The Structure and Dynamics of Fish Isotopic and Trophic Niches in Natural Lakes and Constructed Fisheries Offsets in the Alberta Oil Sands

by

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Abstract

Habitat offsets, where damages to natural ecosystems caused by socio-economic development projects are compensated for by the construction or restoration of other ecosystems, can contribute to biological conservation when implemented properly. But, large uncertainties remain surrounding our ability to construct ecosystems that offer high quality habitat and sustainably provide desired ecosystem functions and services. Trophic structure and dynamics maintain biodiversity and sustain ecosystem function by limiting competition between consumers and alleviating prey species from damaging levels of predation. The application of food web theory has improved outcomes in ecological restoration and conservation, and has the potential to do the same for habitat offsetting. In this thesis I asses trophic structure and seasonal trophic dynamics of an offset, and examine how stable isotope analysis can be improved for use on sensitive species and in multi-season studies.

In the Alberta oil sands, unavoidable destruction of fish habitat from open-pit mining is offset with the construction of small lakes on or near mine sites. To investigate trophic structure in constructed offsets, I sampled the first offset lake constructed in the Alberta oil sands, Horizon Lake, and eight natural lakes for comparison. I measured stable carbon and nitrogen isotope ratios in the tissues of fish, and used these values to estimate metrics of trophic structure. Despite the unique fish assemblage found in the offset lake, its trophic structure metrics are within the range of variation detected in natural lakes. The offset lake was most similar in terms of habitat to natural lakes that are relatively small and deep, but its trophic structure was more like large lakes with diverse fish assemblages. I recommend trophic structure continue to be examined in these and other offsets to further our understanding of their effectiveness in compensating for habitat loss and their potentially unique ecology.

Seasonal variation in environmental conditions and resource availability may play an underappreciated role in the maintenance of biodiversity, especially in ecosystems that

experience drastic seasonal changes. High-latitude and high-altitude aquatic ecosystems switch between an open water state in the summer and an ice-covered state in the winter. This large environmental change is associated with reduced primary and secondary productivity, but it is not clear how consumers such as fishes respond. To address this knowledge gap, I examined the trophic dynamics of fish populations in three natural lakes and one constructed offset habitat. I used stable isotope analysis and stomach content analysis to assess if fish were 1) maintaining the same diet across seasons, 2) changing their diet seasonally, or 3) going dormant seasonally. Most fishes fed all year-round. Some populations displayed clear seasonal changes in their diet, others displayed less drastic diet shifts, and one population maintained the same diet year-round. Only one population went seasonally dormant in the winter. Flexible foraging and a diversity of seasonal trophic responses among fish populations are likely contributing to the maintenance of biodiversity within these ecosystems. Evidence of winter activity by fishes in the offset is promising, and suggests the habitat is providing over-wintering habitat for fishes.

Stable isotope analysis is an important ecological method with many applications, but it often requires lethal sampling to obtain tissue samples. In addition, most stable isotope research is performed in the summer, leaving a gap in our understanding of whether isotope data collected in different seasons can be interpreted using the same methods. To address this, I investigated how lethal (muscle) and non-lethal (fin) tissues differ in their stable carbon and nitrogen isotope ratios, and whether inter-tissue differences change with season. I found that muscle and fin differ in carbon and nitrogen stable isotope ratios, but whether season affects this relationship is species- and isotope-dependent. I recommend accounting for differences in tissue types whenever possible, and accounting for season of capture when research questions are highly sensitive to variation in isotope ratios.

Overall, this thesis demonstrates how ecological study of constructed offsets can advance our understanding of human modified ecosystems, basic ecological principles, and

ecological methods. As offsetting grows as a practice across Canada and around the world, using these large-scale projects to further these objectives is imperative for improving the practice of offsetting and represents an enormous opportunity for advancing ecological research.

Preface

This thesis is an original work by Karling N. Roberts. The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Office, Project Name “Oil Sands Compensation Lakes”, Animal Use Protocol 00001547.

Chapters 2 and 3 have been prepared for submission to scientific journals.

Chapter 2: Roberts, K.N., Poesch, M. Trophic structure in a newly created lake ecosystem: Opportunities to improve monitoring and assessment in large scale habitat offsets.
In preparation for submission to *Biological Conservation*. K.N.R. designed the study with input from M.P. K.N.R. led field and lab work, data analysis, and manuscript writing. MP contributed to manuscript writing and editing.

Chapter 3: Roberts, K.N., McMeans, B., Poesch, M. Boreal freshwater fishes use multiple strategies in response to seasonal ice coverage in lakes. In preparation for submission to *Ecology*. K.N.R. designed the study with input from M.P. and B.M. K.N.R. led field and lab work, data analysis, and manuscript writing. All co-authors contributed to manuscript writing and editing.

Chapter 4 of this thesis has been published in *The Journal of Fish Biology*. The paper was “Highly Commended” in the competition for the FSBI Huntingford Medal. The content presented in this thesis is the same as that which was published, except for changes to formatting and the correction of a few typos.

Chapter 4: Roberts, K. N., Lund, T., Hayden, B., & Poesch, M. (2021). Season and species influence stable isotope ratios between lethally and non-lethally sampled tissues in freshwater fish. *Journal of Fish Biology*, 1–13. https://doi.org/10.1111/jfb.14939. K.N.R. and T.L. designed the study with input from M.P. and H.B.. K.N.R. led field, lab work, and data analysis with help from T.L., and led manuscript writing. All co-authors contributed to manuscript writing and editing.

This thesis is dedicated to my grandparents,
Stewart, Hazel, and Ruth,
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Chapter 1 Introduction

1.1 Offsetting Habitat Loss

Healthy and abundant freshwater ecosystems are central to human well-being. Lakes, rivers, wetlands, and reservoirs support biodiversity and ecosystem services upon which human life depends (e.g.: for drinking water, food, and income) and that greatly improve quality of life (e.g.: for recreation, aesthetics, and spiritual and artistic inspiration) (Béné *et al*., 2016; Costanza *et al*., 2014; Lynch *et al*., 2016; Youn *et al*., 2014). Yet, human activities have vastly altered and degraded freshwater ecosystems resulting in a global freshwater biodiversity crisis (Darwall *et al*., 2018; Dudgeon *et al*., 2006; Reid *et al*., 2018). The extinction rate of North American freshwater animals is 1000 times higher than the background extinction rate and is on par with that of tropical forests (Ricciardi and Rasmussen, 1999). Between 1989 and 2008 there was an 92% increase in the number of imperiled and extinct freshwater fish taxa in North America (Jelks *et al*., 2008). Reports from the World Wide Fund for Nature show an 83% decline in the abundance of animals dependent on freshwater between 1970 and 2014 (WWF, 2018). These losses threaten the ability of freshwater ecosystems to reliably provide ecosystem services such as culturally, ecologically, and economically important fisheries (Lynch *et al*., 2016).

Stopping and reversing the loss of species is a large and urgent challenge. It will require multiple, combined actions from all sectors at international, national, regional, and local scales (WWF, 2018). Habitat offsetting is one type of action aimed at addressing this goal. Offsetting is the practice of compensating for environmental damage by protecting, restoring, or enhancing an existing habitat, or by constructing a new habitat where one did not previously exist (McKenney and Kiesecker, 2010). It is increasingly common around the world (Madsen *et al*., 2010), with at least 56 nations having legislation that supports offsetting (OECD, 2016). Examples of such legislation include the Canadian Fisheries Act, the European Water Framework Directive, and the US Clean Water Act, all of which pertain specifically to freshwater ecosystems. These legislative frameworks encourage offsetting as the fourth and last step of a "mitigation hierarchy" – where plans for projects that impact the environment must be modified such that they first *avoid* creating impacts, then *minimize* the duration, intensity, or extent of impacts, and thirdly develop plans to *restore* habitat. As the last step of the hierarchy, *offsets* that compensate for residual, unavoidable damages may be required (BBOP, 2012). Through

proper implementation of the mitigation hierarchy and offsetting techniques, habitat offsets have the potential to contribute to the conservation of biodiversity and ecosystem services.

When offsetting damage to freshwater ecosystems, there is the possibility to put a body of water on the landscape where one did not previously exist (e.g.: dug-out ponds, reservoirs), or to move an aquatic ecosystem by redirecting flow (e.g.: stream diversions, ditches). In this way, a new aquatic ecosystem can be constructed, at another time or place, to replace one destroyed by human activity. Examples of constructed freshwater habitat offsets include streams (e.g.: Enders *et al*., 2007; Courtice *et al*., 2014; Palmer and Hondula, 2014), river side channels (Scruton *et al*., 2005), wetlands (e.g.: Hill *et al*., 2013; Brown and Veneman, 2001; Kettlewell *et al*., 2008), and ponds (Pickett *et al*., 2013). These large-scale constructed offsetting projects merit caution and proper consideration of their risks and uncertainties (Maron *et al*., 2016) but offer opportunities for improving and testing our understanding of aquatic ecology (Jones *et al*., 2003).

Despite the widespread adoption of offsetting policy and implementation of offsetting projects around the world, significant uncertainties remain surrounding their use (Bull *et al*., 2013; Curran *et al*., 2014; Harper and Quigley, 2005; Quetier *et al*., 2014). One of the major uncertainties is whether humans can reliably construct self-sustaining ecosystems that function in the same way as their natural counterparts. Freshwater habitat offsets often achieve some, but not all, offsetting goals (Harper and Quigley, 2005; Theis *et al*., 2020). For example, a constructed stream in northern Canada meant to offset habitat destruction caused by open pit mining provided habitat connectivity and spawning habitat for arctic grayling (*Thymallus arcticus*) but supported only 37% of the grayling biomass found in similar natural streams (Jones *et al*., 2003). Similarly, constructed wetlands that offset highway construction in western USA supported similar or higher densities of amphibian larvae as natural wetlands, but dried up prior to metamorphosis, resulting in no recruitment (Swartz *et al*., 2020). When all types of freshwater habitat offsetting projects are examined, less than 60% result in high levels of ecosystem function (Theis *et al*., 2020). Yet, offsetting policies continue to be implemented, replacing natural habitat with constructed or human modified habitats. Offsetting habitats merit a more comprehensive assessment of their ecology, along with a more thorough analysis of how they compare to their natural counterparts. These are fundamental actions in developing offsets as a reliable tool for conserving biodiversity and ecosystem services.

1.2 Trophic Structure and Dynamics

Trophic structure characterizes food webs using metrics such as food chain lengths, trophic positions, organism diets and diet breadth, functional feeding groups and functional diversity, and stable isotope niches and diversity (Perkins *et al*., 2014; Loch *et al*. 2020). Studying trophic structure offers information on the interactions among organisms within an ecosystem, which play a role in the maintenance of biodiversity (Jiang *et al*., 2009; Rooney et al. 2006; Rooney and McCann 2012) by helping to prevent prey extirpations or dominance by a superior competitor (Kratina *et al*., 2012). Assessing trophic structure in human disturbed and modified ecosystems offers a more holistic view of the system (Fraser *et al*., 2015), and can be complimentary to information that is more commonly collected during habitat monitoring projects such as physical habitat characteristics or population density and diversity of primary producers and other low trophic level organisms (Ruiz-Jaen and Aide, 2005; Hale *et al*., 2019).

Trophic dynamics include variation in consumer diets that occur in space, as they move through their environment (McCann *et al*. 2005), and in time, as consumers grow and mature (ontogenetic shifts), seasons change, or prey and predator population densities change (Warren, 1989). Seasonal changes to the environment and their associated effects on resource availability and consumer behavior have the potential to drive large, re-occurring, and important trophic dynamics within ecosystems (McMeans *et al*. 2015). But most ecosystem types have a dominant season for ecological research, meaning that the other season(s) and the differences between seasons are less understood. Research on the impacts of seasonal variation in consumer trophic dynamics and the importance of these shifts is still needed.

In high altitude and high latitude freshwater ecosystems, the understudied season is the winter, when water bodies become covered in a layer of ice. Winter in freshwater ecosystems is characterized by distinct environmental changes. Temperatures drop to 0-4ºC, light intensity decreases, and material and gas exchange between water, land, and air are cut off (Shuter *et al*., 2012). These seasonal changes may cause consumers at multiple trophic levels to change their diet seasonally or go seasonally dormant. Such dynamics prevent the overexploitation of resources and the loss of consumers by allowing consumers to switch to an alternate resource or stop exploiting a resource if it becomes scarce or overly vulnerable (McMeans *et al*., 2020). This is one way that temporal trophic dynamics have stabilizing properties, by preventing

extreme fluctuations in population densities and potential extirpations, and encouraging ecosystems to return to a stable state after a perturbation (McCann, 2000).

The importance of trophic structure and temporal dynamics to conservation is highlighted by drastic changes in community composition and the loss of ecosystem functions after human induced disruption of food webs. These disruptions may come in the form of predator or other consumer removals (Terborgh, 1988; Dirzo and Miranda, 1990, Estes *et al*. 1989, Myers *et al*. 2007), the introduction of invasive species (Olsen *et al*., 1991; Roemer *et al*., 2002), the loss of habitat (Brook *et al*., 2003; Terborgh *et al*. 2001), or the addition of nutrients (Smith *et al*., 1999). These disruptions can lead to species losses and ecological phase shifts that can be difficult to undo (Estes *et al*., 1989) but reversal of disturbance effects is possible if food web structure can be restored (Lepak *et al*., 2006; Painter *et al*., 2018; Beschta and Ripple, 2019). Integrating food web theory into ecological applications such as alien species control, restoration, and conservation is becoming more common (Memmott, 2009; Fraser *et al*., 2015). Doing the same for habitat offsetting could improve the ability of constructed habitat offsets to meet offsetting targets, support biodiversity, and provide ecosystem services.

1.3 Fisheries Habitat Offsetting in the Alberta Oil Sands

In Canada, the province of Alberta contains a large oil deposit in the form of oil sands, in which bitumen hydrocarbons are found within a mixture of sand, water, and clay. Much of this deposit is located deep underground and is extracted using *in situ* methods, where oil sands deposits are extracted without removing the overlying rock and dirt. But, a portion of the deposit located in the lower Athabasca River watershed is surface mineable. In this region, companies construct large scale open-pit mines and oil sands processing facilities. Construction and operation of these sites result in the loss and degradation of fish habitat. Such losses need to be offset to meet the requirement for No Net Loss of fish habitat or fish productivity legislated by the Canadian *Fisheries Act.* In the oil sands region, this has been accomplished by constructing new fish habitat in the form of lakes on or near the mine sites. The offsetting targets for these lakes are double the habitat area or fisheries productivity that was lost due to habitat disruption (Golder Associates ltd., 2004). There are multiple offset lakes on the landscape in the Alberta oil sands region, and others in the planning process (Ruppert *et al*., 2018). The monitoring requirements for each offset lake are determined by the Department of Fisheries and Oceans. Conditions to meet the monitoring requirements are developed by environmental consulting

companies with input from the mining company and approved by the Department of Fisheries and Oceans. The monitoring programs are relatively extensive compared to many other habitat construction and restoration projects (Bernhardt *et al*., 2005), examining water quality, habitat characteristics, and the diversity and biomass of multiple trophic levels from plankton and macrophytes to fishes. But these programs do not explicitly examine trophic structure and dynamics, and comparisons with natural lakes in the region are limited or not performed. These offset lakes offer an opportunity to examine trophic structure and dynamics in constructed ecosystems and make comparisons with analogous natural ecosystems.

1.4 Stable Isotope Analysis

A common approach for assessing trophic structure and dynamics is with stable isotope analysis. In ecology, stable isotope analysis measures the ratio of heavy isotopes to light isotopes of certain elements in the tissue of organisms and uses estimates of how those ratios change as matter flows through a food web to infer information about the diet and behaviour of the organisms (Fry, 2006). Commonly measured stable isotopes for ecological studies include oxygen, hydrogen, sulfur, and, most commonly, carbon and nitrogen. Carbon stable isotope ratios (^{13}C:^{12}C) relate to the sources of primary production that support a consumer (Fry *et al*., 1978). For example, in lakes, phytoplankton in the pelagic zone typically have lower ^{13}C:^{12}C ratios compared to benthic algae in the littoral zone (France, 1995). Nitrogen stable isotope ratios (^{15}N:^{14}N) increase incrementally from prey to consumer, and thus offer information on the trophic position of a consumer (Vander Zanden and Rasmussen, 1999; Post, 2002). For example, carnivorous species typically have higher ^{15}N:^{14}N ratios compared to herbivores from the same ecosystem (Minagawa and Wada, 1984). When the isotope ratios of consumers and their potential prey are known, one can infer what prey are consumed and in what proportions (Moore and Semmens, 2008). For example:

- **Isotopic niche** can be measured by the variation in stable isotope ratios between individuals within a species as an index of dietary niche breadth (Bearhop *et al*., 2004).
- **Diet overlap** or potential competition between species can be measured by the similarity or difference in stable isotope ratios between species from the same ecosystem (Mumby *et al*., 2017).
- **Niche diversity** can be measured by the variation in stable isotope values of multiple species within a community (Layman *et al*., 2007).

Using these and other stable isotope-based metrics of trophic structure, stable isotopes have been used to understand the effects of invasive species (Jackson *et al*., 2012), monitor recovery after invasive species control (Lepak *et al*., 2006), and monitor or assess habitat restoration projects (Loch *et al*., 2020). Similar insights and benefits could be gained by applying stable isotope analysis to better understand the trophic structure and dynamics of constructed habitat offsets.

Over four decades, the use of stable isotopes to understand the trophic niches of organisms and communities has rapidly advanced. Research has improved our understanding of the relationship between consumer diet, physiology, and their tissue stable isotope ratios (e.g.: Pinnegar and Polunin, 1999; Kiljunen *et al*., 2006; Elsdon *et al*., 2010) and stable isotope-specific statistical tools and metrics have been developed (e.g.: Layman *et al*., 2007, Jackson *et al*., 2011, Kadoya *et al*., 2012; Swanson *et al*., 2015, Eckrich *et al*., 2018). Alongside these advancements, our awareness of multiple sources of variation that impact stable isotope ratios in the environment and in the tissues of organisms (potentially resulting in misinterpretations of stable isotope data) has also increased (Boecklen *et al*., 2011; Bond and Diamond, 2011). Potential sources of variation include season of capture (Perga and Gerdeaux, 2005) and the tissue examined (Tieszen *et al*., 1983), among many other potential sources of variation that merit further investigation (See Boecklen *et al*., 2011 for a review of variation sources in stable isotope analysis). The season when samples are collected might impact an organism's stable isotope ratios due to seasonal variation in temperature (Bosley *et al*., 2002) and seasonal fasting or nutritional deficits (Hobson *et al*., 1993). Within an organism, tissue types will vary in their stable isotope ratios due to differences in tissue turn-over rates (Vander Zanden *et al*., 2015), isotopic routing (Kelly and del Rio, 2010), and lipid content (Skinner *et al*., 2016). In fish, white dorsal muscle is the most common tissue for stable isotope analysis and is considered a reliable indicator of the resources assimilated by fish over the weeks to months preceding sampling (Pinnegar and Polounin, 1999). Other popular tissues include liver and blood for examining resource assimilation over shorter periods of time (days to weeks) (Hayden *et al*., 2014), and fins, scales, and epidermal mucous for non-lethal sampling (Winter *et al*., 2019). Further research is required on the impacts of these sources of variation, and how multiple sources of variation interact, to advance stable isotope analysis as a reliable tool in ecological studies.

Stable isotope analysis commonly requires lethal or invasive tissue sampling, limiting its application with sensitive species and ecosystems, or when lethal sampling is not permitted. The use of non-lethal and less invasive tissues such as fins, scales, or mucous can be challenging because much of our understanding of fish isotope ecology is based on muscle. Our ability to interpret the stable isotope ratios of non-lethal tissue samples in fish has improved through the development of tissue conversion relationships (Kelly et al., 2006; Winter *et al*., 2019). But such studies are limited to a small number of species, and most have been performed in the summer months or under summer-like laboratory conditions. The variation introduced into stable isotope ratios from non-lethal tissues in most freshwater fish species and in fish collected in the winter remains largely unknown. For stable isotope analysis to continue to improve as a tool for studying trophic structure and dynamics, research that improves our understanding of how to account for sources of variation and that permits non-lethal sampling is required.

1.5 Thesis Objectives

My thesis objectives are to examine the trophic structure and dynamics of a constructed freshwater habitat offset, compare the offset to natural ecosystems, improve our knowledge of trophic structure and dynamics in boreal lakes, and advance the use of stable isotope analysis as a tool for ecological studies and monitoring. I sampled the first constructed fisheries offsetting habitat in the Alberta oil sands, Horizon Lake (Wāpan Sākahikan), and up to eight natural lakes for comparison. In chapter 2 I assess the trophic structure of the fish assemblage in Horizon Lake and eight natural lakes. I estimate metrics of trophic structure based on fish carbon and nitrogen stable isotope ratios. The natural lakes can be categorised into three groups, referred to as assemblage types, based on their fish species. These assemblage types are characteristic of small lakes in the region (Robinson and Tonn, 1989) and are realistic targets for the fish species compositions of fisheries habitat offsets in the Alberta oil sands. I assess the relationship between species assemblages and their trophic structure by comparing trophic structure metrics among the natural lakes.

In chapter 3 I examine trophic dynamics in Horizon Lake and natural lakes. I categorise how the trophic niches of boreal freshwater fish respond seasonally between open water in the summer and ice cover in the winter. I compare the seasonal trophic responses of fish populations in Horizon Lake to what is observed in natural lakes.

In chapter 4 I advance the ecological application of stable isotope analysis to the study of fish. For three common boreal fish species, I test whether fish tissues that can be sampled non-lethally can be used as alternatives to lethally sampled tissues. I generate mathematical conversion relationships between lethal and non-lethal tissues for carbon and nitrogen stable isotopes, assess how tissue type impacts the estimation of common metrics of trophic structure, and determine whether season of capture needs to be accounted for when interpreting isotope values and metrics of trophic structure from non-lethal fish tissues.

