The Abiotic and Biotic Controls of Arctic Lake Food Webs: A Multifaceted Approach to Quantifying Trophic Structure and Function

Stephen L. Klobucar
Utah State University

Follow this and additional works at: https://digitalcommons.usu.edu/etd
Part of the Terrestrial and Aquatic Ecology Commons

Recommended Citation
https://digitalcommons.usu.edu/etd/7293

This Dissertation is brought to you for free and open access by the Graduate Studies at DigitalCommons@USU. It has been accepted for inclusion in All Graduate Theses and Dissertations by an authorized administrator of DigitalCommons@USU. For more information, please contact rebecca.nelson@usu.edu.
THE ABIOTIC AND BIOTIC CONTROLS OF ARCTIC LAKE FOOD WEBS:
A MULTIFACETED APPROACH TO QUANTIFYING
TROPHIC STRUCTURE AND FUNCTION

by

Stephen L. Klobucar

A dissertation submitted in partial fulfillment of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Aquatic Ecology

Approved:

Phaedra Budy, Ph.D.
Major Professor

Jereme Gaeta, Ph.D.
Committee Member

Nancy Huntly, Ph.D.
Committee Member

Chris Luecke, Ph.D.
Committee Member

Sarah Null, Ph.D.
Committee Member

Laurens H. Smith, Ph.D.
Interim Vice President for Research and Dean of the School of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah
2018
ABSTRACT

The abiotic and biotic controls of arctic lake food webs:
A multifaceted approach to quantifying
trophic structure and function

by

Stephen L. Klobucar, Doctor of Philosophy
Utah State University, 2018

Major Professor: Dr. Phaedra Budy
Department: Watershed Sciences

Understanding the abiotic and biotic factors controlling food webs is critical to effectively and efficiently guide management and conservation of aquatic ecosystems. In the Arctic, where the climate is rapidly warming, quantifying the extent abiotic and biotic factors determine trophic structure and function is increasingly important. I used a multifaceted approach of modeling, experimentation, and observation to quantify trophic structure and function in arctic lakes.

My research focused on fish communities and populations of arctic char *Salvelinus alpinus* in lakes of northern Alaska. First, I predicted the availability and biomass of important invertebrate prey (i.e., zooplankton) for fishes under different thermal regimes. I observed variable responses across seasons and prey species, but overall, invertebrate prey will increase due to warmer lake temperatures, allowing for concomitant increases in predator consumption. Next, I investigated the morphological and genetic diversity of arctic char populations across lake ecosystems of different fish size structures. I determined size structure of arctic char is determined primarily by biotic factors including basal primary production and arctic char density.
Following these analyses, I evaluated and quantified the trophic structure (e.g., diet, trophic position) of these char populations. I found char diets are relatively similar across populations with and without other predators, indicating differences in size structure is likely driven by density dependence, rather than distinct genetic or morphological differences. Finally, I tested and confirmed environmental DNA (eDNA) can be used to obtain quantitative estimates of both species presence and population abundance, an important finding for monitoring arctic fish populations that would otherwise be logistically challenging.

Overall, my research demonstrates the trophic structure of arctic lake ecosystems is controlled by an interaction of both abiotic and biotic factors, with the latter appearing more important. While these interactions, and trophic structure may shift in a warming climate, it is possible that organisms inhabiting arctic lakes can adapt rapidly. Managing these ecosystems for subsistence and conservation needs can be achieved across broad scales, but owing to the sheer number and diversity of lakes in the Arctic, specific goals may need to be targeted to the smaller spatial scale of lake group.
The Arctic is warming faster than any other region of the globe. To conserve and manage many thousands of lakes across arctic landscapes, scientists need to understand historic and present conditions within these lakes to predict how the lakes, and the organisms that inhabit them, may respond to a changing climate. The goal of my research was to improve our understanding of what physical, chemical, and biological factors contribute to: 1) how lake food webs are assembled; and, 2) how these food webs may change in the future. First, I used long-term observations and lab experiments to determine how fish food, including zooplankton and snails, may respond to a warming climate. I then used field measurements of arctic char (Salvelinus alpinus) body characteristics, genetic samples, and fish diets to investigate if, and potentially why, populations of arctic char across a series of lakes achieve different maximum body sizes. Finally, as a method of monitoring population-level changes of fish abundance, I collected samples of arctic char DNA in lake water to test if estimated arctic char population abundances within a given lake correspond to the amount of DNA collected.

Fish will require more food to eat as their metabolism increases with warming lake temperatures. Based on a thirty-year period of record, I determined zooplankton abundance increases in warmer years, indicating there is likely to be enough food for fishes in the future. Accordingly, zooplankton and snail abundance and development was also faster in warmer treatments of my lab experiments. My field observations indicated these are important prey items for arctic char. Small arctic char eat more zooplankton and large arctic char eat more snails, and
these observations were consistent whether or not other predators are found in the particular lake. Similarly, my analyses did not indicate morphological or genetic differences between small and large arctic char within the same lake, suggesting arctic char size structure is determined by biological characteristics, including primary productivity and arctic char density. Indeed, estimates of arctic char population abundances across a series of lakes followed a gradient of arctic char densities, and my DNA sampling corresponded with this gradient.

As there are thousands of lakes across the Arctic, my research demonstrates lake food webs, and the fishes within them, are likely to adapt to a warming climate. However, biological, chemical, and physical properties of these lakes can vary widely such that management and conservation plans may need to be developed at relatively small spatial scales across a large landscape.
ACKNOWLEDGMENTS

My dissertation research would not have been possible without the guidance, support, and assistance of a great number of people and organizations near and far. First and foremost, I am extremely grateful of my academic advisor, Phaedra Budy. The guidance she provided, and the opportunities she afforded me, allowed me to become a better scientist, a somewhat better communicator, and an overall better person. My Ph.D. committee: Jereme Gaeta, Nancy Huntly, Chris Luecke, and Sarah Null provided their valuable time, support, and insight throughout this process-- thank you, my research presented here, and my future research endeavors will be better for it.

Without support from The National Science Foundation, the USGS Utah Cooperative Fish and Wildlife Research Unit, the Ecology Center at Utah State University, the Utah State University School of Graduate Studies, and the U.S. Fish and Wildlife Service Arctic National Wildlife Refuge, my graduate research and education would not have been possible. An immense amount of logistical support was provided by the ENTIRE staff of Toolik Field Station, and the Alaska Department of Fish and Game helped with permitting. The NSF Arctic Long-Term Ecological Research program, particularly George Kling, Anne Giblin, and Dan White, provided data, support, and countless intellectual conversation throughout my research that greatly improved my research.

I am one of the lucky ones that can state they were a member of the Budy Fish Ecology Lab. Along with Phaedra, Gary Thiede and Peter MacKinnon have been there, from start to finish, in any and all capacities, to support my research endeavors, as well as guided and misguided social forays. My labmates, past and present, helped to improve various aspects of my research and provided distractions as necessary. I am indebted to all of the FEL. Additionally, the greater research community of the Department of Watershed Sciences at Utah State University fostered an exceptional learning environment, and it was a pleasure to continually be on the
winning side of the annual WATS Turkey Bowl. I thank Brian Bailey and Enid Kelley for all of
their help along the way, and keeping the Department functioning efficiently and effectively, with
strong leadership from Peter Wilcock.

My dissertation research is part of a greater team effort, particularly in the field.
Assistance was provided, often times on a volunteer basis, by the ‘Lounge One Crew’ including:
Robert Al-Chokhachy, Hank Baker, Nick Barrett, Phaedra Budy, Jereme Gaeta, Deanna
Klobucar, Jim Klobucar, Keaton Molt, Clint Muhlfeld, Brett Roper, Gary Thiede, and Dan White.
The Marine Biological Laboratory and the NSF Arctic Long-Term Ecological Research program
supported two Research Experience for Undergraduates summer technicians, Jamie Goethlich
and Levi Simmons, who were tremendous help in the field and lab. Additional laboratory
assistance was provided by many hard-working FEL technicians: T. Arnold, T. Hafen, E.
Haroldsen, A. Huish, K. Nichols, T. Larkin, R. Peterson, and R. West.

Last, but certainly not least, I thank my family and friends for their ongoing support and
encouragement. I would not be in this position in my life without the love and support of my
parents, Jim and Barb, as well as my grandparents, who always encouraged me to do what makes
me happy and instilled a work ethic that made my research possible. I am grateful for my parents’
many long haul trips to Utah, fieldwork trips to Alaska with my father, as well as their
willingness to always accommodate my dog when I was away in the field. My brother, James,
and sister, Amanda…’Beezer’, can always be counted on for support and a much needed laugh. I
would be remiss to not also thank my dog, Trout, for not only always being happy to see me and
providing needed distractions throughout this process, but also playing an instrumental role (some
would argue larger than Phaedra) in bringing me and Deanna (and Banjo) together. To Deanna,
thank you for putting up with me, supporting me in my struggles, and loving me unconditionally.
I look forward to continuing our adventures in The Last Frontier and beyond.

Stephen L. Klobucar
CONTENTS

ABSTRACT......................................................................................................................... iii
PUBLIC ABSTRACT ......................................................................................................... v
ACKNOWLEDGMENTS ...................................................................................................... vii
LIST OF TABLES ............................................................................................................... xi
LIST OF FIGURES ............................................................................................................ xii

CHAPTER

1. INTRODUCTION ........................................................................................................... 1
   References ....................................................................................................................... 6

2. A CHANGING MENU IN A CHANGING CLIMATE: USING EXPERIMENTAL AND
   LONG-TERM DATA TO PREDICT INVERTEBRATE PREY BIOMASS AND
   AVAILABILITY IN LAKES OF ARCTIC ALASKA ...................................................... 10
   Abstract......................................................................................................................... 10
   Introduction..................................................................................................................... 11
   Methods ......................................................................................................................... 15
   Results............................................................................................................................ 22
   Discussion...................................................................................................................... 25
   References....................................................................................................................... 31

3. INVESTIGATING THE MORPHOLOGICAL AND GENETIC DIVERSITY OF ARCTIC
   CHAR (SALVELINUS ALPINUS) POPULATION IN DISTINCT GROUPS OF FOOTHILL
   LAKES IN ARCTIC ALASKA ...................................................................................... 46
   Abstract......................................................................................................................... 46
   Introduction..................................................................................................................... 47
   Methods ......................................................................................................................... 51
   Results............................................................................................................................ 55
   Discussion...................................................................................................................... 57
   References....................................................................................................................... 61

4. ASSESSING THE ABIOTIC AND BIOTIC FACTORS THAT STRUCTURE LAKE FOOD WEBS
   WITH POPULATIONS OF ARCTIC CHAR (SALVELNIUS ALPINUS) IN ARCTIC ALASKA
............................................................................................................................................ 78
   Abstract......................................................................................................................... 78
   Introduction..................................................................................................................... 79
   Methods ......................................................................................................................... 82
   Results............................................................................................................................ 86
   Discussion...................................................................................................................... 88
   References....................................................................................................................... 92
5. AT THE FOREFRONT: EVIDENCE OF THE APPLICABILITY OF USING ENVIRONEMENTAL DNA TO QUANTIFY THE ABUNDANCE OF FISH POPULATIONS IN NATURAL LENTIC WATERS WITH ADDITIONAL SAMPLING CONSIDERATIONS .................................................................................................................. 109
Abstract ......................................................................................................................... 109
Introduction ..................................................................................................................... 110
Methods ........................................................................................................................... 111
Results .............................................................................................................................. 114
Discussion ......................................................................................................................... 114
References ......................................................................................................................... 118

6. SUMMARY AND CONCLUSIONS .................................................................................. 125
References ......................................................................................................................... 128

APPENDICES .................................................................................................................... 129
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 General additive mixed model backward selection results. All candidate models included a smoother of day of year (DOY) and the random effect of year</td>
<td>40</td>
</tr>
<tr>
<td>3.1 Lake morphometry and fish community composition (AC = arctic char, AG = arctic grayling, BT = burbot, LT = lake trout, SS = slimy sculpin) for study lakes</td>
<td>68</td>
</tr>
<tr>
<td>3.2 Summary of fish captured and measured for morphological traits during 2016 – 2017 from study lakes on the North Slope, Alaska</td>
<td>69</td>
</tr>
<tr>
<td>3.3 Summary statistics of PERMANOVA analyses to determine best predictors of arctic char size structure across the Fog and LTER lake groups on the North Slope, Alaska, for arctic char captured 2016 -2017</td>
<td>70</td>
</tr>
<tr>
<td>4.1 Physical and chemical conditions of study lakes near Toolik Field Station, Alaska</td>
<td>98</td>
</tr>
<tr>
<td>4.2 Catch summary for arctic char in study lakes near Toolik Field Station, Alaska</td>
<td>99</td>
</tr>
<tr>
<td>4.3 Model summary for linear mixed effects model predicting relationship of arctic char trophic position with fish length</td>
<td>100</td>
</tr>
<tr>
<td>4.4 Summary of fish sampled for diets and isotopes by lake group and species, and average diet proportion for each size of char or other species (pooled), in study lakes near Toolik Field Station, Alaska, 2014 – 2016</td>
<td>101</td>
</tr>
<tr>
<td>4.5 Schoener’s α index for diet overlap between diets of arctic char (AC) in Fog lakes (by size class), compared with arctic char in LTER lakes (by size class), arctic grayling (AG), lake trout (LT) for fish sampled in study lakes near Toolik Field Station, Alaska, 2014 – 2015</td>
<td>102</td>
</tr>
<tr>
<td>5.1 Summary of five northern Alaska study lakes and conditions during each eDNA sampling period in 2016</td>
<td>120</td>
</tr>
<tr>
<td>5.2 Summary of abundance (number of fish), density by area (fish·ha-1), and density by volume (10-3; fish·m-3) estimates for arctic char (Salvelinus alpinus) populations in five study lakes in northern Alaska</td>
<td>121</td>
</tr>
<tr>
<td>5.3 Summary statistics of linear models fit to predict eDNA concentration from known metrics of relative fish abundance across five lakes in northern Alaska</td>
<td>122</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Abundance of adult Daphnia, Daphnia eggs, and total Daphnia as measured in three treatments across three mesocosm experiments conducted at Toolik Field Station, Alaska, in 2015</td>
<td>41</td>
</tr>
<tr>
<td>2.2</td>
<td>Juvenile snails hatched, eggs produced, and total new snail abundance as measured in three treatments across three mesocosm experiments conducted at Toolik Field Station, Alaska, in 2015</td>
<td>42</td>
</tr>
<tr>
<td>2.3</td>
<td>Change in adult snail biomass as measured in three treatments across mesocosm experiments conducted at Toolik Field Station, Alaska, in 2015</td>
<td>43</td>
</tr>
<tr>
<td>2.4</td>
<td>Top: Generalized additive model lake temperature predictions at a depth of 3 m in Toolik Lake, Alaska, for a warm year and cold year as a function of day of year. Bottom: Generalized mixed effects additive model predictions of standing stock zooplankton biomass in Toolik Lake, Alaska, for a warm year and cold year as a function of day of year</td>
<td>44</td>
</tr>
<tr>
<td>3.1</td>
<td>Map of the study area in northern Alaska</td>
<td>71</td>
</tr>
<tr>
<td>3.2</td>
<td>Length-frequency histogram of arctic char captured in the Fog and LTER lakes 2014 – 2017</td>
<td>72</td>
</tr>
<tr>
<td>3.3</td>
<td>Examples arctic char found in the closed ‘Fog’ and open ‘LTER’ lakes near Toolik Field Station, Alaska</td>
<td>73</td>
</tr>
<tr>
<td>3.4</td>
<td>Principal component analyses between size classes in Lake Fog3 and Lake LTER348</td>
<td>74</td>
</tr>
<tr>
<td>3.5</td>
<td>Uncorrected morphological traits measured for arctic char in Fog and LTER lakes, 2016 – 2017</td>
<td>75</td>
</tr>
<tr>
<td>3.6</td>
<td>Logistic regression models of size class designation from model-based clustering of raw morphometric traits</td>
<td>76</td>
</tr>
<tr>
<td>3.7</td>
<td>Back-calculated size-at-age for arctic char in Fog and LTER lakes</td>
<td>77</td>
</tr>
<tr>
<td>4.1</td>
<td>Length-frequency of all arctic char sampled in Fog lakes and LTER lakes near Toolik Field Station, Alaska, 2014 – 2015</td>
<td>103</td>
</tr>
<tr>
<td>4.2</td>
<td>Linear mixed effects relationship between length and arctic char trophic position for all lakes and by lake group</td>
<td>104</td>
</tr>
</tbody>
</table>
4.3 Average diet proportion for arctic char in Fog lakes and LTER lakes
(by size class: small, medium, and large in study lakes
near Toolik Field Station, AK, 2014 – 2015

4.4 Average diet proportion for arctic char, arctic grayling, and lake trout
in LTER lakes near Toolik Field Station, Alaska, 2014 – 2015

4.5 SIBER ellipses representing arctic char trophic niche space (by size class)
for individual stable isotope measurements for each Fog lake studied near
Toolik Field Station, Alaska

4.6 SIBER ellipses representing arctic char trophic niche space (by size class or
species) for individual stable isotope measurements for each LTER lake
studied near Toolik Field Station, Alaska

5.1 Mean eDNA copies for all lakes pooled (E5, Fog1, Fog2, Fog3, Fog5) by
season for shallow and deep samples

5.2 Relationships between fish abundance, density by area, density by volume and
mean eDNA concentration across five study lakes in northern Alaska sampled
in 2016
CHAPTER 1
INTRODUCTION

Food webs and local communities are structured and regulated by abiotic and biotic factors and investigating the relative role of these factors has long been a focus of ecology (e.g., Paine 1966) that continues today (e.g., Wisz et al. 2013). Interactions between consumers and their resources create food web dynamics that can vary in complexity (Polis and Strong 1996) and are influenced by key abiotic factors (e.g., ecosystem size; Paszkowski and Tonn 2000) and biotic factors (e.g., species richness, predation; Arnott and Vanni 1993). Accordingly, the relative role of abiotic and biotic factors can have variable control when determining the distribution of species (Meier et al. 2010). Early work proposed that local communities are resultant from a series of filters (e.g., geographical, physiological), primarily influenced by abiotic conditions, that reduce the global pool of fauna to discrete, fine-scale communities (Smith and Powell 1971), and many species distribution models still focus on abiotic factors (Boulangeat et al. 2012). In fresh waters, the relative role of abiotic and biotic factors in structuring community assemblages is likely influenced by ecosystem size (Jackson et al. 2001), but biotic factors (e.g., piscivory) have been widely shown to have nearly equally as strong direct and indirect effects on fish communities (Robinson and Tonn 1989; He and Kitchell 1990; Gilliam and Fraser 2001).

However, determining the extent that abiotic and biotic factors influence lake trophic structure and function remains at the forefront of ecology, especially in a changing climate.

Freshwater ecosystems, which can act as sentinels of climate change, are often studied to determine how physical, chemical, and biological processes interact and are affected by a warmer climate (Rosenzweig et al. 2008; Williamson et al. 2009). At high latitudes, where warming is occurring faster than any other region on the globe and freshwaters cover as much as 48% of the land surface, aquatic ecosystems face increased risks (Reist et al. 2006; Riordan et al. 2006). Arctic lakes are generally less productive and diverse than temperate water bodies (Kling et al.
1992; Luecke et al. 2014). The timing and magnitude of seasonal processes will be altered as the climate continues to warm (Smol and Douglas 2007; Prowse et al. 2011). These factors, coupled with changes in periods of ice cover in the Arctic (e.g., shorter or longer growing seasons), suggests arctic lakes may be particularly sensitive to climate change (Kling 2009; Moss 2012), but the response of lakes to climate change may not be homogeneous across the landscape.

Community openness and connectivity in arctic lakes varies from completely closed (isolated lakes) to extremely open (strings of lakes connected by flowing streams). Previous studies showed that lake morphometry and landscape position predict the presence or absence of fish, and provided a prediction of fish and invertebrate species composition of those lakes sampled at a coarse scale (e.g., Hershey et al. 1999). Relative to closed lakes, lakes open to contemporary colonization and fish movement are characterized by different fish communities, trophic structures, and pathways of energy flow (biomass). Thus, the mobility of fishes, and overall connectivity of lakes across the landscape is likely to influence trophic structure and function, which can affect broad subsistence management and conservation actions.

Under a warmer climate, altered stream hydrology, increased lake temperatures, and longer growing seasons will have varying effects on open versus closed lakes. Open lakes may be more resilient to climate change effects because species can move from unfavorable to favorable habitats, but that resilience requires movement corridors to remain navigable to fishes with varying movement tendencies and swimming abilities. For example, highly-mobile arctic grayling may serve to recolonize lakes after a disturbance, and whether or not grayling are available as a trophic subsidy may determine the food-web response to warmer lake temperatures. Fish will likely grow faster and need more food under warmer temperatures (Budy & Luecke 2014), potentially enhancing the importance of trophic subsidies from streams in open lakes. Closed lakes have strong intra-specific population cycles, cannibalism, and cohort organization (e.g., Power 1978, Finstad et al. 2006). In contrast, open systems may be buffered by immigration, emigration, and interspecific interactions and thus be more stable over time (e.g.,
a ‘portfolio effect’, Schindler et al. 2010). Considered collectively, these characteristics indicate that climate change and disturbance should have much more pronounced impacts on fish population structure, dynamics, and probability of persistence in closed systems relative to open systems.

Beyond understanding the basic ecology behind the structure and function of lake food webs, understanding arctic lake food webs are important to guide management and conservation. Locally, these lakes provide important subsistence fisheries to Inupiat and Nunamiut Eskimo communities, and the effects of a warmer climate pose pertinent ecological and socio-economic questions that must be addressed in order to adapt and provide sustainable solutions. In 2001 and 2002, over 60% and 76% of households in Kaktovik used fish for subsistence, accounting for over 15,000 total pounds harvested (Alaska Department of Fish and Game 2012). In 2011, over 88% and 91% of households in Anaktuvuk Pass and Selawik utilized fish, respectively. For Anaktuvuk Pass, non-salmon fish accounted for over 89% of fish harvested in 2011, nearly 5,200 lbs. Furthermore, in 2002, residents of Anaktuvuk Pass harvested fish for subsistence in every month of the year except for January and February and over 50% of sites fished were lake ecosystems (Pedersen and Hugo 2005). In order to be sustainable, this level of subsistence harvest may have to be locally adapted as the climate warms.

The primary purpose of my dissertation research was to investigate and address existing knowledge gaps in our understanding how abiotic and biotic factors affect arctic lake food webs, particularly regarding trophic structure and function, with consideration of current and future climatic conditions. My research objectives were to: 1) quantify changes to invertebrate prey biomass and availability as a result of a warming climate (Chapter 2); 2) measure morphometric and genetic characteristics across populations of arctic char to better understand the adaptive capacity of these population when subjected to environmental change (Chapter 3); 3) evaluate fish community and trophic dynamics, specifically for arctic char, in contrasting abiotic and
biotic environments (Chapter 4); and, 4) assess the applicability of a novel survey method to detect changes in lake fish abundance across spatial and temporal scales (Chapter 5).

In Chapter 2, I used a multifaceted approach of empirical modelling, using long-term field observations, and laboratory experiments to address prey availability to predators in arctic lake ecosystems. As temperatures increase toward metabolic optima, fishes may experience increased consumption, growth, and survival (Elliot and Elliot 2010; Jeppesen et al. 2010; Budy and Luecke 2014). However, this overall increase in fish vital metrics would require lower trophic levels respond at a similar rate (i.e., they will require more food; see Winder and Schindler 2004). In this chapter, I used a laboratory mesocosm experiment and measured metrics of abundance for snails (*Lymnaea elodes*) and zooplankton (*Daphnia middendorffiana*) across three time periods (early, mid, and late season), and across three temperature and photoperiod treatments (control, increased temperature, increased temperature*photoperiod). Additionally, I used additive mixed effects models related to long-term trends in zooplankton biomass across a range of observed temperatures to predict biomass and availability in a warmer climate. This research improves our understating of the interactive effects of temperature and seasonality affect invertebrate prey biomass and my results have important implications for lake food webs, and in particular, fishes in terms of food availability.

In Chapter 3, I describe the morphological and genetic diversity of arctic char and quantify the differences within and across a subset of postglacial lakes. Postglacial lakes are often viewed as ideal systems to study adaptive processes such as resource polymorphism (e.g., Schluter 1996; Snorrason and Skulason 2004). Characteristics of postglacial lakes including relatively low species diversity and productivity, and overall high habitat segregation (e.g., littoral, pelagic) contribute to these systems as advantageous to investigate polymorphism in arctic char (Pielou 2008; Klemetsen 2010). In this chapter, I measured nine morphometric traits of arctic char that relate their ability obtain and eat prey (see Chapter 4 below). Additionally, I investigated the extent that char in these populations are genetically distinct. This research
improves our understanding of how abiotic and biotic factors may affect the adaptability and ultimate success of arctic char in a warmer climate.

