Comparing habitat use and mercury accumulation in Arctic Grayling (*Thymallus arcticus*) from two northern ecosystems

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

An improved understanding of the distribution and habitat requirements for mountain stream populations of Arctic Grayling (*Thymallus arcticus*) is necessary to assess their vulnerability to environmental stressors, establish thresholds for development activities, and evaluate population distribution trends over time. Life stage-specific habitat use may be a particularly sensitive indicator of environmental change, but baseline data is lacking, especially for young-of year (YOY) Arctic Grayling. Occupancy-based survey methods were used in the Little Nahanni River watershed in 2015 to quantify Arctic Grayling occupancy across the landscape. Data on a suite of associated habitat variables were also collected to explain YOY occupancy, and to relate to detection efficiency during field surveys, as detection efficiency is an often-neglected aspect of field sampling that affects our understanding of species-habitat relationships. Occupancy modelling has revealed that stream temperature ($>8°C$) and elevation (<1150 masl) best explain YOY occupancy in the Little Nahanni River. Increasing percent boulder substrate and percent riffle decreased the detection efficiency during surveys. By accounting for imperfect detection, my research helps to better quantify habitat that is important for a sensitive life stage of Arctic Grayling and establishes a baseline against which results from future monitoring efforts can be compared. Similar occupancy methods can be used by industry and regulatory organizations to increase standardization and efficiency of sampling in remote areas, and assess changes in Arctic Grayling distribution that may reflect changes in water quality and stream features.

Abiotic and biotic habitat features can also affect bioaccumulation of contaminants, such as mercury, which have increased in remote northern regions since industrialization, bioaccumulating in fish tissues consumed by northerners. Given that mercury bioaccumulation is habitat-specific, differences in life history type and associated habitat features may affect

mercury concentrations in populations of Arctic Grayling. I assessed differences in mercury bioaccumulation between fluvial Arctic Grayling in mountain streams and adfluvial Arctic Grayling in barrenland tundra streams. Overall, mercury bioaccumulation was higher in adfluvial Arctic Grayling from tundra barrenland streams compared to fluvial, mountain populations. Differences in mercury concentration between these populations of Arctic Grayling may be due to differences in methylation rates caused by a number of habitat factors, such as increased water temperatures, as well as differences in baseline methyl-mercury (MeHg) concentrations that may reflect a difference in foraging strategies by each respective population. As well, trophic interactions and the role of terrestrial prey items may influence mercury concentrations in fish tissue, however; further investigation is required to quantify these potential effects. All mercury concentrations in Arctic Grayling sampled during my study were below guidelines for human consumption of fish.

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(Equation 2.1)

$$\hat{c} = \chi^2_{obs} \Big/ \bar{\chi}^2_B$$

(Equation 2.2) z-score $= \frac{x_{i-}\mu}{\sigma}$

(Equation 2.3) (model iterations) $X = \frac{n}{r*(n-r)}$

(Equation 3.1) adjusted $\delta^{15}N$ (or $\delta^{13}C$) $= \delta^{15}N_{fish/invertebrate} - \delta^{15}N_{baseline}$

(Equation 3.2) Fulton's condition factor (K): $K = \left(\frac{W}{L^3}\right) \times 100$

(Equation 3.2) Relative condition factor (K$_r$): $K_R = \frac{W}{\exp(a+b \; x \; Ln(L))}$

Chapter 1

Introduction

1.1 General Introduction

Northern Canada contains vast, intact freshwater ecosystems that are undergoing rapid environmental change (IPCC, 2007). Stressors, such as climate change, land use, and contaminant deposition, are altering northern aquatic ecosystems, and effects are revealing the complexity of interactions that result in altered ecosystem function (Schindler et al., 1995; Ficke et al., 2007; Post et al., 2009). For example, the accelerated warming that is predicted for northern regions will likely have profound effects on Holarctic aquatic ecosystems (Rouse et al., 1997; IPCC, 2007), including effects on distribution, habitat use, and life history of fishes (e.g., Reist et al., 2006). Fish species can be particularly useful for detecting and assessing changes in aquatic environments, because they are susceptible to human-induced stressors (Whitney et al., 2016), and are valued by a variety of stakeholders. Fishes of the family Salmonidae, including Arctic Grayling, are often considered to be useful sentinel species, because they are sensitive to changes in the environment and typically require specialized habitats to complete specific life stages (Beer & Anderson, 2011).

Arctic Grayling (*Thymallus arcticus*, Pallas 1776) is a cold-water, stenothermic fish species within the *Thymallus* genus (Salmonidae family). Arctic Grayling are best known for their sail-like dorsal fin (Figure 1.1) and their sensitivity to changes in water quality (e.g., turbidity; Birtwell et al., 1984). Growing to a maximum length of approximately 50 cm, the body is covered in large scales that are often perceived as having a blue hue, with black circular markings occurring most often on the cheek and trailing behind the gill plate toward the caudal

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fin (Scott & Crossman, 1973). Arctic Grayling are iteroparous and show spawning site fidelity (Northcote, 1995; Deegan et al., 1999). Habitat use and life history characteristics vary among populations, and seasonal migrations can be complex. Arctic Grayling can use riverine and lacustrine waterbodies to complete different life stages - adfluvial, fluvial, and lacustrine populations have been described (Vincent, 1962; Scott & Crossman, 1973; de Bruyn & McCart, 1974; Kaya, 1991).

Figure 1.1. Illustration of an adult male Arctic Grayling from Baker Creek, Northwest Territories (NWT; Paul Vecsei, 2008).

1.2 Arctic Grayling Distribution

Fishes in the genus *Thymallus* span North America and Eurasia, with Grayling, *Thymallus thymallus*, distributed throughout European countries and Arctic Grayling distributed across Holarctic regions of Canada, Alaska, and Asia (Scott & Crossman, 1973). Arctic Grayling colonized North America between 3-5 MYA, after migrating through the Bering land bridge from their origin in eastern Siberia (Stamford & Taylor, 2004). Current distributions in North America originated from three glacial refugia: North Beringia, South Beringia and Nahanni refuges. This separation in refugia led to distinct genetic lineages (Stamford & Taylor, 2004).

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Historically, the distribution of Arctic Grayling stretched southward from the eastern Lake Superior in Ontario to the Missouri River basin. However, major extirpations due to overexploitation and habitat disturbance have led to significant range retraction (Buhl & Hamilton, 1991; Kaya, 1991). Canadian populations are currently only found in the western provinces, from Manitoba to British Columbia, and the three Canadian territories (Northcote, 1995; Stewart et al., 2007). Most populations occur in sub-Arctic or Arctic ecoregions. Populations of Arctic Grayling have also been introduced to various cold-water lakes and rivers across Canada and the US, mainly for sport fishing purposes (Beauchamp, 1990; Peterson & Ardren, 2009). Adaptations to a variety of aquatic ecosystem types and plasticity in life history have allowed the species to populate cold-water lakes, and, barrenland and mountain streams in boreal, mountain and tundra environments.

1.3 Life History

Like many species within the family Salmonidae, Arctic Grayling display plasticity in life history traits. This plasticity allows them to inhabit a variety of aquatic ecosystems, including lakes, rivers and streams (Jones et al., 2010). Similar to other Salmonids, Arctic Grayling require different habitats for spawning, rearing, feeding, and overwintering. Three different life history strategies have been described for Arctic Grayling, according to habitats utilized during different life stages: lacustrine, adfluvial and fluvial (Scott & Crossman, 1973).

Lacustrine populations of Arctic Grayling are strictly lake-dwelling for all life stages. Though relatively less common, lacustrine populations of Grayling, including European and Arctic Grayling, have been documented across their distributional range (Peterman, 1972; Northcote, 1995; Peterson & Ardren, 2009; Amundsen et al., 2010). There is no geographic

pattern to lacustrine population distribution; however, new populations have been introduced as part of stocking efforts for sport-fishing in the United States (Peterson and Ardren, 2009).

Adfluvial populations of Arctic Grayling utilize a combination of lentic and lotic environments to complete various life stages (Watry & Scarnecchia, 2008). Adults use lake habitats for feeding in summer and as a refuge from ice formation during winter (West et al., 1992). Mature adfluvial Arctic Grayling also use lotic environments - rivers, streams or stream outlets - for spawning in the spring and during seasonal migrations in spring and fall (Buzby & Deegan, 2004). Less is known about habitat use and seasonal migrations for young-of-year (YOY) and juvenile Arctic Grayling. YOY and juvenile Arctic Grayling use spawning streams as rearing habitat, since lotic environments provide drift-feeding opportunities and may provide refuge from predators (Schlosser, 1987; Hughes, 1999). Important habitat characteristics have been identified for YOY in adfluvial, barrenland populations of Arctic Grayling. Preferred habitat characteristics include shallow depths (< 30cm for small YOY), presence of midstream cover (for YOY > 25mm) and reduced water velocity (< 0.2m/s; Jones & Tonn, 2004; Baker et al., 2017). Evidence suggests that YOY Arctic Grayling show high fidelity to natal streams (Craig & Poulin, 1975; Buzby & Deegan, 2000), migrating to overwintering areas after completing their first summer feeding season (Craig & Poulin, 1975). However, our understanding of habitat use during the overwintering stage of Arctic Grayling life-history is lacking.

Fluvial Arctic Grayling populations have been documented in Alaska, Montana, and Canada (McPhail & Lindsey, 1970; Scott & Crossman, 1973; Jessop & Lilley, 1975; Kaya, 1991; Lohr, 1996). Fluvial populations of Arctic Grayling remain in lotic systems for all life stages, often migrating between streams and rivers with differing environmental characteristics to carry out

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different life stages (Watry & Scarnecchia, 2008). Fluvial populations typically migrate in spring

to streams dominated by gravel or silt substrate for spawning; these streams can vary in water

velocity (0.34 – 1.50 m/s; Tack 1971; Stewart et al., 2007). Streams used by adult fluvial Arctic

Grayling for feeding in summer have similar velocities (0.61 – 1.08 m/s) with greater water

depths (1.10 – 1.52 m) than spawning streams (Evans et al., 2002). In feeding streams, Arctic

Grayling disperse in an ordered manner with larger, dominant, Arctic Grayling maintaining

feeding territories in lower-order (higher elevation) streams (Hughes, 1999). Fluvial populations

of Arctic Grayling use higher order and lower elevation streams and rivers for overwintering

(West et al., 1992). In general, fish populations in remote regions of the sub-Arctic are

understudied, and as such there is limited quantifiable information about habitat use by fluvial

Arctic Grayling in this area, particularly for YOY.

1.4 Fluvial Reproduction and Early Development

Adult Arctic Grayling are iteroparous and reach sexual maturity between 3 and 8 years of

age (Craig & Poulin, 1975; Buzby & Deegan, 2004). Similar to other iteroparous Salmonids,

such as Bull Trout (*Salvelinus confluentus*) and Atlantic Salmon (*Salmo salar*), Arctic Grayling

may skip reproductive events to conserve bioenergetic resources (Fleming, 1998; Buzby &

Deegan, 2004). Skipped spawning can improve survivorship and protect against year class

failure (Johnston & Post, 2009). Long migrations, sometimes over 100 km in length, between

overwintering and spawning streams can contribute to lowered individual fitness and thus result

in skipped reproduction (West et al., 1992). Migration events are driven by Arctic Grayling

preference for natal spawning streams, which can vary slightly across their geographic range

depending on population, regional stream morphology and climatic events (Craig & Poulin,

1975; Northcote, 1995; Buzby & Deegan, 2000). However, general trends in suitable spawning habitat have been documented across many North American populations of Arctic Grayling.

Arctic Grayling are spring spawners that can begin migrations under the river ice, completing their spawning event when water temperatures reach between 4 to 5 °C in May or early June (Peterman, 1972; Jones et al., 2003a). Spawning streams contain clear water with small gravel and sand substrates and a water velocity typically less than 1.50 m/s (Stewart et al., 2007). Males migrate into lower-order streams, two weeks in advance of females, and defend spawning territories (Beauchamp, 1990). Females deposit their adhesive negatively-buoyant eggs over clean gravel where flowing water moderate's temperature for optimized egg development. Eggs fall into interstitial spaces of the substrate where they incubate for two to four weeks before the eggs hatch as alevins in time for the summer feeding period (Kaya, 1991; Mantua et al., 2010).

The alevin stage is considered an embryonic stage of fish development; newly hatched Arctic Grayling are supported by their yolk-sac and emerge from the substrate after $3 - 5$ days (McPhail & Lindsey, 1970; Deegan et al., 2005). Alevins use an inflated air bladder to emerge to the surface during evening hours and become free-swimming fry (Bardonnet & Gaudin, 1990). Free-swimming fry can be as small as 2 mm in length and are referred to as YOY during their first year of life (Kreuger, 1981). YOY are particularly susceptible to changes in the environment (e.g., temperature and velocity), and as such, the YOY age class is a critically sensitive life stage. Fry generally feed in shallow slack water, along the periphery of streams. They prey on small invertebrates, and prey size gradually increases as the YOY grow larger (McPhail & Lindsey, 1970; Deegan et al., 2005). Adfluvial and fluvial Arctic Grayling populations show different behavioural tendencies as early as three weeks old; adfluvial/lacustrine populations start moving

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downstream to seek refuge in still waters whereas fluvial Arctic Grayling show upstream rheotaxis (Kaya, 1991).

Habitat preferences exhibited by fluvial populations of Arctic Grayling vary across tundra, coastal, boreal, and mountain ecosystems (Table 1.1). YOY habitat is better characterized for Arctic Grayling in inland mountain systems than in tundra, coastal and boreal regions. Inland mountain fluvial systems differ from coastal or tundra ecosystems, in that they consist of high gradient, cold-water streams that are not in coastal habitats and often show high variability in substrate type among streams. Qualitative descriptions of YOY habitat in mountain coastal drainage populations include shallow, calm side pools and channels with coarse sand and gravel (de Bruyn & McCart, 1974). Although data are limited, YOY Arctic Grayling in mountain streams have been found in water velocities < 0.16m/s (Krueger, 1981), and in association with in-stream boulder cover and shallow pool habitats (Stuart & Chislett, 1979; Kreuger, 1981).

Achieving sufficient growth in the first year is critical for Arctic Grayling, for holding position in flowing environments and achieving migrations between habitats used during different life-history stages. Stream characteristics, such as presence of groundwater, may play a role in growth and help moderate stream temperatures in cold-water mountain streams. Currently, the role of groundwater and how it influences YOY Arctic Grayling habitat choice is poorly understood, but groundwater can provide important warm-water refugia for fish species in cold-water mountain streams (Dunmall et al., 2016). Studies suggest that YOY Arctic Grayling may out-migrate from natal streams to downstream riverine habitats in order to find flowing water over winter months (West et al., 1992). Other studies suggest that groundwater may play a significant role by providing flowing, oxygenated water for overwintering in natal streams (Craig & Poulin, 1975; Ford et al., 1995).

Table 1.1 Quantifiable habitat characteristics preferred by YOY Arctic Grayling identified in previous scientific literature.

Population Type	Location	Life Stage	Variable	Value	Source
fluvial	Montana, mountain/agricultural streams	all age classes	depth width velocity temperature	0.284 m 12.21 m 0.21m/s 13.7°C	Liknes & Gould, 1987
adfluvial	Montana, mountain stream	YOY	substrate	silt/sand (0.062 – 2.0m)	Deleray, 1991
fluvial	Montana, artificial stream	YOY	depth	< 0.31m	McClure & Gould, 1991
Unknown	Alaska, tundra stream	YOY	velocity temperature	0.15-0.25m/s <12°C	Deegan et al., 2005
adfluvial	NWT, barrenland stream	small YOY (15-21mm)	depth velocity	~0.10-0.20m <0.10m/s	Jones & Tonn, 2004
adfluvial	NWT, barrenland stream	large YOY (38-57mm)	depth substrate	~0.25-1.0m fines	Jones & Tonn, 2004
adfluvial	NWT, barrenland stream	YOY	water velocity depth	0.05-0.20m/s < ~ 0.5m	Baker et al., 2017

Habitat use and migration of YOY Arctic Grayling in northern mountain fluvial systems is poorly understood. Qualitative descriptions are often used in the literature, with little reference to quantifiable habitat characteristics. Quantifying habitat use by YOY is critical to the development of effective monitoring and conservation plans in the face of increasing anthropogenic threats.

1.5 Threats

Arctic Grayling are considered secure globally; however, this species is vulnerable at more regional and local levels (NatureServe, 2016). Arctic Grayling populations can become locally sensitive to various pressures, such as overfishing, habitat disturbance, habitat degradation, and habitat fragmentation (Kaya, 1991; Clark, 1992; Northcote, 1995). Effects of stressors are most profound when they alter habitats important for critical life stage events, such as spawning, rearing and overwintering (Reist et al., 2006).

Mineral and hydrocarbon exploration and development are altering the remote landscape of northern Canada and are becoming more common as initiatives to promote economic growth in the region (see Cott et al., 2015). Valuable resources, such as natural gas and metals, are driving development further into pristine areas, and effects of these developments can impair natural ecosystem function. Mining developments often include landscape disturbances such as road construction, aggregate removal, and water diversion that can negatively affect sensitive fish species (Cott et al., 2015). Natural resource development can affect fish habitat by increasing water temperature (Kondolf, 1997), turbidity (McLeay et al., 1987) or altering natural stream flow (see Poff & Zimmerman, 2010). Fish species, like Arctic Grayling, that show fidelity to streams for various life stages and are more vulnerable to anthropogenic impacts than transient (Buzby & Deegan, 2000). As cold-water stenotherms, Arctic Grayling are particularly sensitive to changes in stream temperature and water clarity during sensitive life stages. YOY Arctic Grayling require temperatures below 16°C to avoid increases in their metabolic rate (Deegan et al., 2005). Turbidity greater than 20 NTU that results from upstream mining operations or road construction can greatly reduce the ability of adult Arctic Grayling to detect prey and can force juvenile and YOY Arctic Grayling to migrate out of suitable habitat to avoid turbid water

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(Birtwell et al., 1984; Scannell, 1988; Kemp et al., 2011). Mining operations may also affect water discharge regimes by diverting or modifying stream flow, which can interfere with fish migrations (Mitchell & Cunjack, 2007). Lower baseline discharges can also increase water temperatures and alter fish behaviour (Daufresne et al., 2003). Such effects of mining and other natural resource development may be further compounded by climate change, creating cumulative stressors on Arctic Grayling populations.

Contiguity of migratory pathways for fluvial populations of Arctic Grayling are threatened by land use changes (West, 1992). Arctic Grayling show high site fidelity for all life stages (spawning, rearing feeding and overwintering), which make them especially vulnerable to habitat alteration (Buzby & Deegan, 2000). Stream characteristics, like riparian vegetation, can be important habitat for Arctic Grayling and their prey (Kawaguchi & Nakano, 2001). Arctic Grayling, like other Salmonids, also show affinity for particular sediment size and types for spawning and rearing. Stream bed alternation and sedimentation from logging activities has been implicated for extirpations of Arctic Grayling in Ontario and Michigan (Stewart et al., 2007). Although land use changes occur at a significantly lower rate in the NWT than in southern parts of their range, Arctic Grayling should be considered vulnerable to development-induced habitat alteration, especially in areas with road or trail access.

Overfishing is a growing concern for government organizations such as Parks Canada and Fisheries and Oceans Canada (DFO) in more remote regions of northern Canada. Overfishing has been partly responsible for declines and extirpations of Arctic Grayling populations in several watersheds in Alaska (Buzby & Deegan, 2000), Alberta (Berry, 1998), Michigan (Vincent, 1962) and Ontario (Scott & Crossman, 1973). Overfishing has also reduced stocked populations and impeded stocking efforts in the Chena River, Alaska (Buzby & Deegan, 2004).

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Arctic Grayling is considered a prized sport fish associated with northern regions, and the species attracts fish enthusiasts and promotes tourism in the NWT (McPhail & Lindsey, 1970; Scott & Crossman, 1973). Newly developed roads and increased access to remote areas as a result of natural resource development can expose Arctic Grayling to increased fishing pressure leading to overfishing.

Air temperatures are increasing, and more frequent extreme weather events are projected, with effects of climate change being even more pronounced at northern latitudes (IPCC, 2007). The effects of climate change on northern fishes are unclear, however projections suggest that cold-water fishes, including most Salmonids, will experience some of the greatest changes in environmental conditions. Climate change can influence stream thermal regimes (Reist et al., 2006; Dallas, 2016), alter benthic invertebrate abundance and diversity (Wrona et al., 2006), as well as disrupt migratory cues for fish (Swanberg, 1997; Reist et al., 2006). In high elevation streams, climate change has the potential to shift elevational thresholds because of warming temperatures, creating opportunities for other competing species (Wiens, 2016). Presumably, cascade effects on food web structure involving Arctic Grayling can occur as a result of climate change, although effects are difficult to predict. Regardless of uncertainty, cold-water Salmonids are among the most-likely organisms to reflect impacts from climate change, and more baseline information for inland freshwater ecosystems is needed to help predict and characterize impacts.

1.6 Study Rationale

The impetus for this research stems from existing knowledge gaps about Arctic Grayling, specifically what habitat characteristics are associated with critical life stages of the fluvial life history type of the species. Literature about YOY Arctic Grayling and associated habitat use is sparse and is often associated with adfluvial populations in coastal (West et al., 1992) or barrenland habitats (Jones et al., 2003b; Baker et al., 2017). Aquatic ecosystems in inland mountainous regions can be markedly different in terms of terrain and climatic characteristics when compared to coastal or barrenland regions, as such YOY Arctic Grayling in mountainous regions may have different habitat requirements. It has been implied that Arctic Grayling in mountain streams remain fluvial for all life-stages (Liknes & Gould, 1987).

Arctic Grayling have been proposed as an indicator species for both biotic and abiotic changes in northern aquatic systems (Stewart et al., 2007; Wedekind & Küng, 2009). This research will strengthen our understanding of YOY Arctic Grayling habitat use. Improved knowledge regarding habitat use of Arctic Grayling will assist resource managers determine if habitat and/or habitat use is changing over time in response to natural resource development or climate change. Due to the scarcity of literature on this topic, decision makers in government, industry and First Nations are often under-informed about ecological consequences of development projects. Developing our understanding of habitat use by Arctic Grayling in mountainous regions will help further the scientific knowledge about this sensitive fish species and will allow more effective conservation and monitoring plans. Parks Canada and Fisheries and Oceans Canada have expressed interest in refining their monitoring guidelines and protocols for northern Salmonids, using Arctic Grayling as a sentinel species.

Arctic Grayling occupy many ecoregions and habitats, and this diversity in habitat use results in variability in contaminant accumulation. In addition to assessing habitat use, my research will examine the bioaccumulation of mercury in Arctic Grayling from two distinct remote northern regions. Bioaccumulation of mercury varies in response to location and habitat use of fish species (Chételat et al., 2015) and thus, understanding habitat use by Arctic Grayling can enable better understanding of mercury bioaccumulation in this species. Mercury is a bioaccumulating neurotoxin that is harmful for humans if exposure is high enough (see AMAP, 2011). The subsistence diet of Indigenous communities in the interior of NWT is largely comprised of wild game and fish, with fish supplementing the diets of approximately 40% of the NWT population (GNWT, 2014). Further, 25% of the population of the NWT participates in recreational fishing (Fisheries and Oceans Canada, 2012), where the Arctic Grayling is a prized game fish. Informing Indigenous communities and the general public about potential mercury bioaccumulation is important for maintaining human and wildlife health. It would benefit these groups to understand if mercury accumulation in Arctic Grayling is a human health concern. Co-management boards, such as the Sahtu Renewable Resources Board and Dehcho Consensus Team have shown interest in better understanding the fish and freshwater health in their regions to determine if mining will impact bioaccumulation of contaminants in the region.

1.7 Habitat Characterization using Occupancy Analysis

Little work has been done to quantify YOY habitat use northern mountain streams in a manner that also allows for effective, replicable survey methods for future monitoring. Research and modelling completed for sub-Arctic barrenland, adfluvial populations of YOY Arctic Grayling have identified specific habitat characteristics, such as water velocity (~2-10 cm/s), depth (~10 – 50 cm), stream slope (> 2 degrees) and greater amount of adjacent wetland that are preferred (Jones et al., 2003b; Baker et al., 2017). By applying similar survey and modelling methods to mountain systems, habitat use can be directly compared between ecoregions (barrenland versus mountainous) and life history types of YOY Arctic Grayling (fluvial versus adfluvial populations).

A common method for assessing habitat use is distribution modelling. Distribution models relate a given species presence to corresponding habitat features and can be useful for predicting species distribution in relation to habitat features. This can be especially useful in remote regions, where cost of work and time requirements often limit study (Albanese et al., 2007).

Occupancy analysis is a type of distributional modelling used for quantifying species' habitat use while accounting for imperfect detection (MacKenzie et al., 2002). With previous modelling approaches (e.g., resource selection functions), it was impossible to discern whether absence of a species truly meant the spatial unit of study was unoccupied (species absence) or whether the species simply was not detected (false absence). Typical occupancy modelling is based on a two-state model (presence-absence) and detection efficiency (i.e., probability of detection), which is most traditionally determined using temporally replicated surveys (MacKenzie et al., 2002). Results from occupancy models provide estimates of probability of occupancy (ψ), as well as probability of detection (p), which is less than 1 (1 represents a perfect

method of detection). Environmental covariates, either abiotic or biotic, can be related to both probability of occupancy and detection (MacKenzie & Royle, 2005). By accounting for imperfect detection, more realistic habitat use can be predicted from model outputs (MacKenzie et al., 2002; Kéry & Schmidt, 2008).

There are several advantages to using an occupancy model approach for assessing habitat use in remote regions. First, presence-absence data are quicker to produce than abundance data. In addition, although the occupancy model approach most commonly uses temporal replicates to generate estimates of detection probability, spatial replicates can also be used. Using spatial replicates can maximize efficiency in regions that are difficult to access (Charbonnel et al., 2014), and if a species is sparse, sampling more sites with less temporal replication can produce more robust results (MacKenzie & Royle, 2005). Spatial replication in remote areas can also be highly effective when the study species is restricted to linear transects of habitat, such as streams (Hines et al., 2010; Parry et al., 2013; Charbonnel et al., 2014).

Occupancy modelling requires that four assumptions be met:

i) **Closure** – sites must remain occupied or unoccupied for a defined sampling period. Individual fish may move in or out of the sampling unit, but overall the state of the sampling unit (unoccupied or occupied) cannot change during a user-defined 'season' of sampling.

ii) **Site independence** – a site is not occupied as a result of being in close proximity to another occupied site. If this assumption is violated, an auto-correlation structure can be implemented into the model.

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iii) **Proper species identification –** ensures that data collected in the field does not lead to false presence or absence of a target fish species, which would inaccurately inform model outputs.

iv) **No unmodeled heterogeneity** – ensures that all variability in fish occupancy and detectability is explained by environmental variables collected and included in the model. To avoid violating this assumption, practitioners must select hypothesis-driven and scientifically relevant covariates to measure.

