Response of Stream Biofilm Function to Pulsed Increases in Velocity and Nutrients: An Artificial Stream Study

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Abstract

Pulsed increases in flow within streams alter abiotic conditions such as nutrient concentrations and velocity which can influence the function of stream ecology, including biofilms. A 31-day artificial stream experiment at Thames River Experimental Stream Sciences (TRESS) Centre, London, Ontario, assessed the response of stream biofilm function (decomposition, primary production, community respiration) to individual and combined increases in velocity and phosphorus associated with a 48-hour hydrological pulse event. There was some evidence of an interactive subsidy effect of increased phosphorus and velocity on algal productivity. Decomposition increased as a result of increased phosphorus but there was no synergistic interaction of phosphorus and velocity. Ecosystem respiration did not show a substantive response to velocity or phosphorus increases. This study contributes to furthering ecological knowledge of the effect of hydrological pulses on biofilm function and to progressing understanding of the potential ecological implications of alterations to nutrient loading and stream hydrology.

Keywords: Biofilm, ecosystem function, hydrological pulse event, velocity, phosphorus, artificial stream, algae, stream metabolism, decomposition, chlorophyll-a.
Summary for Lay Audience

Flow is an ecologically important variable within streams. Increases in flow (e.g. following rainfall) can change several ecologically important variables within streams. For example, increases in flow can reduce light availability, increase the physical stress experienced by organism due to higher water velocities and alter resource availability such as nutrients. Two of the most ecologically influential changes during high flow events are increases in water velocity and increased nutrient availability, as nutrients can be transported into streams following rainfall. Changes to water velocity and nutrient concentrations influence stream biofilms. Biofilms are communities of algae, bacteria and fungi that grow on surfaces like rocks within most streams. Biofilms influence key ecological processes in streams such as energy availability and nutrient cycling. For example, algae in biofilms use nutrients, such as phosphorus, and energy from light to photosynthesize and grow (primary production). Bacteria and fungi in biofilms break down organic matter like dead leaves (decomposition). Such ecological processes are called ‘functions’. Primary production and decomposition are important functions, providing a pathway for energy into stream food webs.

This study assessed the response of biofilm function, including primary production and decomposition, to increases in velocity and nutrients (phosphorus) during a 48-hour high flow event. The study was conducted in nine outdoor artificial streams in London, Ontario, which provided a controlled environment to assess and compare the effect of individual and combined pulsed increases in phosphorus and velocity. Primary production in biofilms was greatest when velocity and phosphorus were increased simultaneously. The individual increase in phosphorus had a greater effect on primary productivity compared to the velocity increase. Increased phosphorus, both individually and in combination with velocity, appeared to increase decomposition compared with the individual increases in velocity.

This study, and further related research, is important to understand the effect of high flow events on biofilm function, which influences processes like the energy supply to stream food webs. Understanding the ecological effects of high flow events may allow for assessment of human impacts on streams, such as the effects of alterations to flow and nutrient delivery to streams caused by certain land use changes.
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1 Introduction and Literature Review

The condition and biodiversity of stream ecosystems are declining at an unprecedented rate in human history (EASAC, 2009). In recognition of the importance of freshwater ecosystems, and the stress anthropogenic activities exert on these systems, several jurisdictions have introduced legislation (e.g. European Water Framework Directive (European Commission, 2019); United States Clean Water Act (United States Environmental Protection Agency, 2019)) to protect and rehabilitate stream ecosystems. We thus need clear understanding of the linkages between environmental drivers and the ecological conditions of aquatic systems in order to develop more effective management strategies (Cattaneo et al., 1993; Riseng et al., 2004).

1.1 Ecological Importance of Stream Flow

Flow is a master variable within stream ecosystems (Poff et al., 1997, Hart & Finelli, 1999) that can affect the structure and functioning of stream ecology (Bunn & Arthington, 2002; Bernhardt et al., 2017). Alterations to the hydraulic regime of a stream can affect its ecology through multiple mechanisms. For example, flow can impact the life history of individual species, influence the susceptibility of a system to invasive species and alter the connectivity of a stream (Bunn & Arthington, 2002). Flow can also influence the physical and chemical conditions within streams, thus shaping the biotic community and ecological processes (Bunn & Arthington, 2002; Baker et al., 2004). Flow related variables, such as high flow disturbance events that increase scour and abrasion, shear stress, water velocity, turbulence, as well as the transport and delivery of energy and materials, influence the distribution of aquatic species and ecological processes at a range of spatial and temporal scales (Biggs et al., 2005; Bunn & Arthington, 2002; dos Santos Fonseca et al., 2013; Junk et al., 1989). Developing a better understanding of the biological effects of changes in physical and chemical conditions associated with changes in flow will improve understanding of stream ecology and the potential impacts of flow alterations (Hart & Finelli, 1999).
1.1.1 Hydrological Regime

Variability in flow occurs at multiple temporal scales from longer periods of years or decades, to shorter monthly and daily variability (Biggs et al., 2005; Baker et al., 2004). The characteristics of flow variability in a stream over time is referred to as the flow regime (Poff et al., 1997). Large flood disturbance events with infrequent return periods disproportionately influence the high-level structure and functional characteristics of stream ecosystems (Biggs et al., 2005; Uehlinger & Naegeli, 1998). In particular, shear forces, bed movement and abrasion associated within higher flow events is a key control on the physical characteristic of a stream and can impact or remove benthic organisms within a stream (Uehlinger & Naegeli, 1998; Cobb et al., 1992). Variability in flow that occurs at shorter time scales of weeks to days tends to be smaller than that associated with events with long return periods, yet these smaller more frequent variations are still ecologically important (Biggs et al., 2005). Variability in flow at smaller time scales includes increases in discharge resulting from rain events or smaller melt events. These smaller often sub-catastrophic variations in flow alter the abiotic conditions such as water velocity, delivery of resources and potentially bed stability, which is often reflected by the distribution and ecological functioning of stream biota (Bunn & Arthington, 2002; Biggs & Close, 1989).

The hydrological regime of streams is influenced by numerous factors including the regional climate, catchment topography, catchment size, geology, vegetation, land use, channel morphology and human water usage (Baker et al., 2004). The accumulation of these factors influences both the pathways and delivery rate of water to streams, as well as the characteristics of flow within streams (Baker et al., 2004). Some streams have low variability in flow over time, with a more consistent baseflow. In contrast, ‘flashier’ streams exhibit greater temporal variability in flow. Flashy streams respond quickly to rainfall events with faster rising and falling limbs on the hydrographs and are characterised by higher peak flows but lower baseflows (Baker et al., 2004). Streams located within catchments that exhibit high levels of runoff following rainfall events are generally flashier. Runoff occurs when the rates of interception and infiltration are lower than the rate of rainfall (Sharp & Sawden, 2014). Infiltration of water into ground water
and the subsequent inputs to streams as baseflow is relatively slow. In contrast, surface and subsurface runoff changes rapidly and is therefore the primary cause of short-term hydrological variability, such as floods and hydrological pulse events (Sharp & Sawden, 2014). In the context of this study, a hydrological pulse event will refer to increases in stream discharge caused by increased precipitation or snowmelt that enters the stream within hours through surface or shallow subsurface flow (sensu Kaushal et al., 2014). These short duration increases in flow are the focus of this study and will hereafter be referred to as hydrological pulse events.

1.1.2 Abiotic Effects of Hydrological Pulse Events

Hydrological pulse events cause many abiotic changes to streams, which act as important controls of biota and ecosystem processes in streams (Meyer et al., 1999; Biggs et al., 1999a; Junk et al., 1989; Hart & Finelli, 1999). Two of the most ecologically influential abiotic changes that can occur during hydrological pulse events are the increases in nutrient concentrations and velocity (Biggs & Close, 1989; Bondar-Kunze et al., 2016).

Alterations to discharge, such as those associated with hydrological pulse events, can increase stream velocities (Cronin et al., 2007). Changes to velocity can cause an array of direct hydrodynamic changes to stream environments, as well as indirect affects to streams, such as inducing sediment transport, that influence organisms within streams (Hart & Finelli, 1999). Extreme flow events are common disturbance events within streams where disturbance is defined as an unpredictable and temporally discrete perturbation event which derives from a source external to the community and causes a loss of organisms that is rapid when compared to the biomass accrual duration (Uehlinger & Naegeli, 1998; Biggs et al., 1999a).

Hydrological pulse events are also an important control on the delivery of resources such as nutrients and organic matter into streams (Wold & Hershey, 1999; Davies & Bothwell, 2012). Runoff is the dominant hydrological pathway for nutrient loading to streams, particularly phosphorus (Peterjohn and Correll, 1984; Biggs & Close, 1989). Accordingly, the loading of nutrients into streams, including nitrogen and phosphorus, can increase during hydrological pulse events and can lead to increases in nutrient
concentration (Kaushal et al., 2014; Humphrey & Stevenson, 1992; Meyer et al., 1988). For example, Biggs and Close (1989) studied the effect of flow and nutrients on biofilms within nine streams in New Zealand and found that the concentrations of several nutrient parameters, including dissolved reactive phosphorus and total phosphorus, were correlated with flow. Additions of nitrogen and phosphorus can be ecologically influential within stream environments as these are commonly the limiting nutrients for autotrophs, such as algae, and microbial heterotrophs (Johnson et al., 1997; Tank & Dodds, 2003). Increased algal biomass can impair ecosystem function, water quality and the ecosystem services associated with streams (Carpenter et al., 1998). Phosphorus, particularly soluble reactive phosphorus, which is the most bioavailable fraction of phosphorus, is often identified as the single most important nutrient limiting algal growth in freshwaters and is increasingly becoming the focus of management efforts to limit anthropogenic increases in nutrient loading (Jarvie et al., 2017; Withers & Jarvie, 2008). Phosphorus loading to streams occurs naturally from sources such as the weathering and erosion of soils and inputs from riparian vegetation to streams (Withers & Jarvie, 2008); however, anthropogenic activity such as agricultural or urban land use can increase the delivery of phosphorus to streams within runoff (Carpenter et al., 1998). Alterations to phosphorus loading may alter key ecosystems processes, thus potentially change energy flows and nutrient cycling with streams (Ferreira et al., 2015).

1.2 Benthic Biofilms.

Hydrological pulses are a major factor controlling biofilms within streams (Biggs & Close, 1989). Stream biofilms, often called periphyton, refers to the community of eukaryotic algae, bacteria, cyanobacteria, fungi and protozoa that grow on the benthic substrate and other surfaces (e.g., woody debris) within streams (Biggs & Close, 1989). Biofilms are ubiquitous within streams (Hondzo & Wang, 2002) and influence ecologically important processes such as energy flows and nutrient cycling (Battin et al., 2003; Allan & Castillo, 2007; Saravia et al., 1998; Tanks & Dodds, 2003; Hoellein et al., 2013).
Biofilms consist of autotrophs and heterotrophs, which both play an important role in the basal energy supply to the food web, representing an important source of carbon to stream ecosystems (Frost et al., 2002; Battin et al., 2003; Hoellein et al., 2013). For example, biofilms are a key food resource to aquatic organisms, such as invertebrates and fish (Lambert et al., 1992; Cattaneo et al., 1993; Feminella & Hawkins, 1995; Sigee, 2005). Autotrophs, also referred to as primary producers, acquire energy from sunlight. Autotrophs within biofilms, which include algae, certain bacteria and certain protists (Allan & Castillo, 2007), are a major contributor to primary production within streams (Biggs, 1996; Battin et al., 2003). Thus, biofilms commonly represent an important autochthonous energy source to stream ecosystems (Allan & Castillo, 2007). In contrast, fungi, certain bacteria and certain protists within biofilms are heterotrophs, consuming dead or living organic matter to obtain energy (Allan & Castillo, 2007). Allochthonous inputs of organic matter, such as leaf litter, constitute a significant portion of the carbon and energy sources within streams, particularly within forested catchments (Fisher & Likens, 1973). The consumption of dead organic matter within streams by heterotrophs like bacteria and fungi can improve the accessibility of nutrients and energy from dead organic matter for other consumers (Allan & Castillo, 2007; Cummins et al., 1989).

Algae, fungi and bacteria within biofilms commonly represent the primary site for the uptake of inorganic nutrients such as phosphorus within freshwater environments (Sigee, 2005; Allan & Castillo, 2007). Uptake of inorganic nutrients by biofilms immobilise bioavailable nutrients (Vymazal, 1988) and provides an important pathway for inorganic nutrients to enter the food web (Sigee, 2005). Biological uptake of nutrients is thus an important, yet often overlooked, aspect of nutrient cycling within streams as it influences the transport pathways of nutrients through aquatic systems (Withers & Jarvie, 2008).

Despite the ecological importance of biofilms, there are relatively few studies on biofilm communities compared with phytoplankton communities within freshwater environments (Hondzo & Wang, 2002, Hansson, 1992). Biofilms have a short life cycle and reproduce rapidly meaning that biofilm communities often reflect short-term alterations to the stream environment (Bondar-Kunze et al., 2015; Biggs, 1996). The structure and function of biofilms has been shown to be influenced by a range of environmental conditions (e.g.
temperature, light, water chemistry, flow velocity, grazers, colonisation history) (Allan & Castillo, 2007; Sigee, 2005). Accordingly, field studies on biofilms commonly find substantive spatial and temporal variability in community structure and biomass (Morin & Cattaneo, 1992). The range of influential variables can be categorised into three broad factors that determine the distribution and function of biofilms: 1) hydrological impacts, 2) biological pressures, and 3) water chemistry (Biggs & Close, 1989; Rosemond et al., 1993; Riseng et al., 2004; Peterson, 1996). In particular, the frequency and intensity of hydrological pulse events can play an important role in influencing biofilm dynamics (Biggs & Close, 1989; Tett et al., 1978) as increases in velocity and nutrient concentrations during storm events are key factors controlling the structure, accrual and function of stream biofilms (Riseng et al., 2004; Biggs & Close, 1989; McCormick & Stevenson, 1991; Biggs, 2000).

1.3 Ecological Function

Studies on stream ecology, including some studies on disturbance and nutrient enrichment, have traditionally focused on structural indices such as measures of water quality or taxonomic composition of organisms (e.g. macro-invertebrates, algae or fish) (Riseng et al., 2004; Gessner & Chauvet, 2002; Biggs et al., 1999a, Barbour et al., 1996; Young et al., 2008; Tiegs et al., 2013). However, the importance of ecosystem function is increasingly recognised (Naeem et al., 2002; Tiegs et al., 2013; Bunn et al., 1999; Young et al., 2008; Death et al., 2009; Gessner et al., 1999). Function or functioning is a concept of growing popularity within the field of freshwater ecology, although the term has a broad array of definitions and is often used ambiguously (Jax, 2005). Function or functioning can broadly refer to four different meanings according to Jax (2005). Functioning can mean the interaction of multiple organisms within a whole ecosystem, the specific role of different species or groups of organisms (e.g. identifying certain species of benthic macroinvertebrates as shredders) or it can refer to an ‘ecosystem service’ that the ecosystem provides which usually benefits humans. Within this thesis, I adopt a descriptive definition where function is synonymous with process and refers to the interaction of two objects or entities that cause a state change over time (Jax, 2005).
Biota are not passive by-products reflecting the physical and geochemical conditions on earth (Naeem et al., 2002). Rather, biotic function plays a crucial role in influencing the abiotic conditions at varying scales (Butcher et al., 1992). For example, the metabolic activity and growth of biota contributes to global biogeochemical cycling on earth (Naeem et al., 2002). Certain ecological functions are critical to support the supply of energy or nutrients to the ecosystem (Bunn et al., 1999). Therefore, maintaining ecological diversity within aquatic systems, as well as the ecosystem services associated with freshwater environments is dependent on ecosystem functioning (Meyer et al., 1999). Common ecological functions assessed within streams include decomposition of organic matter, metabolism (gross primary production and ecosystem respiration), invertebrate drift and coarse particulate organic matter retention (Death et al., 2009; Lepori et al., 2005).

