

The Importance of Marine-derived Nutrients from Anadromous Fishes to Atlantic Rivers

by

Kurt M. Samways

Master of Science, University of Regina, 2008

A Dissertation Submitted in Partial Fulfillment
of the Requirements for the Degree of

Doctor of Philosophy

in the Graduate Academic Unit of Biology

Supervisor: Richard A. Cunjak, Ph.D., Biology

Examining Board: Mary McKenna, Ph.D., School of Graduate Studies
Wendy Monk, Ph.D., Biology
Michael Duffy, Ph.D., Biology
David Lentz, Ph.D., Earth Sciences

External Examiner: Joseph Rasmussen, Ph.D., Biological Sciences, Water Institute for
Semi-arid Ecosystems (WISE), University of Lethbridge

This dissertation is accepted by the
Dean of Graduate Studies

THE UNIVERSITY OF NEW BRUNSWICK

August 17, 2017

© Kurt M. Samways, 2017

ABSTRACT

With the dramatic declines in Atlantic anadromous fishes over the past century it is important to identify the relative roles marine-derived nutrients (MDNs) delivered by these fishes play in influencing freshwater food web dynamics. Rivers in New Brunswick and Nova Scotia, Canada containing Atlantic salmon (*Salmo salar*), alewife (*Alosa pseudoharengus*), rainbow smelt (*Osmerus mordax*), or sea lamprey (*Petromyzon marinus*) as the primary anadromous species, were chosen to study their effects on *i*) primary production/productivity; *ii*) trophic interactions; and *iii*) resource quality. To understand the linkages between freshwater and marine ecosystems, observational studies, experimental frameworks, and analytical techniques (including stable isotope and fatty acid analysis) were employed.

Biofilm communities followed a predictable response pattern to MDN inputs, regardless of the fishes spawning strategy, timing, or MDN load being delivered. Biofilm community standing crop and gross primary productivity were greater in sites receiving MDN subsidies than reference sites. The ^{13}C and ^{15}N data showed that MDNs were incorporated into all trophic levels (biofilm, invertebrates, and salmon parr) across streams with anadromous fish spawning. Community-wide niche space (i.e. the trophic diversity among food webs) shifted toward the marine-nutrient source, however the total ecological niche space (i.e. magnitude of trophic diversity) did not always increase with MDN inputs. Exposure to MDN resources from spawning Atlantic salmon led to improved nutritional quality for all biota, as indicated by increased lipid stores in all trophic levels and incorporation of fatty acids. The variability in fatty acid profiles was accredited to inherent differences between trophic groups combined with assimilation of

marine-derived fatty acids in the MDN treatments. Precipitous declines in fish populations have resulted in a net loss in MDN loading to a point that may no longer sustain elevated levels of productivity needed for sustaining large fish populations.

The current trend of declining anadromous fish populations in Atlantic Canada means fewer nutrient-rich marine subsidies for stimulating trophic production in these river systems. Marine-derived subsidies (nutrients and lipids) benefit multiple trophic levels of freshwater organisms as well as provide a cross-ecosystem spatial subsidy. In order to maintain ecosystem function and productivity, it is critical to include MDNs for effective ecosystem management and river restoration strategies.

DEDICATION

To my father, Ron Samways,
and in the memory of my mother,
Maxine Samways (1948-2014).

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. Rick Cunjak, for taking a chance on someone from the prairies no less to work, in part, on marine organisms. Through stimulating discussions, telling stories, and fundamentally changing the way I think, Rick has broadened my horizons and definitely made me a better scientist. I also thank my supervisory committee, Donald Baird, Joseph Culp, and Kerry MacQuarrie for their open door policy and sharing their time and expertise throughout my studies.

A large part of my studies was comprised of fieldwork and no graduate career could be endured without the help of so many. In particular, Michelle Charest, you helped me beyond any level imaginable. Whether sitting on the bank of a river in the rain sorting invertebrates, spending countless hours in a lab analyzing samples, or trekking for kilometers, you were always there to offer a helping hand (especially if it meant procrastinating). Emily Corey, you too went above and beyond anything I could have dreamed. From turning blue from the cold, working with a torn ACL, or volunteering to help knowing you would only get a few hours sleep, you did not hesitate your services. Despite both of your best efforts to ruin my PhD, I somehow managed to succeed. I am truly grateful for all for all your help. We have shared experiences to last a lifetime and I will take those memories with me wherever the road may take me.

To the rest of my lab mates, Guillaume Dauphin, Aaron Fraser, Heather McCracken, Katrina Chu, Michelle Lavery, Adrian Hards, and Sherr Vue, I thank you for all your help and I couldn't ask for a better group of people to share my time with. I also

thank the Stable Isotope in Nature Laboratory (Anne McGeachy, David Soto, Brian Hayden, Mireille Savoie, and Christine Paton), Mactaquac Biodiversity Facility (Trevor Goff, Danielle MacDonald, Stephanie Ratelle, John Whitelaw, and staff), Environmental Quality Analysis Laboratory (Vince Ignatiuk), Institute of Environmental change and Society (Bjoern Wissel), UNB Microscopy and Microanalysis Facility (C. Susan Belfry), Kabir Tosho, Sarah Lehnert, Catherine Desjardins, and Crystal Lewis. There are so many people in the greater UNB and CRI community to thank, that I could not possibly list them all.

I would like to thank my family for all their love and support. Mom and Dad, you neglected me as a child, instead of taking me to theme parks, you made me spend my summers camping and exploring nature. You instilled a life-long love and appreciation for the natural environment.

Tamara, my partner in life, thank you for putting up with me through all the extensive fieldwork, late night writing sessions, and being there for me, or not, as appropriate. I could not have completed this without your immeasurable love and support.

Finally, I wish to acknowledge my sampling companion, Blitz. You couldn't wait to join us in the field, the only one that loved the long drives, and always made the hikes more enjoyable. Seeing you find absolute joy in the perfect stick made even the worst day instantly better.

Table of Contents

ABSTRACT.....	ii
DEDICATION.....	iv
ACKNOWLEDGEMENTS.....	v
Table of Contents.....	vii
List of Tables	xi
List of Figures.....	xiv
Chapter 1	1
General Introduction	1
1.1 Objectives	5
1.2 Dissertation Overview	6
1.3 Literature Cited	8
Chapter 2	13
Spatiotemporal responses of algal, fungal, and bacterial biofilm communities in Atlantic rivers receiving marine-derived nutrient inputs	13
2.1 Abstract.....	14
2.2 Introduction.....	15
2.3 Methods.....	19
2.3.1 Study sites	19
2.3.2 Experimental design and sample collection.....	19
2.3.3 Laboratory analyses	21
2.3.4 Data analysis	22
2.4 Results.....	25
2.5 Discussion	27
2.6 Acknowledgments.....	36
2.7 Literature Cited	36
Chapter 3	59
Aquatic food web dynamics following incorporation of nutrients derived from Atlantic anadromous fishes	59
3.1 Abstract.....	60
3.2 Introduction.....	60

3.3	Methods.....	64
3.3.1	Site Descriptions	64
3.3.2	Sample Collection and Processing.....	65
3.3.3	Data Analysis	67
3.4	Results.....	69
3.4.1	Temporal Isotopic Enrichment	69
3.4.2	Reliance on Marine-derived Nutrients.....	72
3.5	Discussion	74
3.6	Acknowledgements.....	82
3.7	References.....	82
3.7.1	Electronic References	88
Chapter 4		97
	Increases in benthic community production and metabolism in response to marine-derived nutrients from spawning Atlantic salmon (<i>Salmo salar</i>)	97
4.1	Summary	98
4.2	Introduction.....	99
4.3	Methods.....	102
4.3.1	Study site.....	102
4.3.2	Stream channels	102
4.3.3	Mesocosms.....	104
4.3.4	Benthic metabolism	105
4.3.5	Biofilm growth.....	106
4.3.6	Marine-derived nutrient inputs	107
4.3.7	Changes in production	110
4.3.8	Data analysis	110
4.4	Results.....	111
4.4.1	Biofilm growth.....	111
4.4.2	Benthic metabolism	112
4.4.3	Marine-derived nutrient inputs	112
4.4.4	Changes in production	113
4.5	Discussion	113
4.6	Acknowledgments.....	120

4.7 Literature Cited	121
Chapter 5	132
Effects of spawning Atlantic salmon (<i>Salmo salar</i>) on total lipid content and fatty acid composition of river food webs	132
5.1 Abstract	133
5.2 Introduction	134
5.3 Methods	137
5.3.1 Study Site	137
5.3.2 Stream-channels	138
5.3.3 Mesocosms	140
5.3.4 Sample collection	141
5.3.5 Lipid extraction and separation	142
5.3.6 Data Analysis	142
5.4 Results	144
5.4.1 Growth	144
5.4.2 Total lipid	145
5.4.3 Fatty acid profiles	145
5.5 Discussion	151
5.6 Acknowledgements	157
5.7 Literature Cited	157
Chapter 6	179
The ecological toll from declining fish populations: Anadromous fishes function as nutrient import/export specialists	179
6.1 Abstract	180
6.2 Introduction	181
6.3 Methods	185
6.3.1 Equilibrium Spawning Population	185
6.3.2 Marine-derived nutrient loading models	190
6.3.3 Sensitivity Analysis	196
6.4 Results	197
6.4.1 Equilibrium Spawning Population	197
6.4.2 Marine-derived nutrient loading models	198

6.4.3 Sensitivity Analysis	199
6.5 Discussion	200
6.6 Acknowledgements.....	206
6.7 Literature Cited	206
Chapter 7	225
Synthesis	225
7.1 Significance of Dissertation.....	226
7.2 Is Nutrient Enrichment the Only Ecological Role of Anadromous Fishes?	229
7.3 Management Implications.....	230
7.4 Future Directions	231
7.5 Literature Cited	233
Appendix 1	236
Net increase estimation	236
Appendix 2	237
Area bounded by 2 algal abundance curves and the representative trapezoids between two sampling intervals (i.e. t_2 and t_1) used to estimate net increase in a downstream area of a river receiving marine-derived nutrients from spawning anadromous fish.	237
Appendix 3	238
Mean (SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (‰) isotope values for samples collected from all rivers for both upstream and downstream sites. Highlighted regions indicate periods of spawning.	238
Appendix 4	243
Permissions to Reprint from Journals	243
Appendix 5	245
Contribution of Authors in Chapters 2-6	245
Appendix 6	247
Permissions to Reprint from Co-Authors	247
Curriculum Vitae	

List of Tables

Table 2.1. Characteristics of study systems in New Brunswick and Nova Scotia, Canada. NS = no spawning occurred, NA = not available, BS = broadcast spawner, NB = nest builder.....	43
Table 2.2. Structure of mixed-model analyses of variance used to evaluate variation in algal and fungal biomass and bacterial density.....	45
Table 2.3. Results of the mixed-model analyses of variance evaluating variation in up- and downstream biofilm algal, fungal, and bacterial standing stock. The <i>F</i> - and <i>p</i> - values in the fungal group for factors time and site(barrier) × time are not computable because there are 0 df for the residuals (the demoninator of the <i>F</i> -ratios). The number of replicates/site is 1. Significant p-values are in bold.	46
Table 2.4. Effect of marine-derived nutrients on accrual of algae (chlorophyll), fungal (ergosterol), bacteria, all groups combined (overall) and stable-isotope enrichment of ¹⁵ N. Response variables reported as mean effect size; values in parentheses are upper and lower 95% confidence intervals.	48
Table 2.5. Mean (SD) δ ¹³ C and δ ¹⁵ N (‰) isotope values for biofilm samples collected from up- and downstream sites prior to anadromous fish spawning.	49
Table 2.6. Results of mixed-model analyses of variance in up- and downstream δ ¹⁵ N stable-isotope values. The <i>F</i> -and <i>p</i> -values for factors time and site(barrier) × time are not computable because there are 0 df for the residuals (the denominator of the <i>F</i> -ratios). The number of replicates per site is 1.	50

Table 4.1. Productivity – marine-nutrient models associated with Atlantic salmon spawning at the Mactaquac Biodiversity Facility, New Brunswick, Canada.	125
Table 5.1. Growth (mean $\pm$ SD) of wild Atlantic salmon parr in stream-channels receiving marine-derived nutrients (MDNs) from spawning Atlantic salmon and control treatments over the 116 day experimental period.....	163
Table 5.2. Fatty acid composition of initial stream-channel biofilm, invertebrate, and Atlantic salmon parr tissue samples. Values (mean $\pm$ SD) are percentages of total fatty acids (nd = not detected; na = ratio cannot be calculated as the denominator is zero; asterisk = replicate samples were pooled).	164
Table 5.3. Fatty acid composition of adult Atlantic salmon gametes. Values are percentages of total fatty acids. See Table 1 for explanation of symbols.....	166
Table 5.4. Fatty acid composition of stream-channel biofilm tissues. Values (mean $\pm$ SD) are percentages of total fatty acids. The “assimilation period” is defined as the period when greatest MDN incorporation occurred (83 day period encompassing the adult introduction, spawning, and brief post adult removal periods; sampling times 1-3). See Table 1 for explanation of symbols.	167
Table 5.5. Fatty acid composition of mesocosm biofilm tissues. Values (mean $\pm$ SD) are percentages of total fatty acids. See Table 1 for explanation of symbols.....	168
Table 5.6. Fatty acid composition of invertebrate tissues. Values (mean $\pm$ SD) are percentages of total fatty acids. See Table 1 for explanation of symbols.....	169
Table 5.7. Fatty acid composition of Atlantic salmon parr muscle tissue. Values (mean $\pm$ SD) are percentages of total fatty acids. See Table 1 for explanation of symbols.	170

Table 6.1. Results of the sensitivity analysis where s_i represents the percent change in the model output for a $\pm 10\%$ change in a given parameter.	214
--	-----

List of Figures

Figure 2.1. Map of New Brunswick and Nova Scotia, Canada, showing the location of the 6 study system used for assessing biofilm responses to marine-derived nutrient inputs from anadromous fishes.	52
Figure 2.2. Mean (± 1 SD) biofilm algal biomass (as chlorophyll) (A-F), fungal biomass (G-L), and bacterial density (M-R) responses to marine-derived nutrients from spawning anadromous fishes at upstream baseline and downstream treatment sites in Doctor's Brook (A, G, M), Knoydart Brook (B, H, N), Indiantown Brook (C, I, O), Oromocto River (D, J, P), Cross Creek (E, K, Q), and Rocky Brook (F, L, R) in New Brunswick and Nova Scotia. Shaded areas represent periods of anadromous fish spawning (spawning did not occur in Doctor's Brook). See Table 1.1 for primary spawning fish species in each river. * indicates a statistically significant difference ($p < 0.05$) between down- and upstream sites.	53
Figure 2.3. Mean (± 1 SD) standardized $\delta^{15}\text{N}$ stable-isotope signatures of biofilm in Doctor's Brook (A), Knoydart Brook (B), Indiantown Brook (C), Oromocto River (D), Cross Creek (E), and Rocky Brook (F). Changes in $\delta^{15}\text{N}$ are represented as the difference between down- and upstream (baseline) response values ($\Delta\delta^{15}\text{N}$, Eq. 2). Shaded areas represent periods of anadromous fish spawning (spawning did not occur in Doctor's Brook). See Table 1.1 for primary spawning fish species in each river. * indicates a statistically significant difference ($p < 0.05$) between down- and upstream sites.	55

Figure 2.4. Net increase in algal biomass (as chlorophyll) (A), fungal biomass (B), and bacterial density (C) as a function of Rainbow Smelt spawning density for 3 rivers in 2009-2010. Net increase over the entire sampling season was estimated as the area between the up- and downstream curves. 57

Figure 3.1. Community-wide convex hull total areas (TA) are based on the distribution of aquatic community groups isotope values in niche space for rivers representing a) no anadromous fish (Doctor's Brook), b) rainbow smelt (Knøydart Brook), c) alewife (Oromocto River), d) sea lamprey (Cross Creek), and e) Atlantic salmon (Rocky Brook). Convex hulls illustrate how community niche space shifts from pre-spawn (light orange: downstream treatment; light blue: upstream reference) to spawning periods (dark red: downstream treatment; dark blue: upstream reference) in both treatment and reference sites. Black circles represent the marine-derived nutrient subsidies for each river represented by the isotope values of the corresponding anadromous fish eggs..... 89

Figure 3.2. Temporal $\Delta\delta^{13}\text{C}$ changes between upstream reference and downstream treatment sites from pre-spawning conditions in aquatic community groups for rivers representing a) no anadromous fish (Doctor's Brook), b) rainbow smelt (Knøydart Brook), c) alewife (Oromocto River), d) sea lamprey (Cross Creek), and e) Atlantic salmon (Rocky Brook). Mean $\Delta\delta^{13}\text{C}$ ($\pm$ one standard deviation) values for biofilm (red triangles), Heptageniidae scrapers (dark peach circles), Simuliidae filter feeders (light beige diamonds), Perlidae predators (light grey squares), e) Pteronarcyidae shredders (purple inverted triangles), and resident salmonids (Atlantic salmon parr and brook trout) (black asterisk). 91

Figure 3.3. Temporal $\Delta\delta^{15}\text{N}$ changes between upstream reference and downstream treatment sites from pre-spawning conditions in aquatic community groups for rivers representing a) no anadromous fish (Doctor's Brook), b) rainbow smelt (Knoydart Brook), c) alewife (Oromocto River), d) sea lamprey (Cross Creek), and e) Atlantic salmon (Rocky Brook). Mean $\Delta\delta^{15}\text{N}$ ($\pm$ one standard deviation) values for biofilm (red triangles), Heptageniidae scrapers (dark peach circles), Simuliidae filter feeders (light beige diamonds), Perlidae predators (light grey squares), e) Pteronarcyidae shredders (dark purple upside down triangles), and resident salmonids (Atlantic salmon parr and brook trout) (black asterisk). 93

Figure 3.4. Seasonal variation in the proportion of marine-derived nutrient resources in fin tissue stable isotope ratios of resident salmonid fishes (Atlantic salmon parr and brook trout), in a) Doctor's Brook, b) Knoydart Brook, c) Oromocto River, d) Cross Creek, and e) Rocky Brook. Gradations represent the 50%, 75%, and 95% credibility intervals. Shaded areas represent periods of anadromous fish spawning (there was no spawning in Doctor's Brook by anadromous rainbow smelt). 95

Figure 4.1. Biofilm accrual on artificial substrata, measured as chlorophyll *a*, receiving marine-derived nutrients (MDNs) from spawning Atlantic salmon and control treatments in: a) stream channels and b) mesocosms. Experimental system: stream channels, circles; mesocosms, triangles. Treatments: MDN subsidies, shaded symbols; controls, open symbols. Values with an * denote a statistically significant difference ($P < 0.05$) between stream channels/mesocosms with MDN inputs and the controls. Shaded areas denote the period of spawning (8–23 November 2011).

Adults were introduced into the stream channels on 7 October 2011 and removed on 10 December 2011.	126
Figure 4.2. Seasonal patterns of gross primary production (GPP) of biofilm on artificial substrata receiving marine-derived nutrients (MDNs) from spawning Atlantic salmon and control treatments in: a) stream channels and b) mesocosms. See Fig. 4.1 for explanation of symbols.	127
Figure 4.3. Seasonal patterns of the P/R ratio of biofilm on artificial substrata receiving marine-derived nutrients (MDNs) from spawning Atlantic salmon and control treatments in: a) stream channels and b) mesocosms. See Fig. 4.1 for explanation of symbols.	128
Figure 4.4. Accumulation of marine-derived nitrogen (solid line) and phosphorous (dashed line) inputs from 22 Atlantic salmon spawning in stream channels at the Mactaquac Biodiversity Facility, New Brunswick, Canada.	129
Figure 4.5. Increase in algal productivity (chlorophyll mg m^{-2}) as a function of marine-derived nitrogen inputs from spawning Atlantic salmon in: a) stream channels and b) mesocosms. Marine-derived nitrogen inputs are from adult Atlantic salmon spawning in stream channels at the Mactaquac Biodiversity Facility, New Brunswick, Canada.	130
Figure 4.6. Increase in algal productivity (chlorophyll mg m^{-2}) as a function of marine-derived phosphorous inputs from spawning Atlantic salmon in: a) stream channels and b) mesocosms. Marine-derived phosphorous inputs are from the same source as noted in Figure 4.5.	131

Figure 5.1. Schematic drawing of the experimental stream-channels. V: water inflow, adjustable with a valve; O: water outflow via an overflow weir regulating the water level in the stream-channel with a mesh panel (wire mesh size 2.5 mm) to block fish emigrations; Ri: riffle habitats; Ru: run habitats; P: pool habitats. Arrows indicate direction of flow..... 171

Figure 5.2. Seasonal patterns in mean % lipid ($\pm$ one standard deviation) in a) stream-channel biofilm, b) invertebrates, and c) Atlantic salmon parr (muscle) receiving marine-derived nutrients (MDNs) from spawning Atlantic salmon and control treatments. MDN treatments: shaded symbols; controls: open symbols. Values with an * denote a statistically significant difference ($p < 0.05$) between stream-channels with MDN inputs and the controls for the same sampling period. Shaded areas denote the period of spawning (November 8 – 23, 2011). Adult salmon were introduced into the stream-channels on October 7, 2011 and removed on December 10, 2011..... 172

Figure 5.3. Principle component analysis (PCA) plots of fatty acids in biofilm (green), invertebrates (black), Atlantic salmon parr (blue) and Atlantic salmon eggs (green star). Treatments: MDN subsidies: shaded symbols, controls: open symbols. Sampling times: triangle (October 6, 2011), square (November 22, 2011), diamond (December 9, 2011), circle (December 28, 2011), and inverted triangle (January 25, 2012). The eigenvectors (represented by an individual fatty acid and corresponding vector length) reflect the importance of that fatty acid's contribution to distinguishing between the first two principal component axes. 174

Figure 5.4. Percent average dissimilarity in fatty acid profiles as calculated using Similarity Percentage (SIMPER) analysis based on a Bray-Curtis similarity matrix. For each group (biofilm, invertebrates, and salmon parr), at each sampling period (0 – 4), dissimilarity represents the differences between treatment and control stream-channels. Dissimilarity was also compared between treatment (T) and control (C) stream-channels for between group differences (e.g. between biofilm and invertebrates denoted as biofilm:inverts).....	175
Figure 5.5. Temporal changes in the fatty acids (mean $\pm$ 1 SD) identified using Similarity Percentage (SIMPER) analysis which accounted for 68.8% of the differences between biofilm (a-e), invertebrates (f-j), and Atlantic salmon parr (k-o) in stream-channels receiving marine-derived nutrients from spawning Atlantic salmon (shaded symbols) and control stream-channels (open symbols). Fatty acids identified were 16:0 (a, f, k), 16:1(n-7) (b, g, l), 18:1(n-9) (c, h, m), 20:5(n-3) (d, i, n), and 22:6(n-3) (e, j, o). The dashed line represents the fatty acid value of the eggs; see figure 2 for explanation of the rest of the symbols. Shaded areas denote the period of spawning (November 8 – 23, 2011).....	176
Figure 5.6. Box and whisker plots of the Euclidean distance between the group centroid and each sample within that group: for a) biofilm, b) invertebrates, and c) Atlantic salmon parr. For stream-channels receiving marine-derived nutrients (MDNs) from spawning Atlantic salmon and control treatments, sampling periods were divided into initial (time 0), assimilation period (times 1, 2, and 3) and final (time 4).....	178
Figure 6.1. Map of the Miramichi River catchment, New Brunswick, Canada.	215

Figure 6.2. Atlantic salmon (diamond symbols and black line) and gaspereau (collective term for alewives and blueback herring) (square symbols and blue line) landings, in the Miramichi River. Values with associated error bars represent the maximum and minimum landing estimates surrounding the mean (symbol) as historic data for these time periods was commonly recorded as a range rather than absolute values..... 216

Figure 6.3. Figure 3: Landings and escapement for a) Atlantic salmon and b) gaspereau. Diamond symbols with the solid black line represent landings. For Atlantic salmon the triangles with solid blue line represent total salmon escapement, circles and large dashed grey line represents one-sea winter spawners, and the squares and small dashed light blue line represents multi-sea winter spawners. For gaspereau the triangles with solid blue line represent total gaspereau escapement, circles and large dashed grey line represents alewife spawners, and the squares and small dashed light blue line represents blueback herring spawners..... 218

Figure 6.4. Comparison of the estimated nitrogen and phosphorous flux from nutrient loading models between Atlantic salmon landings and escapement data in the Miramichi River..... 219

Figure 6.5. Comparison of the estimated nitrogen and phosphorous flux from nutrient loading models between gaspereau landings and escapement data in the Miramichi River..... 220

Figure 6.6. Estimated nitrogen and phosphorous flux from nutrient loading models based on Atlantic salmon landings in the Miramichi River..... 221

Figure 6.7. Estimated nitrogen and phosphorous flux from nutrient loading models based on gaspereau landings in the Miramichi River. 222

Figure 6.8. Conceptual representation of relative marine-derived nutrient (MDN) inputs to a river from rainbow smelt (dark blue), alewives (yellow), sea lamprey (light blue), and Atlantic salmon (red). Shaded areas represent the relative contribution over their freshwater residency, with the dark areas indicating the period of spawning. Dashed lines represent this relative contribution in a historical context.	223
---	-----

Figure 6.9. Distribution of active harvest plots across the entire Miramichi River catchment between 2001 and 2014; includes all industrial freehold, public, and private land (based on Hansen et al. 2013 and Linke et al. 2017).	224
---	-----

Chapter 1

General Introduction

Ecosystem productivity is fueled by nutrient subsidies and cycling from within an ecosystem or supplemental contributions (Polis et al. 2004). Pacific salmon migrating from the ocean to fresh water to spawn, deliver marine-derived nutrients (MDNs) (e.g. nitrogen, phosphorous, fatty acids) in the form of excretory metabolites, gamete release, and carcass decomposition (Bilby et al. 1996, Wipfli et al. 1998, Gende et al. 2002). These MDNs provide an important seasonal marine resource to freshwater and riparian ecosystems (Durbin et al. 1979, Schindler et al. 2003, Johnston et al. 2004, Walters et al. 2009) and have the potential to increase stream ecosystem productivity (Naiman et al. 2002, Mitchell and Lamberti 2005); the loss of these MDN may reduce stream productivity (Stockner and Ashley 2003). In the Pacific Northwest, MDN subsidies delivered by Pacific salmon (*Oncorhynchus* spp.) have resulted in increases in biofilm biomass (Wipfli et al. 1998, Johnston et al. 2004, R  egg et al. 2012) and productivity (Levi et al. 2013), macroinvertebrate abundance and biomass (Wipfli et al. 1998, Chaloner et al. 2004, Lessard and Merritt 2006, Lessard et al. 2009), and increases in juvenile salmon growth (Wipfli et al. 2003) and abundance (Chaloner et al. 2002, Wipfli et al. 2010). Although less studied, MDNs have been found to have widespread effects at multiple trophic levels in Atlantic river systems, including biofilm (Walters et al. 2009), zooplankton (Durbin et al. 1979), macroinvertebrates (Jardine et al., 2009; Walters et al., 2009), and fish (Garman and Macko, 1998; MacAvoy et al. 2009).

Many anadromous fishes in Atlantic Canada do not die after spawning, but collectively their release of excretory by-products and gametes may result in quantities of MDN comparable to those of Pacific salmon (Jardine et al. 2005, 2009). Rainbow smelt (*Osmerus mordax*) are iteroparous and return to the sea after spawning. In early spring,

they spawn in small streams just above the head of tide (McKenzie 1964, Chase 2009) laying small, adhesive demersal eggs. Alewives (*Alosa pseudoharengus*) also are iteroparous, spawning in late spring, depositing nonadhesive, demersal eggs in headwater lakes or lake-like areas in rivers (Mullen et al. 1986, Mather et al. 2012). Therefore nutrient subsidies to lotic portions of the river are primarily through excretory products and decomposition of unsuccessful spawners. Sea lamprey (*Petromyzon marinus*), an obligate semelparous species, build redds and spawn in early summer in similar habitats to Atlantic salmon (Scott and Scott 1988). Atlantic salmon (*Salmo salar*), like their Pacific counterparts, spawn in the autumn, but they are iteroparous. It should be noted that these are not the only anadromous fishes found in Atlantic rivers, but rather the ones chosen for this study.

In Atlantic Canada, freshwater systems generally are nutrient limited (Elliot et al. 1998). The dramatic decline of anadromous fishes in the past century and the subsequent reduction of MDNs may have had profound effects on aquatic production, particularly in nutrient-poor systems. Biofilm is a complex of autotrophic and heterotrophic organisms embedded in a polysaccharide matrix and attached to submerged organic and inorganic surfaces (Lock et al. 1984). The algal, fungal, and bacterial community groups bound within the polysaccharide matrix exchange nutrients and metabolic byproducts with the boundary layer (Wetzel 2001). Algae produce assimilable photosynthetic organic compounds used by bacteria (Romaní and Sabater 2001) and fungi (Findlay 2010) and bacteria have a synergistic relationship with fungi, however the mechanisms are unknown (Bengtsson 1992). Fungi produce particulate organic matter biologically available to algae and bacteria (Findlay 2010) and algae incorporate micronutrients secreted by

bacteria (Nalewajko et al. 1980). Biofilms are the foundation of stream food webs, and contribute significantly to overall primary productivity. Therefore, they are the basis for bottom-up effects of MDN. The storage and release of nutrients from the substratum acts as a mechanism by which nutrients are made available to primary producers after anadromous fish have emigrated (O'Keefe & Edwards, 2002), which enables the biofilm to increase metabolic functioning and keep food-web productivity high during an otherwise slow period of metabolism. Petticrew, Rex & Albers (2011) demonstrated that MDNs are retained and stored within the substratum, lengthening the period of nutrient availability. Any changes in stream nutrient dynamics (e.g. marine-nutrient subsidies) will be reflected on the biofilm community, either directly on particular organisms or as a cascading indirect effect on multiple members of the community.

Stable isotope analysis of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) is an effective tool for identifying marine resources in freshwater food webs and allows for dietary inferences to be made due to the predictability of isotopic relationships between consumers and their food (e.g. Bilby et al. 1996, Garman and Macko 1998, Chaloner et al. 2002, Jardine et al. 2009, Reisinger et al. 2013). Stable isotope composition reflects both trophic structure and function, making inferences about food web dynamics possible (Finlay et al. 1999). The predictable fractionation patterns of $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ are well documented and have been useful to identify food sources and trophic position, respectively (Post 2002). Marine environments are typically enriched in the heavy isotopes of nitrogen (^{15}N) and carbon (^{13}C), whereas freshwater systems are typically depleted, facilitating the use of stable isotope analysis to trace MDNs through aquatic food webs (Kline et al. 1990). Isotopic enrichment primarily provides indirect evidence

of MDNs, the use of mixing models (e.g. SIAR) can increase the understanding of the importance of MDNs by identifying their relative contributions to a consumer's diet (Phillips and Gregg 2001, Moore and Semmens 2008). This better estimates the proportion of diet attributable to direct and indirect sources and pathways of incorporation.

Other biomarker methods such as lipid and fatty acid analysis can help identify the ecophysiological importance of MDNs. Similar to stable isotope analysis, when sources have distinct fatty acid compositions, fatty acid profiles can be used to characterize freshwater and marine food webs (Henderson and Tocher 1987) and resources consumed by freshwater biota (Heintz et al. 2004). This is possible because, unlike other dietary nutrients, fatty acids are not generally degraded and retain their basic form in consumer tissues (Budge et al. 2006). Lipids supply energy and essential nutrients that are critical for metabolic functions, growth and reproduction (Müller-Navarra et al. 2000) and are widely considered to be among the most important nutritional compounds. Fatty acid profiles are not only an effective biomarker for studying trophic transfer (Iverson et al. 2004) but also provide information as to their importance as essential nutrients. Therefore lipid analysis represents surplus energy and a measure of nutritional condition or an increase in the *quality* of resources (Fraser 1989).

1.1 Objectives

The goal of my dissertation was to understand the importance of returning anadromous fishes on freshwater ecology and trophic interactions. Specifically the objectives were: 1) to investigate whether MDNs from spawning anadromous fishes

resulted in changes in the biofilm constituents in Atlantic rivers; 2) evaluate how different timing and spawning strategies in anadromous fishes influence resource subsidization through indirect and direct trophic pathways within freshwater food webs; 3) determine the effects of spawning Atlantic salmon on stream productivity; 4) investigate whether Atlantic salmon are a significant source of marine fatty acids to freshwater food webs; 5) determine the significance (current and historic) of MDN as a source of nutrients for increasing freshwater productivity and how life history and spawning strategy affect this MDN flux. To address these objectives the following studies were conducted:

- biofilm community responses to MDN inputs (Chapter 2)
- timing and response of MDN uptake into aquatic food webs (Chapter 3)
- changes in freshwater production and productivity associated with MDNs (Chapter 4)
- uptake and storage of marine-derived fatty acids in freshwater organisms (Chapter 5)
- comparative analysis of MDN inputs of pre-exploitation relative to present day (Chapter 6)

1.2 Dissertation Overview

My dissertation is focused on identifying the relative roles MDNs from a variety of anadromous fishes play in regulating freshwater food web dynamics. To achieve this I combine observational studies, experimental frameworks, and analytical techniques to understand the linkages between freshwater and marine ecosystems, as presented in four chapters (Chapters 2-5). I also looked at marine-nutrient contributions from a historical perspective (Chapter 6).

In CHAPTER 2 (published in *Freshwater Science* (2015) 34:881-896), I focused on biofilm community group responses to MDNs from four species of anadromous fish (rainbow smelt, alewife, sea lamprey, and Atlantic salmon). I measured the spatiotemporal changes in the abundance and composition of biofilm algae, fungi, and bacteria in six Atlantic rivers with contrasting influx of MDN. I hypothesized that with exposure to MDNs, algal, fungal, and bacterial communities would experience similar stimulated growth patterns, which would be maintained through the season relative to comparable river reaches not receiving MDN.

In CHAPTER 3 (in review *Journal of Fish Biology*), the focus was to evaluate how different timing and spawning strategies in anadromous fishes influence resource subsidization through indirect and direct trophic pathways within freshwater food webs in Atlantic rivers. I measured changes in nitrogen isotopic composition ($\delta^{15}\text{N}$) in biofilm, macroinvertebrates and resident salmonids to characterize temporal dynamics of MDN incorporation between stream reaches with and without MDN inputs. I predicted that the pathway of MDN incorporation into the tissues of freshwater organisms would differ depending on the anadromous species spawning. Furthermore, I postulated that the tissue isotopic composition of resident stream fish would shift to reflect a diet high in MDNs during the period of anadromous fish spawning.

In CHAPTER 4 (published in *Freshwater Biology* (2015) 60:1647-1658), assuming biofilm is under some degree of nutrient limitation, I investigated whether spawning Atlantic salmon alleviates such nutrient limitation, thereby increasing biofilm community metabolism and biomass. I predicted that MDNs from spawning Atlantic salmon would relieve the 'bottom-up' constraints and stimulate productivity. CHAPTER

5 (published in *Ecosphere* DOI: 10.1002/ecs2.1818) focused on whether Atlantic salmon are a significant source of marine fatty acids to freshwater food webs. Biofilm, macroinvertebrates and Atlantic salmon parr were analyzed for total lipid content and fatty acid profiles to determine if in addition to increasing freshwater production, marine-derived subsidies can also increase the *quality* of food resources by increasing energy stores in constituents of aquatic ecosystems. I predicted that access to marine subsidies would lead to increased lipid stores in consumers through direct consumption of eggs rather than via indirect consumption and trophic transfer.

In CHAPTER 6, I compared the net MDN contribution from Atlantic salmon and alewives in the Miramichi River, NB Canada, at the current (low) population levels and at their population equilibriums (with no exploitation). I predicted that with the precipitous declines in fish populations (i.e. 96% decline in Atlantic salmon and a 76% decline in alewives), net MDN loading has shifted to a point that may no longer sustain elevated levels of productivity for sustaining large fish populations. In CHAPTER 7 I summarize the results from CHAPTERS 2- 6 to make generalized predictions about how MDNs from Atlantic anadromous fishes influence stream ecosystems in relation to prevalent environmental conditions.

1.3 Literature Cited

- Bengtsson, G. 1992. Interactions between fungi, bacteria, and beech leaves in a stream mesocosm. *Oecologia* 89:542-549.
- 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.

- Budge, S. M., S. J. Iverson, and H. N. Koopman. 2006. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. *Marine Mammal Science* 22:759-801.
- Chaloner D. T., M. S. Wipfli, and J. P. Caouette. 2002. Mass loss and macroinvertebrate colonisation of Pacific salmon carcasses in south-eastern Alaskan streams. *Freshwater Biology* 47:263-273.
- Chaloner, D. T., G. A. Lamberti, R. W. Merritt, N. L. Mitchell, P. H. Ostrom, and M. S. Wipfli. 2004. Variation in responses to spawning Pacific salmon among three south-eastern Alaska streams. *Freshwater Biology* 49:587-599.
- Chase, B. C. 2009. The spawning habitat of anadromous rainbow smelt: trouble at the tidal interface. Pages 859-862 *in* Challenges for diadromous fishes in a dynamic global environment.
- Durbin, A. G., S. W. Nixon, and C. A. Oviatt. 1979. Effects of the spawning migration of the alewife, *Alosa pseudoharengus*, on freshwater ecosystems. *Ecology* 60:8-17.
- Elliott, S. R., T. A. Coe, J. M. Helfield, and R. J. Naiman. 1998. Spatial variation in environmental characteristics of Atlantic salmon (*Salmo salar*) rivers. *Canadian Journal of Fisheries and Aquatic Sciences* 55:267-280.
- Findlay, S. 2010. Stream microbial ecology. *Journal of North American Benthological Society* 29:170-181.
- Finlay, J.C., M. E. Power, and G. Cabana. 1999. Effects of water velocity on algal carbon isotope ratios: Implication for river food web studies. *Limnology and Oceanography*, 44:1198-1203.
- Fraser, A. 1989. Triacylglycerol content as a condition index from fish, bivalve, and crustacean larvae. *Canadian Journal of Fisheries and Aquatic Sciences*. 46:1868-1873.
- Garman G.C. and S. A. Macko. 1998. Contribution of marine-derived organic matter to an Atlantic coast, freshwater, tidal stream by anadromous clupeid fishes. *Journal of the North American Benthological Society*, 17:277-285.
- Gende S.M., R. T. Edwards, M. F. Willson, and M. S. Wipfli . 2002. Pacific salmon in aquatic and terrestrial ecosystems. *Bioscience*, 52:917-928.
- Heintz, R. A., B. D. Nelson, J. Hudson, M. Larsen, L. Holland, and M. Wipfli. 2004. Marine subsidies in freshwater: effects of salmon carcasses on lipid class and fatty acid composition of juvenile coho salmon. *Transactions of the American Fisheries Society* 133:559-567.
- Henderson, R. J., and D. R. Tocher. 1987. The lipid composition and biochemistry of freshwater fish. *Progress in Lipid Research* 26:281-347.
- Iverson, S. J., C. Field, W. D. Bowen, and W. Blanchard. 2004. Quantitative fatty acid signature analysis: a new method of estimating predator diets. *Ecological Monographs* 74: 211-235.
- Jardine, T. D, D. F. Cartwright, J. P. Dietrich, and R. A. Cunjak. 2005. Resource use by salmonids in riverine, lacustrine and marine environments: evidence from stable isotope analysis. *Environmental Biology of Fishes* 73: 309-319.
- Jardine, T. D, J-M Roussel, S. C. Mitchell, and R. A. Cunjak. 2009. Pathways of marine nutrient and organic matter entry from anadromous fishes into Atlantic coast streams. Pages 427-445 *in* A. J. Haro, K. L. Smith, R. A. Rulifson, C. M. Moffitt,

- R. J. Klauda, M. J. Dadswell, R. A. Cunjak, J. E. Cooper, K. L. Beal, and T. S. Avery (editors). Challenges for Diadromous Fishes in a Dynamic Global Environment. American Fisheries Society Symposium 69. Bethesda, Maryland.
- 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.
- Kline, T. C., J. J. Goering, O. A. Mathisen, P. H. Poe, and P. L. Parker. 1990. Recycling of elements transported upstream by runs of Pacific salmon: 1. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ evidence in Sashin Creek, southeastern Alaska. *Canadian Journal of Fisheries and Aquatic Sciences*. 47:136-144.
- Lessard, J. L., and R. W. Merritt. 2006. Influence of marine-derived nutrients from spawning salmon on aquatic insect communities in southeast Alaskan streams. *Oikos* 113:334-343.
- Lessard J. L., R. W. Merritt, and M. B. Berg. 2009. Investigating the effect of marine-derived nutrients from spawning salmon on macroinvertebrate secondary production in southeast Alaskan streams. *Journal of the North American Benthological Society* 28:683-693.
- Levi, P. S., J. L. Tank, J. R  egg, 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.
- Lock, M. A, R. R. Wallace, J. W. Costerton, R. M. Ventullo, and S. E. Charlton. 1984. River epilithon: toward a structural-functional model. *Oikos* 42:10-22.
- MacAvoy S. E., G. C. Garman, and S. A. Macko. 2009. Anadromous fish as marine nutrient vectors. *Fishery Bulletin*. 107:165-174.
- Mather, M. E., H. J. Frank, J. M. Smith, R. D. Cormier, R. M. Muth, and J. T. Finn. 2012. Assessing freshwater habitat of adult anadromous alewives using multiple approaches. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science*, 4:188-200.
- McKenzie, R. A. 1964. Smelt life history and fishery in the Miramichi River, New Brunswick. Fisheries Research Board of Canada, St Andrews, NB. Bulletin No. 144.
- Mitchell, N. L., and G. A. Lamberti. 2005. Responses in dissolved nutrients and epilithon abundance to spawning salmon in southeast Alaska streams. *Limnology and Oceanography* 50:217-227.
- Moore, J. W. and B. X. Semmens. 2008. Incorporating uncertainty and prior information into stable isotope mixing models. *Ecology Letters*. 11: 470–480.
- Mullen, D. M., C. W. Fay, and J. R. Moring. 1986. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (North Atlantic) - alewife/blueback herring. U.S. Fish and Wildlife Service, Biological Report 82(11.58).
- M  ller-Navarra, D. C., M. T. Brett, A. M. Liston, and C. R. Goldman. 2000. A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. *Nature* 403:74-77.
- 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* 5:399-417.
- Nalewajko, C., K. Lee and P. Fay. 1980. Significance of Algal Extracellular Products to Bacteria in Lakes and in Cultures. *Microbial Ecology*. 6:199-207.
- Petticrew E.L., J. F. Rex, and S. J. Albers. 2011. Bidirectional delivery of organic matter between freshwater and marine systems: the role of flocculation in Pacific salmon streams. *Journal of the North American Benthological Society*. 30:779–786.
- Phillips, D. L. and J. W. Gregg. 2001. Uncertainty is source partitioning using stable isotopes. *Oecologia*. 127:171-179.
- 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.
- Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, 83, 703-718.
- O’Keefe T.O. 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.
- Reisinger, A. J., D. T. Chaloner, J. Rüeegg, 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.
- Romaní, A. M. and S. Sabater. 2001. Structure and activity of rock and sand biofilms in a Mediterranean stream. *Ecology*. 82:3232-3245.
- Rüeegg, 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.
- Schindler D. E., M. D. Scheuerell, J. W. Moore, S. M. Gende, T. B. Francis, and W. J. Palen. 2003. Pacific salmon and the ecology of coastal ecosystems. *Frontiers in Ecology and the Environment* 1:31-37.
- Scott, W. B. and M. G. Scott. 1988. Atlantic fishes of Canada. *Canadian bulletin of Fisheries and Aquatic Sciences* No. 219.
- Stockner, J. G., and K. I. Ashley. 2003. Salmon nutrients: closing the circle. Pages 3-15 in J. G. Stockner, editor. *Nutrients in salmonid ecosystems: sustaining production and biodiversity*. American Fisheries Society, Symposium 34, Bethesda, MD.
- Walters A. W., R. T. Barnes, and D. M. Post. 2009 Anadromous alewives (*Alosa pseudoharengus*) contribute marine-derived nutrients to coastal stream food webs. *Canadian Journal of Fisheries and Aquatic Sciences* 66:439-448.
- Wetzel, R. G. 2001. *Limnology: Lake and River Ecosystems*. 3rd edition. Academic Press, Orlando, Florida.
- Wipfli, M. S., J. Hudson, and J. Caouette. 1998. Influence of salmon carcasses on stream productivity: response of biofilm and benthic macroinvertebrates in southeastern Alaska, U.S.A. *Canadian Journal of Fisheries and Aquatic Sciences* 55:1503-1511.
- Wipfli, M.S., J. Hudson, and J. Caouette. 2003. Marine subsidies in freshwater ecosystems: salmon carcasses increase the growth rates of stream-resident salmonids. *Transactions of the American Fisheries Society* 132:371-381.

Wipfli, M. S., J. P. Hudson, J. P. Caouette, N. L. Mitchell, J. L. Lessard, R. A. Heintz, and D. T. Chaloner. 2010. Salmon carcasses increase stream productivity more than inorganic fertilizer pellets: a test on multiple trophic levels in streamside experimental channels. *Transactions of the American Fisheries Society*. 139:824–839.

Chapter 2

Spatiotemporal responses of algal, fungal, and bacterial biofilm communities in Atlantic rivers receiving marine-derived nutrient inputs

Kurt M. Samways¹, Zoraida J. Quiñones-Rivera², Peter R. Leavitt², and Richard A. Cunjak¹

¹Canadian Rivers Institute and Department of Biology, University of New Brunswick,
P.O. Box 4400, 10 Bailey Avenue, Fredericton, New Brunswick E3B 5A3 Canada

²Limnology Laboratory and Department of Biology, University of Regina, 3737 Wascana
Parkway, Regina, Saskatchewan S4S 0A2 Canada

Published: *Freshwater Science*. 2015. 34(3):881–896. DOI: 10.1086/681723. © 2015 by
*The Society for Freshwater Science. Reproduced with the kind permission of The
University of Chicago Press.*

2.1 Abstract

Spawning anadromous fishes can enhance primary and secondary production in streams by providing marine-derived nutrients (MDN), but little is known of their effects on individual biofilm constituents (algae, fungi, and bacteria). To address this issue, we measured spatial and temporal changes in the abundance and composition of biofilm algae, fungi, and bacteria in 6 Atlantic rivers with contrasting influx of MDN. Each river had a natural barrier to anadromous fish migration, which established an upstream control and downstream treatment area, with Atlantic Salmon (*Salmo salar*), Alewife (*Alosa pseudoharengus*), Rainbow Smelt (*Osmerus mordax*), or Sea Lamprey (*Petromyzon marinus*) as the primary anadromous species. In areas receiving MDN, increases in algal and fungal biomass and bacterial density were maintained throughout the season. Algal communities showed the largest (2–10×) increase in abundance over control sites, and Rainbow Smelt spawning streams had the greatest accrual. The river with Alewife showed the largest (2×) increase in fungal biomass during the spawning period. Changes in bacterial density were greatest (2× increase) in the Rainbow Smelt spawning streams. An increase in spawning density (i.e., nutrient load) supported greater biomass accumulation, but the system appeared to saturate quickly. Biofilm in Rainbow Smelt and Alewife streams were enriched up to 2.5‰ in $\delta^{15}\text{N}$ during spawning, whereas a 1‰ enrichment occurred in the Sea Lamprey and Atlantic Salmon streams compared with system baseline values. The degree to which MDN drive productivity and the mechanism of how these nutrients are incorporated to increase productivity and ultimately fish production remain unknown.

2.2 Introduction

Nutrient subsidies and cycling drive productivity in freshwater ecosystems. Anadromous fishes migrating from the ocean to fresh water to spawn deliver marine-derived nutrients (MDN) in the form of excretion metabolites, gamete release, and carcass decomposition (Bilby et al. 1996). These MDN may constitute a significant nutrient subsidy to recipient fresh waters (Durbin et al. 1979, Johnston et al. 2004, Walters et al. 2009), which is important for stimulating and maintaining productivity (Naiman et al. 2002), and the loss of these MDN may reduce stream productivity (Stockner and Ashley 2003). In the Pacific Northwest, Pacific salmon runs have declined significantly, precipitating declines in productivity (Schindler et al. 2005). Although these reductions are well documented in western North America, MDN contributions to freshwater productivity in Atlantic rivers have received little investigation (Nislow et al. 2004, Post and Walters 2009).

In the Pacific Northwest, MDN subsidies delivered by Pacific salmon (*Oncorhynchus* spp.) have resulted in increases in biofilm biomass (Wipfli et al. 1998, Johnston et al. 2004, R  egg et al. 2012) and productivity (Levi et al. 2013), although in some cases disturbance from spawning salmon has decreased biofilm biomass (Moore and Schindler 2008). Semelparous Pacific salmon contribute MDN through carcass loading of all spawners, gametes, and excretory products (Wipfli et al. 1998). Many of these studies were focused on the effects of dissolved MDN and organic matter delivered by anadromous fishes on biofilm accrual, but in several studies, consumption of carcass and egg material also was an important driver of productivity. Changes in macroinvertebrate abundance and biomass (Wipfli et al. 1998, Chaloner et al. 2004,

Lessard and Merritt 2006, Lessard et al. 2009), and increases in juvenile salmon growth (Wipfli et al. 2003) and abundance (Chaloner et al. 2002, Wipfli et al. 2010) also have been observed with salmon-derived inputs. MDN inputs from Atlantic anadromous species are not as well explored as those on the Pacific coast, but a number of investigators have shown that they are important to freshwater ecosystems (Durbin et al. 1979, Jonsson and Jonsson 2003, Nislow et al. 2004, Jardine et al. 2009, Nislow and Kynard 2009, Walters et al. 2009, Guyette et al. 2013, 2014). The diverse life histories and spawning strategies of Atlantic anadromous fishes probably influence the relative effects of MDN from partial carcass loading, gametes, and excretory products on stream productivity.

Unlike Pacific salmon, Rainbow Smelt (*Osmerus mordax*) are iteroparous and return to the sea after spawning. In early spring, they spawn in small streams just above the head of tide (McKenzie 1964, Chase 2009). Excretory products provide dissolved nutrients immediately available for uptake by biofilm communities (Vanni 2002), and the small, demersal eggs are readily available for direct consumption by benthic invertebrates and fish. Unsuccessful eggs, shells from hatched eggs, and limited carcasses provide nutrients and organic matter through breakdown and subsequent nutrient release (Parmenter and Lamarra 1991). Alewives (*Alosa pseudoharengus*) also are iteroparous. They spawn in late spring, deposit nonadhesive, demersal eggs in headwater lakes or lake-like areas in rivers (Mullen et al. 1986, Mather et al. 2012). Other than in the lentic spawning areas, nutrient subsidies to lotic portions of the river are primarily through excretory products and decomposition of unsuccessful spawners. Sea Lamprey (*Petromyzon marinus*), the only obligate semelparous species that we sampled, build

redds and spawn in early summer in similar habitats to Atlantic Salmon (Scott and Scott 1988). In addition to excretory products and gamete release, Sea Lamprey potentially contribute large amounts of MDN and organic matter through the body mass of dead spawners (Nislow and Kynard 2009). Atlantic Salmon (*Salmo salar*), like their Pacific counterparts, spawn in the autumn, but they are iteroparous, so metabolic by-products and gametes are the primary sources of MDN.

In Atlantic Canada, freshwater systems generally are nutrient limited (Elliot et al. 1998). Many anadromous fishes in Atlantic Canada do not die after spawning, but collectively their release of excretory by-products and gametes may result in quantities of MDN comparable to those of Pacific salmon (Jardine et al. 2005, 2009). The dramatic decline of these fishes in the past century and the subsequent scarcity of these MDN may have profound effects on aquatic production, particularly in nutrient-poor systems. Nutrient availability plays a primary role in stream dynamics, and the spatial and temporal availability of these resources can influence community structure and ecosystem functioning.

Biofilm, in the context of our study, is the matrix of autotrophic and heterotrophic organisms growing on the surface of organic and inorganic substrates in streams and may be particularly sensitive to changes in MDN influx. Stream biofilms are a complex community of algae, fungi, and bacteria within a self-excreted polysaccharide matrix (Lock et al. 1984). In addition to light availability (Rosemond et al. 2000), constraints on the productivity of these communities are frequently related to the amount of nutrients available to periphytic algae (Carr et al. 2005, Chaloner et al. 2007, Fisher, Wold and Hershey 1999, Romaní and Sabater 1999) and bacteria (Haack and McFeters 1982,

Sobczak 1996, Sobczak and Burton 1996, Romani and Sabater 2001). Studies of periphytic fungi are limited (Porter-Goff et al. 2010) and tend to be focused on epixylic, rather than epilithic biofilm (Tank and Webster 1998, Tank and Dodds 2003). Most of the research on the effects of MDN on biofilm has been centered on Pacific salmon (e.g., Wipfli et al. 1998, Collins et al. 2011, Holtgrieve and Schindler 2011, R  egg et al. 2012, Levi et al. 2013) and evaluation of whole-community responses, rather than algal, fungal, and bacterial community responses separately. Many comparative studies on assemblage structure of biofilm communities were not focused on all 3 of these components (algae, fungi, bacteria), and few published studies have addressed the interactions among all 3 of these primary biofilm groups (Porter-Goff et al. 2010). Biofilms are the foundation of stream food webs, and contribute significantly to overall primary productivity. Therefore, they are the basis for bottom-up effects of MDN. Any changes in stream ecosystem dynamics will be reflected on the biofilm community, either directly on particular organisms or as a cascading indirect effect on multiple members of the community. These shifts in food web productivity in response to environmental change are evidence of the dynamic nature of biofilm.

The focus of our research was to investigate whether MDN from spawning anadromous fishes resulted in changes in the biofilm constituents in Atlantic rivers. The objectives of this study were to: 1) quantify changes in biofilm algae, fungi, and bacteria, and 2) assess spatial and temporal dynamics in biomass accumulation as a function of spawning density. We used stable-isotope analysis of C ($\delta^{13}\text{C}$) and N ($\delta^{15}\text{N}$) to assess whether biofilm assimilated the MDN. The algal response to nutrient inputs is well established in the literature, so we predicted that with exposure to MDN, algal, fungal,

and bacterial communities would demonstrate similar stimulated growth patterns, and that this standing-stock accrual would be maintained through the season relative to comparable river reaches not receiving MDN.

2.3 Methods

2.3.1 Study sites

We compared biofilm accrual in 6 streams in New Brunswick and Nova Scotia, Canada, chosen for study in 2009 and 2010 based on the anadromous fish species present and timing of the spawning run (Fig. 2.1). We selected rivers to include MDN transport primarily by Rainbow Smelt (Knoydart Brook and Indiantown Brook), Alewife (Oromocto River), Sea Lamprey (Cross Creek), or Atlantic Salmon (Rocky Brook), although minor contributions by other anadromous fishes were present in each system (Table 2.1). Doctor's Brook served as a control system with no MDN inputs, although prior to 2009, Rainbow Smelt commonly spawned in this river (e.g., Jardine et al. 2009).

2.3.2 Experimental design and sample collection

For each river, 2 reference sites (no anadromous fish) were selected upstream of a natural barrier (impassable waterfall), and 3 sites receiving MDN were selected downstream of the barrier. The upstream sites and Doctor's Brook allowed for controls at 2 scales of spatial resolution. Biofilm samples were collected during prespawning, spawning, and postspawning periods in up- and downstream sites following a Before-After-Control-Impact (BACI) study design (e.g., Stewart-Oaten et al. 1986, Mitchell and Lamberti 2005). This upstream–downstream approach has been used to evaluate the

effects of MDN in several studies (e.g., Wipfli et al. 1999, Chaloner et al. 2004, Mitchell and Lamberti 2005, Collins et al. 2011). We assumed that anadromous fish spawning did not affect stream processes upstream of the natural barrier. Therefore, we compared measurements from downstream sites to the corresponding upstream sites through time to track incorporation of MDN into the food web.

We used artificial substrates for colonizing biofilm under natural stream conditions and for subsequent sampling. These substrates were 10- × 10-cm slate tiles, preconditioned to remove organics by combustion at 550°C for 12 h in a muffle furnace. We attached 14 tiles to a plastic mesh in a 2 × 7 rectangular grid with a tile-width separation between tiles. We randomly placed and secured to the stream bed 3 tile grids/site and incubated them for 3 wk before any anadromous fish spawning.

We collected biofilm samples after colonization occurred and included prespawning, spawning, and postspawning periods. At each site, we collected 1 tile randomly from each tile grid and placed it in a container with stream water. We scraped biofilm from the upper surface of each tile until we exposed bare tile. We used subsamples in 4 different analyses: 1) biomass of algae (measured as chlorophyll pigments), 2) biomass of fungus (as ergosterol), 3) density of bacteria (direct count), and 4) MDN assimilation (stable-isotope analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$).

Increased $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable-isotope signatures would suggest incorporation of MDN into the biofilm. To ensure that these isotopic changes were the result of incorporation of dissolved MDN rather than eggs and other marine-origin particulates embedded in the biofilm matrix, we placed 3 additional tiles, affixed to the substrate, inside enclosures at each downstream site in Knoydart Brook. These enclosures excluded

anadromous fish and particulates >243 μm (mesh size of the enclosure). We also sampled tiles postspawn, as previously described, for stable-isotope analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

2.3.3 Laboratory analyses

For algal analysis, we filtered 3 replicate samples of biofilm material onto glass-fiber filters (Whatman GF/C, Maidstone, UK) in the field, stored the filters in dark containers (to minimize pigment degradation), and kept them on ice until they could be frozen at the laboratory. We thawed frozen filters and extracted biofilm pigments in a standard mixture of acetone:methanol (80:15 by volume). We measured algal biomass by quantifying concentrations of algal pigments using a standard trichromatic protocol (Jeffrey and Humphrey 1975).

We extracted and quantified ergosterol to estimate fungal biomass (Gessner 2005). We filtered samples onto glass-fiber filters and froze them for preservation as described above. We quantified the unreplicated samples using the high-performance liquid chromatographic (HPLC) protocol described by Gessner (2005).

We placed biofilm samples (5 mL) collected for bacterial counts in sterile glass vials and preserved them with Lugol's iodine solution (Pomroy 1984). We counted bacteria with a modified 4,6-diamidino-2-phenylindole (DAPI) stain and epifluorescence microscopy protocol, adapted from Porter and Feig (1980) and Tumber et al. (1993). Modifications included heating the sample at 65°C for 15 min, acidifying the sample to a pH of 2.0 to 2.2 with 1 M formic acid, and adding 600 μL of sodium thiosulfate solution to clear the sample and 500 μL sodium pyrophosphate, which acts as a surfactant to dissolve any bacteria attached to the biofilm.

We oven-dried biofilm samples collected for stable isotope analysis at 60°C for 48 h, ground them to a homogenous powder, and weighed them in Sn cups. Stable-isotope analysis of C and N was done in the Stable Isotopes in Nature Laboratory (SINLAB), Canadian Rivers Institute, University of New Brunswick. Samples were analyzed with either a Costech 4010 Elemental Combustion System (Costech, Milan, Italy) or a Carlo Erba NC2500 Elemental Analyzer (Carlo Erba, Milan, Italy) interfaced through either a Thermo Finnigan ConFlo II or ConFlo III elemental analyzer (Thermo Finnigan, Bremen, Germany) to a continuous-flow mass spectrometer (Thermo Finnigan Delta Plus or Delta Plus XP). Isotope values, reported in standard δ -notation, were measured in units of parts per thousand (‰) and calculated using the following formula:

$$(2.1) \quad \delta X = (R_{sample} - R_{standard}) - 1$$

where X is the stable isotope of interest (^{13}C or ^{15}N), and R is the heavy:light isotope ratio.

Stable-isotope values were calibrated to Vienna PeeDee Belemnite (C) and atmospheric N using known laboratory standards: acetanilide, nicotinamide, (Elemental Microanalysis Ltd., Okehampton, UK), ammonium sulfate (N1, N2; International Atomic Energy Agency [IAEA], Vienna, Austria), sucrose (IAEA-CH6), polyethylene foil (IAEA-CH7), and peach leaf (National Institute of Standards and Technology [NIST] 1547; Gaithersburg, Maryland). Duplicate determination of actual samples agreed to within 0.2‰.

2.3.4 Data analysis

We expected biofilm accrual to vary naturally through time, and we assumed that

all sites within a river would experience similar seasonal changes. We measured algal, fungal, and bacterial biomass/density and isotope ratios before anadromous fish spawning and assumed that these values were background or baseline conditions. To account for inherent differences in background isotope values and compare changes in isotopic incorporation correctly among down- and upstream sites, we standardized all ^{15}N values to baseline values by subtracting the prespawning ^{15}N isotope values ($\delta^{15}\text{N}_0$) obtained for each sampling location from postspawning values ($\delta^{15}\text{N}_i$):

$$(2.2) \quad \Delta\delta^{15}\text{N} = (\delta^{15}\text{N}_i - \delta^{15}\text{N}_0)$$

We evaluated the effect of sampling up- or downstream of a spawning barrier and time on algal biomass, fungal biomass, bacterial density, and stable-isotope ratios using mixed-model analyses of variance (ANOVAs). The ANOVA model included the fixed factors barrier (above or below), date (sampling period), and the random factor site (2 or 3 replicates above or below the barrier, respectively). For the algal biomass and bacterial density analyses, the level of replication was the samples (3/site). However, samples for fungal biomass were unreplicated, so the replication unit for this analysis was the site. The replication unit for the stable-isotope analysis also was the site. The appropriate denominators for F-ratios were identified following Underwood (1997) (Table 2.2). We conducted multiple comparisons of significant fixed sources of variation with Tukey's test.

We calculated effect size to express the general response of algae, fungi, and bacteria to MDN. We calculated cumulative effect sizes and 95% confidence intervals (CI) for response variables chlorophyll, ergosterol, bacterial counts, and $\delta^{15}\text{N}$ as

described by Janetski et al. (2009). We calculated effect sizes for all constituents in all rivers and in each river, and for each group (algae, fungi, bacteria) in all rivers and in each river. We also calculated the effect size for $\delta^{15}\text{N}$ stable-isotope data in all rivers and in each river.

We fit the relationship between Rainbow Smelt spawning density and net algal, fungal, and bacterial increase to a Monad model (McIntire 1973, Borchardt 1996). This model is used frequently to represent the relationship between periphyton growth and nutrient concentration. We built this relationship using Rainbow Smelt because we had comprehensive spawning estimates for the entire river and individual study sites, whereas spawning estimates for the other rivers were not as rigorous and may have led to spurious results. Rainbow Smelt spawning estimates for Doctor's, Knoydart, and Indiantown Brooks for 2009 and 2010 were compared to net increases in algal and fungal biomass and bacterial density.

We estimated the net increase in abundance from the plots of biomass/density through time as the area between the up- and downstream accrual curves. We calculated area as the weighted average of abundance for each sampling period using the trapezoid rule (see Appendix 1, Appendix 2 for details). This approach yielded 1 time-integrated value of the net increase in accrual relative to the system baseline (upstream of barrier). Assuming abundance is an independent, normally distributed, random variable, the square root of the sum of the variances can be used to calculate the standard deviation for the weighted-average estimate.

2.4 Results

Standing crop of constituent groups of the biofilm (epilithon) varied seasonally, and downstream sites had greater algal and fungal biomass and bacterial density than upstream reference sites (Fig. 2.2A– R, Table 2.3). Algal, fungal, and bacterial abundance all increased significantly after rivers received MDN (Fig. 2.2A– R). In all rivers, except Doctor's Brook (no anadromous fish), fungal biomass (Fig. 2.2G– L) and bacterial density (Fig. 2.2M– R) reached a maximum followed by a decline before stabilizing or increasing again. Algal biomass showed this pattern in only 3 rivers (Fig. 2.2C, D, F), and had a more linear pattern through time in 1 of the Rainbow Smelt rivers (Knoydart Brook; Fig. 2.2B) and the Sea Lamprey river (Cross Creek; Fig. 2.2E). The Alewife river (Oromocto River) supported the greatest fungal biomass during spawning and the greatest seasonal increase of fungal biomass of any river (Fig. 2.2J). It also had the greatest seasonal increase in bacterial density (Fig. 2.2P). Knoydart Brook showed the largest seasonal increase in algal biomass of any river (Fig. 2.2B). Only Doctor's Brook, which had no anadromous Rainbow Smelt spawn in 2009 and 2010, showed no difference in biofilm standing crop between the up- and downstream sites.

MDN had an overall positive effect on community standing stock (cumulative effect size 1.522, 95% CI = 0.945–1.657; Table 2.4). Bacteria had the greatest cumulative effect size of the 3 groups (1.092, 95% CI = 0.949–1.647). Of the 5 rivers receiving MDN, the greatest effect was on Knoydart Brook (1.033, 95% CI = 0.851– 1.777) and the lowest was on Cross Creek (0.687, 95% CI = 0.532–1.043). Doctor's Brook, which did not receive MDN, had a negative cumulative effect size (– 0.015, 95% CI = – 0.272– 0.208). Mean cumulative effect sizes for algal, fungal, and bacterial responses differed

among rivers. Indiantown Brook had the largest effect size for the algal group (0.687, 95% CI = 0.422–0.999), whereas Rocky Brook had the greatest effect size for the fungal group (1.061, 95% CI = 0.720–1.913), and Knoydart Brook had the greatest effect size for the bacterial group (2.010, 95% CI = 1.595–2.961). Marine-derived nutrient inputs resulted in positive cumulative effect sizes for all rivers combined (1.213, 95% CI = 0.840–1.776), with Rocky Brook having the greatest effect size (3.170, 95% CI = 2.570–3.653; Table 2.4).

Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values were similar between up- and downstream sites before spawning (Table 2.5). Spawning anadromous fish delivered MDN that were enriched in ^{13}C and ^{15}N (e.g., -18.56 and 15.70 , respectively, for smelt eggs). Trends in isotopic enrichment of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were similar during periods of MDN input, so we report only results for $\delta^{15}\text{N}$. Isotope values for $\delta^{15}\text{N}$ varied seasonally, and downstream sites had greater values than did upstream reference sites (Fig. 2.3A–F, Table 2.6). In all rivers with anadromous fishes, we detected a significant enrichment of ^{15}N in the biofilm during spawning (Fig. 2.3B–F). By the end of July, $\delta^{15}\text{N}$ values returned to baseline in rivers with spring (Rainbow Smelt and Alewife) and early summer (Sea Lamprey) spawning fishes (Fig. 2.3B–E). In Rocky Brook where spawning by Atlantic Salmon occurs in the autumn, isotope values also declined post-spawn, but we were unable to determine whether these values returned to baseline levels because sites were inaccessible during winter (Fig. 2.3F). The seasonal pattern of ^{15}N incorporation closely tracked the seasonal changes in fungal biomass, especially for the Oromocto River and Rocky Brook. Stable-isotope values in Knoydart Brook (Fig. 2.3B) and Indiantown Brook (Fig. 2.3C) became enriched in ^{15}N , reached a maximum during

spawning, and became depleted after spawning. This pattern was also observed in algal biomass, but the pattern was less pronounced. $\delta^{15}\text{N}$ values did not differ between up- and downstream sites at Doctor's Brook (no anadromous fish spawning) (Fig. 2.3A). Prespawning data showed no spatial patterns in isotope values among sites within streams. Therefore, we attributed significant increases in $\delta^{15}\text{N}$ at downstream sites relative to upstream sites to MDN (Fig. 2.3B– F). $\delta^{15}\text{N}$ isotope values did not differ between tiles in- or outside enclosures ($F(2,12) = 0.002$, $p = 0.965$).

