University of Alberta

RESPONSES OF AQUATIC INVERTEBRATE ASSEMBLAGES
TO AN IRON TREATMENT AIMED AT REDUCING
INTERNAL PHOSPHORUS LOADING

by

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Abstract

This mesocosm study investigates changes in aquatic invertebrate assemblages in response to iron application, a remediation treatment that inhibits internal phosphorus loading in culturally-eutrophied freshwaters. To determine the effects of ferric chloride application on aquatic invertebrates, I analyzed the changes in abundance, biomass, and diversity of zooplankton and zoobenthos assemblages over a logarithmic scale of ferric chloride treatment doses applied to 12 experimental mesocosms installed in Nakamun Lake, AB. Zooplankton abundance and biomass significantly decreased immediately following iron treatment, likely through acidification, co-precipitation with the flocculant, and declines in phytoplankton populations. However, zooplankton assemblages recovered within one month after application. I detected no iron-induced changes in zoobenthos assemblages, perhaps because taxa present in hypereutrophic lakes are typically pollution-tolerant. My results indicate that ferric chloride application has only minor and short-term effects on aquatic invertebrate assemblages, so this remediation treatment may be a suitable tool for rehabilitation of eutrophied lakes.

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Eutrophication

Causes and symptoms

Eutrophication is the process in which increased nutrient loading increases growth of primary producers within a water body (Brönmark & Hansson, 2005). Eutrophication can be a natural process: as organic material accumulates, an aquatic ecosystem gradually shifts into a terrestrial ecosystem (Wetzel, 2001). This natural process is extremely slow and it would take thousands of years for an oligotrophic (i.e., nutrient-poor) lake to naturally become eutrophic (Schindler & Vallentyne, 2008). Many lakes, particularly in northern temperate regions, have become unnaturally eutrophic through nutrient enrichment from anthropogenic sources, such as human sewage or fertilizer run-off from agricultural areas (Wetzel, 2001). This so-called cultural eutrophication can cause lakes to become eutrophic in only a few years (Schindler & Vallentyne, 2008) and is one of the largest global problems affecting freshwaters worldwide (Smith & Schindler, 2009).

Cultural eutrophication drastically changes the water chemistry and community composition of water bodies, resulting in numerous undesirable side effects on both ecosystems and humans. Greater nutrient supplies increase the growth of primary producers, such as phytoplankton and macrophytes, because they are often growth-limited by nutrient availability (Brönmark & Hansson, 2005). Although the early stages of the eutrophication process typically result in an increase in biomass of periphytic (substrate-associated) algae and macrophytes (higher aquatic plants), as eutrophication progresses, phytoplankton quickly outcompete these submersed plants and become the dominant primary producers (Brönmark & Hansson, 2005). Cyanobacteria, or "blue-green algae", can often outcompete other phytoplankton and become the dominant primary producer due to the ability of some species to fix atmospheric nitrogen (Brönmark & Hansson, 2005) and adjust their buoyancy (Spencer & King, 1987). Cyanobacteria can also outcompete other phytoplankton because some species are poor quality food for herbivorous grazing zooplankton because they clog filtering appendages

(Lampert, 1982; DeMott *et al*., 2001) and are low in nutrients (Porter & Orcutt, 1980; Brett & Müller-Navarra, 1997). Uncontrolled algal biomass, particularly cyanobacteria, ultimately results in decreased water transparency, shoreline and surface scums, and unpleasant water taste and odour.

Excessive algal growth also induces considerable changes in other biotic assemblages within the lake ecosystem. The collapse and subsequent bacterial degradation of phytoplankton blooms lowers dissolved oxygen concentrations and increases ammonia concentrations, which can result in fish kills (Carpenter *et al*., 1985; Chorus *et al*., 2000; Zhou *et al*., 2002) and declines in oxygen-sensitive benthic invertebrates (Wiederholm, 1984). Eutrophication also adversely affects aquatic biota by decreasing biodiversity and disrupting ecosystem functioning (reviewed in Ansari *et al*., 2011). In addition, cyanobacteria blooms are of specific concern because certain species can produce neuro- and hepato- (liver) toxins, which can harm aquatic biota (e.g., Lampert, 1987; Reinikainen *et al*., 1999), domestic animals and wildlife (Sivonen & Jones, 1999) and even humans (Chorus *et al*., 2000; Zhou *et al*., 2002). Given the numerous consequences of cultural eutrophication on humans and natural ecosystems, measures to rehabilitate these polluted water bodies are of high priority to freshwater managers.

Internal and external phosphorus loading

Although there has been much debate over the nutrient(s) that limit algal growth, phosphorus (P) is most often the limiting nutrient on a long-term scale (Schindler, 1974, 1977; Schindler *et al*., 2008a). In light of this extensive body of research, management efforts focus on improving water quality by reducing external P inputs from within the catchment of the impacted water body. External P inputs can originate from point sources such as sewage plants or industrial effluent, or from non-point sources such as agricultural runoff, urban runoff, or septic system effluent. Although point sources are easier to regulate than non-point sources, all anthropogenic external P inputs must be reduced for a water body to recover. Lakes in the North American prairies are generally naturally

mesotrophic to mildly eutrophic because they lie in catchments geologically rich in phosphorus (Prepas & Trew, 1983) but the substantial contributions of external P loading from sewage effluent and agricultural and livestock-holding operations and other anthropogenic sources has caused severe cultural eutrophication across the Canadian prairie provinces (Alberta, Saskatchewan, and Manitoba). As of 2012, around 70% of lakes in the boreal and grassland regions in Alberta are considered to be either eutrophic or hypereutrophic (Alberta Environment, 2012).

Although culturally-eutrophied lakes can sometimes recover rapidly after external P inputs are reduced (Cooke *et al.,* 1993), other lakes do not recover quickly because P continues to be released from the lake sediments (internal loading). Internal P loading is the result of decades of excessive external nutrient loading, causing organic matter (and therefore nutrients) to accumulate within the sediments, and allowing P to be mobilized and released from the sediments to the overlying water under certain conditions (reviewed in Cooke *et al*., 2005). In some cases, eutrophied lakes can take decades to recover to conditions similar to those prior to human-induced eutrophication when only external P-loading is reduced because of this high internal P loading (Jeppesen *et al.,* 1999; Søndergaard *et al*., 2001). Internal loading can be the major source of P in freshwater systems: in some prairie lakes, contributing up to 90% of the total P pool (Prepas & Vickery, 1984). Therefore, a technique to inhibit internal P loading will help restore prairie lakes to their natural state more quickly than external P reductions alone.

Prairie lakes usually have low iron (Fe) concentrations (Schindler *et al.,* 2008b), which may be a potential reason for why they are slow to recover from reductions in external nutrient loading. Inorganic Fe is a naturally occurring element in fresh water bodies and plays an integral role in P cycling (Mortimer, 1941), appearing to be an important factor in internal P loading (Schindler & Vallentyne, 2008). Mortimer (1941) provides the generally accepted basic cycle of Fe and P in lakes, which is controlled by the redox state of Fe. In oxygenated, neutral to alkaline conditions, redox potential is high and iron is oxidized to the ferric (Fe^{3+}) form (Equation 1.1), which sorbs P and causes sediments to have

high P retention, mainly through sorption to $Fe(OH)_3$ but also through the formation of ferric complexes ($FePO_4$) (Andersen, 1975; Lijklema, 1977). When anoxia develops, (e.g., during periods of thermal stratification) Fe is used as an electron acceptor instead of oxygen, causing Fe to be reduced to its ferrous (Fe^{2+}) form (Cooke *et al.,* 2005). In the reduced state Fe is soluble, causing the release and mobilization of previously iron-bound P (Mortimer 1941).

$$Fe^{2+} + ¼\ O_2 + 2\ OH^- + ½\ H_2O \rightarrow Fe(OH)_{3\,(s)} \qquad (1.1)$$

This simplified description of the Fe and P cycle indicates that by ensuring water remains oxygenated and that Fe is available for P sorption, internal P loading should easily be terminated. However, the redox potential of Fe is sensitive to changes in pH, availability of sulfur compounds, and even lake trophic status (reviewed in Cooke *et al*., 2005). The conclusions of Mortimer's (1941) work have been questioned (e.g., Golterman, 2001) and some research suggests that P release can be inhibited through iron enrichment of sediments even under anoxic conditions (Quaak *et al.,* 1993; Boers *et al.,* 1994).

Iron as a remediation tool

The mechanisms of internal P loading in the presence of Fe, and the effectiveness of iron salts as a potential remediation tool, are now being examined for culturally-eutrophied lakes in the Canadian prairies (Orihel *et al*., in prep.). Iron salts (e.g., ferric chloride or ferric sulfate) have been applied to entire lakes (Foy, 1985; Boers *et al.,* 1992) and reservoirs (Hayes *et al.,* 1984; Deppe *et al.,* 1999; Perkins & Underwood, 2000) in western European countries, as well as to a few lakes in North America (Walker *et al.,* 1989; Engstrom, 2005; R. Carignan, Université de Montréal, unpublished data). These studies have shown varying effectiveness of iron salts both at reducing internal P loading, and in the longevity of the treatment (reviewed in Cooke *et al*., 2005).

The evaluation of success of these whole-lake Fe application studies has been defined by changes in nutrients (P) and phytoplankton; very few studies have investigated the effects of iron treatment on other biota. Iron application can be a technique suitable for lake rehabilitation, but only if the treatment does not

adversely affect aquatic food webs, and consequently ecosystem function. Therefore, it is essential to investigate how iron application affects biotic assemblages other than phytoplankton alone. The few studies directly assessing the responses of biota to Fe treatment have typically focused only on a few zooplankton species (e.g., Foy & Fitzsimons, 1987; Daldorph, 1999; Randall *et al*., 1999; Van Anholt *et al*., 2002) and ignored community-level responses. This thesis investigates the effects of iron application (in the form of iron(III)chloride) on aquatic invertebrate assemblages by performing an in-situ mesocosm study and assessing the changes in abundance, biomass, and taxonomic composition of zooplankton and benthic macroinverebrates.

Potential effects of iron application on aquatic invertebrates

Aquatic invertebrates are important constituents of aquatic ecosystems and provide a measure of secondary productivity. Zooplankton are an integral component of aquatic food webs because they are closely coupled to phytoplankton, and are often the main source of food for higher organisms such as planktivorous fish (Brönmark & Hansson, 2005). Benthic macroinvertebrates are important in decomposing organic matter and cycling essential nutrients such as nitrogen (N) and phosphorus (P) back into the water column (Lindeman, 1942), and are also an important component of lake food webs (Vadeboncoeur *et al*., 2002), sometimes as a major prey resource supporting fish production in North American lakes (Schindler *et al*., 1997; Vander Zanden & Vadeboncoeur 2002). Many benthic insects only carry out their larval stages in aquatic habitats before emerging as adult insects and carry out the remainder of their life in terrestrial ecosystems. Through the emergence of adult insects, benthic secondary production in lakes can transport energy and nutrients from lakes to terrestrial systems. This exported energy derived from aquatic systems contributes to terrestrial ecosystem function; emergent insects can be important energy subsidies for aerial insectivores such as birds (Davies, 1976; McCarty, 1997) and bats (Sullivan *et al*., 1993), as well as for terrestrial arthropod insectivores in the riparian zone (Paetzold & Tockner, 2005).

Current literature indicates that the most apparent consequences of iron application on aquatic invertebrates are via direct toxicity from dissolved iron, acidification (decrease in pH), and indirect effects from the formation of iron precipitates. If iron application is successful at substantially reducing internal P loading then the eutrophication process could be reversed, sometimes referred to as "oligotrophication" (Stockner *et al*., 2000). Oligotrophication would result in the alteration of phytoplankton assemblages, and in response it could potentially alter other biotic assemblages (e.g., zooplankton and macroinvertebrates).

Iron toxicity

Iron is an essential trace element, vital for the survival of biota because it is involved in many metabolic processes, such as oxygen transport, DNA synthesis, and electron transport (Gurzau *et al*., 2003). However, excess ferrous (Fe^{2+}) iron also poses a risk of overload due to acute and/or chronic exposures (Gurzau *et al*., 2003). In the ferrous form iron remains soluble, but dissolved iron concentration is difficult to determine in surface waters because iron speciation is sensitive to changes in dissolved organic matter, sulfur compounds, pH, and redox potential. Total iron can be measured to avoid these complexities (Linton *et al*., 2007) because it encompasses the concentration of all forms of dissolved Fe. Unfortunately, the absolute iron concentration that negatively affects biota is difficult to determine at a community scale, because species have different iron-toxicity sensitivities (reviewed in Vuori, 1995), laboratory toxicity experiments have shown highly variable adverse effects thresholds even within a single species (e.g., Maltby *et al*., 1987), and toxicity thresholds determined in laboratory bioassays are often very different in the field (Gerhardt & Westermann, 1995; Vuori, 1995).

For these reasons, the literature on direct iron toxicity on aquatic invertebrates is limited, and generally restricted to macroinvertebrates. Some field surveys have shown that highly sensitive insect taxa disappear at dissolved (Rasmussen & Lindegaard, 1988) and total (Linton *et al*., 2007) iron concentrations between 0.2 and 0.3 mg L^{-1}. Field surveys also indicate that

zoobenthos richness is minimally affected at total iron concentrations <1 mg L^{-1} (Linton *et al*., 2007; Peters *et al*., 2011) and that taxon richness and relative abundance of zoobenthos decreases at dissolved and total iron concentrations >1 mg L^{-1} (Rasmussen & Lindegaard, 1988). These results support that the current U.S. Environmental Protection Agency's water quality guidelines of <1 mg L^{-1} (US EPA) as sufficient to protect most aquatic invertebrates. In Canada, however, the surface water quality guideline for total iron concentration in freshwaters is much lower, at 0.3 mg L^{-1} (CCME, 1999).

Iron concentrations within the water column would be expected to remain low after Fe application to an alkaline, oxic, eutrophic water body because Fe would be bound in ferric hydroxide precipitates. Because ferrous iron remains soluble at pH < 5.5 it is considered toxic under acidic conditions (McDonald *et al*., 1989). For that reason, water pH should stay above 5.5 during iron application to avoid iron toxicity to invertebrates.

It is possible, however, that benthic macroinvertebrates in lakes suited for iron treatment may be less susceptible to direct iron toxicity than taxa found in less eutrophic lakes because the taxa present are likely more pollution-tolerant. Rasmussen and Lindegaard (1988) suggest that the taxa most tolerant to high iron pollution are also tolerant to organic enrichment (i.e., eutrophication). In addition, the uptake of Fe^{2+} is regulated by biota because it is a bioavailable ion (Locke & Nichol, 1992), and Fe^{2+} does not accumulate in whole body loads when exposed for short periods of time (Gerhardt, 1994). Furthermore, aquatic invertebrates can develop tolerance to metal pollution at sub-lethal concentrations (Maltby *et al*., 1987; Klerks & Weis, 1987). It is thus unlikely that Fe^{2+} will pose a direct toxic threat to the aquatic biota as long as total Fe concentrations within the water column after iron application do not exceed the recommended guidelines for the respective jurisdiction.

Acidification

Although direct Fe toxicity seems unlikely, Fe treatment could still have indirect effects on aquatic biota. Acidification is the most significant drawback to

iron dosing (Cooke *et al*., 2005). When ferric chloride or other iron salts are added to water, they immediately dissociate to form ferric hydroxide, leaving free chloride anions (Equation 1.2).

$$FeCl_3 + 3\ H_2O \leftrightarrow Fe(OH)_{3\ (s)} + 3\ H^+ + 3\ Cl^- \qquad (1.2)$$

In hard water lakes with high alkalinity, bicarbonate (HCO_3^-) prevents hydrochloric acid (HCl) from forming and buffers the lake water from acidification. As long as bicarbonate is available, the excess hydrogen ions combine with bicarbonate (Equation 1.3) and pH is relatively unaffected.

$$H^+ + HCO_3^- \leftrightarrow H_2CO \leftrightarrow H_2O + CO_2 \qquad (1.3)$$

However, once the bicarbonate ions are exhausted, as might occur after high iron(III)chloride doses, the excess hydrogen ions combine with free chloride ions and hydrochloric acid (HCl) is formed.

This strong acid quickly decreases the water pH, which can have detrimental effects on freshwater biota, and particularly zooplankton. Because rotifer (Berzins & Pejler, 1987) and crustacean (e.g., Price & Swift, 1985; Brett, 1989; Havens *et al*., 1993; Doka *et al*., 2003) assemblages display species-specific pH tolerances, they are useful indicators of acidification. Water pH between 5.5 and 6.0 is the threshold in which shifts between acid-sensitive and acid-tolerant zooplankton species occur (Schindler *et al*., 1991; Havens *et al*., 1993; Doka *et al*., 2003). In the zoobenthos assemblages, molluscs and crustaceans are particularly acid-sensitive because low pH reduces the ability of these organisms to uptake the calcium essential for their shells and exoskeletons (Malley, 1980). Acidification impacts on macroinvertebrates are generally observed at pH < 6.0 (e.g., Barmuta *et al*., 1990; Griffiths, 1992; Orendt, 1999).

Aluminum toxicity in freshwater biota is also commonly associated with acidification in freshwater systems because the concentration and toxicity of aluminum increases at lower pH levels (reviewed in Havas & Rosseland, 1995). However, aluminum toxicity is likely not of concern for my study because increased aluminum toxicity in acidified lakes is often a result of atmospheric acid deposition, causing aluminum to leach from the watershed into the lake (reviewed in Havas & Rosseland, 1995). In my treatment, acidification is a result of an in-

situ iron treatment, rather than catchment-wide precipitation and is therefore an internal process unaffected by the surrounding catchment.

Physical Fe-hydroxides effects

Laboratory studies do not agree on which form of iron is most toxic to aquatic invertebrates (Peters *et al*., 2011), but several studies have suggested that the physical effects of ferric hydroxide precipitates are more detrimental to aquatic biota than dissolved ferrous iron (e.g., Rousch *et al*., 1997; Regerand *et al*., 2005). Although influenced by other chemical parameters, the oxidation of soluble ferrous (Fe^{2+}) iron to particulate ferric (Fe^{3+}) iron hydroxides generally occurs under oxic conditions and neutral pH. Because dissolved organic carbon (DOC) can act as a chelating agent with iron, high DOC concentrations in eutrophic lakes can encourage the oxidation of iron and the formation of ferric hydroxides (Cooke *et al*., 2005).

In macroinvertebrates, ferric hydroxide precipitates have been observed to interfere with oxygen transfer (Gerhardt, 1992; Vouri & Kukkonen, 1996; Regerand *et al*., 2005) and induce starvation by coating and reducing the permeability of the gut (Gerhardt, 1992). Precipitation of Fe^{3+} hydroxide flocculant can also reduce the quantity and quality of zoobenthos food resources (e.g., periphyton), and destabilize habitat substrates in lotic systems (Rasmussen & Lindegaard, 1988; Koryark *et al*., 1972). Rapid accumulation of iron precipitates at the water-sediment interface may affect zoobenthos if oxygen becomes depleted before they are able to move back up to the interface, and thereby become "smothered" within the floc layer.

An organism's sensitivity to metal toxicity can vary at different stages over its life cycle (Gauss *et al*., 1985; Williams *et al*., 1986; Rousch *et al*., 1997). Because the benthic fauna in the profundal zone of eutrophic lakes are typically dominated by chironomid midges (Wetzel, 2001), sampling emerging adult insects can capture the major portion of macroinvertebrate secondary production. The successful emergence of chironomids would indicate that they were able to complete the aquatic portion of their life cycle. However, sub-lethal Fe effects

(e.g., changes in fitness or body condition) are still possible even if emergence is unaffected.

In addition to the negative physical flocculation effects, chronic Fe^{3+} exposure can also cause changes in behavior that can reduce growth and survival. When exposed to high iron concentrations, both *Gammarus pulex* (Amphipoda) (Maltby & Crane, 1994) and *Leptophlebia marginata* (Ephemeroptera) (Gerhardt, 1994) show reduced feeding rates. *L. marginata* also exhibit reduced avoidance behavior, which may reduce its ability to escape predation (Gerhardt, 1994). Studies of behavioural changes in response to Fe application are lacking in the literature.

Similar to macroinvertebrates, zooplankton are also more commonly affected by ferric precipitates than by ferrous iron. Small-scale studies suggest that zooplankton that filter-feed (e.g., daphniids) are affected most by iron application through the physical properties of solid ferric hydroxide, which decreases the daphniids' filtering efficiency (Randall *et al.,* 1999) and interfere with nutrient absorption (Van Anholt *et al.,* 2002). Chronic exposure to ferric hydroxides can decrease total zooplankton biomass (Holz & Hoagland, 1996) and lower reproductive success in *Daphnia* species (Randall *et al.,* 1999; Van Anholt *et al.,* 2002; Sotero-Santos *et al.*, 2007).

In contrast to zoobenthos, who are chronically exposed to iron precipitates because the floc settles in the benthic invertebrates' habitats, zooplankton are exposed to the solid ferric hydroxide precipitates for a short period of time. However, zooplankton can potentially be precipitated during iron flocculation and buried within the sediments. Although co-precipitation of zooplankton with iron flocculant has not been confirmed from previous studies, zooplankton have been found to be precipitated with aluminum flocculants (Wold *et al.*, 2005).

Oligotrophication

Although there is little literature discussing the response of littoral or profundal macrozoobenthos to nutrient reductions (i.e., "oligotrophication"), some studies indicate that macrozoobenthos communities respond to

oligotrophication in the reverse order as the eutrophication process. Specifically, the abundance of hypereutrophic chironomid and oligochaete taxa, as well as the total macrozoobenthos abundance decreases (Köhler *et al*., 2005), and richness and diversity of zoobenthos increases (Mastrantuona & Sforza, 2008). Because the eutrophication process increases sedimentation and decreases light penetration, benthic eutrophic habitats become less complex as macrophyte abundance declines and substrates become homogenous. As the reverse process occurs, the benthic macroinvertebrate assemblages are likely to be driven by increasing habitat heterogeneity if macrophytes re-establish from the increased light penetration and decreased sedimentation. Increased habitat heterogeneity and complexity is likely to increase zoobenthos species heterogeneity (Donohue *et al*., 2009).

Dipteran emergence can be used as an endpoint to quantify secondary productivity. Increased abundance of emergent insects is often positively correlated to increases in primary productivity (McCarty, 1997) and with higher chlorophyll *a* concentrations (Davies, 1980; Welch *et al*., 1988; Blumenshine *et al*., 1997). Nutrient sequestration from iron addition could therefore lead to reductions in emergence, as secondary productivity would be expected to decline in response to oligotrophication.

Zooplankton are likely to respond to oligotrophication quicker than zoobenthos, because zooplankton are strongly dependent on phytoplankton populations and have shorter generation times than benthos. If zooplankton are able to resist co-precipitating with the iron flocculant, they might still die from starvation if all of the phytoplankton precipitated out. Zooplankton species have variable starvation times ranging from 0.4 - 5 days for rotifers (Kirk, 1997) and 4 - 11 days for cladocerans (Threlkeld, 1976). Other studies have shown zooplankton abundance declines after iron application due to reductions in their food resources (Holz & Hoagland, 1996; Randall *et al*., 1999). The few studies on zooplankton responses to oligotrophication in the literature have been inconsistent (Jeppesen *et al*., 2005a). However, Jeppesen *et al*.'s (2005b) synthesis indicates that over the long term, oligotrophication of shallow lakes typically leads to

reduction in total zooplankton biomass, increased zooplankton to phytoplankton biomass ratio, and an increase in the mean body size of cladocerans.

Summary of reviewed literature and study rationale

To limit the adverse effects of ferric chloride application on aquatic biota, the review of relevant literature suggests that the water column pH should not drop below 5.5 or 6. These pH values appear to be thresholds at which acidification effects become apparent in pelagic and profundal biota, and at which iron will remain in the more toxic, ferrous form. After iron application, keeping total Fe concentration below the applicable (e.g., CCME) water quality guidelines should ensure that there are minimal impacts on aquatic biota, while also meeting jurisdictional guidelines.

In general, there is a lack of specific research on how ferric chloride application affects aquatic biota. The evaluation of potential negative effects of iron treatment on aquatic invertebrates is a critical part of determining optimal remediation strategies, so that remediation attempts do not inadvertently cause negative impacts on aquatic biodiversity and ecosystem function. The objective of this thesis is to determine the effects of different iron application concentrations on invertebrate assemblages, by examining changes in abundance, biomass, and taxonomic composition of zooplankton and macroinvertebrate assemblages. By experimentally altering water column nutrient concentrations, this study also contributes to the growing body of literature investigating how aquatic invertebrate assemblages respond to oligotrophication.

