

**Freshwater Macroinvertebrate Responses to
Multiple Stressors: Interactions Between Fine
Sediment Grain Size and Flow Velocity Reduction**

Matthew Roy Ward

A thesis submitted for the degree of

Master of Science

at the University of Otago, Dunedin

New Zealand

December 2017

Abstract

Agricultural development has resulted in the degradation of freshwater ecosystems worldwide. Disentangling the individual and combined effects of agricultural stressors is crucial for future land-use management and restoration efforts. Two key stressors impacting streams and rivers draining agricultural catchments are deposited fine sediment and water abstraction. The effects of fine sediment grain size combined with water abstraction on benthic invertebrate communities have not yet been investigated. I addressed this knowledge gap by conducting a manipulative experiment in 60 outdoor stream mesocosms using four fine sediment treatments (no added sediment, silt: 0-0.125mm, fine sand: 0.125-0.250mm, coarse sand: 1-2mm) combined with three flow velocity treatments, simulating varying levels of water abstraction (fast: 26.5cm/s; medium: 13.9cm/s; slow: 0.0cm/s).

In Chapter 2, I determine the effects of fine sediment and reduced flow velocity on the benthic, drifting, and emerging stream macroinvertebrate communities (55 invertebrate response variables). There were 24 significant responses to sediment (44% of all variables; 83% negative responses) and 27 to flow velocity (49%; 85% negative), and 3 complex interactions (5%; and 20% additive multiple-stressor responses). My results imply that deposited fine sediment (of varying sizes) and reduced flow velocity can have pervasive detrimental impacts on stream invertebrate communities, and that the combined effects of these two stressors may be mainly additive. Effects of fine sediment were often negative regardless of grain size, especially for sediment-sensitive invertebrate taxa (e.g. benthic EPT taxon richness). The negative effects of fine sediment were also often worse at the smaller two grain sizes (e.g. benthic taxon richness and evenness). Finer sediments may reduce benthic habitat, food, and oxygen availability to a greater degree than larger sediments, and cause more damage to sensitive individuals through abrasion, smothering, or burial.

In Chapter 3, I use biological traits to determine how stream invertebrate communities responded to the two stressors, by analysing the responses of 8 biological traits (consisting of 33 trait modalities). Biological trait modalities showed 25 significant responses to sediment (76% of all variables; 60% negative responses) and 20 to flow velocity (61%; 60% negative). There were 12 complex interactions between stressors (36%; and also 12 additive multiple-stressor responses). Results indicated that fine sediment (of varying grain sizes) and reduced flow velocity often have pervasive negative effects on the functional composition of benthic

invertebrate communities, individually and in a multiple-stressor context. My results also provide insights into the mechanisms driving stressor responses (e.g. prevalence of Crawlers decreased as fine sediment size decreased, likely due to reduced interstitial habitat availability).

My key findings are that regardless of grain size, fine sediment can negatively affect benthic freshwater invertebrate communities, and the effects of fine sediment may be augmented by reduced flow velocities. Furthermore, sediment grain size matters for some invertebrate taxa and biological traits, and the severity of sediment effects can increase as sediment particle size decreases. Management decisions should seek to avoid fine sediment inputs, and minimize water abstraction, and strategies must be informed by knowledge of the individual and interactive effects of multiple agricultural stressors.

Acknowledgements

I would like to take this opportunity to express my sincere gratitude and appreciation to the outrageously talented, intelligent, and beautiful people who have performed above and beyond any kind of expectations, and have been an incredible source of inspiration, knowledge, support, and confidence for me throughout my journey in completing this thesis. None of this would have been possible without you. Always and forever, thank-you from the top to the bottom of my joyous heart.

Firstly, I would like to acknowledge the *crème de la crème* of supervisors, Christoph Matthaei and Jeremy “Jay” Piggott. I feel so privileged and lucky to have been taken under your wing. Your confidence in me, and patience throughout all my struggles and sloth-like tendencies has been unwavering, and your insight, feedback, and encouragement have been thorough, thought provoking, and uplifting. I couldn’t have asked for a better duo to guide me through this process.

I was fortunate enough to share this experience with a diverse and talented crew, whose company I spent many hours with in the field and laboratory: fellow Master’s student Lisa Carlin, and international recruits Johanna Blöcher, Abel Csabai, Stephanie Gobet, and Ricardo Taniwaki. I will always be indebted and grateful for your hard work, long hours, and friendship. I would not be at all surprised to see any of your names headlining ecological masterpieces in the future, or to see you excel in any field you pursue.

Nicky McHugh, Murray McKenzie, and Kim Garrett worked wonders behind the scenes in the Zoology department. How you guys manage to do what you do is beyond me; I am in awe of your technical abilities and the time management skills you possess. I am so grateful for your teachings and your guidance.

Fellow Zoology staff and students, Andreas Bruder, Haseeb Randhawa, Carolyn Burns, Trent Rasmussen, Scott Morrison, Rohan Menzies, Stephanie De Hamel, Julia Hunn, Matt Jarvis, Jason Augspurger, and the many other faces who drifted in and out of the Zoology building – I will always cherish the memories I have of the guidance, friendship, laughs, and songs we sang.

My amazing friends – my university family – Troy Negus, Joshua La Pine, Paul Winter, Vivienne Rjinberg, and Sophie Sparrow. You have been there since the beginning of my university experience, and I am so grateful you are all still here at the end.

I would also like to thank Katie Rood, the best friend, adventure buddy, and inspiration any shark-bag wearing dude could hope to walk through life with. I am so excited to see where the future takes us.

Finally, and most importantly, I would like to dedicate this thesis to my loving family: Mum, Dad, Rebecca, and Shannon. Thank-you for your years of endless encouragement, love, support, hugs, company, kindness and wisdom. Thank-you for always being there, and for making me who I am today. Words can't express how much I love you all.

Table of Contents

Abstract	i
Acknowledgements	iii
List of Figures	viii
List of Tables	x
CHAPTER 1 <i>General Introduction</i>	1
1.1 A Freshwater Perspective.....	2
1.2 Multiple Stressors.....	3
1.3 Agricultural Stressors	4
1.3.1 Fine Sediment.....	5
1.3.2 What, Exactly, is Fine Sediment?.....	6
1.3.3 Water Abstraction, Reduced Flow and Reduced Current Velocity.....	8
1.4 Freshwater Benthic Invertebrates.....	9
1.5 Thesis Outline	12
CHAPTER 2 <i>Macroinvertebrate Benthic, Drift, and Emergence Community Responses to Fine Sediment Grain Size and Water Abstraction</i>	13
2.1 Introduction	14
2.2 Materials and Methods	17
2.2.1 Study Location and Experimental System.....	17
2.2.2 Experimental Design.....	18
2.2.3 Benthic Invertebrate Communities	22
2.2.4 Invertebrate Sampling and Response Variables	22
2.2.5 Data Analysis	25
2.3 Results.....	27
2.3.1 Fine Sediment and Flow Velocity.....	27
2.3.2 Benthic Invertebrate community.....	29
2.3.3 Benthic Invertebrate Body Size	32
2.3.4 Benthic Invertebrate Community Composition and Common Taxa	33
2.3.5 Invertebrate Drift	37
2.3.6 Invertebrate Drift Body Size.....	40
2.3.7 Invertebrate Drift Community Composition and Common Taxa.....	41
2.3.8 Insect Emergence	44
2.3.9 Insect emergence body size	44
2.3.10 Insect Emergence Community Composition and Common Taxa.....	44
2.4 Discussion	49
2.4.1 Physicochemical Measures	49

2.4.2	The Two Stressors Compared	49
2.4.3	Fine Sediment	50
2.4.4	Water Abstraction and Flow Velocity	52
2.4.5	Multiple-Stressor Effects	55
	Additive Effects	55
	Complex Interactive Effects	56
2.4.6	Management Implications.....	56
CHAPTER 3 <i>Macroinvertebrate Biological Trait Responses to Fine Sediment Grain Size and Water Abstraction</i>		59
3.1	Introduction	60
3.2	Methods and Materials	64
	3.2.1 Study Location and Experimental System.....	64
	3.2.2 Experimental Design	64
	3.2.3 Invertebrate Sampling and Response Variables	65
	3.2.4 Relative Abundance of Biological Traits in the Invertebrate Community	65
	3.2.5 Data Analysis.....	66
3.3.	Results	69
	3.3.1 Physicochemical Measures	69
	3.3.2 Biological Trait Responses to Stressor Treatments	69
	3.3.3 Life History Traits	70
	Maximum Potential Size.....	70
	Maximum Reproductive Cycles per Year.....	73
	Life Duration of Adults.....	75
	3.3.4 Resistance and Resilience Traits	77
	Oviposition Site	77
	3.3.5 General Biological Characteristics Traits.....	79
	Attachment to Substrate of Aquatic Stages (excluding eggs).....	79
	Feeding Habits	81
	Dietary Preferences	83
	Respiration of Aquatic Stages (excluding eggs).....	85
3.4	Discussion	87
	3.4.1 The Stressors Compared.....	87
	3.4.2 Predicted Responses of Traits to Stressors	88
	3.4.3 Deposited Fine Sediment.....	90
	3.4.4 Water Abstraction and Reduced Flow Velocity	92
	3.4.5 Fine Sediment Size	94
	3.4.6 Multiple-Stressor Effects	96

Additive Effects	96
Complex Interactive Effects	96
3.4.7 Management Implications	98
CHAPTER 4 <i>General Discussion</i>	101
4.1 Effects of Deposited Fine Sediment of Varying Grain Sizes and Water Abstraction..	102
4.2 Fine Sediment.....	103
4.3 Fine Sediment Grain Size.....	104
4.4 Water Abstraction and Reduced Flow Velocity.....	105
4.5 Stressor Interactions	106
4.6 Management Implications and Limitations.....	107
References.....	110

List of Figures

Figure 2.1: The <i>ExStream System</i> on location at the Kauru River, Otago, New Zealand.	18
Figure 2.2: Slow flow velocity mesocosms on day 0 following sediment addition: a) slow control; b) small, 0 – 0.125 mm; c) medium, 0.125 – 0.250 mm; d) large, 1 – 2 mm.	21
Figure 2.3: Experimental set-up on the final day before samples were collected, with both drift and emergence nets in place.	23
Figure 2.4: Averages of physicochemical variables across the experimental treatments (sediment depth measured every three days for 14 days, sediment cover and flow velocity every three days for 28 days during the manipulative period). Error bars (SEs) show the variation between replicates (n = 5 for each treatment combination). Text in indicates direction of significant stressor main effects or interactive effects (Flow velocity: S, slow; M, medium; F, fast; Sediment: C, control, S, small (0 – 0.125 mm); M, medium (0.125 – 0.250 mm); L, large (1 – 2 mm).	28
Figure 2.5: Averages of benthic invertebrate community variables across the experimental treatments sampled on day 28. Error bars (SEs) show the variation between replicates (n = 5 for each treatment combination). Significant stressor main effects or interactive effects are labeled (Flow velocity: S, slow; M, medium; F, fast; Sediment: C, control, S, small (0 – 0.125 mm); M, medium (0.125 – 0.250 mm); L, large (1 – 2 mm).	31
Figure 2.6: Body size patterns and (averages \pm SEs) of the benthic invertebrate assemblage and two common taxa (Chironomidae excluding Tanypodinae and <i>Deleatidium</i> spp.) sampled on day 28. For more details see Fig. 2.5.	32
Figure 2.6: Average abundance (\pm SEs) of the eight common taxa in the benthic samples collected on day 28 that responded to one or both stressors. For more details see Fig. 2.5.	35
Figure 2.7: Average abundance (\pm SEs) of the five common taxa in the benthic samples collected on day 28 that responded to neither stressor. For more details see Fig. 2.5.	36
Figure 2.8: Averages of the invertebrate drift community-level variables (\pm SEs) across the drift samples collected over the final 3 days of the experiment (days 25-28). For more details see Fig. 2.5.	39
Figure 2.9: Body size patterns (averages \pm SEs) of the total drift assemblage, and two common taxa (Chironomidae excluding Tanypodinae and <i>Deleatidium</i> spp.) collected in drift samples over the final 3 days of the experiment (days 25 – 28). For more details see Fig. 2.5.	40
Figure 2.10: Drift propensity patterns (averages \pm SEs) of the six common drifting invertebrate taxa that showed a significant response to the sediment or flow velocity treatments, collected over the final 3 days of the experiment (days 25 – 28). Note the wide range of drift propensities across taxa (from <0.01 to >10). For more details see Fig. 2.5.	42
Figure 2.11: Drift propensity patterns (averages \pm SEs) of the three common drifting invertebrate taxa that showed no significant response to either experimental stressor, collected over the final 3 days of the experiment (days 25 – 28). For more details see Fig. 2.5 and Fig. 2.10.	43
Figure 2.12: Averages (\pm SEs) of emerged insect community-level variables, mean body size of all emerged adult insects and sizes of two common taxa (<i>Deleatidium</i> spp. and Chironomidae) across the experimental treatments collected over the final 3 days of the experiment (days 25 – 28) For more details see Fig. 2.5.	47
Figure 2.13: Emergence propensities of three common insect taxa (Chironomidae; <i>Austrosimulium</i> spp., <i>Deleatidium</i> spp.) across the experimental treatments collected over the final 3 days of the experiment (days 25 – 28) For more details see Fig. 2.5.	48
Figure 2.14: Averages for benthic EPT abundance and benthic <i>Deleatidium</i> spp. abundance (\pm SEs) sampled on day 28 and for the drift propensity patterns of EPT and <i>Deleatidium</i> (\pm SEs) across the drift samples collected over the final 3 days of the experiment (days 25-28).	53

Figure 3.1: Mean relative abundances of trait modalities in the benthic invertebrate community for the trait 'Maximum Potential Size' across the experimental treatments sampled on day 28. Error bars (SEs) show the variation between replicates (n = 5 for each treatment combination). Significant stressor main effects (post-hoc test rankings) or interactive effects are indicated in each plot (Flow velocity: S, slow; M, medium; F, fast; Sediment: C, control, S, small (0 – 0.125 mm); M, medium (0.125 – 0.250 mm); L, large (1 – 2 mm). '*' indicates cases where a stronger interaction between stressors overrode the main effect in question.	72
Figure 3.2: Relative abundances of trait modalities in the benthic invertebrate community for the biological trait 'Maximum Reproductive Cycles per Year' across the experimental treatments sampled on day 28 (averages ± SEs). For more details see Fig. 3.1.	74
Figure 3.3: Relative abundances of trait modalities in the benthic invertebrate community for the biological trait 'Life Duration of Adults' across the experimental treatments sampled on day 28 (averages ± SEs). For more details see Fig. 3.1.	76
Figure 3.4: Relative abundances of trait modalities in the benthic invertebrate community for the biological trait 'Oviposition Site' across the experimental treatments sampled on day 28 (averages ± SEs). For more details see Fig. 3.1.	78
Figure 3.5: Relative abundances of trait modalities in the benthic invertebrate community for the biological trait 'Attachment to Substrate of Aquatic Stages (excluding eggs)' across the experimental treatments sampled on day 28 (averages ± SEs). For more details see Fig. 3.1.	80
Figure 3.6: Relative abundances of trait modalities in the benthic invertebrate community for the biological trait 'Feeding Habits' across the experimental treatments sampled on day 28 (averages ± SEs). For more details see Fig. 3.1.	82
Figure 3.7: Relative abundances of trait modalities in the benthic invertebrate community for the biological trait 'Dietary Preferences' across the experimental treatments sampled on day 28 (averages ± SEs). For more details see Fig. 3.1.	84
Figure 3.8: Relative abundances of trait modalities in the benthic invertebrate community for the biological trait 'Respiration of Aquatic Stages (excluding eggs)' across the experimental treatments sampled on day 28 (averages ± SEs). For more details see Fig. 3.1.	86

List of Tables

Table 1.1: Examples of prior research using varying definitions of fine sediment, in New Zealand and abroad (adapted from Clapcott <i>et al.</i> 2011)	7
Table 2.1: Summary (P-values and effect sizes) of ANOVAs comparing physiochemical variables between experimental treatments. Rankings for <i>post hoc</i> tests in cases with significant <i>between-subjects</i> effects are given in columns 4 and 6. Sediment treatments: C, control; S, small (0 - 0.125 mm); M, medium (0.125 - 0.250 mm); L, large (1 - 2 mm). Flow treatments: S, slow; M, medium; F, fast. Significant P-values are indicated in bold print, and effect sizes (in parentheses) are given for all results where $P < 0.10$	28
Table 2.2: Summary (P-values and effect sizes) of (M)ANOVAs comparing benthic invertebrate responses between experimental treatments. Multivariate P-values are for the Pillai's Trace statistic. Rankings for <i>post hoc</i> tests in cases with significant between-subjects effects are given in columns 4 and 6. Sediment treatments: C, control; S, small (0 - 0.125 mm); M, medium (0.125 - 0.250 mm); L, large (1 - 2 mm). Flow treatments: S, slow; M, medium; F, fast. Significant P-values (with effect sizes in parentheses) are indicated in bold print, and effect sizes (in parentheses) are given for all results where $P < 0.10$	30
Table 2.3: Summary (P-values and effect sizes) of (M)ANOVAs comparing drifting invertebrate responses, and propensities of common drifting invertebrate taxa, between experimental treatments. See Table 2.2 for further details.	38
Table 2.4: Summary (P-values and effect sizes) of (M)ANOVAs comparing emerging invertebrate responses, and propensities of common emerged invertebrate taxa, between experimental treatments. See Table 1 for further details.	46
Table 2.5: Numbers and percentages of interpretable significant main effects and interactions for all invertebrate response variables combined. Means of effect sizes \pm standard errors are also shown.	49
Table 3.1: Traits identified in the review by Clapcott <i>et al.</i> (2017, Table 5) as responding to the agricultural stressors deposited fine sediment and water abstraction.	63
Table 3.2: Benthic invertebrate biological traits and their categories (adapted from Clapcott <i>et al.</i> 2017). ...	68
Table 3.3: Summary (P-values and effect sizes) of the between-subjects results of the MANOVA comparing biological trait responses between experimental treatments. Rankings for <i>post hoc</i> tests in cases with significant between-subjects effects are given in columns 4 and 6. Sediment treatments: C, control; S, small (0 - 0.125 mm); M, medium (0.125 - 0.250 mm); L, large (1 - 2 mm). Flow treatments: S, slow; M, medium; F, fast. Significant P-values are indicated in bold print, and effect sizes (in parentheses) are given for all results where $P < 0.10$. '*' indicates cases where an interaction between stressors prevented a ranking for the corresponding main effect (effect size of interaction term larger than size of a main effect/s, see Methods).	71
Table 3.4: Numbers and percentages of interpretable significant main effects and interactions for all trait response variables combined. Means of effects sizes \pm standard errors are also shown.	87
Table 3.5: Traits identified in the review by Clapcott <i>et al.</i> (2017, Table 5) as responding to the agricultural stressors deposited fine sediment and water abstraction, and their observed responses to applied stressors (indicated by post-hoc test rankings). All observed responses were significant at $P = 0.05$, except for the ranking for the trait modality Size 5 ($P = 0.054$). The average relative frequency of each trait modality is expressed as a percentage ($n = 60$).	89
Table 4.1: Numbers and frequencies (in %) of interpretable significant main effects and complex (non-additive) interactions for all invertebrate response variables for Chapter 2 (benthic, drift, and emergence community and common taxa metrics), and Chapter 3 (biological traits). Means of effect sizes \pm standard errors are also shown.	103

CHAPTER 1

General Introduction

1.1 A Freshwater Perspective

Freshwater ecosystems are a highly diverse yet delicate component of Earth's biosphere. Although freshwater ecosystems cover less than 1% of the Earth's surface and hold only 0.01% of the total water supply, they contain 6% of all known species (Dudgeon *et al.* 2006). Freshwater ecosystems have been heavily impacted by human expansion and population growth (Ricciardi & Rasmussen, 1999; Sala *et al.* 2000, Dudgeon *et al.* 2006). This is of increasing concern to managers, especially considering the global importance of freshwater as a resource (Kenny *et al.* 2009, Ormerod *et al.* 2010). Various ecosystem assessments have indicated that freshwater ecosystems are highly sensitive to anthropogenic and environmental pressures, including habitat destruction, intensified land-use, water pollution, introduction of invasive species, climate change and over-exploitation of resources (Sala *et al.* 2000; Allan, 2004; Dudgeon *et al.* 2006; Ormerod *et al.* 2010). In fact, Sala *et al.* (2000) identified freshwater ecosystems as the most sensitive biome to climate change, changes in land-use and invasion by exotic species, and the rates of biodiversity decline in freshwater ecosystems are faster than in even the most affected terrestrial ecosystems. Clearly, we are facing a global freshwater crisis.

The freshwater situation in New Zealand is not "100% pure" (as in the promotional slogan propagated by the previous government), either. While the water quality in New Zealand is still good compared to many other countries (Davies-Colley 2013), in recent decades there has been a marked decrease in water quality as farming intensity and land development have increased (Davies-Colley 2013; Foote *et al.* 2015; Ministry for the Environment & Stats NZ 2017). A report published by the Ministry for the Environment & Stats NZ (2017) highlighted some of the key issues and threats facing our waterways: native fish, plant and invertebrate species are under immense pressure and many are at risk of extinction; more than half of New Zealand's total consented water volume is allocated to irrigation; the extent of wetland cover in New Zealand is 10% of the original extent before human settlement; 90% of monitored lowland pastoral and urban rivers are unsafe for recreational use; and 62% of New Zealand's monitored waterways are unsafe to swim in due to pollution. Urban waterways are generally New Zealand's most polluted freshwater systems (Ministry for the Environment & Stats NZ 2017); however, high intensity agricultural land uses, which require large amounts of fertilizer and irrigation, have had the greatest negative impact on New Zealand's rivers in recent decades (Julian *et al.* 2017). As the human population continues to rise in New Zealand in coming

decades, there will likely be an increase in farming intensity, which will lead to an increase of pollutant inputs into streams lakes and rivers, as well as increased water usage, and stock densities (Foote *et al.* 2015).

A disciplined, research-based effort is required to manage, repair and restore freshwater ecosystems, as further human population growth, climate change and the demand for freshwater as a resource will result in increased stress on these threatened habitats, and the sensitive species contained within. Understanding the interactions and outcomes of anthropogenic and environmental stressors is of utmost importance for establishing a management framework to prevent further losses and environmental disasters.

1.2 Multiple Stressors

A stressor is a variable that exceeds its normal range of variation as a result of human activity, and affects ecosystems (individual taxa, community composition or ecosystem function) either negatively or positively (Piggott *et al.* 2015b, modified from Townsend, Uhlmann, & Matthaei, 2008). Piggott *et al.* (2015b) emphasises the importance of including that stressors can have positive impacts in the definition of the term; what is detrimental to one species may be beneficial to another.

Multiple stressors can interact with simple or complex outcomes (Folt *et al.* 1999; Crain *et al.* 2008). Some stressors provide a subsidy (positive effect) at low levels, but have negative effects at higher levels (Odum *et al.* 1979; Wagenhoff *et al.* 2011). In simple interactions, the effect of all stressors combined is equal to the sum or product of the individual effects, whereas in complex interactions, the combined effect of stressors can be more-than (synergistic) or less-than (antagonistic) predicted from single effects (Crain *et al.* 2008). Due to the complexity of stressor interactions, it is often difficult to predict the effects multiple stressors will have in ecosystems based on the findings of single-stressor research alone (Paine *et al.* 19988; Townsend *et al.* 2008).

It is of increasing importance for managers to mitigate and minimise the negative effects of multiple stressors in freshwater environments, and to understand when multiple stressors working in concert will produce complex outcomes (as opposed to simple outcomes). However, the formation of a general theory of how multiple stressors interact began less than

20 years ago and is still being refined (Folt *et al.* 1999; Crain *et al.* 2008; Townsend *et al.* 2008; Piggott *et al.* 2015b; Jackson *et al.* 2016; Kroeker *et al.* 2017). Although multiple stressors often occur and interact simultaneously, if interactions are not taken into account in assessment of risk, conflicting interpretations and false conclusions may be drawn.

Recent multiple-stressor research in freshwaters has utilised a combination of field surveys (e.g. Yuan & Norton 2004; Lange *et al.* 2014), laboratory (e.g. Chen *et al.* 2004; Boone *et al.* 2005), mesocosm (e.g. Wagenhoff *et al.* 2012; Elbrecht *et al.* 2016) and field experiments (e.g. Matthaei *et al.* 2006; Ramezani *et al.* 2014) to gain further understanding into the effects of a number of individual stressors and, more importantly, the interactions between these stressors. Townsend *et al.* (2008) discussed the benefits of each approach, and Swanson (2004) concluded that a combination of all three methods would be most beneficial for managers to assess risks associated with multiple-stressors. Surveys are a good starting point when assessing the effects of multiple stressors because they often allow for identification of the key stressors in a system. While field experiments have high ecological realism, and are the most effective method for disentangling and isolating multiple stressor effects, they are also the costliest in terms of time and effort required, and achieving successful replication can be challenging (Belanger *et al.* 2002, Townsend *et al.* 2008). In contrast, laboratory experiments can be quickly and easily replicated, with the drawback of low ecological realism. Outdoor mesocosm experiments, such as the experiment conducted in this thesis, strike a balance between high realism and control, and allow for high numbers of experimental replicates. However, care must be taken in extrapolating results obtained from mesocosm experiments to field conditions, due to temporal and spatial constraints resulting from the nature of these experiments (Piggott *et al.* 2015a).

1.3 Agricultural Stressors

Agricultural industries are the largest users of freshwater worldwide (Allan, 2004), and the intensity of agricultural practises has increased in recent decades to supply the demands of the growing human population. Agricultural intensification is a major contributor to decreased stream health and function in New Zealand (Matthaei *et al.* 2010; Scarsbrook *et al.* 2016). Previous research has indicated that all aspects of stream health, including water quality, habitat, and biological communities, decline because of high intensity agricultural activity

(Allan, 2004). As farming intensity increases, the presence and adverse effects of multiple anthropogenic stressors in freshwater systems also increases (Matthaei *et al.* 2006; Wagenhoff *et al.* 2011; Lange *et al.* 2014). In rivers and streams, such stressors include water abstraction, removal of riparian strip vegetation, bank erosion, increased salinity, and increased input of pollutants such as fine sediment, nutrients, and pesticides (e.g. Allan 2004; Matthaei *et al.* 2010; Statzner & Beche, 2010; Wagenhoff *et al.* 2011; Magbanua *et al.* 2013; Elbrecht *et al.* 2016). My thesis will focus on the effects of two key agricultural stressors, deposited fine sediment and water-abstraction-induced reductions in flow velocity, on stream benthic macroinvertebrate communities, a key component of stream ecosystems (see below).

1.3.1 Fine Sediment

Scientists have become increasingly aware that fine sediment is a master stressor in freshwater ecosystems (Wood & Armitage, 1997; Scarsbrook *et al.* 2016), and often augments the effects of other anthropogenic stressors (Matthaei *et al.* 2010; Piggott *et al.* 2012). The input of fine sediment into freshwater systems is a naturally occurring process, and in the absence of anthropogenic effects, fine sediment is a temporary feature of streams and rivers (Suttle *et al.* 2004). However, changes to land use in catchments (e.g. residential development or industrial practices, tree plantations, and agricultural activities) often lead to an increased input of fine sediment into streams and rivers through catchment erosion, removal of riparian strip vegetation and direct livestock access to waterways (Wood & Armitage, 1997; Allan, 2004). Biological responses to increased fine sediment are generally negative, and these effects are apparent at all trophic levels of stream food webs, affecting periphyton (Magbanua *et al.* 2013), fish (Kemp *et al.* 2011; Bowerman *et al.* 2014), and invertebrates (Townsend *et al.* 2008; Jones *et al.* 2012).

Deposited fine sediment has mainly negative effects on invertebrate community metrics and community composition (Larsen *et al.* 2009), and reduces richness and abundance of sediment-sensitive EPT species (larval mayflies, stoneflies and caddis flies; see e.g. Matthaei *et al.* 2006; Piggott *et al.* 2012). This is likely because fine sediment homogenises the benthic substratum, filling interstitial spaces which stream invertebrates use as habitat and refugia, and clogs invertebrate respiratory organs, increasing mortality and reducing fitness (Waters, 1995; Wood & Armitage, 1997; Jones *et al.* 2012, Extence *et al.* 2013; Glendell *et al.* 2014). Increased

fine sediment input in streams reduces light penetration and consequently algal biomass and phytoplankton production, reducing food availability for algal grazers (Wood & Armitage, 1997; Dolédec *et al.* 2011). Suspended sediment may scour or smother algal periphyton, reducing the quality of this food resource for benthic invertebrates (Henley *et al.* 2000; Statzner & Beche, 2010). However, abundance of certain benthic invertebrates can also increase in response to increased fine sediment, through increased population sizes of sediment-tolerant taxa (e.g. *Chironomidae*, *Oligochaeta*, and *Potamopyrgus antipodarum*) (Matthaei *et al.* 2006). Further, in streams with increased fine sediment deposits, there is often a greater representation of resilience traits such as deposition of eggs below the water surface, burrowing, and reduced body size (Dolédec *et al.* 2006; Townsend *et al.* 2008; Lange *et al.* 2014). This suggests a high sensitivity of early life stages to fine sediment, either through abrasive contact or smothering (Quinn & Stroud, 2002; Townsend *et al.* 2008; Dolédec *et al.* 2011).

1.3.2 What, Exactly, is Fine Sediment?

An important question to ask at this point is ‘what is fine sediment?’ Wentworth (1922) stated in his original work classifying sediment grain sizes that “in no other science does the problem of terminology present so many difficulties as in geology”. Freshwater ecologists seem to have eagerly latched on to this statement: in the literature, there is no consensus on how to define fine sediment, and in fact, the definition and use of fine sediment as a measured or manipulated variable varies widely from study to study (see Table 1.1). A common definition of fine sediment in freshwater ecology is “inorganic particles < 2mm in diameter”. However, perhaps “fine sediments” (plural) would be a more appropriate term to use if the upper bounds are set at < 2 mm, as this grain size range encompasses seven different grain size categories as defined in the Wentworth Scale: fine sand (<0.25 mm), very fine sand (<0.125 mm), silt (coarse <0.063 mm; medium <0.031 mm; fine <0.016 mm; very fine <0.008 mm), and clay (<0.004 mm).

To date, the role grain size of fine sediment plays in determining the severity or prevalence of negative effects on stream ecosystems, and the benthic macro-invertebrates inhabiting sediment affected patches, is still largely unknown (but see Conroy *et al.* 2018). As a result of the loose use of the term ‘fine sediment’ in previous experiments and surveys in running waters, there are a number of unanswered questions, including the following: (1) Are macroinvertebrate communities equally affected by fine sediment 2mm or less, and (2) are

smaller grain sizes worse? A survey of seven Appalachian streams with varying substratum compositions and grain sizes identified consistent negative relationships between fine sediment < 0.25 mm in diameter and EPT taxon richness (Kaller & Hartman 2004), and it has been known for a while that the substratum particle composition (ranging from cobbles to fine sediments) plays a role in determining invertebrate community structure (e.g. Moss *et al.* 1987). Ryder (1989) suggested that fine sediment impacts would differ between grain sizes, and a review of sediment effects on river biota in New Zealand stated that a greater understanding of the effects differing sediment grain sizes have on invertebrates is needed for modelling purposes (Crowe & Haye 2004). A recent laboratory experiment (Conroy *et al.* 2018) tested the responses of 6 stream invertebrate taxa to burial by four sediment size classes (ranging from 0.125 mm – 2 mm) at two varying depths, and found that buried taxa struggled to emerge from the smaller sediment size classes compared to the coarser particles. While this study provides valuable insight into the potential mechanisms driving negative-sediment response for these few species, the response of entire stream invertebrate communities to deposited fine sediment of varying sizes is still unknown. To the best of my knowledge, the effects that various specific grain sizes of deposited fine sediment have on entire benthic macroinvertebrate community assemblages in New Zealand, and the biological traits expressed by these communities, has not been investigated experimentally; my thesis identifies and disentangles these effects.

Table 1.1: Examples of prior research using varying definitions of fine sediment, in New Zealand and abroad (adapted from Clapcott *et al.* 2011).

Sediment Size Used	Source
< 4 mm	Dunning (1998)
< 2 mm	Ryder (1989); Quinn & Hickey (1990); Suren & Jowett (2001); Matthaei <i>et al.</i> (2006); Townsend <i>et al.</i> (2008); Piggot <i>et al.</i> (2012, 2015a, 2015b); Elbrecht <i>et al.</i> (2016); Mathers <i>et al.</i> (2017); Beermann <i>et al.</i> (2018)
'Sand'	Cotton & James (2003)
< 0.5 mm	Suren (2005)
< 1 mm	Townsend <i>et al.</i> (2008); Doretto <i>et al.</i> (2018)
< 0.06 mm	Glendell <i>et al.</i> (2014)

1.3.3 Water Abstraction, Reduced Flow and Reduced Current Velocity

Another key pressure exerted on freshwater ecosystems is reduced flow rate, as a consequence of diversion of waterways, damming, or water abstraction for irrigation purposes (Allan 2004). Water abstraction may affect biological response variables in stream communities as often as sediment addition (Matthaei *et al.* 2010, Elbrecht *et al.* 2016; Beerman *et al.* 2018). Periods of low flow are a naturally occurring process in streams due to seasonal and climatic variation (Smakhtin, 2001; Dewson *et al.* 2007). However, water abstraction results in uncharacteristic and extended periods of low flow, reducing stream wetted width and depth, and reducing habitat availability downstream of the abstraction site (Gore, 1977). Deviations from natural flow regimes can generate artificial droughts, and the effects of naturally occurring droughts are amplified by water abstraction (Dewson *et al.* 2007). The effects of water abstraction and reduced flow on streams are especially pronounced in arid areas of the world (Matthaei *et al.* 2010). Water abstraction alters temperature regimes in streams, which in turn disrupts life history patterns of invertebrates and fish, and may lead to increased mortality (Meier *et al.* 2003; Piggott *et al.* 2012). Connections between streams can be partially or completely severed as a result of reduced flow; however, waterways to facilitate irrigation may also increase connectivity, enhancing the spread of invasive species (Allibone, 2000).

Reduced flow (which generally results in slower current velocities) also enables increased settlement of fine sediment and organic matter on the stream bed (Castella *et al.* 1995; Dewson *et al.* 2007). This alters the relative frequency of functional feeding groups, notably increasing abundance of collector-gatherer taxa, and in turn increasing abundance of predatory invertebrates which feed on these species (Brooks *et al.* 2011; Dolédec *et al.* 2011). The effects of water abstraction are augmented by fine sediment addition (Matthaei *et al.* 2010). In combination, these two stressors can result in changes in benthic invertebrate community composition through decreased abundances of common invertebrate taxa, reductions in abundance of sensitive EPT taxa, and reduced expression of algal grazing traits (Dewson *et al.* 2007; Townsend *et al.* 2008; Dolédec *et al.* 2011; Lange *et al.* 2014). In the face of future climate change and intensified agricultural intensity in New Zealand and elsewhere, there is an urgent need for further studies investigating the effects increased water abstraction have on stream communities, especially in conjunction with other major agricultural stressors (Lange *et al.* 2014).

