

Multiple agricultural stressors and stream benthic algae: the effects of fine sediment particle size and flow velocity reduction on community composition and biological traits

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Dedicated to the memory of
William Worsfold
A true visionary in the field of land management

Abstract

There are many challenges facing freshwater systems that originate from urban development, climate change and agriculture. Agricultural intensification is one of the leading causes of freshwater biodiversity decline worldwide. Effective management of the ecological consequences of agricultural intensification requires understanding the effects of multiple stressors operating in agricultural streams. This is because interactions, especially synergisms and antagonisms, can lead to situations where management actions may not lead to a proportional recovery of impacted streams. Previous research has shown that fine sediment and water abstraction are pervasive stressors that can interact and strongly affect stream communities. However, the individual and interactive effects of different grain sizes of deposited fine sediment and abstraction-induced flow velocity reduction have yet to be investigated.

To study the effects and interactions of fine sediment particle size and flow velocity reduction, I performed an experiment in 60 outdoor stream mesocosms. This experiment addressed the above knowledge gap for benthic algal population, community and biological trait responses. I used four fine sediment treatments (no sediment added versus three different grain sizes: 0-0.125mm, 0.125-0.250mm, 1-2mm) combined with three flow velocities simulating increasing levels of water abstraction (fast, medium, slow). Biological response variables included four algal community-level metrics, absolute abundances of 15 common taxa (Chapter 2) and relative abundances of 25 algal trait categories (Chapter 3).

Algal community metrics and common taxa (Chapter 2) showed 16 significant responses to sediment addition, three significant responses to flow velocity reduction and 11 non-additive interactions between the two stressors (interaction frequency 58% of all community/taxon metrics). The majority of these responses to sediment addition and flow velocity reduction were positive. There were also some negative responses, namely the blue-green alga *Phormidium* spp. and the diatom *Gomphonema parvulum*. The three flow velocity reduction responses were all negative. Algal traits (Chapter 3) showed 14

significant responses to sediment, two responses to flow velocity and 12 interactions (48% of trait variables). Sediment addition caused seven positive responses and eight negative responses. The two flow reduction responses were both positive.

These findings allow several interesting conclusions. Firstly, added fine sediment with different particle sizes was a more pervasive stressor than flow velocity reduction. Secondly, fine sediment and flow velocity reduction interacted frequently to produce complex response patterns that could not be predicted based on the single-stressor effects involved. This point was illustrated by algal trait interactions that often overrode the main stressor effects, especially for flow velocity reduction. Thirdly, facilitation (an interaction that benefits at least one species and harms neither) was probably more prevalent with the addition of different-sized fine sediment rather than when flow velocity was reduced. My findings also highlight the considerable potential of using biological algal traits for detecting both simple and complex multiple-stressor effects. My main conclusion for freshwater managers is that fine sediment, regardless of its particle size, was a very pervasive stressor in my study, and that consequently efforts should prioritize the reduction of fine sediment inputs to the stream environment. Furthermore, care should be taken when implementing management strategies in situations where multiple stressors operate simultaneously because of the numerous interactions observed.

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Chapter One:

1 General Introduction

1.1 Land use effects on freshwater ecosystems (fine sediment particle and water abstraction)

In the last 200 years, human activity has played an increasingly large role in transforming the environment on which all organisms rely to survive and thrive (Scheffer *et al.*, 2001). These transformations include invasive species, hydrological alterations, habitat fragmentation, increased inputs of nutrients, chemicals and pollutants, and agricultural intensification (Woodward *et al.*, 2010). Chief amongst the pressures behind these transformations are industrialization, the growth of the human population, increase of human consumption, and globalisation of society and the economy (Malmqvist & Rundle, 2002; Pettifor, 2006). All of these transformations have affected the freshwater environment significantly.

Freshwater (especially clean freshwater) is one of the world's most important resources in regard to biological life. For humans, it is not only necessary for life, it is also the basis on which our food supply (both nomadic and agricultural) depends (Lowe & Pan, 1996; Malmqvist & Rundle, 2002). Since freshwater forms the basis of the food supply (especially surplus food supply in our modern society), it is crucial to society, the economy and industry (Lowe & Pan, 1996; Malmqvist & Rundle, 2002). It also serves auxiliary functions, for example transport and recreation (Lowe & Pan, 1996; Malmqvist & Rundle, 2002). Similarly, freshwater ecosystems and the biodiversity they contain hold both practical and intrinsic worth (Lowe & Pan, 1996; Malmqvist & Rundle, 2002; Geist, 2011). Freshwater biodiversity has been particularly affected by human-induced transformations, with noticeable declines in both the range and abundance of many freshwater species (Dudgeon *et al.*, 2006; Vörösmarty *et al.*, 2010). This is because of a disproportionately high species richness, high levels of interaction with human systems and the magnification of these impacts due to human factors (settlement patterns) and natural factors (limited capacity for dilution) (Dudgeon, 2010; Ormerod *et al.*, 2010; Vörösmarty *et al.*, 2010). Future trends suggest that these factors are likely to intensify with an increasing human population, an increased demand for luxury goods, a globalized economy, and global climate change (Dudgeon *et al.*, 2006; Piggott *et al.*, 2015a; Piggott *et al.*, 2015b; Piggott *et al.*, 2015c; Piggott *et al.*, 2015d).

All of this suggests that these transformations will continue to produce various ‘stressors’ affecting freshwater ecosystems. A ‘stressor’ can be defined as ‘a variable that exceeds its normal range of variation and adversely affects individual taxa, community composition or ecosystem functioning’ (Townsend *et al.*, 2008). Agricultural intensification is a particularly important producer of freshwater stressors, and consequently one of the leading causes associated with the decline in native freshwater biodiversity worldwide (Allan, 2004; Parkyn & Wilcock, 2004; Vörösmarty *et al.*, 2010; Bierschenk *et al.*, 2012). It often produces stressors such as elevated nutrient levels, pesticides, herbicides, hydrological alterations (flow velocity and flow discharge) and excessive fine deposits and suspended sediment (Niyogi *et al.*, 2003; Townsend *et al.*, 2008; Magbanua *et al.*, 2013a; Piggott *et al.*, 2015b&c; see chapters 2, 3, 4 & 5 in Salis 2016). These stressors have become more widespread and serious in New Zealand, especially over the last 20 years. This change coincides with the shift from extensive sheep and beef farming to the more intensive and profitable dairy farming, with the addition of 2.9 million dairy cows from 1994 to 2014 (Monaghan *et al.*, 2007b; Statistics New Zealand, 2014; Foote *et al.*, 2015). Similarly, there has been a diversification and increase into deer farming in some regions, which releases particularly high amounts of fine sediment into streams (Matthaei *et al.*, 2006; Niyogi *et al.*, 2007 from Environmental Management).

Fine sediment, both in its suspended and deposited forms, is one of the most pervasive stressors affecting freshwater ecosystems in inhabited areas (Allan, 2004; Schofield *et al.*, 2004; Matthaei *et al.*, 2006; Jones *et al.*, 2014). Naturally, fine sediment is present in fluvial systems and there are many pathways by which sediment enters a river, for example from channel sources (the bed and banks of the stream) and erosion of soil in the catchment (Williamson *et al.*, 1992; Foote *et al.*, 2015). With agricultural intensification and some subsequent poor land management, channel source erosion increases due to bank instability (removal of plants) and trampling by stock. At the same time, erosion from the catchment increases due to vegetation removal during initial deforestation (Allan, 2004; Buck, 2004). Once in the stream, sand grains (size 0.2-0.5 mm) are the first to be entrained (drawn in and transported) by the stream current while clays (< 0.063 mm), silts

and larger substratum particles may require greater velocities and shear stress in order entrain them. This is due to submergence within the laminar sublayer (Wood & Armitage, 1997). Suspended fine sediment (especially smaller particles) is often associated indirectly with light attenuation (through increased turbidity affecting primary production) and directly with damage to cells through physical abrasion associated with substratum movement (Wood & Armitage, 1997). Abrasion and clogging of the gills also negatively influences macroconsumers such as macro-invertebrates and fish (Schofield *et al.*, 2004; Elbrecht *et al.*, 2016). In benthic algae, cell damage is more likely to happen due to larger, unconsolidated sediment grains such as sand because they move more often than clay particles. Deposition generally occurs at low stream flows, but this can be altered by areas of high turbulence (Wood & Armitage, 1997; Yamada & Nakamura, 2002). It is the deposition of clays and silts that often leads to some of the more severe impacts of increased fine sediment (Graham, 1990; Jones *et al.*, 2014). These impacts can include the smothering of the original periphyton communities, a change of substratum composition, reduced habitat heterogeneity (at high levels) for stream algae and the reduction of food quality (through the increase in the inorganic content of biofilms) for stream invertebrates (Jones *et al.*, 2014). It can also hamper the attachment of periphyton. All of these effects may be made even more severe by water abstraction (Lange *et al.*, 2016; see chapter 5 in Salis, 2016).

Water abstraction (for irrigation or hydropower generation) is another stressor present in the agricultural environment which often has adverse effects on freshwater ecosystems, especially when acting in combination with increased fine sediment (Matthaei *et al.*, 2010; see chapter 5 in Salis, 2016). Water abstraction can influence stream communities through decreased water discharge which, in turn, affects stream temperature, number of drying events, increased boundary thickness near the substratum and current velocity (Lemly, 1982; Dewson *et al.*, 2007). A primary factor involved is current velocity (and its subsequent reduction), which influences benthic algae, macro-invertebrates, and fish through numerous pathways (Leprieur *et al.*, 2006; Matthaei *et al.*, 2010; Lange *et al.*, 2014; see chapter 5 in Salis, 2016). For benthic algae (the focus of my MSc research project), current velocity can directly affect cells through altering shear stress, nutrient uptake rates, metabolism

and reproduction (Stevenson *et al.*, 1996; Peterson, 1996; Passy, 2007a; Passy & Larson, 2011). At fast current velocities, there is high shear stress (from drag), increased metabolism (through higher nutrient transport to cells), increased photosynthesis, respiration and reproduction (Allan & Castillo, 2007; Passy & Larson, 2011; Tang *et al.*, 2013). The life processes mentioned before may be especially important in high-density biofilms where nutrient-depleted conditions may occur (Stevenson, 1997). The reduction of current velocity could have a negative effect on these processes, thus affecting the algal communities. This negative effect, however may have exceptions in certain environments, such as in nutrient-poor waters. The lower shear stress of reduced current velocity, would increase algal immigration rates and may decrease emigration rates leading to higher algal densities (Stevenson, 1997). Similarly, lower velocities may allow periphyton to recycle resources and enzymes, which could be beneficial in nutrient-poor conditions. Indirect effects of reduced current velocity are numerous and include effects on habitat (composed of substrate size and stability), light availability (through water depth) and other ecosystem players (macro-invertebrate grazers and fish) (Stevenson, 1997). The former is the result of water velocity determining which sediment particles are entrained, transported and deposited (depending on size/mass and cohesion). Very high water velocities (e.g. during floods) can transport every streambed substratum particle size including cobbles and boulders (e.g. Matthaei *et al.*, 1999, Matthaei & Huber, 2002). The continuous reduction of flow velocity means that only a narrow range of very small particle sizes (sand, silts and clays) are entrained and transported. Slow flow velocity also means that the larger of these particle sizes (i.e. unconsolidated sand) are deposited first. This ability of a current to entrain, transport, and deposit different sediment sizes suggest that it is probable that these stressors interact in accordance with multiple-stressor theory.

1.2 Multiple stressors in freshwaters

Managing and monitoring freshwater systems impacted by agricultural intensification has proven to be a major problem globally. Effective management of this problem requires knowledge of the effects of both individual stressors and the interactions produced when two or more stressors are acting in concert on stream

ecosystems (Brown *et al.*, 2013). This is because single-stressor studies cannot account for ‘ecological surprises’ and the complex responses that often occur when interactions are present (Folt *et al.*, 1999). These interactions, in the form of synergisms and antagonisms, are particularly important in an agricultural context where there are many altered variables. There are additive effects which are the sum of the individual effects, and then there are non-additive effects like synergisms and antagonisms. Synergisms can be either positive or negative (Piggott *et al.*, 2015c). According to this model, positive synergisms occur when “the result is greater than the sum of the individual effects and greater than individual effects in the same direction. Conversely, a negative synergism occurs when a result is more negative than predicted additively. Synergisms are a concern because rates of ecosystem decline might be underestimated if just the individual stressors are considered (Brown *et al.*, 2013). They also can cause a more rapid decline in local ecosystems than additive stressor effects and antagonistic interactions. Antagonisms, in the same way as synergisms, can be divided into both positive and negative forms (Piggott *et al.*, 2015c). Positive antagonisms occur when a result is less positive than the sum of the individual effects or less than the effect of a single stressor in the same direction, whereas negative antagonisms occur when a result is less negative than predicted additively. Based on the existing information available, antagonisms may be more common than synergisms in freshwater systems, and they can lead to situations where management actions may not lead to a proportional recovery (Brown *et al.*, 2013; Jackson *et al.*, 2016; Piggott *et al.*, 2015c). For example, reducing a stressor in an antagonistic situation may yield smaller benefits. It is therefore necessary to know the types of stressors, their individual effects and any interactions present in a system before expending limited management resources (Brown *et al.*, 2013).

Investigating multiple-stressor effects and interactions is often difficult and determining the mechanistic pathways by which these stressors act can be problematic (Downes, 2010). There are three main approaches to investigating multiple stressors. These are laboratory experiments, field surveys and outdoor experiments (ranging from field manipulations to mesocosm experiments) (Townsend *et al.*, 2008). Laboratory experiments are good for investigating multiple

stressors because there is a high degree of control on experimental and external variables. This allows for causation to be established, but the laboratory setting means that there are considerable limitations when trying to apply the results to a real situation. These experiments are often used in toxicology assessments where individual responses to stressors are investigated (Maltby, 1999). Alternatively, field surveys can provide realism, however they cannot establish causation, investigate mechanistic pathways through which stressors act or suggest effective management approaches (Downes, 2010). A good way of reconciling these two approaches has been the development of controlled outdoor field experiments. If designed well, these experiments can approach the realism of a field survey and combine this with a strong element of variable control, and allow for manipulated stressors and biological response variables to be directly measured (see chapter 1 in Salis, 2016). While field experiments cost more to perform than laboratory experiments, they provide both a realistic setting and the ability, if designed well, to establish causation. In my research project, a field experiment was conducted in stream mesocosms in order to investigate the effects of two key agricultural stressors, deposited fine sediment and reduced flow velocity (simulating water abstraction), on freshwater benthic algae.

1.3 Freshwater benthic algae

Freshwater benthic algae are a diverse assemblage of photosynthetically active protists and cyanobacteria (Lowe & Pan, 1996; Lange *et al.*, 2016). They are autotrophs who inhabit biofilms, respond to landscape and in-stream environmental variables and play a central role in the physical, chemical and biological process of the stream ecosystem (Lowe & Pan, 1996; Lange *et al.*, 2016). They are the primary source of energy in many streams, stabilize and bind stream substrata, oxygenate the water and control the flow of nutrients and chemicals such as phosphorus into the water column (Lowe & Pan, 1996; Gerbersdorf *et al.*, 2008; Jones *et al.*, 2014). Landscape variables (which determine in-stream variables) such as topography, land use, slope and vegetation are important in determining broad-scale patterns within a system (Biggs, 1996; Allan, 2004). These landscape variables are altered most drastically by initial deforestation and then again by later agricultural intensification (Allan, 2004).

In-stream variables (that are determined by landscape variables) control regulating processes of periphyton biomass accrual and loss (Biggs, 1996). Periphyton biomass accrual is controlled mainly by nutrients and light, and is also influenced by water temperature (via metabolism and growth). Biomass loss is the result of grazing (by invertebrates and sometimes fish) and physical disturbance, the latter of which results from substratum instability, abrasion, and high water velocity (Biggs, 1996; Jones *et al.*, 2014). The degree to which biomass accumulates and is lost in a periphyton species or community is related to the biological traits present in that community or species (Passy 2007b; Lange *et al.*, 2016; see chapters 4 & 5 in Salis, 2016). For example, light and nutrient acquisition (and thus biomass accumulation) is related to pigment composition, motility and nitrogen fixation, whereas biological traits associated with disturbance and grazing (both of which cause biomass loss) include life form, attachment mode and life history (Biggs *et al.*, 1998; Litchman & Klausmeier, 2008; Lange *et al.*, 2016). Consequently, changes in periphyton community structure and trait biological composition can be measured through indicators and connected with changes to landscape variables.

1.4 Biological monitoring (structural and functional indicators)

Regular monitoring is an essential part of assessing environmental health in order to develop and review management practices. More specifically, it is necessary in order to identify baseline and reference conditions as well as track changes during freshwater management (Park & Hwang, 2016; Park, 2016). For freshwater ecosystems, the two most prominent types of monitoring are chemical and biological monitoring, due to their moderate retrieval cost and relevancy of the information provided (Lowe & Pan, 1996; Karr & Chu, 1997). While chemical monitoring is useful, biological monitoring is necessary in dealing with any water quality issue. There are several reasons for this including range of stressors monitored, accuracy and ability to detect and measure the effects of multiple stressors (Lowe & Pan, 1996; Karr & Chu, 1997). Firstly, biological monitoring can detect diffuse sources of pollutants and stressors, such as fine sediment and water abstraction which cannot be usually detected by chemical monitoring (Lowe & Pan, 1996; Karr & Chu, 1997). Thirdly, biological monitoring can pick up significant

discrete pollution events that chemical monitoring may miss because biological communities integrate the conditions which have been present for several weeks or months (Chapman, 1996). Lastly, it allows scientists to investigate the complex responses such as synergisms and antagonisms (Karr & Chu, 1997). Biological monitoring can use structural and functional indicators to investigate these stressors, preferably together (Chapman, 1996; Karr & Chu, 1997; Yates *et al.*, 2014). These indicators tend to be better at assessing specific human activities (e.g. wastewater treatment) rather than broad landscape changes (e.g. agriculture), but they are still relatively effective for both purposes (Yates *et al.*, 2014). Structural biological indicators consist of community metrics (e.g. chlorophyll, species diversity, species evenness), and population abundance and trait abundance. Functional indicators include indices such as stream metabolism, decomposition rates, stable isotopes, litter breakdown and trait abundance (Gessner & Chauvet, 2002; Niyogi *et al.*, 2003; Yates *et al.*, 2014; Lange *et al.*, 2016). The use of structural indicators has been associated more with cultivation, agriculture and sensitivity to water abstraction while functional indicators have been associated with wastewater treatment and urban land cover (Death *et al.*, 2009; Yates *et al.*, 2014). Therefore, it is beneficial to use a combination of both types of bio-indicators to investigate the effect of stressors on benthic algae (Yates *et al.*, 2014).

Benthic algae make excellent water quality monitoring organisms. Their many useful indicator attributes include their importance in the food web and their predominantly sessile lifestyle (they cannot move far enough to avoid pollution). Further, they are species-rich with a range of biological traits, short lifestyles, easily sampled and relatively easy to handle and identify (Chapman, 1996; Lowe & Pan, 1996; McCormick & Stevenson, 1998; Johnson *et al.*, 2006; Resh, 2008; Whitton & Potts, 2012). Benthic algae typically reflect changes in stream condition through distinct changes in assemblage composition, rather than diversity (Kutka & Richards, 1996; Hirst *et al.*, 2002; Gessner & Chauvet, 2002). In my MSc research project, I will make use of these positive attributes in combination with structural indicators to investigate the individual and interactive effects of fine sediment particle size and flow velocity reduction on stream benthic algal communities.

1.5 Thesis outline and aims

The predominate aim of my thesis was to determine the individual and combined effects of sediment particle size and flow velocity reduction on benthic algal communities. It was used to determine the pervasiveness of stressors and mechanisms by which these stressors act on benthic algal communities. Auxiliary aims include exploring the usefulness of Lange *et al's* (2016) trait based framework which seeks to provide a comprehensive system to identify stressor mechanism pathways. To satisfy these aims, I conducted (with the Stream Team) one mesocosm experiment in Autumn 2015. This experiment resulted in two data chapters.

Chapter 2- Community indices and population abundances are the traditional and most common approaches to investigating the effects and interactions of stressors. These approaches have demonstrated this when investigating the general effects of fine sediment addition and water abstraction on benthic algal communities. In this chapter, I will focus on the effects and interactions of sediment particle size and flow velocity reduction on benthic algae. This will be explored using the responses of species diversity, taxon richness, species evenness, chlorophyll a, and the most common 15 species abundances.

Chapter 3- Trait based frameworks are starting to complement community indices and population abundances. Some previous research has suggested that this trait based framework can be used to investigate effects and interactions of stressors. Most importantly these traits based frameworks may provide a better way to investigate mechanistic pathways of stressors. In this chapter, the Lange *et al.*, (2016) trait based framework is used to explore the effect and mechanistic pathways of sediment grain size and flow velocity reduction on benthic algal communities.

The final chapter of this thesis, is the General Discussion, which will discuss and integrate the findings of my two data chapters. This chapter discusses the overall impact of sediment particle size and flow velocity reduction on benthic algal communities and includes interactions and the probable mechanistic explanations

behind them. Furthermore, it evaluates the use of the trait based framework proposed by Lange *et al.*, (2016) to investigate the mechanistic pathways by which these stressor act and interact. Finally, it discusses the implications of these findings in terms of management and future research.

Chapter Two:

**2 Algal community and population responses to
sediment particle size and flow velocity
reduction**

2.1 Introduction

A major challenge facing environmental managers is how to account for multiple stressors operating in the freshwater environment (Vörösmarty *et al.*, 2010; Ormerod *et al.*, 2010; Nõges *et al.*, 2016; Jackson *et al.*, 2016). These stressors originate from human activities such as urban development, climate change and agriculture (Allan 2004). Many studies over the last decade have identified fine sediment and flow reduction as significant stressors in streams and rivers impacted by agricultural development (see e.g. reviews by Wood & Armitage, 1997; Dewson *et al.*, 2007; Kemp *et al.*, 2011). Previous studies have revealed many single-stressor effects on stream biota as well as several complex interactions between stressors (Matthaei *et al.*, 2010; Elbrecht *et al.*, 2016; Lange *et al.*, 2016; see chapters 4 & 5 in Salis, 2016). Assessing the composition and structure of biological communities is an important, efficient and sensitive measure of freshwater ecosystem health in relation to land use changes (Death *et al.*, 2009; Yates *et al.*, 2014). The use of the benthic periphyton community (henceforth called algal communities for simplicity) structure for this purpose is based on the idea that each algal species has its own stressor limit that is derived from their unique ecological niche and evolutionary history (Vinebrooke *et al.*, 2004; Crain *et al.*, 2008).

Fine sediment has been proven to be a pervasive stressor for stream algal communities in agricultural catchments (Magbanua *et al.*, 2013a; Wagenhoff *et al.*, 2013; Jones *et al.*, 2014; Lange *et al.*, 2016). Sediment affects benthic algae in its deposited and suspended forms and impacts directly and indirectly on algal community metrics (e.g. taxon richness, biomass, total cell density and evenness) and community composition (e.g. abundances of common taxa). Indirectly, deposited fine sediment has been associated with reduced grazing pressure from macro-invertebrates (Wood & Armitage, 1997; Allan, 2004). Directly, it increases habitat heterogeneity for benthic algae, decreases light attenuation, increases substratum instability, exaggerates abrasion and contributes to the smothering of the original periphyton community (leading to anoxia) (Schofield *et al.*, 2004; Izagirre *et al.*, 2009; Piggott *et al.*, 2012; Wagenhoff *et al.*, 2013; Jones *et al.*, 2014). Increased suspended fine sediment concentrations have been associated with higher water turbidity, increased scouring/abrasion and increased nutrient uptake

(from suspended sediment) (Francoeur & Biggs, 2006; Piggott *et al.*, 2012). Previous studies have revealed taxon-specific algal responses such as increases in the density of certain taxa, for example non-filamentous algae, motile diatoms and some diatoms. Moreover, these taxa sometimes displayed a subsidy-stress relationship with deposited fine sediment (Wagenhoff *et al.*, 2013). A decline in the diatom *Gomphonema minutum* and algal biomass accrual has also been recorded as well as a reduction in filamentous and adnate/prostrate forms (Piggott *et al.*, 2012; Piggott *et al.*, 2015b). The negative effects of increasing fine sediment levels on periphyton have been shown to interact with stream flow, with reductions in algal biomass more marked at reduced or higher flow than at normal flow (Francoeur and Biggs, 2006; Matthaei *et al.*, 2010).

Sediment particle size can also play a potentially important, but less well researched, role in the biological effects of fine sediment on algae. Silt particles (sediments less than 63 µm in size) are known to be important for contaminant absorption (especially heavy metals) and later transportation. This is because of their large surface area and geochemical composition (Thoms, 1987; Stone & Droppo, 1994; Wood & Armitage, 1997). It is therefore conceivable that agrochemicals such as herbicides may be more potent when occurring in streams together with silt particles than with coarser fine sediment above 63 µm. Furthermore, settlement works differently for silts and clays than for particles larger than 0.1 mm. Firstly, clays and silts tend to form floccules (aggregations of particles), and this discourages their detachment from the base substratum (Graham, 1990; Wood & Armitage, 1997). Secondly, these very small particles are protected by their submergence within the laminar sublayer (Richards, 1982; Wood & Armitage, 1997). Sand (2 mm-0.063 mm) is especially effective at scouring (through physical abrasion) and damaging diatom cells (Delgado *et al.*, 1991; Francoeur & Biggs, 2006; Jones *et al.*, 2014). This is because sand grains lack the cohesive forces of larger particles or silts, causing them to move frequently (Jewson *et al.*, 2006; Jones *et al.*, 2014). They also tend to be infertile compared to silts and therefore do not provide the same subsidy-stress relationship through adding nutrients to stream ecosystems that many silts and clays do (Jones *et al.*, 2014).

