SPATIAL AND TEMPORAL ASSESSMENTS OF ZOOPLANKTON COMMUNITY AND STABLE ISOTOPE INDICATORS: DEVELOPING A PREDICTIVE UNDERSTANDING OF ECOLOGICAL DYNAMICS IN BOREAL LAKES

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by
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Mohamed Anas Mohamed Usoof, candidate for the degree of Doctor of Philosophy in Biology, has presented a thesis titled, *Spatial and Temporal Assessments of Zooplankton Community and Stable Isotope Indicators: Developing a Predictive Understanding of Ecological Dynamics in Boreal Lakes*, in an oral examination held on December 11, 2018. The following committee members have found the thesis acceptable in form and content, and that the candidate demonstrated satisfactory knowledge of the subject material.

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ABSTRACT

Developing a predictive understanding of processes that regulate lake ecosystem dynamics is crucial in the face of environmental perturbations affecting boreal western Canada, particularly atmospheric pollutant deposition from oil sands operations in Alberta and climate change. Based on spatial and temporal analyses of zooplankton communities and their δ¹³C and δ¹⁵N values, my research sought to 1) assess the biological responses to climate and industrial deposition and 2) develop a holistic understanding of regulatory processes of these indicator dynamics to better assess future impacts. First, a spatial assessment of 97 lakes detected significant correlations of zooplankton community composition to environmental gradients vulnerable to industrial deposition, together with intriguing spatial patterns. Yet, unraveling industrial impacts from natural variability was precluded by lack of regional baselines. The effects of both environmental gradients and dispersal on species composition were scale-dependent, emphasizing the need for explicit consideration of spatial scale in regional bioassessments. Second, a multi-lake paleolimnological study revealed that trajectories of zooplankton community compositional turnover during the past century were largely asynchronous among lakes, indicating dominant local effects over climatic forcing. This cautions against regional extrapolation of site-specific results, highlighting the need for intensive replication in regional bioassessments. Specific changes in trajectories corresponding to intensified oil sands development were not detected, suggesting minimal industrial impacts. Third, evaluation of spatial patterns in δ¹³C and δ¹⁵N of taxon-specific zooplankton from a 233-lake survey revealed that relative effects of lake/catchment-specific factors (water chemistry, hydromorphology and land cover) on stable isotope (SI) variation were more important than potential regional factors.
(lithology, atmospheric nitrogen deposition). The relative importance of specific lake/catchment-specific predictors of δ\textsuperscript{13}C varied among taxa, likely due to contrasting dietary niches and resulting differences in allochthonous input. This suggests δ\textsuperscript{13}C may respond taxon-specific to limnological changes. Fourth, analyses of δ\textsuperscript{13}C and δ\textsuperscript{15}N time series of taxon-specific zooplankton subfossils during the 20\textsuperscript{th} century demonstrated that they can better reflect environmental impacts on carbon/nutrient fluxes and trophic dynamics in different lake habitats compared to bulk sediment. Beyond indicating limited current biological impacts of industrial deposition and establishing crucial regional baselines, my research provided a comprehensive understanding of regulatory processes of zooplankton communities and SI dynamics, with broad ecological implications for developing indicator frameworks to assess future environmental perturbations in boreal regions.
LIST OF PAPERS

Research chapters (Chapters Two-Four) of this thesis are written as manuscripts with the intent to publish each of them. Hence, there is some overlap among chapters in introductory material and methods. Citations are as follows;


Anas, M.U.M., K.A. Scott and B. Wissel. Water chemistry, landscape and spatial controls of $\delta^{13}C$ and $\delta^{15}N$ of zooplankton taxa in boreal lakes: one size doesn’t fit all. Submitted to the Freshwater Biology.


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CHAPTER 1. GENERAL INTRODUCTION

1.1. Threats to lake ecosystems in boreal western Canada

Threats to lake ecosystems in boreal western Canada have been of recent interest, particularly in relation to the rapid and extensive growth of the energy sector in western Canada (Aherne and Shaw, 2010). The boreal region across northeast Alberta and northwest Saskatchewan has been variably exposed to deposition of atmospheric pollutants (sulfur and nitrogen oxides, trace metals, polycyclic aromatic compounds) from oil sands operations in northern Alberta (Kirk et al., 2014; Makar et al., 2018; Percy, 2013), especially since intensification of crude oil production ca. 1980 (from 0.1 to 2.65 million barrels per day from 1980 to 2017) (Canadian Association of Petroleum Producers, 2018). Among them, deposition of acid precursors i.e. sulfur (S) and nitrogen (N) oxides in both wet and dry forms can result in acidification of lakes (Hazewinkel et al., 2008), while deposition of reactive N species in nutrient-limited lake systems can lead to increased primary production (Baron et al., 2011; Curtis et al., 2010). In addition, atmospheric deposition of toxic pollutants released from oil sands activities including trace metals and polycyclic aromatic compounds may also adversely impact lake biota (Kirk et al., 2014; Kurek et al., 2013; Laird et al., 2013).

So far, there is no consensus among recent studies of lakes in the region with respect to the spatial scale and degree of impact caused by industrial pollutant deposition. Field observations showed that S and N deposition rates generally decline with distance from the industrial center (Fenn et al., 2015; Proemse et al., 2012; Watmough et al., 2014). Accordingly, recent synoptic lake assessments based on chemical and biological criteria (Anas et al., 2014a; Jeffries et al., 2010; Parsons et al.,
2010; RAMP, 2012; Scott et al., 2010) and paleolimnological studies (Curtis et al., 2010; Hazewinkel et al., 2008; Laird et al., 2013) provided little evidence of chronic acidification. In contrast, model-based predictions revealed critical load exceedances across a broad geographical area, warning that large numbers of lakes are extremely vulnerable to acidification at the current level of industrial emissions (Cathcart et al., 2016; Makar et al., 2018). While most paleolimnological studies did not find a fertilizing effect of industrial N deposition on primary production in lakes (Laird et al., 2017; Mushet et al., 2017; Summers et al., 2016), Wolfe (2016) showed that N deposition led to further oligotrophication of phosphorus-limited lakes through modification of nutrient export from catchments. Conflicting evidences also exist on spatial extent of the depositions of trace metal and polycyclic aromatic compounds from oil sands operations and consequent impacts on aquatic ecosystems (Ahad et al., 2015; Bari et al., 2014; Jautzy et al., 2013; Kelly et al., 2010, 2009; Kirk et al., 2014; Kurek et al., 2013; Laird et al., 2013; Summers et al., 2017). These findings together with anticipated increase in oil sands production over the next decades (by ~1.5 million barrels per day; Canadian Association of Petroleum Producers (2018)) requires continued lake assessments based on robust ecological criteria.

Besides potential impacts of industrial emissions, lake ecosystems in northeast Alberta and northwest Saskatchewan are also vulnerable to effects of climate variability. Meteorological records indicate this region has become warmer and drier during the 20\textsuperscript{th} century (Kurek et al., 2013; Schindler and Donahue, 2006; Zhang et al., 2000). Recent paleolimnological assessments in the region suggested that increased primary and secondary productivity over the past century detected in lakes were likely induced by
this climate variability, through changes in internal and external nutrient loading, stratification length and intensity, degree days and ice-free period (Curtis et al., 2010; Hazewinkel et al., 2008; Kurek et al., 2013; Laird et al., 2017, 2013; Moser et al., 2002; Mushet et al., 2017; Summers et al., 2017, 2016). Moreover, evidence exists of increased frequency of forest fires in the region associated with warmer and drier conditions (Ahad et al., 2015; Evans et al., 2016), leading to altered nutrient and organic matter fluxes to lakes and consequent impacts on lake ecosystem functioning (Mceachern et al., 2002; McEachern et al., 2000; Scrimgeour et al., 2001; Tonn et al., 2004). Regional climate models project further intensification of the warming trend in the years ahead, while associated increases in evapotranspiration and declines in snowpack, river flows and glaciers can further aggravate the drier conditions, despite expected increase in precipitation (Kerkhoven and Gan, 2011; Schindler and Donahue, 2006). Therefore, future lake studies on oil sands impacts should consider the possibility of cumulative effects with climate change and attempt to explicitly separate the effects of individual stressors (Environment Canada, 2011).

1.2. Assessing spatial and temporal variability

In general, both atmospheric deposition of industrial pollutants and climate change are spatial-temporal phenomena because they vary through space and time. Both empirical and model-based estimates indicate atmospheric pollutant deposition from oil sands operations vary spatially across northern Alberta and Saskatchewan (Bari et al., 2014; Fenn et al., 2015; Makar et al., 2018; Proemse et al., 2012; Watmough et al., 2014), with likely changes through time due to increased industrial emissions (Hazewinkel et al., 2008; NPRI, 2018). On the other hand, climate change is likely
homogenous across the particular geographical area and hence, can be considered merely as a temporal phenomenon within the context of evaluating impacts of industrial pollutant deposition (Laird et al., 2017). Therefore, combined spatial and temporal assessments are necessary to achieve a regional understanding of long-term lake dynamics in response to industrial deposition and climate variability (Leavitt et al., 2009; Magnuson and Kratz, 2000).

Multiple-lake studies across broad geographical regions largely benefit when a landscape perspective is incorporated (Leavitt et al., 2009; Magnuson and Kratz, 2000; Soranno et al., 2010). In general, landscape approaches are aimed to understand causes and consequences of spatial heterogeneity by examining relationships between spatial patterns and ecological processes (Turner, 1989). A landscape approach in regional lake studies involves taking explicitly into account the position of lakes in the landscape and thereby considering the effects of spatial connectivity among them (that allow fluxes of energy, materials and organisms across ecosystem boundaries), spatial scale and spatial heterogeneity across the region (Magnuson and Kratz, 2000; Read et al., 2015; Soranno et al., 2010; Webster et al., 2000). Quantification of these spatially-contingent effects can be instrumental for developing a better predictive understanding of lake dynamics across northern Alberta and Saskatchewan.

One major challenge in regard to determining the scope of impacts on lake ecosystems in northwest Saskatchewan and northeast Alberta due to oil sands operations and climate change is the lack of long-term monitoring data. Without such data, it is difficult to establish trajectories of limnological changes since pre-oil sands development periods, let alone distinguish between ecosystem state changes attributable to different
stressors. Fortunately, studies of lake sediments can provide an effective means for reconstructing limnological responses to environmental changes over time scales inaccessible by contemporary lake studies (Anderson, 1993; Leavitt et al., 2009). Although it is difficult to deduce cause-effect relationships from sedimentary studies alone, carefully designed paleolimnological analyses combined with robust quantitative approaches can provide important insights into underlying regulatory mechanisms (Anderson, 1993; Battarbee, 2000; Fritz, 2008; Leavitt et al., 2009; Simpson and Anderson, 2009).

1.3. Zooplankton communities as ecological indicators

The use of compositional changes in biotic assemblages to evaluate environmental changes is a long-standing practice in ecology. Without disturbances, the species composition of an ecosystem generally remains stable for many decades, perhaps even centuries (Gannon and Stemberger, 1978). Disturbance-induced changes in physicochemical environment can alter community structure not only through direct physiological effects, but also indirectly via modified biotic interactions (e.g., competition, predation) (Brett, 1989; Gannon and Stemberger, 1978; Wilhm and Dorris, 1968).

Crustacean zooplankton communities have been widely used to assess environmental perturbations in lakes ecosystems due to their desirable indicator properties. Zooplankton species have different physiological sensitivities and thresholds to abiotic factors such as pH, temperature, salinity, dissolved oxygen, turbidity and heavy metals (Havens and Hanazato, 1993; Steiner, 2004; Wissel et al., 2011, 2003), and their high taxonomic diversity allows for substantial differences in community structure
that can be expected in response to variations in these factors (Gyllström et al., 2005; Havens and Hanazato, 1993). In addition, as a critical energetic link between pelagic predators and primary producers, zooplankton also respond to changes in bottom-up (productivity) and top-down (predation) processes induced by environmental perturbations (Anas et al., 2014b; Brett, 1989; Gyllström et al., 2005; McQueen et al., 1986). Given relatively short generation times of most zooplankton species, changes in recruitment success due to altered environmental conditions are rapidly expressed as changes in population size and community structure (Marmorek and Korman, 1993). Further, as zooplankton occur at higher densities and are more homogenously distributed in lakes, they are fairly easy to sample and more representative than fish or benthos (Marmorek and Korman, 1993). Finally, chitinous exoskeletons of cladoceran zooplankton are well preserved in lake sediments, which allows reconstructing historical changes in community structure over long time scales (Korhola and Rautio, 2001).

One of the particularly well studied interactions between zooplankton communities and the environment is the impact of anthropogenic acidification on boreal lake ecosystems in eastern Canada, yielding predictable and consistent responses to decline in pH and other associated chemical parameters (Derry and Arnott, 2007; Keller et al., 2002; Sprules, 1975; Yan and Strus, 1980). The most consistent responses, as supported by both field surveys and controlled experiments, include the decline in species richness (Holt et al., 2003; Keller and Pitblado, 1984; Yan and Strus, 1980) and increased importance of few acid-tolerant species (in terms of frequency of occurrence and relative abundance) at the expense of several acid-sensitive species (Derry and Arnott, 2007; Havens et al., 1993; Keller and Pitblado, 1984; Sprules, 1975; Yan and
Strus, 1980). These responses were resulted from both direct (acute or chronic) toxicological effects of altered water chemistry and indirect effects through altered biotic interactions including changes in phytoplankton interactions, predation regime and competitive relationships (Reviewed by Brett, 1989; Marmorek and Korman, 1993). These well-established responses provide an excellent opportunity to use zooplankton community changes as a biological criterion to track impacts of acidic deposition on boreal lakes across western Canada.

1.4. Stable isotopes of zooplankton as ecological indicators

The naturally occurring carbon (C) and N stable isotope (SI) ratios of contemporary zooplankton and their chitinous fossil remains (i.e. exoskeletons and resting eggs) can reflect the food web consequences of environmental perturbation. Given the small isotopic fractionation of C between diet and consumer (DeNiro and Epstein, 1978; Fry and Sherr, 1984), C SI (δ¹³C) variation of zooplankton is reflective of the relative importance of different basal resources (i.e. phytoplanktonic, benthic, littoral, terrestrial) to the food web (del Giorgio and France, 1996; Grey et al., 2000; Wilkinson et al., 2013), inorganic C sources (atmospheric, geogenic, respired) used by primary producers, and magnitude of inorganic C limitation for primary production (France et al., 1997; Perga and Gerdeaux, 2004; Raven et al., 1994). Therefore, δ¹³C variation of zooplankton has the potential to record changes in above factors due to environmental perturbations, for instance, changes in terrestrial organic matter, carbon dioxide and methane concentrations in lakes in response to climate change (Perga et al., 2016; Persaud et al., 2009; Schilder et al., 2015a), altered contribution of atmospheric C for
primary producers due to eutrophication (Frossard et al., 2013b) and dietary shifts of zooplankton in response to biological invasions (Maguire and Grey, 2006).

On the other hand, animals are consistently $^{15}\text{N}$-enriched by 2 - 4‰ relative to their diet (Minagawa and Wada, 1984) and hence, N SI ratios ($\delta^{15}\text{N}$) of consumers are indicative of their trophic position (Cabana and Rasmussen, 1994; Fry, 1988). Therefore changes in $\delta^{15}\text{N}$ of zooplankton relative to an isotopic baseline can be indicative of shifts in trophic position due to environmental changes (Griffiths et al., 2010; Perga et al., 2010). On the other hand, if trophic position of zooplankton remained stable, spatial and temporal variability of their $\delta^{15}\text{N}$ can be reflective of differences in $\delta^{15}\text{N}$ basal resources, which can be induced by environmental perturbations such as changes in N sources (e.g. atmospheric deposition, agricultural, urban) (Cabana and Rasmussen, 1996; Elliott et al., 2007; Vander Zanden et al., 2005) and loading to lakes (Lake et al., 2001; Peterson et al., 2007; Vander Zanden et al., 2005), and alterations in biogeochemical N processing (nitrogen fixation, denitrification, ammonification) in lakes and watersheds (Bedard-Haughn et al., 2003; Cabana and Rasmussen, 1996; Kendall, 1998).

1.5. Objectives and relevance

The overall purpose of my thesis research was to evaluate the regulatory factors of spatial and temporal variability of zooplankton communities and their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in lakes across northeast Alberta and northwest Saskatchewan, with a special emphasis on potential impacts of oil sands emissions and climate change. My research was based on a series of neo- and paleolimnological studies over spatial extents of hundreds of square kilometers and centennial time scales, respectively. Such broad spatial and temporal perspectives are crucial to develop a predictive understanding of
regulatory processes of zooplankton community and SI dynamics (Leavitt et al., 2009). The specific goals of different research projects (written as separate chapters) as described below.

Chapter two is conducting a broad-scale spatial assessment of lakes across northeast Alberta using crustacean zooplankton communities as biological indicators. This study evaluates the effects of natural environmental gradients and industrial emissions on structuring crustacean zooplankton communities of the region, while taking into account the influences of spatially contingent dispersal processes and spatial scale. This chapter has been published in the journal Ecological Indicators (Anas et al., 2017).

Chapter three presents a spatio-temporal assessment of cladoceran (a subgroup of zooplankton) community dynamics in lakes of northwest Saskatchewan based on a multi-lake paleolimnological study. The goal of this study was to examine the spatial synchrony in cladoceran compositional turnover among six lakes during the past century, in relation to likely effects of climate change, atmospheric deposition of industrial pollutants and other local factors. This chapter will be submitted to the Journal of Paleolimnology.

Chapter four employs SI analysis of taxon-specific zooplankton samples from a broad-scale lake survey in northwest Saskatchewan to identify the landscape-level controls of $\delta^{13}C$ and $\delta^{15}N$ variation of zooplankton taxa. Specifically, I assessed the variation in $\delta^{13}C$ and $\delta^{15}N$ of several zooplankton taxa in relation to water chemistry parameters, hydromorphological and land cover variables, and potential spatial factors including atmospheric pollutant deposition from oil sands operations. This chapter is in review at the journal Freshwater Biology.
In chapter five, I analyzed $\delta^{13}C$ and $\delta^{15}N$ time series of chitinous fossil remains of zooplankton taxa representing pelagic, littoral and benthic habitats during the 20th century in five lakes of northwest Saskatchewan. The purpose of this study was to examine if historical isotopic trends of zooplankton fossil remains would better reflect habitat-specific food web responses to environmental perturbations than that of bulk sedimentary organic matter derived from heterogeneous sources. This chapter will be submitted to the journal *Freshwater Science*.

My research identifies the current level of biological impacts of environmental perturbations and establishes crucial regional baselines for assessing future impacts. In addition, my work provides a holistic understanding of regulatory processes of zooplankton community and SI dynamics, while identifying strengths and constraints for using them as biological criteria in lake assessments for environmental changes.
CHAPTER 2. SCALE-DEPENDENT EFFECTS OF NATURAL ENVIRONMENTAL GRADIENTS, INDUSTRIAL EMISSIONS AND DISPERSAL PROCESSES ON ZOOPLANKTON METACOMMUNITY STRUCTURE: IMPLICATIONS FOR THE BIOASSESSMENT OF BOREAL LAKES

2.1. Abstract

Environmental controls were traditionally considered as sole determinants of community assembly for freshwater bioassessment studies, whereas potential importance of dispersal processes and spatial scale have received limited attention. We conducted a bioassessment of lakes across northeast Alberta, Canada using crustacean zooplankton to develop a framework for evaluating if and how atmospheric emissions from the nearby Athabasca Oil Sands Region could impact their community assemblages. We quantified the effects of environmental gradients and spatially contingent dispersal processes for determining zooplankton community composition of 97 lakes at two spatial scales (regional and sub-regional) using constrained ordination, spatial modeling and variance partitioning techniques. Our findings indicated that effects of both environmental gradients and dispersal processes on species composition were scale-dependent. Zooplankton community composition was significantly correlated to environmental parameters that are directly and indirectly sensitive to industrial deposition including nitrate, sulphate, dissolved organic carbon, base cation, chloride, trace metal concentrations and predation regime, indicating their potential to track future environmental impacts. The relative importance of these environmental predictors varied with spatial scale, yet unraveling the effects of natural environmental heterogeneity vs.
industrial deposition on this scale-dependency was not possible due to lack of regional baseline information. Inferred dispersal effects were not important in shaping zooplankton communities at the sub-regional scale, but had limited, yet significant influence on species composition at the regional scale, emphasizing the need for cautious interpretation of broad-scale community patterns. Beyond establishing crucial regional baselines, our study highlights the necessity for explicit incorporation of dispersal effects and spatial scale in bioassessment of lakes across landscapes.

2.2. Introduction

A robust bioassessment system requires thorough understanding of the ecological processes structuring biotic communities, whereas the metacommunity concept provides a sound mechanistic framework for explaining the relative importance of such processes (Heino, 2013; Leibold et al., 2004; Logue et al., 2011). A metacommunity represents a set of local communities connected via species dispersal, such that local communities are structured by interactions between local processes (abiotic environmental conditions and biotic interactions) and regional (dispersal) processes (Leibold et al., 2004; Logue et al., 2011). Dispersal limitation can be particularly important in freshwater habitats which are isolated in a matrix of inhospitable terrestrial landscapes (Alahuhta and Aroviita, 2016). Traditionally, mainly local environmental conditions were considered to shape community structure in freshwater bioassessments (Heino, 2013), while the simultaneous role of dispersal processes has only recently been established (Alahuhta and Aroviita, 2016; Anas et al., 2014a; Gray and Arnott, 2011).

Evaluating effects of local and regional processes on community assembly is confounded by their scale-dependency, whose importance in structuring biotic
communities has increasingly been recognized (Menge and Olson, 1990). For example, heterogeneity of environmental factors that shape communities may vary among spatial scales (Borcard et al., 2004). Furthermore, relative importance of dispersal limitation in structuring freshwater biotic communities may also vary with spatial scale (Alahuhta and Heino, 2013; Cottenie, 2005; Declerck et al., 2011; Havel and Shurin, 2004). Hence, ‘scale’ can confound our understanding of underlying ecological processes (Wiley et al., 1997) and should be a critical consideration in bioassessments (Alahuhta and Heino, 2013; Heino, 2013).

Lentic crustacean zooplankton communities possess several attributes that make them effective biological indicators of environmental conditions. First, substantial differences in zooplankton community composition can be expected in response to environmental variation because of their high taxonomic diversity (Gyllström et al., 2005; Havens and Hanazato, 1993). Moreover, zooplankton composition is responsive to variations in abiotic factors such as pH, temperature, salinity, dissolved oxygen, turbidity and heavy metals due to differential physiological tolerances of species (Havens and Hanazato, 1993; Steiner, 2004; Wissel et al., 2011, 2003). Given the intermediate trophic position of zooplankton in lake food webs, they are also indirectly impacted by modifications in bottom-up (algal resources) and top-town (predation) processes caused by environmental perturbations (Anas et al., 2014b; Brett, 1989; McQueen et al., 1986; Stenson and Eriksson, 1989). Furthermore, most zooplankton taxa have relatively short generation times that allow changes in recruitment success resulted from altered environmental conditions to be rapidly reflected as changes in community structure. Finally, zooplankton are cost-effective indicators because they are easy to collect and
more representative than fish or benthos due to higher densities and more homogenous distributions in lakes (Marmorek and Korman, 1993). Consequently, zooplankton assemblages are common indicators to assess the impacts of anthropogenic perturbations of lakes, including acidification (Brett, 1989; Havens and Hanazato, 1993; Marmorek and Korman, 1993), metal contamination (Valois et al., 2010; Yan and Strus, 1980; Zhou et al., 2008) and eutrophication (Gannon and Stemberger, 1978; Haberman and Haldna, 2014).

However, divergent evidences persist on dispersal ability of lentic zooplankton (Bohonak and Jenkins, 2003), which is a prerequisite for species to track environmental changes across landscapes (Anas et al., 2014a; Heino, 2013). Zooplankton disperse passively among lakes in both live and dormant forms, either via overland (e.g., wind and animal vectors) or watercourse routes (Gray and Arnott, 2011; Havel and Shurin, 2004). Several studies provided empirical evidence for the importance of dispersal as a determinant of compositional variation of zooplankton communities in lakes (Beisner et al., 2006; Gray and Arnott, 2011; Strecker et al., 2008), whereas others did not find a significant effect (Anas et al., 2014a; Kurek et al., 2011; Pinel-Alloul et al., 1995; Shurin et al., 2009). This discrepancy may be related to varying spatial extents used in different studies (ranging from tens to thousands of kilometers), which emphasizes the importance of a scale-dependent framework to evaluate the effect of dispersal on zooplankton compositional variability across a geographic area (Declerck et al., 2011).

Rapid expansion in oil sand operations in northern Alberta, Canada (from 0.1 to 1.5 million barrels per day from 1980 to 2010) (Canadian Association for Petroleum Producers, 2011) has led to concerns on potential ecological consequences (Schindler,
2010). One such concern is potential impacts of elevated emissions of atmospheric pollutants from oil sands mining and processing (Percy, 2013) on surrounding boreal lake ecosystems. Among them, deposition of acid precursors i.e. sulfur (S) and nitrogen (N) oxides in both wet and dry forms can lead to acidification of lakes (Hazewinkel et al., 2008), while N deposition in nutrient-limited lake systems can increase primary productivity (Baron et al., 2011; Curtis et al., 2010). Moreover, atmospheric deposition of toxic pollutants associated with oil sands activities, such as trace metals and polycyclic aromatic compounds can also have detrimental effects on lake ecosystems (Kurek et al., 2013; Laird et al., 2013). The vulnerability of lakes to acidification varies across northeast Alberta, as determined by the geographic coincidence of elevated S and N deposition and geologically sensitive terrain (Environment Canada, 2011; Saffran and Trew, 1996; Watmough et al., 2014). On the other hand, potential nutrient enrichment of lakes due to N deposition can be much more localized across the region, mostly depending on local characteristics such as catchment area, proportion of forested area in the catchment, lake morphometry, etc., in addition to deposition levels (Baron et al., 2011; Caraco et al., 2003). To this point however, findings from current monitoring activities (principally the Regional Aquatic Monitoring Program, RAMP) and other studies of lakes in northeast Alberta (Curtis et al., 2010; Hazewinkel et al., 2008; Kurek et al., 2013; Parsons et al., 2010; Summers et al., 2016) have not led to any consensus with respect to the spatial scale and degree of impact caused by industrial deposition. Several recent studies indicated that atmospheric fallout of industrial pollutants declines exponentially with the distance from industrial center, with varying deposition patterns for different pollutants (Bari et al., 2014; Fenn et al., 2015; Watmough et al., 2014).
Notwithstanding this attenuation, likely ecological responses to industrial deposition have been detected in remote systems (Anas et al., 2014a; Laird et al., 2013; Wolfe, 2016).

A comprehensive bioassessment program using zooplankton was recently implemented in lakes encompassing a broad geographic radius around oil sands operations in northeast Alberta as part of the Clean Air Regulatory Agenda (CARA) lakes monitoring program of the Canadian government, of which thorough baseline characterization is a primary objective (Environment Canada, 2011). The goal of this study was to evaluate the effects of natural environmental gradients, industrial emissions and dispersal processes for structuring crustacean zooplankton communities of the region. Specifically, we first identified environmental correlates of zooplankton species composition at two spatial scales: within the entire study region and within five physiographic sub-regions (located within the study region) and secondly, assessed their relationships to natural environmental heterogeneity and industrial deposition. Thirdly, we quantified the relative roles of dispersal processes vs. environmental control on zooplankton compositional variation at different spatial scales. We expected, based on evidence from previous studies of zooplankton communities (Declerck et al., 2011; Havel and Shurin, 2004), a greater importance of dispersal processes at the regional scale compared to the sub-regional scale. Finally, we discussed the implications of dispersal effects for bioassessment of lakes subject to industrial impacts.

2.3. Methods

Study area

The study domain consisted of five physiographic sub-regions in northeast Alberta
(55° to 60° latitude; -113° to -108° longitude), namely Athabasca Plain (AP), Tazin River Plain (TRP), Birch Mountains (BM), Muskeg River Uplands (MRU) and Stony Mountains (SM) (Fig. 2.1). Of these, AP is part of the Boreal Shield ecozone and TRP is part of the Taiga Shield ecozone, while all other regions were located in the Boreal Plain ecozone. AP and TRP are characterized by Archaean crystalline bedrock of the Precambrian Shield and Brunisolic soils in outwash deposits, with jack pine forest and lowland peatlands. BM, MRU and SM are underlain by Cretaceous shales and sandstones draped predominantly by Luvisolic soils. Mixed coniferous forest interspersed with peatlands is typical to these regions. The landscape changes from undulating to hummocky across the study area. Altitude across the regions is between 200 and 825 m a.s.l (Turchenek and Lindsay 1982). The hydrological variability across the area is influenced by bog cover, thaw features in bogs, permafrost thawing, elevation and watershed morphometry, while most lakes in the area are located in headwater catchments (Gibson et al., 2015). We refer to Turchenek and Lindsay (1982) and Gibson et al. (2015) for more detailed descriptions of geological characteristics and hydrological conditions of the survey domain, respectively. Meanwhile, the Athabasca Oil Sands Region is located primarily in MRU.

The study area is characterized by a low subarctic climate with long, cold winters and short, cool summers. The average January (coldest month) and July (warmest month) temperatures for the area (recorded at Fort McMurray airport) are -19.5 and 16.7°C, respectively. Mean annual precipitation is ~437mm, of which 73% is falling as rain between June and September, while the remainder is falling as snow from October to March (Environment Canada, 2017; Hatfield Consultants Ltd. et al., 2015).
Fig. 2.1. Sampling domain and locations of the 97 study lakes belonging to five physiographic sub-regions in northern Alberta, showing their distribution across two eozones (AP = Athabasca Plain; TRP = Tazin River Plain; BM = Birch Mountains; MRU = Muskeg River Uplands; SM = Stony Mountains; AOSR = Athabasca Oil Sands Region)
Lake selection

The lake selection was aimed to represent the five different physiographic sub-regions mentioned above. For this, we used a comprehensive, digital inventory of lakes for the survey domain (applying GIS technology to the digital National Topographic Database (NTDB) at the 1:50,000 scale). A stratified-random criterion similar to Jeffries et al. (2010) was used to select candidate lakes from each sub-region, where strata were defined by lake size (≥1-2, >2-5, >5-10, >10-50, >50-100, >100-500 ha and >500 ha). Then random selections of candidate lakes were made from each strata in each sub-region, in proportion to the average ratios of lake densities between strata (Environment Canada, 2011). Only a subset of 97 lakes from this selection was sampled for zooplankton due to logistic constraints including weather for safe net lowering, water depth, and time during a helicopter day. However, the resulting subset is representative of the original selection in terms of number of study lakes selected from each sub-region and their allocation among size strata.

Sampling

Target lakes were visited during the last two weeks of September 2012, the time of fall turn-over. Sample collection was performed from a helicopter on floats, at a visually-defined mid-lake position. The helicopter was at idle rotation during sampling, causing minimal disturbance. Physical measurements including water depth and a thermal profile were recorded on site. A 2 L bulk water sample for chemical analyses was taken at a depth of 1 m using a submersible pump equipped with weighted Tygon tubing. Based on in-situ temperature profiles, this sampling depth was a good representation of the whole water-column. Zooplankton samples were collected using a conical plankton net (mesh
size=76 micron, diameter=0.3 m) that was towed from near bottom to the surface.

_Laboratory analysis_

pH and specific conductance were analyzed at a temporary field station, followed by taking aliquots and processing (filtering, preserving, etc. for subsequent chemical analyses) for major ion, nutrient and metal analyses. Samples were then express-shipped on ice to the National Laboratory for Environmental Testing (NLET) in Burlington, Ontario where chemical analyses were carried out using standard methods (APHA, 2005; EPA, 1994). NLET is accredited to the standard ISO/IEC 17025 by Canadian Association for Environmental Analytical Laboratories (CALA) and participates in national and international QA-QC programs to assure production of high quality chemical analyses. In addition, we used charge balance calculations to independently verify the data quality.

Crustacean zooplankton composition of each sample were identified and enumerated to species level. Entire samples were examined to identify species, whereas taxonomic identifications were based on Aliberti et al. (2009), Hebert (1995), Pennak (1978) and Sandeckock and Scudder (1996). When zooplankton were highly abundant, samples were divided (multiple times as necessary) with a Folsom plankton splitter, and two subsamples of approximately 150-200 individuals each were counted.

_Data analysis_

_Variability in limnological characteristics_

Performing statistical tests to determine significant differences in multivariate means of limnological parameters among physiographic sub-regions was not permitted
because the multivariate variances of sub-regions were not homogenous \( (F = 16.5, \ p < 0.05, \ \text{Appendix A: Fig. A1}) \) according to the PERMDISP2 test (Anderson, 2006). Hence, we performed Kruskal-Wallis tests to examine if median values of individual limnological parameters were significantly different among geographical sub-regions, followed by multiple comparisons of median values (Conover, 1999). Holm’s correction was used to adjust the alpha level \((0.05)\) for multiple comparisons (Holm, 1979).