Ecosystem functions can be highly integrative and sensitive to a broad range of environmental and anthropogenic induced variability (Bunn et al., 1999; Young et al., 2008). For example, ecosystem level process commonly responds to alterations in nutrients, light, sediment or organic matter supply, which may result from anthropogenic disturbance at a catchment scale (Bunn et al., 1999). There is currently a dearth of understanding on how key ecological functions, such as decomposition and primary production, may respond to stressors (e.g. Death et al., 2009). In particular, studies focusing on ecosystem function response to pulsed increases in nutrients and velocity associated with hydrologic events are lacking.

1.3.1 Decomposition

The decomposition of organic matter is a major energy pathway within freshwater ecosystems. Organic detritus within streams can be autochthonous, originating from within the stream, or allochthonous, originating from an external source (Webster & Benfield, 1986). Allochthonous inputs of organic matter, such as leaf litter, constitute a significant portion of the carbon and energy sources within streams, particularly within the upper reaches of forested catchments (Fisher & Likens, 1973; Abelho, 2001; Wallace et al., 1997). Riparian vegetation within small, forested streams often limits
photosynthesis as light is reflected or absorbed by the canopy. However, riparian vegetation can also provide large inputs of organic matter (Ferreira et al., 2015).

Decomposition of organic matter in a stream is a complex process that can be summarised into three overlapping processes: 1) leaching of soluble compounds, 2) microbial colonisation and degradation and 3) fragmentation, physical abrasion and invertebrate shredding (Abelho, 2001; dos Santos Fonseca et al., 2013). Leaching of soluble compounds is generally rapid and may vary with factors such as temperature, leaf type and leaf condition (Maloney & Lamberti, 1995). Decomposition is, however, primarily a biological process (Ferreira et al., 2015). Microbial degradation, driven by heterotrophic bacteria and fungi, is a key mechanism driving decomposition (Rader et al., 1994; Grattan & Suberkropp, 2001). Fungi are commonly recognised as the dominant driver of decomposition within streams, with studies finding that fungi have greater biomass and higher production than bacteria (Weyers & Suberkropp, 1996; Baldy et al., 1995). Indeed, Gulis & Suberkropp (2003) conducted a field experiment on leaf decomposition and found fungal biomass was between one and two orders of magnitude greater than bacteria. These microbes enhance decomposition by macerating or metabolising organic matter (Abelho, 2001). Additionally, the colonisation of organic matter with microbes can enhance the palatability for shedder invertebrates promoting fragmentation. Decomposition via fragmentation can also be stimulated by physical abrasion (dos Santos Fonseca et al., 2013).

There are a multitude of variables that can influence decomposition rates, including nutrient concentration, the availability and types of organic matter inputs, substrate characteristics, temperature, flow, biological community and pH (Young et al., 2008; Abelho, 2001; Maloney & Lamberti, 1995; Lepori et al., 2005; Webster & Benfield, 1986). These factors vary naturally with changes in climate and with longitudinal position along a stream, yet anthropogenic activities also alter these influential variables meaning that decomposition can exhibit high spatial and temporal variability (Suberkropp & Chauvet, 1995; Young et al., 2008; Gessner & Chauvet, 2002). Therefore, understanding anthropogenic effects requires understanding of natural variability and potential interactions of multiple environmental factors. There have been a relatively
large number of studies on the breakdown of organic matter within low order streams, most of which focused on the breakdown of leaves (Abelho, 2001). Such studies suggest that both velocity and nutrients can play an important role in organic matter decomposition; however, both factors are understudied in relation to effects of short-term increases related to hydrological events (Grattan & Suberkropp, 2001; Gessner et al., 1999). Moreover, studies assessing the effect of multiple interacting factors on decomposition, such as the combined effects of nutrient and velocity are few (Ferreira et al., 2015).

1.3.1.1 Velocity Effects on Decomposition.

Increases in velocity can affect decomposition by increasing the physical fragmentation of organic matter (dos Santos Fonseca et al., 2013; Gessner et al., 1999). Increases in velocity can cause physical shear stress to organic matter and lead to higher levels of abrasion, thus increasing decomposition (dos Santos Fonseca et al., 2013; Gessner et al., 1999). The contribution of physical fragmentation to the decomposition of organic matter within streams is understudied and commonly overlooked (Gessner et al., 1999). A study by Lepori et al. (2005) found that velocity during high flow events was the most important factor influencing course particulate organic matter breakdown within restored and unrestored streams in Sweden, suggesting velocity can be an important factor. The physical fragmentation of course particulate organic matter may reduce the availability of organic matter to the localised biota within streams as fragmentation may promote leaching and finer particulate matter are more easily transported downstream (Webster & Benfield, 1986; Wallace et al., 1982). Therefore, understanding the relative contribution of different mechanisms in decomposition may be important for understanding energy availability within streams and the drivers of key ecosystem processes.

1.3.1.2 Nutrient Effects on Decomposition

Although the limiting effect of nutrients on algal growth rates and primary production are well documented, the effect of nutrients on decomposition rates are relatively understudied (Grattan & Suberkropp, 2001). There is a growing body of literature that suggests limiting nutrients, primarily nitrogen and phosphorus, are an important factor
influencing decomposition (Elwood et al., 1981; Suberkropp & Chauvet, 1995; Gulis & Suberkropp, 2003; Boulton & Quinn, 2000; Ferreira et al., 2015; Meyer & Johnson, 1983), although some studies have found varying results (Grattan & Suberkropp, 2001).

Many studies have focused on the relative importance of nitrogen and phosphorus on decomposition rates. A meta-analysis on decomposition by Ferreira et al. (2015) found that studies on decomposition that involved increases in both nitrogen and phosphorus resulted in higher decomposition rates compared to individual increases in nitrogen or phosphorus, suggesting potential co-limitation. Additionally, decomposition was affected by nutrient enrichment to a greater extent when the background nutrient level was low (Ferreira et al., 2015). The effect that temporal variability in nutrients exerts on decomposition has received relatively little attention (Ferreira et al., 2015).

Field studies tend to find a weaker association between nutrients and decomposition, compared with laboratory or mesocosm studies (Ferreira et al., 2015). A field study by Suberkropp and Chauvet (1995) conducted in eight streams in Alabama, U.S.A. found that the fungi associated with leaf decomposition appear to receive a large portion of their nitrogen and phosphorus from the surrounding water suggesting nutrient concentration within the water are important. Contrastingly, several field studies on leaf litter decomposition have failed to find an effect of nutrients (Abelho & Graça, 2006; Baldy et al., 2007; Abelho et al., 2010).

A laboratory study by Suberkropp (1998) found that the loss of leaf litter weight associated with decomposition by fungi was stimulated by increases in potassium nitrate and potassium phosphate within the surrounding water; however, fungal growth was present within treatments where no potassium nitrate and potassium phosphate was present in the water, suggesting fungi were able to obtain required nutrients from leaf litter. Ferreira et al. (2015) suggests that the mining of nutrients from organic matter by microorganism requires the synthesis of extracellular enzymes which is metabolically costly, mean that nutrients derived from the water column commonly represent an important exogenous resource for microbial activity. Nevertheless, studies assessing the effect of nutrients on leaf decomposition are confounded by the fact that the leaves are a source of multiple nutrients as well as carbon. The relative importance of surrounding
water verses the leaf tissue as a source of nutrients is unknown and may be influenced by the relative concentrations and ratios of nutrients. Additionally, leaf type and condition are important factors in determining the availability of nutrients from the leaves, which may influence the demand for nutrients by microbial decomposers (Grattan & Suberkropp, 2001; Ferreira et al., 2015). Similarly, dos Santos Fonseca et al. (2013) found that the type of organic matter can influence the affect that velocity has on decomposition rate.

Many studies now use standardised cotton strips to assess decomposition to eliminate the influence that leaf condition or composition exerts on measures of decomposition (Tiegs et al., 2013; Boulton & Quinn, 2000). A decomposition assay by Tiegs et al. (2013) assessed the loss of tensile strength of standardised cellulose cotton strip within 45 streams that exhibited low variation in nutrient concentration and found a weak relationship between the decomposition and the concentration of soluble reactive phosphorus and ammonia. The weaker associations between nutrients and decomposition observed in the field is potentially a result of confounding environmental variables (Ferreira et al., 2015). Therefore, combining the use of cotton strips as a standardised organic matter, and the use of artificial streams may facilitate the development of a clearer understanding of the effects of nutrients, albeit at the expense of realism.

1.3.2 Stream Metabolism

Algal growth and stream metabolism are two key ecological functions that influence energy pathways and nutrient dynamics in streams (Demars et al., 2015). Nutrient supply and disturbance are two of the primary factors controlling the productivity and accrual of biofilm biomass (Biggs, 2000). Stream metabolism is a measure of the daily variation in the level of gross primary productivity and ecosystem respiration within a stream. Gross primary productivity reflects the amount of solar energy that is converted into organic energy via photosynthesis (Bernhardt et al., 2017). Ecosystem Respiration is a measure of the amount of organic energy that is dissipated because of the metabolic activity of autotrophic and heterotrophic organisms (Bernhardt et al., 2017). Thus, stream metabolism indicates the amount of organic carbon that is produced and consumed within a stream, providing information on the food base available within an ecosystem (Young.
et al., 2008). Additionally, metabolism is important for nutrient dynamics (Hall & Tank, 2003; Lupon et al., 2016).

The energy flow through stream ecosystems is determined by the level of primary production within the channel and the inputs of allochthonous organic carbon derived from upstream or from the riparian zone (Uehlinger & Naegeli, 1998). Energy flow can be calculated as the combination of both gross primary productivity and ecosystem respiration (Uehlinger & Naegeli, 1998). Measures of net daily metabolism (gross primary productivity – ecosystem respiration) or gross primary productivity to respiration ratios indicate whether a stream is net heterotrophic or autotrophic.

The metabolism of streams is largely influenced by stream biofilms (McIntire, 1975; Tank & Dodds, 2003) which tend to respond quickly to changes in abiotic factors (Hill et al., 2009). Stream metabolism is thus highly dynamic and sensitive to multiple environmental factors (Bernhardt et al., 2017), including hydrology, nutrients, light, substrate and temperature (Beaulieu et al., 2013; Young et al., 2008; Griffiths et al., 2013; Bunn et al., 1999). Traditionally, there has been a large focus on assessing spatial variability in stream metabolism, with relatively little focus on temporal changes, such as those driven by disturbance (Uehlinger & Naegeli, 1998).

1.3.2.1 Primary Productivity

Stream metabolism is commonly measured based on the characteristics of diel changes in oxygen concentration, with increases in dissolved oxygen during daylight conditions owing to primary production and decreases at night owing to the persistence of respiration in the absence of primary productivity (Bernhardt et al., 2017). Measures of stream metabolism are increasingly easy to record owing to the development of more accurate and affordable dissolved oxygen loggers (Grace & Imberger, 2006; Bernhardt et al., 2017; Young et al., 2008). Estimates can be made directly from measurements of oxygen taken throughout the day (Odum, 1956) or modelled based on oxygen curves from sub daily scale (Demars et al., 2015).
1.3.2.1.1 Chlorophyll-\textit{a} and Algal Growth

In addition to measures of primary productivity based on changes in dissolved oxygen concentrations, studies on the productivity of algae within biofilms commonly measure changes in the concentration of chlorophyll-\textit{a} within biofilms to assess the algal growth (Davies and Bothwell, 2012; Wold & Hershey, 1999; Atkinson \textit{et al}., 2008; Biggs, 2000; Morgan \textit{et al}., 2006). Measures of the photosynthetic pigment chlorophyll-\textit{a} using spectrometry enable an estimate of algal biomass within biofilms without incorporating organic matter such as detritus and or biomass from heterotrophs with biofilm (Biggs, 2000). The accrual of biofilm is controlled by the rate of uptake of limiting nutrients by cells, light availability and temperature (Biggs, 2000). The loss of biofilm biomass is a result of sloughing resulting from the physical drag force exceeds the tensile strength of the biofilm or the strength of the mat attachment, aging of the biofilm community, physical disturbance events such as floods and grazing press from biota such as macroinvertebrates (Biggs, 2000).

1.3.2.1.2 Velocity Effects on Algal Productivity and Algal Growth

Increases in velocity and the resulting changes to near bed hydrodynamics have been shown to be an important control on the structure, function and biomass dynamics of biofilms (Biggs & Hickey, 1994; Biggs \textit{et al}., 1998; Biggs & Stokseth, 1996; Poff \textit{et al}., 1990). Biggs and Close (1989) conducted a field study on the relative importance of flow and nutrients on biofilm biomass dynamics and suggested hydrological factors are at least as influential as nutrients. Biofilm biomass dynamics are dependent on growth and detachment processes (Ateia \textit{et al}., 2016). Velocity has the potential to affect counteracting processes relating to biofilm growth (Biggs & Stokseth, 1996). Velocity can enhance the mass transfer of nutrients from the water column into biofilm mats, stimulating metabolic activity and promoting biomass accrual (Biggs & Stokseth, 1996; Horner \textit{et al}., 1990). Yet, velocity can also increase the loss of biofilm biomass via bed movement, abrasion and drag induced sloughing (Biggs \textit{et al}., 1998; Larned \textit{et al}., 2004; Biggs, 1996). However, the effect of increased velocity on biofilm communities is not fully understood (Biggs \textit{et al}., 1999a).
There is a growing body of evidence highlighting the impact high flow disturbance events can have on stream metabolism (Acuña et al., 2004; Acuña et al., 2011; Uehlinger & Naegeli, 1998; Atkinson et al., 2008; O’Connor et al., 2012; Beaulieu et al., 2013). Hydrological disturbance is a major factor controlling the loss of biofilms from substrate in streams and can act as a successional reset mechanism (Biggs et al., 1999a; Bernhardt et al., 2017). The increase in velocity associated with hydrological pulse events increases the shear stress exerted on the stream bed and biofilm community (Biggs & Stokseth, 1996; Cronin et al., 2007; O’Connor et al., 2012). Shear stress refers to the force applied to the bed per unit area and can be estimated from discharge when the channel geometry, channel slope and bed roughness are known (Cronin et al., 2007). The effect that short duration increases in velocity has on biofilms is highly dependent on bed stability (Peterson, 1996; Atkinson et al., 2008). Increases in velocity that reach critical shear stress cause bed sediments to move. The movement of bed sediments have been shown to cause large disturbances to biofilm communities and depress primary productivity (Uehlinger, 2006; Uehlinger, 2000; Cronin et al., 2007). Bed movement can remove, bury or prevent the establishment of primary producers on bed sediment by destroying benthic habitat and increasing abrasion (Atkinson et al., 2008; Uehlinger & Naegeli, 1998; Bernhardt et al., 2017; Biggs et al., 1999a; Biggs & Close, 1989; McCormick & Stevenson, 1991; Horner et al., 1990). Streams where bed movement events occur frequently tend to be less productive (Uehlinger, 2006). Bed sediment movement is therefore an important factor influencing biofilm function and biomass dynamics (Biggs et al., 1999a; Uehlinger, 2000).