1.8 Linking habitat use and life history with mercury bioaccumulation

Mercury is a contaminant of particular concern in northern regions. Fish are the biggest source of mercury to humans, and many northerners rely on fish as a subsistence food source (e.g., AMAP, 2011; see Wheatley & Paradis, 1995). Concentrations of mercury increase up the food chain and can cause harmful neurotoxic effects for humans (see AMAP, 2011). Data from this study will allow a comparison of mercury concentrations in adfluvial Arctic Grayling from a barrenland system to fluvial Arctic Grayling from a mountainous system. Mercury concentrations in fish are affected by a variety of variables that should vary between barrenland and mountainous ecosystems, and between adfluvial and fluvial Arctic Grayling, including: water temperature, dissolved oxygen, dissolved organic carbon, primary productivity, fish growth rates, and fish trophic position (see AMAP, 2011).

Mercury is deposited in remote, northern environments via long-range atmospheric transport from industrialized areas, but point sources from development operations, such as mining, also contribute (Fitzgerald et al., 1998; Morel et al., 1998; Stern et al., 2012). Despite a reduction in anthropogenic emissions of mercury in recent decades (AMAP, 2011), permafrost thaw

(Rydberg et al., 2010) and increased forest fire activity (Kelly et al., 2006) may contribute to increased mercury deposition in northern aquatic ecosystems. Upon deposition into aquatic ecosystems, mercury can be reduced and methylated from Hg (II) to MeHg. Methyl mercury is the organic form of mercury that accumulates in fish tissue by binding to amino acid groups. MeHg biomagnifies in food chains with each trophic transfer (Morel et al., 1998; Hoffman et al., 2002) and comprises a high percentage of total mercury (THg) in most fish (Bloom, 1992; Forsyth et al., 2004). THg can, therefore, be used as a surrogate for measuring MeHg, as laboratory analysis for THg is more time and cost-effective than MeHg analysis (Bloom, 1992). Higher MeHg concentrations tend to occur more often in northern ecosystems as a result of the longer-lived and slow-growing fishes typical of the region (see Morel et al., 1998; Evans et al., 2005). Mercury concentrations in stream systems, as in lake environments, increase with higher trophic position in biota (Evans et al., 2005; Chasar et al., 2009). However, direct comparisons of mercury concentrations in fluvial vs. adfluvial or barrenland vs. mountain populations Arctic Grayling have not been undertaken. Trophic position, as well as mechanisms within the biogeochemical cycle of mercury, may affect differences in mercury concentrations between the two life history and landscape types.

1.9 Objectives

Objective 1:

To better understand and quantify young-of-year habitat use in mountain stream ecosystems using occupancy modelling.

Due to the remoteness of mountainous regions of NWT and the consequent high costs of performing field research, data on habitat use by fluvial Arctic Grayling in northern mountain systems are largely unavailable. To address this knowledge gap, an occupancy modelling approach was used to quantify YOY habitat use in stream habitats in the Nahanni Mountains of the NWT. Following MacKenzie et al. (2002), the probability of YOY Arctic Grayling occupying streams was assessed using the occupancy modeling estimation and information theoretic approach. Ecological covariates were selected *a priori* to explain habitat occupancy and detection efficiency and were based on previous literature and personal knowledge of stream fish habitat use in mountain ecosystems.

Hypothesis:

YOY Arctic Grayling presence is dependant on landscape-level habitat characteristics that promote survival in cold-water mountain ecosystems and will vary with life-stage.

Predictions:

i) YOY Arctic Grayling have limited swimming ability and as such their habitat use in mountain systems will be best predicted by characteristics that determine water flow such as stream slope and elevation.

ii) Stream-specific habitat variables that explain flow dynamics (e.g., water velocity) and in-stream cover (e.g., overhanging vegetation) will negatively affect detection efficiency during surveys in mountain systems.

Objective 2:

To quantify and compare mercury concentrations in Arctic Grayling from fluvial mountain populations to concentrations in adfluvial barrenland tundra populations.

Mercury concentrations can vary within species in response to landscape variables that affect methylation, food web structure, habitat use, and bioaccumulation rates, and between populations with different life histories and growth rates. Linear statistical models were used to compare THg concentration between a mountain fluvial and barrenland adfluvial populations of Arctic Grayling. Fish captured in two ecosystems: Little Nahanni River and Kennady Lake, representing a mountain fluvial tundra adfluvial populations respectively.

Hypothesis:

Ecosystem processes will influence nutrient availability and ecosystem productivity that will, in turn, affect fish growth rates and ultimately mercury bioaccumulation in Arctic Grayling.

Prediction:

      i)   Arctic Grayling mercury concentrations will be higher in Kennady Lake than the Little Nahanni River as adfluvial populations feed in a more complex lentic environment, resulting higher trophic position for fish from Kennady Lake.

Chapter 2

Young of year (YOY) Arctic Grayling occupancy in the Little Nahanni River, NWT

2.1 Introduction

Resource development and climate-related impacts are predicted to alter aquatic ecosystem function in remote northern regions at a rate that may outpace resource management and mitigation efforts (Lemly, 1994; Schindler, 2001). This is concerning for northern residents, especially northern Indigenous communities, who rely on healthy aquatic ecosystems for subsistence hunting and fishing (Berkes, 1990). To enable better long-term management outcomes, regulators, industry, and communities alike are looking to better understand past and current ecological conditions, so as to better predict what may happen in future. Currently, quantitative baseline data are lacking for many northern aquatic ecosystems, making it difficult to determine and predict changes in ecosystem function (Reist et al., 2006).

Research in remote northern streams has been limited, mostly due to the logistic difficulties and high cost of northern research. These high latitude ecosystems are projected to experience intensified impacts from climate change and other anthropogenic stressors at a faster rate than the global average (Prowse et al., 2006). In northern Canada natural resource development is increasing at a rapid pace and these activities can impact northern fishes (Cott et al., 2015). Anthropogenic activities, such as resource extraction, can alter biotic and abiotic characteristics in northern streams, and are predicted to negatively affect northern fishes by altering habitat use and restricting distribution (Reist et al., 2006). Northern stream fishes are dependent on specific habitat characteristics, such as in-stream cover as flow refugia to lower energetic costs and maximize fitness (e.g. Jones & Tonn, 2004). Alterations of critical habitat features that result

from natural resource development, and temperature alterations from climate change can have significant effects on stream fish populations (Allan & Flecker, 1993; Wood & Armitage, 1997). For example, increased sedimentation and turbidity from placer mining can significantly decrease feeding opportunities and affect gill function of Arctic Grayling in Yukon Territory (McLeay et al., 1987). Climate and development-induced increases in stream temperatures can also negatively affect cold-water fishes by increasing mortality over extended warming periods (Becker & Genoway, 1979; Mantua et al., 2010). Stream fishes with migratory life histories, such as Arctic Grayling (*Thymallus arcticus* Pallas 1776), may be especially vulnerable to habitat alteration, as these fishes require multiple intact habitats and migratory corridors (Reist et al., 2006).

Arctic Grayling is a freshwater salmonid species that is found in remote regions of northern North America. The distribution of Arctic Grayling ranges west from the Hudson Bay coast throughout the mainland Canadian territories and Alaska to the western coast of British Columbia, and south to Montana (Scott & Crossman, 1973). This cold-water fish inhabits streams, rivers, and lakes, mainly in Arctic and sub-Arctic regions of Canada (Vincent, 1962; Scott & Crossman, 1973; de Bruyn & McCart, 1974; Kaya, 1991). Residents of northern communities value Arctic Grayling as an opportunistic food source and recreational sport fish. Arctic Grayling are considered a sentinel species that have specific habitat requirements and are sensitive to changes in their environment, particularly during the young-of-year (YOY; Age-0) life stage (Birtwell et al., 1984, Deegan et al., 1999). Streams associated with YOY Arctic Grayling often have high invertebrate production, which promotes rapid growth and lipid storage in developing fishes that is necessary for over-winter survival (Deegan et al., 2005; Heim et al., 2016). After their first summer, YOY Arctic Grayling typically migrate and overwinter in larger

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streams, rivers or lakes that remain unfrozen during winter periods (Buzby & Deegan, 2004). It is not currently known whether groundwater inputs play a role in providing overwintering habitat for YOY Arctic Grayling, providing young fish with a warm-water thermal refuge during winter months.

Arctic Grayling have three history strategies: lacustrine (lake-dwelling), adfluvial (move between rivers and lakes) and fluvial (live in streams and rivers), with the latter to strategies being most prevalent (de Bruyn & McCart, 1974). Adfluvial populations use lentic environments for feeding and overwintering, and lotic environments for spawning, rearing and juvenile development (Beauchamp, 1990; West, 1992; Jones & Tonn, 2004; Baker et al., 2017). Habitat preferences for adfluvial Arctic Grayling have been studied in Alaska, Montana, and in Canadian barrenland tundra ecosystems (Table 2.1). Habitats used by fluvial YOY Arctic Grayling have been described as shallow, low-flow environments with fine sand or gravel sediments, and the use of side channels or wet, grassy areas has also been noted (de Bruyn & McCart, 1974; Northcote, 1995; Stewart et al., 2007). Quantitative data regarding habitat use by fluvial YOY Arctic Grayling is sparse (Table 2.1), and this is particularly true for northern mountain environments, which are difficult and expensive to sample.

Table 2.1. Quantified habitat characteristics for YOY Arctic Grayling identified in previous scientific literature.

Population Type	Location	Life Stage	Variable	Value	Source
fluvial	Montana, mountain/agricultural streams	all age classes	depth width velocity temperature	0.284 m 12.21 m 0.21m/s 13.7°C	Liknes & Gould, 1987
adfluvial	Montana, mountain stream	YOY	substrate	silt/sand (0.062 – 2.0mm)	Deleray, 1991
fluvial	Montana, artificial stream	YOY	depth	< 0.31m	McClure & Gould, 1991
adfluvial	NWT, barrenland stream	small YOY (15-21mm)	depth velocity	~10-20cm <0.10m/s	Jones & Tonn, 2004
adfluvial	NWT, barrenland stream	large YOY (38-57mm)	depth substrate	~25-100cm fines	Jones & Tonn, 2004
Unknown	Alaska, tundra stream	YOY	velocity temperature	0.15-0.25m/s <12°C	Deegan et al., 2005
adfluvial	NWT, barrenland stream	YOY	water velocity depth	0.05-0.20m/s < ~ 0.5m	Baker et al., 2017

Arctic Grayling are particularly vulnerable during their YOY life stage compared to adults, when the young fish are more susceptible to environmental perturbations such as high turbidity and have limited sustained swimming ability to avoid adverse conditions (Birtwell et al., 1984; McLeay et al., 1987; Deegan et al., 2005). Developing quantitative models of YOY Arctic Grayling habitat use is critical for long-term management and conservation of the species. While these models exist for adfluvial Arctic Grayling in barrenland systems (Jones et al., 2004; Baker et al., 2017), quantitative descriptions of habitats used by fluvial, YOY Arctic Grayling are lacking, particularly at larger landscape scales.

Various models can be used to relate presence or abundance of an organism to habitat characteristics. Distribution models associate presence of an animal with habitat characteristics, making it easier to search, monitor or manage a species by identifying habitat preferences (Guisan & Zimmermann, 2000). Distribution models have added value in areas where animals are sparse and abundance measures are unfeasible. One such family of models - resource selection functions - are useful in assessing habitat use (Jones & Tonn, 2004). A shortcoming of these models is that it is assume that if an organism is present there is a 100% probability that it will be detected - they lack the ability to account for imperfect detection. Models that fail to account for imperfect detection may not allow for valid inferences about true habitat use (MacKenzie et al., 2002; Kéry & Schmidt, 2008). Occupancy models are a type of distribution model that explicitly accounts for probability of detection, and variables that affect detection.

Occupancy models assess the proportion of an area used by a given organism or community of organisms, account for imperfect detection, and relate probability of occupancy and detection to user-specified covariates (MacKenzie et al., 2002). Occupancy models can allow for inferences on habitat use and population trends in habitats where abundance measures are not feasible (Bailey et al., 2004). The basic framework for occupancy modeling relies on presence/absence data for an organism from a given population in a defined spatial area (MacKenzie, 2005). Environmental (e.g., glare, flow) and methodological (e.g., sampler, sampling equipment) covariates can be related to both the probability of occupancy and detection efficiency during occupancy analysis (MacKenzie & Royle, 2005). The most basic occupancy models are two-state models (presence-absence) where detection efficiency (i.e., probability of detection) is estimated using temporally replicated surveys. Spatial replication can also be used to estimate detection efficiency. In spatially replicated occupancy studies, multiple, spatially

discrete sites are surveyed once. Detection efficiency is then estimated by grouping multiple sites into larger units, and assuming that probability of occupancy among sites is equal (Charbonnel et al., 2014). In remote regions, spatial replication can help reduce time and cost of sampling programs, because the need to re-visit sites (as required for temporal replication) is eliminated (Charbonnel et al., 2014). To avoid effects of auto-correlation in spatially replicated models, sites must be randomly selected and separated by some biologically relevant distance, and the distance should incorporate knowledge of the mobility of the study species. Alternatively, an auto-correlation structure can be incorporated into the model (Kendall & White, 2009).

The main objective of this research was to quantify fluvial YOY Arctic Grayling habitat use in a remote mountain stream ecosystem in the Northwest Territories (NWT), Canada. My aim was to use an occupancy-based approach that would allow creation of a repeatable, reliable survey method that was efficient and feasible for resource managers and other stakeholders to use in future. I predict that YOY Arctic Grayling habitat use in mountain systems will be best predicted by characteristics that determine water flow such as stream slope and elevation. Landscape level characteristics, like elevation, have explained habitat use by Bull Trout (*Salvelinus confluentus*) in similar ecosystems (Dunham et al., 2003). Better scientific understanding of YOY Arctic Grayling habitat use will help inform decision makers, promote conservation efforts, inform monitoring strategies, and provide baseline data for rehabilitation of fish habitat. By using spatial replication and accounting for imperfect detection in an occupancy framework, I aim to establish a baseline against which can be used as a time and cost-effective method to measure changes in habitat use by YOY Arctic Grayling in sensitive mountain stream ecosystems. The results presented herein are specific to the YOY life stage of Arctic Grayling, and to northern mountain streams. This research focuses on one mountainous watershed as a

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representation of habitat use by YOY Arctic Grayling across a broader range of northern

mountain ecosystems.

2.2 Methods

2.2.1 Study area

The study area encompasses the Little Nahanni River watershed, which is located in a sub-

Arctic region in the NWT, along the southwestern border with Yukon Territory (Fig 2.1). The

Little Nahanni watershed is part of the headwater system for the South Nahanni River, which

drains into the larger Mackenzie River Basin (Ootes et al., 2013). The watershed falls within

Sahtu and Dehcho First Nations traditional territories and is partially protected by both the

Nahanni and Nááts'ihch'oh National Park Reserves (Irwin, 1993).

The mountainous, sub-Arctic region of the Little Nahanni watershed is part of the

Mackenzie and eastern Selwyn mountain ranges, which are considered the northernmost extent

of the Canadian Rockies (Ootes et al., 2013). The watershed varies in elevation from

approximately 800 to 2200 meters above sea level (masl), and is categorized as the Taiga

Cordillera Ecozone, consisting of wetland, boreal forest and alpine tundra (Ponomarenko &

Quirouette, 2015). Vegetation across the landscape is determined by elevation, topography and

substrate. At higher elevations (approximately > 1700 masl), common alpine vegetation, such as

lichen (*Cladonia rangiferina*) and mountain avens (*Dryas octopetala*) dominates, but at lower

elevations (between 800 – 1700 masl) stands of stunted white spruce (*Picea glauca*), alpine fir

(*Abies lasiocarpa*), willow (*Salix sp.*) and dwarf birch (*Betula nana*) dominate the landscape

(Ponomarenko & Quirouette, 2015). Temperatures for the Selwyn mountain range can range

from -51°C in winter to 28°C in summer (Environment Canada). The region receives 644 mm of

precipitation annually, with most precipitation falling as rain in the summer months (Jackson, 1987). As with much of the Yukon and NWT, the watershed does not have a defined dry period of the year, resulting in summer stream flows that are highly variable and influenced by montane climate patterns.

Stream characteristics vary considerably in Little Nahanni River watershed. High elevation and steep grades in the region lead to a matrix of well-defined stream channels in mountainous terrain. Summer stream temperatures vary based on contributions from surface-water, groundwater, glaciers, and springs (Mochnacz et al., 2013). Sandstones, shale and granite each add complexity to stream geomorphology (Jackson, 1987). Gravel-bottomed habitats in the streams play an important role in facilitating spawning for many northern fishes, including Arctic Grayling (Evans et al., 2002). Overall, variability in stream temperature, morphology, substrate, and vegetation result in different stream habitats that Arctic Grayling use during various life stages.

Habitat conditions and the distribution of fishes in tributaries of the Little Nahanni River were poorly documented prior to this study. As a result, reconnaissance surveys were conducted in 2014 to determine the feasibility of research planned for 2015. Four sub-watersheds of varying catchment size were chosen for further study based on the reconnaissance work: Dozer (497 km^2), Fork (341 km^2), Guthrie (275 km^2) and March (263 km^2) creeks were surveyed in 2015 (Fig. 2.1). Species of fish found in the tributaries included Arctic Grayling (most abundant), Slimy Sculpin (*Cottus cognatus*), Burbot (*Lota lota*), Lake Trout (*Salvelinus namaycush*) and Lake Chub (*Couesius plumbeus*).
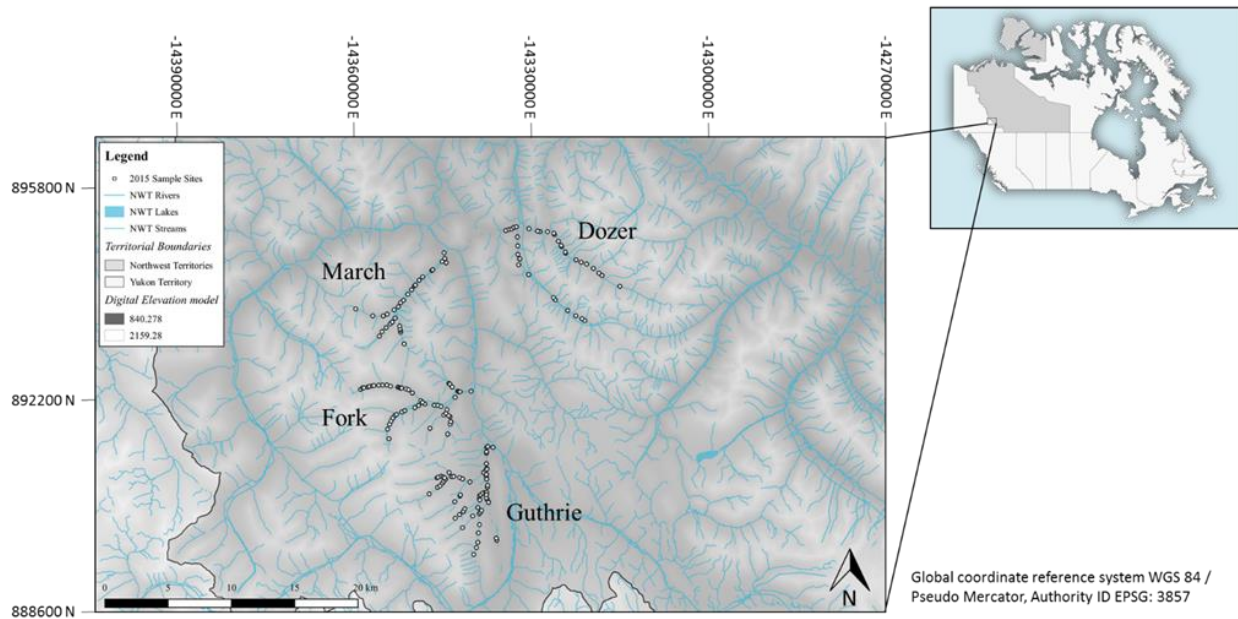
Figure 2.1. The Little Nahanni River watershed, NWT Canada. Four sub-watersheds contain sites that were surveyed for YOY Arctic Grayling in June-August 2015.

2.2.2 Occupancy terms and considerations

In occupancy modeling, spatial or temporal replication in surveys facilitates the estimation of detection efficiency (MacKenzie & Royle, 2005). Spatial replicates – sites in contiguous stream sections with similar habitat characteristics – were used in this study for several analytical and practical reasons. Spatial replication reduces survey costs by eliminating the need to revisit the same site multiple times (temporal replication), increases space-for-time efficiency, and improves our understanding of fish distribution and habitat use across a broader landscape (MacKenzie & Royle, 2005; Charbonnel et al., 2014). In this study, spatial replicates ('sites') were randomly assigned within 'patches'. A 'patch' was defined as a contiguous and homogeneous section of stream with similar environmental characteristics (substrate size etc.), consistent water discharge, and constant Strahler stream order. Patches ranged in size from approximately 500 m to 3000 m, depending on in-flow and out-flow of other streams, or if impassable barriers were encountered. Sites were 100 m in length and embedded within patches.

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Since sites were used as spatial replicates to assess detection efficiency, and patches are considered a homogeneous section of stream, occupancy could only be assessed at the patch level (Charbonnel et al., 2014). The rationale for site size (100 m) was to capture fish movement and dispersal patterns (Dunham et al., 2003) that are typical of Arctic Grayling (Armstrong, 1986), and to allow for sufficient habitat categorization of the patches.

Occupancy modelling has four main assumptions that must be considered in the study design and analysis: (i) closure; (ii) site independence; (iii) proper species identification; and, (iv) no unmodeled heterogeneity. 'Closure' means that the habitat units of interest maintain their occupancy status, either occupied or unoccupied, throughout the entirety of the defined sampling period (Rota et al., 2009). In the context of this spatially replicated occupancy design, 'closure' means that fish should be available for detection over all sites within a patch, given that one site within the patch is occupied (Charbonnel et al., 2014). With site independence, I assumed that a site was not occupied as a result of being near another occupied site, and that occupancy was purely related to habitat variables present, rather than proximity to an occupied site (MacKenzie & Royle, 2005; Canessa et al., 2015). I further assumed that all fish were correctly identified to species. The assumption that there is no unmodeled heterogeneity means that all variability in fish occupancy and detectability is explained by covariates that are assessed in the model. It is unreasonable to expect this assumption to be completely fulfilled, however, biological theories and hypothesis-driven decisions should be made to conform with this assumption (Dorazio et al., 2006). To meet occupancy model assumptions: i) stream patches were long enough (500 m – 3000 m) to accommodate within-patch summer movement by YOY Arctic Grayling (Buzby & Deegan, 2000); ii) site independence was maintained using a downstream-to-upstream sampling technique in combination with a 10 m buffer between sites; iii) team members were trained at

identifying YOY Arctic Grayling; and, (iv) the 29 habitat variables chosen for assessment best represented habitat characteristics that previous authors found to be important for determining YOY Arctic Grayling habitat use (de Bruyn & McCart, 1974; Liknes & Gould 1987; Deleray, 1991; McClure & Gould 1991; Northcote, 1995; Jones & Tonn, 2004; Deegan et al., 2005; Baker et al., 2017).

2.2.3 Survey design

This study was conducted in 2015 during the summer season. Three sampling periods were used to evaluate YOY Arctic Grayling distribution and habitat use in the region (Table 2.2). Sampling periods were not designed as a form of temporal replication, but rather resulted from timing constraints on field work (e.g., helicopter availability). I assumed that all sites had equal probability of being occupied in each of the three sampling events during the summer period. Arctic Grayling show variability in YOY emergence timing based on environmental factors, such as temperature, which controls egg development (Kaya, 1991; Mantua et al., 2010). In the Little Nahanni River, larval emergence can occur from late June to early August (Mochnacz, Fisheries and Oceans Canada, pers. comm.), making it plausible that YOY Arctic Grayling would occupy natal streams in all of the three sampling periods. Data from all three sampling periods were used in the occupancy analysis.

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Table 2.2. Three sampling periods and range of dates for surveys conducted to detect YOY Arctic Grayling in the Little Nahanni River, NWT in 2015.

Sampling Period	Start Date	End Date
1	July 5, 2015	July 10, 2015
2	July 27, 2015	August 5, 2015
3	August 27, 2015	August 31, 2015

The four sub-watersheds included in this study, Dozer, Fork, Guthrie and March creeks, were subdivided based on habitat criteria, and categorized into 35 patches (Table 2.3). One hundred and eighty-three sites were randomly distributed throughout the 35 patches (Fig. 2.1). Sites were assigned using a pre-defined suite of broad-scale stream characteristics that have been identified by Fisheries and Oceans Canada (DFO) as providing potentially suitable habitat for YOY Arctic Grayling (Mochnacz, Fisheries and Oceans Canada, pers. comm.). The criteria for selecting sites included: stream catchment area > 400 ha, stream grade <15%, stream Strahler Order < 4 and elevation <1600 m above sea level. Sample sites were randomly distributed using a Generalized Random Tessellation Stratified design (GRTS; Stevens & Olsen, 2004). GRTS uses a randomized-hierarchical grid that arrays sites throughout a stream network to achieve spatial representation. The GRTS design was conducted by an independent contractor for DFO, as part of a larger salmonid monitoring program in northern Canada. Each site was subsequently sampled one time to determine fish occupancy and evaluated for habitat characteristics (see below).

Table 2.3. The four sub-watersheds of Little Nahanni River, NWT that were sampled in 2015 and the corresponding number of patches and sites sampled across three sampling periods.

Sub-watershed	Number of Patches	Number of sites per sub-watershed	Mean number of sites per patch
Guthrie	14	64	4.6
Fork	9	47	5.2
Dozer	6	36	6.0
March	6	36	6.0

Sampling for YOY Arctic Grayling at each site was achieved by electrofishing. A Smith-Root Inc. LR-24 Electrofisher was used with a standard 11" anode ring. Netters used a standard 16" x 16" electrofishing dipnet. A pulsed DC waveform with a frequency of 30Hz and a 12% duty cycle was used. Voltages ranged from 185 to 475 v, and were adjusted based on the quick-set function of the unit, which assessed the conductance of the stream and adjusted the voltage setting accordingly. Each survey team consisted of two personnel wearing polarized glasses; one crew member operated the backpack electrofisher while the other netted. Fishers began at the downstream end of the 100 m site and moved towards the upstream extent of the site. Surveys were conducted in a 'zig-zag' pattern from downstream to upstream. The dipnet was placed on the downstream side of the anode to capture shocked fish that were flowing downstream (as per Kimmel & Argent, 2006). All in-stream habitat types (e.g., riffle, pools) were sampled equally (Meador et al., 2003). Fish that were observed outside of the effective shock range (1 to 2 meters) or that were shocked but not captured were considered a positive detection if both survey personnel could positively identify that it was an Arctic Grayling. On average, 411 seconds of electrofishing were employed at each site. Fish processing in the field included species identification and fork length measurements (mm).