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Chapter 2: Changes in zooplankton assemblages following iron treatment

Introduction

Many lakes in the Canadian prairie provinces suffer from cultural eutrophication as a result of increased nutrient input from anthropogenic activities (Schindler & Vallentyne, 2008). Lake restoration techniques often focus on reducing phosphorus (P) loading from sources that are external to the water bodies (e.g., agricultural runoff), but prairie lakes often have high internal P loads due to the high return of P accumulated in their sediments (Schindler & Comita, 1972). If only external P-loading is reduced, lakes can take decades to recover to conditions similar to those prior to human-induced eutrophication because of this high internal P loading (Jeppesen *et al.,* 1999; Søndergaard *et al*., 2001), including prairie lakes (Schindler, 2012). One potential reason for prairie lakes being slow to recover from external nutrient reductions is their low iron (Fe) concentrations. Iron is a naturally occurring element in fresh water bodies, and its presence limits the availability of P in the water column because Fe binds with P and limits its availability (Mortimer, 1941), so the application of Fe salts (e.g., ferric sulfate or ferric chloride) may prove an effective method to restore natural iron conditions and reduce internal P loading. To test this hypothesis, Orihel *et al*., (in prep) added ferric chloride in a wide range of concentrations to in-situ mesocosms. As part of these studies, I assessed the potential of iron addition for damaging the food chain by analyzing changes in zooplankton assemblages.

Although Fe is an essential trace element required for the survival of biota, excess ferrous (Fe^{2+}) iron also poses a risk of overload due to acute and/or chronic exposures (Gurzau *et al*., 2003). In the ferrous form, iron remains soluble and has been shown to cause DNA damage and disrupt cell membrane function (Gurzau *et al*., 2003). Ferrous iron remains soluble at pH < 5.5 and is therefore considered toxic under acidic conditions (McDonald *et al*., 1989). Under oxic and neutral pH conditions, iron rapidly becomes oxidized to solid ferric (Fe^{3+}) hydroxides. Small-scale studies suggest that filter-feeding zooplankton are affected most by iron application through the physical properties of solid ferric hydroxide, by decreasing their filtering efficiency (Randall *et al*., 1999), interfering with nutrient

absorption (Van Anholt *et al*., 2002), and reducing food resources (Holz & Hoagland 1996; Randall *et al*., 1999). Chronic exposure to ferric hydroxides can decrease total zooplankton biomass (Holz & Hoagland, 1996) and lower reproductive success in *Daphnia* species (Randall *et al*., 1999; Van Anholt *et al*., 2002; Sotero-Santos *et al*., 2007). Despite these potential negative effects, iron salts have been applied to entire lakes (e.g., Foy & Fitzsimons, 1987; Boers *et al*., 1992) and reservoirs (e.g., Daldorph, 1999; Perkins & Underwood, 2000) with relatively little knowledge on how it might affect zooplankton assemblages. Where this literature does exist, it typically focuses only on daphniids (e.g., Foy & Fitzsimons, 1987; Daldorph, 1999; Randall *et al*., 1999; Van Anholt *et al*. 2002) and ignores community-level responses.

Research is also sparse on the responses of zooplankton assemblages to reduced nutrient availability, sometimes termed "oligotrophication". Although responses to reduced nutrient loading in other biotic assemblages, such as phytoplankton and fish, are fairly consistent (reviewed in Jeppesen *et al*., 2005b), zooplankton responses to oligotrophication are comparatively underrepresented in the literature and the few observed responses have been inconsistent (Jeppesen *et al*., 2005a). Major reductions in external P loading have resulted in reductions in biomass of total zooplankton and *Daphnia* (Moss *et al*., 2005; Phillips *et al*., 2005), slight increase in biomass of total zooplankton and *Daphnia* (Jeppesen *et al*., 2005a), or minor to no effects (Jeppesen *et al*., 2002; Straile & Geller 2007). Jeppesen *et al*.'s (2005b) synthesis indicates that over the long term, oligotrophication of shallow lakes typically produces reductions in total zooplankton biomass, increased zooplankton to phytoplankton biomass ratio, and an increase in the mean body size of cladocerans. These changes are strongly affected by reductions in planktivorous fish as a result of increased piscivorous fish abundance (Jeppesen *et al*., 2005b). Therefore, the direct responses of zooplankton to nutrient reductions may be easier to determine in a more controlled oligotrophication study where fish are excluded.

The effectiveness of iron salts at controlling internal P loading is being examined as a potential remediation tool for lakes in the Canadian prairies (Orihel

et al., in prep.). The evaluation of potential negative effects of iron treatment on zooplankton is a critical part of determining optimal remediation strategies, so that remediation attempts do not inadvertently cause negative impacts on aquatic biodiversity and ecosystem function. Thus, the objectives of the present study are to determine the effects of different iron application concentrations on zooplankton assemblages by examining changes in abundance, biomass, and taxonomic composition of crustacean and rotifer zooplankton both directly after treatment and throughout the subsequent open-water season. By altering water column nutrient concentrations, this study will also contribute to the growing body of literature investigating how zooplankton assemblages respond to oligotrophication.

Materials and methods

Study site

My experiment was conducted in Nakamun Lake (latitude 53.886, longitude -114.206), a shallow, polymictic water body located in the boreal transition zone in central Alberta, Canada (Fig. 2.1). The surrounding watershed of Nakamun Lake is 299 km^2; the lake has a surface area of 3.5 km^2, maximum depth of 8 m, average depth of 4.5 m, and a water residence time of 21 years (Mitchell & Prepas, 1990). The high total phosphorus (TP) and chlorophyll-*a* (chl-*a*) concentrations combined with low Secchi depth (Table A.1; Appendix A) are indicative of hypereutrophication. The lake often becomes hypoxic in winter months and fish kills are frequent (Mitchell & Prepas, 1990). Nakamun Lake has hard water with high alkalinity, due to high concentrations of sodium, calcium, and bicarbonate ions (Table A.1). Further details describing Nakamun Lake water-quality characteristics are reported in Orihel *et al*. (in prep) and in Appendix A.

Experimental set-up

Fifteen large experimental units (mesocosms) were constructed in May 2009 and installed in a small sheltered bay on the northeast shore of the lake. The

mesocosms consisted of a floating collar, 2 m in diameter, and a 6 m cylindrical polyethylene curtain that extended from the floating collar to the lakebed. The bottom of each curtain was buried ~ 1.5 m within the sediments and weighted in place with sandbags (Fig. 2.2). The mesocosms were installed in the limnetic zone and were located at the depth that represented the average depth (4.5 m) of Nakamun Lake. Natural zooplankton assemblages from the lake were retained within the mesocosms when the curtain walls were dropped vertically from the surface to the sediments in May 2009. Fish were removed by vertically hauling up a circular seine net (1.75 m in diameter with a 4 mm mesh size); because *Gammarus lacustris* can be an active predator on zooplankton (Anderson & Raasveldt, 1974; Wilhelm & Schindler, 1999) the quantity of *G. lacustris* brought up with the net was estimated and unusually high numbers were also removed.

Iron application

Ferric chloride ($FeCl_3$) was applied over three days from June 9-11, 2009. Appropriate iron doses were determined by literature review and on the basis of substantial reductions in pore water P concentrations in previous sediment-core incubation experiments (Orihel *et al*., in prep.). Single-dose treatments increased on a logarithmic scale, ranging from 2.25 g m^{-2} to 225 g m^{-2}. Ferric chloride was diluted with lake water, applied as a liquid just below the water surface, and then stirred with a long oar for two minutes to promote mixing. Twelve of the mesocosms received iron treatment, while three mesocosms did not and were left as controls. Lake reference samples were collected in close proximity to the mesocosms to compare control mescosms to natural lake assemblages. Further details describing the experimental set up and a pictorial representation of the mesocosm set-up are shown in Figs. 2.1 and 2.2.

Sample collection and laboratory analyses

Water chemistry analyses and light profiles were performed bi-weekly from June 9 until October 16. Vertical profiles measuring pH, temperature, specific conductivity, and dissolved oxygen (DO) were performed using a

Hydrolab Series 5 Datasonde Multiprobe. Total phosphorus (TP), total nitrogen (TN), dissolved organic carbon (DOC) and chlorophyll *a* (chl-*a*) samples were collected and analyzed at the University of Alberta Biogeochemical Analytical Services Laboratory. Secchi depth and light profiles were taken on alternate weeks as the water chemistry samples, the latter with a LI-COR LI-250A light meter.

Zooplankton samples were collected on four dates: before iron application (June 3), and 4 days (June 15), 34 days (July 15), and 76 days (Aug 26) after iron application. Samples were collected during the daylight from all mesocosms, as well as from one lake reference site (Fig. 2.1). Nocturnal and daytime zooplankton samples were also collected within 24 hours in a subset of the mesocosms (treatment levels of 0, 2.25, 12.0, 42.2, and 225 g m^{-2} ferric chloride) on day 20 (July 1) and day 69 (August 19). Integrated zooplankton samples were collected from the surface to 4 m deep using a 7.6 cm diameter flexible plastic tube (Pennak, 1962) for a total volume sampled of 13.9 L. The samples were immediately sieved with a 63 μm mesh-sized plankton net and narcotized with methanol, and then preserved in 4% sugared and buffered formalin within ~2 hours.

Zooplankton were enumerated and identified to the lowest possible taxonomic level, species when possible, using a dissecting stereo-microscope (Leica MZ9.5) and compound microscope (Nikon). For easier identification, zooplankton were sieved through a 180 μm mesh in the laboratory to divide the samples into two size classes: 63-180 μm (containing rotifers and some juvenile crustacean taxa) and >180 μm (containing crustacean taxa only). After enumeration and identification of these two size classes, the data from each site were combined again for statistical analyses. One sample from Day 76 was mishandled during laboratory processing which caused substantial loss of the sample; therefore, it was not included in any quantitative analyses (i.e., abundance and biomass estimates) but was still used in qualitative assessments (i.e., species composition and diversity). Taxonomic keys used for zooplankton identification were Ward & Whipple (1959), supplemented by the keys of Brooks (1957),

Smirnov (1971), Brandlova *et al*., (1972), Ruttner-Kolisko (1974), Kiefer (1978), and Thorp & Covich (2001).

When possible, crustaceans >180 μm were completely enumerated, but sub-sampling was performed on samples that contained large numbers of specimens. The rotifers and juvenile crustaceans (63-180 μm) were sub-sampled until 500 organisms were counted. Sub-sampling was performed by bringing the sample to a known volume, then sub-sampling with an automatic pipette while the sample was being mixed. Densities were then back calculated to the total volume of water filtered.

To calculate biomass, lengths were measured from the first 30 individuals of each species observed in a sample. Lengths of zooplankton were measured directly using Openlab™ 4.0.1 (Improvision®) for crustacean taxa and an ocular micrometer on the compound Nikon microscope (100X magnification) for rotifers. Using length measurements from individual organisms, weights were calculated from published length-weight regression equations for both crustaceans (Bottrell *et al*., 1976; McCauley, 1984) and rotifers (Ruttner-Kolisko, 1974; Stemberger & Gilbert, 1987). For each sample, a mean individual weight was calculated by averaging the estimated weights generated from the length-weight regression equation, because it is important to average weights and not lengths (Bird & Prairie, 1985). Biomass for each taxonomic group was calculated by multiplying the counts for that sample by the mean individual weight.

Statistical analyses

Data were tested for normality and transformed when necessary to meet normality assumptions prior to further parametric statistical tests. Skewness and kurtosis were considered acceptable after transformations (Zar, 1999). Differences in community composition were analyzed using two approaches: community composition among treatment groups and sample dates was compared with multivariate ordination analyses and analysis of diversity indices (see below). Changes in abundance, biomass and diversity values of zooplankton taxa across iron treatment levels were assessed using linear regression. An average and

standard error of the three controls was calculated and was used as a single datum in regression analyses. Because the primary objective was to assess the effect of iron treatment on zooplankton assemblages, the lake reference site was not included in the regressions. Analysis of covariance (ANCOVA) was used to determine temporal changes in diversity values in relation to iron treatment level.

Non-parametric ecological indices used to assess alpha diversity of the macroinvertebrate assemblages were richness, Shannon-Weaver diversity (H'), and Pielous's evenness (J) because they are some of the most commonly used indices in community ecology and aquatic studies (Washington, 1984; Karydis & Tsitsis, 1996). Although the utility of diversity indices, in particular H', has been extensively debated in the ecological literature (Barrantes & Sandoval, 2009; Spatharis *et al*., 2011), we considered them useful for this study as all samples were taken from the same bay in close proximity to one another, and the primary goal was not to assess absolute diversity, but to assess relative changes in community composition over a gradient of iron treatment levels.

Zooplankton assemblage structure was examined using multivariate ordination analyses (PC- ORD, version 5.10; MjM Software Design, Gleneden Beach, Oregon) to determine how iron treatment (Day 4 only) and seasonality (all sample dates) influenced zooplankton species composition. A linear ordination technique, principal component analysis (PCA) was selected to describe the treatment effect of iron on zooplankton (Day 4). PCA was determined to be appropriate after preliminary direct gradient analysis (DCA) indicated that the response gradient lengths were small. Gradient lengths <4 indicate that taxa display a linear response, while gradient lengths >4 indicate that some taxa may display a unimodal response (Borcard *et al*., 2011). The DCA results indicated that gradient lengths of the first two axes were 1.54 and 1.05; therefore, the linear PCA technique was deemed suitable for this data. For the seasonal ordination (all sample dates), DCA results indicated that the gradient lengths were greater; gradient lengths of the first two axes were 3.26 and 2.91. Although these lengths were <4 and could still be appropriate for linear ordination techniques, visual inspection indicated that some taxa displayed a unimodal response. Therefore,

nonmetric multidimensional scaling (NMS) was determined to be more appropriate for these data because this unconstrained ordination technique does not require that any assumptions of response be met, meaning taxa may display both linear and unimodal responses. Prior to ordination analyses, rare species (comprising less than 5% of the total) were removed, and raw abundances were log (x+1) transformed (McCune & Grace, 2002). The environmental matrix used in the ordinations included integrated concentrations of TP, TN, chl-*a*, DO, iron, and specific conductivity taken within one week of zooplankton sampling. Iron treatment level (g m^{-2}) was also included in the environmental variable matrix. A multiple response permutation procedure (MRPP) was used to test for differences in assemblages between *a priori* groups directly after treatment (Day 4), defined as control mesocosms (C01, C02, and C03), low (2.3, 3.4, 5.2, and 7.9 g m^{-2}), medium (12, 18, 28, and 42 g m^{-2}), and high (64, 97, 148, and 225 g m^{-2}) iron treatments. MRPP was also used to determine the significance of seasonality (i.e., sample date) on structuring zooplankton assemblages.

With the exception of ordination analyses, all other statistical analyses were performed in the R statistical program (version 2.12.2; R Development Core Team, Vienna, Austria). Regression analyses and ANCOVA analyses were performed within the MASS library and the vegan library was used to calculate diversity indices. Sigmaplot version 12.0 was used for graphical representations of the data.

Results

Water quality parameters

Despite the high alkalinity of Nakamun Lake, iron application still caused the pH to decrease 3 units, from 8.5 to 5.5, in the highest-dosed mesocosm during initial iron application (Fig. 2.3). Although the high alkalinity in the lake was able to buffer this drop in pH for most treatments (Table A.2; Appendix A), the highest iron treatment was slower to recover and took 36 days to return to a neutral pH of 7 (Fig. 2.3). The application of ferric chloride was successful as a short-term oligotrophication treatment within the mesocosms. Average TP, TN, and chl-*a*

significantly decreased as a function of iron treatment level during the open water season. A detailed analysis of changes in water chemistry parameters and phytoplankton assemblages is described in Orihel *et al*., (in prep).

Zooplankton assemblage structure and comparison of controls to lake reference

There were no significant differences in zooplankton assemblage composition between the zooplankton assemblages sampled during the daylight compared to those sampled at night (Artym, unpublished data), indicating that daytime sampling of zooplankton is a sufficient sampling method in this shallow, hypereutrophic lake. Because zooplankton sampling during daylight was conducted at a higher frequency than nocturnal sampling, this thesis examines zooplankton assemblages that were sampled in the daytime.

A total of 64 zooplankton taxa were collected, consisting of one calanoid, 3 cyclopoid, 6 cladoceran, and 54 rotifer taxa. Dominant crustacean zooplankton (making up greater than 5% of the overall abundance) were *Skistodiaptomus oregonensis* (Lilljeborg), *Cyclops bicuspidatus thomasi* (Forbes), *Cyclops vernalis (*Fischer), *Ceriodaphnia* spp., *Chydorus sphaericus* (Müller), *Bosmina longirostris* (Müller), *Diaphanosoma birgei* (Korinek), and *Daphnia pulex* (Linnaeus). Dominant rotifers that made up greater than 5% of the overall rotifer abundance were *Conochilus unicornis* (Rousselet), *Polyarthra* spp., *Keratella cochlearis* (Gosse), *Keratella quadrata* (Müller), and *Keratella testudo* (Ehrenberg)/ *hiemalis* (Carlin). Species present in the mesocosms were very similar to those in the lake; all crustacean taxa that were captured were present in both the mesocosms and the lake reference, but the calanoid *S. oregonensis* was more abundant in the lake than in the mesocosms. Several rare rotifer taxa captured in a small proportion of the mesocosms were not found in the lake reference samples. Taxon richness, diversity, and evenness from the lake samples were comparable to those of the control mesocosms throughout the open water season, although these indices became much more variable among the three mesocom controls as the experiment progressed (Table 2.1). Total zooplankton abundance and estimated biomass were also comparable between the control

mesocosms and the lake reference, with the exception of total biomass being considerably higher on the June sampling dates in the lake than the controls (Table 2.1).

Zooplankton abundance and biomass

Although there was some variability in total zooplankton abundance and biomass among the mesocosms prior to iron application, this variability was not significant (June 3; Table 2.2). However, zooplankton abundance and biomass were negatively affected by iron treatment; both the total abundance and total biomass significantly decreased immediately following ferric chloride application (linear regression; $r^2 = 0.505$, df = 11, p = 0.006 and $r^2 = 0.559$, df = 11, p = 0.003, respectively; Fig. 2.4a,b; Table 2.2). The most significant declines in abundance and biomass were apparent in the rotifer and cyclopoid assemblages (Fig. 2.5; Table 2.3), which were the predominant taxa in the zooplankton community at the time of iron application. Although these declines were significant when analyzed as linear responses, the most substantial declines were observed in the highest-dosed mesocosm (Fig. 2.4a,b), indicating that it is possible that the zooplankton assemblages were responding in a threshold manner, as opposed to a linear response. However, replication and a wider range of high iron doses would be required to test this observation. Biomass and abundance increased one month following iron application (Fig. 2.4c) and there was no significant relationship between iron treatment level and total biomass or abundance 34 or 76 days following ferric chloride application (Fig. 2.4c,d; Table 2.2). Similar to the lack of response of total zooplankton abundance to iron application one month after iron application, there was also no relationship between iron treatment level and abundance of any particular taxonomic group (calanoid, cyclopoid, cladoceran, or rotifer) 34 or 76 days after iron application (Table 2.3).

Zooplankton diversity and assemblage structure

Diversity indices (richness, diversity, and evenness) were similar among mesocosms prior to ferric chloride application (all p-values >0.68; Table 2.2).

Unlike the significant declines in abundance and biomass directly after iron application, there was no significant change in zooplankton richness ($r^2 = 0.061$, df = 11, p = 0.414), diversity ($r^2 = 0.042$, df = 11, p = 0.504), or evenness ($r^2 = 0.070$, df = 11, p = 0.384) 4 days after iron application (Fig. 2.6a,b,c; Table 2.2). There was no relationship between richness, diversity, or evenness and level of iron treatment 34 or 76 days after iron application either (all p-values >0.125; Fig. 2.6a,b,c; Table 2.2). However, there was a significant seasonal increase in diversity, richness, and evenness in the zooplankton assemblages (ANCOVA; all r^2 values >0.33, df=48, all p-values <0.001). The proportion of crustacean taxa, particularly cladocerans, became greater as the experiment progressed into late summer (Fig. 2.5c,d).

The PCA performed on the zooplankton assemblages 4 days following iron application suggests that the zooplankton assemblage composition was initially affected by iron treatment, as indicated by the target treatment level (abbreviated "Treat") and the treatment tracer, chloride ("Cl") and directly opposing vectors associated with the first axis (Fig. 2.7a). Although the first two axes were determined to be significant and accounted for 71% of the total variance, strongly correlated environmental parameters ($r^2 > 0.5$) were associated with the first axis only. Samples treated with the highest rates of iron application are positively associated with the treatment vector, and negatively correlated with pH (Fig. 2.7a). Almost all species scores were related to lower treatment levels and higher pH and there were no taxa associated with the higher-dosed mesocosms with low pH (Fig. 2.7b). There was lack of distinction between acid-tolerant and acid-sensitive taxa within the ordination (i.e., no species were positively related to pH). The MRPP cluster analysis comparing iron treatment groups indicated that there was notable clustering among treatment level groups determined *a priori* (p = 0.01), and there was no overlap between the highest treatment and the low treatment or control groups (Fig. 2.7a; Table 2.4).

When zooplankton community composition is analyzed temporally, however, the variation among mesocosms is much greater compared to directly after treatment. NMS ordination analysis shows that the variation in assemblage

structure increases with seasonality, because the clustering among mesocosm scores becomes broader with each progressive sample date (Fig. 2.8a). Phosphorus ("TP") and phytoplankton biomass ("Chl *a*") are positively associated with zooplankton assemblages from later sample dates, as are crustacean taxa (Fig. 2.8b). MRPP cluster analysis comparing sample dates was highly significant; overall and all pairwise comparisons were significant (all p-values <0.001), indicating that assemblage structure within each sample date was more similar than between dates. This suggests that seasonality was a stronger influence on zooplankton assemblage structure than were the initial effects of iron application.

Zooplankton responses to oligotrophication

Although changes in water quality parameters and phytoplankton assemblages indicate that mesocosms that received high levels of iron treatment shifted towards a less nutrient-rich state (Orihel *et al*., in prep), the zooplankton did not show signs of response to oligotrophication. The initial decrease in biomass and abundance is considered a direct treatment effect and not an indirect response to decreased P loading. There was no significant change in the ratio of zooplankton to phytoplankton biomass in relation to iron application 34 or 76 days after application (linear regression; all p-values > 0.05; Table 2.2). Furthermore, there were no significant seasonal shifts in cladoceran taxa in relation to iron treatment level (all p-values > 0.05; Table 2.6).

Discussion

Zooplankton responses to iron treatment

The results of my mesocosm experiment indicate that this type of iron application minimally impacted zooplankton assemblages in the limnetic zone of Nakamun Lake. The most substantial effect of iron application on the zooplankton assemblages was the significant decline in both total abundance and biomass directly following high rates of iron application. The lack of significance in relation to iron treatment level one month following application indicates that the

zooplankton assemblages were able to recover from the effects of the treatment and the response was short-lived. Several potential mechanisms may have caused the temporary declines in zooplankton abundance and biomass.

Acidification is the most negative side effect of iron dosing (Cooke *et al*., 2005) and is likely the most threatening effect of iron application on zooplankton. Both rotifer (e.g., Bērziņš & Pejler, 1987) and crustacean (e.g., Price & Swift, 1985; Havens *et al*., 1993; Doka *et al*., 1997) assemblages display species-specific pH tolerances and are therefore useful indicators of acidification. pH between 5.5 and 6.0 is often recognized as an important threshold in which shifts between acid-sensitive and acid tolerant species occur (Schindler *et al*., 1991; Havens *et al*., 1993; Doka *et al*., 2003). Because the pH dropped below 6.0 (to pH 5.5 from 8.5) only in the high-dose treated mesocosm (225 g m^{-2}) responses to acidification would be expected to occur in this enclosure only. However, the zooplankton within my study lake may be more sensitive when the pH is substantially lowered. Some zooplankton taxa can develop acid-adaptive tolerances (Fischer *et al*., 2001) and zooplankton that have previously experienced acidification can tolerate greater drops in pH (Derry & Arnott 2007). The high alkalinity in Nakamun lake buffers natural acid inputs, so the zooplankton would not have been previously exposed to acidic water and would not have developed acid-tolerances. The rapid acidification pulse may have also increased the severity of zooplankton responses to acidification because they may not have had a sufficient acclimation period.

Acidification typically alters zooplankton assemblage composition; the number of zooplankton species (i.e., richness) and biodiversity both decline with acidification (reviewed in Brett, 1989), but in this study there were no significant changes in richness, diversity, or evenness of zooplankton assemblages following iron application. In addition, the ordination analysis directly after treatment did not show strong differentiation between acid-sensitive and acid-tolerant taxa in relation to iron treatment level. Zooplankton species tolerant to low pH (e.g., *B. longirostris* (Havens *et al*., 1993)) were present in considerably lower numbers in the highest-dose treatment compared to the other treatments. The lack of

association between acid-sensitive and acid-tolerant taxa with level of iron-dosing, and the absence of change in zooplankton assemblage composition indicate that zooplankton assemblage composition was not substantially altered by the acidification induced by the iron treatment.