Water abstraction for irrigation or hydroelectricity production reduces stream discharge and current velocity, which in turn can increase the frequency and severity of droughts in streams (Jones *et al.*, 2014; Lange *et al.*, 2016). Water abstraction has previously been shown to interact with fine sediment, often enhancing the strength of negative biological responses to sediment (Matthaei *et al.*, 2010; see chapter 5 in Salis 2016). Slow to intermediate current velocities tend to benefit current-sensitive benthic algal species, while fast current velocities tend to stimulate the growth (through increased nutrient uptake) of tolerant, motile or attached high-profile algae such as the diatom genera *Synedra* and *Gomphonema* (Passy & Larson, 2011). Fast flow velocities may also remove algal biomass, especially unattached forms such as the diatoms *Melosira* and *Fragilaria*, through scour (physical abrasion) (Passy & Larson, 2011). This process can be of considerable importance when unconsolidated sand particles are the dominant substratum size in a stream and when the algal community affected has low biomass (Delgado *et al.*, 1991; Francoeur & Biggs, 2006; Jones *et al.*, 2014). Furthermore, fast flow velocities may slow down biomass accumulation at the beginning of succession, but may ultimately generate a larger biomass in mature communities (Passy & Larson, 2011).

As can be seen from the previous paragraphs, there are several existing studies that have focused on the individual effects of fine sediment particle size or flow velocity reduction on stream benthic algae. However, to my knowledge, there are no studies that have investigated the interactive effects of current velocity reduction and fine sediment particle size on benthic algae. The present study addresses this knowledge gap using 60 circular stream mesocosms in an outdoor experimental system (*ExStream System*). This system offers an excellent compromise between the realism of a stream survey and the strict control of a laboratory experiment (see e.g. Wagenhoff *et al.*, 2012, 2013; Piggott *et al.*, 2015a; Piggott *et al.*, 2015b). The system provides high statistical power, very good control of all manipulated factors, and allows natural immigration and emigration of stream algae, bacteria and invertebrates. Moreover, the mesocosms experience the same light, temperature,

chemical and weather conditions as the river from which the water feeding the system is sourced.

Against this background, the aim of my experiment was to test the following hypotheses regarding periphyton population and community processes.

- 1) The addition of larger sediment grain sizes (1-2 mm) will result in stronger negative effects on periphyton community metrics and population abundances compared to smaller sediment grain sizes (0-0.125 mm) (Delgado *et al.*, 1991; Francoeur & Biggs 2006; Jewson *et al.*, 2006; Jones *et al.*, 2014). More specifically:
 - a. Algal biomass and total cell density may decline with the addition of large-sized sediment but not with the addition of smaller particles (Stevenson *et al.*, 2006);
 - b. Taxon richness, algal taxon richness and evenness may decrease with the addition of large-sized sediment;
 - c. Abundances of certain resistant taxa (such as *Melosira* and Cyanobacteria) may increase or remain stable with the addition of large-sized sediment, whereas species that are vulnerable to sand abrasion (such as *Cocconeis* and other diatoms) may decline (Stevenson *et al.*, 2006).
- 2) The reduction of flow velocity (simulating water abstraction) may have a similarly strong effect (compared to sediment particle size) on community metrics and population abundances (Matthaei *et al.*, 2010).
 - a. Algal biomass, total cell density, taxon richness and evenness may increase with flow velocity reduction (Matthaei *et al.*, 2010);
 - b. Fast flow velocity (the default) may benefit rheophilic algal taxa such as *Melosira* spp., *Fragilaria* spp. and *Cocconeis* spp. (Stevenson *et al.*, 2006; Passy & Larson, 2011);
 - c. Flow velocity reduction may benefit *Gomphonema* spp. and some other velocity-sensitive species (Passy & Larson, 2011).

- 3) The responses of algal community metrics and abundances of common taxa to the addition of fine sediment may be more frequent and severe at reduced flow velocities (Matthaei *et al.*, 2010; see chapter 5 in Salis, 2016). This is because fine sediment is generally more likely to settle and persist at reduced flow velocities.

2.2 Methods

2.2.1 Study Site

The experiment was performed from 7th February to 31st March (late summer/ early autumn) 2015. The mesocosm setup was located near the banks of the Kauru River, a 5th-order stream located in North Otago, New Zealand (170°44.60 East, 45°6.50 South, 98 m a.s.l). The catchment is in the partial rain shadow of the Southern Alps and receives a mean annual rainfall of 755 mm (Otago Regional Council, 2013). Mean annual discharge of the river is approximately 1.29m³ s⁻¹, as recorded at a gauging station 300 meters upstream from the experimental site (Otago Regional Council, 2013). The natural vegetation in the catchment consists of native tussock; however, exotic pasture is also present, especially in the lower reaches. This is primarily the result of low-intensity farming (sheep and beef cattle). The river is nutrient-poor and is known to contain diverse algal and invertebrate communities (e.g. Lange *et al.*, 2011; Piggott *et al.*, 2012; Piggott *et al.*, 2015a; Piggott *et al.*, 2015b).

2.2.2 Experimental Design

A full-factorial design was used to determine the effects of multiple fine sediment and flow velocity levels on the stream periphyton using 60 flow-through, circular mesocosms (volume 3.5 L, bed surface area 450 cm²; Microwave Ring Moulds, Interworld, Auckland, New Zealand). The experiment comprised a 25-day colonization period (day -25 to day 0) and a four-week manipulative period (day 0 to day 27). Four fine sediment treatments (no added sediment, coarse sand, fine sand, silt) were used. The particle sizes in the sediment addition treatments were 1-2 mm for coarse sand (henceforth called “large sediment”), 0.125 mm – 0.250 mm for fine sand (“medium sediment”) and < 0.125 mm for silt (“small sediment”). Sediment treatments were crossed with three levels of flow velocity reduction,

which consisted of fast (35 cm/s; the default treatment), moderate (17 cm/s) and slow velocity (0 cm/s), resulting in 12 treatment combinations. For each treatment combination there were five replicates. The mesocosms were filled with stones from dry sections of the Kauru riverbed. The stones were cleaned and sieved in order to remove fine sediment and organic matter. This base sediment consisted of 500 ml of 2-20 mm gravel along with 16 stones (placed on the surface) with a maximum width of 20 mm. This substratum composition was similar to that of the stream beds in many small Otago streams that are situated in sheep and beef farmland areas (Matthaei *et al.*, 2006; Townsend *et al.*, 2008).

2.2.3 Physicochemical Variables

Four main physicochemical variables (sediment size, sediment cover and depth, flow velocity and nutrient concentrations) were measured during the preparatory or manipulative phase. Firstly, during the preparatory phase, sediment grain size distribution was determined using Endicott sieves. This process involved pouring 100 g of the sediment into a sieve tower comprising sieves with different sized apertures (stacked with the largest (1-2 mm) on top and the smallest (0-0.63 mm) at the bottom. The machine then shook the sieves for five minutes. After shaking, the sediment of different particle sizes was placed in large storage containers which corresponded to the three different sediment sizes. The sieves (with sediment) were weighed and the mass of each sieve deducted. Then exactly 500 g of sediment pertaining to the different particle size categories was placed in containers.

Secondly, upon initiation of the experiment, sediment cover (%) and depth (using a ruler) was measured during the experiment. Further, visual sediment cover (%) estimates were collected every third day for the entire experiment. Sediment depth was measured every third day for the first 14 days of the manipulative phase. These measurements were suspended after 14 days because of the possibility that they might disturb the benthic communities. Thirdly, flow velocity was monitored and re-calibrated using a portable hand-held flow meter (Schiltknecht MiniAir2 device (Gossav, Switzerland)) in order to ensure flow velocity consistency throughout the manipulative period. Lastly, nutrient concentrations were collected at the start of the manipulative phase, two weeks and four weeks later. This was done by washing

out the sampling bottle and then washing out the 50 mL sampling syringe three times and taking a 50 mL sample from each mesocosm. These nutrient samples were measured using standard methods in an auto-analyser (APHA, 1998).

2.2.4 Fine Sediment

The added fine sediment was sourced from the Manuherikia River catchment in Central Otago, New Zealand. The sediment was low in organic nitrogen (0.05 mg/kg) and phosphorus (617 mg/kg) (Alexandra dust, Clyde Scree Supplies Ltd, Central Otago, New Zealand). It was added to the mesocosms as a one-off dump in order to simulate a situation in which large amounts of fine sediment are moved into a waterway in single rainfall event causing surface runoff. This form of sediment addition was realistic because surface runoff during rainfall events is the primary source of sediment to farmland streams (Matthaei et al., 2006). Previous experiments in the same setup demonstrated that this sediment stayed in the mesocosms throughout a three-week manipulative period (Wagenhoff *et al.*, 2013). Approximately 500 g of different sized sediment was added to the mesocosms on the 4th of March 2015 (day 0). It was added in such a way as to create an even distribution around the mesocosm and to minimise the loss of sediment suspended in the water column. This was done by stopping water input when the sediment was added and adding the sediment slowly and evenly within each mesocosm.

2.2.5 Flow Velocity

During the preparatory period, flow velocity was set in all mesocosms to 26.2 cm/s (\pm 1.6 cm/s, SD, n = 60) and recalibrated at least every 48 hours. During the manipulative period, velocity in each of the three treatments was controlled through a different mechanism. Fast flow was achieved by adding a jet to the terminal end of the pipe feeding each mesocosm and pointing the jet outlet towards the mesocosm wall (to maximise circular current velocity). Moderate flow was accomplished by removing the jet but still facing the pipe outlet toward the mesocosm wall. Slow flow was achieved by removing the jet and turning the pipe outlet towards the bottom of the mesocosm (to minimize circular current velocity).

2.2.6 Algal Sampling

Two main types of algal sampling technique were used to sample benthic algae at the end of the experiment they differed depending on the substratum in the mesocosm (see e.g. Magbanua *et al.*, 2013a; Wagenhoff *et al.*, 2013). All mesocosms that contained added fine sediment were sampled using the top 2 mm of surface sediment within the area defined by a plastic ring (about 25 mm high) with a diameter of 27 mm. This surface sediment was sucked up using a cut-off 3 mL pipette, with two sub-samples (from random surface locations covered by fine sediment) making up one sample. In control mesocosms, three randomly chosen surface stones were selected and the same plastic ring was used to define the area sampled on each rock. The periphyton from each mesocosm was transferred to a sterile 100 mL Astraline container. These containers were stored on ice in the dark in the field, transported to the laboratory on the same day and frozen at -20 °C on arrival.

2.2.7 Algal Laboratory Procedures

Sample preparation consisted of thawing overnight and proceeding through several steps, depending on the type of sample. In the laboratory, “Stone” samples (3 stones per sample) were scrubbed twice on each stone surface (approx. 40 seconds) in order to remove all algae. “Sediment” samples did not require this additional step. After the algae had been deposited into a plastic 100 mL Astraline container, the sample was homogenized for one minute using a blender. The sample volume was then measured and made up to a known volume (100 mL for both stone and sediment samples). The sample was then sub-sampled for taxonomic identification and analysis of algal community composition, with 19 mL of homogenized sample being placed in a 50 mL falcon tube with 1 mL (5 %) formalin (for preservation). A further 20 mL was used for chlorophyll *a* analysis.

Chlorophyll *a* was analysed as a proxy for algal biomass. This analysis followed standard methods (Biggs & Kilroy, 2000) and consisted of filtering a 10 or 20 mL sample on to a fine glass fibre filter (47 mm) in order to concentrate the sample. Those filters were then placed in a 15 mL falcon tube and then extracted using ethanol (90%) that had been boiled at 78 °C. This ethanol mixture was then left to extract overnight in the refrigerator. The ethanol extracts were then centrifuged and

run through a plate reader twice in order to determine absorbance (750 nm and 665 nm). For algal community composition analysis, about 300 cells were identified at 400x magnification to the lowest practicable taxonomic level using an inverted microscope (Zeiss Axiovert 25, Jena, Germany). Algal filaments could not be identified by cell number and were thus counted as 10 µm increments equalling one 'cell' (as in Lange *et al.*, 2016). Most algal taxa were identified to species, and some to genus or family, using the Stream Periphyton Monitoring Manual (Biggs & Kilroy, 2000) and the photographic reference collection of stream algae from the Kauru River (K. Lange, unpublished).

2.2.8 Statistical Analysis

The statistical analysis for this thesis was conducted in SPSS 22.0 (IBM SPSS Inc., IL, Chicago, USA). Exploratory data analysis indicated that the community response variables did not need any transformations whereas population abundance response variables required a 4th root transformation to improve homogeneity of variances and normality of these data. The two fixed factors in the ANOVAs and MANOVAs were sediment type and flow velocity. A block factor was included to account 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). Because the experiment focused on determining differences between individual sediment treatments and flow velocity levels, the two stressors were modelled as categorical predictors. Note that this analysis may underestimate the actual frequency of two-way interactions somewhat because of the larger number of degrees of freedom required to model interaction effects compared to the two factor main effects (Cottingham *et al.*, 2005).

Physico-chemical analysis consisted of investigating nutrient levels, flow velocity, sediment depth, and sediment cover (presented in the results section). With the exception of nutrient levels (measured three times), the analysis was performed on overall means in each mesocosm during the full manipulative period (1 value per mesocosm).

To investigate effects of sediment addition and flow velocity manipulation on benthic algae, the community indices and population abundances were investigated. Community indices were analysed using individual ANOVAs for each community-level response variable. To investigate the responses of benthic algal community composition to stressors, MANOVAs were used. These were based on the abundances of the 15 most common taxa. These taxa were determined by their percentage contributions to the total cell count (across all samples with a cut-off point of 0.9 %) and by the percentage of samples in which the species was present (at least in 50 % of all samples). The Pillai's Trace test statistic was used to determine the multivariate stressor effects, and the between-subjects effects of the MANOVA were used to determine stressor effects on the individual common taxa. Significant between-subjects effects for both stressor main effects (sediment and flow velocity) were further investigated using pairwise comparisons with Tukey's HSD *post hoc* tests.

Results presented below include those for the factors sediment and flow velocity plus their interactions. The results from the block factor are not presented because they merely relate to background variation that is irrelevant to my research objectives. The significance level for all tests was $P < 0.05$, and all responses described in the Results were significant unless indicated otherwise. Standardized effect sizes (partial eta-squared values, range 0-1, Nakagawa, 2004; Garson, 2012) are presented for all findings with $P < 0.05$.

The presence of significant interactions between sediment particle size and flow velocity (the two experimental factors) requires careful interpretation of factor main effects. As recommended by Quinn & Keough (2002), significant individual main effects are interpreted in the presence of a significant interaction only when the interaction effect size was smaller than the size of the corresponding main effect(s).

2.3 Results

2.3.1 Physiochemistry

Chemical habitat variables included the nutrient variables DIN (Dissolved Inorganic Nitrogen) and DRP (Dissolved Reactive Phosphorus). There were no significant differences between the sediment or flow velocity treatments for either nutrient variable (Table 2.1). Physical habitat variables included sediment depth (mm), sediment cover (%), and flow velocity (cm/s). On day 0, the average across all three sediment addition treatments was 96.4 ± 2.9 % sediment cover and 9.5 ± 2.5 mm sediment depth. On the same day, small-sized sediment had a cover of 95.5 ± 3.0 % and a depth of 8.8 ± 2.2 mm. Medium-sized sediment had a cover of 96.3 ± 3.2 %, with a depth of 10.0 ± 2.2 mm, while large-sized sediment had a cover of 97.3 ± 2.0 % and a depth of 9.9 ± 2.7 mm. Sediment depth was greater in mesocosms with added fine sediment regardless of grain size compared to control mesocosms (Table 2.1, **Fig. 2.1**). Sediment cover was also lower in control mesocosms compared to all sediment addition treatments, especially in mesocosms with large-grained added sediment (Table 2.1, **Fig. 2.1**). Sediment cover also responded to flow velocity treatments, with fast flow (the default) having lower sediment cover than moderate (half velocity reduction) or slow velocity (full reduction). Further, flow velocity and sediment effects interacted for sediment cover, with the increase in sediment cover due to added sediment being reduced by fast flow, especially for medium and small-sized sediment (**Fig. 2.1**). Flow velocity was highest in “fast” mesocosms, intermediate in “moderate” flow velocity and slowest in “slow” flow velocity treatments, with a very large effect size of almost 1.0 (Table 2.1, **Fig. 2.1**). This parameter also had an almost significant, weak sediment main effect ($P = 0.053$, effect size = 0.155). Overall, flow velocity tended to be slightly higher in medium-sized sediment mesocosms than in mesocosms without added sediment.

Table 2-1: Summary (P-values and effect sizes) of (M)ANOVAs of physico-chemical variables between the experimental treatments P-values for multivariate results are for the Pillai's Trace statistic. For univariate results, rankings for post hoc tests in cases with significant between subjects effects are given in columns 4 and 6. Sediment treatments: C, control; L, large (1-2 mm); M, medium (0.125 – 0.250 mm); S, small (0 – 0.125 mm). Flow velocity treatments: F, fast (default); M, medium; S, slow. Significant P-values (with effect sizes in parentheses; partial eta squared, > 0.1 = small; > 0.3 = medium; > 0.5 = large) are indicated in bold print.

Dependant variable	Sediment particle size	Ranking	Flow Velocity	Ranking	Sediment x Flow
DIN	0.710		0.779		0.888
DRP	0.638		0.122		0.119
Sediment depth	<0.001 (0.921)	C < (S=M) < L	0.080 (0.106)		0.377
Sediment cover	<0.001 (0.997)	C < (S=M) <L	<0.001 (0.780)	F < (M=S)	<0.001 (0.712)
Flow velocity	0.053 (0.155)	C<M (P = 0.06)	<0.001 (0.995)	S < M <F	0.512

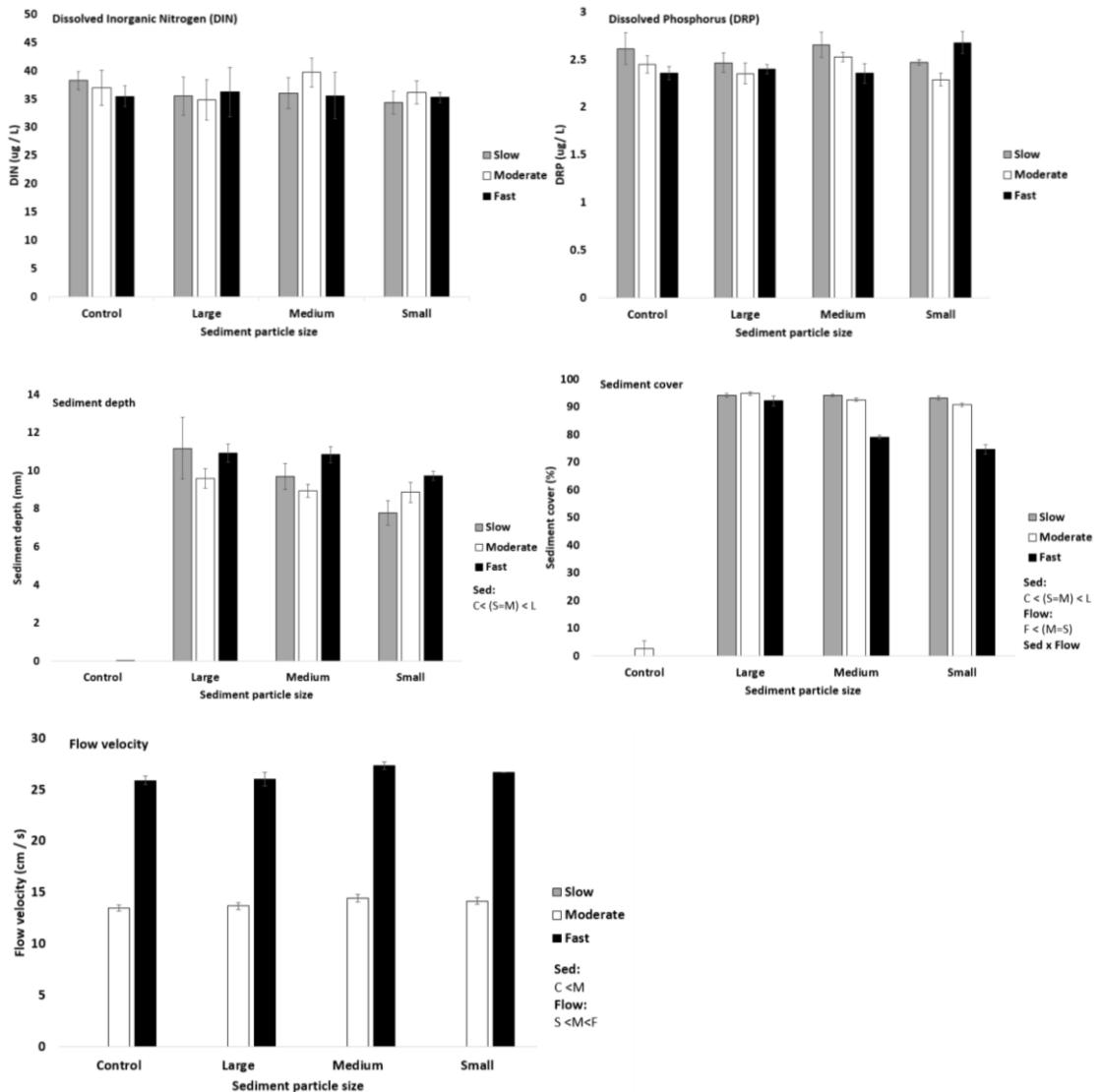


Figure 2-1: Mean response of physico-chemical variables including the nutrient variables DIN and DRP (ug/L) and the physical variables sediment depth (mm), sediment cover (%), and flow velocity (cm/s) to the experimental treatments. Error bars represent standard errors ($n=5$ for each treatment combination).

2.3.2 Community metrics

Algal biomass (measured as Chl a) and total algal cell density responded significantly to the fine sediment treatments, whereas the flow velocity treatments affected algal evenness, total cell density, and taxon richness via a velocity by sediment interaction (Table 2.2, Fig. 2.2). Algal biomass was greater in mesocosms with added large or medium-sized sediment compared to control mesocosms. Total cell density was higher in mesocosms with added small or medium sediment than in those with large sediment or in controls. This metric also had an almost

significant, weak flow velocity main effect ($P = 0.052$, effect size = 0.12), but this was overridden by a stronger sediment by flow interaction. In this interaction, the positive effects of small or medium added sediment were strongest at fast or moderate flow velocity (Table 2.2, **Fig. 2.2**). Algal taxon richness showed no significant stressor main effects but flow velocity and sediment effects interacted, with a positive effect of flow velocity reduction on richness occurring only in mesocosms with medium-sized added sediment (Table 2.2, **Fig. 2.2**). For community evenness, both significant factor main effects were overridden by a strong interaction in which reduced current velocity increased evenness in all three sediment addition treatments but reduced evenness when sediment was absent (Table 2.2, **Fig. 2.2**).

Table 2-2: Summary (P-values and effect sizes) of (M)ANOVAs comparing algal community responses between the experimental treatments. P-values for multivariate results are for the Pillai's Trace statistic. For univariate results, rankings for *post hoc* tests in cases with significant between subjects effects are given in columns 4 and 6. Sediment treatments: C, control; L, large (1-2 mm); M, medium (0.125 – 0.250 mm); S, small (0 – 0.125 mm). Flow velocity treatments: F, fast (default); M, medium; S, slow;. Significant P-values (with effect sizes in parentheses; partial eta squared, > 0.1 = small; > 0.3 = medium; > 0.5 = large) are indicated in bold print. Effects marked with x were overridden by the interaction.

Dependant variable	% Total cell count	Sediment particle size	Ranking	Flow Velocity	Ranking	Sediment x Flow
Community indices						
Total cell density		<0.001 (0.50)	(C=L) < (M=S)	0.052 (0.12)	x	0.010 (0.30)
Chlorophyll a (per m ²)		0.023 (0.19)	C<(L=M)	0.10		0.20
Algal taxon richness		0.142		0.917		0.045 (0.240)
Algal evenness		<0.001 (0.45)	x	0.036 (0.13)	x	<0.001 (0.510)

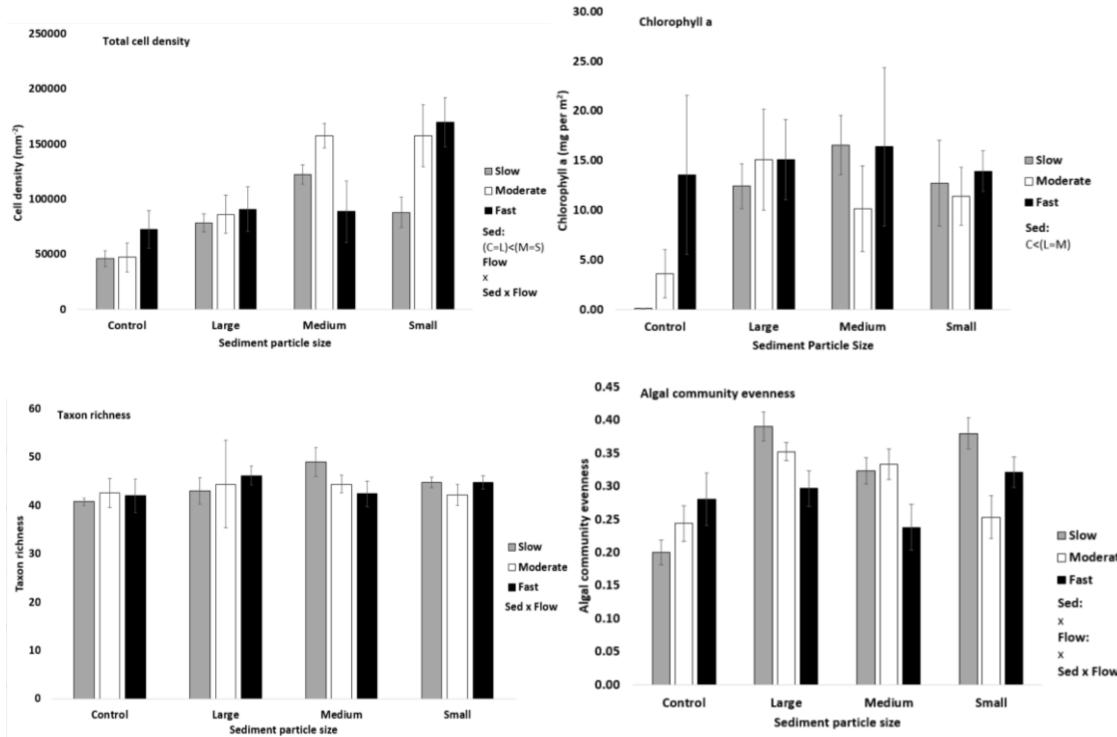


Figure 2-2: Mean response of the algal community-level metrics total cell density, chlorophyll a, taxon richness and community evenness to the sediment and flow velocity treatments. Error bars represent standard errors ($n=5$ for each treatment combination). Effects marked with x were overridden by the interaction.