2.2.4 Covariates

Habitat features at each site were assessed at the same time that fishing was conducted (Table 2.4). In occupancy models, habitat characteristics are used to explain variation in fish occupancy, and in this study these variables were collected and/or aggregated at the 'patch' scale. Habitat and other covariates used to explain detection efficiency were collected and analyzed at the 'site' scale. Some habitat variables were assessed for effects on both fish occupancy and detection efficiency (e.g., % boulder).

For further information on covariate collection, please refer to Appendix A, where detailed field methods are outlined.

Table 2.4. Habitat variables assessed as potential covariates to explain YOY Arctic Grayling habitat use and detection efficiency in the Little Nahanni River.

Variable	Occupancy	Detection efficiency	Model Abbreviation	Units	Method of measurement[1]
photoperiod	yes	no	photoperiod	minutes	n/a
air temperature	yes	no	air_temp	°C	point
water temperature	yes	no	water_temp	°C	point
YSI water temperature	yes	no	ysi_temp	°C	point
wetted width	yes	yes	width	cm	point
average depth	yes	yes	depth	cm	point
average velocity	yes	yes	velocity	m/s	point
discharge	yes	no	discharge	(m^3/s)	point
slope	yes	no	slope	na	point
elevation	yes	no	elevation	masl	point
stream order	yes	no	order	strahler	n/a
boulder	yes	yes	boulder	%	site
cobble	yes	yes	cobble	%	site
gravel	yes	no	gravel	%	site
sand/silt	yes	no	fines	%	site
overhanging riparian vegetation	yes	yes	rip._veg.	%	site
run	yes	no	run	%	site
riffle	yes	yes	riffle	%	site
pool	yes	no	pool	%	site
cascade	yes	no	cascade	%	site
aquatic vegetation	yes	yes	aq._veg	%	site
coarse woody debris	yes	yes	cwd	%	site
undercut banks	yes	yes	ucb	%	site
boulder cover	yes	no	bld._cov.	%	site
eddies	yes	yes	eddies	%	site
pH	yes	no	ph	pH units	point
specific conductivity	yes	no	sp.con.	μS/cm	point
dissolved oxygen	yes	no	do	mg/L	point
efisher	no	yes	efisher	personnel	n/a
netter	no	yes	netter	personnel	n/a
efisher settings	no	yes	volts	voltage	n/a
visibility	no	yes	VisObs	0-3	site
cloud cover	no	yes	CloudCov	%	site
effort	no	yes	effort	seconds	site
effort area	no	yes	effort_per_sq._m	seconds/m^2	site
previous knowledge	no	yes	prev.knowledge	0-2 rank	n/a

[1] Point measurement: the covariate of interest was measured at one lateral transect that spanned the wetted width of the stream, perpendicular to the flow; this was considered representative of the site or patch (e.g., discharge). Site measurement: the covariate of interest was assessed throughout the entire site and then an estimate was assigned based on the surveyor evaluation (e.g., substrate cover). Habitat covariates were evaluated after the site was fished to avoid disturbing fish in the stream and to allow surveyors to observe all habitat heterogeneity throughout a site prior to habitat evaluations.

2.2.5 Statistical analysis

There are two occupancy (ψ) states: presence ($\psi = 1$) and absence ($\psi = 0$). Detection

efficiency (*p*) can theoretically vary between 0 (fish are never detected when they are present)

and 1 (fish are always detected when they are present). Following methods in MacKenzie et al.

(2002), the probability of occupancy for YOY Arctic Grayling was assessed using the occupancy

modeling estimation and information theoretic approach. The Akaike Information Criterion

(AIC) was used to find the most parsimonious model that linked occupancy and detection

efficiency parameters to non-random explanatory variables using the logit-link function

(MacKenzie et al., 2006). Models in an *a priori* model set were ranked using AIC (Burnham &

Anderson, 1998; MacKenzie et al., 2006). Model fit, occupancy parameters, detection efficiency

parameters, habitat covariates and ultimately final model selection were assessed using the

occupancy modelling software PRESENCE (v10.7).

2.2.6 Global model selection

An *a priori* model set was prepared that adequately addressed competing hypotheses I had

about Arctic Grayling habitat use. There are several parameterizations of occupancy models,

including: simple-single season, correlated detections with fixed detection efficiency, staggered

entry, multi-method, and standard correlated detection models. A chi-square (χ^2) goodness of fit

test was conducted to examine overdispersion ($\hat{c}$; variance inflation factor calculated using

parametric bootstrapping (n=1000)) for each model type, and these results, when combined with

AIC rankings, were used to select the global model best suited for further analysis (Cooch,

2012).

2.2.7 Variable collinearity

Hypothesized covariates of detection efficiency and occupancy were normalized using z-scores (Burnham & Anderson, 2004). Pearson product moment correlations were then performed to identify collinear variables. Keeping collinear habitat and detection variables in the model can lead to overdispersion (Berry & Feldman, 1985; Haynes et al., 2014). Correlations were performed using R Studio and Microsoft Excel, and covariates were ranked as highly correlated (i.e., $|r| \geq 0.70$, $p \leq 0.05$; Berry and Feldman 1985), moderately correlated ($0.60 \leq |r| \geq 0.70$, $p \leq 0.05$) or less correlated ($|r| \leq 0.60$, $p \geq 0.05$). If variables were colinear, one of the variables was removed from further analysis based on biologically informed decision making or if one variable was nested in another. Moderately correlated variables were used in the analysis but were considered for removal later during the modelling process - dependent on AIC ranking. All less correlated variables were further considered in models of occupancy and detection efficiency.

2.2.8 Detection-specific model variables

Normalized detection efficiency covariates were added to the global model to determine their influence on detection efficiency (one covariate in each model). AIC rankings were then compared amongst models and to the original psi(.),p(.) model (no covariates). Variables that were in the top 50% of AIC rankings were selected for future model-building activities, and all iterations of two and three covariate combinations were considered. A maximum of three detection efficiency covariates were used to avoid overparameterization of the model, as assessed by the numerical convergence feature in the program PRESENCE.

2.2.9 Occupancy-specific model variables

Normalized occupancy variables were added (one in each model) to the top AIC-ranked model fitted with detection efficiency covariates. As described above, occupancy covariates were measured or calculated at the patch scale using predominantly mean values. Minimum values were used when ecologically relevant (e.g., elevation). AIC was used to determine the best predictive model. Variables present in the top models were then further investigated with a sequential model building technique that used all iterations of two and three covariate combinations to avoid overparameterization. Models were then evaluated using AIC rankings and weights.

2.2.10 Final model selection

AIC weights, slopes (β coefficients), and standard errors were investigated for models with ΔAIC < 2 (Burnham & Anderson, 2002). 'Pretending' variables were then identified as those with a β coefficient and standard error that overlapped with zero (MacKenzie 2006; Arnold, 2010; Dextrase et al., 2014) – indicating that the covariate in question had low predictive power. Additionally, some model β values could not "numerically converge", meaning that models were over parameterized or the standard errors for the β coefficients were too high for the model to predict. Models that did not converge and pretending variables were removed from the model selection process. Model-averaging was employed using all models that were within delta AIC <2 (with pretending variables and non-converged models removed). Model averaging requires the estimation of unconditional standard error and 95% confidence intervals using weighted averages (w_i) (Buckland et al., 1997). Unconditional standard error and confidence intervals were calculated for occupancy and detection efficiency covariates using the delta transformation

method (Cooch, 2012; Falke et al., 2012). The delta transformation allows for standard error and 95% confidence intervals to be transformed from the linear (logistic scale) value to the parameter value.

2.3 Results

2.3.1 Fish collection

Five hundred and forty (540) Arctic Grayling were detected during the course of this study (Table 2.5). Arctic Grayling YOY were captured in seven patches within Dozer (n=1) and Guthrie (n=6) sub-watersheds. No Arctic Grayling YOY were captured in either the Fork or March sub-watersheds. Overall, the mean number of fish per patch was highest in Dozer Creek, despite only one patch being occupied in that creek.

Table 2.5. Number of Arctic Grayling caught per patch in four sub-watersheds of the Little Nahanni River, NWT in 2015.

Sub-watershed	Total fish per sub-watershed	Number of patches occupied	Mean number fish (± SD) per patch	Minimum number of fish per patch	Maximum number of fish per patch
Dozer	377	1	63 (± 154)	0	377
Fork	0	0	0	0	0
Guthrie	163	6	12 (± 22)	0	70
March	0	0	0	0	0

2.3.2 Selection of global model

To determine the best global model structure for this dataset, a variety of potential model

types were evaluated strictly using occupancy (presence/absence) data without habitat covariates.

Two global models, simple single season and multi-method, sufficiently fulfilled requirements of

the goodness-of-fit test ($\hat{c}$ = 1.21) (Table 2.6). The simple single season model had a

considerably lower AIC value than the multi-method model (ΔAIC = 5.48); thus, a simple season

model was used for all further model-building and analysis. Other global model choices did not

numerically converge, because the occupancy data were sparse or over parameterized for the

global model type (Table 2.6).

Table 2.6. Global model types were investigated using $\hat{c}$ to assess global model fit to the occupancy data collected for YOY Arctic Grayling in the Little Nahanni River, NWT, in 2015. ψ represents the probability of occupancy, p represents the probability of detection, and theta represents the probability of occupancy by accounting for differences in occupancy as a function of how spatial unit occupancy relates to each other (e.g., are occupied sites correlated in adjacent sites).

Model Type	Model Name	$\hat{c}$	AIC	deltaAIC	AIC wgt	Model Likelihood
Simple single season	ψ(.),p(.)	1.21	76.23	0	0.9393	1
Multi-method	ψ(.),theta(.),p(m)	1.31	81.71	5.48	0.0646	0.0646
Correlated detections with fixed p	ψ, th(),th1(),p(0.25),th0pi()	Convergence not achieved	NA	NA	NA	NA
Correlated detections	ψ, th(),th1(),p(),th0pi()	Convergence not achieved	NA	NA	NA	NA
Staggered entry	ψ(.),e(.),d(.),p(full)	Convergence not achieved	NA	NA	NA	NA

2.3.3 Covariates

Data for 28 *a priori* occupancy covariates were collected in the field (Table 2.7). As described above, covariates were removed from further analysis if the variable exhibited a Pearson correlation value greater than 0.70 (Appendix A; Table A1) or if they were not included in the top 50% of models (using AIC rankings) tested to explain occupancy. Dissolved oxygen and pH were removed as occupancy variables because of unreliable YSI readings at a subset of sites, which was likely a result of calibration issues in the field. Interestingly, neither sampling period nor date influenced where fish were caught, as assessed using calendar day and photoperiod as covariates in the occupancy analysis. In total, 15 occupancy and 10 detection efficiency variables were removed from further analysis (Appendix A; Table A1). Variables that remained in the analysis are listed in (Table 2.8).

Table 2.7. Occupancy covariates associated with YOY Arctic Grayling in the Little Nahanni River, NWT in 2015. Italicized values were removed from further analysis based on model performance and collinearity with other variables.

Covariate	YOY Arctic Grayling Observed			YOY Arctic Grayling Not Observed		
	Mean	Min	Max	Mean	Min	Max
calendar day	213.12 ± 21.79	186.00	243.00	212.39 ± 18.45	186.00	243.00
photoperiod (mins)	1049.82 ± 103.79	873.00	1162.00	1043.37 ± 97.49	873.00	1172.00
air temperature (°C)	12.80 ± 3.08	4.00	19.00	14.50 ± 5.15	1.50	24.00
water temperature (°C)	9.17 ± 2.33	6.00	17.00	6.77 ± 1.95	2.48	13.00
wetted width (cm)	439.61 ± 261.94	146.00	1064.00	428.56 ± 213.11	55.00	1432.00
avg. depth (cm)	35.25 ± 14.51	11.63	68.50	29.08 ± 9.73	9.25	51.25
avg. velocity (m/s)	0.70 ± 0.25	0.23	1.31	0.86 ± 0.30	0.11	1.93
discharge (m³/s)	0.65 ± 0.46	0.12	1.39	0.92 ± 0.75	0.15	2.73
boulder (%)	10.79 ± 15.80	0.00	70.00	29.21 ± 19.70	0.00	80.00
cobble (%)	42.12 ± 24.50	0.00	85.00	49.45 ± 18.23	0.00	85.00
gravel (%)	28.70 ± 19.37	1.00	70.00	15.95 ± 10.36	0.00	60.00
sand/silt (%)	18.42 ± 23.65	0.00	89.00	5.51 ± 7.24	0.00	70.00
run (%)	43.61 ± 37.73	0.00	100.00	13.59 ± 18.54	0.00	80.00
riffle (%)	39.24 ± 36.19	0.00	100.00	55.69 ± 24.60	0.00	100.00
pool (%)	14.24 ± 10.24	0.00	40.00	12.87 ± 7.57	0.00	60.00
cascade (%)	2.94 ± 9.67	0.00	50.00	17.79 ± 22.03	0.00	100.00
aquatic vegetation (%)	5.61 ± 11.51	0.00	50.00	4.75 ± 9.54	0.00	50.00
overhanging riparian vegetation (%)	17.42 ± 18.70	1.00	70.00	21.78 ± 22.49	0.00	90.00
woody debris (%)	6.09 ± 6.48	0.00	20.00	7.10 ± 10.00	0.00	60.00
undercut banks (%)	50.30 ± 31.87	0.00	95.00	25.39 ± 20.89	0.00	95.00
boulder cover (%)	4.61 ± 8.36	0.00	40.00	9.37 ± 8.63	0.00	40.00
eddies (%)	5.82 ± 5.82	0.00	20.00	5.57 ± 5.25	0.00	25.00
slope	0.03 ± 0.03	0.01	0.01	0.060 ± 0.029	0.010	0.135
elevation (masl)	1046.80 ± 53.12	946.00	1143.00	1235.37 ± 140.56	914.00	1543.00
stream order (strahler)	2.38 ± 0.82	1.00	3.00	2.02 ± 0.72	1.00	3.00
YSI water temperature (°C)	8.38 ± 2.26	5.60	13.00	5.97 ± 1.57	2.48	10.60
pH	8.20 ± 0.31	7.23	8.43	8.05 ± 0.21	7.43	8.37
specific conductivity	271.06 ± 114.86	93.80	519.50	242.16 ± 64.04	113.70	366.70
dissolved oxygen	10.71 ± 1.53	7.15	13.41	11.61 ± 1.36	9.78	14.31

Sites with YOY Arctic Grayling present had water temperatures that were 2.4°C warmer on average than sites without Arctic Grayling (Table 2.7). Differences in variables that reflect flow dynamics (e.g., velocity) were negligible between sites where Arctic Grayling were present and not present. Silt and gravel were more dominant at sites where YOY Arctic Grayling were

observed, whereas cobble and boulder were more dominant in unoccupied streams. Run habitat was dominant at sites with YOY Arctic Grayling, whereas riffle habitat was dominant at sites without Arctic Grayling. In-stream habitat variables appeared to be similar between streams that did and did not contain YOY Arctic Grayling, except for undercut banks, which were more prevalent in streams with YOY Arctic Grayling. On average, elevation was approximately 200 masl lower at sites with YOY Arctic Grayling than at sites without YOY Arctic Grayling. Elevation was moderately correlated with several flow dynamics variables like discharge (r = 0.65), velocity (r = 0.60) and slope (-0.53) (Appendix A, Table A.5.). Alternatively, elevation was not correlated with water temperature (r = 0.03).

Table 2.8. All occupancy and detection efficiency variables assessed in further simple single season occupancy analysis.

Parameter type	Variables Included	Parameter type	Variables Included
occupancy	air temperature	detection efficiency	avg. depth
	avg. depth		avg. velocity
	avg. velocity		boulder
	slope		riffle
	elevation		aquatic vegetation
	stream order		eddies
	boulder		efisher
	gravel		cloud cover
	silt/sand		effort
	% riffle		previous knowledge
	aquatic vegetation		
	coarse woody debris		
	undercut banks		
	boulder cover		
	water temperature		
	specific conductivity		

2.3.4 Model building and selection

The simple single season model identified a naïve occupancy estimate of 0.20. If detection efficiency is 100%, then naïve occupancy = modeled occupancy; that is, naïve occupancy represents the proportion of sites that were known to be occupied by YOY Arctic Grayling. Naïve occupancy estimates were calculated using up to five spatially replicated surveys per patch.

The top model for detection efficiency (occupancy was held constant ($\psi(.)$) included the following covariates: percent boulder, with beta value $\beta = -2.807 \pm 1.091$, and percent riffle, beta value $\beta = -1.122 \pm 0.509$ (Table 2.9). Detection efficiency was negatively related to both percent boulder (Figure 2.2) and percent riffle (Figure 2.3), and was highest in streams with no boulder substrate, and with no riffle habitat. For comparison purposes, the second top model (ΔAIC > 2) and the simple single season global model AIC values are also listed in Table 2.9. All other models performed poorly in comparison to the top model. A total of 55 models were assessed; 10 models with individual covariates, and 45 models using all combinations of two covariates.

Table 2.9. Detection efficiency variables fit to simple single season occupancy analysis for YOY Arctic Grayling in the Little Nahanni River, NWT during the summer (July-August) of 2015.

Model Name	AIC	ΔAIC	AIC wgt	Model Likelihood	no.Par.	-2*LogLike
$\psi(.),p$(Boulder/Riffle)	51.98	0	0.4143	1.0000	4	43.98
$\psi(.),p$(Boulder/AquaticVeg)	54.27	2.29	0.1318	0.3182	4	46.27
$\psi(.),p(.)$	76.23	24.25	0	0.0000	2	72.23
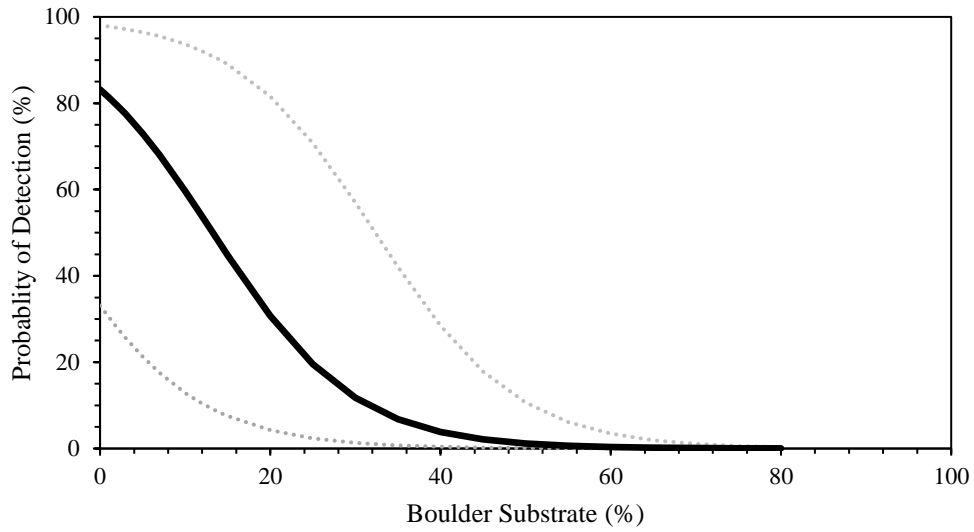
Figure 2.2. Relationship between probability of detecting YOY Arctic Grayling and percent boulder substrate present in a stream in the Little Nahanni River, NWT during the summer (July-August) of 2015. Logistic curve represents the true relationship on a probability scale, with 95% confidence intervals.
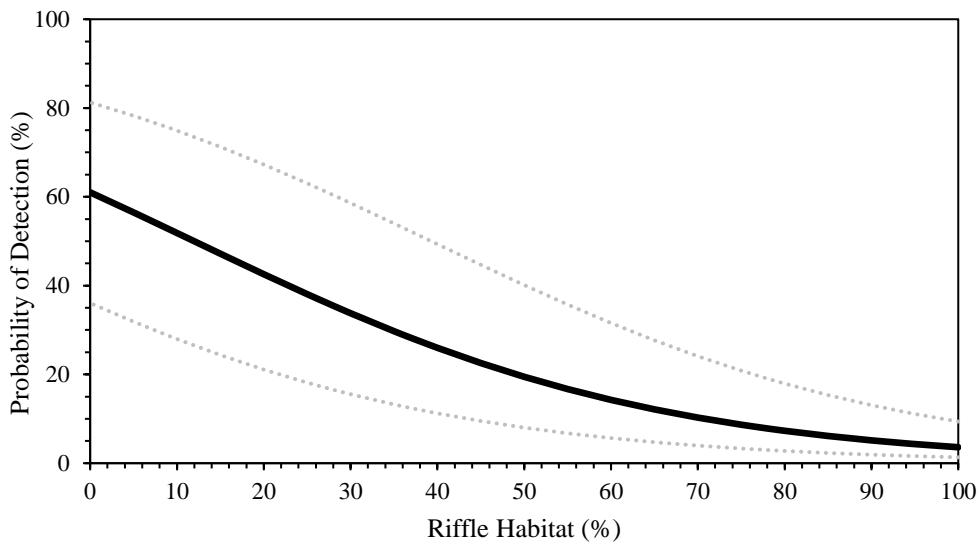
Figure 2.3. Relationship between probability of detecting YOY Arctic Grayling and percent riffle habitat present in a stream in the Little Nahanni River, NWT during the summer (July-August) of 2015. Logistic curve represents the true relationship on a probability scale, with 95% confidence intervals.

After important detection efficiency variables were identified, a model set that included 15

covariates of occupancy was tested [ψ(.),p(Boulder/Riffle)] (Table 2.8). A maximum of three

covariates was included in each model tested, which resulted in 575 models in the set. Individual

occupancy covariates examined in the model [ψ(.),p(Boulder/Riffle)] were, from strongest

predicting capability to weakest: elevation, water temperature, undercut banks, depth, slope,

fines, boulder, air temperature, velocity, riffle, gravel, stream order, specific conductivity,

boulder cover, aquatic vegetation, and coarse woody debris. Six models had delta AIC values Δ<

2 relative to the top model (Table 2.10), and elevation was in all of these models. Water

temperature was included in six of seven of the top models.

Table 2.10. Seven of 575 models evaluated to explain and predict occupancy of YOY Arctic Grayling in the Little Nahanni River, NWT during summer 2015. All seven models included had Δ AIC< 2.

Model Rank	Model	AIC	ΔAIC	AIC wgt	Model Likelihood	K.
1	ψ(elevation/watertemp), p(Boulder/Riffle)	39.56	0	0.2805	1	6
2	ψ(elevation/watertemp/ucb), p(Boulder/Riffle)	41	1.44	0.1365	0.4868	7
3	ψ(elevation/watertemp/velocity), p(Boulder/Riffle)	41.16	1.6	0.126	0.4493	7
4	ψ(elevation/watertemp/slope), p(Boulder/Riffle)	41.18	1.62	0.1248	0.4449	7
5	ψ(elevation/ucb), p(Boulder/Riffle)	41.19	1.63	0.1242	0.4426	6
6	ψ(elevation/watertemp/depth), p(Boulder/Riffle)	41.54	1.98	0.1042	0.3716	7
7	ψ(elevation/watertemp/boulder), p(Boulder/Riffle)	41.55	1.99	0.1037	0.3697	7

Other covariates of occupancy that were included in the top seven models included

percent undercut banks, velocity, slope, depth and percent boulder. To investigate whether these

additional variables were 'pretending' variables (high standard error overlaps with 0 and negates

influence in modelling habitat use); (MacKenzie et al., 2006; Robles & Ciudad, 2012), model averaging was used to investigate beta values and unconditional standard error on the logit scale (Table 2.11). Beta values and unconditional standard errors overlapped for all covariates, indicating their poor predictive value for occupancy. As such, model averaging efforts were abandoned, and the top model [ψ(elevation/watertemp), p(Boulder/Riffle)] was selected for further interpretation and analysis.

Table 2.11. Beta coefficient estimates on the logit scale with unconditional standard error calculated using model averaging for the top seven models representing YOY Arctic Grayling summer habitat use in the Little Nahanni River, NWT in 2015. Overlap beta and SE represents the standard error associated to covariates using model averaging and whether their variability overlaps with zero. Y=yes, N=no

Model Rank	Covariate	Beta Coefficienct	Uncond. SE	Overlap Beta and SE
1	elevation	-6.15	13.05	Y
2	elevation	-5.96	13.05	Y
3	elevation	-6.91	13.05	Y
4	elevation	-8.35	13.05	Y
5	elevation	-8.36	13.05	Y
6	elevation	-6.27	13.05	Y
7	elevation	-6.27	13.05	Y
1	water temp	4.87	7.98	Y
2	water temp	3.68	7.98	Y
3	water temp	4.52	7.98	Y
4	water temp	6.01	7.98	Y
6	water temp	4.86	7.98	Y
7	water temp	5.02	7.98	Y
1	ucb	1.31	2.75	N
2	ucb	4.68	2.75	N
1	velocity	-0.97	0.63	N
1	slope	0.86	0.68	N
1	depth	-0.19	2.12	Y
1	boulder	0.21	0.22	Y

*ucb = undercut banks

Elevation and temperature were the best predictors of YOY Arctic Grayling occupancy in the Little Nahanni River watershed. Elevation was negatively related to occupancy of YOY Arctic Grayling with a beta value of $\beta = -6.148 \pm 3.957$. Probability of occupancy of YOY Arctic Grayling decreased from lower to higher elevations (Figure 2.4; min elevation = 946 masl, max elevation = 1534 masl). Water temperature was positively related to occupancy of YOY Arctic Grayling with beta value $\beta = 4.873 \pm 3.104$, and occupancy of YOY Arctic Grayling increased from lower to higher temperatures (Figure 2.5; min temp = 2.5°C, max temp = 17°C). Occupancy of YOY Arctic Grayling was, thus, highest in lower elevation streams (<1150 masl) with higher stream temperatures (> 8°C). Elevation had the highest beta value, suggesting that it is likely the best predictor of habitat use. The corresponding beta values were converted from the logit scale to the probability scale to produce Figures 2.4 and 2.5.
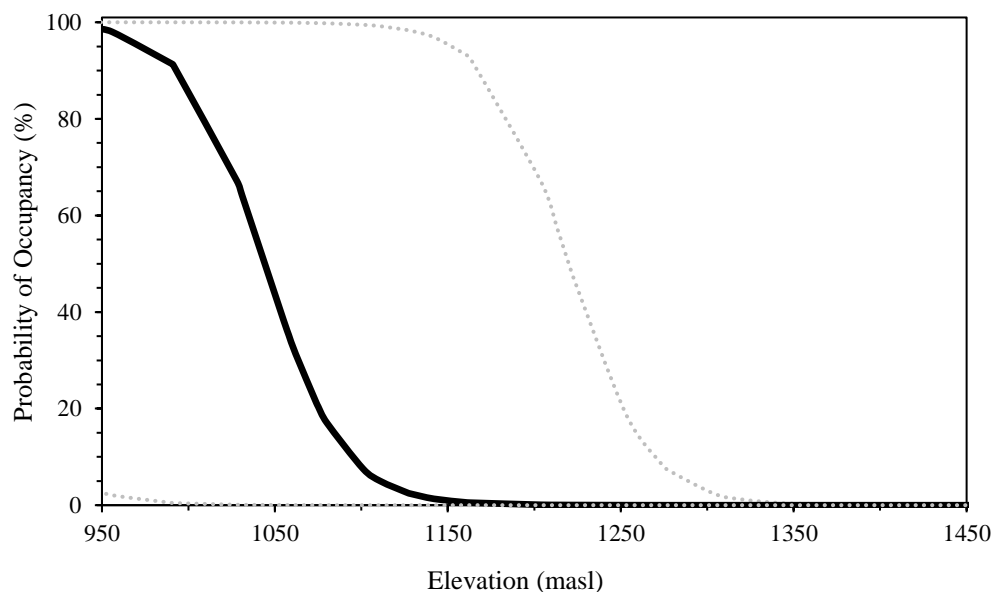
Figure 2.4. Effects of elevation (masl) on the probability of occupancy for YOY Arctic Grayling in Little Nahanni River, NWT during the summer (July-August) of 2015. The logistic curve represents the true relationship on a probability scale, with 95% confidence intervals.
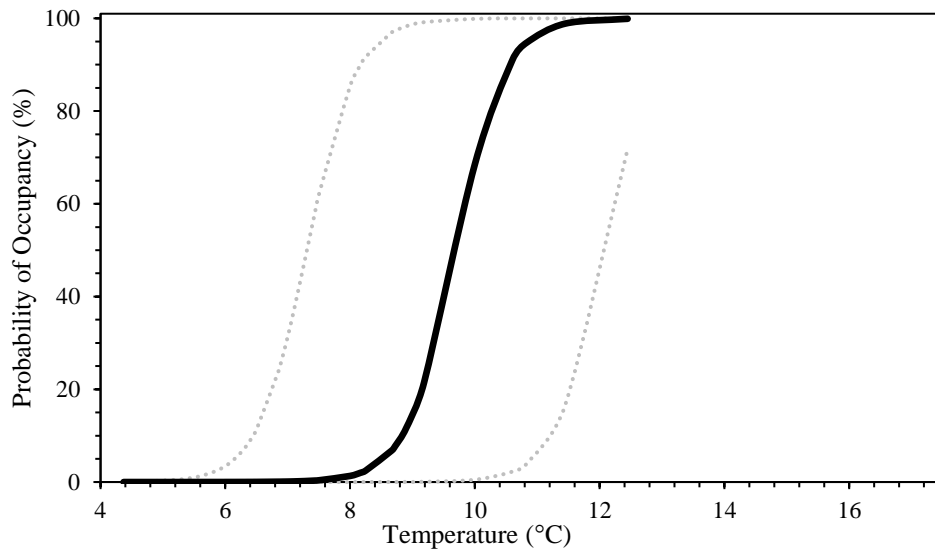
Figure 2.5. Effects of water temperature (°C) on the probability of occupancy by YOY Arctic Grayling in Little Nahanni River, NWT during the summer (July-August) of 2015. Logistic curve represents the true relationship on a probability scale, with 95% confidence intervals.

