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Relationships between Didymosphenia geminata occurrence, blooms and environmental conditions

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Relationships between *Didymosphenia geminata* occurrence, blooms and environmental conditions

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Relationships between *Didymosphenia geminata* occurrence, blooms and environmental conditions" submitted by Lisa Maureen Corbett in partial fulfilment of the requirements of the degree of Master of Science.

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Abstract

Algal blooms have often been attributed to an increase of nutrients that results in increases in maximum algal biomass. However, *Didymosphenia geminata*, a diatom blooming in Canadian streams for the past 30 years, blooms in oligotrophic streams. I examined the environmental conditions and algal nutrient use of low nutrient Rocky Mountain streams where *D. geminata* experiences significant blooms. I determined that the local variables of low water velocity, low water temperature and low concentrations of total phosphorus were important predictors of *D. geminata* cell abundance. The conditions that promote algal blooms were not clearly established, as occurrence of blooms was not strongly related to an increase or decrease in phosphorus concentration. These results suggest there may be a complex combination of nutrients and environmental conditions that promote blooms that were not identified within the scope of this study. However, as I had no consistent conditions that related to the presence of *D. geminata* blooms, an alternative hypothesis is that there may be multiple strains of *D. geminata.* There may be strains that are able to bloom and others that cannot. This study has opened many doors into further work that needs to be conducted on *D. geminata* before we can fully understand the factors that cause this diatom to create noxious algal blooms.

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Chapter One: **General Introduction**

Invasive species can restructure ecosystems (Baxter et al. 2004). Biological invasions occur when an organism is introduced outside its native range. Introductions may be due to human activities and range from unintentional (e.g., transport on equipment, ballast water etc.) to accidental (e.g., spread of purple loosestrife, *Lythrum salicaria* L.) to intentional (e.g., many stocked fish species such as Rainbow trout (*Oncorhynchus mykiss*) in numerous Alberta ponds). Aquatic invasions have occurred globally and led to demonstrable changes in ecological function. For example, the zebra mussel (*Dreissena polymorpha*) invasion of the Great Lakes has altered nutrient cycling (Johannsson et al. 2000), water clarity (Idrisi et al. 2001) and food web structure and function (Vanderploeg et al. 2002). *Bythotrephes longimanus,* a crustacean zooplankton, has invaded many North American lakes and affected community structure by reducing total zooplankton species richness and abundance (Strecker and Arnott 2008, Strecker et al. 2006). Aquatic invasions may alter ecological processes such as competition and predation, nutrient cycling, and energy cycling (Mack et al. 2000, Vitousek 1990). The effects following invasion can propagate through entire ecosystems because responses can occur at all trophic levels (Crooks 2002) potentially changing fundamental ecological properties such as the dominant species in the community, nutrient cycling, primary production and the physical features of the ecosystem itself (Mack et al. 2000, Vitousek 1990).

Algal blooms, a rapid accumulation of algal cells, represent another change in ecosystem structure that can restructure an ecosystem. Algal blooms often occur following an increase in anthropogenic nutrient input or other form of ecosystem enrichment (Sunda et al. 2006, Schindler 1977). When the bloom alters ecosystem function it is referred to as an environmentally degrading algal bloom (Sunda et al. 2006), and is often associated with toxic or unpalatable algae that reduce zooplankton grazing and disrupt nutrient and energy flow to higher trophic levels (Turner and Tester 1997). The amount of algae that constitutes a nuisance algal bloom is hard to define; Winter et al. (2011) defined blooms as "excessive growth of algae to a level that prompts complaint". In New Zealand, freshwater blooms have been quantified by saying, >30% coverage with green filamentous algae, >60% with a diatom mat, >35 g/m^2 of ash-free dry mass (AFDM) or 120 mg/ m^2 of chlorophyll a would constitute an algal bloom (Biggs 2000b).

1.1.1 Didymosphenia geminata

In Western Canada, a microscopic alga has recently displayed characteristics of an invasive algal bloom. This species *Didymosphenia geminata* (*D. geminata*), is a single-celled diatom. *D. geminata* is common in freshwater environments. It has an apical porefield that often secretes an inordinate amount of muccopolysaccharide stalk, which can produce up to 250 times more biomass than the diatom cell itself (Larned et al. 2006) and can have devastating effects in streams and Rivers (Larned et al. 2006, Campbell 2005, Kilroy et al. 2005a, b, c, d). The stalk is largely composed of polysaccharides that

are not easily degraded and lack nutritional value to other organisms in the community (Spaulding and Elwell 2007, Larned et al. 2006). The stalks continue to grow when the diatom divides, thus causing the material to accumulate rapidly and cover a large extent of the stream substrate (Spaulding and Elwell 2007, Spaulding, USGS unpublished data), which leads to homogenization of the sediment and a build up of stalk material.

Homogenized sediments can create problems for many aquatic species that require a heterogeneous habitat in which to nest or hide (Larned et al. 2006). Many fish species such as Alberta's endangered bull trout (*Salvelinus confluentus*) require space between cobbles to build their redds and could be very negatively impacted by sediment homogenization (Larned et al. 2006). Reduced numbers of brown trout (*Salmo trutta*) have been observed in South Dakota where *D. geminata* now blooms (Larson and Carreiro 2008). Community shifts in benthic invertebrate community composition may also occur directly due to the build up of stalks, where only species such as chironomids are able to consume the stalk material and survive (Larson and Carreiro 2008, Larned et al. 2007, Larned et al. 2006) Shifts in macroinvertebrate benthic community can result in preferred fish prey (e.g., caddisflies, stoneflies and mayflies) being replaced by less favoured prey (e.g., Chironomidae) once *D. geminata* has invaded (Larson and Carreiro 2008, Larned et al. 2007, E. Stoermer in Campbell 2005).

D. geminata blooms have been common in Europe for over a century (Schmidt-Nielsen and Printz 1915 in Bothwell et al. 2009, Lindstrom and Skulberg 2008). *D. geminata* in its unicellular form is considered native to North American and Asian

streams (Cleve 1894-96 in Bothwell et al. 2009, Lord 1866 in Bothwell et al. 2009). *D. geminata* exists in many Alberta streams and beginning in 2004 blooms began and reached nuisance levels (Bowman 2008, Kirkwood et al. 2007). The first reports of blooms in Western Canada occurred in 1989 on Vancouver Island (Sherbot and Bothwell 1993) and by 1994, 13 watersheds on Vancouver Island experienced blooms (Bothwell et al. 2009). Blooms have also been noted in Rapid Creek, South Dakota (Larson and Carreiro 2008, Shearer and Erickson 2006), Iceland (Jonsson 2000), the Bow River, Calgary (Bowman 2008, Kirkwood 2007), the Yukon River, Yukon (Barraclough 1995 cited in Bothwell et al. 2009) the Kootenai River, Montana (Holderman and Hardy 2004), and Quebec (Simard and Simoneau 2008), suggesting a rapid expansion of *D. geminata* in the stream ecosystem or a change in the *D. geminata* cell itself. Of the *D. geminata* appearances, the most worrisome is the rapid invasion of New Zealand streams. *D. geminata* was first noticed in 2004 and within 18 months had spread to 12 streams (Biggs et al. 2005, Kilroy 2004) and today is present in more than one hundred New Zealand Rivers.

D. geminata blooms may cause a strain on local and national economic and ecological resources by impacting fisheries, hydropower and tourism. *D. geminata* stalks can build up to such a large extent that the build-up impacts water flow through canal systems and a large effort is required to remove bloom remnants (Pryfogle et al. 1997). Streams that were once considered clear and pristine become unappealing following *D. geminata* invasion, which is detrimental to eco-tourism that promotes beautiful rivers. New Zealand tourism has lost millions of dollars following *D. geminata* invasion

(Branson 2006). Loss of tourism is also a potential concern for Canadian National Parks that rely on tourism and have recently experienced large blooms. Despite the negative impacts *D. geminata* has caused, there remains a significant lack of knowledge of *D. geminata's* basic ecology. Due to this lack of knowledge, management attempts to mitigate the effects of *D. geminata* blooms may be ineffective.

What is now causing *D. geminata* blooms? Environmental conditions in bloom locations may not have changed substantially (Bothwell et al. 2009), yet *D. geminata* thrives in many streams. The appearance of *D. geminata* on Vancouver Island in 1989 closely followed the introduction of felt bottomed waders, which became commercially available in 1988 (Kilroy 2008). The moisture retained by such waders has the capacity to allow *D. geminata* cells to remain viable for hours or days after leaving a river (Kilroy 2008, 2006). The transmission of *D. geminata* on waders is consistent with the observation that the locations of new blooms follow a trail of popular fishing paths. Blooms on Vancouver Island were most often along popular Steelhead salmon (*Oncorhynchus mykiss)* fishing routes (Bothwell et al. 2009), the blooms in the Bow River are along top trout fisheries, invasions in Quebec are along popular salmon runs (Simard and Simoneau 2008) and the first invasions in New Zealand were in trout streams (Kilroy 2004). These invasions collectively suggest that fishermen may be vectors for *D. geminata*, transporting it to areas where it can thrive.

Once a vector has transported *D. geminata* to a new location it is very difficult to remove and scouring an area of *D. geminata* appears to be followed by rapid

recolonization (Lee et al. 2008). The factors associated with the establishment and development of a bloom remain unknown. Bothwell et al. (2009) suggested that there might be a genetic strain of *D. geminata* that will bloom. *D. geminata* blooms also seem to flourish in areas of flow regulation (Kirkwood et al. 2009). Tail waters are viewed as 'hot-spots' for growth as *D. geminata* is more likely to bloom below a dam than above (Kirkwood et al. 2009). The water coming from dam spillways that promotes *D. geminata* blooms is typically hypolimnetic discharge that is cool, nutrient poor, and stable (Kirkwood et al 2007).

Blooms in nutrient poor waters are perhaps the most puzzling aspect of *D. geminata* ecology. Eutrophication usually promotes algal blooms (Dodds et al. 2002, Biggs 2000, Schindler 1977). Sentinel experiments in Lake 226 in Ontario demonstrated that increased phosphorus inputs resulted in significant increases in chlorophyll a and primary production in lakes. In contrast, although the frequency of *D. geminata* cell division may have a positive relationship with phosphorus (Kilroy and Bothwell 2012), *D. geminata* bloom formation occurs when concentrations of soluble reactive phosphorus (SRP) are very low and often below analytical detection limits (e.g. < 0.05 µg/L) (Bothwell et al. 2009, Bowman 2008, Kirkwood et al. 2007). Ironically, *D. geminata* blooms have even been described as an indicator of pristine water quality (Kirkwood et al. 2007) because it blooms in oligotrophic or ultra-oligotrophic water. Filamentous green algae (*Zygogonium* sp. and *Mougeotia* sp.) have been known to bloom in temperate oligotrophic lakes (Turner et al. 1995) while other diatoms (*Aulacoseiria bailacalensisi)* and Chrysoflagellates have been observed to bloom under ice cover (Richardson et al.

2000, Watson et al. 2001). These blooms all occurred in stable water columns or under winter stratification; *D. geminata* appears to be the first recorded bloom to occur in oligotrophic lotic systems.