2.3.3 Population abundance

The MANOVA on the cell densities of the 15 most common algal taxa (see Table 2.3 for names and % contributions to the total cell count of each taxon) indicated that periphyton community composition differed across sediment and flow velocity treatments, and that sediment and velocity treatments interacted (Table 2.3). The *between-subjects* results of the MANOVA showed that these overall patterns were caused by 14 significant responses of individual taxa to the sediment treatments, seven responses to flow velocity reduction and eight interactions between the two stressors. Many of the significant factor main effects were overridden by stronger interactions (four flow reduction main effects) (Table 2.3).

Table 2-3: Summary (P-values and effect sizes) of (M)ANOVAs comparing algal population responses of *Gomphonema minutum*, *Fragilaria vaucheriae*, *Cymbella kappii*, *Merismopedia* spp., *Gomphonema clavatum*, *Rhopalodia novae-zealandiae*, *Gloeocystis* spp., *Coccconeis* *placentula*, *Melosira varians*, *Encyonema minuta*, *Achnanthidium minutissimum*, *Gomphonema parvulum*, *Phormidium* spp., *Nitzschia palea*, and *Epithemia sorex* between the experimental treatments. P-values for multivariate results are for the Pillai's Trace statistic. For univariate results, rankings for *post hoc* tests in cases with significant between subjects effects are given in columns 4 and 6. Sediment treatments: C, control; L, large (1-2 mm); M, medium (0.125 – 0.250 mm); S, small (0 – 0.125mm). Flow velocity treatments: F, fast (default); M, medium; S, slow;. Significant P-values (with effect sizes in parentheses; partial eta squared, > 0.1 = small; > 0.3 = medium; > 0.5 = large) are indicated in bold print. All taxon variables were 4th-root transformed before analysis (see Methods). Effects marked with x were overridden by the interaction.

Dependant variable	% Total cell count	Sediment particle size	Ranking	Flow Velocity	Ranking	Sediment x Flow
(MANOVA, 15 common taxa)		<0.001 (0.73)		<0.001 (0.69)		<0.001 (0.48)
<i>Gomphonema minutum</i>	16.9	0.041 (0.17)	L<M	0.152		0.201
<i>Fragilaria vaucheriae</i>	9.4	<0.001 (0.50)	C< (L=M=S)	0.459		0.147
<i>Cymbella kappii</i>	5.5	<0.001 (0.37)	(C=L)<M<S	<0.001 (0.30)	x	0.004 (0.33)
<i>Merismopedia</i> spp.	4.5	<0.001 (0.57)	L<M<S; C<S	<0.001 (0.32)	S< (F=M)	0.007 (0.31)
<i>Gomphonema clavatum</i>	4.4	<0.001 (0.30)	C< (L=M=S)	0.344		0.018 (0.28)
<i>Rhopalodia novae-zealandiae</i>	3.9	<0.001 (0.40)	C< (L=M=S)	0.003 (0.23)	x	0.005 (0.33)
<i>Gloeocystis</i> spp.	3.2	<0.001 (0.37)	(C=S=M) < L	0.018 (0.16)	x	0.005 (0.33)
<i>Coccconeis</i> <i>placentula</i>	2.7	0.009 (0.22)	C<S	0.586		0.461
<i>Melosira varians</i>	2.7	0.053 (0.16)	M<L (P = 0.076) C<L (P = 0.079)	0.688		0.180

Table 2-3 Continued

Dependant variable	% Total cell count	Sediment particle size	Ranking	Flow Velocity	Ranking	Sediment x Flow
<i>Encyonema minuta</i>	2.6	<0.001 (0.48)	(C=L) < (S=M)	<0.001 (0.30)	S < (F=M)	0.054 (0.23)
<i>Achnanthidium minutissimum</i>	2.3	<0.001 (0.49)	C < (L=S=M)	0.467		0.080
<i>Gomphonema parvulum</i>	2.0	0.006 (0.24)	(S=L) < C	0.023 (0.15)	S < F	0.865
<i>Phormidium spp.</i>	1.9	0.004 (0.25)	(S=L=M) < C	0.263		0.123
<i>Nitzschia palea</i>	1.9	<0.001 (0.55)	(C=L) < (M=S)	0.617		0.006 (0.32)
<i>Epithemia sorex</i>	2.0	0.137		0.026 (0.150)	x	0.025 (0.27)

Gomphonema minutum was more abundant in mesocosms with medium-sized added fine sediment than in those with large added sediment (Table 2.3, Fig. 2.3). *Fragilaria vaucheriae* was less abundant in control mesocosms than in those with added large, medium, or small sediment. Similarly, *Cocconeis placentula* was more abundant in small-sediment mesocosms compared to controls (Table 2.3, Fig. 2.3), and *Achnanthidium minutissimum* was less common in control mesocosms than in those with added sediment regardless of grain size (Table 2.3, Fig. 2.3). By contrast, *Gomphonema parvulum* was more abundant in control mesocosms than in those with added small or large sediment (Table 2.3, Fig. 2.3). This species was also more abundant at fast than at slow flow. *Melosira varians* showed an almost significant, weak sediment main effect ($P = 0.053$, effect size = 0.16). This species tended to be more abundant in mesocosms with large-sized added sediment than in those with medium added sediment or in controls (Table 2.3, Fig. 2.3). *Phormidium spp.* was also more abundant in control mesocosms compared to those with added sediment of any size (Table 2.3, Fig. 2.3).

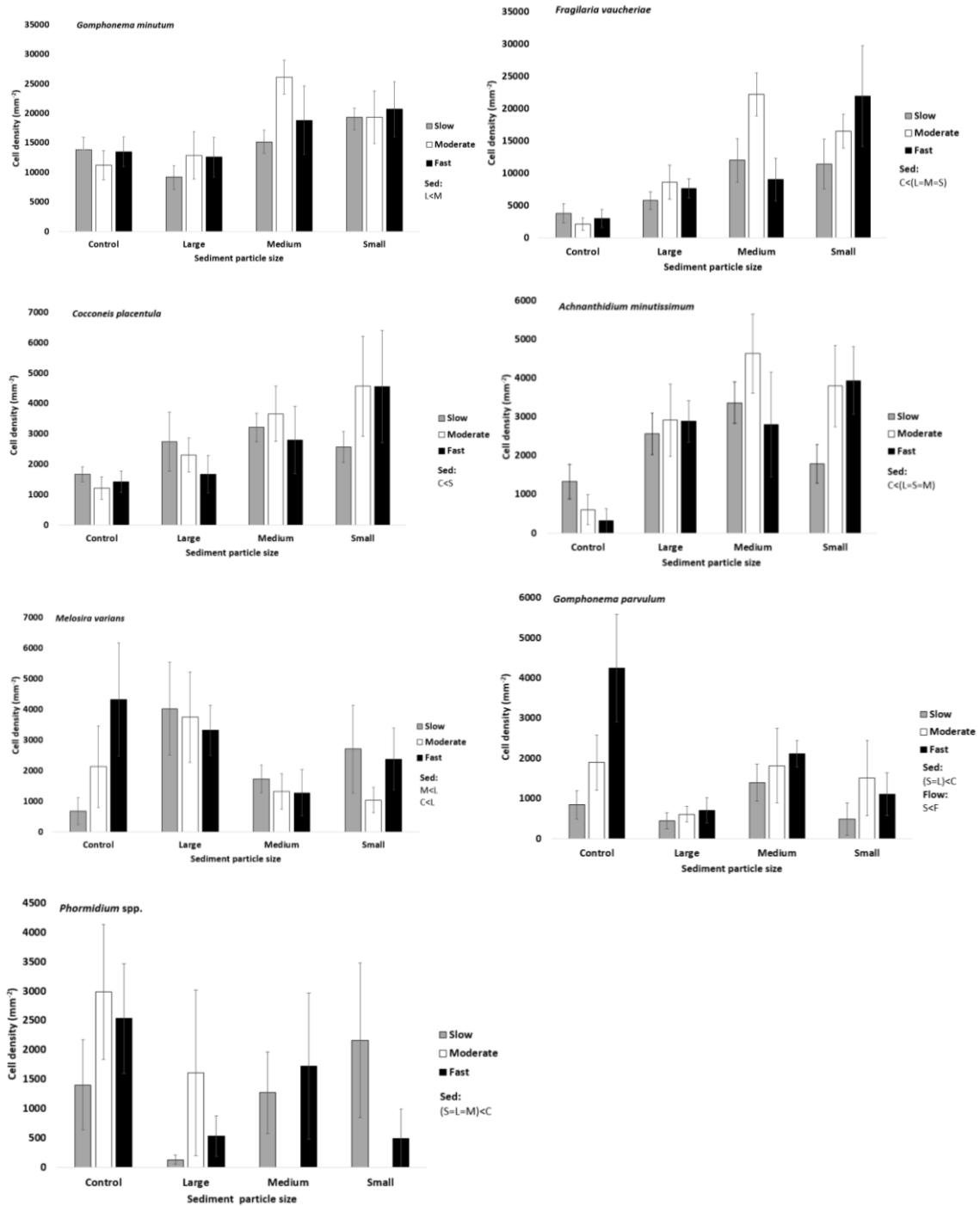


Figure 2-3: Mean densities of *Gomphonema minutum*, *Fragilaria vaucheriae*, *Cocconeis placentula*, *Achnanthidium minutissimum*, *Melosira varians*, *Gomphonema parvulum* and *Phormidium* spp. in the experimental treatments. All seven responded significantly to sediment as a main effect, with no interactions between sediment and flow velocity. Error bars represent standard errors ($n=5$ for each treatment combination).

Cymbella kappii density was highest in small-sediment mesocosms, intermediate in medium-sediment mesocosms, and lowest in large-sediment or control mesocosms (Table 2.3, **Fig. 2.4**). This species was also more abundant at fast or moderate flow velocities than at slow velocity, but this factor main effect was overridden by a slightly stronger sediment by velocity interaction. In this interaction, the negative effect of flow velocity reduction was weakest in mesocosms with medium-sized added sediment. *Merismopedia* spp. was more abundant in small-sediment mesocosms than in large-sediment or control mesocosms (Table 2.3, **Fig. 2.4**). The taxon also responded negatively to flow velocity reduction, with higher densities occurring at fast and moderate than at slow flow. Flow velocity and sediment effects interacted for this taxon, with the positive effect of added medium or small sediment being much stronger at fast and especially moderate flow than at slow flow. Overall, density of *Gomphonema clavatum* increased with the addition of any type of fine sediment (large, medium or small) (Table 2.3, **Fig. 2.4**). However, flow velocity and sediment effects interacted for this species, with the positive effect of added sediment being strongest at moderate flow for large and medium sediment but strongest at fast flow for small sediment. Overall, *Rhopalodia novae-zealandiae* was more abundant in mesocosms with added fine sediment of any size compared to controls (Table 2.3, **Fig. 2.4**). Flow velocity and sediment effects interacted for this species, overriding a weaker velocity main effect, with the positive effect of added sediment being much stronger in mesocosms with slow flow than with fast or moderate flow. *Gloeocystis* spp. was more abundant in large-sediment mesocosms compared to no added sediment, medium-sediment and small-sediment mesocosms (Table 2.3, **Fig. 2.4**). Flow velocity and sediment effects interacted for this taxon, overriding a weaker velocity main effect, with responses to current velocity reduction varying from negative (for small fines) to positive (for medium fines) depending on the grain size of the added sediment. *Encyonema minuta* was more abundant in medium and small-sediment mesocosms compared to large-sediment mesocosms or controls. This species was also more abundant at fast or moderate flow than at slow flow (Table 2.3, **Fig. 2.3**). Further, there was an almost significant ($P = 0.054$, effect size = 0.23) flow velocity by sediment interaction, in which the positive effects of medium and small added sediment tended to be weaker at slow flow velocity.

Nitzschia palea was more abundant in mesocosms with small or medium added sediment than in those with no sediment or large sediment added (Table 2.3, **Fig. 2.4**). Flow velocity had no effect on its own but velocity and sediment interacted for this species, with the positive effects of medium or small sediment addition being strongest at moderate flow velocity. Finally, *Epithemia sorex* showed no sediment main effect and a weak flow velocity main effect that was overridden by a stronger flow velocity and sediment interaction (Table 2.3, **Fig. 2.4**). This interaction indicated that flow velocity reduction only had a positive effect when combined with added sediment of any grain size.

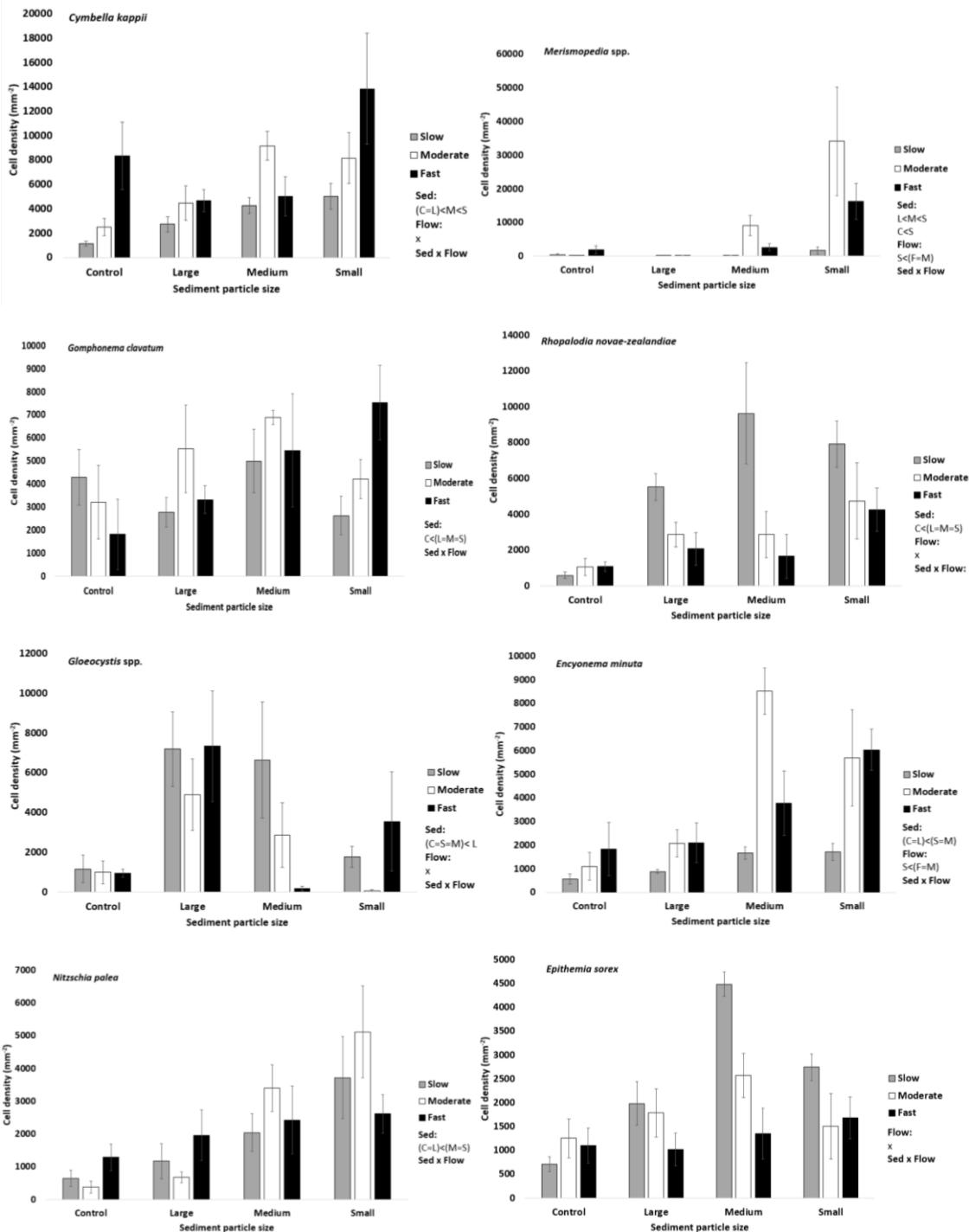


Figure 2-4: Mean densities of *Cymbella kappii*, *Merismopedia spp.*, *Gomphonema clavatum*, *Rhopalodia novae-zealandiae*, *Gloecystis spp.*, *Encyonema minuta*, *Nitzschia palea* and *Epithemia sorex* in the experimental treatments. All seven showed significant interactions between sediment and flow velocity. Error bars represent standard errors ($n=5$ for each treatment combination). Effects marked with x were overridden by the interaction.

2.3.4 Overview of algal community composition response patterns

The four algal community metrics responded significantly to sediment addition in 50 % of all possible cases, whereas flow velocity reduction caused no significant responses at the community level. Interactions occurred for 75 % of community responses. Of the 15 common algal taxa, 93 % responded to sediment addition (one marginal effect) and 20 % to flow velocity reduction while interactions occurred at a rate of 53 % (one marginal effect). Consequently, while the common taxa more frequently showed significant main effects of the two stressors, they showed relatively fewer interactions between stressors than the community metrics. Of the two stressors investigated, fine sediment addition (with different grain sizes) was the more pervasive stressor, although flow velocity also had frequent effects. Thus, periphyton response variables demonstrated 17 significant responses (out of 19 possible ones) to sediment treatments, whereas flow velocity caused nine significant responses. Furthermore, many of the factor main effects were overridden by a stronger sediment flow velocity interaction. This occurred for one sediment main effect and six flow velocity reduction effects. Interactive effects were displayed by three community-level metrics and eight common taxa (one marginal interaction), and these interactions were all synergistic.

2.4 Discussion

2.4.1 Effects of added fine sediment with different particle sizes on benthic algae

In my first hypothesis, I predicted that the addition of relatively large sediment grain sizes (1-2 mm) may have a more negative, and more pervasive, effect on periphyton community metrics and population abundances than smaller sediment grain sizes (0-0.125 mm) (Delgado *et al.*, 1991; Francoeur & Biggs, 2006; Jewson *et al.*, 2006; Jones *et al.*, 2014). As part of this hypothesis there were three sub-hypotheses. Firstly, that algal biomass and total cell density may decline with the addition of large-sized sediment but not with the addition of smaller particles (Stevenson *et al.*, 2006). Secondly, that taxon richness, algal taxon richness and community evenness may decrease with the addition of large-sized sediment. Lastly, that the abundance of certain resistant taxa (such as *Melosira*, *Epithemia sorex*, *Phormidium* spp, and *Merismopedia* spp.) may increase or remain stable with the addition of large-sized sediment, whereas species that are vulnerable to sand abrasion (such as *Cocconeis*

placentula and other diatoms) may decline (Stevenson *et al.*, 2006). There was little evidence to support my overarching hypothesis that abrasive effects of sand (i.e. relatively large fine sediment particles) would have a more pervasive and negative effect on the periphyton than the smothering effects of smaller particles. In total, there were only two negative responses to the sediment addition treatments, and neither was specific to large-sized particle addition. Furthermore, there were more positive (13 response variables out of 20) than negative responses to sediment addition regardless of grain size, and often the response to addition of large-sized particles was not significantly different from the control (no sediment addition). With regards to my first sub-hypothesis, there was no evidence from chlorophyll a (a proxy of algal biomass), algal community evenness and total cell density (another proxy of algal biomass) to suggest a decline with the addition of large sediment sized particles. In fact, chlorophyll a and algal community evenness increased with large-sized added sediment, and total cell density increased with small and medium-sized sediment while there was no difference between large-sized sediment treatments and control. With regards to the second sub-hypothesis, taxon richness displayed no response to sediment as a main factor while algal evenness increased with the addition of large sediment. Lastly, there was partial evidence to support my third sub-hypothesis. *Melosira varians*, *Epithemia sorex*, *Phormidium* spp. and *Merismopedia* spp. were expected to increase or remain stable with the addition of large sized sediment. *Melosira varians* and *Epithemia sorex* did not respond to sediment addition of any size, while *Merismopedia* spp. responded positively to small-sized added sediment but the large-sized sediment treatment was no different from the control. In other words, in these species the response to large-sized added sediment was neutral. Against expectations, the blue-green alga *Phormidium* spp. declined. With regards to *Cocconeis placentula* and other diatoms there was no decrease in their abundance with large-sized sediment addition, with the exception of *Gomphonema parvulum*. In spite of providing limited support for my hypotheses, these are interesting results which could provide fertile areas for future related research. The following discussion of these results often uses ecological information from Biggs & Kilroy (2000) (for species cell sizes); Passy (2007b); Piggott *et al.*, 2015b; Lange *et al.*, 2016, and chapters 3, 4 & 5 in Salis (2016).

It is important to note that the predictions in Hypothesis 1 were tentative and based on circumstantial evidence from previous studies. This was because no previous studies have specifically investigated effects of fine sediment particle size in relation to periphyton ecology and often only mentioned it as part of a habitat assessment. Secondly, in the existing related studies the bed particle size ranges used were usually far greater than in my experiment (Kutka & Richards, 1996; Gainswin *et al.*, 2006; Francoeur & Biggs, 2006; Ahn *et al.*, 2013). These studies often used particle sizes ranging from boulders down to silts rather than different grain sizes within the fine sediment category. Thirdly, many studies classified all particles from 2 mm to 63 µm as ‘sand’ and everything smaller as ‘clays’ or simply defined fine sediment as <2 mm without making a distinction (Delgado *et al.*, 1991; Jewson *et al.*, 2006; Jones *et al.*, 2014; Mustonen *et al.*, 2016; see chapters 1, 2 & 3 in Salis, 2016). For example, one study classified clays as < 0.5 mm (Izagirre *et al.*, 2009). These broad classifications mean that the effects of all but part of the small-sized sediment in my experiment (0-0.125 mm) would have been masked by this ‘pooling’ in the earlier studies.

The two negative responses to fine sediment addition came from the diatom *Gomphonema parvulum* and the blue-green alga *Phormidium* spp. *Gomphonema parvulum* responded negatively to addition of small or large but not medium-sized sediment while *Phormidium* spp. responded negatively to added fine sediment regardless of particle size. Conversely, positive responses came from the other taxa, along with the community metrics total cell density and chlorophyll a. Of those positive responses to sediment addition, four responded to added fine sediment regardless of size, three responded more strongly to addition of the small particle size, and one taxon (*Gloeocystis* spp) responded positively only to the addition of large-sized particles. A more complex response was observed for two taxa and one community metric (*Encyonema minuta*, *Nitzschia palea* and total cell density), which all responded positively to added small or medium-sized but not to large sediment particles. Lastly, chlorophyll a responded positively to addition of large- or medium-sized but not small-sized particles.

The two negatively responding taxa, *Phormidium* spp. and *Gomphonema parvulum*, are interesting cases. *Phormidium* spp. (cell length 1-3 µm, width 1-2.5 µm) declined with the addition of any type of added fine sediment. This response pattern is inconsistent with previous studies on this genus that focused on fine sediment addition without particle size differentiation. These studies generally found a positive relationship between fine sediment levels and *Phormidium* abundance, often attributed to phosphorus release from clay sediments (Izagirre *et al.*, 2009; Wagenhoff *et al.*, 2013; Wood *et al.*, 2015; see chapters 4 & 5 in Salis 2016). In my experiment, *Phormidium* declined in abundance across all sediment particle sizes, including clay-sized particles, in contrast to previous research. One possible explanation for this difference could be shading by other algal species that proliferated with the addition of fine sediment, especially epiphytic algae such as *Cocconeis placentula* (10 - 90 µm length, 8-40 µm wide; dimensions for all algal taxa used in this discussion are based on Biggs & Kilroy, 2000). This species lives on top of other algal species in order to avoid the nutrient and light limitation present in the basal layers of the periphyton mat (Passy, 2007b). This epiphytic growth behaviour of *Cocconeis placentula* may subsequently block light and nutrients for the host species as these mats can become quite dense (Passy, 2007b). Alternatively, the instability provided by the addition of fine sediment might reduce the abundance of *Phormidium*. Since *Phormidium* is a filamentous taxon, addition of fine sediment may also be harmful in the absence of a nutrient-rich environment because of smothering (as *Phormidium* is unable to enter crevices between sediment particles) and slower reproduction (by fragmentation). The latter trait means this taxon is slow to disperse and recolonize compared to other species after sediment addition.

Gomphonema parvulum (10-25µm long) declined in abundance with the addition of large or small-sized sediment. *G. parvulum* has a medium-strength attachment to the substratum with a stalk and has been classified as low profile (Piggott *et al.*, 2015b; Law *et al.*, 2014; see chapters 4 & 5 in Salis, 2016). Low-profile species tend to be able to tolerate disturbance and sediment movement but are often sensitive to burial (Passy, 2007b). It is therefore surprising that this species declined when large-sized particles were added. It is possible that this might be the result of abrasion by particles in suspension (which damage cells when passing) rather than

an effect of the sediment instability per se. Small-sized particles could easily still act negatively on this species because it is attached to the substratum, thus burial would still be a hazard with silts and clays regardless of the lifestyle or attachment strength (Passy, 2007b).

