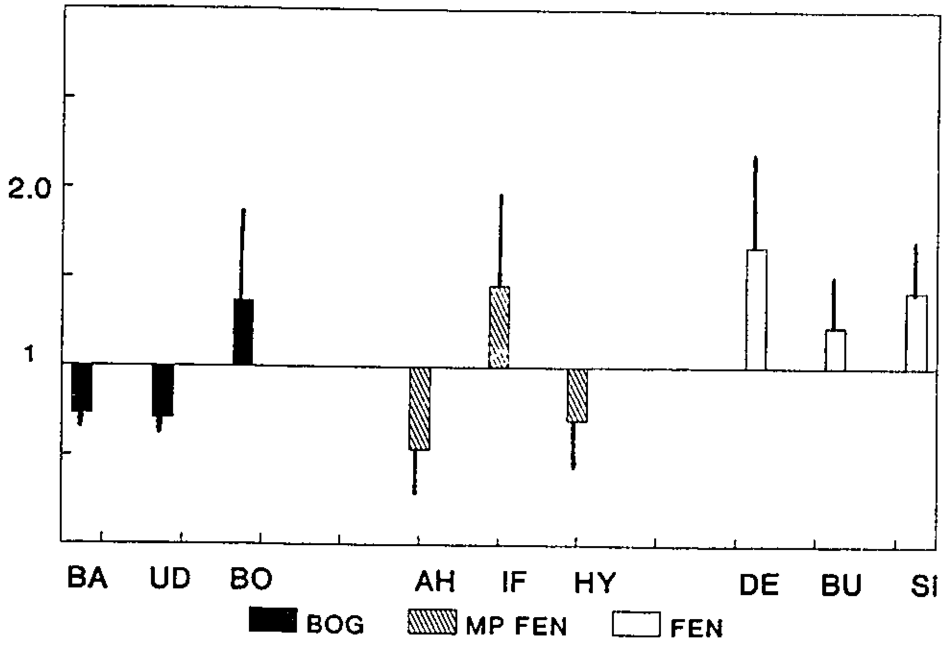
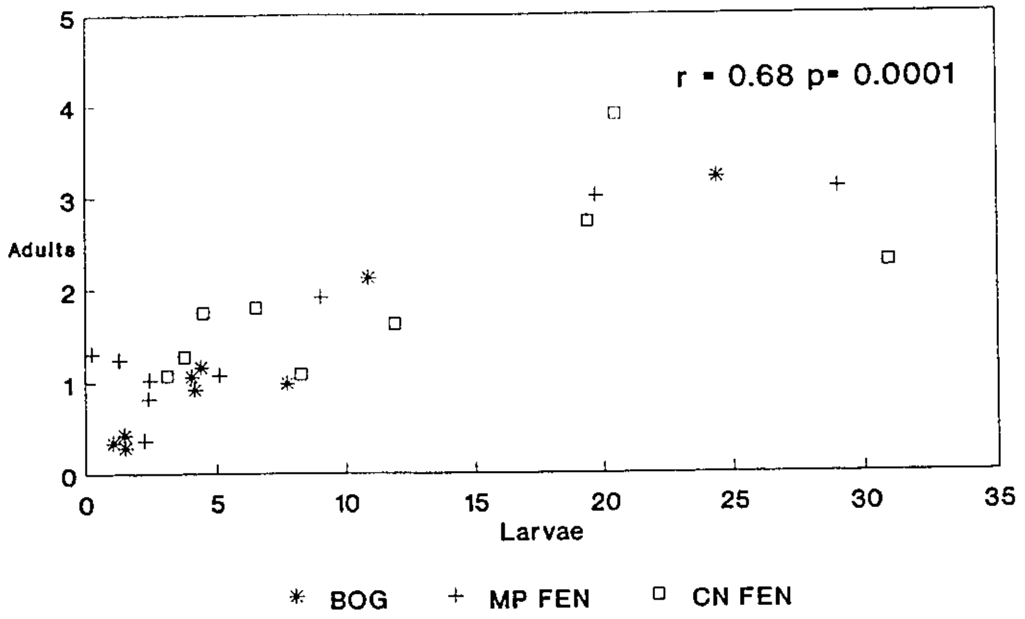


FIGURE 3.7 Mean number of Chironomid larvae·m^{-2} versus adult·m^{-2}·day^{-1}. r=correlation coefficient and p=alpha value.



Adult data based on number emerged
over 24 hour per m2

TABLE 3.1. Mean metal concentration (± S. E.) in larval Chironomidae collected by Ekman grab from three types of peatland in Central Ontario. Values are in $\mu g \cdot g^{-1}$ dry weight. Nested ANOVA F Values are for peatland type and peatland. Different letters indicate differences at $p=0.05$ for Tukey's Multiple Comparison Test.

	Metal $\mu g \cdot g^{-1}$ (dry weight)					
	Zn	Cu	Al	Ca	Mn	Fe
PEATLAND TYPE						
Bog	76.02 b	20.19 a	280.56 b	898.65 c	9.37 c	1211.73 c
	(2.98)	(1.38)	(66.11)	(62.31)	(0.79)	(60.56)
n	27	26	26	26	27	27
Fen	92.94 a	16.25 a	296.69 b	1223.88 b	31.77 a	2521.54 a
	(3.69)	(1.40)	(58.78)	(93.72)	(2.71)	(212.37)
n	32	35	32	35	34	34
MP Fen	99.99 a	20.83 a	566.82 a	1500.04 a	23.38 b	1894.79 b
	(7.53)	(2.12)	(126.19)	(120.25)	(2.61)	(167.92)
n	26	26	27	24	27	27
Nested ANOVA						
Peatland Type:						
F Value	9.41	1.20	4.61	30.97	46.02	40.62
p Value	0.0002	0.3080	0.0129	0.0001	0.0001	0.0001
Peatland:						
F Value	4.03	6.29	6.51	21.92	5.06	12.18
p Value	0.0015	0.0001	0.0001	0.0001	0.0002	0.0001

TABLE 3.2. Mean metal concentration (± S. E.) in larval Chironomidae collected by Ekman grab from nine peatlands in Central Ontario. Values are in $\mu g \cdot g^{-1}$ dry weight. Nested ANOVA F Values are for peatland. Different letters indicate differences at p=0.05 for Tukey's Multiple Comparison Test.

	Metal $\mu g \cdot g^{-1}$ (dry weight)					
	Zn	Cu	Al	Ca	Mn	Fe
BOGS						
BA	71.73 c	26.07 ab	311.75 b	1271.28 cd	14.27 cd	1335.20 de
	(4.12)	(0.99)	(126.90)	(48.81)	(0.53)	(52.20)
UD	79.97 abc	17.17 abc	421.51 ab	779.58 ef	7.70 cd	1368.37 de
	(6.07)	(3.34)	(150.39)	(63.48)	(0.71)	(120.54)
BO	76.36 bc	17.01 abc	124.08 b	613.38 f	6.13 d	931.63 e
	(5.37)	(0.98)	(19.41)	(24.43)	(0.56)	(61.03)
MINERAL POOR FENS						
AH	122.14 a	19.95 abc	1001.63 a	2455.51 ab	30.64 ab	2744.98 b
	(16.05)	(1.18)	(318.02)	(487.34)	(4.43)	(325.86)
IF	101.72 abc	13.45 c	326.68 ab	1051.13 cde	22.65 ab	1549.38 cd
	(11.55)	(3.00)	(73.85)	(107.96)	(4.47)	(161.34)
HY	79.59 abc	29.00 a	372.15 ab	1488.73 bc	17.75 b	1522.85 cd
	(8.14)	(2.74)	(114.40)	(181.94)	(4.05)	(213.60)
FENS						
DE	112.72 ab	10.14 c	778.34 a	2107.16 a	43.80 a	4179.38 a
	(11.02)	(3.03)	(165.08)	(123.11)	(5.28)	(486.22)
BU	83.75 abc	20.70 abc	178.27 b	938.86 de	26.48 ab	2165.89 bc
	(2.29)	(1.60)	(19.52)	(61.68)	(3.34)	(114.15)
SI	90.67 abc	14.96 abc	131.91 b	1001.80 cde	29.47 ab	1719.68 cd
	(4.11)	(1.91)	(33.36)	(71.01)	(4.98)	(223.63)
Nested ANOVA						
F Value	5.73	6.47	2.26	3.39	3.48	2.96
p Value	0.0001	0.0001	0.0334	0.0025	0.0019	0.0065

TABLE 3.3. Pearson product correlation coefficients for larval metal concentrations ($\mu g \cdot g^{-1}$) and peatland water chemistry. Correlations significant at $p=0.05$ marked with *. Bonferoni adjusted p value 0.002.

	pH	Alkalinity	DOC	LOI
Zn				
r	0.36	0.90	0.44	-0.76
p	0.35	0.82	0.27	0.03 *
Cu				
r	-0.23	-0.21	-0.77	0.35
p	0.56	0.59	0.03 *	0.40
Al				
r	-0.04	-0.27	0.08	-0.65
p	0.91	0.47	0.85	0.08
Mn				
r	0.51	0.57	0.62	-0.71
p	0.16	0.11	0.10	0.05 *
Fe				
r	0.33	0.34	0.50	-0.69
p	0.39	0.38	0.21	0.06
Ca				
r	0.09	-0.06	0.10	-0.66
p	0.82	0.87	0.82	0.08

LOI is % loss on ignition, DOC is dissolved organic carbon in the surface water in $mg \cdot L^{-1}$ and alkalinity is in $\mu eq \cdot L^{-1}$.

TABLE 3.4. Mean metal concentration (± S. E.) in *Libellula* nymphs (Order: Odonata) collected by Ekman grab from nine peatlands in Central Ontario. Values are in $\mu g \cdot g^{-1}$ dry weight. One-way ANOVA F values for peatland. Different letters indicate differences at p=0.05 for Tukey's Multiple Comparison Test.