*Environmental control and dispersal effects at regional scale*

To evaluate unique and shared effects of different sets of environmental parameters on compositional variation among zooplankton communities across the entire study region, we followed the approach of Pinel-Alloul et al. (1995). A range of water chemistry parameters can influence zooplankton community composition (Havens and Hanazato, 1993; Steiner, 2004; Wissel et al., 2003). Yet, these associations may be confounded by lake morphometry, which can also impact species composition directly (Keller and Conlon, 1994). Further, water chemistry can control the planktivore predation regime of lakes (Anas et al., 2014b; Arnott and Vanni, 1993; Keller and Conlon, 1994), which itself can be a major predictor of zooplankton composition (Anas et al., 2014b; Hall et al., 1976; Zaret, 1980). Thus, to distinctly identify zooplankton-environment associations, we conducted separate analyses with two subsets of environmental factors: 1) water chemistry variables (CHEM) and 2) predation regime and lake morphometry variables i.e., variables which can influence or can be influenced by water chemistry variables (PRED_MORPH).

The CHEM variable subset included pH, base cations, chloride (Cl), sulphate \((\text{SO}_4)\), nitrate \((\text{NO}_3-N)\), ammonium \((\text{NH}_4-N)\), total nitrogen \((\text{TN})\), total phosphorus \((\text{TP})\),
soluble reactive phosphorus (SRP), dissolved organic carbon (DOC) and two compressed variables representing the variability in trace metal concentrations. We chose to retain [base cation] over alkalinity which were highly, positively correlated.

Several trace metals were selected (from all the trace metal data available) for statistical analyses due to the following reasons; (1) Zinc (Zn), arsenic (As), antimony (Sb), chromium (Cr), beryllium (Be), lead (Pb), nickel (Ni), selenium (Se), silver (Ag) and thallium (Tl) are considered as priority pollutants (EPA, 1977). (2) Aluminum (Al), iron (Fe) and manganese (Mn) were relatively more abundant in study lakes. (3) Vanadium (V) is a metal commonly associated with pollution due to oil refineries (Khalaf et al., 1982). Given the practical difficulty in including all above mentioned trace metal variables individually in the analyses, we first conducted Principal Component Analysis (PCA) using standardized (scaled to unit variance) trace metal variables and used the first two resulting principal components (trace metal-1 and trace metal-2, which explained 72% of variability) as explanatory variables in the CHEM variable subset.

PCA evaluates the correlation structure within the original data matrix and represents a significant portion of its variance in terms of new orthogonal variables (principal components), which can be used as traditional variables for further statistical analyses.

The PRED_MORPH variable subset consisted of maximum water depth, lake surface area and a nominal variable for planktivore predation regime. The nominal variable defining predation regime of lakes (fishless vs. ‘potentially’ fish-bearing) was characterized by the respective presence / absence of larvae of Chaoborus americanus (Anas et al., 2014a). Due to its large size, strong pigmentation and lack of diurnal vertical migration, C. americanus can only persist in the absence of fish or with very low
planktivorous fish predation (Garcia and Mittelbach, 2008; Sweetman and Smol, 2006; Von Ende and Dempsey, 1981). Nevertheless, lakes without *C. americanus* are not necessarily fish-bearing (Keller and Conlon, 1994). In addition, used gear size (for zooplankton sampling) could have potentially missed the presence of *C. americanus* if they occurred in very low densities, although unlikely since this species often develops high densities in fishless lakes (Anas et al., 2014b). Hence, our results were construed only in relation to fishless conditions.

To test for statistically significant associations between zooplankton species abundances and the CHEM and PRED_MORPH subsets, Redundancy Analyses (RDA) were conducted. RDA is a direct gradient analysis that performs well with linear species-environmental relationships (ter Braak and Prentice, 2004). Preceding the analyses, all variables (with the exceptions of pH, trace metal-1, trace metal-2 and predation regime) were log$_{10}$-transformed to achieve normality and homoscedasticity. Species abundance data were Hellinger transformed, which allow the use in Euclidean-based RDA by giving low weights to rare species with many zeros and low counts (Legendre and Gallagher 2001). Species that were encountered in fewer than 5% of the study lakes were not included in the analysis (Leps and Smilauer, 2003). All cyclopoid copepod species were considered as a single taxonomic group in order to improve the model. After testing the significance of global models containing all variables of CHEM and PRED_MORPH (p<0.05), forward selection (p<0.05, 999 Monte Carlo permutations) with double-stopping criterion (Blanchet et al., 2008) was performed to identify the most parsimonious sets of significant predictors from each explanatory variable set. This was followed by testing collinearity among selected predictors (VIF>4). Finally, we tested if
individual canonical axes of the selected models (which only included significant predictors) were significant \((p<0.05)\) using permutation tests with 1000 iterations and only significant axes were retained for interpretation (Borcard et al., 2011). This procedure was applied to all the subsequent RDAs.

Spatial analysis combined with variation partitioning (see below) was performed to test the relative importance of spatially-contingent dispersal processes vs. environmental gradients on variation in zooplankton community composition among lakes. Spatial structures of communities that are uninfluenced by environmental gradients are considered to represent dispersal effects (Beisner et al., 2006; Cottenie et al., 2003; Heino, 2013). Accordingly, by including spatial factors, we were able to quantify the among-lake variation in zooplankton assemblages related to dispersal effects.

We tested for among sub-region differences in species composition using RDA, in order to identify significant, broad-scale spatial structures in communities of the region. The application of this test was permitted as multivariate variances were homogeneous among sub-regions \((\text{PERMDISP2 test}; F = 0.59, p>0.05)\) (Anderson, 2006). Sub-regions were used as a categorical explanatory variable (SPACE) in RDA with Hellinger-transformed species abundances.

Variance partitioning in RDA was performed to quantify the unique and shared fractions of variation in species composition explained by CHEM, PRED_MORPH and SPACE (sub-regions). We used only the reduced subsets of significant variables from each of explanatory variable sets (identified by the above RDAs) in this analysis (Borcard et al., 2004; Peres-Neto et al., 2006), while Hellinger-transformed species abundances were used as the response matrix. The results were expressed in terms of
adjusted R², which is an unbiased estimate of explained variation as it accounts for number of explanatory variables and sample size (Peres-Neto et al., 2006). Among different fractions of variation quantified, the fraction shared by CHEM and PRED_MORPH variable sets indicated the extent to which the effects of predation regime and morphometric factors on species composition were indirectly associated with the effects of water chemistry. Further, the fraction of among sub-region variation shared with CHEM and PRED_MORPH variable subsets represented the spatial variation induced by environmental factors, whereas pure spatial variation was attributed to the effect of dispersal processes. Given the lack-of-fit of data to model inherent to constrained ordination techniques, we interpreted contributions of different fractions in a relative sense rather than in absolute terms (Økland, 1999). In addition, we also identified the significant (p<0.05) explanatory variables of species composition in different combined or unique fractions in variance partitioning by performing corresponding RDAs and partial RDAs. Subsequently, to identify those environmental variables that contributed to broad-scale spatial structures (among sub-region variability) in species composition, we performed a series of variance partitioning among CHEM, PRED_MORPH and SPACE by eliminating one CHEM or PRED_MORPH variable at a time and checked if shared variation with SPACE was changed due to elimination of particular variable.

Environmental control and dispersal effects at sub-regional scale

In order to identify significant environment-species relationships in each sub-region, we applied the same procedure used at the regional scale (described above) to each sub-region separately. However, the spatial analysis technique used was different
from the regional-scale approach. We conducted eigenvector-based spatial modeling to detect spatial structures in species composition occurring in each sub-region (Borcard and Legendre, 2002; Dray et al., 2006). For each sub-region, a set of spatial variables (i.e., eigenvectors) was derived from Cartesian coordinates (X, Y) of the study lakes using distance-based Moran's Eigenvector Mapping (dbMEM) method (Dray et al., 2006). A truncation distance equal to the longest link between neighboring lakes in a minimum spanning tree linking all survey lakes of a sub-region was used, which is a standard practice in this analysis. The generated spatial variables (dbMEM variables) represent a spectral decomposition of spatial relationships among study lakes, which can be directly related to the spatial patterns in species distributions (Borcard et al., 2011). Subsequently, a forward-selection criterion in a RDA (as described above) was applied to select the smallest subset of significant variables from positive dbMEM variables (SPACE). Finally, we performed variance partitioning to decompose the species compositional variation among CHEM, PRED_ MORPH and SPACE variable subsets.

All statistical analysis were performed in R version 3.2.0 (R Core Team, 2015) using the following packages and functions; (i) ‘agricolae’ (de Mendiburu, 2014) for Kruskal-Wallis test and post-hoc tests (ii) ‘FactoMineR’ (Husson et al., 2015) for PCA, (iii) ‘vegan’ (Oksanen et al., 2015) for RDA, variance partitioning and associated permutation tests and (iv) ‘PCNM’ function (Dray et al., 2009) to generate dbMEM variables.

2.4. Results

Limnological characteristics

There were several notable differences in limnological characteristics between
sub-regions located on the Precambrian Shield (AP and TRP) and those located off-shield (MRU, BM and SM). Maximum depth varied greatly in sub-regions located on the Shield with a greater proportion of deeper lakes, whereas off-Shield sub-regions mostly contained more shallow lakes (Table 2.1). In addition, [Cl] was significantly higher (p<0.05) in on-Shield vs. off-Shield sub-regions. On the other hand, pH was substantially more variable (from acidic to basic ranges) in off-Shield sub-regions, while varying narrowly around circumneutral values in on-Shield sub-regions. The acidic conditions detected in off-Shield sub-regions were likely due to natural organic acidity (associated with high [DOC]) in combination with low base cation concentrations (Halsey et al., 1997; Western Resource Solutions, 2006).

BM was distinct from other sub-regions in terms of several water chemistry parameters. With respect to major acid anions, not only was [SO₄] significantly higher (p<0.05) in BM, but variations in both [SO₄] and [NO₃ N] were also larger than for any other sub-region (Table 2.1). In addition, both [SRP] and [TP] were significantly higher (p<0.05) and more variable in BM relative to other sub-regions. Furthermore, median concentrations of several priority metals (i.e. As, Cu, Ni, Se) were significantly higher (p<0.05) in BM than all other sub-regions (Appendix A: Fig.A2), and variations in [Ag], [Al], [As], [Be], [Cd], [Cr], [Cu], [Fe], [Ni], [Pb], [Se], [Tl], [Zn] and [V] were greater within this vs. other sub-regions. The reason for the distinct water chemistry in BM lakes compared to other sub-regions is not yet ascertained. However, several studies identified distinct bedrock geology (associated with volcanogenic origin) in this sub-region (Eccles, 2011; Sabag, 2008; Turchenek and Lindsay, 1982), which can influence water chemistry of lakes via groundwater sources (Hatfield Consultants Ltd. et al., 2015).
Table 2.1. Limnological characteristics of study lakes of five sub-regions located across northeast Alberta.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Statistic</th>
<th>MRU</th>
<th>BM</th>
<th>SM</th>
<th>AP</th>
<th>TRP</th>
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<tr>
<td>Number of lakes</td>
<td>n</td>
<td>15</td>
<td>16</td>
<td>24</td>
<td>23</td>
<td>19</td>
</tr>
<tr>
<td>Elevation (m)</td>
<td>Range</td>
<td>400-650</td>
<td>350-850</td>
<td>500-800</td>
<td>200-450</td>
<td>200-400</td>
</tr>
<tr>
<td>Surface area (ha)</td>
<td>Mean</td>
<td>313.86</td>
<td>371.51</td>
<td>795.69</td>
<td>129.22</td>
<td>726.62</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>57.46a</td>
<td>110.75a</td>
<td>102.80a</td>
<td>44.03a</td>
<td>111.42a</td>
</tr>
<tr>
<td></td>
<td>5th, 95th Percentile</td>
<td>10.87, 1383.83</td>
<td>28.70, 1795.70</td>
<td>15.45, 2387.35</td>
<td>11.26, 549.77</td>
<td>14.43, 4510.00</td>
</tr>
<tr>
<td>Maximum depth (m)</td>
<td>Mean</td>
<td>1.53</td>
<td>3.83</td>
<td>3.57</td>
<td>5.91</td>
<td>8.85</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>1.60c</td>
<td>2.00bc</td>
<td>1.60bc</td>
<td>4.10ab</td>
<td>7.00a</td>
</tr>
<tr>
<td></td>
<td>5th, 95th Percentile</td>
<td>1.10, 1.18, 1.60bc</td>
<td>0.93, 1.24, 1.43</td>
<td>1.60, 4.10ab</td>
<td>11.48, 7.00ab</td>
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</tr>
<tr>
<td>Fishless lakes</td>
<td>%</td>
<td>40</td>
<td>56</td>
<td>25</td>
<td>22</td>
<td>26</td>
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<tr>
<td>pH</td>
<td>Mean</td>
<td>7.06</td>
<td>7.09</td>
<td>6.86</td>
<td>7.64</td>
<td>7.58</td>
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<tr>
<td></td>
<td>Median</td>
<td>7.64a</td>
<td>6.97a</td>
<td>6.96a</td>
<td>7.81a</td>
<td>7.62a</td>
</tr>
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<td></td>
<td>5th, 95th Percentile</td>
<td>4.76, 5.03, 6.69</td>
<td>5.10, 7.06</td>
<td>11.48, 26.50</td>
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<tr>
<td>Alkalinity (μeq L⁻¹)</td>
<td>Mean</td>
<td>1.44</td>
<td>0.77</td>
<td>0.64</td>
<td>0.97</td>
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<td>1.50a</td>
<td>0.28a</td>
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<td>0.88a</td>
<td>0.60a</td>
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<td></td>
<td>5th, 95th Percentile</td>
<td>0.04, 0.06, 0.08</td>
<td>0.01, 0.28, 0.42</td>
<td>0.08, 0.15a</td>
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<tr>
<td>Base cation (mg L⁻¹)</td>
<td>Mean</td>
<td>28.36</td>
<td>19.35</td>
<td>14.06</td>
<td>18.30</td>
<td>15.66</td>
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<tr>
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<td>Median</td>
<td>26.89a</td>
<td>14.52a</td>
<td>8.11a</td>
<td>17.24a</td>
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<td>5th, 95th Percentile</td>
<td>4.02, 3.74, 1.90</td>
<td>3.74, 2.65, 8.89</td>
<td>1.73, 23.74</td>
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<td>Cl (mg L⁻¹)</td>
<td>Mean</td>
<td>1.14</td>
<td>0.37</td>
<td>0.26</td>
<td>1.55</td>
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<td>Median</td>
<td>0.13b</td>
<td>0.15b</td>
<td>0.15b</td>
<td>0.42a</td>
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<td></td>
<td>5th, 95th Percentile</td>
<td>0.08, 0.09, 0.07</td>
<td>0.07, 0.15, 0.31</td>
<td>0.48, 9.45</td>
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<td>Substance</td>
<td>Unit</td>
<td>Mean</td>
<td>Median</td>
<td>5th, 95th Percentile</td>
<td>5th, 95th Percentile</td>
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<tr>
<td>( \text{SO}_4 ) (mg L(^{-1}))</td>
<td></td>
<td>0.39</td>
<td>0.26(^c)</td>
<td>0.11, 1.05</td>
<td>1.05</td>
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<td>0.76, 15.93</td>
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<td>0.02, 1.51</td>
<td>1.51</td>
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<td></td>
<td></td>
<td>0.77</td>
<td>0.63(^b)</td>
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<tr>
<td>( \text{NO}_3)-N ((\mu)g L(^{-1}))</td>
<td></td>
<td>5.67</td>
<td>3.00(^a)</td>
<td>3.00, 174.70</td>
<td>174.70</td>
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<td></td>
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<td>12.50(^a)</td>
<td>3.00, 148.90</td>
<td>148.90</td>
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<td>5.00(^a)</td>
<td>3.00, 10.60</td>
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<td>( \text{NH}_4)-N ((\mu)g L(^{-1}))</td>
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<td>36.00(^a)</td>
<td>3.00, 168.20</td>
<td>168.20</td>
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<td>53.31</td>
<td>26.00(^a)</td>
<td>3.00, 147.00</td>
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<td>53.17</td>
<td>17.00(^a)</td>
<td>3.00, 90.00</td>
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<td>13.10(^a)</td>
<td>3.00, 148.90</td>
<td>148.90</td>
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<td>9.00(^a)</td>
<td>3.00, 168.20</td>
<td>168.20</td>
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<td>TN (mg L(^{-1}))</td>
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<td>0.83(^a)</td>
<td>0.62, 1.63</td>
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<tr>
<td></td>
<td></td>
<td>0.82</td>
<td>0.83(^a)</td>
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<td>0.94</td>
<td>0.82(^a)</td>
<td>0.46, 103.32</td>
<td>103.32</td>
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<td>0.78</td>
<td>0.53(^a)</td>
<td>0.4, 18.53</td>
<td>18.53</td>
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<tr>
<td>TP (mg L(^{-1}))</td>
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<td>0.02(^bc)</td>
<td>0.01, 0.01</td>
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<td>0.07(^a)</td>
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<td>0.03</td>
<td>0.03(^ab)</td>
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<td>0.04(^c)</td>
<td>0.02, 0.02</td>
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<tr>
<td>SRP ((\mu)g L(^{-1}))</td>
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<td>0.56</td>
<td>0.2(^b)</td>
<td>0.5, 0.45(^b)</td>
<td>0.4(^b)</td>
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<td>26.59</td>
<td>11.1(^a)</td>
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<td>0.4(^b)</td>
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<td>1.15</td>
<td>0.45(^b)</td>
<td>0.19, 0.2(^b)</td>
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<td>0.19(^b)</td>
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<td>DOC (mg L(^{-1}))</td>
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<td>13.8, 23.55(^ab)</td>
<td>23.55(^ab)</td>
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<td></td>
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<td>23.55(^ab)</td>
<td>11.15, 18.05(^ab)</td>
<td>18.05(^ab)</td>
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<td></td>
<td>19.69</td>
<td>18.05(^ab)</td>
<td>9.33, 7.80(^c)</td>
<td>7.80(^c)</td>
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<td>9.64</td>
<td>7.80(^c)</td>
<td>3.31, 14.6(^b)</td>
<td>14.6(^b)</td>
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<td></td>
<td></td>
<td>16.65</td>
<td>14.6(^b)</td>
<td>8.22, 8.22</td>
<td>8.22</td>
<td></td>
</tr>
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<td></td>
<td></td>
<td>22.46</td>
<td>14.6(^b)</td>
<td>8.22, 8.22</td>
<td>8.22</td>
<td></td>
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<td></td>
<td></td>
<td>19.69</td>
<td>7.80(^c)</td>
<td>3.31, 14.6(^b)</td>
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<td></td>
<td>16.65</td>
<td>8.22(^b)</td>
<td>3.31, 8.22</td>
<td>8.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>24.93</td>
<td>8.22(^b)</td>
<td>3.31, 8.22</td>
<td>8.22</td>
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</tr>
</tbody>
</table>

Note: Median values of the sub-regions indicated by same letters are not significantly different according to Kruskal-Wallis test followed by multiple comparisons (adjusted \( p < 0.05 \)).
Evidence for impacts of industrial deposition in BM has not been provided by previous studies (Curtis et al., 2010; Hazewinkel et al., 2008; Summers et al., 2016; Wieder et al., 2010). BM also had the highest prevalence of fishless lakes, probably as a result of more acidic conditions (associated with higher [SO\textsubscript{4}] and [DOC]) and higher trace metal concentrations compared to other sub-regions and, physical isolation of lakes because of differences in elevation, leading to lack of fish migration routes.

*Environmental predictors of species composition: regional scale*

Several significant environmental predictors of species composition of lakes in the study region (i.e. all sub-regions together) were identified by RDAs of species composition with CHEM and PRED\_MORPH. Significant CHEM predictors (p<0.05) of species composition included [SO\textsubscript{4}], [NO\textsubscript{3}-N], [DOC], [base cation] and [Cl] (Fig.2.2a). From PRED\_MORPH, on the other hand, predation regime was identified as the only significant predictor (p<0.05) of species composition (Fig.2.2b). Variance partitioning revealed that most of the variation explained by predation was shared by the effects of significant chemical predictors on species composition identified above (Table 2.2). This shared variation mostly resulted from the positive association of [SO\textsubscript{4}], [NO\textsubscript{3}-N] and [DOC] with potentially fishless conditions across the study region.

*Spatial structuring of species composition: regional scale*

Our analyses indicated that broad-scale spatial structures of species composition in the study region were largely induced by environmental factors. RDA between species composition and SPACE (which included sub-regions as sole explanatory variable) revealed that BM was significantly distinct (p < 0.05) from all other sub-regions along RDA axis 1(Fig.2.2c), whereas AP was significantly different (p < 0.05) from MRU and
Fig. 2.2. Redundancy analysis (RDA) biplots of different sets of predictors versus crustacean zooplankton composition of the study region; (a) zooplankton species versus CHEM; (b) Zooplankton species versus PRED_MORPH; (c) Zooplankton species versus
sub-regions; Only significant (p<0.05) predictors retained in each independent model by the forward selection criterion are presented; Biplots are scaled to optimally display the relationships among variables; Dpul = *Daphnia pulex*; Dmen = *Daphnia mendotae*; Damb = *Daphnia ambiguа*; Dros = *Daphnia roseа*; Dbra = *Diaphanosoma brachуrum*; Clac = *Ceriodaphnia lacustris*; Blon = *Bosmina longirostris*; Hgib = *Holopedium gibberum*; Csp = *Chyдорus sphaericus*; Alep = *Agлodiaptomus leptopus*; Hsep = *Heterocope septentrionalis*; Lmin = *Leptodiaptomus minutes*; Sore = *Skistodiaptomus oregonensis*; Elac = *Epischura lacustris*; Cycl = *Cyclopoid spp.*
SM sub-regions along RDA axis 2. According to variance partitioning, most of this spatially structured species variation (i.e. among sub-region variation) was shared by significant CHEM and PRED_MORPH variables (12% out of 16%) (Table 2.2). Further analyses showed that distinction of BM from other sub-regions with regard to species composition was related to variations in [SO$_4$], [NO$_3$-N], [DOC], [base cation], [Cl] and predation. Among them, [SO$_4$] and [Cl] solely contributed to broad-scale spatial variation, given that they were not identified as significant predictors in partial RDA between species composition and significant environmental factors after partialling out broad-scale spatial (among- sub-region) variability (Table 2.2). On the other hand, the remaining 4% of species variation explained by the pure spatial component (i.e. spatially structured variation independent of environmental influence) was statistically significant (p<0.001), yet it was less important relative to the 25% of the variation explained by significant environmental factors. This component mostly represented the difference in species composition between AP vs. MRU and SM.

*Environmental predictors of species composition: sub-regional scale*

RDAs between species composition and CHEM identified significant relationships (p<0.05) for all sub-regions except SM. The relationships between species composition and [NO$_3$-N], [base cation] and [DOC] in MRU, BM and TRP were similar to those detected at regional scale (Fig.2.3a, b and d). In addition, the effects of trace metal concentrations on species composition were important in MRU and AP (Fig.2.3a and c).

As evident from RDAs between species composition and PRED_MORPH, a significant relationship (p<0.05) between predation and species composition (similar to
Table 2.2. Variance partitioning of zooplankton species composition of the study region using CHEM, PRED_MORPH and SPACE variable subsets.

<table>
<thead>
<tr>
<th>Explanatory variable set/fraction</th>
<th>Significant variables</th>
<th>$R^2_{adj}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHEM</td>
<td>[SO$_4$], [NO$_3$-N], [DOC], [base cation], [Cl]</td>
<td>20.0***</td>
</tr>
<tr>
<td>PRED_MORPH</td>
<td>predation status†</td>
<td>13.0***</td>
</tr>
<tr>
<td>SPACE</td>
<td>sub-region†</td>
<td>16.0***</td>
</tr>
<tr>
<td>CHEM+PRED_MORPH</td>
<td>[SO$_4$], [NO$_3$-N], [DOC], [base cation], [Cl], predation status</td>
<td>25.0***</td>
</tr>
<tr>
<td>CHEM</td>
<td>SPACE</td>
<td>[NO$_3$-N], [DOC], [base cation]</td>
</tr>
<tr>
<td>PRED_MORPH</td>
<td>SPACE</td>
<td>predation status†</td>
</tr>
<tr>
<td>SPACE</td>
<td>CHEM+ PRED_MORPH</td>
<td>sub-region†</td>
</tr>
<tr>
<td>CHEM∩PRED_MORPH</td>
<td>-</td>
<td>8.0$^{nt}$</td>
</tr>
<tr>
<td>CHEM∩SPACE</td>
<td>-</td>
<td>12.0$^{nt}$</td>
</tr>
<tr>
<td>PRED_MORPH∩SPACE</td>
<td>-</td>
<td>5.0$^{nt}$</td>
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<tr>
<td>CHEM∩PRED_MORPH∩SPACE</td>
<td>-</td>
<td>5.0$^{nt}$</td>
</tr>
<tr>
<td>Unexplained</td>
<td>-</td>
<td>71.0$^{nt}$</td>
</tr>
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</table>

**Note:** CHEM= variation explained by chemical factors; PRED_MORPH = variation explained by predation regime and lake morphometry; SPACE= spatial variation; CHEM+PRED_MORPH = total environmental variation; CHEM| SPACE = pure (spatially unstructured) variation explained by chemical factors; PRED_MORPH| SPACE= pure (spatially unstructured) variation explained by predation regime and lake morphometry; SPACE| CHEM+ PRED_MORPH = pure spatial variation; CHEM∩PRED_MORPH = shared variation explained by chemical factors variable set and predation regime and lake morphometry variable set; CHEM∩SPACE = spatially unstructured variation;
structured variation explained by chemical factors; $PRED\_MORPH\cap SPACE$ = spatially structured variation explained by predation regime and lake morphology;

$CHEM\cap SPACE + PRED\_MORPH\cap SPACE - CHEM\cap PRED\_MORPH\cap SPACE =$ spatially structured environmental variation; Unexplained = variation unexplained by chemical factors, predation regime and lake morphometry and spatial variables; $R^2_{adj} =$ Adjusted $R^2$; *** $p \leq 0.001$; nt = not testable; † Both predation status and sub-regions were categorical variables, while all the other explanatory variables were log10-transformed.
Fig. 2.3. Redundancy analysis (RDA) biplots of crustacean zooplankton composition versus CHEM of four physiographic sub-regions; (a) MRU; (b) BM; (c) AP; (d) TRP; Only significant (p<0.05) predictors retained in each independent model by the forward selection criterion are presented; Significant CHEM predictors were not identified for SM; Biplots are scaled to optimally display the relationships among variables; For zooplankton taxa codes, see legend of Fig. 2.2.
that of regional scale) was identified in all sub-regions except AP (Appendix A: Fig.A3a, b, c and e). In addition, maximum depth was a significant predictor of species composition in MRU and AP (Appendix A: Fig.A3a and d). Variance partitioning indicated that the majority of variability in species composition explained by significant PRED_MORPH variables was shared by significant CHEM variables in all cases except AP (Table 2.3).

Spatial structuring of species composition: sub-regions

RDAs between species composition and SPACE (which included dbMEM variables) identified significant spatial structures within sub-regions (p<0.05) only for MRU and AP. Variance partitioning showed that most of the species variation explained by spatial variables (12% of 15%) was shared by significant environmental factors in MRU (Table 2.3), while the variation explained by the purely spatial component (3%) was not significant (p>0.05). In AP, the variation explained by spatial structures (10%) was completely shared by significant environmental factors.

2.5. Discussion

This study showed that compositional variation of zooplankton communities in northeast Alberta lakes was largely influenced by environmental gradients. Significant environmental predictors of species composition were [SO$_4$], [NO$_3$-N], [DOC], [base cation], [Cl], trace metal concentrations, maximum lake depth and predation regime, but their relative importance varied with spatial scale. The effect of purely spatially contingent processes (dispersal limitation) was significant only at the regional scale, yet less important relative to environmental control.
Table 2.3. Variance partitioning of zooplankton species composition of five sub-regions using CHEM, PRED_MORPH and SPACE variable subsets.

<table>
<thead>
<tr>
<th>Explanatory variable set/fraction</th>
<th>R\textsuperscript{2} adj (%)</th>
<th>MRU</th>
<th>BM</th>
<th>SM</th>
<th>AP</th>
<th>TRP</th>
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<tr>
<td>CHEM</td>
<td>47.0***</td>
<td>8.0*</td>
<td>ns</td>
<td>32.0***</td>
<td>15.0**</td>
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<tr>
<td>PRED_MORPH</td>
<td>34.0***</td>
<td>12.0*</td>
<td>14.0**</td>
<td>10.0*</td>
<td>22.0**</td>
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</tr>
<tr>
<td>SPACE</td>
<td>15.1*</td>
<td>ns</td>
<td>ns</td>
<td>10.0**</td>
<td>ns</td>
<td></td>
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<td>CHEM+PRED_MORPH</td>
<td>60.0***</td>
<td>13.0*</td>
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<td>36.0***</td>
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<td>CHEM</td>
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<td>SPACE</td>
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<td>CHEM+ PRED_MORPH</td>
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<td>CHEM∩PRED_MORPH</td>
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<td>6.0</td>
<td>nt</td>
</tr>
<tr>
<td>CHEM∩SPACE</td>
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<td>nt</td>
<td></td>
<td></td>
<td>10.0</td>
<td>nt</td>
</tr>
<tr>
<td>PRED_MORPH∩SPACE</td>
<td>12.0</td>
<td>nt</td>
<td></td>
<td></td>
<td>6.0</td>
<td>nt</td>
</tr>
<tr>
<td>CHEM∩PRED_MORPH∩SPACE</td>
<td>12.0</td>
<td>nt</td>
<td></td>
<td></td>
<td>6.0</td>
<td>nt</td>
</tr>
<tr>
<td>Unexplained</td>
<td>37.0</td>
<td>nt</td>
<td>87.0</td>
<td>nt</td>
<td>86.0</td>
<td>nt</td>
</tr>
</tbody>
</table>

Note: CHEM = variation explained by chemical factors; PRED_MORPH = variation explained by predation regime and lake morphometry; SPACE = spatial variation; CHEM+PRED_MORPH = total environmental variation; CHEM| SPACE = pure (spatially unstructured) variation explained by chemical factors; PRED_MORPH| SPACE = pure (spatially unstructured) variation explained by predation regime and lake morphometry; SPACE| CHEM+ PRED_MORPH = pure spatial variation; CHEM∩PRED_MORPH = shared variation explained by chemical factors variable set.
and predation regime and lake morphometry variable set; CHEM∩SPACE = spatially structured variation explained by chemical factors; PRED_MORPH∩SPACE = spatially structured variation explained by predation regime and lake morphometry;

CHEM∩SPACE+ PRED_MORPH∩SPACE- CHEM∩PRED_MORPH∩SPACE= spatially structured environmental variation; Unexplained = variation unexplained by chemical factors, predation regime and lake morphometry and spatial variables; R$^2_{adj}$ (Adjusted R$^2$) value is not provided when at least one of the explanatory variable sets used to estimate the particular fraction was not significant (p>0.05); *** p≤0.001; ** 0.001<p≤0.01; * 0.01<p≤0.05; ns = not significant (p>0.05); nt = not testable.
**Environmental predictors of species composition**

The observed environment-species relationships are mostly expected, as supported by empirical evidences from other studies. Certain water chemistry parameters (e.g. acid-base status and trace metal concentrations) likely influenced the species composition directly due to different physiological sensitivities of species to these conditions. On the other hand, the effects of chemical parameters such as [SO$_4$], [NO$_3$-N] and [DOC] on the species composition are likely indirect, via their influences on top-down (predation) and bottom-up (primary production) processes in lakes. A detailed account of environment-species relationships identified in the present study, together with their ecological interpretations is provided in the supplementary discussion (Appendix A). Ultimately, our findings indicate that compositional variation in zooplankton communities has the potential to reflect natural and/or anthropogenically induced variability in environmental conditions of lakes across northeast Alberta.

**Scale-dependency of environmental predictors**

Our study clearly identified that environmental predictors of zooplankton community composition in northeast Alberta lakes are scale-dependent. The effects of [SO$_4$] and [Cl] on species composition were important at the regional scale only. Conversely, the effects of trace metal concentrations and lake depth were significant only at the sub-regional scale (only in MRU and AP). Meanwhile, [NO$_3$-N], [DOC], [base cation] and predation regime influenced species composition not only at the regional scale, but also at the sub-regional scale with varying degrees of importance in different sub-regions. For instance, the effect of [NO$_3$-N] was more pronounced in MRU and BM, while [DOC] was more influential in sub-regions located on the Precambrian
Shield (i.e. AP and TRP). In addition, the effect of [base cation] was particularly strong in MRU.