1.4 Freshwater Benthic Invertebrates

Macroinvertebrates are a key component of freshwater ecosystems. Freshwater benthic communities are characterised by a diverse and connected network of taxa, and each unique taxon varies in its preference, sensitivity and tolerance to physical, chemical, and biological stream processes (Quinn & Hickey, 1990; Covich *et al.* 1999). Aquatic invertebrates are easy to sample, and doing so requires minimal equipment (Stark, 1985; Quinn & Hickey, 1990). Consequently, the structural composition of stream macroinvertebrate communities is widely used as a biomonitoring tool (Stark, 1985; Johnson *et al.* 1993; Stark *et al.* 2001). However, as species composition in freshwater invertebrate communities varies on geographic scales, the effects of multiple stressors may be better understood through analysis of the biological traits expressed by invertebrate communities, because these traits reflect functional interactions between organisms and environmental pressures (Dolédec *et al.* 2011). In my thesis, multiple-stressor effects on benthic invertebrate communities will be determined using both structural measures (community-level metrics, common taxa) and functional measures (biological traits).

Commonly used structural invertebrate metrics rely on accurate taxonomic identification (Dolédec *et al.* 2006). The present study will utilise a number of different community-level and population-level metrics. Thus, I will investigate how community taxon richness and evenness (Simpson's indices), abundance and richness of EPT taxa (Ephemeroptera, Plecoptera, and Trichoptera), the New-Zealand specific MCI (Macroinvertebrate Community Index) and common individual invertebrate taxa respond to stressor effects. Both EPT indices are widely used globally because the taxa within these orders are generally highly sensitive to abiotic and biotic stressors (Lenat 1988; Dolédec *et al.* 2006; Matthaei *et al.* 2006). The New Zealand MCI ranks species based on their sensitivity and tolerance to organic pollution (Stark, 1985; Quinn & Hickey, 1990). This index is commonly used in stony-bedded streams and riffles or runs, and it will be of interest to know if this index is of any use in sediment-clogged mesocosms. Use of these community metrics allows for in-depth bio-assessment of benthic invertebrate community responses to the impacts of multiple stressors. Low taxonomic diversity and abundance of sensitive species is often associated with decreased habitat heterogeneity and increased physiological stress; however, the natural absence of certain pollution-sensitive species from streams may reduce the effectiveness of these community measures (Covich *et al.* 1999).

Stream communities can also be assessed through analysis of the relative abundances of a suite of biological traits expressed by invertebrate assemblages, and this approach may help elucidate causative mechanisms of functional and structural community composition (Usseglio-Polatera *et al.* 2000). Research has indicated that the use of functional metrics (i.e. biological traits), as opposed to traditional taxonomic metrics, can be as effective at stream and reach scales in detecting community responses to stressors (Richards *et al.* 1997; Stutzner *et al.* 1997). Traits are typically collated into trait categories (also called trait modalities) for data collection and analysis. For example, the trait Maximum Potential Size comprises 5 modalities (invertebrates with a maximum size of ≤ 5 mm (SIZE1), $> 5-10$ mm (SIZE2), $> 10-20$ mm (SIZE3), $> 20-40$ mm (SIZE4) and > 40 mm (SIZE5)). Dolédec *et al.* (2006) outline the potential advantages of using biological traits over traditional benthic invertebrate metrics. Unlike species distributions which can vary over even narrow geographic scales, biological traits are ‘tactics’ which are shared among all benthic invertebrates, albeit in variable combinations (Usseglio-Polatera *et al.* 2000). Unique assemblages of taxa, regardless of geographic closeness, can therefore be assessed using the same trait dataset (Stutzner *et al.* 2001; Vandewalle *et al.* 2010). Invertebrate traits have evolved in response to environmental variables acting upon organisms (Bunn & Arthington, 2002). Relative frequency of traits, and therefore functional community composition, varies in response to environmental stressors (Dolédec *et al.* 2006; Dolédec *et al.* 2011; Lange *et al.* 2014). Analysis of relative trait abundance can be applied to identify physiological and behavioural characteristics, as well as life history stages sensitive to stressors.

Traits in a dataset can be chosen specifically for analysis of certain focal stressors, to reflect the key physicochemical or biotic factors present at the study site or sites (Dolédec *et al.* 2006). For example, Townsend & Hildrew (1994) chose to focus on biological traits relating to resistance and resilience in a paper investigating invertebrate responses to physical disturbances, such as droughts and floods. In contrast, Dolédec *et al.* (2011) selected certain traits and their categories based on prior knowledge that these traits were susceptible to influence by catchment land-use intensity. In my thesis, I will utilise a broad range of invertebrate traits related to general biology (e.g. feeding, respiration), resistance and resilience (e.g. oviposition site), and life history (e.g. aspects of movement, reproduction and longevity), to disentangle the effects of deposited fine sediment grain size and simulated water abstraction on invertebrate community trait expression. This wide range of biological traits will be used, based on recommendations that extensive trait datasets are more beneficial in statistical

analyses to determine community responses to stressors (Lange *et al.* 2014), and that certain specific traits are sensitive to land-use and stressor effects at stream and catchment scales (Clapcott *et al.* 2017).

1.5 Thesis Outline

My thesis investigates the individual and interactive responses of stream invertebrate communities to two key agricultural stressors affecting running waters, deposited fine sediment and water abstraction, in an outdoor mesocosm experiment. To my knowledge, this is the first experiment globally to investigate structural, dynamic and functional responses of entire stream invertebrate communities (benthos, drift and emergence, biological traits) to varying grain sizes of deposited fine sediment and varying levels of water abstraction (simulated by reducing current velocity).

Chapter 2 will investigate how the benthic, drifting, and emerging invertebrate communities respond to the sediment and current velocity treatments, by analysing the responses of common community-level metrics such as diversity and evenness, total taxon richness, EPT richness and abundance, New Zealand's MCI, multivariate community composition and the abundances of individual common taxa.

Chapter 3 will explore how the benthic invertebrate community responds to the two stressors by analysing how the relative abundances of the biological traits expressed by the communities shift in relation to which stressor combinations are impacting the community.

A concluding General Discussion (Chapter 4) will compare and contrast the two approaches of studying benthic invertebrate communities, as well as discuss the implications of my findings in the wider context of freshwater resource management.

CHAPTER 2

Macroinvertebrate Benthic, Drift, and Emergence Community Responses to Fine Sediment Grain Size and Water Abstraction

2.1 Introduction

Agricultural industries are the largest users of freshwater worldwide (Allan, 2004), and agricultural intensification is the major contributor to decreased stream health and function in New Zealand (Matthaei *et al.* 2010; Scarsbrook *et al.* 2016). Previous research has indicated that all aspects of stream health, including water quality, habitat, and biological communities decline as a result of agricultural activities (Allan, 2004). As farming intensity increases, the effect and presence of multiple anthropogenic stressors in freshwater systems such as rivers and streams also increases (Matthaei *et al.* 2006; Wagenhoff *et al.* 2011; Lange *et al.* 2014). This results from individual and interactive effects of these multiple stressors. The present study will investigate the individual and interactive effects of two key agricultural stressors; deposited fine sediment and water abstraction.

Manipulative experiments are highly important for resource managers, land-owners, industries and conservation ecologists, in their efforts to disentangle and elucidate cause-and-effect relationships between multiple stressors (Swanson, 2004; Matthaei *et al.* 2010). Multiple-stressor experiments are essential for ecological risk assessment to evaluate the likelihood and severity that ecosystems may be impacted by anthropogenic stressors. It is crucial that future research experiments focus on disentangling the interactive effects of multiple stressors and acknowledge the complexity and unpredictability of multiple-stressor interactions (Folt *et al.* 1999; Townsend *et al.* 2008; Ormerod *et al.* 2010) – managers may simply get it wrong when it comes to reparation efforts, if stressors interact in unexpected ways to produce ‘ecological surprises’ (Paine *et al.* 1998).

Deposited fine sediment is a major stressor in running water ecosystems (Wood & Armitage, 1997), and often augments the effects of other anthropogenic stressors (e.g. Matthaei *et al.* 2010; Piggott *et al.* 2012). The input of fine sediment into freshwater systems is a naturally occurring process, and in the absence of anthropogenic effects, fine sediment is a temporary feature of streams and rivers (Suttle *et al.* 2004). Modifications to the landscape through agricultural activity leads to an increased input of fine sediment into streams and rivers through catchment erosion, removal of riparian strip vegetation and direct livestock access to waterways (Wood & Armitage, 1997; Allan, 2004). Benthic macroinvertebrate responses to increased fine sediment are generally negative (Waters, 1995; Townsend *et al.* 2008; Matthaei *et al.* 2010; Jones *et al.* 2012; Piggott *et al.* 2012). However, there is some inconsistency in the scientific literature as to how to define ‘fine’ sediment. Thus, fine sediment has been described

as particles < 0.06 mm (e.g. Glendell *et al.* 2014), < 0.5 mm (Suren 2005), < 1 mm (Townsend *et al.* 2008), and < 2 mm (Angradi 1999, Zweig & Rabeni 2001). It is evident there is no universally accepted definition or consensus regarding the actual grain size range of fine sediment and, thus far, there have been few studies which have investigated whether this grain size plays a role in determining the severity or prevalence of its negative effects. The latter is one of the main questions my study will aim to address. A recent laboratory experiment (Conroy *et al.* 2018) tested the responses of 6 stream invertebrate taxa to burying by four sediment size classes (ranging from 125 μm – 2 mm) at two varying depths, and found that buried taxa struggled to emerge from the smaller sediment size classes compared to the coarser particles. While this study provides valuable insight into the potential mechanisms driving negative-sediment response for these few species, the response of entire stream invertebrate communities to deposited fine sediment of varying sizes is still unknown.

Another key pressure agriculture exerts on running water ecosystems is reduced flow rate, a consequence of water abstraction for irrigation purposes (Allan, 2004). Water abstraction may affect biological responses of stream communities as often as added fine sediment (Matthaei *et al.* 2010; Elbrecht *et al.* 2016; Beermann *et al.* 2018). Periods of low flow are a naturally occurring process in streams due to seasonal and climatic variation (Smakhtin, 2001; Dewson *et al.* 2007). However, water abstraction generates artificial droughts, resulting in uncharacteristic and extended periods of low flow, reducing stream wetted width and depth, and reducing habitat availability downstream of the abstraction site (Gore, 1977; Dewson *et al.* 2007). The effects of water abstraction and reduced flow on streams are especially pronounced in arid areas of the world (Dewson *et al.* 2007). Water abstraction also alters temperature regimes in streams, which in turn disrupts life history patterns of invertebrates and fish, and may lead to increased mortality (Meier *et al.* 2003; Piggott *et al.* 2012).

Further, reduced flow enables increased settlement of fine sediment and organic matter on the streambed (Castella *et al.* 1995; Dewson *et al.* 2007), and the adverse ecological effects of water abstraction can be augmented by elevated levels of fine sediment (Matthaei *et al.* 2010). In combination, these two stressors can result in drastic changes in benthic invertebrate community composition through decreased invertebrate taxon richness and reductions in the abundances of sediment-sensitive taxa, ultimately resulting in a community composition more tolerant of increased environmental pressures (Dewson *et al.* 2007; Townsend *et al.* 2008; Dolédec *et al.* 2011).

In the present experiment, I use streamside mesocosms to investigate how reduced flow velocity (simulating water abstraction) and added deposited fine sediment of different grain sizes interact to affect structural (benthic) and dynamical (drift and emergence) macroinvertebrate communities. Experimental treatments comprised three levels of flow velocity (simulating no to high water abstraction) and four fine sediment size classes (ranging from 0-2 mm in diameter) in a fully crossed design. This was the first experiment investigating the combined effects of these two stressors on stream communities under controlled yet field-realistic experimental conditions. Based on previous research investigating the effects of deposited fine sediment and reduced flow velocity, I have developed six general hypotheses:

(1) Fine sediment addition will have pervasive, and mainly negative, effects on the benthic stream invertebrate community. This is because fine sediment causes changes to the streambed substratum (which degrades habitat quality and changes algal food resources) and physical damage to sensitive invertebrates through mechanisms such as scouring and clogging of sensitive external organs (Wood & Armitage 1997, Matthaei *et al.* 2006, Larsen & Ormerod 2010, Jones *et al.* 2012; Wagenhoff *et al.* 2012).

(2) The smaller the size of the deposited fine sediment, the more severe the negative impacts will be on the benthic invertebrate communities. Finer sediment classes are more likely to persist on the stream bed and become more embedded, resulting in greater homogenisation and clogging of the bed, as well as causing more harm to sensitive individuals through burial, abrasion, reduced oxygen and reduced refugia (Waters 1995; Wood & Armitage 1997; Jones *et al.* 2012, Extence *et al.* 2013; Glendell *et al.* 2014; Conroy *et al.* 2018).

(3) Reduced flow velocity will also have pervasive, and mainly negative, effects on the benthic invertebrate community, as the community structure will shift to a composition more tolerant of slow flows and sensitive taxa preferring faster flows (e.g. EPT taxa) will exit the experimental mesocosms (Dewson *et al.* 2007, Matthaei *et al.* 2010, Elbrecht *et al.* 2016).

(4) The effects of the experimental stressor regimes on benthic invertebrate populations will be mirrored by the corresponding invertebrate drift and/or emergence propensities. Thus, reductions in taxon-specific benthic abundances should be reflected in the dynamic communities, by increased drift or emergence propensity patterns of these taxa, or vice versa (Magbanua *et al.* 2013 Piggott *et al.* 2015c, Magbanua *et al.* 2016).

(5) Negative fine sediment effects will be more frequent and more severe at reduced flow velocity (Matthaei *et al.* 2010; Beermann *et al.* 2018). This is because sediment is more likely to settle and persist at reduced flow velocities, and sensitive invertebrates that prefer faster currents may find it harder to deal with high sediment loads when flow velocity conditions and oxygen availability are suboptimal.

(6) Moreover, this negative synergistic interaction should be stronger for smaller fine sediment sizes because this sediment should cause the strongest negative effects and is most likely to be washed out by faster flow velocities (see Hypothesis 2 and Conroy *et al.* 2018).

2.2 Materials and Methods

2.2.1 Study Location and Experimental System

The experiment was conducted from 7 February to 1 April 2015 (Austral summer/early autumn) in 60 circular stream mesocosms installed at the Kauru River, a fifth-order stream in the Otago province of New Zealand (170°44.60 East, 45°6.50 South, 98 m a.s.l; Piggott *et al.* 2012). The river contains diverse and abundant invertebrate and algal communities (Liess *et al.* 2009; Herrmann *et al.* 2012), and the water is relatively nutrient-poor (Magbanua *et al.* 2013).

The *ExStream System* (experimental stream mesocosm system, Figure 2.1) comprises 128 circular flow-through mesocosms (outer diameter 0.25 m, volume 3.5 L, bed surface area 450 cm²; Microwave Ring Moulds, Interworld, Auckland, New Zealand), installed on a double-layered scaffold situated close to the banks of the Kauru River. Due to the particular nature of this experiment (a desire for higher flow rates and current velocities than has been achieved in previous studies using the System), only 60 mesocosms in half the set-up (64 mesocosms) were used. All mesocosms naturally receive high numbers of immigrating stream organisms (invertebrates, algae, and microbes), and water chemistry, temperature, and light conditions are the same as in the nearby river (Wagenhoff *et al.* 2012; Magbanua *et al.* 2013; Piggott *et al.* 2015a). For a detailed description of the *ExStream System* see Piggott *et al.* (2015a). A short video about the research done in the System since 2007 is available at <https://vimeo.com/243219546>.



Figure 2.1: The *ExStream* System on location at the Kauru River, Otago, New Zealand.

2.2.2 *Experimental Design*

Fine sediment cover and flow velocities were manipulated in 60 circular flow-through mesocosms. Three fine sediment addition treatments (grain sizes 0 - 0.125 mm; 0.125 – 0.250 mm; 1 – 2 mm; see below for details) plus a control with no added sediment were crossed with three flow velocity treatments (fast, medium, slow) in a full-factorial design (12 treatment combinations, 5 replicates of each treatment combination). Sediment and flow treatments were randomly assigned within four spatial blocks (each consisting of 15 mesocosms fed by one header tank in the setup) on 5th March 2015. One full set of the treatment combinations was randomly assigned within each block. The final 12 treatment combinations were randomly assigned across the four blocks (to 3 mesocosms per block).

The experiment lasted almost 8 weeks, with a 25-day pre-colonization period (day -25 to day 0) followed by a four-week manipulative period (day 0 to day 28) when all stressor treatments were implemented. Fine sediment was added once on day 0 and remained in the mesocosms for the duration of the experiment. Flow velocity treatments were implemented on day 0 following sediment addition, and were applied continuously for 28 days.

Prior to turning on the flow on day -25, the 60 mesocosms were filled with dry river stones (500 ml of 2-20 mm gravels, plus 16 surface stones with a maximum width of 20 mm) simulating the beds of small sheep/beef farmland streams in the Otago region (Matthaei *et al.* 2006; Townsend *et al.* 2008). This substratum was obtained from a dry section of the Kauru

River floodplain, and was washed and sieved before it was deposited in the mesocosms to remove any fine sediment or organic matter. Current velocities in the mesocosms prior to stressor implementation were standardised across all mesocosms and kept as similar as possible for the duration of the colonisation period (mean 26.2 ± 1.6 cm/s, $n = 60$). Each mesocosm received approximately 2.8 L/min, checked on day -1. Current velocities were recorded by taking a reading of flow velocity near the wall of the mesocosm directly opposite the water inflow using a propeller flow meter (Schiltknecht, MiniAir 20, Gossau, Switzerland). Water exited the mesocosms through the central circular outflow, and flowed back to the river floodplain across pastoral land.

Flow was turned on in the channels on 7th February 2015, and the experimental set-up was left for 25 days to allow for natural colonization by invertebrates and algae. This method of colonization has been shown to be highly effective and standardised across all mesocosms (see e.g. Wagenhoff *et al.* 2012). Flow velocity in each mesocosm was checked and adjusted where needed at least once every 48 hours during the colonization period.

On day -7 (1 week prior to implementing stressor treatments), natural colonization of the mesocosms was augmented by adding one standard load of invertebrates into each of the mesocosms, to introduce invertebrate species underrepresented in the drift (Wagenhoff *et al.* 2012, Piggott *et al.* 2015c). Invertebrates were collected from the nearby river through standardised kick-net sampling (3 minutes, mesh size 250 μ m, frame size 60 x 40 cm), moving from downstream to upstream in run sections of the river. All kick-net sampling was conducted by the same person undergoing the same methodology, ensuring a high level of consistency.

Kick-net samples were collected from 0.36 m² patches of the river, comparable to the combined surface area of eight mesocosms (8 x 0.045 m²). Invertebrate kick samples were transported in a bucket to the set-up where they were divided into eight equal parts using a circular rotating sub-sampler (Waters, 1969), and these eight parts were randomly assigned to eight of the 60 mesocosms (1 load per mesocosm). Before each standard load of invertebrates was added to a mesocosm, the inflow jets were closed and kept shut for 2 minutes subsequently, to allow the added invertebrates to settle and avoid being washed out by the circular flow.

On day 0 (4th March 2015), the manipulative phase of the experiment began. Fine sediment was added to the designated mesocosms in a standardized and controlled process. Flow to the mesocosms was turned off during sediment addition and settlement (no longer than 5 minutes) to minimise the loss of sediment suspended in the water column. Fine sediment was

added in 500 g standardised loads per mesocosm. The fine sediment used was sourced from tributaries of the Manuherikia River in Central Otago, New Zealand, and was low in organic nitrogen (0.05 mg/kg) and phosphorus (617 mg/kg) (Spectrachem Analytical, Clyde Scree Supplies Ltd, Otago, New Zealand). Prior to addition, the sediment was dried and divided into three size classes in the laboratory using a sieve tower on a mechanical shaker, which separated the sediment contents into specific size classes through the sieve layers. These size classes will henceforth be referred to as: ‘small’ fine sediment (‘silt’ according to the Wentworth Particle Scale; 0 – 0.125 mm); ‘medium’ fine sediment (sand, 0.125 – 0.250 mm); and ‘large’ fine sediment (coarse sand, 1 – 2 mm) (see Figure 2.2).

Four randomly positioned sediment depth estimates (using a ruler) were collected from each mesocosm every three days during the first 14 days of the manipulative period, but were then suspended due to the possibility that these measurements were disturbing the benthic communities. Visual sediment cover percentage estimates were collected every three days throughout the duration of the manipulative period.

Mean sediment cover percentages and depths achieved on day 0 in the 45 mesocosms with added sediment were similar to natural deposited fine sediment levels observed in high-intensity farmland streams in the South Island of New Zealand (Matthaei *et al.* 2006; Wagenhoff *et al.* 2011; Clapcott *et al.* 2011). The means across all three addition treatments on day 0 (shortly after sediment addition) were 96.4 ± 2.9 % sediment cover (mean \pm SE, $n = 45$) and 9.5 ± 2.5 mm sediment depth (mean \pm SE, $n = 45$). The corresponding values per sediment grain size category on day 0 were: small 95.5 ± 3.0 %, 8.8 ± 2.2 mm; medium 96.3 ± 3.2 %, 10.0 ± 2.2 mm; large 97.3 ± 2.0 %, 9.9 ± 2.7 mm (means \pm SE, $n = 15$).

Flow velocity was manipulated at three levels, and these will henceforth be referred to as: ‘fast’ (26.5 ± 1.4 cm/s; mean \pm SE, $n = 20$), ‘medium’ (13.9 ± 0.7 cm/s; mean \pm SE, $n = 20$) and ‘slow’ (0.0 ± 0.0 cm/s; mean \pm SE, $n = 20$, means averaged over the duration of the manipulative period, flows recalibrated daily and one reading taken every 3 days). It is important to note that the fast flow treatment was the default or natural flow velocity, and the medium and slow treatments were implemented to simulate varying levels of water abstraction, which result in reduced in-stream current velocities (Dewson *et al.* 2007; Matthaei *et al.* 2010).

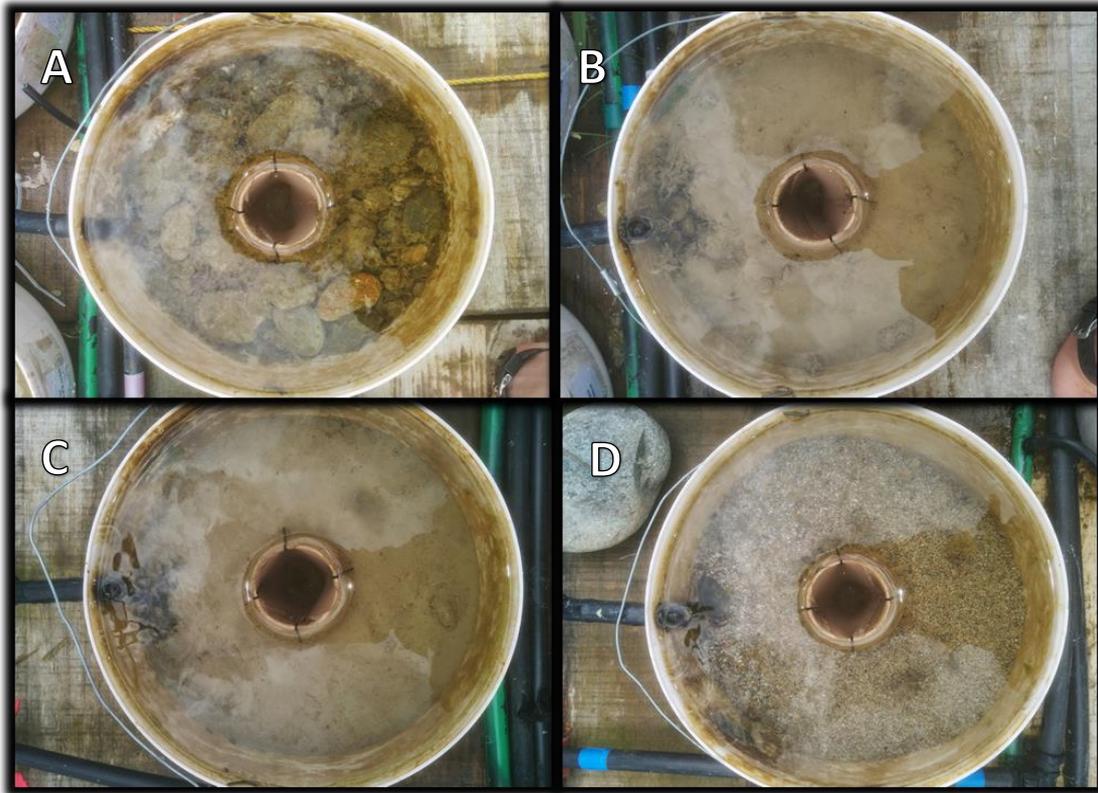


Figure 2.2: Slow flow velocity mesocosms on day 0 following sediment addition: A) slow control; B) small, 0 – 0.125 mm; C) medium, 0.125 – 0.250 mm; D) large, 1 – 2 mm.

During the colonization period, all mesocosms had an inflow jet attached at the terminal end of the piping, which was angled perpendicular to the mesocosm wall to establish fast flow velocities. During the manipulative phase, this jet remained for the fast flow velocity treatment. For the medium velocity treatments, the jet was removed and the nozzle remained perpendicular to the mesocosm wall, resulting in reduced circular current velocity. For the slow velocity treatments, the jet was removed and the nozzle was angled straight down to ensure minimal circular flow. While current velocities differed among flow treatments, all treatments still received the same volume of water (2.8 L/min, checked on day -1). Current velocities were recorded every three days during the experimental period, and flow rates in each mesocosm were checked daily and re-adjusted where needed.

2.2.3 Benthic Invertebrate Communities

Because sampling the benthic community in each mesocosm is a destructive process, benthic sampling was conducted once (see details below), by elutriating the substratum in each mesocosm and retrieving the entire benthic community on the final day of the experiment. The benthic invertebrates which exited the system by emerging as winged insects and the invertebrates which drifted out of or through the mesocosms (e.g. to find more favourable habitat or to escape predators) were also sampled (details see below). By sampling all benthic invertebrates which resided in or exited the mesocosms during the final 72 hours of the experiment (benthic, emerging, and drifting invertebrates), it is possible to determine how the manipulated stressors affect dynamic in-stream processes involving stream invertebrates. Previous experiments using the *ExStream* setup have established that, prior to implementing treatments in the mesocosms, benthic invertebrate communities are similar and invertebrate drift into the mesocosms can be assumed to be equal among blocks and individual experimental units (see Magbanua *et al.* 2013). Differences in final invertebrate community compositions among treatments can therefore be interpreted as a result of treatment effects on natural in-stream processes (immigration, emigration, mortality and reproduction) (Piggott *et al.* 2015c; Magbanua *et al.* 2016).

2.2.4 Invertebrate Sampling and Response Variables

Emerged invertebrates were collected in emergence hoods (hair nets tied down with elastic, and suspended by a wire frame; approx. mesh size < 250 μm) from days 25-28 during a 72-hour period, mirroring the sampling methods of Piggott *et al.* (2015c). Drifting invertebrates were also collected during this 72-hour period in drift nets (consisting of 40 denier panty-hose secured with bobby pins; approx. mesh size < 250 μm) secured to the circular outflow of the channels (See Figure 2.3). The nets for collecting emergence and drift samples were selected based on two main criteria: firstly, the nets needed to last 72 hours in wet conditions; secondly, the mesh size of the netting needed to be small enough so that emerged or drifting invertebrates would not pass through.

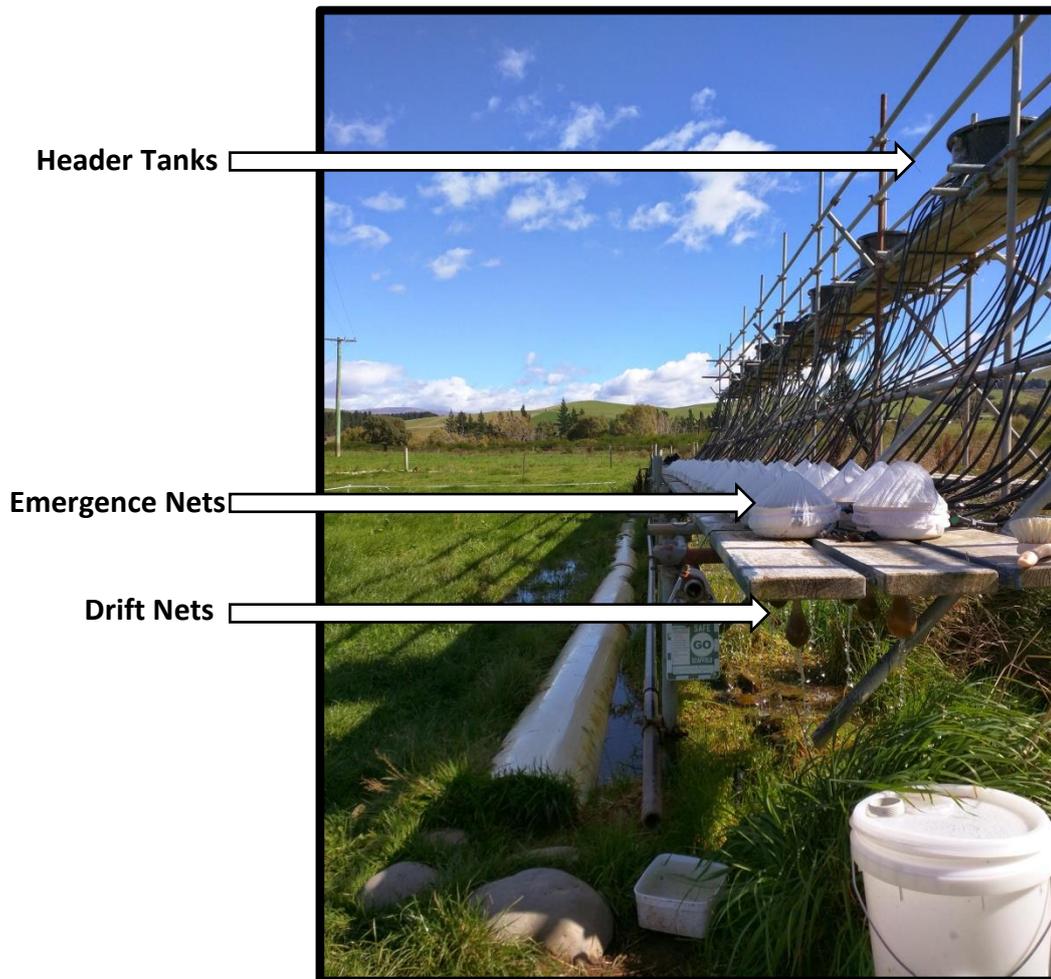


Figure 2.3: Experimental set-up on the final day before samples were collected, with both drift and emergence nets in place.

On day 28, emergence hoods were removed and stored individually in plastic containers, and were immediately transferred to an on-site freezer. Drift nets were removed and transferred into individual plastic containers and stored in 70% ethanol immediately after sampling. Following the removal of these nets, flow to each mesocosm was halted, and all benthic invertebrates were elutriated from the substratum and retrieved in a sieve (mesh size 0.250 mm), and then stored in 70% ethanol before being transported back to the laboratory.

Invertebrate emergence (days 25-28), drift (days 25-28) and benthic samples (day 28) were identified to the lowest practicable taxonomic level. All individuals were counted, and body lengths were measured (excluding Oligochaeta which often fragment, and Cladocera (family Chydoridae) which were of uniform size and too numerous) to the nearest mm

(maximum body length without cerci; cases removed where present) under a dissecting microscope (Olympus SZ51, 8-409, Tokyo, Japan). Each individual was counted in the emergence and drift samples. Because the benthic samples were too large to process in their entirety, each benthic sample was divided into 1/4 and 3/4 subsamples using a rotating subsampler (Waters 1969). These were stored in 70% ethanol and stained with Rose Bengal. The 1/4 subsample for each mesocosm was fully processed, and the remaining 3/4 of each benthic sample was scanned for rare taxa (which were included in taxon richness counts). Drifting invertebrates were removed from the panty-hose mesh in the lab, stored in 70% ethanol and stained with Rose Bengal. Any emerged adult invertebrates found in the drift samples were included in the emergence counts.

Twenty-one benthic invertebrate community response variables were calculated: (i) total benthic invertebrate abundance, (ii) benthic taxon richness, (iii) benthic EPT richness (number of taxa in the insect orders Ephemeroptera, Plecoptera and Trichoptera), (iv) benthic EPT abundance, (v) Simpson's diversity index, (vi) Pielou's evenness, (vii) New Zealand's Macroinvertebrate Community Index (MCI; Stark 1985), and (viii-xxi) a MANOVA of the abundances of the 13 most common taxa present in >50% of the samples and comprising >99% of the total invertebrate community (across all samples). Pupating individuals were included in benthic counts.

Fifteen drift-specific invertebrate responses were calculated: (i) total invertebrate drift propensity (number of invertebrates drifting during the final 3 days divided by final benthic invertebrate abundance; Townsend & Hildrew, 1976), (ii) drift taxon richness, (iii) EPT drift propensity, (iv) drift EPT richness, (v) Simpson's diversity index, (vi) Pielou's evenness, and (vii-xv) a MANOVA of the drift propensities of the 10 most common drifting taxa present in >50% of the samples and comprising >99% of total numbers of drifting invertebrates (across all samples).

Furthermore, nine emergence-specific responses were calculated: (i) total emergence propensity (numbers emerging during the final three days/final benthic invertebrate abundance), (ii) emergence taxon richness, (iii) emergence EPT richness, (iv) Simpson's diversity index, (v) Pielou's evenness, (vi-ix) a MANOVA of emergence propensities of the mayfly *Deleatidium* spp. and the dipterans Chironomidae and *Austrosimulium* spp. (numbers emerging during the final three days/final benthic abundance +1).

Finally, for the benthic, drifting and emerging invertebrates, (i) the total mean body size, and (ii) mean body sizes of *Deleatidium* spp. and (iii) Chironomidae were calculated. It has been predicted previously that smaller individuals are likely to be more impacted by in-stream contaminants, such as fine sediment, than larger individuals (Townsend & Thompson 2007).

2.2.5 Data Analysis

All statistical analyses were conducted in SPSS 22.0 (IBM SPSS Inc., IL, Chicago, USA). Exploratory data analysis revealed that no transformations of response variables were needed because the assumptions of homogeneity of variances and normality were not violated. Sediment type and flow velocity (plus their interaction) were the two fixed factors in the ANOVAs and MANOVAs used, and a block factor was also included (for the four mesocosm header tank blocks). The resulting model was intercept (d.f. 1) + sediment type (3) + flow velocity (2) + sediment x flow velocity (6) + block (3). I selected the type III sum of squares, as this is the appropriate method for analysing this type of design in SPSS (Garson, 2012). Because I was interested in differences between each individual sediment treatment and flow velocity level, I modelled both stressors as categorical (rather than continuous) predictors. Due to the larger number of degrees of freedom required to model the interaction term compared to the factor main effects, this analysis may somewhat underestimate the frequency of two-way interactions in the data (Cottingham *et al.* 2005).