To determine if the top model variables were explaining the same variation in the data, the relationship between stream temperature and elevation were evaluated, which was found to be not significant (simple linear regression, $F_{(35)} = 1.14$, $P = 0.29$).

2.4 Discussion

Results from this study improve our current knowledge and basic understanding of ecology of fluvial Arctic Grayling, specifically for the YOY life stage. Across their range, Arctic Grayling have complex life histories and use a variety of habitat types. In the mountain streams investigated in this study, there is a higher probability for habitat use by YOY Arctic Grayling at elevations less than 1150 masl, and at water temperatures greater than 8°C. These results support my hypothesis that YOY Arctic Grayling occupancy is affected by landscape scale habitat variables in fluvial mountain systems.

Results from a recent study -using similar methods- on an adfluvial population of Arctic Grayling in a barrenlands landscape showed that stream occupancy by YOY Arctic Grayling was best predicted by water velocity and depth (Baker et al. 2017). While velocity and water depth were included in two of the top seven models in my study, effects of these parameters on occupancy were weak when compared to effects of elevation and temperature. Differences in predictor variables between my study and the Baker et al. (2017) study are perhaps not surprising. Barrenland tundra ecosystems show little change in elevation when compared to mountain systems, and in the Baker et al. (2017) study, water temperatures in all barrenlands streams were > 8°C. Overall, my results help to clarify the differences in habitat use by Arctic Grayling in two populations with fluvial and adfluvial life history strategies, and that region or landscape-specific models are needed to better predict habitat use by Arctic Grayling across its distributional range.

2.4.1 Occupancy variables

Occupancy of YOY Arctic Grayling increased with water temperature in the Little Nahanni River, and results indicated YOY Arctic Grayling did not occupy streams with water temperature < 8°C. Previous literature has not identified a lower temperature threshold for YOY Arctic Grayling, but upper temperature limits are reached around 12°C to 16°C (Deegan et al., 2005). In my study, streams occupied by YOY Arctic Grayling were on average 2.4°C warmer than streams that were not occupied. Previous studies have identified that YOY Arctic Grayling have faster growth rates in years when stream temperatures are higher in Arctic tundra ecosystems, but it is difficult to isolate temperature as the individual explanatory variable (Deegan et al., 1999). It has also been shown that the metabolic rate of YOY Arctic Grayling significantly increases at temperatures above 12°C (Deegan et al., 2005). Modestly warmer water temperatures (2-3°C) may confer a bioenergetic advantage (Elliott & Elliott, 2010). YOY Arctic Grayling in Little Nahanni River may select habitats with water temperatures > 8°C to ensure bioenergetically favourable conditions that allow rapid growth in their first year. Faster growth would help YOY Arctic Grayling improve swimming performance in fast-flowing mountain streams, especially when Arctic Grayling may have to travel to reach suitable overwintering habitat (West et al., 1992). YOY Arctic Grayling in this study area usually reach a length of 75 mm by the end of their first summer (M. McPherson, University of Alberta, unpublished data). Other Salmonids like Arctic Charr (*Salvelinus alpinus*) show preference for relatively colder water temperatures as fish age (Siikavuompio et al., 2013), which may be relevant for Arctic Grayling as well. Further research into size- and age-dependent temperature preferences of fluvial Arctic Grayling is required to help elucidate the role of temperature in structuring populations in mountainous fluvial landscapes.

Elevation best predicted occupancy of YOY Arctic Grayling in my study system, and it thus appears to be a useful landscape-level characteristic for modelling YOY Arctic Grayling habitat use in mountain streams. As remote sensing and digital elevation models provide accurate elevation estimates, remote predictions of YOY Arctic Grayling habitat use should be possible when designing future monitoring of these remote mountain populations. YOY Arctic Grayling were most associated with elevations less than 1150 masl, which is relatively low elevation for this region. While Arctic Grayling are known to partition feeding habitat based on an elevational gradient, in which larger Arctic Grayling use more upstream sites for feeding (Hughes, 1999), YOY Arctic Grayling have not explicitly been associated previously with low elevation natal streams. In a geographically similar system to Little Nahanni River, YOY Bull Trout in the nearby Prairie Creek watershed are most associated with low Strahler stream order at higher elevations (2nd and 3rd order reaches; Mochnacz, DFO, pers. comm.). Arctic Grayling are also present in the Prairie Creek watershed occupying lower elevation streams in the watershed. It is plausible that, in the areas where Bull Trout and Arctic Grayling overlap, these two species have co-evolved to occupy different elevational niches in order to spatially partition resources in oligotrophic streams. This has been observed in other Salmonids, where Cutthroat Trout (*Oncorhynchus clarkii*) and Bull Trout were found to be associated with higher elevation streams (>1500masl), whereas Brook Trout (*Salvelinus fontinalis*) and Rainbow Trout (*Oncorhynchus mykiss*) utilized lower elevation streams (<1500 masl) (Paul & Post, 2001), indicating a spatial partitioning of resources. Elevation-based partitioning of habitats by fish species and life history stages in the Little Nahanni River deserves further research attention.

The river continuum concept explains that flowing water ecosystems sort in a particular manner and that abiotic and biotic characteristics can be predictably categorized into distinct

sections of streams (Vannote et al., 1980). Often, stream characteristics upstream can affect downstream attributes in a predictable manner based on the abiotic characteristics and biotic organisms that are present. Abiotic factors can be correlated to one another in a similar fashion. Traditionally, lower elevations have been associated with increased stream temperatures (e.g., Brunger Lipsey et al., 2005). However, this study found that elevation and temperature were not correlated significantly and that the negative relationship between the variables was not significant as observed in other studies. This suggests that each covariate is acting independent of eachother as predicting covariates for YOY Arctic Grayling. One potential explanation is the role of groundwater in the Little Nahanni River and how groundwater can provide warm-water refugia in an otherwise cold-water ecosystem.

Influences of groundwater on spatial distribution of fishes in remote northern ecosystems are understudied, but groundwater may play a critical role in providing suitable habitat for Arctic Grayling. Although I did not address the role of groundwater directly, the warm stream temperatures (max= 17.5°C) that I observed indicate that some streams may be warmed by groundwater or thermal springs. Groundwater in northern regions helps to stabilize water temperatures in winter months, prevents ice formation and can help provide relatively warmer water temperatures for fish growth in cold, stream environments (Dunmall et al., 2016). Further investigation into the role of groundwater and thermal springs by YOY Arctic Grayling may reveal their dependency on groundwater-influenced streams for growth or as overwintering habitat between their first and second year of growth.

While results of my study suggests that YOY Arctic Grayling are bound by a cold-water threshold (8°C) and occupy elevations <1150 masl, several other biological and sampling-related factors should be considered when making inferences with these data. For example, a number of

streams with elevation <1150masl were not sampled because they were inaccessible, for example

due to fast flows that were unsafe to wade in. Thus, not all streams with an elevation <1150masl

are necessarily suitable for YOY Arctic Grayling. However, utilizing elevation as a first-cut

landscape-level predictor should help prioritize areas for more in-depth study and enable better

predictions of suitable habitat for YOY Arctic Grayling.

2.4.2 Detection efficiency

Based on AIC ranking, modelled occupancy estimates for YOY Arctic Grayling better

predicted suitable habitat when % boulder and % riffle were included as variables that affected

detection efficiency. Visual obstructions and natural camouflage inherently make fish surveys

difficult in some systems (Albanese et al., 2007), especially when looking for with small fish in

lotic systems (King & Crook, 2002). Both % boulder and % riffle habitat reduced probability of

detection of YOY Arctic Grayling, presumably by allowing fish to hide, avoid shock, or avoid

capture.

Percent boulder decreased probability of detection, likely by restricting netting ability and

visibility during surveys. This is consistent with other electrofishing literature that identifies

boulder and undercut banks as habitats that allow concealment and avoidance of capture

(Peterson et al., 2004). Boulder substrate occurred in 95% of my study sites, including in lower

elevation streams. Arctic Grayling seek boulder cover as a velocity refuge in flowing streams in

a similar manner to other salmonids, which likely lowers energetic expenditures during drift

feeding events (Quinn & Kwak, 2000).

Percent riffle habitat also affected detection efficiency. Riffles are faster flowing, white

water sections that are important for many biotic and abiotic process in streams, including

oxygenation of water and provision of habitat for specialized invertebrate and fish communities (Gorman & Karr, 1978). In riffle habitats, YOY Rainbow Trout use interstitial spaces for feeding and as refugia from flow, and this results in their concealment (Meyer & Griffith, 1997). YOY Arctic Grayling exhibit similar behaviour, especially when fry emerge (McPhail & Lindsey 1970; Kaya, 1991). Ultimately, reduced visibility in riffle habitats results in lower detection efficiencies, which traditional distribution models fail to account for in their evaluations of habitat use (Bozek & Rahel, 1991; Elith & Leathwick, 2009).

2.4.3 Occupancy modelling approach

Remote areas of northern Canada are difficult to access and are logistically challenging environments for conducting field research. Using an occupancy modeling framework allowed greater spatial coverage than would have been possible using more traditional habitat models; sampling time was reduced by using presence/absence surveys, and by using spatial replication to determine detection efficiency (Charbonnel et al., 2014). Identifying landscape (elevation) and local (stream temperature) covariates can be useful for applying the model in a monitoring context (Dextrase et al., 2014). My results demonstrated the utility of spatial replication in occupancy studies, strengthening arguments by Charbonnel et al. (2014) that spatial replication is useful and provides meaningful model outputs for organisms in linear systems (e.g., streams). Other authors have expressed apprehension with respect to using spatial replication, and have found that strict adherence to assumptions in temporal replicate studies is necessary (e.g., Rota et al., 2009). However, results from my research and other studies (e.g., Baker et al., 2017) demonstrate that use of spatial replication mitigates constraints and limitations associated with

field research in remote landscapes, and that modifying assumptions can still produce statistically robust results.

Making inferences from highly variable data is challenging in field studies and can be difficult to evaluate even with robust statistical methods, such as occupancy models. The top model that explained YOY Arctic Grayling occupancy produced two important habitat characteristics that independently predicted occupancy (temperature and elevation) and two habitat characteristics that affected detection efficiency (boulder and riffle). While more study is needed to improve the precision around the parameter estimates of each variable, the results of my study identify and narrow down variables that future research should further investigate in other populations of Arctic Grayling. Future research should also consider the other variables that were included in models within two delta AIC of the top model, including undercut banks, velocity, and slope. These variables were removed from further consideration in the present study because of high uncertainty associated with their effects (which reflected high variability in the data), but both the initial results from model ranking and results of previous studies indicate that these may be important habitat features, especially when considering smaller spatial scales. Undercut banks are important habitat features for other salmonid species, such as Bull Trout, which use them for predator and capture avoidance (Peterson et al., 2004). Velocity was also found to be a key habitat covariate in predicting occupancy of YOY Arctic Grayling in barrenland systems, as small fish are unable to maintain position in high water velocities (Pearsons et al., 1992; Jones & Tonn, 2004; Baker et al., 2017).

2.4.4 Research Implications

Results from my research will help resource managers monitor and protect Arctic Grayling in sensitive northern mountain stream ecosystems. Habitat models, such as the one I created, can be especially useful in remote regions such as NWT, where data are sparse and difficult and expensive to obtain and maximizing time and cost efficiency of sampling is of paramount importance. The ecology of fishes at northern latitudes is less understood relative to southern latitudes. Broadly characterizing fish habitat use in northern mountain regions, using elevation, and refining models with stream-specific covariates, such as stream temperature, can be informative and useful for various resource management applications. However, the results of my models also identify further questions about how landscape and stream-specific habitat characteristics affect occupancy of YOY Arctic Grayling, because it is evident by study comparisons that habitat predictor variables for YOY Arctic Grayling differ between ecosystems (Baker et al., 2017). It is clear that Arctic Grayling show great plasticity and use a variety of habitats to complete life stages depending on life history type (i.e., adfluvial vs. fluvial) and such differences should be considered in management decisions.

While elevation was highlighted as the most important predictor variable at the landscape scale, the importance of water temperature in cold-water systems was also highlighted through my findings. YOY Arctic Grayling showed affinity for warmer-water areas. Stream temperature can be monitored using passive methods such as autonomous temperature loggers. Use of continuously recording loggers can help establish baseline water temperatures, assess presence and/or importance of groundwater inputs, and identify changes in water temperature from natural resource development and climate change, all of which could directly affect YOY Arctic Grayling.

Results from this research can help resource managers, industry, researchers, and First Nations understand and monitor sensitive fish species and their habitats. Threats such as habitat disturbance or overfishing should be monitored closely for susceptible species like Arctic Grayling. Degradation of spawning and rearing habitats can have negative consequences for future adult Arctic Grayling populations. An occupancy study design, that provides time- and cost-savings, may be useful to detect anthropogenic impacts that would otherwise be challenging to assess using conventional survey methods (e.g., abundance measures). As well, changes in fish occupancy can be useful for First Nations and government, to inform future fishery management decisions and can help indicate changes in freshwater health. Future monitoring and research on juvenile and adult Arctic Grayling can help continue to monitor habitat use and distribution of Arctic Grayling.

2.4.5 Future directions

Improved scientific understanding of YOY Arctic Grayling habitat can have significant bearing in determining critical habitat required by Arctic Grayling during sensitive life stages. Parks Canada and DFO are interested in applying results of this research directly into broad-scale monitoring programs. Both stakeholders are interested in identifying YOY Arctic Graying habitat because it is a life stage that is particularly vulnerable to anthropogenic impacts from development, increased park use, and climate change. Part of the impetus of this study was the stated interest of both Nahanni and Nááts'ihch'oh Park Reserves in identifying fish-bearing streams and habitats used in the Little Nahanni River watershed, to better understand and monitor the potential impacts of mine development on fisheries in the region. Parks Canada

could use this modelling approach to assess changes in Arctic Grayling habitat and use these results as an ecological baseline for their monitoring program.

Future research should assess whether landscape-level predictors, such as elevation and stream temperature, are important for other salmonid species in Arctic and sub-Arctic watersheds. Groundwater inputs may provide critical overwintering habitats for Arctic Grayling; however, little is known about the importance of groundwater in structuring habitat use for fluvial fishes in these mountain ecosystems. Expanding this framework of research to include other sensitive salmonid species will allow better understanding of the role of water temperature and other habitat covariates on distribution of other cold-water fishes and will overall contribute to conservation plans for salmonid populations in northern mountains. DFO has expressed interest in incorporating findings from this model, to produce a wide-scaling northern salmonid model.

As a result of this research, habitat requirements are now better understood for YOY Arctic Graying in mountain systems, which is important for territorial and federal regulators when assessing resource development projects. An additional concern, especially for northern residents, is how contaminants in freshwater organisms like Arctic Grayling and other subsistence fishes can be affected by development in the area. Heavy metal contaminants, such as Mercury, are already a concern in the north due to atmospheric transport and resource extraction industries. As northern communities become more accessible the exploration and subsequent development of base metal and hydrocarbon deposits will increase (Cott et al., 2015) and fossil fuel and mining activities increase, there is the potential for increasing bioaccumulation of contaminants in freshwater biota (Streets et al., 2011). Establishing a

baseline understanding of habitat use, contaminant concentrations and their interactions is critical for all stakeholders.

2.4.6 Conclusion

Using an occupancy modelling approach, I have identified elevation and water temperature as important habitat characteristics for YOY Arctic Grayling in mountain system. My findings differ from the recent results presented by Baker et al. (2017), who showed that water velocity and depth are the most important predictors for occupancy of adfluvial YOY Arctic Grayling in barrenlands streams (Baker et al., 2017). This key finding highlights that life-history type and ecoregion can play a role in determining important spawning and rearing habitat for Arctic Grayling. When applying predictive habitat models for monitoring, population information like life-history and ecoregion are critical for accurate prediction. Elevation may be particularly useful for resource managers to apply in predictive modelling because it can be remotely sensed. Using remote sensing to model elevation and its influence on Arctic Grayling distribution can help narrow search efforts and provide further cost-savings during monitoring and conservation efforts of YOY habitat. As well, using an occupancy-based approach can provide resource managers with the survey methods required to determine change in habitat use by YOY Arctic Grayling, while providing a feasible survey method required for working in remote northern environments.

Chapter 3

Comparing mercury bioaccumulation in Arctic Grayling in two sub-Arctic ecosystems: mountain versus barrenland habitats

3.1 Introduction

Mercury point sources are rare in northern Canada, yet mercury concentrations in biota and the abiotic environment of Arctic and sub-Arctic ecosystems have increased since the 1940's (see Lucotte et al., 1995; AMAP, 2011). Mercury released to the atmosphere in more southerly regions via coal combustion, mining and other anthropogenic activities, is subject to long-range atmospheric transport, and can be deposited in remote northern environments (see Fitzgerald et al., 1998; Morel et al., 1998; Durnford et al., 2010; Stern et al., 2012). Mercury transported to remote regions can ultimately be delivered to aquatic environments and undergo methylation to form methyl mercury (MeHg). MeHg can bioaccumulate and biomagnify in high-trophic level consumers such as fish (Kidd et al., 1995). Ingesting fishes with high levels of mercury can pose serious health risks to humans and wildlife (AMAP 2011).

The effects of climate change on mercury biomagnification and bioaccumulation are complex. Northern ecosystems are especially vulnerable to climate change (IPCC, 2007) and climate change may be affecting mercury cycling in northern regions by mobilizing mercury, increasing methylation rates, and altering trophic transfer (AMAP 2011). Despite a reduction in anthropogenic emissions of mercury in recent decades, increased permafrost thaw (Rydberg et al., 2010) and forest fire activity (Kelly et al., 2006) are contributing to increased mercury deposition in some northern aquatic ecosystems. Recent models predict that northern permafrost contains almost twice the amount of MeHg found in other natural environments, such as the ocean and atmosphere (Schuster et al., 2018). Fish are the largest source of mercury to humans,

and many northerners rely on fish as a food source (e.g., AMAP, 2011; see Wheatley & Paradis, 1995). In northern regions, Indigenous groups show an increased likelihood of being exposed to mercury through traditional harvesting of fish and mammals (Kuhnlein et al., 1995; Braune et al., 1999). Causes of spatial variation in fish mercury concentrations among northern regions are poorly understood, and as such, current models cannot accurately predict what regions have high MeHg concentrations in fish, just that mercury is generally correlated positively with latitude (Evans et al., 2005; Lavoie et al., 2013).

MeHg accumulation in fish reflects a complex interaction of several abiotic and biotic variables operating at spatial scales ranging from within-system habitats to landscapes. Along with many other variables, mercury concentrations in fish can respond to variability in water temperature, redox conditions, water chemistry, composition of the prey community, presence and distribution of methylating environments, lotic vs lentic habitat use, pelagic vs littoral habitat use, water residence times, and catchment sizes (see AMAP, 2011). Understanding how broad-scale differences in habitat use affect mercury bioaccumulation in fishes that show plasticity in life history and ecology will enable better predictions of effects of climate change on fish mercury levels, and ultimately better inform northern residents and regulators.

Arctic Grayling is a cold-water stenothermic fish species within the *Thymallus* genus (Salmonidae family). Habitat use and life history vary among populations, and seasonal migrations can be complex; Arctic Grayling use riverine and lacustrine waterbodies to complete different life stages (Vincent, 1962; Scott & Crossman, 1973; de Bruyn & McCart, 1974; Kaya, 1991; see Chapter 2). Similar to other Salmonids, Arctic Grayling require different habitats for spawning, rearing, feeding, and overwintering. Arctic Grayling have adopted three different life history strategies: lacustrine, adfluvial and fluvial. Lacustrine populations use lakes for all life

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stages (Vincent, 1962), adfluvial populations use streams for spawning and rearing and lakes for summer feeding and overwintering (de Bruyn & McCart, 1974), and fluvial populations use flowing stream environments for all life stages (Liknes & Gould, 1987). As factors that affect methylation (e.g., redox conditions; Fleming et al., 2006; Hammerschmidt et al., 2010) and bioaccumulation (e.g., growth rates, feeding ecology; Jardine et al., 2015; Clayden et al., 2013) of mercury can differ among habitats, it is likely that mercury concentrations also differ among life history types of Arctic Grayling; however, these differences have not been directly investigated.

Previous studies have generally found that mercury levels in Arctic Grayling are below human consumption guidelines (< 0.5 μg/g ww). Total mercury (THg) levels in adfluvial Arctic Grayling ranged from 0.078 to 0.264 mg/kg (wet weight) in two rivers in western Alaska (Jewett et al., 2003). Adfluvial Arctic Grayling were found to have lower THg than Lake Trout (*Salvelinus namaycush*) in four Alaskan lakes, which was thought to be partly due to differences in diet (Allen-Gil et al., 1997). Arctic Grayling fed primarily on surface invertebrates, whereas Lake Trout feed on a more complex diet including benthic invertebrates and fishes. Allen-Gil et al. (1997) inferred differences in feeding habits and diet between Arctic Grayling and Lake Trout that affected THg concentrations, and these authors suggested that surface-feeding Arctic Grayling were exposed to less THg in their diet than benthivorous and piscivorous Lake Trout.

THg concentrations in fish are affected by a variety of variables that likely differ between adfluvial, barrenland tundra populations of Arctic Grayling and fluvial, mountain populations of Arctic Grayling. These variables include: water temperature, dissolved oxygen, dissolved organic carbon, primary productivity, fish growth rates, and fish trophic position (see AMAP, 2011). In this study, I compared THg concentrations, age, and trophic position between adfluvial

and fluvial Arctic Grayling in barrenlands and mountain streams, respectively. I also compared MeHg concentrations in invertebrates between these two northern ecosystems. To achieve this, THg concentrations and stable isotope ratios of carbon and nitrogen in Arctic Grayling muscle tissue, as well as MeHg concentrations in benthic invertebrates, were compared between two ecosystems: Kennady Lake, representing the barrenland adfluvial population and Little Nahanni River, representing the mountain fluvial population. I hypothesized that THg concentration in Arctic Grayling would differ between Kennady Lake and Little Nahanni River as a function of differences life history strategies (adfluvial versus fluvial), trophic ecology (e.g., prey selection), and ecosystem processes (e.g., methylation rates). I predicted that barrenland, adfluvial Arctic Grayling would have higher mercury levels than mountain, fluvial Arctic Grayling, partially because of slower fish growth rates and higher rates of methylation in the former. I further predicted that at a similar life stage and size, adfluvial Arctic Grayling in the barrenlands would have higher THg levels due to feeding at a higher trophic position. The trophic food web in Kennady Lake is more complex than Little Nahanni River, providing an opportunity for Grayling to consume small prey fish and benthic invertebrates that may have increased mercury concentrations because of the lake environment that is more conducive to mercury methylation.

3.2 Methods

3.2.1 Study sites

The Kennady Lake watershed is a barrenland chain-lake ecosystem that supports an adfluvial life history type of Arctic Grayling. Kennady Lake is a headwater lake located approximately 280 km northeast of Yellowknife, Northwest Territories (NWT), 63°26′04″N 109°11′10″W (Fig. 3.1). It is considered part of the Mackenzie River basin, with the outflow running southwest into Great Slave Lake and ultimately into the Mackenzie River. Kennady Lake is currently the site of a diamond mining project (Gahcho Kue) operated by DeBeers Canada Inc., and is typical of larger lakes in the area, with a surface area of 11.8 km^2 and a maximum depth of 18 m. Large areas of the lake have been dewatered for mine development, reducing downstream flow from Kennady Lake. Data used in this study were collected before mining commenced. Hydrological linkages in the watershed are representative of tundra systems, with meandering streams that flow through small, shallow ponds (<4m) and lakes (Baker et al., 2017). The area surrounding Kennady Lake is low-relief, sub-Arctic tundra with low shrubs and sparse black spruce and dwarf birch in riparian areas. Kennady Lake is in the sub-Arctic tundra shield ecozone (Ecosystem Classification Group 2012). The watershed is comprised of hummocky glacial till covering continuous permafrost, except for deep lakes, such as Kennady Lake, where talik links the lake with groundwater (De Beers Canada 2010a). Kennady Lake has characteristics typical of an oligotrophic lake, with low nutrient concentrations, specific conductivity and total alkalinity (De Beers Canada 2010b) (Table 3.1).