The eleven algal species and two community metrics that displayed overall positive responses to fine sediment addition showed a wide variety of responses to specific sediment particle sizes. *Fragilaria vaucheriae*, *Achnanthidium minutissimum*, *Gomphonema clavatum*, and *Rhopalodia novae-zealandiae* all responded to sediment addition regardless of particle size. *Fragilaria vaucheriae* (cell size 30 µm) and *Achnanthidium minutissimum* (5-25 µm) are both relatively small diatoms, and this means that these species may take advantage of the increase in habitat heterogeneity with sediment addition. While *F. vaucheriae* is a high-profile and *A. minutissimum* a low-profile species, the common factor is a small cell size which may provide four advantages. Firstly, a higher surface area to volume ratio may mean a faster nutrient uptake rate and a faster growth rate (Biggs *et al.*, 1998; Passy, 2007b). Secondly, their small size may also allow both of these species to form colonies on a single fine sediment particle (sediment grain sizes in my experiment were 1-2 mm for large, 0.125-0.250 mm for medium and < 0.125 mm for small fine sediment). This behaviour may subsequently reduce the effects of particle movement on the cell. Thirdly, regardless of sediment instability or burial this species may simply be capable of rapid replacement (Biggs, 1996). Lastly, smaller-celled species can exploit interstitial spaces that may give protection from abrasion, competition (from large-celled species) and grazing. Additionally, *A. minutissimum* has a high attachment to the substratum which potentially means that this cell might be able to resist abrasion and movement associated with sediment addition (Passy, 2007b). *F. vaucheriae*, on the other hand, may be able to use erect or mucilaginous stalks to avoid burial. *Gomphonema clavatum* (cell size 25-100 µm) and *Rhopalodia novae-zealandiae* (up to 200 µm) also shared this response. *G. clavatum* is a large, high-profile species that has a long stalk. The presence of a stalk may mean that this species may be able to avoid burial and take advantage of the improved resource competition provided by its high-profile nature and the elimination of some low-profile species. By contrast, longer stalked species still have attachment but are

more susceptible to drag and sediment instability because of their height in the water and their weaker attachment (Passy, 2007b; Passy & Larson, 2011; Law *et al.*, 2014). *R. novae-zealandia* can glide and may therefore be able to move through the sediment and avoid some of the smothering effects of clay particles (Wagenhoff *et al.*, 2013; Lange *et al.*, 2016). Furthermore, its mobility may also allow this species to recolonise the substratum faster than its non-motile counterparts after sediment influxes and substratum instability (Wagenhoff *et al.*, 2013; Lange *et al.*, 2016). It is also a good competitor and so might benefit from the reduction in the abundance of other species.

Three algal taxa responded most positively to addition of small-sized sediment, these were *Cymbella kappii*, *Cocconeis placentula*, and *Merismopedia* spp. Underlying the increase in abundance in most diatom species is the increase in habitat heterogeneity that tends to benefit stalked/mucilage and motile forms (Piggott *et al.*, 2015b). *C. kappii* (cell size 25-35 µm) is a high-profile species while *C. placentula* is a low-profile (adnate and prostrate) form, with both having a medium to high attachment to the substratum (Passy 2007b; Schneck *et al.*, 2011). Low-profile forms can typically tolerate disturbance associated with sediment addition but may be subject to burial (Passy, 2007b). However, *C. kappii* may be able to overcome burial by rapid replacement while *C. placentula* typically lives as an epiphyte on filamentous algae (as discussed earlier), not the stream bottom, and therefore may not be affected by burial. *Merismopedia* spp. (cell size 3-6 µm) is a very small, colonial taxon that can move through fine sediment (using drift motility). It therefore may be able to avoid burial and move to more suitable habitats. Furthermore, burial may reduce competition with other species.

One algal taxon responded positively to the addition of large-sized sediment particles; this was *Gloeocystis* spp. (cell size about 10 µm). *Gloeocystis* spp. may prosper in large-sized sediment because of its small size which, similar to other small diatoms, may allow it to recolonise faster than larger species (Krejci & Lowe, 1986; Miller *et al.*, 1987; Biggs, 1996).

Two common algal taxa and one community metric responded positively to addition of small or medium-sized sediment. These were *Encyonema minuta*, *Nitzschia palea*, and total cell density (a proxy of algal biomass). *Encyonema minuta* (15-30 µm) is a small-sized diatom that forms mucilage tubes, has low attachment and is part of the high-profile guild (Biggs & Kilroy, 2000; Passy, 2007b; Law *et al.*, 2014). Small-grained fine sediments have been reported to be very stable (Graham, 1990; Wood & Armitage, 1997). High-profile algal species tend to prefer more stable environments in which the algae are less likely to be dislodged or be subject to abrasion (Krejci & Lowe, 1987; Round & Bukhityarova, 1996; Passy, 2007b). Furthermore, *Encyonema minuta* may overcome burial by clays and silts through the tall mucilage tubes (Biggs, 2000; Schneck *et al.*, 2011). *Nitzschia palea* (15-60 µm) has an elevated raphe on a keel which allows it to be motile (Lowe, 2011). It can therefore move through the fine sediment to a less clogged area if it is buried by very small particles (Bahls, 1993; Lowe, 2011; Schneck *et al.*, 2011). Lastly, the results for total algal cell density reflect the fact that the majority of the common taxa preferred the addition of medium or small-sized sediment particles. They could also indicate that this preference was not just restricted to the common taxa but also extended to those that were relatively rare.

Gomphonema minutum responded in an unusual manner by not displaying a difference between the control and any of the sediment added mesocoms. Instead, this study showed that this species preferred medium sediment over large sized sediment. Previous studies (that did not differentiate between sediment particle sizes) suggested that *G. minutum* responded negatively to sediment addition (Piggott *et al.*, 2015b; see chapter 4 in Salis, 2016). Given this unusual result and the relatively weak effect size this response is hard to interpret in any biologically meaningful way.

Chlorophyll a (a widely used proxy of algal biomass) responded positively to addition of large or medium-sized sediment but not to small-sized sediment. This is an interesting result, in light of the fact that as particle size became smaller, total

algal cell density (another commonly used proxy of algal biomass) increased fairly consistently whereas chlorophyll a remained similar to controls when the smallest fine sediment was added. This difference suggests that there was a shift from larger-sized algal cells to smaller-sized cells as sediment particle size decreased from sand to clays. This trend can also be seen in the common algal taxa where many of the smaller-sized species preferred medium or small fine sediment

A key factor driving many of the algal responses above may be the armouring of the stream bed by the algae themselves. Algae typically live in a biofilm and often form dense mats that incorporate fine sediment into them. This often has a stabilizing effect on sediments because the biofilm binds sand and clay particles in with the algal matrix (Paterson & Hagerhey, 2001; Jones *et al.*, 2014). Additionally, it provides a layer between the water flow and the stream bed and therefore protects substratum particles underneath from further entrainment (Droppo *et al.*, 2001; Passy, 2007b, Jones *et al.*, 2014; Piqué *et al.*, 2016). For example, Piqué *et al.*, (2016) reported that bedload rates of coarse sand (0.5-1 mm) were reduced by 5 times in the presence of biofilm. Given that the velocities used in the Piqué *et al.*, (2016) experiment (Control; Low flow = 5.76 cm/s, High flow=18.59 cm/s, Biofilm; Low flow= 7.05 cm/s, High flow= 19.32) were within the range of those in my own experiment, the impacts of abrasion, burial and shear stress on various indices and taxa in this experiment are likely to be similar. However, anecdotally (as observed when sieving the sediment) there appeared to be a higher proportion of sand than clay. Therefore, benthic algae may be subject more to abrasion by sand than burial in our mesocom experiment. Furthermore, the similarity in flow velocities indicates that the flow velocities used in my experiment were probably fast enough to move large-sized fine sediment. Additionally, there may be further effects of biofilm development which could allow for stability-preferring species to establish and live alongside instability-preferring species.

Since my experiment is the first to investigate the ecological effects of added fine sediment with different particle sizes (instead of a single type of fine sediment comprising a wider range of particle sizes), it is hard to relate my findings to previous research and provide sound conclusions. It is therefore recommended that

the explanations offered here should be taken in a speculative manner that need to be built upon by further investigations. These investigations should preferably include studies that take into account changes in periphyton communities that occur on a seasonal or inter-annual scale. In previous related mesocosm experiments and surveys in the same setup (Wagenhoff *et al.*, 2013; Magbanua *et al.*, 2013a; Piggott *et al.*, 2015b; see chapter 3 in Salis, 2016), the study season and/or year had considerable effects on the species present, the abundances of those species and their responses to agricultural stressors such as added fine sediment and/or nutrient enrichment.

2.4.2 Effect of flow velocity reduction on benthic algae

My second hypothesis predicted that flow velocity reduction should have a similarly strong effect as the sediment treatments on periphyton community indices and taxon abundances (Matthaei *et al.*, 2010). As part of this hypothesis there were three sub-hypotheses. Firstly, that algal biomass, total cell density, taxon richness and evenness may increase with flow velocity reduction (Matthaei *et al.*, 2010). Secondly, fast flow velocity (the default) may benefit rheophilic algal taxa such as *Melosira* spp., *Fragilaria* spp. and *Cocconeis* spp. (Stevenson *et al.*, 2006; Passy & Larson 2011). Lastly, flow velocity reduction may benefit *Gomphonema* spp. and some other velocity-sensitive species (Passy & Larson, 2011). There was little evidence to suggest that that flow velocity reduction had a similarly strong and pervasive effect as the sediment treatments on periphyton community indices and taxon abundances. For the overarching hypothesis, there were fewer responses to flow velocity reduction than to sediment addition (nine significant responses to flow velocity versus 16 significant responses to sediment particle size). Furthermore, six velocity effects were overridden by stronger interactions (one effect was marginal). They include algal evenness *C. kappii*, *R.novae-zealandiae*, *Gloeocystis* spp. and *E. sorex*. The other three responses displayed a pattern whereby the flow velocity effect size was smaller (and in some cases considerably smaller) than the sediment particle effect size (mean sediment particle effect size = 0.38, flow velocity mean effect size = 0.21, interaction effect size = 0.32). There was only partial evidence to support the first sub-hypothesis. Algal biomass, total cell density, taxon richness, and community evenness did not, as a main effect, increase with flow velocity

reduction. With regards to the second sub-hypothesis, fast flow velocity (the default) did not benefit *Melosira* spp., *Fragilaria* spp. or *Cocconeis* spp. All these species showed no significant response to flow velocity. There was also little evidence to support the third sub-hypothesis, that flow velocity reduction may benefit *Gomphonema* spp. and some other velocity-sensitive species (Passy & Larson, 2011). *G. parvulum* were less abundant at full flow reduction (the slow velocity treatment) and there was no response from *G. clavatum*.

My experiment revealed one major negative pattern in response to flow velocity reduction. There were three taxa which were more abundant at fast flow velocity (the default) and half velocity reduction (the medium velocity treatment) than at full velocity reduction (the slow treatment). These were *Merismopedia* spp. *E. minuta*, and *G. parvulum*. *Merismopedia* spp and *E. minuta* are loosely attached species, however, the patterns displayed by these species suggest a preference for a certain current velocity. This is consistent with the idea put forward by many studies that relatively fast (but not too fast) current velocities can stimulate the metabolism, increase photosynthesis and allow greater diffusion of nutrients from the water column (because of a reduced boundary layer thickness) (Horner *et al.*, 1990; Stevenson *et al.*, 1996; Ghosh & Guar, 1998). It has been suggested that a current velocity of 15 cm/s or greater is required to affect the diffusion gradient and increase nutrient uptake rates (Whitford & Schumacher, 1964), whereas velocities of around 60 cm/s may be required for the effect of current velocity to become destructive (Horner *et al.*, 1990). Perhaps, in this case, half flow reduction allows for some stimulation by the current but without the required energy to dislodge these cells. *G. parvulum* also displayed the same response. Previous studies have suggested that species belonging to the genus *Gomphonema* typically have medium attachment to the substratum using a stalk (see chapter 3 in Salis, 2016). Therefore, it is possible that these species can resist some shear stress associated with higher current velocities while taking advantage of the higher metabolism and photosynthesis levels provided by faster flows (Stevenson *et al.*, 1996; Ghosh & Guar, 1998). Furthermore, the *Gomphonema* genus has varied stalk length with some species having a short stalk (low profile) while others have a longer stalk (high profile) (Passy, 2007b). Shorter stalks provide better attachment to the substratum, which makes them more resistant to dislodgement by the current (Passy, 2007b).

However, short-stalked species also experience light and nutrient limitation (Passy, 2007b; Passy & Larson, 2011). The species *G. parvulum* have short stalks which gives them better attachment to the substratum. Given that this species seemed to prefer moderate current velocities over full flow velocity reduction, this preference is probably the result of current stimulation (through the reduction in boundary layer thickness).

A caveat of my experiment is the range of current velocities present. This range is representative of current velocities at normal discharges in small agricultural streams in southern New Zealand (see e.g. Matthaei *et al.*, 2006). However, my experiment does not include the extreme effects that are possible within a periphyton community in real streams, especially in systems that are commonly affected by floods. These include biotic processes such as facilitation (an interaction that benefits at least one species and harms neither) between the high-profile guild and the low-profile guild as well as community shifts (Horner *et al.*, 1990; Passy, 2007b; Passy & Larson, 2011). Furthermore, an important process overlooked might be immigration of high profile (stalked) sensitive species, which is a threshold process (Horner *et al.*, 1990) linked to fast flows. For example, in one previous study at current velocities above 60 cm/s, there was a community shift which was characterized by reduced diatom diversity, reduced presence of sensitive species and the dominance of *Achnanthidium minutissimum* (Passy, 2001). A further study, in which the maximum current velocity was 30 cm/s (just below my fastest current velocity of 35 cm/s), suggested that at this speed current velocity was probably more stimulating than destructive (Passy & Larson, 2011). This conclusion in combination with the results may have numerous management implications (as explored further in the General Discussion).

2.4.3 Interactions between sediment particle size and flow velocity reduction

My third hypothesis postulated that interactive responses to fine sediment addition should be more frequent and severe at reduced flow velocities (because fine sediment is more likely to settle and persist at slow flows). The algal community metrics and common taxa that showed significant stressor interactions were total cell density, taxon richness, community evenness, *Nitzschia palea*, *Merismopedia*

spp., *Rhopalodia novae-zealandiae*, *Gomphonema clavatum*, *Gloeocytis* spp., *E. sorex*, and *Cymbella kappii*. All of these indices and common taxa showed synergistic interactions, and added fine sediment and flow velocity reduction interacted to produce stronger positive outcomes for taxon richness, *R. novae-zealandiae*, and *E. sorex*. Consequently, this hypothesis was partly supported, but the predominant direction of the synergistic responses (positive rather than negative) was unexpected. These findings have various implications for management initiatives (as highlighted in the General Discussion).

Total cell density and taxon richness all showed a conditional response in which there was a flow velocity reduction effect in the presence of a certain sediment particle size. Total cell density (a proxy of algal biomass) was highest in the medium and small sediment treatments, especially when combined with fast or moderate current velocity. This potentially suggests that the combination of relatively stable clay sediment with some current-induced stimulation of nutrient uptake and metabolism enhances algal biomass (Labiod *et al.*, 2007; Ahn *et al.*, 2013). Studies have suggested that current velocity can have a profound effect on algal biomass and cell density (Horner *et al.*, 1990; Ahn *et al.*, 2013). However, this effect is typically the opposite of my results, with cell densities increasing with decreasing velocities (Keithan & Lowe, 1985; Lamb & Lowe, 1987). For example, Keithan & Lowe (1985) reported in a two stream survey that cell densities increased with a decrease in current velocity (the fastest velocity was between 50-100 cm/s while the slowest velocity was between 17-33 cm/s in pools accounting for rainfall events). My experiment did not have the fastest current velocity range and therefore the comparison is tenuous, however, this overall trend of increasing density with declining current velocity, seen in many taxon results in my study, but only when there was no added fine sediment. It is possible that when fine sediment is added, the smothering present in slower-velocity sections becomes an additional stressor that reduces abundance while in the faster-velocity sections the sediment may be washed out rapidly.

Algal taxon richness was highest in mesocosms with larger clay/small sand substratum (the medium sediment treatment) in combination with slow current

velocity (full velocity reduction). This response pattern suggests that a greater number of species can coexist under these particular microhabitat conditions. This could be the result of minimal entrainment and subsequent particle movement. Furthermore, the presence of medium-sized fine sediment potentially means that many competitors that would otherwise dominate the community are reduced or eliminated and thus allow the proliferation of a larger number of other, less competitive species. This finding is consistent with those of chapters 4 & 5 in Salis (2016) in which two experiments were conducted in the same mesocosm setup. It was different from Passy (2007b) who suggested that peak species diversity often occurs at moderate flow velocities, through the process of facilitation. It is possible, however, that in my experiment the facilitation process took place primarily along the sediment particle size gradient rather along the current velocity gradient.

Algal community evenness was highest at slow flow velocity, but only when combined with added sediment, especially at the medium and large grain sizes. Evenness decreased with velocity reduction when no sediment was added. This is an unusual response to changes in flow velocity. It suggests that the community is the most even (i.e. there are few or no dominant species) in an environment where there is increased habitat heterogeneity and an environment that favours a wide range of biological traits (i.e. low abrasion/scouring and low smothering). However, a decline of evenness signals that, with no sediment added, certain species become more dominant while others decline. These species that dominate are most likely to be either *G. parvulum* or *Phormidium* spp. which were the only two abundant species that preferred no added sediment. Alternatively, the species which were drastically less abundant with no sediment added were *F. vaucheriae* and *A. minutissimum*, which could be outcompeted or shaded out by *G. parvulum* or *Phormidium* spp. in low-sediment environments. Interestingly, a previous experiment in the same mesocosm setup (see chapter 5 in Salis, 2016), which investigated (as one of four manipulated stressors) flow velocity reduction and added fine sediment (< 2mm diameter, mean particle size= 0.2mm), found the opposite pattern. In that experiment, in which 8 out of the 12 same species were dominant, sediment addition decreased algal community evenness, especially when combined with flow velocity reduction. On the other hand, my findings for algal community metrics agree

partially with the observation of Keithan & Lowe (1985) that algal community evenness and taxon richness tend to be lower under the same conditions as total cell density.

Two common algal taxa (*Nitzschia palea* and *Merismopedia* spp.) displayed an interaction pattern in which abundance considerably increased in mesocosms with added medium and small-sized sediment when there was no flow reduction (fast flow) or half flow reduction (moderate flow). *N. palea* and *Merismopedia* spp. are both motile species that can either glide (*N. palea*) or drift (*Merismopedia*) and move to avoid burial by sediment and move into interstitial spaces (Passy, 2007b; Jones *et al.*, 2014). This trait may allow them to survive while other species are buried or washed away, thus eliminating other species and reducing competition. Furthermore, the acquisition of resources may be further enhanced by the current that provides a stimulatory effect.

G. clavatum showed a complicated interaction whereby the positive effect of added sediment was strongest at moderate flow for large and medium sediment but strongest at fast flow for small sediment. Given that this species is a large, stalked, high-profile taxon, this possibly suggests an abrasion-nutrient uptake trade-off whereby abrasion from sand is not too strong or absent and nutrient uptake (due to boundary layer reduction) is present. The further reduction in sediment size to clay particles may mean that this species suffered from burial or nutrient limitation at moderate flow velocities but may be able to take advantage of lower depositional conditions, lower abrasion rates and a subsequent release from the trade-off between increased abrasion (generally negative) and increased nutrient uptake rates (generally positive) associated with increased current velocity, resulting in higher net growth rates.

The two diatoms *Rhopalodia novae-zealandiae* (cell size up to 200 µm) and *Epithemia sorex* (20-60 µm) displayed quite similar interactive response patterns. *R. novae-zealandiae* generally increased in abundance with added sediment of all sizes, however, additional flow reduction (both full and half) increased abundance

considerably more in all mesocosms with sediment addition. *E. sorex* preferred full flow reduction with all sized sediment addition, but especially with added sediment of medium or small particle size. *R. novae-zealandiae* has the ability to glide as noted in the Appendix of Salis (2016) and this means it can move to avoid burial. This ability, combined with the fact that this species is a good competitor (large cell volume, small surface area and slow growth), means that this species can tolerate sediment addition and then take advantage of flow reduction (provides conditions of nutrient limitation) which reduces the nutrients going to other taxa. This, in turn, may allow this species to benefit from a further reduction in competition. However, at faster velocities where there is a higher nutrient uptake rate, smaller-sized species may take over as they have faster growth and reproduction rates. *E. sorex* has low attachment to the substratum, reproduces via fission and has the ability to fix nitrogen. The attachment to the substratum means that this species would be more susceptible to burial, however the ability to reproduce using fission means that this drawback could be overcome because fission is typically fast and allows for the successful dispersal and recolonization in a disturbed environment (Lange *et al.*, 2016). Furthermore, the ability to fix nitrogen may allow *E. sorex* to overcome any nutrient limitation associated with full flow reduction and low current stimulation. This would provide a distinct advantage in this environment. Additionally, the large size of both of these cells means that at faster flows they would be disproportionately exposed to abrasion by fine sediment and therefore might prefer slower velocities (Ghosh & Gaur, 1998).

Cymbella kappi and *Gloeocytis* also showed synergistic responses to sediment addition and flow velocity reduction, but both response patterns were very complicated and hard to interpret in any biologically meaningful way.

While the main focus of this chapter has been on the abiotic processes that may influence algal communities, there are several important biotic processes that are likely to have also played a role in the observed responses. These include competition between algal species and herbivory by macroinvertebrates. Competition between algal species is a significant process in algal communities and is often linked to light, nutrients and space (Passy, 2007b; Lange *et al.*, 2011).

However, flow velocity and fine sediment can mediate competition by eliminating species not suited to the conditions, thus potentially allowing more tolerant species access to resources (Passy, 2007b; Passy & Larson, 2011). Herbivory is another factor that may influence algal responses to fine sediment particle size and flow velocity reduction (Poff & Ward, 1995; Wood & Armitage, 1997; Dewson *et al.*, 2007). It is possible that the combination of very fine sediment and fast flow velocity could remove or exclude certain benthic invertebrate grazers (Poff & Ward, 1995). A parallel study conducted in the same mesocosm experiment (M. Ward, unpublished data) observed a decline in the predominant grazer, larvae of the mayfly *Deleatidium* spp., with the addition of all three fine sediment sizes. Furthermore, no flow velocity reduction and full velocity reduction also led to a decline in *Deleatidium* spp. abundances. In both these cases, grazing pressure on algal communities can be expected to be reduced. Other studies and reviews substantiate this trend. Fine sediment is known to have an adverse effect on many benthic macroinvertebrates in streams (reviewed by e.g. Wood & Armitage, 1997). Additionally, Poff & Ward (1995) demonstrated experimentally that at flow velocities of 40 cm/s grazers were initially less dense and were less effective consumers than at 20 cm/s. The combination of these two stressors may lead to a decline in grazers and subsequent lower grazing pressure. Therefore, both these processes should be noted as potential explanations for some of my findings.

Chapter Three:

3 Stream algal trait responses to sediment addition and flow velocity reduction

3.1 Introduction

Agricultural intensification produces many stressors that threaten biological communities in freshwater ecosystems (Allan, 2004; Ormerod *et al.*, 2010; Bierschenk *et al.*, 2012; Nõeges *et al.*, 2016; Jackson *et al.*, 2016). In running waters, these stressors include nutrients, pesticides, fine sediment and hydrological alterations (Townsend *et al.*, 2008; Magbanua *et al.*, 2013a; Wada *et al.*, 2014; Piggott *et al.*, 2015b; see chapters 4 & 5 in Salis, 2016). Stressors often have interactive effects and can produce complex responses in biological communities (Piggott *et al.*, 2015b; Jackson *et al.*, 2016), including benthic algae in running waters (Wagenhoff *et al.*, 2013; Piggott *et al.*, 2015b). Benthic algal communities in streams and rivers comprise protists and cyanobacteria that are photosynthetically active primary producers and are important in the functioning of a healthy ecosystem (Biggs *et al.*, 1998).

Stream algal communities are particularly affected by deposited fine sediment through shading, substratum change, increased habitat heterogeneity and the smothering of substrata which predominately has a negative effect on non-motile, long-lived and chain-forming diatoms (Jones *et al.*, 2014). These effects may be more complicated when considering the unique properties of different-sized fine sediment particles. For example, very small particles such as clay and silt (< 63 µm) are less likely to affect periphyton through abrasion and more likely to act through reduced light penetration (Graham, 1990; Parkhill & Gulliver, 2002; Jones *et al.*, 2014). Therefore, it is of interest to separate the effects of relatively coarser fine sediment (e.g. fine sand particles; < 2000 to > 63 µm) from those of even finer sediment particles (e.g. clay). There is, to my knowledge, no experiment that has investigated and compared the specific effects through which different-sized fine sediment particles act on benthic algal communities.

Benthic algal communities are also affected by stream water abstraction for irrigation (Lange *et al.*, 2016). Water abstraction often results in the reduction of stream discharge, the reduction of flow velocity, an increase in water temperature, more frequent low flows, more frequent stream drying events and accumulation of fine sediment (through increased deposition) (Dickman *et al.*, 2005; Dewson *et al.*,

2007; Jones *et al.*, 2014). Given the close links between water abstraction and instream sedimentation, algal community responses may differ depending on the size of the fine sediment particles and the degree of water abstraction involved. However, based on algal community and taxon responses (e.g. taxon richness, abundances of common taxa) alone it may be difficult to identify the mechanisms causing the responses at different levels of these two stressors. Therefore, it is hoped that the use of a new, comprehensive, trait-based framework for benthic stream algae (developed by Lange *et al.*, 2016) will help illuminate the mechanisms by which these two stressors affect benthic algae.

A significant challenge currently facing freshwater managers is finding an indicator framework that is accurate, informative, practical, and useful in the identification of the effects produced by stressor mechanisms (Berthon *et al.*, 2011; Law *et al.*, 2014). Currently, community structure indices and population abundances are the most common approach used to indicate the ecological health and potential threats to a stream. While relatively cheap and easy, this approach has not been very illuminating or accurate when trying to understand the mechanisms by which many of these stressors act on community and ecosystem processes (Young & Collier, 2009; Death *et al.*, 2009; Law *et al.*, 2014).

