THE INFLUENCE OF MARINE-DERIVED NUTRIENT SUBSIDIES ON STREAMS AND LAKES AT IZEMBEK NATIONAL WILDLIFE REFUGE

A Thesis

Submitted to the Graduate School

of the University of Notre Dame

in Partial Fulfillment of the Requirements

for the Degree of

Master of Science

by

Sarah G. Winikoff

Jennifer L. Tank, Director

Graduate Program in Biological Sciences

Notre Dame, Indiana

August 2014

© Copyright by

Sarah G. Winikoff

2014

All rights reserved

THE INFLUENCE OF MARINE-DERIVED NUTRIENT SUBSIDIES ON STREAMS AND LAKES AT IZEMBEK NATIONAL WILDLIFE REFUGE

Abstract

By

Sarah G. Winikoff

Although the effects of Pacific salmon spawners have been well-studied in the Pacific Northwest, their influence remains relatively unexplored across portions of their range including the North Pacific Rim and along the Alaska Peninsula. In my first data chapter, I examined whether the influence of salmon on streams and lakes at Izembek National Wildlife Refuge (INWR) was similar to previously studied salmon-bearing ecosystems. At INWR, the impact of salmon was smaller than elsewhere, likely due to small run sizes and regional environmental characteristics including very low background molar nitrogen to phosphorous ratios. Nevertheless, I found that upstream and downstream reaches within a single watershed can respond differently to salmon migration, suggesting that salmon differentially influence freshwater ecosystems depending on landscape position. In my second data chapter, I examined whether marine-derived nutrient subsidies from salmon and waterbirds differ in their effects on lake ecosystems. In general, lakes receiving subsidies had higher nutrient concentrations than lakes with no significant subsidies, and water column chl-*a* was positively correlated to

nutrient availability. However, biofilm nutrient limitation differed between lakes receiving salmon versus waterbird subsidies. In summary, both source and environmental context strongly influences the effect of marine-derived nutrient subsidies in streams and lakes at INWR.

CONTENTS

Figures....iv

Acknowledgments....vii

Chapter 1: Introduction....1
1.1 Izembek National Wildlife Refuge....1
1.1.1 Location, Climate, and Geologic History....1
1.1.2 Becoming Izembek National Wildlife Refuge....3
1.2 Subarctic Maritime Tundra and Resource Subsidies in Lakes and Streams....7
1.3 Research Goals and Thesis Outline....10
1.4 Literature Cited....11

Chapter 2: Pacific Salmon (*Oncorhynchus* spp.) Influence State Variables and Rates in Subarctic Tundra Streams and Lakes....17
2.1 Abstract....17
2.2 Introduction....18
2.3 Methods....21
2.3.1 Site Description....21
2.3.2 Water Quality....26
2.3.3 Ecosystem Responses....26
2.3.4 Statistical Analyses....29
2.4 Results....30
2.4.1 Water Quality....30
2.4.2 Ecosystem Responses....34
2.5 Discussion....39
2.5.1 Lakes and Streams at INWR Had a Low Nutrients and N:P Ratio Despite Salmon Runs....39
2.5.2 Salmon Subsidies Increased Water Column Productivity....42
2.5.3 Benthic Biofilms at INWR Lakes and Streams Were N-Limited....43
2.5.4 The Balance between Enrichment and Disturbance by Salmon Could Depend on Position in the Watershed....44
2.6 Acknowledgments....46
2.7 Literature Cited....46

Chapter 3: Enrichment of Subarctic Tundra Lakes Receiving Multiple Resource Subsidies....51

3.1 Abstract 51
3.2 Introduction 52
3.3 Materials and Methods 55
3.3.1 Site Description 55
3.3.2 Water Quality 57
3.3.3 Ecosystem Structure 58
3.3.4 Biofilm Nutrient Limitation 58
3.3.5 Isotopic Analysis 60
3.3.6 Statistical Analyses 61
3.4 Results 64
3.4.1 Water Quality 64
3.4.2 Ecosystem Structure 64
3.4.3 Biofilm Nutrient Limitation 67
3.4.4 Isotopic Analysis 68
3.5 Discussion 71
3.5.1 Lakes Receiving Subsidies Had Higher Nutrient Concentrations than Reference Lakes 71
3.5.2 Phytoplankton Biomass was Positively Correlated with the Availability of Inorganic Nutrients 72
3.5.3 Biofilm Nutrient Limitation Reflected the Relative Availability of N and P in Lakes 74
3.5.4 Macroinvertebrates and Benthic Sediments Were Enriched with Marine-Derived N at Waterbird Lakes but Not at Salmon-Bearing Lakes 76
3.6 Conclusions 77
3.7 Acknowledgments 79
3.8 Literature Cited 79

Chapter 4: Conclusions 85
4.1 Summary of Research Results 85
4.2 Enrichment and Disturbance by Multiple Taxa 90
4.3 Literature Cited 96

FIGURES

Figure 1.1 Photograph of upland tundra dotted with lakes and wetlands at Izembek National Wildlife Refuge. 4

Figure 1.2 Photograph demonstrating the close proximity of lakes and streams to Izembek Lagoon which is protected from Bering Sea storms by barrier islands. 5

Figure 2.1 Locations of stream and lake study sites at Izembek National Wildlife Refuge located in Southwest Alaska (U.S.A.). Salmon-bearing watersheds (including streams and lakes) included Frosty, Sophie, Blue Bill, and Red Salmon. Isolated control lakes included Goose, Rescue, and Kayak. 23

Figure 2.2 Estimated salmon escapement (1000's of fish) declined sharply in 2010 and 2011. Aerial surveys for Blue Bill and Red Salmon were not gathered in 2013. These data were provided by the Alaska Department of Fish and Game commercial salmon annual management reports. 24

Figure 2.3 Water column ammonium concentrations in isolated control lakes, salmon-bearing lakes, upstream, and downstream sites during the 2012 (left) and 2013 (right) seasons. Error bars represent standard error among field replicates. Note that the scale is different for concentration of NH_4^+-N at control lakes (range, 0 to 10) compared to salmon-bearing lakes and streams (range, 0 to 15). 32

Figure 2.4 Water column SRP concentrations in isolated control lakes, salmon-bearing lakes, upstream, and downstream sites during the 2012 (left) and 2013 (right) seasons. Error bars represent standard error among field replicates. Note that the scale is different for concentration of SRP at control lakes (range, 0 to 15) compared to salmon-bearing lakes and streams (range, 0 to 30).....33

Figure 2.5 Molar N (as dissolved inorganic N) and P (as SRP) ratios before and during the salmon run at INWR salmon-bearing sites, shown with values reported in a meta-analysis by Janetski and colleagues (2009) and values reported from Southeast Alaska by Levi and colleagues (2011). The line represents the Redfield ratio of 16 N to 1 P. 35

Figure 2.6 Water column chlorophyll-*a* concentrations in isolated control lakes, salmon-bearing lakes, upstream, and downstream sites during the 2012 (left) and 2013 (right) seasons. Error bars represent standard error among field replicates. Note that the scale is different for concentration of chl-*a* at control lakes (range, 0 to 20) compared to salmon-bearing lakes and streams (range, 0 to 200).....36

Figure 2.7 Benthic biofilms at salmon-bearing lakes and streams were often N-limited but never P-limited. Nutrient limitation status of chlorophyll-*a* (chl-*a*), gross primary production (GPP), and community respiration (CR) before and during the salmon run for the 2012 (left) and 2013 (right) seasons 38

Figure 2.8 Whole-stream gross primary production (GPP) and ecosystem respiration (ER) at the upstream (US) and downstream (DS) reaches in the Red Salmon, Blue Bill, and Frosty watersheds for the 2012 (left) and 2013 (right) seasons. The scale for GPP ranges from 0 to 5, while the range for ER ranges from 0 to -35. No data were available (NA) for Red Salmon downstream during the 2012 salmon run. Error bars represent standard error among field replicates 40

Figure 3.1 Location of lake study sites at Izembek National Wildlife Refuge located in Southwest Alaska (U.S.A.). Letters indicate the site name. Salmon bearing lakes include; Red Salmon (RS), Blue Bill (BB), and Sophie (Sop). Waterbird sites include; Horseshoe (Hors), Molly (Mol), and Shane (Sha) lakes. Finally, reference lakes include; Goose (Goo), Kayak (Kay), and Rescue (Res) lakes.....56

Figure 3.2 Mean water column dissolved inorganic nitrogen (DIN) concentrations in salmon-bearing lakes (dark grey), waterbird lakes (light grey), and reference lakes (white) prior to the fall salmon and waterfowl migrations. Error bars represent standard error among sites receiving similar subsidies. Letters indicate statistically different groups 65

Figure 3.3 Mean water column chlorophyll-*a* concentrations in salmon-bearing lakes (dark grey), waterbird lakes (light grey), and reference lakes (white) prior to the fall salmon and waterfowl migrations. Error bars represent standard error among sites receiving similar subsidies. Letters indicate statistically different groups. 66

Figure 3.4 Water column chlorophyll-a concentrations plotted against dissolved inorganic nitrogen (DIN) and soluble reactive phosphorous (SRP) concentrations in salmon-bearing lakes (dark grey), waterbird lakes (light grey), and reference lakes (white) prior to the fall salmon and waterbird migrations. Solid line represents the line of best fit.....67

Figure 3.5 Benthic biofilm nutrient limitation status of chlorophyll-a (chl-*a*), gross primary production (GPP), and community respiration (CR) for each lake, where lakes are grouped by the type of subsidy that they received (i.e.,

Waterbird, Salmon, or Reference). Each slice of the pie chart represents the nutrient limitation status of a single lake.....68

Figure 3.6 Isotopic ratios of $\delta^{13}C$ and $\delta^{15}N$ of lake sediments (grey), macrophytes (white), and macroinvertebrates (black). Plotted points represent mean values ± standard error. The dotted lines are for reference purposes between the 3 panels. Riparian vegetation mainly included *Rosaceae, Sanguisorba stipulata* and *Rosaceae, Potententilla anserina*. Submerged vegetation mainly included *Ranunculus longirostris* and *Ranunculus richardsonii*, but also included *Potamogeton richardsonii*, *Myriophyllum farwellii*, and *Nitella* spp. Benthic invertebrate consumers included snails, amphipods, isopods, and aquatic insects (*Trichoptera, Limnephilidae*; *Diptera, Chironomidae*; *Diptera, Tipulidae*).....70

Figure 4.1 Conceptual model showing salmon-mediated enrichment and disturbance in aquatic ecosystems. As enrichment (blue) increases, disturbance (red) decreases and vice versa.....92

Figure 4.2 Conceptual models showing salmon-mediated and waterbird-mediated enrichment and disturbance in aquatic ecosystems. As enrichment (blue) increases, disturbance (red) decreases and vice versa.....93

Figure 4.3 Conceptual model showing the hypothetical interaction between salmon- and waterbird-mediated enrichment and disturbance in aquatic ecosystems. As enrichment (blue) increases, disturbance (red) decreases and vice versa. Any given ecosystem exists at the intersection of the enrichment-disturbance diagrams (bolded box), where some components of the ecosystem are enriched or disturbed by both salmon and waterbirds (dark blue and dark red, respectively), while other components of the ecosystem are enriched by one group and disturbed by the other (purple).....94

Figure 4.4 Conceptual model showing the hypothetical interaction between salmon- and waterbird-mediated enrichment and disturbance in aquatic ecosystems. Notice that as waterbirds have more of an enrichment effect, the fraction of the ecosystem receiving dual-disturbance decreases and the fraction receiving dual-enrichment increases.....95

ACKNOWLEDGMENTS

This research would not have been possible without the support of Dr. Jennifer Tank. Throughout my graduate degree, Jen has allowed me to pursue interesting research topics, pushed me to achieve more than I could have ever imagined, and challenged me to see the world through a new lens. In addition, she has taught me what it means to stand up for myself, and showed me how to make tough decisions. For these reasons and many more, I offer my thanks to Dr. Jennifer Tank.

Next, I would like to thank my committee members: Dr. Gary Lamberti and Dr. Stuart Jones. Gary has been like a second advisor to me. He has challenged me to think at the ecosystem level and has taught me to design projects with the statistics in mind. More than once he has reminded me that the simplest solution is often the best, and his unbridled enthusiasm for science is a constant reminder of why I want to be an ecologist. Stuart has inspired me to think small. His guidance has emphasized the value of the microbe and motivated me to incorporate molecular biology into my research. In addition, he has provided excellent feedback that has shaped my research over the past three years.

Many thanks also go out to the current and former Tank and Lamberti Lab members that made this thesis possible. Specifically, I would like to thank Drs. Peter Levi, Janine Rüegg, Sarah Roley, Natalie Griffiths, and Laura Johnson for being patient

mentors and graciously offering me their guidance when I did not know enough to ask for help. In addition, I owe a great deal to Alex Reisinger, Robert Davis, Brittany Hanrahan, Arial Shogren, Martha Dee, Carmella Vizza, Nathan Evans, Ursula Mahl, and Mike Bruseke for providing me with valuable input at every stage of my thesis. Furthermore, I would like to thank Joe Mueller, Audrey Kelley, and Karen Huang for helping me prepare supplies for the field and process samples in the lab.

I would also like to thank all of the folks who helped me in the field; without them, I literally could not have collected any samples. In particular, I want to thank Jennifer Gregory and James Smith, both of whom seemed to enjoy backpacking even more than me. In addition, a debt of gratitude goes out to all of the Fish and Wildlife Service staff who provided me with both logistical and emotional support; specifically, Trent Liebich, Leticia Melendez, Doug Damberg, Spencer Berg, Shane Watson, and Stacey Lowe.

I personally owe a thank you to the GLOBES program at the University of Notre Dame for graciously providing me with a fellowship and a number of opportunities to broaden my academic experiences with interdisciplinary trainings in communications, policy, economics, and ethics. I would also like to thank the Center for Aquatic Conservation at the University of Notre Dame for providing me with a fellowship that allowed for the completion of my masters research. Finally, I would like to extend my thanks to the U.S. Fish and Wildlife Service and Izembek National Wildlife Refuge for providing the financial support that made this research possible.

I am fortunate to have made some lifelong friends over the past three years. In particular Ursula Mahl, Rob Davis, Carmella Vizza, Trent Liebich, and Leticia Melendez have provided me with more support than I could have ever hoped for, both as a scientist and as a friend. I also found mentors who helped me realize that my calling is teaching. Kristen Lewis, Jennifer Robichaud, and Ron and Barb Hellenthall have been both friends and mentors to me, they ignited my passion for teaching, and more than anything they believed in me. Thank you.

Last but not least, I would like to thank my family. My mom (Gale), dad (Arlan), and sister (Andrea) have always been there for me, even when I did not want them. Their signature brand of tough love followed by crying and hugs has helped me cope with my personal and professional insecurities. In addition, I want to thank my in-laws, Sara and Steve, who send me a card just about every month telling me to hang in there and offering to let me stay over at their place whenever I want a change of scenery. Finally, I want to thank my husband Stuart Winikoff. Stuart is my missing piece. He makes me a better and more patient person. He also makes me a better scientist. Plus, he is very good at weighing filters.

Thank you all for your part in making this thesis possible.

CHAPTER 1:

INTRODUCTION

"To a biologically oriented individual, there is no better place on earth, and few that will match it. It is alive with creatures. The Alaska brown bear, caribou, river otters, sea otters, waterfowl of several different types, seals, [you are] just never away from wildlife there"

– Bob "Sea Otter" Jones, writing about Izembek National Wildlife Refuge

1.1 Izembek National Wildlife Refuge

1.1.1 Location, Climate, and Geologic History

Izembek National Wildlife Refuge (INWR) is located at the western terminus of the Alaska Peninsula and provides 417,533 acres of critical habitat for a variety of unique and cherished plant and animal species (U.S. Fish and Wildlife Service, 2013). Weather fronts from the Bering Sea and Pacific Ocean collide over the thin strip of land dividing the two bodies of water, resulting in nearly constant cloud cover with heavy wind and rain that shape the landscape. Barrier islands separate and shelter Izembek Lagoon, which supports the largest continuous eelgrass bed in the world, from Bering Sea storm fronts (Ward et al. 1997). The wind and ocean have shaped the landscape by maintaining cold

winters and cool, windswept summers that prevents the growth and reproduction of trees and helps maintain tundra habitat (Hopkins 1959, but see also Talbot et al. 2006).

With Bering Sea tundra to the north and Aleutian Heath Meadow to the south, INWR straddles two distinct ecoregions (Bailey 1995). However, the landscape as a whole is best characterized as an expanse of rolling tundra frequently interspersed with wetlands, ponds, and streams. While there is some debate about whether the region should be characterized as Arctic (Bliss and Matveyana 1991) or Boreal (Tuhkanen 1984 in Talbot et al. 2000), the flora suggest that the region is climatically boreal and maritime (Talbot et al. 2000, 2006).

Izembek NWR has geologically young bedrock and overlying soils. Across much of the refuge, volcanic rock in addition to alluvial and glacial moraine from the Quaternary period form the underlying bedrock (Wilson et al. 1999). Subsequently the soils of INWR are only between 1,000-10,000 years old (Wilson et al. 1997). These young soils have more available phosphorous (P) from rock weathering than older, more well developed soils (Chadwick et al. 1999). Conversely, nitrogen (N) accumulates in soils over longer periods of time, such that older soils have more available N (Yano et al. 2013). Still, both short-term and long-term erosional processes (e.g., river flow and glacial till) can liberate bioavailable nutrients in older soils (Vitousek et al. 2003). At INWR, inorganic P is likely more available than inorganic N, both on land and in freshwater habitats such as ponds, lakes, and streams; this availability likely reflects the age of the underlying bedrock and the nature of the subsequent soil development, setting the stage for the biotic community.

1.1.2 Becoming Izembek National Wildlife Refuge

Izembek NWR is unique because of the local and regional environmental context that attracts and supports abundant and diverse wildlife. The 34,662 hectare Izembek Lagoon is home to the largest single stand of eelgrass on earth at 15,000-16,000 hectares (Ward et al. 1997). Eelgrass beds are some of the most productive aquatic ecosystems on earth, and are able to support robust estuarine assemblages including diatoms, epiphytes, mollusks, gastropods, copepods, amphipods, crabs, juvenile salmonids, and other fish that attract migratory waterfowl, seabirds, and shorebirds which aggregate in eelgrass beds to feed and rest (Phillips 1984). During the fall, many species of waterfowl fatten-up before their annual migration south by eating eelgrass seeds and blades, as well as resident mollusks. Shorebirds and seabirds also feed in the eelgrass beds, consuming gastropods, crustaceans, and small fish (Phillips 1984). In addition to Izembek Lagoon, the numerous wetlands that dot the inland landscape offer an ideal habitat for staging migratory waterfowl and shorebirds to rest and feed before migrating to breeding and wintering areas around the world (Fig. 1.1; U.S. Fish and Wildlife Service 2013). For example, nearly 90% of the global population of Pacific Brant (*Branta bernicla*) and Emperor Geese (*Chen canagica*) pass through INWR each fall and spring, staging in the lagoon and wetlands. In the fall, Brant may spend up to 8 weeks feeding in Izembek Lagoon and resting in the wetlands near the lagoon before they migrate south for the winter. In addition, a handful of Brant remain at INWR over the winter (U.S. Fish and Wildlife Service, 2013). Furthermore, the expansive upland tundra at INWR provides ideal breeding and rearing habitat for many species of waterfowl, shorebirds, and seabirds (U.S. Fish and Wildlife Service, 2013). Likewise, the lakes, streams, and the lagoon (Fig.

1.2) provide critical spawning and rearing habitat for all 5 species of Pacific salmon (*Oncorhynchus* spp.). Still, INWR is more than just habitat for birds and fish; the area is also home to wolverines (*Gulo gulo*), wolves (*Canis lupus*), caribou (Rangifer tarandus), and river otters (*Lontra canadensis*). In addition, brown bear (*Ursus arctos*) reach some of their highest densities in the world at INWR, with 1 bear for every 2.25 square miles (U.S. Fish and Wildlife Service, 2013).

Figure 1.1 Photograph of upland tundra dotted with lakes and wetlands at Izembek National Wildlife Refuge.

Figure 1.2 Photograph demonstrating the close proximity of lakes and streams to Izembek Lagoon which is protected from Bering Sea storms by barrier islands.

Recognizing the valuable natural resources supported by Izembek Lagoon and its contributing watershed, Izembek National Range was established in 1960, and 20 years later, the U.S. Congress and passed the Alaska National Interest Lands Conservation Act (ANILCA), which re-designated Izembek as a National Wildlife Refuge. The purposes of INWR, as stated by ANILCA, include:

i. *To conserve fish and wildlife populations and habitats in their natural diversity including, but not limited to, waterfowl, shorebirds, and other migratory birds, brown bears, and salmonids;*
ii. *To fulfill the international treaty obligations of the United States with respect to fish and wildlife and their habitats;*
iii. *To provide, in a manner consistent with the purposes set forth in subparagraphs (i) and (ii), the opportunity for continued subsistence uses by local residents, and*
iv. *To ensure, to the maximum extent practicable and in manner consistent with the purposes set forth in subparagraph (i), water quality and necessary water quantity within the refuge*.

In addition to protecting the land and water that support Izembek flora and fauna, the U.S. Congress also recognized the value of biota at Izembek to Alaskan natives, both culturally and as critical food resources, deliberately protecting the right to practice subsistence harvest (ANILCA). Today, INWR is internationally recognized as a wetland of importance and is protected both nationally as a refuge, and internationally by the Ramsar Convention.

1.2 Subarctic Maritime Tundra and Resource Subsidies in Lakes and Streams

Nutrient subsidies generally enhance primary and secondary productivity in freshwater ecosystems (Polis et al. 1997), and the source of subsidies can be from land, water, or air. For example, terrestrial subsidies to freshwater ecosystems may be in form of leaf litter fall (Webster et al. 1999), dead animals or excrement (Polis et al. 1997), debris deposited during a flood (Khan et al. 2013), and soil organic matter (Tockner et al. 1999). Subsidies can also enter a freshwater ecosystem from other aquatic ecosystems through several available mechanisms, such as the movement of water from upstream to downstream (Vannote et al. 1980) and the movement of anadromous Pacific salmon (*Oncorhynchus* spp.) from marine environments to their natal habitats (Gende et al. 2002, Naiman et al. 2002). Previous research has demonstrated the importance of salmon as nutrient subsidies to freshwater ecosystems (e.g., Chaloner et al. 2002, Johnston et al. 2004, Mitchell and Lamberti 2005, Janetski et al. 2009, Rüegg et al. 2011, Levi et al. 2012, Reisinger et al. 2013). However, for salmon, the net effect of the nutrient subsidy on the recipient freshwater ecosystem is often mediated by local and regional environmental characteristics including temperature, light availability, presence of large woody debris, substrate size, background nutrient availability, and salmon spawner density (Moore et al. 2004, Moore and Schindler 2008, Tiegs et al. 2008, Janetski et al. 2009, Holtgrieve et al. 2010, Tiegs et al. 2011, Rüegg et al. 2012).

The native range of Pacific salmon extends across nearly the entire state of Alaska (Augerot and Foley 2005) and there has been a substantial amount of research on salmon-derived nutrient subsidies (SDN) in some ecotones in Alaska and the Pacific Northwest (i.e., Pacific coast mountain forest, Cascade mixed forest, and redwood forest; Bailey

1995). However, across large portions of their range the role of salmon in freshwater lakes and streams has yet to be fully investigated. For example, 42% of Alaska is tundra (Bailey 1995), but very little is known about how Pacific salmon influence ecosystem structure and function (i.e., state variables and rates, respectively) in low gradient, tundra streams outside of the Wood River system in Alaska (but see Morris and Stanford 2011). Izembek NWR is a unique tundra ecosystem where the role of resource subsidies in lakes and streams has not yet been explored. While the role of salmon-derived nutrients in freshwater ecosystems has been well-studied elsewhere in Southwest Alaska, those studies occurred closer to mainland Alaska, were regionally constrained, and possessed much larger salmon runs than those present at INWR (e.g., Moore and Schindler 2004, Moore et al. 2007, Holtgrieve and Schindler 2011). Investigating how salmon influence ecosystem structure and function in previously unstudied tundra systems will provide new insights about the interactions between salmon and their environment, which has important implications for conservation efforts throughout the Pacific salmon range now and in the future (Varnavskaya et al. 1994, Rogers and Schindler 2008).

At INWR, in addition to salmon subsidies, waterbirds (here defined as waterfowl, shorebirds, and seabirds) may also serve as a source of nutrients for freshwater ecosystems. Previous literature investigating the role of waterbird subsidies on aquatic ecosystem structure and function has largely been conducted in temperate regions where geese can significantly impact freshwaters (Kitchell et al. 1999, Jefferies 2000, Olson et al. 2005). Among the studies that have considered waterbird nutrients in lakes very few have occurred at high latitudes. However, the studies that have directly considered waterbird nutrient subsidies in lakes and streams found that waterbird-derived nutrients

can support a substantial portion (28-38%) of the biomass of stream biota and can increase ecosystem productivity (Izaguirre et al. 1998, Harding et al. 2004, Brimble et al. 2009).

In the Arctic, there is growing interest in the role of waterbirds as vectors for the transfer of environmental toxins (Evenset et al. 2004, 2007, Blais et al. 2005, Brimble et al. 2009), and some studies have used differences in dissolved nutrient concentrations and stable isotope enrichment as an indicator of the impact of waterbirds on Arctic lakes (Evenset et al. 2007, Keatley et al. 2009). However, waterbirds are not the only potential source of nutrient enrichment to Arctic lakes and streams. Arctic tundra ecosystems are underlain by permafrost, and the increasing depth of annual permafrost thaw is projected to release phosphates that have been stored in frozen soils to freshwater lakes and streams (Hobbie et al. 1999). Previous research at Toolik Lake, Alaska suggests that phosphorous enrichment in Arctic tundra could alter aquatic ecosystem productivity and community assemblages (Hobbie et al. 1999). At INWR, the subarctic maritime tundra is not underlain by permafrost, and the refuge receives large annual migrations of waterbirds. Since environmental context influences the net effect that other subsidies have on aquatic ecosystems (i.e., salmon), it is important to understand how waterbirds influence ecosystem structure and function across a broad range of ecoregions and environmental conditions.

1.3 Research Goals and Thesis Outline

The goal of my thesis research was to establish the baseline structural and functional conditions in freshwater lakes and streams at INWR. In particular, my goal was to understand how local environmental conditions and resource subsidies, both via salmon and waterbirds, may play a role in shaping the state variables and rates in Izembek streams and lakes.

Chapter 2: I began by examining how Pacific Salmon (*Oncorhynchus* spp.) influence stream ecosystem structure and function in a portion of their range (e.g., tundra habitat at INWR) that had not been previously considered. I designed my research to quantify how streams and lakes change in the presence of salmon spawners and how the local environmental context can influence the net ecosystem effect of salmon. I predicted that at INWR salmon would primarily have a disturbance effect where salmon actively spawn, while the downstream environment would be enriched by salmon-derived nutrients transported downstream.

