WATERSHED CONTROLS ON BIOGEOCHEMICAL PROCESSES IN AQUATIC ECOSYSTEMS

Kathi Jo Jankowski

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

Doctor of Philosophy

University of Washington
2014

Reading Committee:
Daniel E. Schindler, Chair
Gordon W. Holtgrieve
M. Claire Horner-Devine

Program Authorized to Offer Degree:
School of Aquatic and Fishery Sciences

University of Washington

Abstract

Watershed Controls on Biogeochemical Processes in Aquatic Ecosystems

Kathi Jo Jankowski

Chair of the Supervisory Committee:
Professor Daniel E. Schindler
School of Aquatic and Fishery Sciences

Freshwater ecosystems provide critical services to sustain human livelihoods, such as fisheries, agriculture and clean water, yet face increasing demands from rapidly expanding populations and globalizing societies. In addition, the threats to ecosystem services, such as climate and land use change, are increasingly large-scale and diffuse. The productivity and sustainability of freshwater ecosystem services rely on fundamental biogeochemical processes such as photosynthesis and nutrient cycling. Yet, biogeochemistry has traditionally been done at scales that do not match the spatial or temporal scale of modern issues like climate change. Therefore, it is increasingly clear that we need techniques that enable us to understand how the biogeochemical processes that support critical ecosystem services play out at scales that match these threats.

In this dissertation, I address this gap by using novel approaches to evaluate aquatic carbon (C) and nitrogen (N) cycling at watershed scales. In chapter 2, I evaluate changes in N fixation and N cycling in small urban lakes in response to a gradient of human nutrient loading.

I paired an isotope mass balance model with nutrient stoichiometry to estimate loads of nutrients from background, human and atmospheric sources; estimate N fixation at the ecosystem scale in response to eutrophication; and track the importance of N from various sources in supporting lake food webs. In chapter 3, I tie these changes in lake chemistry to shifts in bacterial community composition. Using a molecular approach, I examined how bacterial communities changed in response to eutrophication and whether the response to eutrophication differed among lake habitats. I found that surface and deep bacterial communities responded differently to eutrophication and were increasingly distinct as eutrophication progressed because of heterogeneity in resource distribution.

My last two chapters focus on understanding how C cycling in boreal stream ecosystems will respond to warming temperatures associated with climate change. Using a mesocosm approach (chapter 4) and an ecosystem-scale Bayesian model (chapter 5), I examine how variation in watershed geomorphology influenced the temperature dependence of C metabolism in streams of the Wood River watershed in southwest Alaska. I found the response of stream respiration to temperature was not universal, but instead varied substantially among streams within a single watershed in southwest Alaska. Temperature sensitivity was hierarchically controlled by geomorphology; specifically, the sensitivity of stream respiration to increasing temperature was highest in streams draining flat watersheds. I found that this was linked to the quality and quantity of available carbon substrates.

Together these results show that by using approaches that integrate over larger spatial and temporal scales, we can better understand how large-scale stressors like climate and land use change will influence aquatic ecosystems.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

A PhD dissertation although authored by an individual, is the product of the generosity of many people, programs, and funding sources that contribute to making research happen. Many have contributed to my dissertation, and I am deeply grateful.

First and foremost, I would like to thank my advisor, Daniel Schindler. Not only has he taught me many of the fundamental skills required to be a scientist, but also the value of pursuing my curiosity, questioning the status quo, and enjoying myself while doing so. I am also extremely grateful to the members of my committee – Gordon Holtgrieve, Claire Horner-Devine, and Mike Brett. Gordon provided many hours of his time, and consistently valuable feedback on ideas and drafts. Claire provided encouragement to try new things in the microbial world and taught me a great deal of ecology along the way. Mike consistently helped me think through things in a new way and provided a novel perspective on my work.

I am indebted to many people and organizations that provided funding to train me as a scientist. I received fellowships to fund my research and coursework from the School of Aquatic & Fishery Sciences (SAFS), the Seattle ARCS Foundation, EPA STAR, Washington Lakes Protection Association, and the UW Graduate School. Funding from the National Science Foundation, the Gordon and Betty Moore Foundation, and the Bullitt Foundation to Daniel Schindler covered many of my research expenses. I am grateful to the Wood Tikchik State Park and UBC Malcolm Knapp Research Forest for enabling my field research.

I feel very fortunate to have been part of the community of SAFS and especially the Alaska Salmon Program. I am so thankful to have shared time with the many accomplished, driven, and creative people that make up these two programs. I learned a great deal from

working with them in the field, hearing about their work, and sharing ideas at TGIT. SAFS hosts an astounding group of scientists.

There are several individuals who gave me field, analytical or statistical help during my tenure at UW. I would to thank the following, specifically: Teresa Z'Amar, Jackie Carter, Kathy Krogslund, Aaron Morello, Dongsen Xue, Gregory Korshin, Arni Litt, Anna Coogan, Rachel Lange, Kendra Boymer, Mackenzie Consoer, Sydney Clark, and Katyana Vert-Pre.

The Schindler lab – past and present – has been an extraordinary part of my time at UW. I feel grateful to have been part of such a diverse and impressive research group. I learned a great deal from my time spent with lab members in lab meetings, discussions, in the field, and reading their papers. I made friends and colleagues that will last a career.

Lastly, my friends and family have provided a critical personal support system throughout the duration of my PhD. I made many good friends while in Seattle who kept me sane, kept me in shape, and helped me enjoy the outdoors. My family inspired me to keep going, gave me confidence that I could, and provided support in more ways than I can count.

DEDICATION

To all of my science teachers - for inspiring me to pursue this path and my curiosity.

Chapter 1. INTRODUCTION

Human societies depend on the services provided by freshwater ecosystems, such as fisheries, agriculture, and clean water to sustain our livelihoods (Kareiva et al. 2011). Recent population estimates project that there will be 9 billion people on the planet by 2050 (Gerland et al. 2014), placing increasing pressure on ecosystems to provide food and clean water to sustain these populations. As our populations have grown and societies have globalized, the types of perturbations that we have caused such as climate and land use change have broader and more diffuse implications for ecosystem functioning. These changes are rapidly shifting the composition and functioning of landscapes and threaten the ability of ecosystems to provide these services (e.g., Foley et al. 2005).

Many of the services provided to us from ecosystems - like clean water, good soil for crop production, and productive fisheries - are based on fundamental biogeochemical processes like the cycling of nutrients and photosynthesis (Millennium Ecosystem Assessment 2005). Therefore, in order to understand how large-scale changes will influence critical ecosystem services, we need to quantify how they will influence fundamental processes of energy production and nutrient cycling in ecosystems at broader scales. Traditionally, biogeochemistry has been done at small scales, either through laboratory experiments or small-scale plot manipulations (Levin 1992), however, these broader threats necessitate that we develop techniques to understand and measure how aquatic systems function at watershed scales.

While we cannot assume each individual ecosystem within a watershed will respond the same to a given driver, how do we understand the variation? Do results of small-scale studies match what we find at the landscape scale? Thus, the challenge to addressing large-scale threats to ecosystems lies in translating what we have learned at smaller scales to a broader landscape

(Levin 1992). For instance, we know that watersheds filter large-scale drivers so that processes play out differently across space and time, and that large-scale processes such as urban development and climate change are not likely to affect all systems in the same way based on their background geology, hydrology and climate (Soranno et al. 2014). However, because of the multiple scales at which these drivers and biogeochemical processes operate, understanding the spatial complexity of biogeochemical patterns and processes across aquatic ecosystems has proven difficult (Levin 1992, McGuire et al. 2014) and remains a serious impediment towards understanding how ecosystems fundamentally work. Therefore, there is a great need for robust means of predicting local scale variation in how climate and land use will play out at watershed scales.

In recent years, aquatic ecology has increasingly focused on the unifying influence that watershed features and hydrological regimes have on aquatic ecosystem function (Poole et al. 2010). It has long been recognized that aquatic communities and ecological processes are dependent on their watershed context. For example, Bormann et al. (1968) provided a classic example of terrestrial-aquatic connectivity at Hubbard Brook by showing that clear-cutting forests substantially increased river flow and nutrient loads in resident streams. Vannote et al. (1980) further formalized the links between rivers and their watersheds through the River Continuum Concept that linked the downstream continuum of physical forms in river networks to organic matter inputs, ecosystem productivity, and food web configuration. While this has been a reasonable guiding principle for understanding landscape-scale processes in river networks for many years, we have come to appreciate that there is much more nuance to how watershed form influences river function and the importance of geomorphology in that relationship (Montgomery 1999). Watershed geomorphology (e.g., river network characteristics,

floodplain topography, and channel patterns) influences how aquatic ecosystems function through affecting the patterns and rates of ecosystem processes and through determining the connectivity between surface and subsurface water (Poole 2008, Poole et al. 2010). For example, some recent papers have explored how the chemistry of different elements scale differently across river networks – some are tightly controlled by downstream flow dynamics (e.g., nitrate) and patterns in others more closely reflect local geology and topography (e.g., dissolved organic matter; McGuire et al. 2014). In addition, the spatial configuration of geomorphic features and human alterations obviously influences ecological processes, and we are just starting to appreciate these subtleties at the macroscale in river landscapes (McCluney et al. 2014).

Climate change is one example of a major threat to the structure and functioning of ecosystems that necessitates a broader scale understanding of biogeochemical processes. Climate conditions are mostly structured by mechanisms that operate at very broad spatial scales, but efforts to downscale climate models to a manageable scale are complex (Bindoff et al. 2014). Yet most ecological and biogeochemical processes operate at scales much smaller than even a single climate model grid cell (Bindoff et al. 2014). Climate change is anticipated to alter aquatic biogeochemistry in a number of ways. First, temperature is a fundamental control on biological rates and thus anticipated changes in average temperatures and thermal regimes will impact rates the processes involved in nutrient cycling and decomposition of organic matter. While increases in stream temperature have been widely observed (Kaushal et al. 2010), how air temperature translates to stream temperature changes in diverse landscapes is still being worked out (Hilderbrand and Kashiwagi 2014), which makes linking biogeochemical responses to changing air temperatures challenging. Second, anticipated shifts in the magnitude and timing of

precipitation with climate change will cause changes in water sources (snow vs. rain; Berghuijs et al. 2014) and hydrological regimes (Poff et al. 2010, Davis et al. 2013, Dulière et al. 2013). However, recent work has shown that while climate warming is expressed and experienced differently in rivers and streams, physical features of their watersheds can constrain how physical and chemical processes such as temperature (Lisi et al. 2013), dissolved oxygen dynamics (Helton et al. 2012), and nutrient transport (McGuire et al. 2014) play out, as well as control the timing of how organisms use those ecosystems (Schindler et al. 2013).

Some of the variation in how aquatic ecosystems respond to changes in climate and land use change will depend on their species and how these respond to these perturbations (Woodward et al. 2010). Organisms can utilize the heterogeneity in how climate is expressed across a watershed to their advantage, however, and therefore will respond differently to the same drivers (e.g., behavioral thermal adaptation, Sunday et al. 2014). For example, recent work has shown that there is meaningful variation in the temperature sensitivity in physiological and ecological traits among organisms (Dell et al. 2011), such that negative traits (predator-avoidance) are less temperature sensitive than positive or neutral traits (e.g., foraging rate), which has obvious implications for predator-prey dynamics and community composition. In addition, climate has the potential to alter food web dynamics because of the differential temperature sensitivities of autotrophic and heterotrophic (i.e., consumer) production (Yvon-Durocher et al. 2010): as temperatures warm, heterotrophic production increases disproportionately more than autotrophic production leading to changes in top-down vs. bottom-up controls on food webs (O'Connor et al. 2009). These organism responses are well-studied in macro-organisms, but many important questions remain to be asked with microbial communities, whose ecology is linked directly to biogeochemical processes but which exist at a much different

scales and experience heterogeneity differently. We should not necessarily expect that microbial communities will have the same responses as macro-organism communities. Because of the large reservoir of metabolic diversity in microbes, the potential for genetic exchange, and the widespread use of dormancy (Jones and Lennon 2010), microbial communities have the potential to be much more functionally resilient than macro-organisms.

Thus, to explore possible scenarios for aquatic ecosystem responses to further land-use changes and responses to climate change, there will be great utility in developing general relationships that link climate and land use driven changes in temperature and hydrology to organism and ecosystem responses at the watershed scale. There is a need for approaches that integrate across time (e.g., stable isotope tracers, genetics, paleo-ecology) and space (remote sensing, modeling). Thus, in this dissertation I explored the utility of some of these approaches for understanding how landscape-scale patterns in land use change and climate warming influence biogeochemical processes in aquatic ecosystems.

In Chapter 2, I explored how nutrient inputs from human sources altered lake nitrogen cycles along a watershed urbanization gradient in the Puget Sound, WA area. I combined a Bayesian isotope mixing model approach ($\delta^{15}N$) with lake nutrient stoichiometry (N:P ratios) to track the sources of nitrogen along this urbanization gradient. I found a more complex response than expected than a simple increase in human nutrient inputs, however. I showed that as nutrients from human sources increased, the N: P ratio of lake nutrients declined and induced N limitation at the ecosystem scale, but that this deficit in nitrogen was made up by increasing N fixation (as expected, Schindler 1977). Further, we developed an approach to use stable N isotopes to estimate contribution of N to nutrient cycles, relative to human and watershed sources. The isotopic signature of N fixation was apparent in biota from POM to zooplankton,

suggesting that the novel source of N was utilized throughout the food web. This study demonstrated a novel approach for simultaneously quantifying the magnitude of nutrient input to lakes and resulting changes in N cycles at the ecosystem scale. In addition, because we did the estimation in a Bayesian framework, we were also able to quantify the uncertainty in our estimates, making this a useful approach for surveying the effects of human influence at the landscape scale. Last, this study demonstrated clear implications for the energy base of lake food webs in watersheds with increasing urban development.

In Chapter 3, I examined the implications of lake nutrient enrichment for the diversity and composition of resident microbial communities. While the response of diversity and community composition to nutrient enrichment has been widely studied for macro-organisms in lakes (e.g. Dodson et al. 2000, Vonlanthen et al. 2012), much less is understood for the bacterial communities that mediate the fundamental ecosystems processes at the base of lake food webs. Therefore, I asked how bacterial communities changed in response to increasing nutrient enrichment in the same set of lakes from Chapter 2. In addition, I examined whether the response was uniform among habitats within the lake (surface and deep layers) or whether bacterial communities capitalized on heterogeneous conditions within the lake. I found that while richness increased with nutrient enrichment as expected, the response varied among habitats within the lake and that communities became less similar between surface and deep layers as nutrient enrichment progressed. The greatest increase in richness occurred in the deep layer, which I hypothesized was linked to the greater quantity of nutrients in that habitat and anoxic conditions that allowed microbes with diverse forms of metabolism to coexist. This also likely drove the differences in the response of surface vs. deep bacteria to nutrient enrichment. Interestingly I also found that “rare” bacteria only present in high nutrient lakes drove the

increase in richness I observed and augmented a core community that existed among all lakes. This chapter showed that bacterial communities responded differently among lake habitats to the large-scale stressor of nutrient enrichment by taking advantage of how that stressor was expressed heterogeneously among lake habitats.

In Chapters 4 and 5, I shifted environments to explore the question of how the large-scale stressor of climate warming might be expressed in streams in a pristine boreal watershed of southwest Alaska. Here I asked the following questions: 1) how does aquatic ecosystem metabolism respond to climate warming and 2) what aspects of watershed geomorphology affect the sensitivity of aquatic metabolic processes to changing temperature. Specifically, I explored whether there was variation across the landscape in how ecosystem respiration (ER) rates responded to warming temperatures. In Chapter 4, I performed a mesocosm experiment in 11 streams that varied in watershed geomorphic features. I incubated benthic sediments from each stream at 5 different temperatures and compared the differences in temperature sensitivity of ER, or the rate at which a given biological process responds to temperature, across streams. I found that watershed slope was negatively related to temperature sensitivity, such that ER in flat streams responded more strongly to temperature than did respiration in steep streams. This resulted from the relationship of watershed slope with the quality of carbon substrates available in streams: flat streams had more low quality carbon substrates, which caused ER to respond more to temperature. This link between carbon quality and the temperature sensitivity of ER had been shown widely in terrestrial systems, but not previously in aquatic ecosystems. In Chapter 5, I scaled this result up by adapting an ecosystem metabolism model (Holtgrieve et al. 2010) in order to capture temperature sensitivity at the whole stream scale rather than just in a sample of benthic sediments. I found that the results were in line with what I found in the small-scale

experiments: 1) the temperature sensitivity of ER was higher in flat streams than in steep ones and 2) this was related to the quality of carbon substrates available for decomposition. However, the strength of these relationships was more attenuated for the ecosystem scale studies compared to the mesocosm studies. The reasons for this are not understood at the moment, but I suspect that much of the ER measured by the ecosystem approach occurred in the hyporheic zone (Poole et al. 2008), which is typically more buffered from daily temperature variation and from terrestrial organic matter subsidies. These two chapters demonstrated how warming can be expressed heterogeneously in streams across a watershed, but that watershed geomorphology provides unifying features such that the small scale response can be scaled to the watershed. In addition, these results can inform how we may anticipate processes to play out in more impacted systems, such as urban landscapes, as well.

Many of the threats to the integrity of ecological systems such as global climate change, urban development or ocean acidification operate at scales greater than a single ecosystem. Thus, we have moved from a "point-source" world to one in which we must understand how diffuse, large-scale stressors play out differently across ecosystems (Soranno and Schimel 2014). Our understanding of how these large-scale stressors influence ecological processes has grown as we have tried to respond and manage these issues (e.g., fisheries management and climate prediction, Great Lakes stressors and nutrient management). It has forced us to ask questions such as: Does what we see at the small-scale average out into something more predictable or do we have to consider all the variation and manage locally? How can we make meaningful management-scale predictions when there is a mismatch between the scale of the mechanism and the pattern of interest? Will our management actions have equal effects across diverse landscapes? Recent work has developed more quantitative ways to answer some of these

questions (Soranno et al. 2014), which will be is crucial for adequately addressing the environmental and management challenges of the 21st century. This dissertation intends to contribute to that body of work.

REFERENCES

Berghuijs et al. 2014. A precipitation shift from snow towards rain leads to a decrease in streamflow. Nature Climate Change 4: 583-586.

Bindoff, N., P. Stott, K.M. AchutaRao, M. Allen, N. Gillett, D. Gutzler, K. Hansingo, G. Hegerl, Y. Hu, S. Jain, I. Mokhov, J. Overland, J. Perlwitz, R. Sebbari, and X. Zhang, 2014: Detection and attribution of climate change: from global to regional. In: Climate Change 2014: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. [Stocker, T.,et al. (ed.)]. pp. 12.

Bormann, F.H., G.E. Likens, D.W. Fisher and R.S. Pierce. 1968. Nutrient loss accelerated by clear-cutting of a forest ecosystem. Science 159(3817): 882-884.

Dell, A. I., S. Pawar, and V. M. Savage. 2011. Systematic variation in the temperature dependence of physiological and ecological traits. Proceedings of the National Academy of Sciences USA 108(26): 10591-10596.

Dodson SI, Arnott SE, Cottingham KL (2000) The relationship in lake communities between primary productivity and species richness. Ecology 81: 2662-2679.

Dulière, V., Y. Zhang, and E.P. Salathé. 2013. Changes in 20th century extreme temperature and precipitation over the western United States from regional climate model simulations and observations. *Journal of Climate* 26(21):8556-8575, http://dx.doi.org/10.1175/JCLI-D-12-00818.1.

Foley, J. A., R. DeFries, G. P. Asner, C. Barford, G. Bonan, S. R. Carpenter, F. S. Chapin, M. T. Coe, G. C. Daily, H. K. Gibbs, J. H. Helkowski, T. Holloway, E. A. Howard, C. J. Kucharik, C. Monfreda, J. A. Patz, I. C. Prentice, N. Ramankutty, and P. K. Snyder. 2005. Global consequences of land use. Science 309: 570-574.

Gerland, P., A. E. Raftery, H. Sevcikova, N. Li, D. Gu, T. Spoorenberg, L. Alkema, B. K. Fosdick, J. Chunn, N. Lalic, G. Bay, T. Buettner, G. Hellig, and J. Wilmoth. 2014. World population stabilization unlikely this century. Science 346(6206): 234-237.

Gillooly, J. F., J. H. Brown, G. B. West, V. M. Savage, and E. L. Charnov. 2001. Effects of size and temperature on metabolic rate. Science **293**:2248-2251.

Helton, A. M., G. C. Poole, R. A. Payn, C. Izurieta, and J. A. Stanford. 2012. Scaling flow path processes to fluvial landscapes: An integrated field and model assessment of temperature and dissolved oxygen dynamics in a river-floodplain-aquifer system, Journal of Geophyshyical Research 117, G00N14, doi:10.1029/2012JG002025.

Hilderbrand, R. H. and M. T. Kashiwagi. 2014. Regional and local scale modeling of stream temperatures and spatio-temporal variation in thermal sensitivities. Environmental Management 54(1): 14-22.

Holtgrieve, G. W., D. E. Schindler, T. A. Branch, and Z. T. A'Mar. 2010. Simultaneous quantification of aquatic ecosystem metabolism and reaeration using a Bayesian statistical model of oxygen dynamics. Limnology and Oceanography **55**:1047-1063.

Jones, S. E. and J. T. Lennon. 2010. Dormancy contributes to the maintenance of microbial diversity. Proceedings of the National Academy of Sciences of the United States of America **107**:5881-5886.

Kareiva, P., H. Tallis, T.H. Ricketts, G.C. Daily and S. Polasky. 2011. Natural Capital: Theory and Practice of Mapping Ecosystem Services. Oxford University Press, New York, NY. USA.

Kaushal, S. S., G. E Likens, N. A Jaworski, M. L Pace, A. M Sides, D. Seekell, K. T Belt, D. H Secor, and R. L Wingate. 2010. Rising stream and river temperatures in the United States. Frontiers in Ecology and the Environment. doi:10.1890/090037

Levin, S.A. 1992. The problem of pattern and scale in ecology: The Robert H. MacArthur award lecture. Ecology 73(6): 943-1967.

Lisi, P. J., D. E. Schindler, K. T. Bentley, and G. R. Pess. 2013. Association between geomorphic attributes of watersheds, water temperature, and salmon spawn timing in Alaskan streams. Geomorphology 185:78–86.

McCluney, K. E., N. L. Poff, M. A. Palmer, J. H. Thorp, G. C Poole, B. S Williams, M. R Williams, and J. S Baron. 2014. Riverine macrosystems ecology: sensitivity, resistance, and resilience of whole river basins with human alterations. Frontiers in Ecology and the Environment 12(1): 48-58.

McGuire, K. J., C. E. Torgersen, G. E. Likens, D. C. Busoe, W. H. Lowe, and S. W. Bailey. 2014. Network analysis reveals multiscale control on streamwater chemistry. Proceedings of the National Academy of Sciences USA. www.pnas.org/cgi/doi/10.1073/pnas.1404820111

Millennium Ecosystem Assessment. 2005. Ecosystems and Human Well-being: Synthesis., Island Press,Washington, DC.

Montgomery, D. R. 1999. Process Domains and the River Continuum. Journal of the American Water Resources Association **35**:397-410.

O'Connor, M. I., M. F. Piehler, D. M. Leech, A. Anton, and J. F. Bruno. 2009. Warming and resource availability shift food web structure and metabolism. PLoS Biol 7(8): e1000178. doi:10.1371/journal.pbio.1000178

Poff NL, Pyne MI, Bledsoe BP, Cuhaciyan CC, Carlisle DM. 2010. Developing linkages between species traits and multi-scaled environmental variation to explore vulnerability of stream benthic communities to climate change. J. N. Am. Benthol.Soc. 29:1441–58.

Poole, G. C., S. J. O'Daniel, K. L. Jones, W. W. Woessner, E. S. Bernhardt, A. M. Helton, J. A. Stanford, B. R. Boer, and T. J. Beechie (2008), Hydrologic spiralling: The role of multiple interactive flow paths in stream ecosystems. River Research and Applications 24: 1018–1031. doi:10.1002/rra.1099.

Poole, G. C. 2010. Stream hydrogeomorphology as a physical science basis for advances in stream ecology. Journal of the North American Benthological Society 29(1): 12-25.

Schindler, D. W. 1977. Evolution of phosphorus limitation in lakes. Science **195:** 260-262.

Schindler, D.E., J.B. Armstrong, K.T. Bentley, K. J. Jankowski, P.J. Lisi, and L.X. Payne. 2013. Riding the crimson tide: mobile terrestrial consumers track phenological variation in spawning of an anadromous fish. Biology Letters 9 (3): Article Number 20130048

Soranno, P.A., and D.S. Schimel. Macrosystems ecology: Big data, big ecology. 2014. Guest editorial in the special issue on Macrosystems Ecology in Frontiers in Ecology and the Environment. 12(1):3.

Soranno, P.A., K.S. Cheruvelil, E. Bissell, M. Tate Bremigan, J.A. Downing, C.E. Fergus, C. Filstrup, N.R. Lottig, E.N. Henry, E.H. Stanley, C.A. Stow, P.-N. Tan, T. Wagner, K.E. Webster. 2014. Cross-scale interactions: Quantifying multi-scaled cause-effect relationships in macrosystems. Frontiers in Ecology and the Environment. 12(1):65-73.

Sunday, J. M., A. E. Bates, M. R. Kearney, R. K. Colwell, N. K. Dulvy, J. T. Longino, and R. B. Huey. 2014. Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. Proceedings of the National Academy of Sciences USA 111(5): 5610-5615.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. River Continuum Concept. Canadian Journal of Fisheries and Aquatic Sciences **37**:130-137.

Vonlanthen P, Bittner D, Hudson AG, Young KA, Muller R, et al. (2012) Eutrophication causes speciation reversal in whitefish adaptive radiations. Nature 482: 357-U1500.

Woodward, G., D. M. Perkins, and L. E. Brown. 2010. Climate change and freshwater ecosystems: impacts across multiple levels of organization. Philosophical Transactions of the Royal Society B-Biological Sciences **365**:2093-2106.

Yvon-Durocher, G., J. M. Caffrey, A. Cescatti, M. Dossena, P. del Giorgio, J. M. Gasol, J. M. Montoya, J. Pumpanen, P. A. Staehr, M. Trimmer, G. Woodward, and A. P. Allen. 2012. Reconciling the temperature dependence of respiration across timescales and ecosystem types. Nature **487**:472-476.

Yvon-Durocher, G., J. I. Jones, M. Trimmer, G. Woodward, and J. M. Montoya. 2010. Warming alters the metabolic balance of ecosystems. Philosophical Transactions of the Royal Society B-Biological Sciences **365**:2117-2126.

Chapter 2: Assessing non-point source nitrogen loading and nitrogen fixation in lakes using δ15N and nutrient stoichiometry*

ABSTRACT

Runoff from human-dominated watersheds has greatly altered nitrogen (N) and phosphorus (P) cycling in lakes. Nutrients from human sources are distinct from those from undisturbed ecosystems in several ways including lower N:P ratios, which can drive ecosystems to N limited conditions, and enriched stable N isotope ratios. In this study, we used these distinct characteristics to estimate shifts in N sources to 27 lakes across a human density gradient in western Washington. We compared an N stable isotope two-source mixing model with a mixing model that coupled N stable isotopes to N:P stoichiometry and included N fixation. We found that a two-source mixing model (human and watershed sources) did not explain observed variation in δ^{15}N of particulate organic matter (POM) and primary consumers ($R^2 = 0.60$) as well as model that included a third N source (N fixation; $R^2 = 0.72$). When fixed N was facultatively added to the ecosystem below a critical N:P ratio, the more complex mixing model captured the observed patterns in POM and primary consumer δ^{15}N among lakes extremely well. In lakes with P concentrations > 20 μg L^{-1} (N:P mass ratio < 15.3), N-fixation became an increasingly important component of the N cycle, accounting for > 50% of lake N budgets. This model provides a novel way to estimate the contribution of non-point N sources and N fixation to lakes in watersheds subject to human nutrient inputs.

**** Full citation:*** *Jankowski, K. J., D. E. Schindler, and G. W. Holtgrieve. 2012. Assessing nonpoint-source nitrogen loading and nitrogen fixation in lakes using δ^{15}N and nutrient stoichiometry. Limnology and Oceanography 57(3): 671-683.*

INTRODUCTION

Human activities such as urbanization and agriculture increase loading of nitrogen (N) and phosphorus (P) to aquatic ecosystems (Carpenter et al. 1998; Grimm et al. 2008), which can cause widespread water quality and ecosystem health problems (Smith and Schindler 2009). Nutrient supply and recycling in lake ecosystems depends on a variety of factors, including point sources, land use patterns (Carpenter et al. 1998), food web structure (Elser et al. 1990), and rates of P and N recycling from sediments (Schindler et al. 1987; Levine and Schindler 1992; Nürnberg 1998). Human development often alters the source, frequency, and form of nutrients entering aquatic ecosystems. In particular, the increasing importance of non-point nutrient sources has made estimating inputs from specific sources particularly challenging. Yet, the ability to assess how watershed sources affect lake nutrient cycling remains critical to managing nutrient loading and identifying mechanisms for restoring eutrophic lakes (Smith and Schindler 2009).

Stable isotopes have been widely used to track human nutrient inputs to aquatic ecosystems (Cabana and Rasmussen 1996; McClelland et al. 1997; Vander Zanden et al. 2005). Human-derived nutrients are enriched in ^{15}N relative to other watershed sources, providing the opportunity to track human input of N into aquatic ecosystems and to follow its movement through food webs (Peterson and Fry 1988; Vander Zanden and Rasmussen 1999). Several studies have used isotopes as effective tracers of human N sources to aquatic environments (Cabana and Rasmussen 1996; Steffy and Kilham 2004; Bannon and Roman 2008) or to differentiate the sources of N inputs to a variety of aquatic ecosystems (Diebel and Vander Zanden 2009). For example, Vander Zanden et al. (2005) developed several isotope mixing models to identify the N sources most likely responsible for primary consumer $\delta^{15}N$, and found

that $\delta^{15}N$ was most strongly linked to riparian and watershed land use rather than in-lake N processing. In addition, Diebel and Vander Zanden (2009) were able to link primary consumer $\delta^{15}N$ in streams directly to landscape-level variables such as fertilizer application, manure spreading or the density of wetlands in individual catchments.

Stable N isotope signatures in biota are the product of a series of biological and chemical transformations such as N fixation, denitrification, nitrification, uptake and excretion. Therefore, it is challenging to constrain mixing models to account for these multiple processes and often necessitates using multiple tracers (Fry 2006). However, in addition to having a unique isotopic signature, human-derived nutrients also have N: P stoichiometry distinct from other watershed sources (Downing and McCauley 1992; Hecky et al. 1993). For example, runoff from forested watersheds is relatively rich in N compared to wastewater sources, while human sources are richer in P than runoff from intact watersheds (Downing and McCauley 1992). Low N:P ratios in human nutrient sources are a product of wastewater treatment processes that are typically more effective at the removal of N than P due to the primary role of nitrification-denitrification reactions in the treatment process (Heaton 1986).

Human sources of nutrients create additional complexity in lake nutrient cycles. Anthropogenic inputs increase both P and N loading to lakes but are richer in P than most other sources and typical algal requirements. Thus, the input of anthropogenic nutrients creates high demand for N and can induce substantial N fixation in lakes (Schindler 1977). Below a threshold ratio of N:P, N fixation is up-regulated to meet the ecosystem demand generated by excess P inputs from humans. This adds atmospheric N to the ecosystem through N fixation, which is depleted in ^{15}N relative to human and watershed sources (Fry 2006). As a result, human nutrient sources to aquatic ecosystems have both unique N stable isotope characteristics and N:P

stoichiometry -- two predictable characteristics that have not been coupled in quantitative assessments of nutrient loads to lakes.

Here we developed a linked stoichiometry – N stable isotope model (N fixation model) that simultaneously integrates N isotope mixing and stoichiometric constraints on N fixation to estimate the relative importance of human, watershed, and atmospheric sources of N to lakes. We use N and P stoichiometry of nutrient loads from watershed and human sources to estimate N and P loads to lakes and compare the N fixation model with a two-source isotope mixing model to explain the variation in the observed $\delta^{15}N$ in nutrient pools as lakes become eutrophied along an urbanization gradient. These analyses show that a simple isotope mixing model does a poor job at accounting for the inputs of human N to ecosystems. However, when this model is coupled with a simple stoichiometric model that accounts for the contrasts between the N:P ratios from humans and the watershed and their effects on in-lake N:P ratios, we can estimate N fixation and provide a better fit to N stable isotope characteristics of lakes along a eutrophication gradient. This approach provides a simple but powerful means to estimate the relative importance of various sources of N in human impacted ecosystems.

METHODS

We surveyed 27 lakes in the Greater Seattle region that spanned a large gradient of shoreline development and anthropogenic P loading (Moore et al. 2003), ranging from lakes within protected watersheds in the University of British Columbia's Malcolm Knapp Research Forest to urban lakes with 100% shoreline development. These lakes are physically similar: all are summer stratified, with a mean depth of 8 m (± 0.9 SE), average surface area of 2.97 km^2 (± 0.52 SE) and lie between 35-1121 meters above sea level (Moore et al. 2003, Francis et al. 2011). All lakes have some combination of stocked or native populations of rainbow trout (*Oncorhynchus gairdnerii*), cutthroat trout (*Oncorhynchus clarkii*), largemouth bass

(*Micropterus salmoides*), and similar assemblages of zooplankton including abundant representatives from the genera *Ceriodaphnia*, *Cyclops*, *Diaptomus*, *Daphnia*, and *Bosmina* (Francis et al. 2011).

Limnological data

The lakes were sampled in mid-summer of 1998 for a number of physical, biological, and chemical characteristics including N stable isotope ratios in key food web components. We took measurements of temperature and oxygen at 1 m depth intervals at the deepest point in each lake with a YSI Incorporated Sonde 6600 V2 (YSI Incorporated). Shoreline development intensity was calculated as the percent of shoreline containing residential or recreational development within 10 m of shore (Francis and Schindler 2009).

We measured total N (TN) and total P (TP) concentrations by collecting duplicate water samples from the epilimnion, from the thermocline depth, and within 3 m of the lake bottom with a Van Dorn bottle. Unfiltered water was collected in acid-washed polyethylene bottles, frozen, and transported to the Marine Chemistry Laboratory at the School of Oceanography, University of Washington. TN and P were determined using persulfate digestion followed by colorimetric analysis on a Technicon model AAII auto-analyzer (Bran Luebbe).

We used chlorophyll *a* concentration from the water column as a surrogate for algal community biomass. We took two samples from the epilimnion, thermocline and hypolimnion of each lake using a Van Dorn bottle. Samples were transported back to the lab, filtered onto 47 mm diameter, 0.7 μm GF/F filters (Whatman) and frozen until analysis. Chlorophyll *a* was then extracted with methanol, determined fluorometrically and corrected for pheophytin content (Wetzel and Likens 1991).

Nitrogen stable isotope analysis

Surface particulate organic matter (POM) was collected by filtering 350 mL water samples onto pre-combusted (550ºC for 1 h) 47 mm GF/F filters, after pre-filtering through a 25 mm mesh to remove zooplankton, and then frozen. Zooplankton were collected as a pooled sample from the epilimnion, thermocline and hypolimnion using a 30 L Schindler-Patalas trap and preserved in 50% ethanol solution (Francis et al. 2011). Isotope analyses were conducted on bulk zooplankton samples after being dried at 60ºC and ground to a fine powder, and on POM using entire GF/F filters. Benthic macroinvertebrates were collected by sweep net in less than 1 m of water in the littoral zone of at least three sites per lake. Individual organisms were sorted to family and combined across sites to produce two composite samples for each lake. POM, zooplankton and invertebrates were analyzed for N isotopic composition at the Alaska Stable Isotope Laboratory at the University of Alaska Fairbanks, with a Carlo Erba NC2500 elemental analyzer interfaced to a Finnigan Delta Plus isotope ratio mass spectrometer. The ratio of ^{15}N to ^{14}N was expressed in conventional notation, relative to air, according to

$$\delta^{15}N = \frac{(R_{sample} - R_{standard})}{R_{standard}} \times 1000 \quad (1)$$

where $R_{sample} = {}^{15}N:{}^{14}N$ in the sample, and $R_{standard}$ is $^{15}N:{}^{14}N$ in air and equal to 0.003663 (Peterson and Fry 1987).

Two-source isotope mixing model

We developed a simple, two-source mixing model that accounted for isotope mixing between human N sources and watershed N sources and fit it to the observed $\delta^{15}N$ values of POM in these lakes. This model incorporates two N sources distinct in $\delta^{15}N$ and N:P ratio: 'human' N and 'watershed' N. Human N is defined as the N from wastewater and septic systems, which is enriched in ^{15}N. Watershed N is considered the combined contribution of atmospheric deposition and terrestrially-derived N from a forested watershed in the absence of

human development. Both ratios are considered to apply to the portion of N that is available for uptake. The mixing equation is as follows:

$$\delta^{15}N_{mix,i} = \frac{\delta^{15}N_w N_{w,i} + \delta^{15}N_h N_{h,i}}{N_{w,i} + N_{h,i}} \quad (2)$$

where $\delta^{15}N_{mix,i}$ is the isotopic ratio of the resultant mixture for each lake (denoted by i). $\delta^{15}N_w$ is the isotopic ratio of watershed N (in delta units) and $\delta^{15}N_h$ is the isotopic ratio of human N and are assumed to be constant among lakes. $N_{w,i}$ is the mass of N from watershed sources and $N_{h,i}$ is the mass of N from human sources. We assumed that the proportion of N from watershed sources was constant among lakes, but that $N_{h,i}$ varied according to human influence. Because human-derived N tends to be enriched in ^{15}N compared to watershed sources, this model produces a smooth asymptotic function describing the changes in the $\delta^{15}N$ of the ecosystem as human inputs become increasingly important.