Because zooplankton are closely coupled to phytoplankton, their main food source, zooplankton assemblages respond to phytoplankton alterations in a cascading manner (Carpenter *et al*., 1985); therefore, zooplankton can also indirectly respond to iron application that significantly alter nutrients available to phytoplankton. However, the immediate and substantial declines in zooplankton from the mesocosms receiving the high doses of iron application, particularly the highest treated mesocosm (225 g m^{-2}), suggests that the zooplankton were responding to factors other than changes in phytoplankton. Directly following iron application, Secchi depth increased with higher levels of iron treatment and up to 4.5 m in the highest iron dose (Orihel *et al*., in prep), where the sediment bed could be observed from water surface (pers. obs.). This immediate increase in water transparency, along with the significant declines in chl *a* and zooplankton, indicate that pelagic plankton was co-precipitated with the iron floc and settled to the sediments. Although co-precipitation of zooplankton with iron hydroxide has not been confirmed from previous studies, zooplankton have been found to precipitate out with aluminum flocculants (Wold *et al*., 2005). Alternatively, if the zooplankton were able to resist co-precipitating with the iron flocculant, there is potential that they died from starvation if all of the phytoplankton precipitated out. Zooplankton species have variable starvation times ranging from 0.4 - 5 days for rotifers (Kirk, 1997) and 4 - 11 days for cladocerans (Threlkeld, 1976).

My results indicate that the zooplankton were able to recover relatively quickly, within one month after treatment. It is possible that the assemblages recovered even faster, but my sampling frequency could not capture the exact recovery time. Potential mechanisms of recovery could be from rescue from egg banks or dormant stages within the sediments or due to recovery of phytoplankton following iron application. pH also rebounded quickly (> 7.0 in all mesocosms

within one month), so if sensitive zooplankton species were indeed affected by the drop in pH, they would recover shortly after application.

Despite these significant immediate effects, the variation among treatments observed directly after iron application was much smaller than the seasonal variation in zooplankton assemblages, particularly in late summer, two months after iron application. Seasonal variation in zooplankton is expected as the species complete their life cycles. Decreases in juvenile crustacean taxa (e.g., nauplii and copepodites) over the course of the summer are expected as they are recruited to adult cohorts. These larger crustaceans also increase predation pressure on the rotifer assemblages; therefore, seasonal decreases in rotifer abundance are also expected. Seasonal variation in zooplankton species composition was also driven by seasonal changes in nutrients and phytoplankton, as phytoplankton biomass naturally peaks in late summer. The substantial seasonal variability can also be attributed to the prolonged duration that the assemblages had been segregated from the lake and each other, creating unique communities dependant on the assemblages captured at the time of the experimental set up.

Oligotrophication

Zooplankton assemblages did not clearly respond to reduced nutrient loading in this experiment. Other treatments to control internal P loading (e.g., alum or calcium addition) have been shown to have a variety of effects on zooplankton such as reduced growth and reproduction rates of *Daphnia* (Lürling & Tolman, 2010), lower total zooplankton abundance and biomass (Schumaker *et al*., 1993), changes in zooplankton assemblage composition (Schumaker *et al*., 1993; Van Oosterhout & Lürling, 2011), or no detectable changes at all (Özkundakci *et al*., 2011; Moore & Christensen, 2009). Zooplankton assemblages in my study did not respond in any ways that are clearly comparable to those in other published studies investigating reductions in external nutrient loading either. There was no significant change to large grazers such as *Daphnia* and the zooplankton:phytoplankton biomass did not increase (reviewed in Jeppesen *et al*.,

2005b). However, responses in zooplankton to reduced nutrient loading are not as clear as for other taxa such as phytoplankton or fish (Jeppesen *et al.,* 2005b), likely because zooplankton abundances and composition are so intimately related to food availability and predation pressure and are therefore only indirectly influenced by nutrient reductions. In addition, zooplankton responses to reduced nutrient loading may not be apparent for several years after initial ferric treatment (Daldorph, 1999). Therefore, the short temporal scale of my study (a single open-water season) may not have been long enough to capture zooplankton responses related to oligotrophication.

Predation

I attempted to entirely remove fish from the mesocosms prior to the start of my experiment, but small Brook Stickleback (*Culaea inconstans* Kirtland) were observed in some mesocosms throughout the duration of the experiment. Although fish were not at all observed throughout the duration of the experiment in several mesocosms, up to three sticklebacks were observed within a single mesocosm (pers. obs.). When detected, attempts were made to catch and remove fish from the mesocosms, but it is possible that not all fish were removed. Fish predation most likely varied among treatments and contributed to the observed variability in zooplankton assemblages among mesocosms as the experiment progressed.

The pelagic invertebrate predator, *Chaoborus*, was also observed in the mesocosms in low densities. *Chaoborus* is an important predator of several small- and medium-bodied crustacean zooplankton taxa (Peckarsky, 1984). With the exception of a single specimen, adult *Chaoborus* were completely absent from the catch from the submerged emergence traps installed within each mesososm, and at 3 lake reference sites (refer to Chapter 3 of this thesis for emergence trap details). Although *Chaoborus* spp. in Alberta have one or 2 generations a year (Clifford, 1991), the lack of captured emergent insects may indicate that they only emerged in the spring, before the traps were installed. Alternatively, *Chaoborus* abundance could have been extremely low and therefore a low capture rate would

be expected. *Chaoborus* spp. present throughout the duration of my experiment within the mesocosms likely remained as larvae. Nocturnal zooplankton sampling with a Wisconsin-style plankton net (30 cm diameter, sampled from surface to 3.5 m deep) in September 2009 estimated *Chaoborus* spp. densities to range from 0 to 12 per m^3.

The generalist feeder, *G. lacustris,* has also been shown to be an active predator on zooplankton (Anderson & Raasveldt, 1974; Wilhelm & Schindler, 1999) and can alter zooplankton community structure in mesocosms of similar size and volume as my experiment (Wilhelm *et al*., 2000). However, most studies investigating *G. lacustris* predation on zooplankton are conducted in alpine systems (e.g., Wilhelm & Schindler 1999; Wilhelm *et al*., 2000; Weidman *et al*., 2011) with much simpler food webs and at much higher abundances (e.g., 200 – 400 m^{-2}; Wilhelm *et al*., 2000) than in my hypereutrophic study lake. In my study, *G. lacustris* were often observed along the mesocosm walls (pers. obs.), likely feeding on the periphyton rather than on zooplankton. Nocturnal sampling in September (sampling same as *Chaoborus* discussed above) estimated that *G. lacustris* ranged from 0 to 152 individuals per m^3 with an average of 32 per m^3. Water mites (particularly *Piona spp*.) are also predators of cladocerans (Clifford, 1991) and were present within the enclosures.

Although efforts were made to control predation on zooplankton, there was likely considerable variability in predation pressure among mesocosms from both fish and predacious invertebrates. Iron application could potentially affect zooplankton composition by indirectly altering predation pressure (e.g., increased predation from visual predators due to higher water clarity). However, it is unlikely that these changes in predation pressure would be the sole cause for the significant declines in zooplankton abundance and biomass directly after treatment. *C. inconstans*, *Piona* spp., *Chaoborus* spp., and *G. laustris* generally select for various crustacean zooplankton taxa; therefore, if predation were of principal importance crustacean abundance would decline with iron treatment level, but rotifer abundance would be unchanged, or even increase due to predation release from predatory crustacean taxa. Therefore, the significant

decrease in rotifer abundance indicates that predation is not the main driver behind initial reductions in zooplankton abundance. However, the uncontrolled variability of predators likely contributed to the variability in zooplankton abundance, biomass, and composition as the experiment progressed.

Application of mesocosm results to natural lakes

Comparing the zooplankton assemblages from the control mesocosms to the lake reference assemblages allows us determine whether the mesocosm enclosures themselves affected zooplankton, and hence the degree to which we can safely extrapolate from mesocosms to the lake environment. The lack of significant relationship between zooplankton abundance, biomass, or diversity values and designated treatment level prior to iron application indicates that the zooplankton assemblages were similar among mesocosms before iron was applied and there were no confounding effects prior to treatment. The abundance, biomass, and diversity values of the zooplankton assemblages were also similar between the lake reference site and the control mesocosms throughout the experiment, indicating that there was a minimal mesocosm effect influencing the zooplankton assemblages exposed to my treatment. However, the variability in these endpoints increased as the experiment progressed in all mesocosms (including the controls), indicating that the effects of the mesocosms became greater the longer that the assemblages within the mesocosms were segregated from the natural lake communities. Given that there were minimal differences among all mesocosms prior to iron application and between the lake and the control mesocosms, my conclusions on the safety of iron as a remediation technique are interpretable and reliable. However, the substantial variability among mesocoms as the experiment progressed indicate that further research is needed to determine long-term effects of ferric chloride on the zooplankton community, and at a larger scale.

Although this study provides a broader and more comprehensive analysis of the effect of iron application on zooplankton assemblages than previous microcosm-scaled studies, discrepancies between findings from mesocosms and

whole-lake manipulations have often been noted (e.g., Carpenter, 1996; Schindler, 1998). These discrepancies are usually due to the spatial and temporal scale limitations imposed by mesocosms. Given that the mesocosms were located in the limnetic zone of the lake, the iron treatment was only applied on the zooplankton assemblages associated with this habitat. Some crustacean zooplankton species utilize littoral macrophyte beds as refuge from predation and exhibit horizontal migration when predation is not as threatening (Laurisden *et al*., 1999). Because the mesocosms were installed during the day, when predation risk from visual predators is highest, it may be that these species were not captured within the mesocosms. Furthermore, littoral zooplankton could be much more sensitive to iron treatment than the assemblages we assessed. The small spatial scale of the mesocosms could affect food webs (and therefore species composition) by altering predatory behavior (e.g., Wilhelm *et al*., 2000) and physical refugium availability. Furthermore, the responses of other assemblages (e.g., fish, macrophytes, etc.) to iron application were not taken into consideration in this study. Therefore future research should investigate the effects of iron application on all biotic assemblages before extrapolating to a whole-lake manipulation.

General conclusions and recommendations

I observed minimal changes in the abundance, biomass, and taxonomic composition of zooplankton assemblages in relation to iron application. Although zooplankton biomass and abundance significantly decreased directly after high rates of iron application, the response was short-lived and zooplankton assemblages had recovered within one month of iron application. The initial treatment effect was likely caused by a combination of stressors, including acidification, reductions in phytoplankton, and co-precipitation of plankton with the iron floc as it settled. The minimal immediate changes in zooplankton assemblages receiving low and medium rates of iron application indicate that these doses do not adversely affect pelagic zooplankton. There was substantial seasonal variation in zooplankton assemblages, but these differences were likely caused by variations in predation pressure, changes in phytoplankton

composition, and the differences among initial biotic communities, rather than directly by the iron application. Although my results did not indicate a clear response of zooplankton to reduced internal P loading (oligotrophication), my temporal scale may not have been adequate to capture oligotrophication effects, or the influence of the mesocosms themselves may have concealed these effects. Overall, the results of my mesocosm experiment indicate that this type of iron application minimally impacts zooplankton assemblages in the limnetic zone. Further research is recommended to determine the full effects of iron application on other biota such as fish before a whole-lake application is performed.

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Table 2.1. Minimum, maximum, and average abundance (no. m^{-2}), biomass (mg dw m^{-2}), richness (no. taxa), diversity (Shannon H'), and evenness (Pielou's J) of zooplankton from the three control mesocosms and the lake reference site before iron application and 4, 34, and 76 days after iron application.

		Controls			Lake
		Min	Max	Ave	
Before (June 3)					
	Abundance	140	184	169	145
	Biomass	77	106	95	176
	Richness	12	14	13	11
	Diversity (H')	0.85	0.94	0.90	1.01
	Evenness (J)	0.33	0.38	0.34	0.42
Day 4 (June 15)					
	Abundance	147	198	175	111
	Biomass	26	65	51	145
	Richness	12	14	13	16
	Diversity (H')	1.05	1.33	1.23	1.51
	Evenness (J)	0.42	0.52	0.48	0.54
Day 34 (July 15)					
	Abundance	122	436	240	168
	Biomass	103	223	161	109
	Richness	16	17	16	15
	Diversity (H')	1.38	2.00	1.69	1.75
	Evenness (J)	0.49	0.72	0.61	0.65
Day 76 (August 26)					
	Abundance	140	336	225	386
	Biomass	180	1070	485	397
	Richness	14	20	16	17
	Diversity (H')	1.39	1.78	1.58	1.33
	Evenness (J)	0.46	0.66	0.57	0.47

Table 2.2. Regression summary statistics for the abundance (no. m^{-2}), biomass (mg dw m^{-2}), richness (no. taxa), diversity (Shannon H'), evenness (Pielou's J), and zooplankton to phytoplankton biomass ratio (Zoop:Chl *a*) of total zooplankton in relation to iron treatment before iron application and 4, 34, and 76 days after iron application.

	Analysis	p-value*	r^2	F-stat	Slope	Intercept	df
Before (June 3)							
	Abundance (log x+1)	0.733	0.011	0.123	0.121	0.689	1,11
	Biomass (log x+1)	0.319	0.090	1.090	0.249	0.203	1,11
	Richness	0.783	0.007	0.079	-0.205	11.957	1,11
	Diversity (H')	0.688	0.015	0.170	-0.022	0.909	1,11
	Evenness (J)	0.789	0.007	0.075	-0.005	0.366	1,11
	Zoop:Chl *a*	0.862	0.005	0.016	0.003	1.520	1,11
Day 4 (June 15)							
	Abundance (log x+1)	**0.006**	**0.505**	**11.240**	**-0.623**	**3.988**	**1,11**
	Biomass (log x+1)	**0.003**	**0.559**	**13.940**	**-0.567**	**3.039**	**1,11**
	Richness	0.414	0.061	0.721	-0.761	12.723	1,11
	Diversity (H')	0.504	0.042	0.478	0.077	1.102	1,11
	Evenness (J)	0.384	0.070	0.824	0.044	0.437	1,11
	Zoop:Chl *a*	0.846	-0.096	0.040	-0.002	1.392	1,11
Day 34 (July 15)							
	Abundance (log x+1)	0.302	0.096	1.171	-0.598	4.329	1,11
	Biomass (log x+1)	0.336	0.084	1.015	0.265	0.064	1,11
	Richness	0.126	0.200	2.749	-1.819	17.905	1,11
	Diversity (H')	0.381	0.070	0.832	-0.139	1.715	1,11
	Evenness (J)	0.701	0.014	0.156	-0.022	0.593	1,11
	Zoop:Chl *a*	0.814	0.006	0.059	<0.001	1.490	1,11
Day 76 (August 26)							
	Abundance (log x+1)	0.991	0.000	0.000	0.003	1.272	1,10
	Biomass (log x+1)	0.888	0.002	0.021	-0.020	1.392	1,10
	Richness	0.489	0.045	0.514	-1.046	19.372	1,11
	Diversity (H')	0.381	0.070	0.832	-0.139	1.715	1,11
	Evenness (J)	0.237	0.125	1.564	0.056	0.485	1,11
	Zoop:Chl *a*	0.987	<0.001	<0.001	<0.001	1.286	1,10

*Bold values indicate significant results at $\alpha = 0.05$.

Table 2.3. Regression summary statistics for the abundance (no. m^{-2}) and biomass (mg dw m^{-2}) of zooplankton taxa (calanoids, cyclopoids, cladocerans, and rotifers) and total zooplankton in relation to iron treatment before iron application and 4, 34, and 76 days after iron application.

		Abundance						Biomass					
Date	**Assemblage**	**p-value***	**r^2**	**F-stat**	**Slope**	**Int.**	**df****	**p-value***	**r^2**	**F-stat**	**Slope**	**Int.**	**df****
Before (June 3)	Calanoida (log x+1)	0.954	<0.001	0.004	0.025	1.246	1,11	0.855	0.003	0.035	0.055	1.783	1,11
Before (June 3)	Cyclopoida (sqrt)	0.388	0.068	0.808	0.081	0.716	1,11	0.286	0.103	1.258	1.388	3.825	1,11
Before (June 3)	Cladocera (log x+1)	0.158	0.172	2.292	-0.484	1.494	1,11	0.286	0.103	1.259	-0.336	0.916	1,11
Before (June 3)	Rotifera (log x+1)	0.964	0.000	0.002	0.014	1.223	1,11	0.835	0.004	0.046	0.052	2.117	1,11
Before (June 3)	ALL ZOOPLANKTON	0.733	0.011	0.123	0.121	0.689	1,11	0.319	0.090	1.090	0.363	3.896	1,11
Day 4 (June 15)	Calanoida (log x+1)	0.779	0.008	0.083	-0.163	1.400	1,11	0.385	0.069	0.817	-0.253	1.221	1,11
Day 4 (June 15)	Cyclopoida (sqrt)	**0.020**	**0.401**	**7.366**	**-0.196**	**2.490**	**1,11**	**0.004**	**0.547**	**13.280**	**-1.080**	**4.180**	**1,11**
Day 4 (June 15)	Cladocerana (log x+1)	0.119	0.206	2.861	-0.603	1.610	1,11	0.171	0.163	2.145	-0.404	1.342	1,11
Day 4 (June 15)	Rotifera (log x+1)	**0.014**	**0.439**	**8.592**	**-0.532**	**3.221**	**1,11**	**0.046**	**0.315**	**5.506**	**-0.439**	**1.555**	**1,11**
Day 4 (June 15)	ALL ZOOPLANKTON	**0.006**	**0.505**	**11.240**	**-0.623**	**3.988**	**1,11**	**0.005**	**0.527**	**12.270**	**-0.932**	**4.320**	**1,11**
Day 34 (July 15)	Calanoida (log x+1)	0.709	0.013	0.147	0.107	1.194	1,11	0.245	0.120	1.506	0.534	0.829	1,11
Day 34 (July 15)	Cyclopoida (sqrt)	0.962	0.000	0.002	-0.004	1.327	1,11	0.113	0.213	2.968	1.149	3.395	1,11
Day 34 (July 15)	Cladocera (log x+1)	0.620	0.023	0.260	-0.097	1.547	1,11	0.660	0.018	0.205	-0.183	3.191	1,11
Day 34 (July 15)	Rotifera (log x+1)	0.786	0.007	0.077	-0.087	1.588	1,11	0.224	0.131	1.661	0.174	0.619	1,11
Day 34 (July 15)	ALL ZOOPLANKTON	0.302	0.096	1.171	-0.598	4.329	1,11	0.336	0.084	1.015	0.319	4.210	1,11
Day 76 (Aug 26)	Calanoida (log x+1)	0.591	0.030	0.309	-0.143	1.413	1,10	0.304	0.105	1.173	-0.459	1.737	1,10
Day 76 (Aug 26)	Cyclopoida (sqrt)	0.631	0.024	0.246	0.028	1.107	1,10	0.359	0.085	0.924	1.182	2.833	1,10
Day 76 (Aug 26)	Cladocera (log x+1)	0.773	0.009	0.088	-0.035	1.418	1,10	0.632	0.024	0.244	-0.398	4.569	1,10
Day 76 (Aug 26)	Rotifera (log x+1)	0.791	0.007	0.074	-0.042	1.472	1,10	0.963	0.000	0.002	-0.017	1.347	1,10
Day 76 (Aug 26)	ALL ZOOPLANKTON	0.991	0.000	0.000	0.003	1.272	1,10	0.888	0.002	0.021	-0.103	5.287	1,10

*Bold values indicate significant results at $\alpha = 0.05$. **One sample from Day 76 was mishandled during laboratory processing so was not included in these analyses. Intercept is abbreviated as "Int.".

Table 2.4. Multiple response permutation procedure (MRPP) summary statistics from the principal component analysis (PCA) performed on log (*x*+1) transformed zooplankton abundance (no. m^{-2}) four days after iron application. Groups compared were defined *a priori* as controls (3 sites), low (2.3, 3.4, 5.2, and 7.9 g Fe m^{-2}), medium (12, 18, 28, and 42 g Fe m^{-2}), and high (64, 97, 148, and 225 g Fe m^{-2}) iron treatments. *Significant p-values at $\alpha = 0.05$ are in bold; α not adjusted for multiple comparisons. T = test-statistic; A = within group agreement (measure of variance). Corresponding PCA ordination is displayed in Figure 2.7.

Group comparisons			*p-value	T	A
All groups			**0.010**	-2.759	0.113
Control	vs	Low	0.281	-0.554	0.031
Control	vs	Medium	0.052	-1.829	0.073
Control	vs	High	**0.027**	-2.512	0.141
Low	vs	Medium	0.420	-0.010	0.001
Low	vs	High	**0.023**	-2.643	0.136
Medium	vs	High	0.087	-1.460	0.068

Table 2.5. Multiple response permutation procedure (MRPP) summary statistics from the non-metric multidimensional scaling (NMS) ordination analysis performed on log (*x*+1) transformed zooplankton abundance (no. m^{-2}) before iron application and 4, 34, and 76 days treatment. *Significant p-values at $\alpha = 0.05$ are in bold; α not adjusted for multiple comparisons. T = test-statistic; A = within group agreement (measure of variance). Corresponding NMS ordination is displayed in Figure 2.8.

Group comparisons			*p-value	T	A
All groups			**<0.001**	-22.618	0.196
Before	vs	Day 4	**<0.001**	-11.685	0.133
Before	vs	Day 34	**<0.001**	-15.804	0.224
Before	vs	Day 76	**<0.001**	-14.113	0.163
Day 4	vs	Day 34	**<0.001**	-12.882	0.133
Day 4	vs	Day 76	**<0.001**	-13.494	0.146
Day 34	vs	Day 76	**<0.001**	-7.017	0.063

Table 2.6. Regression summary statistics for the relative total proportion of crustacean taxa in relation to iron treatment 34 and 76 days after iron application. There were no significant results at $\alpha = 0.05$.

	Day 34 (July 15)						*Day 76 (August 26)*					
Taxa	**p-value**	**r^2**	**F-stat**	**Slope**	**Int.**	**df**	**p-value**	**r^2**	**F-stat**	**Slope**	**Int.**	**df**
Calanoida												
Skistodiaptomus oregonensis	0.123	0.203	2.796	0.005	-0.002	1,11	0.167	0.166	2.192	-0.002	0.006	1,10
Cyclopoida												
Cyclops bicuspidatus thomasi	0.132	0.194	2.643	0.025	-0.014	1,11	0.469	0.049	0.563	-0.016	0.056	1,10
Cyclops vernalis	0.179	0.158	2.061	0.017	0.008	1,11	0.628	0.022	0.249	0.006	0.010	1,10
Mesocyclops edax	0.086	0.244	3.545	0.005	-0.001	1,11	0.227	0.130	1.636	-0.004	0.009	1,10
Cladocera												
Diaphanosoma birgei	0.749	0.010	0.108	<0.001	0.003	1,11	0.487	0.045	0.523	0.018	0.007	1,10
Daphnia pulex	0.672	0.017	0.189	0.003	0.001	1,11	0.336	0.084	1.012	0.003	<0.001	1,10
Daphnia galeata mendotae	0.666	0.018	0.197	-0.001	<0.001	1,11	0.907	0.001	0.014	<0.001	0.002	1,10
Ceriodaphnia spp.	0.552	0.033	0.377	0.001	<0.001	1,11	0.645	0.020	0.224	0.009	0.011	1,10
Chydorus sphaericus	0.119	0.206	2.861	-0.006	0.002	1,11	0.342	0.082	0.988	-0.004	0.009	1,10
Bosmina longirostris	0.672	0.017	0.189	-0.023	0.141	1,11	0.867	0.003	0.029	-0.078	0.668	1,10

*One sample from Day 76 was mishandled during laboratory processing so was not included in these analyses. Intercept is abbreviated as “Int.”.

Table 2.7. Taxa abbreviations used in PCA (Fig. 2.7b) and NMS (Fig. 2.8b) ordinations.