These works improve our understanding of the ecology of constructed offset habitats and of boreal lake fish communities, while advancing stable isotope analysis as a tool for ecological studies and monitoring. The natural lakes examined here offer a baseline for the assessment of future constructed fisheries offsets in the Alberta oil sands. Examining trophic structure and dynamics in constructed fisheries offsets and advancing the methods for doing so can improve fishery conservation. This in necessary in Canada, where impacts from a growing resource extraction industry will be compensated for with offsetting, and globally, as over 56 nations use or plan to use habitat offsets to conserve biodiversity and natural resources.

Chapter 2 Trophic Structure in a Newly Created Lake Ecosystem: Opportunities to Improve Monitoring and Assessment in Large Scale Habitat Offsets

2.1 Abstract

Habitat offsets compensate for the loss of natural habitat due to anthropogenic activity by creating, restoring, or protecting habitat at another time or place. This process is gaining popularity worldwide, but large uncertainties remain surrounding the ability to construct habitats that support desirable ecological structure. In the Alberta oil sands, Canada, the loss of fish habitat due to the construction of open pit mines is compensated with the construction of reservoirs placed on or near mine sites. I examined the trophic structure of the fish assemblage in the first of such offsets and in eight natural lakes. I used stable isotope analysis of fish muscle and baseline organisms to calculate metrics of trophic structure and compared metrics between the natural lakes and the offset lake. Trophic structure in the offset lake was within the range of variation detected in the natural assemblages. Across all metrics, the offset lake most closely resembled the trophic structure found in lakes that support multiple large-bodied fisheries species, despite having a different species composition and different habitat characteristics. A unique feature of the offset lake is that its top trophic position is occupied by three fish species, compared to one species in natural lakes with piscivores. Examining trophic structure metrics, as done here, can be useful in determining how closely habitat offsets resemble natural systems. Ultimately the success and resiliency of offset lakes will depend on the structure of their food webs and their ability to mimic natural ecosystems.

2.2 Introduction

Habitat loss caused by anthropogenic activities is mitigated through four steps, known as the mitigation hierarchy – 1) avoid creating impacts, 2) minimise the duration, intensity, or extent of impacts, 3) restore degraded or destroyed ecosystems, and 4) compensate for residual impacts that could not be avoided, minimised, or restored through offsetting (BBOP, 2012). Offsets compensate for habitat loss by protecting, restoring, or enhancing a different ecosystem from that which is being harmed, or by constructing an ecosystem at a time or location separate from where impacts occur (McKenney and Kiesecker, 2010). Offsets have the potential to contribute to conservation and restoration, but there are serious theoretical (e.g.: Spash, 2015; Moren-Mateos *et al.,* 2015; Maron *et al*., 2016) and practical (e.g.: Bell, 2016; Sonter *et al*., 2020; Coker *et al*., 2018; Theis and Poesch 2022) challenges to their appropriate implementation.

Choosing how to monitor offsets and judging offsetting success is one challenge associated with habitat offsets (e.g., Moilanen *et al*., 2009; Bas *et al*., 2016; Simmonds *et al*., 2020). This is especially true for out-of-kind offsets, where the type of habitat that is constructed differs from the type of habitat that was lost (Bull *et al*., 2015). Success criteria and monitoring requirements are mandated under various frameworks globally (GIBOP, 2019) and often focus on habitat area, species composition, or productivity. But, achieving targets based on these factors does not guarantee that an offset will achieve full ecological function (Theis *et al*., 2020), and failing to meet these targets does not guarantee that the offset failed ecologically. Restoration ecologists recommend including trophic structure in the assessment of habitat restoration projects (Vander Zanden *et al*., 2006; Lake *et al*., 2007; Fraser *et al*., 2015). Trophic structure highlights whether key ecological interactions are developing, instead of assuming they are occurring based on the populations of organisms detected (Palmer *et al*., 1997; Weinstein *et al*., 2005; Howe and Simenstad, 2011). Application of this recommendation to the monitoring and assessment of habitat offsets will improve understanding of the ecology of offsets and their value to ecological conservation.

Alberta, Canada, contains the fourth largest proven crude oil reserve is the world (Alberta Government, 2022). A portion of this reserve is surface mineable and located in the Lower Athabasca Watershed. In this region, mining companies are constructing reservoirs, hereafter called offset lakes, on or near their open pit mine sites to satisfy the offsetting requirements outlined in the Canadian *Fisheries Act* (1985) for the destruction of fish habitat. Offset lakes are

an example of out-of-kind offsetting, where the loss of fish habitat due to draining and diverting streams and wetlands from the mine sites is compensated by the construction of a different type of fish habitat – lakes.

The first offset lake constructed in the Alberta oil sands, Horizon Lake (Wāpan Sākahikan), was constructed in the winter of 2008 and filled in the spring of 2008. Horizon Lake was constructed by damming the Tar River valley with an earthen dam, using the natural contour of the land to create the lake. Surficial materials, such as muskeg, were removed within the reservoir footprint to reduce the peak mercury loading potential. Enhanced habitat features such as boulder gardens, riparian plants, and fallen trees were placed throughout and around the lake. It was designed to support populations of large-bodied sport fishes and features shallow littoral bays and a deep pelagic zone where fish can overwinter (Ruppert *et al.*, 2018). Due to provincial management decisions aimed at supporting a species of locally sensitive fish that colonized the lake, arctic grayling *Thymallus arcticus* (Pallas 1776), it was ultimately never stocked with sport fishes. The lake drains through a diversion ditch system (which is currently impassable to fish) into the lower Tar River. When the mine is no longer active an outlet will be constructed to connect the lake to the Athabasca River and provide additional fish habitat. This will achieve the remaining required habitat offsetting for the Horizon Oil Sands project. The lake has been colonized by fish populations from the Tar River upstream from the earthen dam, and fathead minnow *Pimephales promelas* (Rafinesque 1820) and brook stickleback *Culaea inconstans* (Kirtland 1840) that were introduced from a nearby lake. This has resulted in a unique fish assemblage for the region that may include up to nine fish species identified during fish monitoring surveys: fathead minnows, brook stickleback, arctic grayling, trout-perch *Percopsis omiscomaycus* (Walbaum 1792), lake chub *Couesius plumbeus* (Agassiz 1850), white sucker *Catostomus commersonii* (Lacépède 1803), longnose sucker *Catostomus catostomus* (Forster 1773), slimy sculpin *Cottus cognatus* (Richardson 1836), and burbot *Lota lota* L. 1758.

Fish assemblages in freshwater ecosystems are non-random combinations of species that share similar environmental tolerances and can co-exist (Giam and Olden, 2016; Jackson *et al*., 1992; Jackson and Harvey, 1993). Traditional theories of community assembly expected interspecies competition to be a driving factor determining the composition of species assemblages (Diamond, 1975; M'Closkey, 1978), but there is little evidence of this in freshwater ecosystems (Matthews, 1982; Jackson *et al*., 2001; Giam and Olden, 2016). Environmental conditions such as habitat morphology and oxygen concentrations (Mehner *et al*., 2005); degree

of isolation from other water bodies (Spens *et al*., 2007; Drakou *et al*., 2009; Ohman *et al*., 2006); and predator-prey interactions (Robinson and Tonn, 1989; Englund *et al*., 2009) are the most important factors shaping freshwater fish assemblages in temperate and boreal ecosystems (Tonn and Magnuson, 1982; Tonn *et al*., 1990; Jackson *et al*., 2001). In human made and modified ecosystems, management decisions such as if and how to stock fish also influence fish assemblages (Zhao *et al*., 2016; Ruppert *et al*., 2018).

Fish assemblages of small lakes in the Athabasca Watershed fall into three categories, as identified by Robinson and Tonn (1989). *Minnow* assemblages are characterized by species that are sensitive to predation, especially fathead minnow and brook stickleback, and the absence of large-bodied, piscivorous fish species. They occur in isolated, shallow lakes where piscivores, especially northern pike *Esox Lucius* L. 1758, cannot overwinter. *Pike* assemblages are characterized by northern pike and may contain other fish species that can co-exist with them. They occur in lakes that are deep enough for northern pike to overwinter in, or shallow lakes that are connected to a habitat refuge for overwintering. *Pike-perch* assemblages are characterized by northern pike and yellow perch *Perca flavescens* (Mitchill 1814), and fishes that can co-exist with them. They tend to occur in larger lakes than *Minnow* and *Pike* assemblages.

In this study I assess the trophic structure of fish assemblages in Horizon Lake, a constructed offset lake, and eight natural lakes in the Alberta oil sands region. Due to health and safety and other concerns, I did not receive authorization to sample other offset lakes in the area, and the data from monitoring reports that I received for those lakes were not comparable to our study and could not be included here. The natural lakes' fish assemblages align with the three fish assemblage types identified by Robinson and Tonn (1989) and are realistic target fish assemblages for offset lakes. I estimate niche sizes, niche overlap, trophic position, and resource use of the fish populations in each lake. I compare these metrics of trophic structure between the natural assemblage types and between the natural lakes and the offset lake. I also examine how habitat characteristics differ between the offset and the natural assemblage types, and whether habitat characteristics are related to fish assemblage trophic structure.

2.3 Methods

2.3.1 Study Sites and Habitat Assessments

Horizon Lake and eight natural lakes located in the Lower Athabasca watershed were sampled between August and October 2017. The natural lakes were classified into the three assemblage types identified by Robinson and Tonn (1989) for small lakes in the Athabasca watershed based on the species captured during sampling. The lakes cover a range of physical and chemical characteristics typical of lakes in the region (Table 2.1). Water samples were collected one time from each lake during sampling and were sent to the Natural Resource Analytical Laboratory at the University of Alberta for measurement of total organic carbon (TOC), total nitrogen (TN), and total phosphorus (TP). TOC and TN were measured using a Shimadzu TOC-L CPH Model Total Organic Carbon Analyzer with an ASI-L and TNM-L (Shimadzu Corporation, Jiangsu China). TOC was measured as non-purgeable organic carbon by fist acidifying the sample with 1M HCl, sparging the sample, injecting the sample into a combustion tube at 720°C with platinum catalyst beads where a redox reaction occurs that evolves CO_2, which is measured by a non-dispersive infrared detector for carbon. TN was measured by combusting the sample to NO and NO_2, then reacting it with ozone to form NO_2 in an excited state and measuring the photon emissions with a Chemiluminescence detector. TP was measured using inductively coupled plasma-optical emission spectroscopy with a Thermo iCAP6300 Duo inductively coupled plasma-optical emission spectrometer (Thermo Fisher Corp., Cambridge, United Kingdom). Surface area measurements came from shapefiles of surface waterbodies available from the Government of Alberta. The maximum depth of each lake was measured using a transducer during hydroacoustic surveys that measured the depth of the lake along transects spread 50m apart. Secchi depth was measured on the shady side of the boat by taking the average of the depth at which the secchi disk was no longer visible and the depth at which it re-appeared. I used linear discriminant analysis with assemblage type as the grouping variable to assess how lake physical and chemical characteristics (referred to as habitat characteristics) relate to assemblage type.

Table 2.1. Study lake characteristics.

Lake	Code	Lat	Lon	SA[1] (ha)	Max Depth (m)	Secchi[2] (m)	TOC[3] (mg/L)	TN[4] (mg/L)	TP[5] (mg/L)
Horizon*	HZ	57.385	-111.962	76.7	18	1.87	24.39	0.53	0.033
Calumet	CA	57.416	-111.763	69.9	1.5	0.5	65.09	2.42	0.10
Unnamed 5	U5	55.797	-111.292	36.5	4	1.45	23.09	0.51	-
Unnamed 2	U2	55.483	-111.476	24.3	15	1.97	24.25	0.71	-
Unnamed 1	U1	55.485	-111.535	109.4	7	1.25	15.53	0.79	0.041
Steepbank	SB	55.478	-111.575	193.5	17	1.65	13.36	0.53	0.015
Goodwin	GW	55.418	-111.657	864.6	15	2.15	12.21	0.37	0.012
Kirby	KB	55.475	-110.761	536.5	10	2.35	12.8	0.32	0.051
Wappau	WP	55.496	-111.597	460.8	7	0.9	17.4	0.9	0.015

* Offset lake

[1] SA = surface area

[2] Secchi = secchi depth

[3] TOC = total organic carbon

[4] TN = total nitrogen

[5] TP = total phosphorus

Blank field (-) were below detection limits

2.3.2 Sample Collection

I collected fish from the nine study lakes using gill nets, minnow traps, angling, and boat electrofishing. I used multiple gear types to target the whole fish assemblage and account for species specific (Poos *et al*., 2007) and habitat specific (Neufeld *et al*., 2016) gear selectivity. Up to 10 individuals per species captured in each lake were euthanized with an overdose of MS-222. The capture, handling, and euthanasia of fish followed Canadian Council on Animal Care guidelines for the use of fish in research. Sampling and collections were performed with permission from Alberta Environment and Parks under Research License 17-1803 Amended and 17-0403 Amended, and with ethics approval from the University of Alberta Research Ethics Office under Animal Use Protocol 00001547.

To calculate estimates of trophic position and resource use, consumer isotope values must be standardized against a common baseline collected from each lake (Vander Zanden and Rasmussen, 1999; Post, 2002). As a littoral baseline, I collected snails from logs, rocks, vegetation, or sediment by hand or with a kick net from three random locations around the margin of each lake (Post, 2002). For an offshore baseline, I collected zooplankton using a

zooplankton net with 80µm mesh and a diameter of 25cm. The net was deployed to 2m off the bottom near the deepest region of each lake and was pulled up vertically through the water column. Two hauls were performed in each lake and the samples were combined for storage. In lake Unnamed 5 there was no difference between zooplankton and snail carbon and nitrogen stable isotope ratios, so chironomids (Vander Zanden and Rasmussen, 1999) were collected from the deepest area of the lake using an Eckman grab as an alternate offshore baseline. Baseline organisms were collected at the same time as fish collections on each lake. Samples were stored on ice, frozen in the field using electric coolers, and then stored at -20ºC until further processing.

2.3.3 Stable Isotope Analysis

Approximately 1g of boneless, skinless, white dorsal muscle was taken from below the dorsal fin of each sampled fish. Snails were removed from their shells and rinsed with distilled water an separated into individual samples. Zooplankton samples were thawed and separated into five sub-samples. Chironomids were rinsed with distilled water and separated into individual samples. Between one and ten chironomids were included in a sample to ensure sufficient mass for stable isotope analysis. Stable isotope analysis was performed on four to six samples of each baseline per lake. Samples were freeze dried at -54ºC for 24-48h. Most samples dried within 24h, but zooplankton samples required more time (up to 48h) because of the relatively large volume of water in the samples. After freeze drying, I homogenized the samples into a fine powder.

Carbon and nitrogen stable isotope ratios are reported as $\delta^{13}C$ and $\delta^{15}N$: the ratios of ^{13}C:^{12}C and ^{15}N:^{14}N of the sample divided by the ratios of ^{13}C:^{12}C and ^{15}N:^{14}N found in international standards - VPD Belemnite for ^{13}C and air for ^{15}N. Results are then multiplied by 1000, giving units of ‰ (per-mil).

I weighed 0.4±0.05mg of dried tissue into 4 mm tin capsules and sent them to the Natural Resources Analytical Laboratory (NRAL), University of Alberta, for stable carbon and nitrogen isotope analysis. NRAL analyzed samples using a Delta V Advantage Isotope Ratio Mass Spectrometer coupled to a ThermoScientific Flash 2000 Elemental Analyser (ThermoScientific, Waltham U.S.A.). Protein was run every 12 samples as an in-house $\delta^{13}C$ (-27.03 ± 0.02‰) and $\delta^{15}N$ (6.02 ± 0.02‰) standard to check for instrument error. 27 fish muscle samples were run in

duplicate to test our homogenization process. The mean difference and standard deviation between duplicate samples was 0.1 ± 0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$.

$\delta^{13}C$ of fish muscle and baseline samples were not mathematically corrected for lipids. C:N ratios of fish muscle were near or below 3.5, indicating low lipid content that does not need correcting (Post *et al.*, 2007; Logan *et al.*, 2008). C:N ratios of baseline samples were greater than 3.5, potentially indicating high lipid content that could decrease baseline organisms' $\delta^{13}C$ values relative to the primary producers they are meant to represent, if those lipids are synthesized de novo by the organism (DeNiro and Epstein, 1977). But, mathematical lipid correction of invertebrates may be inappropriate because: 1) high C:N ratios in invertebrates can be caused by high levels of the storage molecule glycogen (Kiljunen *et al.*, 2006); 2) lipids accumulated in a consumer by directly routing lipid molecules from their food into their tissues do not decrease a consumer's $\delta^{13}C$ value relative to their diet (Arostegui *et al.*, 2019); and 3) most published mathematical lipid corrections perform poorly on whole invertebrates and bulk zooplankton samples because the assumption that lipid free muscle has a C:N ratio of 3 does not apply (Kiljunen *et al.*, 2006; Logan *et al.*, 2008). For these reasons I did not correct for lipids in baseline organisms' $\delta^{13}C$ values.

2.3.4 Assessment of Fish Assemblage Trophic Structure

To assess the trophic structure of fish assemblages, I examined: 1) the size of species isotopic niches, 2) isotopic niche overlap, 3) species trophic positions and fish assemblage food chain lengths, and 4) species reliance on littoral vs offshore resources and the diversity of basal resources used by the assemblage. To account for small sample sizes (one offset lake, eight natural lakes divided into three assemblage types) that were not amenable to inferential statistical analyses I used Bayesian approaches to calculate estimates of trophic structure metrics. The Bayesian models incorporate multiple sources of variation into metric estimates by producing a distribution of values. Trophic structure metrics were estimated as follows:

Species isotopic niche size and overlap

I assessed species' isotopic niche sizes and amount of overlap in each assemblage based on the space occupied in an isotope bi-plot, with $\delta^{13}C$ plotted on the x-axis and $\delta^{15}N$ plotted on the y-axis. Niche size was estimated using 95% standard ellipse areas. Niche overlap was estimated by calculating the mean probability of finding individuals of the other species within

the 95% standard ellipse area of each species in an assemblage. 95% standard ellipse areas and overlap probabilities were calculated using the Bayesian model in the R package nicheROVER (version 0.1.1, Swanson *et al*., 2015). Mean 95% standard ellipse areas and overlap probabilities were based on posterior distributions of metric estimates formed by 1000 Monte Carlo draws.

Species trophic position and littoral resource use

I estimated species trophic positions and littoral resource reliance by calculating estimates of trophic position and alpha (proportion of littoral basal resource use) using the Bayesian model within the R package tRophic Position (version 0.7.7, Quezada-Romegialli *et al*., 2018). This method incorporates variance for important parameters used to calculate trophic position and resource use, such as trophic discrimination factors and the isotopic values of baseline organisms. I included the isotope values of littoral and offshore baselines in the two-baseline model and assumed the trophic position of baseline organisms was 2. I used trophic discrimination factors with means and standard deviations of 3.4±0.98 for $\delta^{15}N$ and 0.39±1.3 for $\delta^{13}C$ (Post, 2002). The model was run with 5 parallel chains and 10 000 iterations in the adaptive phase, a burn-in period of 10 000 iterations, 10 000 iterations in the sampling phase, and a thinning interval of 10. This resulted in 1001 samples per chain. Mean trophic position and mean alpha of each population were estimated as the mean of the posterior distribution of trophic position and alpha estimates, respectively.

Fish assemblage trophic structure and habitat characteristics

I used the following calculations as whole-assemblage metrics of trophic structure:

1) **Mean isotopic niche size** was calculated by averaging the means of isotope niche size estimates across all species in an assemblage.
2) **Isotopic niche size range** was calculated by taking the difference between the species with the largest and the species with the smallest isotope niche size in an assemblage.
3) **Mean isotopic niche overlap** was calculated by averaging the mean overlap probabilities of all the species in an assemblage.
4) **Isotopic niche overlap range** was calculated by taking the difference between the species with the highest and the species with the lowest mean overlap probability.
5) **Maximum trophic position**, an estimate of food chain length, was the mean of the posterior distribution of trophic position estimates for the highest trophic position species in an assemblage.

6) **Mean alpha** was calculated by averaging the mean percent littoral resource use estimates across all the species in an assemblage.
7) **Alpha range**, an estimate of resource use diversity, was calculated as the difference in mean percent littoral resource use between the species with the highest and lowest percent littoral resource use estimates in each assemblage (Layman *et al*., 2007).

I assessed how trophic structure differed between the assemblage types by running a linear discriminant analysis with assemblage type as a grouping variable on the whole-assemblage trophic structure metrics. I used a discriminant analysis because this method maximizes the difference between known or hypothesized groups along metrics of interest. I used an indirect gradient analysis to examine how habitat characteristics related to the trophic structure metrics by adding vectors of the habitat characteristics to the ordination created from the trophic structure metrics. Data analysis and visualization was performed using R statistical software (R version 4.1.2; R Core Team, 2021).

2.4 Results

2.4.1 Assemblage Types and Habitat Characteristics

In total, 33 fish populations including 12 species were sampled from the nine study lakes. Of the nine species known to occur in Horizon Lake, eight were captured during our sampling and six had large enough sample sizes to be included in the present study. Two natural lakes were classified as *Minnow* assemblages, three as *Pike* assemblages, and three as *Pike-perch* assemblages (Table 2.2). Lake surface area was the most important habitat characteristic separating the fish assemblage types, with *Pike-perch* lakes having the largest surface areas. *Minnow* lakes had relatively high nutrient concentrations and low water clarity. *Pike* lakes were relatively deep with relatively small surface areas. Habitat characteristics in the offset lake were most like *Pike* lakes, but were unique, with the offset lake lying outside of the polygons created by all natural lakes and by each natural assemblage type. (Figure 2.1).