None of the parameters on the right side of Eq. 2 were known a priori. However, incorporating measured nutrient concentrations and information on nutrient stoichiometry provided additional constraints on N_w and N_h. N_w and N_h are expected to vary among lakes in constant proportion to TP loading from watershed and human sources ($P_{w,i}$ and $P_{h,i}$, respectively):

$$N_{w,i} = P_{w,i} r_w \quad (3)$$

$$N_{h,i} = P_{h,i} r_h \quad (4)$$

where r_w is the N:P of watershed-derived nutrients, and r_h is the N:P of human-derived nutrients, which were assumed constant among lakes (Table 2.1). In protected watersheds, we assumed the largest nutrient inputs generally came from inflowing streams because the watershed: lake area ratios were large (mean ratio = 18.0). Therefore, we used N:P ratios of stream nutrient

concentrations, which have N:P mass ratios that range between 30-110 (mean = 67) in streams draining forested areas in this region (Kiffney et al. 2003; Brett et al. 2004; Tallis 2005).

The relative load of human-derived nutrients ($N_{h,i}$) was assumed to be reflected in the measured hypolimnetic TP concentrations from each study lake. We assumed the relative input of N and P from watershed sources scaled proportionally among lakes varying in watershed size and volume, but that the proportional increase in $N_{h,i}$ was reflected in the increase in hypo TP above background concentrations. In other words, a lake's TP concentration should increase with external P loading from human sources (eutrophication), and any increase in hypolimnetic TP concentration above the concentrations observed in reference lakes was assumed to proportional to the relative contributions of human-derived nutrients. Other studies have demonstrated that the in-lake P concentration is a good indicator of external load when internal load is minimal (Nürnberg 1984; Sondegaard et al. 1999; Nürnberg and LaZerte 2004). The lakes included in this study had oxic hypolimnia during the majority of the summer (King County Small Lakes Program unpubl. data), and therefore low potential for significant internal loading (Nürnberg 1984). We also sampled lakes early in early summer (June and July) to minimize the inputs of internal loading. However, we tested this assumption and its effect on model results by removing lakes under various minimum hypolimnetic oxygen thresholds (as a proxy for potential internal P loading) and comparing results when using hypolimnetic vs. epilimnetic P concentration as an estimate of the relative magnitude of P loading (Sondergaard et al. 1999). Thus, the relative mass of P from human sources was calculated as follows:

$$P_{h,i} = \frac{P_{hyp,i} - P_w}{P_w} \tag{5}$$

with $P_{hyp,i}$= P concentration by mass measured in the hypolimnion, P_w = minimum watershed P, which was considered constant across all lakes and derived from minimum value found in

undisturbed lakes (2.7 μg L^{-1}), which varied little among undeveloped lakes (n = 4, standard deviation (SD) = 2.0 μg L^{-1}). Finally, the relative TN (L_N) and P (L_P) loads were calculated as follows based on the stoichiometry of watershed and human inputs:

$$L_{P,i} = P_{w,i} + P_{h,i} \tag{6}$$

$$L_{N,i} = P_{w,i} r_w + P_{h,i} r_h \tag{7}$$

Mixing model with N fixation

We expanded the simple two source mixing model to include a third potential N source to each lake: N derived from N fixation (N_{fix}). The mixing equation was amended as follows:

$$\delta^{15}N_{mix} = \frac{\delta^{15}N_{w,i}N_{w,i} + \delta^{15}N_{h,i}N_{h,i} + \delta^{15}N_{fix,i}N_{fix,i}}{N_{w,i} + N_{h,i} + N_{fix,i}} \tag{8}$$

We added a stoichiometric constraint to this model such that when N is limiting in a given lake (i.e., when in-lake N:P ratio drops to a threshold value), N fixation is up-regulated to meet the N demand generated by excess P loading from human sources. We represented this threshold value by r_{crit} that defined the point below which the ecosystem became limited by the availability of N. We assigned atmospherically-derived N a fixed value of -1‰ (Fry 2006), and added this source to the mixture only after nutrient load stoichiometry fell below r_{crit}:

$$\begin{aligned} &\text{if } r_i < r_{crit}, \text{ then } N_{fix,i} = L_P r_{crit} - L_N \\ &\text{else } N_{fix,i} = 0 \end{aligned} \tag{9}$$

where, r_i = the ratio of L_N and L_P for individual lakes. TN and TP loads were calculated in the same manner as above (*see* Eqs. 6-7), but TN load calculations were modified to include N from N fixation:

$$L_{N,i} = N_{w,i} + N_{h,i} + N_{fix,i} \tag{10}$$

To evaluate whether N incorporated into the base of the food web was transferred to higher trophic levels, we also fit the N fixation model to the observed $\delta^{15}N$ of amphipods, zooplankton and damselflies. These taxa were useful indicators of the overall importance of N from different sources to the broader ecosystem since they occupied different trophic levels and distinct habitats within these lakes. We fit both models to data on these taxa by reformulating the model to include a trophic fractionation constant (F):

$$\delta^{15}N_{mix} = \frac{\delta^{15}N_{w,i}N_{w,i} + \delta^{15}N_{h,i}N_{h,i} + \delta^{15}N_{fix,i}N_{fix,i}}{N_{w,i} + N_{h,i} + N_{fix,i}} + F$$

Bayesian estimation of model parameters

Bayesian analysis was used to estimate values for the threshold N:P ratio (r_{crit}), $\delta^{15}N_h$, and $\delta^{15}N_w$, r_w, and r_h. Bayesian methods allow for the comparison of model predictions to data and generate posterior probability distributions of model parameters by accounting for the likelihood of observing a set of data given a certain model and the prior probability of that model (Hilborn and Mangel 1997). This approach allowed us to assess how parameter uncertainty influenced model results and to estimate posterior probabilities of model parameters.

We designated prior distributions for model parameters as shown in Table 2.1. We used uniform priors distributions on r_{crit} and $\delta^{15}N_w$ and normally distributed priors on $\delta^{15}N_h$, r_w, and r_h. Uniform priors identify a range of values of equal probability, whereas the probability of values in a normal prior varies in accordance with a specified mean and standard deviation. The uniform prior for r_{crit} (1-40) was derived from literature values for optimal N:P ratios for algal taxa, which have been found to range from 4-38 (Smith 1982). The uniform prior distribution on $\delta^{15}N_w$ spanned from -4 to 0.5‰. The range was derived from reference sites in our study, which are similar to background values measured in several other studies in freshwater lakes and rivers

(Wayland and Hobson 2001; deBruyn et al. 2003; Anderson and Cabana 2006). We based the normal prior distribution of $\delta^{15}N_h$ on Leavitt et al. (2006) who measured $\delta^{15}N$ values of treated human wastes and found a mean of 16 (SD = 2). This value is well-supported by several other studies that have evaluated $\delta^{15}N$ signatures of sewage and septic outflows (10-20‰; Heaton 1986; Cabana and Rasmussen 1996; Mayor et al. 2002; Weiskel and Howes 1992). The normal prior on r_w was based on the measurements of Brett et al. (2004), who surveyed several forested streams in this region and found a mean of 67:1 by mass (SD = 20). Finally, we included a normal prior on r_h with a mean of 5 (SD = 1) based on the range of variation for N:P ratios of human wastewater cited the literature, which typically range from 2 – 10 (Canter and Knox 1985; Downing and McCauley 1992; Mayer et al. 2002; McCray et al. 2005).

Bayesian estimation of model parameters was performed using the Gibbs Sampling method of Markov Chain Monte Carlo with Just Another Gibbs Sampler (JAGS; http://sourceforge.net/projects/mcmc-jags/) in R through the 'runjags' and 'R2jags'packages (Denwood 2010; R Development Core Team 2010; Su and Yajima 2010). The models used a normal likelihood when determining quality of fits to the data. Three Markov chains were generated for each analysis. Chains were 10000 iterations with a 5000 iteration burn-in. Estimated posterior distributions were considered to have converged on the underlying true distribution if the autocorrelation among saved parameter draws was < 0.05 for all model parameters. We report the mean and 95% credible intervals for each parameter estimate and show the posterior distributions for both models.

We then compared the simple and N fixation models for each taxon with the estimated parameters using the Deviance Information Criterion. Deviance Information Criterion (DIC) is a penalized likelihood criterion for evaluating support among competing models. DIC is similar to

Akaike's Information Criterion (AIC), but formulated for models with Bayesian estimated parameters by Markov Chain Monte Carlo. The equation to calculate is: DIC = pD + Dbar, where Dbar is a measure of how well the model fits to the data and pD is a penalty for the effective number of parameters in the model. As with AIC, models with the lowest DIC best explain the data, models within 1-2 units of the model with the lowest overall DIC are reasonably equivalent in explaining the data, and models within 3-7 units have considerably less empirical support (Spiegelhalter et al. 2002).

RESULTS

The lakes in this study spanned a broad range in limnological conditions and shoreline development levels (Table 2.2). Nutrient concentrations characterized these lakes from oliotrophic to eutrophic (epilimnetic TP range from 3-60 μg L^{-1}). There was an increase in POM $\delta^{15}N$ ($\delta^{15}N_{POM}$) associated with several variables reflective of increasing human influence and eutrophication. Values for $\delta^{15}N_{POM}$ ranged from -3.1 to 6.5‰ and increased with TP concentration in the epi-, meta-, and hypolimnion (Fig. 2.1). The trend was not linear, however; $\delta^{15}N_{POM}$ values were roughly constant in lakes with TP concentrations greater than 20 μg L^{-1} in all three cases.

The N fixation model fit the observed $\delta^{15}N_{POM}$ data better than the two-source mixing model (Table 2.3, Fig. 2.2). The two-source model predicted a smooth but saturating increase in $\delta^{15}N_{POM}$ with increased P (Fig. 2.2B) and estimated $\delta^{15}N_w$ of –3.4‰ (95% credible limits: -4.3 to -2.4‰), $\delta^{15}N_h$ of 12.6‰ (8.7-16.8‰). This model estimated a r_w similar to the prior mean of 50 (21.8-85.1) and r_h of 5.5 (3.7-7.3). However, the two-source model did not capture the shape of the relationship observed between $\delta^{15}N_{POM}$ and P concentration, generally underestimating $\delta^{15}N_{POM}$ at low P concentrations and over-estimating $\delta^{15}N_{POM}$ at high P inputs (Fig. 2.2B).

The N fixation model also predicted an increase in $\delta^{15}N_{POM}$ with increases in P, but successfully captured the abrupt leveling off of $\delta^{15}N_{POM}$ at P concentrations greater than about 20 μg L^{-1} (Fig. 2.2A). The model estimated an r_{crit} value of 15.3 (9.6-22.7, mass ratio), the point at which fixed N was added to the ecosystem to maintain a minimum N:P stoichiometry. The N fixation model predicted a higher $\delta^{15}N_h$ value of 16.9‰ (13.2-20.6‰) and a $\delta^{15}N_w$ value of –3.3‰ (-4.2 to -2.4‰). The model predicted an r_w of 48.5 (22.4-76.2) and an r_h of 5.7 (4.0-7.5). The addition of this third N source to the mixture substantially improved the model fit to the data (Table 2.3, Fig. 2.2). DIC evaluation of the model fits to the data showed that the N fixation model (DIC = 81.5, Table 2.3) fit the data substantially better than the two-source mixing model (DIC = 100.2).

The posterior distributions for parameters estimated by both models were normally distributed about the means (Fig. 2.3). The posterior distribution for r_w was the widest of all estimated parameters. This likely reflects the actual variation in the relative background input of N and P to lakes in the region and fits with variation observed by other studies. This estimate could be better constrained with more direct measurements of watershed inputs of N and P to lakes.

Potential for internal P loading

Our predictions of $\delta^{15}N_h$ and $\delta^{15}N_w$ were robust to the exclusion of lakes with the highest potential for internal P loading (Table 2.3). When we removed lakes with hypolimnetic $O_2 < 1$ mg L^{-1}, we estimated a $\delta^{15}N_h$ of 17.0‰ (13.3-20.7‰) and a $\delta^{15}N_w$ of -3.3‰ (-4.3 to -2.4‰). Estimates when removing lakes with hypolimnia < 2 mg O_2 L^{-1} were nearly identical, with an estimated $\delta^{15}N_h$ of 16.9‰ (13.0-20.7‰) and $\delta^{15}N_w$ of -3.3‰ (-4.3 to -2.4‰). The predicted value and uncertainty of r_{crit} increased slightly when lakes with hypolimnetic O_2 concentrations <

1 and < 2 mg L^{-1} were removed. We estimated an r_{crit} of 15.4 (9.7-22.3) after removing < 1mg L^{-1} lakes and an r_{crit} of 15.7 (9.6-23.7) after removing < 2 mg O_2 L^{-1} lakes. However, lakes with low dissolved oxygen (DO) values were not exclusive to lakes with high TP concentrations, i.e., those producing the asymptote (Fig. 2.1), and the new estimates were similar to the mean of the posterior of the original model r_{crit} (Table 2.3).

We also fit the N fixation model using epilimnetic TP concentration as a proxy for external P load. The asymptotic trend in $\delta^{15}N_{POM}$ with increasing TP was not as pronounced using epilimnetic concentrations as for hypolimnetic concentrations (Fig. 2.1), however, $\delta^{15}N_{POM}$ values above a TP concentration, although more variable, remained roughly constant. Means of parameter estimates were similar, but posterior distributions (data not shown) were slightly wider when using epilimnetic TP. The two-source model estimated a $\delta^{15}N_h$ of 15.1‰ (10.8-19.0‰), a $\delta^{15}N_w$ of -3.5‰ (-4.5 to -2.5‰), an r_w of 41.2 (19.1-68.2) and an r_h of 5.6 (3.7-7.4). The N fixation model estimated r_{crit} of 15.2 (8.9-23.2), $\delta^{15}N_h$ of 17.1‰ (13.4-20.9‰), a $\delta^{15}N_w$ of -3.7‰ (-4.7 to -2.7‰), an r_w of 29.3 (9.6-53.7) and an r_h of 5.8 (3.9-7.6). Again, the N fixation model (DIC = 86.8) fit the data substantially better than the two-source model (DIC = 98.4).

Isotope signatures of consumers

Aquatic consumers followed the same increasing, but saturating, trend in $\delta^{15}N$ with increasing hypolimnetic TP (Fig. 2.4). Observed amphipod $\delta^{15}N$ ranged from 0.01-9.1‰, zooplankton had the widest range in $\delta^{15}N$ of -0.14-12.9‰, and damselfly $\delta^{15}N$ values fell between 1.6-10.8‰. The N fixation model fit the data better than the two-source model and estimated similar parameter values in all cases (Table 2.3), with R^2 values of 0.61, 0.42 and 0.63 for amphipods, zooplankton and damselflies, respectively. Using the estimates for r_{crit}, $\delta^{15}N_h$ and $\delta^{15}N_w$, r_w and r_h generated by the POM model, we estimated values for trophic fractionation (i.e.,

the offset from $\delta^{15}N_{POM}$) of 4.1‰ for amphipods, 4.2‰ for zooplankton and 4.6‰ for damselflies. Overall, the N fixation model provided extremely good fits to our observations of $\delta^{15}N$ in lake consumers (Table 2.3).

Ecosystem N budget

We used the N fixation model to estimate the proportional contribution of watershed N, human N and N fixation to lake N budgets as P loads increased (Fig. 2.5). The proportion of N from watershed sources decreased as human inputs increased to lakes in this study. It decreased from 100% of the lake N load to 10% in lakes with the highest nutrient concentrations. Human N load increased from 0% of the N budget to 36% in the highest nutrient lakes and N fixation increased from 0% to 53% of the N load across the nutrient loading gradient. Atmospheric N, therefore, became the dominant component of the N budget as P loading to lakes increased, demonstrating a switch in lake biota's use of terrestrial N sources to a heavy reliance on N from atmospheric sources in lakes with hypolimnetic P concentrations greater than ~ 50 $\mu g\ L^{-1}$.

DISCUSSION

We evaluated shifts in the sources of N to lakes with increasing levels of human nutrient input. We compared two isotope mixing models and found that a model that included a term for N derived from N-fixation and N:P stoichiometry best explained the patterns in $\delta^{15}N$ data for multiple taxa (Fig. 2.2, Table 2.3). Our comparison of a more traditional N isotope mixing approach with a mixing model including stoichiometry demonstrated the utility of incorporating this additional information in evaluating lake nutrient cycles. To our knowledge, no other modeling attempts of lake N cycles have paired these two approaches to quantify changes in lake N cycling, despite the large body of literature on each subject. Most studies that have used

natural abundance stable isotopes to examine the contribution of human nutrient inputs to aquatic ecosystem N budgets typically have accounted for 2 sources (depleted background δ^{15}N vs. enriched human δ^{15}N; Wayland and Hobson 2001; Savage and Elmgren 2004) as we did in our initial two-source model. Although we estimated reasonable values for δ^{15}N of the two sources, this model did not fit the trend in the data since it did not account for subsequent in-lake N cycling and interactions with the P cycle. There are well-known drawbacks to this two-source approach: the signatures of human N and background N are the result of multiple competing N cycling processes which differentially fractionate N isotopes, therefore, the true value is difficult to determine (Weiskel and Howes 1992; Leavitt et al. 2006) and this approach does not account for the altered stoichiometry of human nutrient inputs, whose low N:P ratio causes increased N limitation and often induces higher rates of N fixation. Both of these processes alter the isotopic signature of the biota so they may no longer reflect a simple mixture of background and human N inputs.

To address these issues, we used another widely recognized characteristic of human nutrient sources, their low N:P ratio, which also allowed us to account for the addition of atmospheric N. The N:P composition of nutrient sources to lakes is a major determinant of N:P in nutrient pools (Downing and McCauley 1992). The low relative load of N to P in human nutrient sources is well-documented to change N limitation and cycling in lakes (Schindler 1977, Smith 1983, Howarth et al. 1988). In response to high P inputs from human nutrients, lakes become N limited and cyanobacterial N fixer populations often increase to meet the N demand (Schindler 1977). Our estimate of a threshold N:P value, 15.3, reflects the mass ratio of N and P in nutrient loads necessary to up-regulate N fixation in these lakes. Our estimate is higher than the classic Redfield mass ratio of 7:1 (Redfield et al. 1963), however, other studies have shown

that organisms in habitats outside of the open ocean do not consistently have C:N:P reflective of the Redfield ratio (Sterner et al. 2008). POM in small lakes, such as those in our study, has been found to vary in N:P from 7.4 – 18.1 (Hecky et al. 1993; Sterner et al. 2008) and the optimal ratio of N:P for individual algal taxa has been found to range from 4 - 38 (Rhee 1978; Smith 1982). Additionally, Smith (1983) found that N-fixing cyanobacteria dominated lake phytoplankton communities at an N:P mass ratio of < 29:1, suggesting that organisms experience intense N limitation well above the classic Redfield N:P ratio. In fact, the lakes receiving nutrient loads below this threshold in our study have seen increased dominance by cyanobacteria in recent years (Moore et al. 2003; King County Small Lakes Program unpubl. data). The use of isotopes in combination with stoichiometry enabled us to understand under what conditions organisms experienced N limitation and the level of P loading necessary to induce an N fixer response at the whole-ecosystem scale.

Both models predicted similar values for $\delta^{15}N_w$ (Table 2.3). The $\delta^{15}N_w$ value of -3.3‰ that we estimated was based on the values measured in the undeveloped lakes in our study and was assumed to integrate the $\delta^{15}N$ of runoff from riparian areas and atmospheric deposition. The Puget Sound region is largely dominated by secondary growth forests that have high abundances of alders (Bechtold et al. 2003). As a result, the $\delta^{15}N$ of runoff from these areas is likely close to the $\delta^{15}N$ of fixed N, as our model predicts. Although the $\delta^{15}N$ of precipitation and deposition in this area is not well documented, studies from other regions have shown values of $\delta^{15}N$ for precipitation and deposition close to our estimated value of -3.3‰. For example, Heaton (1986) found an average value for $\delta^{15}NO_3$ in precipitation of -3‰ and Wankel et al. (2010) found an average $\delta^{15}NO_3$ in deposition of -1.7‰. We also measured $\delta^{15}NO_3$ (Sigman et al. 2001) in three of the undeveloped lakes in this study and found a similar range of values (-1.3 to -0.23‰).

Despite observing maximum $\delta^{15}N$ of about 6‰ in POM, the N fixation model estimated $\delta^{15}N_h$ of ~16.9‰, which is within a reasonable range of what other studies have measured or estimated for $\delta^{15}N$ values of human wastewater inputs and urban runoff (10-20‰, Heaton 1986; 11‰, Cabana and Rasmussen 1996; 21-31‰, Vander Zanden et al. 2005; 16.2‰, Leavitt et al. 2006). N from human sources becomes enriched in ^{15}N primarily through wastewater treatment processes; denitrifying bacteria preferentially take up and reduce $^{14}NO_3$ thus leaving the residual NO_3^- pool in wastewater effluent enriched in ^{15}N (Heaton 1986). Uncertainty in this estimate could stem from both variability in wastewater treatment processes (Leavitt et al. 2006) and in the fractionation that takes place en route to the lake (Weiskel and Howes 1992). For example, a study linking primary consumer $\delta^{15}N$ in lakes to riparian and watershed scale land use produced distinctly different estimates of $\delta^{15}N$ for human N inputs (Vander Zanden et al. 2005). They estimated a value of 21‰ using a model considering whole watershed scale land use variables and 31‰ when considering riparian land use.

Although this value could be further constrained by direct measurement of human nutrient inputs, we aimed to provide an efficient and simple means of estimating relative proportions of N sources to lakes, and their stable isotope signatures. Our approach produced values that compared well with other estimates based on alternative approaches. While we assumed that the isotope signatures of various nutrient sources did not vary among lakes, it is likely that they do, particularly for human-derived N inputs whose N isotope signatures will vary according to the efficiency of septic operations, fertilizer applications, and other processes that likely vary among watersheds. However, this variation in the isotope signal of human N should be reflected in our posterior estimates of $\delta^{15}N_h$ (Fig. 2.3).

Lake N budgets and N in food webs

While many studies have used the enriched $\delta^{15}N$ of human nutrients to track human nutrient inputs to aquatic ecosystems and their relative contribution to N budgets (Cabana and Rasmussen 1996; McClelland et al. 1997; Steffy and Kilham 2004), it has typically been difficult to compare the contribution of human N and fixed N across ecosystems. A novelty of the model developed here is that it combines the use of $\delta^{15}N$ as a tracer of human input with the N:P stoichiometry of human nutrients to predict relative N loads from multiple sources to lakes, including N fixation. We calculated the relative inputs from watershed, human, and atmospheric N sources and found that N fixation contributed an increasing proportion of N to overall lake budgets as human P loading increased (Fig. 2.5). It has been widely observed that when nutrient loads reach a threshold N: P ratio, the prevalence of N fixing cyanobacteria increases, N-fixation becomes an increasingly important proportion of N of lake N budgets (Schindler 1977; Scott et al. 2008) and can form up to 80% of the TN budget in eutrophic lakes (Howarth et al. 1988). Other studies have documented similar contributions of N fixation to N budgets in eutrophic lakes using a variety of methods (Howarth et al. 1988; Patoine et al. 2006; Scott et al. 2008). For example, a recent study by Patoine et al. (2006) using isotope mass-balance models examined how lake landscape position influenced N fixation as a proportion of lake N budgets. They found that although N fixation was a negligible component of N budgets in headwater sites, it made up to 77% of the N supply in more fertile, depositional downstream sites. They also found that $\delta^{15}N$ in POM was positively correlated with the abundance of cyanobacteria in their lakes, which is also true of the lakes in this study (Moore et al. 2003; King County Small Lakes Program unpubl. data).

N-fixation is difficult to measure at a whole ecosystem scale. Traditionally, others have used mass-balance approaches (Hellstrom 1996; Jonsson and Jansson 1997), estimated whole ecosystem scale N fixation from lab assays (Horne and Goldman 1972; Findlay et al. 1994; Mugidde et al. 2003) or small in situ mesocosm experiments (Bergmann and Welch 1990). N fixation, like all biogeochemical processes, is a spatially and temporally patchy process, thus small-scale experiments may misrepresent its extent. There is also growing appreciation for the potential N fixation in benthic habitats (Marcarelli and Wurtsbaugh 2009) that are difficult if not impossible to quantify in microcosms. Thus, an approach to measuring N fixation that integrates over space and time has become increasingly needed to understand how eutrophication changes the importance of N-fixation in aquatic ecosystems.

The combination of isotopes with stoichiometry allows not only an assessment of the importance of N fixation across time and space but also its importance within the food web and to multiple consumers. We observed the same trend in $\delta^{15}N$ across a gradient of increasing trophic status in multiple consumers from different trophic levels. This demonstrates that $\delta^{15}N_{POM}$ reflects the ecosystem level mixture of N from different sources, and suggests that N entering the ecosystem via N fixation is used throughout the food web (Fig. 2.4). The consumers included in this study (amphipods, zooplankton, and damselflies) occupy different trophic levels in lakes, and accordingly, we saw enrichment of these taxa with ^{15}N. We estimated a trophic fractionation relative to POM of 4.1‰, 4.2‰ and 4.6‰ for amphipods, zooplankton, and damselflies, respectively, which scale closely with an expected trophic fractionation of 3.4‰ (Minagawa and Wada 1884). The high estimated value for damselflies is likely reflective of omnivory by this taxon (Merritt, Cummins, and Berg 2008). There is significant variation among the diets of the three consumers considered in this study, and the three together integrate

across benthic and pelagic habitats within the lakes. For example, amphipods are benthic detritivores while zooplankton are pelagic herbivores or omnivores. Further, because these organisms are considerably longer lived than the organisms included in the POM samples, they provide a more temporally integrated estimate of the inputs of N from various sources to the ecosystem.

Uncertainties

An additional process that may contribute to the asymptotic trend that we observed is uptake fractionation of inorganic N. Marine and lab studies have demonstrated $\delta^{15}N$ of phytoplankton will asymptote as the availability of surface water NO_3^- increases (Altabet and Francois 1994; Sigman et al. 1999; Altabet 2006). In other words, at low concentrations of surface water NO_3, $\delta^{15}N_{POM}$ would directly reflect $\delta^{15}NO_3$, whereas at non-limiting concentrations algae will preferentially take up lighter isotopes of N thereby depleting their ^{15}N and enriching the residual NO_3 pool. However, studies in freshwater ecosystems have found little evidence for this relationship (Owen et al. 1999; Lehmann et al. 2004; Leavitt et al. 2006). They suggest that because primary productivity is generally P-limited in lakes (rather than N-limited as in marine systems), POM $\delta^{15}N$ is controlled by external N loading rather than by N assimilation (Lehmann et al. 2004). In addition, NO_3^- is undetectable in P-rich lakes in this study (i.e., lakes most depleted in ^{15}N) suggesting that N remains limiting to primary production at short time scales and fractionation is negligible (McClelland and Valiela 1997). In addition, we tested for uptake fractionation in a subset of six lakes across a range of external nutrient loading by measuring $\delta^{15}NO_3$ (Sigman et al. 2001) and comparing those values to $\delta^{15}N_{POM}$. We found that there was negligible difference between $\delta^{15}NO_3$ and $\delta^{15}N_{POM}$ (mean difference = -0.4‰, SD = 2.0‰). They were linearly related (Fig. 2.6, slope = 1.0, $R^2 = 0.64$, $p = 0.04$), with an intercept

not different from zero ($p = 0.55$), indicating little to no uptake fractionation in lakes across a wide range in trophic status. This relationship has also been shown in other freshwater studies (Leavitt et al. 2006, Gu 2009).

Another uncertainty of this model is our use of hypolimnetic TP to estimate external P load. In lakes with oxic hypolimnia (> 0.5 mg O_2 L^{-1}, Nürnberg 1984), P is largely retained in the sediments, and hypolimnetic TP can be a good estimation of external P loading (Nürnberg 1984; Schindler et al. 1987). However, eutrophic or hyper-eutrophic lakes commonly develop anoxic hypolimnia in response to nutrient loading, subsequent high productivity, and high rates of decomposition (Wetzel 2001). Under oxic conditions, iron will bind phosphate in the sediments, however, low oxygen availability leads to iron reduction and increased P solubility. However, because our sampling occurred shortly after summer thermal stratification, we expected that internally regenerated P was minimal in the study lakes. Nevertheless, we re-fit the model without lakes that had measured hypolimnetic DO concentrations under various thresholds (< 1 mg O_2 L^{-1} and < 2 mg O_2 L^{-1}). Despite removing these lakes, the trend in $\delta^{15}N$ with TP concentration was consistent (Fig. 2.1C) and our estimates of N source contributions were similar to the original model (Table 2.3). In fact, the lakes with potential for internal loading were not the lakes with highest P concentration (Fig. 2.1C). Additionally, the trend was consistent (although more variable) when using epilimnetic TP as a proxy for P loading (data not shown). Furthermore, we also observed the same relationship between P concentrations and $\delta^{15}N$ in several taxa with distinctly different life-spans that integrate N budgets over various time scales, which suggests that our estimates of N input to these systems are not substantially confounded by internal P loading.

Last, by using a Bayesian approach to estimate the parameters in our models and to compare model performance, we explicitly assessed uncertainties in our analyses. The posterior distributions of the model parameters reflect the degree to which individual parameters are defined by the data and the priors used in the analysis. Inspection of Figure 2.4 shows that all model parameters were clearly constrained, except for N:P ratio of watershed derived nutrients in the absence of humans whose 95% credible interval spanned from 22.4 to 76.2. At present we do not know whether this large range in parameter space reflects inherent model uncertainty or real variation in the N:P ratio of non-human nutrients loaded from watersheds across this region.

The Seattle, Washington metropolitan region is the most populous and urbanized area in the U.S. Pacific Northwest. Its high rate of urban expansion and sharp rural-urban gradient makes it an ideal place to study the effects of human development and nutrient loading on lake ecosystems (Moore et al. 2003; Alberti et al. 2007). We evaluated how the contributions of N from human, watershed and atmospheric sources to lake N budgets changed along this urbanization gradient. We found a major shift in the relative contribution of N from watershed, human and atmospheric sources and that N fixation made up a much larger fraction of the N budgets in more developed and nutrient-rich lakes. We were able to detect these shifts in N loading from external sources by developing models incorporating nutrient stoichiometry with natural abundance stable N isotopes. We acknowledge that our broad groupings of N sources integrate across the complexity of N cycling and fractionation that takes place from source to uptake, yet despite this over-simplification, we successfully captured the trend in the data using a fairly simple model.

The increased reliance of lake biota on atmospheric N as nutrient inputs increase in a single lake has been documented on numerous occasions (Schindler 1977; Smith 1982; Scott et

al. 2008). However, the relative magnitude of its contribution has been difficult to compare across systems. Recent debate on whether N limitation dominates in eutrophic systems is partially driven by the difficulty of measuring whether N-fixation is able to satisfy N demand induced by chronic inputs of P to lakes (Elser et al. 2007; Sterner 2008; Xu et al. 2010). The N fixation model considered here provides a novel means for cross-system comparison of the magnitude and uncertainty of N fixation and can contribute to the understanding of human effects on nitrogen supply and demand in lakes.

Table 2.1. Description of parameters and data used in mixing models: Measured variables, fixed constants, estimated parameters and their associated prior distributions.

Measured variables	Fixed model constants	Estimated parameters	Prior distributions
$\delta^{15}N_{POM}$ (‰)	$P_w = 0.0027$	$\delta^{15}N_h$	normal: $\mu = 16$‰, $\sigma = 2$
$\delta^{15}N_{amph}$ (‰)	$\delta^{15}N_{fix} = -1$‰	$\delta^{15}N_w$	uniform: -4 - 0.5‰
$\delta^{15}N_{damsel}$ (‰)		r_{crit}	uniform: 1-40
$\delta^{15}N_{zoop}$ (‰)		r_w	normal: $\mu = 67$, $\sigma = 20$
$P_{hyp,i}$		r_h	normal: $\mu = 5$, $\sigma = 1$
		F	normal: $\mu = 3$, $\sigma = 1$

Table 2.2. Ranges of lake nutrient and limnological characteristics for 27 study lakes in Puget Sound region of Washington State in 1998 (na = not applicable, nm = not measured, DOC = dissolved organic carbon).

	Epilimnion	Metalimnion	Hypolimnion
Total P (μg L^{-1})	3.2 - 59.0	3.8 - 43.0	2.7 - 76.0
Total N (μg L^{-1})	10.9 - 98.9	70.4 - 89.1	51.7 - 1648.6
Chlorophyll *a* (μg L^{-1})	0.5 - 17.0	0.92 - 49.8	nm
TN:TP (by mass)	9.4 - 70.5	3.4 - 86.7	11.3 - 89.0
Secchi depth (m)	1.5 - 10.5	na	na
DOC (mg L^{-1})	0.014 - 0.234	0.01 - 0.229	nm
$\delta^{15}N_{POM}$ (‰)	-3.34 - 6.46	nm	nm
Zooplankton $\delta^{15}N$ (‰)	-0.14 - 12.94	nm	nm

Table 2.3. Estimated parameter values based on the mean of the posterior distribution and goodness of fit for each model and taxa considered. Values shown in parentheses are bounds of 95% credible interval for parameter estimates.

Model	Taxa	N	r_{crit}	$\delta^{15}N_h$	$\delta^{15}N_w$	r_w	r_h	DIC	Dbar	pD	R^2
N fixation	POM	22	15.3 (9.6, 22.7)	16.9 (13.2, 20.6)	-3.3 (-4.2, -2.4)	48.1 (22.4, 76.2)	5.7 (4.0, 7.5)	81.50	79.70	1.8	0.72
Two-source	POM	22		12.6 (8.7, 16.8)	-3.4 (-4.3, -2.4)	49 (21.8, 85.1)	5.5 (3.7, 7.3)	100.2	98.30	1.9	0.60
N fixation (< 1 mg L^{-1} DO)	POM	20	15.4 (9.7, 22.3)	17 (13.3, 20.7)	-3.3 (-4.3, -2.4)	45.4 (16.5, 75.0)	5.7 (3.9, 7.5)	75.30	73.50	1.8	0.73
Two-source (< 1 mg L^{-1}DO)	POM	20		13.2 (9.0, 17.2)	-3.4 (-4.4, -2.3)	53.6 (23.4, 88.5)	5.4 (3.7, 7.3)	94.30	92.50	1.8	0.59
N fixation (< 2 mg L^{-1} DO)	POM	16	15.7 (9.6, 23.7)	16.9 (13.0, 20.7)	-3.3 (-4.3, -2.4)	46.9 (17.5, 77.6)	5.7 (4.0, 7.5)	66.20	64.40	1.9	0.73
Two-source (< 2 mg L^{-1} DO)	POM	16		14.3 (10.5, 18.2)	-3.4 (-4.4, -2.5)	58.6 (30.1, 91.7)	5.3 (3.6, 7.2)	79.80	78.30	1.5	0.61
N fixation	Amphipods	27	21.3 (11.9, 35.2)	16.7 (13.1, 20.5)	-3.8 (-4.8, -2.9)	52.1 (22.5, 85.2)	5.6 (3.7, 7.3)	103.4	101.8	1.6	0.61
Two-source	Amphipods	27		12.3 (8.1, 16.5)	-3.6 (-4.5, -2.6)	62.1 (18.3, 103.1)	5.1 (3.1, 7.1)	127.7	125.9	1.8	0.34
N fixation	Zooplankton	26	21.2 (11.4, 36.1)	16.8 (13, 20.6)	-3.8 (-4.8, -2.8)	46.3 (15.1, 80.4)	5.6 (3.8, 7.5)	127.1	128.6	1.5	0.42
Two-source	Zooplankton	26		13.1 (9.0, 17.2)	-3.6 (-4.6, -2.7)	60.2 (18.3, 100.5)	5.2 (3.3, 7.2)	150.0	148.2	1.6	0.18
N fixation	Damselfly	27	17.2 (9.9, 26.6)	17 (13.3, 20.7)	-3.6 (-4.6, -2.7)	42.5 (10.8, 73.6)	5.7 (3.9, 7.5)	101.7	103.3	1.7	0.63
Two-source	Damselfly	27		11.3 (7.2, 16)	-3.6 (-4.5, -2.6)	34.6 (5.6, 84.1)	5.5 (3.6, 7.4)	124.3	121.1	3.2	0.46

FIGURE LEGENDS

Figure 2.1. Relationship of $\delta^{15}N_{POM}$ with (A) epilimnetic, (B) metalimnetic, and (C) hypolimnetic TP concentration across lakes (n = 22). Open circles and triangles denote lakes with hypolimnetic DO concentrations of < 1 mg L^{-1} and < 2 mg L^{-1}, respectively.

Figure 2.2. (A) Fit of two-source mixing model (R^2=0.61) and (B) N fixation model (R^2=0.72) to $\delta^{15}N_{POM}$ data from all lakes (n=22). Dashed lines show credible intervals.

Figure 2.3**.** Posterior distributions two-source model parameters: (A) $\delta^{15}N_h$, (B) $\delta^{15}N_{w,}$ (C) r_w, (D) r_h and N fixation model parameters: (E) r_{crit}, (F) $\delta^{15}N_h$, (G) $\delta^{15}N_{w,}$ (H) r_w, (I) r_h,

Figure 2.4. Trends and fit of N fixation model to $\delta^{15}N$ of (A) amphipod larvae (n=27, R^2=0.61), (B) zooplankton (n=26, R^2=0. 42), and (C) damselfly larvae (n=27, R^2=0.63).

Figure 2.5. Shifts in the percent of N load to lakes from N fixation, watershed, and human sources with increases in TP load. Solid line = watershed N, dotted line = human N, dashed line = N fixation.