Given the vicinity of our study lakes to the oil sands region, it is crucial to assess the effects of industrial deposition vs. natural environmental heterogeneity on varying importance of environmental predictors across spatial scales and in different sub-regions. At the regional scale, broad-scale patterns in species composition were likely induced by natural environmental heterogeneity across the region i.e., natural variability between BM and other sub-regions (discussed above). In contrast, associations between industrial deposition and fine-scale environmental predictors were likely more important at the sub-region scale. Recent empirical evidence indicated that atmospheric deposition of N, S, trace metals and base cations (derived from fugitive dust sources in the mines) declines exponentially with distance from the oil sands region (Bari et al., 2014; Watmough et al., 2014). In addition, several paleolimnological studies provided evidence for temporal changes in lake ecosystems located closer to industrial development, which were likely associated with industrial emissions (Curtis et al., 2010; Kurek et al., 2013; Laird et al., 2013). MRU being closest to the industrial center and AP being located in a highly geologically sensitive terrain (Environment Canada, 2011; Whitfield and Watmough, 2012), are most vulnerable to effects of atmospheric deposition.

Interestingly, NO$_3$-N, base cation and trace metal concentrations were significantly related to species composition in MRU, and trace metal concentration was identified as significant predictor of species composition in AP. In addition to metal contamination from airborne deposition, acidic deposition may promote speciation of metals in lakes, rendering them more toxic (Sullivan, 2002). It should be noted however that
concentrations of most trace metals considered here were below the guidelines for protection of aquatic life (Canadian Council of Ministers of the Environment, 2007). Besides direct deposition of base cations, the highly pronounced effect of [base cation] in MRU might also be related to acidic deposition in its initial stages, which promotes leaching of base cations to lakes from catchment soils (Sullivan, 2002). Furthermore, significantly greater environmental heterogeneity in MRU compared to other sub-regions (Appendix A: Fig. A1) may have resulted from industrial impacts (Sousa, 1984; Warren et al., 2007). However, such effects of industrial deposition in northeast Alberta are speculative at this point as disentanglement of industrial effects from prevailing natural variability was not possible due to lack of baseline (pre-oil sands development) data for the region.

Scale-dependency of dispersal processes

Our results indicated that the effect of dispersal is likely only important at the regional scale. The variation explained by the pure spatial component (which is reprehensive of the effect of dispersal processes) was significant (p<0.05) only at the regional scale, whereas it was either non-existent or statistically non-significant (p>0.05) at the sub-regional scale. Several other studies also indicated that the importance of dispersal-limitation in structuring lentic zooplankton communities increases with spatial scale (Cottenie, 2005; Declerck et al., 2011; Havel and Shurin, 2004; Ng et al., 2009). Non-existence / non-significance of the pure spatial component at the sub-regional scale is indicative of a moderate dispersal level, which is sufficient to deliver species to environmentally favourable sites (Cottenie, 2005; Leibold et al., 2004; Ng et al., 2009; Shurin et al., 2009). In other words, dispersal rates are neither too low hindering arrival
of species to environmentally suitable sites (dispersal limitation), nor excessively high sustaining populations even in environmentally unsuitable sites (mass effects). In contrast, co-occurrence of significant, exclusive spatial and environmental components at the regional scale suggests that environmental control is partially constrained by dispersal limitation at the broad spatial scale (Ng et al., 2009). This is not surprising as transport of passive dispersers such as zooplankton is more likely to occur over short than long distances (Havel and Shurin, 2004; Ng et al., 2009) and consequently, species may be unable to reach favourable sites that meet their niche requirements over broader spatial scales (Cottenie, 2005; Declerck et al., 2011; Ng et al., 2009). However, pure spatial (dispersal) processes were much less important relative to environmental control at the regional scale of our study (4 vs. 25% of species variation respectively), which is in line with findings of other broad-scale zooplankton surveys (Anas et al., 2014a; Kurek et al., 2011; Pinel-Alloul et al., 1995; Shurin et al., 2009), indicating environmental control is the dominant process shaping lentic zooplankton assemblages even at broad spatial scales. Ultimately, our findings suggest that zooplankton communities are appropriate to trace changes in environmental conditions associated with disturbances both at sub-regional and regional scales. Yet community patterns at the regional scale should be interpreted with caution, as they might be partially influenced by dispersal limitation besides environmental changes.

Three potential caveats in our spatial analysis should be noted. First, some of the pure spatial variation (attributed to dispersal effects) might actually be induced by unmeasured environmental parameters (Borcard et al., 2011; Cottenie, 2005). This possibility is rather low because most ecologically meaningful explanatory variables
were included in our analyses. Nevertheless, such possibility would only further support our conclusion that dispersal limitation is less important relative to environmental control in the present study (Anas et al., 2014a; Cottenie, 2005). Second, all or part of the pure spatial variation may also result from historical effects or barriers to dispersal (Borcard and Legendre, 1994; Heino, 2013). Finally, as zooplankton can disperse passively either through overland (e.g., wind and animal vectors) or hydrological routes (Havel and Shurin, 2004), both overland distances and hydrological connectivity between lakes can be important for zooplankton dispersal across a landscape (Beisner et al., 2006; Cottenie et al., 2003; Shurin et al., 2009). Unfortunately, hydrologic connectivity data were unavailable for this study.

Besides spatial dispersal (discussed above), “temporal dispersal” i.e. dispersal from long-lived diapausing eggs may also influence zooplankton community responses to environmental changes. Diapausing eggs laid at the times in the distant past may introduce species to the community that can thrive in the altered environmental conditions in future (Hairston, 2002, 1996). In addition, egg banks provide the extended generation overlap necessary to maintain the coexistence of competing species over prolonged durations, which in turn can maintain the species diversity under fluctuating environmental conditions as different species are favored at different times (Brendonck and Meester, 2003; Hairston, 1996). Therefore, temporal dispersal may counteract the impact of spatial dispersal limitation on community responses to environmental changes.

2.6. Conclusions

Our results provide crucial baseline information against which future ecological changes in northeast Alberta lakes due to industrial deposition can be assessed.
Zooplankton communities of the region were significantly correlated to environmental gradients that are susceptible to industrial deposition (including SO$_4$, NO$_3$-N, base cation and trace metals), indicating their potential to track future environmental changes. Future enhanced atmospheric deposition in association with expansions of oil sands industry in combination with geological and geo-morphological processes may result in broad-scale structuring of these environmental parameters across the region (Gray and Arnott, 2011; Pinel-Alloul et al., 1995), subsequently inducing broad-scale spatial patterns in zooplankton composition. However, the expected community responses can be partially suppressed by dispersal limitation at the broad scale. Therefore, caution needs to be exercised when interpreting broad-scale community patterns in relation to potential impacts of industrial deposition.

In addition, our findings indicate that effects of both environmental gradients and dispersal processes on zooplankton community composition in lakes across northeast Alberta were scale-dependent. The use of a single spatial scale would have led to incomplete understanding on these ecological processes. Therefore, the present study highlights the importance of explicitly considering the effects of dispersal processes and spatial scale on community assembly in bioassessment of lakes across landscapes.

2.7. Acknowledgements

We thank Jonathan Keating and Mike McAulay for excellent technical support. Wood Buffalo Helicopters provided transportation and Lloyd Lake Lodge provided accommodation for the field crew. The National Laboratory for Environmental Testing (NLET) performed the chemical analyses. Fariborz Norouzian and Kara Chan were involved in the data processing. We are grateful to anonymous reviewers for their
constructive comments. This work was supported by the Clean Air Regulatory Agenda (CARA) of Environment and Climate Change Canada and the University of Regina.
CHAPTER 3. ASYNCHRONY OF ZOOPLANKTON COMMUNITY DYNAMICS IN BOREAL LAKES DURING THE 20TH CENTURY: LOCAL FACTORS OVERRIDE CLIMATIC FORCING

3.1. Abstract

Regional-scale, extrinsic forces such as climatic factors may lead to synchronous limnological dynamics among lakes, whereas dominance of lake-specific forces can lead to idiosyncratic behaviour. Consensus on synchrony of biological properties among lakes is lacking, with most studies of biological synchrony being limited to decadal to multi-decadal time scales, using aggregate population or community measures. Here, we used time series of fossil cladoceran remains to investigate synchrony among zooplankton assemblages at the community level in six boreal lakes of central Canada during the 20th century. We identified two common cladoceran compositional turnover (CCT) patterns across all study lakes, which likely arose in response to climate-induced changes in acidity and productivity. Despite similarities between lakes with respect to geomorphological and limnological characteristics, trajectories of compositional turnover were significantly different among lakes in terms of timing, direction and pace of change. Such widespread asynchrony indicates dominant, confounding effects of local factors over climatic forcing of cladoceran dynamics, cautioning against regional extrapolation of lake-specific cladoceran dynamics. Any specific changes in trajectories of CCT corresponding to intensified development of the nearby oil sands industry (post ca.1980) were not detected in study lakes, indicating minimal impacts of atmospheric deposition of industrial pollutants on cladoceran dynamics. Our findings highlight the
need for intensive replication and improved understanding of effects of local processes for zooplankton-based bioassessments of regional impacts.

3.2. Introduction

The tendency of ecosystem properties to behave similarly among spatially distinct locations over time, a phenomenon referred to as spatial synchrony or temporal coherence (Kratz et al., 1998; Magnuson et al., 1990), has been of high interest in lake ecosystem studies (Angeler and Johnson, 2012; Magnuson et al., 1990; Rusak et al., 1999; Vogt et al., 2011). Analysis of temporal coherence provides an effective means of identifying the nature of forcing factors influencing lake dynamics across landscapes. A high temporal coherence between spatially distinct locations indicates a primary role of extrinsic factors operating at a regional scale (e.g. climate) in regulating limnological variables (Baines et al., 2000; Magnuson et al., 1990; Rusak et al., 1999). In contrast, lack of coherence indicates a greater importance of local-scale factors, which can be both intrinsic (e.g. trophic interactions) or extrinsic (e.g. spatially varying atmospheric contaminant deposition from a point source) to lake ecosystems (Das et al., 2009; Keller and Pitblado, 1986; Rusak et al., 2008). Determination of relative roles of these forces is essential to develop a predictive understanding of lake dynamics across landscapes (Baines et al., 2000).

In contrast to consistent findings of strong to moderate coherence in abiotic variables among lakes (Baines et al., 2000; Kratz et al., 1998; Magnuson et al., 1990; Vogt et al., 2011), conflicting evidence exist on synchrony of biological variables. Several limnological studies detected weaker synchrony for biological variables (e.g. chlorophyll concentration, fish and plankton abundances) compared to those of physical
(e.g. water temperature, water level) and chemical parameters (e.g. pH, alkalinity, dissolved organic carbon and nutrient concentrations) with direct mechanistic linkages to regional extrinsic factors (e.g. climate), indicating that influences of local factors such as trophic interactions are more important (Arnott et al., 2003; Baines et al., 2000; George et al., 2000; Kratz et al., 1998; Magnuson et al., 1990; Patoine and Leavitt, 2006). On the other hand, others showed strong synchrony in biological variables among lakes, suggesting regional factors (mainly climate forcing) can induce biological synchrony even through indirect and complex mechanisms (e.g. via trophic interactions) (Angeler and Johnson, 2012; Anneville et al., 2005; Kent et al., 2007; Rusak et al., 1999; Vogt et al., 2011).

Biological synchrony across lakes has been typically examined for aggregate population or community measures such as chlorophyll concentration, abundance/biomass of individual taxa and species richness (Arnott et al., 2003; Magnuson et al., 1990; Rusak et al., 1999; Vogt et al., 2011), while few recent studies evaluated synchrony at the community composition level (Angeler and Johnson, 2012; Anneville et al., 2005; Kent et al., 2007). Community-level approaches can be more effective than population-based studies to assess synchrony as they reveal overall patterns by synthesising sometime disparate population responses of individual taxa (Angeler, 2009). In addition, multivariate measures of compositional turnover that take taxonomic identity into account may be more efficient than univariate measures in detecting temporal community responses to environmental forcing (e.g. species richness, community evenness, Shannon diversity), as they can simultaneously capture species substitution and changes in abundances over time (Angeler and Johnson, 2012).
Most studies of biological synchrony in lakes were limited to decadal to multi-decadal time scales (e.g. Arnott et al. 2003; Anneville et al. 2005; Rusak et al. 2008; Vogt et al. 2011; Angeler and Johnson 2012) given the lack of long-term monitoring data spanning longer time periods. In fact, strength of biological synchrony between sites may vary with time scale of investigation, as different mechanisms can drive synchrony at different time scales (Keitt and Fischer, 2006; Sheppard et al., 2016; Vogt et al., 2011). To this end, Patoine and Leavitt (2006) demonstrated the potential of using fossil algal pigments archived in lake sediment to assess synchrony at centennial or longer time scales. Similarly, analysis of chitinous fossil remains of Cladocera offers an excellent opportunity for centennial-scale investigation of biological synchrony in response to climatic forcing from a community perspective, as most zooplankton species are sensitive to direct physiological effects of elevated temperature and ultraviolet radiation, as well as indirect effects through food-web interactions (e.g. changes in food availability, predation and competition) (Adrian and Deneke, 1996; Alric et al., 2017; Jeppesen et al., 2014; Rusak et al., 2008; Straile and Adrian, 2000; Winder and Schindler, 2004).

Here, we used time series of fossil cladoceran remains to investigate synchrony among zooplankton communities in six boreal lakes of central Canada during the 20th century. Recent paleolimnological studies suggested a major role of climate in regulating biological changes in lakes of this region over the past century (Curtis et al., 2010; Hazewinkel et al., 2008; Kurek et al., 2013; Laird et al., 2017, 2013; Mushet et al., 2017; Summers et al., 2017, 2016). In addition, lakes in this region are variably exposed to deposition of atmospheric pollutants (sulphate, nitrogen oxides, trace metals, polycyclic
aromatic compounds) from oil sands operations in northern Alberta, especially since ca. 1980 with the intensification of bitumen extraction and upgrading in the Athabasca oil sands region (AOSR) (Kirk et al., 2014; Makar et al., 2018; Percy, 2013). However, consensus is still lacking regarding the spatial scale and degree of impact of industrial deposition on lake ecosystems (Anas et al., 2017, 2014a; Kurek et al., 2013; Laird et al., 2013; Parsons et al., 2010; Summers et al., 2016). We hypothesized that cladoceran compositional turnover (CCT) would be highly synchronous among lakes prior to1980, in consistently with climatic impacts detected in prior studies in the region. We expected CCT to be idiosyncratic post-1980 in response to varying intensities of atmospheric pollutant deposition among basins (i.e. local scale effect). Beyond identifying the potential impacts of AOSR emissions on CCT, our findings may have broader implications for zooplankton-based bioassessment of lakes across boreal landscapes.

3.3. Methods

Study lakes

The study lakes are a subset of 10 lakes in northwest Saskatchewan described in Laird et al. (2013). The lakes were originally selected from a dataset of 259 lakes surveyed by the provincial government from 2007 to 2009 within ~ 300 km east of Fort McMurray (Scott et al., 2010). Of the 10 lakes cored, the six examined here (Fig.3.1) were chosen to include a range of distances from center of Athabasca AOSR (85, 122, 138, 160, 196, 221 km) representing varying vulnerabilities to atmospheric deposition of industrial pollutants (Fenn et al., 2015). Yet, geographical vicinity of the study lakes (i.e. distance between lakes < 143 km) places them within a similar climatic regime
Fig. 3.1. Sampling domain and locations of six study lakes in northwest Saskatchewan.

AOSR = Athabasca oil sands region
characterized by increased temperature over the past ~50 years (Kurek et al., 2013; Zhang et al., 2000).

The selected lakes are headwater lakes with relatively undisturbed catchments, and all except lake 10W are underlain by the Precambrian Shield. They are mostly shallow (maximum depth < 10 m) and small (< 45 ha), with the exception of relatively large lake 15J (Table 3.1). The climatic exposure index (ratio of lake area to maximum depth or lake area^2/lake volume; Rusak et al. 1999), which is an important determinant of extent of lake exposure to climatic factors (Kratz et al., 1998; Magnuson et al., 1990), varied by two orders of magnitude among lakes. The lakes are circumneutral (pH range 6.8-7.3), while being sensitive to acidic deposition (organic acid adjusted acid neutralizing capacity < 200 μeq L^{-1}; Scott et al. 2010). Furthermore, they are largely oligotrophic as reflected by low total phosphorous (TP; range 6.3-16.2 μg L^{-1}), total nitrogen (TN; range 286-694 μg L^{-1}) and chlorophyll a (range 2.0-7.7μg L^{-1}) concentrations. The ranges of dissolved organic carbon (DOC) concentration (2.5-7.1 mg L^{-1}) and water color (2.1-19.7 mg L^{-1} Pt) indicate varying importance of allochthonous organic matter inputs in these lakes (Wetzel, 1983). We refer to Scott et al., (2010) and Anas et al., (2014) for detailed descriptions of survey domain, water chemistry and hydromorphological attributes of study lakes.

**Sediment coring**

Sediment cores were retrieved in March 2010 using a Glew gravity corer with a 7.62 cm internal diameter tube. The length of the cores varied from 24 to 60 cm, which were sectioned into 0.5 cm intervals upon return to the base camp. Samples were shipped
### Table 3.1. Limnological characteristics of cored lakes

<table>
<thead>
<tr>
<th>Variable</th>
<th>10W</th>
<th>12D</th>
<th>15J</th>
<th>7A</th>
<th>8H</th>
<th>9D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevation (m)</td>
<td>535.00</td>
<td>516.00</td>
<td>544.00</td>
<td>517.00</td>
<td>551.00</td>
<td>529.00</td>
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<tr>
<td>Distance to AOSR (km)</td>
<td>85.14</td>
<td>196.28</td>
<td>221.00</td>
<td>160.90</td>
<td>121.70</td>
<td>138.38</td>
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<tr>
<td>Lake area (ha)</td>
<td>39.00</td>
<td>43.00</td>
<td>243.00</td>
<td>20.00</td>
<td>5.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Maximum depth (m)</td>
<td>6.82</td>
<td>6.36</td>
<td>6.26</td>
<td>4.00</td>
<td>4.09</td>
<td>8.96</td>
</tr>
<tr>
<td>Volume (m$^3$)</td>
<td>1,346,447</td>
<td>1,354,815</td>
<td>7,605,625</td>
<td>399,250</td>
<td>98,592</td>
<td>127,602</td>
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<td>Lake area/mean depth (m)</td>
<td>115,792</td>
<td>133,975</td>
<td>776,330</td>
<td>99,812</td>
<td>23,575</td>
<td>6,358</td>
</tr>
<tr>
<td>Chlorophyll a (μg L$^{-1}$)</td>
<td>4.51</td>
<td>7.71</td>
<td>2.61</td>
<td>4.07</td>
<td>5.30</td>
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<td>DIN (μg L$^{-1}$)</td>
<td>66.50</td>
<td>24.63</td>
<td>20.67</td>
<td>21.88</td>
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<td>286.75</td>
<td>342.00</td>
<td>494.00</td>
<td>571.50</td>
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<td>TP (μg L$^{-1}$)</td>
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<td>7.67</td>
<td>10.00</td>
<td>16.25</td>
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<td>DOC (mg L$^{-1}$)</td>
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<td>3.47</td>
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<td>8.58</td>
<td>19.67</td>
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<td>Specific conductivity (μS cm$^{-1}$)</td>
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<td>36.67</td>
<td>20.75</td>
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<td>Gran Alkalinity (mg L$^{-1}$ CaCO$_3$)</td>
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<td>2.81</td>
<td>3.99</td>
<td>1.58</td>
<td>2.03</td>
<td>1.96</td>
</tr>
<tr>
<td>ANC$_{oaa}$ (μeq L$^{-1}$)</td>
<td>48.37</td>
<td>66.31</td>
<td>80.63</td>
<td>36.13</td>
<td>45.67</td>
<td>46.15</td>
</tr>
</tbody>
</table>

Note: ANC$_{oaa}$ = Organic acid adjusted acid neutralizing capacity (i.e. ANC + 3.43 x [DOC])
to Queen’s University, Kingston, Ontario where they were stored at 4 °C in a cold room until further analyses.

Core chronologies

Details on radiometric dating (using $^{210}$Pb activities) and age models of the cores are described in Laird et al. (2013). Background level of $^{210}$Pb was reached at 20 cm in 15J and by 12-16 cm in all other lakes. In general, all cores examined here showed robust depth-time chronologies. The sediment accumulation rates of 7A, 8H and 15J were sufficient to achieve ~1-5 years temporal resolution between two adjacent samples (spaced 0.5 cm apart), while temporal resolutions ranged between ~1-9 years for remaining lakes (Laird et al., 2013).

Subfossil cladoceran analysis

Samples were prepared for cladoceran analysis following standard methods (Korhola and Rautio, 2001) with only minor deviations. For each interval, a sediment subsample of ~1 g was deflocculated in hot 10% KOH solution (~100 mL) for ~2 hours, then rinsed through 53 μm sieve. Subsequently, the residue retained in the sieve was transferred to a 5 mL vial using deionized water, along with few drops of ethanol and safranin solution to prevent fungal growth and to stain cladoceran remains, respectively. For preparation of slides, aliquots of uniformly mixed suspension were pipetted onto the glass slides, allowed to dry and mounted using glycerine gelly and a cover slip. Entire slides were enumerated under bright-field illumination at 400x magnification, while taxonomic identifications of fossil cladoceran remains were based on multiple sources (Bos, 2001; Korosi and Smol, 2012a, 2012b; Szeroczyńska and Sarmaja-Korjonen, 2007). Individual cladoceran remains (headshields, carapaces, postabdomen,
postabdominal claws, etc.) were tabulated separately and the number of individuals for each taxon was estimated based on the most abundant remain type (Frey, 1986). At least 100 cladoceran individuals were enumerated per sediment interval (based on multiple slides when necessary) to obtain a representative estimate of the cladoceran assemblage (Kurek et al., 2010).

Data analysis

To summarise the main variation in cladoceran species assemblages across all study lakes, a principal components analysis (PCA) was performed on Hellinger-transformed (Legendre and Gallagher, 2001) and centered (by partialling out among-lake variability) species abundances. Species rare in all study lakes (i.e. occurred in < 5% of intervals) were excluded from the analysis (Leps and Smilauer, 2003). PCA evaluates the correlation structure within the original data matrix and represents a significant portion of its variance in terms of new orthogonal variables (principal components), which can be used as traditional variables for further statistical analyses. Based on the broken-stick model (Borcard et al., 2011), the first two principal components (PC-1 and PC-2, which collectively explained 34% of the variation) were selected to represent the variation of the data.

Subsequently, generalized additive models (GAMs) were used to test if temporal trends in PC-1 and PC-2 scores were significantly different among study lakes by modifying the approach of Rose et al. (2012). GAMs are a semi-parametric regression form, which models non-linear relationships between predictors and response in terms of smooth functions of predictors (Wood, 2017). For each lake, we fitted an ordered-factor-smooth interaction model of the form:
\[ y_{ij} = \alpha_0 + \alpha_{1j}\text{(lake}_j) + f_1(\text{time}_i) + f_2(\text{time}_i) + \varepsilon_i, \varepsilon_i \sim N(0, \sigma^2), \]

where lake is an ordered factor variable representing six study lakes, whilst using a pre-defined lake as the reference level. Here response \( y_{ij} \) was modelled as the mean value of the response in the reference lake (model intercept \( \alpha_0 \)), plus the difference between mean response of the \( j \)th lake and \( \alpha_0 (\alpha_{1j}) \), plus the centered smooth function of time (sediment age in years AD) for the reference lake \( (f_1) \), plus the difference between smooth function of time for the \( j \)th lake and \( f_1 (f_{2j}) \), plus model residuals \( (\varepsilon_i) \) which are assumed to be Gaussian distributed with mean 0 and variance \( \sigma^2 \). In other words, the model tests i) if the trend (change over time) of PC-1 or PC-2 scores for a selected reference lake was statistically significant and ii) if trends (and means) of PC-1/PC-2 of other study lakes were significantly different from that of the reference lake. We iterated the model using each study lake as the reference level and contrasted other study lakes against it. We interpreted a significant difference \( (p < 0.05) \) in at least one of PC-1 and PC-2 trends between a pair of lakes as lack of synchrony in CCT.

Our GAM-based approach has a couple advantages over conventional correlation-based approaches for assessing synchrony between two time series. First, the synchrony / asynchrony between two time series may vary through time (Post and Forchhammer, 2004; Sheppard et al., 2016), which can be detected by plotting the difference in trends of two time series along with corresponding 95% point-wise confidence intervals against time (see results section). In such plot, inclusion of zero within the confidence interval during a particular time period indicates that trends are not significantly different \( (p > 0.05) \) or they are synchronous within the specific time frame. The corresponding F-test provides an overall evaluation of whether trends were
significantly different for a substantial duration within the time period of investigation. Second, our approach simultaneously compares timing, direction and pace of change in trends between two time series. Yet, this procedure can be tedious when large number of pair-wise comparisons is involved.

All statistical analysis were performed in R version 3.4.3 (R Core Team, 2017) using the packages ‘vegan’ (Oksanen et al., 2015), ‘mgcv’ (Wood, 2017; Wood et al., 2016), ‘ggplot2’ (Wickham, 2009) and ‘ggvegan’ (Simpson, 2017).

3.4. Results

Cladoceran species associations

Despite differences in cladoceran species composition among lakes (Appendix B: Fig.B1), PCA revealed two common CCT patterns across all study lakes. Along PCA axis 1 (PC-1, 22% of the variation), relative abundances of Paralona pigra, Acantholeberis curvirostris and Alona affinis were negatively related to abundances of Alona quadrangularis and Chydorus brevalabris (Fig.3.2a). PCA axis 2 (PC-2, 12% of the variation) mainly represented the negative association of Bosmina longirostris and Alona excisa with Alona guttata and Alonella nana (Fig.3.2a).

Temporal trends in cladoceran composition

GAM analysis revealed significant trends in cladoceran composition of study lakes represented by PC-1 and PC-2 scores. GAMs, which explained 65% (adjusted R²) of temporal variability in PC-1 scores, identified statistically significant trends (p < 0.05) in all lakes except 12D (Fig.3.3a). PC-1 temporal trends were more pronounced for 7A and 10W compared to other lakes. On the other hand, significant trends in PC-2 scores
Fig.3.2. Principal components analysis (PCA) biplots of cladoceran species composition of study lakes; (a) Correlation biplot displaying relationships among species; (b) Distance biplot showing Euclidean distances between time points (years) in terms of species composition; See Appendix B: Fig.B2 for distance biplots of individual lakes;

Blong = *Bosmina longirostris*; Dpule = *Daphnia pulex* complex; Acurv = *Acantholeberis curvirostris*; Aharp = *Acroperus harpae*; Aaffi = *Alona affinis*; Agutt = *Alona gutta*; Aquad = *Alona quadrangularis*; Aexcis = *Alonella excisa*; Anana = *Alonella nana*; Cbrev = *Chydorus brevilabris*; Cling = *Chydorus linguilabris*; Dauti = *Disparalona acutirostris*; Euryc = *Eurycercus* sp.; Laton = *Latona* sp.; Mdisp = *Monospilus dispar*; Ograc = *Ophryoxus gracilis*; Ppigr = *Paralona pigra*; Scrys = *Sida crystallina Americana*
Fig. 3.3. Temporal trends of cladoceran compositional turnover in study lakes; (a) Trends of PC-1 scores; (b) Trends of PC-2 scores; Solid lines denote the fitted trends (smooth functions) and shaded regions represent the point-wise approximate 95% confidence intervals; Dashed vertical blue line denotes the onset of intensified oil sands production.
were detected by GAMs only for lakes 9D and 12D (Fig. 3.3b), with 45% of temporal variation explained. A linear and relatively intense PC-2 historical trend was detected for 9D, contrary to non-linear and more subtle trend detected for 12D.

*Synchrony in cladoceran compositional turnover*

The PCA distance biplot (Fig. 3.2b) provides a visual indication of lack of temporal coherence in CCT among study lakes. The time points (years; represented by colours) are mostly randomly scattered across the ordination space of PCA distance biplot. This is in contrast to expected systematic distribution of time points across the ordination space (indicated by a smooth colour gradient across the plot) if compositional changes were coherent among lakes.

Results of GAM analysis further supported asynchrony in CCT among study lakes. Despite qualitative similarities in temporal trends of PC-1 scores (representing CCT patterns) between lakes (in terms of nature of change; Fig. 3.3), they were significantly different (p < 0.05) between all lake pairs except between 9D and 12D and between 9D and 15J (Table 3.2 and Fig. 3.4a). Significant differences (p < 0.05) in temporal trends in PC-2 scores were detected for half of the pair-wise comparisons among lakes (Table 3.3 and Fig. 3.4b). Overall, significant differences (p < 0.05) were detected for at least one of PC-1 and PC-2 trends for all lake pairs. In any case where significant trend differences were detected between lakes, the divergence of trends was not exclusive to post 1980s period i.e. intensification of oil sands production (Fig. 3.4).
Table 3.2. Model summaries for time series of PC-1 scores

<table>
<thead>
<tr>
<th>Covariate</th>
<th>EDF</th>
<th>Ref. DF</th>
<th>F-statistic</th>
<th>p-value</th>
</tr>
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<tr>
<td><strong>Lake-specific smoothers</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Trend\textsubscript{7A}</td>
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<td>0.52</td>
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</tr>
<tr>
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<td>1.73 \times 10^{13}</td>
</tr>
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Note: EDF = Effective degrees of freedom; Ref. DF = Reference degrees of freedom
Table 3.3. Model summaries for time series of PC-2 scores

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<th>p-value</th>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0.21</td>
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<tr>
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<td>22.42</td>
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<td>0.35</td>
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<tr>
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</tr>
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</tr>
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Note: EDF = Effective degrees of freedom; Ref. DF = Reference degrees of freedom
Fig. 3.4. Differences in trends of cladoceran compositional turnover between lake pairs; (a) Differences in trends of PC-1 scores between lakes; (b) Differences in trends of PC-2 scores between lakes; Solid lines denote the estimated differences in trends (difference smooths) and shaded regions represent the point-wise approximate 95% confidence intervals.
3.5. Discussion

We identified two independent CCT patterns in our study lakes along the first two PCA axes, which appeared to arise from different ecological affinities of species. Despite similarities between lakes with respect to geomorphological and limnological characteristics, trajectories of CCT were significantly different among lakes in terms of timing, direction and pace of change. Such widespread asynchrony indicates dominant, confounding effects of local factors over climatic forcing of CCT, which may have important implications for zooplankton-based bioassessments across broad geographical regions.

Cladoceran compositional turnover patterns

It is impossible to unambiguously identify the mechanistic drivers of observed CCT patterns due to lack of historical data for the study region, yet certain speculations can be made based on literature. The CCT pattern represented by PC-1 (Fig.3.2a) i.e. increased abundances of A. curvirostris, P. pigra and A. affinis (Hann and Turner, 2000; Havens, 1991; Korosi and Smol, 2012c; Nilssen and Sandoy, 1990), typically known to be acid-tolerant species with simultaneous decrease in abundances of A. quadrangularis and C. brevilabris, characteristic of alkaline conditions (Hann and Turner, 2000; Korosi and Smol, 2012c; Krause-Dellin and Steinberg, 1986) indicates a possible role of acid-base status in regulating community dynamics via direct, physiological effects. It is also possible that pH changes induced the same community pattern indirectly via shifts in macrophyte composition (Korosi and Smol, 2012c). For instance, increased abundances of Sphagnum species with increasing acidity (Lacoul et al., 2011) may have favored Sphagnum colonizers such as A. curvirostris and P. Pigra (Fryer and Biological, 1980;
Kattel et al., 2006; Korosi and Smol, 2012c; Nilssen and Sandoy, 1990). Alternatively, the same turnover pattern may also be related to changes in primary productivity, given that *A. quadrangularis* and *C. brevilabris* are characteristic of highly productive conditions (Amsinck et al., 2006; Bos and Cumming, 2003; Jeppesen et al., 2001; Whiteside, 2009), whereas *P. pigra* and *A. affinis* are typically associated with oligotrophic conditions (Amsinck et al., 2006; Bos and Cumming, 2003; Brodersen et al., 1998; Jeppesen et al., 2001; Whiteside, 2009). The negative association of *B. longirostris* and *A. excisa* abundances with those of *A. nana* and *A. guttata* (represented by PC-2; Fig.3.2a) may also be influenced by acid-base status, as the former set of species are known to be acid-tolerant (Adamczuk, 2016; Brett, 1989; DeSellas et al., 2007; Hann et al., 1994; Krause-Dellin and Steinberg, 1986; Nilssen and Sandoy, 1990), whereas species of the latter group are acid-sensitive (Bjerring et al., 2009; Hann et al., 1994; Hann and Turner, 2000; Korosi and Smol, 2012c; Nilssen and Sandoy, 1990; Paterson, 1994).