Chapter 3: In analyzing the results from my first field season at INWR, I hypothesized that lakes retained salmon-derived nutrients over multiple growing seasons and that those nutrients were stimulating primary productivity. In addition, many INWR lakes that did not receive salmon were visited by dense aggregations of waterbirds in the spring and fall. For my second data chapter, I examined whether the source of a nutrient subsidy (salmon or waterbird) influences how the subsidy is incorporated into lake ecosystems. Specifically, I examined the differences between lakes receiving subsidies predominantly from salmon, compared to lakes with waterbird subsidies, and finally with reference lakes that did not receive subsidies. Based on the preliminary data from my first

year at INWR, I predicted that lakes receiving salmon and waterbird subsidies would have similar abiotic and biotic characteristics demonstrating enrichment.

Chapter 4: In my two data chapters I found that (1) The response of streams and lakes at INWR to salmon-derived nutrient subsidies is influenced by salmon run size, the initial availability of nutrients, and the relative availability of phosphorous compared to nitrogen (N:P ratio), (2) in low productivity ecosystems, researchers can gain valuable insight into the effects of salmon in streams by measuring whole-stream metabolism in multiple stream reaches within a single watershed, and (3) subsidies from salmon may have long-term effects on nutrient availability and phytoplankton biomass, but at lakes receiving waterbird subsidies patterns of nutrient limitation and marine-derived nutrient enrichment in the aquatic food web were more similar to what we expected from salmon-bearing lakes given the relative availability of nitrogen in the water column. With the results from my two data chapters, I use the opportunity in Chapter 4 to consider the broader implications of my research for the scientific community.

1.4 Literature Cited

Augerot, X. and D. Foley. 2004. Atlas of pacific salmon: the first map-based status assessment of salmon in the North Pacific. University of California Press, Portland, OR.

Bailey, R.G. 1995. Description of the Ecoregions of the United States. 2nd edition. USDA Forest Service, Washington DC.

Blais, J.M., L.E. Kimpe, D. McMahon, B.E. Keatley, M.L. Mallory, M.S.V. Douglas, and J.P. Smol. 2005. Arctic seabirds transport marine-derived contaminants. Science 309: 445.

Bliss, L.C., and N.V. Matveyana. 1991. Circumpolar arctic vegetation. Pages 59-86 in F.S. Chapin III, R.L. Jeffries, J.F. Reynolds, G.R. Shaver, and J. Svoboda (editors). Arctic ecosystems in a changing climate: an ecophysiological perspective. Academic Press, San Diego, CA.

Brimble, S.K., J.M. Blais, L.E. Kimpe, M.L. Mallory, B.E. Keaatley, M.S.V. Douglas, and J.P. Smol. 2009. Bioenrichment of trace elements in a series of ponds near a northern fulmar (Fulmarus glacialis) colony at Cape Vera, Devon Island. Canadian Journal of Fisheries and Aquatic Sciences 66:949–958.

Chadwick, O.A., L.A. Derry, P.M. Vitousek, B.J. Huebert, and L.O. Hedin. 1999. Changing sources of nutrients during four million years of ecosystem development. Nature 397:491–497.

Chaloner, D.T., K.M. Martin, M.S. Wipfli, P.H. Ostrom, and G.A. Lamberti. 2002. Marine carbon and nitrogen in southeastern Alaska stream food webs: evidence from artificial and natural streams. Canadian Journal of Fisheries and Aquatic Science 59: 1257-1265.

Evenset, A., J. Carroll, G.N. Christensen, R. Kallenborn, D. Gregor, and G.W. Gabrielsen. 2007. Seabird guano is an efficient conveyer of persistent organic pollutants (POPs) to Arctic lake ecosystems. Envrionmental Science and Technology 41:1173–1179.

Evenset, A., G.N. Christensen, T. Skotvold, E. Fjeld, M. Schlabach, E. Wartena, and D. Gregor. 2004. A comparison of organic contaminants in two high Arctic lake ecosystems, Bjørnøya (Bear Island), Norway. The Science of the total environment 318:125–41.

Gende, S.M., R.T. Edwards, M.F. Willson, and M.S. Wipfli. 2002. Pacific salmon in aquatic and terrestrial ecoystems. Bioscience 52(10):917-928.

Harding, J.S., D.J. Hawke, R.N. Holdaway, and M.J. Winterbourn. 2004. Incorporation of marine-derived nutrients from petrel breeding colonies into stream food webs. Freshwater Biology 49:576–586.

Hobbie, J.E., B.J. Peterson, N. Bettez, L. Deegan, W.J. O'Brien, G.W. Kling, G.W. Kipphut, W.B. Bowden, and A.E. Hershey. 1999. Impact of global change on biogeochemistry and ecology of an Arctic freshwater system. Polar Research 18(2):207-214.

Holtgrieve, G.W., and D.E. Schindler. 2011. Marine-derived nutrients, bioturbation, and ecosystem metabolism: reconsidering the role of salmon in streams. Ecology 92:373–385.

Holtgrieve, G.W., D.E. Schindler, C.P. Gowell, C.P. Ruff, P.J. Lisi. 2010. Stream geomorphology regulates the effects on periphyton of ecosystem engineering and nutrient enrichment by Pacific salmon. Freshwater Biology 55:2598-2611.

Hopkins, D.M. 1959. Some characteristics of the climate in forest and tundra regions in Alaska. Arctic 12:214–220.

Izaguirre, I., A. Vinocur, G. Mataloni, and M. Pose. 1998. Phytoplankton communities in relation to trophic status in lakes from Hope Bay (Antarctic Peninsula). Hydrobiologia 370:73–87.

Janetski, D.J., D.T. Chaloner, S.D. Tiegs, and G.A. Lamberti. 2009. Pacific salmon effects on stream ecosystems: a quantitative synthesis. Oecologia 159:583–595.

Jeffries, R.L. 2000. Allochthonous inputs: integrating population changes and food-web dynamics. Trends in ecology & evolution 15:19–22.

Johnston, N.T., E.A. Macisaac, P.J. Tschaplinski, and K.J. Hall. 2004. Effects of the abundance of spawning sockeye salmon (*Oncorhynchus nerka*) on nutrients and algal biomass in forested streams. Canadian Journal of Fisheries and Aquatic Sciences 61:384-403.

Keatley, B.E., M.S.V. Douglas, J.M. Blais, M.L. Mallory, and J.P. Smol. 2009. Impacts of seabird-derived nutrients on water quality and diatom assemblages from Cape Vera, Devon Island, Canadian High Arctic. Hydrobiologia 621:191–205.

Khan, N.S., B.J. Horton, K.L. McKee, D. Jerolmack, F. Falcini, M.D. Enache, C.H. Vane. 2013. Tracking sedimentation from the historic A.D. 2011 Mississippi river flood in the deltaic wetlands of Louisiana, USA. Geology 41(4):391-394.

Kitchell, J.F., D.E. Schindler, B.R. Herwig, D.M. Post, M.H. Olson, and M. Oldham. 1999. Nutrient cycling at the landscape scale : The role of diel foraging migrations by geese at the Bosque del Apache National Wildlife Refuge , New Mexico. Limnology and Oceanography 44:828–836.

Levi, P.S. 2012. The response of stream ecosystem function to salmon subsidies. Doctoral Dissertation, University of Notre Dame.

Mitchell, N.A., and G.A. Lamberti. 2005. Responses in dissolved nutrients and epilithon abundance to spawning salmon in southeast Alaska streams. Limnology and Oceanography 50(1):217-227.

Moore, J.W., and D.E. Schindler. 2004. Nutrient export from freshwater ecosystems by anadromous sockeye salmon (*Oncorhynchus nerka*). Canadian Journal of Fisheries and Aquatic Sciences 61:1582–1589.

Moore, J.W., and D.E.Schindler. 2008. Biotic disturbance and benthic community dynamics in salmon-bearing streams. Journal of Animal Ecology 77(2):275-284.

Moore, J.W., D.E. Schindler, J.L. Carter, J. Fox, J. Griffiths, and G.W. Holtgrieve. 2007. Biotic control of stream fluxes: spawning salmon drive nutrient and matter export. Ecology 88:1278–1291.

Moore, J.W., D.E. Schindler, and M.D. Scheuerell. 2004. Disturbance of freshwater habitats by anadromous salmon in Alaska. Oecologia 139(2):298-308.

Morris, M.R., and J.A. Stanford. 2011. Floodplain succession and soil nitrogen accumulation on a salmon river in southwestern Kamchatka. Ecological Monographs 81:43–61.

Naiman, R.J., R.E. Bilby, D.E. Schindler, and J.M. Helfield. 2002. Pacific salmon, nutrients, and the dynamics of freshwater and riparian ecosystems. Ecosystems 5(4):399-417.

Olson, M.H., M.M. Hage, M.D. Binkley, and J.R. Binder. 2005. Impact of migratory snow geese on nitrogen and phosphorus dynamics in a freshwater reservoir. Freshwater Biology 50:882–890.

Phillips, R.C. 1984. Ecology of eelgrass meadows in the Pacific Northwest: a community profile. FWS/OBS-84/24, US Fish and Wildlife Service.

Polis, G.A., W.B. Anderson, and R.D. Holt. 1997. Toward and integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annual Review of Ecology and Systematics 28:289–316.

Reisinger, A.J., D.T. Chaloner, J. Rüegg, S.D. Tiegs, and G.A. Lamberti. 2013. Effects of spawning Pacific salmon on the isotopic composition of biota differ among southeast Alaska streams. Freshwater Biology 58:938–950.

Rogers, L.A., and D.E. Schindler. 2008. Asynchrony in population dynamics of sockeye salmon in southwest Alaska. Oikos 117:1578–1586.

Rüegg, J., D.T. Chaloner, P.S. Levi, J.L. Tank, S.D. Tiegs, and G.A. Lamberti. 2012. Environmental variability and the ecological effects of spawning Pacific salmon on stream biofilm. Freshwater Biology 57:129–142.

Rüegg, J., S.D. Tiegs, D.T. Chaloner, P.S. Levi, J.L. Tank, and G.A. Lamberti. 2011. Salmon subsidies alleviate nutrient limitation of benthic biofilms in Southeast Alaska streams. Canadian Journal of Fisheries and Aquatic Science 68:277-287.

Talbot, S.S., S.L. Talbot, J.W. Thomson, and W.B. Schofield. 2000. Lichens of Izembek National Wildlife Refuge , Westernmost Alaska Peninsula. The Bryologist 103:379–389.

Talbot, S.S., S.L. Talbot, and B. Wilfred. 2006. Vascular flora of Izembek National Wildlife Refuge , Westernmost Alaska Peninsula , Alaska. Vascular 108:249–293.

Tiegs, S.D. 2008. Timber harvest transforms ecological roles of salmon in Southeast Alaska rain forest streams. Ecological Applications 18(1): 4-11.

Tiegs, S.D., P.S. Levi, J. Rüegg, D.T. Chaloner, J.L. Tank, G.A. Lamberti. 2011. Ecological effects of live salmon exceed those of carcasses during an annual spawning migration. Ecosystems 14:598-614.

Tockner, K., D. Pennetzdorfer, N. Reiner, F. Schiemer, and J.V. Ward. 1999. Hydrological connectivity, and the exchange of organic matter and nutrients in a dynamic river-floodplain system (Danube, Austria). Freshwater Biology 41:521-535.

Tuhakanen, S. 1984. A circumboreal system of climate phytogeographical regions. Acta Biotanica Fennica 127:1-50.

U.S. Fish and Wildlife Service. 2013. Izembek National Wildlife Refuge land exchange/road corridor: biological environment.

U.S. Senate and House of Representatives, 96th Congress. 1980. Public Law 96-487. Alaska National Interest Lands Conservation Act (ANILCA). Washington: Government Printing Office.

Vannote, R.L., G.W. Minshall, K.W. Cummins, J.R. Sedell, and C.E. Cushing. 1980. The river continuum concept. Canadian Journal of Fisheries and Aquatic Science 37:130-137.

Varnavskaya, N.V., C.C. Wood, R.J. Everett, R.L. Wilmot, V.S. Varnavsky, V.V. Midanaya, T.P. Quinn. 1994. Genetic differentiation of subpopulations of sockeye salmon (*Oncorhynchus nerka*) within lakes of Alaska, British Columbia, Kamchatka, Russia. Canadian Journal of Fisheries and Aquatic Sciences 51: 147-157.

Vitousek, P., O. Chadwick, P. Matson, S. Allison, L. Derry, L. Kettley, A. Luers, E. Mecking, V. Monastra, and S. Porder. 2003. Erosion and the Rejuvenation of Weathering-derived Nutrient Supply in an Old Tropical Landscape. Ecosystems 6:762–772.

Ward, D.H., C.J. Markonb, and D.C. Douglas. 1997. Distribution and stability of eelgrass beds at Izembek Lagoon, Alaska. Aquatic Botany 58:229–240.

Webster, J.R., E.F. Benfield, T.P. Ehrman, M.A. Schaeffer, J.L. Tank, J.J. Hutchens, and D.J. D'Angelo. 1999. What happens to allochthonous material that falls into streams? A synthesis of new and published information from Coweeta. Freshwater Biology 41:687-705.

Wilson, F.H., R.L. Detterman, and G.D. Dubois. 1999. Digital data for geologic framework of the Alaska Peninsula, Southwest Alaska, and the Alaska Peninsula terrane. USGS open –file report 99-317:41.

Wilson, F.H., F.R. Weber, T.M. Dochat, T.P. Miller, and R.L. Detterman. 1997. Revised geologic map of the Cold Bay and False Pass quadrangles, Alaska Peninsula. USGS open-file report 97-866:34.

Yano, R., G.R. Shaver, E.B. Rastetter, A.E. Giblin, and J.A. Laundre. 2013. Nitrogen dynamics in arctic tundra soils of varying age: differential responses to fertilization and warming. Oecologia 173:1575–1586.

CHAPTER 2:

PACIFIC SALMON (ONCORHYNCHUS SPP.) INFLUENCE STATE VARIABLES AND RATES IN SUBARCTIC TUNDRA STREAMS AND LAKES[1]

2.1 Abstract

Migrations of Pacific salmon (*Oncorhynchus* spp.) provide nutrient subsidies to freshwater ecosystems and salmon-derived nutrients can stimulate ecosystem productivity in low-nutrient systems. Nevertheless, the net effect of salmon on their natal lakes and streams is often mediated by local (e.g., sediment size, riparian vegetation, salmon run size) and regional (e.g., geology, climate) environmental characteristics. To examine the impact of salmon migration on ecosystem structure and function (i.e., state variables and rates, respectively) in the Southwest Alaska tundra landscape, we measured water column nutrients and chlorophyll-a, quantified whole-stream metabolism, and deployed nutrient diffusing substrata (NDS) amended with inorganic nitrogen and phosphorus in three streams and 6 lakes in Izembek National Wildlife Refuge. Dissolved inorganic nutrient concentrations remained at, or near detection limits both before and during the salmon migration, but water column chlorophyll-*a* concentrations substantially increased in response to salmon. Benthic biofilms were almost exclusively limited by nitrogen availability, and nutrient limitation was seldom alleviated by salmon regardless of whether we measured autotrophic or heterotrophic biofilm response. Whole-stream

[1] This chapter is in preparation for the Canadian Journal of Fisheries and Aquatic Science with co-authors J.L. Tank, U.H. Mahl, G.A. Lamberti, P.S. Levi, and T.A. Liebich.

gross primary production (GPP) generally decreased when salmon were present, while ecosystem respiration (ER) varied in its response (disturbance vs. enrichment) over space within a single watershed and from year to year. We suggest that in the subarctic tundra, these very low-nutrient freshwaters are potentially sensitive to salmon enrichment, but that salmon run characteristics influence the magnitude of response (i.e., enrichment or disturbance) in their natal streams and lakes, and the net salmon effect depends on local and regional environmental conditions. Furthermore, we show how the use of both structural and functional metrics can provide information about the variable role of salmon as ecosystem engineers.

2.2 Introduction

It is well accepted that migrating Pacific salmon (*Oncorhynchus* spp.) increase lake and stream ecosystem productivity by providing high quality nutrient subsidies when they spawn and die (e.g., Bilby et al. 1996, Janetski et al. 2009). However, the magnitude of salmon enrichment depends on the density of salmon spawners (Johnston et al. 2004, Janetski et al. 2009), carcass retention (Schuldt and Hershey 1995, Johnston et al. 2004), and local and regional environmental characteristics (e.g., substrate size, discharge) (Moore and Schindler 2008, Mitchell and Lamberti 2005, Tiegs et al. 2008, Janetski et al. 2009, Rüegg et al. 2012). Research has also demonstrated that environmental characteristics influence the degree to which salmon physically disturb their natal ecosystem during spawning via redd construction (Moore et al. 2004, Moore and Schindler 2008, Tiegs et al. 2009). The balance between salmon disturbance and

enrichment can be difficult to predict, especially among regions, watersheds, and years (e.g., Rüegg et al. 2012), but some of this ambiguity may stem from spatial limitations of the study design or the response metrics employed to assess enrichment or disturbance. For example, salmon can be a source of both enrichment and disturbance within a single watershed, but the measured net effect may be dictated by the relative position of the study reach (i.e., upstream or downstream) compared to salmon spawners (Albers and Petticrew 2012).

Recent research has investigated which local and regional environmental factors control the ecosystem-scale response to salmon, as well as temporal variability in those factors. The majority of studies have quantified the salmon response via structural metrics such as dissolved nutrient concentrations (e.g., Naiman et al. 2002, Levi et al. 2011), benthic or water column chlorophyll-a (chl-a)(Mitchell and Lamberti 2005, Moore and Schindler 2008, Rüegg et al. 2012), sediment size distribution and mobilization (Gottesfeld et al. 2004, Moore et al. 2004, Albers and Petticrew 2012), and macroinvertebrate biomass (Moore and Schindler 2008). In contrast, ecosystem functional metrics that integrate local and regional variability over time may be more effective at discerning the ecosystem response to salmon, but these have been less frequently measured. Recent studies have demonstrated the sensitivity of functional metrics to salmon spawning runs, including nutrient assimilation (Bilby et al. 1996, Reisinger et al. 2013), nutrient limitation status (Rüegg et al. 2011), and ecosystem metabolism (Holtgrieve and Schindler 2011, Levi et al. 2013). Understanding how salmon influence ecosystem function over space and time provides critical information about ecosystem-wide influences of salmon disturbance and enrichment.

Although Pacific salmon have North American ranges that extend from California to the North Slope in Alaska, much of the research on salmon in freshwater ecosystems has been limited to relatively few geographic locations that are largely dominated by high gradient, forested streams and rivers (i.e., Oregon, Washington, and Southeast Alaska). While there is a substantial body of research on open-canopy lakes and streams on the Wood River in Southwest Alaska, few other low-gradient, open-canopy systems have been considered. Furthermore, very little research has been done on the role of salmon in freshwaters along the North Pacific rim (but see Thompson 2007 and Morris and Stanford 2011). In tundra ecosystems of the Alaska Peninsula, the majority of salmon-bearing streams occur in low-gradient (Danielson and Gesch 2011), open-canopy, tundra ecosystems (Bailey 1995). Furthermore, the Alaska Peninsula region is responsible for ~10% of the Alaska sockeye salmon (*Oncorhynchus nerka*) commercial harvest (Alaska Department of Fish and Game 2013), yet few studies have explored how salmon influence ecosystem structure and function (i.e., state variables and rates, respectively) in this region.

We chose to work at Izembek National Wildlife Refuge (INWR) to quantify the ecosystem response of low-gradient, open-canopy, tundra systems to salmon spawners, and to determine whether salmon have an enrichment or disturbance effect on these subarctic tundra streams and lakes along the Alaska Peninsula. Using a combination of structural and functional metrics, we tracked changes in dissolved inorganic nutrient concentrations, water column and benthic chlorophyll-a (chl-a), benthic nutrient limitation status, and whole-stream metabolism before and during the salmon run in streams and lakes at INWR. We also explored whether disturbance and enrichment from

salmon in lakes and upstream reaches of streams influenced structure and function downstream. We predicted that (i) salmon would have a disturbance effect where they spawn, but an enrichment effect downstream of these spawning sites (sensu Albers and Petticrew 2012), and that (ii) quantifying both structural and functional metrics would provide a clearer understanding of the ecosystem response to salmon (e.g., enrichment or disturbance) than either type of metric alone.

2.3 Methods

2.3.1 Site Description

Located at the western terminus of the Alaska Peninsula, the landscape of INWR is dominated by maritime tundra along the coasts and alpine tundra at higher elevations. The refuge provides crucial overwintering and breeding habitat for multiple species of protected waterfowl, seabirds, and shorebirds, and habitat for caribou (*Rangifer tarandus*), brown bear (*Ursus arctos*), and all five species of Pacific salmon (*Oncorhynchus* spp.). The environmental characteristics at INWR allowed us to study the impact of Pacific salmon on low-gradient, open-canopy, tundra streams and lakes. Furthermore, at INWR lake, stream, and estuary ecosystems are all in close proximity, presenting a unique opportunity to evaluate the influence of salmon on ecosystem structure and function along the lake-stream continuum.

We studied salmon-bearing streams and lakes in three INWR watersheds over two years. In 2012, we sampled three salmon-bearing streams and two salmon-bearing lakes; one stream was glacially-fed (Frosty) and primarily received runs of chum salmon

(*Oncorhynchus keta*), while two watersheds had lake-fed streams (Blue Bill and Red Salmon; Fig. 2.1) and primarily received runs of sockeye salmon (*Oncorhynchus nerka*). We did not have an upstream control reach, as there were no physical barriers to salmon migration upstream of the salmon-bearing lakes. Instead we performed limited sampling at two isolated control lakes (Goose and Rescue) that have never received salmon, which acted as a reference condition to salmon-bearing lakes. In summer 2013, we constrained our stream survey by excluding Frosty due to a change in permitting, and instead expanded our lake survey to include an additional salmon-bearing lake (Sophie) and isolated control lake (Kayak; Fig. 2.1). Though the salmon-bearing systems have historically received high returns of salmon, the salmon escapements were atypically low in the years prior to and during our sampling (Fig. 2.2).

Within each stream, we sampled two reaches receiving salmon (Upstream and Downstream) to compare how the effects of salmon on ecosystem structure and function vary along the lake-stream continuum. In lake-fed streams, the upstream reach was located immediately downstream of the lake outlet. At Frosty stream, the upstream reach was located where chum salmon (*Oncorhynchus keta*) had been observed actively spawning in previous years. In all cases, the downstream reach was located downstream of the upstream reach. We selected stream reaches based on accessibility, minimum lateral inflow within the reach, position within the watershed, and maximal separation between study reaches. The reach lengths varied, from 178 to 407 m, to allow for a minimum water travel time of 10 minutes within each reach for metabolism estimation (Table 2.1). The distance between upstream and downstream reaches was equal to or greater than the reach lengths. All streams had open canopies but differed in

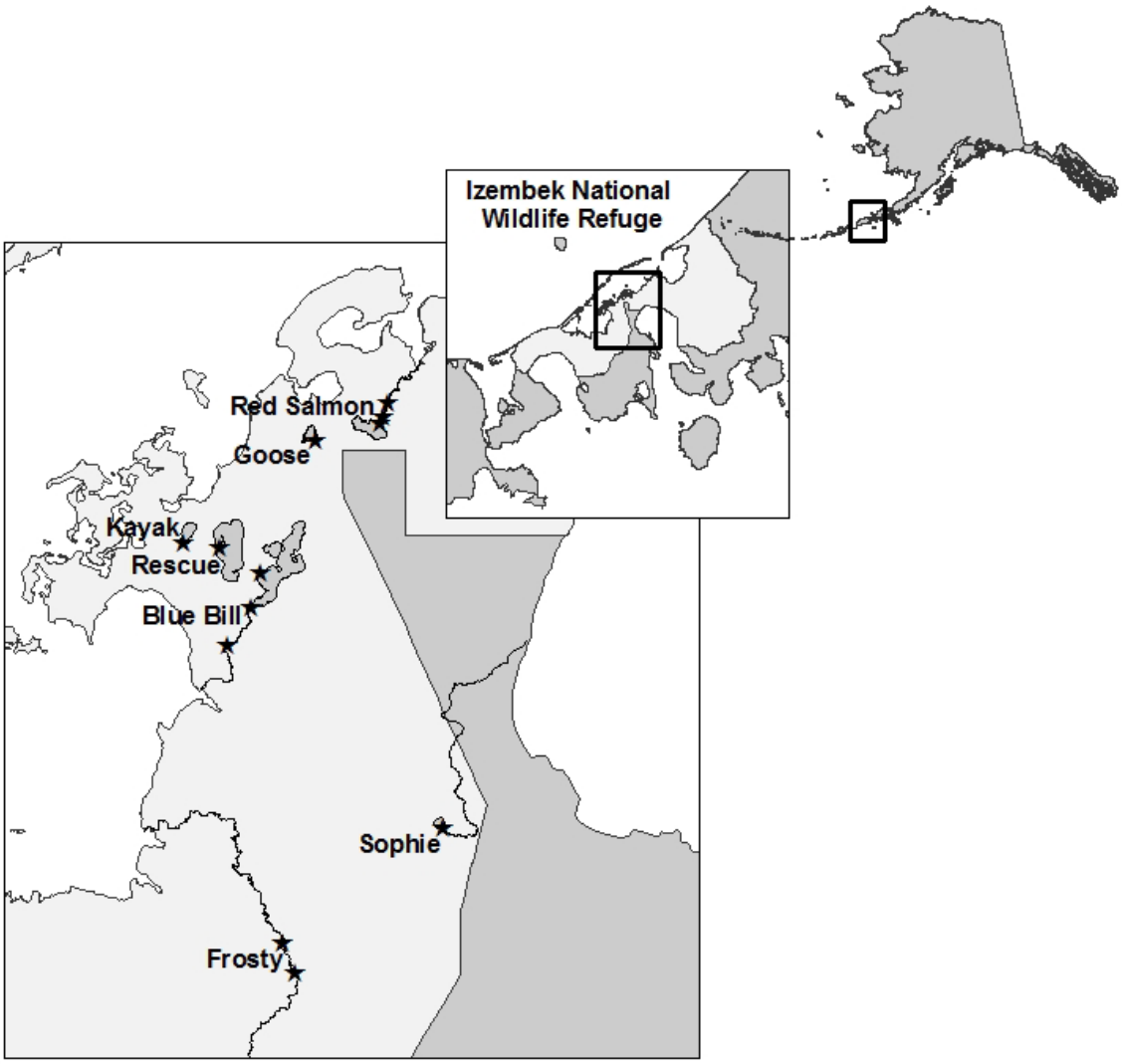

Figure 2.1 Locations of stream and lake study sites at Izembek National Wildlife Refuge located in Southwest Alaska (U.S.A.). Salmon-bearing watersheds (including streams and lakes) included Frosty, Sophie, Blue Bill, and Red Salmon. Isolated control lakes included Goose, Rescue, and Kayak.