In Chapter 4, I used field observations in two contrasting series of lakes to determine how abiotic and biotic factors structure lake food webs and fish communities, and in turn, how these factors affect fish diet and trophic position. In arctic Alaska, community openness and connectivity in lakes varies from completely closed (isolated lakes) to extremely open (strings of lakes connected by flowing streams), generally as a function of past glacializations and concordant geomorphic changes (Hershey et al. 1999; Hershey et al. 2006). In this chapter, I quantify predator diet and trophic position in two series of lakes: 1) lakes that are “closed,” defined by little to no surface water connection, and thus no fish emigration or immigration between lakes; and, 2) lakes that are “open,” defined by the presence of inlet and outlet streams that allow potential fish movements, at least seasonally. This research improves our understanding of how both abiotic and biotic interactions affect trophic structure and will help guide subsistence management and conservation decisions for culturally and ecologically important fish species.

In Chapter 5, I used field measurements of environmental DNA (eDNA) in conjunction with population estimates derived from long-term observations, to assess the application of obtaining quantitative estimates of relative species abundance using rapid and minimally invasive sampling. Methodologies of eDNA sampling are rapidly evolving and improving (e.g., Furlan et al. 2016), especially with regard to species detection. A next logical step towards advancing eDNA techniques would be to achieve estimates of fish abundance and biomass. In this chapter, I investigated the relationship between eDNA concentration and arctic char (Salvelinus alpinus) abundance in five well-studied natural lakes, and additionally, I examined the effects of different temporal (e.g., season) and spatial (e.g., depth) scales on eDNA concentration. The research improves our understanding of using eDNA rapidly assess relative fish abundance, especially in remote locations, and can guide future studies to improve and expand eDNA methods while informing research and management using rapid and minimally invasive sampling.
References


CHAPTER 2

A CHANGING MENU IN A CHANGING CLIMATE: USING EXPERIMENTAL AND
LONG-TERM DATA TO PREDICT INVERTEBRATE PREY BIOMASS AND
AVAILABILITY IN LAKES OF ARCTIC ALASKA

Abstract

1. Changes in seasonality associated with climate warming (e.g., temperature, growing season duration) are likely to alter invertebrate prey biomass and availability in aquatic ecosystems through direct and indirect influences on physiology and phenology, particularly in arctic lakes. However, despite warmer thermal regimes, photoperiod will remain unchanged such that potential shifts resulting from longer and warmer growing seasons could be limited by availability of sunlight, especially at lower trophic levels. Thus, a better understanding of warming effects on invertebrate prey throughout the growing season (e.g., early, peak, late) is important to understand arctic lake food web dynamics in a changing climate.

2. Here, we use a multi-faceted approach to evaluate prey availability to predators in lakes of arctic Alaska. In a laboratory mesocosm experiment, we measured different metrics of abundance for snails (*Lymnaea elodes*) and zooplankton (*Daphnia middendorffiana*) across three time periods (early, mid, and late growing season), and across three temperature and photoperiod treatments (control, increased temperature, increased temperature*photoperiod). Additionally, we used generalized additive models (GAMs) and generalized additive mixed effects models (GAMMs) to relate long-term empirical observations of zooplankton biomass (1983 – 2015) to observed temperature regimes in

---

1 This chapter is co-authored by Stephen L. Klobucar, Jereme W. Gaeta, and Phaedra Budy
2 © 2018. The authors. The full text of this article is published in Freshwater Biology 00:1-13. It is available online at https://doi.org/10.1111/fwb.13162
an arctic lake. We then simulated zooplankton biomass for the warmest temperature observations across the growing season to inform likely zooplankton biomass regimes under future change.

3. We observed variable responses by snails and zooplankton across experiments and treatments. Early in the growing season, snail development was accelerated at multiple life stages (e.g., egg and juvenile). In mid-season, in accordance with warmer temperatures, we observed significantly increased *Daphnia* abundances. However, in the late season, *Daphnia* appeared to be limited by photoperiod. Confirming our experimental results, our models of zooplankton biomass showed an increase of nearly 20% in warmer years. Further, these model estimates could be conservative as the consumptive demand of fishes may increase in warmer years as well.

4. Overall, our results highlight the importance of interactive effects of temperature and seasonality. Based primarily on temperature, we can readily predict the response of fish metabolism in warmer temperatures. However, in this context, we generally require a better understanding of climate-driven responses of important invertebrate prey resources. Our results suggest invertebrate prey biomass and availability is likely to respond positively with climate change based on temperature and seasonality, as well as proportionally to the metabolic requirements of fish predators. While further research is necessary to understand how other food web components will respond climate change, our findings suggest that the fish community at the top of arctic lake food webs will have adequate prey base in a warming climate.

1 | INTRODUCTION

Freshwater ecosystems, which can act as sentinels of climate change, are often studied to determine how physical (e.g. hydrologic regime), chemical (e.g., nutrient availability), and biological processes (e.g. primary production) interact and are affected by a warmer climate
(Rosenzweig et al., 2008; Williamson, Saros, Vincent, & Smol, 2009). At high latitudes, where warming is occurring faster than in any other region on the globe and freshwaters cover as much as 48% of the land surface, aquatic ecosystems face increased risks (Reist et al., 2006; Riordan et al., 2006). Arctic lakes are generally less productive and diverse than temperate water bodies (Kling, O’Brien, Miller, & Hershey, 1992; Luecke et al., 2014), and the timing and magnitude of seasonal processes will be altered as the climate continues to warm (Prowse, Alfredsen, Beltaos, Bonsal, Bowden, et al., 2011; Smol & Douglas, 2007). These factors, coupled with changes in periods of ice cover in the Arctic (e.g., shorter or longer growing seasons), suggests arctic lakes may be particularly sensitive to climate change (Kling, 2009; Moss, 2012). Accordingly, understanding future changes to arctic lakes is increasingly important for conserving the ecosystem services these lakes provide (e.g., habitat, water storage, fish production; Pederson & Hugo, 2005; Adrian et al., 2009).

Air temperatures of Alaska’s North Slope have been rising since the 1950s, but these changes have not occurred in all seasons and should be further considered across long-term periods of observation (e.g., Kaufman et al., 2009; Sheng Hu et al., 2003). A recent analysis of arctic climate in Alaska showed no significant long-term trend in summer or winter monthly average air temperatures, but identified potentially important trends in the spring months (colder over time) and in the fall months (warmer over time; Hobbie et al., 2017). Future projections of climate warming indicate reductions in ice duration and thickness (Prowse Alredsen, Beltaos, Bonsal, Duguay, et al., 2011) that could modify seasonal lake temperature regimes (Cahill, Gunn, & Futter, 2005) and overall photosynthetic production (Melles et al., 2007). Annual rates of production are limited by light availability due to a relatively short ice-free season (Karlsson, Jonsson, & Jansson, 2005) such that total production in arctic lakes is considerably lower than in similarly oligotrophic lakes from lower latitudes (i.e. ultra-oligotrophic). In spite of the importance of photoperiod, ample paleolimnological evidence suggests lake warming and longer
growing seasons have increased pelagic production in high latitude lakes where warming has occurred (Micheluttia, Wolfe, Vinebrooke, Rivard, & Briner, 2005).

Arctic fishes will be directly affected by higher temperatures and shifts in food availability driven by changes at lower trophic levels (Woodward, Perkins, & Brown, 2010). As temperatures increase toward metabolic optima, fishes may experience increased consumption, growth, and survival (Budy & Luecke 2014; Elliot & Elliot 2010; Jeppesen et al., 2010). However, this overall increase in fish vital metrics would require lower trophic levels to respond at a similar rate (i.e. they will require more food; see Winder & Schindler, 2004). Alternatively, fish metabolism, and ultimately, survival may respond negatively to deeper and more stable stratification due to lower hypolimnetic oxygen concentrations (Jacobsen, Stefan, & Pereira, 2010; Jankowski, Livingstone, Buhrer, Forster, & Niederhauser, 2006). Even fairly small increases in pelagic productivity increase rates of microbial respiration in the hypolimnion and decrease oxygen concentrations (Daniels, Kling, & Giblin, 2015). If fish are forced to remain in the warmer epilimnion due to oxygen stress, or if temperatures rise significantly without concordant increases in food availability, fish respiration rates could exceed energetic inputs from food consumption and ultimately result in lower vital rates. As such, even minor changes in the timing and duration of ice-free days and the associated thermal regime could manifest in substantial differences in annual fish growth (Kristensen, Jørgensen, Larsen, Forchhammer, & Christoffersen, 2006) and other vital rates that strongly co-vary with growth and body size (e.g. survival, fecundity, timing of spawning; Wedekind & Kung, 2010).

The timing and seasonality of warming can also determine the magnitude and direction of effects on important invertebrate prey for fishes (Feuchtmayr et al., 2010; Wagner & Benndorf, 2007). Chironomids and snails are dominant components of the zoobenthos and diets of many arctic fishes (e.g. Goyke & Hershey, 1992), while zooplankton are also an important pelagic food source in many arctic lakes (e.g. Luecke et al., 2014; O’Brien et al., 1997). In littoral areas of lakes, snail densities have been shown to increase up to 5-fold with increased nutrients (Hershey,
1990, 1992), and zooplankton biomass and production is driven by phytoplankton production and temperature (O’Brien et al., 1992). For example, cladocerans produce more eggs in fertilized lakes during warm but not cool summers (Luecke, unpublished data). Under warming conditions, biomass and abundance of these important benthic and pelagic prey could increase in accordance with increased temperature and/or primary productivity (Straile & Geller, 1998). Alternatively, increased stratification could result in epilimnetic food limitation due to nutrient depletion from decreased mixing (George, 2000). Accordingly, increased predation pressure from fish, owing to increased metabolism in warmer conditions, could offset increased snail and zooplankton abundance (McDonald, Hershey, & Miller, 1996; Vadadi-Fulop, Sipkay, Meszaros, & Hufnagel, 2012).

Examining long-term measurements of environmental change can be useful to identify and quantify invertebrate prey dynamics within food webs (e.g. Hampton, Gray, Izmest’eva, Moore, & Ozersky, 2014), while experimental measurements can distinguish responses to more rapid changes owing to alterations in phenology and life history expression (e.g. Geerts et al., 2015). As temperature and photoperiod are important determinants of production rates and timing of emergence, species-specific plasticity of phenology can determine the success of many invertebrate prey species (Gilbert & Schroder, 2004; Gyllström & Hansson, 2004). However, while climate change will increase lake temperatures and growing seasons, photoperiod, often an important cue for life stage shifts, will remain fixed (Gilg et al., 2012). Thus, in the late season, photoperiod induced diapause of zooplankton species could influence timing despite changing temperature regimes (e.g. Chinery and Williams, 2003; Marcus, 1982).

In this study, we investigated potential changes to invertebrate prey biomass and availability in lakes of arctic Alaska as a result of a warming climate. We used laboratory mesocosm experiments to quantify changes of abundance and biomass for zooplankton and snails and used long-term observations to predict zooplankton biomass across a range of temperatures. For our laboratory experiments, we hypothesized (a) invertebrate prey abundance would increase
across the growing season with warmer temperatures; and (b) invertebrate prey would continue increased production in the late season when subjected to increased, yet unnatural, photoperiod. For our long-term modeling, we hypothesized (c) zooplankton biomass would increase in warm years relative to cold or average years. Our multi-faceted approach distinguishes between changes in timing of warming and phenology with regard to important invertebrate prey resources. Overall, our results illustrate how food webs in arctic lakes may respond to future warming, and whether prey will be available for fishes to satisfy their metabolic demands.

2 | METHODS

2.1 | Study site

Our research was conducted in lakes near Toolik Field Station (68°37.796’N, 149°35.834’W), home of the Arctic Long Term Ecological Research project (http://arc-lter.ecosystems.mbl.edu/), in the northern foothills of the Brooks Mountain Range, Alaska. Lakes in this region were formed by glaciers over three periods approximately 12 – 25, 60 – 100 and 250 – 300 ka in age (Hamilton, 2003). Generally, the lakes are shallow (maximum depths of 3 – 30 m) and ultra-oligotrophic (chlorophyll-α concentrations <5 µg/L; Kling et al., 1992) and nearby lakes are often particularly similar in regard to chemical and biological properties. Fish community composition is broadly determined by landscape factors (e.g. lake depth, surface water connectivity), but overall, fish species richness is low (Hershey et al., 1999, 2006).

2.2 | Predicting seasonal invertebrate biomass from laboratory mesocosm experiments

We conducted controlled mesocosm experiments during the 2015 growing season at Toolik Field Station, Alaska, to investigate potential impacts of climate warming on seasonal phenology of invertebrate species known to be important fish prey (e.g. Merrick, Hershey, & McDonald, 1991). In a heated incubation facility, we used insulated, recirculating water baths (350 L maximum volume) equipped with 1/3 HP chillers and temperature regulators, along with 1,000-W grow lights and light timers, to simulate future environmental conditions in the Arctic.
Within three separate water bath chambers, we installed an open top, clear, polycarbonate container (c. 200 L) partitioned into six equal-size replicates (n = 12 or 18 total replicates per experiment; c. 58 x 29 x 19 cm). We filled the water baths with epilimnetic water from Toolik Lake screened through 80-µm mesh. To allow water flow and exchange, but keep organisms in their respective replicate, evenly-spaced 12.7-mm holes were drilled in the container walls and each partition (n = 8 – 12/replicate wall), and the holes were covered with 80-µm mesh. We draped black mesh over the top of the water bath to dampen the grow light and mimic natural light conditions; PAR was c. 105 µE·m⁻²·s⁻¹ at the water surface and 40 µE·m⁻²·s⁻¹ at the bottom of the container. These values are representative of summer measurements in surrounding lakes between depths of 2.0 – 5.0 m (Gettel, 2006), and we did not observe notable temperature or evaporative effects as a results of the dampening mesh.

We conducted three separate experiments throughout the growing season, and each experiment lasted 31 days: early season (22 June – 22 July), mid-season (23 July – 22 August), and, late season (24 August – 23 September). At the beginning of each experiment, we collected zooplankton Daphnia middendorffiana (hereafter, Daphnia) and snails Lymnae elodes (hereafter, snails) from nearby lakes to populate each replicate. To efficiently initiate each experiment, we collected Daphnia from Lake E6 (68°38.611’N, 149°26.425’W) and snails from Lake E1 (68°37.5774’N, 149°33.3114’W) and Toolik Lake. These lakes have abundant populations of Daphnia (E6) and snails (E1), whereas although present, in Toolik Lake, densities of Daphnia middendorffiana and Lymnae elodes are low due to increased levels of fish predation in Toolik Lake, relative to Lakes E6 and E1 (making collection for experiments difficult). Nonetheless, the species used in our experiment are also present and naturally occurring in Toolik Lake and most lakes in this area (O’Brien, Buchanan, & Haney, 1979; Yurista & O’Brien, 2001). Additionally, Lakes E6 and E1 are in close proximity to Toolik Field Station (<6 and <1 km, respectively) and have similar temperature regimes and chlorophyll a concentration to Toolik Lake. For example, in July 2015, the mean epilimnetic temperature of Toolik Lake was 12.8°C, while Lake E6 was
13.0°C, and mean chlorophyll a concentration in Toolik Lake was 2.9 µg/L while Lake E6 was 3.6 µg/L (Arctic LTER, unpublished data). Accordingly, we populated each replicate to natural densities representative of surrounding lakes; we added 20 Daphnia (c. 0.6 ind/L; O’Brien et al., 1997; Arctic LTER, unpublished data) and 4 – 6 snails, weighed and marked individually (c. 0.5 g/m²; Hershey, 1990), per replicate. For substrate, we added 2-3 clean rocks from Toolik Lake (dried and frozen over winter) to cover the bottom, and added 1.0 mg of scraped benthic algae to each replicate as an initial food source. Using a Hydrolab DS5 multiprobe sensor, we periodically monitored physical and chemical parameters (e.g. chl-a, dissolved oxygen) during each experiment. At the end of each experiment, the baths were drained to a level sufficient to remove rocks and collect all Daphnia in a modified aquarium net with 80 µm mesh. During the draining of the mesocosms, we noted many Daphnia eggs were released and we subsequently collected these free eggs in the remaining water. In the lab, we enumerated the total number of individuals in each replicate, including any clonal or resting eggs, and measured individual total length from a subset of Daphnia in each replicate (n = 25). We collected all initial snails, weighed them individually, and noted any mortalities. Additionally, we collected and enumerated any juvenile snails or snail eggs sacs. Thus, our primary response variables for Daphnia were counts of individuals and eggs, which we added together to represent a measure of overall Daphnia abundance. For snails, our primary response variables were adult survival, change in adult individual biomass (% change in g), as well as counts for juvenile snails and eggs, which we summed to represent overall snail abundance.

Throughout the course of each experiment, we used a control treatment, in which natural temperature and light conditions were mimicked, and one or two experimental treatments (increased temperature and increased temperature × photoperiod). We were limited by total available water baths and therefore could not run a full factorial design. As such, and because photoperiod alone will not change in a warming climate, we did not run a photoperiod treatment, but rather, examined the interactive effect of increased temperature and photoperiod. By
including a treatment with increased photoperiod we were able to assess between physiological
cues of temperature and light in the absence of predation and under constant food availability. For
the early season experiment, we used two water baths, a control and an experimental (increased
temperature) treatment. As the sun does not set at high latitudes during this time period, we held
light constant throughout the experiment (24-hour photoperiod). Water temperatures in each bath
started at 11°C. We warmed the control approximately 0.25°C/day until reaching a typical
epilimnetic average temperature of 14°C, and we warmed the increased temperature treatment
approximately 0.50°C/day until reaching 18°C. These rates of warming follow a typical seasonal
trajectory observed for Toolik Lake (e.g. days of warming to maximum temperature), and both
temperatures were held constant for the duration of the experiment once the maximum
temperature for each treatment was achieved. For the mid-season experiment, we used all three
water baths, which started at 14°C (control) or 18°C (increased temperature and increased
temperature × photoperiod). For the control and increased temperature treatments, photoperiod
decreased 10 or 15 minutes per day, such that the photoperiod at the end of the experiment was
18.5 hr, while the increased temperature × photoperiod treatment remained at 24 hr throughout
the duration of the experiment. Photoperiod was decreased based on natural observations for a
given day of year during the experiment and rounded to the closest 5-min interval (10 or 15 min)
as limited by the precision of our light timers. Starting on August 7, we allowed the water baths to
cool by approximately 0.25°C/day such that the control was 10°C at the end of the experiment,
while the experimental treatments were 14°C. For the late season experiment, the control started
at 10°C and ended at 4°C, while the experimental treatments started at 14°C and ended at 8°C.
Again, we held photoperiod constant at 24 hr for the increased temperature × photoperiod
treatment, while the control and increased temperature treatments decreased 10 – 15 min per day
from 18 hr at the beginning of the experiment to 12 hr at the end of the experiment.

We used a one-way ANOVA to compare the control, increased temperature, and
increased temperature × photoperiod treatments (mid- and late season) followed by pairwise
comparisons using t tests with Bonferroni adjustment of p values to compare between treatments. For the early season experiment, we used Student’s t test. We used R statistical package (version 3.3.2; R Core Team, 2016) for all analyses.

2.3 | Predicting seasonal zooplankton biomass from long-term data

2.3.1 | Data

To predict zooplankton biomass from long-term observations, we used data from Toolik Lake, Alaska, compiled for ice-free periods (approximately June through September) from 1983 to 2015. These data are typically collected weekly during the summer months as part of the Arctic Long-Term Ecological Research program (ARC LTER; detailed methods can be found at http://arc-lter.ecosystems.mbl.edu/). The number of observations in each year varied with length of ice-free period, and some years in the period of record were incomplete (1 – 16 observation/year; total n = 234; mean n = 7.8 observation/year). Observation dates ranged from June 12 to September 26. We did not have data for years 1999, 2000, and 2007.

Zooplankton were collected via two duplicate vertical tows of a Wisconsin-style net at a station located in the deepest area of the lake. Tow depth during the period of record ranged from 4.0 to 20.0 m, and we excluded all observations of tow depth < 12.0 m (n = 29 observations). Zooplankton counts by species were obtained from the ARC LTER database, and we calculated species-specific biomass estimates (mg/L) from length-weight relationships (McCauley, 1984; Yurista, 1999). Duplicate samples from the same observation date were averaged. Vertical temperature profiles (°C) were often measured in concert with zooplankton sampling events; as we were interested in temperature related trends, we excluded the biomass estimates when depth-specific temperature data were not available (n = 27 observations). To address common seasonal trends in zooplankton biomass, we limited our biomass estimates to the sum of the four most prevalent species of zooplankton during the period of record (Cyclops scutifer 47% total biomass, Diaptomus pribilofensis 31% total biomass, Daphnia longiremis 8% total biomass, Heterocope
septentrionalis 6% total biomass). We applied a natural log-transformation to total biomass data to normalize the distribution, and we removed three estimates determined to be outliers (>5 times the overall mean; final n = 175 observations; mean n = 5.8 observation/year).

### 2.3.2 Generalized additive modelling

We used generalized additive mixed models (GAMMs; Hastie & Tibshirani, 1990; Wood, 2006; Zuur, Ieno, Walker, Saveliev, & Smith, 2009) to capture the non-linear dynamics of zooplankton biomass across the growing season. Due to the observed unimodal pattern of these data, we a priori decided to model zooplankton biomass across day of year (DOY) using a cubic regression spline smoothing function. Lake temperatures also exhibit a nonlinear pattern throughout the ice-free period, and our primary interest was to predict how zooplankton biomass might change in a warmer climate. Therefore, we included temperature as a covariate to fit seasonal trends across the growing season, and to predict zooplankton biomass across the growing season under various temperature scenarios.

Zooplankton biomass was modeled across DOY and temperature using a generalized additive mixed effects model taking into account the hierarchical structure of repeated samples within a year. We fitted a generalized additive mixed effects model to predict zooplankton biomass considering day of year (fit as a smoother) with the random effect of year (categorical) (Equation 1):

\[
\text{Log Biomass}_{ik} = \alpha + \beta_1 + s(\text{Day of Year}_i) + \text{Temperature}_j + a_k + \varepsilon_{ik},
\]

\[
a_k \sim N(0, \sigma_a^2),
\]

\[
\varepsilon_{ik} \sim N(0, \sigma_e^2),
\]

where \( \text{Log Biomass}_{ik} \) is the predicted zooplankton biomass for an observation on day \( i \), during year \( k \), in which \( s(\text{Day of Year}_i) \) is a cubic regression spline smoothing function, \( \text{Temperature}_j \) is the predicted lake temperature at depth \( j \) from Equation (2), \( a_k \) is a categorical random effect among years and \( \varepsilon_{ik} \) are the residuals. The random effects \( a_k \) and residuals \( \varepsilon_{ik} \) are normally
distributed with a mean of 0 and variance $\sigma^2$. These analyses were performed using the `gam()` function in the “mgcv” package version 1.8-9 (Wood, 2011) of R version 3.3.2 (R Development Core Team, 2016).

To characterize the effect of variable annual temperatures, and predict zooplankton biomass in a warmer climate, we used the range of observed lake temperatures during the period of record as potential scenario end members. For the overall observed ice-free period, we binned observations by 5-day windows ($n = 21$) and selected the warmest and coldest temperature within each bin, and repeated the process for depths 1, 3, 5, 8 m. We then fit generalized additive models for each depth to develop continuous estimates of temperature at depth across the growing season (Equation (2)):

$$ \text{Temperature}_j = \alpha + s(\text{Day or Year}_i) + \varepsilon_j, $$

$$ \varepsilon_j \sim N(0, \sigma^2), $$

where Temperature$_j$ is the depth-specific temperature for observation $j$ in which $s(\text{Day or Year}_i)$ is a cubic regression spline smoothing function, $\alpha$ is the intercept, and $\varepsilon_j$ is the normally distributed residual error with a mean of 0 and variance $\sigma^2$. From these models, we predicted depth-specific daily temperatures from DOY 163 – 269 (June 12 to September 26).

