Aquatic and terrestrial foraging by a subarctic herbivore: the beaver

Heather Milligan
Department of Natural Resource Sciences
McGill University, Macdonald Campus
Montréal, Québec

August 2008

A thesis submitted to McGill University in partial fulfillment of the requirements
of the degree of Masters in Science

Abstract

Freshwater and terrestrial ecosystems are tightly linked by food web interactions. Naturally occurring carbon and nitrogen stable isotopes provide a tool to quantify nutrient flows across ecological boundaries, however their application to freshwater-terrestrial systems has been limited. This thesis evaluated whether stable isotope analysis can be effective in differentiating freshwater and terrestrial vascular plants at the base of subarctic food webs and found freshwater plants to be consistently enriched in their isotopic signatures relative to terrestrial plants. Stable isotope approaches were then used to investigate spatial and temporal variability in the diet of a population of subarctic beavers (*Castor canadensis*). Freshwater macrophytes contributed more to the diets of beavers than previously reported. During the winter, beavers from pond habitats consumed more aquatic vegetation than beavers from stream habitats which relied more heavily on food hoards of terrestrial vegetation. Aquatic foraging may enable beavers to persist at the northern periphery of their range by reducing foraging pressure on the subarctic terrestrial ecosystem where their preferred tree species are scarce.

Résumé

Les écosystèmes d'eau douce et terrestres sont liés par les interactions trophiques. Les isotopes stables naturels de carbone et d'azote offrent une méthode pour quantifier les transferts de nutriments entre les frontières écologiques, mais leurs applications aux systèmes d'eau douce-terrestres sont encore limitées. Cette thèse évalue l'efficacité des isotopes stables pour distinguer les plantes vasculaires d'eau douces et terrestres qui forment la base des chaînes alimentaires subarctiques. Nous avons trouvé qu'en général les plantes aquatiques avaient des signatures isotopiques enrichies par rapport aux plantes terrestres. Nous avons ensuite employé les techniques d'isotopes stables pour évaluer la variabilité spatiale et temporale dans le régime alimentaire d'une population de castors (*Castor canadensis*) subarctiques. Les macrophytes aquatiques semblent avoir une place plus importante dans le régime alimentaire des castors en comparaison avec la littérature disponible. Durant l'hiver, les castors qui habitaient les lacs ont consommé plus de végétation aquatique par rapport aux castors qui habitaient dans les rivières, ceux-ci comptant plutôt sur les provisions de végétation terrestre. L'accumulation de provisions constituées de plantes aquatiques peut permettre aux castors de persister à la limite de leurs aires de distribution où les arbres préférés des castors sont rares. Ainsi, ce phénomène pourrait réduire la pression des herbivores sur ces écosystèmes terrestres à faible productivité.

Contributions of Authors

This thesis consists of two manuscripts. In these studies, the candidate developed the initial research ideas and sampling methodologies and was responsible for data collection, management, and analysis. The writing of both manuscripts was the responsibility of the candidate.

As the thesis supervisor, Murray M. Humphries provided guidance and support during the preparation, field work, analysis, interpretation, and writing. Both chapters are co-authored by MM Humphries. Chapter 1 is also co-authored by Troy D. Pretzlaw who coordinated the field collection and analysis for the subset of the data collected in Old Crow Flats, Yukon.

Acknowledgements

I am greatly indebted to the many people and organizations who have generously contributed to the research in this thesis and who have broadened my perspectives during the course of this research. To those I may have overlooked in my haste, I hope I have shown my gratitude in other ways. *Chiniskumitin*.

The research in this thesis is part of a larger collaborative effort initiated by the Cree Nation of Wemindji from James Bay, Quebec, and a multi-disciplinary group of university researchers. The central focus of this research project is to study and promote approaches to environmental protection that take into account the community's commitment to safeguard Cree hunting, fishing, and trapping as a way of life in response to industrial development. The primary goal of the Paakumshumwaau-Wemindji Protected Area Project is the creation of an integrated marine and terrestrial protected area on portions of Wemindji's territory that will enhance Cree land tenure and be built on Cree values of respect and relationship. A further aim of the project is to foster meaningful engagement and an exchange of knowledge between community members and academic scholars that is beneficial to both. During the course of my thesis research, I had the privilege to work and live closely out on the land with several families from the community. Many community members shared their ecological expertise and support during the development of this project as research partners, guides, research assistants, informants, sample providers, and friends. Our conversations often stemmed from a shared enthusiasm and respect for the natural world, especially animals. I learned more about the ecology of beavers and the relationship between people and environment during these exchanges than from the body of scientific literature.

Foremost, I would like to thank the *amiskuchimaauch* (beaver hunting bosses) Fred Stewart, Leonard Asquabaneskum, and Jimmy Matches for the permission to study in their family territories, for sharing their expertise, and for being involved with this project since its conception. I would also like to thank their extended families on Porcupine Road who shared their backyard and gave us a home in the community. I cannot thank Ronnie and Hilda Asquabaneskum and Jimmy and Minnie Matches and their families enough for

everything they did for us by sharing their *miichiwaahp* (teepee). From collecting spruce boughs and firewood, to keeping us well fed on bannock and goose, they made us feel welcome and created a place for us to socialize and have fun. To everyone in the backyard gang, thank you very much for the warm memories.

I am also grateful to all the Cree hunters who provided samples for the purpose of this study: Leonard Asquabaneskum, Rene Atsynia, Sinclair Georgekish, Rocky Georgekish, Ashley Matches, Jimmy Matches, Sinclair Mayappo, Bill Stewart, George Stewart, Henry Stewart, and Roy Stewart. I also want to thank them and the following people for sharing aspects of natural history and ecology, the significance of the places we visited, as well as ensuring we traveled and camped safely and respectfully: Fred Blackned, Sam Georgekish, Beverly Mayappo, and Dorothy Stewart. Thank you to the Council of the Cree Nation of Wemindji for the permission to conduct our research and to Ross Miniquaken and Edward Georgekish from the Cree Trappers' Association of Wemindji for all of their help. Without all of your involvement, this project would not have been possible.

I am very fortunate to work with the dedicated and passionate team of people in the Paakumshumwaau-Wemindji Protected Area Project. I learned so much about different research approaches from their perspectives as community leaders and social scientists. Their research and feedback helped broaden my understandings of stewardship and the role that scientists can have in community-based conservation. A special mention must also be made to Katherine Scott for all her administrative and logistical help.

The following organizations kindly provided research funding: SSHRC Community-University Research Alliance and Aboriginal Research grants to Paakumshumwaau-Wemindji Protected Area Project, Government of Canada's IPY program, NCE ArcticNet, the Northern Scientific Training Program, and NSERC Northern Research Chairs program. The Centre for Indigenous Peoples' Nutrition and Environment provided lab space. I am also grateful for several scholarships awarded by: NSERC Post-graduate Scholarship, Association of Canadian Universities for Northern Studies Northern

Resident Scholarship, McGill University, Yukon Outdoors' Club Scholarship, Vancouver Yukoners' Association Scholarship, and Yukon Foundation.

I cannot express how grateful I am to everyone who became part of the beaver research team. Thank you to the core field team for persevering through days with little rest, inclement weather, biting insects, rapids, overturned canoes, and soakers with a warm sense of humor: Hugo Kitching, Brianna Linklater-Georgekish, Kimberly Matches, Stacy Matches, Marina Milligan, Nicholas Mirotchnick, and Jason Samson. To our guides Leonard Asquabaneskum, Ronnie Asquabaneskum, Ashley Matches, Jimmy Matches, Edward Georgekish, James Shashaweskum, Fred Stewart, and Henry Stewart, it was an honour to have you show us your family territory and share your knowledge. Thank you also to the McGill researchers and associates who joined us on some of our adventures: Marcel Blondeau, Zohrin Jivraj, Wren Nasr, Florin Pendea, Jesse Sayles, Katherine Scott, and Chris Wellen. I will never forget our campfire moments and sharing the beauty of the Wemindji land and seascape with all of you. Far removed from the glory of field work, Dan Brown and Caroline Trudeau diligently prepared samples in the laboratory.

I owe a lot to my supervisor Murray Humphries for this opportunity to begin a career as a northern ecologist. Murray gave me an incredible amount of freedom, trust, and encouragement with my graduate research. That freedom allowed me to further develop my skills as a researcher and build more confidence as a supervisor. I often took that freedom to dabble in my other non-thesis related interests in ethnoecology, education, and collaboration. Those explorations deeply enriched my graduate experience and development as a person. I am thankful to learn from someone so open-minded and positive. I also received the patient guidance of Colin Scott who shared his experience of living on the land with people from Wemindji and helped create the opportunity for us to study wildlife with the Paakumshumwaau-Wemindji Protected Area Project. During the development of this project, Peter Outridge and my committee members Jim Fyles and Rene Gregory-Eaves also gave thoughtful advice.

I am privileged to be part of the very diverse, energetic, and supportive group of people from the Humphries Ecology and Ecology Lab: Jean-François Aublet, Brian Bell, Elad

Ben-Ezra, Thomas Doniol-Valcroze, Quinn Fletcher, Paul Jensen, Manuelle Landry-Cuerrier, Meghan Larivée, Marianne Marcoux, Troy Pretzlaw, Sonja Ostertag, Jason Samson, and Sarah Woods. Thank you all for sharing your unique strengths as ecologists during the process of my studies, enduring long lab meetings, editing manuscripts, stimulating our discussions, cooking great food, and for your friendship. You gave me a home in Montreal in both the metaphorical and literal sense.

Furthermore, I want to thank my friends and family who have not been previously mentioned for the support they radiated over several thousand kilometers over the past two and a half years: Kathleen Aikens, Megan Haddock, Heather Callaghan, Krystal Cooper, Jessie Cowperthwaite, Tim Irvin, and Catharine Pendrel. To my sister Marina, I am so glad we were able spend a summer in Wemindji getting closer by sharing our tent, our canoe, our work, and our Cree name. To my parents Pat and Karen, I am forever grateful for your love and support. From my earliest memories, you nurtured my sense of awe and respect for nature. As that eight year old girl inspiring to be a conservation biologist, I could never have imagined that my endeavors would lead me to such amazing adventures in such amazing places with such amazing people. Mom and Dad, I dedicate this to you.

Table of Contents

List of Tables

Chapter 1

Chapter 2

List of Figures

Chapter 1

Chapter 2

General Introduction

The freshwater-terrestrial interface represents an abrupt transition in habitat and species composition. Although distinct food webs occur within each habitat, they are also tightly linked. Trophic interactions (such as the movement of nutrients, detritus, prey, and consumers across habitats) that integrate these apparently distinct food webs can often be difficult to observe directly. Naturally occurring carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes provide a tool to quantify nutrient flows across ecological boundaries in food webs. These methodologies have proved insightful in understanding the linkages of marine and terrestrial food webs. However, their application to freshwater-terrestrial systems has been limited. In this thesis, I first evaluate whether stable isotope analysis can be effective in differentiating freshwater and terrestrial vascular plants at the base of subarctic food webs. I then use stable isotope analysis to investigate spatial and temporal dynamics in the dietary niche of a generalist herbivore to better understand freshwater-terrestrial ecosystem linkages.

Literature Review

Freshwater-Terrestrial Ecosystem Linkages

Freshwater and terrestrial ecosystems differ dramatically in their physical environments and have apparently distinct food webs. These food webs, however, are interdependent and tightly linked. Terrestrial organic matter can be the major driver of productivity in freshwater ecosystems (Polis et al. 1997; Polis et al. 2004; Pace et al. 2004). Nutrient transfers from freshwater to terrestrial ecosystems are less understood, but could be substantial (Polis et al. 2004; Ballinger and Lake 2006). Nutrients are predicted to flow from more to less productive habitats (DeAngelis 1980; Huxel and McCann 1998; Nakano and Murakami 2001) and herbivory rates and population densities of organisms are higher in freshwater ecosystems compared to adjacent terrestrial ecosystems (Cyr and Pace 1993; Cyr et al. 1997; Cebrian and Lartigue 2004; Shurin et al. 2006). These freshwater-terrestrial linkages can also be dynamic over time. For example, Nakano and

Murakami (2001) discovered that peak insect emergence was staggered between freshwater and terrestrial ecosystems, which led to reciprocal exchange of nutrients across habitats.

Flexible foraging behaviour of generalist animals that feed in both freshwater and terrestrial ecosystems can exert critical influences on food webs. For example, herbivores that feed in freshwater and terrestrial ecosystems, such as North American beavers (*Castor canadensis*), moose (*Alces alces*), muskrat (*Ondatra zibethicus*), and waterfowl, have the potential to affect the stability of both ecosystems dynamics by switching between habitats (de Ruiter et al. 2005; McCann et al. 2005). This may enable generalist herbivores to persist in marginal freshwater and/or terrestrial habitats by altering the abundance or succession of vegetation. These processes may be especially apparent in high latitude ecosystems where both freshwater and terrestrial ecosystems can be characterized by low productivity and extreme seasonality. Although the interdependence of freshwater and terrestrial food webs is recognized, the majority of researchers examine food web dynamics within each ecosystem (reviewed in Polis et al. 2004; Stergiou and Browman 2005).

Stable Isotopes in Freshwater-Terrestrial Ecology

The analyses of carbon and nitrogen stable isotopes provide a framework to determine the diet of animals and thus quantify nutrient transfers between freshwater and terrestrial ecosystems. Isotopes are atoms with the same number of protons and electrons, but differ in the number of neutrons. Carbon and nitrogen both have two stable (non-radioactive) isotopes that vary in their abundance in nature. The lighter isotope is typically present in greater abundance than the heavier isotope (Michener and Lajtha 2007). Isotopic composition is reported relative to internationally accepted standards and expressed in parts per thousand deviations from that standard by:

$$\delta X(‰) = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where X is ^{13}C or ^{15}N, R is the ratio of heavy-to-light ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) isotope.

Carbon and nitrogen stable isotope ratios in animal tissues have been applied to food web studies for over 30 years to reconstruct animal diets and trace nutrients flows across habitats (Fry et al. 1978; DeNiro and Epstein 1978 and 1981; Rounick et al. 1982). The foods that animals eat may have distinctive isotopic signatures. Plants with different photosynthetic modes have different $\delta^{13}C$ values and foods from marine sources are enriched in their $\delta^{13}C$ and $\delta^{15}N$ values compared to terrestrial and freshwater sources (DeNiro and Epstein 1978 and 1981; Chisolm et al. 1982; Schoeninger et al. 1984). Animals incorporate these $\delta^{13}C$ and $\delta^{15}N$ ratios from their food into their tissues after fractionation (slight changes in isotopic signatures due to digestion, metabolism, and assimilation; Peterson and Fry 1987; Gannes et al. 1997; Kelly 2000; McCutchan et al. 2003; Vanderklift and Ponsard 2003). The time frame represented by these tissues depends on their metabolism (Tieszen et al. 1983; Kelly 2000; Phillips and Eldridge 2006). Researchers have used different animal tissues to examine daily to life-time records of diet (e.g. Hobson and Clark 1992; Hobson and Sease 1998; Cerling et al. 2006).

Isotopic variability in primary producers and detritus at the base of food webs determine whether stable isotope techniques can be effective at reconstructing the diets of consumer animals. Isotopic variation among freshwater algae and terrestrial detritus is well established in studies that trace freshwater and terrestrial nutrient flows in stream ecosystems (reviewed in Rounick and Winterbourn 1986; Fry 1991; France 1995a and b; Michener and Lajtha 2007). Vascular freshwater plants are hypothesized to have more positive $\delta^{13}C$ and $\delta^{15}N$ values than terrestrial plants (Osmond et al. 1981; Keely and Sandquist 1992; France 1995a and b; Dawson et al. 2002; Michener and Lajtha 2007), however that differentiation has been considered too small and variable to be effective natural tracers in food webs (France 1995a and b; Michener and Lajtha 2007). These assumptions are based on global values, but need to be re-evaluated within localities, where signatures are often more distinct (Michener and Lajtha 2007).

Beavers as Freshwater-Terrestrial Herbivores

North American beavers are generalist mammalian herbivores that feed on both freshwater and terrestrial vegetation. The terrestrial component of their diet has been well described in numerous studies, however, little is known of the aquatic contribution to their diet. The terrestrial diet of beavers is quite general and consists of the leaves, twigs, and bark of many species of woody and herbaceous plants (Aleksiuk 1970; Northcott 1971; Jenkins and Busher 1979; Svendsen 1980). Despite this generality, they are selective and a small number of species account for the majority of their diet. The number of species found in beaver diets decreases from their southern range limit to their northern rage limit where many preferred hardwood species do not occur (birch: *Betula* spp.; cherry: *Prunus* spp.; dogwood: *Cornus* spp.; hazel: *Corylus* spp.; maple: *Acer* spp.; mountain ash: *Sorbus* spp.; oak: *Quercus* spp.; and poplar: *Populus* spp.; Aleksiuk 1970; Dennington and Johnson 1974; Belovsky 1984; Novak 1987; Gallant et al. 2004; Jarema 2006). During the summer months at northern latitudes, their diet often consists of herbaceous plants and the leaves and twigs of willow (*Salix* spp.; Aleksiuk 1970; Northcott 1971; Jenkins and Busher 1979). Beavers are also shown to concentrate most of their foraging within 60 m of shore and be more selective further from shore, largely due to increased predation risk (Donkor and Fryxell 1999). They are likely to travel further in search of their preferred terrestrial trees such as aspen (*Populus* spp.). During the fall, they gather the branches and logs woody plants including aspen, willow, and alder (*Alnus* spp.; Aleksiuk 1970; Northcott 1971; Slough 1978; Jenkins and Busher 1979). Beavers gather these branches in a pile beside their lodge which is eaten throughout the winter when they live under the ice. Thus, although beavers live exclusively under the ice during the winter and essentially are excluded from the terrestrial ecosystem, they have potential access to terrestrial vegetation year round.

Beavers have year round access to the freshwater ecosystem, yet the aquatic contribution to their diet has rarely been quantified. The dietary contribution of aquatic plants in ice-covered months is largely unknown, but several anecdotal references exist of beavers hoarding the rhizomes of pond lilies (*Nuphar spp.* and *Nymphaea* spp.; Dennington and

Johnson 1974; Slough 1978; Jenkins and Busher 1979; Bearskin et al. 1989; Ray et al. 2001). Belovsky (1984) and Doucet and Fryxell (1993) modeled the optimal dietary contribution of aquatic and terrestrial plants using energetic constraints and concluded that beavers should consume approximately 12 to 24% aquatic vegetation during the summer. Several behavioural studies indicate the contribution of aquatic plants in the summer diet can be more substantial (Aleksiuk 1970; Northcott 1972; Svendsen 1980; Doucet and Fryxell 1993; Fryxell 2001; Parker et al. 2007). The investigation of seasonal shifts in the aquatic and terrestrial diets of beavers could provide insight into linkages of freshwater and terrestrial ecosystems mediated by their herbivory.

As flexible herbivores that can switch feeding between freshwater and terrestrial habitats, beavers could potentially affect the stability of freshwater and terrestrial ecosystem dynamics. Beaver colonies can considerably modify their environments by their foraging activity and by building dams and canals in their long-term territories (reviewed in Naiman et al. 1988; Rosell et al. 2005). As a result, beavers are coined as 'ecosystem engineers' (Jones et al. 1994; Rosell et al. 2005). Beaver foraging affects succession and species composition of riparian terrestrial communities (Rosell et al. 2005). Beavers can replace mid-succession with early-succession trees by opening up canopies, increasing wetlands, and permitting the regeneration of shade intolerant trees (Donkor and Fryxell 1999). Beavers can also suppress the regeneration of preferred trees (such as aspen and willow) by intense foraging (Barnes and Mallik 2001). Beaver herbivory of aquatic plants can also shift species composition of aquatic plants (Parker et al. 2007) and the creation of beaver ponds can alter their succession (Ray et al. 2001). The terrestrial debris from beaver food caches can also supply nutrients and structure to aquatic invertebrate and vertebrate communities which enhances biodiversity (France 1997). Therefore, beaver foraging activities can play an important role in regulating the succession and composition of freshwater and terrestrial plant communities.

Research Objectives

In this thesis, I quantify the seasonal contribution of freshwater and terrestrial vegetation in the diet of a herbivore in order to better understand the role of herbivory in linking subarctic ecosystems. In the first chapter of this thesis, I examine the potential for differentiating freshwater and terrestrial vascular plants using stable isotope analysis from two distant subarctic regions: 1) Wemindji Territory, Quebec, and 2) Old Crow Flats, Yukon Territory, Canada. In the second chapter, I use this methodology to quantify spatial and seasonal variation in the freshwater and terrestrial diet of a population of beavers near Wemindji, northern Quebec, in order to better understand freshwater-terrestrial ecosystem linkages mediated by herbivory.