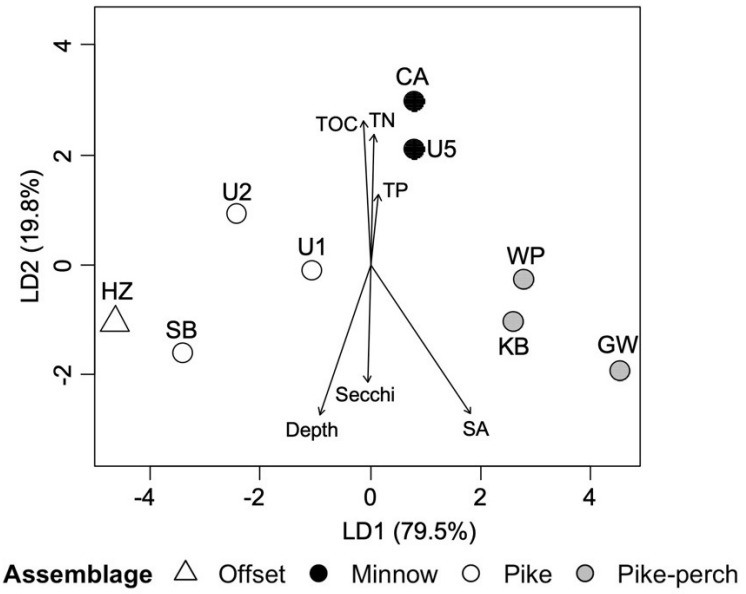

Figure 2.1. Linear discriminant analysis of lake habitat characteristics with lakes grouped by assemblage type. Symbols represent individual lakes and are labelled with lake two-letter codes: Horizon Lake (HZ), Steepbank Lake (SB), Unnamed 2 (U2), Unnamed 1 (U1), Calumet Lake (CA), Unnamed 5 (U5), Wappau Lake (WP), Kirby Lake (KB), and Goodwin Lake (GW). Vectors demonstrate the direction and strength of the relationship between habitat characteristics and the new linear discriminant variables (LD1 and LD2). Habitat characteristics are total organic carbon concentration (TOC), total nitrogen concentration (TN), total phosphorus concentration (TP), lake maximum depth (Depth), Secchi depth (Secchi), and lake surface area (SA).

2.4.2 Isotopic Niche Size and Overlap

Mean isotopic niche sizes, isotopic niche size range, mean isotopic niche overlap, and isotopic niche overlap range of fish populations in the offset lake were like fish populations in some *Minnow* and *Pike-perch* assemblages with four or more species. Fish populations in *Pike* assemblages had smaller mean isotopic niche sizes and niche size ranges with less overlap and smaller overlap ranges (Figure 2.2). This included northern pike, with northern pike populations in the *Pike* assemblages having smaller niches and less niche overlap than pike populations in *Pike-perch* assemblages. Mean isotopic niche sizes (mean 95% standard ellipse areas) varied from 1.4 to 32.5 across all lakes. Fish populations in the three *Pike* assemblages had consistently small mean isotopic niche sizes (between 1.4 and 13.4) compared to fish populations in *Minnow* assemblages (between 3.8 and 26.4), fish populations in *Pike-perch*

assemblages (between 5.0 and 32.5) and fish populations in the offset lake (between 2.6 and 24.8) (Figure 2.3a).

The mean isotopic niche overlap (mean probability of individuals of other species occurring within the niche of a focal species) varied from 1.3 to 76.6% across all lakes. Fish populations in *Pike* assemblages had consistently less niche overlap and smaller niche overlap ranges (range: 1.3 to 35.1%) compared to fish populations in *Minnow* assemblages (range: 20.6 to 72.3%), *Pike-perch* assemblages (range: 11.0 to 73.8%), and the offset lake (range: 5.0 to 76.6%). Fish populations in the offset lake had the widest isotopic niche overlap range, and was most similar to *Pike-perch* assemblages (Figure 2.3b).

2.4.3 Trophic Position

Mean trophic position of fish populations ranged from 3.0 to 4.9 across all lakes. In *Pike* and *Pike-perch* assemblages northern pike had the highest trophic position and in *Minnow* assemblages brook stickleback, fathead minnow, and white suckers shared the highest trophic position. In the offset lake the highest trophic position was shared by arctic grayling, trout-perch, and lake chub. Maximum trophic position varied within and among the assemblage types. In general *Pike* assemblages (maximum trophic positions between 3.8 and 4.9) and *Pike-perch* assemblages (maximum trophic positions between 3.9 and 4.7) had higher maximum trophic positions and more variation in maximum trophic position than *Minnow* assemblages (maximum trophic position between 3.5 and 3.9). Maximum trophic position in the offset lake was 3.9 - at the low range of maximum trophic position detected in *Pike* and *Pike-perch* assemblages and at the high range of maximum trophic position detected in *Minnow* assemblages (Figure 2.3c).

2.4.4 Littoral Resource Use

Mean alpha values (the proportion of littoral basal resource use) of fish populations varied from 9.1% to 84.7% across all sampled populations from the nine lakes. Fish populations in *Minnow* assemblages (mean alpha values between 62.0 and 84.7%) relied more heavily on littoral resources while fish populations in *Pike* assemblages (mean alpha values between 9.1 and 61.6%) and *Pike-perch* assemblages (mean alpha values between 27.0 and 68.1%) tended to have intermediate levels of littoral resource use. Fish populations in the offset lake had intermediate alpha values (between 26.1% and 47.9%), similar to *Pike* and *Pike-perch*

Table 2.2. Fish assemblages, sample sizes (N), and mean and standard deviation (SD) of $\delta^{13}C$ and $\delta^{15}N$ for fish populations and littoral and offshore baselines.

					$\delta^{13}C$		$\delta^{15}N$	
Lake	Assemblage	Species	Code	N	Mean	SD	Mean	SD
HZ	Offset	Arctic grayling (*Thyllamus arcticus*)	ARGR	5	-28.9	0.5	9.0	0.5
		Brook stickleback (*Culaea inconstans*)	BRST	10	-29.2	0.7	6.2	0.5
		Lake chub (*Couesius plumbeus*)	LKCH	8	-30.0	1.0	8.6	0.8
		Longnose sucker (*Catastomus catastomus*)	LNSC	8	-29.2	0.9	5.8	1.0
		Trout-perch (*Percopsis omiscomaycus*)	TRPR	9	-30.7	1.0	8.9	0.7
		White sucker (*Catatosmus commersonii*)	WHSC	9	-30.3	1.2	7.1	1.3
		Littoral baseline		5	-24.9	1.5	2.1	0.6
		Offshore baseline - zoopplankton		5	-33.7	0.5	2.8	0.2
CA	Minnow	Brook stickleback (*Culaea inconstans*)	BRST	6	-23.8	1.1	8.0	0.2
		Fathead minnow (*Pimephales promelas*)	FTMN	9	-23.6	0.3	7.7	0.2
		Littoral baseline		5	-24.2	2.6	3.3	0.3
		Offshore baseline - zoopplankton		5	-27.6	0.8	2.2	0.1
U5	Minnow	Brook stickleback (*Culaea inconstans*)	BRST	10	-32.2	1.0	9.8	1.1
		Finescale dace (*Chrosomus neogaeus*)	FNDC	10	-29.0	1.9	8.1	0.8
		Fathead minnow (*Pimephales promelas*)	FTMN	10	-31.6	1.6	8.3	1.1
		White sucker (*Catatosmus commersonii*)	WHSC	7	-31.1	1.1	9.5	0.5
		Littoral baseline		5	-27.7	2.1	3.0	0.9
		Offshore baseline - chironomids		5	-40.1	0.7	3.8	0.2
U2	Pike	Brook stickleback (*Culaea inconstans*)	BRST	3	-32.2	0.6	6.9	0.5
		Northern pike (*Esox lucius*)	NRPK	10	-33.6	0.5	9.7	0.7
		Littoral baseline		4	-31.0	1.3	1.6	0.3
		Offshore baseline - zoopplankton		5	-35.6	0.5	4.7	0.3
U1	Pike	Northern pike (*Esox lucius*)	NRPK	10	-27.1	0.4	11.8	1.0
		Spottail shiner (*Notropis hudsonius*)	SPSH	10	-28.2	0.2	9.8	0.5
		White sucker (*Catatosmus commersonii*)	WHSC	10	-27.3	1.0	9.8	0.4
		Littoral baseline		5	-22.7	0.9	3.3	0.3
		Offshore baseline - zoopplankton		5	-30.0	1.4	1.5	0.5
SB	Pike	Northern pike (*Esox lucius*)	NRPK	10	-25.8	0.5	11.6	0.9
		Spottail shiner (*Notropis hudsonius*)	SPSH	10	-27.9	0.5	9.8	0.2
		White sucker (*Catatosmus commersonii*)	WHSC	10	-24.8	1.7	9.1	0.5
		Littoral baseline		5	-23.0	2.3	5.1	0.3
		Offshore baseline - zoopplankton		5	-28.4	0.4	5.7	0.3
GW	Pike-perch	Lake whitefish (*Coregonus clupeaformis*)	LKWH	10	-25.2	2.2	11.2	0.9
		Northern pike (*Esox lucius*)	NRPK	9	-24.9	0.7	12.9	0.8
		White sucker (*Catatosmus commersonii*)	WHSC	10	-24.8	1.2	10.4	0.6
		Yellow perch (*Perca flavescens*)	YLPR	10	-24.6	3.1	10.4	2.3
		Littoral baseline		5	-22.8	1.6	3.6	0.4
		Offshore baseline - zoopplankton		6	-27.3	0.6	7.1	0.5

Table 2.2 (Con't)

Lake	Assemblage	Species	Code	N	$\delta^{13}C$ Mean	$\delta^{13}C$ SD	$\delta^{15}N$ Mean	$\delta^{15}N$ SD
KB	Pike-perch	Lake whitefish (*Coregonus clupeaformis*)	LKWH	10	-26.5	0.8	11.5	0.8
		Northern pike (*Esox lucius*)	NRPK	10	-25.9	0.7	12.3	1.1
		White sucker (*Catatosmus commersonii*)	WHSC	7	-24.8	1.1	10.4	0.5
		Yellow perch (*Perca flavescens*)	YLPR	10	-25.0	1.0	10.9	1.2
		Littoral baseline		5	-23.5	1.3	4.6	0.7
		Offshore baseline - zoopplankton		5	-28.3	0.2	6.9	0.7
WP	Pike-perch	Northern pike (*Esox lucius*)	NRPK	9	-26.3	0.5	11.6	0.8
		Spottail shiner (*Notropis hudsonius*)	SPSH	10	-25.3	1.1	9.5	0.8
		Trout-perch (*Percopsis omiscomaycus*)	TRPR	8	-25.0	1.5	10.5	0.7
		White sucker (*Catatosmus commersonii*)	WHSC	10	-25.9	1.8	8.9	1.1
		Yellow perch (*Perca flavescens*)	YLPR	10	-26.2	1.4	9.2	1.5
		Littoral baseline		5	-24.3	1.9	4.6	1.6
		Offshore baseline - zoopplankton		5	-29.5	0.2	0.6	0.3

assemblages (Figure 2.3d). Alpha range (basal resource diversity) was largest but the most variable in *Pike* assemblages (alpha ranges between 10.1 and 52.1%), intermediate in *Pike-perch* assemblages (alpha ranges between 12.6% and 35.4%), and lowest in *Minnow* assemblages (alpha ranges between 6% and 22.7%). In the offset lake alpha range was intermediate among the natural lakes at 21.8%.

2.4.5 Assemblage Type Trophic Structure and Relationship with Habitat Characteristics

When the trophic metrics are considered together, isotopic niche overlap range, mean isotopic niche overlap, isotopic niche size range, and mean isotopic niche size are the most important trophic metrics for separating between the assemblage types. *Pike-perch* assemblages have relatively large and *Pike* assemblages have relatively small mean isotopic niche size, mean isotopic niche overlap, isotopic niche size range, and isotopic niche overlap range. One *Minnow* assemblage (Calumet Lake) is different from the other lakes because it has low maximum trophic position, trophic position range, and alpha range values, and relatively high alpha values. Trophic structure in the offset lake is the most similar to the *Pike-perch* lakes, but is unique to all the natural lakes as it lays outside of the polygon formed by the natural lakes (Figure 2.4).

Indirect gradient analysis revealed some potential relationships between lake habitat characteristics and metrics of trophic structure. Nutrient concentrations were positively related to

alpha and negatively related to alpha range, maximum trophic position, mean isotopic niche size, and isotopic niche size range. Water clarity, lake maximum depth, and lake surface area were negatively related to alpha and positively related to alpha range, maximum trophic position, mean isotopic niche size, and isotopic niche size range. Mean isotopic niche overlap and isotopic niche overlap range were not related to any of the habitat characteristics (Figure 2.4).

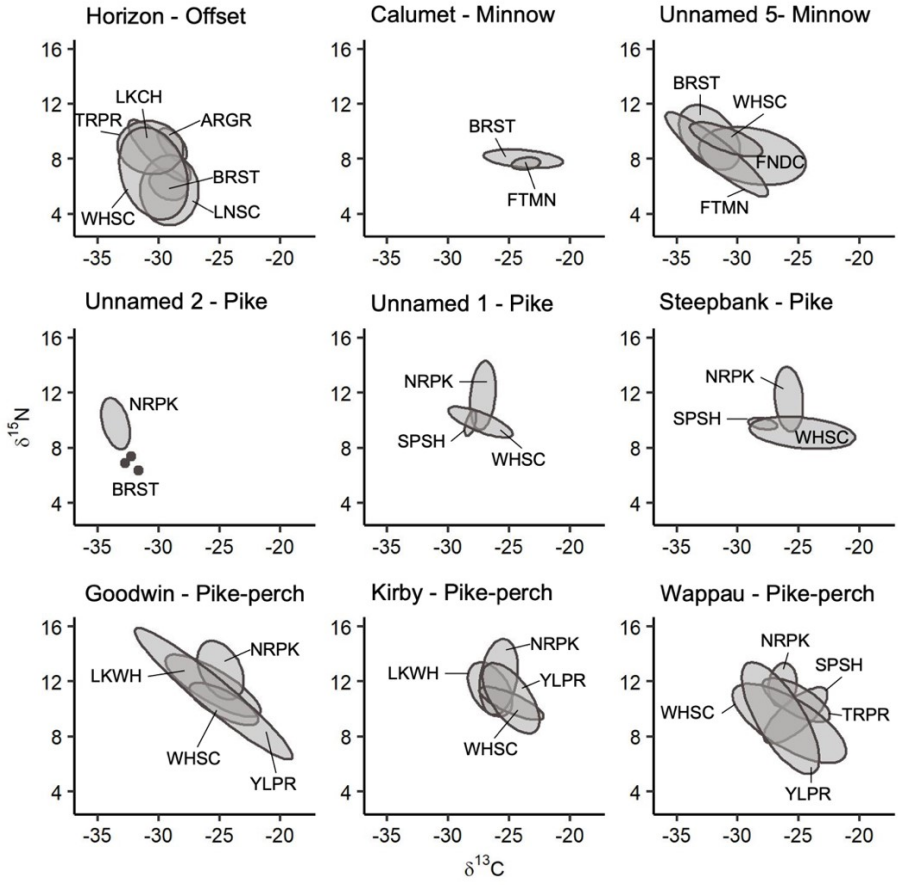

Figure 2.2. 95% ellipses representing species isotopic niches in the nine study lakes. Plots are labeled with lake names and assemblage types. Species isotopic niches are labeled with four-letter species codes: lake chub (LKCH), trout-perch (TRPR), arctic grayling (ARGR), brook stickleback (BRST), white sucker (WHSC), longnose sucker (LNSC), fathead minnow (FTMN), finescale dace (FNDC), northern pike (NRPK), spottail shiner (SPSH), lake whitefish (LKWH), yellow perch (YLPR).

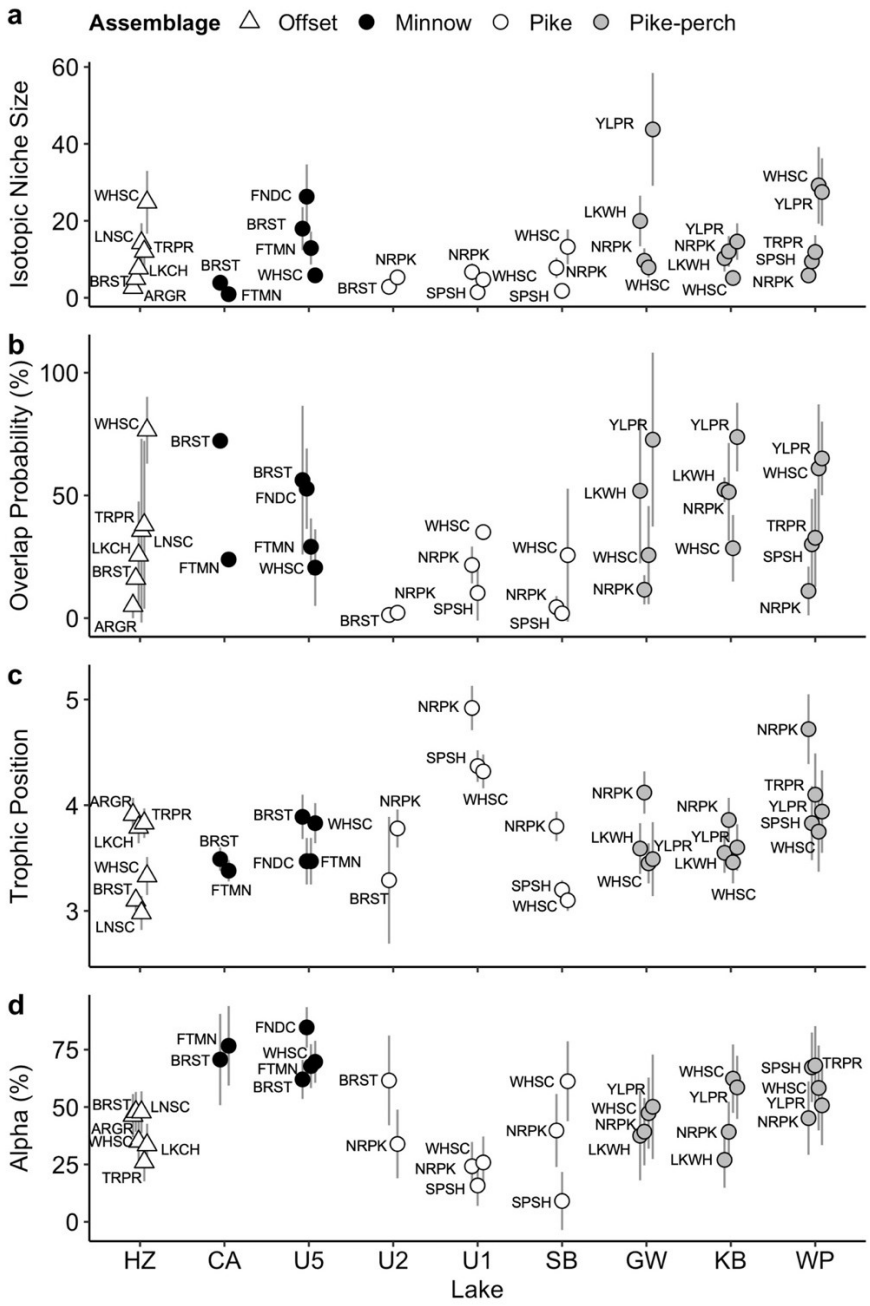

Figure 2.3. a) Mean isotopic niche sizes and standard deviations based on 1000 random draws from the posterior distribution of niche size estimates. b) Mean and standard deviation of the probability of finding individuals of other species within the 95% isotopic niche of each species. c) Mean trophic position and standard deviation based on the posterior distribution of trophic position estimates. d) Mean alpha values (percent littoral resource use) and standard deviation based on the posterior distribution of alpha estimates. Lakes are ordered by assemblage type (Offset, Minnow, Pike, Pike-perch) and identified with two-letter codes: Horizon Lake (HZ), Steepbank Lake (SB), Unnamed 2 (U2), Unnamed 1 (U1), Calumet Lake (CA), Unnamed 5 (U5), Wappau Lake (WP), Kirby Lake (KB), and Goodwin Lake (GW) and are

ordered by assemblage type (left to right): Offset, Minnow, Pike, Pike-perch. Points are labelled with the corresponding four-letter species codes: lake chub (LKCH), trout-perch (TRPR), arctic grayling (ARGR), brook stickleback (BRST), white sucker (WHSC), longnose sucker (LNSC), fathead minnow (FTMN), finescale dace (FNDC), northern pike (NRPK), spottail shiner (SPSH), lake whitefish (LKWH), yellow perch (YLPR).

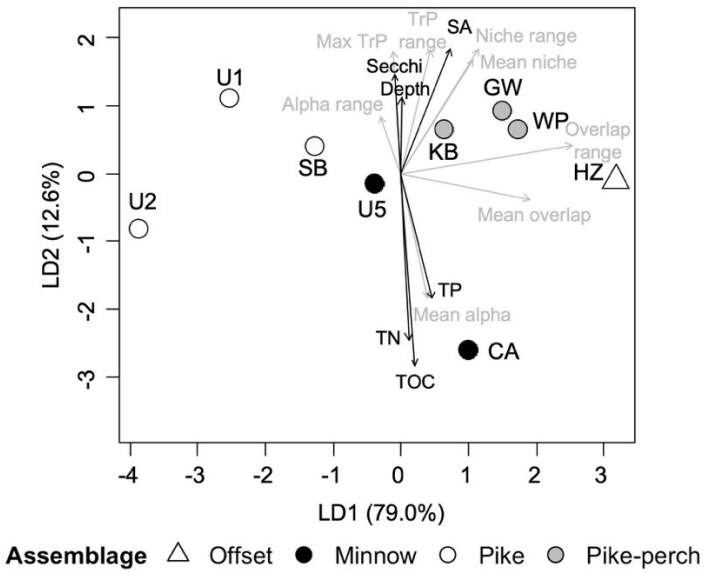

Figure 2.4. Linear discriminant analysis of trophic structure metrics with lakes grouped by assemblage type with an indirect gradient analysis of habitat characteristics on the trophic structure metrics. Symbols represent individual lakes and are labelled with lake two-letter codes: Horizon Lake (HZ), Steepbank Lake (SB), Unnamed 2 (U2), Unnamed 1 (U1), Calumet Lake (CA), Unnamed 5 (U5), Wappau Lake (WP), Kirby Lake (KB), and Goodwin Lake (GW). Grey vectors demonstrate the direction and strength of the relationship between trophic structure metrics and the new linear discriminant variables (LD1 and LD2). Trophic structure metrics are mean alpha (percent littoral resource use; Mean alpha), alpha range (Alpha range), maximum trophic position (Max TrP), trophic position range (TrP range), mean isotope niche size (Mean niche), isotopic niche size range (Niche range), mean isotopic niche overlap (Mean overlap), range isotopic niche overlap (Overlap range). Black vectors represent the strength and direction of the relationship between habitat characteristics and trophic structure metrics. Habitat characteristics are total organic carbon concentration (TOC), total nitrogen concentration (TN), total phosphorus concentration (TP), lake maximum depth (Depth), Secchi depth (Secchi), and lake surface area (SA).