We selected the best model incorporating depth-specific temperatures using a backwards stepwise procedure using BIC. We combined the zooplankton model (Equation (1)) with our thermal extremes (Equation (2)) to simulate seasonal zooplankton biomass under the coldest and warmest observed thermal conditions. GAMM scenario predictions with nonoverlapping ±2 SE estimates are considered significantly different.
3 | RESULTS

3.1 | Predicting seasonal invertebrate biomass from laboratory mesocosm experiments

3.1.1 | Zooplankton

We observed distinct seasonal patterns across and within the three experiments in overall *Daphnia* abundance (counts of [i.e. the sum] of all *Daphnia* individuals and eggs) and when considering individuals and eggs separately (Figure 2.1). However, we did not observe differences in *Daphnia* individual size across experiments and treatments. For example, in the early season experiment, mean length (mm ± 2 SE) in the control was 1.71 ± 0.10 and 1.67 ± 0.09 (t = 0.59, df = 220, p = 0.55). During the early season experiment, in contrast to our hypotheses, total *Daphnia* abundance was significantly greater in the control relative to the increased temperature treatment (t = 2.96, df = 5.4, p = 0.03). Replicate *Daphnia* individual counts in the control ranged from 208 to 410 individuals (mean = 346.8) and *Daphnia* egg counts ranged from 1 to 107 eggs (mean = 26.8), while in the increased temperature treatment counts ranged from 248 to 294 individuals (mean = 265.5) and 0–12 eggs, respectively (mean = 4.3). During the mid-season experiment, which also included the increased temperature × photoperiod treatment, our *Daphnia* egg and individual counts varied significantly across treatments (F = 5.72, df = 2, p = 0.01); however, in the increased temperature and increased temperature × photoperiod treatments, we observed significantly greater *Daphnia* abundance relative to the control, which supported our first hypothesis (mean = 376 and 342 vs. 191; p = 0.01 and 0.05). In the late season, relative to the first two experiments, we observed decreased overall *Daphnia* abundance across all treatments, and overall abundance in the increased temperature × photoperiod treatment was slightly increased relative to the control (mean = 83.5 vs. 47.7; p = 0.12). However, in accordance with our second hypothesis, the counts for individual *Daphnia* were significantly greater in the increased temperature*photoperiod treatment relative to the control (mean = 69.7 vs. 27.2; p = 0.04). Counts for individual *Daphnia* were variable in the increased temperature...
treatment (n = 17–106 individuals), but included the two most numerous replicates (n = 102, 106 individuals).

3.1.2 | Snails

Nearly all snail production occurred during the early season experiment (> 99%; Figure 2.2). While overall abundance of juvenile snails and eggs did not differ between the control and increased temperature treatment (t = -0.85, df = 6.9, p = 0.42), development of snail offspring occurred significantly quicker in the increased temperature treatment. At the end of the early season trail, in accordance with our first hypothesis, we observed more juvenile snails in the increased temperature treatment (mean = 50.7 vs. 19; P < 0.01).

Adult snail biomass and survival was variable within and across treatments and experiments (Figure 2.3). In the early season experiment, “change in biomass (%)” was significantly greater in the control relative to the increased temperature treatment (mean % change = +2.45 vs. – 14.93; t = 4.58, df = 16.5, p < 0.001), while survival was relatively low for both treatments (40% for control and increased temperature treatment). In the mid-season experiment, change in biomass was significantly different across treatments (f = 12.09, df = 2, p < 0.001). In the increased temperature × photoperiod treatment, change in biomass was significantly greater relative the control (mean % change = +7.47 vs. +0.30; p = 0.04), but snails in the increased temperature treatment lost significantly more biomass relative to the control (mean % change = -12.30 vs. +0.30; p = 0.005). Adult snail survival was relatively high for all treatments following the mid-season experiment (control = 92%, increased temperature = 79%, increased temperature × photoperiod = 79%). Adult snail biomass exhibited a similar pattern for the late season experiment (f = 5.05, df = 2, p = 0.009). In the increased temperature × photoperiod treatment, biomass was elevated relative to the control (mean % change = +9.37 vs. +2.08; p = 0.07) while overall biomass decreased in the increased temperature treatment relative to the control (mean % change = -1.94 vs. +2.08; p = 0.22). Adult snail survival also remained
high for all treatments following the late-season experiment (control = 96%, increased temperature = 96%, increased temperature × photoperiod = 92%).

3.2 | Predicting seasonal zooplankton biomass from long-term data

Using a backwards stepwise model selection process, our best model to predict zooplankton biomass included a temperature effect for depth-specific temperatures at 3 m (R² adjusted = 24.9, n = 173; Table 2.1). This top model produced an intercept of 12.94 ± 0.29, significant smoother of DOY (edf = 3.46, p <0.001). While the temperature coefficient (0.03 ± 0.02) was not statistically significant (t = 1.31, p = 0.19), the model predicted change in zooplankton biomass across temperature may be biologically significant. Indeed, statistical significance can be attributed to a lack of pattern or high uncertainty. To this end, we simulated zooplankton biomass (plus uncertainty) across day-of-year under high and low temperature regimes (including uncertainty) to evaluate whether the observed range of temperature regimes may have ecological consequences in arctic lakes (Figure 2.4).

Using generalized additive models (GAMs) models as described in Equation (1), our predicted temperatures at 3 m explained 96.7% of the deviance in observations for a cold year (R² adjusted = 94.9%, n = 21) and 93.6% of the deviance for a warm year (R² adjusted = 91.8%, n = 21; Figure 2.4). Across our modeled period (DOY 163 – 269), the mean temperature at 3 m (°C ± 2 SE) was 9.62 ± 0.41 for an average year, 7.33 ± 0.38 for a cold year, and 11.70 ± 0.62 for a warm year. At maximum temperature differences, a warm year was 2.85°C warmer than an average year and a cold year was 4.36°C colder than an average year.

When applying these predicted temperatures to GAMM models as described in Equation (2), we predicted standing stock of available zooplankton biomass for an average, cold, and warm year (Figure 2.4). Across our modeled period, mean available daily zooplankton biomass (mg/L ± 2 SE) for a given day was 17.27 ± 1.16 in an average year, 15.99 ± 1.18 for a cold year, and 18.60 ± 1.18 for a warm year. Thus, across a cold year, daily available zooplankton biomass could be as
much as 19.6% less than an average year, while across a warm year, daily available zooplankton biomass could be as much as 18.5% greater than an average year, which supports our third hypothesis. At maximum temperatures for each scenario, daily available zooplankton biomass (mg/L ± 2 SE) in an average year is predicted as 26.26 ± 1.10, 23.90 ± 1.13 for a cold year, and 28.74 ± 1.13 for a warm year.

4 | DISCUSSION

In a future, warmer climate, changes to invertebrate prey availability and biomass, coupled with changes in seasonality (e.g., ice-on/ice-off dates) will create responses that cascade through lake food webs (Striale, 2002). We used laboratory mesocosm experiments coupled with long-term observations of zooplankton biomass to predict the response of invertebrate prey to a warmer climate. In our experiments, we generally confirmed our hypotheses of increased invertebrate biomass and availability, however, these increases were inconsistent across experiments and treatments. Changes in snail biomass and numbers, as well as zooplankton biomass, varied across treatments of increased temperature and increased temperature × photoperiod. In our models, we predicted ecologically important differences in total zooplankton biomass based on lake temperature.

We found increased temperature had a positive effect on snail development in the early season, indicating that earlier ice-off of arctic lakes would result in an increase in the snail populations earlier in the growing season, which could continue through the growing season. Benthic prey items are often important for fishes in colder periods prior to summer, when pelagic (e.g. zooplankton) and terrestrial items are less numerous (Amundsen & Knudsen, 2009). In temperate lakes, earlier spring break-up of lake ice has already been observed (Magnuson et al., 2000) and could be expected in the Arctic in response to a warmer climate. On the other hand, increased insulation of lake ice by increased snow cover under warmer winter conditions could negate earlier ice-off (Meehl et al., 2007). In either scenario, the cumulative effects of lake
warming across continuous years should be further studied for this species in particular as it may require more than one year to complete its life cycle. Nonetheless, our findings suggest the timing of ice-off and early season warming could directly affect the life history and abundance of snails in arctic lakes at the population level, but warming could affect individual biomass of adults.

In all experiments, adult snails lost weight in increased temperature treatments, and biomass was significantly decreased relative to the control. In the early season, this could arise from alternative energy allocation due to reproduction and a loss of biomass from oviposition of eggs (e.g. earlier development in warmer temperatures; Leicht, Jokela, & Seppala, 2013). In the mid- and late season, as well as the early season, decreased snail biomass in increased temperature treatments could also be an artifact of increased metabolism and food limitation (Britton & McMahon, 2004). While we did not specifically measure food availability (e.g. periphyton) in our experiment, adult snail biomass in increased temperature × photoperiod treatments increased, suggesting in the absence of light limitation, food may not have been limiting. However, as light (e.g. length of day) will not change, snails could be adversely affected by warmer temperatures if food is limiting (i.e. our adult snail biomass observations in control vs. increased temperature treatment), which could be determined by run-off and nutrient availability during thaw events (Hobbie and Chapin, 1996).

Aside from light limitation, primary production is likely to increase in a warmer climate if nutrients increase (e.g. Trochine, Guerrieri, Liboriussen, Lauridsen, & Jeppesen, 2014 but see Daniels et al., 2015). However, the timing of altered nutrient availability, and thus primary productivity, could vary seasonally and affect dynamics of the pelagic food web. When earlier ice-off results in earlier spring algal blooms, zooplankton, especially *Daphnia*, shift towards earlier emergence and increased abundance (e.g. Caceres & Schwalbach, 2001; Preston & Rusak, 2010). Further, earlier warming that results in strengthened summer stratification may decrease nutrient flux into surface waters from deeper water accumulations, which could limit phytoplankton and zooplankton during typically peak periods (e.g. mid-summer; Winder &
Sommer, 2012). Accordingly, when turnover occurs later in the season, nutrients, and thus phytoplankton, could be available later in the season for zooplankton (Noges et al., 2010). Many studies have focused on earlier ice-off and spring blooms of both phytoplankton and zooplankton (e.g. Adrian, Wilhelm, & Gerten, 2006; Preston & Rusak, 2010); however, ice-on and late season production is less understood (but see Dokulil & Herzig, 2009).

In more temperate, eutrophic systems, *Daphnia* respond readily with warmer early season temperatures (Adrian et al., 2006; Schalau, Rinke, Straile, & Peeters, 2008). In our experiment, *Daphnia* exhibited decreased abundance in the early season under warmer temperatures. As our experimental set up did not include an increase in early season primary production, *Daphnia* may not have responded as expected, as food quantity and quality is a dominant driver of zooplankton dynamics (Van Geest, Spierenburg, Van Donk, & Hessen, 2007). As with any mesocosm experiment, we cannot rule out potential influences of the mesocosm environment (e.g., laboratory setting, lake water) on our outcomes (Carpenter, 1996). Our control represents the conditions in Toolik Lake as best replicated in the laboratory, but cannot replicate conditions found in nature. For example, other experimental studies have observed zooplankton increases with temperature are usually in accordance with nutrient loading, a factor not manipulated here (Fuechtmayr et al., 2010). Nonetheless, the lack of difference in chl-*a* among treatments and control, and the similarity to Toolik Lake water gives us some confidence in our experimental results, in terms of treatments relative to control.

In our mid-season experiment, we observed increased *Daphnia* abundance relative to the control with increased temperatures (increased temperature and increased temperature × photoperiod treatments), which is supported by long-term data as well (Figure 2.4). Chlorophyll-*a* measurements were similar to the early season, but observed numeric responses of *Daphnia* could illustrate grazer control of phytoplankton. Due to relatively short development periods, *Daphnia* can respond rapidly and increased abundance can occur in just a few days (Goss & Bunting, 1983), while we might not expect similar responses in multistage species (Adrian et al.,
Further, an energetics study of *D. middendorffiana* from the Toolik Lake region suggested optimal performance at 14°C, which was the maximum temperature of our increased temperature and increased temperature*photoperiod treatments (Yurista, 1999). Should temperatures increase beyond 14°C under a future climate, this particular species could be limited physiologically if they do not rapidly adapt (but see Geerts et al., 2015; Przytuska, Bartosiewicz, Rautio, Dufrense, & Vincent, 2015).

At Toolik Lake, a 5°C increase in air temperature is predicted to result in 2°C increase in epilimnetic lake temperature and a seven-week increase in the ice-free growing season (Hobbie et al., 1999). For our experiment in the late season, overall *Daphnia* abundance in all treatments decreased relative to early and mid-season experiments; however, increased temperature and light appears to increase biomass in the late season (increased temperature × photoperiod; Figure 2.1). Although zooplankton responds to early-season temperature, food availability, and light (e.g. Adrian et al., 2006; Winder & Sommer, 2012), we require a better understanding of how late season dynamics could affect zooplankton abundance and/or senescence. Recent late season sampling of Toolik and other surrounding lakes suggest primary production persists longer into autumn than previously thought (Budy, LTER unpublished data), which could be fueled by nutrient fluxes from lake turnover (e.g. Noges et al., 2010). Our experimental results suggest light and/or temperature may prolong zooplankton availability. However, the increased *Daphnia* abundance we observed in the treatments relative to the control could signify increased male production in preparation for sexual resting egg production, which may be influenced by a temperature, light, or the interaction of temperature and light (Korpelainen, 1986). Further, species-specific adaptations to warmer autumn periods could result in increased overall biomass or further annual development (e.g. copepods; Gerten & Adrian, 2002), potentially leading towards increased zooplankton biomass into winter and the next season (e.g. Dokulil & Herzig, 2009).
From our statistical models of historic observations, we observed a typical unimodal peak of zooplankton biomass in summer for Toolik Lake, similar to other high latitude lakes (e.g. De Senerpont Domis et al., 2013; Figure 2.4). The warm year we modeled from observations over the last 30 years resulted in a 2.9°C increase in lake temperature relative to an average year at a depth of 3 m (Figure 2.4). This increase in temperature is predicted to increase zooplankton biomass by up to nearly 20% in a warm year relative to an average year, and 25% relative to a cold year. However, because Toolik Lake contains fish, a paradox exists by using observed data of standing stock zooplankton biomass integrated throughout the water column as large-bodied zooplankton are often absent from systems with fishes (Yurista & O’Brien, 2001). In other words, because fish eat zooplankton, changes to total production of zooplankton in a warmer year are likely masked by increases in consumption by fish to meet their metabolic demands.

In warmer years across the period of record, increased fish consumption due to increased lake temperatures and metabolic demand may not quantitatively represent zooplankton abundance (e.g., production). Using simple egg ratio production models (Edmondson, 1968; Palohiemo, 1974) and our modeled temperatures, we could expect abundance of some species to increase further. In warmer years relative to a colder years, *Daphnia* abundance could increase up to 29.2% and up to 40.5% for *Cyclops scutifer*. Our laboratory experiments, at similar temperature increase (c. 4°C), indicate the *Daphnia* could increase by nearly 50%. Thus, our model predictions of zooplankton biomass increases in warm years may be conservative based on actual increased fish consumption of increased zooplankton production. In previous work, we used similar “cold” and “warm” year observations, with scenarios of 5°C increased water temperatures to model the increase in consumption of fish based on bioenergetics (Budy & Luecke, 2014). Bioenergetics estimates of fish consumptive demand increased 23%–34% relative to a cold year and 10%–13% relative to a warm year. Our predictions of potential zooplankton biomass increases fall within this realm, especially if these estimates are conservative. However, further
mechanistic study of species-specific responses would improve our understanding of abundance versus selectivity for invertebrate prey biomass as fish food.

Overall, we show abundance and thus availability of arctic lake invertebrates will likely respond positively to a warmer climate based primarily on temperature and seasonality. As invertebrates are extremely important prey for fish in arctic lakes, especially given the presence of relatively few prey fish, our findings suggest that top-down effects, such as increased consumptive demand of fish in warmer arctic lakes, could be buffered by increased production at intermediate trophic levels. Accordingly, changes in lake temperature are not likely to occur without changes in nutrient inputs (De Senerpont Domis et al., 2013; Wrona et al., 2006), and thus, bottom-up effects could provide further food web resilience to a changing arctic climate (Budy, Giblin, Kling, White, & Luecke, in prep). Additionally, in fishless lakes, increased invertebrate prey is likely to be more available for other invertebrate planktivores (e.g., Chaoborus), but the response of invertebrate predators, regardless of food availability, remains understudied. However, species-specific responses will likely vary, and given some disconnect between mesocosm experiments and natural systems, future work with in-situ and whole ecosystem manipulation will lend further credence to our study (DeBoeck et al., 2015). Beyond our work here, important questions remain regarding the availability and accessibility to aquatic habitats under climate change, which could have unknown effects across trophic levels. If surface waters become disconnected between lakes (e.g., seasonal drying of streams), or if temperature/oxygen squeezes become more common or severe in lakes, shifting climate and hydrological regimes are likely to disrupt food web interactions by limiting food resources and/or predator access to these resources (Hobbie & Kling, 2014). As lakes in arctic Alaska provide valuable subsistence fish resources for local communities (e.g. Pederson & Hugo, 2005) and harbor highly adapted natives species (e.g. Gilg et al., 2012), continuing to understand potential changes brought about by a warming climate is important for the species that inhabit them, as well as the ecosystem services they provide.
References


Table 2.1. General additive mixed model backward selection results. All candidate models included a smoother of day of year (DOY) and the random effect of year. The number following ‘Temp’ in model is the depth (m) of the temperature. Shown with Bayesian Information Criterion (BIC) value with ΔBIC is the difference in BIC values from the most supported model, and LL is the log-likelihood.

<table>
<thead>
<tr>
<th>Model</th>
<th>BIC</th>
<th>ΔBIC</th>
<th>LL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp3</td>
<td>376.32</td>
<td>0</td>
<td>-172.73</td>
</tr>
<tr>
<td>Temp1</td>
<td>377.07</td>
<td>0.75</td>
<td>-173.11</td>
</tr>
<tr>
<td>Temp1 + Temp3</td>
<td>384.98</td>
<td>8.66</td>
<td>-174.52</td>
</tr>
<tr>
<td>Temp1 + Temp3 + Temp5</td>
<td>392.32</td>
<td>16</td>
<td>-175.66</td>
</tr>
<tr>
<td>Temp1 + Temp3 + Temp5 + Temp8 (full)</td>
<td>401.72</td>
<td>25.4</td>
<td>-177.83</td>
</tr>
</tbody>
</table>
Figure 2.1. Abundance of adult *Daphnia* (top), *Daphnia* eggs (middle), and total *Daphnia* (adults + eggs; bottom) as measured in three treatments across three mesocosm experiments (Early = 22 June – 22 July, Mid = 23 July – 22 August, Late = 24 August – 23 September) conducted at Toolik Field Station, Alaska, in 2015. Open circles are observations outside of the range of the whiskers (±1.5*interquartile range). Note: there was not an increased temperature*photoperiod treatment for the early season experiment because sunlight is unlimited.
Figure 2.2. Juvenile snails hatched (top), eggs produced (middle) and total new snail abundance (juveniles + eggs; bottom) as measured in three treatments (control = white, increased temperature = light gray, increased temperature*photoperiod = N/A) across three mesocosm experiments (Early = 22 June – 22 July, Mid = 23 July – 22 August, Late = 24 August – 23 September) conducted at Toolik Field Station, Alaska, in 2015. Open circles are observations outside of the range of the whiskers (±1.5*interquartile range). Note: there was not an increased temperature*photoperiod treatment for the early season experiment, and no snails were produced during the late season experiment.
Figure 2.3. Change in adult snail biomass (% change, g) as measured in three treatments (control = white, increased temperature = light gray, increased temperature*photoperiod = dark gray) across three mesocosm experiments (Early = 22 June – 22 July, Mid = 23 July – 22 August, Late = 24 August – 23 September) conducted at Toolik Field Station, Alaska, in 2015. Adult snail survival is represented by black stars. Open circles are observations outside of the range of the whiskers (±1.5*interquartile range). Note: there was not an increased temperature*photoperiod treatment for the early season experiment.
Figure 2.4. Top: Generalized additive model predictions of lake temperature at a depth of 3 m in Toolik Lake, Alaska, for a warm year (red; $R^2 = 0.91$) and cold year (blue; $R^2 = 0.94$) as a function of day of year ($p < 0.001$) based on minimum and maximum observed temperatures within five day windows ($n = 21$) across the period of record (Date = June 12 – Sept 26; DOY = 163 - 269). Dashed lines are ± 2se. Gray points are all temperature observations from 1987 – 2015 ($n = 175$).

Bottom: Generalized mixed effects additive model predictions ($R^2 = 0.25$, edf = 3.64) of standing stock zooplankton biomass in Toolik Lake, Alaska, for a warm year (red) and cold year (blue) as a function of day of year ($p < 0.001$) and lake temperature ($p = 0.19$) at 3 m with random effect of sample year. Dashed lines are ± 2se. Gray points are all observed measures of zooplankton biomass from 1987 – 2015 ($n = 175$).
CHAPTER 3
INVESTIGATING THE MORPHOLOGICAL AND GENETIC DIVERSITY OF ARCTIC CHAR (*Salvelinus alpinus*) POPULATION IN DISTINCT GROUPS OF FOOTHILL LAKES IN ARCTIC ALASKA\(^3\)

Abstract

Polymorphism allows divergent morphs (e.g., phenotypes) of the same species to coexist by minimizing intraspecific competition, especially when resources are limiting. Arctic char are described as one of the most versatile vertebrates in the world, and accordingly, morphologically and genetically divergent morphs are extremely common. In the face of a changing climate, populations of char can be expected to adapt to changing conditions to maximize fitness and persistence; however, to be successful, these adaptive changes must minimally match the rate of environmental change. In this study, we investigated the morphological and genetic diversity of seven populations of arctic char across two distinct lake groups with different size structures (e.g., mean, maximum total length). Across all char, using model-based clustering of morphometric traits corrected for allometry, we did not detect morphological differences within and across char populations. Genetic analyses showed different genetic structures between lakes groups, but within group and individual lakes, arctic char genetic structure was similar regardless of total length. Accordingly, further cluster analyses did identify three main size classes of arctic char based on uncorrected morphometric traits. We used PERMANOVA analyses to identify factors that determine observed arctic char size structures, and significant predictors included Secchi depth, arctic char density, and lake group. We also observed different growth patterns between lake groups. Larger char occurred in lakes with shallower Secchi depths and lower arctic char densities. As the climate warms, more productive lakes (e.g., shallower Secchi depths) with less

---

\(^3\) This chapter is co-authored by Stephen L. Klobucar, Jessica Rick, Elizabeth Mandeville, Catherine Wagner, and Phaedra Budy
dense char populations may be more stable, and hold the potential capacity for each of these characteristics to increase. However, the adaptability of char could be reduced should landscape connectivity, and thus, potential gene flow and trophic subsidies be reduced. Our findings provide some of the first descriptions of evolutionary characteristics of char population in arctic Alaska, and will be important to consider for the persistence of these populations for subsistence and conservation decisions.

1 | INTRODUCTION

Complex selection pressures acting between and among species, and influenced by environmental factors and resources availability, can determine the adaptive potential and persistence of populations (Reznick & Ghalambor, 2001). Phenotypic plasticity allows for morphological and physiological responses to spatial and temporal variation in the environment, and thus, species’ evolution may stem from plasticity (Agrawal, 2001; Schulte et al., 2011). However, smaller populations, especially in smaller ecosystems (e.g., small, isolated lakes) may have limited adaptive potential due to genetic bottlenecks or other inherent factors (e.g., dispersal capabilities) that can limit the effectiveness of different phenotypes of the same species (e.g., ‘morphs’; DeWitta et al., 1998; Willi et al., 2006).

Resource polymorphism allows for morphs of the same species to occur in the same environment, especially when resources are limiting (Svanbäck & Persson 2004). This polymorphism, which may arise from genetic factors or phenotypic plasticity (Andersson, 2003), can aid in maintaining genetic diversity and adaptive potential in changing environments while minimizing intraspecific competition (Ghalambor et al., 2007; Smith & Skúlason 1996). However, in many cases, phenotypic divergence that creates these distinct morphs may also precede genetic divergence (Schluter, 2000). For example, in temperate lakes, pumpkinseed sunfish (Lepomis gibbosus) can exhibit high degrees of intraspecific variation in jaw morphology based on the availability of a primary prey (gastropods), yet common garden experiments show
these differences more driven by plasticity rather than rapid evolution (Mittelbach et al., 1999; Robinson & Wilson, 1996). Thus, considerable differences in morphology, life-history, and behavior of fishes often results from differences in food and habitat selection (e.g., Power et al., 2005), and these differences can be even more profound within isolated lakes (Skúlason & Smith, 1995).

In high latitudes, arctic char (Salvelinus alpinus) occupy many circumpolar lakes and exhibit widespread polymorphism across their range (Jonsson & Jonsson, 2001). With widespread polymorphic populations distributed across the Arctic and Neararctic, it is highly unlikely that differentiation is explained by chance or unknown ecological factors (Robinson & Wilson, 1994). In fact, Klemetsen (2013) regarded arctic char as “the most variable vertebrate on Earth.” Feeding ecology of arctic char, and thus intraspecific competition, is often attributed to the divergence of separate morphs based on habitat (e.g., littoral, pelagic, profundal) and diet (e.g., planktivorous, piscivorous), and 1 – 4 distinct char morphs can occur within the same lake (Jonsson & Jonsson, 2001; Klemetsen, 2010). However, the presence of separate morphs, and the exact number of morphs, is highly variable from lake to lake due to both abiotic (e.g., ecosystem size, habitat availability) and biotic factors (e.g., prey availability, genetic diversity). The degree of habitat segregation, and accordingly, polymorphism, has been shown to be positively related to overall ecosystems size including lake depth, surface area, and volume (Recknagel et al., 2017); meanwhile, interactions with other species can directly or indirectly affect char trophic dynamics and survival (Eloranta et al., 2013). Water temperature can also influence overall food availability and rate of food consumption such that faster growing individuals may mature sooner (Hindar & Jonsson, 1993). Overall, however, the development or persistence of a particular morph can be highly variable and unpredictable.