Most studies assessing the effect of increased flow on stream biofilms and metabolism focus on catastrophic high flow events that mobilised a large portion of the bed sediments (Uehlinger, 2006; Uehlinger, 2000; Cronin et al., 2007; Hart et al., 2013). Less research has focused on the effect of sub-catastrophic flows. For example, Cronin et al. (2007) suggests that the effect of flow on metabolism is proportional to the amount of bed movement; however, this assumes that bed stability is the only cause of depressions in metabolism. Other factors associated with increases in velocity may contribute to reductions in metabolism such as the removal of autotrophs from biofilms due to the increased drag force (Lau & Liu, 1993), the abrasive scouring force of suspended
sediment (Horner et al., 1990; Francoeur & Biggs, 2006) or light reductions through increased turbidity (O’Connor et al., 2012). Increases in shear stress increases the drag effect exerted on the biofilm community which can causes the biofilm community to tear or slough from the substrate depending on the age and susceptibility of the biofilm community (Lau & Liu, 1993; Biggs & Close, 1989; Biggs & Thomsen, 1995; Tuchman, 1996; Biggs & Stokseth, 1996). Epilithic algae taxa commonly attach to rocks by either basal cell and stalk or by mucilaginous secretions which reduces their susceptibility to being dislodged during higher flow events (Allan & Castillo, 2007). Biofilm communities established under low velocities conditions tend to be less resistant to increases in velocity compared with communities that develop under higher velocities (Peterson, 1996). This may be due to a higher biomass present within biofilms developed under lower flow. A mesocosm study by Poff et al. (1990) found that periphytic biomass was 30 to 40 times higher within treatments that receive lower velocities over a 42 day experiment compare to higher velocity treatments, with the low velocity community being characterised by a large three-dimensional structure. Therefore, increases in velocity that do not cause bed movement can still reduce primary productivity, thus the potential for high gross primary productivity is greater in streams with more stable flow and clearer water (Acuña et al., 2011).

The scale of the impact of higher flows on gross primary productivity (resistance) and the associated recovery time (resilience) is affected by multiple variables including season and the magnitude of the flow event and the associate disturbance caused (Biggs et al., 1999b). Recovery of gross primary productivity following bed movement events has been found to be faster in summer compared to winter (Uehlinger & Naegeli, 1998; Uehlinger, 2006). Cronin et al. (2007) suggest the recovery time following a large flood event where the majority of the bed is mobilised may take upwards of 10 days. Similarly, O’Connor et al. (2012) reported a threshold response in the impact and recovery rates of metabolism following increases in flow, whereby large floods that mobilised the full bed caused large prolonged depressions in gross primary productivity lasting up to 15 days, whereas the recovery from smaller events was faster, lasting approximately 5 days.
Contrastingly, increased velocity below a community and site-specific threshold can remove waste products or detritus from biofilms leading to an increase in the productivity of algae within biofilms (Saravia et al., 1998; Biggs et al., 1998; Horner et al., 1990). Flow can also influence the delivery of nutrients to biofilms, potentially enhancing nutrient supply to depleted areas (Saravia et al., 1998; Larned et al., 2004; Biggs et al., 1998). Larned et al. (2004) suggest there are three main mechanisms controlling nutrient uptake by biofilms. At the largest scale, nutrients are delivered via turbulent diffusion from the main channel to the benthos, a process that is rarely limiting apart from within the slowest streams. The second mechanism affecting nutrient uptake is molecular diffusion through the inner diffusive boundary layer of the laminar sublayer to reach the cells within the biofilm (Bondar-Kunze et al., 2016). Laminar sublayer, also referred to as the viscous sublayer, is the layer immediately adjacent to the substrate (Saravia et al., 1998). The diffusive boundary layer is the inner portion of the laminar sublayer that is closest to the benthos (Larned et al., 2004). The final mechanism is the transport of nutrients through cell boundaries and into the cells. Alterations to the thickness of the diffusive boundary layer resulting from alterations to velocity can promote nutrient uptake. Additionally, flow can also influence the spatial and temporal delivery of algal propagules to the benthos (Peterson & Stevenson, 1989). Simultaneous alterations to flow velocity and nutrients have the potential to impact the structure and function of biofilm communities. There is, however, a lack of studies determining the relative importance of chemical and hydrological determinants of biofilm growth (Biggs & Close, 1989).

1.3.2.1.3 Nutrient Effects on Algal Productivity and Algal Growth

Understanding of the effects if nutrient enrichment on metabolism and algal productivity within stream systems is lagging in comparison to understanding within lakes (Dodds et al., 2016; Van Nieuwenhuyse & Jones, 1996; Biggs, 2000), yet has received increasing attention (Guasch et al., 1995). While nutrients are commonly not the single direct control of in stream primary productivity (Bernhardt et al., 2017; Mulholland et al., 2001), nutrient concentrations are a major determining factors of gross primary productivity and benthic algal biomass within streams (Bondar Kunze et al., 2016; Van Nieuwenhuyse & Jones, 1996). For example, biomass of biofilms during the accrual
period has been shown to be significantly correlated with dissolved reactive phosphorus (Biggs & Close, 1989). Accordingly, some eutrophic streams can exhibit large diel changes in oxygen, ranging from super saturated in the day to near anoxic at night (Morgan et al., 2006).

The relationship between nutrients and primary producers is not straighthforward and developing quantifiable relationships between nutrients and algal response has proved difficult (Izagirre et al., 2008). Factors such as light, hydrology and the background nutrient concentration can determine the effect that potential increases in nutrient concentrations have on primary productivity (Morgan et al., 2006). For example, a study conducted by Mulholland et al. (2001) in eight streams across different biomes within North America found that gross primary productivity was most strongly correlated with photosynthetically active radiation, with soluble reactive phosphorus having a secondary effect. Similarly, Bernardt et al. (2017) suggest that increases in nutrient loads resulting from anthropogenic land use change have a lesser effect on metabolism relative to the associated loss of riparian shade and alterations to the hydrological regime.

The background nutrient concentration is another important factor influencing the effect of nutrient increases on gross primary productivity. Evidence suggests that under low concentrations, phosphorus or nitrogen can limit primary productivity in streams (Guasch et al., 1995; Elwood et al., 1981; Grimm & Fisher, 1986). In contrast, increases in nutrients in streams where productivity is not nutrient limited may have little effect (Tank & Dodds, 2003). For example, Bushong and Bachmann (1989) conducted field experiments where nitrogen and phosphorus were added to agricultural streams and found algal communities were rarely limited by either nutrient. Hill et al., (2009) found a hyperbolic relationship between phosphorus and algal growth and stream metabolism, suggesting at higher concentration the limiting effect will decrease. Therefore, stream metabolism may be sensitive to anthropogenic alterations to pulsed nutrient loading and increased velocity (Frankforter et al., 2009; Bunn et al., 1999), particularly in low nutrient systems.
1.3.2.2 Respiration

1.3.2.2.1 Velocity Effects on Respiration

Ecosystem respiration responds differently to high flow events than does gross primary productivity (Cronin et al., 2007). Studies have found that increases in flow can both reduce (Uehlinger & Naegeli, 1998; Uehlinger, 2006) and increase ecosystem respiration (Acuña et al., 2011). Increases in flow may cause a localised export or import of organic matter potentially affecting ecosystem respiration (Bernhardt et al., 2017; Naegeli et al., 1995). Ecosystem respiration is more resistant to disturbance caused by increases in flow as, unlike gross primary productivity, ecosystem respiration can occur within deeper sediments that do not receive light and are less susceptible to disturbance compared to surface sediments (Atkinson et al., 2008; Uehlinger, 2000; Cronin et al., 2007; Naegeli & Uehlinger, 1997; Uehlinger & Naegeli, 1998; Fellows et al., 2001). Indeed, Atkinson et al. (2008) found that after a high flow event that disturbed surface sediments and increased abrasion the highest metabolic activity shifted from surface sediments to the deeper hyporheic zone. The hyporheic zone can play an important role in stream metabolism, contributing up to 96% of ecosystem respiration (Naegeli & Uehlinger, 1997). Several field studies have found that gross primary productivity was impacted by increases in flow to a greater extent compared to ecosystem respiration, causing the metabolic status of the streams to shift towards a more heterotrophic status post hydrological pulse event (Acuña et al., 2011; Uehlinger & Naegeli, 1998; Uehlinger et al., 2003; Uehlinger, 2006).

1.3.2.2.2 Nutrient Effects on Ecosystem Respiration

Research on nutrient limitation within streams commonly focuses on autotrophs, predominantly algae; however, heterotrophs are a key component determining the metabolism of streams (Tanks & Dodds, 2003). Several studies have found that increases in nutrients contribute to an increase in ecosystem respiration. For example, Uehlinger (2006) found that ecosystem respiration declined over a 15 year period during which nitrate and soluble reactive phosphorus concentrations also declined. Similarly, Mulholland et al. (2001) found that ecosystem respiration within eight different biomes
in North America was correlated with the size of the transient storage zone and soluble reactive phosphorus concentrations. Increases in algal abundance that occurs under nutrient enriched conditions can cause an increase in autotroph respiration (Morgan et al., 2006). Additionally, heterotrophs in streams can be nutrient limited meaning that nutrient enrichment may increase ecosystem respiration (Suberkropp & Chauvet, 1995; Johnson et al., 2009). This said, the substrate type may also influence the likelihood of inorganic nutrient limitation of heterotrophs. Johnson et al. (2009) used nutrient diffusing substrates within 72 streams to assess nutrient limitation of biofilms and found that limitation was common on organic substrate yet was rare on inorganic substrate, citing low carbon availability on inorganic substrate as a possible reason for this disparity.

1.4 Artificial Stream Experiments

There is an increasing focus on field-based studies aimed at assessing the influence of catchment-scale anthropogenic activities on stream ecology (Allan, 2004). Collection of data from the field can be challenging and time consuming (e.g. Izagirre et al., 2008). Additionally, identifying cause and effect relationships of different variables in the field is challenging as studies can be impeded by the influence of environmental variability (Wagenhoff et al., 2012; Biggs 1996; Bernhardt et al., 2017), a lack of replication and co-variation of and interaction between variables (Culp et al., 2000; Niyogi et al., 2006). Biofilm structure and function is influenced by multiple proximal factors including temperature, light, water chemistry (e.g. nutrients, pH and dissolved contaminants), substrate, velocity, scouring during floods and grazing by organisms such as invertebrates (Allan & Castillo, 2007; Aristi et al., 2016). These proximal factors are influenced by catchment scale variability including vegetation, land use, geology, and topography (Biggs, 1996).

Artificial streams are increasingly being used to compliment field studies and further the understanding of the effect of environmental factors on stream ecology (Lamberti & Steinman, 1993; Culp et al., 2009). Artificial streams are constructed channels with a controlled flow of water that enables the assessment of physical, chemical or biological properties of streams (Lamberti & Steinman, 1993). Artificial stream experiments allow...
researchers to manipulate environmental variables and replicate experimental treatments (McIntire, 1975), whilst maintaining consistent ambient physiochemical conditions such as the substrate, water chemistry, light, flow and temperature (Culp et al., 2009; Lamberti & Steinman, 1993). Consequently, data derived from artificial streams studies tends to be more compatible with inferential statistics (Lamberti & Steinman, 1993). Therefore, artificial streams allow for the isolation and manipulation of individual or multiple variables to determine their effects under known, replicable and controlled conditions.

There are, however, several limitations associated with conducting experiments within an artificial stream compared to a field-based approach. For instance, the increase in the control of natural variability tends to be offset by a reduction in the scope and realism of the study compared to field studies owing to the simplification of the system (Lamberti & Steinman, 1993). Additionally, artificial streams commonly do not replicate the hyporheic zone or habitat heterogeneity of natural streams, which can play an important role in ecological processes such as stream metabolism (Cardinale et al., 2002; Naegeli & Uehlinger, 1997). Therefore, the results of individual artificial stream studies often need to be combined with several related studies in order to process understanding of natural systems (Lamberti & Steinman, 1993).
2 Aim and Hypotheses

My thesis aims to assess the response of stream biofilm function (i.e. decomposition, primary production, respiration and algal growth rates) following increases in velocity and phosphorus associated with a hydrological pulse event.

2.1 Hypotheses

Primary productivity

It is expected that increases in velocity during the hydrological pulse will remove biofilm as a result of increased drag force, which will reduce the biomass and growth rate of benthic algal and lead to a reduction in primary productivity. In contrast, the pulsed increase in phosphorus concentration will increase algal growth rate and primary productivity as autotrophs will be phosphorus limited and will be able to respond to the short duration increase in phosphorus availability. Simultaneous increases in velocity and phosphorus will have an antagonistic effect on algal productivity and algal biomass whereby increased velocity will cause a decrease in productivity, yet increased phosphorus will increase productivity.

Community Respiration

I predict that community respiration will also decrease following a pulsed increase in velocity as organism and organic matter will be removed from the biofilm. It is expected that the pulsed increase in phosphorus within the phosphorus level will lead to an increase in the rate of respiration as both primary producers and heterotrophic organism may be phosphorus limited prior to the pulse event. The combination of pulsed increases in both velocity and phosphorus are predicted to have antagonistic effects, whereby phosphorus enhances ecosystem respiration yet velocity removed organisms from the biofilm and decrease ecosystem respiration.

Sestonic chlorophyll-a

I expect sestonic chlorophyll-a will increase rapidly following the pulsed increase in velocity owing to loosely attached algae being removed from the biofilm and lifted into
suspension. The pulsed increase in phosphorus in the absence of velocity increases will not influence sestonic chlorophyll-\textit{a}.

\textit{Decomposition}

The pulsed increases in velocity will increase decomposition owing to an increase in fragmentation associated with the higher velocity. Decomposition will increase following the pulsed increase in the phosphorus concentration, as fungi and bacteria associated with the decomposition of organic matter will be phosphorus limited. I predicted there will be an additive effect of a combined increase in velocity and phosphorus on decomposition, whereby decomposition will be greatest when both velocity and phosphorus increase during a hydrological event.
3 Methods

3.1 Artificial Stream Facility

The experiment was conducted at the Thames River Experimental Stream Sciences (TRESS) Centre in London, Ontario (43° 0'57.39"N, 81°14'52.51"W) over a 31-day period in autumn 2018 (October 12 to November 12). TRESS is an outdoor artificial stream facility consisting of nine artificial streams that can be controlled and manipulated to assess the effect of physical and chemical conditions on biological communities. Each artificial stream is made of thermo-molded, food-grade plastic with a channel width of 0.24 m and a total channel length of approximately 6 m, incorporating three 180-degree bends (Figure 3.1).

![Diagram of artificial stream channels](image)

Figure 3.1. One of the artificial stream channels in both aerial schematic (left) and at the TRESS site (right).

The water is sourced from the local drinking water supply, which derives from Lake Huron (City of London, 2018). Monitoring of drinking water during 2018 found that pH averages 7.85 – 8.07, phosphorus <3 µg L⁻¹ and Nitrate + Nitrite ranged from 105-826 µg L⁻¹ (City of London, 2017). Source water was filtered through charcoal filters to remove chlorine before entering a 1000L head tank. Water is pumped from the head tank to 50 L
holding tanks below each artificial stream, from which water is pumped into the artificial streams at specified rates before draining back into the 50 L holding tank, forming a partially recirculating system with a residency time of approximately 2 hours. The facility enables water with controlled chemistry to be pumped into each artificial stream at specified rates.