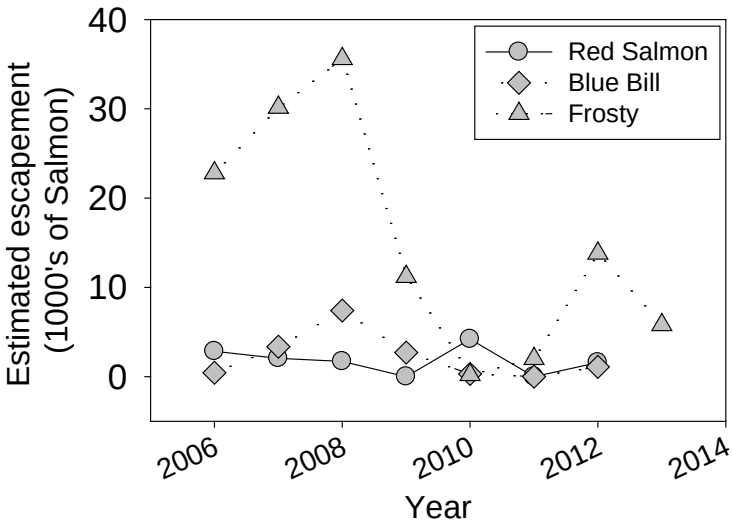

Figure 2.2 Estimated salmon escapement (1000's of fish) declined sharply in 2010 and 2011. Aerial surveys for Blue Bill and Red Salmon were not gathered in 2013. These data were provided by the Alaska Department of Fish and Game commercial salmon annual management reports.

geomorphology and channel characteristics (Table 2.1). Riparian vegetation varied somewhat between sites but generally consisted of grasses, sedges, mosses, lichens, and some forbs. In addition, Frosty stream had stands of alder bushes growing along stretches of the riparian zone. Salmon-bearing lakes had similar geomorphology and riparian vegetation to adjacent streams. We selected control lakes that were in close proximity to salmon-bearing lakes but were hydrologically isolated from salmon-bearing streams and lakes. The control lakes also had similar geomorphology and riparian vegetation to salmon-bearing lakes.

TABLE 2.1

PHYSICAL CHARACTERISTICS OF STREAM REACHES AT IZEMBEK NATIONAL WILDLIFE REFUGE

Stream Site		**Reach Length (m)**	**Mean Width (m)**	**Mean Depth (cm)**	**Mean Discharge (m^3 s^{-1})**	**D_{50}[a] (mm)**	**D_{90}[b] (mm)**	**Stream Gradient %**
Watershed	**Position**							
Red Salmon	*Upstream*	178	3.19	47	0.13 (*0.01*)	2.0	16.0	0.094
	Downstream	274	4.01	27	0.13 (*0.004*)	6.0	32.0	
Blue Bill	*Upstream*	211	3.55	51	0.20 (*0.01*)	2.0	23.0	0.164
	Downstream	250	3.78	46	0.23 (*0.01*)	0.001	6.0	
Frosty	*Upstream*	348	8.44	35	1.62 (*0.23*)	45.0	128.0	0.893
	Downstream	407	10.60	35	2.46 (*0.83*)			

[a] Substrate size at the 50th percentile.

[b] Substrate size at the 90th percentile.

Note: Discharge was the mean value calculated over 2 years. Substrate size at Frosty stream was similar between upstream and downstream reaches. Gradient was approximated using contour maps extracted from digital elevation maps in ArcGIS 10.1

.

2.3.2 Water Quality

We collected water samples to quantify concentrations of ammonium (NH_4^+-N), nitrate (NO_3^--N), and soluble reactive phosphorous (SRP) before and during the annual salmon migration. We collected three replicate 60-ml water samples at each site every 7-14 days throughout the field season, filtered stream water through a 0.7 μm glass fiber filter (GFF, Pall Corporation, Port Washington, NY, USA) in the field, saving the filters for later chl-*a* analysis. We froze all filtrate and filters for later analysis in the laboratory at the University of Notre Dame, where we measured nutrient concentrations on a Lachat Flow Injection Autoanalyzer (Loveland, Colorado, USA) using the phenol-hypochlorite method for NH_4^+-N (Solorzano 1969), cadmium reduction method for NO_3^--N (APHA 1995), and the ascorbic acid method for SRP (Murphy and Riley 1962). Dissolved inorganic nitrogen (DIN) concentrations are expressed as the sum of NH_4^+-N and NO_3^- -N concentrations on each sample date.

2.3.3 Ecosystem Responses

To assess the combined effects of salmon-related enrichment and disturbance on primary producers (i.e. algae), we measured chl-*a* before and during the salmon run. We sampled water column chl-*a* by filtering a known volume of stream water through a 0.7 μm GFF. We measured benthic chl-*a* by collecting 3 rocks or sediment grab samples that were representative of the substrate within the study reach, storing them in dark containers, and transporting the samples to the laboratory. We removed epilithic biofilms from the rocks using a wire bristle brush and distilled water, and filtered a known volume of the benthic slurry onto a 0.7 μm GFF. We froze the samples for later chl-*a* analysis,

which we completed using the methanol extraction method, followed by fluorometry (Wetzel and Likens 2000). We also measured ash-free dry mass (AFDM) of benthic biofilms using the loss-upon-combustion method (Steinman et al. 2006) for biofilm samples collected using the same methods as for chl-*a*.

We deployed nutrient diffusing substrata (NDS) to determine whether the availability of inorganic nitrogen (N) and/or phosphorous (P) limited productivity of benthic biofilms both before and during the salmon run. We constructed NDS by filling polyethylene cups (30 ml) with 2% agar that was amended with 0.5M of NH_4Cl (N treatment), KH_2PO_4 (P treatment), NH_4Cl and $KH_2PO_4^-$ (N+P treatment), or no nutrients (Control;Tank et al. 2006). In 2013, we changed our P source to NaH_2PO_4 because we observed P-inhibition in 2012 and thought our cation choice could be influencing biofilm growth (Lehman 1976). We covered agar with two types of substrata to select for particular biofilm constituents: (1) fritted glass disks selected for autotrophic biofilms, and (2) cellulose cloth selected for heterotrophic biofilms (Tank and Dodds 2003, Johnson et al. 2009). We deployed five replicate nutrient treatments for each substratum at each site, attaching NDS cups to plastic L-bars in groups of 8 randomly ordered cups, such that each bar acted as a block containing all treatments (n=40 cups per site). We secured the L-bars to the stream benthos in riffles and lake littoral zones. In 2012, we deployed NDS at all salmon-bearing lake and stream sites (n=8) before and during the salmon run. During the salmon run, NDS from two sites were destroyed by bears, decreasing our replicate sites to n=6. In summer 2013, we successfully deployed NDS before and during the salmon run at all salmon-bearing sites (n=5). After 16-20 days, we retrieved the NDS and transported substrata to the laboratory in individual 50 mL

centrifuge tubes filled with unfiltered stream water in the dark. In the lab, we quantified autotrophic activity by measuring gross primary production (GPP), and heterotrophic activity by measuring community respiration (CR) on each individual disk, then we individually packed disks in aluminum foil and froze them for later chl-*a* extraction using the methanol extraction method and fluorescence, as an index of algal biomass.

We measured GPP and CR on NDS disks using a modified light-dark bottle method (Johnson et al. 2009). Before each incubation, we refilled individual centrifuge tubes containing substrata with unfiltered site water, ensuring that there were no air bubbles in the tubes. We conducted all incubations for a minimum of 3 hours at room temperature, performing light incubations under 24 inch full-spectrum grow lights (Lights of America; model F17T8/GL, 1093 lumens) to maintain consistent light conditions, in order to compare biofilm nutrient limitation across multiple streams (Johnson et al. 2009).

We also measured whole-system metabolism, as GPP and ecosystem respiration (ER) in each of the three streams, using diel fluctuations in dissolved oxygen (DO) and water temperature measured using MiniDOT data loggers equipped with optical DO sensors (Precision Measurement Engineering Inc., City, California USA). In 2012, we deployed MiniDOTs in the upstream and downstream reaches of each stream for 5-10 day periods before and during the salmon run. During the 2012 salmon run, bears damaged our data loggers at the downstream site in Red Salmon, and we were not able to collect metabolism information for that period. In 2013, we deployed MiniDOTs continuously in the upstream reaches of Red Salmon and Blue Bill streams. However, modeled GPP and ER were discontinuous at times due to interference by resident biota

(i.e., fishes taking shelter in sensor housings). We also developed a stage-discharge relationship using water depth data logged every 10 minutes using capacitance meters (Odyssey, Christchurch New Zealand) combined with periodic discharge measurements, which we estimated using a Marsh-McBirney Flo-Mate flow meter (Hach Company, Loveland, Colorado, USA) and the partial summation method. Due to disturbance by bears, in 2013 we used weekly measurements of mean reach depth, width, and discharge. We calculated GPP and ER using the two-station open-channel method and inverse modeling approach in R 2.15.2 using the chron package (James and Hornik 2014) to fit diel patterns in dissolved oxygen, temperature, and photosynthetically active irradiance (Roley et al. in press).

2.3.4 Statistical Analyses

We began by comparing isolated control lakes to salmon-bearing lakes using multi-way repeated measures analysis of variance (rmANOVA, α=0.05). We used rmANOVAs to identify whether water column nutrient concentrations, water column chl-*a*, and benthic chl-*a* differed significantly between isolated control lakes and salmon-bearing lakes. When we found significant differences between lake types, we performed a Tukey's HSD post hoc comparisons test (α=0.05) to identify whether the significant differences were driven by trends before and/or during the salmon run.

Next, we used a multi-way rmANOVA (α=0.05) to examine the effects of *salmon presence* (before vs. during salmon) and *position* in the lake-stream continuum (Salmon Lake, Upstream, Downstream). We used *watershed* as a blocking factor to account for variation between Blue Bill, Red Salmon, Frosty, and Sophie watersheds. We utilized the

multi-way rmANOVA to identify whether significant differences existed in time (*salmon presence*) and in space (*position*) for nutrient concentrations, water column chl-*a*, benthic chl-*a*, and whole-system metabolism. When we found significant differences in space, we performed a Tukey's HSD post hoc comparisons test (α=0.05) to identify whether the significant differences were driven by trends at Salmon Lakes, Upstream sites, or Downstream sites.

We assessed nutrient limitation status on NDS using a two-way analysis of variance (ANOVA) based on the presence or absence of N or P (Tank and Dodds 2003), assessing the impacts of N and P for each response metric (GPP, CR, and chl-*a*), on each substrate type (inorganic and organic) separately. Although we were testing for nutrient limitation, it is also possible for nutrient inhibition to occur, therefore, we also inspected the directional response of any significant N or P effects.

For all analyses, we transformed data when necessary to meet assumptions of normality and equal variance. All statistical analyses were performed using R 2.15.2. Repeated measures ANOVA and post-hoc analyses were performed using the nlme and multcomp packages in R (Pinheiro et al. 2012, Hothorn et al. 2008).

2.4 <u>Results</u>

2.4.1 Water Quality

Salmon-bearing lakes had significantly higher dissolved nutrient concentrations than isolated control lakes, regardless of whether salmon were present or absent. In 2012, isolated control lakes had significantly lower concentrations of NH_4^+ (Fig. 2.3), NO_3^-, and

SRP (Fig. 2.4) than salmon-bearing lakes (rmANOVA, $P<0.01$) both before and during the salmon run (Tukey's HSD, $P<0.01$ for both). In salmon-bearing lakes, NH_4^+ concentration increased significantly during the salmon run (rmANOVA, $P<0.01$, Followed by Tukey's HSD, $P<0.01$). In 2013, DIN and SRP concentrations were significantly lower at isolated control lakes than at salmon-bearing lakes (rmANOVA, $P<0.01$ for both) both before and during the salmon run (Tukey's HSD, $P<0.01$ for all).

In 2012, salmon presence significantly influenced NH_4^+ concentrations in salmon-bearing lakes and streams (i.e., Blue Bill, Red Salmon, Frosty; rmANOVA, $P<0.01$). During the salmon run, NH_4^+ concentration increased 1.4 to 4.0 fold (Fig. 2.3). In contrast, SRP concentrations generally decreased during the salmon run, though the trend was not statistically significant (Fig. 2.4). The position in the lake-stream continuum (i.e., upstream vs. downstream sites) did not significantly influence nutrient concentrations and there were no significant differences among salmon-bearing watersheds (e.g., Blue Bill, Red Salmon, Frosty, Sophie). In 2013, no differences were found in inorganic nutrient concentrations in response to the presence of salmon, or along the lake-stream continuum (Fig. 2.3 and 2.4). However, Red Salmon watershed had markedly lower SRP concentrations than at Blue Bill or Sophie.

We compared nutrient concentrations and molar N:P values from our salmon-bearing lakes and streams to values from a previous meta-analysis of salmon-bearing streams plus 6 salmon-bearing streams in Southeast Alaska (Janetski et al. 2009 and Levi et al. 2011, respectively). In previous studies, the average DIN concentration increased 2-fold when salmon were present, from a mean of 71 μg N L^{-1} before salmon, to 156 μg N

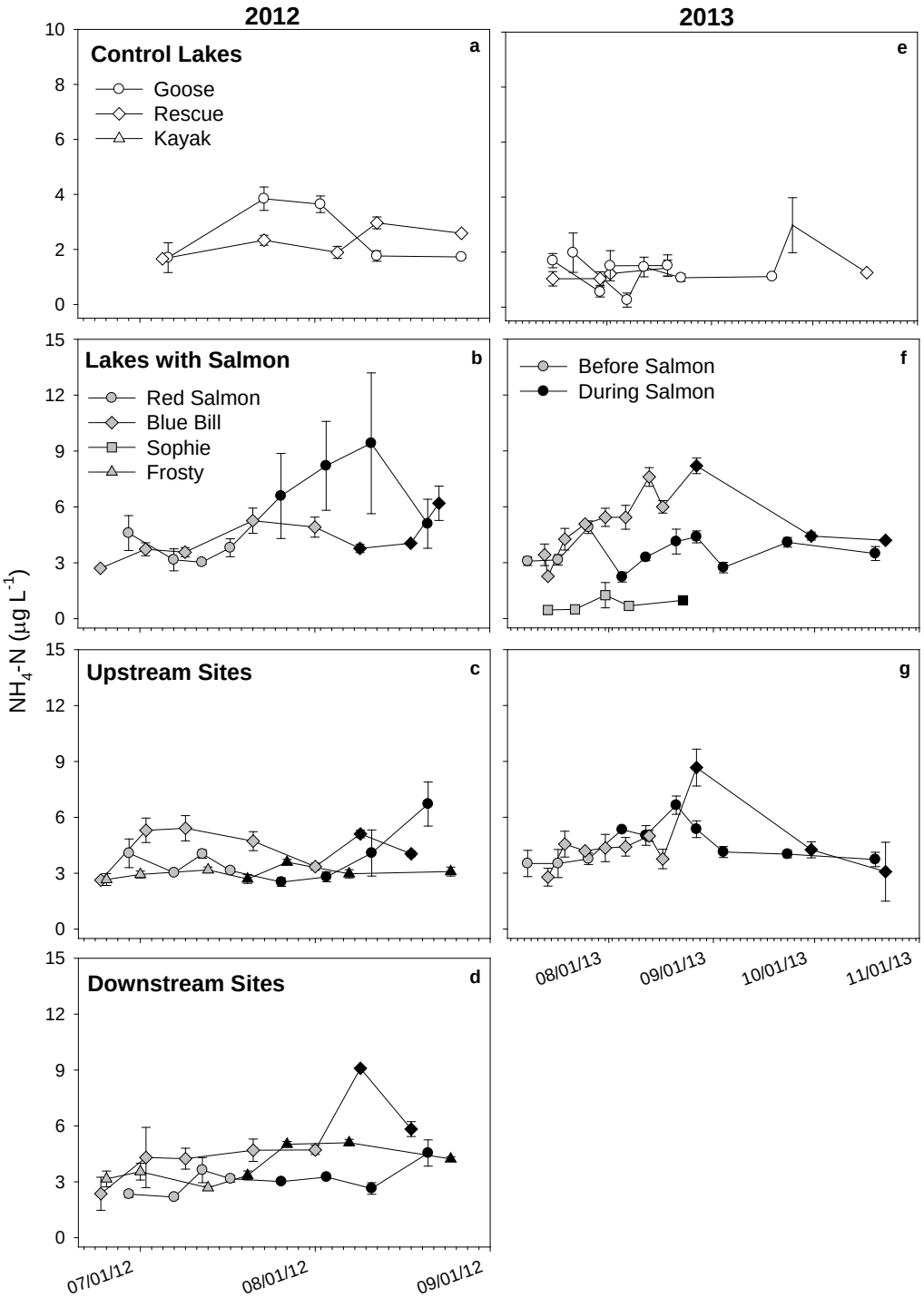

Figure 2.3 Water column ammonium concentrations in isolated control lakes, salmon-bearing lakes, upstream, and downstream sites during the 2012 (left) and 2013 (right) seasons. Error bars represent standard error among field replicates. Note that the scale is different for concentration of NH_4^+-N at control lakes (range, 0 to 10) compared to salmon-bearing lakes and streams (range, 0 to 15).

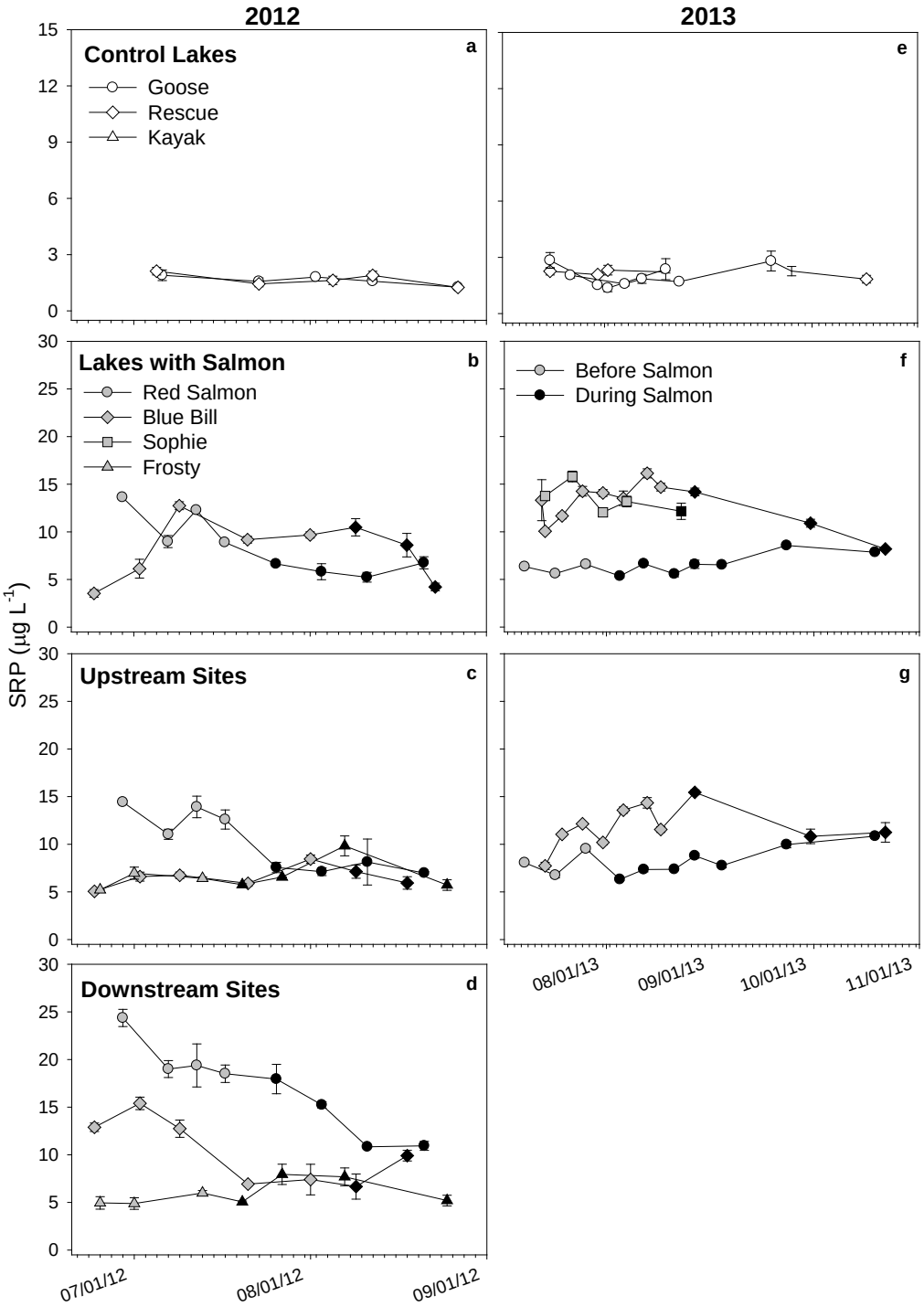

Figure 2.4 Water column SRP concentrations in isolated control lakes, salmon-bearing lakes, upstream, and downstream sites during the 2012 (left) and 2013 (right) seasons. Error bars represent standard error among field replicates. Note that the scale is different for concentration of SRP at control lakes (range, 0 to 15) compared to salmon-bearing lakes and streams (range, 0 to 30).

L^{-1} during the salmon run. Likewise, the mean SRP concentration increased 4-fold when salmon were present from 2 μg P L^{-1} to 8 μg P L^{-1} during the salmon run. By comparison, DIN at INWR increased by less than 10% during the salmon run and SRP concentrations decreased by about 12%. When we translated DIN and SRP concentrations to molar N:P ratios, we found that literature values for salmon-bearing streams almost always had N:P ratios at or above the Redfield ratio (16:1, Redfield 1958), whereas N:P ratios in salmon-bearing lakes and streams at INWR were much lower (average N:P = 1.9:1, Fig. 2.5), suggesting that at INWR lakes and streams are more likely to be limited by N availability, while elsewhere salmon-bearing ecosystems are more likely to be limited by P availability.

2.4.2 Ecosystem Responses

Water column chl-*a* concentrations increased significantly in response to salmon, with salmon-bearing lakes having higher concentrations of water column chl-*a* than control lakes. In 2012, salmon-bearing lakes had higher concentrations of chl-*a* (1.3 to 51 times higher, rmANOVA, $P<0.02$) than control lakes only during the salmon run (Tukey's HSD, $P<0.05$), while concentrations were higher both before and during salmon in 2013 (rmANOVA, $P<0.01$, Tukey's HSD, $P<0.02$ and $P=0.01$, respectively). Across all salmon-bearing watersheds there was temporal variability in the chl-*a* response to salmon. For example, in the Red Salmon watershed water column chl-*a* increased when salmon arrived, while at Blue Bill and Frosty, water column chl-*a* decreased slightly after the arrival of salmon (Fig. 2.6). In 2013, salmon presence was the only factor that was significantly related to water column chl-*a* concentration in salmon-bearing

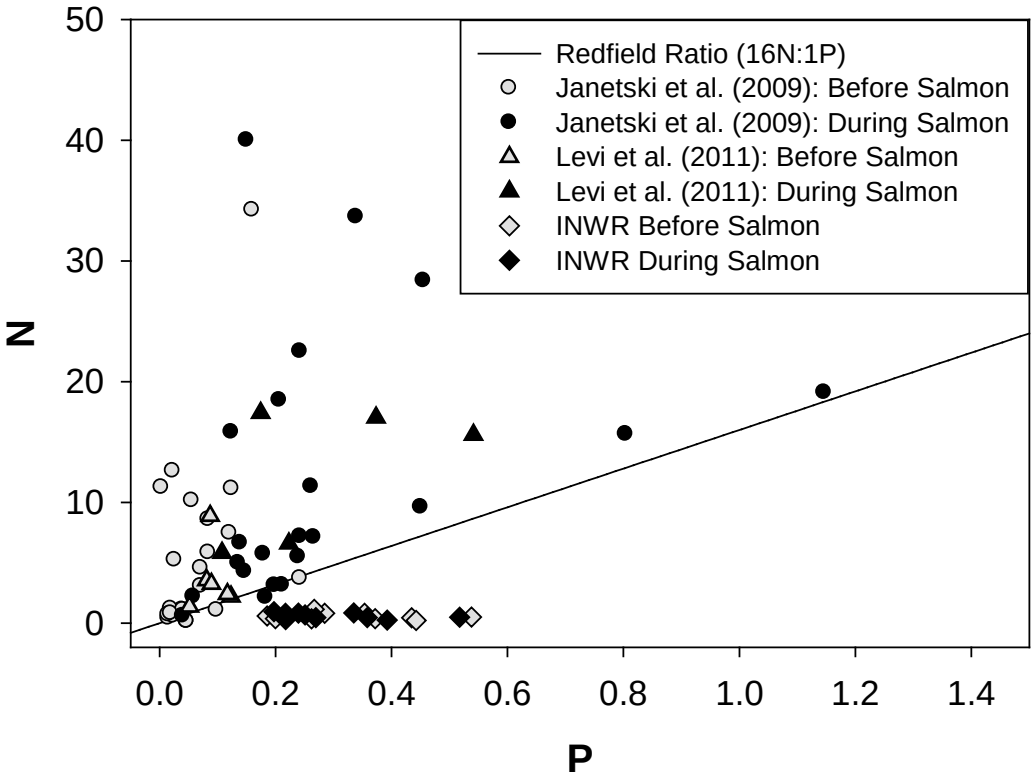

Figure 2.5 Molar N (as dissolved inorganic N) and P (as SRP) ratios before and during the salmon run at INWR salmon-bearing sites, shown with values reported in a meta-analysis by Janetski and colleagues (2009) and values reported from Southeast Alaska by Levi and colleagues (2011). The line represents the Redfield ratio of 16 N to 1 P.

watersheds (rmANOVA, $P<0.01$), and concentrations increased during the salmon run (Fig. 2.6). Notably, among all the watersheds receiving salmon, Blue Bill consistently had the highest concentrations of water column chl-*a* both before and during the salmon run in both 2012 and 2013.