1.1.2 Resource Competition

Nutrients are a major resource that control the dynamics of algal growth and biomass. Increased nutrient supply typically results in increased plant growth and algal blooms, a process known as eutrophication. Eutrophication can lead to the degradation of water quality, the loss of natural species, and a change in ecosystem structure (Carpenter et al. 1998). Carbon, nitrogen and phosphorous are primary limiting nutrients for algae because, they are often in short supply relative to algal demand. Algal species have different nutrient requirements to attain maximal growth (Redfield 1958) yet, an average across species is C:N:P, 106:16:1 (Redfield Ratio) (Redfield 1958). When species require several nutrients, the nutrient that is present in the environment in the least amount relative to the cellular requirements for growth will limit the growth of the species (Leibigs "Law of the Minimum") (von Leibig, 1855 in van de Ploeg et al. 1999). Therefore, in aquatic systems there will often be nutrients that limit growth.

Aquatic systems vary considerably in their nutrient concentrations. For example, some alpine lakes are extremely nutrient poor, whereas urban ponds may receive an immense quantity of urban runoff containing higher nutrient concentrations. If a nutrient is added and net primary production increases, than the system is assumed to have been

nutrient limited. Nutrient limitation of the net primary productivity may generate shifts in community structure (D'Elia et al. 1986, Howarth and Cole 1985, Goldman et al. 1979, Edmondson 1970). Even if one algal species is growing at maximal rate (e.g. molar ratios approach those of the Redfield Ratio), the addition of a nutrient could cause the dominance of a different algal species. For example, a system dominated by N-fixing algae may switch to a green algae dominated system with the addition of Nitrogen. This system would then be considered N-limited. As long as net primary productivity can increase, the system is considered nutrient limited.

Surveys conducted at the experimental lakes area (ELA) in Ontario have clearly demonstrated that primary productivity is P-limited in freshwater lakes (Schindler 1977). The fact that many freshwater lakes are P-limited would suggest that freshwater streams may be P-limited. However, P-limitation in freshwater streams has not been extensively confirmed. Nutrient limitation in streams is difficult to examine because many other constraints (e.g., water velocity and flood) also affect algal accrual (Kirkwood et al. 2007, Biggs 2000, Grimm and Fisher 1986). Because more carbon stalk material is required to resist the forces of flow, relationships between total biomass and chlorophyll content are more variable in streams than in lakes (Van Nieuwenhuyse and Jones 1996). Chlorophyll and nutrient data from nearly 1000 lotic systems were compiled and nutrient availability was discovered to explain ~40% of benthic autotrophic biomass (Dodds et al. 2002). However, there is disagreement over which nutrients limit stream systems. Some studies identify P-limitation (Bowman et al. 2007, Bothwell, 1985) while others suggest a pattern of co-limitation (Francoeur 2001, Borchardt et al. 1994). A review of nutrient limitation

of streams globally emphasizes that nutrient co-limitation may, in fact, be much more common than previously realized (Dodds et al. 2002, Scrimgeour and Chambers 2000)

Although a pattern of nutrient co-limitation appears possible in streams, phosphorus still plays a major role in controlling productivity. In oligotrophic lakes, the hypolimnion is oxygenated and most phosphorus remains trapped in the sediments. However, in a eutrophic lake, bottom waters can become anoxic and phosphorus is released back into water as it dissociates from iron ($Fe_3(PO_4)_2 \rightarrow 2Fe^{3+} + 2PO_4^{3-}$). In contrast, rivers are unlikely to have substantial amounts of anaerobic sediment and therefore should not release large amounts of phosphorus into the water from sediment decomposition. Instead, phosphorus "spirals" down the channel (Newbold et al. 1981, 1983). As phosphorus is taken up and released back into the water column, it moves further downstream. For example, Correll (1958) added phosphate to a stream and measured increased phosphorus downstream. Peterson et al. (1985) added phosphate to a stream and periphyton chlorophyll concentration increased for 10 km downstream. These phosphorus addition studies, as well as many others, indicate that phosphorus is important in controlling productivity in many streams and rivers (Elwood et al 1981, Stockner and Shortreed 1978).

To predict the equilibrium outcomes of algal growth rates as a function of nutrient concentrations at steady state, a simple model can be used (Monod 1950). The "Monod" model considers the effect of nutrient uptake and utilization on growth rate and describes the range of concentrations over which a nutrient limits algal growth (Monod 1950).

Chemostat experiments are run to equilibrium where the supply of the nutrient is equal to its consumption, which determines a phytoplankton species' growth rate at a particular nutrient supply rate. Many species respond to nutrient limitation with compositional and physical changes to cells (Goldman 1980). Different algal species have different growth responses to low levels of the same nutrient such that there is interspecific competition for limited nutrients and means competitors cannot coexist. Under steady state conditions, when two species are limited by the same nutrient, the ability of one species to more efficiently take up the limiting nutrient will determine the ultimate success of that species (Tilman et al. 1982, Tilman 1980, 1977, Tilman and Kilham 1976). Levine (1983) observed that most phytoplankton species were present at N:P ratios between 20:1 and 11:1 and once ratios dropped below 11:1 nitrogen fixing cyanobacteria dominated.

While a species may be limited by one nutrient, it is rather simplistic to assume communities are limited by only one resource. Community structure can be altered by different ratios of many nutrients (Tilmans resource ratio hypothesis) (Tilman 1977). The amount of nutrient required to reach an equilibrium state will be different for each species, but the species that requires the lowest amount of nutrient to reach equilibrium should displace its competitors when that nutrient is limited. Dominance will change depending on which nutrient is limited in the system, which, in the case of lotic systems, varies over space and time.

Diatoms require silica to build their frustule and early experiments were conducted to determine which species (*Cyclotella meneghiniana, Fragillaria* sp.*, Synedra*

sp. *and Asterionella formosa*) dominated under different ratios of silica and phosphorus. As was expected, when Si:P was low, large diatoms (*Fragillaria* sp*., Synedra* sp. *and Asterionella formosa)* were rare and the relatively small *Cyclotella meneghiniana* dominated. When Si:P was high, *Fragillaria, Synedra* and *Asterionella* dominated as these species were no longer Si-limited and were better competitors for phosphorus than *Cyclotella meneghiniana* (Tilman 1980, Tilman 1977, and Tilman and Kilham 1976)

When a resource can be acquired from many sources, or by many methods, the method or source that is least energetically costly to the organism should be used (Gutschick 1981) because this method will conserve energy that can be used to acquire other limited resources, or to conduct other cellular processes. In a competitive environment, there should be a strong selection towards those organisms able to acquire resources in an optimal fashion. However, when a nutrient is limited the optimal option may be the most energetically costly.

1.1.2.1 Nitrogen Limitation

Organisms require nitrogen to produce proteins and nucleic acids, yet the largest global N reserve (79%) is in the form of N_2, which most plants cannot take up. N_2 must first be converted into an amine group ($-NH_2$) through nitrogen fixation. However, when nitrogen limits growth, nitrogen fixation can adjust the environmental nitrogen supply close to the availability of other nutrients (Schimel et al. 1997). A comparison of 17 lakes

around the world found that where environmental N:P ratios were below 29:1 (N-limited), blue-green algae were seen to dominate (Smith, 1983). Blue-green algae are capable of nitrogen fixation, are able to maintain high growth rates in nitrogen deficient waters and outcompete other algal species. However, when phosphorus becomes limiting blue-green algae are often out-competed. At lake 227 at the ELA lakes in Ontario, at N:P levels of 30:1 dominance of cyanobacteria was not observed but as N:P ratios were dropped over time, cyanobacteria dominance began to occur (Schindler 1977).

1.1.2.2 Phosphorus Limitation

Phosphorus is integral to energy metabolism of all life forms, and is often very limited in freshwater systems (Schindler 1977). Phosphorus is not found in a gaseous state and cycles mainly between soil, water and sediment following release from weathering rocks. The phosphorus cycle is slow as P cycles between sediments and living organisms. Only orthophosphate (PO_4^{3-}) is taken up directly by autotrophs; any other forms of phosphorus must first be converted into PO_4^{3-} prior to uptake.

Organisms have few options when phosphate supply becomes low; they can adopt different pathways to take up inorganic phosphorus more efficiently (Wagner and Falkner 2001) or use the organic phosphorus present in the environment. Organic phosphorus is an organic compound that contains phosphorus such as: nucleic acids, phospholipids, sugar phosphates, organic condensed phosphates etc. Organic phosphorus represents the largest single pool of phosphorus in many aquatic systems. Organic phosphorus is

bioavailable to phytoplankton (Cotner and Wetzel 1992) and its turnover rate may be of great importance to them (Baldwin et al. 2003, Hudson et al. 2000).

Phytoplankton may take up PO_4^{3-} preferentially when PO_4^{3-} and organic phosphorus sources are present (Hong et al. 1995). However, when quantities of PO_4^{3-} are not readily available, organisms may use dissolved organic phosphorus (DOP) as an alternative source of phosphorus (Havens et al. 2001, Hong et al. 1995, Koborki and Taga 1980). There is an energetic cost to employ mechanisms to acquire organic phosphorus, for example to produce phosphatases to cleave the P from its organic backbone. So organisms likely only use those mechanisms when they provide a competitive advantage.

Uptake of dissolved organic phosphorus (DOP) across the plankton cell membranes does not occur but the activation of cell-surface-associated phosphorus monoester hydrolase enzymes converts DOP into $PO_4^{3-,}$ which can then pass through membranes (Hernandez et al. 2003, Strojsova et al. 2003, Whitton et al. 1991, McComb et al. 1979). The best-known phosphorus monoester hydrolase enzyme (phosphomonoesterase) in aquatic algae is the non-specific alkaline phosphatase (AP) (Pick 1987, Pettersson 1980), which has been observed in times of phosphorus limitation (Chrost et al. 1984, Francko 1983, Healy 1975). High levels of PO_4^{3-} inhibit AP (Berman 1970), so when the PO_4^{3-} concentration is no longer limited, AP activity declines, which suggests that the energetic costs of the enzyme activity are no longer worth expending. The ability to exploit DOP may be crucial for the survival of species in P-limited systems.

All alkaline phosphatases act on the cell surface. Therefore, during times of severe P-limitation more surface area can be created to increase space for enzyme activity, resulting in increased production of orthophosphate. In times of P-limitation, some bacteria, cyanobacteria and algae have been seen to form long multicellular hairs that may lack chlorophyll and sometimes even DNA (Whitton 1988). These hairs are often sites of high surface phosphatase activity (Mahasneh et al. 1990, Whitton 1988). Is there an advantage to localizing enzyme activity to one part of the organism? Localization of enzyme activity may be a strategy that *D. geminata* employs. *D. geminata* enzyme activity appears to be localized in the stalks, and not on the diatoms frustule (Ellwood and Whitton 2007). Such localization could indicate that the stalks are areas of organic phosphorus hydrolyzation and the resulting orthophosphate would then be taken up a central tube to provide a phosphorus source for the *D. geminata* cell (Ellwood and Whitton 2007). Whitton and Neal (2011) found that *D. geminata* associated with watersheds draining high peat sites had high levels of surface phosphatase activity, which suggests high levels of organic phosphorus usage. Sundareshwar et al. (2011) also highlight the importance of iron in the uptake of phosphorus by *D. geminata*. They suggest that the muccopolysaccharide stalk absorbs both iron and phosphorus. Through enzymatic and bacterial processes with iron, a positive feedback exists where the amount of phosphorus available is increased which, in turn, increases the amount of stalk material. Thus different methods of phosphorus acquisition may confer a competitive advantage to *D. geminata* in phosphorus-limited systems.