Figure 2.6**.** Comparison of $\delta^{15}NO_3$ with $\delta^{15}N_{POM}$ in subset of six lakes. Solid line is regression line (R^2 = 0. 59, p = 0.04)

Figure 2.1.

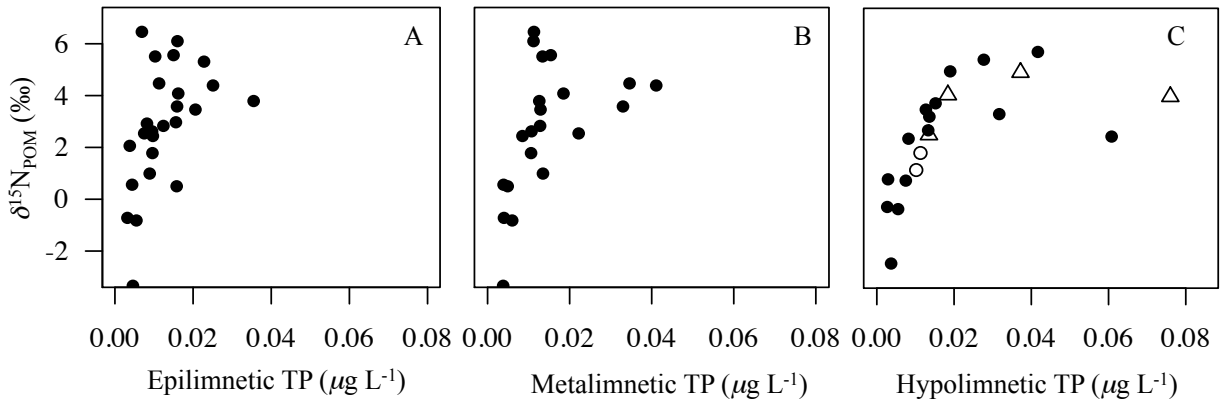

A
B
C
6
4
2
0
-2
$\delta^{15}N_{POM}$ (‰)
0.00
0.02
0.04
0.06
0.08
Epilimnetic TP (μg L^{-1})
Metalimnetic TP (μg L^{-1})
Hypolimnetic TP (μg L^{-1})

Figure 2.2.

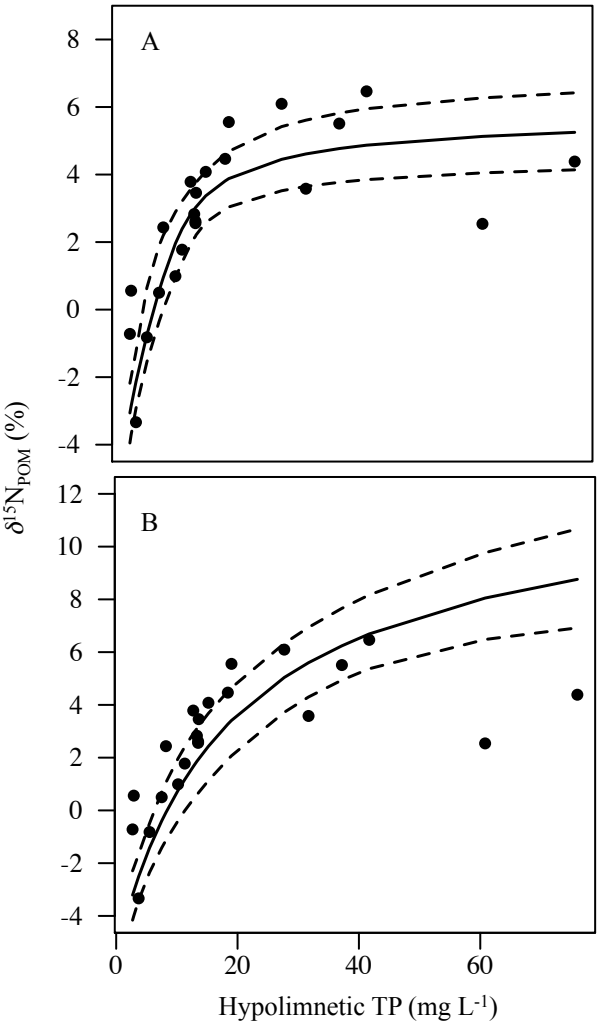

A
B
8
6
4
2
0
-2
-4
12
10
8
6
4
2
0
-2
-4
0
20
40
60
$\delta^{15}N_{POM}$ (%)
Hypolimnetic TP (mg L^{-1})

Figure 2.3.

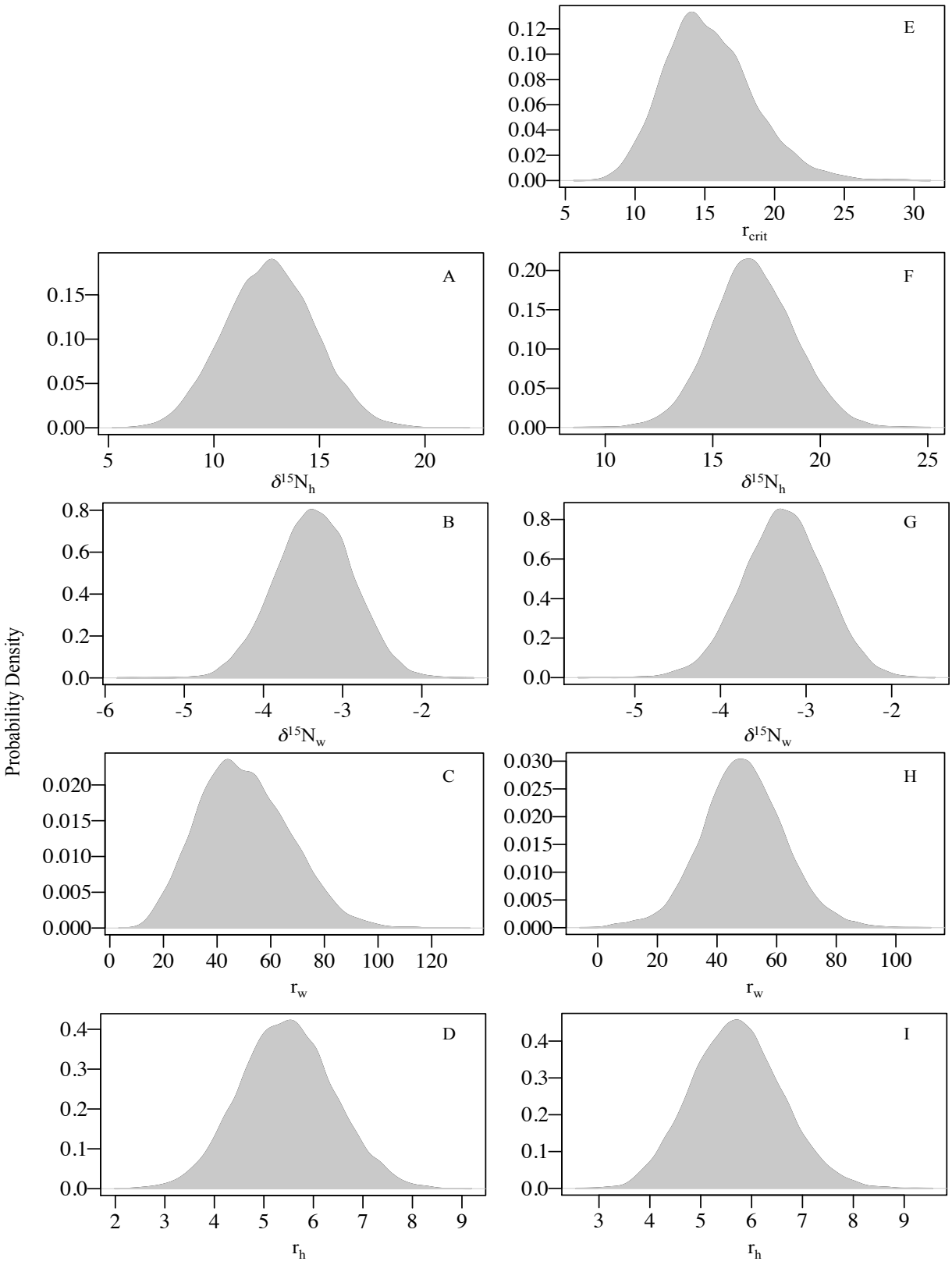

Probability Density
A
B
C
D
E
F
G
H
I
$\delta^{15}N_h$
$\delta^{15}N_w$
r_w
r_h
r_{crit}

Figure 2.4.

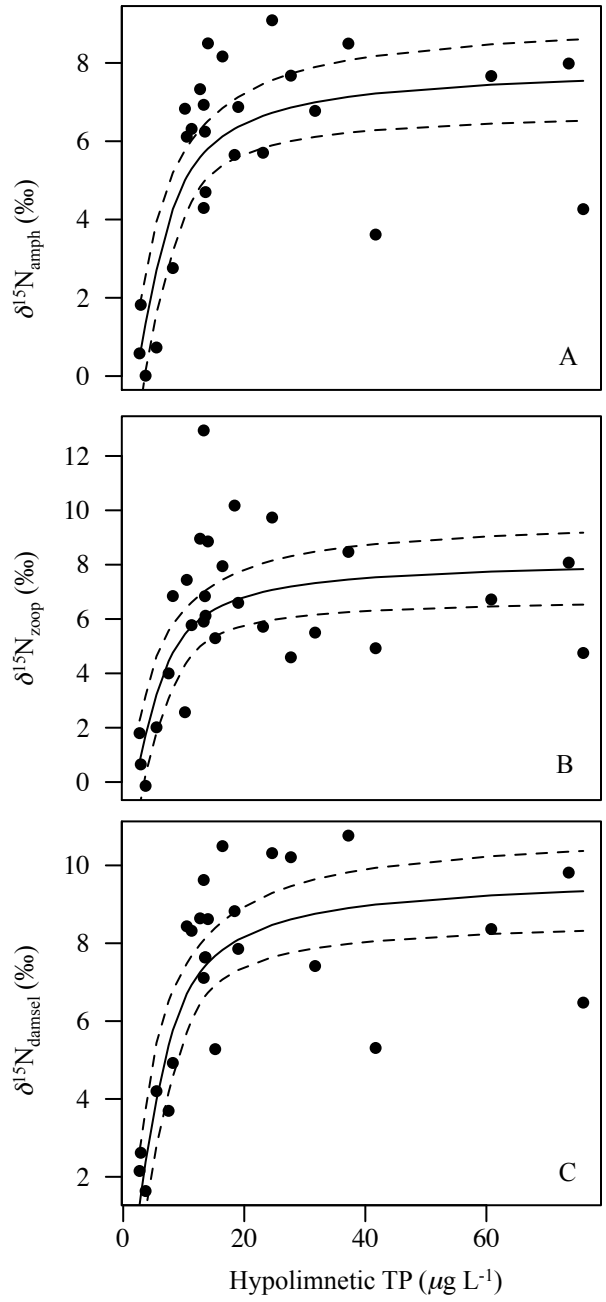

$\delta^{15}N_{amph}$ (‰)
$\delta^{15}N_{zoop}$ (‰)
$\delta^{15}N_{damsel}$ (‰)
A
B
C
Hypolimnetic TP (μg L^{-1})

Figure 2.5.

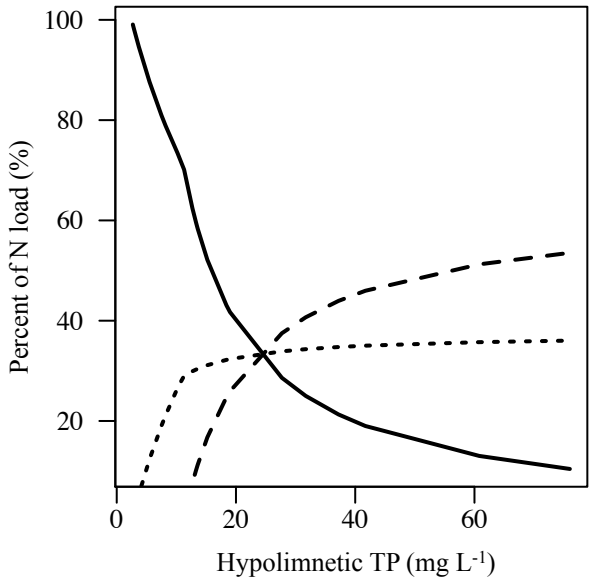
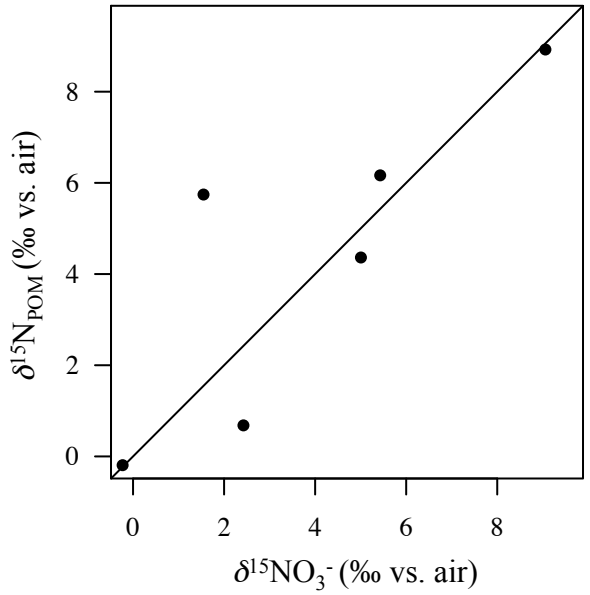

100
80
60
40
20
Percent of N load (%)
0
20
40
60
Hypolimnetic TP (mg L-1)

Figure 2.6.



REFERENCES

Alberti, M., D. Booth, K. Hill, B. Coburn, C. Avolio, S. Coe, and D. Spirandelli. 2007. The impact of urban patterns on aquatic ecosystems: An empirical analysis in Puget lowland sub-basins. Landscape Urban Plan. **80:** 345-361.

Altabet, M. A., and R. Francois. 1994. Sedimentary nitrogen isotopic ratio as a recorder for surface ocean nitrate utilization. Global Biogeochem. Cy. **8:** 103-116.

Altabet, M. A. 2006. Isotopic tracers of the marine nitrogen cycle: Present and past, p. 251-293. In J. Volkman [ed.], The Handbook of Environmental Chemistry 2N. Springer-Verlag.

Anderson, C., and G. Cabana. 2005. delta N-15 in riverine food webs: effects of N inputs from agricultural watersheds. Can. J. Fish. Aquat. Sci **62:** 333-340.

Bannon, R. O., and C. T. Roman. 2008. Using stable isotopes to monitor anthropogenic nitrogen inputs to estuaries. Ecol. Appl. **18:** 22-30.

Bechtold, J. S., R. T. Edwards, and R. J. Naiman. 2003. Biotic versus hydrologic control over seasonal nitrate leaching in a floodplain forest. Biogeochemistry **63:** 53-71.

Bergmann, M. A., and H. E. Welch. 1990. Nitrogen-fixation by epilithic periphyton in small arctic lakes in response to experimental nitrogen and phosphorus fertilization. Can. J. Fish. Aquat. Sci. **47:** 1545-1550.

Brett, M. T., G. B. Arhonditsis, S. E. Mueller, D. M. Hartley, J. D. Frodge, and D. E. Funke. 2005. Non-point-source impacts on stream nutrient concentrations along a forest to urban gradient. Environ. Manage. **35:** 330-342.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multi-model inference: A practical information-theoretic approach, 2nd ed. Springer-Verlag.

Cabana, G., and J. B. Rasmussen. 1996. Comparison of aquatic food chains using nitrogen isotopes.

P. Natl. Acad. Sci. USA. **93:** 10844- 10847.

Canter L.W., and R.C. Knox RC. 1985. Septic tank system effects on ground water quality. CRC Press.

Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecol. Appl. **8:** 559-568.

deBruyn, A. M. H., D. J. Marcogliese, and J. B. Rasmussen. 2003. The role of sewage in a large river food web. Can. J. Fish. Aquat. Sci. **60:** 1332-1344.

Denwood, R. J. 2010. runjags: Run Bayesian MCMC Models in the BUGS syntax from within R. R package version 0.9.9-1. http://sourceforge.net/projects/mcmc-jags

Diebel, M. W., and M. J. Vander Zanden. 2009. Nitrogen stable isotopes in streams: effects of agricultural sources and transformations. Ecol. Appl. **19:** 1127-1134.

Downing, J. A., and E. McCauley. 1992. The nitrogen - phosphorus relationship in lakes. Limnol. Oceanogr. **37:** 936-945.

Elser, J. J., M. E. S. Bracken , E. E. Cleland, D. S. Gruner, W. S. Harpole, H. Hillebrand, J. T. Ngai, E. W. Seabloom, J. B. Shurin, and J. E. Smith. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. **10:** 1135-1142.

Elser, J. J., H. J. Carney, and C. R. Goldman. 1990. The zooplankton-phytoplankton interface in lakes of contrasting trophic status - an experimental comparison. Hydrobiologia **200:** 69-82.

Findlay, D. L., R. E. Hecky, L. L. Hendzel, M. P. Stainton, and G. W. Regehr. 1994. Relationship between N-2-fixation and heterocyst abundance and its relevance to the nitrogen budget of lake-227. Can. J. Fish. Aquat. Sci. **51:** 2254-2266.

Francis, T. B., and D. E. Schindler. 2009. Shoreline urbanization reduces terrestrial insect subsidies to fishes in North American lakes. Oikos **118:** 1872-1882.

Francis T. B., D. E. Schindler, G. W. Holtgrieve, E. R. Larson, M. D. Scheuerell, B. X. Semmens, and E. J. Ward. 2011. Habitat structure determines resource use by zooplankton in temperate lakes. Ecol. Lett. **14**: 364-372.

Fry, B. 2006. Stable Isotope Ecology. Springer-Verlag.

Grimm, N.B., S. H. Faeth, N. E. Golubiewski, C. L. Redman, J. G. Wu, X. M. Bai, J. M. Briggs. 2008. Global change and the ecology of cities. Science **319:** 756-760.

Heaton, T. H. E. 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere - a review. Chem. Geol. **59:** 87-102.

Hecky, R. E., P. Campbell, and L. L. Hendzel. 1993. The stoichiometry of carbon, nitrogen, and phosphorus in particulate matter of lakes and oceans. Limnol. Oceanogr. **38:** 709-724.

Hilborn, R., and M. Mangel. 1997. The ecological detective: Confronting models with data. Princeton Univ. Press.

Horne, A. J., and C. R. Goldman. 1972. Nitrogen-fixation in clear-lake, California .1. Seasonal-variation and role of heterocysts. Limnol. Oceanogr. **17:** 678-692.

Howarth, R. W., R. Marino, J. Lane, and J. J. Cole. 1988. Nitrogen-fixation in fresh-water, estuarine, and marine ecosystems. 1. Rates and importance. Limnol. Oceanogr. **33:** 669-687.

Jonsson, A., and M. Jansson. 1997. Sedimentation and mineralisation of organic carbon, nitrogen and phosphorus in a large humic lake, northern Sweden. Arch. Hydrobiol. **141:** 45-65.

Kiffney, P. M., J. S. Richardson, and J. P. Bull. 2003. Responses of periphyton and insects to experimental manipulation of riparian buffer width along forest streams. J. Appl. Ecol. **40:** 1060-1076.

Leavitt, P. R., C. S. Brock, C. Ebel, and A. Patoine. 2006. Landscape-scale effects of urban nitrogen on a chain of freshwater lakes in central North America. Limnol. Oceanogr. **51:** 2262-2277.

Leggett, M. F., O. Johannsson, R. Hesslein, D. G. Dixon, W. D. Taylor, and M. R. Servos. 2000. Influence of inorganic nitrogen cycling on the delta N-15 of Lake Ontario biota. Can. J. Fish. Aquat. Sci. **57:** 1489-1496.

Lehmann, M. F., S. M. Bernasconi, J. A. Mckenzie, A. Barbieri, M. Simona, and M. Veronesi. 2004. Seasonal variation of the delta C-13 and delta N-15 of particulate and dissolved carbon and nitrogen in Lake Lugano: Constraints on biogeochemical cycling in a eutrophic lake. Limnol. Oceanogr. **49:** 415-429.

Marcarelli, A. M., and W. A. Wurtsbaugh. 2009. Nitrogen fixation varies spatially and seasonally in linked stream-lake ecosystems. Biogeochemistry **94:** 95-110.

Mayer, B, E. W. Boyer, C. Goodale, N. A. Jaworski, N. Van Breemen, R. W. Howarth, S. Seitzinger, G. Billen, K. Lajtha, K. Nadelhoffer, D. Van Dam, L. J. Hetling, M. Nosal, and K. Paustian. 2002. Sources of nitrate in rivers draining sixteen watersheds in the northeastern U.S.: Isotopic constraints. Biogeochemistry **57/58**: 171-197.

Mcclelland, J. W., I. Valiela, and R. H. Michener. 1997. Nitrogen-stable isotope signatures in estuarine food webs: A record of increasing urbanization in coastal watersheds. Limnol. Oceanogr. **42:** 930-937.

McCray, J. E., S. L. Kirkland, R. L. Siegrist, and G. D. Thyne. 2005. Model parameters for simulating fate and transport of on-site wastewater nutrients. Ground Water **43:** 628-639.

Merritt, R. W., K. W. Cummins, and M. B. Berg. 2008. Introduction to the aquatic insects of North America, 4th ed. Kendall Hunt.

Minagawa, M., and E. Wada. 1984. Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}N$ and animal age. Geochim. Cosmochim. Acta **48:** 1135–1140.

Moore, J. W., D. E. Schindler, M. D. Scheuerell, D. Smith, and J. Frodge. 2003. Lake eutrophication at the urban fringe, Seattle region, USA. Ambio. **32:** 13-18.

Mugidde, R., R. E. Hecky, L. L. Hendzel, and W. D. Taylor. 2003. Pelagic nitrogen fixation in Lake Victoria (East Africa). J. Great Lakes Res. **29:** 76-88.

Nürnberg, G. K. 1984. The prediction of internal phosphorus load in lakes with anoxic hypolimnia. Limnol. Oceanogr. **29:** 111-124.

Nurnberg, G. K. 1998. Prediction of annual and seasonal phosphorus concentrations in stratified and polymictic lakes. Limnol. Oceanogr. **43:** 1544-1552.

Nürnberg, G. K., and B. D. LaZerte. 2004. Modeling the effect of development on internal phosphorus load in nutrient-poor lakes. Water Resour. Res. **40**: 1-9.

Patoine, A., M. D. Graham, and P. R. Leavitt. 2006. Spatial variation of nitrogen fixation in lakes of the northern Great Plains. Limnol. Oceanogr. **51:** 1665-1677.

Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Ann. Rev. Ecol. Syst. **18:** 293-320.

R Development Core Team. 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Savage, C., and R. Elmgren. 2004. Macroalgal (*Fucus vesiculosus*) delta N-15 values trace decrease in sewage influence. Ecol. Appl. **14:** 517-526.

Schindler, D. W. 1977. Evolution of phosphorus limitation in lakes. Science **195:** 260-262.

Schindler, D. W., R. H. Hesslein, and M. A. Turner. 1987. Exchange of nutrients between sediments and water after 15 years of experimental eutrophication. Can. J. Fish. Aquat. Sci. **44:** 26-33.

Scott, J. T., R. D. Doyle, S. J. Prochnow, and J. D. White. 2008. Are watershed and lacustrine controls on planktonic N-2 fixation hierarchically structured? Ecol. Appl. **18:** 805-819.

Sigman, D. M., M. A. Altabet, R. Francois, D. C. Mccorkle, and J. F. Gaillard. 1999. The isotopic composition of diatom-bound nitrogen in Southern Ocean sediments. Paleoceanography **14:** 118-134.

Sigman, D. M., K. L. Casciotti, M. Andreani, C. Barford, M. Galanter, and J. K. Bohlke. 2001. A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. Analytical Chemistry **73:** 4145-4153.

Smith, V. H. 1983. Low nitrogen to phosphorus ratios favor dominance by blue-green-algae in lake phytoplankton. Science **221:** 669-671.

Smith, V. H. 1990. Nitrogen, phosphorus, and nitrogen-fixation in lacustrine and estuarine ecosystems. Limnol. Oceanogr. **35:** 1852-1859.

Smith, V. H., and D. W. Schindler. 2009. Eutrophication science: where do we go from here? Trends Ecol. Evol. **24:** 201-207.

Sondergaard, M., J. P. Jensen, and E. Jeppesen. 1999. Internal phosphorus loading in shallow Danish lakes. Hydrobiologia **408:** 145-152.

Spiegelhalter, D. J., N.G. Best, B. R. Carlin, and A. van der Linde. 2002. Bayesian measures of model complexity and fit. Journal of the Royal Statistical Society Series B-Statistical Methodology **64**: 583-639.

Steffy, L. Y., and S. S. Kilham. 2004. Elevated delta N-15 in stream biota in areas with septic tank systems in an urban watershed. Ecol. Appl. **14:** 637-641.

Sterner, R. W. 2008. On the phosphorus limitation paradigm for lakes. Int. Rev. Hydrobiol. **93:** 433-445.

Sterner, R. W., T. Anderson, J. J. Elser, D. O. Hessen, J. M. Hood, E. McCauley, and J. Urabe. 2008. Scale-dependent carbon: nitrogen : phosphorus stoichiometry in marine and freshwaters. Limnol. Oceanogr. **53:** 1169-1180.

Su, Y., and M. Yajima. 2010. R2jags: A Package for Running jags from R. R package version 0.02-11. http://CRAN.R-project.org/package=R2jags.

Tallis, H. M. 2005. Human impacts on the coupling of terrestrial and marine ecosystems. Ph.D. thesis. University of Washington.

Vander Zanden, M. J., and J. B. Rasmussen. 1999. Primary consumer delta C-13 and delta N-15 and the trophic position of aquatic consumers. Ecology **80:** 1395-1404.

Vander Zanden, M. J., Y. Vadeboncoeur, M. W. Diebel, and E. Jeppesen. 2005. Primary consumer stable nitrogen isotopes as indicators of nutrient source. Environ. Sci. Technol. **39:** 7509-7515.

Wankel, S. D., Y. Chen, C. Kendall, A. F. Post, and A. Paytan. 2010. Sources of aerosol nitrate to the Gulf of Aqaba: Evidence from delta N-15 and delta O-18 of nitrate and trace metal chemistry. Mar. Chem. **120:** 90-99.

Wayland, M., and K. A. Hobson. 2001. Stable carbon, nitrogen, and sulfur isotope ratios in riparian food webs on rivers receiving sewage and pulp-mill effluents. Can. J. Zoo. **79:** 5-15.

Weiskel, P. K., and B. L. Howes. 1992. Differential transport of sewage-derived nitrogen and phosphorus through a coastal watershed. Environ. Sci. Technol. **26:** 352-360.

Wetzel, R. W., and G. E. Likens. 1991. Limnological analyses, 2nd ed. Springer-Verlag.

Wetzel, R. W. 2001. Limnology: Lake and river ecosystems. 3rd ed. Academic Press.

Xu, H., H. W. Paerl, B. Q. Qin, G. W. Zhu, and G. Gao. 2010. Nitrogen and phosphorus inputs control phytoplankton growth in eutrophic Lake Taihu, China. Limnol. Oceanogr. **55:** 420-432.

Chapter 3: Resource availability and spatial heterogeneity control bacterial community response to nutrient enrichment in lakes*

Abstract

The diversity and composition of ecological communities often co-vary with ecosystem productivity. However, the relative importance of productivity, or resource abundance, versus the spatial distribution of resources in shaping those ecological patterns is not well understood, particularly for the bacterial communities that underlie most important ecosystem functions. Increasing ecosystem productivity in lakes has been shown to influence the composition and ecology of bacterial communities, but existing work has only evaluated the effect of increasing resource supply and not heterogeneity in how those resources are distributed. We quantified how bacterial communities varied with the trophic status of lakes and whether community responses differed in surface and deep habitats in response to heterogeneity in nutrient resources. Using ARISA fingerprinting, we found that bacterial communities were more abundant, richer, and more distinct among habitats as lake trophic state and vertical heterogeneity in nutrients increased, and that spatial resource variation produced habitat specific responses of bacteria in response to increased productivity. Furthermore, changes in communities in high nutrient lakes were not produced by turnover in community composition but from additional taxa augmenting core bacterial communities found in lower productivity lakes. These data suggests that bacterial community responses to nutrient enrichment in lakes vary spatially and are likely influenced disproportionately by rare taxa.

***Full Citation:** *Jankowski, K. J., D. E. Schindler, and M. C. Horner-Devine. 2014. Resource availability and spatial heterogeneity control bacterial community response to nutrient enrichment in lakes. PLOS ONE 9(1): e86991*

Introduction

Ecosystem productivity is an important driver of the diversity and composition of ecological communities. Much attention has been given to understanding how communities change with increased productivity, due to the desire to understand how species and their threats are distributed globally [1] and the widespread increase in nutrient enrichment and primary productivity of many ecosystems [2]. Productive ecosystems often support high species richness [3], as evidenced by diversity hotspots in ecosystems such as marine upwelling zones [4] and tend to host distinct communities from low productivity ecosystems. Productivity is thought to promote changes in species richness and composition due to the increased energy available to support the coexistence of multiple species and trophic levels [5,6], as well as by promoting shifts to species that dominate in productive environments. However, productivity is not always a good predictor of species richness [7], and the mechanisms behind observed richness and compositional changes in response to increased ecosystem productivity remain obscure [8].

Spatial or temporal heterogeneity in resource availability can also facilitate the coexistence of species in many environments [9,10], and is commonly used to explain why species richness varies with ecosystem productivity [11-13]. Yet, the relative importance of resource availability and heterogeneity in influencing patterns of species richness and composition in productive ecosystems remains unclear for many ecological communities [8,14-16], especially for prokaryotes. Bacteria are a fundamental component of food webs and provide the foundation for overall ecosystem functioning, yet we know relatively little about how bacterial communities respond to increases in productivity in most ecosystems [17-19]. In addition, bacteria have unique characteristics, such as metabolic flexibility and dormancy that might make their response to productivity and resource heterogeneity unique. In addition,

bacteria can acquire new functional capacities through the exchange of genetic material [20], thus, taxonomic richness may be unresponsive to changes in productivity [21].

Lakes vary widely in productivity and the heterogeneity of resource distribution in response to variation in nutrient loading from human and watershed sources [15,22]. Increased primary production, or trophic status, in lakes is associated with changes in species richness and composition of many ecological communities, including bacteria [23,24]. The richness of macroorganisms often declines at richer trophic state, due to the dominance of phytoplankton that are less palatable or toxic to consumers [25], declines in littoral productivity [26], and changes to the physical and chemical characteristics of the lake environment [22]. Therefore, changes associated with increased lake trophic status often negatively impact diversity of lake communities, change their composition, and lead to the dominance of a few species through homogenization of food resources and reduction in habitat availability [15,27]. Bacterial communities are known to shift in response to increased lake trophic status [28], but the fundamental mechanisms and importance of resource distribution in mediating those changes have not been fully explored.

Nutrient enrichment in lakes tends to magnify the vertical differences in physical and chemical characteristics such as nitrogen (N), phosphorus (P) and dissolved oxygen (DO) among lake strata [22]. However, existing studies of the response of bacterial communities to eutrophication have only evaluated the responses of surface communities or the integrated water column rather than habitat-specific responses [28,29]. In stratified lakes, the surface layer (epilimnion) is typically warm, nutrient-poor, and productive, whereas the deep layer (hypolimnion) is cooler, richer in nutrients, and often low in dissolved oxygen (DO). These differences may be especially important when considering how the response of lake bacteria may

differ from eukaryotic communities since vertical differences in physical and chemical conditions are known to structure bacterial communities in stratified lakes [30-32]. For example, low DO in the hypolimnion promotes the use of diverse energy pathways by bacteria such as denitrification and sulfate reduction that are not energetically advantageous in the oxic epilimnion, and therefore, could promote higher diversity of bacterial communities in the entire water column in response to increased trophic status. Therefore, bacterial communities are likely less similar among lake strata in high productivity (eutrophic) than in low productivity (oligotrophic) lakes.

In addition, although several studies have observed changes in bacterial communities with increased lake trophic status [24,29,33], few have identified which type of bacterial taxa are responsible for driving shifts in overall composition [34,35]. For example, while there is increasing evidence that some taxa flourish in high productivity lakes [36], it is unclear whether taxonomic changes result from a complete turnover in the community [37], an increase in the relative abundance of a few key taxa [38], or the increased presence of previously rare or novel taxa that augment a core community of taxa present in low nutrient lakes. For example, a study that evaluated how dominant, common and rare taxa responded to another important disturbance in lakes, lake mixing, found that shifts in the bacterial community were driven by the increased dominance of a few taxa [38].

We evaluated how bacterial abundance, taxonomic richness, and composition changed among and within lakes along a gradient of increasing trophic state. In particular, we quantified the amount of variation in the bacterial response that was explained by trophic state, resource heterogeneity, and their combination. Second, we evaluated whether communities associated with different lake habitats (specifically the epi-, meta- and hypolimnion) responded differently

to increased trophic status than communities assessed in the surface layer or integrated at the whole-lake scale. Finally, we evaluated which taxa were responsible for changes in community characteristics; specifically, we asked whether patterns were driven by turnover in the community or by additional taxa augmenting a core community present across all lakes. Thus, in this study we were able to address whether changes in the observed number of taxa and composition of bacterial communities followed the same patterns as eukaryotic communities in response to productivity in lakes and whether the distribution or abundance of resources was more important in shaping those patterns.

METHODS

We sampled 21 lakes in the Puget Sound region of western Washington (USA) and southern British Columbia (Canada, Figure 3.1) that spanned a large gradient of anthropogenic nutrient loading and productivity [39]. We sampled during the summer-stratified period of July and August 2008. Therefore, our samples reflected the communities that had developed following two to three months of stratified conditions within the water column [31]. As previously described [39], the lakes included in this study were physically similar. Twenty of the 21 lakes were monomictic, and one lake was too shallow to develop thermal stratification. No permissions were required to access 17 of these lakes since they were accessible via a public boat launch. We obtained permission from the University of British Columbia to access the remaining four lakes, which were on the property of their Malcolm Knapp Research Forest. No endangered or protected species were involved in this research.

All bacterial community samples and measurements of lake environmental characteristics were collected over the deepest point in each lake. Water samples for nutrient and chlorophyll *a* analyses were collected from the epilimnion (surface), metalimnion (thermocline depth), and

hypolimnion (within 3 m of the lake bottom) with a van Dorn bottle. Total N (TN) was determined using the perchloric acid digestion method [40] followed by analysis with automated colorimetry on a Lachat autoanalyzer (Lachat Instruments, Loveland, CO, USA). Total P (TP) concentration was determined colorimetrically after persulfate digestion and reaction with molybdate and stannous chloride [40]. Water samples for inorganic N and P determination were pre-filtered through a 0.2 mm Supor filters (Supor-200, Pall Gelman, East Hills, NY) and then analyzed colorimetrically using the same methods as above without a pre-digestion step. Chlorophyll *a* concentration was determined fluorometrically (Turner Designs, Sunnyvale, California) and used as a surrogate for algal community biomass. Temperature, dissolved oxygen (DO), and pH measurements were taken at 1-m depth intervals with a YSI sonde 6600 (YSI Integrated Systems & Services, Yellow Springs, OH, USA). Other physical lake data such as mean and maximum depth, lake area, and drainage area were obtained from the King County Water and Land Resources Division and the Washington Department of Ecology.

Two water samples for bacterial community analysis were collected from the epilimnion, metalimnion and hypolimnion of each lake with a Van Dorn Bottle. Two 300-mL samples were pooled and bacteria collected on 0.2-μm filters (Supor-200, Pall Gelman, East Hills, NY). Filters were frozen immediately and stored at -80 ˚C until further processing. DNA was extracted from replicate filters using the Qiagen DNEasy Blood and Tissue Mini-kit (Qiagen, Valencia, CA). Samples for bacterial cell enumeration were preserved with 2% formalin, filtered onto a 0.2 μm black polycarbonate filter, stained with 4', 6-diamidino-2-phenylindole (DAPI), and viewed with a Nikon Eclipse 80i digital microscope at 1000x magnification.

Bacterial community composition and observed richness were assessed using automated ribosomal intergenic spacer analysis [41]. ARISA generates fingerprints of the microbial

community based on the length heterogeneity in the intergenic spacer region between the 16S and 23S rRNA genes, which varies among organisms. ARISA has similar limitations as other PCR-based fingerprinting approaches [41] and tends to only survey dominant taxa in a community, thus our assessment of bacterial community composition is really a comparison of the community of dominant taxa among lakes. However, ARISA has been shown to give a robust, high-resolution view of bacterial assemblages in aquatic ecosystems [42,43], to generate results that are consistent with more high resolution techniques [42,44], and can represent species-level taxonomic resolution (98-99% sequence similarity; [42]). The 16S-23S intergenic region was amplified using the polymerase chain reaction (PCR) from the total extracted DNA using 6-FAM-labelled universal 1406-F primer (5′ TGYACACACCGCCCGT-3′) and bacterial specific primer 23S-R(5′-GGGTTBCCCCATTCRG-3′) [41,45]. PCRs were conducted on a Mastercycler gradient thermocycler (Eppendorf, New York). PCR products were pooled, quantified, and analyzed on a MegaBACE 1000 automated capillary sequencer (GE Healthcare Corporation, New Jersey). Operational taxonomic units (OTUs) were generated by binning ARISA fragments into successively larger length bins based on their size and eliminating fragments that were <150 and >1300 bp [42]. We used peak area to estimate relative abundance of OTUs in our samples [43], which we considered to be the ratio of the peak area of an OTU in a sample to the total peak area of the sample. We also converted the peak area matrix to presence-absence to assess the composition of bacterial communities in the ARISA profiles by occurrence patterns. We calculated observed richness from ARISA profiles by summing the number of OTUs observed in each sample, hereafter referred to as profile richness. We found no differences in bacterial community patterns using peak height vs. peak area.

