

MULTIPLE STRESSOR RESPONSES OF STREAM BENTHOS TO NUTRIENT ENRICHMENT AND INORGANIC SEDIMENTATION

by

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DEDICATION

To Rose, Taryn, and Afton

ABSTRACT

There is a need to investigate freshwater ecosystem degradation as a function of multiple, often coinciding factors (*stressors*). Artificial stream systems known as *mesocosms* provide a useful means of disentangling the contributions of individual stressors acting in a multiple stressor environment while also investigating interactions between these factors. This thesis examines the impacts of locally relevant levels of nutrient loading and inorganic sedimentation on benthic invertebrate assemblages characteristic of northern temperate streams. Experimental manipulations were performed using a mesocosm system consisting of 96 self-contained artificial streams. Key questions addressed included: (1) what are the individual contributions of sedimentation and nutrient loading when simultaneously acting on benthic environments; and (2) do multiple-stressor impacts simply match the effect of the most deleterious stressor or are stressors worse in combination than alone? Complex multiple-stressor relationships were observed between nutrients and fine sediment, and combined effects could not be inferred from information on single-stressor effects. Several individual invertebrate taxa, such as Lepidostomatidae caddisflies, responded positively to additions of nutrients and fine sediment, although the apparent subsidy from single factors was often suppressed under multiple-stressor conditions. Sediment functioned exclusively as a habitat variable, and the effects on individual taxa were dependant on their specific habitat requirements (niches). Nutrients drove shifts in periphytic algal composition, from low-profile diatom dominated communities to stands of high-profile diatoms with an overstory of mostly filamentous green algae. Moderate nutrient enrichment was related to increased organism density, particularly among herbivores, although densities of several taxa declined with high enrichment, especially when coupled with sediment addition. This suggests that in highly enriched systems the food resource subsidy is confounded by habitat

related stress, as excessive algal accumulation smothers the substrate and clogs interstitial spaces. Therefore, in highly impacted streams stressor mechanism overlap is probable, as both algal accumulation and sedimentation contribute to deterioration of benthic/hyporheic habitat. Given that these stressors strongly co-vary along agricultural land use gradients, it should be anticipated that with increased agricultural intensity the cooperative action of nutrients and sediment will amplify, leading to ecological impacts that far exceed that of either driver individually. Pollution standards and management practices should reflect this precept and the independent management of common non-point source pollutants should be phased out in favour of comprehensive, multiple-stressor strategies.

PREFACE

The following thesis has been prepared in article format. Chapter 1 is an introduction to the thesis and Chapter 3 is a general discussion of the project. The following article is also included:

Chapter 2: **Multiple stressor responses to nutrient enrichment and inorganic sedimentation in stream mesocosms.** The authors on this paper will be J.W. Chase, G.A. Benoy and J.M. Culp.

The above authors are the main contributors to the creation, execution and composition of the included article and are thus represented.

This thesis has been formatted in the Freshwater Biology journal style.

DECLARATION

This thesis has been composed in its entirety by the candidate, and no part of this work has been submitted for any other degree.

Candidate

J.W. Chase

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TABLE OF CONTENTS

DEDICATION	ii
ABSTRACT.....	iii
PREFACE	v
ACKNOWLEDGEMENTS	vi
TABLE OF CONTENTS.....	vii
LIST OF TABLES.....	ix
LIST OF FIGURES.....	x
1 INTRODUCTION.....	1-12
1.1 NON-POINT SOURCE AGRICULTURAL POLLUTION OF TEMPERATE STREAM SYSTEMS	2
1.2 ECOSYSTEM RESPONSE TO MULTIPLE STRESSORS	5
1.3 STRESSOR COVARIANCE.....	5
1.4 CHARACTERIZING STRESSOR RELATIONSHIPS	6
1.4 OBJECTIVE	9
1.5 OVERALL SIGNIFICANCE OF WORK	9
1.6 LITERATURE CITED	10
2 INVESTIGATING MULTIPLE STRESSOR RESPONSES TO NUTRIENT ENRICHMENT AND INORGANIC SEDIMENTATION IN STREAM MESOCOSMS	13-47
2.0 ABSTRACT.....	13
2.1 INTRODUCTION	14
2.2 MATERIALS AND METHODS	16
2.2.1 <i>Experimental Design</i>	16
2.2.2 <i>Establishment of Treatments</i>	17
2.2.3 <i>Response parameters</i>	20
2.2.4 <i>Analytical approach</i>	21
2.3 RESULTS.....	24
2.3.1 <i>Periphytic algae</i>	24
2.3.2 <i>Benthic invertebrate assemblages</i>	27
2.3.3 <i>Univariate taxonomic responses</i>	30
2.3.4 <i>Adult insect emergence</i>	33

2.4	DISCUSSION.....	35
2.4.1	<i>Benthic community shift</i>	35
2.4.2	<i>Adult Insects</i>	39
2.4.3	<i>Conclusions</i>	40
2.5	ACKNOWLEDGEMENTS	41
2.6	LITERATURE CITED	41
3	GENERAL DISCUSSION.....	48-52
3.1	TOWARDS THE DEVELOPMENT OF POLLUTION STANDARDS.....	49
3.2	PERSPECTIVES FOR FUTURE RESEARCH	50
3.2.1	<i>Approaches to multiple stressor investigation</i>	50
3.3	SYNTHESIS	51
3.4	LITERATURE CITED	52
	APPENDIX A - 2010 Pilot Experiment	53
	APPENDIX B - 2011 Experimental Layout	56
	CURRICULUM VITAE	

LIST OF TABLES

CHAPTER 1: INTRODUCTION	1-12
None	
CHAPTER 2: INVESTIGATING MULTIPLE STRESSOR RESPONSES TO NUTRIENT ENRICHMENT AND INORGANIC SEDIMENTATION IN STREAM MESOCOSMS	13-47
Table 2.1. Mean temperature and water chemistry parameters in nutrient treatments measured over duration of the 2011 mesocosm experiment.	20
Table 2.2. Periphytic algae responses in mesocosms treated with combinations of nutrients and deposited fine sediment.	26
Table 2.3. PerMANOVA on Bray-Curtis dissimilarities of benthic invertebrate assemblages in mesocosms treated with combinations of nutrients and deposited fine sediment.	28
Table 2.4. Pair-wise distances between centroids for simple main effects of nutrient and sediment treatments on benthic invertebrate assemblages. Significant multivariate distances differentiated with non-parametric t-tests represented by bold font and asterisks.	29
Table 2.5. Univariate taxonomic responses to Nutrient and Sediment treatments, with significant trends in bold and direction of single factor responses in parentheses.....	31
Table 2.6. Adult insect responses to Nutrient and Sediment treatments, with significant trends in bold and direction of single factor responses in parentheses.	34
CHAPTER 3: GENERAL DISCUSSION.....	48-52
None	
APPENDIX A - 2010 Pilot Experiment	53-55
Table A-1. Non-parametric MANOVA on Bray-Curtis dissimilarities of benthic invertebrate assemblages from mesocosms in the 2010 pilot experiment.	54
APPENDIX B - 2011 Experimental Layout	56-57
None	

LIST OF FIGURES

CHAPTER 1: INTRODUCTION	1-12
Figure 1.1. Predicted response under the null hypothesis for three model frameworks described by Folt et al. (1999), where, for example, single stressors generate 20% and 30% reductions in survival.	8
CHAPTER 2: INVESTIGATING MULTIPLE STRESSOR RESPONSES TO NUTRIENT ENRICHMENT AND INORGANIC SEDIMENTATION IN STREAM MESOCOSMS	13-47
Figure 2.1. Schematic representation of single nutrient treatment, depicting nesting of tables and sediment treatments replicated within each table.	17
Figure 2.2. Mesocosm system: A) schematic diagram showing water delivery; B) modular table containing 8 mesocosm streams; C) pump house with Pulsar metering pumps each supplying a single mesocosm table; and D) individual mesocosm stream with emergence trap removed.	18
Figure 2.3. Biomass (mg cm^{-2}) of diatoms and green algae (mean \pm 1 SE) in nutrient treatments; total chlorophyll-a represented by a dashed line (other algae groups not shown).	26
Figure 2.4. Relative cell density of diatom ecological guilds (mean \pm 1 SE) in nutrient treatments.	27
Figure 2.5. Invertebrate assemblage dispersion (\pm 1 SE) in response to nutrients (Bray-Curtis dissimilarities).	30
Figure 2.6. Densities (\pm 1 SE) of selected macroinvertebrates and EPT in response to Nutrient and Sediment treatment combinations. Significant nutrient trends represented by dark lines, while faded lines represent non-significant trends. Sediment treatments differentiated by Tukey's HSD indicated by asterisks.	33
Figure 2.7. Body lengths of male Baetidae (mean \pm 1 SE) in nutrient and sediment treatments of 2011 mesocosm experiment (inset: photograph indicating portion of body measured).	35
CHAPTER 3: GENERAL DISCUSSION	48-52
Figure 3.1. Conceptual diagram of a regional nutrient standard associated with a critical biological change point (threshold), adjusted according to relevant sedimentation intensity category.	50

APPENDIX A - 2010 Pilot Experiment 53-55

 None

APPENDIX B - 2011 Experimental Layout 56-57

Figure B-1. Layout of nutrient and sediment treatments on mesocosm tables in
 2011 experiment.....57

1 INTRODUCTION

Agriculture is one of the foremost contributing factors to the global decline in ecological integrity of running waters (Allan 2004; Matson, *et al* 1997). Throughout history, human societies have tended to congregate around waterways, resulting in extensive modification of landscapes. Clearing of land for the purposes of crop cultivation and livestock grazing, including direct manipulation of watercourses, has drastically altered natural abiotic and biotic characteristics of streams and rivers (Meyer & Turner 1992). Non-point (diffuse) source pollutants, such as eroded sediments, nutrients, and pesticides, are among the most pervasive and ubiquitous forms of agriculturally-derived threats to fluvial ecosystems (Allan 2004). Stream catchments in North America have been greatly modified for agricultural utility (Naiman & Turner 2000) and Atlantic Canada is no exception.

Though there is an immense body of research examining the impacts of individual threats to aquatic life, the degradation of ecosystems is rarely the result of single stressors (Ling, *et al* 2009). Therefore, a multiple stressor approach to investigating ecosystem decline should be applied. This is especially relevant for research involving agricultural pollution of streams, as the presence of any one diffuse source pollutant tends to be accompanied by others (Matthaei, Piggott & Townsend 2010). Considering the apparent complexity of agriculturally-driven impairment of fluvial environments, there is great value in employing a range of approaches to precisely describe relationships between agricultural practices and biological responses (Culp & Baird 2006). Traditional field-based studies have extensively documented general relationships between agriculture and stream systems, though there has been a marked resistance to assign definitive explanations of causality of specific phenomena (Adams 2003). Laboratory based approaches, including various bioassays and analytical

techniques, have the potential to isolate cause-and-effect relationships in highly controlled environments, although results from these studies have only limited applicability to the characterization of relationships in natural systems (Adams 2003). The use of artificial stream systems provides an attractive middle ground between field and lab studies, allowing for controlled examination of the influence of individual or multiple stressors on entire (or nearly entire) biotic communities in an environment that reflects natural habitats (Culp, Lowell & Cash 2000).

In an attempt to more intimately understand the relationship between New Brunswick's streams and the agricultural landscapes they drain, this project examined the influence of multiple stressors, which typically occur as diffuse source pollutants, on benthic communities. Ten litre, circular artificial stream systems referred to as "mesocosms" were employed whereby community responses to various combinations of sediment and nutrient treatments were monitored. Results from this research were anticipated to reveal new information regarding the contributions of individual drivers as well as interactions between stressors.

1.1 NON-POINT SOURCE AGRICULTURAL POLLUTION OF TEMPERATE STREAM SYSTEMS

Agriculture is the dominant human activity in many temperate watersheds, and diffuse source pollutants, such as eroded sediment and nutrient runoff, are often considered the leading drivers of water quality decline in agricultural streams (Quinn & Stroud 2002).

According to the definition of Wagenhoff et al. (2011), anthropogenic inputs are considered "stressors" when they exceed naturally occurring in-stream variance. Furthermore, "multiple-stressor effect" refers to any case where several stressors are both present and active, and

includes both additive and multiplicative stressor relationships (Townsend, Uhlmann & Matthaei 2008).

Though transportation of soils and sediments is a natural geomorphological process, unnatural proportions of material can be moved in response to anthropogenic activity through direct agricultural runoff, modification and removal of riparian vegetation, and stream channelization (Rabeni, Doisy & Zweig 2005). For stream environments, the implications of greater sediment loading can be loosely partitioned into two components: increased turbidity due to suspended particulate matter and increased sediment deposition and subsequent modification of streambed composition (Henley, *et al* 2000). The ecological repercussions of additional suspended particulate matter include reduction in primary productivity due to limited ultraviolet penetration (Bruton 1985), scouring of benthic habitat (Steinman & McIntire 1990), abrasion and coating of animal respiratory surfaces (Goldes, *et al* 1988) and decreased oxygen availability (Henley, *et al* 2000). The implications of increased deposited sediments are largely related to infilling of interstitial invertebrate habitat and diminished exchange of gases, nutrients and organic material within the hyporheic zone (Rabeni, Doisy & Zweig 2005). Homogenization of surface substrate due to excessive fine sediment deposition has also been associated with depletion of fish spawning habitat (Sutherland, Meyer & Gardiner 2002).

Nutrient enrichment of running waters is another major stressor associated with agriculture. Inorganic nitrogen and phosphorus are the primary nutrients of concern with respect to ecological disruption of streams (eutrophication), although external inputs of organic carbon can also be a significant issue (Dodds 2006). Agricultural soils, which are often naturally high in nutrients, commonly become further enriched through manure production and application of fertilizers (Carpenter, *et al* 1998). Phosphorus and nitrogen (as nitrate) can

then be transported by groundwater or surface runoff, much of which ends up in streams. Accumulated nutrients can also be transported with soils through erosion (Carpenter, *et al* 1998) or released from stream sediments through direct disturbance of streambeds (Schoellhamer 1996). Dissolved nutrients can be quickly assimilated by primary producers, particularly if the added nutrient is of otherwise limited availability to organisms, initiating a bottom-up increase in biomass (subsidy) at multiple trophic levels (Biggs 2000; Hadwen & Bunn 2005). However, nutrient enrichment is known to disproportionately benefit some organisms more than others, with alters community structure and function. In severe cases, the proliferation of autotrophs can lead to oxygen depletion and deleterious modification of important habitat for higher organisms (Carpenter, *et al* 1998; Mainstone & Parr 2002).