In contrast to water column measurements, benthic chl-*a* did not differ between salmon-bearing lakes and control lakes, but salmon presence did influence benthic chl-*a* between lakes and streams in salmon-bearing watersheds. In 2012, salmon presence significantly influenced benthic chl-*a* in salmon-bearing watersheds (rmANOVA,

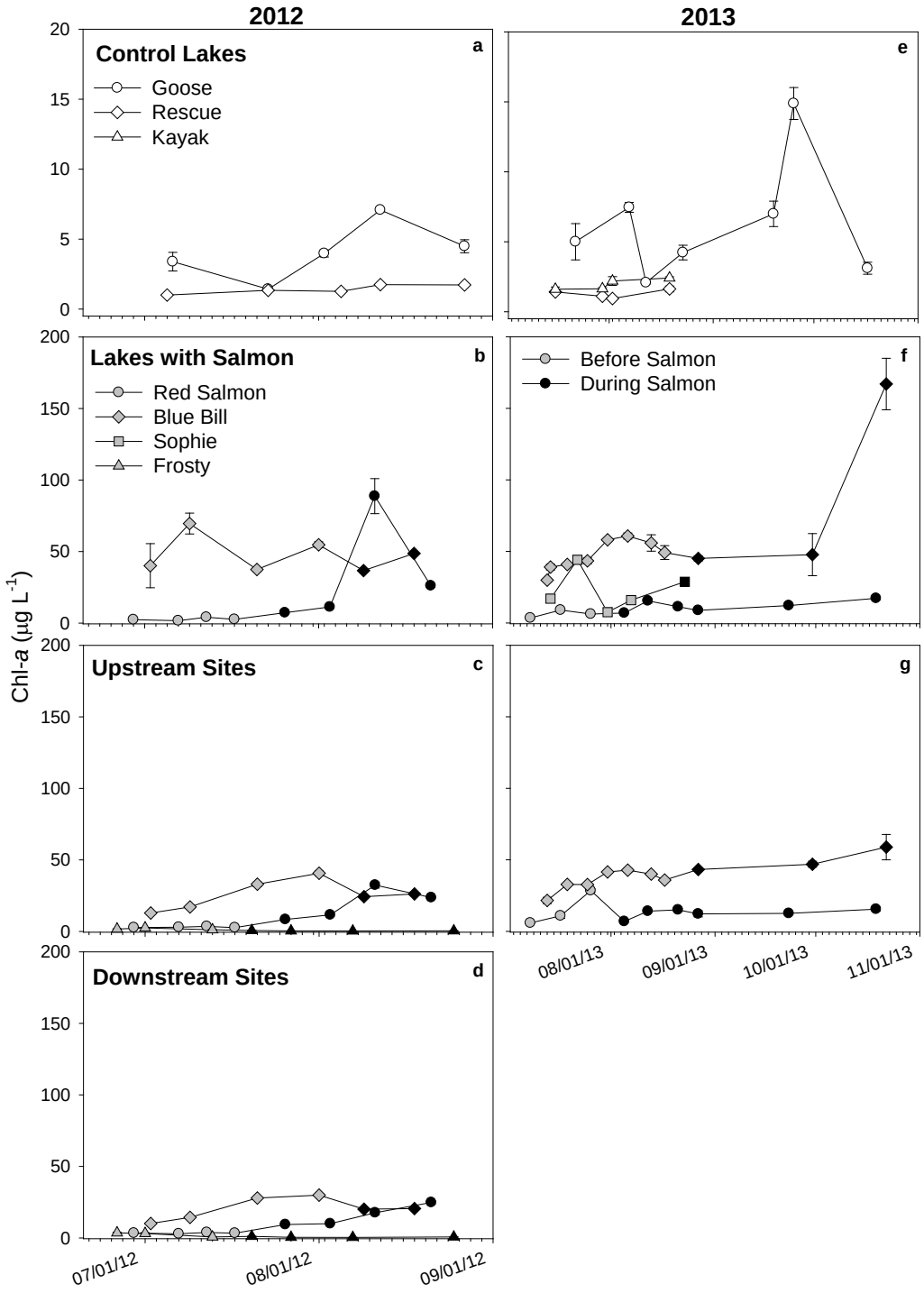

Figure 2.6 Water column chlorophyll-*a* concentrations in isolated control lakes, salmon-bearing lakes, upstream, and downstream sites during the 2012 (left) and 2013 (right) seasons. Error bars represent standard error among field replicates. Note that the scale is different for concentration of chl-*a* at control lakes (range, 0 to 20) compared to salmon-bearing lakes and streams (range, 0 to 200).

P=0.04), but the direction and magnitude of the effect depended on position in the lake-stream continuum (rmANOVA, *P*=0.04). For instance, benthic chl-*a* increased during the salmon run at salmon-bearing lakes, and decreased at upstream and downstream sites. In 2013, benthic chl-*a* was not influenced by salmon presence, watershed position, or watershed identity.

The nutrient limitation of benthic biofilms varied in space and time, but the presence of salmon did not alleviate limitation in these low-nutrient systems. Using chl-*a* as the response metric for fritted glass disks, in 2012 the biofilm autotrophs were N-limited at all salmon-bearing lake and stream sites prior to the salmon run (Fig. 2.7). During the salmon run, the percentage of N-limited sites decreased modestly from 100% before salmon to 83% during salmon, reflecting a change in limitation status at one site. In contrast, biofilm GPP on glass disks were generally not nutrient limited before the salmon run, and the proportion of nutrient limited sites did not change substantially during the salmon run. Heterotrophic biofilms on organic substrata were N-limited at the majority of sites both before (88%) and during (67%) the salmon run, but heterotrophic limitation did decrease during salmon (Fig. 2.7). In 2013, chl-*a* concentrations suggested that autotrophic biofilms were N-limited at the majority of sites before and during the salmon run on fritted glass substrata, while biofilm GPP was N-limited at less than half of sites regardless of salmon status (Fig. 2.7). Heterotrophic biofilms on organic substrata were N-limited at 100% of salmon-bearing lake and stream sites prior to the salmon run, and at 80% of sites during the salmon run.

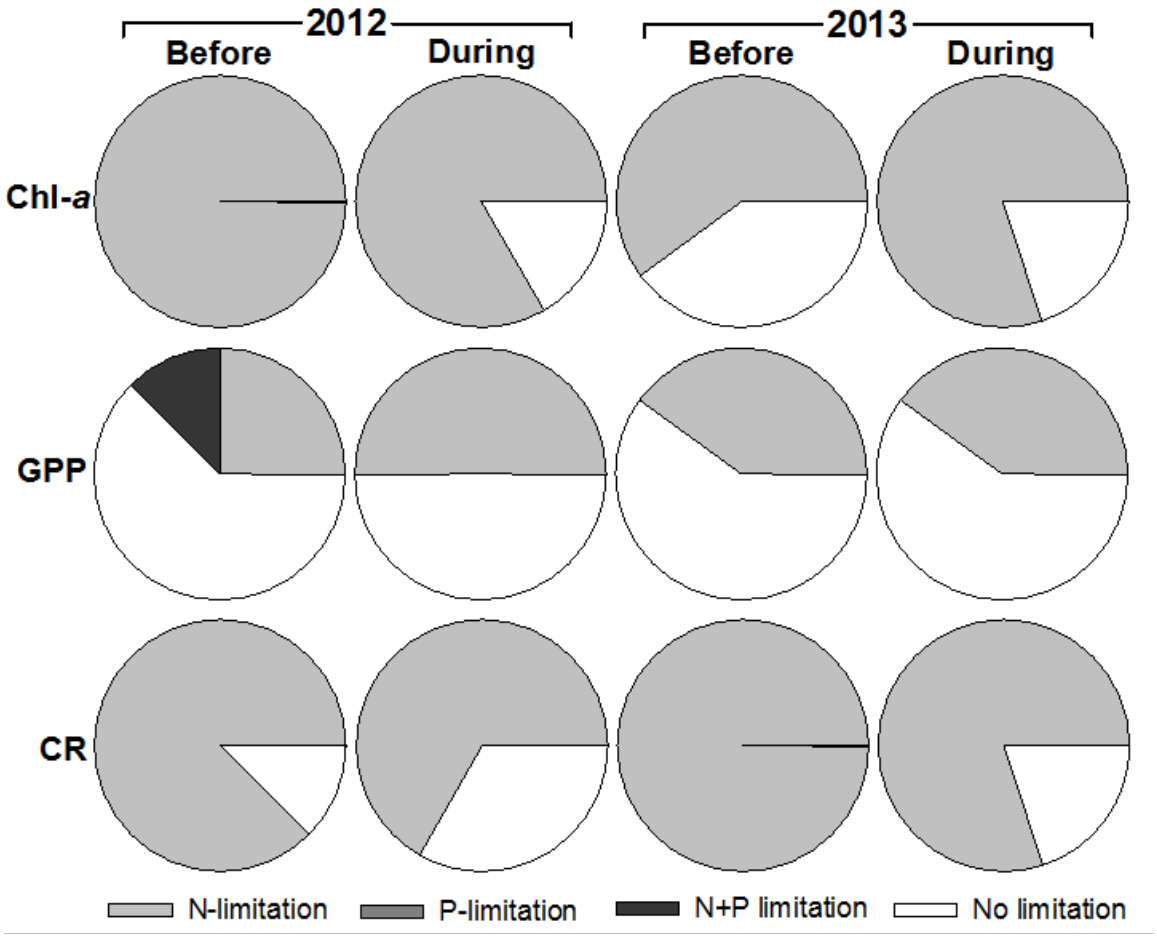

Figure 2.7 Benthic biofilms at salmon-bearing lakes and streams were often N-limited but never P-limited. Nutrient limitation status of chlorophyll-*a* (chl-*a*), gross primary production (GPP), and community respiration (CR) before and during the salmon run for the 2012 (left) and 2013 (right) seasons.

Using the whole-stream metabolism data measured before and during salmon, in 2012 and 2013, we found that salmon-bearing streams at INWR were typically heterotrophic at both upstream and downstream reaches, before and during the salmon run (Fig. 2.8). In 2012, both GPP (rmANOVA, P=0.04) and ER (rmANOVA, P< 0.01) changed when salmon were present, but the magnitude and direction of that change was watershed-specific. At Red Salmon, GPP increased during the salmon run, while at Blue Bill and the glacially-fed Frosty, GPP decreased during salmon. In 2012, there was a

significant interaction between the effect of salmon and watershed position for ER (rmANOVA, $P<0.01$), suggesting that location in the watershed mediated responses to salmon. More specifically, at upstream sites, ER decreased during the salmon run, while at downstream sites, ER increased during the salmon run (Fig. 2.8). In 2013, GPP decreased significantly at both Red Salmon and Blue Bill when salmon were present (rmANOVA, $P<0.01$), which is consistent with the overarching pattern of disturbance that we found in 2012 (Fig. 2.8). Unlike 2012, in 2013 ER did not change substantially during the salmon run, which could be the result of year-to-year variability of the population of salmon spawners (Varnavskaya et al. 1994).

2.5 Discussion

2.5.1 Lakes and Streams at INWR Had Low Nutrients and N:P Ratio Despite Salmon Runs

Lakes and streams at INWR had some of the lowest reported DIN concentrations for salmon-bearing ecosystems, while SRP was comparatively high. Furthermore, N:P ratios did not change substantially when salmon were present, suggesting that salmon-derived N was quickly incorporated by biota, and therefore not reflected in water chemistry. In 2012, we observed a concurrent increase in NH_4^+ concentrations and a decrease in SRP during the salmon run, which suggests that the salmon-related N enrichment could have increased biotic P uptake by the microbes and algae. It is well established that salmon spawners increase stream water N, and that the magnitude of

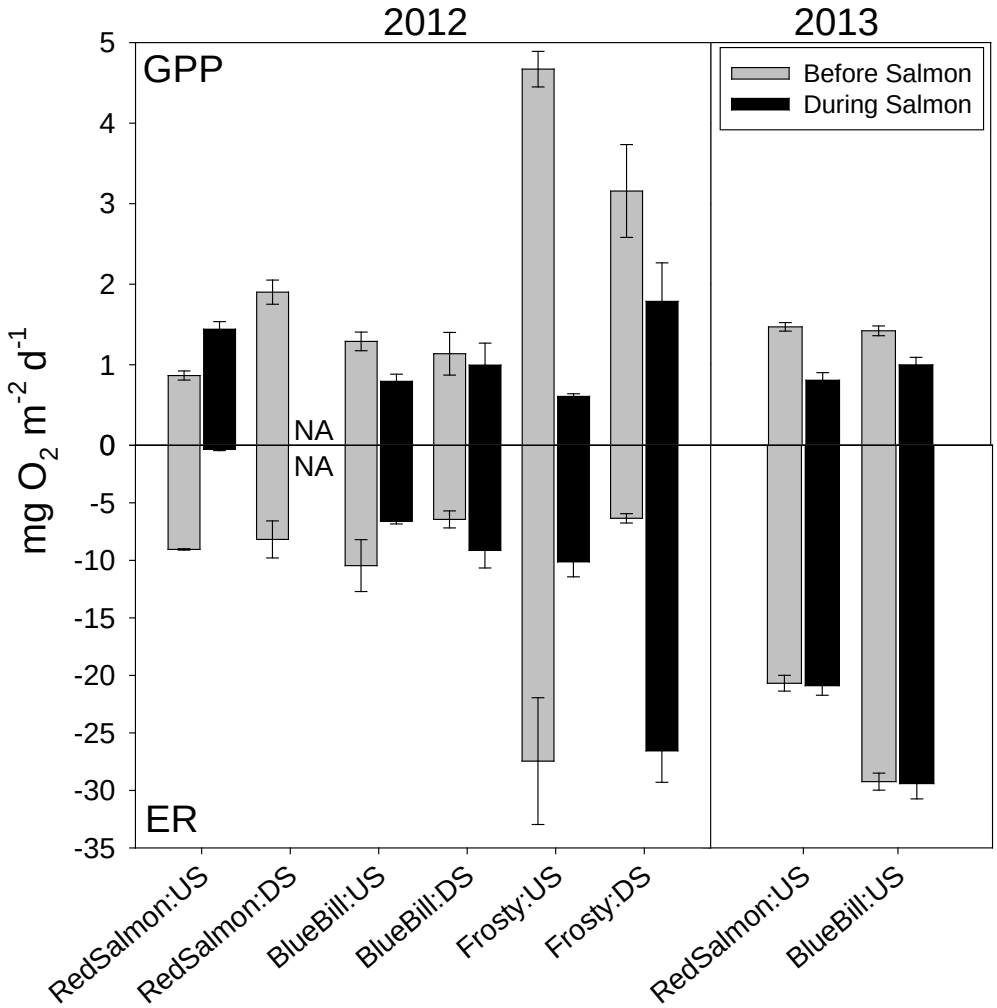

Figure 2.8 Whole-stream gross primary production (GPP) and ecosystem respiration (ER) at the upstream (US) and downstream (DS) reaches in the Red Salmon, Blue Bill, and Frosty watersheds for the 2012 (left) and 2013 (right) seasons. The scale for GPP ranges from 0 to 5, while the range for ER ranges from 0 to -35. No data were available (NA) for Red Salmon downstream during the 2012 salmon run. Error bars represent standard error among field replicates.

these increases corresponds with salmon spawner density (Johnston et al. 2004, Janetski et al. 2009, Levi et al. 2011). Although the increase in DIN concentration at INWR during the salmon run was measurable and statistically significant, it was small. The small increase in N may reflect low salmon spawner density, which is consistent with differences in regional salmon harvest across Alaska (Alaska Department of Fish and Game 2013), and/or biotic N demand may be so high in these low-nutrient systems that the observed increases appear minor. Even though N concentrations remained low before and during the salmon run, subsequent declines in P availability during the salmon run suggest that even small salmon runs can have important effects on nutrient availability.

At INWR, salmon-bearing lakes had higher concentrations of DIN and SRP than isolated control lakes both before and during the salmon run, which could suggest that salmon-derived nutrients (SDN) were retained in lakes after the salmon migration. In lakes and streams receiving large salmon runs, adult spawners contribute substantial N and P to aquatic ecosystems (Naiman et al. 2002). Sedimentary records (Finney et al. 1998, Finney et al. 2000) and isotopic food web studies (Kline et al. 1990, Bilby et al. 1996, Reisinger et al. 2013) have shown ecosystem retention and incorporation of salmon-derived N into aquatic food webs. For example SDN can be stored in epilithic biofilms (Shuldt and Hershey 1995, Holtgrieve and Schindler 2011), macroinvertebrates (Kline et al. 1990, Reisinger et al. 2013), and resident fishes (Bilby et al. 1996). However, when juvenile salmonids migrate to the marine environment they may remove as much or more nutrients than were provided by adult spawners, particularly when runs are small (Moore and Schindler 2004). Despite unusually small salmon runs in the years leading up to our study, our observations over 2 years in INWR lakes are consistent with

results in the Kuparuk River, Alaska, where biota slowly released N incorporated during a tracer study over a period of 2 years (Peterson et al. 1997). At INWR, elevated nutrient levels in lakes receiving salmon compared to isolated control lakes could suggest that SDN are retained or recycled over multiple years.

2.5.2 Salmon Subsidies Increased Water Column Productivity

Elevated chl-*a* concentrations in salmon-bearing lakes compared to isolated control lakes may also reflect a salmon enrichment effect. Across all salmon-bearing sites, concentrations of water column chl-*a* were higher during the salmon run than before salmon, which is consistent with the predicted effects of nutrient enrichment on a bottom-up trophic cascade (Carpenter et al. 2001). In addition, we cannot rule out that increased water column chl-*a* may be a result of salmon disturbance dislodging benthic biofilms during redd construction and other mating activities (Moore et al. 2004, Tiegs et al. 2009). If adult spawners had a disturbance effect on the benthos that increased water column chl-*a*, then we would expect to observe the reciprocal decrease in benthic chl-*a* concentrations; however, we saw no clear inverse relationship between water column and benthic chl-*a*.

Benthic biofilms generally reflect the net effect of both enrichment and disturbance from salmon spawners in stream ecosystems (Tiegs et al. 2009), but biofilms can also vary substantially over space and time (Rüegg et al. 2012). At INWR, we did not see a significant salmon effect on biofilms in 2013, but in 2012 benthic chl-*a* was likely influenced by salmon, although the direction of that effect differed between lakes and streams. Specifically, salmon had an enrichment effect in lakes, and a disturbance effect

in streams, driven in particular by trends at Frosty stream. Since sockeye salmon (*O. nerka*) typically spawn and die in lakes, including the lakes of INWR, they often have a net enrichment effect in lake ecosystems (Finney et al. 2000, Chen et al. 2011). Conversely, in streams (particularly at Frosty) SDN and carcasses were often flushed downstream, which is consistent with observations and results from other studies (Moore and Schindler 2004, Cak et al. 2008). These differences in water residence time likely influence the magnitude of salmon-mediated enrichment in lakes and streams.

2.5.3 Benthic Biofilms at INWR Lakes and Streams Were Consistently N-Limited

Salmon-derived nutrient enrichment did not alleviate N-limitation in autotrophic or heterotrophic biofilms. Using chl-*a* as an indicator of autotrophic biofilm enrichment, we found that nearly all of our sites were N-limited, and SDN enrichment did not alleviate the nutrient limitation, although we observed more nutrient limitation than for GPP. These results suggest that the biomass of primary producers, rather than their activity, may be a more sensitive response metric in these systems. Similar to the autotrophic response, nearly all heterotrophic biofilms were strongly N-limited regardless of salmon presence or absence. In contrast, in 7 Southeast Alaska streams, nutrient limitation by N and P was almost completely alleviated during the salmon run (Rüegg et al. 2011). Our results contrast with previous work in Southeast Alaska, suggesting that local and regional environmental characteristics including salmon run size, riparian vegetation, and regional differences in N and P availability may influence nutrient limitation and the response of these ecosystems to salmon spawners.

2.5.4 The Balance between Enrichment and Disturbance by Salmon Could Depend on Position in the Watershed

The streams at INWR were heterotrophic, with ER exceeding GPP by as much as 10-times before salmon and 20-times during the salmon run, although both GPP and ER responded differently to the presence of salmon. While GPP typically decreased in response to salmon, the response of ER was influenced by the longitudinal position of the reach in the lake-stream-estuary continuum. Furthermore, whole-stream metabolism in INWR streams was comparable to forested sites in Southeast Alaska where streams were generally heterotrophic, both before and during the salmon run (Levi et al. 2013). In contrast, salmon-bearing streams in the Wood River system of Southwest Alaska were autotrophic before salmon, but heterotrophic during and after the salmon run (Holtgrieve and Schindler 2011). Although studies of metabolism in salmon-bearing streams are limited, they all suggest that streams become more heterotrophic during the salmon run.

An in depth examination of each metric indicates that salmon at INWR generally had a disturbance effect on GPP, decreasing production by 13-87% compared to rates before salmon presence. Previous studies have found salmon presence can have either enrichment (Levi et al. 2013) or disturbance effects on GPP (Holtgrieve and Schindler 2011), but increases in ER in response to salmon presence have been observed across multiple ecoregions (Holtgrieve and Schindler 2011, Levi et al. 2013). Furthermore, our study builds on previous research by including the additional aspect of position in the watershed, where we considered two stream reaches that may be influenced by both salmon enrichment and disturbance. We found that the effect of salmon on ER varied between upstream and downstream reaches; upstream ER decreased when salmon were

present, whereas ER increased in downstream reaches during the salmon run. While the ER response was consistent among upstream and downstream reaches, GPP did not exhibit a consistent response to salmon presence in the upstream and downstream reaches. Salmon spawners dislodge small substrates, fine sediments, and associated organic matter, which can be suspended in the water column, and then exported to downstream locations or adjacent areas in the benthos (Moore et al. 2004, Tiegs et al. 2009, Holtgrieve et al. 2010). The sediments, organic matter, dislodged biofilms, and nutrients that are exported from lakes and upstream reaches can then influence productivity downstream (Vannote et al. 1980, Albers and Petticrew 2012). At INWR, the downstream export of nutrients and organic matter may have stimulated ER at downstream sites. Similarly, at upstream sites, the disturbance and export of fine sediments, organic matter, and periphyton may have led to the observed decline in ER and could also account for decreased GPP at Blue Bill and Frosty streams. Furthermore, it is likely that salmon dislodge periphyton and disturb sediments as they make their way upstream as well, which could account for declines in downstream GPP.

Our data suggests that salmon can have contrasting effects within a single stream ecosystem, and that the effect may depend on the longitudinal location of a stream reach in the lake-stream-estuary continuum. While most previous studies have focused on how salmon influence a single stream reach within the complex network of lakes and streams that comprise a watershed (but see Albers and Petticrew 2012), our study demonstrates how studying multiple stream reaches within a watershed can provide novel insights into how salmon influence overall ecosystem structure and function. Furthermore, our data suggests that using reach-scale metrics of ecosystem function may provide information

about salmon-mediated disturbance and enrichment that would otherwise be overlooked by traditional metrics of ecosystem structure.

2.6 Acknowledgements

This research was made possible by the field, technical, and logistical support provided by Izembek National Wildlife Refuge and the US Fish and Wildlife Service. In particular, we are grateful for field help provided by Jennifer Gregory, James Smith, Leticia Melendez, Spencer Berg, Stacey Lowe, and Jon Arkley. We also thank Joe Mueller and Audrey Kelley for their assistance processing samples. Funding for this research was provided by a US Fish and Wildlife Service cooperative agreement (F12AC00370).

2.7 Literature Cited

Alaska Department of Fish and Game. 2013. Alaska Commercial Salmon Harvests and Exvessel Values. Accessed 7 May 2014. http://www.adfg.alaska.gov/index.cfm?adfg=CommercialByFisherySalmon.exvesselquery

Albers, S.J., and E.L. Petticrew. 2012. Ecosystem response to a salmon disturbance regime: implications for downstream nutrient fluxes in aquatic systems. Limnology and Oceanography 57(1):113-123.

APHA. 1995. Standard methods for the examination of water and wastewater, 19th edition. American Public Health Association, Washington D.C.

Bailey, R.G. 1995. Description of the Ecoregions of the United States. 2nd edition. USDA Forest Service, Washington DC.

Bilby, R.E., B.R. Fransen, and P.A. Bisson. 1996. Incorporation of nitrogen and carbon from spawning coho salmon into the trophic system of small streams : evidence from stable isotopes. Canadian Journal of Fisheries and Aquatic Sciences 53:164–173.

Cak, A.D., D.T. Chaloner, and G.A. Lamberti. 2008. Effects of spawning salmon on dissolved nutrients and epilithon in coupled stream-estuary systems of Southeastern Alaska. Aquatic Sciences 70:169-178.

Carpenter, S.R., J.J. Cole, J.R. Hodgson, J.F. Kitchell, M.L. Pace, D. Bade, K.L. Cottingham, T.E. Essington, J.N. Houser, and D.E. Schindler. 2001. Trophic cascades, nutrients, and lake productivity: whole-lake experiments. Ecological Monographs 71(2):163-186.

Chen, G., D.T. Selbie, B.P. Finney, D.E. Schindler, L. Bunting, P.R. Leavitt, and I. Gregory-Eaves. 2011. Long-term zooplankton responses to nutrient and consumer subsidies arising from migratory sockeye salmon *Oncorhynchus nerka*. Oikos 120: 1317-1326.

Danielson, J.J., D.B. Gesch. 2011. Global multi-resolution terrain elevation data (GMTED2010). Open-file report 2011-1073.

Finney, B.P. 1998. Long-term variability of Alaskan sockeye salmon abundance determined by analysis of sediment cores. North Pacific Anadromous Fish Commission. Bull. No. 1:388-395.

Finney, B.P., I. Gregory-Eaves, J. Sweetman, M.S.V. Douglas, J.P. Smol. 2000. Impacts of climatic change and fishing on Pacific salmon abundance over the past 300 years. Science 290:795-799.

Gottesfeld, A.S., M.A. Hassan, J.F. Tunnicliffe, and R.W. Piorier. 2004. Sediment dispersion in salmon spawning streams: the influence of floods and salmon redd construction. Journal of the American Water Resources Association (JAWRA) 40(4):1071-1086.

Holtgrieve, G.W., and D.E. Schindler. 2011. Marine-derived nutrients, bioturbation, and ecosystem metabolism: reconsidering the role of salmon in streams. Ecology 92(2): 373-385.

Holtgrieve, G.W., D.E. Schindler, C.P. Gowell, C.P. Ruff, P.J. Lisi. 2010. Stream geomorphology regulates the effects on periphyton of ecosystem engineering and nutrient enrichment by Pacific salmon. Freshwater Biology 55:2598-2611.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous Inference in General Parametric Models. Biometrical Journal 50(3):346-363.

James, D., and K. Hornik. 2014. chron: chronological objects which can handle dates and times. R package version 2.3-45.

Janetski, D.J., D.T. Chaloner, S.D. Tiegs, and G.A. Lamberti. 2009. Pacific salmon effects on stream ecosystems: a quantitative synthesis. Oecologia 159:583-595.

Johnston, N.T., E.A. Macisaac, P.J. Tschaplinski, and K.J. Hall. 2004. Effects of the abundance of spawning sockeye salmon (*Oncorhynchus nerka*) on nutrients and algal biomass in forested streams. Canadian Journal of Fisheries and Aquatic Sciences 61:384–403.

Johnson, L.T., J.L. Tank, and W.K. Dodds. 2009. The influence of land use on stream biofilm nutrient limitation across eight North American ecoregions. Canadian Journal of Fisheries and Aquatic Sciences 66:1081-1094.

Kline, T.C., J.J. Goering, O.A. Mathisen, and P.H. Poe, and P.L. Parker. 1990. Recycling of elements transported upstream by runs of Pacific salmon: $\delta^{15}N$ and $\delta^{13}C$ evidence in Sashin creek, Southeastern Alaska. Canadian Journal of Fisheries and Aquatic Science. 47: 136-144.

Lehman, J.T. 1976. Ecological and nutritional studies on *Dinobryon* Ehrenb.: seasonal periodicity and the phosphate toxicity problem. Limnology and Oceanography 21(5):646-658.

Levi, P.S., J.L. Tank, S.D. Tiegs, J.Rüegg, D.T. Chaloner, and G.A. Lamberti. 2011. Does timber harvest influence the dynamics of marine-derived nutrients in Southeast Alaska streams? Canadian Journal of Fisheries and Aquatic Science 68:1326-1329.