	Metal $\mu g \cdot g^{-1}$ (dry weight)					
	Zn	Cu	Al	Ca	Mn	Fe
BOGS						
BA	81.85 a	19.61 a	75.49 a	1142.10 a	6.53 a	842.16 a
	(17.49)	(1.21)	(13.84)	(191.20)	(1.92)	(221.94)
n	5	5	4	5	4	4
UD	79.61 a	16.98 a	276.05 a	1259.57 a	12.26 a	768.20 a
	(4.89)	(2.25)	(98.04)	(167.07)	(4.49)	(124.81)
n	7	6	7	7	6	6
MINERAL POOR FENS						
AH	102.38 a	44.76 b	320.60 a	5136.79 b	22.35 a	1852.99 b
	(10.55)	(8.21)	(31.79)	(945.55)	(5.10)	(322.86)
n	6	6	6	5	3	6
IF	100.21 a	14.43 a	289.65 a	1167.71 a	6.51 a	624.11 a
	(1.73)	(2.31)	(7.61)	(99.18)	(2.37)	(136.16)
n	4	4	4	4	3	3
FENS						
BU	74.41 a	17.82 a	94.07 a	1561.31 a	12.13 a	613.89 a
	(5.97)	(0.89)	(15.36)	(99.69)	(1.85)	(90.93)
n	10	10	10	8	8	9
One-Way ANOVA						
F Value	1.06	10.07	3.06	27.79	2.38	4.66
p Value	0.3968	0.0001	0.0375	0.0001	0.0879	0.0070

TABLE 3.5. Pearson product correlation coefficients for *Libellula* nymph metal concentrations ($\mu g \cdot g^{-1}$) and peatland water chemistry. Correlations significant at p=0.05 marked with *. Bonferoni adjusted p value 0.002.

	pH	Alkalinity	DOC	LOI
Zn				
r	0.13	-0.46	0.33	-0.53
p	0.83	0.43	0.67	0.46
Cu				
r	0.04	-0.18	0.34	-0.83
p	0.94	0.77	0.66	0.17
Al				
r	0.14	-0.49	0.93	-0.78
p	0.82	0.40	0.07	0.22
Mn				
r	0.26	0.03	0.65	-0.97
p	0.67	0.95	0.34	0.04 *
Fe				
r	-0.06	-0.31	0.36	-0.84
p	0.09	0.61	0.64	0.16
Ca				
r	0.19	-0.10	0.45	-0.89
p	0.31	0.87	0.54	0.11

LOI is % loss on ignition, DOC is dissolved organic carbon in the water column in $mg \cdot L^{-1}$ and alkalinity is in $\mu eq \cdot L^{-1}$.

TABLE 3.6. Pearson Product Correlation Coefficients for Chironomidae Larvae and *Libellula* Nymphs Metal Concentrations ($\mu g \cdot g^{-1}$). Correlations significant at p=0.05 marked with *. Bonferoni adjusted p value 0.0016.

	Zn	Cu	Al	Ca	Mn	Fe
ALL PEATLANDS						
r	-0.01	0.02	0.36	0.70	0.30	0.11
p	0.96	0.18	0.05 *	0.0001 *	0.16	0.57
BA						
r	0.50	0.41	0.28	0.32	0.28	-0.41
p	0.20	0.20	0.29	0.25	0.29	0.24
UD						
r	-0.16	0.33	0.43	0.24	0.38	-0.23
p	0.30	0.21	0.14	0.25	0.19	0.27
AH						
r	-0.63	-0.24	-0.03	0.78	N/A	-0.47
p	0.07	0.28	0.38	0.17		0.17
IF						
r	-0.62	0.03	0.82	0.32	0.60	0.95
p	0.15	0.40	0.16	0.32	0.24	0.08
BU						
r	0.21	0.40	0.43	0.68	0.11	-0.24
p	0.23	0.10	0.10	0.02	0.33	0.22

TABLE 3.7. Bioconcentration Factors for detritivore Chironomidae larvae and predator *Libellula* nymph. Calculations based on: mean metal concentration in nymphs / mean metal concentration in larvae.

	Metal					
	Zn	Cu	Al	Ca	Mn	Fe
BOGS						
BA	1.14	0.75	0.24	0.90	0.46	0.63
UD	0.99	0.99	0.65	1.61	1.59	0.56
MINERAL POOR FENS						
AH	0.84	2.24	0.32	2.09	0.73	0.67
IF	0.98	1.07	0.89	1.11	0.29	0.40
CIRCUMNEUTRAL FENS						
BU	0.89	0.86	0.53	1.66	0.46	0.28

TABLE 3.8. Mean estimated removal of metals by insect emergence in $\mu g \cdot m^{-2}$ for each peatland. Calculated as number of larvae$\cdot m^{-2}$ * individual adult dry weight in grams * mean adult metal concentration in $\mu g \cdot g^{-1}$ for each of three sites per peatland (± standard errror).

	MEAN NO. LARVAE . m^{-2}	MEAN ADULT DRY WT (mg)	Zn	Cu
			μg·g (S.E.)	
BOGS				
BA	340.39 (88.52)	0.11 (0.03)	5.52 (2.58)	0.73 (0.29)
UD	184.85 (43.57)	0.37 (0.19)	7.08 (1.76)	0.72 (0.24)
BO	980.51 (153.2)	0.43 (0.14)	69.29 (25.25)	11.21 (5.22)
MINERAL POOR FENS				
AH	93.19 (74.17)	0.09 (0.02)	2.38 (1.95)	0.14 (0.13)
IF	112.94 (16.5)	0.10 (0.02)	1.43 (0.51)	0.16 (0.05)
HY	800.09 (240.49)	0.17 (0.03)	17.15 (7.86)	2.76 (1.67)
CIRCUMNEUTRAL FENS				
DE	174.45 (4.40)	0.90 (0.01)	2.23 (0.13)	0.24 (0.03)
BU	595.77 (212.95)	0.14 (0.20)	10.67 (2.46)	8.15 (6.15)
SI	55.64 (6.32)	0.39 (0.02)	2.52 (0.38)	0.44 (0.08)

n=3 except AH (Arrowhead where n=2)

TABLE 3.8. Estimated removal of metals continued.

	Al	Fe	Mn	Ca
		$\mu g \cdot g$ (S.E.)		
BOGS				
BA	2.14 (0.87)	35.92 (14.35)	0.92 (0.39)	59.63 (23.86)
UD	7.98 (4.81)	85.22 (17.58)	0.91 (0.12)	74.28 (12.95)
BO	90.77 (45.15)	575.58 (203.71)	8.06 (2.86)	738.03 (285.81)
MINERAL POOR FENS				
AH	0.90 (0.52)	14.00 (11.00)	0.72 (0.62)	15.83 (13.12)
IF	1.70 (0.83)	9.32 (4.13)	0.32 (0.09)	16.63 (5.63)
HY	8.25 (3.94)	72.23 (33.00)	3.90 (1.86)	160.38 (104.25)
CIRCUMNEUTRAL FENS				
DE	4.84 (1.67)	44.11 (12.63)	5.24 (1.75)	26.73 (1.39)
BU	17.58 (7.96)	150.77 (26.97)	20.98 (5.33)	204.57 (63.62)
SI	1.84 (0.48)	24.58 (0.44)	1.25 (0.43)	30.19 (2.91)

CHAPTER 4.0

DISCUSSION

4.1 Metal concentrations in Larval Chironomidae and Odonata Nymphs

To determine whether metal concentrations in larval chironomids and *Libellula* nymphs differ among peatlands, metal concentrations will be considered individually. In making comparisons with concentrations reported in the literature, this discussion will draw frequently on findings from similar studies conducted on lakes or streams because of the lack of data on invertebrates in peatland environments.

Lakes and streams are chemically very different from the peatlands selected in this study. One primary difference is in the relative concentrations of various water chemistry variables. In a study conducted on lakes in the same area, DOC levels ranged from 2.4 to 5.2 $mg.L^{-1}$ (Krantzberg, 1987); however, levels of 3.8 to over 11 $mg.L^{-1}$ were found in the peatlands. Other studies found that organic matter in the sediment, as measured by percent LOI ranged from 32 to 44 percent (Krantzberg, 1987) and 4 to 40 percent (Bendell-Young, 1990) in lakes from the same area. In contrast, percent LOI in the peatlands varied from 31 to 88 percent (Appendix 7.1 for percent LOI and mean DOC in surface waters). DOC levels and percent organic matter in the sediment can have considerable influence on metal bioavailability (reviewed in National Research Council of Canada, 1988).

4.1.1 Larval Chironomidae

The following section will examine metal concentrations in chironomid larvae in an attempt to determine where the greatest metal accumulation occurs. This information will aid in the assessment of the effects of acidification upon metal bioavailability in peatlands.

Zn:

Although Zn is an important constituent of many enzymes and an essential element for all organisms (Mathis and Cummings, 1973), it has been shown to be toxic to fish and invertebrates when present at high levels (Dixit and Witcomb, 1983). Zinc is a transitional metal and is a ubiquitous contaminant. Evans et al., (1983) demonstrated that Zn concentrations in the Muskoka-Haliburton area were elevated in sediments as compared with natural or background levels. These authors concluded that atmospheric deposition onto surface waters was responsible. Zinc present in aquatic systems may be mobilized under conditions of low pH (reviewed in Campbell and Stokes, 1985).

Given the range of environmental conditions (water chemistry and dominant vegetation in this study) differences in larval Zn concentrations were expected to occur among peatlands. Metal concentrations in larvae did vary among peatlands, with one circumneutral fen (Arrowhead) having significantly greater larval

Zn concentrations than larvae from two bogs (Bat and Bor)(Table 3.2). In addition, the bog Bat had significantly lower larval Zn concentrations than the circumneutral fen Deer (Scheffe's, p=0.05).