Abbreviation	Full name
Calanoida	
Ca_naup	Calanoid nauplii (N1-N6)
S_ore	*Skistodiaptomus oregonensis* (Lilljeborg)
Cyclopoida	
Cy_naup	Cyclopoid nauplii (N1-N6)
Cy_cop	Cyclopoid copepodites (C1-C5)
C_bic	*Cyclops bicuspidatus thomasi* (Forbes)
C_ver	*Cyclops vernalis* (Fischer)
Cladocera	
D_juv	Daphnia juveniles
B_lon	*Bosmina longirostris* (Müller)
C_sph	*Chydorus sphaericus* (Müller)
Cerio	*Ceriodaphnia spp.*
D_birg	*Diaphanosoma birgei (Korinek)*
D_gal	*Daphnia galeata* (Sars) *mendotae* (Birge)
D_pul	*Daphnia pulex* (Linnaeus)
Rotifera	
A_bri	*Asplanchna brightwelli* (Gosse)
A_fissa	*Anuraeopsis fissa* (Gosse)
B_bid	*Brachionus bidentata* (Anderson)
B_plic	*Brachionus plicatilis* (Müller)
B_quad	*Brachionus quadridentatus* (Hermann)
Bdell	*Bdelloida* spp.
C_dos	*Conochiloides dossuarius* (Hudson)
C_pel	*Collotheca pelagica* (Rousselet)
C_uni	*Conochilus unicornis* (Rousselet)
G_styl	*Gastropus stylifer* (Imhof)
K_coch	*Keratella cochlearis* (Gosse)
K_quad	*Keratella quadrata* (Müller)
K_tes	*Keratella testudo* (Ehrenberg) *hiemalis* (Carlin)
L_cla	*Lecane elasma* (Harring and Myers)
L_pat	*Lepadella patella* (Müller)
M_bul	*Monostyla bulla* (Gosse)
M_ste	*Monostyla stenroosi* (Meissner)
P_pat	*Platyias patulus* (Müller)
P_sul	*Pompholyx sulcata* (Hudson)
Poly	*Polyarthra* spp.
S_obl	*Synchaeta oblonga* (Ehrenberg)
T_cyl	*Trichocerca cylindrica* (Imhof)
T_mul	*Trichocerca multicrinis* (Kellicott)
T_rou	*Trichocerca rousseleti* (Voigt)

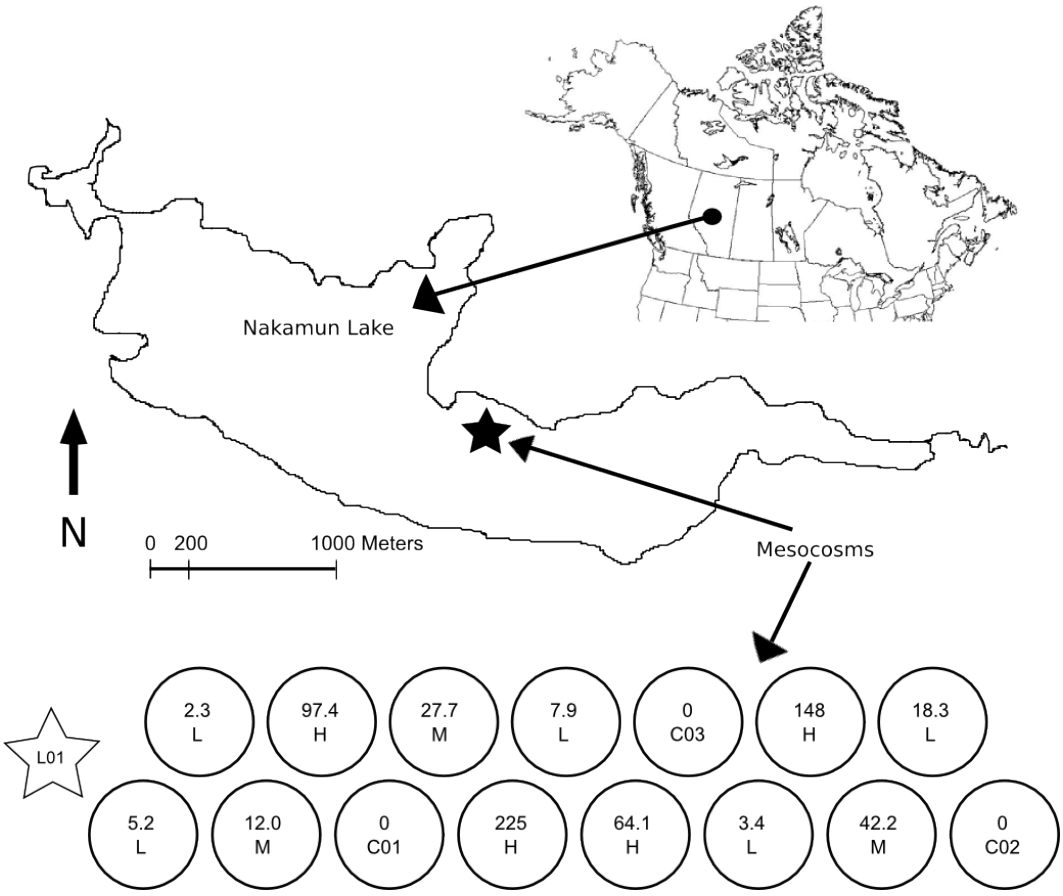

Figure 2.1. Map of study site and a pictorial representation of the experimental set-up. Experimental mesocosms were installed in a sheltered bay near the north shore of Nakamun Lake, at a depth of 4.5 m. Mesocosms are depicted with an open circle and lake reference location (L01) is depicted with and open star. Iron treatment levels are in g m^{-2} and the treatment group is indicated below as controls (C01, C02, and C03), low (L), medium (M), or high (H).

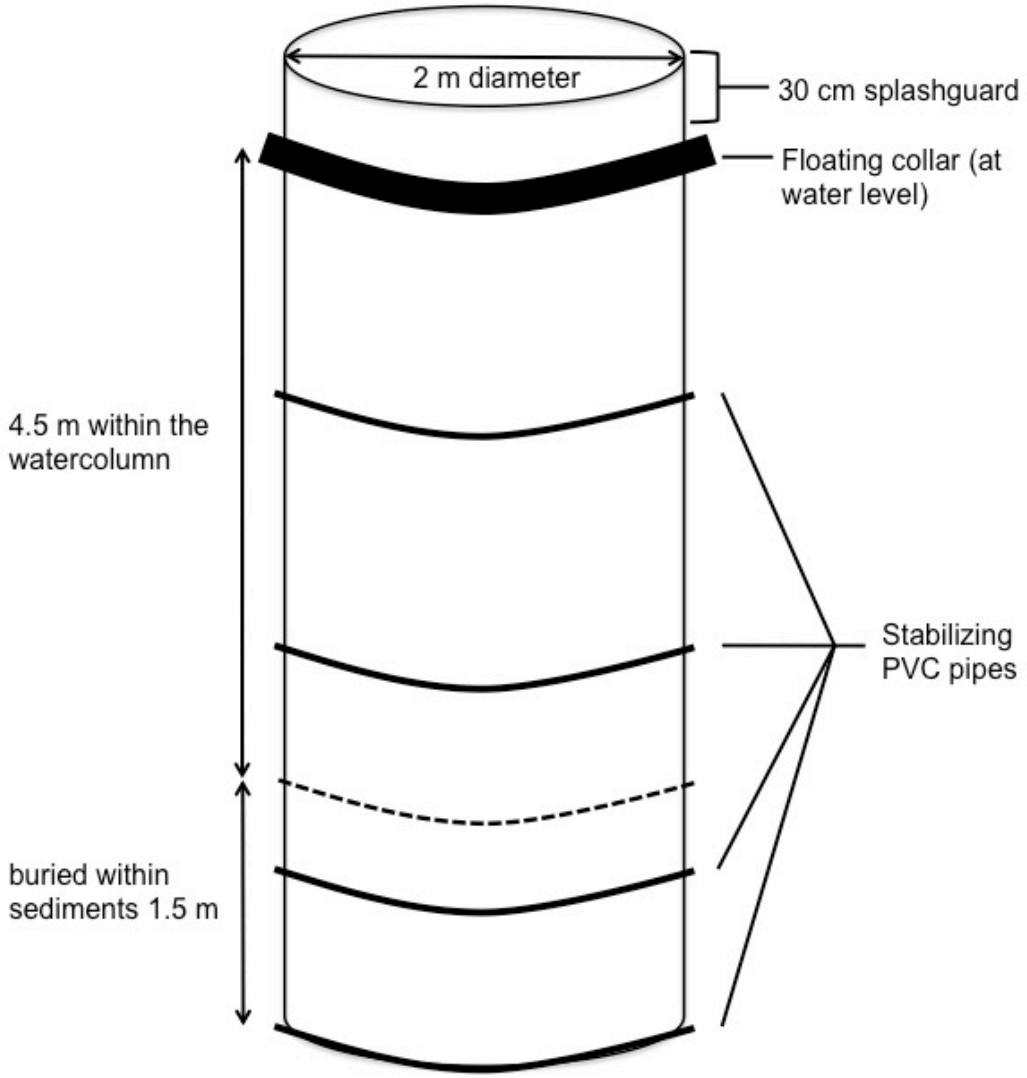

Figure 2.2. Schematic representation of the mesocom design. Fifteen experimental mesocosms were installed in small sheltered bay on the north side of Nakamun Lake in May 2009.

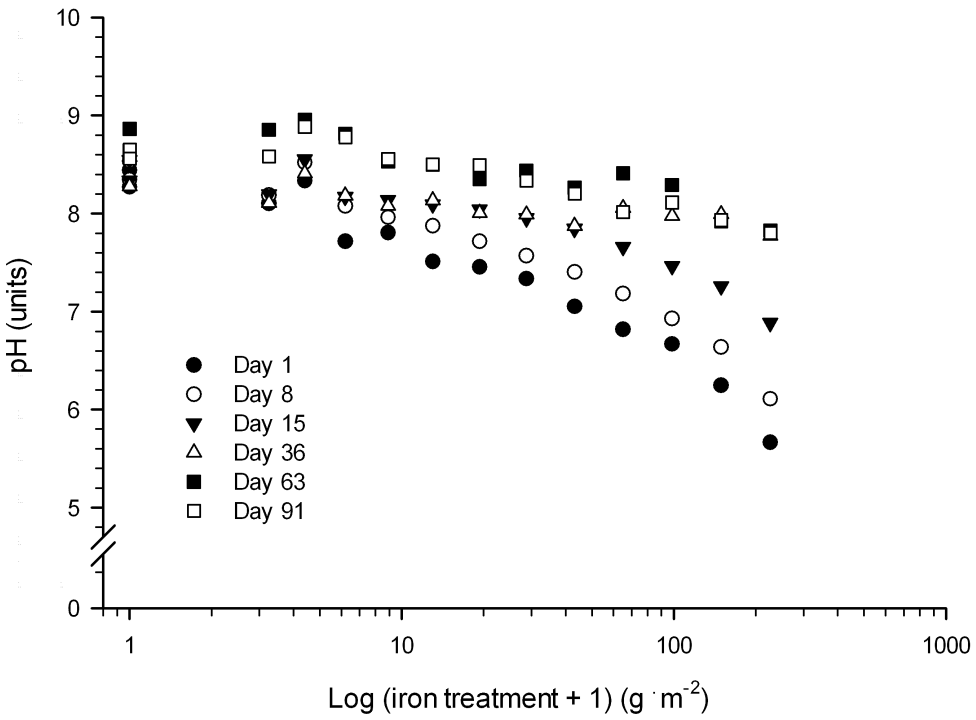

Figure 2.3. Mean water pH in mesocosms after iron application on days 1, 8, 15, 36, 63, and 91 (June 12, June 19, June 26, July 17, August 31, and September 10, 2009, respectively). The average pH was calculated from measurements taken at 0.5 m intervals from the surface to 4 m. Water pH dropped in relation to level of iron application. Average water column pH was neutral (pH > 7) by day 36 in all mesocosms.

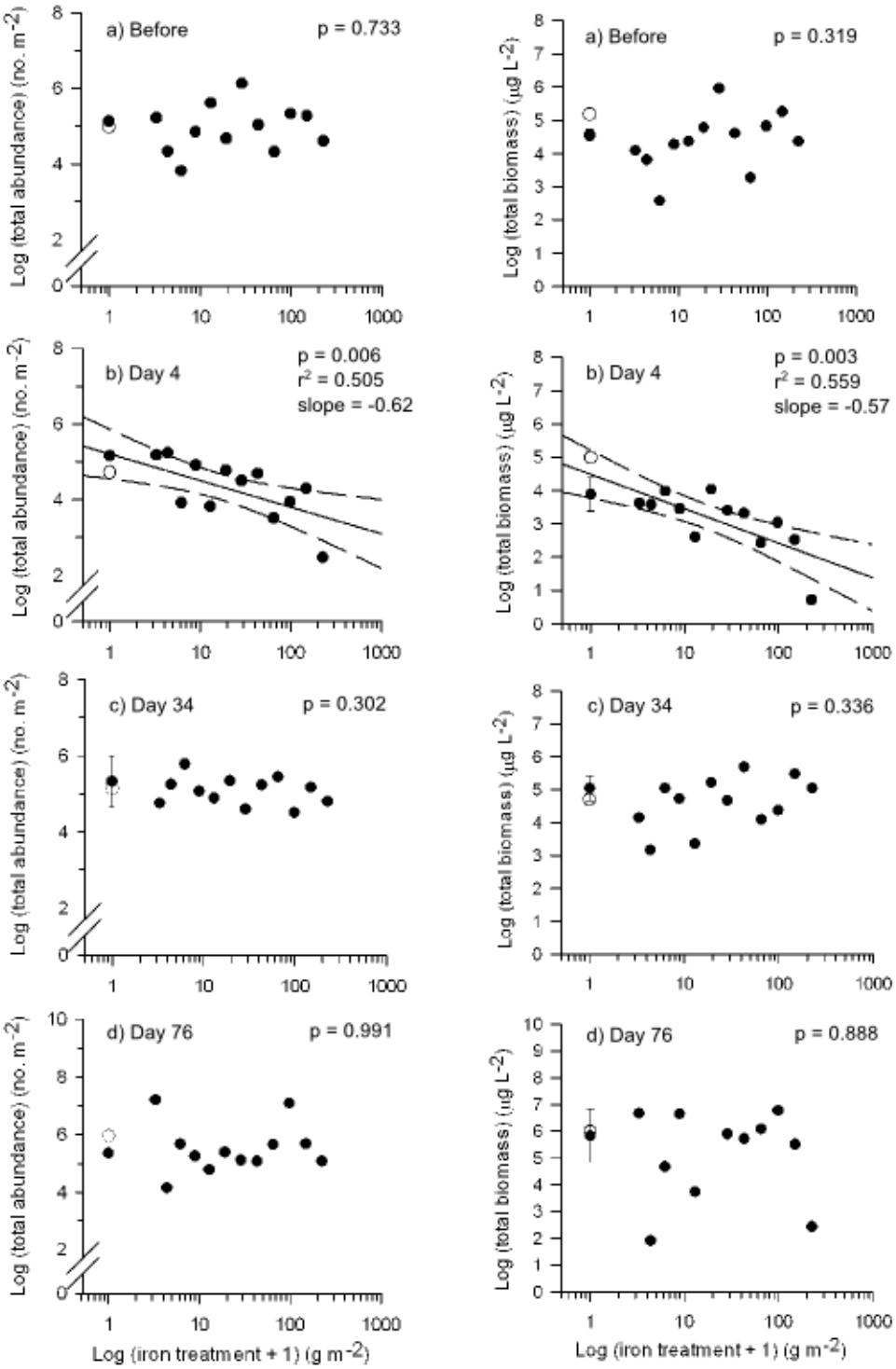

Figure 2.4a,b,c,d. Linear regression analyses for the total abundance (no. m^{-2}; left figures) and biomass (mg dw m^{-2}; right figures), of zooplankton in relation to iron treatment before (a), 4 days (b), 34 days (c), and 76 days (d) after iron application. The three control treatments were averaged and all other treatments were single-doses; therefore, standard error is associated with the control treatments only. The lake reference site is shown with open circles, but was not included in the regression analyses. Regressions summary statistics are given in Table 2.2. One sample from Day 76 was mishandled during laboratory processing so was not included in these analyses.

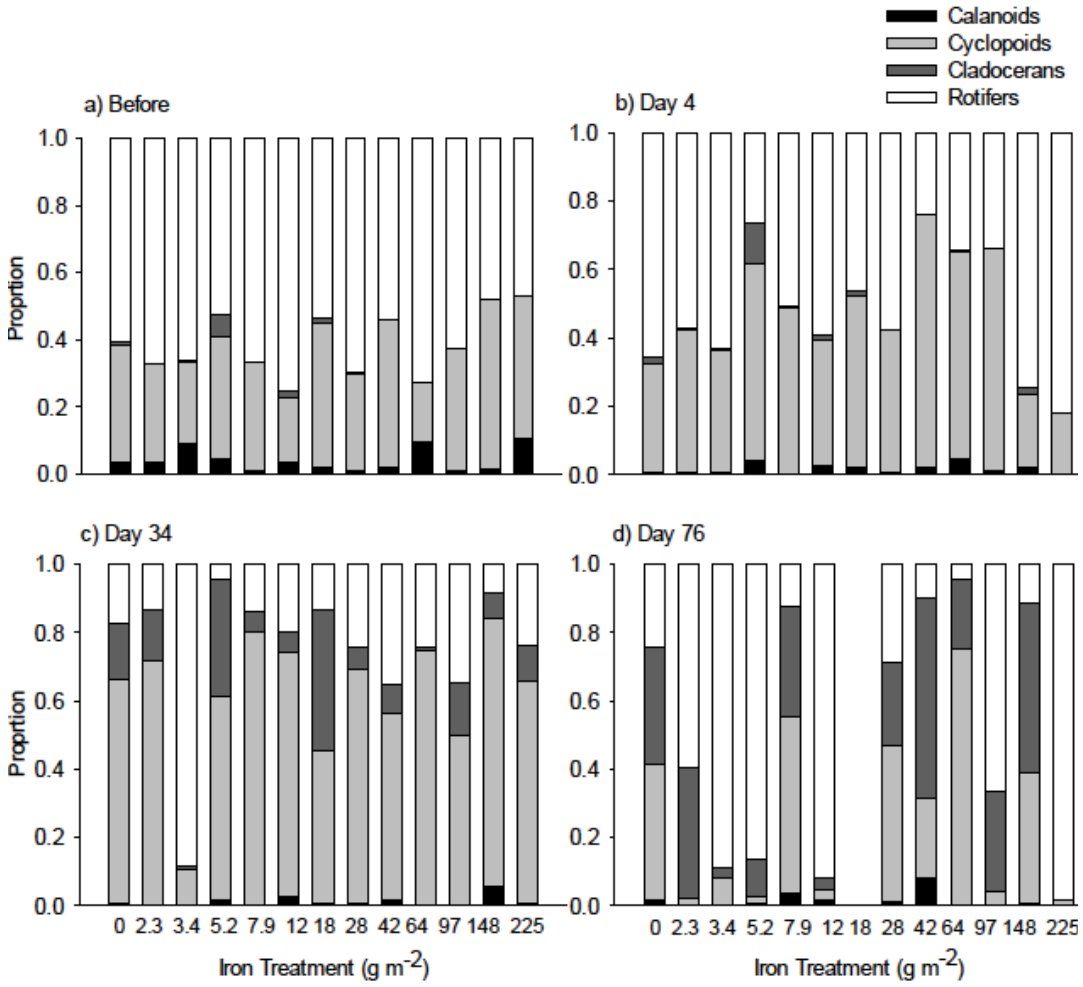

Figure 2.5a,b,c,d. The proportion of the total zooplankton sample made up by rotifers (white), cladocerans (dark gray), cyclopoids (light gray) and calanoids (black) before iron application (a) and 4 (b), 34 (c) and 76 (d) days after iron application.

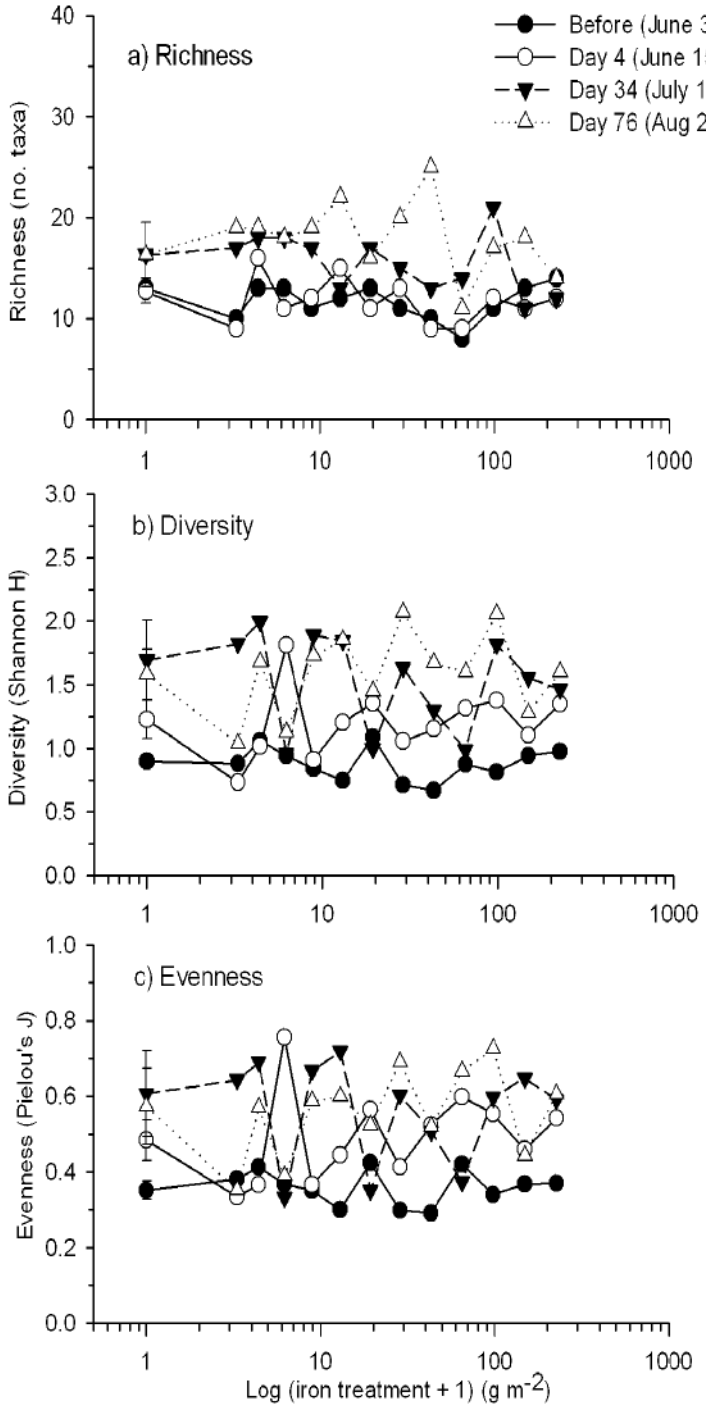

Figure 2.6a,b,c. Richness (no. taxa; top panel), diversity (Shannon H'; middle panel), and evenness (Pielou's J; bottom panel) of zooplankton assemblages in relation to iron treatment level before iron application (closed circles) and 4 days (open circles), 34 days (closed triangles), and 76 days (open triangles) after iron application. There was no significant relationship between indices and iron treatment; regressions summary statistics are given in Table 2.2.

a) **Mesocosms (sites)** **b) Species**

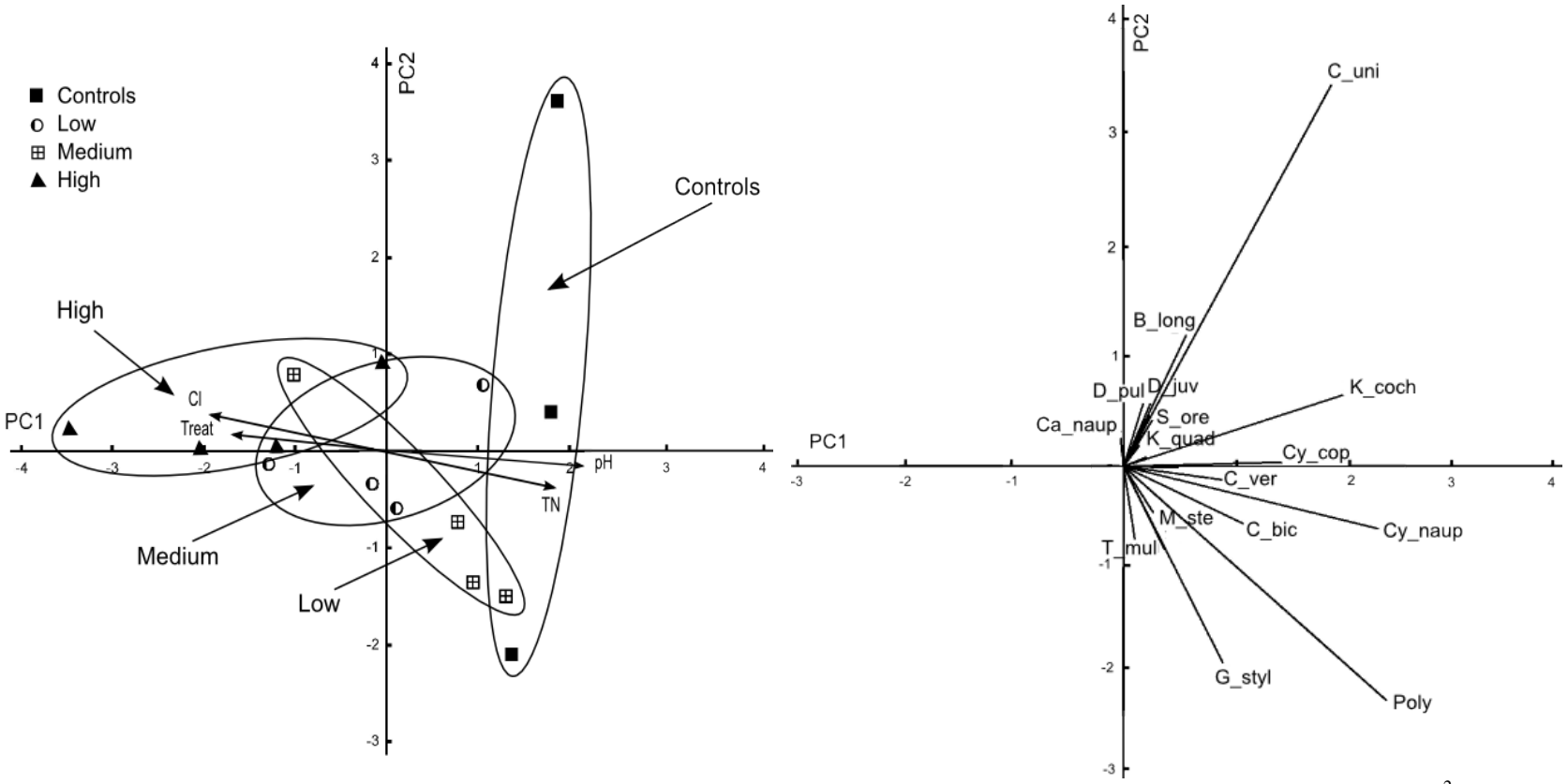

Figure 2.7a,b. Principal component analysis (PCA) performed on log (*x*+1) transformed zooplankton abundance (no. m^{-2}) four days after iron application showing site scores (a; left figure) and species scores (b; right figure). Groups (left figure) were defined *a priori* as controls (3 sites), low (2.3, 3.4, 5.2, and 7.9 g Fe m^{-2}), medium (12, 18, 28, and 42 g Fe m^{-2}), and high (64, 97, 148, and 225 g Fe m^{-2}) iron treatments. Environmental vectors ($r^2 > 0.5$) strongly associated with taxa composition were pH, total nitrogen (TN), chloride (Cl), and iron treatment level. Multiple response permutation procedure (MRPP) summary statistics are given in Table 2.4. Taxa abbreviations are explained in Table 2.7.