I also wanted to ensure that the sediment and flow treatments were implemented successfully. To determine this, I ran individual ANOVAs for sediment depth, percentage sediment cover, and flow velocity. Sediment depth data was collected over the first 14 days of the manipulative period, whereas sediment cover estimates and flow velocity measurements were collected every three days for the entire 28 days of the manipulative period. For these ANOVAs, each variable was averaged for each mesocosm across the entire manipulative period, giving one data value per mesocosm for each of the three physical measures.

To assess effects of sediment addition and flow velocity manipulation on benthic, drift, and emergence invertebrate community-level variables, I performed individual ANOVAs for each community-level response variable. Further, to assess the effects of the stressors on the

community compositions of the abundant benthic, drifting, and emerging invertebrates (these “common” taxa had to be present in at least half of all samples and contributed over 99% of the total invertebrate community for each method of sampling), I performed MANOVAs with the multivariate equivalent of the model above. Multivariate stressor effects were determined using the Pillai’s Trace statistic, and effects on each of the common taxa were determined by examining the *between-subjects* effects for each individual taxon.

When *between-subjects* tests were significant, pairwise comparisons were performed for the factors sediment type and flow velocity using post hoc tests (Tukey’s HSD), and the rankings for these tests are presented in the tables alongside all significant findings. As the results for the block factor were irrelevant for my research objectives, these are not presented in the results. The level of significance for all tests was $P < 0.05$, and all response patterns described in the results were significant (plus one case where $P = 0.05$, see below). In the Results tables, I have presented standardized effect sizes (partial η^2 values, range 0.0-1.0; Garson, 2012) for all findings with $P < 0.10$ to allow readers to evaluate the biological importance of results (Nakagawa, 2004).

In cases where significant interactions between two or more experimental factors are present, interpretation of the main effects of the factors involved must be done with care. Consequently, I followed the recommendation of Quinn & Keough (2002) and interpreted individual main effects in the presence of a significant interaction only when the effect size of this interaction was smaller than the size of the corresponding significant main effect(s).

2.3 Results

2.3.1 *Fine Sediment and Flow Velocity*

Three physicochemical variables were analysed to determine if the experimental stressor applications were successful: fine sediment depth (mm), fine sediment cover (visual estimate, 0-100%), and flow velocity (cm/s). Each variable was averaged for each mesocosm across the entire manipulative period (see Methods). Findings for all three variables indicated that the stressor manipulations were highly effective (see Table 2.1, Figure 2.4), with very strong effect sizes (0.71-1.0) and directions of stressor main effects and interactions that conformed to the predictions in my hypotheses (see Introduction).

Sediment depth was lower in controls (0.00 ± 0.01 mm; mean \pm SD) than in all three sediment addition treatments. Sediment depth was equal in medium (9.8 ± 1.3 mm) and large sediment treatments (11.2 ± 3.6 mm) but slightly higher in large than in small sediment treatments (8.8 ± 1.3 mm). Average sediment depth was not affected by flow velocity or a velocity by sediment interaction.

Sediment cover differed between sediment types and flow velocities, and also showed a sediment by velocity interaction. Overall, sediment cover was greatest in the large sediment treatment (93.8 ± 2.4 %; mean \pm SD), intermediate in medium (88.6 ± 7.1 %) and small sediment addition treatments (86.2 ± 8.9 %), and lowest in controls (0.9 ± 3.5 %). Overall, sediment cover was also lower in fast velocity mesocosms (61.5 ± 37.1 %) compared to slow (70.4 ± 41.7 %) and medium velocity mesocosms (70.2 ± 40.2 %). Further, fast flow velocity reduced sediment cover more strongly in medium, and especially in small, sediment treatments than in the large sediment treatment (see Figure 1). The effect size for this interaction was not quite as strong as those of the two corresponding stressor main effects, indicating that both these main effects remain valid (see Methods).

Flow velocity was highest in fast flow velocity mesocosms (26.5 ± 1.4 cm/s; mean \pm SD), intermediate in medium velocity treatments (13.9 ± 0.7 cm/s), and lowest in slow velocity treatments (0.0 ± 0.0 cm/s). Flow velocity was only slightly affected by the sediment treatments ($P = 0.05$, effect size 0.15) and showed no velocity by sediment interaction.

Table 2.1: Summary (P-values and effect sizes) of ANOVAs comparing physicochemical variables between experimental treatments. Rankings for *post hoc* tests in cases with significant *between-subjects* effects are given in columns 4 and 6. Sediment treatments: C, control; S, small (0 - 0.125 mm); M, medium (0.125 - 0.250 mm); L, large (1 - 2 mm). Flow treatments: S, slow; M, medium; F, fast. Significant P-values are indicated in bold print, and effect sizes (in parentheses) are given for all results where P < 0.10.

Dependent Variable	Sediment	Rankings	Flow	Rankings	Sediment x Flow
Sediment depth	<0.001 (0.92)	L > M > C; S > C	0.08 (0.11)		0.38
Sediment cover	<0.001 (1.0)	L > (M = S) > C	<0.001 (0.78)	(M = S) > F	<0.001 (0.71)
Flow velocity	0.05 (0.15)		<0.001 (1.0)	F > M > S	0.51 (0.11)

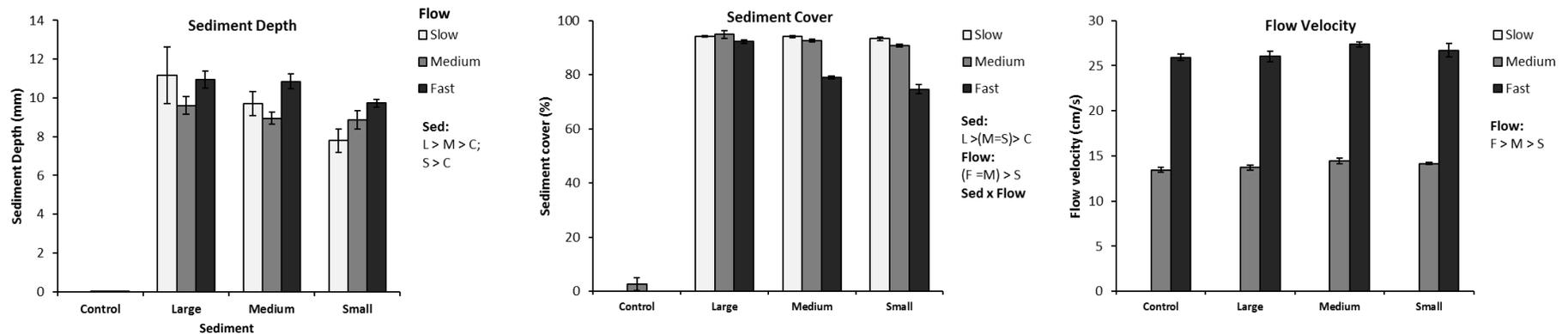


Figure 2.4: Averages of physicochemical variables across the experimental treatments (sediment depth measured every three days for 14 days, sediment cover and flow velocity every three days for 28 days during the manipulative period). Error bars (SEs) show the variation between replicates (n = 5 for each treatment combination). Text in indicates direction of significant stressor main effects or interactive effects (Flow velocity: S, slow; M, medium; F, fast; Sediment: C, control, S, small (0 – 0.125 mm); M, medium (0.125 – 0.250 mm); L, large (1 – 2 mm)).

2.3.2 *Benthic Invertebrate community*

Of the seven benthic invertebrate community-level metrics studied, five responded to the sediment addition treatments, five to the flow velocity manipulations, and there was a single interaction between sediment and flow velocity (see Table 2.2).

Total benthic invertebrate abundance was higher in mesocosms with fast flow velocity than in mesocosms with slow velocity; however, there was no effect of the sediment treatments and no flow by sediment interaction (Table 2.2, Figure 2.5). In contrast to total abundance, invertebrate taxon richness was higher in mesocosms with slow flow than in those with medium flow (Figure 2.5). Taxon richness was also higher in mesocosms without added sediment than in mesocosms with medium-sized sediment.

Overall, EPT abundance was highest in mesocosms without added sediment and much lower across all three sediment addition treatments (Figure 2.5). EPT abundance was also higher in mesocosms with fast flow than in those with medium or slow flow. Sediment and flow effects interacted for this variable, with the negative effect of sediment addition generally being stronger at slow or (especially) medium flow velocity than at fast velocity. This interaction was slightly weaker than the main effect of flow velocity and much weaker than the sediment main effect; therefore, both main effects remain reliable (see Methods).

EPT taxon richness was also higher in controls compared to all mesocosms with added sediment; however, EPT richness did not differ among flow velocity treatments or show a sediment by velocity interaction (Figure 2.5). Finally, the MCI showed no response to either stressor (Figure 2.5).

Simpson's community diversity was higher in control mesocosms than in mesocosms with small added sediment (Figure 2.5). Diversity was also higher in mesocosms with fast velocity compared to slow and medium flow treatments. Pielou's evenness showed similar response patterns as diversity (Figure 2.5): invertebrate communities in mesocosms with no added sediment or the largest added sediment size were more even than communities in mesocosms with the two smaller sediment sizes. Communities in mesocosms with fast flow were also more even compared to communities in mesocosms with medium and slow flow velocity.

Table 2.2: Summary (P-values and effect sizes) of (M)ANOVAs comparing benthic invertebrate responses between experimental treatments. Multivariate *P*-values are for the Pillai's Trace statistic. Rankings for *post hoc* tests in cases with significant between-subjects effects are given in columns 4 and 6. Sediment treatments: C, control; S, small (0 - 0.125 mm); M, medium (0.125 - 0.250 mm); L, large (1 - 2 mm). Flow treatments: S, slow; M, medium; F, fast. Significant *P*-values (with effect sizes in parentheses) are indicated in bold print, and effect sizes (in parentheses) are given for all results where $P < 0.10$.

Dependent variable	%	Sediment	Ranking	Flow	Ranking	Sediment x Flow
Total invertebrate abundance	100	0.74		0.02 (0.16)	F > S	0.13
Invertebrate taxon richness		0.02 (0.20)	C > M	0.02 (0.15)	S > M	0.09 (0.21)
Total EPT abundance		<0.001 (0.66)	C > (L = M = S)	<0.001 (0.33)	F > (M = S)	<0.001 (0.32)
EPT richness		<0.001 (0.27)	C > (L = M = S)	0.15		0.7
Invertebrate diversity (Simpson's)		0.04 (0.25)	C > S	<0.001 (0.26)	F > (M = S)	0.12
Invertebrate evenness (Pielou's)		<0.001 (0.42)	(C = L) > (M = S)	0.01 (0.17)	F > (M = S)	0.12
Macroinvertebrate community index (MCI)		0.54		0.83		0.53
Invertebrate body size		0.02 (0.19)	S > L	0.32		0.16
<i>Deleatidium</i> spp. body size		0.01 (0.23)	S > C	0.19		0.06 (0.23)
Chironomidae body size (excl. Tanypodinae)		<0.001 (0.29)	(C = M) > L	0.22		0.53
Community composition (MANOVA 13 taxa)	99.4	<0.001 (0.57)		<0.001 (0.68)		0.26
Cladocera (Chydoridae)	45	0.34		0.36		0.24
Oligochaeta	34.2	<0.001 (0.54)	(L = M = S) > C	<0.001 (0.27)	(F = M) > S	0.07 (0.22)
Chironomidae (excl. Tanypodinae)	8.2	<0.001 (0.29)	(C = L) > M	<0.001 (0.72)	F > M > S	0.5
<i>Deleatidium</i> spp.	4.4	<0.001 (0.64)	C > (L = M = S)	<0.001 (0.33)	(F = S) > M	0.02 (0.27)
Copepoda	2.6	<0.001 (0.42)	C > M; L > (M = S)	<0.001 (0.30)	S > (F = M)	0.07 (0.22)
Nematoda	1.1	0.12		0.17		0.73
<i>Oxyethira</i> spp.	0.9	0.4		0.04 (0.13)	M > S	0.47
Tanypodinae	0.8	<0.001 (0.53)	C > (L = M = S)	0.99		0.23
<i>Psilochorema</i> spp.	0.6	<0.001 (0.36)	(C = L) > (M = S)	0.02 (0.15)	F > S	0.19
Conoesucidae	0.6	0.08 (0.14)		0.47		0.15
<i>Potamopyrgus antipodarum</i>	0.5	0.89		0.99		0.59
Hydrobiosidae (excl. <i>Psilochorema</i> spp.)	0.3	0.06 (0.15)		<0.001 (0.24)	F > (M = S)	0.5
<i>Hydora</i> spp. (larvae)	0.2	0.58		0.5		0.93

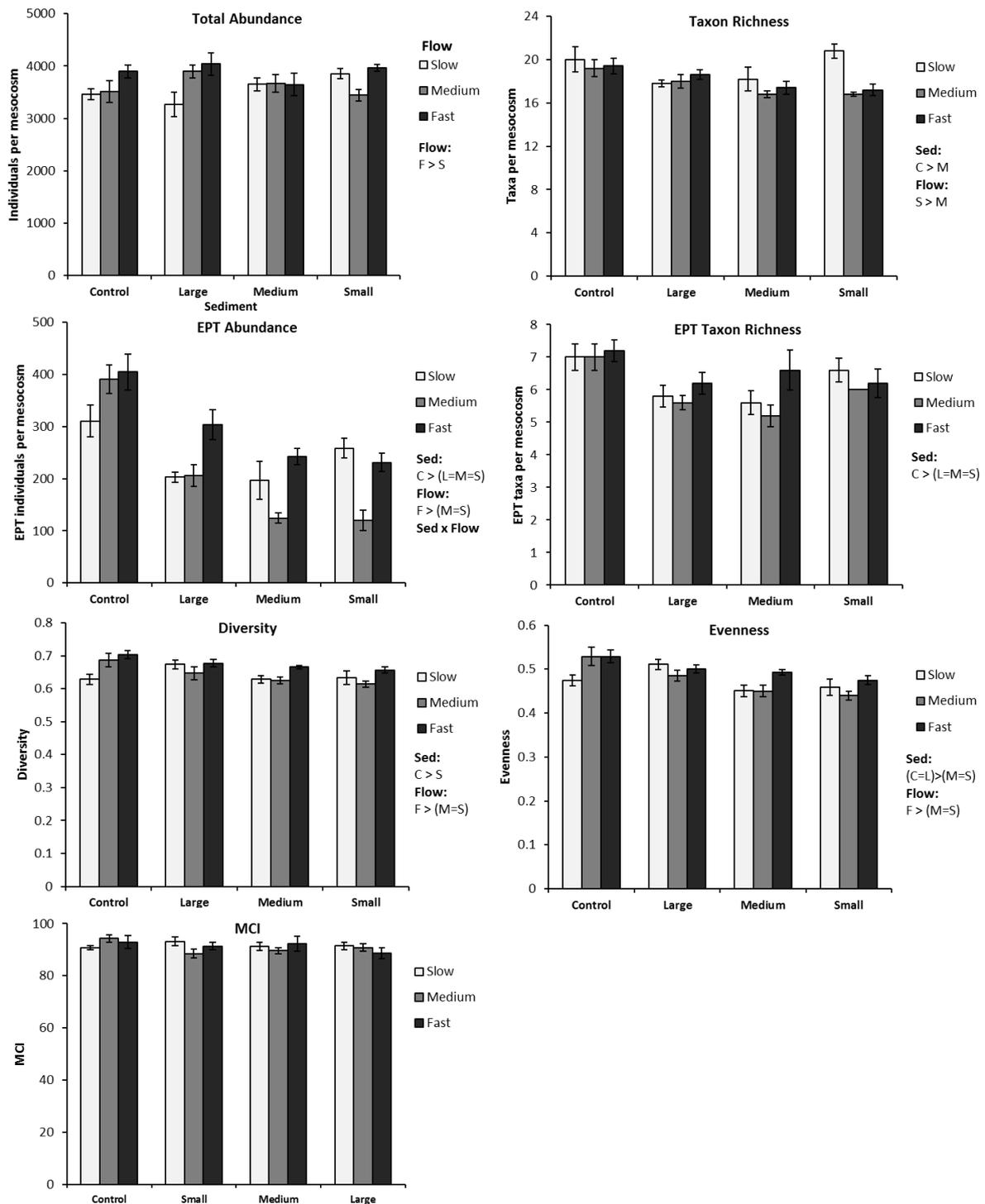


Figure 2.5: Averages of benthic invertebrate community variables across the experimental treatments sampled on day 28. Error bars (SEs) show the variation between replicates ($n = 5$ for each treatment combination). Significant stressor main effects or interactive effects are labeled (Flow velocity: S, slow; M, medium; F, fast; Sediment: C, control, S, small (0 – 0.125 mm); M, medium (0.125 – 0.250 mm); L, large (1 – 2 mm)).

2.3.3 Benthic Invertebrate Body Size

Three benthic invertebrate size metrics were studied. Although all three responded to sediment addition, none of them were affected by flow velocity, and there were no interactions between the two stressors (Table 2.2).

The average size of all benthic invertebrates per sample was larger in mesocosms with small added sediment compared to those with large sediment (Table 2.2, Figure 2.6). Similarly, the average body length of the mayfly nymph *Deleatidium* spp. was greater in small sediment treatments compared to controls (Figure 2.6). Finally, mean body size of Chironomidae larvae (excluding Tanytopodinae) was larger in control and medium sediment treatments compared to the large sediment treatment.

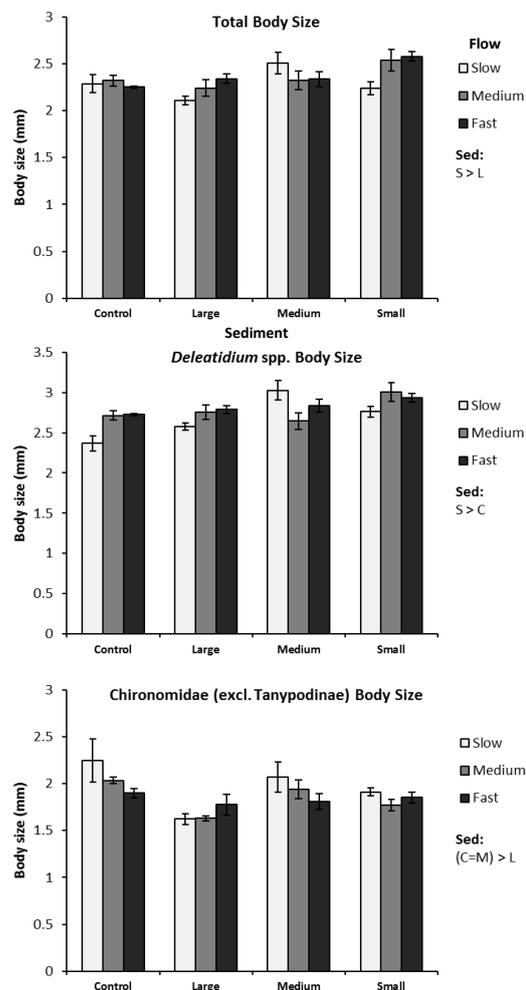


Figure 2.6: Body size patterns and (averages \pm SEs) of the benthic invertebrate assemblage and two common taxa (Chironomidae excluding Tanytopodinae and *Deleatidium* spp.) sampled on day 28. For more details see Fig. 2.5.

2.3.4 Benthic Invertebrate Community Composition and Common Taxa

I identified 55,381 invertebrates belonging to 36 taxa (Table 2.2) in the benthic invertebrate subsamples (1/4 processed per sample, $n = 60$). This equates to 221,524 invertebrates when extrapolated to the entire samples, with an average of 3692 (SE = 54.6) individuals and a mean taxon richness of 18.4 taxa (SD = 0.25) per benthic sample.

Thirteen of the 36 taxa identified occurred in more than 50% of the mesocosms. When combined these common taxa made up 99.4% of the total number of invertebrates recorded (see Table 2.2 for the percentages for each taxon). The MANOVA on the abundances of these 13 common taxa indicated that benthic invertebrate community composition differed across sediment and flow velocity treatments, whereas the sediment by velocity interaction was not significant (Table 2.2). The *between-subjects* results of the MANOVA identified the univariate responses that caused these overall patterns. Five taxa responded to both sediment and flow velocity (Oligochaeta, Chironomidae excluding Tanypodinae, the mayfly larva *Deleatidium* spp., Copepoda, and the caddisfly larva *Psilochorema* spp.), one taxon responded only to sediment (Tanypodinae) and two were affected only by flow velocity (Hydrobiosidae excluding *Psilochorema* spp., and the caddisfly larva *Oxyethira* spp.) (Figure 2.6). Five of the 13 taxa were unaffected by the sediment or flow velocity treatments (the Cladoceran family *Chydoridae*, Nematoda, caddisfly larvae of the family Conoesucidae, the New Zealand mud snail *Potamopyrgus antipodarum*, and larvae of the beetle *Hydora*, Figure 2.7). Significant interactions among stressors only occurred for one taxon (*Deleatidium* spp.).

Oligochaeta preferred mesocosms with added sediment of any size over the control treatments, and medium and fast flow velocity treatments compared to slow flow (Figure 2.6). Chironomidae (excluding Tanypodinae) preferred the control and large sediment treatments over medium-sized sediment (Figure 2.6). They also showed a consistent negative response to flow velocity reduction, decreasing in abundance from fast to medium and further to slow velocity. *Deleatidium* spp. and Tanypodinae responded strongly negatively to sediment addition; both were more abundant in control mesocosms than in any of the mesocosms with added sediment (Figure 2.6). Overall, *Deleatidium* also responded to flow velocity, but inconsistently, and was found in higher abundance in slow or fast treatments compared to medium velocity. Sediment and flow velocity effects interacted for *Deleatidium*, with the negative effect of sediment addition generally being stronger at slow or (especially) medium flow velocity than at fast velocity (similar to the interaction pattern for total EPT abundance).

This interaction was weaker than the corresponding main effects of flow velocity or sediment, indicating that both main effects remain valid (see Methods).

Copepoda were more abundant in large sediment than in medium or small sediment addition treatments, and more common in control than in medium sediment treatments (Figure 2.7). Copepoda also preferred mesocosms with slow flow velocity over medium or fast velocity. *Psilochorema* spp. was more abundant in control and large sediment treatments than in medium or small sediment treatments, and this taxon preferred fast flow over slow flow (Figure 4). *Oxyethira* spp. did not respond to sediment and showed an inconsistent response to flow velocity, preferring medium flow over slow flow (Figure 2.7). Hydrobiosidae (excluding *Psilochorema* spp.) also failed to respond to sediment and were more abundant in fast velocity treatments than in mesocosms with slow or medium flow (Figure 2.7).

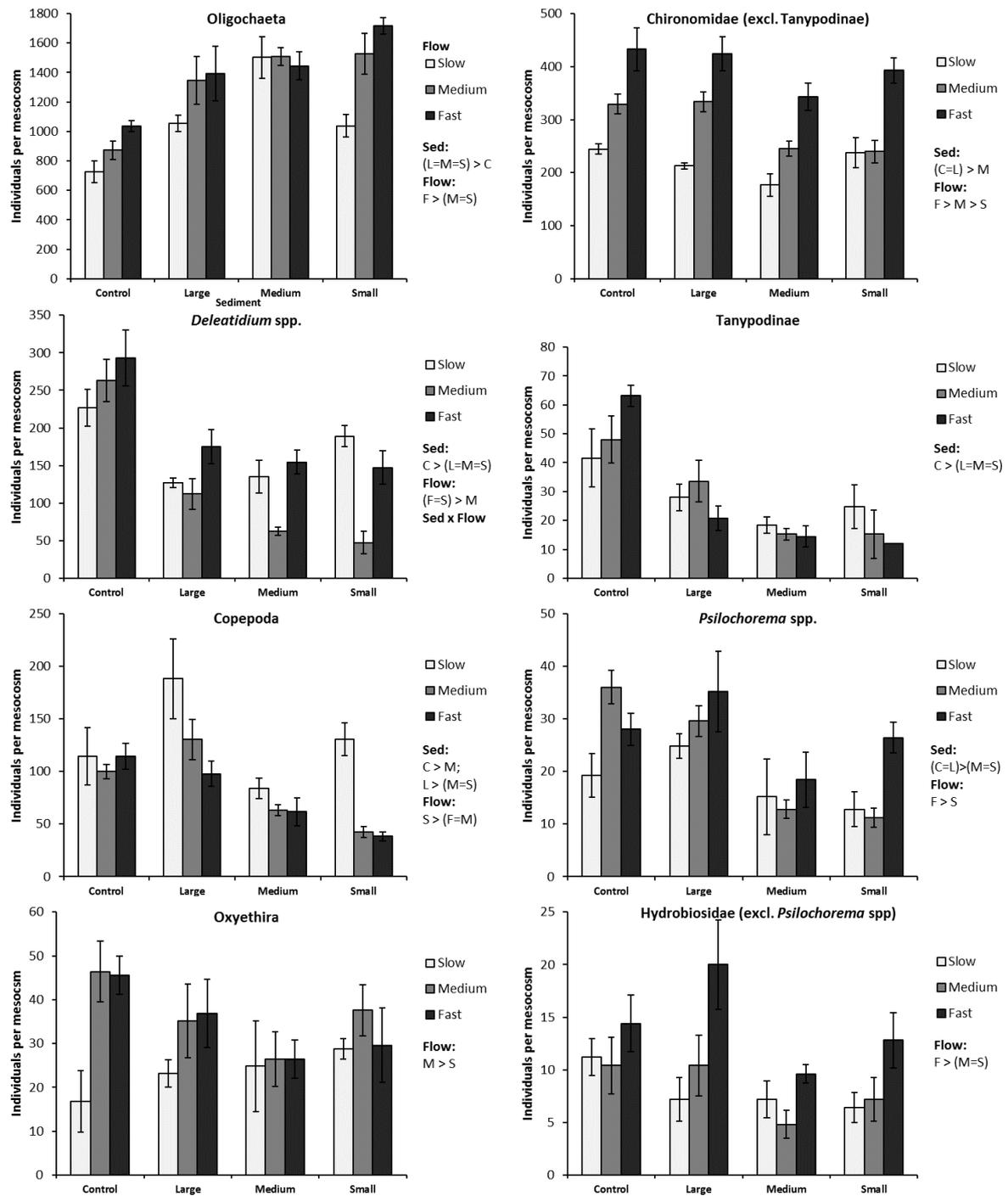


Figure 2.6: Average abundance (\pm SEs) of the eight common taxa in the benthic samples collected on day 28 that responded to one or both stressors. For more details see Fig. 2.5.

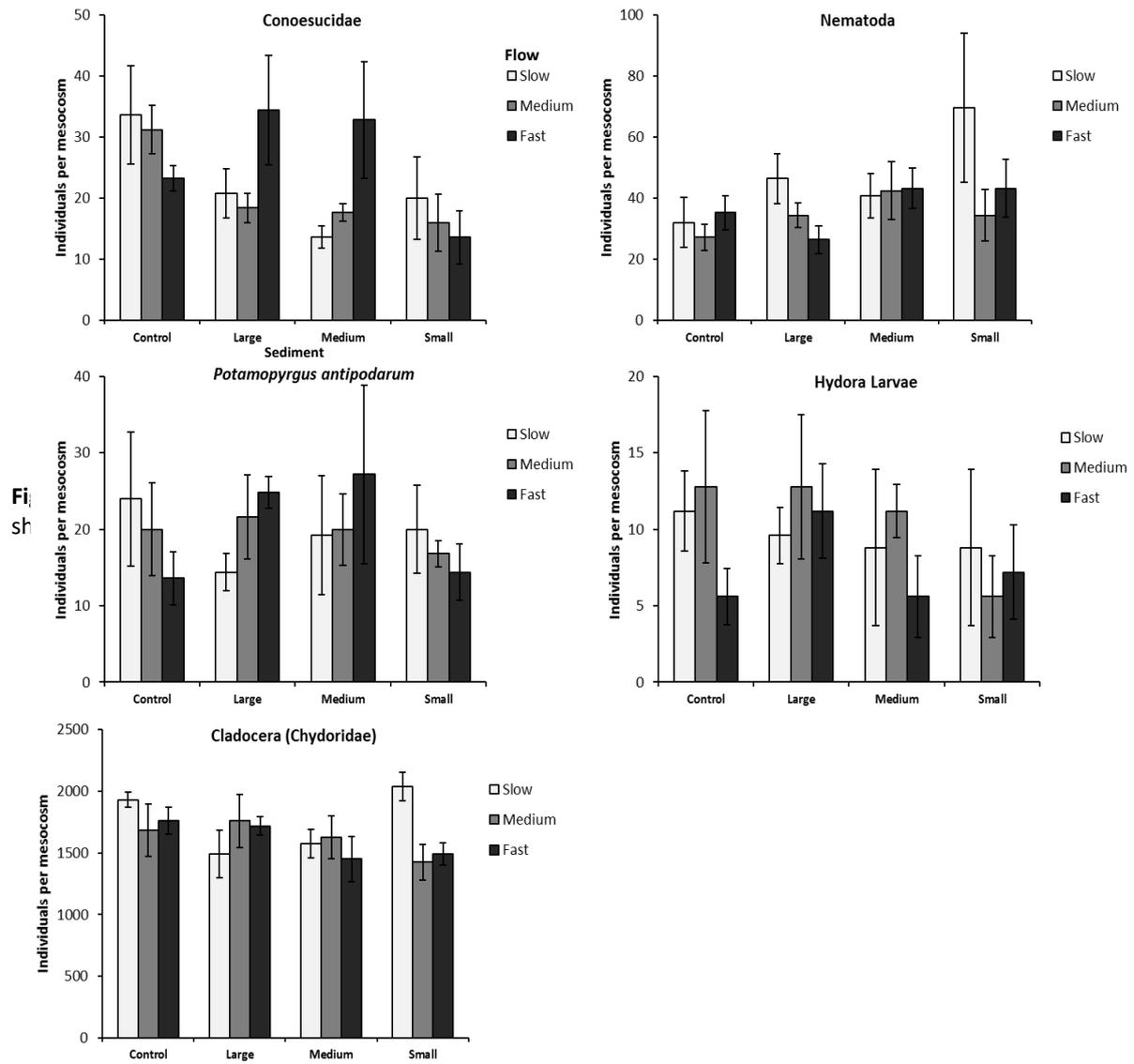


Figure 2.7: Average abundance (\pm SEs) of the five common taxa in the benthic samples collected on day 28 that responded to neither stressor. For more details see Fig. 2.5.

2.3.5 *Invertebrate Drift*

Of the six drift community-level metrics studied, only one was affected by sediment addition, whereas two responded to the flow velocity manipulations, and sediment and flow velocity interacted once (Table 2.3, Figure 2.8).

Overall, total drift propensity and EPT drift propensity were both higher in mesocosms with medium flow velocity compared to those with slow or fast flow (Figure 2.8). Moreover, EPT drift propensity was higher in small sediment treatments than in large sediment treatments or in controls. EPT drift propensity was also higher in mesocosms with medium sediment than in controls. Further, sediment and flow velocity effects interacted, with EPT drift propensity being highest at medium flow velocity in all sediment addition treatments, but not in controls (where drift propensity at slow flow was as high). This interaction was weaker than the main effect of either stressor, indicating that these main effects remain valid (see Methods). Total drift propensity did not differ between sediment treatments. Drift taxon richness, drift EPT richness, Simpson's diversity and Pielou's evenness of the drift community were all unaffected by the sediment or flow treatments (Figure 2.8).

Table 2.3: Summary (P-values and effect sizes) of (M)ANOVAs comparing drifting invertebrate responses, and propensities of common drifting invertebrate taxa, between experimental treatments. See Table 2.2 for further details.

Dependent Variable	%	Sediment	Ranking	Flow	Ranking	Sediment x Flow
Total drift propensity	100	0.97		< 0.001 (0.31)	M > (F = S)	0.53
Drift taxon richness		0.87		0.41		0.57
EPT drift propensity		< 0.001 (0.41)	S > (L = C); M > C	< 0.001 (0.56)	M > (F = S)	0.02 (0.27)
Drift EPT richness		0.92		0.15		0.67
Invertebrate diversity (Simpson's)		0.63		0.31		0.24
Invertebrate evenness (Pielou's)		0.73		0.36		0.48
Invertebrate drift body size		0.87		0.01 (0.19)	F > S	0.33
<i>Deleatidium</i> spp. drift body size		0.21		0.63		0.80
Chironomidae drift body size (excl. Tanypodinae)		0.67		< 0.001 (0.43)	(F = M) > S	0.22
<u>Drift community composition (MANOVA 9 taxa)</u>	98.6	0.24		< 0.001 (0.48)		0.61
Cladocera (Chydoridae)	62.2	0.77		0.01 (0.20)	M > S	0.37
Chironomidae (excl. Tanypodinae)	14.6	0.04 (0.17)	S > L	0.01 (0.17)	M > F	0.38
Oligochaeta	6.1	0.37		0.01 (0.18)	(F = M) > S	0.44
<i>Deleatidium</i> spp.	5.0	0.09 (0.13)		< 0.001 (0.24)	M > (F = S)	0.29
<i>Austrosimulium</i> spp.	3.9	0.60		< 0.001 (0.23)	M > (F = S)	0.40
Conoesucidae	3.2	0.02 (0.20)	S > (L = M = C)	0.71		0.99
<i>Oxyethira</i> spp.	1.8	0.49		0.52		0.41
Copepoda	1.1	0.08 (0.14)		0.26		0.59
Hydrobiosidae (excl. <i>Psilochorema</i> spp.)	0.7	0.44		0.10 (0.10)		0.99

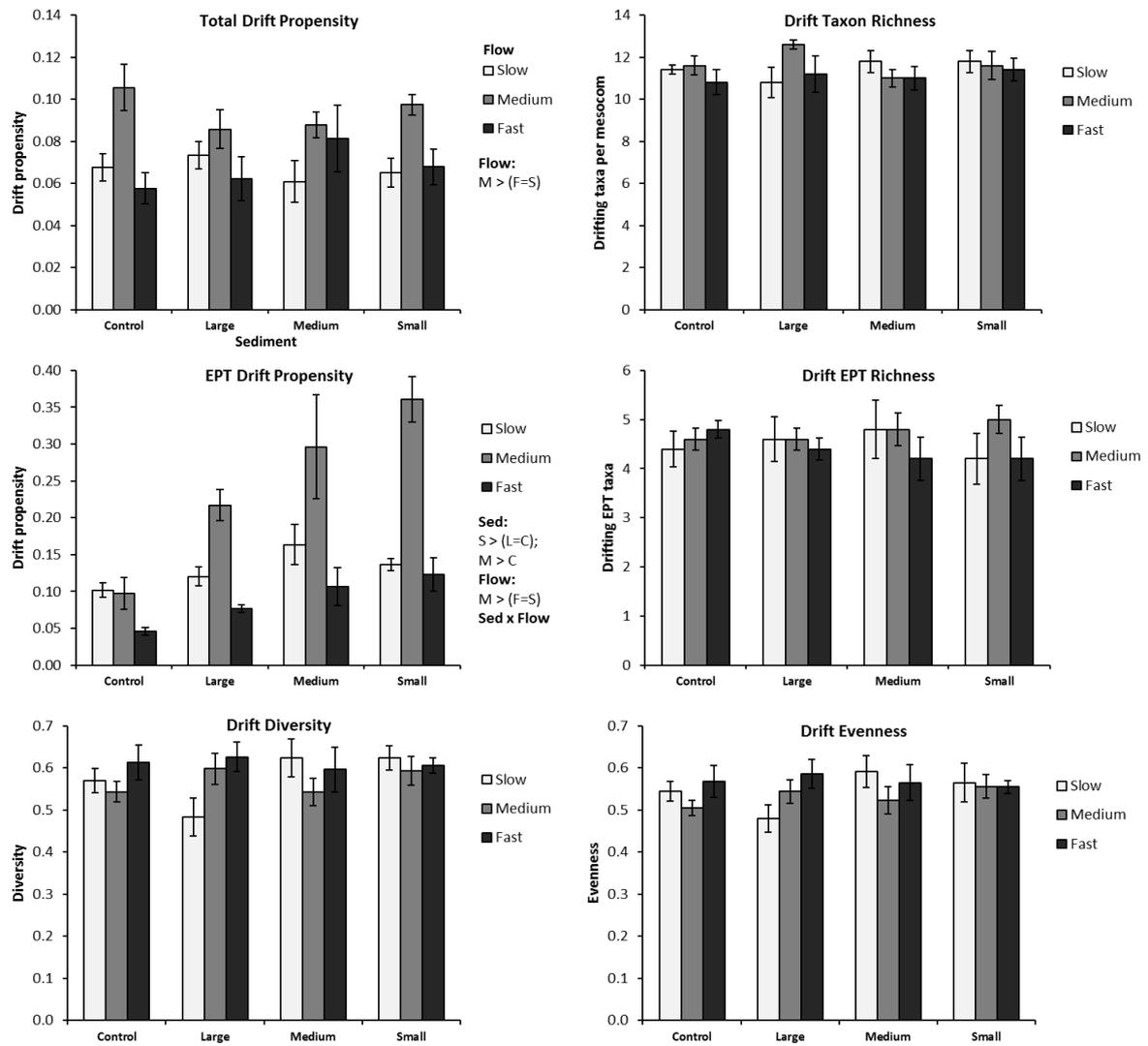


Figure 2.8: Averages of the invertebrate drift community-level variables (\pm SEs) across the drift samples collected over the final 3 days of the experiment (days 25-28). For more details see Fig. 2.5.