In the same area (Muskoka-Haliburton), Bendell-Young and Harvey (1988) noted lower Zn concentrations in chironomids from acidified lakes versus circumneutral lakes. Krantzberg (1987) also noted lower body burdens (concentration$\cdot$individual^{-1}) in larvae from lakes of higher acidity. Because larvae in lower pH environments have greater concentrations of H+ ions, it is believed that the ions compete for binding sites on the insect exoskeleton. Metals adsorbed to the insect exoskeleton would be reduced in acidified systems (Krantzberg and Stokes, 1987). The trends noted in these studies were not observed here. Though, the range of concentrations found in this study were consistent with concentrations observed by Bendell-Young (1990).

There are two possible explanations for the lack of distinct differences in chironomid Zn concentrations among peatlands: one relates to abiotic factors, the other to biotic factors. It is known that acidification results in the increased mobility of metals in lakes (National Research Council of Canada, 1988). However, trace metals may be associated with a variety of forms of organic matter. Components of sediments, such as humic and fulvic acids, are capable of binding Zn (Allen et al., 1990).

Although the binding is greater with other metals, such as Cu, Zn concentrations in larvae were in fact found to be negatively correlated with the organic content of the peat, as measured by percent LOI (r=-0.76, p=0.03, Table 3.3). Zinc concentrations in larvae were highest where organic matter was lowest, in Arrowhead mineral poor fen, and lowest where organic matter was highest, Bat bog.

A second potential explanation for the lack of clear trends relates to the organism itself. Krantzberg (1987) and Hare and Campbell (1992) noted that insect larvae from more contaminated systems (in the Sudbury area, for example) could regulate Zn (i.e. maintain a constant concentration). According to Krantzberg and Stokes (1989), tolerance to Zn is frequently encountered in invertebrates from contaminated sites. This tolerance is related to the ability of the organism to sequester metals in membrane-bound vesicles. In an experiment performed by these authors, larvae from contaminated and uncontaminated sites maintained constant Zn concentrations regardless of sediment Zn concentrations. Krantzberg and Stokes (1988) hypothesized that Zn was stored in metal-containing granules by *Chironomus*. The structures and the content of the granules were believed to differ among taxa and environments (Krantzberg and Stokes, 1990). In addition, Hare et al., (1991b) found that in the mayfly *Hexagenia* gut tissues were a major storage site for metals that are taken up from sediment consumed as food. Varying amounts of

Zn-containing sediment in the organism's gut may also obscure trends.

Cu:

Copper is a transitional element and is essential for all living organisms. Although a common element in natural waters, Cu can be toxic at high concentrations. Cu enters aquatic environments from soils and mineral deposits (National Research Council of Canada, 1979), however these background values are exceeded by atmospheric deposition of Cu. According to Wong et al., (1984) atmospheric inputs to the Algonquin Park area of Ontario are responsible for an overall two fold enrichment of Cu in recently deposited sediment.

Larvae from the bog Bat (which had the highest organic matter) had significantly greater concentrations of Cu than larvae from two of the fens, Ideal and Deer (Scheffe, p=0.05). In contrast, Krantzberg (1987) noted that body burdens of Cu, like Zn burdens, were lower in larvae from acidified lakes in the Wanapetei area (near Sudbury's smelting activities). This finding was confirmed by Bendell-Young and Harvey (1988), who noted that larval Cu concentrations were lower in larvae from acidified lakes as compared to circumneutral lakes.

A possible explanation for the lack of agreement with the findings from the lakes may relate to the higher concentrations of organic matter in the peatlands. In this study DOC in the surface waters was negatively correlated (r=-0.77, p=0.03) with Cu levels in larvae. The binding capacity of organic matter to metal ions is especially strong with Cu (Allen et al., 1990). Mobilized in low pH systems, Cu in humic waters may be complexed by organic matter and rendered unavailable to organisms (Walker et al., 1985, Allen et al., 1990). Further, this ameliorating effect of DOC on the availability of Cu could potentially mask differences in metal availability based on other water chemistry parameters.

To further obscure trends between abiotic factors and concentrations of Cu in larvae, Cu may also be regulated by the organism. Krantzberg and Stokes (1988) noted that Cu was not accumulated in direct proportion to sediment or water Cu. According to these authors, tolerance was achieved either through regulation or through internal storage in a detoxified form. According to Darlington and Gower (1990), who examined Cu in the trichopteran *Plectrocnemia conspersa*, Cu is stored in granules. The granules are usually intracellular, homogeneous and spherical, with a diameter of between 0.5 and 2.0 μm (Brown, 1982). The granules are believed to provide a mechanism for removing the Cu from circulation. By examining two populations of larvae, one from a contaminated site near Sudbury, the other from a less contaminated site near Dorset, Ontario, Krantzberg and Stokes (1988) found that larvae from the contaminated site were tolerant to Cu. When larvae from both sites were placed in spiked sediments, larvae from the less contaminated sites accumulated Cu five times more rapidly than the tolerant population (Krantzberg and Stokes, 1989).

Mn:

Manganese, a transitional metal, is an essential element for organisms owing to its importance in enzymatic reactions. It is mainly soil-derived in aquatic systems (National Research Council of Canada, 1988). Although there may be metallurgical sources of Mn in the atmosphere, there is no evidence that this is an important source in surface waters (National Research Council of Canada, 1988). The direct toxic effects of Mn on biota are unknown, but there is some evidence that Mn may have an indirect effect on Ca homeostasis (National Research Council of Canada, 1988). Mn may compete with Ca for cellular binding sites. Fraser and Harvey (1981) observed a correlation between low Ca and high Mn in the bone of the common white sucker (*Catostomus commersoni*) and suggested that Mn inhibited Ca uptake. This correlation was also observed in crayfish (Decapoda) by Bendell-Young and Harvey (1991b).

The fens in this study receive hydrological inputs from a variety of sources, such as ground water and surface water. This peatlands receive water that has percolated from soils which may have elevated levels of Mn. Because of this, Mn levels were expected to be greater in these systems. In contrast, because the bog environments permit little input except from atmospheric sources, Mn levels were expected to be lower (Bendell-Young and Pick, 1992 submitted). Chironomids burrow in layers of surficial sediment (Oliver, 1971); as a result, the larvae are exposed to metals contained in porewaters and sediment, which reflect ground water concentrations. Mn levels were greater (Tukey's, p=0.05) in larvae from both mineral poor and circumneutral fens than in bog larvae (Table 3.2). Krantzberg and Stokes (1988) noted that surface adsorption of Mn was lower than Zn and Cu in low pH systems. In fact, in the low pH systems surface adsorption accounted for a mere 0-9 percent of larval body burden. At pH 5.1 or greater the adsorption was between 39 and 46 percent. This may have played an additional role in reducing Mn levels in the bog larvae.

As with Zn, a negative correlation was observed between larval Mn concentrations and organic matter in the peat (r= -0.71, p.0.05). Where low larval Mn levels occurred, peat organic matter was high. Bendell-Young and Harvey (1991) also found that Mn concentrations in chironomid larvae from lakes of varying acidity correlated negatively with organic matter in the sediments, as measured by percent loss on ignition (r= -0.66, p=0.002).

In addition, as with Cu and Zn, Krantzberg and Stokes (1989) found that chironomid larvae from contaminated sites (near Sudbury) could regulate Mn. Mn regulation has been reported in a number of other invertebrates (reviewed in National Research Council of Canada, 1988). By examining chironomids of various sizes and ages, Krantzberg (1989) also found concentrations to be constant. These findings suggest that the metal is under homeostatic control.

Fe:

Fe, a transitional metal, is an essential element to all organisms. It is primarily soil-derived in aquatic systems (National Research Council of Canada, 1988). Although non-toxic, the presence of Fe in the peat may reduce availability of other metals. There is some evidence to suggest that Fe can protect organisms from metal uptake. In a review paper, Luoma (1989) concluded that Fe oxides appear to modify the uptake of other metals from the sediment. Krantzberg (1987) suggested that Fe may have modified the availability of Al and Cu to *Chironomus*. The sources of Fe in aquatic systems are predominantly surface water and ground water inputs. Fe concentrations were expected to be greater in the fen systems than in the bogs, which have reduced hydrologic inputs.

In this study larvae from two of the circumneutral fens, Deer and Buck, had significantly greater Fe than larvae from the bogs (Tukey's, p=0.05). This finding supports the claim that hydrogeological influences (i.e. the presence of obvious surface water inputs in fens and the absence of inputs in bogs) are important; however, it does not fully explain the trends observed.

Bendell-Young and Harvey (1991a) found that larval Fe concentrations were negatively correlated with organic matter (r= -0.56 p.0.014). This study did not observe a significant negative correlation with organic matter. According to Kerndorff and Schnitzer (1980), of 11 metals tested Fe sorbed more readily with soil derived humic acids than the other metals.

The members of the tribe Chironomini in this study contain a pigment chemically similar to haemoglobin. According to Krantzberg and Stokes (1989), Fe metabolism is closely linked to haemoglobin synthesis in *Chironomus*. These authors suggest that Fe accumulation in larvae may be related to greater haemoglobin production. In acidified systems, it is thought that higher haemoglobin concentrations buffer the acidity and enable larvae to survive by becoming acid-tolerant (Krantzberg and Stokes, 1989). Although larvae from the acidic bogs could potentially use higher Fe concentrations, this metal may not be abundant in this type of peatland because of hydrogeological conditions.