Statistical Analyses

We used a principal components analysis (PCA) to summarize physical and chemical variation related to trophic status among lakes. We found that lakes varied little in relevant physical characteristics (lake area and mean depth), and thus we used the first axis of the resulting PCA as a multivariate proxy for increasing lake trophic state (Figure 3.2A). Although we did not measure primary productivity directly, other studies have found good agreement between primary productivity and chlorophyll *a* and nutrient concentrations in lakes with similar concentrations as lakes in this study [46].

To quantify vertical heterogeneity in chemical and physical variables within each lake (e.g., TN, TP, temperature, and DO), we used the standard deviation of measurements among lake strata (Table S1). We then performed a PCA that included only the standard deviations of these physical and chemical variables to establish a gradient of resource heterogeneity among lakes.

To compare the influence of increasing trophic state ("trophic status"), depth variation in resource availability ("resource heterogeneity"), and their combination (trophic status and resource heterogeneity) on the bacterial community, we then took the scores from the first principal component (PC 1) of each PCA and regressed them against metrics of bacterial abundance, ARISA profile richness, and an index of community similarity among ARISA profiles (see below for description). The combined trophic status and resource heterogeneity model contained two predictor variables: PC 1 of the trophic status PCA, and PC1 of the resource heterogeneity PCA. All variables were transformed to meet the assumptions of normality prior to the PCA. We evaluated the support for each of the three candidate models describing the relationships between environmental conditions and the bacteria community

attributes using Akaike's Information Criteria adjusted for small sample sizes [47]. The model with the lowest AIC_c was considered the best model, and models within 2 AIC_c units of one another were considered to be equally good [47]. In addition, we calculated AIC weights (w_i) for each individual model, which estimates a probability that model *i* is the best model given the set of models we considered. Finally, to evaluate overall importance of the individual variables, trophic status and resource heterogeneity, we calculated w_i for each term across the three models we compared (Table 1).

Bacterial community similarity among samples was assessed using Sorensen's coefficient for occurrence data [48] and the Chao-Sorensen abundance estimator for relative abundance data [49]. We assessed the overall similarity of communities within a lake by using an average dissimilarity value among ARISA profiles from all two-layer comparisons. We used a constrained analysis of principal coordinates (CAP) to evaluate if changes in community composition were associated with increasing trophic state [50] since it allowed us to use the Chao-Sorensen similarity index.

Finally, we investigated whether changes in the bacterial community with increased lake trophic status were realized by shifts in "widespread" or "narrowly distributed" taxa. We assessed how the relative contribution of widespread OTUs (taxa observed in the majority of lakes) changed with trophic state and habitat heterogeneity. We considered widespread taxa to be those that were observed in 90% of lakes in our study (but see Table S2 for evaluation of different thresholds). We then assessed whether the occurrence and relative abundance of these taxa changed across the lake trophic gradient and with increasing heterogeneity (i.e., PC1 of trophic status and resource heterogeneity PCAs). All analyses were done in R Version 2.14.0 [51] using the vegan package [52].

RESULTS

Lake characteristics

Productivity-related variables such as TP, TN, and chlorophyll a explained a substantial portion of the environmental variation among lakes in this study (59%; Figure 3.2, Table 3.2). Epilimnetic TP concentrations ranged from 4.6 μg L^{-1} to over 30 μg L^{-1}, and chlorophyll *a* ranged from 0.23 to 10.2 μg L^{-1}, thus the lakes ranged from oligotrophic to eutrophic [22]. Environmental conditions did not change similarly in each layer with increased trophic state; for example, the epilimnion was less variable among lakes than either the metalimnion or hypolimnion in most environmental characteristics (Table 3.4). As a result, conditions within the water column were more heterogeneous as trophic state increased (R^2=0.57, Figure 3.2C). We observed the most significant differences in TN and TP concentrations among layers as trophic state increased (Figures 3.2B and C). TN and TP were correlated with the availability of NH_4 (r=0.71) and PO_4 (r=0.86), respectively. Thus, nutrient availability was variable within the water column. Finally, the percent change in nutrient concentrations was greater in the hypolimnion than in either the epi- or metalimnion (Table 3.4).

Did lake bacterial communities shift in response to increasing trophic state?

Bacterial communities at the whole-lake scale shifted significantly in association with increasing lake trophic state (Figure 3.3, Figure 3.6). Average bacterial abundance (R^2=0.46, Table 3.1, Figure 3.2A) and ARISA profile richness increased linearly with our proxy for lake eutrophication (R^2=0.30, Table 3.1, Figures 3.3A & E) and ranged from 66 to 106 OTUs per lake. Our CAP model showed that bacterial community composition shifted with increased lake trophic status and shifts were strongly associated with increasing chlorophyll *a* (r = 0.99) and epilimnetic TN (r = 0.76) concentration (Figure 3.6). The CAP model explained 29% of the total

variation in community composition among lakes. The first CAP axis captured the majority of that explained variation (71.4%), indicating that community composition changed in response to increased lake trophic state.

Did the responses of the bacterial community vary among habitats in lakes?

Bacterial communities associated with surface and deep habitats displayed different patterns of abundance, and the richness and composition of ARISA profiles changed significantly as lake trophic state increased (Figure 3.3, Figure 3.6). Average bacterial abundance increased with trophic state ($R^2 = 0.46$), was highest in the metalimnion (ANOVA; $F=5.6$, $p = 0.006$), but increased significantly in all layers across the trophic gradient (Figure 3.3). The richness of ARISA profiles also varied significantly among layers (ANOVA, $F=10.6$, $p < 0.001$), but only increased notably in the hypolimnion in response to trophic state ($R^2 = 0.15$, Figure 3.3H). Profile richness was highest on average in the hypolimnion (55 ± 7 SD), which also had the largest range of observed richness, ranging from 37 OTUs in Gwendoline Lake, an oligotrophic lake, to 71 OTUs found in more nutrient-rich Geneva Lake. Therefore, increases in the profile richness in the hypolimnion accounted for the increases we observed in overall lake richness ($R^2=0.39$).

When all lake communities were considered together, there were significant, but small, compositional differences among epi-, meta- and hypolimnetic communities (ANOSIM, $R = 0.16$, $p=0.001$), and surface and deep communities shared the fewest taxa (data not shown). Furthermore, surface communities were significantly less variable than deep communities across the trophic gradient (Homogeneity of dispersion, $p<0.001$), and surface and deep communities within a given lake tended to become less similar to one another as trophic state increased ($R^2 =$

0.12). However, heterogeneity in nutrient concentrations among strata explained slightly more of that variation than trophic state alone (R^2=0.17, Figure 3.4, Table 1).

In all cases, the heterogeneity model or the trophic status plus heterogeneity model explained more variation in bacterial communities among lakes than the trophic status model alone (Table 1). We found that greater vertical heterogeneity of nutrient availability (Figure 3.2) was strongly related to increased abundance, ARISA profile richness, and decreasing similarity of communities among lake strata (Table 3.1, Figure 3.4). Total abundance and observed richness were both more strongly related to increases in the heterogeneity of P and N than increases in their concentrations alone or to differences in temperature and DO among strata, which other studies have shown to be associated with heterogeneity in bacterial community composition (Table 3.1; [31,32]). Therefore, although we saw an increase in observed richness with increased trophic state (R^2=0.30), observed richness was more closely linked to greater heterogeneity of nutrients within the water column (R^2=0.41). Overall, the AIC_c shows that the heterogeneity model had the most support, and that, in fact, the heterogeneity term had the most weight across models (Table 3.1). Thus, as measured here, bacterial communities exhibited habitat-specific responses to lake eutrophication, and spatial variation in resource availability often influenced bacterial community composition more than simple increases in nutrient concentration and productivity (Figures 3.4 and 3.5).

Which taxa accounted for changes in community composition with eutrophication?

We observed a total of 221 OTUs across all lakes and found that some of those taxa were widespread among lakes. We observed that while a core community of 11 OTUs was present and detected using ARISA in ~90% of lakes in this study ("widespread taxa"), and while still present across lakes, made up a decreasing proportion of both the number (R^2=0.23) and relative

abundance of taxa in lakes as lake trophic state increased (R^2=0.35, Figure 3.5). Specifically, while these widespread taxa comprised 35-40% of the relative abundance of the community in the more homogenous oligotrophic lakes, they were less than 15% in the more heterogeneous eutrophic lakes. These trends were robust to using different thresholds to define "widespread" (e.g., present in 70-90% of lakes), and strengthened as we considered thresholds up to 90% (Table 3.3). We only observed four OTUs that were present in > 90% of lakes, which likely were ubiquitous taxa that would be present regardless of lake trophic state [36]. In addition, we found that the increasing representation of previously low abundance or new taxa in response to trophic status was explained most by increasing heterogeneity among habitats (Table 3.1). Although the AIC_c values for all three models were very close, the AIC value and variable w_i's suggest that heterogeneity explained the declining contribution of the widespread OTUs we observed to the overall lake community (Table 3.1). This suggests that either novel, previously low abundance, (i.e., below the detection limit of ARISA), or dormant taxa [35] increased in their relative importance as nutrient status of lakes increased, and that chemical heterogeneity in the lake environment most likely facilitated the increased prevalence of these taxa in the lake community.

DISCUSSION

We found that the abundance and richness of bacterial communities increased as lake nutrient status increased, in parallel to what has been reported in other studies [28,33,45]. However, we found even stronger relationships between the overall abundance and number of OTUs observed and the spatial heterogeneity in nutrient conditions among lake layers as trophic status increased. Additionally, because environmental conditions among lake strata diverged and communities associated with these habitats responded differently to lake trophic status (Figure 3.3), bacterial communities as measured by ARISA fingerprinting were much less similar among

lake strata as lake trophic state increased (Figure 3.4, Table 3.1). Furthermore, our analyses demonstrated that a core bacterial community of dominant bacteria did not change systematically in high nutrient lakes but rather that increasing habitat heterogeneity, specifically due to large changes in conditions in the deepest layer of lakes, provided additional habitat for previously low abundance or absent taxa that became detectable in more eutrophic conditions. Our study shows that the response of bacterial communities to increased productivity in lakes may differ from that of other lake organisms as a result of spatial heterogeneity in resources specifically affecting the ecology of bacteria and supports the idea that the recruitment of rare or dormant bacterial taxa in lake communities may facilitate much of this response (e.g., [35]).

Effects of trophic state on lake bacterial communities

We observed significant increases in bacterial abundance and changes in the richness and composition of ARISA profiles at the whole-lake scale in response to increases in lake trophic status. As expected, abundance was positively related to increases in nutrient availability (TN and TP) and chlorophyll *a* (Table 3.1) across the entire range of ecosystem productivity we observed. High abundances of bacteria are often correlated with high rates of bacterial productivity [46], which can be nutrient-limited in oligotrophic lakes. We also observed increased ARISA profile richness as trophic state increased (Figure 3.3E), suggesting that more taxa coexisted as dominant types (and thus able to be detected by ARISA) as resource availability increased. As has been observed for macroorganisms in lakes [23], other studies have shown lake bacterial richness to exhibit a range of linear and unimodal responses to productivity depending on the spatial and taxonomic resolution of the study [17,18,29]. We likely observed a linear increase in observed richness since our lakes represented a relatively modest gradient in nutrient loading and chlorophyll *a* compared to the global distribution of lake

productivity [22], thus, we may have only captured the initial upward slope of a unimodal response across the global range of lake trophic states. Alternatively, is also possible that we observed a consistently increasing number of taxa rather than a unimodal trend due an effect of increased sampling; in other words, because we took discrete samples from each lake habitat and ran a separate ARISA analysis on each sample rather than on one integrated sample from each lake, we were able to detect more taxa than previous studies [23,29].

Bacterial community response to resource heterogeneity

Bacterial community shifts were most strongly associated with changes in the heterogeneity of the lake environment in all cases (Table 3.1). Specifically, we observed more OTUs and decreased similarity among bacterial communities in surface and deep habitats in response to heterogeneity in N and P concentrations (Figures 3.2B and 3.3). Environmental conditions within lakes changed to different degrees among the epi-, meta- and hypolimnion (Table 3.4), which translated to habitat specific responses of bacterial communities in each layer across lakes (Figure 3.3). For instance, although bacterial abundance increased in all layers, we only observed increases in richness in the hypolimnion with increased trophic status (Figure 3.3H), which also had the largest range in nutrient concentrations across the trophic gradient (Figure 3.6). Interestingly, communities in the surface layer varied less across the trophic gradient than communities in the hypolimnion, suggesting that studies that evaluate trends in bacterial communities in response to eutrophication miss an important aspect of the bacterial response.

While our study design does not allow us to definitely conclude whether patterns in bacterial communities were related more to increased trophic state or to habitat heterogeneity because the two were correlated (Figure 3.2C), we found strong support for our heterogeneity

model (Table 3.1) and patterns suggesting that both were important for bacterial communities. For instance, we observed lower richness (66 OTUs) and higher similarity of communities among lake strata (Sorensen similarity = 0.86) in the only lake in our study that did not stratify into discrete habitats. Although this lake had similar nutrient concentrations as eutrophic lakes, environmental conditions were more homogeneous within the water column in this lake suggesting that heterogeneity in resource availability influenced bacterial richness more than nutrient concentration alone in all lakes in our study.

While changes in bacterial communities in response to increasing lake trophic status have been widely observed [28,29,45] as have differences in communities among lake strata [30,31,53] previous studies have not linked these observations to evaluate the combined effects of lake resource availability and resource heterogeneity on the richness and composition of lake bacterial communities. Thus, while similar communities may inhabit eutrophic lakes [36], communities become increasingly distinct from one another as surface and deep habitat conditions diverge and biotic interactions change [54]. Furthermore, these findings contrast with studies of other lake organisms, such as zooplankton and fish, which find increased productivity reduces diversity as a result of homogenization of food resources and loss of habitat [15,26,27]. Thus, our study demonstrates that the type of heterogeneity that influences communities varies among macro- and microorganisms and that the response to a large-scale environmental change is expressed differently among habitats within ecosystems.

In addition, our results suggest that changes in bacterial communities with increased trophic status may be more strongly related to vertical differences in nutrient concentrations than dissolved oxygen, temperature, and light, which are typically thought to structure differences among communities in different strata within lakes [24,30,31,53]. We tested this by regressing

axis 1 and 2 of our environmental PCA (Figure 3.6), which represented differences among layers in nutrient concentrations and DO/temperature, respectively. We found that in all cases axis 2 (variation in DO and temperature) did not have a strong relationship with any bacterial response measure. Few studies have tested for this and those that have, have often focused on a single lake or small set of lakes [30,53]. Thus, these results suggest that increased vertical differences in nutrient concentrations may be more important in structuring the bacterial community than vertical differences in DO and temperature as the trophic state of lakes increases.

Were widespread or narrowly distributed taxa responsible for shifts in community composition?

We found that habitat heterogeneity played an important role in shaping the bacterial response to increasing lake trophic status through enabling the increased contribution of new or previously low abundance taxa in lake communities. Furthermore, we found that increased resource heterogeneity simultaneously allowed for the retention of a core group of taxa that were widespread among lakes while also providing new habitat and resources for these previously unobserved or low abundance taxa (Figure 3.5). Thus, our results support the general notion of the importance of rare taxa in microbial communities [35,55] and suggest that bacterially-relevant habitat heterogeneity may be an important mechanism driving the bacterial response to increased lake ecosystem productivity. Additionally, this pattern may be common to many types of planktonic communities [34]. For instance, a study of the response of a lake phytoplankton community to eutrophication and recovery found that there were phytoplankton that were consistently present through time, but that temporally rare taxa were most responsive to changes in lake nutrient status and drove changes in community composition [56].

While the use of ARISA allowed us to screen the bacterial community in a large number of lakes and has been shown to have species-level taxonomic resolution, as with so many

methods used to sample bacterial communities, there are limitations associated with using this approach [57]. For example, ARISA underestimates the total richness of the bacterial community and has biases such as preferential amplification of abundant organisms [41]. For example, many of the additional taxa that we observed in more eutrophic lakes could have been present at low abundances in oligotrophic lakes, and therefore below the detection limit of ARISA. However, increased detection of these taxa in eutrophic lakes suggests that they are at higher abundances and thus may be more functionally important in those communities. In addition, other studies have shown that ARISA captures similar patterns in diversity among communities as more high-resolution techniques such as clone libraries [58]. Further, although sequencing and clone library techniques would have allowed us to identify specific taxa, the higher costs associated with those techniques would have limited our ability to sample the entire trophic gradient in our study. Finally, our results are comparable to other studies using similar techniques [24,33,35], and recent studies of bacterial responses to nutrient additions that have used high throughput sequencing techniques suggest that using a more thorough sampling approach likely would not likely reveal a different trend in how richness and composition respond to increased ecosystem productivity (e.g., [19,44]). Thus, the fact that we observed such striking trends using this approach suggests that higher resolution sampling would have only strengthened observed patterns.

In summary, we showed that bacterial community composition changed and was richer and more heterogeneous within lakes as trophic status increased. In contrast to trends in macroorganisms whose diversity is often negatively associated with increases in lake productivity [15,23,27], we showed that the high degree of heterogeneity in bacterial resources in eutrophic lakes promoted higher richness as a result of differentiation of bacterial taxa among

lake habitats. We found that eutrophication alters the drivers of bacterial community differences within lakes from physical and redox related variables to changes in nutrient availability. Furthermore, our results suggest that rare or dormant taxa may be most responsible for changes in bacterial communities with increased lake trophic state [35,59]. This "seed bank" of taxa has increasingly been recognized to be important in responding to changes in many types of ecosystems [60], and understanding the role of rare and dormant taxa an important frontier for understanding the processes that regulate how microbial communities respond to ecosystem change in general.

FIGURE LEGENDS

Figure 3.1. Map of study sites. Lakes included in the study were located in the Puget South Basin in Washington state, USA and British Columbia, Canada. Lakes are indicated by black points.

Figure 3.2. Principal component analyses (PCAs) showing environmental variation across lakes in this study. Panel A shows PCA results based on trophic state variables, panel B shows PCA results based on Heterogeneity variables (standard deviation among depths in variables) and panel C shows the correlation between the trophic state PC 1 scores and heterogeneity PC 1 scores (R^2=0.57). Arrows show significant variables ($p < 0.05$) and values in parentheses show percentages of total environmental variation among lakes explained by each axis. 'EpiTN' = epilimnetic TN, 'EpiTP' = epilimnetic TP, 'HypoTP' = Hypolimnetic TP, 'HypoDO' = hypolimnetic DO, and DO = epilimnetic DO. '.std' indicates that the standard deviation of measurements of the specified variable among layers. Triangles = eutrophic, stars = mesotrophic, and squares = oligotrophic lakes.

Figure 3.3. Relationships of increasing trophic state (PC 1 scores) with abundance and richness. A) Whole-lake average abundance (R^2=0.46, $p < 0.001$), B) Epilimnetic abundance ($R^2 = 0.52$, $p < 0.001$), C) Metalimnetic abundance ($R^2 = 0.33$, $p = 0.007$), D) Hypolimnetic abundance ($R^2 = 0.40$, $p = 0.003$), E) Whole-lake average richness ($R^2 = 0.30$, $p = 0.003$),, F) Epilimnetic richness ($R^2 = -0.06$, $p = 0.98$), G) Metalimnetic richness ($R^2 = -0.03$, $p = 0.49$), and H) Hypolimnetic richness ($R^2 = 0.15$, $p = 0.05$).

Figure 3.4. Bacterial communities were less similar among lake strata as chemical heterogeneity increased (R^2=0.17, $p = 0.05$). Similarity of communities was based on the average Chao Sorensen Dissimilarity from comparisons of relative abundance of taxa among three depth strata.

Figure 3.5. Relationship of habitat heterogeneity with the proportional representation of widespread taxa in each lake. Heterogeneity was measured as the PC 1 scores of 'heterogeneity' model. Filled circles show the total proportion of relative abundance made up by widespread taxa ($R^2 = 0.35$, $p = 0.007$) and open circles show widespread taxa as a proportion of the total number of taxa observed ($R^2 = 0.23$, $p = 0.03$).

Figure 3.6. Constrained analysis of principal coordinates (CAP) of bacterial community composition with environmental variables. CAP explains 29% of variation in community composition among lakes. Arrows show variables significantly related to bacterial community composition ($p < 0.05$). Values in parentheses show percentages of total variation in bacterial community composition explained by each axis. 'EpiTN' = epilimnetic TN, 'EpiTP' = epilimnetic TP, and DO = epilimnetic DO. Triangles = eutrophic, stars = mesotrophic, and squares = oligotrophic lakes.

Table 3.1. Comparison of models evaluating the effects of trophic state, heterogeneity, and their combination on bacterial communities. Relationship of trophic state, heterogeneity, and their combination with abundance, richness, dissimilarity of bacterial communities among lake habitats ("Dissimilarity"), and the proportional abundance of common taxa. 'n' = sample size and 'k' = number of parameters in each model. 'w_i' refers to the AIC_c weight calculated for each model and the weights for the individual terms 'T' and 'H' across all models.

	Model	n	k	R^2	AIC_c	ΔAIC_c	w_i*	w_i of T^+	w_i of H^{++}
ABUNDANCE									
	Trophic State	21	3	0.46	612.2	9.1	0.01	0.34	0.83
	Heterogeneity	21	3	0.66	603.1	0.0	0.50		
	PC1 T + PC1 H	21	4	0.69	603.3	0.0	0.33		
RICHNESS									
	Trophic State	17	3	0.30	129.3	2.9	0.16	0.33	0.84
	Heterogeneity	17	3	0.41	126.4	0.0	0.67		
	PC1 T + PC1 H	17	4	0.40	129.2	2.8	0.17		
DISSIMILARITY									
	Trophic State	17	3	0.12	-23.8	0.9	0.35	0.46	0.65
	Heterogeneity	17	3	0.17	-24.7	0.0	0.54		
	PC1 T + PC1 H	17	4	0.12	-21.5	3.1	0.11		
WIDESPREAD TAXA									
	Trophic State	18	3	0.29	-36.9	1.5	0.26	0.44	0.74
	Heterogeneity	18	3	0.35	-38.4	0.0	0.56		
	PC1 T + PC1 H	18	4	0.35	-36.1	2.3	0.18		

* AIC_c weight, $^{+}$Trophic State, $^{++}$ Heterogeneity

Table 3.2. Results of eutrophication and heterogeneity Principal Components Analyses (PCAs). Correlation coefficients of significant variables are shown in parentheses. Variable names followed by ".std" signify the standard deviation of measurements of those variables among lake strata. "PC" = principal component.

Principal Component	Percent Variance Explained	Significance	Significiant Variable Loadings
Trophic State			
PC 1	59.0	< 0.001	Epi TP (0.94) Epi TN (0.91) Chl a (0.83) Hypo TP (0.78) Hypo DO (-0.66)
PC 2	19.0	0.968	Epi DO (0.83)
Heterogeneity			
PC 1	51.7	0.005	TP.std (0.97) TN.std (0.95)
PC 2	36.0	0.002	DO.std (0.80) Temp.std (0.89)

Table 3.3. Model results from comparing effects of trophic state, heterogeneity, and the combination of the two (T+H) on the relative abundance of widespread taxa in lakes. Table shows results of model when using different threshold values for defining widespread (percent of lakes present). 'k' = number of parameters and 'n' = sample size.

Percent of Lakes Present	n	k	R^2	AIC_c	ΔAIC_c	w_i	w_i of T	w_i of H
72								
Trophic State	18	3	0.03	-22.4	6.6	0.03	0.28	0.97
Heterogeneity	18	3	0.34	-29.0	0.0	0.72		
PC1 T + PC1 H	18	4	0.35	-26.9	2.1	0.26		
78								
Trophic State	18	3	0.05	-34.9	3.4	0.13	0.27	0.87
Heterogeneity	18	3	0.22	-38.3	0.0	0.73		
PC1 T + PC1 H	18	4	0.17	-34.9	3.4	0.14		
83								
Trophic State	18	3	0.15	-39.7	3.2	0.14	0.28	0.86
Heterogeneity	18	3	0.30	-42.9	0.0	0.72		
PC1 T + PC1 H	18	4	0.25	-39.6	3.3	0.14		
89								
Trophic State	18	3	0.29	-36.9	1.5	0.27	0.44	0.73
Heterogeneity	18	3	0.35	-38.4	0.0	0.56		
PC1 T + PC1 H	18	4	0.35	-36.0	2.4	0.17		
94								
Trophic State	18	3	-0.05	-44.6	0.0	0.48	0.57	0.52
Heterogeneity	18	3	-0.06	-44.4	0.2	0.43		
PC1 T + PC1 H	18	4	-0.12	-41.2	3.3	0.09		

Table 3.4. Range of values of chemical and physical characteristics of lake habitats.

	Epilimnion (n = 19)				**Metalimnion** (n = 20)				**Hypolimnion** (n = 21)			
	Range	**Mean**	**S.D.**	**C.V.**	**Range**	**Mean**	**S.D.**	**C.V.**	**Range**	**Mean**	**S.D.**	**C.V.**
Total P ($\mu g\ L^{-1}$)	4.6 – 30.2	7.0	4.3	0.61	5.0 - 145	23.1	29.8	1.29	3.8 - 168	37.9	44.2	1.17
Total N ($\mu g\ L^{-1}$)	175 - 900	455	189.0	0.42	161 – 2,898	623	577.3	0.93	178 – 1,377	638	305	0.48
Chl a ($\mu g\ L^{-1}$)	0.23 – 10.2	2.6	2.4	0.92	0.4 – 48.5	10.5	13.5	1.29	0 - 177	14.4	37.9	2.63
NO_3^- ($\mu g\ L^{-1}$)	0.21- 642.9	55.8	145.6	2.61	0 - 725.2	88.9	179.7	2.02	0 - 611.7	109.9	187.2	1.70
NH4 ($\mu g\ L^{-1}$)	3.5- 19.6	8.1	5.3	0.65	3.11 - 28.6	8.3	7.0	0.84	1.7 - 751.1	151.9	219	1.44
PO_4^{3-} ($\mu g\ L^{-1}$)	0.09- 2.84	1.4	0.78	0.56	0.1 - 9.9	1.8	2.1	1.17	0.17 - 70.8	9.6	18.8	1.96
Temp (° C)	17.3 - 25.5	21.6	2.2	0.10	10 – 21.3	14.9	2.8	0.19	0.63 – 19.0	7.2	3.5	0.49
DO ($mg\ L^{-1}$)	7.9 – 12.0	9.0	1.0	0.11	1.5 – 14.5	9.4	3.5	0.37	0.3 – 10.7	3.5	3.7	1.06

Figure 3.1.

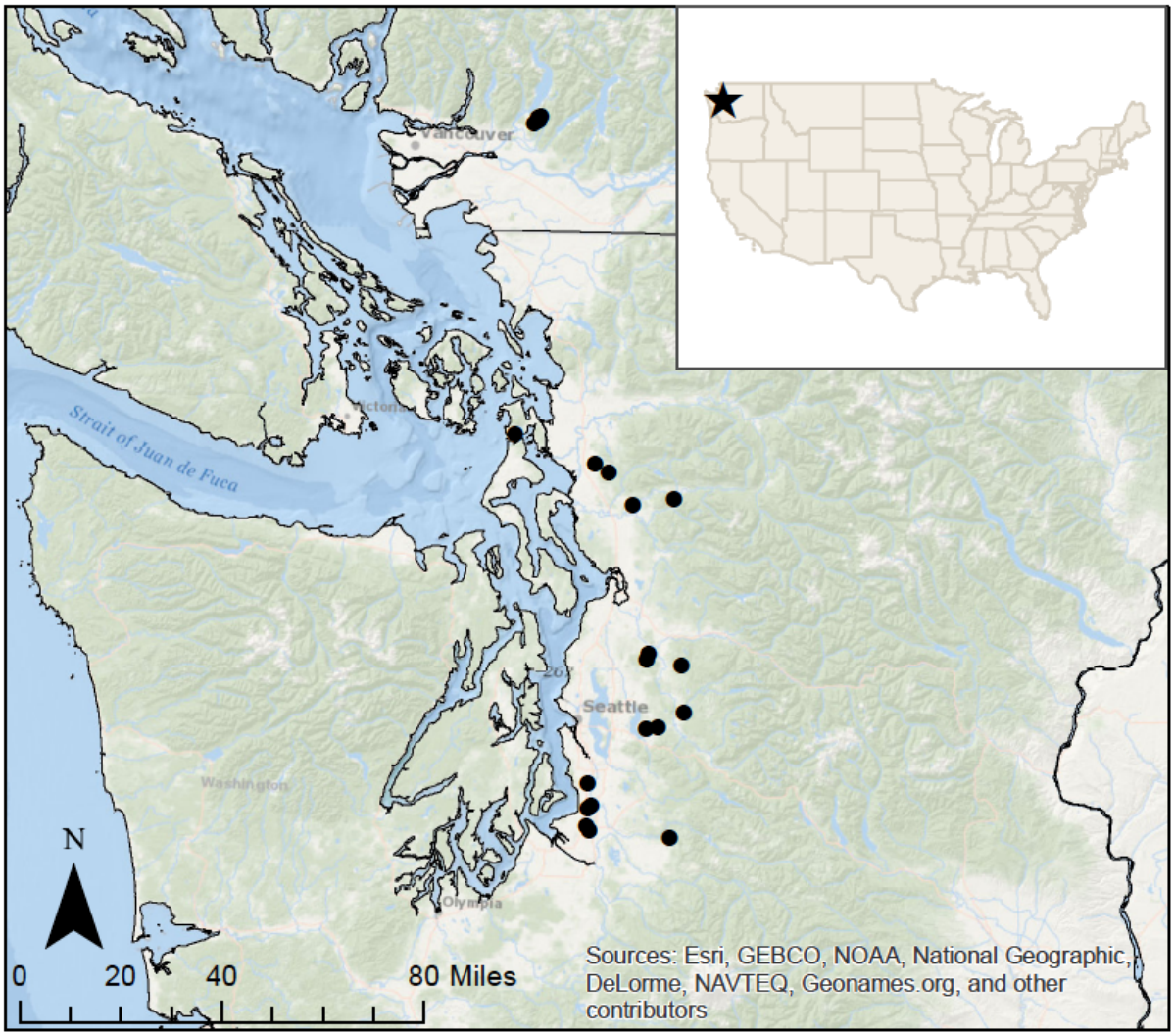

Vancouver
Strait of Juan de Fuca
Seattle
Washington
N
0
20
40
80 Miles
Sources: Esri, GEBCO, NOAA, National Geographic, DeLorme, NAVTEQ, Geonames.org, and other contributors

Figure 3.2.

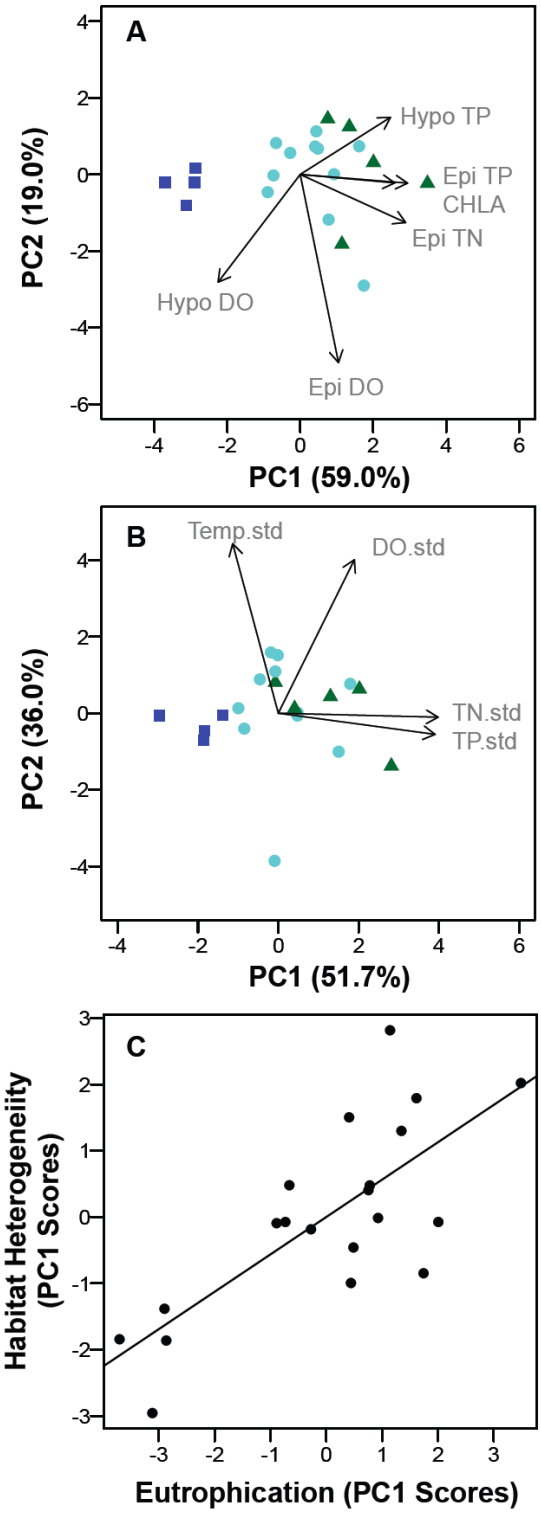

A
Hypo TP
Epi TP
CHLA
Epi TN
Hypo DO
Epi DO
PC2 (19.0%)
PC1 (59.0%)
B
Temp.std
DO.std
TN.std
TP.std
PC2 (36.0%)
PC1 (51.7%)
C
Habitat Heterogeneiity
(PC1 Scores)
Eutrophication (PC1 Scores)

Figure 3.3.

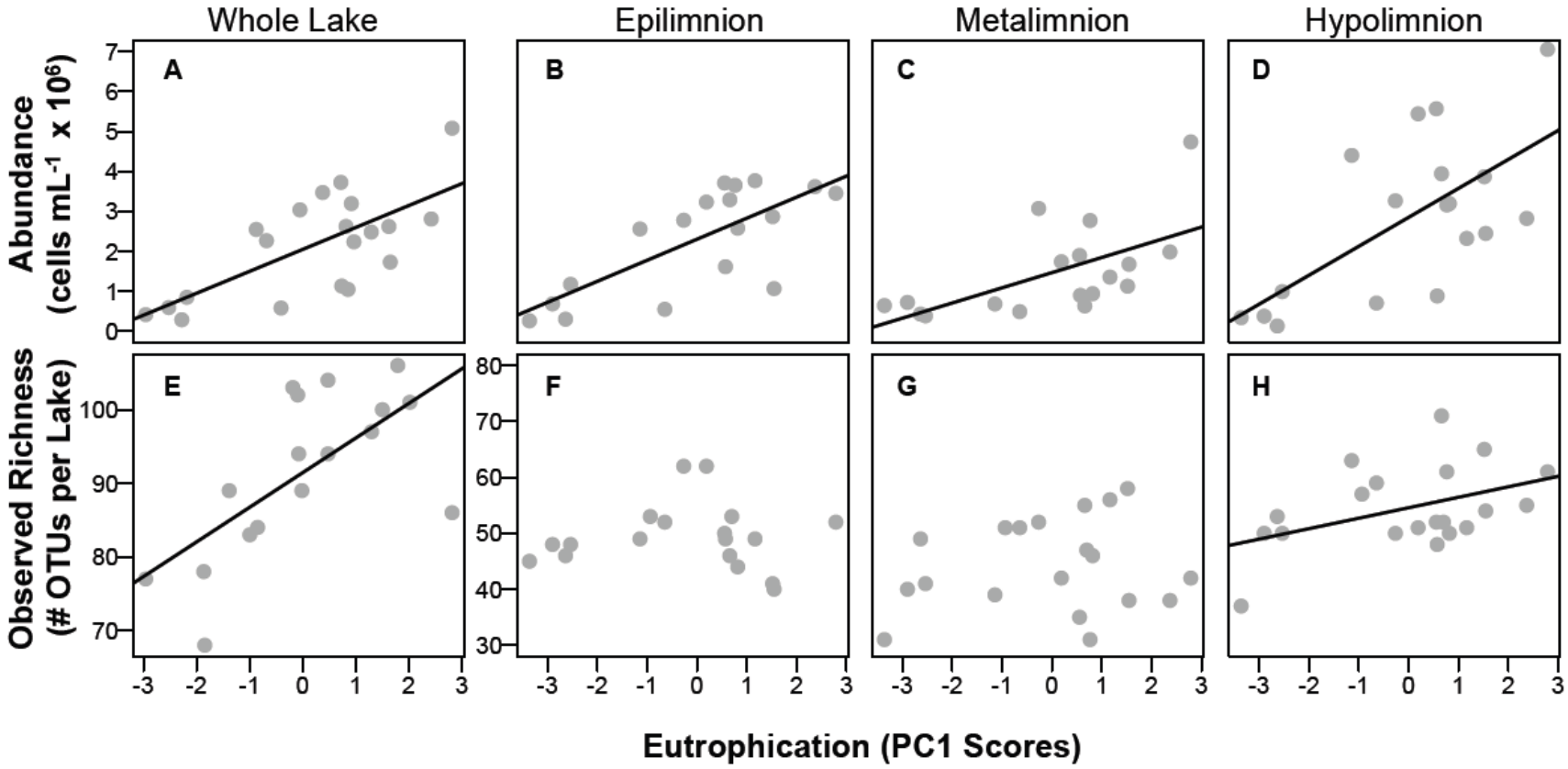

Whole Lake
Epilimnion
Metalimnion
Hypolimnion
A
B
C
D
E
F
G
H
Abundance (cells mL-1 x 10^6)
Observed Richness (# OTUs per Lake)
Eutrophication (PC1 Scores)

Figure 3.4.