Many critical questions about stream ecosystem responses to agricultural influences have yet to be addressed (Ormerod, *et al* 2010). However, there has been a considerable amount of effort made recently to understand agricultural impacts within Canadian watersheds. Between 2004 and 2008 the National Agri-Environmental Standards Initiative (NAESI), a collaborative effort between Environment Canada and Agriculture and Agri-Food Canada, developed environmental performance standards for various pollutants of Canada's agricultural streams (Bowerman, *et al* 2009). The standards put forth were based on biotic community composition observed along pollution intensity gradients in streams and rivers across several agricultural regions; however, they should be considered preliminary tools to establish a framework for current policy decisions and future research. The NAESI overarching synthesis report (Bowerman, *et al* 2009) calls for further validation of the standards developed for nutrient and fine sedimentation levels in agricultural streams, as well as interactive behaviour of these ecological stressors in real-world conditions.

1.2 ECOSYSTEM RESPONSE TO MULTIPLE STRESSORS

A major challenge inherent to modern ecological research is the underrepresentation of multiple-stressor studies, despite the fact that ecological decline tends to be driven by multiple factors (Ormerod, *et al* 2010). Accurate causal diagnoses are vital precursors to sound management of environmental stressors; hence the investigation of multiple-stressor influences on stream biota is of immense value (Bunn, *et al* 2010).

Rigorous studies of multiple interacting stressors have only been near the forefront of ecological research for about a decade (Townsend, Uhlmann & Matthaei 2008). Investigation of the influence of temperature, food availability and toxin exposure on cladoceran zooplankton by Folt *et al.* (1999) is one of the earliest experimental inquiries of multiple-stressor interactions in freshwater biology. Of late, several studies have centered on multifactorial explanation of fluvial ecosystem decline (Adams 2003). Though research on the subject is only beginning, it is generally accepted that ecosystem degradation can result from a plethora of mechanisms and that individual contributions and interactions of agriculturally-derived stressors can vary considerably (Townsend, Uhlmann & Matthaei 2008). Also, combinations of stressors can have additive (Fausch, Baxter & Murakami 2010), synergistic (Townsend, Uhlmann & Matthaei 2008; Folke, *et al* 2004), and even antagonistic (Matthaei, Piggott & Townsend 2010) relationships. Furthermore, spatial and temporal variation of ecosystem responses to multiple stressors has also been revealed (Voeroesmary, *et al* 2010; Molinos & Donohue 2010).

1.3 STRESSOR COVARIANCE

In natural stream systems stressors tend to co-vary, which complicates the interpretation of their effects (Quinn & Stroud 2002; Adams 2003). This is especially common

in agricultural settings where pollutants such as nutrients and sediment are often generated from similar farming practices (Quinn & Stroud 2002). Moreover, the longitudinal element of rivers means that directional (downstream) accumulation of pollutants is an inherent property of fluvial systems. Failure to acknowledge stressor covariance in multiple regression analysis leads to models fraught with (multi)collinearity – correlation among predictor variables – and, ultimately, inaccurate parameter estimations (Quinn & Keough 2002). Though there are ample techniques for both detection and correction of collinearity the manipulation of stressors in factorial mesocosm experiments eliminates natural co-variation. Therefore, any non-independence among predictor variables can be fully attributed to statistical interactions and collinearity becomes a nonissue.

1.4 CHARACTERIZING STRESSOR RELATIONSHIPS

There does not yet exist a universal framework by which to describe relationships between stressors (interactions). Folt et al. (1999) addressed this dilemma, stating that in cases where *“effects of stressors are worse in combination than alone... there are several ways of defining “worse than” which affect interpretation of the effects of multiple stressors.”* They described three models for the combined effect of stressors: the *simple comparative effects*, *additive effects*, and *multiplicative effects* models. Model framework selection is dependent on the particular hypothesis and prior knowledge of stressor mechanisms.

Preceding any investigation of multiple stressor effects it is worth considering what model framework best suits the stressors of concern. Studies that employ multifactorial Analysis of Variance (ANOVA) (Underwood 1997) often need to confirm whether or not the effect of one independent factor is consistent across levels of another. The interaction term of an ANOVA measures the relationship between predictor variables according to an additive null

hypothesis, and therefore conforms to the *additive effects* paradigm. The *additive effects model* tests the hypothesis that the effect of stressors in combination is equal to the sum of the effects of each stressor alone (Figure 1.1). One may then assign relationships such as synergism and antagonism, when the combined response is greater than or less than (respectively) the sum of the individual effects. Though heavily used, the *additive effects model* can lead to misinterpretation of interactions when individual effects are large, due to zero truncation of count data (Quinn & Keough 2002). As highlighted by Pennings (1996), synergisms cannot be detected if the sum of individual effects exceeds 100% (ex: 0% survival of a population of organisms). In contrast, the *multiplicative effects model* tests the null hypothesis that stressors in combination elicit an effect equivalent to the product of each single stressor response (also referred to as multiplicative additivity or log-additivity). The *multiplicative effects model* is particularly applicable when stressor mechanisms are similar, as with combinations of pesticides, when similar physiological or ecological components of a system are acted upon. Synergisms are assigned more often under the multiplicative effects framework than under the more conservative additive effects model. Finally, the *comparative effects model* tests the null hypothesis that stressors in combination generate an effect equal to that of the single worst stressor. The *comparative effects* framework is well suited for cases where a single stressor effect is so large that additional, secondary stressors are expected to have little to no impact.

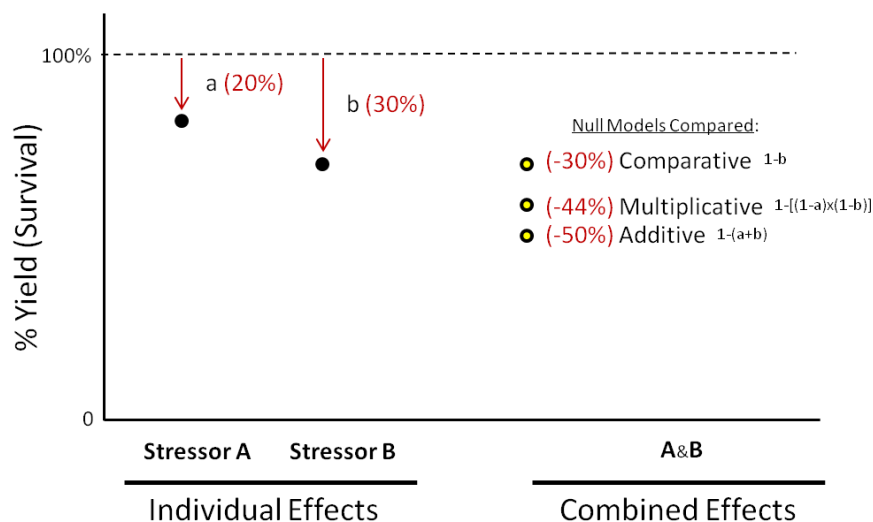


Figure 1.1. Predicted response under the null hypothesis for three model frameworks described by Folt et al. (1999), where, for example, single stressors generate 20% and 30% reductions in survival.

Standards for agriculturally derived pollutants have traditionally focused on single factors, independent of other possible coinciding stressors. It could therefore be argued that, by their nature, management strategies assume that streams are not influenced by multiple stressors simultaneously. Such an assumption would be acceptable if, when stressors happen to co-occur, the combined response is no greater than that generated by the most severe stressor. This project was therefore framed around the hypothesis that multiple stressors in concert would result in a greater decline in ecosystem integrity than that generated by sedimentation or nutrient loading alone. This hypothesis was therefore tested against the null model associated with the comparative effects approach described by Folt et al. (1999) (Figure 1.1).

1.4 OBJECTIVE

This project was specifically intended to expose causal mechanisms underlying changes in benthic community composition in response to combinations of deposited sediment and dissolved nutrients. The general hypothesis that the deleterious ecological effects of sedimentation and nutrient loading are greatest when streams are subjected to both stressors was also addressed. With the use of artificial stream mesocosms, assemblage composition within multiple trophic levels was characterized in communities driven by single and multiple stressor gradients of sediment and nutrients (see chapter 2). Relevant functional attributes of primary producers (growth form; palatability) and macroinvertebrates (motility; susceptibility to smothering; trophic position) were then considered in an attempt to elucidate how changes in habitat and water quality lead to shifts in assemblages of aquatic organisms.

1.5 OVERALL SIGNIFICANCE OF WORK

Globally, water resources are threatened (Voeroesmart, *et al* 2010) and there is an imminent need to establish causality of ecological problems occurring amid a backdrop of numerous stressors. This project increased current understanding of freshwater pollutants that naturally co-vary along agricultural land use gradients. However, these experiments did not merely fill a gap with intuitive findings, but will make a vital contribution to the much larger conquest that is multiple stressor research. The development of emerging approaches for identifying thresholds in multiple-stressor environments, such as the application of mesocosms, will be of immediate benefit to the greater scientific community. Finally, novel insight in to statistical and ecological interactions between variables influential to ecological change has been revealed.

1.6 LITERATURE CITED

Adams S.M. (2003) Establishing causality between environmental stressors and effects on aquatic ecosystems. *Human and Ecological Risk Assessment*, 9, 17-35.

Allan J.D. (2004) Landscapes and riverscapes: The influence of land use on stream ecosystems. *Annual Review of Ecology Evolution and Systematics*, 35, 257-284.

Biggs B.J.F. (2000) Eutrophication of streams and rivers: dissolved nutrient-chlorophyll relationships for benthic algae. *Journal of the North American Benthological Society*, 19, 17-31.

Bowerman M., Maclean L., Villeneuve S. & Roberts E.S. (2009) National Agri-Environmental Standards Initiative Overarching Report. National Agri-Environmental Standards Initiative Synthesis Report No. 1. 1-239.

Bruton M.N. (1985) The Effects of Suspensoids on Fish. *Hydrobiologia*, 125, 221-241.

Bunn S.E., Abal E.G., Smith M.J., Choy S.C., Fellows C.S., Harch B.D., Kennard M.J. & Sheldon F. (2010) Integration of science and monitoring of river ecosystem health to guide investments in catchment protection and rehabilitation. *Freshwater Biology*, 55, 223-240.

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

Culp J.M., Lowell R.B. & Cash K.J. (2000) Integrating mesocosm experiments with field and laboratory studies to generate weight-of-evidence risk assessments for large rivers. *Environmental Toxicology and Chemistry*, 19, 1167-1173.

Culp J.M. & Baird D.J. (2006) Establishing cause-effect relationships in multi-stressor environments. In: *Methods in Stream Ecology* (Eds F.R. Hauer & G.A. Lamberti), pp. 835-854. Elsevier, Oxford, UK.

Dodds W.K. (2006) Eutrophication and trophic state in rivers and streams. *Limnology and Oceanography*, 51, 671-680.

Fausch K.D., Baxter C.V. & Murakami M. (2010) Multiple stressors in north temperate streams: lessons from linked forest-stream ecosystems in northern Japan. *Freshwater Biology*, 55, 120-134.

Folke C., Carpenter S., Walker B., Scheffer M., Elmqvist T., Gunderson L. & Holling C.S. (2004) Regime shifts, resilience, and biodiversity in ecosystem management. *Annual Review of Ecology Evolution and Systematics*, 35, 557-581.

Folt C.L., Chen C.Y., Moore M.V. & Burnaford J. (1999) Synergism and antagonism among multiple stressors. *Limnology and Oceanography*, 44, 864-877.

- Goldes S.A., Ferguson H.W., Moccia R.D. & Daoust P.Y. (1988) Histological Effects of the Inert Suspended Clay Kaolin on the Gills of Juvenile Rainbow-Trout, *Salmo-Gairdneri* Richardson. *Journal of Fish Diseases*, 11, 23-33.
- Henley W.F., Patterson M.A., Neves R.J. & Lemly A.D. (2000) Effects of sedimentation and turbidity on lotic food webs: A concise review for natural resource managers. *Reviews in Fisheries Science*, 8, 125-139.
- Ling S.D., Johnson C.R., Frusher S.D. & Ridgway K.R. (2009) Overfishing reduces resilience of kelp beds to climate-driven catastrophic phase shift. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 22341-22345.
- Mainstone C.P. & Parr W. (2002) Phosphorus in rivers - ecology and management. *Science of the Total Environment*, 282, 25-47.
- Matson P.A., Parton W.J., Power A.G. & Swift M.J. (1997) Agricultural intensification and ecosystem properties. *Science*, 504-509.
- Matthaei C.D., Piggott J.J. & Townsend C.R. (2010) Multiple stressors in agricultural streams: interactions among sediment addition, nutrient enrichment and water abstraction. *Journal of Applied Ecology*, 47, 639-649.
- Meyer W.B. & Turner B.L. (1992) Human-Population Growth and Global Land-use Cover Change. *Annual Review of Ecology and Systematics*, 23, 39-61.
- Molinos J.G. & Donohue I. (2010) Interactions among temporal patterns determine the effects of multiple stressors. *Ecological Applications*, 20, 1794-1800.
- Naiman R.J. & Turner M.G. (2000) A future perspective on North America's freshwater ecosystems. *Ecological Applications*, 10, 958-970.
- Ormerod S.J., Dobson M., Hildrew A.G. & Townsend C.R. (2010) Multiple stressors in freshwater ecosystems. *Freshwater Biology*, 55, 1-4.
- Pennings S.C. (1996) Testing for synergisms between chemical and mineral defenses - A comment. *Ecology*, 77, 1948-1950.
- Quinn G.P. & Keough M.J. (2002) *Experimental design and data analysis for biologists*.
- Quinn J.M. & Stroud M.J. (2002a) Water quality and sediment and nutrient export from New Zealand hill-land catchments of contrasting land use. *New Zealand Journal of Marine and Freshwater Research*, 36, 409-429.
- Quinn J.M. & Stroud M.J. (2002b) Water quality and sediment and nutrient export from New Zealand hill-land catchments of contrasting land use. *New Zealand Journal of Marine and Freshwater Research*, 36, 409-429.

Rabeni C.F., Doisy K.E. & Zweig L.D. (2005) Stream invertebrate community functional responses to deposited sediment. *Aquatic Sciences*, 67, 395-402.

Schoellhamer D.H. (1996) Anthropogenic sediment resuspension mechanisms in a shallow microtidal estuary. *Estuarine Coastal and Shelf Science*, 43, 533-548.

Steinman A.D. & McIntire C.D. (1990) Recovery of Lotic Periphyton Communities After Disturbance. *Environmental management*, 14, 589-604.

Sutherland A.B., Meyer J.L. & Gardiner E.P. (2002) Effects of land cover on sediment regime and fish assemblage structure in four southern Appalachian streams. *Freshwater Biology*, 47, 1791-1805.