2.5 Discussion

Examining trophic structure in large scale constructed offsets and comparing them to analogous natural ecosystems will be important for understanding their ecology and long-term sustainability. In a constructed lake in the Alberta oil sands, individual metrics of trophic structure were within the range of variation detected in natural lakes representing three natural fish assemblage types, despite the unique fish assemblage found in the offset (Ruppert *et al*., 2018). When all trophic structure metrics were considered together, the trophic structure in the offset lake was unique, but most closely resembled *Pike-perch* assemblages. A notable difference between the trophic structure of the offset lake and *Pike-perch* assemblages is the number of fish species occupying the top trophic position and the ecological traits of the species occupying this niche (Eakins, 2022). In *Pike-perch* assemblages this spot is consistently filled by one species, northern pike – a large bodied piscivore, while in the offset lake three species share the top trophic position – small to medium sized invertivore-carnivores. This is more like *Minnow* assemblages, where two or three species occupy the top trophic position. Trophic structure in the offset lake resembled one of the *Minnow* assemblages, lake Unnamed 5, in many trophic structure metrics as well, except that fish populations in Unnamed 5 rely more on littoral resources than fish populations in the offset lake. Fish populations in *Minnow* assemblages relied more heavily on littoral resources than populations in *Pike* and *Pike-perch* assemblages as well.

Habitat characteristics have a limited ability to explain the patterns of trophic structure observed in the natural and offset lakes. The offset lake was most similar to *Pike* lakes in regard to habitat characteristics but was quite different from *Pike* assemblages and more similar to *Pike-perch* assemblages in trophic structure. The most important trophic structure metrics separating the assemblage types was niche overlap and niche overlap range, but the habitat characteristics examined here did not relate to them. But, greater reliance on littoral resources by *Minnow* assemblages is related to shallow lake depth, relatively low water transparency, and relatively high nutrient concentrations. In *Pike* lakes, *Pike-perch* lakes, and the offset lake with deep pelagic areas, fish assemblages used both littoral and pelagic resources. Factors other than habitat characteristics, such as species richness, species ecological traits, other environmental variables, or the interactions among these factors were likely affecting trophic structure.

Large scale constructed offsets provide excellent opportunities for studying ecosystem structure and dynamics. In this study I examine how trophic structure varies across three natural fish assemblage types in addition to assessing the offset. I observed that fish populations in *Pike* assemblages consistently had smaller isotopic niches and less isotopic niche overlap compared to *Minnow* assemblages, *Pike-perch* assemblages, and the offset lake. Differences in fish species' functional roles between assemblage types could impact trophic structure. Smaller niches with less overlap in *Pike* assemblages could be due to relatively low species richness (2-3 species) combined with relatively high interspecific functional diversity, with northern pike being large-bodied piscivores, spottail shiners *Notropis hudsonius* (Clinton 1824) and brook sticklebacks being small bodied invertivore-planktivores, and white suckers being large bodied benthivores (Eakins, 2022). In comparison, *Pike-perch* assemblages, *Minnow* assemblages, and the offset lake had more functional redundancy among their species. Instead of partitioning resources, which would result in smaller niches and less overlap, it appears that increases in fish species richness in these northern boreal lakes is accompanied by increases in the niche sizes and niche overlap of their fish populations. This finding agrees with past studies that found competition was not a dominant factor determining species compoisition (Matthews, 1982; Jackson *et al*., 2001; Giam and Olden, 2016).

Globally, multiple offsetting frameworks aim to create habitat for high trophic level organisms such as fish. High trophic levels structure ecological communities and provide ecosystem services such as fishing and hunting opportunities, tourism, invasive species mitigation, and pest control (Hammerschlag *et al*., 2019). Attracting and retaining these species in restored and constructed habitats is therefore critical to ensuring these projects hold ecological and social value (Loch *et al*., 2020). Examining trophic structure and whether trophic interactions or energy sources have changed highlights the ecological impacts of human modification and places emphasis on high trophic level organisms (Jordan and Arrington, 2014; Wootton, 2012). I demonstrate that metrics of trophic structure of a constructed offset with a unique fish assemblage are within the range of values measured in natural lakes that support popular fisheries species, yet when all the metrics are examined together, the trophic structure of the offset is unique. This result that could not be assumed without directly investigating the fish assemblage trophic structure. Within the restoration ecology literature there are increasing calls for the inclusion of trophic structure in monitoring and assessment (Ruiz-Jaen and Aide, 2005, Fraser *et al*., 2015) and I support this messaging for habitat offsetting.

Comparing offsets to analogous natural habitats complements common monitoring practices aimed at assessing whether offsets have met their legal offsetting targets, (e.g.: achieving No Net Loss of fish habitat productive capacity but creating double the habitat area or fish production as was lost). Large scale offsetting projects will most often involve the construction of a single new habitat, not supporting replicated study designs that facilitate inferential statistical analyses. To account for the limitation of surveying only one constructed habitat offset, I aimed to capture the potential natural variation in lake fish assemblages by sampling eight natural lakes and used a Bayesian statistical approach to integrate sources of variation into calculations of trophic structure metrics. Ecological studies of future offsets could be improved by using a study design analogous to a Before-After-Control-Impact (BACI) design – where both the habitat that will be lost and natural reference sites that are analogous to the offset that will be created are monitored before impacts, and the offset habitat and the natural reference sights are monitored after construction (Block *et al*., 2001).

2.6 Conclusion

Constructing large scale habitat offsets to compensate for environmental damage is an increasingly common practice and understanding their ecology can improve the practice of habitat offsetting. In this study I examine the trophic structure of fish assemblages from the first constructed offset lake in the Alberta oil sands and eight natural lakes that support common fish assemblages for the region. Individual metrics of trophic structure measured in the offset lake were within the range of variation detected in natural lakes, and were similar to *Pike-perch* assemblages, which support multiple large-bodied fisheries species, and a *Minnow* assemblage, which supports small-bodied and benthivorous fish species, across most metrics. The fish assemblage in the offset lake is unique for the region, and when all metrics of trophic structure and habitat characteristics were assessed together, both the habitat and the trophic structure are unique compared to the natural lakes. Similar to the field of restoration ecology, monitoring and assessment of offsets can be improved by monitoring natural, analogous habitats to those created for offsetting and by examining trophic structure. These actions will connect offsetting with the big-picture goal of creating habitats that support biodiversity, complex ecosystems, and ecosystem services (such as fisheries, recreation, or simply offer a beautiful natural space to visit), in addition to meeting technical targets laid out in harm authorizations.

Chapter 3 Boreal Freshwater Fishes Use Multiple Strategies in Response to Seasonal Ice Coverage in Lakes

3.1 Abstract

Temporal trophic dynamics can support the long-term sustainability of ecological communities by reducing competition between consumers and releasing prey populations from predation at regular intervals. In this study I investigate the seasonal trophic responses of 13 fish populations (including 10 species) from four boreal lakes and compare the responses of fish in a constructed habitat offset to three natural lakes. I used carbon and nitrogen stable isotopes and stomach content analysis to look for evidence of seasonal diet maintenance, diet changes, or dormancy. All three trophic responses were detected, with seasonal diet change being the most common followed by diet maintenance and dormancy, while five populations fed in all seasons but displayed inconclusive evidence as to their seasonal trophic response. Fish populations in three natural lakes used diet change and diet maintenance and fish populations in the constructed habitat offset used diet change and dormancy. Flexibility in seasonal trophic responses could help support the sustainability of the offset's unique fish assemblage. For offsetting projects, understanding seasonal cycles in the type of ecosystem being constructed can guide design and management such that these cycles are preserved and promoted.

3.2 Introduction

Spatial and temporal food web dynamics stabilize ecological communities, supporting vital ecosystem functions and maintaining biodiversity (McCann *et al*., 2005). The temporal axis may be particularly important in ecosystems that experience large fluctuations in environmental conditions, such as in ecosystems with distinct seasons (McMeans *et al*., 2015). For example, high-latitude and high-altitude aquatic systems are in an open water state in the summer and an

ice-covered state in the winter. This includes approximately half of the world lakes (~50 000 000 lakes) (Verpoorter *et al*., 2014; Kirillin *et al*., 2011; Shuter *et al*., 2012), with ice cover often occurring for many months. Boreal lakes in North America (between 50-60º latitude) experience between 140 to 190 days of winter ice cover in a year (Kirillin *et al*., 2011), meaning resident organisms must respond annually to the seasonal changes associated with spending approximately half of the year in an open water state and half in an ice-covered state.

Winter ice cover has distinct impacts on the lake environment. It reduces light penetration, cuts off gas and material exchanges between water, land, and air, and is accompanied by a reduction in water temperature (Shuter *et al*., 2012). Traditionally, researchers expected these environmental changes to cause a decrease in primary and secondary productivity (Sommer *et al*., 1986) and thus a decrease in available dietary resources for fishes. Recent findings suggest that primary and secondary productivity under lake ice is higher than traditionally expected (although still less than what is observed in ice-free seasons) (Sommer *et al*., 2012; Hampton *et al*., 2017). In addition, it is known that many consumers continue to feed and hunt throughout the winter (McMeans *et al*., 2015). This calls into question how consumer diets in ice covered lakes respond to these seasonal fluctuations in environmental conditions and resource availability.

McMeans *et al*. (2015) propose three potential trophic responses to seasonal changes in resource availability: 1) Coupling – switching between relatively abundant resources seasonally, 2) Dormancy – reducing or stopping foraging and feeding seasonally, and 3) Omnivory – feeding upon greater or fewer trophic positions seasonally. I build on this framework to describe three potential trophic responses of boreal lake fishes to seasonal changes between open water and ice-covered conditions: 1) Diet maintenance – feeding on a similar diet in all seasons; 2) Diet change – feeding on different diets in different seasons, potentially via coupling or omnivory; and 3) Dormancy – feeding in some seasons and not in other seasons. Seasonal diet maintenance, diet change, and dormancy have been observed in tropical fishes responding to shifts between wet and dry seasons (Prejs and Prejs, 1987; Novakowski *et al*., 2008; McMeans *et al*., 2019). Less information is available on the seasonal trophic responses of freshwater fish in high-latitude and high-altitude ecosystems due to the challenges of sampling during the winter when water bodies are ice-covered (but see Hayden *et al*., 2014). How the diet and feeding behavior of fish changes in seasonally ice-covered lakes remains largely unknown.

In the Alberta oil sands, companies are compensating for the destruction of fish habitat due to the creation of open-pit mines by constructing small reservoirs as habitat offsets on or near mining lease sites. The long-term sustainability and value to society of constructed habitat offsets and other human-modified ecosystems, such as habitat restoration and enhancement projects, depends on our ability to create ecosystems with ecological structure and dynamics that resemble natural ecosystems. Given the important role of temporal trophic dynamics in structuring and stabilizing ecosystems, examining these dynamics in constructed offsets will provide information on the ecology of these novel ecosystems.

Our objectives are to look for evidence of diet maintenance, diet change, or dormancy in boreal freshwater fishes as responses to seasonal ice coverage and compare the responses of fishes in a constructed fisheries offset to natural lakes. I sampled populations from four boreal lakes in northeastern Alberta: three natural lakes and the first constructed offset lake in the Alberta oil sands, Horizon Lake. I use stable isotope- and stomach content-based evidence to examine how boreal fishes respond to seasonal variation in their environment.

3.3 Methods

3.3.1 Study Sites and Sample Collection

Fish were collected from four lakes in northeastern Alberta: Horizon Lake (57.587087, -111.963826), an unnamed lake (55.797951, -111.295620), Goodwin Lake (55.417562, -111.655308), and Steepbank Lake (55.477482, -111.579530). The four lakes vary in size, depth, and water quality (Table 1). All lakes experience approximately six months of open water (May – October) and six months of ice coverage (November – April). None of the lakes experienced winterkill during our study period.

Horizon Lake is a reservoir, constructed as a fisheries habitat offset on Canadian Natural Resources Limited's Horizon Oil Sands site near Fort McKay, Alberta. It was constructed and filled in 2008 and supports a unique fish community for the region (Ruppert *et al*., 2018). This allowed us to investigate seasonal trophic responses in a more diverse range of fishes than would have otherwise been possible.

Sampling took place over four sampling periods representing three seasons: March 2017 and February 2018 (winter), May-June 2017 (spring), and August-September 2017 (fall). Fish were captured with gillnets, minnow traps, and angling in the spring, fall, and winter, as well as fyke nets and electrofishing in the spring and fall. Captured fish were identified to species, weighed, and had their lengths measured. In each season up to ten individuals per population (species within a lake) were euthanized with an overdose of MS-222 prior to stable isotope and stomach content analysis. Euthanized fish were stored on ice until they could be stored frozen at -20°C until dissected.

Fish collections were done following guidelines developed by the Canadian Council on Animal Care for the use of fish in research. Fish collections were authorized by Alberta Environment and Parks under Research Licenses 16-444, 16-1819, 17-1803, 17-0403, 17-1802, and 17-0443, and with ethics approval from the University of Alberta Research Ethics Office under Animal Use Protocol 00001547.

In each season one water sample was collected from each of the sampling lakes using a VanDorne sampler. Water was collected from 1m off the bottom, the thermocline or the mid-way point of the water column if no thermocline was present, and 1m below the water surface (in the spring and fall) or below the surface ice (in the winter). Equal parts of each water sample were combined to create one integrated water sample for each lake in each season. Water samples were stored frozen until they could be analyzed at the Natural Resource Analytical Laboratory at the University of Alberta for measurement of total organic carbon (TOC), total nitrogen (TN), and total phosphorus (TP). TOC and TN were measured using a Shimadzu TOC-L CPH Model Total Organic Carbon Analyzer with an ASI-L and TNM-L (Shimadzu Corporation, Jiangsu China). TOC was measured as non-purgeable organic carbon by fist acidifying the sample with 1M HCl, sparging the sample, injecting the sample into a combustion tube at 720°C with platinum catalyst beads where a redox reaction occurs that evolves CO_2, which is measured by a non-dispersive infrared detector for carbon. TN was measured by combusting the sample to NO and NO_2, then reacting it with ozone to form NO_2 in an excited state and measuring the photon emissions with a Chemiluminescence detector. TP was measured using inductively coupled plasma-optical emission spectroscopy with a Thermo iCAP6300 Duo inductively coupled plasma-optical emission spectrometer (Thermo Fisher Corp., Cambridge, United Kingdom). Surface area measurements came from shapefiles of surface waterbodies available from the Government of Alberta. The maximum depth of each lake was measured using a transducer

during hydroacoustic surveys that measured the depth of the lake along transects spread 50m apart. Secchi depth was measured on the shady side of the boat by taking the average of the depth at which the secchi disk was no longer visible and the depth at which it re-appeared.

Table 3.1. Lake physical and water quality characteristics.

Lake	Lake code	Lat	Lon	Surface area (ha)	Max depth (m)	Season	TOC[1] (mg/L)	TN[2] (mg/L)	TP[3] (mg/L)
Horizon*	HZ	57.385	-111.962	76.7	18	Fall	24.39	0.53	0.033
						Winter	26.00	0.56	0.014
Unnamed	UN	55.797	-111.292	36.5	4	Spring	23.13	0.49	0.018
						Fall	23.09	0.51	-
						Winter	28.74	0.68	0.045
Steepbank	SB	55.478	-111.575	193.5	17	Spring	11.85	0.5	-
						Fall	13.36	0.53	0.015
						Winter	14.59	0.61	0.005
Goodwin	GW	55.418	-111.657	864.6	15	Spring	12.49	0.34	0.028
						Fall	13.01	0.38	-
						Winter	16.74	0.56	0.012

* Offset lake

[1] TOC = Total organic carbon

[2] TN = total nitrogen

[3] TP = Total phosphorus

Blank fields (-) were below detection limit

3.3.2 Stable Isotope Analysis

I used liver tissue for stable isotope analysis. Liver tissue has a fast isotopic turnover rate, making it appropriate for the study of seasonal dietary changes (Logan *et al*., 2006; Buchheister and Latour, 2010; Barton *et al*., 2019). Because it is metabolically active it is representative of fishes' diets during the winter, unlike other commonly used tissues (Perga and Gerdeaux, 2005). Liver tissue was freeze dried for 24h and homogenized using a mortar and pestle or a metal rod while keeping the sample inside a glass scintillation vial.

Fractionation of carbon isotopes during lipid synthesis elevates $\delta^{13}C$ values of lipids relative to a consumer's diet. To account for this, lipid normalization via chemical extraction or mathematical corrections is recommended when analysing lipid-rich tissues such as liver (Skinner *et al*., 2016). When sufficient liver tissue was available, samples were divided into two and lipids were extracted from one of the sub-samples using a 2:1 methanol:chloroform bath (Bligh and Dyer, 1959). First, samples were freeze dried for 24h and homogenized to a fine powder using a mortar and pestle. The powdered tissue was covered with the chloroform:methanol solution and the sample tube was inverted several times. The samples were left to settle for 15 minutes, centrifuged at 2000rpm for 5 minutes, and the solution was decanted from the sample. This process was repeated 2-5 times per sample, until the solution appeared clear after soaking the sample for 15 minutes. Lipid extracted samples were dried at 60ºC overnight and were re-homogenized prior to being weighed for stable isotope analysis.

I weighed 0.2±0.05mg or 1±0.05mg of dried liver into 4mm tin capsules and sent them to the Natural Resources Analytical Laboratory (NRAL), University of Alberta, or the Biogeochemical Analytical Service Laboratory (BASL), University of Alberta, respectively, for carbon and nitrogen stable isotope analysis. NRAL used a Delta V Advantage Isotope Ratio Mass Spectrometer coupled to a ThermoScientific Flash 2000 Elemental Analyser and ran protein every 12 samples as an in-house $\delta^{13}C$ (-27.03 ± 0.02‰) and $\delta^{15}N$ (6.02 ± 0.02‰) standard to check for instrument error. BASL used a Vario Pyrocube elemental analyzer coupled to an Elemental IsoPrime vision continuous-flow isotope ratio mass spectrometer and ran NIST 8415 whole egg powder SRM every 20 samples as an in-house $\delta^{13}C$ (-23.99 ± 0.01‰) and $\delta^{15}N$ (6.89 ± 0.2‰) standard to test for instrument error. I ran 38 repeat samples to test our homogenization processes. The mean difference and standard deviation for $\delta^{13}C$ was 0.1 ± 0.3‰ and $\delta^{15}N$ was 0.1 ± 0.2‰.

Stable isotope analysis was run on lipid extracted and untreated samples, and I used $\delta^{13}C$ values from the lipid extracted samples and $\delta^{15}N$ values from untreated samples. Samples that did not have sufficient mass to be split in two were run with either the lipids extracted or untreated. Species with some liver samples that were too small to split into two samples were brook stickleback *Culaea inconstans* (Kirtland 1840), finescale dace *Chrosomus neogaeus* (Cope 1867), fathead minnow *Pimephales promelas* (Rafinesque 1820), lake chub *Couesius plumbeus* (Agassiz 1850), trout-perch *Percopsis omiscomaycus* (Walbaum 1792), white sucker *Catostomus commersonii* (Lacépède 1803), and yellow perch *Perca flavescens* (Mitchill 1814). I

used samples from these species that had stable isotope values measured on untreated and lipid extracted fractions to develop arithmetic lipid corrections. Following Post *et al*. (2007), I used the linear relationship between the C:N ratio and the difference between untreated $\delta^{13}C$ values and lipid extracted $\delta^{13}C$ values to develop the arithmetic correction. The C:N ratios of brook stickleback samples were higher than for all other species, so I developed two arithmetic lipid corrections: one for brook stickleback and another for the remaining species. For brook sticklebacks, the arithmetic lipid correction took the form of:

$$\delta^{13}C_{cor} = 0.14 * C:N + 1.16 + \delta^{13}C$$

For the remaining species, the arithmetic lipid correction took the form of:

$$\delta^{13}C_{cor} = 0.71 * C:N - 0.71 + \delta^{13}C$$

I found a significant difference between the $\delta^{15}N$ values of samples when they were untreated vs lipid extracted (mean difference = -0.24, paired t-test, $p < 0.05$). To correct the $\delta^{15}N$ values of small mass samples that were only run with lipids extracted, I subtracted 0.24 from the lipid-extracted $\delta^{15}N$ value to estimate the untreated $\delta^{15}N$ value.

3.3.3 Stomach Content Analysis

Sampled fishes had their stomachs removed and frozen. I identified and enumerated diet items taxonomically to Order or Family and recorded when stomachs were empty. Diet items were then grouped into the following 11 categories: fish, terrestrial invertebrates, benthic macroinvertebrates, *Chaoborus*, zooplankton, eggs, bryozoa, phytoplankton, filamentous algae, detritus, and unknown. Stomach content items included in each of the 11 groups included:

- **Fish**: all fish species or fish body parts.
- **Terrestrial invertebrates**: all terrestrial invertebrates, including emerged adult invertebrates with juvenile aquatic stages.
- **Benthic macroinvertebrates**: all invertebrates that live on or near the lake bottom.
- ***Chaoborus****:* juvenile stages of Chaoboridae flies which perform a characteristics diel migration from the deep, profundal areas of lakes to the pelagic zone.
- **Zooplankton**: copepods, cladocerans, and Anostracoda.
- **Eggs**: fish and invertebrate eggs.
- **Bryozoa**: bryozoans.
- **Phytoplankton**: phytoplankton.
- **Filamentous algae**: filamentous algae known to occur in the littoral zone.
- **Detritus**: sediment, decaying aquatic and terrestrial vegetation.