2.3.6 Invertebrate Drift Body Size

Of the three drift-community size metrics analysed (mean body length of all drifting invertebrates and body lengths of *Deleatidium* spp. and Chironomidae excluding Tanypodinae), two responded to flow velocity manipulations; however, all three were unaffected by sediment addition (Table 2.3, Figure 2.9). The size of *Deleatidium* spp. was also similar across flow velocity treatments (Figure 2.9). By contrast, mean size of drifting invertebrates was larger in mesocosms with fast flow velocity than in those with slow flow, and drifting Chironomidae were larger in fast and medium flow treatments than in mesocosms with slow flow (Figure 2.9).

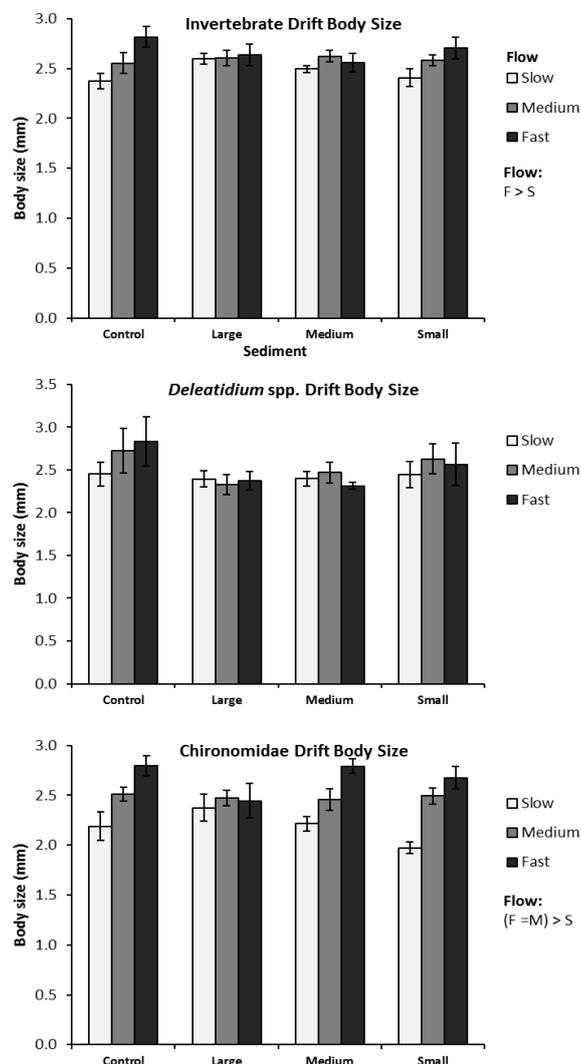


Figure 2.9: Body size patterns (averages \pm SEs) of the total drift assemblage, and two common taxa (Chironomidae excluding Tanypodinae and *Deleatidium* spp.) collected in drift samples over the final 3 days of the experiment (days 25 – 28). For more details see Fig. 2.5.

2.3.7 Invertebrate Drift Community Composition and Common Taxa

I retrieved and counted 16,527 invertebrates from 28 taxa over the final three sampling days of the experiment (Table 2.3). There was an average of 275.5 individuals per drift sample (SE = 10.3), and an average of 11.4 taxa per sample (SE = 0.17).

Nine of the 28 taxa identified made up 98.6% of the total number of invertebrates caught in the drift nets (see Table 2.3 for the percentages for each taxon). Drift propensities (number of invertebrates drifting during the final 3 days divided by final benthic invertebrate abundance; see Methods) of these nine common taxa varied by three orders of magnitude, from < 0.01 to > 10 (see Figures 2.10 & 2.11). The MANOVA on the drift propensities of the common taxa indicated that community composition of drifting invertebrates differed across flow velocity treatments, whereas the sediment treatments and the sediment by velocity interaction was not significant (Table 2.3). The *between-subjects* results of the MANOVA showed that these overall patterns were due to drift propensities of five taxa responding to flow velocity (Chydoridae, Chironomidae excluding Tanypodinae, Oligochaeta, *Deleatidium* spp., and *Austrosimulium* spp.), whereas only two taxa were affected by sediment (Chironomidae excluding Tanypodinae, Conoesucidae) and (Figure 2.10). Drift propensities of three taxa were unaffected by either stressor (*Oxyethira* spp., Hydrobiosidae (excluding *Psilochorema* spp.), and Copepoda) (Figure 2.11). There were no interactions between sediment and flow for common drifting taxa.

Drift propensity of Chironomidae (excluding Tanypodinae) was higher in the small sediment than in the large sediment treatment, and higher in mesocosms with medium flow velocity than in mesocosms with fast velocity (Figure 2.10). Conoesucidae drift propensity was higher in mesocosms with the smallest sediment size compared to all other sediment treatments including controls (Figure 2.10).

Drift propensity was higher in medium flow velocity than in slow velocity mesocosms for Cladocera (Chydoridae), and higher at fast and medium flow compared to slow flow for Oligochaeta (although this taxon generally had a very low drift propensity). For both *Deleatidium* spp. and *Austrosimulium* spp., drift propensity was higher in medium flow velocity treatments than in slow or fast velocity treatments (Figure 2.10).

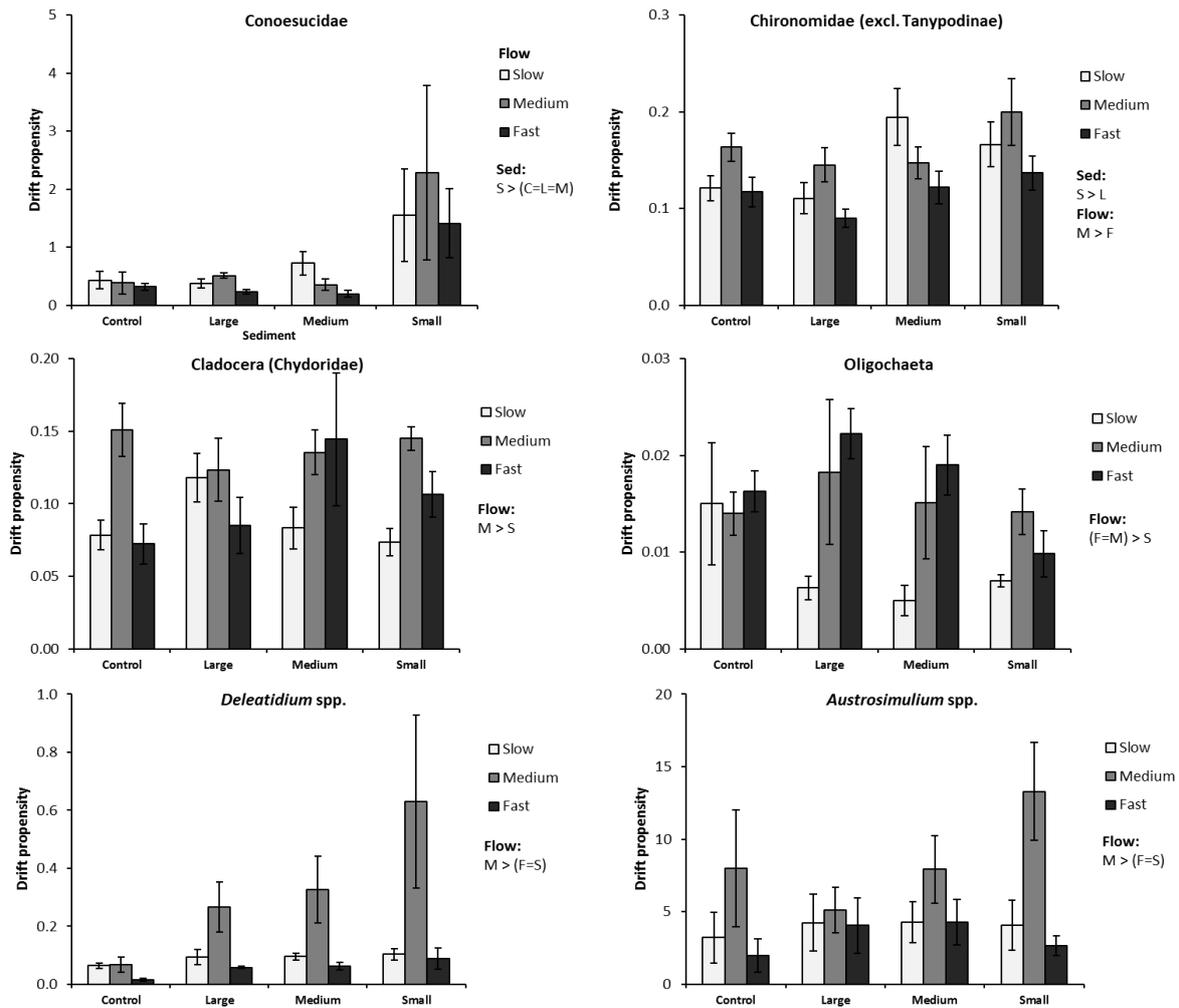


Figure 2.10: Drift propensity patterns (averages \pm SEs) of the six common drifting invertebrate taxa that showed a significant response to the sediment or flow velocity treatments, collected over the final 3 days of the experiment (days 25 – 28). Note the wide range of drift propensities across taxa (from <0.01 to >10). For more details see Fig. 2.5.

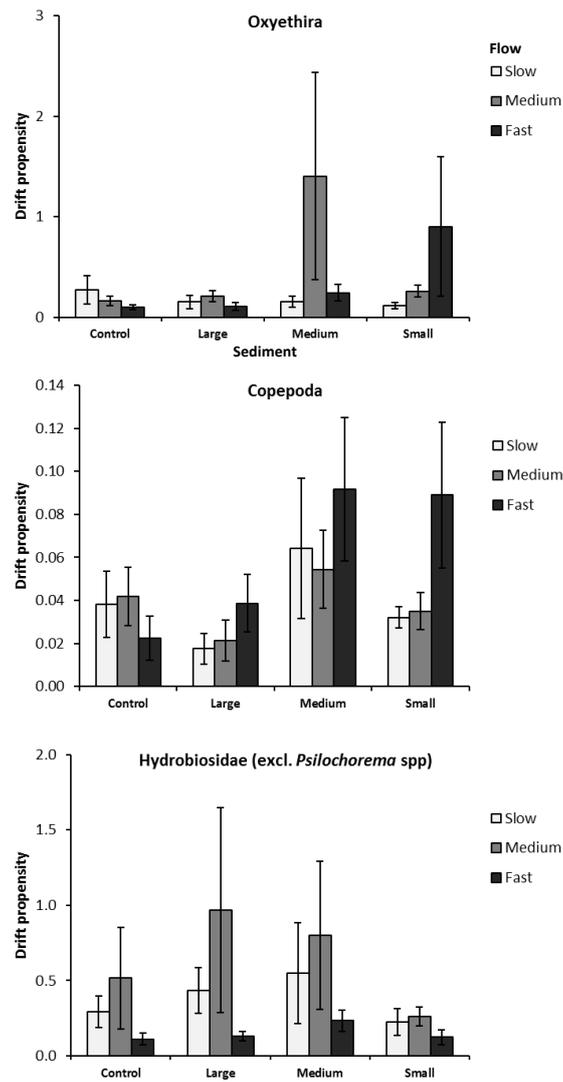


Figure 2.11: Drift propensity patterns (averages \pm SEs) of the three common drifting invertebrate taxa that showed no significant response to either experimental stressor, collected over the final 3 days of the experiment (days 25 – 28). For more details see Fig. 2.5 and Fig. 2.10.

2.3.8 *Insect Emergence*

Of the five emergence community-level metrics analysed, two were affected by sediment addition (Table 2.4, Figure 2.12). All five were unaffected by the flow velocity manipulations, and there were no interactions between stressors. Simpson's diversity of the emergence community and emerged EPT richness were both higher in controls than in mesocosms with small or large added sediment. Total emergence propensity, emerged taxon richness and evenness of the emergence community were unaffected by either stressor.

2.3.9 *Insect emergence body size*

Average body size of all emerged adult insects and body length of emerged adult Chironomidae did not differ among sediment treatments (Table 2.4, Figure 2.12). However, the average size of all emerged insects was larger in mesocosms with fast velocity than in slow velocity treatments, and emerged Chironomidae were larger in medium and fast flow treatments than in mesocosms with slow flow. Body size of emerged adult *Deleatidium* spp. was greater in control mesocosms than in all three sediment addition treatments but did not differ among flow velocity treatments.

2.3.10 *Insect Emergence Community Composition and Common Taxa*

I identified 1,613 emerged insects from four different taxa in all emergence samples collected over the final 3 days of the experiment (Table 2.4, Figure 2.13). There was an average of 26.8 emerged individuals per mesocosm (SE = 1.35), and an average of 1.9 taxa per emergence sample (SE = 0.10).

The total number of emerged insects was low compared to the benthic and drift samples, and only three taxa were common (see Table 2.4 for percentage contributions of each of these taxa). Total emergence propensity did not differ among sediment or flow velocity treatments (Table 2.4). However, the MANOVA on the emergence propensities of the three common taxa indicated that community composition of emerging invertebrates differed across sediment and flow velocity treatments, whereas the sediment by velocity interaction was not significant (Table 2.4). The *between-subjects* results of the MANOVA revealed that responses of two of

the three common taxa caused these overall patterns. Emergence propensity of Chironomidae was higher in small and medium sediment treatments compared to controls, and also higher in the medium velocity than in fast velocity treatments (Figure 2.13). Emergence propensity of *Deleatidium* spp. was higher in control treatments compared to mesocosms with large added sediment (where no *Deleatidium* individuals emerged) (Figure 2.13). By contrast, emergence propensity of the black fly *Austrosimulium* spp. did not differ across sediment or flow velocity treatments (Figure 2.13).

Table 2.4: Summary (P-values and effect sizes) of (M)ANOVAs comparing emerging invertebrate responses, and propensities of common emerged invertebrate taxa, between experimental treatments. See Table 1 for further details.

Dependent Variable	%	Sediment	Ranking	Flow	Ranking	Sediment x Flow
Total emergence propensity		0.21		0.20		0.36
Emergence taxon richness		0.09 (0.13)		0.67		0.66
Emergence EPT richness		< 0.001 (0.26)	C > (L = S)	0.68		0.16
Invertebrate diversity (Simpson's)		0.01 (0.24)	C > (L = S)	0.09 (0.10)		0.41
Invertebrate evenness (Pielou's)		0.49		0.66		0.33
Invertebrate emergence body size		0.87		0.01 (0.19)	F > S	0.33
<i>Deleatidium</i> spp. emergence body size		< 0.001 (0.28)	C > (L = M = S)	0.41		0.20
Chironomidae emergence body size		0.14		0.01 (0.20)	(F = M) > S	0.71
<u>Community composition (MANOVA 3 taxa)</u>	98.7	0.001 (0.19)		0.02 (0.15)		0.09 (0.17)
Chironomidae	94.9	< 0.001 (0.30)	(M = S) > C	0.01 (0.21)	M > F	0.27
<i>Austrosimulium</i> spp.	2.85	0.16		0.61		0.30
<i>Deleatidium</i> spp.	0.93	0.01 (0.21)	C > L	0.18		0.07 (0.22)

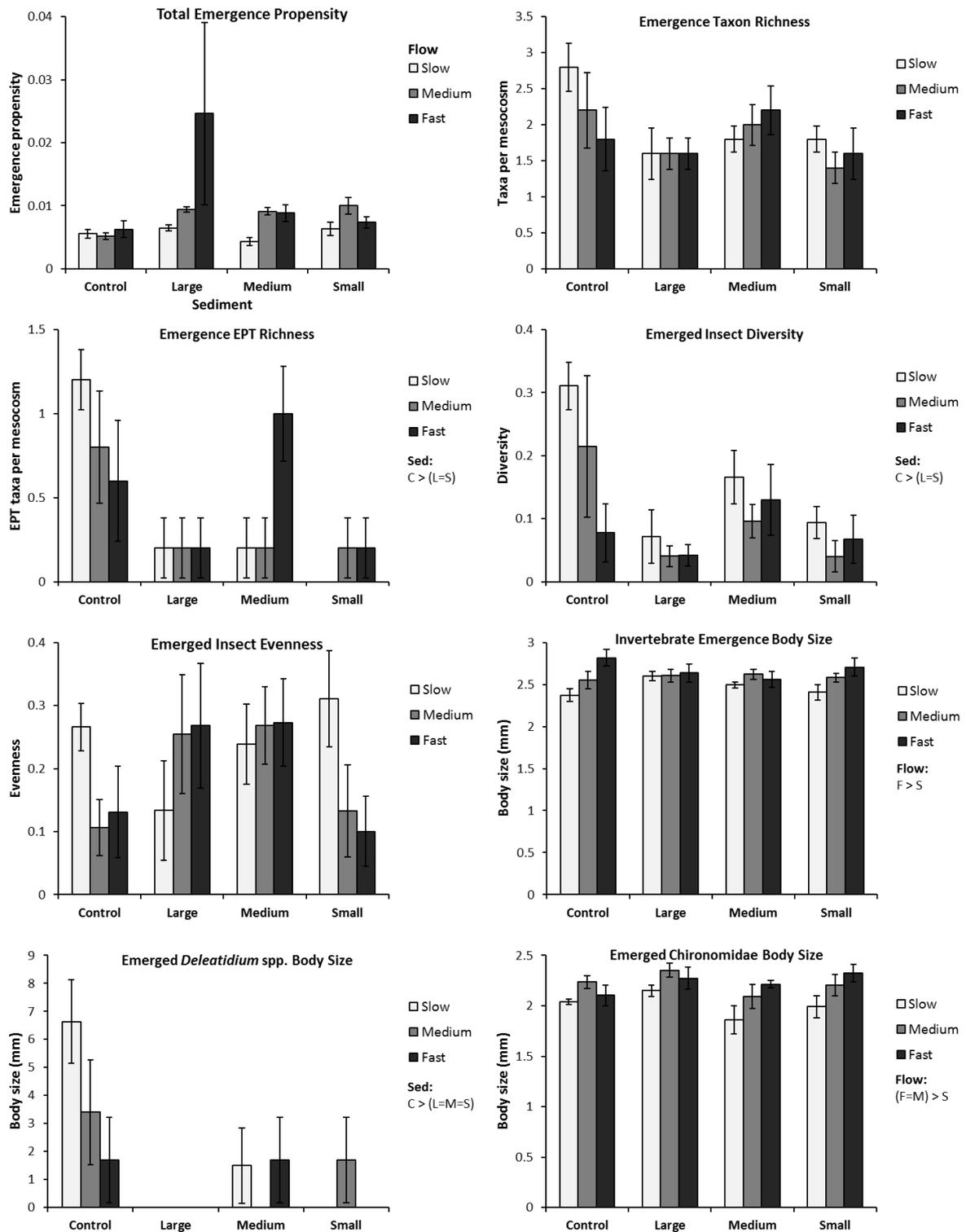


Figure 2.12: Averages (\pm SEs) of emerged insect community-level variables, mean body size of all emerged adult insects and sizes of two common taxa (*Deleatidium* spp. and Chironomidae) across the experimental treatments collected over the final 3 days of the experiment (days 25 – 28) For more details see Fig. 2.5.

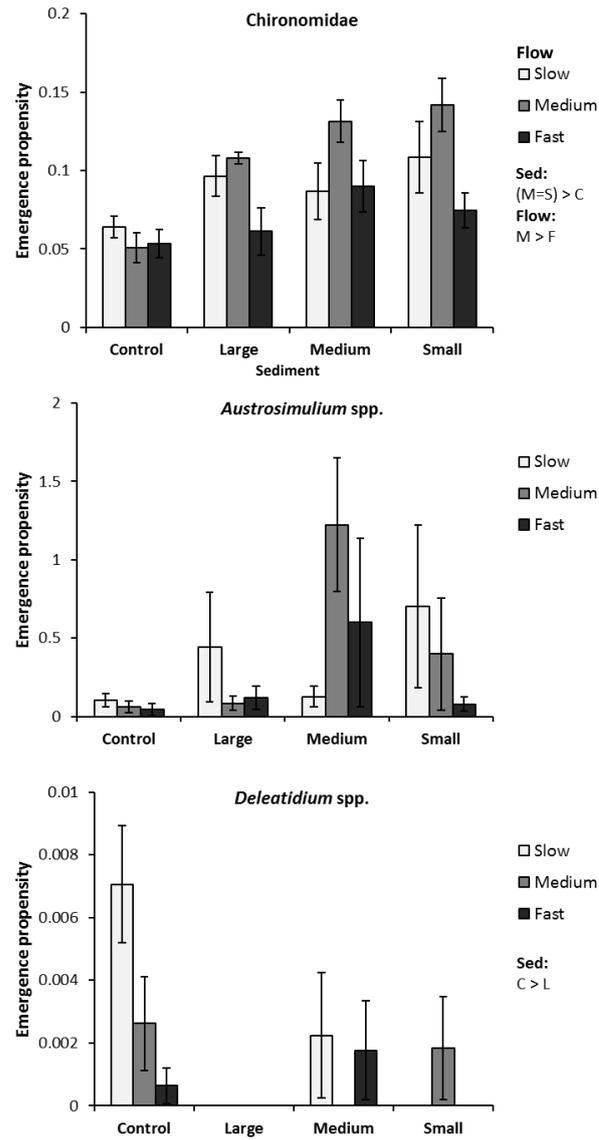


Figure 2.13: Emergence propensities of three common insect taxa (*Chironomidae*; *Austrosimulium* spp., *Deleatidium* spp.) across the experimental treatments collected over the final 3 days of the experiment (days 25 – 28) For more details see Fig. 2.5.

2.4 Discussion

2.4.1 Physicochemical Measures

The experimental treatment combinations resulted in visually different habitats (see Figure 2). Stressor application in this experiment was conclusively successful and, as was evident from the macroinvertebrate community results, the implementation of the twelve different treatment combinations had strong effects on the benthic communities. Flow velocity treatments were stable throughout the manipulative period, and sediment retention was high for all sediment addition treatments. Sediment cover was reduced under fast flow conditions, especially for the two smaller sediment grain sizes. This was expected, as smaller sediment particles are more likely to be re-suspended and flushed out from a given reach by fast flow velocities in real streams (Clapcott *et al.* 2011).

2.4.2 The Two Stressors Compared

Added fine sediment and flow velocity reduction affected 44% and 49%, respectively, of all invertebrate response variables as valid main factor effects (i.e. when the main effect size was larger than any interaction effect sizes; Quinn & Keough, 2002). Fine sediment was not the more pervasive stressor, but the mean effect sizes indicate that it was the stronger of the two stressors (Table 2.5). Fine sediment effects were mainly negative (83% of 24 cases), as were the effects of reduced flow velocity (85% of 27 cases). These results are consistent with those seen in three previous experiments (Matthaei *et al.* 2010; Elbrecht *et al.* 2016; Beermann *et al.* 2018), in which observed negative responses of reduced flow and/or flow velocity were as common, if not more common, than the negative effects of deposited fine sediment. As indicated by the results of the community MANOVAs, both deposited fine sediment and flow velocity are key determinants of macroinvertebrate community composition.

Table 2.5: Numbers and percentages of interpretable significant main effects and interactions for all invertebrate response variables combined. Means of effect sizes \pm standard errors are also shown.

	Sediment	Flow	Sediment x Flow
Overall (55 variables)	24 (44%)	27 (49%)	3 (5%)
Community MANOVA (3 variables)	2	3	0
Mean effect size (significant effects)	0.34 \pm 0.03	0.28 \pm 0.03	0.29 \pm 0.01

2.4.3 Fine Sediment

My first hypothesis predicted that sediment would be a pervasive stressor with mainly negative effects on benthic invertebrate response variables, and my results strongly supported this prediction. Fine sediment addition negatively affected several key community and bioindicator metrics. These include benthic taxon richness, invertebrate diversity and evenness, abundance and richness of benthic EPT taxa, EPT drift propensity, emergence EPT richness, diversity of emerged invertebrates, abundance of 5/13 most common benthic invertebrate taxa (Chironomidae (excluding Tanypodinae), *Deleatidium* spp., Copepoda, Tanypodinae and *Psilochorema* spp), and reduced benthic larval densities, emerged invertebrate body size, and emergence propensity of the mayfly *Deleatidium* spp. The negative impact of deposited fine sediment on this wide range of invertebrate variables add weight to the findings of previous studies and reviews which determined that deposited fine sediment is a master stressor in stream ecosystems, and has strong negative impacts on sensitive benthic invertebrate taxa (e.g. Waters 1995; Wood & Armitage 1997; Matthaei *et al.* 2006; Townsend *et al.* 2010; Piggott *et al.* 2015c; Elbrecht *et al.* 2016; Conroy *et al.* 2018).

My second hypothesis predicted that the smaller the size of the deposited fine sediment, the more severe the negative impacts would be on the benthic invertebrate communities. My findings indicate that the size of fine sediments does indeed matter in some cases; however, for key sensitive species, the presence of deposited fine sediment of any grain size is detrimental. Thus, total benthic abundance and taxonomic richness of larval EPT taxa (mayflies, stoneflies, caddisflies) as well as the benthic abundances of Tanypodinae and *Deleatidium* spp. (the most common EPT taxon) were all negatively impacted by fine sediment regardless of grain size. EPT taxa are highly sensitive to most forms of in-stream pollution. In particular, they are negatively impacted by the presence of fine sediment in the environment; fine sediment homogenizes the benthic habitat, buries individuals, fills interstitial spaces EPT taxa use for protection from predators and other stressors, limits availability of food and oxygen, and reduces life expectancy by damaging sensitive external organs (Waters 1995; Wood & Armitage 1997; Jones *et al.* 2012; Conroy *et al.* 2018). In contrast, benthic Oligochaeta responded in the opposite fashion to this stressor – they were far more abundant in mesocosms treated with all size classes of fine sediment compared to the controls. Benthic invertebrate communities are highly susceptible to change under environmental pressures and stressors, and

these sediment-specific responses show that what may be detrimental to certain taxa can be an opportunity to thrive for other, better-adapted taxa.

There were many other consistent responses to sediment size across the benthic, drift, and emergence communities. Several invertebrate response variables were most affected by either of the two smallest sediment sizes. Also in several cases, the effects of the large sediment size were no different from the controls without added sediment. Response variables which showed these patterns include benthic community evenness, benthic abundance of the caddis *Psilochorema* spp., drift propensity of EPT individuals, as well as the drift propensities of two common taxa (Chironomidae (excluding Tanypodinae) and cased caddisflies of the family Conoesucidae). Further, benthic diversity was most reduced in the mesocosms with the smallest sediment size added, and emergence EPT richness and diversity were negatively affected by only the small sediment.

The two smaller sediment size classes were not as persistent in the benthos as the larger sediment size, but the negative effects of these smaller grain sizes were more consistent and more pervasive. Although the exact mechanisms driving these results are unclear for all metrics, there are numerous potential reasons for these findings. The finer sediment sizes may have resulted in a more compact benthic habitat, proving more difficult for burrowing species to escape from (or penetrate into) (Conroy *et al.* 2018). The finer sediment particles may also have limited potential habitat and refugia considerably more than the larger sediment grains, and consequently limited oxygen and food availability in the substratum, resulting in more harm to individuals through clogging or abrasion of external organs.

Interestingly, and in contrast to predictions by Townsend & Thompson (2007) and observations in a previous experiment (Piggott *et al.* 2015c), the average benthic invertebrate body size was larger in mesocosms with the smallest added sediment. A similar pattern was seen for the sediment-sensitive mayfly *Deleatidium* spp., whose larvae were also larger in the smallest sediment treatments. A possible explanation for this is that smaller individuals may have been more susceptible to the negative impacts of fine sediment particles, whereas larger individuals may have been more resistant to potential negative impacts of the stressor.

2.4.4 Water Abstraction and Flow Velocity

My third hypothesis predicted that reduced flow velocity, simulating varying levels of water abstraction, would have pervasive and predominantly negative effects on the invertebrate communities. My results strongly supported this hypothesis.

Mesocosms with the default fast flow velocity (simulating no water abstraction) had higher total invertebrate abundance and EPT abundance than mesocosms with medium or slow flow. Although total benthic taxon richness was highest in mesocosms with slow flow, the benthic communities were more even and diverse at fast flow compared to medium or slow flow. This indicates that the slow velocity mesocosms had a few additional resident individuals boosting taxon richness, but because all these were rare this result is not indicative of a more healthy or resilient community. These results highlight the importance of flow velocity in determining overall benthic community structure and composition. My study is not the first time similar invertebrate responses have been observed (see e.g. review by Dewson *et al.* 2007; Matthaei *et al.* 2010; Elbrecht *et al.* 2016). Nevertheless, the mounting evidence indicating that water abstraction has detrimental impacts on stream macroinvertebrate communities should be taken into account by those responsible for approving water consents and implementing large-scale irrigation schemes in catchments already under stress from other environmental factors.

Flow velocity also had some consistent, but unexpected, effects on the drift community. Thus, drift propensities of four common drifting invertebrates (Cladocera (Chydoridae), Chironomidae (excluding Tanypodinae), *Deleatidium* spp., *Austrosimulium* spp.) were higher at medium flow velocity compared to slow or fast flow velocity, or to both. Total drift propensity and drift propensity of EPT taxa also followed this pattern. This leads to the question of why it was more common for invertebrates to drift at medium flow velocity, rather than at slow velocity, and why this trend was so consistent.

My fourth hypothesis predicted that the effects of the experimental stressor regimes on benthic invertebrate populations would be mirrored by the corresponding invertebrate drift and/or emergence propensities. Thus, reductions in taxon-specific benthic abundances should be reflected by increased drift or emergence propensity patterns of these taxa, or vice versa (Magbanua *et al.* 2013; Piggott *et al.* 2015c). This hypothesis was largely supported and explains why such low benthic abundances were seen at medium flow velocity for some response variables. Figure 2.14 shows the benthic abundance of EPT individuals and *Deleatidium* (sampled on day 28) and the corresponding drift propensities for EPT individuals

and *Deleatidium* across the drift samples collected over the final 3 days of the experiment (days 25-28). The drop in benthic *Deleatidium* and EPT abundances at medium flow velocity is contrasted by higher drift propensities at medium flow velocity, suggesting that the former was a consequence of the latter. Drift densities were higher in this experiment than in previous studies using the *ExStream* experimental design system in New Zealand (Bruder *et al.* 2017) and in Germany (Beermann *et al.* 2018). The higher drift propensities at medium flow velocity for EPT and *Deleatidium*, as well as the other invertebrate variables that followed similar trends, remain somewhat puzzling, but there are some possible explanations for these patterns.

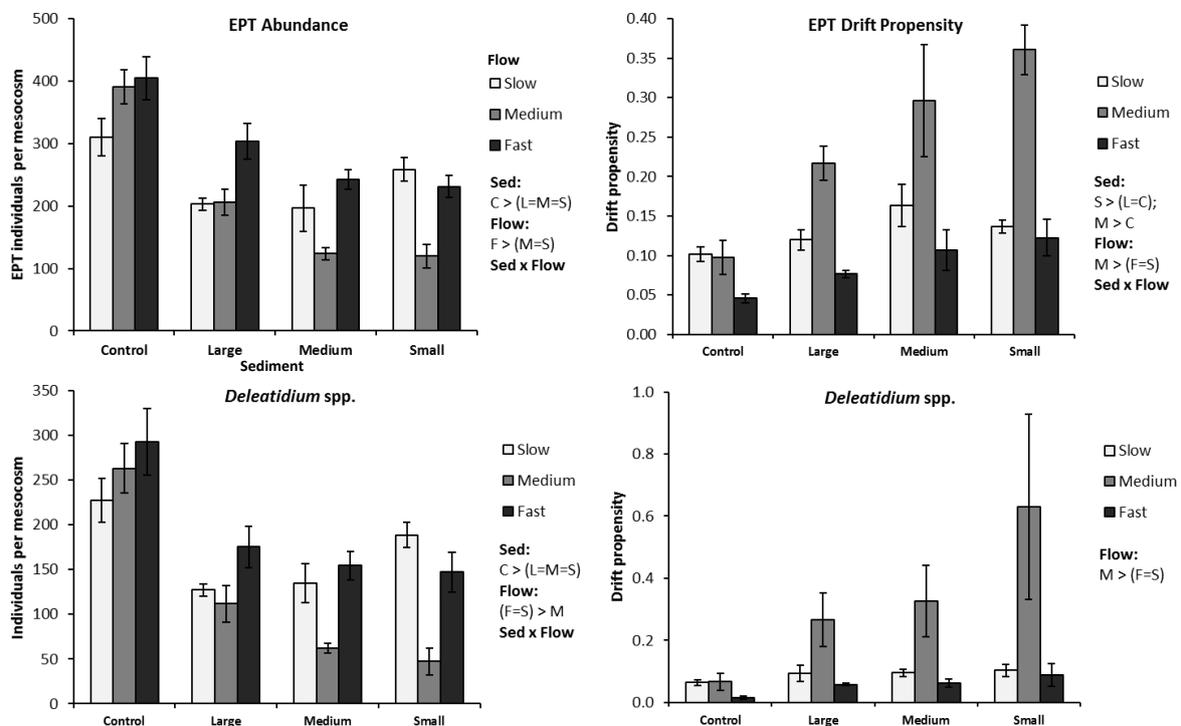


Figure 2.14: Averages for benthic EPT abundance and benthic *Deleatidium* spp. abundance (\pm SEs) sampled on day 28 and for the drift propensity patterns of EPT and *Deleatidium* (\pm SEs) across the drift samples collected over the final 3 days of the experiment (days 25-28).