The concept of using algal biological traits as indicators has been adopted from other fields of ecology (Biggs *et al.*, 1998; Passy, 2007a). In 2016, a comprehensive trait-based framework was developed and trialled as a new tool for investigating multiple stressors and their drivers (Lange *et al.*, 2016). The overarching premise of all biological trait-based systems is that the geographic distribution and the abundance of a species is related to its ability (assemblage of traits) to withstand habitat filters at all scales (Townsend & Hildrew, 1994; Poff *et al.*, 1997). Trait-based systems have to include a mechanistic relationship to evolutionary processes, have a foundation in abiotic factors, be reflective of processes affecting organisms and be easily measurable (Poff *et al.*, 1997; Weithoff, 2003; Kruk *et al.*, 2010; Lange *et al.*, 2016). Early attempts to develop a trait-based system for stream algae include the growth form system developed by Biggs *et al.*, (1998) and the ecological guild system devised by Passy *et al.*, (2007a). The former system was based on multiple, and often

redundant, traits that were related to resource acquisition (pigment composition, nitrogen fixation and mobility) and disturbance resistance (life forms, attachment mode and life history) (Biggs *et al.*, 1998; Passy *et al.*, 2007a; Statzner & Beche, 2010). The latter system attempted to reduce this redundancy by grouping traits into three different ecological guilds consisting of low profile, high profile and motile (Passy, 2007a). However, the required integration of many traits and the need for expert knowledge of species in order to assign taxa to an ecological guild still made this task difficult for non-experts and contradicted the idea that trait-based systems should be based on criteria that are easily measurable by all users (Lange *et al.*, 2016). Further criticisms included the restrictiveness of the three (and later five) algal guilds used (Schneck *et al.*, 2011; Lange *et al.*, 2016). To address these limitations, the recently published trait framework by Lange *et al.*, (2016) has expanded the guild system and proposes that the use of morphological, physiological, behavioural and life history traits can reveal the drivers of stressors operating in the agricultural stream environment. The framework proposes the use of seven algal traits including cell size, life form, attachment to the substratum, nitrogen fixation, motility, reproduction and spore formation. These seven traits are broken down into 25 trait categories covering a wide range of potential characteristics (e.g. nano-sized cells, colonial life form, high attachment, gliding motility, no spore formation) that may be affected by in-stream stressors related to agricultural intensification.

Two previous experiments in the same stream mesocosm system as the one used in my thesis, described in chapters 4 & 5 in Salis (2016), were the first to use the Lange *et al.*, (2016) trait framework to study effects of multiple agricultural stressors (nutrient enrichment, fine sediment addition, the nitrification inhibitor DCD and streamflow velocity reduction) on stream algae. However, to my knowledge my study is the first experiment to investigate the responses of biological algal traits to deposited fine sediment of different grain sizes, and how different degrees of abstraction-induced flow velocity reduction may interact with and modify the effects of these different sediment grain sizes. The experiment will also be used to infer the mechanisms behind the observed algal responses, thus potentially providing further experimental evidence that trait-based approaches can be helpful

for understanding multiple-stressor effects on stream communities. I tested three hypotheses:

- 1) The addition of larger sediment grain sizes (1-2 mm) will result in stronger negative effects on certain periphyton traits compared to smaller sediment grain sizes (0-0.125 mm) (Francoeur & Biggs, 2006; Jones *et al.*, 2014). These negative effects may happen in traits including macro-sized cells, very large sized cells, filamentous life forms, attached algae, fragmentation reproducing life forms, and non-spore producing species. There have been no previous studies that have investigated fine sediment particle size effects on stream algae using the Lange *et al.*, (2016) framework; therefore, this hypothesis is speculative and is based on the Chapter 2 hypothesis for sediment particle size.
- 2) Flow velocity will have pervasive effects on the relative abundances of algal traits:
 - a. Reduced flow velocities will favour smaller-sized (nano-macro), unicellular, nitrogen fixing, colonial, tightly attached and gliding species (see chapter 5 in Salis, 2016);
 - b. Fast flows will favour very large, filamentous, drift-motile, fission-reproducing and non-spore-forming (see chapter 5 in Salis, 2016);
- 3) The responses of algal traits to the addition of fine sediment of any grain size will be more frequent and severe at reduced flow velocities (Matthaei *et al.*, 2010; see chapter 5 in Salis, 2016). This is because fine sediment is generally more likely to settle and persist at reduced flow velocities.

3.2 Methods

3.2.1 Experimental Design

The experiment was performed in late summer/early autumn and comprised a 25-day colonization period (day -25 to day 0) followed by a four-week manipulative period (day 0 to day 27). It involved a full-factorial design of 60 flow-through,

circular stream mesocosms (volume 3.5 L, bed surface area 450 cm²; Microwave Ring Moulds, Interworld, Auckland, New Zealand) (see Chapter 2 for more information about the experimental setup). Four different fine sediment treatments (no added sediment, coarse sand, fine sand, silt) were used. The particle sizes in the sediment addition treatments were 1-2 mm for coarse sand (henceforth called “large sediment”), 0.125 mm – 0.250 mm for fine sand (“medium sediment”) and < 0.125 mm for silt (“small sediment”). Sediment treatments were crossed with three levels of flow velocity reduction, which consisted of fast (35 cm/s; the default treatment), moderate (17 cm/s) and slow velocity (0 cm/s), resulting in 12 treatment combinations. For each treatment combination there were five replicates. The mesocosms were filled with stones from dry sections of the Kauru River bed. This base sediment consisted of 500 mL of 2-20 mm gravel along with 16 stones (placed on the surface) with a maximum length of 20 mm.

3.2.2 Algal Sampling and Trait Analysis

The technique used to sample benthic algae at the end of the experiment differed depending on the substratum in the mesocosms (see e.g. Magbanua *et al.*, 2013a; Wagenhoff *et al.*, 2013). Mesocosms without added fine sediment were sampled using a technique which included three standard circular areas in added sediment mesocosms. All mesocosms that contained added fine sediment were sampled using the top 2 mm of surface sediment in the same defined core area. This surface sediment was sucked up using a cut-off 3 mL pipette, with two sub-samples (from random surface locations covered by fine sediment) making up one sample. In control mesocosms, three randomly chosen surface stones were selected. This core was defined using a plastic ring (about 25 mm high) with a diameter of 27 mm. The periphyton was transferred to a sterile 100 ml Astraline container and preserved on ice, in the field. The containers were transported in the dark to the -20 °C freezer and frozen on arrival.

Algal trait analysis was built on the community composition analysis in which about 300 cells were identified at 400x magnification to the lowest practicable taxonomic level using an inverted microscope (Zeiss Axiovert 25, Jena, Germany). Algal filaments could not be identified by cell number and were thus counted as 10 µm

increments equalling one ‘cell’ (see e.g. Lange *et al.*, 2016). Most algal taxa were identified to species, and some to genus or family, using the Stream Periphyton Monitoring Manual (Biggs & Kilroy, 2000) and the photographic reference collection of stream algae from the Kauru River (K. Lange, unpublished). Various sources of information were used to find the trait for the various algal species, including Algaebase, Lange 2016 trait and species list, and the appendix from the PhD thesis by Salis (2016). Traits from the seven categories described in Lange *et al.*, (2016) were assigned to species, using these information sources. Traits were assigned on a presence or absence system. Within these categories, the species were grouped by trait type, and then the relative abundance of each trait type was calculated (i.e the % of each trait type within a category (out of 100 %).

3.2.3 Statistical Analysis

The data analysis for this chapter was conducted in SPSS 22.0 (IBM SPSS Inc., IL, Chicago, USA). The initial exploratory analysis suggested that the algal trait data did not need any transformations. The two fixed factors in the ANOVAs and MANOVAs were sediment type and flow velocity (with the interaction). A block factor was included to account for the four mesocosm header tank blocks. This resulted in a model whereby the intercept was (d.f. 1) + sediment type (3) + flow velocity (2) + sediment x flow velocity (6) + block (3).

The experiment that was performed was interested in differences between the four individual sediment treatments and the three individual flow velocity levels. Therefore, both stressors were modelled as categorical (rather than continuous) predictors. This analysis may underestimate the frequency of two-way interactions because of the larger number of degrees of freedom required to model interaction effects compared to the two main factor effects (see Cottingham *et al.*, 2005).

To investigate effects of sediment addition and flow velocity manipulation on benthic algae, the experiment investigated algal trait relative abundance. To investigate the responses of algal traits to stressors, MANOVAs were used. The experiment used the responses of the 23 trait types described in Lange *et al.*, (2016)

and two extra traits (because they might show interesting responses) to investigate the effects of both stressors and their interactions. Pillai's Trace statistic was used to determine the multivariate stressor effects, and the between-subjects effects for each taxon were used to determine stressor effects on common taxa. All significant between-subjects main effects of the two factors were investigated further by pairwise comparisons using Tukey's HSD *post hoc* tests. The test results and rankings are presented in tables with all other significant results.

The results presented were for the factors sediment particle size and flow velocity (i.e those directly related to the questions posed). The results for the block factor were not presented because they were irrelevant to the research objectives. The significance level for all tests was $P < 0.05$, and the significant responses were described in the results (including results with $P = 0.05$). Standardized effect sizes were presented in the results for all findings with $P < 0.05$.

The presence of significant interactions between sediment particle size and flow velocity (the two experimental factors) required a careful interpretation of the main effect of the factors. As recommended by Quinn & Keough (2002); Piggott *et al.*, 2015b; and Piggott *et al.*, 2015c, significant individual main effects were interpreted (in the presence if a significant interaction) only when the interaction effect size was smaller than the corresponding main effect(s) size.

3.3 Results

3.3.1 Algal trait relative abundance

The MANOVA on the 25 algal traits indicated that trait composition differed across sediment and flow velocity treatments and that the sediment by velocity interaction was significant (Table 3.1). The between-subjects results of the MANOVA showed that these overall patterns were caused by 19 significant responses of individual trait categories to the sediment addition treatments, nine responses to flow velocity reduction and 12 interactions between the two stressors. Many of the significant factor main effects were overridden by stronger interactions (three sediment main effects and seven flow reduction main effects) (Table 3.1).

The five cell size metrics were influenced by both sediment addition and flow velocity reduction. Relative abundance of nano-sized species was higher in mesocosms with added small sediment than in mesocosms with large sediment or in controls (no added sediment) (Table 3.1., **Fig. 3.1**). The flow velocity reduction effect was overridden by a stronger interaction between flow velocity and sediment. The positive effect of fast or medium velocities was much weaker in mesocosms with large added sediment than in the other three sediment treatments. Micro-sized species increased in abundance with the addition of any size of sediment but were unaffected by flow velocity or stressor interactions (Table 3.1, **Fig. 3.2**). Meso-sized species were most abundant in control mesocosms, intermediately abundant in mesocosms with medium sediment, and rarest in mesocosms with small or large sediment (Table 3.1, **Fig. 3.1**). These species were unaffected by flow velocity as a main effect but showed a velocity by sediment interaction, displaying an increase with flow velocity reduction in mesocosms without added sediment whereas the opposite pattern occurred in mesocosms with added sediment of any size. Macro-sized species were unaffected by sediment addition or stressor interactions but were more common at slow velocity compared to medium and fast flow velocities (Table 3.1, **Fig. 3.2**). Very large species were more abundant in mesocosms at slow velocity compared to medium or fast flow velocities and also showed a significant, but fairly weak (effect size 0.23), overall sediment main effect. However, this main effect was overridden by a slightly stronger, marginally significant sediment by velocity interaction ($P = 0.050$, effect size = 0.24). This interaction occurred because the overall positive effect of flow velocity reduction was absent in mesocosms without added sediment (Table 3.1, **Fig. 3.1**).

Table 3-1: Summary (P-values and effect sizes) of MANOVAs comparing algal trait responses of Nano-sized species ($5 \leq 100 \mu\text{m}^3$), Micro-sized species ($100 \leq 300 \mu\text{m}^3$), Meso-sized species ($300 \leq 600 \mu\text{m}^3$), Macro-sized species ($600 \leq 1500 \mu\text{m}^3$), Very Large sized species ($>1500 \mu\text{m}^3$), Colonial species, Filamentous species, Unicellular species, Low attachment species, Medium attachment species, Nitrogen-fixing species, and High attachment species, Non-nitrogen-fixing species, Attachment motility species, Drift motility species, Glide motility species, Fission reproducing species, Fragmentation reproducing, No spore producing species, Akinete spore species, Autospore producing species, Monospore producing species, Zygospore producing species, Restspore producing species, and Zoospore producing species between the experimental treatments. 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 – 0.125mm); M, medium (0.125 – 0.250 mm); L, large (1-2 mm). Flow treatment; S, slow; M, medium; F, fast. Significant P-values (with effect sizes in parentheses; partial eta squared, > 0.1 = small; > 0.3 = medium; > 0.5 = large) are indicated in bold print. Effects marked with x were overridden by the interaction.

Dependent variable	%	Sediment	Ranking	Flow	Ranking	Sediment x Flow
MANOVA on trait composition (25 traits) <i>(Wilks' Lambda statistic)</i>		<0.001 (0.85)		0.03 (0.68)		0.007 (0.63)
Nano	15. 1	<0.001 (0.32)	L < S C < S	0.007 (0.2)	x	0.041 (0.24)
Micro	24. 6	<0.001 (0.35)	C < (S=M=L)	0.427		0.290
Meso	34. 2	<0.001 (0.53)	(L=S) < M < C	0.145		0.017 (0.28)
Macro	10. 1	0.098		<0.001 (0.33)	(F=M) < S	0.108
Very Large	12. 9	0.008 (0.23)	x	<0.001 (0.35)	(F=M) < S	0.050 (0.24)
Colonial	21. 7	<0.001 (0.40)	L < S C < M	0.024 (0.15)	x	0.013 (0.29)

Table 3-1 Continued

Dependent variable	%	Sediment	Ranking	Flow	Ranking	Sediment x Flow
Filamentous	13.3	<0.001 (0.53)	(S=M) < (L=C)	0.175		0.004 (0.34)
Unicellular	65.0	0.054 (0.16)	L < M (P = 0.028)	0.318		0.117
Low attachment	50.2	<0.001 (0.45)	C<(S=M=L)	0.910		0.101
Medium attachment	42.2	<0.001 (0.50)	(S=M=L) <C	0.679		0.153
High attachment	6.9	0.011 (0.22)	x	0.088 (0.10)		0.022 (0.27)
Nitrogen fixing	7.9	0.442		<0.001 (0.37)	x	<0.001 (0.42)
Non- Nitrogen fixing	91.6	0.891		<0.001 (0.36)	x	<0.001 (0.45)
Attachment motility	67.5	0.004 (0.25)	S < (C=L)	0.573		0.117
Drift motility	16.1	0.114		0.013 (0.18)	x	0.033 (0.25)
Glide motility	15.7	0.006 (0.24)	x	0.008 (0.19)	x	0.001 (0.39)
Fission reproduction	89.3	<0.001 (0.42)	(C=L) < (M=S)	0.108		0.045 (0.24)
Fragmentation Reproduction	10.2	<0.001 (0.39)	(S=M) <(L=C)	0.108		0.062 (0.23)
No spore production	85.5	<0.001 (0.48)	(C=L) < (M=S)	0.048 (0.13)	x	0.018 (0.28)
Akinete spore production	5.8	0.356		0.435		0.332
Autospore production	3.2	<0.001 (0.47)	(S=C=M) < L	0.103		0.646
Monospore production	0.1	0.047 (0.16)	(S=M=L) < C	0.333		0.348
Zygospor production	2.0	0.008 (0.23)	(M=S) (L=C)	0.136		0.479
Restspore production	0.0	0.202		0.997		0.503
Zoospore production	1.3	0.003 (0.26)	(S=M) (L=C)	0.219		0.208

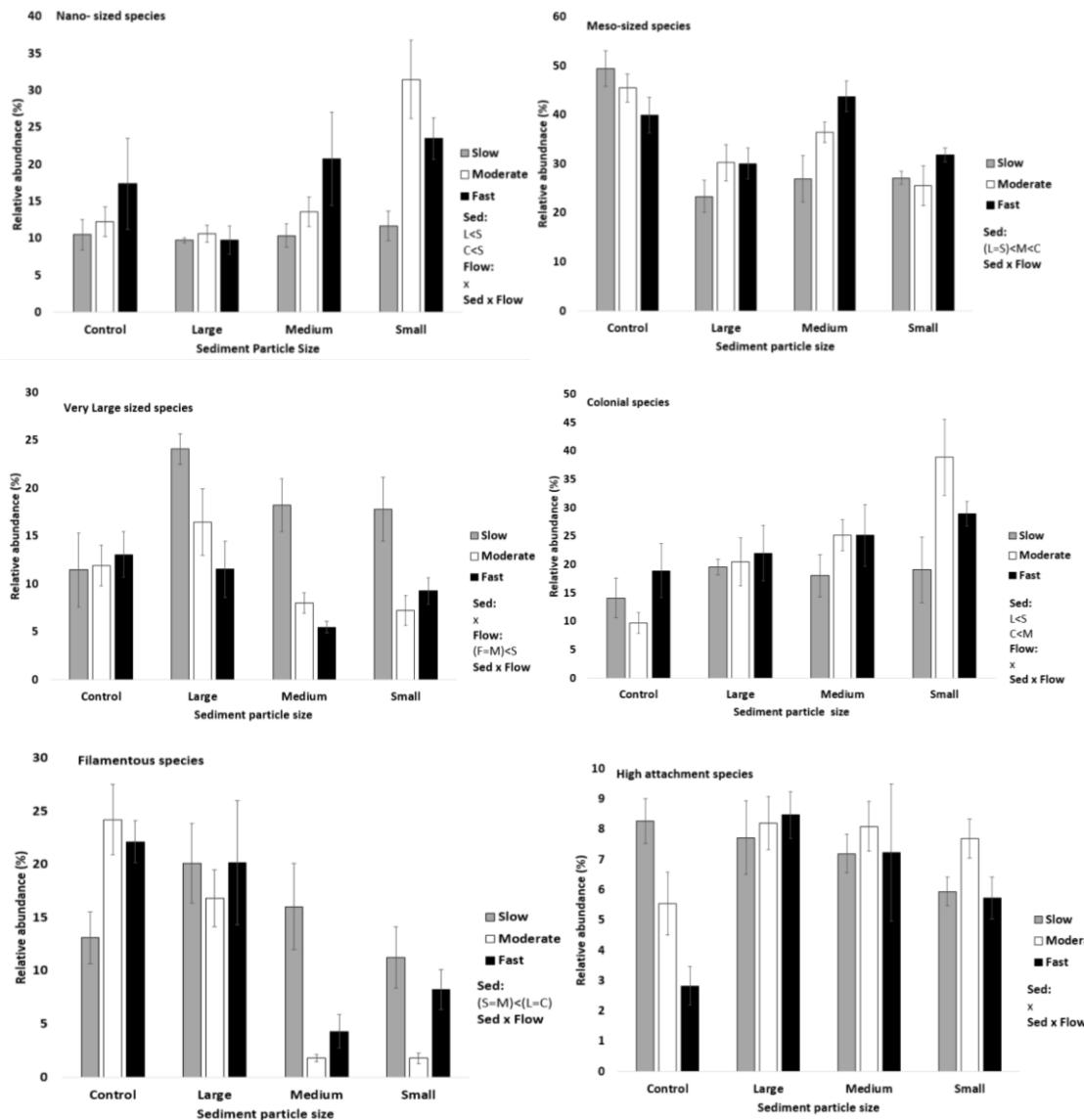


Figure 3-1: Mean relative abundances (%) of Nano-sized species, Meso-sized species, Very large sized species, Colonial species, Filamentous species and High attachment species. All these showed a significant interaction between sediment particle size and flow velocity reduction. Error bars represent standard errors ($n = 5$ for each treatment combination). Effects marked with x were overridden by the interaction.

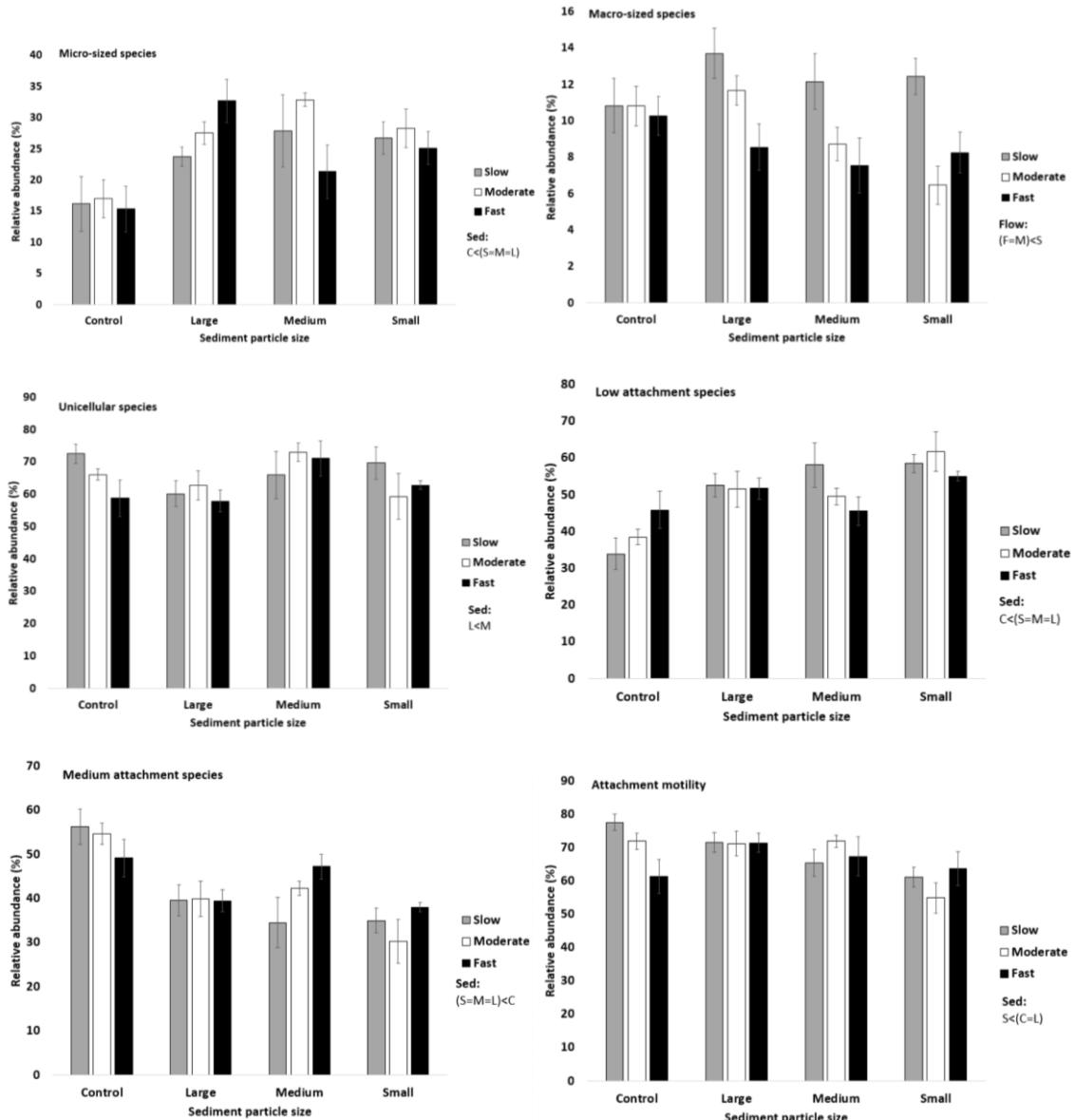


Figure 3-2: Mean relative abundances (%) of Micro-sized species, Macro-sized species, Unicellular species, Low attachment species, Medium attachment species, Attachment motility species. All these showed no interaction between sediment particle size and flow velocity reduction. Error bars represent standard errors (n= 5 for each treatment combination).

The trait life form also displayed various responses to sediment and flow velocity reduction. Colonial life forms were more abundant in small-sediment than in large-sediment mesocosms and in medium-sediment mesocosms than in controls, while the flow reduction effect was overridden by a stronger interaction between sediment and flow velocity. In this, the positive effect of added small sediment was

strongest in mesocosms with moderate flow velocity (Table 3.1, **Fig. 3.1**). Filamentous life forms were less abundant in medium- and small-sediment mesocosms compared to control or large-sediment mesocosms. These species were unaffected by flow velocity as a main effect but showed a velocity by sediment interaction, displaying an abundance increase in slow-velocity mesocosms with medium or small added sediment whereas the opposite pattern occurred in mesocosms without any added sediment (Table 3.1, **Fig. 3.1**). Unicellular life forms showed an almost significant, weak sediment main effect ($P = 0.054$, effect size = 0.16). This trait tended to be more abundant in mesocosms with medium-sized added sediment than in those with large added sediment (Table 3.1, **Fig. 3.2**).

The attachment strength of algal cells also showed a number of different responses to sediment and flow velocity reduction. Low-attachment species were less abundant in control (no sediment added) mesocosms than in those with added sediment of any size (Table 3.1, **Fig. 3.2**). Medium-attachment species, by contrast, showed exactly the opposite pattern (Table 3.1, **Fig. 3.2**). High-attachment species showed a velocity by sediment interaction, displaying an increase in abundance with flow velocity reduction only in mesocosms without added sediment (Table 3.1, **Fig. 3.1**).

The ability to fix nitrogen responded in various ways to flow velocity reduction. Nitrogen-fixing species were unaffected by the sediment treatments and flow reduction as a main effect but showed a velocity by sediment interaction, displaying an increase in abundance at slow velocity in mesocosms with added sediment of any size but showing the opposite pattern in controls (Table 3.1, **Fig. 3.3**). Non-nitrogen fixing species were unaffected by sediment addition as a main effect but showed a velocity by sediment interaction, decreasing in abundance at slow velocity in mesocosms with added sediment regardless of size while showing the opposite pattern in controls (Table 3.1, **Fig. 3.3**).