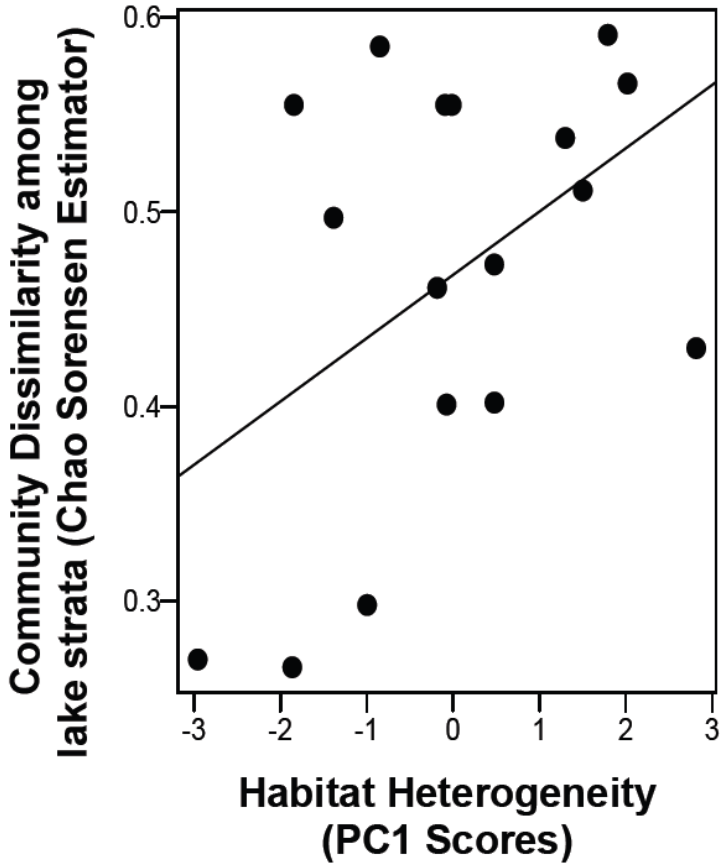

Community Dissimilarity among lake strata (Chao Sorensen Estimator)
0.6
0.5
0.4
0.3
-3
-2
-1
0
1
2
3
Habitat Heterogeneity (PC1 Scores)

Figure 3.5.

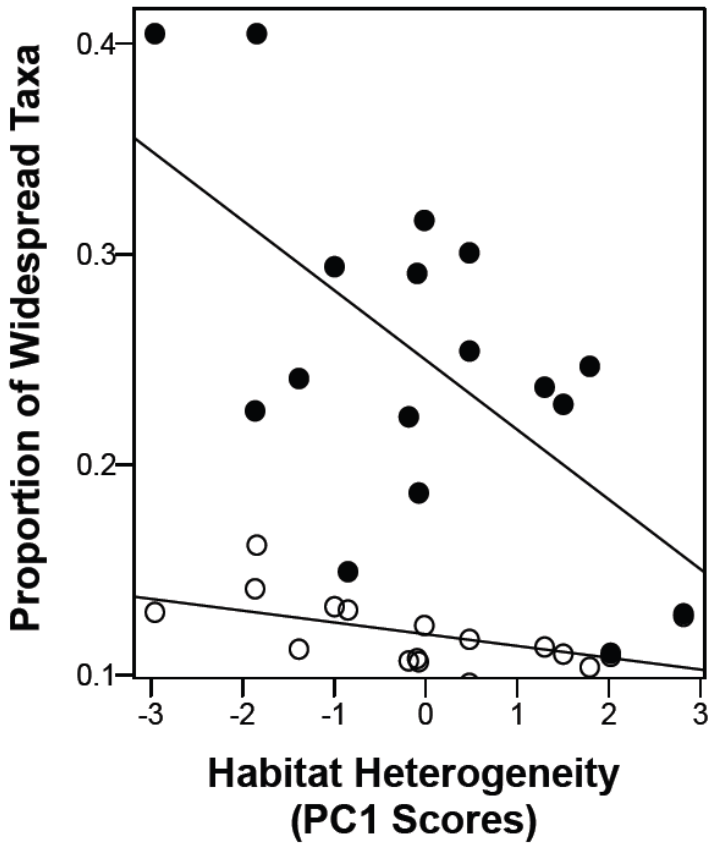
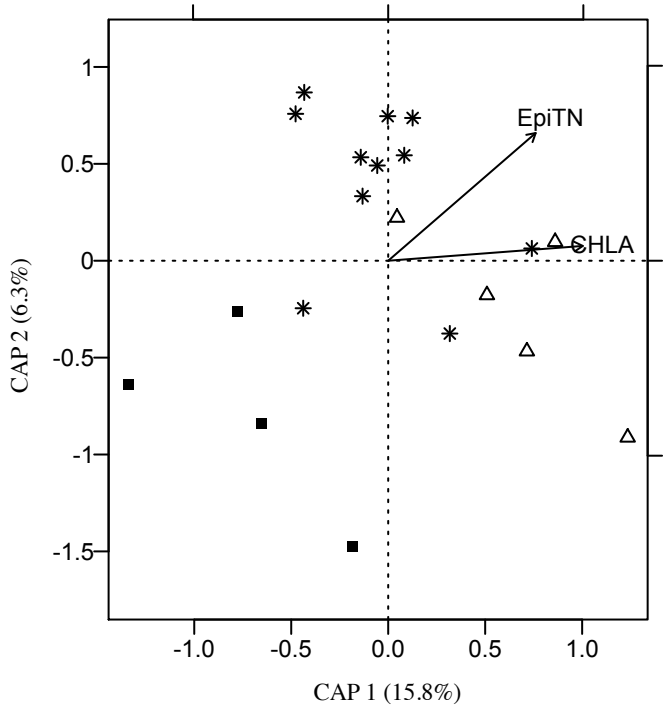

Proportion of Widespread Taxa
0.4
0.3
0.2
0.1
-3
-2
-1
0
1
2
3
Habitat Heterogeneity
(PC1 Scores)

Figure 3.6.



REFERENCES

1. Reid WV, Mooney HA, Cropper A, Capistrano D, Carpenter SR, et al. (2005) Ecosystems and human well-being: synthesis. Washington, D.C.: Island Press.
2. Carpenter SR, Caraco NF, Correll DL, Howarth RW, Sharpley AN, et al. (1998) Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecological Applications 8: 559-568.
3. Rozenzweig ML (1995) Species diversity in space and time. Cambridge, U.K.: Cambridge University Press.
4. Ribalet F, Marchetti A, Hubbard KA, Brown K, Durkin CA, et al. (2010) Unveiling a phytoplankton hotspot at a narrow boundary between coastal and offshore waters. Proceedings of the National Academy of Sciences of the United States of America 107: 16571-16576.
5. Wright DH (1983) Species-energy theory - An extension of species-area theory. Oikos 41: 496-506.
6. Mittelbach GG, Steiner CF, Scheiner SM, Gross KL, Reynolds HL, et al. (2001) What is the observed relationship between species richness and productivity? Ecology 82: 2381-2396.
7. Adler PB, Seabloom EW, Borer ET, Hillebrand H, Hautier Y, et al. (2011) Productivity Is a Poor Predictor of Plant Species Richness. Science 333: 1750-1753.
8. Cardinale BJ, Hillebrand H, Harpole WS, Gross K, Ptacnik R (2009) Separating the influence of resource 'availability' from resource 'imbalance' on productivity-diversity relationships. Ecology Letters 12: 475-487.
9. Hutchinson GE (1961) The paradox of the plankton. American Naturalist 95: 137-145.

10. Tilman D (1982) Resource Competition and Community Structure. Princeton, New Jersey: Princeton University Press.

11. Chase JM, Leibold MA (2002) Spatial scale dictates the productivity-biodiversity relationship. Nature 416: 427-430.

12. Shurin JB, Winder M, Adrian R, Keller W, Matthews B, et al. (2010) Environmental stability and lake zooplankton diversity - contrasting effects of chemical and thermal variability. Ecology Letters 13: 453-463.

13. Reich PB, Frelich LE, Voldseth RA, Bakken P, Adair EC (2012) Understorey diversity in southern boreal forests is regulated by productivity and its indirect impacts on resource availability and heterogeneity. Journal of Ecology 100: 539-545.

14. Stevens MHH, Carson WP (2002) Resource quantity, not resource heterogeneity, maintains plant diversity. Ecology Letters 5: 420-426.

15. Barnett A, Beisner BE (2007) Zooplankton biodiversity and lake trophic state: Explanations invoking resource abundance and distribution. Ecology 88: 1675-1686.

16. Eilts JA, Mittelbach GG, Reynolds HL, Gross KL (2011) Resource Heterogeneity, Soil Fertility, and Species Diversity: Effects of Clonal Species on Plant Communities. American Naturalist 177: 574-588.

17. Horner-Devine MC, Leibold MA, Smith VH, Bohannan BJM (2003) Bacterial diversity patterns along a gradient of primary productivity. Ecology Letters 6: 613-622.

18. Smith VH (2007) Microbial diversity-productivity relationships in aquatic ecosystems. Fems Microbiology Ecology 62: 181-186.

19. Bowen JL, Ward BB, Morrison HG, Hobbie JE, Valiela I, et al. (2011) Microbial community composition in sediments resists perturbation by nutrient enrichment. Isme Journal 5: 1540-1548.

20. Madigan MT, Martinko JM, Stahl DA, Clark DP (2010) Brock biology of microorganisms. San Francisco: Benjamin Cummings.

21. Green JL, Bohannan BJM, Whitaker RJ (2008) Microbial biogeography: From taxonomy to traits. Science 320: 1039-1043.

22. Wetzel RC (2001) Limnology: lake and river ecosystems. San Diego: Academic Press.

23. Dodson SI, Arnott SE, Cottingham KL (2000) The relationship in lake communities between primary productivity and species richness. Ecology 81: 2662-2679.

24. Longmuir A, Shurin JB, Clasen JL (2007) Independent gradients of producer, consumer, and microbial diversity in Lake Plankton. Ecology 88: 1663-1674.

25. Smith VH (2003) Eutrophication of freshwater and coastal marine ecosystems - A global problem. Environmental Science and Pollution Research 10: 126-139.

26. Vadeboncoeur Y, Jeppesen E, Vander Zanden MJ, Schierup HH, Christoffersen K, et al. (2003) From Greenland to green lakes: Cultural eutrophication and the loss of benthic pathways in lakes. Limnology and Oceanography 48: 1408-1418.

27. Vonlanthen P, Bittner D, Hudson AG, Young KA, Muller R, et al. (2012) Eutrophication causes speciation reversal in whitefish adaptive radiations. Nature 482: 357-U1500.

28. Kolmonen E, Haukka K, Rantala-Ylinen A, Rajaniemi-Wacklin P, Lepisto L, et al. (2011) Bacterioplankton community composition in 67 Finnish lakes differs according to trophic status. Aquatic Microbial Ecology 62: 241-U249.

29. Korhonen JJ, Wang JJ, Soininen J (2011) Productivity-Diversity Relationships in Lake Plankton Communities. Plos One 6.

30. De Wever A, Muylaert K, Van der Gucht K, Pirlot S, Cocquyt C, et al. (2005) Bacterial community composition in Lake Tanganyika: Vertical and horizontal heterogeneity. Applied and Environmental Microbiology 71: 5029-5037.

31. Shade A, Jones SE, McMahon KD (2008) The influence of habitat heterogeneity on freshwater bacterial community composition and dynamics. Environmental Microbiology 10: 1057-1067.

32. Garcia SL, Salka I, Grossart HP, Warnecke F (2013) Depth-discrete profiles of bacterial communities reveal pronounced spatio-temporal dynamics related to lake stratification. Environ Microbiol Rep 5: 549-555.

33. Lindstrom ES (2000) Bacterioplankton community composition in five lakes differing in trophic status and humic content. Microbial Ecology 40: 104-113.

34. Galand PE, Casamayor EO, Kirchman DL, Lovejoy C, Karl DM (2009) Ecology of the rare microbial biosphere of the Arctic Ocean. . Proceedings of the National Academy of Sciences of the United States of America 106: 22427-22432.

35. Jones SE, Lennon JT (2010) Dormancy contributes to the maintenance of microbial diversity. Proceedings of the National Academy of Sciences of the United States of America 107: 5881-5886.

36. Newton RJ, Jones SE, Eiler A, McMahon KD, Bertilsson S (2011) A Guide to the Natural History of Freshwater Lake Bacteria. Microbiology and Molecular Biology Reviews 75: 14-49.

37. Bell T, Bonsall MB, Buckling A, Whiteley AS, Goodall T, et al. (2010) Protists have divergent effects on bacterial diversity along a productivity gradient. Biology Letters 6: 639-642.

38. Shade A, Chiu CY, McMahon KD (2010) Seasonal and Episodic Lake Mixing Stimulate Differential Planktonic Bacterial Dynamics. Microbial Ecology 59: 546-554.

39. Jankowski KJ, Schindler DE, Holtgrieve GW (2012) Assessing non-point source nitrogen loading and nitrogen fixation in lakes using δ15N and nutrient stoichiometry. Limnology and Oceanography 57: 671-683.

40. Eaton A, Greenberg AE, Rice EW (2005) Standard Methods for the Examination of Water and Wastewater: American Public Health Association.

41. Fisher MM, Triplett EW (1999) Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. Applied and Environmental Microbiology 65: 4630-4636.

42. Brown MV, Schwalbach MS, Hewson I, Fuhrman JA (2005) Coupling 16S-ITS rDNA clone libraries and automated ribosomal intergenic spacer analysis to show marine microbial diversity: development and application to a time series. Environmental Microbiology 7: 1466-1479.

43. Yannarell AC, Triplett EW (2005) Geographic and environmental sources of variation in lake bacterial community composition. Applied and Environmental Microbiology 71: 227-239.

44. Logue JB, Langenheder S, Andersson AF, Bertilsson S, Drakare S, et al. (2012) Freshwater bacterioplankton richness in oligotrophic lakes depends on nutrient availability rather than on species-area relationships. ISME J 6: 1127-1136.

45. Yannarell AC, Kent AD, Lauster GH, Kratz TK, Triplett EW (2003) Temporal patterns in bacterial communities in three temperate lakes of different trophic status. Microbial Ecology 46: 391-405.

46. Pace ML, Cole JJ (1994) Comparative and experimental approaches to top-down and bottom-up regulation of bacteria. Microbial Ecology 28: 181-193.

47. Burnham KP, Anderson DR (2002) Model selection and multi-model inference: A practical information-theoretic approach. New York: Springer-Verlag.

48. Legendre P, Legendre L (1998) Numerical Ecology. Amsterdam: Elsevier Science B.V.

49. Chao A, Chazdon RL, Colwell RK, Shen TJ (2005) A new statistical approach for assessing similarity of species composition with incidence and abundance data. Ecology Letters 8: 148-159.

50. Legendre P, Anderson MJ (1999) Distance-based redundancy analysis: Testing multispecies responses in multifactorial ecological experiments. Ecological Monographs 69: 1-24.

51. R Core Team (2012) R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

52. Oksanen J, Guillaume Blanchet F, Kindt R, Legendre P, Minchin PR, et al. (2012) vegan: Community Ecology Package. R package version 2.0-5 ed.

53. Dorigo U, Fontvieille D, Humbert JF (2006) Spatial variability in the abundance and composition of the free-living bacterioplankton community in the pelagic zone of Lake Bourget (France). FEMS Microbiol Ecol 58: 109-119.

54. Kent AD, Jones SE, Yannarell AC, Graham JM, Lauster GH, et al. (2004) Annual patterns in bacterioplankton community variability in a humic lake. Microbial Ecology 48: 550-560.

55. Sogin ML, Morrison HG, Huber JA, Mark Welch D, Huse SM, et al. (2006) Microbial diversity in the deep sea and the underexplored "rare biosphere". Proceedings of the National Academy of Sciences of the United States of America 103: 12115-12120.

56. Schindler DE, Chang GC, Lubetkin S, Abella SEB, Edmonson WT (2003) Rarity and functional importance in a phytoplankton community. . In: Kareiva P, Levin SA, editors. The importance of species: perspectives on expendability and triage. Princeton, New Jersey: Princeton University Press.

57. Bent SJ, Forney LJ (2008) The tragedy of the uncommon: understanding limitations in the analysis of microbial diversity. ISME J 2: 689-695.

58. Kovacs A, Yacoby K, Gophna U (2010) A systematic assessment of automated ribosomal intergenic spacer analysis (ARISA) as a tool for estimating bacterial richness. Res Microbiol 161: 192-197.

59. Lyons KG, Brigham CA, Traut BH, Schwartz MW (2005) Rare species and ecosystem functioning. Conservation Biology 19: 1019-1024.

60. Lennon JT, Jones SE (2011) Microbial seed banks: the ecological and evolutionary implications of dormancy. Nature Reviews Microbiology 9: 119-130.

Chapter 4: Temperature sensitivity of community respiration rates in streams is associated with watershed geomorphic features*

ABSTRACT

Aquatic ecosystems play an important role in the global carbon (C) cycle and represent an important source of greenhouse gases to the atmosphere. However, little attention has been paid to quantifying how aquatic community respiration (CR), a major source of CO_2, will respond to warming temperatures expected under climate change, and whether landscape features affect temperature modulation of CR. We quantified how temperature sensitivity of CR varied among streams in southwestern Alaska, a region with one of the fastest warming trends globally. We incubated sediments from streams spanning a geomorphic gradient and estimated the degree to which CR responded to increased water temperature as described by Arrhenius kinetics. As expected, CR increased with temperature across all streams, and the average temperature sensitivity was similar to theoretical predictions for heterotrophic metabolism. However, temperature sensitivity was significantly higher in streams with higher quantity but lower quality organic C sources, conditions that were strongly associated with watershed geomorphic characteristics. These results suggest that basic geomorphic features of landscapes will control the rates at which C is lost or sequestered from watersheds under new climate regimes.

***Full Citation:** Jankowski, K.J., D.E. Schindler, and P.J. Lisi. Temperature sensitivity of community respiration rates in streams is associated with watershed geomorphic features. Ecology, *in press*. http://dx.doi.org/10.1890/14-0608.1

INTRODUCTION

It is increasingly recognized that aquatic ecosystems play an important role in the carbon (C) cycle of their watersheds (Richey et al. 2002, Aufdenkampe et al. 2011) with potential global consequences (Cole et al. 2007, Battin et al. 2008, Raymond et al. 2013). Streams and rivers represent a substantial source of greenhouse gases to the atmosphere (Butman and Raymond 2011), in part through the mineralization of organic matter to CO_2 by the respiration of aquatic consumers (Mayorga et al. 2005). However, only recently has attention been given to understanding how community respiration (CR) rates in aquatic systems respond to increasing temperatures associated with climate change (Sobek et al. 2005, PERKINS 2012, Yvon-Durocher et al. 2012); most work comes from terrestrial ecosystems. Recent studies suggest that the temperature sensitivity of CR, (i.e. the degree to which CR increases with warming temperatures) of aquatic CR may in fact be higher and more variable than terrestrial CR (Yvon-Durocher et al. 2012). However, the key sources of variation in temperature sensitivity of CR in aquatic ecosystems remain unclear, limiting our ability to understand how watersheds will function as either sources or sinks of C in response to ongoing climate change.

Temperature is a principal control of CR, yet there is substantial debate about whether there is a universal value for temperature sensitivity of CR among ecosystems (Conant et al. 2011). Some have suggested that temperature sensitivity is inherently invariant among ecosystems, and that variation in other controls of respiration rate (e.g., organic C, nutrients) mask a universal temperature dependence across organisms and ecosystems (Gillooly et al. 2001, Perkins et al. 2012). In particular, the Metabolic Theory of Ecology (MTE; Brown et al. 2004) proposes that metabolism scales consistently with temperature among organisms and ecosystems due to the consistent biochemistry of metabolism among microbes and plants that form the basis for ecosystem metabolism. Therefore, temperature sensitivity is often expressed as an enzyme

activation energy (E), and predictions of this universal scaling rate typically fall around 0.63 eV (which corresponds to a $Q_{10} = 2.4$; Gillooly et al. 2001). While there is evidence that the MTE is supported at the global scale (Yvon-Durocher et al. 2012), several studies have shown substantial site-scale variation (Acuña et al. 2008, Craine et al. 2010) that is potentially meaningful for watershed-scale carbon sequestration estimates (Richey et al. 2002).

Studies in terrestrial and marine ecosystems suggest that deviation from the intrinsic temperature sensitivity reflects spatial variability in resource availability and quality (Davidson and Janssens 2006, López-Urrutia and Morán 2007). Because microbial respiration, which dominates CR in aquatic ecosystems (del Giorgio et al. 1997) is a series of enzymatic reactions, enzyme kinetics have been applied to understand the basis for this relationship, otherwise known as the "C-quality-temperature hypothesis" (first proposed by Bosatta and Ågren 1999, Craine et al. 2010). This hypothesis states that because the activation energy is greater for more complex C substrates, rates of respiration based on recalcitrant C substrates, such as the organic C loaded to streams from peatlands and soils, will increase more with temperature (i.e., will have a higher temperature sensitivity) than will respiration based on labile C sources (e.g., Ylla et al. 2012). This relationship has important implications for how ecosystem respiration will scale with climate-driven changes in the hydrological and thermal characteristics of ecosystems.

Temperature dependence of CR has important implications for mineralization of C pools in river basins, which vary widely in both quantity and quality across the diverse landscapes that rivers drain (Weyhenmeyer et al. 2012). Rivers are heavily influenced by the size, slope, and topography of their watersheds that hierarchically control channel form (Montgomery 1999), light (Finlay et al. 2011), temperature (Caissie 2006), nutrients (Helton et al. 2010), and the quantity and quality of organic C input (Battin et al. 2008) important to CR and other ecosystem

processes. Broad scale watershed characteristics also regulate the potential for storage and retention of C in the river channel and, thus, the opportunity for microbes to access and metabolize C substrates (Battin et al. 2008). Yet there has been little research linking geomorphic features of watersheds to thermal dependence of CR.

We hypothesized that watershed geomorphology is predictably associated with the temperature sensitivity of CR in streams draining them by influencing the availability and quality of nutrients and organic matter. We assessed whether stream chemistry was related to watershed geomorphic features in streams of the Wood River basin in southwest Alaska and investigated whether the temperature sensitivity of CR among streams was consistent with the predictions of the MTE (Brown et al. 2004). We then examined the variation in the temperature sensitivity of CR among streams, and assessed whether this variation was associated with the chemical and geomorphic features of their watersheds.

METHODS

This study was conducted in 11 streams of the Wood River basin (59°20'N, 158°40'W) in the Bristol Bay region of southwest Alaska during July and August 2011 (see Lisi et al. 2013 for further characterization of streams in this basin). The majority of the Wood River basin is within the Wood-Tikchik State Park and is comprised of five large, interconnected lakes fed by numerous small streams that drain southwards through the Wood River into Bristol Bay. This region is one of the fastest warming on Earth (Maurer et al. 2007), and is characterized by extensive peatlands with the potential to release substantial amounts of C to the atmosphere with ongoing climate warming. The Wood River basin has considerable variation in geomorphic conditions among sub-watersheds that produces a distinct gradient in watershed slope and average elevation among streams (Lisi et al. 2013).

To characterize variation in thermal conditions among streams, water temperatures were monitored at 90-min intervals throughout the summer from June – September 2011 at the mouth of each stream using I-button temperature recorders (Maxim Integrated Products, Sunnyvale, CA). Water samples for total nitrogen (TN), total phosphorus (TP), dissolved organic C (DOC), total dissolved nitrogen (TDN) and C: N ratio were taken from streams at the same time as sediments were collected for incubations to estimate CR. TN was determined using perchloric acid digestion followed by analysis with automated colorimetry. TP concentration was determined colorimetrically after persulfate digestion and reaction with molybdate and stannous chloride (American Public Health Association 2012). Samples for DOC and TDN analysis were acidified to pH 2 with hydrochloric acid and frozen until analysis on a TOC/TN Analyzer (Shimadzu, Kyoto, Japan). Estimates of stream periphyton biomass and benthic organic matter (as ash-free dry mass, AFDM, which we also used as a proxy for microbial biomass when testing for the effect of microbial biomass on CR and temperature sensitivity) were determined by scrubbing six rocks per stream (the primary substrate in these streams). We quantified chlorophyll content per unit rock surface area via flourometry after extraction in methanol as described in Holtgrieve et al. (2010) and AFDM by loss on ignition at 500 °C for 30 minutes. Benthic chlorophyll a and AFDM were highly correlated ($r = 0.84$), therefore, both terms were not included in models together (see below for model description).

Stream benthic substrates, which in this system consist of post-glacial gravel, were collected from 11 streams arrayed along a distinct geomorphic gradient to quantify the temperature sensitivity of oxygen consumption rates (CR). We collected 360 rocks of the median particle size (D50, Wolman 1954) from each stream (Pess 2009). Thirty rocks were placed into individual 13 L dark high-density polyethylene buckets ("chambers"; US Plastic Corp.), which

were subsequently filled with stream water and covered with airtight lids after eliminating any headspace. Two replicate chambers were incubated in a water bath at each of five temperatures distributed approximately evenly from ~4 – 22 °C, representing the range of stream summer temperatures observed among streams in this system (Lisi et al. 2013). Chambers were allowed to equilibrate to the incubation temperature for 1-2 hours, an initial oxygen concentration and water temperature were taken using a YSI 6000MS V2 sonde (YSI, Inc., Yellow Springs, Ohio), and chambers were then sealed. The chambers were gently shaken periodically throughout the incubation period and a final oxygen concentration was taken after four hours. After the incubation, the surface area of all stream particles in each chamber was measured. CR was calculated as (final $[O_2]$ – initial $[O_2]$)/ incubation period (h) and reported as mg O_2 consumed per hour per unit surface area of sediments (mg O_2 L^{-1} h^{-1} cm^{-2}).

Statistics and model fitting

We used two principal components analyses (PCAs) to summarize variation among streams in their physical, chemical, and geomorphic conditions. The first PCA summarized variation in stream chemical and physical conditions among streams (variables included: TN, TP, TDN, Chl a, DOC, C:N, seasonal temperature average and range). The second PCA was taken from Lisi et al. (2013) and included watershed geomorphic data on a larger set of streams from the Wood River basin to quantify variation among watershed geomorphic characteristics for streams in this study; variables included watershed slope, elevation, stream particle size, watershed area, and lake area (Figure A1). We regressed the resulting composite axes (principal components 1 and 2) from the stream chemistry PCA against the watershed geomorphology principal components (Table 4.1) to evaluate how variation in stream chemistry was co-varied with watershed geomorphic characteristics. In addition, we used the axes of these PCAs to

evaluate how variation among temperature sensitivity values was explained by composite variation in stream chemistry or watershed geomorphology (Table 4.5).

We quantified the temperature sensitivity of CR using the Arrhenius equation as described in Brown et al. (2004). We fit the linear form of these equations (Eq. 1) by regressing the natural log of measured rates of CR against a standardized temperature predictor, which set the intercept of the model equal to the rate of respiration at a specific temperature (T_c = 20 °C) according to:

Eq 1. $$\ln CR(T) = -E\left(\frac{1}{kT} - \frac{1}{kT_c}\right) + \ln CR(T_c)$$

In this equation, *k* represents the Boltzmann constant (in eV K^{-1}; 1 eV = 96.5 KJ mol^{-1}) and T is the temperature in degrees Kelvin. The intercept (ln $CR(T_c)$) is hypothesized to reflect the total biomass of organisms in the ecosystem and allows for the comparison of respiration rates at a standard temperature across streams (Allen et al. 2005). The temperature sensitivity is given by the slope of this relationship and represented by the activation energy (*E*). *E* is a measure of the minimum amount of chemical energy necessary for a chemical reaction to occur and is measured in eV.

We estimated *E* and ln ($CR(T_c)$) with linear mixed modeling using the lme4 package in R (Bates et al. 2013; R Development Core Team, 2012). All models considered included temperature as a fixed effect and a random effect of stream and water bath on the intercept. We then considered more complex alternative models including temperature and several stream-specific environmental parameters as fixed effects (see Table 4.5; DOC concentration, C:N, TN, TP, average temperature, seasonal and diel temperature range, principal component axes from above) to evaluate how these influenced the temperature sensitivity of CR. We tested both

interactive and additive fixed effects. A significant interaction of an environmental predictor with temperature indicates that it altered the slope of the relationship between respiration rate and temperature across all streams (i.e., the temperature sensitivity, *E*). However, if only the additive main effect was included in a model, that variable only influenced the intercept of the relationship (ln $CR(T_c)$). Models to compare fixed effects were fit by maximum likelihood and compared with AIC_c. The significance of individual parameters was assessed using likelihood ratio tests. The random effects structure for the model was selected by using the restricted maximum likelihood approach (REML; Zuur et al. 2009) and evaluated with AIC_c (Burnham and Anderson 2002). The final parameter estimates for the best models were computed using REML (Zuur et al. 2009).

Finally, we estimated a river basin-scale temperature sensitivity value that accounted for potential variation in temperature sensitivity among streams. To do so, we used the watershed slope - *E* relationship generated from our dataset of streams (R^2=0.32, Figure 4.2B) to estimate an *E* value for 40 additional streams in the Wood River where we had values for watershed slope which accounted for ~50% of the total watershed area of the Wood River. We used watershed slope to calculate *E* rather than stream chemistry because slope data are readily available from GIS and slope captured ~56% of stream chemistry variation in this study (i.e. $PC1_{chem}$). In addition, we accounted for each stream's contribution to a basin-scale temperature sensitivity by, first, weighting each stream's estimated *E* value by its proportional contribution to the total water discharge of the 51 streams in our expanded dataset ("flow-weighted"; Lisi et al. 2013), and, second, by the proportional DOC load (concentration*discharge) from the 15 streams for which we had DOC concentration data ("DOC-weighted"). We used a Monte Carlo approach to generate a mean and standard deviation for each stream's *E* value by taking 10,000 random

draws from the prediction interval of the original regression between watershed slope and *E* value. For streams for which we had no discharge data, we estimated a discharge value using its watershed area based on a regression between watershed area and discharge for 12 streams where we had seasonal discharge measurements (R^2=0.97). We then accounted for error in the flow-weighted *E* estimate by multiplying 10,000 individual random draws from the error around each stream's original *E* value (as above) by random draws from within the prediction interval of the watershed area-discharge relationship. The DOC-weighted value was generated similarly to the flow-weighted estimate, but we assumed that the measurement error in the DOC value was trivial (<10%) therefore, only accounted for error in the original *E* value.

RESULTS

Streams in the Wood River Basin varied substantially in physical and chemical conditions. We found that the first axis of the stream chemistry PCA ($PC1_{chem}$) primarily captured differences among streams in DOC and N concentrations (61% of variation) whereas the second axis of this analysis ($PC2_{chem}$) captured differences in benthic chlorophyll a concentrations (20% of variation; Figure 4.3, Tables 4.1). Moreover, stream chemistry varied predictably with watershed features. Specifically, $PC1_{geo}$ (associated with watershed elevation, slope, and stream particle size) explained 50% of the variation in stream chemistry (i.e. $PC1_{chem}$). For example, watershed slope alone explained 68% of the variation in DOC concentration among streams. The addition of $PC2_{geo}$ (associated with lake and watershed area) improved model performance to explain 77% of the observed variation in stream chemistry ($PC1_{chem}$, Figure 4.1).

As expected, CR increased with temperature across all streams in this study (R^2 = 0.40, Figure 4.2A, Table 4.5, Figure 4.4). When considering a model with only temperature as a fixed uniform effect, we estimated an average temperature sensitivity value (*E*) across all streams of

0.70 eV (SE = 0.06; $Q_{10,\ 5\text{-}15°C}$ = 2.7, S.E. = 2.5-3.1), which is within the range predicted by the MTE (0.40-0.74 eV; Gillooly et al. 2001), but higher than the assumed universal value (Gillooly et al. 2001). When we assessed whether differences in stream physical and chemical conditions influenced the temperature sensitivity of CR and we found that the two best models included DOC and C: N ratio as covariates. These models were indistinguishable based on their AIC_c values ($\Delta AIC_c \sim 2$), therefore, we considered them to have equal explanatory power. Second, we found that both DOC concentration and C: N were strongly and positively associated with *E* among streams, suggesting that both stream DOC concentration and C: N ratio influence the temperature sensitivity of CR. We illustrate these relationships in Figures 4.2C & D by plotting the estimated *E* value for each stream against the best environmental predictors. Chlorophyll a concentration was also included in one of the best models, suggesting that C sources in general were key predictors of temperature sensitivity (Table 4.5). Estimated stream intercept values were significantly positively related to TN concentration, but were not related to other stream or watershed characteristics (data not shown). Last, central to our core hypothesis, including DOC concentration and C: N improved the model fit over a model with temperature alone, and increased the estimated value of *E* (*E* = 0.75 eV; $Q_{10,5\text{-}15°C}$ = 2.9, Table 4.5).

Models with geomorphic variables as covariates were comparable to the best models based on their AIC_c values ($\Delta AIC_c < 6.0$). In addition, stream-specific temperature sensitivity was positively correlated with $PC1_{geo}$ (R^2 = 0.35), such that watershed slope was negatively related to *E* and explained the most variation of any watershed geomorphic variable we considered (R^2 = 0.32, Figure 4.2B).

Last, we found that the basin-scale unweighted average *E* among the 51 streams was 0.82 eV (S.E. = 0.04; $Q_{10,\ 5\text{-}15°C}$ = 3.3; Table 4.5), the discharge-weighted average was 0.82 eV (S.E. =

0.06) and the DOC load weighted average among the 15 streams was 0.84 eV (S.E. = 0.08; $Q_{10, 5\text{-}15°C.}$ = 3.4). Thus, all approaches for scaling up to the river basin generated higher estimates of *E* than the average of our 11 focal streams, largely a result of the dominance of streams draining flat watersheds throughout the basin.

DISCUSSION

We found substantial variation in the sensitivity of CR to increasing temperature among streams in the Wood River basin (Figures 4.2 and 4.4). CR was positively related to temperature but the steepness of this relationship varied predictably with stream chemistry and watershed physical features. (Figure 4.2, Table 4.5). Further, we found that the average temperature sensitivity of aquatic CR increased as stream DOC concentration and C: N increased (Figures 4.2C & D). Thus, the thermal scaling of CR varied predictably across the river basin in response to DOC concentration and quality (as C:N ratio) in streams which are, in turn, regulated by watershed geomorphic features.

It has been suggested that there is a universal temperature dependence of community respiration (Gillooly et al. 2001, Yvon-Durocher et al. 2012), reflecting the consistency in the basic biochemistry of metabolism among organisms (Allen et al. 2005). The average temperature sensitivity among all streams in this study, *E* = 0.70 eV (S.E. = 0.06; Q_{10} of ~2.7), was within the range of values that have been observed in both terrestrial and aquatic ecosystems (0.24-1.08; Acuña et al. 2008, Perkins et al. 2012, Yvon-Durocher et al. 2012) and similar to that predicted by the MTE (*E*~0.63, Gillooly et al. 2001).

Despite the consistency of this average result with predictions of the MTE (Gillooly et al. 2001, Brown et al. 2004) and with findings from other studies at the global scale (Yvon-Durocher et al. 2012), we observed a large degree of variation in *E* among individual streams

(Figure 4.2A, Table 4.4). Slight differences in the value of this slope can translate to substantial differences in respiration rate (Gilooly et al. 2001). For example, the *E* values that we estimated for individual tributaries spanned the entire range included in a recent global meta-analysis of temperature sensitivity in rivers and streams (0.57-1.08; Yvon-Durocher et al. 2012). In fact, the only other study to evaluate the variation in temperature sensitivity among individual tributaries at the river basin scale, also found a similar magnitude of variation (0.24-0.86; Acuña et al. 2008). Taken together, these data suggest that using a universal value to describe the temperature sensitivity of C processing across a river basin does not accurately reflect the variation in C metabolism that occurs as rivers drain heterogeneous landscapes with spatial variation in geomorphic and chemical conditions.

We found that including concentration of DOC and C quality (as C: N ratio) in our model modified estimates of *E* (Table 4.5). ıt is well established that the DOC content and quality, and nutrient availability in aquatic ecosystems are important modifiers of CR (del Giorgio et al. 1997; Fontaine et al. 2007). Our results suggest that these factors also modify the temperature sensitivity of CR, which agrees with studies of soils and marine systems showing that the temperature sensitivity of CR can be regulated by environmental factors such as substrate supply, substrate quality, and nutrient concentrations (Davidson and Janssens 2006, López-Urrutia and Morán 2007, Billings and Ballantyne 2013). A recent global meta-analysis found that most ecosystem types had similar temperature sensitivity values at the seasonal scale, but terrestrial and aquatic systems diverged at longer time scales (Yvon-Durocher et al. 2012). This might reflect that the variety of C substrates is greater in aquatic systems, which are more dependent on allochthonous C subsidies than terrestrial systems (Cole and Caraco 2001). Interestingly, the MTE also predicts that heterotrophic metabolism is more temperature sensitive than primary

production (Gillooly et al. 2001, Allen et al. 2005), which has also been supported by aquatic mesocosm experiments (Yvon-Durocher et al. 2010). Other studies have also shown that *E* of heterotrophic respiration of photosynthetically-derived, labile C often reflects the lower temperature sensitivity of photosynthesis (~0.32 eV; Allen et al. 2005) Thus, our higher estimates of *E* also suggest that heterotrophs in these systems depend more on recalcitrant, terrestrial C sources rather than algal-derived C. This is further supported by the positive relationship we observed between *E* and the C:N ratio among streams in this study (Figure 4.2D). The dependence on substrate supply and quality that we found within a river basin has important implications for the fate of watershed C given the large pools of C of varying quality that await decomposition on boreal landscapes and suggests that landscape-scale variation in C quantity and quality will affect the response of CR in streams to warming.