Due to the greatly reduced flow velocity in the slow flow treatments, individuals may have struggled to drift out of the mesocosms. Excluding the portion of the mesocosms very close to the inflow jet, flow velocity in the slow treatments was a consistent 0.0 cm/s (at least as measured with the Schiltknecht propeller flow meter, which can reliably measure current speeds of 2 cm/s or more). Thus, although individuals may not have favoured their habitat, they

may have been unable to enter the drift in search of new habitat. Individuals may have also temporarily sought refuge in the slow flow mesocosms, waiting for a chance to drift in the event of increased flow velocity. However, *Deleatidium* are very mobile, and can swim and crawl effectively, so this explanation may be dubious.

Higher drift propensities in the medium flow velocity treatments compared to the fast treatments may have been caused by individuals preferring habitats with higher flow velocity than was present in the medium flow treatments, and these individuals may have entered the drift in search of more favourable conditions. It was also observed that at fast flow, compared to medium or slow velocity, a larger amount of the smaller fines were removed or at least rearranged, as the rapid circular flow in the mesocosms pushed this fine sediment towards the centre of the mesocosms. This process may have somewhat alleviated the negative effects of the deposited fine sediment as refugia were formed around the edges of the mesocosms, resulting in a reasonably high-quality habitat that did not induce increased emigration rates via drift.

Drift propensity varied by three orders of magnitude across the common invertebrate taxa in the mesocosms, and although trends in drift propensity were clearly evident, some were much more marked. For example, *Oligochaeta* and *Deleatidium* spp. both showed significant responses to reductions in flow velocity (*Oligochaeta* drift propensity higher at fast or medium flow velocity, *Deleatidium* drift higher at medium flow). However, the largest drift propensity measure for *Oligochaeta* was <0.05 % of the total benthic population over the final three days of the experiment. By contrast, for *Deleatidium* drift propensities measured were as high as 60 % of the total benthic population leaving the mesocosms over the final three days. This probably reflects differences in drift response behaviour, as *Oligochaeta* are not a drifting organism, whereas *Deleatidium* actively drift. *Oligochaeta* likely ended up in the drift samples because of sloughing and being washed out from mesocosms with fast or medium flow velocity, whereas most *Deleatidium* individuals found in drift samples had probably actively left the mesocosms to find more favourable habitat.

It is also worth noting that the two ‘dynamic’ drift propensity metrics for the benthic community (total drift propensity and EPT drift propensity) responded to the experimental stressors, whereas the standard community measures (drift taxon richness, drift EPT richness, diversity, and evenness) did not detect the response that the drift community showed to the implemented stressors. This difference highlights the usefulness of utilising a dynamic metric such as drift propensity when interpreting drift community patterns.

2.4.5 *Multiple-Stressor Effects*

Interactions between sediment and flow were not common. There were three stressor interactions (3/55 cases overall, 5 % of all response variables); two in the benthic community (2/23 cases) and one in the drift community (1/20 cases). My fifth hypothesis predicted that negative fine sediment effects would be more frequent and more severe at reduced flow velocity, and my sixth hypothesis predicted that this negative synergistic interaction should be stronger for smaller fine sediment sizes because this sediment should cause the strongest negative effects. The results weakly supported hypothesis 5 but did not support hypothesis 6.

Additive Effects

Fourteen of the 55 studied invertebrate variables responded to both stressors; however, eleven of these variables showed no interactions between the two stressors, resulting in additive multiple-stressor effects. For example, benthic abundance, drift propensity, and emergence propensity of Chironomidae (excluding Tanypodinae) were all affected by both stressors, but without interactions. A possible reason for these results is that Chironomidae were not identified to the species level, due to time constraints during sample processing. The Chironomidae family in New Zealand is a diverse and cryptic group, and larvae of different species are often indistinguishable unless viewed under a high-powered microscope, with emphasis on determining the shape and structure of their mouthparts. I was unable to take these extra steps during processing of my invertebrate samples due to the large number of Chironomids that needed to be identified (4517 individuals in processed benthic sub-samples). Therefore, potential patterns for individual species or genera within this diverse family and their responses to the manipulated stressors, including potential interactions between stressors, may have been missed. This is an important limitation of my study. As noted by Elbrecht *et al.* (2016), future studies utilising DNA community barcoding techniques and advances in sequencing technology, as opposed to standard visual identification methods, may allow for faster and more accurate identification of individual species responses in taxonomically challenging freshwater invertebrate groups (e.g. Chironomidae) to multiple-stressor applications.

Complex Interactive Effects

Although interactions between stressors were uncommon, the response variables which showed complex interactions included an invertebrate community metric used worldwide in stream biomonitoring and a pollution-sensitive mayfly taxon that is common and widespread throughout New Zealand. In the benthic community, fine sediment addition and flow velocity treatments interacted for EPT abundance and abundance of *Deleatidium* larvae in a similar manner, with the negative effect of added sediment generally being stronger at slow or (especially) medium flow velocity than at fast velocity. These two interactions support my prediction in hypothesis 5, in that the effects of sediment were worsened at reduced flow velocity. However, due to the low frequency of interactions of this nature in general, the hypothesis was only weakly supported overall. These two stressor interactions are a result of a ‘double negative’ interaction (negative effect of both added sediment and reduced flow velocity) that resulted in an effect which is less negative than would have been expected by adding the effect of the two stressors together. Piggott *et al.* (2015b) described interactions of this type as negatively antagonistic and is driven by one of the two stressors having a stronger effect than the other – in this case, fine sediment was the main driver in these interactions. The strength of this effect was not a result of fine sediment grain size, however, as EPT abundance and *Deleatidium* abundance were greater in controls than at all three added sediment size classes, and therefore, these interactions reject hypothesis 6.

In the drift community, sediment and flow velocity effects interacted for EPT drift propensity, which was highest at medium flow velocity in all sediment addition treatments but not in controls. As was the case for the previous two interactions, sediment grain size was not the cause for this interaction, also offering no support for hypothesis 6. The main driver behind the interaction for EPT drift propensity was the strong effect of flow velocity, as opposed to deposited fine sediment for benthic EPT abundance. Possible explanations for the higher drift propensity at medium flow velocity have been postulated above.

2.4.6 Management Implications

Extrapolations of the results in this study to in-field settings should be done with care, due to the experiment’s relatively short nature (4-week manipulative period) and the small spatial scale of the stream mesocosms. However, the *ExStream System* naturally receives high

numbers of immigrating stream organisms (invertebrates, algae, and microbes), and water chemistry, temperature, and light conditions are the same as in the nearby river (Wagenhoff *et al.* 2012; Magbanua *et al.* 2013; Piggott *et al.* 2015a). Further, invertebrate emigration rates (and thus presumably also immigration rates) were higher than in previous experiments (Bruder *et al.* 2017), and were as high as in real small farmland streams in New Zealand (Hansen & Closs, 2007).

Nevertheless, my experiment has demonstrated the severe negative impacts that environmental stressors can have on benthic macroinvertebrate communities. Fine sediment and water abstraction are evidently master stressors in stream ecosystems, and the effects of these environmental pressures will only increase with future climate change (Piggott *et al.* 2015a). In New Zealand, already degraded catchments are threatened by further increases in agricultural land-use intensity, and measures need to be taken rapidly to ensure that stream-side erosion and fine sediment inputs into streams are minimised, large irrigation projects are re-considered, and more sustainable agricultural futures are explored. Streams in catchments with high-intensity agriculture are typically already threatened by multiple stressors (Allan, 2004; Lange *et al.* 2014), and increasing water abstraction in these areas will likely have worse effects on macroinvertebrate communities and other measures of stream health than in streams not already affected by multiple stressors.

Essentially, it is key that managers consider that in stream systems, stressors often work in unison, interactions between stressors can be unpredictable, and outcomes of interactions may be worse than predicted single-stressor effects (Townsend *et al.* 2008, Ormerod *et al.* 2010). In the case of agricultural stressor impacts on sensitive benthic macroinvertebrate species and invertebrate community composition, single-stressor effects and stressor interactions are consistently negative at high intensity (see Townsend *et al.* 2008; Matthaei *et al.* 2010; Wagenhoff *et al.* 2011, 2012; Piggott *et al.* 2012; Magbanua *et al.* 2013, 2016; Elbrecht *et al.* 2016; Beermann *et al.* 2018), and managers should prepare and plan for these interactive outcomes in future scenarios.

The knowledge that individual taxa (e.g. *Deleatidium*, Taypodinae), and sensitive community response variables, namely the total abundance of EPT taxa and EPT taxon richness, are negatively impacted by deposited fine sediment of all tested grain sizes, and often interacting with increasing water abstraction, is a novel discovery. This is just a single study investigating the response of benthic invertebrate communities to fine sediment grain size, and replication on larger scales and in other catchments is necessary to provide further context and

robustness to the results. Nevertheless, regarding the question as to which fine sediment grain size is worst, the results of my study indicate that, especially for pollution-sensitive species, all fine sediment < 2 mm has profound negative effects. Consequently, stream management strategies should prioritise the mitigation and reduction of all fine sediment inputs into streams, as doing so is crucial to maintaining sensitive community components and preserving overall stream health. Sediment inputs can be prevented by maintaining buffer strips at stream edges, fencing to prevent livestock entering waterways, and replanting of riparian strips (Carling *et al.* 2001; Quinn *et al.* 2009; Daigneault *et al.* 2017).

CHAPTER 3

Macroinvertebrate Biological Trait Responses to Fine Sediment Grain Size and Water Abstraction

3.1 Introduction

Freshwater ecosystems have been heavily impacted by human expansion and population growth (Ricciardi & Rasmussen, 1999; Sala *et al.* 2000, Dudgeon *et al.* 2006). This is of increasing concern to managers, especially considering the global importance of freshwater as a resource (Kenny *et al.* 2009, Ormerod *et al.* 2010). Agricultural industries are the largest users of freshwater worldwide (Allan, 2004), and the intensity of agricultural practises has increased in recent decades to supply the demands of the growing human population. Agricultural intensification is the major contributor to decreased stream health and function in New Zealand (Matthaei *et al.* 2010; Scarsbrook *et al.* 2016). Previous research has indicated that all aspects of stream health, including water quality, habitat, and biological communities, decline because of high intensity agricultural activity, and associated agricultural stressors (Allan, 2004).

Stressors are variables that exceed their normal range of variation as a result of human activity, and affect ecosystems (individual taxa, community composition or ecosystem function) either negatively or positively (Piggott *et al.* 2015b, modified from Townsend *et al.* 2008). Multiple stressors can interact with simple or complex outcomes (Folt *et al.* 1999; Crain *et al.* 2008), and the outcomes of stressor interactions may be worse than predicted single-stressor effects (Townsend *et al.* 2008, Ormerod *et al.* 2010). It is of increasing importance for managers to understand when multiple stressors working in concert will produce complex outcomes (as opposed to simple outcomes), and mitigate and minimise the negative effects of multiple stressors in freshwater environments.

The ‘habitat templet theory’ (Townsend & Hildrew 1994) predicts that populations exposed to environmental disturbance and multiple stressors should reflect an increased representation of biological traits linked to resistance (enabling populations to withstand disturbance) and resilience (enabling populations to return to a pre-disturbance state). The present experiment will simulate the impacts on running-water ecosystems of high-intensity farming practices commonly seen in New Zealand. In streams and rivers draining agricultural catchments, deposited fine sediment and reduced flow velocity are two major anthropogenic stressors that can interact in complex ways to affect benthic invertebrate communities (Matthaei *et al.* 2006; Elbrecht *et al.* 2016; Chapter 2 of this thesis).

The input of fine sediment into freshwater systems is a naturally occurring process, and in the absence of anthropogenic effects, fine sediment is a temporary feature of streams and

rivers (Suttle *et al.* 2004). Modifications to the landscape through agricultural activity leads to an increased input of fine sediment into streams and rivers through catchment erosion, removal of riparian strip vegetation, and direct livestock access to waterways (Wood & Armitage, 1997; Allan, 2004). Benthic macroinvertebrate responses to increased fine sediment are generally negative (Waters, 1995; Townsend *et al.* 2008; Matthaei *et al.* 2010; Jones *et al.* 2012; Piggott *et al.* 2012). However, there is some inconsistency in the scientific literature as to how to define 'fine' sediment. Thus, fine sediment has been described as particles < 0.06 mm (e.g. Glendell *et al.* 2014), < 0.5 mm (Suren 2005), < 1 mm (Townsend *et al.* 2008), and < 2 mm (Angradi 1999, Zweig & Rabeni 2001). There is no universally accepted definition or consensus regarding the actual grain size range of fine sediment, and there have been no studies which have investigated whether this grain size plays a role in determining the severity or frequency of its negative effects on benthic invertebrate trait prevalence.

Another key pressure agriculture exerts on running water ecosystems is reduced flow rate, a consequence of water abstraction for irrigation purposes (Allan, 2004). Water abstraction may affect biological responses of stream communities as often as added fine sediment (Matthaei *et al.* 2010; Elbrecht *et al.* 2016; Beermann *et al.* 2018). Water abstraction generates artificial droughts, resulting in uncharacteristic and extended periods of low flow, reduces stream wetted width and depth, and reduces habitat availability downstream of the abstraction site (Gore, 1977; Dewson *et al.* 2007). The effects of water abstraction and reduced flow on streams are especially pronounced in arid areas of the world (Dewson *et al.* 2007). Water abstraction also alters temperature regimes in streams, which in turn disrupts life history patterns of invertebrates and fish, and may lead to increased mortality (Meier *et al.* 2003; Piggott *et al.* 2012).

Further, reduced flow enables increased settlement of fine sediment and organic matter on the streambed (Castella *et al.* 1995; Dewson *et al.* 2007), and the adverse ecological effects of water abstraction can be augmented by elevated levels of fine sediment (Matthaei *et al.* 2010). In combination, these two stressors can result in drastic changes in benthic invertebrate community composition through decreased invertebrate taxon richness and reductions in the abundances of sediment-sensitive taxa, ultimately resulting in a community composition more tolerant of increased environmental pressures (Dewson *et al.* 2007; Townsend *et al.* 2008; Dolédec *et al.* 2011). Previous research has indicated that the use of functional metrics (e.g. biological traits), as opposed to traditional metrics (e.g. taxon richness), can be as effective at

stream and reach scales in detecting community responses to stressors (Richards *et al.* 1997; Statzner *et al.* 1997).

To the best of my knowledge, the present study is the first to investigate how deposited fine sediment of different grain sizes and reduced flow velocity interact to affect the relative abundances of benthic invertebrate biological traits in streamside mesocosm communities. In a recent literature review by Clapcott *et al.* (2017), 14 modalities (categories) of 8 biological traits were identified as suitable functional metrics for detecting effects of deposited fine sediment and water abstraction on trait representation in benthic stream invertebrate communities (data in the review obtained from: Richards *et al.* 1997; Doledec *et al.* 2006; Townsend *et al.* 2008; Pollard & Yuan, 2010; Doledec *et al.* 2011; Lange *et al.* 2014). I have investigated the responses of these 14 trait modalities in my experiment (Hypothesis 1).

I have also expanded the trait set to include all other modalities in these 8 traits (33 total), following a recommendation from Lange *et al.* (2014) who suggested examining all available traits and trait subsets when focusing on rarely studied stressors such as water abstraction, as trait responses to these stressors are still largely unknown. Including all modalities for the chosen traits will allow determining the robustness of the pre-determined trait-set given in Clapcott *et al.* (2017), by testing the predicted responses of these traits (see Table 3.1).

In addition to the 15 predicted responses taken from Table 5 in Clapcott *et al.* (2017) for the 14 trait modalities in Table 3.1, I made the following three predictions regarding the effects of fine sediment grain size and stressor interactions:

(1) The smaller the size of the added deposited fine sediment, the more severe the negative impacts will be on the relative abundances of sediment-sensitive biological traits represented in the benthic invertebrate community. This is because smaller fine sediment classes are more likely to persist on the stream bed and become more embedded, resulting in greater homogenisation and clogging of the bed, as well as causing more harm to sensitive species through abrasion (Waters, 1995; Wood & Armitage, 1997; Jones *et al.* 2012, Extence *et al.* 2013; Glendell *et al.* 2014). Conversely, it is predicted that biological traits that tolerate or favour deposited fine sediment will be more positively affected by smaller sediment sizes.

(2) Negative fine sediment effects on traits which are sediment-intolerant will be more frequent and more severe at reduced flow velocity (Matthaei *et al.* 2010; Elbrecht *et al.* 2016), because sediment is more likely to settle and persist at reduced flow velocities.

(3) This negative synergistic interaction should be stronger for smaller fine sediment sizes because this sediment should cause the strongest negative effects (see Hypothesis 1) and is most likely to be washed out by faster flow velocities.

Table 3.1: Traits identified in the review by Clapcott *et al.* (2017, Table 5) as responding to the agricultural stressors deposited fine sediment and water abstraction.

Stressor	Trait	Trait modality in NZ trait database	Predicted response to stressor increase
Fine sediment	Clinging taxa relative richness	Crawler	Negative
	Merovoltine (≥ 3 -year life cycle)	Semivoltine	Negative
	Multivoltine	Plurivoltine	Negative
	Large body size	SIZE4 (>20-40mm)	Negative
		SIZE5 (>40mm)	Negative
	Scrapers	Scraper	Negative
	Burrowers	Burrower	Positive
Lay unattached eggs at water Surface	Surface	Negative	
Water abstraction	Scrapers	Scraper	Negative
	Respiration of aquatic stages through Gills	Gill	Negative
	Deposit Feeders	Deposit Feeder	Positive
	Predators	Predator	Positive
	Moderate dietary preference	Moderate Specialist	Positive
	Respiration of aquatic stages through Tegument	Tegument	Positive
	Life duration of adults (>365 days)	LDA5 (>365 Days)	Positive

3.2 Methods and Materials

3.2.1 Study Location and Experimental System

The experiment was conducted in Austral summer/early autumn in 60 circular flow-through stream mesocosms installed on the bank of the Kauru River, a fifth-order stream in the Otago province of New Zealand (170°44.60 East, 45°6.50 South, 98 m a.s.l.; Piggott *et al.* 2012). The river contains diverse and abundant invertebrate and algal communities (Liess *et al.* 2009; Herrmann *et al.* 2012), and the water is relatively nutrient-poor (Magbanua *et al.* 2013). The mesocosms naturally receive high numbers of immigrating stream organisms (invertebrates, algae and microbes), and water chemistry, temperature and light conditions are the same as in the nearby river (Wagenhoff *et al.* 2012; Magbanua *et al.* 2013; Piggott *et al.* 2015a). For a detailed description of the *Ex-Stream System* see Piggott *et al.* (2015a).

3.2.2 Experimental Design

Three fine sediment addition treatments (grain sizes 0 - 0.125 mm; 0.125 – 0.250 mm; 1 – 2 mm; see below for details) plus a control with no added sediment were crossed with three flow velocity reduction treatments (fast, medium, slow) in a full-factorial design (12 treatment combinations, 5 replicates of each treatment combination).

Fine sediment was added to the mesocosms in standardised loads of 500 g, resulting in deposited fine sediment levels equivalent to those found occurring in streams flowing through high-intensity agricultural catchments (Matthaei *et al.* 2006, Clapcott *et al.* 2011). Mean sediment cover percentages and depths achieved on Day 0 in the 45 mesocosms with added sediment fell within the range of deposited fine sediment levels observed in farmland streams in southern New Zealand (Matthaei *et al.* 2006; Wagenhoff *et al.* 2011). The size classes will henceforth be referred to as: ‘small’ fine sediment (‘silt’ according to the Wentworth particle scale scale; 0 – 0.1.25 mm); ‘medium’ fine sediment (sand, 0.125 – 0.250 mm); and ‘large’ fine sediment (coarse sand, 1 – 2 mm).

Flow velocity was manipulated at three levels, and these will henceforth be referred to as: ‘fast’ (26.5 ± 1.4 cm/s; mean \pm SE, $n = 20$), ‘medium’ (13.9 ± 0.7 cm/s; mean \pm SE, $n = 20$) and ‘slow’ flow velocity (0.0 ± 0.0 cm/s; mean \pm SE, $n = 20$, means averaged over the duration of the manipulative period based on one measure per mesocosm every 3 days). It is important

to note that the fast flow treatment was essentially the default or natural flow velocity (simulating riffle/run conditions in a small stream), and the medium and slow treatments were implemented to simulate varying levels of water abstraction, which result in reduced in-stream current velocities (Dewson *et al.* 2007; Matthaei *et al.* 2010).

Sediment and flow treatments were randomly assigned within four spatial blocks (each consisting of 15 mesocosms). One full set of all 12 treatment combinations was randomly assigned within each block. The final 12 treatment combinations were randomly assigned across the four blocks (to 3 mesocosms per block). A 25-day pre-colonization period was followed by a 28-day manipulative period when all stressor treatments were implemented. For more details see Chapter 2.

3.2.3 Invertebrate Sampling and Response Variables

At the conclusion of the experimental period, a single benthic invertebrate sample was collected from each mesocosm. Invertebrates were elutriated from the entire substratum and retrieved in a sieve (mesh size 250 μm) and stored in 70% ethanol. Because the benthic samples were too large to process in their entirety, each of these samples was divided into 1/4 and 3/4 subsamples using a rotating subsampler (Waters 1969). The 1/4 subsample for each mesocosm was fully processed (all invertebrates identified to the lowest practical taxonomic level), and the remaining 3/4 of the sample was scanned for rare taxa.

3.2.4 Relative Abundance of Biological Traits in the Invertebrate Community

The information for eight biological traits with 33 categories reflecting invertebrate Life History (three traits for body size, reproduction and development), Resistance and Resilience (one trait for oviposition site), and General Biological Characteristics (four traits for locomotion, feeding and respiration) were obtained from the most recent version of the New Zealand freshwater macroinvertebrate trait database (Clapcott *et al.* 2017; see Table 3.2). This is based on genus or higher-level identifications and utilizes a fuzzy-coding approach which assigns an affinity score from 0 to 3 to each taxon for each category of a given trait (see Doledec *et al.* 2006; Lange *et al.* 2014). A score of 0 indicates no affinity of a taxon to a trait category, whereas a score of 3 indicates a strong association for a given modality. Affinity scores were

treated as a frequency function and standardized so that the sum for a given taxon and trait was equal to 1. The functional composition of the benthic invertebrate community in each mesocosm was expressed as trait relative abundance. I calculated this relative abundance by multiplying the affinity scores for each trait by the abundance of taxa expressing the trait in the mesocosm communities (see Doledec *et al.* 2011).

For certain taxa, the New Zealand freshwater macroinvertebrate trait database has been recently updated (Clapcott *et al.* 2017) to include species-specific affinity scores. Where possible I used these high-resolution scores, but many of my taxa could not be identified accurately to the species level without genetic analysis or specialist consultation, due to small sizes of many individuals and similar morphology between larvae of species groups. In these cases, genus- or family-level scores were used. For a few taxa, the resolution had to be coarser still (e.g. order-level for Cladocera and Ostracoda), but this was a limitation of the database for these groups which are taxonomically understudied in New Zealand. Another limitation of the database is that it does not indicate whether given affinity scores are for stream/river or pond/lake taxa. This limitation was particularly relevant for the trait ‘Attachment to Substrate of Aquatic Stages’ and its modality ‘Swimmer’. In the end I chose to omit this modality because some Burrower/Crawler taxa present in the experimental mesocosms would have been falsely assigned affinity scores for swimming, probably due to the inclusion of lake/pond taxa in the database. For example, the benthic Cladoceran family Chydoridae, which was common in the mesocosms, consists of Crawlers, not Swimmers (personal communication with C. Burns, 2015).

3.2.5 Data Analysis

All statistical analyses were conducted in SPSS 22.0 (IBM SPSS Inc., IL, Chicago, USA). Exploratory data analysis revealed that no transformations of trait response variables were needed because the assumptions of homogeneity of variances and normality were not violated. I wanted to ensure that the fine sediment and flow velocity treatments were implemented successfully. To determine this, I ran individual ANOVAs for sediment depth, percentage sediment cover, and flow velocity. Results and discussion regarding the physico-chemical data can be found in Chapter 2.

Sediment type and flow velocity (plus their interaction) were the two fixed factors in a single MANOVA computed for the trait data (all 34 modalities), and a block factor was also included (for the four mesocosm header tank blocks). The resulting model was intercept (d.f. 1) + sediment type (3) + flow velocity (2) + sediment x flow velocity (6) + block (3). I selected the type III sum of squares, as this is the appropriate method for analysing this type of design in SPSS (Garson, 2012). Because I was interested in differences between each individual sediment treatment and flow velocity level, I modelled both stressors as categorical (rather than continuous) predictors. Due to the larger number of degrees of freedom required to model the interaction term compared to the factor main effects, this analysis may somewhat underestimate the frequency of two-way interactions in the data (Cottingham *et al.* 2005).

Multivariate stressor effects on community-level trait modality composition were determined using the Pillai's Trace statistic, and effects on individual trait modalities were determined by examining the *between-subjects* effects of the MANOVA. When *between-subjects* tests were significant, pairwise comparisons were performed for the factors sediment type and flow velocity using post hoc tests (Tukey's HSD). The rankings for these tests are presented in Table 3.3 for all significant findings. As the results for the block factor were irrelevant for my research objectives, these are not presented below. The level of significance for all tests was $P < 0.05$, and all response patterns described in the results were significant, except for three identified cases where $P = 0.05$. I present standardized effect sizes (partial η^2 values, range 0-1; Garson, 2012) for all findings with $P < 0.10$, to allow readers to evaluate their biological relevance (Nakagawa, 2004). In cases where significant interactions between two or more experimental factors are present, interpretation of the main effects of the factors involved must be done with care. Consequently, I followed the recommendation of Quinn & Keough (2002) and interpreted individual main effects in the presence of a significant interaction only when the effect size of this interaction was smaller than the size of the corresponding significant main effect(s).

Table 3.2: Benthic invertebrate biological traits and their categories (adapted from Clapcott *et al.* 2017).

Biological Traits		Trait Modality	Trait Acronym	
Life History	Maximum Potential Size	≤ 5 mm	SIZE1	
		> 5 - 10 mm	SIZE2	
		> 10 - 20 mm	SIZE3	
		> 20 - 40 mm	SIZE4	
		>40	SIZE5	
	Maximum Reproductive Cycles per Year	Univoltine	UNIVOLTINE	
		Semivoltine	SEMIVOLTINE	
		Plurivoltine	PLURIVOLTINE	
	Life Duration of Adults	≤ 1 day	LDA1	
		> 1 - 10 days	LDA2	
		> 10 - 30 days	LDA3	
		> 30 - 365days	LDA4	
		> 365 days	LDA5	
	Resistance and Resilience	Oviposition Site	Water Surface	SURFACE
			Submerged	SUBMERGED
Terrestrial			TERRESTRIAL	
Eggs Endophytic			EGGSEND0	
General Biological Characteristics	Attachment to Substrate of Aquatic Stages (excluding eggs)	Crawlers (epibenthic)	CRAWLER	
		Burrowers (infauna)	BURROWER	
		Attached	ATTACHED	
	Feeding Habits	Scrapers	SCRAPER	
		Deposit Feeders	DEPOSITFEEDER	
		Filter Feeders	FILTERFEEDER	
		Predator	PREDATOR	
		Algal piercer	ALGALPIERCER	
		Shredders	SHREDDER	
	Dietary Preferences	Specialist (strong preferences)	SPECIALIST	
		Moderate Specialist	MODERATE	
		Generalist (weak preferences)	GENERALIST	
	Respiration of Aquatic Stages (excluding eggs)	Tegument	TEGUMENT	
		Gill	GILL	
		Aerial	AERIAL	
Plastron		PLASTRON		

3.3. Results

3.3.1 *Physicochemical Measures*

Three physicochemical variables were analysed to determine if the experimental stressor applications were successful: fine sediment depth (mm), fine sediment cover (visual estimate, 0-100%), and flow velocity (cm/s). Each variable was averaged for each mesocosm across the entire manipulative period (see Methods). All three variables indicated that the stressor manipulations were highly effective, and directions of stressor main effects and interactions conformed to predictions (for more in-depth analysis of these physicochemical measures see the Results section in Chapter 2) Flow velocity treatments were stable throughout the duration of the experiment, and sediment retention was high for all sediment sizes. Sediment cover was reduced at high flow conditions, especially for the two smaller sediment grain sizes.

3.3.2 *Biological Trait Responses to Stressor Treatments*

I analysed the responses of eight biological traits to experimental stressors. These eight traits consisted of 33 categories, or modalities (Table 3.3). The traits were split into three main groups: Life History traits (Maximum Potential Size, Maximum Reproductive Cycles per Year, Life Duration of Adults), Resistance and Resilience traits (Oviposition Site), and General Biological Characteristics (Attachment to Substrate of Aquatic stages (excluding eggs), Feeding Habits, Dietary Preferences, Respiration of Aquatic Stages (excluding eggs)). The MANOVA on the relative abundances of these 33 trait modalities indicated that the trait composition of the benthic invertebrate community differed across sediment (Pillai's Trace P -value = <0.001 ; effect size 0.79) and flow velocity treatments (P = <0.001 ; effect size 0.79), whereas the sediment by velocity interaction was not significant (P = 0.47). The between-subjects results of the MANOVA identified the responses of the individual trait modalities that caused these overall patterns.

Fine sediment treatments affected the relative abundances of 25 of the 33 trait modalities, and flow velocity reduction treatments affected the prevalence of 26 modalities (Table 3.3). In total, 16/25 of the trait modalities which responded to sediment showed negative responses, and 14/26 flow velocity reduction responses were negative. The relative abundances

of 12 trait modalities (including three borderline cases where $P = 0.50$; see Table 3.3) were affected by an interaction between the fine sediment and flow velocity treatments.

3.3.3 Life History Traits

I analysed three Life History Traits, which consisted of 13 trait modalities: Maximum Potential Size (5 modalities), Maximum Reproductive Cycles per Year (3), and Life Duration of Adults (5). Sediment treatments affected 11/13 trait modalities for life history traits, and flow treatments affected 10/13 trait modalities (Table 3.3). Further, five life history trait modalities were affected by interactions between the two stressors.

Maximum Potential Size

This trait is divided into five modalities: invertebrates with a maximum size of ≤ 5 mm (SIZE1), $>5-10$ mm (SIZE2), $>10-20$ mm (SIZE3), $>20-40$ mm (SIZE4) and >40 mm (SIZE5) (Figure 3.1). The prevalence of three size modalities were affected by sediment treatments, two modalities were affected by flow velocity treatments, and two modalities were affected by interactions between stressors (Table 3.3, Figure 3.1).

Invertebrates with a maximum size ≤ 5 mm were more prevalent in control mesocosms (no added sediment) than in mesocosms with added small or medium fine sediment (Fig. 3.1). This invertebrate size category was also more common in mesocosms with slow flow than in medium and fast flow velocity treatments. Invertebrates $>5-10$ mm were more common in controls than in all three sediment addition treatments and showed an interaction between sediment and flow velocity that overrode the weaker flow velocity main effect. This size category responded positively to reduced flow velocity in controls and (most markedly) when small fines were added, but this effect was absent when large or medium fines were added. Invertebrates $>10-20$ mm were more prevalent in all sediment addition treatments than in controls, and in fast and medium flow than in slow flow treatments. Invertebrates $>20-40$ mm were generally rare and did not respond to either stressor. Invertebrates >40 mm, which were even rarer, did not respond to added fine sediment or flow velocity, but showed an interaction between stressors. This size category was positively affected by small sediment treatments, but only at the slowest flow velocity.

Table 3.3: Summary (P-values and effect sizes) of the between-subjects results of the MANOVA comparing biological trait responses between experimental treatments. Rankings for *post hoc* tests in cases with significant between-subjects effects are given in columns 4 and 6. Sediment treatments: C, control; S, small (0 - 0.125 mm); M, medium (0.125 - 0.250 mm); L, large (1 - 2 mm). Flow treatments: S, slow; M, medium; F, fast. Significant P-values are indicated in bold print, and effect sizes (in parentheses) are given for all results where $P < 0.10$. ‘*’ indicates cases where an interaction between stressors prevented a ranking for the corresponding main effect (effect size of interaction term larger than size of a main effect/s, see Methods).