- **Unknown**: unidentifiable diet items.

3.3.4 Evidence of Trophic Responses

I developed stable isotope and stomach content-based metrics as evidence of the three seasonal trophic responses. Because of the known shortcomings of both data types (Nielsen *et al*., 2017), I required a population to display evidence of a particular trophic response from both data types to suggest that the population is likely using that response type. Diet maintenance was evidenced by no significant differences in carbon and nitrogen stable isotope values between seasons and similar stomach contents between seasons (seasonal changes in mean relative abundance of diet items are less than 20%). Diet change was evidenced by significant differences in carbon and/or nitrogen stable isotope values between seasons and changes in stomach contents between seasons (at least one diet item changes by more than 20% between at least two seasons). I selected a threshold of 20% for classifying variation in stomach contents as diet changes vs. diet maintenance to allow some natural variation in capture rates of prey types, but to still recognize that relatively small changes in diet can be ecologically significant (Takimoto *et* al., 2002; Kondoh, 2003) (e.g.: requiring a diet change of 50% could miss ecologically important changes in consumer diet). The species included in this study are considered warm to cold water species (with preferred water temperatures between 11°C and 30°C (Magnuson *et al*., 1979)) whose high productivity season is in the summer or fall (Shuter *et al*., 2012). I assume that seasonal trophic dormancy occurs in winter if populations use this strategy. Winter dormancy was evidenced by significantly higher nitrogen stable isotope values in winter compared to the spring or fall, as I expect $\delta^{15}N$ to increase if a fish is metabolising their energy reserves (Doi *et al*., 2017), and more empty stomachs in the winter compared to the spring or fall.

3.3.5 Data Visualization and Analysis

Outliers in the stable isotope data were investigated via visual assessment using boxplots, where outliers are more than 1.5 times the interquartile range above or below the upper or lower quartile, respectively. ANOVA is sensitive to outliers in the dataset. I suspected that samples were contaminated, impacted by their processing or storage, or were impacted by errors during mass spectrometry if they were outliers in both $\delta^{13}C$ and $\delta^{15}N$. Thus these samples were removed from the data set, resulting in the removal of eight fish from the dataset.

The $\delta^{13}C$ and $\delta^{15}N$ values of each population were compared across sampling seasons visually and using one-way ANOVAs. When a population was sampled in three seasons and significant differences between seasons were detected, Tukey's HSD test was performed to identify which seasons differed.

The proportion of a population's diet coming from different diet item categories was assessed by calculating the mean relative abundance each category contributed to each season's diet. Seasonal variation in diet was assessed visually using bar plots. I compared the proportion of empty stomachs between seasons for each population.

3.4 Results

Ten species were captured in multiple seasons across the three natural lakes and one constructed offset lake, resulting in 13 populations with large enough sample sizes to assess seasonal trophic responses (Table 3.2). I found evidence of all three of the proposed trophic responses across the 13 populations (Figure 3.1). Diet change was evidenced by six populations and was the most common response. Populations from all four lakes displayed the diet change response type. Diet maintenance and dormancy were each evidenced by one population. The seasonal trophic response of five populations could not be categorized due to inconsistency between the stable isotope-based evidence and the stomach content-based evidence. The populations had significant differences in $\delta^{13}C$ and/or $\delta^{15}N$ between seasons (Table 3.3), but similar stomach contents between seasons. Three populations had no significant difference in $\delta^{13}C$ or $\delta^{15}N$ between seasons, but stomach contents changed between seasons. Out of five fish populations sampled in the constructed offset, Horizon Lake, two displayed seasonal diet changes, one displayed dormancy, and two displayed inconclusive evidence. Out of eight fish populations sampled in natural lakes, four displayed seasonal diet changes, one displayed diet maintenance, and three displayed inconclusive results.

Stable isotope and stomach content-based evidence suggested that lake chub and longnose suckers *Catostomus Catostomus* (Forster 1773) from Horizon Lake, finescale dace and white suckers from Unnamed Lake, northern pike *Esox Lucius* L. 1758 from Steepbank Lake, and northern pike from Goodwin Lake change their diet seasonally (Figure 3.1). These populations had significant differences in $\delta^{13}C$ and/or $\delta^{15}N$ between at least two seasons (Table 3.3) and a change in the mean relative abundance of 20% or greater for at least one diet item between at

least two season. They displayed little or no seasonal variation in the proportion of empty stomachs, suggesting these population were not using seasonal dormancy.

Stable isotope and stomach content-based evidence suggested that yellow perch from Goodwin Lake maintain their diets across seasons (Figure 3.1). This population had no difference in $\delta^{13}C$ or $\delta^{15}N$ between any of the seasons (Table 3.3) and stomach contents remained consistent across seasons. They displayed little or no seasonal variation in the proportion of empty stomachs, suggesting they were not using seasonal dormancy.

Stable isotope and stomach content-based evidence suggested that fathead minnows in Horizon Lake go dormant in the winter (Figure 3.1). $\delta^{15}N$ increased significantly in the winter (Table 3.3), and stomach contents showed an increase in the proportion of empty stomachs in the winter.

Brook stickleback and trout-perch from Horizon Lake, brook stickleback and fathead minnow from Unnamed Lake, and lake whitefish *Coregonus clupeaformis* (Mitchill 1818) from Goodwin Lake could not be categorized into a seasonal trophic response because of inconclusive evidence. Brook stickleback and trout-perch from Horizon Lake and lake whitefish from Goodwin Lake had significant differences in $\delta^{13}C$ or $\delta^{15}N$ between seasons, but stomach contents were similar between seasons. Brook stickleback and fathead minnow from Unnamed Lake had no difference in $\delta^{13}C$ or $\delta^{15}N$ between seasons, but stomach contents changed between seasons. These populations did not have a higher proportion of empty stomachs in the winter compared to the spring or fall, and thus I did not suspect they were using dormancy as a strategy.

Table 3.2. Species captured and sample sizes (n) for stable isotope analysis (SIA) and stomach content analysis (SCA).

Lake	Season	Species	n SIA	n SCA
Horizon	Spring	Trout-perch	9	5
	Fall	Brook stickleback	10	10
		Fathead minnow	4	10
		Lake chub	10	10
		Longnose sucker	8	10
		Trout-perch	10	10
	Winter	Brook stickleback	7	4
		Fathead minnow	5	5
		Lake chub	10	10
		Longnose sucker	10	9
		Trout-perch	10	10
Unnamed	Spring	Brook stickleback	10	5
		Finescale dace	10	10
		Fathead minnow	6	10
	Fall	Brook stickleback	10	10
		Finescale dace	10	10
		Fathead minnow	5	10
		White sucker	10	10
	Winter	Brook stickleback	10	10
		Finescale dace	10	10
		Fathead minnow	6	10
		White sucker	7	7
Steepbank	Spring	Northern pike	10	9
	Fall	Northern pike	10	10
	Winter	Northern pike	10	10
Goodwin	Spring	Lake whitefish	10	4
		Northern pike	10	5
		Yellow perch	10	10
	Fall	Lake whitefish	10	10
		Northern pike	9	9
		Yellow perch	8	10
	Winter	Lake whitefish	10	10
		Northern pike	9	10
		Yellow perch	10	10

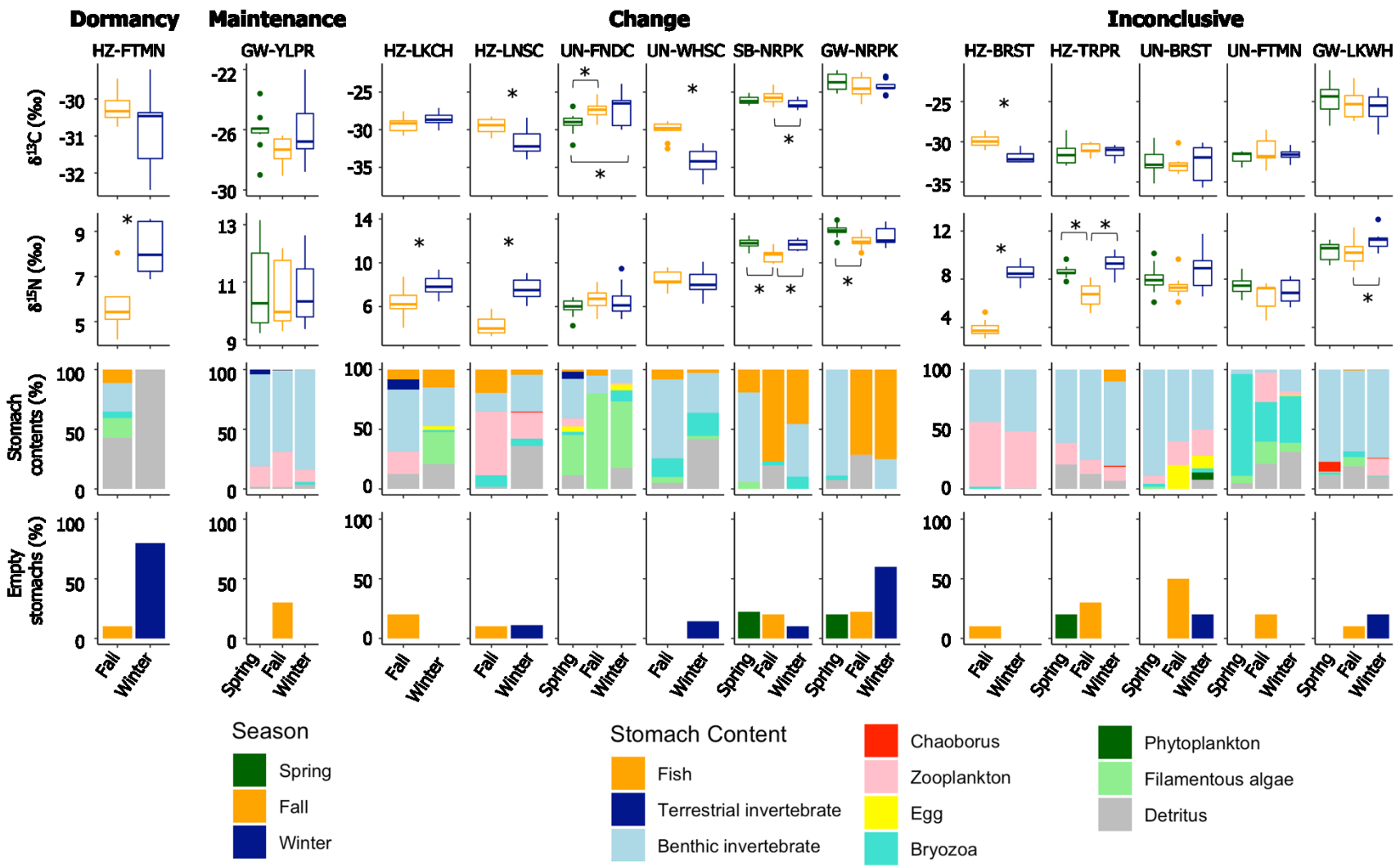
Dormancy
Maintenance
Change
Inconclusive
HZ-FTMN
GW-YLPR
HZ-LKCH
HZ-LNSC
UN-FNDC
UN-WHSC
SB-NRPK
GW-NRPK
HZ-BRST
HZ-TRPR
UN-BRST
UN-FTMN
GW-LKWH
δ13C (‰)
δ15N (‰)
Stomach contents (%)
Empty stomachs (%)
Fall
Winter
Spring
Season
Spring
Fall
Winter
Stomach Content
Fish
Terrestrial invertebrate
Benthic invertebrate
Chaoborus
Zooplankton
Egg
Bryozoa
Phytoplankton
Filamentous algae
Detritus

Figure 3.1. Stable isotope and stomach content-based evidence of seasonal trophic responses for study populations using dormancy, maintenance, and diet change or with inconclusive results. Asterisks denote significant differences between seasons (alpha=0.05). Each column of figures is for a study population and are labelled as “two-letter lake code”-“four-letter species code”. Two letter lake codes are: Horizon Lake (HZ), Unnamed Lake (UN), Steepbank Lake (SB), and Goodwin Lake (GW). Four letter species codes are fathead minnow (FTMN), brook stickleback (BRST), yellow perch (YLPR), lake chub (LKCH), longnose sucker (LNSC), trout-perch (TRPR), finescale dace (FNDC), white sucker (WHSC), northern pike (NRPK), and lake whitefish (LKWH).

3.5 Discussion

The importance of seasonal dynamics to ecosystem health and long-term sustainability has been hypothesized (McMeans *et al*., 2015) and the observed for tropical (McMeans *et al*., 2019; Prejs and Prejs, 1987), temperate (McMeans *et al*., 2020), and subarctic (Amundsen and Knudsen, 2009; Hayden *et al*., 2014) freshwater fishes. Here I provide evidence that multiple species with a diversity of traits, from multiple lakes with different physical characteristics employ similar types of trophic dynamics. Fish populations in the three natural lakes used two of the proposed trophic responses, with seasonal diet change being the most common followed by diet maintenance. Fish populations in the constructed habitat offset mainly indicated seasonal diet changes with one population using dormancy.

Evidence from a wide range of biomes and habitat types suggests that most freshwater fishes are generalist feeders that display flexible foraging in response to changes in prey availability (Little *et al*., 1998; Gerdeaux *et al*. 2002; Xu *et al*., 2012). Our study aligns with this, with seasonal diet change being the most common response observed. Among populations that use diet change, there is evidence of both omnivory, feeding on greater or fewer trophic positions seasonally, and coupling, switching between relatively abundant resources in different seasons (McMeans *et al*., 2015). This was the case for fish populations in both the natural lakes and Horizon Lake. Evidence of these trophic dynamics within Horizon Lake is promising since diet subsidies and adaptive foraging can stabilize complex food webs (Takimoto *et* al., 2002; Kondoh, 2003; Kratina *et al*., 2012), supporting the long-term goal of producing a sustainable ecosystem that supports desired functions and services.

Seasonally stable diets have also been observed in various habitats and species (Gimenes *et al*., 2013; Novakowski *et al*., 2008) and were observed in one population. Populations classified

as using diet change make large enough seasonal changes in their diet, behavior, and physiology to result in a significant difference in their stable isotope ratios and display some changes in their stomach contents between seasons. In contrast, the population classified as using diet maintenance did not make large enough changes in diet, behavior, or physiology to result in a significant difference in their stable isotope ratios and display small changes in their stomach contents. I classified these populations into discrete categories, but the seasonal trophic responses of fishes may be more or equally well understood as lying on a continuum of full diet coupling (completely different diets between seasons), to omnivory (seasonal diet subsidies), to full diet maintenance (diet remains the same across seasons). This proposition is supported by the number of populations displaying inconclusive evidence – where stable isotope and gut content analysis do not align to suggest the same seasonal trophic response to being employed. Populations may use different trophic responses or trend towards a different trophic response in different years. Multi-year studies on seasonal trophic responses could highlight whether a particular seasonal trophic response is characteristic of some species or populations, or if populations commonly adjust their trophic responses based on environmental conditions, resource availability, or other factors.

Traditionally, it was expected that in the dark, cold water under lake ice productivity would decrease substantially, and so would the activity of many consumers. This view has been challenged by the observation of fish and other organisms that remain active under ice cover (Amundsen and Knudsen, 2009; Shuter et *al*., 2012; Hayden *et al*., 2014; McMeans *et al*. 2020) and our findings also challenge this notion. I observe only one population, fathead minnow in Horizon Lake, using dormancy, while the other population of fathead minnow continues to feed over winter. The difference between these two populations could be due to predation risk (Prejs and Prejs, 1987) since fathead minnows are very sensitive to predation (Savino and Stein, 1989). Habitat structure in the littoral zone that alleviates predation pressure such as macrophytes and woody debris (Savino and Stein, 1989; Sass *et al*., 2006) may not be accessible in the winter due to ice, increasing predation risk relative to open water seasons. In Unnamed Lake, the assemblage lacks piscivorous species, and thus predation risk is low. In Horizon Lake, several species including lack chub and arctic grayling are able to consume small fish, therefore predation risk for fathead minnows could outweigh the benefits of actively feeding throughout the winter.

The current study takes advantage of indirect evidence of habitat use and fish behavior. Direct observation (ex: using cameras, hydroacoustics, or observing an experimental setup) is time-consuming and logistically challenging, particularly in the winter, but would provide additional evidence on what strategies fishes use in response to seasonality. In our study, dormancy could be under-represented because dormant fish may be less active and thus more difficult to catch (Dupuch and Magnan, 2011). A fourth option for responding to seasonal environmental changes that was not examined here is migration. In three out of four lakes some species were excluded from the study because they were not caught during the winter. This could be indirect evidence of dormancy or migration by these species. Future studies could employ additional methods to confirm the use of diet changes and maintenance by many populations and illuminate whether and under what conditions dormancy and migrations are used.

3.6 Conclusion

Natural habitats are increasingly degraded by anthropogenic disturbances such as habitat loss and alteration, climate change, and invasive species. In response, potentially positive anthropogenic impacts such as habitat restoration and habitat construction are occurring. Understanding the temporal dynamics of such ecosystems will help us evaluate both destructive and constructive anthropogenic impacts on ecosystems. The current study is novel because it examines multiple species from multiple lakes across multiple seasons, including the difficult to access ice covered season, and includes a constructed offsetting habitat. Evidence of temporal dynamics within the offset that are similar to what is observed in natural ecosystems is promising, while evidence of dormancy in one species may be informative about the direct and indirect interactions occurring within the offset. For restoration or offsetting projects, understanding seasonal cycles in the type of ecosystem you aim to create or modify can guide design and management, such that these cycles are preserved and promoted. For example, it could encourage managers to construct appropriate over-wintering habitats and include habitat that supports important winter food sources for fishes of interest.

Table 3.3. ANOVA and Tukey test results for comparisons of $\delta^{13}C$ and $\delta^{15}N$ between seasons. Bold text denotes significant differences between seasons.

			ANOVA					Tukey test				
Dependent variable	Lake	Species	df Season	df Residuals	F	P	Season pair	Difference	Lower	Upper	P adj	Notes
$\delta^{13}C$	**SB**	**NRPK**	**2**	**27**	**3.49**	**0.05**	Fall-Spring	0.32	-0.48	1.12	0.59	
							Winter-Spring	-0.52	-1.32	0.28	0.26	
							Winter-Fall	**-0.84**	**-1.64**	**-0.04**	**0.04**	
	GW	NRPK	2	25	1.18	0.32						
		LKWH	2	27	0.68	0.52						
		YLPR	2	25	2.09	0.15						
	U5	FTMN	2	14	0.49	0.63						
		FNDC	**2**	**27**	**4.43**	**0.02**	**Fall-Spring**	**1.85**	**0.04**	**3.67**	**0.04**	
							Winter-Spring	**1.92**	**0.10**	**3.74**	**0.04**	
							Winter-Fall	0.07	-1.75	1.88	1.00	
		BRST	2	27	0.08	0.93						
		WHSC	**1**	**15**	**29.91**	**0.00**	**Fall-winter**					2 seasons
	HZ	**BRST**	**1**	**15**	**25.86**	**0.00**	**Fall-Winter**					2 seasons
		FTMN	1	7	0.80	0.40						
		TRPR	2	26	0.74	0.49						
		LKCH	1	18	2.12	0.16						
		LNSC	**1**	**16**	**10.51**	**0.01**	**Fall-winter**					2 seasons
$\delta^{15}N$	SB	**NRPK**	**2**	**27**	**13.32**	**0.00**	**Fall-Spring**	**-1.12**	**-1.71**	**-0.53**	**0.00**	
							Winter-Spring	-0.12	-0.71	0.47	0.86	
							Winter-Fall	**1.00**	**0.41**	**1.59**	**0.00**	
	GW	**NRPK**	**2**	**25**	**4.76**	**0.02**	**Fall-Spring**	**-1.02**	**-1.85**	**-0.19**	**0.01**	
							Winter-Spring	-0.58	-1.41	0.25	0.21	
							Winter-Fall	0.44	-0.41	1.29	0.41	
		LKWH	**2**	**27**	**4.20**	**0.03**	Fall-Spring	-0.12	-1.09	0.85	0.95	
							Winter-Spring	0.92	-0.05	1.89	0.07	
							Winter-Fall	**1.04**	**0.07**	**2.01**	**0.03**	
		YLPR	2	25	0.20	0.89						
	U5	FTMN	2	14	1.10	0.36						
		FNDC	2	27	1.13	0.34						
		BRST	2	27	2.66	0.09						
		WHSC	1	15	0.36	0.56						
	HZ	**BRST**	**1**	**15**	**173.00**	**0.00**	**Fall-Winter**					2 seasons
		FTMN	**1**	**7**	**6.60**	**0.04**	**Fall-winter**					2 seasons
		TRPR	**2**	**26**	**26.91**	**0.00**	**Fall-Spring**	**-1.94**	**-2.87**	**-1.00**	**0.00**	
							Winter-Spring	0.65	-0.28	1.59	0.21	
							Winter-Fall	**2.59**	**1.68**	**3.50**	**0.00**	
		LKCH	**1**	**18**	**8.05**	**0.01**	**Fall-winter**					2 seasons
		LNSC	**1**	**16**	**54.06**	**0.00**	**Fall-Winter**					2 seasons

Chapter 4 Season and Species Influence Stable Isotope Ratios Between Lethally and Non-lethally Sampled Tissues in Freshwater Fish

4.1 Abstract

The field of stable isotope ecology is moving away from lethal sampling (internal organs, muscle) towards non-lethal sampling (fins, scales, epidermal mucous) of fish. Lethally and non-lethally sampled tissues often differ in their stable isotope ratios due to differences in metabolic turnover rate and isotopic routing. If not accounted for when using non-lethal tissues, these differences may result in inaccurate estimates of resource use and trophic position derived from stable isotopes. To address this, I tested whether tissue type, season, and their interaction influence the carbon and nitrogen stable isotope ratios of different species of fishes, and whether estimates of species trophic position and resource use are affected by tissue type, season, and their interaction. I developed linear conversion relationships between two fin types and dorsal muscle, accounting for seasonal variation. I focused on three common temperate freshwater fishes: northern pike *Esox lucius,* yellow perch *Perca flavescens,* and lake whitefish *Coregonus clupeaformis.* I found that fins were enriched in ^{13}C and depleted in ^{15}N compared to muscle in all three species, but the effect of season and the interaction between tissue type and season was species and isotope dependent. Estimates of littoral resource use based on fin isotope ratios were between 13% and 36% greater than estimates based on muscle across species. Season affected this difference for some species, suggesting the potential importance of using season-specific conversions when working with non-lethal tissues. Fin and muscle stable isotopes produced similar estimates of trophic position for northern pike and yellow perch, but fin-based estimates were 0.2-0.4 trophic positions higher than muscle-based estimates for lake whitefish. The effect of season was negligible for estimates of trophic position

in all species. Strong correlations existed between fin and muscle $\delta^{13}C$ and $\delta^{15}N$ values for all three species, thus linear conversion relationships were developed. The results of this study support the use of non-lethal sampling in stable isotope studies of fishes. I suggest researchers use tissue conversion relationships and account for seasonal variation in these relationships when differences between non-lethal tissues and muscle, and seasonal effects on those differences, are large relative to the scale of isotope values under investigation and/or the trophic discrimination factors under use.