Our results demonstrate the importance of the geomorphology of the river network in modifying the response of CR to temperature. Watersheds control several physical and chemical factors that influence the metabolism of C by bacterial communities (Battin et al. 2008) and, thus, ultimately regulate ecosystem metabolism and how it scales with temperature. While this study is only representative of a single season, we found strong support that geomorphic features influenced temperature sensitivity indirectly through regulating stream C and N concentrations and their ratio among streams (Figures 4.1 & 4.2). Watershed geomorphic characteristics explained a substantial proportion (~77%) of variation in stream chemistry, and the majority of that variation was a function of watershed slope (~56%). Watershed slope was negatively related to temperature sensitivity (Figure 4.2B) such that CR in streams in steep watersheds was less sensitive to temperature than in streams with flat watersheds. This result likely derives from the effect of watershed slope on accumulation of C on the landscape; steep watersheds do not

tend to accumulate C in soils and wetlands whereas streams in flat watersheds accumulate C as peat in the watershed or within the stream itself in transient storage zones (Battin et al. 2008). Our results show that the connectivity of streams to their watersheds will influence the ecosystem scale responses to temperature and has the potential to alter rates of CO_2 export from watersheds.

While we only tested the influence of increasing average temperatures, recent studies have suggested that metabolism can also be sensitive to changes in thermal regimes (Vasseur et al. 2013). We tested this by including diel and seasonal stream temperature ranges as potential fixed effects in our model, and neither were included in any top models. Capturing more realistic scenarios of changing thermal regimes would require a more complicated experimental design than we used in this study. Nevertheless, our results demonstrate that watershed physical and chemical conditions modulate the sensitivity of CR to changing temperature; the nuances of which have yet to be worked out.

In summary, we show that the responses of C processing in river networks to warming are heterogeneous and will be expressed differently among tributaries of river basins, thereby having the potential to affect how watersheds sequester and release CO_2 in response to changing climate. Our results suggest that thermal scaling of CR across river basins can be constrained by accounting for geomorphic features of watersheds, particularly in attributes such as average slope that are readily obtainable from standard GIS applications.

Figure Legends

Figure 4.1. A) Relationship of stream chemistry with watershed geomorphic characteristics (R^2=0.77). PC axes refer to PC axes from geomorphic and chemical PCAs shown in Figure 2.

Figure 4.2. A) Community respiration rate (CR) as a function of incubation temperature across all streams and experimental replicates in the study. Bold black line is fitted regression line from mixed effects model of relating respiration rate to temperature (R^2= 0.40) across all observations (n=94). Dashed lines represent fitted lines from models fit to data from 11 individual streams in the study. Relationship of temperature sensitivity (*E*) estimated for individual streams (n=11) with B) watershed slope (R^2=0.32), C) DOC (mg L^{-1}; R^2=0.46), and D) C: N ratio (R^2=0.58).

Figure 4.3. Principal Components Analysis of stream chemical and physical characteristics of stream sites included in this study. 'TDN' = total dissolved nitrogen, 'DOC' = dissolved organic carbon, 'TN' = total nitrogen, 'N:P' = nitrogen to phosphorus ratio of water, 'chla' = chlorophyll a concentration, 'CN' = carbon to nitrogen ratio. 'PC' = principal component and value in parentheses indicates the percentage of variance explained by each axis. All vectors and axes shown are significant.

Figure 4.4. Variation in the response of CR to temperature across all 11 streams in the study. Lines shown are the model fit for the individual stream (thin line) and the average relationship when all streams were considered together (thick line, Figure 2A in main text).

Table 4.1. Results from stream chemistry PCA analysis. The stream chemistry PCA is based on stream chemical and physical data collected from the 11 streams at the time of this study. 'CN' = carbon: nitrogen ratio, 'N:P' = nitrogen: phosphorus ratio, 'DOC' = dissolved organic carbon, 'TN' = total nitrogen concentration, and 'Chl a' = chlorophyll a of sediments.

Principal Component	Percent Variance Explained	Significance	Significant Variable Loadings
Stream Chemistry			
PC1	61.0%	< 0.001	C:N (-0.92) N:P (0.78) DOC (-0.75) TN (0.71)
PC2	20.0%	0.982	Chl a (0.74)

Table 4.2. Estimates of intercept and slope values for regression fits to data from individual streams. Values in parentheses are standard errors of the parameter estimates.

Stream	Intercept ($mg\ O_2\ L^{-1}\ h^{-1}\ cm^{-2}$)	E value (eV)	R^2
Elva	-6.92 (0.07)	0.38 (0.05)	0.85
C	-7.15 (0.20)	0.50 (0.12)	0.89
Hidden	-7.61 (0.19)	0.62 (0.20)	0.56
Teal	-6.17 (0.26)	0.62 (0.19)	0.48
LTR	-6.58 (0.12)	0.73 (0.11)	0.83
Rainbow	-7.37 (0.34)	0.75 (0.75)	0.79
Pick	-6.47 (0.37)	0.90 (0.27)	0.54
Whitefish	-6.28 (0.29)	0.94 (0.26)	0.71
Lynx	-6.50 (0.22)	1.13 (0.19)	0.80
Bear	-7.06 (0.15)	1.25 (0.27)	0.81
Stovall	-6.72 (0.39)	1.36 (0.47)	0.60

Table 4.3. Range of values of chemical, physical, and geomorphic parameters of streams sampled for this study

Variable	**Range**
TN (μg L^{-1})	185.7 – 569.4
TP (μg L^{-1})	1.9 – 21.4
N:P	13.6 – 298.5
TDN (μg L^{-1})	0.1 – 0.9
DOC (mg L^{-1})	1.8 – 3.9
C:N	3.2 – 27.3
Chl a (μg cm^{-2})	0.4 - 4.7
Seasonal Temperature Range (ºC)	2.0 – 12.5
Average Temperature (ºC)	4.7 – 12.6
Watershed Elevation (m.a.s.l.)	42.7 – 493.5
Watershed Slope	2.3 – 25.9
Watershed Area (km^2)	1.9 – 80.7
Lake Area (km^2)	0 – 8.1
Substrate Size (D84)	19.0 – 168.0

Table 4.4. Means and standard deviations of unweighted E values for the expanded dataset of streams. Estimates of proportional discharge and DOC load are given for each stream. A watershed scale flow and DOC weighted E estimate was generated by multiplying the estimated E value by the proportional flow or DOC value for each stream and then summing the resulting values across all 51 streams.

Stream	Mean *E* value	Std. Deviation of *E* value	Proportional Discharge	Std. Deviation of Flow Estimate	Proportional DOC load
6th	0.98	0.32	0.0002	0.0038	
7th	0.94	0.31	0.0008	0.0038	
A	0.62	0.32	0.0002	0.0038	
Allah	0.70	0.32	0.0064	0.0037	0.014
Bear	0.84	0.31	0.0096	0.0037	0.044
Berm	0.73	0.31	0.0009	0.0038	
C	0.83	0.31	0.0008	0.0038	
Cabin	0.63	0.32	0.0030	0.0038	
Cold	0.74	0.31	0.0128	0.0037	
Cottonwood	0.50	0.35	0.0066	0.0037	
Eagle	0.95	0.32	0.0024	0.0038	
Elva	0.56	0.33	0.0222	0.0037	0.054
Fenno	0.83	0.31	0.0278	0.0037	
Grant	0.97	0.31	0.0907	0.0057	
Hansen	0.94	0.31	0.0013	0.0038	
Happy	0.85	0.31	0.0127	0.0036	
Hidden	0.80	0.31	0.0054	0.0038	0.011
Ice	0.88	0.31	0.0681	0.0049	
Joe	0.58	0.33	0.0072	0.0037	
K3	0.59	0.33	0.0134	0.0037	
Kema	0.98	0.32	0.0144	0.0037	0.032
LTR	0.60	0.33	0.0590	0.0045	0.141
Lynx	0.80	0.31	0.0183	0.0037	0.068
Mission	0.91	0.31	0.0019	0.0038	
Moose	1.02	0.32	0.0692	0.0049	0.278
Nerka Bear	1.06	0.33	0.0071	0.0037	
Pick	0.95	0.31	0.0142	0.0037	0.045
Pike	1.07	0.33	0.0375	0.0039	0.106
Rainbow	0.56	0.34	0.0478	0.0041	
Sam	0.68	0.32	0.0067	0.0037	
SilverSalmon	1.02	0.32	0.0229	0.0037	
Stovall	1.05	0.33	0.0221	0.0037	0.093

Teal	0.95	0.31	0.0072	0.0037	0.024
Uno	0.84	0.31	0.0122	0.0037	0.026
Whitefish	1.05	0.33	0.0067	0.0037	0.031
Yako	0.91	0.31	0.0092	0.0037	0.033
LTC	0.54	0.34	0.0309	0.0038	
N6	0.66	0.32	0.0038	0.0038	
MooseSpit	0.61	0.33	0.0052	0.0037	
B12	0.60	0.33	0.0113	0.0037	
Youth	0.78	0.31	0.0579	0.0045	
Lilly	0.82	0.31	0.0083	0.0037	
Sunshine	0.75	0.31	0.1180	0.0071	
LittleWhitefish	1.02	0.32	0.0003	0.0039	
K10	0.77	0.31	0.0478	0.0041	
K11	0.81	0.31	0.0216	0.0037	
Arcana	0.97	0.31	0.0218	0.0037	
Belt	1.05	0.33	0.0166	0.0037	
N4	0.65	0.32	0.0031	0.0038	
Coffee	1.06	0.33	0.0013	0.0038	
Bug	0.99	0.32	0.0034	0.0038	

Figure 4.1

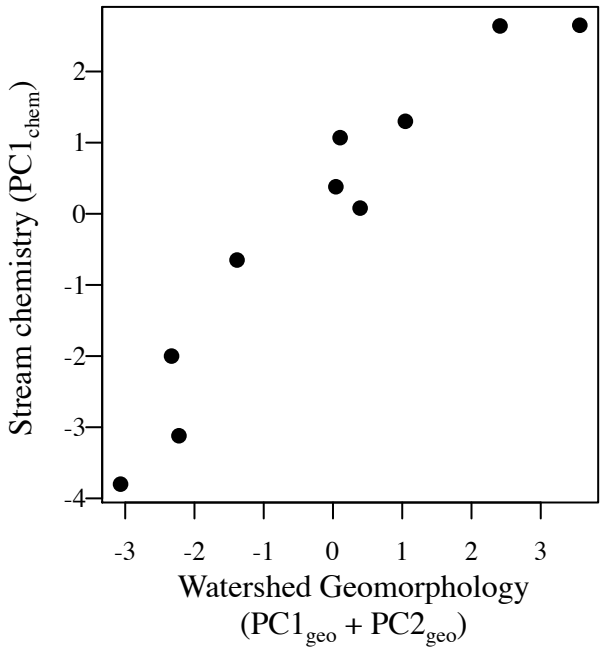

Stream chemistry ($PC1_{chem}$)
2
1
0
-1
-2
-3
-4
-3
-2
-1
0
1
2
3
Watershed Geomorphology
($PC1_{geo}$ + $PC2_{geo}$)

Figure 4.2.

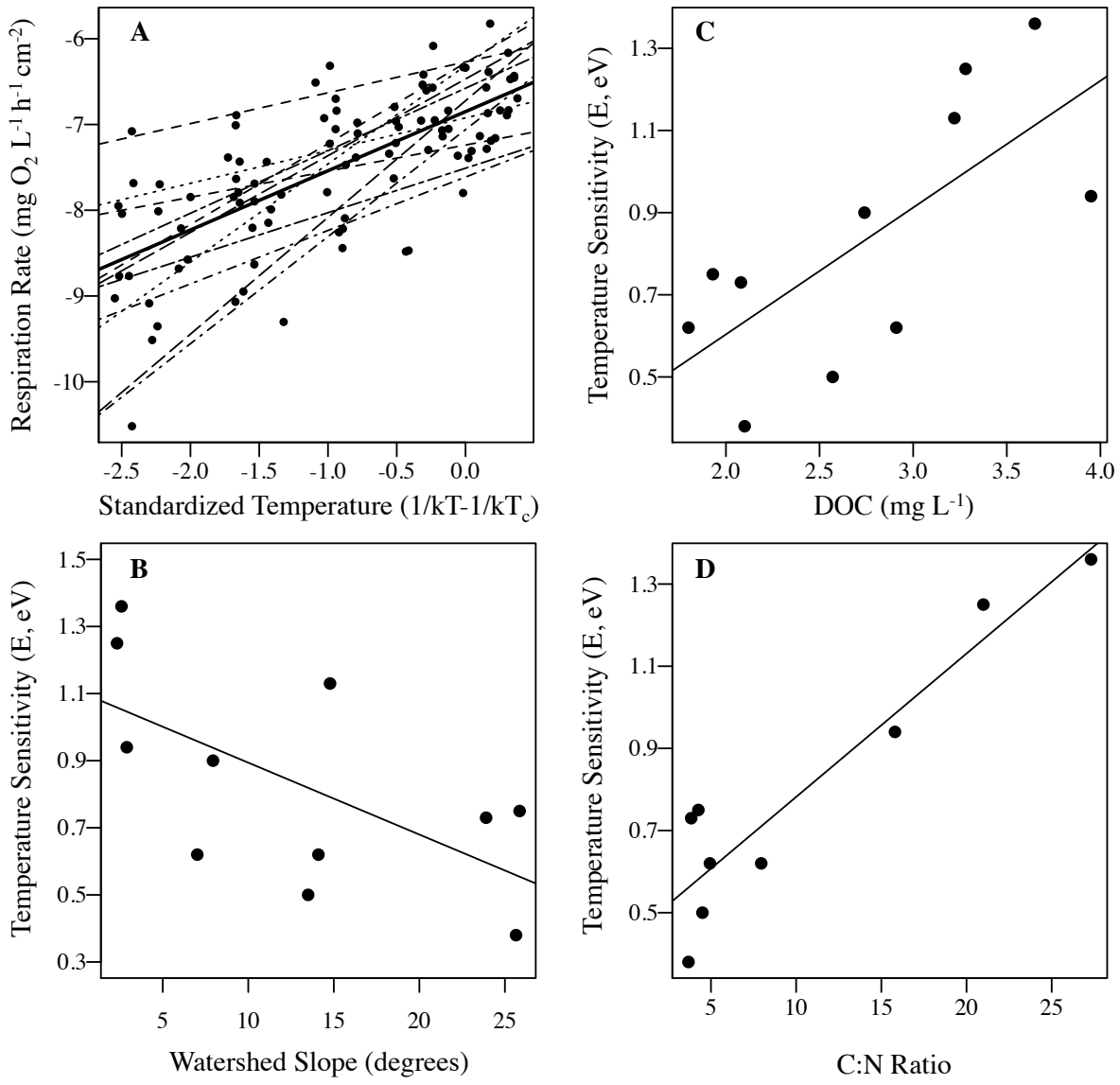

A
Respiration Rate (mg O_2 L^{-1} h^{-1} cm^{-2})
-6
-7
-8
-9
-10
-2.5
-2.0
-1.5
-1.0
-0.5
0.0
Standardized Temperature (1/kT-1/kT$_c$)
C
Temperature Sensitivity (E, eV)
1.3
1.1
0.9
0.7
0.5
2.0
2.5
3.0
3.5
4.0
DOC (mg L^{-1})
B
Temperature Sensitivity (E, eV)
1.5
1.3
1.1
0.9
0.7
0.5
0.3
5
10
15
20
25
Watershed Slope (degrees)
D
Temperature Sensitivity (E, eV)
1.3
1.1
0.9
0.7
0.5
5
10
15
20
25
C:N Ratio

Figure 4.3.

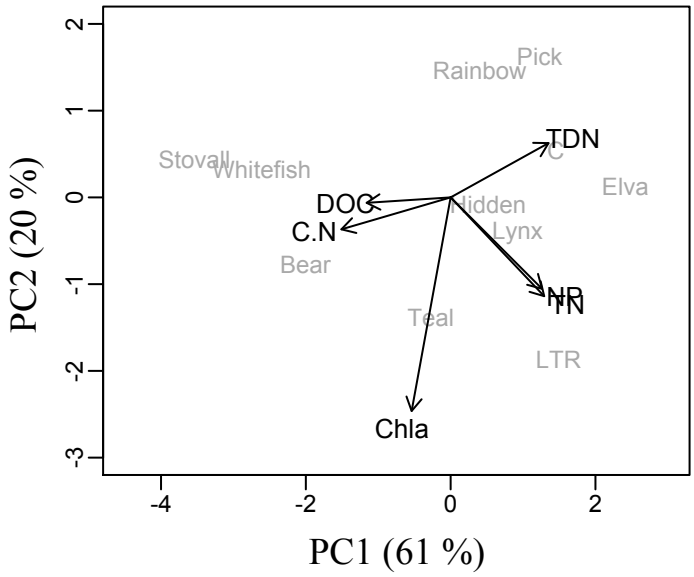

Pick
Rainbow
TDN
Stovall
Whitefish
Elva
DOC
Hidden
C.N
Lynx
Bear
NP
TN
Teal
LTR
Chla
PC2 (20 %)
PC1 (61 %)
2
1
0
-1
-2
-3
-4
-2
0
2

Figure 4.4.

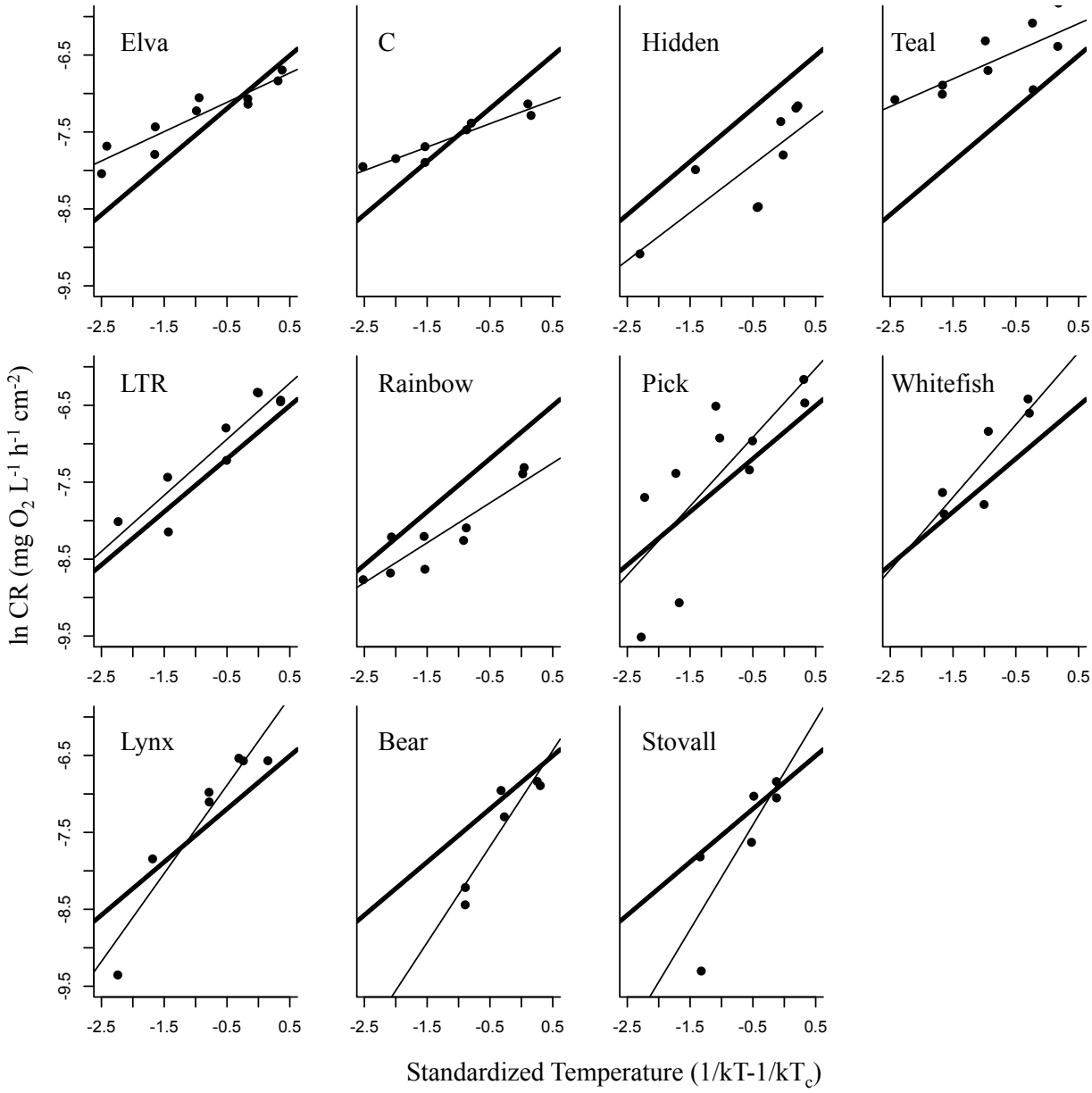

Elva
C
Hidden
Teal
LTR
Rainbow
Pick
Whitefish
Lynx
Bear
Stovall
ln CR (mg O2 L-1 h-1 cm-2)
Standardized Temperature (1/kT-1/kTc)
-6.5
-7.5
-8.5
-9.5
-2.5
-1.5
-0.5
0.5

Table 4.5. Range of models predicting the effect of temperature and its interaction with environmental factors on rates of respiration in streams in this study. Individual terms were evaluated with likelihood ratio tests and models were compared using AIC_c. E = the model-specific estimated slope, B_o = the estimated intercept, N = sample size, k = number of parameters, and AIC_c w_i is the model weight for each model considered.

General model form for all models considered with random effects: R ~ T + T*X1…T*Xn + (1 | Stream) + (1 | WaterBath)							
Models Considered	**E**	**B_o**	**N**	**k**	**AIC_c**	**ΔAIC_c**	**AIC_c w_i**
R~T*DOC+CN	0.75	-6.82	91	9	155.7	0	0.6
R~T*DOC+T*CN	0.76	-6.82	91	10	157.7	2.0	0.2
R~T+DOC+CN	0.71	-6.85	91	8	158.1	2.4	0.1
R~T*PC1.chem	0.79	-6.82	91	8	158.6	2.9	0.1
R~T*DOC+T*CN+Chl	0.75	-6.84	91	11	158.6	2.9	0.1
R~T*DOC	0.74	-6.85	91	8	159.3	3.6	0.1
R~T	0.70	-6.86	91	6	159.9	4.2	0.0
R~T*CN	0.75	-6.83	91	8	160.8	5.1	0.0
R~T*DOC+T*CN +T*RangeT+Chl	0.77	-6.84	91	13	161.3	5.6	0.0
R~ T*RangeT	0.72	-6.86	91	8	161.4	5.7	0.0
R~T*PC1.geo	0.76	-6.84	91	8	162.7	7.0	0.0
R~T*Chl	0.70	-6.89	91	8	163.7	8.0	0.0
R~T*AFDM	0.69	-6.87	91	8	163.7	8.0	0.0
R~T*DOC+T*CN+T*Chl+T*RangeT	0.76	-6.85	91	14	163.9	8.2	0.0
R~T*AvgT	0.71	-6.87	91	8	164.1	8.4	0.0
R~T*Biomass	0.69	-6.87	91	8	164.6	8.9	0.0
R~T*DOC+T*CN+T*Chl +T*RangeT+T*Biomass	0.71	-6.82	91	16	166.7	11.0	0.0
R~T*DOC+T*CN+T*Chl+T*RangeT+T*AvgT+T*Biomass	0.68	-6.79	91	18	171.2	15.5	0.0
R~T*DOC+T*CN+T*Chl+T*TP+T*RangeT+T*AvgT+T*Biomass	0.68	-6.76	91	20	176.5	20.8	0.0
R~T*DOC+T*CN+T*Chl+T*TN+T*TP+T*RangeT+T*AvgT+T*Biomass	0.70	-6.73	91	22	181.9	26.2	0.0

REFERENCES

Acuña, V., A. Wolf, U. Uehlinger, and K. Tockner. 2008. Temperature dependence of stream benthic respiration in an Alpine river network under global warming. Freshwater Biology 53:2076–2088.

Allen, A., J. Gillooly, and J. Brown. 2005. Linking the global carbon cycle to individual metabolism. Functional Ecology:202–213.

Aufdenkampe, A. K., E. Mayorga, P. A. Raymond, J. M. Melack, S. C. Doney, S. R. Alin, R. E. Aalto, and K. Yoo. 2011. Riverine coupling of biogeochemical cycles between land, oceans, and atmosphere. Frontiers in Ecology and the Environment 9: 53-60.

Battin, T., L. Kaplan, S. Findlay, H. CS, E. Marti, P. AI, N. JD, and S. F. 2008. Biophysical controls on organic carbon fluxes in fluvial networks. Nature Geoscience 1:95–100.

Billings, S. a, and F. Ballantyne. 2013. How interactions between microbial resource demands, soil organic matter stoichiometry, and substrate reactivity determine the direction and magnitude of soil respiratory responses to warming. Global Change Biology 19:90–102.

Bosatta, E., and G. Ågren. 1999. Soil organic matter quality interpreted thermodynamically. Soil Biology and Biochemistry 31:1889–1891.

Brown, J., J. Gillooly, A. Allen, V. Savage, and G. West. 2004. Toward a metabolic theory of ecology. Ecology 85:130–137.

Butman, D., and P. Raymond. 2011. Significant efflux of carbon dioxide from streams and rivers in the United States. Nature Geoscience 4:839–842.

Caissie, D. 2006. The thermal regime of rivers: a review. Freshwater Biology 51:1389–1406.

Cole, J., Y. Prairie, and N. Caraco. 2007. Plumbing the global carbon cycle: integrating inland waters into the terrestrial carbon budget. Ecosystems 10:171–184.

Cole, J. J. and N. F. Caraco. 2001. Carbon in catchments: connecting terrestrial carbon losses with aquatic metabolism. Marine and Freshwater Research **52**:101-110.

Conant, R. T., M. G. Ryan, G. I. Ågren, H. E. Birge, E. A. Davidson, P. E. Eliasson, S. E. Evans, S. D. Frey, C. P. Giardina, F. M. Hopkins, R. Hyvönen, M. U. F. Kirschbaum, J. M. Lavallee, J. Leifeld, W. J. Parton, J. Megan Steinweg, M. D. Wallenstein, J. Å. Martin Wetterstedt, and M. A. Bradford. 2011. Temperature and soil organic matter decomposition rates - synthesis of current knowledge and a way forward. Global Change Biology **17**:3392-3404.

Craine, J. M., N. Fierer, and K. K. Mclauchlan. 2010. Widespread coupling between the rate and temperature sensitivity of organic matter decay. Nature Geoscience 3:854–857.

Davidson, E., and I. Janssens. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440.

Finlay, J. C., J. M. Hood, M. P. Limm, M. E. Power, J. D. Schade, and J. R. Welter. 2011. Light-mediated thresholds in stream-water nutrient composition in a river network. Ecology 92:140–50.

Fontaine, S., S. Barot, P. Barré, N. Bdioui, B. Mary, and C. Rumpel. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature* **450**, 277–280

Gillooly, J., J. Brown, G. West, S. VM, and C. EL. 2001. Effects of Size and Temperature on Metabolic Rate. Science 293:2248–2251.

del Giorgio, P., J. Cole, and A. Cimbleris. 1997. Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems. Nature 385:148–151.

Helton, A., G. Poole, J. Meyer, W. Wollheim, B. Peterson, P. Mulholland, E. Bernhardt, J. Stanford, C. Arango, L. Ashkenas, L. Cooper, W. Dodds, S. Gregory, R. Hall, S. Hamilton,

S. Johnson, W. McDowell, J. Potter, J. Tank, P. JD, S. Thomas, H. Valett, J. Webster, and L. Zeglin. 2010. Thinking outside the channel: Modeling nitrogen cycling in networked river ecosystems. Frontiers in Ecology and the Environment 9:229–238.

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

Lisi, P. J., D. E. Schindler, K. T. Bentley, and G. R. Pess. 2013. Association between geomorphic attributes of watersheds, water temperature, and salmon spawn timing in Alaskan streams. Geomorphology 185:78–86.

López-Urrutia, Á., and X. Morán. 2007. Resource limitation of bacterial production distorts the temperature dependence of oceanic carbon cycling. Ecology 88:817–822.

Maurer, E.P., L. Brekke, T. Pruitt, and P.B. Duffy. 2007. Fine-resolution climate projections enhance climate change impact studies. Eos Transcations AGU 88: 504.

Mayorga, E., A. K. Aufdenkampe, C. a Masiello, A. V Krusche, J. I. Hedges, P. D. Quay, J. E. Richey, and T. a Brown. 2005. Young organic matter as a source of carbon dioxide outgassing from Amazonian rivers. Nature 436:538–41.

Montgomery, D. R. 1999. Process Domains and the River Continuum. Journal of the American Water Resources Association **35**:397-410.

Perkins, D., G. Yvon-Durocher, B.O.L Demars, J. Reiss, D. Pilcher, N. Friberg, M. Trimmer, and G. Woodward. 2012. Consistent temperature dependence of respiration across ecosystems contrasting in thermal history. Global Change Biology 18:1300–1311.

Raymond, P.A., J. Hartmann, R. Lauerwald, S. Sobek, C. McDonald, M. Hoover, D. Butman, R. Striegl, E. Mayorga, C. Humborg, P. Kortelainen, H. Dürr, M. Meybeck, P. Ciais, P. Guth. 2013. Global carbon dioxide emissions from inland waters. Nature 503: 355-359.

Richey, J., J. Melack, and A. Aufdenkampe. 2002. Outgassing from Amazonian rivers and wetlands as a large tropical source of atmospheric CO2. Nature 6416:6413–6416.

Sobek, S., L. J. Tranvik, and J. J. Cole. 2005. Temperature independence of carbon dioxide supersaturation in global lakes. Global Biogeochemical Cycles **19**.

Vasseur, D.A., J.P. DeLong, B. Gilbert, H.S. Greig, C.D.G. Harley, K. McCann, V. Savage, T.D. Tunney, and M.I. O'Connor. 2014. Increased temperature variation poses a greater risk to species than climate warming. Proceedings of the Royal Society B. 281: 20132612.

Weyhenmeyer, G. A., M. Fröberg, E. Karltun, M. Khalili, D. Kothawala, J. Temnerud, and L. J. Tranvik. 2012. Selective decay of terrestrial organic carbon during transport from land to sea. Global Change Biology 18:349–355.

Ylla, I., A. M. Romaní, and S. Sabater. 2012. Labile and recalcitrant organic matter utilization by river biofilm under increasing water temperature. Microbial ecology 64:593–604.

Yvon-Durocher, G., J. M. Caffrey, A. Cescatti, M. Dossena, P. Giorgio, J. M. Gasol, M. Montoya, J. Pumpanen, P. A. Staehr, M. Trimmer, G. Woodward, and A. P. Allen. 2012. Reconciling the temperature dependence of respiration across timescales and ecosystem types. Nature 487:472–476.

Yvon-Durocher, G., J. I. Jones, M. Trimmer, G. Woodward, and J. M. Montoya. 2010. Warming alters the metabolic balance of ecosystems Warming alters the metabolic balance of ecosystems Philosophical Transactions of the Royal Society Biological Sciences 365: 2117–2126.

Chapter 5: Watershed geomorphology modifies the sensitivity of aquatic ecosystem metabolism to temperature

ABSTRACT

Aquatic systems are understood to play an important role in the global carbon cycle, but our understanding of how carbon cycling in aquatic systems will respond to hydrological and thermal changes associated with climate change remains uncertain. While the potential for a major positive feedback between climate warming and CO_2 flux from ecosystems has been well studied in terrestrial systems, much less is known about how CO_2 producing processes in aquatic systems, such as ecosystem respiration, will shift with increasing temperatures. Therefore, using a process-based model of oxygen dynamics, we evaluated how the temperature sensitivity of ecosystem respiration (ER) varied in 23 boreal streams in the Wood River watershed of southwestern Alaska in 2010-2013. We found that variation in temperature sensitivity was strongly linked to watershed geomorphology: ER in flat, wetland-dominated streams was much more sensitive to temperature than streams draining steeper watersheds. Further, we show that the link between watershed geomorphology and temperature sensitivity was due to the difference in the quality of carbon substrates between steep and flat watersheds. Our results have implications for how climate driven shifts in hydrology and temperature will influence R in aquatic systems, which can impact fundamental processes such as CO_2 flux, food web dynamics and carbon transport and storage in river networks.

INTRODUCTION

How biogeochemical processes respond to changing temperature regimes at landscape scales remains one of the biggest uncertainties in understanding how aquatic ecosystems will respond to ongoing climate change. While our understanding of how changing air temperatures will influence aquatic temperature regimes has advanced in recent years (Hilderbrand and

Kashiwagi 2014, Luce et al. 2014), it remains challenging to quantify and predict the effect of temperature on biogeochemical processes at ecosystem and landscape scales (Soranno et al. 2014). Stream and river temperatures have been widely observed to increase (Kaushal et al. 2010), which has implications for the rates of many biogeochemical processes and organism physiology in aquatic ecosystems.

For example, while there has been increasing recognition of the importance of aquatic ecosystems to watershed carbon cycles, (Kling et al. 1991, Richey et al. 2002, Raymond et al. 2103), knowledge of the watershed scale controls of aquatic carbon cycling processes remains weak. In particular, our understanding of how changes in temperature regimes will alter rates of ecosystem metabolic processes and, therefore, aquatic contributions to carbon cycles at scales ranging from watersheds to the globe, remains limited. The largest reservoir of organic carbon on land is stored in terrestrial soils, thus most efforts to understand how decomposition will respond to warming have been pursued in terrestrial ecosystems (Conant et al. 2011). However, much of the CO_2 emitted from freshwater systems is derived from terrestrial sources. CO_2 produced by microbial decomposition of organic matter in soils is transported via groundwater into the aquatic environment where it is diffused back to the atmosphere. In addition, the decomposition of terrestrial organic matter in aquatic environments produces substantial amounts of CO_2 (e.g., Ward et al. 2013, Butman & Raymond 2013). Therefore, by not accounting for aquatic processing of C, we are potentially missing a major flux of carbon and underestimating how temperature and other climate change processes may alter watershed C balances.

One expectation for how ecosystem metabolic processes respond to changing temperature has been formalized as the Metabolic Theory Ecology (Brown et al. 2004). MTE assumes that temperature dependence of most biochemical processes is described by a universal relationship due to the consistent biochemistry involved in the metabolism across organisms and ecosystems {Gillooly, 2001 #214;Allen, 2005 #267}. However, it remains unclear whether the temperature response is universal or whether there are meaningful deviations from this universal value as a result of differences in resource supply (Fissore et al. 2013), resource quality (Davidson and

Janssens 2006, Huey & Kingsolver 2013), nutrient availability (Lopez-Urrutia and Moran 2007), (Berggren et al. 2010), and microbial community composition (Allison et al. 2010), which all vary across time and space in ecosystems.

In addition, recent work has shown that there may be a fundamental difference in how aquatic and terrestrial ecosystems respond to warming temperatures. A recent meta-analysis showed that over annual time scales, the temperature sensitivity of ecosystem respiration (ER) – defined as the rate at which ER increases with temperature -- was higher and more variable among aquatic than terrestrial systems (Yvon-Durocher et al. 2012). In fact, studies of ER in aquatic systems have led to varying conclusions about the consistency of temperature dependence among systems (Sobek et al. 2005, Acuna et al. 2008, Yvon-Durocher et al. 2010, Demars et al. 2011, Yvon-Durocher et al. 2012, Jankowski et al. 2014). In cases where temperature dependence has been found to vary among systems, resource quantity and quality appear to be a key control on temperature sensitivity of ER (e.g., Lopez-Urrutia and Moran 2007). Respiration of lower quality substrates responds more strongly to temperature increases than respiration based of easily degradable, labile carbon substrates. This hypothesis, known as the "Carbon-Quality Temperature Hypothesis" (Ågren and Bosatta 1999) suggests that because the activation energy is greater for more complex C substrates, rates of respiration based on recalcitrant C substrates -- such as the organic matter loaded to streams from peatlands and soils -- will increase more with temperature (i.e., will have a higher temperature sensitivity) than will respiration based on labile C sources (e.g., Ylla et al. 2012). Much of the organic matter fueling aquatic ER is derived from the terrestrial subsidies to aquatic environments (del Giorgio et al. 1997), which tend to be more recalcitrant than autotrophic sources. Thus, the difference between terrestrial and aquatic systems could reflect the greater variety in quality and timing of substrates available for aquatic ER than terrestrial ER.

Therefore, while theory predicts that temperature sensitivity would be universal across habitats in a given watershed, it is highly likely that the observed temperature sensitivity of

respiration would vary across a watershed in response to factors such as carbon quantity and quality (Acuña et al. 2008, Jankowski et al. 2014). In aquatic systems, watershed hydrogeomorphology controls the expression of ecosystem processes (Beechie et al. 2013, Poole et al. 2010, Montgomery 1999), including factors that are expected to influence the response of R to temperature. For instance, geomorphic features that control transient storage zones influence the tendency for rivers to store, process or transport carbon downstream (Helton et al. 2012, Aufdenkampe et al. 2011, Battin et al. 2008). Hydrological regimes and floodplain connectivity vary within a river network from headwaters to mainstem channels, thereby influencing the connectivity among aquatic and terrestrial flow paths, the timing and magnitude of terrestrial organic matter subsidies to the stream (Poole et al. 2010), and the composition microbial communities that decompose them (Widder et al. 2014). Further, thermal regimes, nutrients, and light availability are also tightly linked to watershed hydrogeomorphology (Finlay et al. 2011, Helton et al. 2012, Lisi et al. 2013). Therefore, geomorphic features of watersheds likely modulate the responses of aquatic ecosystem metabolism to changes in stream thermal regimes, but there are few studies that have linked our understanding of how watershed geomorphology influences ecosystem processes to how those processes will respond to warming at the basin scale.