Townsend C.R., Uhlmann S.S. & Matthaei C.D. (2008) Individual and combined responses of stream ecosystems to multiple stressors. *Journal of Applied Ecology*, 45, 1810-1819.

Underwood A.J. (1997) *Experiments in ecology: their logical design and interpretation using analysis of variance*. Cambridge University Press, Cambridge, U.K.

Voeroesmarty C.J., McIntyre P.B., Gessner M.O., Dudgeon D., Prusevich A., Green P., Glidden S., Bunn S.E., Sullivan C.A., Liermann C.R. & Davies P.M. (2010) Global threats to human water security and river biodiversity. *Nature*, 467, 555-561.

Wagenhoff A., Townsend C.R., Phillips N. & Matthaei C.D. (2011) Subsidy-stress and multiple-stressor effects along gradients of deposited fine sediment and dissolved nutrients in a regional set of streams and rivers. *Freshwater Biology*, 56, 1916-1936.

2 INVESTIGATING MULTIPLE STRESSOR RESPONSES TO NUTRIENT ENRICHMENT AND INORGANIC SEDIMENTATION IN STREAM MESOCOSMS

2.0 ABSTRACT

There is a present need to investigate freshwater ecosystem degradation as a function of multiple, often coinciding factors (*stressors*). Non-point source impacts on streams in the form of sedimentation and nutrient loading are significant threats to fluvial ecosystem integrity in North American watersheds. This study employed artificial stream systems (*mesocosms*) to disentangle the contributions of dissolved nutrients and deposited fine sediment acting in a multiple stressor environment while also investigating interactions between these factors. 96 circular mesocosms were used in a fully crossed experiment measuring the impact of three substrate compositions (0, 25, and 50 % fines <2 mm) and four nutrient concentrations (20, 40, 80, and 150 $\mu\text{gL}^{-1}[\text{N}]$) on benthic macroinvertebrate assemblages. Permutational MANOVA (PerMANOVA) of whole macroinvertebrate assemblages indicated substantial multiple stressor shifts in structural composition, while univariate modeling revealed that nutrient and sediment subsidies related to single factors were suppressed by an additional stressor. Stressor mechanism overlap was evident at higher treatment levels, as moderate nutrient enrichment increased nutritional resources but high nutrient concentrations lead to substrate smothering by periphytic algae, contributing to habitat degradation originating from inorganic sedimentation. My study is consistent with a growing body of research demonstrating that anthropogenic nutrient loading and sedimentation interact to deteriorate lotic systems beyond levels attributable to either single stressor. Management practices and pollution standards need to incorporate relationships between stressors like nutrients and sedimentation that tightly co-vary in natural settings.

2.1 INTRODUCTION

Excess nutrients and sediments are pervasive, non-point source pollutants of fluvial environments (Allan 2004). Several recent studies have centered on multifactorial explanation of fluvial ecosystem decline (see review by Adams (2003)). Ecosystem degradation can result from a plethora of mechanisms and individual contributions and interactions of stressors can vary considerably (Fausch, Baxter & Murakami 2010; Folke, *et al* 2004; Molinos & Donohue 2010; Townsend, Uhlmann & Matthaei 2008). Previous investigation of the relationship between nutrients and sediment in multiple-stressor contexts has raised serious concern about stressor interactions, meaning that ecological consequences not predictable from knowledge of single-stressor effects alone should be expected (Townsend, *et al* 2008; Lemly 1982; Wagenhoff, *et al* 2011).

Anthropogenic increases in nutrient availability in streams and rivers typically trigger rapid proliferation of algae, which would otherwise be limited by phosphorus and/or nitrogen (Dodds 2006; Biggs 2000). Subsequently, significant structural and functional shifts can be observed at higher trophic levels, with even minor enrichment (Hadwen & Bunn 2005). Though eutrophication is often associated with higher productivity of whole food webs, competition for habitat and oxygen can ultimately result in decreased abundance and diversity of indigenous, sensitive invertebrates and fishes (Carpenter, *et al* 1998; Mainstone & Parr 2002). In contrast, the implications of sediment loading can be loosely partitioned into two components: increased turbidity due to suspended particulate matter and increased sediment deposition and subsequent modification of streambed composition (Henley, *et al* 2000; Jones, *et al* 2011). For most cobble-bottomed streams in temperate biomes, sedimentation acts primarily as a stressor (Niyogi, *et al* 2007), leading to decline in Ephemeroptera, Plecoptera and Trichoptera (EPT) and allowing pollution-tolerant taxa, such as Oligochaeta, to represent a

more substantial proportion of benthic assemblages (Jones, *et al* 2011; Larsen, Vaughan & Ormerod 2009).

Because nonpoint source stream pollutants tend to co-vary along land use gradients (J. M. Quinn & Stroud 2002), traditional field-based studies that involve modeling relationships between biological data and environmental vectors are often unable to correctly describe stressor relationships. *In situ* paired gradients of specific pollutants functioning in both single and multiple stressor conditions occur infrequently and thus experimental manipulations of real streams or artificial habitats have become vital diagnostic practices in multiple stressor research (Townsend, *et al* 2008; J. M. Culp & Baird 2006).

This experiment represents the first inquiry of potential effects of nutrient-sediment interactions within ranges of stressor intensities reported recently in Canadian streams (Benoy, *et al* 2012; Chambers, *et al* 2009; J. M. Culp, *et al* 2009). Artificial streams, hereafter referred to as mesocosms, have proven useful in the assessment of community responses to multiple stressors (Adams 2003; J. M. Culp & Baird 2006; J. M. Culp, Podemski & Cash 2000). This experiment uses mesocosms to simultaneously manipulate replicate streams, in order to characterize the combined effects of sedimentation and nutrient loading on lotic ecosystems. More specifically, the objective was to quantify the degree to which taxonomic shifts at multiple trophic levels over a single stressor gradient are modified by the addition of a second stressor. Because the traditional management strategy of regulating stream pollutants individually carries the implicit assumption that stressors do not co-occur to produce cumulative effects, combined responses more pervasive than that of either individual driver, according to the '*comparative effects*' model outlined by Folt *et al.* (1999), are of particular interest (see Chapter 1). It was anticipated that this would apply to most community metrics sensitive to both nutrients and sediments.

2.2 MATERIALS AND METHODS

2.2.1 *Experimental Design*

The 21-d experiment was carried out from July 20 to August 10, 2011 at Agriculture and Agri-Food Canada's Potato Research Centre (Fredericton, New Brunswick). The facility is completely exposed to ambient environmental temperature, light, and meteorological conditions. Multiple years of water chemistry tests of groundwater available from the on-site well show consistently high levels of phosphorus and low levels of nitrogen, with constant concentrations of approximately $80 \mu\text{g L}^{-1}$ [TP] and $20 \mu\text{g L}^{-1}$ [inorganic N]. Therefore, biological productivity of the mesocosm streams is primarily limited by nitrogen, and all nutrient treatments involve manipulation of nitrogen concentrations only. Three sediment levels (0, 25, and 50 % fines (<2mm)) were replicated within 3 modular "tables" (J. M. Culp & Baird 2006; J. M. Culp, *et al* 2003) for each of 4 nutrient concentrations (20, 40, 80, and $150 \mu\text{g L}^{-1}$ [N]) in a fully crossed, partially-nested design (Figure 2.1). A total of 96 mesocosm streams were in used, evenly distributed across 12 modular tables. The specific aim of this experiment was to examine multiple stressor responses of communities over regionally relevant gradients of nutrients and deposited fine sediment, represented by treatment levels expected to highlight critical thresholds along response profiles.

		Single Nutrient Treatment Example: 20 $\mu\text{g L}^{-1}$		
		Table 1	Table 2	Table 3
Sediment	0% Fines	n = 3	n = 3	n = 3
	25% Fines	n = 3	n = 3	n = 3
	50% Fines	n = 2	n = 2	n = 2

Figure 2.1. Schematic representation of single nutrient treatment, depicting nesting of tables and sediment treatments replicated within each table.

2.2.2 Establishment of Treatments

Individual mesocosm streams consist of 10 litre polyethylene, open-top cylinders. Each stream is equipped with a plastic stirrer mounted to a small electric motor, which is connected to a regulated DC power supply (Circuit-Test, PS-3330), allowing current velocity to be maintained at approximately 7 cm s^{-1} . Untreated groundwater is pumped to large ($\sim 700\text{L}$) polyethylene head tanks, where nutrients (NaNO_3) are constantly added according to desired treatment. Pulsar® diaphragm metering pumps are used to deliver water to reservoir tanks beneath each table, from which it is evenly distributed to each stream via a manifold fed by a small centrifugal pump (March: LC-3CP-MD). When the system is operating the streams are constantly spilling through $400 \mu\text{m}$ Nitex® mesh covers in to a polyethylene collection tray, drained back in to the reservoir tank and re-circulated within the table. Excess water drains from the top of the reservoir tank and is removed from the system. Hydraulic residence time at the tables is approximately 2 hours. Shade cloth is installed over each mesocosm table, reducing light levels by approximately 60% to simulate depth and prevent excessive water temperatures during the day. Further information including photographs of our artificial stream systems can be found in figure 2.2 or in Alexander *et al.* (2008) and Pestana *et al.* (2009).

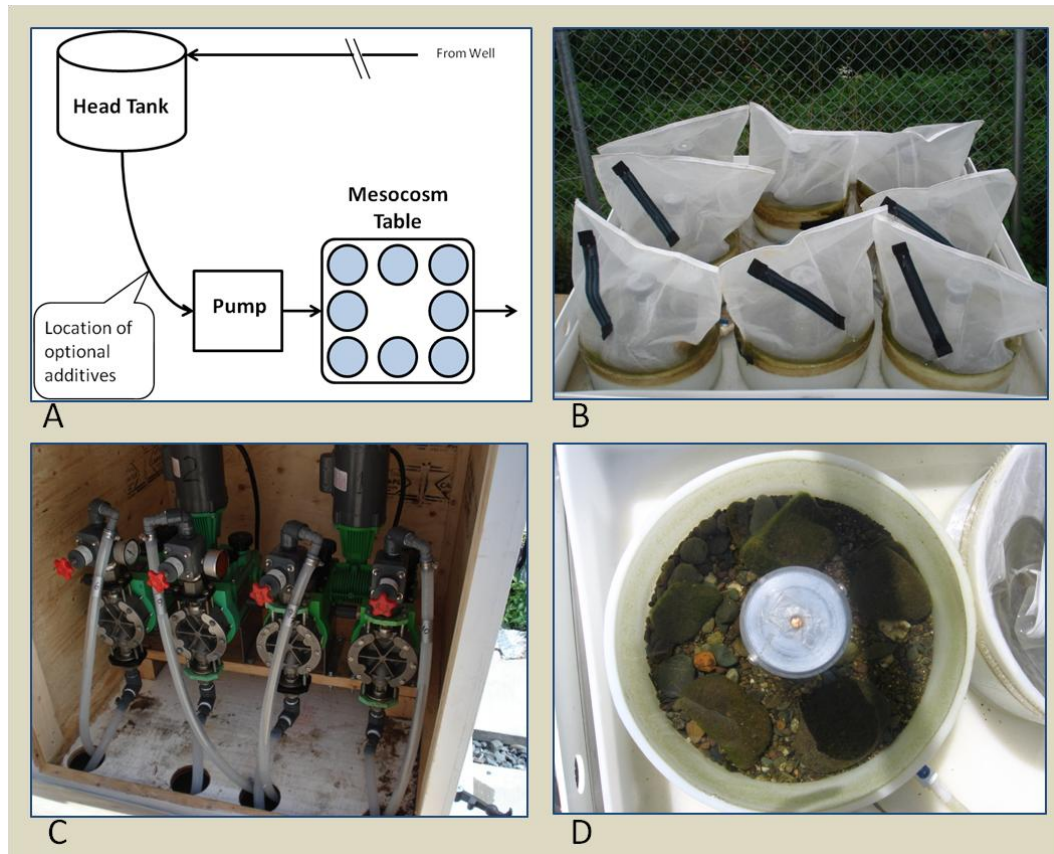


Figure 2.2. Mesocosm system: A) schematic diagram showing water delivery; B) modular table containing 8 mesocosm streams; C) pump house with Pulsar metering pumps each supplying a single mesocosm table; and D) individual mesocosm stream with emergence trap removed.

Mesocosm substrate material consisted of 250 ml of coarse sand ranging in size from 2 mm to 4 mm mixed evenly with 750 ml of gravel ranging in size from 4 mm to 30 mm, as fractionated by standard test sieves. Five relatively flat, ~10 cm cobbles were placed above the gravel substrate to simulate natural armoring and provide a sampling surface for algae. Sediment treatment streams received fine sand ranging in size from 0 mm to 2 mm - mixed evenly into the substrate matrix; moderate and high sediment treatments contained 260 ml and 520 ml of fine sediment, respectively. Visual estimations of streambed surface coverage by fine sediment in each sediment treatment were 0 %, 10-20 %, and 40-50 %. All substrate material was collected from the Nashwaak River, New Brunswick, Canada (N 46.18609;

W66.61061 (WGS84)), sieved on site and transported to the mesocosm facility in large coolers where it was kept moist until use. Material was set in place and water circulation through the mesocosm system was initiated 5 days prior to the beginning of the experiment to facilitate algal colonization. Nine 20 ml samples of the fine sand (<2mm) were air dried and combusted at 500°C for 4 hours, confirming that the organic content was consistently less than 1%.

Benthic invertebrates were collected immediately prior to the beginning of the experiment from a site approximately 8 kilometers downstream from the sediment source on the Nashwaak river using U-nets (area = 0.06 m², mesh size = 250 µm) (Scrimgeour, Culp & Glozier 1993). Multiple samples were collected from a riffle, homogenized in a pail and subdivided using a splitter; generating 4 subsamples for every 5 U-net samples (approximately 20% overstock to account for potential mortality incurred during capture and transport). Each mesocosm stream, which is comparable in area to a U-net sample, was inoculated with one subsample within 5 hours of collection. Subsamples from pooled invertebrate collections used to populate mesocosms were allocated evenly across tables and within levels of nutrient and sediment (refer to pilot study analysis in Appendix A for justification of sample allocation). Thus, the nuisance variation due to spatial heterogeneity of the riffle where invertebrates were collected was simply coupled with residual error, and therefore required no additional model term.

In order to characterize the nutrient status of the source invertebrate population, analysis of a water sample taken on July 13, 2011 from the stream site where invertebrates were collected indicated that the Nashwaak River is of low to moderate nutrient status, with nitrates (NO₃ + NO₂) concentration of approximately 35 µg L⁻¹ and 8 µg L⁻¹ total phosphorus (unfiltered). Fine sediment coverage within the riffle, estimated visually (Sutherland, Culp &

Benoy 2010), ranged between 0 and 15 percent. During the experiment, chemical analysis of water supplying one table per nutrient treatment confirmed that a strong nitrogen gradient was maintained (Table 2.1). Water temperature, recorded constantly at each table, remained consistent across tables and nutrient treatments.