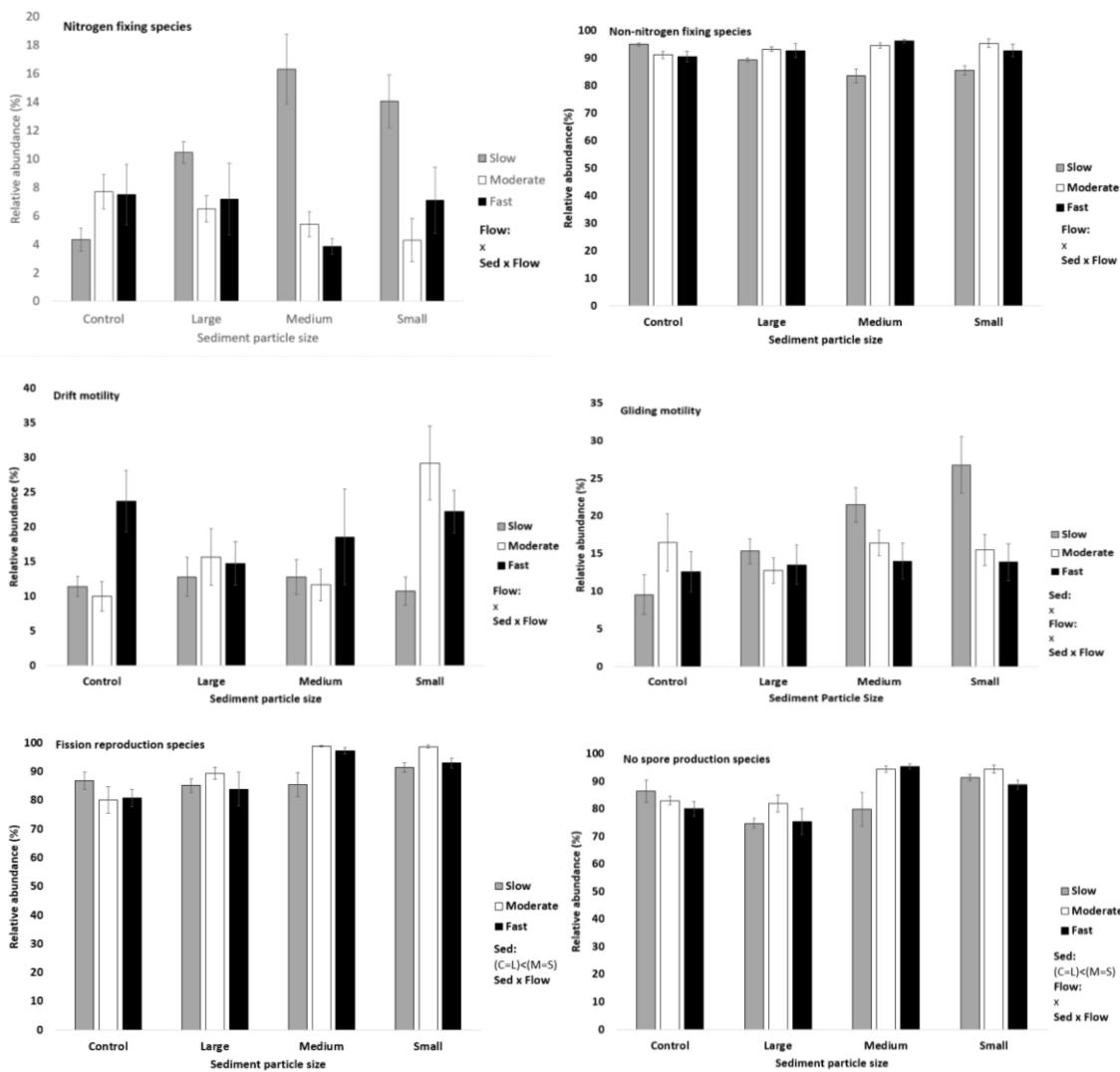


Figure 3-3: Mean relative abundances (%) of Nitrogen fixing species, Non-nitrogen-fixing species, Drift motility species, Gliding motility species, Fission reproducing species, and No spore forming species. All these showed a significant interaction between sediment particle size and flow velocity reduction. Error bars represent standard errors (n=5 for each treatment combination). Effects marked with x were overridden by the interaction.

The degree and method of motility was another algal trait that displayed various responses to sediment and flow velocity reduction. Species that are attached to the substratum (i.e. cannot move) were less abundant in small-sediment mesocosms than in those with large added sediment or without added sediment (Table 3.1, **Fig. 3.2**). Drifting species were unaffected by sediment addition as a main effect but showed a velocity by sediment interaction, displaying the highest abundance at fast flow velocity in all sediment treatments except for in mesocosms with large added

sediment (Table 3.1, **Fig. 3.3**). Gliding species showed an interaction which overrode the weaker main effects of both sediment particle size and flow velocity reduction. A positive effect of added sediment (which became larger as sediment particle size decreased) occurred under slow-flow velocity conditions but not at medium or fast flow (Table 3.1, **Fig. 3.3**).

The method of reproduction also responded to the sediment treatments. Species that reproduce using fission were more abundant in small- and medium-sediment mesocosms than in large-sediment mesocosms or controls. These species were unaffected by flow velocity as a main effect but showed a velocity by sediment interaction, displaying a decrease in abundance in slow-velocity mesocosms with medium-sized added sediment while showing in opposite pattern in mesocosms without added sediment (Table 3.1, **Fig. 3.3**). Species that reproduce using fragmentation were less common in small- and medium-sediment mesocosms than in those with large sediment or in controls (Table 3.1, **Fig. 3.4**).

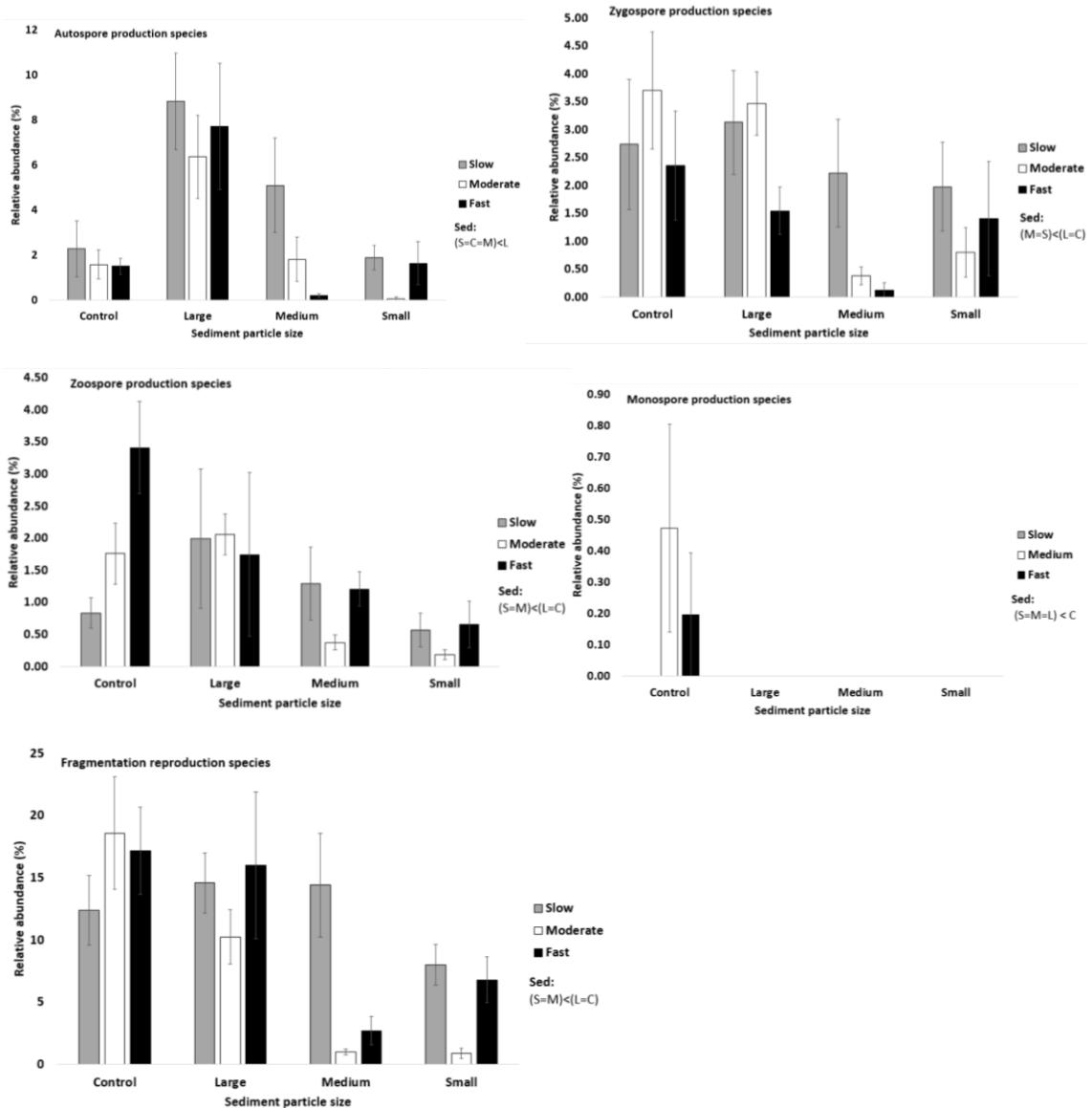


Figure 3-4: Mean relative abundances (%) of Autospore producing species, Zygospor e producing species, Zoospore producing species, Monospore producing species and Fragmentation reproduction species. All these showed no interaction between sediment particle size and flow velocity reduction. Error bars represent standard errors ($n=5$ for each treatment combination).

Lastly, the type of reproductive spore also responded to both sediment particle size and flow velocity reduction. Non-spore producing species were more abundant in small and medium-sediment mesocosms than in those with large sediment or without added sediment. Flow velocity and sediment effects interacted for this trait category, with the positive effect of added medium-sized sediment being much weaker in mesocosms with slow flow velocity than in the other two velocity

categories, whereas the opposite pattern occurred in mesocosms with no added sediment (Table 3.1, **Fig. 3.3**). Autospore-producing species were more abundant in large-sediment mesocosms than in the other three sediment treatments (Table 3.1, **Fig 3.4**). Monospore-producing species were more abundant in control mesocosms than in any of the sediment addition treatment. Zygospore-producing species were less abundant in small-sediment mesocosms than in those with medium, large or no added sediment (Table 3.1, **Fig. 3.4**). Similarly, zoospore-producing species were more common in mesocosms with large added sediment or no added sediment than in mesocosms with small or medium added sediment (Table 3.1, **Fig. 3.4**). Akinetes and rest spores were the only two algal trait categories which did not respond to the experimental treatments.

3.3.2 Overview of biological trait response patterns

Of the two stressors investigated in this chapter, fine sediment was the more pervasive stressor, although flow velocity also had frequent effects. The evidence to suggest this included that periphyton response variables demonstrated 19 significant responses (out of 25 possible ones) to sediment treatments. This is compared to flow velocity which demonstrated nine significant responses. Interactive effects between sediment particle size and flow velocity reduction occurred in 12 trait categories (one marginal category). Furthermore, many of the significant factor main effects, especially for flow velocity reduction, were overridden by stronger interactions (three sediment main effects and seven flow reduction main effects).

3.4 Discussion

3.4.1 Effects of added sediment with different particle sizes on benthic algal traits

In hypothesis 1, I predicted that the addition of relatively large fine sediment grain sizes may have a more negative and widespread effect on relative algal trait abundances of macro-sized cells, very large sized cells, filamentous life forms, attached algae, fragmentation-reproducing life forms, and non-spore producing species than the small grain sizes. There was no evidence in support of this hypothesis because the effects of added large sediment were not negative or as

widespread in these traits as the effects of smaller sediment sizes. This result is not totally unexpected as the hypothesis proposed had to be rather tentative. Firstly, this is because there have been no specific studies on the effects of fine sediment grain size on stream periphyton. Secondly, to date there have been only two studies (one stream survey and two experiments) that employed the Lange *et al.*, (2016) algal trait framework, which both focused on the effects of fine sediment defined simply as < 2 mm diameter. Therefore, not much is known about how biological traits of stream algae may react to a range of fine sediment particle sizes.

In my experiment, there was a variety of responses to the addition of different-sized particles to the mesocosms. The addition of sediment produced positive results in seven algal trait categories including nano cell size species, micro cell size species, colonial species, fission reproduction species, no spore producing species, autospore producing species and low attachment species. Of these positive responses, the most common response was a preference for small-sized sediment particles. Moreover, there were eight negative responses including attachment motility, filamentous species, fragmentation reproduction, zygospore production, zoospore production, medium attachment, monospore species, and meso sized species. Of these negative responses, the most common response was a preference for small- and medium-sized sediment particles.

The positive responses to sediment addition were mixed (in terms of responses to the three different sediment grain sizes) but overall there was a distinct preference for small-sized sediment. Nano and micro sized cells preferred small-sized sediment particles to the control and also large sized sediment. This is probably because this quick growth rate allows small species to recolonise new and easily disturbed sediment faster (Biggs *et al.*, 1998; Passy, 2007b; Lange *et al.*, 2016; see chapter 4 in Salis, 2016).

Colonial species preferred medium-sized sediment particles to the control and also preferred small-sized sediment to large-sized sediment. This is surprising because a previous study (which did not distinguish between sand and clay sediment sizes) suggested that the addition of fine sediment resulted in a negative response of this

trait category (see chapters 4 & 5 in Salis, 2016). The colonial species present in this study (especially the most numerous species *Merismopedia* spp. and *Fragilaria vaucheriae*) had three other algal traits in common. These were small sized cells (nano and micro) which had low attachment and reproduced via fission. These were traits which also responded positively to the addition of any sized sediment. Given this observation, it is possible to suggest that these three traits may allow colonial species to live in the unstable environment provided by added fine sediment. Furthermore, the particular preference for small and medium-sized sediment may reflect the fact that, of the sediment addition treatments, this sediment size may allow colonial species to form a matrix in an unstable environment and potentially provide further protection from significant erosion (Piqué *et al.*, 2016). This is because small and medium-sized sediment may provide a degree of stability (unlike large-sized sediment).

Fission reproduction and no spore production were also associated with colonial species and responded positively to the addition of medium (small sand) and small (clay) sized particles. Fission reproduction typically occurs in small unicellular cells and is a faster form of reproduction than fragmentation (Biggs *et al.*, 1998; Passy, 2007a; Litchman & Klausmeier, 2008; Lange *et al.*, 2016; see chapters 4 & 5 in Salis, 2016). It is therefore probably more suited to the successful dispersal and recolonization in a disturbed environment (Biggs *et al.*, 1998; Lange *et al.*, 2016). Similarly, the no spore formation trait also responded positively to small sand and clay addition. This is surprising since the ability to produce spores and not non spore production is typically associated with surviving unfavourable conditions (Lange *et al.*, 2016). However, it is possible that the no spore formation trait responded positively because of its strong links to other traits such as small cell size (nano and micro) and fission reproduction. These traits are not independent as non-spore formation has been linked to diatoms which are often small and unicellular/colonial (Lange *et al.*, 2016; see chapters 4 & 5 in Salis, 2016). These combinations have been observed in two previous experiments (see chapters 4 & 5 in Salis, 2016). Given this common observation, it would be useful to try and determine the degree to which these traits overlap.

Given the positive response of no spore formation to sediment addition, it is interesting to note the positive response of autospores to the addition of large sediment sizes. This response is unlikely to be the result of a link to other traits such as colonial life form, low attachment, small size or fission reproduction because these traits preferred small and medium sized sediment. Autospores are asexual spores that are non-motile (South & Whittick, 2009). The size and inability to move may mean that these spores are cheaper to produce by colonial species. Therefore, while autospores may not last long in an unstable habitat, the potential quantity and short reproduction time of these cells may give them an advantage in an unstable environment. Since very little is known about autospores and their advantages, the above explanation should be treated as speculative.

The low attachment trait responded positively to the addition of all sizes of fine sediment. This is unsurprising because several previous studies have indicated that stream algal species with low attachment (motile and mucilaginous stalked taxa) have preferred added fine sediment because they are not as vulnerable to burial and instability that accompanies high levels of sediment deposition (Pringle, 1990; Burkholder, 1996; Schneck *et al.*, 2011; Wagenhoff *et al.*, 2013; Piggott *et al.*, 2015b).

The negative responses to sediment addition followed a similar pattern, where the majority of responses were to medium and small sediment sizes. Attachment motility showed a negative response to the addition of small sized sediment. This is unsurprising because species with attachment to the surface cannot move to avoid unfavourable conditions such as burial by sediment, especially small-sized sediment such as clays which can smother attached communities (Jones *et al.*, 2014; Lange *et al.*, 2016).

Filamentous species (*Phormidium* spp. and *Melosira varians*) declined with medium and small-sized sediment. This is interesting because the existing literature suggests that filamentous species primarily react to agriculture through nutrients (Lange *et al.*, 2016). However, my study and a similar previous study suggests that there may be a negative reaction to fine sediment as well (see chapters 4 & 5 in Salis, 2016).

The response by this trait was probably heavily influenced by *Phormidium* spp. which is thought to dislike the instability present with added sediment. This dislike is probably caused by the slower reproduction rate (because of larger propagule size) of filamentous algae through fragmentation. This may be particularly disadvantageous in an unstable and inhospitable environment (Blinn *et al.*, 1998; Lange *et al.*, 2016). This idea is supported by the observation in my experiment that the fragmentation reproduction trait displayed the same response. Furthermore, previous related research has suggested a relationship between filamentous species and fragmentation reproduction (Lange *et al.*, 2016).

An interesting pattern appeared with zygospor production (motile and sexual) and zoospore production (motile and asexual). Zygospores and zoospores responded negatively to the addition of medium and small sediment sizes (South & Whittick, 2009). This is an interesting pattern because it contradicts previous research and knowledge which suggests that the production of spores, especially sexual zygospores is beneficial in inhospitable environments (Lange *et al.*, 2016). The literature suggests that a combination of altered metabolic rates, heavy cell walls and reduced exchange with the external environment are thought to provide benefits in situations of chemical, nutrient and sediment stress (Fryxell, 1983; Lange *et al.*, 2016). This ability to develop spores is typically found in species that reproduce using fragmentation and it enables them to disperse and reproduce more successfully in harsh environments (De Bie *et al.*, 2012; see chapter 3 in Salis, 2016). However, my experiment produced a counterintuitive result and the exact cause is uncertain. The most likely reasons are probably spore viability time and trait links. Firstly, spore viability may be an issue because it is possible that, while these spores may be viable in a stressed situation for a short while (days), there is the potential that burial by clays for a long time period may invalidate the spores. Secondly, the species making up the traits could be species that reproduce using fragmentation. This is a likely factor because spore formation has been linked to fragmentation reproduction, which is a slower form of reproduction (Lange *et al.*, 2016).

Medium attachment species and monospore producing species responded negatively to all sizes of added sediment. Logically, this is not surprising because

species with attachment to the surface cannot move to avoid unfavourable conditions such as burial by sediment. There could be an alternative potential explanation for this observation. Many of the less common species that had medium attachment may have been filamentous and reproduced using fragmentation. These traits are linked and have both been associated with a negative response to sediment addition. Whether the total abundance of these less common species could overwhelm the effect of those unicellular species with quicker fission reproduction is questionable, but it is a possibility. Monospore-producing species were generally very rare (0.011136 %) and occurred only in 3 of the 60 samples, therefore it is hard to tell whether the fact that they occurred only in control mesocosms is biological meaningful.

Meso sized cells declined with all sediment sizes but were most affected by the addition of large-sized sediment (sand) and small-sized sediment (clays and silts). The species that displayed this trait have been previously categorized as high profile (Passy, 2007b). They often form long colonies that can extend vertically into the boundary layer and are vulnerable to abrasion and dislodgement (Passy 2007b). Therefore, it is possible that with large-sized sediment, these cells may suffer from increased abrasion while clay sized particles may become trapped in and on the biofilm matrix, thus influencing light levels (Jones *et al.*, 2014). Furthermore, small sediment may smother these species as they are unable to move. Medium-sized sediment may have a more mitigated effect because of its more cohesive nature compared to large-sized sediment (which is less able to be entrained) and its potential ability to allow enough space to not completely smother these diatoms (allowing them to access nutrients and light through their stalks). Medium-sized sediment may also eliminate many low-profile forms with no stalks (reducing competition) with this smothering.

3.4.2 Effects of flow velocity reduction on benthic algae

In hypothesis 2, I predicted that flow velocity will have pervasive effects on the relative abundances of algal traits. Two sub-hypotheses were also formed. Firstly, I predicted that reduced flow velocities will favour smaller-sized (nano-macro), unicellular, nitrogen fixing, colonial, tightly attached and gliding species (see

chapter 5 in Salis, 2016). Secondly, fast flows should favour very large, filamentous, drift-motile, fission-reproducing and non-spore-forming (see chapter 5 in Salis, 2016). There was no evidence to support any of these hypotheses. In fact, there was evidence against these hypotheses regarding the response of macro and very large sized cells.

The only traits which responded to flow velocity reduction were macro and very large-sized cells. They both displayed a positive response to full flow velocity reduction. This response, while surprising from the perspective of chapter 5 in Salis (2016), is logically consistent with the idea that larger cells would at faster current velocities be subject to higher shear stress (compared to small-sized cells) and benefit less from an increase in nutrient uptake rates provided by a thinner boundary layer (because of the smaller surface area to volume ratio). Therefore, larger cells should prefer slower flow velocities (Passy, 2007b). Furthermore, larger cells might be more subject to friction and abrasion from sediment during normal flow conditions than under flow reduction conditions.

There were only two significant responses to flow velocity reduction as a main effect, but there were many interactions between sediment particle and flow velocity (see next section). This suggests a heavy influence of sediment particle size on the effects of flow reduction for algal traits.

3.4.3 Interactions between sediment and flow velocity reduction

In hypothesis 3, I predicted that the responses of algal traits to the addition of fine sediment of any grain size will be more frequent and severe at reduced flow velocities. The trait responses observed suggest that there is some evidence to support this hypothesis. There were 12 significant sediment by flow velocity interactions (one interaction was marginally significant), of 25 possible interactions. Ten interactions were stronger than one or both of the stressor main effects for the trait in question and therefore overrode these main effects.

The trait interactions were all complex (non-additive), with both antagonisms and synergisms occurring, plus an interesting group of trait categories that displayed complex interaction patterns. Antagonisms occurred for nano-sized species, non-spore reproducing species, and high attachment species. Synergisms occurred for colonial species, gliding species, drift motility species, and very large sized cells. The category which displayed both interaction types consisted of meso-sized species, fission-reproducing species, filamentous species, non-nitrogen fixing species and nitrogen fixing species.

Antagonistic interactions were found for nano-sized species, non-spore reproducing species, and high attachment species. Nano-sized species experienced an interaction in which the positive effect of faster velocities was weakened with the addition of large-sized sediment. It is possible that in this situation, the nutrient uptake enhancement effect promoted by a faster current is reduced by the increased abrasion from sand grain particles that may cause cell damage. Furthermore, large-sized sediment at faster flow velocities may be more unstable and therefore may prevent the development of a biofilm matrix that could prevent erosion (Piqué *et al.*, 2016). Non-spore producing species displayed an interaction whereby the positive effect of added medium sediment was weaker in mesocosms with slow flow velocity. This response is similar to fission reproduction suggesting that these two traits may be linked in some way. The production of spores is typically only seen in filamentous species that reproduce using fragmentation. Therefore, as for fission reproduction it is possible that this trait also responded negatively to the more stable conditions present (which favour filamentous species) when flow velocity is reduced and the particles are not entrained. High attachment displayed a unique response with a decrease in abundance with default flow velocities, but only in mesocosms without added sediment. Species that have high attachment to the substratum tend to be low profile while high profile and motile species tend to have a weaker attachment to the substratum (Biggs *et al.*, 1998; Passy, 2007b; Schneck *et al.*, 2011). It is possible that this response is not entirely related to the high attachment trait but rather to competition for light and nutrients (Biggs *et al.*, 1998; Passy, 2007b). It could be that under control conditions (no sediment/normal flow velocity) in my experiment, the high profile taxa (low/medium attachment species with stalks) benefited from the

stimulatory effect of normal flow which provided a higher nutrient environment. This proliferation of the high profile taxa means that highly attached species (often associated with the low profile guild) suffered from nutrient and light deprivation (Passy, 2007b). However, the reduction of flow velocity may have led to a reduction of this stimulatory effect. Subsequently, the high profile guild declined and allowed the low profile guild to increase in relative abundance due to less shading and better competitive abilities of the low profile taxa (Biggs *et al.*, 1998; Passy, 2007b). The addition of sediment may reduce this flow velocity effect because many of the low profile, highly attached species may predominantly live on other filamentous algae and are more resistant to removal by abrasion thus avoiding the worst impacts of fine sediment (Biggs *et al.*, 1998).

Synergisms occurred for colonial species, gliding species, drift motility species and large-sized species. Colonial life forms displayed an interesting interaction where the positive effect of added small sediment was much stronger in mesocosms with medium flow velocity. Drift motility displayed a complex synergistic interaction. This trait category displayed an increase in abundance in fast-flow mesocosms with no added sediment, small sized sediment and medium sized sediment. Gliding species displayed an interaction where the positive effect of added small sized sediment, medium sized sediment, and large sized sediment was much stronger in mesocosms with slow flow velocity than in the other two flow velocity categories. Furthermore, the positive effect of slow flow velocities becomes larger as sediment particle size decreased. All of these traits deal with small-sized sediment and its associated smothering as well as the nutrient stimulatory effect. Smothering (burial) would be an issue that can be avoided by algae possessing these traits and may benefit them with the elimination of many other species through burial (smothering) and thus reduce competition. Other species, which cannot move to a more favourable habitat, may not get enough nutrients, space and light so species that can actively move or can colonise fast would have a competitive advantage. Colonial species may be able to avoid the worst effects through fast colonisation and reproduction as mentioned in the previous sediment addition section. Alternatively, gliding species and drift species can actively move through the sediment in order to access resources and gain from the stimulus and boundary layer effect. This

response in motile species has often been observed where there has been high fine sediment cover (Schneck *et al.*, 2011; Wagenhoff *et al.*, 2013; Piggott *et al.*, 2015b). Drift motility species (for example *Merismopedia* spp.) displayed a similar response pattern as was reported in chapter 5 of Salis (2016) who found a negative response of this trait to flow velocity reduction as well as a negative response to fine sediment addition (defined as particles < 2 mm). For this trait, there was a more complex synergistic interaction in my experiment. With regards to the first part of this interaction, a normal (fast) flow velocity might have the ability to stimulate the metabolism and reduce the boundary layer thickness in a relatively stable environment that many benthic algal species prefer (Horner *et al.*, 1990; Stevenson *et al.*, 1996; Ghosh & Guar, 1998). However, the addition of large- and medium-sized sediment muted this response which suggests, potentially, that scour and abrasion might be limiting abundance even with the stimulatory effect still in place. Alternatively, colonial life forms may benefit from the stimulatory effect without severe abrasion and instability because the current may not be fast enough to move the sediment at medium flow velocity. Gliding species have a different problem. Their low-strength to non-existing attachment, which allows them to move through deposited sediment to avoid burial, also makes these species susceptible to dislodgement by both shear stress and abrasion at faster current velocities. This may make them less able to benefit from a nutrient-stimulus effect of faster flows.