Al:

Al is a nonessential and toxic element in organisms (National Research Council of Canada, 1986). An abundant element in soils and bedrock, Al is generally low in non-acidic drainage basins owing to its low solubility (Spry and Winner, 1991). However, the weathering and leaching rate of aluminum can be increased by acidification (National Research Council of Canada, 1986). Considerable research has focused on the impact of Al on ion regulation in fish. It is believed that particulate Al on the gills is absorbed to the mucus and physically interferes with respiration (reviewed in National Research Council of Canada, 1986). Little research has focused on the accumulation of Al at lower trophic levels.

It is known that, in contrast to metals such as Cd, Hg and Pb, aquatic invertebrates do not accumulate higher concentrations of Al in acidified waters, despite higher Al levels in the water column (Ormerod et al., 1988). Given that H+ concentrations are expected to affect the solubility of Al and its biological uptake, results of many studies remain unclear (reviewed in Campbell and Stokes, 1985). No clear trend with Al concentrations in larvae and pH was observed in the present study (Table 3.2). In fact, this metal showed a great deal of intra-peatland variability.

Decreases in Al concentrations with decreasing pH were observed in chironomids by Bendell-Young and Harvey (1988) and in black flies (Simuiilidae) and mayflies (Ephemeroptera) by Hall et al., (1988). Herrmann (1990) studied Al in a number of invertebrate taxa from acidified and non-acidified streams. In contrast to these studies, the majority of taxa from the acidified streams contained more Al than taxa from the higher pH streams (Herrmann, 1990). Organisms referred to as 'shredders' contained the highest Al concentrations (Herrmann, 1990).

Al readily complexes with organic acids resulting in reduced Al bioavailability to aquatic invertebrates (Wren and Stephenson, 1991). In high DOC (50 $mg \cdot L^{-1}$) peatlands of North Central Minnesota, Helmer et al., (1990) found 80 to 90 percent of dissolved Al complexed with organic matter. This was particularly true in low pH peatlands in which metal solubility was high and organic content was high. Bendell-Young and Harvey (1991a) found low larval Al concentrations where sediments were high in organic matter ($r=-0.67$, $p=0.008$). In this study the relationship was not significant; however, larvae from Arrowhead and Deer, fens in which organic content in the peat was lowest, also had the highest Al concentrations.

Ca:

Ca is an alkaline earth metal and an essential macronutrient. Aluminum is known to decrease Ca uptake (reviewed in National Research Council of Canada, 1988). Ca-metal interactions and the amelioration of metal toxicity by Ca have also been reported in several invertebrates species (reviewed in Krantzberg and Stokes, 1989).

In low alkalinity and Ca lakes Al toxicity has been found to increase. This relationship has recently been shown with black ducklings exposed to 0.1 percent dietary Al in combination with low dietary Ca (0.3 percent) (Sparling 1991). It is therefore important to assess whether Ca concentrations in prey items such as chironomids are reduced in the presence of high Al.

In the present study, Ca concentrations in larvae did not follow expected trends (Table 3.2). Although larvae from the bogs did not have consistently lower Ca concentrations, the concentrations were consistently low. Perhaps trends were not observed because of problems in the methodology (i.e. high co-efficient of variation in the standards).

Krantzberg and Stokes (1988) found that Ca burdens in chironomid larvae were highest in pH 4.4 populations. These authors claim that higher Ca contents reflect an adaptive response, with the Ca protecting against H+ toxicity. Metal tolerant populations of chironomid larvae were more efficient at retaining Ca than non-tolerant larvae populations.

Summary

Zinc and Cu concentrations in larvae were not elevated in the low pH peatlands. This may be due to biotic factors, such as larvae metal regulation and tolerance, and also to abiotic factors, such as the ameliorating effects of high organic matter in the peat. In fact, the effect of organic matter on metal bioavailability was important. Larval Zn and Mn concentrations were negatively correlated with organic matter and larval Cu was negatively correlated with DOC in the surface waters.

Mn and Fe concentrations were lower in bog larvae than in fen larvae. This may be a result of hydrogeological factors, such as reduced surface water and ground water inputs in the bogs. Also, because larval Ca concentrations were not reduced in the fens, where Mn concentrations were high, it appears that Mn did not interfere with Ca uptake. It is also difficult to assess whether Fe truly modified the uptake of metals. Iron concentrations were elevated in the fens, however there is no clear evidence to suggest that uptake was reduced in these peatlands as a result.

4.1.2 Odonata Nymphs

To address the second question of this study: are concentrations of metals in *Libellula* different among peatlands, it is important first to distinguish this insect from the chironomid. The two insects have very different life histories.

First, *Libellula* is predacious on a number of benthic invertebrates, including Diptera larvae. This feeding strategy may have important implications for metal accumulation (Smock, 1983). The potential effects of feeding habits on metal accumulation will be discussed below. Secondly, because *Libellula* nymphs increase greatly in size and have 13 instar stages, metals adsorbed to their exoskeleton may not accumulate to the extent that they do in the four instar chironomid larvae. Hare et al., (1991a) noted that the proportion of trace elements adsorbed to the exoskeleton can vary according to the age of the exoskeleton. Recently moulted individuals are expected to have lower adsorbed metal concentrations than organisms about to moult. Thirdly, the chironomid larvae examined in this study are primarily sedentary , whereas *Libellula* are not. Sedentary organisms generally have higher bioconcentration factors than non-sedentary because concentrations are more indicative of exposure (reviewed in National Research Council of Canada, 1988). Nevertheless, similarities in benthic lifestyles may override the life history differences mentioned above.

Smock (1983) examined metal concentrations in organisms of different trophic levels because it is an ideal way to look at the effect of feeding habits on metal concentrations. Further, one can also determine whether the acidification of circumneutral fens into acidic bogs will result in greater metal accumulation in invertebrate predators in acidified peatlands.

Zn concentrations in *Libellula* nymphs were not significantly different among the peatlands (F=1.06, p=0.3968, Table 3.4, complete ANOVA in Appendix 7.5c). In a study examining metal concentrations in Trichoptera, Iivonen et al., (1992) noted that larval Zn and Cu concentrations were not dependent upon pH. It is interesting to note that, unlike Zn concentrations in chironomid larvae, Zn concentrations in nymphs were unaffected by varying water chemistry parameters. There are two possible explanations for this trend: either greater Zn availability in the more acidified sites was masked by higher organic content in the peat, or as observed in other studies, Zn levels were under homeostatic control as they did not increase despite environmental variables.

Al concentrations in *Libellula* nymphs, though varying among peatlands, were not found to be statistically different (Tukey's, p=0.05). Mn concentrations in nymphs were not significantly different among peatlands (F=2.38, p=0.0879), although the trend loosely followed the trend discussed below.

Cu, Fe and Ca concentrations displayed the same trend in *Libellula* nymphs. Concentrations in nymphs did not vary among peatlands, except in the mineral poor fen Arrowhead where nymphal levels far exceeded the levels in the other four peatlands (Tukey's, p=0.05). Copper, Fe and Ca concentrations in *Libellula* nymphs from Arrowhead were greater than in nymphs from the other peatlands (Table 3.4).

Arrowhead is unique among the peatlands in that it had considerably lower percent organic matter in the peat. It is therefore possible that the absence of the ameliorating effect of organic matter led to greater metal bioavailability. With nymphal Mn concentrations, this relationship is statistically significant. Concentrations of Mn in nymphs were lowest in the peatlands where the organic content of the peat was highest ($r= -0.97$, $p=0.04$). In pH, alkalinity, vegetation and concentrations of DOC in the surface waters, Arrowhead had intermediate values.

Metal concentrations in chironomid larvae and *Libellula* nymphs were not correlated. Two exceptions to this were Ca and Al concentrations, which were positively correlated with chironomid concentrations (for Ca $r=0.70$, $p=0.0001$ and Al $r=0.36$, $p=0.05$, Table 3.6). It appears that although the two organisms, chironomids and *Libellula*, inhabit the same benthic environment, metal availability was determined by different factors.

These findings highlight the importance of selecting several organisms in assessing metal availability. Chironomids appeared to regulate both Zn and Cu, whereas only Zn appeared to be regulated by *Libellula*. The ameliorating effect of organic matter in reducing metal availability was only significant with Mn concentrations in *Libellula*. These trends may have occurred because of the differences in life history between the detritivorous chironomid larvae and the predacious *Libellula* nymph.

4.2.0 Metal Transfer from Chironomidae Larvae to Adults

Mean concentrations of metals (in $\mu g \cdot g^{-1}$ dry weight) in adult chironomids can be found in Appendix 7.6. The objective of this section is to determine whether metals concentrated in larval tissues are lost or retained upon emergence. When possible, results will be compared to similar data derived from insect research on lakes and streams. A distinction will be made between purged and non-purged organisms in this section because the gut contents of organisms may, in some cases, obscure differences between metal concentrations in larvae and adults. Nevertheless, metal concentrations in this study's non-purged organisms were in the same range as metal concentrations from studies where organisms were purged (compare concentrations with Krantzberg, 1988 and Timmermans and Walker, 1989).

Chironomids may lose a considerable amount of metals upon emergence. First, metals adsorbed to the larval and pupal exoskeletons are lost upon emergence. And second, prior to entering the pupal stage, the contents of the guts are also purged (Wood, M., Personal Communication, 1992). As the moulting of the cuticle includes the loss of the cuticular lining of the gut, metals adsorbed to the gut cuticle would also be lost. One may therefore expect metal concentrations to be considerably lower in adults than in larvae since only internally incorporated metals would remain.

In spite of the loss of adsorbed and gut-associated metals, concentrations were not consistently lower in chironomid adults. Metals stored in internal organs and metals required for growth (for example, Zn and Cu) were not lost. A substantial weight loss was observed from the larvae to the adult stage (Appendix 7.7), which concentrated the metals in the lower weight adult. This weight loss from the larval to adult chironomid stage has also been observed by Larsson (1984), who noted a weight reduction from a mean of 27.8 mg (larvae) to 7.3 mg (adults). As expected, the weight loss was not the same in all peatlands because of variations in larval and adult weights.

