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ARCTIC STREAM CHARACTERISTICS AND DIET ANALYSIS CHOICE IMPACT CONDITION AND DIET ESTIMATES OF NINESPINE STICKLEBACK (*Pungitius pungitius)*

by

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B.Sc. (Env.), University of Guelph, 2018

THESIS

Submitted to the Department of Biology

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in partial fulfilment of the requirements for the

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ABSTRACT

Ninespine stickleback (*Pungitius pungitius*) are ubiquitous in circumpolar freshwaters, but their ecological role is not well understood. Little research has been conducted on the influence of environmental variables on ninespine stickleback in stream environments, and while they are understood to be generalist feeders, their diet in stream environments is equally understudied. Determining diet is difficult due to biases inherent in all standard diet analysis methods. Morphological gut-content analysis (M-GCA), DNA metabarcoding of gut contents (D-GCA), and stable isotope analysis (SIA) are currently three of the most frequently conducted diet analyses; and while combinations of these methods are commonly used to counteract their biases, limited analyses have compared all three. The aims of this thesis were to address these knowledge gaps by determining the impacts of tundra stream characteristics on ninespine stickleback condition and abundance, characterizing their diet in these streams, and assessing the relative benefits and disadvantages of the above-mentioned diet analysis techniques for determining the diet of small stream fishes. The impacts of environmental factors (e.g. temperature, nutrient concentrations, prey and predator/competitor abundance) on condition and abundance were present but limited, likely due to both the tolerant nature of ninespine stickleback and carry-over effects from over-wintering environments. The generalist nature of ninespine stickleback was confirmed by M-GCA and D-GCA results which described a high occurrence of abundant stream invertebrates in the gut, namely Orthocladiinae and Chironominae. In contrast, SIA estimated Arachnida and Tanypodinae to be the most significant contributors to diet over a longer period, suggesting a diet shift over the summer due to either a change in stream invertebrate

community composition, or prior feeding in a different environment. Biases of each diet analysis technique were consistent with prior reports, with M-GCA being biased towards hard-bodied organisms, and D-GCA being biased towards soft-bodied organisms. The findings of this thesis contribute to a growing understanding of ninespine stickleback ecology in tundra streams and indicate the importance of studying connections with lentic over-wintering environments in future research. Finally, this research complements other research being conducted in the Greiner Lake watershed on tundra stream food-web dynamics and stream metabolism.

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LIST OF ABBREVIATIONS

AIC_C	Akaike Information Criterion
ANOVA	analysis of variance
BOLD	Barcode of Life Data System
BLAST	basic local alignment search tool
CABIN	Canadian Aquatic Biomonitoring Network
CCDB	Canadian Centre for DNA Barcoding
CPUE	catch per unit effort
CV	coefficient of variation
D%	relative abundance
D-GCA	DNA metabarcoding of gut contents
F%	frequency of occurrence
K_{rel}	LeCren's relative condition factor
Masl	metres above sea level
M-GCA	morphological gut content analysis
NLET	National Laboratory for Environmental Testing
NMDS	non-metric multidimensional scaling
PCA	principal component analysis
PCR	polymerase chain reaction
PERMANOVA	permutational multivariate analysis of variance
QA/QC	quality assurance / quality control
SEA_B	Bayesian standard ellipse area
SFS	Society of Freshwater Science
SIA	stable isotope analysis
SIMPER	similarity percentage
TDP	total dissolved phosphorus

CHAPTER 1: INTRODUCTION

1.1 – Ninespine stickleback ecology

The ninespine stickleback (*Pungitius pungitius*; Figure 1.1) is a ubiquitous fish species found in Arctic freshwater environments (Von Hippel et al., 2016). It is phenotypically plastic with body size, growth rate and lifespan varying depending on abiotic environmental conditions (Kuparinen et al., 2011) and predator presence or absence (DeFaveri et al., 2014; Herczeg et al., 2012). This species is tolerant of extreme environmental conditions, most notably high salinity (Nelson, 1968) and hypoxia (Lewis et al., 1972). As a generalist feeder it primarily consumes benthic macroinvertebrates, zooplankton, and occasionally fish larvae and fry (Hynes, 1950; Laske et al., 2017). While ninespine stickleback are ubiquitous in Arctic freshwaters, most research has been undertaken in lentic (standing water) environments (i.e., Gallagher & Dick, 2011; Laske et al., 2017). However, Mcfarland et al. (2018) conducted a lotic (flowing water) food-web study within a lower order watershed consisting of a series of lakes and streams that included both ninespine stickleback and Arctic grayling (*Thymallus arcticus*). They found that stickleback accounted for almost 90% of Arctic grayling diet by mass, thus displaying the importance stickleback can have for larger piscivorous fish in such systems. Moreover, this unique study provided

Figure 1.1 - Adult ninespine stickleback (*Pungitius pungitius*). Mature fish are usually < 6cm.

baseline diet information, reporting that zooplankton and dipteran larvae were predominant diet items of stream-dwelling ninespine stickleback in this Arctic system (Mcfarland et al., 2018; Figure 1.2).

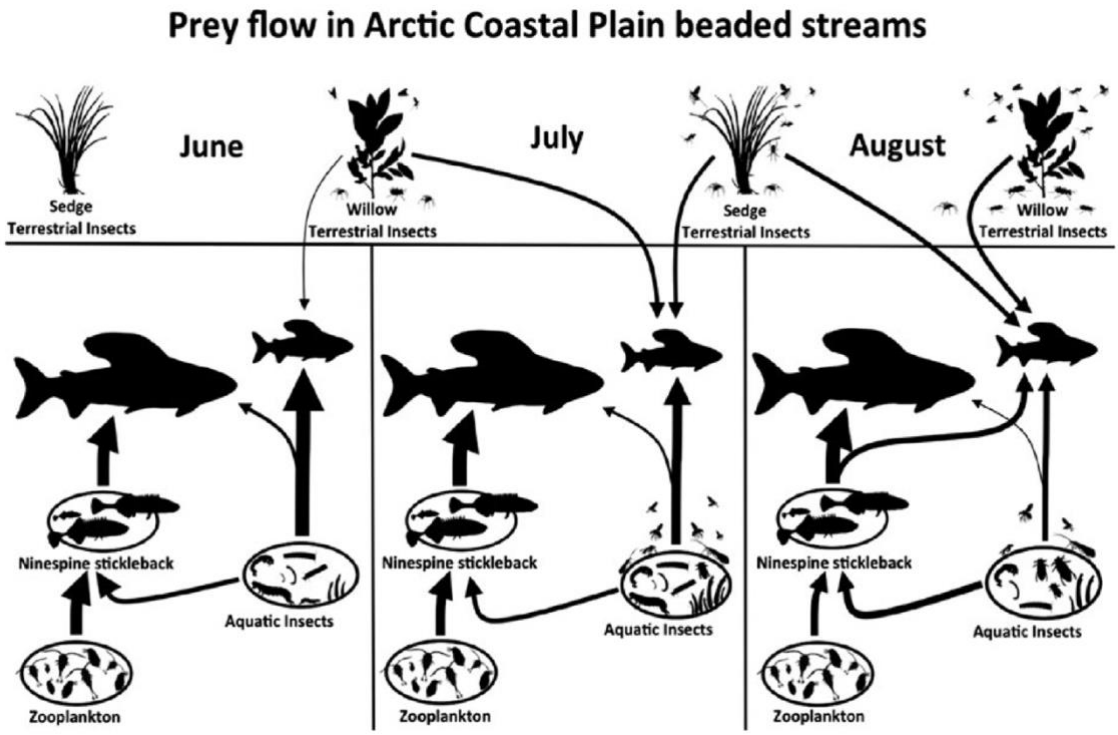

Figure 1.2 Feeding relationships between Arctic grayling, ninespine stickleback, and invertebrates from a beaded stream in the Arctic Coastal Plain in Alaska. Arrow size represents the amount of biomass flowing between groups in each feeding relationship (McFarland et al. 2018, reproduced with permission from the author).

With the Arctic changing rapidly due to climate change, it is expected that stream ecosystems will be impacted by a variety of processes including increased groundwater and sediment inputs to streams, and modified water chemistry (Bowden et al., 2008; Prowse et al., 2006a; Wrona et al., 2016). These environmental changes are predicted to impact primary production, as well as fish and their macroinvertebrate food resources by modifying habitat and causing mortality of some species (Allan & Castillo, 2007; Benke

& Wallace, 2003; Kemp et al., 2011). Given the dramatic changes that these stream ecosystems will experience, it is timely and important to fill knowledge gaps on the role of ninespine stickleback role in Arctic stream food webs.

1.2 – Environmental factors impacting fish condition and abundance

Condition factor and abundance can be used as indicators of fish population health (Whitfield & Elliott, 2002). Condition (K) is a measure of the average relative weight of individuals in a population based on their length (Froese, 2006). At a given length, heavier fish of a given species may indicate a favourable habitat (greater condition), whereas thinner individuals relative to the same length indicate less favourable habitat (lesser condition; Blackwell et al., 2000). Moreover, depending on tolerances of taxa in the community, fish abundance can be related to habitat quality with better habitats generally supporting larger populations (Whitfield & Elliott, 2002). Factors that impact the condition and abundance of fish in lotic ecosystems are primarily abiotic factors such as temperature and nutrient concentration, and biotic factors like bottom-up and top-down control of food webs as well as competition (Behrens & Lafferty, 2007; Peterson et al., 1993; Whitfield & Elliott, 2002). Ninespine stickleback condition and abundance have both been found to negatively correlate with warmer temperatures (Guderley & Foley, 1990; Khalsa et al., 2021), while both the presence/absence of interspecific competition and predation are suspected to impact growth strategies (Herczeg et al., 2012). Greater nutrient concentrations can support larger fish populations with greater condition by increasing food (invertebrate) productivity and availability (Krohn et al., 1997; Peterson et al., 1993). Collectively, the influences of various environmental factors can combine to produce cumulative effects

on fish populations, which makes determining direct impacts of environmental variables on metrics such as condition and abundance challenging (Peterson et al., 1993; Whitfield & Elliott, 2002).

1.3 – Diet analysis methodology

Diet of ninespine stickleback is poorly understood, however, further assessment of fish diet is complicated by the various biases inherent in standard methods for diet analysis (Nielsen et al., 2018). Three common diet analysis methods include morphological gut content analysis (M-GCA), DNA metabarcoding of gut contents (D-GCA), and stable isotope analysis (SIA) of both consumer and suspected prey tissues. M-GCA involves the visual identification of gut contents, and the enumeration of diet items through a variety of methods (e.g., numeric, mass, volumetric; Hyslop, 1980). D-GCA involves the use of DNA metabarcoding methods to identify the taxonomic identities of prey items contained within consumer guts (Deiner et al., 2017; Jakubavičiūtė et al., 2017). SIA involves sampling tissue from the consumer and from the suspected prey to determine the average Carbon and Nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$ respectively) of the tissues sampled (Nielsen et al., 2018). When organic tissue is consumed and assimilated into a consumer's tissue, $\delta^{13}C$ changes (fractionates) very little, as opposed to $\delta^{15}N$, which fractionates at a consistent rate (Deniro & Epstein, 1981; Perkins et al., 2014). These isotope ratios and approximate fractionation rates can then be used in mathematical models to estimate the proportional contribution of each prey group to the diet of a consumer (Figure 1.3; Lancaster & Waldron, 2001; Parnell et al., 2013).

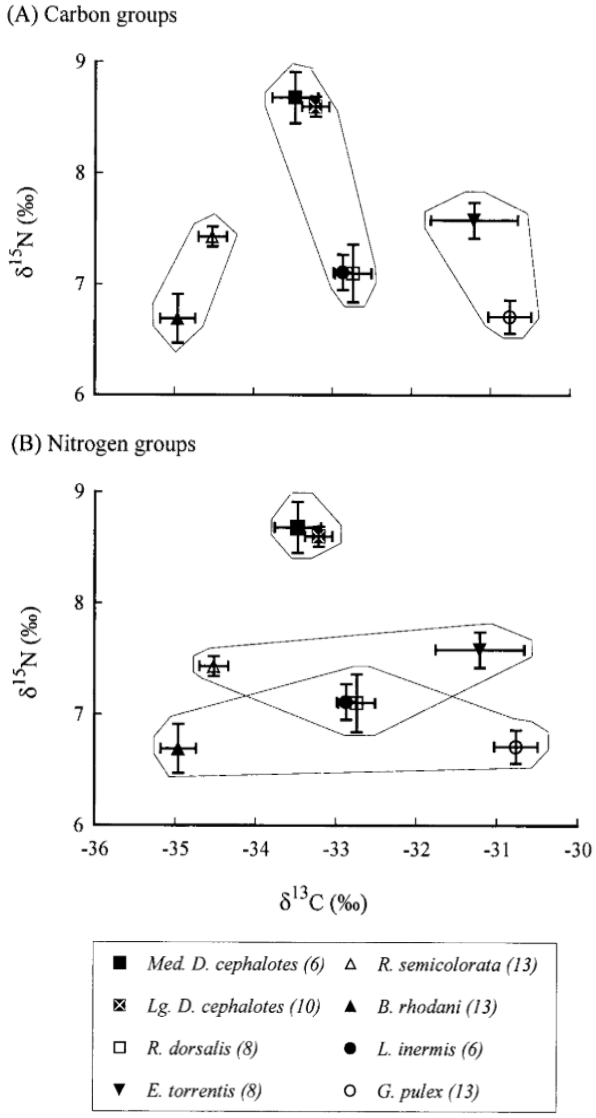

Figure 1.3 - Example of two dual isotope biplots. Squares represent predators, triangles are grazers/collectors, and circles are detritivores/collectors. Groupings created in each plot represent species that are not significantly different in terms of (A) carbon, and (B) nitrogen. (Lancaster & Waldron, 2001; reproduced with permission from the author).

While each method provides information on diet, they each have biases and drawbacks. M-GCA and D-GCA only establish a snapshot of what an organism has eaten within the past few hours to days (depending on digestion rates; Hyslop, 1980; Lee et al., 2018) and differ in identification biases for different types of organisms (Berry et al., 2015; Martins et al., 2021). SIA provides diet information over a longer time scale but relies on the assumption that estimated prey types are correct and important prey items have not been omitted (Nielsen et al., 2018; Post, 2002). Because of these various identification biases and drawbacks, diet analysis methods are often combined in a complementary fashion (Nakamura et al., 2020; Pacioglu et al., 2019; Whitaker et al., 2019). Given that DNA metabarcoding is a novel approach, only recently has the combination of all three become available and has yet to be applied to fish. This presents an opportunity to compare the results of these three diet analysis methods on the same small fish individuals for the first time while also filling a knowledge gap about ninespine stickleback diet in Arctic streams.

1.4 – Objectives and Hypotheses

This thesis aims to: **1)** determine ninespine stickleback abundance and condition and how tundra stream characteristics impact these metrics, **2)** characterize the diet of ninespine stickleback in Arctic tundra streams, and **3)** assess the relative benefits and disadvantages of each technique for the dietary study of small stream fishes. Chapter 2 examines ninespine stickleback abundance and condition, and correlations among these fish metrics and tundra stream characteristics, while Chapter 3 assesses ninespine stickleback diet and how diet estimates vary among M-GCA, D-GCA and SIA methods.

The predictions made in Chapter 2 are:

i) stickleback condition and catch per unit effort (CPUE) are correlated with water temperature;

ii) greater stickleback condition and CPUE are associated with greater total dissolved phosphorus (TDP) concentrations (i.e., used as a proxy of overall lotic productivity);

iii) greater stickleback condition and CPUE are associated with greater macroinvertebrate abundance; and

iv) ninespine stickleback condition and CPUE are negatively correlated with competitor/predator (i.e., Arctic charr) CPUE.

The predictions made in Chapter 3 are:

i) M-GCA would identify hard-bodied organisms more frequently than D-GCA;
ii) D-GCA would identify soft-bodied organisms more frequently than M-GCA; and
iii) SIA would estimate sub-families of Chironomidae as the primary contributors to long-term diet.

1.5 – Study area and study design

This study took place in the Greiner Lake (69° 13.145' N, 104° 51.911' W) watershed, a tundra environment located northeast of Cambridge Bay, Nunavut on the south side of Victoria Island (Figure 1.4). The watershed is comprised of gently sloping hills underlain by permafrost, with a variety of dwarf shrubs, grasses, sedges and low-lying flowering plants covering the landscape (NASA, 2015). A network of lakes and interconnected streams cover a large portion of the landscape, flowing in a complex pattern across the tundra towards Greiner Lake (NASA, 2015). These streams' food-

webs are relatively short, containing algal and macrophyte primary producers, invertebrates, ninespine stickleback, and Arctic charr (*Salvelinus alpinus*). Streams across the tundra were sampled via helicopter in both 2018 (n = 14) and 2019 (n = 17) for benthic macroinvertebrates and fish, as well as a suite of environmental data that included stream morphology, hydrology, and water chemistry. Streams were selected prior to visiting the watershed to encompass the spatial and physical variability of streams in the watershed.