Table 2.1. Mean temperature and water chemistry parameters in nutrient treatments measured over duration of the 2011 mesocosm experiment.

Nominal Nutrient Treatment [N]	Temperature °C	Measured Water Chemistry ($\mu\text{g L}^{-1}$)				
		Total P	Total N	NO ₃ +NO ₂	NH ₃	# Samples
20 $\mu\text{g L}^{-1}$	15.10	85.4	55.7	5.3	11.7	3
40 $\mu\text{g L}^{-1}$	15.09	85.5	65.3	17.0	4.7	3
80 $\mu\text{g L}^{-1}$	15.28	85.5	106.7	39.0	4.3	3
150 $\mu\text{g L}^{-1}$	15.20	85.8	143.0	94.5	0.0	2

2.2.3 Response parameters

Periphyton biomass was approximated by ethanol extraction and quantification of chlorophyll-a ($\mu\text{g cm}^{-2}$) (as in Culp *et al.* (2003)) from three combined 9.3 cm^2 samples scraped from cobbles within each mesocosm stream. Additionally, species-level identification and enumeration of algae by Bio-Limno Research & Consulting Inc., Halifax, Nova Scotia was completed for 36 mesocosms based on one 18.4 cm^2 sample per sediment treatment from every table. This was done using the traditional Utermöhl settling technique (Utermöhl 1931) and an inverted microscope equipped with phase contrast, at magnifications ranging between 165X and 750X.

Benthic invertebrates were collected at the end of the experiment by swirling the contents of each stream in a large pail and pouring water and suspended organic material through 500 μm mesh sieves. This process was repeated until all invertebrates were removed from the sediment. Remaining sediment from 5 mesocosms was checked for residual

invertebrates confirming that more than 99% of macroinvertebrates were captured during the swirling process. Samples were preserved in 10% formalin for a minimum of three days and then transferred to 70% ethanol. All invertebrates were enumerated and identified to Family or Order (oligochaete and nematode worms) at 16x magnification with the aid of a Leica[®] M80 dissection microscope and standard keys (Merritt, Cummins & Berg 2008; Wiggins 1996).

Emergent adult insects were captured by the mesh covers and collected every 2 days using GAST[®] 1HAB-25B vacuum pump equipped with a Bug-Vac[®] #2 insect aspirator. Specimens were preserved immediately in ethanol and later identified to family. Body length (excluding eyes, antennae, and cerci) of abundant taxa was measured using a Leica[®] M80 dissection microscope equipped with an ocular micrometer (reticle).

2.2.4 Analytical approach

A partly-nested factorial design, commonly referred to as a split-plot (Winer, Brown & Michels 1991), was used because nutrient treatments were delivered to whole tables (“plots”), and sediment treatments were replicated within tables. Therefore, nutrient was analyzed as a whole-plot factor, and sediment was analyzed as a sub-plot factor (Gotelli & Ellison 2004). Typical linear models for split-plot designs include an interaction between the sub-plot factor (sediment in this case) and plot (Underwood 1997) as follows:

$$\mathbf{y} = \mu + \text{Nutrient}_i + \text{Sediment}_j + \text{Nut} \times \text{Sed}_{ij} + \text{Table}_k(\text{Nut}_i) + \text{Sed} \times \text{Table}(\text{Nut}_i)_{jk} + \mathbf{e}_{l(ijk)} \quad (1)$$

Nutrient, Sediment and their Interaction were analyzed as fixed factors and all other factors were considered random. To increase the probability of detecting fixed effects, the Sediment x Table(nutrient) interaction term was excluded when P was greater than 0.25, according to recommendation of Quinn & Keough (2002), effectively pooling sub-plot error with residual variance:

$$\mathbf{y} = \boldsymbol{\mu} + \text{Nutrient}_i + \text{Sediment}_j + \text{Nut} \times \text{Sed}_{ij} + \text{Table}_k(\text{Nut}_i) + \mathbf{e}_{i(j)k} \quad (2)$$

Periphyton communities were analyzed in three steps. First, the influence of experimental treatments on whole periphyton assemblages was evaluated with nonparametric multivariate analysis of variance (PerMANOVA) (M. J. Anderson 2001a; McArdle & Anderson 2001) using the PERMANOVA+ add-on (M. J. Anderson, Gorley & Clarke 2008) for the PRIMER statistical package – version 6 (Primer-E, Plymouth, UK). Densities (individuals cm⁻²) were fourth root transformed to down-weight the influence of highly abundant taxa (Thorne, Williams & Cao 1999), and analysis was performed on Bray-Curtis dissimilarities between samples (mesocosm streams). The PERMDISP routine (M. J. Anderson 2006) in PRIMER was used prior to PerMANOVA to test for violations of the assumption of homogeneity of dispersion of samples within levels of each experimental factor (*p*-values derived by permuting residuals). PERMDISP was also used on an untransformed Bray-Curtis matrix to test for experimental treatment effects on true dispersion of samples within treatment levels because data transformation can hide effects on dispersion (community dissimilarity among samples) itself (Houseman, *et al* 2008). PerMANOVA was performed using the model in equation 2 because periphyton was only sampled from one stream per sediment treatment within each table; thus the Sediment x Table(nutrient) effect could not be measured. A random subset of 4999 permutations was used for the generation of pseudo *F* distributions of the null model for each PerMANOVA and PERMDISP significance test. Due to the hierarchical nature of the experimental design, residuals were permuted under a reduced model according to Anderson (2001b). Type II (conditional) sums-of-squares was selected so as not to prioritize either experimental treatment. Second, linear mixed effects models (Bolker, *et al* 2009; Crawley 2007) for three major taxonomic divisions – diatoms (Bacillariophyceae), green algae (Chlorophyta) and ‘other’ (includes trace amounts of cyanobacteria, Chrysophyceae, and

Euglenophyceae) – as well as overall algal biomass (Chlorophyll *a*) were fit with Restricted Maximum Likelihood Estimation (REML) using the nlme package (Pinheiro, *et al* 2012) in the R statistical program (version 2.14.0 R Development Core Team, Vienna, Austria). The linear mixed effects function in the nlme package assumes Gaussian error distributions and does not require specification of a link function. Type II (conditional) ANOVA tables with Wald's Chi-Square significance tests for fixed effects were then generated for each model using the car R package (Fox & Weisberg 2011)). Anderson-Darling tests of residuals were performed using the nortest R package (Gross 2012) to confirm that errors were normally distributed. Square root and fourth root transformations (with inclusion of a constant for values <1) were applied as necessary to normalize residual distributions of response variables found to be significantly skewed using the Anderson-Darling test. Third, the relative representation of three general ecological diatom 'guilds' proposed by Passy (2007) were evaluated with the REML procedure in R. These guilds include low profile, high profile, and motile taxa, which have been shown to respond strongly to changes in water chemistry (Lange, *et al* 2011), substrate roughness (Schneck, Schwarzbald & Melo 2011), and even chemical toxins (Rimet & Bouchez 2011). Since several taxa found were not included in Passy (2007) or the supplementary table associated with Passy and Larson (2011), several species were assigned to guilds based on the supplementary table associated with Lange *et al.* (2011).

Benthic invertebrate responses to experimental treatments were evaluated with PerMANOVA on Bray-Curtis dissimilarities of fourth-root transformed benthic invertebrate densities, using the previously described procedure. Pair-wise inter-centroid distances were then calculated and simple main effects (G. P. Quinn & Keough 2002) multiple comparison tests for nutrient and sediment were also performed using the PerMANOVA+ add-on.

Detection of significant fixed effects on whole assemblages was followed by univariate analysis

of 4th root transformed densities of taxa present in at least 50% of samples, modeled using the REML approach described previously. Additionally, several other metrics, including total combined abundance of the insect orders Ephemeroptera, Plecoptera, and Trichoptera (EPT), number of EPT families (EPT Richness), and total number of taxa (Total Richness), were modeled using the same procedure as with univariate invertebrate densities. Finally, simple main effects were assessed with Tukey's Honestly Significant Difference (HSD) multiple comparisons procedure for all univariate metrics that significantly responded to both nutrients and sediments. Multiple comparisons were carried out using the stats R package. It should be noted that REML, and to a certain extent PerMANOVA, perform relatively well with unbalanced designs (M. J. Anderson, *et al* 2008; Littell 2002). That stated, given that our design was complete (no missing cells) and evenly replicated we were confident that the quality of our analyses would not be compromised by the slightly unbalanced replication of sediment treatments.

Adult insect emergence across experimental treatments was also examined using linear mixed effects modeling procedure. Densities, sex ratios and total body length of insect families that emerged from at least 50% of mesocosm streams were analyzed. Sex ratios were analyzed as a continuous response variable approximated by dividing the number of males of a given family + 1 by the number of females + 1. Because mayflies exhibit strong sexual dimorphism (Soldan 1981), body length was analyzed for each sex independently.

2.3 RESULTS

2.3.1 *Periphytic algae*

Chlorophyll *a* extractions from periphyton scrapings collected at the end of the 2011 mesocosm experiment reflected a strong positive response to dissolved nutrients (Figure 2.3)

with no significant response to fine sediment addition (Table 2.2). PerMANOVA on Bray-Curtis dissimilarities of 4th root transformed algal densities (cells cm⁻²) confirmed that nutrients were also influential to the overall structure of the periphyton community and sediment was not (Table 2.2). Sediment effects were not anticipated because periphyton was collected from the upper surface of cobbles and was therefore not in direct contact with the settled fines. Furthermore, turbulence and shear stress were not great enough in the mesocosms for resuspension of fine sediment. PERMDISP analysis on untransformed algal densities did not indicate any effect of nutrients ($F_{3,32} = 0.565, p = 0.679$) or sediments ($F_{2,33} = 0.206, p = 0.846$) on within-group dispersion.

Without augmentation of background nutrient concentrations (20 µg L⁻¹ [N]), periphytic algal communities were dominated primarily by low profile diatoms (Figure 2.3; Figure 2.4); 30-75% of which were *Achnanthydium minutissimum*. Moderate nutrient enrichment (40 µg L⁻¹ [N]) resulted in relatively even proportions of greens and diatoms, with diatom assemblages consisting of relatively equal amounts of low and high growth forms. High nutrient enrichment (80 and 150 µg L⁻¹ [N]) allowed green algae to proliferate, and, in the most enriched streams (150 µg L⁻¹ [N]), high profile taxa, predominantly *Synedra rumpens*, to dominate diatom assemblages. Chlorophyte biomass in nutrient enriched streams consisted almost entirely of filamentous taxa such as *Mougeotia sp.* (Biggs & Price 1987) and *Stigeoclonium sp.* (Biggs & Smith 2002). Trace amounts of other algal groups, including cyanobacteria, Chrysophyceae, and Euglenophyceae, were present in several streams but followed no discernible pattern related to nutrient or sediment conditions. Motile taxa represented roughly 10-15 percent of diatoms and did not vary significantly across experimental treatments.

Table 2.2. Periphytic algae responses in mesocosms treated with combinations of nutrients and deposited fine sediment.

		Transformation	Nutrient <i>P</i>	Sediment <i>P</i>	Interaction <i>P</i>
Whole Community					
	PermDISP	$^4\sqrt{\quad}$	0.970	0.751	---
	PerMANOVA	$^4\sqrt{\quad}$	0.012	0.892	0.483
Biomass					
	Chl-A ($\mu\text{g cm}^{-2}$)	$^4\sqrt{\quad}$	<0.001	0.436	0.255
	Greens (mg cm^{-2})	$^4\sqrt{\quad}$	<0.001	0.938	0.771
	Diatoms (mg cm^{-2})	$^4\sqrt{\quad}$	<0.001	0.369	0.531
	Others (mg cm^{-2})	$^4\sqrt{\quad}$	0.561	0.836	0.153
Ecological Guilds					
	% Low Profile	none	0.035	0.663	0.630
	% High Profile	none	0.040	0.387	0.334
	% Motile	none	0.152	0.872	0.799

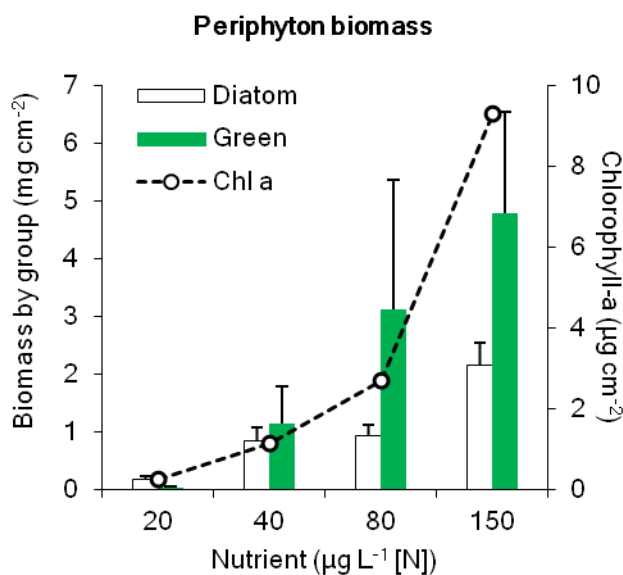


Figure 2.3. Biomass (mg cm^{-2}) of diatoms and green algae (mean ± 1 SE) in nutrient treatments; total chlorophyll-a represented by a dashed line (other algae groups not shown).

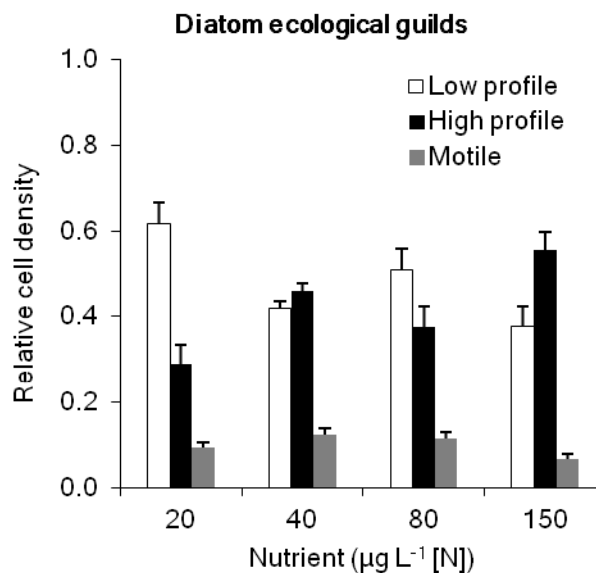


Figure 2.4. Relative cell density of diatom ecological guilds (mean \pm 1 SE) in nutrient treatments.