Net increase in algal biomass was related to Rainbow Smelt spawning density ($R^2 = 0.784$; Fig. 2.4A). Algal abundance increased 4× in rivers/years with Rainbow Smelt spawning than without, independent of spawning density, river, or year (Fig. 2.4A). Net increase in fungal biomass was 3× greater with Rainbow Smelt spawning ($R^2 = 0.759$; Fig. 2.4B), whereas bacterial density was 226× greater with Rainbow Smelt spawning ($R^2 = 0.724$; Fig. 2.4C).

2.5 Discussion

We tested the effects of MDN from anadromous fishes on biofilm in several rivers in Atlantic Canada. Our data show that biofilm communities follow a predictable response pattern to MDN inputs, regardless of the spawning strategy, timing, or MDN load being delivered. Biofilm community standing stocks from corresponding rivers were greater at downstream sites receiving MDN subsidies than at upstream reference sites, a general pattern observed for most biofilm groups in most river systems receiving MDN. The Oromocto River and Indiantown Brook had the most pronounced patterns. For most rivers, the seasonal maxima (before the period of decline) of algal biomass were

inconsistent between streams, whereas for fungi and bacteria, seasonal peaks generally were synchronous with the period of spawning. In all rivers with anadromous fishes, biofilm was significantly enriched in ^{15}N during the spawning period and variable for periods thereafter. This isotopic response provides additional evidence that increases in biofilm standing stock were the result of MDN incorporation (Bilby et al. 1996).

Variation in spawning strategy, timing, and spawning abundance influenced the direction and magnitude of the effect of MDN on biofilm community standing stock. The presence of spawning Rainbow Smelt resulted in a net increase in algal biomass. However, a maximum biomass accumulation threshold was reached despite increases in spawning density, a response also reported by Wipfli et al. (1999).

Biofilm group standing stocks generally followed the predicted temporal pattern of algal biomass in streams (Biggs 1988, 1996) with accrual reaching a maximum level in spring followed by a decline through the summer and another increase in autumn. The summer decline has been associated with light limitation as the canopy closes (Allan and Castillo 2008). The lower canopy cover of Knoydart Brook compared with the other study streams would have allowed more light to reach the substrate and may explain the lack of a summer decline in algal biomass at this site.

The similar responses of algae, fungi, and bacteria to MDN indicate that MDN and organic matter from anadromous fish provide resources that directly increase the turnover of the entire biofilm community. Marine-derived N and P contribute more to the autotrophic (algae) response, whereas particulate organic matter contributes more to the heterotrophic (fungi and bacteria) responses. These mixed autotrophic and heterotrophic groups have a relationship that allows the community as a whole to sustain high standing

stock (Carr et al. 2005) through synergistic interactions between biofilm groups supplying dietary nutrients to one another (Bengtsson 1992). The autotrophic and heterotrophic components contribute organic material to the river environment. Algae release photosynthetic products; bacteria release micronutrients, CO₂, and enzymes (Wetzel 1993); and fungi release particulate organic matter (Findlay 2010); all of which help facilitate stream processes, such as primary production and decomposition. The integrated structure of biofilm makes it physiologically beneficial to be closely coupled with the metabolism of the associated communities (Wetzel 1993). The mechanisms underlying the coinciding increases in standing stock observed in all rivers with anadromous fish probably occur via a mix of direct and indirect pathways connecting MDN and biofilm groups through direct consumption of MDN and organic matter, increasing turnover and metabolic by-products.

Seasonal peaks in algal, fungal, and bacterial standing stock were less pronounced in the Sea Lamprey stream (Cross Creek) than in the other streams. The smaller magnitude of change may be a result of the spawning strategy or nutrient availability from Sea Lamprey. Redd construction during spawning can significantly alter the stream bed and displace and reduce biomass and density of biofilm communities (Minakawa and Gara 1999, Moore and Schindler 2008). Sea Lamprey are semelparous, so they potentially contribute large amounts of MDN through carcass decomposition (Nislow and Kynard 2009), with gametes and excretory products also contributing. Sea Lamprey were observed spawning (a total of 17 redds counted; KMS, personal observation) within the study sites, but no carcasses were found. Thus, the small changes in community standing stock could be the result of minimal loading from carcass breakdown. In contrast, the

large increase in fungal abundance in the Oromocto River occurred during the upstream migration of Alewife to the spawning grounds in the headwater lake. During the migratory period, the river bed was covered by a layer of scales, in some places 2 to 5 cm thick. This river was the only one with noticeable carcass loading. The observed fungal accrual in the Oromocto River probably was a result of this organic-matter loading and associated decomposition (Tiegs et al. 2011).

Other species may deliver proportionally more nutrients in a form that is more readily available for uptake by biofilm. Unlike nutrient subsidies from carcasses, which have to be mobilized before uptake, nutrients from excretory products (e.g., NH_3) are available for immediate uptake. The greatest increase in algal abundance was in the Rainbow Smelt streams, Knoydart and Indiantown Brooks. Rainbow Smelt spawn in early spring typically after the ice has left the stream (early May at Knoydart and Indiantown Brooks). Spawning occurred only in the lower 350 m of the streams and lasted ~ 4 wk, so nutrients from excretory products were readily available to drive biofilm growth. MDN from spawning Atlantic Salmon increased biofilm group standing stocks at a time (late summer and autumn) when accrual usually is slow (Liboriussen and Jeppesen 2009). The prolonged residence time of Atlantic Salmon in Rocky Brook made MDN contributions from excretory products more significant to nutrient loading and production than previously thought, especially because dissolved nutrients are readily assimilated by biofilm (Post and Walters 2009).

Historically, these and other species would have spawned in the same river system, resulting in multiple MDN pulses, differing in type (dissolved vs particulate), amount delivered, and timing of delivery (seasonality, frequency, magnitude, or

duration). For example, one species might deliver MDN primarily in dissolved form (i.e., excretory products) optimal for autotrophs, whereas subsequent species might deliver primarily particulate nutrients (body material or substrate disturbance) more available to heterotrophic components of the biofilm. Thus, sequential MDN pulses might allow different members of the biofilm community to respond in a way that optimizes the total uptake of MDN.

Increased MDN loading can alleviate nutrient limitation for autotrophic groups and, at least in part, for heterotrophic groups (Rüegg et al. 2011). Based on the data from the 3 streams with Rainbow Smelt, large increases in net algal and fungal biomass and bacterial density occurred at the lowest spawning density observed (5 fish/m²) and did not increase significantly at higher spawning densities. This result suggests that a trophic capacity was reached between 0 and 5 fish/m². Spawner biomass and density differed among rivers (896–575,140 kg, 0.02– 5.51 fish/m², respectively), and Rainbow Smelt had the highest spawning density. Greater Rainbow Smelt spawning densities did not increase biofilm standing crops above a production threshold, spawning densities of the other species were much lower than the lowest densities observed with smelt, and changes in biofilm community abundance were similar across all fish species. Therefore, we can assume that increases in biofilm standing stock are not solely dependent on spawner density. Wipfli et al. (1999) found a similar response pattern of chlorophyll *a* and salmon carcass loading over a 2-mo period, but speculated that long-term effects (i.e., spawning periods > 2 mo) would differ as a result of system-specific storage capacity. Johnston et al. (2004) found that chlorophyll *a* concentration increased with salmon carcass abundance, but reached a maximum at 10× baseline levels. Increased N loading can

saturate lotic systems when N loading is sustained (Bernot and Dodds 2005). In our study system, larger nutrient loads from greater spawning populations appeared to exceed the system's productive capacity. An alternative explanation is density-dependent feedback. As biofilm standing stock increases, a thick layer of photosynthetically active algal cells on the outside of the matrix can shade inner cells, thereby limiting cell turnover and growth (Guasch et al. 1995).

During the spawning period, biofilm was enriched in ^{15}N at downstream sites relative to reference sites. This result suggests a strong relationship between autotrophic and heterotrophic production and MDN inputs. Assimilation of marine-derived ^{15}N in these rivers reflects direct uptake of dissolved nutrients and sorption and subsequent breakdown of marine-derived particles in the biofilm matrix (Bilby et al. 1996, Claeson et al. 2006, Verspoor et al. 2010, Harding et al. 2014). MDN were detected in the biofilm immediately upon the start of spawning, and the same general pattern of rapid isotopic enrichment followed by a return to baseline values was observed in 4 of the 5 rivers receiving MDN. Rocky Brook was the exception, but we were unable to follow the full temporal isotopic pattern because postspawning (winter) samples were not available. Differences in uptake by algal, fungal, and bacterial components of biofilm are difficult to distinguish using marine-derived ^{13}C and ^{15}N because all groups incorporate the same nutrient source with the same isotopic signature rather than discrete sources, such as allochthonous and autochthonous material, which have differing isotopic signatures (Parkyn et al. 2005). Differential responses of autotrophs and heterotrophs to nutrient subsidies may explain the variable isotope patterns observed in our study. Both groups can incorporate dissolved nutrients, but autotrophs are unlikely to consume particulate

marine-derived matter directly (Fenchel et al. 1998). For example, the similarities between the temporal patterns of fungal abundance and the change in $\delta^{15}\text{N}$ in the Oromocto River could be the result of breakdown and incorporation of marine-derived particulate matter (scales and carcasses) into new tissues by fungi (the primary decomposers between the 2 heterotrophic groups).

Effect sizes for biofilm groups and $\delta^{15}\text{N}$ within and between our study rivers receiving MDN were similar to those observed for effects of Pacific salmon on stream biofilm (measured as chlorophyll *a*) (Janetski et al. 2009). Effect sizes on algae were comparable among study rivers receiving MDN where net algal accrual reached a threshold with increased spawning density (Wipfli et al. 1999), thereby limiting the effect size of MDN on algae. Overall, mean effect sizes were similar between the algal and fungal groups, but effect sizes were much more variable for fungi. Spawning strategy affects the amount of organic matter that is delivered/released into the water column to be incorporated by the heterotrophic biofilm groups. A large amount of particulate organic matter associated with egg membranes is released upon larval emergence of broadcast-spawning Rainbow Smelt. Spawning Alewife release large amounts of organic matter in the form of scales and carcasses during their upstream migration to headwater lakes. Redd construction during Sea Lamprey and Atlantic Salmon spawning can displace and resuspend sediment, thereby increasing the amount of organic matter available in the stream. The effect size on $\delta^{15}\text{N}$ was greatest with MDN from spawning Atlantic Salmon. Atlantic Salmon have a prolonged residence time in fresh water during their spawning period, so biofilm is exposed to MDN from Atlantic Salmon for a much longer period than MDN from other fishes. Thus, marine-derived N incorporation can occur over an

extended period of time, which maintains the marine $\delta^{15}\text{N}$ signature in the biofilm community longer and leads to a large mean effect size.

The incubation time of the artificial substrates could have been a potential source of among-river variation in patterns of biofilm standing stock. A 2- to 4-wk incubation period is suitable for biofilm colonization (Bernhardt and Likens 2004). However, although mature algal and bacterial communities can be accurately described after a 28-d incubation period on natural substrates, 2 mo may be needed to reach a climax community on artificial substrates (Lamberti and Resh 1985). A longer prespawning biofilm incubation time might have reduced variation between rivers. Variation in flow regimes between rivers also might explain the variation in community standing stock because higher flows support lower biofilm standing stock (Ghosh and Gaur 1998). Grazing by macroinvertebrates also may have affected temporal patterns in biofilm standing stock if nutrient-enriched biofilm attracted grazers that consumed the new growth stimulated by MDN (Tank and Dodds 2003, Parkyn et al. 2005). If grazing affected MDN-driven biofilm accrual, we would have expected standing stocks at downstream sites to be similar to those at upstream sites, although biofilm accrual might have occurred even in the presence of grazers if bottom-up stimulation by MDN overwhelmed the grazing effect. Our results suggest that nutrient subsidies were more likely than grazers to be responsible for the divergence in biofilm standing stock between sites.

Biofilm standing stock did not increase above a threshold level with increased MDN loads, but standing stock remained above baseline conditions for up to 6 mo after MDN inputs ceased, a pattern also observed by Bellmore et al. (2014). Excess nutrients

that are not incorporated into the biofilm probably are sequestered in the sediment. Petticrew et al. (2011) showed that MDN are retained and stored in the substrate, and that this storage lengthens the period of nutrient availability and uptake to maintain high productivity levels. A potential mechanism to help explain why abundance levels remained elevated without continued MDN inputs is the inability of epilithic biofilm to access excess nutrients stored in the substrate unless the substrate is disturbed and nutrients are resuspended. Without direct access to stored MDN, seasonal increases in biofilm standing stock would be limited, even in areas with large spawning populations. However, substrate-stored nutrients would be available for microbial loop-mediated trophic transfer and uptake, maintaining increased net abundance.

It is widely accepted that returning adult Pacific salmon increase freshwater productivity (e.g., Bilby et al. 1996, Wipfli et al. 1999, Naiman et al. 2009). Resource availability plays a primary role in stream dynamics, and the spatial and temporal availability of resources can influence community structure and ecosystem functioning (Nowlin et al. 2008). Our study illustrates the importance of seasonal MDN inputs from multiple anadromous fish species to biofilm algae, fungi, and bacteria in Atlantic rivers. MDN inputs result in considerable increases in biofilm standing stock. Nutrient enrichment of autotrophic and heterotrophic groups may extend to higher trophic levels (Chaloner et al. 2007) via energy and nutrient transfer by invertebrates and fish (Bilby et al. 1996) or via the microbial loop for nutrients stored in the substrate (Rex and Petticrew 2010, Petticrew et al. 2011). A better understanding of the biological processes that regulate MDN uptake and storage is needed to understand nutrient saturation and production limits. We need to understand the degree to which MDN drive productivity

and the mechanisms by which these nutrients are incorporated into biofilm to increase productivity and, ultimately, increase fish production in Atlantic Canada.

2.6 Acknowledgments

We appreciate the help of Michelle Charest in the field and in the laboratory, especially with the bacterial counts. We thank Emily Corey for her help in the field and for generating the map of the study locations, and Aaron Fraser, Kabir Tosho, Sarah Lehnert, Catherine Desjardins, and Crystal Lewis for their field and laboratory assistance. Vincent Ignatiuk and staff at the University of Regina are gratefully acknowledged for their help with chlorophyll and ergosterol analyses, and Myriam Barbeau and James Watmough provided invaluable assistance with the statistical analysis. Furthermore, we thank 2 anonymous referees for their valuable comments on this manuscript. Financial support was provided by grants from the National Sciences and Engineering Research Council of Canada and the Canada Research Chairs Program awarded to RAC.

2.7 Literature Cited

- Allan, J. D., and M. M. Castillo. 2008. Stream ecology: structure and function of running waters. 2nd edition. Kluwer Academic Publishers, Boston, Massachusetts.
- Bellmore, J. R., A. K. Fremier, F. Mejia, and M. Newsom. 2014. The response of stream periphyton to Pacific salmon: using a model to understand the role of environmental context. *Freshwater Biology* 59:1437–1451.
- Bengtsson, G. 1992. Interactions between fungi, bacteria and beech leaves in a stream microcosm. *Oecologia* 89:542–549.
- Bernhardt, E. S., and G. E. Likens. 2004. Controls on periphyton biomass in heterotrophic streams. *Freshwater Biology* 49:14–27.
- Bernot, M. J., and W. K. Dodds. 2005. Nitrogen retention, removal, and saturation in lotic ecosystems. *Ecosystems* 8:442–453.
- Biggs, B. J. F. 1988. Artificial substrate exposure times for periphyton biomass estimates in rivers. *New Zealand Journal of Marine and Freshwater Research* 22:507–515.
- Biggs, B. J. F. 1996. Patterns in benthic algae of streams. Pages 31–56 in R. J. Stevenson, M. L. Bothwell, and R. L. Lowe (editors). *Algal ecology: freshwater benthic*

- ecosystems. Academic Press, San Diego, California.
- 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.
- Borchardt, M. A. 1996. Nutrients. Pages 183–227 in R. J. Stevenson, M. L. Bothwell, and R. L. Lowe (editors). *Algal ecology: freshwater benthic ecosystems*. Academic Press, San Diego, California.
- Carr, G. M., A. Morin, and P. A. Chambers. 2005. Bacteria and algae in stream periphyton along a nutrient gradient. *Freshwater Biology* 50:1337–1350.
- Chaloner, D. T., G. A. Lamberti, A. D. Cak, N. L. Blair, and R. T. Edwards. 2007. Inter-annual variation in responses of water chemistry and epilithon to Pacific salmon spawners in an Alaskan stream. *Freshwater Biology* 52:478–490.
- Chaloner, D. T., G. A. Lamberti, R. W. Merritt, N. L. Mitchell, P. H. Ostrom, and M. S. Wipfli. 2004. Variation in responses to spawning Pacific salmon among three south-eastern Alaska streams. *Freshwater Biology* 49:587–599.
- Chaloner D. T., M. S. Wipfli, and J. P. Caouette. 2002. Mass loss and macroinvertebrate colonisation of Pacific salmon carcasses in south-eastern Alaskan streams. *Freshwater Biology* 47:263–273.
- Chase, B. C. 2009. The spawning habitat of anadromous rainbow smelt: trouble at the tidal interface. Pages 859–862 in A. J. Haro, K. L. Smith, R. A. Rulifson, C. M. Moffitt, R. J. Klauda, M. J. Dadswell, R. A. Cunjak, J. E. Cooper, K. L. Beal, and T. S. Avery (editors). *Challenges for diadromous fishes in a dynamic global environment*. Symposium 69. American Fisheries Society, Bethesda, Maryland.
- Claeson, S. M., J. L. Li, J. E. Compton, and P. A. Bisson. 2006. Response of nutrients, biofilm, and benthic insects to salmon carcass addition. *Canadian Journal of Fisheries and Aquatic Sciences* 63:1230–1241.
- 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 North American Benthological Society* 30:831–839.
- Durbin, A. G., S. W. Nixon, and C. A. Oviatt. 1979. Effects of the spawning migration of the alewife, *Alosa pseudoharengus*, on freshwater ecosystems. *Ecology* 60:8–17.
- Elliott, S. R., T. A. Coe, J. M. Helfield, and R. J. Naiman. 1998. Spatial variation in environmental characteristics of Atlantic salmon (*Salmo salar*) rivers. *Canadian Journal of Fisheries and Aquatic Sciences* 55:267–280.
- Fenchel, G. M., T. H. King, and T. H. Blackburn. 1998. *Bacterial biogeochemistry: the ecophysiology of mineral cycling*. Academic Press, San Diego, California.
- Findlay, S. 2010. Stream microbial ecology. *Journal of North American Benthological Society* 29:170–181.
- Fisher Wold, A. K., and A. E. Hershey. 1999. Effects of salmon carcass decomposition on biofilm growth and wood decomposition. *Canadian Journal of Fisheries and Aquatic Sciences* 56:767–773.
- Gessner, M. O. 2005. Ergosterol as a measure of fungal biomass. Pages 189–195 in M. A. S. Graça, F. Bärlocher, and M. O. Gessner (editors). *Methods to study litter*

- decomposition: a practical guide. Springer, Dordrecht, The Netherlands.
- Ghosh, M., and J. P. Gaur. 1998. Current velocity and the establishment of stream algal periphyton communities. *Aquatic Biology* 60:1–10.
- Guasch, H., E. Martí, and S. Sabater. 1995. Nutrient enrichment effects on biofilm metabolism in a Mediterranean stream. *Freshwater Biology* 33:373–383.
- Guyette, M. Q., C. S. Loftin, and J. Zydlewski. 2013. Carcass analog addition enhances juvenile Atlantic salmon (*Salmo salar*) growth and condition. *Canadian Journal of Fisheries and Aquatic Sciences* 70:860–870.
- Guyette, M. Q., C. S. Loftin, J. Zydlewski, and R. Cunjak. 2014. Carcass analogues provide marine subsidies for macroinvertebrate and juvenile Atlantic Salmon in temperate oligotrophic streams. *Freshwater Biology* 59:392–406.
- Haack, T. K., and G. A. McFeters. 1982. Nutritional relationships among microorganisms in an epilithic biofilm community. *Microbial Ecology* 8:115–126.
- Harding, J. N., J. M. Harding, and J. D. Reynolds. 2014. Movers and shakers: nutrient subsidies and benthic disturbance predict biofilm biomass and stable isotope signatures in coastal streams. *Freshwater Biology* 59:1361–1377.
- 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.
- Janetski, D. J., D. T. Chaloner, S. D. Tiegs, and G. A. Lamberti. 2009. Pacific salmon effects on stream ecosystems: a quantitative synthesis. *Oecologia (Berlin)* 159:583–595.
- Jardine, T. D., D. F. Cartwright, J. P. Dietrich, and R. A. Cunjak. 2005. Resource use by salmonids in riverine, lacustrine and marine environments: evidence from stable isotope analysis. *Environmental Biology of Fishes* 73:309–319.
- Jardine, T. D., J-M Roussel, S. C. Mitchell, and R. A. Cunjak. 2009. Pathways of marine nutrient and organic matter entry from anadromous fishes into Atlantic coast streams. Pages 427–445 in A. J. Haro, K. L. Smith, R. A. Rulifson, C. M. Moffitt, R. J. Klauda, M. J. Dadswell, R. A. Cunjak, J. E. Cooper, K. L. Beal, and T. S. Avery (editors). *Challenges for diadromous fishes in a dynamic global environment*. Symposium 69. American Fisheries Society, Bethesda, Maryland.
- Jeffrey, S. W., and G. F. Humphrey. 1975. New spectrophotometric equation for determining chlorophylls *a*, *b*, *c1*, *c2* in higher plants, algae and natural phytoplankton. *Biochemie und Physiologie der Pflanzen* 167:191–194.
- 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.
- Jonsson, B., and N. Jonsson. 2003. Migratory Atlantic Salmon as vectors for the transfer of energy and nutrients between freshwater and marine environments. *Freshwater Biology* 48:21–27.
- Lamberti, G. A., and V. H. Resh. 1985. Comparability of introduced tiles and natural substrates for sampling lotic bacteria, algae and macroinvertebrates. *Freshwater Biology* 15:21–30.
- Lessard, J. L., and R. W. Merritt. 2006. Influence of marine-derived nutrients from

- spawning salmon on aquatic insect communities in southeast Alaskan streams. *Oikos* 113:334–343.
- Lessard J. L., R. W. Merritt, and M. B. Berg. 2009. Investigating the effect of marine-derived nutrients from spawning salmon on macroinvertebrate secondary production in southeast Alaskan streams. *Journal of the North American Benthological Society* 28:683–693.
- Levi, P. S., J. L. Tank, J. R  egg, 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.
- Liboriusson, L., and E. Jeppesen. 2009. Periphyton biomass, potential production and respiration in a shallow lake during winter and spring. *Hydrobiologia* 632:201–210.
- Lock, M. A, R. R. Wallace, J. W. Costerton, R. M. Ventullo, and S. E. Charlton. 1984. River epilithon: toward a structural-functional model. *Oikos* 42:10–22.
- Mather, M. E., H. J. Frank, J. M. Smith, R. D. Cormier, R. M. Muth, and J. T. Finn. 2012. Assessing freshwater habitat of adult anadromous alewives using multiple approaches. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science* 4:188–200.
- McIntire, C. D. 1973. Periphyton dynamics in laboratory streams: a simulation model and its implications. *Ecological Monographs* 43:399–420.
- McKenzie, R. A. 1964. Smelt life history and fishery in the Miramichi River, New Brunswick. Bulletin No. 144. Fisheries Research Board of Canada, St Andrews, New Brunswick.
- Minakawa, N., and R. I. Gara. 1999. Ecological effects of a chum salmon (*Oncorhynchus keta*) spawning run in a small stream of the Pacific Northwest. *Journal of Freshwater Ecology* 14:327–335.
- Mitchell, N. L., and G. A. Lamberti. 2005. Responses in dissolved nutrients and epilithon abundance to spawning salmon in southeast Alaska streams. *Limnology and Oceanography* 50:217–227.
- Moore, J. W., and D. E. Schindler. 2008. Biotic disturbance and benthic community dynamics in salmon-bearing streams. *Journal of Animal Ecology* 77:275–284.
- Mullen, D. M., C. W. Fay, and J. R. Moring. 1986. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (North Atlantic) - alewife/blueback herring. Biological Report 82(11.58). US Fish and Wildlife Service, Washington, DC.
- 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* 5:399–417.
- Naiman, R. J., J. M. Helfield, K. K. Bartz, D. C. Drake, and J. M. Honea. 2009. Pacific salmon, marine-derived nutrients and the characteristics of aquatic and riparian ecosystems. Pages 395–425 in A. J. Haro, K. L. Smith, . A. Rulifson, C. M. Moffitt, R. J. Klauda, M. J. Dadswell, R. A. Cunjak, J. E. Cooper, K. L. Beal, and T. S. Avery (editors). Challenges for diadromous fishes in a dynamic global environment. Symposium 69. American Fisheries Society, Bethesda, Maryland.
- Nislow, K., J. Armstrong, and S. McKelvey. 2004. Phosphorus flux due to Atlantic

- Salmon (*Salmo salar*) in an oligotrophic upland stream: effects of management and demography. *Canadian Journal of Fisheries and Aquatic Sciences* 61:2401–2410.
- Nislow, K. H., and B. E. Kynard. 2009. The role of anadromous sea lamprey in nutrient and material transport between marine and freshwater environments. Pages 485–494 in A. J. Haro, K. L. Smith, R. A. Rulifson, C. M. Moffitt, R. J. Klauda, M. J. Dadswell, R. A. Cunjak, J. E. Cooper, K. L. Beal, and T. S. Avery, editors. *Challenges for diadromous fishes in a dynamic global environment*. Symposium 69. American Fisheries Society, Bethesda, Maryland.
- Nowlin, W. H., M. J. Vanni, and L. H. Yang. 2008. Comparing resource pulses in aquatic and terrestrial ecosystems. *Ecology* 89:647–659.
- Oromocto River Watershed Association. 2014. Oromocto River Watershed Gaspereau Study. Final Report. Prepared by Parish Geomorphic Ltd. and Oromocto River Watershed Association, Oromocto, New Brunswick. (Available from: <http://www.oromoctowatershed.ca/wp-content/uploads/2014/05/Oromocto-River-Watershed-Alewife-Study-Final.pdf>)
- Parkyn, S. M., J. M. Quinn, T. J. Cox, and N. Broekhuizen. 2005. Pathways of N and C uptake and transfer in stream food webs: an isotope enrichment experiment. *Journal of North American Benthological Society* 24:955–975.
- Parmenter, R. R., and V. A. Lamarra. 1991. Nutrient cycling in a freshwater marsh: the decomposition of fish and waterfowl carrion. *Limnology and Oceanography* 36:976–987.
- Petticrew, E. L., J. F. Rex, and S. J. Albers. 2011. Bidirectional delivery of organic matter between freshwater and marine systems: the role of flocculation in Pacific salmon streams. *Journal of North American Benthological Society* 30:779–786.
- Pomroy, A. J. 1984. Direct counting of bacteria preserved with Lugol iodine solution. *Applied and Environmental Microbiology* 47:1191–1192.
- Porter, K. G., and Y. S. Feig. 1980. The use of DAPI for identifying and counting aquatic microfloral. *Limnology and Oceanography* 25:943–948.
- Porter-Goff, E. R., C. W. Boylen, and S. A. Nierzwicki-Bauer. 2010. Periphyton dynamics along a stream with a gradient of human impact. *Journal of Freshwater Ecology* 25:385–394.
- Post, D. M., and A. W. Walters. 2009. Nutrient excretion rates of anadromous alewives during their spawning migration. *Transactions of the American Fisheries Society* 138:264–268.
- Reid, J. 2010. Fisheries Management Program, 2010 Program Review. International Paper Canada Holdings, Rocky Brook Camp, P.O. Box 354, Boiestown, New Brunswick, E6A 2A2.
- Rex, J. F., and E. L. Petticrew. 2010. Salmon-derived nitrogen delivery and storage within a gravel bed: sediment and water interactions. *Ecological Engineering* 36:1167–1173.
- Romaní, A. M., and S. Sabater. 1999. Effects of primary producers on the heterotrophic metabolism of a stream biofilm. *Freshwater Biology* 41:729–736.
- Romaní, A. M. and S. Sabater. 2001. Structure and activity of rock and sand biofilms in a Mediterranean stream. *Ecology* 82:3232–3245.

- Rosemond, A. D., P. J. Mulholland, and S. H. Brawley. 2000. Seasonally shifting limitation of stream periphyton: response of algal populations and assemblage biomass and productivity to variation in light, nutrients, and herbivores. *Canadian Journal of Fisheries and Aquatic Sciences* 57:66–75.
- 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 Sciences* 68:277–287.
- Schindler, D. E., P. R. Leavitt, C. S. Brock, S. P. Johnson, and P. D. Quay. 2005. Marine-derived nutrients, commercial fisheries, and production of salmon and lake algae in Alaska. *Ecology* 86:3225–3231.
- Scott, W. B., and M. G. Scott. 1988. Atlantic fishes of Canada. Canadian Bulletin of Fisheries and Aquatic Sciences No. 219. University of Toronto Press, Toronto, Ontario.
- Sobczak, W. V. 1996. Epilithic bacterial responses to variations in algal biomass and labile dissolved organic carbon during biofilm colonization. *Journal of North American Benthological Society* 15:143–154.
- Sobczak, W. V., and T. M. Burton. 1996. Epilithic bacterial and algal colonization in a stream run, riffle, and pool: a test of biomass covariation. *Hydrobiologia* 332:159–166.
- Stewart-Oaten, A., W. W. Murdoch, and K. R. Parker. 1986. Environmental impact assessment: “pseudoreplication” in time? *Ecology* 67:929–940.
- Stockner, J. G., and K. I. Ashley. 2003. Salmon nutrients: closing the circle. Pages 3–15 in J. G. Stockner (editor). *Nutrients in salmonid ecosystems: sustaining production and biodiversity*. Symposium 34. American Fisheries Society, Bethesda, Maryland.
- 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., and J. R. Webster. 1998. Interaction of substrate and nutrient availability on wood biofilm processes in streams. *Ecology* 76:2168–2179.
- Tiegs, S. D., P. S. Levi, J. Rüegg, D. T. Chaloner, J. L. Tank, and G. A. Lamberti. 2011. Ecological effects of live salmon exceed those of carcasses during an annual spawning migration. *Ecosystems* 14:598–614.
- Tumber, V. P., R. D. Robarts, M. T. Arts, M. S. Evans, and D. E. Caldwell. 1993. The influence of environmental factors on seasonal changes in bacterial volume in two prairie saline lakes. *Microbial Ecology* 26:9–20.
- Underwood, A. J. 1997. *Experiments in ecology: their logical design and interpretation using analysis of variance*. Cambridge University Press, Cambridge, UK.
- Vanni, M. J. 2002. Nutrient cycling by animals in freshwater ecosystems. *Annual Review of Ecological and Systematics* 33:341–370.
- Verspoor, J. J., D. C. Braun, and J. D. Reynolds. 2010. Quantitative links between Pacific salmon and stream periphyton. *Ecosystems* 13:1020–1034.
- Walters, A. W., R. T. Barnes, and D. M. Post. 2009. Anadromous alewives (*Alosa*

- pseudoharengus*) contribute marine-derived nutrients to coastal stream food webs. Canadian Journal of Fisheries and Aquatic Sciences 66:439–448.
- Wetzel, R. G. 1993. Microcommunities and microgradients: linking nutrient regeneration, microbial mutualism, and high sustained aquatic primary production. Netherlands Journal of Aquatic Ecology 27:3–9.
- Wipfli, M. S., J. Hudson, and J. Caouette. 1998. Influence of salmon carcasses on stream productivity: response of biofilm and benthic macroinvertebrates in southeastern Alaska, U.S.A. Canadian Journal of Fisheries and Aquatic Sciences 55:1503–1511.
- Wipfli, M.S., J. Hudson, and J. Caouette. 2003. Marine subsidies in freshwater ecosystems: salmon carcasses increase the growth rates of stream-resident salmonids. Transactions of the American Fisheries Society 132:371–381.
- Wipfli, M. S., J. P. Hudson, J. P. Caouette, N. L. Mitchell, J. L. Lessard, R. A. Heintz, D. T. Chaloner. 2010. Salmon carcasses increase stream productivity more than inorganic fertilizer pellets: a test on multiple trophic levels in stream-side experimental channels. Transactions of the American Fisheries Society 139:824–839.
- Wipfli, M. S., J. Hudson, D. T. Chaloner, and J. P. Caouette. 1999. The influence of salmon spawner densities on stream productivity in southeast Alaska. Canadian Journal of Fisheries and Aquatic Sciences 56:1600–1611.

Table 2.1. Characteristics of study systems in New Brunswick and Nova Scotia, Canada. NS = no spawning occurred, NA = not available, BS = broadcast spawner, NB = nest builder.

Variable	Doctor's Brook (Nova Scotia)	Knoydart Brook (Nova Scotia)	Indiantown Brook (New Brunswick)	Oromocto River (New Brunswick)	Cross Creek (New Brunswick)	Rocky Brook (New Brunswick)
Location	45°47'N	45°42'N	46°49'N	45°51'N	46°15'N	46°36'N
	62°07'W	62°14'W	65°47'W	66°28'W	66°38'W	66°37'W
Drainage area (km ²)	25	24	70	2025	200	150
Mean canopy cover	65%	60%	50%	45%	55%	40%
Mean depth (m)	0.23	0.21	0.25	0.29	0.24	0.48
Mean width (m)	5.56	3.83	9.17	18.87	6.79	16.52
Mean discharge (m ³ /s)	0.47	0.40	1.62	3.77	0.98	4.17
Primary anadromous species	Rainbow Smelt ^a	Rainbow Smelt	Rainbow Smelt	Alewife	Sea Lamprey	Atlantic Salmon

Spawning strategy	BS	BS	BS	BS	NB	NB
Life-history type	Iteroparous	Iteroparous	Iteroparous	Iteroparous	Semelparous	Iteroparous
Spawning period	Early May ^a	Early May	Early May	Late May	Early June	Oct–Nov
Estimated biomass of run in 2010 (kg WM)	NS	1875 ^b	1219 ^b	575 140 ^c	NA	896 ^c
Estimated spawner density of run in 2010 (no./m ²)	NS	5.41 ^b	44.74 ^b	0.02 ^c	0.08 ^d	0.01 ^e

^a Rainbow Smelt historically spawned in Doctor's Brook, but have not returned as of 2008

^b Estimated from egg deposition (unpublished data; personal observation)

^c Oromocto River Watershed Association Inc. (2014)

^d Estimate based on observed spawning in 2 locations (unpublished data; personal observation)

^e Reid (2010)

Table 2.2. Structure of mixed-model analyses of variance used to evaluate variation in algal and fungal biomass and bacterial density.

Source of variation	Effect type	Denominator of F -
		ratio
Barrier B_i	Fixed	$MS_{S(B)}$
Site(barrier) $S_k(B_i)$	Random	MS_e
Time T_j	Fixed	$MS_{S(B)T}$
Barrier $\times$ time BT_{ij}	Fixed	$MS_{S(B)T}$
Site(barrier) $\times$ time $S_k(B_i)T_j$	Random	MS_e
Residual e_l	Random	

Table 2.3. Results of the mixed-model analyses of variance evaluating variation in up- and downstream biofilm algal, fungal, and bacterial standing stock. The F - and p -values in the fungal group for factors time and site(barrier) $\times$ time are not computable because there are 0 df for the residuals (the demoninator of the F -ratios). The number of replicates/site is 1. Significant p -values are in bold.

River	Source of variation	Algae			Fungi			Bacteria		
		df	F	p	df	F	p	df	F	p
Doctor's Brook	Barrier	1	0.23	0.65	1	1.18	0.42	1	3.14	0.14
	Site(barrier)	5	1.92	0.31	5	4.32	0.06	5	1.53	0.27
	Time	6	0.48	0.80	6			6	0.12	0.99
	Barrier $\times$ time	5	182.52	<0.001	5	287.47	<0.001	5	164.46	<0.001
	Site(barrier) $\times$ time	30	15.13	<0.001	30			30	308.07	<0.001
	Residual	96			0			96		
Knoydart Brook	Barrier	1	19.76	0.007	1	42.05	<0.001	1	37.62	<0.001
	Site(barrier)	5	6.16	0.01	5	1.92	0.20	5	13.19	0.001
	Time	8	0.98	0.54	8			8	0.07	0.99
	Barrier $\times$ time	5	83.24	<0.001	5	377.42	<0.001	5	92.67	<0.001
	Site(barrier) $\times$ time	40	0.89	0.66	40			40	6.78	<0.001
	Residual	120			0			120		
Indiantown Brook	Barrier	1	8.99	0.03	1	83.77	<0.001	1	9.08	0.03
	Site(barrier)	5	2.98	0.11	5	32.45	<0.001	5	8.59	0.01
	Time	6	11.81	0.008	6			6	12.40	0.007
	Barrier $\times$ time	5	132.49	<0.001	5	763.69	<0.001	5	106.38	<0.001
	Site(barrier) $\times$ time	30	6.01	<0.001	30			30	0.74	<0.001
	Residual	96			0			96		
Oromocto River	Barrier	1	6.51	0.05	1	40.78	0.001	1	48.45	<0.001
	Site(barrier)	5	11.98	0.002	5	14.36	0.001	5	9.24	0.004
	Time	8	3.38	0.98	8			8	0.23	0.97
	Barrier $\times$ time	5	91.81	<0.001	5	957.38	<0.001	5	94.50	<0.001
	Site(barrier) $\times$ time	40	2.34	<0.001	40			40	4.44	<0.001
	Residual	120			0			120		
Cross Creek	Barrier	1	12.09	0.04	1	67.99	0.003	1	7.84	0.07

Rocky Brook	Site(barrier)	3	26.93	<0.001	3	33.58	<0.001	3	7.19	0.02
	Time	6	0.93	0.57	6			6	1.86	0.32
	Barrier $\times$ time	3	175.96	<0.001	3	509.49	<0.001	3	78.19	<0.001
	Site(barrier) $\times$ time	18	1.57	0.09	18			18	1.25	0.25
	Residual	64			0			64		
	Barrier	1	12.49	0.04	1	103.42	<0.001	1	13.21	0.04
	Site(barrier)	3	32.14	<0.001	3	18.52	0.001	3	15.93	0.001
	Time	8	1.49	0.40	8			8	4.82	0.11
	Barrier $\times$ time	3	271.34	<0.001	3	348.82	<0.001	3	187.49	<0.001
	Site(barrier) $\times$ time	24	0.62	0.91	24			24	1.73	0.04
	Residual	80			0			80		

Table 2.4. Effect of marine-derived nutrients on accrual of algae (chlorophyll), fungal (ergosterol), bacteria, all groups combined (overall) and stable-isotope enrichment of ^{15}N . Response variables reported as mean effect size; values in parentheses are upper and lower 95% confidence intervals.

River	Chlorophyll	Ergosterol	Bacteria	Overall	$\delta^{15}\text{N}$
Doctor's Brook	0.037(−0.075, 0.148)	−0.206 (−0.444, 0.027)	0.133 (−0.581, 0.744)	−0.015 (−0.272, 0.208)	0.135 (−0.275, 0.210)
Knoydart Brook	0.455 (0.073, 0.951)	0.963 (0.478, 1.785)	2.010 (1.595, 2.961)	1.033 (0.851, 1.777)	1.098 (0.852, 1.769)
Indiantown Brook	0.687 (0.422, 0.999)	0.310 (0.048, 0.598)	1.465 (1.051, 2.185)	0.766 (0.560, 1.212)	1.140 (0.228, 5.368)
Oromocto River	0.560 (0.379, 0.763)	0.582 (0.065, 1.455)	1.336 (0.828, 2.367)	0.790 (0.581, 1.381)	1.604 (0.928, 4.490)
Cross Creek	0.630 (0.252, 0.901)	0.441 (0.227, 0.568)	1.041 (0.745, 1.687)	0.687(0.532, 1.043)	1.693 (0.831, 3.331)
Rocky Brook	0.588 (0.485, 0.876)	1.061 (0.720, 1.913)	0.916(0.748, 1.400)	0.844 (0.707, 1.178)	3.170 (2.570, 3.653)
All Rivers	0.475 (0.358, 0.649)	0.474 (0.325, 0.858)	1.092 (0.949, 1.647)	1.522(0.945, 1.657)	1.213 (0.840, 1.776)

Table 2.5. Mean (SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (‰) isotope values for biofilm samples collected from up- and downstream sites prior to anadromous fish spawning.

Stream	$\delta^{13}\text{C}$ (‰)		$\delta^{15}\text{N}$ (‰)	
	Upstream	Downstream	Upstream	Downstream
Doctor's Brook	-27.15 (0.73)	-28.30 (0.41)	2.43 (0.58)	2.61 (0.48)
Knoydart Brook	-27.30 (2.53)	-29.15 (1.10)	3.47 (0.73)	3.95 (0.80)
Indiantown Brook	-30.29 (0.34)	-30.25 (0.48)	6.90 (0.30)	6.83 (0.21)
Oromocto Brook	-27.15 (1.26)	-26.53 (0.55)	10.67 (1.25)	8.51 (2.43)
Cross Creek	-30.12 (1.06)	-29.31 (0.93)	4.44 (0.45)	3.61 (0.63)
Rocky Brook	-31.03 (1.14)	-28.49 (1.70)	4.09 (0.61)	4.31 (0.67)

Table 2.6. Results of mixed-model analyses of variance in up- and downstream $\delta^{15}\text{N}$ stable-isotope values. The F - and p -values for factors time and site(barrier) $\times$ time are not computable because there are 0 df for the residuals (the denominator of the F -ratios). The number of replicates per site is 1.

River	Source of variation	$\delta^{15}\text{N}$		
		df	F	p
Doctor's Brook	Barrier	1	2.22	0.10
	Site(Barrier)	5	1.20	0.48
	Time	6		
	Barrier $\times$ time	5	134.66	<0.001
	Site(barrier) $\times$ time	30		
	Residual	0		
Knoydart Brook	Barrier	1	70.83	<0.001
	Site(barrier)	5	65.40	<0.001
	Time	8		
	Barrier $\times$ time	5	189.79	<0.001
	Site(barrier) $\times$ time	40		
	Residual	0		
Indiantown Brook	Barrier	1	81.26	<0.001
	Site(barrier)	5	62.13	<0.001
	Time	6		
	Barrier $\times$ time	5	144.18	<0.001
	Site(barrier) $\times$ Time	30		
	Residual	0		
Oromocto River	Barrier	1	100.06	<0.001
	Site(Barrier)	5	83.32	<0.001
	Time	8		
	Barrier $\times$ time	5	214.25	<0.001

	Site(barrier) $\times$ time	40		
	Residual	0		
Cross Creek	Barrier	1	23.69	0.009
	Site(Barrier)	3	17.21	0.02
	Time	6		
	Barrier $\times$ Time	3	63.32	<0.001
	Site(barrier) $\times$ time	18		
	Residual	0		
Rocky Brook	Barrier	1	107.51	<0.001
	Site(barrier)	3	31.36	0.002
	Time	8		
	Barrier $\times$ time	3	364.76	<0.001
	Site(barrier) $\times$ time	24		
	Residual	0		

Figure 2.1. Map of New Brunswick and Nova Scotia, Canada, showing the location of the 6 study system used for assessing biofilm responses to marine-derived nutrient inputs from anadromous fishes.

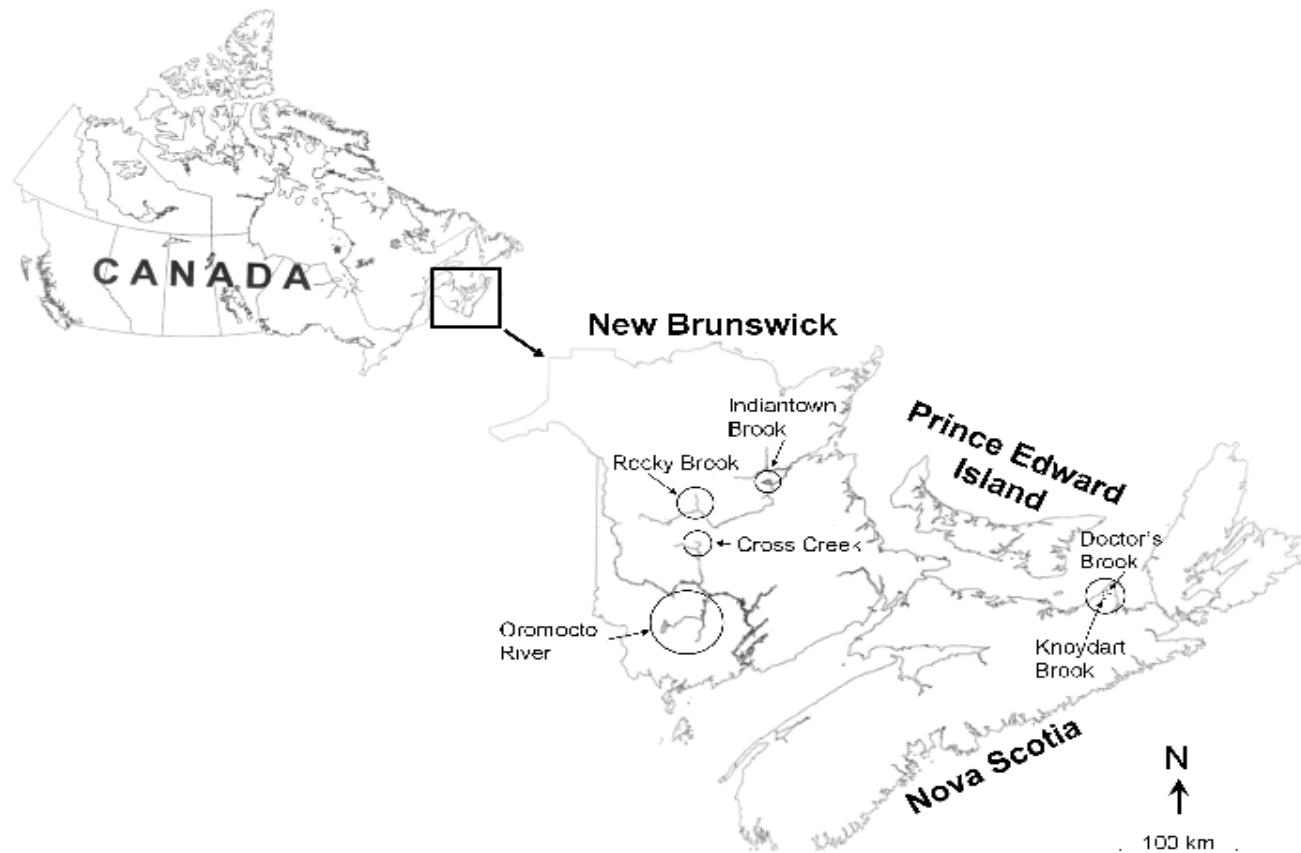


Figure 2.2. Mean (± 1 SD) biofilm algal biomass (as chlorophyll) (A-F), fungal biomass (G-L), and bacterial density (M-R) responses to marine-derived nutrients from spawning anadromous fishes at upstream baseline and downstream treatment sites in Doctor's Brook (A, G, M), Knoydart Brook (B, H, N), Indiantown Brook (C, I, O), Oromocto River (D, J, P), Cross Creek (E, K, Q), and Rocky Brook (F, L, R) in New Brunswick and Nova Scotia. Shaded areas represent periods of anadromous fish spawning (spawning did not occur in Doctor's Brook). See Table 1.1 for primary spawning fish species in each river. * indicates a statistically significant difference ($p < 0.05$) between down- and upstream sites.

[Figure on following page]

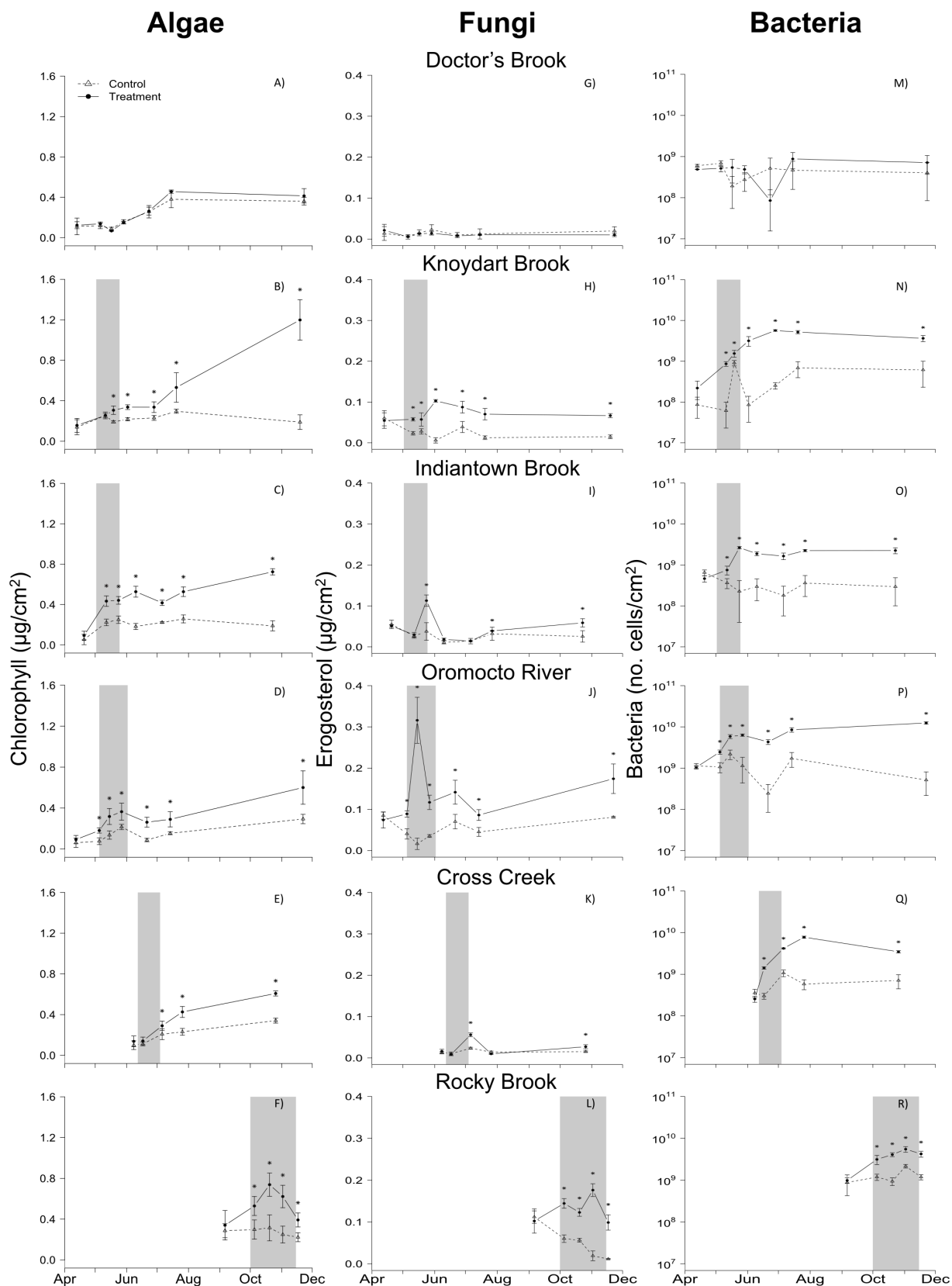


Figure 2.3. Mean (± 1 SD) standardized $\delta^{15}\text{N}$ stable-isotope signatures of biofilm in Doctor's Brook (A), Knoydart Brook (B), Indiantown Brook (C), Oromocto River (D), Cross Creek (E), and Rocky Brook (F). Changes in $\delta^{15}\text{N}$ are represented as the difference between down- and upstream (baseline) response values ($\Delta\delta^{15}\text{N}$, Eq. 2). Shaded areas represent periods of anadromous fish spawning (spawning did not occur in Doctor's Brook). See Table 1.1 for primary spawning fish species in each river. * indicates a statistically significant difference ($p < 0.05$) between down- and upstream sites.

[Figure on following page]

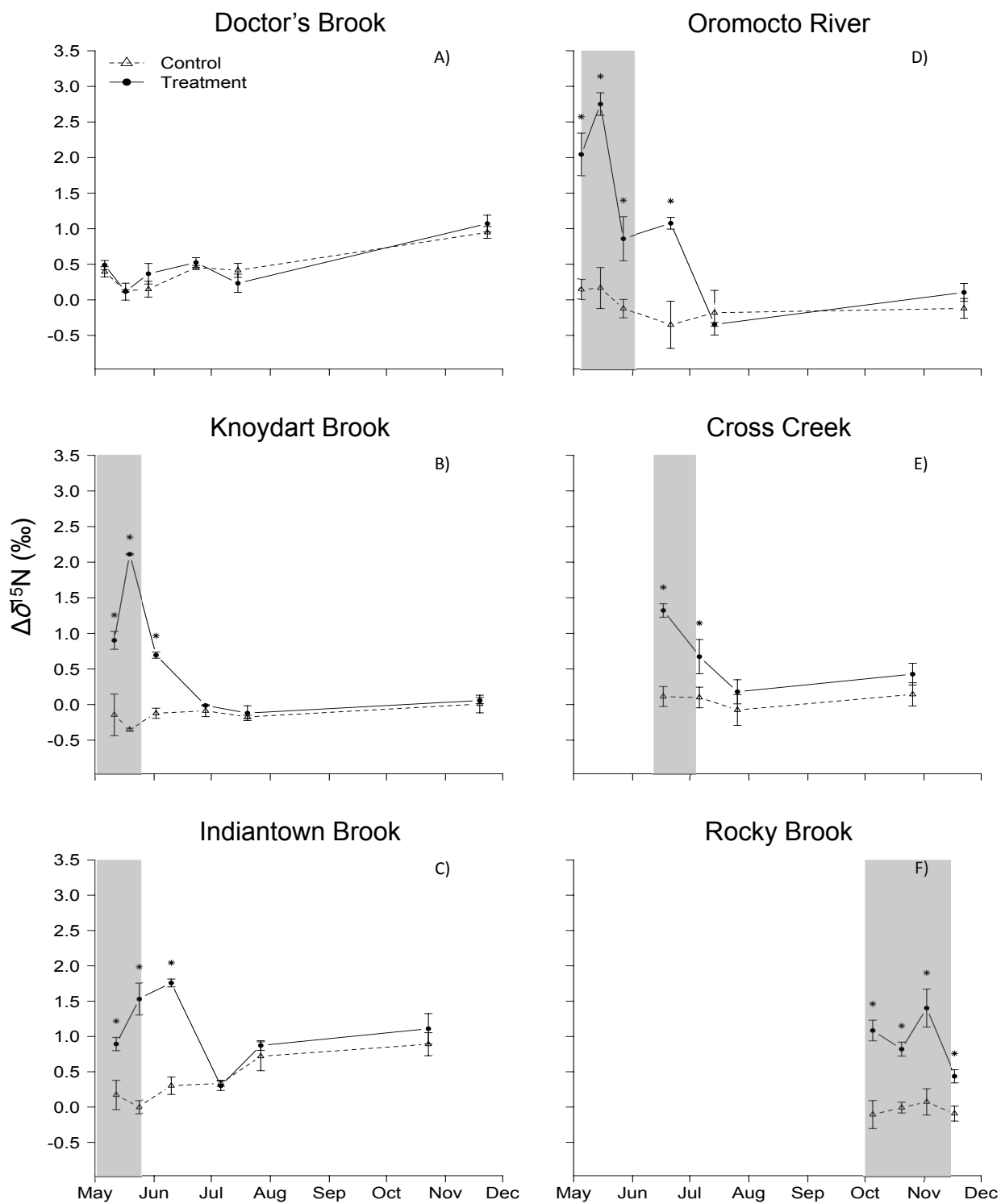
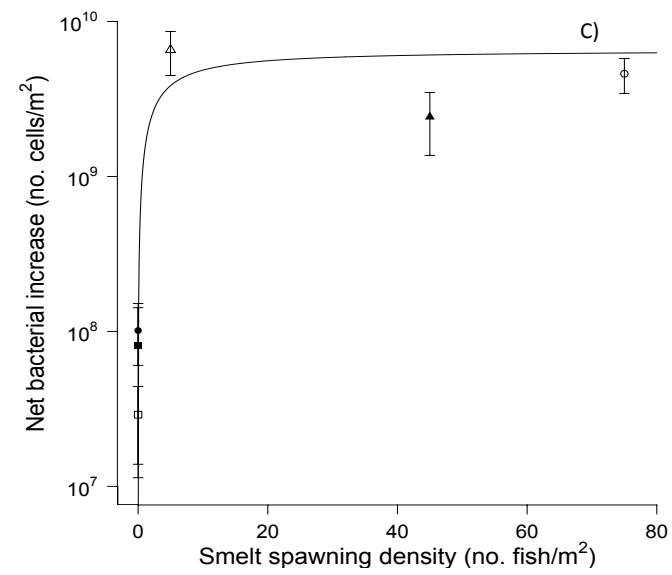
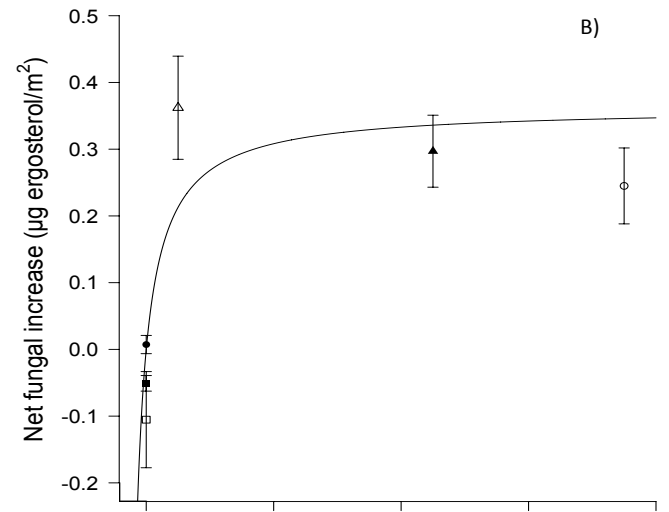
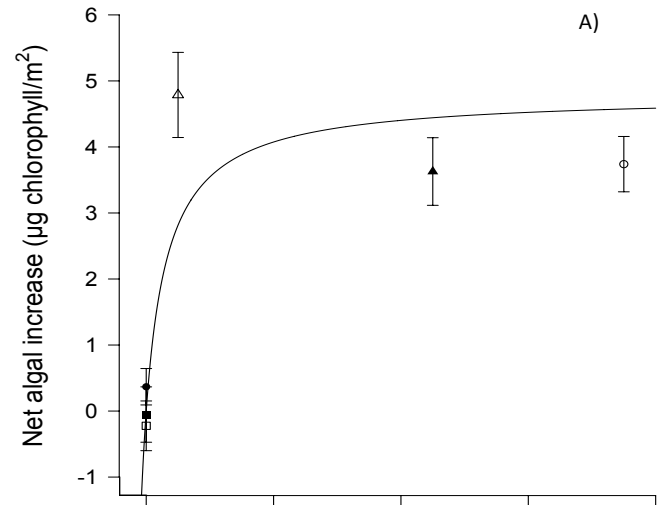


Figure 2.4. Net increase in algal biomass (as chlorophyll) (A), fungal biomass (B), and bacterial density (C) as a function of Rainbow Smelt spawning density for 3 rivers in 2009-2010. Net increase over the entire sampling season was estimated as the area between the up- and downstream curves.

[Figure on following page]



Chapter 3

Aquatic food web dynamics following incorporation of nutrients derived from Atlantic anadromous fishes

Kurt M. Samways¹, David X. Soto^{1,2}, and Richard A. Cunjak¹

¹Canadian Rivers Institute and Department of Biology, University of New Brunswick,
P.O. Box 4400, 10 Bailey Avenue, Fredericton, New Brunswick E3B 5A3 Canada

²Department of Earth and Environmental Sciences, KU Leuven, B-3001 Leuven,
Belgium

In Review: *Journal of Fish Biology*. © The Fisheries Society of the British Isles.

Reproduced with the kind permission from The Fisheries Society of the British Isles.

3.1 Abstract

Regardless of the spatial resolution or the pathway of incorporation, annual nutrient pulses from spawning rainbow smelt (*Osmerus mordax*), alewife (*Alosa pseudoharengus*), sea lamprey (*Petromyzon marinus*), and Atlantic salmon (*Salmo salar*) lead to isotopic enrichment at all trophic levels (biofilm, 1.2-5.4‰; macroinvertebrates, 0.0-6.8‰; fish, 1.2-2.6‰). Community-wide niche space shifted toward the marine-nutrient source, however the total ecological niche space did not always increase with MDN inputs. The time-integrated marine-nutrient resource contribution to the diet of Atlantic salmon parr and brook trout ranged between 33.1-47.0% during anadromous fish spawning periods. The high degree of spatio-temporal heterogeneity in marine-nutrient subsidies from anadromous fishes lead to both direct and indirect pathways of MDN incorporation into stream food webs. This suggests that organisms at many trophic levels derive a substantial proportion of their energy from marine resources when present. The current trend of declining anadromous fish populations means fewer nutrient-rich marine subsidies being delivered to rivers, diminishing the ability to sustain elevated riverine productivity.

3.2 Introduction

Nutrient resource availability has a primary role in structuring freshwater food web communities and driving stream ecosystem functions. Nutrient subsidies to lotic systems are important because they provide an important energy source and are very often capable of alleviating resource limitation to productivity (Polis *et al.*, 1997). Many aquatic ecosystems experience temporary periods of greatly increased nutrient resource

availability. These nutrient pulses affect population dynamics at multiple trophic levels by attenuating “bottom-up” constraints on productivity via increased resource supply to freshwater food webs (Ostfeld & Keesing, 2000).

The migration of anadromous fishes delivers energy and nutrients of marine origin into rivers. These marine-derived nutrients (MDNs) of carbon, nitrogen and phosphorous are assimilated in the ocean and subsequently delivered by spawning anadromous fishes in the form of excretory metabolites, gametes, and carcass decomposition (Bilby *et al.*, 1996; Naiman *et al.*, 2002; Samways *et al.*, 2015). Marine-derived nutrients are assimilated into freshwater food webs via two predominant pathways: (i) indirect incorporation through the sorption and remineralization of dissolved MDN into the biofilm and subsequent trophic transfer (Wipfli *et al.*, 1999; Guyette *et al.*, 2014; Samways *et al.*, 2015) or (ii) direct consumption of marine-derived organic matter such as eggs and carcasses (Naiman *et al.*, 2002). Tracing the pathways of these MDNs through freshwater food webs is challenging because it requires an understanding of the mode and timing of nutrient delivery.

Studies on Pacific salmon (*Oncorhynchus* spp.) have demonstrated the importance of MDNs for maintaining enhanced levels of stream productivity by increasing nutrient availability (Naiman *et al.*, 2002), whereby the loss of these MDNs may reduce stream productivity (Stockner & Ashley, 2003). These beneficial effects have been observed at multiple trophic levels including biofilm (Wipfli *et al.*, 1998; Johnston *et al.*, 2004; Rüegg *et al.*, 2012), macroinvertebrates (Wipfli *et al.*, 1998, 1999; Chaloner *et al.*, 2004; Lessard and Merritt, 2006; Lessard, 2009), and fish (Johnston *et al.*, 1990; Wipfli *et al.*,

2003, 2010). However, in some cases, disturbance from spawning Pacific salmon can also decrease biofilm biomass (Moore & Schindler, 2008).

Marine-derived nutrients from anadromous fishes along the northwest Atlantic coast play an important role in freshwater productivity (Durbin *et al.*, 1979; Jonsson & Jonsson, 2003; Nislow *et al.*, 2004; Jardine *et al.*, 2009; Nislow & Kynard, 2009; Walters *et al.*, 2009; Guyette *et al.*, 2013), with many anadromous species spawning in the same rivers (Garman & Macko, 1998), at least historically. The diverse life histories and spawning modes of Atlantic anadromous fishes can potentially influence the relative effects of MDNs from partial carcass loading, gametes, and excretory products on aquatic ecosystems in a manner distinct from the Pacific northwest. In Atlantic rivers receiving MDNs, the bottom-up transfer of energy and nutrients from biofilm to fish may be of greater importance for maintaining production and ecosystem function than previously thought (Kiffney *et al.*, 2014). Species such as rainbow smelt (*Osmerus mordax*), alewives (*Alosa pseudoharengus*), sea lamprey (*Petromyzon marinus*), and Atlantic salmon (*Salmo salar*), contribute significant MDN subsidies through the release of gametes, excretory products, and partial carcass loading (Durbin *et al.*, 1979; Jonsson & Jonsson, 2003; Nislow *et al.*, 2004; Jardine *et al.*, 2009; Nislow & Kynard, 2009; Walters *et al.*, 2009; Samways *et al.*, 2015), in amounts comparable to those of Pacific salmon (Parmenter & Lamarra, 1991; Jardine *et al.*, 2005b, 2009).

Differences in timing and spawning mode displayed by Atlantic anadromous species represent spatial and temporal heterogeneity in MDN subsidies across the landscape. Iteroparous rainbow smelt spawn in early spring, laying demersal, adhesive eggs in small streams and tributaries, often just above the head of tide (McKenzie, 1964;

Chase, 2009). Alewives, also iteroparous, spawn in late spring in lakes and/or lentic areas within rivers, where they deposit demersal, non-adhesive eggs (Mullen *et al.*, 1986; Mather *et al.*, 2012). Sea lamprey, the only obligate semelparous species, spawn in early summer where they construct nests in riffles to lay and bury their eggs (Scott & Scott, 1988). Atlantic salmon are iteroparous and spawn in late autumn in similar habitats as sea lamprey (Bardonnnet & Baglinière, 2000). Collectively these MDN inputs should be measurable as variable ecological responses within freshwater communities and potentially altering food web dynamics.

Stable isotope analysis of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) is often used to study marine-nutrient dynamics and trophic interactions (Bilby *et al.*, 1996; Chaloner *et al.*, 2002; Jardine *et al.* 2009; Reisinger *et al.*, 2013; Weber & Brown, 2013). Marine environments are typically enriched in the heavy isotopes of carbon (^{13}C) and nitrogen (^{15}N), whereas freshwater systems are typically depleted (Kline *et al.*, 1990). Enriched isotope ratios in anadromous fish relative to freshwater biota (Charles *et al.*, 2004) provide a means of tracing marine C and N through the food web, with temporal pulses of isotopic enrichment indicating incorporation of MDNs by consumers. Stable isotope mixing models provide an opportunity to identify a time-integrated assessment of the contributions of MDNs to a consumer's diet (Phillips & Gregg, 2001).

The focus of our research was to evaluate how different timing and spawning modes in anadromous fishes influence resource subsidization through indirect and direct trophic pathways within food webs of Atlantic rivers. The objectives of this study were to (i) determine how MDNs transported to five rivers by Atlantic salmon, alewife, rainbow smelt, or sea lamprey affect short-term resource dynamics of lotic communities,

and (ii) determine the relative contribution and temporal shifts of MDNs to the diet of freshwater resident fishes (salmon parr and brook trout). We predicted that MDNs would be incorporated into the tissues of freshwater organisms, however the pathway of incorporation would differ depending on the anadromous species and the nature of the spawning; primarily direct consumption in rivers with readily accessible eggs and carcasses (e.g. Knoydart Brook with rainbow smelt) and indirect assimilation when excretory products are the primary nutrient source (e.g. Oromocto River with alewife). We also predicted that the tissue isotopic composition of resident stream fish would shift to reflect a diet high in MDNs during the period of spawning by anadromous fish.