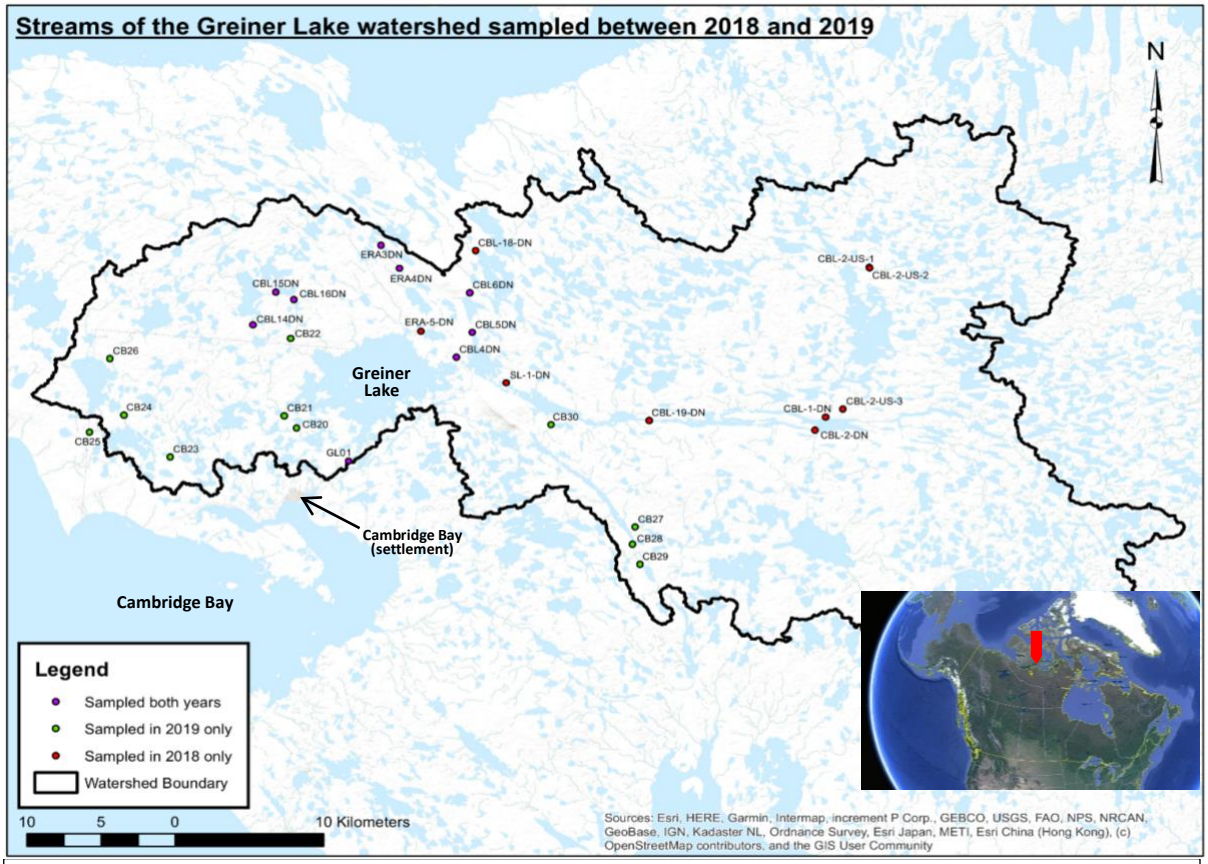

Figure 1.4 – Map of the Greiner Lake watershed, northeast of Cambridge Bay, Nunavut. Streams sampled in 2018 are represented red, streams sampled in 2019 are represented in green, and those sampled in both years are represented in purple. Black line indicates the watershed boundary. CB25 was not included in any analyses, due to it being outside the watershed boundaries.

CHAPTER 2: IMPACTS OF TUNDRA STREAM CHARACTERISTICS ON NINESPINE STICKLEBACK (*Pungitius pungitius*) CONDITION AND ABUNDANCE

2.1 – Abstract

Ninespine stickleback (*Pungitius pungitius*) are ubiquitous in many circumpolar aquatic environments, yet their ecological role in streams is not well understood. The objectives of this study were to describe ninespine stickleback populations in tundra streams of the Greiner Lake watershed (Cambridge Bay, Nunavut, Canada) and determine what environmental variables explain ninespine stickleback condition and catch per unit effort (CPUE) in tundra streams. Ninespine stickleback CPUE varied from 0.0-12.2 individuals min^{-1} across all sites and years, with an average of 3.72 ± 3.98 in 2018 and 1.74 ± 2.42 in 2019. Average relative condition was 1.03 ± 0.18 across all sites and years. The condition and catch per unit effort of stickleback from these streams were tested against average stream temperature, total dissolved phosphorus (TDP) concentrations, total macroinvertebrate abundance in a 3-min CPUE kick sample, and Arctic charr (*Salvelinus alpinus*) CPUE. Abiotic variables ranked as more important in determining stickleback CPUE and condition than biological variables related to prey availability or competitor/predator interactions. CPUE was best explained by variation in TDP and temperature and average condition correlated negatively with temperature. AIC rankings compared to other models and low correlation coefficients suggest both factors have minor relationships to environmental variables likely due to both the tolerance of ninespine stickleback to a wide range of environmental conditions and carry-over effects from over-wintering environments. This study provides evidence to explain the drivers

of ninespine stickleback condition in Arctic freshwaters and should be followed up with research conducted over a longer time-scale that includes sampling of both immediately prior to freeze-up and after break-up.

2.2 - Introduction

The Arctic is warming rapidly due to climate change (Bintanja, 2018), with climate-induced shifts in weather having led to distinct alterations in freshwater environments including: increasing stream temperatures, groundwater flow and nutrient levels, and changes in invertebrate community structure (Lento et al., 2013; Prowse et al., 2006). Such changes are likely to have significant implications for stream and lake resident biota, including fish (Ficke et al., 2007), with shifts in species range, life history patterns, and genetics all occurring as temperatures continue to warm (Casselman, 2002; Chu et al., 2005; Golden et al., 2021). The complex ways in which climate change may act directly (e.g., via water temperature changes) or indirectly (e.g., via permafrost thaw and fluxes in nutrient inputs to streams) on fish combined with the limited existing understanding of fish and climate interactions in the Arctic makes the prediction of probable effects difficult (Reist et al., 2006). This is especially problematic when information used to guide management of species in a changing Arctic climate is derived from studies done in vastly different geographical locations.

Ninespine stickleback (*Pungitius pungitius*), which are highly abundant within freshwaters of the circumpolar Arctic (Von Hippel et al., 2016), are a species of freshwater fish that are both understudied in the Arctic and expected to be impacted by climate change. Ninespine stickleback are generalists that prey primarily on macroinvertebrates and zooplankton (Laske et al., 2017), but have also been known to

occasionally feed on larval stickleback and fish eggs (Hynes, 1950). They provide an important energy linkage between lower trophic levels (macroinvertebrates/zooplankton) and higher level predators such as piscivorous fish and shorebirds (Griswold & Smith Jr, 1973; Von Hippel, 2008). Ninespine stickleback have been found to control macroinvertebrate populations through predation and fall prey to larger piscivorous fish species like Arctic charr (*Salvelinus alpinus*) and Arctic Grayling (*Thymallus arcticus*) (Gallagher & Dick, 2010; Laske, et al., 2017; Mcfarland, et al., 2018). Arctic charr predate on ninespine stickleback, but have been found to rarely do so prior to reaching a length threshold of approximately 20-30 cm (Gallagher & Dick, 2010), below which they feed almost entirely on invertebrates. Thus, ninespine stickleback may have both a competitive and prey relationship with other fish species. Ecological relationships such as these may explain why three-spined stickleback (*Gasterosteus aculeatus*) presence/absence has consequences for food-web trophic diversity and macroinvertebrate community structure, with food-webs in Greenland streams being longer where stickleback are present and the relative abundances of filter feeders and collector gathers varying with their presence or absence (González-Bergonzoni et al., 2014).

While ninespine stickleback are known to be tolerant to extreme environmental conditions, and have a wide thermal range based on their global distribution (Markovic et al., 2021), the optimal temperatures for critical life-history functions (e.g. growth, spawning) remain unknown. Phenotypic plasticity suggests that many populations of ninespine stickleback are locally adapted (Tufts, 2018), which may increase their susceptibility to rapidly shifting environmental conditions. For example, rearing temperature increases of 3^{o}C have been shown to quicken growth and lower age at

maturation, which are known correlates of fitness important for predicting population responses to environmental change (Kuparinen et al., 2011). Further, temperature increases have been associated with declines in fish condition suggested to have resulted from reduced prey consumption (Guderley & Foley, 1990). Despite their abundance in Arctic freshwaters and noted probable sensitivity to climate change, little baseline research has been conducted on the ecological role of ninespine stickleback or the environmental drivers of their condition, especially in Arctic streams. While lentic (lake/pond) studies are useful, adaptations to lotic (stream/river) environments often differ as a result of the flowing water environments (Statzner, 2008), thus it is pertinent to specifically study the ecological role of ninespine stickleback in streams. Fish populations are influenced by water temperature, food abundance, predation, and competition (Peterson et al., 1993; Reist et al., 2006; Sih et al., 1985); given the importance of these variables for determining growth, survival, and reproductive success. Most fish also have a narrow optimal temperature range for physiological processes and may choose to move to areas where temperature is more preferable (Beitinger & Fitzpatrick, 1979; Reist et al., 2006). In rivers, increased concentrations of nutrients such as phosphorus can lead to greater macroinvertebrate production, which may in turn increase the condition and abundance of a population if it is food-limited (Milbrink et al., 2008; Peterson et al., 1993). Abundance and condition of fish species can be influenced by increased competition or predation risks, causing a reallocation of time and energy to competitive behaviours and survivorship (Robertson, 1996; Sih et al., 1985; Walsh et al., 2012).

This study sought to test the predictions that: (1) stickleback condition and catch per unit effort (CPUE) are correlated with water temperature; (2) greater stickleback condition and CPUE are associated with greater total dissolved phosphorus (TDP) concentrations (i.e., used as a proxy of overall lotic productivity); (3) greater stickleback condition and CPUE are associated with greater macroinvertebrate abundance, and (4) ninespine stickleback condition and CPUE are negatively correlated with competitor/predator (i.e., Arctic charr) CPUE. These hypotheses were examined by assessing the relationship of ninespine stickleback condition and CPUE to key environmental stream variables in multiple streams of the Greiner Lake watershed.

2.3 - Methods

2.3.1 - Study area

The Greiner Lake (69° 13.145' N, 104° 51.911' W) watershed is located on southern Victoria Island, Nunavut and has numerous populations of ninespine stickleback (Johnson, 1962). Within the watershed, terrestrial plant communities are comprised primarily of dwarf shrubs, grasses, sedges, and mosses, with low-lying flowering plants and sedges occurring in moderate and drier soils (NASA, 2015). The underlying surficial geology of the area is primarily carbonate rock, with the Paleozoic limestone and dolomite rock commonly found across Victoria Island (NASA, 2015). The topography is relatively flat, and mostly comprised of low, gently sloping hills, with the watershed surface underlain by permafrost and an active layer less than 1m deep (NASA, 2015). Greiner Lake sits at approximately 15 m above sea level (masl), with the highest point in the watershed being Mount Pelly, an esker peaking at approximately 200 masl (Johnson, 1962; NASA, 2015). The watershed is comprised of a network of streams that

interconnect lakes that drain into Greiner Lake, and subsequently into the marine waters of Cambridge Bay via Freshwater Creek (NASA, 2015). Lakes and streams in the watershed are alkaline (pH = 8.0-8.6) due to the underlying carbonate bedrock (NASA, 2015). Highest average daily air temperatures occur in July (8.9 °C) and August (6.8 °C), and decline to a low of -32.5 °C in February (Environment Canada, 2019). The region experiences low mean annual precipitation (100-150 mm), and stream flow is primarily driven by the melting of snow and ice built up over the winter season (Environment and Climate Change Canada, 2019; Poff & Ward, 1989). Biota within streams are generally limited to primary producers, macroinvertebrates, occasional zooplankton, ninespine stickleback and Arctic charr.

2.3.2 – Sample design

Streams were selected prior to visiting the watershed based on best estimates to capture variation in both stream order and lake proximity across the watershed. Streams analyzed were visited once in both July and August of 2018 (n = 14) and 2019 (n = 17), with 6 streams sampled in both years. A suite of physical-chemical and hydrological data was collected on each site visit to comprehensively describe stream environmental characteristics. Macroinvertebrates and fish were sampled in August coincident with environmental sampling.

2.3.3 - Field Methods

2.3.3.1 – Fish and macroinvertebrate sampling

Ninespine stickleback, Arctic charr, and macroinvertebrates were collected in August of each field season. Macroinvertebrates were collected using the Canadian Aquatic Biomonitoring Network (CABIN) protocol for kick-net sampling, where the

collector placed a 400-µm mesh kick-net in the water facing upstream and disturbed the substrate while moving upstream in a zig-zag pattern for 3 minutes (Environment Canada, 2012). Macroinvertebrates were preserved in 95% ethanol immediately after capture. Ninespine stickleback and Arctic charr were collected with 5mm mesh handheld dipnets and a Smith-Root LR-24 electro-fisher (Smith-Root Inc., Vancouver, WA) using a continuous, zig-zag pattern of fishing along an approximately 50 m reach. Fishing effort (seconds) was recorded for catch per unit effort (CPUE) computations. Fish were counted, euthanized, and put into whirl-paks on-site, and frozen at a constant temperature (-20°C) upon return to the lab at the end of the day. Where catch exceeded n = 130, a randomized sub-sample of 130-150 fish were retained for further analysis, and the rest were returned to the stream.

2.3.3.2 – Environmental variable sampling

Physical-chemical and hydrological variables were collected as follows. In July, Onset U20-001-04 water level loggers (Onset Computer Corporation, Bourne, MA) and a Zebra-Tech LTD D-Opto dissolved oxygen logger (Zebra-Tech LTD, Nelson, NZ) were affixed to rebar with sensor-heads submerged approximately 5 cm above the substrate surface at each study site. Deep sections of water with continuous flow at mid-channel were selected for logger deployment to accommodate seasonal hydrological flux and ensure loggers remained submerged for the duration of the field season. Loggers took measurements at regular intervals throughout the field season (every hour in 2018, every 15 minutes in 2019). A calibrated YSI ProDSS-2 Multiparameter Meter (YSI Incorporated, Yellow Springs, OH) was used to measure water temperature (^{o}C), conductivity (S m^{-1}), dissolved oxygen concentrations (mg L^{-1}), and pH. Stream velocity

($m\ s^{-1}$) was determined using a SonTek FlowTracker1 (SonTek / Xylem Inc., San Diego, CA). Ten wetted widths (m) spaced evenly along each sample reach were measured using a surveyor measuring tape, with detailed depth (cm) transects being completed at every second transect using a ruler. Substrate characteristics were determined using a modified Wolman pebble count (pieces of substrate were randomly selected and their b-axis measured) for 100 stones at each reach (Wolman, 1954). Rock counts were conducted once for each reach under the assumption that substrate composition changes little over the course of the summer due to low variability in snowmelt driven streamflow and minimal precipitation (Poff & Ward, 1989). Water samples were collected and analyzed for nutrients (i.e.: total nitrogen, dissolved organic/inorganic carbon, total/total dissolved phosphorus), major ions (alkalinity, pH, conductivity), and trace metals (i.e.: iron, copper, zinc) using Environment Canada's standard operating procedures for sampling via hand dipping, where bottles were first rinsed and then filled with stream water at mid-depth (Environment and Climate Change Canada, 2018).

2.3.4 - Lab Methods

In the laboratory all fish were thawed, measured for total length (mm), blotted dry and weighed to the nearest 0.001 g using a Fisher Science Education Model SLF103 scale (Thermo Fisher Scientific, Waltham, MA). All macroinvertebrates were sorted and assessed following the CABIN lab protocol by a Society of Freshwater Science certified taxonomist (Environment Canada, 2014). A Marchant (1989) box was used to sub-sample macroinvertebrates, after which cells of the Marchant box were randomly selected, and the invertebrates within each cell were sorted and identified under a Olympus SZX16 stereo microscope (Olympus, Tokyo, Japan) until at least 300

individuals had been counted. Quality assurance and quality control (QA/QC) were done on 20 % of the samples by a different taxonomist and confirmed with an average sorting accuracy of >95 %. Water samples were analyzed at Environment Canada's National Lab for Environmental Testing (NLET) for major ions, trace metals and nutrients following the standard operating procedures and QA/QC protocols for each variable (see Environment and Climate Change Canada (ECCC), 2020).

2.3.5 - Statistical Analysis

All statistical analyses were completed using R statistical software (*version 4.0.4,* R Core Team, 2021). A principal component analysis (PCA) was conducted to examine the variables that varied most and best explained the inter-site variation in measured physical-chemical variates and physical descriptors. Variables were screened for significant correlations (Pearson's $r > 0.7$) to avoid inclusion of highly correlated variables that might give rise to statistical issues associated with multicollinearity.

Fish samples from all sites and years were aggregated and used to estimate a standard length-weight relationship ($W = aL^b$) for the region which was used to estimate LeCren's relative condition factor (LeCren, 1951; Froese, 2006) for each stickleback using the equation:

$$K_{rel} = W/aL^b$$

where *W* and *L* define weight and length, respectively, and *a* and *b* are the estimated model parameters. The function measures the deviation of individual fish from the regional weight-length relationship, thereby describing the condition of the individual with respect to the mean expected condition for fish in the region at a given length.

Comparisons between relative condition and environmental variables were conducted using only streams where greater than 10 stickleback were caught to capture within-site variation. Only adult stickleback (>=28 mm of total length, age-1 and older) were used for the relative condition analysis due to the notable change in the weight-length relationship among young-of-the-year fishes (Froese 2006), and the observed variation in juvenile stickleback condition associated with scale sensitivity. Ninespine stickleback catch per unit effort (CPUE) was calculated for each site by dividing the number of stickleback caught by the seconds spent electrofishing at each site. Arctic charr CPUE was calculated the same way. Given that no more than one Arctic charr >15 cm in length was found in any stream, Arctic charr were not partitioned into different size classes to differentiate competitors and predators. Average temperature was calculated using all temperature readings taken by the depth loggers at each stream from deployment to the date at which fish were sampled. TDP concentrations were measured from water samples taken at the August fish collection date and processed by Environment Canada`s certified NLET laboratory. Whole sample estimates based on sub-sample counts divided by the proportion of Marchant box cells counted for the sub-sample were used as an index of total macroinvertebrate abundance at each site.