The Little Nahanni River supports the fluvial life history type of Arctic Grayling and is a mountain stream system located in southwest NWT along the border with Yukon Territory, (62°12′52.1″N 128°46′01.5″W) (Fig. 3.1). This watershed is part of the headwater system for the

South Nahanni River, which drains into the larger Mackenzie River Basin (Ootes et al., 2013). Approximately one-third of the Little Nahanni River watershed is protected by both the Nahanni and Nááts'ihch'oh National Park Reserves. There are numerous mineral claims in the area by various mining companies interested in the estimated 185 million tonnes of lead-zinc-tungsten deposits in the region (Ootes et al., 2013).

The mountainous Little Nahanni watershed is part of the Mackenzie and eastern Selwyn Mountain range, which is considered the northern extent of the Canadian Rockies (Ootes et al., 2013). The watershed varies in elevation from approximately 800 to 2200 meters above sea level (masl) and is categorized as a taiga cordillera ecozone, consisting of wetland, boreal forest and alpine tundra (Ponomarenko & Quirouette, 2015). Vegetation across the landscape is determined by elevation, topography and substrate. At higher elevations (approximately > 1700 masl), common alpine vegetation, such as lichen (*Cladonia rangiferina*) and mountain avens (*Dryas octopetala*), dominates, but at lower elevations (between 800 – 1700 masl) stands of stunted white spruce (*Picea glauca*), alpine fir (*Abies lasiocarpa*), willow (*Salix sp.*) and dwarf birch (*Betula nana*) dominate the landscape (Ponomarenko & Quirouette, 2015). Annual seasonal temperatures for the Selwyn mountain range can range from -51°C in winter to 28°C in summer. The region receives 644mm of precipitation annually, with most precipitation falling as rain in the summer months (Jackson, 1987). Most streams in the area are surface-water fed, although groundwater, glaciers, and springs also contribute to base stream flow (Mochnacz et al., 2013). Mountain streams in the Selwyn Mountain Range are generally faster-flowing systems than tundra, barrenland streams at Kennady Lake (Table 3.1).

Figure 3.1. Map of North America (Image: W. Ricketts 1999) depicting the sub-Arctic ecoregion (dark grey). Sub-Arctic mountain (left) and barrenland, tundra (right) streams contain fluvial and adfluvial populations of Arctic Grayling, respectively. Photo left, credit: Morag McPherson, photo right, credit: Sarah Lord.

Table 3.1. Range of values associated with habitat data collected from Kennady Lake and Little Nahanni River during summer months. The habitat data are representative of the different ecosystems, and do not represent the habitat preferred by adult Arctic Grayling.

Stream Parameter	Kennady Lake Range		Little Nahanni River Range	
water temperature (°C)	8.60	22.40	2.48	17.00
stream width (cm)	4.00	80.25	1.50	14.32
avg. depth (cm)	1.00	100.00	9.25	68.50
avg. velocity(m/s)	0.01	0.55	0.11	1.93
discharge(m^3/s)	0.01	0.31	0.12	2.73
pH	6.45	7.45	7.23	8.43
specific conductivity (µS/cm)	12.90	16.30	113.70	519.50
dissolved oxygen (mg/L)	9.00	11.00	7.15	14.31

3.2.2 Sample collection and preparation

As part of the diamond mine project development at Kennady Lake, a partial fish-out was performed in 2014 and 2015. Adult Arctic Grayling were obtained opportunistically from these activities. Using a lake fish-out protocol (Tyson et al., 2011), gill nets of varying mesh sizes were used to capture Arctic Grayling in Kennady Lake. In suitable conditions, dissections took place in the field, otherwise, fish were frozen, shipped and dissections were completed in university labs prior to further analyses. Arctic Grayling were captured in the Little Nahanni River during summers 2015 and 2016 with either a Smith-Root Inc. LR-24 electrofisher or angling. Fish were subsequently frozen whole, using a portable electric freezer, until they could be transported to a suitable laboratory for dissection.

Ultimately, there were 27 and 24 adult Arctic Grayling (all > 148 mm fork length) collected from Kennady Lake and the Little Nahanni River, respectively. Fish were processed for fork length (mm) and wet weight (g). Sex (male/female) and maturity (immature/mature) were visually assessed for each fish. Sagittal otoliths were collected from Arctic Grayling at both sites

for aging analysis. Structures were removed, cleaned, dried, and frozen until analysis could be completed. Dorsal muscle tissue with skin removed was taken from fish. Samples were freeze-dried for a minimum of 48 hours in a Labconco Freezone 2.5 Liter Freeze Dry System at -54 °C and 10 mTorr (Labconco, Kansas City, Missouri, USA). Freeze-dried tissue was then homogenized using a mortar and pestle, until a fine powder consistency was achieved. Between each sample grinding, equipment was rinsed with Milli-Q water and ethanol. Samples were stored in acid washed scintillation vials prior to being analyzed for THg and stable isotope ratios (N and C). Arctic Grayling from Kennady Lake were shipped directly to University of Waterloo for further processing. Whole Arctic Grayling from Little Nahanni River were sent to collaborators at the University of Alberta for further processing and then samples were shipped to University of Waterloo for THg and stable isotope analysis.

In addition to Arctic Grayling, forage fishes and benthic invertebrates from Kennady Lake and Little Nahanni River were also collected, analyzed, and used in quantifications of biomagnification and food web dynamics in each study system. Prey fish species, including Slimy Sculpin (*Cottus cognatus*), Ninespine Stickleback (*Pungitius pungitius*) and Lake Chub (*Couesius plumbeus*) were caught using gill nets in Kennady Lake. Prey fish in Little Nahanni River include Slimy Sculpin and juvenile Burbot (*Lota lota*), and were captured opportunistically during electrofishing surveys for Arctic Grayling. Prey fishes were frozen whole, and dissected in the lab at University of Waterloo. Samples were prepared by removing the head and gastrointestinal tract, in accordance with USGS prey fish preparation for THg analysis (Scudder et al., 2008).

Invertebrate samples from both Little Nahanni River and Kennady Lake were collected at the same time as fishes. Sampling was conducted using the kick and sweep method (D-net;

400μm mesh). An Ekman dredge was also used in Kennady Lake to obtain benthic samples from deeper areas of the lake. Invertebrates were sorted in the lab to the lowest feasible taxonomic level - family, suborder or order - depending on the taxa. Invertebrates selected for further analysis were chosen based on three factors: i) taxa occurred at both study sites, ii) sufficient mass available for both THg and stable isotope analysis, and, iii) the organism should represent the lowest possible trophic level, preferably a shredding, grazing or filtering organism. Two families of invertebrates were represented in samples collected from both the Little Nahanni River and Kennady Lake, Chironomidae (Diptera) and Phryhaneidae (Trichoptera). These samples were thus used to establish an isotopic baseline (e.g., Post, 2002), and to compare MeHg concentrations between study sites. When necessary, individual organisms and sub-sites were pooled to achieve adequate sample mass for laboratory analyses. Three sub-sites were combined for Chironomidae from the Little Nahanni River, and four sub-sites were combined for Chironomidae at Kennady Lake. In addition, three sub-sites were combined for Phryhaneidae at Kennady Lake. Invertebrates were freeze-dried whole for a minimum 24 hours in a Labconco Freezone 2.5 Liter Freeze Dry System at -54 °C and 10 mTorr (Labconco, Kansas City, Missouri, USA). Samples were then either homogenized using a mortar and pestle, or by grinding in the sample scintillation vial. Samples were stored in acid-washed vials prior to analyses for MeHg concentrations and stable isotope ratios (N and C).

3.2.3 Mercury

MeHg comprises the majority of THg in fish (Bloom, 1992; Forsyth et al., 2004). THg is, therefore, often used as a surrogate for MeHg, as laboratory analysis for THg can yield comparable results using simpler laboratory analyses. Jewett et al. (2003) showed that 95% of THg in Arctic Grayling (N = 10) was in the MeHg form. All Arctic Grayling in this study were analyzed for THg concentration. THg analyses were completed at the Biotron Experimental Climate Change Research Centre at Western University. THg analysis was performed on a Milestone DMA-80 Direct Mercury Analyzer. A certified reference material (DORM-4: Fish protein certified reference material from the National Research Council, Ottawa, Ontario) was analyzed at the beginning of each run and after every 10 fish samples analyzed. Duplicate THg analyses were completed for approximately 10% of all samples to quantify machine precision (Mean Δ Hg = 0.0092 ± 0.016 SD, n = 28).

Analyses for MeHg on 38 prey fish samples were achieved using cold vapour atomic fluorescence spectrophotometry (Tekran 2700; modified EPA method 1631). A certified reference material (DORM-4: fish protein certified reference material from the National Research Council, Ottawa, Ontario) was analyzed (~10% of samples, mean recovery rate = 87% ± 3.04 SD). Duplicate MeHg analyses were completed for ~20% of prey fish analyzed (Relative percent difference (RPD) = 11% ± 9.4 SD, n = 8).

Invertebrates were analyzed for MeHg using a cold vapour atomic fluorescence spectrophotometer (Tekran 2700) at Western University - Analytical Service Lab in the Biotron Experimental Climate Change Research Centre. Duplicates were not available for MeHg analyses, as sample mass was limited.

3.2.4 Stable isotope ratios

Homogenous powder from fish and invertebrate samples were transferred into an aluminum cup and weighed using a Mettler-Toledo Analytical Microbalance (model XPO5DR) (Mettler-Toledo). The aluminum cup was weighed with powder, conformed into a cube necessary for stable isotope sample analysis, and weighed again to confirm sample weight. Sample weights ranged from 0.30-0.35 mg. Fish and invertebrate samples were analyzed for C and N stable isotope ratios at the Environmental Isotope Laboratory at the University of Waterloo. The equipment used was a 4010 Elemental Analyzer (Fisons Instruments) coupled to a Delta XL (Thermo Fisher Scientific) continuous flow isotope ratio mass spectrometer. Duplicates were run every 10th sample. Carbon and nitrogen values are reported as elemental isotope ratios in delta notation as $\delta^{13}C$ and $\delta^{15}N$. Both are relative values to the reference materials Vienna Pee Dee Belemnite (VPDB) and atmospheric nitrogen, respectively, and are reported as parts per mil (‰). Analyses are quality controlled for instrument precision using international reference materials and in-house EIL standards, which are analyzed in each run. Ammonium sulfate (IAEA-N1 and IAEA-N2) is used as the laboratory international reference material for $\delta^{15}N$, in conjunction with in-house standard material ammonium sulfate (EIL-3 and JSEC-01). In the case of $\delta^{13}C$, international standard cellulose (IAEA-CH-3) and in-house standard cellulose (EIL-72) is used as a quality control standard. The standards are cross-calibrated to organic materials and the reference materials VPDB and atmospheric nitrogen. Standard reference materials were included as 20% of each analytical run and duplicate samples fell within quality control standards of 0.2‰ for $\delta^{13}C$ and 0.3‰ for $\delta^{15}N$.

Baseline corrected values of $\delta^{13}C$ and $\delta^{15}N$ for Arctic Grayling and prey fishes were achieved using $\delta^{13}C$ and $\delta^{15}N$ values for Chironomidae as the only in-common and most

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abundant baseline invertebrate across both sites (see equation 3.1; e.g., Swanson et al., 2003). Other invertebrates that were collected in Kennady Lake and Little Nahanni River were used to assess biomagnification and trophic ecology in each respective system.

3.2.5 Aging analysis

Ages of Arctic Grayling for Kennady Lake and Little Nahanni River were evaluated using whole or thin sections of otoliths viewed under a microscope. Annuli appear as alternating opaque and translucent bands, which represent one summer and winter, respectively (see Panfili et al., 2002). For simplicity, fish ages are based on calendar year, assuming fish hatch January 1. Aging analysis was completed for Arctic Grayling from Kennady Lake (n = 21) and Little Nahanni River (n = 20). Some aging structures were either (a) not collected for each individual fish or (b) were not in suitable condition for aging analysis. Thus, sample sizes for age estimates are smaller than the total number of fish evaluated for THg concentration in tissue. Aging was completed at AAE Tech Services, with duplicate age estimates completed for 50% of the samples by two different aging technicians (duplicate age estimate rate = 100% agreement).

3.2.6 Water Analysis

Dissolved THg concentrations in water were compared between Kennady Lake and Little Nahanni River, "clean-hands dirty-hands" sampling techniques were used to collect water samples from three sites in the Little Nahanni River system in September 2016 (St. Louis et al., 1994). Samples were preserved with 1% (by volume) ultra-trace HCl and transported to the Biotron at Western University. Water samples were analyzed using Cold Vapour Atomic Fluorescence - Digestion, Method Ref. modified from EPA 1631, Lab Method ID - TM.0811.

Initial and ongoing precision and recovery values ranged from 105% - 110% and 100% - 111% respectively. Concentrations of THg in water from Kennady Lake were evaluated as part of an Environmental Impact Statement at six sites between 1995 and 2010 (De Beers, 2010b).

3.2.7 Statistical analyses

Statistical analyses were completed using IBM SPSS Statistic software version 2.1 or Microsoft Excel 2016. All Arctic Grayling collected from Kennady Lake (n=27) and Little Nahanni River (n=24) were used during THg analysis. Significance level for analyses was set at $\alpha = 0.05$. All THg concentrations for Arctic Grayling are converted and reported as wet weight (ww), which is consistent with THg literature. All THg data were $\log_{10}$ transformed prior to statistical analysis. A series of possible covariates for Hg in fish, including fork length, wet weight, age, condition factor, $\delta^{15}N$, $\delta^{13}C_{adj}$, condition factor, age and C:N ratio, were related to Arctic Grayling THg concentration and were compared between sites using general linear models (e.g., t-test, linear regression). Covariates that were compared among sites include: fork length, wet weight, age, condition factor, THg, $\delta^{13}C_{adj}$, $\delta^{15}N$ and C:N ratio. Because length and weight were highly correlated ($r^2 = 0.96$), and fish weights were recorded at different stages (e.g., in field versus in lab), analyses relating fish THg concentrations to fish size were completed using fork length. When comparing differences in THg between systems, values of $\delta^{13}C$ and $\delta^{15}N$ were adjusted ($\delta^{13}C_{adj}$ and $\delta^{15}N_{adj}$) using the baseline benthic invertebrate Chironomidae, to account for differences in basal carbon and nitrogen in the system. Analyses involving age were completed using the reduced sample sizes available for analysis (Kennady Lake, $n = 21$; Little Nahanni River, $n = 20$).

Relationships between fish weight and length provide important inferences about a fishes condition. As well, weight-at-length relationships can be used to evaluate growth patterns in fish

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species like Arctic Grayling (Le Cren, 1951). Based on a non-linear power line of fit, slopes of weight-length relationships indicated isometric growth for Arctic Grayling captured in Kennady Lake (slope = 2.86) and allometric growth for Arctic Grayling captured in Little Nahanni River (slope = 3.18). Condition factor was calculated using Fulton's condition factor (K) (equation 3.2).

Analyses of covariance were used to compare THg levels in Arctic Grayling between sites; fork length was a covariate and least-squares means THg levels were compared at a fork length of 239 mm, as this was the estimated marginal mean common between populations. Heterogeneity of variance and normality of the residual assumptions were assessed. Associations between covariates and $\log_{10}$ THg concentration were then assessed using correlation matrices (r). General linear models, including analyses of covariance, were then used to further assess relationships between $\log_{10}$ THg concentration (wet weight) and covariates between the sites.

3.3 Results

On average, Arctic Grayling captured at Kennady Lake were approximately 68 mm longer and weighed 140 g more than Arctic Grayling from Little Nahanni River. Data were log-transformed prior to analysis, with the exception of stable isotope values. Mean THg concentration was higher in Kennady Lake (mean = 0.06 ± 0.02) than Little Nahanni River (mean = 0.02 ± 0.01). All 51 Arctic Grayling collected from the study systems had THg concentrations that were well below the commercial sale guideline in Canada, which is 0.5 µg/g wet weight (Figure 3.2).

Table 3.2. Descriptive statistics derived from Arctic Grayling in Kennady Lake and Little Nahanni River to assess mercury concentration differences among sites. Unadjusted values are displayed for δ^{13}C and δ^{15}N.

Covariate	Kennady Lake (n=27)			Little Nahanni River (n=24)		
	Mean (± St. Dev.)	Min	Max	Mean (± St. Dev.)	Min	Max
Length (mm)	288.07±47.02	188	370	220.38±76.39	148	369
Weight (g)	318.04±137.48	77	650	177.92±193.94	35	610
Age (years)	3.81±1.08	2	5	4.83±3.4	2	11
THg (µg/g ww)	0.06±0.02	0.03	0.10	0.02±0.01	0.01	0.04
δ^{13}C (‰)	-22.41±0.8	-23.95	-20.05	-31.91±1.72	-35.05	-28.42
δ^{15}N (‰)	8.06±0.42	7.10	8.82	8.11±1.53	6.48	11.20
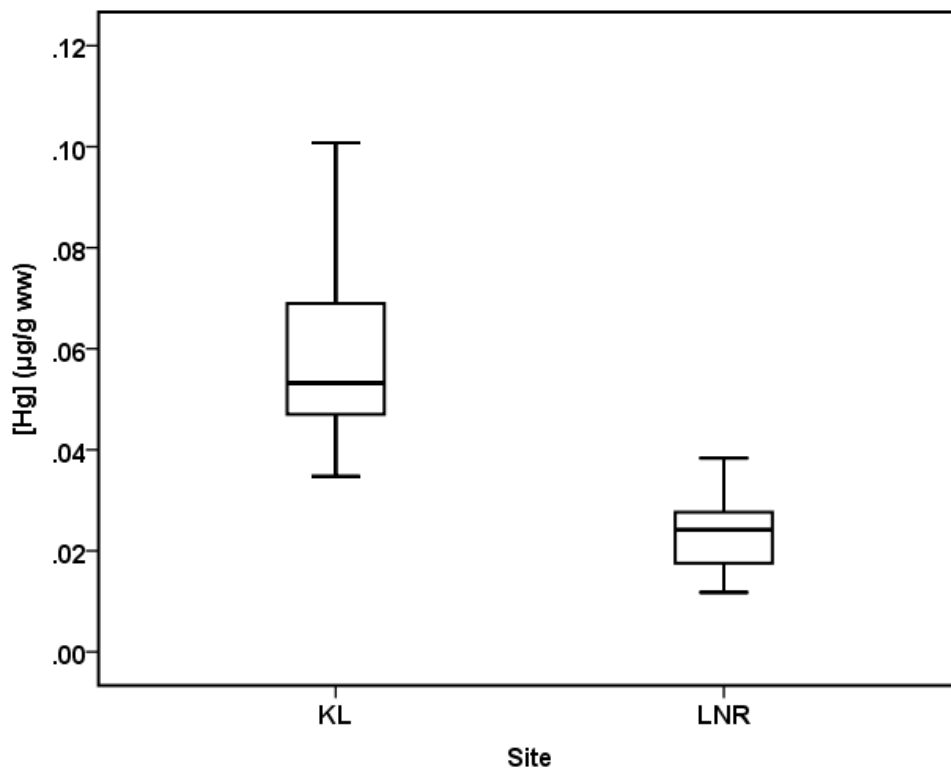
Figure 3.2. Box plot of total mercury (THg) concentrations (ww) in Arctic Grayling captured from Kennady Lake (KL), NWT in 2014 and Little Nahanni River (LNR), NWT, 2015 and 2016. All measured mercury concentrations are well below the Canadian commercial sale guideline (0.5µg/g ww). Arctic Grayling from Kennady Lake, NWT had significantly higher THg than Arctic Grayling from Little Nahanni River, NWT (Pooled samples t-test, $t_{(49)} = 9.579$, $P < 0.05$).

THg in muscle tissue of adult Arctic Grayling was significantly higher in fish from Kennady Lake than in fish from Little Nahanni River (Figure 3.2; pooled samples t-test, $t_{(49)} = 9.579$, $P < 0.05$). A variety of covariates, listed in Table 3.3, were investigated as potential explanatory variables for the difference in mercury between sites.

Fork length and weight of Arctic Grayling were highly correlated ($r = 0.98$) in both study systems. Fork length was used to estimate size-standardized THg concentrations in each of Kennady Lake and Little Nahanni River. Arctic Grayling at Kennady Lake were significantly longer than at Little Nahanni River (Pooled samples t-test, $t_{(49)} = 4.32$, $P < 0.05$) (Figure 3.3). Log_{10} THg concentration was significantly related to fork length at Kennady Lake (linear regression, $t_{(25)} = 3.73$, $P < 0.05$), but was not significantly related to fork length at Little Nahanni River (linear regression, $t_{(22)} = 1.77$, $P = 0.09$) (Figure 3.4).
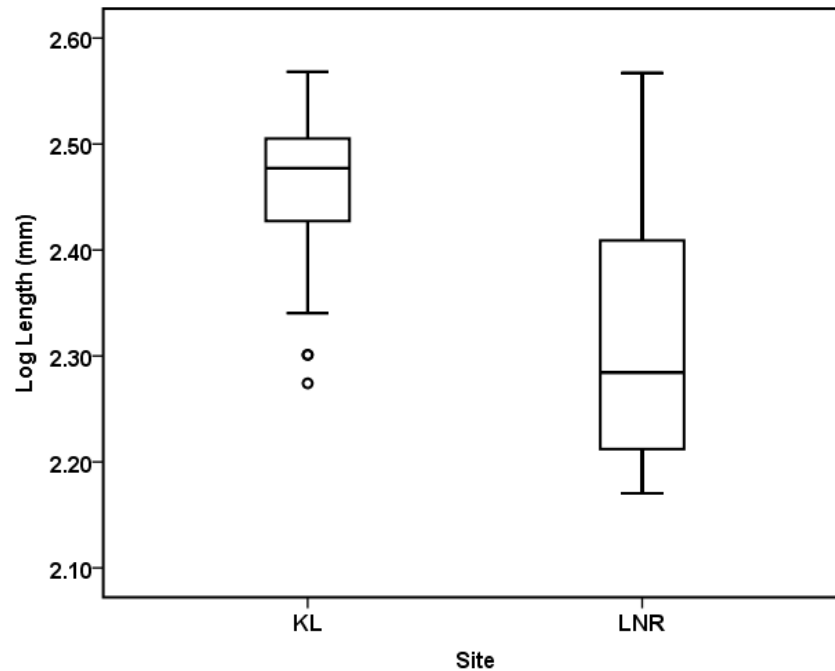
Figure 3.3. Boxplot of adult Arctic Grayling fork length (mm; log10 transformed). Arctic Grayling from Kennady Lake were significantly longer than those from Little Nahanni River (Pooled samples t-test, $t_{(49)} = 4.32$, $P < 0.05$). Outliers are represented by black circles included in the t-test. Boxes represent the 25th, 50th, and 75th percentiles, and whiskers represent the 5th and 95th percentiles.

76
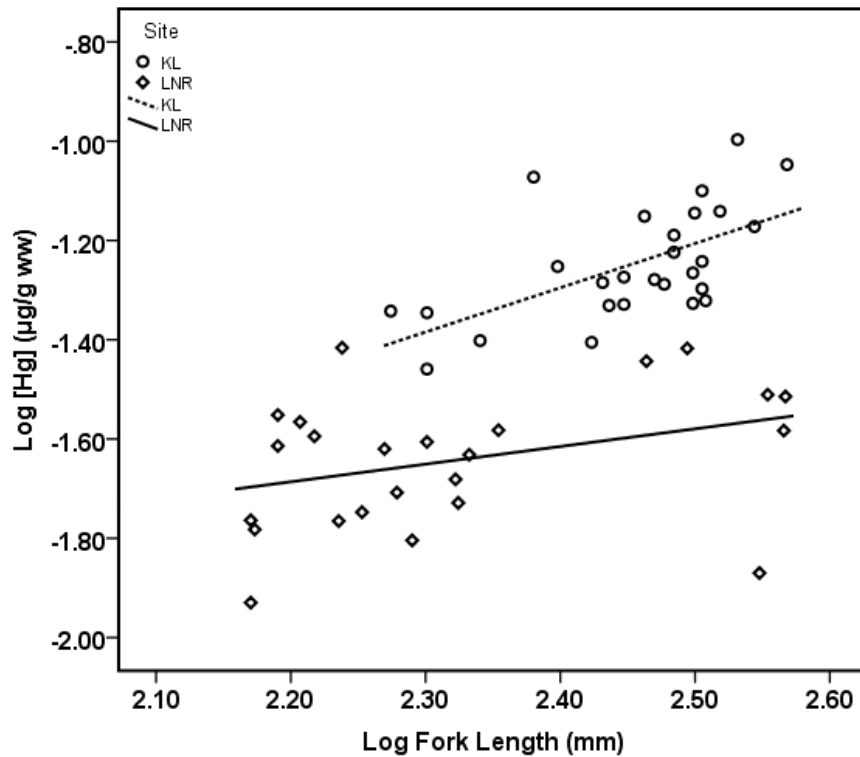
Figure 3.4. Linear regressions of Log_{10} total mercury (THg) concentration (ww) and $\log_{10}$ fork length (mm) for Kennady Lake (KL), NWT, 2014 and Little Nahanni River (LNR), NWT, 2015 and 2016. Log_{10} Total mercury (THg) was significantly and positively related to $\log_{10}$ fork length (mm) at Kennady Lake, NWT (t $_{(25)} = 3.73$, P < 0.05) but not at Little Nahanni River, NWT (t $_{(22)} = 1.77$, P = 0.09). At a fork length of 239 mm ($\log_{10}$ fork length (mm) = 2.38), Total mercury (THg) concentrations in Arctic Grayling were significantly higher at Kennady Lake, NWT than at Little Nahanni River, NWT.

To investigate whether Arctic Grayling had higher THg at Kennady Lake because they were larger, I performed an analysis of covariance; site was the independent variable, Log_{10}THg was the dependent variable, and $\log_{10}$ fork length was the covariate. Heterogeneity of slopes was not observed – i.e., the interaction term of site* Log Fork Length was not significant (ANCOVA, F $_{(1, 47)} = 2.54$, P = 0.12). Mercury concentrations were significantly related to log fork length (mm) (ANCOVA, $F_{(1, 48)} = 10.89$, P < 0.05) and differed significantly between sites (ANCOVA, $F_{(1, 48)} = 75.78$, P < 0.05). Tests of normality, including the Kolmogorov-Smirnov test (test statistic $_{(51)} =$

.09, P = 0.20), as well as qualitative assessments of Q-Q and residual*predicted plots, indicated

normality of residuals from the ANCOVA analysis. Estimated least squares means using type III

sum of squares produced an estimate of Log_{10} THg (wet weight) to be -1.28 ($\pm$ 0.02 SE) and -

1.61 ($\pm$ 0.03 SE) for Kennady Lake and Little Nahanni River, respectively. Estimates were made

at the mean level of the covariate (log fork length of 2.39), and were significantly different ($F_{(48)}$

= 75.78, P < 0.05). That is, Arctic Grayling at Kennady Lake had higher THg than at Little

Nahanni River, even when the effect of larger fish size at Kennady Lake was accounted for.

To further investigate why Arctic Grayling at Kennady Lake had higher THg than at Little

Nahanni River, correlations between THg and seven additional covariates were assessed (Table

3.3). In general, correlations between THg and covariates were stronger for Kennady Lake then

for Little Nahanni River.