3.3 Methods

3.3.1 Site Descriptions

Five rivers in Atlantic Canada (New Brunswick and Nova Scotia) were chosen for study in 2010 (see Samways *et al.* (2015) for map of study sites). Each river supported anadromous fish populations and was selected to represent MDN transport primarily by rainbow smelt (Knoydart Brook), alewife (Oromocto River), sea lamprey (Cross Creek), or Atlantic salmon (Rocky Brook), although minor contributions by other anadromous fishes may have been present in each river system (Samways *et al.*, 2015). Doctor's Brook served as a reference system with no MDN inputs, although prior to 2009, rainbow smelt commonly spawned in this river (Jardine *et al.*, 2009).

Each river, including Doctor's Brook, contained a natural barrier (e.g. impassable waterfall) to upstream fish migration. Two sites a minimum of 5 km upstream of the barrier (no anadromous fish) and three sites downstream of the barrier were selected as

reference and treatment (marine-nutrient enriched) sites, respectively. Study sites were located in riffle-type habitats with a mean flow of 0.49 m s^{-1} (ranging from 0.05 to 1.62 m s^{-1} throughout the study period) and had a similar substrate composition of small cobble to boulders. Stable isotope ratios in samples from the upstream sites, and all sites in Doctor's Brook, served as references (absence of marine-derived C and N) at two scales of spatial resolution (within and between river systems) whereas isotope ratios in samples collected in the treatment sites downstream of the barrier represented a mixture of marine and freshwater sources. This upstream-downstream approach is commonly used to evaluate the effects of MDNs (Wipfli *et al.*, 1999; Chaloner *et al.*, 2004; Mitchell & Lamberti, 2005; Collins *et al.*, 2011). Stream samples were collected at least three weeks prior to any spawning (pre-spawn), as well as throughout the spawning and post-spawning periods in both the upstream and downstream sites, following a Before-After-Control-Impact (BACI) study design (e.g. Stewart-Oaten *et al.*, 1986; Mitchell & Lamberti, 2005; Collins *et al.*, 2011). Under the assumption that anadromous fish spawning did not affect stream processes upstream of the barrier (Stewart-Oaten *et al.*, 1986), we compared stable isotope ratios from downstream sites to upstream sites to track the level of enrichment or depletion due to MDN uptake.

3.3.2 Sample Collection and Processing

Samples of biofilm, benthic macroinvertebrates, and fish ($n = 3\text{-}10$ individuals per taxonomic group, per sampling round) were collected for stable isotope analysis from each site in each river from April to November 2010. Biofilm samples were collected from the upper surface of three rocks, chosen haphazardly, by scraping until bare rock

was exposed. Prior to stable isotope analysis, biofilm samples were centrifuged and then manually cleaned to remove inorganic components. Benthic macroinvertebrates were sampled using a D-frame kick-net, and identified to family. Specific macroinvertebrates were selected for analysis according to their main functional feeding mode, specifically scrapers (Ephemeroptera: Heptageniidae), predators (Plecoptera: Perlidae), filter-feeders (Diptera: Simuliidae) and shredders (Plecoptera: Pteronarcyidae). Electrofishing was used to collect fish (Atlantic salmon parr and brook trout (*Salvelinus fontinalis*); these were anesthetized and an upper caudal fin clip was sampled for stable isotope analysis. Fin tissue was selected because it can be collected non-lethally (Kelly *et al.*, 2006) and it correlates closely with muscle tissue (Jardine *et al.*, 2005a, 2011). All samples were placed on ice until they could be frozen at the lab. Prior to stable isotope analysis, samples were oven-dried at 60°C for 48 hours, ground to a homogenous powder and weighed into tin cups.

Stable isotope analysis of carbon and nitrogen was performed at the Stable Isotopes in Nature Laboratory (SINLAB), Canadian Rivers Institute, University of New Brunswick on a Costech 4010 elemental analyzer interfaced through a Conflo III to a DeltaPlus XP continuous-flow mass spectrometer (Thermo Finnigan; Bremen, Germany). Isotope values, reported in standard δ -notation, were calculated by:

$$(3.1) \quad \delta X = \left(R_{\text{sample}} / R_{\text{standard}} \right) - 1$$

where, X represents the stable isotope of interest (i.e. ^{13}C or ^{15}N) and R is the ratio of heavier to lighter isotope ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$, respectively) in the sample and the international standard. The isotope values are thus reported in parts per thousand (‰) deviations from the international standard (Vienna Pee Dee Belemnite, VPDB, and

atmospheric nitrogen, AIR, respectively). Calibrated in-house standards were analyzed within each analytical run to normalize the isotope measurements. Analytical precision, measured as the standard deviation of replicate standards, was better than ± 0.2 ‰ for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. Duplicate determination of actual samples agreed to within ± 0.2 ‰ for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$.

3.3.3 Data Analysis

Changes in isotopic composition through time were assessed to characterize temporal dynamics of MDN incorporation into freshwater food webs, as turnover rates of ^{13}C and ^{15}N are relatively fast, especially in benthic organisms of streams (Jardine *et al.*, 2014). We assumed each food web component in treatment sites within a given river would experience similar natural seasonal changes as those in reference sites. The convex hull total area (TA) encompassing isotopic values for all community members (biofilm, Heptageniidae, Perlidae, Simuliidae, Pteronarcyidae, and fish) provided an indication of ecological niche space (Layman *et al.*, 2007). We plotted the TA values for both the upstream reference and downstream treatment sites for each river prior to anadromous fish spawning and during the peak of spawning to represent the shift in the food web as a result of MDNs. The TA values were calculated using the Stable Isotope Bayesian Ellipses in R (SIBER) package (Jackson *et al.*, 2011).

We measured stable isotope ratios in samples prior to anadromous fish spawning and assumed these to represent initial ‘background’ conditions. To account for inherent temporal variability and compare changes in stable isotope values between treatment and reference sites, the difference between downstream and upstream response values was

used. The standardized values representing seasonal patterns of change from reference conditions were calculated as:

$$(3.2) \quad \Delta\delta X_i = (\delta X_{di} - \delta X_{d0}) - (\delta X_{ui} - \delta X_{u0})$$

where $\Delta\delta X_i$ is the change in the response value (i.e. abundance, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$) at time i , δX_{di} is the downstream response value at post-spawn time i , δX_{d0} is the downstream pre-spawn response value, δX_{ui} is the upstream response value at post-spawn time i , δX_{u0} is the upstream pre-spawn response value.

We evaluated the effect of MDN incorporation upstream or downstream of a spawning barrier with time using mixed-model ANOVAs. The ANOVA model included the fixed factors Barrier (upstream or downstream), Date (sampling round), Organism and the random factor Site (two or three replicates above or below the barrier, along the length of the river). The appropriate denominators for F-ratios were determined following Underwood (1997). Multiple comparisons of significant fixed sources of variation were conducted using Tukey's test. Bonferroni correction was applied to the p -values to account for the error associated with multiple comparisons. Statistical tests were performed using R along with the R package *nlme* (Pinheiro *et al.*, 2012; R Core Team, 2014).

The Bayesian stable-isotope mixing model (SIAR) was used to estimate the reliance of resident salmonids (Atlantic salmon parr and brook trout) on marine-nutrient resources using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Parnell *et al.*, 2010; Parnell & Jackson, 2013). The mean ($\pm$ SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of pteronarcyid (shredder) stoneflies from each sampling period were used to define, isotopically, the freshwater resources. This taxon

has a long developmental time and strict diet which makes it well suited as an isotopic baseline for top predators (Jardine *et al.*, 2014) and unlike the other invertebrates sampled, the Pteronarcyidae specimens did not incorporate MDNs (see results) which would otherwise confound the mixing model. The mean ($\pm$ SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of eggs from the predominant anadromous fish species for each river were used as a proxy to define the marine isotopic signal. We used trophic discrimination values of $0.4 \pm 1.3\text{‰}$ for $\delta^{13}\text{C}$ and $3.4 \pm 1.0\text{‰}$ for $\delta^{15}\text{N}$ (Post, 2002). The models were run using Markov chain Monte Carlo (MCMC) chains with 500,000 iterations and 50,000 of burn-in.

3.4 Results

3.4.1 Temporal Isotopic Enrichment

Stable isotope values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ varied seasonally in both downstream treatment and upstream reference sites (Appendix 3). In the downstream treatment sites in all rivers receiving MDNs from spawning anadromous fishes, we detected a significant enrichment of both ^{13}C and ^{15}N in samples during spawning and post-spawning periods by anadromous fishes (relative to pre-spawning samples), in the biofilm, macroinvertebrates (Heptageniidae scrapers, Perlidae predators, and Simuliidae filter-feeders) and in resident salmonid fin tissues (all comparisons had an F -value ≥ 8.197 and a p -value(*adj.*) ≤ 0.001); Pteronarcyidae shredders were the only group where a significant isotopic enrichment was not detected. Isotopic enrichment coincided with anadromous fish spawning, however the magnitude and duration of enrichment varied between rivers.

As only juvenile fish were sampled, growth of new tissue is the main isotopic

driver and anabolic processes help to assimilate the marine resources quickly, whereas catabolic turnover is small and not as important here. This rapid isotopic turnover in fish fin tissue was observed with rainbow smelt spawning in a river in Prince Edward Island, Canada (Sean Landsman; personal communication). Additionally, Heady and Moore (2012) also observed fin tissue turnover times in the order of three weeks in rainbow trout, *Oncorhynchus mykiss*.

Community niche space did not change in Doctor's Brook, with no MDN inputs, between the pre-spawn and spawning sampling periods in either the upstream reference or downstream treatment sites (Fig 1a). By contrast, in downstream treatment sites in all rivers receiving MDNs, community niche space shifted between pre-spawn and spawning sampling periods in the direction of the marine-nutrient subsidies, while in upstream reference sites community niche space did not change (Fig 1 b-e). In Knoydart Brook, Cross Creek and Rocky Brook, the TA increased from 2-10 times from the pre-spawn period (Fig 1b,d,e), whereas in the Oromocto River the TA decreased 3 times during the spawning period (Fig 1c).

In Doctor's Brook, with no MDN inputs, there were no significant changes in $\delta^{13}\text{C}$ values between the pre-spawn and post-spawn sampling periods for any organisms sampled in either the upstream reference or downstream treatment sites (Fig 2a). In Knoydart Brook, biofilm showed the greatest mean enrichment in ^{13}C (8.38 ‰) of any group in any river (Fig 2b). As with the biofilm, Heptageniidae also reached maximum enrichment (5.70 ‰) three weeks into the spawning period. In contrast, Simuliidae did not reach maximum enrichment (4.26 ‰) until after spawning occurred and rainbow smelt larvae had hatched and migrated back to the marine environment. Perlidae and

resident fish tissue reached maximum enrichment (3.73 and 3.62 ‰; respectively) two weeks into the spawning period, remaining elevated into June (Fig 3b). In the Oromocto River, Heptageniidae (6.05 ‰), biofilm (5.33 ‰), Simuliidae (5.03 ‰), and Perlidae (3.90 ‰) all reached maximum ^{13}C enrichment within two weeks of the spawning period (Fig 2c), with Simuliidae maintaining enrichment into June and Heptageniidae remaining enriched into July. Fish tissue did not reach maximum enrichment (2.64 ‰) until three weeks into the spawning period. In Cross Creek where early summer spawning lamprey predominated, larval Simuliidae and fish tissue reached maximum ^{13}C values (4.22 and 4.17 ‰; respectively) two weeks into the spawning period and remained elevated into August (Fig 2d). Biofilm, Perlidae, and Heptageniidae also became enriched two weeks into the spawning period (3.54, 2.71, and 2.53 ‰; respectively), with biofilm maintaining enrichment into July, while Perlidae and Heptageniidae declined to baseline levels (Fig 2d). In Rocky Brook (autumn spawning salmon stream), groups did not display maximum ^{13}C enrichment until four to six weeks into the spawning period (Fig 2e). Fish tissue became significantly enriched two weeks into the spawning period, reaching maximum enrichment (1.81 ‰) at the beginning of November and remaining elevated after spawning had ceased. Simuliidae reached maximum enrichment after four weeks (2.34 ‰), whereas biofilm, Perlidae, and Heptageniidae (3.59, 3.12, and 2.47 ‰; respectively) reached maximum enrichment in mid-November, after the spawning period (Fig 2e).

As with $\delta^{13}\text{C}$, there were no significant changes in $\delta^{15}\text{N}$ values in Doctor's Brook between the pre-spawn and post-spawn sampling periods for any organisms sampled in either the upstream reference or downstream treatment sites (Fig 3a). In Knoydart Brook,

the Simuliidae showed the greatest mean enrichment in ^{15}N (6.71 ‰) of any group in any river, however maximum enrichment did not occur until just after spawning (Fig 3b). In contrast, Perlidae, biofilm and Heptageniidae reached maximum enrichment (4.27, 1.63 and 2.18 ‰, respectively) two to three weeks into the rainbow smelt spawning period. Knoydart Brook also had the greatest mean ^{15}N enrichment for resident fish compared to any other river (5.62 ‰) and enrichment lasted until into July (Fig 3b). In the Oromocto River, the biofilm, Heptageniidae, Perlidae, and Simuliidae all reached maximum ^{15}N enrichment two weeks into the alewife spawning period (5.59, 4.81, 3.00, 2.22 ‰; respectively) (Fig 3c). The Heptageniidae in the Oromocto River had the greatest sustained enrichment in any river, lasting into July, well after the spawning period. Mean ^{15}N enrichment (2.37 ‰) in fish in the Oromocto River did not occur until three weeks into the spawning run (Fig 3c). In Cross Creek, all groups sampled (except the shredders) had their highest level of ^{15}N enrichment two weeks into the sea lamprey spawning period, with the Perlidae having the greatest enrichment (4.76 ‰), followed by biofilm (2.49 ‰), Simuliidae (2.29 ‰), Heptageniidae (1.71 ‰) and resident fish (1.11 ‰) (Fig 3d). In contrast, most of the groups sampled in Rocky Brook did not display maximum ^{15}N enrichment until four weeks into the Atlantic salmon spawning period (Fig 3e). Mean ^{15}N enrichment was greatest for the resident fish compared to any other river (4.56 ‰), whereas enrichment for the biofilm (2.72 ‰), Simuliidae (2.28 ‰), Perlidae (1.89 ‰), and Heptageniidae (1.50 ‰) were much lower (Fig 3e).

3.4.2 Reliance on Marine-derived Nutrients

The SIAR mixing model results, based on Atlantic salmon parr and brook trout fin

tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values, indicated a reliance on marine-nutrient subsidies during anadromous fish spawning periods (Fig 4). Resident brook trout and salmon parr derived a high percentage of their nutrients (overall mean = 39.9%; mean range: 33.1-47.0%) from marine subsidies during the period of anadromous fish spawning. No differences in modal MDN resource use estimates among were detected in Doctor's Brook (no MDN's present); values ranged between 1.3% and 8.7%, with no significant difference between sampling periods (95% credibility intervals, CI, between 0.0% and 14.8% for all sampling times) (Fig 4a). In Knoydart Brook, there was increased assimilation of marine nutrients in May (modes range between: 22.3-41.2%) during rainbow smelt spawning. Combined with the 95% CI for MDN use for each sampling period during spawning (16.2-48.7%), these data indicated a reliance on marine-nutrient subsidies by resident salmonids (Fig 4b). Resident fish in the Oromocto River also displayed a reliance on MDN resources in May (mode: 38.1%; 95% CI 30.4-45.1%) coinciding with alewife spawning and extending at least to the end of June (Fig 4c; mode: 22.9%; 95% CI 14.5-30.3%). Marine-nutrient resource use in Cross Creek occurred in mid-June, at the beginning of the lamprey spawning period (mode: 33.1%; 95% CI 27.1-37.9%) and was still evident by late July (Fig 4d). For Rocky Brook, the SIAR mixing model indicated a reliance on marine-nutrient subsidies starting in late October (mode: 20.7%; 95% CI 15.4-26.6%), continuing into November (mode: 47.0%; 95% CI 34.4-57.5%), and was still evident in mid-November as spawning ended and sampling was discontinued (mode: 30.9%; 95% CI 25.3-35.5%) (Fig 4e).

3.5 Discussion

Our data showed that MDNs were incorporated into all trophic levels across streams with spawning Atlantic salmon, alewife, rainbow smelt, and sea lamprey. Biofilm, macroinvertebrates, and resident salmonids were more enriched in ^{13}C and ^{15}N in downstream sites receiving MDN subsidies than in upstream reference sites during and soon after spawning by anadromous fishes. Community-wide niche space shifted toward the marine-nutrient source, however TA did not always increase with MDN inputs. Community-wide metrics, such as TA, represent the extent of trophic diversity within multiple, similarly comprised food webs, especially when examining temporal changes and/or exposure to environmental gradients (Layman *et al.*, 2007).

The magnitude and temporal patterns of isotopic enrichment differed amongst rivers depending on the timing and spawning mode of the anadromous fish. In rivers receiving MDNs, peak $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were synchronous with the period of spawning, a general pattern observed for all trophic groups, with the exception of the Pteronarcyidae shredders for which there was no response to MDNs. The SIAR mixing model displayed a strong pattern of reliance on MDN resources in the diet of resident top predators during anadromous fish spawning periods. The contribution of MDNs to community food webs is indicative of short-term resource use and reflects the opportunistic use of marine-nutrient subsidies in various Atlantic rivers.

Overall, isotopic enrichment within each group was similar between rivers and consistent with previous studies investigating the effects of MDNs from Pacific salmon (Kline *et al.*, 1990; Bilby *et al.*, 1996; Wipfli *et al.*, 1999; Chaloner *et al.*, 2002; Reisinger *et al.*, 2013) and Atlantic anadromous fishes (Walters *et al.*, 2009; Guyette *et al.*, 2014;

Samways *et al.*, 2015). Regardless of the species of anadromous fish present, spawning mode, or time of year, MDNs are readily incorporated into stream food webs. Only the Pteronarcyidae shredders in our study streams showed no evidence of MDN uptake based on isotopic enrichment, a result also observed in streams of the Pacific Northwest (Claeson *et al.*, 2006). Other studies, however, have demonstrated a 3.5-4.0‰ enrichment in the pteronarcyid group (Bilby *et al.*, 1996; Walters *et al.*, 2009; Guyette *et al.*, 2014). Given that all of our study rivers were in well forested areas with high leaf-litter contribution and retention, the lack of MDN assimilation by shredder invertebrates may be the result of sufficient allochthonous resources allowing shredders to concentrate their feeding on leaf litter (Claeson *et al.*, 2006). Such evidence of enrichment among trophic levels and rivers provide insight into the importance of these anadromous species to stream ecosystem function.

Our study also goes one step further, distinguishing how differences in anadromous life history and spawning mode influence the predominant pathway of MDN uptake. Anadromous fishes deliver dissolved MDNs through milt, ovarian fluid, and excretory products, thereby elevating the dissolved nutrient pool and subsequently stimulating in-stream production (Wipfli *et al.*, 1998; Rüegg *et al.*, 2012; Samways & Cunjak, 2015). Biofilm incorporates these readily available dissolved nutrients as shown by the relatively quick isotopic enrichment during the first three weeks of the spawning period of spring and summer spawning anadromous fishes (smelt, alewife, lamprey). However, the uptake by biofilm is much lower in Rocky Brook, the fall spawning stream, likely a function of cold water temperatures slowing uptake. The predominance of this bottom-up attenuation pathway relative to direct consumption depends on the availability

of marine-derived material (primarily eggs and carcass tissues), which is associated with the anadromous species present.

The availability and relative abundance of MDNs from rainbow smelt resulted in the greatest increase in community niche space of any river. Rainbow smelt contribute large amounts of MDNs and organic matter through excretory products, non-viable eggs, shells from hatched eggs and limited carcass decomposition, which are incorporated through direct consumption, and indirectly via breakdown and subsequent nutrient release. Limited spawning habitat in Knoydart Brook (based on in-stream characteristics, such as suitable substrate, flow, and amount of structure or barriers) resulted in smelt egg deposition up to 122,677 eggs/100 cm² (Charest, 2010) and led to the highest spawning density (5.41 individuals/m²) observed in any of the study rivers (Samways *et al.*, 2015). This, combined with the fact that smelt lay adhesive eggs ‘en masse’ on top of substrate, provided MDNs in forms readily available for direct consumption by fish and predatory invertebrates, as well as indirect pathways through uptake of dissolved nutrients by the biofilm and subsequent trophic transfer. After a 20 day incubation period (McKenzie, 1964; personal observation), smelt larvae hatch and migrate back to the sea, mobilizing large amounts of particulate marine-derived matter from the egg casings, consumed by filter-feeders. This is likely a key pathway for trophic transfer of MDNs to stream fish and macroinvertebrate predators beyond the spawning period. Because of the demersal nature of smelt spawning, biofilm is closely coupled to the mobilization of nutrients from decomposing eggs, and the subsequent uptake of these nutrients (Wetzel, 1993), extends biofilm enrichment beyond the spawning period. Isotope values remain elevated in the predatory Perlidae and resident fishes likely through trophic transfer via indirect bottom-

up processes.

Alewives contribute large quantities of MDNs to rivers through excretory products and their large spawning biomass (Durbin *et al.*, 1979; Post & Walters, 2009; West *et al.*, 2010). Nutrients from excretory products (e.g. ammonia) are available for immediate uptake by the biofilm (Vanni, 2002), unlike nutrient subsidies from eggs and carcasses, which have to be mobilized before uptake. The sizeable quantity of excretory products from the large spawning population in the Oromocto River (e.g. 575,140 alewives in 2010; (Oromocto River Watershed Association Inc., 2014)) resulted in biofilm displaying the greatest increase in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values of any river. The Heptageniidae scrapers in the Oromocto River had one the largest increases in isotopic enrichment of any river through feeding on the greatly enriched biofilm, and the influence persisted well past the spawning period. Alewives are predominantly lake spawners, therefore nutrient subsidies to lotic portions of the river are primarily through excretory products with limited opportunity for direct consumption (no eggs and partial carcass loading). However passing obstacles such as rapids or falls can increase mortality, making carcasses a more significant resource in these areas.

Being the only obligate semelparous species in our study, the pathway of incorporation of MDNs from sea lamprey likely depends on carcass loading and retention within the stream (Weaver *et al.*, 2015). Post-spawning salmonid carcasses (in western North America) are retained in streams or accumulate in slow moving areas through downstream displacement (Williams *et al.*, 2010). Although we did observe sea lamprey spawning, we did not observe many carcasses remaining in the study areas; therefore we deemed that carcass loading was not a major vector of marine subsidies in our rivers.

Rather, direct consumption of isotopically enriched lamprey eggs was the predominant pathway of MDN incorporation into the food web of Cross Creek. MDN's likely found their way into the diets of resident fish through the consumption of sea lamprey eggs and invertebrate prey enriched through trophic transfer. This was reflected in the overall increase in community niche space post-spawning, and the shift in isotopic space towards the MDN source (i.e. sea lamprey eggs). Summer marks the period of slow growth for both brook trout and Atlantic salmon parr (Carlson *et al.*, 2007; Davidson *et al.*, 2010; Xu *et al.*, 2010). The relatively low isotopic change in the resident salmonids was likely due to reduced feeding rates (Carlson *et al.*, 2007; Xu *et al.*, 2010), leading to reduced tissue formation and isotopic turnover. The persistence of MDNs in the system post-spawn may have been the result of prolonged nutrient loading from the breakdown of lamprey carcasses retained in the stream.

Isotopic enrichment in the Rocky Brook fauna clearly demonstrated the flow of MDNs between spawning Atlantic salmon, biofilm, macroinvertebrates and resident stream salmonids. The energetically and nutrient rich salmon eggs offer a valuable resource and salmonids are known to directly consume salmon eggs (Thomaz *et al.*, 1997; Cunjak & Therrien, 1998; Bently *et al.*, 2012). The relatively fast ^{15}N enrichment of fin tissue of resident fishes during the period of salmon spawning suggests a direct consumption of eggs. In Rocky Brook, the peak of Atlantic salmon spawning occurs in late October, a period marked by a decline in stream productivity (Liboriusson & Jeppesen, 2009), likely a function of rapidly declining water temperatures. However, through the incorporation of MDN subsidies, biofilm biomass can actually increase (Wipfli *et al.*, 1998; Johnston *et al.*, 2004; Walters *et al.*, 2009; Samways *et al.*, 2015).

Such a response may explain the increase in isotope values in the biofilm and subsequently, in the Heptageniidae scrapers that feed on biofilm components.

The Oromocto River and Knoydart Brook provide extreme examples of how MDN's and spawning mode combine to contract or expand niche space; respectively. A marked reduction in niche width was also observed as a result of habitat fragmentation (Layman et al. 2007) and differential habitat use (Quevedo et al. 2009). In this study, the change in niche width appears to be associated with differential resource use/availability rather than habitat related. In Knoydart Brook, the expansion of niche width was a function of the increase in diversity of available resources, as represented by the increased variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Aquatic organisms were able to exploit the various forms of MDNs available (e.g. dissolved, gametes, particulate) through rainbow smelt spawning resulting in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values varying by as much as 8.2 and 7.4 ‰, respectively. In the Oromocto River, with alewives passing through on their way to spawn in the headwater lake, the decrease in community niche space and shift to a marine source was the result of trophic transfer through the consumption of organisms (e.g. biofilm) capable of exploiting marine resources in the dissolved nutrient pool. This resulted in a food web with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values only varying by as much as 3.3 and 4.2 ‰, respectively, and within the assumed values of fractionation for trophic transfers (Post 2002, Layman *et al.*, 2005). Historically, these and other anadromous species would have spawned within the same rivers, resulting in temporally pulsed marine-nutrient subsidies that were spatially distributed within river systems and released to consumers in the recipient habitat(s). These sequential MDN pulses allow different consumers within the system to respond and exploit the resources to enhance growth, which in turn may

have helped keep resources at an elevated level and increased production (Holt, 2008). By studying each anadromous species separately, as in this study, a pattern became obvious relating spatio-temporal uptake of MDNs in Atlantic river food webs. Due to the high degree of spatio-temporal heterogeneity in the quantity and quality of marine-nutrient subsidies among different anadromous fish species, with different spawning modes, the importance of multiple nutrient pulses to rivers may be greater than previously considered.

This study provides evidence that anadromous fishes are important vectors of pulsed marine-nutrient subsidies to freshwater ecosystems. Determining how pulsed marine nutrient subsidies permeate through food webs and influence ecosystem process and function is a significant challenge in ecology (Ostfeld & Keesing, 2000). Identifying the impacts of MDNs on freshwater communities includes the evaluation of bottom-up influences as well as integrating the timing, abundance and life history mode of anadromous fishes into the mechanism of MDN incorporation and transfer. Stable isotope analysis revealed that stream organisms were enriched in marine isotopes. As well, there was evidence of community wide marine-resource assimilation, suggesting organisms at multiple trophic levels derive a substantial proportion of their energy from these pulses of marine resources. The patterns and processes of MDN incorporation and transfer in streams are mediated by the spatial, temporal and life history characteristics of the anadromous species delivering the pulsed resource subsidy (Polis *et al.*, 1997; Bently *et al.*, 2012; Samways *et al.*, 2015). Our findings also revealed shifts in energy flow between indirect (bottom-up) and direct resource consumption, which would regulate the relative contribution of marine resource use to the freshwater food web. Two pathways

of MDN incorporation were identified: (1) direct consumption of marine-derived organic matter by macroinvertebrates and fish, and (2) indirect consumption through uptake and trophic transfer of dissolved nutrients. While MDNs delivered from some species greatly enhance productivity via bottom-up trophic pathways (e.g. alewife), other anadromous species promote the direct consumption of eggs and partial carcass loading (e.g. rainbow smelt, sea lamprey, Atlantic salmon). The results indicated that both pathways were involved in incorporating MDNs into stream food webs, a response also reported in other studies (Bilby *et al.*, 1996; Chaloner *et al.*, 2002; Reisinger *et al.*, 2013). It is difficult to make definitive statements about the relative importance of individual pathways as it depends both on the organism assimilating the marine-nutrients and the anadromous species delivering the marine subsidy. The current trend of declining anadromous fish populations means fewer nutrient-rich marine subsidies for stimulating trophic production in these river systems. Consequently, decreased riverine productivity may be self-perpetuating as fewer returning adults diminish the ability of a river to produce (and sustain) subsequent generations (Bilby *et al.*, 1996). This study provides insight into the pathways by which MDNs are assimilated into freshwater food webs and therefore a better understanding of how these nutrients can affect the long-term productivity of these rivers. Restoration strategies focused on a single species may not be as effective as including multiple anadromous species. Therefore, in order to maintain ecosystem function and productivity, it is critical to include anadromous fishes (and the marine subsidies they deliver) for effective ecosystem management and river restoration strategies.

3.6 Acknowledgements

The authors greatly appreciate the extensive help and effort put forth by Aaron Fraser, Michelle Charest, Emily Corey, Kabir Tosho, Sarah Lehnert, Catherine Desjardins, and Crystal Lewis in the field. We also thank Anne McGeachy, Christine Paton and Mireille Savoie for their help with the stable isotope analysis. Brian Hayden is gratefully acknowledged for his help with the SIAR mixing models.

3.7 References

- Anderson, W. B., Wait, D. A. & Stapp, P. (2008). Resources from another place and time: responses to pulses in a spatially subsidized system. *Ecology* **89**, 660-670. Doi: 10.1890/07-0234.1
- Bardonnnet, A. & Baglinière, J.-L. (2000). Freshwater habitat of Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 497-506. Doi: 10.1139/f99-226.
- Bently, K. T., Schindler, D. E., Armstrong, J. B., Zhang, R., Ruff, C. P. & Lisi, P. J. (2012). Foraging and growth responses of stream-dwelling fishes to inter-annual variation in a pulsed resource subsidy. *Ecosphere* **3**, 113. DOI: 10.1890/ES12-00231.1
- Bilby, R. E., Fransen, B. R. & Bisson, P. A. (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. Doi: 10.1139/f95-159.
- Carlson, S. M., Hendry, A. P. & Letcher, B. H. (2007). Growth rate differences between resident native brook trout and non-native brown trout. *Journal of Fish Biology* **71**, 1430-1447. doi:10.1111/j.1095-8649.2007.01615.x.
- Chaloner, D. T., Lamberti, G. A., Merritt, R. W., Mitchell, N. L., Ostrom, P. H. & Wipfli, M. S. (2004). Variation in responses to spawning Pacific salmon among three south-eastern Alaska streams. *Freshwater Biology* **49**, 587-599. DOI: 10.1111/j.1365-2427.2004.01213.x.
- Chaloner, D. T., Wipfli, M. S. & Caouette, J. P. (2002). Mass loss and macroinvertebrate colonisation of Pacific salmon carcasses in south-eastern Alaskan streams. *Freshwater Biology* **47**, 263-273. DOI: 10.1046/j.1365-2427.2002.00804.x.
- Charest, M.A. (2010). Estimation of rainbow smelt, *Osmerus mordax*, population in Knoydart Brook and the incorporation of rainbow smelt egg nutrients into the food web. Honours thesis, University of New Brunswick, Fredericton, Canada.
- Charles, K., Roussel, J.-M. & Cunjak, R. A. (2004). Estimating the contribution of sympatric anadromous and freshwater resident brown trout to juvenile production. *Marine and Freshwater Research* **55**, 185-191. DOI: 10.1071/MF03173.

- Chase, B. C. (2009). The spawning habitat of anadromous rainbow smelt: trouble at the tidal interface. In *Challenges for diadromous fishes in a dynamic global environment*. (Haro, A. J., Smith, K. L., Rulifson, R. A., Moffitt, C. M., Klauda, R. J., Dadswell, M. J., Cunjak, R. A., Cooper, J. E., Beal, K. L. & Avery, T. S., eds), pp. 859-862. American Fisheries Society, Symposium 69, Bethesda, Maryland.
- Claeson, S. M., Li, J. L., Compton, J. E. & Bisson, P. A. (2006). Response of nutrients, biofilm, and benthic insects to salmon carcass addition. *Canadian Journal of Fisheries and Aquatic Sciences* **63**, 1230-1241. Doi: 10.1139/f06-029.
- Collins, S. F., Moerke, A. H., Chaloner, D. T., Janetski, D. J. & Lamberti, G. A. (2011). Response of dissolved nutrients and periphyton to spawning Pacific salmon in three northern Michigan streams. *Journal of the North American Benthological Society* **30**, 831-839. DOI: 10.1899/10-164.1.
- Cunjak, R. A. & Therrien, J. (1998). Inter-stage survival of wild juvenile Atlantic salmon, *Salmo salar* L. *Fisheries Management and Ecology* **5**, 209-224. DOI: 10.1046/j.1365-2400.1998.00094.x.
- Davidson, R. S., Letcher, B. H. & Nislow, K.H. (2010). Drivers of growth variation in juvenile Atlantic salmon (*Salmo salar*): an elasticity analysis approach. *Journal of Animal Ecology* **79**, 1113-1121. DOI: 10.1111/j.1365-2656.2010.01708.x.
- Durbin, A. G., Nixon, S. W. & Oviatt, C. A. (1979). Effects of the spawning migration of the alewife, *Alosa pseudoharengus*, on freshwater ecosystems. *Ecology* **60**, 8-17. DOI: 10.2307/1936461.
- Garman, G. C. & Macko, S. A. (1998). Contribution of marine-derived organic matter to an Atlantic coast, freshwater, tidal stream by anadromous clupeid fishes. *Journal of the North American Benthological Society* **17**, 277-285. DOI: 10.2307/1468331.
- Guyette, M. Q., Loftin, C.S. & Zydlewski, J. (2013). Carcass analog addition enhances juvenile Atlantic salmon (*Salmo salar*) growth and condition. *Canadian Journal of Fisheries and Aquatic Sciences* **70**, 860-870. Doi: 10.1139/cjfas-2012-0496.
- Guyette, M. Q., Loftin, C. S., Zydlewski, J. & Cunjak, R. (2014). Carcass analogues provide marine subsidies for macroinvertebrate and juvenile Atlantic salmon in temperate oligotrophic streams. *Freshwater Biology* **59**, 392-406. DOI: 10.1111/fwb.12272.
- Heady, W. N. & Moore, J. W. (2012). Tissue turnover and stable isotope clocks to quantify resource shifts in anadromous rainbow trout. *Oecologia* **172**, 21-34. DOI: 10.1007/s00442-012-2483-9.
- Holt, R. D. (2008). Theoretical perspectives on resource pulses. *Ecology* **89**, 671-681. DOI: 10.1890/07-0348.1.
- Jackson, A. L., Parnell, A. C., Inger, R. & Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER – Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology* **80**, 595-602. DOI: 10.1111/j.1365-2656.2011.01806.x.
- Jardine, T. D., Cartwright, D. F., Dietrich, J. P. & Cunjak, R. A. (2005a) Resource use by salmonids in riverine, lacustrine and marine environments: evidence from stable

- isotope analysis. *Environmental Biology of Fishes* **73**, 309-319. DOI: 10.1007/s10641-005-2259-8.
- Jardine, T. D., Gray, M. A., McWilliam, S. M. & Cunjak, R. A. (2005b). Stable isotope variability in tissues of temperate stream fishes. *Transactions of the American Fisheries Society* **134**, 1103-1110. DOI: 10.1577/T04-124.1.
- Jardine, T. D., Hadwen, W. L., Hamilton, S. K., Hladysz, S., Mitrovic, S. M., Kidd, K. A., Tsoi, W. Y., Spears, M., Westhorpe, D. P., Fry, V. M., Sheldon, F. & Bunn, S. E. (2014). Understanding and overcoming baseline isotopic variability in running waters. *River Research and Applications* **30**, 155-165. DOI: 10.1002/rra.2630.
- Jardine, T. D., Hunt, R. J., Bradley, J., Pusey, B. J. & Bunn, S. E. (2011). A non-lethal sampling method for stable carbon and nitrogen isotope studies of tropical fishes. *Marine and Freshwater Research* **62**, 83-90. doi: 10.1071/MF10211.
- Jardine, T. D., Roussel, J.-M., Mitchell, S. C., & Cunjak, R. A. (2009). Pathways of marine nutrient and organic matter entry from anadromous fishes into Atlantic coast streams. In *Challenges for diadromous fishes in a dynamic global environment* (Haro, A. J., Smith, K. L., Rulifson, R. A., Moffitt, C. M., Klauda, R. J., Dadswell, M. J., Cunjak, R. A., Cooper, J. E., Beal, K. L. & Avery, T. S. eds), pp. 427-445. American Fisheries Society, Symposium 69, Bethesda, Maryland.
- Johnston, N. T., MacIsaac, E. A., Tschaplinski, P. J. & Hall, K. J. (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. Doi: 10.1139/f03-172.
- Johnston, N. T., Perrin, C. J., Slaney, P. A. & Ward, B. R. (1990). Increased juvenile salmonid growth by whole-river fertilization. *Canadian Journal of Fisheries and Aquatic Sciences* **47**, 862-872. Doi: 10.1139/f90-099.
- Jonsson, B. & Jonsson, N. (2003). Migratory Atlantic salmon as vectors for the transfer of energy and nutrients between freshwater and marine environments. *Freshwater Biology* **48**, 21-27. DOI: 10.1046/j.1365-2427.2003.00964.x
- Kelly, M. H., Hagar, W. G., Jardine, T. D. & Cunjak, R. A. (2006). Nonlethal sampling of sunfish and slimy sculpin for stable isotope analysis: How scale and fin tissue compare with muscle tissue. *North American Journal of Fisheries Management* **26**, 921-925. DOI: 10.1577/M05-084.1.
- Kiffney, P. M., Buhle, E. R., Naman, S. M., Pess, G. R. & Klett, R. S. (2014). Linking resource availability and habitat structure to stream organisms: an experimental and observational assessment. *Ecosphere* **5**, 39. DOI: 10.1890/ES13-00269.1.
- Kline, T. C., Goering, J. J., Mathisen, O. A., Poe, P. H. & Parker, P. L. (1990). Recycling of elements transported upstream by runs of Pacific salmon: 1. d15N and d13C evidence in Sashin Creek, southeastern Alaska. *Canadian Journal of Fisheries and Aquatic Sciences* **47**, 136-144. Doi: 10.1139/f90-014.
- Layman, C. A., Arrington, D. A., Montaña, C. G. & Post, D. M. (2007). Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology* **88**, 42-48. DOI: 10.1890/0012-9658(2007)88[42:CSIRPF]2.0.CO;2.
- Layman, C.A., Winemiller, K.O., Arrington, D.A. & Jepsen, D.B. (2005). Body size and trophic position in a diverse tropical food web. *Ecology* **86**, 2530-2535. DOI: 10.1890/04-1098.

- Lessard, J. L., Merritt, R. W. & Berg, M. B. (2009). Investigating the effect of marine-derived nutrients from spawning salmon on macroinvertebrate secondary production in southeast Alaskan streams. *Journal of the North American Benthological Society* **28**, 683-693. DOI: 10.1899/08-141.1.
- Lessard, J. L. & Merritt, R. W. (2006). Influence of marine-derived nutrients from spawning salmon on aquatic insect communities in southeast Alaskan streams. *Oikos* **113**, 334-343. DOI: 10.1111/j.2006.0030-1299.14512.x.
- Liboriussen, L. & Jeppesen, E. (2009). Periphyton biomass, potential production and respiration in a shallow lake during winter and spring. *Hydrobiologia* **632**, 201-210. DOI: 10.1007/s10750-009-9840-7.
- Mather, M. E., Frank, H. J., Smith, J. M., Cormier, R. D., Muth, R. M. & Finn, J. T. (2012). Assessing freshwater habitat of adult anadromous alewives using multiple approaches. *Marine and Coastal Fisheries* **4**, 188-200. DOI: 10.1080/19425120.2012.675980.
- McKenzie, R. A. (1964). Smelt life history and fishery in the Miramichi River, New Brunswick. *Fisheries Research Board of Canada, St Andrews, NB Bulletin* No. 144.
- Mitchell, N. L. & Lamberti, G. A. (2005). Responses in dissolved nutrients and epilithon abundance to spawning salmon in southeast Alaska streams. *Limnology and Oceanography* **50**, 217-227. DOI: 10.4319/lo.2005.50.1.0217.
- Moore, J. W. & Schindler, D. E. (2008). Biotic disturbance and benthic community dynamics in salmon-bearing streams. *Journal of Animal Ecology* **77**, 275-284. DOI: 10.1111/j.1365-2656.2007.01336.x.
- Mullen, D. M., Fay, C. W. & Moring, J. R. (1986). Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (North Atlantic) - alewife/blueback herring. *U.S. Fish and Wildlife Service, Biological Report* **82**, 11.58.
- Naiman, R. J., Bilby, R. E., Schindler, D. E. & Helfield, J. M. (2002). Pacific salmon, nutrients, and the dynamics of freshwater and riparian ecosystems. *Ecosystems* **5**, 399-417. DOI: 10.1007/s10021-001-0083-3.
- Nislow, K., Armstrong, J. & McKelvey, S. (2004). Phosphorus flux due to Atlantic salmon (*Salmo salar*) in an oligotrophic upland stream: effects of management and demography. *Canadian Journal of Fisheries and Aquatic Sciences* **61**, 2401-2410. Doi: 10.1139/f05-006.
- Nislow, K. H. & Kynard, B. E. (2009). The role of anadromous sea lamprey in nutrient and material transport between marine and freshwater environments. In *Challenges for diadromous fishes in a dynamic global environment* (Haro, A. J., Smith, K. L., Rulifson, R. A., Moffitt, C. M., Klauda, R. J., Dadswell, M. J., Cunjak, R. A., Cooper, J. E., Beal, K. L. & Avery, T. S. eds), pp. 485-494. American Fisheries Society, Symposium 69, Bethesda, Maryland.
- Ostfeld, R. S. & Keesing, F. (2000). Pulsed resources and community dynamics of consumers in terrestrial ecosystems. *Trends in Ecology & Evolution* **15**, 232-237.
- Parmenter, R. R. & Lamarra, V. A. (1991). Nutrient cycling in a freshwater marsh: the decomposition of fish and waterfowl carrion. *Limnology and Oceanography* **36**, 976-987. DOI: 10.4319/lo.1991.36.5.0976.

- Parnell, A. C., Inger, R., Bearhop, S. & Jackson, A. L. (2010). Source partitioning using stable isotopes: coping with too much variation. *PLOS One* **5**, 1-5. doi: 10.1371/journal.pone.0009672.
- Phillips, D. L. & Gregg, J. W. (2001). Uncertainty in source partitioning using stable isotopes. *Oecologia* **127**, 171-179. DOI: 10.1007/s004420000578.
- Polis, G. A., Anderson, W. B. & Holt, R. D. (1997). Toward an integration of landscape and food web ecology: The dynamics of spatially subsidized food webs. *Annual Review of Ecology and Systematics* **28**, 289-316. DOI: 10.1146/annurev.ecosys.28.1.289.
- Post, D. M. & Walters, A. W. (2009). Nutrient excretion rates of anadromous alewives during their spawning migration. *Transactions of the American Fisheries Society* **138**, 264-268. DOI: 10.1577/T08-111.1.
- Post, D. M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* **83**, 703-718. DOI: 10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2.
- Quevedo, M., Svanbäck, R. & Eklöv, P. (2009). Intrapopulation niche partitioning in a generalist predator limits food web connectivity. *Ecology* **90**, 2263-2274. DOI: 10.1890/07-1580.1.
- Reid J. Fisheries Management Program, 2010 Program Review. (2010). International Paper Canada Holdings. Print Available: Rocky Brook Camp, P.O. Box 354, Boiestown, New Brunswick E6A 2A2 Canada. (last accessed 15 June 2014).
- Reisinger, A. J., Chaloner, D. T., Rüeegg, J., Tiegs, S. D. & Lamberti, G. A. (2013). Effects of spawning Pacific salmon on the isotopic composition of biota differ among southeast Alaska streams. *Freshwater Biology* **58**, 938-950. DOI: 10.1111/fwb.12098.
- Rüeegg, J., Chaloner, D. T., Levi, P. S., Tank, J. L., Tiegs, S. D. & Lamberti, G. A. (2012). Environmental variability and the ecological effects of spawning Pacific salmon on stream biofilm. *Freshwater Biology* **57**, 129-142. DOI: 10.1111/j.1365-2427.2011.02703.x.
- Samways, K. M. & Cunjak, R. A. (2015). Increases in benthic community production and metabolism in response to marine-derived nutrients from spawning Atlantic salmon (*Salmo salar*). *Freshwater Biology* **60**, 1647-1658. DOI: 10.1111/fwb.12597.
- Samways, K. M., Quiñones-Rivera, Z. J., Leavitt, P. R. & Cunjak, R. A. (2015). Spatio-temporal responses of algal, fungal and bacterial biofilm communities in Atlantic rivers receiving marine-derived nutrient inputs. *Freshwater Science* **34**, 881-896. DOI: 10.1086/681723.
- Scott, W. B. & Scott, M. G. (1988). *Atlantic fishes of Canada*. No. 219. University of Toronto, Toronto: Canadian Bulletin of Fisheries and Aquatic Sciences.
- Stewart-Oaten, A., Murdoch, W.W. & Parker, K. R. (1986) Environmental impact assessment: "Pseudoreplication" in time? *Ecology* **67**, 929-940. DOI: 10.2307/1939815.
- Stockner, J. G. & Ashley, K. I. (2003). Salmon nutrients: closing the circle. In *Nutrients in salmonid ecosystems: sustaining production and biodiversity* (Stockner J. G., ed), pp. 3-15. American Fisheries Society, Symposium 34, Bethesda, MD.

- Thomaz, D., Beall, E. & Burke, T. (1997). Alternative reproductive tactics in Atlantic salmon: factors affecting mature parr success. *Proceedings of the Royal Society B: Biological Sciences* **264**, 219-226. DOI: 10.1098/rspb.1997.0031.
- Underwood, A. J. (1997). *Experiments in Ecology: their Logical Design and Interpretation using Analysis of Variance*. Cambridge, UK: Cambridge University Press.
- Vanni, M. J. (2002). Nutrient cycling by animals in freshwater ecosystems. *Annual Review of Ecology and Systematics* **33**, 341-370. DOI: 10.1146/annurev.ecolsys.33.010802.150519.
- Walters, A. W., Barnes, R. T. & Post, D. M. (2009). Anadromous alewives (*Alosa pseudoharengus*) contribute marine-derived nutrients to coastal stream food webs. *Canadian Journal of Fisheries and Aquatic Sciences* **66**, 439-448. Doi: 10.1139/F09-008.
- Weaver, D. M., Coghlan, S. M. Jr., Zydlewski, J. & Hogg, R. S. (2015). Decomposition of sea lamprey *Petromyzon marinus* carcasses: temperature effects, nutrient dynamics, and implications for stream food webs. *Hydrobiologia* **760**, 57-67. DOI: 10.1007/s10750-015-2302-5.
- Weber, M. J. & Brown, M. L. (2013). Continuous, pulsed and disrupted nutrient subsidy effects on ecosystem productivity, stability, and energy flow. *Ecosphere* **4**, 27. DOI: 10.1890/ES12-00354.1.
- West, D. C., Walters, A. W., Gephart, S. & Post, D. M. (2010). Nutrient loading by anadromous alewife (*Alosa pseudoharengus*): contemporary patterns and predictions for restoration efforts. *Canadian Journal of Fisheries and Aquatic Sciences* **67**, 1211-1220. Doi: 10.1139/F10-059.
- Wetzel, R. G. (1993). Microcommunities and microgradients: linking nutrient regeneration, microbial mutualism, and high sustained aquatic primary production. *Netherlands Journal of Aquatic Ecology* **27**, 3-9. doi:10.1007/BF02336924.
- Williams, K. L., Griffiths, S. W., McKelvey, S. & Armstrong. (2010). Deposition of Atlantic salmon *Salmo salar* carcasses in a Scottish upland catchment. *Journal of Fish Biology* **77**, 927-934. doi: 10.1111/j.1095-8649.2010.02725.x.
- Wipfli, M. S., Hudson, J. & Caouette, J. (1998). Influence of salmon carcasses on stream productivity: response of biofilm and benthic macroinvertebrates in southeastern Alaska, U.S.A. *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 1503-1511. Doi; 10.1139/f98-031.
- Wipfli, M. S., Hudson, J. & Caouette, J. (2003). Marine subsidies in freshwater ecosystems: salmon carcasses increase the growth rates of stream-resident salmonids. *Transactions of the American Fisheries Society* **132**, 371-381. DOI: 10.1577/1548-8659(2003)132<0371:MSIFES>2.0.CO;2.
- Wipfli, M. S., Hudson, J. P., Caouette, J. P., Mitchell, N. L., Lessard, J. L., Heintz, R. A. & Chaloner, D. T. (2010). Salmon carcasses increase stream productivity more than inorganic fertilizer pellets: a test on multiple trophic levels in streamside experimental channels. *Transactions of the American Fisheries Society* **139**, 824-839. DOI: 10.1577/T09-114.1.

- Wipfli, M. S., Hudson, J. P., Chaloner, D. T. & Caouette, J. P. (1999). The influence of salmon spawner densities on stream productivity in Southeast Alaska. *Canadian Journal of Fisheries and Aquatic Sciences* **56**, 1600-1611. Doi: 10.1139/f99-087.
- Xu, C., Letcher, B. H. & Nislow, K. H. (2010). Context-specific influence of water temperature on brook trout growth rates in the field. *Freshwater Biology* **55**, 2253-2264. DOI: 10.1111/j.1365-2427.2010.02430.x.

3.7.1 Electronic References

- Oromocto River Watershed Association Inc. (2014). Oromocto River Watershed Gaspereau Study. Final Report. Prepared by Parish Geomorphic Ltd. and Oromocto River Watershed Association Inc; Available [URL:http://www.oromoctowatershed.ca/wp-content/uploads/2014/05/Oromocto-River-Watershed-Alewife-Study-Final.pdf](http://www.oromoctowatershed.ca/wp-content/uploads/2014/05/Oromocto-River-Watershed-Alewife-Study-Final.pdf). (last accessed 15 April 2017).
- Parnell, A. & Jackson, A. (2013). siar: Stable Isotope Analysis in R. R package version 4.2.2. Available: <https://cran.r-project.org/web/packages/siar/index.html>. (last accessed 15 April 2017).
- Pinherio, J., Bates, D., DebRoy, S., Sarkar, D. & the R Development Core Team. (2012) nlme: Linear and Nonlinear Mixed Effect Models. R package version 3.1-103. Available: <http://cran.r-project.org/package=nlme>. (last accessed 8 October 2015).
- R Core Team. (2014). R: A Language and Environment for Statistical Computing [Internet]. R Foundation for Statistical Computing, Vienna, Austria. Available: <http://www.R-project.org/>. (last accessed 8 October 2015).

Figure 3.1. Community-wide convex hull total areas (TA) are based on the distribution of aquatic community groups isotope values in niche space for rivers representing a) no anadromous fish (Doctor's Brook), b) rainbow smelt (Knoydart Brook), c) alewife (Oromocto River), d) sea lamprey (Cross Creek), and e) Atlantic salmon (Rocky Brook). Convex hulls illustrate how community niche space shifts from pre-spawn (light orange: downstream treatment; light blue: upstream reference) to spawning periods (dark red: downstream treatment; dark blue: upstream reference) in both treatment and reference sites. Black circles represent the marine-derived nutrient subsidies for each river represented by the isotope values of the corresponding anadromous fish eggs.

[Figure on following page]

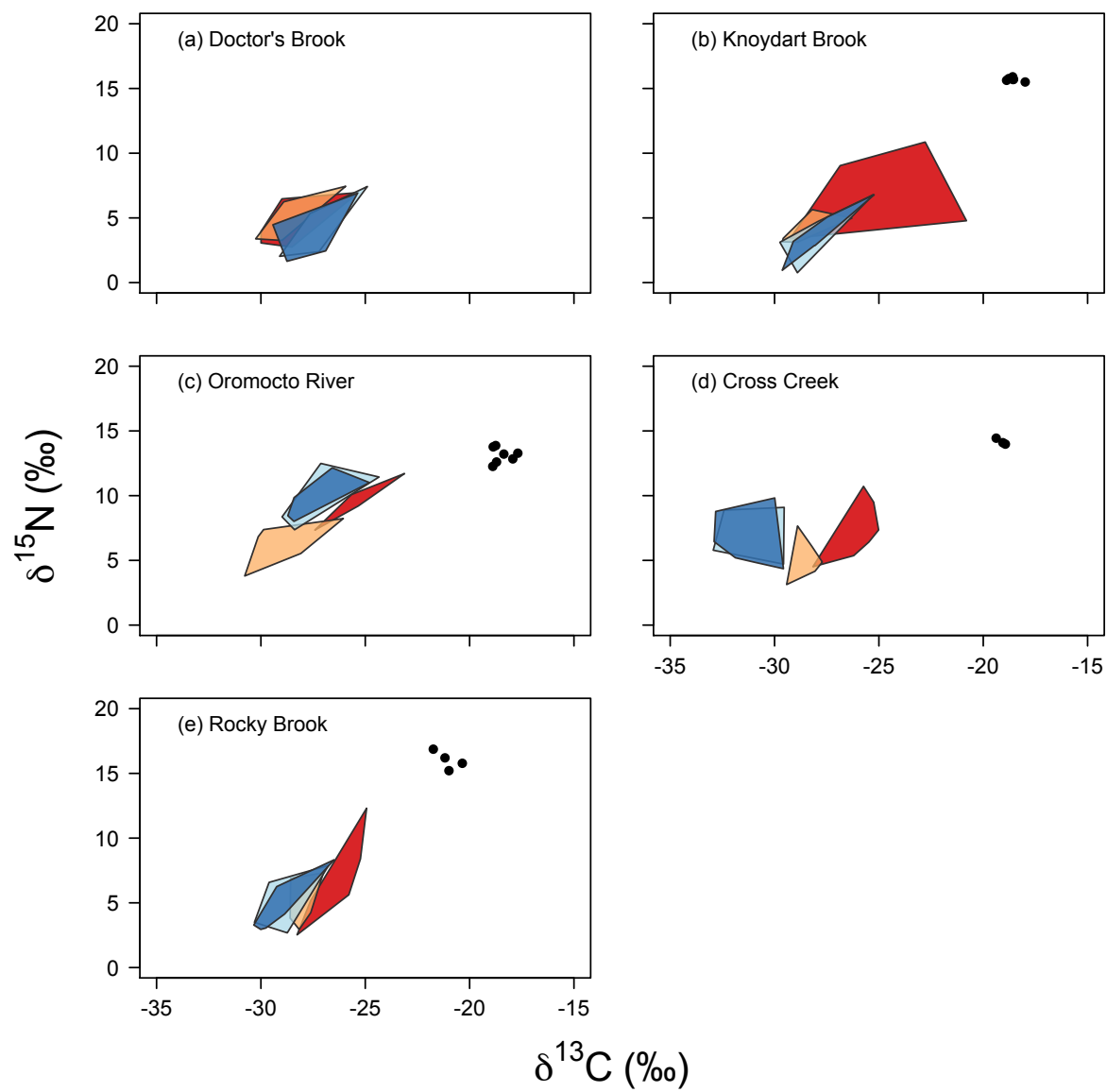


Figure 3.2. Temporal $\Delta\delta^{13}\text{C}$ changes between upstream reference and downstream treatment sites from pre-spawning conditions in aquatic community groups for rivers representing a) no anadromous fish (Doctor's Brook), b) rainbow smelt (Knoydart Brook), c) alewife (Oromocto River), d) sea lamprey (Cross Creek), and e) Atlantic salmon (Rocky Brook). Mean $\Delta\delta^{13}\text{C}$ ($\pm$ one standard deviation) values for biofilm (red triangles), Heptageniidae scrapers (dark peach circles), Simuliidae filter feeders (light beige diamonds), Perlidae predators (light grey squares), e) Pteronarcyidae shredders (purple inverted triangles), and resident salmonids (Atlantic salmon parr and brook trout) (black asterisk).

[Figure on following page]

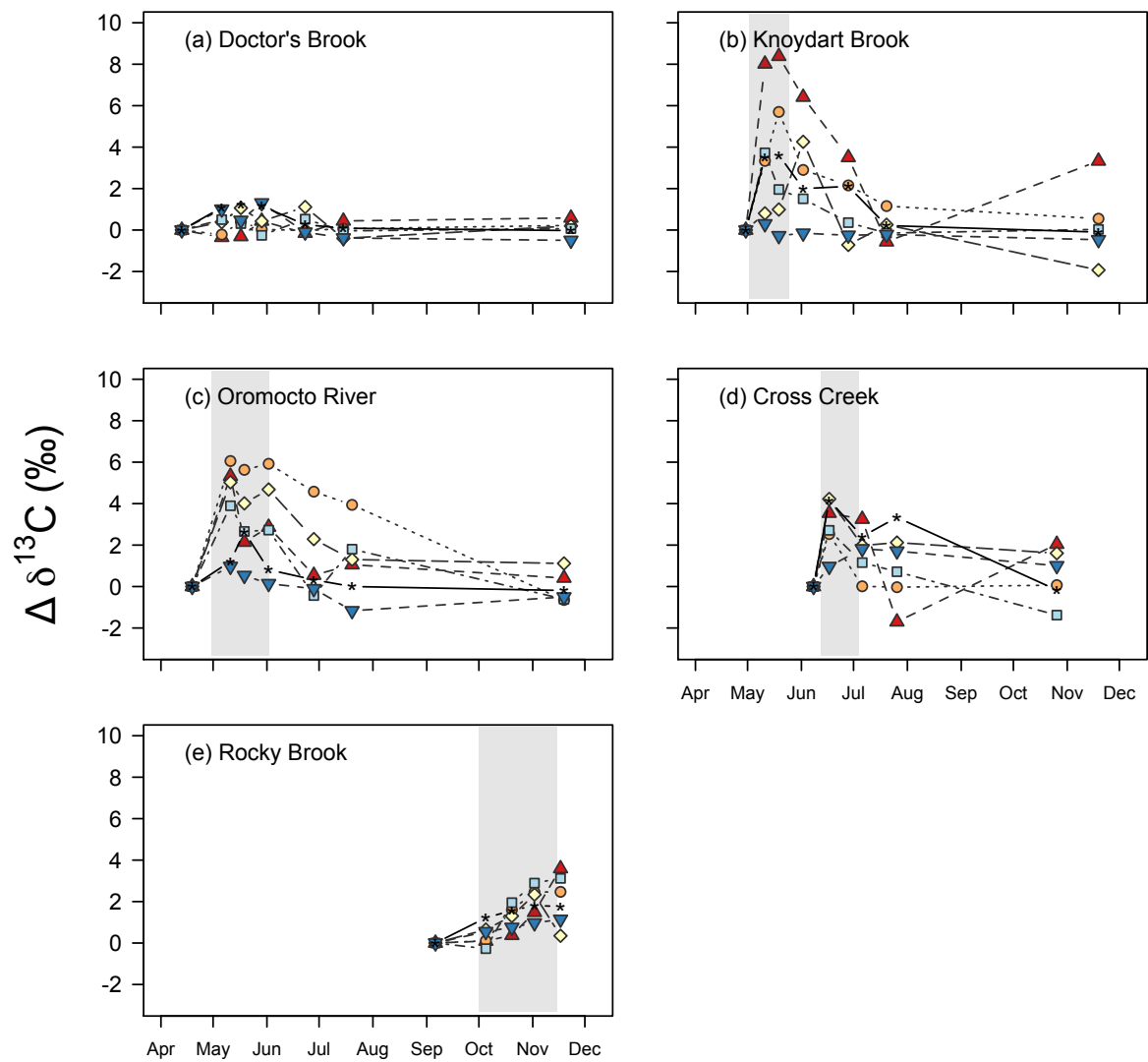


Figure 3.3. Temporal $\Delta\delta^{15}\text{N}$ changes between upstream reference and downstream treatment sites from pre-spawning conditions in aquatic community groups for rivers representing a) no anadromous fish (Doctor's Brook), b) rainbow smelt (Knoydart Brook), c) alewife (Oromocto River), d) sea lamprey (Cross Creek), and e) Atlantic salmon (Rocky Brook). Mean $\Delta\delta^{15}\text{N}$ ($\pm$ one standard deviation) values for biofilm (red triangles), Heptageniidae scrapers (dark peach circles), Simuliidae filter feeders (light beige diamonds), Perlidae predators (light grey squares), e) Pteronarcyidae shredders (dark purple upside down triangles), and resident salmonids (Atlantic salmon parr and brook trout) (black asterisk).

[Figure on following page]

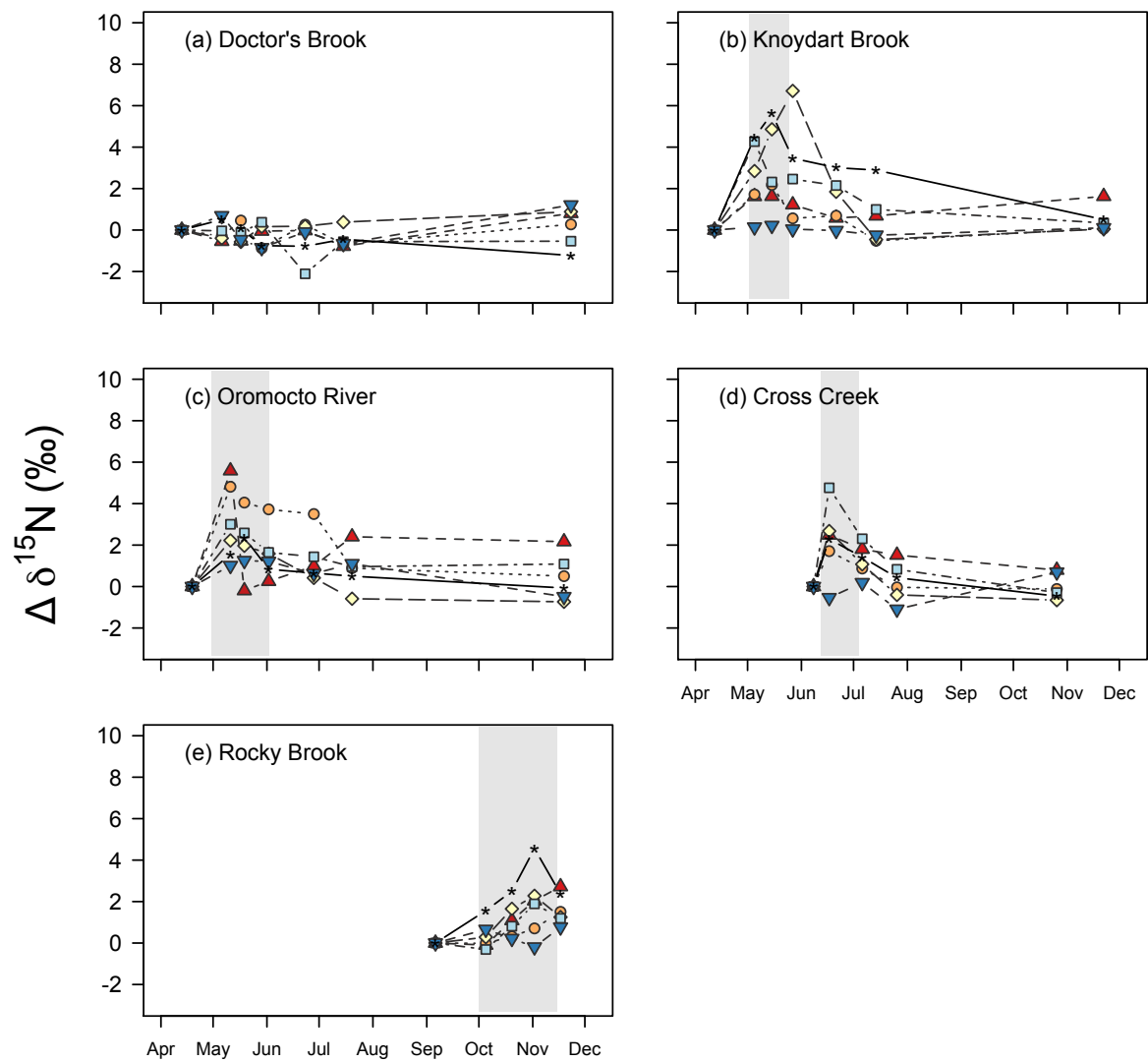
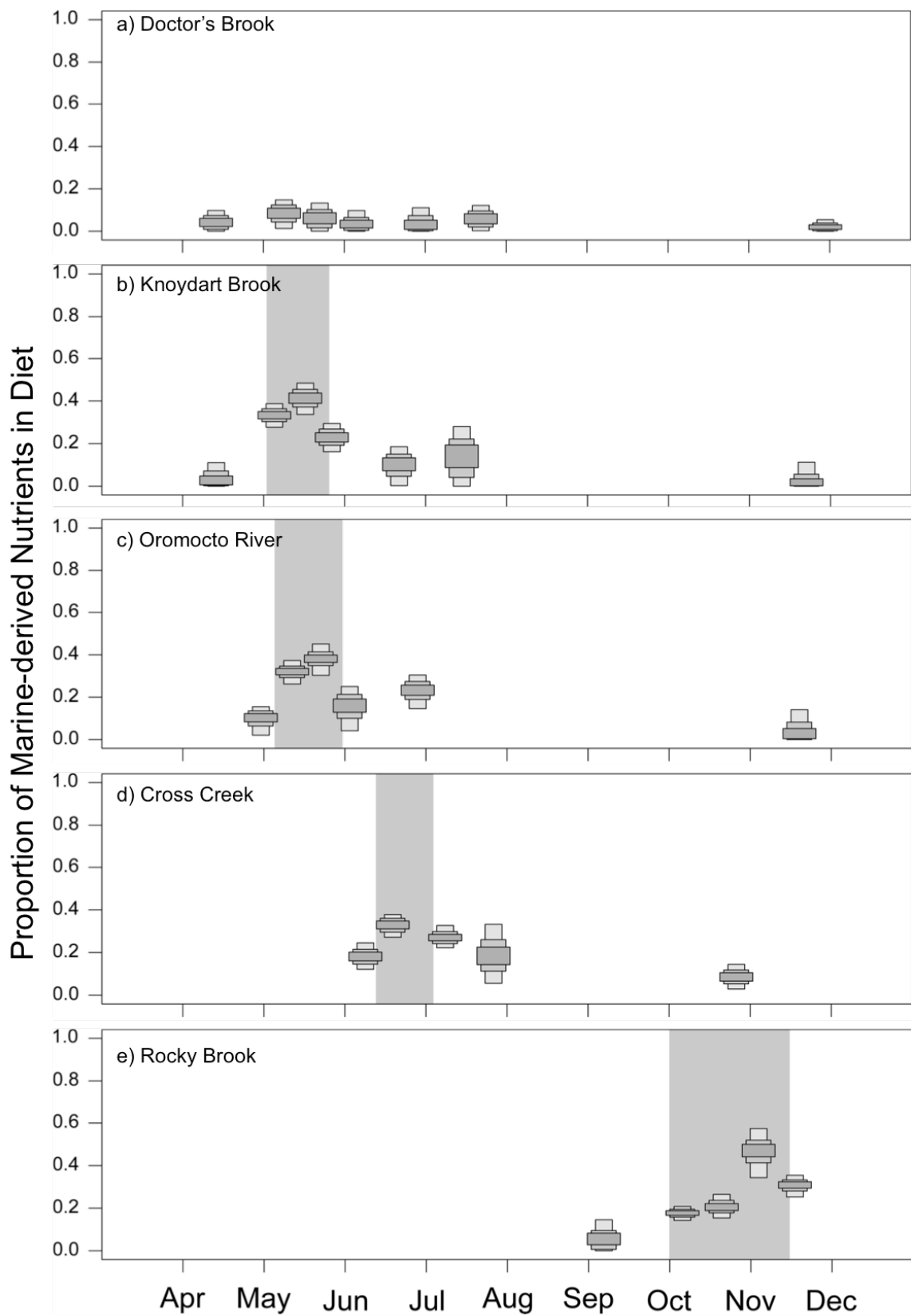


Figure 3.4. Seasonal variation in the proportion of marine-derived nutrient resources in fin tissue stable isotope ratios of resident salmonid fishes (Atlantic salmon parr and brook trout), in a) Doctor's Brook, b) Knoydart Brook, c) Oromocto River, d) Cross Creek, and e) Rocky Brook. Gradations represent the 50%, 75%, and 95% credibility intervals. Shaded areas represent periods of anadromous fish spawning (there was no spawning in Doctor's Brook by anadromous rainbow smelt).