The ratios of adult concentrations to larval concentrations indicated three main trends.

1) Zn, Mn and Ca

Zn, Mn and generally Ca were greater in adults and therefore readily transferred to the adult life stage (Figure 3.1). Bendell-Young and Harvey (1988) noted that 100 percent of chironomid larval Zn concentrations were transferred to the adult stage. Timmermans and Walker (1989) found Zn burdens in

Chironomus anthracinus adults to be slightly higher than in the purged larvae. However, those same authors found Zn burdens to be lower in the adults of another species, *Stictochironomus histrio*. Krantzberg and Stokes (1988) observed that 20 percent of the Zn burden in purged *Chironomus* was lost during metamorphosis. In a study in which nymphs were not purged, Jop (1991) noted that adult forms of four Ephemeroptera species had significantly lower Zn burdens than larval forms.

Bog larval Zn ratios were higher than ratios from two of the fens. This observation suggests that metals accumulated in bog larvae may be more readily transferred to the adult form than metals accumulated in fen larvae. According to Krantzberg and Stokes (1988), a greater proportion of metals may be internally incorporated as pH decreases. This may explain why metals are not readily lost upon emergence in the bogs.

Mn is also transferred to the adult stage during metamorphosis (Figure 3.2). In two of the three fens, adult concentrations were between seven and 11 times higher than concentrations observed in the larvae. This finding implies that Mn may be stored internally, in great quantities, and that it may therefore not be lost. In contrast, other studies found that Mn was lost upon emergence. Krantzberg and Stokes (1988) noted greater Mn burdens in purged chironomid larvae than in chironomid adults, with the exception of one lake with a pH of 4.4. In lakes with pH greater than 5.1, Krantzberg and Stokes (1988) found that between 39 and 46 percent of Mn burdens in larvae were lost. This finding was corroborated by Bendell-Young and Harvey (1988), who found only 10 percent of larval Mn transferred to the adult stage. It is unknown why the findings of the present study do not agree with these studies.

Ca was greater in chironomid adults in six of the nine peatlands (Figure 3.3). This may be expected because of its role as a macronutrient. No similar data were available for comparison. This finding however is relevant and will be highlighted in section 4.3.0.

2) Fe and Al

Ratios calculated for Fe and Al indicate that the metals are, in most cases, effectively lost upon emergence (Figure 3.4 and 3.5). Because these metals are soil-derived, one would expect them to be lost upon emergence (however, Mn is soil-derived and was not lost). This loss would occur as metals adsorbed to the insect exoskeleton from the surrounding peat are lost with the shed exoskeleton. The trend with Fe and Al is consistent with the findings of Bendell-Young and Harvey (1988), who noted only 10 percent of Fe and Al concentrations transferred from larvae to adults.

The loss of Fe upon emergence may relate to the role of this element in the larval form. Fe concentrations may be greater in the larvae because of the requirement for the haemoglobin pigment which aids in pH and low O_2 tolerance. Since the adult chironomid is concerned with reproduction, the Fe requirement may be minimal. The loss of Fe in *Chironomus* adults upon emergence was also observed by Krantzberg and Stokes (1988).

With the exception of the fen Buck and the bog Bor, Al was lost upon emergence. This trend was expected. According to Frick and Herrmann (1990) and Krantzberg and Stokes (1988), Al concentrations in aquatic insects are largely associated with the surfaces of insects. Aluminum is deposited on the exoskeleton of the insects, which is then shed upon emergence. Adsorption of Al may, however, vary with environmental conditions such as pH. Krantzberg and Stokes (1988) found that *Chironomus* adults lost less Al during emergence in lakes at pH 4.4 than in higher pH systems. This trend was not observed in this study.

3) Cu

Although most Cu ratios were near 1, there appeared to be a split trend (Figure 3.6) possibly due to biotic variables. The ratios followed a trend loosely based on pH: metals were transferred to the adults in the fens and lost in the more acidified systems. This trend was also observed by Krantzberg and Stokes (1988), who found that surface adsorption of Cu in *Chironomus* increased with increasing pH.

Timmermans and Walker (1989) reported that Cu burdens were similar in purged larvae and adults of two species of Chironomidae. One species, *Chironomus anthracinus*, had slightly higher burdens in adults; the other species, *Stictochironomus histrio*, had lower burdens in adults. Bendell-Young and Harvey (1988) found 100 percent of larval Cu concentrations transferred to adults. In contrast, Krantzberg and Stokes (1988) observed a 70 percent loss of Cu during metamorphosis, with adult burdens lower than purged larval burdens.

Timmermans and Walker (1989) examined Cu in the pupae, exuviae, larvae and adults of *Hexagenia* in order to locate where the loss of Cu occurred. These authors did not recover Cu in either pupae or shed exuviae, a finding which suggests that *Hexagenia* may have an effective excretory mechanism between the larval and the pupal stage. Copper was not simply lost upon shedding of the exoskeleton.

Summary

Mn, Zn and generally Ca were readily transferred from the chironomid larvae to the adult stage. In fact, Mn concentrations were several times greater in adults than in larvae. This finding implies that these metals are not lost, but may be effectively removed from the peat and from the aquatic system through emerging adults.

In contrast, Al (except two bogs), Fe, and in the more acidic peatlands, Cu, were lost upon emergence. Accumulated from the peat, these metals will not be available in great quantities to the terrestrial system. Examining metal loss and retention in chironomid adults is important to assessing possible transfer to terrestrial and aquatic predators. This transfer depends on several factors and will be discussed in section 4.3.0.

4.3.0 Metal Export via Emerging Chironomidae

Importance as Food

Chironomids may be an important vector in the transfer of anthropogenic metals from peat to terrestrial ecosystems because they are important prey items for a number of waterfowl and bird species. Sjoberg and Danell (1982) studied the feeding activity of ducks in relation to chironomid emergence. These authors noted that during peak emergence periods duck species, such as the tufted duck (*Aythya fuligula*) and the goldeneye (*Bucephala clangula*), fed intensely on chironomids at the water surface.

Food supply on the water surface, or in emerging vegetation, is critical for other duck species, such as mallards (*Anas platyrhnchos*). Mallard ducklings typically do not feed below the water surface in the first two weeks of life and therefore rely on emerging insects. Juvenile waterfowl are great consumers of aquatic insects having high protein requirements for growth and feather development (Murkin and Batt, 1987). Invertebrate densities may be important factors in waterfowl habitat selection during the spring breeding season. According to McNicol et al., (1987) some species of insectivorous waterfowl, such as common goldeneye (*Bucephala clangula*) and hooded mergansers (*Lophodytes cucullatus*), may select fish-less peatlands, acidic bogs, for example, in an attempt to avoid competition with fish for insect prey.

Chironomids may also be important food for a number of terrestrial bird species, such as tree swallows (*Tachycineta bicolor*), red-winged blackbirds (*Agelaius phoeniceus*), kingbirds (*Tyrannus tyrannus*), and the like. These terrestrial birds rely heavily on aquatic systems for food and may also be adversely affected by changes in food quality or contaminant levels in emerging insects.

Food quality may be adversely affected by low pH. Metals that are mobilized in low pH systems, such as Al, may pose a threat to waterfowl. Ormerod and Tyler (1987) examined reproductive success in dippers (*Cinclus cinclus*) breeding near acidified streams. The study noted strong evidence of population reduction, moderate shell thinning and a significant reduction in egg mass near acidified streams. According to Tyler and Ormerod (1992), these effects may have been caused by Ca scarcity in the pre-breeding period. Sparling (1991) conducted an experimental study on black ducks (*Anas rubripes*) and mallards (*Anas* *platyrhnchos*) to examine further the Ca-Al interaction in waterfowl diets. While the ducks were exposed to a variety of prepared diets varying in Al, Ca and P concentrations, Sparling (1991) found evidence that Al binds with Ca, thereby preventing Ca metabolism. This interaction between Al and Ca may be especially detrimental to waterfowl (and birds) during the spring breeding season because the female birds have high Ca requirements at this time (Murkin and Batt, 1987).

Several studies have found reduced survival or breeding success in avian species near acidic lakes (reviewed in Blancher and McAuley, 1987). Nyholm (1981) measured Al in pied flycatchers (*Ficedula hypoleuca*) and found that Al contaminated birds breeding near acidic lakes had smaller clutches and showed defective eggshell formation. Bone Ca deposits had been depleted during the incubation phase. Nyholm (1981) claimed that Al in prey from nearby lakes was responsible because it disrupted the metabolism of Ca during eggshell formation. Blancher and McNicol (1988) observed reduced breeding success in tree swallows (*Tachycineta bicolor*) near acidic peatlands. Three hypotheses were proposed: increased metal uptake causing related toxic effects, changes in aquatic food abundance and reduced calcium availability. The first two mechanisms were considered unlikely (Blancher and McNicol, 1992). Also in acidic peatlands, Glooschenko et al., (1986) noted that Al in peatland waters accounted for a small portion of the variation in the number of eggs and in nestling growth in eastern kingbirds (*Tyrannus tyrannus*). Given the above effects, it is therefore important to determine whether greater exports of toxic metals and lower Ca exports will occur in emerging chironomids from the lower pH systems.

Export

To compare the estimated export of metals among peatlands, export values were calculated for a standardized area. The calculations reflect both peatlands where metal concentrations in chironomids are elevated and peatlands where chironomid productivity is great. According to Menzie (1980), significant amounts of contaminants can be transported out of the aquatic systems depending on the level of insect production, on bioconcentration factors and on the proportion of biomass that leaves the system as adults. If a given system contains chironomids with high metal levels, it will be of toxicological importance if it is also accompanied by high productivity.