1.1.3 Goals of Thesis

The goal of this thesis was to determine which environmental variables, of those measured, support *D. geminata* blooms. In particular, focus was placed on the role of organic phosphorus as a driver for *D. geminata* blooms. The study of *D. geminata* blooms occurred in two parts. First, I conducted a survey to uncover environmental conditions that were associated with *D. geminata* populations at bloom and non-bloom sites throughout pristine mountain streams. This was done in 2009 by conducting a large-scale survey of streams in the Canadian Rockies. This gave me a picture of the environment in which *D. geminata* thrives. Then, in 2009 and 2010 I conducted an experiment in seven mountain streams to gain a closer look at which nutrient *D. geminata* was using.

Each main data chapter has been written with publication in mind. There may appear to be redundancy within the introductions from one chapter to the next, but each introduction is meant to describe the research in enough depth so that each chapter could be understood without the aid of this introductory chapter.

Chapter Two: ***Didymosphenia geminata*** **within Canadian Rocky Mountain streams**

2.1 Introduction

Eutrophication, the process of organic enrichment, often occurs following an excessive input of nutrients, most commonly, Nitrogen or Phosphorus (Sunda et al., 2006, Dodds et al. 2002, Biggs 2000, Schindler 1977, Nixon 1995). This enhanced growth of algae can result in deterioration of water quality, which often results in a change in ecosystem structure function. When an algal bloom significantly alters the structure and function of an aquatic ecosystem, the bloom is referred to as an environmentally degrading algal bloom (Sunda et al. 2006). These blooms can cause harm due to the production of toxins, or due to an accumulation of biomass that alters food web dynamics (Gobler et al. 2002, Johansson and Graneli 1999a, Nielsen et al. 1990). A large algal bloom may alter habitat and trophic structure by depleting oxygen as the algae decays (Mahoney and Steimle 1979, Cho 1979, Grindley and Taylor 1962), reducing food and space for co-occurring organisms, destroying habitat and shading out submerged vegetation (Onuf 1996, Onuf 1994). Blooms caused by the diatom algae, *Didymosphenia geminata* (*D. geminata*) exhibit characteristics of an environmentally degrading algal bloom.

Algal blooms caused by *D. geminata* have become recognizable worldwide. Blooms on Scandinavian river bottoms were reported a century ago (Schmidt-Nielsen and Printz 1915, cited in Lindstrom and Skulberg 2008) but recently the geographic range

of *D. geminata* blooms has drastically increased. The first reports of blooms in Western Canada occurred in 1989 on Vancouver Island (Sherbot and Bothwell 1993). However, blooms have been also noted in Rapid Creek, South Dakota (Larson and Carreiro 2008, Shearer and Erickson 2006), the Bow River, Calgary (Bowman 2008, Kirkwood 2007), the Yukon River, Yukon (Barraclough 1995 cited in Bothwell et al. 2009) the Kootenai River, Montana (Holderman and Hardy 2004), and Quebec (Simard and Simoneau 2008), suggesting a rapid expansion of *D. geminata* to streams throughout North America. Since *D. geminata*s' first recorded appearance in the Canadian Rocky Mountains in the 1990s the spread of *D. geminata* throughout these mountains has increased (Bowman 2008, Kirkwood et al. 2007). As concerns regarding *D. geminatas'* disruption of stream ecosystems rise, knowledge regarding *D. geminata* infected streams and the diatoms' environmental preference has become increasingly important.

Control of most algal blooms often involves alteration of nutrient inputs. However, contrary to most algal blooms (Sunda et al. 2006, Dodds et al. 2002, Biggs 2000, Schindler 1977), *D. geminata* cells show a positive relationship with dissolved phosphorus levels, but the amount of stalk material increases as dissolved phosphorus levels decrease (Kilroy and Bothwell 2012, Bothwell and Spaulding 2008, Kirkwood et al. 2007). In particular, it is found blooming in streams with low, almost undetectable levels of phosphorus (Bothwell and Kilroy 2011, Kirkwood et al. 2009, Lindstrom and Skulberg 2008). Ironically, *D. geminata* blooms have been described as an indicator of pristine water quality (Kirkwood et al. 2007, Jonsson et al. 2000, Sherbot and Bothwell 1993). *D. geminata* also blooms in streams with low water velocity (Tomas et al. 2010,

Kirkwood et al. 2007, Larned et al. 2006, Kilroy et al. 2005d), flow regulation (Kirkwood et al. 2009), and very cold water temperatures (Lindstrom and Skulberg 2008, Kirkwood et al. 2007, Blanco and Ector 2002).

When *D. geminata* blooms, the muccopolysaccharide stalk it creates can produce up to 250 times more biomass than the diatom cell itself, which can completely homogenize a streambed (Spaulding and Elwell 2007, Larned et al. 2006). The stalk is largely composed of polysaccharides that are not easily degraded and lack nutritional value to other organisms in the community (Spaulding and Elwell 2007, Larned et al. 2006, 2007). In infected streams, stalk features cause trophic shifts in invertebrate communities (Larson and Carreiro 2007, Larned et al. 2006, 2007, Campbell 2005). The shift in community is generally away from invertebrates easily grazed by higher trophic levels (e.g. Mayflies, Stoneflies, Caddisflies) to less desirable groups (e.g. Chironomids). This is indicative of an unhealthy stream environment. Shifts in invertebrate communities and sediment homogenization (changes in structure) raise concerns for the long-term survival of fish populations (changes in function) within infected streams (Larned et al. 2006). Coincidentally, it appears that anglers are likely the main vectors that transport *D. geminata* cells from one stream to another, through attachment to their felt-soled waders (Bothwell et al. 2009, Kilroy et al. 2009).

The pristine conditions of Canadian Rocky Mountain streams would seem to provide an ideal habitat for *D. geminata* blooms. Canadian Rocky Mountain streams experience a high volume of tourist traffic, which would provide many potential vectors

for diatom transport. They are also cold, clear, oligotrophic streams, which should be ideal for *D. geminata* bloom development (Lindstrom et al. 2008, Kirkwood et al. 2007, Larned et al. 2006). The goal of this research was to evaluate variation in stream characteristics at 76 sites within the Canadian Rocky Mountains, to test relationships between environmental variables and *D. geminata* cells and blooms. Regional variables were those that varied over the sampling region such as anthropogenic use of sites and latitude. Local variables included environmental variables, such as water velocity and water temperature, that were stream specific and could vary from stream to stream.

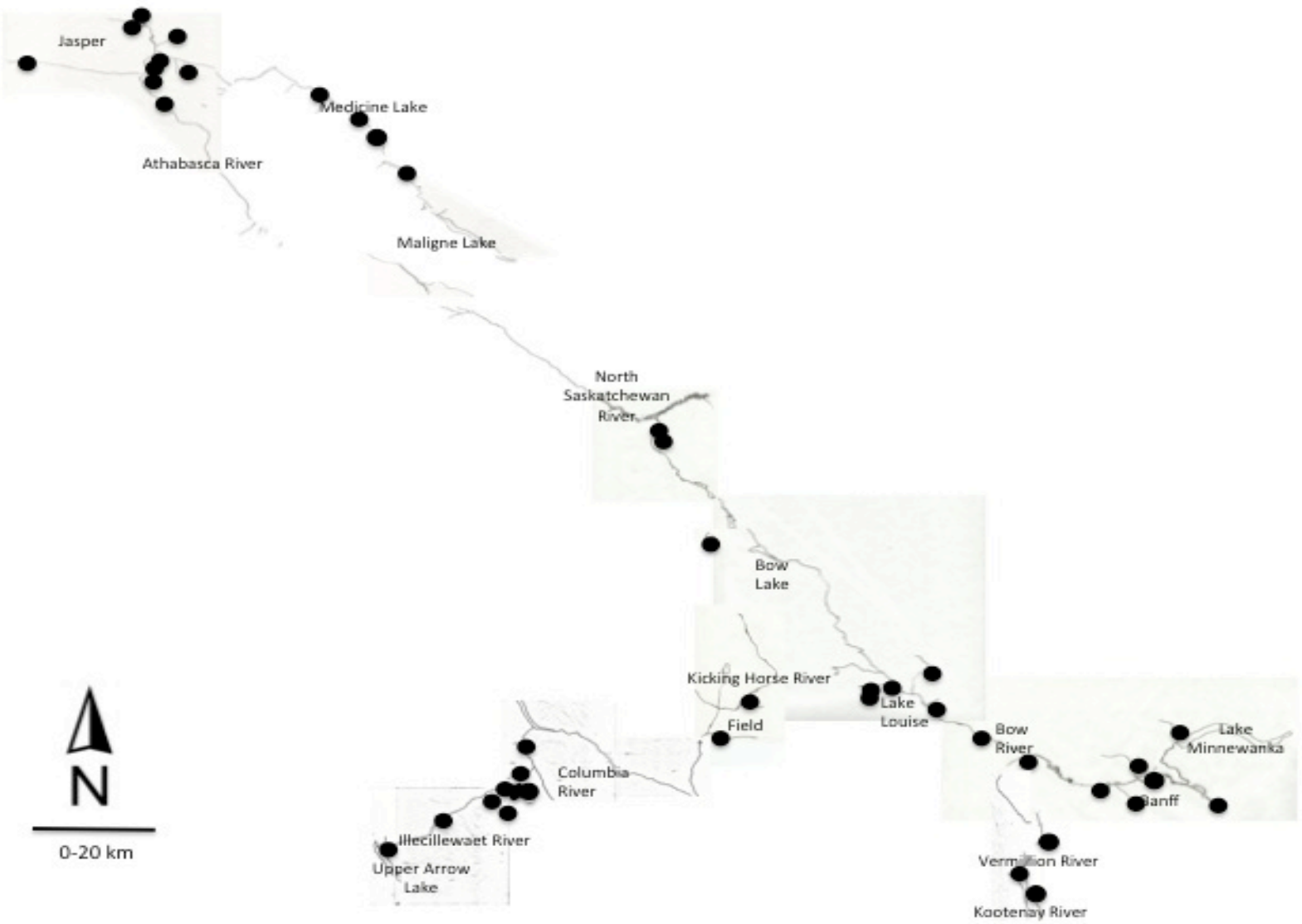
The hypotheses of this study were that i) the presence of *D. geminata* cells would vary between sites and their presence would be determined by regional variables, ii) the abundance of *D. geminata* cells would be driven by local stream variables, iii) local environmental conditions would determine whether a stream containing *D. geminata* cells would bloom, and iv) local environmental conditions would determine the biomass of bloom material. I predicted that streams with high levels of anthropogenic activity would contain *D. geminata* cells, as opposed to streams that were very remote where no *D. geminata* was expected. I also predicted that low nutrient, low water velocity and low temperature water would be associated with *D. geminata* blooms.

2.2 Methods

2.2.1 Study sites

Periphyton samples were collected during August/September 2009. Seventy-six sites on first to fifth order streams in the Rocky Mountains of Alberta and British Columbia were sampled (Figure 2.1). Streams had circum-neutral to slightly alkaline pH, moderate to high slope and during the fall and were typically clear (water turbidity < 20 NTU). Concentrations of total phosphorus (TP) and total nitrogen (TN) are typically very low (TP <0.002 to 0.01 mg/L, TN <0.05 to 0.2 mg/L). Streams are ice covered during November to April and open during the summer months. Larger streams support rainbow trout (*Oncorhynchus mykiss*), bull trout (*Salvelinus confluentus*) and mountain whitefish (*Prosopium williamsoni*) populations, which provide angling opportunities. All streams were located within National Park boundaries, and covered a range of tourist and recreation intensities.