[Figure on following page]



Chapter 4

Increases in benthic community production and metabolism in response to marine-derived nutrients from spawning Atlantic salmon (*Salmo salar*)

Kurt M. Samways¹ and Richard A. Cunjak¹

¹Canadian Rivers Institute and Department of Biology, University of New Brunswick,
P.O. Box 4400, 10 Bailey Avenue, Fredericton, New Brunswick E3B 5A3 Canada

Published: *Freshwater Biology*. 2015. 60:1647–1658. DOI:10.1111/fwb.12597. © 2015
John Wiley & Sons Ltd. *Reproduced with the kind permission from John Wiley & Sons
Ltd.*

4.1 Summary

1. Atlantic salmon (*Salmo salar*) and other anadromous fishes represent a major vector for transporting marine-derived nutrients (MDNs) to Atlantic rivers. Marine-derived nutrient subsidies may be key for maintaining ecological processes and ecosystem function in river basins.
2. Stream channels and mesocosms designed to approximate natural river systems were used to measure the response of stream productivity in two treatments, one with marine-derived nutrients from spawning Atlantic salmon and one without marine-nutrient subsidies (control). Biofilm biomass (measured as chlorophyll *a*) and benthic metabolism (measured as the net change in dissolved oxygen) were evaluated from artificial substrata encompassing pre-spawning, spawning and postspawning periods. We calculated marine-nutrient contributions from spawning salmon using a simple empirical process model.
3. We found that biofilm accrual was significantly greater with MDN inputs, with mesocosms having higher biomass ($34.8 \pm 1.2 \text{ mg chl } a \text{ m}^{-2}$) and growth rates ($0.071 \pm 0.002 \text{ mg m}^{-2} \text{ day}^{-1}$) than the stream channels ($24.4 \pm 4.8 \text{ mg chl } a \text{ m}^{-2}$ and $0.052 \pm 0.003 \text{ mg m}^{-2} \text{ day}^{-1}$, respectively). Both control mesocosms and stream channels had significantly lower biomass ($10.4 \pm 0.7 \text{ mg chl } a \text{ m}^{-2}$ and $6.3 \pm 2.5 \text{ mg chl } a \text{ m}^{-2}$, respectively) and growth rates ($0.042 \pm 0.008 \text{ mg m}^{-2} \text{ day}^{-1}$ and $0.029 \pm 0.009 \text{ mg m}^{-2} \text{ day}^{-1}$, respectively) than treatment channels. Despite having a lower biofilm biomass, stream channels yielded a significantly greater final gross primary production of $2343.5 \text{ mg C m}^{-2} \text{ day}^{-1}$. The presence of MDNs in the stream channels shifted biofilm

metabolism from heterotrophy ($P/R = 0.283$) to autotrophy ($P/R = 2.422$) within 112 days.

4. Spawning Atlantic salmon contributed a total of 20.98 g m^{-2} of nitrogen and 1.04 g m^{-2} of phosphorous to each stream channel, through excretion and gametes alone. There was a strong predictable positive relationship between the increase in productivity and the amount of marine-derived nitrogen and phosphorous delivered.

5. This study highlights the importance of marine-derived nutrients from Atlantic salmon for driving and maintaining freshwater productivity. Marine-nutrient subsidies relieve the 'bottom-up' constraints on stream productivity by facilitating the production of enough energy to support the food web, lessening the reliance on outside energy sources.

4.2 Introduction

Anadromous fish can be significant transporters of nutrients and energy to inland waters. The spawning of anadromous fish delivers biological materials in the form of excretory products, gametes and carcasses that are enriched in nutrients (e.g. nitrogen and phosphorous) (Wipfli et al. 1999). Perhaps not as dramatic as the Pacific Northwest, marine-derived nutrients (MDNs) have been found to have widespread effects in Atlantic rivers. Incorporation of MDNs has been observed at multiple trophic levels including biofilm (Walters et al. 2009), zooplankton (Durbin et al. 1979), macroinvertebrates (Jardine et al. 2009, Walters et al. 2009) and fish (Garman and Macko 1998, MacAvoy et al. 2009). Although biofilm biomass responds positively to increases in spawner abundance, productivity may plateau despite increasing nutrient inputs (Wipfli et al. 1999).

Epilithic biofilm is the foundation of most stream food webs and is therefore the basis for 'bottom-up' processes. Biofilms are the matrix of autotroph and heterotroph communities growing on the surface of both organic and inorganic substrata in streams and are the primary producers of stream benthos (Costerton et al. 1995). Nutrients, primarily nitrogen and phosphorous, are often limiting in streams, and changes in nutrient regimes should first affect the algae, fungi and bacteria communities in these systems. Marine-derived nutrients delivered by spawning anadromous fishes may be key for maintaining enhanced levels of stream productivity by increasing the availability of limiting nutrients (Durbin et al. 1979, Jonsson and Jonsson 2003, Nislow et al. 2004), thereby stimulating biofilm production (Wipfli et al. 1998). Excretory products provide nutrients in forms that are immediately assimilable by biofilm (Vanni 2002), whereas eggs and carcasses provide 'slow-release' nutrients (Parmenter and Lamarra 1991). Atlantic anadromous fishes are generally iteroparous, and therefore, MDN inputs from excretion, gamete release and partial carcass loading likely differ in their relative importance to stream productivity compared with semelparous species, such as most Pacific salmonids.

Direct measures of stream metabolism provide an integrative measure of ecological processes and ecosystem function. Stream metabolism and biofilm abundance can serve as indicators of ecosystem responses to anadromous fishes because both metabolism and abundance respond to fluctuating nutrient regimes. Via uptake and retention of nutrients (including MDNs) and conversion to organic matter (Sabater et al. 2002), biofilms contribute to the energy pool. Gross primary production (GPP) is a measure of the total organic matter produced plus the amount of energy lost through

respiration (R), and the GPP/R ratio (also expressed as P/R) is commonly used as an index of stream production. Energy in stream ecosystems is either autochthonous ($P/R > 1$) or allochthonous ($P/R < 1$) in origin. Chlorophyll *a* (chl *a*) measurements are commonly used as surrogate measures of primary productivity (Morin et al. 1999). Together, GPP and biofilm biomass (measured as chl *a* abundance) can be used to predict changes in stream productivity as a function of nutrient loading.

Establishing a direct cause-and-effect relationship between productivity changes and MDN inputs remains elusive due to the dynamic properties of streams and fish populations. The importance of MDNs from Atlantic salmon (*Salmo salar*) and other anadromous fishes in determining freshwater productivity varies with species distribution, community regulation and ecosystem and landscape processes (Wipfli et al. 1999). Although many studies have documented the effects of MDNs on productivity (Wipfli et al. 1998, 1999, Naiman et al. 2002, Jonsson and Jonsson 2003, Walters et al. 2009), few studies quantify spawner nutrient loading and the processes that govern ecosystem function (Johnston et al. 2004). By constructing empirical models of nutrient contributions from spawning anadromous fishes, the effects of MDNs on stream productivity can be assessed.

The goal of this study was to determine the effects of spawning Atlantic salmon on stream productivity. We took a comparative experimental approach examining productivity in stream channels with and without MDN inputs. Specifically, we examined patterns of change in biofilm community metabolism in relation to MDN inputs: (i) in stream channels with complete stream assemblages, including spawning Atlantic salmon, and (ii) in adjacent mesocosms experiencing only dissolved nutrient inputs (no fish or

macroinvertebrates present). We used direct measures of biofilm community metabolism, measures of biofilm biomass and estimates of marine-nutrient loading to quantify changes in productivity. We predicted that MDNs from excretion and gametes provided by spawning Atlantic salmon would stimulate productivity, relieving the ‘bottom-up’ constraints on productivity currently found within nutrient-limited systems.

4.3 Methods

4.3.1 Study site

This study was conducted at the Mactaquac Biodiversity Facility, a federal Atlantic salmon mitigation facility on the St. John River, New Brunswick, Canada, approximately 120 km from the river mouth. Experiments spanned from August 2011 to January 2012. Daily mean water temperatures were 5.4 °C (minimum 0.4; maximum 14.8 °C), calculated from hourly water temperature recordings in each tank using VEMCO Minilog temperature data loggers (VEMCO, Bedford, NS, Canada).

4.3.2 Stream channels

Four, 121-square-metre conventional concrete Swede ponds were converted to semi-natural, flow-through stream channels, supplied with continuous flow-through water from the St. John River. A 1.30-m-high, hollow octagon was placed in the centre of each pond (around the drain), to help direct flow with two drain screens located on the wall furthest from the water input. The resulting stream channel was 3.1 m wide and 31 m long (measured as circumference at mid-stream width) for a rearing area of 96.1 m². Substratum added to each stream channel consisted of a 25-cm base layer of small gravel

(1.9–3.8 cm), with a 15-cm top layer consisting of a mixture of small, medium (3.8– 7.6 cm) and large (>7.6 cm) gravels. Large rocks (>20 cm) were added throughout the stream channel to provide suitable refuge for parr. Substratum was sculpted into riffles, runs and pools, which also helped to direct and amplify water velocities. The average water depth was 45 cm, although depth ranged from 12 cm in the riffle to 60 cm in the pools, providing a rearing volume of 39.15 m³. Water velocities ranged between 15 and 110 cm s⁻¹ depending on the location in the stream (i.e. pool, riffle, run).

Stream channels were constructed to support realistic semi-natural stream assemblages. Each stream channel was seeded with a representative macroinvertebrate community that was collected from a nearby tributary, equal to 2× the stream channel area, to supplement natural colonisation via drift. Biofilm growth in each stream channel supported the diverse aquatic macroinvertebrate population that served as the natural food source for salmon parr. Prior to the addition of parr, invertebrate densities in each stream channel were evaluated to ensure a similar community composition and adequate food supply. Wild Atlantic salmon parr were collected from the Tobique River, a tributary of the St. John River, with 115 parr representing 0+ to 2+ age classes, stocked into each stream channel. Artificial substrata were 4.5 by 4.5 cm slate tiles, pre-conditioned to remove organics by combusting at 550 °C for 12 h in a muffle furnace. Tiles were randomly placed and secured to the channel bed prior to the addition of adult salmon allowing the biofilm to colonise the tiles under natural conditions. Biofilm response measurements from each treatment channel were compared to corresponding control channels.

Wild Atlantic salmon smolts were collected in May 2009 from the Campbell

River, a tributary of the Tobique River, reared in the facility and fed a marine-based diet (primarily herring) to produce mature adults after 2 years (Jones et al. 2010). Twenty-four (12 male and 12 female) of these ‘wild-exposed’ adult Atlantic salmon were introduced into each of two treatment stream channels on 7 October 2011, with spawning occurring from 8 until 23 November. This stocking density is within the range of what is observed in many spawning locations in rivers with self-sustaining populations. The average size of introduced fish was 1.53 kg (range 0.99–2.16 kg) for the males and 2.49 kg (range 1.82–3.61 kg) for the females. Adults remained in the channels until 10 December, at which time they were removed to simulate post-spawning out-migration. Hatching of eggs in the substratum was estimated to be 90% complete by 10 March 2012, and emergence of fry began on 15 April 2012.

4.3.3 Mesocosms

In addition to the stream channels, eight mesocosms were used to assess the effects of the dissolved MDN pool only on biofilm growth and primary production. Partial flow-through mesocosms consisted of eight, 10-L polyethylene, open-top cylinders; similar to those used by Pestana et al. (2009). Mesocosms were designed to mimic the stream channels by encompassing a portion of the substratum (including artificial substrata), while excluding invertebrates and salmon parr. Water from each stream channel was pumped with a small centrifugal pump (March: LC-3CP-MD) through a 243 μm mesh (to exclude invertebrates), into a manifold, which supplied two replicate mesocosms. Current velocity inside the mesocosms was maintained at 7 cm s^{-1} , the average current velocity within the stream channels. Mesocosm substrata consisted of

biofilm-colonised rocks and artificial substrata, devoid of any invertebrates. Mesh covers on each mesocosm were used to preclude aerial invertebrates from colonising as well as to maintain similar light intensities reaching the substratum as in the stream channels.

4.3.4 Benthic metabolism

Samplings occurred between October 2011 and January 2012: prior to the adults being introduced (6 October), during spawning (22 November), after spawning but prior to removal (9 December) and twice after adults were removed (28 December and 25 January). At each sampling event, we randomly sampled two tiles from each pond and one tile from each mesocosm for estimates of GPP. Benthic metabolism was assessed from the net change in dissolved oxygen within a biological oxygen demand (BOD) chamber (dimensions: $17 \times 17 \times 9$ cm). Each BOD chamber was fitted with a dissolved oxygen (DO) sensor (OxyGuard stationary probe, Point Four Systems Inc., Coquitlam, BC, Canada) and a recirculating water pump to reduce biofilm boundary layer effects and ensure adequate water flow across the oxygen sensor, resulting in a total volume of 1.775 L. DO probes were attached to a PT4 (Point Four Systems Inc.) multichannel data logger, which recorded DO at 20-s intervals. Collected tiles were immediately placed into separated BOD chambers, filled with water from the experimental pond of origin. BOD chambers were incubated for approximately 2 h under light and dark conditions (1 h each) in a temperature-controlled room under growth lamps to measure productivity and respiration, respectively.

The two parameters directly measured by gas exchange were the net change in DO in the light (daytime) (balance between photosynthesis and respiration) and the dark

(night-time) representing community respiration. The mean DO change in the dark was taken as the rate of community respiration per hour and multiplied by 24 for total daily respiration (R_{24}). This assumed that respiration rates were constant. Gross primary production was calculated from the daylight DO production plus the DO consumed by respiration during that period of time (respiration rate multiplied by daylight hours). Net daily metabolism (NDM), calculated as the difference between GPP and R_{24} , is the net DO change per day from biological activity. By subtracting R_{24} , any error associated with the photoperiod respiration is removed (Bott 2006). The P/R ratio (GPP/R_{24}) was also calculated and is an expression, in relative terms, of the balance between these metabolic processes. Changes in DO measurements through time ($\text{mg O}_2 \text{ l}^{-1} \text{ h}^{-1}$) were converted to units of carbon ($\text{mg C m}^{-2} \text{ h}^{-1}$) by multiplying by the chamber volume, dividing by substratum surface area and, assuming that in both photosynthesis and respiration one mole of C is equivalent to one mole of O_2 , multiplying by 12 mg C/32 mg O_2 (gram equivalent weight of carbon/gram equivalent weight of oxygen).

4.3.5 Biofilm growth

Measurement of algal pigments from artificial substrata provides a measure of biofilm accrual and growth rate. After benthic metabolism was measured, the biofilm from the upper surface of each tile was scraped, until bare tile was exposed, and placed in a collection vessel with water from which the tile was collected. Three replicate samples of the biofilm material were filtered onto 1.2- μm glass fibre filters (Whatman GF/C), stored in dark containers (to minimise pigment degradation) and frozen. Biofilm pigments were extracted using a standard mixture of acetone:methanol (80 : 15 by

volume) and filtered through a 0.22-µm lock-on syringe filter to remove any particulates.

Algal abundance was determined by quantifying concentrations of biofilm algal pigments using a standard trichromatic protocol (Jeffrey and Humphrey 1975).

4.3.6 Marine-derived nutrient inputs

Due to logistical restraints at the Biodiversity Facility, the experiment concluded at the end of January; however, we were able to monitor growth and survival of the offspring to emergence. This allowed us to estimate the MDN inputs from the adult Atlantic salmon using a mass balance approach adapted from Johnston et al. (2004). The predicted amount of MDNs deposited by live spawners on the day of the t th observation is

$$(4.1) \quad MDN(t) = \frac{NEx(t) + NEgg(t) + NMilt(t)}{T}$$

where $NEx(t)$ is the nutrient contribution from the excretion by live spawners, $NEgg(t)$ is the nutrient contribution of the eggs, $NMilt(t)$ is the nutrient contribution of the milt, and T is the retention time of the water in the stream channel (1.55 h); calculated as the discharge entering the stream channel from the inflow pipe, divided by the volume of water in the tank. Carcasses were not included in this nutrient budget as any mortalities were removed immediately to minimise the possible spread of infection within the facility. As all introduced adults were accounted for and no mortalities were allowed to remain, the net MDN inputs from the adult carcass mass were zero and therefore not included in the nutrient budget.

Johnston et al. (2004) assumed a P excretion rate of zero based on non-feeding *Oncorhynchus* spp.; however, this seemed inappropriate as breeding individuals undergo dramatic physiological changes (Gende et al. 2002). During the spawning migration,

Atlantic salmon spend extended periods (1–5 months) in fresh water without feeding. Because excretion rates can decline when fish are not feeding (Vanni 2002), using excretion rates measured over short time periods may not be appropriate. Instead, we adopted the approach of comparing the weight to elemental composition of the fish before and after spawning (Durbin et al. 1979). As metabolic rates of sockeye salmon (*Oncorhynchus nerka*) during spawning increase 8% for females and 61% for males (Brett 1995) and excretion rates are a function of metabolism and not feeding (Wood 1995), excretion rates used in our model were increased during this period accordingly. The nutrient contribution from excretory products is

$$(4.2) \quad NEx(t) = \sum_{i=t_0}^t Wt_{GF}(t) \eta \varepsilon$$

where $Wt_{GF}(t)$ is the gonad-free wet weight of all individuals on day t , η is the nutrient loss during spawning (35.1% of N and 22.7% of P lost, estimated from Jonsson et al. 1990 and Jonsson and Jonsson 2003), and ε be the nutrient content of an unspawned adult (3% N and 0.47% P, Lyle and Elliott 1998).

The breakdown of gametes from spawning Atlantic salmon releases labile nutrients into the water column. Nutrients released from milt enter the nutrient pool directly upon spawning, whereas nutrients from eggs are released as the eggs die and subsequently decompose. The number of live eggs at time t is

$$(4.3) \quad Egg(t+1) = Egg(t) + qR(t) - mEgg(t)$$

where $Egg(t)$ is the amount of eggs in grams, q is the number of eggs per redd defined by

$$(4.4) \quad q = \left(\frac{Fecundity}{Redds_{total}} \right) \times Wt_{egg}$$

where *Fecundity*, based on the work of Marshall and Penney (1983) on Atlantic salmon in the St. John River, is

$$(4.5) \quad Fecundity = \sum_{i=1}^n e^{6.06423+0.03605(fork\ length(i))}$$

$Redds_{total}$ is the average number of redds produced by spawning Atlantic salmon in stream channels at the Mactaquac Biodiversity Facility (46 redds per stream channel, K.M. Samways, unpubl. data), Wt_{egg} is the average weight of an egg from wild-exposed Atlantic salmon from the Tobique River (0.202 g, unpubl. data), $R(t)$ is the number of new redds on day t defined by

$$(4.6) \quad R(t) = s(t) \times Redds_{total}$$

where $s(t)$ is the spawning rate of fish in stream channels (based on a Poisson distribution with lambda (k) = 5.4532 of spawning activity within stream channels, K.M. Samways., unpubl. data), and $mEgg$ is the egg mortality rate at time (t) (0.0122; estimated from egg survival assuming a constant exponential mortality rate). Let the biomass of dead eggs Mt be

$$(4.7) \quad M(t + 1) = (1 - e^{-d})M(t) + mEgg(t)$$

where d is the decomposition rate (based on Garmin 1992); therefore, the release of nutrients from dead eggs ($NEgg$) at time t will be

$$(4.8) \quad NEgg(t + 1) = \Phi e^{-d}M(t)$$

where Φ is the quantity of nutrient per unit weight of egg (0.23 by wet weight for nitrogen, Johnston et al. (2004), and 0.014 for phosphorous, Nislow et al. (2004), using the average egg weight of 0.202 g). The contribution of milt to the nutrient pool is

$$(4.9) \quad NMilt(t) = \left(\sum_{i=1}^n Wt_{male}(i) \times GSI_{male} \times Prop_{male} \times \mu \right) \times s(t)$$

where Wt_{male} is the weight of the i^{th} male, GSI_{male} is the gonad-somatic index (0.0608, Fleming et al. 2000), $Prop_{male}$ is the proportion of wet weight of testes lost during spawning (0.68, Idler and Clemens 1959), and μ is the quantity of nutrient per unit weight of milt (0.20 for nitrogen and 0.012 for phosphorous based on the same N:P ratio as eggs; see Johnston et al. 2004).

4.3.7 Changes in production

The rate at which production, as measured by algal abundance, changes with MDN inputs was determined from the plot of the change in production with MDN inputs for both stream channels and mesocosms. The change in production for a given time interval was estimated, from the plot of algal biomass through time, as the area between the MDN and control treatment productivity curves. Area was calculated as weighted average of algal biomass for each sampling period using the trapezoid rule, see Samways et al. (2015) for detailed description. This approach gave a single, time-integrated value of the change in productivity relative to the control treatments.

4.3.8 Data analysis

We evaluated the effect, with or without MDN inputs, from Atlantic salmon spawning and time on algal abundance, GPP and P/R from both stream channels and mesocosms using mixed-model ANOVAs. For algal abundance, the replication unit was the replicate samples (3 per site). Replicate samples for GPP were the number of tiles collected per treatment, and therefore, the replication unit for this analysis was two. The ANOVA

model included the fixed factors treatment (with or without Atlantic salmon spawning), date (sampling round) and the random factor stream channel or mesocosm. The appropriate denominators for F-ratios were determined following Underwood (1997). Multiple comparisons of significant fixed sources of variation were conducted using Tukey's test.

4.4 Results

4.4.1 Biofilm growth

Biofilm biomass and accrual, expressed as chlorophyll *a*, was significantly greater in treatments with (MDN treatment) than without (control treatment) Atlantic salmon spawning for both stream channels and mesocosms ($F_{24,80} = 19.524$, $P < 0.0001$ and $F_{24,80} = 24.312$, $P < 0.0001$, respectively) (Fig. 4.1). Mesocosms had, on average, 21.5 and 19.5% more biofilm than stream channels for both MDN ($F_{24,80} = 58.712$, $P < 0.0001$) and control treatments ($F_{24,80} = 55.035$, $P < 0.0001$), respectively. Biofilm biomass increased steadily after spawning and was greatest at the end of the experiment (late January) with mesocosms reaching $34.8 \pm 1.2 \text{ mg chl } a \text{ m}^{-2}$ and stream channels reaching $24.4 \pm 4.8 \text{ mg chl } a \text{ m}^{-2}$. Exponential growth rates of biofilm in mesocosms (Fig. 4.1b) were 27% higher in the MDN treatments and 31% higher in the control treatments in comparison with the stream channels (Fig. 4.1a). Exponential growth rate was highest during the spawning period for both stream channels ($0.052 \pm 0.003 \text{ mg m}^{-2} \text{ day}^{-1}$) and mesocosms ($0.071 \pm 0.002 \text{ mg m}^{-2} \text{ day}^{-1}$), whereas in the control treatments peak exponential growth rates did not occur until approximately 15 days later ($0.034 \pm 0.002 \text{ mg m}^{-2} \text{ day}^{-1}$ for stream channels; $0.047 \pm 0.003 \text{ mg m}^{-2} \text{ day}^{-1}$ for mesocosms).

4.4.2 Benthic metabolism

Seasonal trends of GPP showed significant increases in the metabolic response of biofilm after the spawning period of Atlantic salmon (Fig. 4.2). Rates of GPP were significantly greater in both stream channels and mesocosms receiving MDNs ($F_{24,40} = 11.994$, $P < 0.001$ and $F_{24,40} = 11.824$, $P < 0.001$, respectively) (Fig. 4.2). Stream channels with a final mean GPP of $2343.5 \text{ mg C m}^{-2} \text{ day}^{-1}$ was 29% greater than the GPP in mesocosms ($1146.9 \text{ mg C m}^{-2} \text{ day}^{-1}$; $F_{24,40} = 13.131$, $P < 0.022$). There was no significant difference in the GPP between stream channel and mesocosm control treatments ($F_{24,40} = 13.134$, $P = 0.978$).

Stream channels and mesocosms with MDN inputs also had higher P/R ratios ($F_{4,24} = 3.816$, $P = 0.015$ and $F_{4,24} = 2.235$, $P = 0.045$, respectively) than in the control treatments, although the differences were not significant until the December sampling period (Fig. 4.3). P/R ratios in stream channels were 20% greater than in mesocosms ($F_{1,6} = 63.497$, $P = 0.001$), while there were no significant differences in the P/R ratios between the control treatments ($F_{1,6} = 2.039$, $P = 0.227$). The P/R ratio showed the same overall seasonal pattern as algal productivity in the stream channels, reaching a maximum ratio value of 2.422 ± 0.229 , whereas the P/R ratio in the mesocosms reached a maximum value of 1.353 ± 0.137 by the end of December, remaining stable thereafter.

4.4.3 Marine-derived nutrient inputs

Spawning Atlantic salmon contributed considerable amounts of nutrients through gametes and excretion (Fig 4.4). Stream channels received both particulate and dissolved MDN additions, whereas the mesocosms only received dissolved nutrients. Adult salmon

contributed a total of 2016 g of nitrogen (N) and 100 g of phosphorous (P) to each stream channel between 7 October 2011 and 16 April 2012. Nitrogen and phosphorous from excretory products entered the nutrient pool as soon as the adults were introduced to the stream channels (Fig 4.4). Excretory products contributed 11% of the total marine-derived nitrogen inputs ($9.95 \text{ g N fish}^{-1}$) and 23% of the total marine-derived phosphorous inputs ($1.01 \text{ g P fish}^{-1}$). Eggs contributed 69% of the marine-derived phosphorous ($6.27 \text{ g P female}^{-1}$) and 56% of the marine-derived nitrogen ($103.01 \text{ g N female}^{-1}$) inputs. Milt provided 8% of the total marine-derived phosphorous ($0.73 \text{ g P male}^{-1}$) and 33% of the total marine-derived nitrogen inputs ($59.46 \text{ g N male}^{-1}$).

4.4.4 Changes in production

Strong relationships were found between productivity and MDN inputs in both stream channels and mesocosms. Over the range of marine-nutrient inputs from 0 to 1660 g nitrogen and 0 to 79 g phosphorous (the duration of the study), the productivity data were best fitted using an exponential function for nitrogen (both stream channels and mesocosms) and phosphorous (stream channels) and a linear model for phosphorous in the mesocosm (Table 4.1). There was a strong positive relationship between the increase in productivity and MDN inputs from nitrogen (Fig. 4.5) and phosphorous (Fig. 4.6). The rate of change in algal productivity was 97.5% greater in the mesocosms, for both nitrogen and phosphorous inputs, compared with the stream channels.

4.5 Discussion

This study tested the direct effects of MDN subsidies from spawning Atlantic salmon on primary production. We found that biofilm accrual was greater with MDN

inputs, and despite only being exposed to dissolved MDNs, mesocosms had higher biomass and growth rates than the stream channels. Both stream channels and mesocosms showed significant increases in GPP in response to MDNs. The presence of spawning salmon in the stream channels switched biofilm metabolism from heterotrophy ($P/R < 0.5$) to autotrophy ($P/R > 2$). The marine-nutrient model shows that Atlantic salmon subsidise spawning areas with substantial quantities of nutrients from excretion and gametes alone. There was a predictable relationship between the increase in primary productivity and the amount of marine nutrients delivered. These results show that MDNs are an important driver of benthic processes.

Algal biomass increased with MDN inputs in both mesocosms and stream channels, as in previous studies (Wipfli et al. 1999; Johnston et al. 2004). Algal biomass was greater in mesocosms, which only received dissolved MDNs and excluded fish and macroinvertebrates. Grazing by benthic macroinvertebrates can be very effective at reducing algal standing crop (Elwood et al. 1981), and in the absence of these grazers, algal accrual was able to proceed unimpeded in the mesocosms. Grazing, which did occur in the stream channels, can also temporarily mask the response of algae to increases in nutrient inputs by reducing standing crop (Elwood et al. 1981) and may also explain the lag in algal biomass accrual in the stream channels. The lag in algal biomass abundance also coincided with the spawning period. Redd construction during spawning can significantly disturb the streambed and in the process displace and reduce the abundance of benthic communities (Minakawa and Gara 1999; Moore and Schindler 2008). The small artificial substrata, which were similar in size to the natural substratum, were exposed to the same disturbances associated with the digging of redds, leading to the

reduction in benthic production. Upon adult removal, streambed disturbance ceased; therefore, the rate of biofilm growth increased rapidly.

Community structure and differential responses of autotrophic and heterotrophic biofilm groups to nutrient subsidies may explain the differences in primary productivity rates observed between the mesocosms and stream channels. Differences in productivity rates may be attributable to differences in biofilm community composition. Stream channels may have had a more balanced biofilm community (i.e. algal, fungal and bacteria groups), whereas algae may have dominated the mesocosms. For example, by the end of the experiment, there was an obvious proliferation of filamentous algae on the substrata in the mesocosms; by contrast, there was a noticeable lack of filamentous algae on stream channel substrata. Although all biofilm groups readily incorporate dissolved nutrients, particulate marine matter is probably only consumed by heterotrophs (Fenchel et al. 1998). A more balanced biofilm community in the stream channels would have been able to access a larger nutrient pool (dissolved and particulate MDNs), leading to increased nutrient cycling and uptake rates resulting in the greater productivity rates observed.

As GPP is a measure of productivity and therefore a rate of how much new carbon is fixed, and algal abundance is a measure of production and therefore of standing crop, the two are not necessarily linked at all times. GPP was significantly greater in both stream channels and mesocosms with MDN inputs over controls by the end of the experiment. After 64 days of MDN inputs, GPP rates did not differ between mesocosms and stream channels; however, after 92 days, GPP was significantly greater in the stream channels. A study by Holtgrieve and Schindler (2011) found that GPP declined by an

order of magnitude during sockeye salmon spawning, but then increased after spawning. GPP did not decrease or increase during Atlantic salmon spawning in our study, but did increase significantly in the period after spawning. GPP has been shown to increase as nutrient levels rise (Bernot et al. 2010). Even though mesocosms had a higher standing crop, biofilm in the stream channels was metabolically more active, with increased turnover rates, nutrient absorption, photosynthetic activity and reproduction (McIntire 1973). The increase in GPP relative to ecosystem respiration in the stream channels indicates a shift from the dependency on external energy inputs.

The P/R (productivity to respiration) ratio is a simple index of the relative importance of energy supplied through in-stream photosynthesis (autotrophy) versus energy derived from the breakdown and use of imported organic matter (heterotrophy). When $P/R > 1$, the system is primarily supported by in-stream production and there is a net addition of energy to the system; however, when respiration exceeds production, $P/R < 1$, the system relies on organic matter originating outside the stream (Allan and Castillo 2008). The stream channels shifted from being heterotrophic ($P/R = 0.229$) before adult salmon were introduced, to being autotrophic ($P/R = 2.422$) by the end of the experiment. Although the mesocosms receiving MDNs had a significantly higher P/R ratio than the control mesocosms, primary productivity did not increase enough to shift these systems to autotrophy.

Marine-nutrient subsidies from excretory products, milt and egg decomposition and mineralisation relieve the ‘bottom-up’ constraints on stream productivity by producing enough energy to support the food web, lessening the reliance on outside energy sources. Biofilm biomass, measured as chl *a*, GPP and P/R, increased

continuously into the winter with MDN inputs. Biomass accrual (Francoeur et al. 1999) and metabolism (McIntire and Phinney 1965) are typically lowest in the winter. The storage and release of nutrients from the substratum acts as a mechanism by which nutrients are made available to primary producers over the winter (O’Keefe and Edwards 2002), which enables the biofilm to increase metabolic functioning and keep food-web productivity high during an otherwise slow period of metabolism. Petticrew et al. (2011) demonstrated that MDNs are retained and stored within the substratum, lengthening the period of nutrient availability.

Biofilm in the stream channels would not have had direct access to marine nutrients stored in the substratum. However, nutrients from decomposing eggs in the substratum become available to biofilm through microbial loop-mediated mineralisation and trophic transfer (Petticrew et al. 2011). Assuming that MDNs from the decomposition of eggs are highly labile and therefore rapidly exploited by the biofilm community, little dissolved marine-nutrient matter would be exported from the substratum. Increased availability due to close proximity of this nutrient source, coupled with the slow release associated with decomposition, allowed for productivity rates to increase in the stream channels rather than declining as in the mesocosms. Biofilm in the mesocosms would not have been exposed to these dissolved nutrients until they were mobilised into the water column. Marine-derived nutrients from Atlantic salmon may facilitate shifts between alternate stable states that persist longer than the spawning period.

The mass-balance model illustrates how different salmon products contribute to the marine-nutrient pool. To understand the magnitude and direction of marine-nutrient

flux, the process model should include MDNs leaving the system via smolt emigration. Within each stream channel, approximately 6205 fry emerged and were released. Fry to smolt survival estimates for Atlantic salmon ranged from 1.8 to 11.5% depending on age at smoltification (Cunjak & Therrien 1998). Using a 2.5% N and 0.45% P content for Atlantic salmon smolts (Lyle and Elliott 1998) and an average mass of 27.19 g for smolts from the stream channels, the amount of nutrients leaving the stream channels via smolt emigration would have been between 77.44 and 494.76 g of N and 13.67 and 87.31 g of P. Even at high survival rates, there would be more nutrients being imported than exported. However, these mass-balance estimates do not include nutrient inputs from carcass retention, and given the iteroparous life history of Atlantic salmon, probably a minor factor in these rivers.

The inclusion of carcasses to the mass-balance model would result in a surplus of marine nitrogen and phosphorous to the stream channel systems. Stream channels had, on average, nine adult mortalities, which is consistent with spawning mortality estimates by Chadwick et al. (1978). These carcasses would have resulted in 500 g of N and 78 g of P being added to the nutrient pool. Therefore, the 2516 g of N and 123 g of P from carcasses, excretion and gametes combined would have been greater than the total nutrient export through smolt emigration. Studies by Lyle and Elliott (1998) and Jonsson and Jonsson (2003) also found a positive net import of MDNs with Atlantic salmon spawning. Nislow et al. (2004) found similar results of marine-nutrient loading, but only when retention rates were greater than 20%; under certain mortality and return rates, they predict a net export of MDNs. The surplus of marine nutrients deposited by migratory salmon throughout river systems may be required to maintain increased production,

especially in areas where other nutrient sources are unavailable.

We derived a predictive model that demonstrates the effects of MDN inputs from salmon spawning on increases in primary productivity in streams. Assimilation of MDNs resulted in increased productivity, measured as the difference in chlorophyll *a* between treatment and control stream channels. Johnston et al. (2004) showed a similar curvilinear relationship between marine-nutrient inputs from sockeye salmon and algal abundance. Although there is a clear relationship between MDN inputs and productivity, there is probably a trophic capacity at which productivity no longer increases with continued marine-nutrient inputs. These effects on production are not specific to Atlantic salmon as a study on anadromous fishes demonstrated that biofilm biomass increased with rainbow smelt (*Osmerus mordax*) spawning; however, it did not increase above a production threshold, even with increased spawning density (Samways et al. 2015). On the west coast of North America, algal abundance peaked approximately 75–125 days from the beginning of the salmon carcass decomposition period, followed by a decline (Johnston et al. 2004). Empirical models relating nutrients to algal abundance are often location specific and therefore can be inadequate for general predictions (Bowman et al. 2007); however stream channels are representative of natural spawning rivers and not a specific site or locality and therefore serve as a model system for assessing the effects of MDNs from anadromous fishes.

The ecological response to marine-nutrient subsidies in a particular reach is a function of net nutrients transferred by all anadromous species present. Historically in Atlantic rivers, multiple anadromous species would have spawned in the same river (e.g. rainbow smelt (*Osmerus mordax*), alewife (*Alosa pseudoharengus*), blueback herring

(*Alosa aestivalis*), American shad (*Alosa sapidissima*), sea lamprey (*Petromyzon marinus*)) bringing with them MDN subsidies that differ in magnitude, timing, spawning strategy and distribution. These sequential MDN pulses would have resulted in a large marine-nutrient pool that would have been temporally very stable, allowing for increased levels of productivity to be maintained. Our nutrient model provides insights into the timing and magnitude of ecosystem responses from marine-nutrient inputs of a single species. In turn, a multi-species model would encompass temporal ecosystem responses from recurrent nutrient pulses.

Studies by Lyle and Elliott (1998), Jonsson and Jonsson (2003) and Nislow et al. (2004) have quantified the potential nutrient loadings by Atlantic salmon. Our work has expanded on these findings by demonstrating the influence that these marine nutrients have on stream metabolism and the potential mechanisms and routes of nutrient and material exchange. Marine-derived nutrients appear to be a significant nutrient component of rivers with anadromous fishes, as they subsidise productivity to a level that may not be maintained by local nutrient supply. Understanding the extent to which MDNs increase productivity is very important especially in the light of increasing concerns surrounding issues of nutrient loading and eutrophication. Marine-derived nutrients should be part of river restoration and ecosystem management strategies to rehabilitate freshwater habitats of anadromous fishes in order to maintain ecosystem function and productivity.

4.6 Acknowledgments

The authors greatly appreciate the help and support of Trevor Goff, Danielle

MacDonald, Stephanie Ratelle, John Whitelaw and staff at the Mactaquac Biodiversity Facility. We also thank Michelle Charest, Emily Corey, Paula Thoms and Sherr Vue for their help in the field and in the laboratory. James Watmough is gratefully acknowledged for his invaluable assistance with the statistical analysis and modeling. Financial support was provided by grants from the National Sciences and Engineering Research Council of Canada and the Canada Research Chairs Program awarded to R.A. Cunjak.

4.7 Literature Cited

- Allan J.D. & Castillo M.M. (2008) *Stream Ecology: Structure and Function of Running Waters*, 2nd ed. Kluwer Academic Publishers, Boston, MA.
- Bernot M.J., Sobota D.J., Hall R.O., Mulholland P.J., Dodds W.K., Webster J.R. et al. (2010) Inter-regional comparison of land-use effects on stream metabolism. *Freshwater Biology*, **55**, 1874-1890.
- Bott T. L. (2006) Primary productivity and community respiration. In: *Methods in Stream Ecology* (Eds F. R. Hauer & G. A. Lamberti), pp. 663-690. 2nd Edition. Academic Press, San Diego, CA, USA.
- Brett J.R. (1995) Excretion. In: *Physiological Ecology of Pacific Salmon* (Eds C. Groot, L. Margolis, & W.C. Clarke), pp. 381-438. UBC Press, Vancouver, British Columbia, Canada.
- Bowman M.F., Chambers P.A. & Schindler D.W. (2007) Constraints on benthic algal response to nutrient addition in oligotrophic mountain rivers. *River Research and Applications*, **23**, 858-876.
- Chadwick E.M.P., Porter T.R. & Downton P. (1978) Analysis of growth of Atlantic salmon (*Salmo salar*) in a small Newfoundland river. *Journal of the Fisheries Research Board of Canada*, **35**, 60-68.
- Costerton J.W., Lewandowski Z., Caldwell D.E., Korber D.R. & Lappinscott H.M. (1995) Microbial biofilms. *Annual Review of Microbiology*, **49**, 711-745.
- Cunjak R.A. & Therrien J. (1998) Inter-stage survival of wild juvenile Atlantic salmon, *Salmo salar* L. *Fisheries Management and Ecology*, **5**, 209-223.
- Durbin A.G., Nixon S.W. & Oviatt C.A. (1979) Effects of the Spawning Migration of the Alewife, *Alosa Pseudoharengus*, on Freshwater Ecosystems. *Ecology*, **60**, 8-17.
- Elwood J.W., Newbold J.D., Trimble A.F. & Stark R.W. (1981) The role of phosphorus in a woodland stream measured with a ³²P material balance method. *Oikos*, **23**, 295-303.
- Fenchel G.M., King T.H. & Blackburn T.H. (1998) *Bacterial biogeochemistry: the ecophysiology of mineral cycling*. Academic Press, San Diego, California.

- Fleming I.A., Hindar K., Mjølnerød I.B., Jonsson B., Balstad T. & Lamberg A. (2000) Lifetime success and interactions of farm salmon invading a native population. *Proceedings of the Royal Society of London B*, **267**, 1517-1523.
- Francoeur S.T., Biggs B.J.F., Smith R.A. & Lowe R.L. (1999) Nutrient limitation of algal biomass accrual in streams: seasonal patterns and a comparison of methods. *Journal of the North American Benthological Society*, **18**, 242-260.
- Garman G.C. & Macko S.A. (1998) Contribution of marine-derived organic matter to an Atlantic coast, freshwater, tidal stream by anadromous clupeid fishes. *Journal of the North American Benthological Society*, **17**, 277-285.
- Garmin G. (1992) Fate and potential significance of postspawning anadromous fish carcasses in an Atlantic coastal river. *Transactions of the American Fisheries Society*, **121**, 390-394.
- Gende S.M., Edwards R.T., Willson M.F. & Wipfli M.S. (2002) Pacific salmon in aquatic and terrestrial ecosystems. *Bioscience*, **52**, 917-928.
- Holtgrieve G.W. & Schindler D.E. (2011) Marine-derived nutrients, bioturbation, and ecosystem metabolism: reconsidering the role of salmon in streams. *Ecology*, **92**, 373-385.
- Idler D.R. & Clemens W.A. (1959) The energy expenditures of Fraser River sockeye salmon during the spawning migration to Chilko and Stuart Lakes. *International Pacific Salmon fisheries Commission*. Progress Report No. 6.
- Jardine T.D., Roussel J.M., Mitchell S.C. & Cunjak R.A. (2009) Detecting marine nutrient and organic matter inputs into multiple trophic levels in streams of Atlantic Canada and France. In: *Challenges for Diadromous Fishes in a Dynamic Global Environment* Pages 427-445 in (Eds A.J. Haro, K.L. Smith, R.A. Rulifson, C.M. Moffitt, R.J. Klauda, M.J. Dadswell, R.A. et al.), pp.427-445, Symposium 69. American Fisheries Society, Bethesda, Maryland.
- Jeffrey S.W. & Humphrey G.F. (1975) New spectrophotometric equation for determining chlorophylls *a*, *b*, *c1*, *c2* in higher plants, algae and natural phytoplankton. *Biochemie und Physiologie der Pflanzen*, **167**, 191-194.
- Johnston N.T., MacIsaac E.A., Tschaplinski P.J. & Hall K.J. (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.
- Jones, R.A., Anderson, L., Gibson, A.J.F., and Goff, T. (2010). Assessments of Atlantic Salmon Stocks in South Western New Brunswick (Outer Portion of SFA 23): An Update to 2008. *Canadian Aquatic Fisheries Science Advisory Council*, Research Document, 2010/118. Ottawa, Canada.
- Jonsson N., Jonsson B. & Hansen L.P. (1990) Partial segregation in the timing of migration of Atlantic salmon of different ages. *Animal Behaviour*, **40**, 313-321.
- Jonsson B. & Jonsson N. (2003) Migratory Atlantic salmon as vectors for the transfer of energy and nutrients between freshwater and marine environments. *Freshwater Biology*, **48**, 21-27.
- Lyle A.A. & Elliott J.M. (1998) Migratory salmonids as vectors of carbon, nitrogen and phosphorus between marine and freshwater environments in north-east England. *Science of the Total Environment*, **210**, 457-468.

- MacAvoy S.E., Garman G.C. & Macko S.A. (2009) Anadromous fish as marine nutrient vectors. *Fishery Bulletin*, **107**, 165-174.
- Marshall T.L. & Penney G.H. (1983). Spawning and river escapement requirements for Atlantic salmon of the Saint John River, New Brunswick. *Canadian Aquatic Fisheries Science Advisory Council*, Research Document, 83/66. Ottawa, Canada.
- McIntire C.D. (1973) Periphyton dynamics in laboratory streams: a simulation model and its implications. *Ecological Monographs*, **43**, 399-420.
- McIntire C.D. & Phinney H.K. (1965) Laboratory studies of periphyton production and community metabolism in lotic environments. *Ecological Monographs*, **35**, 237-258.
- Minakawa N. & Gara R.I. (1999) Ecological effects of a chum salmon (*Oncorhynchus keta*) spawning run in a small stream of the Pacific Northwest. *Journal of Freshwater Ecology*, **14**, 327-335.
- Morin A., Lamoureux W. & Busnarda J. (1999) Empirical models predicting primary productivity from chlorophyll *a* and water temperature for stream periphyton and lake and ocean phytoplankton. *Journal of the North American Benthological Society*, **18**, 299-307.
- Moore J.W. & Schindler D.E. (2008) Biotic disturbance and benthic community dynamics in salmon-bearing streams. *Journal of Animal Ecology*, **77**, 275-284.
- Naiman R.J., Bilby R.E., Schindler D.E. & Helfield J.M. (2002) Pacific salmon, nutrients, and the dynamics of freshwater and riparian ecosystems. *Ecosystems*, **5**, 399-417.
- Nislow K., Armstrong J. & McKelvey S. (2004) Phosphorus flux due to Atlantic salmon (*Salmo salar*) in an oligotrophic upland stream: effects of management and demography. *Canadian Journal of Fisheries and Aquatic Sciences*, **61**, 2401-2410.
- O'Keefe T.O. & Edwards R.T. (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.
- Parmenter R.R. & Lamarra V.A. (1991) Nutrient cycling in a freshwater marsh: the decomposition of fish and waterfowl carrion. *Limnology and Oceanography*, **36**, 976-987.
- Pestana J.L.T., Alexander A.C., Culp J.M., Baird D.J., Cessna A.J. & Soares A.M. (2009) Structural and functional responses of benthic invertebrates to imidacloprid in outdoor stream mesocosms. *Environmental Pollution*, **157**, 2328-2334.
- Petticrew E.L., Rex J.F. & Albers S.J. (2011) Bidirectional delivery of organic matter between freshwater and marine systems: the role of flocculation in Pacific salmon streams. *Journal of North American Benthological Society*, **30**, 779-786.
- Sabater S.H., Guash H., Romání A. & Muñoz I. (2002) The effect of biological factors on the efficiency of river biofilms in improving water quality. *Hydrobiologia*, **469**, 149-156.
- Samways K. M., Quiñones-Rivera, Z. J., Leavitt P. R. & Cunjak R. A. (2015) Spatio-temporal responses of algal, fungal and bacterial biofilm communities in Atlantic rivers receiving marine-derived nutrient inputs. *Freshwater Science*, (accepted, scheduled for 34/3 issue).

- Underwood A.J. (1997) *Experiments in Ecology: their Logical Design and Interpretation using Analysis of Variance*. Cambridge University Press, Cambridge, UK.
- Vanni M.J. (2002) Nutrient cycling by animals in freshwater ecosystems. *Annual Review of Ecological Systems*, **33**, 341-370.
- Walters A.W., Barnes R.T. & Post D.M. (2009) Anadromous alewives (*Alosa pseudoharengus*) contribute marine-derived nutrients to coastal stream food webs. *Canadian Journal of Fisheries and Aquatic Sciences*, **66**, 439-448.
- Wipfli M.S., Hudson J. & Caouette J. (1998) Influence of salmon carcasses on stream productivity: response of biofilm and benthic macroinvertebrates in southeastern Alaska, USA. *Canadian Journal of Fisheries and Aquatic Sciences*, **55**, 1503-1511.
- Wipfli M.S., Hudson J., Chaloner D.T. & Caouette J.P. (1999) The influence of salmon spawner densities on stream productivity in Southeast Alaska. *Canadian Journal of Fisheries and Aquatic Sciences*, **56**, 1600-1611.
- Wood C.M. (1995) Excretion. In: *Physiological Ecology of Pacific Salmon* (Eds C. Groot, L. Margolis, & W.C. Clarke), pp. 381-438. UBC Press, Vancouver, British Columbia, Canada.

Table 4.1. Productivity – marine-nutrient models associated with Atlantic salmon spawning at the Mactaquac Biodiversity Facility, New Brunswick, Canada.

Treatment	Nitrogen		Phosphorous	
	Model	R ²	Model	R ²
Stream-channel	$P = 1.09e^{(0.0024N)}$	0.982	$P = 1.44e^{(0.0499P)}$	0.930
Mesocosm	$P = 1.25e^{(0.0027N)}$	0.971	$P = 1.78e^{(0.0553P)}$	0.894

Figure 4.1. Biofilm accrual on artificial substrata, measured as chlorophyll *a*, receiving marine-derived nutrients (MDNs) from spawning Atlantic salmon and control treatments in: a) stream channels and b) mesocosms. Experimental system: stream channels, circles; mesocosms, triangles. Treatments: MDN subsidies, shaded symbols; controls, open symbols. Values with an * denote a statistically significant difference ($P < 0.05$) between stream channels/mesocosms with MDN inputs and the controls. Shaded areas denote the period of spawning (8–23 November 2011). Adults were introduced into the stream channels on 7 October 2011 and removed on 10 December 2011.

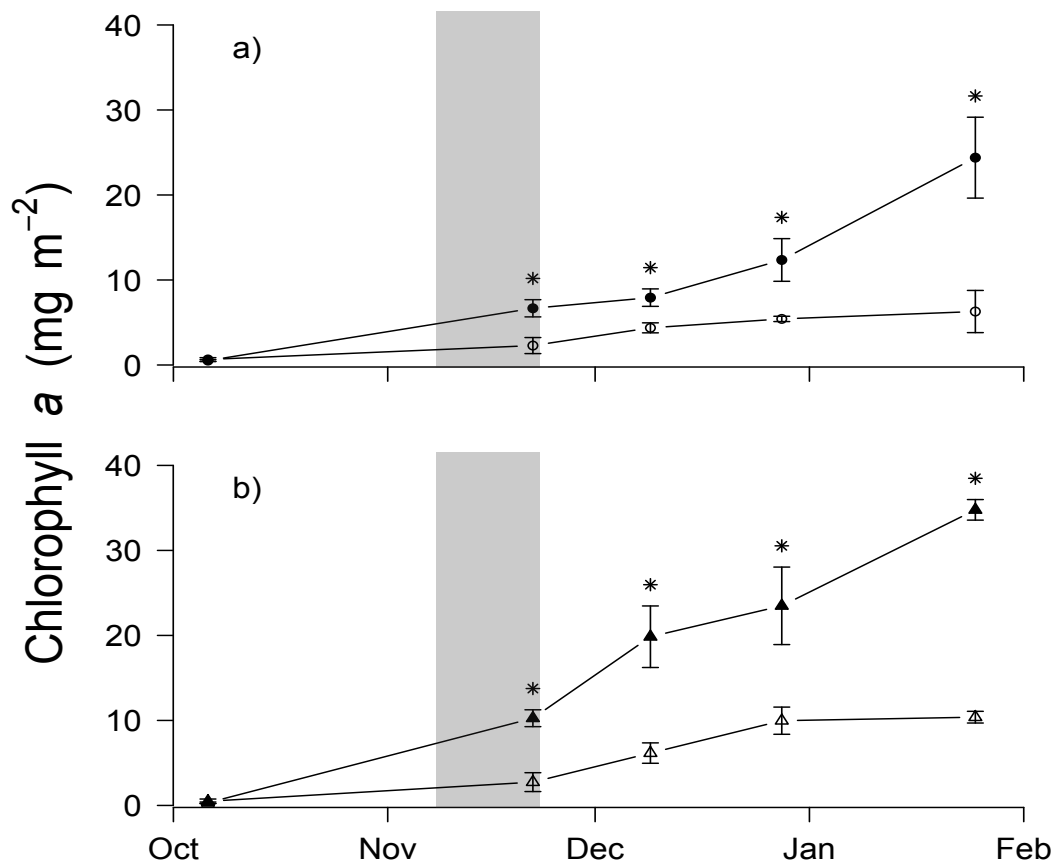


Figure 4.2. Seasonal patterns of gross primary production (GPP) of biofilm on artificial substrata receiving marine-derived nutrients (MDNs) from spawning Atlantic salmon and control treatments in: a) stream channels and b) mesocosms. See Fig. 4.1 for explanation of symbols.

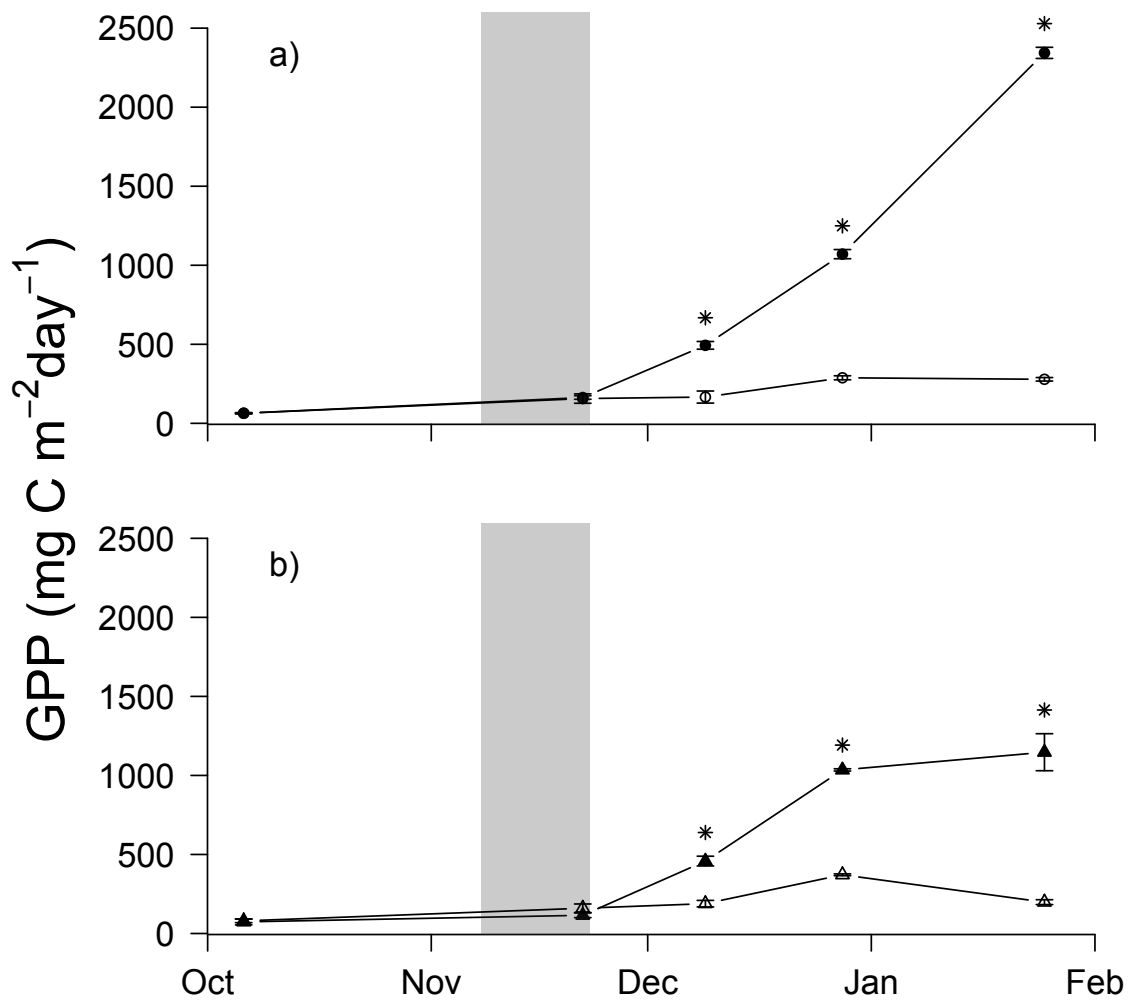


Figure 4.3. Seasonal patterns of the P/R ratio of biofilm on artificial substrata receiving marine-derived nutrients (MDNs) from spawning Atlantic salmon and control treatments in: a) stream channels and b) mesocosms. See Fig. 4.1 for explanation of symbols.

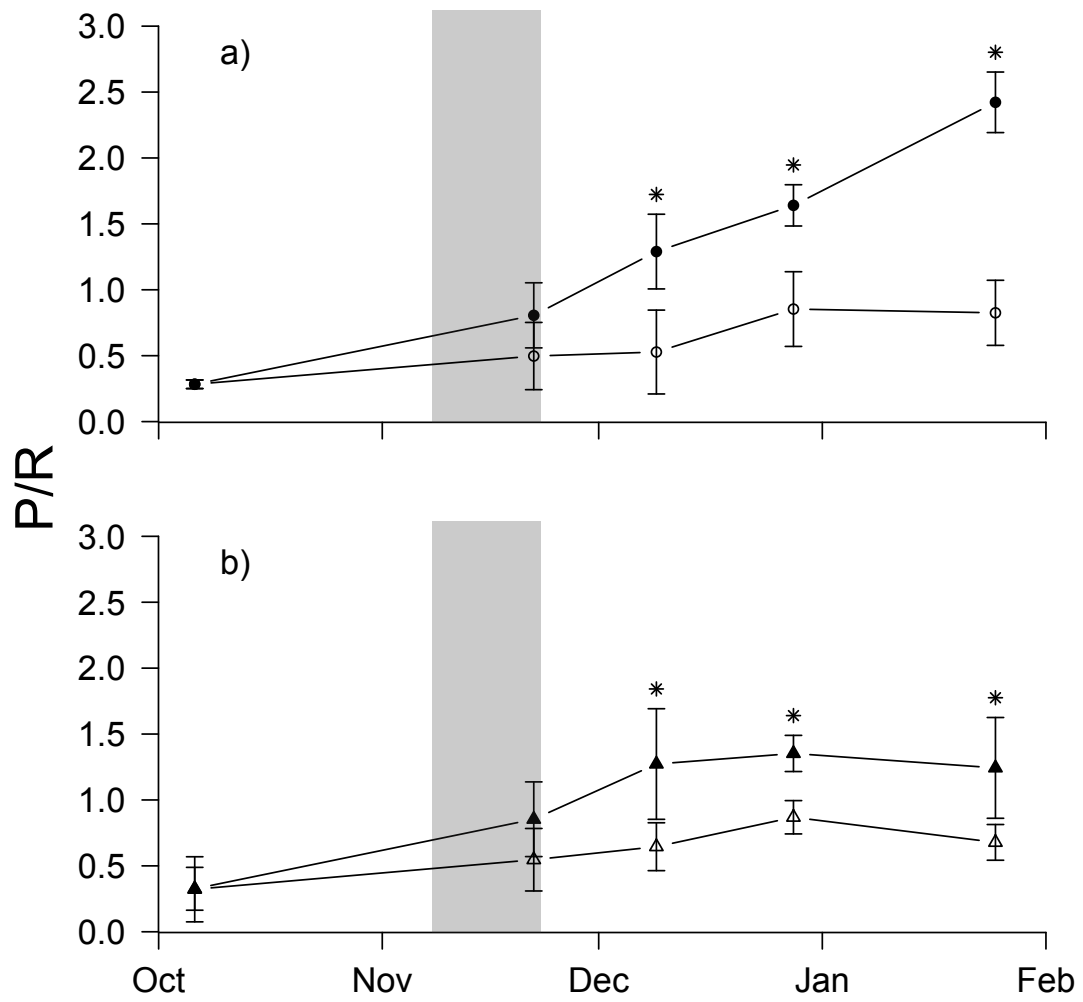


Figure 4.4. Accumulation of marine-derived nitrogen (solid line) and phosphorous (dashed line) inputs from 22 Atlantic salmon spawning in stream channels at the Mactaquac Biodiversity Facility, New Brunswick, Canada.

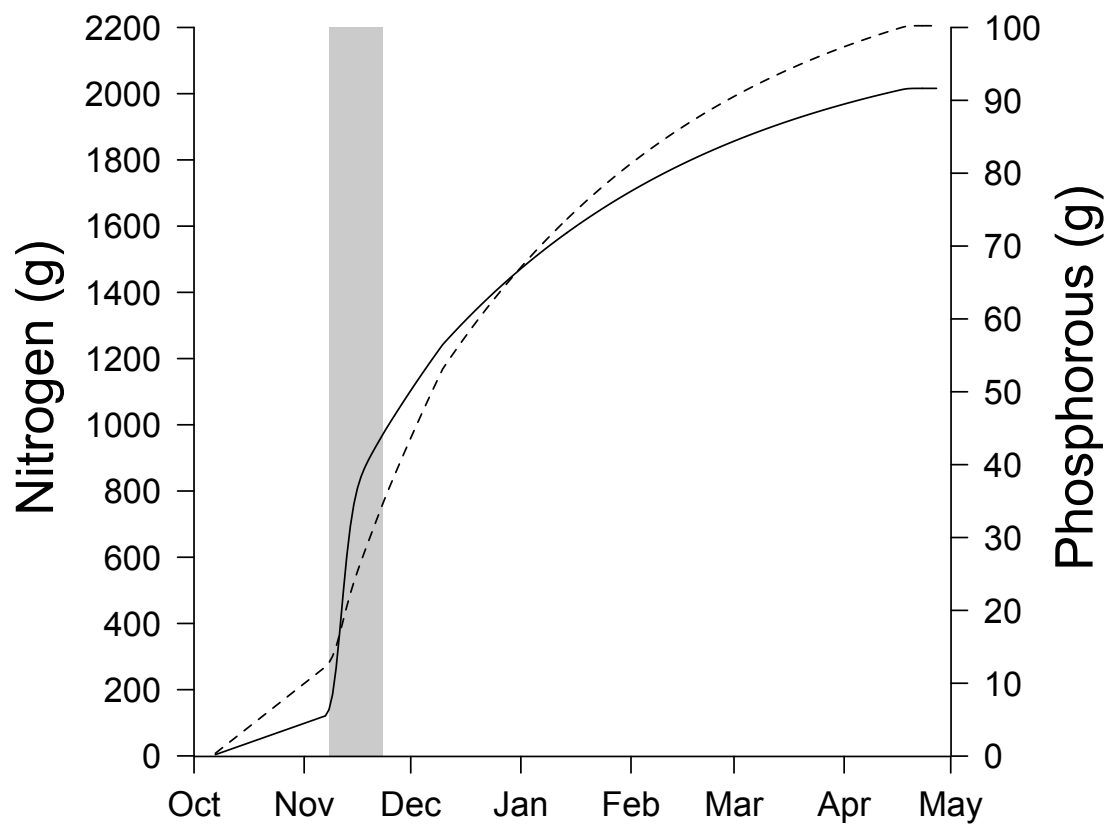


Figure 4.5. Increase in algal productivity (chlorophyll mg m^{-2}) as a function of marine-derived nitrogen inputs from spawning Atlantic salmon in: a) stream channels and b) mesocosms. Marine-derived nitrogen inputs are from adult Atlantic salmon spawning in stream channels at the Mactaquac Biodiversity Facility, New Brunswick, Canada.

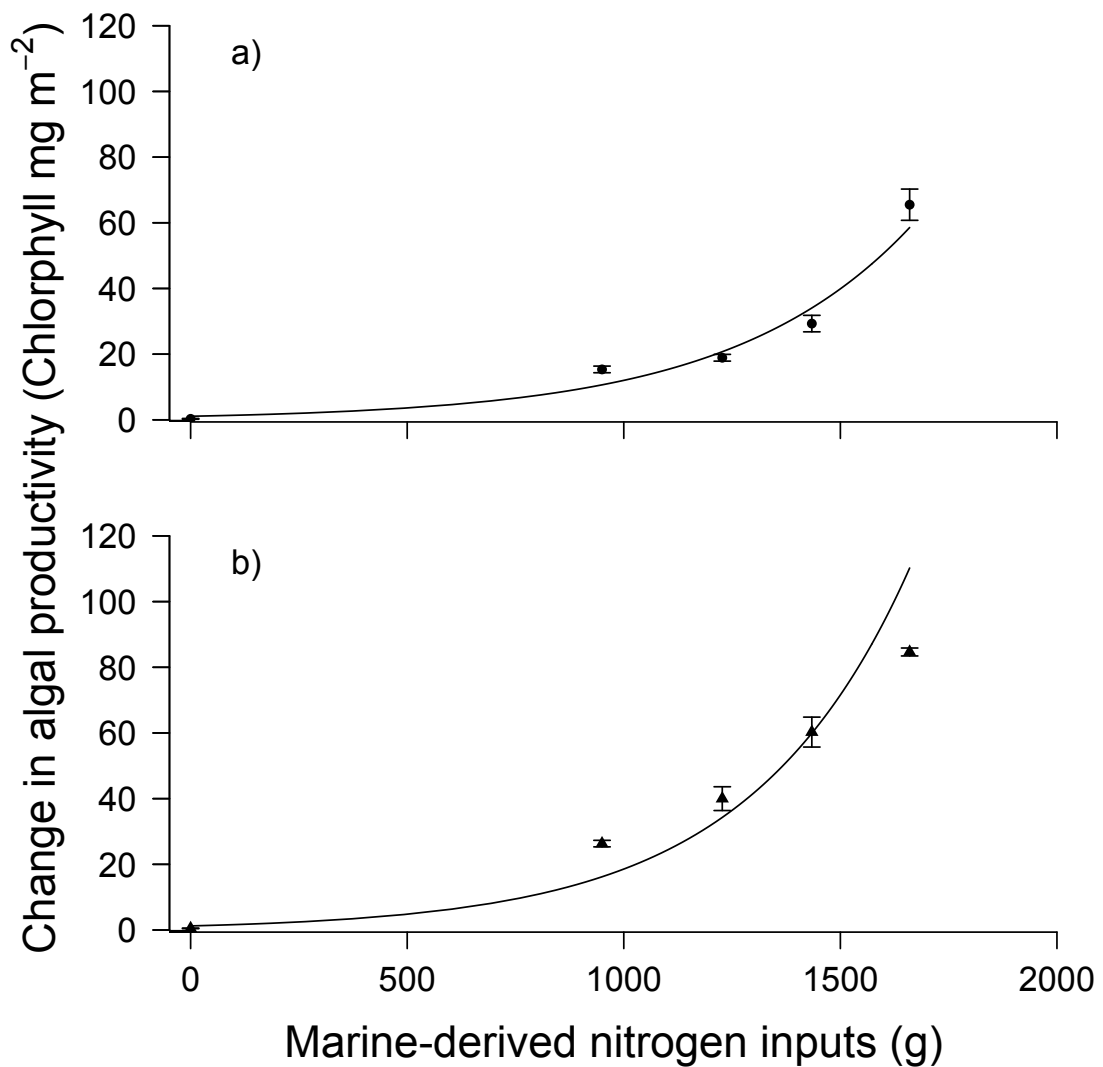
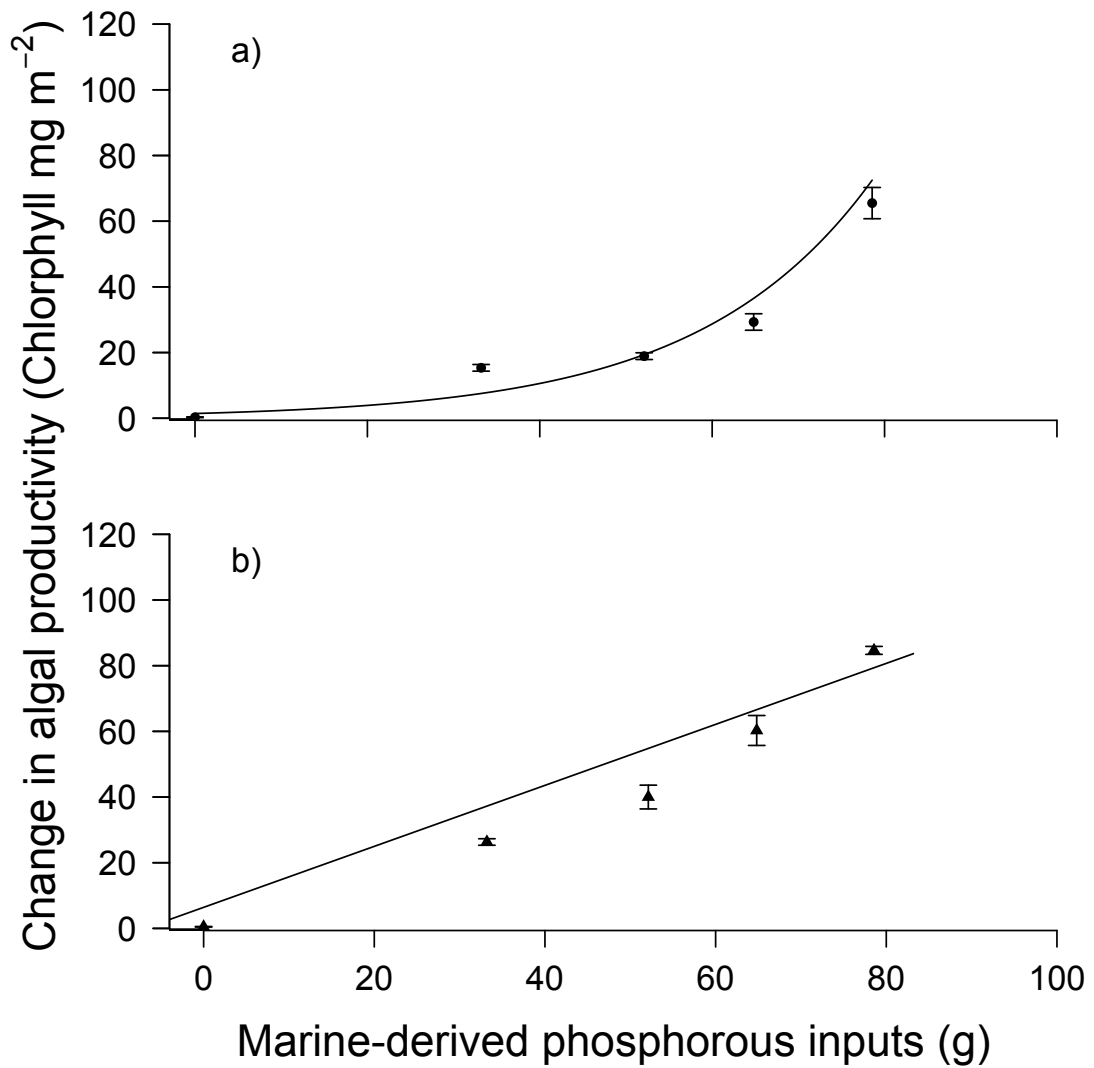


Figure 4.6. Increase in algal productivity (chlorophyll mg m^{-2}) as a function of marine-derived phosphorous inputs from spawning Atlantic salmon in: a) stream channels and b) mesocosms. Marine-derived phosphorous inputs are from the same source as noted in Figure 4.5.