Biological Trait	Trait Modality	Sediment	Ranking	Flow	Ranking	Sediment x Flow	
<i>Life History</i>	Maximum Potential Size	SIZE1 ≤5mm	0.001 (0.30)	C > (M = S)	0.003 (0.23)	S > F	0.22
		SIZE2 >5-10mm	<0.001 (0.69)	C > (L = M = S)	0.004 (0.21)	*	0.02 (0.27)
		SIZE3 >10-20mm	<0.001 (0.45)	(L = M = S) > C	0.004 (0.23)	(F = M) > S	0.11
		SIZE4 >20-40mm	0.07 (0.14)		0.23		0.59
		SIZE5 >40mm	0.07 (0.14)		0.08 (0.10)		0.03 (0.25)
	Maximum Reproductive Cycles per Year	Semivoltine	<0.001 (0.66)	C > (L = M = S)	<0.001 (0.30)	*	0.01 (0.30)
		Univoltine	<0.001 (0.33)	(M = S) > C	0.007 (0.20)	(F = M) > S	0.11
		Plurivoltine	<0.001 (0.32)	C > (M = S)	0.007 (0.20)	S > (F = M)	0.12
	Life Duration of Adults	LDA1 ≤1 Day	<0.001 (0.67)	C > (L = M = S)	<0.001 (0.28)	(F = S) > M	0.03 (0.26)
		LDA2 >1-10 Days	<0.001 (0.64)	C > L > M; C > S	<0.001 (0.49)	F > (M = S)	0.13
		LDA3 >10-30 Days	0.005 (0.24)	C > (M = S)	<0.001 (0.26)	S > (F = M)	0.25
		LDA4 >30-365 Days	<0.001 (0.68)	(M = S) > L > C	<0.001 (0.39)	(M = S) > F	0.059 (0.23)
		LDA5 >365 Days	<0.001 (0.51)	(L = M = S) > C	0.057 (0.12)		0.07 (0.22)
<i>Resistance and Resilience</i>	Oviposition Site	Surface	<0.001 (0.72)	C > (L = M = S)	<0.001 (0.30)	(F = S) > M	0.02 (0.28)
		Submerged	<0.001 (0.71)	(L = M = S) > C	<0.001 (0.34)	(M = S) > F	0.03 (0.25)
		Terrestrial	<0.001 (0.40)	C > (M = S); L > M	<0.001 (0.45)	F > M > S	0.93
	Eggs Endophytic	0.37		0.12		0.26	
<i>General Biological Characteristics</i>	Attachment to Substrate of Aquatic Stages (excluding eggs)	Crawler (epibenthic)	<0.001 (0.67)	C > L > M; C > S	0.004 (0.22)	*	0.009 (0.31)
		Burrower (infauna)	<0.001 (0.67)	M > L > C; S > C	0.003 (0.22)	*	0.007 (0.31)
		Attached to Substrate	0.04 (0.16)	C > M	0.54		0.79
	Feeding Habits	Scrapers	<0.001 (0.57)	C > (L = M = S)	0.003 (0.23)	*	0.02 (0.28)
		Deposit Feeders	<0.001 (0.53)	M > L > C; S > C	0.005 (0.21)	*	0.055 (0.23)
		Filter Feeders	0.008 (0.23)	C > M	0.002 (0.24)	S > (F = M)	0.23
		Predators	0.002 (0.28)	(L = M = S) > C	0.003 (0.22)	(F = M) > S	0.14
		Algal Piercers	0.36		0.04 (0.13)	M > S	0.52
	Dietary Preferences	Shredders	0.11		0.59		0.46
		Specialist	0.001 (0.28)	(M = S) > C	<0.001 (0.31)	(F = M) > S	0.10 (0.21)
		Moderate Specialist	<0.001 (0.48)	(L = M = S) > C	0.002 (0.24)	(F = M) > S	0.059 (0.23)
	Respiration of Aquatic Stages (excluding eggs)	Generalist	<0.001 (0.39)	C > (L = M = S)	<0.001 (0.28)	S > (F = M)	0.07 (0.22)
		Tegument	0.73		0.005 (0.21)	S > F	0.64
Gill		0.44		0.02 (0.16)	F > S	0.51	
Aerial		0.001 (0.29)	C > (M = S)	<0.001 (0.39)	F > (M = S)	0.29	
	Plastron	0.62		0.25		0.88	

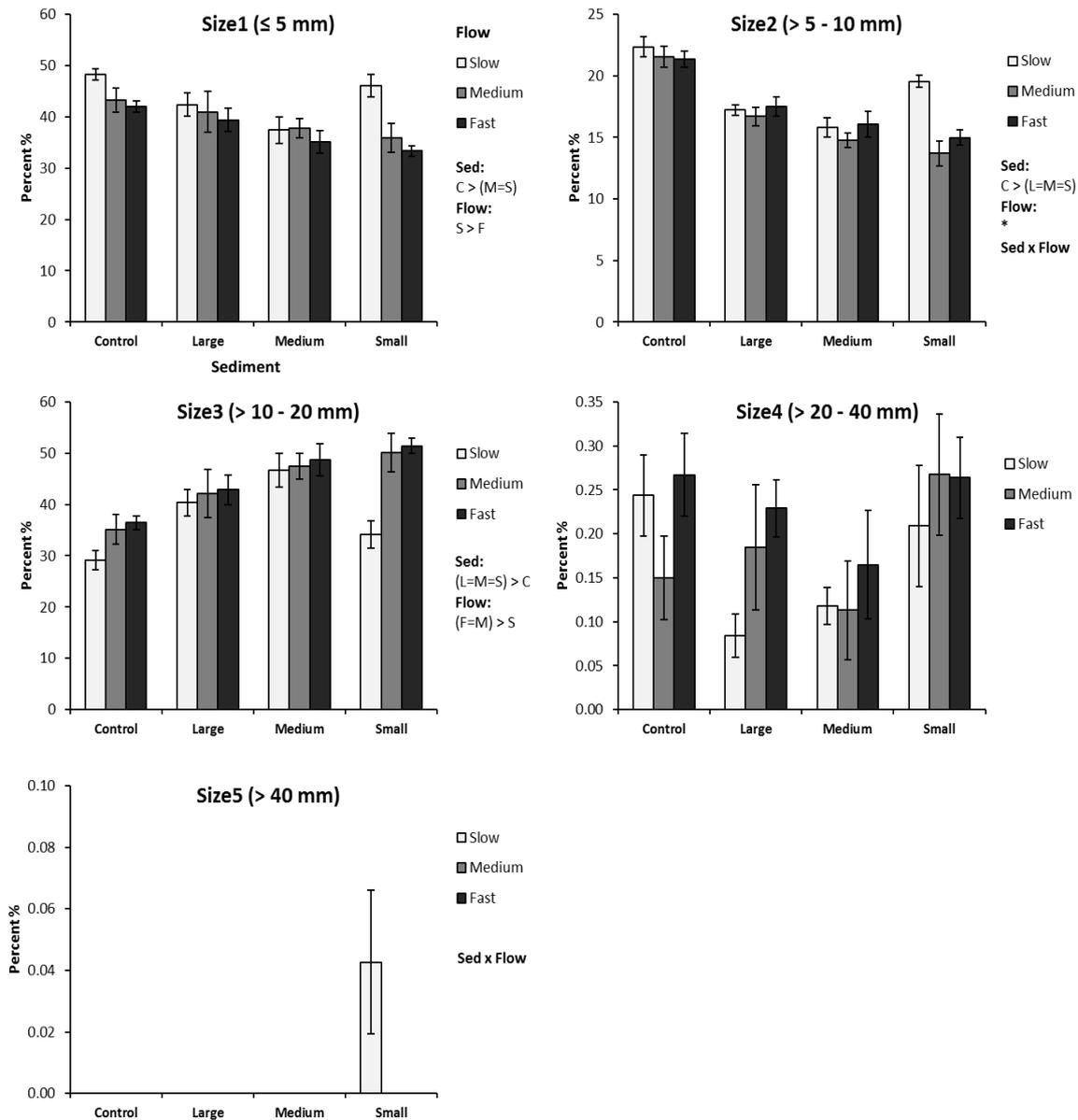


Figure 3.1: Mean relative abundances of trait modalities in the benthic invertebrate community for the trait 'Maximum Potential Size' across the experimental treatments sampled on day 28. Error bars (SEs) show the variation between replicates ($n = 5$ for each treatment combination). Significant stressor main effects (post-hoc test rankings) or interactive effects are indicated in each plot (Flow velocity: S, slow; M, medium; F, fast; Sediment: C, control, S, small (0 – 0.125 mm); M, medium (0.125 – 0.250 mm); L, large (1 – 2 mm). '*' indicates cases where a stronger interaction between stressors overrode the main effect in question.

Maximum Reproductive Cycles per Year

The three modalities for this trait reflect different breeding strategies: Semivoltine (reproduction cycle greater than 1 year), Univoltine (one reproductive cycle per year), and Plurivoltine (>1 reproductive cycles per year). All three modalities were significantly affected by the sediment and flow velocity treatments, and there was a single interaction (Table 3.3, Figure 3.2).

Semivoltine taxa were relatively rare overall and more abundant in controls than in mesocosms with deposited fine sediment regardless of grain size (Fig. 3.2). This trait modality was also affected by an interaction between sediment and flow velocity which overrode the similar-sized velocity main effect. In this interaction, Semivoltine taxa were less common at medium flow compared to fast or slow flow, but only when fine sediment (of any size) was present in the mesocosms. Univoltine organisms were more prevalent in mesocosms with added medium or small sediment than in controls, and also in fast or medium flow mesocosms than in slow flow mesocosms. Conversely, Plurivoltine invertebrates were more common in controls than in medium or small sediment addition treatments, and also at slow flow compared to fast or medium flow.

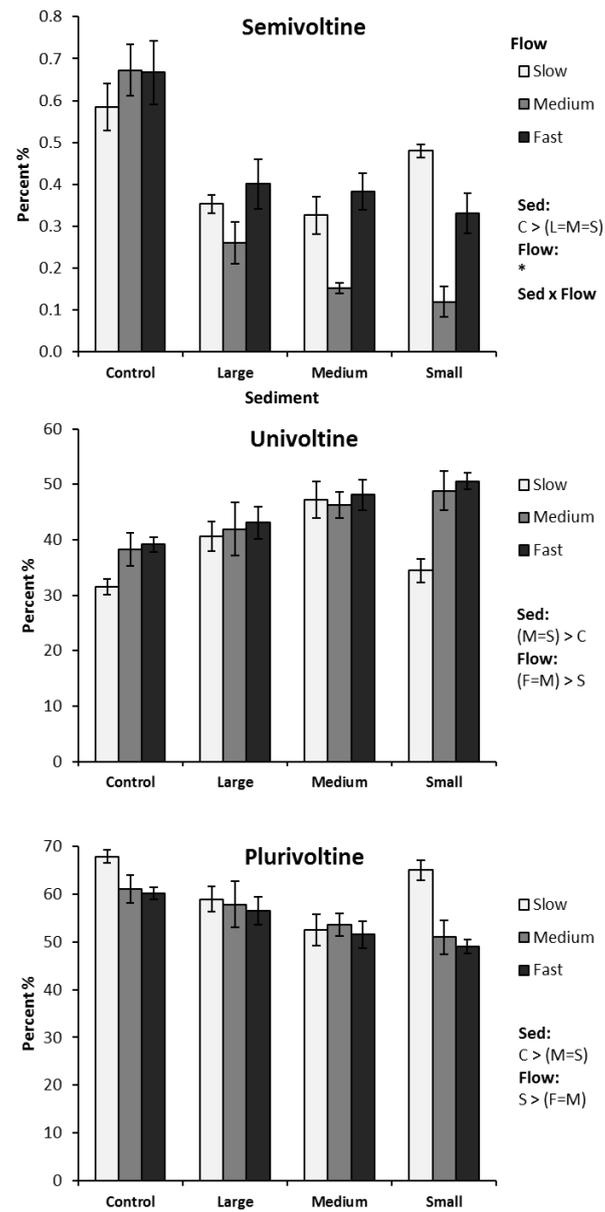


Figure 3.2: Relative abundances of trait modalities in the benthic invertebrate community for the biological trait 'Maximum Reproductive Cycles per Year' across the experimental treatments sampled on day 28 (averages \pm SEs). For more details see Fig. 3.1.

Life Duration of Adults

This trait comprises five modalities: invertebrate adults that live ≤ 1 day (LDA1), $>1-10$ days (LDA2), $>10-30$ days (LDA3), $>30-365$ days (LDA4), and >365 days (LDA5). The prevalence of two life duration modalities were affected by sediment and flow velocity treatments (Table 3.3, Figure 3.3).

The LDA1 modality was more prevalent in controls than in all sediment addition treatments, and also at fast or slow flow compared to medium flow (Fig. 3.3). Sediment and flow velocity treatments interacted for this modality, with the negative effect of added sediment (regardless of size) being strongest at medium flow velocity. This interaction was slightly weaker than the main effect of flow velocity and much weaker than the sediment main effect; therefore, both main effects remain valid. LDA2 prevalence decreased from controls to all three sediment addition treatments, especially in medium-sized sediment mesocosms. This modality was more prevalent at fast flow compared to slow or medium flow. LDA3 was more common in controls than in medium or small sediment addition treatments, and higher at slow compared to fast or medium flow velocity. LDA4 prevalence was positively affected by added fine sediment of all three sizes, and prevalence of this trait modality decreased consistently from medium and small to large sediment to control mesocosms. Further, LDA4 prevalence was greater at slow or medium flow than at fast flow. This modality was also affected by an almost-significant interaction between sediment and flow ($P = 0.059$): the positive effect of added sediment was generally weakest at slow flow. This interaction was weaker than the main effects of flow velocity and sediment, therefore these main effects remain reliable. Lastly, LDA5 prevalence was higher in all sediment addition treatments than in controls, and this modality did not respond to flow velocity.

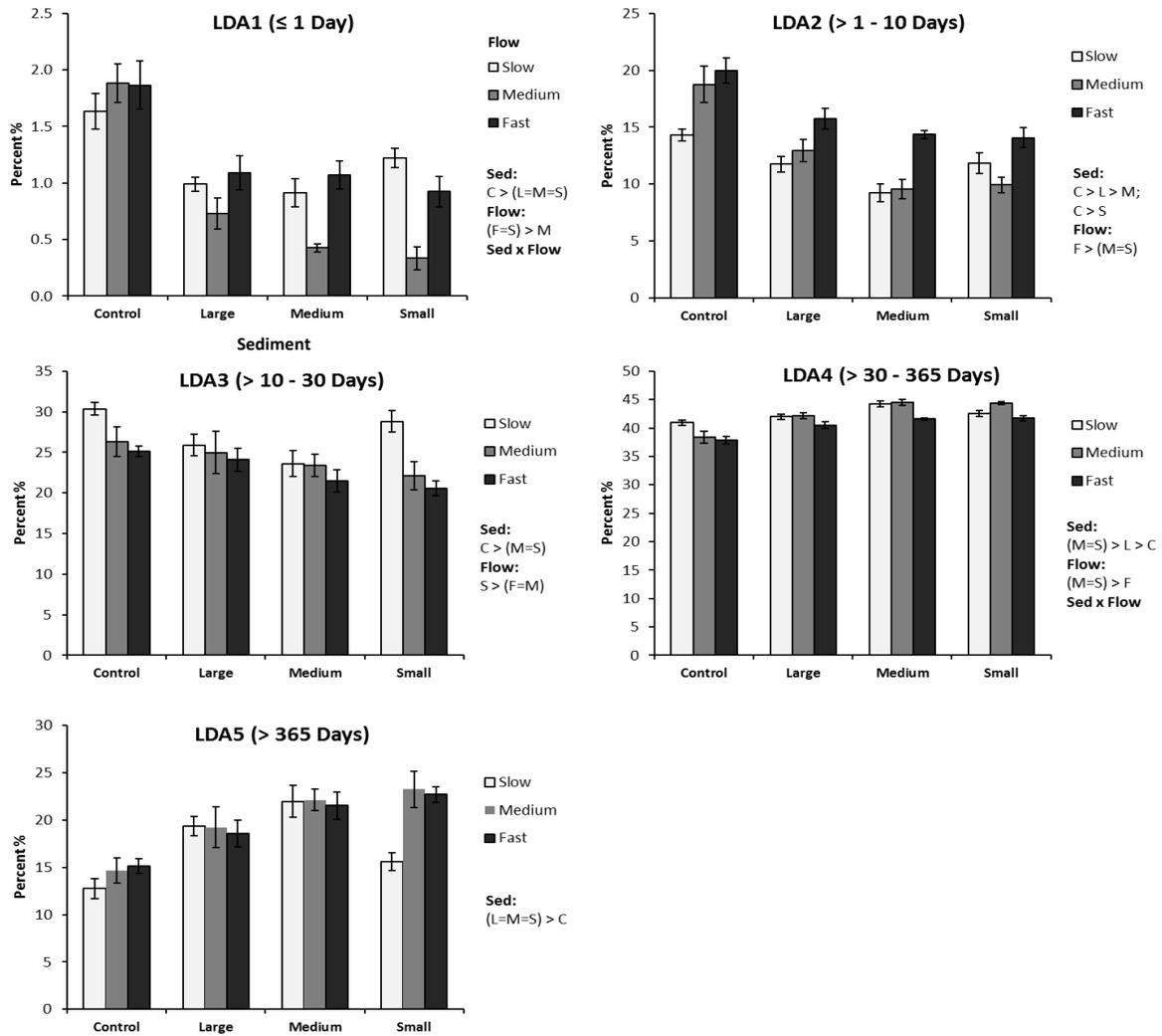


Figure 3.3: Relative abundances of trait modalities in the benthic invertebrate community for the biological trait 'Life Duration of Adults' across the experimental treatments sampled on day 28 (averages \pm SEs). For more details see Fig. 3.1.

3.3.4 Resistance and Resilience Traits

Oviposition Site

In the Resistance and Resilience category, I used one biological trait, Oviposition Site, which consists of four trait modalities: Surface (water surface), Submerged (below water surface), Terrestrial, and Eggs Endophytic (eggs inserted into substrate/plant tissue). Three of these modalities were affected by both sediment and flow treatments. Furthermore, interactions between stressors affected two modalities (Table 3.3, Figure 3.4).

Surface-laying invertebrates were more abundant in controls than in all sediment addition treatments, and at fast and slow flow compared to medium flow (Fig. 3.4). This modality was also affected by an interaction between sediment and flow velocity: Surface layers were less common at medium flow velocity, but only when fine sediment (regardless of grain size) was present in the mesocosms. This interaction was slightly weaker than the main effect of flow velocity and much weaker than the sediment main effect; therefore, both main effects remain reliable. The majority of the benthic invertebrate community lay their eggs under the water surface (Submerged), and prevalence of these invertebrates was higher in all sediment addition treatments than in controls. Submerged egg layers were also more common at slow or medium flow than at fast flow. Sediment and flow interacted for this trait modality, with the positive effect of added sediment generally being weakest at slow flow velocity. This interaction did not affect the reliability of the flow velocity and sediment main effects. Terrestrial egg layers were more common in controls than in small or medium sediment addition treatments, and also more common in large compared to medium sediment treatments. They also showed a consistent negative response to flow velocity reduction, decreasing in abundance from fast to medium and further to slow velocity. Finally, endophytic egg layers, which were generally very rare in the community, did not respond to either stressor.

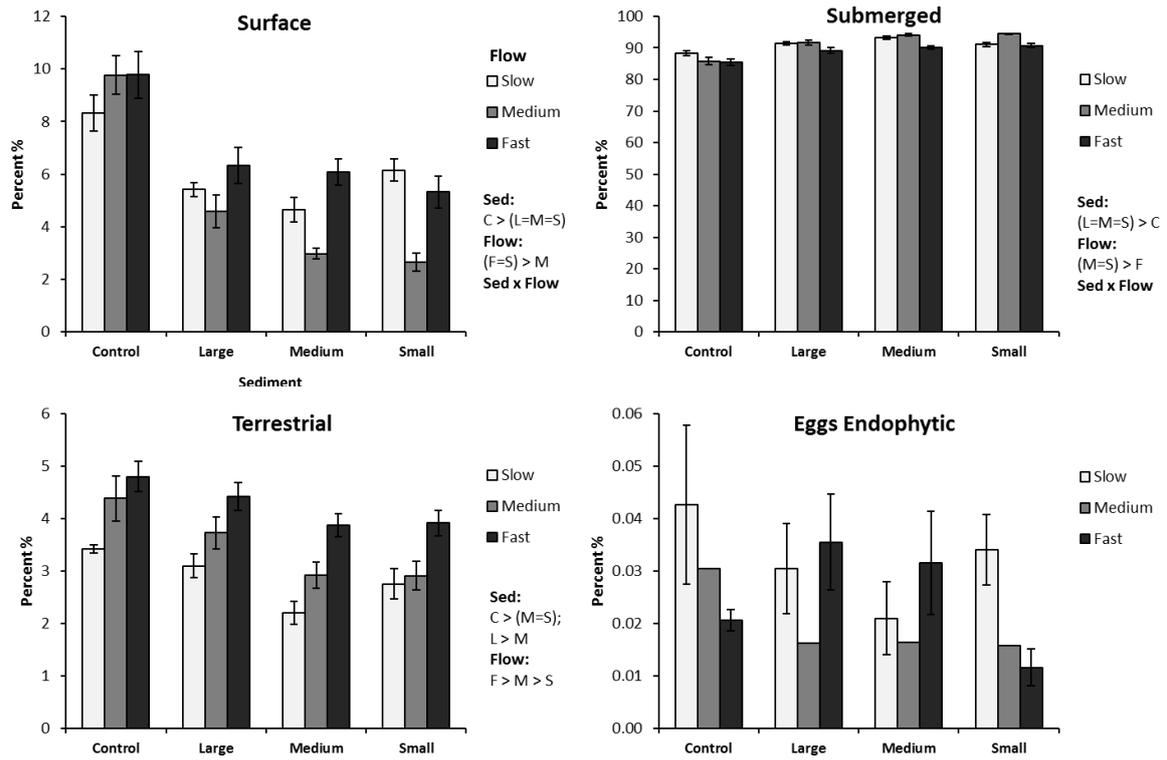


Figure 3.4: Relative abundances of trait modalities in the benthic invertebrate community for the biological trait 'Oviposition Site' across the experimental treatments sampled on day 28 (averages \pm SEs). For more details see Fig. 3.1.

3.3.5 General Biological Characteristics Traits

I analysed four traits with 16 modalities in this trait category: Attachment to Substrate of Aquatic Stages (excluding eggs) (3 modalities), Feeding Habits (6), Dietary Preferences (3), and Respiration of Aquatic Stages (excluding eggs) (4). Sediment treatments affected 11/17 trait modalities, flow velocity treatments affected 13/17 modalities, and there were five interactions between stressors in this trait group (Table 3.3).

Attachment to Substrate of Aquatic Stages (excluding eggs)

This trait consists of three modalities: Crawler (epibenthic), Burrower (infauna), and Attached to Substrate. All three modalities for this trait were affected by sediment treatments, and there were two interactions (Table 3.3, Figure 3.5).

Prevalence of Crawlers decreased consistently from controls, to large and to medium sediment addition treatments, and also from controls to small sediment mesocosms (Fig. 3.5). Sediment and flow velocity reduction treatments interacted for this trait modality, overriding a weaker flow velocity main effect: only in mesocosms with small fine sediment, Crawlers increased at the slowest flow velocity. Burrowers generally showed a strong preference for fine sediment. Their prevalence increased consistently from controls, to large to medium sediment mesocosms, and also from controls to small sediment mesocosms. Treatment effects interacted for this modality, and the slightly stronger interaction effect overrode the main effect for flow velocity. The generally positive effect of fine sediment was weakest at slow flow in mesocosms with small added sediment. Invertebrates which are firmly Attached to the Substrate, which were generally rare in the community, were more prevalent in controls than in mesocosms with medium fine sediment. This modality did not respond to the flow velocity treatments.

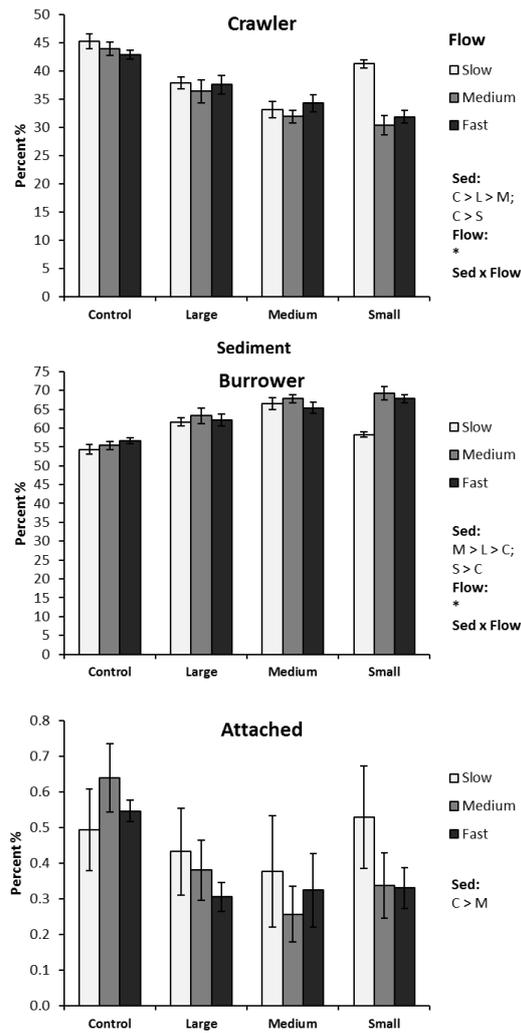


Figure 3.5: Relative abundances of trait modalities in the benthic invertebrate community for the biological trait 'Attachment to Substrate of Aquatic Stages (excluding eggs)' across the experimental treatments sampled on day 28 (averages \pm SEs). For more details see Fig. 3.1.

Feeding Habits

The Feeding Habits of benthic invertebrates were categorised into six trait modalities: Scraper (scrapers or grazers), Deposit Feeder, Filter Feeder, Predator, Algal Piercer and Shredder. Four feeding habit modalities were affected by sediment treatments, two modalities were affected by flow velocity treatments, and two modalities were affected by interactions between stressors (Table 3.3, Figure 3.6).

Scrapers were more abundant in controls than in all three fine sediment addition treatments (Fig. 6). Sediment and flow velocity interacted for this trait modality, with a positive effect of reduced flow velocity occurring in control channels but not when sediment (regardless of size) was added, and this interaction overrode the weaker main effect of flow velocity. Deposit Feeders generally showed a strong preference for fine sediment. Their prevalence increased consistently from controls, to large to medium sediment mesocosms, and also from controls to small sediment mesocosms. This modality was also affected by an almost-significant interaction between sediment and flow ($P = 0.055$): the generally positive effect of fine sediment was weakest at slow flow in mesocosms with small added sediment. The slightly stronger interaction effect overrode the main effect for flow velocity. Filter Feeders were more abundant in controls than in medium sediment addition treatments, and also more prevalent at slow flow than at fast or medium flow. Predators were more abundant in all sediment addition treatments compared to controls, and more common at fast and medium flow than at slow flow. Algal Piercers, which were fairly rare in the community, were more common in medium than in slow flow velocity mesocosms. Finally, Shredders were extremely rare and did not respond to any of the stressor treatments.

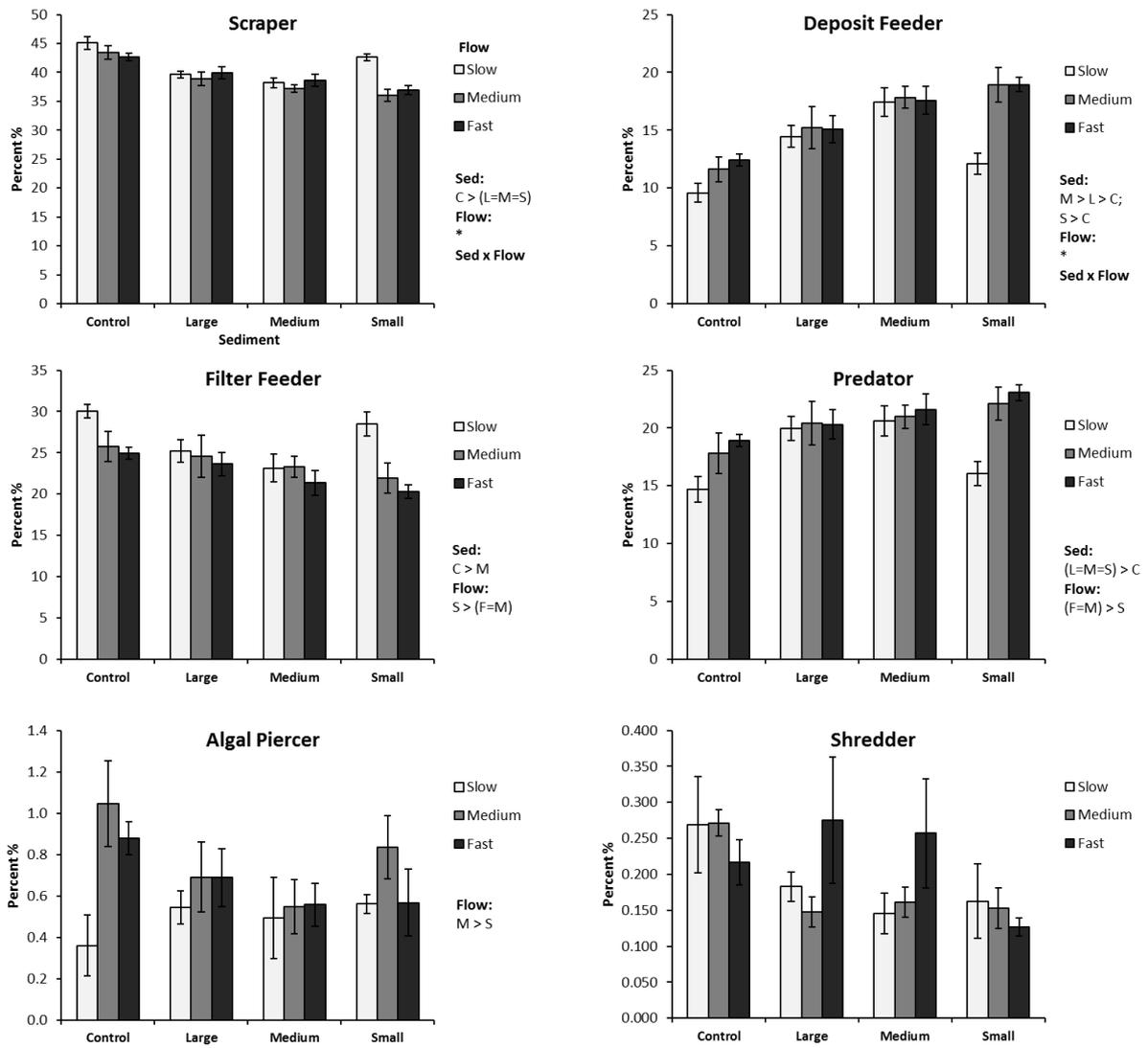


Figure 3.6: Relative abundances of trait modalities in the benthic invertebrate community for the biological trait 'Feeding Habits' across the experimental treatments sampled on day 28 (averages \pm SEs). For more details see Fig. 3.1.

Dietary Preferences

This biological trait consists of three modalities: Specialist (strong preferences), Moderate Specialist and Generalist (weak preferences). All three modalities were affected by both sediment and flow velocity treatments, and there was a single interaction (Table 3.3, Figure 3.7).

Strong Specialists and Moderate specialists responded similarly to the stressors (Fig. 3.7). Strong Specialists were more prevalent in small and medium sediment addition treatments compared to controls, and Moderate specialists were more abundant in all sediment addition treatments compared to controls. These two modalities were more common at fast or medium flow than at slow flow. Prevalence of Moderate specialists was also affected by an almost-significant stressor interaction ($P = 0.059$). In this, the negative effect of slow flow was strongest when combined with small added sediment. This interaction did not affect the reliability of the two stressor main effects. Generalists were more common in controls than in all sediment addition treatments, and also at slow flow compared to medium or fast flow.

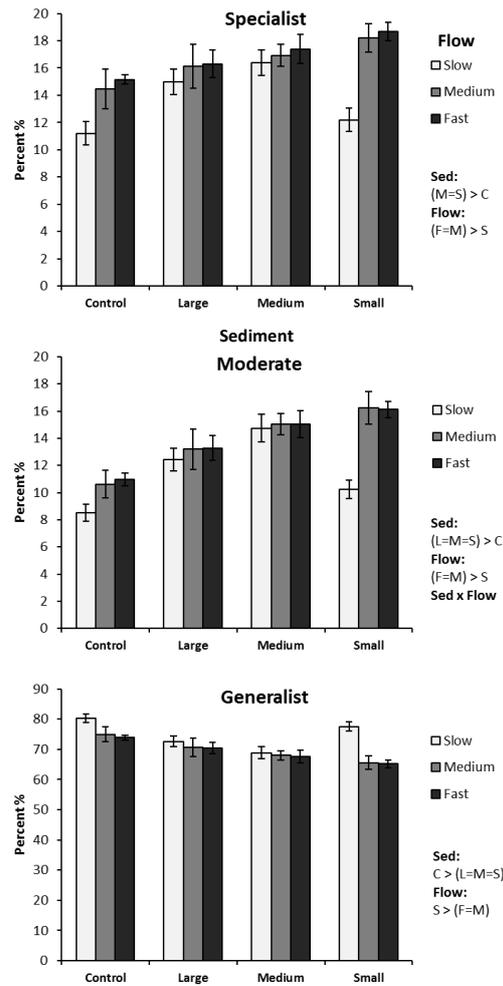


Figure 3.7: Relative abundances of trait modalities in the benthic invertebrate community for the biological trait 'Dietary Preferences' across the experimental treatments sampled on day 28 (averages \pm SEs). For more details see Fig. 3.1.

Respiration of Aquatic Stages (excluding eggs)

This trait is categorised into four modalities: Tegument, Gill, Aerial, and Plastron. One of the trait modalities was affected by sediment treatments, and three were affected by flow velocity treatments (Table 3.3, Figure 3.8).

Invertebrates respiring through their Tegument, which comprised the majority of the community, were more common in slow flow than in fast flow mesocosms and did not respond to the sediment treatments (Fig. 3.8). Invertebrates utilising external Gills to respire, conversely, were more abundant at fast flow compared to slow flow but were also unaffected by sediment. Aerial respiring individuals, who contributed only 1-2% of the community, were more common in controls than in small or medium sediment addition treatments. This modality was also more prevalent at fast flow than at medium or slow flow. Prevalence of the Plastron respiration strategy, which was very rare in the community, was not affected by either stressor.

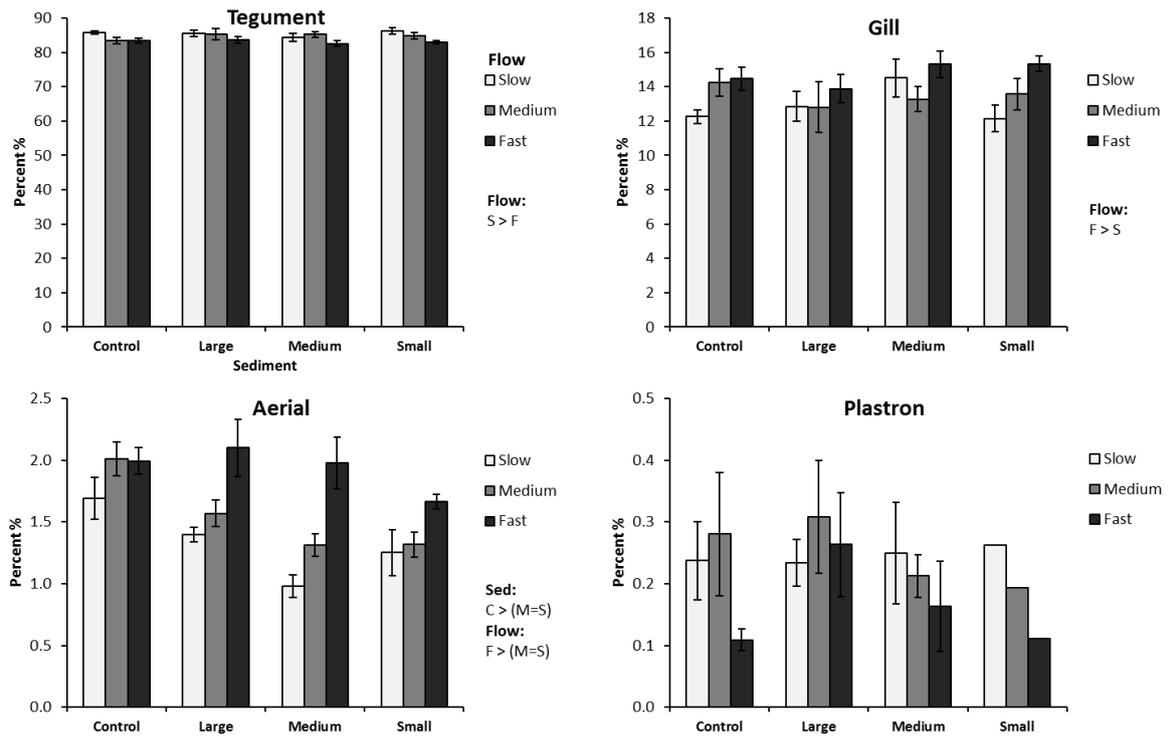


Figure 3.8: Relative abundances of trait modalities in the benthic invertebrate community for the biological trait 'Respiration of Aquatic Stages (excluding eggs)' across the experimental treatments sampled on day 28 (averages \pm SEs). For more details see Fig. 3.1.