References Cited

Aleksiuk M (1970) The seasonal food regime of arctic beavers. Ecology 51:264-270

Ballinger A, Lake PS (2006) Energy and nutrient fluxes from rivers and streams into terrestrial food webs. Marine and Freshwater Research 57:15-28

Barnes BM, Mallik AU (2001) Effects of beaver, *Castor Canadensis*, herbivory on streamside vegetation in a northern Ontario watershed. Canadian Field-Naturalist 115:9-21

Bearskin D, Lameboy G, Matthew R, Pepabano J, Pisinaquan A, Ratt W, Rupert D (1989) Cree trappers speak. James Bay Cree Cultural Education Centre, Chisasibi

Belovsky GE (1984) Summer diet optimization by beaver. American Midland Naturalist 111:209-222

Cebrian J, Lartigue J (2004) Patterns of herbivory and decomposition in aquatic and terrestrial ecosystems. Ecological Monographs 74:237-259

Cerling TE, Wittemyer G, Rasmussen HB, Vollrath F, Cerling CE, Robinson TJ, Douglas-Hamilton I (2006) Stable isotopes in elephant hair document migration patterns and diet changes. Proceedings of the National Academy of Sciences of the United States of America 103:371-373

Chisolm BS, Nelson DE, Schwarcz HP (1982) Stable-carbon isotope ratios as a measure of marine versus terrestrial protein in ancient diets. Science 216:1131-1132

Cyr H, Pace ML (1993) Magnitude and patterns of herbivory in aquatic and terrestrial ecosystems. Nature 361:148-150

Cyr H, Peters RH, Downing JA (1997) Population density and community size structure: comparison of aquatic and terrestrial systems. Oikos 80:139-149

Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP (2002) Stable isotopes in plant ecology. Annual Review of Ecology and Systematics 33:507-559

DeAngelis DL (1980) Energy flow, nutrient cycling, and ecosystem resilience. Ecology 61:764-771

Dennington M, Johnson B (1974) Studies of beaver habitat in the Mackenzie Valley and northern Yukon. Canadian Wildlife Service Report 74-39

DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42:495-506

DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta 45:341-351

de Ruiter PC, Wolters V, Moore JC (2005) Dynamic food webs: multispecies assemblages, ecosystem development and environmental change. Elsevier Academic Press, Burlington

Donkor NT, Fryxell JM (1999) Impact of beaver foraging on structure of lowland boreal forests of Algonquin Provincial Park, Ontario. Forest Ecology and Management 118:83-92

Doucet CM, Fryxell JM (1993) The effect of nutritional quality on forage preference by beavers. Oikos 67:201-208

France RL (1995a) Critical examination of stable isotope analysis as a means for tracing carbon pathways in stream ecosystems. Canadian Journal of Fisheries and Aquatic Sciences 52:651-656

France RL (1995b) Source variability in δ15N of autotrophs as a potential aid in measuring allochthony in freshwaters. Ecography 18:318-320

France RL (1997) The importance of beaver lodges in structuring littoral communities in boreal headwater lakes. Canadian Journal of Zoology 75:1009-1013

Fry B, Joern A, Parker PL (1978) Grasshopper food web analysis: use of carbon isotope ratios to examine feeding relationships among terrestrial herbivores. Ecology 59:498-506

Fry B (1991) Stable isotope diagrams of freshwater food webs. Ecology 72:2293-2297

Fryxell JM (2001) Habitat suitability and source-sink dynamics of beavers. Journal of Animal Ecology 70:310-316

Gallant D, Bérubé CH, Tremblay E, Vasseur L (2004) An extensive study of the foraging ecology of beavers (*Castor canadensis*) in relation to habitat quality. Canadian Journal of Zoology 82:922-933

Gannes LZ, O'Brien DM, Martinez del Rio C (1997) Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology 78:1271-1276

Hobson KA, Clark RG (1992) Assessing avian diets using stable isotopes. 1. Turnover of C-13 in tissues. Condor 94: 181-188

Hobson KA, Sease JL (1998) Stable isotope analyses of tooth annuli reveal temporal dietary records: an example using steller sea lions. Marine Mammal Science 14:116-129

Huxel GR, McCann K (1998) Food web stability: the influence of trophic flows across habitats. American Naturalist 152:460-469

Jarema SI (2006) The abundance and distribution of beavers (*Castor canadensis*) in Québec, Canada. M.Sc. Thesis, McGill University, Montreal

Jenkins SH, Busher PE (1979) *Castor canadensis*. Mammalian Species 120:1-8

Jones CG, Lawton JH, Shachak M (1994) Organisms as ecosystem engineers. Oikos 69:373-386

Keeley JE, Sandquist DR (1992) Carbon: freshwater plants. Plant, Cell and Environment 15:1021-1035

Kelly JF (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Canadian Journal of Zoology 78:1-27

McCann KS, Rasmussen JB, Umbanhowar J (2005) The dynamics of spatially coupled food webs. Ecology Letters 8:513-523

McCutchan JH, Lewis WM, Kendall C, McGrath CC (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102:378-390

Michener R, Lajtha K (2007) Stable isotopes in ecology and environmental science 2nd edn. Blackwell Publishing, Malden

Naiman RJ, Johnston CA, Kelley JC (1988) Alteration of North-American streams by beaver. BioScience 38:753-762

Nakano S, Murakami M (2001) Reciprocal subsidies: dynamic interdependence between terrestrial and aquatic food webs. Proceedings of the National Academy of Sciences of the United States of America 98:166-170

Northcott TH (1971) Feeding habits of beaver in Newfoundland. Oikos 22:407-410

Northcott TH (1972) Water lilies as beaver food. Oikos 23:408-409

Novak M (1987) Beaver. In: Novak M, Baker JA, Obbard ME, Malloch B (eds) Wild furbearer management and conservation in North America. Ontario Trapping Association, North Bay pp 283-312

Osmond CB, Valaane N, Haslam SM, Uotila P, Roksandic Z (1981) Comparisons of $\delta^{13}C$ values in leaves of aquatic macrophytes from different habitats in Britain and Finland; some implication for photosynthetic processes in aquatic plants. Oecologia 50:117-124

Pace ML, Cole JJ, Carpenter SR, Kitchell JF, Hodgson JR, Van de Bogert MC, Bade DL, Kritzberg ES, Bastviken D (2004) Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. Nature 427:240-243

Parker JP, Caudill CC, Hay HE (2007) Beaver herbivory on aquatic plants. Oecologia 151:616-625

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18:293-320

Phillips DL, Eldridge PM (2006) Estimating the timing of diet shifts using stable isotopes. Oecologia 147:195-203

Polis GA, Anderson WB, Holt RD (1997) Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annual Review in Ecology and Systematics 28:289-316

Polis GA, Power ME, Huxel GR (2004) Food webs at the landscape level. University of Chicago Press, Chicago

Ray AM., Rebertus AJ, Ray HL (2001) Macrophyte succession in Minnesota beaver ponds. Canadian Journal of Botany 79:487-499

Rosell F, Bozser O, Collen P, Parker H (2005) Ecological impact of beavers *Castor fiber* and *Castor canadensis* and their ability to modify ecosystems. Mammal Review 35:248-276

Rounick JS, Winterbourn MJ, Lyon GL (1982) Differential utilization of allochthonous and autochthonous inputs by aquatic invertebrates in some New Zealand streams: a stable carbon isotope study. Oikos 39:191-198

Rounick JS, Winterbourn MJ (1986) Stable carbon isotopes and carbon flow in ecosystems. BioScience 36:171-177

Schoeninger MJ, DeNiro MJ, Tauber MS (1984) Stable nitrogen isotope ratios of bone collagen reflect marine and terrestrial components of prehistoric human diet. Science 220:1381-1383

Shurin JB, Bruner DS, Hillebrand H (2006) All wet or dried up? Real differences between aquatic and terrestrial food webs. Proceedings of the Royal Society B: Biological Sciences 273:1-9

Slough BG (1978) Beaver food cache structure and utilization. Journal of Wildlife Management 42:644-646

Stergiou KI, Browman HI (2005) Imbalances in the reporting and teaching of ecology from limnetic, oceanic, and terrestrial eco-domains. Marine Ecology-Progress Series 304:292-297

Svendsen GE (1980) Seasonal change in feeding patterns of beaver in southeastern Ohio. Journal of Wildlife Management 44:285-290

Tieszen LL, Boutton TW, Tesdahl KG, Slade NG (1983) Fractionation and turnover of stable carbon isotopes in animal tissues: implications for δ^{13}C analysis of diet. Oecologia 57:32-37

Vanderklift MA, Ponsard S (2003) Sources of variation in consumer-diet δ^{15}N enrichment: a meta-analysis. Oecologia 136:169-182

Chapter 1

Consistent Carbon and Nitrogen Isotopic Differentiation of Freshwater and Terrestrial Plants in Subarctic Ecosystems

Manuscript formatted for submission to the journal *Oecologia*

Authors:

Heather E. Milligan (email: heather.milligan@mail.mcgill.ca)
Natural Resource Sciences, Macdonald campus, McGill University
21 111 Lakeshore Road, Ste-Anne-de-Bellevue, QC
Canada, H9X 3V9

Troy D. Pretzlaw (email: troy.pretzlaw@mcgill.ca)
Natural Resource Sciences, Macdonald campus, McGill University
21 111 Lakeshore Road, Ste-Anne-de-Bellevue, QC
Canada, H9X 3V9

Murray M. Humphries (email: murray.humphries@mcgill.ca)
Natural Resource Sciences, Macdonald campus, McGill University
21 111 Lakeshore Road, Ste-Anne-de-Bellevue, QC
Canada, H9X 3V9

Abstract

Naturally occurring carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes can trace trophic interactions across ecological boundaries. We examined the potential for $\delta^{13}C$ and $\delta^{15}N$ signatures to distinguish freshwater macrophyte and terrestrial plant sources at the base of food webs. We collected freshwater macrophytes and terrestrial plants that are consumed by vertebrate herbivores from inland, coastal, river, and lake habitats of subarctic ecosystems. We consistently observed more positive isotopic values in freshwater macrophytes than terrestrial plants (+ 1.8 to 10.4‰ $\delta^{13}C$ and + 0.7 to 3.0‰ $\delta^{15}N$) and could classify 86% (±14%) of plant isotopic signatures into freshwater and terrestrial groups with cluster analyses. We are the first to show site-specific $\delta^{13}C$ and $\delta^{15}N$ separation between freshwater macrophytes and terrestrial plants, which could be applied to studies that trace nutrient transfers and food web interactions across freshwater-terrestrial interfaces.

Keywords

Food webs, Macrophytes, Aquatic, Stable isotopes, Herbivores

Introduction

The freshwater-terrestrial interface represents an abrupt transition in habitat and species composition. Trophic linkages across this interface can exert critical influences on freshwater and terrestrial food webs but the majority of researchers examine interactions within each habitat (reviewed in Polis et al 1997; Nakano and Murakami 2001; Polis et al 2004). Trophic interactions across freshwater and terrestrial habitats can be difficult to observe directly. Naturally occurring carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes provide a tool to trace nutrients across ecological boundaries in food webs (Peterson and Fry 1987). The ability to discriminate marine and terrestrial sources with $\delta^{13}C$ and $\delta^{15}N$ signatures is well established due to differential pathways of carbon and nitrogen uptake in marine and terrestrial autotrophs at the base of food webs (DeNiro and Epstein 1978 and 1981; Chisolm et al 1982; Schoeninger et al 1984; Hobson 1999; Kelly 2000). The same processes affect the isotopic signatures of freshwater and terrestrial autotrophs and therefore should provide a means to discriminate freshwater and terrestrial sources in food webs.

Freshwater autotrophs often exhibit more variable and positive $\delta^{13}C$ signatures than terrestrial autotrophs as a result of photosynthetic differences in freshwater and terrestrial ecosystems. Terrestrial autotrophs sequester atmospheric CO_2 during photosynthesis and their $\delta^{13}C$ signatures vary depending on the photosynthetic pathway involved (Dawson et al 2002). For example, terrestrial autotrophs using C_3 or C_4 photosynthetic pathways often exhibit $\delta^{13}C$ signatures around -27‰ (range -32 to -22‰) and -16‰ (range -23 to -9‰), respectively (Rounick and Winterbourn 1986; Finlay and Kendall 2007). On the other hand, the $\delta^{13}C$ signatures of freshwater autotrophs can vary from -51 to -8‰ (Osmond et al 1981; Finlay and Kendall 2007), which is partly attributed to freshwater autotrophs having variable inorganic carbon sources. Carbon sources for emergent aquatics include atmospheric CO_2 and can vary depending on pH levels for submergent aquatics (aqueous CO_2 at pH < 6.4, HCO_3^{-} at pH 6.4 to 10.3, and CO_3^{2-} at pH > 10.3; Finlay and Kendall 2007). The boundary layer of water surrounding freshwater autotrophs also restricts the diffusion of inorganic carbon and limits the ability of these autotrophs to discriminate against the heavier isotope (^{13}C; Keeley and Sandquist 1992; Finlay and Kendall 2007). This results in a more positive $^{13}C/^{12}C$ ratio and higher $\delta^{13}C$ signature for the organism. In turbulent waters, this boundary layer is reduced, resulting in more negative $\delta^{13}C$ signatures (Osmond et al 1981; France 1995b), and contributing to the variability observed in the $\delta^{13}C$ signatures of freshwater autotrophs.

Freshwater autotrophs often also exhibit more positive $\delta^{15}N$ signatures than terrestrial autotrophs but the mechanisms affecting this dissimilarity are less understood. Freshwater and terrestrial autotrophs are likely to differ in their organic and inorganic sources of $\delta^{15}N$, but nitrogen cycling systems are complex and less well studied than carbon cycling systems (Finlay and Kendall 2007). Terrestrial autotrophs can vary -9 to 8‰ $\delta^{15}N$ whereas freshwater autotrophs can vary -3 to 10‰ $\delta^{15}N$ (France 1995c; Finlay and Kendall 2007). A global review by France (1995c) found that the mode $\delta^{15}N$ for freshwater autotrophs (3‰) was 4‰ more positive than that of terrestrial autotrophs (-1‰).

Although stable isotope analyses may provide a means to discriminate freshwater and terrestrial autotrophs, their differentiation has been considered too small and variable to be effective natural tracers in food webs (France 1995a; Finlay and Kendall 2007). These assumptions based on global values should be revisited since freshwater and terrestrial autotrophs often exhibit more distinct isotopic compositions at specific sites and most applications of stable isotopes in food webs occur within relatively small geographic locations (Finlay and Kendall 2007). Furthermore, the majority of studies that contributed to global ranges in isotopic signatures of freshwater and terrestrial autotrophs examined $\delta^{13}C$ and $\delta^{15}N$ separately, but their potential for resolution is much higher in combination (see reviews in France 1995a and c; Finlay and Kendall 2007). A substantial body of research has applied $\delta^{13}C$ and $\delta^{15}N$ values to trace freshwater and terrestrial nutrient flows in stream ecosystems (reviewed in Rounick and Winterbourn 1986; Fry 1991; France 1995a and c; Finlay and Kendall 2007). These studies primarily used freshwater algae and terrestrial detritus as primary producers, which has limited application for many food web studies. Freshwater angiosperm macrophytes are important primary producers for a suite of invertebrate and vertebrate herbivores (Cyr and Pace 1993; Cebrian and Lartigue 2004) yet few researchers have examined the isotopic signatures of freshwater macrophytes (but see Osmond et al 1981; Keeley and Sandquist 1992; France 1995d; Finlay and Kendall 2007).

Our objective was to evaluate whether $\delta^{13}C$ and $\delta^{15}N$ signatures can be used to differentiate freshwater macrophytes and deciduous terrestrial plants that are important food sources of vertebrate herbivores in subarctic ecosystems. We collected vegetation from two geographically distant regions in Canada, one in Yukon Territory and the other in James Bay, Quebec. Since the James Bay site was proximate to the coast and potentially influenced by marine nutrients (such as marine nutrients transported inland by anadromous fish), we examined coastal and inland variability in plant isotopic signatures. Due to the variability of $\delta^{13}C$ signatures in freshwater plants associated with stream flow, we also examined the variability of isotopic signatures in river and lake habitats. To our knowledge, this is the first study to quantify site-specific differentiation of both $\delta^{13}C$ and $\delta^{15}N$ signatures of freshwater macrophytes and terrestrial plants.

Methods

Study Areas

Our study areas were 16 locations in Old Crow Flats, a 6170 km^2 basin of thermokarst lakes in northern Yukon Territory (68° 05’ N, 140° 05’ W), and 23 locations in Wemindji, a 5000 km^2 network of rivers and lakes in eastern James Bay, Quebec (53° 0’ N, 78° 48’ W; Fig. 1). Old Crow Flats is at the boreal tree line, with the predominant terrestrial vegetation being willow shrubs (*Salix* spp.) and intermittent spruce (*Picea mariana* and *P. glaucus*; Yukon Ecoregions Working Group 2004). The aquatic ecosystem is a network of shallow, clear lakes and ponds that are slightly basic (pH 9.0). Freshwater vegetation is submergent consisting primarily of pondweeds (*Potamogeton* spp.) and milfoil (*Myriophyllum* spp.). Terrestrial vegetation in Wemindji is composed primarily of black spruce (*Picea mariana*) lichen forests with the understory vegetation consisting mainly of ericaceous, alder (*Alnus* spp.) and willow shrubs (Dignard et al 1991; Parisien and Sirois 2003). Freshwater ecosystems are a network of ponds, marshes, lakes, creeks, and rivers with headwaters 100 km inland that drain into James Bay. Freshwater ecosystems are shallow, darkly stained, and slightly acidic (pH 5.5). Freshwater macrophytes are emergent and submergent consisting primarily of sedge (*Carex* spp.), water lilies (*Nuphar variegatum*), and burreed (*Sparganium* spp.).

Sample Collections

In order to compare $\delta^{13}C$ and $\delta^{15}N$ signatures of freshwater and terrestrial vegetation in food webs, we collected the portions of common plants that are typically consumed by local vertebrate herbivores. In particular, we collected the leaves, bark, rhizomes, or stems of freshwater macrophytes and deciduous terrestrial plants that are frequently consumed by herbivores such as North American beaver (*Castor canadensis*), moose (*Alces alces*), muskrat (*Ondatra zibethicus*), and waterfowl (Aleksiuk 1970; Northcott 1971; Fraser et al 1980; Jelinski 1989; MacCracken et al 1993; Baldassarre and Bolen 2006). We identified plants to the species level when possible or to the genus level when several sympatric species were similar in their ecology and identifying features. We use the term vegetation type in this paper to describe the different portions of plants from the same species or genus. We considered any plants that typically grew in water as a freshwater plant. All our terrestrial plants were collected from riparian habitats near the high water line.

Our methods of collection and sample preparation differed slightly among three collection periods and therefore are analyzed separately. We collected vegetation from Old Crow Flats in July-August 2007. Three replicate samples per vegetation type were collected at each location, then combined in approximately equal portions into a composite sample, and then air dried in silica pouches. We collected vegetation from Wemindji in June-August 2006 and July-August 2007. In 2006, we collected one sample per vegetation type at each location. We air-dried samples and then removed debris with distilled water in a sonicator for 30 minutes before drying them for a minimum of 24 hours at 50 °C. In 2007, we collected three replicate samples per vegetation type at each location, including near the coast (within 15 km east of James Bay) and further inland (75 to 110 km east of James Bay). We dried the replicate samples for a minimum of 24 hours at 50° C and then combined an equal mass (measured with an analytical scale) of each replicate into a composite sample for isotopic analysis.

Stable Isotope Analyses

To prepare samples for isotopic analysis, we powdered samples with a mortar and pestle or a coffee grinder. We sub-sampled approximately 0.5 to 2 mg for continuous-flow isotope ratio mass spectrometry analysis at the Geochemistry and Geodynamics Research Centre Stable Isotopes Laboratory, l'Université de Québec a Montréal, and the Stable Isotopes in Nature Laboratory, University of New Brunswick, using a Carlo Erba NC 1500 or a Carlo Erba NC 2500 interfaced with a Micromass Isoprime or a Thermo-Finnigan Delta Plus Mass Spectrometer, respectively. Isotopic signatures are expressed in delta notation (δ) as ratios relative to PeeDee Belemnite carbonate (carbon) and atmospheric N_2 (nitrogen) standards as follows: $\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$, where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The typical precision obtained by repeated analyses of primary standards at both labs was <0.10‰ for $\delta^{13}C$ and <0.20‰ for $\delta^{15}N$. A subset of samples ($n = 44$) were analyzed twice with an average difference of 0.10‰ for $\delta^{13}C$ and 0.14‰ for $\delta^{15}N$. We use the term enriched to describe isotopic signatures that are more positive and the term depleted to describe isotopic signatures that are more negative.

Statistical Analyses

We compared mean $\delta^{13}C$ and $\delta^{15}N$ signatures of freshwater and terrestrial vegetation types for each collection and all collections pooled together with a series of *t*-tests. Since vegetation types differed in their isotopic signatures and represent different food types for different herbivores, we kept them separate in our analyses. We used mean values of each vegetation type to avoid pseudoreplication. We did not assume that variances were equal and therefore used the most conservative of the *t*-tests generated. We tested for equality of variances using Levene's test. All distributions were normal with a Kolmogorov-Smirnov test.

We compared mean $\delta^{13}C$ and $\delta^{15}N$ signatures of freshwater and terrestrial vegetation types from inland and coastal habitats from the Wemindji 2007 collection with a series of *t*-tests. Similarly, we compared mean $\delta^{13}C$ and $\delta^{15}N$ values of freshwater and terrestrial vegetation types from river (and creek) and lake (and pond) habitats from the Wemindji 2007 collection. We excluded vegetation types without replicate isotopic values from our analyses of pooled collections and habitat differences.

We tested our ability to correctly classify freshwater and terrestrial vegetation types by their $\delta^{13}C$ and $\delta^{15}N$ signatures with a series of cluster analyses. We used 2-group *k*-means cluster analyses with Euclidean distances to classify isotopic signatures of vegetation types into freshwater and terrestrial clusters. We calculated the percentage of vegetation types that were correctly classified as freshwater and terrestrial plants. All tests were performed using a combination of SPSS 11 and SYSTAT 11.