A


Jasper
Medicine Lake
Athabasca River
Maligne Lake
North Saskatchewan River
Bow Lake
Kicking Horse River
Lake Louise
Field
Bow River
Lake Minnewanka
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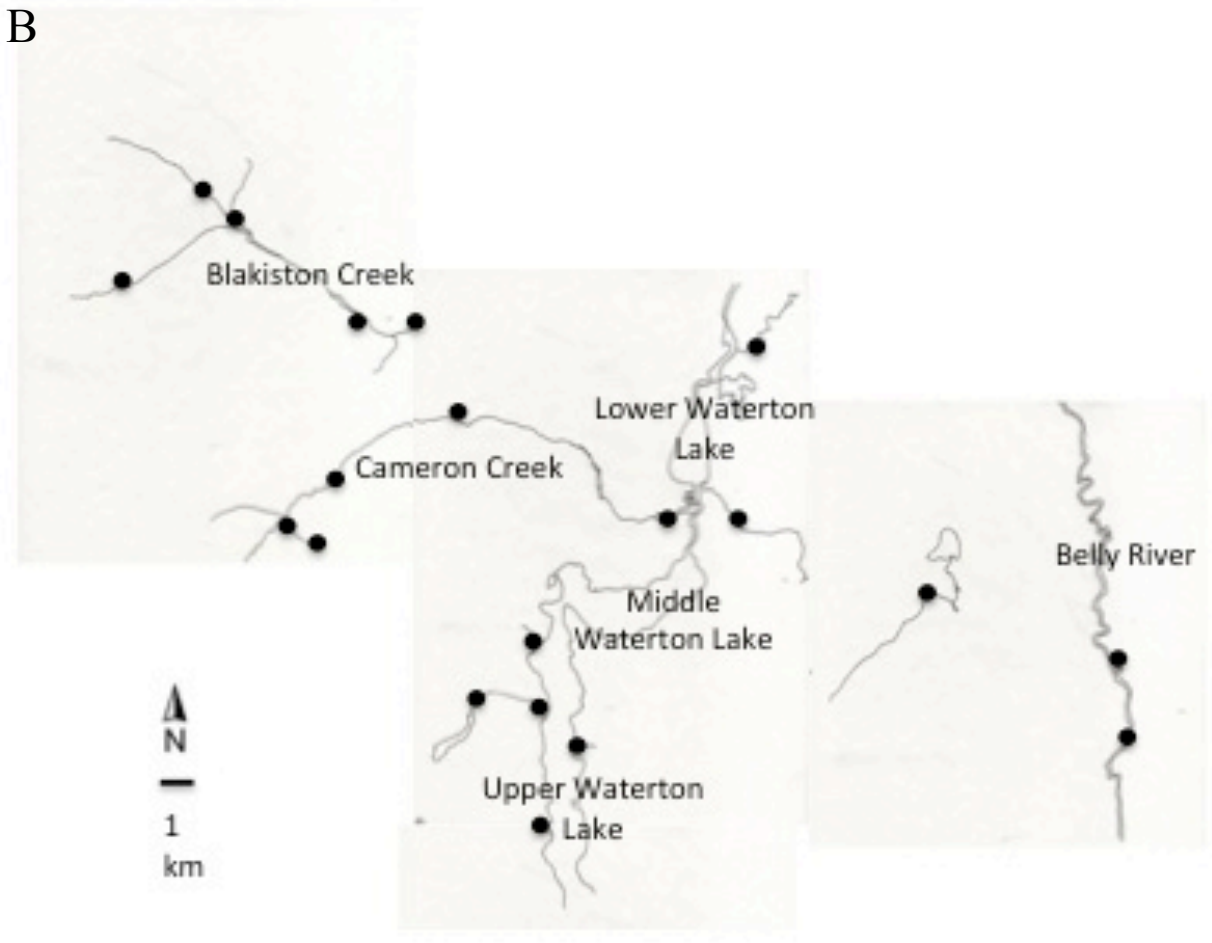

Figure 2.1. The 76 sites within the Canadian Rocky mountains sampled over the summer of 2009. The black dots represent a sampling location. (A) Banff, Lake Louise, Kootenay, Yoho, Glacier/Revelstoke and Jasper National Park sites, (B) Waterton Lakes National Park sites. The arrow with the N points north, and the solid black line gives the map scale.

At each site, stream attributes and *D. geminata* presence and abundance were sampled (Table 2.1). Sites were chosen from a subset of Parks Canada existing monitoring sites to provide a range of stream order, stream type, substrate type, area use, public access, algal community composition and nutrient concentrations.

Thirteen environmental variables, which were thought to potentially explain variance in the occurrence and abundance of *D. geminata* at each site, were quantified (Table 2.1) and seven were used within my model selection analysis. The variables were identified from a more extensive suite of variables from an understanding of *D. geminata* biology (Bothwell and Kilroy 2011, Kirkwood et al. 2009, Whitton et al. 2009, Bothwell et al. 2009, Lindstrom and Skulberg 2008, Bothwell and Spaulding 2008, Kirkwood et al. 2007, Blanco and Ector 2002). Environmental variables were divided into those that define regional conditions and those that define local conditions (Table 2.1). Regional conditions are those that would not change over a large area, whereas local conditions are those that can vary between streams or sampling locations within streams.

Table 2.1. Environmental variables used to predict the occurrence and abundance of *D. geminata* at 76 sites within streams of the Alberta and British Columbian Rocky Mountains.

Variable	Abbreviation	Units	Range
Regional Variables			
Elevation	Elev	m	576 - 2449
Latitude	Lat	°	-118.45 - -113.68
Stream Order*	Order	-	1-6

Stream Type	Type	-	fast / slow riffle, fast/slow run, pool or cascade
Local Variables			
Water Velocity*	Velocity	m/s	0.08-1.23
Total Nitrogen*	TN	mg/L	0.01 (min. detection) - 0.26
Total Phosphorus*	TP	mg/L	0.002 (min. detection) – 0.047
Water Temperature*	Temp	°C	2.25 - 18.2
Dissolved Oxygen	DO	mg/L	0.75 – 14.19
Stream Depth	Depth	m	0.046 - 1.46
Stream Width	Depth	m	0.56-121.33
Angling Pressure	Fish	-	rare, occasional, frequent
Site Access*	Access	-	highway, paved road, gravel road, marked trail or unmarked trail.

Note: '*'indicates variables that were used in model selection. All others were correlated to a combination of these variables and were dropped from these analyses to avoid co-linearity.

2.2.2 Physico-chemical Attributes

Anthropogenic activity was evaluated as site use and angling pressure, was evaluated by Parks Canada staff that were familiar with each study area. Site use was determined on a scale of one to five, with one being rarely used and five being highly frequented. Angling pressure was determined to be frequent, occasional or rare. Mean water velocity was calculated at 60% water depth using a velocity meter (Global Water

Flow Probe (FP111)). Water temperature (+/- 1 °C) was measured using a glass thermometer. Five stream widths and depths were taken randomly at each site. Dissolved oxygen was measured with a YSI Dissolved Oxygen Meter. Triplicate water samples were taken at each site in acid-washed, double-distilled water rinsed 1L Nalgene bottles. Bottles were filled to the top to leave as little air as possible and stored on ice, prior to being sent to an Environment Canada Lab location for total phosphorus (TP) and total nitrogen (TN) analysis. SRP, TDP and NO_2^- were not measured, as levels were often below the detection limits of my equipment.

At each site, three cobbles (0.05 - 0.1 m) were chosen randomly within 0.5 - 2.5 m from shore and 0.2-0.7 m depth. Three 14.3 cm^2 circles were traced on each cobble following a PVC template and the algal material within the traced circle was mechanically dislodged from the cobble. Dislodging occurred with a combination of scalpels, tweezers and toothbrushes to ensure all material was removed from the desired area. The algal material from one circle was placed in a 125 mL borosilicate bottle with distilled water and Lugol's acid-iodine fixative. The sample was placed in the dark, for later *D. geminata* identification and enumeration. The material from a second circle was wrapped in aluminum foil and kept on ice during transport to the lab and then stored at –20 °C prior to chlorophyll *a* analyses. The material from a third circle was collected in a small plastic bottle and used for ash-free dry mass (AFDM) determination.

All chlorophyll *a* samples were extracted into methanol and processed following the methods of Thompson et al. (1999), without correction for phaeophytin content.

Chlorophyll *a* was analyzed with a Spectromax Gemini XS dual-scanning microplate spectroflurometer (Molecular Devices, Sunnyvale, California USA) at an excitation wavelength of 440 nm. All AFDM samples were processed according to Biggs and Kilroy (2000).

An entire 14.3 cm^2 sample was resuspended in 100 mL of water, then 10 mL sub-samples, from the well mixed 100 mL samples, were poured into setting chambers for cell identification and enumeration using a Leica DM IRB inverted microscope (Leica Microsystems, Wetzlar, Germany) Cells were counted at a 100X magnification. *D. geminata* cells where identified by the diacritical feature of 2-5 stigmata and the conspicuous size of 60-150 μm.

2.2.3 Statistical Analyses

Descriptive statistics were calculated using Microsoft Excel (2011). Statistical analyses were conducted using the statistics package R (v2.8.1) (R development core team 2008). Maps (1:200,000) were traced by hand from maps located in the Canadian Rockies Backroad Mapbook (2011). Any chemistry data that were less than detection limits were removed from analysis and all analyses used a critical $\alpha = 0.05$.

The 13-predictor variables were reduced to seven after correlations were uncovered between some variables. These seven predictor variables were used to test 10 *a priori* models representing multiple combinations of variables. Environmental variables

were either measured during stream surveys (i.e. water velocity, water temperature, TP and TN) or derived from queries of geographic information system (GIS) databases (latitude, stream access and stream order).

Spatial autocorrelation was assessed using a Mantel test. Generalized linear models (GLM) and analyses of covariance (ANCOVA) were used to quantify relationships between occurrence and abundance of *D. geminata* and selected environmental variables (Table 2.2) by completing two sets of analyses. First, models of *D. geminata* occurrence and abundance from the 76 Canadian Rocky Mountain stream sites were derived. Second, data from the 76 sites were used for a closer look at the relationships between *D. geminata* bloom sites and non-blooms sites and their corresponding environmental matrix.

2.2.3.1 Generalized Linear Models (GLMs)

GLMs were conducted on binary (presence or absence of *D. geminata* cells or blooms) and continuous (amount of *D. geminata* cells and amount of AFDM) data and their relationships to environmental variables. Binomial, negative binomial and Gaussian GLMs for the binary analyses were conducted to determine the amount of *D. geminata* cells and the amount of AFDM respectively. For the amount of AFDM, log (x+1) transformation of data reduced variance and therefore over dispersion.

The ability of 10 *a priori* models to explain the variation in occurrence and abundance of *D. geminata* in the Canadian Rocky Mountain streams was quantified (Table 2.2). These models were derived from combinations of the seven environmental variables (two to four variables) resulting in the final models evaluated. Correlations among predictor variables were evaluated prior to inclusion in models to avoid co-linearity, and variables were excluded when correlations were significant (p <0.05). Elevation, stream depth and stream width were all correlated to stream order (rho =-0.34, p =0.0063; rho =0.63, p <0.001 and rho =0.73, p <0.001 respectively). Site access and angling pressure (rho =0.59, p =1.93x10^{-8}), dissolved oxygen and temperature (r =-0.44, p <0.001) and latitude and stream type (rho =0.26, p =0.025) were also correlated. As a result, the final predictor variables were: stream order, temperature, site access and latitude, TP, TN and stream type.