Larval density (Table 3.8) varied among the peatlands, with BO bog having the greatest density at $980.51 \cdot m^{-2}$ and SI circumneutral fen having the lowest at $55.64 \cdot m^{-2}$. Larvae were relatively abundant in two of the three bogs and abundant in one of each of the circumneutral and mineral poor fens.

If bogs produce greater chironomid densities, high densities coupled with intermediate metal concentrations may lead to greater metal export from bogs. However, as stated earlier, this study did not find a clear trend of higher chironomid densities in bogs. The greatest export of Zn, Cu, Al, Fe and Ca occurred at Bor, a bog where metal and Ca levels were indeed intermediate and where chironomid biomass was greatest (Table 3.8). The two fens, Hydro and Buck, also had relatively high export values that were related to high biomass. It would appear that high metal export values are not restricted to bogs. In addition, there is no clear indication that the food quality would be more reduced in the bogs.

Manganese export was highest in the fen Buck, where Mn levels were high and there was relatively high biomass. Though the biomass was greater in Bor, Mn export may have been reduced because of the lower Mn levels found in organisms from this system.

It is clear that export is not related to water chemistry variables such as pH. This confirms findings by Bendell-Young and Harvey (1988) that Al, Zn and Cu export through emerging chironomids is not related to pH. This may be because export depends upon two very distinct variables (i.e. biomass and metal concentration). However in some cases emerging insects can have an impact on sediment contamination, by removing small amounts of contaminants as they emerge. Fairchild et al., (1992) observed a small, but biologically significant, removal of sediment loads of 2,3,7,8-Tetrachlorodibenzofuran by emerging insects. According to Menzie (1980), sediment contamination concentrations can decline owing to removal by aquatic insects, but this generally occurs in systems where high levels of production are accompanied by high bioconcentration factors. Export values found in this study are several substantially lower than values calculated for Al, Zn and Cu by Bendell-Young (1990). This may be explained by the lower chironomid production observed in the peatlands.

Export quantities may be of minor importance to the removal of pollutants from systems. However, these quantities may represent an additional source of pollution to insectivorous predators, a factor generally overlooked (Timmermans and Walker, 1989). This is especially true in the case of terrestrial birds that have a strong trophic dependence on freshwater systems but are easily overlooked in an assessment of the effects of acidification on aquatic systems.

Insects that do not fall prey to predation return their contaminant load to the environment. According to Fairchild et al., (1992), the contaminant is then in a highly bioavailable form that likely moves into a food chain rather than returning to the sediment.

4.4.0 Metal Concentrations in Chironomidae larvae and Odonata Nymphs: Biomagnification or Biominification

As fish communities decline in acidified environments, rapid increases may occur in the number of invertebrate predators, such as Odonata (Henrikson et al., 1982, Hilton, 1987). Hence, it is important to consider the role of *Libellula* in food chain transfer of contaminants. The Odonata living in bogs may be able to cope with further reductions in pH because they are already adapted to a low pH environment. Also, in less acidic environments where fish are present, Odonata are important prey (Benke, 1978, Hilton, 1987) and therefore represent an additional link between the benthic and pelagic food chain. There is also the potential for transfer of contaminants to the terrestrial food chain with emerging odonates.

There are many possible factors that account for differences in metal concentrations among organisms: trophic level, feeding habit (Smock 1983) and association with the substrate (Timmerman et al., 1989). According to Krantzberg (1985), metals associated with detrital materials are more available to biota than those associated with prey species. Sediment-dependent organisms, such as chironomid larvae, may ingest relatively greater amounts of sediment than do species in the other feeding categories (Smock, 1983).

According to Benke (1978), the predominant prey for Odonata are chironomids and mayflies. This finding was also confirmed by Larson and House (1990), who examined Odonata in Newfoundland bogs. These authors noted that both chironomids and Odonata were abundant in the bogs and that chironomids were a major portion of the diet of all Odonata species. In stomach contents of two Odonata genera, *Ladona* and *Epitheca*, Benke (1978) found that Chironomidae comprised greater than 80 percent of all prey items.

Bioconcentration factors were near 1 for Zn, therefore Zn was not biomagnified in the *Libellula* nymphs (Table 3.7). Concentrations were, in fact, similar to those found in chironomid larvae. In contrast, Timmermans et al (1989) found that Zn concentrations in the chironomid predator *Hygrobates*, the water mite, were five times the concentrations observed in the chironomid larvae. This finding may relate to the manner in which this particular predator feeds.

Hare et al., (1991b) noted that in two predators, *Hexagenia* and *Sialis*, substantial quantities of Zn were in the gut contents as opposed to being absorbed. Nott and Nicolaidou (1990) examined biomagnification of Zn and Mn in sediment-feeding (*Cerithium vulgatum)* and carnivorous whelks (*Murex trunculus*). These authors claimed that metals were detoxified by the sediment-feeding *C. vulgatum* through incorporation into membrane-bound granules. Results of an x-ray microprobe indicated that granules were not destroyed in the digestive system of the predator *M. trunculus*. The granules therefore benefit both predator and prey. This finding is important as it not only confirms tolerance and detoxification of Zn and Mn in a sediment-feeding organism, but also suggests that this detoxification for the initial consumer is passed on to predators.

It is important to consider more than just trophic position when considering the biomagnification of metals (Timmermans et al., 1989). The manner in which the prey is consumed is also important. Timmermans et al., (1989) examined metal accumulation from *Chironomus* to two predators, caddisfly larvae and *Hygrobates*. Caddisfly larvae ate the *Chironomus* larvae from one end and in doing so therefore spill gut contents, thereby reducing their metal exposure. In contrast, *Hygrobates* first pierced the larvae, ingested the digestive fluids, and then discarded the empty exoskeleton (Timmermans et al., 1989). This feeding behaviour may explain why *Hygrobates* had greater Cu and Zn concentrations than non-predacious organisms. *Hygrobates* was the only predator (of six) to have greater Cu than the deposit feeders.

Prey handling can influence metal uptake in terms both of the amount of metal transferred and of the efficiency of this transfer. Odonata consumed the chironomid prey in a manner similar to that of the caddisfly larvae described above (personal observation). Therefore, the possibility that internal body fluids and/or gut contents were lost upon predation should not be excluded. This may have provided an additional means of reducing metal exposure to predators. In addition, physiological differences in the digestive tract, chemical differences, for example, may result in different reactions of metals as they pass through the gut (Krantzberg, 1985).

Concentrations of Cu in nymphs suggest that, like Zn, neither biomagnification nor biominification occurred (as BCFs were near 1), with the exception of nymphs in Arrowhead fen. Timmermans et al. (1989) also found no biomagnification of Cu in the predators of chironomids, *Hygrobates* and *Hexagenia*. The absence of clear biomagnification was also observed in terrestrial insect predators by Laskowski (1991). Bioconcentration factors of 1.26 to 1.83 were found. Laskowski (1991) concluded that it was easier for organisms to regulate body levels of nutritional heavy metals (such as Zn and Cu). Kiffney and Clements (1991) examined bioaccumulation of Zn and Cu in a number of benthic organisms. These authors found that Grazers and shredders generally had metal levels exceeding those in predators. In addition, Hare et al., (1991a) found that concentrations of Cu in purged organisms from Lake St. Joseph were greater in *Chironomus* than in the two predators *Hexagenia* and *Sialis* (concentrations of 21.2, 18 and 14 $\mu g \cdot g^{-1}$ respectively).

Aluminum and Fe concentrations were found to be lower in the predators, suggesting that biominification occurred. The lack of biomagnification of Al in invertebrate predators was also observed at pH 4.0 and 6.0 by Herrmann (1990) in insects from streams. Herrmann (1990) noted that the predators *Isoperla*, *Plectrocnemia* and *Rhyacophila* had lower Al concentrations than 'shredders'. Smock (1983) examined Fe concentrations in a number of predacious and non-predacious aquatic insects and found that concentrations were highest in the sediment-dependent chironomids.

With the exception of one bog, (Upper Dawson, Mn also biominified. Detoxification of Mn by sediment-dwellers and the absence of biomagnification were also found in a review of marine organisms by Nott and Nicolaidou (1990). Mn may increase in food webs, resulting in weak biomagnification; however, the metal has generally been found to biominify (reviewed in National Research Council of Canada, 1988).

An interesting trend was observed with Ca concentrations in the nymphs. Concentrations were greater in *Libellula* than in chironomid larvae from four of five sites. This difference may be a result of different metabolic requirements for Ca in the predator, or it may simply be a result of more effective mechanism by the organism for retaining the nutrient.

Summary

It is important to determine which metals readily biomagnify and which are lost upon trophic transfer. It is also important to establish in which type of environment the greatest biomagnification occurs and to determine whether this finding is toxicologically significant. Cu and Zn are readily available to benthic organisms, but the results of this study indicate that levels accumulated are not biomagnified in higher trophic levels. Indeed, the two organisms appear to have similar Cu and Zn concentrations regardless of their feeding habits and habitat.

Many studies have found that metal concentrations are lower in higher trophic level organisms (reviewed in Smock, 1983). According to National Research Council of Canada (1988), biomagnification has been adequately demonstrated for only Hg. Biominification occurs more frequently. In this study, Al, Mn, and Fe concentrations were generally lower in *Libellula* nymphs than in chironomid larvae. As these three metals are derived from the soil and from watershed processes, the chironomid, building tubes in the peat, may obtain greater metal concentrations because of it is more intimately associated with the sediment than *Libellula*. The greater Ca concentrations found in the *Libellula* nymphs are toxicologically significant because they implies that invertebrate predators in peatlands retain slightly higher Ca concentrations than their chironomid prey in spite of environmental conditions.

CHAPTER 5.0

IMPLICATIONS AND SUMMARY

This section will present a summary of the findings of this study. The implications of these findings for the natural and anthropogenic acidification of peatlands will also be discussed.