Possibly, changes in acid-base status and productivity reflected by cladoceran community dynamics were induced by climatic forcing. As the changes in cladoceran communities occurred through the past century (i.e. not restricted to post-1980 after intensification of oil sands production) and these remote systems were not being subjected to other major anthropogenic influences operating at centennial scales, regional climate variability (Zhang et al., 2000) is likely the main extrinsic forcing for temporal changes of limnological parameters. Climatologic factors may influence acid-base status of lakes through several mechanisms. Increased water residence times due to warmer and drier conditions can lead to greater retention and evapo-concentration of
base cations in lakes (Baines et al., 2000; Schindler, 1997; Vogt et al., 2011; Webster et al., 2000). Furthermore, increased duration of ice cover with low winter temperatures may prolong organic matter degradation, resulting in enhanced carbonic acid formation under ice (Vogt et al., 2011). On the other hand, increased runoff and groundwater inputs with precipitation changes can lead to base cation dilution (Adrian et al., 2009; Baines et al., 2000; Hessen et al., 2017; Magnuson et al., 1990). Climatic factors may also influence primary production in lakes through changes in internal and external nutrient loading, stratification length and intensity, degree days and ice-free period (Adrian et al., 2009; Baines et al., 2000; Finlay et al., 2015; Patoine and Leavitt, 2006).

**Asynchrony in cladoceran compositional turnover**

Contrary to our expectation, CCT patterns are largely asynchronous among study lakes, suggesting that any climate signal in cladoceran temporal dynamics is likely obscured by dominant lake-specific effects. As discussed above, climatic regulation of cladoceran community dynamics likely involved indirect and complex pathways (i.e. climate → changes in acidity/productivity [→ changes in microhabitat structure] → changes in cladoceran dynamics), where local factors may have introduced noise to the climatic signal at each link of the pathway (Blenckner, 2005). For instance, hydrogeomorphological differences among lakes may lead to differential climatic influence on acid-base status and productivity (Adrian et al., 2009; Baines et al., 2000; Schindler, 1997; Webster et al., 2000). In addition, lake-specific characteristics such as lake topography, wave exposure, water level fluctuations, light penetration and sediment quality may obscure the effects of climate-induced acidity/productivity changes on macrophyte composition (Alahuhta et al., 2011). In addition to above potential
mechanisms, cladoceran dynamics in lakes may have been at least partially influenced by other lake-specific factors (e.g. predation and other food-web dynamics), which may have responded individually to climatic forcing (Jeppesen et al., 2014; Perga et al., 2015; Woodward et al., 2010).

Past studies detected higher coherence in limnological variables between lakes that were more similar to each other in terms of lake morphometry (Kratz et al., 1998; Magnuson et al., 1990), fertility (Rusak et al., 2008) and landscape position (Webster et al., 2000). However, our findings did not show a clear association between synchrony between lakes and lake similarity with respect to the above characteristics. Although some lake pairs in our study were highly similar to each other in terms of morphometric factors such as lake area, maximum depth and climatic exposure (e.g. 10W and 12D) and variables related to fertility including chlorophyll a and DOC concentrations (e.g. 15J and 9D), the trajectories of CCT were still largely idiosyncratic as detected for other lake pairs. Meanwhile, the effect of landscape position on synchrony among study lakes should be minimal as these headwater systems (Scott et al., 2010) are of similar hydrological order (Webster et al., 2000). However, differences among lakes with respect to detailed hydrological features (e.g. groundwater inputs), which could also influence the similarity of climate signal among lakes (Webster et al., 2000), are presently poorly known.

**Impacts of industrial deposition from AOSR**

We did not detect any specific changes in trajectories of CCT corresponding to intensified industrial development ca. post 1980 in any of the study lakes, indicating a limited effect of atmospheric deposition of industrial pollutants on cladoceran
community dynamics. Furthermore, as asynchrony in CCT was not exclusive to ca. post 1980 (in contrast to our expectation), unravelling the effects of industrial deposition from climatic forcing is impossible. While speculative, such conclusion is in agreement with other paleolimnological proxies from the same sediment cores, including diatom assemblages, scaled-crysophytes, trace metals (Laird et al., 2013) and polycyclic aromatic hydrocarbons (Ahad et al., 2015), as well as other paleolimnological studies in the region (Hesjedal, 2017; Laird et al., 2017; Mushet et al., 2017), which indicated only limited industrial impacts on these lakes.

3.6. Conclusions

Our findings indicate possible climatic influence on CCT in our study lakes via indirect pathways, yet trajectories of CCT are largely asynchronous among lakes. This is in agreement with the notion that confounding effects of local factors are dominant when climatic forcing of particular parameter is indirect (Arnott et al., 2003; Magnuson et al., 1990; Vogt et al., 2011). Such lack of synchrony cautions against extrapolating site-specific results across broader regions (Huttunen et al., 2014; Magnuson et al., 1990), which has two important implications for using zooplankton communities as biological indicators to assess regional impacts. First, intensive replication is necessary for zooplankton-based bioassessments. Second, improved understanding of local processes influencing zooplankton community dynamics is required to better predict their responses to regional stressors. It remains to be explored if asynchrony in zooplankton compositional turnover is widespread in other geographical areas with different geomorphological, hydrological and climatological settings.
3.7. Acknowledgements

We thank Alison Walker, Buddhine Meegahage and Steve Wilkie for technical support. We are grateful to Oil Sands Quest Inc. for allowing us to use of their facilities during the fieldwork. This work was supported by the Saskatchewan Ministry of Environment and University of Regina.
CHAPTER 4. WATER CHEMISTRY, LANDSCAPE AND SPATIAL CONTROLS OF δ^{13}C AND δ^{15}N OF ZOOPLANKTON TAXA IN BOREAL LAKES: ONE SIZE DOESN’T FIT ALL

4.1. Abstract

Del^{13}C and δ^{15}N of zooplankton are common proxies to infer carbon and nitrogen sources and their processing in lake food-webs. Yet, their broad-scale environmental controls are poorly understood due to lack of landscape perspective, limited taxonomic resolution, small sample sizes/spatial extents in previous synoptic surveys. Here we evaluated the variation in δ^{13}C and δ^{15}N of taxon-specific zooplankton (nine species/genus) from 233 boreal lakes in relation to water chemistry (CHEM), hydromorphological and land cover variables (HYDR_LAND) and spatial position of the lakes in the landscape (SPATIAL). CHEM variables associated with allochthonous inputs (color/dissolved organic carbon) and nutrient concentrations had respective negative and positive associations with δ^{13}C of most taxa. Del^{13}C variation explained by significant HYDR_LAND variables (%peat area, drainage basin area:lake area ratio, shoreline development index and lake area) was largely shared by significant CHEM variables, indicating their likely indirect effects. Together, this suggests that δ^{13}C variation of zooplankton is reflective of differences in δ^{13}C of lake primary producers due to environmental influences. Del^{15}N variation of most taxa was significantly related to pH, total phosphorous concentration (CHEM variables) and water retention time (a HYDR_LAND variable), with their relationships being largely independent. Del^{15}N variation of zooplankton was likely induced by effects of environmental variables on biogeochemical nitrogen transformation processes and phytoplankton^{15}N fractionation.
Specific CHEM and HYDR_LAND predictors of $\delta^{13}$C and $\delta^{15}$N and direction of their effects (i.e. positive or negative) were largely similar among taxa. Yet, their degree of importance varied among taxa particularly for $\delta^{13}$C, likely due to contrasting feeding selectivity and resulting differences in allochthon. This suggests $\delta^{13}$C of different taxa may respond differently to changes in limnological gradients due to environmental perturbations affecting boreal regions. The relative effects of lake/catchment-specific factors on $\delta^{13}$C and $\delta^{15}$N variation of zooplankton were more important than modeled SPATIAL factors attributable to potential regional factors (lithology, soil properties, atmospheric nitrogen deposition). Hence, before inferring any regional effects in stable isotope studies, importance of lake/catchment-specific factors needs to be explicitly quantified.

4.2. Introduction

Lake ecosystems are regulated by range of forcing factors, such as climate, hydrogeomorphology, land cover and land use (Anas et al., 2015; Leavitt et al., 2009), that interact at varying spatial and temporal scales. While conventional site-based or experimental studies have limited potential to evaluate hierarchical effects of these factors on ecosystem processes (Leavitt et al., 2009; Soranno et al., 2010), recent studies conducted over broad spatial scales provided powerful insights into their unique and interactive effects (e.g. Quinlan et al., 2003; Soranno et al., 2010; Read et al., 2015). Accordingly, spatially-explicit approaches are required to quantify the relative importance of forcing factors operating at different scales, i.e. relative effects of lake/catchment-specific factors (e.g. water chemistry, land cover, morphology) versus regional factors (e.g. climate, geology, atmospheric deposition) on ecosystem processes.
Effects of lake/catchment-specific factors and regional factors are often reflected in the among-lake variation of biological and chemical proxies. In this context, carbon and nitrogen stable isotope (SI) analyses of aquatic fauna have become a powerful tool in aquatic sciences. Because carbon SI ratios ($\delta^{13}C$) are transferred from diet to consumers with little modification (DeNiro and Epstein, 1978), $\delta^{13}C$ variation of consumers is associated with relative dietary importance of basal resources (i.e. pelagic, benthic, littoral, terrestrial) (del Giorgio and France, 1996; Grey et al., 2000), inorganic carbon sources (atmospheric, geogenic, respired) of primary producers, and magnitude of inorganic carbon limitation for primary production (France et al., 1997; Raven et al., 1994). Therefore, among-lake $\delta^{13}C$ variation of consumers can be related to water chemistry parameters associated with autochthonous primary production (e.g. nutrient and chlorophyll concentrations) and allochthonous carbon inputs (e.g. water color, dissolved organic carbon concentration, partial pressure of CO$_2$) (France et al., 1997; Grey et al., 2000; Jones et al., 1999; Persaud et al., 2009). Hydromorphological and land-cover attributes, such as catchment area and slope, lake area, drainage ratio, water retention time, vegetation type and coverage can influence above chemical parameters (Kortelainen, 1993; Rasmussen et al., 1989; Xenopoulos et al., 2003) and may indirectly be correlated with $\delta^{13}C$ variation of consumers. In addition, regional differences in bedrock geology may influence $\delta^{13}C$ of inorganic carbon used by aquatic primary producers over broad geographical scales (Campeau et al., 2017; Lennon et al., 2006) and by extension, that of consumers.

Nitrogen SI ratios ($\delta^{15}N$) of consumers are indicative of their trophic position within lakes, given a consistent $^{15}N$ enrichment (2 to 4‰) relative to their diet (Cabana
and Rasmussen, 1994). Among-lake $\delta^{15}$N variation of consumers within a trophic level can reflect $\delta^{15}$N variability of basal resources induced by lake/catchment-specific factors as well as regional factors. For instance, among-lake $\delta^{15}$N variation of phytoplankton can be related to nutrient loads to lakes that influences phytoplankton $^{15}$N fractionation (Meyers and Teranes, 2001). In addition, nitrogen isotopic fractionation occurs during catchment and in-lake biogeochemical transformations (e.g. ammonification, nitrification, denitrification). Hence, among lake differences in these processes induced by lake/catchment-specific factors such as pH, water retention time, and terrestrial vegetation characteristics can impact $\delta^{15}$N of organic and inorganic nitrogen pools (Bunting et al., 2010; Grey et al., 2001; Kendall, 1998; Robinson, 2001; Rudd et al., 1988), which are eventually incorporated into the pelagic food web (Karlsson et al., 2004). Regional differences in nitrogen sources with distinct isotopic values (e.g. atmospheric deposition, agricultural, urban) can correspond to $\delta^{15}$N variation in inorganic nitrogen assimilated by lacustrine primary producers, which is passed on to consumers (Cabana and Rasmussen, 1996; Elliott et al., 2007; Vander Zanden et al., 2005). However, the relative importance of such lake/catchment-specific and regional factors on regulating the SI variation of consumers has not been explicitly studied.

Zooplankton, as key secondary producers in lake ecosystems, play an important role in supporting the predator chains (Detmer et al., 2017). Analyses of their dietary contributions to predators such as fish are often based on SI analyses of carbon and nitrogen (Cabana and Rasmussen, 1994; Persaud and Dillon, 2010; Vander Zanden and Rasmussen, 1999). While among-lake variability in $\delta^{13}$C and $\delta^{15}$N of these secondary producers can be induced by a range of forcing factors (as mentioned above), their
effects may vary among taxa with different feeding modes and/or habitat specializations due to likely dietary differences (Kling et al., 1992; Matthews and Mazumder, 2003; Persaud et al., 2009). For instance, factors controlling primary production can be strongly correlated to δ¹³C variation of selective feeders of phytoplankton relative to non-selective feeders, whereas factors related to allochthonous inputs may be more influential for the latter. Meanwhile, the relative dietary importance of different zooplankton taxa to different predators changes with behavioural and structural characteristics of both prey and predators (O’Brien, 1979). Therefore, an improved understanding of taxon-specific controls of zooplankton SI values is required to better use SI to assess how these different secondary producers support predator chains in among-lake comparisons (Grey et al., 2002).

Over the past two decades, several synoptic SI surveys of lake zooplankton were conducted (del Giorgio and France, 1996; France et al., 1997; Grey et al., 2000; Jones et al., 1999; Karlsson et al., 2004, 2003; Mohamed and Taylor, 2009; Persaud et al., 2009; Rautio and Vincent, 2007). However, SI variability of zooplankton (primarily δ¹³C) was evaluated mostly in relation to autochthonous and allochthonous carbon inputs (e.g., water color, concentrations of dissolved organic carbon (DOC), nutrients and chlorophyll, isotopic composition of dissolved and particulate organic matter) or their derivatives, resulting in limited understanding of landscape-level controls (i.e. lake/catchment-specific and regional) of isotopic variability in zooplankton. Furthermore, broad-scale patterns can be obscured by minor sources of variability in studies with smaller sample sizes and/or conducted at small spatial scales (Kendall et al., 2010). Hence, the generality of patterns detected by previous studies should be assessed.
across a larger number of systems (compared to 12-52 lakes in above mentioned studies) encompassing broad environmental gradients (Wilkinson et al., 2013), while testing for their inter- and spatial dependencies. Finally, previous studies relied on isotopic composition of either bulk or coarse taxonomic resolution of zooplankton (but see Persaud et al., 2009), which likely masks potential taxon-specific differences in environment-SI relationships due to differential feeding modes and habitat specializations (Berggren et al., 2014; Kling et al., 1992; Matthews and Mazumder, 2003; Persaud et al., 2009).

Here, we present the results of a synoptic 233-lake survey across the boreal region of northwest Saskatchewan, Canada to evaluate landscape-level controls of $\delta^{13}C$ and $\delta^{15}N$ of several zooplankton taxa ($\delta^{13}C_{ZOOP}$ and $\delta^{15}N_{ZOOP}$). We analyzed $\delta^{13}C_{ZOOP}$ and $\delta^{15}N_{ZOOP}$ variations in relation to a large suite of lake/catchment-specific factors including water chemistry (CHEM), hydromorphological and land cover (HYDR_LAND) variables as well as spatial position of the lakes in the landscape (SPATIAL). Our previous work showed that several CHEM variables including alkalinity, color, DOC, nutrient loads and chlorophyll concentrations are largely influenced by HYDR_LAND attributes and to a lesser degree by spatial position of these lakes (Anas et al., 2014a; Scott et al., 2010). Effects of spatial position alone on SI variation, i.e. independent of its effects through lake/catchment-specific factors, can be attributable to the effects of regional-scale factors that were not explicitly measured in the present study (Anas et al., 2014a; Beisner et al., 2006). For instance, spatial variations in bedrock geology, soil properties (Shewchuk, 1982; Whitfield and Watmough, 2012) and potential atmospheric nitrogen deposition from nearby Athabasca
oil sands operations (Makar et al., 2018) across the region may influence among-lake SI variability at the food-web base (Aravena et al., 1992; Elliott et al., 2007).

Based on existing knowledge of limnological and regional characteristics of the study domain, δ\(^{13}\)C and δ\(^{15}\)N variation in zooplankton and other aquatic organisms (see above) and dietary niche differences among zooplankton taxa (Barnett et al., 2007; Berggren et al., 2014; Matthews and Mazumder, 2003), we hypothesized that 1) lake/catchment-specific (CHEM + HYDR_LAND) controls of SI composition vary among zooplankton taxa with different dietary niches, 2) across all taxa, lake/catchment-specific factors are more important than regional factors in regulating δ\(^{13}\)C and δ\(^{15}\)N, and 3) of the variation explained by lake/catchment-specific factors in all taxa, a major fraction is shared by effects of CHEM and HYDR_LAND. Subsequently, we discuss implications of our findings in the context of isotope-based ecological studies.

4.3. Methods

Lake survey

The study lakes were part of a broad-scale, lake assessment program in the boreal region of northwest Saskatchewan conducted by the provincial government from 2007 to 2009. In total 244 headwater lakes were surveyed during fall turnover, from which 57 were sampled in all three years, 84 were sampled twice and 103 were sampled once. The replication resulted from staggered lake sampling over three years aimed to establish a spatially-distributed statistical assessment of headwater lakes in the region. Of the 244 lakes, 233 had adequate zooplankton samples for SI analysis (Fig.4.1). When the same lake was surveyed in multiple years, we only selected data from a single year (according to the preferential order of 2009>2008>2007; as most lakes were sampled in
2009 followed by 2008 and 2007) to avoid temporal variability included in the analyses. We refer to Scott et al., (2010) and Anas et al., (2014) for detailed descriptions of survey domain, lake selection criteria, derivation of catchment and hydromorphological attributes of study lakes, collection and processing of water and zooplankton samples, analytical procedures for water chemistry parameters and zooplankton taxonomy.

**Study lakes**

The study lakes represented broad gradients in chemical, hydromorphological and land-cover attributes (Table 4.1), mostly with skewed distributions indicated by substantial discrepancies between mean and median values. Lake area (LA) and maximum lake depth (MaxD) varied by more than an order of magnitude (~15-360 ha and 1-20 m respectively). Catchment area (CA) also varied substantially (~70-4,080 ha), with primarily forest-dominant land covers (median 72%). Trophic state of these lakes ranged from oligotrophic to slightly eutrophic (based on [TP], [TN] and [Chl a]), but most lakes were oligotrophic (median [TP], [TN] and [Chl a] = 11 µg L⁻¹, 405 µg L⁻¹ and 4 µg L⁻¹, respectively) (Forsberg and Riding, 1980). TN in lakes was mostly organic indicated by high concentration of dissolved organic nitrogen (DON) relative to dissolved inorganic nitrogen (DIN) (median [DON] and [DIN] = 311 µg L⁻¹ and 23 µg L⁻¹, respectively). In general, the study lakes were slightly colored (median color 16.4 mg L⁻¹ Pt) and pH was marginally acidic to circum-neutral (~6.1-7.7).

**SI analysis**

As zooplankton samples were preserved in 10% formalin, they were filtered through a 55 µm sieve and rinsed three times with deionized water to remove any formalin residue. The effects of formalin preservation on zooplankton SI values are
Fig. 4.1. Sampling domain and locations of 233 study lakes in northwest Saskatchewan
Table 4.1. Limnological characteristics of the 233 study lakes located across northwest Saskatchewan.

<table>
<thead>
<tr>
<th>Variable/s</th>
<th>Abbreviation</th>
<th>Mean</th>
<th>Median</th>
<th>5&lt;sup&gt;th&lt;/sup&gt; percentile</th>
<th>95&lt;sup&gt;th&lt;/sup&gt; percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hydromorphological and land cover variables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lake area (ha)</td>
<td>LA</td>
<td>172.15</td>
<td>65.97</td>
<td>14.52</td>
<td>360.23</td>
</tr>
<tr>
<td>Maximum lake depth (m)</td>
<td>MaxD</td>
<td>7.02</td>
<td>4.71</td>
<td>1.32</td>
<td>20.85</td>
</tr>
<tr>
<td>Lake volume (m&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>LV</td>
<td>8.36 x 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>1.49 x 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>1.70 x 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>165 x 10&lt;sup&gt;7&lt;/sup&gt;</td>
</tr>
<tr>
<td>Water retention time (years)</td>
<td>RT</td>
<td>2.93</td>
<td>2.00</td>
<td>0.42</td>
<td>8.85</td>
</tr>
<tr>
<td>Shoreline development index</td>
<td>SDI</td>
<td>3.42</td>
<td>3.05</td>
<td>2.27</td>
<td>5.79</td>
</tr>
<tr>
<td>Catchment area (ha)</td>
<td>CA</td>
<td>5160.21</td>
<td>629.00</td>
<td>72.00</td>
<td>4077.40</td>
</tr>
<tr>
<td>Drainage basin area to lake area ratio</td>
<td>DBA:LA ratio</td>
<td>16.60</td>
<td>7.22</td>
<td>1.93</td>
<td>50.81</td>
</tr>
<tr>
<td>Catchment area slope (%)</td>
<td>CAS</td>
<td>3.38</td>
<td>3.10</td>
<td>1.00</td>
<td>7.30</td>
</tr>
<tr>
<td>% peat land cover in the catchment</td>
<td>% peat</td>
<td>13.10</td>
<td>8.60</td>
<td>0.20</td>
<td>40.08</td>
</tr>
<tr>
<td>% forest land cover in the catchment</td>
<td>% forest</td>
<td>67.05</td>
<td>72.00</td>
<td>18.28</td>
<td>92.84</td>
</tr>
<tr>
<td>% burnt land cover in the catchment</td>
<td>% burnt</td>
<td>14.69</td>
<td>3.80</td>
<td>0.00</td>
<td>67.86</td>
</tr>
<tr>
<td>Elevation (m)</td>
<td>Elev</td>
<td>456.44</td>
<td>483.00</td>
<td>298.20</td>
<td>541.40</td>
</tr>
</tbody>
</table>
### Water chemistry variables

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll a (µg L(^{-1}))</td>
<td>Chla</td>
<td>6.66</td>
<td>4.24</td>
<td>1.14</td>
<td>19.69</td>
</tr>
<tr>
<td>Total phosphorus (µg L(^{-1}))</td>
<td>TP</td>
<td>16.99</td>
<td>11.00</td>
<td>4.00</td>
<td>52.40</td>
</tr>
<tr>
<td>Total nitrogen (µg L(^{-1}))</td>
<td>TN</td>
<td>478.55</td>
<td>405.00</td>
<td>191.00</td>
<td>1060.80</td>
</tr>
<tr>
<td>Dissolved organic nitrogen (µg L(^{-1}))</td>
<td>DON</td>
<td>310.53</td>
<td>311.50</td>
<td>309.89</td>
<td>311.13</td>
</tr>
<tr>
<td>Dissolved inorganic nitrogen (µg L(^{-1}))</td>
<td>DIN</td>
<td>41.68</td>
<td>23.00</td>
<td>11.50</td>
<td>130.40</td>
</tr>
<tr>
<td>Particulate nitrogen (µg L(^{-1}))</td>
<td>PN</td>
<td>126.34</td>
<td>89.00</td>
<td>29.60</td>
<td>382.80</td>
</tr>
<tr>
<td>Dissolved organic carbon (mg L(^{-1}))</td>
<td>DOC</td>
<td>7.11</td>
<td>6.20</td>
<td>2.06</td>
<td>14.58</td>
</tr>
<tr>
<td>Dissolved inorganic carbon (mg L(^{-1}))</td>
<td>DIC</td>
<td>2.68</td>
<td>1.60</td>
<td>0.60</td>
<td>7.68</td>
</tr>
<tr>
<td>Particulate carbon (µg L(^{-1}))</td>
<td>PC</td>
<td>1251.69</td>
<td>833.20</td>
<td>334.60</td>
<td>3694.08</td>
</tr>
<tr>
<td>Color (mg L(^{-1}) Pt)</td>
<td>Color</td>
<td>33.84</td>
<td>16.36</td>
<td>2.98</td>
<td>125.48</td>
</tr>
<tr>
<td>Gran alkalinity (µeq L(^{-1}))</td>
<td>GAlk</td>
<td>10.55</td>
<td>5.83</td>
<td>1.43</td>
<td>31.86</td>
</tr>
<tr>
<td>Specific conductivity (µS cm(^{-1}))</td>
<td>SpCond</td>
<td>25.38</td>
<td>16.00</td>
<td>8.00</td>
<td>64.60</td>
</tr>
<tr>
<td>pH</td>
<td>pH</td>
<td>6.98</td>
<td>7.02</td>
<td>6.13</td>
<td>7.72</td>
</tr>
</tbody>
</table>
likely minimal (Rennie et al., 2012; Sarakinos et al., 2002). We only selected zooplankton taxa abundant enough in the samples for stable isotope analysis (0.2-0.5 mg dry weight). Individuals belonging to each taxa were hand-picked with forceps under a dissecting microscope, packed in tin capsules, and dried at 50°C to constant weight.

All tin capsules were combusted in an Elemental Combustion System (Costech EA) that led into a Thermo-Finnigan Delta V isotope ratio mass spectrometer (IRMS). Bovine liver and wheat flour were used as internal laboratory standards. Carbon and nitrogen isotopes are reported in the conventional δ notation (‰), relative to Vienna Pee-Dee-Belemnite and atmospheric N₂ for carbon and nitrogen stable isotopes, respectively (Cooper and Wissel, 2012). Samples that were split in the laboratory and analyzed as duplicates (n=82) usually gave isotopic compositions that agreed within 0.2‰ range for both δ¹³C and δ¹⁵N.

In addition, we also analyzed carbon and nitrogen SI of dissolved organic matter (δ¹³C_{DOM} and δ¹⁵N_{DOM}) for a subset of 54 lakes representing the DOC/color gradient. 0.45 μm-filtered lake water samples (stored refrigerated in gas tight containers) were concentrated by freeze drying, and SI analysis was performed using the above procedure.

Despite the possibility that ¹³C-depleted lipids (relative to protein) in zooplankton could lead to lighter isotopic values (Kling et al., 1992), we chose not to mathematically correct δ¹³C_{ZOOP} for lipid content. First, lipids in zooplankton are primarily accumulated from dietary sources, with little de novo synthesis (Arts, 1998; Grosbois et al., 2017) and hence, mathematical lipid normalization of δ¹³C would exclude an important dietary source to zooplankton (Matthews and Mazumder, 2005). Second, lipid correction of δ¹³C is only required when variation in lipid content can confound the comparison of
δ¹³C values of zooplankton with other species (e.g. phytoplankton, fish) or among different zooplankton taxa (Matthews and Mazumder, 2003; Post et al., 2007). Here we evaluate the among-lake δ¹³C variation of each zooplankton taxon separately, where among-lake variation in lipid content of a given taxon is likely due to influences of environmental parameters (e.g. factors related to food availability) (Arts, 1998; Grosbois et al., 2017). Finally, carbon:nitrogen ratios (indicator of lipid content and main variable in lipid correction models) were largely constant among zooplankton taxa. Consequently, the correlations of carbon:nitrogen with δ¹³CZOOP of taxa were weak (Pearson’s correlation coefficient -0.28 to 0.38), contrary to the expected strong negative correlation if δ¹³CZOOP was largely impacted by lipid content (Matthews and Mazumder, 2005).

Data analysis

To distinctly identify environment-SI relationships for zooplankton taxa and DOM, we performed separate analyses with two predictor variable sets i.e. CHEM and HYDR_MORPH. The CHEM variable set consisted of eight parameters that can influence isotopic composition at the food-web base, namely pH, [DIC], [TN], [TP], [Chl a], [DOC], color and [DIN]. Meanwhile, the HYDR_LAND variable set included MaxD, LA, LV, RT, CA, shoreline development index (SDI), catchment area slope (CAS), elevation (Elev), %-peat, %-forest and %-burnt land cover in the catchment. Preceding the subsequent analyses, all variables except pH were either \( \log_{10} (x+0.01) \) or \( \log_{10} \)-transformed to achieve normality and homoscedasticity.

We performed multiple linear regressions to quantify significant relationships of above variables with carbon/nitrogen isotopic composition of each zooplankton taxon and DOM. We used the information-theoretic approach to select best explanatory
models for each case (Burnham and Anderson, 1998), while following the approach of Donald et al. (2015) to address multicollinearity among predictors. First, we fitted a global model for carbon/nitrogen SI composition of each taxon with all potential predictors (separately for CHEM and HYDR_LAND) and checked for multicollinearity. In the absence of multicollinearity (VIF < 4), candidate models with all combinations of predictors were developed. Otherwise, we developed a separate set of candidate models for each conflicting predictor, which included all combinations with other non-conflicting predictors. For example, given the multicollinearity between color and DOC, we developed a set of candidate models which contained all combinations of color with other predictors except DOC and another set of models which included all combinations DOC with other predictors except color. Other multicollinear variable groups were pH/[DIC], [TN]/[TP]/[Chl a], MaxD/RT and LV/LA/CA (Appendix C: Fig.C1). Then, all candidate models generated for each case were ranked by Bayesian Information Criteria (BIC) (Schwarz, 1978), which is conservative (compared to Akaike Information Criteria) for selecting the best and most parsimonious model among a set of nested candidate models (Forster, 2000). In addition, we calculated BIC model weights from raw BIC values for each model, which is an alternative criterion for best model selection when the difference between raw BIC values of two competing models is small (Burnham and Anderson, 1998). The best performing model for each case was selected based on the lowest raw BIC value and/or BIC model weight. Subsequently, we tested the significance of selected models and predictors and estimated the unbiased variation in isotopic composition explained by the model (adjusted R²). Then, the relative contribution of each significant predictor to the overall model was calculated by
using the method proposed by Genizi (1993), which can estimate the relative importance of predictors even when they are correlated.

We performed 2-step spatial modeling to detect spatial structures in $\delta^{13}C_{ZOOP}$ and $\delta^{15}N_{ZOOP}$ across the landscape. First, we modeled linear spatial trends in SI composition of each taxon by performing a trend-surface analysis using Cartesian coordinates $(X, Y)$ coordinates of the study lakes (Borcard and Legendre, 2002). Detrended SI data (residuals of trend surface models) or raw SI data (when linear trends were not present) were used in the second step of the analysis, an eigenvector-based spatial modeling approach to identify more complex spatial structures (Borcard and Legendre, 2002; Dray et al., 2006). For this, a set of spatial variables (i.e. eigenvectors) was generated from Cartesian coordinates of the lakes using a distance-based Moran's Eigenvector Mapping (dbMEM) method (Dray et al., 2006). A truncation distance equal to the longest linkage between neighboring lakes in a minimum spanning tree connecting all study lakes was used, which is a customary practice in this analysis. The generated spatial variables (dbMEM variables) represent a spectral decomposition of spatial relationships among lakes (Dray et al., 2006), which can be directly related to the spatial structures in SI composition of zooplankton taxa and environmental parameters. Subsequently, we applied a forward-selection in regression analysis to select the minimum subset of significant variables from dbMEM variables (SPATIAL).

We performed variance partitioning to quantify the unique and shared fractions of variation in carbon/nitrogen isotopic composition of zooplankton taxa and DOM explained by CHEM, HYDR_LAND and SPATIAL. Only the subsets of significant predictors from each of the variable sets (identified by the above regression analyses)
were used in this analysis (Peres-Neto et al., 2006). Among different fractions of variation quantified, first, the fraction shared by CHEM and HYDR_LAND variable subsets indicated the likely indirect effects of hydromorphological and land-cover variables on isotopic composition of zooplankton via their effects on water chemistry. Second, the spatially structured isotopic variation (SPATIAL) shared with CHEM and HYDR_LAND variable subsets represented the isotopic variation induced by local environmental factors that were spatially autocorrelated. Third, the variation in isotopic composition explained by CHEM and HYDR_LAND variables that was not shared by spatial factors (i.e. pure environmental variation) represented the localized/spatially unstructured effects of lake/catchment-specific factors (processes occurring at finer spatial scales that are not detectable at the resolution employed in spatial modeling). Finally, we assumed that pure spatial variation i.e. spatially structured isotopic variation independent of influence of local environmental factors (CHEM and HYDR_LAND) represents the effects of unmeasured regional-scale factors such as spatial variations in bedrock geology, soil properties (Shewchuk, 1982; Whitfield and Watmough, 2012) and potential atmospheric nitrogen deposition from nearby Athabasca oil sands operations (Makar et al., 2018) across the region. Given that exact spatial patterns of these factors are largely unknown, our spatial modeling approach has an advantage over the conventional approach of using a priory-defined, fixed regional-scale unit to successfully capture the variation induced by potential regional factors that may be operating at varying spatial scales.