3.2 Nutrient Supply and Stream Velocity

The concentration of phosphorus and nitrogen being delivered to the nine artificial streams was controlled throughout the experiment. A 1000 L mixing tank was used to achieve required concentrations of nutrients (ammonium nitrate (NH$_4$NO$_3$) and potassium phosphate (KH$_2$PO$_4$)).

The nutrient solution was drip fed into the head tank at a specified rate using a Pulsatron (Pulsafeeder Pulsatron Series A+) pump to achieve baseflow concentrations throughout the experiment. An aquarium propeller pump (Aqueon Circulation 950 Aquarium Water Pump) was installed in the head tank to ensure the nutrients were well mixed. Pulsafeeder Duplex pumps (Pulsar 25HJ) supplied the nutrients and water solution from the head tank to a 50 L holding tank below each artificial stream at a rate of 0.0475L·s$^{-1}$. Additional Pulsatron pumps were used to add phosphorus into the pipes that delivered water and nutrients to the holding tanks below the artificial streams to achieve the higher phosphorus concentration associated with a hydrological pulse event. The pulsatron pumps were calibrated daily through the experiment, while the Pulsafeeder Duplex pumps were calibrated approximately every two days to ensure that the nutrient supply was consistent and accurate.

A Goulds Centrifugal pumps (3656LH) below each artificial stream was used to circulate water from the 50 L holding tank to the artificial stream, thus determining the flow condition in the artificial streams. The flow rate of the Goulds pumps was controlled by an A/C inverter; however, flow rate was calibrated manually using an impeller flow metre which was placed at the inflow of the artificial stream. The artificial streams drained back into the holding tank to form a partially recirculating system which reduced the demand for water and further ensured that nutrients were well mixed before water
entered the channels. The velocity conditions within the artificial stream channels was measured with an impeller flow meter. Near bed velocity measurements were taken at 25%, 50% and 75% of the channel width within the middle of second, third and fourth channel of the artificial streams, an average velocity was then calculated.

### 3.3 Controlled Variables

Effort was made to minimise variation in controlled environmental variables between artificial streams. Variation in temperature and light between artificial streams was minimised by ensuring artificial streams were near each other and in a location with minimal and consistent shade throughout the day. Shade cloth was placed over each artificial stream to limit light intensity and to prevent external interference or inputs, such as animals, birds, insects or leaves. The shade cloth reduced the level of light entering the artificial stream by approximately 42%. Temperature and light were recorded throughout the experiment in each artificial stream at 15-minute intervals using HOBO loggers (HOBO pendant Temp/Light,64K), which were placed in the final channel of each artificial stream. Photosynthetically active radiation was recorded every ten minutes throughout the experiment using four Odyssey PAR loggers; two loggers were placed within two of the artificial streams and two loggers were placed on the perimeter fence of the facility. Throughout the experiment nitrogen was maintained at 1500 µg L⁻¹, which is above the limiting level for algae growth relative to the concentration of phosphorus (Rhee, 1978), thus isolating phosphorus as the limiting nutrient for algae growth. Cobble substrate with a d50 of approximately 5 cm was placed in the last three channels of each artificial stream to replicate a local stream habitat.

### 3.4 Biofilm

Prior to the start of the experiment, the cobble substrate was placed into a combination of mesh bags and metal cages and anchored in run habitats of Medway Creek in London, Ontario (43° 0'46.72"N, 81°16'50.40"W). Substrate remained in Medway Creek for 28 days to allow colonisation by the local biofilm community. After 28 days, the rock bags and metal cages were removed from Medway Creek and transported to the TRESS center before being distributed evenly among the nine artificial streams. To increase the initial
diversity of the biofilm community additional biofilm samples were collected from five local streams by scrubbing the surface from five cobbles into sampling containers. The five streams were in the Upper Thames River watershed ([43° 5′40.82″N, 81°10′6.03″W], [42°59′40.68″N, 81° 7′0.28″W],[ 43° 1′51.40″N, 80°59′54.45″W] and [43° 3′30.50″N, 80°59′38.14″W]) in southern Ontario and exhibited a range of soluble reactive phosphorus concentrations. The inoculum was homogenised, and aliquots were added into each artificial stream at the start of the experiment. Collecting multiple samples of biofilm from different streams has been utilised in other artificial stream studies on algae (Rier and Stevenson, 2006) to create a diverse pool of local algal species which can potentially colonise the artificial stream depending on the conditions.

3.5 Experiment Treatments

The velocity and phosphorus concentration were applied at two intensities; velocity was either at baseflow velocity (approximately 0.06 m s\(^{-1}\)) or an increased event velocity (approximately 0.20 m s\(^{-1}\)), while phosphorus concentration was either at a baseflow concentration (10 ug L\(^{-1}\)) or a higher event concentration (300 ug L\(^{-1}\)).

The experiment began with an initial colonisation period (18 days), where velocity and phosphorus concentration were both set at baseflow levels. Mid-way through the experiment, all artificial streams were exposed to a simulated 48-hour hydrological pulse event (days 19 and 20) (Table 3.1), following which conditions returned to baseflow conditions for the remainder of the experiment. The hydrological pulse event consisted of one of three sets of phosphorus and velocity conditions. Three artificial streams were assigned to each set of conditions or level. The first level, hereafter referred to as velocity level, involved an increase in velocity to the event velocity, with phosphorus concentration remaining at the baseflow level. The second level, referred to as ‘phosphorus’ level, involved an increase in phosphorus concentration to the event level with velocity remaining at the baseflow level. Finally, the third exposure hereafter called the ‘combined’ level involved increases in both velocity and phosphorus concentrations to event levels.
Table 3.1. The duration and levels of velocity and phosphorus concentration in the three treatments.

<table>
<thead>
<tr>
<th>Level</th>
<th>Parameter</th>
<th>Day 1 to 18</th>
<th>Day 19 to 21</th>
<th>Day 22 to day 31</th>
</tr>
</thead>
<tbody>
<tr>
<td>Velocity</td>
<td><em>Velocity (m s⁻¹)</em></td>
<td>0.06</td>
<td>0.2</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td><em>Phosphorus (ug L⁻¹)</em></td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Phosphorus</td>
<td><em>Velocity (m s⁻¹)</em></td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td><em>Phosphorus (ug L⁻¹)</em></td>
<td>10</td>
<td>300</td>
<td>10</td>
</tr>
<tr>
<td>Combined</td>
<td><em>Velocity (m s⁻¹)</em></td>
<td>0.06</td>
<td>0.2</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td><em>Phosphorus (ug L⁻¹)</em></td>
<td>10</td>
<td>300</td>
<td>10</td>
</tr>
</tbody>
</table>

3.6 Sampling Strategy

Prior to the start of the experiment, 100 unglazed ceramic tiles measuring 4.8 cm by 4.8 cm were placed on top of the cobble substrate within the artificial stream channels to provide a clean surface of known area for biofilm to colonise during the experiment. The colonised tiles were then used to sample indicators of ecosystem function (benthic chlorophyll-*a* for algal growth rates and metabolism). Indicators of stream ecosystem function were measured as follows.

3.6.1 Algal Growth

Chlorophyll-*a* was measured as an indicator of algal biomass abundance (Davies and Bothwell, 2012; Morgan *et al.*, 2006; Van Nieuwenhuyse & Jones, 1996). Average algae growth rate within each artificial stream was estimated by dividing the change in chlorophyll-*a* concentration at consecutive sampling intervals by the number of days between intervals, to provide a growth rate per day. Chlorophyll-*a* was sampled five times throughout the experiment, once before and four times after the pulse (Table 3.2).
Chlorophyll-\(a\) was sampled by scraping biofilm off tiles using a syringe with a brush attached to the end of the plunger (figure 3.2a), enabling a consistent 5.31 cm\(^3\) circular area to be sampled (figure 3.2b). At each sampling interval a total of nine tiles were sampled per artificial stream. A tile was selected from the upper, middle and lower portion of the last three channels of each artificial stream to incorporate the effect of spatial variability within the artificial stream on the sampled community. Two 5.31 cm\(^2\) areas was brushed from each tile, a brushed area from three tiles were combined in a sample pot, equating to six sample pots for chlorophyll-\(a\) per artificial stream per sampling event. Samples were transported to the Western StrEAMS. Laboratory and stored in a freezer at -12 °C before analysis.

**Table 3.2. Timing at which parameters were sampled through the 31-day experiment.**

<table>
<thead>
<tr>
<th>Sampled Parameter</th>
<th>Day of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>18</td>
</tr>
<tr>
<td>Chlorophyll-(a)</td>
<td>X</td>
</tr>
<tr>
<td>(Used for algal biomass and growth rate)</td>
<td></td>
</tr>
<tr>
<td>Benthic Metabolism</td>
<td>X</td>
</tr>
</tbody>
</table>
In the laboratory, three of the six chlorophyll-\textit{a} samples collected during each sampling event were randomly selected and analysed to determine the chlorophyll-\textit{a} content per area of the tile scraped using the hot ethanol extraction method (Castle \textit{et al}., 2011). For this method, samples were initially filtered through GF/C filters within a room lit only by a green bulb light. Filter papers were then submerged in 10 ml of 90\% ethanol within a 50 ml centrifuge tubes. The samples were placed in an 80 °C water bath for 7 minutes then left to cool to room temperature for a minimum of 30 minutes. A fluorometer (Turner Designs 10-AU Field Fluorometer with Red PMT Chlorophyll-\textit{a} optical kit) was then used to estimate the chlorophyll-\textit{a} concentrations of the sample solution. The chlorophyll-\textit{a} concentration was then calculated per cm$^2$ by accounting for the total 15.93 cm$^2$ area scraped from the three tiles. Once processed, an average of the three samples taken from each artificial stream during each sampling event was calculated providing one replicate per artificial stream and three replicates per treatment per sampling time point.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure32.png}
\caption{A syringe with a brush attached to the plunger (A), was used to sample a consistent circular area of biofilms from the tiles (B).}
\end{figure}
3.6.2 Sestonic Chlorophyll-a

Grab samples of approximately 500 ml of stream water were taken from the final channel of the artificial stream and analysed for chlorophyll-a concentration using the same procedure as described in section 3.6.1 (algal growth). Sestonic chlorophyll-a samples were collected before the pulse started, as well as 5 minutes, 30 minutes, 6 hours and 28 hours after the initiation of the pulse event. Samples were also collected on the day after the pulse stopped and on the final day of the experiment. Two samples were taken in the first 30 minutes of the exposure as it was expected that the increase in velocity would dislodge the majority of loosely attached algae within a short time period. Sestonic chlorophyll-a samples were filtered and processed in the laboratory using the hot ethanol extraction method described for the benthic chlorophyll-a samples; however, the concentration of chlorophyll-a was given per volume of sample by dividing the concentration per 1 ml of processed sample by the volume of the grab sample.

3.6.3 Benthic Metabolism

Benthic metabolism was sampled in each artificial stream (n=9) before and after the pulse exposure, as well as at the end of the experiment. Metabolism was measured using two 2.6 L sealed plastic chambers, which allowed isolation of a known substrate area within a known water volume. One chamber was clear (light chamber) allowing to assess metabolism under light conditions, the other chamber was covered (dark chamber) allowing assessment of metabolism in the dark (figure 3.3). Eight tiles with a representative biofilm community were selected from each artificial stream, providing a known and consistent area of biofilm community. Four tiles, with a combined upper surface area of 92.13 cm², were placed in the dark chamber and four were placed in the light chamber. The chambers were filled with water from the artificial stream and a D-OPTO oxygen logger was placed in each chamber. Chambers were then sealed with an airtight lid and placed in the first channel of the artificial stream to maintain water temperature and light conditions. Dissolved oxygen and temperature within the chambers were monitored by the D-OPTO’s at 5-minute intervals for approximately 4 hours during the middle of the day. The rate of change in dissolved oxygen levels was used to
calculate the metabolism of the biofilm community in the artificial stream at that time period. As conditions such as flow, temperature and water chemistry within the chambers differed from those in the artificial stream, the metabolism measurements were indicative of the metabolic potential of the biofilms rather than the realized rates in the artificial stream.

The rate of change in the concentration of dissolved oxygen was calculated by fitting a linear regression line through the dissolved oxygen data and measuring the slope of the line. The rate of change of dissolved oxygen was then used to calculate parameters of benthic metabolism (net metabolism, gross primary productivity and community respiration) based on the following equation (Grace and Imberger, 2006):

Figure 3.3. Benthic metabolism was calculated by placing tiles with a known surface area of biofilm within dark (left) and light (right) chambers and measuring changes in dissolved oxygen.

The rate of change in the concentration of dissolved oxygen was calculated by fitting a linear regression line through the dissolved oxygen data and measuring the slope of the line. The rate of change of dissolved oxygen was then used to calculate parameters of benthic metabolism (net metabolism, gross primary productivity and community respiration) based on the following equation (Grace and Imberger, 2006):
\[ \Delta \text{DO} = \text{GPP} - \text{CR} \]

Where:

\( \Delta \text{DO} \) = the rate of change in dissolved oxygen observed during the experiment.

\( \text{GPP} \) = gross primary productivity

\( \text{CR} \) = community respiration.

The rate of change in the dark chamber was used to calculate the rate of community respiration only, as the absence of light prevents primary production. The light chamber was used to calculate net metabolism under light conditions. The rate of gross primary productivity within the light chamber was then derived by subtracting the community respiration values of the dark chambers from the net metabolism values provided by the light chambers. Each benthic metabolism parameter was calculated as a rate per surface area unit by dividing the volumetric rate of change of dissolved oxygen by the known surface area. Community respiration was calculated based on equation 2 (Grace and Imberger, 2006).

Equation 2

Community respiration \( (\text{g } \text{O}_2 \text{m}^{-2} \text{ d}^{-1}) \)

\[ = \text{mean } R \times \text{chamber volume } \times 24 \]

\[ \text{/ photosynthetically active surface area } / 1000 \]

Where:

\( R \) = mean respiration rate in \( \text{mg } \text{O}_2 \text{ L}^{-1} \text{ hr}^{-1} \)

Chamber volume is given in L

Photosynthetically active surface area in m².
Gross primary productivity was calculated as the rate of change in dissolved oxygen within the light chamber plus the change in dissolved oxygen due to respiration calculated on the dark chamber (Grace and Imberger, 2006):

\[
\text{Gross primary productivity (g O}_2\text{m}^{-2}\text{ d}^{-1}) = \Sigma \text{GPP}_\text{flux} \times \text{chamber volume} \times 24 / \text{surface area} / 1000
\]

Where:

\(\Sigma \text{GPP}_\text{flux} = \Sigma (\text{rate of change in dissolved oxygen} - \text{respiration})\) is in mg O\(_2\) m\(^{-1}\) hr\(^{-1}\)

Chamber volume is in L

R is the average rate of respiration

Photosynthetically active surface area in m\(^2\)

Net daily metabolism could then be calculated as follows (Grace and Imberger, 2006):

\[
\text{NDM} = \text{GPP} - \text{CR}
\]

NDM is net daily metabolism in g O\(_2\) m\(^{-2}\)d\(^{-1}\)

GPP is gross primary productivity

CR is community respiration.
3.6.4 Decomposition

Decomposition was estimated using the cotton strip assay outlined by Tiegs et al. (2013). The cotton strip assay is a standardized method which uses artist canvas to assess decomposition in aquatic environments based on the loss of tensile strength of the fabric. Following this procedure, pieces of cellulose-based artist canvas (Fredrix-brand unprimed 12-oz. heavy-weight cotton) were cut into cotton strips measuring 8 cm in length and consisting of 27 individual threads width wise (approximately 2.5 cm). Five cotton strips were then attached to small weights and placed in the final channel of each artificial stream for the duration of the experiment. At the end of the experiment the five cotton strips were removed and soaked in 70% ethanol for 10 minutes to prevent further decomposition. The strips were then dried in an oven for 24 hours at 40°C. The tensile strength of the strips was measured by placing each strip within the grips of a test stand (Mark-10 G1008) which pulled the strip apart at a rate of 2 cm min⁻¹. A tensiometer (Mark-10, Model M3-100) recorded the peak tension (lbF) during the process of pulling the strip.