Multiple linear regression models for explaining observed variation in stickleback condition and CPUE were estimated and ranked using the corrected Akaike Information Criterion (AICc). Candidate explanatory variables included: (**i**) average water temperature, (**ii**) TDP concentration, (**iii**) total macroinvertebrate abundance, and (**iv**) Arctic charr CPUE. The model with the lowest AICc value was considered the "best" model. Models within 7 ΔAICc of the best model were considered plausible (Anderson,

2008). AICc analyses and ranking were completed using the "AICcmodavg" package in R (Mazerolle, 2020). ANOVAs were used to investigate differences in environmental variables between years/sites prior to regressions (Zar, 2010). ANOVAs and linear regressions were carried out using base R routines (R Core Team, 2020).

2.4 – Results

2.4.1 – Stream characteristics

A PCA conducted using stream variables including average depth, average width, total dissolved phosphorus (TDP), dissolved organic carbon (DOC), conductivity, total macroinvertebrate abundance, Arctic charr CPUE explained 63.5 % of total variance (PC1 = 43.7 %, PC2 = 19.8 %; Figure 2.1). Site variation along PC1 was best explained by conductivity, average temperature, dissolved organic carbon (DOC), TDP and average stream width; while variation along PC2 was best explained by macroinvertebrate abundance, average stream depth and Arctic charr CPUE. In August of both years, streams ranged between 0.28-31.7 m wide, and 0.07-0.4 m of average depth (Table 2.1). August specific conductance ranged between 229 and 683 in 2018, and 226 and 893 in 2019. There was no significant difference ($F_{1,29} = 3.36$, $p = 0.08$) in average total macroinvertebrate abundance between years (9038 ± 5262 in 2018 and 6290 ± 2957 in 2019). Total dissolved phosphorus concentrations ranged between 0.004 and 0.007 mg L^{-1} in 2018 and 0.004 and 0.006 mg L^{-1} in 2019. Watershed average stream temperatures (July to August) ranged between 7.2 and 11.3 °C in 2018, and 7.3 and 12.6 °C in 2019. Approximately half of the streams sampled each year yielded Arctic charr, and CPUE ranged between 0 and 2.38 fish min^{-1}. Mean Arctic charr CPUE did not differ significantly between years ($F_{1,29} = 0.14$, $p = 0.71$) averaging 0.325 ± 0.738 in 2018 and

0.243 ± 0.447 in 2019. TN was not included in the PCA due to high correlation with conductivity (Pearson's r > 0.7), while DO and pH were not considered for inclusion in the PCA due to their minimal observed among-site variation (10.8-12.6 mg L^{-1}, coefficient of variation (CV) = 3.73% and 7.9-8.5, CV = 1.81%, respectively across both years).

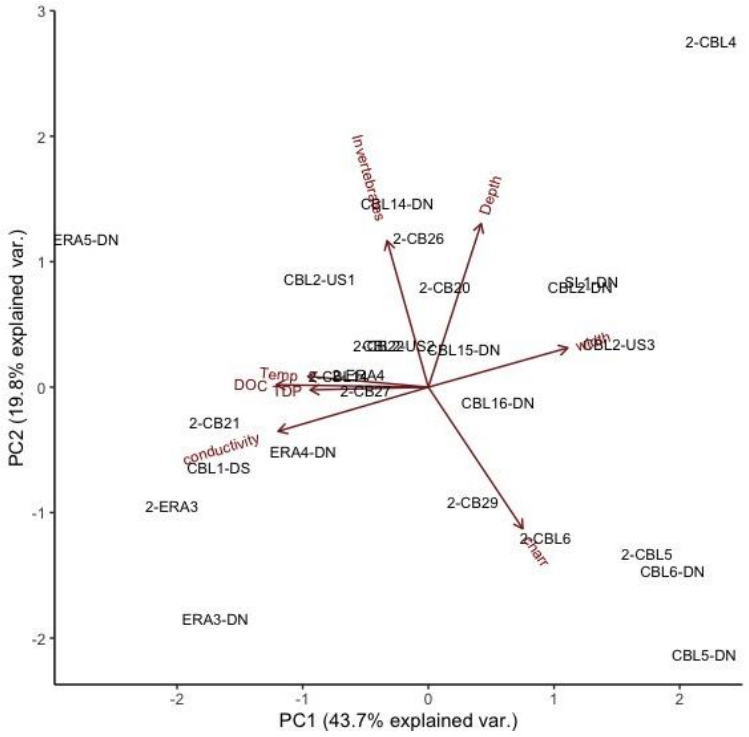

Figure 2.1 – Principal component analysis (PCA) of environmental variables measured at each stream (average depth, average width, total dissolved phosphorus (TDP), conductivity, total macroinvertebrate abundance (Invertebrates), Arctic charr catch per unit effort (charr), dissolved organic carbon (DOC), and average temperature). Stream characteristics associated with each site are listed in Table 2.1.

Table 2.1 – Summary of stream characteristics measured in the Greiner Lake watershed including physical-chemical characteristics, hydrological characteristics, and biota. Site names preceded by a "2-" were sampled in 2019, those without were sampled in 2018.

Site	Arctic charr CPUE (fish min^{-1})	Macroinvertebrate abundance (# of individuals)	Average Temp (°C)	Average depth (m)	Average velocity ($m\ s^{-1}$)	Average width (m)	Specific conductance ($\mu S\ cm^{-1}$)	DOC	TDP ($mg\ L^{-1}$)
CBL14-DN	0	13220	9.61	0.289	0.077	0.31	448	6.1	0.004
CBL15-DN	0	11460	8.93	0.119	0.130	6.28	229	3.7	0.006
CBL16-DN	0	5698	9.77	0.147	0.053	8.56	302	4.7	0.004
CBL5-DN	2.3789	4970	7.22	0.121	0.155	16.02	287	3.8	0.004
ERA3-DN	0.2559	1460	9.10	0.073	0.212	0.28	674	9.4	0.005
ERA4-DN	0.094	6400	10.30	0.139	0.257	0.42	523	6.4	0.005
ERA5-DN	0	18920	10.69	0.195	0.182	2.64	683	9.5	0.007
CBL2-DN	0.0744	6640	9.30	0.241	0.213	21.10	273	3.9	0.005
CBL2-US1	0	19020	8.76	0.101	0.221	9.34	397	7.9	0.006
CBL2-US2	0	11680	8.03	0.143	0.136	3.00	323	5.6	0.006
CBL2-US3	0	5633	8.27	0.178	0.156	27.90	276	3.8	0.005
CBL1-DS	0.0685	7340	11.33	0.101	0.143	1.02	514	7.2	0.006
CBL6-DN	1.677	4591	7.31	0.147	0.105	16.62	301	3.5	0.004
SL1-DN	0	9500	7.32	0.198	0.315	21.88	261	6.5	0.004
2-CBL5	1.4057	2266	8.98	0.162	0.253	17.00	280	3.5	0.004
2-CBL6	1.2212	3410	9.32	0.144	0.365	18.69	298	3.8	0.006
2-ERA3	0	3120	10.21	0.137	0.170	0.42	639	9.1	0.006
2-ERA4	0	6520	9.82	0.211	0.317	0.76	517	6.0	0.004
2-CB20	0.0588	6580	10.50	0.263	0.378	7.90	275	4.1	0.006
2-CB21	0	7040	10.75	0.142	0.352	3.49	499	8.2	0.006
2-CB22	0	6860	10.35	0.200	0.580	5.48	320	5.6	0.006
2-CBL14	0	5552	10.36	0.212	0.330	0.51	429	5.4	0.006
2-CBL16	0.0632	3819	NA	0.268	0.183	10.40	298	5.0	0.005
2-CB27	0.1703	5338	12.59	0.177	0.406	10.50	348	5.3	0.005
2-CB28	0.0594	3980	NA	0.158	0.459	1.67	603	8.2	0.005
2-CB29	0.47	3124	9.23	0.151	0.305	0.67	362	4.1	0.005
2-CB30	0.6857	12020	NA	0.175	0.223	16.50	325	4.2	0.005
2-CB23	0	9720	NA	0.200	0.388	2.11	414	5.1	0.006
2-CB24	0	8760	11.19	NA	NA	NA	893	9.6	0.006
2-CB26	0	7040	10.75	0.319	0.429	1.46	326	6.1	0.004
2-CBL4	0	11780	7.39	0.405	0.194	31.70	226	3.7	0.004

2.4.2 – Ninespine stickleback populations

Stickleback were caught at all but three sites, but where stickleback were present the number collected varied from 1 to nearly 150. CPUE did not differ significantly between 2018 and 2019 across all stream sites ($F_{1,29} = 2.92$, $p = 0.10$), nor were there significant differences between years for sites sampled both years ($F_{1,5} = 0.07$, $p = 0.80$). However, variation in average CPUE within years was great (3.72 ± 3.98 fish min^{-1} in 2018, CV = 107%; 1.74 ± 2.42 fish min^{-1} in 2019, CV = 140%; Table 2.2) and a significant difference was found among sites sampled both years ($F_{1,5} = 7.74$, $p = 0.02$). Stickleback sampled in 2019 were significantly smaller in length than those sampled in 2018 (31.2 ± 9.6 mm and 47.8 ± 6.9 mm, respectively; $F_{1,16} = 18.25$, $p \leq 0.01$; Table 2.2), though no significant differences in mean length were found between years within sites sampled both years ($F_{1,4} = 2.48$, $p = 0.19$). Stickleback of lengths 44-46 mm and 54-56 mm were collected most frequently in 2018, with fish between 40-60 mm occurring more than 5x as frequently as those below 35 mm in length. On the contrary, in 2019 stickleback with lengths between 20-22 mm were collected most frequently, with stickleback below 35 mm in length occurring 5x more frequently than stickleback between 40-60 mm in length (Figure 2.2). Most populations sampled in 2018 lacked stickleback in the smaller size-classes (20-22 mm/32-34 mm) found predominantly in the 2019 samples. The average condition of adult stickleback (>28 mm) across the watershed did not differ significantly between years ($F_{1,16} = 0.21$, $p = 0.65$). Given the lack of overall variation between years, data from both years were pooled for subsequent analyses.

Table 2.2 – Summary of ninespine stickleback samples taken from streams of the Greiner Lake watershed between 2018 and 2019. Catch per unit effort (CPUE) was calculated by dividing the sample size by the number of minutes the electro-fisher was used at each site. Relative condition values are the mean ± SE of all fish from that site. Mean summary statistics were omitted for outliers and streams with n < 10.

Site	Year	n	CPUE (fish min^{-1})	Mean relative condition	Mean length (mm)	Min length (mm)	Max length (mm)
2-CB20	2019	31	1.82	0.97 ± 0.24	28.3	12	61
2-CB23	2019	42	3.04	0.99 ± 0.31	24.4	12	60
2-CB26	2019	5	0.39	N/A	N/A	26.5	66.5
2-CB30	2019	36	2.47	1.15 ± 0.17	49.9	14	77
2-CBL14	2019	99	5.94	1.04 ± 0.18	22.9	12	60
2-CBL16	2019	6	0.38	N/A	N/A	12	57
2-CB21	2019	75	8.12	0.86 ± 0.06	23.6	17.5	56
2-CB22	2019	8	0.66	N/A	N/A	19	63
2-CB28	2019	3	0.18	N/A	N/A	43	65
2-CB29	2019	1	0.08	N/A	N/A	61	61
2-CBL4	2019	15	0.89	1.12 ± 0.25	34.9	18.5	57
2-CBL5	2019	4	0.30	N/A	N/A	57	69
2-CBL6	2019	3	0.16	N/A	N/A	46.5	71
2-ERA3	2019	35	4.85	1.03 ± 0.08	33.8	21	61
2-ERA4	2019	3	0.23	N/A	N/A	24	66
CBL1-DS	2018	111	7.60	1.02 ± 0.20	55.1	36	73
CBL14-DN	2018	31	3.08	1.02 ± 0.13	39.4	19	55
CBL15-DN	2018	136	11.10	0.97 ± 0.20	50.0	32	71
CBL16-DN	2018	14	1.27	0.93 ± 0.10	54.0	40	65
CBL2-DN	2018	31	2.31	1.10 ± 0.15	45.5	33.5	60.5
CBL2-US1	2018	38	2.79	1.00 ± 0.14	46.2	29	77
CBL2-US2	2018	149	12.20	1.06 ± 0.15	44.2	30	68
CBL2-US3	2018	18	1.33	1.12 ± 0.15	57.9	49	67
CBL6-DN	2018	32	1.99	1.01 ± 0.16	48.6	28	64
ERA3-DN	2018	48	6.14	1.05 ± 0.15	34.3	17	53
ERA4-DN	2018	5	0.47	N/A	N/A	51	60
ERA5-DN	2018	3	0.51	N/A	N/A	24.5	24.5
SL1-DN	2018	16	1.25	1.21 ± 0.14	50.2	39	58
CBL5-DN	2018	0	0.00	N/A	N/A	N/A	N/A
2-CB27	2019	0	0.00	N/A	N/A	N/A	N/A
2-CB24	2019	0	0.00	N/A	N/A	N/A	N/A

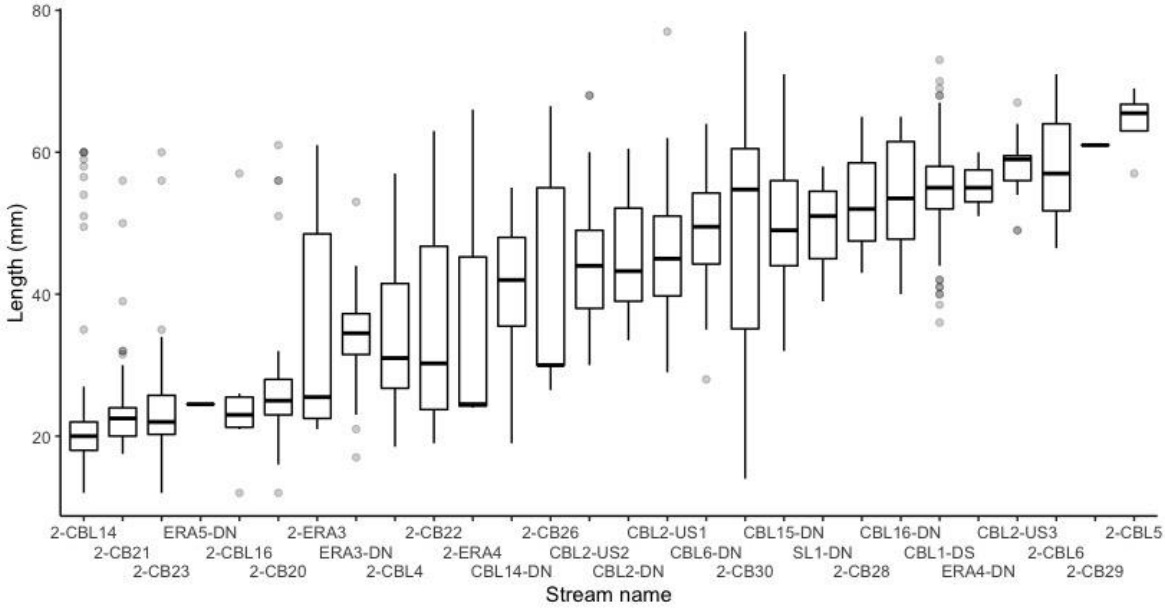

Figure 2.2 – Length distributions of ninespine stickleback caught via electro-fishing at each site in 2018 and 2019, organized by median, including outlier sites. Each stream with a "2-" preceding its name was sampled in 2019 (the "2-" referring to second sample year), and each stream without was sampled in 2018. Site labels on the x-axis are offset and stacked in groups of three sites.

2.4.3 – Influence of environmental variables on ninespine stickleback

2.4.3.1 – Relative condition

There was a significant difference in K_{rel} between sites ($F_{17,642} = 4.63$, $p = <0.01$; Figure 2.3). Relative condition had a significant, negative correlation with average water temperature (adj. $R^2 = 0.31$, $p = 0.01$; Figure 2.4). No significant relationships were found between relative condition and TDP, invertebrate abundance or Arctic charr presence (adj. $R^2 = 0.13$, $p = 0.07$; adj. $R^2 = -0.05$, $p = 0.71$; adj. $R^2 = -0.06$, $p = 0.84$; Figure 2.4). AIC_c ranking estimated "TDP" to be the best model for estimating ninespine stickleback relative condition, but 12 other predictive models were within 7 ΔAIC_c of the best model, including the null model which was estimated to be the second most

plausible model by <2 ΔAIC_c (see Table 2.3). Variable importance weights based on the sum of the Akaike weights for each model that included the variable indicated TDP concentrations and temperature were more important variables (importance weights = 0.41 and 0.35 respectively) for explaining variation in stickleback relative condition than Arctic charr CPUE and invertebrate abundance (importance weights = 0.24 and 0.17 respectively).