Results

Freshwater-Terrestrial $\delta^{13}C$ and $\delta^{15}N$ Signatures in Subarctic Ecosystems

The mean $\delta^{13}C$ and $\delta^{15}N$ values of freshwater vegetation types were consistently enriched compared to terrestrial vegetation types despite overlap in isotopic signatures (Table 1 and Fig. 2). The pooled means of freshwater vegetation types were significantly enriched by 4.8‰ $\delta^{13}C$ and 2.7‰ $\delta^{15}N$ relative to terrestrial vegetation types (Table 2). Freshwater vegetation (n = 114) ranged from -32.3 to -4.4‰ for $\delta^{13}C$, whereas terrestrial vegetation (n = 148) ranged from -32.0 to -25.8‰ for $\delta^{13}C$ (Table 1). Freshwater vegetation ranged from -5.39 to 7.42‰ for $\delta^{15}N$, whereas terrestrial vegetation ranged from -6.5 to 9.8‰ for $\delta^{15}N$. Despite this overlap, ellipses delineating standard deviation of bivariate means clearly demonstrate that freshwater and terrestrial vegetation differed in their isotopic signature overall and within sample collections.

Freshwater and terrestrial vegetation types differed consistently in their $\delta^{13}C$ and $\delta^{15}N$ signatures in each collection, regardless of slightly different sampling procedures and widely spaced geographic locations (Fig. 2). In Old Crow Flats, $\delta^{13}C$ signatures of freshwater vegetation types were relatively enriched by 10.4‰ to terrestrial vegetation whereas there was no differentiation in $\delta^{15}N$ signatures (Table 2). The two collections from Wemindji were similar in their isotopic signatures (Fig. 2). In both years, freshwater vegetation types in Wemindji were enriched approximately 2‰ in $\delta^{13}C$ and 3‰ in $\delta^{15}N$ relative to terrestrial vegetation (Table 2). Freshwater vegetation in Old Crow Flats, Wemindji 2007, and all collections pooled together were more variable in their $\delta^{13}C$ signatures compared to terrestrial vegetation (Table 2).

Freshwater-Terrestrial $\delta^{13}C$ and $\delta^{15}N$ Signatures of Inland and Coastal Habitats

Freshwater vegetation types were enriched in their $\delta^{13}C$ and $\delta^{15}N$ signatures compared to terrestrial vegetation types in both inland and coastal habitats (Table 2 and Fig. 3). Freshwater and terrestrial vegetation types differed slightly more in their $\delta^{13}C$ signatures in coastal habitats. Coastal terrestrial vegetation types were 0.9‰ more depleted in $\delta^{13}C$ than those inland (t-test = $3.08_{(16)}$, $P<0.01$) and coastal freshwater vegetation types were more variable in $\delta^{13}C$ than those inland (Levene's test $F=5.37_{(1,15)}$, $P<0.05$). *Nuphar variegatum* was enriched by approximately 2‰ $\delta^{13}C$ at coastal habitats (leaves t-test = $11.5_{(4)}$, $P<0.001$; rhizome t-test = $3.04_{(5)}$, $P<0.05$) and *Salix* spp. bark was enriched by approximately 1‰ $\delta^{13}C$ at inland habitats (t-test = $2.60_{(14)}$, $P<0.05$), but no other freshwater or terrestrial vegetation types differed in their $\delta^{13}C$ or $\delta^{15}N$ signatures between inland and coastal habitats (t-tests $P>0.07$).

Freshwater-Terrestrial $\delta^{13}C$ and $\delta^{15}N$ Signatures of River and Lake Habitats

Freshwater vegetation types were enriched in their $\delta^{13}C$ and $\delta^{15}N$ signatures compared to terrestrial vegetation types in both river and lake habitats (Table 2 and Fig. 3). Freshwater and terrestrial vegetation types differed slightly more in their $\delta^{13}C$ signatures in lake habitats. Freshwater vegetation from lakes was more variable in $\delta^{13}C$ than from rivers (Levene's test $P<0.05$). *Sparganium* and *Potamogeton* spp. from lake habitats appeared to be enriched in their $\delta^{13}C$ signatures compared to river habitats, but these lake-river differences were not significant (*t*-tests $P=0.26$ and $P=0.06$). Freshwater and terrestrial vegetation growing near rivers differed more in their $\delta^{15}N$ signatures. The $\delta^{15}N$ signatures of vegetation types did not differ between river and lake habitats (*t*-test $P=0.44$).

Classification of Freshwater-Terrestrial Vegetation Types

Cluster analyses of $\delta^{13}C$ and $\delta^{15}N$ signatures correctly classified 86% (± 14% SD) of vegetation types into freshwater or terrestrial groups (Table 4). Most terrestrial plants and submergent freshwater plants were correctly classified in all collections and habitats. The majority of misclassified freshwater macrophytes were emergent plants living near the shoreline (see Table 4 for a complete list of misclassified vegetation types). Cluster analyses using $\delta^{13}C$ and $\delta^{15}N$ separately correctly classified 72% (± 7% SD) of vegetation types into freshwater or terrestrial groups with $\delta^{13}C$ and 83% (± 11% SD) with $\delta^{15}N$.

Discussion

Freshwater-Terrestrial $\delta^{13}C$ and $\delta^{15}N$ Signatures in Subarctic Ecosystems

Carbon and nitrogen stable isotope analyses in combination provide a useful tool in differentiating freshwater macrophytes and terrestrial plants in subarctic ecosystems. Our comparisons of freshwater macrophytes and terrestrial plants from forests in northern Yukon and Quebec show a freshwater enrichment of 1.8 to 10.4‰ for $\delta^{13}C$ and 0.7 to 3.0‰ for $\delta^{15}N$ relative to terrestrial vegetation. No site-specific comparisons exist of the isotopic compositions of freshwater macrophytes and terrestrial plants, however a global review by Finlay and Kendall (1998) suggests that our observed differences are within typical ranges. They reported that $\delta^{13}C$ values of freshwater macrophytes are potentially enriched ranging from 0 to 7‰ $\delta^{13}C$ and $\delta^{15}N$ values are potentially depleted by 12‰ $\delta^{15}N$ or enriched by 13‰ $\delta^{15}N$, relative to C_3 terrestrial plants. We found that $\delta^{15}N$ signatures of freshwater macrophytes were enriched relative to terrestrial plants in Wemindji but not Old Crow Flats. The overlap in freshwater and terrestrial $\delta^{15}N$ signatures from Old Crow Flats may be partially driven by several small samples of terrestrial plants (*Chamerion angustifolium, Erigeron acris*, and *Salix alaxensis*) that were collected from drained lake basins. Although the lakes had drained 20 years prior, the soil chemistry may reflect lacustrine sources. The analysis of $\delta^{13}C$ and $\delta^{15}N$ together was necessary to consistently differentiate freshwater and terrestrial plants qualitatively with bivariate scatter plots and statistically with cluster analyses.

The isotopic signatures of freshwater and terrestrial vegetation from Old Crow Flats and Wemindji generally fell within the range of previous studies. Freshwater vegetation was more variable than terrestrial vegetation, which is supported by a review by Rounick and Winterbourn (1986). Our $\delta^{13}C$ signatures of terrestrial plants were similar to other studies of C_3 plants whereas some freshwater macrophytes were 5‰ more positive than the range (-50 to -10‰) compiled by Rounick and Winterbourn (1986). Our $\delta^{15}N$ signatures fell within the range of previous studies (France 1995c; Finlay and Kendall 2007). Isotopic signatures of vegetation at the genus and species level generally fell within reported ranges (Osmond et al 1981; Keeley and Sandquist 1992; McArthur and Moorhead 1996; Ben-David et al 2001). The $\delta^{13}C$ values of several macrophytes (*Myriophyllum spicatum, Potamogeton* spp., and *Sparganium* spp.*)* from Old Crow Flats were enriched by 5 to 10‰ compared to other studies (Osmond et al 1981; Keeley and Sandquist 1992). We may have observed enriched isotopic values due to different environmental conditions or due to organisms attached to our samples. We intentionally did not rinse our 2007 samples so that they better represented what would be consumed by herbivores.

Although we were able to discriminate the mean $\delta^{13}C$ and $\delta^{15}N$ signatures of freshwater and terrestrial plants, they overlapped considerably in their isotopic values. Nutrient flows across the freshwater-terrestrial interface could contribute to some of this overlap in isotopic values (reviewed in Polis et al 2004). If that is the case, we should expect freshwater macrophytes growing near the shoreline to be an isotopic intermediate between macrophytes from deeper water and terrestrial plants, which was supported with our cluster analyses. We consistently discriminated submergent macrophytes that grow in deeper water (ex: *Nuphar variegatum*, *Potamogeton* spp., and *Sparganium* spp.) from terrestrial plants. Emergent macrophytes growing along the shoreline were sometimes misclassified with terrestrial plants (ex: *Carex* spp., *Comarum palustris*, and *Typha latifolia*). Emergent aquatics can also sequester atmospheric and aqueous CO_2 (Osmond et al. 1981) and therefore should have intermediate $\delta^{13}C$ values compared to submergent aquatics and terrestrial plants. We might have found more isotopic differentiation between ecosystems had we excluded emergent aquatics and sampled terrestrial plants further upland.

Freshwater-Terrestrial $\delta^{13}C$ and $\delta^{15}N$ Signatures of Inland and Coastal Habitats

We observed an isotopic separation between freshwater and terrestrial vegetation in both inland and coastal sites of Wemindji even though the community composition and certain genera shifted in their isotopic signatures. We observed coastal $\delta^{13}C$ enrichment for some freshwater vegetation types, which may indicate a slight marine nutrient influence in the aquatic ecosystem (reviewed in Kelly 2000). Overall there is little difference between coastal and inland isotopic values. Our ability to discriminate freshwater and terrestrial vegetation with $\delta^{13}C$ and $\delta^{15}N$ with cluster analyses was comparable in inland and coastal habitats.

Freshwater-Terrestrial $\delta^{13}C$ and $\delta^{15}N$ Signatures of River and Lake Habitats

We observed an isotopic separation between freshwater and terrestrial vegetation in both river and lake habitats even though community composition and certain genera shifted in their isotopic signatures. Freshwater macrophytes and riparian plants were depleted in $\delta^{13}C$ in rivers relative to lakes. Osmond and colleagues (1981) found similar depletions of $\delta^{13}C$ for riverine freshwater macrophytes and attributed lower $\delta^{13}C$ signatures to increased water flow and smaller boundary layers affecting CO_2 uptake. We likely observed the same mechanism since $\delta^{13}C$ signatures of *Potamogeton* spp. and *Sparganium* spp. appeared to shift between rivers and lakes while *Carex* spp. did not. In our study areas, *Carex* spp. grew along the shoreline in slow moving water in both lakes and rivers. *Potamogeton* spp. and *Sparganium* spp. grew in slightly deeper water with faster flow in rivers, which likely reduces their boundary layer. Overall there is little difference between river and lake isotopic values. Our ability to discriminate freshwater and terrestrial vegetation with $\delta^{13}C$ and $\delta^{15}N$ with cluster analyses was comparable in rivers and lake habitats.

Utility of $\delta^{13}C$ and $\delta^{15}N$ in Freshwater-Terrestrial Food Web Studies

The isotopic differences we observed between freshwater macrophytes and terrestrial plants were small but have potential to be applied to food web studies. Primary producers from marine and terrestrial systems typically differ in ^{13}C content by 7.3 to 18.6‰ and in ^{15}N content by 6.4 to 9.2‰ when applied to food web studies (Anderson and Polis 1998; McCutchan et al 2003). We observed much smaller differences between freshwater and terrestrial plants (1.8 to 10.4‰ $\delta^{13}C$ and 2.7 to 3.0‰ $\delta^{15}N$), potentially because marine autotrophs sequester carbon from enriched bicarbonate sources and $\delta^{15}N$ becomes enriched with ocean depth (Peterson and Fry 1987; Kelly 2000). The differences we observed are enough to determine freshwater and terrestrial plant dietary sources in food webs. Our freshwater and terrestrial plants had distinct isotopic signatures with analyses of variance and cluster analyses and therefore can be applied to food web analyses that determine the dietary contribution from several sources using linear mixing models (Phillips and Greg 2003). The level of $\delta^{13}C$ and $\delta^{15}N$ enrichment from diet to consumer varies and can reduce the ability of ecologists to distinguish dietary sources (Gannes et al 1997), but is likely not to impede the ability to distinguish freshwater and terrestrial sources in food webs. Meta-analyses of $\delta^{13}C$ and $\delta^{15}N$ diet-consumer enrichment values indicate that freshwater and terrestrial organisms have similar enrichment values and the standard error typically associated with enrichments (<0.4‰) is less than our observed differences (McCutchan et al 2003; Vanderklift and Ponsard 2003).

We were able to consistently discriminate freshwater and terrestrial plant sources in subarctic food webs regardless of temporal and spatial shifts in the isotopic signatures of some taxa. Temporal and spatial variation of isotopic signatures within a species can limit the utility of stable isotope analysis to discriminate sources in food webs (McArthur and Moorhead 1996; Cloern et al 2002; Post 2002). Our study indicates these factors do not impede our ability to discriminate freshwater and terrestrial sources at a specific site. Repeated sampling at our Wemindji sites indicates a temporal consistency in the differences between freshwater and terrestrial plant species. Furthermore, we found spatial consistency of isotopic signatures within freshwater and terrestrial plant species regardless of habitat differences.

The combination of $\delta^{13}C$ and $\delta^{15}N$ stable isotope analysis proved useful in differentiating freshwater macrophytes and terrestrial plants in subarctic ecosystems. We found consistent isotopic differentiation of freshwater and terrestrial plants from two subarctic forests in northern Canada that grow in habitats with very different hydrology, pH, water clarity, and vegetation cover. The site-specific analysis of both $\delta^{13}C$ and $\delta^{15}N$ was necessary to consistently distinguish freshwater and terrestrial plants. Our study reveals the potential utility of $\delta^{13}C$ and $\delta^{15}N$ signatures in tracing nutrient flows, animal movements, and food web interactions across freshwater-terrestrial boundaries.

Acknowledgements

We thank the Cree Nation of Wemindji and the Vuntut Gwitchin First Nation for their permission and participation. We also thank P Abel, B Bell, M Gay, B Linklater-Georgekish, K Matches, M Milligan and N Mirotchnick for fieldwork. Funding was provided by SSHRC Community-University Research Alliance and Aboriginal Research grants to Paakumshumwaau-Wemindji Protected Area Project, Government of Canada's

IPY program, NCE ArcticNet, the Northern Scientific Training Program, and NSERC Northern Research Chairs program. While preparing the manuscript, HEM was supported by NSERC Post-graduate Scholarship and Association of Canadian Universities for Northern Studies Northern Resident Scholarship. This study complies with Canadian laws.

References

Aleksiuk M (1970) The seasonal food regime of arctic beavers. Ecol 51:264-270

Anderson WB, Polis GA (1998) Marine subsidies of island communities in the Gulf of California: evidence from stable carbon and nitrogen isotopes. Oikos 81:75-80

Baldassarre GA, Bolen EG (2006) Waterfowl ecology and management, 2nd ed. Krieger Publishing Company, Malabar

Ben-David M, Schochat E, Adams LG (2001) Utility of stable isotope analysis in studying foraging ecology of herbivores: examples from moose and caribou. Alces 37:421-434

Cebrian J, Lartigue J (2004) Patterns of herbivory and decomposition in aquatic and terrestrial ecosystems. Ecol Monogr 74:237-259

Chisolm BS, Nelson DE, Schwarcz HP (1982) Stable-carbon isotope ratios as a measure of marine versus terrestrial protein in ancient diets. Science 216:1131-1132

Cloern JE, Canuel EA, Harris D (2002) Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system. Limnol Oceanogr 47:713-729

Cyr H, Pace ML (1993) Magnitude and patterns of herbivory in aquatic and terrestrial ecosystems. Nature 361:148-150

Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP (2002) Stable isotopes in plant ecology. Annu Rev Syst 33:507-559

DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42:495-506

DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45:341-351

Dignard N, Lalumière R, Reed A, Julien M (1991) Habitats of the northeast coast of James Bay. Canadian Wildlife Service Occasional Paper No 70, Ottawa

Finlay JC, Kendall C (2007) Stable isotope tracing of temporal and spatial variability in organic matter sources to freshwater ecosystems. In: Michener RH, Lajtha K (eds) Stable isotopes in ecology and environmental science, 2nd edn. Blackwell Publishing, Malden, pp 283-333

France RL (1995a) Critical examination of stable isotope analysis as a means for tracing carbon pathways in stream ecosystems. Can J Fish Aquat Sci 52:651-656

France RL (1995b) Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. Limnol Oceanogr 40:1310-1313

France RL (1995c) Source variability in $\delta^{15}N$ of autotrophs as a potential aid in measuring allochthony in freshwaters. Ecography 18:318-320

France RL (1995d) Stable isotopic survey of the role of macrophytes in the carbon flow of aquatic foodwebs. Vegetatio 124:67-72

Fraser D, Arthur D, Morton JK, Thompson BK (1980) Aquatic feeding by moose *Alces alces* in a Canadian lake. Holarc ecol 3:218-223

Fry B (1991) Stable isotope diagrams of freshwater food webs. Ecol 72:2293-2297

Gannes LZ, O'Brien DM, Martinez del Rio C (1997) Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecol 78:1271-1276

Hobson KA (1999) Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120:314-326

Jelinski DE (1989) Seasonal differences in habitat use and fat reserves in an arctic muskrat population. Can J Zool 67:305-313

Keeley JE, Sandquist DR (1992) Carbon: freshwater plants. Plant Cell Environ 15:1021-1035

Kelly JF (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Can J Zool 78:1-27

MacCracken JG, Ballenberghe VV, Peek JM (1993) Use of aquatic plants by moose: sodium hunger or foraging efficiency? Can J Zool 71:2345-2351

McArthur JV, Moorhead KK (1996) Characterization of riparian species and stream detritus using multiple stable isotopes. Oecologia 107:232-238

McCutchan JH, Lewis WM, Kendall C, McGrath CC (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102:378-390

Nakano S, Murakami M (2001) Reciprocal subsidies: dynamic interdependence between terrestrial and aquatic food webs. Proc Natl Acad Sci USA 98:166-170

Northcott TH (1971) Feeding habits of beaver in Newfoundland. Oikos 22:407-410

Osmond CB, Valaane N, Haslam SM, Uotila P, Roksandic Z (1981) Comparisons of $\delta^{13}C$ values in leaves of aquatic macrophytes from different habitats in Britain and Finland; some implication for photosynthetic processes in aquatic plants. Oecologia 50:117-124

Parisien MA Sirois L (2003) Distribution and dynamics of tree species across a fire frequency gradient in the James Bay region of Quebec. Can J For Res 33:243-256

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Ann Rev Ecol Syst 18:293-320

Phillips DL, Gregg JW (2003) Source partitioning using stable isotopes: coping with too many sources. Oecologia 136:261-269

Polis GA, Anderson WB, Holt RD (1997) Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annu Rev Ecol Syst 28:289-316

Polis GA, Power ME, Huxel GR (2004) Food webs at the landscape level. University of Chicago Press, Chicago

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecol 83:703-718

Rounick JS, Winterbourn MJ (1986) Stable carbon isotopes and carbon flow in ecosystems. Bioscience 36:171-177

Schoeninger MJ, DeNiro MJ, Tauber MS (1984) Stable nitrogen isotope ratios of bone collagen reflect marine and terrestrial components of prehistoric human diet. Science 220:1381-1383

Vanderklift MA, Ponsard S (2003) Sources of variation in consumer-diet $\delta^{15}N$ enrichment: a meta-analysis. Oecologia 136:169-182

Yukon Ecoregions Working Group (2004) Old Crow Basin and Old Crow Flats. In: Smith CAS, Meikle JC, Roots CF (eds) Ecoregions of the Yukon Territory: biophysical properties of Yukon landscapes. Agriculture and agri-foods Canada PARC Technical Bulletin No 04-01, Summerland, pp 107-122

Table 1 Summary of $\delta^{13}C$ and $\delta^{15}N$ signatures for each freshwater and terrestrial vegetation type from Old Crow Flats, Yukon Territory, and Wemindji, James Bay Quebec. *n* indicates the number of samples analysed, which consists of one individual plant per location in 2006 and a composite of three individual plants per location in 2007 (see text for details).