Table 2.2. Summary of a priori candidate models used to evaluate relationships between the occurrence of *D. geminata* cells and blooms and environmental variables, including metrics of anthropogenic activity in the Canadian Rocky Mountains. K is the number of independent variables.

Model	Variables	K
Regional Variables		
1	Lat + Order + Access	3
2	Lat + Order	2
Local Variables		
3	Velocity + TP + TN + Temp	4
4	TP + TN	2
5	Velocity + Temp	2
6	Velocity + TP	2
Regional + Local Variables		
7	Access + Velocity + TP	3
8	Lat + Velocity + TP	3
9	Lat + Velocity + TP + TN	4
10	Order + Velocity + Temp + TP	4

Aikaike's Information Criterion corrected for small sample sized (AICc) was used to select the most parsimonious candidate model (Burnham and Anderson 1998, Anderson et al. 2000). Each model was evaluated by the difference in AIC_c values from the most parsimonious model (Δ_i) that was weighted against other models. The top three models were selected but only models that had a $\Delta_i < 10$ were considered statistically meaningful (Anderson et al. 2001). For all models with variables that had Akaike's weights ≥ 0.01, model averaged estimates of coefficients and associated p-values were

calculated. This was done to assess the variables' relative contributions to the variance observed in *D. geminata* growth.

2.2.3.2 Differences between bloom and non-bloom sites

The GLMs indicated a difference in the response of *D. geminata* cells and *D. geminata* blooms to the environmental variables evaluated. Therefore, additional analyses were conducted to look at interactions between sites where *D. geminata* bloomed and sites where *D. geminata* cells were present but did not bloom. This analysis was conducted to evaluate relationships between environmental variables identified as significant and their respective *D. geminata* bloom status.

2.3 Results

Of the 76 sites sampled within the Alberta and British Columbian Rocky Mountains, *D. geminata* was detected at 67 (88%), and of those 67 sites with *D. geminata* cells, 23 (34%) had *D. geminata* blooms. Blooms occurred along the Bow River from the Bow Lake outflow and into the Banff town site. However within the Banff town site, there were two sites with no blooms and one site where *D. geminata* was not detected (Figure 2.2). These sites flowed into the Bow River. In Waterton Lakes National Park there were two streams within the same tributary that had differences in D. *geminata* abundance. There was no evidence of *D. geminata* blooms along the heavily visited Red

Rock Creek, but in a remote site along the Blakiston Creek a large *D. geminata* bloom was observed (Figure 2.2).

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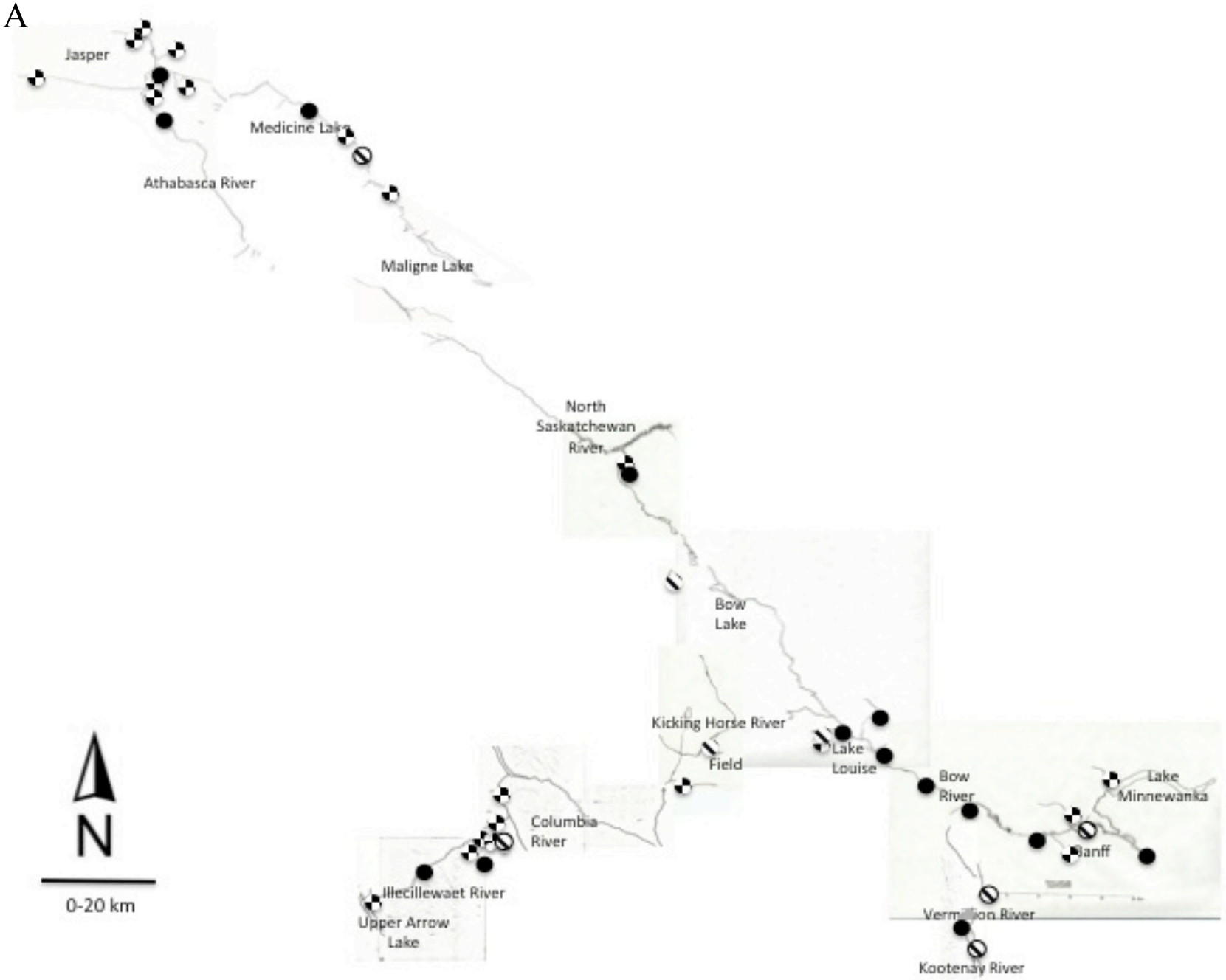

Jasper
Medicine Lake
Athabasca River
Maligne Lake
North Saskatchewan River
Bow Lake
Kicking Horse River
Field
Lake Louise
Bow River
Lake Minnewanka
Banff
Columbia River
Illecillewaet River
Upper Arrow Lake
Vermillion River
Kootenay River
N
0-20 km

B

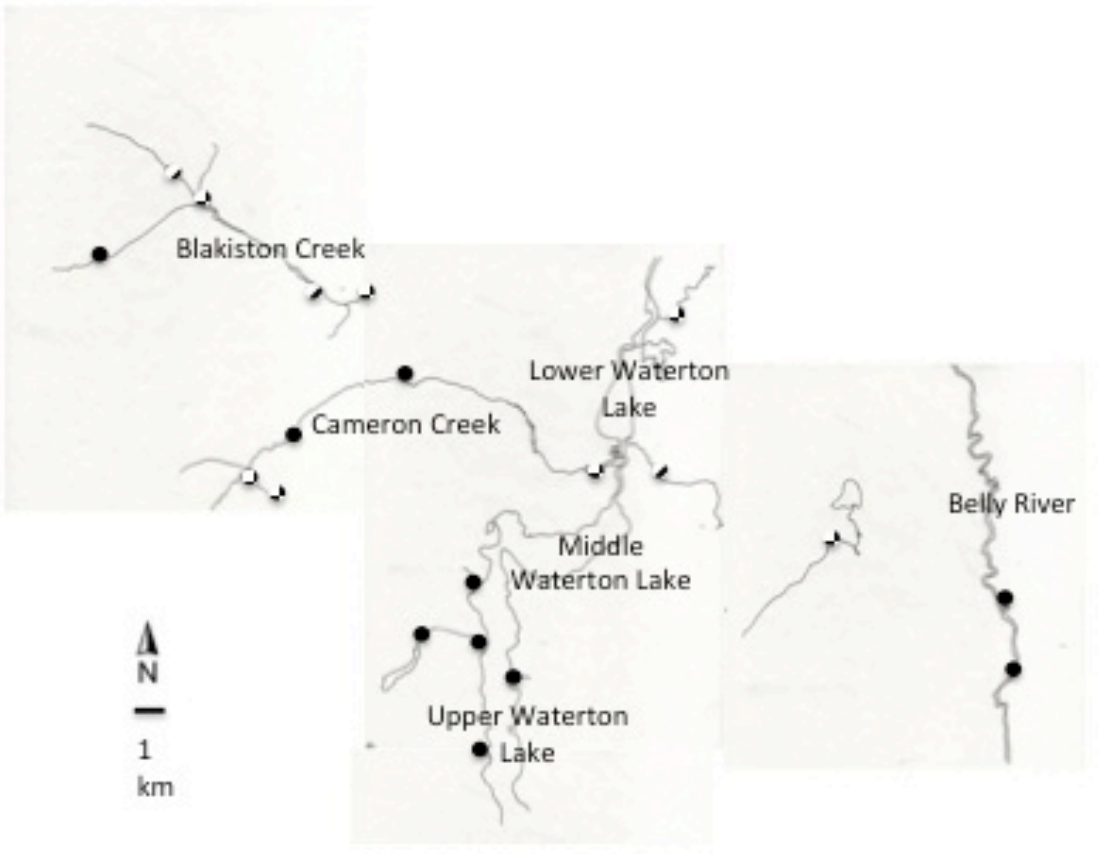


Figure 2.2. The 76 sites sampled within the Canadian Rocky mountains over the summer of 2009. The black dots represent sites where *D. geminata* blooms were found, the checkered dots are sites where *D. geminata* cells were found but there was no evidence of a bloom and the circle with lines are sites where no *D. geminata* was located. (a) Banff, Lake Louise, Kootenay, Yoho, Glacier/Revelstoke and Jasper National Park sites, (b) Waterton Lakes National Park sites. The arrow with the N points N, and the solid black line gives the map scale.

Mantel tests revealed no spatial autocorrelation regarding where *D. geminata* cells were present or absent (p =0.37), where *D. geminata* blooms occurred (p =0.11), or the amount of AFDM (p =0.30). However, there was spatial autocorrelation to *D. geminata* cell abundance (p =0.011).

2.3.1 *D. geminata* cell presence and abundance

A combination of regional and local variables is the most parsimonious model for the presence or absence of *D. geminata* cells (Table 2.3). Based on AIC_c, the simplest most parsimonious model for presence or absence of *D. geminata* cells included: stream order, water velocity, water temperature and TP. Although this model best fit the data, there was no significant relationship between these variables and the presence or absence of *D. geminata.* The driver behind the presence or absence of *D. geminata* cells could not be determined from the data. *D. geminata* was found in a range of streams that ranged in order from one to five, water velocities that ranged from 0.11 m/s to 1.36 m/s, temperatures that ranged from 2.25 °C to 12.54 °C and TP concentrations that ranged from < 2.0 μg/L to 42 μg/L.