The loss of tensile strength was determined by comparing to that of reference strips. A group of 50 reference cotton strips were exposed to a simulated field deployment in which strips were soaked in distilled water before being cleaned with ethanol then dried in the oven for 24 hours at 40 °C (Webb, 2018). The data was corrected for temperature by calculating the loss of tensile strength per degree day using temperature data from HOBO loggers which were placed in the final channel of each artificial stream. The mean of the loss of tensile strength per degree-day was then calculated from the five cotton strips from each artificial stream using equation 5. The average from the three artificial streams within each treatment was then used as a replicate (n=3).

\[ \text{Tensile Loss (\%)} = \frac{(\text{Tensile Strength Reference} - \text{Tensile Strength Treatment})}{\text{Tensile Strength Reference}} \times 100 \]

\[ \text{Degree Day} \]
3.7 Data Analysis

A one-way analysis of variance (ANOVA) was applied to assess for a treatment effect on the decomposition data. A two-way analysis of variance was used to assess whether there was a statistically significant interaction between time and treatment for the chlorophyll-a, growth rate and metabolism data. Data were tested for homogeneity of variance and normality of residuals using the Levene's and the Kolmogorov-Simonov tests, respectively. If data failed to meet the assumptions of homogeneity of variance or normality for parametric analysis, log base ten or square root transformations were applied. The P value for all analysis was set to 0.1 owing to the small sample size (n=3) which limits the power of statistical analysis, thus increasing the likelihood of type two error. Data was analysed in R software 3.4.3 (R Core Team, 2019), utilising psych (Revelle, 2018) and dplyr packages (Wickham, 2017). Graphs were created in SigmaPlot version 12.3 (Systat Software, 2013).
4 Results

4.1 Experimental Conditions

Water temperature within the nine artificial streams averaged 12.49°C (SD=2.08 °C) and ranged from 6.27°C to 20.33°C (figure 4.1).

![Box plot showing temperature variation in artificial streams.]

Figure 4.1. Temperature within the nine artificial streams recorded at 15 minute intervals throughout the experiment.

4.2 Benthic Chlorophyll-α

Prior to the pulse starting (day 18) benthic chlorophyll-α samples within the phosphorus level artificial streams averaged 3.41 µg cm⁻² (SD=0.67 µg cm⁻²) which was 21% greater
than the combined level (2.81 µg cm\(^{-2}\), SD=0.64 µg cm\(^{-2}\)) and nearly 8% greater than the velocity level (3.17 µg cm\(^{-2}\), SD= 0.59 µg cm\(^{-2}\))(figure 4.2).

On the day after the pulse exposure ended (day 22), the average chlorophyll-\(a\) had increased within all artificial streams. The highest biofilm chlorophyll-\(a\) concentration was the combined treatment (6.31 µg cm\(^{-2}\), SD= 0.31 µg cm\(^{-2}\)), while the velocity had the lowest average (5.29 µg cm\(^{-2}\), SD= 1.55 µg cm\(^{-2}\)). Chlorophyll-\(a\) concentration in the phosphorus level at this time averaged 5.71 µg cm\(^{-2}\) (SD= 0.71 µg cm\(^{-2}\)).

The greatest difference in chlorophyll-\(a\) concentrations between levels was recorded on day 25, four days after the pulse exposure. The combined level had the highest average chlorophyll-\(a\) of 11.79 µg cm\(^{-2}\) (SD=2.09 µg cm\(^{-2}\)). The phosphorus level artificial streams had an average of 9.47 µg cm\(^{-2}\) (SD=1.48 µg cm\(^{-2}\)). In comparison, the velocity level artificial streams had a lower average concentration of 7.08 µg cm\(^{-2}\) (SD= 1.25 µg cm\(^{-1}\)), 40% lower than the combined level and 25% lower than the phosphorus level.

After 29 days of the experiment (8 days after the pulse) all three levels reached peak chlorophyll-\(a\) concentration. The relative differences between the averages of the three levels reduced (figure 4.2). The combined level had the highest peak chlorophyll-\(a\) concentration of 13.77 µg cm\(^{-1}\) (SD=2.95 µg cm\(^{-1}\)), 8% higher than the velocity level (12.72 µg cm\(^{-1}\), SD=4.47 µg cm\(^{-1}\)) and 14% higher than the phosphorus level (12.04 µg cm\(^{-1}\), SD=3.13 µg cm\(^{-1}\)).

On the final day of the experiment (day 31) the phosphorus level had the highest average concentration (9.93 µg cm\(^{-1}\), SD= 1.93 µg cm\(^{-1}\)), followed by the combined level (9.39 µg cm\(^{-1}\), SD=2.15 µg cm\(^{-1}\)). The velocity level averaged 7.50 µg cm\(^{-1}\), (SD=0.78 µg cm\(^{-1}\)), 24% lower than the phosphorus level and 20% lower than the combined level. A two-way ANOVA did not find a significant interaction between time and level for benthic chlorophyll-\(a\) (F=0.907, p=0.524).
Figure 4.2. Mean chlorophyll-a concentrations from sampled tiles within the three levels (velocity [white]; combined [dark grey]; phosphorus [light grey]) measured at five time points during the 31-day experiment. Error bars indicate standard deviation.

4.3 Algal Growth Rates

Mean algal growth rates ranged between 0.17 µg cm$^{-2}$ d$^{-1}$ (SD=0.04 µg cm$^{-2}$ d$^{-1}$) and 0.20 µg cm$^{-2}$ d$^{-1}$ (SD=0.04 µg cm$^{-2}$ d$^{-1}$) in the three levels during the first 18 days of the experiment (figure 4.3).

Between days 18 and 22, which included the pulse exposure, the algal growth rate within the velocity level averaged 0.53 µg cm$^{-2}$ d$^{-1}$ (SD=0.24 µg cm$^{-2}$ d$^{-1}$). The phosphorus level growth rate was nearly 9% higher for this period, averaging 0.58 µg cm$^{-2}$ d$^{-1}$ (SD= 0.34
µg cm$^{-2}$ d$^{-1}$), whereas the combined treatment was 64% greater than the velocity level, averaging 0.87 µg cm$^{-2}$d$^{-1}$ (SD=0.10 µg cm$^{-2}$ d$^{-1}$).

During the period after the pulse (day 22 to 25), the average growth rate within the velocity level was 0.56 µg cm$^{-2}$d$^{-1}$, (SD=0.26 µg cm$^{-2}$ d$^{-1}$). At the same time the phosphorus level growth rate had increased to 1.25 µg cm$^{-2}$ d$^{-1}$, 110% higher than the velocity level. The combined level had the highest average growth rate of 1.83 µg cm$^{-2}$d$^{-1}$ which was 207% higher than the velocity level and 46% higher than the phosphorus level, representing the highest growth rate recorded in any level during the experiment.

Contrastingly, between day 25 and 28, the velocity level had the highest growth rate of the three levels, averaging 1.41 µg cm$^{-2}$d$^{-1}$, which was 286% greater than the combine treatment growth level (0.49 µg cm$^{-2}$d$^{-1}$, SD= 1.25) and at 219% higher than the phosphorus level (0.64 µg cm$^{-2}$d$^{-1}$, SD= 1.25 µg cm$^{-2}$ d$^{-1}$) at this stage.

During the last two days of the experiment chlorophyll-α concentrations decreased in all levels, as demonstrated by the negative growth rates (figure 4.3). The decrease was greatest in the velocity level which had an average growth rate of -1.74 µg cm$^{-2}$d$^{-1}$ (SD=1.24). In comparison the combined level growth rate was -1.46 cm$^{-2}$ d$^{-1}$ (SD=1.24 µg cm$^{-2}$ d$^{-1}$), 19% higher than the velocity level. The phosphorus level averaged -0.703 µg cm$^{-2}$d$^{-1}$ (SD=1.69 µg cm$^{-2}$ d$^{-1}$), nearly 148% higher than the velocity level, this said the coefficient of variation was particularly high at this stage in the velocity and phosphorus level 70% and 240%, respectively.

Despite the differences outlined above, a two-way ANOVA conducted on algal growth rate found no significant interaction between the treatment levels and time (F=10.71, p=0.409).
Figure 4.3. Mean algae growth rate per day within the three levels (velocity [white]; combined [dark grey]; phosphorus [light grey]) estimated from the change in chlorophyll-a concentration between sampling intervals.

4.4 Sestonic Chlorophyll-a

There was a rapid increase in sestonic chlorophyll-a concentration within the velocity level and the combined level in the sample taken 5 minutes after the initiation of the pulse, which had declined in the samples taken 6 hours after the pulse initiation (figure 4.4).

Prior to the start of the pulse exposure sestonic chlorophyll-a in the three levels averaged between 0.40 μg L\(^{-1}\) (SD= 0.05 μg L\(^{-1}\)) and 0.44 μg L\(^{-1}\) (SD=0.09 μg L\(^{-1}\)). Five minutes
after the initiation of the pulse the average sestonic chlorophyll-\(a\) in the velocity level increased by greater than 1600\% from 0.42 \(\mu g\) L\(^{-1}\), to 6.81 \(\mu g\) L\(^{-1}\). The average in the combined level also increased by over 900\% to 4.01 \(\mu g\) L\(^{-1}\), whereas sestonic chlorophyll-\(a\) in the phosphorus level averaged 0.85 \(\mu g\) L\(^{-1}\) (SD= 0.19 \(\mu g\) L\(^{-1}\)). 30 minutes after the pulse initiation the velocity level and combined level both declined to averages of 3.80 \(\mu g\) L\(^{-1}\) (SD=0.864) and 3.684 \(\mu g\) L\(^{-1}\) (SD=1.25 \(\mu g\) L\(^{-1}\)), respectively. The artificial streams in the phosphorus level averaged 0.76 \(\mu g\) L\(^{-1}\) (SD=0.29 \(\mu g\) L\(^{-1}\)) at 30 minutes post pulse.

The average (\(n=3\)) chlorophyll-\(a\) concentrations within the grab samples collected 6 hours after the start of the pulse had declined by 2.75 \(\mu g\) L\(^{-1}\) in the velocity level and by 2.68 \(\mu g\) L\(^{-1}\) in the combined level, to averages of 1.05 \(\mu g\) L\(^{-1}\) (SD=0.32 \(\mu g\) L\(^{-1}\)) and 1.01 \(\mu g\) L\(^{-1}\) (SD=0.54 \(\mu g\) L\(^{-1}\)) respectively. In comparison, sestonic chlorophyll-\(a\) in the phosphorus level averaged 0.50 \(\mu g\) L\(^{-1}\) (SD=0.29 \(\mu g\) L\(^{-1}\)) at the same time point. The final set of samples taken during the pulse, 28 hours after the pulse began, showed a further decline in the average chlorophyll-\(a\) concentration in the combined level and the velocity level to 0.51 \(\mu g\) L\(^{-1}\), (SD=0.37 \(\mu g\) L\(^{-1}\)) and 0.82 \(\mu g\) L\(^{-1}\) (SD=0.122), respectively. The phosphorus level averaged 0.39 \(\mu g\) L\(^{-1}\) (SD=0.11 \(\mu g\) L\(^{-1}\)).
Figure 4.4. Sestonic chlorophyll-α before the pulse (day 18), during the pulse (after 5 minutes, 30 minutes, 6 hours and 28 hours), after the pulse (day 22) and at the end of the experiment (velocity [white]; combined [dark grey]; phosphorus [light grey]).

After the hydrological pulse event, sestonic chlorophyll-α within the three levels was similar, both after the pulse (day 22) and at the end of the experiment (day 31), with ranges comparable to the level average range of 0.40 μg L⁻¹ (SD= 0.05 μg L⁻¹) to 0.44 μg L⁻¹ (SD=0.09 μg L⁻¹) prior to the pulse. On day 22 the averages of the three levels ranged between 0.37 μg L⁻¹ (SD=0.15 μg L⁻¹) in the combined level and 0.48 μg L⁻¹ (SD= 0.09 μg L⁻¹) in the phosphorus level. At the end of the experiment the averages of the three levels ranged between 0.39 μg L⁻¹, (SD=0.11 μg L⁻¹) and 0.44 μg L⁻¹ (SD=0.13 μg L⁻¹) in the phosphorus and combined levels, respectively.

A two-way ANOVA conducted on the sestonic chlorophyll-α found a significant interaction between time and exposure level (F=4.974, p=<0.001). A post hoc tukey HSD
test showed that the significant difference between levels at sampling intervals occurred in the first (5 minute) and second (30 minute) sampling interval after the pulse began, where the velocity ($F=1.626$, $p=<0.001$ and $F=1.082$, $p=<0.001$, respectively) and the combined levels ($F=1.071$, $p=<0.001$, $F=1.043$, $p=<0.001$, respectively) were both significantly different compared to the phosphorus level.

4.5 Metabolism

4.5.1 Gross Primary Productivity

Before the pulse exposure gross primary productivity in the nine mesocosms averaged 2.52 g O$_2$ m$^{-2}$ d$^{-1}$ (SD=0.35 g O$_2$ m$^{-2}$ d$^{-1}$) (figure 4.5). On the day after the pulse exposure (day 22), gross primary productivity was highest in the combined level, averaging 4.24 g O$_2$ m$^{-2}$ d$^{-1}$ (SD=0.78 g O$_2$ m$^{-2}$ d$^{-1}$). The phosphorus level averaged 3.70 g O$_2$ m$^{-2}$ d$^{-1}$ (SD=1.11 g O$_2$ m$^{-2}$ d$^{-1}$), whereas the velocity level averaged 3.72 g O$_2$ m$^{-2}$ d$^{-1}$ (SD=0.18 g O$_2$ m$^{-2}$ d$^{-1}$), which was 13% and 12% lower than the combined level.

On the final day of the experiment (day 31), the phosphorus level (5.00 g O$_2$ m$^{-2}$ d$^{-1}$, SD=0.63 g O$_2$ m$^{-2}$ d$^{-1}$) had overtaken the combined level by less than 2% (4.92 g O$_2$ m$^{-2}$ d$^{-1}$, SD=0.32 g O$_2$ m$^{-2}$ d$^{-1}$) as the level with the highest gross primary productivity, with the highest average throughout the experiment. The velocity level averaged (4.08 g O$_2$ m$^{-2}$ d$^{-1}$, SD=0.20 g O$_2$ m$^{-2}$ d$^{-1}$), 23% lower than the phosphorus level and 21% lower than the combined level. Nevertheless, a two-way ANOVA found no statistically significant interaction between the three levels and time ($F=1.511$, $p=0.241$).

4.5.2 Community Respiration

Before the pulse exposure, community respiration within the three levels averaged -0.59 g O$_2$ m$^{-2}$ d$^{-1}$ (SD=0.24 g O$_2$ m$^{-2}$ d$^{-1}$), with the velocity level having the highest rate (-0.72 O$_2$ m$^{-2}$ d$^{-1}$, SD=0.20 O$_2$ m$^{-2}$ d$^{-1}$) and the phosphorus level having the lowest rate (-0.53 O$_2$ m$^{-2}$ d$^{-1}$, SD=0.36 O$_2$ m$^{-2}$ d$^{-1}$) (Figure 4.5).