						δ^{13}C (‰)		δ^{15}N (‰)	
Collection	Ecosystem	Vegetation	Type	Code	*n*	Mean ± SD	Range	Mean ± SD	Range
Old Crow Flats 2007	Freshwater	Bryophyta	stem	BR	1	-28.0		3.7	
		Callitriche hermaphroditica	leaf	CH	1	-12.7		-1.6	
		Carex spp.	leaf	CA	1	-27.2		3.0	
		Ceratophyllum demersum	leaf	CD	1	-21.7		3.0	
		Comarum palustre	leaf	CP	2	-25.7 ± 1.4	-26.7 to -24.7	5.1 ± 3.2	2.9 to 7.4
		Equisetum fluviatile	stem	EF	1	-25.0		2.5	
		Myriophyllum spicatum	leaf	MS	3	-8.7 ± 4.7	-13.7 to -4.4	1.8 ± 2.1	0.4 to 4.2
		Potamogeton richadsonii	leaf	PR	3	-12.2 ± 3.7	-16.0 to -8.7	2.0 ± 0.6	1.4 to 2.6
		Potamogeton spp.	leaf	PO	9	-8.6 ± 1.6	-10.4 to -5.4	3.3 ± 3.7	-5.4 to 7.0
		Sparganium spp.	leaf	SP	2	-9.8 ± 0.7	-10.3 to -9.4	3.4 ± 0.2	3.2 to 3.5
	Terrestrial	*Betula* spp.	leaf	BE	2	-26.9 ± 1.0	-27.6 to -26.2	-3.5 ± 3.1	-5.6 to -1.3
		Chamerion angustifolium	leaf	CM	2	-30.4 ± 0.8	-31.0 to -29.9	5.9 ± 4.6	2.6 to 9.2
		Erigeron acris	leaf	ER	1	-29.2		3.3	
		Poacea spp.	leaf	POA	1	-25.8		2.5	
		Rubus chamaemorus	leaf	RU	1	-28.7		2.6	
		Salix alaxensis	leaf	SL	7	-28.6 ± 1.4	-30.6 to -26.1	2.9 ± 2.4	-0.6 to 5.9
		Salix spp.	leaf	SA	11	-28.6 ± 0.9	-30.1 to -27.4	1.1 ± 4.8	-4.9 to 9.8
Wemindji 2006	Freshwater	*Carex* spp.	leaf	CA	3	-27.7 ± 1.3	-29.1 to -26.8	1.2 ± 2.5	-1.5 to 3.4
		Eleocharis palustris	stem	EP	3	-28.2 ± 1.2	-29.0 to -26.8	0.9 ± 1.9	-1.3 to 2.4
		Equisetum fluviatile	stem	EF	3	-26.6 ± 1.4	-27.7 to -25.1	2.0 ± 3.1	-1.6 to 4.0
		Nuphar variegatum	leaf	NU-L	3	-26.3 ± 0.7	-26.8 to -25.5	3.0 ± 2.4	0.5 to 5.4
		Nuphar variegatum	rhizome	NU-R	3	-26.3 ± 1.2	-27.3 to -25.0	-0.3 ± 1.2	-1.1 to 1.1
		Potamogeton spp.	leaf	PO	3	-28.2 ± 0.2	-28.4 to -28.0	1.8 ± 1.0	0.7 to 2.8
		Sparganium spp.	leaf	SA	3	-28.5 ± 0.7	-29.0 to -27.7	1.9 ± 1.2	0.5 to 2.7
	Terrestrial	*Alnus* spp.	bark	AL-B	3	-30.7 ± 1.0	-31.7 to -29.8	-2.5 ± 0.1	-2.7 to -2.4
		Alnus spp.	leaf	AL-L	3	-30.8 ± 0.2	-31.0 to -30.6	-1.7 ± 0.1	-1.8 to -1.6
		Chamerion angustifolium	leaf	CM	3	-30.4 ± 0.3	-30.7 to -30.0	-3.8 ± 1.5	-5.5 to -2.7
		Populus tremuloides	bark	PT-B	3	-28.6 ± 1.0	-29.5 to -27.6	-2.3 ± 3.7	-6.5 to 0.0
		Populus tremuloides	leaf	PT-L	3	-29.2 ± 1.1	-30.5 to -28.3	0.2 ± 4.2	-4.6 to 3.0
		Salix spp.	bark	SA-B	3	-28.1 ± 1.1	-29.2 to -27.0	-1.1 ± 2.3	-3.3 to 1.4
		Salix spp.	leaf	SA-L	3	-28.8 ± 1.2	-30.1 to -27.8	0.7 ± 2.8	-1.6 to 3.8
Wemindji 2007	Freshwater	*Carex* spp.	leaf	CA	12	-27.2 ± 0.6	-28.0 to -26.0	2.4 ± 1.6	-0.4 to 4.9
		Comarum palustre	leaf	CP	4	-27.9 ± 0.5	-28.6 to -27.5	0.5 ± 1.5	-1.6 to 2.0
		Eleocharis palustris	stem	EP	3	-28.7 ± 0.6	-29.3 to -28.1	2.1 ± 0.5	1.6 to 2.5
		Equisetum fluviatile	stem	EF	4	-25.8 ± 2.3	-28.8 to -23.2	2.1 ± 1,1	0.9 to 3.4
		Hippuris vulgaris	leaf	HV	3	-28.5 ± 3.3	-32.3 to -26.6	1.8 ± 1.0	0.6 to 2.6
		Iris versicolor	leaf	IV	2	-27.7 ± 1.7	-28.9 to -26.5	1.4 ± 1.0	0.7 to 2.1
		Nuphar variegatum	leaf	NU-L	7	-26.1 ± 1.0	-27.2 to -24.9	1.0 ± 1.4	-0.5 to 2.9
		Nuphar variegatum	rhizome	NU-R	7	-25.7 ± 1.1	-27.5 to -24.4	0.2 ± 1.0	-1.2 to 1.7
		Potamogeton spp.	leaf	PO	7	-24.3 ± 5.2	-31.6 to -16.8	1.1 ± 2.1	-1.9 to 3.8
		Scirpus atrocinctus	leaf	SC	5	-27.6 ± 1.0	-28.8 to -26.2	1.7 ± 1.8	-1.4 to 3.0
		Sparganium spp.	leaf	SP	12	-27.8 ± 2.2	-31.7 to -24.1	1.8 ± 1.6	-0.9 to 3.5
		Typha latifolia	leaf	TL	3	-29.0 ± 0.8	-29.9 to -28.3	1.3 ± 0.7	0.7 to 2.1
	Terrestrial	*Alnus* spp.	bark	AL-B	14	-29.6 ± 1.3	-31.6 to -28.2	-1.9 ± 0.5	-2.7 to -1.1
		Alnus spp.	leaf	AL-L	10	-28.5 ± 1.0	-30.1 to -26.7	-1.5 ± 0.2	-1.8 to -1.2
		Chamerion angustifolium	leaf	CM	12	-29.7 ± 0.6	-31.0 to -28.9	-3.0 ± 1.5	-5.1 to 0.1
		Heracleum maximum	leaf	HM	3	-29.7 ± 0.8	-30.6 to -29.2	0.4 ± 1.5	-1.3 to 1.5
		Populus balsamifera	bark	PB-B	3	-29.4 ± 2.3	-32.0 to -27.6	-2.1 ± 1.0	-3.2 to -1.1
		Populus balsamifera	leaf	PB-L	3	-29.8 ± 0.7	-30.5 to -29.2	-2.0 ± 0.9	-2.9 to -1.2
		Populus tremuloides	bark	PT-B	7	-28.7 ± 0.8	-30.3 to -27.8	-1.2 ± 1.8	-4.1 to 1.2
		Populus tremuloides	leaf	PT-L	6	-29.2 ± 0.6	-30.2 to -28.7	-0.7 ± 2.3	-3.3 to 2.3
		Prunus pensylvanica	bark	PP-B	4	-28.4 ±2.1	-30.8 to -25.8	0.1 ± 3.1	-3.2 to 3.4
		Prunus pensylvanica	leaf	PP-L	6	-28.2 ± 1.2	-28.9 to -25.8	-1.4 ± 2.9	-3.9 to 3.5
		Rubus idaeus	leaf	RU	3	-30.2 ± 1.1	-31.2 to -29.1	-2.3 ± 1.4	-3.7 to -1.0
		Salix spp.	bark	SA-B	17	-28.3 ± 0.9	-29.9 to -26.7	-0.6 ± 1.4	-3.8 to 1.5
		Salix spp.	leaf	SA-L	14	-27.9 ± 0.8	-29.1 to -25.9	-0.2 ± 1.5	-3.0 to 2.0

Table 2 Comparison of mean $\delta^{13}C$ and $\delta^{15}N$ signatures of freshwater and terrestrial vegetation types from each collection and all collections pooled. Vegetation types without replicate isotopic values were excluded from the analyses of pooled collections.

			$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		
Collection	Ecosystem	*n*	Mean ± SD	Difference	T-Test	Mean ± SD	Difference	T-Test
Pooled Collections	Freshwater	14	-24.1 ± 6.5	4.8	$2.74_{(13)}$, $P < 0.05^a$	1.7 ± 0.6	2.7	$6.37_{(17)}$, $P < 0.001^a$
	Terrestrial	15	-28.9 ± 0.8			-1.0 ± 1.5		
Old Crow Flats 2007	Freshwater	10	-18.0 ± 8.2	10.4	$3.26_{(15)}$, $P < 0.01^a$	2.6 ± 1.8	0.7	$0.42_{(9)}$, $P = 0.67$
	Terrestrial	7	-28.3 ± 1.5			2.1 ± 2.8		
Wemindji 2006	Freshwater	7	-27.4 ± 1.0	2.1	$3.74_{(11)}$, $P < 0.01$	1.5 ± 1.0	3.0	$4.21_{(10)}$, $P < 0.01$
	Terrestrial	7	-29.5 ± 1.1			-1.5 ± 1.6		
Wemindji 2007	Freshwater	12	-27.2 ± 1.4	1.8	$4.01_{(16)}$, $P < 0.001^a$	1.4 ± 0.7	2.7	$7.89_{(20)}$, $P < 0.001$
	Terrestrial	13	-29.0 ± 0.7			-1.3 ± 1.0		

[a] Levene's test $P < 0.05$

Table 3 Comparison of mean δ^{13}C and δ^{15}N signatures of freshwater and terrestrial vegetation types from inland, coastal, river, and lake habitats from the Wemindji 2007 collection. Vegetation types without replicate isotopic values were excluded from analyses.

			δ^{13}C (‰)			δ^{15}N (‰)		
Habitat	Ecosystem	*n*	Mean ± SD	Difference	T-Test	Mean ± SD	Difference	T-Test
Inland	Freshwater	9	-27.2 ± 1.0	1.3	$3.14_{(14),}$ $P<0.01$	1.7 ± 0.8	3.2	$5.96_{(12)}$, $P<0.001$
	Terrestrial	9	-28.4 ± 0.7			-1.5 ± 1.4		
Coast	Freshwater	8	-26.4 ± 1.8	2.9	$4.47_{(8),}$ $P<0.00^{a}$	1.4 ± 1.0	2.4	$4.55_{(18),}$ $P<0.001$
	Terrestrial	12	-29.3 ± 0.6			-1.0 ± 1.2		
River	Freshwater	7	-27.9 ± 1.0	1.5	$3.31_{(10),}$ $P<0.01$	1.6 ± 0.7	3.2	$7.25_{(15),}$ $P<0.001$
	Terrestrial	10	-29.4 ± 0.8			-1.6 ± 1.0		
Lake	Freshwater	10	-26.5 ± 2.2	2.2	$3.10_{(10),}$ $P<0.05^{a}$	1.4 ± 0.8	2.6	$5.88_{(14)}$, $P<0.001$
	Terrestrial	9	-28.7 ± 0.6			-1.2 ± 1.1		

[a] Levene's test $P<0.05$

Table 4 Classification of freshwater and terrestrial vegetation types based on 2-group *k*-means cluster analyses of δ^{13}C and δ^{15}N signatures. All vegetation types, other than those listed as misclassified, were correctly identified as aquatic or terrestrial species based on isotopic signatures.

			Misclassified Vegetation Types	
Collection	*n*	% Accurate Classification	Freshwater	Terrestrial
Pooled Collections	29	62	*Carex* spp. *Comarum palustre* *Eloecharis palustre* *Equisetum fluviatile* *Hippuris vulgaris* *Iris versicolor* *Nuphar variegatum* (leaf and rhizome) *Scirpus atrocinctus* *Sparganium* spp. *Typha latifolia*	
Old Crow Flats 2007	17	71	Bryophyta *Carex* spp. *Ceratophyllum demersum* *Comarum palustre* *Equisetum fluviatile*	
Wemindji 2006	14	86		*Populus tremuloides* (leaf) *Salix* spp. (leaf)
Wemindji 2007	25	100		
Inland	18	89		*Prunus pensylvanica* (bark and leaf)
Coast	20	95	*Typha latifolia*	
River	17	100		
Lake	19	84	*Comarum palustre* *Eloecharis palustris* *Typha latifolia*	

Fig. 1 Study sites in Old Crow Flats, Yukon, and Wemindji, Quebec, including insets showing land (white) and water (black) configurations in the two localities.

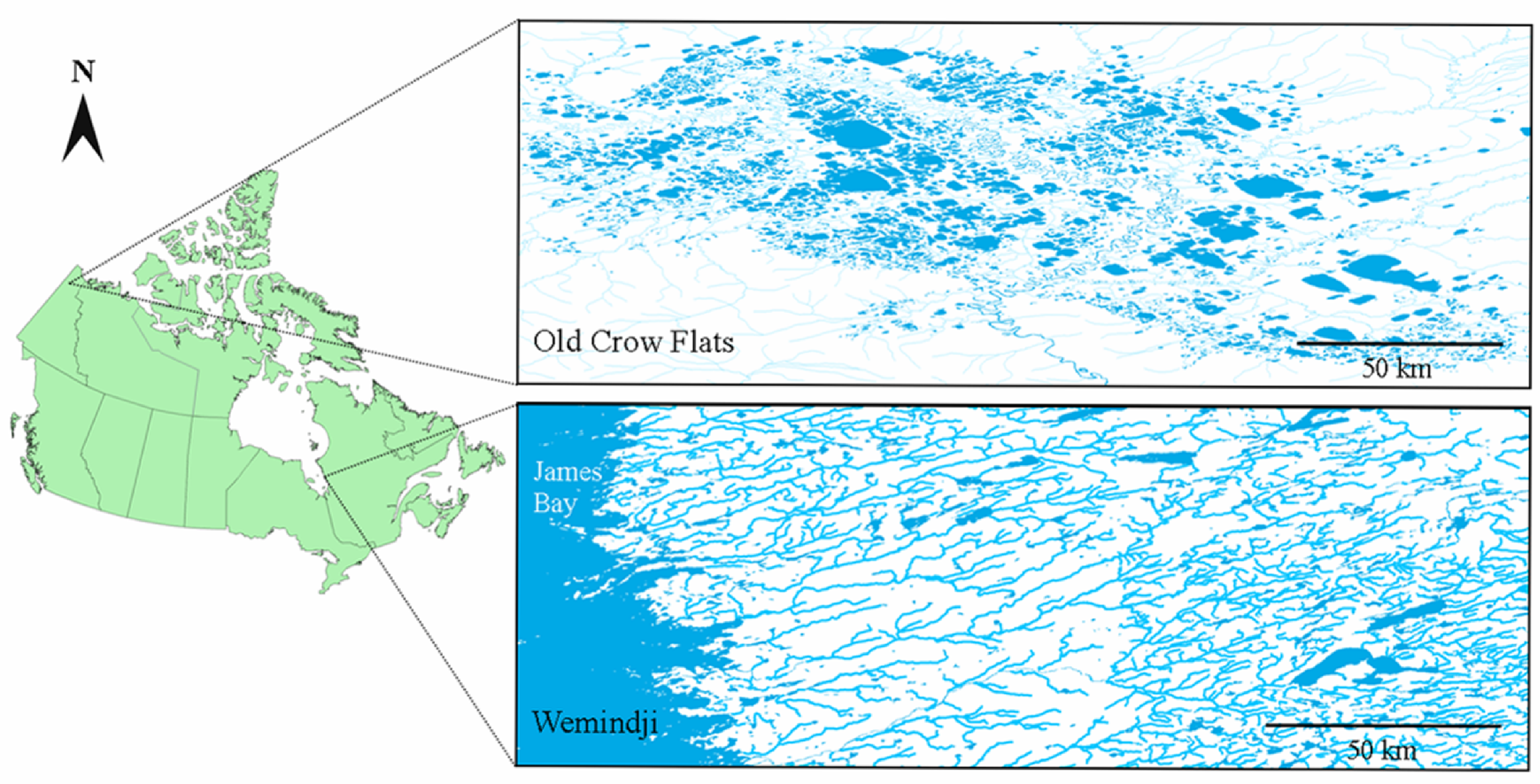


Fig. 2 Mean ± SE $\delta^{13}C$ and $\delta^{15}N$ signatures of freshwater and terrestrial vegetation types pooled or separated by locality and years. Dotted and solid ellipses show standard deviations for mean isotopic values of freshwater and terrestrial vegetation types, respectively. Vegetation type codes are described in Table 1. Vegetation types without replicate isotopic values are not shown in the pooled collection.

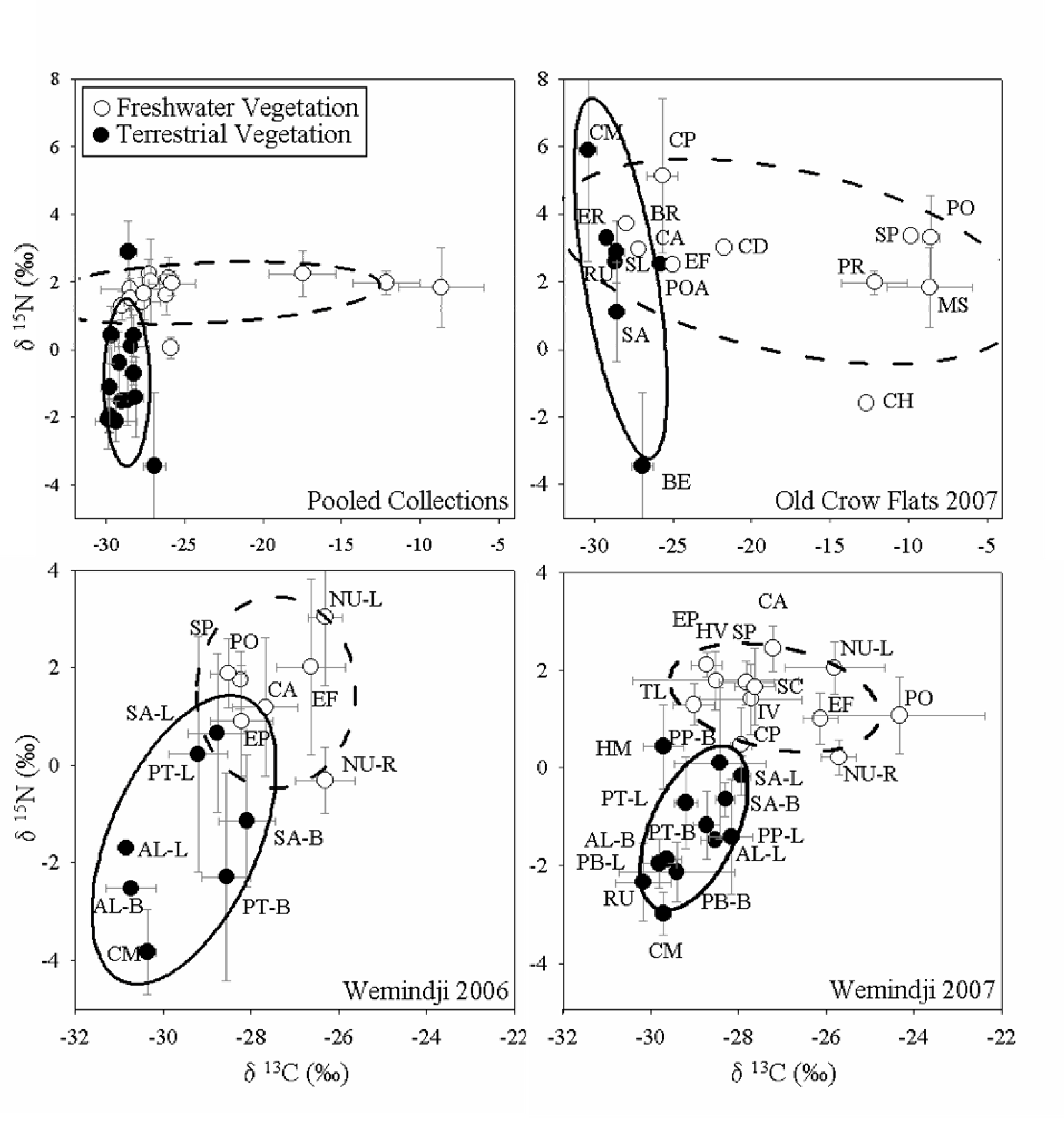
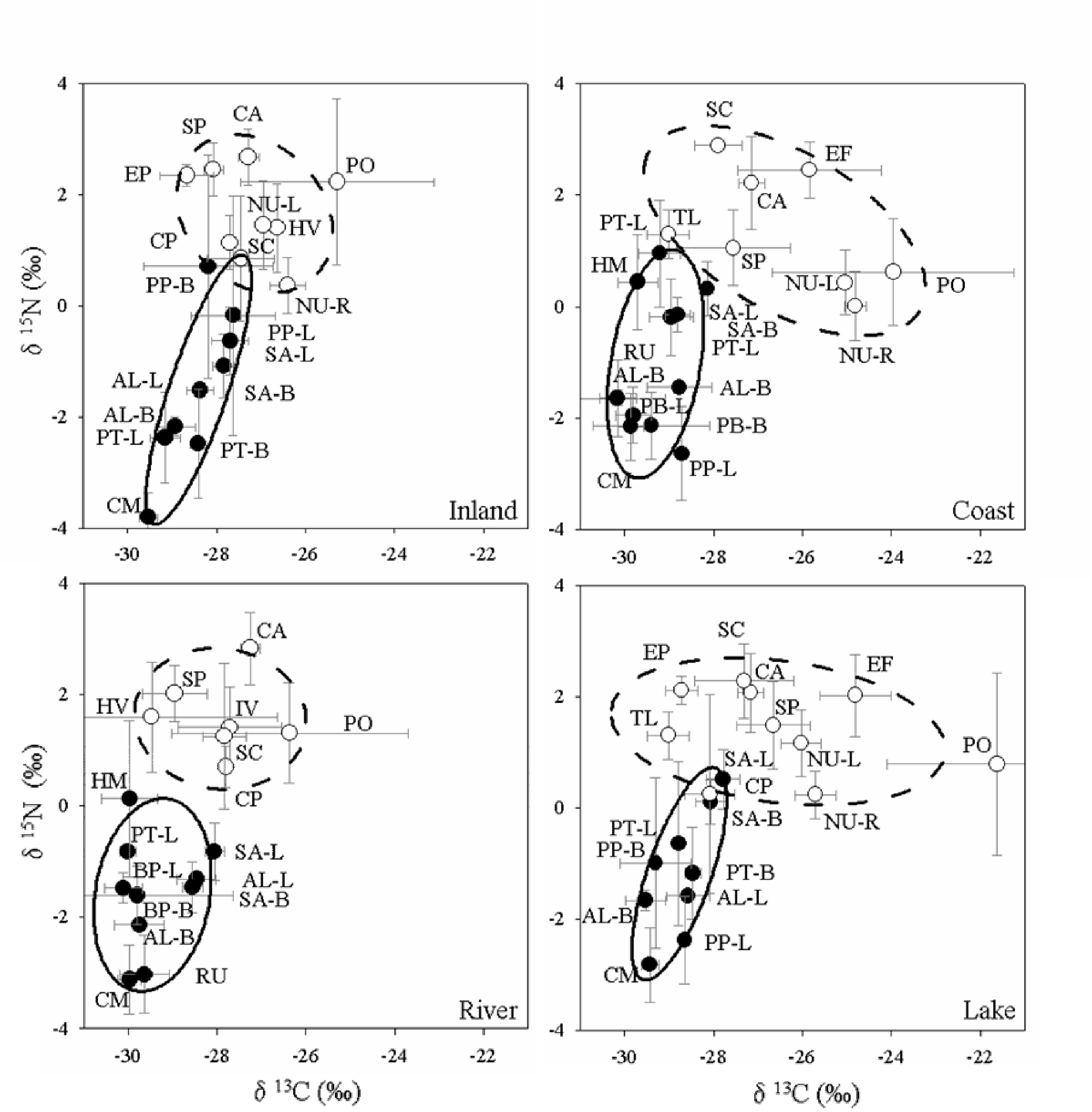


Figure 3 Mean ± SE $\delta^{13}C$ and $\delta^{15}N$ signatures of freshwater (open circles) and terrestrial (closed circles) vegetation types in inland, coastal, river, and lake habitats from the Wemindji 2007 collection. Dotted and solid ellipses show standard deviations for mean isotopic values of freshwater and terrestrial vegetation types, respectively. Vegetation type codes are described in Table 1. Vegetation types without replicate isotopic values are not shown.