Chapter 5

Effects of spawning Atlantic salmon (*Salmo salar*) on total lipid content and fatty acid composition of river food webs

K. M. Samways¹, T. J. Blair², M. A. Charest¹, and R. A. Cunjak¹

¹University of New Brunswick, Department of Biology and Canadian Rivers Institute, Fredericton, New Brunswick E3B 5A3 Canada.

²Fisheries and Oceans Canada, Biological Effects Section, St Andrews Biological Station, St Andrews, New Brunswick E5B 2L9 Canada.

Published: *Ecosphere*. Accepted April 4, 2017. DOI: 10.1002/ecs2.1818. Reproduced with the kind permission from John Wiley & Sons Ltd.

5.1 Abstract

Returning Atlantic salmon subsidize rivers with marine-derived nutrients (MDNs), potentially altering food web dynamics, shifting to reliance on marine-derived resources. Stream-channels designed to simulate natural river systems, one with marine-derived nutrients from spawning Atlantic salmon (*Salmo salar*) and one without marine-nutrient subsidies (control), were used to determine if fatty acids delivered by spawning Atlantic salmon are incorporated by freshwater biota. Changes in fatty acid profiles between stream-channels with and without MDN inputs for biofilm, macroinvertebrates and Atlantic salmon parr were used to characterize temporal dynamics of MDN incorporation during pre-spawning, spawning and postspawning periods. There were no differences between control and treatment stream-channels prior to the addition of Atlantic salmon. Spawning Atlantic salmon had a positive effect on total lipid content in all trophic levels (biofilm, 4.33%; macroinvertebrates, 7.54%; salmon parr, 2.59%), relative to controls. Fatty acid profiles between the MDN treatment and control stream-channels were similar for each biotic group prior to the introduction of adult Atlantic salmon; however, following adult salmon introductions, the fatty acid profiles of the biofilm, invertebrates and salmon parr differed by as much as 36.7, 30.3, and 22.9%, respectively between the MDN treatment stream-channels and the controls. Fatty acid profiles of the invertebrates and salmon parr from the MDN treatment stream-channels tracked the fatty acid profile of the salmon eggs. Proportions of DHA and EPA varied with diet in a predictable manner related to dietary fatty acid proportions. These responses demonstrate a change in the food web structure resulting from the establishment of this new, marine-based, basal resource. The positive effects of MDNs increasing freshwater productivity are

complimented by marine-based lipids that represent surplus energy and an increase in the *quality* of resources, thereby contributing to the diversity and health of freshwater ecosystems.

5.2 Introduction

Anadromous fish can be important vectors of pulsed nutrients and energy to freshwater ecosystems. Fish returning from the ocean to spawn subsidize rivers with marine-derived nutrients (MDNs) (i.e. nitrogen and phosphorous) through the excretion of metabolites, gamete release, and carcass decomposition (Bilby et al. 1996, Wipfli et al. 1999). The effects of these marine-nutrient subsidies on freshwater food webs are well documented in the Pacific Northwest, with MDNs increasing biofilm productivity (Levi et al. 2013) and biomass (Wipfli et al. 1998, Johnston et al. 2004, R  egg et al. 2012), macroinvertebrate abundance and biomass (Wipfli et al. 1998, 1999 Chaloner et al. 2004, Lessard and Merritt 2006, Lessard et al. 2009), and juvenile salmon abundance (Chaloner et al. 2002, Wipfli et al. 2010) and growth (Johnston et al. 1990, Wipfli et al. 2003). Whereas Pacific salmonids are semelparous, Atlantic anadromous fishes are generally iteroparous so carcasses are less likely to be a significant source of marine-nutrients making MDN inputs from excretion and gamete release relatively more important to food web processes. Nevertheless, several studies have demonstrated the importance of MDNs from Atlantic anadromous fishes to freshwater ecosystems (Durbin et al. 1979, Jonsson and Jonsson 2003, Nislow et al. 2004, Jardine et al. 2009, Nislow and Kynard 2009, Walters et al. 2009, Guyette et al. 2013, 2014, Samways and Cunjak 2015, Samways et al. 2015).

Freshwater consumers can derive a substantial proportion of their energy from marine resources when present, and may therefore maintain stream productivity. Marine-derived nutrient subsidies facilitate the production of enough in-stream energy to support food web processes, thereby relieving the bottom-up constraints on stream productivity (Ostfeld and Keesing 2000, Samways and Cunjak 2015). Dissolved MDNs provide energy to consumers through uptake by the biofilm (a complex community of algae, fungi, and bacteria within a self-excreted polysaccharide matrix) and subsequent trophic transfer (Wipfli et al. 1999, Guyette et al. 2014, Samways et al. 2015). However, the availability of nutrient rich eggs and partial carcass loading can lead to shifts in the energy flow from indirect consumption to direct consumption of marine-derived organic matter by macroinvertebrates and fish (Naiman et al. 2002).

Stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) are effective biomarkers for identifying marine sources consumed by stream organisms (e.g. Bilby et al. 1996, Garman and Macko 1998, Chaloner et al. 2002, Jardine et al. 2009, Reisinger et al. 2013). The use of stable isotope mixing models (e.g. SIAR – Stable Isotope Analysis in R) can increase the understanding of the importance of MDNs by identifying their relative contributions to a consumer's diet (Phillips and Gregg 2001). Changes in stable isotope ratios can identify protein synthesis and the metabolic breakdown of lipids (Doucett et al. 1999), but they provide limited direct information about the nutritional state of organisms. Methods such as lipid and fatty acid analysis can help identify the ecophysiological importance of MDNs.

Lipids are among the most important organic compounds that affect the health and fitness of aquatic organisms. They improve the nutritional status of aquatic food

webs by supporting the physiological development and health of organisms (Müller-Navarra et al. 2000, Kainz and Fisk 2009). In addition to increasing freshwater production, MDN subsidies can also increase the *quality* of food resources by increasing energy stores in constituents of aquatic ecosystems. Therefore, lipid stores represent surplus energy and a measure of nutritional condition (Fraser 1989). Fatty acids (FAs) are a group of compounds that comprise the majority of lipids found in all organisms. Similar to stable isotope analysis, when sources have distinct fatty acid compositions, fatty acid profiles can be used to characterize resources consumed by freshwater biota (Heintz et al. 2004) and characterize freshwater and marine food webs (Henderson and Tocher 1987). This is possible because, unlike other dietary nutrients, fatty acids are not generally degraded and retain their basic form in consumer tissues (Budge et al. 2006). Many of these fatty acids are essential for key regulatory roles including membrane structure and function (Bell et al. 1996), brain (Brodtkorb et al. 1997) and gonad development (Pickova et al. 1999), and salmonid parr-smolt transformation (Bell et al. 1997). Fatty acids cannot be synthesized by freshwater organisms at sufficient rates to meet their basic biochemical requirements; therefore, they must be supplied through diet. Returning Atlantic salmon provide many of these essential fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), to freshwater ecosystems through gamete release and carcass deposition.

As part of a larger study using semi-natural stream channels to investigate the effects of MDNs from spawning Atlantic salmon, the focus of this research was to investigate whether Atlantic salmon are a significant source of marine fatty acids to freshwater food webs. Understanding trophic-specific differences with respect to

incorporating and/or processing dietary lipids, particularly from Atlantic anadromous species has not been directly demonstrated. This is particularly important in the context of nutrient dynamics in Atlantic rivers given the iteroparous life history and relatively limited biomass contributions from Atlantic salmon (relative to their Pacific counterparts).

The objectives of this study were to (1) determine if fatty acids delivered to rivers and streams by spawning Atlantic salmon are incorporated by freshwater biota, (2) examine how exposure to marine subsidies relates to lipid content and fatty acid profiles of consumers, and (3) determine if food web structure changes with a new, marine-based, basal resource. We hypothesized that access to marine subsidies would lead to increased lipid stores in consumers through direct consumption of eggs rather than via indirect consumption and trophic transfer because dissolved nutrients are not likely to contain fatty acids.

5.3 Methods

5.3.1 Study Site

This study was conducted between August 2011 and January 2012 at the Mactaquac Biodiversity Facility (45°57'31.36"N, 66°50'38.72"W), an Atlantic salmon mitigation facility on the St. John River (SJR) operated by Fisheries and Oceans Canada. It is located approximately 120 km from the river mouth, near the city of Fredericton, New Brunswick, Canada.

5.3.2 Stream-channels

Four, 121 m² conventional concrete Swede ponds were converted to semi-natural, flow-through stream-channels, supplied with continuous flow-through water from the SJR (Figure 5.1). To direct flow and establish a stream-channel a 1.30 m high, hollow octagon with drainage screens was placed in the centre of each pond. These stream-channels were 3.1 m wide and 31 m long (measured as circumference at mid-stream width) resulting in a rearing area of 96.1 m². Substrate, which was sculpted to produce natural stream features of riffles, runs, and pools, consisted of 25 cm base layer of small gravel (1.9 – 3.8 cm), with a 15cm top layer consisting of a mixture of small, medium (3.8– 7.6 cm), and large (>7.6 cm) gravels. Large rocks (>20 cm) were added throughout the stream channel to provide suitable refuge for juvenile salmon (parr). These features also helped to direct and amplify water velocities. All stream channels were constructed equally with the same number of riffles, runs, pools, cover, etc. to minimize any asymmetry in surface area between stream-channels. Substrate symmetry ensures changes in biofilm production, resource use, and growth rates are the result of MDN inputs rather than boundary layer effects. Water depth ranged from 12 cm in the riffles to 60 cm in the pools, with an overall average depth of 45 cm, providing a rearing volume of 39.15 m³. Water velocities ranged between 15 cm/s in the pools to 110 cm/s in the riffles and daily mean water temperatures were 5.4 °C (minimum 0.4; maximum 14.8 °C).

Biofilm growth in the stream-channels supported a diverse macroinvertebrate population that served as the natural food source for salmon parr. Invertebrate samples were collected from a nearby tributary using a standardized (3 minute) kick net (400 µm

mesh) sampling effort, identified to family and density and community composition evaluated (Reynoldson et al. 2002). Each stream-channel was seeded with a representative invertebrate community, equal to 2× the stream-channel area, to supplement the natural colonization via drift. Prior to the addition of parr, each stream-channel was sampled in the same manner as the wild collections to ensure comparable invertebrate densities and compositions, as well as an adequate food supply. Wild Atlantic salmon parr, were collected from the Tobique River (a tributary of the SJR), with 115 parr (0+ to 2+ age-classes) stocked into each stream-channel. Plastic mesh baskets (15 cm x 30 cm x 30 cm) were filled with substrate and inserted level with the surface bottom of the stream-channel. These invertebrate collection baskets permitted quantifiable sampling of invertebrates, while minimizing disturbance to the adult salmon and parr. Fixed to each basket was a 4.5 by 4.5 cm slate tile (pre-conditioned to remove organics by combusting at 550 °C for 12hr in a muffle furnace) to collect biofilm from a standardized area. These baskets were randomly placed and secured to the channel bed prior to the addition of adult salmon, allowing the biofilm to colonize the tiles under natural conditions.

In May of 2009, wild Atlantic salmon smolts were collected from the Campbell River, a tributary of the Tobique River, and reared in the Mactaquac facility to produce mature adults in 2011 (Jones et al. 2010). These adults were reared on a marine-based diet, primarily herring; therefore, we classify nutrient subsidies delivered by these fish as marine-derived. On October 7, 2011, 24 of these “wild-exposed” adult Atlantic salmon (12 male and 12 female) were introduced into each of two treatment stream-channels, with the majority of spawning occurring November 8-23. The average size of introduced

fish was 1.53 kg (range 0.99-2.16 kg) for the males and 2.49 kg (range 1.82-3.61 kg) for the females. The resultant spawning density is within the range observed in spawning locations in rivers with self-sustaining populations (personal observation; K.M. Samways, R.A. Cunjak). Adults remained in the stream-channels until December 10, 2011, when they were removed to simulate post-spawning out-migration. Although Atlantic salmon are iteroparous, a portion did die during the experimental period which would have provided carcass inputs. Due to facility regulations, all mortalities had to be removed to minimize any potential spread of disease. Hatching of eggs in the substrate was estimated using a standard cumulative degree-days formula and a lower thermal limit to salmon growth of 0 °C (Chezik et al. 2014). This was estimated to be 90% complete by March 10, 2012 and emergence of salmon fry began on April 15, 2012.

5.3.3 Mesocosms

To assess the effects of only the dissolved MDN pool on fatty acid uptake in the biofilm, eight partial flow-through mesocosms were used. These were located immediately adjacent to the stream-channels. Each mesocosm was a 10L polyethylene, open-top cylinder, similar to those used by Pestana et al. (2009). Mesocosms contained substrates consisting of biofilm-colonized rocks and artificial substrates devoid of any invertebrates. Mesh covers were used to preclude aerial invertebrates from colonizing the mesocosms, as well as to maintain similar light intensities reaching the substrate as in the stream-channels. Water from each stream-channel was pumped with a small centrifugal pump (March: LC-3CP-MD) through a 243µm mesh (to exclude fish and invertebrates), into a manifold, which supplied two replicate mesocosms. Current

velocity inside the mesocosms was maintained at $0.07 \text{ m}\cdot\text{s}^{-1}$, the average current velocity within the stream-channels.

5.3.4 Sample collection

Sampling occurred between October 2011 and January 2012 (time 0, October 6, 2011; time 1, November 22, 2011; time 2, December 9, 2011; time 3, December 28, 2011; and time 4, January 25, 2012) to capture pre-spawn, spawning and post-spawn effects. At each sampling event, biofilm, invertebrates, and salmon parr were collected for lipid and fatty acid analysis. The primary invertebrate families found were Gammaridae, Perlidae, and Ephemerellidae. Atlantic salmon eggs and milt were also collected to serve as representative MDN sources. Benthic macroinvertebrates were sampled by retrieving two of the plastic mesh baskets, sorting through the material within, and identifying to family. Biofilm was collected from the affixed tiles from the two baskets and one tile from each of the replicate mesocosms. The upper surface of each tile was scraped, until bare tile was exposed, and rinsed with water from which the tile was collected. Biofilm samples were centrifuged to isolate the organic material. Electrofishing was used to collect salmon parr, which were euthanized prior to dissection of muscle tissue by placing the fish in a concentrated clove oil solution until cessation of respiration, followed by a blow to the dorsal side of the skull. Dissection revealed that stomach contents contained invertebrates, while the stomach contents of many parr from treatment stream-channels also contained salmon eggs. All samples were placed on ice until they could be frozen at -80°C in the lab. Prior to lipid and fatty acid analysis, samples were freeze-dried and ground to a homogenous powder.

5.3.5 Lipid extraction and separation

Lipids were extracted following the method of Folch et al. (1957) using a 2:1 (v/v) chloroform:methanol solvent mixture. Adding 3.75 ml of 0.88% potassium chloride separated the mixture into two phases. The lower phase was isolated, filtered through glass wool and Na₂SO₄ anhydrous crystals, evaporated under nitrogen and the total lipid weighed. Pooling of replicate biofilm samples was done to ensure enough material was available for analysis. Following a modified method of Morrison and Smith (1964), the lipid samples were transmethylated by incubation with 2 ml of 7% BF₃(MeOH) and 0.5 ml of toluene at 100 °C for 1 hr. The fatty acid methyl esters were isolated on Silica Gel 60 high performance thin layer plates (HPTLC) using a developing solution of hexane:diethyl ether:acetic acid (90:10:1 v/v/v) solvent. The silica plates were sprayed with a 2,4,7 dichlorofluorescein_(MeOH) solution to visualize the methyl ester spots under UV light. The fatty acid methyl esters were separated and analyzed using an Agilent 7890A model gas liquid chromatograph (Santa Clara, California, USA.), as described by Nanton and Castell (1998). Within each analytical run, the Supelco Omegawax® standard from Sigma-Aldrich (St. Louis, Missouri, USA) and GLC-463 standard from Nu-Chek Prep, Inc. (Elysian, Minnesota, USA) were analyzed to calibrate fatty acid retention times.

5.3.6 Data Analysis

We used the statistical program PRIMER with the PERMANOVA (Permutational Multivariate Analysis of Variance) add-on (McArdle&Anderson 2001) to quantify how fatty acid profiles varied between groups and over time. For all analyses, an α of 0.05

was used. Fatty acid profiles were analyzed with a PERMANOVA with data arcsin square root transformed and a resemblance matrix was calculated using Bray-Curtis coefficients (Clarke et al. 2006). In the PERMANOVA, Treatment (2 levels; with and without adult salmon), Sampling Time (5 levels), and Trophic Group (4 levels; salmon parr, invertebrates, biofilm, salmon eggs) were fixed factors; Tank (4 levels) nested within Treatment, and Sample (the error term) were random factors. A total of 56 fatty acids per sample were used in the PERMANOVA. As there were no significant differences between replicate tanks ($p=0.526$), PERMANOVAs were re-analyzed with the variable Tank removed. Due to a significant three-way interaction between fixed factors (Treatment x Sampling Time x Trophic Group), a separate PERMANOVA was conducted for each Trophic Group (as data permitted) as a *post hoc* analysis to assess temporal dynamics. Similarity Percentage (SIMPER) analysis, which measures the top 90% of contributing variables (e.g. fatty acids) (Clarke 1993), was used to calculate the average sample dissimilarity of each fatty acid to the separation between treatment and control stream-channels.

Principal components analysis (PCA) was used to visualize the fatty acid profiles between groups and identify those fatty acids that accounted for the major sources of variation within the dataset, therefore minimizing redundancy. PCA was conducted using fatty acid data for all individuals in all groups. The fatty acids with the highest absolute loadings on the first two PC axes were selected, following the procedures outlined in Olden and Poff (2003). The number of fatty acids selected for each axis was weighted by the proportion of the variance explained by that PC relative to all PCs retained. For example, based on all groups the first PC explained 68.4% of the total

95.9% variance explained by the first five PCs – 4 of the 47 fatty acids were selected from PC1 (as they accounted for 68% of the loading values). We employed a minimum threshold of three fatty acids selected per axis.

The Euclidean distance for each sample to the group centroid was calculated to provide a metric of feeding diversity within the food web (Layman et al. 2007). The centroid was the mean PC1 and PC2 value for all samples in a particular group (e.g. pre-spawning invertebrates). We evaluated the effect of being exposed to salmon spawning to not being exposed, time and age class on length and weight of salmon parr using an analysis of variance (ANOVA).

5.4 Results

5.4.1 Growth

Average mass of salmon parr in the different cohorts ranged from 2.9 – 16.9 g at the time of stocking to 2.9 – 22.8 g at the last sampling period (Table 5.1). Growth, in terms of length, was significant ($p=0.044$) in the 2+ parr in the MDN treatment with increases in length of 0.45 ± 0.07 cm fish⁻¹. Growth, in terms of weight, was significant ($p<0.04$) for the 1+ and 2+ parr in the MDN treatment stream-channels (Table 5.1). The 1+ and 2+ parr in the controls lost weight from initial stocking. The weight of 0+, 1+, and 2+ parr in the MDN treatment was on average by 14.7, 7.2, and 32.5 % greater, respectively, over the 116 day experiment.

5.4.2 Total lipid

Total lipid content varied temporally throughout the experiment, with MDN treatments having a greater lipid content compared with the controls (Figure 5.2). We detected a significant increase in total lipid content in the biofilm (both stream-channels and mesocosms), invertebrates (primarily Isopoda) and salmon parr (all comparisons had an $F\text{-value} \geq 6.75$ and a $p\text{-value} \leq 0.006$). Greater total lipid content coincided with salmon spawning for the invertebrates and salmon parr; however, differences in the biofilm did not occur until after the adults were removed (Figure 5.2a). The magnitude of differences in total lipid content varied among trophic groups. Biofilm in stream-channels with MDN inputs had up to a 4.33% higher lipid content than stream-channels without MDNs (Figure 5.2a). Invertebrate total lipid was larger than both biofilm or salmon parr and the only trophic group to show increases in lipids in both treatment and controls; however the greatest increases were in MDN treatment stream-channels (7.54%). For salmon parr, like biofilm, controls showed little to no change in lipid content over time, whereas in the MDN treatment, lipid content was greater immediately after spawning.

5.4.3 Fatty acid profiles

The PCA results based on the fatty acid profiles distinguished between trophic groups and Figure 5.3 represents the two-dimensional ordination displaying the patterns of inter-correlation among the 56 fatty acids for the combined set of 140 samples. The first principal component axis accounted for most of the variation between trophic groups (59.9%) and differentiated fish from the invertebrate and biofilm groups. The second

principal component axis, which accounted for 10.3% of the variation, clearly separated the salmon parr and invertebrate groups between the MDN treatment and control groups for sampling periods following adult salmon introductions (sampling times 1-4).

Although biofilm displayed more temporal variability in both the MDN treatment and control stream-channels, there were differences between the MDN treatment and control groups between sampling periods 1-3. There were no differences in fatty acid profiles within a trophic group between the MDN treatment and control stream-channels prior to the addition of adult salmon (sampling time 0). For salmon parr the MDN treatment groups separated into three groups: 1) the initial “baseline” period (before adult introduction; sampling time 0); 2) an “assimilation period” when the greatest MDN incorporation occurred (83 day period encompassing the adult introduction, spawning, and brief post adult removal periods; sampling times 1-3); and 3) final sampling period (46 days post adult removal; sampling time 4) (Figure 5.3).

A total of 56 fatty acids were detected and identified in the tissues analyzed, with 23 of those selected to demonstrate differences between trophic groups because the relative levels were greater than 0.1% of total fatty acids in at least one trophic group (Table 5.2). PERMANOVA showed that fatty acid compositions varied significantly between trophic groups prior to the introduction of adult Atlantic salmon ($F_{8, 16}=4.593$, $p=0.001$); however, pair-wise tests revealed no significant differences between MDN treatments and controls ($p\geq 0.237$). There did not appear to be any significant differences between the fatty acid profiles in biofilm from either stream-channels or mesocosms at the initial sampling period; however, this could not be tested statistically as replicate samples had to be pooled for analysis. Therefore, only initial biofilm samples for the

stream-channel are reported. Saturated fatty acids accounted for > 60% of the total fatty acids in biofilm but only 27-28% in the invertebrates and salmon, with 16:0 and 18:0 accounting for most of the total in each trophic group (Table 5.2). The monounsaturated fatty acids (monoenes) accounted for approximately 20 - 30% of the total fatty acid composition in both the invertebrates and biofilm and only 15% in the salmon, with the proportion of 16:1(n-7) being greatest in the invertebrate group. In contrast to the biofilm and invertebrates, the fatty acid composition of salmon parr was dominated by (n-3) fatty acids (nearly 50%) with 22:6(n-3) making up half of this group (Table 5.2), while the invertebrates contained ~24% (n-3) and the biofilm contained 5% (n-3). SIMPER analysis identified an average dissimilarity of only 4.48-8.45% between MDN treatment and control groups for stream-channel biofilm, invertebrates, or salmon parr at the time of initial sampling (Figure 5.4), whereas mesocosm biofilm had an average dissimilarity of 16.43%. Fatty acid compositions of Atlantic salmon eggs and milt more closely resembled those of the salmon parr than either the biofilm or invertebrates (Table 5.3, Figure 5.4).

Unlike the initial sampling period where differences in the fatty acid profiles occurred between stream biota and not between treatment and control groups, large variations between treatment and control groups were detected following adult introduction (sample times 1-4). PERMANOVA showed a significant difference between the fatty acid profiles of both stream-channel and mesocosm biofilm between MDN treatment and controls ($F_{4, 11}=1.623, p=0.034$; and $F_{4, 11}=1.747, p=0.026$; for stream-channels and mesocosms, respectively). Within stream-channels, the proportions of 18:0 and total saturated fatty acid groups decreased in both MDN treatment and

controls for biofilm compared to the initial proportions; however, the decrease was much greater in the MDN treatment stream-channels (Tables 5.2 and 5.4). Temporally, 16:0 declined significantly by the end of the spawning (assimilation) period followed by an increase post-spawning, with MDN treatments approximating levels closer to the levels in salmon eggs than controls (Figure 5.5). The increase in biofilm 16:1(n-7) was greater in the controls than MDN treatments; however, both treatments maintained levels greater than the salmon eggs (Figure 5.5). Total (n-6) fatty acids doubled during the assimilation period in the MDN treatments, largely due to an increase in 18:2(n-6), but not in the controls and then fell back to initial levels at the final sampling period. The total amount of (n-3) fatty acids increased twice as much in the MDN treatments as in the controls during the assimilation period. However, by the final sampling period, the total amount of (n-3) in the controls returned to initial levels, whereas (n-3) in the MDN treatments remained elevated (Table 5.4). The 22:5(n-3) and 22:6(n-3) fatty acids were not detected in the biofilm in the controls during the duration of the experiment, whereas they were detected in the MDN treatments post adult introduction. SIMPER analysis identified the highest average dissimilarity between the MDN treatment and control stream-channels during the adult-in period (time 2 and 3; 27.23 and 21.87 %, respectively) (Figure 5.4). The biofilm in the mesocosms also exhibited temporal trends in fatty acid composition. However, unlike in the stream-channels, the saturated and monounsaturated groups decreased more in the controls than in the treatments (Table 5.5). The percentage of (n-3) in the biofilm increased three times as much in the MDN treatments than in the controls during the assimilation period.

As in the biofilm, fatty acid compositions of invertebrates displayed qualitatively similar temporal changes in fatty acid composition relative to the initial sampling period. PERMANOVA showed a significant difference between fatty acid profiles of invertebrates in the MDN treatments and controls ($F_{4, 11}=2.412, p=0.001$) post adult introduction. In the assimilation and final sampling periods, the percentage of total saturated fatty acids remained unchanged from the initial sampling period; however, the level of 16:0 decreased and the level of 18:0 increased in the MDN treatments (Table 5.6). The percentage of total monounsaturated fatty acids also did not differ from the start of the study, but 16:1(n-7) decreased and 18:1(n-9) increased in the MDN treatments, both tracking the percentages in the salmon eggs (Figure 5.5). The percentage of total (n-6) fatty acids increased in the MDN treatments. The amount 22:5(n-3) in the controls remained relatively unchanged throughout the experiment, while in the treatments it decreased, tracking the profile of the eggs (Figure 5.5). The amount of 22:6(n-3) in both the controls and treatments remained relatively unchanged throughout the experiment (Figure 5.5). SIMPER analysis showed the average dissimilarity between the MDN treatment and controls increased as much as 12.21% from the initial sampling period and remained elevated for the duration of the study (Figure 5.4).

At all sampling periods after the introduction of adult Atlantic salmon to the stream-channels the percentage of total saturated fatty acids increased and the percentage of total monounsaturated fatty acids increased in MDN treatments (Table 5.7). PERMANOVA showed a significant difference between fatty acid profiles of salmon parr muscle tissue between MDN treatments and controls ($F_{4, 90}=9.520, p=0.001$) post

adult introduction. Increased sample size of salmon parr allowed for pair-wise comparisons (unique permutations ≥ 992) which showed MDN treatment fish sampled post adult introductions were significantly different from the initial sampling period and that samples from the assimilation period were significantly different from the final samples ($F_{19} \geq 4.732$, $p=0.001$). SIMPER analysis identified that the greatest average dissimilarity (14.69-15.68%) between the MDN treatment and control fatty acid profiles of fish muscle was during the adult-in period (times 2 and 3) (Figure 5.4). Average dissimilarity fell after the adults were removed, but remained high relative to pre-adult profiles. In the MDN treatments, salmon muscle tissue fatty acids generally tracked the values of the eggs (Figure 5.5). For example, fatty acids 16:1(n-7) and 18:1(n-9) each decreased and increased, respectively in the MDN treatments. The percentages of total (n-6) fatty acids increased and total (n-3) decreased in the MDN treatments, with both 20:5(n-3) and 22:6(n-3) decreasing to approach the levels in the salmon eggs (Figure 5.5).

The variation of resource use, measured as the median Euclidean distance to group centroid, was lowest in the invertebrates and greatest in the salmon parr (Figure 5.6). For the invertebrates and salmon parr, median Euclidean distance values did not differ between the initial and assimilation sampling periods; however, the variability around the median within the assimilation periods was smaller in the MDN treatments. Initial biofilms samples were unreplicated; therefore, Euclidean distances could not be calculated. Median Euclidean distance in stream-channel biofilm during the assimilation period was two times larger in the controls than in the MDN treatments (Figure 5.6a). In addition to possible trophic shifts in resource use, there appears to be some natural

temporal variation, most notably in the final samples of fish (Figure 5.6c) as both the MDN treatment and controls have a greater median Euclidean distance to the centroid and a greater variability surrounding the median.

5.5 Discussion

Exposure to MDN resources from spawning Atlantic salmon led to improved nutritional quality for all biota, as indicated by increased lipid stores and incorporation of fatty acids. Increased lipid reserves were observed in all trophic levels (biofilm, invertebrates, and salmon parr) with the greatest increases occurring in the invertebrates. Fatty acid profiles readily distinguished between biofilm, invertebrates and Atlantic salmon parr at all sampling periods, a result also found in other studies involving salmonids (e.g. Heintz et al. 2010, Volk and Kiffney 2012). The variability in fatty acid profiles was accredited to inherent differences between trophic groups combined with assimilation of marine-derived fatty acids in the MDN treatments. During the initial sampling period, overlap between individuals from the MDN treatments and controls indicated feeding on the same food source. Separation during the assimilation and final sampling periods indicated a shift wherein the two groups were using different resources. The establishment of this new resource, relatively enriched in marine lipids (e.g. salmon eggs), resulted in changes in the nutritional and chemical composition of the available foods as a result of uptake of MDN's. These results demonstrated the existence of MDN effects on consumer condition at multiple trophic levels. Biofilm total lipid increased with MDN inputs in both the stream-channels and mesocosms, although total lipid was greater in the stream-channels and a lag in total lipid coincided with salmon spawning. In

a related experiment, significant differences in biofilm production and productivity also followed a lag period post-spawning (Samways and Cunjak 2015). During spawning, redd construction can significantly reduce localized benthic community abundance through streambed disturbance (Minakawa and Gara 1999, Moore and Schindler 2008). Once adults were removed and streambed disturbance ceased, biofilm production and productivity increased rapidly due to increased MDN loading (Samways and Cunjak 2015). Increased biofilm lipid stores post-spawning were likely due to indirect effects from increased biofilm metabolic activity from dissolved marine-nutrient loading (i.e. nitrogen and phosphorous from excretory products), rather than direct consumption of marine lipids. The 12% increase in total lipid content in the MDN invertebrate group was the greatest increase of all biota. Salmon parr showed the lowest increase in total lipid of all the groups. The fish collected represented a combination of females, non-precocious males, and precocious males spanning 0+, 1+, and 2+ age cohorts; therefore, the variation observed in total lipid stores likely reflected the different biological requirements associated with the different life stages (Cunjak and Therrien 1998).

Docosahexaenoic acid and eicosapentaenoic acid are considered essential fatty acids for salmon and other marine or anadromous fish because *in vivo* production of these fatty acids is relatively limited (Sargent et al. 1989, 1995, 2002). Salmon need these essential fatty acids for optimal nervous system development and function, for production of eicosanoids which promote immune function, and for reproductive function (Tocher 2003). Salmon parr in the treatment stream-channels were clearly following the fatty profiles of the salmon eggs and invertebrates (e.g. greater 18:2(n-6), lower DHA and EPA). Although in the treatment stream-channels during the assimilation period, parr

had DHA proportions low relative to the total fatty acid mass, the mean concentration of DHA on a per total fish mass basis was higher (0.31 ± 0.03 g DHA/g total fish mass) in the treatments than the controls (0.22 ± 0.02 g DHA/g total fish mass). These approximations are based on parr having a higher fat (9.12% vs 6.45%) and DHA (25.67% vs 19.60%) content in the treatment vs control stream-channels respectively. By the end of the experiment, parr mass in the treatment stream-channels had increased making the difference in concentration of DHA even greater (0.25 ± 0.02 and 0.10 ± 0.01 g DHA/g total fish mass in treatment and control stream-channels, respectively). The accumulation and retention of DHA delivered by adult salmon helps to ameliorate constraints put on parr by the low levels of DHA found in aquatic ecosystems. Freshwater invertebrates essentially lack DHA (Hanson et al. 1985, Ghioni et al. 1996) due to a dietary shortage, making little of this fatty acid available to higher trophic levels. In contrast, EPA is found in abundance in freshwater invertebrates and is an important eicosanoid precursor (Ghioni et al. 1996). Unlike the invertebrates, salmon eggs are a resource rich in DHA.

Marine-derived subsidies from spawning Atlantic salmon had positive impacts on the growth of salmon parr. Previous studies have also shown the positive impacts of marine subsidies on juvenile salmonid growth (Bilby et al. 1998, Wipfli et al. 2003, Guyette et al. 2013). In stream-channels without any marine-derived resources, 1+ and 2+ salmon parr actually lost 7.49 and 2.77 % of their body mass, respectively, over the 116 day experiment, a result also observed in juvenile coho salmon in artificial stream reaches (Wipfli et al. 2003). Increases in body mass also coincided with increases in lipid stores.

One of our main objectives of this study was to demonstrate a change in the food web structure resulting from the establishment of a new, marine-based, basal resource. Fatty acid profiles between the MDN treatment and control stream-channels for each biotic group were relatively similar prior to the introduction of adult Atlantic salmon (average dissimilarity ranging between 4.5 to 8.5 %). Following adult salmon introductions, the fatty acid profiles of the biofilm, invertebrates and salmon parr in the MDN treatment stream-channels differed from the controls by as much as 27.3, 20.8, and 15.7 %, respectively. In general, the fatty acid profiles of the invertebrates and salmon parr from the MDN treatment stream-channels tracked the fatty acid profile of the salmon eggs. The temporal composition of many of these fatty acids demonstrates that invertebrates and salmon parr in the MDN treatment stream-channels were consuming marine-derived egg material. Studies on Pacific salmon have also demonstrated that salmon parr and invertebrates feed directly on salmon eggs (Bilby et al. 1998, Gresh et al. 2000). Marine-subsidies and in particular dissolved MDNs, increase biofilm biomass; however, the response to MDN inputs of autotrophic and heterotrophic groups within the biofilm community differs (Samways et al. 2015).

The community-wide metric of feeding diversity (as measured by the Euclidean distance) also indicates a shift to a new basal resource in the MDN treatment stream-channels. Given that the controls did not have adult salmon, the only diet available to the salmon parr had to be a non-MDN source, whereas salmon parr in the MDN treatments had two available food sources (invertebrates and salmon eggs). Overlap in fatty acid profiles between individuals from the MDN treatments with individuals from the controls (as is the case in the initial sampling period) and a similar mean Euclidean distance to the

centroid, indicated they were feeding on the same food source. As there was no overlap in fatty acid profiles between the MDN treatments and controls during the assimilation period, salmon parr from each of the groups were feeding on different resources. The low mean Euclidean distance (low variability) in the MDN treatments during the assimilation period, coupled with similar fatty acid profiles of the salmon parr and salmon eggs during the assimilation period, indicates that parr appear to be feeding on this new MDN-source, perhaps even targeting the eggs (Thomaz et al. 1997, Cunjak and Therrien 1998). During the final sampling period, salmon parr in the MDN treatments had 1.40 times more variability in their feeding diversity than parr in the controls indicating that the salmon parr diet was shifting back from predominantly MDN-influenced to one that included more invertebrates.

Although representative of natural rivers, it is important to recognize the potential limitations of applying the results from experimental stream-channels to a natural setting. The adult Atlantic salmon used in this study were fed a marine-based diet typical of those used in aquaculture with a lipid content of 20%. Some plant-based and poultry products were also present in the feed, which could influence the fatty acid profiles of consumers of the marine-subsidies. Effects of MDNs on biofilm abundance (measured as chlorophyll a) showed increases up to $0.4 \mu\text{g cm}^{-2}$ in natural rivers with wild Atlantic salmon spawning (Samways et al. 2015), compared to increases up to $2.4 \mu\text{g cm}^{-2}$ in stream-channels with hatchery-reared spawning Atlantic salmon (Samways and Cunjak 2015). Therefore, caution should be taken applying these results, as the variation in fatty acid composition in biota in natural systems is unknown. However, this study made use of naturally occurring stream flora and fauna, including wild salmon parr and marine-

nutrient additions from naturally spawning salmon, rather than bags of nutrients (Wipfli et al. 2010); therefore, it serves as a model system for assessing the effects of marine subsidies from Atlantic salmon.

Increased lipid and essential fatty acid stores are important for alleviating the energy demands for growth and storage in salmon parr. Heintz et al. (2004) found that when exposed to marine-subsidies from carcasses, juvenile coho salmon increased their mass-specific energy stores by 25-fold and suggested that this would be particularly beneficial for overwintering success (Berg and Bremset 1998). Bull et al. (1996) demonstrated that the appetite of Atlantic salmon parr is regulated according to long-term energy requirements rather than short-term needs, with winter being the period imposing the greatest energy demands (Cunjak et al. 1998, Næsje et al. 2006). With Atlantic salmon spawning occurring in late fall, the availability of lipid-rich eggs coincides with the increased physiological demands associated with winter (i.e. cold acclimation, Cunjak 1988). If early winter is a physiological stressful period that depletes lipid stores (Cunjak 1988), then consuming eggs over other available resources should be an adaptive trait to acquire adequate energy reserves to ensure survival through the winter (Cunjak et al. 1998).

These marine-derived subsidies (nutrients and lipids) benefit multiple trophic levels of freshwater organisms as well as a cross-ecosystem spatial subsidy (Fox et al. 2014). The bottom-up effects of MDNs (from nitrogen and phosphorous loading) are complimented by the direct consumption of marine-based tissues, increasing productivity and therefore contributing to freshwater ecosystems diversity and health.

5.6 Acknowledgements

The authors greatly appreciate the help and support of Trevor Goff, Danielle MacDonald, Stephanie Ratelle, John Whitelaw and staff at the Mactaquac Biodiversity Facility. We also thank Emily Corey, Paula Thoms, Sherr Vue, and Aaron Fraser for their help in the field and in the lab. Financial support was provided by grants from the National Sciences and Engineering Research Council of Canada and the Canada Research Chairs Program awarded to R.A. Cunjak.

5.7 Literature Cited

- Bell, J. G., B. M. Farndale, J. R. Dick, and J. R. Sargent. 1996. Modification of membrane fatty acid composition, eicosanoid production, and phospholipase A activity in Atlantic salmon (*Salmo salar*) gill and kidney by dietary lipid. *Lipids* 31:1163-1171.
- Bell, J. G., D. R. Tocher, B. M. Farndale, D. I. Cox, R. W. McKinney, and J. R. Sargent. 1997. The effect of dietary lipid on polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*) undergoing parr-smolt transformation. *Lipids* 32:515-55.
- Berg, O. K., and G. Bremset. 1998. Seasonal changes in the body composition of young riverine Atlantic salmon and brown trout. *Journal of Fish Biology* 52:1272-1288.
- 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.
- Bilby R. E., B. R. Fransen, P. A. Bisson, and J. K. Walter. 1998. Response of juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead (*Oncorhynchus mykiss*) to the addition of salmon carcasses to two streams in southwestern Washington, U.S.A. *Canadian Journal of Fisheries and Aquatic Sciences* 55:1909-1918.
- Brodtkorb, T., G. Rosenlund, and Ø. Lie. 1997. Effects of dietary levels of 20:5n-3 and 22:6n-3 on tissue lipid composition in juvenile Atlantic salmon, *Salmo salar*, with emphasis on brain and eye. *Aquaculture Nutrition* 3:175-187.
- Budge, S. M., S. J. Iverson, and H. N. Koopman. 2006. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. *Marine Mammal Science* 22:759-801.
- Bull, C. D., N. B. Metcalfe, and M. Mangel. 1996. Seasonal matching of foraging to anticipated energy requirements in anorexic juvenile salmon. *Proceedings of the Royal Society of London B* 263:13-18.

- Chaloner, D. T., G. A. Lamberti, R. W. Merritt, N. L. Mitchell, P. H. Ostrom, and M. S. Wipfli. 2004. Variation in responses to spawning Pacific salmon among three south-eastern Alaska streams. *Freshwater Biology* 49:587-599.
- Chaloner D. T., M. S. Wipfli, and J. P. Caouette. 2002. Mass loss and macroinvertebrate colonisation of Pacific salmon carcasses in south-eastern Alaskan streams. *Freshwater Biology* 47:263-273.
- Chezik, K., N. Lester, and P. A. Venturelli, 2014. Fish growth and degree-days I: selecting a base temperature for a within-population study. *Canadian Journal of Fisheries and Aquatic Science* 71:47-55.
- Clarke, K. R. 1993. Nonparametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* 18:117-143.
- Clarke, K. R., P. J. Somerfield, and M. G. Chapman. 2006. On resemblance measures for ecological studies, including taxonomic dissimilarities and a zero-adjusted Bray–Curtis coefficient for denuded assemblages. *Journal of Experimental Marine Biology and Ecology* 330:55-80.
- Cunjak, R. A. 1988. Physiological consequences of overwintering in streams: the cost of acclimatization. *Canadian Journal of Fisheries and Aquatic Sciences* 45:443-452.
- Cunjak, R. A., and J. Therrien. 1998. Inter-stage survival of wild juvenile Atlantic salmon, *Salmo salar* L. *Fisheries Management and Ecology* 5:209-223.
- Cunjak, R. A., T. D. Prowse, and D. L. Parrish. 1998. Atlantic salmon (*Salmo salar*) in winter: “the season of parr discontent”? *Canadian Journal of Fisheries and Aquatic Sciences* 55(S1):161-180.
- Doucett, R. R., R. K. Booth, and R. S. McKinley. 1999. Effects of the spawning migration on the nutritional status of anadromous Atlantic salmon (*Salmo salar*): insights from stable-isotope analysis. *Canadian Journal of Fisheries and Aquatic Sciences* 56:2172-2180.
- Durbin, A. G., S. W. Nixon, and C. A. Oviatt. 1979. Effects of the spawning migration of the alewife, *Alosa pseudoharengus*, on freshwater ecosystems. *Ecology* 60:8-17.
- Folch, J., M. Lees and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* 226:497-509.
- Fox, C. H., R. El-Sabaawi, P. C. Paquet, T. E. Reimchen. 2014. Pacific herring *Clupea pallasii* and wrack macrophytes subsidize semi-terrestrial detritivores. *Marine Ecology Progress Series* 495:49-65.
- Fraser, A. 1989. Triacylglycerol content as a condition index from fish, bivalve, and crustacean larvae. *Canadian Journal of Fisheries and Aquatic Sciences* 46:1868-1873.
- Garman G. C., and S. A. Macko. 1998. Contribution of marine-derived organic matter to an Atlantic coast, freshwater, tidal stream by anadromous clupeid fishes. *Journal of the North American Benthological Society* 17:277-285.
- Ghioni, C., J. G. Bell, and J. R. Sargent. 1996. Polyunsaturated fatty acids in neutral lipids and phospholipids of some freshwater insects. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 114:161-170.

- Guyette, M. Q., C. S. Loftin, and J. Zydlewski. 2013. Carcass analog addition enhances juvenile Atlantic salmon (*Salmo salar*) growth and condition. *Canadian Journal of Fisheries and Aquatic Sciences* 70:860-870.
- Guyette, M. Q., C. S. Loftin, J. Zydlewski, and R. Cunjak. 2014. Carcass analogues provide marine subsidies for macroinvertebrate and juvenile Atlantic salmon in temperate oligotrophic streams. *Freshwater Biology* 59:392-406.
- Gresh, T., J. Lichatowich, and P. Schoonmaker. 2000. An estimation of historic and current levels of salmon production in the northeast Pacific ecosystem: evidence of a nutrient deficit in the freshwater systems of the Pacific northwest. *Fisheries* 25:15-21.
- Hanson, B. J., K. W. Cummins, A. S. Cargill, and R. R. Lowry. 1985. Lipid content, fatty acid composition and the effect of diet on fats of aquatic insects. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 80:257-276.
- Heintz, R. A., B. D. Nelson, J. Hudson, M. Larsen, L. Holland, and M. Wipfli. 2004. Marine subsidies in freshwater: effects of salmon carcasses on lipid class and fatty acid composition of juvenile coho salmon. *Transactions of the American Fisheries Society* 133:559-567.
- Heintz, R. A., M. S. Wipfli, and J. P. Hudson. 2010. Identification of marine-derived lipids in juvenile coho salmon and aquatic insects through fatty acid analysis. *Transactions of the American Fisheries Society* 139:840-854.
- Henderson, R. J., and D. R. Tocher. 1987. The lipid composition and biochemistry of freshwater fish. *Progress in Lipid Research* 26:281-347.
- Jardine, T. D., J.-M. Roussel, S. C. Mitchell, and R. A. Cunjak. 2009. Pathways of marine nutrient and organic matter entry from anadromous fishes into Atlantic coast streams. Pages 427-445 in A. J. Haro, K. L. Smith, R. A. Rulifson, C. M. Moffitt, R. J. Klauda, M. J. Dadswell, R. A. Cunjak, J. E. Cooper, K. L. Beal, and T. S. Avery, editors. *Challenges for diadromous fishes in a dynamic global environment*. Symposium 69. American Fisheries Society, Bethesda, Maryland, USA.
- 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.
- Johnston, N. T., C. J. Perrin, P. A. Slaney, and B. R. Ward. 1990. Increased juvenile salmonid growth by whole-river fertilization. *Canadian Journal of Fisheries and Aquatic Sciences* 47:862-872.
- Jones, R. A., L. Anderson, A. J. F. Gibson, and T. Goff. 2010. Assessments of Atlantic Salmon Stocks in South Western New Brunswick (Outer Portion of SFA 23): An Update to 2008. Canadian Aquatic Fisheries Science Advisory Council, Research Document, 2010/118. Ottawa, Canada.
- Jonsson, B., and N. Jonsson. 2003. Migratory Atlantic salmon as vectors for the transfer of energy and nutrients between freshwater and marine environments. *Freshwater Biology* 48:21-27.

- Kainz, M. J., and A. T. Fisk. 2009. Integrating lipids and contaminants in aquatic ecology and ecotoxicology. Pages 93-113 in M. T. Arts, M.T. Brett, and M.J Kainz, editors. *Lipids in Aquatic Ecosystems*. Springer. New York, USA.
- Layman, C. A., D. A. Arrington, C. G. Montaña, and D. M. Post. 2007. Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology* 88:42-48.
- Lessard, J. L., and R. W. Merritt. 2006. Influence of marine-derived nutrients from spawning salmon on aquatic insect communities in southeast Alaskan streams. *Oikos* 113:334-343.
- Lessard J. L., R. W. Merritt, and M. B. Berg. 2009. Investigating the effect of marine-derived nutrients from spawning salmon on macroinvertebrate secondary production in southeast Alaskan streams. *Journal of the North American Benthological Society* 28:683-693.
- 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.
- McArdle, B. H. and M. J. Anderson. 2001. Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology* 82:290-297.
- Minakawa N., and R. I. Gara. 1999. Ecological effects of a chum salmon (*Oncorhynchus keta*) spawning run in a small stream of the Pacific Northwest. *Journal of Freshwater Ecology* 14:327-335.
- Moore J. W., and D. E. Schindler. 2008. Biotic disturbance and benthic community dynamics in salmon-bearing streams. *Journal of Animal Ecology* 77:275-284.
- Morrison, W. R. and L. M. Smith. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *Journal of Lipid Research* 5:600-608.
- Müller-Navarra, D. C., M. T. Brett, A. M. Liston, and C. R. Goldman. 2000. A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. *Nature* 403:74-77.
- Næsje, T. F., E. B. Thorstad, T. Forseth, M. Aursand, R. Saksgård, and A. G. Finstad. 2006. Lipid class content as an indicator of critical periods for survival in juvenile Atlantic salmon (*Salmo salar*). *Ecology of Freshwater Fish* 15:572-577.
- 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* 5:399-417.
- Nanton, D. A. and J. D. Castell. 1998. The effects of dietary fatty acids on the fatty acid composition of the harpacticoid copepod, *Tisbe* sp., for use as a live food for marine fish larvae. *Aquaculture* 163:251-261.
- Nislow, K., J. Armstrong, and S. McKelvey. 2004. Phosphorus flux due to Atlantic salmon (*Salmo salar*) in an oligotrophic upland stream: effects of management and demography. *Canadian Journal of Fisheries and Aquatic Sciences* 61:2401-2410.
- Nislow, K. H., and B. E. Kynard. 2009. The role of anadromous sea lamprey in nutrient and material transport between marine and freshwater environments. Pages 485-494 in A. J. Haro, K. L. Smith, R. A. Rulifson, C. M. Moffitt, R. J. Klauda, M. J.

- Dadswell, R. A. Cunjak, J. E. Cooper, K. L. Beal, and T. S. Avery, editors. Challenges for diadromous fishes in a dynamic global environment. Symposium 69. American Fisheries Society, Bethesda, Maryland, USA.
- Olden, J. D., and N. L. Poff. 2003. Redundancy and the choice of hydrologic indices for characterizing streamflow regimes. *River Research and Applications* 19:101-121.
- Ostfeld, R. S., and F. Keesing. 2000. Pulsed resources and community dynamics of consumers in terrestrial ecosystems. *Trends in Ecology and Evolution* 15:232-237.
- Pestana J. L. T., A. C. Alexander, J. M. Culp, D. J. Baird, A. J. Cessna, and A. M. Soares. 2009. Structural and functional responses of benthic invertebrates to imidacloprid in outdoor stream mesocosms. *Environmental Pollution* 157:2328-2334.
- Phillips, D. L., and J. W. Gregg. 2001. Uncertainty in source partitioning using stable isotopes. *Oecologia* 127:171-179.
- Pickova, J., A. Kiessling, A. Pettersson, and P. C. Dutta. 1999. Fatty acid and carotenoid composition of eggs from two anadromous Atlantic salmon stocks of cultured and wild origin. *Fish Physiology and Biochemistry* 21:147-156.
- 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.
- Reynoldson, T. B., C. Logan, T. Pascoe, S. P. Thompson. 2002. CABIN (Canadian Aquatic Biomonitoring Network) Invertebrate Biomonitoring Field and Laboratory Manual. National Water Research Institute, Environment Canada, Burlington, Ontario.
- 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.
- Samways, K. M., and R. A. Cunjak. 2015. Increases in benthic community production and metabolism in response to marine-derived nutrients from spawning Atlantic salmon (*Salmo salar*). *Freshwater Biology* 60:1647-1658.
- Samways K. M., Z. J. Quiñones-Rivera, P. R. Leavitt, and R. A. Cunjak. 2015. Spatio-temporal responses of algal, fungal and bacterial biofilm communities in Atlantic rivers receiving marine-derived nutrient inputs. *Freshwater Science* 34:881-896.
- Sargent, J. R., J. G. Bell, M. V. Bell, R. J. Henderson, and D. R. Tocher. 1995. Requirement criteria for essential fatty acids. *Journal of Applied Ichthyology* 11:183-198.
- Sargent, J. R., R. J. Henderson, and D. R. Tocher. 1989. The lipids. Pages 153-218 in J. E. Halper, editor. *Fish Nutrition*, 2nd ed. Academic Press. New York, USA.
- Sargent, J. R., D. R. Tocher, and J. G. Bell. 2002. The lipids. Pages 181-257 in J. E. Halper, editor. *Fish Nutrition*, 3rd ed. Academic Press. San Diego, USA.
- Thomaz, D., E. Beall, and T. Burke. 1997. Alternative reproductive tactics in Atlantic salmon: factors affecting mature parr success. *Proceedings: Biological Sciences* 264:219-226.
- Tocher, D. R. 2003. Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews in Fisheries Science* 11:107-184.

- Volk, C., and P. Kiffney. 2012. Comparison of fatty acids and elemental nutrients in periphyton, invertebrates, and cutthroat trout (*Oncorhynchus clarki*) in conifer and alder streams of western Washington state. *Aquatic Ecology* 46:85-89.
- Walters, A. W., R. T. Barnes, and D. M. Post. 2009. Anadromous alewives (*Alosa pseudoharengus*) contribute marine-derived nutrients to coastal stream food webs. *Canadian Journal of Fisheries and Aquatic Sciences* 66:439-448.
- Wipfli, M. S., J. Hudson, and J. Caouette. 1998. Influence of salmon carcasses on stream productivity: response of biofilm and benthic macroinvertebrates in southeastern Alaska, U.S.A. *Canadian Journal of Fisheries and Aquatic Sciences* 55:1503-1511.
- Wipfli, M. S., J. P. Hudson, J. P. Caouette, N. L. Mitchell, J. L. Lessard, R. A. Heintz, and D. T. Chaloner. 2010. Salmon carcasses increase stream productivity more than inorganic fertilizer pellets: a test on multiple trophic levels in streamside experimental channels. *Transactions of the American Fisheries Society* 139:824-839.
- Wipfli, M. S., J. P. Hudson, D. T. Chaloner, and J. P. Caouette. 1999. The influence of salmon spawner densities on stream productivity in Southeast Alaska. *Canadian Journal of Fisheries and Aquatic Sciences* 56:1600-1611.
- Wipfli, M. S., J. P. Hudson, D. T. Chaloner, and J. P. Caouette. 2003. Marine subsidies in freshwater ecosystems: salmon carcasses increase the growth rates of stream-resident salmonids. *Transactions of the American Fisheries Society* 132:371-381.

Table 5.1. Growth (mean $\pm$ SD) of wild Atlantic salmon parr in stream-channels receiving marine-derived nutrients (MDNs) from spawning Atlantic salmon and control treatments over the 116 day experimental period.

	Age 0+		Age 1+		Age 2+	
	MDNs	Control	MDNs	Control	MDNs	Control
Initial length (cm fish ⁻¹)	6.5 $\pm$ 0.5	7.5 $\pm$ 0.8	10.2 $\pm$ 0.2	10.0 $\pm$ 0.4	11.6 $\pm$ 0.3	11.1 $\pm$ 0.1
(Range)	(6.1 - 6.7)	(6.2 - 8.2)	(10.0 - 10.3)	(9.6 - 10.3)	(11.4 - 11.8)	(11.0 - 11.2)
Final length (cm fish ⁻¹)	6.8 $\pm$ 0.6	7.7 $\pm$ 0.8	10.4 $\pm$ 0.1	10.0 $\pm$ 0.3	12.1 $\pm$ 0.4	11.3 $\pm$ 0.1
(Range)	(6.1 - 7.0)	(6.5 - 8.5)	(10.3 - 10.5)	(9.7 - 10.5)	(11.8 - 12.3)	(11.2 - 11.3)
Length change (cm fish ⁻¹)	0.25 $\pm$ 0.17	0.23 $\pm$ 0.15	0.20 $\pm$ 0.10	0.02 $\pm$ 0.05	0.45 $\pm$ 0.07	0.15 $\pm$ 0.07
Initial weight (g fish ⁻¹)	3.0 $\pm$ 0.5	4.3 $\pm$ 1.3	10.4 $\pm$ 0.9	10.6 $\pm$ 0.3	15.2 $\pm$ 2.4	12.0 $\pm$ 0.9
(Range)	(2.9 - 3.7)	(3.0 - 5.5)	(9.4 - 10.6)	(10.4 - 11.0)	(13.5 - 16.9)	(11.4 - 12.6)
Final weight (g fish ⁻¹)	3.5 $\pm$ 0.8	4.5 $\pm$ 1.2	10.7 $\pm$ 0.1	9.8 $\pm$ 0.7	20.9 $\pm$ 2.7	11.7 $\pm$ 1.0
(Range)	(2.9 - 4.7)	(3.0 - 5.6)	(10.7 - 11.0)	(9.0 - 10.6)	(18.9 - 22.8)	(10.9 - 12.4)
Weight change (g fish ⁻¹)	0.46 $\pm$ 0.40	0.26 $\pm$ 0.34	0.70 $\pm$ 0.60	-0.79 $\pm$ 0.49	5.68 $\pm$ 0.33	-0.33 $\pm$ 0.19

Table 5.2. Fatty acid composition of initial stream-channel biofilm, invertebrate, and Atlantic salmon parr tissue samples. Values (mean $\pm$ SD) are percentages of total fatty acids (nd = not detected; na = ratio cannot be calculated as the denominator is zero; asterisk = replicate samples were pooled).

Fatty Acid	Biofilm*		Invertebrates		Atlantic Salmon Parr	
	Control	Treatment	Control	Treatment	Control	Treatment
12:0	nd	nd	0.23 $\pm$ 0.02	0.12 $\pm$ 0.01	0.04 $\pm$ 0.05	0.00 $\pm$ 0.00
14:0	3.61	3.80	2.04 $\pm$ 0.20	2.08 $\pm$ 0.16	1.11 $\pm$ 0.75	0.55 $\pm$ 0.22
15:0	1.38	1.24	0.24 $\pm$ 0.02	0.23 $\pm$ 0.01	0.12 $\pm$ 0.05	0.11 $\pm$ 0.02
16:0	35.48	32.75	18.92 $\pm$ 0.46	19.60 $\pm$ 0.76	19.28 $\pm$ 1.15	19.09 $\pm$ 0.96
17:0	1.05	0.89	0.51 $\pm$ 0.02	0.47 $\pm$ 0.02	0.44 $\pm$ 0.14	0.59 $\pm$ 0.11
18:0	22.54	19.36	4.83 $\pm$ 0.08	4.70 $\pm$ 0.32	6.19 $\pm$ 0.37	6.40 $\pm$ 0.26
22:0	0.85	0.67	0.28 $\pm$ 0.05	0.28 $\pm$ 0.04	0.11 $\pm$ 0.03	0.14 $\pm$ 0.02
24:0	nd	nd	nd	nd	0.02 $\pm$ 0.04	0.00 $\pm$ 0.00
Σ Saturates[†]	65.70	59.32	27.98 $\pm$ 0.56	28.30 $\pm$ 0.47	27.8 $\pm$ 1.51	27.32 $\pm$ 1.11
16:1(n-7)	4.20	4.77	14.57 $\pm$ 0.51	14.05 $\pm$ 1.23	2.79 $\pm$ 0.59	2.79 $\pm$ 0.36
18:1(n-9)	8.98	9.56	9.85 $\pm$ 1.22	8.85 $\pm$ 0.43	6.28 $\pm$ 0.86	6.41 $\pm$ 0.65
18:1(n-7)	1.76	1.89	8.49 $\pm$ 0.71	8.36 $\pm$ 0.29	4.22 $\pm$ 0.94	4.92 $\pm$ 0.78
22:1(n-9)	6.55	6.51	0.03 $\pm$ 0.01	nd	0.11 $\pm$ 0.16	0.01 $\pm$ 0.01
Σ Monoenes[‡]	21.49	23.66	34.24 $\pm$ 0.52	32.39 $\pm$ 0.05	15.17 $\pm$ 2.96	14.66 $\pm$ 0.95
16:2(n-4)	nd	nd	1.37 $\pm$ 0.09	1.38 $\pm$ 0.21	0.47 $\pm$ 0.17	0.47 $\pm$ 0.14
16:3(n-4)	nd	nd	1.14 $\pm$ 0.01	1.46 $\pm$ 0.55	0.25 $\pm$ 0.14	0.18 $\pm$ 0.11
16:4(n-1)	nd	nd	0.31 $\pm$ 0.05	0.28 $\pm$ 0.06	0.17 $\pm$ 0.18	0.05 $\pm$ 0.02
18:2(n-6)	2.75	2.65	3.87 $\pm$ 0.30	3.84 $\pm$ 0.99	2.65 $\pm$ 0.39	2.78 $\pm$ 0.28
20:4(n-6)	nd	0.44	1.61 $\pm$ 0.32	1.53 $\pm$ 0.30	1.81 $\pm$ 0.36	1.89 $\pm$ 0.37
Σ (n-6)[§]	2.75	3.09	6.25 $\pm$ 0.67	6.17 $\pm$ 1.39	5.81 $\pm$ 0.41	5.96 $\pm$ 0.45
16:4(n-3)	0.72	0.58	0.27 $\pm$ 0.26	0.36 $\pm$ 0.26	0.25 $\pm$ 0.14	0.32 $\pm$ 0.15
18:3(n-3)	2.53	2.26	7.84 $\pm$ 0.64	8.84 $\pm$ 0.20	5.59 $\pm$ 2.66	7.30 $\pm$ 0.96
18:4(n-3)	nd	0.99	1.02 $\pm$ 0.03	1.12 $\pm$ 0.02	0.80 $\pm$ 0.27	0.92 $\pm$ 0.17
20:5(n-3)	1.18	1.77	13.56 $\pm$ 0.09	13.93 $\pm$ 0.68	12.18 $\pm$ 0.87	11.45 $\pm$ 0.87
22:5(n-3)	nd	nd	nd	nd	4.09 $\pm$ 0.73	4.11 $\pm$ 0.47
22:6(n-3)	nd	nd	0.48 $\pm$ 0.17	0.38 $\pm$ 0.08	22.76 $\pm$ 1.34	22.36 $\pm$ 1.83
Σ (n-3)[¶]	4.43	5.60	23.5 $\pm$ 0.05	24.94 $\pm$ 0.57	46.86 $\pm$ 4.01	47.93 $\pm$ 1.68
(n-3)/(n-6)	1.61	1.81	3.77 $\pm$ 0.41	4.16 $\pm$ 1.03	8.09 $\pm$ 0.91	8.08 $\pm$ 0.67
20:5(n-3)/22:6(n-3)	na	na	29.71 $\pm$ 10.35	37.57 $\pm$ 9.79	0.53 $\pm$ 0.02	0.51 $\pm$ 0.05
20:4(n-6)/22:6(n-3)	na	na	3.41 $\pm$ 0.55	4.03 $\pm$ 0.08	0.07 $\pm$ 0.01	0.08 $\pm$ 0.01

(from previous table)

† Totals include saturated fatty acids in table plus: 8:0, 10:0, 11:0, 13:0, 7Me16:0, 20:0, 21:0, 23:0

‡ Totals include monounsaturated fatty acids in table plus: 14:1(n-7), 14:1(n-5), 15:1(n-5), 16:1(n-5), 17:1(n-7), 20:1(n-11), 20:1(n-9), 20:1(n-7), 22:1(n-11), 24:1(n-9)

§ Totals include n-6 fatty acids in table plus: 16:2(n-6), 18:3(n-6), 20:2(n-6), 20:3(n-6), 22:2(n-6), 22:4(n-6), 22:5(n-6)

¶ Totals include n-3 fatty acids in table plus: 20:3(n-3), 20:4(n-3), 21:5(n-3)

Table 5.3. Fatty acid composition of adult Atlantic salmon gametes. Values are percentages of total fatty acids. See Table 1 for explanation of symbols.

Fatty Acid	Adult Atlantic Salmon	
	Eggs	Milt
12:0	nd	nd
14:0	1.37	0.84
15:0	0.18	0.16
16:0	13.80	22.76
17:0	0.23	0.21
18:0	7.47	5.33
22:0	nd	nd
24:0	nd	nd
Σ Saturates[†]	23.54	29.48
16:1(n-7)	4.66	0.97
18:1(n-9)	20.15	7.79
18:1(n-7)	3.55	5.17
22:1(n-9)	0.10	0.07
Σ Monoenes[‡]	29.75	14.45
16:2(n-4)	0.24	nd
16:3(n-4)	0.09	nd
16:4(n-1)	0.07	0.28
18:2(n-6)	8.89	4.17
20:4(n-6)	1.82	4.39
Σ (n-6)[§]	13.17	9.68
16:4(n-3)	nd	nd
18:3(n-3)	0.76	0.16
18:4(n-3)	0.63	0.13
20:5(n-3)	7.88	18.70
22:5(n-3)	4.87	3.94
22:6(n-3)	15.22	21.62
Σ (n-3)[¶]	30.60	44.84
(n-3)/(n-6)	2.32	4.63
20:5(n-3)/ 22:6(n-3)	0.52	0.87
20:4(n-6)/22:6(n-3)	0.12	0.20

Table 5.4. Fatty acid composition of stream-channel biofilm tissues. Values (mean $\pm$ SD) are percentages of total fatty acids. The “assimilation period” is defined as the period when greatest MDN incorporation occurred (83 day period encompassing the adult introduction, spawning, and brief post adult removal periods; sampling times 1-3). See Table 1 for explanation of symbols.