Model averaging was only conducted on the presence or absence of *D. geminata* cells model set. All other model sets did not have appropriate Akaike's weights. With reference to model-averaged estimates for *D. geminata* presence or absence of cells, I obtained the following ranking of contribution of the main variables: latitude (coefficient = -0.004, p = 0.004) > TN (coefficient = 0.109, p = 0.009) > velocity (coefficient = 0.48,

$p = 0.074$) > TP (coefficient = 11.97, $p = 0.088$) > temperature (coefficient = 0.0149, $p = 0.396$).

There was, however, an effect of local variables on the abundance of *D. geminata* cells present at a site (Table 2.3). The most parsimonious model, based on AICc scores, included four environmental variables that showed that the abundance of *D. geminata* cells was significantly negatively related to stream velocity, stream order and TP levels (Table 2.3). The abundance of *D. geminata* cells was highest in stream orders 2 and 3, and lowest in stream orders 5 and 6 (Figure 2.3). Higher *D. geminata* cell abundances were also found in low water velocities and low TP levels.

Table 2.3. A summary of the three most parsimonious GLMs' for presence or absence of *D. geminata* cells and blooms and the three most parsimonious GLMs' for *D. geminata* cells and amount of AFDM.

Model	Variable	Coefficient	SE	AIC_c	Δ_i	w_i
Presence or Absence of *D. geminata* cells						
10	Intercept	-1.66	2.37	35.94	0	0.92
	Order	0.49	0.45			
	Velocity	2.053	1.91			
	Temperature	0.032	0.20			
	TP	54.60	65.03			
6	Intercept	1.041	0.90	41.36	5.42	0.061
	Velocity	0.96	1.43			
	TP	22.25	63.70			
3	Intercept	-0.23	1.74	43.52	7.59	0.021
	Velocity	1.31	1.60			
	TP	11.36	72.15			
	TN	6.98	10.35			
	Temperature	0.066	0.18			

Abundance of *D. geminata* cells (cells/cm^2)						
10	Intercept	15.85***	1.80	354.99	0	1.00
	Order	-1.71***	0.32			
	Velocity	-2.14*	0.98			
	Temperature	0.081	0.13			
3	TP	-273.96***	41.47	496.09	141.10	2.29×10^{-31}
	Intercept	12.48***	1.53			
	Velocity	-3.31**	1.08			
	TP	-170.98***	44.88			
	TN	-11.58	7.37			
	Temperature	0.014	0.15			
6	Intercept	11.14***	0.72	547.88	192.89	1.30×10^{-42}
	Velocity	-2.27	0.97			
	TP	-202.93***	40.69			
Presence or Absence of *D. geminata* blooms						
10	Intercept	3.47	2.68	31.36	0	1.00
	Order	-0.60	0.44			
	Velocity	-0.85	1.93			
	Temperature	0.14	0.21			
	TP	-416.38	267.37			
3	Intercept	0.42	1.63	47.36	16.00	0.00034
	Velocity	0.19	1.30			
	TP	-113.78	143.93			
	TN	-8.60	9.18			
	Temperature	0.030	0.16			
6	Intercept	0.23	0.91	47.66	16.30	0.00029
	Velocity	-0.048	1.11			
	TP	-164.74	163.026			
Amount of log(AFDM (mg/cm^2)+1)						
10	Intercept	4.64***	0.79	61.90	0	1.00
	Order	-0.24	0.14			
	Velocity	-0.78 ~	0.41			
	Temp	-0.14*	0.058			
	TP	-18.91	16.21			
3	Intercept	3.60***	0.60	84.037	22.14	1.56×10^{-5}
	Velocity	-0.83 ~	0.41			
	TP	-7.21	16.59			
	TN	1.14	2.84			
	Temperature	-0.13	0.58*			

6	Intercept	2.51***	0.32	100.78	38.89	$3.60x10^{-9}$
	Velocity	-0.53	0.43			
	TP	-0.91	17.3			

Note: * indicate significant deviations from the null model. '~' is a p-value of <0.1,'*' is a p-value of <0.05, '**' is a p-value of <0.01 and '***' is a p-value of <0.0001. Δ_i is the change in AIC_c scores from the lowest AIC_c score and w_i are the AIC_c weights.

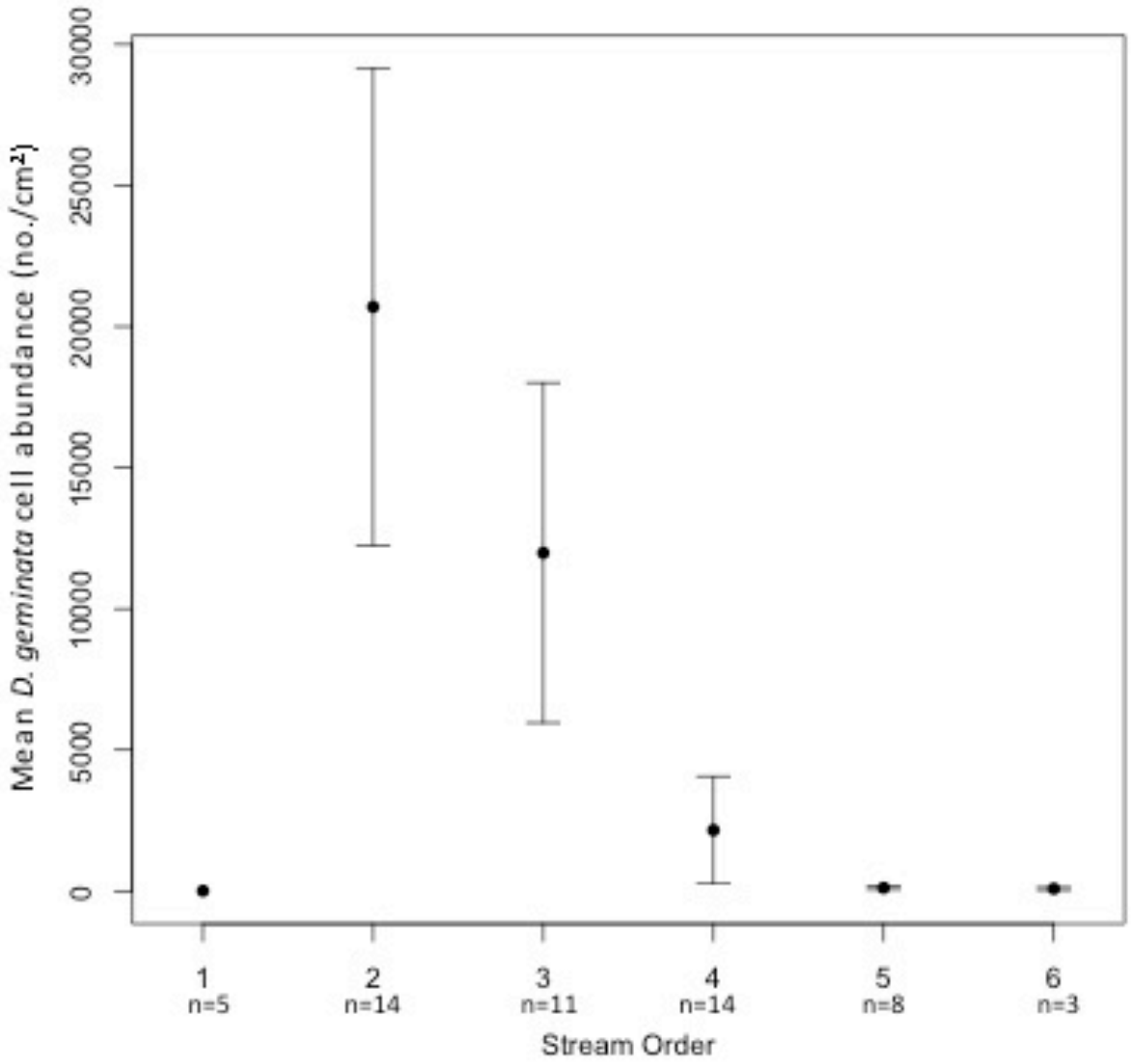


Figure 2.3. Mean *D. geminata* cell abundance (no. /cm^2) in 1st to 6th order rivers in Canadian Rocky mountain streams at all sites. Error bars represent 95% confidence intervals. N indicates the amount of streams at that stream order level.

2.3.2 *D. geminata* bloom presence and AFDM abundance

The most parsimonious model, based on AIC_c scores, included: stream order, water velocity, water temperature and TP (Table 2.3), a combination of both regional and local variables. Although this model best fit the data, there was no significant relationship between the presence or absence of *D. geminata* blooms and any of the environmental variables. The driver behind what causes a *D. geminata* colony to bloom remains unknown as blooms were observed across a range of stream orders (2 to 5), water velocities (0.17 m/s to 0.76 m/s), temperatures (3.7 °C to 12.03 °C) and TP concentrations (<0.002 mg/L to 0.008 mg/L).

There was an effect of local variables on the amount of AFDM present at a site (Table 2.3). The most parsimonious model based on AIC_c scores included four environmental variables and showed that the amount of AFDM was significantly positively related to temperature, and slightly negatively related to water velocity (Table 2.3).

2.3.3 Relationship between *D. geminata* bloom sites and non-bloom sites

Variables that had a significant relationship with *D. geminata* growth in the previous subset of models were analyzed to identify differences between bloom sites and sites with *D. geminata* cells and no blooms. There was a significant negative relationship between log TP ((mg/L)+1) and log *D. geminata* cells ((cells/cm^2)+1) ($Z_{3,31}$=-3.407,

p<0.001) in bloom and non-bloom sites (Figure 2.4). *D. geminata* cells did not bloom at TP>0.008 mg TP/L, whereas *D. geminata* cells in non-bloom sites occurred if TP ≥ 0.047 mg TP/L. I note that TP was at analytical detection limits (0.002 mg TP/L) at 27 sites sampled and these cases were excluded from analyses. In general, increased TP corresponded to lower *D. geminata* cell abundance wherever *D. geminata* occurred.

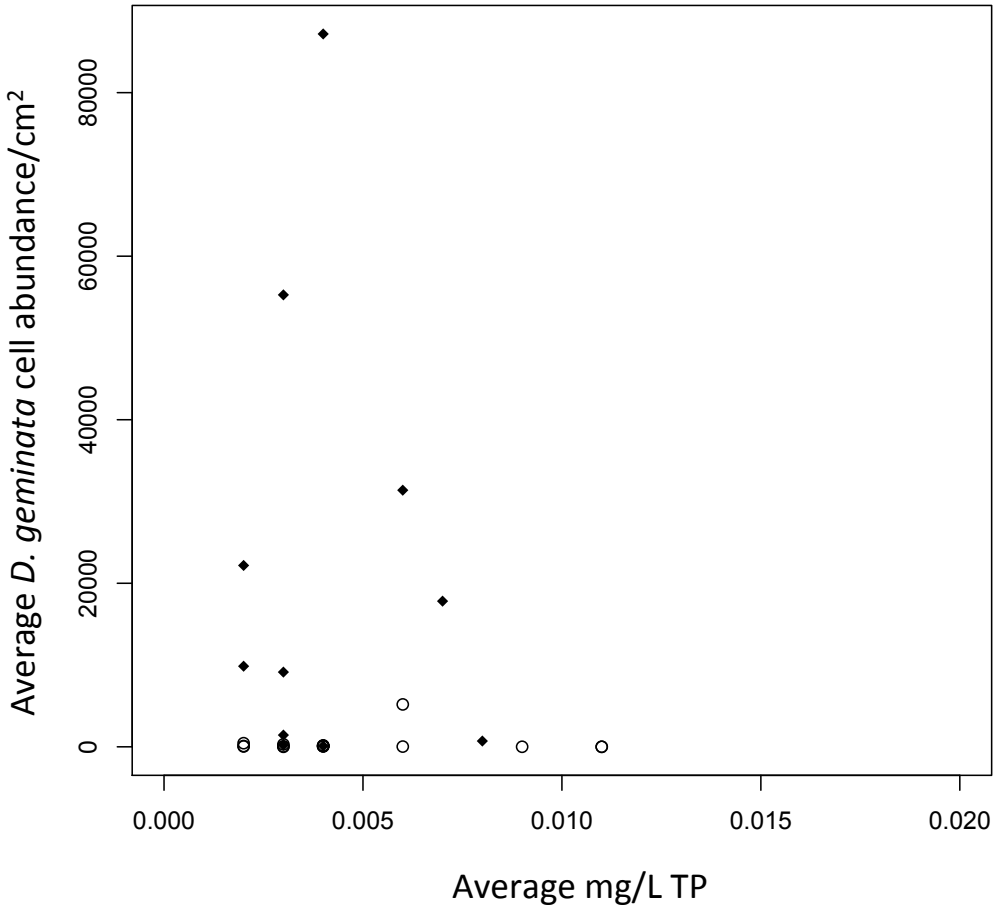


Figure 2.4. Relationship between mean density of *D. geminata* cells (no./cm^2) and mean concentration of total phosphorus (mg / L). Solid diamonds indicate sites with *D. geminata* blooms and open circles indicate sites with *D. geminata* cells but no blooms.