Connecting Statement

Results from the previous chapter demonstrate that carbon and nitrogen stable isotopic values differ between subarctic freshwater and terrestrial vascular plants. This establishes that stable isotopic analysis can be an effective tool in quantifying nutrient transfers across freshwater and terrestrial ecosystem boundaries and the contribution of freshwater and terrestrial vegetation in the diets of herbivores. The next chapter examines dietary niche variation in beavers with stable isotopic analysis. Specifically, it examines spatial and seasonal variation in aquatic and terrestrial contributions to beaver diets.

Chapter 2

Seasonal Importance of Riparian Shrubs and Aquatic Macrophytes in the Diet of Subarctic Beavers from Stream and Pond Habitats

Manuscript in preparation for submission to *Oikos*

Authors:

Heather E. Milligan (email: heather.milligan@mail.mcgill.ca)
Natural Resource Sciences, Macdonald campus, McGill University
21 111 Lakeshore Road, Ste-Anne-de-Bellevue, QC
Canada, H9X 3V9

Murray M. Humphries (email: murray.humphries@mcgill.ca)
Natural Resource Sciences, Macdonald campus, McGill University
21 111 Lakeshore Road, Ste-Anne-de-Bellevue, QC
Canada, H9X 3V9

Abstract

Aquatic foraging may allow subarctic herbivores to persist at the northern limits of their range when their preferred terrestrial vegetation is scarce. Beavers (*Castor canadensis*) are generalist herbivores that can feed on terrestrial shrubs and aquatic macrophytes, however no studies have investigated the aquatic contribution to their diet during the winter when they live under the ice. We used carbon and nitrogen stable isotope analysis, which can differentiate freshwater and terrestrial dietary sources, to investigate spatial and seasonal dietary variability in a population of beavers from northern Quebec, Canada. We surveyed freshwater and terrestrial vegetation from stream and pond habitats near beaver colonies and collected vegetation and beaver hairs for isotopic analysis. Pond habitats had four times more aquatic vegetation than stream habitats. Stream habitats had more than twice as many terrestrial shrubs than pond habitats. Isotopic mixing models estimated the dietary contribution of aquatic macrophytes for beavers was approximately 60 to 80%. During the seasonal change from autumn to winter, beavers from ponds consumed more aquatic vegetation than beavers from streams which relied more heavily on food caches of terrestrial shrubs. Our study is the first to examine dietary variability between beavers from stream and pond habitats and to quantify aquatic feeding during the winter. Aquatic foraging may enable beavers to persist at the northern periphery of their range by reducing foraging pressure on the subarctic terrestrial ecosystem where their preferred tree species are scarce. Intrapopulation variability in aquatic and terrestrial foraging implies that beavers from stream and pond habitats play differential roles in mediating nutrient transfers across freshwater and terrestrial systems in the boreal forest.

Introduction

High latitude terrestrial ecosystems are characterized by low primary productivity and extreme seasonality. The growing season for vegetation is short and interspersed by prolonged periods of snow and ice cover that last most of the year. Within the boreal forest, which is the predominant subarctic biome, a vast majority of the biomass present in the ecosystem consists of coniferous trees and mosses, both of which are slow-growing and low in nutrients (Larsen 1980; Shurin et al. 2006). Thus, the conditions in boreal forests make it a difficult existence for many herbivores.

Boreal forests do offer herbivores an abundance of freshwater ecosystems, which are important for boreal productivity and diversity for several reasons. Rivers and lakes create riparian habitats that promote the growth of understory shrubs and other vascular plants, which are the preferred terrestrial forage of most boreal herbivores (reviewed in Naiman and Décamps 1997). Freshwater ecosystems are characterized by thermal inertia (remaining warmer than the surrounding air in winter and cooler than surrounding air in summer), which creates attractive thermal conditions for flora and fauna capable of living on or in the water that could not withstand the temperature variability on land (Cyr and Cyr 2003). Due to the higher density of water than air, aquatic plants can allocate less biomass to structural support and more to photosynthetic tissues, rendering aquatic plants with faster growth rates and potentially higher nutritional values to herbivores than many terrestrial plants (Lodge 1991; MacCracken et al. 1993; Cebrian and Lartigue 2004; Shurin et al. 2006). Consequently, herbivory rates are generally higher in aquatic ecosystems than terrestrial ecosystems (Cyr and Pace 1993; Cebrian and Lartigue 2004; Shurin et al. 2006) and freshwater vegetation may be vital for the diet of several boreal herbivores.

The North American beaver (*Castor canadensis*) is a generalist herbivore that blurs the boundary between freshwater and terrestrial ecosystems by flooding forests and dragging terrestrial vegetation into the water. Beavers are large, amphibious rodents that inhabit streams and ponds ranging from Mexico to the arctic tundra (Novakowski 1967; Jenkins and Busher 1979). Beavers thus occupy a wide range of habitats including tropical ecosystems, which offer a wide array of trees, to the forest-tundra transition at the southern boundary of the arctic, where only patchily distributed, stunted, coniferous trees are present (Aleksiuk 1970; Dennington and Johnson 1974; Belovsky 1984). The ability of beavers to 'engineer ecosystems' by building dams and felling trees allows them to adapt to a wide range of conditions (Rosell et al. 2005). Habitat and diet selection studies of beaver typically identify hydrology as a critical physical feature and terrestrial vegetation as a critical trophic feature of suitable beaver habitat (Jenkins and Busher 1979). In particular, studies of beaver diet selection usually survey cut and uncut stems of shrubs and trees in the vicinity of beaver colonies to establish preferred and non-preferred types of terrestrial vegetation. These studies have repeatedly shown that beaver prefer deciduous over coniferous vegetation and, where available, prefer deciduous trees and shrubs close to the shore (Dennington and Johnson 1974; Jenkins and Busher 1979; Belovsky 1984; Donkor and Fryxell 1999).

During autumn, northern populations of beavers gather branches and twigs of shrubs and trees into a pile beside their lodge so that they can access a food cache from under the ice during the winter (Novakowski 1967; Aleksiuk 1970; Slough 1978; Jenkins and Busher 1979). The long winter is thought to be a limiting factor for many northern beavers. In some northern populations, winter food caches do not appear to contain enough energy to sustain the colony (Novakowski 1967; Aleksiuk and Cowan 1969), leading to speculation about possible behavioural (e.g., huddling, augmentation of lodge insulation) and physiological adaptations (e.g., facultative heterothermy, metabolic depression, augmentation of fur insulation) that would allow beavers to survive the winter on limited rations of terrestrial vegetation (Novakowski 1967; Aleksiuk and Cowan 1969; Smith et al. 1991). Northern beavers also frequently relocate to unoccupied territories when, as a result of overexploitation, there is insufficient terrestrial forage to sustain the colony (Aleksiuk 1970).

Aquatic macrophytes could be an important alternative to terrestrial vegetation in the diet of beavers, particularly at high latitudes where the local abundance of preferred deciduous tree species is often limited, and during winter when access to terrestrial vegetation is restricted by ice cover. Aquatic foraging may also reduce the costs associated with foraging since beavers expend significant amounts of energy while on land felling trees where they are more susceptible to predators (Belovsky 1984; Doucet and Fryxell 1993; Basey and Jenkins 1995). However, because aquatic foraging by beavers is much more difficult to observe and quantify than their terrestrial foraging, particularly during winter, very few studies have evaluated the importance of aquatic vegetation in the diet of beaver. The few studies that have observed beavers foraging indicate that herbivory on aquatic macrophytes can be extensive (Aleksiuk 1970; Northcott 1972; Jenkins and Busher 1979; Svendsen 1980; Ray et al. 2001) and an exclosure study suggests beavers can remove 60% of the aquatic biomass (Parker et al. 2007). Captive beavers preferred pond lily leaves (*Nymphaea odorata*) to all other forage types except aspen (*Populus tremuloides*) in a feeding experiment (Doucet and Fryxell 1993). Several accounts report beavers hoarding the rhizomes of pond lilies (*Nymphaea* and *Nuphar* spp.) in their food caches, however their overall contribution in the winter diets is unknown (Dennington and Johnson 1974; Slough 1978; Jenkins and Busher 1979; Ray et al. 2001).

The analysis of stable isotope ratios is a method that allows researchers to quantify the seasonal contribution of terrestrial and aquatic sources in the diets of animals. Naturally occurring carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes provide a tool to trace nutrients flows across ecological boundaries in food webs (Peterson and Fry 1987). Freshwater plants are predicted to have more positive and variable $\delta^{13}C$ and $\delta^{15}N$ values than terrestrial vegetation (France 1995; Finlay and Kendall 2007). The isotopic signatures of animal tissues provide an integrated record of their diet that could differentiate freshwater and terrestrial dietary sources and provide a measure of dietary variability (Rounick and Winterbourne 1986; France 1995; Bearhop et al. 2004). Aquatic macrophytes that are potential food items of beavers were shown to consistently have more positive $\delta^{13}C$ and $\delta^{15}N$ values than terrestrial deciduous plants (Chapter 1). Thus, the contribution of aquatic and terrestrial plants in the diets of beavers can be estimated with isotopic dietary models (Phillips and Gregg 2003).

Our objective was to examine spatial and seasonal dietary niche variability in a population of beavers from northern Quebec, Canada. We hypothesize that beavers are able to persist in subarctic regions with low quality terrestrial vegetation by foraging on available aquatic macrophytes, particularly in pond habitats during winter. We predict that beavers from pond habitats feed on more aquatic vegetation than beavers from stream habitats since aquatic vegetation should be more abundant in the former than the latter (Northcott 1972). Furthermore, we predict beavers will rely on aquatic vegetation more during winter than autumn, because their access to terrestrial vegetation in winter is restricted to their food cache by ice cover. To test our predictions, we first surveyed freshwater and terrestrial vegetation near beaver colonies to document whether stream and pond habitats differ in available food sources. Then we compared the stable isotope signatures of beaver hair samples collected from stream and pond habitats during autumn and winter, in relation to isotopic signatures of aquatic and terrestrial vegetation collected in the same habitats (Chapter 1). In order to estimate the seasonal dietary contribution of aquatic vegetation, we used isotopic mixing models (IsoSource; Phillips and Gregg 2003). To our knowledge, we are the first to examine dietary variability in beavers from stream and pond habitats and to quantify the aquatic diet of beavers during the winter.

Methods

Study Area

Our study was conducted within the traditional territory of the Wemindji Cree First Nation, a 5000 km^2 network of rivers and lakes in eastern James Bay, Quebec (53° 0' N, 78° 48' W). Wemindji is located about 350 km south of the northern range limit of beavers in eastern Quebec (Jarema et al. *in press*). The daily minimum temperature near Wemindji is above the freezing point for approximately four months of the year (129 days; Environment Canada 2008). The terrestrial vegetation is composed primarily of black spruce (*Picea mariana*) lichen forests with the understory consisting mainly of ericaceous, alder (*Alnus* spp.) and willow shrubs (*Salix* spp.; Dignard et al 1991). Freshwater ecosystems are a network of streams, rivers, ponds, marshes, and lakes with headwaters approximately 100 km inland that drain into James Bay. Freshwater ecosystems are shallow, darkly stained (Secchi depth 1 m), and slightly acidic (pH 5.5).

Aquatic macrophytes are emergent and submergent consisting primarily of water sedges (*Carex aquatilus* and *C. utriculata*), pond lilies (*Nuphar variegatum*), burreeds (*Sparganium* spp.), and horsetail (*Equisetum fluviatile*). We use the term stream and pond to describe freshwater habitats with flowing water (streams and rivers) and standing water (ponds, marshes, and lakes), respectively.

Beaver Forage Plant Surveys

As central place foragers, beavers concentrate most of their foraging activities near their lodge (Basey and Jenkins 1995). We surveyed freshwater vegetation by canoe in the vicinity of active beaver lodges in stream (*n* = 23) and pond (*n* = 25) habitats during July and August 2006 and 2007. We surveyed aquatic vegetation cover within 50 m of the lodge by establishing 10 transects running perpendicular to the shore every five metres. We recorded the percent cover of plant species visible from the water surface within 1 x 5 m plots at five metre intervals from shore until no more aquatic vegetation grew, we reached halfway of the opposite shore, or we reached 50 m (Fig. 1). We judged the borders of the plots by the length by our canoe (five metres) and one metre markings on the gunwale. We used the same survey design to sample aquatic vegetation along the shoreline at 1 km (both sides of the stream) or 500 m (pond) in both directions from the lodges in order to have 3 to 4 replicate surveys per beaver colony.

We also surveyed terrestrial vegetation near active beaver lodges in stream (*n* =12) and pond (*n* =27) habitats. We recorded vegetation cover in 2 m radius plots from the shoreline up to 60 m inland at 20 m intervals with three replicates located 20 m apart from each other (Fig. 1). We only surveyed up to 60 m inland because beavers are known to concentrate most of their terrestrial foraging near the shoreline due to the risk of predation on land (Fryxell 2001). We also surveyed terrestrial vegetation at 1 km (stream) or 500 m (pond) along the shoreline in both directions from the lodges to have 2 to 3 replicate surveys per beaver colony.

Isotopic Sample Collection

In order to investigate spatial and temporal variability in beaver diets with $\delta^{13}C$ and $\delta^{15}N$ isotopic analysis, we collected samples of forage plants and beaver hairs from stream and pond habitats. We collected the portions of aquatic and terrestrial plant species that are typically consumed by beavers, such as the leaves, bark, and rhizomes during the summer months of 2006 and 2007. Freshwater plants from stream and pond habitats in Wemindji were shown to have more positive $\delta^{13}C$ and $\delta^{15}N$ signatures compared to terrestrial plants (Fig 2; for more detail see Chapter 1). These isotopic signatures within plant species were fairly consistent across stream and pond habitats, however aquatic macrophytes had more variable $\delta^{13}C$ signatures in ponds. We also collected beaver hair samples from trappers. Beavers were trapped from mid October 2006 to mid March 2007. Trappers provided information of trapping date and habitat characteristics (pond, river, stream, lake, etc). Since the diet-to-hair fractionation (changes in isotopic signatures due to digestion, metabolism, and assimilation) values for beavers are not established and most fractionation values from the literature are based on muscle tissues, we also collected frozen beaver heads from trappers in Wahnapitae, Ontario (46° 45’ N, 80° 45’ W) in 2007 to measure isotopic variability between muscle and hair tissues in the same individuals.

Isotopic Sample Preparation

Beavers have one annual moult during the late summer and then begin to grow a new coat in the late autumn that grows continuously throughout the winter (Ling 1970). Assuming the coat grows at a relatively constant rate, the base portion of the hair represents the most recent dietary assimilation. We cut beaver guard hairs at 8 to 10 mm from the skin and retained the base portion of the hair (roughly half to two thirds of the average hair length) to sample their more recent diet with stable isotope analysis. For those beavers trapped from mid October to December, we estimate that the hair samples represent autumn diet (during the period that stream and lake ice develops). We estimate that samples from January to mid March represent winter diet (during the period that beavers live under the ice). To clean hair of debris, we sonicated samples for 30 minutes in de-ionized water followed by a rinse. We then soaked hair in 2:1 chloroform:methanol overnight, followed by a rinse in de-ionized water, to remove remaining oils. We dried samples at 50 °C for 48 hours and cut the hairs into a fine powder with scissors. To prepare beaver muscle tissues for stable isotope analysis, we dried frozen beaver muscle tissues at 50 °C for 48 hours and ground them into a fine powder with a mortar and pestle. The muscle tissues represent an integrated record of beaver diet from approximately the last several weeks of foraging (Darimont and Reimchen 2002).

Isotopic Analyses

We sub-sampled approximately 0.2 mg of hair and muscle samples for analysis on continuous-flow isotope ratio mass spectrometry at the Stable Isotopes in Nature Laboratory, University of New Brunswick, with a Carlo Erba NC 2500 interfaced with a Thermo-Finnigan Delta Plus Mass Spectrometer. Isotopic signatures are expressed in delta notation (δ) as ratios relative to PeeDee Belemnite carbonate (carbon) and atmospheric N_2 (nitrogen) standards as follows: $\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$, where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The typical precision obtained by repeated analyses of primary standards was <0.10‰ for $\delta^{13}C$ and <0.14‰ for $\delta^{15}N$. A subset of samples (n =8) were analyzed twice with an average difference of 0.19‰ for $\delta^{13}C$ and 0.10‰ for $\delta^{15}N$. We use the term enriched to describe isotopic signatures that are more positive and the term depleted to describe isotopic signatures that are more negative.

Statistical Analyses

We calculated the average percent cover of freshwater and terrestrial plants at each beaver colony. Since proportional data is not normally distributed, we then we compared the mean arcsine square root transformation of those values between stream and pond habitats with a series of *t*-tests. To investigate spatial variability in beaver diets, we compared $\delta^{13}C$ and $\delta^{15}N$ values of beaver hair between stream and pond habitats with *t*-

tests. We investigated seasonal variability in beaver diets from stream and pond habitats by comparing isotopic values of hair from autumn and winter with *t*-tests. We tested for individual isotopic variation among hair and muscle tissues with paired *t*-tests. For all our analyses, we tested for equality of variances using Levene's test. All distributions were normal with a Kolmogorov-Smirnov test. All tests were performed using a combination of SPSS 11 and SYSTAT 11.

Beaver Dietary Modeling

To identify the contribution of riparian shrubs and aquatic macrophytes in the diets of beavers, we used isotopic mixing models (IsoSource; Phillips and Greg 2003). Given that potential dietary sources are distinct in their isotopic signatures, these models assess the similarity in isotopic composition among an animal's tissues and its food sources after accounting for diet-to-consumer fractionation. Inputs to these models include the isotopic values of dietary sources and the consumers. We included the two most common terrestrial and aquatic forage plants for beavers as isotopic sources in our dietary model: alder bark and leaves, willow bark and leaves, water lily roots, and water sedge leaves (Novakowski 1965 and 1967; Aleksiuk 1970; Slough 1978; Doucet and Fryxell 1993; Samson *unpublished data*; *personal observation*). In our IsoSource models, we examined all possible combinations of the models using source increments of 1‰ and mass balance tolerance values of 0.1%, which incorporate uncertainty to the models with a magnitude similar to measurement error and source variability in isotopic values (Phillips and Greg 2003).

We adjusted beaver hair isotopic values to account for diet-to-consumer fractionation. To determine the fractionation value, we measured the individual variability between hair and muscle tissues (Table 1). Since we observed a significant muscle-to-hair enrichment in $\delta^{13}C$ but not $\delta^{15}N$ values, we adjusted our fractionation values by +1.1‰ for $\delta^{13}C$ and 0‰ for $\delta^{15}N$ for muscle-to-hair fractionation. We then incorporated diet-to-muscle fractionation values for mammalian herbivores based on meta-analyses of captive rearing experiments from the literature (+1.3‰ for $\delta^{13}C$ and +3.0‰ for $\delta^{15}N$; McCutchan et al.

2003; Vanderklift and Ponsard 2003). Therefore we subtracted hair values by 2.4‰ for $\delta^{13}C$ and 3.0‰ for $\delta^{15}N$ to account for dietary fractionation in our models. These diet-to-hair fractionation values are also very similar to a controlled feeding study of another boreal rodent (red-backed voles; *Clethrionomys gapperi*) fed a medium protein level herbivore diet (+2.4‰ for $\delta^{13}C$ and +2.8‰ for $\delta^{15}N$; Sare et al. 2005).

Results

Aquatic and terrestrial vegetation availability

Aquatic vegetation near beaver colonies was nearly four times more abundant in pond habitats than stream habitats (Fig. 3; $t=3.91_{46}$; $P<0.001$). The mean vegetation cover for all aquatic plants from 0 to 50 m from the shoreline at pond sites was 4.6% (±5.5% SD), whereas stream sites had 1.2% cover (±2.0% SD). Ponds had slightly more vegetation cover than streams from 0 to 5 m from the shoreline ($t=2.07_{46}$; $P<0.05$), where the predominant pond plants were water sedges (11.1%), pond lilies (1.7%), burreeds (1.1%) and horsetails (0.7%) and the predominant stream plants were water sedges (4.8%) and burreeds (3.6%). Ponds had more vegetation cover than streams at all other five metre intervals from 5 m to 50 m from the shoreline (*t*-tests=$P<0.001$). Water sedges and burreeds were dominant in both streams and ponds from 5 to 15 m and pond lilies were dominant in ponds from 5 to 50 m from the shoreline. Aquatic vegetation was more variable in ponds than streams from 5 to 50 m from the shoreline (Levene's tests=$P<0.05$).