On day 22, after the pulse, the average community respiration between the three levels was 0.659 g O$_2$ m$^{-2}$ d$^{-1}$ (SD=0.076), with the velocity level having the lowest average rate -0.57 g O$_2$ m$^{-2}$ d$^{-1}$ (SD=0.08 O$_2$ m$^{-2}$ d$^{-1}$), and the combined level having the highest average
rate \((-0.72 \text{ O}_2 \text{ m}^{-2} \text{ d}^{-1}, \text{SD}=0.09 \text{ O}_2 \text{ m}^{-2} \text{ d}^{-1})\). On the final day of the experiment, the average between the three levels was \(-0.57 \text{ O}_2 \text{ m}^{-2} \text{ d}^{-1} \text{ (SD}=0.11 \text{ O}_2 \text{ m}^{-2} \text{ d}^{-1})\), with the lowest average rate being the phosphorus level \((-0.46 \text{ O}_2 \text{ m}^{-2} \text{ d}^{-1}, \text{SD}=0.25 \text{ O}_2 \text{ m}^{-2} \text{ d}^{-1})\) and the highest average rate being with the combined level \((-0.69 \text{ O}_2 \text{ m}^{-2} \text{ d}^{-1}, \text{SD}=0.24 \text{ O}_2 \text{ m}^{-2} \text{ d}^{-1})\). There was no significant interaction between time and level for community respiration \((F=0.880, p=0.495)\).

### 4.5.3 Net Daily Metabolism

Average net metabolism before the pulse exposure (day 18) ranged between \(1.84 \text{ O}_2 \text{ m}^{-2} \text{ d}^{-1} \text{ (SD}=0.36 \text{ O}_2 \text{ m}^{-2} \text{ d}^{-1})\) in the combined level and \(2.02 \text{ O}_2 \text{ m}^{-2} \text{ d}^{-1} \text{ (SD}=0.16 \text{ O}_2 \text{ m}^{-2} \text{ d}^{-1})\) in the velocity level (figure 4.5). After the pulse exposure (day 22) the average net metabolism had increased in all three levels, averaging \(3.02 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1} \text{ (SD}=1.25 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1})\) in the phosphorus level, the combined level averaged \(3.52 \text{ O}_2 \text{ m}^{-2} \text{ d}^{-1} \text{ (SD}=0.87 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1})\), while the velocity level averaged \(3.14 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1} \text{ (SD}=0.20 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1})\). At the end of the experiment (day 31) net metabolism was highest in the phosphorus level and the combined level which averaged of \(4.54 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1} \text{ (SD}=0.47 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1})\) and \(4.23 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1} \text{ (SD}= 0.27\), respectively. In comparison, the velocity level averaged \(3.52 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1} \text{ (SD}=0.16 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1})\), which was 29% lower than the phosphorus level and 20% lower than the combined level. However, a two ANOVA found no significant interaction between time and level for net metabolism \((F=1.248, p=0.326)\).
Figure 4.5. Benthic metabolism (gross primary productivity [no hatch] and community respiration [cross hatches]) before (day 18) and after (day 22) the pulse and at the end of the 31 day experiment (velocity [white]; combined [dark grey]; phosphorus [light grey]).

4.6 Decomposition

The phosphorus and combined levels had average decomposition rates of 0.218 % loss of tensile strength per degree day (SD= 0.00738 % loss of tensile strength per degree day) and 0.220 % loss of tensile strength per degree day (SD= 0.0129 % loss of tensile strength per degree day), respectively. In comparison, the velocity level had the smallest average at 0.189 % loss of tensile strength per degree day, (SD= 0.0257 % loss of tensile strength per degree day; figure 4.6 bar graph of the decomp data), over 13% lower than
the phosphorus and combined levels. However, a one-way ANOVA showed no statistically significant difference between the three levels (F = 3.135, P = 0.117).

Figure 4.6. Loss of tensile strength in cotton strips per degree day between the three treatment levels (n=3).
5 Discussion

5.1 Statistical Power and Pseudoeplication.

The results of my study and subsequent statistical analysis using one-way and two-way ANOVAs identified only a single statistically significant interaction, which was the increase in sestonic chlorophyll-\(a\). It is possible that the alterations to velocity and phosphorus concentration during the two-day hydrological pulse event were not of a magnitude or duration great enough to cause a significant alteration to biofilm which is discussed in further detail later in the discussion.

The ability of my study to detect effects of phosphorus and velocity may have been limited by the small number of artificial streams included in the study, which equated to low statistical power for statistical analysis. Statistical power refers to the probability of a statistical test being able to reject a false null hypothesis (Nakagawa, 2004, Cohen, 1992). Statistical power can be calculated by subtracting beta from one, where beta or type two error is the probability of a test failing to reject the null hypothesis when the alternative hypothesis is true.

Statistical power analysis assesses four related variables associated with statistical inferences which are significance criterion (\(\alpha\)), sample size (\(n\)), statistical power and effect size (Cohen, 1992). A larger sample size increasing the statistical power of analysis, that is, the ability to detect smaller effect sizes and the significance criterion that can be used (Cohen, 1992). My study used nine mesocosms with three artificial streams serving as replicates within each of the three levels. Although multiple samples were taken from each artificial stream for decomposition and benthic chlorophyll-\(a\) during each sampling event, these samples were averaged to provide a single value for each artificial stream. Using multiple samples from each artificial stream as individual units of replication would increase the sample size and the statistical power of analyses. However, this approach was not utilised owing to the potential for criticism and false conclusions associated with pseudoreplication.
Hurlbert (1984) brought the idea of pseudoreplication into mainstream usage within the field of ecology to provide certain methodological guidelines for experimental design and analysis. Pseudoreplication is concerned with the use of inappropriate experimental designs or sampling procedures relative to the hypothesis being tested (Hurlbert, 1984). More specifically, pseudoreplication occurs when inferential statistics are used to test for treatment effects without the use of suitable treatment replicates, or if replicates are not statistically independent (Hurlbert, 1984). According to some definitions, experimental replicates must not be isolated spatially or temporally, nor can they be physically interconnected otherwise replicates are deemed non-independent (Hurlbert, 1984; Schank and Koehnle, 2009).

The definition of experimental units is therefore at the heart of determining whether a study is pseudoreplicated. Schank and Koehnle, (2009) describe experimental units as the recipient things that experimental manipulations or treatments are applied to. Hurlburt (1984) provides a stricter definition whereby an experimental unit is defined based on spatiotemporal proximity, physical boundaries and physical connectedness. According to the Hurlburt (1984) definition, each artificial stream in my study is an individual unit of replication, meaning that my study has a maximum sample size (N) of nine. This said, each of the artificial streams shared a common water source, therefore it could be suggested that each artificial stream within the three levels was spatially correlated and falsely defined as being independent meaning the levels were only replicated once (Davies and Gray, 2015). Using an N of nine limited the power of statistical analysis meaning there was an increased probability of type two error, failing to reject a false null hypothesis. The significance criterion or alpha level is selected prior to statistical analysis and determines the amount of risk of type one error within the analysis, which is the probability of rejecting the null when the null is true. An alpha of 0.1 was selected for analysis in this study, rather than the typical 0.05, which increased the probability of type one error but reduced the probability of type two error. Increasing the alpha to 0.1 was done to reduce the disparity between the relative probabilities of type one and type two error.
Substantially increasing the number of artificial stream replicates to increase the statistical power of this study was unfeasible owing to the associated financial and logistical costs. Artificial stream facilities like TRESS provide multiple benefits such as controlling environmental variables and reducing the resource and logistical demands of field studies, yet the experimental design and facility size limits the power of statistical analysis of this study when adopting the stricter definitions of replication units associated with pseudoreplication.

Since the publication by Hurlburt (1984) on pseudoreplication there has been much debate and concern surrounding the implication of this influential concept (e.g. Oksanen, 2001, Davies and Gray, 2015). Schank and Koehnle (2009) critique the definition of replication unit suggesting the definition of a unit based on spatial relationships or physical boundaries is an arbitrary and empirically based definition of statistical dependency rather than a logic-based definition. Moreover, there are currently no clear criteria for determining experimental boundaries (Schank and Koehnle, 2009). Schank and Koehnle (2009) suggest that statistical dependency can be accounted for in statistical models or through controlling physical conditions. While each artificial stream is a distinct physical unit, each unit within each level was seeded with the same biofilm community, received the same treatments and received the same environmental variable controls.

If the experimental units were not defined by physical boundaries, then there would be potential to define the tile or cotton strip as the experimental unit. Consequently, the sample size for analysis of benthic chlorophyll-\(a\) and decomposition would increase from an N of nine to an N of 27 and 45, respectively, thus increasing statistical power. This said, benthic chlorophyll-\(a\) samples were composite samples where each of the three samples per artificial stream were the average taken from scraping three areas from separate tiles into a single sample. When the artificial stream is the unit of replication, using composite samples for chlorophyll-\(a\) allows to better account for spatial heterogeneity within the artificial stream and provide a more accurate representation. If the tile was to be considered the unit of replication then the composite samples would potentially be breaching a new pseudoreplication error of sacrificial pseudoreplication.
Sacrificial pseudoreplication refers to the averaging of samples, which reduces the variability within the sampling unit increasing the likelihood of identifying statistically significant differences between groups.

Despite potentially committing sacrificial pseudoreplication, a two-way ANOVA was conducted on the benthic chlorophyll-\(a\) data using each of the three samples collected from each of the artificial stream (\(N=27\)), to explore the effect that the limited sample replication had on the results of analysis. Even with the higher sample size the two-way ANOVA did not find a significant time treatment interaction (\(F=1.166, p=0.325\)).

Similarly, a one-way ANOVA was conducted on the decomposition data using each strip as a unit of replication (\(N=45\)) and found a statistically significant difference between levels (\(F=14.03, p<0.001\)). Therefore, the results of the analysis conducted on the decomposition strips differs depending on the definition of the experimental unit.

The following discussion of the results of this study will use the analysis outlined in the results section in which the individual artificial streams were used as the units of replication, rather than the test results of analysis using individual tiles or cotton strips as units of replication, to avoid accusations of pseudoreplication. The fact that only one end point showed a statistically significant result may be attributed to the low statistical power associated with this study, which is largely a result of the low sample size. The sample size could not be increased as adding more artificial streams to TRESS would be logistically challenging and costly. Sample size cannot be increased with the current number of artificial streams owing to potential accusations of committing pseudoreplication based on certain definitions of units of replication. As the statistical power was low, the chance of type two error was higher. Therefore, the discussion will focus primarily on the percentage differences of each parameter which were outlined in the results section, rather than solely on the ANOVA results. Utilising the percentage differences will provide a more qualitative approach but will prevent the likelihood of omitting biological effects that were not detected by the statistical analysis which was limited by low power.
5.2 Discussion of Results

The results of this experiment suggest that there was a potential interaction between the pulsed increase in velocity and phosphorus leading to a subsidy response in algal productivity. Overall, the increase in phosphorus had a greater effect on biofilm function compared with the increase in velocity. The increase in velocity in the combined and velocity level was followed by a large increase in the sestonic chlorophyll-\(a\) which suggests that algae within the biofilm was removed from the cobble substrate and potentially the tile substrate. There did not, however, appear to be an associated decline in the amount of benthic chlorophyll-\(a\) on the tile substrate on the day after the exposure event within the combined treatment, which was higher than the phosphorus level. Similarly, velocity did not appear to negativity effect biofilm function in terms of metabolism, growth rate or decomposition as demonstrated by the fact that the combined level and phosphorus level tended to have comparable functional rates higher than the velocity level averages.

5.3 Interactive Subsidy Response

There was some evidence of an interactive subsidy effect of increased velocity and phosphorus on algal productivity (gross primary productivity, growth rate) within the combined level, despite the lack of statistically significant results. Algal growth rates within the combined level for the three days following the pulse were 46% higher than the phosphorus level and 207% higher than the velocity level. Similarly, the benthic chlorophyll-\(a\) was highest in the combined level after day 25, 25% higher than the phosphorus level and 66% higher than the velocity level. However, a two-way ANOVA using an N of 27 on log transformed data failed to identify an interaction between time and level (\(F=1.166, p=0.325\)). There was little evidence that respiration was enhanced in a similar way by the interaction between the pulsed increase in velocity and phosphorus.

Previous studies both in the field and within artificial streams have found evidence of a subsidy effect of increased velocity on biofilm biomass (Horner et al., 1990; Biggs et al., 1998; Humphrey & Stevenson, 1992). Increases in velocity can enhance biomass accrual by removing detritus from the biofilm and enhancing the mass transfer of nutrients.
through the boundary layer and into biofilm mats (Biggs and Stokseth, 1996). An experiment by Horner et al. (1990) conducted in laboratory channels assessed the response of benthic algal biomass to different increments of fixed velocity at several different concentrations of phosphorus. Horner et al. (1990) found a subsidy stress effect of velocity where increases in the fixed velocity from 0.1 m s\(^{-1}\) to 0.6 m s\(^{-1}\) caused an increase in biomass accrual; however, increased velocity beyond 0.6 m s\(^{-1}\) led to a decrease in biomass. The response of algae to certain velocities was also dependent on nutrient concentration, with increases in phosphorus from a background level to 7.5 µg L\(^{-1}\) leading to increased biomass accrual (Horner et al., 1990). Similarly, Hoellein et al. (2013) suggests that the stimulation of metabolism in streams following nutrient enrichment is dependent upon nutrients being delivered from the main water column to the benthos, which is partially dependent on velocity. However, under higher nutrient concentrations, the potential for an interactive subsidy effect may be diminished as the nutrient availability to cells may be optimal and not dependent on the increased delivery associated with higher velocity (Biggs et al., 1998). The phosphorus concentration within this study was increased from a relatively low baseflow of 10 µg L\(^{-1}\) to 300 µg L\(^{-1}\), and the response of algal growth observed suggests phosphorus availability to cells within the biofilms was sub-optimal prior to the pulse. While studies on the combined effects of increases in velocity and nutrients on biofilms have commonly focused on catastrophic high flow events (Uehlinger, 2006; Uehlinger, 2000; Cronin et al., 2007; Hart et al., 2013), or fixed velocity increased (Horner et al., 1990), my study provides evidence that relatively short (48 hour) pulses of velocity and phosphorus may generate an interactive subsidiary effect on algal.

The potential for a subsidiary effect of velocity on biofilm productivity may be dependent on the algal community within the biofilm. Biggs et al. (1998) suggests that dense coherent biofilms, such as those dominated by mucilaginous diatoms are more resistant to sloughing induced by shear stress yet may also have lower mass transfer rates of nutrients owing to the biofilms dense structure. Consequently, increases in flow may increase the rate of nutrient delivery to cells within the biofilm and enhance biomass accrual. In contrast, long filamentous green algal has a high open matrix and high surface area enhancing nutrient uptake yet increasing susceptibility to higher drag forces associated
with increased velocity (Biggs et al., 1998). Therefore, future studies on the interactive effects of velocity and nutrients on biofilm function should consider coarse taxonomy to aid interpretation of functional findings.