Very large species displayed a marginally significant sediment by velocity interaction. In this, the overall positive effect of flow velocity reduction was absent in mesocosms without added sediment. Without added sediment, very large species are exposed to the same reduced amount of abrasion and instability at all flow velocities. In mesocosms with added fine sediment (regardless of grain size), these very large cells may be subject to higher abrasion and instability under normal flow velocity conditions than at reduced flow velocities.

There were five trait categories (meso-sized species, fission-reproducing species, filamentous species, non-nitrogen fixing species and nitrogen fixing species) which showed interactions that were hard to explain biologically because they were neither clearly antagonistic or synergistic.

Meso-sized species displayed an abundance increase with flow reduction without added sediment, whereas the opposite pattern occurred in mesocosms with added sediment of any size. Potentially, meso-sized species would be more susceptible to drag and shear stress because of their larger cell size; however, the minor influence of this effect might be because many of the species that made up this trait category had medium attachment. Therefore, reduced flow velocity might reduce this shear stress. With the addition of sediment, scouring and burial probably lowered the abundance at all flow velocities, but this decline was less severe at medium-sized sediment and fast velocity. Potentially, the medium-sized sediment did not bury the species at fast velocities, while abrasion might have been less of an issue (slightly more cohesive sediment than sand particles). This, combined with medium attachment and fission reproduction, may mean that this trait could increase so long as there was current stimulus effect.

Fission-reproducing species displayed an increase in abundance in slow-velocity mesocosms with no added fine sediment. Filamentous species displayed a higher abundance in control and large-sized sediment mesocosms, except at slow flow current velocity. Without medium and small fine sediment, the environment for benthic algae is relatively stable. Nutrient limitation is prevalent in this condition, for different reasons and with different effects for fission species and filamentous species. Under conditions of flow velocity reduction, the current stimulus decreases which induces low nutrient uptake rates. This can lead to competition with other species for those nutrients and the potential build-up of the periphyton matrix leads to nutrient limitation within the matrix (especially for low-profile species) (Biggs *et al.*, 1998; Passy, 2007b). In this environment, fission-reproducing species can disperse to avoid this nutrient limitation or can compensate if they are very small (higher surface area to volume ratio). Filamentous species (which reproduce using fragmentation) remain relatively abundant. This suggests that filamentous species can possibly outcompete and shade these fission reproducing species. Alternatively, filamentous species can potentially escape the nutrient limited environment using their long filaments. The latter is probable since filamentous species also tend to float on top of the substratum above the other diatoms (Biggs *et al.*, 1998; Lange *et*

al., 2016). At normal flow velocity (no reduction), filamentous species can take advantage of the increased metabolic and nutrient uptake rates associated with faster current velocities, without the additional abrasion. With flow velocity reduction, this effect may be lost and therefore the slower reproducing species declines.

The addition of fine sediment led to an increase in abundance for fission-reproducing algae, except in slow-velocity mesocosms. Filamentous species displayed a decrease in abundance with added medium- and small-sized sediment, although this appeared to be marginally ameliorated under full flow velocity reduction. Medium and small-sized fine sediment addition brings extra challenges, such as abrasion by medium-sized sediment and smothering by small-sized sediment, as well as instability from both. This is potentially demonstrated by fission, fragmentation and filamentous traits, in which with or without sediment, the relative abundance of slow-flow mesocosms remained relatively stable. At fast and moderate flow velocities, abrasion and instability would be enhanced. Fission-reproducing species (as discussed in previous sections) may be able to recolonise disturbed sediment particles faster because theirs is a quicker form of reproduction that is adapted to an unstable and disturbed environment. The exception for fission-reproducing species is the addition of medium sized sediment slow flow velocity where this beneficial effect is ameliorated. This is probably because at medium sediment size and slow flow the substratum is very stable and favours the establishment of filamentous species. Filamentous species may take longer to recover from sediment addition because of their slower reproduction (using fragmentation) and colonization. Once established again (at a lower biomass), filamentous species may only reach a significant biomass (lower than in mesocosms without added sediment) at full flow velocity reduction, perhaps because of decreased sediment instability and abrasion. At faster flow velocities, nutrient uptake remains somewhat similar but instability and abrasion might affect the filamentous connection with the substratum. Additionally, the slower reproduction and colonisation cycle may mean that constant sediment instability and abrasion could lead to lower biomass at these faster velocities.

Non-nitrogen fixing species displayed a decrease in abundance in slow-velocity mesocosms with all added sediment sizes. Simultaneously, they increased in abundance in mesocosms with slow flow velocity and no added sediment. Nitrogen-fixing species increased in abundance in slow-velocity mesocosms with all added sediment sizes. Simultaneously, they decreased in mesocosms with slow flow velocity and no added sediment. Sediment addition may bury species while flow velocity reduction both eliminates the nutrient stimulus effect and increases sediment deposition. These results may produce an environment in which nutrients are a major limiting factor and those species that can fix nitrogen thrive and outcompete non-nitrogen fixing species (Biggs *et al.*, 1998), resulting in the decline of the latter trait category. The increase in non-nitrogen fixing species in conditions of slow velocity and no added sediment was probably due to a reduction in competition from nitrogen-fixing species.

Nitrogen fixers, while performing well in nutrient-limited environments, also need light which is another resource potentially in short supply, especially when there is attenuation by a thick periphyton matrix (Bhaya *et al.*, 2000). Furthermore, the ability to fix nitrogen is costly (in energy). Therefore, non-nitrogen fixers may have an advantage in not having to expend additional energy for an ability that is not useful in this situation. Nitrogen-fixing species are able to produce nitrogen and can therefore ameliorate nutrient limitation and outcompete non-nitrogen fixing species. In slow flow and no sediment conditions, however, the nitrogen-fixing ability may not be helpful due to limited light and therefore represent an unhelpful, energy-costly appendage. This may mean as described above that non-nitrogen fixing species may be able to outcompete nitrogen-fixing species under such conditions.

Chapter Four:

4 General Discussion

4.1 Stressor responses

In this thesis, I explore the individual and combined effects of fine sediment particle size and flow velocity reduction (to simulate one consequence of water abstraction) on benthic algae communities and biological traits. Chapter 2 investigates the effects of these stressors on algal community indices and population abundance. It demonstrated that the majority of significant responses, especially taxonomic responses, to fine sediment addition was largely positive (17 significant responses out of 19 response variables), 13 positive significant responses (with one response variable which was overridden by an interaction). Of these positive responses, the two prevalent responses were related to all three fine sediment particle sizes or to the smallest added sediment size (7 of 12 responses). There were two negative significant responses. Chapter 2 also demonstrated the less common responses to flow velocity reduction, with seven of 19 algal variables responding significantly (with four of these responses being overridden by interactions). The three species (that were not overridden) responded negatively to flow reduction, especially full flow reduction. Furthermore, algal community metrics showed proportionally fewer responses to both stressors than the population abundance metrics (Table 4.1). There were 11 interactions which were very interesting for two reasons. Firstly, the interactions overrode many of the stressor main effects (because they were stronger), especially for flow velocity reduction. Secondly, the interactions were all synergistic (i.e the combined effect of both stressors was greater than the sum of the individual effects involved), and this has important implications for management.

Table 4-1: Number and frequency (in % of all possible cases of significant effects of the investigated stressors (sediment particle size and flow reduction) on algal community indices and common taxa and on algal biological traits.

Study	Number of responses	Sediment addition treatments	Flow velocity reduction	Interactions
Chapter 2: Algal community indices and common taxa (19 response variables in total)	37 significant responses out of 57 possible responses	17 significant responses of 19 possible responses (89 %) 1 borderline (positive) 1 overridden 2 negative 13 positive	9 significant responses of 19 possible responses (47 %) 6 overridden 3 negative	11 significant responses of 19 possible responses (58 %) 1 borderline (antagonism) All synergistic
Community metrics	8 significant responses out of 12 possible responses	3 significant responses out of 4 possible responses (75%) 1 overridden	2 significant responses out of 4 possible responses (50%) 2 overridden	3 significant responses out of 4 possible responses (75%)
Common taxa (population abundance)	29 significant responses out of 45 possible responses	14 significant responses out of 15 possible responses (93%) 1 borderline (positive)	7 significant responses out of 15 possible responses (47%) 4 overridden	8 significant responses out of 15 possible responses (53%) 1 borderline (antagonism)
Chapter 3: Algal biological traits (25 response variables in total)	40 significant responses (algal traits) out of 75 possible responses.	19 significant responses out of 25 possible responses (76 %) 3 overridden 1 borderline (positive) 8 negative 7 positive	9 significant responses out of 25 possible responses (36 %) 7 overridden 2 negative	12 significant responses out of 25 possible responses (48 %) 4 synergistic 3 antagonistic 5 complex

In Chapter 3, which investigated algal biological traits, there is a more complicated story. For fine sediment addition (with different particle sizes), there were seven significant positive responses and eight negative responses (of 25 trait response variables in total). A further five significant responses were varied and response shapes could not be generalized. Of the positive responses, five were to small-sized sediment, plus two positive responses to medium-sized sediment). Of the eight negative responses, four were to medium- or small sized sediment. Chapter 3 shows significant responses of flow velocity reduction were less common (9 of 25 trait variables), with only two responses occurring which both favoured full flow velocity reduction. There were 12 interactions which were very interesting. The interactions were both synergistic (i.e. the combined effect of both stressors was greater than the sum of the two individual stressor effects involved) and antagonistic (i.e. the combined effect was smaller than the sum of the individual effects). Furthermore, some traits displayed both these interaction types depending of the particle size of the added fine sediment. All these responses have important implications for management (discussed later in this section). When combined, the findings from both Chapters 2 & 3 suggest that most algal species and biological traits responded to the addition of fine sediment with different particle sizes in a predominantly positive way.

While the spatial and temporal limits of this experiment do restrict the applicability of the knowledge gained, this experiment has provided a good insight into the effects of sediment particle size and flow reduction, as well as their interactions. In general, added fine sediment was a very pervasive stressor, with frequent significant effects on benthic algae. These included predominantly positive effects on community metrics and population abundances and a mixture of positive and negative effects on algal traits. The predominant individual effect of fine sediment of different particle sizes was the opposite of what was predicted. Some previous studies had hinted that many of the responses should be related to large sediment sizes and predominantly negative (Delgado *et al.*, 1991; Francoeur & Biggs, 2006; Jewson *et al.*, 2006; Jones *et al.*, 2014). However, my experiment suggests that the opposite may be true, especially in the case of algal community indices and population abundance. The overwhelmingly positive response to small-sized fine sediment in

my study suggests that species (many of which were among the 15 most common taxa) have traits that lead to a faster reproduction rate (e.g. small size, high nutrient uptake efficiency). Furthermore, these species possibly also benefit from lower competition with larger sized algal species and filamentous species. Whether the predominantly positive significant effect of small-sized fine sediment on a majority of these 15 species is a sign of good stream health is another matter because the proliferation of certain species (*Encyonema minuta* and *Nitzschia palea*; Biggs & Kilroy, 2000) can indicate a highly impacted stream.

The few negative responses highlighted the variety of mechanisms that fine sediment acted upon. In the community chapter these came from *Phormidium* spp. and *Gomphonema parvulum*, while the trait chapter highlighted some of the processes through the negative responses of filamentous life form, fragmentation reproduction, meso-sized, medium attachment species, attachment motility, Zygospore production, and Zoospore production. The bed instability present with the additional sediment is unfavourable to filamentous species which reproduces using fragmentation which leads to a slower colonisation rate. Similarly, this may be the case for Zygospore production and Zoospore production which is associated with fragmentation reproduction. This is demonstrated by *Phormidium* spp. Meso-sized species and attachment motility emphasized the role of burial as species with this trait cannot move to avoid unfavourable conditions. Medium attached species emphasized the role of abrasion of sand-sized sediment (1-2 mm treatment) on larger-sized cells while small-sized particles probably acted through smothering and reducing light levels in the biofilm. Whether the response is positive or negative, it is most likely that any facilitation operated along the sediment particle size gradient rather than the flow velocity reduction gradient (see chapter 2). For example, certain algal species that prefer unstable environments may initially form a biofilm which stabilises the fine sediment and subsequently allows other algal species which prefer some stability to develop.

Flow velocity reduction was also an influential stressor for benthic algae, especially when it interacted with fine sediment particle size. While the flow velocity reduction and sediment size interaction was no surprise, there were many main effects from

flow velocity reduction. In this experiment, flow velocity reduction main effects occurred in benthic algal communities at a similar percentage as other stressors studied in previous experiments (Piggott *et al.*, 2015b; see chapter 5 in Salis, 2016). Previous research has suggested that the flow regime (and flow velocity) is an important driver in stream ecosystems, especially in determining the physical habitat in the stream (Poff *et al.*, 1997; Bunn & Arthington, 2002). Typically, the flow regime acts through physical force and shear stress, especially at higher velocities. There is a lot of literature which suggests that there is a threshold at which shear stress becomes the major factor influencing algal communities. At this stage the effects of algal removal from the substrate would outweigh the still present beneficial stimulatory effect. This shear stress threshold is probably met in this experiment, because a previous experiment reported the operation of shear stress and the subsequent dislodgement of algae from the substrate within a similar range of current velocities (Passy, 2007b; Piqué *et al.*, 2016). Therefore, the fast (35 cm/s) and moderate (17 cm/s) current velocities would have caused algal dislodgement while the slow (0 cm/s) current velocity is unlikely to meet this threshold and therefore should have little algal removal through shear stress (but may act through a reduction in the nutrient stimulator effect, autogenic sloughing and enhanced deposition). These current velocity treatments were realistic for small farmland streams in New Zealand (Matthaei *et al.*, 2010), and there is some evidence to suggest that this ‘shear stress threshold’ is slightly different for each species and trait category (Peterson *et al.*, 1990; Peterson & Stevenson, 1992; Peterson, 1996). Overall, shear stress is probably a major force acting in my experiment and may disproportionately affect larger algal cells, especially at the fastest current velocity. Faster flow velocity (from 15 cm/s to 60 cm/s, Passy, 2001; Whitford & Schumacher, 1964) can also have a stimulatory effect by reducing the boundary layer thickness and thus increasing the availability of nutrients and oxygen (Passy, 2001; Whitford & Schumacher, 1964; Horner *et al.*, 1990; Hurd & Stevens, 1997; Eriksson, 2001). Conversely, flow velocity reduction acts through boundary layer effects that reduce the nutrient and oxygen uptake rates. In my experiment, this stimulatory effect (or lack thereof) could play a large role in influencing population abundance, community indices and algal traits. However, the impact of the stimulatory effect may vary depending on the species or trait characteristic.

While sediment particle size and flow velocity reduction present direct forms of stress through main effects and interactions, there are also indirect ways in which these stressors can affect algal communities and traits. Herbivory by grazing invertebrates and competition among algal species were probably involved in creating some of the responses observed. Herbivory could play a role in many algal responses, through the release of grazing pressure on algal abundances. Competition among algal species for resources and space may have played a role in both single-stressor responses and stressor interactions. This may be the case for *N. palea*, *Merismopedia* spp, high attachment, non-nitrogen fixing, and nitrogen-fixing species. *N. palea* and *Merismopedia* spp may benefit from a reduction in competition when other species are smothered. High attachment species (low profile guild) face competition (for light and resources) from high profile species, especially when there is normal flow velocity and no added fine sediment. Fine sediment addition and flow velocity reduction increases sediment deposition. This situation may produce an environment in which nutrients are a major limiting factor and those species that can fix nitrogen thrive and outcompete non-nitrogen fixing species (Biggs *et al.*, 1998).

While these responses are interesting, it is appropriate to note the limitations of this study. These were especially prominent at the temporal scale because there was only one sampling event at the end of the experiment. This means that successional changes in algal biomass, community structure and trait abundance in response to the manipulated stressors were not documented.

4.2 Stressor interactions

In the two data chapters (Chapter 2 & 3) of this thesis, I investigated the interactive effects of added fine sediment with different particle sizes and flow velocity reduction. There were three main findings. Firstly, there were slightly less significant interactions found for the 25 algal biological traits than for the four community-level metrics and the population abundance of the 15 most common taxa (Table 4.1). This consisted of a 10 % difference between the community-level

metrics and common taxa abundance results and the biological trait results. Secondly, many interactions overrode the main factor effects. In both my data chapters, a considerable number of interactions overrode the corresponding stressor main effect(s), especially for flow velocity reduction main effects (Table 4.1). This suggests that the interaction was often more important than the main effect and that the two stressors were acting in strong non-additive fashion. Given the literature this is not surprising. It is well known that current velocity influences which sediment particles sizes will be entrained, transported or deposited. Similarly, it is known that sediment particle size, in turn, can influence current velocity through friction and turbulence (often associated with grain roughness) (Wood & Armitage, 1997; Yamada & Nakamura, 2002; see chapter 5 in Brierley & Fryirs, 2012).

Thirdly, the majority of significant stressor interactions were synergistic. The interactions observed for algal community metrics and population abundances (Chapter 2) were all synergistic while algal trait interactions (Chapter 3) were both synergistic and antagonistic. The overall predominance of synergisms in my experiment was surprising considering that antagonisms are thought to be prevalent in multiple-stressor interactions (Crain *et al.*, 2008; Darling & Côté, 2008; Piggott *et al.*, 2015c). Furthermore, Piggott *et al.*, 2015c suggested that synergy may be the exception rather than the rule in two-way stressor interactions. None of these previous studies used the Lange *et al.*, (2016) algal trait framework or investigated sediment particle size and current velocity, but Piggott *et al.*, (2015b) did investigate algal community metrics and common taxa with fine sediment as one of three stressors. Given that my community metrics and common algal taxa all displayed synergistic interactions (when an interaction was present), therefore my study can suggest that the antagonistic trend may not be as prevalent as previously thought. One explanation for this difference could be that the nature of the sediment particle size and current flow velocity combination may produce more synergistic interactions than the combination of other stressors. The prevalence of synergisms is concerning in unrestored multiple-stressor situations, because there may be an amplified negative effect on biota. On the other hand, a primarily synergistic response may be beneficial if a restoration were to take place because the reduction

of a master stressor may lead to a more favourable outcome than expected (Crain *et al.*, 2008; Piggott *et al.*, 2015c). There were also a minority of antagonistic responses, which may present a different challenge. The antagonistic responses present are concerning for restoration management because the reduction in a stressor may not lead to a proportional recovery, unless it is the more pervasive stressor that is reduced (Crain *et al.*, 2008; Piggott *et al.*, 2015c). Additionally, some traits displayed both responses depending on the addition of any fine sediment or a particular sediment grain size.

4.3 Management implications

The objective of the management and monitoring of streams is to restore them to a healthy state and the diversity and stability benthic algal communities is important in this process. The management implications of my findings are similar to those of previous studies that have investigated fine sediment effects on stream algal communities and its interactions with other stressors (Piggott *et al.*, 2015b; see chapter 7 in Salis, 2016; Lange *et al.*, 2016). The conclusion of all these studies was that fine sediment was a very pervasive stressor, having the most frequent stressor main effects and many interactions, and that consequently efforts should be made to limit fine sediment inputs to the stream environment. This is because of repeated pervasiveness of fine sediment impacts that have been demonstrated in many different studies. Despite my study having similar spatial and temporal limitations to most other mesocosm studies, some management implications can be deduced. My study highlights the complexity of stressors interactions and confirms the pervasiveness of effects caused by very small sized clay and silt particles (the smallest sediment added in my experiment) that has been speculated about in some previous studies (e.g. Graham, 1990; Wood & Armitage, 1997; Jones *et al.*, 2014).

While small sand and clay particles had the most pervasive effects (16 of the 45 significant responses to my sediment manipulations; Table 4.1), there are few sediment-size-specific actions that can be undertaken to keep clays from entering streams. Firstly, it is hard to separate clays from sand particles in the field. Other than through changes in land management, there are no known technologies to prevent clay particles (or fine sediment particles of any other size) from entering

streams. Secondly, even if such a technology was developed, it would probably be too costly to deploy. Therefore, the most realistic way would be through land management practices (Foote *et al.*, 2015). Channel source and catchment erosion is very hard to control completely but steps can be taken to minimise the amount of sediment entering a waterway (Williamson *et al.*, 1992; Wood & Armitage, 1997; Foote *et al.*, 2015). Firstly, stock can be removed from within a stream and also from adjacent land near a stream. Secondly, the establishment of riparian buffers has been shown to be effective in reducing in-stream sediment (Blanco-Canqui *et al.*, 2004; Muenz *et al.*, 2006). One study (Albert *et al.*, 1981) found that riparian buffer strips were more effective in reducing the amount of coarser sediment (approx 0.200-2 mm) entering waterways and concurrently, less effective in reducing finer sediment (approx. 0.063-0.200 mm) transportation (Barling & Moore, 1994). These fine particles often remain in the surface runoff water and can therefore move into the stream unimpeded (Barling & Moore, 1994; Collins *et al.*, 2013). Farm plans can be used to significantly reduce the amount of fine sediment entering rivers. For example, Schierlitz, Dymond & Shepherd (2006) suggested that in the Manawatu region, 500 farm plans in the highest-priority farms (10% of intensive farms within sensitive catchments) could lead to an approximate reduction of mean sediment discharge by 50% into the Manawatu River in the North Island of New Zealand. These measures, including an Integrated Catchment plan, can reduce sediment transport into the stream and should provide the best restoration outcomes in the long term (Hughes & Quinn, 2014).

While fine sediment should remain the primary focus for freshwater resource managers, high levels of water abstraction should also be accounted for where this is a problem. Many running-water species can withstand some flow reduction but not the severe flow reduction associated with high- intensity water abstraction (especially in dry regions) (Dewson *et al.*, 2007; Matthaei *et al.*, 2010; Lange *et al.*, 2016). Furthermore, water abstraction is a stressor that can be managed in an easier way through water consents, bylaws and water pricing. A factor that should be taken to account in any flow management regime is the fact that benthic algae tend to adapt to a particular flow regime, especially in terms of community composition, community matrix structure and intra-specific physical structure (Keithan & Lowe,

1985; Ghosh & Gaur, 1998; Neif *et al.*, 2017). For example, thick, cohesive cyanobacterial mats were more resistant to flow velocity increases than diatom communities (Peterson *et al.*, 1994) and may therefore take longer to respond to current velocity changes (lag time in response). This means that flow regulation should take into account that there may be a short lag period (of about 1 week Neif *et al.*, 2017) between flow regulation and benthic algal community change. This would be more prevalent in the case of changing from a fast-velocity algal community structure to a slow-velocity structure. Fast-velocity community structures tend to be prostrate, pioneer, small, fast colonisers and have high growth rates while at slower current velocities algal cells tend to grow upright and favour large and motile species (Keithan & Lowe, 1985; Lamb & Lowe, 1987). Therefore, a significant increase in flow velocity might considerably change algal communities in streams currently subjected to high water abstraction rates and which may suffer from desiccation.

Many previous reviews and studies in running waters have recommended that care should be taken when implementing management strategies in situations where multiple stressors operate simultaneously because these stressors often interact (e.g. Matthaei *et al.*, 2010; Brown *et al.*, 2013; Magbanua *et al.*, 2013a; Piggott *et al.*, 2015a; Piggott *et al.*, 2015b; Piggott *et al.*, 2015c; Piggott *et al.*, 2015d; see chapter 7 in Salis, 2016; Lange *et al.*, 2016; Neif *et al.*, 2017). I put forward the same recommendation in this thesis and suggest that, in areas where water abstraction is a contentious issue, it may be sensible for managers to prioritize reducing one stressor initially (probably fine sediment). Afterward, repeated monitoring of algal community changes should take place to detect potential changes in community composition, before considering reducing water abstraction.

4.4 Algal trait framework

I had hypothesized that the Lange *et al.*, (2016) algal trait framework would be a more effective way, compared to community indices and population abundance, to infer mechanisms through which fine sediment size and flow velocity reduction affect benthic algal communities in streams. If this was the case, this framework would be a significant improvement on algal community indices in trying to

understand the mechanisms behind the responses. The results of my experiment were useful in the sense that they contrasted with the findings of related previous studies. Therefore, I could propose some new ideas for responses to sediment particle size, flow reduction and their interactions.

Overall, my hypothesis was partially supported and did provide insights into some the mechanisms operating in the system. There was a relatively clear association between added fine sediment with the smallest grain size and the responses of a certain set of algal biological traits. For example, traits associated with a faster reproduction rate (a useful characteristic in unstable situations) benefited from small sediment addition while gliding (motile) species had their response to fine sediment overridden by the interaction with flow velocity. Furthermore, those species that had a slower reproduction rate tended to respond negatively to small sediment addition. This also suggests that when added by itself, small sediment typically acted through abrasion and instability, rather than through burial. This distinct pattern was also discerned for flow velocity reduction for the only two non-overridden main effects in the cell size trait. Both macro and very large-sized cells benefited from full flow velocity reduction. This response pattern suggests that even though larger-sized cells are less effective at sequestering nutrients than small-sized cells (and thus would benefit from the faster current and nutrient stimulus), the negative effect of increased shear stress and abrasion may override this. Furthermore, it provides some evidence that flow velocity may have acted through shear stress. The interaction of the two stressors often changed the degree to which the mechanisms appeared to operate. For example, in filamentous species, the prominent negative effect of sediment addition of any size, which probably acts through sediment instability, was ameliorated by full flow reduction (slow flow). Often burial and the nutrient stimulatory effect (or lack of) became more prominent as demonstrated by non-spore producing species which responded in a more pronounced positive manner to sediment instability (at high and medium flow velocities) but seemed to weaken with full flow reduction (when burial became a significant mechanism). The trait framework also highlighted how interactions and main effects could influence biological processes present, especially competition between algae for nutrients and light. For example, competition between non-

nitrogen fixing and nitrogen-fixing species might be mediated by burial and the nutrient stimulus effect which affects access to light and nutrients. Another potentially interesting insight of my study was that the algal traits that benefited from fine sediment addition (and often its interaction with medium and fast flow velocities) could be described as traits that are favourable in a precarious environment. Often the life history (of species such as *E. minuta*, *Merismopedia* spp., *N. palea*, *F. vaucheriae* and *A. minutissimum*) was of rapid population turnover, small size and lower energy investment, which favours the unstable environment created by current land management practices. This could, over time, make stream ecosystems (while still modified) more robust to further human modification (such as urbanization or land use changes).