Levi, P.S., J.L. Tank, J. Rüegg, D.J. Janetski, S.D. Tiegs, D.T. Chaloner, and G.A. Lamberti. 2013. Whole-stream metabolism responds to spawning Pacific salmon in their native and introduced ranges. Ecosystems 16: 269-283.

Mitchell, N.A., and G.A. Lamberti. 2005. Responses in dissolved nutrients and epilithon abundance to spawning salmon in southeast Alaska streams. Limnology and Oceanography 50(1):217-227.

Moore, J.W., and D.E. Schindler. 2004. Nutrient export from freshwater ecosystems by anadromous sockeye salmon (*Oncorhynchus nerka*). Canadian Journal of Fisheries and Aquatic Science 61:1582-1589.

Moore, J.W., and D.E.Schindler. 2008. Biotic disturbance and benthic community dynamics in salmon-bearing streams. Journal of Animal Ecology 77(2):275-284.

Moore, J.W., D.E. Schindler, and M.D. Scheuerell. 2004. Disturbance of freshwater habitats by anadromous salmon in Alaska. Oecologia 139(2):298-308.

Morris, M.R., and J.A. Stanford. 2011. Floodplain succession and soil nitrogen accumulation on a salmon river in southwestern Kamchatka. Ecological Monographs 81:43–61.

Murphy, J., and J.P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Cheimica Acta 27:31-36.

Naiman, R.J., R.E. Bilby, D.E. Schindler, and J.M. Helfield. 2002. Pacific salmon, nutrients, and the dynamics of freshwater and riparian ecosystems. Ecosystems 5(4):399-417.

Peterson, B.J., M. Bahr, and G.W. Kling. 1997. A tracer investigation of nitrogen cycling in a pristine tundra river. Canadian Journal of Fisheries and Aquatic Science. 54:2361-2367.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and the R development core team. 2012. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-105.

Redfield, A.C. 1958. The biological control of chemical factors in the environment. American Scientist 46(3):205-221.

Reisinger, A.J., D.T. Chaloner, J. Rüegg, S.D. Tiegs, and G.A. Lamberti. 2013. Effects of spawning Pacific salmon on the isotopic composition of biota differ among Southeast Alaska streams. Freshwater Biology 58:938-950.

Roley, S.S., J.L. Tank, N.A. Griffiths, R.O. Hall Jr., and R.T. Davis. The influence of floodplain restoration on whole-stream metabolism in an agricultural stream: insights from a 5-year continuous data set. Freshwater Science. *in press*.

Rüegg, J., S.D. Tiegs, D.T. Chaloner, P.S. Levi, J.L. Tank, and G.A. Lamberti. 2011. Salmon subsidies alleviate nutrient limitation of benthic biofilms in Southeast Alaska streams. Canadian Journal of Fisheries and Aquatic Science 68:277-287.

Rüegg, J., D.T. Chaloner, P.S. Levi, J.L. Tank, S.D. Tiegs, and G.A. Lamberti. 2012. Environmental variability and the ecological effects of spawning Pacific salmon on stream biofilm. Freshwater Biology 57:129-142.

Schuldt, J.A., and A.E. Hershey. 1995. Effect of salmon carcass decomposition on lake Superior tributary streams. Journal of North American Benthological Society 14(2):259-268.

Solorzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnology and Oceanography 14:799–801.

Steinman, A.D., G.A. Lamberti, P.R. Leavitt. 2006. Biomass and pigments of benthic algae. Pages 357-379 in F.R. Hauer and G.A. Lamberti (editors). Methods in stream ecology 2nd edition. Elsevier Inc., San Diego, CA.

Tank, J.L., and W.K. Dodds. 2003. Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. Freshwater Biology 48:1031-1049.

Tank, J.L., M.J. Bernot, and E.J. Rosi-Marshall. 2006. Nitrogen limitation and uptake. Pages 213-238 in F.R. Hauer and G.A. Lamberti (editors). Methods in stream ecology 2nd edition. Elsevier Inc., San Diego, CA.

Thompson, A.M. 2007. Amphipods are a strong interactor in the food web of a brown-water salmon river. University of Montana, Missoula, Montana.

Tiegs, S.D. 2008. Timber harvest transforms ecological roles of salmon in Southeast Alaska rain forest streams. Ecological Applications 18(1): 4-11.

Tiegs, S.D., E.M. Campbell, P.S. Levi, J. Rüegg, M.E. Benbow, D.T. Chaloner, R.W. Merritt, J.L. Tank, and G.A. Lamberti. 2009. Separating physical disturbance and nutrient enrichment caused by Pacific salmon in stream ecosystems. Freshwater Biology 54:1864-1875.

Vannote, R.L., G.W. Minshall, K.W. Cummins, J.R. Sedell, and C.E. Cushing. 1980. The river continuum concept. Canadian Journal of Fisheries and Aquatic Science 37:130-137.

Varnavskaya, N.V., C.C. Wood, R.J. Everett, R.L. Wilmot, V.S. Varnavsky, V.V. Midanaya, T.P. Quinn. 1994. Genetic differentiation of subpopulations of sockeye salmon (*Oncorhynchus nerka*) within lakes of Alaska, British Columbia, Kamchatka, Russia. Canadian Journal of Fisheries and Aquatic Sciences 51: 147-157

Wetzel, R.G., and G.E. Likens. 2000. Composition and biomass of phytoplankton. 3rd edition. Springer, New York, NY.

CHAPTER 3:

ENRICHMENT OF SUBARCTIC TUNDRA LAKES RECEIVING MULTIPLE RESOURCE SUBSIDIES[1]

3.1 Abstract

Freshwater ecosystems are strongly influenced by their surrounding landscape and often rely on allochthonous resource subsidies to provide nutrients and energy to support productivity. Nutrient subsidies from migratory animals such as anadromous fish and waterbirds can stimulate primary productivity, but the net effect may be mediated by local environmental characteristics such as light availability and riparian vegetation. Additionally, subsidies can be retained and recycled within aquatic ecosystems over long periods of time. We examined the influence of Pacific salmon (*Oncorhynchus* spp.) and migratory waterbirds at 9 subarctic tundra lakes in Izembek National Wildlife Refuge, Alaska. Specifically, we measured differences in water column nutrients, water column and benthic chlorophyll-*a*, and nutrient limitation status of benthic biofilms using nutrient diffusing substrata. Dissolved nutrient concentrations were at or near the analytical detection limits in all lakes, but concentrations were higher in salmon-bearing lakes than in waterbird lakes or isolated control lakes. Water column chlorophyll-*a* concentrations were positively correlated with inorganic nitrogen and phosphorous availability. When

[1] This chapter is in preparation for publication with co-authors J.L. Tank, U.H. Mahl, M.M. Dee, and C. Vizza.

nutrient limitation occurred, benthic biofilms were primarily nitrogen-limited. Our data suggest that in oligotrophic subarctic tundra lakes, resource subsidies from migratory species such as salmon and waterbirds may have important implications for ecosystem structure (i.e., state variables).

3.2 Introduction

Nutrient subsidies generally enhance primary and secondary productivity in freshwater ecosystems (Polis et al. 1997). Previous research has demonstrated the importance of salmon as a nutrient subsidy in freshwater ecosystems that receive salmon runs (e.g., Mitchell and Lamberti 2005), but across large portions of their range (i.e., the Pacific North Rim and the Arctic), the role of Pacific salmon (*Oncorhynchus* spp.) in nutrient enrichment has yet to be fully investigated. Additionally, across much of their range, Pacific salmon co-occur with migratory waterbirds including waterfowl, seabirds, and shorebirds. Waterbirds aggregate in estuaries, lakes, and ponds to overwinter, breed and rear young in the summer, or rest and accumulate fat stores before or after the annual migration in spring and fall (Sedinger 1997). Waterbirds are important vectors for nutrients to terrestrial ecosystems (Ellis 2005 and citations therein), but relatively few studies have considered how they influence nutrient availability and productivity in freshwater ecosystems(Kitchell et al. 1999, Olson et al. 2005, Keatley et al. 2009). Since waterbirds are an efficient vector for the transport of environmental toxins to freshwater ecosystems via excrement, feathers, and dead chicks (Blais et al. 2005, Evenset et al.

2007, Brimble et al. 2009), it seems likely that they can also provide a subsidy of allochthonous nutrients.

Previous studies of waterbirds as nutrient subsidies have largely focused on their differential effects (including disturbance) in terrestrial ecosystems, while a limited number of studies have investigated the role of waterbirds in freshwater ecosystems (Izaguirre et al. 1998, Wainright et al. 1998, Harding et al. 2004, Evenset et al. 2007, Brimble et al. 2009, Keatley et al. 2009). On land, excrement, feathers, and dead chicks can augment the supply of nutrients to soils near breeding colonies of birds (Cocks et al. 1999, Harding et al. 2004, Kameda et al. 2006, Rush et al. 2011, Zwolicki et al. 2013). However, changes in soil pH and nutrient concentrations (Wait et al. 2005, Kazama et al. 2013, Zwolicki et al. 2013), and increases in physical disturbance and nitrogen toxicity near waterbird colonies (Odasz 1994, Ellis 2005 and citations therein) can alter plant communities and decrease species richness (Wooton et al. 1991, Tomassen et al. 2005, Wait et al. 2005). Nevertheless, continued interest exists in the role of waterbirds as vectors for the transport of nutrients and contaminants (Blais et al. 2005, Evenset et al. 2007), particularly in the Arctic and Antarctic (Izaguirre et al. 1998, Mallory et al. 2006). For example, concerns about the transfer of environmental toxins to freshwater ecosystems have underscored the value of using stable isotopes to track the sources and fates of orthinogenic (i.e., bird-derived) inputs to lakes (Izaguirre et al. 1998, Mallory et al. 2006, Brimble et al. 2009, Keatley et al. 2009).

While many studies in natural ecosystems have found that lakes and streams receiving orthinogenic subsidies are enriched in available nitrogen and phosphorous that stimulates primary productivity (Izaguirre et al. 1998, Kitchell et al. 1999, Harding et al.

2004, Olson et al. 2005), other studies have found little or no enrichment effect (Pettigrew et al. 1998, Unckless and Makarewicz 2007). Some of the discrepancies could be attributed to the high settling rate of waterbird excrement paired with local and regional environmental variables that prevent or encourage lake turn-over including wind and seasonal differences in air temperature. Several studies have detected orthinogenic lake enrichment by monitoring the concentration of nutrients and the density of primary producers across a gradient of waterbird influenced systems (Izaguirre et al. 1998, Payne and Moore 2006, Brimble et al. 2009, Keatley et al. 2009), but the sources and fates of orthinogenic subsidies can also be tracked using stable isotope techniques (Harding et al. 2004). The accumulation of ^{15}N can be used to track the transfer of marine-derived nutrients to terrestrial and freshwater ecosystems by waterbirds (Payne and Moore 2006), in the same manner as it has been frequently used in salmon research (Bilby et al. 1996, Finney 1998, Hilderbrand et al. 1999, Helfield and Naiman 2001, Chaloner et al. 2002, Hocking and Reynolds 2011, Reisinger et al. 2013).

Coastal regions of Alaska and Canada provide habitat for large populations of migratory waterbirds and Pacific salmon spawners, but to our knowledge, no previous studies have attempted to understand the balance between salmon and waterfowl subsidies occurring in the same geographic region. Izembek National Wildlife Refuge (INWR), located on the Alaska Peninsula, provides critical spawning habitat for all five species of Pacific salmon and is home to millions of migratory waterbirds that feed, nest, and overwinter at the refuge. At INWR, we had the unique opportunity to study how salmon and waterbird subsidies interact to influence lake ecosystems. The objective of this research was to understand how salmon and waterbirds can variably impact

freshwater lakes at INWR. Specifically, we wanted to know whether (1) water column nutrients, algal biomass, and biofilm nutrient limitation status differ between lakes receiving subsidies compared to reference lakes that do not receive subsidies, (2) lakes differ if they receive predominantly a salmon subsidy alone versus only the waterbird subsidy, and (3) subsidies are detectable in lake food webs through isotopic analysis. To answer these questions we quantified water column nutrient concentrations and algal biomass, determined benthic nutrient limitation, and quantified the isotopic enrichment by marine-derived nutrients in lake food webs.

3.3 Materials and Methods

3.3.1 Site Description

We conducted our study at INWR, which is located at the western terminus of the Alaska Peninsula (Fig. 3.1) where the landscape is dominated by maritime tundra along the coasts and alpine tundra at higher elevations. The refuge provides crucial overwintering and breeding habitat for multiple species of protected waterfowl, seabirds, and shorebirds (hereafter called waterbirds), and also supports populations of all 5 species of Pacific salmon (*Oncorhynchus* spp.). Waterbirds aggregate in INWR lakes, where they both forage and rest; some lakes support denser aggregations of birds than others. Many lakes are hydrologically isolated from the marine environment, but some lakes have a stream outflow that connects to an estuary, thereby providing habitat for salmon spawners. We examined how the legacy of salmon and waterbird subsidy

retention influences nutrient availability and ecosystem function prior to the fall migration of either group of organisms.

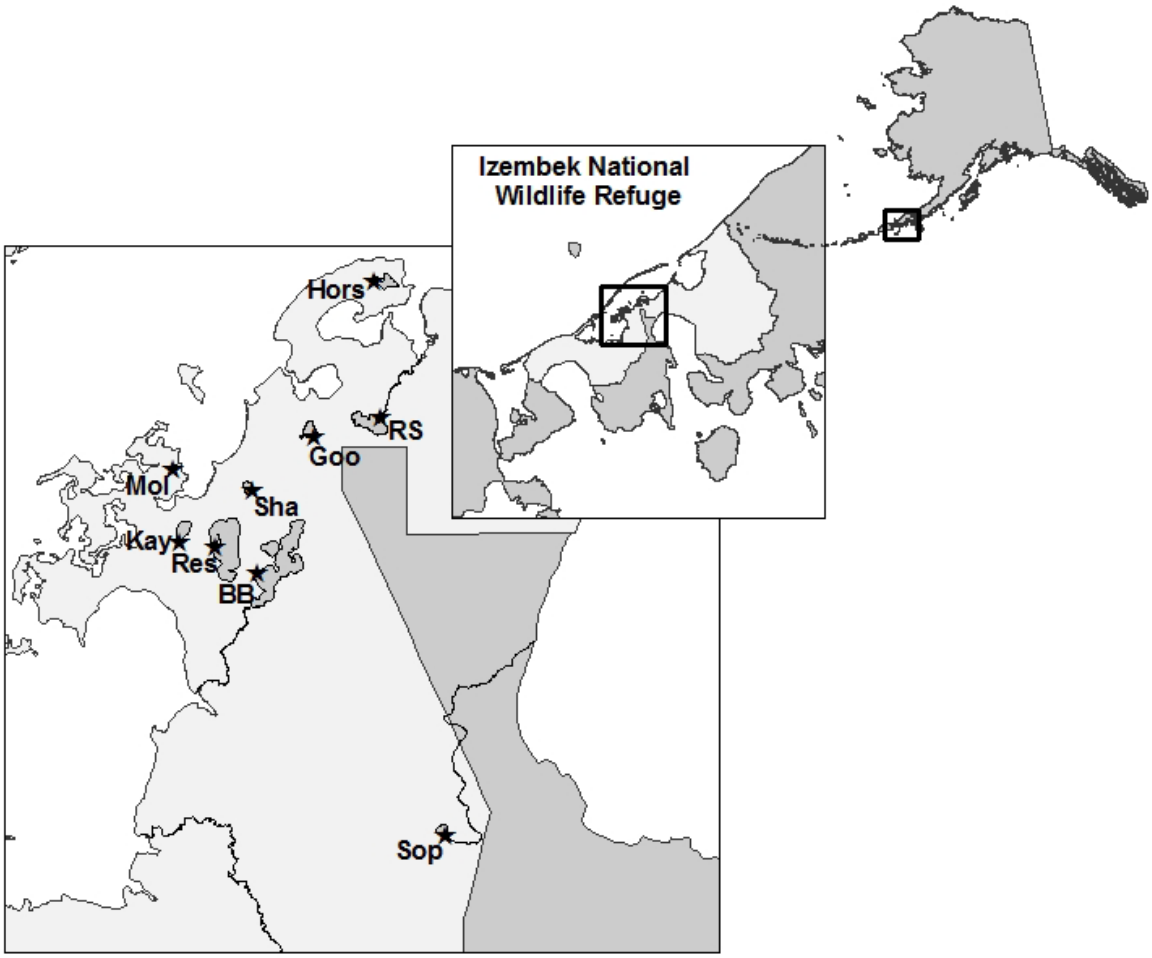

Figure 3.1 Location of lake study sites at Izembek National Wildlife Refuge located in Southwest Alaska (U.S.A.). Letters indicate the site name. Salmon bearing lakes include; Red Salmon (RS), Blue Bill (BB), and Sophie (Sop). Waterbird sites include; Horseshoe (Hors), Molly (Mol), and Shane (Sha) lakes. Finally, reference lakes include; Goose (Goo), Kayak (Kay), and Rescue (Res) lakes.

To study the effects of salmon and waterbird subsidies on lakes at INWR, we compared 3 waterbird and 3 salmon-bearing lakes to 3 reference lakes that received little or no subsidies (Fig. 3.1). We selected waterbird lakes based on recommendations from local hunters, who identified lakes that consistently receive dense aggregations of birds.

We selected salmon-bearing lakes based on fish counts from the Alaska Department of Fish and Game along with corroboration from local anglers. We are confident that waterbird lakes did not receive salmon spawners because they have no stream connections, but we acknowledge that salmon-bearing lakes could not be completely isolated from visits by waterbirds; some lakes were visited regularly by ducks and seagulls, but local hunters assured us that the density of birds was substantially lower than at lakes where they harvested Brant (i.e., waterbird lakes).

We measured differences in concentrations of water-column inorganic nutrients, and water column and benthic chlorophyll-*a* (chl-*a*) concentrations at each site between 2 and 7 times during July and August 2013, prior to salmon and waterbird migrations. We also assessed nutrient limitation status of benthic biofilms at all sites using nutrient diffusing substrata (NDS), and we conducted synoptic sampling of lake food webs by collecting sediments, macrophytes, and macroinvertebrates for isotopic analysis to assess whether marine enrichment was detectable at lakes receiving subsidies compared to reference lakes.

3.3.2 Water Quality

During each sampling visit, we collected 3 replicate 60 ml water samples from the littoral zone to quantify ammonium (NH_4^+-N), nitrate (NO_3^--N), and soluble reactive phosphorous (SRP) concentrations. In the field, we filtered water through a 0.7 μm glass fiber filter (GFF, Pall Corporation, Port Washington, NY, USA) for later chl-*a* analysis. We froze the filtrate and filters for later analysis in the laboratory at the University of Notre Dame. We measured water column nutrients on a Lachat Flow Injection

Autoanalyzer (Loveland, Colorado, USA) using the phenol-hypochlorite method for NH_4^+-N (Solorzano 1969), cadmium reduction method for NO_3^--N (APHA 1998), and the ascorbic acid method for SRP (Murphy and Riley 1962). We added NH_4^+-N and NO_3^--N concentrations together to express dissolved inorganic nitrogen (DIN).

3.3.3 Ecosystem Structure

We measured water column and benthic chl-*a* concentrations to quantify the net effect of salmon and waterbird nutrient enrichment on primary producers (i.e., algae). We sampled water column chl-*a* by filtering a known volume of water through a 0.7 µm GFF filter. We sampled benthic chl-*a* by collecting 3 rocks, or representative sediment samples when no rocks were available, to reflect the dominant substrate in the lake littoral zone. We stored substrate in the dark and transported it back to the laboratory where we removed benthic biofilms by scrubbing using a wire bristle brush and distilled water. We then filtered a known volume of the benthic slurry onto a 0.7 µm GFF, and froze the filters for later chl-*a* analysis, which we completed using the methanol extraction method and fluorometric analysis (Wetzel and Likens 2000). We also measured biofilm ash-free dry mass (AFDM) using the loss-upon-combustion method (Steinman et al. 2006).

3.3.4 Biofilm Nutrient Limitation

We deployed NDS to determine whether the availability of inorganic nitrogen (N) and/or phosphorous (P) limited productivity of benthic biofilms at lakes receiving subsidies compared to reference lakes. We constructed NDS by filling 30 ml

polyethylene cups with a 2% agar solution amended with 0.5M of NH_4Cl (N treatment), NaH_2PO_4 (P treatment), NH_4Cl and $NaH_2PO_4^-$ (N+P treatment), or no nutrients (Control; Tank et al. 2006). We covered agar with two different substrata to select for different components of biofilm growth. We used cups with fritted glass disks to select for autotrophic constituents (i.e., algae) and cellulose cloth to select for heterotrophic (i.e., fungi and bacteria) biofilms (Tank and Dodds 2003, Johnon et al. 2009). As each lake, we deployed five replicates for each nutrient treatment for each substratum, attaching NDS cups to plastic L-bars in groups of 8 randomly ordered cups, such that each bar acted as a block containing all treatments (n=40 cups per site). We secured L-bars to the bottom of each lake in the littoral zone. After 16-18 days, we retrieved the NDS and transported substrata to the laboratory in individual 50 ml centrifuge tubes that were filled with unfiltered stream water and kept in the dark. In the laboratory, we measured oxygen production and consumption, in light and dark centrifuge tubes and calculated gross primary production (GPP) by algae on fritted glass disks, and community respiration (CR) by fungi and bacteria on cellulose cloth. For GPP and CR incubations, we used a modified light-dark bottle method (Johnson et al. 2009). Before each incubation, we refilled individual centrifuge tubes containing NDS substrata with unfiltered lake water from each site, ensuring that there were no air bubbles in the tubes. We also included tubes with no NDS as a control for GPP and CR of lake water only. We conducted all incubations for a minimum of 3 hours at room temperature; light incubations were made using 4, 24 inch full-spectrum grow lights (Lights of America; model F17T8/GL, 1093 lumens) to maintain consistent light conditions across all samples (Johnson et al. 2009). After incubations for GPP and CR, we individually packed

colonized NDS fritted glass disks in aluminum foil and froze them for later chl-*a* analysis as described above, which we used as an indicator of algal biomass (Wetzel and Likens 2000).

3.3.5 Isotopic Analysis

At each lake, in the summer prior to salmon and waterbird arrival, we collected sediment, macrophyte, and invertebrate samples for subsequent stable isotope analysis for $\delta^{15}N$ and $\delta^{13}C$ signatures. Because taxonomic composition of macrophytes and invertebrates varied among lakes, we collected samples from as many taxa as possible, focusing our efforts on those taxa that were most common. We collected lake sediments from the top 5 cm of the lake littoral zone and froze sediments for later analysis. We collected riparian vegetation in the saturated zone within 0.5 meters of the lake edge. We collected emergent and submerged macrophytes opportunistically, bagged the plants in individual plastic baggies, and stored frozen until analysis. We collected invertebrates opportunistically using nets and benthic sampling, with subsequent hand-picking, and preserved invertebrate taxa in 60% isopropyl alcohol. In the laboratory, we dried plant and animal matter at 60 °C, followed by grinding to a fine powder, storing ground samples in individual pre-ashed scintillation vials. We analyzed samples for isotopic composition using a Carlo Erba Elemental Analyzer (CE Elantech, Inc., Lakewood, NJ, USA) coupled to a Finnigan Delta+ Mass Spectrometer (Thermo Scientific, Waltham, MA, USA) at the Center for Environmental Science and Technology (CEST) at the University of Notre Dame. We normalized sample values using an Acetanilide standard

($CH_3CONHC_6H_5$) for both C and N, and calculated stable isotope ratios of N ($\delta^{15}N$) and C ($\delta^{13}C$) using the following equation:

$$\delta^{15}N \text{ or } \delta^{13}C = [(R_{samples}/R_{standard}) - 1] \times 1000$$

where R is the ratio of ^{15}N:^{14}N or ^{13}C: ^{12}C for $\delta^{15}N$ and $\delta^{13}C$, respectively. We report isotopic composition, expressed as δ values, in parts per thousand (‰).

3.3.6 Statistical Analyses

We used repeated measures analysis of variance (rmANOVA; α=0.05) to determine whether concentrations of water column nutrients, water column chl-*a*, and benthic chl-*a* differed significantly between salmon-bearing, waterbird, and reference lakes. When we found significant differences between lakes receiving different subsidies, we performed a Tukey's HSD post hoc comparisons test (α=0.05) to identify whether the significant differences were driven by trends at salmon-bearing, waterbird, or reference lakes. To assess which environmental factors were correlated with chl-*a* concentrations, we performed a Pearson's correlation analysis using all samples from each lake. We compared water column and benthic chl-*a* to a variety of physiochemical lake characteristics including temperature, pH, specific conductance, light attenuation (measured as depth to 99% incident light extinction), mean substrate size, and inorganic N and P concentrations (Table 3.1).

We assessed nutrient limitation status from NDS incubations using a two-way analysis of variance (ANOVA) based on the presence or absence of N and P (Tank and Dodds 2003). Although we were testing for nutrient limitation, it is also possible for nutrient inhibition to occur, and we considered the directional response of any significant

N or P effects. We individually determined the effect of added N and P for each response metric (GPP, CR, and chl-*a*) separately on each substrate (fritted glass disks or cellulose cloth). We only report GPP and chl-*a* for inorganic substrata and CR on organic substrata, which select for autotrophic and heterotrophic biofilm constituents, respectively (Johnson et al. 2009).

We used one-way analysis of variance (ANOVA; α=0.05) to determine whether ^{15}N and ^{13}C isotopic signatures differed significantly among salmon-bearing, waterbird, and reference lakes. When we found significant differences between lakes receiving different subsidies, we performed a Tukey's HSD post hoc comparisons test (α=0.05) to identify whether the significant differences were driven by trends at salmon-bearing, waterbird, or reference lakes.

We transformed data to meet assumptions of normality and equal variance when necessary. All statistical analyses were performed using R 2.15.2. Repeated measures ANOVA and post hoc analyses were performed using the nlme and multcomp packages in R (Pinheiro et al. 2012, Hothorn et al. 2008). Pearson's correlation analyses were done using the Hmisc package in R (Harrell et al. 2014).