Differences in metal concentrations were observed in chironomid larvae collected in the nine peatlands. Concentrations of Cu and Zn varied, but were not a function of pH or alkalinity. Larval Zn, however, was negatively correlated with organic matter in the peat and larval Cu with DOC in the surface waters. Greater concentrations of Mn and Fe were found in the fen larvae. In addition, Mn concentrations were negatively correlated with organic matter in the peat. Differences in concentrations of Mn and Fe among peatlands were attributed to differences in hydrogeological conditions. Finally, larval Al and Ca concentrations did not appear to follow a clear trend with pH or alkalinity. These findings do not clearly indicate an additional risk for larval predators as acidification accelerates the transition of circumneutral fens to acidic bogs. In some cases, metal concentrations in larvae were reduced owing to the presence of organic matter.

Concentrations of Zn, Cu, Fe, Ca and, to an extent, Mn, were greatest in the aquatic predator *Libellula* from the mineral poor fen Arrowhead. This peatland was intermediate in pH, alkalinity, DOC, vegetation, etc,. The peatland did, however, have substantially lower organic matter in the peat than the others.

These findings stress the importance of organic matter in sequestering metal ions in aquatic systems. Although bogs are generally more acidic and have lower alkalinities than fens, the presence of organic matter in these systems may prevent greater metal accumulation in organisms.

Zinc, Mn and Ca concentrations were generally greater in chironomid adults than in larvae, indicating that these metals were not lost upon emergence, but rather retained. There was some evidence that greater transfer of Zn occurred in the bogs. If Zn transfer could be increased with acidification, Zn contamination of chironomids could be an important source of metal pollution, which is capable of crossing the aquatic terrestrial barrier. Iron and Al concentrations were greater in chironomid larvae than in adults. Since these metals were lost upon emergence, it appears that larval chironomids may be capable of trace metal elimination. This finding also has interesting biological and toxicological consequences. The trend with Cu in larvae and adults was split because Cu was greater in the adults in the high pH systems and greater in the larvae in the low pH systems. As peatlands acidify, the chironomid larvae may be capable of greater Cu elimination prior to emergence.

Metal export calculations were based on a number of variables, including adult metal concentrations and chironomid density. For a given area, the greatest transfer of Cu, Zn, Al, Fe and Ca occurred from the bog Bor (Table 3.8). Although adults from this peatland had intermediate metal concentrations, productivity was high, which resulted in greater potential transfer. Because the abundance and availability of chironomids can have a marked influence on peatland use by waterfowl and terrestrial predators, organisms feeding on adults from this peatland may obtain greater metal loads than predators feeding elsewhere. The export of Mn was greatest in the fen Buck, where Mn concentrations were high and productivity was intermediate. Because high export of metals is possible given either high productivity or high concentrations, peatlands where both variables are high would therefore be of particular concern.

Bioconcentration factors were calculated to determine whether biomagnification of metals occurred in *Libellula* nymphs. Chironomid larvae and *Libellula* had relatively similar concentrations of both Zn and Cu, indicating no biomagnification. Biominification was observed with Al, Fe and Mn. Because these metals are primarily soil-derived, they may have been higher in the chironomid larvae as a result of its more benthic lifestyle. The absence of biomagnification in invertebrate predators implies that organisms consuming insect predators are not at a greater risk of metal contamination. Ca concentrations were greatest in *Libellula*, a finding which may reflect a greater need for the nutrient in this organism.

Ca concentrations in nymphs may, however, still be insufficient to meet the needs of breeding waterfowl and terrestrial birds. In acidic peatlands, Ca-rich prey, such as Mollusca, are reduced or absent. Ca concentrations in the more tolerant aquatic invertebrates, such as chironomids and Odonata, may be all that remains available.

CHAPTER 6.0

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APPENDIX 7.1 Relevant water chemistry for the nine peatlands located in the Muskoka-Haliburton area, Ontario. Samples collected spring, 1990. Percent LOI (loss on ignition of sediments) and DOC (dissolved organic carbon in water) from Bendell-Young et al., (1992).

Peatland	Ca ($mg.L^{-1}$)	DOC ($mg.L^{-1}$)	% LOI
Bat	0.49	5.1	88
Upper Dawson	1.66	7.1	54
Bor	2.46	6.15	71
Arrowhead	2.69	7.0	18.9
Ideal	2.76	6.4	70
Hydro	3.77	3.7	54
Deer	4.87	9.9	31
Buck	4.42	n/a	n/a
Simp	6.2	11.4	34

With the exception of DOC where n=2, all values are means for n=4.

APPENDIX 7.2 Detection Limits ($\mu g \cdot mL^{-1}$) of the ICP-AS for four separate runs.

METAL	RUN 1	2	3	4
Al	0.01	0.004	0.043	0.02
Ca	0.008	0.001	0.025	0.008
Cd	0.002	0.003	0.002	0.006
Cu	0.005	0.009	0.006	0.005
Fe	0.006	0.048	0.006	0.011
Mn	0.004	0.001	0.001	0.006
Zn	0.004	0.002	0.007	0.001

Run #1 December, 1991; Runs #2,3 and 4 May, 1992.

APPENDIX 7.3 Observed and expected values for NBS Standard Reference Material (bovine liver tissue). Precision as indicated by coefficients of variation (C.V.) for Chironomidae and *Libellula*.

	Ca	Cu	Fe	Mn	Zn
Observed Values (g·mL^{-1})	131	154	193	9.8	108
(± S.D.)	16	6	19	0.9	4
Expected Values (μg·mL^{-1})	120	140	194	9.9	123
(± S.D.)	7	7	20	0.1	8
C.V.					
Chironomids	25.69	5.60	11.46	10.43	7.11
Libellula	11.80	5.00	11.32	9.08	3.39

Coefficients of Variation = standard deviation/mean * 100.

N.B. Al values not certified

APPENDIX 7.4 Results of Regression of Libellula metal concentration ($\mu g \cdot g^{-1}$) versus body length (mm) for each metal in a circumneutral fen, Buck. All data were log transformed.

Metal	F value	p value	r^2	Slope	
Zn	0.0500	0.8280	0.0063	0.23	n.s.
Cu	0.5600	0.4759	0.0654	0.37	n.s.
Al	4.5700	0.0650	0.3635	-2.85	n.s.
Ca	2.4470	0.1688	0.2897	-0.83	n.s.
Mn	n/a				
Fe	0.8600	0.3845	0.1094	-1.39	n.s.

n.s. not significant at p 0.05 level

APPENDIX 7.5a. ANOVA results in tabular form. One-Way Nested ANOVA for larval Chironomidae metal concentrations among peatland types and peatlands.

NESTED ANOVA - LOG Zn In Larvae by Peatland Type and Peatland

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.39	0.05	5.07	0.0001
Error	76	0.74	0.01		
Corrected Total	84	1.14			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TYPE	2	0.16	0.08	8.19	0.0006
PEATLAND (TYPE)	6	0.24	0.04	4.03	0.0015

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TYPE	2	0.18	0.09	9.41	0.0002
PEATLAND(TYPE)	6	0.24	0.04	4.03	0.0015

NESTED ANOVA - LOG Cu In Larvae by Peatland Type and Peatland

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	3.01	0.38	4.77	0.0001
Error	78	6.14	0.08		
Corrected Total	86	9.15			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TYPE	2	0.03	0.02	0.21	0.8075
PEATLAND (TYPE)	6	2.97	0.50	6.29	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TYPE	2	0.19	0.09	1.20	0.3080
PEATLAND(TYPE)	6	2.97	0.50	6.29	0.0001

NESTED ANOVA - LOG Al In Larvae by Peatland Type and Peatland

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	6.00	0.75	6.29	0.0001
Error	76	9.07	0.12		
Corrected Total	84	15.08			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TYPE	2	1.35	0.68	5.67	0.0051
PEATLAND (TYPE)	6	4.65	0.77	6.50	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TYPE	2	1.10	0.55	4.61	0.0129
PEATLAND(TYPE)	6	4.65	0.78	6.50	0.0001

APPENDIX 7.5a. ANOVA results continued...

NESTED ANOVA - LOG Ca In Larvae by Peatland Type and Peatland

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	2.12	0.26	22.91	0.0001
Error	76	0.88	0.01		
Corrected Total	84	2.99			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TYPE	2	0.60	0.30	25.88	0.0001
PEATLAND (TYPE)	6	1.52	0.25	21.92	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TYPE	2	0.72	0.36	30.97	0.0001
PEATLAND(TYPE)	6	1.52	0.25	21.92	0.0001

NESTED ANOVA - LOG Mn In Larvae by Peatland Type and Peatland

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	5.57	0.70	15.02	0.0001
Error	79	3.66	0.05		
Corrected Total	87	9.23			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TYPE	2	4.16	2.08	44.90	0.0001
PEATLAND(TYPE)	6	1.41	0.23	5.06	0.0002

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TYPE	2	4.27	2.13	46.02	0.0001
PEATLAND(TYPE)	6	1.41	0.23	5.06	0.0002

NESTED ANOVA - LOG Fe In Larvae BY Peatland Type amd Peatland

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	2.50	0.31	18.54	0.0001
Error	79	1.33	0.02		
Corrected Total	87	3.84			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TYPE	2	1.27	0.63	37.61	0.0001
PEATLAND(TYPE)	6	1.23	0.21	12.18	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TYPE	2	1.37	0.69	40.62	0.0001
PEATLAND(TYPE)	6	1.23	0.21	12.18	0.0001

APPENDIX 7.5b. ANOVA results in tabular form. One-Way Nested ANOVA for larval Chironomidae metal concentrations among peatlands and peatland sites.