We evaluated how the temperature sensitivity of aquatic ecosystem respiration varied among the tributaries of a boreal river basin in southwest Alaska. We interpreted daily dynamics in dissolved oxygen concentrations with a statistical process model to estimate rates of ecosystem respiration and its sensitivity to thermal variation. Specifically, we addressed the following questions: 1) Does the temperature sensitivity of aquatic R vary significantly among tributaries within a single river basin?, 2) If so, does watershed geomorphology constrain the metabolic response of aquatic R to temperature?, and 3) Does accounting for variability in temperature sensitivity of aquatic R have the potential to alter our estimates of changes in CO_2 flux in response to warming temperatures?

METHODS

Field site

This study was conducted in 23 2nd-3rd order streams of the Wood River basin (59°20'N, 158°40'W) in the Bristol Bay region of southwest Alaska during the summers of 2010-2013 (see Lisi et al. 2013 for further characterization of streams in this basin). The majority of the Wood River basin is within the Wood-Tikchik State Park and is comprised of five large, interconnected lakes fed by numerous small streams that drain through the Wood River into Bristol Bay. This region is one of the fastest warming on Earth (Maurer et al. 2007), and is characterized by extensive peatlands with the potential to release substantial amounts of C to the atmosphere with ongoing climate warming. The Wood River basin has considerable variation in geomorphic conditions among sub-watersheds that produces a distinct gradient in watershed slope and elevation among streams (Lisi et al. 2013).

Stream metabolism and the temperature sensitivity of respiration

We estimated the temperature sensitivity of ecosystem respiration by fitting a statistical model of ecosystem metabolism to daily changes in stream dissolved oxygen concentration, water temperature, and irradiance data (Holtgrieve et al. 2010). Water temperature and [O2] were recorded at a single station near the outflow of each stream for a minimum of two days at 10-min intervals with a YSI 6600 V2 sonde equipped with a optical dissolved oxygen (ROx) sensor. Sensor [O2] measurements were calibrated to [O2] measurements on a subset (n=10 streams, 3 replicates per stream) as determined by Winkler titrations at the time of sonde deployment or retrieval. Irradiance at the water surface was measured directly in 10-min intervals at one of two stations no more than 30km away with a HOBO® universal weather station PAR sensor (Onset Computer Corporation, Bourne, MA, USA).

We modified a mass-balance model of ecosystem metabolism describing oxygen

dynamics and fit it to these data to estimate rates of gross primary production (GPP), ecosystem respiration (R), and gas exchange (Holtgrieve et al. 2010). The model simulates changes in stream oxygen concentrations through estimating light-dependent oxygen production via photosynthesis, temperature-dependent oxygen consumption via respiration, and oxygen exchange between the stream and the atmosphere dependent on the gas transfer velocity and concentration gradient:

$$\frac{dO_2}{dt} = \left[k\left(\left[O_{2,sat}\right] - \left[O_2\right]\right) - R + P\right]/D$$

where $[O_2]$ is the dissolved oxygen concentration (mg m^{-3}), $[O_{2,sat}]$ is the dissolved oxygen concentration at atmospheric equilibrium, R is the instantaneous respiration rate, P is the instantaneous rate of photosynthesis (both in units of mg O_2 m^{-2} h^{-1}), and D is the average mixed layer depth (m). The first term in the above equation is the net effect of gas exchange, which is the gas transfer velocity, *k*, times the O_2 concentration gradient.

We modified this model to allow for variable temperature dependence of R according to Arrhenius kinetics:

$$(T, t) = R_{ref} * e^{\frac{-E_b(T-T_{ref})}{kTT_{ref}}}$$

where *R (T, t)* is the rate of respiration at a given time *t* and temperature *T* (°K). *E* is the activation energy of respiration in eV and describes the slope of R with temperature, i.e., the temperature dependence of respiration, and therefore is our key parameter of interest. k is the Boltzmann constant (8.62 x 10-5 J $°K^{-1}$), and R_{ref} is respiration at a standard temperature (T_{ref}). The temperature data were centered to the average temperature of each individual stream dataset to reduce the correlation between the slope, *E*, and intercept, R_{ref}.

The model was also adjusted to allow variation in the substrate supporting heterotrophic

respiration (Schindler et al., *in prep*), which could influence the overall temperature sensitivity of oxygen consumption rates (Davidson et al. 2012, Jankowski et al. 2014). We considered the potential for two substrate pools to support R: respiration of ambient carbon substrates or "background R" (R_b), and respiration of a pool of labile organic matter produced via recent photosynthesis (R_p; Sadro et al. 2011).

$$R_{total}(t) = R_b(t) + R_p(t)$$

R_p was modeled as an exponentially declining fraction of photosynthesis that occurred in previous time steps, formulated as:

$$R_p\ (T, t) = \exp\ (-\frac{E_p}{kT}) \sum_{i=1}^{n} P[I(t-i)] * \exp\ (\beta_i)$$

According to this formulation we assume that carbon produced by some portion of *n* previous time steps of photosynthesis was directly consumed and respired by heterotrophs. The slope of an exponential decay function (β) describes the rate at which photosynthetically-derived organic matter is metabolized or washed out of the system. The greater the value of β , the lower the influence of photosynthetically-derived carbon on R.

R_b and R_p were considered to have different sensitivities to temperature. We set the *E* value for R_p at the theoretical E value for photosynthesis, E_p = 0.32 eV (Allen et al. 2005) and then estimated E_b for R_b from the data. In all streams, we compared two models for R: one that considered a proportional contribution of R from two substrate pools and an alternative model for each stream that accounted for a single pool of substrate as modified by a single temperature sensitivity (Table of models, parameters). Where the model converged on a clearly defined β, (i.e., there was strong evidence for two-sources of respiration) we calculated a model-integrated value of E, Et, which accounted for the proportional contribution of R_p and R_b to the overall

estimate of R as follows:

$$E_t = p_b * E_b + p_p * E_p$$

where p_b and p_p (equal to 1-pb) are the proportions of R_b and R_p integrated over a 24-hour period. In the cases where the model did not converge a β, we assumed that there was no evidence for a two-stage R (R_b and R_p) and fit the data with a single source model (R_b only).

Model parameters were estimated in a Bayesian context (Holtgrieve et al. 2010). Priors were specified on a subset of model parameters -- k_{20}, E_b, $O_{2\ init}$, $O_{2\ init,\sigma}$, -- all of which were given a uniform distributions (a range of equally probable values). P_{max}, α_{PI}, R_{ref}, and β were estimated without prior information. Posterior probability distributions for each of the parameters were estimated though the implementation of a Markov Chain Monte Carlo (MCMC) algorithm in the software program AD Model Builder (ADMB; Fournier et al. 2012). We implemented three MCMC chains with unique starting values to be sure the chains fully explored the posterior parameter space. To test for model convergence, we used the Gelman-Rubin diagnostic which tests for within vs. among chain variance, tested the saved draws for all parameters for autocorrelation of 5% using the acf function in the CODA package of R (R Foundation), and visually examined parameter traces (Plummer et al. 2006). The fit of one-source vs. two-source models were assessed by using the Deviance Information Criterion. The Deviance Information Criterion (DIC) is a penalized likelihood criterion for evaluating support among competing models. DIC is similar to Akaike's Information Criterion (AIC), but formulated for models with Bayesian estimated parameters by Markov Chain Monte Carlo. The equation to calculate is: DIC = pD + Dbar, where Dbar is a measure of how well the model fits to the data and pD is a penalty for the effective number of parameters in the model. As with AIC, models with the lowest DIC best explain the data, models within 1-2 units of the model with the

lowest overall DIC are reasonably equivalent in explaining the data, and models within 3-7 units have considerably less empirical support (Spiegelhalter et al. 2002).

We performed a number of simulations to test how well the model performed under a variety of conditions (Supplemental Section 5.1). We varied the magnitude of daily changes temperature and oxygen concentration (through changing k) and also evaluated if there was an interaction between the daily temperature range and the magnitude of the expected E (see Appendix 2). These exercises showed that the model was able to reliably estimate E_t across a broad range of parameterizations of the model.

Environmental data

In order to compare temperature sensitivity estimates to environmental characteristics, we collected samples at the time of sonde deployment to measure stream chemical and physical conditions. Water samples for total nitrogen (TN), total phosphorus (TP), dissolved organic C (DOC), total dissolved nitrogen (TDN) and C:N ratio were taken from streams at the time of sonde deployment. TN was determined using perchloric acid digestion followed by analysis with automated colorimetry. TP concentration was determined colorimetrically after persulfate digestion and reaction with molybdate and stannous chloride (American Public Health Association 2012). Samples for DOC and TDN analysis were filtered through a 0.7 um glass fiber filter (Whatman) acidified to pH 2 with hydrochloric acid and frozen until analysis on a TOC/TN Analyzer (Shimadzu, Kyoto, Japan). Estimates of stream periphyton biomass were determined by scrubbing six rocks per stream (the primary substrate in these streams). We quantified chlorophyll *a* content per unit rock surface area via flourometry after extraction in methanol as described in Holtgrieve et al. (2010). Seasonal water temperature data were taken from Lisi et al. (2013) who measured stream temperatures at the mouth of each stream at 90-min intervals throughout each summer in our study from June – September using I-button

temperature recorders (Maxim Integrated Products, Sunnyvale, CA). Flow measurements were taken and discharge was calculated at the time of sampling for all streams in the dataset. In addition, pressure gages (Onset Computer Corporation, Bourne, MA, USA) were deployed in a subset of streams ranging in watershed characteristics from which we calculated seasonal trends and averages. Watershed geomorphic characteristics for streams in this study were taken from Lisi et al. (2013). Variables included watershed slope, elevation, stream particle size, watershed area, and lake area.

We evaluated the influence of watershed geomorphic features and stream environmental conditions on the temperature sensitivity of stream respiration by regressing our estimated E_t value from the metabolism model described above against watershed geomorphic and stream chemistry variables. We tested the controls on E_t across all years in our dataset (2010-2013) and within each year independently (Tables 5.2 and 5.3). When evaluating environmental and geomorphic controls across all years, we used a mixed effects modeling framework to account for repeated measurements among years by including a random effect of year on the intercept as below:

$$E_t \sim \beta_1 X_1 + \beta_2 X_2 \ldots + \beta_n X_n + (1|\text{Year}) + \varepsilon_i$$

We considered several alternative models to explain variation in E_t among streams in this river basin including several stream-specific environmental parameters as fixed effects (DOC, C:N, TN, TP, chlorophyll *a*, average temperature, seasonal and daily temperature range, discharge) to evaluate how these influenced the temperature sensitivity of ER. Models to compare fixed effects were fit by maximum likelihood and compared with AIC_c. For year-specific assessment of environmental controls on E_t, we used step-wise multiple regressions and model selection by AIC_c to compare the influence of all of the same environmental parameters listed above on E_t.

To understand how temperature sensitivity was expressed across a larger set of 53 streams in the Wood River watershed, we used the relationship between watershed slope and temperature sensitivity derived from our smaller dataset of streams to extrapolate to the entire river basin. We then calculated an average watershed temperature sensitivity value for this dataset of streams. We also calculated watershed-scale values that accounted for each stream's contribution to the watershed water and carbon loads. To do so, we weighted each individual stream by its relative proportion of the total discharge and DOC load as in Jankowski et al. (2014).

Lastly, we were interested in how our estimates of temperature sensitivity could translate into changes in rates of ER under warmer stream temperatures. Therefore, we simulated a two-degree warming increase in average temperature across the streams in the watershed. We then used the temperature sensitivity values estimated for each stream to compare how ER at current temperatures compared to rates of ER with a two-degree increase in average stream temperature. We then evaluated how the percent change from current to warmed temperatures varied with watershed slope.

RESULTS

Temperature dependence of ER was variable among sites and years, with values for E_t ranging between 0 and 1.93 (average of 0.35 eV, Table 5.1). The range and mean of E_t varied among years (significant "Year" effect in mixed effects model, p=0.03) with the highest average value and largest range in 2010 and the lowest in 2012 (Table 5.1). In most cases, the two-source model fit the data either the same or better than the one source model (Table 5.2). Where the model did not converge on a reasonable β value, the one and two source models fit the data equally well, since there was no support for R_p. Typically estimating E_b with a one vs. two-source model did not change the estimate for E_b, but when it did, the estimate of E_b increased

slightly since E_p captured some of the temperature dependency. Using a two-source model did influence the model-integrated value of E_t because it was weighted according the proportional contribution from R_p.

Geomorphic features

Geomorphic conditions had clear effects on our estimates of temperature sensitivity of ER across all years of the study (Figure 5.1). The best geomorphic predictor of temperature sensitivity was watershed slope: respiration in streams draining flat watersheds were substantially more sensitive to thermal conditions than were streams draining steep watersheds. Furthermore, the strength of the relationship of E_t with watershed slope changed among years (Figure 5.1, likelihood ratio test for significance of 'year' random effect, $p = 0.07$) but the direction of this relationship was consistent. The relationship of temperature sensitivity with watershed slope was strongest in 2010 and 2011 but declined in 2012 and 2013 (Figure 5.1).

Stream characteristics

When we considered which environmental factors influenced the variation in temperature sensitivity across all years in our dataset, models including C: N, average summer temperature, TP and chlorophyll *a* were best supported by the data (Table 5.3, Figure 5.2), but only C.N ratio was present in all five of the best models. We also tested each year independently to evaluate whether variation in E_t was controlled by the same environmental factors each year. We found that, in general, carbon substrate availability and quality (DOC, C:N, chlorophyll *a*) were included in the best models across all years, but which was most strongly related to E_t varied by year (Table 5.4).

Temperature sensitivity at the river basin scale

We used the relationship between watershed slope and temperature sensitivity generated from our dataset of study streams to estimate a watershed scale value for the Wood River basin.

We expanded our dataset to 53 streams for which we had watershed slope data and estimated a temperature sensitivity value only based on the slope, and also weighted by discharge and DOC load. We found that for all years together the watershed scale value was 0.24, 0.18, and 0.25 eV respectively, so all values were lower than the average generated by our dataset of streams (0.35 eV, Table 5.5). This is likely because > 38% of the streams in this expanded dataset had watershed slope values > 15 degrees, which our data suggest to be a potential threshold in the relationship between temperature sensitivity and slope (data not shown). Above this value, most streams are estimated to have temperature sensitivities less than 0.14 eV.

Simulating effects of warming on ER

Lastly, we compared rates of ER under a 2° increase in temperature with the rate of ER under current average temperatures for all the streams in our study. We found that the percent increase in ER under a warming scenario was greatest for streams that drained flatter watersheds (slope < 10 degrees). This suggests that stream watershed geomorphology can give us some information about how stream ER in a given river basin will respond to rising temperatures.

DISCUSSION

We found that there was wide variation in temp sensitivity across streams within a single river basin in SW Alaska. The variation we observed in temperature sensitivity was consistently negatively related to watershed slope, although the strength of that relationship varied among years (Figure 5.1). Our data suggest that the relationship of watershed geomorphic features and temperature sensitivity arises from how watershed slope controls the availability and quality of carbon substrates for respiration (Figure 5.2, Table 5.3). Thus, this study is an important advance in understanding how aquatic ER responds to temperature at the ecosystem scale and the factors control that variation at a scale that can be incorporated into watershed models to explore how aquatic ecosystems will respond to ongoing climate change.

We found that watershed slope consistently explained the most variation in temperature sensitivity (Figure 5.1), and that R in flat streams was more sensitive to temperature than R in steep streams. This result was consistent with our previous findings in this watershed (Jankowski et al. 2014), and strengthens our conclusions since this study was done at the ecosystem scale and over the course of several years. The relationship between watershed slope and temperature sensitivity likely derives from the effect of watershed slope on accumulation of C on the landscape: steep watersheds do not tend to accumulate C in soils and wetlands whereas streams in flat watersheds accumulate C as peat in the watershed or within the stream itself in transient storage zones (Battin et al. 2008). We found both the quantity and quality (as C: N) were strongly linked to watershed slope (Figure 5.2, Tables 5.3 and 5.4). This is consistent with several studies of streams in boreal watersheds that have shown wetland cover and altitude to be linked to high DOC concentrations and CO2 flux in streams (Wallin et al. 2010), Laudon et al. 2011, Agren et al 2010).

It has been recognized for over 30 years that even simple measures such as stream order can give some information about ecosystem function and food web structure (Vannote et al. 1980). More recently, work has focused on understanding how river geomorphology influences variables that control variation temperature sensitivity such as organic carbon processing and storage (Battin et al. 2008), stream temperature (Lisi et al. 2013), hydrological regime (Helton et al. 2012, Widder et al. 2014), as well as light and nutrient availability (Finlay et al. 2011). Thus, it is a logical extension to expect that river geomorphology will constrain how metabolism scales with climate warming as well. Interestingly, the one other study that has examined temperature dependence in streams at the network scale, showed that temperature dependence was a function of the antecedent thermal conditions (previous 15 days), which varied predictably between

headwater and downstream reaches (Acuña et al. 2008), thus suggesting that in their system, geomorphic features control temperature sensitivity as well. Although we recognize that rates of R and GPP are not only sensitive to temperature, they also respond to factors such as nutrients, light, biofilm community composition (e.g., Bernot et al. 2010) many of these factors are jointly controlled by watershed geomorphology (Beechie et al. 2013). As a result, geomorphic features provide a unifying feature to make predictions about how carbon stored in river networks will respond to climate warming (Figure 5.5).

While we found that temperature sensitivity consistently declined with increasing watershed slope, there were significant differences among years on the relationship of slope with temperature sensitivity (Figure 5.1). There are a number of potential reasons for these differences. First, there were significant differences in rainfall, hydrology, and temperature among years in our study (Figure 5.3) that would drive changes in carbon substrates, development of stream biofilms (Timoner et al. 2014) and thermal regimes among years (Lisi et al., in prep). Interestingly, 2012 was the wettest year in the dataset and 2013 was the warmest, and those two years both have lower E values on average and less variation among streams (as well as a weaker relationship with watershed slope). There are many factors that could complicate understanding the hydrological controls on temperature sensitivity, however, such as the strength and timing of storm events that affect algal growth, flow rate, deposition, etc. In addition, studies linking landscape features with DOC and CO_2 flux (e.g., Ågren et al. 2010) have found that DOC and CO_2 flux could increase proportionally more in response to rain events in steep, low DOC streams than in streams draining wetlands.

In addition, we saw greater variation in temperature sensitivity among the flatter streams in our study than those draining steep watersheds. We hypothesize that greater variation

observed in flat streams could be because those streams are dominated more by floodplain and aquifer flow paths that create lags and complexity that alter residence time and retention (e.g., Helton et al. 2012). As a result, flatter streams may have greater variation in temperature and the timing and magnitude organic matter delivery to the main stream channel where we measured metabolism.

Despite this variability, there was a clear trend of increasing temperature sensitivity with increasing stream C: N ratio and benthic chlorophyll a (Figure 5.2, Table 5.2). This was similar to previous findings (Jankowski et al. 2014) and the results of several terrestrial studies that have linked temperature sensitivity to the quality of the substrate being metabolized (Davidson and Janssens 2006, Conant et al. 2011), Davidson et al. 2012). In fact, a recent study showed that the magnitude of the response of soil R to temperature was linked to the C: N ratio of soils from arctic to tropical regions (Karhu et al. 2014). The reason for this derives from enzyme kinetics and the link between activation energy and temperature; more complex carbon substrates are associated with higher enzyme activation energies, and the rates of these enzyme-mediated reactions respond more strongly to temperature than respiration based on more labile carbon (Davidson and Janssens 2006, Ylla et al. 2012). There are several other potential causes of variation in temp sensitivity of microbial metabolism among sites such as substrate quantity, thermal acclimation or adaptation of microbial communities (Bradford 2013), and reliance on other forms of metabolism for microbial energy production (e.g., methanogenesis; Yvon-Durocher et al. 2014). For example, it has been suggested the link between C: N ratio and temperature sensitivity results from the fact that high C: N ratios indicate N limitation and several N cycle processes (e.g., N fixation) are more sensitive to temperature than those in the C cycle (Karhu et al. 2014).

There are numerous ways to assess the quality of carbon substrates, and many technologies exist to probe much further into the identity of the carbon substrates in a given organic matter pool than a simple C: N ratio. Thus, we recognize that C: N is a coarse measure of carbon quality. In fact, recent work has shown that even terrestrial molecules that are traditionally viewed as recalcitrant (lignin) can in fact vary in quality (Ward et al. 2013). Thus, in 2013, we did also measure SUVA, which is an index of aromatic content of organic matter, and we found that it was positively correlated with C:N ratio ($r = 0.48$, data not shown), indicating that indeed high C:N ratios correlate with the presence of lower quality carbon substrates. Nonetheless, our results support an increasing body of work showing that the quality of organic carbon substrates is strongly linked to the temperature response of microbial respiration (Ågren and Bosatta, Davidson and Janssens 2006, Conant et al. 2011, Karhu et al. 2014).

While we found wide variation in the value of E among streams in our study, the average value across all streams and years was 0.35 eV (S.D = 0.43; $Q_{10,\ 5\text{-}15°C}$ ~1.7; Table 5.1). This suggests that, first, temperature sensitivity was highly variable, and second, in general, ER was less temperature sensitive than expected from mesocosm experiments where we found an average E value in this system of 0.71 eV (Jankowski et al 2014). The value we estimated at the ecosystem scale is lower than the theoretical value for heterotrophic respiration (0.65; Figure 5.4; Gilooly et al. 2001, Brown et al. 2004), the average observed in other studies in stream ecosystems (Acuna et al. 2008, Demars et al. 2009, Perkins et al. 2012), and is, in fact, closer to the temperature sensitivity value for photosynthesis (E = 0.32 eV, Allen et al. 2005). Other studies have shown that this lower temperature sensitivity of heterotrophic-based respiration results from a close link between heterotrophic respiration and autotrophic sources of carbon

(Yvon-Durocher et al. 2012). From our previous study using a mesocosm approach, we had concluded that much of the carbon driving respiration was terrestrially derived because of it's higher temperature sensitivity and C:N ratio. However, our results at the ecosystem scale to do not seem to agree (Figure 5.4). This could be for a number of reasons. For example, because we estimated temperature sensitivity at the ecosystem scale, our estimate reflects respiration occurring in several components of the stream channel, including the hyporheic zone and fringing riparian areas (Fellows et al. 2001). Previous studies have shown that areas of the channel with higher residence times, such as floodplain and hyporheic sediments often have distinct thermal regimes from open channel areas (Stanford and Ward 1988?, Helton et al. 2012). Therefore, if most of the stream's "metabolic machinery" operates subsurface and is buffered against temperature variation, this could influence our estimate of temperature sensitivity. We used open channel temperature data to estimate E, which preliminary data from our system has shown to have a wider daily swing in temperature than subsurface sediments. Thus, if respiration is in fact occurring hyporheically and experiencing a smaller range in temperature, the response is in fact more sensitive per unit temperature change than we estimated based on open channel temperatures. This result points to the need to understand how hydrology controls the specific sites of ecosystem metabolism relative to the amount of temperature variation that occurs at those scales.

These results have implications for understanding how stream respiration -- an important source of CO_2, driver of food web dynamics, and control on stream transport of C -- will respond to a shifting mosaic of hydrology and temperature. Climate change has many implications for thermal and hydrological regimes in stream ecosystems, for example: changing precipitation regimes (IPCC 2013), shifts in water sources (snow vs. rain; Berghuijs et al. 2014), melting

permafrost (Zarnetske et al. 2008), and "browning" (Monteith et al. 2007). Our study is novel in providing insight into how interactions between changing temperatures and hydrologically-driven shifts in organic matter substrates will influence aquatic R. Further, we were able to estimate temperature sensitivity in the field and at the ecosystem scale, both more robust approaches to understanding how R varies with temperature than more traditional, but simplifying experimental approaches. We provide an important direction for developing a framework for scaling up from single streams to river basins based on simple geomorphic rules.

Figure Legends

Figure 5.1. The relationship of temperature sensitivity with watershed slope for each year in the dataset: A) 2010, $R^2 = 0.53$, n = 14, B) 2011, $R^2 = 0.74$, n = 13, C) 2012, $R^2 = 0.27$, n = 14, and D) 2013, $R^2 = 0.08$, n = 12.

Figure 5.2. Temperature sensitivity was positively related to the C:N ratio of the dissolved constituents in the water column for the years in which we had C:N data, 2011-2013($R^2 = 0.36$, n = 52).

Figure 5.3. Variation among years in our dataset (2010-2013) in A) Lake level at time of sampling, B) cumulative rainfall, C) Average summer temperature, D) summer temperature range, E) DOC and F) C:N. (DOC and C:N not sampled in 2010).

Figure 5.4. Mean and variation of temperature sensitivity when using experimental ("bucket"), ecosystem ("Model") and Metabolic Theory of Ecology ("MTE") approaches to estimate its value.

Figure 5.5. Relationship between watershed slope and the percent change in ER under a 2° warming scenario vs. current temperatures (R^2=0.28).

Table 5.1. Average and range of estimated temperature sensitivity for four years in this study (2010-2013).

Year	**Average E_t**	**Range**	**N**
2010	0.58	0 - 1.93	16
2011	0.30	0 – 1.29	11
2012	0.20	0 – 0.76	14
2013	0.32	0 – 0.89	13

Table 5.2. Comparison of one and two-source R model fits to the data for each stream included in this dataset. 'N.A.' indicates cases in which the model would not converge or parameter could not be estimated.

Stream	Year	Best Model	beta	DIC Two Source	DIC One Source	Difference
Hidden	2011	OneSource	-1.63	-624.9	-631.7	-0.01
N.Bear	2011	OneSource	-19.8	-658.2	-710.7	-0.08
Hope	2013	OneSource	-2.14	-1173.2	-1177.8	0.00
Joe	2013	OneSource	NA	N.A.	-229.5	
Yako	2013	OneSource	NA	N.A.	-1341.2	
Yako	2010	OneSource	-25	N.A.	-593	
Happy	2013	OneSource	-23	N.A.	-1807.3	
6th	2010	Same	-30	-582.9	-582.3	0.00
7th	2010	Same	-25	-809.8	-810.2	0.00
Fenno	2010	Same	-200	-641.7	-641.7	0.00
Hidden	2010	Same	-26.8	-642	-642.1	0.00
Hope	2010	Same	-24.9	-720.7	-720.6	0.00
Lynx	2010	Same	-28	-627.2	-626.1	0.00
Moose	2010	Same	-2.05	-720.7	-717.9	0.00
Stovall	2010	Same	-24.6	-643.9	-644.3	0.00
Fenno	2011	Same	-24	-1096.5	-1096.5	0.00
Berm	2012	Same	-26	-1035.5	-1035.4	0.00
Bug	2012	Same	-26	-1097.7	-1098.2	0.00
Hope	2012	Same	-20	-960.5	-960.7	0.00
Moose	2012	Same	-22	-1256	-1256	0.00
N.Bear	2012	Same	-2.57	-666.4	-666.4	0.00
Pick	2012	Same	-30	-596.3	-596.4	0.00
Stovall	2012	Same	-23.5	-514.4	-515.5	0.00
Berm	2013	Same	-24	-1151.9	-1151.9	0.00
Moose	2013	Same	-20	-1253.3	-1253.4	0.00
N.Bear	2013	Same	NA	-1249.1	-1249	0.00
Pick	2013	Same	-27	-596.3	-596.4	0.00
Stovall	2013	Same	-24	-515.3	-515.5	0.00
Bear	2010	Same	-25	-450	-450	0.00
Rainbow	2011	Same	-28	-2108	-2108	0.00
Hidden	2012	Same	-18	-909	-909	0.00
Rainbow	2013	Same	-27	-1588	-1588	0.00
Eagle	2013	TwoSource	NA	-813	N.A.	
Bug	2011	TwoSource	-1.5	-602.8	-597.4	0.01
C	2010	Twosource	-1.86	-1066.4	-1054.9	0.01

Eagle	2010	TwoSource	-1.57	-360.9	-344.4	0.05
Hansen	2010	Twosource	-1.65	-613.6	N.A.	
Pick	2010	TwoSource	-24	-586.5	-581.5	0.01
Whitefish	2010	TwoSource	-1.69	-718.2	-669.5	0.07
7th	2011	TwoSource	-2.38	-1523.2	-1358.6	0.11
C	2011	TwoSource	-1.32	-1011	-966.5	0.04
Elva	2011	TwoSource	-1.9	-650.3	-643.4	0.01
Stovall	2011	TwoSource	-1.56	-543.2	-519.1	0.04
Teal	2011	TwoSource	-1.68	-447.5	-436.7	0.02
Whitefish	2011	TwoSource	-0.98	-629.5	-614.3	0.02
7th	2012	TwoSource	-2.6	-709.8	-517.3	0.27
Eagle	2012	TwoSource	-1.4	-634.9	-581.8	0.08
Teal	2012	TwoSource	-3.1	-306.7	-135.6	0.56
Whitefish	2012	TwoSource	-20	-626.5	-610.6	0.03
Yako	2012	TwoSource	-1.43	-772.5	N.A.	
7th	2013	TwoSource	-1.2	-709.5	-518.2	0.27
Hansen	2013	TwoSource	-1.32	-520.3	N.A.	
Hidden	2013	TwoSource	-1.7	-442	-415.3	0.06
Whitefish	2013	TwoSource	-1.5	-779.6	-515.5	0.34
Yuno	2010	TwoSource	-2.2	-674	-671	0.00
Berm	2011	TwoSource	-0.77	-1704	-1608	0.06
Pick	2011	TwoSource	-2.8	-1277	-1275	0.00

Table 5.3. Comparison of the effects of chemical and physical variables on the temperature sensitivity of stream ER. Models only include data from 2011-2013 because no DOC or C:N data were available in 2010. 'DOC' = dissolved organic carbon, 'C:N' = carbon to nitrogen ratio of dissolved organic matter, 'AvgT' = averaged summer stream temperature, 'RangeT' = range of summer stream temperature, 'TP' = total phosphorus, 'TN' = total nitrogen, 'and Chl' = benthic chlorophyll *a*.

Model	**N**	**k**	**AIC**	**AICc**	**DAICc**
E~C:N+AvgT+TP	40	6	13.5	15.3	0.0
E~C.N+AvgT	40	5	14.2	15.4	0.1
E~C:N+TP	40	5	15.3	16.5	1.2
E~C:N+AvgT+TP+Chl	40	7	14.1	16.7	1.4
E~C:N	40	4	17.2	17.9	2.6
E~DOC+C:N+AvgT+TP+Chl	40	8	15.8	19.4	4.1
E~DOC+C:N+AvgT+RangeT+TP+Chl	40	9	17.1	21.9	6.6
E~AvgT	40	4	23.3	24.0	8.7
E~DOC+C:N+AvgT+RangeT+TP+TN+Chl	40	10	18.6	24.8	9.5
E~DOC	40	4	25.6	26.3	11.0
E~TP	40	4	31.9	32.6	17.3
E~RangeT	40	4	33.9	34.6	19.3
E~AvgTN	40	4	39.9	40.6	25.3

Table 5.4. Stream environmental characteristics that influenced the value of temperature sensitivity for streams in each year considered independently. Abbreviations same as above.

Model - 2010	**N**	**k**	**AIC**	**AICc**	**ΔAICc**
E~AvgChl	14	3	26.1	27.1	0
E~AvgT+AvgChl	14	4	25.5	27.7	0.6
E~AvgT+AvgChl+Q	14	5	26.4	30.4	3.3
E~AvgT+RangeT+AvgChl+Q	14	6	27.4	34.1	7.0
E~AvgT+RangeT+AvgTN+AvgChl+Q	14	7	29.3	39.8	12.7
E~AvgT+RangeT+AvgTP+AvgTN+AvgChl+Q	14	8	31.2	47.2	20.1
Model - 2011					
E~C:N+AvgTN+AvgChl	13	5	-11.3	-4.6	0.0
E~C:N+AvgChl	13	4	-7.9	-4.5	0.2
E~C:N+AvgT+AvgTN+AvgChl	13	6	-11.3	0.7	5.3
E~C:N	13	3	3.8	5.3	9.9
E~C:N+AvgT+RangeT+AvgTN+AvgChl	13	7	-11.4	9.6	14.2
E~AvgChl	13	3	13.2	14.7	19.3
E~C:N+AvgT+RangeT+AvgTN+AvgChl+Q	13	8	-12.6	24.7	29.4
E~C:N+AvgT+RangeT+AvgTP+AvgTN+AvgChl+Q	13	9	-13.3	58.7	63.3
E~DOC+C:N+AvgT+RangeT+AvgTP+AvgTN+AvgChl+Q	13	10	-11.6	168.4	173.0
Model - 2012					
E~DOC+AvgTP	14	4	-8.1	-5.4	0
E~DOC+C:N+AvgTP	14	5	-6.9	-1.9	3.5
E~AvgTP	14	3	0.26	1.5	6.9
E~DOC+C:N+AvgTP+AvgChl	14	6	-5.2	3.4	8.8
E~DOC	14	3	3.9	5.1	10.5
E~DOC+C:N+AvgTP+AvgTN+AvgChl	14	7	-3.4	10.6	16.0
E~DOC+C:N+RangeT+AvgTP+AvgTN+AvgChl	14	8	-1.8	20.6	26.0
E~DOC+C:N+RangeT+AvgTP+AvgTN+AvgChl+Q	14	9	0.19	36.2	41.6
E~DOC+C:N+AvgT+RangeT+AvgTP+AvgTN+AvgChl+Q	14	10	2.2	62.2	67.6
Model - 2013					
E~C:N+AvgT+Chl	12	5	-3.14	2.6	0
E~AvgT	12	3	1.71	3.0	0.5
E~C:N+AvgT	12	4	0.78	3.8	1.2
E~C:N+AvgT+Q+Chl	12	6	-1.14	8.9	6.3
E~C:N	12	3	9.2	10.5	8.0
E~Chl	12	3	10.12	11.5	8.9
E~C:N+AvgT+RangeT+Q+Chl	12	7	-2.36	14.4	11.9
E~DOC+C:N+AvgT+RangeT+Q+Chl	12	8	-0.77	27.2	24.7
E~DOC+C:N+AvgT+RangeT+AvgTN+Q+Chl	12	9	0.78	48.8	46.2
E~DOC+C:N+AvgT+RangeT+AvgTP+AvgTN+Q+Chl	12	10	2.48	92.5	89.9

Table 5.5. Estimated E_t values for broader dataset of streams from Wood River basin (n = 53) for which watershed slope data were available. "Unweighted" estimates were generated simply from the watershed slope value, whereas "flow-weighted" estimates were weighted by each stream's seasonal discharge and "DOC-weighted" estimates were weighted by the DOC load.

Model	**Average E - unweighted**	**Average E – flow-weighted**	**Average E – DOC-weighted**
Experimental Approach (Jankowski et al. 2014, n = 11)	0.83	0.82	0.82
E~Slope – All years in this study	0.25	0.24	0.18
E~Slope - 2010	0.28	0.26	0.17
E~Slope – 2011	0.25	0.22	0.15
E~Slope – 2012	0.06	0.05	0.01
E~Slope – 2013	0.25	0.24	0.21

Figure 5.1.

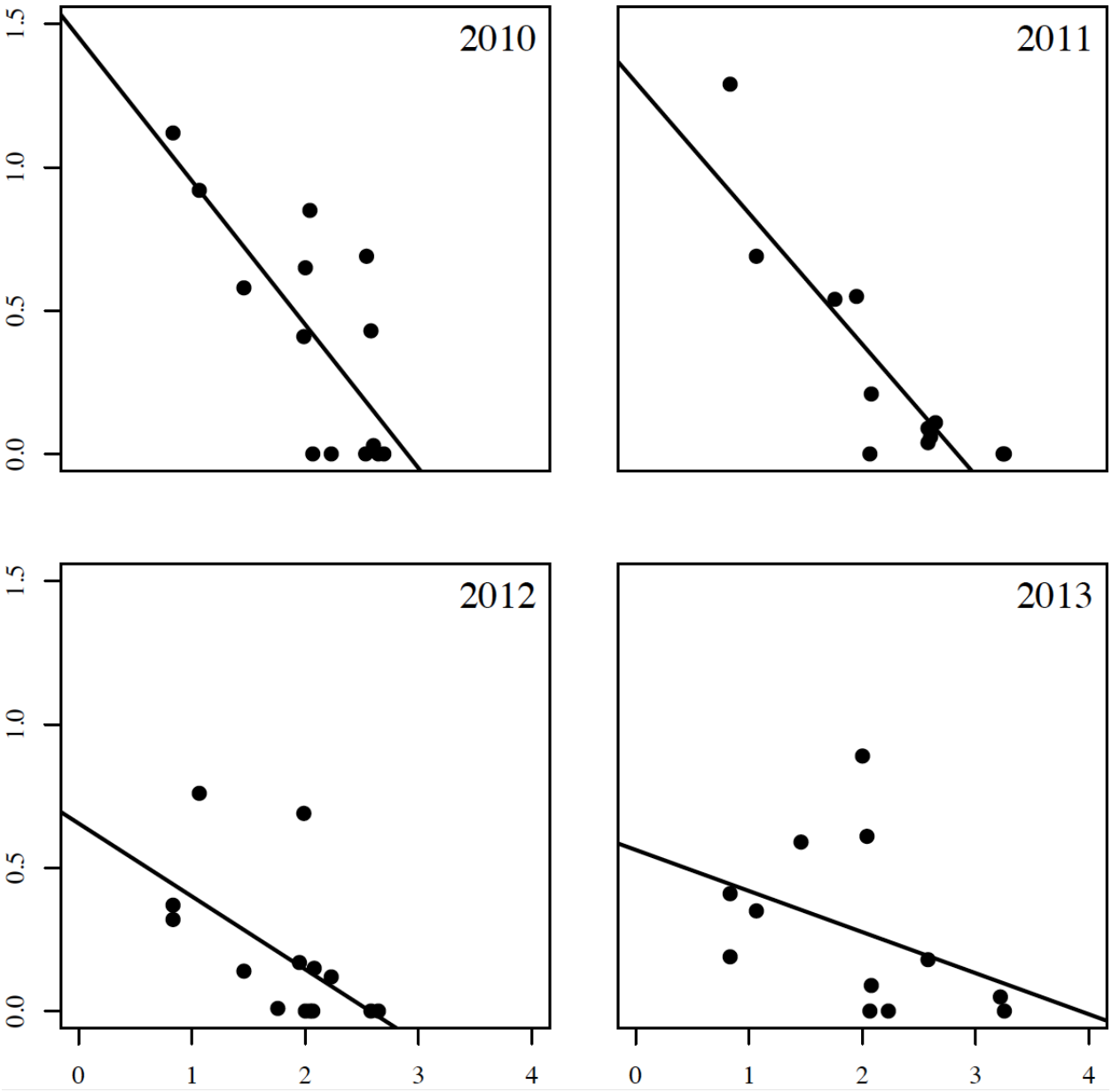


Figure 5.2.