While functional traits, such as jaw morphology or fin anatomy, are likely related to foraging behavior and success in many populations of char (e.g., Arbour, et al., 2011; Bryce et al., 2016), genetic distinction can occur due to divergence from these traits or differing life histories
(e.g., May-McNally et al., 2015; Skúlason et al., 1996). Accordingly, contrasting allopatric and sympatric divergence can result in genetic consequences, such as variable allelic richness, which helps structure different life histories (Praebel et al., 2016). In genetically distinct populations, differences in allometry can have functional consequences that relate to behavior and life history, such as predator avoidance (e.g., Knutsdotter Simonsen et al., 2017). Overall, the high diversity of arctic char populations and associated trophic dynamics (e.g., Klobucar et al., in prep; see Chapter 4) contribute as ‘non’-ideal models for genomic research such that identifying underlying genetics for phenotypes in nature may help to predict evolutionary pathways under future environments (Elmer, 2016;Violle et al., 2014).

Postglacial lakes are often viewed as ideal systems to study adaptive processes such as resource polymorphism (e.g., Schluter, 1996; Snorrason & Skúlason, 2004). Relatively low species diversity and productivity, and overall high habitat segregation (e.g., littoral, pelagic), contribute to these lakes systems as advantageous to investigate polymorphism in arctic char (Klementsen, 2010; Pielou, 2008). In postglacial lakes, colonization and adaptation has occurred relatively recently, as recent as 10,000 years ago (e.g., Skúlason, Snorrason, Noakes, Ferguson, & Malmquist, 1989). Across the foothills of the Brooks Mountain range in arctic Alaska, repeated glaciations (e.g., 12 – 25 ka, 60 – 100 ka, and 250 – 300 ka years ago) left the landscape littered with literally thousands of kettle lakes (Hamilton, 2003). The age of the glacial landscape affects physical, chemical, and biological characteristics that may underpin morphological segregation of arctic char populations across the landscape (Hershey et al., 1999; Luecke et al., 2014). For example, older lakes that typically have decreased surface area and depth due to sedimentation may not have clearly segregated habitats zones (e.g., pelagic versus profundal). These type of distinct habitats likely foster different char morphs, especially relative to newer lakes on more recently glaciated landscapes. Older lakes are also less connected to other surrounding surface waters, such that barriers to genetic flow exists, and populations of char in these lakes are more isolated than populations in younger, more connected lakes. However, as the Arctic continues to
warm, loss of surface water connectivity between lakes (e.g., seasonal drying of streams) or temperature/oxygen squeeze in lakes may disrupt access to habitats that create or maintain char polymorphism (Hobbie & Kling, 2014).

In the face of a warming climate, the distribution and abundance of many organisms, including fishes in northern Alaska are likely to shift (Ehrlen & Morris, 2015). The fate of such species is uncertain, as rates of adaptation are expected to be slower than that of climate change (Etterson & Shaw, 2001). Species living near physiological limits are likely to have less adaptive capacity (Hoffman & Sgro, 2011). Along the southernmost limit of arctic char in the UK, population declines of char have already been attributed to climate change (Winfield et al., 2010). In addition to concerns over ecological changes to these natural lake ecosystems, there are important considerations for humans.

In northern Alaska, char and other fishes represent a critical subsistence resource for Native communities (e.g., Pedersen & Hugo, 2005). As such, a better understanding of the factors that potentially structure the morphological and genetic diversity of Alaskan arctic char populations is important in order to project the success (or failure) of these populations and fisheries in a warmer climate. In this study, we examine potential morphological and genetic differences between arctic char populations within and across two geographically close but otherwise contrasting lake groups in the foothills of the Brooks Range, Alaska. These lakes exhibit a range of abiotic and biotic characteristics, and we use these gradients to help explain the diversity of arctic char populations and size structures. First, we test for morphological differences between the arctic char populations of varying size structures, and determine the factors potentially contributing to differences in char morphology and/or size structures. Secondly, we use a genotyping by sequencing (GBS) approach to determine the extent that these populations of arctic char may be genetically distinct across morphs (or size structures) and lake groups. Overall, our findings provide some of the first descriptions of char morphological and
genetic diversity in northern Alaska, and further, contribute to our understanding of how abiotic and biotic factors can structure arctic char populations.

2 | METHODS

2.1 | Study site

Our research was conducted in lakes near Toolik Field Station (68°37.796’N, 149°35.834’W), home of the Arctic Long Term Ecological Research project (http://arclter.ecosystems.mbl.edu/), in the northern foothills of the Brooks Mountain Range, Alaska. Lakes in this region were formed by glaciers over three periods approximately 12 – 25 ka, 60 – 100 ka, and 250 – 300 ka in age (Hamilton, 2003). Generally, the lakes are shallow (maximum depths of 3 – 30 m) and ultra-oligotrophic (chlorophyll-α concentrations <5 µg·L⁻¹; Kling et al., 1992). Fish community composition is broadly determined by landscape factors (e.g., lake depth, surface water connectivity), but overall, fish species richness is low (Hershey et al., 1999; Hershey et al., 2006).

We measured arctic char morphometry from 7 lakes in two distinct lake complexes (Figure 3.1). One series of lakes (n = 4; the ‘Fog lakes’) are isolated lakes with no surface water connectivity between the lakes and contain arctic char as the only apex predator. The other series of lakes (n = 3; the ‘LTER lakes’) are defined by increased surface water connectivity between them (e.g., inlet and outlet steams) and contain arctic char as well as arctic grayling (Thymallus arcticus), lake trout (Salvelinus namaycush), and burbot (Lota lota) as potential competing predators. While these lake complexes are located in close proximity (~5 km), they are situated on different glacial landscapes (Fog lakes = Itkillik II, 12 – 25 ka; LTER lakes = Itkillik I > 53 ka), which, in combination with contrasting connectivity, could represent different colonization periods and potential for historic gene flow. The LTER lakes are found in a headwater sub-basin of the Sagavanirktok River drainage whereas the Fog lakes are located in the main drainage of Sagavanirktok. Notably, arctic char populations in these lakes vary greatly in size structure.
(Figure 3.2) and the abiotic and biotic characteristics across the lakes represent natural gradients (see Table 3.1).

2.2 Arctic char morphometric traits and growth

We sampled arctic char in 2016 (May – Sept) and 2017 (May) via gill nets and hook-and-line sampling. For each arctic char, we photo-documented the fish on a grid board for later trait measurement and clipped a portion of the anal fin for genetic analyses. We placed each fish flat, oriented head to the left, and photographed the fish from approximately 60 cm directly above the fish prior to releasing the char. In the lab, we made morphometric measurements including: snout length (SL), eye width (EW), maxilla length (ML), head depth (HD), head length (HL), body depth posterior (BDP), body depth anterior (BDA), post pelvic fin length (PPF) and caudal peduncle depth (CP), using the software program ImageJ (e.g., Skoglund et al., 2015; Figure 3.3).

To account for allometric size differences, we first log-transformed measurements to reduce heterogeneity in variance, and then size-adjusted our measurement using an allometric growth formula (e.g., Senar et al., 1994):

\[
\log_{10} Y_i = \log_{10} M_i + b (\log_{10} L_m - \log_{10} L_i)
\]

where \(Y_i\) is the size-adjusted trait value, \(M_i\) is the measured trait value, \(b\) is the slope of the measured trait (\(\log_{10} M_i\)) against total length (\(\log_{10} L_i\)), \(L_i\) is the measured total length, and \(L_m\) is the mean total length for all fish (e.g., all char for comparisons across lakes, all char within a lake for within lake comparisons).

We collected otoliths from a subset of arctic char captured during our gill net and hook-and-line sampling (\(n = 18\) in both Fog and LTER lakes) to further examine growth and size-at-age of arctic char across the study systems. Otoliths were mounted with glue on a slide and sanded to expose annual growth rings. We measured annual growth along a radius from the origin to the edge perpendicular to the growth rings and back-calculated length-at-age using the
biological intercept method (Campana, 1990). We calculated the biological intercept by using an observed linear relationship of log-transformed annual growth and otolith age for the five youngest fish collected and used an average length at hatch of 17 mm (Nordeng, 1983).

2.3 | Statistical analyses

To test for morphological differences, we performed model-based clustering on the size-adjusted trait measurements using the ‘mclust’ package (Version 5.4; Scrucca et al., 2016) in R version 3.5.0 (R Core Team 2018), which we also used for all other statistical analyses. We expected distinct char morphs, as a result of habitat or trophic segregation, to have distinct body and head shapes that best suit the ecology of a given morph, and thus, would not explained by allometry alone (e.g., Jonsson & Jonsson, 2001; Skúlason, Noakes, & Snorrason., 1989). Our preliminary analyses indicated different arctic char size structures within and between in the Fog and LTER lake groups (Figure 3.2), suggesting potential morphological differences. However, if our initial size-adjusted clustering analyses did not reveal morphological differentiation, we would reanalyze the trait measurements without size-adjustments (e.g., raw trait measurements) to classify the observed char size classes within and across lake groups.

Following these cluster models, we used PERMANOVA analyses (adonis.II) in the ‘RVAideMemoire’ package (Version 0.9-69; Herve, 2018) to determine the abiotic and biotic factors that may determine either: 1) the potential drivers of distinct arctic char morphs; or, 2) the potential drivers of arctic char size classes and growth patterns. We considered PERMANOVA predictors significant at the significance level of 0.05, and included abiotic factors of: maximum lake depth, mean lake depth, lake surface area, lake volume; and, biotic factors of: char abundance, fish density, and secchi depth (as an index of primary production) (see Table 3.1). We first tested for a potential effect of lake group, which incorporates lake connectivity and fish species richness, as a categorical predictor of arctic char size structure. We also tested the abiotic and biotic factors above, singularly and additively, to construct the best fitting model with
significant predictors and that minimized residuals sums of squares. Next, we used binary logistic regression models for each lake group to determine the total length at which char transition between size classes (e.g., ‘small’ to ‘medium’, ‘medium to large’) as determined by our clustering models.

2.4 | Genetics

For each arctic char we photo-documented for the two lake groups, we also collected a fin clip from the anal fin for later genetic analyses. Fin clips were stored in 95% ethanol and shipped to the Wagner Laboratory at the University of Wyoming for genotyping by sequencing analyses. To determine whether lakes with bimodal size distributions of fish exhibited genetic divergence between putative ecotypes, we generated a genotyping-by-sequencing dataset (Elshire et al., 2011; Parchman et al., 2012). We extracted DNA from archived fin clips using a QIAcube DNA extraction robot using the DNEasy Blood & Tissue kit (Qiagen, Inc.), according to the manufacturer’s instructions. We then prepared reduced-representation genomic libraries using a protocol (Parchman et al., 2012) that starts by digesting DNA with two restriction enzymes, MseI and EcoRI. Following the restriction digest, we ligated unique nucleotide barcodes to each individual fish’s DNA. To increase the template for sequencing, we then amplified barcoded DNA using PCR. Prior to sequencing at the University of Texas Genome Sequencing and Analysis Facility (UT-GSAF), the genomic library was size-selected using BluePippin (Sage Science) to retain only fragments 250-400 base pairs in length. The genomic library was sequenced on the Illumina Hiseq 4000 (SE, 1x150).

Prior to population genetic analyses, we completed several bioinformatics steps necessary to processing data. First, we filtered common contaminants (PhiX, E. coli, and leftover barcodes, primers, and adaptors from library preparation) from our data using bowtie2. We then matched sequences to individual fish using a custom barcode parsing Perl script. All data was assembled to the Atlantic salmon genome (Lien et al., 2016) using bwa (Li & Durbin, 2009), and we then
identified variable sites in the assembly using samtools and bcftools (Li, 2011; Li et al., 2009). We then filtered SNPs by minor allele frequency and amount of missing data using VCFTOOLS (Danecek et al., 2011) to allow no more than 50% missing data, and retained only SNPs with minor allele frequency greater than 0.05. We used this dataset to generate a genetic covariance matrix of individuals within each lake with a bimodal size distribution, and to perform a principal components analysis (prcomp in R).

3 | RESULTS

From May 2016 – May 2017, we photo-documented a total 233 arctic char including 116 from the Fog lakes and 117 from the LTER lakes (Table 3.2). Between lake complexes, arctic char were generally larger in the LTER lakes; however, based on model-based clustering, we did not detect different morphs from the nine measured traits across all size-corrected fish data (BIC = 8632.8; ΔBIC > 25 over models with more clusters). Additionally, within lakes exhibiting bimodal size distributions (Lakes Fog3 and LTER348), we did not identify separate morphometric classifications within either of these lakes using cluster analyses (Fog3 BIC = 2912.3, ΔBIC > 8 over models with more clusters; LTER348 BIC = 2295.1, ΔBIC > 18 over models with more clusters).

Our analysis of genetic structure corroborated the model-based, morphometric clustering analyses. We did not observe genetic separation between size classes in these lakes (Figure 3.4). However, between lake groups, the char populations were genetically distinct. Genotyping-by-sequencing resulted in 382,537,258 150 base pair reads, which we then assembled to the Atlantic salmon genome after bioinformatic processing steps similar to (Mandeville et al., 2017; Underwood, Mandeville, & Walters, 2016). We then removed individuals with fewer than 10,000 assembled reads and identified variable genetic sites for use in downstream analyses. We used a genetic dataset composed of 7,241 SNPs to assess the relationship between size of putative char ecotypes and intraspecific genetic variation in two lakes exhibiting a bimodal size distribution of
fish. For Lakes Fog3 and LTER348, we constructed a genotype covariance matrix among individuals using data from all 7,241 SNPs, and then did a principal components analysis on the genotype covariance matrix (Figure 3.4). We identified differentiation among individuals within each lake, but genetic differentiation did not correspond to size of individuals.

When not corrected for allometry, we detected three distinct size classes across all fish sampled using model-based clustering (e.g., ‘small’, ‘medium’, ‘large’; BIC = 11199.7, ΔBIC > 218 over models with fewer clusters; Table 3.2). Accordingly, all morphometric trait measurements scaled with size class, and in general, morphological traits for each size class were larger in the LTER lakes when compared to those in the Fog lakes (Figure 3.5). All of the small size class fish were found in the Fog lakes, and nearly all (n = 45 of 47) came from Lake Fog3 specifically.

Arctic char size structure appeared to be influenced more by biotic factors than abiotic factors (Table 3.3). We found a significant multivariate effect of Secchi depth (p = 0.006) and fish density (p = 0.005), followed by lake group (p = 0.010) in our PERMANOVA of char size classes across lake ecosystems. When tested as the primary predictor, lake group was not significant (p = 0.268); however, this predictor (e.g., as a random effect) was significant when coupled with the other predictors of Secchi depth and fish density. No single abiotic predictor was significant (Table 3.3).

Accordingly, further analyses indicated size and growth differences between lake groups. In the Fog lakes only, the average size of arctic char in the small size class was 159.8 mm (range = 117 – 210 mm), and our logistic regressions showed that these small char would transition to the medium size class at 208.4 mm (e.g., probability of classification switch from small to medium char = 0.5; Figure 3.6). Despite not clustering into a distinct morph based on morphology or genetics, our analyses of growth using otoliths indicated small char in Lake Fog3 exhibit significantly slower growth rates and smaller size-at-age (Figure 3.7). In both the medium and large size classes, mean arctic char were larger in the LTER lakes (mean TL of medium char
= 406.6 mm, range = 223 – 543 mm; mean TL of large char = 547.2 mm, range = 432 – 601 mm) relative to Fog lakes (mean TL of medium char = 335.8 mm, range = 192 – 436 mm; mean TL of large char = 444 mm, range = 424 – 457 mm; Table 2. As such, the size at which individuals would transition from ‘medium’ to ‘large’ was greater in the LTER (512.2 mm) lakes relative to the Fog lakes (432.1 mm; e.g., probability of classification switch from medium to large char = 0.5; Figure 3.6). The larger sizes of arctic char in the LTER lakes was further supported by larger size-at-age and increased growth rates when compared to the Fog lakes (Figure 3.7).

4 | DISCUSSION

Understanding the potential morphological divergence and polymorphism of arctic char, including the genetic diversity of local populations, is critical to understanding the adaptability of these populations to changing environmental conditions. The persistence of arctic char populations across the landscape, especially in a warming climate, may depend on phenotypic plasticity and genetic diversity. Here, we investigated and quantified the morphological and genetic diversity of arctic char populations across two contrasting lake complexes in northern Alaska. Surprisingly, despite strong phenotypic differences (e.g., coloration, maximum size), we did not detect differences of morphological traits or genetic diversity within lakes or lakes groups. However, between lake groups at the watershed scale, we noted significant differences in genetic and size structure. Overall, primary production and arctic char density were significant predictors of size structure variation between lake groups. Genetic differences are likely driven by landscape position as the lake groups in our study are found on different glacial landscapes, and thus, the respective char populations were likely isolated as a group of lakes during different glacial periods.

While arctic char polymorphism has been widely studied for this Holarctic species in Scandinavian regions, and to some extent, the lower latitudes of Alaska, little is known regarding char polymorphism and ecology for populations in lakes of arctic Alaska. Further, the body of
knowledge regarding char polymorphism is often centered on singular, large (surface area 10 to >500 km$^2$), and deep (maximum depth 100 to >200 m) bodies of water (e.g., Arbour et al., 2011; Power et al., 2005; Skoglund et al., 2015). In contrast, we studied multiple lakes, singularly and in combination, that are relatively small (<0.3 km$^2$), shallow (generally < 20 m), and generally representative of thousands of lakes located in the foothills of the Brooks Mountain Range, Alaska. As such, we capitalized on groups of postglacial lakes located in close proximity, yet on different glacial landscapes, which exhibit relative gradients of abiotic and biotic factors in order to further investigate char polymorphism in these types of lake ecosystems.

Based on age of the glacial landscapes, it is possible that char in LTER lakes have had up to five times longer to differentiate, relative to Fog lake populations (Hamilton, 2003). Despite the differences in age of lake groups, we did not find strong evidence of genetic differentiation within any of the lakes. If there are dimorphic ecotypes within these lakes, they are not genetically divergent, as genetic structure was not related to see structure in either Lake Fog3 or LTER348. If ecotypes had persisted stably through time, we would expect to see differentiation between large-bodied and small-bodied fish. Interestingly, in other areas, lakes of similar ages to our study lakes exhibit genetically distinct populations. In Loch Rannoch, Scotland (~12ka in age), divergent traits of genetically distinct char were not coupled with the age of lineage divergence; however, Loch Rannoch (surface area = 17 km$^2$; maximum depth = 134 m) is also much larger than our study lakes here (Bryce et al., 2016). In Iceland, char populations in a series of lakes (~10ka in age), exhibit varying degrees of phenotypic and genetic differentiation, and divergent morphs are likely due to intralacustrine divergence (Gislason et al., 1999). Additional predators in the LTER lakes could have excluded ‘dwarf’ char in these lakes, resulting in a single genetic (and morphological) population, whereas in the Fog lakes, spawning habitat availability may limit genetic divergence.

Morphometric differences described for arctic char are often attributed to foraging strategies (e.g., Floro-Larsen et al., 2016; Malmquist et al., 1992). Limnetic morphs feed in open
water environments and typically have a fusiform body with shorter fins, while benthic morphs (or ‘giant’ morphs) have chunky body shapes and much larger pectoral fins (Malmquist, 1992). In our other work (see Chapter 4), we quantified high trophic overlap (e.g., diet, niche space) between size classes of char in the Fog lakes. In contrast to the findings herein that char of different size classes are morphologically and genetically similar, our diet study suggests greater potential for morph differentiation within char of LTER lakes. We observed, in general, larger char appearing to reside in the littoral zone and consuming increased proportions of littoral prey (e.g., snails), while smaller char appeared more pelagic in diet and consumed more zooplankton and other aquatic macroinvertebrates (e.g., Trichoptera; Chapter 4). In this study, we show that any potential diet or habitat segregation, in either lake group, does not result in measureable differences of arctic char morphology. We found only one cluster of char when analyzing all size-corrected morphological traits, and further, we did not find differences when applying model-based clustering to head traits (e.g., snout length, eye width, maxilla length, head depth, head length) or body traits (e.g., body depth posterior, body depth anterior, post pelvic fin length, caudal peduncle depth) separately as groups. However, in at least one lake (Fog3), growth rates were significantly different between two size classes of arctic char, suggesting some trophic morph separation, but these differences were not apparent in our genetic analyses (Figure 3.4).

Indeed, Griffiths (1994) determined that 44% of published arctic char populations were bimodal and included a ‘normal’ and ‘dwarf’ morph within a cohort. An additional potential morphological difference, as qualitatively described by our photo documentation, smaller char (especially in the LTER lakes) exhibited lateral lines that were more pronounced relative to those of giant morphs, which could be a result of open water feeding (e.g., on zooplankton; Montgomery, 1989). We found no other studies that noted differences in lateral line development between char morphs, and this could be an area of future study in regard to char morphology and feeding ecology.
While ecosystem size has previously been attributed to morphometric differentiation (Recknagel et al., 2017), the lakes we studied may not be large enough to allow for this type of differentiation. For example, abiotic factors (e.g., lake surface area) were not significant predictors of arctic char size structure in our PERMANOVA analyses. Due to the relatively small lakes in this study, char populations in these type of systems are likely driven by within lake process and cycles (Budy & Luecke, 2014). Char populations are more densely populated in the Fog lakes (Klobucar et al., 2017; Chapter 4), and these lakes are also generally less productive. In combination, these factors could limit the maximum size char can achieve (Downing & Plante 1993; Naslund et al., 1993; Pechlaner, 1984). In the LTER lakes, it is possible other predators have, over time, selected for faster growing individuals and thereby contribute to lower char densities and larger size structure relative to the Fog lakes where other predators are absent (e.g., Lima, 1998). For example, lake trout and burbot, also present in the LTER lakes, shift to piscivory at a smaller size relative to arctic char (McDonald & Hershey, 1989; Kahlainen & Lehtonen, 2003), and we rarely observe piscivory (or cannibalism) by arctic char in any of these population regardless of lake group (see Chapter 4). The char populations in this study are not exploited by fishing, whereas elsewhere, an increase of char body size with decreased char population density as a result of fishing (Amundsen, 1989).

Overall, we describe the importance of understanding biotic factors that structure populations of an important native fish species. While the lakes we studied are oligotrophic, we still found significant effects of productivity, and accordingly, density dependence. However, as hydrologic and temperature regimes, such as timing of spring breakup and thermal stratification, are predicted to shift seasonally (Prowse et al., 2006), the ability of species to distribute to new and or preferred habitat could be critical and shift towards greater abiotic control. As such, some populations may be more adaptable (or vulnerable) to change. For example, if the more ‘open’ and accessible LTER lakes become more isolated and less connected from reduced surface waters relative to recent history, the movement of nutrients by more mobile species (e.g., arctic


grayling), could affect char populations in these lakes. Accordingly, because there are multiple competing predators in the LTER lakes, as temperature regimes change, species-specific effects may manifest disproportionately. For example, individual lake trout would require much more food than arctic char under scenarios of warming (McDonald et al., 1996; Budy & Luecke, 2014), and we expect food supply to be within the metabolic needs of arctic char, but not lake trout (Klobucar et al., 2018). In other work, we found a shift of lake trout to more littoral habitat, which could further increase competition with arctic char (Zarnetske et al., in prep). In the short term, increases in temperature (and thus, production), may increase population density of the char populations studied here, especially in the Fog lakes. However, population increases could actually lead to future population susceptibility if food does not also increase to meet increased metabolic demand (Budy & Luecke, 2014).

In this study we provide some of the first descriptions of arctic char morphological and genetic diversity in lakes of northern Alaska. As lakes in this region are numerous and diverse (e.g., different abiotic and biotic characteristics), we outline the importance of understanding how the morphological and genetic diversity of different arctic char population can vary over small spatial scales. From a conservation standpoint, it is important to maintain the diversity of arctic char populations (e.g., populations of varying size structures) across the landscape, in order for continued persistence during periods of rapid change that may outpace evolutionary processes. From a subsistence management standpoint, identifying factors that could contribute to population success (or failure) is critical. Overexploitation could quickly and permanently disrupt char production, size structure, and population viability.