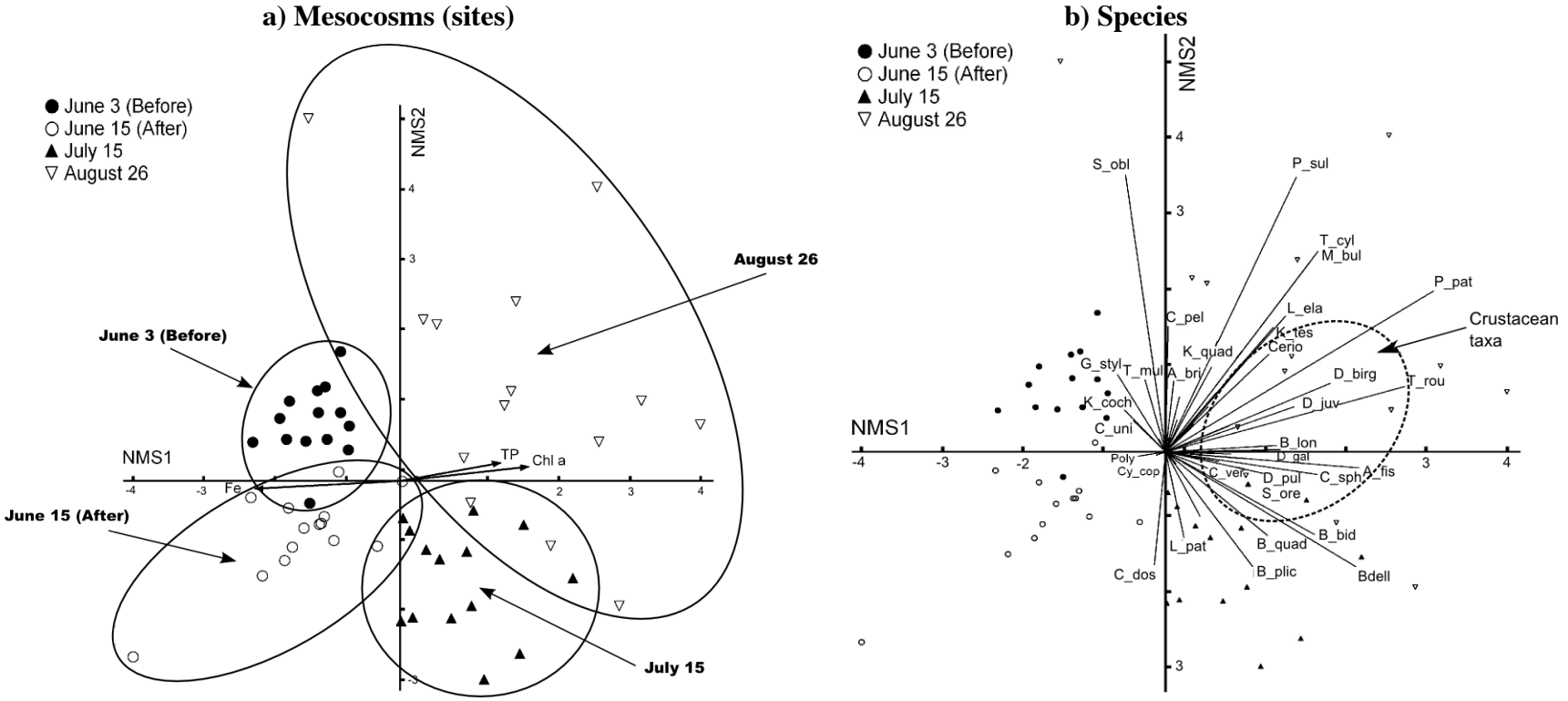

Figure 2.8a,b. Non-metric multidimensional scaling (NMS) ordination analysis performed on log (x+1) transformed zooplankton abundance (no. m^{-2}) before iron application and 4, 34, and 76 days treatment showing site scores (a; left figure) and species scores (b; right figure). Multiple response permutation procedure (MRPP) summary statistics are given in Table 2.5. Sites were grouped by day of experiment (left figure). Environmental vectors moderately associated with taxa composition ($r^2 > 0.3$) were total phosphorus (TP), chlorophyll *a* (Chl a), and iron concentration (Fe). Taxa abbreviations are explained in Table 2.7.

Chapter 3: Changes in macroinvertebrate assemblages following iron treatment

Introduction

Many lakes in the Canadian prairie provinces are suffering from cultural eutrophication, the increased primary productivity (particularly phytoplankton) as a result of anthropogenic activities (Schindler & Vallentyne, 2008). A problem with prairie lakes is that the return of phosphorus (P) from sediments (so called internal loading) is so high, that even when external loading is reduced, recovering from eutrophication requires decades (Schindler, 2012). One potential reason for prairie lakes being slow to recover from external nutrient reductions is their low iron (Fe) concentrations (Schindler *et al*., 2008) because iron (Fe) availability appears to be an important factor in internal P loading (Schindler & Vallentyne, 2008).

Several remediation techniques have been tested for their efficacy in helping water bodies with high internal P loading to recover more quickly (reviewed in Cook *et al*., 2005). The most widely used of these remediation methods involves the application of aluminum (alum) salts to inhibit phosphate release from the sediments and limit its availability within the water column. However, alum salts are most effective at water pH 6.5-8.5 (Cook *et al*., 2005); therefore, this remediation technique is not suitable for highly alkaline water bodies. The application of iron (Fe) salts (most commonly iron(III)chloride, or ferric chloride; $FeCl_3$) may be a suitable technique for systems in which pH is too high for effective alum treatment (Cooke *et al*., 1993; Orihel *et al*., in prep.).

However, iron application can potentially affect aquatic macroinvertebrates through several mechanisms. Fe is an essential trace metal required for all organisms because it is involved with oxygen transport, DNA synthesis, and electron transport (Gurzau *et al*., 2003). Because sulfur interrupts the biogeochemical cycling of iron (Van Der Welle *et al*., 2008), lakes that receive sulfur pollution could be iron deficient and iron application may restore iron concentrations in these lakes to natural conditions. However, excess iron is also a toxicological concern to aquatic biota both in the soluble ferrous (Fe^{2+})

form (Gurzau *et al*., 2003) and in the highly insoluble solid ferric (Fe^{3+}) form (Vuori, 2005).

Like any remediation treatment, iron application will be a useful method to reduce internal P loading only if there are minimal adverse effects on biota. Because the presence or absence of aquatic macroinvertebrates reflects specific physical and chemical parameters of freshwater ecosystems, these invertebrates are useful water quality indicators (Washington, 1984) and their diversity and taxonomic composition can affect ecosystem resilience (Brönmark & Hansson, 2002). These qualities make zoobenthos a useful focal assemblage for testing the possible adverse effects that iron treatment may have on aquatic biota. However, research investigating the effects of iron application on zoobenthos, in both aquatic (e.g., benthic macroinvertebrates) and terrestrial (e.g., emerged adult insects) life stages, is currently lacking.

When dissolved ferric chloride is applied to lake water it undergoes a series of chemical reactions before forming solid precipitates (as ferric hydroxides: $Fe(OH)_3$) that settle throughout the water column and form a flocculent layer at the sediment surface, impeding soluble P from entering the water column (discussed in Mortimer, 1941 and Cook *et al*., 1995). Flocculated Fe-hydroxide precipitates can potentially affect non-selective filter-feeding zoobenthos by decreasing their filtering efficiency, which has been found in lab studies investigating non-selective pelagic filter-feeders (Randall *et al*., 1999). Likewise, iron application may affect benthic invertebrates through the accumulation of a deep flocculent layer. Because this layer is moderately loose, organisms can move within it but may suffocate if oxygen becomes depleted within the layer before they are able to move upwards to the sediment-water interface. Also, iron-hydroxide precipitates can interfere with gas exchange by coating exposed tracheal gills (Gerhardt, 1994; Vouri, 1995; Regerand *et al*., 2005) and can also induce starvation by coating the gut and reducing its permeability to nutrients (Gerhardt 1992).

Hydrolysis of ferric chloride immediately after its application to the water column results in production of hydrochloric acid (HCl), a strong acid (chemical

reactions reviewed in Chapter 1 of this thesis); high rates of iron application thus causes a sharp drop in pH and biota may experience acidification effects from high iron doses. There are many known negative effects of acute and chronic acidification on aquatic invertebrates such as shell thinning in molluscs and crustaceans (Havas & Rosseland 1995), the loss of ability for some crustaceans to moult (Malley & Chang, 1985) and changes in taxonomic composition (Griffiths, 1992; Orendt, 1999), which can result in loss of diversity. At pH < 5 iron remains in the soluble ferrous (Fe^{2+}) state (Maltby *et al*., 1987), a form more toxic to some biota (Gerhardt 1992). Consequently, biota receiving high-iron doses where the pH drops below 5 may also be at higher risk of iron toxicity in addition to acidification impacts. Acidification can be minimized if the ecosystem has high alkalinity to help buffer pH from decreasing.

Because some biotic assemblages are adversely affected by eutrophication, iron application may also help restore healthier zoobenthos assemblages. Zoobenthos abundance, productivity (and/or biomass) (Leach *et al*., 1977), and community composition (Hershey, 1992; Blumenshine *et al*., 1997) can be drastically altered by eutrophication. Although filter feeders within the benthic zone can respond positively to increased deposition of dead phytoplankton through greater biomass (e.g., Jonasson, 1972; Welch *et al*., 1988), this accumulation of dead phytoplankton cells can also negatively impact zoobenthos. The increased deposition of decomposing phytoplankton increases suspended sediments and decreases the physical heterogeneity of benthic habitats (Scheffer *et al*., 1993), which may reduce zoobenthos species diversity (Donohue *et al*., 2009). Furthermore, bacterial decomposition of the settled phytoplankton often causes hypoxia in the benthic zone, which also depletes zoobenthos diversity due to selection for a relatively small number of low-oxygen tolerant species (Wiederholm, 1984). The combination of hypoxia, lower habitat heterogeneity, and increased suspended sediments generated by nutrient enrichment cause shifts in benthic invertebrate species, typically towards domination by oligochaetes and chironomids (Wetzel, 2001).

The objectives of this study were to determine the effects of different iron application concentrations on benthic macroinvertebrate assemblages by examining changes in abundance, biomass, and taxonomic composition in both their aquatic (benthic, all taxa) and terrestrial (emergent adults, insects only) life stages. The evaluation of potential negative effects of iron treatment on zoobenthos is a critical part of determining optimal remediation strategies, so that remediation attempts do not inadvertently cause negative impacts on aquatic biodiversity and ecosystem function.

Materials and methods

Study site and experimental set up

My experiment was conducted in Nakamun Lake (latitude 53.886, longitude -114.206), a shallow, hypereutrophic, polymictic lake located in the boreal transition zone in central Alberta, Canada (Fig. 3.1). Fifteen large experimental units (mesocosms), 2 m in diameter and 4.5 m deep, were installed in the limnetic zone. Natural invertebrate assemblages within the lake were retained within the mesocosms when the curtain walls were dropped vertically from the surface to the sediments in May 2009. Single-dose treatments of ferric chloride, from 2.25 g m^{-2} to 225 g m^{-2} were applied in June 2009. Twelve of the mesocosms received iron treatment, while three mesocosms did not and were left as controls. Lake reference samples were collected in close proximity to the mesocosms to compare control mescosms to natural lake assemblages. A pictorial representation of the experimental set up and the location of lake reference site locations is given in Fig. 3.1. A detailed description of the background water quality of Nakamun Lake and a more detailed description of the mesocosm and experimental set up are included in Chapter 2 of this thesis.

Sample collection and laboratory analyses

Water chemistry analyses and light profiles were performed bi-weekly from June 9 until October 16. Vertical profiles measuring pH, temperature, specific conductivity, and dissolved oxygen were performed using a Hydrolab

Series 5 Datasonde Multiprobe. Total phosphorus (TP), total nitrogen (TN), dissolved organic carbon (DOC) and chlorophyll *a* (chl-*a*) samples were collected and analyzed at the University of Alberta Biogeochemical Analytical Services Laboratory. Secchi depth and light profiles were taken on alternate weeks as the water chemistry samples, the latter with a LI-COR LI-250A light meter.

Emerging adult insects were continuously trapped using a submerged funnel trap with a bottom diameter of 34 cm (Davies, 1984); each trap was attached to a rope strung across the mesocosm collar and hung in the centre of the mesocosm. Traps were installed in the mesocosms on July 1, 2009 and removed September 15, 2009. Captured insects were collected at least twice a week and preserved and stored in 80% ethanol. Traps were scrubbed weekly to reduce periphyton build-up as this has been shown to reduce catch (Welch *et al*., 1988b). Three lake reference sites were also sampled for emergent insects; on the east and west ends of the mesocosms and in between the centre mesocosms (Fig. 3.1). Insects were counted and identified to family and to genus when possible using a stereo dissecting microscope at 50x power and taxonomic literature (McAlpine *et al*., 1981; Townes, 1945). Insects that could not be identified to genus due to poor-quality-specimens or the lack of males (for those taxa in which only males show diagnostic features) were identified only to family. Emergent insect biomass was calculated using published length-weight regressions (Stagliano *et al*., 1998). Total length was measured in dorsal view from the tip of the head to the base of the abdominal apex with an ocular micrometer at 20-50x power for the first 30 specimens in each taxon within a treatment; average lengths of specimens within the same treatment were used after 30 specimens.

Because I did not want to disturb the sediments and the floc layer during the experiment, zoobenthos were only sampled during the final sampling period, 24-26 March 2010, just prior to ice-out and 9 months after treatment. Due to sampling constraints in the field, only one lake benthic reference site was sampled, which was 3 m west of the western-most mesocosm (Fig. 3.1). For this reference site and within each mesocosm, three Ekman grabs of substrate were taken through the ice as evenly spaced as possible. Samples were stored at ≤ 4°C

until sample processing in the laboratory. Within 24 h of sample collection, grab samples were sieved through a 500 μm mesh screen. Retained macrofauna were fixed with 8% formalin for ~48h and then transferred and stored in 80% ethanol.

Zoobenthos were counted and identified using a stereo dissecting microscope at 20-100x power. Individuals were identified to genus or species when possible (Clifford, 1991; Merritt & Cummins, 1996), with the exception of Chironomidae and Sphaeriidae (identified to family), and Cyclopoida, Harpacticoida, and Oligochaeta (identified to order). Nomenclature of zoobenthos follows Clifford (1991). Zoobenthos biomass was calculated using standard length-weight regression equations for Amphipoda, Sphaeriidae, Caenidae, Ceratopogoninae, Chironomidae, (Benke *et al*., 1999), Oligochaeta, Ostracoda, Cyclopoida (Leeper & Taylor, 1998), Hydrachnidia, Diptera (Baumgartner & Rothaupt, 2003), Planorbidae, and Valvatidae (Venturelli, 2004). Biomass conversion equations were not available for Hydrozoa and Physidae; therefore, these specimens were not included in biomass analyses. However, these taxa contributed minimally to the overall total abundance (Table 3.5). Lengths were measured with an ocular micrometer at 20-100x power for the first 30 specimens in each taxon, within a treatment; average lengths of specimens within the same treatment were used after 30 specimens.

Statistical analyses

Data were tested for normality and transformed when necessary to meet normality assumptions prior to further parametric statistical tests. Total emergent insect abundance was square-root transformed and total zoobenthos abundance and emergent insect biomass were both log (x+1) transformed. Skewness and kurtosis were considered acceptable after transformations (Zar, 1999). Emergent insect assemblages in the control mesocosms and lake references were compared with a Welch's t-test due to unequal variance among sample groups (Zar, 1999). Differences in community composition were analyzed using two approaches: taxonomic composition among treatment groups was assessed with nonmetric multidimensional scaling (NMS) and analysis of diversity indices (see details

below). Changes in total macroinvertebrate abundance, biomass, and diversity values across iron treatment levels were assessed using linear regression. An average and standard error of the three controls was calculated and was used as a single datum in regression analyses. Because the primary objective was to assess the effect of iron treatment on macroinvertebrate communities, lake reference sites were not included in the regressions.

Non-parametric ecological indices used to assess alpha diversity of the macroinvertebrate assemblages were richness, Shannon-Weaver diversity (H'), and Pielous's evenness (J) because they are some of the most commonly used indices in community ecology and aquatic studies (Washington, 1984; Karydis & Tsitsis, 1996). Although the utility of diversity indices, in particular H', has been extensively debated in the ecological literature (Barrantes & Sandoval, 2009; Spatharis *et al*., 2011), I considered them useful for this study as all samples were taken from the same bay in close proximity to one another, and the primary goal was not to assess overall diversity, but to assess relative changes in community composition over a gradient of iron treatment levels.

Assemblage structure of zoobenthos and emergent insects was examined with NMS (PC- ORD, version 5.10; MjM Software Design, Gleneden Beach, Oregon). The Sorenson (Bray Curtis) distance measure was used to calculate dissimilarity between sites. Rare taxa making up <1% of total abundance were either excluded from the analysis or grouped at a higher taxon to enable us to discern relationships in community assemblages more readily (McCune & Grace, 2002). This approach reduced the total number of taxa from 22 to 12 for zoobenthos, and 21 to 7 for emergent insects. Zoobenthos and emergent insect raw abundances were log (x+1) transformed prior to ordination analyses to meet normality assumptions (McCune & Grace, 2002). Zoobenthos proportional abundance data were also investigated because proportional data are considered robust for ordination methods (Jackson, 1993). I used Spearman rank correlations to examine the strength of the associations of environmental gradients and taxa with ordination-axis scores. The environmental matrix included average open-water, integrated concentrations of TP, TN, chl-*a*, dissolved oxygen (DO), iron,

and specific conductivity. Iron treatment level (g m^{-2}) was also included in the environmental variable matrix. A multiple response permutation procedure (MRPP) was used to test for differences in assemblages between *a priori* groups, defined as lake references (L01, L02, and L03), control mesocosms (C01, C02, and C03), low (2.3, 3.4, 5.2, and 7.9 g m^{-2}), medium (12, 18, 28, and 42 g m^{-2}), and high (64, 97, 148, and 225 g m^{-2}) iron treatments.

With the exception of ordination analyses, all other statistical analyses were performed in the R statistical program (version 2.12.2; R Development Core Team, Vienna, Austria). Regression analyses and t-tests were performed within the MASS library and the vegan library was used to calculate diversity indices. Sigmaplot version 12.0 was used for graphical representations of the data.

Results

Water quality parameters

Despite the high alkalinity of Nakamun Lake, iron application caused the pH to decrease by 3 units, from 8.5 to 5.5, in the highest-dosed mesocosm during initial iron application (Fig. 2.4; Chapter 2 of this thesis). Although the high alkalinity in the lake was able to buffer this decrease in pH for most treatments (Table A.2; Appendix A), the highest iron treatment was slower to recover and did not return to a neutral pH of 7 until mid-July (Fig. 2.4). The application of ferric chloride was successful as a short-term oligotrophication treatment within the mesocosms. Average TP, TN, and chl-*a* significantly decreased as a function of iron treatment level during the open water season. A detailed analysis of changes in water chemistry parameters and phytoplankton assemblages is described in Orihel *et al*. (in prep) and a brief summary of water chemistry parameters is given in Table A.2 (Appendix A).

EMERGENT INSECTS

Comparisons between lake and mesocosm controls

Abundance and biomass

Although not a main focus of my study, I was interested in testing whether there was a mesocosm effect on the abundance or diversity of benthic and emergent invertebrates. The total abundance (no. of insects captured m^{-2}) was 5x higher in the three lake reference emergence traps (mean = 2,499) than in the three control mesocosms (mean = 472; Fig 3.2a and Table 3.1). Total estimated biomass (mg dry weight m^{-2}) was over twice as high in the lake references (mean = 516) compared to the controls (mean = 214; Fig. 3.2b and Table 3.1). However, because of the high variability among samples, there were no significant differences in emergent insect abundance or biomass between the lake references and control mesocosms at $\alpha = 0.05$ (t-test; $t = 2.708$, $df = 2.079$ $p = 0.109$ and $t = 1.353$, $df = 2.655$ and $p = 0.280$, respectively; Table 3.1). Almost all emergent insects were Chironomidae (Diptera), with a smaller number of other dipteran taxa and a few caenid mayflies. A more detailed description of taxa present is discussed in subsequent paragraphs and is given in Table 3.2. Rare taxa occurred much more frequently in the lake reference sites than within the mesocosms (Table 3.2, Fig. 3.2a) and the number of taxa (richness) was significantly greater in the lake references compared to the controls ($t = 6.364$, $df = 2.560$, $p = 0.012$; Table 3.2). However, differences between H' and J were not detected between the controls and lake references ($t = 1.100$, $df = 3.976$, $p = 0.333$ and $t = -0.490$, $df = 3.592$, $p = 0.654$, respectively; Table 3.2).

Assemblage structure

In addition to differences in community composition among the lake references and the mesocosms, temporal emergence patterns also differed. The lake had a much higher rate of emergence from mid-August to the beginning of September compared to the controls (Fig. 3.3). *Parachironomus abortiva* and *Tanytarsus* spp. emerged at a much higher rate in the lake than the control

mesocosms during this time (Fig. 3.4). However, the controls had a higher emergence rate of *Chironomus plumosus* throughout July (Fig. 3.4). Differences in the community composition and emergence patterns between the lake reference sites and the mesocosms are discussed further in subsequent paragraphs.

Responses among mesocosms

Abundance and biomass

Total abundance of emergent insects in mesocosms ranged from 90 to 1,349 m^{-2} (Table 3.2), and total biomass ranged from 32 to 1,126 mg dw m^{-2}. The total emergent insect abundance and biomass from the highest iron treatment (225 g m^{-2}) was the most similar to the lake references (Fig. 3.2a and b). Within the mesocosms, the mean abundance and biomass of the controls (284 individuals and 472 mg m^{-2}, respectively) were similar to those of the iron treatments (280 and 461 mg m^{-2}, respectively). There was no relationship between total emergent insect abundance or biomass and level of iron treatment (linear regression; r^2 = 0.064, df = 11, p = 0.403 and r^2 = 0.001, df = 11 p = 0.921, respectively; Fig. 3.5a and b; Table 3.3). *C. plumosus* was a major contributor to the total emergent insect abundance in the mesocosms (Fig. 3.2a), and due to its large body size it made up an even higher proportion of the total biomass (Fig. 3.2b).