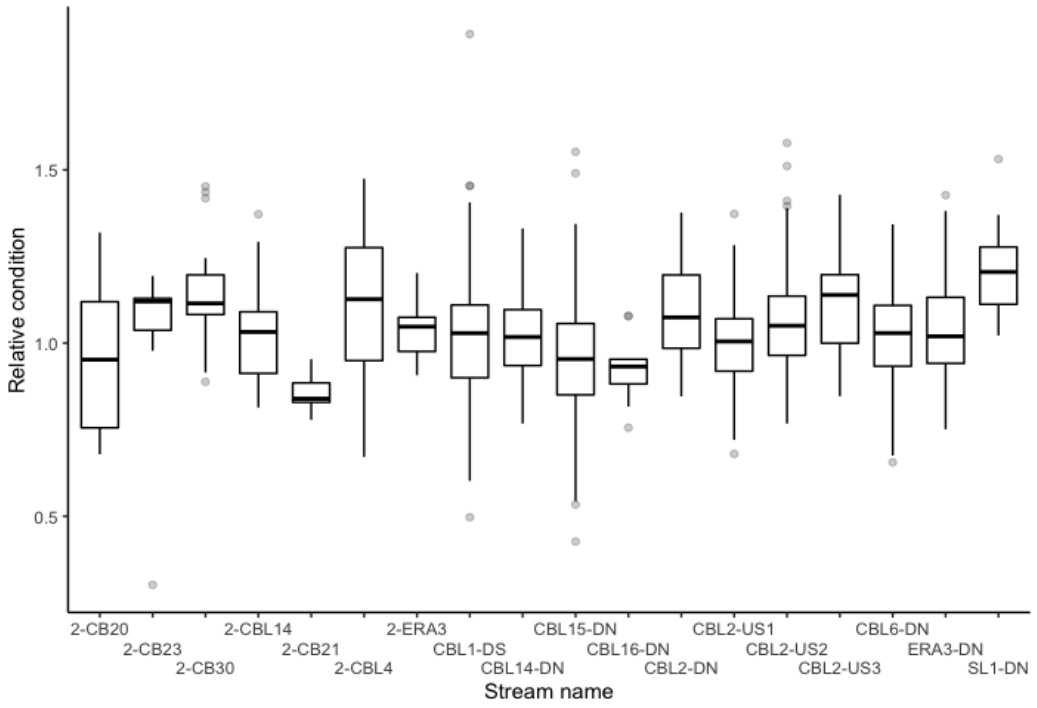

Figure 2.3 – Distribution of ninespine stickleback relative condition at all streams where at least 10 adult (>28 mm in length) stickleback were caught, excluding outlier sites. Each stream with a "2-" preceding its name was sampled in 2019 (the "2-" referring to second sample year), and each stream without was sampled in 2018. Site labels on the x-axis are offset and stacked in groups of three sites.

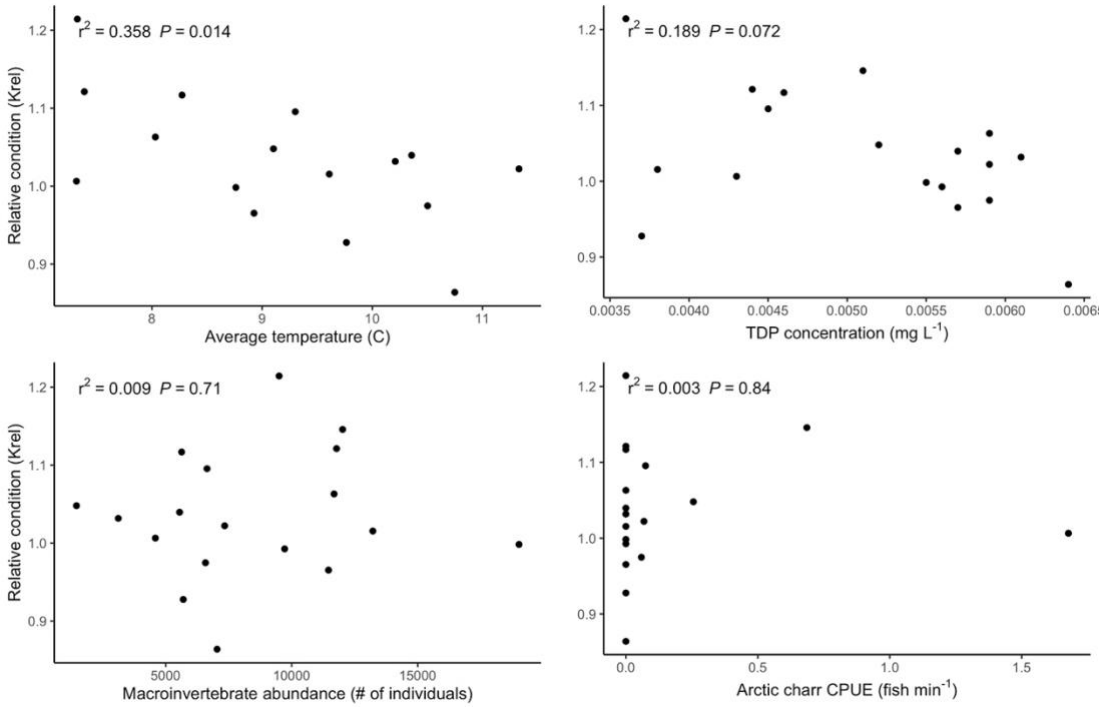

Figure 2.4 – Ninespine stickleback average relative condition at each stream plotted against, from top-left to bottom-right, average temperature, TDP concentration, macroinvertebrate abundance, and Arctic charr catch per unit effort (CPUE).

Table 2.3 – Corrected Akaike Information Criterion (AICc) model selection results for predicting variation in ninespine stickleback relative condition (Krel) against average temperature (Temp), TDP concentration (TDP), macroinvertebrate abundance (Inverts), and Arctic charr catch per unit effort (Charr). ΔAICc represents difference in AICc from the best model. w_i represents the model weight, and K represents the number of fitted parameters. Only models with ΔAICc < 7 are considered plausible and reported. Cumulative importance weights for individual model parameters are reported as the sum of the weights of the models each parameter appeared in.

				Weighted Importance					
Model names	**K**	**AICc**	**ΔAICc**	**wi**	**Cum. Wi**	**TDP**	**Charr**	**Inverts**	**Temp**
TDP	2	-35.63	0.00	0.27	0.27	0.27			
null	1	-34.78	0.85	0.17	0.44				
temp	2	-34.45	1.17	0.15	0.59				0.15
temp+charr	3	-33.46	2.16	0.09	0.68		0.09		0.09
inverts+TDP	3	-32.38	3.25	0.05	0.73	0.05		0.05	
charr+TDP	3	-32.30	3.33	0.05	0.78	0.05	0.05		
inverts	3	-32.02	3.60	0.04	0.82			0.04	
charr	2	-31.91	3.72	0.04	0.86		0.04		
inverts+temp+charr	4	-31.72	3.90	0.04	0.90		0.04	0.04	0.04
temp+TDP	3	-31.36	4.27	0.03	0.93	0.03			0.03
inverts+temp	3	-31.21	4.41	0.03	0.96			0.03	0.03
temp+charr+TDP	4	-29.75	5.87	0.01	0.98	0.01	0.01		0.01
inverts+charr	3	-28.75	6.87	0.01	0.99		0.01	0.01	
				Cum. Importance Weight		**0.41**	**0.24**	**0.17**	**0.35**

2.4.3.2 – Catch per unit effort

Ninespine stickleback catch per unit effort had a significant but weak positive correlation with TDP concentrations (adj. $R^2 = 0.12$, $p = 0.03$; Figure 2.5). No significant relationships were found between ninespine stickleback CPUE and average temperature, invertebrate abundance, or Arctic charr CPUE (adj. $R^2 = -0.04$, $p = 0.83$; adj. $R^2 = <|-0.01|$, $p = 0.33$; adj. $R^2 = 0.03$, $p = 0.17$). AIC_c model ranking suggested the model containing temperature, Arctic charr CPUE and TDP ($w_i = 0.37$) or temperature and TDP ($w_i = 0.35$) as the most plausible model for explaining nine stickleback CPUE (Table 2.4). An additional 5 other models were within 7 ΔAIC_c of the best model. Importance weights indicated average stream temperature and TDP concentration were the most important variables (importance weights = 0.99 and 0.91 respectively) with Arctic charr CPUE and invertebrate abundance being less important (importance weights = 0.52 and 0.19 respectively). Little correlation was found between CPUE and conductivity (adj. $R^2 = -0.03$, $p = 0.94$) or stream width (adj. $R^2 = 0.07$, $p = 0.09$).

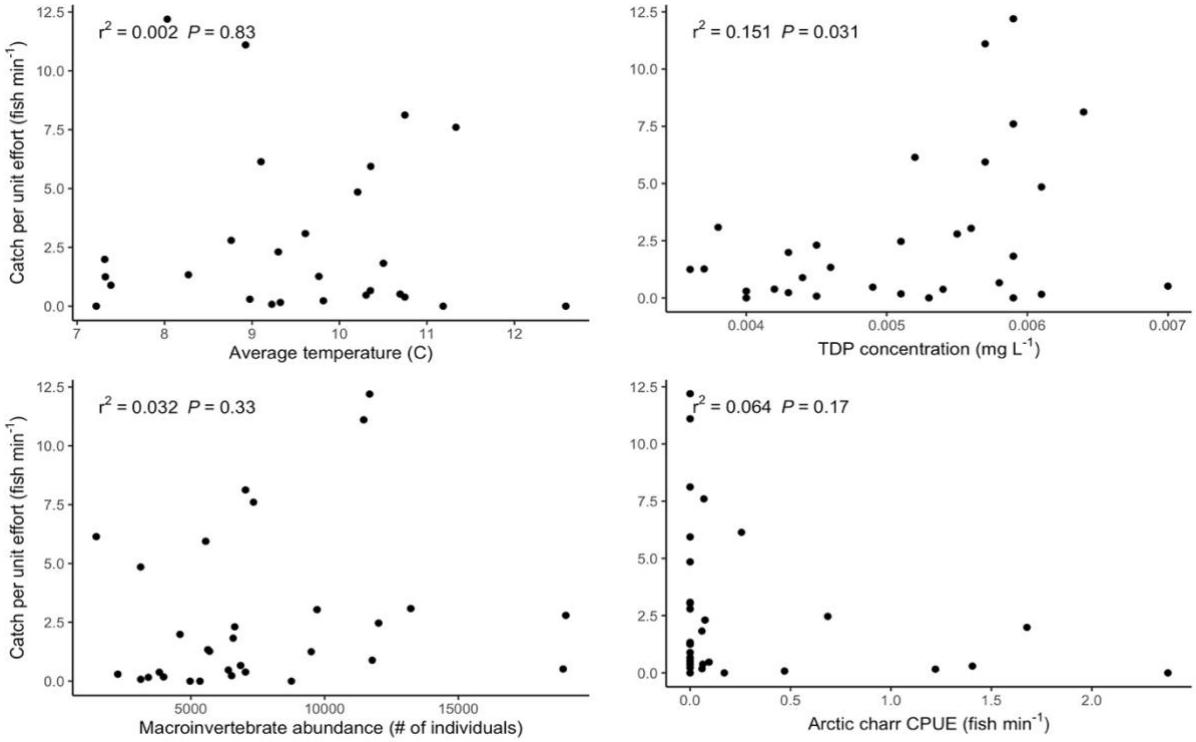

Figure 2.5 – Ninespine stickleback catch per unit effort (CPUE) plotted against, from top-left to bottom-right, average temperature, TDP concentration, macroinvertebrate abundance, and Arctic charr catch per unit effort (CPUE).

Table 2.4 – Corrected Akaike Information Criterion (AICc) model selection results for predicting variation in ninespine stickleback catch per unit effort (CPUE) against average temperature (Temp), TDP concentration (TDP), macroinvertebrate abundance (Inverts), and Arctic charr CPUE (Charr). ΔAICc represents difference in AICc from the best model. w_i represents the model weight, and K represents the number of fitted parameters. Only models with ΔAICc < 7 are considered plausible and reported. Cumulative importance weights for individual model parameters are reported as the sum of the weights of the models each parameter appeared in.

				Weighted Importance					
Model name	**K**	**AICc**	**ΔAICc**	**wi**	**Cum. wi**	**TDP**	**Charr**	**Inverts**	**Temp**
temp+ charr+ TDP	4	145.53	0.00	0.37	0.37	0.37	0.37		0.37
temp+ TDP	3	145.65	0.12	0.35	0.72	0.35			0.35
inverts+ temp+charr+ TDP	5	147.93	2.40	0.11	0.83	0.11	0.11	0.11	0.11
inverts+ temp+TDP	4	148.69	3.16	0.08	0.90	0.08		0.08	0.08
temp+ charr	3	149.86	4.33	0.04	0.95		0.04		0.04
temp	2	150.22	4.69	0.03	0.98				0.03
inverts+ temp	3	152.40	6.87	0.01	0.99			0.01	0.01
null	1	165.74	20.21	0.00					
				Cum. Importance weight		**0.91**	**0.52**	**0.19**	**0.99**

2.5 – Discussion

The ecological role of the ninespine stickleback in Arctic freshwaters is not well understood with a specific lack of baseline knowledge available in literature on their role in streams (Laske et al., 2017). An important aspect of improving understanding their ecological role is to determine how environmental change may affect stickleback populations as climate shifts in Arctic freshwater environments. Here it was predicted

that ninespine stickleback condition and CPUE in streams would be related to average water temperature, nutrient concentrations (TDP), macroinvertebrate abundance, and Arctic char abundance. For streams of the Greiner Lake watershed, there was a weak to moderate association between the measured variables and ninespine stickleback relative condition or abundance measured as CPUE. Based on the importance weights, all variables ranked as important for explaining variation in CPUE, but no variables ranked as more important than the null model for explanation of observed variation in relative condition among the studied streams despite the negative correlation between relative condition and average stream temperature. Abiotic variables (TDP and average stream temperature) ranked as more important than biological variables related to prey availability or competitor/predator interactions. The predicted importance of the abiotic environment for ninespine stickleback underscores the need for further ecological research on Arctic lotic ninespine stickleback populations.

2.5.1 – Temperature

The estimated importance of stream temperature in determining ninespine stickleback CPUE and relative condition may be related to both movement by stickleback to optimal thermal environments, and carry-over effects from over-wintering environments. Fish tend to have a narrow thermal optimal range, but can preferentially move to environments with optimal temperatures (Beitinger & Fitzpatrick, 1979; Coutant, 1987; Reist et al., 2006), and increased stream temperatures can decrease condition based on increases in metabolic rate (Cui & Wootton, 1988). This likely explains the importance of temperature in determining stickleback abundance, and the negative correlation between stickleback relative condition and temperature. Stickleback

populations are known to be more tolerant of extreme environmental conditions than other fish (Lewis et al., 1972; Markovic et al., 2021), but populations still must collectively adapt to local temperature ranges given juvenile sensitivity to extreme low temperatures (Tufts, 2018), which complements our findings dictating that temperature is important in determining where stickleback reside. It is also possible that the predicted lack of importance of temperature by AIC in determining relative condition in relation to the null model may be related to carry over effects from over-wintering environments. Movement from suitable over-wintering sites and subsequent dispersal through the ephemeral connective channels that link streams, ponds and lakes in spring facilitates annual dispersal and colonisation of fish in tundra watersheds (Cameron et al., 1973; Laske et al., 2016). This may further result in carry-over effects (Harrison et al., 2011), particularly for relative condition, that mask the linkages between capture site conditions, and their implications for captured fish. Thus, while temperature has implications for fish growth and condition (Guderley & Foley, 1990; Kuparinen et al., 2011), these small Arctic streams are temporary habitat for ninespine stickleback and further research is required to understand the importance of the summer period occupancy on condition.

2.5.2 – Nutrients and macroinvertebrates

The limited association of TDP and the relative abundance of macroinvertebrate prey with ninespine stickleback CPUE and relative condition may be related to the broad environmental tolerances and generalist feeding strategies of ninespine stickleback. Abilities to tolerate low oxygen (Lewis et al., 1972), high salinity (Nelson, 1968) and to disperse as environmental conditions change appear to facilitate their near ubiquity in Alaskan coastal plain lakes. These qualities are likely beneficial for this species in the

low gradient, hydrologically variable lotic systems of southern Victoria Island. As with temperature, carry-over effects from overwintering habitat (Harrison et al., 2011; Laske et al., 2016) may also mask the linkages between capture site conditions and fish condition. Thus, while nutrient additions have been shown to have strong and persistent effects on the growth and condition of other Arctic fishes through increases in invertebrate production (e.g., Warren et al., 1964; Peterson et al., 1993; Deegan et al.,1999), bottom-up control of ninespine stickleback populations in these streams does not appear strong likely due to the low range of nutrient concentration. The lack of strong local control on ninespine stickleback further suggests predicting their response to climate-triggered changes in stream nutrient additions related to permafrost thaw may be difficult (e.g. Vonk et al., 2015).

2.5.3 – Arctic charr abundance

Given the lack of evidence for bottom-up control of ninespine stickleback populations in the watershed, the low impact of Arctic charr CPUE on ninespine stickleback CPUE and condition may be related to a lack of food limitation for the various fish populations. Given the majority of charr caught in these streams were below 15 cm in length, they likely have a primarily competitive relationship with ninespine stickleback. Organisms in upper trophic levels of an ecosystem are primarily controlled by food limitations and competition (Menge, 2000), but if food is not sufficiently limited, then consumers may be more or less released from the effects of interspecific competition (Lenski, 1984). Thus, while charr presence could likely play a role in determining stickleback CPUE and condition in this watershed, their importance is less significant than that of abiotic watershed factors.

2.5.4 – Conclusions and future research

While bottom-up control and competition/predation are thought to be important in determining ninespine stickleback CPUE and condition (Milbrink et al., 2008; Sih et al., 1985; Hrabik et al., 1998), their importance appears limited in the context of the stream environments of the Greiner Watershed on Victoria Island. While TDP and temperature were determined to be important in estimating stickleback abundance, none of the measured variables were important in estimating average relative condition. Reist et al. (2006) indicate that the quality of over-wintering environments and associated over-wintering mortality may be primary regulators of Arctic fish populations, and carry-over effects from the winter have been shown to impact fish populations, with starvation and thermal stress-related mortality in winter driving summer stream population density (Hurst, 2007; Schlosser, 1998). Such effects of lentic environments may have a significant impact on the ninespine stickleback populations of streams in the Greiner Watershed, and future research should examine the potential importance of overwinter conditions in these lentic habitats on ninespine stickleback ecology of these tundra streams. Studying a smaller number of lotic ninespine stickleback populations over the course of multiple years with the inclusion of their over-wintering environments will provide a more comprehensive understanding of the factors that regulate ninespine stickleback populations in tundra streams, and further elucidate how they may respond to climate-related drivers of ecosystem change.