There were, however, also some problems with using Lange *et al.*'s 2016 trait framework in my study, such as trait interdependency and seasonal variation. Teasing apart different traits and inferring mechanisms was sometimes difficult because there was a lack of trait independence, which is the result of various trade-offs. There is considerable overlap in the trait framework with some of the traits being linked and displaying similar responses. This interdependence has been noted by Litchman *et al.*, (2007), Litchman & Klausmeier (2008), and also by Lange *et al.*, (2016) themselves. Therefore, in some cases it can be hard to tell whether it is the trait in question causing the response or simply another trait which all species in a given dataset have. For example, in my dataset the two traits 'no spore production' and 'fission' seemed to always occur in the same species, and the same was true for 'fragmentation reproduction' and 'filamentous life form'. This means it is hard in these cases to infer which selective pressure related to sediment particle size or flow velocity reduction the trait was responding to, especially since many traits have been rarely investigated in the literature. The result, as noted above, is that the number of potential strategies is limited by different traits and some strategies are impossible to create with certain trait combinations (Litchman & Klausmeier, 2008).

Another issue that has been noted for both trait- and population-based approaches in the context of studying stream benthic algal communities is the effect of season. In a study based on two stream surveys conducted in spring and autumn (see

chapter 3 in Salis, 2016), season had a considerable influence on the algal traits and community present and how stressors influenced traits and populations of common taxa, as well as stressor interactions for these response variables. For example, during an autumn survey the traits nano-sized cells, micro-sized cells, low attachment, drift motility, fission reproducing algae, colonial algae, unicellular algae, and non-spore producing algae had greater relative abundances while in spring very large-sized cells, medium attachment, non-motile, fragmentation-reproducing algae, filamentous algae, akinete-producing algae, and zygosporo- producing algae had greater relative abundances (see chapter 3 in Salis, 2016). Additionally, some relationships with stressors changed depending on season, for instance for life form and reproduction techniques, which were related to sediment and nutrients in autumn but not in spring (see chapter 3 in Salis, 2016). Seasonal differences in responses were also displayed in many community-level metrics and the populations of common algal taxa. However, in chapter 7 of Salis (2016) it was noted that, while the trait framework was not more robust than taxon populations to seasonal changes, it did show complex multiple-stressor patterns, suggesting that it may be better for detecting multiple-stressor effects.

The novelty of the trait framework by Lange *et al.*, (2016), along with the added novelty of investigating effects of different fine sediment sizes, means that it is hard to relate many of my findings to previous work. So far just two stream surveys (Lange *et al.*, 2016; see chapter 3 in Salis, 2016) and two mesocosm experiments (see chapters 4 & 5 in Salis, 2016) have been done using this framework, and none of them investigated sediment particle size. Therefore, the explanations given here are possibilities rather than conclusive statements. The two previous experiments in chapters 4 & 5 of Salis (2016) provided some similar results and insights regarding the effects of fine sediment (defined more broadly as particles < 2 mm) as well as the effect of flow velocity reduction. However, there were differences in the range of stressors used between these two previous experiments and my own. Therefore, while some broader comparisons can be made (as I have done in my Chapter 3), these differences between these studies should be kept in mind.

Finally, the applicability of the algal trait framework is probably limited to the academic setting because of the time and costs required to identify the species and then assign the various biological traits. This sort of time and cost is usually infeasible and unnecessary for regional councils and other management-oriented institutions. Therefore, while useful in academic research, the trait framework is probably not a replacement for community indices or common taxa approaches.

4.5 Future research directions

The future research directions can be divided into more specific trait research and more general recommendations regarding further experiments investigating effects of fine sediment particle size and flow velocity reduction. My recommendations for future trait research include the expansion of the trait framework to other stressors and possibly research into the relationships and links between traits. My general recommendations include extending the experimental current velocity range (to include even faster velocities), and also studying the effect of sediment particle size on herbivory rates and grazing preferences.

My experiment has revealed several areas of future enquiry, especially with regards to the Lange *et al.*, (2016) algal trait framework. As an extension of this research, future research could focus on temperature (as previously explored by Piggott *et al.*, 2015b), herbicides and pesticides (as previously explored by Magbanua *et al.*, 2013a).

Further research should also be done into the relationship between algal traits and the potential significance these inter-dependent traits might have on interpreting the mechanisms by which stressors act. My study is not the first to note that many of the traits are linked and are not independent. Lange *et al.*, (2016) noted this in their survey study; however, very little has been done since to investigate this issue. Therefore, it would be advisable to gain a better insight into these relationships in order to refine the trait framework and understand the implications before further adoption.

General recommendations can also be made from this research. Chapter 2 highlighted an important general recommendation that involved extending the range of current velocities investigated. This could provide insights into both the effect of faster current velocities and the threshold level where algal removal rates negate and outstrip biomass growth from the nutrient stimulus effect. Furthermore, this could be related to the trait framework and the effects of flash flooding in water abstracted systems.

My final recommendation for future research includes a potential investigation into the effects of fine sediment particle size on herbivory rates and grazing habits. Fine sediment may clog invertebrate gills, smother habitat (especially clay particles) and provide additional nutrients to certain grazers (Broekhuizen *et al.*, 2001; Jones *et al.*, 2012; Edwards & Shroufe, 2016). Furthermore, suspended sand may damage body parts and change food availability through abrasion and behavioural changes (Kurtak, 1978; Culp *et al.*, 1986; Jones *et al.*, 2012). For example, the caddisfly larvae *Brachycentrus* feeds by filtering organisms from the flowing water column under normal conditions but graze on periphyton during high suspended sediment loads (Gallepp, 1974; Voelz & Ward, 1992; Jones *et al.*, 2012). This switch is thought to be either the result of particle abrasion or food availability /quality, which are the result of added fine sediment (Jones *et al.*, 2012). Fine sediment may influence the composition, density and distribution of the grazer community, which would then influence periphyton composition and density. Furthermore, grazing effects could be integrated into the Lange *et al.*, (2016) trait framework for both invertebrates and periphyton.

Appendix

Table 1A: List of all algal taxa that occurred in the mesocosms, along with their assigned traits.

Taxon		(1) Cell size	(2) Life form	(3) Attachment	(4) N fix	(5) Motility	(6) Reproduction	(7) Spores
<i>Achnanthidium spp.</i> <i>(minutissimum)</i>	Bacillariophyceae	c1	unicellular	high	0	attached	Fiss	none
<i>Achnanthes oblongella</i>	Bacillariophyceae	?	unicellular	low	0	drift	Fiss	none
<i>Geissleria</i>	Bacillariophyceae	?	unicellular	?	0	?	Fiss	none
<i>Eunophora sp</i>	Bacillariophyceae	?	unicellular	low	0	?	Frag	none
<i>Zygnema</i>	Zygnematophyceae	?	filamentous	medium	0	?	Frag	ZyS
<i>Chroodactylon</i>	Stylonematophycea e	?	filamentous	medium	0	?	Frag	Mono spore
<i>Amphora ovalis</i>	Bacillariophyceae	?	unicellular	low	0	attached	Fiss	none
<i>Actinella</i>	Bacillariophyceae	?	unicellular	medium	0	attached	Fiss	?
<i>Pseudostaurosira brevistriata</i>	Bacillariophyceae	?	unicellular	medium	0	attached	Fiss	none
<i>Encyonema caespitosum</i>	Bacillariophyceae	?	unicellular	low	0	drift	Fiss	none
<i>Cavinula</i>	Bacillariophyceae	?	unicellular	low	0	drift	Fiss	none
<i>Stenopterobia curvula</i>	Bacillariophyceae	?	unicellular	low	0	drift	Fiss	none
<i>Stenopterobia delicatissima</i>	Bacillariophyceae	?	unicellular	low	0	drift	Fiss	none
<i>Navicula margalithi</i>	Bacillariophyceae	?	unicellular	low	0	gliding	Fiss	none
<i>Rhopalodia operculata</i>	Bacillariophyceae	?	unicellular	low	1	attached	Fiss	none
<i>Navicula rhynchocephala</i>	Bacillariophyceae	?	unicellular	low	0	gliding	Fiss	none
<i>Batrachospermum</i>	Bacillariophyceae	?	filamentous	medium	0	attached	Frag	Mono spore
<i>Crucigenia</i>	Chlorophyceae	C1	colonial	low	0	?	Frag	Auto spore
<i>Calothrix</i>	Cyanophyceae	C1	filamentous	?	1	attached	Frag	Akinete s
<i>Klebsoridium</i>	Chlorophyceae	C1	filamentous	medium	0	attached	Frag	ZooS
<i>Nitzschia inconspicua</i>	Bacillariophyceae	C2	unicellular	low	0	gliding	Fission	none
<i>Diatomella balfouriana</i>	Bacillariophyceae	C1	unicellular	low	0	drift	Fission	none
<i>Achnanthidium linearis</i>	Bacillariophyceae	C2	unicellular	high	0	attached	Fiss	none
<i>Staurosirella</i>	Fragilarophyceae	C2	unicellular	medium	0	attached	Fiss	none
<i>Brachysira vitrea</i>	Bacillariophyceae	C2	unicellular	low	0	drift	Fiss	none
<i>Neidium affine</i>	Bacillariophyceae	C2	unicellular	low	0	drift	Fiss	none
<i>Diploneis elliptica</i>	Bacillariophyceae	C2	unicellular	low	0	drift	Fiss	none
<i>Mougeotia</i>	Chlorophyceae	c5	filamentous	low	0	drift	Frag	OoS ZyS
<i>Tetrastrum</i>	Chlorophyceae	C1	colonial	low	0	drift	Fiss	ZoS
<i>Cylindrospermum</i>	Cyanobacteria	C2	filamentous	low	1	drift	Frag	Akinete s

Table 1A Continued

TAXON		(1) Cell size	(2) Life form	(3) Attachment	(4) N fix	(5) Motility	(6) Reproduction	(7) Spores
<i>Coleodesmium</i> sp.	Cyanophyceae	c2	filamentous	medium	1	attached	Frag	none
<i>Achnanthidium minutissimum</i>	Bacillariophycea e	c1	unicellular	high	0	attached	Fiss	none
<i>Epithema adnata</i>	Bacillariophycea e	c5	unicellular	low	1	attached	Fiss	none
<i>Hantzschia amphioxis</i>	Bacillariophycea e	c4	unicellular	low	0	gliding	Fiss	none
<i>Melosira varians</i>	Bacillariophycea e	c5	filamentous	low	0	drift	Fiss	none
<i>Nitzschia cf intermedia</i>	Bacillariophycea e	c5	unicellular	low	0	gliding	Fiss	none
<i>Pinnularia cf gibba</i>	Bacillariophycea e	c5	unicellular	low	0	gliding	Fiss	none
<i>Planothidium lanceolatum</i>	Bacillariophycea e	C2	unicellular	high	1	attached	Fiss	none
<i>Tolyphothrix</i>	Cyanobacteria	?	filamentous	?	0	attached	Frag	Akinete s
<i>Stauroneis</i> sp.	Bacillariophycea e	c2	unicellular	low	0	gliding	Fiss	none
<i>Surirella angusta</i>	Bacillariophycea e	c4	unicellular	low	0	gliding	Fiss	none
<i>Cocconeis placentula</i>	Bacillariophycea e	c4	unicellular	high	0	attached	Fiss	none
<i>Fragilariaformia</i> sp. (<i>viriscens</i>)	Bacillariophycea e	c2	colonial	high	0	attached	Fiss	none
<i>Planothidium</i> spp. (<i>lanceolatum</i>)	Bacillariophycea e	c2	unicellular	high	0	attached	Fiss	none
<i>Reimeria sinuata</i>	Bacillariophycea e	c2	unicellular	high	0	attached	Fiss	none
<i>Rossithidium</i> spp.	Bacillariophycea e	c2	unicellular	high	0	attached	Fiss	none
<i>Achnanthes inflata</i>	Bacillariophycea e	c4	unicellular	low	0	drift	Fiss	none
<i>Cyclotella</i> spp. (<i>meneghiniana</i>)	Bacillariophycea e	c4	unicellular	low	0	drift	Fiss	none
<i>Diatoma hiemale</i> (var. <i>mesodon</i>)	Bacillariophycea e	c4	colonial	low	0	attached	Fiss	none
<i>Diatoma tenuis</i>	Bacillariophycea e	c2	colonial	low	0	attached	Fiss	none
<i>Diatomella parva</i>	Bacillariophycea e	c1	unicellular	low	0	drift	Fiss	none
<i>Epithemia adnata</i>	Bacillariophycea e	c5	unicellular	low	1	attached	Fiss	none
<i>Epithemia sorex</i>	Bacillariophycea e	c4	unicellular	low	1	attached	Fiss	none
<i>Eunotia</i> spp.	Bacillariophycea e	c2	unicellular	low	0	attached	Fiss	none
<i>Fragilaria capucina</i>	Bacillariophycea e	c1	unicellular	low	0	attached	Fiss	none
<i>Fragilaria ungeriana</i>	Bacillariophycea e	c2	unicellular	low	0	attached	Fiss	none
<i>Fragilaria vaucheriae</i>	Bacillariophycea e	c2	colonial	low	0	attached	Fiss	none
<i>Frustulia rhombooides</i> var. <i>crassinervia</i>	Bacillariophycea e	c5	unicellular	low	0	gliding	Fiss	none
<i>Frustulia</i> spp.	Bacillariophycea e	c5	unicellular	low	0	gliding	Fiss	none
<i>Frustulia vulgaris</i>	Bacillariophycea e	c5	unicellular	low	0	gliding	Fiss	none
<i>Gyrosigma</i> sp. (<i>spencerii</i>)	Bacillariophycea e	c4	unicellular	low	0	gliding	Fiss	none

Table 1A Continued

TAXON		(1) Cell size	(2) Life form	(3) Attachment	(4) N fix	(5) Motility	(6) Reproduction	(7) Spores
<i>Hantzschia amphioxys</i>	Bacillariophycea e	c4	unicellular	low	0	gliding	Fiss	none
<i>Karayeria</i> sp.	Bacillariophycea e	c2	unicellular	low	0	gliding	Fiss	none
<i>Melosira varians</i>	Bacillariophycea e	c5	filamentous	low	0	drift	Fiss	none
<i>Navicula avenaceae</i>	Bacillariophycea e		unicellular	low	0	gliding	Fiss	none
<i>Navicula capitoradiata</i>	Bacillariophycea e	c3	unicellular	low	0	gliding	Fiss	none
<i>Navicula cryptocephala</i>	Bacillariophycea e	c3	unicellular	low	0	gliding	Fiss	none
<i>Navicula cryptotenella</i>	Bacillariophycea e	c2	unicellular	low	0	gliding	Fiss	none
<i>Navicula lanceolata</i>	Bacillariophycea e	c3	unicellular	low	0	gliding	Fiss	none
<i>Navicula miniscula</i>	Bacillariophycea e	c1	unicellular	low	0	gliding	Fiss	none
<i>Navicula radiosa</i>	Bacillariophycea e	c4	unicellular	low	0	gliding	Fiss	none
<i>Navicula</i> spp.	Bacillariophycea e		unicellular	low	0	gliding	Fiss	none
<i>Navicula</i> spp. (small)	Bacillariophycea e	c2	unicellular	low	0	gliding	Fiss	none
<i>Nitzschia acicularis</i>	Bacillariophycea e	c2	unicellular	low	0	drift	Fiss	none
<i>Nitzschia amphibia</i>	Bacillariophycea e	c2	unicellular	low	0	gliding	Fiss	none
<i>Nitzschia dissipata</i>	Bacillariophycea e	c2	unicellular	low	0	gliding	Fiss	none
<i>Nitzschia gracilis</i>	Bacillariophycea e		unicellular	low	0	gliding	Fiss	none
<i>Nitzschia linearis</i>	Bacillariophycea e	c5	unicellular	low	0	gliding	Fiss	none
<i>Nitzschia palea</i>	Bacillariophycea e	c2	unicellular	low	0	gliding	Fiss	none
<i>Pinnularia cf interupta</i>	Bacillariophycea e	c4	unicellular	low	0	gliding	Fiss	none
<i>Pinnularia cf subcapitata</i>	Bacillariophycea e		unicellular	low	0	gliding	Fiss	none
<i>Pinnularia gibba</i>	Bacillariophycea e		unicellular	low	0	gliding	Fiss	none
<i>Pinnularia</i> spp. (incl. <i>mesolepta</i> and <i>microstauron</i>)	Bacillariophycea e	?	unicellular	low	0	gliding	Fiss	none
<i>Pinnularia viridis</i>	Bacillariophycea e		unicellular	low	0	gliding	Fiss	none
<i>Rhoicosphenia abbreviata</i>	Bacillariophycea e	c3	unicellular	low	0	attached	Fiss	none
<i>Rhopalodia nova-zealandiae</i>	Bacillariophycea e	c5	unicellular	low	1	gliding	Fiss	none
<i>Sellaphora</i> sp.	Bacillariophycea e	c4	unicellular	low	0	gliding	Fiss	none
<i>Stauroneis anceps</i>	Bacillariophycea e	c2	unicellular	low	0	gliding	Fiss	none
<i>Stauroneis</i> sp. (INCLUDING: <i>anceps</i>)	Bacillariophycea e	c2	unicellular	low	0	gliding	Fiss	none
<i>Surirella angusta</i>	Bacillariophycea e	c4	unicellular	low	0	gliding	Fiss	none
<i>Surirella cf brebissonii</i>	Bacillariophycea e	c4	unicellular	low	0	gliding	Fiss	none
<i>Synedra biceps</i>	Bacillariophycea e	c5	unicellular	low	0	attached	Fiss	none

Table 1A Continued

Taxon		(1) Cell size	(2) Life form	(3) Attachment	(4) N fix	(5) Motility	(6) Reproduction	(7) Spores
<i>Tabellaria flocculosa</i>	Bacillariophycea e	c3	filamentous	low	0	drift	Fiss	none
<i>Ankistrodesmus</i> sp.	Chlorophyceae	c1	colonial	low	0	drift	Fiss	none
<i>Closterium</i> spp.	Chlorophyceae	c5	unicellular	low	0	drift	Fiss	OoS ZyS
<i>Cosmarium</i> spp.	Chlorophyceae	c5	unicellular	low	0	drift	Fiss	OoS ZyS
<i>Geminella</i> spp.	Chlorophyceae	c1	filamentous	low	0	drift	Frag	Akinete s
<i>Gloeocystis</i> spp.	Chlorophyceae	c2	colonial	low	0	attached	Fiss	Auto spore
<i>Microspora</i> spp.	Chlorophyceae	c5	filamentous	low	0	drift	Frag	Akinete s
<i>Mougeotia</i> spp.	Chlorophyceae	c5	filamentous	low	0	drift	Frag	OoS ZyS
<i>Oocystis</i> spp.	Chlorophyceae	c1	unicellular	low	0	drift	Frag	none
<i>Scenedesmus</i> spp.	Chlorophyceae	c1	colonial	low	0	drift	Fiss	Akinete s
<i>Selenastrum</i> sp.	Chlorophyceae	c1	colonial	low	0	drift	Fiss	none
<i>Spirogyra</i> spp.	Chlorophyceae	c5	filamentous	low	0	drift	Frag	OoS ZyS
<i>Staurastrum</i> sp.	Chlorophyceae	c5	unicellular	low	0	drift	Fiss	Zoo spore
<i>Anabaena</i> spp.	Cyanophyceae	c1	filamentous	low	1	drift	Frag	Akinete s
<i>Aphanocapsa</i> spp.	Cyanophyceae	c1	colonial	low	0	drift	Fiss	none
<i>Cylindrospermum</i> sp.	Cyanophyceae	c2	filamentous	low	1	drift	Frag	Akinete s
<i>Lyngbya</i> sp.	Cyanophyceae	c2	filamentous	low	0	drift	Frag	none
<i>Merismopedia</i> spp.	Cyanophyceae	c1	colonial	low	0	drift	Fiss	none
<i>Nostoc</i> spp.	Cyanophyceae	c1	filamentous	low	1	attached	Frag	Akinete s
<i>Oscillatoria</i> sp.	Cyanophyceae	c2	filamentous	low	1	gliding	Frag	Akinete s
<i>Parallelia</i> sp.	Cyanophyceae	?	filamentous	low	1	attached	Frag	none
<i>Phormidium</i> sp.	Cyanophyceae	c1	filamentous	low	0	gliding	Frag	none
<i>Tribonema</i> spp.	Xantophyta	c4	filamentous	low	0	drift	Frag	ZoS
<i>Cymbella aspersa</i>	Bacillariophycea e	c5	unicellular	medium	0	attached	Fiss	none
<i>Cymbella cristula</i>	Bacillariophycea e	c4	unicellular	medium	0	attached	Fiss	none
<i>Cymbella cuspidata</i>	Bacillariophycea e	c4	unicellular	medium	0	attached	Fiss	none
<i>Cymbella kappii</i>	Bacillariophycea e	c3	unicellular	medium	0	attached	Fiss	none
<i>Cymbella tumida</i>	Bacillariophycea e	c4	unicellular	medium	0	attached	Fiss	none
<i>Didymosphenia germinata</i>	Bacillariophycea e	c5	unicellular	medium	0	attached	Fiss	none
<i>Encyonema minuta</i>	Bacillariophycea e	c2	unicellular	medium	0	attached	Fiss	none
<i>Gomphoneis herculeana</i>	Bacillariophycea e	c5	unicellular	medium	0	attached	Fiss	none
<i>Gomphoneis minuta var. cassiaeae</i>	Bacillariophycea e	c5	unicellular	medium	0	attached	Fiss	none

Table 1A Continued

TAXON		(1) Cell size	(2) Life form	(3) Attachment	(4) N fix	(5) Motility	(6) Reproduction	(7) Spores
<i>Gomphonema acuminatum</i>	Bacillariophyceae	c4	unicellular	medium	0	attached	Fiss	none
<i>Gomphonema angustatum</i>	Bacillariophyceae	c3	unicellular	medium	0	attached	Fiss	none
<i>Gomphonema berggrenii</i>	Bacillariophyceae	c3	unicellular	medium	0	attached	Fiss	none
<i>Gomphonema clavatum</i>	Bacillariophyceae	c3	unicellular	medium	0	attached	Fiss	none
<i>Gomphonema lanceolatum</i>	Bacillariophyceae	c3	unicellular	medium	0	attached	Fiss	none
<i>Gomphonema parvulum</i>	Bacillariophyceae	c3	unicellular	medium	0	attached	Fiss	none
<i>Gomphonema</i> spp. (including <i>minutum</i> and <i>EXIGUA</i>)	Bacillariophyceae	c3	unicellular	medium	0	attached	Fiss	none
<i>Gomphonema truncatum</i>	Bacillariophyceae	c3	unicellular	medium	0	attached	Fiss	none
<i>Meridion circulare</i>	Bacillariophyceae	c4	colonial	medium	0	attached	Fiss	none
<i>Synedra acus</i>	Bacillariophyceae	c4	unicellular	medium	0	attached	Fiss	none
<i>Synedra acus / ulna</i>	Bacillariophyceae	c4	unicellular	medium	0	attached	Fiss	none
<i>Synedra delicatissima</i>	Bacillariophyceae	c3	unicellular	medium	0	attached	Fiss	none
<i>Synedra ulna</i>	Bacillariophyceae	c4	unicellular	medium	0	attached	Fiss	none
<i>Synedra ulna</i> var. <i>ramesi</i>	Bacillariophyceae	c4	unicellular	medium	0	attached	Fiss	none
<i>Bulbochaetae</i> sp.	Chlorophyceae	c5	filamentous	medium	0	attached	Frag	OoS ZyS
<i>Cladophora</i> sp.	Chlorophyceae	c5	filamentous	medium	0	attached	Frag	Akinetes
<i>Draparnaldia</i> sp.	Chlorophyceae	c4	filamentous	medium	0	attached	Frag	Akinetes
Filamentous Green spp.	Chlorophyceae	?	filamentous	medium	0	attached	Frag	?
<i>Klebsormidium</i> spp.	Chlorophyceae	c1	filamentous	medium	0	attached	Frag	ZoS
<i>Oedogonium</i> spp.	Chlorophyceae	c5	filamentous	medium	0	attached	Frag	OoS ZyS
<i>Rhizoclonium</i> spp.	Chlorophyceae	c5	filamentous	medium	0	attached	Frag	ZoS
<i>Stigeoclonium</i> spp.	Chlorophyceae	c3	filamentous	medium	0	attached	Frag	Akinetes
<i>Ulothrix</i> sp.	Chlorophyceae	c5	filamentous	medium	0	attached	Frag	Akinetes
<i>Vaucheria</i> spp.	Chlorophyceae	c5	filamentous	medium	0	attached	Frag	OoS ZyS
<i>Chamaesiphon</i> spp.	Cyanophyceae	c1	unicellular	medium	0	attached	Fiss	none
<i>Coleodesmium</i> spp.	Cyanophyceae	c2	filamentous	medium	1	attached	Frag	none
<i>Rivularia</i> spp.	Cyanophyceae	c1	filamentous	medium	1	attached	Frag	none
<i>Spirulina</i> spp.	Cyanophyceae	?	filamentous	medium	0	attached	Frag	none
<i>Audouinella</i> sp.	Rhodophyta	c3	filamentous	medium	0	attached	Frag	none

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