REFERENCES


Zarnetske, P. L., Urban, M.C., Skelly, D.K., Budy, P. & S. L. Klobucar. (In prep). Do climatic changes or biotic interaction explain the condition of Arctic freshwater fishes over 30 years?
Table 3.1. Lake morphometry and fish community composition (AC = arctic char, AG = arctic grayling, BT = burbot, LT = lake trout, SS = slimy sculpin) for study lakes. Lake area and volume < 3 m are proportions of the total area or volume. AC abundance are population estimates from Klobucar et al. 2017 (Fog lakes) and modified Schnabel estimations from mark-recapture (LTER lakes)

<table>
<thead>
<tr>
<th>Group</th>
<th>Lake</th>
<th>Latitude (°N)</th>
<th>Longitude (°W)</th>
<th>Surface area (ha)</th>
<th>Lake volume (m³·10⁵)</th>
<th>Max depth (m)</th>
<th>Mean depth (m)</th>
<th>Lake area &lt; 3m</th>
<th>Lake volume &lt; 3m</th>
<th>Secchi depth (m)</th>
<th>Fish community</th>
<th>AC abundance</th>
<th>AC density (fish·ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fog</td>
<td>Fog1</td>
<td>68.684</td>
<td>149.082</td>
<td>3.5</td>
<td>2.9</td>
<td>19.7</td>
<td>8.4</td>
<td>0.33</td>
<td>0.29</td>
<td>4.9</td>
<td>AC, SS</td>
<td>448</td>
<td>448</td>
</tr>
<tr>
<td></td>
<td>Fog2</td>
<td>68.679</td>
<td>149.091</td>
<td>5.9</td>
<td>4.4</td>
<td>19.8</td>
<td>7.8</td>
<td>0.21</td>
<td>0.34</td>
<td>7.1</td>
<td>AC, SS</td>
<td>163</td>
<td>163</td>
</tr>
<tr>
<td></td>
<td>Fog3</td>
<td>68.673</td>
<td>149.088</td>
<td>3.9</td>
<td>3.1</td>
<td>21.0</td>
<td>7.6</td>
<td>0.30</td>
<td>0.31</td>
<td>6.0</td>
<td>AC, SS</td>
<td>666</td>
<td>666</td>
</tr>
<tr>
<td></td>
<td>Fog5</td>
<td>68.678</td>
<td>149.065</td>
<td>0.7</td>
<td>0.3</td>
<td>9.9</td>
<td>3.5</td>
<td>0.52</td>
<td>0.61</td>
<td>5.0</td>
<td>AC, SS</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>LTER</td>
<td>LTER345</td>
<td>68.623</td>
<td>149.151</td>
<td>30.7</td>
<td>38.2</td>
<td>28.6</td>
<td>12.3</td>
<td>0.16</td>
<td>0.22</td>
<td>1.5</td>
<td>AC, AG, LT, SS</td>
<td>277</td>
<td>277</td>
</tr>
<tr>
<td></td>
<td>LTER347</td>
<td>68.625</td>
<td>149.139</td>
<td>13.5</td>
<td>7.6</td>
<td>17.6</td>
<td>5.6</td>
<td>0.28</td>
<td>0.45</td>
<td>1.8</td>
<td>AC, AG, LT, SS</td>
<td>73</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>LTER348</td>
<td>68.641</td>
<td>149.127</td>
<td>5.7</td>
<td>1.9</td>
<td>9.6</td>
<td>3.2</td>
<td>0.56</td>
<td>0.70</td>
<td>3.7</td>
<td>AC, BT, SS</td>
<td>331</td>
<td>331</td>
</tr>
</tbody>
</table>
Table 2. Summary of fish captured and measured for morphological traits during 2016 – 2017 from study lakes on the North Slope, Alaska. Small, medium, and large size classes were determined via model-based clustering of raw morphometric trait measurements.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean TL ± SE</th>
<th>Range</th>
<th>n</th>
<th>Mean TL ± SE</th>
<th>Range</th>
<th>n</th>
<th>Mean TL ± SE</th>
<th>Range</th>
<th>n</th>
<th>Mean TL ± SE</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
<td></td>
<td></td>
<td></td>
<td>Medium</td>
<td></td>
<td></td>
<td>Large</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All char</td>
<td>233</td>
<td>359.3 ± 8.5</td>
<td>117 - 601</td>
<td>47</td>
<td>269.2 ± 9.4</td>
<td>117 - 457</td>
<td>146</td>
<td>375.6 ± 5.8</td>
<td>192 - 543</td>
<td>40</td>
<td>534.3 ± 7.7</td>
<td>424 - 601</td>
</tr>
<tr>
<td>Closed lakes</td>
<td>116</td>
<td>269.2 ± 9.4</td>
<td>117 - 457</td>
<td>47</td>
<td>269.2 ± 9.4</td>
<td>117 - 457</td>
<td>64</td>
<td>335.8 ± 6.5</td>
<td>192 - 436</td>
<td>5</td>
<td>444.0 ± 6.9</td>
<td>424 - 457</td>
</tr>
<tr>
<td>Fog1</td>
<td>19</td>
<td>343.8 ± 10.4</td>
<td>265 - 453</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>18</td>
<td>337.7 ± 8.9</td>
<td>265 - 400</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fog2</td>
<td>2</td>
<td>253.5 ± 103.5</td>
<td>150 - 357</td>
<td>1</td>
<td>-</td>
<td>150</td>
<td>1</td>
<td>-</td>
<td>357</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fog3</td>
<td>77</td>
<td>232.0 ± 11.1</td>
<td>117 - 436</td>
<td>45</td>
<td>159.0 ± 2.9</td>
<td>117 - 210</td>
<td>30</td>
<td>328.5 ± 10.9</td>
<td>192 - 436</td>
<td>2</td>
<td>427.5 ± 3.5</td>
<td>424 - 431</td>
</tr>
<tr>
<td>Fog5</td>
<td>18</td>
<td>351.4 ± 16.1</td>
<td>209 - 457</td>
<td>1</td>
<td>-</td>
<td>209</td>
<td>15</td>
<td>346.9 ± 13.2</td>
<td>212 - 397</td>
<td>2</td>
<td>455.0 ± 1.0</td>
<td>455 - 457</td>
</tr>
<tr>
<td>Open lakes</td>
<td>117</td>
<td>448.6 ± 8.1</td>
<td>223 - 601</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>82</td>
<td>406.6 ± 7.3</td>
<td>223 - 543</td>
<td>35</td>
<td>547.2 ± 6.1</td>
<td>432 - 601</td>
</tr>
<tr>
<td>LTER345</td>
<td>29</td>
<td>485.4 ± 12.4</td>
<td>304 - 590</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>14</td>
<td>439.1 ± 15.4</td>
<td>304 - 497</td>
<td>15</td>
<td>528.7 ± 10.4</td>
<td>432 - 590</td>
</tr>
<tr>
<td>LTER347</td>
<td>24</td>
<td>447.2 ± 16.9</td>
<td>223 - 570</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>427.8 ± 16.9</td>
<td>223 - 543</td>
<td>4</td>
<td>544.5 ± 12.3</td>
<td>522 - 570</td>
</tr>
<tr>
<td>LTER348</td>
<td>64</td>
<td>432.5 ± 11.6</td>
<td>260 - 601</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>48</td>
<td>388.3 ± 8.5</td>
<td>260 - 532</td>
<td>16</td>
<td>565.2 ± 6.2</td>
<td>518 - 601</td>
</tr>
</tbody>
</table>

Small, medium, and large size classes were determined via model-based clustering of raw morphometric trait measurements.
Table 3.3. Summary statistics of PERMANOVA analyses to determine best predictors of arctic char size structure across the Fog and LTER lake groups on the North Slope, Alaska, for arctic char captured 2016-2017. Sources of variation: lake_group = Lake Group; Nhat = estimated total population of arctic char; ACden_area = arctic char density by area (ind·ha⁻¹); ACden_vol = arctic char density by volume (ind·m⁻³·10⁻⁵); secchi = secchi depth (m); maxZ = maximum lake depth (m); meanZ = mean lake depth (m); SA = surface area (ha); prop3a = proportion of lake area < 3 m; prop3v = proportion of lake volume < 3 m; vol = lake volume (m³·10⁵). Significance codes: (*) P < 0.10, (**) P < 0.05, (***) P < 0.01. For models including more than a single predictor, only the best model’s statistics are displayed.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>SS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>lake_group</td>
<td>1</td>
<td>0.22</td>
<td>1.25</td>
<td>0.268</td>
</tr>
<tr>
<td>Nhat</td>
<td>1</td>
<td>0.21</td>
<td>1.15</td>
<td>0.343</td>
</tr>
<tr>
<td>ACden_area</td>
<td>1</td>
<td>0.18</td>
<td>0.96</td>
<td>0.495</td>
</tr>
<tr>
<td>ACden_vol</td>
<td>1</td>
<td>0.11</td>
<td>0.54</td>
<td>0.809</td>
</tr>
<tr>
<td>secchi</td>
<td>1</td>
<td>0.32</td>
<td>2.00</td>
<td>0.065*</td>
</tr>
<tr>
<td>maxZ</td>
<td>1</td>
<td>0.01</td>
<td>0.03</td>
<td>0.997</td>
</tr>
<tr>
<td>meanZ</td>
<td>1</td>
<td>0.02</td>
<td>0.07</td>
<td>0.990</td>
</tr>
<tr>
<td>SA</td>
<td>1</td>
<td>0.10</td>
<td>0.48</td>
<td>0.646</td>
</tr>
<tr>
<td>prop3a</td>
<td>1</td>
<td>0.08</td>
<td>0.40</td>
<td>0.854</td>
</tr>
<tr>
<td>prop3v</td>
<td>1</td>
<td>0.04</td>
<td>0.17</td>
<td>0.985</td>
</tr>
<tr>
<td>vol</td>
<td>1</td>
<td>0.09</td>
<td>0.44</td>
<td>0.577</td>
</tr>
<tr>
<td>secchi + ACden_area</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>secchi</td>
<td>1</td>
<td>0.53</td>
<td>5.15</td>
<td>0.031**</td>
</tr>
<tr>
<td>ACden_area</td>
<td>1</td>
<td>0.39</td>
<td>3.79</td>
<td>0.045**</td>
</tr>
<tr>
<td>residuals</td>
<td>4</td>
<td>0.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>secchi + ACden_area + lake_group</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>secchi</td>
<td>1</td>
<td>0.28</td>
<td>5.10</td>
<td>0.006***</td>
</tr>
<tr>
<td>ACden_area</td>
<td>1</td>
<td>0.43</td>
<td>7.99</td>
<td>0.005***</td>
</tr>
<tr>
<td>lake_group</td>
<td>1</td>
<td>0.25</td>
<td>4.57</td>
<td>0.010***</td>
</tr>
<tr>
<td>residuals</td>
<td>3</td>
<td>0.16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.1. Map of the study area in northern Alaska.
Figure 3.2. Length-frequency histogram of arctic char captured in the Fog and LTER lakes 2014 – 2017. Middle gray represents the overlap between Fog (dark gray) and LTER (white) catches.
Figure 3.3. Examples arctic char found in the closed ‘Fog’ and open ‘LTER’ lakes near Toolik Field Station, Alaska. Fish are to scale. A) ‘Medium’ char from Lake LTER348 (TL = 330 mm) with colored lines are provided as an illustration of the nine morphometric measurements made on each char in this study: snout length (SL), eye width (EW), maxilla length (ML), head depth (HD), head length (HL), body depth posterior (BDP), body depth anterior (BDA), post pelvic fin length (PPF) and caudal peduncle depth (CP). B) ‘Medium’ char from a closed lake (Lake Fog1; TL = 341 mm) C) ‘Large’ char from Lake LTER348 (TL = 578 mm); D) ‘Medium’ char from Lake LTER348 (TL = 337 mm); and E) ‘Large’ char from Lake LTER348 (TL = 587 mm).
Figure 3.4. Principal component analyses of genetic data between size classes in A) Lake Fog3 and B) Lake LTER348. Note: in the right panel, PC2 is replaced by total fish length (mm).
Figure 3.5. Uncorrected morphological traits measured for arctic char in Fog (light gray) and LTER lakes (dark gray), 2016 – 2017. Note: no char from the LTER lakes clustered into the ‘small’ size class.
Figure 3.6. Logistic regression models of size class designation from model-based clustering of raw morphometric traits. Light gray circles are arctic char from the Fog lakes, while black circles are the LTER lakes. Lines represent the lake group-specific transition probability between the size classes, with the dashed line representing the overall model of arctic char transition from medium to large between Fog and LTER lakes. Triangles represent the length at which the probability of transition between the size classes is equal to 0.5.
Figure 3.7. Back-calculated size-at-age (mm ± SD) for arctic char in Fog and LTER lakes. Fog (white circles) includes model-based size classes of ‘medium’ and ‘large’ (n = 11). Fog3 Dwarfs (gray circles) are the ‘small’ size class and only found in Lake Fog3 (n = 7). LTER lakes include ‘medium’ and ‘large’ size classes (n = 18).
CHAPTER 4

ASSESSING THE ABIOTIC AND BIOTIC FACTORS THAT STRUCTURE LAKE FOOD WEBs WITH POPULATIONS OF ARCTIC CHAR (SALVELNIUS ALPINUS) IN ARCTIC ALASKA

Abstract

Fish communities in arctic Alaska are broadly determined by geomorphic characteristics of the landscape partially determined by surface water connectivity. However, beyond this coarse filter, there is a surprising amount of variation in trophic structure (e.g., top predator, maximum size) across lakes given the relatively low species diversity. Thus, complex interactions between abiotic and biotic factors may ultimately control lake food web dynamics and function. We used field observations of predator diet, niche space, and trophic position from two geographically close but distinct lake groups to investigate food web hypotheses including: 1) arctic char are more densely populated in the absence of other apex predators; 2) arctic char trophic position increases with fish size regardless of char density; 3) arctic char feed at higher trophic positions (e.g., more piscivorous) and achieve greater maximum sizes in the presence of other apex predators; 4) arctic char trophic niche space is narrower in lakes in the presence of other apex predators; and, 5) arctic char resource polymorphism is more prominent in lakes in the absence of other apex predators. Generally, we confirmed hypotheses our first three hypotheses; however, we did not observe piscivory in large arctic char. In contrast to hypothesis #4 and #5, we found that char niche spaces were larger and overlapped less in lake with other predators, which could be driven by abiotic (e.g., ecosystem size) and biotic factors (prey availability and selection). Overall, we provide some of the first descriptions of arctic char trophic dynamics in northern Alaska, and our results increase our understanding of arctic lake trophic structure, including

---

4 This chapter is coauthored by Stephen L. Klobucar and Phaedra Budy
important biotic influences, which are critical for subsistence management and species conservation.

1 | INTRODUCTION

In fresh waters, the relative role of abiotic and biotic factors in structuring community assemblages is likely influenced by ecosystem size (Jackson et al., 2001), but biotic factors (e.g., piscivory) have been widely shown to have nearly equally strong direct and indirect effects on fish communities (Gilliam and Fraser, 2001; He & Kitchell, 1990; Robinson & Tonn, 1989). For many arctic lakes, species distribution and general trophic structure is largely influenced by foundational abiotic filters such as geography and climate, and fishes must necessarily be adapted to short growing seasons. Multiple periods of glaciation and glacial retreat resulted in repeated expansion and contraction of species ranges and fish habitats (Power et al., 2008). These type of environmental extremes have been shown to affect predators, which are longer-lived, to a greater degree than lower trophic levels (Jackson et al., 2001). As such, in lakes of the Arctic, overall species diversity is low. Fishes native to arctic lakes are well-adapted to extreme conditions and possess the ability to grow and reproduce quickly during the brief growing season when lakes are ice-free (lasting approximately 100 days; Wrona et al., 2006). Other adaptations, including large-scale movements (e.g., arctic grayling *Thymallus arcticus*), contribute to individual and population level fitness (Golden, 2016). These movements between lake systems can represent an important subsidies influencing overall lake trophic structure. Accordingly, lake morphometry, landscape position, and surface water connectivity often determines fish presence-absence, and, at a coarse scale, fish community composition (Hershey et al., 1999).

In arctic Alaska, community openness and connectivity among lakes range from completely isolated (‘closed’) to varying degrees of connectedness (strings of lakes connected by flowing streams; ‘open’). Generally, these lake classifications are a function of past glaciations and concordant geomorphic changes (Hershey et al. 1999; Hershey et al. 2006). Fish community
composition of isolated lakes often consists of only arctic char (*Salvelinus alpinus*) and slimy sculpin (*Cottus cognatus*). The trophic structure of these isolated lakes is largely regulated by internal processes (Budy & Luecke, 2014; see also Chapter 3). Alternatively, lakes open to more contemporary fish colonization and movement are characterized by different fish communities, trophic structure, and pathways of energy flow (e.g., biomass; Jones et al., 2017; Laske et al., 2016). Lake trout, generally function as the top predator in these lakes and influence trophic structuring of prey fishes (e.g., Goyke & Hershey, 1992; Hanson et al., 1992) through consumptive and competitive interactions (Zarnetske et al., *in prep*). In fact, it is generally theorized that lake trout exclude arctic char in systems where both of these top predators could occur, and arctic char persist in lakes where lake trout cannot invade (Hershey et al., 2006).

However, we recently began monitoring a group of ‘open’ lakes that contain coexisting populations of both lake trout and arctic char, and, surprisingly, the arctic char are more numerous and generally larger. Thus, it appears in arctic Alaskan lakes that contain arctic char, the function and structure of the food web could be highly dependent on inter- and intraspecific interactions, as well as the physical landscape.

Arctic char are a generalist predator with Holarctic distribution that exhibit a high degree of polymorphic variability among and within populations (e.g., Alekseyev et al., 2002; Klemetsen, 2013). Feeding ecology of arctic char is often attributed to the distinction intraspecific niche partitioning (e.g., littoral, pelagic, profundal) and a variety of diets (e.g., planktivorous, piscivorous) can occur within the same lake (Jonsson and Jonsson, 2001; Klemetsen, 2010). The degree of habitat segregation and char polymorphism may be positively related to overall ecosystems size and lake morphometry including depth (e.g., maximum, mean), surface area, and volume (Recknagel et al., 2017). Alternatively, or in combination with lake physical characteristics, biotic interactions can determine overall resource use, size structure, and trophic position of arctic char (Eloranta et al., 2015; see also Chapter 3).
With a focus on arctic char populations, in this study, we investigated and quantified fish community composition and structure, as well as size structure, specifically in regard to arctic char populations, in two contrasting groups of lakes in arctic Alaska. While relatively close geographically (within 5 km), the lakes in one group of lakes are isolated with no surface water connectivity between the lakes (‘closed’) and contain arctic char as the only apex predator. The other group of lakes are defined by increased surface water connectivity between them (e.g., inlet and outlet steams; ‘open’) and contain arctic char as well as arctic grayling (*Thymallus arcticus*), lake trout (*Salvelinus namaycush*), and burbot (*Lota lota*) as potential competitors. Beyond the physical template that differentially structures these groups of lakes, we would expect the food webs to be structured in ways that allow the persistence of arctic char in each of the lake groups. Thus, across and within these groups of lakes, we tested food web and trophic hypotheses including: 1) arctic char are more densely populated in the absence of other apex predators; 2) arctic char trophic position increases with fish size regardless of char density; 3) arctic char feed at higher trophic positions (e.g., more piscivorous) and achieve greater maximum sizes in the presence of other apex predators; 4) arctic char trophic niche space is narrower in lakes in the presence of other apex predators; and, 5) arctic char resource polymorphism is more prominent in lakes in the absence of other apex predators.

While arctic char are a Holarctic species, they are relatively understudied in Alaska relative to their Scandinavian and Canadian counterparts. We present important findings for char and lake food webs in unexploited systems that may act as sentinels of climate change across the Arctic. As a whole, our study contributes valuable insight regarding trophic structure of lakes in arctic Alaska and sets the stage for subsistence management and conservation decisions for culturally- and ecologically-important fish species.
2 | METHODS

2.1 | Study area

Our research was conducted in lakes near Toolik Field Station (68°37.796’N, 149°35.834’W), home of the Arctic Long Term Ecological Research project (http://arc-lter.ecosystems.mbl.edu/), in the northern foothills of the Brooks Mountain Range, Alaska. We chose two isolated lakes (Lakes Fog1 and Fog2) that have been monitored as part of other studies for many years (e.g., Budy & Luecke, 2014), and to increase sample size and inference, we initiated sampling in 2013 and 2014 on two additional lakes in the Fog lake group (Lakes Fog3 and Fog5, respectively). We chose a nearby, more connected lake group, the LTER lakes (Lakes LTER345, LTER347, and LTER348), to test our trophic structure hypotheses and sampled these lakes, and all Fog lakes, from 2014 – 2016 (Table 4.1). Generally, lakes in the area are shallow (maximum depths of 3 – 30 m) and oligo- to ultra-oligotrophic (chlorophyll-α concentrations <5 µg/L; Kling et al., 1992). Typically, the lakes are ice covered from early October to early June and thermally stratified during summer (June – August).

2.2 | Fish sampling

In each year, we sampled fish using hook-and-line through the ice beginning in May, and throughout the open water via hook-and-line and experimental gill nets (usually 3 – 4 sampling events·lake⁻¹·year⁻¹). We used experimental benthic gill nets (Lester et al. 2009) set perpendicular to shore on the lake bottom, which extended from the littoral zone to pelagic areas, and checked nets every half hour to minimize mortalities. We weighed and measured all fish captured, tagged all fish > 150 mm with a passive integrated transponder (PIT) tag, and clipped the adipose fin for stable isotope analyses. For many captures, we obtained diet information via gastric lavage, and additionally we collected diets (whole stomach) from incidental mortalities. We quantified population abundance in each lake using mark-recapture techniques (e.g., Budy & Luecke, 2014; Klobucar et al., 2017) from PIT tag recaptures. For Lakes Fog1 and Fog2, we used a Huggins
closed-capture model in Program Mark (White & Burnham, 1999), while for all other lakes with shorter mark-recapture time series, we used a modified Schnabel estimate (Krebs, 1999).

2.3 | Diet and stable isotope analyses

In the field, we stored diets in 95% ethanol until analysis in the lab, when we identified stomach contents to the lowest taxonomic group possible. We weighed prey fish individual and for zooplankton and invertebrate prey, obtained blot-dry wet weight en masse to the nearest 0.01 g for each taxonomic group. To determine potential intra- and interspecific competition within lakes, as well as compare fish diets across and with lake groups, we calculated diet overlap using Schoener’s index (Schoener, 1970; Equation 1),

$$\alpha = 1 - 0.5 \cdot (\sum_{i=1}^{n} |p_{xi} - p_{yi}|),$$

where i is a given prey item, p is the mean proportion of i, and x and y are the specified group of predators being compared. The single value α is diet overlap from 0 (no overlap) to 1 (complete overlap), and values greater than 0.6 are indicative of significant diet overlap (Schoener, 1970).

To better compare across lakes, as well as life-stages and physiologies of arctic char, we separated arctic char into ‘small’, ‘medium’, and ‘large’ size classes based on our other work of char morphology and allometry (Klobucar et al., in prep; see Chapter 3).

We used carbon (δ¹³C) and nitrogen (δ¹⁵N) stable isotopes to further explore a time-integrated representation of predator diets and assess potential intra- and interspecific competition within lakes and lake groups. For stable isotope analyses, we used adipose fin clips for arctic char, arctic grayling, and lake trout, and a dorsal fin clip for burbot. Samples were dried for 48 hrs at 70°C, ground into a homogenized powder, and placed into pre-weighed tin capsules. Fish tissues samples were processed at the Washington State University Stable Isotope Core laboratory (prior to 2016) and the Utah State University Stable Isotope Lab (2016) for analysis of δ¹³C and
δ15N, and percent composition of both carbon and nitrogen. Isotopic signatures are reported in δ-notation (Equation 2):

$$
\delta^{13}C \text{ or } \delta^{15}N = \left[ \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \cdot 1000 ,$$

where $R_{\text{sample}}$ is the ratio of $^{13}$C/$^{12}$C or $^{15}$N/$^{14}$N found in the tissue or filter sample, and $R_{\text{standard}}$ is the ratio of $^{13}$C/$^{12}$C or $^{15}$N/$^{14}$N found in the standard sample. The standard for δ13C is PeeDee belemnite and the standard for δ15N is atmospheric nitrogen.

To calculate trophic position of fishes we used (Equation 3):

$$TP_i = \left( \frac{\delta^{15}N_i - \delta^{15}N_{\text{baseline}}}{3.4} \right) + 2,$$

where $TP_i$ is the trophic position of species $i$, $N_i$ is the nitrogen signature of species $i$, and $N_{\text{baseline}}$ is the nitrogen signature for primary consumers (i.e. Vander Zanden & Rasmussen, 1999). We assumed that primary consumers have a trophic position of 2 and a δ15N trophic fractionation value of 3.4 ± 1.1‰ (Minagama & Wada, 1984). We used a mean δ15N value of pelagic zooplankton ($Daphnia$ spp.) and snails ($Lymnaea elodes$) from the littoral zone to represent primary consumers in each lake group (δ15N$_{\text{closed}} = 3.07$‰; δ15N$_{\text{open}} = 3.10$‰).