2.3.2 Benthic invertebrate assemblages

PERMDISP analysis on the Bray-Curtis dissimilarity matrix of 4th root transformed invertebrate densities confirmed that within group multivariate dispersion was consistent across levels of Nutrient and Sediment treatments, although dispersion varied significantly among Tables (whole plots) (Table 2.3). Since the assumption of homogeneity of dispersion was satisfied for the fixed factors, the same matrix was used for non-parametric multivariate analysis of variance (PerMANOVA) examining the influence of experimental treatments on benthic assemblages.

The initial PerMANOVA run using the full linear model (Equation 1) revealed that sediment effects were consistent across tables ($F_{16, 60} = 1.02, p = 0.43$). Thus, the Sediment x Table(nutrient) interaction term was removed from the model (Equation 2), effectively pooling any sub-plot error with residual variance (G. P. Quinn & Keough 2002 pp. 260). The PerMANOVA run with the reduced model (Table 2.3) revealed that whole-plot error (*Table*)

was the most influential experimental factor ($F_{8, 76} = 2.26$, $p = 0.0002$). Thus, it is expected that the probability of failure to detect nutrient and sediment effects (β) was very high and so the critical significance level for all subsequent macroinvertebrate analyses was relaxed to $\alpha = 0.10$ (Peterman 1990), and alpha adjustments (e.g. Bonferroni) were not applied.

Table 2.3. PerMANOVA on Bray-Curtis dissimilarities of benthic invertebrate assemblages in mesocosms treated with combinations of nutrients and deposited fine sediment.

Source	PermDISP (p value)	d.f.	SS	MS	F	P
Nutrient	0.2782	3	0.3663	0.1221	1.1509	0.2588
Sediment	0.6386	2	0.1409	0.0705	1.5029	0.0660
Nutrient \times Sediment		6	0.4096	0.0683	1.4563	0.0120
Table (nutrient)	0.0008	8	0.8488	0.1061	2.2631	0.0002
Residual		76	3.5629	0.0469		
Total		95	5.3285			

Because significant Nutrient*Sediment interaction was detected ($F_{6, 76} = 1.46$, $p = 0.012$), multiple comparisons of simple main effects were performed (Table 2.4). Nutrients directly affected benthic assemblages only at 0% fine sediment, with greatest compositional contrast between 40 and 80 $\mu\text{g L}^{-1}[\text{N}]$ (distance = 18.0, $p = 0.0978$). Significant structural differentiation also occurred between the lowest and highest nutrient concentrations (distance = 1.46, $p = 0.0986$).

Overall compositional differences between sediment levels were more common than with nutrients. Assemblages in the medium sediment treatment (25% fines) showed greatest distinction from other substrate conditions, with particularly strong contrast between 0 and 25 percent fines at moderately high (80 $\mu\text{g L}^{-1}[\text{N}]$) nutrient concentration (distance = 18.3, $p = 0.0006$).

Table 2.4. Pair-wise distances between centroids for simple main effects of nutrient and sediment treatments on benthic invertebrate assemblages. Significant multivariate distances differentiated with non-parametric t-tests represented by bold font and asterisks.

Nutrient (within Sediment)				
Contrasts	0% Fines	25% Fines	50% Fines	
20 versus 150	15.9*	13.1	15.0	
20 versus 80	15.7	15.4	13.9	
40 versus 150	10.2	14.1	18.6	
20 versus 40	13.1	12.4	18.6	
40 versus 80	18.0*	13.5	16.3	
80 versus 150	16.0	7.6	16.2	

Sediment (within Nutrient)				
Contrasts	20 $\mu\text{g L}^{-1}$	40 $\mu\text{g L}^{-1}$	80 $\mu\text{g L}^{-1}$	150 $\mu\text{g L}^{-1}$
0 versus 50	12.3	13.7*	13.3	11.9
0 versus 25	13.3*	8.4	18.3***	8.9
25 versus 50	12.7	16.3*	16.6*	9.8

* $P < 0.1$ *** $P < 0.001$

PERMDISP analysis on untransformed invertebrate densities did not indicate any difference in dispersion among levels of sediment ($F_{2,93} = 2.541$, $p = 0.130$). However, dispersion of mesocosm stream assemblages did vary significantly across nutrient levels ($F_{3,92} = 4.088$, $p = 0.016$), with comparatively high diversity found in the 40 $\mu\text{g L}^{-1}$ [N] treatment (Figure 2.5).

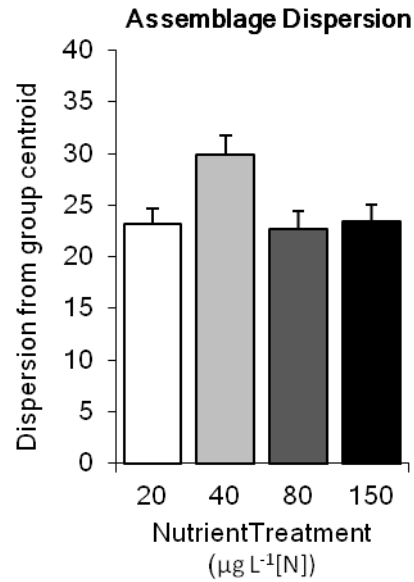


Figure 2.5. Invertebrate assemblage dispersion (± 1 SE) in response to nutrients (Bray-Curtis dissimilarities).

2.3.3 Univariate taxonomic responses

Given the overall significance of the PerMANOVA tests, univariate responses of taxa present in at least 50 percent of samples were modeled (Table 2.5). Of the twelve insect families and two classes of worms examined, five taxa responded to both nutrients and fine sediment addition. In each case, the interaction of nutrients and sediments was significant, and thus, multiple-comparisons of the simple main effects were examined. Total invertebrate richness, and richness of Ephemeroptera, Plecoptera and Trichoptera (EPT) did not significantly vary in response to nutrients ($p = 0.678$; $p = 0.892$) or sediment ($p = 0.962$; $p = 0.487$).

Table 2.5. Univariate taxonomic responses to Nutrient and Sediment treatments, with significant trends in bold and direction of single factor responses in parentheses.

	Transformation	Nutrient <i>P</i>	Sediment <i>P</i>	Interaction <i>P</i>
Invertebrate Density				
Chironomidae	$4\sqrt{}$	0.102	0.595	0.017
Oligochaeta	$4\sqrt{}$	0.036	0.025	0.017
Ephemerellidae	$4\sqrt{}$	0.359	0.485	0.881
Lepidostomatidae	$4\sqrt{}$	0.752	0.399	0.016
Heptageniidae	$4\sqrt{}$	0.672	0.893	0.555
Elmidae	$4\sqrt{}$	0.686	0.978	0.251
Hydropsychidae	$4\sqrt{}$	0.935	0.181	0.611
Simuliidae	$4\sqrt{}$	0.993	0.107	0.078
Leptophlebiidae	$4\sqrt{}$	0.046 (-)	0.944	0.950
Leptohephidae	$4\sqrt{}$	0.496	0.400	0.587
Baetidae	$4\sqrt{}$	0.829	0.161	0.495
Nematoda	$4\sqrt{}$	0.101	0.737	0.002
Psephenidae	$4\sqrt{}$	0.802	0.465	0.224
Athericidae	$4\sqrt{}$	0.154	0.511	0.336
Community Metrics				
EPT	$4\sqrt{}$	0.850	0.388	0.056
EPT Richness	none	0.892	0.487	0.927
Total Richness	none	0.678	0.962	0.163

Densities of Chironomidae followed an erratic pattern over the nutrient gradient with no fine sediment addition (Figure 2.6-A), increasing substantially with low level nutrient enrichment, then declining again at $80 \mu\text{g L}^{-1}[\text{N}]$ only to increase once more at $150 \mu\text{g L}^{-1}[\text{N}]$. However, with increased fine sediment the extremes in the nutrient trends were suppressed, resulting in an overall increase in insect density (survivorship) with slight enrichment, but no change in density beyond $40 \mu\text{g L}^{-1}[\text{N}]$.

At low fine sediment, the nutrient effect detected for Oligochaete worms was similar to the Chironomidae pattern; highest densities were observed with slight ($40 \mu\text{g L}^{-1}[\text{N}]$) and very high ($150 \mu\text{g L}^{-1}[\text{N}]$) enrichment (Figure 2.6-B). Oligochaeta generally responded positively to fine sediment addition, with significant differentiation of sediment treatments at low nutrients ($20 \mu\text{g L}^{-1}[\text{N}]$) and at moderate enrichment ($80 \mu\text{g L}^{-1}[\text{N}]$). Nematode worms also showed a complex multiple-stressor response, with highest densities observed at $80 \mu\text{g L}^{-1}[\text{N}]$ with moderate sedimentation (25% fines) (figure not shown).

Caddisflies of the family Lepidostomatidae generally responded positively to nutrients and fine sediment addition, although densities were lower in streams with high concentrations of nutrients and fine sediment than in those treated with only high nutrients ($150 \mu\text{g L}^{-1}[\text{N}]$) or moderate fine sediment (25% <2mm) (Figure 2.6-C). The effects of experimental treatments on total EPT density were largely related to trends in Lepidostomatidae – the most abundant family of EPT in our mesocosm streams. EPT responded positively to nutrients and (intermediate) sediments as independent drivers; however these effects were lost or reversed with the addition of a second stressor (Figure 2.6-D).

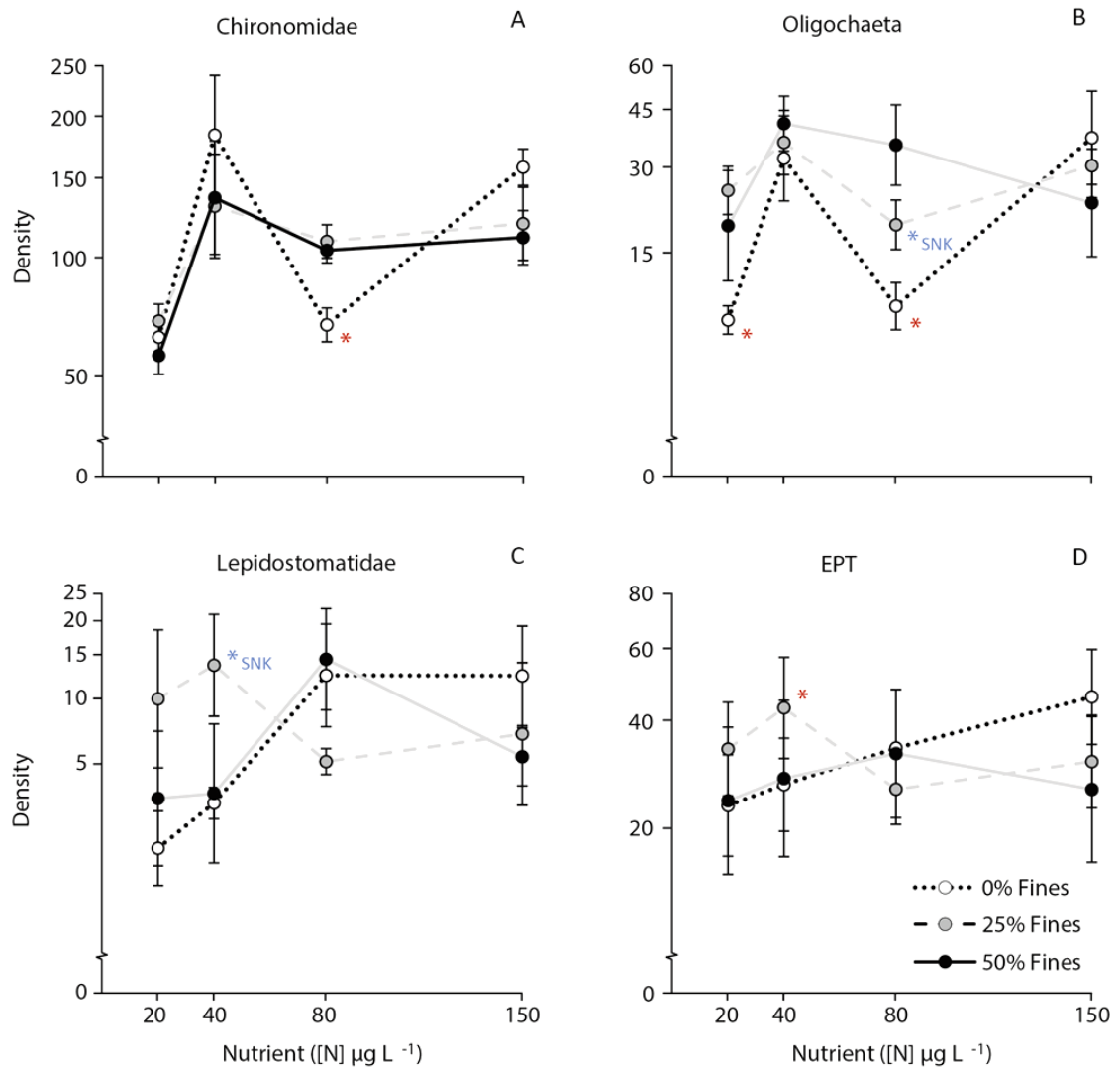


Figure 2.6. Densities (± 1 SE) of selected macroinvertebrates and EPT in response to Nutrient and Sediment treatment combinations. Significant nutrient trends represented by dark lines, while faded lines represent non-significant trends. Sediment treatments differentiated by Tukey's HSD indicated by asterisks.

2.3.4 Adult insect emergence

Approximately 5900 insects emerged as winged adults from mesocosm streams during the 2011 experiment. Chironomidae represented approximately 87 percent of all emergents, while six families of Ephemeroptera accounted for about 13 percent. Twenty-two Trichoptera (from seven families) and nine Plecoptera (all Perlidae) also emerged. Corresponding to the

benthic community, emergence of most insects varied substantially across tables, likely masking some effects of nutrients and sediment (see methods section). Nonetheless, densities of Chironomidae were found to follow a marginally-significant ($p = 0.082$) subsidy-stress pattern over the sediment gradient; with highest emergence at 25% fines (Table 2.6). Adult mayflies of the family Leptophlebiidae increased slightly with 25% fine sediment addition but showed no further increase with higher sediment ($p = 0.076$).

Table 2.6. Adult insect responses to Nutrient and Sediment treatments, with significant trends in bold and direction of single factor responses in parentheses.