Diversity and assemblage structure

There were 21 identifiable taxa captured within the emergent insect traps. With the exception of one *Caenis* sp. (Ephemeroptera), captured emergent insect assemblages were composed of true flies (Diptera), specifically biting (Ceratopogonidae) and non-biting midges (Chironomidae and Chaoboridae). Chironomids made up 97% of the total emergent insects within the mesocosms (Table 3.2) and they were dominated by *C. plumosus*, *Tanytarsus* spp., and *P. abortiva* (Table 3.2, Fig. 3.2a). As discussed previously, rare and unique taxa occurred much more frequently in the lake reference sites than within the mesocosms (Table 3.2, Fig. 3.2a); the only rare taxa that were sampled from within the mesoscoms but not the lake references were *Caenis* sp., *Cricotopus* spp., and *Psectotanypus* spp. (Table 3.2).

There was no response to iron treatment level detected in the diversity indices analyzed (Fig. 3.5c-e). The number of taxa (richness) was not significantly related to iron treatment ($r^2 = 0.197$, df = 1,11, $p = 0.129$; Fig. 3.5c) with the small range of two to seven taxa being distributed randomly among the iron-treated mesocosms (Fig. 3.5c). There was no effect of iron treatment on diversity (H') ($r^2 = 0.069$, df = 1,11, $p = 0.386$; Fig. 3.5d) or evenness (J) ($r^2 = 0.319$, df = 1,11, $p = 0.090$; Fig. 3.5e).

Because taxa that comprised <1% of the total emergent insects captured were excluded, the NMS ordination of the emergent insect assemblages excluded mayflies and ceratopogonids (NMS taxa groupings and abbreviations are given in Table 3.2). The final NMS output was determined in 64 iterations, was 2-dimensional with a stress of 11.714, had a final instability of <0.00001, and had statistically greater structure than randomly created outputs (Monte Carlo test; $p = 0.008$). The incremental r-squared for axes 1 and 2 were 0.755 and 0.142, respectively; combined, the 2 axes explained 89.7% of the variance in the ordination distances.

The NMS indicated that the lake reference assemblages were different from those in the mesocosms (Fig. 3.6). Although the overall MRPP was not significant at $\alpha = 0.05$, there was notable clustering among some groups ($p = 0.060$; Table 3.4), and pairwise comparisons indicated that the lake reference sites were significantly different than the control, low, medium, and high groups ($p < 0.027$ for each comparison; Table 3.4). The MRPP pairwise comparisons among iron-treatment groups were not significant ($p > 0.56$ for all mesocosm comparisons; Table 3.4) and there is extensive overlap among groups in the ordination (Fig. 3.6). The ordination structure was mainly driven by the higher abundances of *P. abortiva* and less common taxa such as Orthocladinae spp. and Tanypodinae spp. in the lake sites (Fig. 3.6). In contrast, *Chironomus* spp. were associated with the mesocosms and not the lake reference sites (Fig. 3.6). The only environmental variable that was highly correlated ($r^2 > 0.50$) was chl-*a*, which was negatively associated with the lake reference sites and positively correlated with the presence of *Chironomus* spp. (Fig. 3.6).

Temporal emergence patterns

Differences in temporal emergence patterns were most obvious between the lake reference sites and the mesocosms. Most insects stopped emerging around mid-August within the mesocosms, but the lake references and the highest iron treatment had high rates of emergence at this time (Fig. 3.3). There was no observable pattern in the timing of emergence among iron-treatment groups (Fig. 3.3b). The late emergence peak in the lake references and the highest treatment was mostly driven by the high emergence rates of *Tanytarsus* spp. in August (Fig. 3.4). *P. abortiva* also had high emergence rates in the lake references later in August, but the timing of emergence was variable among iron treatment groups (Fig. 3.4). *C. plumosus* had much higher emergence rates throughout July, August, and September in the mesocosms than in the lake, and only emerged from the lake at low rates in late summer (Fig. 3.4).

ZOOBENTHOS

Comparisons between lake and mesocosm controls

Both the average total abundance and average total estimated biomass of the control mesocosms (6,268 m^{-2} and 2.02 g dw m^{-2}, respectively) were lower than those of the single lake reference site (11,234 and 5.86 respectively; Table 3.1). However, the diversity indices (richness, evenness, and diversity) were similar between the lake reference and the control mesocosms (Table 3.1). There were no unique taxa in the lake reference sites that did not occur in the mesocosms.

Responses within mesocosms

Abundance and biomass

Total benthic macroinvertebrate abundance ranged from 2,467 to 17,611 m^{-2} within the mesocosms (Fig. 3.7a), with a mean abundance of 6,250 m^{-2}. Total biomass within the mesocosms ranged from 0.95 to 5.85 g dw m^{-2} (Fig. 3.7b) with a mean biomass of 2.81 g dw m^{-2}. There was no relationship between total zoobenthos abundance or biomass and iron treatment level (linear regression; r^2 =

0.115, df = 1,11, p = 0.258 and r^2 = 0.041, df = 1,11, p = 0.505, respectively; Fig. 3.7a, b).

Although care was taken to ensure that the sediment-water interface was captured during sampling, Ekman grabs do not always capture consistent volumes of material, so I assessed relative proportions of macroinvertebrate taxa in addition to absolute abundances. I found no significant relationships between the absolute or relative abundance of any single taxon and iron treatment level (Fig. 3.8).

Assemblage structure

A total of 22 taxa were collected, of which only six occurred at every site (Table 3.5). The number of taxa sampled within each mesocosm ranged from 10 to 16 (Table 3.5). There were 4 unique taxa that occurred only as one individual: *Hydra* sp. (Hydrozoa), *Gammarus lacustris* (Amphipoda), Harpacticoida (Copepoda), and *Nemotelus* sp. (Diptera). All of these unique taxa occurred in the mesocosms. The most abundant taxa (each comprising > 10% of total macroinvertebrate abundance) were Sphaeriidae (Bivalvia), *Candona* sp. (Ostracoda), *Cyclocypris* sp. (Ostracoda), Cyclopoida spp., and Naididae spp. (Oligochaeta). Other common taxa that occurred in more than 70% of the samples included *Valvata tricarinata* (Say) and *Valvata sincera helicoidea* (Dal), (Gastropoda), *Piona* spp. (Hydrachnidia), Candonidae spp. (Ostracoda), Chironomidae spp. (Diptera), and members of the ceratopogonid subfamily Ceratopogoninae spp. (Diptera) (Table 3.5).

There was no effect of iron treatment dose on any diversity indices analyzed (Fig. 3.7c-e). The range in richness in the controls (11 to 16 taxa) was similar to the range in richness across all sites (10 to 16 taxa; Fig. 3.7c). Richness was not related to iron treatment (r^2 < 0.001, df = 1,11, p = 0.989; Fig. 3.7c). The range in H' in the control mesocosms (1.8 to 2.2) nearly encompassed the range in H' across all sites (1.7 to 2.2; Fig. 3.7d) and there was no effect of iron treatment on this diversity index (r^2 = 0.070, df = 1,11 p = 0.382; Fig. 3.7d). The variation in J among control mesocosms was small (0.78 to 0.81; Fig. 3.7e) compared to the

range of J across all treatments (0.65 to 0.89; Fig. 3.7e), but there was no relation between J and iron treatment ($r^2 = 0.059$, df = 1,11, p = 0.425; Fig. 3.7e).

NMS ordinations were performed on both the relative- and absolute-abundance data; however, due to the lack of stability and consistent NMS outputs, a useful ordination was not retained for the zoobenthos assemblages. Non-NMS ordination techniques were not suitable for assessing zoobenthos assemblages because the data do not meet the required assumptions of these more structured techniques.

Discussion

MACROINVERTEBRATE RESPONSES TO IRON TREATMENT

The results of my mesocosm experiment suggest that at the values tested, ferric chloride application has minimal impacts on benthic macroinvertebrate assemblages in the benthic-limnetic zone, and that ecosystem function would not be disrupted as result of changes to the macroinvertebrate assemblages. After iron application I did not detect significant changes in the abundance, biomass, and assemblage structure of zoobenthos or emergent insect assemblages in relation to iron application. It is possible, however, that my ability to detect a significant change in zoobenthos assemblages was hindered by the naturally high heterogeneity in spatial distribution of zoobenthos within the sediments, along with lack of replication of the iron doses. Nonetheless, the lack of response in these invertebrate assemblages to iron application is interesting because there are several risks associated with iron-dosing such as acidification and iron toxicity (both direct and indirect). Given the large range of iron application doses that were tested (2.25 to 225 g m^{-2}) and the substantial changes in water quality parameters and phytoplankton assemblages and biomass (Orihel *et al.*, in prep), I had expected to observe changes in the invertebrate assemblages.

Although direct iron toxicity was not tested in this study, the lack of change in zoobenthos abundance and assemblage structure among treatment levels after 9 months of exposure suggests that the zoobenthos were not severely affected by iron toxicity. Despite the high concentrations of iron applied to my

mesocosms (0.5-50 mg L^{-1}), total Fe concentrations in the water column never exceeded 1 mg L^{-1} during the open water season in any mesocosm (Table A.2: Appendix A). This is lower than the U.S. Environmental Protection Agency (EPA) water quality criterion for fresh waters (<1 mg L^{-1}; US EPA, 1976) and the iron concentrations in which effects have been observed in the field such as decreased taxa richness (Linton *et al*., 2007; Peters *et al*., 2011) and lower abundance of zoobenthos (Rasmussen & Lindegaard, 1988). These low iron concentrations are unlikely to have toxic effects on the benthic invertebrates because the uptake of Fe^{2+} is regulated by biota at low concentrations, (Locke & Nichol, 1992), it does not accumulate in whole body loads when organisms are exposed for short periods of time (Gerhardt, 1994), and there is potential that aquatic invertebrates can develop tolerance to low iron concentrations (Klerks & Weis, 1987; Maltby & Crane, 1994). In addition, benthic macroinvertebrate taxa that are tolerant to organic enrichment (i.e., eutrophication) may be highly tolerant to iron pollution (Rasmussen & Lindegaard, 1988). Therefore, benthic macroinvertebrates within my mesocosms may not have been affected by direct iron toxicity as a result of iron application both because the taxa present were pollution-tolerant, and because total iron concentrations did not exceed those in which significant effects have been observed.

Acidification is likely the most severe risk of iron treatment to aquatic biota, but the short acid-pulse that occurred in my treatment following Fe application likely had minimal effects on the macroinvertebrates. The close proximity of benthic macroinvertebrates to the sediments may have protected them because of the high buffering capacity of the sediments (Havas & Rosseland, 1995). Vertical water-quality profiles support the idea that the sediments may have provided a buffer, because the pH at 4 m (6.24) was much higher than at the surface water pH (5.55) on the first day after iron application. It seems likely that the sediments quickly buffered the acid pulse, preventing the pH from dropping to a level in which impacts are observed (e.g., Barmuta *et al*., 1990; Griffiths, 1992; Orendt, 1999). The presence of molluscs and crustaceans in

all iron treatments also indicates that acidification effects were minimal because these organisms are particularly acid-sensitive (Malley, 1980).

Aluminum (Al) toxicity on freshwater biota is a secondary effect of acidification, but rather than being an in-situ effect, this increase in aluminum toxicity is often a result of acid deposition causing aluminum to leach from the watershed into the acidified lake (reviewed in Havas & Rosseland, 1995). Therefore, aluminum toxicity would not be expected during an in-situ iron application because the acid-pulse occurs within the lake only. My data support this because aluminum concentrations within the mesocosms did not change as a result of iron application and remained very low throughout the duration of the experiment (< 0.014 mg L^{-1} Al; Table A.2; Appendix A), which is much lower than the Al concentrations deemed to be hazardous to aquatic life (e.g., BC water quality criteria for aquatic life < 0.1 mg L^{-1} at pH > 5.5; BC Ministry of Environment, 1988)

In addition to effects from acidification and iron toxicity, I expected oligotrophication-induced changes in the macroinvertebrate assemblages to occur. Previous studies investigating benthic macroinvertebrate responses to reductions in nutrient loading have shown that the abundance of hypereutrophic-adapted chironomid and oligochaete taxa decreases (Köhler *et al*., 2005), and richness and diversity of zoobenthos increases (Mastrantuona & Sforva, 2008). The lack of detectable change in benthic macroinvertebrate abundance and assemblage structure indicates a response to oligotrophication was not detected in this study. However, because of the long generation time of many benthic species, it is possible that such changes could not be captured within the relatively short duration of this study.

Lower nutrient concentrations as a result of iron addition would also be expected to be negatively associated with total dipteran emergence because emergence is often positively correlated with higher nutrient concentrations (McCarty, 1997) and with higher chl-*a* concentrations (Davies, 1980; Welch, 1988a; Blumenshine *et al*., 1997). However, I found that the highest iron treatment with the lowest nutrient and chl-*a* concentrations had the highest

number of insects emerge. This increase in emergent insects may have resulted from greater availability of resources in the benthic zone. Increased water transparency as a result of from iron treatment may have provided more resources by increasing primary productivity in the benthic zone. Also, the initial flocculation drastically decreased phytoplankton (Orihel *et al*., in prep) and zooplankton (discussed in Chapter 2) abundance and biomass within the water column. Therefore, the larval chironomids developing over the spring and summer may have benefitted from an increased energy supply from decreased zooplankton grazing and the increased sedimentation. Jeppesen *et al*., (2007) also suggest that inverse relationships exist in invertebrate biomass in benthic and pelagic habitats. Alternatively, the treatment may have delayed the emergence, but due to the temporal gap between iron application and the trap installations we are unable to quantify this delay.

Oligotrophication would also be expected to increases zoobenthos species diversity (Donohue *et al*., 2009) due to increased macrophyte growth and habitat heterogeneity. Because macrophyte abundance, cover, and richness take several years to increase after nutrient reduction (Jeppesen *et al*., 2005), and minimal macrophyte presence within the mesocosms, it is unlikely that macrophytes (and habitat complexity) increased within this study. On the contrary, the increased suspended sediment loading due to the accumulation of ferric precipitates likely reduced habitat heterogeneity. Due to the high loading of suspended sediments in this hyper-eutrophic lake, the sediments are very loose and are quite similar in particle size and texture to the floccutated iron that accumulated at the water-sediment interface (Wilson, pers. obs.). Subsequently, the precipitates likely did not affect habitat substrate heterogeneity or quality. However, tube-building chironomids may have benefited from the precipitates because many of the chironomids sampled from the highest-treated mesocosm had tubes built out of the iron floc (Wilson, pers. obs.), but I was unable to determine if the precipitates were selected for over the natural sediments to build these tubes. Significant changes in the macrozoobenthos community as a result of increased habitat complexity would likely not occur for several years after continual nutrient

reductions because this shift from phytoplankton- to macrophyte-dominated stable states is often gradual (Jeppesen *et al*., 2005).

APPLICATION OF MESOCOSM RESULTS TO NATURAL LAKES

Comparisons between lake and mesocosm controls

Comparing the macroinvertebrate assemblages from the control mesocosms to the lake reference assemblages allows us determine whether the mesocosm enclosures affected them, and hence the degree to which I can safely extrapolate from mesocosms to the lake environment. The abundance, community composition, and emergence patterns of the emergent insect assemblages were much different between the lake reference sites and the control mesocosms, indicating that there was a strong mesocosm effect influencing the emergent insect assemblages exposed to my treatment. Assemblages within the mesocosms were dominated by *C. plumosus*. This chironomid species is very tolerant to both acidification and hypoxia (reviewed in Armitage *et al*., 1995), which are possible adverse effects of the iron treatment, whereas the lake reference assemblages had much lower abundances of this tolerant species. Because *P. abortiva* is more commonly associated with rooted macrophytes in the littoral zone than with the profundal zone (Armitage *et al*., 1995), it is possible that this species drifted from the littoral zone during emergence, causing the lake assemblages that I captured to have a much higher proportion of this species than assemblages within the mesocosms. These differences limit my conclusions on the safety of iron as a remediation technique and further research is needed to determine how ferric chloride affects the entire emergent insect community at a larger scale.

Zoobenthos within the control mesocosms were much more similar to the lake reference site than were the emergent insect assemblages, but total number of sampled larval dipterans was lower in the mesocosms than the lake. Estimates of larval insect abundance are certain to be low due to the sampling constraints. Most of the larval insects within are Chironomidae, which have a uni- or bivoltine lifecycle in my study area (Clifford, 1991). Most of the univoltine and some of the bivoltine chironomids within the mesocosms would have completed their

lifecycle and emerged as adults during the spring and summer prior to zoobenthos collection. The mesocosms may not have been replenished with eggs from the newly emerged adults because of the 30 cm upright splashguard surrounding the mesocosms (Fig. 2.2). In addition, the chironomid larvae would have been in the early, small instars during my sampling so they may have passed through the sieve or been missed during sample processing, which also contributes to the low dipteran larvae in the samples. Compositional differences between the lake and mesocosms in both the zoobenthos and emergent insect assemblages would also be expected because the organisms retained when the mesocosm walls were dropped into the sediments would influence the assemblages sampled in the mesocosms from that point forward.

Extrapolating to whole lake ecosystems

Although the lack of significant responses in abundance, biomass, and community composition in my study suggests that iron application does not have detrimental effects on freshwater macroinvertebrate assemblages, the constraints of the study scale (both temporal and spatial) must be considered before advocating whole-lake application. For example, I did not want to disturb the sediments during the experiment so zoobenthos sampling was very limited. Zoobenthos were not sampled until the termination of the experiment, which did not allow any initial effects that occurred directly after treatment to be captured. In addition, long-term responses were not investigated. Although the absence of effects 9 months after treatment indicates that any initial effects were short term, long-term effects were not investigated and the long generation time of many macroinvertebrates may have suppressed the expected responses. For example, nutrient enrichment studies often do not detect changes in zoobenthos until several years after nutrient addition (e.g., Hershey *et al*., 1992); thus, effects from oligotrophication may not have been captured within this study.

There were also constraints that restricted emergent insect sampling because there was a gap in sample collection. Because I did not want any devices hanging within the water column inhibiting the iron precipitate from naturally

falling to the sediments, there was a delay in the installation of the submerged funnel traps of almost three weeks after iron application. As a result, the emergence that occurred after ice out was not captured (Wilson, pers. obs.) and I am unable to determine if the high number of insects captured from the highest iron treatment was due to a delay in emergence. Because the period of emergence is one of the most sensitive life stages of insects to acidification (Armitage *et al*., 1995), the timing of iron application could have detrimental effects on emergent insects and their prey that rely on spring emergence events (e.g., McCarty, 1997). Further research is needed to determine whether the high numbers of chironomids emerging at the highest iron dosage was a result of that dosage rather than just being an anomaly, as well as the effect of iron treatment on insect pupae to ensure that iron is applied at the optimal time to minimize effects on emergence.

Given that the mesocosms were located in the limnetic zone of the lake, the iron treatment was only applied to the macroinvertebrate assemblages associated with the benthic zone of this habitat. Littoral macroinvertebrates could be much more sensitive than the assemblages I assessed. The pollution- and hypoxia-tolerant taxa present within the sampled zoobenthos and emergent insect assemblages likely contributed to the lack of effects of the iron treatment on these assemblages. In addition to the temporal and spatial constraints of my study, the responses of other assemblages (e.g., benthic meiofauna, macrophytes, fish, etc.) to iron application also need to be taken into consideration when extrapolating these results to a whole lake.

General conclusions and recommendations

There were minimal differences in the abundance, biomass, and community composition of zoobenthos and emergent insect assemblages in relation to iron application. Because the negative effects of iron precipitates are similar to stresses imposed from nutrient enrichment, the presence of taxa moderately to extremely tolerant of organic pollution within my mesocosms decreased the sensitivity of macroinvertebrate assemblages to the iron application. Littoral macroinvertebrates may be more sensitive than those in the profundal, so

before whole-lake treatments are carried out experimental studies on iron effects on littoral assemblages are needed. Furthermore, the temporal scale of this study limited the assessment of long-term (>9 months) responses within the macroinvertebrate communities. If iron treatment is able to sustain long-term reductions in nutrient and phytoplankton concentrations, I expect the macroinvertebrates to respond to these changes. Increased water transparency would be expected to impact the zoobenthos by allowing photosynthesis to occur at lower depths, thereby increasing benthic primary productivity. Because there was a strong mesocosm effect that did not allow us to assess how iron application affects more sensitive emergent insect taxa, and there was an anomalously high emergence in the highest iron treatment, further research is recommended to determine the full effects of iron application in emergent insects before a whole-lake application is performed.

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Table 3.1. Average, minimum, and maximum abundance (no. m^{-2}), biomass (mg dw m^{-2}), richness, diversity (Shannon H'), evenness (Pielou's J), and Welch's t-test summary statistics of emerged insects and zoobenthos in the lake reference and control sites.

		Lakes			Controls					
	Analysis	**Min.**	**Max.**	**Ave.**	**Min.**	**Max.**	**Ave.**	**p-value***	**df****	**t**
Emergent insects										
	Abundance	1,338	3,878	2,499	326	674	472	0.109	2.079	2.708
	Biomass	310	827	516	164	388	284	0.280	2.655	1.353
	Richness	11	12	11	4	7	5	**0.012**	2.560	6.364
	Diversity (H')	1.01	1.79	1.48	0.89	1.56	1.12	0.333	3.976	1.102
	Evenness (J)	0.41	0.74	0.61	0.55	0.80	0.67	0.654	3.592	-0.488
Zoobenthos										
	Abundance	-----	-----	11,234	2,993	9,192	6,268	-----	-----	-----
	Biomass	-----	-----	5.86	1.48	1.91	2.02	-----	-----	-----
	Richness	-----	-----	15	11	16	13	-----	-----	-----
	Diversity (H')	-----	-----	1.48	0.89	1.56	1.12	-----	-----	-----
	Evenness (J)	-----	-----	0.80	0.77	0.80	0.79	-----	-----	-----

*Significant differences at $\alpha = 0.05$ are in bold.

**Degrees of freedom (df) are not equal because they were approximated using the Welch correction equation.

Table 3.2. Raw abundance (no. m $^{-2}$) of emergent insect taxa and total no. of organisms from the mesocosms and lake reference sites. Taxonomic abbreviations used in the ordination (NMS) analysis, taxa frequency (%), taxa average (mean no. m^{-2}) are also given. Submerged emerging insect traps were installed from July 1 to September 15, 2009.

	NMS	Site abundance (no. m^{-2})																		Freq.	Mean
	abbrev.	L01	L02	L03	C01	C02	C03	2.3	3.4	5.2	7.9	12	18	28	42	64	97	148	225	(%)	(no. m^{-2})
Ephemeroptera																					
Caenidae																					
Caenis spp.	—	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	6	1
Diptera																					
Ceratopogonidae																					
Ceratopogoninae spp.	—	0	11	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	11	1
Chaoboridae																					
Chaoborus spp.	CHAO	146	416	101	34	34	0	11	0	11	0	79	11	34	0	0	0	0	11	61	49
Chironomidae spp.	—	124	371	281	0	11	11	0	0	0	22	11	0	11	11	0	11	0	0	56	48
Chironominae spp.	—	0	0	45	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0	11	3
Chironomini																					
Chironomus pilicornis Fabricius	CMUS	11	0	0	0	56	0	11	0	0	0	0	0	0	0	0	0	0	0	17	4
Chironomus plumosus Linnaeus	CMUS	67	45	0	214	202	90	495	79	135	45	34	45	45	315	22	11	202	708	94	153
Dicrotendipes nervosus Staeger	CMNI	0	0	45	0	0	0	0	0	11	0	0	0	0	0	11	0	0	0	17	4
Endochironomus subtendens Townes	CMNI	0	0	124	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	7
Glyptotendipes spp.	CMNI	0	0	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	1
Harnischia complex	CMNI	11	0	11	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	17	2
Parachironomus abortivus Malloch	PABT	247	630	2574	34	146	0	34	0	22	169	45	0	180	0	157	0	0	0	61	235
Tanytarsini																					
Cladotanytarus viridiventris Malloch	TANY	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	1
Paratanytarsus spp.	TANY	34	34	11	0	11	0	0	0	0	0	0	0	0	0	0	11	0	0	28	6
Tanytarsus spp.	TANY	416	382	382	22	191	281	124	11	79	281	22	259	90	247	304	79	146	618	100	219
Orthocladiinae spp.	ORTH	79	169	101	22	11	0	0	0	11	11	22	0	0	11	34	0	11	11	67	27
Corynoneura spp.	ORTH	11	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0	11	1
Cricotopus spp.	ORTH	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	6	1
Eukiefferiella spp.	ORTH	34	22	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17	4
Krenosmittia spp.	ORTH	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	1
Psectrocladius spp.	ORTH	11	22	22	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0	22	4
Tanypodinae																					
Ablesmyia spp.	TPOD	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	1
Procladius spp.	TPOD	146	157	124	0	11	11	0	0	0	0	45	11	0	0	22	0	0	0	44	29
Psectrotanypus spp.	TPOD	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0	0	0	0	6	1
TOTAL		1338	2282	3878	326	674	416	697	90	270	528	270	337	360	585	573	112	360	1349	---	---

Table 3.3. Regression summary statistics for the abundance (no. m^{-2}), biomass (mg dw m^{-2}), richness, diversity (Shannon H'), and evenness (Pielou's J) of zoobenthos and emerged insects in relation to iron treatment. There were no significant relationships at $\alpha = 0.05$.