To evaluate characteristics trophic overlap of arctic char with other apex predators (open lakes), and within lake size classes of arctic char (closed and open lakes), we used SIBER (Stable Isotope Bayesian Ellipses) in R, which uses Bayesian inference techniques to describe niche and community metrics (Jackson et al., 2011). The SIBER model uses bivariate δ13C and δ15N data to represent isotopic trophic niche space for a species or group. The SIBER model produces ellipses around the centroid that include ± 1 SD. We calculated the percent species overlap at the intersection of ellipse areas. Additionally, as a measure of niche space within lake habitats, we calculated percent littoral contribution using a two-source mixing model (Vander Zanden & Vadeboncoeur, 2002; Equation 4):
percentage contribution of littoral

\[ = \frac{\delta^{13}C_c - \delta^{13}C_p}{\delta^{13}C_i - \delta^{13}C_p}, \]

where \( \delta^{13}C_c, \delta^{13}C_i, \delta^{13}C_p \) are the mean \( \delta^{13}C \) of the consumer, littoral prey, and pelagic prey for each lake group.

To assess arctic char trophic position as a function of length (fixed effect), we used a linear mixed effects model, and used a random effect of group (Fog, LTER) to control for difference between lake groups (Equation 5):

\[
Trophic \ position_{ij} = \alpha + \beta_1 \cdot Group_i + \beta_2 \cdot Length_{ij} + \varepsilon_{ij}, \\
\varepsilon_{ij} \sim N(0, \sigma^2),
\]

where Trophic position\(_{ij}\) is the predicted trophic position for arctic char of a given Length \( j \) in Group \( i \) and \( \varepsilon_{ij} \) is random error that is normally distributed with a mean of 0 and variance \( \sigma^2 \). We also compared between groups (random effect) using a linear mixed effects model using lake as a random effect (Equation 6):

\[
Trophic \ position_{ijk} = \alpha + \beta_1 \cdot Group_{ik} + \beta_2 \cdot Length_{ijk} + \varepsilon_{ijk}, \\
\varepsilon_{ijk} \sim N(0, \sigma^2),
\]

where Trophic position\(_{ijk}\) is the predicted trophic position for arctic char of a given Length \( j \) in Group \( i \), and lake \( k \), and \( \varepsilon_{ijk} \) is random error that is normally distributed with a mean of 0 and variance \( \sigma^2 \). We performed these analyses in R version 3.4.2 (R Core Team 2017) using the ‘nlme’ package (Pinheiro et al. 2017) and assessed model fit using BIC for null, random intercept, and random slope and intercept models.
3 | RESULTS

In total, we sampled 583 individual arctic char across all seven study lakes (n = 360 in closed lakes; n = 223 in open lakes; Table 4.2). Char were significantly larger in the LTER lakes (mean TL ± SE = 468.1 ± 6.37 mm; range = 187 – 670 mm) relative to the Fog lakes (mean TL ± SE = 264.2 ± 3.86; range = 113 – 486 mm; \( t = 27.39 \), df = 383, \( p < 0.001 \); Figure 4.1). We quantified diets from 171 arctic char (n = 116 for Fog lakes; n = 55 for LTER lakes), as well as 10 arctic grayling and 11 lake trout from the LTER lakes. To further quantify diet overlap and niche partitioning, we analyzed stable isotopes from 236 individuals (n = 121 for Fog lakes; n = 115 for LTER lakes), and we also analyzed stable isotopes from 27 arctic grayling, 36 lake trout, and 8 burbot.

Across all lakes, trophic position increased with length (\( t = 2.58 \), df = 233, \( p = 0.01 \); Figure 4.2, Table 4.3). However, by lake group, we only observed a significant increase of trophic position with length in LTER lakes (\( t = 3.33 \), df = 111, \( p = 0.001 \)), where we also captured other apex predator species. Our model for the Fog lakes was not significant (\( t = 0.76 \), df = 116, \( p = 0.45 \)). Across all lakes by size class, arctic char diets were similar for medium and large char, but relatively dissimilar for each of those size classes relative to small char (Schoener’s α between small and medium = 0.55, between medium and large = 0.81, between small and large = 0.40). In general, the proportion of chironomids, trichoptera, and zooplankton in char diets decreased with size, while diet proportions of mollusks increased with fish size (Table 4.4). While we did not obtain any diet information for small arctic char (< 200 mm) in the LTER lakes, diets were of medium char in the LTER lakes were similar to small char in the Fog lakes (Table 4.5; Figure 4.3). Despite noted differences in the relationship between trophic position and total length, we did not observe fish in diets of arctic char in the LTER lakes. Overall, diets of large arctic char were relatively similar between lake groups (Schoener’s α = 0.64). We only observed fish in char diets for medium char in the Fog lakes and instances of piscivory were rare. Conversely, in the LTER lakes, lake trout did consume fish (Table 4.4;
Figure 4.4); however, all of these fish prey we observed were slimy sculpin. Across all predators in the LTER lakes, there was a fair amount of diet overlap (Figure 4.4), especially between medium arctic char and arctic grayling (Schoener’s $\alpha = 0.68$), and medium arctic char and large arctic char (Schoener’s $\alpha = 0.69$).

Regardless of size class, across and within the Fog lakes, arctic char occupied similar trophic niches with considerable overlap (Figure 4.5, Table 4.5). Lake Fog5 was the only lake with a sample size sufficient to estimate niche space for large arctic char, but this space was very narrow. In Lakes Fog1 and Fog3, the niche space of small char was smaller relative to medium char and was almost completely overlapped by medium char niche space (100% and 98.9% overlap, respectively). In contrast, there was more differentiation between medium and large arctic char across the LTER lakes (e.g., % littoral = 76.9 vs 97.2 for medium and large arctic char; $t = 6.61$, df = 77.97, $p < 0.001$). Estimates of trophic niche space showed that only 37.2%, 65.7%, and 53.2% of medium char trophic niche space was overlapped by large char niche space in Lakes LTER345, LTER347, and LTER348, respectively.

Across the LTER lakes, apex predators exhibited significantly different trophic positions except for burbot and lake trout, but these species did not occur in sympatry (e.g., mean TP for large arctic char = 4.00; mean TP for lake trout = 4.51; $t = 6.42$, df = 85.13, $p < 0.001$; Table 4.4). Lake trout maintained the highest trophic position in Lakes LTER345 and LTER347, with minimal overlap with large arctic char (19.5% and 6.9%, respectively). However, lake trout and large arctic char in these lakes were both nearly exclusively littoral (100% and 97.3%, respectively). Arctic grayling niche space fell between medium arctic char and large arctic char in both Lakes LTER345 and LTER347, while burbot overlapped with both size classes of arctic char in Lake LTER348 albeit it at a slightly increased trophic position (4.37 vs 4.00 (large AC) and 3.85 (medium AC); Table 4.4).
4 | DISCUSSION

Understanding the relative role abiotic and biotic factors contribute to structuring lake food webs is important for management and conservation of native fishes. However, anthropogenic influences can mask underlying biology and affect the abiotic (e.g., water quality) and biotic factors (e.g., fish harvest) that contribute to lake trophic structure and function. Here, we investigated arctic lake food web structure, in the absence of human alteration, with a focus on biotic factors across two lake complexes. One series of lakes (the Fog lakes) is closed to other surface water connections and as such contains only arctic char as an apex predator. The second series of lakes (the LTER lakes), within 5 km of the Fog lakes, are more open, with inlet and outlet streams that partially connect the lakes across the landscape. The LTER lakes contain arctic char, surprisingly, in sympatry with lake trout, as well as arctic grayling and burbot as other potential predators.

Across all lakes, arctic char trophic position increased with char length as expected; however, between lake groups, this relationship was only significant for the LTER lakes. Accordingly, arctic char in the LTER lakes were significantly larger than arctic char in Fog lakes, but against our hypotheses, we did not observe evidence of increased piscivory in these larger char. Thus, we generally confirmed our hypotheses #1, #2, and #3—arctic char populations are more densely populated in the absence of other apex predators. When occurring with other predators (e.g., lake trout) char will be larger and feed at higher trophic positions. While we not directly observe piscivory by arctic char in the LTER lakes, we also rarely observed piscivory in the Fog lakes, and never in diets of arctic char greater than 400 mm (Figure 4.3).

Abiotic factors that determine the distribution of arctic fishes across the foothills of the Brooks Mountain Range, Alaska is generally well understood (Hershey et al., 1999; Hershey et al., 2006); however, specific intra- and interspecific interactions likely vary at local scales (e.g., across and within lake complexes). Apex predators that exhibit strong prey preference have been shown to stabilize lake food webs by linking between littoral and pelagic food chains (Post et al.,
Arctic char often segregate between foraging arenas in lakes (e.g., benthic, littoral, pelagic) through resource polymorphism and morphological divergence (Hindar & Jonsson, 1982). As such, char diets may be specialized for specific lake habitats without coupling of pelagic and littoral prey items or habitats (Riget et al., 1986). Similarly, in Norway, when arctic char exist in sympatry with brown trout (Salmo Trutta), coupling of littoral and pelagic food web compartments can be limited as a result of niche partitioning through competitive and consumptive interactions (Eloranta et al., 2013). On the other hand, arctic char, as well as the other predators in the open lakes, are often viewed as generalist consumers, which can also shift prey seasonally (Eloranta et al., 2010), and omnivory is much more likely in upper trophic levels (Thompson et al., 2007). In the Fog lakes, we found little overall diet preference and high overlap between char size classes. This was in opposition to our original hypothesis that resource polymorphism would be more prominent in the Fog lakes as a result of increased intraspecific competition, as well as the absence of other apex predators. However, as the Fog lakes are relatively small and homogenous, habitats may not be segregated enough to show strong habitat selection, and thus, prey preference. For example, the smallest lake (~25 ha) in a study of char in Norway showed similar diets across all char, while in larger lakes (> 1000 ha) char separated by trophic niche area (Knudsen et al., 2006). Additionally, since our study lakes are nutrient poor, intraspecific competition for extremely limited resources may not allow for strong diet selection, and the most successful predators are those that feed opportunistically (e.g., Amundsen, 1995; Jonsson & Jonsson, 2001). Our isotopes results support both of these notions, where mean littoral contribution to δ\(^{13}\)C was similar for all size classes of char in the Fog lakes was relatively low and similar when compared to the LTER lakes.

In the larger LTER lakes, we did observe more separation between diets and habitat preference between medium and large char, as expressed by δ\(^{13}\)C signatures. In general, diet of smaller char in the LTER lakes suggested use of more pelagic habitats, while diets of the largest char were more littoral, consistent with other studies (e.g., Hindar & Jonsson, 1982; Power et al.,
2005). For larger char, increased reliance on littoral prey could be related to greater energy and larger sizes of primary consumers in the littoral zone relative to pelagic zone (Karlsson & Bystrom, 2005). Accordingly, these largest char may exhibit phenotypic adaptations to increased foraging success in shallower, more productive habitats (Chapter 3); however, as the climate warms, the biomass and availability of prey may also effect foraging success in different habitats (Chapter 2).

At the onset of this study, we expected arctic char niche space would be reduced in the LTER lakes relative to the Fog lakes as a result of interspecific competition. However, we observed generally larger and wider niche space of char in the LTER lakes. While we did observe a fair amount of diet overlap between arctic char and lake trout and arctic grayling (Schoener’s $\alpha = 0.49 – 0.68$), less overlap of overall niche space was noted in our isotopic analyses. For example, lake trout niche overlap with large arctic char was less than 20% in both LTER lakes where they co-occur. The LTER lakes may be large enough, and slightly more productive, to allow multiple large predators to coexist, especially in the relatively low densities we observed. In the LTER lakes, snails are the primary food item of large arctic char, whereas lake trout consume more chironomids when arctic char are absent. In comparison, arctic char across the Fog lakes, in the absence of lake trout, generally consume more chironomids than we observed in the LTER lakes. Furthermore, in a nearby lake that contains lake trout without arctic char, diet analyses show that lake trout rely more on snails as a diet item in the absence of arctic char (Klobucar & Budy, unpublished data). Collectively, these results suggest there could be species-specific trade-offs regarding prey selection with and without interspecific competition.

As lake trout are widely believed to be the top predator across the landscape in arctic Alaska (as also indicated by our isotope analyses; Hershey et al. 1999; Hershey et al. 2006), lake trout were thought to play an important role in structuring the populations of arctic char, with which they rarely coexist. However, our diet analyses did not indicate notable differences between arctic char diets in the Fog lakes relative to the LTER lakes. Further, arctic char are
larger and more numerous than lake trout in the LTER lakes. While rare, another study of sympatric populations of arctic char and lake trout in northern Quebec, Canada, revealed similar results (Fraser & Power, 1989). In this study, arctic char were not piscivorous in lakes that also contained lake trout, and arctic char growth rates were faster but their longevity was shorter. In Chapter 3, we noted higher growth rates for char in the LTER lakes than those of char in the Fog lakes. As such, competitive or consumptive pressure from lake trout may have selected for faster growing arctic char. Notably, however, in one LTER lake without lake trout (Lake LTER348), arctic char densities were greatest of the LTER lakes, we still caught large arctic char, and caught more small-medium arctic char as well. As such, other mechanisms by which these populations of arctic char can grow large and coexist with lake trout should be further addressed. Our results suggest arctic char are a generalized consumer relative to other fish species. These generalist characteristics may allow them to succeed in different types of lakes and potentially persist in changing environments (e.g., Laske et al., 2018).

While ecosystem size potentially influences maximum size of arctic char (e.g., Riget et al., 2000), Lake LTER348 was the smallest of the open lakes and most comparable in size to the Fog lakes. However, this lake still contained very large char, including the largest chars sampled in this study. For this particular lake, it is could be possible that char predisposed (genetically or otherwise; see Chapter 3) to faster growth rates colonized this lake while lake trout did not. Alternatively, a historic population of lake trout in Lake LTER348 could have selected for large char, but the population of lake trout has since been extirpated, or the population of burbot in this lake (which are extremely rare in other LTER lakes) could be large enough and piscivorous enough to influence arctic char size structure. Indeed, burbot trophic position is greater than arctic char in Lake LTER348 (Table 4.4). This could potentially be addressed using emerging environmental DNA analyses (Thomsen & Willerslev, 2015), and further study into the LTER lake populations could reveal important genetic divergence and evolutionary adaptations (Chapter
3). The genetic diversity and adaptability of arctic char, in both lake groups, could become increasingly important in a rapidly changing climate (Chapter 4; Gislason et al., 1999).

In addition to warmer temperatures in the arctic, surface water connectivity is likely to become more variable, with seasonal disconnection of currently connected lakes (Prowse et al., 2006), which could have important population and community level effects with regard to lake trophic dynamics as this study indicates. Accordingly, we have shown that biotic factors influence arctic char size structure in these lakes (Chapter 3), and thus, the trophic dynamics and lake food web structure in this study could be driven by similar factors (e.g., primary production, density-dependence). If seasonal subsidies of arctic grayling, particularly important for lake trout growth, no longer reach the LTER lakes, lake trout competition with arctic char may increase. In the Fog lakes, which are already closed to species movements, internal processes are likely to regulate future char population dynamics (Budy & Luecke, 2014), and the direction and magnitude of population abundances is likely to depend on how food resources respond (Klobucar et al., 2018). In northern Alaska, where thousands of fish-bearing lakes are important subsistence resources, understanding the factors that influence lake food web structure is critical for species management and conservation, especially with regard to populations of fishes at the top of these food webs. Apex predators, such as arctic char and lake trout are important for local subsistence (Pedersen & Hugo, 2005). These lakes provide a largely unaltered template to study lake trophic structure explicitly within a framework of inter- and intraspecific community dynamics, especially in a rapidly changing climate.

REFERENCES


Zarnetske, P. L., Urban, M.C., Skelly, D.K., Budy, P. & S. L. Klobucar. (In prep). Do climatic changes or biotic interaction explain the condition of Arctic freshwater fishes over 30 years?
Table 4.1. Physical and chemical conditions of study lakes near Toolik Field Station, Alaska. Secchi depth and chlorophyll concentrations are average measurements from late July 2016.

<table>
<thead>
<tr>
<th>Group</th>
<th>Lake</th>
<th>Latitude (°N)</th>
<th>Longitude (°W)</th>
<th>Surface area (ha)</th>
<th>Max. depth (m)</th>
<th>Mean depth (m)</th>
<th>Secchi depth (m)</th>
<th>Chl-α (µg·L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fog lakes</td>
<td>Fog1</td>
<td>68.684</td>
<td>149.082</td>
<td>3.5</td>
<td>19.7</td>
<td>8.4</td>
<td>4.9</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>Fog2</td>
<td>68.679</td>
<td>149.091</td>
<td>5.9</td>
<td>19.8</td>
<td>7.8</td>
<td>7.1</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>Fog3</td>
<td>68.673</td>
<td>149.088</td>
<td>3.9</td>
<td>21.0</td>
<td>7.6</td>
<td>6.0</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>Fog5</td>
<td>68.678</td>
<td>149.065</td>
<td>0.7</td>
<td>9.9</td>
<td>3.5</td>
<td>5.0</td>
<td>5.2</td>
</tr>
<tr>
<td>LTER lakes</td>
<td>LTER345</td>
<td>68.623</td>
<td>149.151</td>
<td>30.7</td>
<td>28.6</td>
<td>12.3</td>
<td>1.5</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>LTER347</td>
<td>68.625</td>
<td>149.139</td>
<td>13.5</td>
<td>17.6</td>
<td>5.6</td>
<td>1.8</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>LTER348</td>
<td>68.641</td>
<td>149.127</td>
<td>5.7</td>
<td>9.6</td>
<td>3.2</td>
<td>3.7</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 4.2. Catch summary for arctic char in study lakes near Toolik Field Station, Alaska.

<table>
<thead>
<tr>
<th></th>
<th>Fog lakes</th>
<th>Fog1</th>
<th>Fog2</th>
<th>Fog3</th>
<th>Fog5</th>
<th>LTER lakes</th>
<th>LTER345</th>
<th>LTER347</th>
<th>LTER348</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Arctic char</strong></td>
<td>360</td>
<td>71</td>
<td>14</td>
<td>224</td>
<td>51</td>
<td>223</td>
<td>94</td>
<td>38</td>
<td>120</td>
</tr>
<tr>
<td>(n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean TL ± SE (mm)</td>
<td>264.2 ± 3.9</td>
<td>275.3 ± 7.7</td>
<td>330.1 ± 19.3</td>
<td>243.2 ± 4.6</td>
<td>323.1 ± 7.9</td>
<td>468.1 ± 6.4</td>
<td>509.7 ± 5.2</td>
<td>460.7 ± 13.3</td>
<td>444.6 ± 10.1</td>
</tr>
<tr>
<td>Abundance</td>
<td>448 (290 - 693)</td>
<td>163 (105 - 288)</td>
<td>666 (477 - 1073)</td>
<td>75 (55 - 119)</td>
<td>277 (177 - 540)</td>
<td>73 (40 - 196)</td>
<td>331 (227 - 563)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density by area</td>
<td>127 (82 - 197)</td>
<td>29 (19 - 51)</td>
<td>171 (123 - 276)</td>
<td>104 (76 - 164)</td>
<td>7 (6 - 17)</td>
<td>5 (3 - 14)</td>
<td>58 (39 - 98)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arctic grayling</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50</td>
<td>16</td>
<td>34</td>
</tr>
<tr>
<td>(n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean TL ± SE (mm)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>330.6 ± 6.7</td>
<td>304.8 ± 14.6</td>
<td>342.8 ± 35.5</td>
</tr>
<tr>
<td>Range (mm)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>204 -430</td>
<td>232 - 430</td>
<td>204 - 407</td>
</tr>
<tr>
<td>Burbot</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>(n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean TL ± SE (mm)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>410.8 ± 23.1</td>
<td>-</td>
<td>410.8 ± 23.1</td>
</tr>
<tr>
<td>Range (mm)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>272 - 490</td>
<td>-</td>
<td>272 - 490</td>
</tr>
<tr>
<td>Lake trout</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>54</td>
<td>44</td>
<td>10</td>
</tr>
<tr>
<td>(n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean TL ± SE (mm)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>412.6 ± 7.1</td>
<td>414.8 ± 8.3</td>
<td>402.7 ± 13.0</td>
</tr>
<tr>
<td>Range (mm)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>244 - 625</td>
<td>244 - 625</td>
<td>345 - 454</td>
</tr>
</tbody>
</table>
Table 4.3. Model summary (Bayesian information criterion (BIC), Log-Likelihood (LL), standard error (SE), degrees of freedom (DF), t-value ($t$), and p-value ($P$) for linear mixed effects models predicting relationship of arctic char trophic position with fish length. Model for all lakes included a random effect of group (Fog, LTER lakes) and model for each group of lakes included a random effect of lake.

<table>
<thead>
<tr>
<th>Group</th>
<th>Model</th>
<th>BIC</th>
<th>LL</th>
<th>SE</th>
<th>DF</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All lakes</td>
<td>Null</td>
<td>253.6</td>
<td>-118.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Random intercept</td>
<td>250.5</td>
<td>-114.4</td>
<td>0.0003</td>
<td>233</td>
<td>2.58</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Random intercept + slope</td>
<td>260.6</td>
<td>-113.9</td>
<td>0.0003</td>
<td>233</td>
<td>1.91</td>
<td>0.06</td>
</tr>
<tr>
<td>Fog lakes</td>
<td>Null</td>
<td>158.4</td>
<td>-72.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Random intercept</td>
<td>148.4</td>
<td>-64.6</td>
<td>0.0004</td>
<td>116</td>
<td>0.76</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>Random intercept + slope</td>
<td>153.8</td>
<td>-62.5</td>
<td>0.0009</td>
<td>116</td>
<td>-0.49</td>
<td>0.63</td>
</tr>
<tr>
<td>LTER lakes</td>
<td>Null</td>
<td>106.3</td>
<td>-46.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Random intercept</td>
<td>67.5</td>
<td>-24.3</td>
<td>0.0003</td>
<td>111</td>
<td>3.33</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Random intercept + slope</td>
<td>71.9</td>
<td>-21.8</td>
<td>0.0008</td>
<td>111</td>
<td>1.4</td>
<td>0.17</td>
</tr>
</tbody>
</table>
Table 4.4. Summary of fish sampled for diets and isotopes by lake group and species, and average diet proportion for each size of char or other species (pooled), in study lakes near Toolik Field Station, Alaska, 2014 – 2016.

<table>
<thead>
<tr>
<th></th>
<th>Arctic char (Fog lakes)</th>
<th>Arctic char (LTER lakes)</th>
<th>Arctic grayling</th>
<th>Lake trout</th>
<th>Burbot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Small</td>
<td>Medium</td>
<td>Large</td>
<td>Small</td>
<td>Medium</td>
</tr>
<tr>
<td>Number of diets</td>
<td>11</td>
<td>100</td>
<td>5</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>Mean TL ± SE (mm)</td>
<td>177.5 ± 4.2</td>
<td>312.1 ± 5.4</td>
<td>455.2 ± 7.8</td>
<td>-</td>
<td>420.3 ± 15.6</td>
</tr>
</tbody>
</table>

Proportion of prey by weight

- Chironomidae 0.25 0.19 0.20 - 0.03 0.05 0.01 0.16 -
- Fish 0.00 0.04 0.00 - 0.00 0.00 0.00 0.14 -
- Mollusca 0.03 0.40 0.70 - 0.26 0.50 0.03 0.07 -
- Trichoptera 0.58 0.23 0.00 - 0.60 0.34 0.76 0.35 -
- Zooplankton 0.09 0.03 0.00 - 0.06 0.01 0.00 0.00 -
- Terrestrial invertebrates 0.00 0.02 0.00 - 0.00 0.00 0.03 0.04 -
- Aquatic invertebrates 0.00 0.07 0.10 - 0.04 0.10 0.17 0.24 -
- Organic matter 0.05 0.02 0.00 - 0.01 0.00 0.00 0.00 -

<table>
<thead>
<tr>
<th></th>
<th>Arctic char (Fog lakes)</th>
<th>Arctic char (LTER lakes)</th>
<th>Arctic grayling</th>
<th>Lake trout</th>
<th>Burbot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Small</td>
<td>Medium</td>
<td>Large</td>
<td>Small</td>
<td>Medium</td>
</tr>
<tr>
<td>Number of isotopes</td>
<td>29</td>
<td>85</td>
<td>7</td>
<td>0</td>
<td>63</td>
</tr>
<tr>
<td>Mean TL ± SE (mm)</td>
<td>163.6 ± 4.1</td>
<td>302.3 ± 6.1</td>
<td>457.4 ± 5.8</td>
<td>-</td>
<td>390.3 ± 9.4</td>
</tr>
<tr>
<td>Mean δ15N</td>
<td>8.1 ± 0.2</td>
<td>8.4 ± 0.2</td>
<td>9.1 ± 0.3</td>
<td>-</td>
<td>9.4 ± 0.2</td>
</tr>
<tr>
<td>Mean δ13C</td>
<td>-28.8 ± 0.3</td>
<td>-28.2 ± 0.2</td>
<td>-28.1 ± 1.1</td>
<td>-</td>
<td>-27.7 ± 0.2</td>
</tr>
<tr>
<td>Mean TP</td>
<td>3.49 ± 0.05</td>
<td>3.57 ± 0.05</td>
<td>3.77 ± 0.09</td>
<td>-</td>
<td>3.85 ± 0.04</td>
</tr>
<tr>
<td>Mean % Littoral</td>
<td>69.22 ± 0.03</td>
<td>76.61 ± 0.02</td>
<td>74.68 ± 0.12</td>
<td>-</td>
<td>76.91 ± 0.03</td>
</tr>
</tbody>
</table>
Table 4.5. Schoener’s α index for diet overlap between diets of arctic char (AC) in Fog lakes (by size class), compared with arctic char in LTER lakes (by size class), arctic grayling (AG), lake trout (LT) for fish sampled in study lakes near Toolik Fields Station, Alaska, 2014 – 2015.