Table 3.3. Results of Pearson correlation analyses between log_{10} total mercury concentrations in Arctic Grayling and potential covariates at Kennady Lake, Little Nahanni River, and both sites combined. There is a reduced sample population for age-related covariates due to a limited number of aging structures available for analysis.

Covariate	(n)	Kennady Lake		Little Nahanni River		Sites combined	
		r	P	r	P	r	P
fork length	51	**0.57**	**< 0.05**	0.36	0.08	**0.88**	**< 0.05**
weight	51	**0.72**	**< 0.05**	0.30	0.16	**0.85**	**< 0.05**
$\delta^{13}C_{adj}$	51	- 0.38	0.05	-0.08	0.70	**- 0.35**	**< 0.05**
$\delta^{15}N$	51	**0.47**	**< 0.05**	0.17	0.43	**-0.45**	**< 0.05**
C:N	51	- 0.18	0.37	0.16	0.47	- 0.036	0.82
condition factor	51	0.36	0.06	0.07	0.73	**0.65**	**< 0.05**
age	41	**0.71**	**< 0.05**	**0.63**	**< 0.05**	**0.40**	**< 0 .05**
length-at-age	41	**- 0.51**	**< 0.05**	**- 0.54**	**< 0.05**	0.13	0.43

At Kennady Lake, Log_{10} THg concentration in Arctic Grayling was positively correlated with fork length, weight, age, and $\delta^{15}N$, and negatively correlated with length-at-age. There was also a moderate correlation with $\delta^{13}C_{adj}$. Age and length-at-age were the only two covariates that correlated with THg concentration in Arctic Grayling from Little Nahanni River. Log_{10} THg concentration was not correlated to condition factor or C:N ratio at either Kennady Lake or Little Nahanni River, and these variables were thus excluded from analyses designed to further investigate why THg concentrations in Arctic Grayling were different between sites (Table 3.3). Log_{10} THg was significantly correlated with fork length, weight, $\delta^{13}C_{adj}$, condition factor and age.

Age was significantly and positively related to THg concentration at both sites (simple linear regression, $t_{(19)} = 5.03$, $P < 0.05$) and Little Nahanni River (simple linear regression, $t_{(18)} = 2.96$, $P < 0.05$; Figure 3.5). In an ANCOVA in which site was the independent variable, Log_{10} [THg] was the dependent variable, and Log_{10} Age was the covariate, heterogeneity of slopes was not observed (age*site interaction was not significant (ANCOVA, $F = 2.11$, $df = 37$, $P = 0.15$)), [THg] was significantly related to age (ANCOVA, $F_{(2, 37)} = 20.89$, $df = 37$, $P = < 0.05$), and [THg] differed significantly between sites (ANCOVA, $F_{(1, 38)} = 141.98$, $P < 0.05$). Estimated LS means using type III sum of squares produced estimates of Log_{10} [THg] (wet weight) of -1.25 ($\pm$ 0.02 SE) and -1.63 ($\pm$ 0.02 SE) for Kennady Lake and Little Nahanni River, respectively (derived at an average age of 3.5 years). Tests of normality, including the Kolmogorov-Smirnov test, as well as qualitative assessments of Q-Q and residual*predicted plots, indicated normality of residuals from the ANCOVA analysis. Mercury concentrations thus significantly differed between sites even after age correction, and therefore age differences cannot explain differences in mercury.
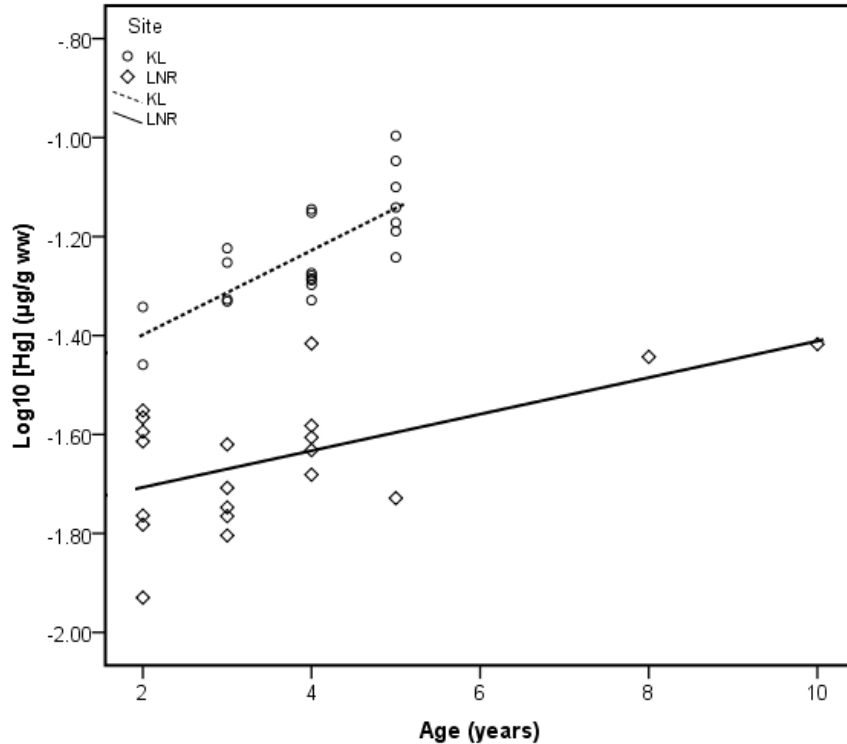
Fig. 3.5. Linear regression of Log_{10} total mercury (THg) concentration (ww) and age (years) in populations of Arctic Grayling in Kennady Lake and Little Nahanni River. Log_{10} THg was significantly and positively related to age (years) at Kennady Lake (SLR, $t_{(19)} = 5.03$, P < 0.05) and at Little Nahanni River (SLR, $t_{(18)} = 2.96$, P < 0.05).

Because length-at-age was significantly and negatively related to THg at both Kennady Lake and Little Nahanni River, an ANCOVA was used to assess whether length-at-age differed between sites and could thus explain the difference between sites in THg in Arctic Grayling. Site was the fixed factor, length was the dependent variable and age was the covariate. The interaction term (Site*Age) was significant (ANCOVA, F = 11.03, df = 37, P < 0.05), indicating that growth rates differed between sites. Arctic Grayling grew faster in Kennady Lake than in Little Nahanni (Figure 3.6), and thus higher THg in Arctic Grayling from Kennady Lake was not caused by slower growth rates in this system.

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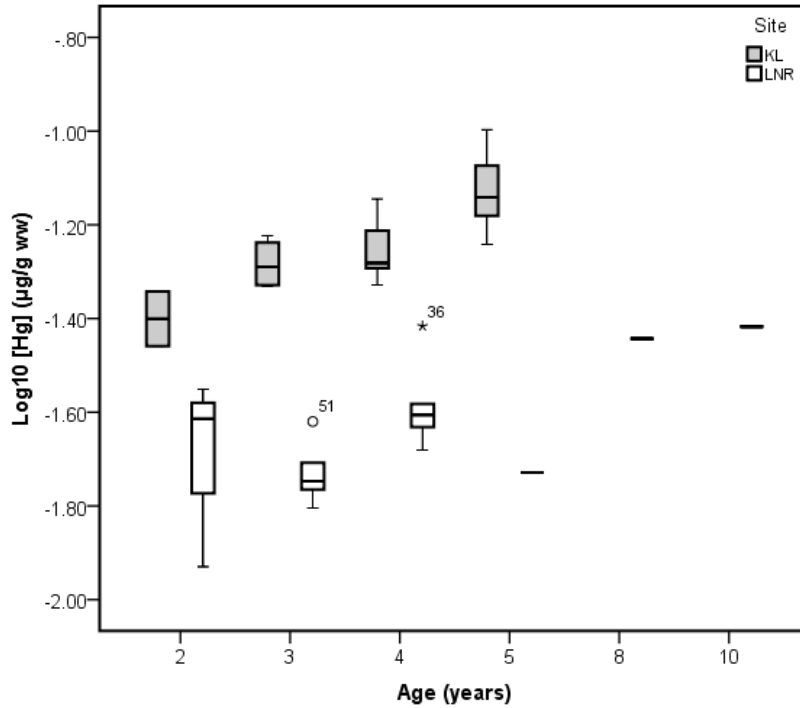
Figure 3.6. Box plots of Log_{10} total mercury (THg) concentration (ww) at each age of Arctic Grayling captured from Kennady Lake and Little Nahanni River. At similar ages, Arctic Grayling from Kennady Lake had higher THg than Arctic Grayling from Little Nahanni River. Outliers (36 and 51) are both from Little Nahanni River; these fish had higher THg at a given age when compared to other fish in the population and were included in additional analyses. Boxes represent the 25th, 50th, and 75th percentiles, and whiskers represent the 5th and 95th percentiles.
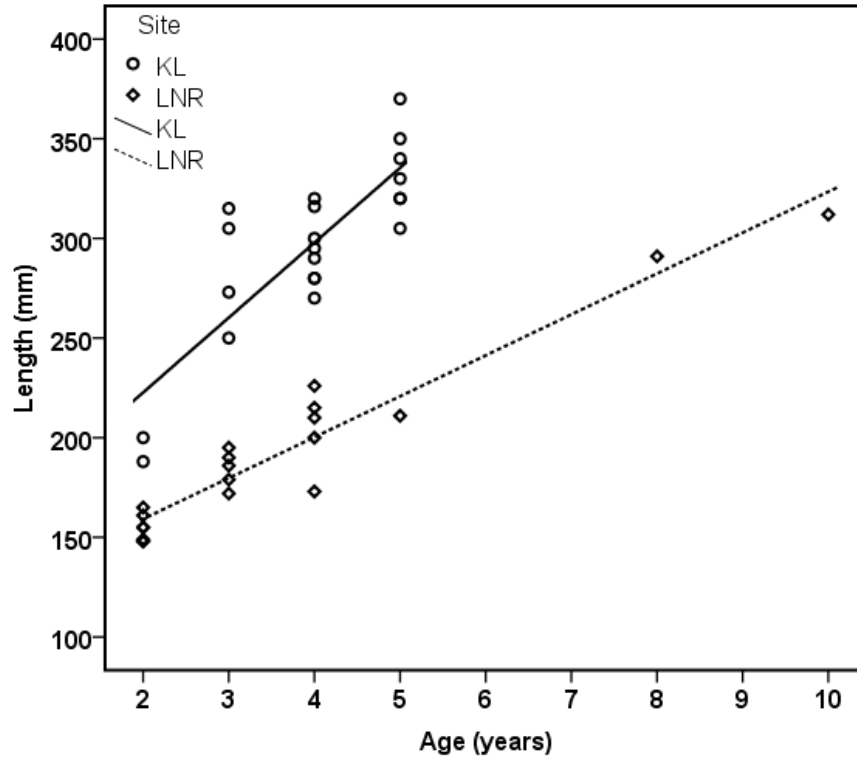
Figure 3.7. Linear regressions between fork length (mm) and age (years) of Arctic Grayling from Kennady Lake (SLR, $F_{(1, 20)} = 41.84$, $P < 0.05$) and Little Nahanni River (SLR, $F_{(1, 19)} = 217.68$, $P < 0.05$).

Log$_{10}$ THg was moderately and negatively related to $\delta^{13}C_{adj}$ at Kennady Lake ($r = -0.38$) and not correlated at Little Nahanni River ($r = -0.08$) (Table 3.3; Figure 3.8). Overall, the relationship between $\delta^{13}C_{adj}$ and THg was negative in both Kennady Lake and Little Nahanni River populations of Arctic Grayling. As previous authors have shown a negative relationship between THg and $\delta^{13}C$ (e.g., Power et al. 2002), a pooled samples t-test was performed to determine whether $\delta^{13}C_{adj}$ differed between sites and thus might explain higher THg in Arctic Grayling from Kennady Lake. Delta^{13}C ratios were adjusted using $\delta^{13}C$ values for Chironomidae as the baseline benthic invertebrate common between Kennady Lake and Little Nahanni River. Delta$^{13}C_{adj}$ did not differ significantly between sites, and thus did not explain differences in THg between sites. To ensure that differences in the relationship between fish size and $\delta^{13}C_{adj}$

between sites did not affect the assessment of mean differences in $\delta^{13}C_{adj}$ between sites, an ANCOVA was performed; Site was the fixed factor, $\delta^{13}C_{adj}$ was the dependent variable, and length was the covariate. The interaction term (Site* Length) was significant (ANCOVA, $F_{(1,47)}$ = 6.23, P < 0.05), indicating that the relationship between $\delta^{13}C$ and length differed between sites. The relationship between $\delta^{13}C_{adj}$ and site was found to be not significant (ANCOVA, $F_{(1,47)}$ = 3.91, P = 0.55). At similar lengths (260mm), $\delta^{13}C_{adj}$ is considered not significantly different between sites, which confirms $\delta^{13}C_{adj}$ does not explain differences in THg between Kennady Lake and Little Nahanni River.
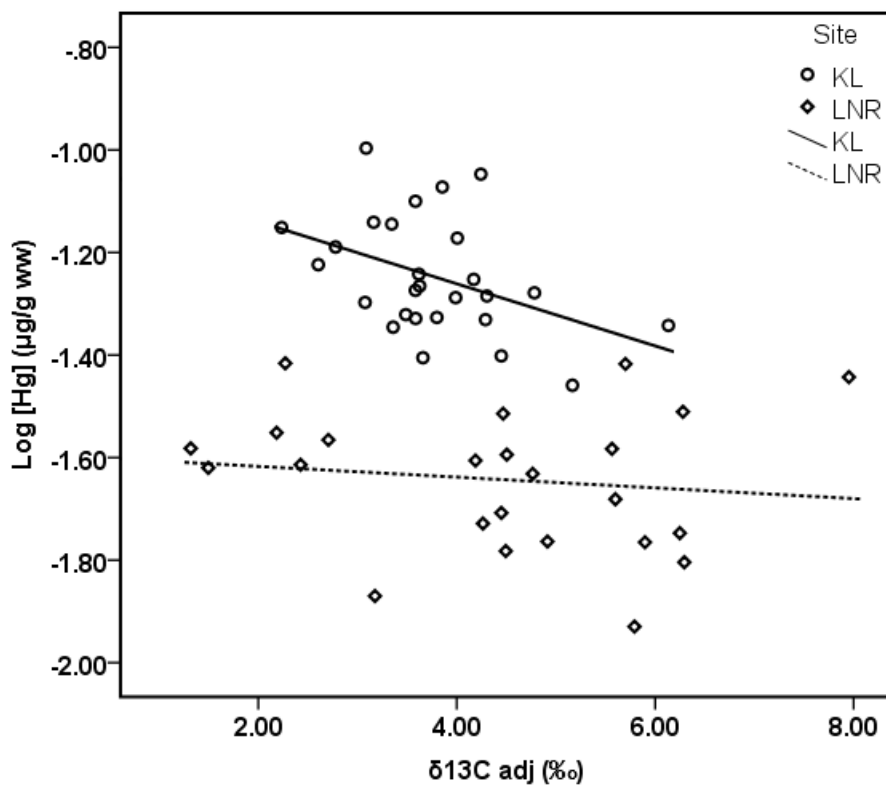
Fig. 3.8. Relationship between Log_{10} Total Mercury (THg) concentration (ww) and $\delta^{13}C_{adj}$ (‰) in Arctic Grayling from Kennady Lake and Little Nahanni River. Log_{10} THg was significantly and negatively related to $\delta^{13}C_{adj}$ (‰) at Kennady Lake (SLR, t $_{(25)}$ = - 2.34, P < 0.05) but the relationship was not significant at Little Nahanni River (SLR, t $_{(22)}$ = - 0.62, P = 0.54).
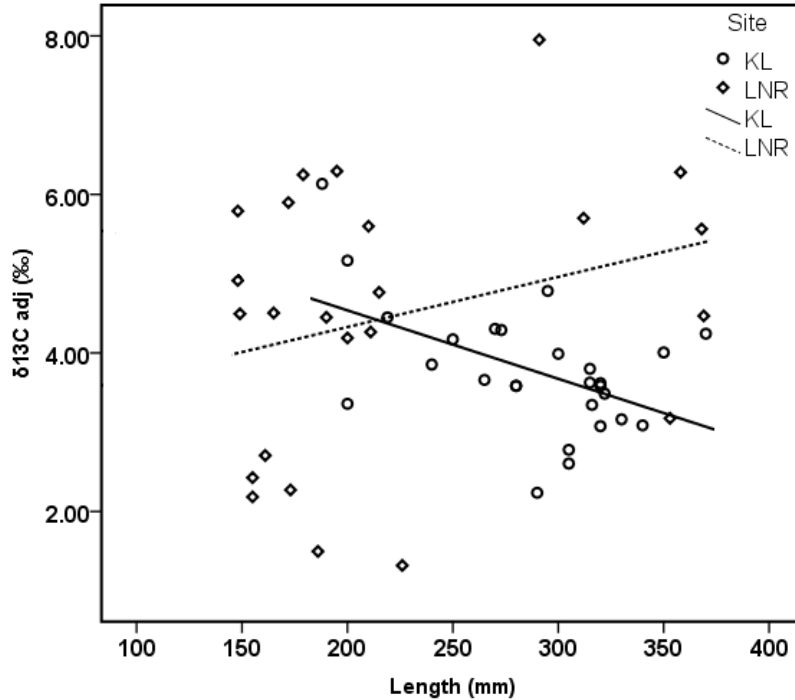
Fig. 3.9. Relationship between $\delta^{13}C_{adj}$ (‰) and length (mm) in Arctic Grayling from Kennady Lake and Little Nahanni River. Length (mm) and $\delta^{13}C_{adj}$ (‰) are negatively and significantly related at Kennady Lake (SLR, $F_{(1, 25)} = 8.65$, $P < 0.05$) but the relationship was not significant in the Little Nahanni River (SLR, $F_{(1, 22)} = 1.89$, $P = 0.18$).

At a similar $\delta^{15}N$, Arctic Grayling from Kennady Lake had higher THg concentration than at Little Nahanni River. Higher THg in Kennady Lake Arctic Grayling at similar values of $\delta^{15}N$ suggests there may be baseline differences in mercury concentration between the two systems. Log_{10} THg in Arctic Grayling was moderately and positively correlated with $\delta^{15}N_{adj}$ at Kennady Lake, but there was no relationship between THg and $\delta^{15}N_{adj}$ in Arctic Grayling at Little Nahanni River (Table 3.3; Figure 3.10). The relationship between $\delta^{15}N_{adj}$ and length in Kennady Lake was positive and significant relationship (SLR, $F_{(1, 25)} = 38.58$, $P < 0.05$). This suggest that Arctic Grayling in Kennady Lake may show an ontogenetic shift in feeding, while feeding by Arctic Grayling in Little Nahanni River appears much more variable across lengths (Figure 3.11).
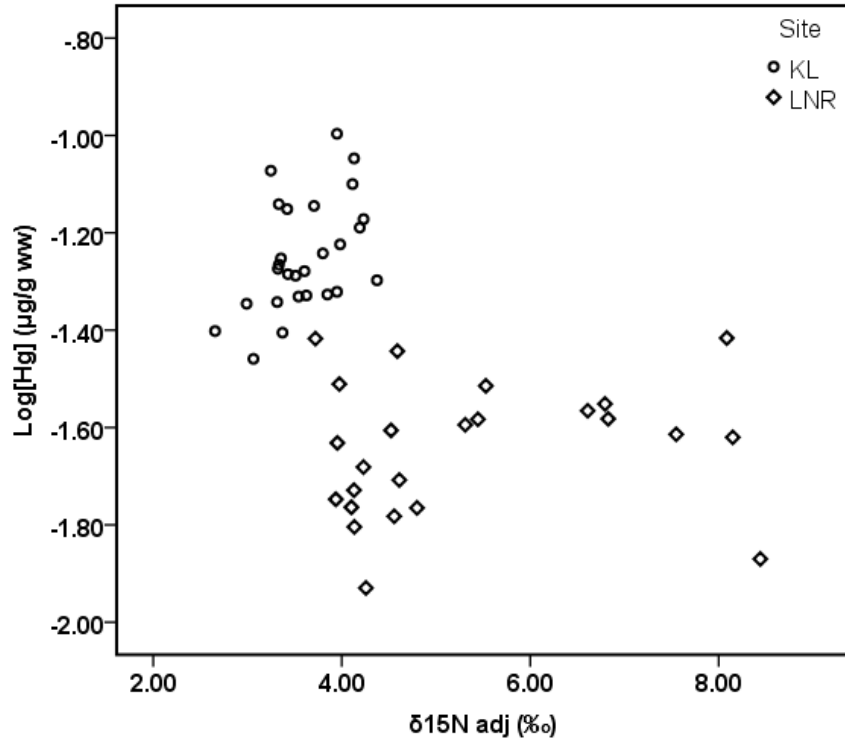
Fig. 3.10. Log_{10} total mercury (THg) concentration (ww) as related to $\delta^{15}N_{adj}$ (‰) for Arctic Grayling in Kennady Lake (KL), NWT, in 2014 and Little Nahanni River (LNR), NWT in 2015 and 2016.

In an ANCOVA, site was the fixed factor, length was the covariate and $\delta^{15}N_{adj}$ was the dependent variable. The interaction term (Site* Length) was not significant (ANCOVA, $F_{(1,47)}$ = 2.26, P = 0.14), indicating regression of slopes was similar. Delta $^{15}N_{adj}$ was significantly related to site (ANCOVA, $F_{(1,47)}$ = 27.96, P < 0.05) but not significantly related to fork length (ANCOVA, , $F_{(1,47)}$ = 0.25, P = 0.62). Tests of normality as well as qualitative assessments of Q-Q and residual*predicted plots, indicated normality of residuals from the ANCOVA analysis. Estimated least squares means using type III sum of squares produced an estimate of $^{15}N_{adj}$ to be 3.57 ($\pm$ 0.22 SE) and 5.38 ($\pm$ 0.24 SE) for Kennady Lake and Little Nahanni River, respectively. Estimates were made at the mean level of the covariate (fork length of 260.75mm) and were significantly different (F $_{(48)}$ = 27.96, P < 0.05). That is, Arctic Grayling at Kennady Lake had lower $^{15}N_{adj}$ than at Little Nahanni River when accounting for larger fish size at Kennady Lake.

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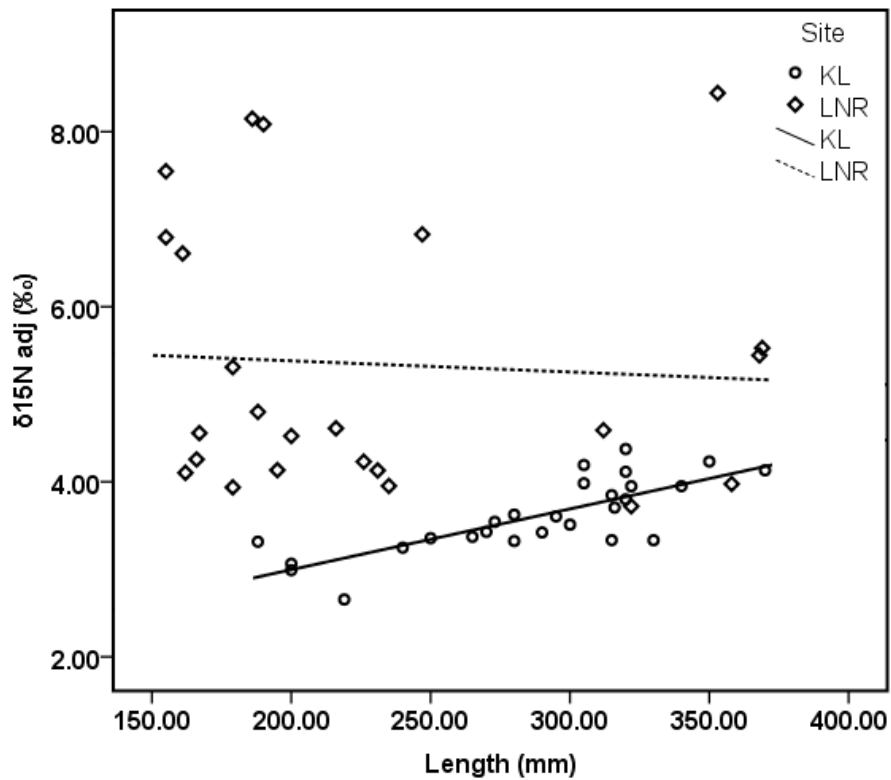
Figure 3.11. The relationship between $\delta^{15}N_{adj}$ (‰) and length for Arctic Grayling in Kennady Lake (KL), NWT, in 2014 and Little Nahanni River (LNR), NWT in 2015 and 2016. $\delta^{15}N_{adj}$ is significantly and positively related to length at Kennady Lake (SLR, $F_{(1, 25)} = 38.58$, $P < 0.05$), but shows no relationship in the Little Nahanni River (SLR, $F_{(1,22)} = 0.01$, $P = 0.92$).
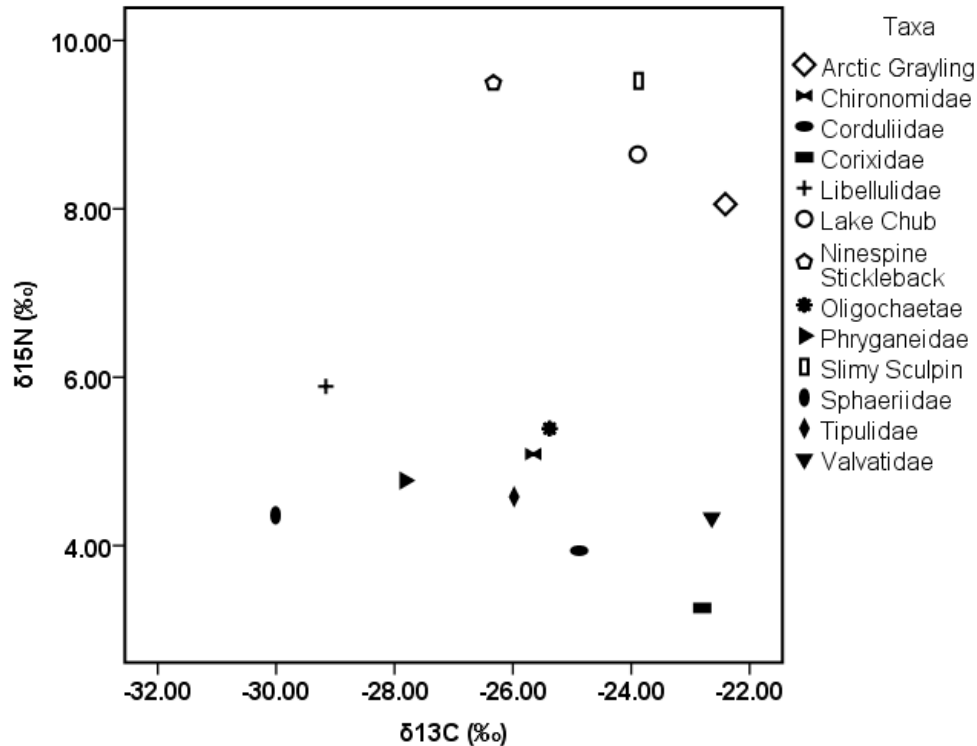
Figure 3.12. Stable isotope biplot illustrating mean values of $\delta^{13}C$ (‰) and (‰) for 13 species of forage fish and benthic invertebrates collected in $\delta^{15}N$ Kennady Lake, NWT.