Terrestrial vegetation commonly found in the diets of beavers was more abundant around beaver colonies from stream habitats compared to ponds habitats (Fig. 4). Alder shrubs were nearly three times more abundant beside stream shorelines than pond shorelines ($t=3.81_{37}$; $P<0.001$) but were similarly abundant further inland from the two types of water bodies (*t*-tests=$P>0.20$). Willow shrubs were nearly twice as abundant within 40 m of the shoreline of streams compared to ponds. That relationship approached significance at the shoreline ($t=1.89_{37}$; $P=0.067$), was significant 20 m inland ($t=3.12_{37}$; $P<0.01$), and not significant further inland (*t*-tests=P>0.11). Other forage species (including less common shrubs and herbaceous plants such as aspen: *Populus tremuloides*; dwarf birch: *Betula pumila*; cherry: *Prunus pensylvatica*; fireweed: *Chamerion angustifolium*; cow parsnip: *Heracleum maximum*; and grasses and sedges) were similar between habitats (*t*-tests=P>0.12). Pond habitats had less shrub cover than streams due to a tendency for more coniferous tree and ericaceous shrub cover at the shoreline (t=2.76$_{37}$; P<0.01) and 20 m inland (t=1.98$_{37}$; P<0.06; other *t*-tests=P<0.18).

Spatial variability in beaver δ^{13}C and δ^{15}N values

Beaver hairs from pond habitats were enriched and more variable in their δ^{13}C values than from stream habitats (Fig. 4; t=3.61$_{87}$; P<0.001 and Levene's test: F=7.52$_{95}$; P<0.01), which suggests a more aquatic dietary signal. The mean δ^{13}C values for pond (*n* = 53) and stream (*n* = 44) beaver hair samples were -24.2‰ (±0.8‰ SD) and -24.7‰ (±0.5‰ SD). Beaver hairs from pond and stream habitats were similar in their mean δ^{15}N values of 2.8‰ (±1.0‰ SD) and 3.0‰ (±0.9‰ SD), respectively (*t*-test: P=0.482 and Levene's test: P=0.579).

Seasonal variability in beaver δ^{13}C and δ^{15}N values

Beaver hairs from stream habitats became depleted in their δ^{15}N values from autumn to winter, which suggests that they shift towards a more terrestrial diet during the winter (Fig. 6 and Table 3). Beaver hairs from pond habitats had a tendency to become enriched in their δ^{13}C values and more variable in their δ^{15}N values from autumn to winter (Table 3). Although this relationship in δ^{13}C was not significant, it suggests that pond beavers shift towards a more aquatic diet during the winter. The isotopic differences between beavers from stream and pond habitats were more predominant during the winter. During the winter, both δ^{13}C and δ^{15}N values were more variable and δ^{13}C values were enriched in pond beavers compared to stream beavers (Table 3; Levene's test: δ^{13}C: F=5.83$_{60}$; P<0.05; δ^{15}N: F=6.44$_{60}$; P<0.05; *t*-test δ^{13}C: t=3.47$_{40}$; P<0.001). During autumn, the isotopic variances were similar between habitats (P>0.17), however stream beavers had enriched δ^{15}N values ($t=2.59_{33}$; $P<0.05$; all other *t*-tests=$P>0.20$). These relationships suggest that the dietary differences between stream and pond beavers are more pronounced during winter than autumn.

Beaver dietary modeling

Our isotopic mixing models indicate that subarctic beavers consume approximately 60 to 80% aquatic vegetation in their diets (Fig. 7; Table 4). Overall, our models estimate that beavers from streams and ponds feed on similar amounts of aquatic vegetation during autumn but during the winter pond beavers shift to a more aquatic diet whereas stream beavers shift towards a more terrestrial diet. Water sedges were more common in the autumn diet, especially in stream beavers, and then disappeared from the diet during the winter. Pond lilies were a major contributor to the diet of beavers, especially during the winter in pond habitats. Terrestrial willow shrubs were generally more present in the diet than alder, except during the winter in pond habitats. The inclusion of other additional common species such as aspen, burreeds, horsetails, and pondweeds (*Potamogeton* spp.) or the use of Bayesian isotopic mixing models (which take into account dietary source and fractionation uncertainty; MixSIR, Moore and Semmens 2008) altered quantitative estimates of dietary sources but did not change our general conclusion that aquatic food sources were more important in pond than stream habitats and during winter than autumn.

Discussion

Spatial and Seasonal Variability in Beaver Diets

Isotopic investigations of intrapopulation dietary variability in subarctic beavers support our hypothesis that aquatic vegetation is a significant contributor to the ability of beaver to persist in habitats of marginal terrestrial quality. We predicted that beavers from pond habitats foraged on more aquatic vegetation than beavers from stream habitats. We observed that pond beavers had more enriched and variable δ^{13}C values, which implies they consume more aquatic vegetation since δ^{13}C values of freshwater vegetation from

Wemindji are also more enriched than terrestrial vegetation and more variable in pond habitats (for more details see Chapter 1). Our second prediction, that beavers rely on aquatic vegetation more during winter than autumn because their access to terrestrial vegetation in winter is restricted to their food cache by ice cover, was only partially supported. During the seasonal shift from autumn to winter, pond beavers had enriched $\delta^{13}C$ values whereas stream beavers had depleted $\delta^{15}N$ values. These seasonal shifts suggest that aquatic vegetation became more prevalent in the diet of beavers from pond habitats in the winter, whereas beavers from stream habitats relied more heavily on their food cache of terrestrial shrubs. Beavers from ponds also appear to have more variability in their diets during the winter than beavers from streams since their $\delta^{13}C$ and $\delta^{15}N$ values were more variable (Bearhop et al. 2004). Our results indicate that the inclusion of aquatic vegetation in the winter diets of beavers from pond habitats enables them to feed on a variety of plants and possibly exist in areas of lower terrestrial quality compared to beavers from stream habitats.

Our field surveys indicate that stream and pond habitats offer a tradeoff for beavers between abundant terrestrial forage along streams and abundant aquatic forage within ponds. Stream habitats in Wemindji territory had approximately two times more terrestrial shrubs and four times less aquatic macrophytes available to beavers compared to pond habitats. Willow and alder, the predominant shrubs in the area, were more abundant in riparian areas surrounding streams. Water sedges and other emergent macrophytes were present in both habitats, but more abundant in ponds. Most of this aquatic vegetation would not be available to beavers during the winter due to fast decomposition rates (Cebrian and Lartigue 2004). The only aquatic macrophytes that are likely to be abundant during the winter grew only in ponds and were pond lilies, whose large rhizomes (4 to 10 cm in diameter) grow in muddy substrate (Northcott 1972). This lack of available aquatic vegetation in streams during the winter likely explains why our isotopic analyses indicate beavers from streams relied more heavily on their winter food cache of shrubs. In ponds, pond lilies typically grew in large beds within 100 m of the lodge, which could possibly be within diving distance from the lodge and available to beavers under the ice during the winter. This might explain the prevalence of pond lilies in our dietary models when other researchers have only occasionally observed pond lilies rhizomes in the food caches (Dennington and Johnson 1974; Slough 1978; Jenkins and Busher 1979; Ray et al. 2001). Overall, our field surveys verified that stream and pond habitats differ in their available food resources for beaver, with ponds being abundant in aquatic vegetation and impoverished in terrestrial vegetation compared to streams.

Beaver Dietary Modeling

Our isotopic dietary models estimated that aquatic plants contributed to approximately 60 to 80% of the diet of beavers, with the lower values of the range from the winter diets of stream beavers and the higher values from the winter diets of pond beavers. Other researchers that examined aquatic herbivory in beavers found similar magnitudes. Svendsen (1980) conducted observational studies during the summers in Ohio and reported that beavers spent up to 50% of their foraging time feeding on aquatic macrophytes. Beaver herbivory also reduced the aquatic biomass by 60% in an experiment that excluded beaver colonies from aquatic vegetation (Parker et al. 2007). These findings are contrary to optimal dietary models based on energetic constraints which predicted that beavers from pond habitats are primarily terrestrial herbivores (Belovsky 1984; Doucet and Fryxell 1993). Belovsky (1984) predicted that aquatic vegetation comprised only 12 to 24% of the dry weight of daily summer energy requirements. Those values would likely appear higher had those percentages been based on the nutritional values of aquatic macrophyte rhizomes instead of leaves, since the rhizomes are thought to be more nutritionally valuable than the leaves (Lodge 1991; Doucet and Fryxell 1993).

The differences in proportions among plant species that were generated by our dietary models correlate well with previous dietary preference studies. Pond lilies were the predominant species in our dietary models. Captive beavers preferred pond lily leaves to all other forage types except aspen in a feeding experiment (Doucet and Fryxell 1993). Our model estimates for pond lilies are likely a bit high, considering we rarely observed pond lilies in stream habitats during our surveys. Our models do estimate that pond beavers consume approximately 25% more pond lilies than stream beavers during both seasons. Water sedges were also a major contributor in the autumn diet in stream habitats. Other northern populations fed extensively on water sedges during the summers (Novakowski 1965). When pond lilies are unavailable in the habitat, water sedges may be an important macrophyte. Our dietary models also estimated that beavers from streams fed on more willow than alder shrubs. These dietary preferences closely match other northern studies where the majority of stems cuts were willow followed by alder (Novakowski 1967; Aleksiuk 1970; Slough 1978). Beavers also consume more of the willow than the alder from their winter food caches (Dennington and Johnson 1974; Slough 1978). Aspen is known as the preferred forage for northern beavers (Jenkins and Busher 1979), however, we only observed it at 30% of our sites during our surveys and less than 5% of the stems cut by beavers were aspen (Samson *unpublished data*).

Although the results of our dietary models correlate well with previous dietary studies, there are several limitations to the approach of dietary modelling with stable isotopes analysis. Foremost, the fractionation value from diet-to-hair is an estimate and could vary with protein content in the diet (Gannes et al. 1997; Sponheimer et al. 2003a and b; Sare et al. 2005). Riparian shrubs and aquatic macrophytes can differ in their nutrient contents and structural composition (Lodge 1991; MacCracken et al. 1993; Shurin et al. 2006) and may be digested, metabolized, or assimilated differently by beavers (Belovksy 1984; Doucet and Fryxell 1993; Gannes et al. 1997). This possible variability in our fractionation values adds a level of uncertainty in our model, however Bayesian models that account for this fractionation uncertainty generated similar results (MixSIR; Moore and Semmens 2008). Second, isotopic variability within dietary sources add a level of uncertainty in our model (Phillips and Gregg 2003; Moore and Semmens 2008). Although we observed considerable variability within plant species, their mean signatures were fairly consistent over repeated sampling events and in stream and pond habitats (Chapter 1) and Bayesian models that account for this variability generated similar results (MixSIR; Moore and Semmens 2008). Third, our dietary model is a simplification of possible dietary combinations (Phillips and Gregg 2003). We observed considerable heterogeneity in available vegetation at our sites and other species may have contributed substantially to the diet, which may explain why our models estimated that pond lilies were important in the diet for stream beavers when they were largely absent from the habitat. Stream beavers could be capturing another un-sampled food source, or a composite of multiple food sources with similar isotopic signatures, that are present in streams. Although our model only included four dietary sources, the inclusion of more species did not improve our resolution or change the general pattern of our results. Fourth, other factors other than diet can alter isotopic values in animals (such as physiology, age, and sex; Gannes et al. 1997; Matthews and Mazumder 2004), which we did not attempt to address in this study.

Another source of variability in our dietary models is the approximation of the time window captured in our samples. The seasons represented in the dietary record of beaver hairs are approximate estimates. Other researchers have applied similar techniques and report finding seasonal dietary shifts towards marine foraging by sectioning the hair of wolves and humans, as well as seasonal changes in the herbivory of elephants (Darimont and Reimchen 2002; Cerling et al. 2006; Knudson et al. 2007). Their methods generated more dietary resolution than sampling the entire hair. It is also possible that our vegetation samples could shift in their isotopic signatures seasonally (McArthur and Moorhead 1996; Matthews and Mazumder 2004). McArthur and Moorhead (1996) sampled riparian shrubs and aquatic macrophytes in spring, summer, and autumn and found some seasonal isotopic shifts within species at certain sites, but not at others. We were unable to sample vegetation during the winter and sampled bark and rhizomes (which are predominant in the winter diet; Aleksiuk 1970) during the summer as a proxy. The isotopic signatures of leaves and bark from willow and alder were similar and therefore we pooled their values for our analyses to meet the criteria for distinct dietary sources isotopic modeling (Gannes et al. 1997; Phillips and Gregg 2003). Although seasonal variability in plants could impede our ability to discriminate dietary sources, we found temporal consistency within species with repeated summer sampling in Wemindji territory (Chapter 1).

Implications of Aquatic Herbivory by Subarctic Beavers

The specialization on riparian shrubs and aquatic vegetation may allow beavers to persist at the northern limits of their species range, albeit at low densities. The density of beaver colonies in Quebec declines with increasing latitude, with high but variable densities across southern Quebec, a sharp decline in density at the 49th parallel, and a long tail of low density reaching as far as the 58th parallel (Jarema et al. *in review*). Wemindji is situated in this long tail of low density near the periphery of the range and near the forest-tundra transition, where preferred deciduous trees are largely absent. Harsh climates and low quality of food resources are thought be the limiting factors at these latitudes, forcing beavers to relocate more frequently because of overexploitation of forage vegetation (Aleksiuk 1970). The short growing season also means that shrubs and seedlings can take longer to reestablish than at southern latitudes and thus take longer before the locality can support a recolonization by beavers. Pond sites may remain occupied longer due to aquatic herbivory and the local depletion of terrestrial shrubs that we observed may reflect longer occupancy periods than for stream localities. Conversely, stream sites may have longer occupancy periods due to the natural abundance of shrubs and beavers can persist in pond sites of marginal terrestrial quality only with the inclusion of aquatic herbivory. In Alaska, the aquatic ecosystem was four times more productive for moose than the terrestrial ecosystem (MacCracken et al. 1993). Aquatic biomass was a better predictor of beaver densities than terrestrial biomass in a population in northern Ontario (Fryxell 2001). A comparison of site occupancy periods in stream and pond habitats may provide further insight into the role of aquatic and terrestrial vegetation in dictating the ability of beavers to persist in marginal habitats.

Aquatic foraging may allow beavers to minimize the energetic costs of foraging and enhance their overwinter survival. The cutting of trees and shrubs requires more time and energy compared to aquatic macrophytes (Belovsky 1984). Therefore aquatic foraging may offer a greater net gain in energy intake. The nutrient values of aquatic macrophytes are often similar to terrestrial forage and in some cases, their mineral contents are higher (Belovsky 1984; Lodge 1991; Doucet and Fryxell 1993; MacCracken et al. 1993; Shurin

2006). Belovsky (1984) and Doucet and Fryxell (1993) concluded that during the summer, beavers preferred terrestrial vegetation, in order to maximize their energetic gains, instead of an aquatic diet, which would minimize their time spent foraging. In autumn, beavers spend a significant amount of their time cutting trees to prepare their food caches (Jenkins and Busher 1979) and aquatic vegetation may offer a quick meal. In winter, the bark from the winter food caches are low in nutrients (Aleksiuk 1970) and fresh aquatic rhizomes may offer important nutrients that reduce the chances of winter starvation for beavers living in pond habitats. Since ponds are typically covered in ice for a longer period than streams and have fewer riparian shrubs, beavers may have difficulty hoarding sufficient amounts of terrestrial vegetation for the winter. Adult beavers in northern climates undergo a significant weight loss during the winter and beavers modify their behaviour and physiology to conserve energy (Novakowski 1967; Aleksiuk and Cowan 1969; Smith et al. 1991). Thus, aquatic vegetation may offer an important secondary food source that enhances their overwinter survival.

Aquatic foraging may also reduce the risk of predation for subarctic beavers. Beavers are not as mobile and cannot detect predators as easily on land as in the water. Predation is a strong selective agent that affects the foraging behaviour of animals and often determines where and what they forage on (reviewed in Lima and Dill 1990). Many animals will forage on less profitable items if they can be consumed in relative safety (Lima and Dill 1990). Beavers are conservative with their foraging on land and typically restrict their terrestrial foraging to within 50 m of the shoreline and will only travel further inland for their preferred trees and shrubs (Donkor and Fryxell 1999). Although several studies have examined their terrestrial foraging behaviour as it relates to predation, none have examined their aquatic foraging (Belovksy 1984; Basey and Jenkins 1995; Donkor and Fryxell 1999). The risk of predation may potentially be higher in individuals within the population that spend more time foraging on land. Svendsen (1980) observed that juvenile beavers spent more time closer to the lodge and consequently foraged on more aquatic macrophytes than adults. Beavers from streams and ponds could vary in the amount of time they spend foraging on land and may have differential rates of predation associated with foraging behaviour.

As flexible herbivores that can switch feeding between aquatic and terrestrial habitats, beavers could play an important role affecting the stability of freshwater and terrestrial ecosystems dynamics in boreal forests. Beavers can considerably modify their environments by their foraging activity and by building dams and canals in their long-term territories (Rosell et al. 2005). As a result, beavers are coined as 'ecosystem engineers'. Beaver foraging affects succession and species composition of riparian terrestrial communities and aquatic macrophytes (Donkor and Fryxell 1999; Rosell et al. 2005; Ray et al. 2001; Parker et al. 2007). Beavers also transport a significant amount of terrestrial nutrients into the aquatic ecosystem with their food caches. The debris from food caches supply nutrients and structure to the aquatic invertebrate and vertebrate community and enhance biodiversity (Rosell et al. 2005). The role of beavers in linking terrestrial and freshwater boreal ecosystems may differ in stream and pond habitats. Beavers from streams potentially transfer more terrestrial nutrients into the aquatic ecosystem since they feed on more terrestrial vegetation during the winter. Beavers living in ponds may also be important agents of dispersal of pond lilies. The seeds of pond lilies do not appear to survive the digestion of fish and birds, however their rhizomes float once uprooted and drift to the shore where they re-establish by vegetative growth (Ray et al. 2001). Beavers frequently carry the rhizomes to feeding areas near their lodge or to their food cache (Ray et al. 2001; *personal observation*). Pond lilies can establish in poor nutrient conditions due to their rhizomes, which draw in nutrients from the sediments (Northcott 1972; Ray et al. 2001). The sediments near the lodge are rich in terrestrial nutrients from partially eaten shrubs (Rosell et al. 2005) and could provide the appropriate conditions for the vegetative growth of partially eaten rhizomes (Ray et al. 2001). Therefore, beaver foraging activities can play an important role in linking freshwater and terrestrial ecosystem dynamics in boreal forests.

Conclusion

We are the first to examine seasonal dietary variability within a subarctic population of beavers from pond and stream habitats. Our results indicate that beaver colonies from stream and pond habitats differ in the amount of riparian shrubs and aquatic macrophytes in their diets. Specifically, during the winter, pond lilies become predominant in the diets of beavers from ponds whereas beavers from streams shift towards a more terrestrial diet of riparian shrubs from their food hoards. Our dietary analyses using stable isotope reveal that aquatic macrophytes are more significant in the diets of beavers than previously shown. In boreal forests, aquatic foraging may enable beavers to persist at the northern limits of their range where trees become scarce. These results have broad implications on the dietary niche and optimal foraging of a subarctic herbivore and their role in linking freshwater and terrestrial ecosystems.

Acknowledgements

We thank the Cree Nation of Wemindji and Cree Trappers' Association of Wemindji for their permission and participation. We thank the following trappers from providing beaver samples and sharing their knowledge: L Asquabaneskum, R Atsynia, S Mayappo, B Stewart, G Stewart, H Stewart, R Stewart, and J Woods. We also thank B Linklater-Georgekish, K Matches, S Matches, M Milligan, and N Mirotchnick for fieldwork, D Brown and C Trudeau for labwork, Centre for Indigenous Peoples' Nutrition and Environment for lab space, and K Scott and C Scott for logistical support. Funding was provided by SSHRC Community-University Research Alliance and Aboriginal Research grants to Paakumshumwaau-Wemindji Protected Area Project, NCE ArcticNet, the Northern Scientific Training Program, and NSERC Northern Research Chairs program. While preparing the manuscript, HEM was supported by NSERC Post-graduate Scholarship and Association of Canadian Universities for Northern Studies Northern Resident Scholarship.

References Cited

Aleksiuk, M. 1970. The seasonal food regime of arctic beavers. – *Ecol.* **51**: 264-270.

Aleksiuk, M. and Cowan, I.M. 1969. Aspects of seasonal energy expenditure in beaver (*Castor canadensis* Kuhl) at the northern limit of its distribution. – *Can. J. Zool.* **47**: 471-481.

Basey, J.M. and Jenkins, S.H. 1995. Influences of predation risk and energy maximization on food selection by beavers (*Castor canadensis*). - *Can. J. Zool.* **73**: 2197-2208.

Bearhop, S, et al. 2004. Determining trophic niche width: a novel approach using stable isotope analysis. – *J. Anim. Ecol.* **73**: 1007-1012.

Belovsky, G.E. 1984. Summer diet optimization by beaver. – *Am. Midl. Nat.* **111**: 209-222.

Cebrian, J. and Lartigue, J. 2004. Patterns of herbivory and decomposition in aquatic and terrestrial ecosystems. – *Ecol. Monogr.* **74**: 237-259.