	Transformation	Nutrient <i>P</i>	Sediment <i>P</i>	Interaction <i>P</i>
Count				
Chironomidae	$4\sqrt{}$	0.331	0.082	(+/-) 0.624
All Mayflies	$4\sqrt{}$	0.614	0.258	0.635
Ephemerellidae	none	0.192	0.814	0.622
Baetidae	none	0.483	0.697	0.730
Leptophlebiidae	none	0.657	0.076	(+) 0.828
Sex Ratio				
Ephemerellidae	$\sqrt{}$	0.107	0.290	0.571
Baetidae	$\sqrt{}$	0.492	0.740	0.110
Leptophlebiidae	$\sqrt{}$	0.633	0.280	0.524
Body Length				
Ephemerellidae	$\sqrt{}$	0.948	0.676	0.756
Ephemerellidae Male	$\sqrt{}$	0.747	0.857	0.173
Baetidae Female	none	0.691	0.251	0.135
Baetidae Male	none	0.001	(+) 0.086	(+) 0.347
Leptophlebiidae	$\sqrt{}$	0.804	0.767	0.827
Leptophlebiidae Male	none	0.751	0.167	0.236

Experimental treatments were not found to significantly alter sex ratios of prominent mayfly families, although given the high probability of effects masking due to between-table

error, the interaction of nutrients and sediment was potentially influential ($p = 0.110$) to Baetidae sex proportions. Both nutrients ($p = 0.001$) and sediment ($p = 0.086$) significantly influenced body length of male Baetidae, which ranged from 3.3 mm to 4.9 mm. The Largest Baetidae males were found in high nutrient ($150 \mu\text{g L}^{-1}[\text{N}]$), high fine sediment (50% fines) combinations (Figure 2.7).

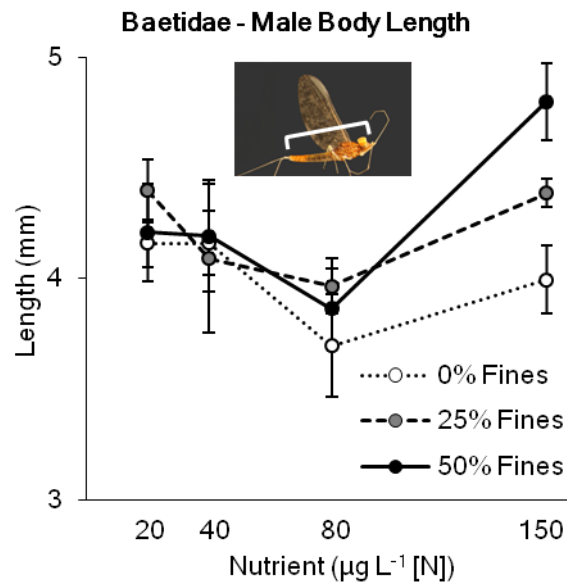


Figure 2.7. Body lengths of male Baetidae (mean \pm 1 SE) in nutrient and sediment treatments of 2011 mesocosm experiment (inset: photograph indicating portion of body measured).

2.4 DISCUSSION

2.4.1 Benthic community shift

Overall, the PerMANOVA test statistics show strong evidence for the general hypothesis that nutrient enrichment and sedimentation concertedly elicit a non-additive, multiple-stressor shift in macroinvertebrate assemblage composition. Nutrient effects were clearly more prominent when fine sediment content was low. Marked compositional difference in macroinvertebrate assemblages was found between the lowest and highest nutrient concentrations, likely corresponding to the shift in periphyton composition from low-

growing diatoms to presumably less palatable green algae. The threshold at which green algae abundance surpassed diatoms occurred between 40 and 80 $\mu\text{g L}^{-1}[\text{N}]$, which also aligns with the most substantial change in macroinvertebrate assemblages. Substrate composition was most influential to macroinvertebrate assemblages within the 80 $\mu\text{g L}^{-1}[\text{N}]$ nutrient treatment. Dispersion of mesocosm communities within treatment groups was generally not influenced by experimental conditions, except for greater dispersion in the 40 $\mu\text{g L}^{-1}[\text{N}]$ nutrient level relative to other nutrient concentrations. However, because dispersion of (untransformed) assemblages was relatively homogeneous across the other three nutrient levels we concluded that the increase at 40 $\mu\text{g L}^{-1}[\text{N}]$ did not warrant further investigation.

The observed shift in periphyton with increased nutrients, from low-profile diatom dominated communities to thick stands of green algae and high-profile diatoms, is generally consistent with the other stream studies (Passy 2007; Lange, *et al* 2011; Hoagland, Roemer & Rosowski 1982). The strong overall dominance of chlorophytes relative to diatoms in enriched streams was somewhat contradictory to several studies that have observed persistent dominance of diatoms (Flecker, *et al* 2002; Stelzer & Lamberti 2001), although chlorophytes dominated across enrichment gradients in the survey conducted by Chetelat *et al.* (1999). Often when nutrient enrichment is sustained indefinitely, autotrophic oxygen consumption creates an environment suitable only for organisms, mainly bacteria, tolerant of hypoxia (Diaz 2001). Thus, this experiment emulated the first stages of biological nutrient assimilation, namely, the events before hypoxia starts to drive autotrophic community structure (Carpenter, *et al* 1998; Conley, *et al* 2009; Watson, McCauley & Downing 1997).

Univariate responses of Chironomidae and Oligochaeta also showed strongest sediment treatment differentiation within the 80 $\mu\text{g L}^{-1}[\text{N}]$ nutrient treatment. Oligochaete worms evidently benefited from sandy substrata, allowing them to capitalize on increased food

availability along the entire enrichment gradient. Chironomidae larvae, which tend to be embedded in fine substrate, often in sand cases (Merritt, *et al* 2008), seemed to also be limited by substrate conditions, though nutrients were primarily responsible for variation in Chironomidae densities. Chironomidae, and to some extent Oligochaeta, increased most substantially with only slight nutrient enrichment, as densities generally leveled off beyond $40 \mu\text{g L}^{-1}[\text{N}]$. It is possible that for these two classes of burrowing detritivores, slight nutrient enrichment equated to nutritional resource subsidy but habitat degradation ensued at higher nutrient concentrations, as substrate smothering by periphyton became the overriding mechanism of nutrient influence. Densities of predators, including Athericidae, Chloroperlidae, Dytiscidae, Empididae, Gomphidae, Perlidae, and Perlodidae, were actually lowest in the $80 \mu\text{g L}^{-1}[\text{N}]$ treatment, though the overall nutrient influence was not significant (one-way nested ANOVA: $F_{3,8} = 2.31, p = 0.152$). Thus, I discount the influence of predators in favor of the nutritional subsidy/habitat modification explanation of Chironomidae and Oligochaete trends.

Conversely, highly motile (crawling) insect larvae of the EPT orders, especially the family Lepidostomatidae, were able to take greater advantage of increased nutritional resources, and were only negatively influenced by algae when substrate heterogeneity was similarly reduced by sedimentation. Most Lepidostomatidae were small (<5mm) and housed in cylindrical cases of fine sand. Thus it was hypothesized that the observed increase in this family with moderate (25%) fine sediment addition reflected their limited ability to construct cases in environments lacking fine sediment. However, the fact that similarly high densities were found at higher nutrients with low fine sediment suggested that Lepidostomatidae – a tiny climbing organism (Merritt, *et al* 2008) - may have also been limited by habitat architecture; high nutrient enrichment caused a marked increase in benthic algal biomass which would potentially infill interstitial spaces to produce habitat conditions not unlike those available with

moderate sediment addition. Therefore, the comparatively lower Lepidostomatidae densities found in high (50%) fine sediment conditions and high nutrients with sediment added imply that optimum habitat conditions are exceeded beyond a sediment threshold (>25% fines) or when both factors coincide.

Increased EPT, particularly Lepidostomatidae, in response to greater resource availability (bottom-up effect) may have, in turn, led to top-down influence on periphytic community structure, possibly explaining the dominance of less palatable greens over diatoms - the food of choice for most herbivorous macroinvertebrates (see review by Hillebrand (2002)). Rosemond *et al.* (1993) highlighted the potential for preferential selection by grazers to alter periphytic community structure even more dramatically than nutrient loading. Furthermore, the review by Feminella and Hawkins (1996) highlighted several examples in which top-down control by herbivorous insect larvae suppressed diatom populations, lending a strong competitive advantage to chlorophytes - specifically the filamentous green *Stigeoclonium* that was dominant in our enriched mesocosm streams.

In general, sediment generated a mix of positive and negative invertebrate responses even among sensitive taxa. I hypothesize that the dominantly negative effects observed in field surveys (Wagenhoff, *et al* 2011; Jones, *et al* 2011; Niyogi, *et al* 2007) are principally related to suspended particulate rather than the subsequent modification of habitat. Also, the tendency for increased invertebrate drift in response to sedimentation has been well documented (J. M. Culp, Wrona & Davies 1986; Suren & Jowett 2001), and since drift was impeded in our experiment, local sediment-induced depletion of invertebrate populations could not occur. However, this inconsistency between *in situ* and *in vitro* phenomena was partially moderated by the fact that recruitment was also absent; catastrophic drift in reaction to sediment deposition (J. M. Culp, *et al* 1986) at actual stream sites would generally be compensated by

upstream recruitment (Bournaud, Tachet & Roux 1987). Wagenhoff, Townsend & Matthaei (2012) examined macroinvertebrate responses to nutrients and fine sediment in a mesocosm experiment similar to ours, except that their system allowed for some drift and recruitment and sediment was added following an initial seeding of streams with invertebrates. Therefore, in contrast to our experiment, invertebrates were directly exposed to suspended material. They observed a strong negative response (in EPT for example) to sediment deposition, which was attributed to drift. Therefore, local declines in invertebrate populations following sedimentation is likely driven almost exclusively by the initial process of particle suspension (turbidity) and deposition, rather than the change in habitat.

2.4.2 Adult Insects

Low EPT emergence rates were anticipated, as adults in these orders tend to emerge during spring and early summer (Merritt, *et al* 2008). Infrequent significant treatment effects on adult insect densities confirms that negative responses observed in larval organisms were not due to emergence. The additive (non interacting) multifactorial response of male Baetidae body length to nutrients and sediments was counterintuitive, given that mayflies are considered pollution-sensitive organisms (Wielgolaski 1975) and that reproductive body size is considered a hallmark of success (Alexander & Culp 2008). Body size of adult Baetidae, especially of males (Alexander, *et al* 2008), has been shown to strongly correlate to nutritional subsidies (Fuller, Roelofs & Fry 1986; Scrimgeour & Culp 1994). However, in my experiment the strong increase in Baetidae size was only realized beyond $80 \mu\text{g L}^{-1}[\text{N}]$, below which body size decreased slightly with nutrients. Predation may have influenced Baetidae body size, as there were more predators (though not significantly so) in low and high nutrient treatments relative to $80 \mu\text{g L}^{-1}[\text{N}]$. Predators in this study were generally small, because exceptionally large predators (such as mature Perlidae nymphs) were removed prior to the start of the

experiment, so predator gape limitation may have allowed larger Baetidae to evade attacks. The observed discrepancy between male and female morphological responses to experimental treatments may have simply been related to differential allocation of resources; males grew longer but females may have, for example, produced more eggs. The positive influence of sedimentation on male Baetidae body size was especially perplexing, and may have also been related to top-down size selection by predators. Peckarsky *et al.* (2002) found that Baetidae male body size did not contribute to mating success, and suggested that increased adult body size was related to selective pressures in larval stages.

2.4.3 Conclusions

My research clearly indicates that at higher nutrient concentrations, stressor mechanism overlap occurs, as primary production contributes to the effect of deposited sediment by altering benthic habitat, leading to less complex hyporheos and increased organic architecture aloft (green algae and high-profile diatoms). Gayraud and Philippe (2001) reported that invertebrate densities were positively related to substrate interstitial space but negatively impacted by increasing organic content in substrates, lending support to the argument that organic material, like fine sediment, can impede the ability of crawling macroinvertebrates to penetrate hyporheic habitat. Pollution-tolerant burrowers are well adapted for homogenous substrates, so unlike larger crawling insects, they respond positively to nutrient-sediment combinations. Wagenhoff *et al.* (2011) also observed such a dichotomy of invertebrate responses to nutrient and sediment combinations in New Zealand streams, where, for example, %EPT was lowest when stressors coincided, while drivers strongly synergized to bolster Oligochaete populations. Though, in my experiment, nutrients and sediment led to increased organism survivorship in several cases, positive effects were generally suppressed when both factors were active. It is therefore expected that in a natural setting these

pollutants would almost certainly co-function to cause the loss of organisms and substantial shifts in community structure and function. I therefore reject the general hypothesis that stressors in combination generate an ecological result comparable to that of the single worst driver. The old model of independently managing agriculturally derived nutrients and sediment should be discouraged, and pollution standards should be adjusted to account for multiple stressor situations.

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2.6 LITERATURE CITED

Adams S.M. (2003) Establishing causality between environmental stressors and effects on aquatic ecosystems. *Human and Ecological Risk Assessment*, 9, 17-35.

Alexander A.C. & Culp J.M. (2008) Insulate or exacerbate? Exploring nutrient masking of contaminant effects. *Integrated Environmental Assessment and Management*, 4, 263-264.

- Alexander A.C., Heard K.S. & Culp J.M. (2008) Emergent body size of mayfly survivors. *Freshwater Biology*, 53, 171-180.
- Allan J.D. (2004) Landscapes and riverscapes: The influence of land use on stream ecosystems. *Annual Review of Ecology Evolution and Systematics*, 35, 257-284.
- Anderson M.J., Gorley R.N. & Clarke K.R. (2008) PERMANOVA+ for PRIMER: Guide to Statistical Software and Statistical Methods. PRIMER-E, Plymouth, UK.
- Anderson M.J. (2006) Distance-based tests for homogeneity of multivariate dispersions. *Biometrics*, 62, 245-253.
- Anderson M.J. (2001a) A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, 26, 32-46.
- Anderson M.J. (2001b) Permutation tests for univariate or multivariate analysis of variance and regression. *Canadian Journal of Fisheries and Aquatic Sciences*, 58, 626-639.
- Benoy G.A., Sutherland A.B., Culp J.M. & Brua R.B. (2012) Physical and Ecological Thresholds for Deposited Sediments in Streams in Agricultural Landscapes. *Journal of environmental quality*, 41.
- Biggs B.J.F. (2000) Eutrophication of streams and rivers: dissolved nutrient-chlorophyll relationships for benthic algae. *Journal of the North American Benthological Society*, 19, 17-31.
- Biggs B.J.F. & Price G.M. (1987) A Survey of Filamentous Algal Proliferations in New-Zealand Rivers. *New Zealand Journal of Marine and Freshwater Research*, 21, 175-191.
- Biggs B.J.F. & Smith R.A. (2002) Taxonomic richness of stream benthic algae: Effects of flood disturbance and nutrients. *Limnology and Oceanography*, 47, 1175-1186.
- Bolker B.M., Brooks M.E., Clark C.J., Geange S.W., Poulsen J.R., Stevens M.H.H. & White J.S. (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution*, 24, 127-135.
- Bournaud M., Tachet H. & Roux A.L. (1987) The Effects of Seasonal and Hydrological Influences on the Macroinvertebrates of the Rhone River France li. *Ecological Aspects*. Archiv fuer Hydrobiologie Supplement, 76, 25-52.
- Carpenter S.R., Caraco N.F., Correll D.L., Howarth R.W., Sharpley A.N. & Smith V.H. (1998) Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications*, 8, 559-568.
- Chambers P.A., Guy M., Dixit S.S., Benoy G.A., Brua R.B., Culp J.M., McGoldrick D., Upsdell B.L. & Vis C. (2009) Nitrogen and Phosphorus Standards to Protect the Ecological Condition of Canadian Streams, Rivers and Coastal Waters. National Agri-Environmental Standards Initiative Synthesis Report. 11.