All statistical analysis were performed using the following packages and functions within R statistical software version 3.2.0 (R Core Team, 2015); (i) ‘MuMIn’
(Barton, 2015) for BIC-based model selection, (ii) ‘relaimpo’ (Grömping, 2006) to calculate relative importance of regressors, (iii) ‘PCNM’ (Dray et al., 2009) to generate dbMEM variables and (iv) ‘vegan’ (Oksanen et al., 2015) for variance partitioning.

4.4. Results

Variations in $\delta^{13}C_{ZOOP}$, $\delta^{15}N_{ZOOP}$, $\delta^{13}C_{DOM}$ and $\delta^{15}N_{DOM}$

Three cladoceran taxa (i.e. *Daphnia* spp., *Holopedium gibberum* and *Bosmina longirostris*), four calanoid (i.e. *Leptodiaptomus minutus*, *Aglodiaptomus leptopus*, *Skistodiaptomus oregonensis* and *Heterocope septentrionali*) and two cyclopoid copepods (*Tropocyclops prasinus mexicanus* and *Diacyclops thomasi*) were selected for the analyses. Their $\delta^{13}C$ and $\delta^{15}N$ values ranged between -36.2 and -22.2‰ and 0.0 and 11.2‰, respectively, which were largely comparable to those reported in other studies (Appendix C: Table C1 and supplementary discussion C1). In general, $\delta^{13}C_{DOM}$ (range -28.2 to -24.9‰) was enriched relative to zooplankton taxa and vice versa for $\delta^{15}N_{DOM}$ (range 0.0 to 3.9‰). Also, SI composition of DOM was less variable compared to zooplankton taxa.

Environmental predictors of $\delta^{13}C_{ZOOP}$

Significant CHEM predictors of $\delta^{13}C_{ZOOP}$ ($p < 0.1$) included color, [DOC], [TN], [TP], [DIC] and [DIN], explaining 12-69% of total variation (Table 4.2). The negative association of $\delta^{13}C$ with variables related to allochthonous inputs (color, [DOC]) and its positive relationship with nutrient concentrations ([TP], [TN], [DIN]) were consistent among taxa. In addition, [DIC] was negatively related to $\delta^{13}C$ in several taxa. Color was the most important predictor of $\delta^{13}C$ in all cladoceran taxa (63-80% of explained
Table 4.2. Results of the best performing model for predicting δ^{13}C values of zooplankton taxa and DOM from CHEM variables based on Bayesian Information Criteria (BIC).

<table>
<thead>
<tr>
<th>Taxon/chemical substance</th>
<th>pH</th>
<th>DIC</th>
<th>TN</th>
<th>TP</th>
<th>Chla</th>
<th>Color</th>
<th>DOC</th>
<th>DIN</th>
<th>Intercept</th>
<th>Model significance (p)</th>
<th>R^2_adj</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cladocera</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daphnia spp.</td>
<td>ns</td>
<td></td>
<td>ns</td>
<td>2.18***</td>
<td>ns</td>
<td>-4.08****</td>
<td>ns</td>
<td>ns</td>
<td>-26.85****</td>
<td>&lt;0.001</td>
<td>0.46</td>
<td>80</td>
</tr>
<tr>
<td>Holopedium gibberum</td>
<td>ns</td>
<td>-1.66**</td>
<td>ns</td>
<td>3.45****</td>
<td>ns</td>
<td>-4.09****</td>
<td>ns</td>
<td>ns</td>
<td>-28.27****</td>
<td>&lt;0.001</td>
<td>0.55</td>
<td>99</td>
</tr>
<tr>
<td>Bosmina longirostris</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-1.70****</td>
<td>ns</td>
<td>2.13****</td>
<td>-28.50****</td>
<td>&lt;0.001</td>
<td>0.35</td>
<td>64</td>
</tr>
<tr>
<td>Calanoida</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptodiaptomus minutus</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>3.84***</td>
<td>ns</td>
<td>-3.32****</td>
<td>ns</td>
<td>ns</td>
<td>-29.31****</td>
<td>&lt;0.001</td>
<td>0.42</td>
<td>98</td>
</tr>
<tr>
<td>Aglodiiaptomus leptomus</td>
<td>ns</td>
<td>-4.07***</td>
<td>ns</td>
<td>2.61*</td>
<td>ns</td>
<td>-5.30****</td>
<td>ns</td>
<td>ns</td>
<td>-25.06****</td>
<td>&lt;0.001</td>
<td>0.58</td>
<td>30</td>
</tr>
<tr>
<td>Skistodiaptomus oregonensis</td>
<td>ns</td>
<td>ns</td>
<td>17.47****</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-10.36****</td>
<td>-4.81****</td>
<td>-60.29****</td>
<td>&lt;0.001</td>
<td>0.58</td>
<td>31</td>
</tr>
<tr>
<td>Heterocope septentrionalis</td>
<td>ns</td>
<td>ns</td>
<td>3.87*</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.08</td>
<td>0.12</td>
<td>18</td>
</tr>
<tr>
<td>Cyclopoida</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tropocyclops prasinus mexicanus</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>13.01****</td>
<td>ns</td>
<td>ns</td>
<td>-8.91****</td>
<td>ns</td>
<td>-55.89****</td>
<td>&lt;0.001</td>
<td>0.63</td>
<td>94</td>
</tr>
<tr>
<td>Diacyclops thomasi</td>
<td>ns</td>
<td>-6.62****</td>
<td>ns</td>
<td>5.17****</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>&lt;0.001</td>
<td>0.69</td>
<td>21</td>
</tr>
<tr>
<td>Chemical substance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOM</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-1.03****</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-25.32****</td>
<td>&lt;0.001</td>
<td>0.44</td>
<td>46</td>
</tr>
</tbody>
</table>
Note: All predictors except pH were log_{10}-transformed. Asterisks indicate significance levels of predictors; **** $p < 0.001$; *** $p < 0.01$; ** $p < 0.05$; * $p < 0.1$; ns = not significant ($p \geq 0.1$); nt = not testable. $R^2_{\text{adj}}$ = adjusted $R^2$. $n$ = number of observations. Values in parentheses denote the relative importance of each significant predictor in the regression model (normalized to sum to 100%) as estimated by Genizi method.
Table 4.3. Results of the best performing model for predicting $\delta^{13}$C values of zooplankton taxa and DOM from HYDR_LAND variables based on Bayesian Information Criteria (BIC).

<table>
<thead>
<tr>
<th>Taxon/chemical substance</th>
<th>Coefficients of predictors</th>
<th>Model significance (p)</th>
<th>$R^2_{adj}$</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MaxD</td>
<td>RT</td>
<td>LV</td>
<td>LA</td>
</tr>
<tr>
<td>Cladocera</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Daphnia</em> spp.</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>3.09*** (28%)</td>
</tr>
<tr>
<td>Holopedium gibberum</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>1.95*** (24%)</td>
</tr>
<tr>
<td><em>Bosmina longirostris</em></td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Calanoida</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Leptodiaptomus minutus</em></td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>1.78*** (23%)</td>
</tr>
<tr>
<td>Aglodiapтомus leptopus</td>
<td>ns</td>
<td>3.88*** (41%)</td>
<td>3.44*** (21%)</td>
<td>ns</td>
</tr>
<tr>
<td>Skistodiaptomus oregonensis</td>
<td>-4.42* (27%)</td>
<td>ns</td>
<td>ns</td>
<td>4.25*** (42%)</td>
</tr>
<tr>
<td>Heterocope septentrionalis</td>
<td>-6.58*** (68%)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Cyclopoida</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Tropocyclops prasinus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>mexicanus</em></td>
<td>-2.79*** (27%)</td>
<td>ns</td>
<td>ns</td>
<td>2.68**** (20%)</td>
</tr>
<tr>
<td><em>Diacyclops thomasi</em></td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Chemical substance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOM</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>
Note: % peat, % forest and % burnt were log_{10}(x + 0.01) transformed, while all other predictors were log_{10}-transformed. Asterisks indicate significance levels of predictors; **** p < 0.001; *** p < 0.01; ** p < 0.05; * p < 0.1; ns = not significant (p ≥ 0.1); R^2_{adj} = adjusted R^2, n = number of observations; Values in parentheses denote the relative importance of each significant predictor in the regression model (normalized to sum to 100%) as estimated by Genizi method.
variation), whereas associations with nutrient concentrations and [DIC] were of minor importance. Similar effects of the above variables were also evident for two calanoid taxa, *L. minutus* and *A. leptopus*. In contrast, [TN] was the predominant predictor of $\delta^{13}C$ (60-100% of explained variation) for the other two calanoids (i.e. *S. oregonensis* and *H. septentrionalis*). Similarly, [TN] was of highest importance for explaining variation in $\delta^{13}C$ (61% of explained variation) in the cyclopoid species *T. prasinus mexicanus*, followed by [DOC]. Finally, [TN], [DIC] and color were more or less equally important predictors of $\delta^{13}C$ of the second cyclopoid *D. thomasi*.

Significant HYDR_LAND predictors ($p < 0.1$) were identified for all zooplankton taxa except *B. longirostris*, explaining 26-47% of total variation in $\delta^{13}C_{ZOOP}$ (Table 4.3). Among them, the negative effect of %-peat on $\delta^{13}C$ was predominant in two cladoceran (*Daphnia* spp. and *H. gibberum*) and all cyclopoid taxa (41-66% of explained variation), while the positive effect of LA and the negative effects of DBA: LA ratio and SDI were relatively less important. The statistical significance and relative importance of the above variables for explaining the variation in $\delta^{13}C$ varied among calanoid taxa. In addition, RT or MaxD (which were highly correlated) were also identified as significant predictors of $\delta^{13}C$ in calanoid taxa.

*Environmental predictors of $\delta^{15}N_{ZOOP}$*

Significant CHEM predictors ($p < 0.1$) including pH, [DIC], [TP] and [DIN] explained 8-64% of total variation in $\delta^{15}N_{ZOOP}$ (Table 4.4). Among them, the positive effect of variables related to acid-base status (pH, [DIC]) on $\delta^{15}N$ was consistent for most taxa. In fact, pH was the sole predictor of $\delta^{15}N$ in two cladocerans, *Daphnia* spp. and *H. gibberum*. In contrast, [DIN] was the only predictor of $\delta^{15}N$ in *B. longirostris*. pH
was also identified as a significant predictor of $\delta^{15}\text{N}$ in all calanoid taxa, yet its effect was only predominant in *L. minutus* and *H. septentrionalis* (100% and 65% of explained variation, respectively). In addition, a significant negative effect of [TP] on $\delta^{15}\text{N}$ was evident for three of four calanoid taxa, i.e. *A. leptopus*, *S. oregonensis* and *H. septentrionalis*, which exceeded the effect of pH in the former two taxa (70% and 65% of explained variation, respectively). Both [DIC] and [TP] were identified as significant predictors of $\delta^{15}\text{N}$ in cyclopoids. The effect of [TP] was more important relative to that of [DIC] in *T. prasinus mexicanus* and vice versa in *D. thomasi*.

Significant HYDR_LAND predictors ($p < 0.1$) explained 13-87% of total $\delta^{15}\text{N}_{\text{ZOOP}}$ variation (Table 4.5). Among them, the positive relationship between $\delta^{15}\text{N}$ and RT (or MaxD which was highly correlated to RT) was consistent among all taxa and it was also the sole or predominant predictor in most cases. In addition, LA was identified as a significant predictor of $\delta^{15}\text{N}$ ($p < 0.05$) in two cladocerans (*Daphnia spp.*, *H. gibberum*) and the calanoid *L. minutus*.

*Spatial structuring of $\delta^{13}\text{C}_{\text{ZOOP}}$ and $\delta^{15}\text{N}_{\text{ZOOP}}$*

Significant spatial structures in $\delta^{13}\text{C}$ ($p < 0.05$) were detected for *H. gibberum*, *L. minutus*, *S. oregonensis*, *T. prasinus mexicanus* and *D. thomasi* (Appendix C: Fig.C2), which explained 14-28% of total $\delta^{13}\text{C}$ variation (Fig.4.2). Significant ($p < 0.05$) spatial structures in $\delta^{15}\text{N}$ were detected only for *Daphnia spp.*, *A. leptopus* and *T. prasinus mexicanus* (Appendix C: Fig.C3), which accounted for 19%, 24% and 13% of total $\delta^{15}\text{N}$ variation, respectively (Fig.4.3).
<table>
<thead>
<tr>
<th>Taxon/chemical substance</th>
<th>Coefficients of predictors</th>
<th>Model significance</th>
<th>( R^2_{\text{adj}} )</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cladocera</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Daphnia</em> spp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>1.17**** (100%)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>DIC</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Dominoidea</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Holopedium</em> gibberum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>1.85**** (100%)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Calanoida</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bosmina longirostris</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>DIC</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Chemical substance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>DOM</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>1.68** (100%)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>
Note: All predictors except pH were log_{10}-transformed. Asterisks indicate significance levels of predictors; **** p < 0.001; *** p < 0.01; ** p < 0.05; * p < 0.1; ns = not significant (p ≥ 0.1); nt = not testable. R^2_{adj} = adjusted R^2. n = number of observations. Values in parentheses denote the relative importance of each significant predictor in the regression model (normalized to sum to 100%) as estimated by Genizi method.
Table 4.5. Results of the best performing model for predicting $\delta^{15}$N values of zooplankton taxa and DOM from HYDR_LAND variables based on Bayesian Information Criteria (BIC).

<table>
<thead>
<tr>
<th>Taxon/chemical substance</th>
<th>Coefficients of predictors</th>
<th>Model significance (p)</th>
<th>$R^2_{\text{adj}}$</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MaxD</td>
<td>RT</td>
<td>LV</td>
<td>LA</td>
</tr>
<tr>
<td>Cladocera</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daphnia spp.</td>
<td>ns</td>
<td>1.35**** (64%)</td>
<td>ns</td>
<td>1.02**** (36%)</td>
</tr>
<tr>
<td>Holopedium gibberum</td>
<td>ns</td>
<td>1.70*** (49%)</td>
<td>ns</td>
<td>1.54**** (51%)</td>
</tr>
<tr>
<td>Bosmina longirostris</td>
<td>ns</td>
<td>2.10*** (100%)</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Calanoida</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptodiaptomus minutus</td>
<td>ns</td>
<td>1.84**** (83%)</td>
<td>ns</td>
<td>0.75** (17%)</td>
</tr>
<tr>
<td>Aglodiaptomus leptopus</td>
<td>-1.02** (9%)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Skistodiaptomus oregonensis</td>
<td>ns</td>
<td>3.19**** (100%)</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Heterocope septentrionalis</td>
<td>ns</td>
<td>3.70**** (94%)</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Cyclopoida</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tropocyclops prasinus mexicanus</td>
<td>ns</td>
<td>2.59**** (100%)</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Diacyclops thomasi</td>
<td>ns</td>
<td>1.25** (21%)</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Chemical substance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOM</td>
<td>ns</td>
<td>1.1**** (48%)</td>
<td>ns</td>
<td>0.99*** (52%)</td>
</tr>
</tbody>
</table>
Note: % peat, % forest and % burnt were $\log_{10}(x + 0.01)$ transformed, while all other predictors were $\log_{10}$-transformed. Asterisks indicate significance levels of predictors; **** $p < 0.001$; *** $p < 0.01$; ** $p < 0.05$; * $p < 0.1$; ns = not significant ($p \geq 0.1$); nt = not testable. $R^2_{\text{adj}} = \text{adjusted } R^2$. $n =$ number of observations. Values in parentheses denote the relative importance of each significant predictor in the regression model (normalized to sum to 100%) as estimated by Genizi method.
Fig. 4.2. Variance partitioning of $\delta^{13}$C of zooplankton taxa using CHEM, HYDR_LAND.
and SPATIAL variable subsets; Dash-lined circles represent null cases; Res = residual variation; **** p < 0.001; *** p < 0.01; ** p < 0.05; * p < 0.1; ns = not significant (p ≥ 0.1); Shared fractions were not testable.
Fig. 4.3. Variance partitioning of δ$^{15}$N of zooplankton taxa using CHEM, HYDR_LAND.
and SPATIAL variable subsets; Dash-lined circles represent null cases; Res = residual variation; **** p < 0.001; *** p < 0.01; ** p < 0.05; * p < 0.1; ns = not significant (p ≥ 0.1); Shared fractions were not testable.
Total, shared and unique effects of CHEM, HYDR_LAND and SPATIAL

Significant CHEM variables explained more variation in $\delta^{13}\text{C}_{\text{ZOOP}}$ than significant HYDR_LAND variables in all taxa except $H.\ septentrionalis$ (Fig.4.2a, b, c, d, e, f, g, h, i). In addition, in most cases a major fraction of the variation in $\delta^{13}$C explained by significant HYDR_LAND variables was shared by significant CHEM variables. Further, in taxa for which significant spatial patterns in $\delta^{13}$C existed, most of the spatially structured variation was shared by CHEM and HYDR_LAND. While the unique variations in $\delta^{13}$C explained by CHEM and HYDR_LAND variable sets were significant in most cases ($p < 0.05$), pure spatial variation was only significant ($p < 0.05$) in $H.\ gibberum$ and $L.\ minutus$.

For $\delta^{15}\text{N}_{\text{ZOOP}}$, significant HYDR_LAND variables were equally or more important than significant CHEM variables in explaining the variation of zooplankton taxa (Fig.4.3a, b, c, d, e, f, g, h, i). Also, the effects of HYDR_LAND variables on $\delta^{15}$N were largely independent from those of CHEM variables as indicated by the minor shared variation relative to the unique variation explained by HYDR_LAND (statistically significant at $p < 0.05$) in most cases. For the three taxa with significant spatial structures in $\delta^{15}$N, the spatially structured variation was mostly shared by effects of CHEM and HYDR_LAND variable sets.

Environmental predictors of $\delta^{13}\text{C}_{\text{DOM}}$ and $\delta^{15}\text{N}_{\text{DOM}}$

Significant ($p < 0.05$) CHEM and HYDR_LAND variables explained 44% and 47% of total $\delta^{13}$C$_{\text{DOM}}$, respectively. Color was the sole CHEM predictor of $\delta^{13}$C (Table 4.2), whereas significant HYDR_LAND predictors included %-peat, DBA:LA ratio and elevation (Table 4.3). Of total variation in $\delta^{15}$N$_{\text{DOM}}$, 18% and 28% were explained by
significant effects (p < 0.05) of pH (CHEM) and RT and LA (HYDR_LAND), respectively (Table 4.4 and 4.5). Most of the variation in δ¹³C and δ¹⁵N explained by significant CHEM variables was shared by significant HYDR_LAND variables (Fig.4.2 and Fig.4.3). Finally, δ¹³C_DOM and δ¹⁵N_DOM were moderately or strongly correlated to those of zooplankton taxa (Table 4.6).

4.5. Discussion

Our comprehensive 233-lake/nine species analyses identified diverse taxon-specific controls of δ¹³C_ZOOP and δ¹⁵N_ZOOP across the boreal study region. Below we discuss plausible mechanistic linkages of δ¹³C_ZOOP and δ¹⁵N_ZOOP to most consistent and prominent CHEM and HYDR_LAND factors, followed by possible reasons for taxon-specific differences. For most taxa, δ¹³C variation explained by significant HYDR_LAND variables was largely shared by significant CHEM variables, indicating confounding effects. In contrast, δ¹⁵N variations explained by CHEM vs. HYDR_LAND were largely independent for most taxa, with the latter being more important. Spatial modeling revealed that spatial structures in δ¹³C_ZOOP and δ¹⁵N_ZOOP largely corresponded to those of CHEM and HYDR_LAND controls, while spatial variation attributable to regional effects was trivial.

*Mechanistic relationships between δ¹³C_ZOOP and lake/catchment-specific factors*

The consistent, negative association of δ¹³C_ZOOP with color/[DOC] indicates indirect influences of terrestrial inputs on δ¹³C of phytoplankton consumed by zooplankton. In general, higher water color represents greater contributions of allochthonous DOC loading (Meili, 1992; Rasmussen et al., 1989). In lakes with high [DOC], a major proportion of DIC is likely generated from respired allochthonous
Table 4.6. Pearson’s correlation coefficients for $\delta^{13}$C and $\delta^{15}$N of DOM versus those of zooplankton taxa.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>$\delta^{13}$C</th>
<th>$\delta^{15}$N</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cladocera</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Daphnia spp.</em></td>
<td>0.84****</td>
<td>0.61**</td>
<td>15</td>
</tr>
<tr>
<td><em>Holopodium gibberum</em></td>
<td>0.82****</td>
<td>0.74****</td>
<td>19</td>
</tr>
<tr>
<td><em>Bosmina longirostris</em></td>
<td>0.5****</td>
<td>0.4***</td>
<td>42</td>
</tr>
<tr>
<td><strong>Calanoida</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Leptodiaptomus minutus</em></td>
<td>0.68**</td>
<td>0.34ns</td>
<td>10</td>
</tr>
<tr>
<td><em>Aglodiatomus leptopus</em></td>
<td>0.82**</td>
<td>0.87**</td>
<td>6</td>
</tr>
<tr>
<td><em>Skistodiatomus oregonensis</em></td>
<td>0.6ns</td>
<td>0.67ns</td>
<td>7</td>
</tr>
<tr>
<td><em>Heterocope septentrionalis</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Cyclopoida</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Tropocyclops prasinus mexicanus</em></td>
<td>0.7****</td>
<td>0.55**</td>
<td>20</td>
</tr>
<tr>
<td><em>Diacyclops thomasi</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: Asterisks indicate significance levels of predictors; **** $p < 0.001$; *** $p < 0.01$; ** $p < 0.05$; * $p < 0.1$; ns = not significant ($p \geq 0.1$). n = number of observations. Correlation coefficients are not provided for taxa with n $\leq$ 3.
organic matter ($\delta^{13}\text{C} \sim -27\%_\text{o} \text{ to } -28\%_\text{o}$ (Peterson and Fry, 1987)), which is $^{13}\text{C}$-depleted compared to atmospheric and geogenic DIC. Photoassimilation of respiratory $^{13}\text{C}$-depleted DIC results in more negative $\delta^{13}\text{C}$ in phytoplankton and subsequently, zooplankton consumers (France et al., 1997; Lennon et al., 2006; Mohamed and Taylor, 2009). Jones et al., (2009) proposed that a negative relationship between $\delta^{13}\text{C}$ of zooplankton and water color (based on 12 Finnish forest lakes) can also be a result of greater contributions of isotopically light, methane-derived carbon to zooplankton diet (via grazing on methane-oxidizing bacteria) in lakes with high [DOC]. However, in agreement with the findings of Lennon et al. (2006) and Mohamed and Taylor (2009), this mechanism is unlikely in our study lakes $\delta^{13}\text{C}_{\text{ZOOP}}$ values in the present study are largely within the range expected for photoautotrophically produced carbon (-35 to -8\%o) (France, 1995a; Meyers and Teranes, 2001; Vuorio et al., 2006) and greater than highly-depleted isotopic values expected from appreciable methanotrophy (<-40\%o) (Jones and Grey, 2011).

The consistent, positive relationship between $\delta^{13}\text{C}_{\text{ZOOP}}$ and nutrient concentrations ([TP] or [TN]) is likely related to differences in pelagic primary production induced by nutrient availability. The epilimnetic DIC pool in boreal lakes declines dramatically with increase in primary production, which can lead to reduced isotopic fractionation and consequent $^{13}\text{C}$ enrichment in phytoplankton (France et al., 1997; Grey et al., 2000; Raven et al., 1994). In addition, increased primary production may result in $\text{CO}_2$ undersaturation in lakes relative to the atmosphere and consequently, net influx of atmospheric $\text{CO}_2$. The assimilation of $^{13}\text{C}$-enriched atmospheric carbon (relative to respiratory carbon) may also contribute to more enriched $\delta^{13}\text{C}$ in
phytoplankton (France et al., 1997; Perga and Gerdeaux, 2004). Ultimately, the $\delta^{13}C$ increase in phytoplankton along the nutrient/primary productivity gradient is likely reflected in zooplankton consumers (France et al., 1997; Grey et al., 2000).

The significant negative association between [DIC] and $\delta^{13}C_{ZOOP}$ taxa could be related to effects of non-metabolic factors on variation in [DIC] among lakes, which in turn may affect isotopic fractionation by phytoplankton. In addition to ecosystem metabolism (as described above), [DIC] can be influenced by underlying geology, DIC inputs from precipitation, groundwater and surface water and physio-chemical determinants of gas exchange between water and atmosphere, such as temperature and pH (Anas et al., 2015). As described above, variation in [DIC] can be reflected in $\delta^{13}C$ values of phytoplankton (and their consumers), due to reduced fractionation against $^{13}C$ with decrease in [DIC] (France et al., 1997; Grey et al., 2000; Raven et al., 1994).

Significant HYDR_LAND variables likely influenced $\delta^{13}C_{ZOOP}$ largely via their effects on carbon loading (terrestrial and atmospheric) and processing in lakes. DOC production in catchment soils and subsequent export to lakes increases with wetland coverage (Kortelainen, 1993; Xenopoulos et al., 2003). In addition, lakes with higher SDI are more strongly connected to riparian habitats due to higher perimeter:area ratio and receive more allochthonous DOC (Schindler and Scheuerell, 2002). Further, LA and DBA:LA ratios are often negatively and positively related to allochthonous [DOC] in lakes, respectively due to greater dilution of allochthonous DOC loading from catchments in larger lakes by precipitation that falls directly onto the lake surface (D’arcy and Carignan, 1997; Rasmussen et al., 1989). Also, greater atmospheric gas-exchange in larger lakes may dilute $^{13}C$-depleted respiratory DIC of allochthonous origin.
with isotopically heavier atmospheric CO₂ (Perga and Gerdeaux, 2004). Finally, higher RT provides more time for photochemical and microbial degradation of allochthonous organic carbon (Anas et al., 2015) and consequent incorporation into pelagic food webs via phytoplankton and/or microbial pathways (described above).

**Mechanistic relationships between \( \delta^{15}N_{ZOOP} \) and lake/catchment-specific factors**

Significant relationships between environmental factors and \( \delta^{15}N_{ZOOP} \) are likely associated with the effects of in-lake biogeochemical nitrogen transformations and catchment processes. Isotope fractionation between product and residual nitrogen pools occurs during microbial decomposition of organic nitrogen to inorganic nitrogen, nitrification and assimilation of inorganic nitrogen by bacteria and phytoplankton (Kendall, 1998; Robinson, 2001). Therefore, rate-determining factors (physical and chemical) of these processes can indirectly influence \( \delta^{15}N \) of organic and inorganic nitrogen pools in lakes, which are eventually incorporated into the pelagic food web (Karlsson et al., 2004). For instance, the positive effect of RT on rates of microbial organic matter decomposition and microbial DIN assimilation in lakes (Anas et al., 2015; Catalán et al., 2016; Saunders and Kalff, 2001) supports the significant and consistent association between RT and \( \delta^{15}N \) of zooplankton taxa in this and other studies (Caraco et al., 1998; Grey et al., 2001). Furthermore, the significant and consistent relationship between pH/DIC and \( \delta^{15}N_{ZOOP} \) likely indicative of the influence of acid-base status on nitrification rates in lakes (Rudd et al., 1988; Strauss et al., 2002). This may be also indicative of negative association between DOM-N inputs from terrestrial sources and \( \delta^{15}N_{DOM} \) i.e. greater DOM-N inputs from nitrogen fixing plants such as *Alnus* spp. and *Shepherdia canadensis* and microorganisms in deciduous forest may result in low
\( \delta^{15}N_{DOM} \) in lakes (Bunting et al., 2010; Engstrom and Fritz, 2006; Wolfe, 2016), while greater terrestrial DOM loadings also lead to lower lake water pH values. Also, the significant positive relationship between TP and \( \delta^{15}N \) for most calanoid and cyclopoid taxa might reflect increased primary productivity with increased phosphorus load in phosphorus-limited or nitrogen/phosphorus co-limited systems, leading to increasing demand for \( \text{NO}_3^- \) relative to supply, reducing \( ^{15}\text{N} \)-discrimination of phytoplankton (Robinson, 2001). Alternatively, enhanced lake productivity provoked an upward shift in trophic position of these taxa, which would result in increasing \( \delta^{15}N_{ZOOPE} \) values along the [TP] gradient (Karlsson et al., 2004; Maguire and Grey, 2006).

**\( \delta^{13}C_{DOM} \) and \( \delta^{15}N_{DOM} \) trends**

The patterns of \( \delta^{13}C_{DOM} \) and \( \delta^{15}N_{DOM} \) provide further support for the above proposed effects of allochthonous inputs on isotopic composition at the base of food webs. DOM in lakes can be autochthonous or allochthonous (Meili, 1992), and as allochthonous contributions increase, \( \delta^{13}C_{DOM} \) becomes more similar to those of C3 terrestrial vegetation i.e. -27‰ to -28‰ (Peterson and Fry, 1987). Del\(^{13}C_{DOM} \) in our study lakes (-25‰ to -28‰) became more terrestrial-like with increase in color, %-peat and DBA:LA ratio, further supporting the inferred associations of these variables to allochthonous DOC loading to lakes. \( \delta^{15}N_{DOM} \) (-0.7‰ to 5‰) largely reflects terrestrial fixed nitrogen and subsequent modification by catchment and in-lake processes (Bunting et al., 2010; Meyers and Teranes, 2001; Talbot, 2001). As discussed above, the significant relationship between \( \delta^{15}N_{DOM} \) and pH may be indicative of negative association between mass flux of terrestrially-fixed DOM-N and \( \delta^{15}N_{DOM} \). In addition, significant relationships of pH and RT with \( \delta^{15}N_{DOM} \) may also indicate their
influences on in-lake biogeochemical processes that alter isotopic ratios of terrestrially-fixed nitrogen (discussed above). The strong correlations between $\delta^{13}C_{\text{DOM}}$ and those of zooplankton taxa, in combination with more depleted $^{13}C_{\text{ZOOP}}$ relative to $\delta^{13}C_{\text{DOM}}$, indicate indirect contributions of DOM to zooplankton diet (via phytoplankton assimilating respiratory carbon). Meanwhile, moderate to strong correlations between $\delta^{15}N_{\text{ZOOP}}$ and $\delta^{15}N_{\text{DOM}}$ corroborate the influence of $\delta^{15}N$ of terrestrial organic matter on isotopic values of zooplankton (France et al., 1997).

*Among-taxon differences in controls of $\delta^{13}C_{\text{ZOOP}}$ and $\delta^{15}N_{\text{ZOOP}}$*

Contrary to our expectation, controls of $\delta^{13}C_{\text{ZOOP}}$ and $\delta^{15}N_{\text{ZOOP}}$ (specific predictors and direction of their effects) were surprisingly similar among taxa, despite their documented differences in feeding strategies and consequent dietary variations (Barnett et al., 2007; Berggren et al., 2014; Persaud et al., 2009). Our findings indicate that $\delta^{13}C_{\text{ZOOP}}$ and $\delta^{15}N_{\text{ZOOP}}$ variations in all taxa are largely reflective of indirect effects of lake/catchment-specific factors on $\delta^{13}C$ and $\delta^{15}N$ of phytoplankton (as discussed above). Contrary to our findings, Persaud *et al.*, (2009) who evaluated $\delta^{13}C_{\text{ZOOP}}$ and $\delta^{15}N_{\text{ZOOP}}$ variations in 15 Canadian boreal lakes using a comparable taxonomic resolution, found that direction of [DOC] and [TP] effects on $\delta^{13}C_{\text{ZOOP}}$ and $\delta^{15}N_{\text{ZOOP}}$ varied among zooplankton taxa. However, despite comparable [DOC] and [TP] gradients and ranges of $\delta^{13}C_{\text{ZOOP}}$ and $\delta^{15}N_{\text{ZOOP}}$ between the studies, lake-specific factors explained much less SI variances of taxa in Persaud *et al.*, (2009) compared to our study. The potential confounding effect of large differences in sample-sizes between the studies may have attributed to these contrasting findings.
However, the degree of importance of different predictors of $\delta^{13}\text{C}_{\text{ZOOP}}$ and $\delta^{15}\text{N}_{\text{ZOOP}}$ varied among taxa. Although the underlying mechanisms for these differences can be only speculated at this point, they can be in fact related to varying importance of taxon-specific dietary pathways. For instance, given the likely relationship between allochthonous inputs (i.e. color) and phytoplankton $\delta^{13}\text{C}$ (discussed above), the dominant effect of color on $\delta^{13}\text{C}_{\text{ZOOP}}$ in all cladoceran and two calanoid taxa, namely *L. minutus* and *A. leptopus* may indicate greater reliance (ingestion and/or assimilation) on phytoplankton sources. Increased reliance on allochthonous sources (in addition to phytoplankton sources) by other calanoid (*S. oregonensis* and *H. septentrionalis*) and cyclopoid taxa (*T. prasinus mexicanus* and *D. thomasi*) (Berggren et al., 2014; Persaud et al., 2009) may have affected their relationship of $\delta^{13}\text{C}_{\text{ZOOP}}$ with color/[DOC].