	Analysis	p-value	r2	F-stat	Slope	Int.	df
Emergent insects							1,11
	SQRT (abundance)	0.403	0.064	0.756	2.541	17.110	1,11
	Log (total biomass)	0.921	0.001	0.010	0.041	5.159	1,11
	Richness (no. taxa)	0.129	0.197	2.690	-1.090	5.505	1,11
	Diversity (H')	0.386	0.069	0.816	-0.121	1.086	1,11
	Evenness (J)	0.319	0.090	1.091	0.074	0.630	1,11
Zoobenthos							1,11
	Log (abundance)	0.258	0.115	1.424	0.213	5.710	1,11
	Total biomass	0.505	0.041	0.476	468	2378	1,11
	Richness (no. taxa)	0.989	0.000	0.000	0.009	12.526	1,11
	Diversity (H')	0.382	0.070	0.829	-0.054	2.058	1,11
	Evenness (J)	0.425	0.059	0.687	-0.021	0.872	1,11

Table 3.4. Multiple response permutation procedure (MRPP) summary statistics from the non-metric multidimensional scaling (NMS) ordination analysis performed on log (*x*+1) transformed emergent insect abundance (no. m^{-2}). Groups compared (defined *a priori*) are lake (3 sites), controls (3 sites), low (2.3, 3.4, 5.2, and 7.9 g Fe m^{-2}), medium (12, 18, 28, and 42 g Fe m^{-2}), and high (64, 97, 148, and 225 g Fe m^{-2}) and all groups. T = test-staistic; A = within group agreement (measure of variance). Corresponding NMS ordination is displayed in Figure 3.6.

Group comparisons			p-value*	T	A
All groups			0.060	-1.671	0.107
Lake	vs	Controls	**0.022**	-2.889	0.268
Lake	vs	Low	**0.009**	-3.463	0.327
Lake	vs	Medium	**0.017**	-2.945	0.221
Lake	vs	High	**0.026**	-2.604	0.314
Control	vs	Low	0.634	0.402	-0.037
Control	vs	Medium	0.953	1.302	-0.130
Control	vs	High	0.576	0.342	-0.027
Low	vs	Medium	0.638	0.411	-0.022
Low	vs	High	0.678	0.572	-0.041
Medium	vs	High	0.661	0.556	-0.038

*Significant p-values at $\alpha = 0.05$ are in bold; α not adjusted for multiple comparisons.

Table 3.5. Raw abundance (no. m^{-2}) of zoobenthos taxa and total no. of organisms from the lake reference and mesoscosms. Taxa frequency (%) and taxa average (mean no. m^{-2}) is also given. Three Ekman grabs were taken just before termination of the experiment in March 2009.

	Site abundance (no. m^{-2})																Freq.	Mean
	L01	C01	C02	C03	2.3	3.4	5.2	7.9	12	18.3	27.7	42.2	64.1	97.4	148	225	(%)	(no. m^{-}
Hydrozoa																		
Hydra sp.	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0	0	6	1
Oligochaeta																		
Naididae spp.	590	295	520	190	1,279	105	1,096	1,307	98	443	654	1,729	1,560	801	590	309	100	669
Dero digitata (Muller)	0	0	0	28	14	0	0	42	0	14	63	0	295	0	105	0	41	33
Gastropoda																		
Physidae																		
Physa spp.	126	0	0	0	0	0	0	0	0	0	28	0	0	0	0	0	12	7
Planorbidae																		
Gyraulus spp.	1,054	1,687	450	0	0	0	485	63	253	28	0	0	0	0	0	0	41	216
Valvatidae																		
Valvata sincera helicoidea (Dal)	252	0	576	28	0	63	211	63	98	148	84	169	42	63	0	14	82	102
Valvata tricarinata (Say)	252	42	141	0	0	28	126	0	197	63	0	84	21	105	0	28	71	59
Pelecypoda																		
Sphaeriidae spp.	675	1,644	759	738	478	295	84	907	337	1,012	1,202	1,883	2,762	632	1,919	1,237	100	961
Hydrachnidia																		
Limnesiidae																		
Limnesia sp.	0	14	14	0	14	0	14	28	0	14	0	0	63	42	0	14	53	13
Pionidae																		
Piona spp.	611	485	829	169	632	422	401	970	351	253	864	857	696	696	274	394	100	512
Unionicolidae																		
Neumania sp.	63	0	14	0	0	0	0	14	28	0	0	0	0	0	0	14	29	7
Copepoda																		
Cyclopoida spp.	2,635	295	1,349	885	703	105	696	590	1,068	1,075	401	14	759	316	1,413	1,026	100	733
Harpacticoida sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	14	0	0	6	1
Ostracoda																		
Candonidae*	126	105	211	0	56	0	0	63	14	0	190	197	28	63	42	28	71	64
Candona sp.	991	379	2,347	148	464	738	654	1,623	1,715	843	1,075	745	1,560	1,771	928	267	100	936
Cyclocypridae																		
Cyclocypris sp.	485	991	1,504	28	70	84	190	190	197	253	759	0	8,539	611	190	28	94	821
Amphipoda																		
Hyalella azteca (Saussure)	21	0	141	0	14	0	0	105	0	0	0	0	0	0	0	0	24	16
Gammarus lacustris (Sars)	0	0	0	0	0	0	0	0	0	0	0	28	0	0	0	0	6	2
Insecta																		
Ephemeroptera																		
Caenis spp.	0	0	42	0	0	0	0	0	14	0	0	0	169	14	0	0	24	14
Diptera																		
Ceratopogoninae spp.	401	611	225	632	379	611	401	569	169	611	675	562	780	274	949	478	100	482
Chironomidae spp.	2,952	63	70	148	211	0	569	401	56	84	105	281	337	253	379	267	94	305
Stratiomyidae																		
Nemotelus sp.	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	6	1
TOTAL	11,234	6,613	9,192	3,007	4,315	2,467	4,926	6,936	4,596	4,842	6,100	6,550	17,611	5,657	6,789	4,104	---	---

*Candonidae species other than *Candona*; Candonidae taxa identifications according to Clifford, 1991.

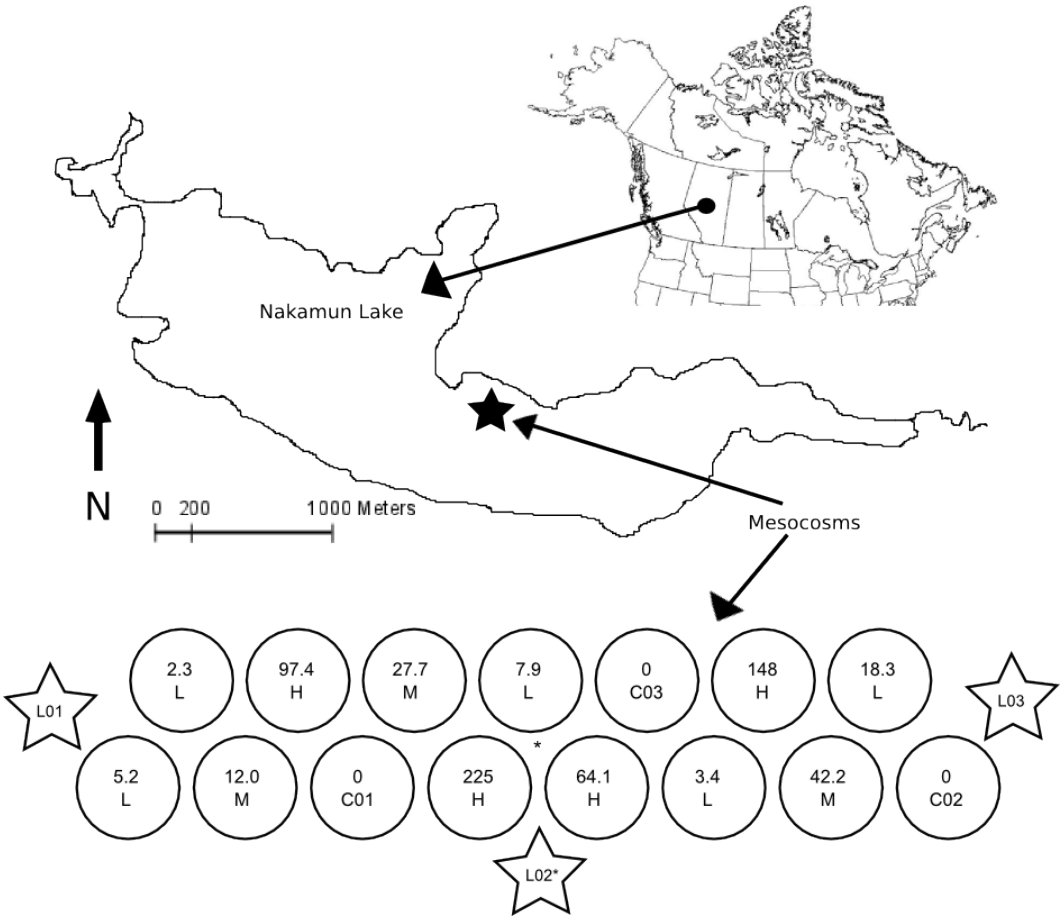

Figure 3.1. Map of study site and a pictorial representation of the experimental set-up. Experimental mesocosms were installed in a sheltered bay near the north shore of Nakamun Lake, at a depth of 4.5 m. Mesocosms are depicted with open circles and lake reference locations are depicted with open stars. Iron treatment levels are in g m^{-2} and the treatment group is indicated below as controls (C01, C02, and C03), low (L), medium (M), or high (H). *The site for the second lake reference (L02) for the emergent insect traps is indicated with an asterisk (*) in the centre of the mesocosms. Zoobenthos lake reference samples were collected at site L01 only.

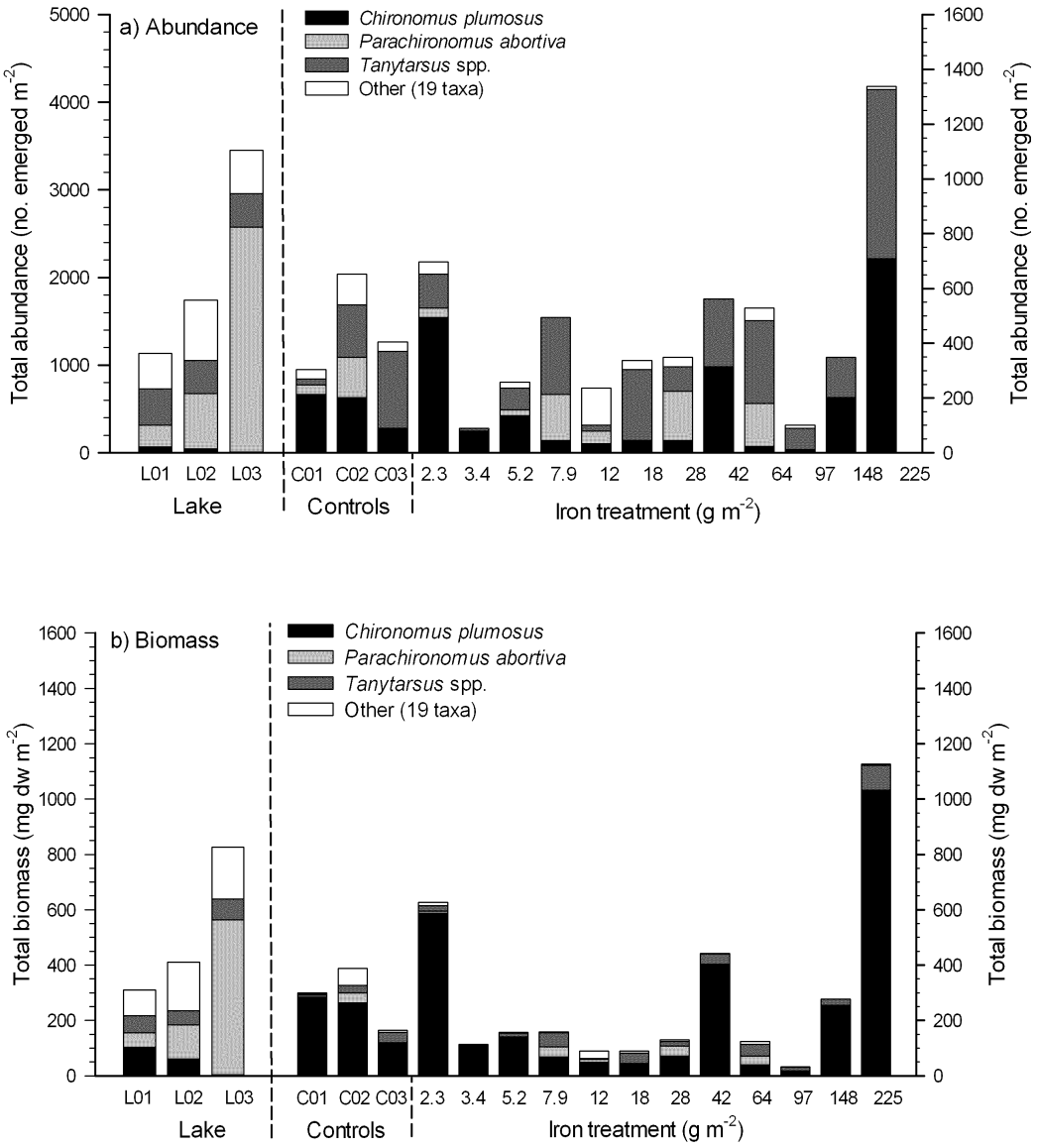

Figure 3.2a,b. Total abundance (no. m^{-2}; top panel) and biomass (mg dw m^{-2}; bottom panel) of emerged insects captured from submerged funnel traps installed from July 1 to September 15, 2009 in the lake and the iron treatments. The proportion that the three most common taxa, *Chironomus plumosus* (black), *Parachironomus abortiva* (light grey), and *Tanytarsus* spp. (dark grey) are displayed; the other 19 taxa are grouped (white). Note that the scale of the y-axis is greater for the abundance within the lakes (top left panel) compared to the iron treatments (top right panel).

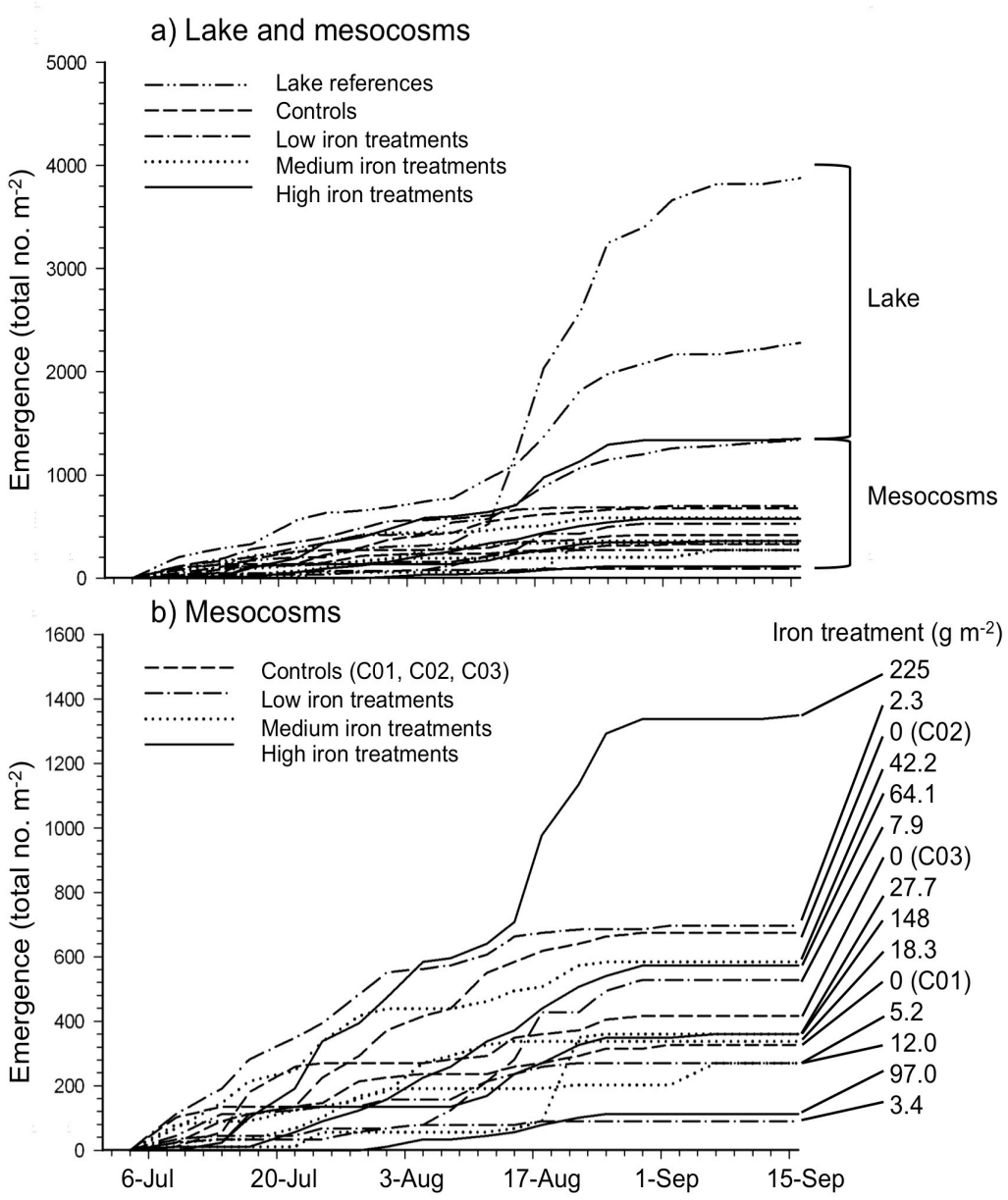

Figure 3.3a,b. Cumulative total (no. m^{-2}) of emerged insects captured from submerged funnel traps installed from July 1 to September 15, 2009 in all sites (top panel) and mesocosms only (bottom panel). More insects emerged from the lake sites than within the mesocosms, with the exception of the highest iron treatment (225 g m^{-2}). Emergence ended around mid-August for most mesocosms, but the high-iron treatment and lake reference sites had high emergence in the last 2 weeks of August. There was no pattern between emergence and iron treatment.

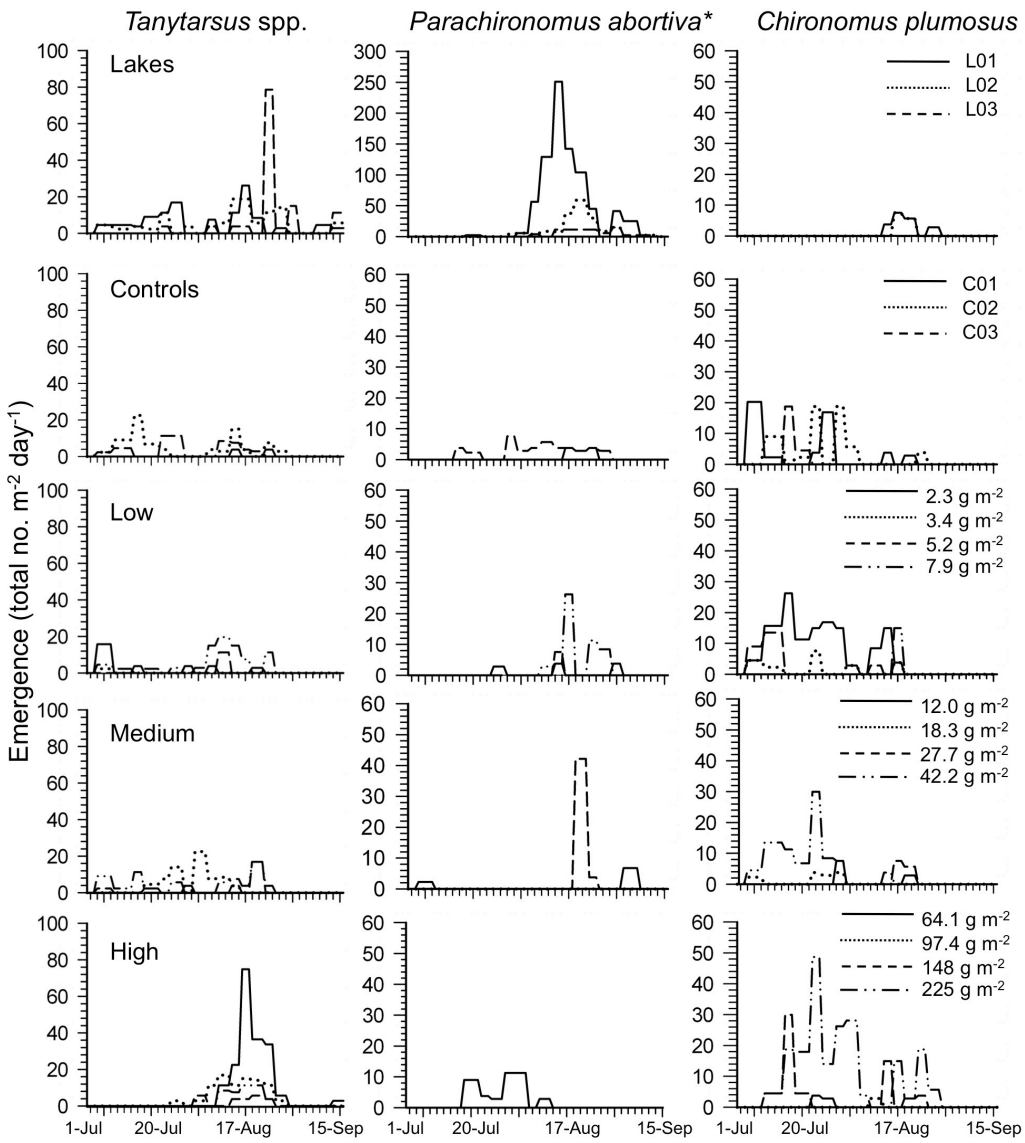

Figure 3.4. Temporal emergence rates (no. m^{-2} day^{-1}) of the three dominant emergent insect species; *Tanytarsus* spp. (left panels), *Parachironomus abortiva* (middle panels), and *Chironomus plumosus* (right panels) from the lake (top panels), control (second panels), low (third panels), medium (fourth panels) and high (bottom panels) sites. Emergence rates of *Tanytarsus* spp. were highest in August in the lake references and highest treatment site. *P. abortiva* also had high emergence rates in the lake references later in August, but the timing of emergence was variable among iron treatment groups. *C. plumosus* had much higher emergence rates throughout July, August, and September in the mesocosms than the lake, and only emerged from the lake at low rates in late summer. *The scale of the y-axes of *P. abortiva* in the lake sites (top middle panel) is 5 times greater than the other *P. abortiva* panels; y-axes scales are the same within the other taxa.