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CHAPTER 3: DIET ANALYSIS METHOD CHOICE AFFECTS THE DIET ESTIMATES OF NINESPINE STICKLEBACK (*Pungitius pungitius*)

3.1 – Abstract

In this study, M-GCA, D-GCA and SIA were conducted on the same ninespine stickleback (*Pungitius pungitius*) individuals to comprehensively describe their diet. In recent literature, multiple diet analyses are often paired together to address the biases and potentially conflicting results of individual analyses. Pairing morphological gut content analysis (M-GCA) and stable isotope analysis (SIA) has become common practice in the last decade, but the recent advances in the application of DNA metabarcoding have opened the possibility for greater combinations of diet analysis techniques. The aims were to categorize their diets in Arctic tundra streams and assess the relative benefits and drawbacks of each technique for the dietary study of small stream fishes. Orthocladiinae and Chironominae were the most frequently occurring taxa identified by both M-GCA and D-GCA and were the most abundant taxa in the stream communities, concurring with prior literature findings of a generalist diet. SIA estimated Arachnida and Tanypodinae to be two of the most significant contributors to diet over a longer period, suggesting a shift in diet over the course of the summer. M-GCA underestimated the frequency of occurrence of soft-bodied Oligochaeta, and D-GCA underestimated the frequency of occurrence of all taxa with hard, identifiable head capsules. Great variation in estimates of taxonomic makeup of diet and niche breadth based on method and metric choice suggest that analysis choice can greatly impact results, and thus it is crucial to choose analyses that best suit proposed research questions.

3.2 – Introduction

Ninespine stickleback (*Pungitius pungitius*) are ubiquitous in circumpolar Arctic watersheds, exhibiting a generalist feeding strategy with diets primarily including abundant stream invertebrates, such as zooplankton and Chironomids, and occasionally fish eggs and/or larval fishes (Gallagher & Dick, 2011; Hynes, 1950; Laske et al., 2017). Their ecological role in Arctic streams is poorly understood, as most ninespine stickleback dietary studies have been conducted in lentic environments (Gallagher & Dick, 2011; Laske et al., 2017). Given the rapid changes occurring and expected in Arctic streams because of climate change (Prowse et al., 2006; Wrona et al., 2016), it is important to characterize the ecological roles of ninespine stickleback as the species is often a top predator in Arctic freshwater ecosystems, or an intermediate energy link for fish like Arctic char (*Salvelinus alpinus*) or grayling (*Thymallus arcticus*; Gallagher & Dick, 2010; Laske et al., 2017; Mcfarland et al., 2018).

Choosing appropriate diet-tracing methods and metrics is non-trivial, as different methods and metrics present differences in the observed importance of prey types and amounts consumed, and thus different information about trophic niche (Nielsen et al., 2018; Wallace Jr., 1981). Recently, pairing of different diet analysis methods has been employed to elucidate potential methodological biases and to more accurately determine diet and the resultant measures based on diet information (Nakamura et al., 2020; Pacioglu et al., 2019; Whitaker et al., 2019). Of the many techniques available, three commonly used currently include: morphological gut-content analysis (M-GCA), DNA metabarcoding of gut-contents (D-GCA), and stable isotope analysis (SIA), but their

relative strengths and weaknesses for application in diet analyses of small stream fishes has not yet been evaluated using a comparative analysis.

In both M-GCA and D-GCA, diet items are identified from the gut of a sample organism, providing a snapshot of what the organism consumed within the past few hours to days (Hyslop, 1980; Lee et al., 2018). M-GCA is the most established method and involves visual examination of gut contents to determine what an organism consumes. In M-GCA, diet is typically quantified by frequency of occurrence (%F) measures, which categorize the percentage of gut samples a diet item was present in within the studied population; and relative abundance (%D), the percentage of gut-contents counted made up by each diet item out of all the gut-contents counted from the sampled fish (Hyslop, 1980). D-GCA on the other hand is carried out using DNA metabarcoding of gut contents, a relatively new method which identifies the presence of mitochondrial DNA that has been replicated and amplified by polymerase chain-reaction (PCR) from samples, and matches it to DNA in a reference library with an associated taxonomic identification (Deiner et al., 2017; Ratnasingham & Hebert, 2007).

While these methods examine the same gut contents, visual identification and metabarcoding each present distinct strengths and weaknesses. M-GCA is beneficial in that it provides visual proof of diet composition. However, the approach requires considerable taxonomic expertise to identify prey that are in various stages of digestive breakdown that can obscure identifications (Baker et al., 2014; Jakubavičiūtė et al., 2017). In contrast, D-GCA can often reliably provide identifications to genus and species even when digestion prevents visual identification of morphological characteristics (Harms-Tuohy et al., 2016; Jakubavičiūtė et al., 2017), and can yield results for analytical

use without extensive taxonomic training. In contrast, relative read abundance of the DNA cannot always reliably provide an accurate portrayal of prey relative abundance in the diet, in which case only occurrence of prey items in the diet of the sample may be determined, providing a limited view of the relative importance of prey contributions (Harms-Tuohy et al., 2016; Jusino et al., 2019). DNA metabarcoding also has greater difficulty matching the DNA of ingested prey as digestion state advances (e.g., Martínez-de la Puente et al., 2013; Moran et al., 2015), and universal primers can have difficulty identifying highly diverse groups, such as zooplankton (Deagle et al., 2014; Zhang et al., 2018). Both D-GCA and M-GCA suffer from biases in their abilities to identify specific prey types. M-GCA is biased towards hard-bodied organisms as their diagnostic characteristics take longer to break down during digestion (Berry et al., 2015; Hyslop, 1980), whereas D-GCA is biased towards soft-bodied organisms as soft-tissue releases DNA more readily than exoskeletal structures (Li et al., 2011; Martins et al., 2021). While pairing the two analyses can counteract such biases, both techniques only provide short-term information on diet, which can create barriers to developing a full understanding dietary niche breadth and/or tropic relationships in northern and remote sampling locations where frequent sampling is infeasible.

Because many organisms undergo seasonal and ontogenetic changes in diet (Ahlbeck et al., 2012; Hayden et al., 2014), samples of M-GCA or D-GCA from a single time period cannot account for temporal shifts in diet or estimate the overall seasonal importance of a given prey item to a consumer. SIA, however, provides information on what an organism has consumed and assimilated over a period of weeks to months, depending on organism growth rate and the tissue from which SIA is determined

(Boecklen, 2011; Hayden et al., 2014; Whiteman et al., 2019). SIA involves sampling tissue from the consumer of interest and suspected prey items from the consumer's habitat to calculate the $\delta^{13}C$ and $\delta^{15}N$ isotope signatures of their tissue. The carbon isotope ratios of organic tissues change little with trophic transfer and may be used to characterize consumer reliance on dietary carbon sources (i.e., primary producers) within an ecosystem (Deniro & Epstein, 1981; Post, 2002) Nitrogen isotope ratios change at a relatively consistent rate with each trophic transfer (2.54-3.4 ‰; Perkins et al., 2014; Vanderklift & Ponsard, 2003) and provide a useful tool for determining organism trophic position within a food-web (Peterson and Fry, 1987). Trophic niche, trophic position, and estimated contributions to diet by prey items can also be calculated using Bayesian stable isotope mixing models and food-webs can be easily visualized using stable isotope biplots (Layman et al., 2007; Layman et al., 2012). While SIA provides an accurate view of an organism's diet over time, the analysis does not provide distinct identification of consumed taxa and may, therefore, discount or miss rarely consumed taxa. Furthermore, SIA relies on an *a priori* understanding of the organism`s diet and sampling of the correct potential prey taxa to make inferences about probable proportional contribution of prey to the diet with mathematically-based models (Pacioglu et al., 2019; Parnell et al., 2013). If an important diet item is overlooked during sampling, the resulting modelling inferences regarding diet could be misleading or wrong. In addition, suspected prey taxa may have similar isotopic ratios, which decreases the ability of Bayesian mixing models to accurately attribute dietary contribution estimates among putative prey taxa (Layman et al., 2012).

Given the distinct advantages and disadvantages of each method, employing two or more methods has the potential of increasing overall confidence in dietary analyses, particularly for species from remote environments where sampling opportunities are limited. For Arctic freshwater species such as ninespine stickleback (*Pungitius pungitius*), dietary studies have been limited, particularly for stream-resident populations, because of such sampling limitations. In this study, M-GCA, D-GCA and SIA methods were used to investigate ninespine stickleback population diets from two Arctic tundra streams with the aims of (1) characterizing their diets in Arctic tundra streams and (2) assessing the relative benefits and disadvantages of each technique for the dietary study of small stream fishes. It was predicted that (i) M-GCA would identify hard-bodied organisms more frequently than D-GCA, (ii) D-GCA would identify soft-bodied organisms more frequently than M-GCA, and (iii) SIA would estimate abundant benthic macroinvertebrate taxa (e.g., sub-families of Chironomidae) as the primary contributors to long-term diet.

3.3 – Methods

3.3.1 – Sample design

Ninespine stickleback and invertebrates were sampled from two streams (2-ERA3 and 2-CB30) within the Greiner Lake watershed (69° 13.145' N, 104° 51.911' W) near Cambridge Bay, Nunavut. These streams were chosen from a set of streams sampled in 2019 based on the greater number of ninespine stickleback found that were determined to be of sufficient size for dissection (n = >15 stickleback >35mm long). The diets of ninespine stickleback sampled from these populations were compared using three different types of dietary analyses: morphological gut-content analysis, DNA

metabarcoding, and stable isotope analysis. The same individual fish were used for each method of diet tracing to ensure that all results were directly comparable.

3.3.2 - Sample collection and processing

Approximately 50 m of each stream was fished in a zig-zag pattern using a Smith-Root LR-24 electro-fisher (Smith-Root Inc., Vancouver, WA) and a hand-held dipnet with a 5 mm mesh. All collected fish were euthanized and preserved in 95% ethyl alcohol immediately upon removal from each stream. Fish were frozen at a constant temperature (-20 °C) prior to shipment to the University of Waterloo for processing. The benthic invertebrate community within each stream was concurrently sampled following the Canadian Aquatic Biomonitoring Network (CABIN) protocol, where the collector places a kick-net with a 400 μm mesh on the substrate facing upstream and disturbs the substrate in front of the net while moving in a zig-zag pattern backwards upstream for three minutes (Environment Canada, 2012). The full contents of the net were preserved in 95 % ethyl alcohol immediately upon capture.

Prior to dissection, all fish were measured to the nearest 1 mm. Only fish equal to or greater than 35 mm in total length were used to ensure sufficient tissue sample material for the required analyses was available from all individuals. To ensure no cross-contamination of gut-contents or DNA, all dissecting tools and materials used for handling digestive tracts and gut-contents were cleaned with Kimwipes (KimTech) and ELIMINase (Decon Labs, Inc), then rinsed with de-ionized water prior to dissection or handling of individual samples. Whole digestive tracts were removed with tweezers via an incision made between the anus and base of the head of each fish. After removal, digestive tracts were preserved in 95% pure ethanol and stored at a constant -20 °C in 1.5

mL snapcap vials. The head and tail of the fish and remaining organs were removed, and the residual whole abdomen dried at a constant 50 °C for a minimum of 72 hours.

Invertebrate kick-net samples were identified to the finest possible taxonomic level and enumerated according to the CABIN lab protocol by a Society of Freshwater Science (SFS) certified taxonomist (Environment Canada, 2014). Invertebrates were sub-sampled with a Marchant (1989) box, after which cells were randomly selected and all invertebrates within the selected cells were identified until at least 300 individuals were counted. Samples were sorted (sorting accuracy >95%) and identified using an Olympus SZX16 stereo microscope (Olympus, Tokyo, Japan). Sorted invertebrates were preserved in 80% ethanol. Unsorted sample material was later examined separately to find Copepoda, Cladocera, and Ostracoda tissue for SIA; with sufficient amounts found for Copepoda and Ostracoda from 2-ERA3, and none from 2-CB30. Three separate bulk sub-samples were created for each chosen taxa with the goal of obtaining 0.3 mg of dried material required for SIA. Non-insect invertebrates were sorted within taxonomic classes (i.e., Arachnida and Oligochaeta). Insects were homogenized within taxonomic families (i.e., Simuliidae and Tipulidae) except for individuals within the family Chironomidae. Due to the large proportion of the benthic community made up by Chironomidae at both 2-ERA3 and 2-CB30, individuals from this family were sorted to sub-family to capture within-family variation in the community.

3.3.3 – Morphological gut content analysis (M-GCA)

Digestive tracts (hereafter referred to as guts) and ethanol were flushed into a small glass dish with 95% ethanol. The gut was pulled open with tweezers, emptied, then placed back into its original vial with fresh 95% ethanol. All contents from each gut

were identified and enumerated under a Nikon SMZ1000 stereo dissecting microscope (Nikon Instruments Inc., Melville, NY) at 20-80x magnification. When a diet item was too small to identify under a dissecting scope (i.e., differentiating between some Diamesinae and Orthocladiinae), the item was pressed onto a microscope slide with deionized water and examined under a Nikon Eclipse 50i stereo compound microscope (Nikon Instruments Inc., Melville, NY) at 100-400x magnification. If no head was attached to the remains, multiple identifications of the taxa would only be counted if enough remains of the identified organism could be found to confirm that there was more than one of these organisms in the gut. If anything found in the gut could not be counted as a diet item (i.e., unidentifiable tissue, plant material, parasites, or sediment), it was counted as "unidentified material". After every 50 identifications, microscope photos were taken of the identifying characteristics of five identified organisms and sent to a SFS certified taxonomist for quality assurance. Photos were taken using a Nikon Digital Sight DS-Fi1 microscope camera and NIS-Elements D 3.1 photography software (Nikon Instruments Inc., Melville, NY). After identifying and enumerating all the contents of each digestive tract, the contents and any remaining digestive tract lining were placed back into the original vial and returned to storage at a constant -20 °C. Results are reported as both the average proportional abundance of each diet item, as well as the proportion of stomachs at each stream in which a taxon was detected. Organisms subsequently referred to as hard-bodied are those with a hard head-capsule and/or other structure(s) on their body (i.e., Chironomidae, Simuliidae, Tipulidae), whereas organisms referred to as soft-bodied are those lacking any hard structures (i.e., Oligochaeta).

3.3.4 – DNA metabarcoding (D-GCA)

DNA metabarcoding of the digestive tract lining and gut-contents were conducted at the Canadian Centre for DNA Barcoding (CCDB) at the University of Guelph following their standard operating procedures (Moran et al., 2019), with DNA extracted using a validated glass fibre plate technique (as in Ivanova et al., 2006). Samples were lysed overnight at 56 °C using 500 μL of invertebrate lysis buffer and 2 mg mL^{-1} of proteinase K (Promega). Arthropod and annelid specific primers (*ZBJ-ArtF1c_t1/ZBJ-ArtR2_t1* and *Prey_AnnelidF1_t1/Prey_AnnelidR1_t1*) were used to extract two DNA replicates for each sample, which respectively targeted 157 and 193 base-pair (bp) fragments of the cytochrome c oxidase subunit I mitochondrial DNA. Two PCR replicates were conducted for each DNA replicate, for a total of 4 replicates for each sample. Pre-cast 2% agarose e-gels (Thermofisher) were used to visualize PCR results. Dual indexing was performed using forward primers tagged with IonXpress 1-96 universal molecular identifiers and reverse primers with unique ion tags. An Ion Torrent S5 Plus (ThermoFisher Scientific, Waltham, MA) was used for DNA sequencing. Resulting sequence reads were associated with their source samples via UMIs (reads lacking a forward primer were excluded), trimmed to remove primer and adapter sequences, and filtered to remove any sequence reads with a quality below QV20 or size below 100 bp. Processed reads were then compared to the Barcode of Life Data System (BOLD) reference library (www.boldsystems.org), identified using the internal basic local alignment search tool (BLAST) algorithm, and converted into unique taxonomic identifications. The BOLD reference library is the largest of its kind and contained all taxa identified within the guts by M-GCA. Due to the high number of identified

sequences with <100 reads per sample; taxonomic identifications were accepted as genuine if they were supported by a minimum of 20 cumulative reads across all replicates for each sample. Identifications were only accepted as accurate to the level of genus due to the relatively small length of most base-pairs used for identification, based on the recommendations of the CCDB (project summary report, Sarah Dolynskyj, 2021). Any *Pungitius* DNA identified was excluded from further analysis. Results are reported as the proportion of stomachs at each stream in which a taxon was detected.