5.4 Effect of Phosphorus on Biofilm Function

Phosphorus concentrations have been shown to be an important factor influencing algal productivity and algal biomass during the accrual phase (Biggs and Close, 1989; Bondar Kunze et al., 2016; Stevenson, 1996). Benthic biofilms within streams receive a constant renewal of nutrients but are only able to assimilate a proportion of water column nutrients (Davies and Bothwell, 2012). The delivery of nutrients to biofilms within streams is temporally variable (Dent and Grimm, 1999), yet there has been little research looking at the effect of pulsed nutrient additions (Davies and Bothwell, 2012). My results indicated that the increased phosphorus concentrations within the phosphorus and combined level appeared to affect biofilm function, although no statistically significant differences were identified. The growth rate of algae within the biofilm was higher in the combined and phosphorus level during the period following the exposure compared with the velocity level, although the peak biomass was comparable between levels. Both gross primary productivity and decomposition at the end of the experiment was higher in the phosphorus and combined levels.

5.4.1 Effect of Phosphorus on Algal Productivity (Gross Primary Productivity, Algal Growth and Chlorophyll-α Concentration)

The findings of my thesis suggest that algal productivity was enhanced by the 48-hour pulsed increase in phosphorus, but the response was not immediate, as no substantive differences were observed between any of the levels on the day after the pulse ended. The chlorophyll-α concentrations within the phosphorus and combined level was 25% and 40% higher than the velocity level four days after the pulse (day 25). Similarly, growth rates in the phosphorus and combined level were 110% and 207% higher, respectively, during the post pulse period (day 22 to day 25) compared to the velocity level. On the day after the pulse exposure, gross primary productivity did not provide clear support for the hypothesis that phosphorus would stimulate productivity. The average of the combined
level was 15\% higher than the velocity level, suggesting the phosphorus addition in the combined level had an effect, yet there was minimal difference between the phosphorus level and velocity level. At the end of the experiment however, the gross primary productivity in the phosphorus and combined level was 21\% and 23\% higher than the velocity level, suggesting the addition of phosphorus had a subsidy effect. Therefore, algal productivity appeared to exhibit a delayed response to the pulsed increase in phosphorus.

Past studies assessing pulse phosphorus additions have found that microalgal are able to uptake phosphorus in excess of immediate growth requirements; however, algal biomass has not always responded to the increases in phosphorus (Davies and Bothwell, 2012; Humphrey & Stevenson, 1992). Davies and Bothwell (2012) found that algal biomass was able to respond to short but frequent (hourly) high concentration pulses of phosphorus. Humphrey & Stevenson, (1992) conducted an artificial stream experiment in which phosphorus concentrations were increased for a 24 hour period and found that phosphorus concentrations within algae increased immediately after the pulse; however, the relative abundance of algal taxon and ash free dry mass weight did not respond to the higher phosphorus levels either immediately after the pulse or 6 days after the pulse when compared to a control. The lack of response in the biofilm biomass within the Humphrey & Stevenson (1992) study is inconsistent with the delayed response in algal biomass and productivity identified in this study. A potential explanation for the difference in response between my thesis and Humphrey & Stevenson (1992) is the disparity in the concentration of phosphorus added during the pulse which was 300 µg L\(^{-1}\) compared to 30 µg L\(^{-1}\), respectively. The response of algal productivity was relatively short lived within my study as during the last two days of the experiment there was a rapid decline in algal biomass which was likely a result of sloughing. Biggs (1996) suggests that time to peak biomass can be faster under nutrient poor conditions, as base cells within the biofilm can become nutrient limited and slough earlier. The baseflow phosphorus concentrations in this study was relatively low which may explain why algal biomass peaked seven days after the pulse then began to decline.
5.4.2 Effect of Phosphorus on Community Respiration

There was no clear effect of the pulsed increase in phosphorus on community respiration. There have been few studies on the effect of pulsed phosphorus additions on community respiration, with the majority of studies assessing the effect of prolonged enrichment. Studies assessing the effect of a prolonged enrichment on community respiration within nutrient poor streams have commonly found that respiration increases with additions of nitrogen and phosphorus (Stelzer et al., 2003; Gulis & Suberkropp, 2003; Tank & Webster, 1998; Elwood et al., 1981; Guasch et al., 1995). Stelzer et al. (2003) found that additions of nitrogen and phosphorus to nutrient poor forest streams using nutrient diffusing substrate caused a significant increase in microbial respiration; however, samples were not tested until 6 to 9 weeks after deployment. Tank & Webster (1998) found that nutrient enrichment increased biofilm respiration on wooden substrate after 63 days but not after 29 days. Similarly, Gulis & Suberkropp (2003) assessed the effect of continuous nutrient additions on the respiration rates of biofilm on leaf matter and found phosphorus additions lead to higher respiration rates; however, the first measurement was taken after two weeks. Therefore, the lack of an effect of phosphorus additions on community respiration in this study may be a result of the short duration of the pulse exposure.

The substrate type on which biofilms develop influences their sensitivity to nutrient enrichment (Johnson et al., 2009; Stelzer et al., 2003). Several studies have found that respiration rates of biofilms on organic substrates are stimulated by nutrient enrichment (Elwood et al., 1981; Stelzer et al., 2003; Tank & Webster, 1998; Niyogi et al., 2003). For example, Niyogi et al. (2003) found that respiration rates were related to nutrient concentrations on organic substrate, with respiration strongly correlating to fungal biomass, suggesting heterotrophic respiration was influential. In contrast, biofilms which develop on inorganic substrate such as rocks have been found to be less sensitive to nutrient enrichment (Johnson et al., 2009). In this study biofilms were sampled from inorganic substrate (non-glazed ceramic tiles), potentially providing an explanation for the lack of an effect of the pulsed increase in phosphorus on community respiration.
The lack of response of ecosystem respiration in my study compared to previous nutrient addition studies may also be due to seasonality. Guasch et al., (1995) found that nutrient enrichment enhanced the amount of respiration to a greater extent during summer compared to winter. It is not possible to measure the direct contribution of autotrophs and heterotrophs to respiration rates (Kosten et al., 2013; Bott, 2006); however, increases in algal biomass can increase respiration rates (Morgan et al., 2006). Griffith et al., (2013) suggests that autotrophic respiration can comprise a high portion of community respiration, particularly during warmer months of spring, summer and autumn. Our study was conducted during a relatively cold autumn and algal biomass was low compared to similar past studies conducted at TRESS during summer (Pearce et al., unpublished). Therefore, future studies either at TRESS or in the field looking at the effect of pulsed phosphorus loading could focus on assessing seasonal variability in the effect of in pulse loading on biofilm function.

Past studies have shown that heterotrophs found in deeper sediments can have a significant contribution to ecosystem respiration (Naegeli & Uehlinger, 1997). The artificial streams, and particularly the benthic chamber method used to calculate metabolism does not include deeper benthic sediments. Metabolism was calculate using 4 tiles that had been placed on surface of the cobble substrate at the start of the experiment. Therefore, the lack of an effect of phosphorus additions on community respiration in this study, which contradicts past field studies on ecosystem respiration, may partly be due to the simplification of the stream environment used in this study. The use of surface tiles may also explain the dominance of gross primary productivity relative to the amount of community respiration recorded in this study, which is contrary to most field studies on ecosystem metabolism in stream which tend to be heterotrophic (Hoellein et al., 2013).

5.4.3 Effect of Phosphorus on Decomposition.

Decomposition and microbial respiration have been found to be significantly related (Niyogi et al., 2003). However, there was a more noticeable effect of the phosphorus additions on decomposition rates within my study compared to ecosystem respiration. This finding supports the growing body of evidence that decomposition can be stimulated by increases in nutrients (Elwood et al., 1981; Suberkropp and Chauvet, 1995; Gulis and
Suberkropp, 2003; Niyogi et al., 2003; Boulton and Quinn, 2000; Ferreira et al., 2015). A review of decomposition studies by Ferreira et al. (2015) found that decomposition tends to be stimulated by nutrient additions to a greater extent in streams where background concentrations is lower, yet enrichment is high. Prior to the initiation of the pulse the concentration of soluble reactive phosphorus was 10 µg L\(^{-1}\), which is relatively low (Dodds et al., 1998) yet was increased to 300 µg L\(^{-1}\) which is characteristic of a eutrophic stream (Dodds et al., 1998). Therefore, the increases in decomposition rates following the phosphorus pulse is consistent with the idea that decomposition within nutrient poor systems is susceptible to increases in nutrients (Ferreira et al., 2015).

Many previous studies looking at the effect of nutrients on decomposition have focused on continuous additions of nutrients (Suberkropp et al., 2010), fewer studies have focused on pulsed loading of nutrients. Indeed, Ferreira et al. (2015) highlight that there is currently a dearth of knowledge relating to the effect that the delivery mechanism of nutrients (i.e. continuous vs pulsed) exerts on decomposition, as well as the influence that stressor interaction has on the effect of nutrient on decomposition. While this study was not focused specifically on the effect of different delivery mechanism of phosphorus on decomposition, the results do suggest that decomposition can be stimulated by pulses in phosphorus. This finding would be strengthened by the inclusion of a control level as data derived from the phosphorus level could be compared with the control where no pulse in either velocity or phosphorus would act as a confounding variable. The increase in decomposition resulting from a pulse of phosphorus is consistent with the findings of past studies on decomposition conducted at the TRESS facility which suggest that the delivery mechanism of phosphorus (i.e. pulse of phosphorus or a continuous press) does not affect decomposition rates (Sauro, 2019).

This said, studies conducted in laboratory streams tended to find that nutrients have a greater effect compared with studies conducted in the field (Ferreira et al., 2015). In the field multiple stressors interact to influence decomposition rates (Young et al., 2008). Artificial streams are a simplification of the natural system which may increase the likelihood of detecting an effect of nutrient enrichment as it reduces the effect of confounding factors (Ferreira et al., 2015). Consequently, studies conducted in artificial
streams such as my study may overestimate the effects of nutrients on decomposition. Therefore, conducting field studies in addition to artificial stream studies would provide a more realistic environment to assess the effect of nutrient enrichment. Nevertheless, these results support the idea that anthropogenic increases to pulsed nutrient loading may alter and degrade the trophic status of streams (Ferreira et al., 2015).

### 5.5 Effect of Velocity on Biofilm Function

In my experiment, the initiation of the pulse exposure was followed by a significant increase in sestonic chlorophyll-\(a\) in the combined and velocity levels after both 5 and 30 minutes. Such an increase in sestonic chlorophyll-\(a\) suggests that the increased shear stress may have removed benthic algae from the biofilm and lifted it into suspension. The rapid increase in sestonic chlorophyll-\(a\) following the increase in velocity is similar to the results of laboratory experiment by Horner et al. (1990) and Biggs and Thomsen (1995). Biggs and Thomsen (1995) assessed the effect of velocity on filamentous algae and found that the majority of the removal of benthic algae occurred within 10 minutes of the beginning of an increase in velocity. Sestonic chlorophyll-\(a\) in this study remained significantly different after 30 minutes of the increase in velocity; however, this may be explained by the partially recirculating artificial stream system used in this study.

While there was an increase in sestonic chlorophyll-\(a\) with increased velocity, there was no noticeable reduction in benthic chlorophyll-\(a\) sampled from the tiles in the velocity and combined level compared to the phosphorus level following the pulse (day 22). Similarly, there was no clear negative effect of velocity on metabolism parameters following the pulse. An absence of an effect on both benthic chlorophyll-\(a\) and metabolism is expected given chlorophyll-\(a\) concentrations have been found to correlate with metabolism parameters such as maximum photosynthesis and community respiration (Biggs et al., 1999a). Thus, the increase in sestonic chlorophyll-\(a\) is almost certainly the result of algae being removed from the cobble substrate in the artificial streams. The lack of a negative effect of velocity on the chlorophyll-\(a\) concentrations sampled from the biofilm on the tiles may be explained by several factors including: the relatively low increase in velocity, the relatively young age or taxonomic composition of
the algae on the tiles prior to the pulse exposure or potentially a rapid regrowth of algae on the tiles following the initiation of the pulse.

 Increases in velocity have been shown to impact biofilm communities through two main mechanisms of increased bed movement or increased drag forces applied to the community (Biggs et al., 1998). The lack of a negative effect of velocity observed in my study can partially be explained by the fact that the increase in velocity did not reach the critical threshold for bed movement, as the cobble substrate and tiles were stable throughout the experiment. The majority of bedload transport within gravel bed rivers is assumed to occur above a critical threshold in flow (Ferguson, 2005). Increases in velocity that reach this critical threshold and cause bed sediment to move have been shown to impact biofilm communities by disturbing benthic habitat and increasing abrasion (Biggs et al., 1999a; Biggs and Close, 1989). Within fluvial geomorphology, the critical threshold for bedload mobilisation is often assessed using calculations of shear stress (Petit et al., 2005); however, Bagnold (1980) proposed that stream power per unit area of the stream could be used to estimate bedload movement in the absence of more specific data. Stream power is derived from discharge, the weight of water and channel slope, and provides an indication of the ability of a stream to transport sediment (Allan and Castillo, 2007; Gartner, 2016). Specific stream power accounts for the channel width and is calculated as follows:
Equation 6.

\[ \omega = \frac{\rho g Q S}{W} \]

Where: \( \omega = \) Specific stream power

\( \rho = \) fluid density

\( g = \) acceleration due to gravity

\( Q = \) discharge

\( S = \) longitudinal slope

\( W = \) channel width

The approximate specific stream power within the velocity and combined level during the hydrological pulse event was calculated as 0.45 W m\(^{-2}\), which is substantial lower than the critical stream power required to mobilised the cobble substrate in the artificial streams (\( d_{50} \approx 5 \) cm) when compared to data from Petit et al. (2005) (Figure 5.1)
Figure 5.1. Critical specific stream power and sediment diameter within gravel bed rivers in Belgium and mountainous boulder rivers in France and Colorado, United States from Petit et al. (2005), approximate stream power and sediment diameter in the artificial streams in red.

Nevertheless, increases in shear stress during hydrological pulse events that do not move bed sediments also have the potential to remove biofilms from substrate, causing a reduction in biomass and function (Biggs et al., 1999a; Biggs and Thomsen, 1995). The results of my study, however, suggest that the increase in drag forces associated with the velocity increase did not remove biofilm.

Velocity was increased from approximately 0.06 m s\(^{-1}\) to 0.2 m s\(^{-1}\) in this study, equating to a 3.33-fold increase. Velocity could not be increased beyond 0.2 m s\(^{-1}\) within the current artificial stream setup as water began to over top the sides of the artificial streams. However, the level of increase in velocity may have been insufficient to cause substantial loss of biofilm from the tiles. An artificial streams experiment by Horner et al. (1990) assessed the effect of sudden velocity increases on biofilm biomass and found that the magnitude of velocity increase was important in determining the amount of biomass lost.
Horner *et al.* (1990) found that increases in flow from an initial 0.2 m s\(^{-1}\) to 0.8 m s\(^{-1}\) (four-fold increase) caused a loss of biomass twice that of the loss associated with an increase from 0.2 to 0.6 m s\(^{-1}\) (three-fold increase). Additionally, the initial velocity in the Horner *et al.* (1990) experiment (0.2 m s\(^{-1}\)) was equal to that of our increased velocity exposure which may suggest the velocity in this study was too low. Multiple studies within the literature that looked to assess the effect of velocity on biofilms have used velocity ranges higher than the low (0.06 m s\(^{-1}\)) and high (0.2 m s\(^{-1}\)) velocities used within this study. For example, a laboratory experiment by Francoeur & Biggs (2006) used a ‘realistic range of water velocities’ of between 0.5 m s\(^{-1}\) and 1.8 m s\(^{-1}\). A similar artificial stream experiment by Ateia *et al.* (2016) assessed the effect of low, medium and high velocities using increments of <0.18 m s\(^{-1}\), 0.18 m s\(^{-1}\) to 0.32 m s\(^{-1}\) and > 0.32 m s\(^{-1}\), respectively. Likewise, a field experiment by Biggs and Stokseth (1996) assessed biomass accrual within multiple patches of two rivers and characterised velocities up to 0.3 m s\(^{-1}\) as low, with velocities over 0.7 m s\(^{-1}\) being class as high. This suggests that the level of velocity that was used in this study was relatively low and insufficient to cause biofilm loss due to the increased drag effect based on the community present. Therefore, future studies conducted at TRESS aimed at assessing the effect of velocity increases during hydrological pulse events could modify the artificial stream facility to enable higher velocities to be achieved.