NESTED ANOVA - RANKED Zn In Larvae by Peatland and Site

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	20506.68	2050.67	4.95	0.0001
Error	74	30662.81	414.36		
Corrected Total	84	51169.50			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
PEATLAND	8	18753.38	2344.17	5.66	0.0001
SITE(PEATLAND)	2	1753.31	876.65	2.12	0.1278

Source	DF	Type III SS	Mean Square	F Value	Pr > F
PEATLAND	8	18999.11	2374.89	5.73	0.0001
SITE(PEATLAND)	2	1753.31	876.65	2.12	0.1278

NESTED ANOVA - RANKED Cu In Larvae by Peatland and Site

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	21563.00	2156.30	5.39	0.0001
Error	74	29605.99	400.08		
Corrected Total	84	51169.00			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
PEATLAND	8	20597.86	2574.73	6.44	0.0001
SITE(PEATLAND)	2	965.15	482.57	1.21	0.3052

Source	DF	Type III SS	Mean Square	F Value	Pr > F
PEATLAND	8	20712.99	2589.10	6.47	0.0001
SITE(PEATLAND)	2	965.15	482.57	1.21	0.3052

NESTED ANOVA - LOG Al In Larvae by Peatland and Site

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	7.88	0.46	4.32	0.0001
Error	67	7.19	0.11		
Corrected Total	84	15.07			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
PEATLAND	8	6.00	0.75	6.99	0.0001
SITE(PEATLAND)	9	1.87	0.21	1.94	0.0607

Source	DF	Type III SS	Mean Square	F Value	Pr > F
PEATLAND	8	1.94	0.24	2.26	0.0334
SITE(PEATLAND)	9	1.88	0.21	1.94	0.0607

APPENDIX 7.5b. ANOVA results continued.

NESTED ANOVA - LOG Ca In Larvae by Peatland and Site

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	2.21	0.13	11.08	0.0001
Error	67	0.79	0.01		
Corrected Total	84	2.99			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
PEATLAND	8	2.12	0.26	22.56	0.0001
SITE(PEATLAND)	9	0.09	0.01	0.87	0.5532

Source	DF	Type III SS	Mean Square	F Value	Pr > F
PEATLAND	8	0.32	0.04	3.39	0.0025
SITE(PEATLAND)	9	0.09	0.01	0.87	0.5532

NESTED ANOVA - LOG Mn In Larvae by Peatland and Site

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	6.03	0.35	7.75	0.0001
Error	70	3.20	0.05		
Corrected Total	87	9.23			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
PEATLAND	8	5.57	0.70	15.22	0.0001
SITE(PEATLAND)	9	0.46	0.05	1.11	0.3652

Source	DF	Type III SS	Mean Square	F Value	Pr > F
PEATLAND	8	1.28	0.16	3.48	0.0019
SITE(PEATLAND)	9	0.46	0.05	1.11	0.3652

NESTED ANOVA - LOG Fe In Larvae by Peatland and Site

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	2.78	0.16	10.77	0.0001
Error	70	1.06	0.15		
Corrected Total	87	3.84			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
PEATLAND	8	2.50	0.31	20.64	0.0001
SITE(PEATLAND)	9	0.27	0.03	1.99	0.0529

Source	DF	Type III SS	Mean Square	F Value	Pr > F
PEATLAND	8	0.36	0.05	2.96	0.0065
SITE(PEATLAND)	9	0.27	0.03	1.99	0.0529

APPENDIX 7.5c. ANOVA results in tabular form. One-Way ANOVA for nymphal *Libellula* metal concentrations among peatlands.

ANOVA - LOG Zn In *Libellula* Nymphs by Peatland

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.34	0.03	1.06	0.3968
Error	26	0.84	0.03		
Corrected Total	30	0.97			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
PEATLAND	4	0.14	0.03	1.06	0.3968

Source	DF	Type III SS	Mean Square	F Value	Pr > F
PEATLAND	4	0.14	0.03	1.06	0.3968

ANOVA - LOG Cu In *Libellula* Nymphs by Peatland

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.59	0.14	10.07	0.0001
Error	25	0.37	0.01		
Corrected Total	29	0.96			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
PEATLAND	4	0.59	0.15	10.07	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
PEATLAND	4	0.59	0.15	10.07	0.0001

ANOVA - LOG Al In *Libellula* Nymphs by Peatland

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.90	0.48	3.00	0.0375
Error	25	3.96	0.16		
Corrected Total	29	5.87			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
PEATLAND	4	1.90	0.48	3.00	0.0375

Source	DF	Type III SS	Mean Square	F Value	Pr > F
PEATLAND	4	1.90	0.48	3.00	0.0375

APPENDIX 7.5c. ANOVA results continued.

ANOVA - LOG Ca In *Libellula* Nymphs by Peatland

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.55	0.39	27.79	0.0001
Error	23	0.32	0.01		
Corrected Total	27	1.87			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
PEATLAND	4	1.55	0.39	27.79	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
PEATLAND	4	1.55	0.39	27.79	0.0001

ANOVA - LOG Mn In *Libellula* Nymphs by Peatland

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.77	0.19	2.38	0.0879
Error	19	1.53	0.08		
Corrected Total	23	2.29			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
PEATLAND	4	0.77	0.19	2.38	0.0879

Source	DF	Type III SS	Mean Square	F Value	Pr > F
PEATLAND	4	0.77	0.19	2.38	0.0879

ANOVA - LOG Fe In *Libellula* Nymphs by Peatland

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.98	0.24	4.66	0.0070
Error	22	1.16	0.05		
Corrected Total	26	2.14			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
PEATLAND	4	0.98	0.24	4.66	0.0070

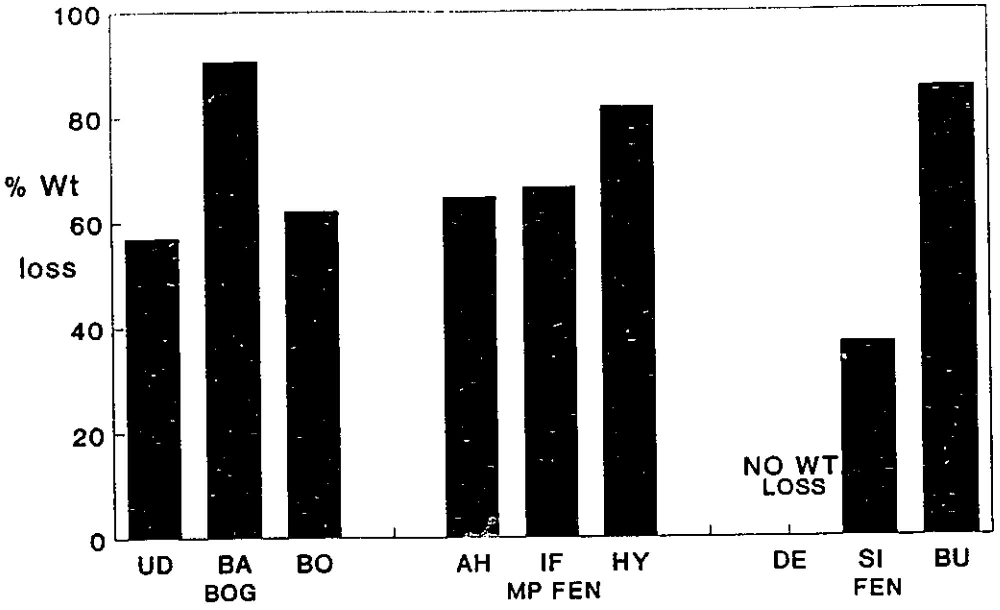
Source	DF	Type III SS	Mean Square	F Value	Pr > F
PEATLAND	4	0.98	0.24	4.66	0.0070

APPENDIX 7.6. Mean metal concentration (± S. E.) in adult Chironomid collected in submerged funnel traps at each peatland.
Values are in $\mu g \cdot g^{-1}$ dry weight.

	Metal $\mu g \cdot g^{-1}$ (dry weight)					
	Zn	Cu	Al	Ca	Mn	Fe
BOGS:						
BA	123.06	19.25	73.68	1496.03	21.18	880.35
	(23.56)	(2.49)	(31.88)	(222.52)	(4.38)	(114.26)
UD	128.08	12.06	192.24	1346.03	17.90	1656.51
	(17.10)	(1.6)	(87.71)	(318.46)	(4.67)	(319.02)
BO	166.02	21.60	188.26	1727.31	19.86	1395.03
	(23.27)	(5.13)	(57.57)	(218.95)	(2.54)	(169.80)
MINERAL POOR FENS:						
AH	241.81	12.64	129.37	1583.04	67.71	1527.92
	(40.36)	(3.87)	(27.24)	(230.40)	(9.91)	(240.70)
IF	125.90	16.37	161.54	1427.27	28.40	753.24
	(18.23)	(2.87)	(68.51)	(288.66)	(4.41)	(141.77)
HY	130.22	21.06	72.57	1143.86	28.65	557.17
	(16.99)	(6.34)	(40.86)	(355.91)	(3.68)	(83.93)
FENS:						
DE	141.99	15.29	293.60	1707.47	338.02	2877.07
	(13.28)	(3.31)	(100.86)	(229.18)	(57.82)	(529.08)
BU	152.46	23.83	207.34	2589.01	291.10	2256.54
	(10.49)	(4.92)	(64.27)	(195.12)	(45.69)	(304.82)
SI	116.86	20.67	87.99	1416.96	67.79	1170.53
	(7.84)	(3.72)	(37.65)	(135.50)	(15.31)	(97.93)

n=9 in BO and SI; n=8 in HY; n=8-9 in BA, IF, DE; n=7-9 in UD and BU;
n=5-6 in AH

APPENDIX 7.7 Percent weight loss (g) of Chironomidae adults upon emergence



• CALCULATION BASED ON MEAN LARVAL AND ADULT WEIGHT/WETLAND