3.3.5 – Stable isotope analysis (SIA)

Dried fish tissue samples were homogenized with a mortar and pestle, and dried invertebrate samples were homogenized within their respective vials via pulverization with a stainless-steel probe. Material was weighed in tin capsules (target weight 0.3 mg) using an analytical balance (XP205 DeltaRange, Mettler-Toledo GmbH, Greifensee, Switzerland). The capsule samples were analyzed for $\delta^{13}C$ and $\delta^{15}N$ stable isotope ratios at the University of Waterloo's Environmental Isotope Lab (EIL) using a Delta Plus Continuous Flow Stable Isotope Ratio Mass Spectrometer (Thermo Finnigan, Bremen, Germany) coupled to a 4010 Elemental Analyser (CNSO 4010, Costech Analytical Technologies Inc., Valencia, USA). Putative prey were similarly analysed. The resulting stable isotope ratios were expressed as ‰ deviation from the international standard reference materials of Vienna PeeDee Belemnite for Carbon (Craig, 1957), and atmospheric nitrogen for Nitrogen (Mariotti, 1983). Duplicates were run for every 12^{th} sample for quality assurance. Internal laboratory standards inserted at the beginning, middle, and end of sample runs were cross-calibrated against International Atomic Energy Agency standards for Carbon (CH3, CH6) and Nitrogen (N1, N2). Reference

materials were used in data normalization and to ensure measurement precision and accuracy, with quality control/assurance check indicating an error for reportable data of no more than 0.2‰ and 0.3‰, respectively, for $\delta^{13}C$ and $\delta^{15}N$.

3.3.6 – Statistical analyses and comparison of methods

All statistical analyses were completed using R statistical software (*version 4.1.0;* R Core Team, 2021). Levins' Index (Levins, 1968) and the standardized Levins' Index (Colwell & Futuyma, 1971) were calculated from both the frequency of occurrence (proportion of guts containing a particular taxon) and relative abundance (proportion of all invertebrates made up by a specific taxon) of invertebrates found in the gut to estimate niche width. The estimated proportional contribution of the different invertebrate taxa to ninespine stickleback diet ($\%Diet_{SI}$) in each stream was estimated using stable isotope mixing models run in the R package "simmr" (A. Parnell, 2021; R Core Team, 2020). A Bayesian estimate of the standard ellipse area (SEA_B) containing 40% of the data was calculated for each stream to estimate niche width using the R package "SIBER" (Jackson et al., 2011). A non-metric multidimensional scaling (NMDS) plot and a permutational multivariate analysis of variance (PERMANOVA) were used to compare dissimilarities between $\%F_{vis}$ and $\%F_{bar}$ at each stream ($\alpha = 0.05$). Similarity percentage (SIMPER) tables were subsequently created to examine which taxa had the greatest dissimilarities in frequency of occurrence between M-GCA and D-GCA. All NMDS, PERMANOVA and SIMPER functions analyses were completed using the "vegan" package and base R routines (Oksanen et al., 2020; R Core Team, 2021). Analyses of variances (ANOVA) and F tests were conducted using base R routines (R Core Team, 2021). Functions from the "tidyverse" package were instrumental to all data

organization, and all figures were created using "ggplot2" (Wickham, 2016; Wickham et al., 2019). All tables were created using the "stargazer" package (Hlavac, 2018).

3.4 – Results

3.4.1 – Stream fish populations and invertebrate community

The abundance and composition of benthic invertebrates varied between the sample sites. An estimated 12080 total macroinvertebrates were caught in kick-net samples at 2-CB30, as opposed to 3220 at 2-ERA3 (based on whole sample estimates of Marchant box sub-sample abundances). Invertebrate communities were dominated by Chironomid larvae (94% of the population at 2-CB30 and 70% at 2-ERA3; Table 3.1). The four taxa making up the largest proportion of the population at 2-CB30 were Orthocladiinae, Chironominae, Diamesinae and Tanypodinae; as opposed to 2-ERA3 with Orthocladiinae, Chironominae, Oligochaeta, and Arachnida being most abundant.

Table 3.1 – Percentage abundance (%) of stream invertebrate community made up by each taxonomic group, and total abundance of invertebrates collected at each stream.

Taxa	2-CB30 (%)	2-ERA3 (%)
Orthocladiinae	61.42	41.93
Chironominae	24.50	24.84
Diamesinae	4.80	0.31
Tanypodinae	3.31	2.80
Oligochaeta	2.32	14.60
Arachnida	1.82	9.32
Tipulidae	1.16	0.93
Baetidae	0.17	0
Nemouridae	0.17	0
Collembola	0	1.24
Ceratopogonidae	0	0.31
Simuliidae	0	2.80
Total invertebrate abundance	**12080**	**3220**

3.4.2 – Morphological gut content analysis (M-GCA)

Differences in invertebrate abundance between streams were reflected in M-GCA relative abundance counts, as a total of 2184 and 528 diet items were identified from stickleback guts from 2-CB30 and 2-ERA3, respectively. There were no empty stomachs at 2-CB30, with each containing an average of 109 ± 72.3 diet items. In contrast, at 2-ERA3, 1 stomach was entirely empty and 5 others were empty aside from unidentifiable material. An average of only 27 ± 54.9 diet items were found in 2-ERA3 guts, with 72.0% of items being accounted for by the high numbers of Copepods (200, 130, and 50) found in three stomachs. Diet items identified as "unidentified material" accounted for 7.4% and 7.8% of the total items, respectively, at 2-CB30 and 2-ERA3.

Based on M-GCA, the most frequently occurring taxa in the guts of ninespine stickleback were Orthocladiinae (95.0%), Copepoda (80.0%), Chironominae (80.0%), and Tanypodinae (65.0%) at 2-CB30; and Chironominae (69.2%), Orthocladiinae (53.8%), Cladocera (53.8%), and Copepoda at 2-ERA3 (38.5%; Figure 3.1). Similarly, the taxa that made up the largest proportion of the diet of each stream population on average were Orthocladiinae (57.8 ± 3.5%), Copepoda (19.2 ± 1.9%), Chironominae (14.2 ± 0.9%), and Tanypodinae (4.6 ± 0.3%) at 2-CB30 and Copepoda (80.4 ± 13.0%), Chironominae (7.2 ± 0.9%), Orthocladiinae (5.6 ± 0.5%), and Cladocera (4.1 ± 0.4%; Figure 3.2) at 2-ERA3. Diamesinae, Simuliidae and Tipulidae were only identified in guts from 2-CB30, though only a single Simuliid was identified. Arachnida and Nemouridae were only identified in guts from 2-ERA3, with a single identification of each. One terrestrial Dipteran was found in guts from each stream. A single fish gonad

was discovered in one gut from 2-CB30 but was removed from the comparative analysis as it was from an unidentified taxon.

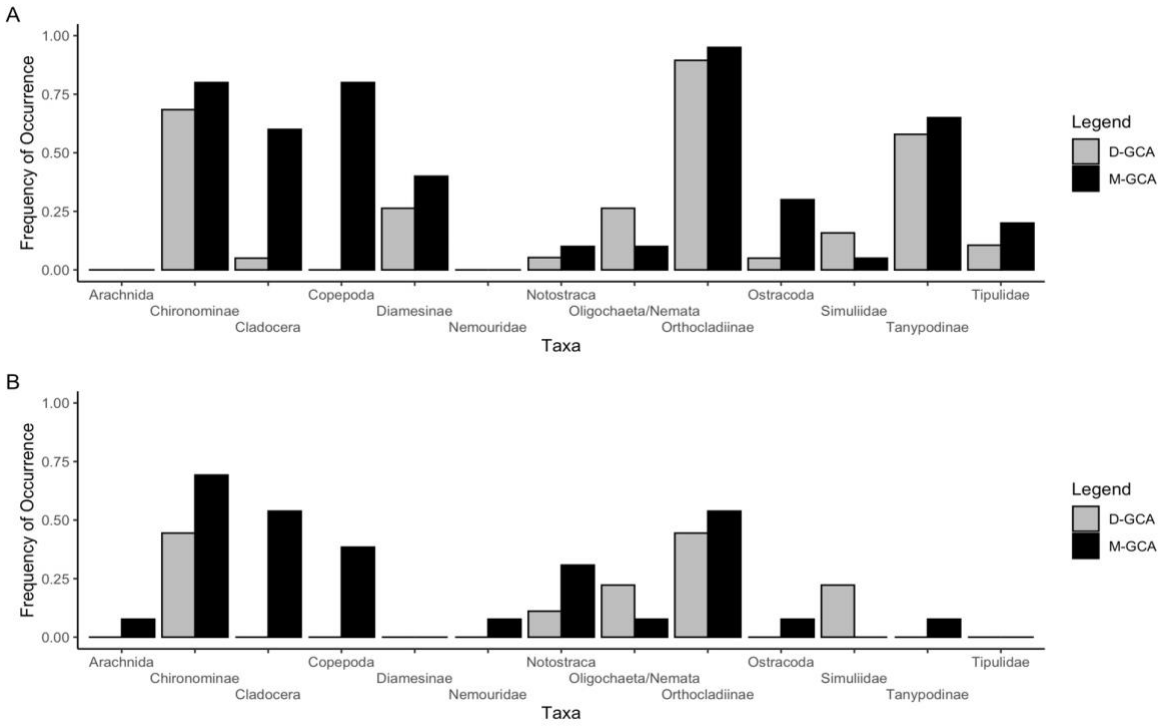

Figure 3.1 – Frequency of occurrence for invertebrate taxa found in the diet of ninespine stickleback at A) 2-CB30 and B) 2-ERA3. Black bars represent the proportion of stomachs where taxa were found using morphological gut-content analysis, and gray bars represent the proportion of stomachs where the taxa were found using DNA analysis of gut-contents.

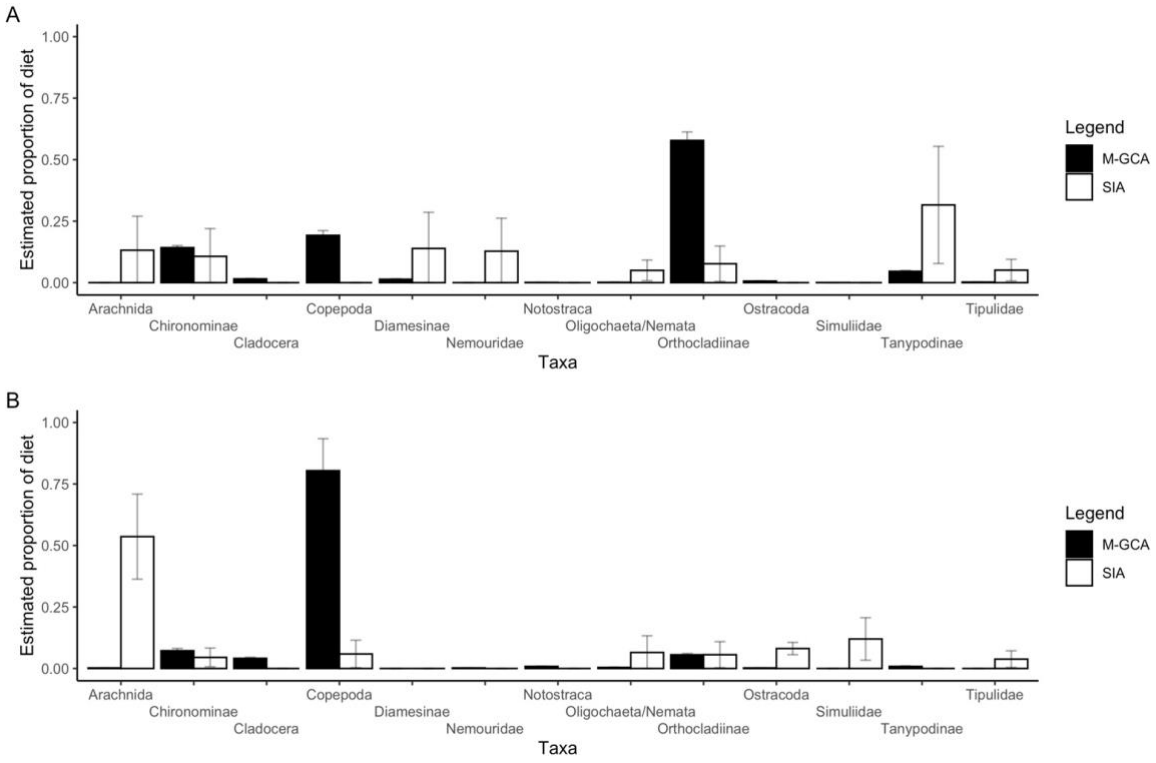

Figure 3.2 – Estimated proportional abundance of various stream invertebrate taxa in the diet of ninespine stickleback at A) 2-CB30 and B) 2-ERA3. Black bars represent the average number of individuals counted via morphological gut-content analysis, and white bars represent estimated proportion of diet from stable isotope analysis. Error bars represent standard deviation.

3.4.3 – DNA metabarcoding (D-GCA)

Across all replicates, a total of 518 successful invertebrate identifications were made from DNA contained in 19 of 20 fish guts from 2-CB30, and a total of 57 successful invertebrate identifications were made from 10 of 19 fish guts from 2-ERA3. A single sample from 2-ERA3 was removed from subsequent analysis as the only invertebrate identification from the gut was a Chironomidae whose taxonomic identification could not be further refined. Based on D-GCA, the taxa that occurred most frequently in stickleback stomachs were Orthocladiinae (89.5%), Chironominae (68.4%), Tanypodinae (57.8%), and Diamesinae/Oligochaeta (26.3%) at 2-CB30; and Orthocladiinae (44.4%), Chironominae (44.4%), Simuliidae (22.2%) and Oligochaeta (22.2%). Within these groups, the most common genera found in stickleback stomachs were *Cricotopus* (Orthocladiinae, 47.4%), *Tanytarsus* (Chironominae, 47.4%), *Procladius* (Tanypodinae, 42.1%), and *Cladopelma* (Chironominae, 36.8%) at 2-CB30 (Table 3.2). At 2-ERA3 the only genus level identification that was identified in more than 1 gut was *Paratanytarsus* (Chironominae, 20%; Table 3.2). Out of the 575 total invertebrate identifications made by DNA barcoding, 75.13% were successfully made to genus or a finer taxonomic level. DNA from Cladocera, Diamesinae, Ostracoda, Tanypodinae, and Tipulidae were only found at 2-CB30 (Figure 3.1).

Table 3.2 - Frequency of occurrence of specific taxonomic identifications made by D-GCA ($\%F_{bar}$). Finest taxonomic level means that this is the lowest taxonomic identification possible by DNA metabarcoding, likely based on DNA quality. Thus, successful identifications of genera within Chironomidae are not included within Chironomidae $\%F_{bar}$ numbers, as "Chironomidae" and "Cricotopus" (a genus of Chironomidae) may both have been identified within one gut based on different qualities of DNA identified in the metabarcoding process. Frequency of occurrence calculations included only guts that had invertebrate DNA identified within them (2-CB30 n=19, 2-ERA3 n = 10).

Taxa	2-CB30	2-ERA3
Chironomidae	0.68	0.20
Cricotopus (Orthocladiinae)	0.47	0.10
Tanytarsus (Chironominae)	0.47	0.00
Orthocladiinae	0.42	0.30
Procladius (Tanypodinae)	0.42	0.00
Cladopelma (Chironominae)	0.37	0.00
Psectrocladius (Orthocladiinae)	0.37	0.10
Nanocladius (Orthocladiinae)	0.32	0.00
Lumbriculus (Oligochaeta)	0.26	0.10
Paratanytarsus (Chironominae)	0.26	0.20
Pseudokiefferiella (Diamesinae)	0.26	0.00
Tanypodinae	0.26	0.00
Corynoneura (Orthocladiinae)	0.21	0.00
Chironominae	0.16	0.10
Orthocladius (Orthocladiinae)	0.16	0.00
Parametriocnemus (Orthocladiinae)	0.16	0.00
Simulium (Simuliidae)	0.16	0.10
Conchapelopia (Tanypodinae)	0.11	0.00
Polypedilum (Chironominae)	0.11	0.00
Tipula (Tipulidae)	0.11	0.00
Eurycercus (Cladocera)	0.05	0.00
Heleniella (Orthocladiinae)	0.05	0.00
Lepidurus (Notostraca)	0.05	0.10
Rheotanytarsus (Chironominae)	0.05	0.00
Synorthocladius (Orthocladiinae)	0.05	0.00
Tokunagaia (Orthocladiinae)	0.05	0.00
Tonnacypris (Ostracoda)	0.05	0.00
Tvetenia (Orthocladiinae)	0.05	0.00
Cladotanytarsus (Chironominae)	0.00	0.10
Enchytraeidae (Oligochaeta)	0.00	0.10
Henlea (Oligochaeta)	0.00	0.10
Hydrobaenus (Orthocladiinae)	0.00	0.10
Metacnephia	0.00	0.10
Simuliidae	0.00	0.10

3.4.4 – Stable Isotope Analysis (SIA)

Based on stable isotope analysis, taxa with the largest estimated proportional contribution to ninespine stickleback diet were Tanypodinae (31.6 ± 23.8%), Diamesinae (13.9 ± 14.7%), Arachnida (13.2 ± 13.8%), and Nemouridae (12.8 ± 13.4%) at 2-CB30, and Arachnida (53.6 ± 17.3%), Ostracoda (9.1 ± 2.5%), Simuliidae pupa (6.6 ± 6.9%) and Oligochaeta (6.5 ± 6.8%) at 2-ERA3 (Table 3.3). $\delta^{13}C$ and $\delta^{15}N$ values of ninespine stickleback tissue averaged -28.36 and 10.57, and -25.28 and 9.10 at 2-CB30 and 2-ERA3 respectively (Figure 3.3). $\delta^{13}C$ among invertebrates sampled at both sites ranged between -27.58 to -24.74 at 2-CB30 and -33.50 to -14.43 at 2-ERA3. $\delta^{15}N$ among invertebrates ranged between 1.87 and 6.05 at 2-CB30 and 0.68 and 6.56 at 2-ERA3. There was greater variation in invertebrate $\delta^{13}C$ sampled at 2-ERA3 (3.68‰) than at 2-CB30 (0.99‰), with the difference being statistically significant ($F = 0.31$, $p < 0.01$). At 2-ERA3, Ostracoda was the only taxonomic group with a distinctly different $\delta^{13}C$ signature than the rest of the invertebrates, without which the standard deviation of all invertebrate samples decreased to 1.76‰. Variation in $\delta^{15}N$ between invertebrate groups was statistically similar ($F = 0.88$, $p = 0.77$) at both sites (2-CB30: 1.30‰, 2-ERA3: 1.47‰).