3.4 Discussion

3.4.1 The Stressors Compared

To my knowledge, the present study is the first to investigate how deposited fine sediment of different sizes and reduced flow velocity interact to affect the relative abundances of benthic invertebrate biological traits in streamside mesocosm communities. Furthermore, no studies have investigated whether fine sediment grain size plays a role in determining the severity or frequency of its negative effects on benthic invertebrate trait prevalence.

Added fine sediment and reduced flow velocity affected 76% and 61%, respectively, of all invertebrate trait response variables as interpretable main factor effects (i.e. when the main effect size was larger than any interaction effect sizes; Quinn & Keogh 2002) (Table 3.4). Fine sediment was the more pervasive stressor, with more negative effects on trait expression. The average effect size for all significant findings also indicates that added fine sediment was a stronger determinant of trait expression than reduced flow velocity. Interactions between stressors were relatively common (33% of the 33 analysed trait responses), considerably more so than for the invertebrate community data from the same experiment (see Chapters 2 and 4).

The results of this study indicate that both fine sediment and water abstraction have strong effects on habitat quality, which in turn largely determine the traits expressed by the invertebrate communities. The frequency and strength of negative sediment effects on trait prevalence may be explained by increased habitat homogeneity and reduced habitat availability, resulting from smothering of the substratum and the clogging of interstitial spaces (Statzner & Beche 2010; Lange *et al.* 2014). Similar to the stream survey conducted by Lange *et al.* (2014) in the Manuherikia River catchment in Central Otago, New Zealand, water abstraction was more often related to general biological traits than life history or resistance and resilience traits, probably reflecting changes in organic matter dynamics and food availability, and overall reductions in habitat quality (Dewson *et al.* 2007).

Table 3.4: Numbers and percentages of interpretable significant main effects and interactions for all trait response variables combined. Means of effects sizes \pm standard errors are also shown.

	Sediment	Flow	Interactions
Overall (33 Variables)	25 (76%)	20 (61%)	12 (36%)
Mean Effect Size (Significant Effects)	0.48 \pm 0.18	0.28 \pm 0.10	0.27 \pm 0.03

3.4.2 Predicted Responses of Traits to Stressors

The results of my experiment offered support for 6/8 of the predicted trait responses from Clapcott *et al.* (2017; see Table 3.5) to added deposited fine sediment (defined as particles < 2 mm in Clapcott *et al.* 2017), but only for 2/7 of the predicted trait responses to flow velocity reduction simulating water abstraction (see Table 3.5 and further discussion below). While my findings are indicative of in-stream processes, care must be taken in extrapolating them to real streams due to the relatively small scale of the mesocosms and limited duration of the experiment. The *ExStream* System allows for high control under field-realistic experimental conditions, but the stream survey by Lange *et al.* (2014), on which all flow-related predictions in Clapcott *et al.* (2017) were based, was conducted at a much larger scale (43 sites spread across an entire river catchment) compared to my experiment. Additionally, the effects of water abstraction on trait expression are understudied compared to sediment effects. The contrasting results of my experiment compared to the survey by Lange *et al.* (2014) only reinforce the notion that further studies need to be conducted on multiple scales to properly understand how water abstraction shapes the traits invertebrate communities express. It was for this reason that I had expanded the limited set of trait response variables used by Clapcott *et al.* (2017) to assess invertebrate responses to stressors to include all modalities for each of these traits.

Table 3.5: Traits identified in the review by Clapcott *et al.* (2017, Table 5) as responding to the agricultural stressors deposited fine sediment and water abstraction, and their observed responses to applied stressors (indicated by post-hoc test rankings). All observed responses were significant at $P = 0.05$, except for the ranking for the trait modality Size 5 ($P = 0.054$). The average relative frequency of each trait modality is expressed as a percentage ($n = 60$).

Stressor	Trait	Trait Modality in NZ Trait Database	Predicted Response to Stressor Increase	Observed Response to Stressor Increase	Prediction Supported	Trait Average % Frequency
Fine Sediment	Large body size	SIZE4 (> 20 – 40 mm)	Negative	No response	No	0.19
	Large body size	SIZE5 (> 40 mm)	Negative	No response	No	0.003
	Merovoltine (≥ 3 -year life cycle)	Semivoltine	Negative	$C > (L = M = S)$	Yes	0.39
	Multivoltine	Plurivoltine	Negative	$C > (M = S)$	Yes	57.1
	Lay unattached eggs at water Surface	Surface	Negative	$C > (L = M = S)$	Yes	6.0
	Clinging taxa relative richness	Crawler	Negative	$C > L > M;$ $C > S$	Yes	37.2
	Burrowers	Burrower	Positive	$M > L > C;$ $S > C$	Yes	62.4
	Scrapers	Scraper	Negative	$C > (L = M = S)$	Yes	39.9
Water Abstraction	Life duration of adults (>365 days)	LDA5 (>365 Days)	Positive	No response	No	18.9
	Scrapers	Scraper	Negative	$S > (F = M)$	No	39.9
	Deposit Feeders	Deposit Feeder	Positive	$(F = M) > S$	No	15.1
	Predators	Predator	Positive	$(F = M) > S$	No	19.7
	Moderate dietary preference	Moderate Specialist	Positive	$(F = M) > S$	No	13.0
	Respiration through Tegument	Tegument	Positive	$S > F$	Yes	84.5
	Respiration through Gills	Gill	Negative	$F > S$	Yes	13.7

3.4.3 Deposited Fine Sediment

It was predicted that invertebrates with large body sizes (SIZE4 and SIZE5; maximum body size between >20-40mm, and >40 mm) would respond negatively to added deposited fine sediment (Table 3.5). A study by Richards *et al.* (1997) in 58 stream sites in Michigan, USA, found that large-bodied invertebrates showed a negative relationship with fine sediments, and this relationship was linked to short intervals between disturbance events and high hydrologic variation within these sites. In the present study, less than 1% of the invertebrates sampled across all mesocosms were associated with the two largest trait modalities that were predicted to respond negatively to fine sediment. At least partly as a result of this rarity (because many zero values increase variation between replicates and reduce statistical power), these modalities showed no response to this stressor, and this prediction was not supported. Only one species identified across all samples, the dobsonfly larvae *Archicauliodes diversus*, was affiliated with the largest body size modality (Size 5). The low prevalence of large invertebrate size modalities in the mesocosms may be limited by the size constraints of the mesocosm habitats, and the lower probability that these larger invertebrates would enter the mesocosms via drifting, due to a 4mm mesh diameter on the water-pump intakes in the river. The smaller two invertebrate size modalities showed a clear negative relationship to deposited fine sediment, whereas the middle size modality (Size 3) was more common in sediment addition treatments than in controls. These response patterns are somewhat unexpected, as previous observations and predictions have suggested that smaller body sizes should indicate stronger resistance and resilience to sediment effects (Townsend & Thompson 2007, Piggott *et al.* 2015c). However, in my experiment the middle size modality (Size 3) was dominated by Oligochaetes, a burrowing taxon that strongly favoured sediment addition (see Chapter 2), and the smaller sizes were dominated by sediment intolerant taxa (e.g. *Deleatidium* spp, Tanypodinae; See Chapter 2), and Cladocera (Chydoridae) which did not respond to added fine sediment.

The prevalence of Semivoltine and Plurivoltine invertebrates were predicted to decrease in mesocosms with added fine sediment (Table 3.5). These predictions were supported by my findings. In a survey of 48 stream sites in east-central Michigan, Richards *et al.* (1997) found a negative correlation between long-lived (Semivoltine) invertebrates and habitats with high percentages of fine sediment, as the presence of fine sediment likely indicates that a site is prone to disturbance events, an environmental condition not conducive to long-lived invertebrates. Further, Richards *et al.* (1997) noted that prevalence of short-lived (Plurivoltine)

taxa also declined in habitats with higher percentages of fine sediments in their survey, suggesting that even short-lived invertebrates capable of rapid reproduction struggle in habitats with high percentages of fine sediments. Semivoltine taxa were rare in my experiment: less than 1% of the invertebrate community was affiliated with this trait. Semivoltine taxa found included the dobsonfly larvae and the stonefly genus *Megaleptoperla*; the mayfly genus *Deleatidium* was also weakly associated with this trait. Some, but not necessarily all, *Deleatidium* species have more than one generation per year, as was observed and documented by Winterbourn (1974) and Huryn (1996). Consequently, this taxon has affiliations with the Semivoltine and Plurivoltine trait modalities. *Deleatidium* is sensitive to most forms of in-stream pollution, and considering the findings of Chapter 2, it is not surprising that this trait showed a consistent negative response to fine sediment of all grain sizes. Plurivoltine taxa were much more common, and invertebrates affiliated with this trait included Cladocera (very common), chironomid larvae, and several snail species (*Potamopyrgus antipodarum* and *Physa acuta*).

The prevalence of invertebrates which lay unattached eggs at the water surface declined in mesocosms with added fine sediment, regardless of grain size, supporting the prediction for this trait modality (Surface) (Table 3.5). By far the most common oviposition site was below the water surface (Submerged), and this modality increased in prevalence in sediment treatments compared to controls, reflecting the decrease in both Surface and Terrestrial oviposition strategies. Invertebrates which lay their eggs at the water surface mostly consisted of EPT taxa, a generally sediment-intolerant group of species commonly used as a bio-indicator for in-stream pollution (see Chapter 2). Doledec *et al.* (2006) suggested that dropping eggs at the water surface is less successful in streams with high sediment loads compared to reference streams, as eggs deposited in this way are more likely to be smothered by sediment.

Similarly, the crawling taxa identified in the mesocosms consisted of many EPT taxa, as well as some mollusc and dipteran species, and the prevalence of this trait modality showed a consistent negative response to added fine sediment, supporting the prediction for this trait modality (Table 3.5). Prevalence of Crawlers was expected to be low in sites with added fine sediments because sediment fills interstitial spaces, an important habitat for crawling taxa (Pollard & Yuan 2010). By contrast, Burrowers have been shown in surveys and experiments to favour habitats high in fine sediment, likely as a result of increased habitat (Richards 1997, Townsend *et al.* 2008, Wagenhoff *et al.* 2012, Lange *et al.* 2014). In my experiment, prevalence of this trait showed a strong positive correlation with added fine sediment, supporting the

prediction for this modality (Table 3.5). Burrowing species included Oligochaetes, and many worm-like fly larvae.

The feeding modality Scraper was predicted to decline in treatments with added fine sediments (Table 3.5). This prediction was supported; Scrapers were less common in all sediment addition treatments compared to controls. Scraping organisms generally prefer larger substratum materials, which provide habitat and a site for algal growth to occur which these invertebrates rely on for grazing (Richards *et al.* 1997). Scrapers were common across all treatments in my experiment, and scraping taxa identified included a diverse group of EPT taxa, molluscs, crustacea and some dipteran larvae. Deposit Feeders and Predators (for which there were no sediment-related predictions in Table 3.5) both increased in prevalence in sediment addition treatments. While the increase in Deposit Feeders is more likely associated with reduced flow velocity (see below), the reasons for increased prevalence of Predators may be two-fold. Firstly, Predators may feed on these Deposit Feeders, and the increase in prey availability may allow for increased Predator prevalence. Secondly, habit homogenisation may have reduced available refugia for prey species, allowing Predators to be more successful and proliferate.

3.4.4 Water Abstraction and Reduced Flow Velocity

It was predicted that the prevalence of the longest-lived adults (LDA 5) would be positively affected by simulated water abstraction (Table 3.5), as long adult life spans allow organisms to escape or persist through periods of low flow and/or drought conditions (Lange *et al.* 2014). Although this trait modality was common in my experiment, it did not respond significantly to the flow velocity treatments, providing no support for this prediction. The lack of response may be explained by the common taxa associated with this trait: Copepoda and Oligochaeta responded to flow velocity individually (see Chapter 2), but only Copepoda showed a positive response to reduced flow velocity, whereas Oligochaeta responded negatively to flow velocity reduction, and Conoesucidae did not respond to flow. Among the adult life duration modalities for which there were no sediment-related predictions in Table 3.5, shorter-lived invertebrates (LDA1 and LDA2) were consistently most common at the fastest flow velocity, whereas the second-longest adult life duration category (LDA4) was more common at both reduced flow velocities. These findings imply that at sites with high water abstraction, the invertebrate

community can be expected to comprise taxa with longer-lived adult stages than at sites without water abstraction.

Three feeding modalities Predator, Deposit Feeders and Scraper, were predicted to respond to water abstraction (Table 3.5). Changes in flow dynamics alter organic matter dynamics, through increased deposition of organic matter and fine sediment, and consequent smothering of surfaces algae may grow on (Dewson *et al.* 2007, Lange *et al.* 2014). These changes were predicted to result in a decrease in Scraper prevalence at increased water abstraction levels (simulated by reduced flow velocity in my experiment), and an increase in prevalence of Deposit Feeders (due to increased deposited organic matter) and Predators (which prey on Deposit Feeders) at slower flows (Lange *et al.* 2014). However, the opposite response patterns were observed. Thus, prevalence of Scrapers increased at slow flow compared to the medium or fast flow treatments, and Deposit Feeders and Predators were more common in the fast and medium treatments compared to slow flow velocity. The prevalence of Predators and Deposit Feeders fluctuated in tandem, and showed similar responses to stressor treatments, indicating that these trait modalities are likely linked through species interactions. Filter Feeders were more common in the slowest flow velocity treatment, an initially confusing result given that these organisms rely on current to deliver food to them (Rabeni *et al.* 2005). Upon closer inspection, however, this trait modality was dominated by Cladocera and Copepoda, which are affiliated with both Filter Feeder and Scraper modalities in the trait database.

The prevalence of Moderate dietary specialists was expected to increase in reduced flow velocity treatments (Table 3.5); however, the opposite trend was observed: Moderate specialists were more common at fast or medium flow velocity than at slow flow. This result contrasts with a strong positive relationship between water abstraction and this trait modality observed by Lange *et al.* (2014) in their stream survey. On the other hand, Doleddec *et al.* (2011) observed a weak negative relationship between Moderate diet prevalence and pastoral land use in another survey of New Zealand streams, and this relationship may in part have been related to increased water abstraction. Regarding the dietary preference modalities for which there were no sediment-related predictions in Table 3.5, strong Specialists responded similarly to the flow velocity treatments as Moderate dietary specialists in my experiment, and Generalists were more common at slow flow than in the two faster flow treatments. This increase in Generalists at slow flow possibly reflects that insects that inhabit temporary waters, or in this

case stream sites subject to high water abstraction, generally exhibit traits of r-selected species, such as Generalist feeding habits, small body size, and short life spans (Williams 1996).

The final two traits that were predicted by Clapcott *et al.* (2017) to respond to simulated water abstraction were respiration strategies. In areas subject to high water abstraction, prevalence of using Gills was predicted to decrease, and prevalence of respiring through the Tegument was predicted to increase (Table 3.5; Lange *et al.* 2014). The results from my experiment support both predictions. The decrease in Gill prevalence in mesocosms with reduced flow velocity likely reflects a preference for habitat conditions with higher flow velocities (and therefore higher amounts of dissolved oxygen delivered per unit of time) (Brooks & Haeusler, 2016). Many of the taxa respiring using Gills were EPT taxa, the total abundance of which was shown to decline under reduced flow velocity conditions in Chapter 2. Invertebrates respiring through the Tegument were extremely common (>80% of the community). While I was able to detect a significant response for this trait modality, it is difficult to elucidate why Tegument respiring taxa preferred slow flow velocities over fast flows. Brooks & Haeusler (2016) investigated the effects of reduced flows on the prevalence of respiration strategies in Kangaroo River, Australia, and found that Tegument respiring individuals were not constrained by oxygen requirements, or flow velocity. Reduced Tegument prevalence has been previously associated with higher temperatures, lower oxygen, and lower flow velocities (Bonada *et al.* 2007).

3.4.5 Fine Sediment Size

My first hypothesis regarding the effects of fine sediment with different grain sizes predicted that the smaller the size of the added deposited fine sediment, the more severe the negative impacts would be on the relative abundances of sediment-sensitive biological traits represented in the benthic invertebrate community. Benthic macroinvertebrates often have specific habitat preferences (Culp *et al.* 1986), and the effects of deposited fine sediment are likely to differ based on particle size, affecting availability and size of important interstitial habitats (Bo *et al.* 2007). Smaller fine sediment classes are more likely to persist on the stream bed and become more embedded, resulting in greater habitat homogenisation and clogging of interstitial spaces, as well as causing more harm to sensitive species through abrasion (Waters, 1995; Wood & Armitage, 1997; Jones *et al.* 2012, Extence *et al.* 2013; Glendell *et al.* 2014). Conversely, I

predicted that biological traits that tolerate or favour deposited fine sediment would be more positively affected by smaller sediment sizes.

My findings indicate that the grain size of fine sediment did indeed matter for some trait modalities; however, for certain other trait modalities the presence of deposited fine sediment of any grain size was detrimental (or positive). Thus, the prevalence of Semivoltine or Plurivoltine invertebrates, Scrapers, Surface egg layers and Generalists were all negatively affected by added sediment regardless of grain size. These trait modalities are indicative of pollution-sensitive taxa; for example, the EPT taxa identified in my experiment generally exhibited these traits. A trait group consisting of some or all of these trait modalities may be useful in the future for assessing fine sediment impacts and those of other environmental stressors, due to the regularity that EPT taxa are affiliated with these modalities and the common occurrence of these traits in reference-quality stony-bedded streams (Richard *et al.* 1997). In contrast, an invertebrate trait set consisting of larger body sizes (SIZE3), longer adult life durations (LDA4, LDA5), Predators, Submerged egg laying and Moderate specialists may be useful as a counter-set of traits that exhibit high resilience and resistance to sediment effects, as these traits responded positively to added fine sediment of all grain sizes in my experiment.

Prevalence of Crawlers, another trait associated with stony beds and pollution-sensitive taxa (Richards *et al.* 1997), decreased as fine sediment grain size decreased. This response pattern makes sense mechanistically; as sediment size decreases, so does the expected habitat availability and ease of movement for these taxa. In contrast, Burrowers and Deposit Feeders were most common in the smaller sediment addition treatments, but still more common in the largest sediment addition treatment than in controls. Again, these results make sense mechanistically as habitat availability should increase under finer sediment conditions for Burrowers, and there should be reduced competition for space and deposited organic matter.

For several trait modalities (SIZE1, LDA3, Plurivoltine and Aerial respiration), the largest sediment size did not have any negative effects. This response pattern indicates that in some cases, relatively large-grained fine sediment (1-2 mm) is tolerable and even allows certain traits to thrive. The two smallest sediment sizes resulted in a decrease in prevalence for these same trait modalities. In contrast, Univoltine and Specialist Feeders were most common in only the small and medium sediment treatments compared to the controls. This suggests that small invertebrates with short life cycles can persist in habitats with coarser sediment sizes, so they

can be considered somewhat sediment-tolerant, but even these trait modalities struggle in the presence of smaller fine sediment classes.

3.4.6 Multiple-Stressor Effects

Interactions between sediment and flow were common (see Table 3.4). My second hypothesis predicted that negative fine sediment effects would be more frequent and more severe at reduced flow velocity, and my third hypothesis predicted that this negative synergistic interaction should be stronger for smaller fine sediment sizes because this type of sediment should cause the strongest negative effects. My findings provided some evidence to support these two hypotheses, but – as is common for interactions between multiple stressors – the interactive response patterns were not always straightforward to interpret.

Additive Effects

Twenty-three of the 33 analysed trait variables responded to both manipulated stressors, and 12 of these 23 variables did so without a significant interaction between the two stressors (see Table 3.3). Such additive multiple-stressor effects (see Piggott *et al.* 2015b) occurred for invertebrates with a maximum body size < 5 mm (SIZE1) and >20 – 40 mm (SIZE4), Univoltine and Plurivoltine reproductive strategies, Adult lifespans >1 – 10 days (LDA2) and >10 – 30 days (LDA3), Terrestrial egg layers, Filter Feeders, Predators, Specialists, Generalists, and the Aerial respiration strategy.

Complex Interactive Effects

Complex interactions between stressors were common (12/33 trait modalities) and evenly spread among life history, resistance and resilience, and general biological traits. Several trait modalities across these categories responded similarly to stressors, indicating these traits are linked either at a species or evolutionary level. It has been known for some time that traits can be linked into trait syndromes (Pianka 1972; Poff *et al.* 2006; Horrigan & Baird 2008), and this likely explains the similar relationships between traits in the communities (Doledec *et al.* 2006). These similar trait responses will be discussed below.

One should note that, due to the larger number of degrees of freedom required to model the interaction term compared to the two factor main effects (9 vs 3 for sediment or 2 for flow velocity), the results of my experiment may somewhat underestimate the actual frequency of significant two-way interactions in the traits data (see Methods and Cottingham *et al.* 2005). This limitation of my study is a consequence of its experimental design because I was interested in the differences between each individual sediment treatment (4 levels) and flow velocity treatment (3 levels); therefore neither stressor could be modelled as a continuous predictor (as recommended by Cottingham *et al.* 2005 for categorical designs with >2 factor levels).

It is also worth noting that disentangling the linkages between traits phylogenetically, to identify certain traits which respond to stressors outside of evolutionary constraints, is a task that could provide further insights into how communities respond to stressors, and doing so was recommended by Poff *et al.* (2006) as an important 'next step' for trait-based research in general.

Three trait modalities - Semivoltine (<1 life cycle per year), Surface egg laying, and short adult lifespans (LDA1 <1 day) - responded strongly negatively to added sediment regardless of grain size, and were especially less abundant in medium flow treatments when fine sediment was present. Two of these trait modalities (Semivoltine and Surface egg laying) were also identified in the review by Clapcott *et al.* (2017) as being sensitive to deposited fine sediment. These three trait modalities are shared by *Deleatidium*, a pollution-sensitive mayfly genus that is common and widespread throughout New Zealand. The observed decrease of *Deleatidium* in the benthic community at medium flow velocity in all three sediment addition treatments was complemented by an increased propensity to enter the drift (see Chapter 2). These results offer some support for my second hypothesis because the negative effects of sediment were augmented at reduced flow velocities.

Three other trait modalities associated strongly with sensitive EPT taxa (Crawler, body length between >5-10mm, Scraper) showed an interaction between sediment addition and reduced flow velocity. These traits were all less abundant across all three sediment addition treatments compared to the controls, but their prevalence increased in the treatment combination of small fine sediment and the slowest flow velocity. These responses are unexpected, as this treatment combination was expected to be the least favourable for sensitive taxa (see hypotheses 2 and 3). Conversely, Burrowers, Deposit Feeders, and Moderate specialists were predicted by Clapcott *et al.* (2017) to favour added fine sediment and reduced

flow velocities, yet for these trait modalities the generally positive effects of fine sediment were weakest in mesocosms where the slowest flow velocity treatment was combined with the smallest sediment size. These results are again somewhat surprising, as it was expected that in this treatment combination, sediment-tolerant traits would thrive. These contrasting patterns of sediment-tolerant and sediment-intolerant traits in this treatment combination are intriguing and indicate that somehow this combination of stressors created an environment more like the controls (no added sediment) than any other treatment combination. The slowest flow velocity combined with the smallest added sediment may have resulted in a densely compact and oxygen-starved benthic habitat (Lancaster & Hildrew 1993). Conversely, this cohesiveness may have created refugia for taxa with sediment-sensitive traits as the firm benthic substratum may have in places essentially become similar to a large 'rock'. At faster flows, the small sediment grains were probably more easily re-suspended, which could be why this pattern was not observed at faster flows. Similarly, the larger sediment grain sizes may be less likely to become so compact due to their size and shape (Gordon, 2004). Furthermore, a similar interaction occurred for the trait modalities Submerged egg-laying and long adult lifespans (LDA4). These modalities were generally favoured by added fine sediment, but the positive effect of sediment was weakest at slow flow. One again, these results only somewhat support my second hypothesis, as interactive patterns of this type were not common.

Finally, the largest larval body size trait (SIZE5) was positively affected by added small sediment, but only at the slowest flow velocity. However, this interaction was caused by the presence of just two individuals of the megalopteran *Archicauliodes diversus* collected across all 60 mesocosms (see Fig. 3.1). While these megalopteran larvae can be voracious predators (Devonport & Winterbourn 1976), I do not believe these two immature individuals (which were both less than 5 mm in length) were able to drive (through the predation pressure they might have exerted) the interactive response patterns for the other trait modalities in this treatment combination discussed earlier on.

3.4.7 Management Implications

As mentioned previously, extrapolations of the results of this study to real stream ecosystems should be done with care, due to the experiment's relatively short nature (4-week manipulative period) and the small spatial scale of the stream mesocosms. However, the *ExStream System* naturally receives high numbers of immigrating stream organisms (invertebrates, algae, and

microbes), and water chemistry, temperature, and light conditions are the same as in the nearby river (Wagenhoff *et al.* 2012; Magbanua *et al.* 2013; Piggott *et al.* 2015a). Further, invertebrate emigration rates (and thus presumably also immigration rates) were higher than in previous experiments (Bruder *et al.* 2017) and are at least as high as in small farmland streams in southern New Zealand (see e.g. Hansen & Closs, 2007; James *et al.* 2009). Further experiments investigating invertebrate trait responses to simulated water abstraction and deposited fine sediment, ideally conducted at larger scales and in other river catchments, would provide further context and robustness to my findings.

The use of invertebrate traits as an analysis tool provides useful insight into in-stream community dynamics and allows us to link the prevalence of traits in a community as a response to stressors through a mechanistic approach (Pollard & Yuan 2010). When testing the sediment and abstraction-related traits compiled by Clapcott *et al.* (2017), I found strong support for the predicted trait responses to fine sediment, whereas for water abstraction only the traits related to respiration strategies responded as predicted. This difference highlights the need for further research into how increasing water abstraction determines trait prevalence in benthic invertebrate communities, using both experimental and field survey settings. The effects of water abstraction on macroinvertebrate trait expression is understudied compared to naturally occurring low flows or dams (Brooks *et al.* 2011), and further research in New Zealand and abroad should focus on how high-intensity water abstraction and irrigation schemes are affecting the health and integrity of stream communities, especially in the face of future climate change and in combination with other environmental stressors. We have limited understanding of how traits respond to multiple stressors, and as such our ability to identify and create a robust trait set is confined by our lack of insight into how traits may interact in nature (Poff *et al.* 2006, Schuwirth *et al.* 2015; Clapcott *et al.* 2017). Furthermore, the relationship between traits and stressors can become confounded if traits respond to multiple features of the environment (Statzner & Beche 2010).

My experiment has demonstrated the severe negative impacts that environmental stressors can have on the relative abundances of a suite of traits expressed by benthic macroinvertebrate communities. Fine sediment and water abstraction are two important stressors affecting agricultural stream ecosystems (Scarsbrook *et al.* 2016), and the results of my study indicate that these stressors often work in unison, and quite unpredictably. Outcomes of stressor interactions may be worse than predicted single-stressor effects (Townsend *et al.* 2008, Ormerod *et al.* 2010), and managers should prepare and plan for these interactive

outcomes in future scenarios. Benthic macroinvertebrate taxa have specific habitat preferences (Culp *et al.* 1986), and my results highlight that fine sediment grain size does determine the prevalence of certain trait modalities in benthic invertebrate communities through habitat alteration. Thus, the negative effects of added fine sediment increased in severity as sediment grain size decreased for Crawlers (with a corresponding positive effect for Burrowers). Further, for three trait modalities (SIZE1, Plurivoltine, and Aerial Respiration) only the smaller sediment grain sizes had a negative effect (opposite positive effect for Univoltine and Specialist Feeders). Alternatively, sediment grain size did not matter in several cases: sediment of all grain sizes had strong negative effects on trait modalities associated with sediment-sensitive taxa (short adult life spans (LDA1), long life cycles (Semivoltine), Surface egg laying, Scrapers), and also positive effects on sediment-tolerant trait modalities (larger body sizes (SIZE4), longer adult life durations (LDA5), Predators, Submerged egg-laying and Moderate specialists).

CHAPTER 4

General Discussion

4.1 Effects of Deposited Fine Sediment of Varying Grain Sizes and Water Abstraction

My research investigated the individual and interactive responses of freshwater invertebrate communities to two key agricultural stressors affecting running water ecosystems – deposited fine sediment and water abstraction. To my knowledge, this was the first experiment that investigated structural, dynamic and functional responses of entire stream invertebrate communities (benthos, drift and emergence, biological traits) to varying grain sizes of deposited fine sediment and varying levels of water abstraction (simulated by reducing flow velocity). Consequently, there are no previous related studies whose results can be directly compared to my grain-size related or interactive findings, but there are several studies that are relevant in a more general context.

My first data chapter (Chapter 2) investigated how the benthic, drifting and emerging invertebrate communities responded to the sediment and current velocity treatments, by analysing the responses of structural and dynamical community-level metrics, including diversity and evenness, total taxon richness, EPT (mayflies, stoneflies, caddisflies) richness and abundance, New Zealand's MCI (Macroinvertebrate Community Index), multivariate community composition and the abundances of individual common taxa in the benthic, drifting and emerging insect communities. Chapter 3 explored how functional measures of the benthic invertebrate community responded to the two stressors by analysing how the relative abundances of the biological traits expressed by the communities shifted in relation to which stressor combinations were impacting the community.

In Chapter 2, I analysed 55 biological response variables across the benthic, drift, and emerging invertebrate communities (Table 4.1). The benthic, drifting and emerging communities were frequently affected by the effects of added fine sediment (44 % of all analysed variables; with 83 % of these being negative responses) and the effects of reduced flow velocity (49 % of all response variables; 85 % negative responses). Complex (non-additive) interactions between stressors were rare when analysing the community in this way (5% of analysed variables), compared to additive responses (20% of analysed variables).

In Chapter 3, I analysed the effects of added fine sediment and reduced flow velocity on the benthic community only, using a set of 8 biological traits consisting of 33 trait modalities (Table 1). Added fine sediment affected 76 % of the studied trait modalities (60 % negative responses), and reduced flow velocity affected 61 % of the trait modalities (60 % negative responses). Complex interactions were much more common in this chapter, affecting 12 trait

modalities (36 % of all analysed trait modalities). There were also 12 additive multiple stressor responses.

Table 4.1: Numbers and frequencies (in %) of interpretable significant main effects and complex (non-additive) interactions for all invertebrate response variables for Chapter 2 (benthic, drift, and emergence community and common taxa metrics), and Chapter 3 (biological traits). Means of effect sizes \pm standard errors are also shown.

	Sediment	Flow	Sediment x Flow
Chapter 2 (55 variables)	24 (44 %)	27 (49 %)	3 (5 %)
Mean effect size (significant effects)	0.34 \pm 0.03	0.28 \pm 0.03	0.29 \pm 0.01
Negative univariate responses	20 (83 %)	23 (85 %)	
Chapter 3 (33 Variables)	25 (76 %)	20 (61 %)	12 (36 %)
Mean effect size (significant effects)	0.48 \pm 0.18	0.28 \pm 0.10	0.27 \pm 0.03
Negative univariate responses	15 (60 %)	12 (60 %)	

4.2 Fine Sediment

Fine sediment was the most pervasive stressor in this experiment (and had the largest mean effect sizes). When looking across both chapters at community and biological trait responses, added fine sediment affected 56 % of all response variables. Added fine sediment resulted in decreased abundance and richness of benthic EPT taxa (larval insects belonging to the orders Ephemeroptera, Plecoptera and Trichoptera), reduced benthic community evenness and diversity, and decreased abundance of 5/13 of the most common benthic taxa. Drift propensity of EPT taxa increased in sediment addition treatments, and emergence propensity of EPT taxa, emergence diversity, and body size of emerged adult *Deleatidium* were all highest in control treatments (no added sediment). The results of my experiment offered support for 6/8 of the predicted trait responses from Clapcott *et al.* (2017) to added deposited fine sediment (defined as particles < 2 mm in Clapcott *et al.* 2017). Furthermore, added fine sediment treatments reduced the prevalence of 15/33 biological trait modalities, including the prevalence of several sediment-sensitive trait modalities (Crawlers, Scrapers, Plurivoltine, Semivoltine, and Surface egg laying). Sediment-intolerant taxa and traits were replaced by Copepods, Oligochaetes, Burrowers, Submerged egg layers and long-lived invertebrates.

These results reaffirm that fine sediment is a master stressor in freshwater ecosystems (Scarsbrook *et al.* 2016), and highlight the importance of mitigating sediment inputs into freshwaters. While sediment inputs into streams are a naturally occurring process (Suttle *et al.*

2004), management strategies, such as increasing riparian vegetation, maintaining buffer strips at stream edges, and fencing to prevent livestock entering waterways (Carling *et al.* 2001; Quinn *et al.* 2009; Daigneault *et al.* 2017), are crucial for ensuring the survival and proliferation of sensitive stream taxa and the overall health of freshwater habitats.

4.3 Fine Sediment Grain Size

As stated previously, ‘fine sediment’ in streams is commonly defined as particles with a grain size of 2mm or less (see e.g. reviews by Clapcott *et al.* 2011; Clapcott *et al.* 2017). In the introductions of my two data chapters, I had posed two questions regarding fine sediment grain size and the effects its presence in freshwater ecosystems has on benthic invertebrate communities: (1) Are stream macroinvertebrate communities equally affected by fine sediment with grain sizes of 1-2 mm, or (2) are smaller grain sizes worse?

To answer the first question, my findings indicate that for sensitive invertebrate community-level metrics, common taxa and biological traits, the presence of deposited fine sediment of any grain size < 2 mm is detrimental. Conversely, for sediment-tolerant taxa and traits, fine sediment of any grain size has positive effects on their abundance and prevalence. Thus, total benthic abundance and taxonomic richness of larval EPT taxa (mayflies, stoneflies, caddisflies) as well as the benthic abundances of Tanypodinae and *Deleatidium* spp. (the most common EPT taxon), and 5 trait modalities (Semivoltine, Plurivoltine, Scrapers, Surface egg layers and Generalists) were all negatively affected by added sediment regardless of grain size. Additionally, benthic abundance of Oligochaeta, and 5 trait modalities (Size3 (>10-20mm), LDA5 (>365 days), Submerged egg-laying, Predators, Moderate Specialist) were more abundant in all sediment addition treatments compared to controls.

Regarding my second question, the two smaller sediment size classes were not as persistent in the mesocosm substrata as the larger sediment size (higher % of fine sediment removed or repositioned by higher flow velocities; see Chapter 2), but the effects of these smaller grain sizes were consistent and pervasive for both community/taxon metrics and biological traits, and were often stronger directionally (positive or negative) than the larger sediment grain size. Thus, small and medium sediment had negative effects on benthic community evenness, benthic abundance of the caddis *Psilochorema* spp., drift propensity of EPT individuals and drift propensities of two common taxa (Chironomidae (excluding

Tanypodinae) and caddisflies of the family Conoesucidae), and decreased prevalence of Size1, LDA3, Plurivoltine and Aerial. Conversely, small and medium sediment had positive effects on the prevalence of Univoltine and Specialist feeders. Further, benthic diversity was most reduced in the mesocosms with the smallest sediment size, and emergence EPT richness and diversity were negatively affected only by the smallest sediment. Finally, prevalence of the trait modality Crawler decreased as fine sediment grain size decreased, and the opposite trend was observed for prevalence of the trait modality Burrower.