Blooms occurred when stream velocities ranged from 0.162 m/s to 0.712 m/s (average velocity of 0.46 m/s). The non-bloom sites ranged from 0.08 m/s to 1.23 m/s with a mean velocity of 0.52 m/s. There was a significant negative relationship between the amount of *D. geminata* cells and the velocity of the stream ($Z_{3,66} = -3.081$, $p = 0.0021$), but this relationship did not differ between bloom sites and non-bloom sites.

Stream temperature ranged from 2.39 °C to 15.2 °C and had an overall significant negative relationship between AFDM at bloom sites ($T_{3,64} = -2.05$, $p = 0.044$). There was a significant difference in this relationship between bloom and non-bloom sites ($T_{3,64} = 2.204$, $p = 0.031$). The relationship observed in bloom sites was positive between AFDM and temperature (Figure 2.6), but negative at non-bloom sites.

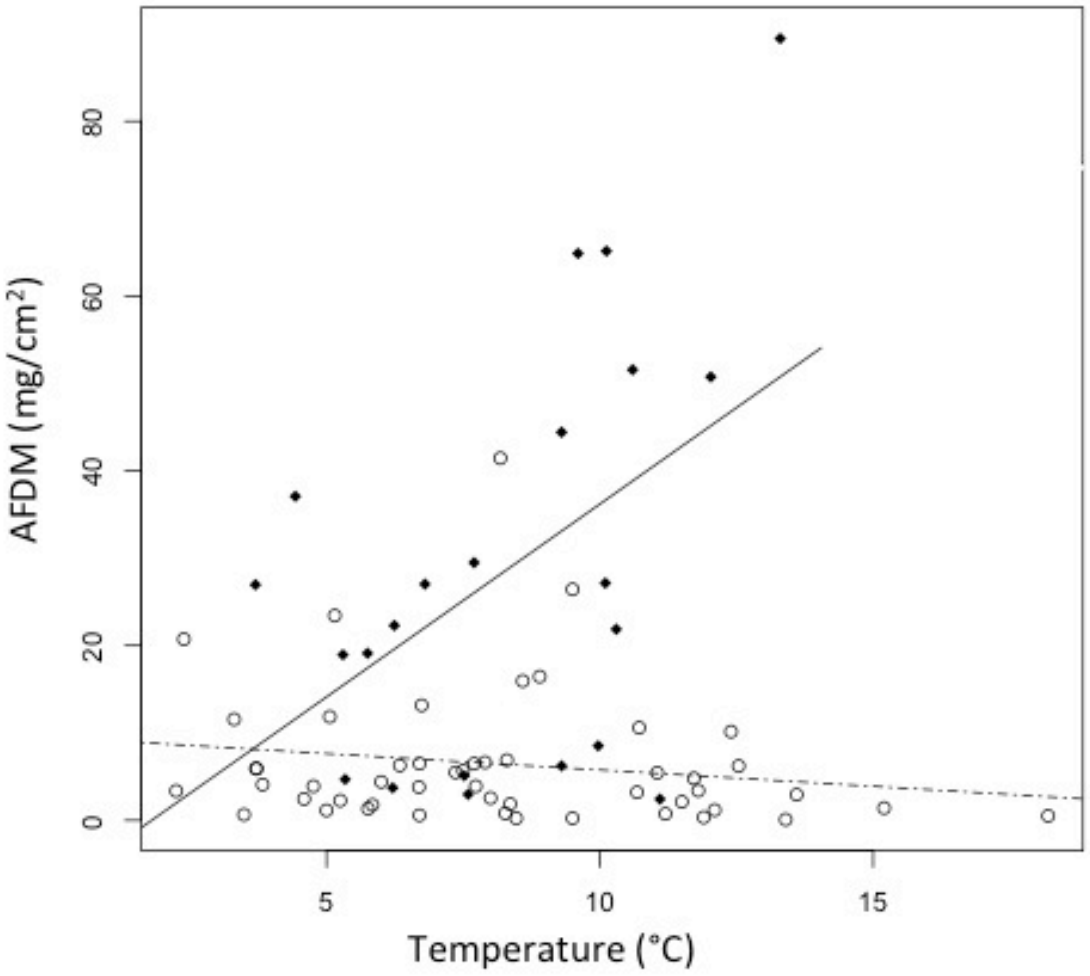


Figure 2.5. Ash-free dry mass (mg/cm^2) by temperature (°C) for all 76 sites sampled in the summer of 2009. Solid diamonds indicate sites with *D. geminata* blooms and open circles indicate sites with *D. geminata* cells but no blooms. Lines are linear regressions for *D. geminata* bloom sites (solid line) or sites with *D. geminata* cells and no blooms (dashed line). The bloom and no-bloom sites have different linear relationships with temperature.

2.4 Discussion

The presence and absence of *D. geminata* blooms at seemingly similar sites leads to a few interesting but not mutually exclusive hypotheses as to why *D. geminata* blooms. The first is that there are specific environmental conditions, or a peculiar set of environmental conditions, that support blooms that are not present at all areas where *D. geminata* cells are located (Bowman 2008, Kirkwood et al. 2007, Kawecka and Sanecki 2003, Noga 2003). The second, as has been suggested before (Bothwell et al. 2009), is that there are different strains of *D. geminata*, one of which is responsible for noxious blooms and may be invasive in the Alberta and British Columbian Rocky mountains.

The fact that I observe *D. geminata* at 67 sites but only observe blooms at 23 sites would indicate that initially the blooms must be driven by local environmental conditions. However, a lack of a strong environmental signal suggests I have not identified the appropriate environmental drivers or, that there is a difference in the diatom itself and not the local environment that cause bloom pattern variation. When relations were explored between bloom and non-bloom sites and environmental indicators a difference between temperature and AFDM was found. The fact that AFDM increases with temperature in bloom sites, but has a negative relationship at non-bloom sites suggests a fundamental difference in responses to temperature. This response may be indicative of different strains or of phenotypic plasticity of the cell's in response to temperature.

2.4.1 *D. geminata* cell presence and abundance

My data indicate that most Alberta and British Columbian streams provide a suitable habitat for *D. geminata* because it was found at almost every site sampled. Model averaged estimates indicate that latitude and TN are important drivers for the presence/absence of *D. geminata* cells. Historically, *D. geminata* has been found in northern latitude streams yet recently its range has been reported to be expanding (Blanco and Ector 2009, Spaulding and Elwell 2007). The streams in my samples are within the northern, circum-boreal range that was historically described (Blanco and Ector 2009). Although *D. geminata* has historically been found in low TN streams (Schweiger et al 2011, Kirkwood et al. 2007), it has been found to be limited by TN (Larned et al. 2006) and therefore higher levels of TN should stimulate algal growth. My results support stimulation of *D. geminata* cell division by TN.

The significant negative relationship between *D. geminata* abundance and stream order is consistent with a Kilroy and Unwin (2011), who found that *D. geminata* invaded sites in New Zealand are strongly related to stream order. However, contrary to my results, they found most streams that were invaded had a stream order > 3 and the occurrence of blooms increased with higher stream order. Streams in the Canadian Rocky Mountains seem to have the inverse relationship, with more cells occurring in lower stream orders. This may be due to the difference in movement of *D. geminata* in the two locations. In New Zealand, *D. geminata* has been a confirmed invasive species that is transferred from site to site. Lower order streams may not be visited as frequently and

therefore *D. geminata* is not transferred to them. They then have to spread upstream, which may take time (Kilroy and Unwin 2011). In the Canadian Rocky Mountain streams, *D. geminata* cells may have always been present within my streams. *Didymo geminata* cells may survive better in lower order streams and passively flow to higher order streams.

Based on the most parsimonious model, lower water velocities and lower TP levels were associated with a higher abundance of *D. geminata* cells. Higher abundance of *D. geminata* in low velocity and high flow stability streams has been identified by other studies (Tomas et al. 2010, Kirkwood et al. 2007, Larned et al. 2006, Kilroy et al. 2005d). The negative relationship between *D. geminata* cell abundance and TP concentration supports previous studies that found Didymo in oligotrophic systems (Kirkwood et al. 2009, Bowman et al. 2007). *D. geminata* seems to have a maximum threshold of phosphorus (0.010 mg/L**)** beyond which the amount of *D. geminata* cells observed decreases. Lindstrom (2008) found *D. geminata* up until 0.2 mg/L at which point it was no longer observed. Although at the higher end of the TP levels, *D. geminata* is observed in its unicellular form with no blooms. This lends support to a study conducted by Kilroy and Bothwell (2012) that suggest that there is a positive relationship with *D. geminata* cells and dissolved phosphorus, but a negative relationship between *D. geminata* blooms and dissolved phosphorus. Phosphorus is vital for algal growth and organisms have few options when phosphate supply becomes low. They can adopt different pathways to take up inorganic phosphorus more efficiently (Wagner and Falkner 2001) or use the organic phosphorus present in the environment. Due to the importance

of phosphorus for growth, they may simply slow their cell division rates thereby allowing more time for stalk development (Kilroy and Bothwell 2012) or *D. geminata* cells may be creating stalks to acquire phosphorus from an alternate source (Elwood and Whitton 2007).

2.4.2 *D. geminata* bloom presence and AFDM abundance

D. geminata was present at 88% of all sites sampled, and formed visible blooms at 34% of sites where *D. geminata* was confirmed. My most parsimonious model indicated that regional (order) and local (water velocity, water temperature and TP) variables were important drivers in whether a *D. geminata* bloom was favoured. However, none of these variables were identified as significant effects.

Many of my phosphorus results were at detection limits (0.002 mg/L), which reduced the power of the analysis. Phosphorus has been identified many times as important for the growth of *D. geminata* cells and blooms (Kilroy and Bothwell 2012, Kirkwood et al. 2009, Elwood and Whitton 2007), and therefore with more sites and a stronger ability to detect low phosphorus levels I may have detected phosphorus as a significant driver of *D. geminata* blooms. Recent studies on *D. geminata* bloom development identify phosphorus as a driver in bloom development (Kilroy and Bothwell 2012.) They suggest that low levels of phosphorus in streams trigger blooms because the rate of cell division is lower thereby allowing more time for stalk development. Elwood and Whitton (2007) suggest that these stalks are created to maximize the amount of

alkaline phosphatase that can react with the dissolved phosphorus in the water column. Each idea maintains that the development of *D. geminata* stalks is driven by oligotrophic conditions. Therefore, further work with phosphorus is important to determine the cause of *D. geminata* bloom development.