Cerling, T.E. et al. 2006. Stable isotopes in elephant hair document migration patterns and diet changes. – *Proc. Natl. Acad. Sci. U.S.A.* **103**: 371-373.

Cyr, H. and Cyr, I. 2003. Temporal scaling of temperature variability from lands to oceans. – *Evol. Ecol. Res.* **5**: 1183-1197.

Cyr, H. and Pace, M.L. 1993. Magnitude and patterns of herbivory in aquatic and terrestrial ecosystems. - *Nature* **361**: 148-150.

Darimont, C.T. and Reimchen, T.E. 2002. Intra-hair stable isotope analysis implies seasonal shift to salmon in gray wolf diet. – *Can. J. Zool.* **80**: 1638-1642.

Dennington, M. and Johnson, B. 1974. Studies of beaver habitat in the Mackenzie Valley and northern Yukon. - *Canadian Wildlife Service Report 74-39*.

Dignard, M. et al. 1991. Habitats of the northeast coast of James Bay. - *Canadian Wildlife Service Occasional Paper No. 70*.

Donkor, N.T. and Fryxell, J.M. 1999. Impact of beaver foraging on structure of lowland boreal forests of Algonquin Provincial Park, Ontario. – *For. Ecol. Manage.***118**: 83-92.

Doucet, C.M. and Fryxell, J.M. 1993. The effect of nutritional quality on forage preference by beavers. - *Oikos* **67**: 201-208.

Environment Canada. 2008. National Climate Archive: La Grande Rivière A, Québec [online] Available from http://www.climate.weatheroffice.ec.gc.ca/index.html

Finlay, J.C. and Kendall, C. 2007. Stable isotope tracing of temporal and spatial variability in organic matter sources to freshwater ecosystems. - In: Michener, R.H. and Lajtha, K. (eds.), *Stable isotopes in ecology and environmental science.* Blackwell Publishing, pp, 283-333.

France, R.L. 1995. Source variability in δ^{15}N of autotrophs as a potential aid in measuring allochthony in freshwaters. - *Ecography* **18**: 318-320.

Fryxell, J.M. 2001. Habitat suitability and source-sink dynamics of beavers. – *J. Anim. Ecol.* **70**: 310-316.

Gannes, L.Z. et al. 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. – *Ecol.* **78**: 1271-1276.

Jarema, S.I. et al. Variation in abundance across a species' range predicts climate change responses in the range interior will exceed at the edge: a case study with North American beaver. – *Glob. Chang. Biol. in press*.

Jenkins, S.H. and Busher, P.E. 1979. *Castor canadensis*. - Mammalian Species 120:1-8.

Knudson, et al. 2007. Seasonality and paleodiet in the Chiribaya polity of southern Peru. – *J. Archaeol. Sci.* **34**: 451-462.

Larsen, J.A. 1980. *The boreal ecosystem*. - Academic Press.

Lima, S.L. and Dill, L.M. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. – *Can. J. Zool.* **68**: 619-640.

Ling, J.K.1970. Pelage and molting in wild mammals with special reference to aquatic forms. – *Q. Rev. Biol.* **45**: 16-54.

Lodge, D.M. 1991. Herbivory on freshwater macrophytes. – Aquat. Bot. 41: 195-224.

MacCracken, J.G. et al. 1993. Use of aquatic plants by moose: sodium hunger or foraging efficiency? – *Can. J. Zool.* **71**: 2345-2351.

Matthews, B. and Mazumder, A. 2004. A critical evaluation of intrapopulation variation of δ^{13}C and isotopic evidence of individual specialization. - *Oecologia* **140**: 361-371.

McArthur, J.V. and Moorhead, K.K. 1996. Characterization of riparian species and stream detritus using multiple stable isotopes. - *Oecologia* **107**: 232-238.

McCutchan, J.H. et al. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. - *Oikos* **102**: 378-390.

Moore, J.W. and Semmens, B.X. 2008. Incorporating uncertainty and prior information into stable isotope mixing models. – *Ecol. Lett.* **11**: 470-480.

Naiman, R.J. and Décamps, H. 1997. The ecology of interfaces: riparian zones. – *Ann. Rev. Ecolog. Syst.* **28**: 621-658.

Northcott, T.H. 1972. Water lilies as beaver food. - *Oikos* **23**: 408-409.

Novakowski, N.S. 1965. Population dynamics of a beaver population in northern latitudes. - Ph.D. Dissertation. University of Saskatchewan.

Novakowski, N.S. 1967. The winter bioenergetics of a beaver population in northern latitudes. – *Can. J. Zool.* **45**: 1107-1118.

Parker, J.P. et al. 2007. Beaver herbivory on aquatic plants. - *Oecologia* **151**: 616-625.

Peterson, B.J. and Fry, B. 1987. Stable isotopes in ecosystem studies. – *Ann. Rev. Ecolog. Syst.* **18**: 293-320.

Phillips, D.L. and Gregg, J.W. 2003. Source partitioning using stable isotopes: coping with too many sources. - *Oecologia* **136**: 261-269.

Ray, A.M. et al. 2001. Macrophyte succession in Minnesota beaver ponds. – *Can. J. Bot.* **79**: 487-499.

Rosell, F. et al. 2005. Ecological impact of beavers *Castor fiber* and *Castor canadensis* and their ability to modify ecosystems. - *Mammal Rev.* **35**: 248-276.

Rounick, J.S. and Winterbourn, M.J. 1986. Stable carbon isotopes and carbon flow in ecosystems. - *BioScience* **36**: 171-177.

Sare, D.T.J. et al. 2005. Tracing dietary protein in red-backed voles (*Clethrionomys gapperi*) using stable isotopes of nitrogen and carbon. – *Can. J. Zool.* **83**: 717-725.

Shurin, J.B. et al. 2006. All wet or dried up? Real differences between aquatic and terrestrial food webs. – *Proc. - Royal Soc. B.* **273**: 1-9.

Slough, B.G. 1978. Beaver food cache structure and utilization. – *J. Wildl. Manag.* **42**: 644-646.

Smith, D.W. et al. 1991. Over-winter activity and body temperature patterns in northern beavers. – *Can. J. Zool.* **69**: 2178-2182.

Sponheimer, M. et al. 2003a. An experimental study of carbon-isotope fractionation between diet, hair, and feces of mammalian herbivores. – *Can. J. Zool.* **81**: 871-876.

Sponheimer, M. et al. 2003b. Nitrogen isotopes in mammalian herbivores: hair $\delta^{15}N$ values from a controlled feeding study. – *Int. J. Osteoarchaeol.* **13**: 80-87.

Svendsen, G.E. 1980. Seasonal change in feeding patterns of Beaver in southeastern Ohio. - *J. Wildl. Manag.* **44**: 285-290.

Vanderklift, M.A. and Ponsard, S. 2003. Sources of variation in consumer-diet $\delta^{15}N$ enrichment: a meta-analysis. - *Oecologia* **136**: 169-182.

Table 1 Beaver $\delta^{13}C$ and $\delta^{15}N$ fractionation values among muscle and hair tissues. Hair was enriched from muscle in their $\delta^{13}C$ values ($t=8.82_{12}$; $P<0.001$) but not for their $\delta^{15}N$ values ($P=0.437$).

		$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		
Tissue	*n*	Mean ± SD	Range	Δ from muscle	Mean ± SD	Range	Δ from muscle
Muscle	13	-24.7 ± 0.5	-25.9 to -24.1		3.3 ± 0.6	2.2 to 4.1	
Fur	13	-23.7 ± 0.5	-24.4 to -22.4	+1.1 ± 0.4	3.4 ± 0.7	2.0 to 4.8	+0.2 ± 0.8

Table 2 Beaver hair $\delta^{13}C$ and $\delta^{15}N$ values showing intrapopulation seasonal differences among beavers living in stream and pond habitats.

			$\delta^{13}C$ (‰)				$\delta^{15}N$ (‰)			
Habitat	Season Trapped	*n*	Mean ± SD	Range	Difference Mean ± SE	*t*-test	Mean ± SD	Range	Difference Mean ± SD	*t*-test
Stream	Autumn	10	-24.7 ± 0.5	-25.2 to -23.7	0.3 ± 0.1	$t=0.17_{42}$; P=0.866	3.7 ± 1.0	1.9 to 5.3	0.9 ± 0.3	$t=3.15_{42}$; P<0.01
	Winter	34	-24.7 ± 0.5	-25.6 to -23.7			2.8 ± 0.7	1.5 to 4.3		
Pond	Autumn	25	-24.4 ± 0.6	-25.3 to -23.3	0.4 ± 0.2	$t=1.80_{51}$; P=0.078	2.9 ± 0.7	1.6 to 4.2	0.1 ± 0.3*	$t=0.44_{51}$; P=0.665
	Winter	28	-24.0 ± 0.9	-25.2 to -21.5			2.8 ± 1.2	-0.5 to 4.6		

* Levene's Test P<0.05

Table 3 Seasonal contribution of riparian shrubs and aquatic macrophytes in the diets of beavers from streams and ponds estimated with IsoSource mixing models. The mean (± SD) isotopic values of dietary sources in the model are shown. The median (1 and 99th percentile) percent of dietary contribution is also shown.

Dietary Source	Isotopic Values (‰)			Dietary Contribution (%)			
		$\delta^{13}C$	$\delta^{15}N$	Stream		Pond	
	n	Mean ± SD	Mean ± SD	Autumn	Winter	Autumn	Winter
Alder	30	-29.5 ± 1.3	-1.8 ± 0.5	8 (0-19)	17 (1-30)	11 (0-23)	13 (6-17)
Willow	37	-28.2 ± 0.9	-0.4 ± 1.6	16 (0-35)	22 (0-52)	19 (0-42)	4 (0-17)
Pond lily	10	-25.9 ± 1.1	0.1 ± 1.0	37 (29-44)	55 (45-64)	63 (55-71)	81 (76-86)
Water sedge	25	-27.3 ± 0.7	2.2 ± 1.8	38 (31-46)	5 (0-14)	6 (0-14)	1 (0-4)

Fig.1 Sampling design of aquatic and terrestrial vegetation surveys near beaver colonies. Aquatic vegetation cover was estimated by establishing 10 transects running perpendicular to the shore every five metres. We recorded the percent cover of plant species visible from the water surface within 1 x 5 m plots (rectangles) at 5 m intervals from shore until no more aquatic vegetation grew, or we reached 50 m. Terrestrial vegetation cover was estimated in 2 m radius plots (circles) from the shoreline up to 60 m at 20 m intervals with three replicates located 20 m apart.

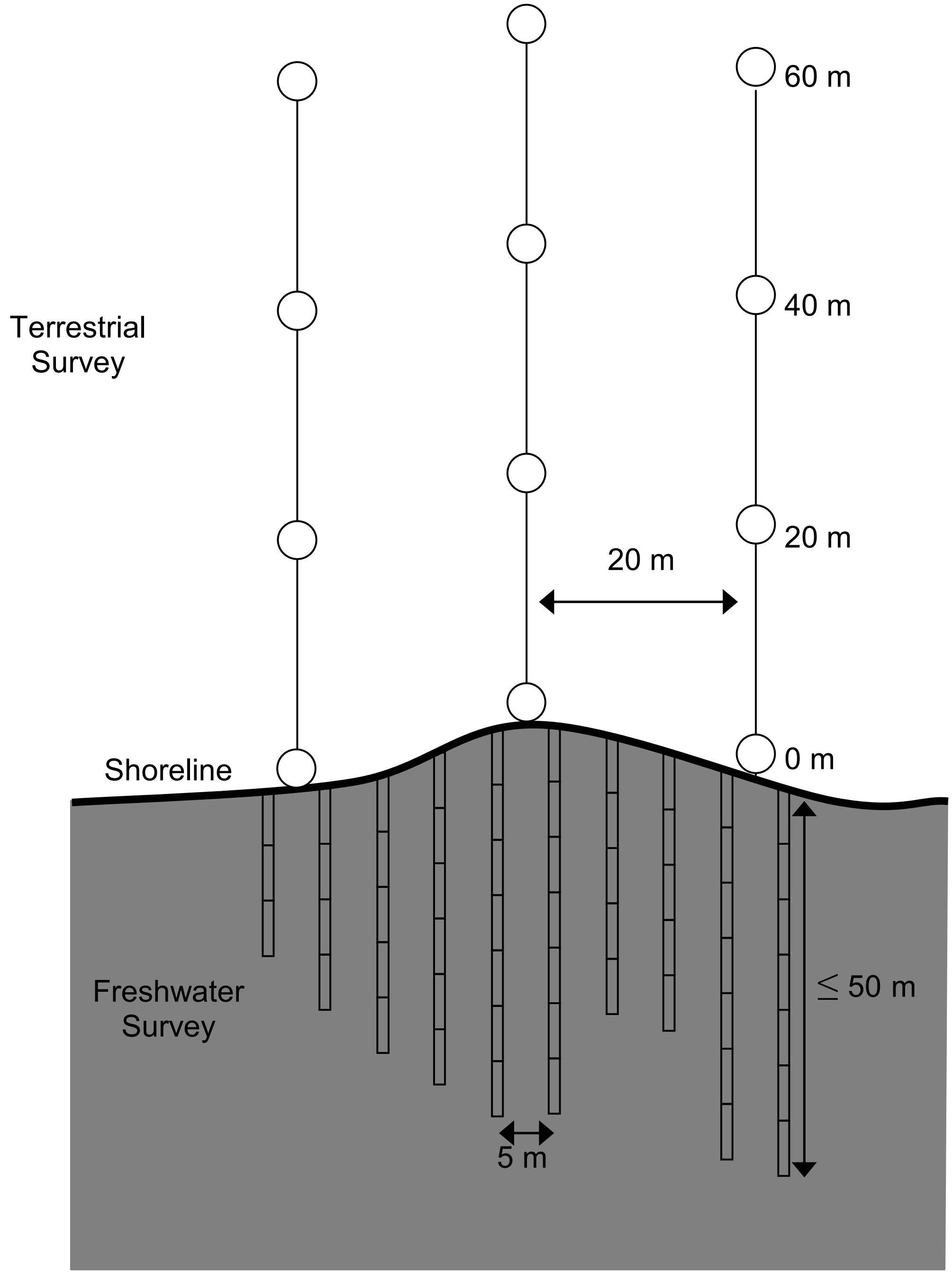


Fig. 2 Mean (± SE) δ^{13}C and δ^{15}N values for freshwater and terrestrial plants in Wemindji territory from stream and pond habitats. Dotted and solid ellipses show standard deviations for mean isotopic values of aquatic and terrestrial vegetation types, respectively. Aquatic plants had more positive δ^{13}C and δ^{15}N values than terrestrial plants. Isotopic signatures were similar between stream and pond habitats except that aquatic vegetation from pond habitats had more variable δ^{13}C values than from stream habitats (redrawn from Chapter 1).

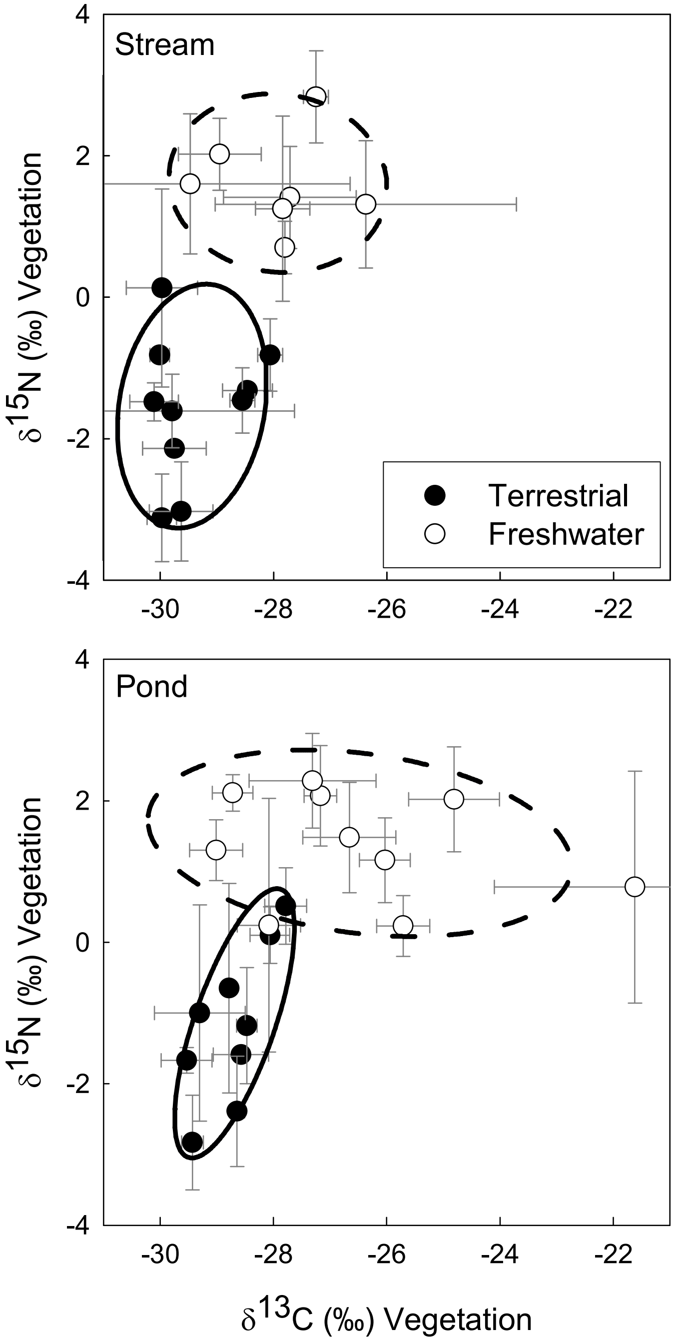


Fig. 3 Mean (± SE) percent of vegetation cover for aquatic plants at 5 m intervals from the shoreline in stream and pond habitats. The aquatic vegetation cover in stream habitats is less abundant than pond habitats. Water sedges are dominant in the littoral zone in both stream and pond habitats while pond lilies are dominant further from the shoreline of ponds.

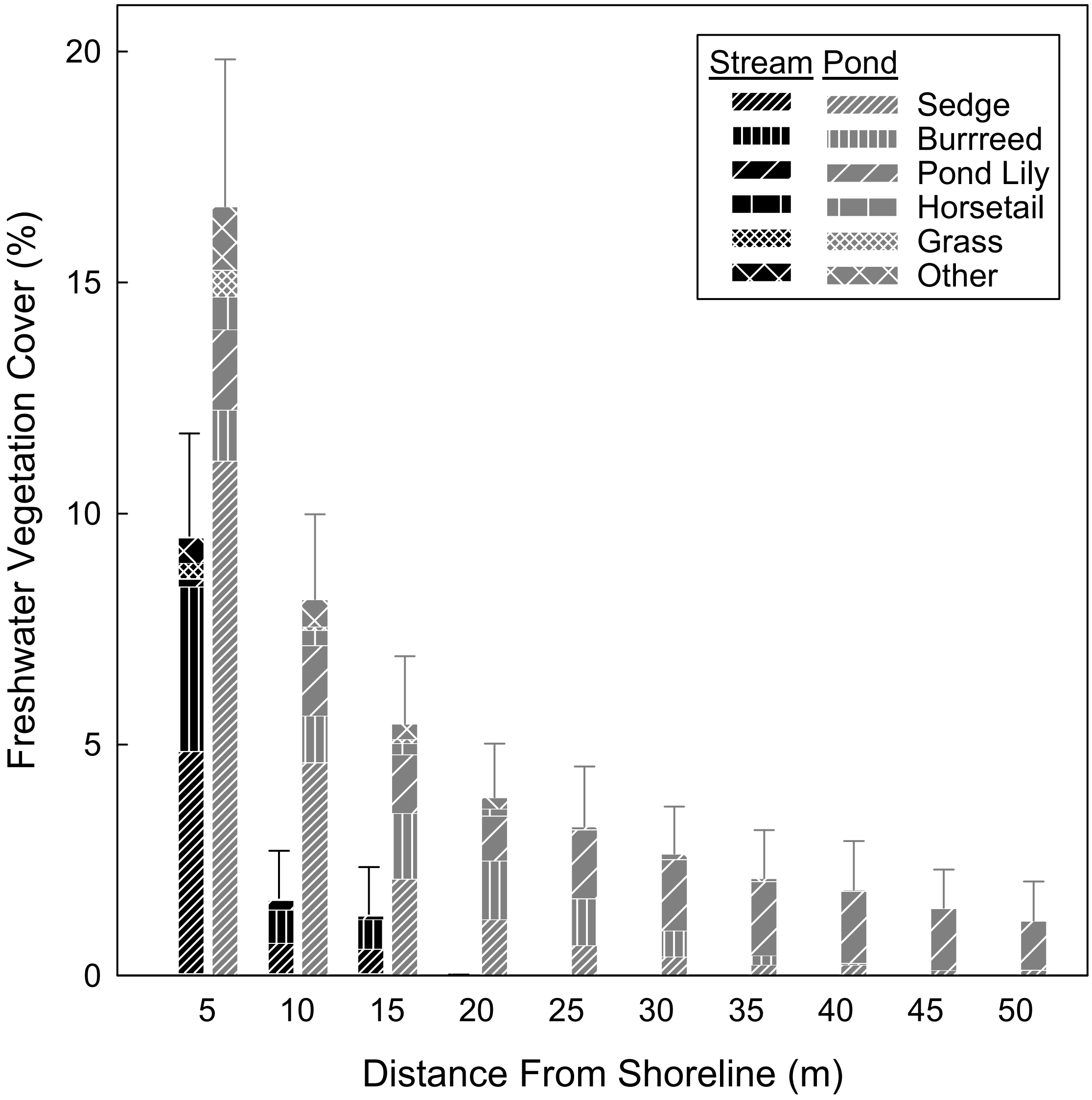


Fig. 4 Mean (± SE) percent of vegetation cover for terrestrial forage plants at 20 m intervals from the shoreline in stream and pond habitats. Alder and willow are more predominant in stream riparian habitats, whereas other forage species are similar in stream and pond habitats.