Fatty Acid	Assimilation Period		Final	
	Control	Treatment	Control	Treatment
12:0	nd	nd	nd	nd
14:0	1.11 $\pm$ 1.41	1.84 $\pm$ 1.31	1.67 $\pm$ 0.77	2.44 $\pm$ 0.16
15:0	0.48 $\pm$ 0.12	0.36 $\pm$ 0.18	0.47 $\pm$ 0.02	0.45 $\pm$ 0.02
16:0	20.37 $\pm$ 2.24	14.33 $\pm$ 3.31	22.35 $\pm$ 1.01	18.1 $\pm$ 0.38
17:0	0.31 $\pm$ 0.14	0.24 $\pm$ 0.04	0.19 $\pm$ 0.07	0.11 $\pm$ 0.01
18:0	12.89 $\pm$ 9.13	4.96 $\pm$ 2.13	3.42 $\pm$ 3.02	1.48 $\pm$ 0.46
22:0	0.27 $\pm$ 0.16	0.16 $\pm$ 0.05	0.22 $\pm$ 0.09	0.09 $\pm$ 0.02
24:0	0.48 $\pm$ 0.10	0.42 $\pm$ 0.36	1.25 $\pm$ 1.23	0.33 $\pm$ 0.06
Σ Saturates[†]	36.45 $\pm$ 7.70	22.76 $\pm$ 3.61	30.05 $\pm$ 2.85	23.41 $\pm$ 1.12
16:1(n-7)	18.45 $\pm$ 5.39	11.92 $\pm$ 4.21	21.89 $\pm$ 3.11	25.77 $\pm$ 0.52
18:1(n-9)	7.66 $\pm$ 1.25	4.05 $\pm$ 0.27	12.33 $\pm$ 5.95	10.11 $\pm$ 2.24
18:1(n-7)	2.57 $\pm$ 0.36	3.44 $\pm$ 0.58	3.41 $\pm$ 1.04	1.93 $\pm$ 0.04
22:1(n-9)	1.03 $\pm$ 0.33	0.57 $\pm$ 0.21	1.05 $\pm$ 1.13	0.30 $\pm$ 0.13
Σ Monoenes[‡]	30.33 $\pm$ 6.66	21.14 $\pm$ 3.91	39.63 $\pm$ 6.95	39.00 $\pm$ 1.61
16:2(n-4)	1.60 $\pm$ 0.71	1.81 $\pm$ 1.04	1.68 $\pm$ 1.36	1.69 $\pm$ 0.52
16:3(n-4)	2.28 $\pm$ 1.43	2.32 $\pm$ 1.98	1.45 $\pm$ 1.68	1.07 $\pm$ 0.58
16:4(n-1)	0.75 $\pm$ 0.46	3.89 $\pm$ 4.19	0.17 $\pm$ 0.03	0.31 $\pm$ 0.11
18:2(n-6)	1.93 $\pm$ 0.41	4.90 $\pm$ 0.69	2.25 $\pm$ 0.24	2.04 $\pm$ 0.54
20:4(n-6)	1.09 $\pm$ 0.25	1.52 $\pm$ 0.41	0.82 $\pm$ 0.05	1.04 $\pm$ 0.24
Σ (n-6)[§]	3.45 $\pm$ 0.98	7.11 $\pm$ 1.02	3.63 $\pm$ 0.18	3.83 $\pm$ 0.73
16:4(n-3)	1.01 $\pm$ 1.25	2.07 $\pm$ 0.12	0.4 $\pm$ 0.35	2.21 $\pm$ 0.49
18:3(n-3)	2.79 $\pm$ 1.91	4.59 $\pm$ 1.81	2.57 $\pm$ 1.02	5.33 $\pm$ 0.72
18:4(n-3)	0.77 $\pm$ 0.54	1.25 $\pm$ 0.76	0.24 $\pm$ 0.12	0.22 $\pm$ 0.10
20:5(n-3)	9.65 $\pm$ 3.43	15.36 $\pm$ 7.44	2.47 $\pm$ 0.19	8.38 $\pm$ 0.64
22:5(n-3)	nd	0.92 $\pm$ 0.51	nd	0.44 $\pm$ 0.09
22:6(n-3)	nd	3.39 $\pm$ 2.25	nd	0.16 $\pm$ 0.02
Σ (n-3)[¶]	14.35 $\pm$ 2.29	27.87 $\pm$ 5.02	5.79 $\pm$ 0.69	16.91 $\pm$ 1.61
(n-3)/(n-6)	4.39 $\pm$ 1.54	3.97 $\pm$ 0.88	1.59 $\pm$ 0.11	4.44 $\pm$ 0.43
20:5(n-3)/ 22:6(n-3)	na	6.65 $\pm$ 4.79	na	52.54 $\pm$ 11.32
20:4(n-6)/22:6(n-3)	na	0.58 $\pm$ 0.31	na	6.63 $\pm$ 2.42

Table 5.5. Fatty acid composition of mesocosm biofilm tissues. Values (mean $\pm$ SD) are percentages of total fatty acids. See Table 1 for explanation of symbols.

Fatty Acid	Assimilation Period		Final	
	Control	Treatment	Control	Treatment
12:0	nd	nd	nd	nd
14:0	0.96 $\pm$ 0.89	1.51 $\pm$ 1.47	2.15 $\pm$ 1.17	3.24 $\pm$ 0.67
15:0	0.34 $\pm$ 0.12	0.32 $\pm$ 0.11	0.71 $\pm$ 0.21	0.55 $\pm$ 0.11
16:0	20.53 $\pm$ 2.31	13.29 $\pm$ 2.60	28.39 $\pm$ 6.83	20.21 $\pm$ 2.43
17:0	0.25 $\pm$ 0.09	0.17 $\pm$ 0.04	0.43 $\pm$ 0.15	0.18 $\pm$ 0.11
18:0	12.43 $\pm$ 8.11	4.51 $\pm$ 2.22	11.95 $\pm$ 5.87	3.21 $\pm$ 2.26
22:0	0.28 $\pm$ 0.1	0.20 $\pm$ 0.06	0.71 $\pm$ 0.27	0.22 $\pm$ 0.12
24:0	0.88 $\pm$ 0.21	0.57 $\pm$ 0.32	1.48 $\pm$ 0.05	0.64 $\pm$ 0.42
Σ Saturates[†]	36.52 $\pm$ 6.75	21.21 $\pm$ 1.51	46.49 $\pm$ 14.61	28.84 $\pm$ 6.16
16:1(n-7)	16.92 $\pm$ 5.57	10.63 $\pm$ 4.42	11.01 $\pm$ 2.98	25.11 $\pm$ 6.89
18:1(n-9)	6.00 $\pm$ 0.5	3.96 $\pm$ 0.24	7.48 $\pm$ 1.76	9.01 $\pm$ 5.13
18:1(n-7)	3.03 $\pm$ 0.42	2.82 $\pm$ 0.62	3.65 $\pm$ 1.13	2.01 $\pm$ 0.05
22:1(n-9)	1.14 $\pm$ 0.75	1.13 $\pm$ 0.64	2.96 $\pm$ 0.51	0.57 $\pm$ 0.24
Σ Monoenes[‡]	27.83 $\pm$ 6.03	19.31 $\pm$ 3.49	26.23 $\pm$ 6.52	37.76 $\pm$ 11.82
16:2(n-4)	2.41 $\pm$ 0.43	3.24 $\pm$ 0.76	1.24 $\pm$ 0.33	1.99 $\pm$ 0.42
16:3(n-4)	2.91 $\pm$ 0.94	4.82 $\pm$ 1.91	1.18 $\pm$ 0.18	1.57 $\pm$ 0.94
16:4(n-1)	1.48 $\pm$ 0.66	1.97 $\pm$ 1.44	0.23 $\pm$ 0.11	0.44 $\pm$ 0.15
18:2(n-6)	1.65 $\pm$ 0.26	4.81 $\pm$ 0.48	2.21 $\pm$ 0.84	2.16 $\pm$ 0.78
20:4(n-6)	1.28 $\pm$ 0.56	1.53 $\pm$ 0.08	0.57 $\pm$ 0.11	1.38 $\pm$ 0.35
Σ (n-6)[§]	3.65 $\pm$ 0.17	7.28 $\pm$ 0.33	3.11 $\pm$ 1.41	4.18 $\pm$ 1.74
16:4(n-3)	0.51 $\pm$ 0.22	1.08 $\pm$ 0.51	0.21 $\pm$ 0.04	0.18 $\pm$ 0.08
18:3(n-3)	1.54 $\pm$ 0.39	2.59 $\pm$ 0.94	2.08 $\pm$ 0.93	1.37 $\pm$ 0.81
18:4(n-3)	1.22 $\pm$ 0.39	1.11 $\pm$ 0.66	0.37 $\pm$ 0.21	0.30 $\pm$ 0.01
20:5(n-3)	10.52 $\pm$ 6.05	17.8 $\pm$ 7.43	3.37 $\pm$ 1.19	8.61 $\pm$ 1.85
22:5(n-3)	nd	1.16 $\pm$ 0.59	nd	0.56 $\pm$ 0.16
22:6(n-3)	nd	3.89 $\pm$ 0.49	0.92 $\pm$ 0.36	0.43 $\pm$ 0.06
Σ (n-3)[¶]	13.89 $\pm$ 6.1	27.70 $\pm$ 8.97	7.05 $\pm$ 1.77	11.53 $\pm$ 2.77
(n-3)/(n-6)	3.75 $\pm$ 1.46	3.80 $\pm$ 1.25	2.38 $\pm$ 0.51	2.86 $\pm$ 0.53
20:5(n-3)/ 22:6(n-3)	na	4.52 $\pm$ 1.51	0.00 $\pm$ 0.00	20.49 $\pm$ 7.32
20:4(n-6)/22:6(n-3)	na	0.39 $\pm$ 0.02	0.13 $\pm$ 0.09	3.29 $\pm$ 1.31

Table 5.6. Fatty acid composition of invertebrate tissues. Values (mean $\pm$ SD) are percentages of total fatty acids. See Table 1 for explanation of symbols.

Fatty Acid	Assimilation Period		Final	
	Control	Treatment	Control	Treatment
12:0	0.47 $\pm$ 0.66	1.67 $\pm$ 1.37	0.34 $\pm$ 0.26	1.02 $\pm$ 0.86
14:0	2.41 $\pm$ 1.61	3.27 $\pm$ 0.91	3.15 $\pm$ 0.49	5.19 $\pm$ 0.48
15:0	0.22 $\pm$ 0.01	0.22 $\pm$ 0.05	0.22 $\pm$ 0.08	0.22 $\pm$ 0.02
16:0	17.2 $\pm$ 2.34	11.99 $\pm$ 1.06	19.43 $\pm$ 1.86	11.89 $\pm$ 0.08
17:0	0.27 $\pm$ 0.07	0.56 $\pm$ 0.15	0.25 $\pm$ 0.06	0.34 $\pm$ 0.16
18:0	4.11 $\pm$ 0.27	7.16 $\pm$ 0.77	2.49 $\pm$ 0.28	4.84 $\pm$ 0.61
22:0	0.28 $\pm$ 0.07	0.25 $\pm$ 0.17	0.08 $\pm$ 0.04	0.19 $\pm$ 0.14
24:0	0.02 $\pm$ 0.03	0.02 $\pm$ 0.04	nd	0.05 $\pm$ 0.02
Σ Saturates[†]	26.02 $\pm$ 3.14	26.23 $\pm$ 1.71	26.75 $\pm$ 0.85	24.91 $\pm$ 1.88
16:1(n-7)	14.12 $\pm$ 1.17	6.91 $\pm$ 0.86	20.56 $\pm$ 0.21	12.63 $\pm$ 0.61
18:1(n-9)	10.44 $\pm$ 0.89	16.16 $\pm$ 1.41	9.41 $\pm$ 0.78	14.63 $\pm$ 0.11
18:1(n-7)	5.6 $\pm$ 0.70	5.33 $\pm$ 1.32	5.26 $\pm$ 0.14	2.82 $\pm$ 0.04
22:1(n-9)	0.04 $\pm$ 0.05	0.05 $\pm$ 0.06	nd	0.02 $\pm$ 0.01
Σ Monoenes[‡]	32.28 $\pm$ 1.77	29.79 $\pm$ 3.90	35.95 $\pm$ 0.72	31.11 $\pm$ 1.01
16:2(n-4)	2.58 $\pm$ 0.10	2.14 $\pm$ 0.88	2.82 $\pm$ 0.38	2.59 $\pm$ 0.35
16:3(n-4)	4.29 $\pm$ 2.11	2.02 $\pm$ 1.51	3.87 $\pm$ 0.95	5.38 $\pm$ 0.27
16:4(n-1)	0.62 $\pm$ 0.17	0.44 $\pm$ 0.16	0.95 $\pm$ 0.29	1.78 $\pm$ 1.07
18:2(n-6)	3.15 $\pm$ 0.67	7.57 $\pm$ 0.71	2.06 $\pm$ 0.47	4.01 $\pm$ 0.46
20:4(n-6)	1.29 $\pm$ 0.73	2.66 $\pm$ 1.01	0.85 $\pm$ 0.09	2.11 $\pm$ 0.55
Σ (n-6)[§]	5.84 $\pm$ 1.31	11.09 $\pm$ 0.60	3.79 $\pm$ 0.62	7.42 $\pm$ 0.64
16:4(n-3)	0.70 $\pm$ 0.33	1.01 $\pm$ 0.63	0.53 $\pm$ 0.02	0.49 $\pm$ 0.42
18:3(n-3)	1.83 $\pm$ 0.92	6.33 $\pm$ 2.18	2.53 $\pm$ 0.23	7.17 $\pm$ 0.39
18:4(n-3)	1.09 $\pm$ 0.03	0.96 $\pm$ 0.54	1.26 $\pm$ 0.24	1.35 $\pm$ 0.38
20:5(n-3)	16.23 $\pm$ 1.98	11.91 $\pm$ 1.67	15.79 $\pm$ 0.48	9.56 $\pm$ 0.16
22:5(n-3)	0.32 $\pm$ 0.15	0.29 $\pm$ 0.27	0.05 $\pm$ 0.04	0.23 $\pm$ 0.07
22:6(n-3)	0.87 $\pm$ 0.66	0.78 $\pm$ 0.78	0.31 $\pm$ 0.23	1.06 $\pm$ 0.68
Σ (n-3)[¶]	21.33 $\pm$ 1.87	21.54 $\pm$ 1.89	20.72 $\pm$ 0.72	20.09 $\pm$ 0.57
(n-3)/(n-6)	3.79 $\pm$ 0.90	1.94 $\pm$ 0.10	5.51 $\pm$ 0.71	2.72 $\pm$ 0.31
20:5(n-3)/ 22:6(n-3)	30.64 $\pm$ 22.68	27.37 $\pm$ 17.64	71.48 $\pm$ 53.14	11.21 $\pm$ 7.01
20:4(n-6)/22:6(n-3)	2.67 $\pm$ 1.98	5.37 $\pm$ 2.80	3.77 $\pm$ 2.59	2.69 $\pm$ 2.24

Table 5.7. Fatty acid composition of Atlantic salmon parr muscle tissue. Values (mean $\pm$ SD) are percentages of total fatty acids. See Table 1 for explanation of symbols.

Fatty Acid	Assimilation Period		Final	
	Control	Treatment	Control	Treatment
12:0	0.01 $\pm$ 0.02	0.01 $\pm$ 0.01	nd	nd
14:0	0.75 $\pm$ 0.13	0.91 $\pm$ 0.01	0.64 $\pm$ 0.13	0.72 $\pm$ 0.15
15:0	0.07 $\pm$ 0.06	0.08 $\pm$ 0.07	nd	nd
16:0	18.79 $\pm$ 1.01	15.45 $\pm$ 0.84	18.88 $\pm$ 0.76	15.55 $\pm$ 0.55
17:0	0.53 $\pm$ 0.01	0.40 $\pm$ 0.04	0.48 $\pm$ 0.09	0.36 $\pm$ 0.09
18:0	6.62 $\pm$ 0.22	4.98 $\pm$ 0.58	5.67 $\pm$ 0.45	6.45 $\pm$ 0.56
22:0	0.11 $\pm$ 0.01	0.06 $\pm$ 0.02	0.1 $\pm$ 0.02	0.06 $\pm$ 0.01
24:0	0.09 $\pm$ 0.04	0.01 $\pm$ 0.01	0.1 $\pm$ 0.01	0.02 $\pm$ 0.01
Σ Saturates[†]	27.5 $\pm$ 1.09	22.36 $\pm$ 1.41	26.36 $\pm$ 1.12	23.59 $\pm$ 0.77
16:1(n-7)	2.87 $\pm$ 0.05	6.11 $\pm$ 0.64	2.49 $\pm$ 0.72	2.98 $\pm$ 0.94
18:1(n-9)	5.34 $\pm$ 0.31	14.92 $\pm$ 0.6	5.32 $\pm$ 1.15	13.63 $\pm$ 2.5
18:1(n-7)	3.64 $\pm$ 0.32	4.03 $\pm$ 0.15	3.31 $\pm$ 0.7	3.57 $\pm$ 0.49
22:1(n-9)	0.02 $\pm$ 0.02	0.11 $\pm$ 0.03	0.03 $\pm$ 0.02	0.08 $\pm$ 0.01
Σ Monoenes[‡]	12.56 $\pm$ 0.24	26.08 $\pm$ 0.94	11.68 $\pm$ 1.67	21.12 $\pm$ 3.09
16:2(n-4)	0.53 $\pm$ 0.10	0.26 $\pm$ 0.03	0.37 $\pm$ 0.09	0.18 $\pm$ 0.09
16:3(n-4)	0.51 $\pm$ 0.07	0.14 $\pm$ 0.02	0.31 $\pm$ 0.15	0.06 $\pm$ 0.03
16:4(n-1)	0.11 $\pm$ 0.01	0.07 $\pm$ 0.01	0.06 $\pm$ 0.05	0.06 $\pm$ 0.02
18:2(n-6)	2.47 $\pm$ 0.19	5.90 $\pm$ 0.43	2.01 $\pm$ 0.49	4.52 $\pm$ 0.49
20:4(n-6)	3.63 $\pm$ 0.62	1.59 $\pm$ 0.34	2.95 $\pm$ 0.57	2.28 $\pm$ 0.17
Σ (n-6)[§]	7.57 $\pm$ 0.58	9.53 $\pm$ 0.25	6.5 $\pm$ 0.56	8.8 $\pm$ 0.57
16:4(n-3)	0.21 $\pm$ 0.08	0.14 $\pm$ 0.04	0.13 $\pm$ 0.12	0.09 $\pm$ 0.05
18:3(n-3)	3.18 $\pm$ 0.89	4.24 $\pm$ 0.46	3.71 $\pm$ 1.29	2.44 $\pm$ 1.13
18:4(n-3)	0.69 $\pm$ 0.09	0.44 $\pm$ 0.04	0.21 $\pm$ 0.2	0.25 $\pm$ 0.14
20:5(n-3)	12.03 $\pm$ 0.56	7.86 $\pm$ 0.48	12.14 $\pm$ 1.21	6.77 $\pm$ 0.55
22:5(n-3)	4.11 $\pm$ 0.15	4.13 $\pm$ 0.22	4.6 $\pm$ 0.42	4.07 $\pm$ 0.37
22:6(n-3)	25.66 $\pm$ 0.98	19.59 $\pm$ 0.18	29.38 $\pm$ 2.94	28.04 $\pm$ 3.54
Σ (n-3)[¶]	47.12 $\pm$ 1.41	37.61 $\pm$ 0.49	51.32 $\pm$ 1.78	42.72 $\pm$ 3.51
(n-3)/(n-6)	6.31 $\pm$ 0.56	3.97 $\pm$ 0.05	7.94 $\pm$ 0.75	4.88 $\pm$ 0.69
20:5(n-3)/ 22:6(n-3)	0.46 $\pm$ 0.03	0.40 $\pm$ 0.02	0.41 $\pm$ 0.06	0.24 $\pm$ 0.02
20:4(n-6)/22:6(n-3)	0.14 $\pm$ 0.01	0.08 $\pm$ 0.01	0.1 $\pm$ 0.01	0.08 $\pm$ 0

Figure 5.1. Schematic drawing of the experimental stream-channels. V: water inflow, adjustable with a valve; O: water outflow via an overflow weir regulating the water level in the stream-channel with a mesh panel (wire mesh size 2.5 mm) to block fish emigrations; Ri: riffle habitats; Ru: run habitats; P: pool habitats. Arrows indicate direction of flow.

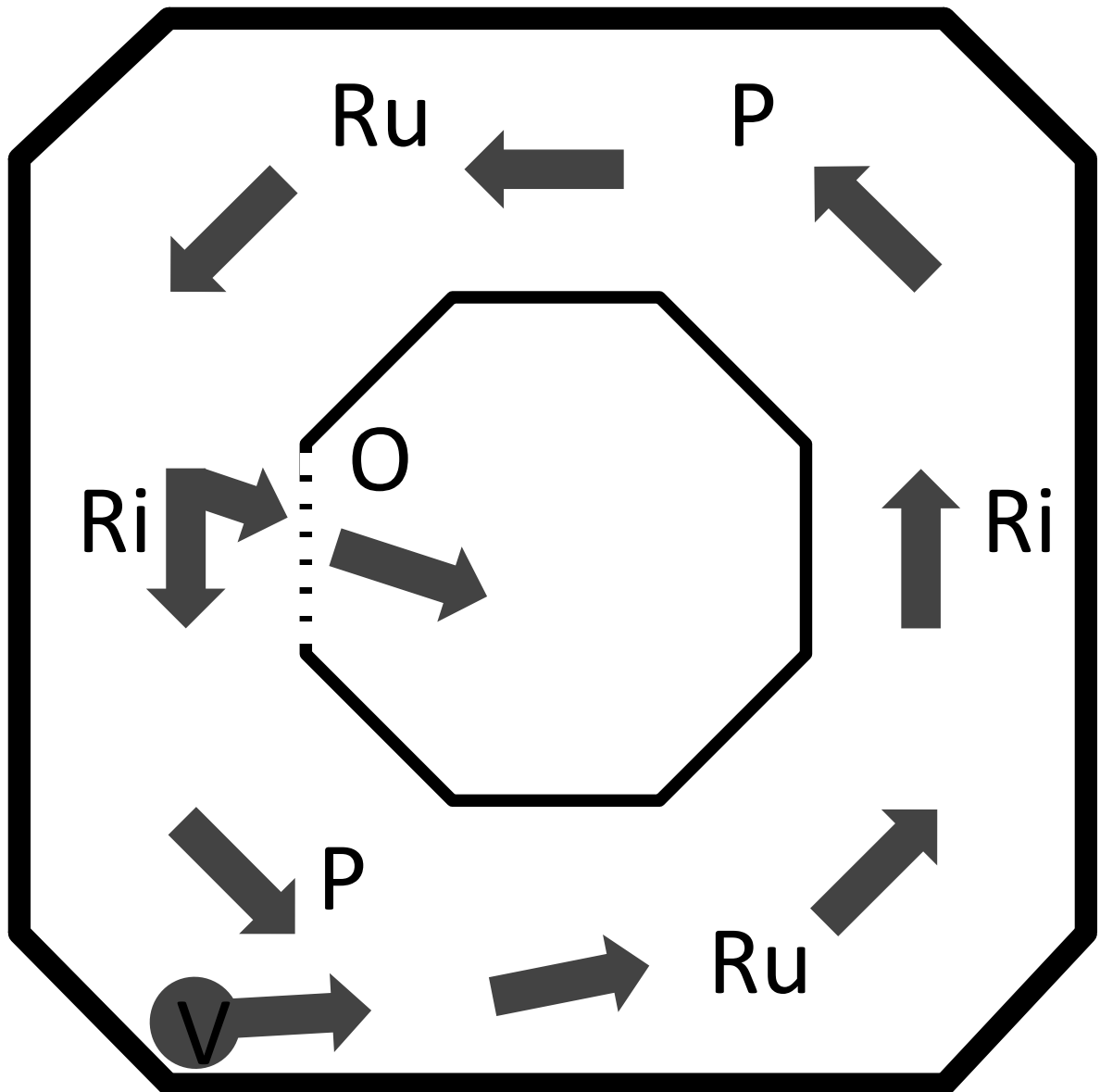


Figure 5.2. Seasonal patterns in mean % lipid ($\pm$ one standard deviation) in a) stream-channel biofilm, b) invertebrates, and c) Atlantic salmon parr (muscle) receiving marine-derived nutrients (MDNs) from spawning Atlantic salmon and control treatments. MDN treatments: shaded symbols; controls: open symbols. Values with an * denote a statistically significant difference ($p < 0.05$) between stream-channels with MDN inputs and the controls for the same sampling period. Shaded areas denote the period of spawning (November 8 – 23, 2011). Adult salmon were introduced into the stream-channels on October 7, 2011 and removed on December 10, 2011.

[Figure on following page]

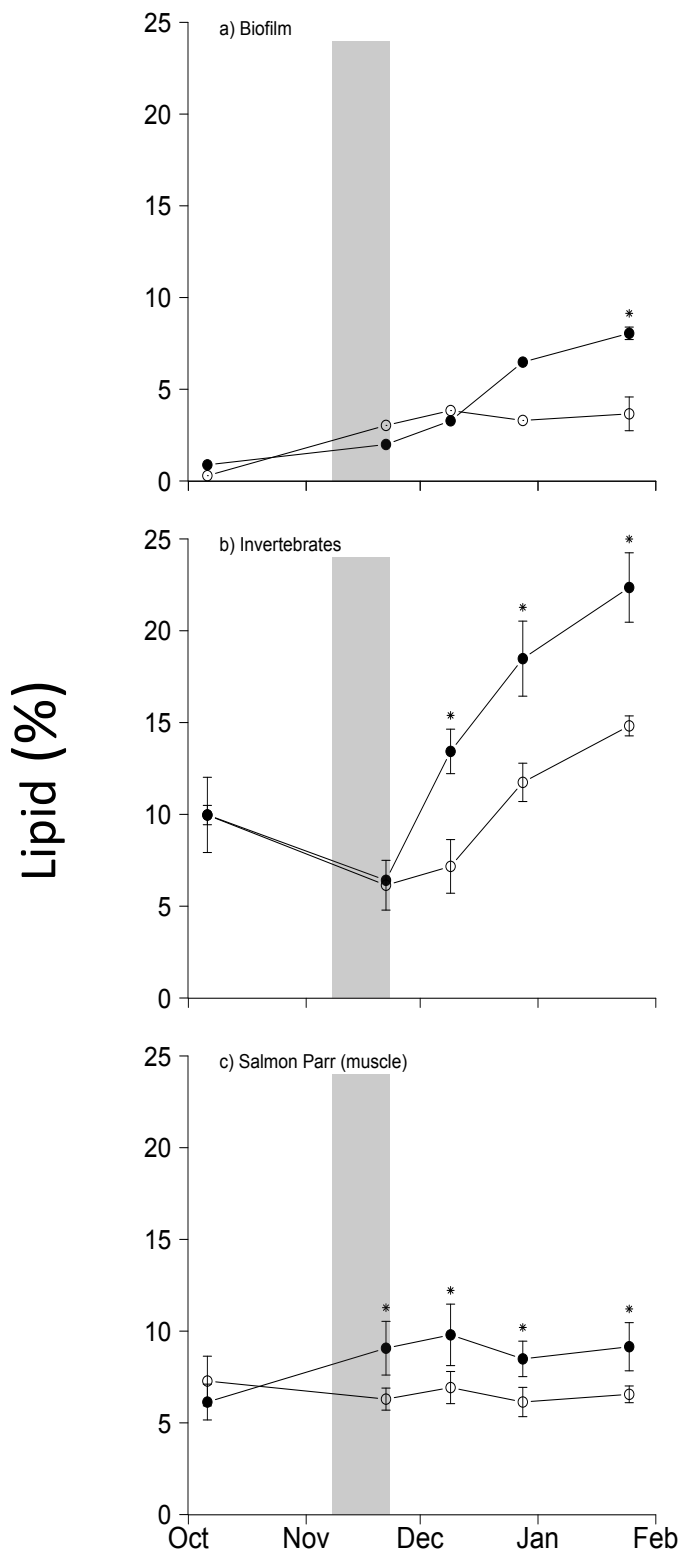


Figure 5.3. Principle component analysis (PCA) plots of fatty acids in biofilm (green), invertebrates (black), Atlantic salmon parr (blue) and Atlantic salmon eggs (green star). Treatments: MDN subsidies: shaded symbols, controls: open symbols. Sampling times: triangle (October 6, 2011), square (November 22, 2011), diamond (December 9, 2011), circle (December 28, 2011), and inverted triangle (January 25, 2012). The eigenvectors (represented by an individual fatty acid and corresponding vector length) reflect the importance of that fatty acid's contribution to distinguishing between the first two principal component axes.

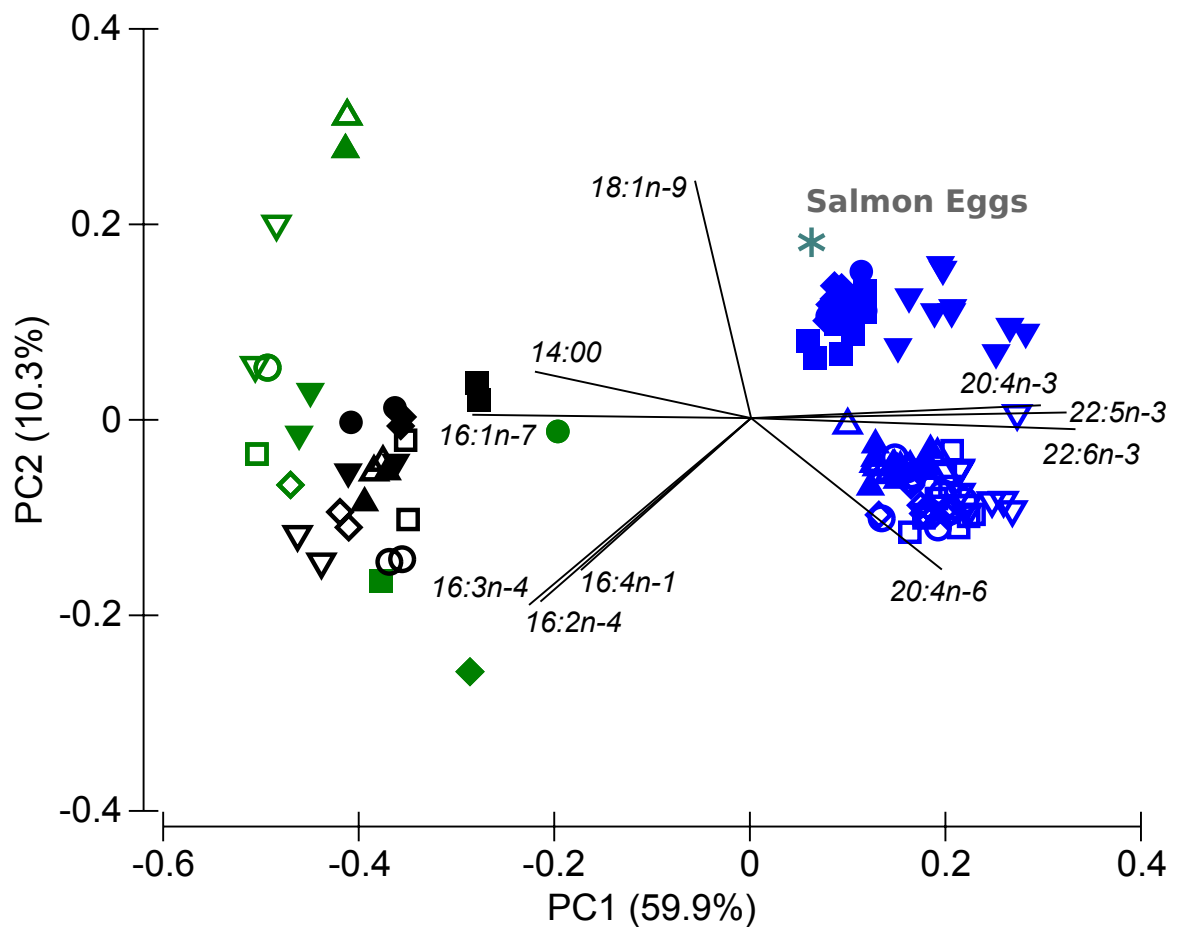


Figure 5.4. Percent average dissimilarity in fatty acid profiles as calculated using Similarity Percentage (SIMPER) analysis based on a Bray-Curtis similarity matrix. For each group (biofilm, invertebrates, and salmon parr), at each sampling period (0 – 4), dissimilarity represents the differences between treatment and control stream-channels. Dissimilarity was also compared between treatment (T) and control (C) stream-channels for between group differences (e.g. between biofilm and invertebrates denoted as biofilm:inverts).

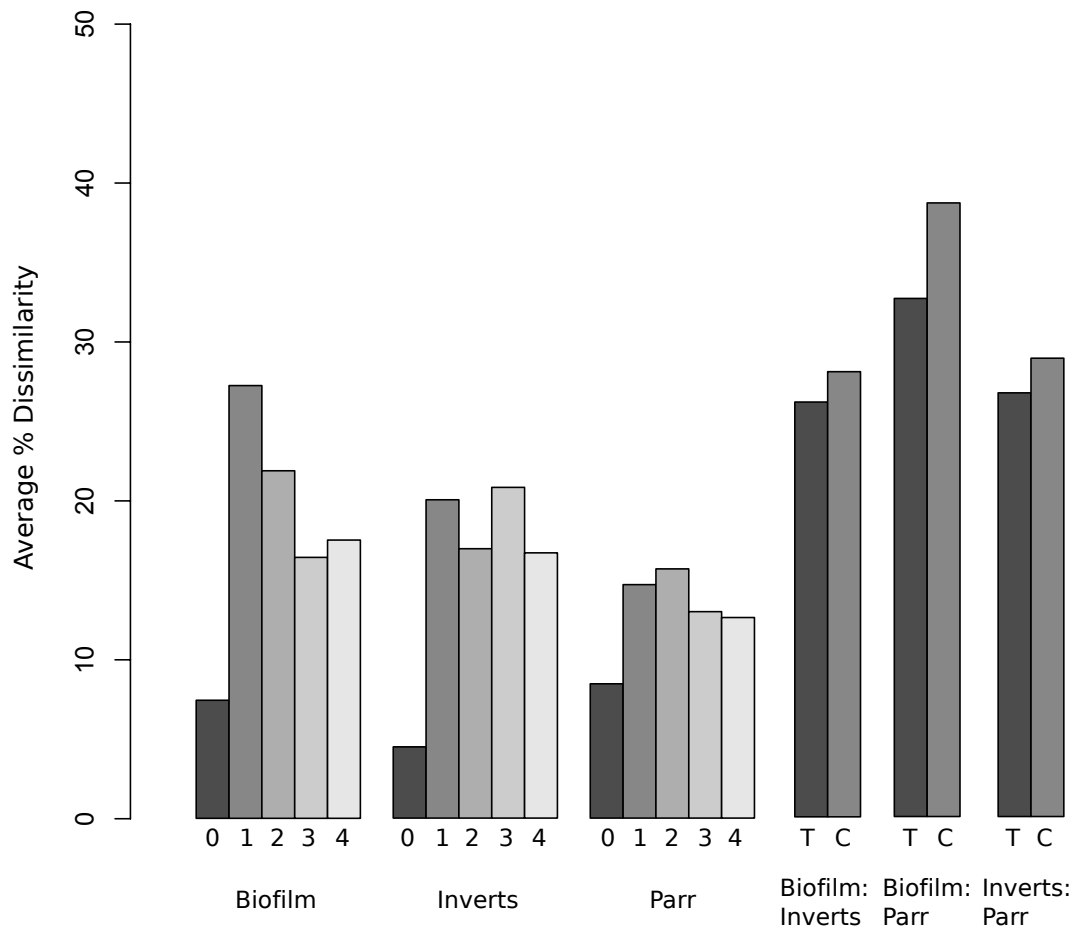


Figure 5.5. Temporal changes in the fatty acids (mean $\pm$ 1 SD) identified using Similarity Percentage (SIMPER) analysis which accounted for 68.8% of the differences between biofilm (a-e), invertebrates (f-j), and Atlantic salmon parr (k-o) in stream-channels receiving marine-derived nutrients from spawning Atlantic salmon (shaded symbols) and control stream-channels (open symbols). Fatty acids identified were 16:0 (a, f, k), 16:1(n-7) (b, g, l), 18:1(n-9) (c, h, m), 20:5(n-3) (d, i, n), and 22:6(n-3) (e, j, o). The dashed line represents the fatty acid value of the eggs; see figure 2 for explanation of the rest of the symbols. Shaded areas denote the period of spawning (November 8 – 23, 2011).

[Figure on following page]

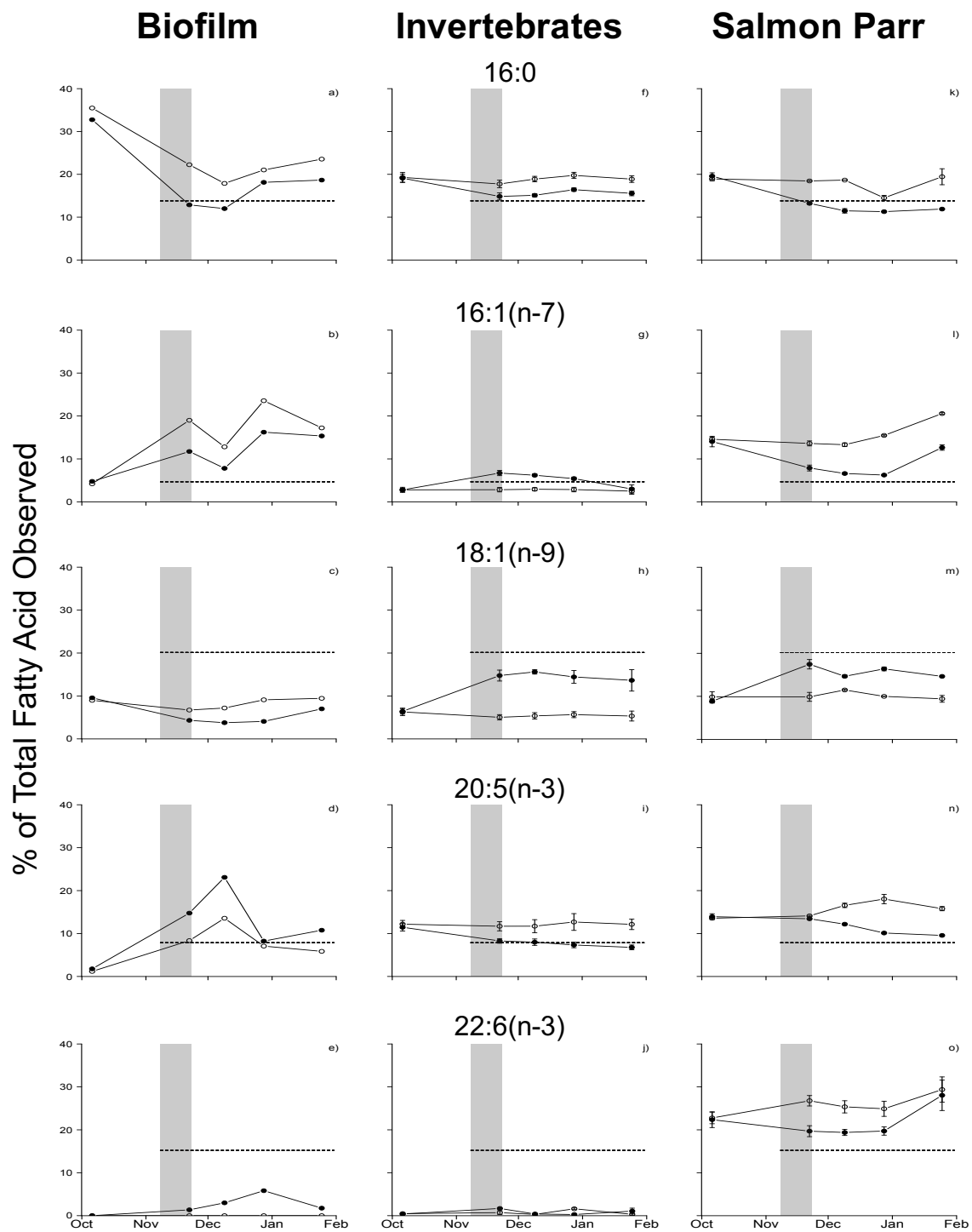
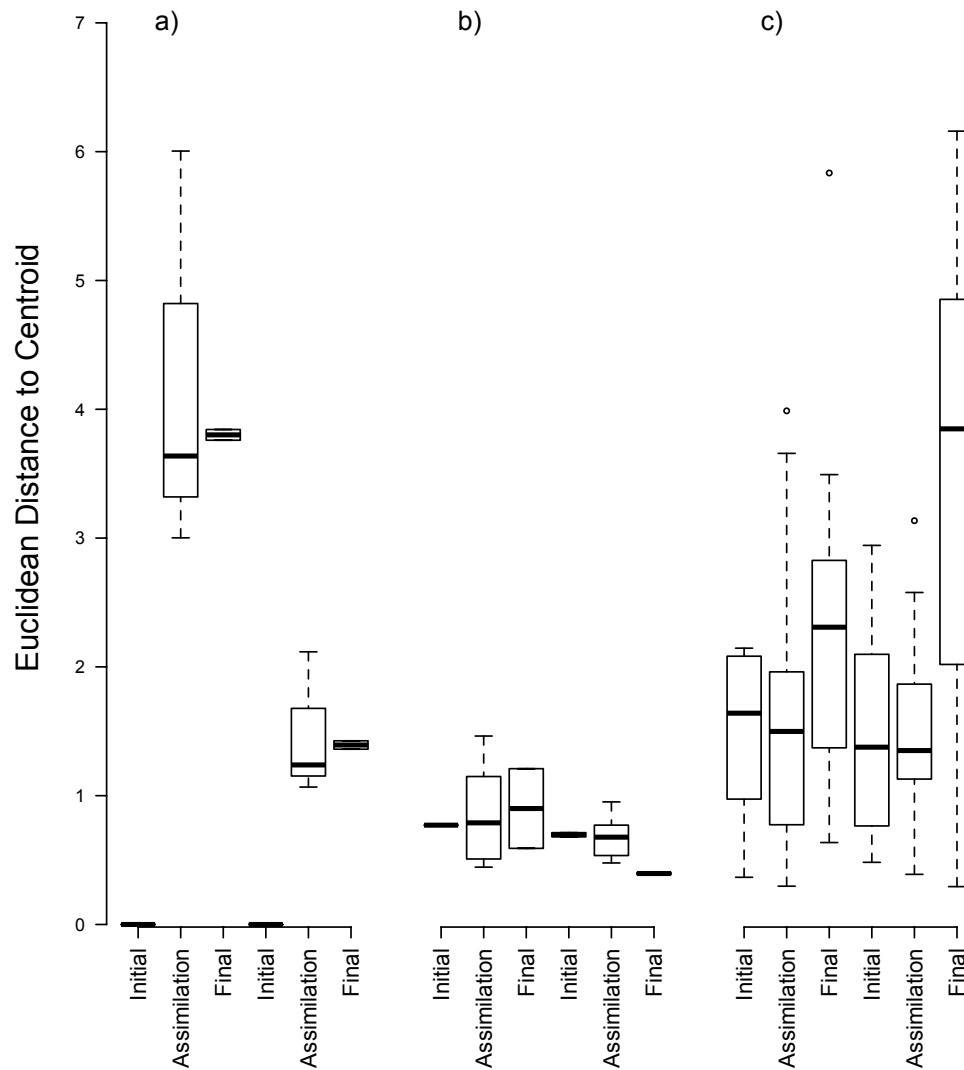


Figure 5.6. Box and whisker plots of the Euclidean distance between the group centroid and each sample within that group: for a) biofilm, b) invertebrates, and c) Atlantic salmon parr. For stream-channels receiving marine-derived nutrients (MDNs) from spawning Atlantic salmon and control treatments, sampling periods were divided into initial (time 0), assimilation period (times 1, 2, and 3) and final (time 4).



Chapter 6

The ecological toll from declining fish populations: Anadromous fishes function as nutrient import/export specialists

Kurt M. Samways¹, Guillaume J.R. Dauphin¹ Richard A. Cunjak¹

¹University of New Brunswick, Department of Biology and Canadian Rivers Institute,
Fredericton, New Brunswick E3B 5A3 Canada.

6.1 Abstract

Anadromous fish deliver marine-derived nutrient (MDN) subsidies to freshwater ecosystems. Since the establishment of commercial fishing in 1765 in the Miramichi River, Atlantic salmon and gaspereau (collective term for alewives and blueback herring) have undergone precipitous declines (i.e. declines of 96% for Atlantic salmon and 64% for gaspereau). Here, we examine the relative importance of nutrient subsidies from Atlantic salmon and gaspereau using the Miramichi River as a model system. We calculated the equilibrium spawning population (point at which the population is neither increasing nor decreasing) as an estimate of the historic or maximum number of fish in the Miramichi River. Applying mass-balance nutrient loading models based on life-history data, we estimated the amount of MDNs being delivered by Atlantic salmon and gaspereau. The equilibrium spawning population estimate in the Miramichi River was 1.6 million Atlantic salmon and 20.25 million gaspereau (12.7 million alewives). Based on these equilibrium spawning population estimates, Atlantic salmon would have imported 189.7 tons of nitrogen (N) and 17.7 tons of phosphorous (P), while only 0.5 tons of N and 0.08 tons of P would have been exported through juvenile out migration. Gasperneau would have imported 121.9 (± 22.9) tons of N and 18.7 (± 3.5) tons of P and exported 129.5 (± 24.2) tons of N and 21.8 (± 4.1) tons of P. Declines in fish populations have led to a greater than 90% reduction in salmon-derived nutrients and greater than 55% reduction in gaspereau-derived nutrients. In general, Atlantic salmon appear to be net nutrient importers and alewives (gaspereau) net nutrient exporters. Diminishing anadromous populations have likely created a situation, whereby cumulative losses in

MDNs may be leading to declines in freshwater productivity, resulting in a decrease in the resilience of these fishes to recover.

6.2 Introduction

Freshwater productivity is driven by nutrients and energy generated from within the ecosystem or by resource subsidies originating in other ecosystems (Polis et al. 2007). Anadromous fish can deliver important marine-derived nutrient (MDN) subsidies to freshwater ecosystems. For example in the Pacific Northwest, salmon (*Oncorhynchus* spp.) deliver marine subsidies in the form of excretory products, gametes and carcasses that are enriched in nutrients (e.g. nitrogen (N) and phosphorous (P)) (Bilby et al. 1996, Wipfli et al., 1999), leading to increases in freshwater productivity (Levi et al. 2003) including juvenile fish growth (Wipfli et al. 2003) and abundance (Chaloner et al. 2002, Wipfli et al. 2010). While not as extensively studied as Pacific salmon, Atlantic anadromous fishes have also been shown to be important contributors of MDNs (Durbin et al. 1979, Jonsson and Jonsson 2003, Jardine et al. 2009, Nislow and Kynard 2009, Walters et al. 2009, Samways et al. 2015).

Biodiversity and species richness play a critical role in maintaining ecosystem productivity, as it can provide a buffer against environmental change (Loreau et al. 2001, Palmer et al. 2004). Atlantic Canada is home to 13 species of diadromous fish, exhibiting a wide variety of life history and spawning strategies (Scott and Scott 1988). These annual migrations deliver MDN subsidies supplementing a food web with a diversity of food resources, which are important for freshwater production, providing a source of energy to higher trophic levels (Chapter 3), and for sustaining life-history processes such

as growth and development (Samways et al. 2017). However, over the last century, eastern North American anadromous fish populations have undergone precipitous declines (Limburg and Waldman 2009), leading to a corresponding decrease in MDN contributions that may explain decreases in freshwater productivity (Samways et al. 2015, Samways and Cunjak 2015).

The Miramichi River in New Brunswick, Canada drains an area of 14,000 km² (Cunjak and Newbury 2005) into the Gulf of St. Lawrence (Fig. 6.1). Because of its size and accessibility to migratory fishes along the entire length, it is currently the largest producer of diadromous fishes in Atlantic Canada (Chaput 1995). Europeans used the Miramichi estuary region throughout the early part of the 16th century, primarily as stopover points to rest and clean their ocean catch, not establishing permanent settlements until the late 1600's. However prior to 1765, only the First Nation People were making use of the within-river fishery resources (Davidson 1947). For example, in the late 18th century, the subsistence exploitation of Atlantic salmon in the Miramichi catchment is estimated between 14 and 64 tonnes annually (calculation based on Rostlund 1952 and Dunfield 1985; respectively).

In 1765 the first commercial fishery (for Atlantic salmon) was established in the Miramichi River, and by the late 1700's a sizeable inland fishery was well established with the principal fisheries being for Atlantic salmon (*Salmo salar*) (see Fig. 6.2 for Atlantic salmon landings), gaspereau (collective term used in Atlantic Canada describing both alewives, *Alosa pseudoharengus* and blueback herring, *Alosa aestivalis*) (see Fig. 6.2 for gaspereau landings), American shad (*Alosa sapidissima*), striped bass (*Morone saxatilis*), rainbow smelt (*Osmerus mordax*), American eel (*Anguilla rostrata*), tomcod

(*Microgadus tomcod*), and brook trout (*Salvelinus fontinalis*) (Perley 1852). In addition to the commercial fishery, local First Nations communities routinely harvested shortnose and Atlantic sturgeon (*Acipenser brevirostrum* and *Acipenser oxyrinchus oxyrinchus*, respectively) as part of their subsistence fishery (Dunfield 1985). This was short lived as much of the commercial fishing in the Miramichi River ceased by 1777 due to a shifting focus on the American revolutionary war (Perley 1852, Dunfield 1985). With commercial fishing once again intensifying post war, catches were bountiful throughout the first decade of the 19th century. However, overfishing caused the vast exports of fish leaving the Miramichi to dwindle to only a few tonnes by 1824 (most of which was salmon) (Perley 1852, Dunfield 1985). By the 1850's species such as Atlantic salmon, gaspereau and striped bass were considered "a matter of history" and "nearly exterminated" (Perley 1852, Monro 1855).

The commercial fishery continued in the Miramichi River, but decreases in the abundance of many anadromous species were very much evident. By the early 1900's approximately 2500 t of diadromous fish were being harvested, of which rainbow smelt, gaspereau, and Atlantic salmon made up 50%, 16%, and 8% of the total catch respectively; the other 26% of the catch being made up of American eel, shad, tomcod, and striped bass (Chaput 1995). By 1984 the commercial salmon fishery was closed and by the early 1990's the total diadromous commercial catch fell to 3100 t, of which rainbow smelt and gaspereau made up 90% of the total (13% and 77% respectively) (Chaput 1995).

The decline of anadromous fishes throughout the 19th and 20th centuries was undoubtedly affected by industrialization and widespread resource use. Forestry, a long-

standing industry in the Miramichi River catchment, contributed to freshwater habitat degradation through blockage of upstream passage from splash dams and the piling of pulpwood in the riverbed, nutrient losses, decreased water quality, altered flow and increased sedimentation, and increased summer water temperatures (Elson et al. 1972). Intensive spraying of DDT (dichlorodiphenyltrichloroethane) between 1952-1967 to control spruce budworm, lead to increased fish mortalities and decreased reproductive success (Elson 1967). As it has a half-life of 13 years, the reservoir of DDT and its derivatives was sufficient to ensure enough recycling through the environment for many, many years even after switching to non-persistent alternatives (Elson et al. 1972). In addition, pollution from mining operations altered freshwater community structure and function (Saunders and Sprague 1967, Buckley and Winters 1983, Schiefer et al. 1992). However, early fishing practices prior to widespread habitat degradation had likely already set in motion the collapse of anadromous fish stocks and that the impacts to rivers that followed (i.e. forestry, pollution, etc.) were merely the final straw.

With much of the overexploitation and subsequent disruption to freshwater productivity occurring decades or even centuries before quantitative stock assessments, it is easy to be “deceived” by the notion that only recent data on composition and abundance are reliable, and that historical data are not good enough to use in rigorous analyses (Pinnegar and Engelhard 2008, Humphries and Winemiller 2009). As a result, we accept the notion that environmental conditions of the immediate past (e.g. last 50-75 years) do not necessarily reflect those of the distant past (Pauly 1995) and that degraded and unsustainable conditions become the accepted management and/or restoration targets (Humphries and Winemiller 2009). Benchmarks based on current rates of MDN loading

by anadromous fishes likely underestimate the historic importance of MDNs in driving and maintaining freshwater productivity.

Here, we examine the relative importance of nutrient subsidies from Atlantic salmon and alewives. Historical records and/or anecdotal data on fish populations go back 300 years, however humans were impacting fish populations prior to this. Because of this, we first calculated estimates of maximum spawning population sizes of both Atlantic salmon and alewives/gaspereau. Then using a mass-balance modelling approach we estimated net nutrient loading based on existing life-history data. Finally we compare current MDN loading to maximum spawning estimates to demonstrate what the maximum MDN inputs would have been and how much has been lost through population declines.

6.3 Methods

6.3.1 Equilibrium Spawning Population

Equilibrium models were used to calculate an estimate of the historic or maximum number of Atlantic salmon and alewife in the Miramichi River. The equilibrium spawning population is defined as the population size at which the components of the life cycle are in balance, such that the population is neither increasing nor decreasing in size (Gibson and Myers 2004). The model is set up by splitting the life cycle into multiple parts, each characterizing a measure of productivity that is influenced by one of more life history parameters (e.g. fecundity, survival, etc.). The application of these models is well suited to anadromous fishes like Atlantic salmon and alewives because they have life cycles that are naturally split into two production stages; the freshwater (egg to smolt/juvenile) and marine (adult) (Gibson et al. 2009).

6.3.1.1 Atlantic salmon equilibrium model

We used a modified version of a model developed by Gibson et al. (2009) for Atlantic salmon in the Tobique River, N.B. to estimate the equilibrium spawning population (ESP_S) in the Miramichi River. The equilibrium spawning population model consists of two components, freshwater production (the relationship between egg deposition and survival to 0+) and marine production (the relationship between adult salmon and 0+ salmon). We acknowledge that the 0+ to smolt life stages occur in freshwater and that marine production maybe better represented by the smolt to egg deposition phase, the data available was such that we had to include part of the freshwater life cycle in the marine production component.

The freshwater production component begins by calculating the number of eggs produced by a 0+ salmon throughout its life (EPY). This 0+ to egg model includes survival from 0+ to smolt, the probability of maturing at sea-age, size-specific fecundity, and survival between spawning events.

$$(6.1) \quad EPY = \sum_{c=1}^2 Egg_c$$

in which

$$(6.2) \quad Egg_1 = (1 - M_{0+,s})P_{fs}(1 - M_{Sea})^c m_1 \left[P_{f1SW} f_{1SW} + \sum_1^{\max s} (1 - M_{Sea1SW})^s (1 - M_{SSp})^s P_{fMSW} f_{MSW} \right]$$

and,

$$(6.3) \quad Egg_2 = (1 - M_{0+,s})P_{fs}(1 - M_{Sea})^c(1 - m_1) \left[\sum_1^{\max s} (1 - M_{SeaMSW})^s (1 - M_{SSp})^s P_{fMSW} f_{MSW} \right]$$

where c is the number of years spent at sea prior to maturity (for this model we assume that all fish mature after their second year at sea), $M_{0+,s}$ is the inter-stage mortality between 0+ and smolt (0.982; Cunjak and Therrien 1998), P_{fs} is the proportion of the smolt population that is female (0.50; assuming a 1:1 male:female smolt ratio), M_{Sea} is the annual mortality between smolt and one sea winter (1SW) salmon at sea (0.969; Aprahamian et al. 2003, Chaput 2003), M_{Sea1SW} is the annual mortality of a 1SW spawner returning to spawn as a MSW (0.75; Chaput et al. 2016), M_{SeaMSW} is the annual mortality of a MSW spawner returning to spawn consecutively (0.40; Chaput et al. 2016), m_1 is the probability of maturing after one winter at sea (0.20; Chaput et al. 2003), P_x is the proportion of the either first-time (P_{f1SW}) or MSW spawners (P_{MSW}) (0.136 and 0.254 respectively; Chaput et al. 1999, Chaput 2010), f_x is the average fecundity of either 1SW (f_{1SW}) or MSW spawners (f_{MSW}) (see below for detailed fecundity calculations), s is the number of previous spawnings, and M_{SSp} is the freshwater spawner mortality rate (0.37; Chadwick et al. 1978). This model assumes all repeat spawning is consecutive, with a maximum of three consecutive spawning events.

As freshwater carrying capacity and maximum survival from egg to 0+ is density dependent (Gibson et al. 2009), the 0+ to egg model was rescaled (EPY^*)

$$(6.4) \quad EPY^* = \frac{(\alpha EPY - 1)R_{asy}}{\alpha}$$

where α is the slope at the origin of the Beverton-Holt function and describes the maximum survival rate between adult and 0+, R_{asy} is the asymptotic density of 0+ parr (number per m²). The Beverton-Holt stock recruitment analysis does not represent 0+ recruitment for the entire Miramich River (as these data were not available) but rather three tributaries (Catamaran Brook, Rocky Brook, Clearwater Brook) of the Little Southwest and Southwest branches. These rivers were used as a proxy population, assuming these values are representative of the population.

Freshwater production (YOY), the number of 0+ salmon produced from egg deposition, is calculated by substituting EPY^* into the freshwater production model described by Quinn and Deriso (1999)

$$(6.5) \quad YOY = \frac{\alpha EPY^*}{1 + \frac{\alpha EPY^*}{R_{asy}}}.$$

The marine production component or equilibrium spawning population size (ESP_S) is where freshwater production equals the inverse of marine production. It is at this point that the lifetime production of an adult by an adult is 1, such that the population neither increases nor decreases in size (Gibson et al. 2009).

$$(6.6) \quad ESP_S = \left(\frac{-YOY}{\alpha \left(\frac{YOY}{R_{asy}} - 1 \right)} \right) h$$

where h is the available juvenile habitat in the Miramichi River catchment (54605100 m²; Amiro 1983).

6.3.1.2 Alewife equilibrium model

The equilibrium spawning population of alewives (ESP_A) in the Miramichi River

was calculated based on Gibson and Myers (2003)

$$(6.7) \quad ESP_A = SSB_{20\%} \times 5 \times Wt_{ASp}$$

where $SSB_{20\%}$ is the life-history based, age-structured population dynamics model used to calculate a spawning biomass of 20% of the equilibrium spawning biomass (Gibson and Myers 2003) and Wt_{ASp} is the average weight of an adult alewife (341.9g; Gibson and Myers 2001, K.M. Samways, unpubl. data). Gibson and Meyers (2003) calculated the $SSB_{20\%}$ as:

$$(6.8) \quad SSB_{20\%} = 0.2 \frac{(\alpha SPR_{F=0} - 1)R_0}{\alpha}$$

where α (slope at the origin) is the maximum rate at which spawners can produce recruits at low population sizes and R_0 is the asymptotic recruitment level. The rate at which recruits produce spawners in the absence of fishing mortality (F) ($SPR_{F=0}$) was based on alewife life history (Gibson and Myers 2003) with age 3 being the earliest age of maturity.

$$(6.9) \quad SPR_{F=0} = \sum_{a_{rec}}^{a_{max}} SS_a w_a$$

where

$$SS_3 = m_3$$

$$SS_4 = SS_3 e^{-M_{ad}} + (1 - m_3) e^{-M_{juv}} m_4$$

$$SS_5 = SS_4 e^{-M_{ad}} + (1 - m_3)(1 - m_4) e^{-2M_{juv}} m_5$$

$$SS_6 = SS_5 e^{-M_{ad}} + (1 - m_3)(1 - m_4)(1 - m_5) e^{-3M_{juv}} m_6$$

•

•

$$SS_{a_{max}} = SS_{a_{max}-1}e^{-M_{ad}} + (1 - p_3)(1 - p_4) \dots (1 - p_{a_{max}-1})e^{-(a_{max}-3)M_{juv}}p_{a_{max}}$$

Here, SS_a is the number of spawners at age a , w_a is the age-specific weight, M_{ad} and M_{juv} are the instantaneous mortality rates for adult and juvenile fish respectively, and m_a is the probability of maturity at age a . See Gibson and Myers (2003) for values used in the calculation and a detailed explanation of the alewife equilibrium spawning biomass.

We applied the average ($\pm$ upper and lower limits) proportion of blueback herring in the Miramichi River gaspereau run (see Chaput and Atkinson 2001) to ESP_A to estimate the equilibrium spawning population of gaspereau.

6.3.2 Marine-derived nutrient loading models

We used a mass balance approach to evaluate net marine-nutrient subsidies from Atlantic salmon and alewives to the Miramichi River. The general model estimates MDN contributions as

$$(6.10) \quad MDN = (NCarc + NEx + NEgg + NMilt) - NJuveniles$$

where $NCarc$ is the nutrient contribution from carcasses as a result of spawner mortality, NEx is the nutrient contribution from excretory products by live spawners while in freshwater, $NEgg$ is the nutrient contribution from eggs, $NMilt$ is the nutrient contribution from milt, and $NJuveniles$ is the export of nutrients by juveniles emigrating from freshwater to the ocean. Separate mass balance models were constructed for Atlantic salmon and alewives to account for differences in life history and spawning strategies between species.

6.3.2.1 Atlantic salmon MDN loading model

We estimated the MDN inputs from Atlantic salmon using a mass balance model approach adapted from Samways and Cunjak (2015) and Johnston et al. (2004). As two size groups of salmon return to the river, the variable $P_{Size}(i)$, proportion of the population that is small (0.68) or large (0.32) (Chaput et al. 1999, Chaput 2010), was used to account for differences in MDN contributions associated with size. The mass of nutrient contributions from spawner mortality ($NCarc_S$) is

$$(6.11) \quad NCarc_S = M_{SSp} \lambda_S N_{SSp} \left(\sum_{i=1}^{I=2} P_{Size}(i) Wt_{SSp}(i) \right)$$

where M_{SSp} is the freshwater spawner mortality (0.37; Chadwick et al. 1978), λ_S is the grams of nutrient per gram of wet weight (0.03 for nitrogen and 0.0047 for phosphorous by wet weight; Lyle and Elliot 1998), N_{SSp} is the number of spawners, and $Wt_{SSp}(i)$ is the average weight of either a small or large salmon (1.57 or 5.86 kg respectively; Reid and Chaput 2012).

Atlantic salmon returning to the Miramichi River exhibit distinct temporal stock components, with early-run fish returning to the river from May to August 31, and late-run fish returning from September 1 onwards (Chaput 2010). Because salmon spend extended periods in freshwater without feeding and excretion rates decline when fish are not feeding (Vanni 2002), excretion rates measured over short periods may not be an appropriate metric. Therefore, we used a comparison of weight to elemental composition before and after spawning to model excretion rates (Durbin et al. 1979). Excretion rates are a function of metabolism and not feeding (Wood 1995) and during spawning,

metabolic rates of salmon can increase 61% for males and 8% for females (Brett 1995); therefore excretion rates used in our model were adjusted accordingly. The nutrient contribution from excretory products (NEx_S) is

$$(6.12) \quad NEx_S = (1 - M_{SSp}) \lambda_S \eta N_{SSp} t \left(\sum_{i=1}^{I=2} \left(\sum_{j=1}^{J=2} \left(\sum_{k=1}^{K=2} Wt_{GF}(i, j) P_{Run}(i, j, k) \right) \right) \right)$$

where η is the % nutrient loss per weight during spawning (35.1% of N and 22.7% of P; estimated from Jonsson et al. 1990 and Jonsson & Jonsson 2003), t is the amount of time spent in freshwater, Wt_{GF} is the gonad-free weight for the i th (small or large salmon) and j th (male or female) proportion of the population, and P_{Run} is the proportion of early or late run fish (k ; 0.55 and 0.45 early and late run, respectively; Chaput et al. 1999).

Gonad-free weight for small and large, males and females is

$$(6.13) \quad Wt_{GF}(i, j) = \left(\sum_{i=1}^{I=2} \left(\sum_{j=1}^{J=4} P_{Size}(i) Wt_{SSp}(i) P_{Sex}(i, j) \right) \right) \left(\sum_{k=1}^{K=2} 1 - \beta(k) \right)$$

where $P_{Sex}(i, j)$ is the proportion of salmon in the population for a given sex/size (small-males, 0.798; large-males, 0.213; small-females, 0.202; large-females, 0.787; Chaput et al. 1999, Chaput 2010) and $\beta(k)$ is the gonad-somatic index for either males (0.050) or females (0.241) (Fleming 1996). As not all eggs successfully develop and hatch into fry, the nutrient contribution from unsuccessful eggs ($NEgg_S$) is

$$(6.14) \quad NEgg_S = (1 - M_{SSp}) \Phi_S P_{Effective\ Female} \left(\left(\sum_{i=1}^{I=2} P_{Size}(i) \varphi(i) \right) - M_{EggS} \right)$$

where ϕ_S is the grams of nutrient per gram of wet weight of an egg (0.23 for nitrogen; Johnston et al. 2004, and 0.014 for phosphorous; Nislow et al. (2004), using the average

egg weight of 0.193 g and 0.300 g for small of small and large salmon respectively; K.M. Samways, unpubl. data), $P_{Effective\ Female}$ is the proportion of eggs successfully laid (0.995; K.M. Samways, unpubl. data), φ is the total amount of eggs produced by females of a given size, and M_{Egg} is the number of eggs that do not result in a smolt leaving the system (modeled as $1 - N_{Smolt}$). The total mass of eggs (φ) produced by females is based on the work of Marshall et al. (1997) on Atlantic salmon fecundity in New Brunswick.

$$(6.15) \quad \varphi = \sum_{i=1}^{I=2} e^{6.06423+0.03605(FL_S(i))} P_{Female}(i) Wt_{EggS}(i)$$

where $FL_S(i)$ is the fork length of either small or large salmon (small, 54.5 cm; large, 80.9 cm; Reid and Chaput 2012), P_{Female} is the proportion of the population that is female (small, 0.213; large, 0.787; Chaput et al. 1999, 2010), Wt_{EggS} is the mean weight of an egg from either small or large females (small, 0.19 g; large, 0.3 g; K.M. Samways, unpubl. data).

$NMilt_S$ is the contribution of milt to the nutrient pool, where $P_{Effective\ Male}$ is the proportion of wet weight of testes lost during spawning (0.68; Idler and Clemens 1959), μ_S is the grams of nutrient per gram of wet weight of milt (0.20 for nitrogen and 0.012 for phosphorous based on the same N:P ratio as eggs; see Johnston et al. 2004), and P_{Male} is the proportion of males in the population of a given size.

$$(6.16) \quad NMilt_S$$

$$= (1 - M_{SSp}) P_{Effective\ Male} \beta_{Male} \mu_S \left(\sum_{i=1}^{I=2} P_{Size}(i) Wt_{SSp}(i) P_{Male}(i) \right)$$

The mass of nutrients exported from the river by salmon smolts ($N_{Juveniles_{Smolt}}$) was modeled as

$$(6.17) \quad N_{Juveniles_{Smolt}} = N_{Smolt} \psi_S Wt_{Smolt}$$

where N_{Smolt} is a density-dependent function that relates egg abundance to smolt survival (see Jonsson et al. 1998, Cunjak and Therrien 1998), and ψ_S is the grams of nutrient per gram of wet weight (0.025 for nitrogen and 0.0045 for phosphorous by wet weight; Lyle and Elliot 1998).

6.3.2.2 Alewife MDN loading model

The mass of nutrients contributed by adult alewife mortality ($NCarc_A$) was modeled as

$$(6.18) \quad NCarc_A = N_{ASp} \times Wt_{ASp} \times M_{ASp} \times \lambda_A$$

where N_{ASp} is the number of spawners, Wt_{ASp} is the average weight of an adult alewife (341.9g; Gibson and Myers 2001, K.M. Samways, unpubl. data), M_{ASp} is the freshwater spawner mortality rate (0.56; Kissil 1974, West et al. 2010), λ_A is the nutrient content per weight (0.0249 for nitrogen and 0.0042 for phosphorous, grams of nutrient per gram of wet weight; Durbin 1979).