TABLE 3.1

PHYSICAL CHARACTERISTICS OF LAKES AT IZEMBEK NATIONAL WILDLIFE REFUGE

Subsidy	**Lake**	**NH4** *$\mu g*L^{-1}$*	**NO3** *$\mu g*L^{-1}$*	**DIN** *$\mu g*L^{-1}$*	**SRP** *$\mu g*L^{-1}$*	**WC Chl-*a*** *$\mu g*L^{-1}$*	**Benthic Chl-*a*** *$mg*m^{-2}$*	**Light Attenuation** *cm*	**Conductivity** *μS*	**pH**	**Temp** *°C*	**D_{50}** *mm*	**Lake SA** *km^2*
Salmon	RedSalmon	3.3	1.2	4.5	6.0	6.4	5.9	419.6	114.5	9.0	9.8	34.4	0.74
	BlueBill	5.0	1.5	6.5	13.3	49.7	5.0	133.5	92.5	8.9	12.1	16.3	2.70
	Sophie	0.7	2.5	3.2	13.7	21.2	14.4	173.2	88.3	8.6	13.1	6.3	0.16
Waterbird	Horseshoe	1.8	0.3	2.1	2.0	10.8	13.8	364.3	101.0	6.2	11.7	33.9	0.32
	Molly	2.6	0.6	3.2	2.4	4.2	16.8	231.6	124.5	7.6	15.3	0.03	0.02
	Shane	2.2	0.9	3.0	3.0	27.4	9.9	77.4	58.7	7.3	13.8	26.5	0.15
Reference	Goose	1.2	1.0	2.3	1.8	4.9	9.8	342.9	64.3	7.8	12.0	1.0	0.28
	Rescue	1.1	0.3	1.4	2.2	1.2	4.0	729.0	82.3	8.2	13.5	23.0	2.30
	Kayak	1.3	0.3	1.5	1.9	1.8	4.3	451.9	66.7	7.7	14.5	13.5	0.36

Note: Physical characteristics of salmon-bearing, waterbird, and reference lakes at Izembek National Wildlife Refuge, Alaska. Light attenuation was the depth at which 1% of incident light remained. D_{50} is the substrate size at the 50th percentile. Lake surface area (SA) was calculated using ArcGIS 10.1.

3.4 Results

3.4.1 Water Quality

We found that inorganic N and SRP concentrations were significantly higher at lakes receiving salmon compared to waterbird and reference lakes. In general, all lakes had very low inorganic nutrient concentrations (Fig. 3.2a and 3.2b); mean NH_4^+-N was 2.1 μg L^{-1}, mean NO_3^--N was 0.9 μg L^{-1}, and SRP was 5.1 μg L^{-1} (Table 3.1). Nevertheless, DIN significantly differed between salmon-bearing, waterbird, and reference lakes (Fig. 3.2a; rmANOVA $P<0.01$), where mean DIN concentrations at waterbird and salmon-bearing lakes were 2- and 3-times higher than reference lakes, respectively. Specifically, salmon-bearing lakes had significantly higher concentrations of DIN than at reference lakes (Tukey's HSD, $P<0.01$). In contrast, NH_4^+-N and NO_3^--N concentrations did not differ among lakes, perhaps due to their extremely low individual values. SRP concentrations were also low, but differed among lakes depending on the subsidy (rmANOVA, $P<0.01$). Specifically, SRP concentrations at salmon-bearing lakes (mean, 11.5 μg L^{-1}) were higher than at waterbird lakes (mean, 2.5 μg L^{-1}) and at reference lakes (mean, 2.0 μg L^{-1}; Fig. 3.2b; Tukey's HSD $P<0.01$ for both).

3.4.2 Ecosystem Structure

Similar to results from nutrient concentrations, water column and benthic chl-*a* were also generally higher at lakes receiving subsidies compared to reference lakes, although substantial variability existed among lakes with similar subsidies. Salmon-bearing lakes had higher chl-*a* concentrations than at waterbird and reference lakes, but the effect of subsidies was only moderately significant (Fig. 3.3, rmANOVA, $P=0.07$).

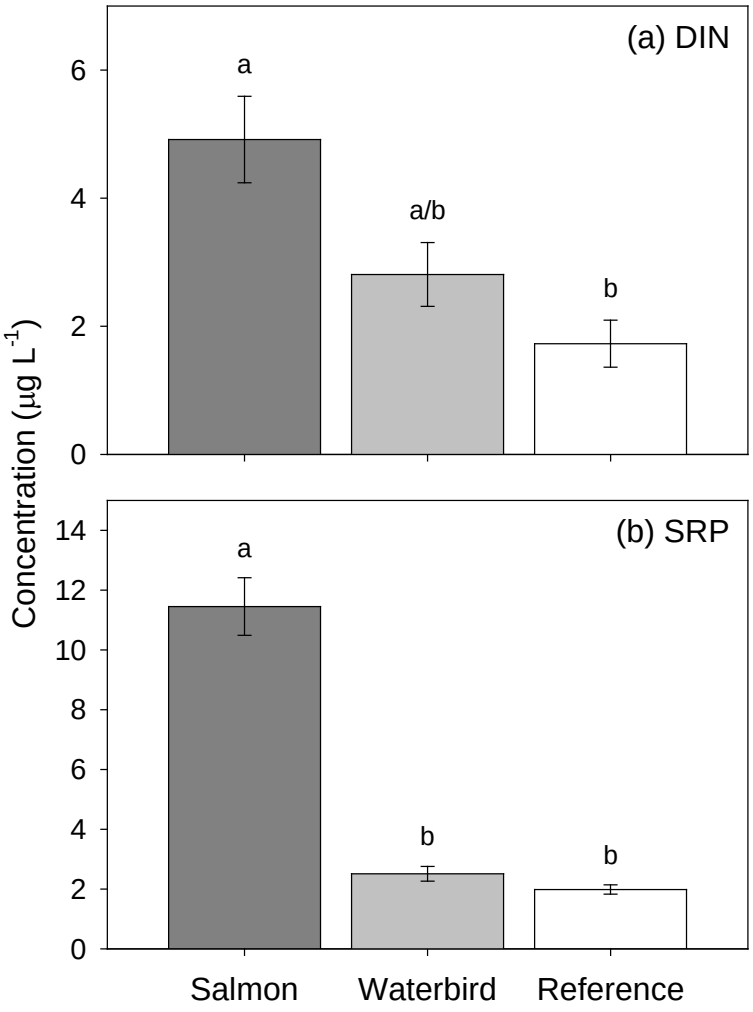

Figure 3.2 Mean water column dissolved inorganic nitrogen (DIN) concentrations in salmon-bearing lakes (dark grey), waterbird lakes (light grey), and reference lakes (white) prior to the fall salmon and waterfowl migrations. Error bars represent standard error among sites receiving similar subsidies. Letters indicate statistically different groups.

Specifically, water column chl-*a* concentrations in salmon-bearing and waterbird lakes were on average 11 and 6 times higher, with means of 29.2 μg L^{-1} and 16.0 μg L^{-1} respectively (Fig. 3.3), than at reference lakes that had a mean chl-*a* concentration of of 2.6 μg L^{-1}. Benthic chl-*a* values at lakes receiving subsidies did not significantly differ from reference lakes (rmANOVA, *P*=0.2), although mean concentrations tended to be higher in lakes receiving subsidies.

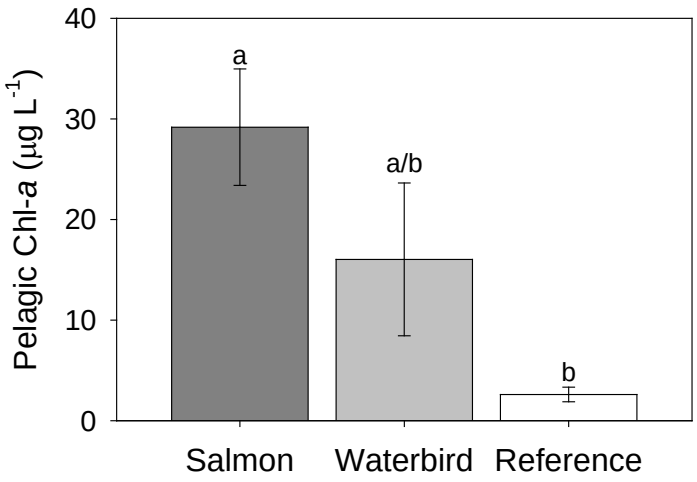

Figure 3.3 Mean water column chlorophyll-*a* concentrations in salmon-bearing lakes (dark grey), waterbird lakes (light grey), and reference lakes (white) prior to the fall salmon and waterfowl migrations. Error bars represent standard error among sites receiving similar subsidies. Letters indicate statistically different groups.

Water column chl-*a* concentrations were positively correlated with NH_4^+-N and NO_3^--N concentrations, separately (Pearson's correlation, *P*<0.01 and *P*=0.02; r=0.64 and r=0.42, respectively), as well as when they were combined as DIN (Fig 3.4a; Pearson's correlation, *P*<0.01; r=0.66). Water column chl-*a* concentrations were also positively

correlated with SRP concentrations (Fig. 3.4b; Pearson's correlation, $P<0.01$; r=0.72). In contrast, light attenuation was negatively correlated with water column c chl-*a* (Pearson's correlation, $P<0.01$; r= -0.65), suggesting that as chl-*a* concentration increased light did not reach as far into the water column. Unlike water column measurements, no meaningful correlations were found between benthic chl-*a* and other phsyiochemical descriptors.

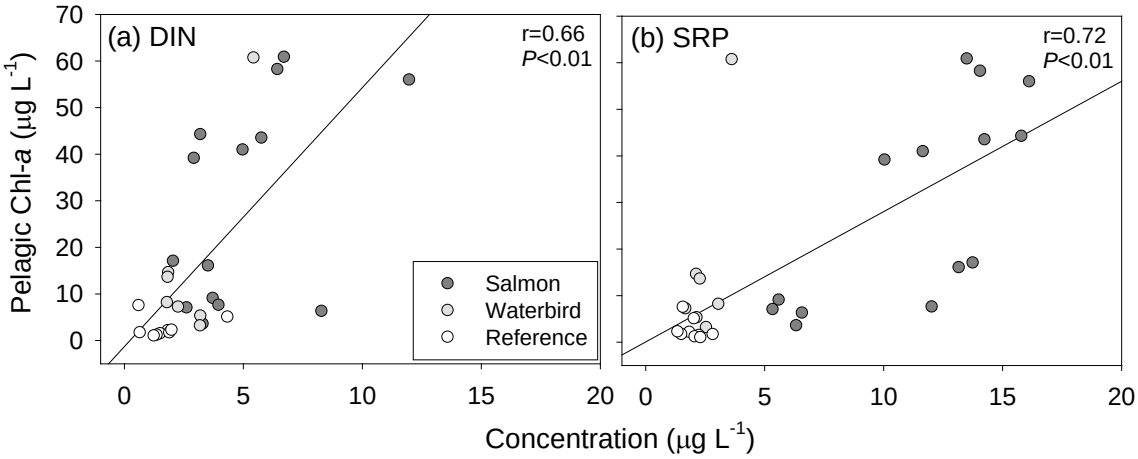

Figure 3.4 Water column chlorophyll-a concentrations plotted against dissolved inorganic nitrogen (DIN) and soluble reactive phosphorous (SRP) concentrations in salmon-bearing lakes (dark grey), waterbird lakes (light grey), and reference lakes (white) prior to the fall salmon and waterbird migrations. Solid line represents the line of best fit.

3.4.3 Biofilm Nutrient Limitation

Using NDS incubations, we assessed the nutrient limitation status of benthic biofilms in all three lake types. Autotrophic constituents (expressed via GPP or chl-*a*) were generally N-limited or not nutrient limited, while the heterotrophic biofilm constituents (expressed via CR) were more consistently N-limited. When we used chl-*a* on fritted glass disks as the response metric, algal biofilms at 1of 3 reference lakes and 1 of 3 salmon-bearing lakes were N-limited, while none of the waterbird lakes were nutrient-limited by either N or P (Fig. 3.5). For GPP, we found that algal biofilms on

fritted glass disks were N-limited in 2 of 3 reference lakes, 1 of 3 salmon-bearing lakes, and again there was no nutrient limitation at the waterbird lakes. In contrast to autotrophs, heterotrophic biofilms on cellulose were N-limited at all salmon-bearing and reference lakes, and not limited by either N or P at waterbird lakes (Fig. 3.5). We were surprised that despite very low SRP concentrations at all lakes (generally <6.0 ug L^{-1}, Fig. 3.2b), we never observed P-limitation on any substrate type at any lake. To summarize, at reference and salmon-bearing lakes, both autotrophic and heterotrophic biofilms showed some N-limitation but never P limitation, while waterbird lakes were never nutrient limited.

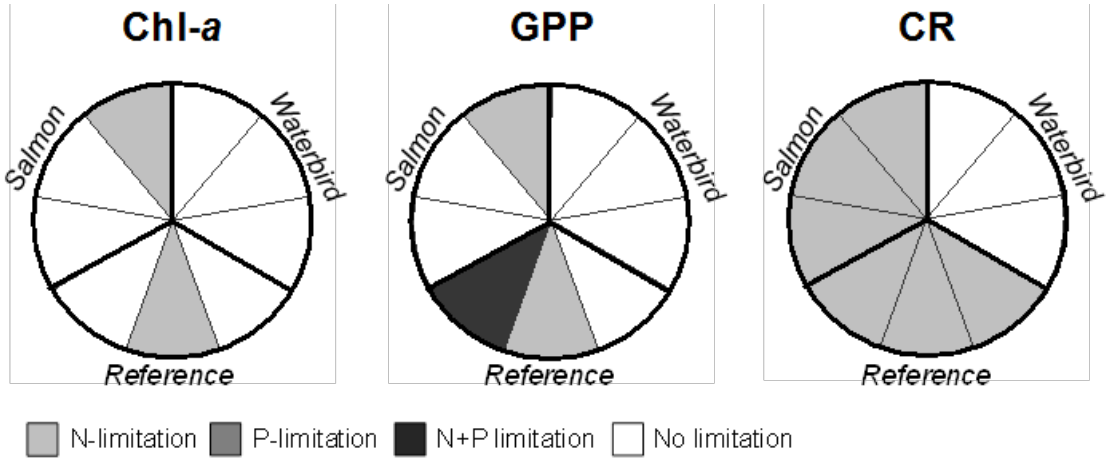

Figure 3.5 Benthic biofilm nutrient limitation status of chlorophyll-a (chl-*a*), gross primary production (GPP), and community respiration (CR) for each lake, where lakes are grouped by the type of subsidy that they received (i.e., Waterbird, Salmon, or Reference). Each slice of the pie chart represents the nutrient limitation status of a single lake.

3.4.4 Isotopic Analysis

We used a synoptic sampling approach at each lake to develop a representative food web using isotopic signatures; in general, we found that the $\delta^{13}C$ signatures were very similar between the 3 lake types, but the $\delta^{15}N$ signature was generally higher for similar food web constituents at waterbird lakes, suggesting that the contribution of

marine-derived N was detectable at higher trophic levels. At the bottom of the food webs, $\delta^{15}N$ values for benthic sediments differed significantly between salmon-bearing, waterbird, and reference lakes (Fig. 3.6, rmANOVA, $P<0.01$). Specifically, there were higher $\delta^{15}N$ signatures at waterbird lakes (mean $\delta^{15}N$ = 3.4‰) than at salmon-bearing and reference lakes (mean $\delta^{15}N$ = 0.7‰ for both). Likewise, benthic macroinvertebrates were significantly more enriched in ^{15}N at waterbird lakes (Fig. 3.6, mean $\delta^{15}N$ = 4.9‰, rmANOVA, $P<0.01$; Tukey's HSD, $p<0.01$) and reference lakes (mean $\delta^{15}N$ = 3.8‰; Tukey's HSD, $P=0.02$) compared to salmon-bearing lakes (mean $\delta^{15}N$= 1.1‰). In contrast to the $\delta^{15}N$ signatures of lake sediments and aquatic invertebrate taxa, the $\delta^{15}N$ signature of riparian (i.e., lakeside) vegetation and emergent sedges did not significantly differ among lake types.

In contrast to ^{15}N, as expected, carbon stable isotopes varied less across lake types in relation to subsidies. Nevertheless, across lakes differences in $\delta^{13}C$ values were found for food web compartments. For example, the $\delta^{13}C$ of benthic sediment was higher at reference lakes (mean $\delta^{13}C$ = -15.1‰) than at lakes receiving subsidies (Fig. 3.6, rmANOVA, $P=0.04$), and the difference was driven by $\delta^{13}C$ values at salmon-bearing lakes (Tukey's HSD, $P=0.03$; mean $\delta^{13}C$= -19.4‰). Unfortunately, we could not accurately compare $\delta^{13}C$ values for invertebrates, because they were influenced by our preservation method (Fanelli et al. 2010, Syvaranta et al. 2011); $\delta^{13}C$ data for higher trophic levels are plotted in Figure 6 for illustrative purposes only.

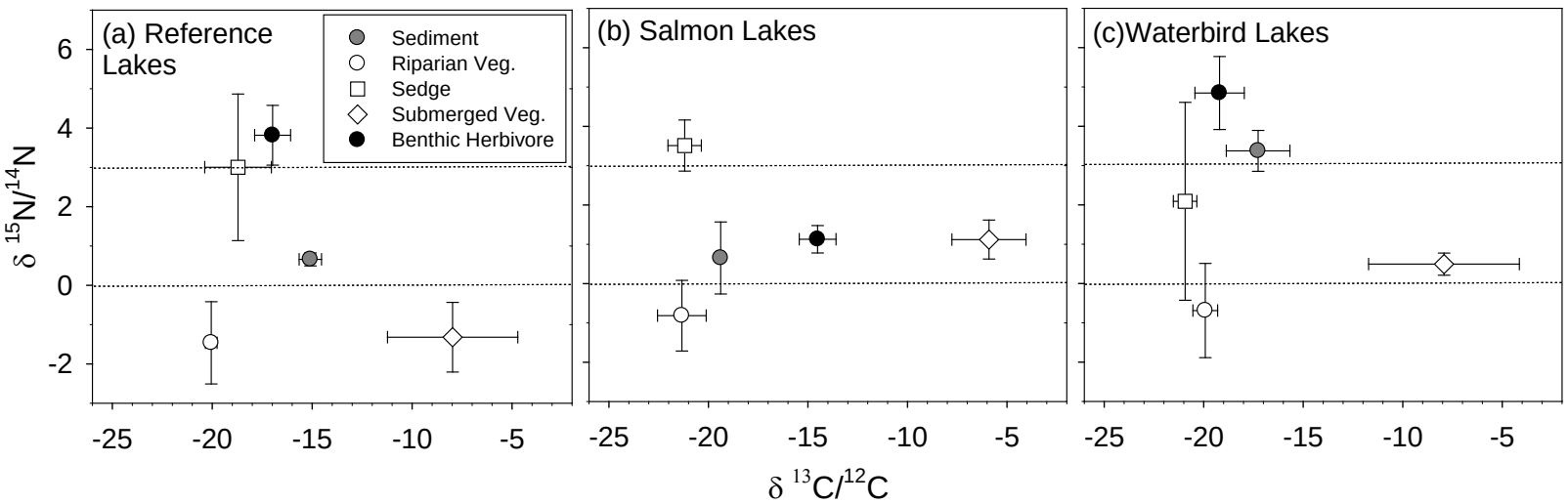

Figure 3.6 Isotopic ratios of $\delta^{13}C$ and $\delta^{15}N$ of lake sediments (grey), macrophytes (white), and macroinvertebrates (black). Plotted points represent mean values ± standard error. The dotted lines are for reference purposes between the 3 panels. Riparian vegetation mainly included *Rosaceae, Sanguisorba stipulata* and *Rosaceae, Potententilla anserina*. Submerged vegetation mainly included *Ranunculus longirostris* and *Ranunculus richardsonii*, but also included *Potamogeton richardsonii*, *Myriophyllum farwellii*, and *Nitella* spp. Benthic invertebrate consumers included snails, amphipods, isopods, and aquatic insects (*Trichoptera, Limnephilidae*; *Diptera, Chironomidae*; *Diptera, Tipulidae*).

3.5 Discussion

3.5.1 Lakes Receiving Salmon Subsidies Had Higher Nutrient Concentrations than Reference Lakes

Salmon-bearing lakes had higher concentrations of DIN and SRP than at reference lakes, suggesting that they may have retained nutrient subsidies over the course of a year and possibly over multiple years. Lakes receiving salmon subsidies had 3- and 6-times higher concentrations of DIN and SRP, respectively, compared to reference lakes, which is consistent with estimates from previous studies that suggest that salmon contribute 25-70% of the annual N budget to salmon-bearing lakes (Naiman et al. 2002), while lakes without salmon have 33% less P than lakes receiving salmon (Kyle et al. 1994). However, mean nutrient concentrations in salmon-bearing lakes at INWR differed from concentrations at other salmon-bearing lakes; on average salmon-bearing lakes at INWR had 25-times less DIN and 6-times more SRP than other salmon-bearing lakes in Alaska (Kyle et al. 1994, Kyle et al. 1997). Likewise, the molar N:P ratio in salmon-bearing lakes was lower at INWR than in other lakes across Alaska. The high availability of P compared to N in INWR lakes could be attributed, in part, to the additional nutrient enrichment provided by waterbird excrement, which is rich in P (Hutchinson 1950, Ganning and Wulff 1969).

Although our data did not show that waterbirds significantly increase nutrient availability in INWR lakes, previous studies have shown that lakes receiving waterbird subsidies have higher concentrations of N and P compared to lakes that are not influenced by birds (Kitchell et al. 1999, Olson et al. 2005). Furthermore, waterbird subsidies appear to have a much larger influence at high latitudes; in the Antarctic, waterbird lakes had 4-

times more DIN and 8-times more P than reference lakes, while waterbird lakes in the Arctic had 40-times more DIN and 87-times more P (Izaguirre et al. 1998, Keatley et al. 2009). By comparison, waterbird lakes at INWR only had 1.6-times more DIN and 1.3-times more P than reference lakes. Previous research has shown that nutrient enrichment is positively correlated with seabird roosting density (Payne and Moore 2006, Keatley et al. 2009) and nutrient availability can be influenced by wind-driven re-suspension of sediments and excrement (Unckless and Makarewicz 2007, Qian et al. 2011), suggesting that the magnitude of the waterbird subsidy likely reflects local and regional environmental characteristics including the availability of suitable habitat for birds, weather patterns, and local topography. Furthermore, taxa-specific differences in life history and behavior are also likely to have an impact on the magnitude of the subsidy with regard to nutrient enrichment (Kitchell et al. 1999).

3.5.2 Phytoplankton Biomass was Positively Correlated with the Availability of Inorganic Nutrients

In general, lakes receiving subsidies at INWR had higher concentrations of water column chl-*a* when compared to reference lakes, but only salmon-bearing lakes had significantly elevated concentrations of phytoplankton biomass. Nevertheless, even reference lakes at INWR were among the most productive lakes in Alaska and had relatively high chl-*a* concentrations, with an average of 3-times more water column chl-*a* than salmon-bearing lakes across Alaska (Kyle et al. 1994, Hume et al. 1996, Kyle et al. 1997). Furthermore, salmon-bearing lakes at INWR had 30-times higher water column chl-*a* concentrations than salmon-bearing lakes elsewhere in Alaska (Kyle et al. 1994,

Hume et al. 1996, Kyle et al. 1997). High levels of phytoplankton biomass in INWR lakes receiving subsidies may reflect a combination of nutrient enrichment by salmon or waterbirds in addition to the contribution of local environmental factors. For example, shallow lakes like the ones in our study, can have high rates of wind-driven re-suspension of benthic sediments and associated nutrients, which can stimulate water column production (Goldman 1960, Douglas and Rippey 2000, Qian et al. 2011).

Although there was substantial variability among waterbird lakes, the mean difference in water column chl-*a* concentrations between waterbird and reference lakes was similar to values documented in the Canadian Arctic. Arctic lakes influenced by waterbird subsidies had 6-times more phytoplankton biomass compared to reference lakes (Keatley et al. 2009); likewise, waterbird lakes at INWR had 6-times more phytoplankton biomass compared to reference lakes; however, the difference was not statistically significant. In contrast, Antarctic lakes with waterbird subsidies had 79-times more phytoplankton biomass relative to reference lakes (Izaguirre et al. 1998), suggesting that influence of waterbird subsidies on phytoplankton biomass may be affected by latitudinal gradient, among other factors.

Algal biomass at INWR lakes reflected the availability of DIN and SRP (Fig. 3.4), which is consistent with previous research that has shown that water column chl-*a* concentrations are strongly influenced by nutrient availability. Other studies have found similar relationships; lakes with higher concentrations of available nutrients had higher water column chl-*a* (Izaguirre et al. 1998, Schmidt et al. 1998, Lim et al. 2005, Keatley et al. 2009). While chl-*a* concentrations mirrored N availability at INWR, phytoplankton productivity elsewhere in Alaska and the Pacific Northwest is generally thought to be

limited by P availability (but see Keatley et al. 2009), which reflects the relatively high molar N:P ratios in lakes across much of the region. At INWR, the positive relationship between water column chl-*a* concentration and both DIN and SRP suggests that N or P availability could limit phytoplankton productivity.

3.5.3 Biofilm Nutrient Limitation Reflected the Relative Availability of N and P in Lakes

Although benthic nutrient limitation is not typically measured in lakes, benthic biofilms can substantially influence whole-lake productivity (Vadeboncoeur et al. 2003) and NDS can accurately reflect nutrient limitation of periphyton in the euphotic zone (Luttenton and Lowe 2006). Researchers have also used substrata suspended from buoys to measure differences in biofilm biomass across a natural gradient of enrichment in a single lake (Payne and Moore 2006). At INWR, autotrophic biofilms at reference and salmon-bearing lakes were often N-limited, whereas heterotrophic biofilms were always N-limited (Fig. 3.5). Previous studies in salmon-bearing lakes in Alaska and Canada have shown that water column productivity was primarily limited by P availability (Stockner and Shortreed 1976, Kyle et al. 1994, Stockner and MacIsaac 1996), which was reflected in molar N:P ratios up to 570 N:1 P in one Alaskan lake (Kyle et al. 1997). In contrast, we never found P-limitation of biofilms at INWR, where our study lakes had molar N:P ratios ranging from 1 N:2 P to 3 N:1 P. Biofilm nutrient limitation is influenced by local differences in N and P availability (Luttenton and Lowe 2006) and, as mentioned previously, INWR lakes have very low inorganic N relative to P-availability compared to other salmon-bearing lakes in Alaska. Therefore, it is not surprising that we found N-

limitation while many other studies have observed P-limitation where there are healthy salmon runs.

While benthic biofilms in reference and salmon-bearing lakes were often N-limited, at waterbird lakes we found that neither autotrophic nor heterotrophic biofilms were limited by the availability of N or P. This general lack of nutrient-limitation was somewhat surprising given the low nutrient concentrations across all of our study lakes, perhaps suggesting that either temperature or light limit productivity instead of nutrient availability. We found no significant differences in temperature between lakes receiving different subsidies, which we would expect if there was a relationship between temperature and biofilm productivity. Furthermore, since we placed NDS in the littoral zone, light should not have been a factor limiting algal biomass or production. However, at many of our sites on windy days it was difficult to see the lake bottom in one meter of standing water because fine particulate matter on the benthos was mixed into the water column by wind and wave action. In addition, at some lakes a substantial portion of the near-shore benthos was covered in fine particulate matter that was easily suspended in the water column upon disturbance. Therefore, it is possible that light availability limited autotrophic biofilm growth despite our best efforts to place substrata in the euphotic zone. However, the availability of light does not adequately explain the lack of nutrient limitation among heterotrophic constituents of the biofilms at waterbird lakes. Therefore, the generalized lack of nutrient limitation in waterbird lakes deserves further study to tease apart which factors control biofilm productivity.