Typically in unproductive, DOC-rich lakes, $\delta^{13}\text{C}$ of allochthonous organic matter is heavier than phytoplankton (Kankaala et al., 2010; Meili et al., 1996; Taipale et al., 2016) and consequently, dilution of phytoplankton-based diet by allochthonous contributions may obscure the depleted $\delta^{13}\text{C}_{\text{ZOOP}}$ signal related to phytoplankton grazing. Hence, the negative relationship of $\delta^{13}\text{C}_{\text{ZOOP}}$ with color/[DOC] (caused by phytoplankton dependence) should be weaker in taxa that increasingly depend on allochthonous resources (through microbial loop) with increased color/[DOC] compared to those feeding more selectively on phytoplankton. This could statistically lead to enhanced relative importance of nutrient loadings as a predictor of $\delta^{13}\text{C}_{\text{ZOOP}}$ in the former group, i.e. with reduced $\delta^{13}\text{C}_{\text{ZOOP}}$ variation explained by color/[DOC], the shared fraction of variation explained by both color/[DOC] and nutrient loading may also be diminished, leading to increased unique variation explained by nutrient loading alone. In addition,
relatively weaker relationships of $\delta^{13}$C of *B. longirostris* to lake/catchment-specific factors compared to other cladoceran taxa (i.e. *Daphnia* spp. and *H. gibberum*) align with the inability of small-bodied *B. longirostris* to ingest larger particles or its greater grazing selectivity on phytoplankton groups (Bogdan and Gilbert, 1982; Demott, 1982).

The above speculations on differences in feeding selectivity among taxa are in accordance with several studies, but contradict others. We are inclined toward the contention that cladocerans more selectively ingest and/or assimilate phytoplankton resources than copepods (Brett et al., 2009; Grey et al., 2001; Persaud et al., 2009; Taipale et al., 2016), which contradicts Matthews & Mazumder, (2003), Karlsson (2007) and Berggren et al. (2014). In addition, our results indicate that feeding selectivity likely varies among species of calanoid copepods, which were likely masked in previous studies due to low taxonomic resolution (Berggren et al., 2014; Persaud et al., 2009).

The major findings of taxon-specific, univariate analyses were further corroborated using multivariate analysis with reduced taxonomic resolution and smaller sample size (Appendix C: Fig.C4 and supplementary discussion C2). This allowed us to evaluate isotopic variation of three broad taxonomic groups i.e. Cladocera, Calanoida and Cyclopoida over identical CHEM, HYDR_LAND and spatial gradients (unlike in univariate analyses for individual taxa). Significant lake/catchment-specific controls of SI variation of each group were generally similar to those identified for most species/genera within the particular group. However, interesting deviations in controls of SI compositions of certain species/genera from their broad taxonomic group were obscured in this analysis. For instance, color is the predominant CHEM predictor of
calanoid δ¹³C according to this analysis, but taxon-specific, univariate analyses identified nutrient loading as the dominant CHEM predictor of δ¹³CZOOP in certain calanoid taxa (i.e. S. oregonensis and H. septentrionalis).

**Shared and unique effects of CHEM, HYDR_LAND and SPATIAL**

Our explicit spatial modeling approach together with variance partitioning quantified the fractions of isotopic variation of zooplankton likely attributable to causal factors operating at different spatial scales. The effects of potential regional factors on isotopic variation of zooplankton taxa (pure spatial variation) were less important compared to lake/catchment-specific factors (combined pure and spatially structured environmental variation). This could be due to limited effects of geological factors on δ¹³C values of geogenic carbon inputs to lakes, minor importance of geogenic carbon at the food-web base, weak spatial gradients in atmospheric nitrogen deposition, and alteration of isotopic values of geogenic carbon or atmospheric nitrogen by in-lake/catchment processes. The greater importance of pure (i.e. spatially unstructured) environmental variation compared to spatially-structured environmental variation is not surprising, given that environmental conditions can be highly different among headwater lakes despite their spatial proximity due to low hydrological connectivity among them (Quinlan et al., 2003).

As hypothesized, δ¹³CZOOP variation explained by HYDR_LAND variables was largely shared by the effects of CHEM variables in most zooplankton taxa. This is not surprising given the above-described effects of HYDR_LAND variables on allochthonous [DOC]/color of lakes, which is a major predictor of δ¹³CZOOP in our study lakes. Those effects of HYDR_LAND variables that did not contribute to variation in
color among lakes (e.g. effect of RT on microbial assimilation of allochthonous DOC and effect of LA on atmospheric CO$_2$ influx) could have led to the minor, yet significant, unique variation in $\delta^{13}$C$_{ZOOP}$ explained by HYDR_LAND in most cases. Conversely, the mechanistic effects of CHEM and HYDR_LAND variables on $\delta^{15}$N$_{ZOOP}$ were mostly independent (as discussed above) and, unlike for $\delta^{13}$C$_{ZOOP}$, explained largely unique fractions of $\delta^{15}$N$_{ZOOP}$ variation.

Implications for isotope-based food-web studies

Our findings emphasize that caution should be exercised when interpreting $\delta^{13}$C$_{ZOOP}$ values in relation to importance of allochthonous subsidies. Many studies from unproductive, DOC-rich lakes have indicated that $\delta^{13}$C of allochthonous organic matter tend to be heavier than phytoplankton (France, 1995b; Grey et al., 2000, 2001; Jones et al., 1999; Kankaala et al., 2010; Meili et al., 1996; Taipale et al., 2016). Therefore, heavier $\delta^{13}$C$_{ZOOP}$ (closer to $\sim$ -27‰) is expected to be indicative of greater allochthony (del Giorgio and France, 1996; France, 1995b; Grey et al., 2001; Jones et al., 1999; Karlsson et al., 2003). However, our findings offer a different lesson. As $\delta^{13}$C$_{ZOOP}$ values of all taxa in highly terrestrially-influenced lakes are systematically depleted (higher color/[DOC]) relative to those in less-influenced lakes, it might be incorrect to directly attribute greater dependence on allochthonous sources to higher $\delta^{13}$C$_{ZOOP}$ values.

There is growing interest using spatial isotopic patterns of biotic and abiotic substances to infer the effects of regional-scale factors on ecosystem functions across broad geographical scales (Kendall et al., 2010), yet such applications are rare in lake studies (e.g. present study; Cabana & Rasmussen, 1996; Vander Zanden et al., 2005). Our study showed that regional factors were less important relative to lake/catchment-
specific factors in regulating zooplankton SI variation. Although this finding may be case-specific, it suggests that lake/catchment-specific factors can have a strong effect on SI variation of lake biota and hence, need to be explicitly accounted for before inferring any regional effects.

**Implications for lake monitoring**

Based on our present and previous work, we suggest SI analysis of zooplankton can complement the common practice of using zooplankton taxonomic composition as indicators of environmental changes in lakes. Our previous work evaluated zooplankton species composition of the present survey lakes, which showed that relative abundances of different zooplankton species are mainly regulated by environmental variables related to acid-base status (lateral effects), productivity (bottom-up effects) and predation regime (top-down effects) (Anas et al., 2014a). This information together with findings of the present SI study may enhance the power of detecting and distinguishing potential impacts of environmental perturbations on lakes across the landscape. For instance, changes in acid-base status in lakes due to atmospheric nitrogen and sulphur deposition would be reflected by contrasting shifts in relative abundances of *L. minutus* and *S. oregonensis* (due to their differential physiological sensitivities to acidity) together with their more depleted δ^{15}N (due to the rate limiting effect of acid-base status on nitrification in lakes; discussed above) relative to baseline data. In addition, changes in their δ^{13}C would indicate if and how atmospheric deposition impacted the carbon metabolism at the food web base (as discussed above). Meanwhile, increased relative abundance of *Daphina pulex* alone would be attributable to enhanced lake productivity and/or declined invertebrate predation due to regional environmental changes, whereas
increased relative abundance of the species together with more enriched $\delta^{13}$C values would provide greater support for the former scenario.

**Caveats**

For some taxa, considerable variation in $\delta^{13}$CZOOP and $\delta^{15}$NZOOP remained unexplained, which could be related to factors that were not accounted for in the present study. For instance, unexplained variation in $\delta^{13}$CZOOP (26-65%) may be related to among-lake differences in phytoplankton composition (Pel et al., 2003), contributions of benthic and littoral subsidies (del Giorgio and France, 1996; Rautio and Vincent, 2007; Wilkinson et al., 2013) and various watershed processes (Aravena et al., 1992), which were likely not correlated to measured lake/catchment-specific factors and spatial position of study lakes. Variations in diet-tissue $^{15}$N fractionation due to nutrient stress, use of internal nitrogen reserves by organisms under starvation conditions (Adams and Sterner, 2000; Karlsson et al., 2004) and shifts in trophic position (Karlsson et al., 2004; Maguire and Grey, 2006) may have contributed to the unexplained variation in $\delta^{15}$NZOOP (12-77%).

**4.6. Conclusions**

To our knowledge, this study is the first to evaluate relationships between SI composition of zooplankton and lake/watershed parameters and other spatially-contingent factors using a landscape approach and large dataset with high taxonomic resolution. Our findings revealed that local lake/catchment-specific factors exert a greater influence than potential regional factors on spatial isotopic variation, indicating they should be explicitly accounted for before inferring any regional effects in SI studies. The effects of HYDR\_LAND variables on $\delta^{13}$CZOOP are mostly indirect, via their effects
on water chemistry, yet had direct effects on $\delta^{15}N_{ZOOP}$. The main limnological gradients influencing $\delta^{13}C_{ZOOP}$ and $\delta^{15}N_{ZOOP}$ variations including allochthonous organic matter inputs, nutrient loadings, pH and water retention time are highly vulnerable to environmental perturbations affecting boreal regions such as climate warming, atmospheric nitrogen and sulphur deposition and land use alterations (Anas et al., 2015; Schindler and Lee, 2010). As relative effects of above environmental gradients on SI composition varied among taxa with different feeding modes, $\delta^{13}C_{ZOOP}$ and $\delta^{15}N_{ZOOP}$ of different taxa may respond differently to these environmental perturbations.

4.7. Acknowledgements

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CHAPTER 5. TAXON-SPECIFIC VARIATION IN $\delta^{13}$C AND $\delta^{15}$N OF SUBFOSSIL INVERTEBRATE REMAINS: INSIGHTS INTO HISTORICAL TROPHODYNAMICS IN LAKE FOOD-WEBs

5.1. Abstract

Carbon and nitrogen stable isotope ratios of sub-fossil invertebrate remains are potentially powerful indicators of nutrient flux, habitat-specific resource utilization, and trophic interactions in lentic food webs, but are rarely estimated for multiple species within lakes. Here we examined historical time series of $\delta^{13}$C and $\delta^{15}$N in remains of individual invertebrate taxa representing pelagic, littoral and benthic habitats during the 20th century in five boreal lakes of central Canada. Differences in centennial means and trends were quantified (i) between invertebrate remains and sedimentary organic matter (SOM), and (ii) among different taxa within each lake to evaluate the coherence of isotope signals during the 20th century. Differences in mean $\delta^{13}$C and $\delta^{15}$N were usually significant ($p < 0.05$) between SOM and invertebrate taxa, and among individual taxa, reflecting selective feeding by invertebrates and differences in trophic position within food webs. In contrast, patterns of historical variance in isotope values varied among lakes with few consistent differences between long-term isotopic trends of SOM and invertebrate remains. In particular, SOM and invertebrate isotopic trends were similar in relatively dystrophic lakes, likely due to the importance of terrestrial carbon in both SOM and invertebrate diets. However, significant SOM-invertebrate trend differences were observed for both $\delta^{13}$C and $\delta^{15}$N in relatively clear-water lakes, possibly reflecting temporal variation in diets or tissue fractionation. Comparison of historical trends in isotope values among taxa revealed few consistent patterns. Together, our findings
suggest that evaluation of taxon-specific δ\textsuperscript{13}C and δ\textsuperscript{15}N can provide valuable insights into
historical tropho-dynamics in lake food webs.

### 5.2. Introduction

Abundance, composition and size of chitinous invertebrate remains preserved in
lake sediments are often used as a paleo-environmental indicators of historical changes
in food-web composition and trophic structure (Jeppesen et al., 2001). Recently, these
analyses have been expanded to include estimates of the elemental composition of sub-
fossils, including carbon and nitrogen stable isotope (SI) ratios as proxies for historical
trophic dynamics (Frossard et al., 2013b; Perga, 2009; Schilder et al., 2017; van
Hardenbroek et al., 2013). The appropriateness of sub-fossil invertebrate SI ratios as
paleolimnological proxies of food-web processes is supported by several lines of
evidence. First, SI ratios of sub-fossil exoskeletons and resting eggs of invertebrates are
predictably related to their whole body values (Perga, 2011; Schilder et al., 2015b), as
well as dietary source material (Frossard et al., 2013a; Heiri et al., 2012; Perga, 2009).
Second, SI ratios of invertebrate remains are largely unaffected by acid and base pre-
treatments usually used for sediment processing (Perga, 2009; van Hardenbroek et al.,
2010). Finally, taphonomic and early diagenetic processes appear to have little effect on
carbon SI ratios (δ\textsuperscript{13}C) of invertebrate remains, although corrections for systematic
changes in nitrogen SI ratios (δ\textsuperscript{15}N) with time may be required (Perga, 2011). Despite
these advances, very little is known of whether SI of sub-fossil remains of individual
taxa record whole-lake or habitat-specific processes (changes in carbon and nutrient
fluxes) or historical variation in trophic positions within the food web (Jeppesen et al.
2001).
The evaluation of $\delta^{13}$C and $\delta^{15}$N stratigraphies of subfossil invertebrate remains is facilitated by understanding of the effects of contemporary food-web processes on isotopic composition of living zooplankton and zoobenthos. Because $\delta^{13}$C is transferred from diet to consumers with little modification (generally < 1%) (DeNiro and Epstein, 1978), variation in $\delta^{13}$C values of invertebrate consumers records: the relative importance of carbon (C) from primary producers in different habitats (planktonic, benthic, littoral, terrestrial) to the food web (del Giorgio and France, 1996; Grey et al., 2000); changes in the proportion of inorganic C sources (atmospheric, geogenic, respired) used in photosynthesis, and/or; variation in the magnitude of inorganic C limitation for primary production (France et al., 1997; Raven et al., 1994). In contrast, $\delta^{15}$N of consumers is often indicative of their trophic position within food webs, with an expected 2-4‰ enrichment over dietary nitrogen (N) sources (Cabana and Rasmussen, 1994). Within a given trophic level (e.g., primary consumers), $\delta^{15}$N variation is related to variation in N sources, mass flux and biogeochemical processing of N (fixation, denitrification, ammonification) in lakes and watersheds (Cabana and Rasmussen, 1996; Vander Zanden et al., 2005). In general, the relation between long-term temporal variation in isotopic values and trophodynamics of individual taxa is less well studied (Maguire and Grey, 2006; Patoine et al., 2006) than those from short-term analyses based on lake surveys (Chapter 2, Matthews and Mazumder 2003; Persaud et al. 2009).

Unlike SI composition of invertebrate remains, analysis of $\delta^{13}$C and $\delta^{15}$N of bulk sedimentary organic matter (SOM) has been commonly incorporated into paleolimnological studies. SOM is a heterogeneous mixture of plant-, animal- and microbial-derived organic matter originated in different habitats of the lake (i.e. littoral,
pelagic and benthic) and received from the watershed (Meyers and Teranes, 2001). Consequently, accurate interpretation of the mechanisms underlying historical changes in isotopic signals of SOM can be difficult due to the potential for multiple pathways influencing both mass fluxes and isotopic value of source materials within the lake and its watershed (Meyers and Teranes, 2001; van Hardenbroek et al., 2014). In particular, because *in situ* primary production is thought to contribute relatively little to total SOM relative to terrestrially-derived C (Doskey and Talbot, 2000; Meyers and Ishiwatari, 1993; Von Wachenfeldt and Tranvik, 2008), and invertebrates often feed selectively (del Giorgio and France 1996; Meili et al. 1996; Doi et al. 2006), it may be particularly difficult to identify how food-web processes influence sedimentary isotope values. In such a case, isotopic trends of sub-fossil invertebrate remains may be better indicators of changes in C and N dynamics within the food webs than are those of SOM (Griffiths et al., 2010; Simon et al., 2017; van Hardenbroek et al., 2014). Yet, in past studies, differences in temporal patterns between SOM and invertebrates were inferred only from pair-wise comparisons of raw values without statistically testing if temporal trends were significantly different (Kattel et al., 2015; Perga et al., 2010; van Hardenbroek et al., 2014).

Comparisons of isotopic trends among taxon-specific invertebrate remains can yield insights into habitat-specific food-web dynamics induced by environmental changes. For instance, δ13C values within a given ecosystem may differ among invertebrate taxa with different habitat affinities (i.e., pelagic, littoral, benthic) and/or feeding modes owing to differences in dietary sources of C (Berggren et al., 2014; Matthews and Mazumder, 2003; Persaud et al., 2009), which may converge or further
diverge over time in response to various environmental changes (del Giorgio and France, 1996; Grey et al., 2001; Vadeboncoeur et al., 2003). For $\delta^{15}\text{N}$, diverging or converging temporal trends between two taxa may indicate variations in their relative trophic positions over time (Patoine et al. 2006; Perga et al. 2010) or shifts in selective feeding on alternate N sources (France, 1995c; Mulholland et al., 2000). Fortunately, recent improvements in technology to lower mass required for analysis (Maddi et al., 2006; Perga, 2009; van Hardenbroek et al., 2010) have allowed investigators to better determine the SI values of individual taxa (Frossard et al., 2013b; van Hardenbroek et al., 2014, 2013; Wooller et al., 2012), although better statistical analysis of the significance of historical trends is still needed.

Here, we quantified time series of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in SOM and fossils from individual invertebrate taxa during the 20th century in five boreal lakes of central Canada to evaluate whether taxon-specific SI analyses improve insights concerning historical changes in lacustrine ecosystems. Specifically, we analyzed chitinous remains of taxa representing different habitats of lakes i.e. *Daphnia* and *Bosmina* spp. (pelagic), *Alona* sp. (littoral) and Chironomidae (benthic) and statistically compared the isotopic trajectories (i) between SOM and invertebrate taxa, and (ii) among invertebrate taxa within each lake. We hypothesized that isotopic trends of invertebrate taxa reflective of food web-specific dynamics would be significantly different from SOM which derives from heterogeneous sources. We also expected that SI trends would be significantly different among invertebrate taxa representing different habitats, as energy and nutrient flux through different habitats can be affected differently by environmental changes (Schindler and Scheuerell, 2002; Vadeboncoeur et al., 2002). To our knowledge, this is

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the first study where taxon-specific SI analyses of invertebrate remains has been performed on multiple study lakes, and in which robust statistical approaches were applied to compare isotopic trends of invertebrate taxa and SOM.

5.3. Methods

Study lakes

Our five study lakes were a subset of 16 lakes in northwest Saskatchewan (Fig. 5.1) described in Mushet et al. (2017), that represent 262 basins surveyed by the provincial government during 2007-2009 and 2011 (Scott et al., 2010). Initially, sites were selected in a factorial design to represent type of nutrient limitation (phosphorus-limited vs. nitrogen-limited) and degree of vulnerability to atmospheric emissions from Athabasca Oil Sands Region (AOSR). Of the 16 lakes cored, the five lakes examined here were chosen on the basis of their elevated densities of fossil invertebrate remains (Hesjedal, 2017) which allowed sufficient sample size to conduct taxon-specific SI analyses. Final lake selection included two N-limited reference lakes (basins 17V, 17F), one N-limited impacted basin (6E), and one P-limited lake within both impacted (13N) and reference regions (17P).

Study lakes are mostly small (surface area < 65 ha) and shallow (maximum depth < 10 m), with the exception of 13N which is both relatively large and deep (Table 5.1). The pH of each basin was circumneutral (range 6.8-7.3), yet alkalinity varied considerably among lakes (range 4.2-16.5 mg L\(^{-1}\) CaCO\(_3\)). Study lakes were largely oligotrophic to mesotrophic, as reflected by low water-column concentration of total

\(^{1}\) Based on molar dissolved inorganic nitrogen to total phosphorus (DIN:TP) ratios: N-limited or N+P co-limited (DIN:TP < 3.5), P-limited (DIN:TP > 7) (Bergström, 2010).
Fig. 5.1. Sampling domain and locations of five study lakes in northwest Saskatchewan.
Table 5.1. Limnological characteristics of five study lakes

<table>
<thead>
<tr>
<th>Variable</th>
<th>Lake 17V</th>
<th>Lake 17P</th>
<th>Lake 17F</th>
<th>Lake 13N</th>
<th>Lake 6E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface area (ha)</td>
<td>62.0</td>
<td>63.0</td>
<td>21.0</td>
<td>150.0</td>
<td>18.3</td>
</tr>
<tr>
<td>Maximum depth (m)</td>
<td>7.4</td>
<td>7.4</td>
<td>8.8</td>
<td>15.4</td>
<td>9.6</td>
</tr>
<tr>
<td>Coring depth (m)</td>
<td>5.4</td>
<td>7.4</td>
<td>7.9</td>
<td>12.2</td>
<td>6.0</td>
</tr>
<tr>
<td>TN (μg L(^{-1}))</td>
<td>290.0</td>
<td>253.0</td>
<td>299.0</td>
<td>253.0</td>
<td>314.0</td>
</tr>
<tr>
<td>TP (μg L(^{-1}))</td>
<td>14.0</td>
<td>7.7</td>
<td>6.5</td>
<td>5.0</td>
<td>16.5</td>
</tr>
<tr>
<td>Chlorophyll a (μg L(^{-1}))</td>
<td>5.4</td>
<td>2.0</td>
<td>3.2</td>
<td>3.1</td>
<td>6.1</td>
</tr>
<tr>
<td>pH</td>
<td>7.1</td>
<td>6.8</td>
<td>7.0</td>
<td>7.1</td>
<td>7.3</td>
</tr>
<tr>
<td>Alkalinity (mg L(^{-1}) CaCO(_3))</td>
<td>7.5</td>
<td>4.2</td>
<td>7.8</td>
<td>11.6</td>
<td>16.5</td>
</tr>
<tr>
<td>DOC (mg L(^{-1}))</td>
<td>3.4</td>
<td>7.1</td>
<td>4.4</td>
<td>7.1</td>
<td>3.4</td>
</tr>
<tr>
<td>Color (mg L(^{-1}) Pt)</td>
<td>4.8</td>
<td>32.8</td>
<td>6.8</td>
<td>26.6</td>
<td>8.34</td>
</tr>
</tbody>
</table>

Note: The values are averages of lake surveys conducted in 2007-2009 and 2011 by Saskatchewan Ministry of Environment (Scott et al., 2010). See text for descriptions of variables.
phosphorous (TP; range 5.0-16.5 μg L\(^{-1}\)), total nitrogen (TN; range 253-315 μg L\(^{-1}\)) and chlorophyll a (range 2.0-6.1 μg L\(^{-1}\)). In 13N and 17P, dissolved organic carbon (DOC) concentration (each 7.1 mg L\(^{-1}\)) and water color (26.2 and 32.8 mg L\(^{-1}\) Pt units, respectively) were considerably higher than in other study lakes, indicating the greater importance of allochthonous dissolved organic matter (DOM) in these lakes (Wetzel, 1983). We refer to Scott et al., (2010) and Anas et al., (2014) for detailed descriptions of survey domain, water chemistry and hydro-morphological attributes of study lakes.

**Sediment coring**

Sediment cores were retrieved in February and April 2014 using a Glew gravity corer with a 7.6-cm internal diameter tube. The length of the cores varied from 36.5 to 48.5 cm, which were sectioned into 0.5-cm intervals upon return to the base camp. Samples were shipped to Queen’s University, Kingston, Ontario where they were stored at 4°C in a cold room until further analyses.

**Core chronologies**

Details on radiometric dating (using \(^{210}\)Pb activities) and age models of the cores are described in Mushet et al. (2017). All cores exhibited robust depth-time chronologies, with the sediment accumulation rates of 17V, 13N and 6E sufficient to achieve a temporal resolution between 2 and 8 years (spaced 0.5 or 1 cm apart, respectively). In contrast, temporal resolutions of cores from lakes 17F and 17P were ~3 - 13 years and 8 - 16 years, respectively, reflecting lower mass accumulation rates (Mushet et al., 2017).
**SI analysis of SOM**

Approximately 3 mg of freeze-dried sediment from each interval were packed in tin capsules, and combusted in an Elemental Combustion System (Costech EA) coupled with a Thermo-Finnigan Delta V isotope ratio mass spectrometer (IRMS). Bovine liver and wheat flour were used as internal laboratory standards. Isotopic ratios are reported in the conventional δ notation (‰), relative to Vienna Pee-Dee-Belemnite (C) and atmospheric N₂ (N) standards (Cooper and Wissel, 2012). Agreement between duplicate sub-samples was within 0.2‰ for each element. Acidification of sediment samples indicated that inorganic C was negligible in all cores (Wolfe, 2016).

**SI analysis of subfossil invertebrate remains**

To recover sufficient subfossil invertebrate remains for SI analysis, sediment samples were first deflocculated in warm 10% KOH for 2 hrs, then thoroughly rinsed with deionized water and concentrated onto a 106-μm mesh sieve (Perga, 2011; van Hardenbroek et al., 2010). Subsequently, the chitinous remains from each taxon were isolated by hand with fine forceps under a dissecting microscope, placed in pre-weighed tin capsules, and dried at 50°C to constant weight. Subfossil remains included headshields and carapaces of *Bosmina* (average 250 remains sample⁻¹, 175 μg dry weight) and *Alona* (average 115 remains, 110 μg dry weight), ephippia (diapausing eggs) of *Daphnia* (average 88 remains, 105 μg dry weight) and head capsules of chironomids (average 7 remains, 32 μg dry weight). Sample sizes were almost always sufficient to provide accurate δ¹³C measurements, yet in some cases were inadequate to obtain reliable δ¹⁵N measurements (mostly *Daphnia* ephippia).
The protocol for stable isotope analysis of invertebrate subfossils was similar to that of SOM, with additional modification of the combustion system to accommodate the small sample mass (Carman and Fry, 2002; Maddi et al., 2006). Briefly, the high temperature-reactor system was minimized by combining combustion and reduction columns into one column with a smaller inner diameter of 8 mm. The single column was filled halfway with elemental copper with a lower reactor temperature of 800°C to prevent melting of the elemental copper. Flow rate was set to 50 mL min⁻¹. Due to the relatively quick oxidation of the elemental copper and narrow zone of optimum temperature, reactors had to be changed about every 100 samples.

Data analysis

Generalized additive models (GAMs) were used to test significant differences of isotopic time series both i) between SOM and different invertebrate taxa and ii) among invertebrate taxa, by modifying the approach of Rose et al. (2012). GAMs are a semi-parametric regression form, which models non-linear relationships between predictors and response in terms of smooth functions of predictors (Wood, 2017). GAMs of Gaussian location-scale family were selected given the considerable differences in variance of δ¹³C and δ¹⁵N values among sample types (SOM, invertebrates) (Fig.5.2), which allows both mean (location) and variance (scale) of the response variable to be modelled as a function of predictors. For each lake, we fitted an ordered-factor-smooth interaction model of the form:

\[ y_{ij} \sim N(\mu_{ij}, \sigma_{ij}^2), \]

\[ \mu_{ij} = \alpha_0 + \alpha_j(\text{sample type}_j) + f_1(\text{time}_i) + f_2(\text{time}_i), \]
\[
\log(\sigma_{ij} - b) = \gamma_0 + \gamma_1 j \text{(sample type)} + \gamma_2 \text{(time interval)},
\]

where sample type is an ordered factor variable representing SOM and invertebrate taxa, while SOM is the reference level. The response \( y_{ij} \) (i.e. \( i \)th \( \delta^{13}C \) or \( \delta^{15}N \) observation of \( j \)th sample type) was Gaussian-distributed with a mean \( \mu_{ij} \) and variance \( \sigma_{ij}^2 \). Here \( \mu_{ij} \) was modelled as the mean value of the response in the reference sample type (SOM) (model intercept \( \alpha_0 \)), plus the difference between mean response of the \( j \)th sample type and \( \alpha_0 \) \( (\alpha_{1j}) \), plus the centered smooth function of time (sediment age in years AD) for the reference sample type SOM \( (f_1) \), plus the difference between smooth function of time for the \( j \)th sample type and \( f_1 \) \( (f_{2j}) \). In other words, the model tests i) if the trend (change over time) of SOM \( \delta^{13}C/\delta^{15}N \) was statistically significant and ii) if both means and trends of \( \delta^{13}C/\delta^{15}N \) of individual invertebrate taxa were significantly different from those of SOM within a given lake.

Subsequently, we processed the GAM output (following Rose et al., 2012) to both generate smooth SI trends for individual invertebrate taxa and estimate differences in SI trends between pairs of taxa, as well as to generate approximate 95% point-wise confidence intervals. We inferred a significant difference in isotopic trends between a pair of taxa when the confidence interval excluded zero for considerable time duration.

Meanwhile, the GAM also modelled \( \sigma_{ij} \) (log scale) as linear effects of the sample type \( \gamma_{1j} \) and the time interval represented by each sediment section \( (\gamma_2) \), plus a constant term (model intercept \( \gamma_0 \)), while \( b \) is a small lower bound on \( \sigma_{ij} \) to avoid any singularities in the model likelihood. This was necessary as variances of sample types were highly different within lakes. Further details of the statistical procedure used here are provided in supplementary methods (Appendix D).
All statistical analysis were performed in R version 3.4.3 (R Core Team, 2017) using the packages ‘mgcv’ (Wood, 2017; Wood et al., 2016) and ‘ggplot2’ (Wickham, 2009).

5.4. Results

δ¹³C of SOM and invertebrate taxa

Across all study lakes, δ¹³C values of SOM ranged from -29.3 to -22.8‰ (Fig. 5.2a), with lakes 17F and 17V exhibiting more enriched δ¹³C values than the other lakes (mean difference of ~3-4‰). For a given taxon, the δ¹³C of invertebrate remains varied less among lakes (-28 and -24‰) than did SI values of SOM (-29.3 to -22.8‰), except *Daphnia* which showed relatively high variation in core-wide mean δ¹³C values (~7‰). Within lakes, ranges of δ¹³C often differed among taxa, although there was no consistent pattern among sites as to which taxon was most variable. GAMs, by modeling both mean and variance components, explained a large proportion of the historical changes in δ¹³C in each lake (explained deviance ranging 91-98%). Sample type (i.e., SOM and invertebrate taxa) was a significant predictor (p < 0.05) of mean δ¹³C i.e. mean δ¹³C of all invertebrate taxa within a lake was significantly different (p < 0.05) from that of SOM in each lake (Fig. 5.2a). For variance of δ¹³C, sample type (i.e., SOM and invertebrate taxa) had a significant effect (p < 0.05) for all lakes, while a significant influence (p < 0.05) of the time interval (represented by each sediment section) was only detected for lakes 17V and 13N.