Nutrient contributions from direct excretion (NEX_A) were modeled using the excretion rate (ε) (0.00002471 for nitrogen and 0.00000217 for phosphorous, grams of nutrient per gram of wet weight per hour; Post and Walters 2009).

$$(6.19) \quad NEX_A = (1 - M_{ASp}) \times N_{ASp} \times Wt_{ASp} \times \varepsilon \times t$$

The mass of nutrients contributed from gametes ($NEgg_A$ and $NMilt_A$) was modeled as

$$(6.20) \quad NEgg_A = (1 - M_{ASp}) \times \frac{1}{2}N_{ASp} \times \gamma \times Wt_{EggA} \times \Phi_A$$

where γ , the average fecundity for a female was based on West et al. (2010), using a mean fork length (FL_A) of 25.5 cm (Gibson and Myers 2001, K.M. Samways, unpubl. data) and was calculated as

$$(6.21) \quad \gamma = (3596 \times FL_A) - 766187$$

Wt_{EggA} is the mass of an egg (0.00012 g; West et al. 2010) and ϕ_A is the nutrient content of an egg (0.03312 for nitrogen and 0.003165 for phosphorous, gram of nutrient per gram of egg; Durbin 1979, West et al. 2010). The nutrient contribution from milt was modeled using the change in the mass of the testes ($Wt_{\Delta Testes}$; see West et al. 2010) to estimate the amount of milt released during spawning and was calculated as

$$(6.22) \quad NMilt_A = (1 - M_{ASp}) \times \frac{1}{2}N_{ASp} \times Wt_{\Delta Testes} \times \mu_A$$

where $Wt_{\Delta Testes}$ was calculated as

$$(6.23) \quad Wt_{\Delta Testes} = (0.157 \times FL_A) - 28.563$$

and μ_A is the quantity of nutrient per unit weight of milt (0.03945 for nitrogen and 0.009495 for phosphorous, gram of nutrient per gram of milt; Durbin 1979, West et al. 2010).

The mass of nutrients exported from the river by young-of-the-year (YOY) alewives ($N_{Juveniles_{YOY}}$) was modeled as

$$(6.24) \quad N_{Juveniles_{YOY}} = N_{ASp} \times Prod_{YOY} \times Wt_{YOY} \times \psi_A$$

where $Prod_{YOY}$ is the number of YOY that survive per spawner, taking density-dependence into consideration (63.9; West et al. 2010). We feel this is a conservative estimate given that other studies have reported juvenile production between 73.88 and

781 juveniles per adult (Havey 1973, Walton 1987, Gibson and Myers 2003), whereas the Mactaquac Headpond on the Saint John River, which is an artificial environment created as a result of damming for hydroelectric power, had an estimated juvenile production of 32.79 per adult (Gibson and Myers 2003). $W_{t_{YOY}}$ is average weight of YOY in the Miramichi River (2.91 g; K.M. Samways, unpubl. data), and ψ_A is the nutrient content of YOY (0.0344 for nitrogen and 0.0058 for phosphorous, gram of nutrient per gram of YOY; based on the same N:P ratio as the adults, see West et al. 2010).

6.3.3 Sensitivity Analysis

A local sensitivity analysis was conducted on both the Atlantic salmon and the alewife nutrient flux models to determine the relative importance of each model parameter to the estimation of net nutrient contribution (Ellner and Guckenheimer 2006). The sensitivity analysis measures the percent change in the model output for a $\pm 10\%$ change in a given parameter, with all other parameters held constant. The centred distance estimate of net MDN flux is

$$(6.25) \quad s_i = \frac{Y(1.1 \times p_i) - Y(0.9 \times p_i)}{0.2 \times Y(p_i)}$$

where s_i is the sensitivity of the model in response to the i th parameter p_i being adjusted, and $Y()$ is the net MDN flux calculated from the output of the model. This is a relative measure of sensitivity among the parameters (i.e. the largest value has the most impact in affecting net nutrient contribution).

6.4 Results

6.4.1 Equilibrium Spawning Population

The equilibrium spawning population estimate for Atlantic salmon (ESP_S) in the Miramichi River was estimated to be 1.6 million individuals, with a biomass of 4709 tons. For alewives, the estimated equilibrium spawning population (ESP_A) was 12.7 million individuals or a biomass of 4327 tons. However, when blueback herring were included, the estimated equilibrium biomass of gaspereau was estimated to be 6922 (± 1300) tons or 20.25 (± 3.8) million individuals. These equilibrium spawning population estimates represent the optimum historic values prior to any significant human impact, compared to recent spawning escapement estimates which are only 0.2% and 1.5% for salmon and alewife respectively (79,970 salmon (DFO 2014) representing 271.8 tons in 2013; 300,000 alewife (Chaput and Atkinson 2001) representing 102.6 tons in 1996). Including blueback herring, results in returns of gaspereau in 1996 that were 700,000 individuals (Chaput and Atkinson 2001) with an estimated biomass of 239.3 tons or 3.5% ($\pm 1\%$) of historic estimates.

Using historical data to model changes at the population level can be problematic (Myers 2000, Dayton et al. 1995), however this data is critical to many ecological questions. Comparisons between landings and escapement for Atlantic salmon and gaspereau (Fig. 6.3) show that the degree of difference between the two metrics can be large at times (e.g. 163 tons and 2987 tons, respectively), however they still serve as a reliable proxy given the differences from historic population estimates are orders of

magnitude greater (it should be noted that commercial fishing for Atlantic salmon ceased in 1984 at which point landings consistently underestimate escapement; Fig. 6.3).

6.4.2 Marine-derived nutrient loading models

Based on the equilibrium spawning population estimates, Atlantic salmon would have imported 189.7 tons of N and 17.7 tons of P, while only 0.5 tons of N and 0.08 tons of P would have been exported through juvenile out migration. Alewife would have imported 76.2 tons of N and 11.7 tons of P, whereas they would have exported 80.9 tons of N and 13.6 tons of P. Including blueback herring resulted in gaspereau importing 121.9 (± 22.9) tons of N and 18.7 (± 3.5) tons of P and exporting 129.5 (± 24.2) tons of N and 21.8 (± 4.1) tons of P. In general, Atlantic salmon appear to be net nutrient importers and alewives (gaspereau) net nutrient exporters.

Nutrient loading estimates based on landings were less variable and generally more conservative than those made using escapement data. Nutrient contributions from Atlantic salmon based on landings (1971-2013) varied between 5.4 and 0.3 tons of N and 0.03 and 0.5 tons of P, representing between 2.8 and 0.16% of optimum historic nutrient contributions, respectively (Fig. 6.4). Using escapement data for the same time, nutrient contributions were between 6.2 and 1.1% of the optimum historic estimates (12.4 and 2.2 tons of N and 1.2 and 0.2 tons of P) (Fig. 6.4). Using either landings or escapement metrics, nutrient exports did not exceed 0.04 tons of N and 0.007 tons of P.

Gaspereau would have imported 38.1 to 43.2 tons of N and 6.0 to 6.6 tons of P between 1982 and 1996 using landings data as a proxy, whereas imports varied between 45.1 and 91.6 tons of N and 6.9 and 14.0 tons of P based on spawning escapement (Fig.

6.5). This equates to 23.8 to 26% and 27.0 to 42.9% of optimum historic nutrient contributions from gaspereau, relative to landings and escapement data respectively. Nutrient export estimates based on landings varied between 41.3 and 45.9 tons of N and 7.0 and 7.3 tons of P (Fig. 6.5). Escapement data resulted in nutrient exports between 48.0 and 97.2 tons of N and 8.1 and 16.4 tons of P.

Marine-derived nutrient loading estimates based on historic landings depict different trends through time. For Atlantic salmon, nutrient imports were still relatively high in the early 1800's (as much as 66.5 tons of N and 6.2 tons of P), falling to 2.8 and 0.3 tons of N and P respectively by the closure of the Atlantic salmon commercial fishery in 1984 (Fig. 6.6). With gaspereau, nutrient imports in the mid-1800' fell to low 1.2 tons of N and 0.2 tons of P (Fig. 6.7). Unlike Atlantic salmon, the gaspereau population was able to recover and nutrient inputs reached an estimated high in 1953 of 77.6 tons of N and 11.9 tons of P, and another high in 1980 (66.3 tons of N and 10.1 tons of P).

6.4.3 Sensitivity Analysis

For the Atlantic salmon MDN nutrient loading model, large salmon fork length and egg weight (wt), were the most sensitive parameters, with sensitivities of 2.89 and 1.51, respectively (Table 6.1). There were seven more parameters that had sensitivities of 0.98 and seven more parameters with sensitivities ranging from 0.91 to 0.51. The proportion of small females had a small impact on the model (sensitivity of 0.25), with sixteen more parameters having little impact on the model (Table 1).

For the alewife MDN nutrient loading model, juvenile survival and juvenile weight had greatest impact on the model (sensitivities of 0.99 and 0.91, respectively

(Table 1). Juvenile P concentration, which is affected by juvenile weight, was also quite sensitive (0.91). Four other parameters had sensitivities between 0.87 and 0.61, while 12 parameters had only a slight impact on the model (Table 1).

6.5 Discussion

Atlantic salmon and alewives in the Miramichi River have undergone precipitous declines from the pre-18th century population levels that once existed. The use of commercial landings was not intended to represent exact figures, but rather a relative metric of change in biomass and population size for Atlantic salmon and alewives in the Miramichi River. The estimated current levels of MDN from Atlantic salmon and gaspereau in the Miramichi River are at 2.1 and 3.3% of historic levels, respectively. This reduction began in the mid 1770's with the onset of commercial fishing, with peak harvests in the mid 1880's, as well as high harvests of gaspereau in 1953, 1979-1980, and 2002. The cumulative loss of MDNs from historical equilibrium population estimates for N and P is 303.3 and 35.4 tons, respectively. This loss of MDNs in the Miramichi River is only reflective of Atlantic salmon and gaspereau, albeit these species are the most abundant in terms of biomass/nutrients.

The annual influx of spawning salmon and gaspereau constitutes a substantial contribution of MDNs to the Miramichi River catchment. However, the outmigration of smolts and especially juvenile gaspereau, results in nutrient export that exceeds net import. Collectively, salmon and alewives historically would have contributed 311.6 tons of N and 36.4 tons of P to the Miramichi River, but gaspereau are net nutrient exporters, making nutrient contributions -129.9 tons of N and -21.9 tons of P, an overall net loss to

the river system. Even when considering other anadromous fishes, the results (i.e. a net export of nutrients) would likely be the same. Rainbow smelt and sea lamprey are two other anadromous species commonly found in the Miramichi River and based on their life histories, would be net nutrient importers. Blueback herring and American shad have a very similar life history to alewives making them nutrient exporters. Striped bass are another common anadromous species found in the Miramichi River, but given that they spawn near the head of tide, they contribute little to freshwater productivity. Combined, anadromous fishes would deliver MDNs spanning the entire year, with two peak periods of MDN inputs (spring and autumn, Fig. 6.8) and historically would have likely resulted in a more balanced input of nutrients to the system, driven by the historically large salmon biomass. Under such a scenario, we believe that a positive feedback loop existed in the Miramichi River wherein increasing numbers of returning adults transported more MDNs, thereby driving increased freshwater productivity and subsequently increasing recruitment (Wipfli et al. 2003). The ecological effects of MDNs from multiple anadromous species may act in an additive or synergistic manner to maintain freshwater productivity and richness. The capacity for anadromous fish populations to recover from perturbations imposed by overfishing and stream disturbance is likely impaired by the declining nutrient status of their nursery streams, which may result in a decrease in the resilience to recover.

Diminishing anadromous populations and cumulative losses in MDNs, have likely shifted the nutrient balance, leading to declines in freshwater productivity and species diversity. This process has been described as a negative feedback loop (Achord et al. 2003, Wipfli et al. 2003). We argue that this is in fact ‘positive feedback’ and not

‘negative feedback’ as it is a process that destabilizes rather than stabilizes. Negative feedbacks result from mechanisms that counteract perturbations and shift populations in the direction of equilibrium (e.g. resource limitations and other negative density dependent processes). The processes that lead to positive synergies, such as mutualistic interactions, positive density dependence (e.g. Allee effects), and synergies (e.g. MDN subsidies) are prime examples of positive feedback, because when perturbed, the perturbations tend to grow rather than dampen, which is indeed the concern.

Annual pulses of MDNs to freshwater ecosystems by anadromous fishes constitute an ecologically important energy and nutrient subsidy to rivers, having ecosystem wide effects. Large, infrequent MDN pulses, such as those from Pacific salmon are known to increase productivity, species richness and abundance (Wipfli et al. 1998, Chaloner et al. 2004, Wipfli et al. 2010, Rüegg et al. 2012, Levi et al. 2013). Temporal MDN pulses from Atlantic anadromous fishes are also known to be very important to freshwater ecosystems (Durbin et al. 1979, Jardine et al. 2009, Nislow and Kynard 2009, Walters et al. 2009, Samways et al. 2015, Samways and Cunjak 2015). Cumulatively, these individual pulses may have been as large, at least historically, as their Pacific counterparts.

With the significant decline of anadromous populations, the once much larger spatio-temporal MDN pulses have been diminished into a series of smaller, infrequent, and/or disrupted MDN pulses such as observed in the Miramichi system (Fig. 6.8). Weber and Brown (2013) demonstrated that the magnitude of nutrient availability and freshwater production was generally greater in pulsed rather than continuous subsidy systems. Having multiple anadromous species spawning in the same river increases the

frequency and magnitude of MDN pulses, maximizing the productivity potential of the river. Atlantic anadromous fishes are commonly viewed as functionally independent, as many studies involving Atlantic MDN inputs focus on single species interactions (e.g. Durbin et al. 1979, Nislow et al. 2004, Nislow and Kynard 2009, Walters et al. 2009). However, their relatedness and potential interdependence may play a bigger role in freshwater productivity than previously thought. Nutrients from anadromous fishes may arrive at a critical period of increased metabolic and freshwater productivity is declining (Hall 1972). Spatio-temporal spawning and life history strategies of Atlantic anadromous fishes ensure that subsequent cohorts are well matched to favourable survival conditions.

The loss of anadromous fishes and their ecosystem functions can result in a decrease in the resilience of the ecosystem to recover. A large adult biomass means increased MDN subsidies, serving as a mechanism of resiliency in freshwater ecosystems (e.g. productivity and richness) by maintaining balanced nutrient loads (Bottom et al. 2009). Significant reductions in salmon and alewife populations from ESP estimates (98% and 88%, respectively) in the Miramichi River have greatly reduced MDN availability by two orders of magnitude. The loss of all or many anadromous fishes results in a decrease in the recovery potential of any single species (i.e. Atlantic salmon) (Worm et al. 2006).

A strong spawning class is expected to sustain recruitment and contribute to subsequent strong year classes (Secor 2007). Most Atlantic anadromous fishes are iteroparous (Scott and Scott 1988), allowing adults to survive over long periods, producing overlapping generations, and an accumulation of adult biomass. The MDN biomass potential stored in a large adult biomass helps to ensure future recruitment.

In many ecosystems, natural drivers of ecosystem function (i.e. nutrient loading) have been replaced by human activities. Anthropogenic nutrient inputs from fertilizer and agricultural run-off, animal waste, industrial effluent and sewage disposal can constitute a significant source of nutrients, and potentially replace natural nutrient subsidies (Polis et al. 1997, Nixon 2003). Despite the size of the Miramich River basin (14,000 km²), most of the human population, and the industrial and commercial activity, are located around the estuary, therefore not a likely significant source of nutrient loading to the river. Forestry on the other hand, is ubiquitous throughout the region and has the potential to be the largest influence on nutrient loading in the catchment. Between 2001 and 2014, an estimated 1,779 km² of forest (12.7% of the catchment) was harvested, primarily following the practice of clearcutting (Fig. 6.9) (Hansen et al. 2013, Linke et al. 2017). Studies have shown that clearcutting adjacent to waterways, even with buffer zones, can increase the concentration of dissolved nutrients (e.g. NO₃⁻, NH₄⁺, PO₄⁻) in stream waters (Jewett et al. 1995, Swank et al. 2001), although increases can be short-lived (Löfgren, et al. 2009) or even absent (even though significant proportion of the catchments were clear-cut) (Palviainen et al. 2015). Based on harvest rates in the Miramichi River catchment from 2001-2014 and nutrient leaching estimates from forestry practices (see Lepistö 2007, Löfgren et al. 2009), forestry activities may have contributed between 7.4 to 35.6 tonnes of N and 0.08 to 1.1 tonnes of P per year over that period. This represents as much as 5 times the amount of N and twice as much P currently being delivered by anadromous fishes, yet only 12% and 4% of the historic marine-derived N and P inputs, respectively. Although forestry is contributing to the

overall nutrient load of the river system, it does not appear to be enough to replace the nutrients lost from declining anadromous populations.

Using equilibrium models based on stock-recruitment parameters allowed us to estimate the adult biomass of Atlantic salmon and alewife populations in the Miramichi River prior to exploitation (i.e. recruitment and mortality are equal). Changes in some of the model parameters could significantly affect the equilibrium population estimates. For example, a 2% increase in at-sea survival would double the equilibrium population estimate. However, these equilibrium population estimates are still orders of magnitude greater than the current populations and therefore conclusions drawn from the decline in nutrient contributions still apply. Similarly, the MDN nutrient loading models had many life-history parameters that were sensitive to changes in their values. Given that the values used were based on estimates from the recent era, they are likely conservative since many of these parameters were much higher historically. For example, in the Atlantic salmon MDN loading model, size was a sensitive parameter. In the 1800's a salmon that was in the 5-8 kg range was considered a grilse or "small salmon" (Perley 1852) whereas Reid and Chaput (2012) recently estimated large, or MSW, fish from the Miramichi River as having an average weight of 5.86 kg. As salmon were much larger historically, their nutrient contributions and relative importance would have been even greater than estimated using our models.

The story of population decline and the potential ecological toll in the Miramichi River is, unfortunately, not unique, but rather common among most Atlantic rivers. With systems that may have been dependent on MDN subsidies to maintain elevated levels of productivity, have we reached a point where populations can no longer recover? Re-

establishing anadromous fish populations (e.g. supplementation programs, reintroductions) could lead to enhanced ecosystem functioning and may serve as a recovery tool for freshwater ecosystems (Samways and Cunjak 2015). Recent recovery strategies in Atlantic Canada rivers are attempting different approaches such as releasing mature adults to spawn of their own volition in rivers and thereby providing much needed MDNs to these systems to help stimulate productivity and increase recruitment success (Clarke et al. 2016).

Historical data analysis is a powerful tool that can be used to better understand ecosystem changes that have occurred through time. Our understanding of MDNs as an ecological driver of community structure and dynamics will improve as we shift our focus from the recovery of a single species to the recovery of an ecosystem. Given the vast geographical area and taxonomically complex nature of the Miramichi River system, the processes identified here, at the catchment scale, may better represent the long-term and/or large-scale biodiversity changes that have taken place (Holyoak et al. 2005).

6.6 Acknowledgements

The authors thank Antoin O’Sullivan for his help with Miramichi River catchment commercial forestry harvest map. Financial support was provided by grants from the National Sciences and Engineering Research Council of Canada and the Canada Research Chairs Program awarded to R.A. Cunjak.

6.7 Literature Cited

Achord, S., Levin, P.S. and Zabel, R.W. 2003. Density-dependent mortality in Pacific salmon: the ghost of impacts past? *Ecology Letters*. 6:335-342.

- Aprahamian, M.W., Smith, K.M., McGinnity, P., McKelvy, S., and Taylor J. 2003. Restocking of salmonids-opportunities and limitations. *Fisheries Research*. 62:211-227.
- Bardonnet, A. and Baglinière, J-L. 2000. Freshwater habitat of Atlantic Salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* 57:497–506.
- Bilby, R.E., Fransen, B.R. and Bisson, P.A. 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.
- Bottom, D.L., Jones, K.L., Simenstad, C.A., and Smith, C.L. 2009. Reconnecting social and ecological resilience in salmon ecosystems. *Ecology and Society*. 14:5.
- Brett, J.R. 1995. Energetics. Pages 3-68 in C. Groot, L. Margolis, and W.C. Clarke (editors). *Physiological Ecology of Pacific Salmon*. UBC Press, Vancouver, BC, Canada.
- Buckley, D.E. and Winters, G.V. 1983. Geochemical transport through the Miramichi Estuary. *Canadian Journal of Fisheries and Aquatic Sciences*. 40 (Suppl. 2):162-182.
- Chadwick, E.M.P., Porter, T.R., and Downton, P. 1978. Analysis of growth of Atlantic salmon (*Salmo salar*) in a small Newfoundland river. *Journal of the Fisheries Research Board of Canada*. 35:60–68.
- Chaloner, D. T., Lamberti, G.A., Merritt, R.W., Mitchell, N.L., Ostrom, P.H., and Wipfli, M.S. 2004. Variation in responses to spawning Pacific salmon among three south-eastern Alaska streams. *Freshwater Biology* 49:587–599.
- Chaloner D T., Wipfli, M.S., and Caouette, J.P. 2002. Mass loss and macroinvertebrate colonisation of Pacific salmon carcasses in south-eastern Alaskan streams. *Freshwater Biology* 47:263–273.
- Chaput, G. 1995. Temporal distribution, spatial distribution, and abundance of diadromous fish in the Miramichi River watershed. Pages 121-139 in E.M.P. Chadwick (editor). *Water, science, and the public: the Miramichi ecosystem*. Canadian Special Publication of Fisheries and Aquatic Sciences. 123.
- Chaput, G. 2003. Estimation of mortality for Atlantic salmon (*Salmo salar* L.). In *Marine Mortality of Atlantic Salmon, Salmo salar L.: Methods and Measures*, pp. 59–82. Ed. by E. C. E. Potter, N. O Maoiléidigh, and G.Chaput. Fisheries and Oceans Canada Canadian Science Advisory Secretariat Research Document, 2003/101. iv + 213 pp.
- Chaput, G. 2010. Assessment of Atlantic salmon (*Salmo salar*) to the Miramichi River (NB) for 1998 to 2009. DFO Canadian Science Advisory Secretariat Research Document. 2010/092. iv + 70 p.
- Chaput, G. and Atkinson, G. 1997. The gaspereau fisheries (*Alosa pseudoharengus* and *A. aestivalis*) of the Miramichi River with updates on the fisheries of the Pokemouche, Tracadie and Richibucto rivers of Gulf New Brunswick. DFO Canadian Stock Assessment Secretariat Research Document. 97/75. iv + 38 p.
- Chaput, G. and Atkinson, G. 2001. The gaspereau fisheries (*Alosa pseudoharengus* and *A. aestivalis*) of the Miramichi River with updates on the fishery of the Richibucto

- River of Gulf New Brunswick. DFO Canadian Stock Assessment Secretariat Research Document. 2001/047. iv + 38 p.
- Chaput, G., Caron, F., and Marshall, L. 2003. Estimates of survival of Atlantic salmon (*Salmo salar* L.) in the first and second years at sea. In Marine Mortality of Atlantic Salmon, *Salmo salar* L.: Methods and Measures, pp. 83-109. Ed. by E. C. E. Potter, N. O Maoiléidigh, and G. Chaput. DFO Canada Canadian Science Advisory Secretariat Research Document, 2003/101. iv + 213 pp.
- Chaput, G., Douglas, S.G., and Hayward, J. 2016. Biological Characteristics and Population Dynamics of Atlantic Salmon (*Salmo salar*) from the Miramichi River, New Brunswick, Canada. DFO Canada Canadian Science Advisory Secretariat Research Document, 2016/029. v + 53 p.
- Chaput, G., Hardie, P., Hayward, J., Shaesgreen, J., and NSPA. 2002. Atlantic salmon smolt production from the Northwest Miramichi River, 1998 to 2000. Canadian Technical Report of Fisheries and Aquatic Sciences. No. 2415. +70 p.
- Chaput, G., Moore, D., Hardie, P., and Mallet, P. 2010. Information on Atlantic salmon (*Salmo salar*) from Salmon Fishing Area 16 (Gulf New Brunswick) of relevance to the development of a COSEWIC status report. DFO Canadian Science Advisory Secretariat Research Document. 2010/064. iv + 50 p.
- Chaput G., Moore, D., Hayward, J., Shaesgreen, J., and Dubee, B. 1999. Stock status of Atlantic salmon (*Salmo salar*) in the Miramichi River, 1998. DFO Canadian Stock Assessment Secretariat Research Document. 99/049. iv + 87 p.
- Chase, B.C. 2009. The spawning habitat of anadromous rainbow smelt: trouble at the tidal interface. Pages 859–862 in A.J. Haro, K.L. Smith, R.A. Rulifson, C.M. Moffitt, R.J. Klauda, M.J. Dadswell, R.A. Cunjak, J.E. Cooper, K.L. Beal, and T.S. Avery (editors). Challenges for diadromous fishes in a dynamic global environment. Symposium 69. American Fisheries Society, Bethesda, Maryland.
- Clarke, C.N., Fraser, D.J., and Purchase, C.F. 2016. Life-long and carry-over effects of early captive exposure in a recovery program for Atlantic salmon. *Animal Conservation*. 19:350-359.
- Cunjak, R.A. and Newbury, R.W. 2005. Atlantic Coast Rivers of Canada. Pages 938-980 in A.C. Benke and C.E. Cushing (editors). *Rivers of North America*. Academic Press. Burlington, Massachusetts.
- Cunjak, R.A. and Therrien, J. 1998. Inter-stage survival of wild juvenile Atlantic salmon, *Salmo salar* L. *Fisheries Management and Ecology*. 5:209-223.
- Davidson, W.H. 1947. William Davidson: 1740-1790. The North Shore Leader. Newcastle, New Brunswick.
- Dayton, P.K., Thrush, S.F., Agardy, M.T., and Hofman, R.J. 1995. Environmental effects of marine fishing. *Aquatic Conservation Marine and Freshwater Ecosystems*. 5:205-232.
- DFO 2006. Integrated Gaspereau Fishery Management Plan, Eastern New Brunswick Area, Gulf Region 2007-2012. Available at: <http://www.dfo-mpo.gc.ca/Library/349477.pdf> (last visited: April 17, 2017).
- DFO 2012. Stock status of Atlantic salmon (*Salmo salar*) in DFO Gulf Region (Salmon Fishing Areas 15 to 18). DFO Canadian Science Advisory Secretariat Science Advisory Report. 2012/040.

- DFO 2014. Stock status of Atlantic salmon (*Salmo salar*) in DFO Gulf Region (Salmon Fishing Areas 15 to 18) to 2013. DFO Canadian Science Advisory Secretariat Science Advisory Report. 2014/057.
- Douglas, S.G., Chaput, G., Hayward, J., and Sheasgreen, J. 2015. Assessment of Atlantic Salmon (*Salmo salar*) in Salmon Fishing Area 16 of the southern Gulf of St. Lawrence to 2013. DFO Canadian Science Advisory Secretariat Research Document. 2015/049. v + 36 p.
- Dunfield, R.W. 1985. The Atlantic salmon in the history of North America. Canadian Special Publication of Fisheries and Aquatic Sciences. 80:181 p.
- Durbin A.G., Nixon, S.W., and Oviatt, C.A. 1979. Effects of the spawning migration of the alewife, *Alosa Pseudoharengus*, on freshwater ecosystems. Ecology. 60:8–17.
- Ellner, S. and Guckenheimer, J. 2006. Dynamic models in biology. Princeton University Press, Princeton, N.J.
- Elson, P.F. 1967. Effects on wild young salmon of spraying DDT over New Brunswick forests. Journal of the Fisheries Research Board of Canada. 24:731-767.
- Elson, P.F., Saunders, J.W., and Zitko, V. 1972. Impact of forest-based industries on freshwater-dependent fish resources in New Brunswick. Fisheries Research Board of Canada Technical Report. 325:26 p.
- Fleming, I.A. 1996. Reproductive strategies of Atlantic salmon: ecology and evolution. Reviews in Fish Biology. 6:379-416.
- Gibson, A.J.F., Jones, R.A., and Bowlby, H.D. 2009. Equilibrium analyses of a population's response to recovery activities: A case study with Atlantic salmon. North American Journal of Fisheries Management. 29:958-974.
- Gibson, A.J.F. and Myers, R.A. 2001. Gaspereau river alewife stock status report. DFO Canadian Science Advisory Secretariat Research Document. 2001/061. iv + 43 p.
- Gibson, A.J.F. and Myers, R.A. 2003. Biological reference points for alewife (*Alosa pseudoharengus*) populations in Atlantic Canada. Canada Technical Report of Fisheries and Aquatic Sciences. No. 2468.
- Gibson, A.J.F. and Myers, R.A. 2004. Estimating reference fishing mortality rates from noisy spawner-recruit data. Canadian Journal of Fisheries and Aquatic Sciences. 61:1771-1783.
- Hall, C. A. S. 1972. Migration and metabolism in a temperate stream ecosystem. Ecology. 53:585–604.
- Hansen, M.C., Potapov, P.V., Moore, R., Hancher, M., Turubanova, S.A., Tyukavina, A., Thau, D., Stehman, S.V., Goetz, S.J., Loveland, T.R., Kommareddy, A., Egorov, A., Chini, L., Justice, C.O., and Townshend, J.R.G. 2013. High-resolution global maps of 21st-century forest cover change. Science. 342:850–853.
- Havey, K.A. 1973. Production of juvenile alewives, *Alosa pseudoharengus*, at Love Lake, Washington County, Maine. Transactions of the American Fisheries Society. 102: 434–437.
- Holyoak, M., Leibold, M.A., Mouquet, N., Holt, R.D., and Hoopes, M. 2005. A framework for large scale community ecology. Metacommunities: spatial dynamics and ecological communities. The University of Chicago Press, Chicago.
- Humphries, P.O. and Winemiller, K.O. 2009. Historical impacts on river fauna, shifting baselines, and challenges for restoration. Bioscience. 59:673-684.

- Hunstman, A.G. 1931. The Maritime salmon of Canada. Bulletin of the Biological Board of Canada. no. 21, 99 pp.
- Jardine, T. D., J-M Roussel, S. C. Mitchell, and R. A. Cunjak. 2009. Detecting Marine Nutrient and Organic Matter Inputs into Multiple Trophic Levels in Streams of Atlantic Canada and France. Pages 427-445 in A.J. Haro, K.L. Smith, R.A. Rulifson, C.M. Moffitt, R.J. Klauda, M.J. Dadswell, R.A. Cunjak, J.E. Cooper, K.L. Beal, and T.S. Avery (editors). Challenges for diadromous fishes in a dynamic global environment. Symposium 69. American Fisheries Society, Bethesda, Maryland.
- Jewitt, K., Daugherty, D., Krause, H.H., Arp, P.A. 1995. Watershed response to clearcutting: effects on soil solutions and stream water discharge in central New Brunswick. Canadian Journal of Soil Sciences. 75:475-490.
- Johnston N.T., MacIsaac, E.A., Tschaplinski, P.J., and Hall, K.J. 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.
- Jonsson, B. and Jonsson, N. 2003. Migratory Atlantic salmon as vectors for the transfer of energy and nutrients between freshwater and marine environments. Freshwater Biology. 48:21-27.
- Jonsson, N., Jonsson, B., and Hansen, L.P. 1990. Partial segregation in the timing of migration of Atlantic salmon of different ages. Animal Behaviour. 40:313-321.
- Jonsson, N., Jonsson, B., and Hansen L.P. 1998. The relative role of density-dependent and density-independent survival in the life cycle of Atlantic salmon *Salmo salar*. Journal of Animal Ecology. 67:751-762.
- Kissil, G.W. 1974. Spawning of anadromous alewife, *Alosa pseudoharengus*, in Bride Lake, Connecticut. Transactions of the American Fisheries Society. 103:312-317.
- LeBlanc, C.H. and Chaput, G.J. 1991. Landings of estuarine fishes in the Gulf of St. Lawrence 1917 - 1988. Canadian Data Report of Fisheries and Aquatic Sciences. 842:101 p.
- Lepistö, A. 2007. Methods for estimating excess nitrogen and phosphorus leakage from forestry in Finland. Pages 18-23 in S. Löfgren (editor). How to estimate N and P losses from forestry in northern Sweden. KSLA Tidskrift 146.
- Levi, P.S., Tank, J.L., Rüegg, J.D., Janetski, J.S., Tiegs, D., Chaloner, D.T., and Lamberti, G.A. 2013. Whole-stream metabolism responds to spawning Pacific salmon in their native and introduced ranges. Ecosystems 16:269-283.
- Limburg, K.E. and Waldman, J.R. 2009. Dramatic Declines in North Atlantic Diadromous Fishes. Bioscience. 59:955-965.
- Linke, J., Fortin, M-J., Courtenay, S., and Cormier, R. 2017. High-resolution global maps of 21st-century annual forest loss: Independent accuracy assessment and application in a temperate forest region of Atlantic Canada. Remote Sensing of Environment 188:164-176.
- Löfgren, S., Ring, E., von Brömssen, C., Sørensen, R., and Högbom, L. 2009. Short-term effects of clear-cutting on the water chemistry of two boreal stream in Northern Sweden: A paired catchment study. Ambio. 38:347-356.
- Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A., Hooper,

- D.U. Huston, M.A., Raffaelli, D., Schmid, B., Tilman, D., and Wardle, D.A. 2001. Biodiversity and Ecosystem Functioning: Current Knowledge and Future Challenges. *Science*. 294:804-808
- Lyle, A.A. and Elliott, J.M. 1998. Migratory salmonids as vectors of carbon, nitrogen and phosphorus between marine and freshwater environments in north-east England. *Science of the Total Environment*. 210:457-468.
- Marshall, T.L., Jones, R.A., and Pettigrew, T. 1997. Status of Atlantic salmon stocks of southwest New Brunswick, 1996. DFO Canadian Stock Assessment Secretariat Research Document. 97/27. iv + 70 p.
- Mather, M.E., Frank, H. J., Smith, J.M., Cormier, R.D., Muth, R.M., and Finn. J.T. 2012. Assessing freshwater habitat of adult anadromous alewives using multiple approaches. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science* 4:188-200.
- McKenzie, R.A. 1964. Smelt life history and fishery in the Miramichi River, New Brunswick. Bulletin No. 144. Fisheries Research Board of Canada, St Andrews, New Brunswick.
- Monro, A. 1855. New Brunswick, with a brief outline of Nova Scotia and Prince Edward Island. Printed by R. Nugent. Halifax, Nova Scotia.
- Mullen, D.M., Fay, C.W., and Moring, J.R. 1986. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (North Atlantic) - alewife/blueback herring. Biological Report 82(11.58). US Fish and Wildlife Service, Washington, DC.
- Myers, R.A. 2000. The synthesis of dynamic and historical data on marine populations and communities; putting dynamics into the Ocean Biogeographical Information System (OBIS). *Oceanography*. 13:56-59.
- Nislow, K., Armstrong, J., and McKelvey, S. 2004. Phosphorus flux due to Atlantic salmon (*Salmo salar*) in an oligotrophic upland stream: effects of management and demography. *Canadian Journal of Fisheries and Aquatic Sciences*. 61:2401-2410.
- Nislow, K.H. and Kynard, B.E. 2009. The role of anadromous sea lamprey in nutrient and material transport between marine and freshwater environments. Pages 485-494 in A.J. Haro, K.L. Smith, R.A. Rulifson, C.M. Moffitt, R.J. Klauda, M.J. Dadswell, R.A. Cunjak, J.E. Cooper, K.L. Beal, and T.S. Avery (editors). Challenges for diadromous fishes in a dynamic global environment. Symposium 69. American Fisheries Society, Bethesda, Maryland.
- Nixon, S. 2003. Are anthropogenic nutrients providing the fertility once brought to the Mediterranean by a great river? *Ambio*. 32:30-39.
- Palmer, M., Bernhardt, E., Chornesky, E., Collins, S., Dobson, A., Duke, C., Gold, B., Jacobson, R., Kingsland, S., Kranz, R., Mappin, M., Martinez, M.L., Micheli, F., Morse, J., Pace, M., Pascual, M., Palumbi, S., Reichman, O.J., Simons, A., Townsend, A., Turner, M. 2004. Ecology for a Crowded Planet. *Science*. 304:1251-1252.
- Palviainen, M., Finér, L., Laurén, A., Mattsson, T., and Högbom, L.. 2015. A method to estimate the impact of clear-cutting on nutrient concentration in boreal headwater streams. *Ambio*. 44:521-531.

- Pauly, D. 1995. Anecdotes and the shifting baseline syndrome of fisheries. *Trends in Ecology and Evolution*. 10:430.
- Perley, M.H. 1852. Reports on the sea and river fisheries of New Brunswick. J. Simpson Printer to the Queen's majesty. Fredericton, New Brunswick.
- Pinnegar, J.K. and Engelhard, G.H. 2008. The 'shifting baseline' phenomenon: A global perspective. *Reviews of Fish Biology and Fisheries*. 18:1-16.
- Polis, G.A., Anderson, W.B. & Holt, R.D. (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.
- Post, D.M. and Walters, A.W. (2009) Nutrient excretion rates of anadromous alewives during their spawning migration. *Transactions of the American Fisheries Society*. 138:264-268.
- Randall, R.G., Pickard, P.R., and Moore, D. 1989. Biological assessment of Atlantic salmon in the Miramichi River, 1988. Canadian Atlantic Fisheries Science Advisory Communication Research Document. 89/73: 36p.
- Reid J.E. and Chaput G. (2012) Spawning history influence on fecundity, egg size, and egg survival of Atlantic salmon (*Salmo salar*) from the Miramichi River, New Brunswick, Canada. *ICES Journal of Marine Science*. 69:1678-1685.
- Rostlund, E. 1952. Freshwater fish and fishing in native North America. (University of California Publications in Geography, Vol. 9). University of California Press, Berkeley and Los Angeles.
- Rüegg, J., Chaloner, D.T., Levi, P.S., Tank, J.L., Tiegs, S.D., and Lamberti, G.A. 2012. Environmental variability and the ecological effects of spawning Pacific salmon on stream biofilm. *Freshwater Biology* 57:129–142.
- Samways K.M. and Cunjak R.A. 2015. Increases in benthic community production and metabolism in response to marine-derived nutrients from spawning Atlantic salmon (*Salmo salar*). *Freshwater Biology*. 60:1647-1658.
- Samways K.M., Quiñones-Rivera, Z. J., Leavitt P.R., and Cunjak R.A. 2015. Spatio-temporal responses of algal, fungal and bacterial biofilm communities in Atlantic rivers receiving marine-derived nutrient inputs. *Freshwater Science*. 34:881-896.
- Samways, K.M., Blair, T.J., Charest M.A., and Cunjak R.A. 2017. Effects of spawning Atlantic salmon (*Salmo salar*) on total lipid content and fatty acid composition of river food webs. *Ecosphere*. DOI: 10.1002/ecs2.1818.
- Saunders, R.L. and Sprague, J.B. 1967. Effects of copper-zinc mining pollution on a spawning migration of Atlantic salmon. *Water Research*. 1:419-432.
- Schiefer, K.L., LeBlanc, C.A., Rodger, B.T., Whyte, R.J., McKee, P.M., and Burt, A.J. 1992. Environmental surveys of the Northwest Miramichi River system following a discharge by Heath Steel Mines, 1991. Report prepared by Beak Consultants Ltd.
- Scott, W.B., and Scott, M.G. 1988. Atlantic fishes of Canada. Canadian Bulletin of Fisheries and Aquatic Sciences No. 219. University of Toronto Press, Toronto, Ontario.
- Secor, D.H. 2007. The year-class phenomenon and the storage effect in marine fishes. *Journal of Sea Research*. 57:91-103.

- Swank, W. T., Vose, J. M., and Elliot, K.J. 2001. Long-term hydrologic and water quality responses following commercial clearcutting of mixed hardwoods on a southern Appalachian catchment. *Forest Ecology and Management*. 143:163-178.
- Vanni M.J. 2002. Nutrient cycling by animals in freshwater ecosystems. *Annual Review of Ecology and Systematics*. 33:341–370.
- Walters, A.W., Barnes, R.T., and Post, D.M. 2009. Anadromous alewives (*Alosa pseudoharengus*) contribute marine-derived nutrients to coastal stream food webs. *Canadian Journal of Fisheries and Aquatic Sciences*. 66:439–448.
- Walton, C. J. 1987. Parent-progeny relationship for an established population of anadromous alewife in a Maine lake. *American Fisheries Society Symposium*. 1:451-454.
- Weber, M. J., and Brown, M. L. 2013. Continuous, pulsed and disrupted nutrient subsidy effects on ecosystem productivity, stability, and energy flow. *Ecosphere*. 4:27
- West, D.C., Walters, A.W., Gephart, S., and Post, D.M. 2010. Nutrient loading by anadromous alewife (*Alosa pseudoharengus*): contemporary patterns and predictions for restoration efforts. *Canadian Journal of Fisheries and Aquatic Sciences*. 67:1211-1220.
- Wipfli, M.S., Hudson, J., and Caouette, J. 1998. Influence of salmon carcasses on stream productivity: response of biofilm and benthic macroinvertebrates in southeastern Alaska, U.S.A. *Canadian Journal of Fisheries and Aquatic Sciences*. 55:1503–1511.
- Wipfli, M.S., Hudson, J., and Caouette, J. 2003. Marine subsidies in freshwater ecosystems: salmon carcasses increase the growth rates of stream-resident salmonids. *Transactions of the American Fisheries Society*. 132:371–381.
- Wipfli, M.S., Hudson, J.P., Caouette, J.P., Mitchell, N.L., Lessard, J.L., Heintz, R.A., and Chaloner, D.T. 2010. Salmon carcasses increase stream productivity more than inorganic fertilizer pellets: a test on multiple trophic levels in streamside experimental channels. *Transactions of the American Fisheries Society*. 139:824–839.
- Wipfli, M.S., Hudson, J.P., Chaloner, D.T., and Caouette, J.P. 1999. The influence of salmon spawner densities on stream productivity in southeast Alaska. *Canadian Journal of Fisheries and Aquatic Sciences*. 56:1600–1611.
- Wood, C.M. 1995. Excretion. Pages 381-438 in C. Groot, L. Margolis, and W.C. Clarke (editors). *Physiological Ecology of Pacific Salmon*. UBC Press, Vancouver, BC, Canada.
- Worm, B., Barbier, E.B., Beaumont, N., Duffy, J.E., Folke, C., Halpern, B.S., Jackson, J.B.C., Lotze, H.K., Micheli, F., Palumbi, S.R., Sala, E., Selkoe, K.A., Stachowicz, J.J., and Watson, R. 2006. Impacts of Biodiversity Loss on Ocean Ecosystem Services. *Science*. 314:787-790.

Table 6.1. Results of the sensitivity analysis where s_i represents the percent change in the model output for a $\pm 10\%$ change in a given parameter.

Atlantic Salmon		Alewife	
Parameter	s_i	Parameter	s_i
Large Fork Length	2.8857696	Juvenile Survival	0.9851330
Large Egg Wt	1.5142193	Juvenile Wt	0.9140893
Number of Adults	0.9760975	Juvenile P Concentration	0.9137931
Proportion of Small Adults	0.9758489	Adult P Concentration	0.8711285
Egg N Concentration	0.9758386	Juvenile N Concentration	0.8343023
Small Adult Wt	0.9757943	Adult N Concentration	0.7884054
Small Egg Wt	0.9756734	Adult Mortality	0.6131067
Proportion of Male Small	0.9756725	Excretion Rate of N	0.1770436
Fecundity of Large	0.9755914	Time in Freshwater	0.1394455
Egg P Concentration	0.9755156	Fecundity	0.1392202
Smolt Survival	0.9090909	Excretion Rate of P	0.1018474
Smolt N Concentration	0.8823529	Adult Wt	0.0310986
Smolt Wt	0.8634146	Number of Adults	0.0307876
Smolt P Concentration	0.7222222	Egg N Concentration	0.0244031
Female GSI	0.6215250	Egg Wt	0.0198426
Adult Mortality	0.5727814	Egg P Concentration	0.0152784
Effective Female	0.5123531	Milt P Concentration	0.0117458
Proportion of Female Small	0.2469078	Milt N Concentration	0.0101442
Adult P Concentration	0.0005649	Wt Change in Testes	0.0050721
Time in Freshwater	0.0002873		
Large Adult Wt	0.0002698		
Proportion of Large Adults	0.0002486		
Adult N Concentration	0.0002336		
Early/Late Run	0.0002332		
Excretion Rate of P	0.0001273		
Small Fork Length	0.0001126		
Excretion Rate of N	0.0000636		
Fecundity of Small	0.0000570		
Proportion of Female Large	0.0000398		
Milt N Concentration	0.0000213		
Effective Male	0.0000211		
Milt P Concentration	0.0000210		
Proportion of Male Large	0.0000201		
Male GSI	0.0000182		

Figure 6.1. Map of the Miramichi River catchment, New Brunswick, Canada
showing its size and accessibility to anadromous fishes.

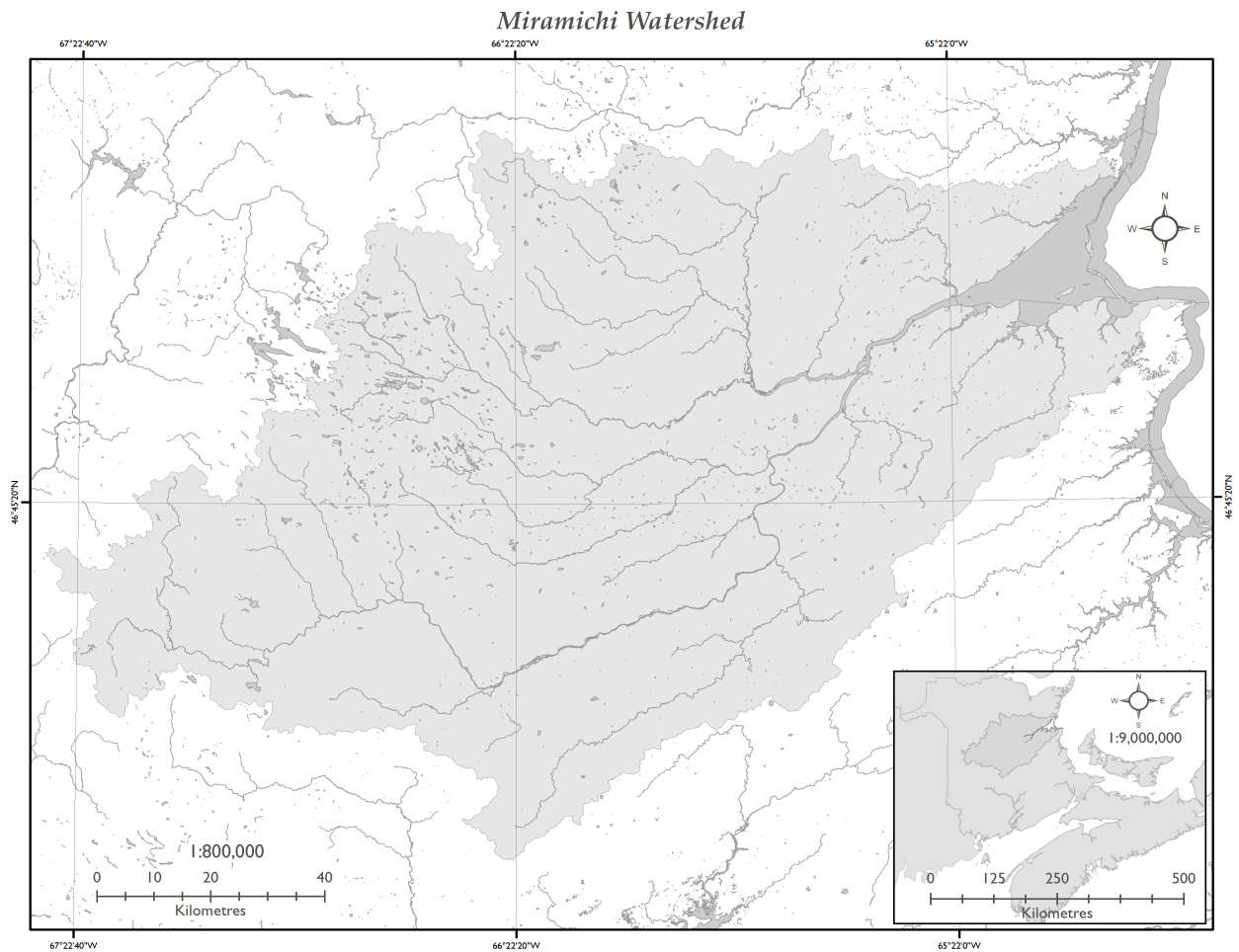


Figure 6.2. Atlantic salmon (diamond symbols and black line) and gaspereau (collective term for alewives and blueback herring) (square symbols and blue line) landings, in the Miramichi River. Values with associated error bars represent the maximum and minimum landing estimates surrounding the mean (symbol) as historic data for these time periods was commonly recorded as a range rather than absolute values.

Atlantic Salmon

1763-1777 – Perley 1852
1784-1788 – Dunfield 1985
1789-1851 – Perley 1852 and Dunfield 1985
1852-1864 – Dunfield 1985
1870-1930 – Huntsman 1931
1931-1946 – Dominion of Canada Fisheries Statistics
1951-1984 – Randall et al. 1989
1970-2010 – DFO 2012
1971-2007 – Chaput et al. 2010
1998-2003 – Chaput et al. 1999
1998-2009 – Chaput 2010
2010-2013 – DFO 2014
1998-2013 – Douglas et al. 2015

Gaspereau

1798-1849 – Perley 1852
1917-1988 – Leblanc and Chaput 1991
1978-1996 – Chaput and Atkinson 1997
1981-2000 – Chaput and Atkinson 2001
1994-2004 – DFO 2006

[Figure on following page]

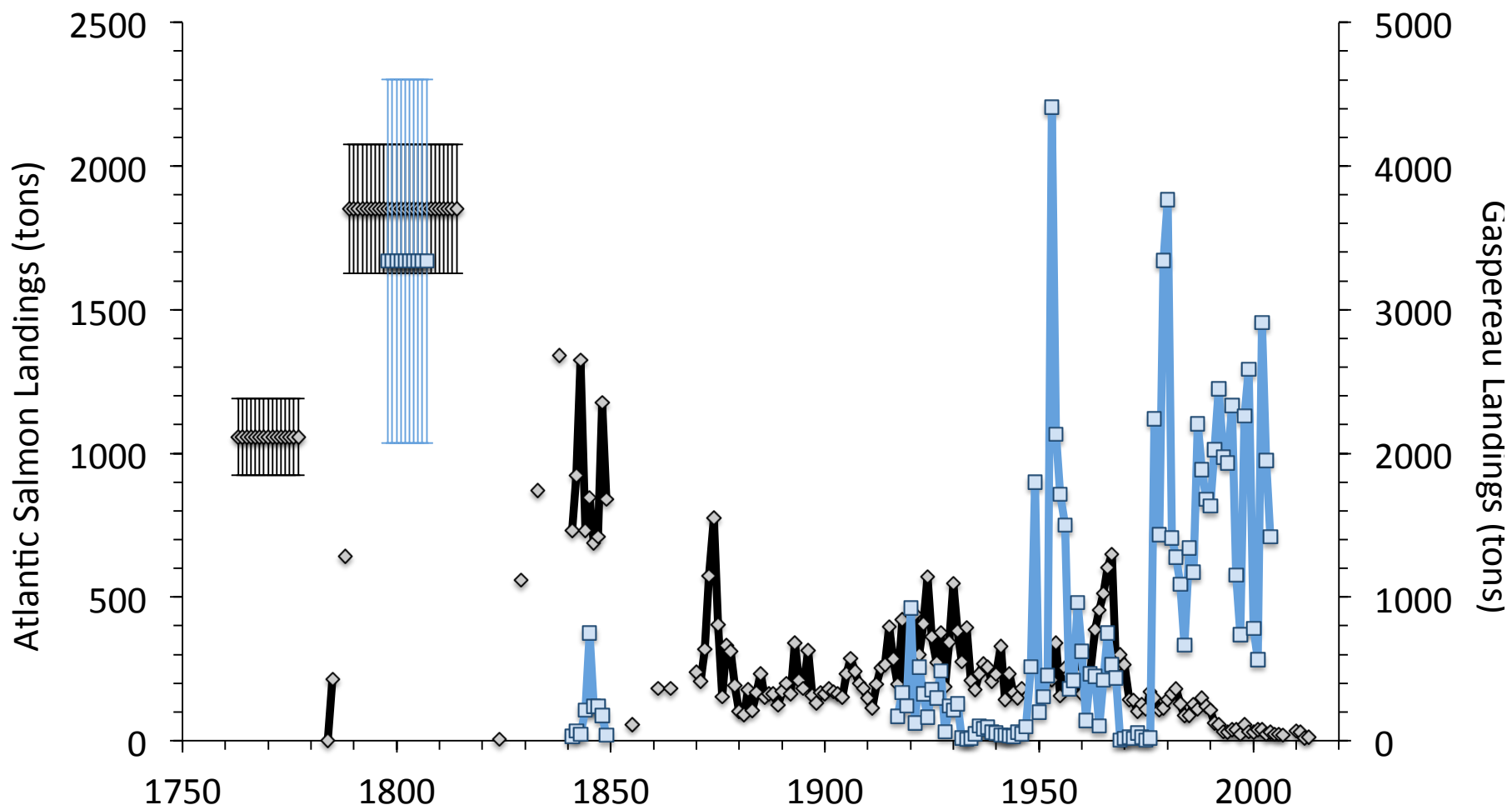


Figure 6.3. Figure 3: Landings and escapement for a) Atlantic salmon and b) gaspereau. Diamond symbols with the solid black line represent landings. For Atlantic salmon the triangles with solid blue line represent total salmon escapement, circles and large dashed grey line represents one-sea winter spawners, and the squares and small dashed light blue line represents multi-sea winter spawners. For gaspereau the triangles with solid blue line represent total gaspereau escapement, circles and large dashed grey line represents alewife spawners, and the squares and small dashed light blue line represents blueback herring spawners.

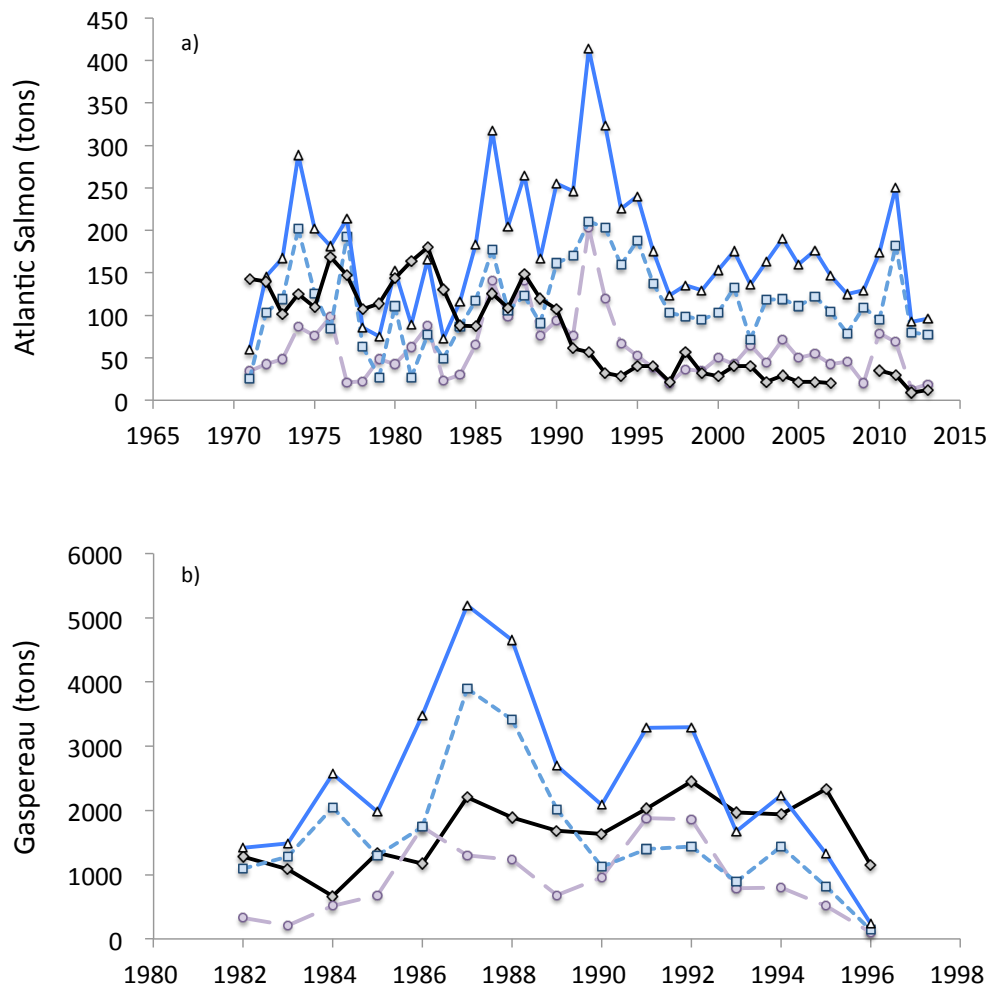


Figure 6.4. Comparison of the estimated nitrogen and phosphorous flux from nutrient loading models between Atlantic salmon landings and escapement data in the Miramichi River.

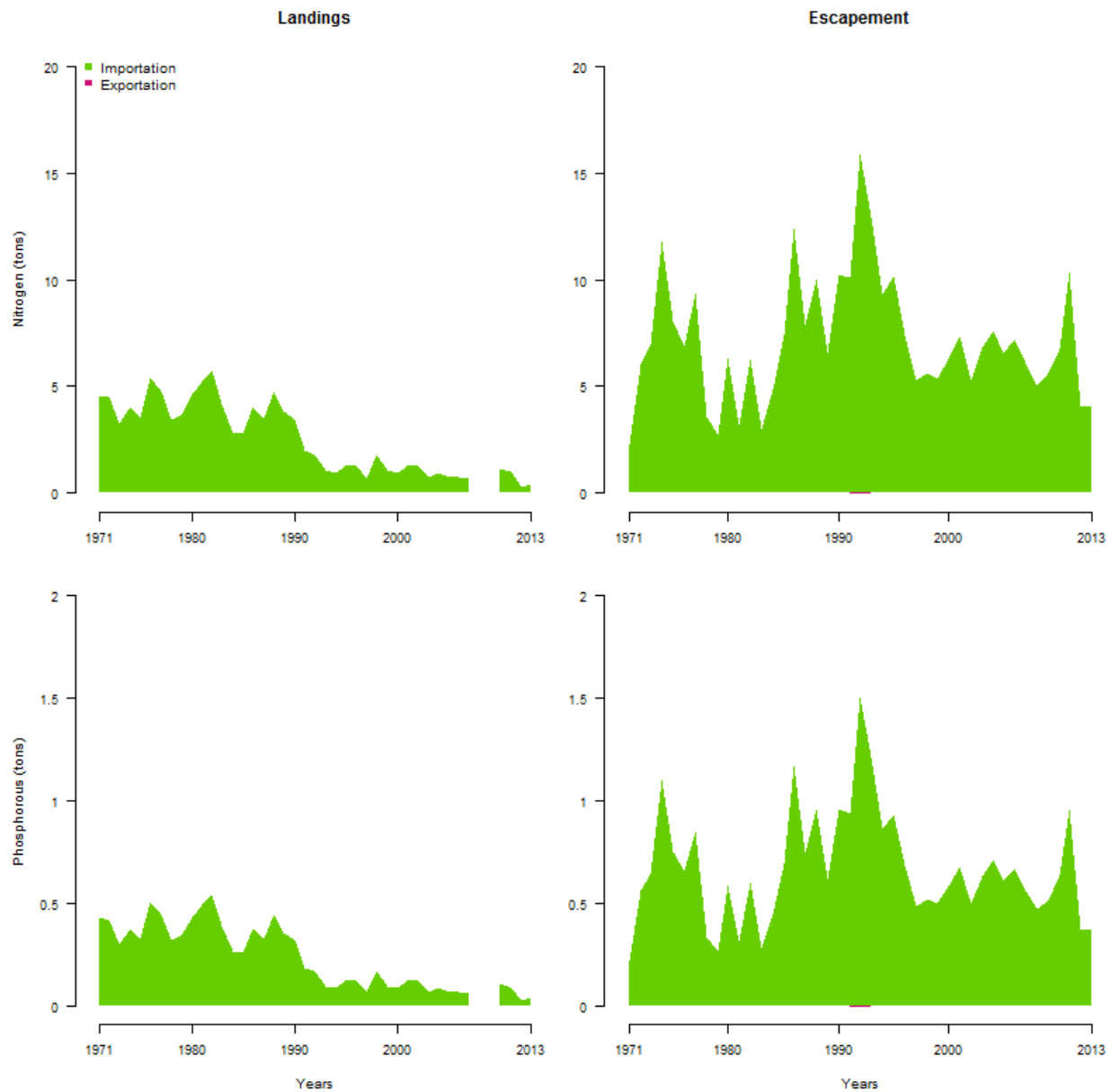


Figure 6.5. Comparison of the estimated nitrogen and phosphorous flux from nutrient loading models between gaspereau landings and escapement data in the Miramichi River.

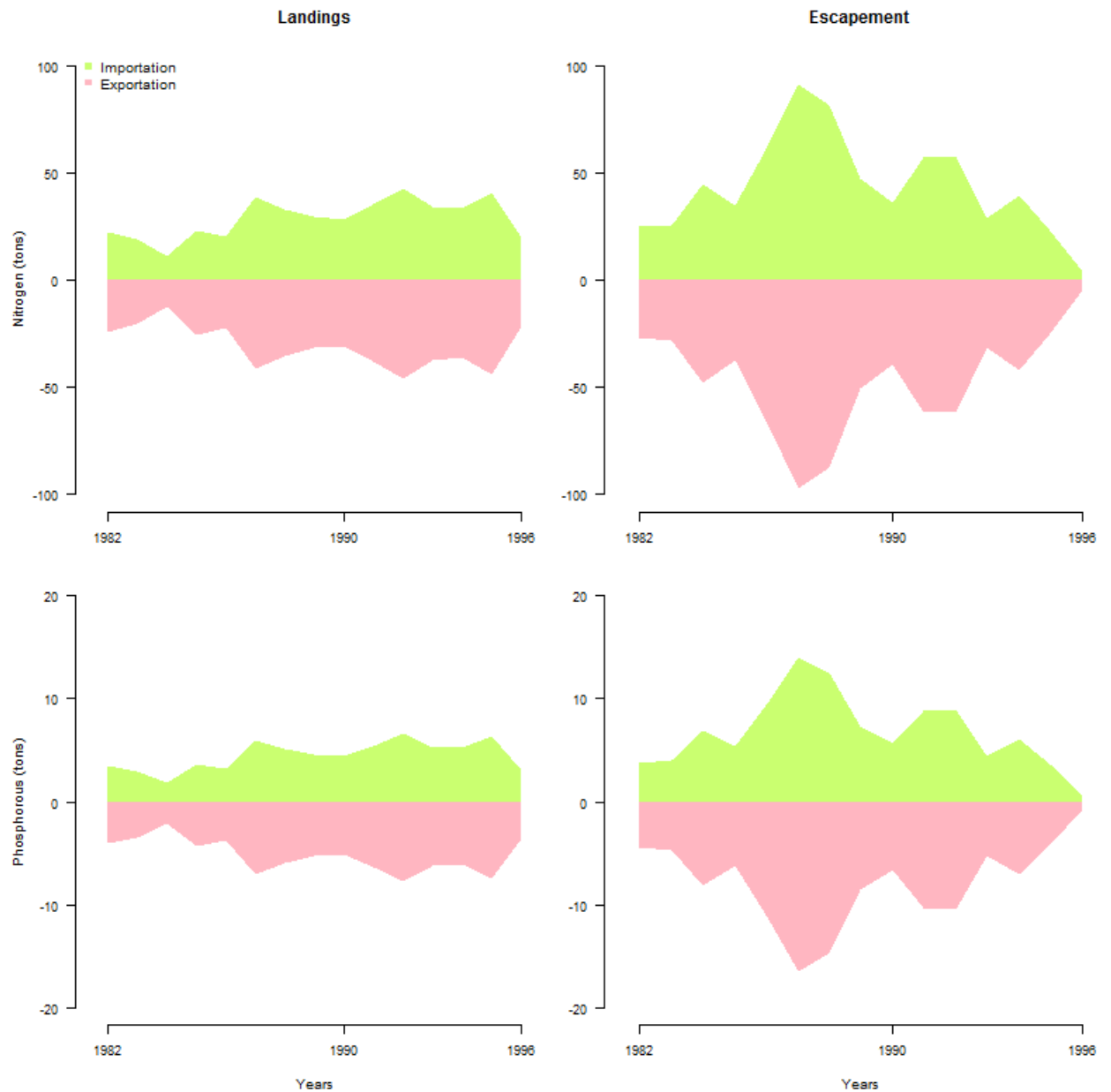


Figure 6.6. Estimated nitrogen and phosphorous flux from nutrient loading models based on Atlantic salmon landings in the Miramichi River. Virgin stock refers to nutrient imports/exports based on the equilibrium spawning population estimates.

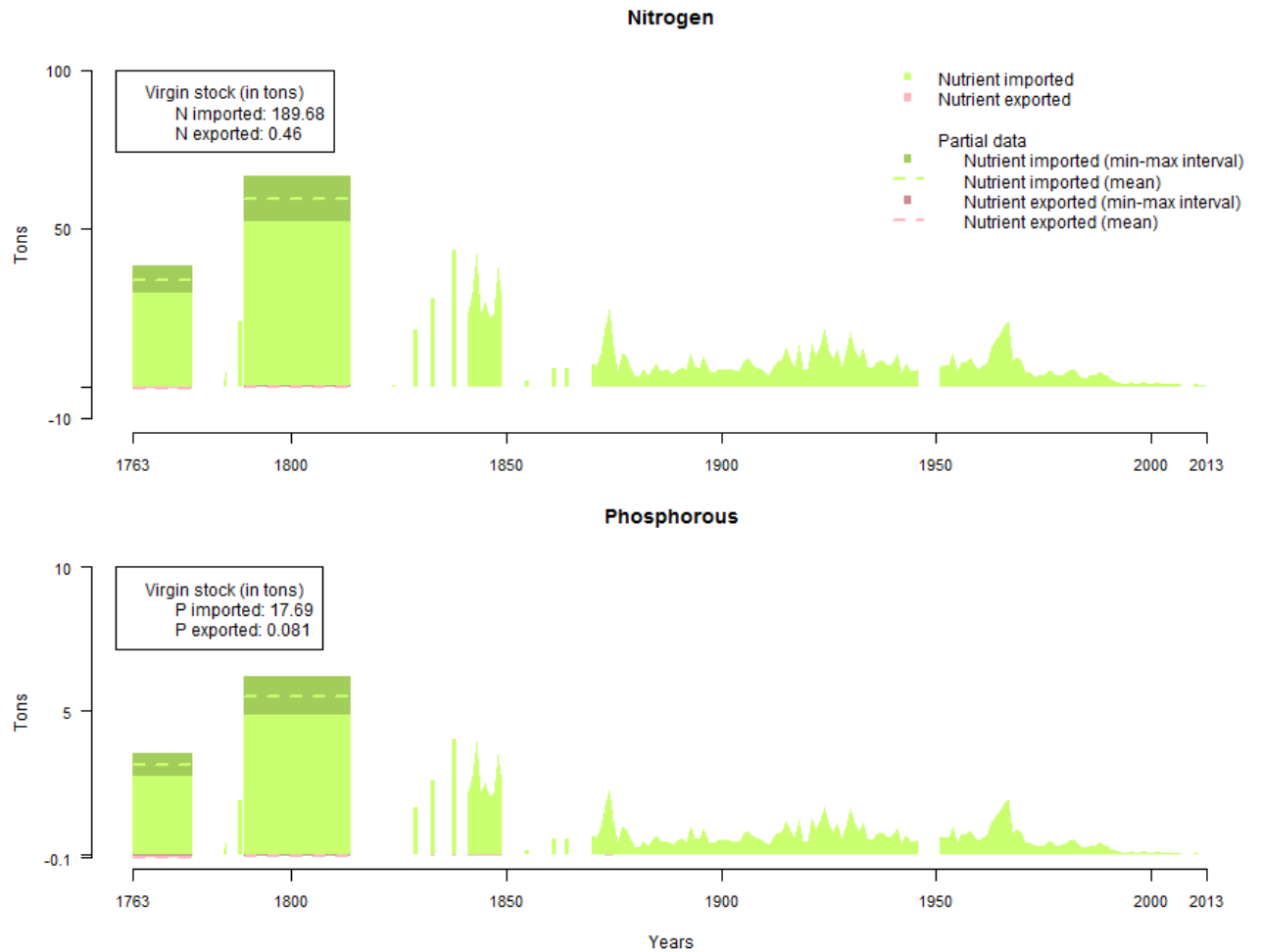


Figure 6.7. Estimated nitrogen and phosphorous flux from nutrient loading models based on gaspereau landings in the Miramichi River. Virgin stock refers to nutrient imports/exports based on the equilibrium spawning population estimates. Values with an asterisk (*) refer to the mean nutrient imports/exports based on the equilibrium spawning population estimates. Minimum and maximum around the mean are, 99.1 and 144.8 ton N imported; 15.2 and 22.2 tons P imported; 105.2 and 153.8 tons N exported; 17.7 and 25.9 tons P exported.