3.5.4 Macroinvertebrates and Benthic Sediments Were Enriched with Marine-Derived N at Waterbird Lakes but Not at Salmon-Bearing Lakes

At INWR, lake sediments and invertebrates at waterbird lakes were significantly enriched with marine-derived N compared to reference lakes (Fig. 3.6), which is consistent with previous studies in Alaska (Payne and Moore 2006), the Arctic (Evenset et al. 2004, Brimble et al. 2009, Keatley et al. 2009), and New Zealand (Harding et al. 2004). At waterbird lakes, sediments and benthic consumers had 5- and 1.3-times higher $\delta^{15}N$ values compared to the same compartments in reference lakes, while salmon-bearing lakes were not enriched in $\delta^{15}N$ compared to reference lakes. The lack of ^{15}N enrichment in salmon-bearing lakes was somewhat surprising given that other studies have demonstrated that the marine-derived N signature from salmon can be tracked in freshwater food webs (Kline et al. 1990, Bilby et al. 1996, Chaloner et al. 2002, Reisinger et al. 2013) as well as in riparian biota (Ben-David et al. 1998, Hocking and Reynolds 2011). Furthermore, at INWR, invertebrate consumers in salmon-bearing lakes did not have an elevated $\delta^{15}N$ signature compared to invertebrates at reference lakes.

Unlike waterbird and reference lakes, salmon-bearing lakes at INWR are open systems with a stream outlet that allows entrance as well as export of organisms, nutrients, and organic matter downstream. The seaward migration of juvenile salmonids constitutes one of the most substantial nutrient losses from salmon-bearing lakes and streams, exporting up to 47% of the marine N imported by adult spawners (Moore et al. 2004). Yet we still observed higher concentrations of N in salmon-bearing lakes compared to waterbird lakes prior to the salmon run, suggesting marine-derived N remains available for trophic transfer. Perhaps the synoptic approach for food web

sampling missed the key primary uptake compartments that utilize the marine-derived N. Furthermore, internal cycling may elevate the $\delta^{15}N$ signal in waterbird lakes, which are hydrologically isolated and represent a relatively closed system compared to salmon-bearing lakes. For example, denitrification concentrates ^{15}N remaining in the lake (Robinson 2001). Denitrification occurs in anoxic environments and requires labile carbon. Waterbird excreta supplies concentrated N as uric acid, can bind to organic molecules in feces and the environment (Ganning and Wulff 1969), and can quickly settle onto the benthos (Unckless and Makarewicz 2007) where the sediment profile can quickly turn anoxic. Therefore, lakes receiving bird excrement may have higher rates of denitrification, which concentrates heavy N isotopes and releases light N isotopes as N_2 gas, thereby influencing the signature of the DIN source remaining for assimilatory uptake into the primary uptake compartments of the food web (i.e., algae and bacteria). Nevertheless, the combination of synoptic food web sampling and subsequent isotopic analyses offer some insight into the variable effect of nutrient subsidies on lake food webs, but clearly more detailed analyses and expanded collections are needed to fully understand the role of marine-derived nutrient subsidies in freshwaters at INWR.

3.6 Conclusions

Our study demonstrates that a history of organism-mediated nutrient subsidies can have a sustained enrichment effect in lakes at INWR, but it is important to use more than one metric to measure the extent of enrichment and it is important to sample across multiple lakes in order to tease apart variations in the subsidy effect. Water column

nutrient concentrations suggested that only salmon provide nutrient subsidies in INWR lakes. Furthermore, differences in water column chl-*a* concentrations also suggested that only lakes receiving salmon had ample enough nutrients to sustain significantly elevated levels of primary productivity. However, the biofilm response to nutrient enrichment varied among lakes and subsidy types in a manner that was inconsistent with the phytoplankton response. For example, at salmon-bearing and reference lakes the heterotrophic biofilm constituents were always N-limited and the autotrophic biofilm constituents were only occasionally nutrient-limited whereas biofilms were never nutrient-limited at waterbird lakes. Although nutrient availability and phytoplankton productivity was elevated at salmon-bearing lakes relative to reference lakes, the incorporation of marine-derived N into lake food webs differed among waterbird and salmon-bearing lakes. While the incorporation of marine-derived N at waterbird lakes was detectable and consistent with results from previous studies, at salmon-bearing lakes the biotic community did not appear to be enriched with marine-derived N, which may reflect small salmon runs in the years prior to our study, but it could also reflect differences in water residence time between lakes with and without stream outlets. While water column nutrient concentrations, phytoplankton biomass, and isotopic analyses indicated that salmon and waterbirds provide nutrient subsidies to lakes at INWR, the mechanism of incorporation and movement of marine-derived nutrients through the ecosystem may differ between salmon-bearing and waterbird lakes.

3.7 Acknowledgements

This research was made possible by the field, technical, and logistical support provided by Izembek National Wildlife Refuge and the US Fish and Wildlife Service. In particular, we are grateful for field help provided by James Smith, Leticia Melendez, Stacey Lowe, Shane Watson, and Doug Damberg. We also thank Joe Mueller and Audrey Kelley for their assistance processing samples. Funding for this research was provided by a US Fish and Wildlife Service cooperative agreement (F12AC00370).

3.8 Literature Cited

APHA. 1995. Standard methods for the examination of water and wastewater, 19th edition. American Public Health Association, Washington D.C.

Ben-David, M., T.A. Hanley, and D.M. Schell. 1998. Fertilization of terrestrial vegetation by spawning Pacific salmon: the role of flooding and predator activity. Oikos 83:47–55.

Bilby, R.E., B.R. Fransen, and P.A. Bisson. 1996. Incorporation of nitrogen and carbon from spawning coho salmon into the trophic system of small streams : evidence from stable isotopes. Canadian Journal of Fisheries and Aquatic Sciences 53:164–173.

Blais, J.M., L.E. Kimpe, D. McMahon, B.E. Keatley, M.L. Mallory, M.S.V. Douglas, and J.P. Smol. 2005. Arctic seabirds transport marine-derived contaminants. Science 309: 445.

Brimble, S.K., J.M. Blais, L.E. Kimpe, M.L. Mallory, B.E. Keaatley, M.S.V. Douglas, and J.P. Smol. 2009. Bioenrichment of trace elements in a series of ponds near a northern fulmar (Fulmarus glacialis) colony at Cape Vera, Devon Island. Canadian Journal of Fisheries and Aquatic Sciences 66:949–958.

Chaloner, D.T., K.M. Martin, M.S. Wipfli, P.H. Ostrom, and G.A. Lamberti. 2002. Marine carbon and nitrogen in southeastern Alaska stream food webs: evidence from

artificial and natural streams. Canadian Journal of Fisheries and Aquatic Science 59: 1257-1265.

Cocks, M.P., J.M. Harris, W.K. Steele, and D.A. Balfour. 1999. The influence of ornithogenic products on the nutrient status of soils surrounding nests on nunataks in Dronning Maud Land, Antarctica. Polar Research 18:19–26.

Douglas, R.W., and B. Rippey. 2000. The random redistribution of sediment by wind in a lake. Limnology and Oceanography. 45(3):686-694.

Ellis, J.C. 2005. Marine birds on land: A review of plant biomass, species richness, and community composition in seabird colonies. Plant Ecology 181:227–241.

Evenset, A., J. Carroll, G.N. Christensen, R. Kallenborn, D. Gregor, and G.W. Gabrielsen. 2007. Seabird guano is an efficient conveyer of persistent organic pollutants (POPs) to Arctic lake ecosystems. Envrionmental Science and Technology 41:1173–1179.

Evenset, A., G.N. Christensen, T. Skotvold, E. Fjeld, M. Schlabach, E. Wartena, and D. Gregor. 2004. A comparison of organic contaminants in two high Arctic lake ecosystems, Bjørnøya (Bear Island), Norway. The Science of the total environment 318:125–41.

Fanelli, E., J.E. Cartes, V. Papiol, P. Rumolo, and M. Sprovieri. 2010. Effects of preservation on the $\delta^{13}C$ and $\delta^{15}N$ values of deep sea macrofauna. Jounal of Experimental Marine Biology and Ecology 395:93-97.

Finney, B.P. 1998. Long-term variability of Alaskan sockeye salmon abundance determined by analysis of sediment cores. North Pacific Anadromous Fish Commission. Bull. No. 1:388-395.

Ganning, B., and F. Wulff. 1969. The effects of bird droppings on chemical and biological dynamics in brackish water rockpools. Oikos 20:274–286.

Goldman, C.R. 1960. Primary productivity and limiting factors in three lakes of the Alaska Peninsula. Ecological Monographs 30:207–230.

Harding, J.S., D.J. Hawke, R.N. Holdaway, and M.J. Winterbourn. 2004. Incorporation of marine-derived nutrients from petrel breeding colonies into stream food webs. Freshwater Biology 49:576–586.

Harrell, F.E., and C. Dupont. 2014. Hmisc: harrel miscellaneous. R package version 3.14-3. http://CRAN.R-project.org/package=Hmisc

Helfield, J.M., and R.J. Naiman. 2001. Effects of salmon-derived nitrogen on riparian forest growth and implications for stream productivity. Ecology 82(9):2403-2409.

Hilderbrand, G.V., T.A. Hanley, C.T. Robbins, and C.C. Schwartz. 1999. Role of brown bears (*Ursus arctos*) in the flow of marine nitrogen into a terrestrial ecosystem. Oecologia 121:546–550.

Hocking, M.D., and J.D. Reynolds. 2011. Impacts of Salmon on Riparian Plant Diversity. Science 331:1609–1612.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous Inference in General Parametric Models. Biometrical Journal 50(3):346-363.

Hume, J.M.B., K.S. Shortreed, and K.F. Morton. 1996. Juvenile sockeye rearing capacity of three lakes in the Fraser river system. Canadian Journal of Fisheries and Aquatic Science 53:719–733.

Hutchinson, G.E. 1950. Survey of contemporary knowledge of biogeochemistry: the biogeochemistry of vertebrate excretion. Bull. American Museum of Natural History.

Izaguirre, I., A. Vinocur, G. Mataloni, and M. Pose. 1998. Phytoplankton communities in relation to trophic status in lakes from Hope Bay (Antarctic Peninsula). Hydrobiologia 370:73–87.

Johnson, L.T., J.L. Tank, and W.K. Dodds. 2009. The influence of land use on stream biofilm nutrient limitation across eight North American ecoregions. Canadian Journal of Fisheries and Aquatic Sciences 66:1081-1094.

Kameda, K., K. Koba, S. Hobara, T. Osono, and M. Terai. 2006. Pattern of natural ^{15}N abundance in lakeside forest ecosystem affected by cormorant-derived nitrogen. Hydrobiologia 567:69–86.

Kazama, K., H. Murano, K. Tsuzuki, H. Fujii, Y. Niizuma, and C. Mizota. 2013. Input of seabird-derived nitrogen into rice-paddy fields near a breeding/roosting colony of the Great Cormorant (*Phalacrocorax carbo*), and its effects on wild grass. Applied Geochemistry 28:128–134.

Keatley, B.E., M.S.V. Douglas, J.M. Blais, M.L. Mallory, and J.P. Smol. 2009. Impacts of seabird-derived nutrients on water quality and diatom assemblages from Cape Vera, Devon Island, Canadian High Arctic. Hydrobiologia 621:191–205.

Kitchell, J.F., D.E. Schindler, B.R. Herwig, D.M. Post, M.H. Olson, and M. Oldham. 1999. Nutrient cycling at the landscape scale : The role of diel foraging migrations by geese at the Bosque del Apache National Wildlife Refuge , New Mexico. Limnology and Oceanography 44:828–836.

Kline, T.C., J.J. Goering Jr., O.A. Mathisen, P.H. Poe, and P.L. Parker. 1990. Recycling of elements transported upstream by runs of Pacific salmon: I. $\delta^{15}N$ and $\delta^{13}C$ evidence in Sashin Creek, Southeastern Alaska. Canadian Journal of Fisheries and Aquatic Science 47:136-144.

Kyle, G.B. 1994. Nutrient treatment of 3 coastal Alaskan lakes: trophic level responses and sockeye salmon production trends. Alaska Fishery Research Bulletin 1:153–167.

Kyle, G.B., J.P. Koenings, and J.A. Edmundson. 1997. An overview of Alaska lake-rearing salmon enhancement strategy: nutrient enrichment and juvenile stocking. Pages 205-227 In A.M. Milner and M.W. Oswood (editors). Freshwaters of Alaska: ecological syntheses. Springer-Verlag, New York, NY.

Lim, D.S.S., M.S.V. Douglas, and J.P. Smol. 2005. Limnology of 46 lakes and ponds on Banks Island, N.W.T., Canadian Arctic Archipelago. Hydrobiologia 545:11–32.

Luttenton, M.R., and R.L. Lowe. 2006. Response of a lentic periphyton community to nutrient enrichment at low N:P ratios. Journal of Phycology 42:1007–1015.

Mallory, M.L., A.J. Fontaine, P.A. Smith, M.O. Wiebe Robertson, and H.G. Gilchrist. 2006. Water chemistry of ponds on Southampton Island, Nunavut, Canada: effects of habitat and ornithogenic inputs. Archiv für Hydrobiologie 166:411–432.

Mitchell, N.A., and G.A. Lamberti. 2005. Responses in dissolved nutrients and epilithon abundance to spawning salmon in southeast Alaska streams. Limnology and Oceanography 50(1):217-227.

Moore, J.W., D.E. Schindler, and M.D. Scheuerell. 2004. Disturbance of freshwater habitats by anadromous salmon in Alaska. Oecologia 139(2):298-308.

Murphy, J., and J.P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Cheimica Acta 27:31-36.

Naiman, R.J., R.E. Bilby, D.E. Schindler, and J.M. Helfield. 2002. Pacific salmon, nutrients, and the dynamics of freshwater and riparian ecosystems. Ecosystems 5(4):399-417.

Odasz, A.M. 1994. Nitrate reductase activity in vegetation below an Arctic bird cliff, Svalbard, Norway. Journal of Vegetation Science 5(6):913-920.

Olson, M.H., M.M. Hage, M.D. Binkley, and J.R. Binder. 2005. Impact of migratory snow geese on nitrogen and phosphorus dynamics in a freshwater reservoir. Freshwater Biology 50:882–890.

Payne, L.X., and J.W. Moore. 2006. Mobile scavengers create hotspots of freshwater productivity. Oikos 115:69–80.

Pettigrew, C.T., B.J. Hann, and L.G. Goldsborough. 1998. Waterfowl feces as a source of nutrients to a prairie wetland: responses of microinvertebrates to experimental additions. Hydrobiologia 362:55–66.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and the R development core team. 2012. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-105.

Polis, G.A., W.B. Anderson, and R.D. Holt. 1997. Toward and integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annual Review of Ecology and Systematics 28:289–316.

Qian, J., S.S. Zheng, P.F. Wang, C. Wang. 2011. Experimental study on sediment resuspension in Taihu lake under different hydrodynamic disturbances. Journal of Hydrodynamics 23(6):826-833.

Reisinger, A.J., D.T. Chaloner, J. Rüegg, S.D. Tiegs, and G.A. Lamberti. 2013. Effects of spawning Pacific salmon on the isotopic composition of biota differ among Southeast Alaska streams. Freshwater Biology 58:938-950.

Robinson, D. 2001. δ^{15}N as an integrator of the nitrogen cycle. Trends in Ecology & Evolution 16:153–162.

Rush, S.A., S. Verkoeyen, T. Dobbie, S. Dobbyn, C.E. Hebert, J. Gagnon, and A.T. Fisk. 2011. Influence of increasing populations of Double-crested Cormorants on soil nutrient characteristics of nesting islands in western Lake Erie. Journal of Great Lakes Research 37:305–309.

Schmidt, D.C., S.R. Carlson, G.B. Kyle, and B.P. Finney. 1998. Influence of carcass-derived nutrients on sockeye salmon productivity of Karluk Lake, Alaska: importance in the assessment of an escapement goal. North American Journal of Fisheries Management 18:743–763.

Sedinger, J.S. 1997. Waterfowl and wetland ecology in Alaska. Pages 155-178 In A.M. Milner and M.W. Oswood (editors). Freshwaters of Alaska: ecological syntheses. Springer-Verlag, New York, NY.

Solorzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnology and Oceanography 14:799–801.

Steinman, A.D., G.A. Lamberti, P.R. Leavitt. 2006. Biomass and pigments of benthic algae. Pages 357-379 in F.R. Hauer and G.A. Lamberti (editors). Methods in stream ecology 2nd edition. Elsevier Inc., San Diego, CA.

Stockner, J.G., and E.A. MacIsaac. 1996. British Columbia lake enrichment programme: two decades of habitat enhancement for sockeye salmon. Regulated Rivers Research and Management 12:547-561.

Stockner, J.G., and K.R.S. Shortreed. 1978. Limnological survey of 35 sockeye salmon (*Onchorhychus nerka*) nursery lakes in British Columbia and the Yukon Territory. Fisheries and Marine Service Technical Report No. 827.

Syväranta, J., A. Martino, D. Kopp, R. Céréghino, F. Santoul. 2011. Freezing and chemical preservatives alter the stable isotope values of carbon and nitrogen of the Asiatic clam (*Corbicula fluminea*). Hydrobiologia 658:383-388.

Tank, J.L., and W.K. Dodds. 2003. Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. Freshwater Biology 48:1031-1049.

Tank, J.L., M.J. Bernot, and E.J. Rosi-Marshall. 2006. Nitrogen limitation and uptake. Pages 213-238 in F.R. Hauer and G.A. Lamberti (editors). Methods in stream ecology 2nd edition. Elsevier Inc., San Diego, CA.

Tomassen, H.B.M., A.J.P. Smolders, L.P.M. Lamers, and J.G.M. Roelofs. 2005. How bird droppings can affect the vegetation composition of ombrotrophic bogs. Canadian Journal of Fisheries and Aquatic Sciences 83:1046–1056.

Unckless, R.L., and J.C. Makarewicz. 2007. The impact of nutrient loading from Canada Geese (*Branta canadensis*) on water quality, a mesocosm approach. Hydrobiologia 586:393–401.

Vadeboncoeur, Y., E. Jeppesen, M.J. Vander Zanden, H.H. Schierup, K. Christoffersen, D.M. Lodge. 2003. From Greenland to green lakes: cultural eutrophication and the loss of benthic pathways in lakes. Limnology and Oceanography 48(4):1408-1418.

Wainright, S.C., J.C. Haney, C. Kerr, A.N. Golovkin, and M.V. Flint. 1998. Utilization of nitrogen derived from seabird guano by terrestrial and marine plants at St. Paul, Pribilof Islands, Bering Sea, Alaska. Marine Biology 131:63–71.

Wait, D.A., D.P. Aubrey, and W.B. Anderson. 2005. Seabird guano influences on desert islands: soil chemistry and herbaceous species richness and productivity. Journal of Arid Environments 60:681–695.

Wetzel, R.G., and G.E. Likens. 2000. Composition and biomass of phytoplankton. 3rd edition. Springer, New York, NY.

Wootton, J.T. 1991. Direct and indirect effects of nutrients on intertidal community structure: variable consequences of seabird guano. Journal of Experimental Marine Biology and Ecology 151:139–153.

Zwolicki, A., K.M. Zmudczynka-Skarbek, L. Iliszko, and L. Stempniewicz. 2013. Guano deposition and nutrient enrichment in the vicinity of planktivorous and piscivorous seabird colonies in Spitsbergen. Polar Biology 36:363–372.

CHAPTER 4:

CONCLUSIONS

4.1 Summary of Research Results

The underlying objective for my research was to establish a baseline of structural and functional characteristics in freshwater lakes and streams at Izembek National Wildlife Refuge (INWR) in Alaska. In my first data chapter, I examined whether the background physiochemical conditions and the effects of salmon in streams and lakes at Izembek NWR were comparable to those found at other salmon-bearing ecosystems throughout the Pacific salmon range. Specifically, I considered the balance between salmon enrichment and disturbance, examining these effects during the salmon run. In my 2nd data chapter, I examined the long-term effects of salmon and waterbird nutrient subsidies in lakes by measuring ecosystem metrics prior to the arrival of migrants. Through my research, I was able to establish a baseline for future studies addressing the role of nutrient subsidies on the ecosystem function of low-nutrient, freshwater ecosystems at INWR.

Specifically, in Chapter 2, I examined whether salmon-bearing lakes and streams at INWR were comparable to other salmon-bearing ecosystems throughout the Pacific salmon range. Although concentrations of dissolved inorganic nitrogen (DIN) were among the lowest reported for salmon-bearing streams, soluble reactive phosphorous

(SRP) concentrations were slightly higher than average values reported elsewherein the Pacific salmon range (Janetski et al. 2009). Elsewhere in Alaska and the Pacific Northwest, inorganic N availability is typically higher compared to soluble reactive P (Janetski et al. 2009, Levi et al. 2011), and the relative surplus of available N suggests that P availability likely limits productivity. At least one study has shown that biofilms in Southeast Alaskan salmon streams were co-limited by both N and P prior to the salmon run, and that N-limitation could be alleviated by the presence of salmon; however, P–limitation persisted during the salmon run (Rüegg et al. 2011). In the freshwater systems at INWR, I predicted that biofilms would primarily be N-limited given that N availability was very low in comparison to background P concentrations. Using nutrient diffusing substrata (NDS) to quantify biofilm nutrient limitation status, I confirmed that autotrophic and heterotrophic biofilms were usually N-limited regardless of salmon presence. *These results suggest that the impacts of salmon-derived nutrient subsidies depends on the environmental context, including the availability of N and P derived from rock weathering, run-off from the terrestrial landscape, and the net effect of internal nutrient recycling*. Given these results, I expected that the balance between disturbance and enrichment at INWR might differ from previously studied salmon-bearing ecosystems in other parts of the Pacific salmon range.

At INWR, salmon generally had an enrichment effect that was similar to the pattern of enrichment found in other parts of the Pacific salmon range (Janetski et al. 2009). Although the magnitude of salmon enrichment was small relative to observations elsewhere, the somewhat “dampened” enrichment effect was not unexpected given the small salmon runs observed in Izembek streams in recent years. Consistent with previous

studies, salmon influenced the concentration of nutrients and chlorophyll-*a* (chl-*a*) in the water column, although the magnitude of these affects were likely influenced by small salmon run sizes (Johnston et al. 2004, Janetski et al. 2009). The increase in the DIN concentration during the salmon run was small but significant. In contrast, P availability decreased slightly during the salmon run, which could be the result of increased P uptake in response to higher N availability. Alternatively, the decreased P availability could be due to an interaction with salmon-induced turbidity via spawning, which could have resulted in lower P concentrations via abiotic sorption dynamics. Unfortunately, my study design could not discern the ultimate cause of P decline, and the origin of this effect remains a question for future research. Nevertheless, concentrations of water column chl-*a* were higher during the salmon run than before salmon, which is consistent with the predicted effects of nutrient enrichment resulting from a bottom-up trophic cascade (Carpenter et al. 2001).

In addition to nutrient and algal biomass effects of salmon-derived nutrient amendments, I also examined the impact of salmon on whole-stream metabolism at INWR. I found that salmon affect metabolism, and this influence is similar to those observed during previous studies in Southwest and Southeast Alaska (Holtgrieve and Schindler 2011, Levi et al. 2013). Consistent with findings elsewhere in Alaska, salmon generally had a disturbance effect on gross primary production (GPP) and an enrichment effect on ecosystem respiration (ER); however, in Izembek streams the influence of salmon on ecosystem respiration (ER) was variable depending on the stream reach location of the stream reach within the watershed. At upstream reaches, ER decreased when salmon were present, while in reaches downstream of salmon spawners, ER

increased during the salmon run, presumably due to the downstream export of salmon-derived nutrients and sediments. While some studies have used control reaches upstream of the study reach to control for seasonal variability in local environmental factors (Cak et al. 2008, Collins et al. 2011), in general, earlier studies have focused on salmon spawners in a single stream reach to study the effect of salmon on a stream ecosystem (Moore and Schindler 2008, Tiegs et al. 2009, Holtgrieve et al. 2010, but see Albers and Petticrew 2012). *My results demonstrate that multiple stream reaches within a single watershed may respond differently to salmon spawners, and expanding a study design to include upper and lower reaches can provide novel insights into how salmon influence ecosystem structure and function at the landscape scale*.

In Chapter 3, I compared the long-term effects of nutrient subsidies on lakes by measuring ecosystem characteristics prior to the arrival of migrants. After my first field season, the data that I collected from INWR lakes suggested that these systems may have retained salmon-derived nutrients over an entire year, with elevated nutrient levels detectable prior to the following year's run, and the nutrients appeared to stimulate primary productivity. However, INWR salmon-bearing lakes were also visited by waterbirds, which can provide an additional source of nutrients to lake ecosystems (Kitchell et al. 1999, Evenset et al. 2007). In addition, many lakes that did not receive salmon did support dense aggregations of waterbirds in the fall and/or spring. *Therefore, the second objective of my thesis was to conduct a preliminary study to examine whether salmon and waterbird subsidies have similar effects on lake ecosystems, as compared to reference lakes receiving little or no subsidies*. It was my goal to perform this research

such that it could establish a framework for future nutrient subsidy research in freshwater ecosystems at INWR.

Prior to the arrival of migrant species, lakes receiving predominantly salmon subsidies had higher concentrations of inorganic nutrients and water column chl-*a* than was observed at waterbird lakes and reference lakes receiving no significant subsidies. Dissolved nutrient concentrations at salmon-bearing lakes were 6-times higher for DIN and 3-times higher for SRP when compared to reference lakes. In contrast, lakes receiving waterbirds did not have significantly higher concentrations of dissolved nutrients than at reference lakes. Although only salmon-bearing lakes had significantly higher nutrient availability, the mean DIN concentration at waterbird lakes was slightly higher than at reference lakes. In addition, water column chl-*a* concentrations were significantly correlated with N and P availability, indicating that lakes receiving substantial subsidies had higher phytoplankton biomass compared to reference lakes. These results are consistent with patterns of enrichment found in both salmon-bearing and waterbird ecosystems, where there is evidence that the magnitude of enrichment is predominantly related to the size of the subsidy (Izaguirre et al. 1998, Kitchell et al. 1999, Johnston et al. 2004, Mallory et al. 2006, Janetski et al. 2009).

Based on these results, I predicted that stable isotope analyses of lake food webs would show more ^{15}N enrichment at lakes receiving salmon subsidies when compared to those receiving waterbird subsides or no nutrient subsidies at all. Contrary to my predictions, waterbird lakes tended to be enriched in ^{15}N. Specifically, at waterbird lakes benthic sediments were significantly enriched with marine-derived N compared to salmon-bearing and reference lakes. Furthermore, macroinvertebrates in waterbird lakes

and reference lakes were significantly enriched in ^{15}N compared to salmon-bearing lakes. In some ways, these results are consistent with other studies conducted in Alaska (Payne and Moore 2006), in the Arctic (Evenset et al. 2004, Brimble et al. 2009), and in New Zealand (Harding et al. 2004), all of which found that waterbird-derived subsidies were evident in stable isotope analyses of aquatic food webs. While the incorporation of marine-derived N at waterbird lakes was detectable and consistent with results from previous studies, at salmon-bearing lakes the biotic community did not appear to be enriched with marine-derived N, which may reflect small salmon runs in the years prior to our study, but it could also reflect differences in water residence time between lakes with and without stream outlets.