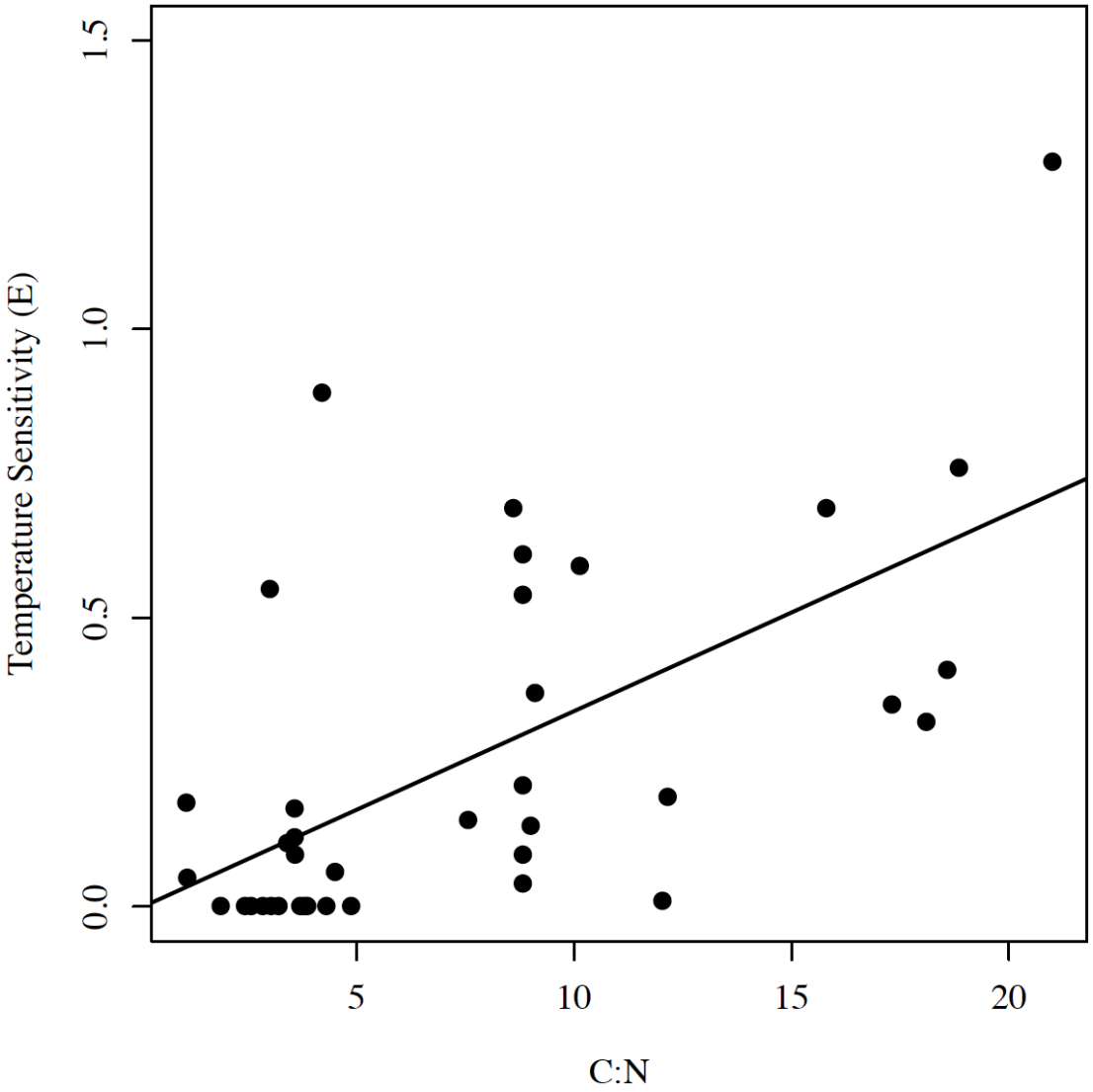


Figure 5.3.

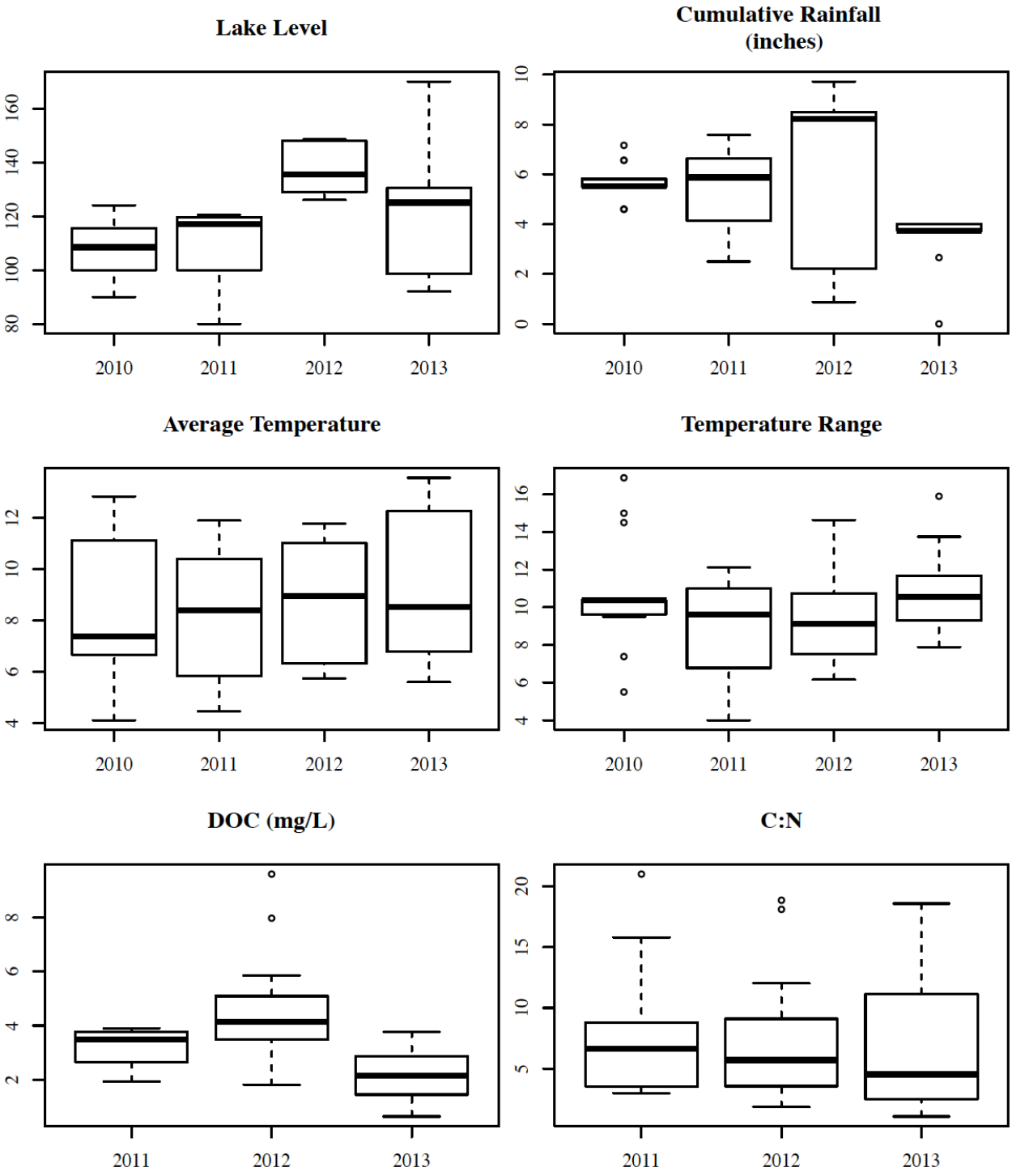

Lake Level
80
100
120
140
160
2010
2011
2012
2013
Cumulative Rainfall
(inches)
0
2
4
6
8
10
2010
2011
2012
2013
Average Temperature
4
6
8
10
12
2010
2011
2012
2013
Temperature Range
4
6
8
10
12
14
16
2010
2011
2012
2013
DOC (mg/L)
2
4
6
8
2011
2012
2013
C:N
5
10
15
20
2011
2012
2013

Figure 5.4.

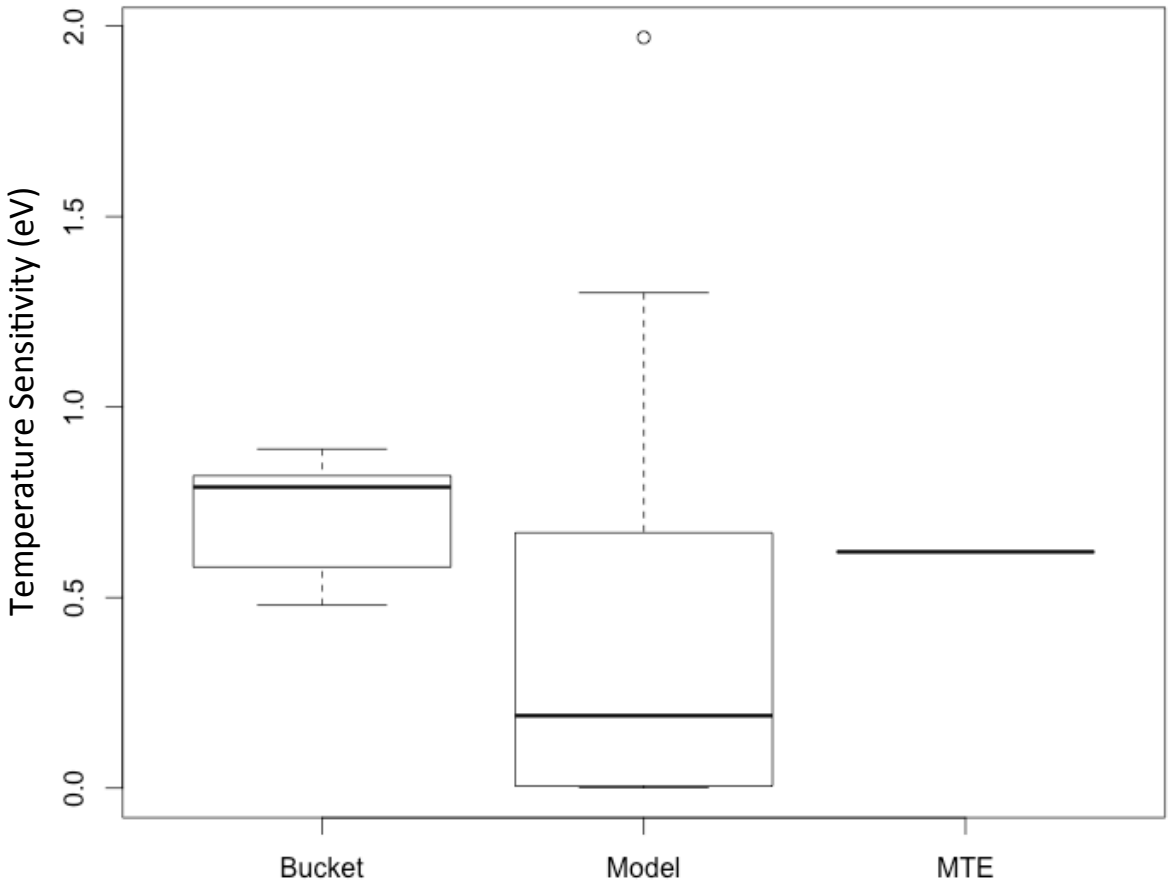
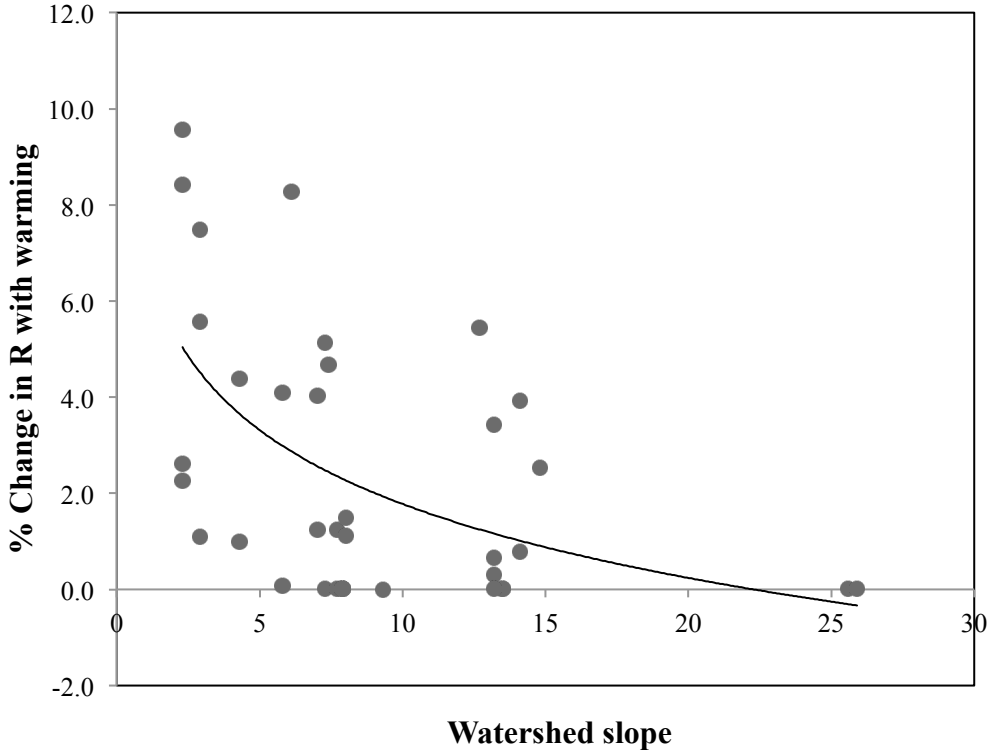


Figure 5.5


12.0
10.0
8.0
6.0
4.0
2.0
0.0
-2.0
% Change in R with warming
0
5
10
15
20
25
30
Watershed slope

Supplemental Section 5.1: Model Validation

We performed a series of simulations to evaluate how well BaMM performed in estimating temperature sensitivity across a range of datasets. We used the model to generate several time-series of dissolved oxygen data with known values of E and then compared the median of the posteriors to the input value. We evaluated how well the model estimated temperature sensitivity under a variety of conditions including varying the magnitude of daily temperature ranges (1-8 °C range), daily swing in oxygen concentration, and respiration (small vs. large CR).

We first tested the effect of varying only the daily range of temperature and kept all other parameters constant. We set the other parameters at values that generated a large daily signal in oxygen dynamics. We found that our ability to estimate E increased as the daily variation in temperature increased (Figure 1), but that if the daily temperature range was > 2°, the 90% credible interval was narrow (+/- 3% of estimated value). All but two streams in our dataset had daily temperature ranges > 2°, thus we feel confident that the temperature range was not a factor that influenced the estimate of E in our dataset.

Second, we tested the effect of the daily change in oxygen concentration on our ability to estimate E. We manipulated the magnitude of the daily swing in oxygen by adjusting the magnitude of gas exchange (changing k_{20}). We set Eb = 0.32 and left all other parameters constant. We simulated all datasets with a 4° temperature range, and evaluated a range of k_{20} from 0.12 – 0.72 m h^{-1}. Varying the magnitude of k_{20} up to a value of 0.53 m h^{-1} did not influence the estimate of E: in all cases we estimated a value of 0.33 eV. The greatest value of k_{20} estimated for our dataset was 0.52 m h^{-1}. However, the scenario in which we set k at 0.72 did not allow us to estimate any of the model parameters accurately.

Lastly, we evaluated the joint effect of the magnitude of temperature change and the value of R (P:R ratios above and below 1) on our ability to estimate E. The largest deviations from our expected value occurred when we gave the model a small daily temperature range (1°) and a small temperature sensitivity value (E = 0.32 eV). The magnitude of R did not seem to influence the estimation on its own, however.

Table S5.1. Effect of changing k on ability to estimate E.

k value	E Input	k estimated	Daily ΔO_2	E estimated	Notes
0.12	0.32	0.12	1.43	0.326	
0.19	0.32	0.19	1.16	0.317	
0.29	0.32	0.29	1.04	0.336	
0.41	0.32	0.42	0.96	0.326	P & R parameters slightly off
0.53	0.32	0.56	0.95	0.329	P & R parameters slightly off
0.72	0.32	0.24	0.93	3.14	P & R parameters off

Table S5.2. Effects of temperature range, magnitude of E and P:R ratio on model ability to estimate E. Largest error occurs where temp range and expected E are small.

Temp Range (°C)	E input	E estimated	Expected R	Expected P:R	% Difference from Expected E
1	0.32	0.03	580	1.5	-90.6
6	0.32	0.30	580	1.5	-14.0
1	1	0.86	296	2.9	-5.0
6	1	1.0	323	2.7	1.0
1	0.32	0.02	1740	0.5	-94.0
6	0.32	0.32	1740	0.5	-16.0
1	1	0.84	1775	0.5	0.0
6	1	1.0	1936	0.5	-0.3

Figure S5.1.

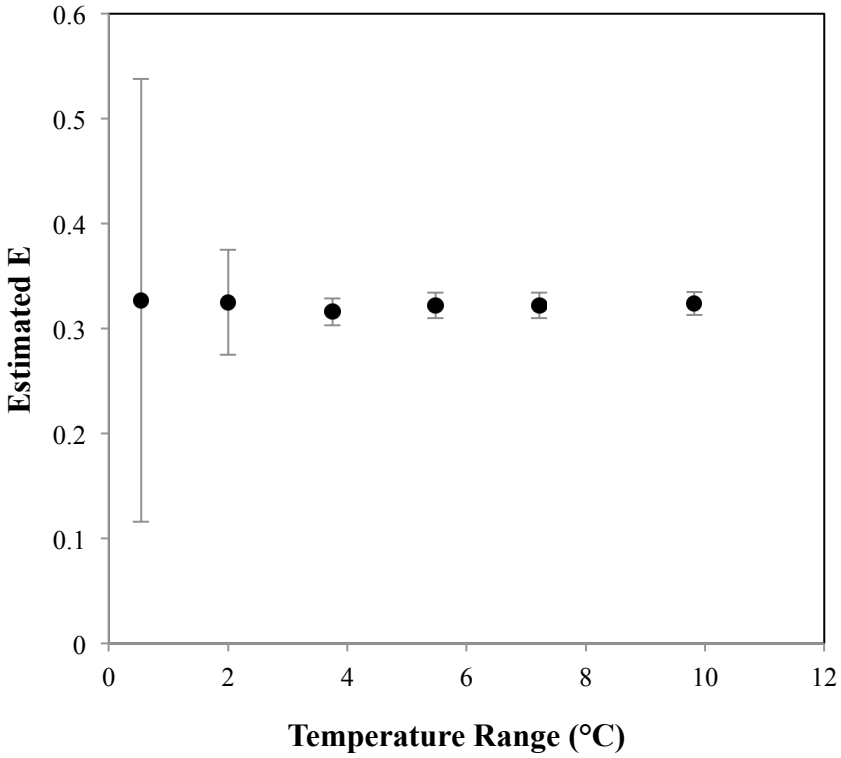
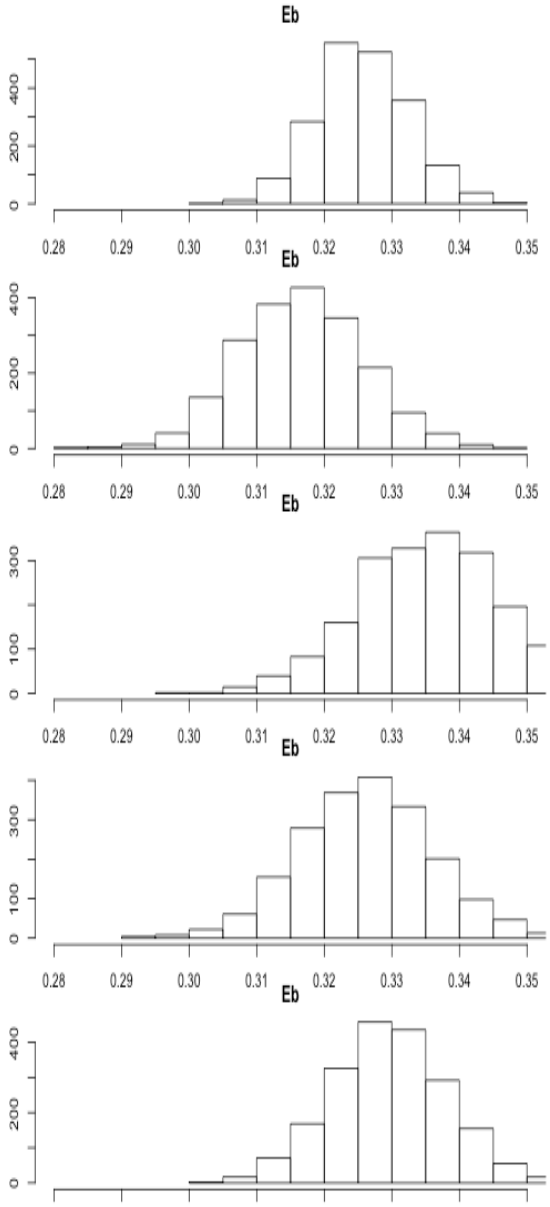


Figure S5.2. Confidence in Eb values estimated under increasing magnitude of gas exchange, which would decrease the daily variation in oxygen concentration (as shown in Table 1 above).



REFERENCES

Acuña, V., A. Wolf, U. Uehlinger, and K. Tockner. 2008. Temperature dependence of stream benthic respiration in an Alpine river network under global warming. Freshwater Biology **53**: 2076-2088.

Ågren, A., I. Buffam, K. Bishop, and H. Laudon. 2010. Modeling stream dissolved organic carbon concentrations during spring flood in the boreal forest: a simple empirical approach for regional predictions. Journal of Geophysical Research 115: doi:10.1029/2009JG001013.

Allen, A. P., J. F. Gillooly, and J. H. Brown. 2005. Linking the global carbon cycle to individual metabolism. Functional Ecology **19**:202-213.

Aufdenkampe, A. K., E. Mayorga, P. A. Raymond, J. M. Melack, S. C. Doney, S. R. Alin, R. E. Aalto, and K. Yoo. 2011. Riverine coupling of biogeochemical cycles between land, oceans, and atmosphere. Frontiers in Ecology and the Environment **9**:53-60.

Battin, T. J., L. A. Kaplan, S. Findlay, C. S. Hopkinson, E. Marti, A. I. Packman, J. D. Newbold, and F. Sabater. 2008. Biophysical controls on organic carbon fluxes in fluvial networks. Nature Geoscience **1**:95-100.

Berggren, M., H. Laudon, A. Jonsson, and M. Jansson. 2010. Nutrient Constraints on Metabolism Affect the Temperature Regulation of Aquatic Bacterial Growth Efficiency. Microbial Ecology **60**:894-902.

Berghuijs, W. R., R. A. Woods, M. Hrachowitz. 2014. A precipitation shift from snow towards rain leads to a decrease in streamflow. Nature Climate Change 4: 583-586. doi:10.1038/nclimate2246

Bernot, M. J., D. J. Sobota, R. O. Hall, P. J. Mulholland, W. K. Dodds, J. R. Webster, J. L. Tank, L. R. Ashkenas, L. W. Cooper, C. N. Dahm, S. V. Gregory, N. B. Grimm, S. K.

Hamilton, S. L. Johnson, W. H. McDowell, J. L. Meyer, B. Peterson, G. C. Poole, H. M. Valett, C. Arango, J. J. Beaulieu, A. J. Burgin, C. Crenshaw, A. M. Helton, L. Johnson, J. Merriam, B. R. Niederlehner, J. M. O'Brien, J. D. Potter, R. W. Sheibley, S. M. Thomas, and K. Wilson. 2010. Inter-regional comparison of land-use effects on stream metabolism. Freshwater Biology **55**:1874-1890.

Bosatta, E., and G. Ågren. 1999. Soil organic matter quality interpreted thermodynamically. Soil Biology and Biochemistry 31:1889–1891.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, and G. B. West. 2004. Toward a metabolic theory of ecology. Ecology **85**:1771-1789.

Conant, R. T., M. G. Ryan, G. I. Ågren, H. E. Birge, E. A. Davidson, P. E. Eliasson, S. E. Evans, S. D. Frey, C. P. Giardina, F. M. Hopkins, R. Hyvönen, M. U. F. Kirschbaum, J. M. Lavallee, J. Leifeld, W. J. Parton, J. Megan Steinweg, M. D. Wallenstein, J. Å. Martin Wetterstedt, and M. A. Bradford. 2011. Temperature and soil organic matter decomposition rates - synthesis of current knowledge and a way forward. Global Change Biology **17**:3392-3404.

Davidson, E. A. and I. A. Janssens. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature **440**:165-173.

Davidson, E. A., S. Samanta, S. S. Caramori And K. Savage. 2012. The Dual Arrhenius and Michaelis-Menten kinetics model for decomposition of soil organic matter at hourly to seasonal time scales. Global Change Biology 18: 371-384. doi: 10.1111/j.1365-2486.2011.02546.x.

Demars, B. O. L., J. R. Manson, J. S. Olafsson, G. M. Gislason, R. Gudmundsdottir, G. Woodward, J. Reiss, D. E. Pichler, J. J. Rasmussen, and N. Friberg. 2011. Temperature and the metabolic balance of streams. Freshwater Biology **56**:1106-1121.

del Giorgio, P. A., J. J. Cole, A. Cimbleris. 1997. Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems. Nature 385(9): 148-151.

Finlay, J. C., J. M. Hood, M. P. Limm, M. E. Power, J. D. Schade, and J. R. Welter. 2011. Light-mediated thresholds in stream-water nutrient composition in a river network. Ecology 92(1): 140-150.

Fournier, D.A., H.J. Skaug, J. Ancheta, J. Ianelli, A. Magnusson, M.N. Maunder, A. Nielsen, and J. Sibert. 2012. AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. Optim. Methods Softw. 27:233-249.

Gillooly, J. F., J. H. Brown, G. B. West, V. M. Savage, and E. L. Charnov. 2001. Effects of size and temperature on metabolic rate. Science **293**:2248-2251.

Hilderbrand, R. H. and M. T. Kashiwagi. 2014. Regional and local scale modeling of stream temperatures and spatio-temporal variation in thermal sensitivities. Environmental Management 54(1): 14-22.

Holtgrieve, G. W., D. E. Schindler, T. A. Branch, and Z. T. A'Mar. 2010. Simultaneous quantification of aquatic ecosystem metabolism and reaeration using a Bayesian statistical model of oxygen dynamics. Limnology and Oceanography **55**:1047-1063.

Jankowski, K.J., D.E. Schindler, and P.J. Lisi. Temperature sensitivity of community respiration rates in streams is associated with watershed geomorphic features. Ecology. *In press.* http://dx.doi.org/10.1890/14-0608.1

Karhu, K., M. D. Auffret, J. A. J. Dungait, D. W. Hopkins, J. I. Prosser, B. K. Singh, J. Subke, P. A.Wookey, G. Ågren, M. Sebastia, F. Gouriveau, G. Bergkvist, P. Meir A. T. Nottingham, N. Salinas & I. P. Hartley. 2014. Temperature sensitivity of soil respiration rates enhanced by microbial community response. Nature 513: doi:10.1038/nature13604

Kling, G. W., G. W. Kipphut, and M. C. Miller. 1991. Arctic lakes and streams as gas conduits to the atmosphere - implications for tundra carbon budgets. Science **251**:298-301.

Laudon, H., M. Berggren, A. Ågren, I. Buffam, K. Bishop, T. Grabs, M. Jansson, and S. Köhler. 2011. Patterns and dynamics of dissolved organic carbon (DOC) in boreal streams: The role of processes, connectivity and scaling. Ecosystems 14:880-893.

Le Que´re´, C. et al. 2009. Trends in the sources and sinks of carbon dioxide. Nature Geosci. 2, 831–836.

Lisi, P. J., D. E. Schindler, K. T. Bentley, and G. R. Pess. 2013. Association between geomorphic attributes of watersheds, water temperature, and salmon spawn timing in Alaskan streams. Geomorphology 185:78–86.

Lopez-Urrutia, A. and X. A. G. Moran. 2007. Resource limitation of bacterial production distorts the temperature dependence of oceanic carbon cycling. Ecology **88**:817-822.

Luce, Charles; Staab, Brian; Kramer, Marc; Wenger, Seth; Isaak, Dan; McConnell, Callie. 2014. Sensitivity of summer stream temperatures to climate variability in the Pacific Northwest. Water Resources Research. doi: 10.1002/2013WR014329

Marotta, H., L. Pinho, C. Gudasz, D. Bastviken, L. J. Tranvik, and A. Enrich-Prast. 2014. Greenhouse gas production in low-latitude lake sediments responds strongly to warming. Nature Climate Change 4: doi: 10.1038/nclimate2222.

Maurer, E.P., L. Brekke, T. Pruitt, and P.B. Duffy. 2007. Fine-resolution climate projections enhance climate change impact studies. Eos Transcations AGU 88: 504.

McCluney, K. E., N. L. Poff, M. A. Palmer, J. H. Thorp, G. C Poole, B. S Williams, M. R Williams, and J. S Baron. 2014. Riverine macrosystems ecology: sensitivity, resistance, and resilience of whole river basins with human alterations. Frontiers in Ecology and the Environment 12(1): 48-58.

McGuire, K. J., C. E. Torgersen, G. E. Likens, D. C. Busoe, W. H. Lowe, and S. W. Bailey. 2014. Network analysis reveals multiscale control on streamwater chemistry. Proceedings of the National Academy of Sciences USA. www.pnas.org/cgi/doi/10.1073/pnas.1404820111

Monteith, D. T., J. L. Stoddard, C. D. Evans, H. A. de Wit, M. Forsius, T. Høgåsen, A. Wilander, B. L. Skjelkvåle, D. S. Jeffries, J. Vuorenmaa, B. Keller, J. Kopácek, and J Vesely. 2007. Dissolved organic carbon trends resulting form changes in atmospheric chemistry. Nature 450: 537-540.

Montgomery, D. R. 1999. Process Domains and the River Continuum. Journal of the American Water Resources Association **35**:397-410.

Perkins, D. M., G. Yvon-Durocher, B.O.L. Demars, J. Reiss, D.E. Pichler, N. Friberg, M. Trimmer, G. Woodward. 2012. Consistent temperature dependence of respiration across systems contrasting in thermal history. Global Change Biology **18**:1300-1311.

Plummer, M., N. Best, K. Cowles, and K. Vines. 2006. CODA: Convergence diagnosis and output analysis for MCMC. R News 6: 7–11.

Raymond, P.A., J. Hartmann, R. Lauerwald, S. Sobek, C. McDonald, M. Hoover, D. Butman, R. Striegl, E. Mayorga, C. Humborg, P. Kortelainen, H. Dürr, M. Meybeck, P. Ciais, P. Guth. 2013. Global carbon dioxide emissions from inland waters. Nature 503: 355-359.

Richey, J. E., J. M. Melack, A. K. Aufdenkampe, V. M. Ballester, and L. L. Hess. 2002. Outgassing from Amazonian rivers and wetlands as a large tropical source of atmospheric CO2. Nature **416**:617-620.

Sadro, S., J.M. Melack, and S. MacIntyre. 2011. Spatial and temporal variability in the ecosystem metabolism of a high-elevation lake: Integrating benthic and pelagic habitats. Ecosystems 14: 1123-1140.

Sadro, S., G.W. Holtgrieve, C.T. Solomon, and G.R. Koch. 2014. Widespread variability in overnight patterns of ecosystem respiration linked to gradients in dissolved organic matter, residence time, and productivity in a global set of lakes. Limnology and Oceanography. 59(5): 1666-1678.

Sobek, S., L. J. Tranvik, and J. J. Cole. 2005. Temperature independence of carbon dioxide supersaturation in global lakes. Global Biogeochemical Cycles **19**.

Soranno, P. A., K. S Cheruvelil, E. G Bissell, M. T Bremigan, J. A Downing, C. E Fergus, C. T Filstrup, E. N Henry, N. R Lottig, E. H Stanley, C. A Stow, P-N Tan, T. Wagner, and K. E Webster. Cross-scale interactions: quantifying multiscaled cause effect relationships in macrosystems. Frontiers and Ecology and the Environment 12(1): 65–73. doi:10.1890/120366

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. River Continuum Concept. Canadian Journal of Fisheries and Aquatic Sciences **37**:130-137.

Wallin, M., I. Buffam, M. Oquist, H. Laudon, and K. Bishop. 2010. Temporal and spatial variability of dissolved inorganic carbon in a boreal stream network: Concentrations and downstream fluxes. Journal of Geophysical Research-Biogeosciences **115**.

Widder, S., K. Besemer, G. A. Singer, S. Ceolae, E. Bertuzzof, C. Quinceg, W. T. Sloang, A. Rinaldof, and T. J. Battin. 2014. Fluvial network organization imprints on microbial co-occurrence networks. Proceedings of the National Academy of Sciences USA 111(35): 12799-12804. www.pnas.org/cgi/doi/10.1073/pnas.1411723111

Ylla, I., A. M. Romaní, and S. Sabater. 2012. Labile and recalcitrant organic matter utilization by river biofilm under increasing water temperature. Microbial ecology 64:593–604.

Yvon-Durocher, G., A. P. Allen, D. Bastviken, R. Conrad, C. Gudasz, A. St-Pierre, N. Thanh-Duc & P. A. del Giorgio. 2014. Methane fluxes show consistent temperature dependence across microbial to ecosystem scales. Nature 507: 488-496. doi:10.1038/nature13164

Yvon-Durocher, G., J. M. Caffrey, A. Cescatti, M. Dossena, P. del Giorgio, J. M. Gasol, J. M. Montoya, J. Pumpanen, P. A. Staehr, M. Trimmer, G. Woodward, and A. P. Allen. 2012. Reconciling the temperature dependence of respiration across timescales and ecosystem types. Nature **487**:472-476.

Yvon-Durocher, G., J. I. Jones, M. Trimmer, G. Woodward, and J. M. Montoya. 2010. Warming alters the metabolic balance of ecosystems. Philosophical Transactions of the Royal Society B-Biological Sciences **365**:2117-2126.

Zarnetske, J.P., M.N. Gooseff, W.B. Bowden, M.J. Greenwald, T.R. Brosten, J.H. Bradford, and J.P. McNamara. 2008. Influence of morphology and permafrost dynamics on hyporheic exchange in arctic headwater streams under warming climate conditions. Geophysical Research Letters 35: L02501, doi:10.1029/2007GL032049.

Appendix 1. Acknowledgements and Collaborators

Chapter 2:

Co-authors: Daniel Schindler and Gordon Holtgrieve

Acknowledgements: We thank two anonymous reviewers for their thoughtful comments on the original manuscript, and Mark Scheuerell, Mike Richlen, Anna Coogan, and Arni Litt for help with field sampling and lab processing of samples. Lucy Flynn helped develop the Bayesian component of our analyses. Mike Brett and Peter Kiffney graciously provided unpublished data on stream chemistry. This work was supported by funding from the University of Washington Royalty Research Fund, the Andrew Mellon Foundation, and the H. Mason Keeler Endowment.

Chapter 3:

Co-authors: Daniel Schindler and Claire Horner-Devine

Acknowledgements: The authors would like to acknowledge M. Dyen, H. Bekris, and A. Coogan for extensive help with field data collection and laboratory analyses. We are grateful to R. Lange, J. Griffiths, J. Armstrong and two anonymous reviewers for helpful reviews on the manuscript.

Chapter 4:

Co-authors: Daniel Schindler and Peter Lisi

Acknowledgements: We thank two anonymous reviewers, Gordon Holtgrieve, and Jennifer Griffiths for helpful reviews of this manuscript. Funding was provided by EPA STAR and ARCS Foundation fellowships to K. Jankowski; NSF and the Bullitt Foundation to D.E. Schindler; and the UW Alaska Salmon Program. We thank the Wood-Tikchik State Park for enabling our research.

Chapter 5:

Co-authors: Daniel Schindler

Acknowledgements: We thank Gordon Holtgrieve and Mike Brett for helpful reviews on this

manuscript. Teresa Z'Amar provided technical assistance with updating and implementing the model. Funding was provided by EPA STAR and ARCS Foundation fellowships to K. Jankowski; NSF and the Bullitt Foundation to D.E. Schindler; and the UW Alaska Salmon Program. We thank the Wood-Tikchik State Park for enabling our research.