4.2 Introduction

Stable isotope analysis is an effective tool for studying the diet, movement, and habitat use of fishes (ex: Gu *et al*., 1996; Robillard *et al*., 2011; Trueman *et al*., 2012). The most used tissue for stable isotope analysis of fishes is white dorsal muscle, which often requires lethal sampling to obtain enough for analysis. The need for lethal sampling precludes the use of stable isotope analysis when studying rare or endangered species, valuable sport fishes, or species in areas where permitting for lethal sampling is difficult to obtain. The reasons for avoiding lethal sampling are compounded and extended to less vulnerable species when multiple sampling events are desired—such as in the case of multi-season or multi-year studies—and when focusing on vulnerable periods in life history—such as during winter, spawning, or migrations.

Tissues that can be sampled non-lethally, such as fins, scales, and epidermal mucous, are alternatives to dorsal muscle for stable isotope analysis (Hette-Tronquart *et al*., 2012; McCloskey *et al*., 2018; Sanderson *et al*., 2009). These tissues have different chemical compositions and are formed through different metabolic processes than dorsal muscle, potentially resulting in different isotope ratios (DeNiro and Epstein, 1977; Macko *et al*., 1986). Since our understanding of the patterns of isotope enrichment and depletion in fishes is predominantly based on white dorsal muscle, understanding the difference introduced into isotope data when using non-lethal tissues is necessary for interpreting isotope data and isotope-based ecological characteristics. Hayden *et al*. (2017) found that estimates of autochthonous resource use in tropical fishes were 15% greater when calculated with fin tissue compared with muscle tissue. Similar differences could be expected for other characteristics estimated from isotopes, such as diet proportions and trophic position (Estrada *et al*., 2005; MacNeil *et al*., 2006; Hobson and Bond, 2012).

Conversion relationships that describe the difference in carbon and nitrogen isotope ratios between tissues exist but need to be developed separately for each species (Willis *et al*., 2013). These relationships have been developed for some fishes, but whether season of collection affects these relationships remains unclear (Hanisch *et al*., 2010). Since the metabolism of ectothermic animals like fishes is temperature dependent, the metabolic processes in one season may differ in another and lead to differences in tissue isotope ratios and isotope discrimination factors (Barnes *et al*., 2007). This is of particular concern in regions with drastic seasonal changes in climate and environment, such as in high-latitude and high-altitude aquatic ecosystems that experience ice cover during the colder winter and open water during the warmer summer. Most ecological studies on high-latitude and high-altitude aquatic ecosystems have occurred in the summer when water bodies are ice-free, leaving a gap in our knowledge of winter ecology (Campbell *et al*., 2005; Hampton *et al*., 2015; McMeans *et al*., 2015). Multi-season and winter-focused stable isotope studies can play a role in filling this knowledge gap and reducing the need for lethal sampling will facilitate this.

Using three species of freshwater fishes— northern pike *Esox lucius* L. 1758, yellow perch *Perca flavescens* (Mitchill 1814), and lake whitefish *Coregonus clupeaformis* (Mitchill 1818)—I investigated whether tissue type, season, and their interaction affect estimates of carbon and nitrogen stable isotope ratios in fishes. I explored how the use of non-lethal tissues affects estimates of resource use and trophic position, and whether this changes with season. I developed linear conversion relationships for estimating muscle $\delta^{13}C$ and $\delta^{15}N$ values from fin $\delta^{13}C$ and $\delta^{15}N$ values for the three species, considering seasonal variation when necessary. I expected dorsal muscle carbon and nitrogen isotope ratios to differ from caudal fin and pectoral fin, and that the magnitude of this difference could change with season due to tissue-specific variations in metabolism and isotopic routing between seasons. Furthermore, I hypothesized that tissue- and season-based differences in carbon and nitrogen isotope ratios would be reflected in estimates of resource use and trophic position.

4.3 Methods

4.3.1 Study Sites

Fishes were collected from two lakes in northeastern Alberta, Steepbank Lake (55.477482, -111.579530) and Goodwin Lake (55.417562, -111.655308), in June 2016 or May 2017

(hereafter referred to as spring), August 2016 or 2017 (hereafter referred to as fall), and February 2017 or March 2018 (hereafter referred to as winter). The surface area of Steepbank Lake is 193ha and the maximum depth is 17m. The surface area of Goodwin Lake is 864ha and the maximum depth is 17m. The lakes experience approximately six months of winter ice cover (November to April) and six months of open water (May to October). Neither lake is known to experience periods of anoxia.

4.3.2 Sample Collection and Processing

Yellow perch and lake whitefish were collected from Goodwin Lake and northern pike were collected from Steepbank Lake. Information on collection dates, gear used, and sample sizes are presented in Table 4.1. Captured fish were identified to species and measured for weight and length. Fish were euthanized with buffered MS-222, stored on ice for 1-4 hours, then frozen to -20°C in portable coolers.

Table 4.1. Sample sizes, capture method, and sampling periods for northern pike *Esox lucius* from Steepbank Lake, yellow perch *Perca flavescens* from Goodwin Lake, and lake whitefish *Coregonus clupeaformis* from Goodwin Lake included in the study.

Species	Lake	Season	Date	Gear	n
Northern pike	Steepbank	Spring	Jun-16	Fyke net	20
		Fall	Aug-16	Gill net	20
		Winter	Feb-17	Angling	12
				Gill net	8
Yellow perch	Goodwin	Spring	May-17	Gill net	11
		Fall	Aug-17	Gill net	11
		Winter	Feb-17	Gill net	8
			Mar-18	Gill net	3
Lake whitefish	Goodwin	Spring	May-17	Gill net	8
		Fall	Aug-17	Gill net	8
		Winter	Feb-17	Gill net	7
			Mar-18	Gill net	1

During dissections, I removed three tissues from each fish to be analysed for carbon and nitrogen stable isotopes: pectoral fin clips, caudal fin clips, and skinless, boneless white dorsal muscle. Whole pectoral fins were removed, placed in coin envelopes, and allowed to dry at room temperature for storage. Caudal fin clips were taken from the margin of the lower lobe of

the caudal fin. Dorsal muscle was dissected from above the lateral line and below the dorsal fin of each fish. Caudal fin clips and dorsal muscle samples were stored frozen in 2mL microcentrifuge tubes. To prepare pectoral fin clips for stable isotope analysis, I removed dried pectoral fins from envelopes and rinsed them with distilled water. Fin clips were taken from the fin margin to maximize the amount of membrane in the sample relative to fin ray (Hayden *et al.*, 2015). Caudal fin, pectoral fin, and dorsal muscle samples were freeze-dried for 24 hours at -54°C. After drying, dorsal muscle samples were homogenized into a powder using a metal rod, and pectoral and caudal fin clips were homogenized by clipping finely with scissors.

4.3.3 Ethics Statement

Fish collections were done following guidelines developed by the Canadian Council on Animal Care for the use of fish in research. Fish collections were authorized by Alberta Environment and Parks under Research Licenses 16-1809, 16-1819, 17-1803, and 17-1820, and with ethics approval from the University of Alberta Research Ethics Office under Animal Use Protocol 00001547.

4.3.4 Stable Isotope Analysis

I weighed 0.2 ± 0.05mg of dried dorsal muscle from northern pike sampled in the spring and fall of 2016 into 4mm tin capsules and sent these to the Natural Resources Analytical Laboratory, University of Alberta, for carbon and nitrogen stable isotope analysis. Samples were analysed using a Delta V Advantage Isotope Ratio Mass Spectrometer coupled to a ThermoScientific Flash 2000 Elemental Analyser. Protein was run every 12 samples as an in-house $\delta^{13}C$ (-27.0 ± 0.02‰) and $\delta^{15}N$ (6.0 ± 0.02‰) standard to check for instrument error. For all other samples, 1±0.05mg of dried tissue (muscle or fins) was measured into 4mm tin capsules and sent to the Biogeochemical Analytical Service Laboratory, University of Alberta, for carbon and nitrogen stable isotope analysis. Samples were analyzed using a Vario Pyrocube elemental analyzer coupled to an Elementar IsoPrime vision continuous-flow isotope ratio mass spectrometer. NIST 8415 whole egg powder SRM was run every 20 samples as an in-house $\delta^{13}C$ (-23.9 ± 0.01‰) and $\delta^{15}N$ (6.9 ± 0.2‰) standard to test for instrument error. 14 repeat samples were run (ten muscle and four fin) to test our homogenization processes (mean difference and standard deviation for $\delta^{13}C$ was 0.2 ± 0.3‰ and $\delta^{15}N$ was 0.2 ± 0.2‰). Muscle $\delta^{13}C$ values were not mathematically lipid corrected because all samples had C:N ratios <3.5 (Table 4.3) indicating low tissue lipid content (Logan *et al.*, 2008; Post *et al.*, 2007). Fin $\delta^{13}C$ values were also not

mathematically lipid corrected despite some samples having C:N ratios >3.5 (Table 4.3), as fin $\delta^{13}C$ values are primarily related to the ratio of fin ray to fin membrane in a particular sample (Hayden *et al*., 2015).

Results are reported using standard δ notation where $\delta^{13}C$ and $\delta^{15}N$ are the ratios of ^{13}C:^{12}C and ^{15}N:^{14}N of the sample divided by the ratios of ^{13}C:^{12}C and ^{15}N:^{14}N found in international standards (VPD Belemnite for ^{13}C and air for ^{15}N), and then multiplied by 1000, giving units of ‰ (per mille).

4.3.5 Data Analysis

Effect of tissue type, season, and their interaction on fish isotope ratios

All data analysis was performed in R (version 3.5.3, R Core Team, 2019). I used linear mixed effects modelling from the R package nlme (version 3.1-137, Pinheiro *et al*., 2018) to test the effects of tissue type, season, and their interaction on $\delta^{13}C$ and $\delta^{15}N$ of northern pike, yellow perch, and lake whitefish. The normal distribution of residuals was confirmed by visual inspection. I looked for outliers using Cleveland dot plots and found that no data points needed to be removed from the data. Each species-isotope combination was modelled separately, thus 6 models were fit in total. Overall, I followed the protocol of Zuur *et al*., (2009) for model fitting and selection. Briefly, I started with the most inclusive linear model, and used a likelihood ratio test and visual inspection of model residuals to determine the optimal variance structure of each model. If residuals were not homogenous across seasons, I included different variances per season to improve model fit and ensured the assumption of homogeneity was not violated. I included individual fish in the random component of the model to account for non-independence of the three tissues within each season. Whether the model included random intercepts for individual fish or random intercepts and random slopes for individual fish was determined using a likelihood ratio test and visual inspection of model residuals. The fixed component of each model included tissue type, season, and their interaction. I assessed the significance of each fixed factor and interaction using P-values ($\alpha = 0.05$) based on F-tests.

Effect of tissue type and season on estimates of littoral resource use and trophic position

Trophic position and littoral resource use were calculated using the R package tRophic Position (version 0.7.6, Quezada-Romegialli *et al*., 2018). I used the Bayesian multi-species model (multiSpeciesTP function) with two isotope tracers and two baselines. I used 5 parallel chains

with 10 000 iterations in the adaptive phase, a burn-in period of 10 000 iterations, 10 000 iterations in the sampling phase and a thinning interval of 10, leaving 1001 samples per chain. Tissue types were treated as different consumers, and season was used as a grouping variable. Separate models were run for each species.

Zooplankton and benthic macroinvertebrates collected from the two lakes were used as pelagic and littoral baselines, respectively, and their trophic positions were assumed to be 2. $\delta^{13}C$ values of baseline organisms increased in the fall compared to the spring and winter. I did not expect this shift to be tracked by the fish tissues under investigation due to their estimated turnover rates of several weeks to months (Boecklen *et al*., 2011; Hesslein *et al*., 1993). Therefore, baseline samples collected in the spring, fall and winter from each lake were pooled together, such that the same set of baselines were used for each season (Figure 4.1). The trophic enrichment factors published by McCutchan *et al*. (2003) were used: $\Delta^{15}N$: 2.9 ± 0.32, $\Delta^{13}C$: 1.3 ± 0.3.

Littoral resource use and trophic position estimates were compared between tissue types and seasons. I explored the need for conversion relationships between muscle and fins by using raw fin isotope values (rather than estimates of muscle isotope values based on fins) to calculate resource use and trophic position. These comparisons will help researchers adjust their data interpretation when using fins instead of muscle when conversion relationships are not available. I compared the same tissue across seasons and the three tissues within each season using means and the pairwiseComparisons function from the tRophic Position package. The pairwiseComparisons function compares posterior distributions and returns a matrix with probabilities that the values for one group are smaller than for another group. I accepted probabilities greater than 0.8 and smaller than 0.2 as evidence that littoral resource use and trophic position estimates from one tissue-season combination were likely smaller or larger, respectively, than another tissue-season combination. Probabilities in between 0.2-0.8 were not considered evidence of a difference in littoral resource use or trophic position estimates between tissue-season combinations.

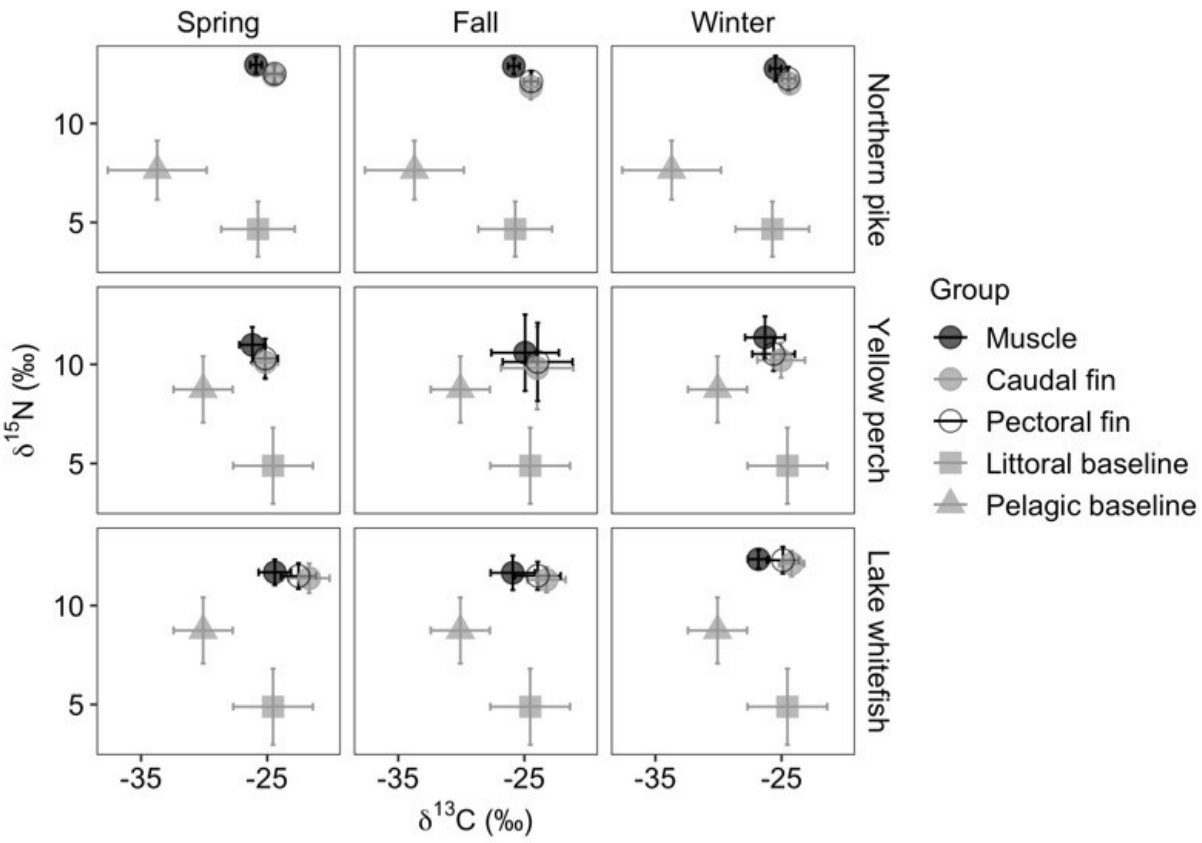

Figure 4.1. Stable isotope biplots showing the mean (points) and standard deviation (bars) for $\delta^{13}C$ and $\delta^{15}N$ of muscle, caudal fin, and pectoral fin from northern pike *Esox lucius*, yellow perch *Perca flavescens*, and lake whitefish *Coregonus clupeaformis,* and the littoral and pelagic baseline organisms used to calculate estimates of littoral resource use and trophic position.

Fin-muscle conversion relationships

I developed linear conversion relationships to estimate muscle $\delta^{13}C$ and $\delta^{15}N$ values from fin $\delta^{13}C$ and $\delta^{15}N$ values using linear regressions. If the linear mixed model of a species $\delta^{13}C$ or $\delta^{15}N$ values detected a significant effect of season or an interaction between tissue type and season, I developed separate conversion relationships for each season. When season and the

interaction between tissue and season were not significant, I combined data from different seasons to develop a single conversion relationship that can be used for fishes caught in any season.

I used linear regression to assess the relationship between $\delta^{13}C$ and $\delta^{15}N$ isotope ratios of fin and muscle. If the slope of the relationship did not differ significantly from 1, and the intercept of the relationship did not differ from 0 (95% confidence intervals for the slope and y-intercept include 0 and 1, respectively), I concluded that there was no difference in $\delta^{13}C$ or $\delta^{15}N$ values of the two tissues. In addition, I used Pearson's correlation co-efficient to assess the correlation between isotope ratios values of fin and muscle.

4.4 Results

4.4.1 Effect of Tissue Type and Season on $\delta^{13}C$ and $\delta^{15}N$

Northern pike

Tissue type, season, and their interaction had significant effects on northern pike $\delta^{13}C$ values (Table 4.2). The difference between muscle $\delta^{13}C$ and fin $\delta^{13}C$ was smaller in the winter than in the spring and fall (Figure 4.2, Table 4.3). Muscle $\delta^{13}C$ was on average 1.4, 1.3, and 1.1‰ less than caudal fin $\delta^{13}C$ and 1.5, 1.4, and 1.0‰ less than pectoral fin $\delta^{13}C$ in the spring, fall and winter, respectively. The best fit linear mixed model included random slopes and intercepts for individual fish and no variance structure.

Tissue type, season, and their interaction had significant effects on northern pike $\delta^{15}N$ values (Table 4.2). The difference between muscle $\delta^{15}N$ and fin $\delta^{15}N$ was largest in the fall, intermediate in the winter, and smallest in the spring (Figure 4.2, Table 4.3). Muscle $\delta^{15}N$ was on average 0.5, 1, and 0.8‰ greater than caudal fin $\delta^{15}N$ and 0.5, 0.8, and 0.5‰ greater than pectoral fin $\delta^{15}N$ in the spring, fall and winter, respectively. The best fit linear mixed model included random intercepts for individual fish and different variances per season. The model was improved by allowing different variances between seasons because the range of $\delta^{15}N$ values detected in the spring was smaller than in the fall and winter.

Table 4.2. Summary of the linear mixed effect models of $\delta^{13}C$ and $\delta^{15}N$ for northern pike *Esox lucius*, yellow perch *Perca flavescens*, and lake whitefish *Coregonus clupeaformis*. Bold text denotes statistically significant parameters (α= 0.05).

Response	Parameter	numDF	denDF	F-value	P-value
		Northern pike			
$\delta^{13}C$	**Intercept**	**1**	**114**	**189112.80**	**<0.0001**
	Tissue	**2**	**114**	**390.51**	**<0.0001**
	Season	**2**	**57**	**3.43**	**0.0392**
	Tissue:Season	**4**	**114**	**3.09**	**0.0185**
$\delta^{15}N$	**Intercept**	**1**	**114**	**39010.92**	**<0.0001**
	Tissue	**2**	**57**	**2.81**	**<0.0001**
	Season	2	114	186.78	0.069
	Tissue:Season	**4**	**114**	**4.07**	**0.004**
		Yellow perch			
$\delta^{13}C$	**Intercept**	**1**	**57**	**6401.48**	**<0.0001**
	Tissue	**2**	**57**	**102.75**	**<0.0001**
	Season	2	30	1.97	0.15
	Tissue:Season	4	57	1.23	0.31
$\delta^{15}N$	**Intercept**	**1**	**57**	**1909.62**	**<0.0001**
	Tissue	**2**	**57**	**167.45**	**<0.0001**
	Season	2	30	0.55	0.58
	Tissue:Season	**4**	**57**	**2.62**	**0.04**
		Lake whitefish			
$\delta^{13}C$	**Intercept**	**1**	**42**	**8358.56**	**<0.0001**
	Tissue	**2**	**42**	**366.70**	**<0.0001**
	Season	**2**	**21**	**7.41**	**0.0037**
	Tissue:Season	4	42	0.19	0.94
$\delta^{15}N$	**Intercept**	**1**	**42**	**11325.7**	**<0.0001**
	Tissue	**2**	**42**	**7.93**	**0.0012**
	Season	2	21	2.89	0.078
	Tissue:Season	4	42	0.64	0.63

Yellow perch

Tissue type had a significant effect on yellow perch $\delta^{13}C$ values but season and the interaction between tissue and season did not (Table 4.2). Muscle $\delta^{13}C$ was on average 1.0, 0.9, and 1.0‰ less than caudal fin $\delta^{13}C$ and 1.0, 1.0, and 0.7‰ less than pectoral fin $\delta^{13}C$ in the spring, fall and winter, respectively (Figure 4.2, Table 4.3). The best fit linear mixed model for yellow perch

$\delta^{13}C$ included random slopes and intercepts for individual fish, and different variances per season. The model was improved by allowing different variances between seasons because the range of $\delta^{13}C$ values detected was largest in the fall, smallest in the spring, and intermediate in the winter.