To begin investigations of food web differences between Kennady Lak and Little Nahanni River, stable isotope biplots were examined. As expected, fish species, including Arctic Grayling, Lake Chub, Ninespine Stickleback and Slimy Sculpin occupy a higher trophic position than benthic invertebrate taxa in Kennady Lake based on $\delta^{15}N$ values (Figure 3.12). Delta^{13}C values for fish species show overlap with invertebrate species that are known to occupy benthic, littoral and pelagic zones. This suggests fish are feeding on a combination of benthic invertebrates from various water column zones, with Arctic Grayling showing a more benthic signature and Ninespine Stickleback feeding more pelagically.
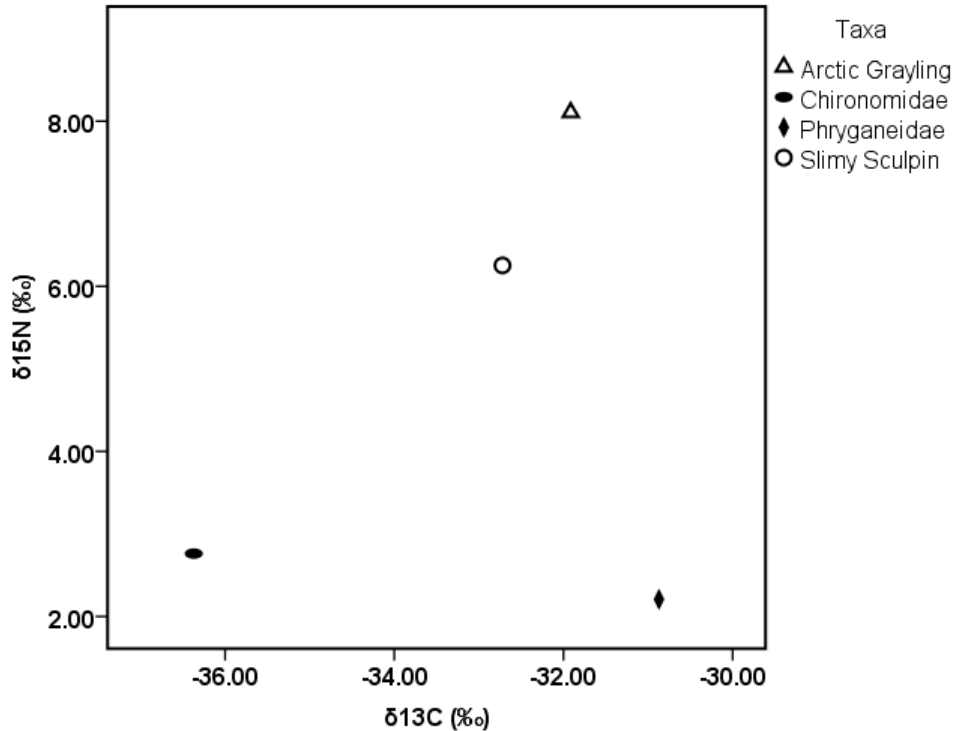
Figure 3.13. Stable isotope biplot illustrating mean values of $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) for four species of fish and benthic invertebrates collected in Little Nahanni River, NWT in 2015 and 2016.

There were four species analyzed from Little Nahanni River that were also captured and analyzed from Kennady Lake. These organisms included two fish species, Arctic Grayling and Slimy Sculpin and two benthic invertebrate taxa, Chironomidae and Phryganeidae. Similar trophic trends are identified in Little Nahanni River as in Kennady Lake, which revealed two distinct groupings; one representing fish and one representing the benthic invertebrate species. As observed in Kennady Lake, fish species in Little Nahanni River are feeding at approximately one trophic level (~3.4 ‰) higher than benthic invertebrates in the system.
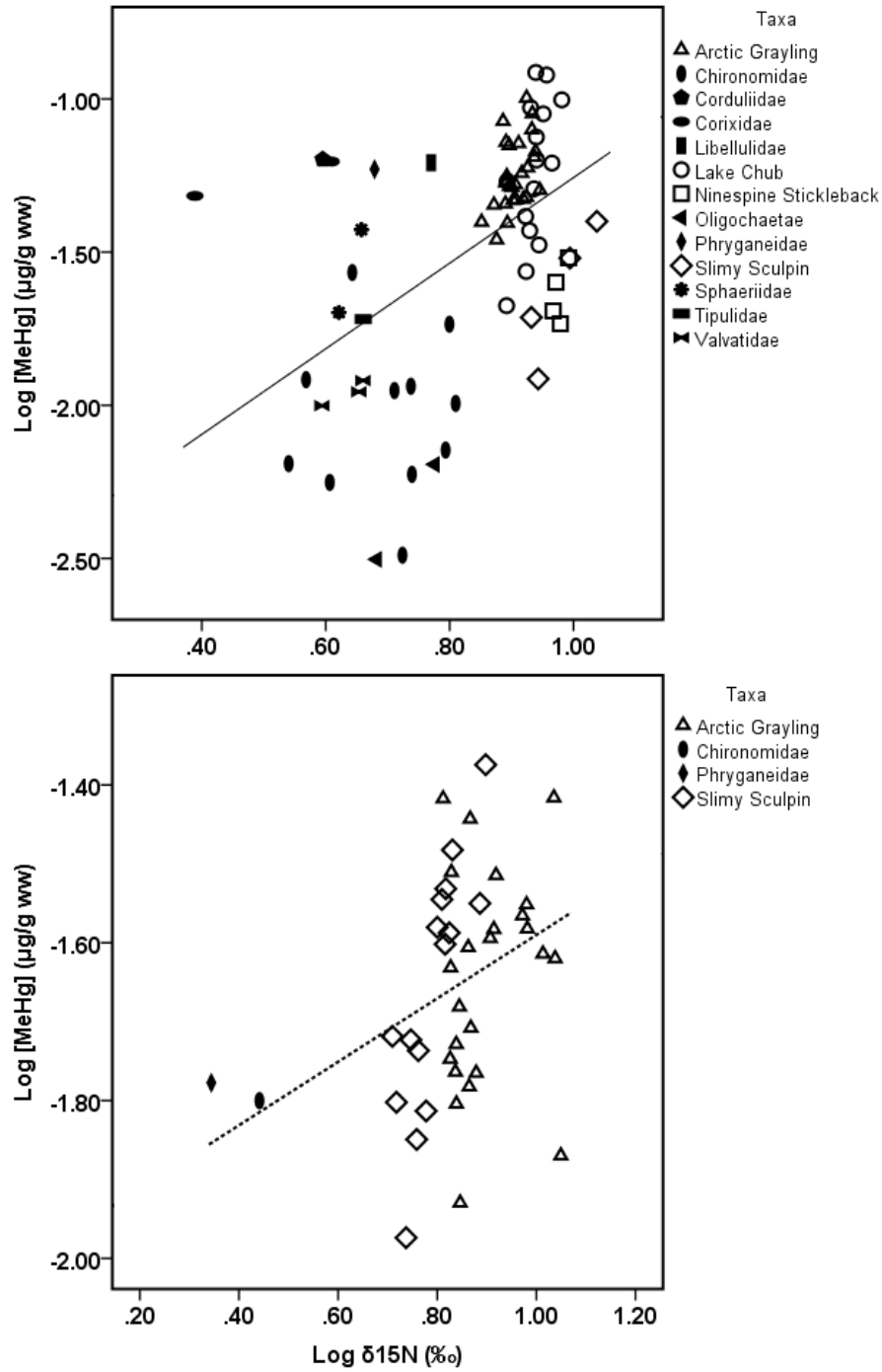
Figure 3.14. Regressions of log mercury concentration (μg/g ww) versus log$_{10}$ δ^{15}N for fishes and benthic invertebrates collected in Kennady Lake, NWT in 2014 (top; slope = 1.4) and Little Nahanni River, NWT in 2015 and 2016 (bottom; slope = 0.40). Methyl-mercury (MeHg) values were used for benthic invertebrate organisms and total mercury (THg) values were used for fish species in regression analyses. Note: scales differ for each site.

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Rates of biomagnification were investigated using regression analyses that revealed a positive and significant relationship between $\log_{10}$ mercury concentration (µg/g ww) and $\log_{10}$ δ^{15}N in both Kennady Lake (SLR, $F_{(1,72)} = 24.19$, $P < 0.05$) and Little Nahanni River (SLR, $F_{(1,39)} = 6.22$, $P < 0.05$). Delta^{15}N and mercury concentrations in all species analyzed at Little Nahanni River, represent biomagnification of mercury from inverts to fish species. I tested for differences in mercury biomagnification rates by conducting an analysis of covariance; site was the independent variable, Log_{10} THg was the dependent variable, and $\log_{10}$ δ^{15}N was the covariate. Heterogeneity of slopes was not observed, with the interaction term of site* $\log_{10}$ δ^{15}N ($F_{(1, 111)} = 2.11$, $P = 0.15$). Therefore, biomagnification rates did not differ between Kennady Lake and Little Nahanni River, and do not explain differences in Arctic Grayling THg between the study systems.
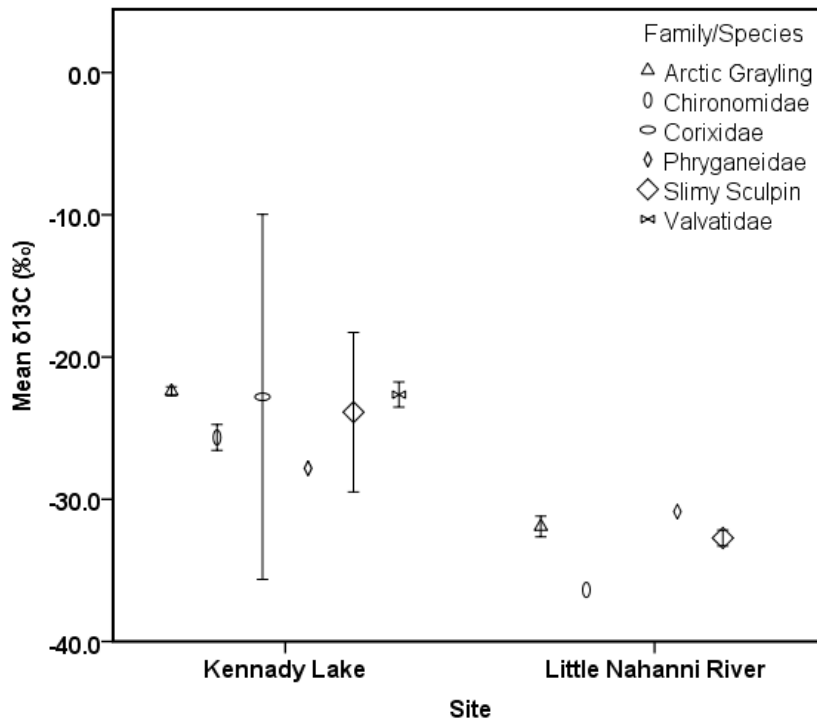
Figure 3.15. Mean δ^{13}C (‰) in fish and benthic invertebrates collected from Kennady Lake, NWT in 2014 and Little Nahanni River, NWT in 2015 and 2016. Where applicable, error bars represent 95% confidence intervals.

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Stable isotope $\delta^{13}C$ can help discern between different prey items that may be consumed. Figure 3.16 shows $\delta^{13}C$ ratios of the fish and invertebrate taxa that were captured in both study systems, as well as additional invertebrate taxa. In Kennady Lake, Arctic Grayling had similar $\delta^{13}C$ ratios to Corixidae and Valvatidae, whereas in Little Nahanni River, Arctic Grayling had similar $\delta^{13}C$ ratios to Phyrganeidae. This suggests that prey item selection may differ between Kennady Lake and Little Nahanni River. If concentrations of mercury in prey items differ between sites, this may explain the difference in mercury concentration in Arctic Grayling between the two sites.
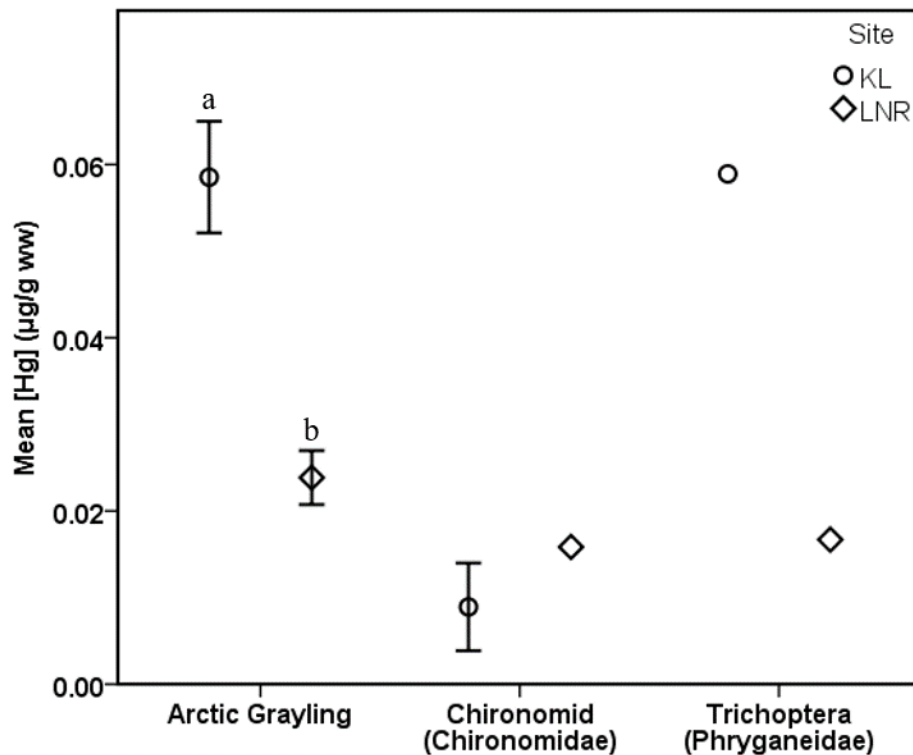
Figure 3.16. Total mercury (THg) concentration (µg/g ww) in Arctic Grayling and methyl-mercury (MeHg) concentration (µg/g ww) in benthic invertebrate families, Chironomidae and Phryganeidae (the only two benthic invertebrate taxa found in both systems) in Kennady Lake (KL) and Little Nahanni River (LNR). Where applicable, error bars represent 95% confidence intervals and letters indicate significant pairwise comparisons.

Two families of invertebrates were captured at both Kennady Lake and Little Nahanni River: Phryganeidae and Chironomidae. Phryganeidae and Chironomidae MeHg concentration values were similar to those found in Arctic Grayling in Little Nahanni River (Figure 3.16. Phryganeidae had higher mercury concentration in Kennady Lake than at Little Nahanni River. Due to having only one sample for most invertebrates, significance testing could not be completed. The results indicate that higher THg in Arctic Grayling at Kennady Lake may reflect differences in MeHg concentrations in some preferred prey items.

Table 3.4. Total dissolved mercury in water samples collected from Kennady Lake, NWT (1995 to 2010) and Little Nahanni River (2016).

Site	Total Dissolved Mercury (ng/L)
Kennady Lake	5.00
Little Nahanni River	0.39

To further evaluate baseline mercury in each study ecosystem, dissolved mercury in water was compared. Samples from Kennady Lake showed considerably higher (1.28 orders of magnitude) dissolved mercury in Kennady Lake than in the Little Nahanni River. Therefore, it appears that there is more mercury available for methylation and uptake by Arctic Grayling in Kennady Lake compared to the Little Nahanni River. Unfortunately, data on MeHg concentrations in water are not available from Kennady Lake, so MeHg concentrations could not be compared between sites.

3.4 Discussion

Direct comparisons of mercury bioaccumulation between adfluvial and fluvial populations of Arctic Grayling from barrenland tundra and mountain ecosystems have not been made in the literature. Each life history type has unique habitat characteristics and trophic interactions that may affect mercury bioaccumulation. Consistent with my hypothesis, mean THg concentrations in Arctic Grayling muscle tissue were significantly higher in the Kennady Lake, adfluvial population (0.06 ± 0.02 μg/g ww) than in the Little Nahanni River, fluvial population (0.02 ± 0.01 μg/g ww). Although mean size of Arctic Grayling was larger in Kennady Lake (adfluvial population), size-corrected THg values were still significantly higher in Kennady lake fish. Differences in baseline MeHg and preferred prey items appear to affect the difference in THg in Arctic Grayling between ecosystems, although further research is necessary. When compared to other contaminant literature, THg concentrations in Arctic Grayling from Kennady Lake (mean = 0.06 ± 0.02 μg/g) and Little Nahanni River (mean = 0.02 ± 0.01 μg/g) are lower than in previously studied adfluvial populations of Arctic Grayling in Alaska (range: 0.078 to 0.264μg/g; Jewett et al., 2003). Given that differences in fish size did not explain differences in THg, other covariates were investigated to help explain variation in THg concentration between sites.

Growth rate of fish can be examined by an age-at-size metric, which has been used to explain differences in THg concentration in previous studies (e.g., Harris & Bodaly, 1998). Fish with increased growth rates exhibit lower THg concentrations, which can be attributed to greater metabolic efficiencies (Kidd et al., 1999; Karimi at al., 2007). In my study, Arctic Grayling from Kennady Lake were larger at a given age (indicating faster growth), however; they also exhibited higher THg concentration than Arctic Grayling from the Little Nahanni River. This result is inconsistent with previous literature (see Morel et al., 1998; Kidd et al., 1999; Evans et al., 2005;

Karimi at al., 2007), and indicates that differences in growth rate do not explain why THg concentrations in Arctic Grayling are higher in Kennady Lake than in Little Nahanni River.

Stable nitrogen isotope ratios (δ^{15}N) indicate trophic position, and when related to THg concentration, δ^{15}N can help illustrate biomagnification of THg in a food web (Kidd et al., 1995). THg was positively related to δ^{15}N$_{adj}$ in Kennady Lake but the relationship was less pronounced in Little Nahanni River Arctic Grayling. Arctic Grayling from Kennady Lake showed higher THg at similar δ^{15}N$_{adj}$ values to fish from Little Nahanni River. Thus, differences in THg concentrations as a function of higher trophic position is not observed like in previous literature (Evans et al., 2005; Chasar et al., 2009). To assess if ontogenetic shifts in diet may alter the interpretation of trophic position, the relationship between δ^{15}N and fork length was assessed. A significant and positive relationship between δ^{15}N$_{adj}$ and fork length was observed in Arctic Grayling from Kennady Lake, while fish from Little Nahanni River showed no discernable trend. This suggests that larger Arctic Grayling from Kennady Lake may shift their diet to alternative prey items, altering their trophic position in comparison to younger, smaller fish. Regardless, at a standardized size (260 mm), δ^{15}N$_{adj}$ was higher in Arctic Grayling from Little Nahanni River than in Kennady Lake, which is contradictory in explaining the increased THg observed in Kennady Lake Arctic Grayling.

Biomagnification is the transfer and retention of THg from one organism to another through consumption. Because THg biomagnifies in food webs, there is often a positive relationship between THg and δ^{15}N (Morel et al., 1998; Hoffman et al., 2002). THg biomagnification was assessed using an ANCOVA and slope coefficients of regressions between THg and δ^{15}N in each system. Slope coefficients were comparable between sites, which represents similar biomagnification rates of THg in Arctic Grayling from Kennady Lake and Little Nahanni River.

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This indicates that Arctic Grayling from Kennady Lake and Little Nahanni River do not differ in their sequestration of methyl mercury, and that differences in mercury must be explained by differences in mercury at more basal levels of the food web.

Qualitative comparisons of isotopic biplots that included potential prey organisms revealed two distinct groupings of similarly feeding organisms in each respective ecosystem. A relatively simple food web was identified in which all fish species feed at least one trophic level higher ($>$ 3.4‰) than benthic invertebrates (Vander Zanden & Rasmussen, 2001; Post, 2002). In comparing $\delta^{13}C$ values and THg between ecosystems, Arctic Grayling showed higher THg concentrations when compared at similar $\delta^{13}C_{adj}$ to Little Nahanni River. A negative relationship between THg concentration and $\delta^{13}C_{adj}$ was observed in Kennady Lake, suggesting higher THg concentrations are found in less enriched $\delta^{13}C_{adj}$ food sources. However, there was not a significant relationship between THg concentration and $\delta^{13}C_{adj}$ in the Little Nahanni River. After standardizing for length, $\delta^{13}C_{adj}$ was found to be similar between Kennady Lake and Little Nahanni River, which does not explain the differences in mercury concentrations between the systems.

Prey species, including prey fish and benthic invertebrates, vary largely in their MeHg concentration due to trophic interactions and basal MeHg that is biologically available for uptake (Riva-Murray et al., 2013). Slimy Sculpin, Phryganeidae and Chironomidae were in-common prey species in Kennady Lake and the Little Nahanni River. Similar $\delta^{15}N$ and THg values between Arctic Grayling and Slimy Sculpin helped elucidate that the two fish species are feeding at a similar tropic level, and above invertebrates. Arctic Grayling are predominantly surface-feeding organisms; in Alaskan lakes, they have been reported to prey exclusively on surface insects (Allen-Gil et al., 1997). Qualitative comparisons of mean $\delta^{13}C$ for fish and benthic

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invertebrates revealed that Arctic Grayling may feed on different carbon sources than the two in-common species investigated in this study. Arctic Grayling from Kennady Lake had similar carbon signatures to Corixidae and Valvatidae, and Corixidae had higher MeHg concentrations in comparison to other invertebrates (Phryganeidae and Chironomidae) from Kennady Lake; feeding on these invertebrates, which were not in-common between systems, may help to explain higher THg concentration in Kennady Lake Arctic Grayling. In a comparison between invertebrate species captured in both systems, there is evidence that Phryganeidae have higher MeHg concentrations in Kennady Lake when compared to Little Nahanni River. In contrast, MeHg concentrations in Chironomidae were similar between the two systems. It is possible that preferred prey items have higher MeHg concentrations in Kennady Lake than in Little Nahanni River. Although lack of stomach content analysis and limited analysis of MeHg in invertebrates precludes strong inference, but overall it appears that benthic invertebrates have higher MeHg in Kennady Lake than in the Little Nahanni River.

Methyl-mercury concentrations in benthic invertebrates in the Little Nahanni River were lower than in similar taxa in Kennady Lake. Riva-Murray et al. (2013) found that MeHg bioaccumulation in stream invertebrates can vary significantly dependant on their functional feeding group (e.g., shredders vs grazers) and habitats at relatively small spatial scales. Thus, determining the preferred prey items for Arctic Grayling in the Little Nahanni River would help explain THg concentrations found in these fish. Carbon signatures of Arctic Grayling in the Little Nahanni River suggest they prefer Phryganeidae as a prey item when compared to Chironomidae. Lower THg in Arctic Grayling in the Little Nahanni River may also be influenced by terrestrial prey item selection by Arctic Grayling. Research by Jardine et al. (2015) found that Arctic Grayling that rely more heavily on terrestrial-based carbon sources are subject

to lower MeHg exposure. Sufficient riparian vegetation and the fluvial nature of mountain streams can deliver terrestrial invertebrates into the trophic food web in Little Nahanni River. A sub-species of Arctic Grayling, the Baikal Grayling (*Thymallus arcticus baicalensis*), show a preference for surface prey items, including terrestrial invertebrates (Olson et al., 2016). Resource partitioning behavior, like that of the Baikal Grayling, can greatly reduce MeHg bioaccumulation in oligotrophic streams (Tsui et al., 2014). Although terrestrial invertebrates were not investigated in this study, similar trends in surface feeding may help explain reduced MeHg concentrations in Arctic Grayling from Little Nahanni River. Future research that better quantifies differences in available prey invertebrates between ecosystems, in particular, invertebrates in the drift and at the surface of the stream or lake should be conducted. Evaluating the differences in MeHg concentration available for biomagnification from aquatic versus terrestrial inputs in these systems may also help explain the observed differences in mercury concentrations between adfluvial and fluvial Arctic Grayling.

Mercury concentrations in fish are related to habitat use (e.g., Power et al. 2002), which could differ significantly across stream and lake habitats used by Arctic Grayling populations for foraging. The relationships between $\delta^{15}N$ and MeHg concentration as well as the carbon signatures of in-common prey items suggest that differences in THg concentrations in Arctic Grayling are a function of differences in baseline MeHg concentrations between Kennady Lake and Little Nahanni River. Water analysis results support that Kennady Lake (5.0ng/L) has dissolved mercury concentrations approximately ten times higher than Little Nahanni River (0.39 ng/L). Higher dissolved mercury can provide sulphur- and iron-reducing bacteria a greater opportunity to methylate mercury, making it biologically available for bioaccumulation (Morel 1998). Basal levels of dissolved and MeHg can be affected by a number of environmental factors

based on physical habitat characteristics and location of the waterbody (Stoken et al., 2016).

Mercury concentrations can respond to variability in ecosystem characteristics, such as water

temperature, redox conditions, water chemistry, composition of the prey community, presence

and distribution of methylating environments, lotic vs lentic habitat use, pelagic vs littoral habitat

use, water residence times, and catchment sizes (see AMAP 2011). Ecosystem characteristics

may have a pronounced effect on THg availability for Arctic Grayling, as adfluvial (lake-

feeding) and fluvial (stream-feeding) populations of Arctic Grayling occupy different foraging

habitats. On average, water temperature in Kennady Lake (~15°C) is higher than Little Nahanni

River (~8°C). Higher water temperatures can also promote methylation and result in higher

MeHg (Evans et al., 2005; St. Louis et al., 2005; Lehnherr et al., 2012), this would be especially

prominent in shallow pockets of water similar to Kennady Lake. Similarly, increased primary

production, as promoted by increases in temperature, can contribute to increased mercury

methylation (Prowse et al., 2006). Unpublished data from Kennady Lake and Little Nahanni

River suggest that primary production is greater in Kennady Lake, as nitrogen, phosphorus, and

chlorophyll a concentrations are higher in that system. Thus, Kennady Lake may be a more

methylating environment than Little Nahanni River. Further investigation into baseline MeHg

availability and the differences in microbial communities present at each site is necessary.

The interactions between Arctic Grayling and their environment, as well as differences in

life history and geographic region can help explain differences in THg concentrations in Arctic

Grayling between the ecosystems. Ecosystem characteristics, climate and geographic location

play a significant role in determining baseline MeHg bioaccumulation rates in a given trophic

food web (Scheuhammer & Graham 1999; see Chétalet et al., 2015). Seasonal climate and

topographic characteristics, like snow-pack and ice melt influence aquatic transport of mercury

across the landscape (Douglas, et al., 2012). Sub-Arctic mountain (Little Nahanni River) and barrenland tundra (Kennady Lake) ecosystems differ largely in their topographic characteristics. The high elevation Selwyn mountain range (Little Nahanni River) has a short snow-free period (July to September), with frequent summer precipitation events that result in flashy stream discharge events. In contrast, the catchment for Kennady Lake is less sloped, and is less flashy in times of snow melt and precipitation. These differences may play an important role in mercury transport and methylation and deserve further investigation.