4.2 Enrichment and Disturbance by Multiple Taxa

Salmon directly and indirectly provide a critical food resource for many organisms that can transport salmon-derived nutrients throughout the landscape (Hansen 1987, Ben-David et al. 1997, Cedarholm et al. 1999, Hilderbrand et al. 1999, Gende et al. 2004), yet few studies have considered how salmon interact with other ecosystem engineers (Sigourney et al. 2006, Hood 2012, Hogg et al. 2014). Furthermore, previous research largely focused on the local effects of a single ecosystem engineer (e.g., salmon) when migrants are present, in addition to immediately before or after migration. However, salmon can have long-term effects that persist after migrants are gone (Hansen 1987, O'Keefe and Edwards 2002, Fellman et al. 2008, Reisinger et al. 2013). This legacy effect of nutrient subsidies was the focus of Chapter 3 of my thesis. Specifically, I

examined structural metrics that reflect the net effect of disturbance and enrichment by contrasting two taxa that provide a subsidy of marine-derived nutrients, in the form of salmon and waterbirds.

My research builds on the existing conceptual model of salmon-mediated enrichment and disturbance (Fig. 4.1), which illustrates how the net effect of salmon on freshwater ecosystems depends on the balance between enrichment (via nutrient excretion and carcass deposition) and disturbance (via the movement of sediments during nest construction; Janetski et al. 2009). In order to account for the effects of multiple taxa on a freshwater ecosystem, we must begin by duplicating the existing model of enrichment and disturbance, and apply the second model to another taxonomic group (i.e., waterbirds; Fig. 4.2). In the waterbird model, ecosystem enrichment could occur when birds and excrement provide a resource subsidy to aquatic food webs (Harding et al. 2004, Olson et al. 2005, Evenset et al. 2007), while disturbance could occur when intense foraging activities alter soil and water chemistry (Ellis 2005 and citations therein, Abraham and Jeffries 2007). In order to illustrate the interaction between salmon and waterbirds, we can rotate the waterbird model by 90° and overlay the image on the salmon model (Fig. 4.3). The representation of enrichment and disturbance by the two taxa on any single ecosystem is illustrated by the box where the two models intersect. In the “interaction model”, different components of the ecosystem are either dual-enriched by both taxa, dual-disturbed, or enriched by one taxa and disturbed by another. Furthermore, the degree of enrichment or disturbance by either taxa can fluctuate along the vertical and horizontal planes. To illustrate this, we can slide the salmon model along the waterbird model, demonstrating different degrees of waterbird enrichment and

disturbance (Fig. 4.4). As the proportion of waterbird enrichment increases the size of the dual-enrichment box also increases, while the size of the dual-disturbance box decreases, illustrating how different components of the ecosystem may shift from being disturbed to being enriched. This conceptual model can serve as a hypothetical framework from which future research can be designed. Specifically, the next step would be to intensively study the enrichment and disturbance effects of both salmon and waterbirds on a single lake ecosystem. Such a study could identify which organisms or ecosystem processes are impacted by each taxon and quantify that effect.

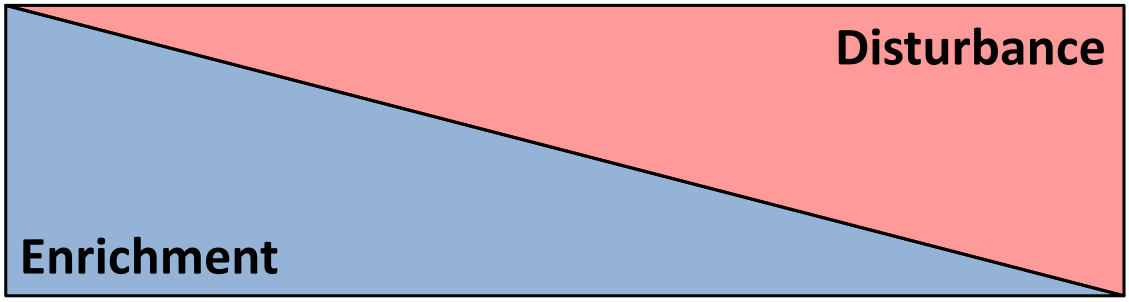

Figure 4.1 Conceptual model showing salmon-mediated enrichment and disturbance in aquatic ecosystems. As enrichment (blue) increases, disturbance (red) decreases and vice versa.

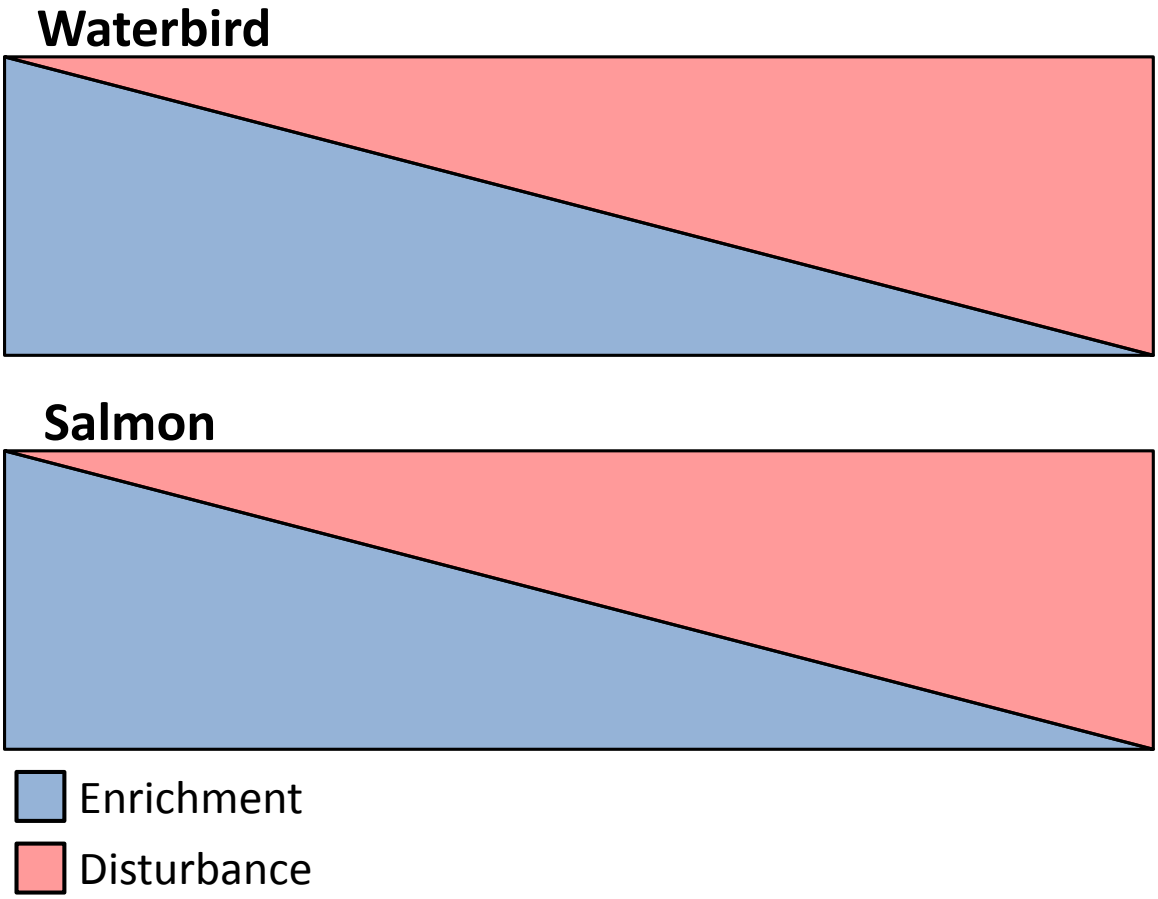

Figure 4.2 Conceptual models showing salmon-mediated and waterbird-mediated enrichment and disturbance in aquatic ecosystems. As enrichment (blue) increases, disturbance (red) decreases and vice versa.

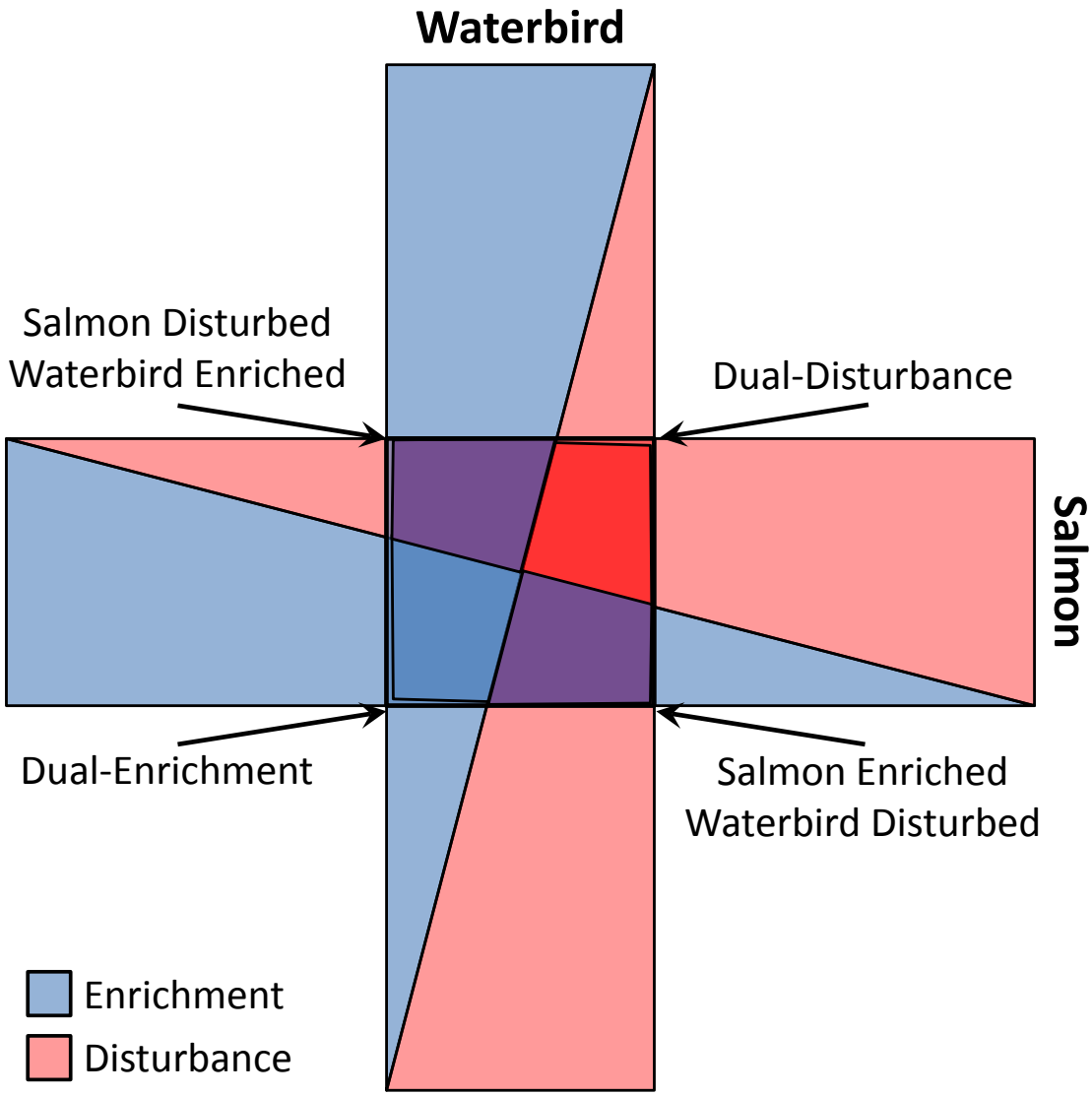

Figure 4.3 Conceptual model showing the hypothetical interaction between salmon- and waterbird-mediated enrichment and disturbance in aquatic ecosystems. As enrichment (blue) increases, disturbance (red) decreases and vice versa. Any given ecosystem exists at the intersection of the enrichment-disturbance diagrams (bolded box), where some components of the ecosystem are enriched or disturbed by both salmon and waterbirds (dark blue and dark red, respectively), while other components of the ecosystem are enriched by one group and disturbed by the other (purple).

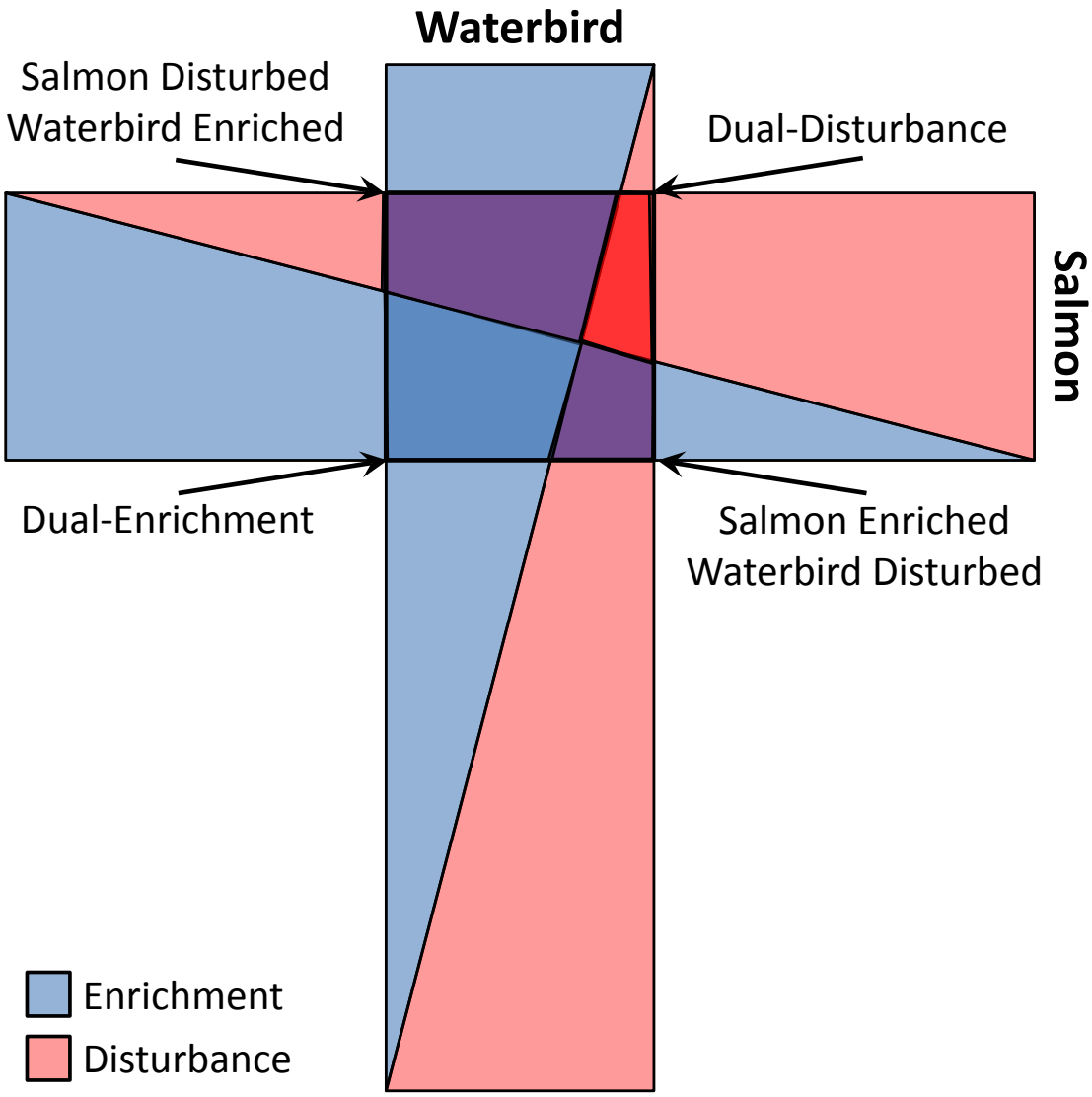

Figure 4.4 Conceptual model showing the hypothetical interaction between salmon- and waterbird-mediated enrichment and disturbance in aquatic ecosystems. Notice that as waterbirds have more of an enrichment effect, the fraction of the ecosystem receiving dual-disturbance decreases and the fraction receiving dual-enrichment increases.

In closing, I conducted a simple internet search to explore the scope of conceptual models for ecosystem engineers such as salmon and waterbirds; most models focus on how a single taxon shapes the local environment. However, in the case of salmon, the taxon can have influence beyond the local scale and into the broader landscape

(Hilderbrand et al. 1999, Hocking and Reynolds 2011, Field and Reynolds 2011). While the net effect of salmon can be influenced by environmental factors (Moore et al. 2004, Tiegs 2008, Rüegg et al. 2012), it may also be modified by interactions with other sources of enrichment and disturbance, such as waterbirds. This proposed conceptual model can be used as a framework from which future investigators can generate new hypotheses and experiments to expand our understanding of the impacts of both salmon-derived and waterbird-derived nutrient subsidies, in addition to their synergistic effects on the freshwater ecosystems that receive them.

4.3 Literature Cited

Abraham, K.F., and R.L. Jefferies. 2007. High goose populations: causes, impacts, and implications. Pages 7-72 in B.D.J. Batt (editor). Arctic ecosystems in peril: report of the arctic goose habitat working group. U.S. Fish and Wildlife Service, Washington D.C. and Canadian Wildlife Service, Ottawa, Ontario.

Albers, S.J., and E.L. Petticrew. 2012. Ecosystem response to a salmon disturbance regime: implications for downstream nutrient fluxes in aquatic systems. Limnology and Oceanography 57(1):113-123.

Ben-David, M., T.A. Hanley, D.R. Klein, and D.M. Schell. 1997. Seasonal changes in diets of coastal and riverine mink: the role of spawning Pacific salmon. Canadian Journal of Zoology 75:803-811.

Brimble, S.K., J.M. Blais, L.E. Kimpe, M.L. Mallory, B.E. Keaatley, M.S.V. Douglas, and J.P. Smol. 2009. Bioenrichment of trace elements in a series of ponds near a northern fulmar (Fulmarus glacialis) colony at Cape Vera, Devon Island. Canadian Journal of Fisheries and Aquatic Sciences 66:949–958.

Cak, A.D., D.T. Chaloner, and G.A. Lamberti. 2008. Effects of spawning salmon on dissolved nutrients and epilithon in coupled stream-estuary systems of Southeastern Alaska. Aquatic Sciences 70:169-178.

Carpenter, S.R., J.J. Cole, J.R. Hodgson, J.F. Kitchell, M.L. Pace, D. Bade, K.L. Cottingham, T.E. Essington, J.N. Houser, and D.E. Schindler. 2001. Trophic cascades, nutrients, and lake productivity: whole-lake experiments. Ecological Monographs 71(2):163-186.

Cedarholm, C.J., M.D. Kunze, T. Murota, and A. Sibatani. 1999. Pacific salmon carcasses: essential contributions of nutrients and energy for aquatic and terrestrial ecosystems. Fisheries Management/Habitat 24(10):6-15.

Collins, S.F., A.H. Moerke, D.T. Chaloner, D.J. Janetski, and G.A. Lamberti. 2011. Response of dissolved nutrients and periphyton to spawning Pacific salmon in three northern Michigan streams. Journal of the North American Benthological Society. 30(3):831-839.

Ellis, J.C. 2005. Marine birds on land: A review of plant biomass, species richness, and community composition in seabird colonies. Plant Ecology 181:227–241.

Evenset, A., G.N. Christensen, T. Skotvold, E. Fjeld, M. Schlabach, E. Wartena, and D. Gregor. 2004. A comparison of organic contaminants in two high Arctic lake ecosystems, Bjørnøya (Bear Island), Norway. The Science of the total environment 318:125–41.

Evenset, A., J. Carroll, G.N. Christensen, R. Kallenborn, D. Gregor, and G.W. Gabrielsen. 2007. Seabird guano is an efficient conveyer of persistent organic pollutants (POPs) to Arctic lake ecosystems. Envrionmental Science and Technology 41:1173–1179.

Fellman, J.B., E. Hood, R.T. Edwards, and D.V. D'Amore. 2008. Return of salmon-derived nutrients from the riparian zone to the stream during a storm in Southeastern Alaska. Ecosystems 11(4):537-544.

Field, R.D., and J.D. Reynolds. 2011. Sea to sky: impacts of residual salmon-derived nutrients on estuarine breeding bird communities. Proceedings of the Royal Society 278(1721):3081–3088.

Gende, S.M., T.P. Quinn, R. Hilborn, A.P. Hendry, and B. Dickerson. 2004. Brown bears selectively kill salmon with higher energy content but only in habitats that facilitate choice. Oikos 104(3):518-528.

Hansen, A.J. 1987. Regulation of bald eagle reproductive rates in Southeast Alaska. Ecology 68(5):1387-1392.

Harding, J.S., D.J. Hawke, R.N. Holdaway, and M.J. Winterbourn. 2004. Incorporation of marine-derived nutrients from petrel breeding colonies into stream food webs. Freshwater Biology 49:576–586.

Hilderbrand, G.V., T.A. Hanley, C.T. Robbins, and C.C. Schwartz. 1999. Role of brown bears (*Ursus arctos*) in the flow of marine nitrogen into a terrestrial ecosystem. Oecologia 121:546–550.

Hocking, M.D., and J.D. Reynolds. 2011. Impacts of Salmon on Riparian Plant Diversity. Science 331:1609–1612.

Hogg, R.S., S.M. Coghlan Jr., J. Zydlewski, and K.S. Simon. 2014. Anadromous sea lampreys (*Petromyzon marinus*) are ecosystem engineers in a spawning tributary. Freshwater Biology 59:1294-1307.

Holtgrieve, G.W., and D.E. Schindler. 2011. Marine-derived nutrients, bioturbation, and ecosystem metabolism: reconsidering the role of salmon in streams. Ecology 92(2): 373-385.

Holtgrieve, G.W., D.E. Schindler, C.P. Gowell, C.P. Ruff, and P.J. Lisi. 2010. Stream geomorphology regulates the effects on periphyton of ecosystem engineering and nutrient enrichment by Pacific salmon. Freshwater Biology 55:2598-2611.

Hood, W.G. 2012. Beaver in tidal marshes: dam effects on low-tide channel pools and fish use of estuarine habitat. Wetlands 32:401-410.

Izaguirre, I., A. Vinocur, G. Mataloni, and M. Pose. 1998. Phytoplankton communities in relation to trophic status in lakes from Hope Bay (Antarctic Peninsula). Hydrobiologia 370:73–87.

Janetski, D.J., D.T. Chaloner, S.D. Tiegs, and G.A. Lamberti. 2009. Pacific salmon effects on stream ecosystems: a quantitative synthesis. Oecologia 159:583-595.

Johnston, N.T., E.A. Macisaac, P.J. Tschaplinski, and K.J. Hall. 2004. Effects of the abundance of spawning sockeye salmon (*Oncorhynchus nerka*) on nutrients and algal biomass in forested streams. Canadian Journal of Fisheries and Aquatic Sciences 61:384–403.

Kitchell, J.F., D.E. Schindler, B.R. Herwig, D.M. Post, M.H. Olson, and M. Oldham. 1999. Nutrient cycling at the landscape scale : The role of diel foraging migrations by geese at the Bosque del Apache National Wildlife Refuge , New Mexico. Limnology and Oceanography 44:828–836.

Levi, P.S., and J.L. Tank. 2013. Nonnative Pacific salmon alter hot spots of sediment nitrification in Great Lakes tributaries. Journal of Geophysical Research Biogeoscience 118:436-444.

Levi, P.S., J.L. Tank, S.D. Tiegs, J.Rüegg, D.T. Chaloner, and G.A. Lamberti. 2011. Does timber harvest influence the dynamics of marine-derived nutrients in Southeast Alaska streams? Canadian Journal of Fisheries and Aquatic Science 68:1326-1329.

Levi, P.S., J.L. Tank, J. Rüegg, D.J. Janetski, S.D. Tiegs, D.T. Chaloner, and G.A. Lamberti. 2013. Whole-stream metabolism responds to spawning Pacific salmon in their native and introduced ranges. Ecosystems 16: 269-283.

Mallory, M.L., A.J. Fontaine, P.A. Smith, M.O. Wiebe Robertson, and H.G. Gilchrist. 2006. Water chemistry of ponds on Southampton Island, Nunavut, Canada: effects of habitat and ornithogenic inputs. Archiv für Hydrobiologie 166:411–432.

Moore, J.W., and D.E.Schindler. 2008. Biotic disturbance and benthic community dynamics in salmon-bearing streams. Journal of Animal Ecology 77(2):275-284.

Moore, J.W., D.E. Schindler, and M.D. Scheuerell. 2004. Disturbance of freshwater habitats by anadromous salmon in Alaska. Oecologia 139(2):298-308.

O'Keefe, T.C., and R.T. Edwards. 2002. Evidence for hyporheic transfer and removal of marine-derived nutrients in a sockeye stream in Southwest Alaska. American Fisheries Society Symposium 33:99-107.

Olson, M.H., M.M. Hage, M.D. Binkley, and J.R. Binder. 2005. Impact of migratory snow geese on nitrogen and phosphorus dynamics in a freshwater reservoir. Freshwater Biology 50:882–890.

Payne, L.X., and J.W. Moore. 2006. Mobile scavengers create hotspots of freshwater productivity. Oikos 115:69–80.

Reisinger, A.J., D.T. Chaloner, J. Rüegg, S.D. Tiegs, and G.A. Lamberti. 2013. Effects of spawning Pacific salmon on the isotopic composition of biota differ among Southeast Alaska streams. Freshwater Biology 58:938-950.

Rüegg, J., S.D. Tiegs, D.T. Chaloner, P.S. Levi, J.L. Tank, and G.A. Lamberti. 2011. Salmon subsidies alleviate nutrient limitation of benthic biofilms in Southeast Alaska streams. Canadian Journal of Fisheries and Aquatic Science 68:277-287.

Rüegg, J., D.T. Chaloner, P.S. Levi, J.L. Tank, S.D. Tiegs, and G.A. Lamberti. 2012. Environmental variability and the ecological effects of spawning Pacific salmon on stream biofilm. Freshwater Biology 57:129-142.

Sigourney, D.B., B.H. Letcher, and R.A. Cunjak. 2006. Influence of beaver activity on summer growth and condition of age-2 Atlantic salmon parr. Transactions of the American Fisheries Society 135:1068-1075.

Tiegs, S.D. 2008. Timber harvest transforms ecological roles of salmon in Southeast Alaska rain forest streams. Ecological Applications 18(1): 4-11.

Tiegs, S.D., E.M. Campbell, P.S. Levi, J. Rüegg, M.E. Benbow, D.T. Chaloner, R.W. Merritt, J.L. Tank, and G.A. Lamberti. 2009. Separating physical disturbance and nutrient enrichment caused by Pacific salmon in stream ecosystems. Freshwater Biology 54:1864-1875.