Tissue type and the interaction between tissue type and season had significant effects on yellow perch $\delta^{15}N$ values but season did not (Table 4.2). Muscle $\delta^{15}N$ was on average 0.9, 0.8, and 0.7‰ greater than caudal fin $\delta^{15}N$ and 0.7, 0.7, 0.8‰ greater than pectoral fin $\delta^{15}N$ in the spring, fall, and winter, respectively (Figure 4.2, Table 4.3). The best fit linear mixed model for yellow perch $\delta^{15}N$ included random intercepts for individual fish and different variances per season. The model was improved by allowing different variances between seasons because the range of $\delta^{15}N$ values detected in the fall was greater than the spring or winter.

Lake whitefish

Tissue type and season had significant effects on lake whitefish $\delta^{13}C$ values but their interaction did not (Table 4.2). Muscle $\delta^{13}C$ values were on average 2.7, 2.7, and 2.6‰ less than caudal fin $\delta^{13}C$ and 1.9, 2.0, and 1.9‰ less than pectoral fin $\delta^{13}C$ values in the spring, fall, and winter, respectively, thus the magnitude of the difference between tissue types is conserved across seasons (Figure 4.2, Table 4.3). $\delta^{13}C$ in the spring was on average 1.5‰ and 2.4‰ greater than in the fall and winter, respectively, across the three tissues. The best fit linear mixed model for lake whitefish $\delta^{13}C$ included random intercepts for individual fish and different variances per season. The model was improved by allowing different variances between seasons because the range of $\delta^{13}C$ values detected in the winter was smaller than in the spring or fall.

Tissue type had a significant effect on lake whitefish $\delta^{15}N$ values but season and the interaction between tissue type and season did not (Table 4.2). Muscle $\delta^{15}N$ was on average 0.4, 0.5, and 0.4‰ greater than caudal fin $\delta^{15}N$ and 0.2, 0.4, and 0.4‰ greater than pectoral fin $\delta^{15}N$ (Figure 4.2, Table 4.3). Visually, it appears that season had a potential weak effect on lake whitefish $\delta^{15}N$ driven by higher $\delta^{15}N$ values in the winter but the model did not detect this effect. The best fit linear mixed model for lake whitefish $\delta^{15}N$ had random slopes and intercepts for individual fish, and no variance structure. Visually, it appears that the variance in the winter is smaller than in the spring and fall; however, including different variances per season did not improve the model (likelihood ratio, L= 0.98, P=0.61), nor the appearance of model residuals.

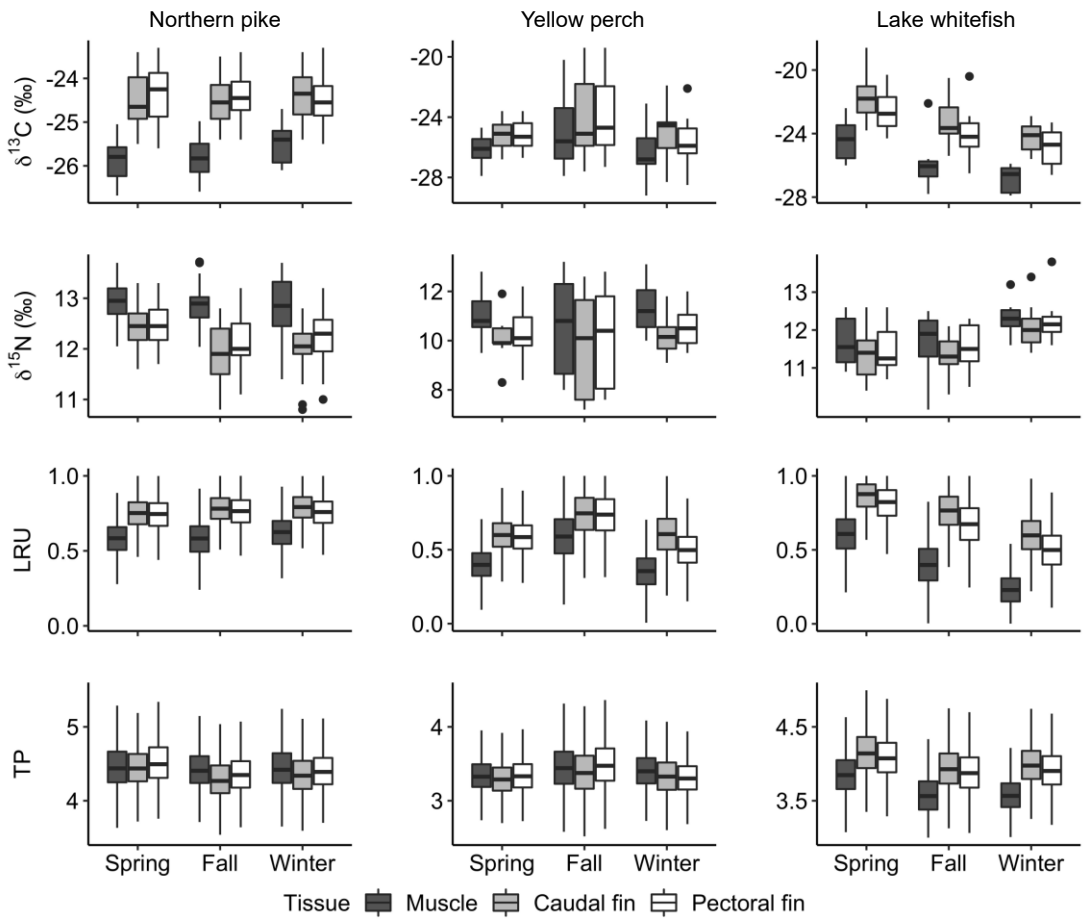

Figure 4.2. Boxplots comparing $\delta^{13}C$, $\delta^{15}N$, littoral resource use estimates (LRU) and trophic position estimates (TP) between muscle, caudal fin and pectoral fin from northern pike *Esox lucius*, yellow perch *Perca flavescens*, and lake whitefish *Coregonus clupeaformis* collected in the spring, fall, and winter. Horizontal lines represent the mode, boxes show the 25th and 75th quartiles, vertical lines show the extent of the data and points represent outliers. Outliers are not shown in the boxplots displaying the Bayesian distributions of estimates for LRU and TP.

4.4.2 Effect of Tissue Type and Season on Estimates of Resource Use and Trophic Position

Northern pike

Both muscle and fin suggested that littoral resources are the main food source for northern pike (Table 4.3). I found no evidence that northern pike littoral resource use estimates differed

between seasons within a tissue type, and the differences in littoral resource use estimates between tissues appear similar across seasons (Figure 4.2, Table S4.1). Mean littoral resource use estimates based on dorsal muscle were 17, 21, and 17% less than those based on caudal fin and 16, 19, and 14% less than those based on pectoral fin in the spring, fall and winter, respectively. There was no evidence of a difference in trophic position estimates between tissues or seasons for northern pike (Figure 4.2, Table 4.3, Table S4.4).

Yellow perch

I found evidence that yellow perch littoral resource use estimates vary between tissues and seasons (Figure 4.2, Table 4.3, Table S4.2). Mean littoral resource use estimates based on dorsal muscle were 20, 14, and 25% less than those based on caudal fin and 19, 14, and 14% less than those based pectoral fin in the spring, fall and winter, respectively. Littoral resource use estimates in the fall were on average 15 and 19% greater than estimates in the spring and winter, respectively, across tissues. There was no evidence of a difference in trophic position estimates between tissues or seasons for yellow perch (Figure 4.2, Table 4.3, Table S4.5).

Lake whitefish

I found evidence that lake whitefish littoral resource use estimates varied between tissues and seasons (Figure 4.2, Table 4.3, Table S4.3). Littoral resource use estimates based on dorsal muscle were 25, 35, and 27% less than estimates based on caudal fin and 20, 27, and 27% less than estimates based on pectoral fin in the spring, fall, and winter, respectively. Littoral resource use estimates in the spring were on average 15 and 32% greater than estimates in the fall and winter, respectively, across tissues.

Table 4.3. Mean and standard deviation (SD) of $\delta^{13}C$, $\delta^{15}N$, C:N ratios, estimates of littoral resource use (LRU), and trophic position estimates (TP) for northern pike *Esox lucius*, yellow perch *Perca flavescens*, and lake whitefish *Coregonus clupeaformis*.

		$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)		C:N		LRU		TP	
Season	Tissue	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
					Northern pike						
Spring	Muscle	-25.9	0.5	13.0	0.4	3.2	0.1	0.58	0.12	4.5	0.3
	Caudal fin	-24.5	0.6	12.4	0.5	3.3	0.1	0.75	0.11	4.5	0.3
	Pectoral fin	-24.4	0.7	12.5	0.5	3.3	0.1	0.74	0.11	4.5	0.3
Fall	Muscle	-25.8	0.5	12.9	0.4	3.2	0.0	0.57	0.13	4.4	0.3
	Caudal fin	-24.5	0.5	11.8	0.6	3.2	0.1	0.78	0.11	4.3	0.3
	Pectoral fin	-24.5	0.6	12.1	0.5	3.3	0.1	0.76	0.11	4.4	0.3
Winter	Muscle	-25.5	0.5	12.8	0.7	3.2	0.0	0.62	0.12	4.5	0.3
	Caudal fin	-24.4	0.6	12.0	0.5	3.4	0.2	0.79	0.1	4.4	0.3
	Pectoral fin	-24.5	0.6	12.3	0.6	3.5	0.1	0.76	0.11	4.4	0.3
					Yellow perch						
Spring	Muscle	-26.2	1.0	11.0	0.9	3.3	0.0	0.4	0.12	3.4	0.2
	Caudal fin	-25.2	1.0	10.1	0.9	3.7	0.3	0.6	0.12	3.3	0.3
	Pectoral fin	-25.2	1.0	10.3	1.0	3.7	0.3	0.59	0.12	3.4	0.3
Fall	Muscle	-24.9	2.7	10.6	1.9	3.2	0.0	0.59	0.17	3.5	0.3
	Caudal fin	-24.0	2.9	9.8	2.1	3.5	0.2	0.73	0.15	3.4	0.4
	Pectoral fin	-24.0	2.8	10.1	2.0	3.4	0.1	0.73	0.15	3.5	0.4
Winter	Muscle	-26.3	1.6	11.4	1.1	3.3	0.1	0.36	0.14	3.4	0.3
	Caudal fin	-25.0	1.9	10.2	0.9	3.7	0.1	0.61	0.16	3.3	0.3
	Pectoral fin	-25.6	1.7	10.5	0.9	3.8	0.2	0.5	0.14	3.3	0.3
					Lake whitefish						
Spring	Muscle	-24.4	1.3	11.7	0.6	3.2	0.1	0.61	0.15	3.9	0.3
	Caudal fin	-21.7	1.6	11.4	0.7	3.1	0.1	0.86	0.11	4.2	0.4
	Pectoral fin	-22.5	1.4	11.5	0.6	3.2	0.1	0.81	0.12	4.1	0.3
Fall	Muscle	-25.9	1.8	11.7	0.9	3.2	0.0	0.4	0.16	3.6	0.3
	Caudal fin	-23.3	1.5	11.3	0.6	3.1	0.1	0.76	0.14	4.0	0.3
	Pectoral fin	-24.0	1.8	11.5	0.7	3.2	0.1	0.67	0.16	3.9	0.4
Winter	Muscle	-26.8	0.8	12.3	0.5	3.2	0.1	0.23	0.11	3.6	0.3
	Caudal fin	-24.2	1.0	12.1	0.6	3.2	0.1	0.6	0.14	4.0	0.3
	Pectoral fin	-24.9	1.3	12.3	0.7	3.2	0.1	0.5	0.15	3.9	0.3

4.4.3 Fin-Muscle Conversion Relationships

All linear regressions between muscle and caudal fin or pectoral fin were significant for all three species and both isotopes (Figure 4.3, 4.4). Pearson correlation coefficients were lowest for northern pike (range: 0.51-0.94) and very high for yellow perch (range: 0.87-0.99) and lake whitefish (range: 0.82-0.97) suggestion strong, positive correlations between $\delta^{13}C$ and $\delta^{15}N$ of fin and muscle for all three species (Table 4.4).

In some cases, the estimated slope and y-intercept were not different from 1 and 0, respectively, suggesting that there is so significant difference between the $\delta^{13}C$ or $\delta^{15}N$ of fin and muscle. This was the case for northern pike $\delta^{15}N$ in the winter, yellow perch $\delta^{13}C$ (all seasons), yellow perch $\delta^{15}N$ in the spring and winter, lake whitefish $\delta^{13}C$ in the fall and winter for caudal fin and in the spring and fall for pectoral fin, and lake whitefish $\delta^{15}N$ for pectoral fin (all seasons) (Table 4.4).

4.5 Discussion

I found that fins had greater $\delta^{13}C$ and lower $\delta^{15}N$ than muscle for all three species, while the effects of season and the interaction between season and tissue type was species- and isotope-specific. Fins produced higher estimates of littoral resource use compared to muscle for all three species, while season affected yellow perch and lake whitefish littoral resource use estimates, but not northern pike. The magnitude of the difference in resource use estimates between tissues varied slightly between seasons, but the direction of the relationship was always consistent. Fins produced slightly higher estimates of trophic position compared to muscle in lake whitefish but not northern pike or yellow perch. Season had a small effect on lake whitefish trophic position, but almost no effect on northern pike or yellow perch. Strong linear relationships existed between fin and muscle $\delta^{13}C$ and $\delta^{15}N$ for all species. I developed linear conversion relationships between fin and muscle $\delta^{13}C$ and $\delta^{15}N$ for the three species and accounted for season of collection for species and isotopes that showed a seasonal effect.

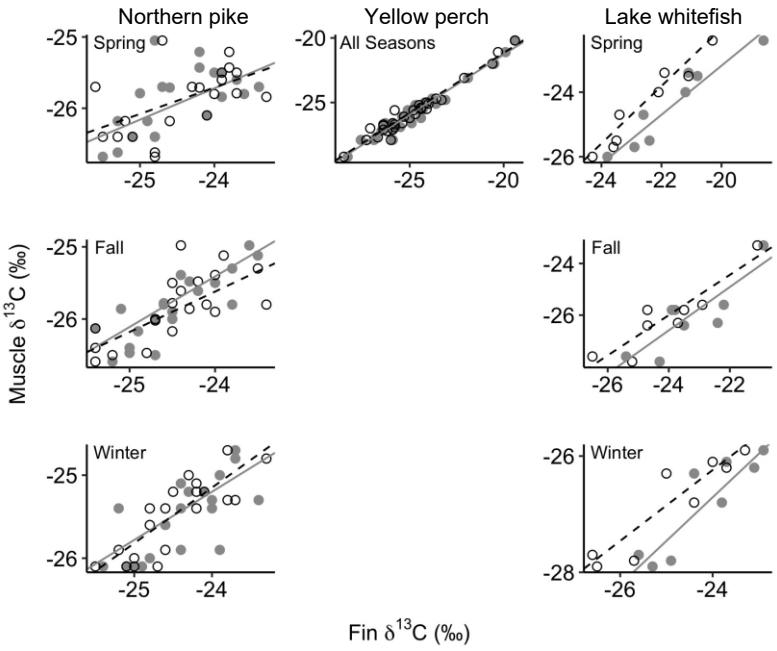

Figure 4.3. Scatterplots showing the relationship between fin (caudal fin = grey points, grey lines; pectoral fins = open points, dashed lines) and muscle δ^{13}C for northern pike *Esox lucius*, yellow perch *Perca flavescens*, and lake whitefish *Coregonus clupeaformis.* Linear regressions use the slope and y-intercept estimates described in Table 4.4.

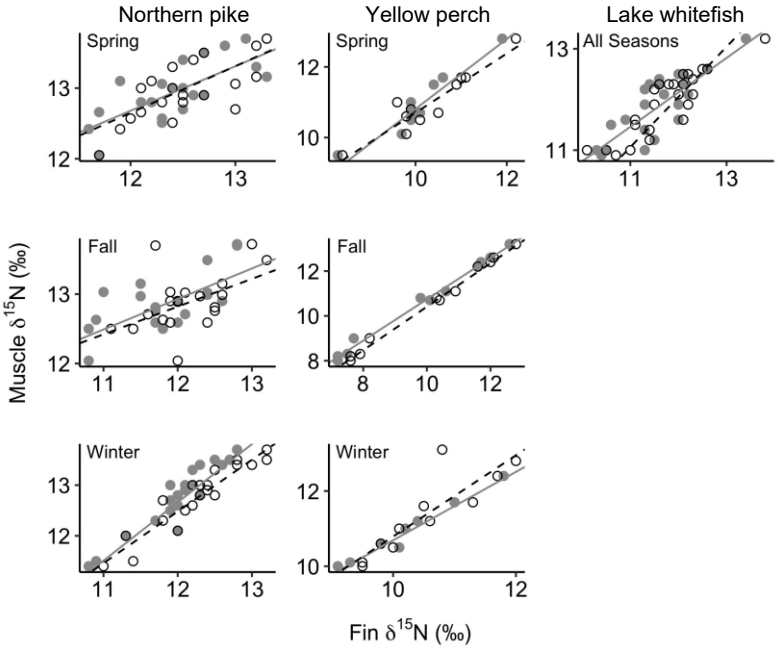

Figure 4.4. Scatterplots showing the relationship between fin (caudal fin = grey points, grey lines; pectoral fins = open points, dashed lines) and muscle δ^{15}N for northern pike *Esox lucius*, yellow perch *Perca flavescens*, and lake whitefish *Coregonus clupeaformis.* Linear regressions use the slope and y-intercept estimates described in Table 4.4.

Table 4.4. Slope estimates and 95% confidence intervals (95% CI), y-intercept estimates and 95% confidence intervals, F-value, P-value, and Pearson's correlation coefficient (r) for the linear regressions predicting muscle $\delta^{13}C$ and $\delta^{15}N$ from caudal fin or pectoral fin $\delta^{13}C$ and $\delta^{15}N$, respectively.

		Slope		Intercept				
Predictor	Season	Estimate	95% CI	Estimate	95% CI	F-value	P-value	r
				Northern pike $\delta^{13}C$				
	Spring	0.44	0.14 - 0.73	-15.16	-22.43 - -7.89	9.62	0.006	0.59
Caudal fin	Fall	0.7	0.47 - 0.94	-8.61	-14.34 - -2.88	39.95	6.00E-06	0.83
	Winter	0.58	0.29 - 0.87	-11.28	-18.18 - -4.38	18.71	0.0004	0.71
	Spring	0.37	0.09 - 0.65	-16.83	-23.72 - -9.94	7.64	0.01	0.54
Pectoral fin	Fall	0.56	0.28 - 0.83	-12.18	-18.95 - -5.41	17.99	0.0005	0.71
	Winter	0.67	0.45 - 0.89	-9.07	-14.43 - -3.71	41.51	5.00E-06	0.83
				Northern pike $\delta^{15}N$				
	Spring	0.63	0.34 - 0.92	5.12	1.49 - 8.76	20.54	0.0002	0.73
Caudal fin	Fall	0.44	0.21 - 0.67	7.65	4.88 - 10.42	15.87	9.00E-04	0.68
	Winter	1.15	0.95 - 1.37	-1.14	-3.68 - 1.38	133.6	9.00E-10	0.94
	Spring	0.66	0.34 - 0.97	4.73	0.75 - 8.71	18.92	0.0004	0.72
Pectoral fin	Fall	0.4	0.067 - 0.74	8.02	3.96 - 12.09	6.35	0.02	0.51
	Winter	1.02	0.84 - 1.20	0.24	-1.98 - 2.47	140.2	6.00E-10	0.94
				Yellow perch $\delta^{13}C$				
Caudal fin	All	0.93	0.85 - 1.01	-2.69	-4.68 - -0.70	566.6	<2.2E-16	0.98
Pectoral fin	All	0.93	0.86 - 1.00	-2.56	-4.35 - -0.77	706	<2.2E-16	0.98
				Yellow perch $\delta^{15}N$				
	Spring	0.99	0.76 - 1.23	0.91	-1.47 - 3.28	92.38	5.00E-06	0.95
Caudal fin	Fall	0.92	0.85 - 0.98	1.54	0.92 - 2.18	1059	1.00E-10	0.99
	Winter	0.91	0.76 - 1.07	1.59	-0.02 - 3.21	201	8.00E-06	0.98
	Spring	0.84	0.60 - 1.08	2.3	-0.16 - 4.75	64.55	2.00E-05	0.94
Pectoral fin	Fall	0.97	0.91 - 1.03	0.71	0.10 - 1.32	1374	4.00E-11	0.99
	Winter	1.08	0.62 - 1.54	-0.01	-4.86 - 4.84	28.3	5.00E-04	0.87
				Lake whitefish $\delta^{13}C$				
	Spring	0.76	0.51 - 1.00	-7.98	-13.28 - -2.66	57.57	0.0003	0.95
Caudal fin	Fall	0.84	0.32 - 1.36	-6.43	-18.47 - 5.62	15.98	7.00E-03	0.85
	Winter	0.75	0.37 - 1.12	-8.71	-17.82 - 0.39	23.76	0.003	0.89
	Spring	0.89	0.65 - 1.12	-4.32	-9.56 - 0.92	88.07	8.00E-05	0.97
Pectoral fin	Fall	0.78	0.44 - 1.12	-7.28	-15.46 - 0.90	31.72	0.001	0.92
	Winter	0.61	0.35 - 0.87	-11.6	-18.00 - -5.13	33.95	1.00E-03	0.92
				Lake whitefish $\delta^{15}N$				
Caudal fin	All	0.68	0.47 - 0.89	3.98	1.54 - 6.43	45.62	9.00E-07	0.82
Pectoral fin	All	0.99	0.85 - 1.14	0.17	-1.50 - 1.84	206.8	1.00E-12	0.88

Fin had higher $\delta^{13}C$ and lower $\delta^{15}N$ relative to muscle in all three species. This is likely due to differences in tissue chemical composition (Hayden *et al*., 2015) and isotopic turnover rates (Fry, 2006) between muscle and fins. This finding is consistent with other fish species (Fincel *et al*., 2012; Jardine *et al*., 2005) and with other populations of northern pike, yellow perch, and lake whitefish (Winter *et al*., 2019, McCloskey *et al*., 2018, Hanisch *et al*., 2010). Species-isotope pairings with stable differences between tissues across seasons (no significant, tissue:season interaction) were yellow perch $\delta^{13}C$ (mean difference between muscle and caudal fin = 1.0‰, muscle and pectoral fin = 0.9‰), lake whitefish $\delta^{13}C$ (mean difference between muscle and caudal fin = 2.6‰, muscle and pectoral fin = 1.9‰), and lake whitefish $\delta^{15}N$ (mean difference between muscle and caudal fin = 0.4‰, muscle and pectoral fin = 0.3‰). The differences between fins and muscle are large enough to affect ecological interpretations of the isotope values depending on the research question of interest (Sanderson *et al*., 2009). I encourage researchers to use fin to muscle conversions relationships when available, and to understand the difference in isotope values that are introduced when tissues other than muscle are used.