<table>
<thead>
<tr>
<th></th>
<th>Fog lakes AC</th>
<th></th>
<th></th>
<th>LTER lakes AC</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Small</td>
<td>Medium</td>
<td>Large</td>
<td>Small</td>
<td>Medium</td>
<td>Large</td>
</tr>
<tr>
<td>Fog lakes AC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>-</td>
<td>0.50</td>
<td>0.24</td>
<td>-</td>
<td>0.71</td>
<td>0.43</td>
</tr>
<tr>
<td>Medium</td>
<td>0.50</td>
<td>-</td>
<td>0.67</td>
<td>-</td>
<td>0.60</td>
<td>0.77</td>
</tr>
<tr>
<td>Large</td>
<td>0.24</td>
<td>0.67</td>
<td>-</td>
<td>-</td>
<td>0.34</td>
<td>0.64</td>
</tr>
<tr>
<td>LTER lakes AC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Medium</td>
<td>0.71</td>
<td>0.60</td>
<td>0.34</td>
<td>-</td>
<td>-</td>
<td>0.69</td>
</tr>
<tr>
<td>Large</td>
<td>0.43</td>
<td>0.77</td>
<td>0.64</td>
<td>-</td>
<td>0.69</td>
<td>-</td>
</tr>
<tr>
<td>LTER lakes AG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.68</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>LTER lakes LT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.49</td>
<td>0.55</td>
<td></td>
</tr>
</tbody>
</table>
Figure 4.1. Length-frequency of all arctic char sampled in Fog lakes (gray; n = 360) and LTER lakes (white; n = 223) near Toolik Field Station, Alaska, 2014 – 2015. Dashed line is the mean length of Fog lakes arctic char (264 mm) and dotted line is the mean length of LTER lakes arctic char (468 mm).
Figure 4.2. Linear mixed effects relationship between length (mm) and arctic char trophic position for all lakes (top) and by lake group (bottom). Blue points and line are Fogs lakes. Red points and line are LTER lakes.
Figure 4.3. Average diet proportion for arctic char in Fog lakes (top) and LTER lakes (bottom) by size class: small (<200 mm), medium (200 – 400 mm), and large (>400 mm) in study lakes near Toolik Field Station, AK, 2014 – 2015. Diet items include chironomids (Chir), mollusks (Moll), trichoptera (Tri), zooplankton (Zoop), fish, terrestrial invertebrates (Terr), organic material (OM), unidentified aquatic invertebrates (Unid AI) and other.
Figure 4.4. Average diet proportion for arctic char (AC), arctic grayling (AG), and lake trout (LT) in LTER lakes near Toolik Field Station, Alaska, 2014 – 2015. Diet items include chironomids (Chir), mollusks (Moll), trichoptera (Tri), zooplankton (Zoop), fish, terrestrial invertebrates (Terr), organic material (OM), unidentified aquatic invertebrates (Unid AI) and other.
Figure 4.5. Stable Isotope Bayesian Ellipses (SIBER) ellipses representing arctic char trophic niche space (by size class) for individual stable isotope measurements for each Fog lake studied near Toolik Field Station, Alaska.
Figure 4.6. Stable Isotope Bayesian Ellipses (SIBER) ellipses representing arctic char trophic niche space (by size class or species) for individual stable isotope measurements for each LTER lake studied near Toolik Field Station, Alaska.
CHAPTER 5

AT THE FOREFRONT: EVIDENCE OF THE APPLICABILITY OF USING ENVIRONMENTAL DNA TO QUANTIFY THE ABUNDANCE OF FISH POPULATIONS IN NATURAL LENTIC WATERS WITH ADDITIONAL SAMPLING CONSIDERATIONS

Abstract

Environmental DNA (eDNA) sampling has proven to be a valuable tool for detecting species in aquatic ecosystems. Within this rapidly evolving field, a promising application is the ability to obtain quantitative estimates of relative species abundance based on eDNA concentration rather than traditionally labor-intensive methods. We investigated the relationship between eDNA concentration and arctic char (Salvelinus alpinus) abundance in five well-studied natural lakes, and additionally, we examined the effects of different temporal (e.g., season) and spatial (e.g., depth) scales on eDNA concentration. Concentrations of eDNA were linearly correlated with char population estimates ($R_{adj}^2 = 0.78$) and exponentially correlated with char densities ($R_{adj}^2 = 0.96$ by area; 0.82 by volume). Across lakes, eDNA concentrations were greater and more homogeneous in the water column during mixis; however, when stratified, eDNA concentrations were greater in the hypolimnion. Overall, our findings demonstrate that eDNA techniques can produce effective estimates of relative fish abundance in natural lakes. These findings can guide future studies to improve and expand eDNA methods while informing research and management using rapid and minimally invasive sampling.

---

5 This chapter is coauthored by Stephen L. Klobucar, Torrey Rodgers, and Phaedra Budy
6 © 2017. The full text of this article is published in the Canadian Journal of Fisheries and Aquatic Sciences 74(12): 2030 – 2034. It is available online at https://doi.org/10.1139/cjfas-2017-0114.
Introduction

Reliable estimates of fish abundance are necessary for making effective conservation and management decisions (Dudgeon et al. 2005). However, obtaining these estimates can be expensive and time consuming, and often requires multiple sampling events (Jerde et al. 2011). Until recently, describing fish populations, even at the presence/absence level, required invasive methods (e.g., gill nets, electrofishing), and these methods can be ineffective or harmful for certain habitats or species, and overall costly and laborious (McDonald 2004). Environmental DNA (eDNA) is increasingly being used as a tool to detect fishes in a more efficient, non-invasive manner (Barnes and Turner 2016; Wilcox et al. 2016). In aquatic systems, organisms release DNA into the environment via life processes (e.g., feces, skin cells, carcasses), and molecular techniques can detect this genetic material from water samples (Ficetola et al. 2008).

Methodologies of eDNA sampling are rapidly evolving and improving (e.g., Furlan et al. 2015), especially with regard to species detection. A next logical step towards advancing eDNA techniques would be to achieve estimates of fish abundance and biomass. Accordingly, there is growing evidence that relates eDNA concentration (e.g., qPCR copies·L$^{-1}$) to fish abundance and/or biomass in laboratory settings (e.g., Klymus et al. 2015) and lotic systems (e.g., Baldigo et al. 2017). However, there is little known about the effectiveness of this application in natural lentic waters. In lakes, the distribution and concentration of eDNA likely varies as a function of processes that affect DNA directly (e.g., degradation due to temperature, light, pH; Strickler et al. 2015) or indirectly via lake physical characteristics that can alter species distribution (e.g., temperature; Takahara et al. 2012) or biological activity that can affect eDNA production (e.g., spawning; Barnes and Turner 2016). Further, with these considerations, location (e.g., spatially, depth of sample) and timing (e.g., season) of sampling is an important consideration in lakes, especially if an estimate of fish abundance is the goal. Here we monopolize on long-term study lakes to verify the application of eDNA to quantify relative abundance of arctic char (*Salvelinus*...
alpinus) in lakes of northern Alaska and examine differences in detection probability and eDNA copy number concentration (hereafter, eDNA concentration) across sites, depth, and season.

Methods

In July and September 2016, we collected depth-specific water samples at spatially-explicit sites in each of five lakes (Lakes E5, Fog1, Fog2, Fog3, and Fog5) near Toolik Field Station, North Slope, Alaska (Table 5.1). Additional study site information can be found in Budy and Luecke (2014; see also http://arc-lter.ecosystems.mbl.edu/). All lakes except Lake Fog5 (only two sites) were divided into quadrants and sites were approximately even-spaced with one site in each quadrant. Each site included a shallow (1.0 m) and deep (approximately 2.0 – 3.0 m from lake bottom) sampling depth (see Table 5.1). During July, the lakes were thermally stratified, while in September, the lakes were isothermal. These lakes are part of the Arctic Long-Term Ecological Research site (http://arc-lter.ecosystems.mbl.edu/), and their fish communities are known to contain only arctic char and slimy sculpin (Cottus cognatus). We have conducted extensive fish sampling via traditional methods (i.e., gill nets, hook-and-line) to quantify population abundance in each lake using mark-recapture techniques (e.g., Budy and Luecke 2014; Table 5.2). For lakes where the times series was >5 years (Lakes E5, Fog1, Fog2), we estimated abundance using a Huggins closed-capture model in Program MARK (White and Burnham 1999). For Lakes Fog3 and Fog5, with shorter mark recapture time series, we used a modified Schnabel estimate (Krebs 1999). Overall, arctic char abundance is relatively low across all lakes, but follows a natural gradient from relatively low to relatively high density. For example, our abundance and density by volume estimates span greater than an order of magnitude (see Table 5.2), such that these lakes provide an excellent template to investigate relationships of eDNA concentration and fish abundance.
To sample for eDNA, at each site, we filtered lake water through vinyl tubing lowered to shallow and deep depths using an in-line peristaltic pump (GeoTech Environmental Equipment, Inc: GeoPump). We used 25 mm nylon net filters with 10 µm pore size, housed in a sterile luer-lock filter holder, and filtered a measured amount of lake water (usually 5L). We used the specific amount of water filtered for each sample to correct for eDNA concentration (e.g., copies·L⁻¹). We also carried 1L of distilled water into the field, and filtered this sample using a clean collection hose to serve as a collection negative control. Between lakes, all equipment was sterilized using 10% bleach solution. Prior to attaching filter holders, we flushed the hoses with lake water to remove bleach residue, and also flushed hoses before starting a new site within the same lake. After filtering, we placed intact filter holders, double-bagged, on ice in a dark container until storage at -80 °C at the field station. We shipped frozen samples overnight from the field station to the Molecular Ecology Lab at Utah State University for DNA extraction and qPCR analyses.

eDNA was extracted using a Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, California). Filters were incubated in 360 µL buffer ATL and 40 µL proteinase K for one hour at 56 °C, with vortexing every 15 minutes. Then, 300 µL buffer AT was added, followed by 300 µL 99% ethanol. Extractions then proceeded following the manufacturers recommendations, with a final elution volume of 100 µL. Each round of extractions included a blank negative control that was later run in qPCR to test for contamination.

Quantitative PCR (qPCR) reactions for arctic char eDNA detection and quantification were carried out using species specific primers and Taqman® Minor Groove Binding probe targeting 145 bp of the mitochondrial gene cytochrome b as described in Rodgers et al. (2017). All samples were initially run in triplicate. For a subset of samples that did not show amplification in the first 3 qPCR replicates (n=10), an additional 3 replicates were run. qPCR reactions were run on an Applied Biosystems QuantStudio three thermocycler (Foster City, California). Each reaction included 7.5 µL Taqman® Environmental Master Mix (Thermo-Fisher; Waltham, MA), 100nM of forward primer, 600nM of reverse primer, 250nM of Taqman® MGB
probe, and 4 μL of template DNA in a total reaction volume of 15 μL. Additionally, each reaction included a VIC labeled Taqman® exogenous internal positive control (Life Technologies, Grand Island, NY) to monitor for PCR inhibition. Samples that showed signs of inhibition (6 samples all from July) were treated with Gene Releaser (Bioventures inc., Murfreesboro, TN) to remove inhibitors, and re-run. qPCR thermal cycling conditions were 10 minutes at 95 °C, followed by 45 cycles of 15 seconds at 95 °C and 1 minute at 60 °C. All qPCR runs included a minimum of 3 no-template negative controls to test for contamination.

For quantification, each qPCR run included a 5-step, 5-fold standard curve run in triplicate. This standard curve was constructed from a serial dilution of a MiniGene plasmid ordered from Integrated DNA Technologies (Coralville, Iowa, USA) containing the target sequence. The plasmid was suspended in 100 µL of IDTE (10 mM Tris, 0.1 mM EDTA) buffer, linearized by digestion with the enzyme PvuI, and then purified with a PureLink PCR Micro Kit (Thermo-Fisher; Waltham, MA) following manufacturer protocol. The product was then quantified and diluted to create reactions of 10, 50, 250, 1250, and 6250 copies for the standard curve. Resulting copy number quantities for each qPCR reaction were converted to eDNA copies per liter of lake water for further analyses, taking into account the number of qPCR replicates that amplified for each sample. Briefly, this conversion was accomplished by multiplying the number of DNA copies per qPCR reaction by the proportion of the total extraction volume run in each qPCR reaction, divided by the water filtration volume. As arctic char are known to occupy all study lakes, we calculated detection probability as the percentage of samples that detected char DNA for each sampling event and depth. Across lakes, we used paired Student’s t-tests to compare eDNA concentration between seasons and depths and we used linear regression models (fit through the origin) to test for a relationship between eDNA concentration and fish abundance. We assessed model fit by evaluating residual-expected value plots and log-transformed eDNA concentrations when necessary to improve fit and appropriately describe the observed relationship (e.g., density by area, density by volume). Due to a relatively low sample size, we
compared relationships using adjusted $R^2$. We used R statistical package (version 3.3.2; R Development Core Team, 2016) for all analyses.

**Results**

We collected a total of 38 eDNA samples across all lakes in both July (stratified) and September (mixis). Across all samples, mean eDNA concentrations (copies·L$^{-1} \pm 2$se) were greater in September than in July (78.26 ± 69.71 vs. 9.38 ± 7.87; $t = 1.96$, df = 37, $p = 0.05$; Figure 5.1), and while variable, eDNA concentrations were generally greater in the deep samples relative to the shallow samples in July (15.77 ± 14.94 vs. 3.00 ± 3.63; $t = 1.66$, df = 18, $p = 0.11$) but not September (84.05 ± 101.84 vs. 72.46 ± 97.95; $t = 0.17$, df = 18, $p = 0.87$).

When pooled across all sites, depths, and lakes, eDNA concentrations were highly correlated with fish abundance (total individuals; $R_{adj}^2 = 0.78$, $F(1,4) = 18.40$, $p = 0.01$) as well as density by area (fish·ha$^{-1}$; $R_{adj}^2 = 0.96$, $F(1,4) = 118.1$, $p < 0.001$) and density by volume (fish·m$^{-3}$) ($R_{adj}^2 = 0.82$, $F(1,4) = 23.17$, $p < 0.01$; Figure 5.2, Table 5.3). Lake-specific eDNA concentrations (copies·L$^{-1} \pm 2$se) were variable across sites and depths (73.25 ± 114.89, 22.93 ± 27.02, 3.70 ± 4.98, 97.17 ± 120.69, 14.80 ± 14.12 for Lakes E5, Fog1, Fog2, Fog3, and Fog5, respectively). Detection probabilities between depths varied between sampling periods with the greatest detection probability in the deeper depths during July, when the lakes were stratified (63.2% deep samples vs. 21.1% shallow samples). In September, there was little difference in detection probabilities between the shallow and deep samples (57.9% deep samples vs. 52.6% shallow samples).

**Discussion**

Our results add to the limited body of knowledge for quantification of fish abundance in natural lentic systems using eDNA. To our knowledge, no other study has related eDNA concentration to lake-wide population estimates of fish abundance under natural conditions,
though others have come to similar conclusions for other metrics of abundance and biomass (e.g., catch-per-unit-effort; Lacoursiere-Roussel et al. 2016). Our study lakes were ideal for addressing this as they are relatively small and closed to emigration and immigration of fishes, with simple and well-known fish communities (e.g., only two species). On the North Slope, Alaska, similar lakes are extremely abundant and can comprise up to 48% of the landscape’s surface. While obtaining lake-specific population estimates for each lake would be logistically challenging and time consuming, we provide a first attempt towards assessing relative abundance of fishes in lesser studied lakes using this study as a baseline. Future work should address the spatial extent of relationships between eDNA concentration and fish abundance across a broader landscape (e.g., multiple watersheds) where environmental variability could be greater.

In our study, natural fish abundance is relatively low, and thus, mean eDNA concentrations and detection probabilities were unsurprisingly also relatively low. Ensuring sufficient detection across sites, depths, and lakes with known fish community species diversity and abundance can require significant sample volumes. Further, reducing the number of false-negatives would likely result in a dramatic reduction in variability across sites, depths, and lakes. However, in our study, during only one sampling period, at one lake, did we fail to detect arctic char (Lake Fog2 in July). Fish density in Lake Fog2 is five-fold lower than the next lowest (Lake Fog5) across our study lakes (~21 fish·ha⁻¹ vs. ~104 fish·ha⁻¹). To achieve near 100% detection probabilities, the minimum volume of water for a single sample using our sampling method would be 25 - 30 L for Lake E5, Fog1, Fog3, and Fog5, while Lake Fog2 would require greater than 40 L (based on the total volume of false negatives from a given lake). Other studies have used much smaller sample volumes to achieve reasonable detection probabilities, but fish abundance in those studies was also much greater (e.g., Baldigo et al. 2017). Further, filter type and pore size can affect eDNA capture, which could potentially decrease the total volume required in our study lakes (Barnes and Turner 2016). To build upon our work here, future studies that aim to estimate fish abundance from eDNA concentration should further consider necessary
sample size (e.g., spatially, volume filtered per sample) and equipment to best achieve these relationships and reduce overall variability, especially in larger lentic systems or with greater fish densities when concentration-abundance relationships may not be exponential.

Various factors could influence differences of eDNA concentration and detection probability between sampling periods and sampling depths in this study. Across high latitudes regions, including the North Slope, Alaska, summer 2016 was the warmest on recent record, such that epilimnetic temperatures during July (18 – 20 °C) were greater than the thermal optimum for arctic char (15.2 °C; Lyytikäinen et al. 1997). In contrast, in average years, epilimnetic temperatures rarely exceed this optimal temperature (Luecke et al. 2014). Water temperatures in July 2016 likely limited thermal habitat for arctic char above the thermocline, such that much of their time was spent in deeper water. Thus, it is reasonable to expect concentrations of eDNA to be higher in samples from deeper depths. With epilimnetic water temperatures in July 2016 approaching 20 °C (Table 5.1), degradation of DNA due to direct and indirect temperature effects (e.g., microbial metabolism) could also limit the total amount of genetic material in epilimnetic waters. Additionally, in lentic systems, settling of genetic material can result in eDNA concentration in deeper the water column (e.g., Turner et al. 2015). Others have observed increased rates of DNA degradation at similar temperatures (Stickler et al. 2015). Degradation due to UV-B exposure could further decrease July epilimnetic eDNA concentrations relative to: 1) July hypolimnetic concentrations (e.g., less photoexposure); and, 2) September epilimnetic eDNA concentrations (e.g., shorter day length). In Arctic regions during July, there are 24-hrs of daylight, while average day length during our September sampling period was approximately 14 hrs. In September, the entire water column was recently mixed and cooler overall (isothermal) which: 1) allows char to move more freely throughout the lake; 2) decreases the rate of degradation of genetic material; and, 3) could re-suspend eDNA that was concentrated in deeper depths throughout the summer period. While we cannot parse these effects in our current study, the increased and more homogeneous eDNA concentrations we observed during September are
likely interactions of physical and biological factors. Overall, when considering physical (e.g., stratification) and biological (e.g., species’ temperature preference) factors, we demonstrate that autumn is better than summer to sample these type of oligotrophic, monomictic lakes for fish eDNA.

Overall, for eDNA studies, there is limited information in regard to sampling depth for natural, true lentic waters. We demonstrated that thermal stratification can affect eDNA concentrations between stratified and isothermal periods (e.g., higher eDNA concentrations in deep samples during the summer). For species detection, many ‘early’ eDNA studies used surface samples (e.g., Jerde et al. 2011 in lotic systems), while others have sampled during isothermal periods to decrease heterogeneity across depths, but without comparison to a stratified period (Lacoursiere-Roussel et al. 2016). Eichmiller et al. (2014) found no difference between surface and subsurface samples in Lake Staring, Minnesota, but sub-surface sampling depths were less than 1 m deeper than the surface. In contrast to our findings, in a controlled lentic setting, African jewelfish were more readily detected from surface water samples than samples taken from the bottom, even though these fishes were located most often near the bottom (Moyer et al. 2014). However, these controlled systems were much smaller, shallower, and warmer, with greater fish densities than the natural Arctic lakes in our study. In deep, natural lakes, especially those that thermally stratify, understanding seasonal depth-specific concentrations is important for future studies and effective sampling design. Nonetheless, due to the remote location of these lakes, a rapid, non-invasive method of assessing relative abundance will allow us to address pressing ecological questions (e.g., lake connectivity) and be important for helping to guide subsistence fishing, as well as larger-scale monitoring of population persistence, especially in a changing climate.
References


Table 5.1. Summary of five northern Alaska study lakes and conditions during each eDNA sampling period in 2016.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Surface area (ha)</th>
<th>Maximum depth (m)</th>
<th>Mean depth (m)</th>
<th>No. of sampling sites</th>
<th>Depth of deep sample (m)</th>
<th>July shallow water temp (°C)</th>
<th>July deep water temp (°C)</th>
<th>Sept. shallow water temp (°C)</th>
<th>Sept. deep water temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E5</td>
<td>68.642</td>
<td>-149.458</td>
<td>10.9</td>
<td>12.9</td>
<td>6.3</td>
<td>4.0</td>
<td>8.0</td>
<td>14.7</td>
<td>6.0</td>
<td>5.6</td>
<td>5.6</td>
</tr>
<tr>
<td>Fog1</td>
<td>68.684</td>
<td>-149.082</td>
<td>3.5</td>
<td>19.7</td>
<td>8.4</td>
<td>4.0</td>
<td>10.0</td>
<td>19.0</td>
<td>4.8</td>
<td>7.4</td>
<td>7.3</td>
</tr>
<tr>
<td>Fog2</td>
<td>68.679</td>
<td>-149.091</td>
<td>5.9</td>
<td>19.8</td>
<td>7.8</td>
<td>4.0</td>
<td>10.0</td>
<td>18.1</td>
<td>5.2</td>
<td>7.4</td>
<td>7.3</td>
</tr>
<tr>
<td>Fog3</td>
<td>68.673</td>
<td>-149.088</td>
<td>3.9</td>
<td>21.0</td>
<td>7.9</td>
<td>4.0</td>
<td>10.0</td>
<td>18.8</td>
<td>4.8</td>
<td>6.7</td>
<td>6.6</td>
</tr>
<tr>
<td>Fog5</td>
<td>68.678</td>
<td>-149.065</td>
<td>0.7</td>
<td>9.9</td>
<td>3.5</td>
<td>2.0</td>
<td>6.0 or 7.0</td>
<td>14.4</td>
<td>5.5</td>
<td>5.7</td>
<td>5.7</td>
</tr>
</tbody>
</table>
Table 5.2. Summary of abundance (number of fish), density by area (fish·ha⁻¹), and density by volume (10⁻³; fish·m⁻³) estimates for arctic char (*Salvelinus alpinus*) populations in five study lakes in northern Alaska. Values in parentheses represent lower and upper 95% confidence intervals for each estimate.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Abundance</th>
<th>Density by area</th>
<th>Density by volume</th>
<th>Time series start</th>
<th>Abundance estimate method</th>
</tr>
</thead>
<tbody>
<tr>
<td>E5</td>
<td>1207 (987 - 1476)</td>
<td>111 (91 - 136)</td>
<td>1.7 (1.4 - 2.1)</td>
<td>1999</td>
<td>Huggins</td>
</tr>
<tr>
<td>Fog1</td>
<td>448 (290 - 693)</td>
<td>127 (82 - 197)</td>
<td>1.6 (1.0 - 2.4)</td>
<td>2011</td>
<td>Huggins</td>
</tr>
<tr>
<td>Fog2</td>
<td>163 (105 - 288)</td>
<td>29 (19 - 51)</td>
<td>0.3 (0.2 - 0.7)</td>
<td>2007</td>
<td>Huggins</td>
</tr>
<tr>
<td>Fog3</td>
<td>666 (477 - 1073)</td>
<td>171 (123 - 276)</td>
<td>2.2 (1.6 - 3.5)</td>
<td>2013</td>
<td>Schnabel</td>
</tr>
<tr>
<td>Fog5</td>
<td>75 (55 - 119)</td>
<td>104 (76 - 164)</td>
<td>3.0 (2.2 - 4.7)</td>
<td>2014</td>
<td>Schnabel</td>
</tr>
</tbody>
</table>
Table 5.3. Summary statistics of linear models fit to predict eDNA concentration from known metrics of relative fish abundance across five lakes in northern Alaska. Bold text signifies the significant relationship that are presented in Figure 2.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Response</th>
<th>$\beta$</th>
<th>$SE$</th>
<th>$p$</th>
<th>$R_{adj}^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish abundance</td>
<td>eDNA copies</td>
<td>0.077</td>
<td>0.018</td>
<td>0.01</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>log(eDNA copies)</td>
<td>0.005</td>
<td>0.001</td>
<td>0.01</td>
<td>0.77</td>
</tr>
<tr>
<td>Fish density (by area)</td>
<td>eDNA copies</td>
<td>0.422</td>
<td>0.106</td>
<td>0.02</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>log(eDNA copies)</td>
<td>0.028</td>
<td>0.003</td>
<td>&lt;0.001</td>
<td>0.96</td>
</tr>
<tr>
<td>Fish density (by volume)</td>
<td>eDNA copies</td>
<td>21967</td>
<td>9108</td>
<td>0.07</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>log(eDNA copies)</td>
<td>1611</td>
<td>335</td>
<td>&lt;0.01</td>
<td>0.82</td>
</tr>
</tbody>
</table>
Figure 5.1. Mean eDNA copies (L$^{-1}$) for all lakes pooled (E5, Fog1, Fog2, Fog3, Fog5) by season (white = July 2016, gray = September 2016) for shallow and deep samples. Combined represents the overall mean between shallow and deep depths. Error bars represent standard error.
Figure 5.2. Relationships between A) fish abundance (top), B) density by area (bottom left), and C) density by volume (bottom right) and mean eDNA concentration (copies·L$^{-1}$) across five study lakes in northern Alaska sampled in 2016. Note: density by area and density by volume relationships are back-transformed from log(eDNA concentration).
CHAPTER 6
SUMMARY AND CONCLUSION

In lakes, food web stability can vary based on community composition and productivity (e.g., Cole et al. 2006), and as these factors shift, changes trophic structure and function is likely to follow (Carpenter and Kitchell 1996). In the Arctic, where productivity and species diversity is low, and direct anthropogenic influences are minimal, understanding the abiotic and biotic factors that contribute to lake trophic structure and function is important for conservation and management. My dissertation research objectives were to: 1) quantify invertebrate prey biomass and availability in response to warming in arctic lakes (Chapter 2); 2) measure morphometric and genetic characteristics across populations of arctic char (Chapter 3); 3) evaluate fish community and trophic dynamics, specifically for arctic char, in contrasting abiotic and biotic environments (Chapter 4); and, 4) assess the applicability of a novel survey method to detect changes in lake fish abundance across spatial and temporal scales (Chapter 5).