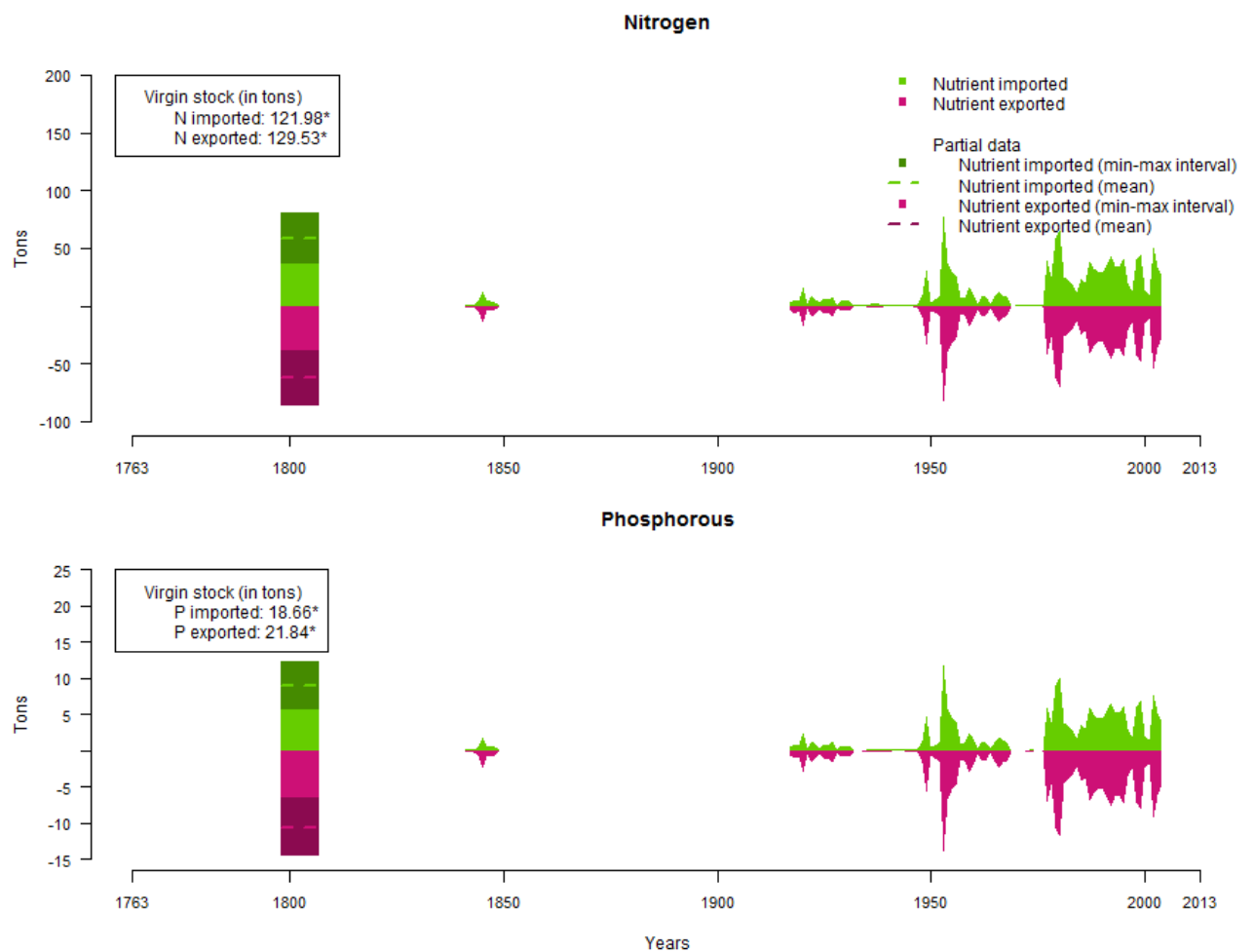


Figure 6.8. Conceptual representation of relative marine-derived nutrient (MDN) inputs to a river from rainbow smelt (dark blue), alewives (yellow), sea lamprey (light blue), and Atlantic salmon (red). Shaded areas represent the relative contribution over their freshwater residency, with the dark areas indicating the period of spawning. Dashed lines represent this relative contribution in a historical context.

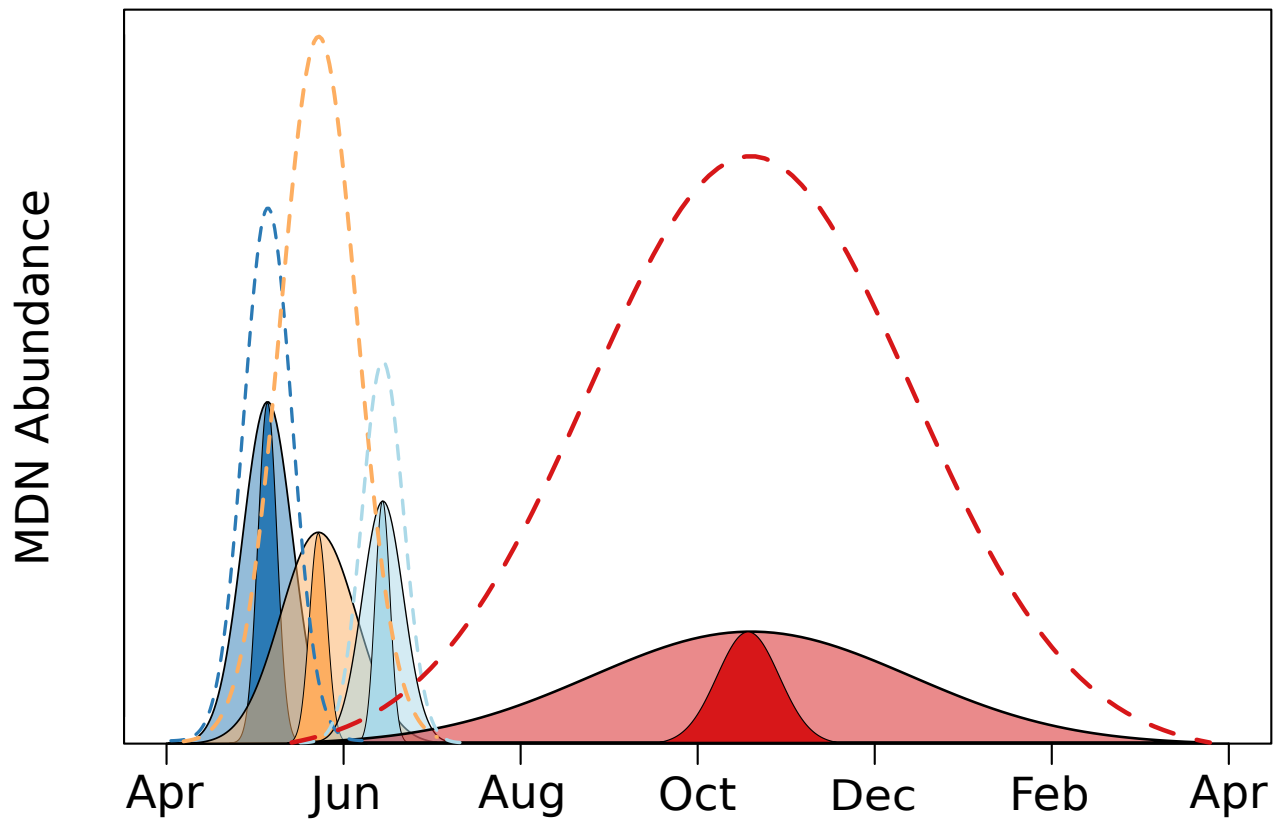
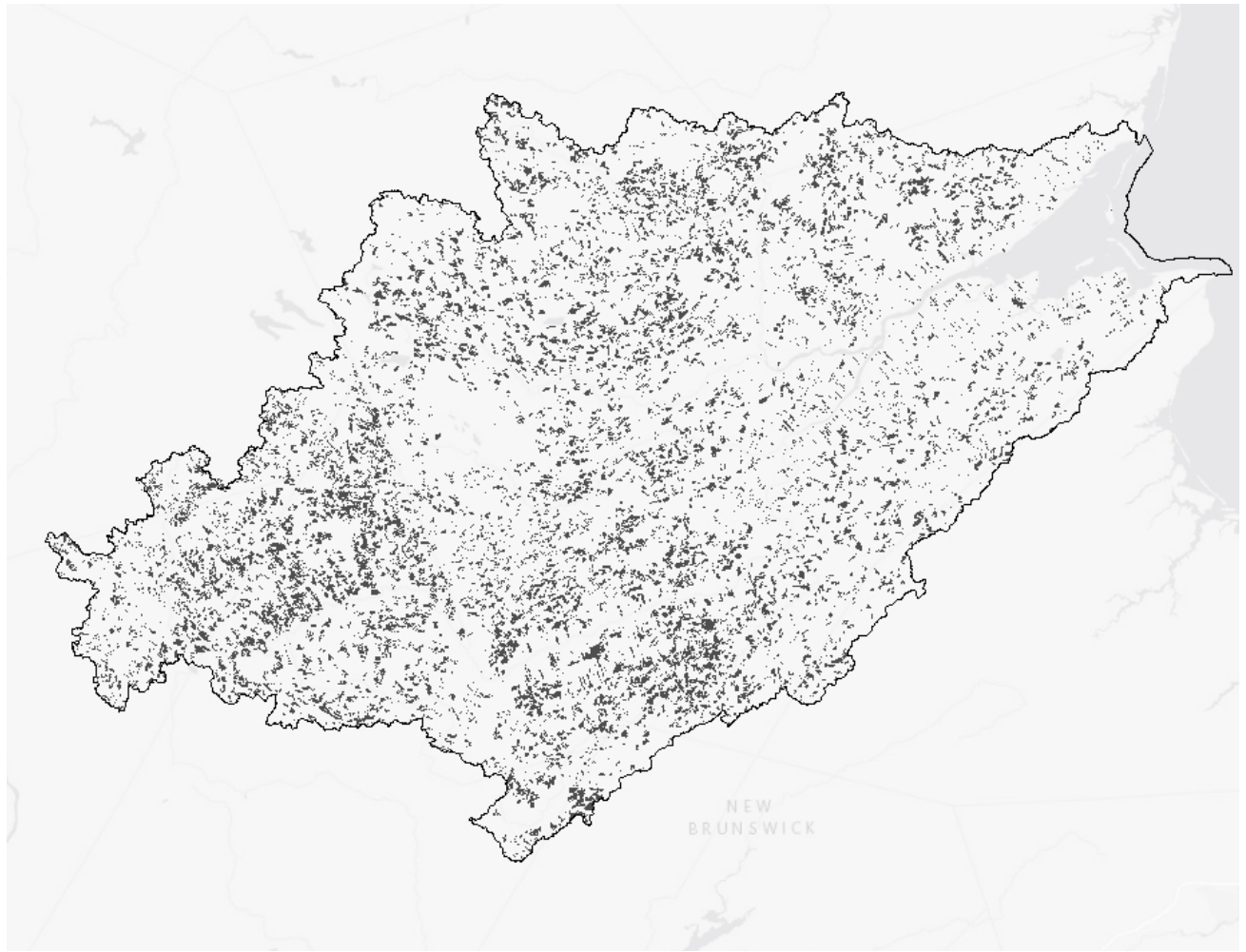


Figure 6.9. Distribution of active harvest plots across the entire Miramichi River catchment between 2001 and 2014; includes all industrial freehold, public, and private land (based on Hansen et al. 2013 and Linke et al. 2017).



Chapter 7

Synthesis

7.1 Significance of Dissertation

The ecological effects of anadromous fishes on freshwater ecosystems are difficult to fully comprehend and require field, experimental, and historical data evaluation to fully explore the question. Multiple factors have led to declines in returns of anadromous fishes (Limburg and Waldman 2009, Hall et al. 2012), reducing the supply and uptake of marine-derived nutrients (MDNs) (Stockner et al. 2000, Stockner 2003). Effects of MDNs have been primarily considered from the perspective of Pacific salmon (Bilby et al. 1996, Wipfli et al. 2002, Chaloner et al. 2004), with much less consideration given to Atlantic anadromous fishes (Nislow et al. 2004, Post and Walters 2009). My dissertation research emphasized that the relative roles of Atlantic anadromous fishes on driving and regulating freshwater food webs is dependent on both life-history dynamics and the synergistic effects of multiple anadromous species present. I present evidence that anadromous fish affect the quantity (i.e. increased biofilm production) as well as the quality (i.e. increased lipid stores and essential fatty acids) of food resources, as well as the mechanisms and trophic pathways of uptake and transfer. The following conclusions are presented to answer the research objectives outlined in Chapter 1.

- 1) With exposure to MDNs, algal, fungal, and bacterial communities exhibited general growth patterns, which were largely maintained through the year.
- 2) The pathway of MDN incorporation into freshwater food webs differed depending on the anadromous species spawning and the diet of resident salmonids shifted to a diet high in MDNs during the period of anadromous fish spawning.

- 3) MDNs from spawning Atlantic salmon relieved 'bottom-up' constraints and stimulated productivity.
- 4) Access to marine subsidies led to increased lipid stores in all trophic levels (biofilm, macroinvertebrates, salmon parr) and the incorporation of essential marine fatty acids.
- 5) Precipitous declines in fish populations have resulted in a net loss in MDN loading to a point that may no longer sustain elevated levels of productivity, inhibiting the production and/recovery of anadromous fish populations.

In this study, biofilm communities followed a general predictable response pattern to MDN inputs, regardless of the species of anadromous fish present (Atlantic salmon, alewife, rainbow smelt, or sea lamprey), spawning strategy employed, timing of subsidy, or MDN load being delivered. Within the biofilm matrix, the seasonal maxima (period before decline) of algae varied by species of anadromous fish present, whereas the seasonal peaks for fungi and bacteria were generally synchronous with the period of spawning. Although spawning rainbow smelt resulted in a net increase in biofilm biomass, a maximum accumulation threshold was reached despite increase in spawning density. Exposure to anadromous fish lead to a predictable relationship between the increase in primary productivity and the amount of MDNs delivered.

Stable isotope analysis demonstrated that all trophic levels (i.e. biofilm, macroinvertebrates, fish) incorporated MDNs when exposed to spawning anadromous fishes. This isotopic response also provides additional evidence that increases in biofilm production and productivity were the result of MDN incorporation, rather than an alternative nutrient source and/or ecological process. The magnitude and temporal pattern

of isotopic enrichment differed depending on the timing and spawning strategy of the anadromous fish present. Where MDNs were present peak isotope enrichments were synchronous with the period of spawning, a general pattern observed for all trophic groups, with the exception of Pteronarcyidae shredders for which there was no observed MDN incorporation. SIAR (Stable Isotope Analysis in R) mixing models demonstrated that the diet of top aquatic predators (e.g. brook trout) relies heavily on MDNs, even when other resources are abundant.

Exposure to MDN resources from spawning Atlantic salmon led to improved nutritional quality for all biota, as indicated by increased lipid stores and incorporation of marine fatty acids. Increased lipid reserves were observed in all trophic levels (biofilm, invertebrates, and salmon parr) with the greatest increases occurring in the invertebrates. The availability of this new resource, relatively enriched in marine lipids (e.g. salmon eggs), resulted in changes in the nutritional and chemical composition of the available foods as a result of uptake of MDN's. These results demonstrated the existence of MDN effects on consumer condition at multiple trophic levels.

Atlantic salmon and alewives in the Miramichi River have undergone declines from the pre-18th century pristine populations that once existed. Pre-exploitation population estimates allow us to appreciate how far present-day freshwater productivity has shifted. By combining historical records, we were able to estimate MDN contributions and how much has been lost through overexploitation. Collectively, salmon and alewives historically would have contributed 260.92 tonnes of N and 28.89 tonnes of P to the Miramichi River, whereas currently the estimated levels of MDN from Atlantic salmon and alewives are at 2.9 and 23.7% of historic levels, respectively. While

Atlantic salmon are net nutrient importers, alewives are net nutrient exporters, resulting in an overall net loss to the system. Historically these and other anadromous fishes (e.g. rainbow smelt, sea lamprey) would have spawned in the same river in a given year, resulting in an overall net import of nutrients to the system. Although the focus of these equilibrium biomass estimates was limited to Atlantic salmon and alewives, the findings indicate the magnitude of MDNs lost. The general conclusions about declining productivity and effects on anadromous populations are widely applicable to maritime rivers.

7.2 Is Nutrient Enrichment the Only Ecological Role of Anadromous Fishes?

Anadromous fishes are important ecosystem engineers, modifying habitat, community structure, and ecosystem processes (Moore and Schindler 2004, Tiegs et al. 2009). The ecological impacts of anadromous fish are complex due to their modification of benthic habitats during spawning, as well as uptake and incorporation of their nutrient contributions via multiple pathways. During spawning anadromous fish can modify the sediment size and bed load of rivers (Kondolf and Wolman 1993), flocculent transport (Rex and Petticrew 2010), abundance of biofilm and invertebrates (Wipfli et al. 1998, Chaloner et al. 2004, Lessard et al. 2009), timing of insect emergence (Moore and Schindler 2010), metabolism (Samways et al. 2015), and nutrient transformations (Levi et al. 2013). The processes of nutrient enrichment and disturbance can occur simultaneously, whereas biofilm biomass can be reduced, while nutrient concentrations increase, switching the metabolic balance of the system (Holtgrieve and Schindler 2011). Anadromous fishes play ecological roles that go beyond supplementing freshwater

ecosystems with MDNs.

7.3 Management Implications

With overexploitation of freshwater ecosystems occurring many decades before quantitative assessments, we must rely on life-history based models to estimate historic populations, still it is hard to imagine what historic levels were truly like (Humphries and Winemiller 2009). Because of this, scientists and resource managers may perceive that the stock characteristics that existed when they began their careers to be the reference condition. Thus, they may be deceived by shifting baselines and accept that degraded and unsustainable conditions of the immediate past reflect those of the distant past (Pauly 1995). Using Atlantic salmon in the Miramichi River as an example, it is clear that the 1980's-1990's (Fig. 6.2) are not a high point in the population's history, yet this is the period often deemed to be the baseline to which conservation targets are set (DFO 2012). Without an adequate understanding of past conditions, the ability to set meaningful targets and management strategies is subject to failure. The effects of historical loss of species and their affect on food webs must be recognized in order to restore aquatic ecosystems.

Attempting to conserve and/or mitigate fish populations without a clear understanding of the interplay and role MDNs play in sustaining productivity will waste time, money and other resources. Restoration strategies focused on a single species may not be as effective as including multiple anadromous species. Anadromous fish maintain the connectivity among systems, thereby preserving ecosystem resilience and functions of great ecological value (Helfield and Naiman 2001). Various attempts have been made

to supplement the loss of MDNs in streams. Inorganic fertilizers have been shown to increase primary production and invertebrate density (Perrin et al. 1987, Sabater et al. 2005, Wipfli et al. 2010); however, this positive response does not reach juvenile salmonids (Wipfli et al. 2010). This may be because inorganic fertilizers lack the carbon-based macromolecules (e.g. fatty acids) that are incorporated by consumers (Wipfli et al. 2010). Carcass additions (Bilby et al. 1998, Wipfli et al. 2004, 2010) and carcass analogues (Wipfli et al. 1998, 2003, 2004, Guyette et al. 2013, 2014) generally benefit biofilm, invertebrates and fishes and have a similar response to MDNs from spawning runs (Minakawa and Gara 1999, Scheuerell et al. 2007). Therefore, in order to maintain ecosystem function and productivity, it is critical to include MDNs for effective ecosystem management and river restoration strategies.

7.4 Future Directions

My dissertation research provides evidence of the importance of MDNs in shaping biofilm communities, food web dynamics, dietary and physiological requirements, nutrient budgets, and historic importance, in understanding the relationship between anadromous fish and freshwater productivity.

Research on systems influenced by anadromous fishes would benefit from a more holistic study approach such as that of my dissertation. Determining how pulsed marine-nutrient subsidies permeate through food webs and influence ecosystem process and function is a significant challenge in ecology (Ostfeld and Keesing 2000). The responses of nutrient enrichment were quantified over short time intervals, however it is important to determine whether effects persist within and across years. Slavik et al. (2004)

demonstrated that changes to community structure from nutrient enrichment requires many years, well outside the scope of many MDN studies.

Direct measures of primary production responses to MDNs demonstrate an increase of *in situ* productivity with anadromous fish spawning. There are a limited number of studies directly addressing whether small-scale increases in biofilm biomass is relevant to higher trophic level production (see Tiegs et al. 2009). Large-scale studies of energy flow, spanning multiple years are needed to evaluate the responses of biomass and production of higher trophic levels and better understand the magnitude and persistence of MDN effects.

Fish require essential fatty acids (FAs). Anadromous fish deliver many of these essential FAs to freshwater ecosystems during their spawning migration. Because these FAs are required by vertebrates for normal, healthy survival, they may limit production at higher trophic levels (Budge et al 2014). Given that juvenile anadromous fish would always be exposed to MDNs and these essential FAs, but due to population declines and extirpations, many systems no longer receive these subsidies. Could this be a reason why stocking programs fail? Many salmon stocking programs have gone to stocking unfed fry. These juveniles are now being placed in a river that no longer has a reliable source of these FAs. Have these systems that once relied on marine subsidies, lost the mechanisms and/or functions to provide what is needed? How do fishes not exposed to MDNs acquire these FAs? Much is known about the synthesis of these FAs in marine phytoplankton, but far less is known about the synthesis in freshwater systems.

Elucidating the interactive effects of MDNs from Atlantic anadromous fishes can enhance our capacity to understand their ecological role. In this dissertation, I have

provided many new insights into the importance of MDNs to freshwater productivity in Atlantic rivers. However, there are still many aspects of marine-nutrient subsidies and anadromous fish ecology that require additional focus in the future in order to fully understand how MDNs affect freshwater food webs.

7.5 Literature Cited

- Bilby, R. E., Fransen, B. R., and Bisson, P. A.. 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.
- Bilby, R. E., Fransen, B. R., Bisson, P. A., and Walter, J. K. 1998. Response of juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead (*Oncorhynchus mykiss*) to the addition of salmon carcasses to two streams in southwestern Washington, U.S.A. *Canadian Journal of Fisheries and Aquatic Sciences* 55:1909–1918.
- Budge, S. M., Devred, E., Forget, M.-H., Stuart, V., Trzcinski, M. K., Sathyendranath, S., and Platt, T. 2014. Estimating concentrations of essential omega-3 fatty acids in the ocean: supply and demand. *ICES Journal of Marine Science*. 71:1885 – 1893.
- Chaloner, D. T., G. A. Lamberti, R. W. Merritt, N. L. Mitchell, P. H. Ostrom, and M. S. Wipfli. 2004. Variation in responses to spawning Pacific salmon among three southeastern Alaska streams. *Freshwater Biology* 49:587–599.
- DFO 2012. Stock status of Atlantic salmon (*Salmo salar*) in DFO Gulf Region (Salmon Fishing Areas 15 to 18). DFO Canadian Science Advisory Secretariat Science Advisory Report. 2012/040.
- Guyette, M. Q., Loftin, C. S., and Zydlewski, J. 2013. Carcass analog addition enhances juvenile Atlantic salmon (*Salmo salar*) growth and condition. *Canadian Journal of Fisheries and Aquatic Sciences* 70:860-870.
- Guyette, M. Q., Loftin, C. S., Zydlewski, J., and Cunjak, R. 2014. Carcass analogues provide marine subsidies for macroinvertebrate and juvenile Atlantic salmon in temperate oligotrophic streams. *Freshwater Biology* 59:392-406.
- Hall, C. H., Jordaan, A., and Frisk, M. G. 2012. Centuries of anadromous forage fish loss: Consequences for ecosystem connectivity and productivity. *Bioscience*. 62:723-731.
- Helfield, J. M. and Naiman, R. J. 2001. Effects of salmon-derived nitrogen on riparian forest growth and implications for stream productivity. *Ecology*. 82:2403-2409
- 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.
- Humphries, P. and Winemiller, K. O. 2009. Historical impacts on river fauna, shifting baselines, and challenges for restoration. 59:673-684.

- Kondolf, G. M. and Wolman, M. G. 1993. The sizes of salmonid spawning gravels. *Water Resource Research*. 29:2275-2285
- Lessard J. L., R. W. Merritt, and M. B. Berg. 2009. Investigating the effect of marine-derived nutrients from spawning salmon on macroinvertebrate secondary production in southeast Alaskan streams. *Journal of the North American Benthological Society* 28:683–693.
- Levi, P.S., Tank, J.L., Tiegs, S.D., Chaloner, D.T., and Lamberti, G.A. 2013. Biogeochemical transformation of a nutrient subsidy: salmon, streams, and nitrification. *Biogeochemistry*. 113:643-655
- Limburg, K. E., and Waldman, J. R. 2009. Dramatic declines in north Atlantic diadromous fishes. *Bioscience*. 59:955-965.
- Minakawa, N., and Gara, R. I. 1999. Ecological effects of a chum salmon (*Oncorhynchus keta*) spawning run in a small stream of the Pacific Northwest. *Journal of Freshwater Ecology* 14:327–335.
- Moore, J. W. and Schindler, D. E. 2004. Nutrient export from freshwater ecosystems by anadromous sockeye salmon (*Oncorhynchus nerka*). *Canadian Journal of Fisheries and Aquatic Sciences*. 61:1582-1589.
- Nislow, K., J. Armstrong, and S. McKelvey. 2004. Phosphorus flux due to Atlantic Salmon (*Salmo salar*) in an oligotrophic upland stream: effects of management and demography. *Canadian Journal of Fisheries and Aquatic Sciences* 61:2401–2410.
- Ostfeld, R. S. and F. Keesing. 2000. Pulsed resources and community dynamics of consumers in terrestrial ecosystems. *Trends in Ecology and Evolution* 15:232–237.
- Pauly, D. 1995. Anecdotes and the shifting baseline syndrome of fisheries. *Trends in Ecology and Evolution* 10: 430.
- Perrin C. J., Bothwell, M. L., and Slaney, P. A. 1987. Experimental Enrichment of a Coastal Stream in British-Columbia - Effects of Organic and Inorganic Additions on Autotrophic Periphyton Production. *Canadian Journal of Fisheries and Aquatic Sciences* 44:1247-1256.
- Post, D. M., and A. W. Walters. 2009. Nutrient excretion rates of anadromous alewives during their spawning migration. *Transactions of the American Fisheries Society* 138:264–268.
- Rex, J. F. and E. L. Petticrew. 2010. Salmon-derived nitrogen delivery and storage within a gravel bed: sediment and water interactions. *Ecological Engineering* 36:1167–1173.
- Sabater S., Acuna, V., Giorgi, A., Guerra, E., Munoz, I., and Romani, A. M. 2005. Effects of nutrient inputs in a forested Mediterranean stream under moderate light availability. *Archiv Fur Hydrobiologie* 163:479-496.
- Scheuerell, M. D., Moore, J. W., Schindler, D. E., and Harvey, C. J. 2007. Varying effects of anadromous salmon on the trophic ecology of two species of resident stream fishes in southwest Alaska. *Freshwater Biology* 52:1944– 1956.
- Wipfli, M. S., Hudson, J., and Caouette, J. 1998. Influence of salmon carcasses on stream productivity: response of biofilm and benthic macroinvertebrates in southeastern Alaska, U.S.A. *Canadian Journal of Fisheries and Aquatic Sciences*. 55:1503–

1511.

- Wipfli, M. S., Hudson, J. P., Caouette, J. P., and Chaloner, D. T. 2003. Marine subsidies in freshwater ecosystems: salmon carcasses increase the growth rates of stream-resident salmonids. *Transactions of the American Fisheries Society*. 132:371–381.
- Wipfli, M.S., Hudson, J. P., and Caouette, J. P. 2004. Restoring productivity of salmon-based food webs: contrasting effects of salmon carcass and salmon carcass analog additions on stream-resident salmonids. *Transactions of the American Fisheries Society*. 133:1440–1454.
- Wipfli, M.S., Hudson, J.P., Caouette, J.P., Mitchell, N.L., Lessard, J.L., Heintz, R.A., and Chaloner, D.T. 2010. Salmon carcasses increase stream productivity more than inorganic fertilizer pellets: a test on multiple trophic levels in streamside experimental channels. *Transactions of the American Fisheries Society*. 139:824–839.

Appendix 1

Net increase estimation

Seasonal productivity was estimated as the area between the up- and down-stream algal biomass curves (Appendix S2.2) using the trapezoid rule. The weighted average of downstream and upstream algal biomass values gives an estimate of the area between the two curves for the period between those two sampling events. Summing all the areas gives an estimate of seasonal productivity (P) between the two curves.

$$P = \sum_i^n (t_{i+1} - t_i) \left(\frac{\Delta C_{i+1} - \Delta C_i}{2} \right)$$

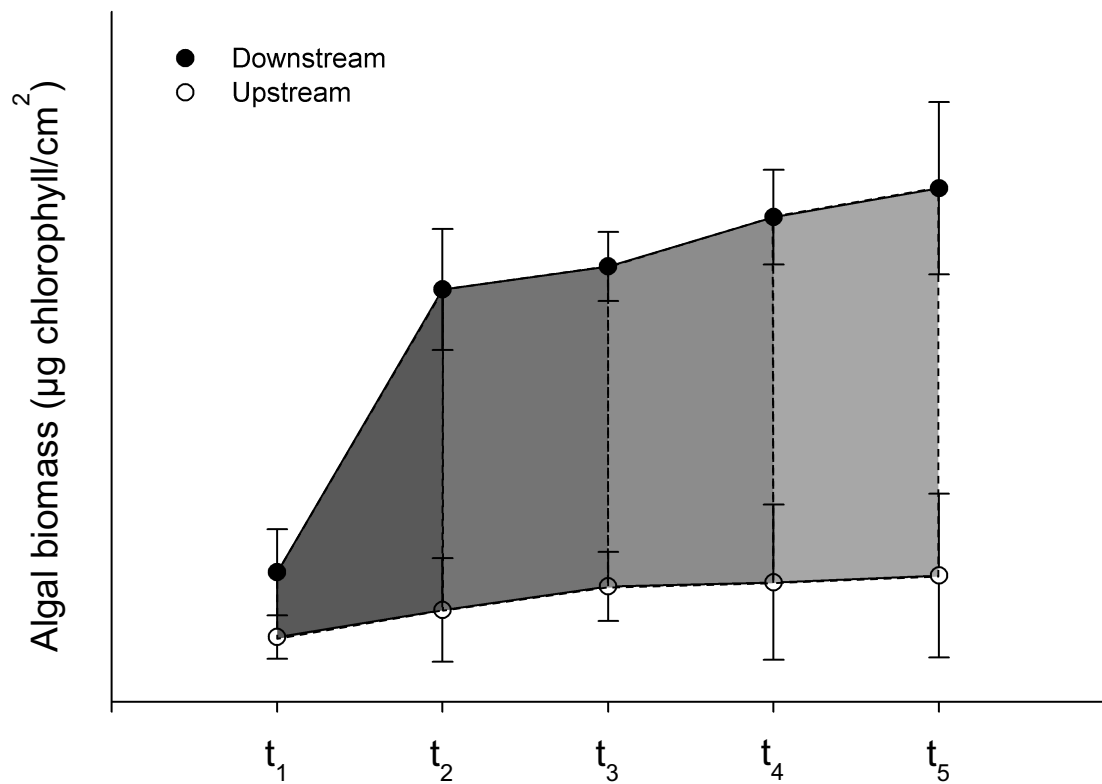
Where n represents the number of sampling events, t is the sampling event for a given i^{th} interval, and ΔC is the difference between the downstream and upstream algal biomass values. Seasonal productivity standard deviation (SD_P) is the square root of the sum of the squared first term multiplied by the square of the mean standard deviation.

$$SD_P = \left(\sum_i^n (t_{i+1} - t_1)^2 \left(\frac{SD_{i+1}^{\Delta C} + SD_1^{\Delta C}}{2} \right)^2 \right)^{\frac{1}{2}}$$

Where n represents the number of sampling events, t is the sampling event for a given i^{th} interval, and $SD^{\Delta C}$ is the standard deviation of the difference between the downstream and upstream algal biomass values.

Appendix 2

Area bounded by 2 algal abundance curves and the representative trapezoids between two sampling intervals (i.e. t_2 and t_1) used to estimate net increase in a downstream area of a river receiving marine-derived nutrients from spawning anadromous fish.



Appendix 3

Mean (SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (‰) isotope values for samples collected from all rivers for both upstream and downstream sites. Highlighted regions indicate periods of spawning.

Group	Date	Doctor' Brook			
		Upstream		Downstream	
		$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Biofilm	10-04-13	-27.15 (0.10)	2.43 (0.58)	-29.82 (0.75)	3.45 (0.23)
	10-05-06	-27.87 (0.38)	2.37 (0.51)	-30.88 (0.47)	2.86 (0.12)
	10-05-17	-27.02 (1.20)	2.56 (0.08)	-30.00 (0.72)	3.07 (0.24)
	10-05-29	-28.03 (0.37)	2.31 (0.08)	-30.26 (0.15)	3.29 (0.12)
	10-06-23	-27.49 (0.12)	2.18 (0.01)	-30.30 (0.13)	3.18 (0.25)
	10-07-15	-27.45 (0.32)	2.72 (0.29)	-29.68 (1.02)	2.97 (0.12)
	10-11-23	-27.76 (0.53)	2.05 (0.30)	-29.84 (0.67)	3.89 (0.09)
Resident Salmonids	10-04-13	-24.91 (0.39)	7.53 (0.06)	-25.94 (0.90)	7.44 (0.13)
	10-05-06	-25.58 (0.11)	7.10 (0.09)	-25.52 (0.39)	7.52 (0.39)
	10-05-17	-25.54 (0.10)	6.98 (0.03)	-25.34 (0.30)	6.96 (0.07)
	10-05-29	-25.60 (0.59)	7.16 (0.01)	-25.47 (0.43)	6.32 (0.18)
	10-06-23	-24.76 (0.01)	7.22 (0.40)	-25.53 (0.38)	6.34 (0.41)
	10-07-15	-24.39 (0.78)	7.35 (0.85)	-25.33 (0.36)	6.82 (0.19)
	10-11-23	-24.44 (0.46)	7.87 (0.26)	-25.49 (0.28)	6.55 (0.20)
Simuliidae	10-04-13	-27.64 (0.35)	5.35 (0.24)	-29.28 (0.47)	5.39 (0.73)
	10-05-06	-27.90 (0.10)	3.95 (0.45)	-29.15 (0.66)	3.59 (0.31)
	10-05-17	-29.42 (0.55)	4.48 (0.32)	-29.99 (0.78)	3.96 (0.21)
	10-05-29	-27.65 (0.37)	5.18 (0.02)	-28.86 (0.71)	5.39 (0.30)
	10-06-23	-28.66 (0.07)	5.39 (0.11)	-29.19 (0.59)	5.62 (0.51)
	10-07-15	-27.87 (0.33)	5.08 (0.14)	-29.91 (0.20)	5.51 (0.31)
	10-11-23	-27.77 (0.72)	4.24 (0.97)	-29.21 (0.23)	5.17 (0.17)
Perlidae	10-04-13	-27.19 (0.57)	4.66 (0.10)	-28.91 (0.59)	6.24 (0.55)
	10-05-06	-27.76 (0.15)	5.19 (0.09)	-28.98 (0.27)	6.73 (0.09)
	10-05-17	-27.58 (0.09)	5.02 (0.24)	-28.99 (0.51)	6.47 (0.14)
	10-05-29	-28.00 (0.45)	4.97 (0.34)	-29.98 (0.43)	6.93 (0.81)
	10-06-23	-27.41 (0.07)	5.67 (0.31)	-28.60 (0.30)	5.14 (0.31)
	10-07-15	-26.89 (0.25)	4.77 (0.82)	-28.65 (0.70)	5.79 (0.65)
	10-11-23	-26.65 (0.29)	5.75 (0.57)	-28.30 (0.84)	6.79 (0.73)

Heptageniidae	10-04-13	-29.00 (0.10)	1.99 (0.07)	-30.25 (0.18)	3.38 (0.37)
	10-05-06	-29.06 (0.25)	1.62 (0.34)	-30.53 (0.45)	2.72 (0.25)
	10-05-17	-28.89 (0.03)	1.49 (0.37)	-29.74 (0.14)	3.35 (0.10)
	10-05-29	-28.73 (0.72)	2.62 (0.18)	-29.95 (0.49)	3.14 (0.77)
	10-06-23	-28.48 (0.51)	0.78 (0.55)	-29.76 (0.17)	2.44 (0.30)
	10-07-15	-28.45 (0.38)	2.55 (0.28)	-29.64 (0.21)	3.27 (0.54)
	10-11-23	-28.87 (0.34)	2.24 (0.17)	-29.90 (0.76)	3.91 (0.27)
Pteronarcyidae	10-04-13	-28.09 (1.26)	4.03 (1.34)	-28.99 (0.39)	3.26 (0.23)
	10-05-06	-28.47 (0.62)	3.34 (0.08)	-28.36 (0.58)	3.26 (0.59)
	10-05-17	-28.08 (0.51)	3.98 (0.88)	-28.51 (0.51)	2.75 (0.02)
	10-05-29	-28.65 (1.05)	4.39 (0.17)	-28.24 (0.71)	2.76 (0.16)
	10-06-23	-27.86 (0.18)	4.16 (0.47)	-28.86 (0.09)	3.29 (0.24)
	10-07-15	-27.41 (0.49)	4.62 (0.28)	-28.70 (1.04)	3.19 (0.74)
	10-11-23	-27.32 (1.27)	3.39 (1.58)	-28.72 (0.23)	3.83 (0.69)

Knoydart Brook					
Group	Date	Upstream		Downstream	
		$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Biofilm	10-04-13	-28.49 (0.02)	3.06 (0.13)	-28.90 (0.41)	3.13 (0.10)
	10-05-05	-29.70 (0.04)	2.88 (0.09)	-22.09 (0.38)	4.57 (0.30)
	10-05-15	-28.78 (0.31)	3.09 (0.16)	-20.80 (0.74)	4.78 (0.17)
	10-05-27	-28.98 (0.63)	2.73 (0.36)	-22.98 (0.38)	4.02 (0.10)
	10-06-21	-29.23 (0.49)	2.14 (0.14)	-26.14 (0.97)	2.79 (0.32)
	10-07-14	-28.78 (0.71)	2.21 (0.15)	-29.76 (0.41)	2.96 (0.25)
	10-11-22	-28.06 (0.71)	1.73 (0.35)	-25.14 (2.37)	3.42 (0.73)
Resident Salmonids	10-04-13	-25.18 (0.06)	6.70 (0.59)	-26.30 (0.50)	4.98 (0.56)
	10-05-05	-25.54 (0.15)	7.02 (0.10)	-23.14 (0.56)	9.78 (0.46)
	10-05-15	-25.29 (0.18)	6.96 (0.12)	-22.78 (0.79)	10.86 (0.83)
	10-05-27	-25.45 (0.15)	7.24 (0.51)	-24.56 (0.51)	8.98 (0.51)
	10-06-21	-26.01 (1.14)	7.04 (0.29)	-25.02 (0.31)	8.33 (0.15)
	10-07-14	-25.43 (0.16)	6.42 (0.01)	-26.32 (1.56)	7.59 (1.01)
	10-11-22	-25.49 (0.39)	7.05 (0.18)	-26.70 (0.65)	5.83 (0.49)
Simuliidae	10-04-13	-28.42 (0.47)	4.18 (0.08)	-28.36 (0.92)	4.60 (0.43)
	10-05-05	-28.55 (1.08)	2.61 (0.70)	-27.62 (0.47)	7.00 (0.51)
	10-05-15	-27.98 (0.05)	2.64 (0.65)	-26.85 (0.20)	9.04 (0.13)
	10-05-27	-27.84 (0.68)	2.81 (0.41)	-23.45 (0.37)	11.07 (0.16)
	10-06-21	-27.07 (0.74)	3.09 (0.02)	-27.65 (0.40)	6.48 (0.96)
	10-07-14	-28.11 (0.18)	2.02 (1.07)	-27.72 (0.19)	3.28 (0.52)

	10-11-22	-27.69 (0.05)	3.39 (0.09)	-29.49 (0.84)	4.98 (0.31)
Perlidae	10-04-13	-27.90 (1.06)	4.86 (0.15)	-28.20 (0.46)	5.64 (0.43)
	10-05-05	-29.18 (0.27)	3.50 (0.21)	-25.74 (0.42)	8.55 (0.39)
	10-05-15	-27.44 (0.42)	5.13 (0.36)	-25.78 (0.83)	8.23 (0.49)
	10-05-27	-27.67 (0.32)	4.08 (0.22)	-26.45 (0.31)	7.32 (0.54)
	10-06-21	-27.48 (0.07)	4.11 (0.14)	-27.41 (0.23)	7.04 (0.15)
	10-07-14	-27.24 (0.23)	3.82 (0.11)	-27.67 (0.26)	5.06 (0.42)
	10-11-22	-27.59 (0.22)	4.15 (0.11)	-27.85 (0.37)	5.28 (0.18)
Heptageniidae	10-04-13	-29.03 (0.13)	0.83 (0.18)	-29.59 (0.11)	3.42 (0.19)
	10-05-05	-28.99 (0.02)	1.19 (0.12)	-26.21 (0.27)	5.50 (0.58)
	10-05-15	-29.76 (0.16)	1.01 (0.13)	-24.62 (0.44)	5.78 (0.53)
	10-05-27	-28.94 (0.08)	1.54 (0.12)	-26.59 (0.67)	4.70 (0.34)
	10-06-21	-28.96 (0.17)	0.91 (0.16)	-27.36 (1.04)	4.19 (0.21)
	10-07-14	-29.10 (0.31)	1.13 (0.14)	-28.50 (0.49)	3.21 (0.37)
	10-11-22	-29.11 (0.18)	1.27 (0.21)	-29.12 (0.26)	3.94 (0.73)
Pteronarcyidae	10-04-13	-29.80 (0.10)	3.17 (0.07)	-29.63 (0.85)	3.16 (0.40)
	10-05-05	-29.51 (0.08)	3.41 (0.07)	-29.04 (0.86)	3.53 (0.23)
	10-05-15	-29.14 (0.57)	3.21 (0.27)	-29.24 (0.48)	3.41 (0.79)
	10-05-27	-29.22 (0.33)	3.54 (0.45)	-29.20 (0.53)	3.56 (0.42)
	10-06-21	-28.90 (0.60)	3.61 (0.54)	-28.98 (0.70)	3.57 (0.54)
	10-07-14	-29.14 (0.32)	3.67 (0.17)	-29.18 (0.36)	3.40 (0.25)
	10-11-22	-28.94 (0.75)	3.57 (0.42)	-29.23 (0.76)	3.66 (0.36)

Oromocto River					
Group	Date	Upstream		Downstream	
		$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Biofilm	10-04-30	-26.86 (0.87)	9.23 (0.55)	-28.09 (0.19)	5.53 (0.43)
	10-05-11	-27.22 (0.86)	9.81 (0.12)	-23.12 (0.43)	11.71 (0.24)
	10-05-19	-27.11 (0.92)	9.54 (0.09)	-26.19 (0.22)	5.66 (0.83)
	10-06-02	-27.43 (0.63)	9.17 (0.04)	-25.79 (0.38)	5.73 (0.37)
	10-06-28	-26.40 (0.20)	8.43 (0.46)	-27.08 (0.17)	5.73 (0.96)
	10-07-20	-26.14 (0.62)	7.23 (0.67)	-26.31 (0.83)	5.94 (0.55)
	10-11-19	-25.99 (0.43)	7.51 (0.36)	-26.80 (1.30)	5.98 (0.98)
Resident Salmonids	10-04-30	-24.34 (0.33)	11.44 (0.33)	-26.05 (0.61)	8.23 (0.11)
	10-05-11	-24.80 (0.47)	11.01 (0.64)	-25.29 (0.51)	9.36 (0.28)
	10-05-19	-25.31 (0.78)	10.61 (0.67)	-24.38 (0.29)	9.77 (0.06)
	10-06-02	-24.65 (0.58)	11.20 (0.14)	-25.55 (0.38)	8.83 (0.63)
	10-06-28	-24.64 (0.96)	11.27 (0.04)	-26.03 (0.30)	8.72 (0.16)

	10-07-20	no data	no data	no data	no data
	10-11-19	-24.09 (0.10)	11.88 (0.10)	-25.99 (0.82)	8.60 (0.19)
Simuliidae	10-04-30	-28.07 (0.15)	9.68 (0.07)	-30.13 (0.87)	6.83 (0.49)
	10-05-11	-28.30 (0.04)	9.83 (0.35)	-25.34 (0.07)	9.20 (0.70)
	10-05-19	-28.24 (0.47)	9.63 (0.15)	-26.30 (0.10)	8.74 (0.21)
	10-06-02	-28.88 (0.07)	9.66 (0.25)	-26.27 (0.59)	8.39 (0.32)
	10-06-28	-27.94 (0.35)	9.13 (0.21)	-27.71 (0.58)	6.69 (0.65)
	10-07-20	-27.48 (0.08)	9.07 (0.39)	-28.24 (0.36)	5.63 (0.26)
	10-11-19	-28.83 (0.01)	9.03 (0.17)	-29.78 (0.48)	5.44 (0.43)
Perlidae	10-04-30	-27.14 (0.37)	12.30 (0.06)	-29.87 (0.42)	7.38 (0.22)
	10-05-11	-26.82 (0.21)	12.01 (0.03)	-25.65 (0.40)	10.09 (0.72)
	10-05-19	-26.63 (0.42)	12.38 (0.40)	-26.70 (0.44)	10.04 (0.62)
	10-06-02	-26.50 (0.04)	11.46 (0.25)	-26.51 (0.58)	8.18 (0.57)
	10-06-28	-25.60 (0.06)	11.14 (0.29)	-28.76 (0.66)	7.65 (0.72)
	10-07-20	-26.31 (0.49)	10.92 (0.05)	-27.23 (0.25)	6.95 (0.23)
	10-11-19	-26.09 (0.38)	10.93 (0.65)	-29.43 (0.08)	7.09 (0.29)
Heptageniidae	10-04-30	-28.36 (0.10)	7.43 (0.49)	-30.78 (0.17)	3.80 (0.68)
	10-05-11	-28.64 (0.85)	8.46 (0.55)	-25.00 (0.53)	9.64 (0.35)
	10-05-19	-29.01 (0.82)	7.74 (0.95)	-25.80 (0.63)	8.15 (0.78)
	10-06-02	-28.47 (0.26)	7.70 (0.02)	-24.97 (0.23)	7.79 (0.26)
	10-06-28	-28.95 (0.46)	8.10 (0.48)	-26.79 (0.80)	7.96 (0.46)
	10-07-20	-28.16 (0.13)	7.96 (0.02)	-26.64 (0.56)	5.22 (0.81)
	10-11-19	-28.11 (0.27)	6.61 (0.99)	-31.18 (0.68)	3.48 (0.47)
Pteronarcyidae	10-04-30	-29.05 (0.02)	8.38 (0.31)	-28.92 (0.28)	6.71 (0.19)
	10-05-11	-28.51 (0.03)	8.01 (0.29)	-27.42 (0.14)	7.34 (0.56)
	10-05-19	-27.90 (0.12)	7.88 (0.05)	-27.24 (2.01)	7.46 (0.23)
	10-06-02	-28.21 (0.66)	7.93 (0.29)	-27.94 (0.10)	7.46 (0.36)
	10-06-28	-27.31 (0.68)	7.66 (0.34)	-27.29 (0.67)	6.61 (0.36)
	10-07-20	-27.07 (0.59)	7.71 (1.00)	-28.11 (0.86)	7.14 (0.54)
	10-11-19	-29.23 (0.85)	7.81 (0.95)	-29.61 (0.53)	5.64 (0.41)

Group	Date	Rocky Brook			
		Upstream		Downstream	
		$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Biofilm	10-09-06	-30.24 (0.02)	3.58 (0.11)	-27.61 (0.55)	4.24 (0.49)
	10-10-05	-30.22 (0.55)	3.99 (0.76)	-27.51 (0.31)	4.55 (0.23)
	10-10-20	-29.25 (0.80)	2.98 (0.24)	-26.26 (0.16)	4.73 (0.10)
	10-11-02	-29.89 (1.00)	2.92 (0.20)	-25.79 (0.26)	5.62 (0.31)

	10-11-17	-30.72 (0.10)	3.21 (0.05)	-24.51 (0.46)	6.59 (0.09)
Resident Salmonids	10-09-06	-26.76 (1.07)	7.89 (0.48)	-27.01 (0.88)	7.32 (0.33)
	10-10-05	-26.76 (0.69)	8.53 (0.54)	-25.80 (0.53)	9.52 (0.26)
	10-10-20	-26.43 (0.46)	8.05 (0.12)	-25.11 (0.30)	9.97 (0.43)
	10-11-02	-26.49 (0.26)	8.32 (0.21)	-24.94 (0.43)	12.31 (0.88)
	10-11-17	-26.76 (0.36)	8.63 (0.54)	-25.25 (0.25)	10.42 (0.47)
Simuliidae	10-09-06	-28.51 (0.33)	4.48 (0.33)	-28.38 (0.45)	4.18 (0.20)
	10-10-05	-28.94 (0.20)	4.84 (0.37)	-28.16 (0.63)	4.82 (0.37)
	10-10-20	-29.37 (0.16)	4.51 (0.33)	-27.93 (0.89)	5.86 (0.51)
	10-11-02	-28.85 (0.25)	4.21 (0.63)	-26.38 (0.22)	6.18 (0.42)
	10-11-17	-29.17 (0.06)	4.60 (0.28)	-28.69 (0.50)	5.55 (0.28)
Perlidae	10-09-06	-29.59 (1.04)	6.65 (0.08)	-28.58 (0.52)	6.89 (0.97)
	10-10-05	-29.37 (1.29)	7.12 (0.09)	-28.63 (0.61)	7.05 (0.69)
	10-10-20	-29.17 (0.25)	6.74 (0.22)	-26.22 (0.84)	7.80 (0.99)
	10-11-02	-29.13 (0.07)	6.26 (0.29)	-25.23 (0.20)	8.40 (0.26)
	10-11-17	-29.51 (0.77)	7.07 (0.42)	-25.38 (0.39)	8.51 (0.33)
Heptageniidae	10-09-06	-28.87 (0.01)	2.63 (0.44)	-28.60 (0.47)	3.82 (0.71)
	10-10-05	-29.34 (0.19)	2.72 (0.29)	-28.96 (0.61)	3.89 (0.14)
	10-10-20	-30.25 (1.03)	2.85 (0.48)	-28.38 (0.55)	4.36 (0.47)
	10-11-02	-30.35 (0.82)	3.27 (0.30)	-27.62 (0.62)	5.16 (0.23)
	10-11-17	-30.41 (0.86)	3.07 (0.12)	-27.67 (0.98)	5.76 (0.17)
Pteronarcyidae	10-09-06	-28.68 (1.61)	3.24 (0.10)	-28.17 (0.16)	2.94 (0.38)
	10-10-05	-28.91 (0.23)	3.07 (0.07)	-27.85 (0.72)	3.43 (0.27)
	10-10-20	-29.41 (0.68)	3.29 (0.82)	-28.13 (0.58)	3.21 (0.10)
	10-11-02	-29.76 (0.31)	3.02 (0.71)	-28.27 (0.25)	2.53 (0.25)
	10-11-17	-29.69 (0.09)	2.80 (0.23)	-28.03 (0.18)	3.27 (0.22)

Appendix 4

Permissions to Reprint from Journals

The permission to reproduce the submitted or published articles in this dissertation was requested from the copyright owners using the following letter. All permissions received and shown below are responses to this request.

Letter requesting permission to reproduce an article in the dissertation:

Dear [*Copyright Owner*],

I am requesting permission to reproduce the article:

[*Scholarly citation of the article*]

in my PhD dissertation at the University of New Brunswick (UNB). The letter of permission will be included as an appendix in the dissertation. The regulations for the preparation and submission of PhD dissertations at UNB require that the format of the text of the article be retyped for the inclusion in the thesis according to UNB standards. The chapter where the article will be located will have a full scholarly citation to the original published article as an introductory note.

Sincerely,

Kurt Samways

Permission from University of Chicago Press (Chapter 2)

March 18, 2017

Hi Kurt,

This use of your article is entirely within Freshwater science and University of Chicago Press authors rights. Feel free to use your paper in the manner you describe.

Congratulations on finishing your degree!

Cheers, Pam

Dr. Pamela Silver
Editor *Freshwater Science*
Distinguished Professor of Biology
Penn State University
School of Science
4205 College Drive
Erie, PA 16563 USA

Permission from Wiley Open Access (Chapter 3)

Authors retain the copyright for their manuscript. All article published in *Ecosphere* are open-access articles distributed under the terms of the Creative Commons Attribution License (CC-BY), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Permission from John Wiley & Sons Ltd (Chapters 4 & 5)

AUTHORS - If you wish to reuse your own article (or an amended version of it) in a new publication of which you are the author, editor or co-editor, prior permission is not required (with the usual acknowledgements).

Appendix 5

Contribution of Authors in Chapters 2-6

Chapter 2 [*Spatiotemporal responses of algal, fungal, and bacterial biofilm communities in Atlantic rivers receiving marine-derived nutrient inputs*]

Kurt M. Samways contributed to the design of the experiment, conducted all the field work, performed all data analysis, and wrote the manuscript; Zoraida J. Quiñones-Rivera contributed to the development of ergosterol and chlorophyll methodology and helped with the analytical procedures; Peter R. Leavitt contributed to the development of ergosterol and chlorophyll methodology, as well as editing of the article; Richard A. Cunjak contributed to the design of the experiment as well as the primary editor.

Chapter 3 [*Aquatic food web dynamics following incorporation of nutrients derived from Atlantic anadromous fishes*]

Kurt M. Samways contributed to the design of the experiment, conducted all the field work, data analysis, and wrote the manuscript; David X. Soto contributed to the stable isotope analysis and interpretation; Richard A. Cunjak contributed to the design of the experiment as well as the primary editor.

Chapter 4 [*Increases in benthic community production and metabolism in response to marine-derived nutrients from spawning Atlantic salmon (*Salmo salar*)*]

Kurt M. Samways conducted all the fieldwork, performed all the analysis and wrote the manuscript; Rick A. Cunjak was the primary editor. Both authors contributed to the design of the study.

Chapter 5 [*Effects of spawning Atlantic salmon (*Salmo salar*) on total lipid content and fatty acid composition of river food webs*]

Kurt M. Samways contributed to the design of the experiment, conducted all the field work, performed all data analysis, sample analysis, and wrote the manuscript; Tammy J. Blair developed the fatty acid methodology, contributed to sample analysis and data interpretation; Michelle A. Charest helped equally with sample analysis, as well as contributed to data interpretation; Richard A. Cunjak contributed to the design of the experiment as well as the primary editor.

Chapter 6 [*The ecological toll from declining fish populations: Anadromous fishes function as nutrient import/export specialists*]

Kurt M. Samways developed the marine nutrient models and wrote the manuscript; Guillaume J.R. Dauphin contributed to the stock-recruitment models and salmon equilibrium population estimate; Richard A. Cunjak contributed with the initial design as well as the primary editor.

Appendix 6

Permissions to Reprint from Co-Authors

The permission to reproduce the submitted or published articles in this dissertation was requested from the co-authors owners using the following letter. All permissions received and shown below are responses to this request.

Letter requesting permission to use co-authored articles in the dissertation:

Dear [*Co-Author*],

I am requesting permission to use reproduce the article(s):

[*Scholarly citation of the article*]

in my PhD dissertation at the University of New Brunswick (UNB). The letter of permission will be included as an appendix in the dissertation. The regulations for the preparation and submission of PhD dissertations at UNB require that the format of the text of the article be retyped for the inclusion in the thesis according to UNB standards. The chapter where the article will be located will have a full scholarly citation to the original published article as an introductory note.

Sincerely,

Kurt Samways

Permission (Z.J. Quiñones-Rivera; Chapter 2)

Yes, you have my permission to use the article in your dissertation.

Zoraida J. Quiñones-Rivera

Permission (P.R. Leavitt; Chapter 2)

This email serves as a formal agreement to allow the listed paper to be used in your PhD.

Peter R. Leavitt

Permission (R.A. Cunjak; Chapters 2,3,4,5, and 6)

You have my permission.

Rick Cunjak

Permission (D. X. Soto; Chapter 3)

Of course, it is totally fine with me that you include our manuscript as a thesis chapter.

David Soto

Permission (T.J. Blair; Chapter 5)



Fisheries
and Oceans

Maritime Region
St. Andrews Biological Station
531 Brandy Cove Road
St. Andrews, NB E5B 2L9

Pêches
et Océans

Région des Maritimes
Station biologique de St. Andrews
531 Brandy Cove Road
St. Andrews (N-B) E5B 2L9

May 05, 2017

Kurt Samways
University of New Brunswick,
Fredericton, NB
E3B 5A3

SUBJECT: Permission to Reproduce Article in PhD Dissertation at the University of New Brunswick.

Dear Mr. Samways:

I hereby grant permission to reproduce the article:

Samways K. M., T. J. Blair, and R. A. Cunjak. 2017. Effects of spawning Atlantic salmon (*Salmo salar*). *Ecosphere*.

In your PhD dissertation thesis at the University of New Brunswick (UNB).

Sincerely,

Tammy Blair
Aquatic Biologist
St. Andrews Biological Station

Bedford Institute of Oceanography
Institut océanographique de Bedford

Marine House

St. Andrews Biological Station
Station biologique de St. Andrews



Permission (M.A. Charest; Chapter 5)

You have my consent to use the manuscript in your thesis.

Michelle Charest

Permission (G.J.R. Dauphin; Chapter 6)

I hereby confirm that I have given my authorization to use our collaborative work in your thesis.

Guillaume Dauphin

Curriculum Vitae

Candidate's full name: Kurt Michael Samways

Universities attended:

University of New Brunswick 2008-2017, Ph.D. (Biology)

University of Regina 2001-2004, M.Sc. (Biology)

University of Regina 1995-2000, B.Sc. (Biology)

Publications:

Samways, Kurt M., Tammy J. Blair, Michelle A. Charest, and Richard A. Cunjak. 2017.

Atlantic anadromous fishes are important vectors of pulsed marine-nutrient subsidies to freshwater ecosystems. *Ecosphere*. – DOI: 10.1002/ecs2.1818.

Samways, Kurt M., David X. Soto, and Richard A. Cunjak. 2017. Aquatic Food Web

Dynamics Following Incorporation of Nutrients Derived from Atlantic Anadromous Fishes. *Journal of Fish Biology* (In Review).

Samways, Kurt M. and Danielle MacDonald. 2017. Where you are raised does matter:

The use of semi-natural rearing ponds as an Atlantic salmon conservation tool. *Canadian Journal of Fisheries and Aquatic Sciences* (In Prep).

Soto, David X., **Kurt M. Samways**, Michael J. Dadswell, Richard A. Cunjak. 2017.

Climate-driven range shifts of Atlantic salmon (*Salmo salar*) revealed by multi-decadal stable isotope trends. *Journal of Animal Ecology* (In Prep).

Samways, Kurt M., Peter Leavitt, Pierre Magnan, Marco A. Rodríguez, and Pedro R.

Peres-Neto. 2015. Convergent polymorphism of brook charr between stream and lake habitats: the case of brook char. *Canadian Journal of Fisheries and Aquatic Sciences*. 72:1406-1414.

Samways, Kurt M. and Richard A. Cunjak. 2015. Increases in benthic community production and metabolism in response to marine-derived nutrients from spawning Atlantic salmon (*Salmo salar*). *Freshwater Biology*. 60:1647-1658.

Vue, Sherr, **Kurt M. Samways** and Richard A. Cunjak. 2015. Assessment of Bioelectrical Impedance Analysis (BIA) for Atlantic Salmon Parr: An Investigation of Temperature Corrections, PIT Tags, and Precision for Application in Field Studies. *Transactions of the American Fisheries Society*. 144: 235-245.

Samways, Kurt M., Ray G Poulin, R Mark Brigham. 2004. Directional Tree Felling by Beavers (*Castor canadensis*). *Northwestern Naturalist*. 85(2): 48-52.

Technical Reports

Inner Bay of Fundy Atlantic Salmon: Marine Research Methods Review and Evaluation. 2016. Prepared for Fisheries and Oceans Canada, by Stantec Consulting Ltd. Solicitation # F5211-150297A.

Conference Presentations:

Linnansaari, T., **Samways, K.**, Babin, A., Helminen, J., O'Sullivan, A., Clarke, C., Jones, R., Curry, R.A., Bernatchez, L., and Cunjak, R.A. Atlantic salmon recovery in New Brunswick; What is on the go to bring salmon back in the Bay of Fundy, the Saint John River and the Miramichi? Quebec City, Quebec. April 2017.

Samways, K.M., Clarke C., and Linnassaari T. Finding your wild side: Restoring inner Bay of Fundy Atlantic salmon through conservation rearing. Canadian Conference for Fisheries Research. Montreal, Quebec. January 2017.

Samways, K.M. Finding your wild side: Restoring inner Bay of Fundy Atlantic salmon through conservation rearing. Department of Fisheries and Oceans Salmon Recovery Team. Moncton, NB. November 2016.

Samways, K.M. Finding your wild side: Restoring inner Bay of Fundy Atlantic salmon through conservation rearing. Cape Breton Highlands National Park. November 2016.

Samways, K.M. What goes around comes around, restoring the Upper Salmon River. ACFFA Aquaculture Research, Science and Technology Forum. October 2016.

Samways, K.M. Restoring ecosystem health through marine reared native adult Atlantic salmon introductions. ACFFA Annual Forum and Research Review. November 2015.

Samways, K.M., T.J. Blair, M.A. Charest, and R.A. Cunjak. Atlantic anadromous fishes are important vectors of pulsed marine-nutrient subsidies to freshwater ecosystems. Contributed Paper to Special Organized Session. Ecological Society of America. August 2015.

Samways, K. M., Margaret Q. Guyette and Richard A. Cunjak. How Marine-derived Nutrients Benefit Atlantic Rivers. Department of Fisheries and Oceans Salmon Planning Group. Amherst, Nova Scotia. Canada. April 2014.

Samways, K.M., M. Q. Guyette and R.A. Cunjak. Importance of Marine-derived Nutrients to Atlantic Rivers. Canadian Model Forest – Salmon Workshop. Amherst, Nova Scotia. March 2014.

- Samways, K.M.,** M. Q. Guyette and R.A. Cunjak. Marine-derived Nutrients from Atlantic Anadromous Fishes. Mount Allison University Departmental Seminar Series. Sackville, Nova Scotia. March 2014.
- Samways, K.M.** and R.A. Cunjak. 2014. An Evaluation of Historic and Current Marine-derived Nutrient Inputs from Multiple Atlantic Anadromous Fishes. Canadian Conference for Fisheries Research. Yellowknife, Northwest Territories. January 2014.
- Samways, K. M.,** D. MacDonald, and S. Ratelle. Where you are Raised Does Matter: The Use of Semi-natural Rearing Ponds as an Atlantic Salmon Conservation Tool. Department of Fisheries and Oceans Salmon Planning Group. Amherst, Nova Scotia. Canada. October 2013.
- Samways, K. M.** and M. Q. Guyette. Marine-Derived Nutrients in Natural and Model Systems in Eastern North America: How Nutrient Subsidies Benefit Resident and Anadromous Fishes. Atlantic Salmon Recovery Workshop. Chamcook, New Brunswick, Canada. September 2013.
- Samways, K. M.,** D. MacDonald, and S. Ratelle. Where you are Raised Does Matter: The Use of Semi-natural Rearing Ponds as an Atlantic Salmon Conservation Tool. Atlantic Salmon Recovery Workshop. Chamcook, New Brunswick, Canada. September 2013.
- Samways, K. M.,** T. J. Blair, M. A. Charest, and R.A. Cunjak. Dietary enrichment of marine-derived lipids in juvenile Atlantic salmon: highly conserved or quickly metabolized? Canadian Conference for Fisheries Research. Windsor, Ontario. January 2013.

Samways, K., B. Graham, and R. Cunjak. How Adult Salmon Deliver Marine Nutrients into River Systems like the Miramichi. Miramichi Research Environmental Conference. Miramichi, NB. March 2012.

Samways, K., B. Graham, and R. Cunjak. Implications of marine-derived nutrients delivered to Atlantic rivers by three species of anadromous fishes. Atlantic Salmon Forum. Bangor, Maine. January 2012.

Samways, K. M., Z. J. Quiñones-Rivera, M. A. Charest, P. R. Leavitt, and R.A. Cunjak. Scratching the surface of stream productivity: compositional changes of biofilm communities in Atlantic rivers receiving marine-derived nutrient inputs. Canadian Conference for Fisheries Research. Moncton, New Brunswick. January 2012.

Vue, Sherr, **K.M. Samways** and R.A. Cunjak. Assessment of Bioelectrical Impedance Analysis on Atlantic Salmon Parr: An Investigation of Temperature Corrections and Potential Sources of Error. Canadian Conference for Fisheries Research. Moncton, New Brunswick. January 2012.

Samways, K., B. Graham, and R. Cunjak. Implications of marine-derived nutrients from anadromous fishes on productivity in Atlantic rivers. 2nd Biennial Symposium of the International Society for River Science: Rivers as Linked Systems. Berlin, Germany. August 2011.

Samways, K., and R. Cunjak. The importance of marine-derived nutrients delivered to Atlantic rivers by anadromous fishes. Biology Department Summer Seminar Series, University of Regina, Regina, Saskatchewan. May 2011.

Samways, K., B. Graham, and R. Cunjak. New Currents in Marine-derived Nutrients:
tracking incorporation within streams. Canadian Conference for Fisheries

Research/Society of Canadian Limnologists. Toronto, Ontario. January 2011.

Samways, K., P. Peres-Neto, P. Leavitt, P. Magnan, and M. Rodríguez. Linking
phenotypic plasticity to differential selective habitat pressures in freshwater
aquatic systems. Departmental Seminar Series, University of Regina, Regina,
Saskatchewan. October 2007.