Table 3.3 - Bayesian mixing model estimations for relative diet contributions to ninespine stickleback by various invertebrate taxa at 2-CB30 and 2-ERA3.

Taxa	2-CB30	2-ERA3
Tanypodinae	0.316 ± 0.238	N/A
Diamesinae	0.139 ± 0.147	N/A
Arachnida	0.132 ± 0.138	0.536 ± 0.173
Nemouridae	0.128 ± 0.134	N/A
Chironominae	0.107 ± 0.113	0.045 ± 0.038
Orthocladiinae	0.077 ± 0.072	0.056 ± 0.053
Tipulidae	0.051 ± 0.044	0.038 ± 0.034
Oligochaeta	0.05 ± 0.042	0.065 ± 0.068
Ostracoda	N/A	0.081 ± 0.025
Simuliidae (pupa)	N/A	0.066 ± 0.069
Copepoda	N/A	0.059 ± 0.056
Simuliidae	N/A	0.054 ± 0.052

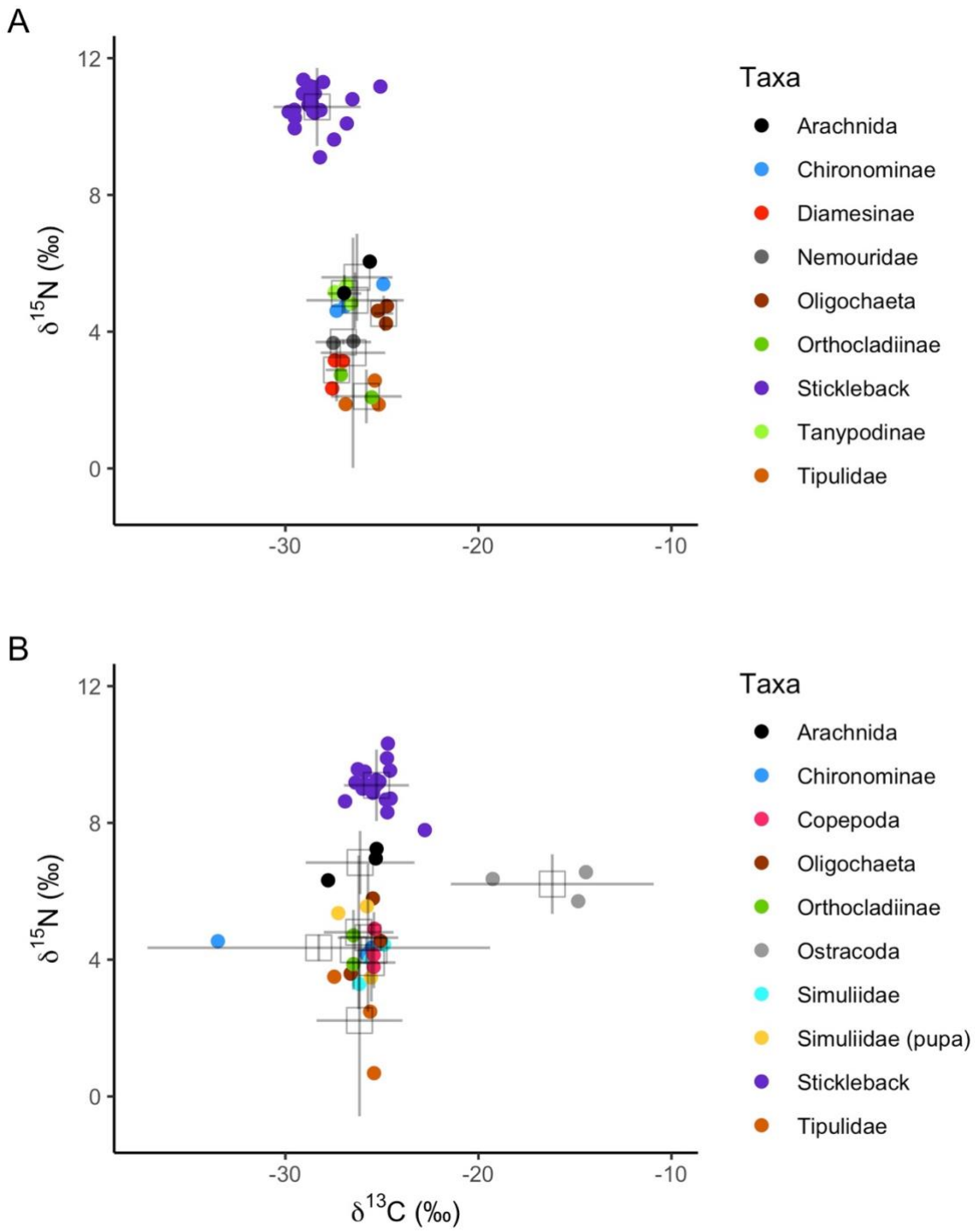

Figure 3.3 – Stable isotope biplots for stickleback and their putative prey from 2-CB30 (panel A) and 2-ERA3 (panel B). Unfilled squares denote the mean $\delta^{13}C$ and $\delta^{15}N$ values for each taxa and bars represent standard deviation.

3.4.5 – Comparative results

3.4.5.1 – Frequency of occurrence differences

NMDS indicated that the occurrence of invertebrate taxa in the gut contents as revealed by M-GCA and D-GCA differed between methods at both sampling sites (Figure 3.4). A PERMANOVA showed a significant difference between M-GCA and D-GCA frequency of occurrence results at 2-CB30 ($F_{1,37} = 8.43$, $p = < 0.01$), but not at 2-ERA3 ($F_{1,20} = 2.06$, $p = 0.10$). A similarity percentages (SIMPER) ranking showed that differences in frequency of occurrence of Copepoda and Cladocera between GCA and D-GCA at 2-CB30 were the most significant drivers of dissimilarity (average dissimilarities 0.106 and 0.072 respectively; Table 3.4). The greatest drivers of dissimilarity between M-GCA and D-GCA frequency of occurrence estimates at 2-ERA3 were Chironominae, Orthocladiinae, Cladocera, and Notostraca; though only the dissimilarity in Cladocera frequency of occurrence was significant (Table 3.5). 1 gut at 2-CB30 and 3 at 2-ERA3 had invertebrates identified in them by M-GCA that were not identified by D-GCA.

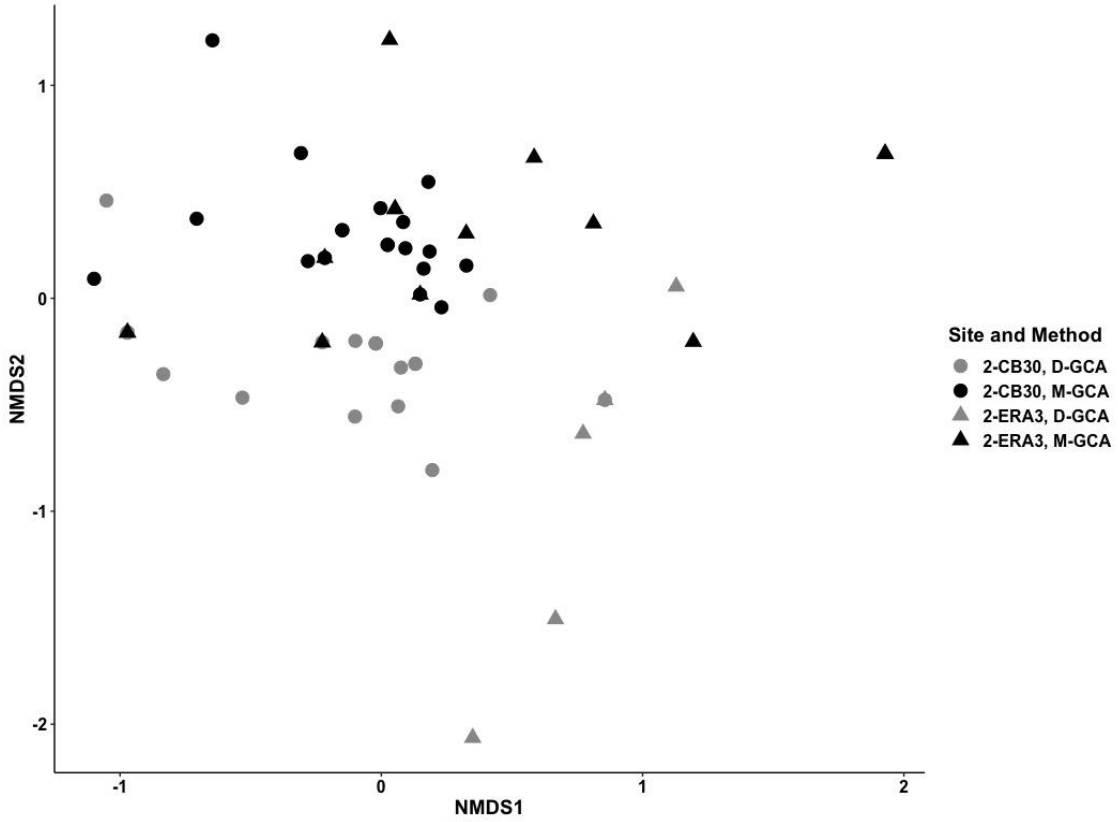

Figure 3.4 – NMDS plot comparing frequency of occurrence of invertebrate taxa as reported by DNA analysis of gut-contents, and frequency of occurrence of taxa as reported by morphological gut-content analysis at both study sites (2-CB30 and 2-ERA3).

Table 3.4 – Similarity percentages (SIMPER) for differences in frequency of occurrence of taxa found between D-GCA and M-GCA at 2-CB30. For each taxa, data columns respectively represent average dissimilarity, standard deviation of contribution to dissimilarity, sd ratio, average proportion of occurrence per group (D-GCA and M-GCA), ordered cumulative contribution to dissimilarity, and p-value. Asterisks represent where difference in frequency of occurrence between methods was significant.

Taxa	Average	sd	ratio	avD-GCA	avM-GCA	cumsum	p-value
Copepoda	0.106	0.066	1.607	0	0.800	0.200	0.0001*
Cladocera	0.072	0.065	1.105	0.053	0.600	0.335	0.0003*
Tanypodinae	0.066	0.071	0.924	0.579	0.650	0.460	1.000
Chironominae	0.059	0.079	0.753	0.684	0.800	0.572	0.998
Diamesinae	0.057	0.068	0.845	0.263	0.400	0.680	0.576
Ostracoda	0.040	0.062	0.647	0.053	0.300	0.756	0.033*
Tipulidae	0.037	0.069	0.535	0.105	0.200	0.825	0.187
Oligochaeta	0.033	0.051	0.653	0.263	0.100	0.889	0.981
Orthocladiinae	0.022	0.058	0.385	0.895	0.950	0.931	0.988
Simuliidae	0.021	0.045	0.470	0.158	0.050	0.971	0.995
Notostraca	0.015	0.038	0.395	0.053	0.100	1	0.275
Arachnida	0	0		0	0	1	1
Nemouridae	0	0		0	0	1	1

Table 3.5 – Similarity percentages (SIMPER) for differences in frequency of occurrence of taxa found between D-GCA and M-GCA at 2-ERA3. For each taxon, data columns respectively represent average dissimilarity, standard deviation of contribution to dissimilarity, sd ratio, average proportion of occurrence per group (D-GCA and M-GCA; avDNA), ordered cumulative contribution to dissimilarity (cumsum), and p-value. Asterisks represent where difference in frequency of occurrence between methods was significant.

Taxa	Average	sd	ratio	avD-GCA	avM-GCA	cumsum	p-value
Chironominae	0.133	0.141	0.941	0.444	0.692	0.181	0.305
Orthocladiinae	0.133	0.153	0.865	0.444	0.538	0.362	0.519
Cladocera	0.112	0.109	1.023	0	0.538	0.514	0.018*
Notostraca	0.102	0.159	0.638	0.111	0.308	0.653	0.145
Copepoda	0.078	0.100	0.774	0	0.385	0.759	0.066
Oligochaeta	0.058	0.102	0.568	0.222	0.077	0.839	0.358
Simuliidae	0.058	0.119	0.485	0.222	0	0.918	0.153
Ostracoda	0.018	0.061	0.286	0	0.077	0.942	0.416
Arachnida	0.014	0.050	0.286	0	0.077	0.961	0.413
Nemouridae	0.014	0.050	0.286	0	0.077	0.981	0.417
Tanypodinae	0.014	0.050	0.286	0	0.077	1	0.416
Tipulidae	0	0		0	0	1	1
Diamesinae	0	0		0	0	1	1

3.4.5.2 – Niche breadth comparisons

The standardized Levins' Index of niche breadth for M-GCA was calculated to be 0.51 and 0.41 for frequency of occurrence at 2-CB30 and 2-ERA3, respectively, and 0.12 and 0.04 for relative abundance (Table 3.6). The standardized Levins' Index of niche breadth for D-GCA frequency of occurrence data was 0.49 and 0.35 for 2-CB30 and 2-ERA3 respectively (Table 3.6). SEA_B was estimated to be 2.21 and 1.46 for 2-CB30 and 2-ERA3 respectively. The standardized Levins' Index calculated from the mixing model estimates were 0.59 and 0.27 for 2-CB30 and 2-ERA3 respectively. All niche breadth indices indicated a wider niche breadth for ninespine stickleback from 2-CB30 than 2-ERA3 (Table 3.6). M-GCA relative abundance data indicated the lowest standardized Levins' Index for both 2-CB30 (0.12) and 2-ERA3 (0.04). Niche breadth estimates are similar among all methods except for M-GCA D%, which estimates a notably smaller niche breadth than the rest. All methods estimated a lower niche breadth at 2-ERA3 compared to 2-CB30. SEA_B showed a proportionally smaller variation in niche width between each stream (2.21 at 2-CB30 is 51% wider than 1.46 at 2-ERA3) than shown by the Levins' index calculation of niche width created using the Bayesian mixing model results (0.59 at 2-CB30 is 119% wider than 0.27 at 2-ERA3).

Table 3.6 – Niche width indices for M-GCA, D-GCA and SIA. Standardized Levins' Index was calculated from frequency of occurrence (F%) and relative abundance (D%) data. SIA based trophic niche area represented as standard ellipse area (SEA_B) is also reported for SIA.

	Standardized Levins' Index				SEA
	M-GCA F%	**M-GCA D%**	**D-GCA F%**	**SIA D%**	**SIA D%**
2-CB30	0.51	0.12	0.49	0.59	2.21
2-ERA3	0.41	0.04	0.35	0.27	1.46

3.5 – Discussion

Ninespine stickleback are known to be generalist feeders that prey primarily on invertebrates (Hynes, 1950; Laske et al., 2017), and while the results of all three diet analyses confirmed this view, there were distinct differences among diet tracing methods with regard to identification of dominant prey items in the diet. Both M-GCA and D-GCA estimated Orthocladiinae and Chironominae to be among the most frequently occurring organisms in the diet. M-GCA estimated zooplankton to occur significantly more frequently than D-GCA, and D-GCA estimated a higher occurrence of soft-bodied prey than M-GCA. M-GCA reported a consistently higher frequency of occurrence of hard-bodied prey than D-GCA, though the difference was not statistically significant. Differences in short and long-term characterizations of diet were evident, with SIA estimated a disproportionately large contribution of Arachnids and Tanypodinae larvae to ninespine stickleback suggestive of a dietary shift having occurred at some point prior to the late summer sampling period. While all methods reported a more taxonomically diverse diet and wider niche breadth at 2-CB30 than 2-ERA3, variation in the taxonomic makeup of diet and niche breadth between methods and indices suggests that the type of analysis and metric chosen will impact the reported results.

3.5.1 – Gut-content analyses

As predicted, M-GCA and D-GCA resulted in taxonomic differences between estimated diet proportions, with the combination of methods providing the most comprehensive picture of diet. Gut content analyses suggested a high frequency of occurrence of Orthocladiinae, Chironominae, zooplankton, and Oligochaeta. D-GCA identified fewer zooplankton when compared to M-GCA. For remote locations, such as

the Arctic, the difference may depend on the absence of complete barcode libraries, as has been noted elsewhere in the literature (Makino et al., 2017), although other studies have similarly reported substantially higher identification success rates using morphological identification when compared to DNA barcoding (Meredith et al., 2021). While zooplankton were exceptionally under-represented in the D-GCA results, the BOLD database has successfully identified zooplankton in many prior studies (Montes-ortiz & Elías-gutiérrez, 2018), thus it is likely that the lack of zooplankton identifications are related to both the diverse nature of the group causing difficulties in primer amplification (Deagle et al., 2014; Zhang et al., 2018), and the leftover exoskeletons being difficult to extra DNA from (Li et al., 2011). M-GCA underestimated the frequency of occurrence of soft-bodied Oligochaeta, and D-GCA underestimated the frequency of occurrence of all organisms with identifiable hard structures (Notostraca, Tipulidae, Diamesinae, etc.). This coincides with recent findings that suggest greater strength of M-GCA in identifying hard-bodied prey due to the greater preservation of visually identifiable hard structures in the gut (Amundsen & Sánchez-Hernández, 2019), and underestimation of soft-bodied organisms due to quicker degradation of identifiable characteristics (Carew et al., 2018; Martins et al., 2021).