Chetelat J., Pick F.R., Morin A. & Hamilton P.B. (1999) Periphyton biomass and community composition in rivers of different nutrient status. *Canadian Journal of Fisheries and Aquatic Sciences*, 56, 560-569.

Conley D.J., Paerl H.W., Howarth R.W., Boesch D.F., Seitzinger S.P., Havens K.E., Lancelot C. & Likens G.E. (2009) ECOLOGY Controlling Eutrophication: Nitrogen and Phosphorus. *Science*, 323, 1014-1015.

Crawley M.J. (2007) *The R Book*. Wiley, Chichester.

Culp J.M., Cash K.J., Glozier N.E. & Brua R.B. (2003) Effects of pulp mill effluent on benthic assemblages in mesocosms along the Saint John River, Canada. *Environmental Toxicology and Chemistry*, 22, 2916-2925.

Culp J.M., Wrona F.J. & Davies R.W. (1986) Response of Stream Benthos and Drift to Fine Sediment Deposition Versus Transport. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, 64, 1345-1351.

Culp J.M. & Baird D.J. (2006) Establishing cause-effect relationships in multi-stressor environments. In: *Methods in Stream Ecology* (Eds F.R. Hauer & G.A. Lamberti), pp. 835-854. Elsevier, Oxford, UK.

Culp J.M., Benoy G.A., Brua R.B., Sutherland A.B. & Chambers P.A. (2009) Total Suspended Sediment, Turbidity and Deposited Sediment Standards to Prevent Excessive Sediment Effects in Canadian Streams. National Agri-Environmental Standards Initiative Synthesis Report No. 13.

Culp J.M., Podemski C.L. & Cash K.J. (2000) Interactive effects of nutrients and contaminants from pulp mill effluents on riverine benthos. *Journal of Aquatic Ecosystem Stress and Recovery*, 8, 67-75.

Diaz R.J. (2001) Overview of hypoxia around the world. *Journal of environmental quality*, 30, 275-281.

Dodds W.K. (2006) Eutrophication and trophic state in rivers and streams. *Limnology and Oceanography*, 51, 671-680.

Fausch K.D., Baxter C.V. & Murakami M. (2010) Multiple stressors in north temperate streams: lessons from linked forest-stream ecosystems in northern Japan. *Freshwater Biology*, 55, 120-134.

Feminella J.W. & Hawkins C.P. (1996) Interactions between stream herbivores and periphyton: A quantitative analysis of past experiments (vol 14, pg 465, 1995). *Journal of the North American Benthological Society*, 15, 141-141.

Flecker A.S., Taylor B.W., Bernhardt E.S., Hood J.M., Cornwell W.K., Cassatt S.R., Vanni M.J. & Altman N.S. (2002) Interactions between herbivorous fishes and limiting nutrients in a tropical stream ecosystem. *Ecology*, 83, 1831-1844.

- Folke C., Carpenter S., Walker B., Scheffer M., Elmqvist T., Gunderson L. & Holling C.S. (2004) Regime shifts, resilience, and biodiversity in ecosystem management. *Annual Review of Ecology Evolution and Systematics*, 35, 557-581.
- Folt C.L., Chen C.Y., Moore M.V. & Burnaford J. (1999) Synergism and antagonism among multiple stressors. *Limnology and Oceanography*, 44, 864-877.
- Fox J. & Weisberg H.S. (2011) *An R Companion to Applied Regression*. Sage, Thousand Oaks, CA.
- Fuller R.L., Roelofs J.L. & Fry T.J. (1986) The Importance of Algae to Stream Invertebrates. *Journal of the North American Benthological Society*, 5, 290-296.
- Gayraud S. & Philippe M. (2001) Does subsurface interstitial space influence general features and morphological traits of the benthic macroinvertebrate community in streams? *Archiv Fur Hydrobiologie*, 151, 667-686.
- Gotelli N.J. & Ellison A.M. (2004) *A Primer of Ecological Statistics*. Sinauer Associates.
- Gross J. nortest: Tests for Normality. R package version 1.0-1. .
- Hadwen W.L. & Bunn S.E. (2005) Food web responses to low-level nutrient and N-15-tracer additions in the littoral zone of an oligotrophic dune lake. *Limnology and Oceanography*, 50, 1096-1105.
- Henley W.F., Patterson M.A., Neves R.J. & Lemly A.D. (2000) Effects of sedimentation and turbidity on lotic food webs: A concise review for natural resource managers. *Reviews in Fisheries Science*, 8, 125-139.
- Hillebrand H. (2002) Top-down versus bottom-up control of autotrophic biomass - a meta-analysis on experiments with periphyton. *Journal of the North American Benthological Society*, 21, 349-369.
- Hoagland K.D., Roemer S.C. & Rosowski J.R. (1982) Colonization and Community Structure of 2 Periphyton Assemblages, with Emphasis on the Diatoms (Bacillariophyceae). *American Journal of Botany*, 69, 188-213.
- Houseman G.R., Mittelbach G.G., Reynolds H.L. & Gross K.L. (2008) Perturbations alter community convergence, divergence, and formation of multiple community states. *Ecology*, 89, 2172-2180.
- Jones J.I., Murphy J.F., Collins A.L., Sear D.A., Naden P.S. & Armitage P.D. (2011) The impact of fine sediment on macro-invertebrates. *River Research and Applications*, 28, 1055-1071.
- Lange K., Liess A., Piggott J.J., Townsend C.R. & Matthaei C.D. (2011) Light, nutrients and grazing interact to determine stream diatom community composition and functional group structure. *Freshwater Biology*, 56, 264-278.

- Larsen S., Vaughan I.P. & Ormerod S.J. (2009) Scale-dependent effects of fine sediments on temperate headwater invertebrates. *Freshwater Biology*, 54, 203-219.
- Lemly A.D. (1982) Modification of Benthic Insect Communities in Polluted Streams - Combined Effects of Sedimentation and Nutrient Enrichment. *Hydrobiologia*, 87, 229-245.
- Littell R.C. (2002) Analysis of unbalanced mixed model data: A case study comparison of ANOVA versus REML/GLS. *Journal of Agricultural Biological and Environmental Statistics*, 7, 472-490.
- Mainstone C.P. & Parr W. (2002) Phosphorus in rivers - ecology and management. *Science of the Total Environment*, 282, 25-47.
- McCordle B.H. & Anderson M.J. (2001) Fitting multivariate models to community data: A comment on distance-based redundancy analysis. *Ecology*, 82, 290-297.
- Merritt R.W., Cummins K.W. & Berg M.B. (2008) An Introduction to the Aquatic Insects of North America. 1158.
- Molinos J.G. & Donohue I. (2010) Interactions among temporal patterns determine the effects of multiple stressors. *Ecological Applications*, 20, 1794-1800.
- Niyogi D.K., Koren M., Arbuckle C.J. & Townsend C.R. (2007) Stream communities along a catchment land-use gradient: Subsidy-stress responses to pastoral development. *Environmental management*, 39, 213-225.
- Passy S.I. (2007) Diatom ecological guilds display distinct and predictable behavior along nutrient and disturbance gradients in running waters. *Aquatic Botany*, 86, 171-178.
- Passy S.I. & Larson C.A. (2011) Succession in Stream Biofilms is an Environmentally Driven Gradient of Stress Tolerance. *Microbial ecology*, 62, 414-424.
- Peckarsky B., McIntosh A., Caudill C. & Dahl J. (2002) Swarming and mating behavior of a mayfly *Baetis bicaudatus* suggest stabilizing selection for male body size. *Behavioral Ecology and Sociobiology*, 51, 530-537.
- Pestana J.L.T., Alexander A.C., Culp J.M., Baird D.J., Cessna A.J. & Soares A.M.V.M. (2009) Structural and functional responses of benthic invertebrates to imidacloprid in outdoor stream mesocosms. *Environmental Pollution*, 157, 2328-2334.
- Peterman R.M. (1990) Statistical Power Analysis can Improve Fisheries Research and Management. *Canadian Journal of Fisheries and Aquatic Sciences*, 47, 2-15.
- Pinheiro J., Bates D., DebRoy S., Sarkar D. & the R Development Core Team (2012) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-103.
- Quinn G.P. & Keough M.J. (2002) Experimental design and data analysis for biologists.

Quinn J.M. & Stroud M.J. (2002) Water quality and sediment and nutrient export from New Zealand hill-land catchments of contrasting land use. *New Zealand Journal of Marine and Freshwater Research*, 36, 409-429.

R Development Core Team R: A language and environment for statistical computing.

Rimet F. & Bouchez A. (2011) Use of diatom life-forms and ecological guilds to assess pesticide contamination in rivers: Lotic mesocosm approaches. *Ecological Indicators*, 11, 489-499.

Rosemond A.D., Mulholland P.J. & Elwood J.W. (1993) Top-Down and Bottom-Up Control of Stream Periphyton - Effects of Nutrients and Herbivores. *Ecology*, 74, 1264-1280.

Schneck F., Schwarzbald A. & Melo A.S. (2011) Substrate roughness affects stream benthic algal diversity, assemblage composition, and nestedness. *Journal of the North American Benthological Society*, 30, 1049-1056.

Scrimgeour G.J. & Culp J.M. (1994) Feeding while Evading Predators by a Lotic Mayfly - Linking Short-Term Foraging Behaviors to Long-Term Fitness Consequences. *Oecologia*, 100, 128-134.

Scrimgeour G.J., Culp J.M. & Glozier N.E. (1993) An Improved Technique for Sampling Lotic Invertebrates. *Hydrobiologia*, 254, 65-71.

Soldan T. (1981) Secondary Sexual Characters in Mayfly Larvae and their Evolutionary Significance (Ephemeroptera). *Acta Entomologica Bohemoslovaca*, 78, 140-142.

Stelzer R.S. & Lamberti G.A. (2001) Effects of N : P ratio and total nutrient concentration on stream periphyton community structure, biomass, and elemental composition. *Limnology and Oceanography*, 46, 356-367.

Suren A.M. & Jowett I.G. (2001) Effects of deposited sediment on invertebrate drift: an experimental study. *New Zealand Journal of Marine and Freshwater Research*, 35, 725-737.

Sutherland A.B., Culp J.M. & Benoy G.A. (2010) Characterizing deposited sediment for stream habitat assessment. *Limnology and Oceanography-Methods*, 8, 30-44.

Thorne R.S., Williams W.P. & Cao Y. (1999) The influence of data transformations on biological monitoring studies using macroinvertebrates. *Water research*, 33, 343-350.

Townsend C.R., Uhlmann S.S. & Matthaei C.D. (2008) Individual and combined responses of stream ecosystems to multiple stressors. *Journal of Applied Ecology*, 45, 1810-1819.

Underwood A.J. (1997) Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge University Press, Cambridge, U.K.

Utermöhl v.H. (1931) Neue Wege in der quantitativen Erfassung des Planktons (Mit besonderer Berücksichtigung des Ultraplanktons). *Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie*, 5, 567-596.

Wagenhoff A., Townsend C.R. & Matthaei C.D. (2012) Macroinvertebrate responses along broad stressor gradients of deposited fine sediment and dissolved nutrients: a stream mesocosm experiment. *Journal of Applied Ecology*, 49, 892-902.

Wagenhoff A., Townsend C.R., Phillips N. & Matthaei C.D. (2011) Subsidy-stress and multiple-stressor effects along gradients of deposited fine sediment and dissolved nutrients in a regional set of streams and rivers. *Freshwater Biology*, 56, 1916-1936.

Watson S.B., McCauley E. & Downing J.A. (1997) Patterns in phytoplankton taxonomic composition across temperate lakes of differing nutrient status. *Limnology and Oceanography*, 42, 487-495.

Wielgolaski F.E. (1975) Biological Indicators on Pollution. *Urban Ecology*, 1, 63-79.

Wiggins G.B. (1996) Larvae of the North American caddisfly genera (Trichoptera), 2nd edition.

Winer B.J., Brown D.R. & Michels K.M. (1991) Statistical Principles in Experimental-Design. McGraw-Hill, New York.

3 GENERAL DISCUSSION

This thesis demonstrates that anthropogenic increases in nutrients and fine sediment drive changes in fluvial communities that exceed the impact of either pollutant independently. Also, the combined effect of stressor combinations is not predictable through indirect inference based on observations of independent effects. Thus, the interactive behaviour of nutrients and sediment must be evaluated directly and accounted for in predictive models.

Invertebrate responses in the 2011 experiment were often non-monotonic, with optimum conditions somewhere between lowest and highest treatment levels, suggesting commonality of subsidy-stress effects along gradients of both nutrients and deposited sediment. It is important to note that optimum conditions, even for sensitive taxa, are not necessarily at lowest stressor intensity. Maximum densities of taxa are likely related to their adaptation to specific niche(s). Of particular interest were situations where organism optima along one gradient shifted with the influence of the second driver (experimental factor), which occurred for total EPT and Lepidostomatidae. I suspect that this is closely linked to the stressor mechanism overlap theory described thoroughly in chapter 2, because if experimental factors were not influencing similar ecosystem (or organism) components, the optimum (or niche) should not have been modified by the second factor. Fine sediment addition obviously modified benthic habitat quality and it was apparent that organisms benefited from moderate increases in fine sediment content. Nutrients were thought to drive macroinvertebrate assemblage changes through two key mechanisms: increased food availability (subsidy) and, at high concentrations, depletion of habitat quality (stress) due to excessive algal growth. Declining food quality for herbivores with increasing nutrients, as periphyton communities

incorporated more filamentous algae, was also suspected to play a role in limiting nutritional subsidies to invertebrates.