The δ¹³C of SOM in all lakes exhibited statistically significant (p < 0.05) declines during the 20th century (Table 5.2, Fig. 5.3), with more prominent trends in lakes 6E and 17F relative to other basins. Trajectories of invertebrate δ¹³C generally declined in each
Fig. 5.2. Comparison of isotopic values among sample types i.e. SOM and invertebrate taxa within study lakes. (a) Boxplots of $\delta^{13}$C values of sample types. (b) Boxplots of $\delta^{15}$N values of sample types. Solid circles (●) indicate the mean values and asterisks (*) denote the invertebrate taxa with significantly different ($p < 0.05$) mean values from that of SOM.
Table 5.2. Model summaries for SOM and invertebrate $\delta^{13}$C time series of study lakes

<table>
<thead>
<tr>
<th>Lake</th>
<th>Covariate</th>
<th>EDF</th>
<th>Ref. DF</th>
<th>$\chi^2$</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>17V</td>
<td>Trend$_{SOM}$</td>
<td>2.41</td>
<td>9.00</td>
<td>36.43</td>
<td>$1.16 \times 10^{-6}$</td>
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<tr>
<td></td>
<td>Trend$_{Daphnia-SOM}$</td>
<td>1.21</td>
<td>5.00</td>
<td>3.74</td>
<td>0.04</td>
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<tr>
<td></td>
<td>Trend$_{Bosmina-SOM}$</td>
<td>1.88</td>
<td>5.00</td>
<td>15.85</td>
<td>$4.91 \times 10^{-5}$</td>
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<tr>
<td></td>
<td>Trend$_{Alona-SOM}$</td>
<td>1.90</td>
<td>5.00</td>
<td>18.60</td>
<td>$1.32 \times 10^{-5}$</td>
</tr>
<tr>
<td></td>
<td>Trend$_{Chironomid-SOM}$</td>
<td>$9.07 \times 10^{-6}$</td>
<td>5.00</td>
<td>0.00</td>
<td>0.51</td>
</tr>
<tr>
<td>17P</td>
<td>Trend$_{SOM}$</td>
<td>1.76</td>
<td>2.00</td>
<td>30.54</td>
<td>$4.05 \times 10^{-8}$</td>
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<td></td>
<td>Trend$_{Daphnia-SOM}$</td>
<td>$6.44 \times 10^{-6}$</td>
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<td>0.00</td>
<td>0.66</td>
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<td></td>
<td>Trend$_{Bosmina-SOM}$</td>
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<td>2.00</td>
<td>0.00</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>Trend$_{Alona-SOM}$</td>
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<td>0.30</td>
<td>0.13</td>
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<td></td>
<td>Trend$_{Chironomid-SOM}$</td>
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<td>0.00</td>
<td>0.17</td>
</tr>
<tr>
<td>17F</td>
<td>Trend$_{SOM}$</td>
<td>3.34</td>
<td>9.00</td>
<td>730.81</td>
<td>$&lt; 2.00 \times 10^{-16}$</td>
</tr>
<tr>
<td></td>
<td>Trend$_{Bosmina-SOM}$</td>
<td>0.96</td>
<td>4.00</td>
<td>27.42</td>
<td>$9.49 \times 10^{-8}$</td>
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<tr>
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<td>Trend$_{Alona-SOM}$</td>
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</tr>
<tr>
<td>6E</td>
<td>Trend$_{SOM}$</td>
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<td>917.35</td>
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</tr>
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<td>Value3</td>
<td>Value4</td>
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Fig. 5.3. $\delta^{13}$C trends of SOM and invertebrate taxa of five study lakes. Solid lines denote the fitted trends (smooth functions) and shaded regions represent the point-wise approximate 95% confidence intervals. The columns represent sample types and the rows represent study lakes.
lake (Fig.5.3), although invertebrate time series exhibited noticeable differences in timing, pattern and degree of change both within and among lakes. In lakes 17V, 17F and 6E, temporal trends of invertebrate taxa varied (p < 0.05) from that of SOM, with the exception of Chironomid in lake 17V (Table 5.2 and Fig.5.4). On the other hand, no significant differences in trends (p< 0.05) between invertebrate taxa and SOM were detected for 13N and 17P.

There were few consistent differences in C source for invertebrates both within and among lakes (6E, 17V) with sufficient fossil density of common taxa (Fig.5.5). For example, the δ^{13}C trajectories of Daphnia and Bosmina differed significantly (p< 0.05) from each other and from other taxa for much of the past 100 years in lake 6E. Similarly, δ^{13}C trends in lake 17V were significantly different (p< 0.05) between Bosmina and Daphnia, Bosmina and Chironomid, Daphnia and Alona, and Alona and Chironomid.

δ^{15}N of SOM and invertebrate taxa

The δ^{15}N of SOM varied from -1.9 to 4.1‰ across all years in individual study lakes, with lakes 13N and 17P exhibiting more enriched δ^{15}N (mean difference ~2.0-3.5‰) with less variability relative to other sites (Fig.5.2b). Overall, δ^{15}N values of invertebrate remains showed a higher variability than that of SOM (-1.9 to 4.1‰), which ranged from -1.4 to 9.7‰ across all study lakes with considerable among-lake differences.

Similar to patterns seen with δ^{13}C, GAM analysis explained most variation in sedimentary δ^{15}N for all parameters in each lake (96-99% deviance explained). Within
Fig. 5.4. Within-lake differences in δ^{13}C trends between invertebrate taxa and SOM. The columns represent pair-wise comparisons and the rows represent study lakes. Solid lines denote the estimated differences in trends (difference smooths) and shaded regions represent the point-wise approximate 95% confidence intervals.
Fig. 5.5. Within-lake differences in δ¹³C trends among invertebrate taxa. The columns represent pair-wise comparisons and the rows represent study lakes. Solid lines denote the estimated differences in trends and shaded regions represent the point-wise approximate 95% confidence intervals.
each site, mean δ\(^{15}\)N values of individual invertebrates were significantly enriched (p < 0.05) relative to SOM for most taxa, except *Daphnia* in 17P (Fig.5.2b). Variance of δ\(^{15}\)N was significantly (p < 0.05) influenced by sample type (SOM and invertebrate taxa), but not by the time interval represented by each sediment section (p > 0.05).

All lakes showed significant (p < 0.05) historical declines in δ\(^{15}\)N of SOM and invertebrate taxa (Table 5.3 and Fig.5.6). Specifically, δ\(^{15}\)N values of SOM and invertebrates declined after ca.1940-1950 in lakes 17V, 17P, 17V and 6E, whereas a continuous, linear decline was detected in 13N over the entire 120 year record. While significant differences (p < 0.05) in δ\(^{15}\)N trends between *Bosmina, Alona* and *Chironomid* and SOM in 6E and between *Alona* and SOM in lakes 17V and 17 F were detected (Table 5.3 and Fig.5.7), there was no similarity in the nature or degree of difference in invertebrate-SOM trends among lakes. Similarly, time series of δ\(^{15}\)N differed significantly (p < 0.05) among most pairs of invertebrates in lakes 6E and 17V (Fig.5.8), with few common difference patterns among pairs of taxa.

5.5. Discussion

The novel combination of taxon-specific isotope time series and GAMs demonstrated that significant differences in mean δ\(^{13}\)C and δ\(^{15}\)N values exist between SOM and invertebrate taxa in all lakes, yet significant differences in long-term trends were only detected in certain basins. Significant differences in mean δ\(^{13}\)C and δ\(^{15}\)N between SOM and invertebrate taxa may be reflective of selective feeding by invertebrates (Jones et al., 2008, 1999) and differences in trophic position within food webs (Minagawa and Wada, 1984; Vander Zanden and Rasmussen, 2001). In contrast, differences in historical trends among sample types (i.e. SOM and invertebrate taxa)
Table 5.3. Model summaries for SOM and invertebrate $\delta^{15}$N time series of study lakes

<table>
<thead>
<tr>
<th>Lake</th>
<th>Covariate</th>
<th>EDF</th>
<th>Ref. DF</th>
<th>$\chi^2$</th>
<th>$p$-value</th>
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<tr>
<td>17V</td>
<td>Trend$_{SOM}$</td>
<td>3.97</td>
<td>9.00</td>
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<tr>
<td></td>
<td>Trend$_{Alona-SOM}$</td>
<td>1.84</td>
<td>5.00</td>
<td>9.96</td>
<td>$2.89 \times 10^{-3}$</td>
</tr>
<tr>
<td></td>
<td>Trend$_{Chironomid-SOM}$</td>
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<td>5.00</td>
<td>0.00</td>
<td>0.56</td>
</tr>
<tr>
<td>17P</td>
<td>Trend$_{SOM}$</td>
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<td>2.00</td>
<td>24.71</td>
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<tr>
<td></td>
<td>Trend$_{Daphnia-SOM}$</td>
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<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Trend$_{Bosmina-SOM}$</td>
<td>$8.81 \times 10^{-7}$</td>
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</tr>
<tr>
<td></td>
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<td>Value</td>
<td>p-value</td>
<td>F-value</td>
<td>Significance</td>
<td></td>
</tr>
<tr>
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<td>-------</td>
<td>---------</td>
<td>---------</td>
<td>--------------</td>
<td></td>
</tr>
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<td>1.00 × 10^{-3}</td>
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</tr>
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</tr>
<tr>
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<td>5.00</td>
<td>5.61</td>
<td>1.00 × 10^{-3}</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 5.6. $\delta^{15}$N trends of SOM and invertebrate taxa of five study lakes. Solid lines denote the fitted trends (smooth functions) and shaded regions represent the point-wise approximate 95% confidence intervals. The columns represent sample types and the rows represent study lakes.
Fig. 5.7. Within-lake differences in $\delta^{15}$N trends between invertebrate taxa and SOM. The columns represent pair-wise comparisons and the rows represent study lakes. Solid lines denote the estimated differences in trends (difference smooths) and shaded regions represent the point-wise approximate 95% confidence intervals.
Fig. 5.8. Within-lake differences in δ¹⁵N trends among invertebrate taxa. The columns represent pair-wise comparisons and the rows represent study lakes. Solid lines denote the estimated differences in trends and shaded regions represent the point-wise approximate 95% confidence intervals.
within lakes were more pronounced for $\delta^{15}$N than $\delta^{13}$C, suggesting that either the biogeochemistry of N (Bunting et al., 2010) or trophic relationships among taxa (Karlsson et al., 2004) was more variable than the sources or cycling of C in these boreal ecosystems. Overall, few differences in SI trends between SOM and invertebrates and among different taxa were noted in lakes with more elevated DOC content, whereas such differences in SI time series were more pronounced in relatively clearwater lakes, possibly reflecting variation in the importance of allochthonous C to invertebrate consumers (Solomon et al., 2008; Tanentzap et al., 2017), as well as a higher degree of habitat diversity or feeding selectivity in transparent ecosystems (Matthews and Mazumder, 2006; Persaud et al., 2009). Together, these findings suggest that the analysis of taxon-specific fossil invertebrate time series provides unique insights into the trophic relationships in lentic ecosystems that cannot be achieved through analysis of bulk sediments alone (See also Wyn et al. 2007; Perga et al. 2010; van Hardenbroek et al. 2014).

Within and among lake variation in $\delta^{13}$C of SOM and invertebrate remains

Overall, the range of SOM $\delta^{13}$C values detected across study lakes (-29.3 to -22.8‰) (Fig.5.2) is indicative of mixed contributions of C from C3 terrestrial plants (-28 to -27‰) and lacustrine primary producers (-35 to -8‰) (Meyers and Teranes, 2001; Vuorio et al., 2006), with among-lake differences likely related to factors that control origin and fate of accumulated C (Supplementary discussion; Appendix D). The ranges of $\delta^{13}$C values for sub-fossils of *Bosmina* (28.1‰ to -23‰) and *Daphnia* (-30.6‰ to -23.5‰) were similar to those recorded for these taxa in a synoptic survey of 233 regional boreal lakes (*Bosmina* -31.4 to -22.9‰; *Daphnia* -35.8 to -24.3‰) (Chapter 2),
suggesting that downcore $\delta^{13}C$ variations in invertebrate $\delta^{13}C$ arise mainly from fluctuations in primary productivity, allochthonous organic matter inputs, and dissolved inorganic carbon (DIC) concentration in regional lakes (Chapter 2). Elsewhere highly negative $\delta^{13}C$ values (~50 to -40‰) of subfossil chironomid head capsules and *Daphnia* ephippia may reflect contributions of $^{13}C$-depleted methonogenic carbon to invertebrate diets (Frossard et al., 2013b; Rinta et al., 2016; Schilder et al., 2017; van Hardenbroek et al., 2013; Wooller et al., 2012), a factor which does not seem to be important in our study (Fig.5.2). Instead, invertebrate $\delta^{13}C$ values herein were usually within the range expected for photoautotrophically-produced C in aquatic and terrestrial ecosystems (-35 to -8‰) (France, 1996; France, 1995a; Meyers and Teranes, 2001; Vuorio et al., 2006).

Significant within-lake differences in mean $\delta^{13}C$ of SOM and chitinous remains (Fig.5.2) are in line with findings of other paleolimnological studies (Kattel et al., 2015; Simon et al., 2017; van Hardenbroek et al., 2014, 2013). In principle, such deviations can arise from preferential ingestion or assimilation of specific fractions of particulate organic matter (POM) or surface sediment by invertebrates (Jones et al., 2008, 1999). Bulk POM is a mixture of several constituents (i.e. algae, detritus, pollen, bacteria and small planktonic organisms) each with a different $\delta^{13}C$ value (del Giorgio and France, 1996; Masclaux et al., 2013; Meili, 1992) and relative contribution to the POM pool. POM is eventually deposited as sediment and, as a result, $\delta^{13}C$ of surface sediment is generally similar to that of POM in overlying waters (Jones et al., 2008; Meili et al., 1996; Meyers and Teranes, 2001). Contemporary studies which detect differences between $\delta^{13}C$ of lacustrine POM or surface sediment and invertebrate consumers (cladocerans and chironomids) often attribute these patterns to the effects of preferential
grazing and/or assimilation of isotopically-distinct fraction within the diet (del Giorgio and France, 1996; Doi et al., 2006; Jones et al., 2008, 1999; Lennon et al., 2006; Meili et al., 1996). This isotopic deviation is likely conserved between invertebrate remains and POM detritus even after burial in lake sediments (Schilder et al., 2015a), assuming that diagenetic alterations of δ^{13}C is negligible or the same in both materials.

Empirical and circumstantial evidence indicates that invertebrate taxa examined here can exhibit feeding selectivity due to physical, sensory and nutritional properties of food items. Properties which affect ingestion include size, shape, flavour, surface characteristics (sheathes, cell projections, flagella, etc.) and nutrient content (Bogdan and Gilbert, 1982; Brett et al., 2009; Butler et al., 1989; Cyr and Curtis, 1999; DeMott, 1986; Knisely and Geller, 1986), while differential digestion in the gut can affect assimilation (Porter, 1973). *Bosmina*, a pelagic suspension-feeder, is known to graze selectively on certain phytoplankton taxa, such as the chlorophytes *Chlamydomonas* and *Cosmarium* (Bleiwas and Stokes, 1985; Bogdan and Gilbert, 1982; Demott, 1982; DeMott, 1986). In contrast, the other pelagic filter-feeder, *Daphnia* is less likely to be selective (Demott, 1982; DeMott and Kerfoot, 1982), despite occasionally-preferentially use of phytoplankton and bacteria (Brett et al., 2009; Grey et al., 2000; Grey and Jones, 1999; Gu et al., 1994; Knisely and Geller, 1986). The feeding ecology of the littoral cladoceran *Alona* is poorly established, although circumstantial evidence suggests they may prefer epiphytic over planktonic algae (Downing, 1981; Sakuma et al., 2004). For detritivorous chironomid larvae, preferential utilization of phytoplankton detritus and methane-oxidizing bacteria in surface sediments has been reported (Doi et al., 2006; Johnson, 1987; Jones et al., 2008; Solomon et al., 2008). Finally, high degrees of
variability in isotope values among invertebrates may also reflect temporal and spatial
differences in the availability of preferred food items (Cole et al., 2011; Solomon et al.,
2008; Tanentzap et al., 2017) or even the presence of different species, tribes,
subfamilies within the broader taxonomic group (Solomon et al., 2008; Tanentzap et al.,
2017; van Hardenbroek et al., 2013).

Differences in $\delta^{13}C$ trends between invertebrate remains and SOM

Patterns of sedimentary $\delta^{13}C$ decline over the 20th century (Fig.5.3) are similar to
those observed of other regional studies of boreal western Canada (Ahad et al., 2011;
Curtis et al., 2010). In principle, such depletion of $^{13}C$ content may arise because of
increased abundance and assimilation of $^{13}C$-depleted respired DIC by primary producers
over the past century (France et al., 1997; Meyers and Teranes, 2001; Rau, 1978).
Alternately, such relatively small declines (1-2‰) may reflect changes in the isotopic
composition of atmospheric CO$_2$ as a result of accelerated release of $^{13}C$-depleted CO$_2$
from fossil fuels, known as Suess Effect (Schelske and Hodell, 1995; Verburg, 2007).
Although a declining trend in SOM $\delta^{13}C$ can also be expected in response to decreased
primary productivity or increase in [DIC] leading to enhanced discrimination against $^{13}C$
(Meyers and Teranes, 2001; Schelske and Hodell, 1995), we believe that these latter
mechanisms are unlikely to occur in the study lakes, particularly as other
paleolimnological proxies from the same sediment cores (i.e. molar C: N ratios of SOM,
algal pigments and scaled-chrysophytes) rather indicate an increase in primary
production over time (Mushet et al., 2017; Wolfe, 2016).

Significant differences between $\delta^{13}C$ trajectories of SOM and invertebrates from
different habitats within lakes suggest that analysis of taxon-specific SI can provide new
insights on the regulation of food-web carbon dynamics of different lake habitats. For instance, variation in phytoplankton $\delta^{13}C$ due to changes in primary productivity, DIC content or DIC source (France et al., 1997; Meyers and Teranes, 2001; Rau, 1978) should be reflected in isotopic values of invertebrate remains if phytoplankton-derived organic matter is grazed or assimilated preferentially by invertebrates. Such fine-scale trophic patterns may be partially or completely masked in analysis of SI in SOM in instances where phytoplankton-derived organic matter is mixed with other sources of POM or dissolved organic matter (DOM) (Davidson and Jeppesen, 2013; Meyers and Teranes, 2001). For the same reason, SOM $\delta^{13}C$ may fail to reflect environmentally-induced variations in epiphytic $\delta^{13}C$ which are faithfully recorded in exoskeletons of littoral herbivore *Alona*, or the incorporation of C from methanogens by benthic chironomids (Schilder et al., 2017; Simon et al., 2017; van Hardenbroek et al., 2014). Finally, historical trends in invertebrate $\delta^{13}C$ may reflect shifts in feeding selectivity of source population due to temporal variations in relative availability of autochthonous vs. allochthonous resources (Demott, 1982; Tanentzap et al., 2017). Under such conditions, the $\delta^{13}C$ values of invertebrate remains may be expected to diverge from those of bulk SOM during periods of high feeding selectivity (and converge as feeding selectivity decreases).

Unexpectedly, trends in $\delta^{13}C$ of invertebrate remains did not vary significantly from SOM in dystrophic lakes (17P and 13N), which may be a result of strong influence of allochthonous inputs on both SOM and invertebrate diets (Fig.5.4). In particular, these sites exhibited not only much higher DOC concentrations and water color (Table 5.1), but also elevated SOM C:N (molar) ratios (ca. 13-15 and 15-17, respectively)
compared to other study lakes (Appendix D: Fig. D1), indicating substantial terrestrial organic matter inputs over the past century (Meyers and Teranes, 2001). Although speculative, we suggest that invertebrate consumers in these lakes were relying more on allochthonous C sources (terrestrial detritus and/or heterotrophic bacteria) than, given they were more abundant than preferred autochthonous foods (Cole et al., 2011; Simon et al., 2017; Solomon et al., 2008; Tanentzap et al., 2017). Allochthonous organic matter may also have largely contributed to SOM in these dystrophic lakes (Dalton et al., 2018; Meyers and Teranes, 2001). Such large contribution of allochthonous sources to both invertebrate diets and SOM may have led to temporal coherence between δ¹³C trends of invertebrate remains and SOM.

**Differences in δ¹³C trends among invertebrate taxa**

The significant among-taxon differences in δ¹³C trends of lakes 17V and 6E indicate that flux of carbon through invertebrates can differ among taxa, habitats and lakes, and that all pathways may have decoupled through time (Fig. 5.5). Dietary sources of C for invertebrates, as well as the associated δ¹³C values of C source can be impacted by habitat-specific or whole-lake environmental changes (Persaud et al., 2009; Vadeboncoeur et al., 2003; van Hardenbroek et al., 2014). In particular, changes in relative availability of different carbon sources (i.e. phytoplankton, epiphyton, terrestrial, methane-derived carbon) can be induced by alteration in water transparency by primary producers (Solomon et al., 2008; Vadeboncoeur et al., 2003), physical turbidity (Scheffer et al., 1993) or DOC influx (Carpenter et al., 1988), leading to shifts in the diets of primary consumers (Solomon et al., 2008; Vadeboncoeur et al., 2003). For instance, a switch from predominantly benthic production to a pelagic regime following
eutrophication can shift the diet of littoral consumers (e.g. Alona) from $^{13}$C-enriched epiphyton ((Hecky and Hesslein, 1995) to $^{13}$C-depleted phytoplankton and suspended detritus, while diet of pelagic consumers (e.g., Bosmina, Daphnia) remain unaffected (Vadeboncoeur et al., 2003). Further, $\delta^{13}$C of littoral epiphyton may respond differently compared to pelagic phytoplankton to temporal changes in DIC content and dominant chemical form of inorganic C ($\text{CO}_2$, $\text{HCO}_3^-$, $\text{CO}_3^{2-}$) due to boundary layer effects (Hecky and Hesslein, 1995; McPherson et al., 2015; Woodland et al., 2012), thereby altering historical trends in $\delta^{13}$C of littoral and pelagic consumers. Differences in degree of feeding selectivity may have contributed to differences in $\delta^{13}$C through time between pelagic taxa Bosmina and Daphnia. For instance, $\delta^{13}$C trajectory of less-selective Daphnia may be more affected by temporal variations in relative availability of autochthonous vs. allochthonous sources compared to more selective Bosmina (Demott, 1982; DeMott and Kerfoot, 1982; Tanentzap et al., 2017).

Time series of $\delta^{13}$C did not differ significantly among invertebrates through time in three lakes (13N, 17P, 17F; Fig.5.5), suggesting the path or process of C assimilation was relatively consistent in food webs of these lakes. Such consistency in the two dystrophic lakes (13N, 17P) may reflect the paramount importance of terrestrial DOM as a food-web C source in all habitats (Solomon et al., 2011), either because of the importance of $^{13}$C-depleted respired DIC to primary producers in all habitats, or, the consistent availability of heterotrophic bacteria as a C source to primary consumers (see above). In lake 17F, both pelagic and benthic habitats were likely autotrophic due to light penetration to the bottom, as indicated by dominance of benthic over planktonic diatoms over the past century (Laird et al., 2017). The unchanged diatom composition in
this lake (Laird et al., 2017) suggests that is limnological conditions have remained largely unaltered through time, with few changes in water transparency and the ratio of benthic to pelagic autotrophy. Therefore, that the dietary sources of C to invertebrates may have remained consistent within 17F’s habitats through time.

*Within and among lake variation in δ15N of SOM and invertebrate remains*

Variation in sedimentary δ15N across the study lakes (~ -2 to 4‰) (Figs.5.2, 5.6) can be related to differences in the mass flux or isotopic ratios of terrestrially fixed DOM-N, as well as subsequent modification following biological uptake by aquatic and terrestrial primary producers (Meyers and Teranes, 2001; Talbot, 2001). Low SOM δ15N values in several study lakes (<2‰) indicate fixed N is the main N source (Meyers and Teranes, 2001; Patoine et al., 2006), which is likely derived from terrestrial sources, as pigments for N2-fixing cyanobacteria were not detected in sediments of these lakes (Wolfe, 2016). Nitrogen fixation by plants such as *Alnus* spp. and *Shepherdia canadensis* and microorganisms in deciduous forest may result in DOM-N inputs with low δ15N into lakes (Bunting et al., 2010; Engstrom and Fritz, 2006; Wolfe, 2016). Among-lake differences in SOM δ15N on the other hand can arise due to subsequent modification of fixed DOM-N by biological (terrestrial and in-lake) processes. For instance, peatlands may act as denitrification hotspots where 14N is preferentially outgassed, resulting in higher δ15N values of residual DOM-N entering the lakes (Lindau et al., 1997; Wray and Bayley, 2007). A detailed account of other potential factors influencing among-lake variability in SOM δ15N is provided in the supplementary discussion (Appendix D). The δ15N values of *Bosmina* exoskeletons and *Daphnia* ephippia recorded in the present study (0.9 to 9.7‰ and -1.4 to 9‰ respectively) were
similar to contemporary values (-1.5 to 9‰ and 0.5 to 7.8‰, respectively) derived from regional lake surveys (Chapter 2), supporting the high likelihood that our observations are within the expected range in response to regional environmental variations.

The significantly higher mean δ¹⁵N of invertebrate remains relative to SOM in our study lakes (Fig.5.7) is characteristic of a 2-4‰ trophic fractionation between diet and consumer (Minagawa and Wada, 1984; Vander Zanden and Rasmussen, 2001), assuming that SOM δ¹⁵N represents available dietary sources (Perga et al., 2010). The δ¹⁵N enrichment relative to SOM varied among invertebrate taxa within each lake (Figs.5.2, 5.7), likely as a result of differences in trophic position of individual taxa within the local aquatic food web, as well as variability in metabolic pathways of protein synthesis (Kling et al., 1992; Nerot et al., 2012; Vander Zanden and Rasmussen, 2001). Meanwhile, among-lake differences in invertebrate δ¹⁵N enrichment relative to SOM may be associated with lake-specific factors that can influence the magnitude of ¹⁵N fractionation by consumers. For instance, reduced availability of N in some food resources (i.e. high C: N ratios) may lead to elevated diet-tissue ¹⁵N fractionation in consumers (Adams and Sterner, 2000). Enriched δ¹⁵N values of consumers can also arise under conditions of low-food quantity or quality as a result of increased turnover of internal nitrogen and preferential excretion of ¹⁴N (Adams and Sterner, 2000; Karlsson et al., 2004)

*Differences in δ¹⁵N trends between invertebrate remains and SOM*

As historical data are not available for much the study region (Wolfe 2016), it is difficult to unambiguously identify the precise mechanism(s) underlying progressive declines in δ¹⁵N of SOM and invertebrate remains. However, given that declines occur
in all lakes, irrespective of the mode of nutrient limitation (N vs. P) and relationship with respect to aerial deposition of nitrogenous pollutants from AOSR (Mushet et al. 2017; Laird et al. 2017), we infer that climate-related processes, such as regional brownification (Anas et al., 2015) leading to increased influx of $^{15}$N-depleted terrestrial DOM-N (Bunting et al., 2010) may underlie the common trend among lakes. This hypothesis is supported by the observations that terrestrially-derived DOM-N is the major N source to these lakes (see above) and that the abundance of mixotrophic algae has increased in these lakes during the past century (Wolfe 2016; Stevenson et al. 2016).

Significant differences between $\delta^{15}$N trends of SOM and invertebrates in lakes 17V, 17F and 6E (Fig.5.7) may have arisen from shifts in dietary sources of N and/or changes in magnitude of isotopic fractionation in invertebrate consumers. For example, $\delta^{15}$N of invertebrate consumers can covary with bacterial contribution to the diet (Grey et al., 2004, 2001; Karlsson et al., 2004; Matthews and Mazumder, 2007). Empirical evidence suggests that bacterial $\delta^{15}$N may differ from other basal resources either due to elevated isotopic fractionation depending on biochemical composition and availability of nitrogen substrate (McGoldrick et al., 2008; Steffan et al., 2015; Yamaguchi et al., 2017) or assimilation of isotopically light nitrogen substrate (e.g. excreted ammonia) (Grey et al., 2004; Macko and Estep, 1984). Alternately, changes in $\delta^{15}$N offset between SOM and invertebrates can reflect variation in trophic position of taxa over decades, assuming that SOM represents an isotopic baseline (Griffiths et al., 2010; Perga et al., 2010). In this case, climatically-induced changes in DOC flux could favour shifts from an algae-dominated to a bacterivorous protozoa-dominated diet (i.e. microflagellates and ciliates) (DeMott and Kerfoot, 1982; Ngochera and Bootsma, 2011; Porter et al., 1985), which is
in turn reflected by temporal changes in $\delta^{15}N$ of invertebrate consumers. Finally, shifts of N-rich algae to N-poor allochthonous food sources may lead to elevated $\delta^{15}N$ in aquatic invertebrates (Maguire and Grey, 2006) as a result of increased diet-tissue $^{15}N$ fractionation (Adams and Sterner, 2000; Karlsson et al., 2004).

*Differences in $\delta^{15}N$ trends among invertebrate taxa*

The presence of significant among-taxon differences in $\delta^{15}N$ trends in lakes 17V and 6E suggests that trophic relationships among taxa may vary in lakes at centennial scales (Fig. 5.8). As noted above, variable offsets in $\delta^{15}N$ among pairs of species may result from habitat-specific changes in resource materials with different $\delta^{15}N$ values, resource limitation, or changes in metabolic processes of individual taxa. In addition, lake-specific changes in the influx of nitrogenous materials may also influence the degree of agreement in $\delta^{15}N$ trends between pairs of species, particularly from different habitats. In particular, the $\delta^{15}N$ of individual taxa may be selectively influenced by dietary shifts or changes in isotopic fractionation while the $\delta^{15}N$ of the other taxon remains unaffected (e.g. *Alona* vs. *Bosmina* in 17V). Alternately, $\delta^{15}N$ variations of both taxa can be influenced by environmental and physiological mechanisms simultaneously, asynchronously or differentially (e.g. *Alona* vs. *Bosmina* in 6E), as has been seen in modern lake time series (Patoine et al. 2006). Regardless of the underlying mechanism, the observation that historical differences between pairs of common species (e.g., *Bosmina* v. *Alona*) vary among lakes (17V, 6E) suggests that future research is needed to evaluate how common this pattern may be among ecosystems.
Impacts of industrial deposition

The study lakes are located downwind of and in near proximity to the AOSR, a major source of atmospheric sulphur and nitrogen oxides, as well as base cations (Fenn et al., 2015; Percy, 2013). Even though the study lakes are less sensitive to acidification due to their high geological buffering capacity (Laird et al., 2017), N-limited (or N-P co-limited) lakes are still vulnerable to increases in primary productivity due to deposition of reactive N (Curtis et al., 2010; Fenn et al., 2015). Consequently, it is feasible that some food-web related changes induced by enhanced lake productivity could be reflected by $\delta^{13}$C and $\delta^{15}$N trajectories of invertebrate remains. However, as we did not detect any changes in trajectories corresponding to intensified industrial development ca. post 1980 in any of the lakes, we believe that atmospheric deposition of industrial pollutants played a negligible role in the patterns recorded in this study. Although speculative, such conclusion is in agreement with other paleolimnological proxies from the same sediment cores (i.e., molar C: N ratios of SOM, diatom assemblages, scaled-chrysophytes and cladoceran composition), which indicated only limited industrial impacts (Hesjedal 2017; Laird et al. 2017; Mushet et al. 2017).

Caveats

Due to methodological constraints, our SI analyses were conducted at a relatively coarse taxonomic resolution relative to modern limnological studies (e.g., Daphnia spp., chironomids, etc.). Consequently, interpretations of taxon-specific isotopic variability may be confounded in part by variation in $\delta^{15}$N and $\delta^{13}$C values among con-specific taxa. For example, although chironomid larvae are generally considered as detrivores, feeding mode may vary among species (e.g. deposit feeding, filter feeding and engulfing), which
may have consequences for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ variability (Griffiths et al., 2010; Solomon et al., 2008; van Hardenbroek et al., 2014). Similarly, differences in daphniid body size can influence the accessibility to both very small and large food particles (Burns, 1968; Kasprzak and Lathrop, 1997). Furthermore, *Daphnia* ephippia may integrate time differently than invertebrate exoskeletons, and may provide a different metric of resource use or trophic position compared to remains deposited as a result of adult death or molting. Ephippia are produced within a very short time frame in response to specific environmental cues such as food limitation, reduced photoperiod and crowding (Kleiven et al., 1992). Hence, they may only provide a temporal snapshot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the source population during their brief period of formation (Griffiths et al., 2010; Perga, 2009). In contrast, invertebrate exoskeletons may provide a relatively long-term representation isotopic composition of the source population.

5.6. Conclusions

Our study demonstrates that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis of taxon-specific invertebrate remains can provide unique insights into historical trophodynamics in lake food webs, different from those derived from an analysis of bulk sediments. Statistically-significant differences in mean values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between SOM and invertebrate remains likely reflected selective feeding or trophic fractionation, and appear to be ubiquitous in study lakes despite limnological differences among lakes. Further, the absence of significant differences in historical trends among trophic levels or taxa in relatively dystrophic lakes suggests a stabilizing role of terrestrial C contributions in food-web processes (cf. Wetzel 1989). On the other hand, the higher variability in SOM-invertebrate SI time series in relatively clear lakes may reflect decadal-scale variation in
in dietary sources of elements or their metabolic processing by invertebrates. That said, it is clear that further refinement of causal mechanisms requires substantial additional work, including additional paleoenvironmental analyses and comparisons with long-term monitoring data. For instance, simultaneous evaluation of $\delta^{13}C$ and $\delta^{15}N$ of chlorins (i.e. chlorophylls and their phaeopigment derivatives) may help to better understand if and how isotopic trends of invertebrate remains are related to changes in aquatic primary productivity (Hayes et al., 1987; Tyler et al., 2010). Based on our results, we suggest that evaluation of isotopic trends of invertebrate remains relative to SOM, will be a particularly informative means of unraveling past food-web carbon, nutrient and trophic dynamics.