Although the exact mechanisms driving these results cannot be identified in this mesocosm experiment that involved realistically simulated stream communities, there are numerous potential reasons for these findings. The finer sediment particles may have limited potential habitats and refugia for the benthic invertebrates considerably more than the larger sediment grains, leading to more severely limited oxygen and less food availability in the substratum and/or resulting in more harm to individuals through burial, clogging or abrasion of external organs (Waters, 1995; Wood & Armitage, 1997; Jones *et al.* 2012, Extence *et al.* 2013; Glendell *et al.* 2014; Conroy *et al.* 2018). For example, in the laboratory experiment by Conroy *et al.* (2018), invertebrate responses to burial by fine sediments were variable, but burial by the finest sediment particles was generally most harmful to four of the five taxa. The results from this laboratory experiment indicated that flattened body-types, extruding gills, or external cases, may be more prone to clogging or mass-bearing by sediment, and escaping burial is worsened by environmental conditions associated with sediment, such as low dissolved oxygen and reduced interstitial habitat (Conroy *et al.* 2018).

4.4 Water Abstraction and Reduced Flow Velocity

Water abstraction, simulated by reduced flow velocity, while not the most pervasive stressor, still affected more than half of all invertebrate response variables (53 %). These results agree with the findings of previous studies that the effects of water abstraction and reduced flow velocity can be as common as fine sediment effects (Matthaei *et al.* 2010, Elbrecht *et al.* 2016; Beerman *et al.* 2018). Total benthic invertebrate abundance, EPT richness, diversity and evenness, and abundance of 5/13 most common benthic taxa were all negatively affected by reduced flow velocity. Total emerged insect size and size of emerged Chironomidae were largest at the highest flow velocity. Effects of flow velocity were common in the drift

community (10/19 response variables), and drift propensities of EPT taxa and 4/9 of the most common drifting taxa were highest at medium flow velocity compared to fast or slow flow. Reduced flow velocity affected the prevalence of 20/33 biological trait modalities; however, when testing the abstraction-related traits compiled by Clapcott *et al.* (2017), only the traits related to respiration strategies responded as predicted. This lack of predictive strength for trait responses to water abstraction highlights the need for further research into how increasing water abstraction determines trait prevalence in benthic invertebrate communities, using both experimental and field survey settings.

4.5 Stressor Interactions

Complex (non-additive) stressor interactions were much less common when analysing the benthic invertebrate community responses to stressors using community-based metrics (3/55 variables responded with significant stressor interactions) compared to using biological traits (12/33).

Although interactions between added fine sediment and reduced flow velocity were uncommon in Chapter 2, the two out of three variables that did interact significantly (benthic EPT abundance, benthic *Deleatidium* spp. abundance, EPT drift propensity) are widely used as biomonitoring tools to assess stream health and water quality in New Zealand and abroad (e.g. Rosenberg & Resh 1996; Townsend *et al.* 2008; Piggott *et al.* 2012; Wagenhoff *et al.* 2012), or an equivalent counterpart is used (e.g. benthic abundance of the common mayfly *Baetis* spp. instead of *Deleatidium* spp. in Europe; Elbrecht *et al.* 2016, Beermann *et al.* 2018). The interactions in Chapter 2 indicated that at reduced flow velocity, the effects of added fine sediment are worsened, regardless of sediment grain size, for sensitive taxa and community metrics.

In Chapter 3, complex interactions between added fine sediment and reduced flow velocity were common and evenly spread among life history, resistance and resilience, and general biological traits. There were several patterns in these stressor interactions, likely reflecting groups of traits (e.g. trait syndromes; Poff *et al.* 2006) which respond similarly to stressors as they are shared by groups of species through evolutionary linkages, or shared by a single species. In some interactions, patterns were the same as in the community metrics chapter, in that the negative effects of sediment were augmented at reduced flow velocities

(e.g. for the trait modalities Semivoltine (<1 life cycle per year), Surface egg laying, and short adult lifespan (LDA1 <1 day), which were all shared by *Deleatidium* spp.). Three other trait modalities associated strongly with EPT taxa identified in the benthic samples (Crawler, body length between >5-10mm (Size2), Scraper) showed an interaction between sediment addition and reduced flow velocity. These traits were all less abundant across all three sediment addition treatments compared to the controls, but their prevalence increased in the treatment combination of small fine sediment and the slowest flow velocity. Conversely, for Burrowers, Deposit Feeders, and Moderate Specialists (traits associated with sediment tolerant species such as Oligochaetes and Copepoda), the generally positive effects of fine sediment were weakest in mesocosms where the slowest flow velocity treatment was combined with the smallest sediment size. The slowest flow velocity combined with the smallest added sediment may have resulted in a densely compact and oxygen-starved interstitial habitat, both unfavourable and impenetrable for burrowing taxa, while creating a refugium on the surface for taxa with sediment-sensitive traits as the firm benthic substratum may have in places essentially become similar to a large 'rock'. These contrasting, and somewhat confusing patterns highlight the unpredictable nature of multiple-stressor interactions, and again reinforce the need for further research in experimental and survey conditions to disentangle the mechanisms driving such multiple stressor interactions.

4.6 Management Implications and Limitations

While my results do not indicate any need for re-evaluation of fine sediment as a stressor for stream invertebrate communities (e.g. re-defining fine sediment as anything other than inorganic particles < 2 mm in diameter), the fact that a number of invertebrate response variables comprising community, taxon and trait metrics were negatively impacted by deposited fine sediment of all tested grain sizes, and that sediment grain size effects often interacted with increasing water abstraction (simulated by flow velocity reduction), are two novel discoveries. Extrapolations of the results of this study to real stream ecosystems should be done with care, due to the experiment's relatively short nature (a 4-week manipulative period) and the small spatial scale of the stream mesocosms. However, as mentioned previously, the *ExStream System* naturally receives high numbers of immigrating stream organisms (invertebrates, algae, and microbes), and water chemistry, temperature, and light conditions are measurably the same as in the nearby river (see e.g. Wagenhoff *et al.* 2012;

Magbanua *et al.* 2013; Piggott *et al.* 2015a). Further, invertebrate emigration rates (and thus presumably also immigration rates) in my study were higher than in previous *ExStream System* experiments (e.g. Bruder *et al.* 2017) and also at least as high as in small farmland streams in southern New Zealand (see e.g. Hansen & Closs 2007; James *et al.* 2009). These numbers indicate that this experimental setup (and the results obtained from my experiment) come with a high degree of realism and applicability to in-field settings. Regardless, I would recommend conducting further experiments investigating benthic invertebrate community responses to simulated water abstraction and deposited fine sediment of different grain sizes, ideally conducted at larger scales and in other river catchments, as this would provide further context and robustness to my findings, and add to our understanding of how we are shaping the distribution and structure of stream communities through our land-use practices.

I can conclude from this experiment that structural and functional metrics were similarly able to effectively detect stream invertebrate community responses to the two applied environmental stressors, and valuable insights can be gained from both approaches. Both data chapters were based on the same benthic community data set, but biological traits responded more frequently than community-based metrics to added fine sediment and reduced flow velocity, and with larger effect sizes (Table 4.1). This may be expected, as a community's functional composition can react faster than taxonomic composition to environmental stress, via sublethal effects such as reduced body size, or reduced reproductive success (Culp *et al.* 2011). Biological traits also have the advantage of providing insights into mechanisms driving stressor effects (Townsend & Hildrew 1994), and being consistent and applicable across spatial and temporal scales (Poff *et al.* 2006, Menezes *et al.*, 2010). Previous multiple-stressor studies have indicated that both trait-based metrics and community measures can respond similarly sensitively to environmental stressors (e.g. Wagenhoff *et al.* 2012, Magbanua *et al.* 2013; Lange *et al.* 2014; Naman *et al.* 2017; Mathers *et al.* 2017), and I would advise that future studies utilise a combination of both community metrics and biological traits to identify individual and multiple stressor effects.

One of the key findings of my study is that, especially for pollution-sensitive community metrics and biological traits, all fine sediment < 2 mm has profound negative effects. Consequently, stream management strategies should prioritise the mitigation and reduction of all fine sediment inputs into streams, not just silts and clays, as doing so is crucial to maintaining sensitive community components and preserving overall stream health. Sediment inputs can be prevented by maintaining buffer strips at stream edges, fencing to

prevent livestock entering waterways, and replanting of riparian strips (Carling *et al.* 2001; Quinn *et al.* 2009; Daigneault *et al.* 2017).

The implementation of these practices is essential, and there is no better time to start than now, to ensure the conservation and restoration of freshwater environments and the species that live in these sensitive and important ecosystems. The measures required to achieve the most desirable outcomes may be complex, require thorough foresight, documentation and planning (Buijse *et al.* 2002; Parkyn *et al.* 2003), and positive effects of restoration practices may require long periods of time to be observed and quantifiable (Davies-Colley *et al.* 2009; Leps *et al.* 2016). Yet, improved measures of river quality and stream health have been observed as a result of freshwater restoration efforts in New Zealand (e.g. Quinn *et al.* 2009; Larned *et al.* 2016; Wright-Stow *et al.* 2017) and overseas (e.g. Kail *et al.* 2005; González *et al.* 2017), and there is hope for the future if we prioritise and encourage the continued development and implementation of freshwater restoration and conservation practises.

References

- 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.
- Allibone, R. M. (2000). *Water abstraction impacts on non-migratory galaxiids of Otago streams*: Department of Conservation Wellington.
- Angradi, T. R. (1999). Fine sediment and macroinvertebrate assemblages in Appalachian streams: a field experiment with biomonitoring applications. *Journal of the North American Benthological Society*, **18**(1), 49-66.
- Beermann, A. J., Elbrecht, V., Karnatz, S., Ma, L., Matthaei, C. D., Piggott, J. J., & Leese, F. (2018). Multiple-stressor effects on stream macroinvertebrate communities: A mesocosm experiment manipulating salinity, fine sediment and flow velocity. *Science of The Total Environment*, **610**, 961-971.
- Belanger, S. E., J. W. Bowling, D. M. Lee, E. M. LeBlanc, K. M. Kerr, D. C. McAvoy, S. C. Christman, & D. H. Davidson (2002). Integration of aquatic fate and ecological responses to linear alkyl benzene sulfonate (LAS) in model stream ecosystems. *Ecotoxicology and Environmental Safety* **52**(2), 150-171.
- Bo, T., Fenoglio, S., Malacarne, G., Pessino, M., & Sgariboldi, F. (2007). Effects of clogging on stream macroinvertebrates: an experimental approach. *Limnologica-Ecology and Management of Inland Waters*, **37**(2), 186-192.
- Bonada, N., Doledec, S., & Statzner, B. (2007). Taxonomic and biological trait differences of stream macroinvertebrate communities between mediterranean and temperate regions: implications for future climatic scenarios. *Global Change Biology*, **13**(8), 1658-1671.
- Boone, M. D., Bridges, C. M., Fairchild, J. F., & Little, E. E. (2005). Multiple sublethal chemicals negatively affect tadpoles of the green frog, *Rana clamitans*. *Environmental Toxicology and Chemistry*, **24**(5), 1267-1272.
- Bowerman, T., Neilson, B. T., & Budy, P. (2014). Effects of fine sediment, hyporheic flow, and spawning site characteristics on survival and development of bull trout embryos. *Canadian Journal of Fisheries and Aquatic Sciences*, **71**(7), 1059-1071.
- Brooks, A. J., Chessman, B. C., & Haeusler, T. (2011). Macroinvertebrate traits distinguish unregulated rivers subject to water abstraction. *Journal of the North American Benthological Society*, **30**(2), 419-435.
- Brooks, A. J., & Haeusler, T. (2016). Invertebrate responses to flow: trait-velocity relationships during low and moderate flows. *Hydrobiologia*, **773**(1), 23-34.
- Bruder, A., Salis, R. K., Jones, P. E., & Matthaei, C. D. (2017). Biotic interactions modify multiple-stressor effects on juvenile brown trout in an experimental stream food web. *Global Change Biology*.
- Buijse, Anthonie D., Hugo Coops, M. Staras, L. H. Jans, G. J. Van Geest, R. E. Grift, Bastiaan Willem Ibelings, W. Oosterberg, & Roozen C.J.M (2002). Restoration strategies for river floodplains along large lowland rivers in Europe. *Freshwater Biology*, **47**(4), 889-907.

- Bunn, S. E., & Arthington, A. H. (2002). Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. *Environmental Management*, **30**(4), 492-507.
- Carling, P., Irvine, B., Hill, A., & Wood, M. (2001). Reducing sediment inputs to Scottish streams: a review of the efficacy of soil conservation practices in upland forestry. *Science of The Total Environment*, **265**(1), 209-227.
- Castella, E., Bickerton, M., Armitage, P. D., & Petts, G. E. (1995). The effects of water abstractions on invertebrate communities in UK streams. *Hydrobiologia*, **308**(3), 167-182.
- Chen, C. Y., Hathaway, K. M., & Folt, C. L. (2004). Multiple stress effects of Vision® herbicide, pH, and food on zooplankton and larval amphibian species from forest wetlands. *Environmental Toxicology and Chemistry*, **23**(4), 823-831.
- Clapcott J, Wagenhoff A, Neale M, Storey R, Smith B, Death R, Harding J, Matthaei C, Quinn J, Collier K, Atalah J, Goodwin E, Rabel H, Mackman J, Young R (2017). *Macroinvertebrate metrics for the National Policy Statement for Freshwater Management*. Prepared for the Ministry for the Environment. Cawthron Report No. 3073. 139 p. plus appendices.
- Clapcott J., Young R., Harding J., Matthaei C.D., Quinn J. & Death R. (2011) *Sediment Assessment Methods: Protocols and Guidelines for Assessing the Effects of Deposited Fine Sediment on In-stream Values*. Cawthron Institute, Nelson, New Zealand, pp. 108.
- Conroy, E., Turner, J., Rymaszewicz, A., Bruen, M., O'Sullivan, J., Lawler, D., & Kelly-Quinn, M. (2018). Further insights into the responses of macroinvertebrate species to burial by sediment. *Hydrobiologia*, **805**(1), 399-341.
- Cottam, D.P. and T.I. James. (2003). *Effects of fine sediment discharges on Card Creek and New River*. The West Coast Regional Council, Greymouth, New Zealand. p. 48.
- Cottingham, K. L., Lennon, J. T., & Brown, B. L. (2005). Knowing when to draw the line: designing more informative ecological experiments. *Frontiers in Ecology and the Environment*, **3**(3), 145-152.
- Covich, A. P., Palmer, M. A., & Cowl, T. A. (1999). The role of benthic invertebrate species in freshwater ecosystems: zoobenthic species influence energy flows and nutrient cycling. *Bioscience*, **49**(2), 119-127.
- Crain, C. M., Kroeker, K., & Halpern, B. S. (2008). Interactive and cumulative effects of multiple human stressors in marine systems. *Ecology Letters*, **11**(12), 1304-1315.
- Crowe A, Hay J 2004. *Effects of fine sediment on river biota*. Cawthron Report No.951
- 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*, **64**(6), 1345-1351.
- Daigneault, A. J., Eppink, F. V., & Lee, W. G. (2017). A national riparian restoration programme in New Zealand: Is it value for money? *Journal of Environmental Management*, **187**, 166-177.

- Davies-Colley, R. J. (2013). River water quality in New Zealand: an introduction and overview. *Ecosystem services in New Zealand: conditions and trends*. Manaaki Whenua Press, Lincoln, 432-447.
- Davies-Colley, R. J., Meleason, M. A., Hall, R. M. J., & Rutherford, J. C. (2009). Modelling the time course of shade, temperature, and wood recovery in streams with riparian forest restoration. *New Zealand Journal of Marine and Freshwater Research*, **43**(3), 673-688.
- Devonport, B., & Winterbourn, M. (1976). The feeding relationships of two invertebrate predators in a New Zealand river. *Freshwater Biology*, **6**(2), 167-176.
- Dewson, Z. S., James, A. B., & Death, R. G. (2007). A review of the consequences of decreased flow for instream habitat and macroinvertebrates. *Journal of the North American Benthological Society*, **26**(3), 401-415.
- Dolédec, S., Phillips, N., Scarsbrook, M., Riley, R. H., & Townsend, C. R. (2006). Comparison of structural and functional approaches to determining landuse effects on grassland stream invertebrate communities. *Journal of the North American Benthological Society*, **25**(1), 44-60.
- Doledec, S., Phillips, N., & Townsend, C. (2011). Invertebrate community responses to land use at a broad spatial scale: trait and taxonomic measures compared in New Zealand rivers. *Freshwater Biology*, **56**(8), 1670-1688.
- Doretto, A., Piano, E., Bona, F., & Fenoglio, S. (2018). How to assess the impact of fine sediments on the macroinvertebrate communities of alpine streams? A selection of the best metrics. *Ecological Indicators*, **84**, 60-69.
- Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z.-I., Knowler, D. J., Lévêque, C., & Stiassny, M. L. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews*, **81**(2), 163-182.
- Dunning, K. J. (1998). *Effects of exotic forestry on stream macroinvertebrates: the influence of scale in North Island, New Zealand streams: a thesis presented in partial fulfilment of the requirements for the degree of Masters of Science in Ecology at Massey University*. Massey University,
- Elbrecht, V., Beermann, A. J., Goessler, G., Neumann, J., Tollrian, R., Wagner, R., . . . Leese, F. (2016). Multiple-stressor effects on stream invertebrates: a mesocosm experiment manipulating nutrients, fine sediment and flow velocity. *Freshwater Biology*, **61**(4), 362-375.
- Extence, C.a, P Chadd, R., England, J., J Dunbar, M., J Wood, P., & D Taylor, E. (2013). The assessment of fine sediment accumulation in rivers using macro-invertebrate community response. *River Research and Applications*, **29**(1), 17-55.
- Folt, C., Chen, C., Moore, M., & Burnaford, J. (1999). Synergism and antagonism among multiple stressors. *Limnology and Oceanography*, **44**(3part2), 864-877.
- Foote, K. J., Joy, M. K., & Death, R. G. (2015). New Zealand dairy farming: milking our environment for all its worth. *Environmental Management*, **56**(3), 709-720.
- Garson, G. D. (2012). *Hierarchical linear modeling: Guide and applications*: Sage.
- Glendell, M., Extence, C., Chadd, R., & Brazier, R. E. (2014). Testing the pressure-specific invertebrate index (PSI) as a tool for determining ecologically relevant targets for reducing sedimentation in streams. *Freshwater Biology*, **59**(2), 353-367.

- González, E., Masip, A., Tabacchi, E., & Poulin, M. (2017). Strategies to restore floodplain vegetation after abandonment of human activities. *Restoration Ecology*, *25*(1), 82-91.
- Gordon, N. D. (2004). *Stream hydrology: an introduction for ecologists*: John Wiley and Sons.
- Gore, J. A. (1977). Reservoir manipulations and benthic macroinvertebrates in a prairie river. *Hydrobiologia*, *55*(2), 113-123.
- Hansen, E. A., & Closs, G. P. (2007). Temporal consistency in the long-term spatial distribution of macroinvertebrate drift along a stream reach. *Hydrobiologia*, *575*(1), 361-371.
- Henley, W., Patterson, M., Neves, R., & 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*(2), 125-139.
- Herrmann, P. B., Townsend, C. R., & Matthaei, C. D. (2012). Individual and combined effects of fish predation and bed disturbance on stream benthic communities: a streamside channel experiment. *Freshwater Biology*, *57*(12), 2487-2503.
- Horrigan, N., & Baird, D. J. (2008). Trait patterns of aquatic insects across gradients of flow-related factors: a multivariate analysis of Canadian national data. *Canadian Journal of Fisheries and Aquatic Sciences*, *65*(4), 670-680.
- Huryn, D. (1996). Temperature-dependent growth and life cycle of *Deleatidium* (Ephemeroptera: Leptophlebiidae) in two high-country streams in New Zealand. *Freshwater Biology*, *36*(2), 351-361.
- James, A. B. W., Dewson, Z. S., & Death, R. G. (2009). The influence of flow reduction on macroinvertebrate drift density and distance in three New Zealand streams. *Journal of the North American Benthological Society*, *28*(1), 220-232.
- Jackson, M. C., Loewen, C. J., Vinebrooke, R. D., & Chimimba, C. T. (2016). Net effects of multiple stressors in freshwater ecosystems: a meta-analysis. *Global Change Biology*, *22*(1), 180-189.
- Johnson, R. K., Wiederholm, T., & Rosenberg, D. M. (1993). Freshwater biomonitoring using individual organisms, populations, and species assemblages of benthic macroinvertebrates. *Freshwater Biomonitoring and Benthic Macroinvertebrates*, 40-158.
- Jones, J., Murphy, J., Collins, A., Sear, D., Naden, P., & Armitage, P. (2012). The impact of fine sediment on macro-invertebrates. *River Research and Applications*, *28*(8), 1055-1071.
- Julian, J. P., de Beurs, K. M., Owsley, B., Davies-Colley, R. J., & Ausseil, A.-G. E. (2017). River water quality changes in New Zealand over 26 years: response to land use intensity. *Hydrology and Earth System Sciences*, *21*(2), 1149.
- Kail, J., & Hering, D. (2005). Using large wood to restore streams in Central Europe: potential use and likely effects. *Landscape Ecology*, *20*(6), 755-772.
- Kaller, M., & Hartman, K. (2004). Evidence of a threshold level of fine sediment accumulation for altering benthic macroinvertebrate communities. *Hydrobiologia*, *518*(1), 95-104.
- Kemp, P., Sear, D., Collins, A., Naden, P., & Jones, I. (2011). The impacts of fine sediment on riverine fish. *Hydrological Processes*, *25*(11), 1800-1821.

- Kenny, J. F., Barber, N. L., Hutson, S. S., Linsey, K. S., Lovelace, J. K., & Maupin, M. A. (2009). *Estimated use of water in the United States in 2005* (No. 1344). US Geological Survey.
- Kroeker, K. J., Kordas, R. L., & Harley, C. D. (2017). Embracing interactions in ocean acidification research: confronting multiple stressor scenarios and context dependence. *Biology Letters*, **13**(3), 20160802.
- Lancaster, J., & Hildrew, A. G. (1993). Characterizing in-stream flow refugia. *Canadian Journal of Fisheries and Aquatic Sciences*, **50**(8), 1663-1675.
- Lange, K., Townsend, C. R., & Matthaei, C. D. (2014). Can biological traits of stream invertebrates help disentangle the effects of multiple stressors in an agricultural catchment? *Freshwater Biology*, **59**(12), 2431-2446.
- Larned, S. T., Snelder, T., Unwin, M. J., & McBride, G. B. (2016). Water quality in New Zealand rivers: current state and trends. *New Zealand Journal of Marine and Freshwater Research*, **50**(3), 389-417.
- Larsen, S., Vaughan, I., & Ormerod, S. (2009). Scale-dependent effects of fine sediments on temperate headwater invertebrates. *Freshwater Biology*, **54**(1), 203-219.
- Lenat, D. R. (1988). Water quality assessment of streams using a qualitative collection method for benthic macroinvertebrates. *Journal of the North American Benthological Society*, **7**(3), 222-233.
- Leps, M., Sundermann, A., Tonkin, J. D., Lorenz, A. W., & Haase, P. (2016). Time is no healer: increasing restoration age does not lead to improved benthic invertebrate communities in restored river reaches. *Science of The Total Environment*, **557-558**(Supplement C), 722-732.
- Liess, A., Lange, K., Schulz, F., Piggott, J. J., Matthaei, C. D., & Townsend, C. R. (2009). Light, nutrients and grazing interact to determine diatom species richness via changes to productivity, nutrient state and grazer activity. *Journal of Ecology*, **97**(2), 326-336.
- Magbanua, F. S., Townsend, C. R., Hageman, K. J., & Matthaei, C. D. (2013). Individual and combined effects of fine sediment and the herbicide glyphosate on benthic macroinvertebrates and stream ecosystem function. *Freshwater Biology*, **58**(8), 1729-1744.
- Magbanua, F. S., Townsend, C. R., Hageman, K. J., Piggott, J. J., & Matthaei, C. D. (2016). Individual and combined effects of fine sediment and glyphosate herbicide on invertebrate drift and insect emergence: a stream mesocosm experiment. *Freshwater Science*, **35**(1), 139-151.
- Mathers, K. L., Rice, S. P., & Wood, P. J. (2017). Temporal effects of enhanced fine sediment loading on macroinvertebrate community structure and functional traits. *Science of The Total Environment*, **599**, 513-522.
- 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**(3), 639-649.
- Matthaei, C. D., Weller, F., Kelly, D. W., & Townsend, C. R. (2006). Impacts of fine sediment addition to tussock, pasture, dairy and deer farming streams in New Zealand. *Freshwater Biology*, **51**(11), 2154-2172.

- Meier, W., Bonjour, C., Wüest, A., & Reichert, P. (2003). Modeling the effect of water diversion on the temperature of mountain streams. *Journal of Environmental Engineering*, **129**(8), 755-764.
- Menezes, S., Baird, D. J., & Soares, A. M. (2010). Beyond taxonomy: a review of macroinvertebrate trait-based community descriptors as tools for freshwater biomonitoring. *Journal of Applied Ecology*, **47**(4), 711-719.
- Ministry for the Environment & Stats NZ (2017). *New Zealand's Environmental Reporting Series: Our fresh water 2017*. Retrieved from www.mfe.govt.nz and www.stats.govt.nz
- Moss, D., Furse, M., Wright, J., & Armitage, P. (1987). The prediction of the macroinvertebrate fauna of unpolluted running-water sites in Great Britain using environmental data. *Freshwater Biology*, **17**(1), 41-52.
- Nakagawa, S. (2004). A farewell to Bonferroni: the problems of low statistical power and publication bias. *Behavioral ecology*, **15**(6), 1044-1045.
- Naman, S. M., Rosenfeld, J. S., Richardson, J. S., & Way, J. L. (2017). Species traits and channel architecture mediate flow disturbance impacts on invertebrate drift. *Freshwater Biology*, **62**(2), 340-355.
- Odum, E. P., Finn, J. T., & Franz, E. H. (1979). Perturbation theory and the subsidy-stress gradient. *Bioscience*, **29**(6), 349-352.
- Ormerod, S., Dobson, M., Hildrew, A., & Townsend, C. (2010). Multiple stressors in freshwater ecosystems. *Freshwater Biology*, **55**(s1), 1-4.
- Paine, R. T., Tegner, M. J., & Johnson, E. A. (1998). Compounded perturbations yield ecological surprises. *Ecosystems*, **1**(6), 535-545.
- Parkyn, S. M., Davies-Colley, R. J., Halliday, N. J., Costley, K. J., & Croker, G. F. (2003). Planted Riparian Buffer Zones in New Zealand: Do They Live Up to Expectations? *Restoration Ecology*, **11**(4), 436-447.
- Pianka, E. R. (1972). r and K selection or b and d selection? *The American Naturalist*, **106**(951), 581-588.
- Piggott, J. J., Lange, K., Townsend, C. R., & Matthaei, C. D. (2012). Multiple stressors in agricultural streams: a mesocosm study of interactions among raised water temperature, sediment addition and nutrient enrichment. *PloS one*, **7**(11), e49873.
- Piggott, J. J., Salis, R. K., Lear, G., Townsend, C. R., & Matthaei, C. D. (2015a). Climate warming and agricultural stressors interact to determine stream periphyton community composition. *Global Change Biology*, **21**(1), 206-222.
- Piggott, J. J., Townsend, C. R., & Matthaei, C. D. (2015b). Climate warming and agricultural stressors interact to determine stream macroinvertebrate community dynamics. *Global Change Biology*, **21**(5), 1887-1906.
- Piggott, J. J., Townsend, C. R., & Matthaei, C. D. (2015c). Reconceptualizing synergism and antagonism among multiple stressors. *Ecology and Evolution*, **5**(7), 1538-1547.
- Poff, N. L., Olden, J. D., Vieira, N. K., Finn, D. S., Simmons, M. P., & Kondratieff, B. C. (2006). Functional trait niches of North American lotic insects: traits-based ecological applications in light of phylogenetic relationships. *Journal of the North American Benthological Society*, **25**(4), 730-755.

- Pollard, A. I., & Yuan, L. L. (2010). Assessing the consistency of response metrics of the invertebrate benthos: a comparison of trait-and identity-based measures. *Freshwater Biology*, **55**(7), 1420-1429.
- Quinn, G. P., & Keough, M. J. (2002). *Experimental design and data analysis for biologists*: Cambridge University Press.
- Quinn, J. M., Croker, G. F., Smith, B. J., & Bellingham, M. A. (2009). Integrated catchment management effects on flow, habitat, instream vegetation and macroinvertebrates in Waikato, New Zealand, hill-country streams. *New Zealand Journal of Marine and Freshwater Research*, **43**(3), 775-802.
- Quinn, J. M., & Hickey, C. W. (1990). Magnitude of effects of substrate particle size, recent flooding, and catchment development on benthic invertebrates in 88 New Zealand rivers. *New Zealand Journal of Marine and Freshwater Research*, **24**(3), 411-427.
- 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**(2), 409-429.
- Rabeni, C. F., Doisy, K. E., & Zweig, L. D. (2005). Stream invertebrate community functional responses to deposited sediment. *Aquatic Sciences-Research Across Boundaries*, **67**(4), 395-402.
- Ramezani, J., Rennebeck, L., Closs, G. P., & Matthaei, C. D. (2014). Effects of fine sediment addition and removal on stream invertebrates and fish: a reach-scale experiment. *Freshwater Biology*, **59**(12), 2584-2604.
- Ricciardi, A., & Rasmussen, J. B. (1999). Extinction rates of North American freshwater fauna. *Conservation Biology*, **13**(5), 1220-1222.
- Richards, C., Haro, R., Johnson, L., & Host, G. (1997). Catchment and reach-scale properties as indicators of macroinvertebrate species traits. *Freshwater Biology*, **37**(1), 219-230.
- Rosenberg, D. M., Reynoldson, T. B., & Resh, V. H. (1996). *Establishing reference conditions for benthic invertebrate monitoring in the Fraser River catchment, British Columbia, Canada*. Vancouver, BC: Environment Canada.
- Ryder, G. I. (1989). *Experimental studies on the effects of fine sediments on lotic invertebrates*. PhD Thesis. University of Otago. Dunedin, New Zealand.
- Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R., & Kinzig, A. (2000). Global biodiversity scenarios for the year 2100. *Science*, **287**(5459), 1770-1774.
- Scarsbrook M., McIntosh A., Wilcock B. & Matthaei C. (2016) Effects of agriculture on water quality. Pages 483-503 in “*Advances in New Zealand Freshwater Science*” (editors C. Pearson, T. Davie, P. Jellyman & J. Harding), Canterbury University Press, Christchurch.
- Schuwirth, N., Kattwinkel, M., & Stamm, C. (2015). How stressor specific are trait-based ecological indices for ecosystem management? *Science of The Total Environment*, **505**, 565-572.
- Smakhtin, V. U. (2001). Low flow hydrology: a review. *Journal of Hydrology*, **240**(3), 147-186.
- Stark, J., Boothroyd, I., Harding, J., Maxted, J., & Scarsbrook, M. (2001). *Protocols for Sampling Macroinvertebrates in Wadeable Streams*: Cawthron Institute.

- Stark, J. D. (1985). Macroinvertebrate community index of water quality for stony streams.
- Statzner, B., Bis, B., Dolédec, S., & Usseglio-Polatera, P. (2001). Perspectives for biomonitoring at large spatial scales: a unified measure for the functional composition of invertebrate communities in European running waters. *Basic and Applied Ecology*, *2*(1), 73-85.
- Statzner, B., & Beche, L. A. (2010). Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? *Freshwater Biology*, *55*(s1), 80-119.
- Statzner, B., Hoppenhaus, K., Arens, M. F., & Richoux, P. (1997). Reproductive traits, habitat use and templet theory: a synthesis of world-wide data on aquatic insects. *Freshwater Biology*, *38*(1), 109-135.
- Suren, A. M. (2005). Effects of deposited sediment on patch selection by two grazing stream invertebrates. *Hydrobiologia*, *549*(1), 205-218.
- 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*(4), 725-737.
- Suttle, K. B., Power, M. E., Levine, J. M., & McNeely, C. (2004). How fine sediment in riverbeds impairs growth and survival of juvenile salmonids. *Ecological Applications*, *14*(4), 969-974.
- Swanson, S.M. (2004) *Multiple Stressors: Literature Review and Gap Analysis. Water Environment Research Foundation Report (Project 00-ECO-2B)*. International Water Association Publishing, London.
- Townsend, C. R., & Hildrew, A. G. (1976). Field experiments on the drifting, colonization and continuous redistribution of stream benthos. *The Journal of Animal Ecology*, 759-772.
- Townsend, C. R., & Hildrew, A. G. (1994). Species traits in relation to a habitat templet for river systems. *Freshwater Biology*, *31*(3), 265-275.
- Townsend, C. R., & Thompson, R. M. (2007). Body size in streams: macroinvertebrate community size composition along natural and human-induced environmental gradients. *Body size: The structure and function of aquatic ecosystems*, 77-97.
- 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*(6), 1810-1819.
- Usseglio-Polatera, P., Bournaud, M., Richoux, P., & Tachet, H. (2000). Biological and ecological traits of benthic freshwater macroinvertebrates: relationships and definition of groups with similar traits. *Freshwater Biology*, *43*(2), 175-205.
- Vandewalle, M., De Bello, F., Berg, M. P., Bolger, T., Dolédec, S., Dubs, F., . . . Lavorel, S. (2010). Functional traits as indicators of biodiversity response to land use changes across ecosystems and organisms. *Biodiversity and Conservation*, *19*(10), 2921-2947.
- 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*(4), 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**(9), 1916-1936.
- Waters, T. F. (1969). Sub-sampler for dividing large samples of stream invertebrate drift. *Limnology and oceanography*, **14**(5), 813-815.
- Waters, T. F. (1995). Sediment in streams. *American Fisheries Society, Monograph*, 7.
- Wentworth, C. K. (1922). A scale of grade and class terms for clastic sediments. *The Journal of Geology*, **30**(5), 377-392.
- Winterbourn, M. (1974). The life histories, trophic relations and production of *Stenoperla prasina* (Plecoptera) and *Deleatidium* sp. (Ephemeroptera) in a New Zealand river. *Freshwater Biology*, **4**(6), 507-524.
- Wood, P. J., & Armitage, P. D. (1997). Biological effects of fine sediment in the lotic environment. *Environmental management*, **21**(2), 203-217.
- Wright-Stow, A. E., & Wilcock, R. J. (2017). Responses of stream macroinvertebrate communities and water quality of five dairy farming streams following adoption of mitigation practices. *New Zealand Journal of Marine and Freshwater Research*, **51**(1), 127-145.
- Yuan, L. L., & Norton, S. B. (2004). Assessing the relative severity of stressors at a watershed scale. *Environmental monitoring and assessment*, **98**(1), 323-349.
- Zweig, L. D., & Rabeni, C. F. (2001). Biomonitoring for deposited sediment using benthic invertebrates: a test on 4 Missouri streams. *Journal of the North American Benthological Society*, **20**(4), 643-657.