In most cases, the effect of season was not significant (yellow perch $\delta^{13}C$, lake whitefish $\delta^{15}N$) or could not be assessed due to significant tissue:season interactions (northern pike $\delta^{13}C$, northern pike $\delta^{15}N$, yellow perch $\delta^{15}N$). The effect of season could only be directly assessed for lake whitefish $\delta^{13}C$. Lake whitefish $\delta^{13}C$ values in the winter were 2.4 to 2.5‰ less than in the spring and 0.9‰ less than the fall across all tissues. These differences may reflect a seasonal change in diet (McMeans *et al*., 2015, Keva *et al*., 2019) to more pelagic or detrital food sources in the winter compared to the spring or fall (France, 1996; Croisetière *et al*., 2009).

Northern pike $\delta^{13}C$, $\delta^{15}N$, and yellow perch $\delta^{15}N$ had statistically significant tissue:season interactions. This means that the difference between tissues varies with season. For northern pike $\delta^{13}C$, the significant interaction is the result of higher $\delta^{13}C$ values in muscle in the winter, resulting in a smaller difference between muscle and fins in this season compared to spring or fall. For northern pike $\delta^{15}N$, the significant interaction is the result of larger differences between muscle and fin in the fall compared to the spring and winter. For yellow perch $\delta^{15}N$, the differences between tissues across seasons are relatively stable, ranging from 0.7 to 0.9‰. The influence of season on the difference between muscle and fin $\delta^{13}C$ and $\delta^{15}N$ could be caused by seasonal change in diet and turnover rates of tissues (Matley *et al*., 2016). Isotopic turnover in tissues is influenced by growth and metabolic rates (Sakano *et al*., 2005), which change

seasonally. Although the interaction between tissue and season was statistically significant for northern pike $\delta^{13}C$, $\delta^{15}N$, and yellow perch $\delta^{15}N$, the influence of season on the differences in $\delta^{13}C$ and $\delta^{15}N$ between tissues was consistently small (ranging from 0.1 to 0.5‰). It is possible that a difference of this magnitude would have only minor effects on results if not accounted for, as I see with the trophic position estimates. Alternatively, in study systems with isotope values that span a narrow range of values or have relatively small trophic discrimination factors, such a difference could influence ecological interpretation. I recommend that researchers assess the sensitivity of their research questions and the ecological system under study, and account for the potential influence of season when results may be sensitive to inaccuracy in the range of 0.1 to 0.5‰.

Fin $\delta^{13}C$ and $\delta^{15}N$ changed resource use estimates by 13-37% compared to muscle for the three species, which is consistent with the change in resource use estimates found by Hayden *et al*., (2017) for tropical fishes. This difference in resource use estimates is congruent with the greater $\delta^{13}C$ values of fins compared to muscle — species with larger differences between fin and muscle $\delta^{13}C$ values have larger differences in estimates of resource use. For studies on resource use in fishes, accounting for the difference between lethal (i.e., muscle) and non-lethal tissues (i.e., fin) may be important.

Although fins were depleted in $\delta^{15}N$ in all three species, estimates of trophic position were the same across tissues in northern pike and yellow perch, but slightly higher (0.2 to 0.4 trophic positions) for fins compared to muscle in lake whitefish. This is likely because the trophic discrimination factors are larger than the difference between $\delta^{13}C$ and $\delta^{15}N$ of fins and muscle (Sanderson *et al*., 2009). Raw fin $\delta^{13}C$ and $\delta^{15}N$ data may be appropriate to use in studies of fish trophic position if the difference between fins and muscle is much smaller than the trophic discrimination factor. Researchers should consider species-specific differences in tissue $\delta^{13}C$, $\delta^{15}N$, and trophic discrimination factors.

The conversion relationships developed herein are similar to previously published conversion relationships for the study species. Our conversion relationships go a step further than prior studies by accounting for seasonal variation in the difference between fin and muscle $\delta^{13}C$ and $\delta^{15}N$. There are also life stage and geographic differences between ours and prior works that may make the use of our relationships or other published relationships more appropriate

depending on the circumstance. For example, conversion relationships presented by Winter *et al*., (2019) for northern pike were developed using juvenile pike caught in the UK. Therefore, the conversion relationships presented therein may be more appropriate for juvenile pike or pike caught in Europe, while our relationships may be more appropriate for adult northern pike or northern pike caught in North America. McCloskey *et al*., (2018) developed δ^{13}C and δ^{15}N fin to muscle conversion relationships for yellow perch caught in Ontario, Canada. McCloskey *et al*., (2018) may be more appropriate for perch caught in eastern North America while ours may be more appropriate for perch caught in Western North America. Hanisch *et al*., (2010) developed conversion relationships for lake whitefish caught in Waterton Lake, Alberta, in October. Given the geographic closeness of our study sites, it is perhaps not surprising that our fall caudal fin to muscle conversion relationship for lake whitefish is quite similar to theirs.

The relationships between muscle and fin δ^{13}C and δ^{15}N within a species can be population specific (Fincel *et al*., 2012). In our study I sampled a single population of each species. This allowed us to better address our main objective – to understand whether season influences tissue isotope relationships – by reducing the noise in the data from sampling multiple populations. I find that season can influence the difference in isotope values, and thus depending on research questions and the sensitivity of the study system to variation in isotopic values, accounting for this seasonal variation could be important. I provide the tissue conversion relationships developed for our study populations and suggest that in instances where developing a population-specific conversion is not possible, our relationship could be used, with the caveat that estimates of muscle δ^{13}C and δ^{15}N from fins will be less accurate than if a population-specific relationship is used.

Some prior studies used wild caught fish that were held in captivity and fed a stable diet prior to sampling for stable isotope analysis (e.g.: Winter *et al*., 2019, McCloskey *et al*., 2018). This holding period allowed their tissues to reach isotopic equilibrium with their captive diet and the environmental conditions of their enclosures (temperature, oxygen, water quality, etc.). Our study, similar to that of Hanisch *et al*., (2010), used tissues from wild caught fish euthanized in the field. This means that their tissue δ^{13}C and δ^{15}N values were affected by their natural diets and other environmental factors that can affect tissue δ^{13}C and δ^{15}N values. Although Winter *et al*., (2019) and McCloskey *et al*., (2018) stated when their fish were captured (in June and October-November, respectively), any impacts on fish isotopic values due to season of collection would have been lost by the time they were sampled for stable isotope analysis. Many

isotope studies aim to gain information on wild populations whose stable isotope ratios are affected by factors such as available dietary resources, seasonal changes in physiology and behaviour (starvation, growth, reproduction, senescence, etc.), and environmental conditions (temperature and oxygen concentration, water quality, etc.). As a result, conversion relationships developed from wild caught populations during the same season of collection may better incorporate these sources of variation.

4.6 Conclusion

Whether tissue and season-based differences in $\delta^{13}C$ and $\delta^{15}N$ will affect the biological interpretation of results will depend on the research questions of interest, the range of $\delta^{13}C$ and $\delta^{15}N$ values covered by the study system, and the size of the tissue and/or seasonal differences relative to trophic discrimination factors. For example, in our study, resource use estimates were more sensitive to the isotopic differences between tissues and seasons, while estimates of trophic position were generally more robust. $\delta^{13}C$ and $\delta^{15}N$ of fin and muscle were highly correlated, allowing us to develop linear conversion relationships for our study populations. I developed separate relationships for each season if the effect of season or the tissue:season interaction was statistically significant. I suggest these conversion relationships can be used on other populations when they present the best alternative to developing population-specific conversions. I recommend that researchers account for tissue-based differences in isotope values, and how season of collection may affect those differences, when appropriate. This includes for species-isotope combinations with differences between non-lethal tissues and muscle, and seasonal effects on those differences, that are large relative to the scale of isotope values under investigation and/or the trophic discrimination factors under use.

Our findings support the use of non-lethal tissues, such as fins, for use in carbon and nitrogen stable isotope studies. Reducing the need for lethal sampling in stable isotope studies will open doors for studying rare, endangered, and data-poor species. By lessening the effects of research on natural populations, I will increase our ability to study species of interest during vulnerable times of the year, such as in the winter, during spawning, or during migrations, and to re-sample populations, such as in multi-season studies.

Chapter 5 Conclusion

This thesis assessed the trophic structure and seasonal dynamics of fish in a constructed fisheries habitat offset in the Alberta oil sands. I compared the offset to natural lakes, and examined the use of stable isotope analysis for multi-season and winter-focused research, including using non-lethal sampling. The trophic structure and dynamics of a constructed fisheries offset were within the range of variation detected in natural lakes. Examining the unique fish assemblage of the offset and multiple natural fish assemblages revealed that the flexible and generalist nature of boreal fishes allows them to adapt to multiple habitat types, a range of water qualities, and to a variety of dietary resources. The three natural fish assemblage types studied here had distinct trophic structure characteristics, which may be in response to habitat quality and interactions among fish with various functional traits. Across a variety of lakes, fishes spanning a range of functional traits displayed similar temporal characteristics in their trophic dynamics. Most populations fed year-round by changing their diets seasonally or maintaining the same diet across seasons while at least one population went seasonally dormant. Advancements were made in our ability to interpret stable isotope data from non-lethally sampled fish tissues and in multiple seasons, by improving our understanding of when and how to account for inter-tissue variation in stable isotope values.

Trophic structure and dynamics relate to the maintenance of biodiversity and ecosystem function (Rooney *et al*., 2006; McMeans *et al*., 2015). The fish assemblage trophic structure of the offset lake had many characteristics that were within the range of variation detected in natural assemblages and was most like natural assemblages with relatively high fish species richness, despite having different species compositions. The seasonal trophic dynamics of fish populations in the offset lake suggested most populations fed year-round, with two populations using diet changes between seasons and two populations using unknown trophic responses, while one population went dormant during the winter. This suggests that the over-wintering habitat in the offset lake supports enough productivity for the fish populations to remain active and feed under lake ice. Fish populations in the offset lake were responding to seasonal changes by altering their diets, either in distinct or subtle ways, similar to what was observed in the natural lakes. The natural assemblage types differed in their trophic structures, which could be valuable to compare with other current and future fisheries offsets in the Alberta oil sands. Future research on the trophic structure and dynamics in other offset lakes could offer insight

into their ecology, how they compare to natural ecosystems, and further our understanding of how species composition and habitat characteristics relate to trophic structure and dynamics.

Stable isotope analysis is a popular method for assessing trophic structure and dynamics in ecological communities but interpreting stable isotope data is challenging because of the multiple sources of variation that impact stable isotope ratios in the tissues of organisms (Boecklen *et al*., 2011). To improve the interpretation of stable isotope data collected non-lethally in fish and in multiple seasons, I investigated how season impacts the difference in carbon and nitrogen stable isotope values between lethally (muscle) and non-lethally (fins) sampled tissues, and in resource use and trophic position estimates calculated with stable isotope ratios from lethally and non-lethally sampled tissues. There were differences in stable isotope values between the tissues for all species, but whether season impacted this difference was species and isotope specific. I recommend accounting for the difference between fin and muscle stable isotope values in most studies, since these differences were large enough to impact the biological interpretation of the data. Since the impact of season was generally small, whether season needs to be accounted for when estimating muscle stable isotope values from fin stable isotope values is study dependent. I recommend accounting for season in tissue conversion relationships when a study includes a narrow range of isotope values or where trophic discrimination factors are small. Following these recommendations can improve the interpretation of stable isotope data and stable isotope-based metrics from non-lethal tissues and reduce the sources of variation in multi-season studies.

The offsetting process involves setting technical targets and assessing whether they have been met. Although using trophic structure and dynamics in that capacity may be too challenging due to their natural variability, they offer valuable insight when assessed alongside common focuses of monitoring such as habitat size and quality, species richness, and population density of various taxonomic groups. In addition to meeting their technical targets, many offsets are meant to be habitats that persist indefinitely and have ecological and social value. Understanding the natural trophic structure and dynamics of analogous natural habitats and comparing offsets to that is a viable way to assess their ecological success (Ruiz-Jaen and Aide, 2005; Fraser *et al*., 2015). In addition, habitat offsets can provide research opportunities to perform more basic scientific studies and develop or advance monitoring methods, offering another way offsetting can be valuable (Jones *et al*., 2017).

Habitat offsetting is a tool that can lessen the negative impacts of habitat loss and degradation. The practice of offsetting has many philosophical, logistical, and ecological challenges (Moren-Mateos *et al*., 2015; Sonter *et al*., 2020; Theis *et al*., 2020) that need to be addressed in each offsetting project. I focused on the ecological challenges, by examining trophic structure and dynamics of an offset and sampling a set of natural lakes for comparison with current and future offset lakes in the Alberta oil sands. I improved understanding of fish assemblage trophic structure and dynamics in offsets, natural boreal lake ecosystems, and the use of stable isotopes to perform such studies. As offsetting continues and grows as a practice in the Alberta oil sands, across Canada, and around the globe, many opportunities to build upon this knowledge will become available. Taking advantage of the numerous constructed habitat offsets to study trophic structure and dynamics and advance research methodologies such as stable isotope analysis will help us understand and address the biodiversity crisis I currently face.

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Appendices

Chapter 4 Supporting Information

Table S4.1. The probability that estimates of littoral resource use differ between tissues and seasons for northern pike *Esox lucius*. Probabilities for comparisons of interest are in black (across tissues within a season, or within tissues across seasons), and probabilities for comparisons not of interest are in grey (different tissues in different seasons). Probabilities in bold are those that surpassed our threshold criteria for evidence of a difference between groups (probabilities equal to or greater than 80%). Tables are interpreted as asking: what is the probability that littoral resource use estimates for the group listed in the first column are smaller than littoral resource use estimates for the group listed in the first row? Probabilities ≧0.8 suggest there is evidence that estimates for groups in the first column are smaller than those for groups in the first row, and probabilities ≦0.2 suggest there is evidence that estimates for groups in the first column are larger than those for groups in the first row. Probabilities close to 0.5 suggest there is no evidence that estimates differ between the groups.

		Spring			Fall			Winter		
		Muscle	Caudal fin	Pectoral fin	Muscle	Caudal fin	Pectoral fin	Muscle	Caudal fin	Pectoral fin
Spring	Muscle	0	**0.848**	**0.828**	0.505	0.896	0.868	0.603	0.901	0.873
	Caudal fin		0	0.485	0.164	0.573	0.529	0.214	0.605	0.517
	Pectoral fin			0	0.161	0.601	0.547	0.237	0.605	0.541
Fall	Muscle				0	**0.892**	**0.861**	0.606	0.904	0.871
	Caudal fin					0	0.45	0.161	0.518	0.445
	Pectoral fin						0	0.195	0.56	0.487
Winter	Muscle							0	**0.859**	**0.803**
	Caudal fin								0	0.432
	Pectoral fin									0

Table S4.2. The probability that estimates of littoral resource use differ between tissues and seasons for yellow perch *Perca flavescens*. See explanation in Table S4.1 caption for interpretation of probabilities.

		Spring			Fall			Winter		
		Muscle	Caudal fin	Pectoral fin	Muscle	Caudal fin	Pectoral fin	Muscle	Caudal fin	Pectoral fin
Spring	Muscle	0	**0.887**	**0.862**	**0.825**	0.949	0.946	0.404	0.855	0.714
	Caudal fin		0	0.462	0.477	0.752	0.746	0.083	0.512	0.285
	Pectoral fin			0	0.514	0.777	0.766	0.098	0.54	0.308
Fall	Muscle				0	0.733	0.729	**0.147**	0.528	0.339
	Caudal fin					0	0.494	0.041	0.278	0.129
	Pectoral fin						0	0.04	0.281	**0.134**
Winter	Muscle							0	**0.884**	0.78
	Caudal fin								0	0.302
	Pectoral fin									0

Table S4.3. The probability that estimates of littoral resource use differ between tissues and seasons for lake whitefish *Coregonus clupeaformis*. See explanation in Table S4.1 caption for interpretation of probabilities.

		Spring			Fall			Winter		
		Muscle	Caudal fin	Pectoral fin	Muscle	Caudal fin	Pectoral fin	Muscle	Caudal fin	Pectoral fin
Spring	Muscle	0	**0.9**	**0.855**	**0.176**	0.772	0.616	**0.023**	0.49	0.303
	Caudal fin		0	0.384	0.014	0.279	0.153	0	**0.079**	0.036
	Pectoral fin			0	0.028	0.392	0.241	0.001	0.138	**0.06**
Fall	Muscle				0	**0.947**	**0.882**	**0.192**	0.826	0.671
	Caudal fin					0	0.335	0.004	0.204	0.102
	Pectoral fin						0	0.018	0.37	0.211
Winter	Muscle							0	**0.98**	**0.922**
	Caudal fin								0	0.309
	Pectoral fin									0

Table S4.4. The probability that estimates of trophic position differ between tissues and seasons for northern pike *Esox lucius*. Probabilities for comparisons of interest are in black (across tissues within a season, or within tissues across seasons), and probabilities for comparisons not of interest are in grey (different tissues in different seasons). Probabilities in bold are those that surpassed our threshold criteria for evidence of a difference between groups (probabilities equal to or greater than 80%). Tables are interpreted as asking: what is the probability that trophic position estimates for the group listed in the first column are smaller than trophic position estimates for the group listed in the first row? Probabilities ≧0.8 suggest there is evidence that estimates for groups in the first column are smaller than those for groups in the first row, and probabilities ≦0.2 suggest there is evidence that estimates for groups in the first column are larger than those for groups in the first row. Probabilities close to 0.5 suggest there is no evidence that estimates differ between the groups.

		Spring			Fall			Winter		
		Muscle	Caudal fin	Pectoral fin	Muscle	Caudal fin	Pectoral fin	Muscle	Caudal fin	Pectoral fin
Spring	Muscle	0	0.504	0.565	0.462	0.345	0.395	0.493	0.404	0.454
	Caudal fin		0	0.561	0.466	0.34	0.412	0.473	0.394	0.449
	Pectoral fin			0	0.414	0.297	0.356	0.423	0.35	0.393
Fall	Muscle				0	0.353	0.434	0.517	0.434	0.483
	Caudal fin					0	0.575	0.647	0.558	0.609
	Pectoral fin						0	0.58	0.488	0.54
Winter	Muscle							0	0.402	0.457
	Caudal fin								0	0.551
	Pectoral fin									0

Table S4.5. The probability that estimates of trophic position differ between tissues and seasons for yellow perch *Perca flavescens*. See explanation in Table S4.4 caption for interpretation of probabilities.

		Spring			Fall			Winter		
		Muscle	Caudal fin	Pectoral fin	Muscle	Caudal fin	Pectoral fin	Muscle	Caudal fin	Pectoral fin
Spring	Muscle	0	0.455	0.491	0.608	0.539	0.647	0.583	0.492	0.465
	Caudal fin		0	0.553	0.647	0.582	0.68	0.625	0.54	0.513
	Pectoral fin			0	0.606	0.54	0.638	0.575	0.492	0.459
Fall	Muscle				0	0.437	0.533	0.456	0.393	0.37
	Caudal fin					0	0.58	0.524	0.45	0.43
	Pectoral fin						0	0.431	0.358	0.339
Winter	Muscle							0	0.422	0.398
	Caudal fin								0	0.471
	Pectoral fin									0

Table S4.6. The probability that estimates of trophic position differ between tissues and seasons for lake whitefish *Coregonus clupeaformis*. See explanation in Table S4.4 caption for interpretation of probabilities.

		Spring			Fall			Winter		
		Muscle	Caudal fin	Pectoral fin	Muscle	Caudal fin	Pectoral fin	Muscle	Caudal fin	Pectoral fin
Spring	Muscle	0	0.758	0.715	0.238	0.581	0.529	0.229	0.631	0.559
	Caudal fin		0	0.435	0.081	0.304	0.269	0.068	0.347	0.276
	Pectoral fin			0	0.108	0.356	0.32	0.094	0.405	0.337
Fall	Muscle				0	0.812	0.773	0.513	0.852	0.806
	Caudal fin					0	0.443	0.174	0.553	0.467
	Pectoral fin						0	0.213	0.602	0.533
Winter	Muscle							0	0.86	0.821
	Caudal fin								0	0.426
	Pectoral fin									0