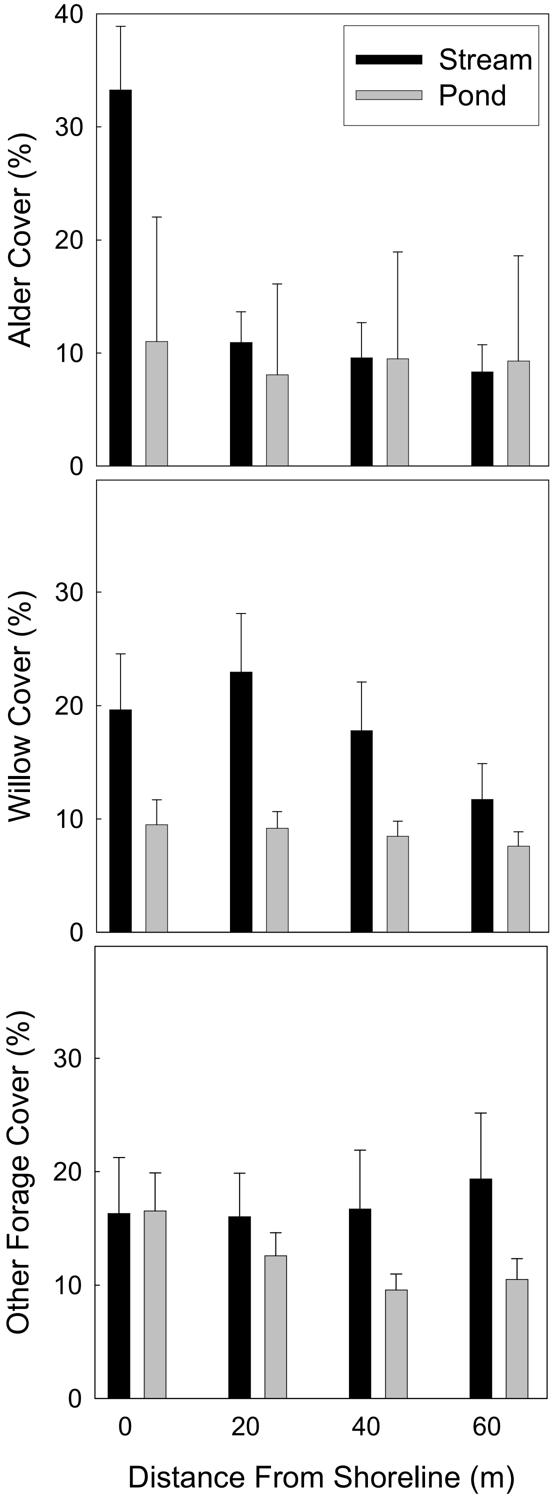


Fig. 5 Values of $\delta^{13}C$ and $\delta^{15}N$ of beaver hair from stream and pond habitats.

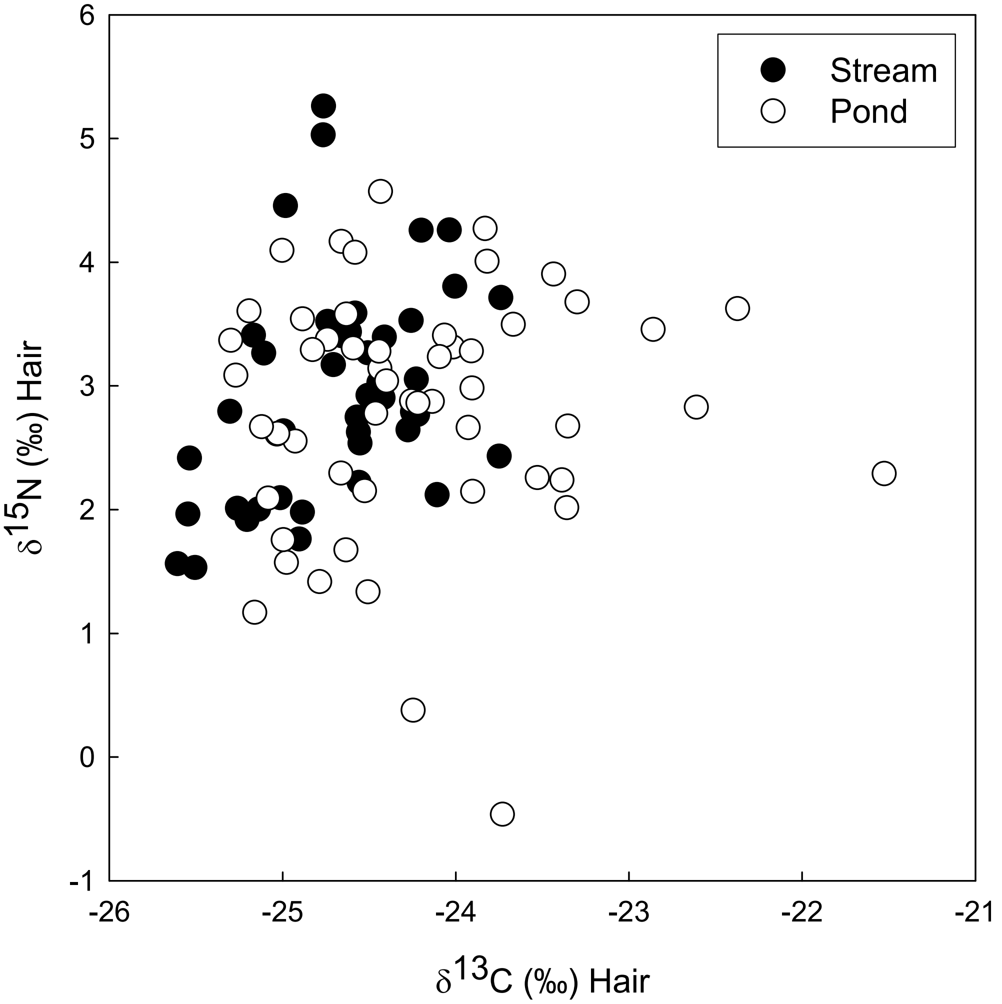


Fig. 6 Values of $\delta^{13}C$ and $\delta^{15}N$ of beaver hair from stream and pond habitats for autumn and winter seasons.

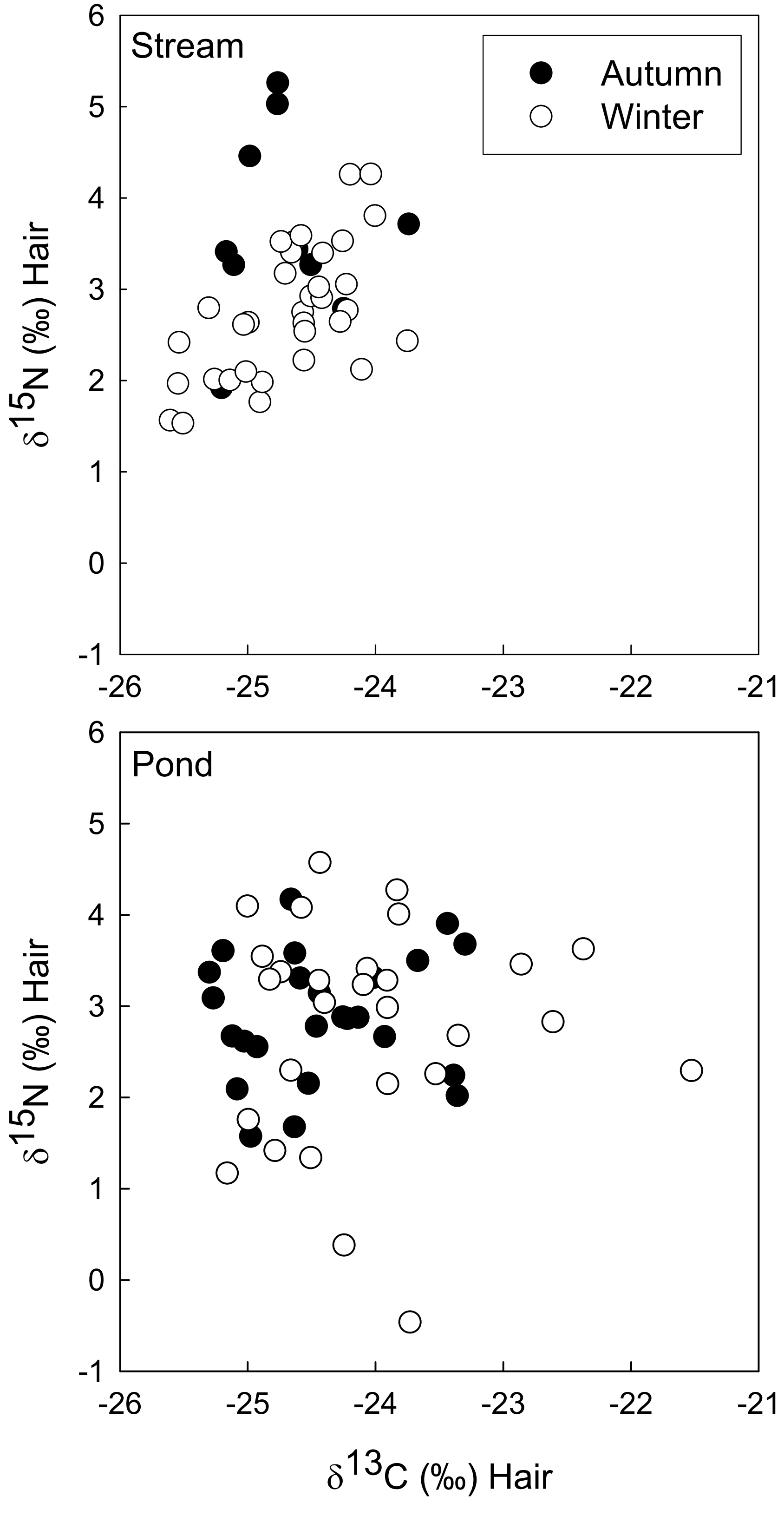
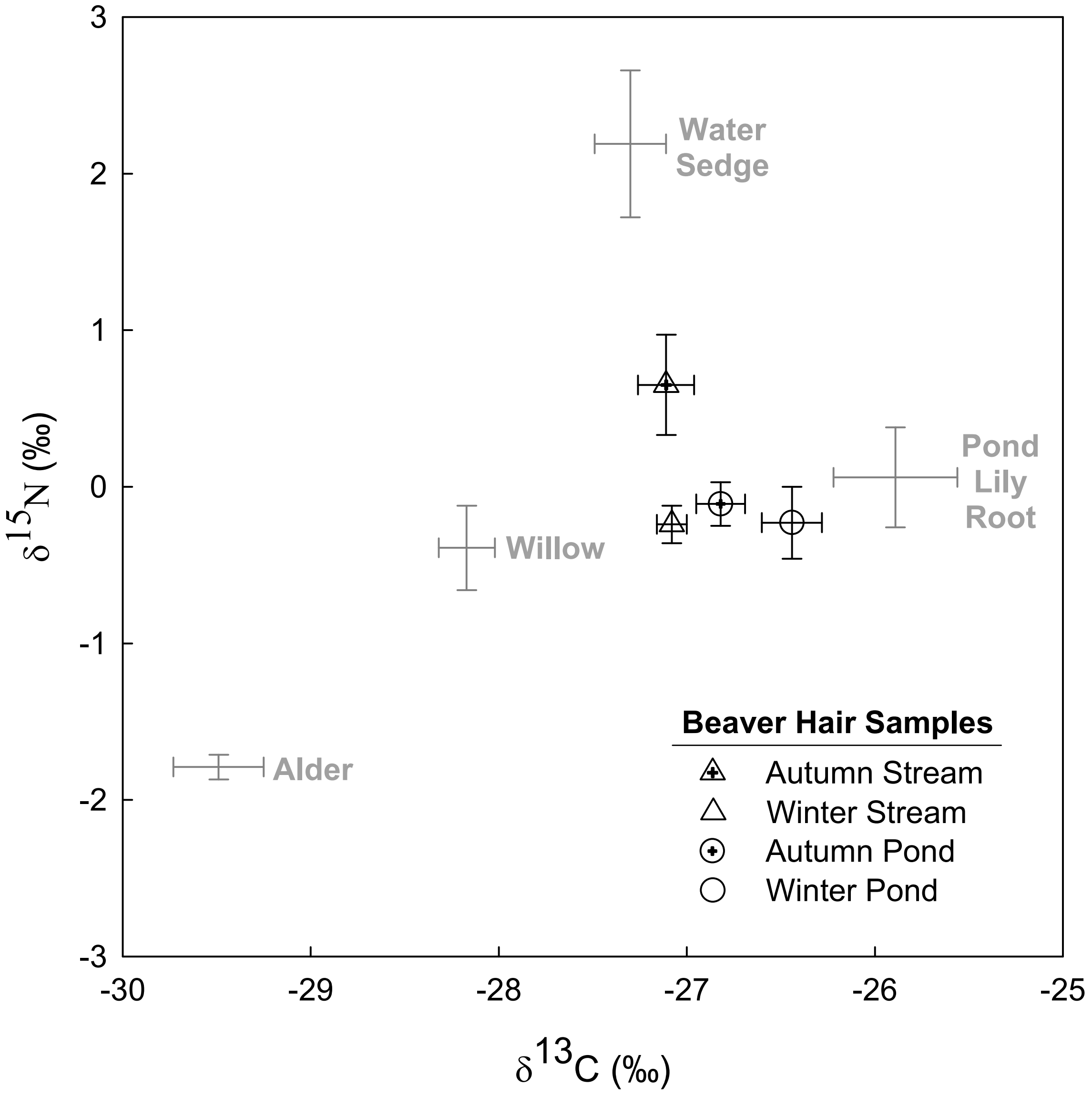


Fig. 7 IsoSource isotopic mixing model of mean (± SE) $\delta^{13}C$ and $\delta^{15}N$ values of beavers and common riparian shrub and aquatic macrophyte dietary sources.



General Conclusion

In this thesis, I examined aquatic and terrestrial foraging by a subarctic herbivore with stable isotope analysis in order to better understand the interactions between freshwater and terrestrial ecosystems in the forest boreal. Food web interactions across the freshwater-terrestrial interface can have a critical influence on both ecosystems but these interactions are often difficult to observe directly. Carbon and nitrogen stable isotope analyses provide a method to quantify interactions across ecological boundaries, however their application to freshwater-terrestrial interfaces has been limited. The first chapter of this thesis developed the methodology to quantify the contribution of vascular freshwater and terrestrial in the diets of subarctic herbivores. The second chapter examined seasonal aquatic and terrestrial dietary shifts in the diets of subarctic beavers in order to better understand their role in mediating freshwater and terrestrial food web dynamics.

This study is the first to quantify site-specific $\delta^{13}C$ and $\delta^{15}N$ values of freshwater and terrestrial vascular plants. I found consistent isotopic differentiation between aquatic macrophytes and deciduous plants in two regions of the Canadian subarctic. Freshwater plants were enriched in their carbon and nitrogen isotopic signatures relative to terrestrial plants in coastal, inland, river, and lake habitats. Although these isotopic differences were consistent across habitats, I found considerable overlap between freshwater and terrestrial plants, possibly due to nutrient exchange across these ecosystems. Aquatic plants from near the shoreline often had intermediate isotopic values between submergent macrophytes and deciduous shrubs. I may have observed more differentiation between freshwater and terrestrial plants had we sampled terrestrial vegetation further inland from the riparian zone.

The ability to differentiate freshwater and terrestrial vascular plants potentially has widespread utility in ecology. Existing freshwater-terrestrial isotopic studies primarily use algae and detritus as the base of food webs (Finlay and Kendall 2007), which has limited application for many systems. Our study can be applied to subarctic food webs where several herbivores feed primarily on freshwater and terrestrial vascular plants (including moose: *Alces alces*; beavers; muskrats: *Ondatra zibethicus*; waterfowl; and insects; Aleksiuk 1970; Fraser et al. 1980; Jelinski 1989; Baldassarre and Bolen 2006). Future research in this area should examine the site-specific isotopic differentiation of freshwater and terrestrial vegetation in other regions to examine the universality of our findings. The combination of $\delta^{13}C$ and $\delta^{15}N$ may be necessary to resolve these patterns. Furthermore, I observed considerable variability within species and among plant tissues. Therefore, other researchers should examine the isotopic variability within plant species from different habitats and seasons in order to clarify potential sources of variability and refine their methodology. The potential application of stable isotope analysis to tracing nutrient flows, animal movements, food web interactions, and diets across freshwater-terrestrial boundaries is substantial.

This study is also the first to compare the seasonal contribution of freshwater and terrestrial plants in the diet of beavers living in stream and pond habitats. Few studies of the foraging ecology of beavers have examined the contribution of aquatic plants in their diets. Stable isotope analysis revealed that aquatic macrophytes contributed more to the diets of beavers than previously reported. I also found that pond habitats had an abundance of aquatic vegetation, but were impoverished in terrestrial forage vegetation compared to stream habitats. During the seasonal change from autumn to winter, beavers from ponds consumed more aquatic vegetation than beavers from stream habitats which relied more heavily on food hoards of terrestrial vegetation. These results indicate that feeding on aquatic vegetation may allow beavers to persist at the northern periphery of their range by reducing herbivory pressure on the subarctic terrestrial ecosystem where preferred tree species are. Beavers from stream and pond habitats may play differential roles in mediating nutrient transfers across terrestrial and freshwater ecosystems.

Future research in this area should examine the ecological differences between beavers from stream and pond habitats to further understand the role of aquatic foraging and their ability to persist in habitats where trees are scarce. Aquatic foraging may allow northern beavers to relieve foraging pressure on the terrestrial system and occupy a territory for longer before having to relocate due to resource overexploitation. Beavers from stream and ponds may differ in the number of years they occupy their territories due to differing amounts and types of available forage. A comparison of site occupancy periods in stream and pond habitats may provide further insight into the role of aquatic and terrestrial vegetation in dictating the ability of beavers to persist in marginal habitats.

Researchers could also use isotopic analyses to examine intraspecific dietary variability of freshwater-terrestrial herbivores across their latitudinal range. The ecological niche within a species is thought to be broader at higher latitudes due to a relaxation of competition associated with a reduction in biodiversity and productivity (MacArthur 1972; Brown 1995; Holt 2003; Vazquez and Stevens 2004). This has rarely been tested, especially in the dietary niche within a species (Vazquez and Stevens 2004). For northern generalist herbivores such as beavers and moose, many of their preferred terrestrial species are not present (Novak 1987; Belovsky 1978; Fraser et al. 1980; MacCracken 1993; MacCracken 1997). They can respond by becoming more generalist terrestrial herbivores, or concentrating on a few abundant species, or foraging more in freshwater ecosystems. A latitudinal gradient in aquatic foraging could have implications for the response of these species to climate change, since freshwater and terrestrial ecosystems differ in their ecosystem properties and dynamics (Rouse et al. 1997; Payette et al. 2001). In turn, these herbivores can also exert critical influences on the dynamics of both ecosystems by feeding from both freshwater and terrestrial ecosystems.

References Cited

Aleksiuk M (1970) The seasonal food regime of arctic beavers. Ecology 51:264-270

Baldassare GA, Bolen EG (2006) Waterfowl ecology and management, 2nd edn. Krieger Publishing Company, Malabar

Belovsky GE (1978) Diet optimization in a generalist herbivore: moose. Theoretical Population Biology 14:105-134

Brown JH, Mehlman DW, Stevens GC (1995) Spatial variation in abundance. Ecology 76:2028-2043

Finlay JC, Kendall C (2007) Stable isotope tracing of temporal and spatial variability in organic matter sources to freshwater ecosystems. In: Michener RH, Lajtha K (eds) Stable isotopes in ecology and environmental sciences, 2nd edn. Blackwell Publishing, Malden, pp 283-333

Fraser D, Arthur D, Morton JK, Thompson BK (1980) Aquatic feeding by moose *Alces alces* in a Canadian lake. Holarctic Ecology 3:218-223

Holt RD (2003) On the evolutionary ecology of species' ranges. Evolutionary Ecology Research 5:159-178

Jelinski DE (1989) Seasonal differences in habitat use and fat reserves in an arctic muskrat population. Canadian Journal of Zoology 67:305-313

MacArthur RH (1972) Geographical ecology. Princeton University Press, Princeton

MacCracken JG, Van Ballenberghe V, Peek JM (1993) Use of aquatic plants by moose: sodium hunger or foraging efficiency? Canadian Journal of Zoology 71:2345-2351

MacCracken JG, Van Ballenberghe V, Peek JM (1997) Habitat relationships of moose on the Copper River Delta in coastal south-central Alaska. Wildlife Monographs 136:1-52

Novak M (1987) Beaver. In: Novak M, Baker JA, Obbard ME, Malloch B (eds) Wild furbearer management and conservation in North America. Ontario Trapping Association, North Bay pp 283-312

Payette S, Fortin MJ, Gamache I (2001) The subarctic forest-tundra: the structure of a biome in a changing climate. BioScience 51:709-718

Rouse WR, Douglas MSV, Hecky RE, Hershey AE, Kling GW, Lesack L, Marsh P, McDonald M, Nicholson BJ, Roulet NT, Smol JP (1997) Effects of climate change on the freshwaters of arctic and subarctic North America. Hydrological Processes 11:873-902

Vasquez DP, Stevens RD (2004) The latitudinal gradient in niche breadth: concepts and evidence. American Naturalist 164:E1-E19