3.1 TOWARDS THE DEVELOPMENT OF POLLUTION STANDARDS

In eastern Canadian stream systems, nutrients tend to create a more broad-scale (diffused) impact than the more heterogeneously-distributed (patchy) effects of sedimentation. Therefore, when both stressors are present it could be argued that nutrient enrichment is the foundational driver, and sedimentation is a moderator (Overton 2001) of nutrient effects, even if the local effect size of sediments is relatively large. Likewise, management actions should be carried out at the scale of nutrient impacts - likely large portions of watersheds ('functional process zones' (Thorp, Thoms & Delong 2006) or sub-watersheds). In the case of agriculturally-derived nutrients and eroded sediments, I propose adjusting previously developed nutrient standards (examples: Chambers, *et al* 2009; United States Environmental Protection Agency 2012) based on the general perceived severity of erosion (categorical: 'Low, Medium or High' sedimentation) for a given watershed. Best management practices (Yang, *et al* 2012) that reduce both nutrient runoff and soil erosion would be aimed at achieving the new adjusted standard.

Nutrient standards need to be developed nationally but be ecoregion-specific (Chambers, *et al* 2011), to reflect variation in geology, landscape morphology, terrestrial vegetation and human activity. The ecological effects of sedimentation are likely more consistent across regions, thus a relatively general correction factor could be applied to ecoregion-specific nutrient standards depending on intensity of sedimentation in watershed portions. The correction factor could be based on a commonly used pollution detection metric, like EPT density. Approaches based on trend surface analysis –assessment of a biotic response

metric over two complete and continuous predictor variables - are increasingly common in multiple-stressor research (Jonker, *et al* 2005; Stueckle, Shock & Foran 2009; Townsend, Uhlmann & Matthaei 2008; Wagenhoff, *et al* 2011), although the complexity of such representations may limit their utility at the management level. I therefore reinforce the suggested use of standards for those stressors with broad-scale impacts based on the response of key biotic indicators (EPT, algal guilds), corrected as appropriate for additional, locally-functioning drivers assigned to simplified severity categories (Figure 3.1).

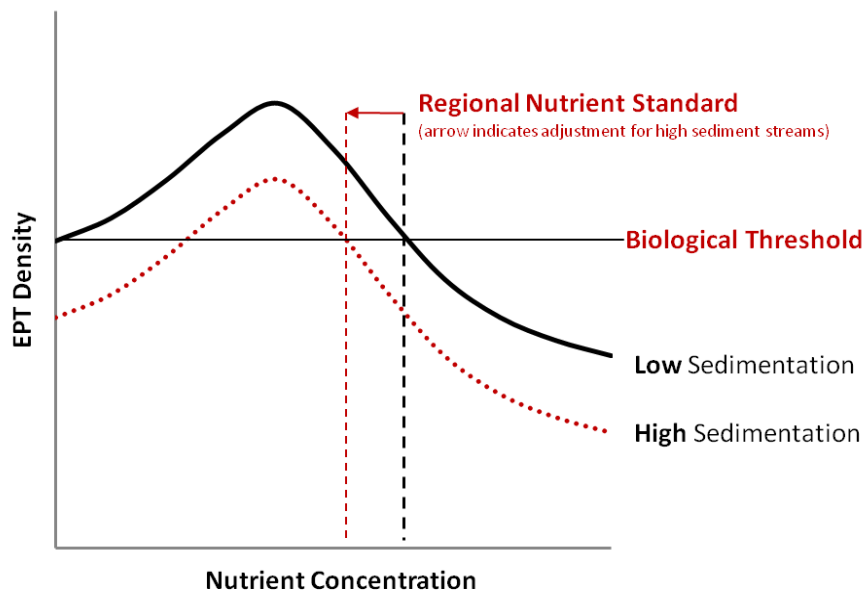


Figure 3.1. Conceptual diagram of a regional nutrient standard associated with a critical biological change point (threshold), adjusted according to relevant sedimentation intensity category.

3.2 PERSPECTIVES FOR FUTURE RESEARCH

3.2.1 *Approaches to multiple stressor investigation*

Short term experiments cannot fully characterize the ecological impact of environmental perturbations like sedimentation and, especially, nutrient loading. They offer novel perspectives of mechanisms and stressor interactions, but need to be thought of as a

complementary diagnostic component of field-based research programs. Certainly management strategies need to rely primarily on in situ observation of environmental impacts, which capture the full temporal scale of ecological phenomena. Studies using artificial environments need to be thoughtfully designed to answer specific questions not easily resolved with field data. In the investigation of stressor interactions, mesocosms have limited but extremely valuable utility. Yet, ecologists should diligently watch for in situ single-stressor gradients, which, when sampled along with systems influenced by multiple stressors, can potentially provide the most valuable insight of all by which to understand stressor relationships. When such paired gradients are not available, manipulative experiments in real streams (example: Townsend, *et al.* 2008) or artificial environments (mesocosms) are appropriate approaches for disentangling co-varying drivers of ecosystem decline.

3.3 SYNTHESIS

Multiple stressors clearly coincide and often strongly co-vary along land use gradients. As I have shown in this thesis, the multifactoral response of biotic communities to combinations of anthropogenic stressors is often highly complex and not easily predicted. The key limitations of my investigation were largely linked to deficient experimental power due to effects masking by random plot error, as well as the short duration of experiments. Nevertheless, the interactive behaviour of nutrient enrichment and sedimentation, both statistically and mechanistically, was obvious from measures of community composition and responses of specific invertebrate taxa. It is therefore pertinent that eutrophication and inorganic sedimentation be considered interconnected ecological drivers and be jointly managed. Future research of the relationships of common agriculturally derived pollutants should couple experimental manipulations of mesocosms with field survey data, paying

particular attention to sites impacted by only one pollutant – as stressor covariance is more likely than not. Finally, multiple stressor research must begin to include investigation targeted at identifying critical thresholds and alternative, stable ecosystem regimes.

3.4 LITERATURE CITED

Chambers P.A., Guy M., Dixit S.S., Benoy G.A., Brua R.B., Culp J.M., McGoldrick D., Upsdell B.L. & Vis C. (2009) Nitrogen and Phosphorus Standards to Protect the Ecological Condition of Canadian Streams, Rivers and Coastal Waters. National Agri-Environmental Standards Initiative Synthesis Report no. 11 .

Chambers P.A., Benoy G.A., Brua R.B. & Culp J.M. (2011) Application of nitrogen and phosphorus criteria for streams in agricultural landscapes. *Water Science and Technology*, 64, 2185-2191.

Jonker M.J., Svendsen C., Bedaux J.J.M., Bongers M. & Kammenga J.E. (2005) Significance testing of synergistic/antagonistic, dose level-dependent, or dose ratio-dependent effects in mixture dose-response analysis. *Environmental Toxicology and Chemistry*, 24, 2701-2713.

Overton R.C. (2001) Moderated multiple regression for interactions involving categorical variables: A statistical control for heterogeneous variance across two groups. *Psychological methods*, 6, 218-233.

Stueckle T.A., Shock B. & Foran C.M. (2009) Multiple Stressor Effects of Methoprene, Permethrin, and Salinity on Limb Regeneration and Molting in the Mud Fiddler Crab (*Uca Pugnax*). *Environmental Toxicology and Chemistry*, 28, 2348-2359.

Thorp J.H., Thoms M.C. & Delong M.D. (2006) The riverine ecosystem synthesis: Biocomplexity in river networks across space and time. *River Research and Applications*, 22, 123-147.

Townsend C.R., Uhlmann S.S. & Matthaei C.D. (2008) Individual and combined responses of stream ecosystems to multiple stressors. *Journal of Applied Ecology*, 45, 1810-1819.

United States Environmental Protection Agency (2012) Ecoregional Nutrient Criteria Documents for Rivers & Streams. 2012.

Wagenhoff A., Townsend C.R., Phillips N. & Matthaei C.D. (2011) Subsidy-stress and multiple-stressor effects along gradients of deposited fine sediment and dissolved nutrients in a regional set of streams and rivers. *Freshwater Biology*, 56, 1916-1936.

Yang Q., Benoy G.A., Chow T.L., Daigle J., Bourque C.P.-. & Meng F. (2012) Using the Soil and Water Assessment Tool to Estimate Achievable Water Quality Targets through Implementation of Beneficial Management Practices in an Agricultural Watershed. *Journal of environmental quality*, 41, 64-72.

Appendix A
2010 Pilot Experiment

Appendix A - Nutrient and sediment combinations in stream mesocosms

Since substrate composition had not previously been manipulated in our system, a pilot experiment examining the combined effects of three sediment levels (0, 15, and 25 percent finer than 2mm, by mass) over low ($20 \mu\text{g L}^{-1}[\text{N}]$) and high ($150 \mu\text{g L}^{-1}[\text{N}]$) nutrient conditions was carried out in the summer of 2010. Non-parametric MANOVA (Anderson 2001a) (refer to section 2.2.4 for more information on PerMANOVA) of benthic invertebrate assemblages showed only slight significance of the main effects of nutrients ($F_{1,6} = 1.596$, $p = 0.106$, power < 0.2) and sediment ($F_{2,12} = 1.545$, $p = 0.109$, power < 0.3) but excessive error associated with differences between modular tables ($F_{6,40} = 4.839$, $p = 0.0002$) (Table A-1). It was hypothesized that much of the between-table variance could be attributed to variation between sets of pooled invertebrate samples used to populate the mesocosms, as individual rounds of samples were allocated to single tables so that the variance associated with having multiple rounds of invertebrate collection and sub-sampling could be measured simultaneously with between-table error. Because this combined source of random variance was uncomfortably large, subsamples from pooled invertebrate collections used to populate mesocosms for the 2011 experiment were allocated evenly across tables and within levels of nutrient and sediment. Thus, the nuisance variation due to spatial heterogeneity of the riffle where invertebrates were collected was simply coupled with residual error, and therefore required no additional model term.

Table A-1. Non-parametric MANOVA on Bray-Curtis dissimilarities of benthic invertebrate assemblages from mesocosms in the 2010 pilot experiment.

Source	d.f.	SS	MS	F	P
Nutrient	1	2585	2585	1.596	0.1056
Sediment	2	1303	652	1.545	0.1092
Nutrient*Sediment	2	535	267	0.646	0.8156
Table (nutrient)	6	9637	1606	4.839	0.0002
Sediment*Table (nutrient)	12	4999	417	1.255	0.0806
Residual	40	13276	332		
Total	63	32572			

Appendix B

2011 Experimental Layout

Appendix B - 2011 Experimental Layout

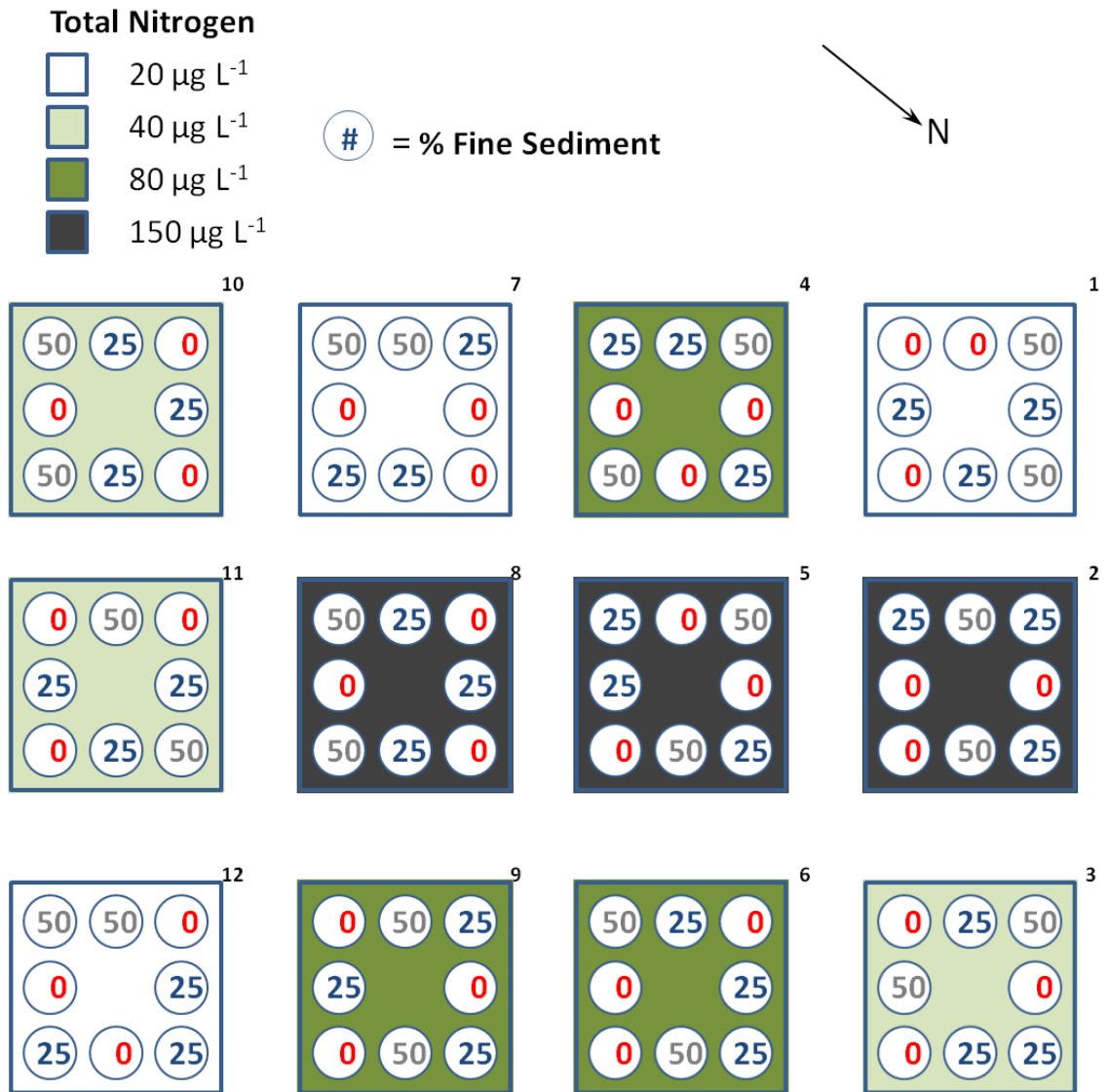


Figure B-1. Layout of nutrient and sediment treatments on mesocosm tables in 2011 experiment.

CURRICULUM VITAE

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MSc Thesis Publications:

Chase, J.W. Benoy, G.A., and J.M. Culp. In review. Multiple stressor responses to nutrient enrichment and inorganic sedimentation in stream mesocosms.

MSc Conference Presentations:

Chase, J.W., Benoy, G.A., and J.M. Culp. 2012. Investigating multiple stressor responses to nutrient enrichment and sedimentation in stream mesocosms. 65th Canadian Conference for Fisheries Research. January 5-6, 2012. Moncton, NB.