The effect that increases in velocity exerts on the biofilm can be dependent on the age, biomass and resistance associated with the algal communities present within the biofilms, particular in relation to smaller disturbance events (Peterson, 1996; Francoeur and Biggs, 2006; Biggs and Thomsen, 1995; Biggs and Stokseth, 1996). For example, biofilms dominated by filamentous algae have been shown to be more susceptible to shear stress increases compared with non-filamentous tightly adhered diatom communities (Biggs and Thomson, 1995; Biggs *et al.*, 1998). Differences between the communities present on the sampled tile substrate compared to the cobble substrate may explain why the increase in sestonic chlorophyll-\(a\) after the initiation of the pulse likely came from the cobbles and the tile biofilm did not appear to decrease. During the colonisation and early accrual stages of succession, biofilms tend to initially be dominated by adnate diatoms which give way to apically attached diatoms which are then followed by green filamentous
algae at later accrual stages where biomass is higher (Biggs, 1996). As the artificial tile substrate was clean at the start of the experiment it is possible that a diatom community, which are less susceptible to increases in velocity, was the first to colonise the tiles and had a greater relative abundant at the start of the pulse. In contrast, the cobble substrate was inoculated within a local stream for 28 days prior to the experiment, meaning that the algae community on the cobbles was at a more mature successional stage than the biofilm community on the sampling tiles. Biofilm communities at later succession stages tend to have higher biomass and cells at the base of the community receive less resources, such as light and nutrients, increasing susceptibility to sloughing (Saravia et al., 1998).

Diatom taxa also tend to be more abundant within biofilms under lower nutrient concentrations, while communities dominated by filamentous algae, blue green algae or cyanobacteria occur within the accrual phase under higher soluble reactive phosphorus concentrations (Horner et al., 1990). The initial phosphorus concentration of 10 ug L\(^{-1}\) used within this study is relatively low, thus potentially favoring diatom taxa (Horner et al., 1990). The initial accrual of biomass on the cobble substrate took place within Medway Creek, which has a catchment area dominated by agriculture and a phosphorus concentration that tends to be higher than the 10 µg L\(^{-1}\) baseflow concentration within the artificial stream experiment (Upper Thames Conservation Authority, 2017). Therefore, communities of filamentous algae may have been more abundant on the cobble substrate compared to the tile substrate. Filamentous algae communities are more susceptible to sloughing under increased velocity (Biggs et al., 1998). For example, a field study by Biggs et al. (1998) on the effect of increased velocity within a nutrient enriched stream on benthic algae communities at peak biomass found that chlorophyll-\(a\) concentrations within filamentous green algae communities decreased when near bed velocity increased above 0.2 m s\(^{-1}\) whereas biomass and chlorophyll-\(a\) derived from mucilaginous diatom communities increased with velocity. This study by Biggs et al. (1998) suggests that the biofilm community present is an important factor not accounted for in this study and also provides further support for the idea that the increase in velocity to 0.2m s\(^{-1}\) was not high enough to initiate sloughing, as discussed above. Therefore, both the succession stage and the community present within the biofilms may have contributed to the lack of an effect of velocity on the biofilms on the sample tiles, thus further investigations into the relative
effects of velocity and phosphorus increases associated with a hydrological pulse events should include course level taxonomy assessments.

An additional factor that may have contributed to the lack of an impact of velocity on algal biomass and productivity is the potential for rapid regrowth following any initial losses. Regrowth after small storm events can be rapid due to the high availability of propagules (Biggs, 1996). Horner et al. (1990) saw a short-lived loss of biofilm following the initiation of a velocity increase within a flume experiment; however, there was no clear decrease in biofilm biomass the following day which they suggest may be the result of a rapid regrowth of biofilm. It is possible that the velocity increase caused a loss of biomass from tiles within this study, however there was over two days in between the initiation of the exposure period and the sampling post exposure which may have allowed for rapid regrowth within the velocity and combined levels, potentially explaining the lack of disparity seen in the chlorophyll-α data between levels. Additionally, the lack of a control level limits our ability isolate the effect of velocity as the phosphorus level does not provide a like for like comparison.

5.5.1 Effect of Velocity on Respiration

Ecosystem respiration within field studies has been found to be more resistant to increases in velocity compared to primary production potential owing to the fact that heterotrophic organisms within deeper sediments are less exposed to velocity compared to surface autotrophs (Acuña et al., 2011; Uehlinger and Naegeli, 1998; Uehlinger et al., 2003; Uehlinger, 2006). In contrast, Biggs et al., (1999a) measured community respiration within an experimental setting and found scouring had a greater effect on community respiration as detritus was removed from the biofilm leading to a relative increase in autotrophy. There was, however, limited evidence of an effect of velocity on community respiration within my study.

5.5.2 Effect of Velocity on Decomposition

Alike the measures of autotrophic function, velocity did not appear to impact decomposition. Indeed, ANOVA tests showed no statistically significant effect of velocity, regardless of whether analysis was conducted with an N of 9 with each artificial
stream being a unit of replication, or an N of 45 with each strip representing a unit of replication. Similarly, the percentage differences showed velocity had little effect. Our results contradict past studies which have shown that increases in velocity can stimulate increases in organic matter decomposition (dos Santos Fonseca et al., 2013). For example, a study conducted in Sweden during autumn found that high velocities occurring during flood events was the most important factor driving detritus breakdown within streams, suggesting that under high flows, physical abrasion and fragmentation was an important driver of decomposition (Lepori et al., 2005). This study by Lepori et al. (2005) suggested that the higher current velocities within channelized streams during floods, which reached 0.54 m s\(^{-1}\), contributed to the higher rates of decomposition compared to restored reaches, where velocity peaked at 0.42 m s\(^{-1}\). It is possible that no such influence of velocity was observed within my study owing to the lower velocity (0.2 m s\(^{-1}\)) associated with the pulse event.
6 Study Limitations and Future Research

There were several factors associated with this study that limited the ability to conclusively address the study aim. The low statistical power associated with this study, which was discussed in section 4.1, may have contributed to only a single statistically significant difference being identified by ANOVA analysis. Each treatment level was replicated three times which equated to low statistical power. Logistical and financial constraints prevented the expansion of the TRESS facility and increasing the units of replication with the current TRESS facility was not done due to potential for criticism of pseudoreplication. Consequently, the discussion focused on more descriptive percentage-based differences rather than inferential statistics. A further limitation was the lack of a control group in which neither velocity nor phosphorus were increased during the pulse exposure. Including a control was not possible in this study as the TRESS facility currently has nine artificial streams. While a control was not essential to address the primary study aim, which focused on the interaction, a control level would have enabled to assess spatial variability within the system and better identify the individual effects of both velocity and phosphorus on the biofilm functional endpoints (Davies and Gray, 2015).

Regardless of the limitation of this study, Lamberti & Steinman (1993) suggest that individual artificial stream experiments commonly contribute little to the progression of understanding of natural systems. Rather, understanding is developed by synthesising the results of a sequence of strategically connected experiments. Therefore, the following section will outline potential future experiments related to velocity and phosphorus that could be conducted at experimental stream facilities, such as TRESS, or in the field to help progress understanding.

A potential area for further investigation is assessment of the influence that algal community composition exerts on the functional response of biofilms to hydrological pulse events. Past studies have found that the response of benthic algae to variations in both velocity and nutrients is influenced by the structure and ages or successional stage of the community (Biggs et al., 1998; Horner et al., 1990). Therefore, an assessment of
course level taxonomy could aid interpretation of functional observations in future studies. Such investigations may also contribute in progressing the current knowledge gap linking structural and function responses to anthropogenic stressors (Sandin and Solimini, 2009).

The applicability and scope of this study is limited by the fact that only a single pulse condition was tested following and preceding a single baseflow condition. In natural streams hydrological pulse events will alter velocity and phosphorus concentrations to varying degrees both temporally and spatially as a result of both natural variability and anthropogenic alterations. Therefore, additional artificial stream studies could assess the effect of differing levels of velocity and phosphorus increases associated with hydrological pulse events. Additionally, the baseflow conditions within streams are also highly variable spatially (e.g. between streams or different reaches, or as a result of land use alterations) and may change seasonally. Therefore, future studies could also assess the effect of hydrological pulse events within streams with different baseflow conditions. For example, an artificial stream experiment on the effect of pulsed increases in velocity and phosphorus could utilise a gradient of nutrient concentrations during baseflow periods before and after the pulse event.

In addition, further studies could investigate the influence of temporal variability at different scales on the effects of hydrological pulse events on biofilm communities. The successional stage of biofilm influences the community structure, function and the resistance (Hoellein et al., 2010). Therefore, the timing and frequency of successive pulse events has the potential to influence the effect of hydrological pulses on biofilm communities. For example, Biggs (1996) found that the frequency of flow disturbance events was an important control on the structure of algal within biofilms in New Zealand rivers. An artificial stream study could investigate the influence that the biofilms’ successional stage exerts on the functional response by comparing the response of biofilms to a consistent pulse event that occurs at a gradient of initiation times following colonisation. Such an investigation may further our understanding of the natural variability of biofilm responses to hydrological pulse events as well as the potential ecological effect that the frequency of hydrological pulse event exerts.
At a larger temporal scale, seasonality also affects biofilm structure and function (Griffith et al., 2013; Guasch et al., 1995; Uehlinger, 2006). For example, seasonality has been shown to influence the response of biofilms to continuous nutrient enrichment (Hoellein et al., 2010; Olapade & Leff, 2005). Additionally, the effects of flow disturbance on stream metabolism has been shown to vary seasonally (Biggs, 1999a). I conducted my study during mid to late autumn, and biomass was lower than similar studies conducted at TRESS using the same baseflow and pulse nutrient concentrations during mid-summer (Pearce et al., unpublished data). However, few studies have investigated the influence of seasonality on the response of biofilms to pulsed increases in velocity and phosphorus. This knowledge gap could be investigated by replicating the experimental design used in my study at different times of the year, such as during summer and comparing the response.

Studies conducted within artificial streams sacrifice realism for environmental control (Lamberti & Steinman, 1993). For example, my study did not account for multiple variables such as increases in turbidity, abrasion associated with suspended sediments or grazing pressure, which have the potential to interact and influence the effect of changes in velocity and phosphorus on biofilm function (Francoeur and Biggs, 2006; Horner et al., 1990; Niyogi et al., 2003; O’Connor et al., 2012; Bernhardt et al., 2017). Artificial streams are also limited in scope and are not representative of a natural stream as they do not account for the influence of multiple groups of organisms such as macrophytes and phytoplankton, which influence stream ecological function (Kaenel et al., 2000). Consequently, artificial stream studies may provide a limited representation of the effect of environmental controls on stream ecosystem function. For example, Ferreira et al. (2015) identified that the effect of nutrients on decomposition is often greater in studies carried out within artificial streams compared to field experiments, which suggests that artificial stream experiments may overestimate the effects of nutrients. Additionally, artificial streams do not account for temporal or spatial variability associated with natural streams. Therefore, results of studies on hydrological pulses events derived from experiments conducted within artificial stream may benefit from comparison and verification using field-based studies.
7 Summary

My thesis investigated the effect of increased phosphorus concentrations and velocity associated with hydrological pulse events on stream biofilm function by conducting a 31-day artificial stream experiment. Only a single statistically significant time treatment interaction was identified by ANOVA analysis which was that of a that of an increase in sestonic chlorophyll-a at the beginning of the pulse exposure following the increase in velocity. The lack of statistically significant results is potentially a result of the low statistical power associated with having three units of replication per level. However, when comparing the percentage differences between the three levels, the results suggest a potential interactive subsidy effect of simultaneous increase in velocity and phosphorus concentration on benthic algal productivity. Measures of benthic metabolism found that gross primary productivity on the day after the pulse exposure was greatest in the combined level, where both velocity and phosphorus were increased compared to the artificial streams where only velocity or phosphorus were increased individually. Similarly, benthic algal biomass and algal growth, both measured using chlorophyll-a, were greater in the combined level in the three-day period after the pulse exposure when compared with the velocity level or the phosphorus level during the same time period.

When considering the effect of individual increases in velocity and phosphorus, the increase in phosphorus appeared to have a greater effect on biofilm function than velocity. Decomposition within the combined and phosphorus level was comparable, and higher than that of the velocity level. Similarly, algal productivity (gross primary productivity, algal biomass and algal growth rates) appeared to show a positive but slightly delayed response to the pulsed increase in phosphorus. In contrast, the pulsed increase in velocity did not have a substantive negative effect on algal productivity despite the increase in sestonic chlorophyll-a following the initiation of the pulse event, nor was decomposition increased as a result of increased fragmentation. The lack of predicted effects of increased velocity were likely a result of the relatively low level of velocity increase which did not reach the critical threshold to initiate bed movement and was insufficient to tear the biofilm from the substrate. Ecosystem respiration appeared to show no substantive response to the treatments.
The results of this study are limited by low statistical power associated with the experiment having nine units of replication. The applicability of the findings of this artificial stream experiment are compromised by the fact that only a single set of velocity and phosphorus concentrations were tested. Moreover, artificial streams are simplified systems which do not account for the spatial and temporal variability of natural streams. Nevertheless, the findings of this study may contribute in progressing understanding of the effects of hydrological pulse events on biofilms and ecosystem function. Pulse and increases in velocity and nutrient loading associated with hydrological pulse events are major factors influencing stream biofilm. Both the hydrological regime of streams and nutrient delivery patterns are heavily influenced by anthropogenic land use alteration such as agriculture and urbanisation (Bondar-Kunze et al., 2016; Hart et al., 2013; Baker et al., 2004). Therefore, understanding how increases in velocity and nutrients may interact to influence key ecological processes within streams is important. This study has demonstrated that simultaneous increases in velocity and phosphorus concentrations may cause an interactive subsidy effect on primary productivity. The potential interactive effects of velocity and phosphorus increases could alter the energy flow within ecosystems and further research is warranted. Further investigation into the effects of hydrological pulse events on biofilm function will progress understanding of biofilm ecology. Additionally, such research may aid in river management including mitigation of the ecological impacts of changes to hydrology and nutrient loading associated with land use alterations and climate change.
References


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Curriculum Vitae

Name: Chris Lucas

Post-secondary: Meadowhead Sixth Form and Sheffield College
2011 - 2013

Education and Degrees:
University of Gloucestershire, Gloucestershire, England
2013- 2016 BSc Hons

Queen Mary University of London, London, England
2016- 2017 MSc Integrated Management of Freshwater Environments

University of Western Ontario, London Ontario.
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Honours and Awards:
University of Gloucestershire Mike Addis Geography Prize 2016.
Runner up in the British Hydrological Society Dissertation Competition 2016.

Related Work
The University of Western Ontario.

- River Ecosystems GEOG3343
- Natural Environments GEOG 2131
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CREATE Internship – August, 2018

Don Catchment Rivers Trust, Doncaster, South Yorkshire, England.

Assisted with community engagement events, stream restoration projects and catchment walkover surveying.