3.4.1 Research implications

The results show that THg concentration in Arctic Grayling are lower than World Health Organization commercial guidelines for THg (< 0.05μg/kg; Lockhart et al., 2005). Harvesting Arctic Grayling would expose northern residents to lower THg concentrations when compared to larger predatory fish that are often targeted through subsistence activities (e.g., Lake Trout; Jewett et al., 2003).

Better understanding mercury sequestration between mountain and barrenland tundra ecosystems is useful for northern residents and scientists to predict areas that are most vulnerable to climate- or development-induced increases in mercury. This study was the first to evaluate mercury concentrations differences between mountain and barrenland streams using Arctic Grayling. Future research should further evaluate the mechanism behind differences in MeHg in invertebrates between mountain streams and barrenland lakes.

The impetus for this research was partly to gain baseline data on mercury and other contaminants in ecosystems from Kennady Lake and Little Nahanni River prior to major development from mining operations. The contaminant burden from diamond (Kennady Lake)

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and zinc/silver mining (Little Nahanni River) are lower than other smelt mining methods, but baseline data will help industry contribute to informed monitoring protocols. This is particularly relevant to adjacent mining projects like Gahcho Kue, De Beers Canada and Selwyn-Chihong, for rehabilitation of their respective mine sites.

3.4.2 Conclusion

Direct comparisons between Arctic Grayling in two contrasting ecosystems provides evidence that mercury bioaccumulation differs with baseline ecosystem processes that affect the amount of baseline MeHg biologically available for uptake by Arctic Grayling. THg concentrations are higher in barrenland tundra, adfluvial populations of Arctic Grayling from Kennady Lake, even with length adjustment. Relationships between THg concentrations in Arctic Grayling and several covariates ($\delta^{15}N_{adj}$, $\delta^{13}C_{adj}$, age and length-at-age) showed that Arctic Grayling THg burdens were most easily explained by basal MeHg in each respective ecosystem. Higher MeHg concentration in benthic invertebrates, specifically Phryganeidae, may help explain higher THg levels in Arctic Grayling from Kennady Lake. Fluvial populations of Arctic Grayling may also incorporate terrestrial invertebrates as prey, which carry lower mercury burden that aquatic invertebrates and may further exacerbate the differences in mercury concentrations between Kennady Lake and Little Nahanni River. Ultimately, Arctic Grayling fall within safe human consumption guidelines regardless of habitat type, although habitats that do not facilitate increased methylation, such as lotic stream environments, appear to produce Arctic Grayling with lower mercury concentrations.

Chapter 4

General conclusion

4.1 Research synopsis

The main objective of this research was to contribute to our current knowledge and understanding of Arctic Grayling habitat use through occupancy modelling and investigate how differences in habitat use may affect mercury bioaccumulation in this species. Overall, knowledge gaps exist regarding Arctic Grayling ecology, partly due to their complex life history and also due to the harsh, remote climates they inhabit across their range.

Results from chapter two of my thesis helped to quantify YOY Arctic Grayling habitat characteristics in fluvial mountain populations using occupancy analysis, which, previously, were described qualitatively or by very few studies (Scott & Crossman 1973; de Bruyn & McCart, 1974; Liknes and Gould, 1987; McClure & Gould 1991). Occupancy models identified water temperature and elevation as important habitat predictors for YOY Arctic Grayling in mountain streams. YOY Arctic Grayling preferred water temperatures greater than 8°C, suggesting that in early development, Arctic Grayling seek warm stream refugia, in an otherwise cold-water ecosystem. The role of groundwater and other warm-water sources (springs) in facilitating YOY Arctic Grayling growth is poorly understood and should be further investigated. Elevation was also identified as an important habitat predictor variable for YOY Arctic Grayling in mountain ecosystems. YOY Arctic Grayling preferred low elevation streams (< 1150masl). Elevation is a particularly useful landscape predictor variable for resource managers, as data can be gathered remotely using digital elevation models and can be used in conjunction with local habitat characteristics to map critical spawning and rearing areas.

Analyses in chapter 3 were aimed at investigating how mercury concentration varies between Arctic Grayling from Kennady Lake and Little Nahanni River; these sites represent a barrenland tundra, adfluvial and mountain, fluvial population, respectively. Occupancy analysis has identified critical YOY rearing habitat in Kennady Lake (Baker et al., 2017) and in Little Nahanni River (Chapter 2). Results from those studies perpetuated further investigations into how Arctic Grayling ecology differs between two populations of the same species. There is a stark contrast between mountain and barrenland, tundra ecosystems, which appears to have had an effect on mercury bioaccumulation in each respective system. After size standardization, Arctic Grayling from Kennady Lake had significantly higher mercury concentrations than adult Arctic Grayling from Little Nahanni River. A variety of covariates, including stable isotopes (δ^{15}N and δ^{13}C) and fish characteristics (age, weight etc.) were analyzed, most of which were not significantly related to mercury, and did not explain differences in concentration between sites. Higher mercury in Arctic Grayling from Kennady Lake may be explained by higher MeHg concentrations at the base of the food chain; I found that invertebrates from Kennady Lake had significantly higher MeHg concentrations than invertebrates from the Little Nahanni River. Higher MeHg at the base of the food web suggests higher MeHg production in Kennady Lake. Mercury was not related to δ^{15}N in Little Nahanni River, suggesting that as Arctic Grayling move up in trophic position, mercury bioaccumulation rates slow, or remain the same. This suggests that bioaccumulation in Little Nahanni River does not biomagnify up the food web as is typically found in other fish species (Kidd et al., 1995). Other explanations suggest that terrestrial prey items may play a significant dietary role in fluvial populations, as lower MeHg concentrations can be associated with terrestrial invertebrates when compared to aquatic organisms. Other variables, not quantified in my analysis, may also drive differences in mercury

concentration between systems. Mercury accumulation is habitat-specific and there are many variables contributing to the deposition, methylation and bioaccumulation of mercury (Evans et al., 2005). The study of contaminants in each respective watershed is a contribution to baseline data and may be especially useful in light of ongoing mining developments in the region.

4.2 Advancing our understanding

Arctic Grayling are a complex organism that utilize a variety habitat types depending on geographic location and life history type. Yet the species is still restricted to mostly northern regions with cold-water environments exhibiting low turbidity and minimal anthropogenic impacts. Arctic Grayling show incredible plasticity that have adapted their life history to a variety of lotic and lentic environments. In comparing YOY habitat from mountain streams to barrenland tundra streams, there are considerable differences in preferred habitat, which are a function of the landscape. Elevation and stream temperatures, which were the strongest predictors of YOY Arctic Grayling occupancy in this study, would be poor predictors of YOY habitat use in barrenland systems. Baker et al., 2017 found, that stream velocity and depth are the most important variables for predicting occupancy of barrenland YOY Arctic Grayling. In the literature, life history type is often only briefly mentioned in describing a given Arctic grayling population. This thesis, in combination with Baker et al., 2017, illustrates complex interactions between habitat use, migration patterns and life history, complicates the role of resource managers in assessing the vulnerability of populations of Arctic Grayling.

4.3 Management considerations

To better manage Arctic Grayling and their habitats, it is critical for resource managers to understand the complexity of Arctic Grayling habitat use across its range. As cold-water salmonids, Arctic Grayling inhabit harsh environments that often depend on unique habitat features, like the presence of warm water natal streams, to sustain populations of Arctic Grayling. Disturbance to critical habitat can result in the loss of entire, remote populations. Information from this thesis and Baker et al., 2017 should be used for broad scale predictions of critical habitats by resource managers, as mining and gas developments continue to grow in northern Canada.

At present, both populations studied in this thesis are vulnerable due to mining development. Kennady Lake has been drained for diamond extraction and road construction in the Little Nahanni River has opened the region up for ore hauling and expansion of mining projects in the future. Critical habitat information developed by this thesis (Chapter 1) should be considered the most quantitative representation of Arctic Grayling spawning and rearing habitat that is represented in in-land mountain populations. Anecdotal evidence suggests that these critical spawning and rearing grounds are highly localized, with specific habitat characteristics that are sparse in mountain ecosystems. These areas need maximum protection from impacts of resource development in order to sustain mountain populations of Arctic Grayling.

4.4 Future study

Chapter 2 provides quantitative results that can be used to predict YOY Arctic Grayling habitat use in mountain streams but has raised questions as to why Arctic Grayling make these habitat choices. In particular, warmer stream temperatures, as preferred by YOY Arctic Grayling,

raise the question of how ground water or warm water springs affect habitat selection by Arctic Grayling. It is common in other salmonids, like Arctic Char, to use groundwater as overwintering refugia (Dunmall et al., 2016), but it is unclear in the literature how groundwater may be used by Arctic Grayling. To assist in answering this question, a baseline dataset of groundwater inputs and thermal conditions (e.g., via thermal imaging) could be helpful.

The predictor variables identified in chapter 1 should be applied to a similar ecosystem with known populations of Arctic Grayling to test model accuracy. Several collaborators, including Parks Canada and Fisheries and Oceans Canada, are planning to implement monitoring programs for Arctic Grayling populations, in which this model could be applied and tested.

Differences in mercury bioaccumulation between mountain and barrenland populations of Arctic Grayling require more explanation through further research. Differences are likely a result of differences in methylation at lower trophic levels that was not assessed through this research. There are several studies that assess mercury bioaccumulation and methylation rates in lentic environments, but very few studies quantify the role of i) methylation processes in streams and ii) the role of terrestrial mercury inputs into mountain stream ecosystems. Both are areas of study that need to be improved, which may help resolve the differences in mercury among ecosystems observed in Chapter 2.

4.5 Final Remarks

The resulting occupancy model (Chapter 2) contributes to our knowledge about Arctic Salmonids, and their habitat use, which was the impetus for this research. Fisheries and Oceans Canada is tasked with monitoring northern salmonid populations, as they may be the most at-risk family of fishes from development and climate-driven changes in the north. Substantial questions

still remain about Arctic Grayling habitat preferences (e.g., ground water) and mechanisms of contaminant bioaccumulation in the species. In collaboration with partner agencies, the methods and results from this thesis can be used in better answering some of these questions across the expansive range of Arctic Grayling.

5.0 References

5.1 Chapter 1 References

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Appendix A.

Table A.1. Occupancy and detection efficiency covariates removed from analysis based on a Pearson correlation coefficient (r) greater than 0.70 or biological relevance.

Parameter type	Correlation Rank	Variables Removed	Correlated variable
occupancy	highly correlated (r > 0.70)	calendar day	photoperiod (r= -0.99)
		discharge	width (r= 0.77), velocity (r= 0.72)
		cobble	water temperature (r= -0.719)
		overhanging riparian vegetation	coarse woody debris (r= 0.828)
		% run	fine sediment (r= 0.738)
		% cascade	boulder sediment (r= 0.787)
		YSI water temp	thermometer water temp (r= 0.784)
	not relevant	photoperiod	
		wetted width	
		pool	
		eddies	
		pH	
		dissolved oxygen	
		DO	
detection efficiency	highly correlated (r > 0.70)	cascade	boulder (r= 0.70)
		effort per sq. m	effort (r= 0.724)
	not relevant	wetted width	
		cobble	
		overhanging veg.	
		coarse woody debris	
		undercut banks	
		netter	
		efisher settings	
		visibility obstructions	

Table A.2. Detection efficiency variables fitted to global model to assess effects of lower detection efficiency during field surveys.

Detection Efficiency	Variable
	% boulder
	% riffle
	% aquatic vegetation
	previous knowledge
	% cloud cover
	electrofisher
	effort
	velocity
	depth
	% eddies

Table A.3. Site matrix used in occupancy analysis. Site data for replicates 6, 7, 8 and 9 replicates was lacking, and therefore did not perform as well in occupancy analyses, resulting in 5 replicates used for final model analysis.

Patch	Replicate								
	1	2	3	4	5	6	7	8	9
10	1673	1833	1769	1721	463	363	100	-	-
23	1652	1732	1700	1640	-	-	-	-	-
48	2000	1346	1166	1102	365	100	1038	-	-
85	956	1320	1612	1376	1148	1332	-	-	-
87	1149	1085	1289	1037	1389	-	-	-	-
95	972	1276	1588	1132	1288	-	-	-	-
97	1116	1268	1244	1020	1084	-	-	-	-
128	P128-New5	768	128	1064	1560	-	-	-	-
69a	95	398	159	-	-	-	-	-	-
15a	1668	1764	2600	1716	1684	1844	1780	-	-
15b	1768	1704	1656	1752	1800	1736	-	-	-
17a	1724	1681	1793	1729	1857	1637	MarchNew	300	-
17b	1852	2000	1692	1740	1788	300	200	-	-
20a	1746	1645	1865	-	-	-	-	-	-
20b	1698	1762	1666	1826	-	-	-	-	-
20c	1634	1869	1650	1810	-	-	-	-	-
21a	1900	1700	1300	400	200	-	-	-	-
21b	3300	3000	500	400	300	200	-	-	-
21c	5200	5000	4900	4600	4500	4300	4200	3870	3600
24a	2200	2100	1600	1500	1100	500	400	-	-
24b	7000	5400	5100	4500	3900	3500	3100	-	-
65a	1041	1089	1025	-	-	-	-	-	-
65b	854	251	481	-	-	-	-	-	-
65c	187	425	1305	977	1213	-	-	-	-
65d	933	1349	1169	460	1481	86	-	-	-
65e	760	353	107	310	751	-	-	-	-
67a	1137	1300	600	400	1549	67a-New	-	-	-
67b	327	630	500	583	171	100	-	-	-
67c	2350	1850	235	467	-	-	-	-	-
67d	2600	697	100	79	-	-	-	-	-
67e	713	600	1201	1325	-	-	-	-	-
67f	15	997	1189	1617	-	-	-	-	-
67g	535	637	1181	1061	1557	-	-	-	-
67i	692	850	-	-	-	-	-	-	-
69b	1405	945	1609	1313	1445	1121	-	-	-

Table A.4. Occupancy matrix used in analysis representing presence (1) or absence (0) of YOY Arctic Grayling during surveys of the Little Nahanni River watershed in 2015.

Site	Replicate								
	1	2	3	4	5	6	7	8	9
10	1	1	0	0	0	1	1	-	-
23	0	0	0	0	-	-	-	-	-
48	0	0	0	0	0	0	0	-	-
85	0	0	0	0	0	0	-	-	-
87	0	0	0	0	0	-	-	-	-
95	0	0	0	0	0	-	-	-	-
97	0	0	0	0	0	-	-	-	-
128	1	1	1	1	1	-	-	-	-
69a	0	0	0	-	-	-	-	-	-
15a	0	0	0	0	0	0	0	-	-
15b	0	0	0	0	0	0	-	-	-
17a	0	0	0	0	0	0	0	0	-
17b	0	0	0	0	0	0	0	-	-
20a	0	0	0	-	-	-	-	-	-
20b	0	0	0	0	-	-	-	-	-
20c	0	0	0	0	-	-	-	-	-
21a	0	0	0	0	0	-	-	-	-
21b	0	0	0	0	0	0	-	-	-
21c	0	0	0	0	0	0	0	0	0
24a	0	0	0	0	0	0	0	-	-
24b	0	0	0	0	0	0	0	-	-
65a	0	0	1	-	-	-	-	-	-
65b	0	0	0	-	-	-	-	-	-
65c	0	0	0	0	0	-	-	-	-
65d	0	0	0	0	0	0	-	-	-
65e	0	0	0	0	0	-	-	-	-
67a	1	1	1	0	1	1	-	-	-
67b	0	0	0	0	0	1	-	-	-
67c	0	0	0	0	-	-	-	-	-
67d	0	0	0	0	-	-	-	-	-
67e	1	1	1	1	-	-	-	-	-
67f	0	0	0	0	-	-	-	-	-
67g	0	0	0	0	0	-	-	-	-
67i	1	1	-	-	-	-	-	-	-
69b	0	0	0	0	0	0	-	-	-

Section A.5. Habitat covariate collection details

A number of different methods were employed in the collection of habitat data. Each site was classified as run, riffle, pool or cascade (Rosgen, 1994). Photoperiod was recorded as the number of daylight minutes in a given day of sampling and was calculated using the difference between sunrise and sunset times according to data available from Environment Canada. Air temperature was recorded in degrees Celsius at each site using a general liquid-filled glass thermometer. Water temperature was measured using both a standard liquid-filled glass thermometer as well as a YSI sonde unit. Wetted width (cm) was a point measurement; the distance from one wetted edge of the stream to the other (perpendicular to the flow) was measured using a 30m tape measure. Water depth (cm) was measured in 20% increments across the wetted width of the stream (taken at 20, 40, 60 and 80% intervals; four total depth measurements) at each site, and averaged (arithmetic mean). Average velocity (m/s) was calculated at each site (detection efficiency) using a modified head-rod method using a standard meter stick. The head-rod calculation uses the difference between actual depth of the stream (reading when the narrow-width of the meter stick is placed parallel to flow) and the head-rod depth (reading when the broad-side of the meter stick is placed parallel to flow) (Fonstad et al., 2005). Velocity was also recorded at the patch scale (occupancy) as a component of the discharge measurement. Velocity (m/s) and discharge (m^3/s) were measured using a Hach FH950 Portable velocity meter mounted on a wading rod. Ten velocity measurements were equally spaced across the wetted width of the stream, at 20% and 60% depth to capture surface and sub-surface variation in flow. Slope was determined at each patch from the elevational change between the end and beginning of each site surveyed using Google Earth Image Landsat / Copernicus and Garmin BaseCamp software. Elevation was determined at each patch from the

elevational reading of the downstream end of each site using Google Earth Image Landsat / Copernicus and Garmin BaseCamp software. Strahler stream order, a stream ranking system based on tributary inflows, was determined using the Natural Resources Canada river and streams layer in ArcGIS. Percent cover of substrate types was estimated using modified Wentworth substrate size classifications: sand/silt (<0.2cm), gravel (0.2 – 6.4cm), cobble (6.4 – 25.6cm), boulder (>25.6cm) and bedrock (Wisniewski 2013). Overhanging vegetation was estimated as percent cover from an overhead view at each site. Aquatic vegetation was evaluated as percent coverage at each site. Coarse woody debris, defined as downed wood that provided velocity refugia and/or in-stream cover for drift-feeding organisms (Langford et al., 2012) was a site measurement that was evaluated as percent cover. Undercut banks were considered as any receding bank line under the top soil layer, and measured as percent cover at each site. Boulder cover was a measure of in-stream cover for fish to use as velocity refuge and was assessed as percent cover where boulders provided slack water at each site. Eddies, which can provide velocity refugia and increased drift-feeding opportunities, were also assessed as percent cover. All water chemistry variables (pH, specific conductivity and dissolved oxygen) were measured using a YSI 556 multi-parameter water quality meter at each site. The YSI unit was calibrated and maintained according to company standards, prior to and during fieldwork.

Data on several variables hypothesized to affect detection efficiency only (not occupancy) were also collected; all detection efficiency variables were collected at the scale of 'site'. Visibility was a ranking of visual obstructions, which included turbulence, physical obstructions, and reflection. Ranks from 0 (no visibility obstructions) to 3 (3 visibility obstructions) were assigned. Cloud cover was measured in percent cover. Effort, measured in seconds, was recorded by the electrofisher as the amount of alternating current applied to the stream during a given

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survey. Effort area (seconds/m) was a calculated measure: effort (seconds) divided by the

amount of stream area (wetted width x 100m stream length). 'Previous knowledge' of the crew

was a categorical variable that was meant to account for surveyor bias: personnel knowledge of a

site was ranked as '0' (no previous visits to patch), '1' (one crew member had surveyed a site

within the same patch) or '2' (both crew members had previously surveyed a site within the same

patch).

Table A.6. Correlation matrix of variables assessed during occupancy analysis to predict YOY Arctic Grayling habitat use in Little Nahanni River in 2015.

	day	photoperiod	time	air temp	water temp	width	depth	velocity	discharge	boulder	cobble	gravel	fines	small sediment	run	riffle	pool	cascade	aq. Veg	rip. veg	cwd	ucb	bld. cov.	eddies	slope	elevation	order	ysi temp	ph	sp. con.	do
day	1																														
photoperiod	-0.99188	1																													
time	-0.04906	0.071405	1																												
air temp	-0.34882	0.312527	0.02594	1																											
water temp	-0.21682	0.216406	0.289153	0.173813	1																										
width	0.110954	-0.05775	-0.05249	-0.11255	-0.060186887	1																									
depth	0.111347	-0.08851	0.006651	-0.16662	0.337534399	0.394434	1																								
velocity	-0.097	0.100958	-0.42316	-0.10538	-0.471655308	0.483895	0.286842	1																							
discharge	-0.04088	0.055037	-0.23397	-0.10557	-0.207597041	0.771941	0.437779	0.721327	1																						
boulder	0.100532	-0.11348	0.222294	-0.01778	-0.17466952	0.247757	-0.06356	0.158887	0.16872	1																					
cobble	0.021761	-0.00456	-0.41532	-0.08855	-0.71916001	0.087175	-0.29259	0.470969	0.201256	-0.20562	1																				
gravel	-0.0172	0.004017	0.225223		0.306274025	-0.27951	-0.04298	-0.25832	-0.33175	-0.58933	-0.07302	1																			
fines	-0.10403	0.109093	-0.03959		0.631973633	-0.14761	0.346681	-0.24577	-0.4341	-0.65495		0.028884	1																		
small sediment	-0.09543	0.092884	0.083056		0.690049902	-0.26881	0.269245	-0.49132	-0.23971	-0.67046	-0.588	0.542991	0.855065	1																	
run	0.09673	-0.10155	0.065804	-0.0666	0.57190021	-0.20014	0.487659	-0.29879	-0.12383	-0.57048	-0.47632	0.40543	0.738344	0.831291	1																
riffle	-0.11341	0.133309	-0.32012	0.040521	-0.64054646	0.216395	-0.39532	0.264626	0.068913	0.741923	-0.22616	-0.59547	0.61847	-0.77994		1															
pool	0.050239	-0.07227	0.243325	-0.02946	0.023433688	0.045335	-0.19118	0.003837	-0.10219	0.786616	-0.29925	-0.38246	-0.26581	-0.42129	-0.14128	-0.10008	1														
cascade	0.010333	0.00451	0.201553	0.289174	0.432201004	-0.15255	0.331412	-0.30474	-0.14926	0.16309	-0.35374	-0.11927	0.549152	0.400205	0.522344	0.108298	-0.2023	1													
aq Veg	-0.13581	0.113799	0.378449	0.039034	-0.102805836	-0.60056	-0.29222	-0.36312	-0.35572	-0.05756	0.058208	0.093114	-0.05689	-0.00064	-0.02631	0.170439	0.017892	0.015803	1												
rip. veg	-0.23615	0.222887	0.45029	0.135502	0.052043228	-0.26933	0.192965	-0.40943	-0.27664	-0.02174	-0.12657	-0.03982	0.155379	0.108738	0.0556	-0.18965	0.232258	0.112754	0.828012	1											
cwd	-0.1661	0.175853	0.071312	-0.00796	0.419056513	-0.40545	-0.23464	-0.45135	-0.38181	0.357394	0.566873	0.660053	0.464998	-0.27956	-0.33643	-0.18955	0.22262	0.130077	0.137501	0.127011	1										
ucb	0.17855	-0.14402	0.250504	-0.00933	0.465605	0.044685	0.067334	0.162933	0.560592	0.035732	-0.031001	-0.39101	-0.48938	-0.39649	-0.2795	0.247161	0.233497	-0.00692	-0.23307	-0.26636	0.313815	1									
bld. cov.	0.093056	-0.00838	0.115069		0.253216853	0.239493	0.308005	0.223984	0.291889	0.265422	-0.12563	-0.25209	-0.14807	0.022021	-0.15106	0.050184	0.171193	0.06274	-0.2009	-0.34416		0.412012	1								
eddies	-0.00475	-0.03421	-0.16467	0.073507	-0.188188814	-0.37842	-0.6051	-0.19753	-0.48873	0.038973	0.175706	0.012071	-0.19683	-0.2673	0.191087	-0.14363	0.17035	-0.13019	-0.03596	-0.20473	-0.29467	0.052863	-0.02947	1							
slope	-0.1445	0.178546	-0.1587	-0.16637	0.540082	0.581686	0.599833	0.652914	-0.01427	0.176338	0.021619	-0.16344	-0.1261	0.017958	0.083571	-0.04587	-0.13452	0.1134	-0.25566	-0.25418	-0.02782	0.041016	0.370515	-0.5257	1						
elevation	0.041905	-0.06947	0.247143	0.157937	0.398486825	-0.39525	-0.16808	-0.303	-0.46045	0.434482	0.171753	-0.22442	-0.48836	0.249559	0.228546	0.418137	-0.13137	0.243309	-0.19787	0.436853	0.346968	-0.32474	0.268366	-0.16568	-0.60037	1					
order	-0.17745	0.194675	0.382054	0.135473	0.784256394	-0.13257	0.384944	-0.21884	-0.38015	0.011326	0.021705	-0.63135	0.690842	0.618009	0.656006	0.327742	-0.06536	0.418137	-0.13452	0.243309	0.342331	0.286784	0.285958	-0.08016	0.155684	0.308083	1				
ysi temp	-0.21192	0.069287	0.155694	0.001914	0.119009954	-0.34894	-0.50006	-0.24888	-0.38015	0.146317	0.072917	-0.26388	-0.22467	0.088278	0.001972	0.015836	-0.23405	-0.01857	0.06555	-0.37004	0.324337	-0.12505	-0.02257	0.164368	-0.33691	-0.07868	-0.21312	1			
ph	-0.01574	0.032103	-0.1484	-0.10534	-0.08394713	0.138555	-0.00473	0.185598	0.268663	0.404849	0.271686	0.072706	0.135209	-0.26388	-0.2467	0.079534	-0.13019	0.243309	-0.03527	0.343231	0.286784	0.079534	-0.08016	0.06555	0.159684	-0.03926	-0.12505	-0.02257	1		
sp. con.	0.032103			-0.1484		0.001914	-0.04073	0.138555	0.185598	0.021705	0.268663	-0.07634	-0.0433	0.12228	0.072706	0.191087	-0.11107	-0.26388	-0.22467	0.088278	0.001972	0.015836	-0.23405	-0.01857	0.324337	-0.12505	0.308083	-0.02257	0.164368	1	
do	0.199915	-0.21976	-0.12651	-0.24433	-0.361456571	0.301548	-0.12043	0.341413	0.290293	0.226469	0.270333	-0.04383	-0.44056	0.292441	-0.28218	-0.0138	0.014697	-0.43911	-0.3865	-0.27947	0.322556	0.033416	0.144491	0.171232	-0.00107	-0.50761	-0.02258	-0.07743			1