Due to the disproportionately high number of Orthocladiinae, Chironominae, and zooplankton found in stickleback guts compared to other taxa, the standardized Levins' Index estimated by M-GCA relative abundance counts suggested a smaller niche than that presented by M-GCA frequency of occurrence data. High relative abundances of Orthocladiinae and Chironominae in both kick-net samples and gut-contents suggest the findings of a smaller niche is directly reflective of the low diversity of these organisms

found in these stickleback's foraging grounds, which complements the established generalist nature of ninespine stickleback diet (Gallagher & Dick, 2011; Hynes, 1950; Laske et al., 2017). The differing views of niche breadth provided by the different methods and metrics, however, support the idea that inclusion of both frequency of occurrence and relative abundance information provide important, but separate, interpretations of diet (Chipps & Garvey, 2007; Laske et al., 2018). While D-GCA was only able to provide a niche metric for frequency of occurrence data, it provided a useful taxonomic perspective missed by M-GCA. Two of the most frequently occurring genera identified by D-GCA (*Cricotopus* and *Cladopelma*) were also not identified in the stream invertebrate community, which raises questions about specific feeding vs sampling location for these ninespine stickleback. While M-GCA identification was not attempted to genus in this study, the advantage of D-GCA in producing finer taxonomic identifications than M-GCA on the gut-contents of small fish is well supported by the findings of Jakubavičiūtė et al. (2017), who showed that DNA metabarcoding was able to more finely identify diet items than M-GCA on the gut contents of threespine stickleback (*Gasterosteus aculeatus*). This is especially useful when the goal is to determine if a specific taxa is being consumed, or in this case where fine taxonomic comparisons can be made to invertebrate community samples. Together, however, the two techniques yielded greater diversity within the diet than either technique alone, a trend noted in other comparitive studies of morphological and DNA-based methods (e.g. Chain et al., 2016; Meredith et al., 2021).

3.5.2 – Long-term diet analysis

In stark contrast to the short-term diet results presented by morphological and DNA metabarcoding gut-content analyses, SIA estimated a significant importance of predatory taxa, specifically Arachnids and Tanypodinae. In fish, muscle tissue stable isotope signatures have been found to turnover at a rate upwards of 3-6 months (Hayden et al., 2014; Nielsen et al., 2018), so this suggests a shift in diet having occurred over the course of the summer. The estimated differences in relative prey contribution to diet were also represented in the wider niche breadth calculated using SIA data, as opposed to M-GCA relative abundance data which were consistent with prey abundance within the stream community. Arachnids and Tanypodinae were less prevalent in the kick-net samples than Orthocladiinae and Chironominae, which were the primary prey identified by M-GCA and D-GCA, but macroinvertebrate taxa are known to go through various seasonal fluxes in abundance (Graeber et al., 2013). While $\delta^{13}C$ signatures were fairly similar across taxa, Arachnids and Tanypodinae had higher $\delta^{15}N$ signatures than Orthocladiinae and Chironominae. Thus, while it is unclear whether ninespine stickleback specifically ate more Arachnids or Tanypodinae earlier in the season, it is clear that their diet shifted away from food with a higher $\delta^{15}N$ signature. Changes in seasonal prey abundance can trigger diet changes in generalist fish (Kreiling et al., 2021) which suggests Arachnids, Tanypodinae, or other predatory taxa may have been more prevalent earlier in the season. For example, the abundances of Chironomid communities associated with lotic mosses are known to be dynamic and are highly influenced by flow regimes (Nolte, 1991). which suggests higher spring availability when bankside mosses and other low-lying areas have hydrological connection to the main stream channel and

allow wider ranging spatial foraging by ninespine stickleback. Similarly, Arachnids have been found to display seasonal variation in density along streams, declining in the May through mid-summer period (Cameron & Buddle, 2017; Kato et al., 2003).

The shift in diet could also be related to movement between different environments on a short time scale, which is supported by the discovery of Chironomid genera by D-GCA that occurred frequently in the diet but were not found in the kick-net samples. Feeding environments would also differ long-term as any ephemeral tundra streams that are not spring-fed freeze solid in the winter (Huryn, 2021), causing stickleback to over-winter in deeper lentic environments. Invertebrate communities in lotic environments tend to differ taxonomically from those in lentic environments based on differing adaptations to flow and lack-there-of (Graeber et al., 2013; Harrison et al., 2004), and can differ in their stable isotope signatures based on their basal carbon and nitrogen sources (Orr et al., 2006). Thus, the stable isotope signatures of these ninespine stickleback may still partially represent their lentic diet as result of the lags associated with tissue isotopic turnover rates and the time it takes a consumer to come to isotopic equilibrium with its prey sources (Tieszen et al., 1983). Given the lack of a clear explanation for the shift in ninespine stickleback diet, further research should be conducted that incorporates multiple sampling dates, particularly early season samples that would be reflective of post-winter isotopic status.

3.5.3 – Conclusions and recommendations

The inclusion of all three dietary analysis methods improved the precision and understanding of ninespine stickleback diets. The findings presented here corroborate prior literature, with M-GCA providing a more accurate picture of diet for organisms

with hard identifiable structures, and D-GCA providing a more accurate picture for soft-bodied organisms and a finer taxonomic view of diet. M-GCA more accurately portrayed consumption of zooplankton than D-GCA and despite variable diet estimates, SIA presented evidence of a probable dietary shift. The inherent temporal constraints and differences in taxonomic resolution facilitated documentation of the disparity between short- and long-term diet analysis results and probable seasonal dietary shifts. In contrast gut-contents based methods, either morphologically or DNA based, refine estimation of the frequency of occurrence of taxa in the gut. The results further highlight the importance of combining analyses and metrics to gain a better understanding of diet. Discrepancies between diet tracing methods are not uncommon (Nielsen et al., 2018), with differences between methods being one of the reasons that multiple methods are frequently used together (Nakamura et al., 2020; Pacioglu et al., 2019; Whitaker et al., 2019). While each technique has its strengths and weaknesses, it is instructive to review the research questions that can be addressed using unique combinations of these methods. If comprehensive description of the diet of an organism is the goal, then methods that examine relative abundance of prey in the diet on both the short- and long-term should be paired, such as SIA and M-GCA, with sampling of the consumer and suspected prey sources carried out multiple times throughout the field season if possible (Davis et al., 2012; Karnovsky et al., 2008). To determine if a consumer is eating a specific organism, then M-GCA and D-GCA should be used, as they are the only methods that can provide certainty regarding the taxonomic identity of prey items and they cover each other's biases. When information on the entire food-web is required, then SIA is most appropriate as it provides long-term diet information, can be used to mathematically

model energy flow through a food-web, and can be easily paired with M-GCA to compare relative abundances of prey in the diet (Rybczynski et al., 2008; Zah et al., 2001). Diet analyses, therefore, need to be carefully selected around proposed project objectives and hypotheses to ensure unbiased conclusions by using individual or complementary methods.

3.6 – Works Cited

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CHAPTER 4: SUMMARY AND SYNTHESIS

4.1 – General summary and synthesis of findings

Despite the ubiquitous presence of ninespine stickleback in the Arctic limited research has previously been conducted on their ecology and diet in Arctic streams. This thesis aimed to address these knowledge gaps by **1)** determining the effects of tundra stream characteristics on ninespine stickleback condition and abundance, **2)** characterizing their diet, and **3)** assessing the relative benefits and disadvantages of each technique for the dietary study of small stream fishes. Chapter 2 addressed the first aim by examining the impacts of temperature, nutrient concentrations, macroinvertebrate abundance, and predator/competitor (Arctic charr) abundance on stickleback condition and abundance. Chapter 3 addressed aims 2 and 3 by utilizing M-GCA, D-GCA and SIA to estimate the diet of ninespine stickleback populations and critically compare the variation in results of these methods. This chapter will summarize and synthesize the findings of each chapter, integrate them into the broader scope of knowledge surrounding ninespine stickleback ecology in the Arctic, discuss the significance of the research, and propose future research based on findings.

Relationships between stickleback condition/CPUE and stream variables (temperature, nutrient concentrations, macroinvertebrate abundance and Arctic charr CPUE) were present but limited, likely due to the tolerance of ninespine stickleback to a wide range of environmental conditions and carry-over effects from over-wintering environments. Overall, abiotic conditions were more important in determining

condition/CPUE than food or competitor/predator abundance, with temperature being most important in determining CPUE and nutrient concentrations most important in determining condition. It may be that stickleback distribute to the most optimal lotic environments in the summer based on abiotic characteristics, while primary local adaptations are to lentic over-wintering environments where they reside most of the year.

As predicted, M-GCA was biased towards hard-bodied prey and D-GCA was biased towards soft-bodied prey, while SIA provided evidence of a diet shift, supporting the idea that combining analyses provides the most comprehensive picture of diet. Differences in diet estimates between gut-content analysis methods were most considerable with zooplankton, where M-GCA identified a high frequency of occurrence in both streams, but D-GCA identified them in only a single gut. Overall, diet estimates described a generalist diet consistent with prior literature findings (Gallagher & Dick, 2011; Hynes, 1950; Laske et al., 2017). Orthocladiinae and Chironominae were the most frequently occurring organisms in the diet, and the most abundant in the stream communities. In contrast, SIA estimated Arachnida and Tanypodinae to be the two most significant contributors to diet. Two potential explanations for this diet shift are a change in the stream invertebrate community composition over the summer, and/or prior feeding in a different environment.

While bottom-up control through greater macroinvertebrate abundance showed limited impact on ninespine stickleback condition and abundance (Chapter 2), it is also clear that ninespine stickleback consumed substantially more food when invertebrate abundance was higher instream (Chapter 3). Given these findings, the diet shift evident in the difference between gut-content analyses findings and SIA diet estimations could

also inversely be due to stickleback exhibiting top-down control on the stream invertebrate community earlier in the season, which has yet to be specifically studied on ninespine stickleback in Arctic streams. Ninespine stickleback have been found to exhibit top-down control on invertebrate communities in Arctic ponds, shifting the community structure towards smaller benthic zooplankton due to initial preferential feeding on large nektonic prey, which was subsequently reflected in a diet of smaller benthic zooplankton that were more available later in the season (Laske et al., 2017). This is consistent with our gut-content analysis findings of highly available stream taxa being highly abundant in the diet, and SIA findings estimating a different diet earlier in the season represented by taxa that were less abundant when fish were collected. The importance of M-GCA is stressed here because of the lack of zooplankton found via D-GCA. It is unclear whether D-GCA had greater difficulty identifying zooplankton due to issues with the universal primers used, lack of DNA in the gut, or a combination of the two; but based on the bias against zooplankton identifications by D-GCA present in our results, this should continue to be paired with M-GCA in the study of small stream fishes to account for this bias, especially for the study of diet in lentic environments where zooplankton are generally more abundant due to slower flow velocity (Špoljar et al., 2012; Statzner, 2008).

Though it is possible a diet shift may have occurred due to top-down control of stream invertebrates, the limited measured impact of environmental factors on ninespine stickleback condition and abundance in these streams suggest it is also likely that stickleback experience carry-over effects due to movement from a lentic overwintering environment. The stream populations of ninespine stickleback examined by McFarland

et al. (2018) spend eight months of the year over-wintering in deeper upstream or downstream lentic environments due to the streams freezing over in winter. Carry-over effects between seasons have been shown for fish, with low-quality diet impacting breeding in later months (Harrison et al., 2011). Given that winter mortality is most often directly related to both starvation and thermal stress (Hurst, 2007) and can be a regulator of fish density in streams (Reist et al., 2006; Schlosser, 1998), it is plausible that the quality of over-wintering habitat in both abiotic conditions and food abundance may have carry-over effects later represented in summer lotic stream populations of ninespine stickleback on Victoria Island. Given the common differences in invertebrate community structure between lentic and lotic environments (Graeber et al., 2013; Harrison et al., 2004), this would likely also coincide with a diet shift. Based on the proposed potential carry-over effects from over-wintering environments and potential diet shifts, there is clearly a knowledge gap associated with the behaviour and dietary patterns of ninespine stickleback in other seasons and habitats that may be important in understanding their ecology. Thus, it is important to focus on more than their summer lotic habitat to gain a full understanding of their ecological role.

4.2 – Broader picture

Ninespine stickleback populations are known to be tolerant to extreme environmental conditions and disturbance (Lewis et al., 1972; Nelson, 1968; Von Hippel et al., 2016). Previous studies have shown that ninespine stickleback can survive in a variety of adverse environmental conditions that are commonly detrimental to many other fish species such as increased sedimentation and acidity (Chiasson, 1993; Lacroix, 1987). This corroborates our findings showing the environmental conditions of streams in the

Greiner Lake watershed did not majorly affect the condition and abundance of ninespine stickleback in these streams. While these environmental factors appear to have limited impact on condition and abundance, they may indirectly impact ninespine stickleback diet. Nutrient enrichment can affect prey composition in fish diet and stimulate growth (Milbrink et al., 2008; Peterson et al., 1993). Temperature was estimated to have the highest correlation with ninespine stickleback abundance in our study. Temperature has also been known to impact fish feeding rates (Behrens & Lafferty, 2007), and both temperature and nutrient enrichment can influence basal food-web structure and function which can impact prey composition in fish diet and stimulate growth (Kreiling et al., 2021; Milbrink et al., 2008; Peterson et al., 1993). All fish have a thermal range that supports optimal metabolic function and when environmental characteristics are not ideal, fish will move to habitat supporting this thermal range (Beitinger & Fitzpatrick, 1979; Reist et al., 2006). Movements between environments can inadvertently lead to a shift in diet composition based on differences in prey community composition between habitats (Garcia et al., 2018; Graeber et al., 2013; Harrison et al., 2004). Thus, the combined effects of environmental conditions on prey communities, fish metabolic function, and fish behaviour may inadvertently have various indirect effects on fish diet. Environmental conditions may also impact differences in diet analysis results. Given the effects of temperature on metabolic function, temperature can effect the speed at which food gets digested (Behrens & Lafferty, 2007) which may affect the time in which diet items last in the gut and can be identified by gut content analyses. Furthermore, the effects of temperature on metabolism also have known effects on both isotopic turnover

rates and discrimination factors (Bloomfield et al., 2011), leading to variation in how diet and associated diet shifts are presented as stable isotope signatures.

Recent research has shown surface-water connectivity to be an important driver of food-web complexity in the Arctic (Laske et al., 2019). There is a large degree of variation in how fish make use of streams in the Arctic throughout the summer, with some entering briefly due to prospecting behaviour, and others spending longer periods in streams to forage or migrate (Haynes et al., 2014; Heim et al., 2019); but leaving during the winter to survive in deeper waters (Mcfarland et al., 2018). The complex relationship in how fish use lentic and lotic environments, the environmental drivers that cause fish to move between them in the Arctic, and the subsequent effects these movements can have on fish suggest that both types of environments may be important for adaptation and survivorship, and therefore should be studied to fully understand the role of ninespine stickleback in these interconnected stream-lake ecosystems. Given the various taxonomic and time-scale biases inherent in each diet analysis technique and the intricacies involved in accurately capturing diet as it shifts, diet analysis combinations should be carefully considered in future research that incorporates multiple environments.

4.3 – Significance of research

Climate change is occurring at an accelerated rate in the Arctic, causing streams to undergo a variety of physical-chemical and hydrological changes that influence fish and invertebrate communities (Prowse et al., 2006; Rouse et al., 1997; Wrona et al., 2016). Consequently, it is pertinent to continue research that will deepen our understanding of Arctic freshwater ecosystems and the ecological role of organisms within them so that predictions can be made about how future shifts will affect ecosystem

dynamics. This study complements and expands on other Arctic stream ecosystem studies that incorporate ninespine stickleback (such as McFarland et al., 2018), and subsequently aids in building an understanding of their ecological role in Arctic freshwater food webs. The diet analysis comparisons will also further aid in the study of Arctic stream food-webs by providing other researchers with evidence of how they compare when used on small Arctic stream fishes. This thesis will support the development of other research projects currently being conducted in the Greiner Lake watershed, including one examining the flow of energy through stream food-webs using stable isotopes, and another examining Arctic stream metabolism. The findings and subsequent speculations presented here will also contribute to the development of important hypotheses that can be applied to freshwater systems across the Arctic.

4.4 – Further research

While correlations between ninespine stickleback condition and CPUE were examined across the whole watershed, a comprehensive diet analysis of ninespine stickleback could only be conducted on populations from two streams due to the sheer amount of work needed to conduct three separate diet analyses. A planned future paper will further synthesize these two perspectives by broadly examining correlations between ninespine stickleback diet and environmental factors in streams across the Greiner Lake watershed, with the goal of answering questions related to how temperature, nutrient availability, and proximity to upstream lakes impact the frequency of occurrence of various taxa in ninespine stickleback diet. Future studies on ninespine stickleback in tundra streams should specifically examine the degree to which they exhibit top-down control on the stream macroinvertebrate community. Future research on lotic stickleback

populations with connected lentic environments should focus on determining how the characteristics of over-wintering environments (i.e., temperature, nutrient concentrations, depth, invertebrate community composition) impact the condition, abundance, and diet of summer stickleback populations. This study should include sampling of the stream and both upstream and downstream lentic environments multiple times throughout the summer (right after thaw, mid-summer, and immediately prior to freeze) to gain a more comprehensive understanding of how ecological role and diet relate to surface-water connectivity. At least M-GCA and SIA should be incorporated into this study to ensure zooplankton are properly represented, with D-GCA on a few samples to account for the bias against soft-bodied prey by M-GCA.

Integrative nature of research

As with all ecological research, the work conducted within this thesis is highly integrative. The physiological effects of temperature on fish metabolism, and the effects of nutrient concentrations on stream productivity incorporate aspects of chemistry; while the stream flow that drives lotic processes is a product of physics. Chapter 3 is particularly integrative as it combines three separate diet analysis methods, two of which examine the molecular nature of tissue (DNA and stable isotopes). Without incorporating aspects of outside fields, it would have been impossible to accomplish this research.

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