5.7. Acknowledgements

We thank Steve Wilkie and Shane O’Neil for technical support. This work was supported by the Natural Sciences and Engineering Research Council of Canada (Grant STPGP/447139-2013), Saskatchewan Ministry of Environment, Saskatchewan Advanced Education, Saskatchewan Water Security Agency, Environment and Climate Change Canada, and University of Regina. This research was conducted in Treaty 8 and 10 territories.
CHAPTER 6. GENERAL CONCLUSIONS

6.1. Summary and synthesis

Potential biological responses to atmospheric pollutant deposition from the Athabasca Oil Sands Region (AOSR) and climate change in lakes across boreal western Canada has been of recent interest. In the face of anticipated expansions in oil sands production (Canadian Association of Petroleum Producers, 2018) and climatic changes (Schindler and Donahue, 2006), a predictive understanding of regulatory processes of biological properties of lakes is necessary to better assess potential future impacts. My research, based on broad-scale spatial and temporal assessments of zooplankton communities and their δ^{13}C and δ^{15}N values, was aimed to assess past and current biological impacts of aforementioned environmental perturbations and more importantly, to develop a holistic understanding of how zooplankton community and stable isotope (SI) dynamics in lakes across the region are regulated.

Impacts of environmental perturbations

Overall, my findings from spatial and temporal assessments of zooplankton compositional and SI variations did not provide evidence for strong and widespread biological impacts of atmospheric pollutant deposition from oil sands operations, which is generally consistent with results of other neo- and paleolimnological investigations based on biological criteria in the region (Anas et al., 2014a; Hazewinkel et al., 2008; Kurek et al., 2013; Laird et al., 2017, 2013; Mushet et al., 2017; Parsons et al., 2010; Summers et al., 2016). The spatial assessment of zooplankton communities (chapter 2) indicated significant correlations of zooplankton compositional changes with environmental gradients that are vulnerable to industrial pollutant deposition (i.e.
variables related to acid base status, productivity and trace metals) together with intriguing spatial patterns. Yet, it was not possible to unambiguously determine if industrial deposition had a role in inducing these changes due to likely confounding effects of natural environmental heterogeneity. Meanwhile, spatial variation in δ\(^{13}\)C and δ\(^{15}\)N of zooplankton taxa (chapter 4) were related to environmental variables that are directly and indirectly sensitive to industrial sulfur and nitrogen deposition (i.e. color, dissolved organic carbon and nutrient concentrations), yet specific spatial patterns of zooplankton SI values across the region did not corroborate any impacts of industrial deposition. In addition, paleolimnological assessments did not detect any changes in trajectories of zooplankton compositional turnover (chapter 3) or SI values of zooplankton subfossil remains (chapter 5) coinciding with industrial intensification of AOSR ca. post 1980 in any of the lakes. Together, this suggest atmospheric pollutant deposition had a negligible effect on temporal biological dynamics of lakes in the region.

The paleolimnological assessments (chapters 3 and 5) suggest that biological responses to climate change may vary not only among lakes, but also among different habitats within lakes. Zooplankton compositional changes during the past century were asynchronous among lakes likely due to stronger effects of local factors over climatic effects, while asynchrony in SI trajectories among zooplankton taxa (from different lake habitats) suggests that climatic factors may have differentially influenced basal food-web dynamics in different habitats. Such inconsistencies in biological responses to climate change limit the ability to forecast future changes (Perga et al., 2015; Reyjol et al., 2014).
Zooplankton compositional variation: ecological implications

My research provides a comprehensive understanding of regulatory processes of zooplankton community dynamics in lakes across boreal western Canada. My findings indicated greater importance of local (lake-specific) factors compared to broad-scale regional factors such as climate and dispersal processes in regulating zooplankton community (spatial and temporal) dynamics. Meanwhile, evaluation of environment-species and species-species associations in relation to known ecology of different zooplankton species suggested that local abiotic factors regulated zooplankton community composition through both direct (physiological effects) and indirect (e.g. altered food-web interactions and microhabitat structure) mechanisms. Ultimately, these insights are crucial to forecast expected zooplankton community responses to future enhanced industrial pollutant deposition and climate change.

In addition, my research has broader implications for designing and implementing zooplankton-based bioassessment studies. The spatial assessment of zooplankton communities (chapter 2) showed that impacts of environmental factors and dispersal processes on community assembly vary with spatial scale. This emphasizes that spatial bioassessments using zooplankton communities should be designed to explicitly incorporate the scale-dependency of environmental and dispersal effects. Meanwhile, temporal assessment of zooplankton communities (chapter 3) indicated lack of spatial synchrony in community dynamics among lakes, owing to dominant effects of lake-specific factors over regional factors. This cautions against extrapolating site-specific results across broad geographic regions (Huttunen et al., 2014; Magnuson et al., 1990),
highlighting the necessity of intensive replication in zooplankton-based bioassessments of regional impacts.

*Zooplankton SI variation: ecological implications*

Spatial isotopic patterns can provide information crucial to revealing dynamics of ecosystem processes at the landscape scale, yet conducting broad-scale SI surveys requires considerable financial and logistic resources (Kendall et al., 2010). The spatial assessment of zooplankton SI (chapter 4) demonstrates that integrating isotope assays with existing environmental monitoring programs not only generates a large set of spatially distributed isotope samples by leveraging existing field sampling resources, but also provides extensive corresponding environmental data at no extra cost. In return, patterns revealed by SI data complemented the lake monitoring program by providing a broad understanding of landscape-level regulatory processes of zooplankton SI variation. This information is crucial to assess the effects of environmental changes on basal food-web dynamics in lake ecosystems through changes in catchment and in-lake processes (Fry, 1999; Kendall et al., 2010). For instance, atmospheric nitrogen and sulphur deposition from oil sands operations together with regional climate change (changes in temperature and hydrologic settings) may not only alter carbon and nutrient export from watersheds to lakes (Anas et al., 2015; Meunier et al., 2016; Wolfe, 2016), but also influence in-lake carbon and nitrogen cycling (Adrian et al., 2009; Anas et al., 2015). These changes may lead to shifts in carbon and nutrient fluxes to food webs (Anas et al., 2015; Meunier et al., 2016), which would be eventually reflected in SI values of zooplankton consumers. As fluxes of carbon and nutrients to zooplankton taxa may vary
depending on their dietary or habitat niches (chapters 4 and 5), $\delta^{13}C$ and $\delta^{15}N$ of different taxa may respond differently to such environmental changes.

6.2. Future work

Lake monitoring and paleolimnological studies alone often may not provide conclusive evidence for cause-effect mechanisms. However, they may provide the basis for deriving ecologically relevant hypotheses that can be tested with additional studies (Keller, 2009; Leavitt et al., 2009). Based on findings of my current work, I propose the following future research directions to further advance the understanding of regulatory mechanisms of zooplankton compositional and SI dynamics.

As both overland (i.e. via wind and animal vectors) and watercourse pathways are important for zooplankton dispersal (Havel and Shurin, 2004), the potential of zooplankton species to disperse among lakes within a landscape may depend on both overland distance and hydrologic connections among lakes (Beisner et al., 2006; Shurin et al., 2009). However, only the former was taken into account in the spatial assessment of zooplankton compositional variation (chapter 2). As study lakes were only random samples from the lake populations of each sub-region, it was assumed that the likelihood of hydrological connectivity among study lakes (within sub-regions) should be lower with an increase in among-lake distances. In addition, as most of the study lakes are headwater systems, they were more likely hydrologically disconnected from each other. However, future lake surveys in the region should deliberately examine the relative importance of overland vs. watercourse routes on zooplankton dispersal at the sub-regional scale. For this, ideally all lakes within the selected geographic areas within each sub-region should be sampled while establishing watercourse distances among them.
Subsequently, zooplankton compositional variation should be explicitly evaluated in relation to overland and watercourse distance matrices.

Spatial and temporal assessments of zooplankton SI variation (chapters 4 and 5) revealed that $\delta^{13}C$ of different taxa responds differently to environmental variations. I speculated that this is a consequence of differences in dietary or habitat niches among taxa and relative availability of preferred autochthonous dietary sources. Examining of $\delta^{13}C$ of autochthonous sources i.e. contemporary phytoplankton or POM samples in the case of spatial (contemporary) assessment and chlorins (i.e. chlorophylls and their phaeopigment derivatives) in the case of temporal (paleoecological) assessment, in parallel with those of taxon-specific zooplankton subfossil remains may help to better elucidate such relationships between isotopic trends of different taxa and variations in autochthonous dietary sources. This may be further validated through an experimental approach, for instance, by changing the autochthonous food supply for selected zooplankton taxa with varying dietary or habitat niches and comparing resulting changes in $\delta^{13}C$ among taxa.

The paleolimnological assessments (chapters 3 and 5) indicated that temporal dynamics of zooplankton composition and SI values were idiosyncratic among lakes and different habitats within lakes, respectively. I argued that such lack of synchrony at the secondary producer level can be at least partially a result of asynchrony at the primary producer level. To test this, future paleolimnological studies should examine the degree of temporal concordance (Bowman et al., 2008; Peres-Neto and Jackson, 2001) of secondary producer attributes (e.g. species composition, SI values) with primary producer attributes (e.g. diatom and chrysophyte species composition, algal pigments, SI
of chlorins). Moreover, an improved study design, for instance, selection of lakes that are similar to each other in terms of lake morphometry and hydrologic setting representing ‘impact’ (i.e. more prone to industrial pollutant deposition) and ‘reference’ (i.e. less vulnerable to industrial pollutant deposition) may help to better detect any climate signal in zooplankton community and SI dynamics and to elucidate industrial impacts from climatic forcing.
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Appendix A: Supplementary material for Chapter Two

Fig. A1. Boxplot of multivariate variances of chemical parameters in five physiographic sub-regions. The multivariate variance for each sub-region was determined by calculating the average distance of group members to group centroid in multivariate space. Mean values of the sub-regions indicated by same letters are not significantly different (p > 0.05) according to PERMDISP2 test (Anderson, 2006) followed by pairwise permutation F-tests.
Fig. A2. Comparison of selected trace metals of 97 study lakes among five physiographic sub-regions. Median values of the sub-regions indicated by same letters are not significantly different (adj.p > 0.05) according to Kruskal-Wallis test followed by multiple comparisons.
Fig. A3. Redundancy analysis (RDA) biplots of crustacean zooplankton composition versus PRED_MORPH of five physiographic sub-regions; (a) MRU; (b) BM; (c) SM;
Only significant \((p<0.05)\) predictors retained in each independent model by the forward selection criterion are presented; Biplots are scaled to optimally display the relationships among variables; For zooplankton taxa codes, see legend of fig.2.2.
Supplementary discussion: Environment – zooplankton relationships in study lakes across northeast Alberta

The significant effect (p<0.05) of predation regime on species composition both at regional and sub-regional scales is mostly expected. As evident from RDAs between species composition and PRED_MORPH, greater abundances of Daphnia pulex were associated with potentially fishless conditions, while higher abundances of Bosmina longirostris were correlated to opposite conditions (along RDA axis 1, Fig.2.2b and Fig.A3a, b, c, e). Small-bodied species (such as B. longirostris) dominate zooplankton communities when large-bodied species are preferentially preyed by visually oriented planktivorous fish (Zaret, 1980). In fishless lakes (or lakes with minimal fish predation) on the other hand, predation pressure on zooplankton is primarily exerted by the invertebrate, gape-limited planktivore Chaoborus, to which large-bodied species such as D. pulex are resistant, whilst small-bodied B. longirostris are highly vulnerable (Anas et al., 2014b; Hall et al., 1976; Von Ende and Dempsey, 1981; Zaret, 1980). In addition, competition from superior, large-bodied species (e.g. D. pulex) can also negatively affect the abundances of small-bodied species in fishless lakes (Anas et al., 2014b; Hall et al., 1976; Vanni, 1986).

The divergence of abundances of D. pulex vs. B. longirostris was also significantly related (p<0.05) to [SO₄], [NO₃-N] and [DOC] both at regional and sub-regional scales as shown by RDAs between species composition and CHEM (along RDA axis 1, Fig.2.2a and Fig.2.3a, b, d). Meanwhile, variance partitioning and partial RDAs revealed that a major proportion of the variation explained by these chemical predictors was shared by the effect of predation regime on species composition identified above.
(Table 2.2 and Table 2.3). These findings collectively indicate that differences in predation regime were at least partly induced by these chemical factors. The acidic properties of $[SO_4]$, $[NO_3-N]$ and $[DOC]$ (both organic and inorganic acidity) can negatively affect fish assemblages in lakes (Power et al., 1973; Schilling et al., 2008; Tammi et al., 2003), while Chaoborus can withstand highly acidic conditions (Sweetman and Smol, 2006).

Besides the shared effect with water chemistry, predation regime also had a unique effect on species composition (Table 2.2). This suggest that predation regime in the region can be associated with factors other than water chemistry parameters, such as topographic features which prevented postglacial colonization of fish, winterkill of fish in shallow lakes, etc. (Drouin et al., 2009; Power et al., 1973).

In addition, $[NO_3-N]$, $[SO_4]$ and $[DOC]$ also had significant unique effects on species composition, after controlling for the shared effects with predation regime (Table 2.2), indicating effects of these variables on zooplankton composition via other indirect pathways. Higher $[NO_3-N]$ can indicate more productive conditions (Bergström, 2010), which may favor high densities of $D. pulex$ (Vanni, 1986). Meanwhile, reduced water clarity associated with high $[DOC]$ may impair predation by visually oriented predators such as fish and thereby enhance refuge availability for large-bodied species such as $D. pulex$ (Wissel et al., 2003). The positive effect of $[SO_4]$ on abundance of $D. pulex$ (beside its indirect effect via predation variability) is mostly unexpected and we could not find any published evidences to support an underlying mechanism.

A significant ($p<0.05$) negative relationship between abundances of Skistodiaptomus oregonensis and those of Leptodiaptomus minutus and/or Holopedium
**gibberum** along the [base cation] gradient was identified by RDAs between species composition and CHEM at regional scale (along RDA axis 2, Fig.2.2a), as well as at sub-regional scale in MRU (along RDA axis 2, Fig.2.3a). This relationship is likely due to different physiological sensitivities of these species to acid-base status. *Skistodiaptomus oregonensis* is a highly acid-sensitive species, whereas both *H. gibberum* and *L. minutus* are acid-tolerant, generalist species which predominate in acidic conditions likely due to reduced competition by *S. oregonensis* (Brett, 1989; Havens and Hanazato, 1993; Marmorek and Korman, 1993). Given that [base cation] was associated with acid-base status of our study lakes, its effect on the negative association between these two groups of species is not surprising.

The effects of trace metal concentrations on species composition were only important at sub-regional scale in MRU and AP, as evident from RDAs between species composition and CHEM. The responses of different species to metal toxicity vary based on their physiological tolerances. Several studies suggest that *Chydorus* species are more tolerant to metal toxicity compared to other cladocerans (Labaj et al., 2015; Valois et al., 2010; Webster et al., 2013), which could explain the greater abundances of *Chydorus sphaericus* relative to *D. pulex* and *B. longirostris* in lakes with higher trace metal-2 values (which represented the variation in [As], [Ni], [Sb] and [Cu]) in MRU (along RDA axis 3, Fig.2.3a). Meanwhile, cladocerans are more sensitive to metal toxicity than copepods in general (Wong et al., 2009; Yan et al., 2004, but see Havens and Hanazato, 1993), which supports the negative and positive effects of trace metal-1 variable (which represented the variation in [Al], [Ag], [V], [Be], [Cu], [Cr] and [Pb]) on *B. lonigirostris* and *L. minutus*, respectively in AP (along RDA axis 1, Fig.2.3c).
References


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Appendix B: Supplementary material for Chapter Three

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12D
Fig. B1. Stratigraphies of cladoceran species relative abundances in six study lakes
Fig. 3.2. Distance biplots of PCA showing Euclidean distances between time points (years) with respect to species composition for each study lake.
Fig. C1. Principal components analysis (PCA) correlation plots for different sets of environmental parameters of 233 study lakes; (a) Correlation plot for water chemistry
(CHEM) variables (axis 1 vs. axis 2); Along axis 1, [TP], [TN], [PN], [PC], [DIN] and [Chla] were correlated to each other, while axis 2 represented correlations among GAlk, SpCond, [DIC] and pH; (b) Correlation plot for CHEM variables (axis 3 vs. axis 4); Color and [DOC] were correlated to each other along axis 3, while axis 4 represented [DIN] variation; (c) Correlation plot for hydromorphological and land cover (HYDR_LAND) variables (axis 1 vs. axis 2); LA, LV and CA are correlated to each other along axis 1, while MaxD and RT correlated to each other along axis 2; Other variables were largely independent from each other; Variables indicated in grey and dash-lined arrows are supplementary variables in the analysis; Standardized (scaled to unit variance) variables were used in the analysis; See Table 4.1 (main text) for description of variables.
Supplementary discussion C1: Variation in $\delta^{13}C$ and $\delta^{15}N$ of zooplankton taxa and DOM of study lakes

The $\delta^{15}N$ values of zooplankton taxa and DOM were more variable than $\delta^{13}C$ as indicated by relatively higher CV values for $\delta^{15}N$ compared to $\delta^{13}C$ (Table C1). In general, the range of $\delta^{13}C$ values of zooplankton detected in the present study is in accord with those of del Giorgio and France (1996), Karlsson et al. (2003) Matthews and Mazumder (2003), Persaud et al. (2009) and Wilkinson et al. (2013), yet less variable than those recorded in Grey et al. (2000) and Jones et al. (1999). Furthermore, the range of $\delta^{13}C$ of DOM in the present study is similar to those of Jones et al. (1999), Karlsson et al. (2003) and Persaud et al. (2009), but in contrast to that of Grey et al. (2000).

Meanwhile, the range of $\delta^{15}N$ of zooplankton taxa of the present study is in agreement with those reported in previous studies (Karlsson et al., 2004; Matthews and Mazumder, 2003; Persaud et al., 2009).
Table C1. Variation in $\delta^{13}C$ and $\delta^{15}N$ of zooplankton taxa and DOM of study lakes

<table>
<thead>
<tr>
<th>Taxon/chemical substance</th>
<th>$\delta^{13}C$ (‰)</th>
<th>$\delta^{15}N$ (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±95% CI</td>
<td>Minimum</td>
</tr>
<tr>
<td>Cladocera</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Daphnia</em> spp.</td>
<td>-30.67±0.56</td>
<td>-35.79</td>
</tr>
<tr>
<td><em>Holopedium gibberum</em></td>
<td>-30.38±0.45</td>
<td>-35.51</td>
</tr>
<tr>
<td><em>Bosmina longirostris</em></td>
<td>-27.45±0.46</td>
<td>-31.37</td>
</tr>
<tr>
<td>Calanoida</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Leptodiaptomus minutus</em></td>
<td>-29.52±0.48</td>
<td>-35.88</td>
</tr>
<tr>
<td><em>Agldiaiptomus leptopus</em></td>
<td>-30.46±0.97</td>
<td>-36.01</td>
</tr>
<tr>
<td><em>Skistodiaptomus</em></td>
<td>-29.20±1.18</td>
<td>-36.2</td>
</tr>
<tr>
<td><em>oregonensis Heterocope</em></td>
<td>-27.56±0.92</td>
<td>-30.98</td>
</tr>
<tr>
<td><em>septentrionalis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclopoida</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Tropocylops prasinus</em></td>
<td>-28.85±0.63</td>
<td>-35.06</td>
</tr>
<tr>
<td><em>mexicanus</em></td>
<td>-28.08±1.03</td>
<td>-31.32</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Chaoborus</em> spp.</td>
<td>-29.55±0.89</td>
<td>-35.12</td>
</tr>
<tr>
<td>DOM</td>
<td>-26.67±0.26</td>
<td>-28.19</td>
</tr>
</tbody>
</table>

Note: 95% CI = 95% confidence interval (based on $t$ distribution). CV = coefficient of variation. n = number of observations.
Fig. C2. Spatial structures in $\delta^{13}$C of zooplankton taxa and DOM across the study region represented in terms of fitted scores of regressions between taxon-specific $\delta^{13}$C and modeled SPATIAL variables.
Fig. C3. Spatial structures in $\delta^{15}N$ of zooplankton taxa across the study region represented in terms of fitted scores of regressions between taxon-specific $\delta^{15}N$ and modeled SPATIAL variables.
Supplementary discussion C2: Environmental and spatial predictors of $\delta^{13}C$ and $\delta^{15}N$ values of major zooplankton groups

Complementary to analyses isotopic variation of individual zooplankton taxa, we also analyzed environment – stable isotope (SI) relationships of three broad taxonomic groups by combining the data for individual zooplankton taxa. Briefly, we first combined data for individual taxa together into three broad taxonomic groups i.e. Cladocera, Calanoida and Cyclopoida, next selected a subset of 61 lakes in which all three groups co-occurred and then, performed a series of multivariate statistical analyses (redundancy analyses, spatial modeling and variance partitioning) on this subset to evaluate environment – spatial – SI relationships.

RDA between $\delta^{13}C$ and $\delta^{15}N$ values of the three major zooplankton groups and CHEM identified significant relationships ($p < 0.05$) along the first two axes, which collectively explained 36% of the total variation in SI composition of zooplankton (Fig.C4a). Along RDA axis 1, $\delta^{13}C$ values of all three groups were negatively related to color. In addition, $\delta^{13}C$ values of cyclopoids were also positively related to total nitrogen (TN) concentration along RDA axis 2. On the other hand, $\delta^{15}N$ values of all taxonomic groups were positively related to pH, while they were negatively related to $[TN]$ along RDA axis 2.

Significant HYDR_LAND variables ($p < 0.05$) explained 20% of the variation in carbon and nitrogen SI composition of the three major zooplankton groups (Fig. C4b). Along RDA axis 1, $\delta^{13}C$ values of all groups were positively associated with lake area (LA) and catchment area slope (CAS), whereas they were negatively related to % peat area in the catchment. They were also negatively related to the water retention time of
lakes (RT) along RDA axis 2. Finally, higher δ^{15}N values of all groups were associated with higher RT along RDA axis 2.

Variance partitioning in RDA revealed total, unique and shared variation in δ^{13}C and δ^{15}N values of three major zooplankton groups explained by CHEM and HYDR_LAND and SPATIAL variable sets (Fig.C4c). The significant CHEM variables were more important than significant HYDR_LAND variables in explaining the δ^{13}C and δ^{15}N variation (36% vs. 20%), whilst most of the variation explained by HYDR_LAND was also shared by CHEM variables (13% out of 20%). Significant (p < 0.05) spatial structures (SPATIAL) explained 22% of isotopic variation (Fig.C4c), of which a major proportion was shared by CHEM and HYDR_LAND variable sets (18% out of 22%). Yet, the remaining 4% of pure spatial variation (i.e. variation independent of influence of other explanatory matrices) was statistically significant (p < 0.05).
Fig. C4. Environmental and spatial predictors of $\delta^{13}$C and $\delta^{15}$N values of three major zooplankton groups for the subset of 61 study lakes; (a) Redundancy analysis (RDA)
biplot between CHEM and SI values of zooplankton groups; (b) RDA biplot between HYDR_LAND and SI values of zooplankton groups; (c) Variance partitioning of carbon and nitrogen SI values of zooplankton groups using CHEM, HYDR_LAND and SPATIAL variable subsets; Only significant predictors retained in each independent model by the forward selection criterion are presented; RDA biplots are scaled to optimally display the relationships among variables; All CHEM and HYDR_LAND variables except pH were either log_{10} or log_{10}(x+0.01) transformed; δ^{13}C_Clad, δ^{13}C_Cala and δ^{13}C_Cycl indicate δ^{13}C values of cladocerans, calanoids and cyclopoids, respectively; δ^{15}N_Clad, δ^{15}N_Cala and δ^{15}N_Cycl indicate δ^{15}N values of cladocerans, calanoids and cyclopoids, respectively.
References


Appendix D: Supplementary material for Chapter Five

Supplementary methods: GAM modeling approach

Generalized additive models (GAMs) were used to test significant differences of isotopic trends (i) between SOM and different invertebrate taxa. GAMs are a semi-parametric regression form, which models non-linear relationships between predictors and response in terms of smooth functions of predictors (Wood, 2017). GAMs of Gaussian location-scale family were selected given the considerable differences in both mean (location) and variance (scale) of $\delta^{13}$C and $\delta^{15}$N values among sample types i.e. SOM and invertebrate taxa, which allows both parameters of the response variable to be modelled as a function of predictors. For each lake, we fitted an ordered-factor-smooth interaction model of the form:

$$y_{ij} \sim N(\mu_{ij}, \sigma_{ij}^2),$$

$$\mu_{ij} = \alpha_0 + \alpha_1j(sample \ type) + f_1(time_i) + f_2j(time_i),$$

$$\log(\sigma_{ij}-b) = \gamma_0 + \gamma_1j(sample \ type) + \gamma_2(time \ interval),$$

where the response $y_{ij}$ i.e. $i$th $\delta^{13}$C or $\delta^{15}$N observation of $j$th sample type (an ordered factor variable representing SOM and invertebrate taxa where SOM is the reference level) is Gaussian distributed with mean $\mu_{ij}$ and variance $\sigma_{ij}^2$, $\mu_{ij}$ was modelled as the mean value of the response in the reference sample type i.e. SOM (model intercept $\alpha_0$), plus the difference between mean response of the $j$th sample type and $\alpha_0$ ($\alpha_1j$), plus the centered smooth function of time (sediment age in years AD) for the reference sample type SOM ($f_1$), plus the difference between smooth function of time for the $j$th sample type and $f_1$ ($f_2j$), $\sigma_{ij}$ was modelled (log scale) as linear effects of the sample type $\gamma_1j$ and the time interval represented by each sediment section ($\gamma_2$), plus a constant term (model intercept $\alpha_0$).
intercept $\gamma_0$), while $b$ is a small lower bound on $\sigma_{ij}$ to avoid any singularities in the likelihood of the model. Note that incorporation of ‘sample type’ as an ordered factor variable in the model allows the smoother for a given invertebrate taxon to be modelled as an additive combination of the smoother for the reference level SOM and the difference smoother for particular taxon. Restricted maximum likelihood (REML) estimation method and thin-plate regression splines were used to parameterize $f_{2j}$ (Simpson and Anderson, 2009; Wood, 2017). We also examined diagnostic plots to check any residual autocorrelation. Among others, this model provided three pieces of information crucial for the present study. First, it statistically tested if mean $\delta^{13}C/\delta^{15}N$ values of each invertebrate taxa were significantly different from that of SOM. Second, it also tested if the trend (change over time) of SOM $\delta^{13}C/\delta^{15}N$ was statistically significant. Finally, it tested if the difference smoother for each invertebrate taxon was statistically significant, i.e. if $\delta^{13}C/\delta^{15}N$ trends of each taxon were significantly different from that of SOM.

In order to test significant differences of isotopic trends among different invertebrate taxa within each lake, we post-processed the results of the above analysis as described in Rose et al. (2012). This involved using the prediction matrix $X_p$ of the fitted GAM which yields the fitted response values ($\hat{y}_p$) for a new set of high resolution data points over the time-scale of each core ($p$) when multiplied by the vector of fitted coefficients i.e. $\alpha_0, \alpha_{1j}, \gamma_0, \gamma_{1j}, \gamma_2$ and penalized coefficient sets of basis functions representing the fitted smooths $f_1$ and $f_{2j}$ ($\hat{\beta}$) i.e.:

$$\hat{y}_p = X_p\hat{\beta}$$
For a given pair of invertebrate taxa to be compared (a and b), the elements of $X_p$ for first the taxon (a) were subtracted from those corresponding to second taxon (b) and then, the columns of the resultant differenced matrix $X_{p(a,b)}$ that were not involved in the comparison were set to zero. Subsequently, the difference between trends for the pair of taxa $D_{p(a,b)}$ was obtained by;

$$D_{p(a,b)} = X_{p(a,b)} \hat{\beta}$$

To determine the uncertainty of estimated difference ($D_{p(a,b)}$), we computed the standard errors for $D_{p(a,b)}$ by using the variance-covariance matrix of the estimated model coefficients $\hat{\beta}$. The standard errors were provided by the diagonal elements of;

$$X_{p(a,b)} \hat{\Sigma}_\beta X_{p(a,b)^T}$$

where $X_{p(a,b)^T}$ is a matrix transpose of $X_{p(a,b)}$. Using these standard errors, point-wise approximate 95% confidence intervals for $D_{p(a,b)}$ were generated. We inferred a significant difference in isotopic trends between a pair of taxa when the confidence interval excluded zero for considerable time duration.

A similar approach was used to generate $\delta^{13}C/\delta^{15}N$ smooth trends for individual invertebrate taxa. The only difference being that instead of subtracting the elements of $X_p$ related to two taxa from one another, the elements of $X_p$ corresponding to the taxon of interest were added to those corresponding to SOM. The estimated isotopic trends for taxa were inferred as statistically significant when the point-wise approximate 95% confidence intervals did not overlap zero for considerable time duration.
Supplementary discussion

*Differences in mean $\delta^{13}$C of SOM among study lakes*

Differences in mean $\delta^{13}$C of SOM among study lakes can be related to number of factors that control origin and fate of accumulated carbon. First, they can be associated with among-lake differences in organic matter sources to bulk sediment (e.g. terrestrial plants, phytoplankton, epiphyton and macrophytes) that likely vary in their $\delta^{13}$C values (Aichner et al., 2010; Brenner et al., 2006; Davidson and Jeppesen, 2013; Meyers and Teranes, 2001). Second, among-lake variation in primary productivity may have contributed these differences, given the greater algal discrimination against $^{13}$C under more productive conditions (Meyers and Teranes, 2001; Schelske and Hodell, 1995). Third, varying importance of dissolved inorganic carbon (DIC) sources (atmospheric, geogenic and respired) with different $\delta^{13}$C values can influence $\delta^{13}$C values of autochthonously-derived organic material (France et al., 1997; Meyers and Teranes, 2001; Rau, 1978). Fourth, among-lake differences in methane production in sediments is another possible factor, as strongly $^{13}$C-depleted methane can lead to a large reduction in SOM $\delta^{13}$C values (Davidson and Jeppesen, 2013; Jones and Grey, 2011). Finally, post-depositional diagenetic effects may alter SOM $\delta^{13}$C differently in lakes with varying SOM composition due to differential diagenesis of their SOM constituents (Meyers and Teranes, 2001).

*Differences in mean $\delta^{15}$N of SOM among study lakes*

Among-lake variability in mean $\delta^{15}$N of SOM is likely a function of anthropogenic and natural impacts on origin and transformation of nitrogen (inorganic and organic) in lakes. First, among-lake variability in $\delta^{15}$N of terrestrially-derived
dissolved organic nitrogen (DOM-N; dominant form of nitrogen in our study lakes; Anas et al. 2014) can be related to differences in terrestrial vegetation characteristics. For instance, peatlands may act as denitrification hotspots where $^{14}$N is preferentially out-gassed, resulting in higher $\delta^{15}$N values of residual DOM-N entering the lakes (Lindau et al., 1997; Wray and Bayley, 2007). In contrast, nitrogen fixation by plants such as Alnus spp. and Shepherdia canadensis and microorganisms in deciduous forest may result in DOM-N inputs with low $\delta^{15}$N into lakes (Bunting et al., 2010; Engstrom and Fritz, 2006; Wolfe, 2016). Second, atmospheric deposition of (likely) $^{15}$N-depleted reactive nitrogen from nearby (i.e., Athabasca Oil Sands Region) and long-range industrial sources and subsequent assimilation by lacustrine primary producers may vary among study lakes (Holtgrieve et al., 2011; Wolfe et al., 2006; Wolfe, 2016). However, the amount of deposited reactive nitrogen transferred from the catchment to the lake is determined by soil nitrogen retention and terrestrial uptake, which may again vary among lakes (Anas et al., 2015; Hobbs et al., 2016; Wolfe, 2016). Third, type and degree of nutrient limitation in lakes may contribute to differences in $\delta^{15}$N of autochthonous organic matter. In phosphorus-limited lakes with sufficient dissolved inorganic nitrogen (DIN) supply to sustain primary production, preferential uptake of $^{14}$N may result in lower $\delta^{15}$N values in autochthonous organic matter relative to the nitrogen source. In lakes with limited DIN supply on the other hand, $\delta^{15}$N of autochthonous organic matter can be similar to the DIN source due to minimal fractionation against $^{15}$N by algae, according to Rayleigh distillation kinetics (Meyers and Teranes, 2001; Talbot, 2001). Finally, isotopic fractionation during other biogeochemical processes occurring in lakes and catchments
(e.g. ammonification, nitrification, denitrification) may also influence $\delta^{15}$N of the DIN pool used by primary producers (Kendall 1998; Robinson 2001, Chapter 2).
Fig. D1. Comparison of SOM C/N ratios among study lakes. Solid circles (●) indicate the mean values. Median values of lakes indicated by different letters are significantly different (adjusted p<0.05) according to Kruskal-Wallis test followed by Dunn’s test for multiple comparisons (Dunn, 1964).
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