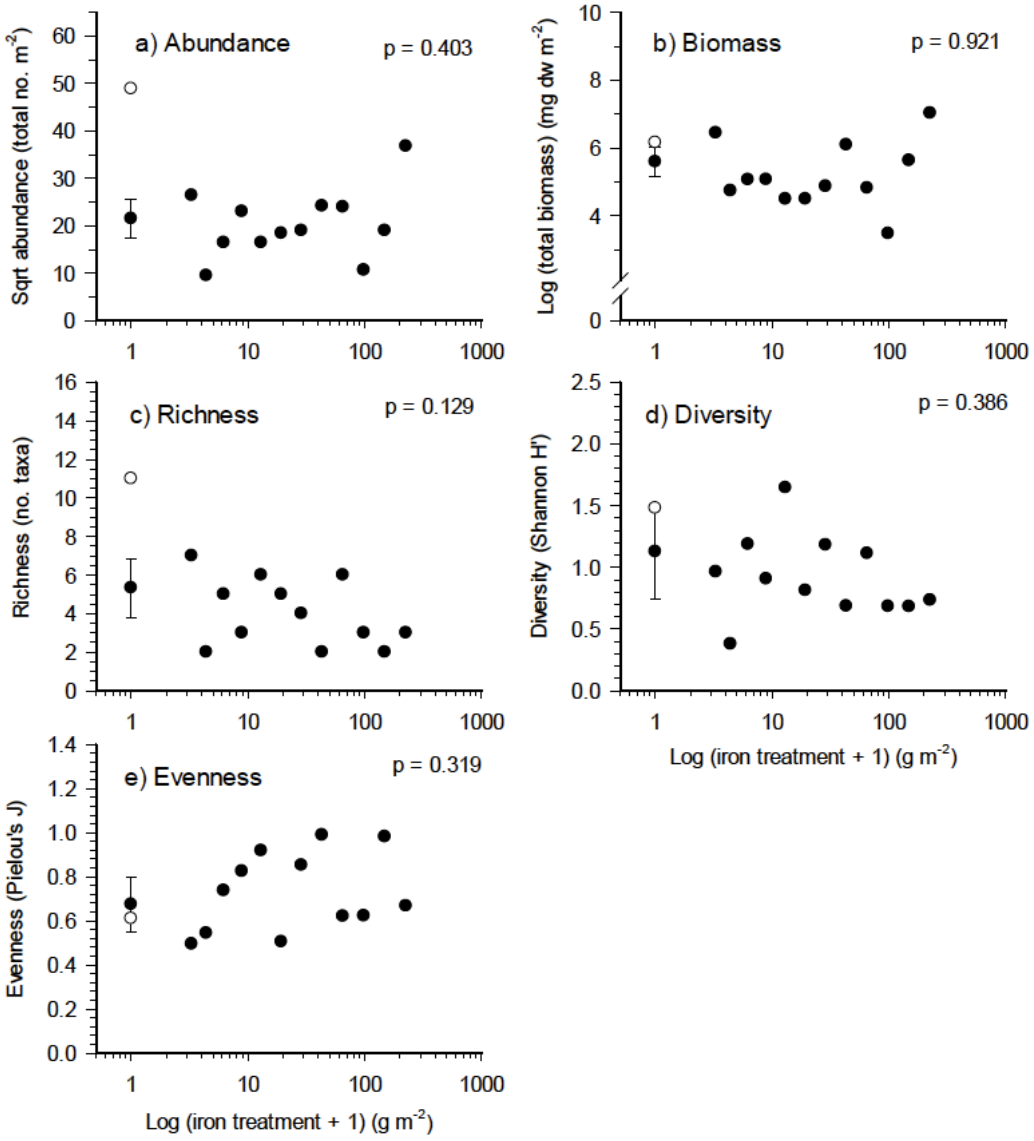

Figure 3.5. Linear regression analyses for the total abundance (no. m^{-2}), biomass (mg dw m^{-2}), richness (no. taxa), diversity (Shannon H'), and evenness (Pielou's J) of emerged insects in relation to iron treatment. The three control treatments were averaged and all other treatments were single-doses; therefore, standard error is associated with the control treatments only. Regressions analyses were not significant; summary statistics are given in Table 3.4. The mean lake reference is shown with open circles, although the lake reference sites were not included in the regression analyses. Emerged insects were captured with submerged funnel traps which were installed from July 1 to September 15, 2009.

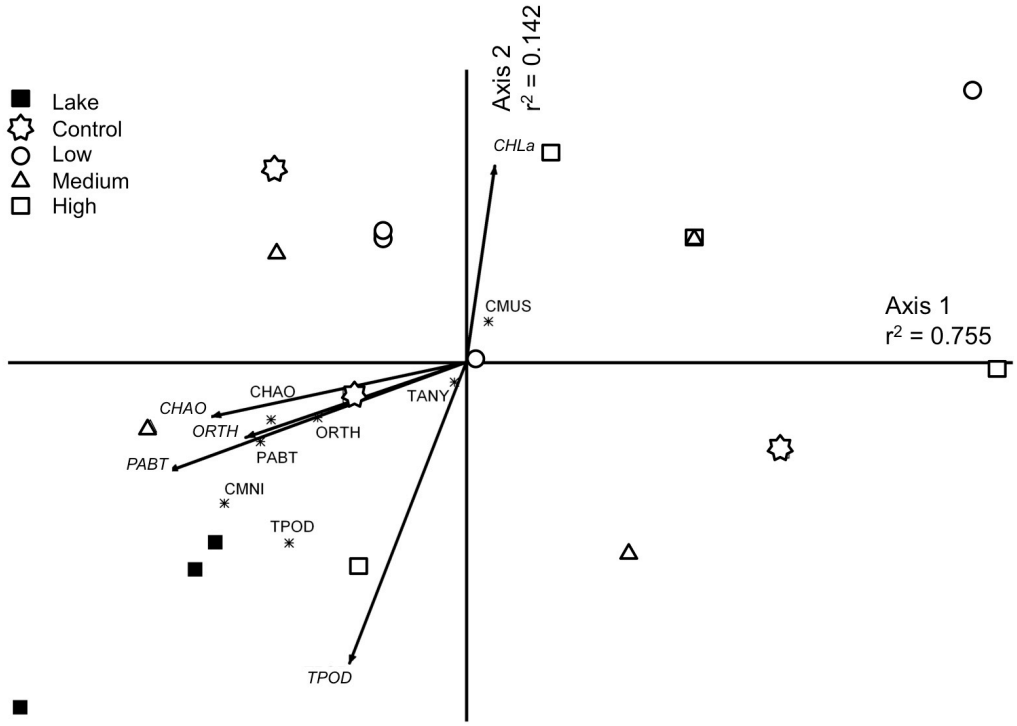

Figure 3.6. Non-metric multidimensional scaling (NMS) ordination of emerged insect abundances. Emergent insects included in the ordination comprised > 1% of the total abundance and data was log (*x*+1) transformed prior to analysis. Significant vectors (italicized) included had a $r^2 \geq 0.5$ and were scaled to 80%. The only highly correlated environmental vector was chlorophyll-*a* (*CHLa*), which was negatively correlated with the lake reference sites. *Chironomus plumosus* was associated with the mesocosm sites and the other taxa were associated with the lake sites. Taxonomic abbreviations are described in Table 3.2. Groups were defined *a priori* as lake references, controls, low (2.3, 3.4, 5.2, and 7.9 g Fe m^{-2}), medium (12, 18, 28, and 42 g Fe m^{-2}), and high (64, 97, 148, and 225 g Fe m^{-2}) iron treatments. Multiple response permutation procedure (MRPP) statistics indicated that the lake reference assemblages were different than other groups. MRPP summary statistics are given in Table 3.4.

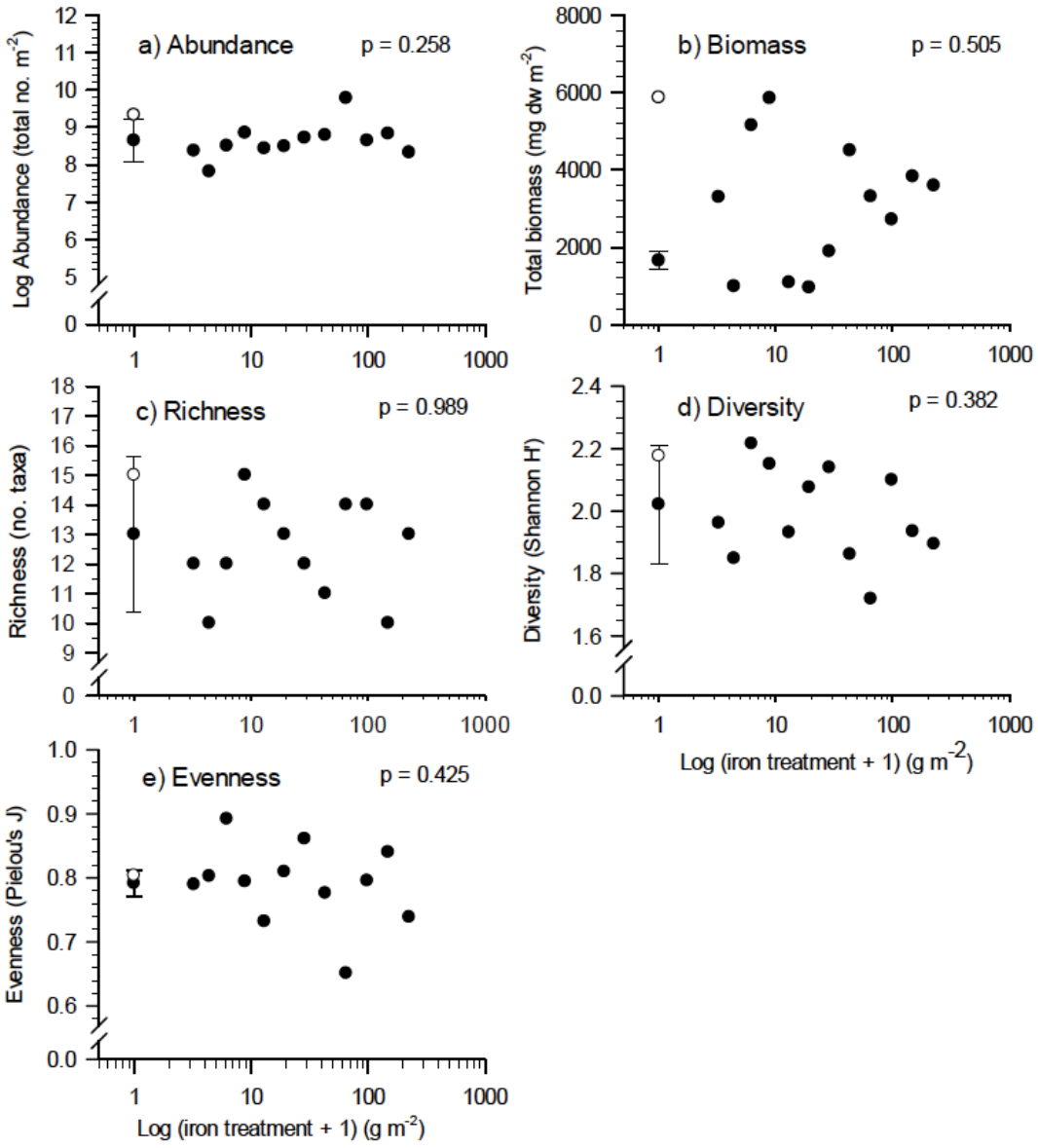

Figure 3.7. Linear regression analyses for the total abundance (no. m^{-2}), biomass (mg dw m^{-2}), richness (no. taxa), diversity (Shannon H'), and evenness (Pielou's J) of zoobenthos in relation to iron treatment. The three control treatments were averaged and all other treatments were single-doses; therefore, standard error is associated with the control treatments only. Regressions analyses were not significant; summary statistics are given in Table 3.4. The mean lake reference is shown with open circles but the lake reference sites were not included in the regression analyses.

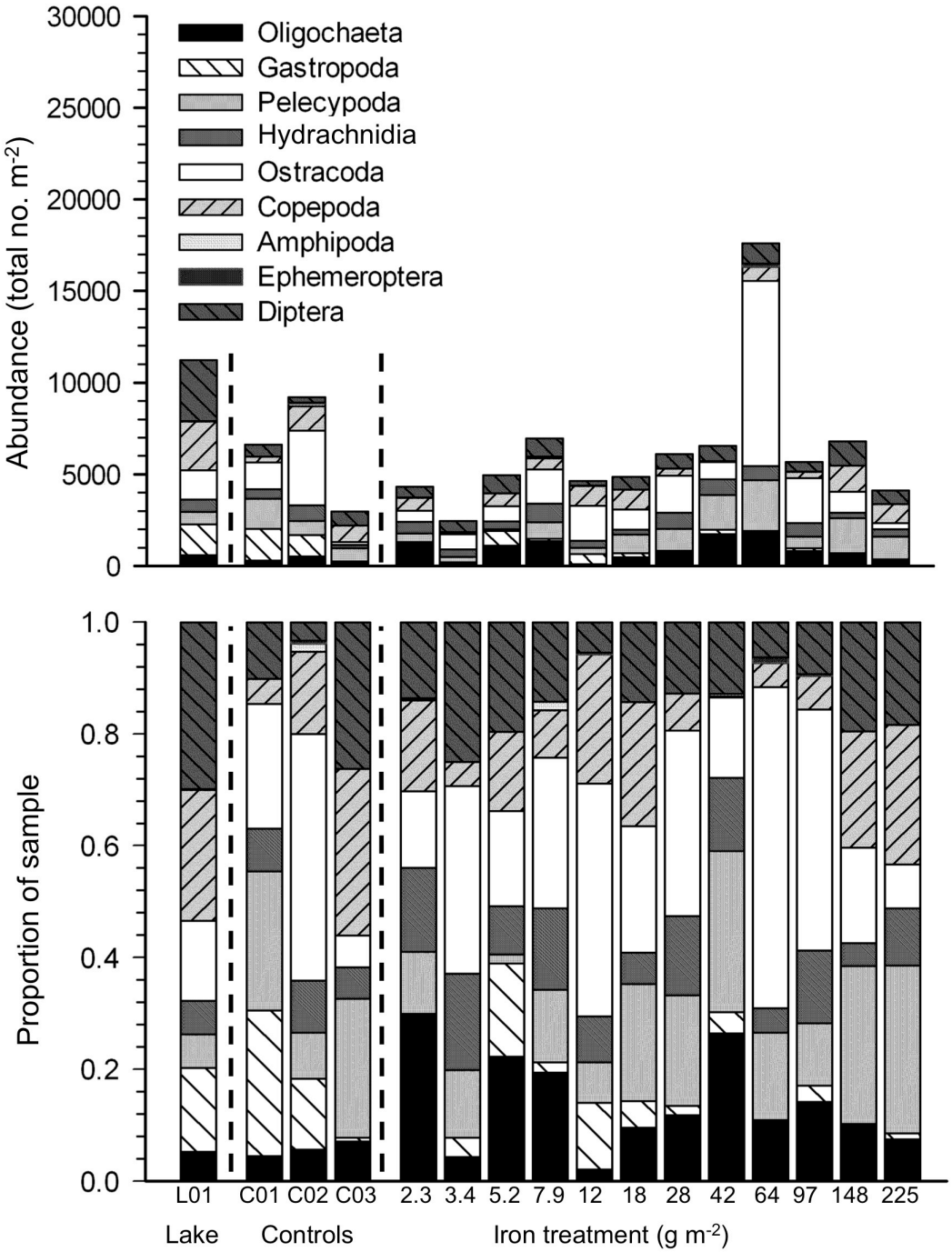

Figure 3.8. Total abundance (no. m^{-2} ; top panel) and relative proportion (bottom panel) of taxa in the zoobenthos assemblages from the lake reference site and mesocosms. Composite samples from three Ekman grabs were taken just before termination of the experiment in March 2010. Total and relative abundance of zoobenthos taxa were variable and not related to iron treatment.

Chapter 4: General conclusions

To date, this is the most detailed study investigating the response of aquatic invertebrate assemblages to the application of iron salts, which are sometimes used to rehabilitate eutrophic water bodies. The most adverse effects of ferric chloride application to aquatic invertebrate assemblages that I observed were the significant declines in both total zooplankton abundance and biomass immediately following high rates of iron application (within 4 days). Similar studies with aluminum or iron salts have concluded that zooplankton abundance and/or biomass decreases following in-situ phosphorus inactivation treatments as a result of reductions in phytoplankton biomass (Schumaker *et al*., 1993; Holz &Hoagland, 1996; Wold *et al*., 2005) and of zooplankton co-precipitation with flocculants (Wold *et al*., 2005). Because of the rapid drop in pH during iron application in this study, acidification likely contributed to the observed declines in zooplankton abundance and biomass, in addition to reductions in phytoplankton, and co-precipitation of plankton with the iron floc as it settled. However, the adverse response to iron application was short-lived and it appeared that zooplankton assemblages had recovered within one month of iron application because by that time there was no longer a relationship between iron treatment level and either zooplankton abundance or biomass. There was substantial seasonal variation in zooplankton assemblages, but these differences were likely caused by variations in predation pressure, changes in phytoplankton composition, and the differences among initial biotic communities, rather than directly by the iron application.

There were minimal differences in the abundance, biomass, and community composition of macroinvertebrates (both zoobenthos and emergent insect assemblages) in relation to iron application. However, there are no other published *in-situ* studies directly investigating community-level responses of macroinvertebrates to iron treatment to aid in the comparison and interpretation of my results. Because the negative effects of iron precipitates are similar to stresses imposed from nutrient enrichment (Rasmussen & Lindegaard, 1988) the natural presence of taxa moderately to extremely tolerant of organic pollution within the

mesocosms likely decreased the sensitivity of macroinvertebrate assemblages to the iron application. The high buffering capacity of the sediments may have acted as a refuge from acidification effects for the benthos (Havas & Rosseland, 1995), also suppressing adverse effects of iron application. The large number of insects that emerged from the highest-treated mesocosm was not expected because increased emergence is generally associated with increased phytoplankton biomass (Davies, 1980; Welch *et al*., 1988; Blumenshine *et al*., 1997), but phytoplankton biomass was lowest in the high-dosed mesocosm (Orihel *et al*., in prep). Further research may help determine if this response was an anomaly or if it was truly related to iron application.

Although other studies indicate that zooplankton (Jeppesen *et al*., 2005; Moss *et al*., 2005; Phillips *et al*., 2005) and zoobenthos (Köhler *et al*., 2005; Mastrantuono & Sforza, 2008) assemblage composition change in response to nutrient reductions (oligotrophication), the results of this study did not indicate any clear response of aquatic invertebrate assemblages to oligotrophication. The temporal scale of my study may not have been adequate to capture oligotrophication effects, or the influence of the mesocosms themselves may have concealed these effects.

Overall, the results of this study indicate that ferric chloride application minimally impacts zooplankton and zoobenthos assemblages in the pelagic and profundal zones of this shallow, hypereutrophic lake. However, there were notable mesocosm effects that did not allow the full assessment of how iron application affects all invertebrate taxa. Before a whole-lake application is performed, further research is recommended to determine the full effects of iron application on the aquatic biota not investigated within this study, particularly, the emerging insect taxa that were not retained in the mesocosms, fish assemblages, and biota in the littoral zone. Further research may also recognize non-lethal effects of iron application on aquatic invertebrates that may have gone undetected in this study, such as lower individual fecundity, decreased condition, or changes in behaviour.

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Appendix A: Water chemistry tables

Table A.1. Historic water quality variables for Nakamun Lake[1]. Averages are from composite samples taken in the euphotic zone during the open water season (May - October) in 1982 or 1983. S.E. = standard error.

	1982			1983		
	Mean	S.E.	n	Mean	S.E.	n
Total phosphorus (µg/L)	97	4.1	36	80	11.2	7
Chlorophyll-*a* (µg/L)	54.7	5.37	36	33.8	18.2	7
Secchi depth (m)	0.8	0.07	36	2.1	0.76	7
pH (range in units)	—	—	—	(7.8-9.5)	—	5
Total alkalinity ($CaCO_3$)(mg/L)	—	—	—	148	2.9	6
Total hardness ($CaCO_3$) (mg/L)	—	—	—	92	3.8	6

[1]SOURCE: Mitchell & Prepas (1990) *Atlas of Alberta Lakes*, 1st edn.

Table A.2. Summary of water quality parameters from 12 iron-treated mesocosms, 3 control mesocosms (C01, C02, C03) and one lake reference (L01) from Nakamun Lake, AB. Monthly means were calculated from bi-weekly composite samples taken from the surface to 4 m during the day.

	Iron Treatment (mg Fe m^{-2})	Temp. (C)*	pH*	Dissolved oxygen (mg L^{-1})*	Total nitrogen (μg L^{-1})	Total phosphorus (μg L^{-1})	Iron (mg L^{-1})	Chlorphyll *a* (μg L^{-1})	Calcium (mg L^{-1})**	Alkalinity (mg L^{-1} as CaCO3)**
June	2.3	18.6	8.1	8.9	1545	30	0.21	12.03	-	-
	3.4	18.5	8.4	10.9	1710	53	0.16	23.96	-	-
	5.2	18.4	8.0	8.7	1550	35	0.19	13.04	-	-
	7.9	18.7	7.9	8.8	1485	28	0.18	10.11	-	-
	12	18.4	7.8	8.9	1475	27	0.21	10.39	-	-
	18	18.5	7.6	8.7	1435	23	0.12	10.24	-	-
	28	18.6	7.5	9.0	1395	23	0.23	11.50	-	-
	42	18.5	7.3	8.4	1355	24	0.12	10.22	-	-
	64	18.4	7.1	9.4	1270	20	0.23	11.93	-	-
	97	18.6	6.9	9.3	1195	19	0.31	10.19	-	-
	148	18.5	6.6	8.7	966	17	0.25	7.80	-	-
	224	18.4	6.1	8.7	741	10	0.22	6.15	-	-
	C01	18.5	8.4	10.0	1695	49	0.22	21.94	-	-
	C02	18.6	8.2	8.2	1515	19	0.15	3.13	-	-
	C03	18.5	8.2	9.1	1505	33	0.14	12.03	-	-
	L01	18.6	8.5	9.4	1570	24	0.18	4.25	-	-
July	2.3	20.9	8.6	10.7	1505	35	0.05	13.34	27.5	193.0
	3.4	20.7	8.7	11.9	1780	55	0.01	30.08	24.0	183.6
	5.2	20.7	8.5	10.6	1660	59	0.01	16.17	26.7	188.7
	7.9	20.8	8.4	9.4	1480	26	0.01	9.76	28.6	193.1
	12	21.1	8.6	10.4	1535	33	0.01	15.31	26.7	188.8
	18	20.8	8.3	9.2	1425	22	0.01	6.57	28.9	186.8
	28	20.8	8.4	9.2	1435	25	0.01	13.12	30.2	185.0
	42	20.7	8.2	8.3	1470	21	0.01	1.89	29.8	176.2
	64	20.7	8.3	9.9	1425	18	0.01	9.17	27.7	164.1
	97	20.9	8.3	9.4	1370	19	0.04	8.99	33.3	146.5
	148	20.9	8.2	8.7	1260	25	0.01	16.02	36.7	120.8
	224	20.7	8.1	9.4	1120	14	0.01	11.16	35.6	80.0
	C01	19.7	8.6	11.8	1765	65	0.00	26.27	23.9	188.5
	C02	20.7	8.3	8.1	1555	23	0.01	3.34	26.3	186.4
	C03	20.8	8.6	10.7	1525	37	0.01	8.35	27.9	195.4
	L01	21.2	8.9	9.8	1620	36	0.12	9.95	22.8	176.7

	Iron Treatment (mg Fe m^{-2})	Temp. (C)*	pH*	Dissolved oxygen (mg L^{-1})*	Total nitrogen (μg L^{-1})	Total phosphorus (μg L^{-1})	Iron (mg L^{-1})	Chlorphyll a (μg L^{-1})	Calcium (mg L^{-1})**	Alkalinity (mg L^{-1} as CaCO3)**
August	2.3	18.7	8.8	11.1	1960	95	0.03	39.02	27.1	187.8
	3.4	18.7	8.9	12.0	2320	128	0.00	65.16	19.7	172.5
	5.2	18.7	8.8	11.6	2590	95	0.00	40.88	22.1	174.9
	7.9	18.7	8.6	11.0	1680	69	0.00	37.08	29.4	193.9
	12	18.7	8.6	10.2	2150	69	0.02	38.92	27.9	188.4
	18	18.7	8.4	9.8	1570	39	0.00	14.80	30.7	191.1
	28	18.7	8.4	9.3	1650	53	0.00	28.76	32.6	188.6
	42	18.6	8.3	9.0	1740	63	0.00	32.14	30.9	179.0
	64	18.6	8.4	9.6	1575	25	0.03	3.50	30.7	161.8
	97	18.7	8.3	9.7	1625	41	0.00	32.01	37.1	153.1
	148	18.7	7.9	8.4	1725	40	0.00	29.64	40.2	129.4
	224	18.7	7.8	8.3	1455	19	0.02	13.74	41.4	92.9
	C01	18.7	8.9	11.7	2320	156	0.02	53.98	21.2	177.0
	C02	18.7	8.2	9.0	1655	53	0.00	16.69	27.7	188.8
	C03	18.6	8.8	12.9	1645	61	0.00	23.42	20.8	179.0
	L01	18.7	8.9	11.3	2310	90	0.00	60.83	21.2	173.0
September	2.3	15.8	8.6	9.5	1965	104	0.00	33.57	-	-
	3.4	15.8	8.9	10.8	2610	129	0.32	82.94	-	-
	5.2	15.8	8.9	10.6	2415	88	0.33	38.90	-	-
	7.9	15.8	8.6	9.5	1925	75	0.00	21.02	-	-
	12	15.8	8.6	10.1	1890	88	0.41	32.00	-	-
	18	15.8	8.6	11.0	1755	43	0.00	18.04	-	-
	28	15.8	8.4	9.6	1685	51	0.00	26.25	-	-
	42	15.8	8.3	8.5	2265	105	0.30	70.87	-	-
	64	15.7	8.1	8.4	1600	33	0.35	2.93	-	-
	97	15.8	8.1	8.5	1630	33	0.04	8.16	-	-
	148	15.8	8.0	7.7	1885	49	0.00	27.94	-	-
	224	15.8	7.8	8.1	1445	31	0.28	16.88	-	-
	C01	15.8	8.8	8.6	2430	193	0.28	75.34	-	-
	C02	15.7	8.4	9.6	1820	54	0.29	36.42	-	-
	C03	15.8	9.0	12.3	1730	54	0.00	15.62	-	-
	L01	15.7	8.6	8.2	2000	51	0.00	11.33	-	-

*Composite values calculated by averaging discrete values taken every 0.5 m. **Only one sample taken (otherwise n=2)