In Chapter 2, I used laboratory mesocosm experiments to quantify abundance and biomass of zooplankton and snails, and long-term observations to predict zooplankton biomass across a range of temperatures. For my experiments, I hypothesized: 1) invertebrate prey abundance would increase across the growing season with warmer temperatures; and, 2) invertebrate prey would continue increased production in the late season when subjected to increased, yet unnatural, photoperiod. For my long-term modeling, I expected increased zooplankton abundance in warm years relative to cold or average years. Overall, I show that abundance biomass of arctic lake invertebrates will likely respond positively to warmer temperatures, depending on the season. I found increased temperatures resulted in more rapid snail development in the early season. As such, earlier ice-off of arctic lakes would likely result in an increase in the snail populations earlier in the growing season. In mid-season, I observed significantly increased Daphnia abundances, while in the late season, Daphnia appeared to be
limited by photoperiod. Accordingly, my model predictions suggest overall zooplankton biomass increases nearly 20% in warmer years. These estimates could be conservative due to increased consumptive demand by fishes, but nonetheless, fall within our expectations of increased fish metabolism (Budy and Luecke 2014).

In Chapter 3, I investigated the morphological and genetic diversity of arctic char populations across two distinct series of lakes. While these lake groups are located in close proximity (~5 km), they are situated on different glacial landscapes and exhibit very different char size structures. Using model-based clustering of morphometric traits corrected for allometry, I did not detect morphological differences within and across char populations. Accordingly, while genetically distinct between lake groups, there were no genetic differences based on size within individual lakes. Thus, I used PERMANOVA analyses to identify factors that determine observed arctic char size structures, and significant predictors included Secchi depth, arctic char density, and lake group. These findings provide some of the first descriptions of evolutionary characteristics of char population in arctic Alaska.

In Chapter 4, I further investigated arctic lake food web structure across these series of lakes. I used field observations of predator diet and trophic position to better understand the role abiotic and biotic factors contribute to structuring lake food webs is important for management and conservation of native fishes. I hypothesized 1) arctic char are more densely populated in the absence of other apex predators (closed lakes), but across lakes, regardless of fish density, arctic char trophic position increases with fish size; 2) to coexist with lake trout, arctic char in open lakes feed at higher trophic positions (e.g., more piscivorous) and achieve greater maximum sizes relative to closed lake systems; 3) due to increased interspecific competition, arctic char trophic niche space is narrower in open lakes relative to closed lakes; but, 4) resource polymorphism is more prominent in closed lakes as a result of increased intraspecific competition and the absence of other apex predators. Generally, I confirmed hypotheses #1 and #2—arctic char populations are more densely populated in the absence of other apex predators, but when occurring with other
predators (e.g., lake trout) char will be larger and feed at higher trophic positions. However, in opposition to hypothesis #3 and #4, I found that arctic char niche spaces were larger and overlapped less in open lakes relative to closed lakes, which could be driven by abiotic (e.g., ecosystem size) and biotic factors (prey availability and selection). These results provide some of the first descriptions of arctic char trophic dynamics in northern Alaska and increase our understanding of arctic lake trophic structure. In combination with Chapter 3, these results provide critical information for subsistence management and species conservation, especially in a changing climate.

In Chapter 5, I used environmental DNA (eDNA) to test this method to obtain quantitative estimates of relative species abundance based on eDNA concentration. I investigated the relationship between eDNA concentration and arctic char abundance in five well-studied lakes, and additionally, examined the effects of depth and season on eDNA concentrations. I found concentrations of eDNA are linearly correlated with char population estimates ($R_{adj}^2 = 0.78$) and exponentially correlated with char densities ($R_{adj}^2 = 0.96$ by area; 0.82 by volume). Across lakes, I found that eDNA concentrations were greater and more homogeneous in the water column during mixis; however, when stratified, eDNA concentrations were greater in the hypolimnion. To date, no other study has related eDNA concentration to lake-wide population estimates of fish abundance under natural conditions. As such, this study provides a baseline for wider population surveying and monitoring, which is especially beneficial in remote northern Alaska.

In sum, my dissertation research improves our understanding of the abiotic and biotic controls of arctic lake food webs, and fills a knowledge gap toward the relative influence of these controls on the trophic structure and function in arctic lakes. Moreover, by using a multifaceted approach of modeling, observation, and experimentation across trophic levels and lake groups, I was able to draw conclusions about the potential adaptability and stability of lake food webs in a
warming climate. Previous work estimated the increases of fish consumption associated with warming temperatures (Budy and Luecke 2014); however, the response of fish food and the ability for fish to meet these consumptive demands were unknown—Chapter 2 of my dissertation addressed this knowledge gap. While fish are important subsistence resources in arctic Alaska, compared to other regions of the world, relatively little was known about adaptive capacity of arctic char in this region—Chapters 3 and 4 of my dissertation addressed this knowledge gap. Accordingly, as populations of arctic fishes are likely to shift (e.g., distribution, abundance) in a changing climate, we lacked the ability to rapidly and effectively measure relative abundances of fish communities across a landscape of thousands of lakes—Chapter 5 of my dissertation addressed this and provides some of the first known proof of concept for this valuable and evolving tool.

References


5 December 2017

Stephen Klobucar
5210 Old Main Hill, Utah State University
Logan, UT 84322-5210
(608)-289-5687, stephen.klobucar@gmail.com

Jessica Rick
Department of Botany, University of Wyoming

Dear Jessica Rick,
I am in the process of preparing my dissertation in the Department of Watershed Sciences at Utah State University.
I am requesting your permission to include our coauthored paper, Investigating morphological and genetic diversity of arctic char (*Salvelinus alpinus*) populations in distinct groups of foothill lakes in arctic Alaska, in my Ph.D. dissertation, as per graduate school requirements. As discussed, I will also submit our manuscript to Ecology and Evolution or a similar journal upon completing my degree. You will be cited as a co-author on the title page, and this will be reflected in my dissertation as well. Please advise me of any changes you require.
Please indicate your approval of this request by signing in the space provided. By signing this letter, you acknowledge that the work leading to the completed paper was primarily my own.
Please contact me with any questions.
Thank you in advance,
Stephen Klobucar

---

I hereby give permission to Stephen L. Klobucar to reprint the following material in his Ph.D. dissertation.

Signature:  
Date: 8/8/18

5 December 2017

Stephen Klobucar
5210 Old Main Hill, Utah State University
Logan, UT 84322-5210
(608)-289-5687, stephen.klobucar@gmail.com

Elizabeth Mandeville
Department of Botany, University of Wyoming
Dear Elizabeth Mandeville,
I am in the process of preparing my dissertation in the Department of Watershed Sciences at Utah State University.
I am requesting your permission to include our coauthored paper, Investigating morphological and genetic diversity of arctic char (Salvelinus alpinus) populations in distinct groups of foothill lakes in arctic Alaska, in my Ph.D. dissertation, as per graduate school requirements. As discussed, I will also submit our manuscript to Ecology and Evolution or a similar journal upon completing my degree. You will be cited as a co-author on the title page, and this will be reflected in my dissertation as well. Please advise me of any changes you require.
Please indicate your approval of this request by signing in the space provided. By signing this letter, you acknowledge that the work leading to the completed paper was primarily my own. Please contact me with any questions.
Thank you in advance,
Stephen Klobucar

I hereby give permission to Stephen L. Klobucar to reprint the following material in his Ph.D. dissertation.
Chapter to be published as: Klobucar, S.L., J.A. Rick, E.G. Mandeville, C.E. Wagner, and P. Budy. Investigating morphological and genetic diversity of arctic char (Salvelinus alpinus) populations in distinct groups of foothill lakes in arctic Alaska, to be submitted to Ecology and Evolution.

Signature: Date: 8/8/18

5 December 2017
Stephen Klobucar
5210 Old Main Hill, Utah State University
Logan, UT 84322-5210
(608)-289-5687, stephen.klobucar@gmail.com

Catherine Wagner
Department of Botany, University of Wyoming

Dear Catherine Wagner,
I am in the process of preparing my dissertation in the Department of Watershed Sciences at Utah State University.
I am requesting your permission to include our coauthored paper, Investigating morphological and genetic diversity of arctic char (Salvelinus alpinus) populations in distinct groups of foothill lakes in arctic Alaska, in my Ph.D. dissertation, as per graduate school requirements. As discussed, I will also submit our manuscript to Ecology and Evolution or a similar journal upon completing my degree. You will be cited as a co-author on the title page, and this will be reflected in my dissertation as well. Please advise me of any changes you require.
Please indicate your approval of this request by signing in the space provided. By signing this letter, you acknowledge that the work leading to the completed paper was primarily my own. Please contact me with any questions.
Thank you in advance,
Stephen Klobucar

I hereby give permission to Stephen L. Klobucar to reprint the following material in his Ph.D. dissertation.
Chapter to be published as: Klobucar, S.L., J.A. Rick, E.G. Mandeville, C.E. Wagner, and P. Budy. Investigating morphological and genetic diversity of arctic char (Salvelinus alpinus) populations in distinct groups of foothill lakes in arctic Alaska, to be submitted to Ecology and Evolution.

Signature: Date: 8/8/18

5 December 2017

Stephen Klobucar
5210 Old Main Hill, Utah State University
Logan, UT 84322-5210
(608)-289-5687, stephen.klobucar@gmail.com

Torrey Rodgers
Department of Wildland Resources, Utah State University

Dear Torrey Rodgers,
I am in the process of preparing my dissertation in the Department of Watershed Sciences at Utah State University.
I am requesting your permission to include our coauthored paper, At the forefront: evidence of the applicability of using environmental DNA to quantify the abundance of fish populations in natural lentic waters with additional sampling considerations, in my Ph.D. dissertation, as per graduate school requirements. Our paper has already been published in the Canadian Journal of Fisheries and Aquatic Sciences, you are a co-author, and this will be reflected in my dissertation as well.
Please indicate your approval of this request by signing in the space provided. By signing this letter, you acknowledge that the work leading to the completed paper was primarily my own. Please contact me with any questions.
Thank you in advance,
Stephen Klobucar

I hereby give permission to Stephen L. Klobucar to reprint the following material in his Ph.D. dissertation.

CURRICULUM VITAE

Stephen L. Klobucar
Department of Watershed Sciences and The Ecology Center
Utah State University, 5210 Old Main Hill, Logan, Utah 84322-5210, USA
Phone: (608) 289-5687; FAX: (435) 797-4025
Email: stephen.klobucar@gmail.com; Website: stephenklobucar.weebly.com

EDUCATION

Ph.D. Ecology. Utah State University, Department of Watershed Sciences and The Ecology Center, Logan, UT. (successful defense December 2017)


PROFESSIONAL APPOINTMENTS

Utah State University, Department of Watershed Sciences
Postdoctoral Research Associate. Logan, UT (Supervisor: Dr. Jereme Gaeta; January 2018 – present)
Focus: Utah Lake ecosystem modeling

RESEARCH and FIELD EXPERIENCE

Utah State University, Department of Watershed Sciences
Ph.D. Graduate Research Assistant. Logan, UT (Advisor: Dr. Phaedra Budy; September 2013 – December 2017)
Dissertation topic: Quantifying the abiotic and biotic controls of arctic lake food webs

University of Canterbury, Department of Biological Sciences
Visiting Ph.D. Student. Christchurch, New Zealand (Host: Dr. Angus McIntosh; January 2017 – April 2017)
Research topic: Assessing regional metrics of stream fish condition with special consideration of climate change

Utah State University, Department of Watershed Sciences
Post-graduate Researcher. Logan, UT (July 2012-August 2013)
Crew & co-crew lead on interagency projects including: native fish assessment and conservation with respect to seasonal movement and habitat usage in large rivers and tributaries (Green, Price, and White Rivers, UT); population assessment of a large reservoir fish community via hydroacoustic and mid-water trawling surveys (Strawberry Reservoir, UT); and, investigating movement, early life history, and food web impacts of invasive burbot (Lota lota) in Flaming Gorge Reservoir, WY/UT.

**Marine Biological Laboratory, Arctic Long Term Ecological Research Network**

Arctic LTER Lakes Research Assistant (interim volunteer). Toolik Field Station, AK (*June – July 2012*)

Assisted with field collection and laboratory processing of limnological and stable isotope samples on remote, long-term monitoring lakes on the North Slope, AK.

**Utah State University, Department of Watershed Sciences**

M.S. Graduate Research Assistant. Logan, UT (*Advisor: Dr. Phaedra Budy; June 2010 – May 2012, converted to PhD*)


**University of Wisconsin, Center for Limnology**


Assisted with a NSF RAPID grant project examining food web interactions following dam and reservoir removal in Big Spring Creek near Briggsville, WI.

**Chase Noland Scholarship in Limnology**

University of Wisconsin, Center for Limnology- University of Notre Dame Environmental Research Center, Land O’ Lakes, WI (*Advisors: Drs. Jim Kitchell & Brian Weidel; May 2009 – August 2009*)

Designed and conducted an independent research project in conjunction with the Trophic Cascade Project.

**Trophic Cascade Project**


Assisted with a whole-lake manipulation investigating increased variance, red-shift of variance, and critical slowing of recovery rate across components of a food web as leading indicators of a regime shift caused by changes in fish community structure.

**PUBLICATIONS**

Yu mentored undergraduate


Klobucar, S.L., T. Rodgers, and P. Budy. 2017. At the forefront: evidence of the applicability of using environmental DNA to quantify the abundance of fish populations in natural lentic waters


IN PRESS


IN PREP

Klobucar, S.L., Y.J. Goethlich, and P. Budy. Does predator density mediate the diet of slimy sculpin (<i>Cottus cognatus</i>) in arctic lakes?


Zarnetske, P.L., M.C. Urban, D.K. Skelly, P. Budy, and S.L. Klobucar. Do climatic changes or biotic interactions explain the condition of Arctic freshwater fishes over 30 years?

TECHNICAL REPORTS


TEACHING EXPERIENCE

**Workshop Presenter**, Western Division of the American Fisheries Society student colloquium (Spring 2015)

Topic: Effectively using conceptual diagrams to share your research

**Teaching Assistant**, Department of Watershed Sciences, Utah State University (Fall 2014)

Course: WATS 6260, WATS Graduate Induction Course

Duties: fish ecology and limnology lectures, course logistics, promote department comradery

**Guest Lecturer**, Department of Watershed Sciences, Utah State University (Spring 2014)

Course: WATS 4500/6500, Limnology: Ecology of Inland Waters, “Introduction to Food Webs”

**Course Grader**, Department of Watershed Sciences, Utah State University (Spring 2014)

Course: WATS 4500/6500, Limnology: Ecology of Inland Waters

**Course Assistant**, Quinney College of Natural Resources, Utah State University (May 2013)

Short Course: Design and Analysis of MARK Re-sight Studies

**Course Assistant**, American Fisheries Society (May 2012)

Short Course: Planning and Executing Successful Rotenone and Antimycin Projects

**Teaching Assistant**, Department of Watershed Sciences, Utah State University (Fall 2011)

Course: WATS 3110, Fish Diversity and Conservation Laboratory

Duties: weekly lab instructor, administer tests and quizzes, field trip coordinator, course grader

**Guest Lecturer**, Department of Watershed Sciences, Utah State University (Fall 2011)

Course: WATS 3100, Fish Diversity and Conservation, “Feeding and Predation Ecology”

UNDERGRADUATE MENTORSHIP

Tyler Arnold, Utah State University, undergraduate research project (Fall 2016 – present)

Topic: Investigating morphometric differences across and among arctic char populations in lakes on the North Slope, Alaska
Levi Simmons, Utah State University, NSF Research Experience for Undergraduates (Summer 2015 – Spring 2016). Received competitive $2,000 USU Undergraduate Research and Creative Opportunity grant.

Topic: Trophic position and niche-space dynamics of apex predators in arctic lakes; currently: Seasonal biologist, Colorado Parks and Wildlife

Jamie Goethlich, Northland College, NSF Research Experience for Undergraduates (Summer 2014)
Topic: A cross-lake comparison of slimy sculpin (Cottus cognatus) diets in fertilized and unfertilized arctic lakes. (undergraduate thesis); currently: M.S. student, Auburn University

Benjamin Wegleitner, University of Wisconsin-Stevens Point, Summer Research Intern (Summer 2011)
Topic: Using multiple metrics to evaluate salmonid growth across an array of high-desert impoundments. (undergraduate thesis); currently: Outreach Assistant, Illinois/Indiana Sea Grant (M.S. from Central Michigan University)

**GRANTS, AWARDS, and HONORS**

**Eugene Maughan Ph.D. Scholarship,** Western Division, American Fisheries Society (2017)-- $2,500

**Graduate Research and Creative Opportunities Grant,** Utah State University (2017)-- $1,000

**Ecology Center International Travel Support,** Utah State University (2017)-- $960

**National Science Foundation,** Office of Polar Programs (2016-2021)-- $999,335 total; $331,737 to date
Collaborative Research: An exploration of the direct and indirect effects of climatic warming on arctic lake ecosystems
(Awarded to P. Budy in my last year of study; however, I played a key role in developing and writing the first two proposals: http://arcticlakewarming.weebly.com/)

**M.S. to Ph.D. Conversion Award,** Utah State University (2013)-- $25,000

**Utah Division of Wildlife Resources,** Sportfish Research (2012-2013)-- $118,017 total
Flaming Gorge Reservoir: Burbot Diet and Distribution-- $82,335
Flaming Gorge Reservoir: Burbot Early Life History [addendum]-- $35,682
(Awarded to P. Budy and post-doc W. Carl Saunders; I co-wrote the grants with WCS)

**John E. Skinner Memorial Scholarship,** American Fisheries Society (2012)-- $800

**Graduate Student Council Travel Award,** Utah State University (2012)-- $250

**RGS Graduate Student Travel Award,** Utah State University (2011, 2012, 2013, 2016, 2017)-- $200 - $300

**Student Travel Award,** Western Division, American Fisheries Society (2011, 2015, 2016, 2017)-- $300 - $630
Graduate Student Senate Research and Project Grant, Utah State University (2011)-- $1,000

Ecology Center Research Support Award, Utah State University (2011, 2016)-- $4,000

Desert Fishes Council Student Travel Award, (2010)-- $250

Chase Noland Scholarship in Limnology, University of Wisconsin-Center for Limnology (2009)-- $5,000

PRESENTATIONS
(First/presenting author and ¥ mentored undergraduates only)

Klobucar, S.L., T.W. Rodgers, and P. Budy. At the forefront: evidence of the applicability of using environmental DNA to quantify the abundance of fish populations in natural lentic waters with additional sampling considerations. Oral presentation. Annual Meeting, Western Division of the American Fisheries Society, May 2017, Missoula, MT.


Klobucar, S.L., and P. Budy. Consequences of seasonal variation in reservoir water level for predatory fishes: linking visual foraging and prey densities. Invited Oral Presentation. Annual Summer Meeting of the Association for the Sciences of Limnology and Oceanography, June 2016, Santa Fe, NM.


Klobucar, S.L., and P. Budy. Understanding how lake populations of arctic char are structured and function with special consideration of the potential effects of climate change. Oral Presentation. 145th Annual Meeting of the American Fisheries Society, August 2015, Portland, OR.


Klobucar, S.L. “A bad day of fishing beats a good day of work”…unless. Invited Oral Presentation. Toolik Field Station Talking Shop Seminar, July 2014, North Slope, AK.


Klobucar, S.L., W.C. Saunders, C. Luecke, and P. Budy. A Lota lota consumption: trophic effects and potential impacts of a novel and voracious predator in Flaming Gorge Reservoir, WY-


SERVICE, OUTREACH, and DEVELOPMENT

Journal Referee
Canadian Journal of Fisheries and Aquatic Sciences, Biological Conservation, Ecological Applications (with PB), North American Journal of Fisheries Management (with PB), PLoS ONE (with PB), Western North American Naturalist, Journal of Applied Ichthyology

USU Department of Watershed Sciences, Water Quality and Environmental Change, Faculty Search Committee
Graduate Student Representative

Graduate Training Series, School of Graduate Studies, Utah State University
Invited Speaker, “Common Grad School Mistakes and How to Avoid Them” (2017)

Trout Unlimited/Cache Anglers, Kid’s Fly Fishing Camp
Aquatic Macroinvertebrate Instructor and Coordinator (2017)

Western Division AFS Student Colloquium, Garden City, UT
Organizer and Host (2015)

USFWS Camp Goonzhii, Arctic Village, AK
K-12 Aquatic Ecology Instructor and Volunteer (2015)

Logan School District, Summer Science Class, Logan, UT

“Science Unwrapped”, Utah State University
Volunteer (2013, 2015)

Getting Started as a Successful Proposal Writer and Academician
Workshop Attendee (2012)

**Ecology Center Seminar Series Graduate Student Committee, Utah State University**
Member (2011-12, 2014-15)

**POPULAR MEDIA and COVERAGE**

*Utah State Magazine, “The Long Drive of Dreams”* (Winter 2016)
https://issuu.com/usuprm/docs/utah_state_magazine_winter_2016/30

*Utah State Magazine, Photograph featured in “Utah State University: Water Expertise at its Source”* (Summer 2015)
https://issuu.com/usuprm/docs/summer_2015_issuu/9


*KSL TV, “KSL Outdoors: 2013 Burbot Bash”* (11 February 2013)
https://www.ksl.com/?sid=24039473

*Salt Lake Tribune, “Burbot Bash: Hunt is on in Utah for predatory eel-like fish”* (4 February 2013)

**AFFILIATIONS**

*Ecological Society of America* (2015 – present)
*Association for the Sciences of Limnology and Oceanography* (2015 – present)
*Cache Anglers- Trout Unlimited* (2014 – present)
*American Fisheries Society* (2010 – present)
*USU Student Subunit President 2014 – 2015*