The most parsimonious GLM model revealed that stream order, water velocity, water temperature and TP are drivers for the amount of AFDM found at a site. However stream order and TP do not have a significant effect on the AFDM. There was a statistically significantly negative relationship between water temperature and AFDM and a slight negative relationship between AFDM and stream velocity.

There was more AFDM in slower streams. In particular, there were no *D. geminata* blooms at velocities above 0.8 m/s, which suggests that *D. geminata* has a maximal threshold velocity. A maximal threshold velocity lends support to studies that found low flow to be an important criterion for *D. geminata* blooms (Tomas et al. 2010, Kirkwood et al. 2007, Larned et al. 2006, Kilroy et al. 2005d). Several studies have shown more than water velocity, stability of the water flow is a better predictor of *D. geminata* blooms (Kirkwood et al. 2009, Biggs 1996). Flow variation was not measured in this study, but may be an important driver of bloom development and merits further study.

The amount of AFDM is significantly higher in waters with low temperatures. However, when the amount of AFDM material was divided into sites where blooms were

observed, and sites where no blooms were observed a different relationship became apparent. There was a negative relationship between temperature and AFDM at sites where no *D. geminata* blooms were observed but a significant positive relationship between *D. geminata* and AFDM at sites where *D. geminata* blooms were observed. *D. geminata* blooms were only observed to a maximum temperature of 14°C. Therefore, this suggests that warmer temperatures will result in more *D. geminata* bloom material up to a threshold temperature of around 14°C.

This temperature threshold supports studies that found *D. geminata* presence up to 23°C but rarely found it in its vegetative state above 18°C (Lindstrom et al. 2008). *D. geminata* blooms have consistently been described as blooming in cold water (Lindstrom and Skulberg 2008, Kirkwood et al. 2007, Blanco and Ector 2002), however there is increasing evidence of blooms appearing in warmer waters (Kumar et al. 2009, Spaulding and Elwell 2007). Kilroy et al. (2006) noted that blooms did not appear in areas where the lowest winter air temperatures are above five °C. This highlights again the importance of D. *geminatas* cold temperature requirements. My sampled streams would definitely support this cold temperature requirement as many of the sample streams would either completely, or partially ice over in the winter as air temperatures drop below zero. The difference in response to water temperatures between blooming and non-blooming *D. geminata* populations lend support to the hypothesis that there are multiple strains of *D. geminata* that have different physiological requirements. It also lends support to the

hypothesis that the *D. geminata* cell is plastic and can change its phenotype when exposed to different temperatures.

It is possible that this study dealt with either multiple strains or a plastic *D. geminata* population, which added levels of complexity to my analyses and masked the behaviour of each individual strain. To fully explore the differences between *D. geminata* populations in the Rocky Mountains, morphological and molecular analyses should be conducted. Support to the different strain hypothesis would require evaluating samples from blooming and non-blooming sites and finding variations within their morphology or genetic design. If this was established, research focus could be placed on the strain-specific bloom sites, their environmental requirements, origin and vectors of transport. If different strains were not established, research could focus on placing identical strains in different environmental conditions to determine if their phenotype was altered which would give support to the hypothesis that the *D. geminata* populations are exhibiting phenotypic plasticity.

2.4.3 Conclusion

Overall, *D. geminata* is present in the majority of Alberta and British Columbian Rocky Mountain streams sampled. *D. geminata* cell presence is driven by regional variables, but once cells are present a combination of the local and regional environmental conditions (stream order, water velocity and TP) may determine the abundance of cells. Blooms do not occur everywhere that *D. geminata* cells are present,

although no differences in local environmental conditions between bloom and non-bloom sites were identified. The amount of AFDM was driven by local variables, but this relationship differed between bloom sites and non-bloom sites. The suite of environmental conditions that cause *D. geminata* blooms may not have been identified in this study, *D. geminata* may exhibit phenotypic plasticity or there may be multiple strains of *D. geminata*. Further work will need to be conducted within the study streams to determine the cause of *D. geminata* blooms.

There were many limitations and constraints to this study that prevented me from fully resolving my questions. First of all, as I mentioned previously, many streams were at the detection limits of the phosphorus analyzing equipment. This reduced the power of the analysis and prevented me from observing the full relationship of phosphorus to *D. geminata.* For future work a more sensitive technique would be necessary. Also, all of the streams were within a similar range of the environmental variables and therefore only represented streams of a similar make-up. For a future study it would be beneficial to include areas of dissimilar environmental make-up. Finally, with only one sampling effort at each site I cannot be positive that the conditions did not change over the season. In order to ascertain that a site does not go from a non-bloom to a bloom site or suddenly have a drastic increase or decrease of *D. geminata* cells, the sites must be sampled numerous times over the season. Only then can one be sure of accurate characterization of the *D. geminata* status at the site.

Given the limitations to the study and lack of information on the causes of *D. geminata* blooms, movement of *D. geminata* through our mountain streams should be prevented. Once *D. geminata* arrives at a low order stream, if the local conditions within in the stream were low water velocity, within the critical temperature range and low TP, the stream could be at risk of a *D. geminata* bloom event. Streams with this suite of characteristics should be heavily monitored. To deter the continued spread of *D. geminata,* I would urge resource managers to educate tourists about the possibility of spreading *D. geminata* cells. However, a strong management plan will be difficult without further research into the *D. geminata* populations in the parks. Genetic analysis should be completed to determine if different *D. geminata* strains are present within the park and what each of their environmental requirements are.

Chapter Three: ***Didymosphenia geminata*** **growth on nutrient diffusing substrata**

3.1 Introduction

Algal growth is influenced by a number of different environmental factors including: light, temperature, oxygen and nutrient levels. Algal blooms develop due to an interaction of these factors that cause a rapid accumulation of algal cells within an aquatic system. Although algal blooms are a natural phenomenon, an increase in frequency, extent, duration and density of the bloom can cause harm to aquatic systems by reducing light and oxygen levels, replacing nutritious algae and releasing toxins. Defining what constitutes an algal bloom is difficult as the type and extent of bloom varies from species to species. Winter et al. (2011) defined algal blooms as "excessive growth of algae to a level that prompts complaint". In New Zealand, algal blooms in lakes and rivers have been quantified by saying a bloom is cover of the streambed by >30% with green filamentous algae, or >60% with diatom mats, or >35 g/m^2 of ash-free dry mass (AFDM), or by 120 mg/ m^2 of chlorophyll a (Biggs 2000b).

Sentinel experiments at Lake 226 in the experimental lakes area (ELA) revealed a positive relationship between algal blooms and phosphorus input (Schindler, 1977), which has since been confirmed many times (Dodds et al. 2002, Biggs 2000, Schindler 1977). Typically, nuisance algal blooms are associated with excessive nutrient inputs. However, in western Canada over the past decade, the diatom *D. geminata* has been creating nuisance blooms in oligotrophic (low nutrient) streams (Kirkwood et al. 2007).

Didymosphenia geminata is a unicellular diatom with an apical porefield that secretes a large amount muccopolysaccharide stalk. The stalk can have up to 250 times more biomass than the diatom cell itself (Larned et al. 2006) and is the cause of devastating ecological and economic effects in streams and rivers. The stalk is largely composed of polysaccharides that resist degradation and lack nutritional value (Spaulding and Elwell 2007, Larned et al. 2006). The stalks divide when the diatom divides, thus causing the material to accumulate rapidly and cover a large extent of the stream substrate (Spaulding and Elwell, 2007, Spaulding, USGS unpublished data, 2006), which leads to homogenization of the sediment and a build up of stalk material. Curiously, *D. geminata* is often found blooming in streams where the soluble reactive phosphorus (SRP) is below analytical detection limits.

Phosphorus is integral to energy metabolism and therefore a requirement for algal species. Organisms have few options when inorganic phosphate (the form of phosphorus that can be taken up through algal cell membranes) supply becomes low; they can adopt different pathways to take up phosphate more efficiently (Wagner and Falkner 2001) or use the organic phosphorus (Org-P) present within the environment. Uptake of Org-P across the cell membrane does not occur, but the activation of cell-surface-associated monoester hydrolase enzymes can convert Org-P into phosphate by cleaving off the organic portion (Hernandez et al. 2003, Strojsova et al. 2003, Whitton et al. 1991). The best-known phosphorus monoester hydrolase enzyme in aquatic algae is alkaline

phosphatase, which has been observed in high levels during times of phosphorus limitation (Chrost et al. 1984, Francko 1983, Healy 1975).

Alkaline phosphatases act on the cell surface, so more cell surface area will create more area for enzymes to work, which will mean a higher rate of phosphate acquisition per cell. Some bacteria and cyanobacteria may create multicellular hairs with high surface phosphatase activity in times of phosphorus limitation (Whitton 1988). Localized enzyme staining on *D. geminata* revealed enzyme activity localized to *D. geminata* stalks and not on the test surface (Elwood and Whitton 2007). This method of phosphorus uptake may provide *D. geminata* with a competitive advantage over other algal species in times of P-limitation and also result in *D. geminata* forming large nuisance blooms in P-limited streams, but the evidence for this has not been tested.

Nutrient limitation of phytoplankton has been experimentally tested in a number of ways. Whole stream nutrient enrichments studies have been used to determine the role of nutrients in the formation of algal blooms (Larson and Carreiro 2008, Schindler 1977). However, whole system manipulations can be costly, logistically challenging and very time consuming. Chemostats have historically been used to determine phytoplankton growth rates across a range of limiting nutrient supply rates and concentrations in the laboratory (Tillman 1997). Ideally, chemostats would be used to assess the effect of different nutrient concentrations on growth of *D. geminata*. However, I was not successful in growing *D. geminata* in controlled laboratory situations and at the time of this study, I was unaware of anyone who had success with *D. geminata* cultures. Since

the completion of this study, there has been some success with culture *D. geminata* (Kuhajek and Wood 2011, Kuhajek et al. 2011).

An alternate method to assess nutrient limitation in rivers and streams uses nutrient diffusing substrata (NDS) (Francoeur 2001). NDS are a smaller scale study where nutrients are released from a medium over time, into a porous substrate upon which the algal cells can colonize and grow. With NDS, replication is easily possible and can be used in the field. NDS have been used successfully in the field many times to answer aquatic nutrient questions (Irvine and Jackson 2006, Francoeur 2001). Significant responses of *D. geminata* to nutrient amendments were found in New Zealand streams (Larned et al. 2006).

The goal of this study was to determine i) if *D. geminata* bloomed in P-limited streams, ii) whether organic phosphorus promotes *D. geminata* blooms, iii) if other nutrients play a role in *D. geminata* growth and, iv) the environmental matrices associated with *D. geminata* blooms. NDS devices were deployed over two years (2009 and 2010) on seven sites on four different streams within the Bow River watershed in Alberta, Canada. NDS manipulations were conducted to determine whether *D. geminata* was N or P limited, and whether of not *D. geminata* could grow on organic phosphorus. Nutrients used were combination of inorganic phosphorus, organic phosphorus and nitrogen.

3.2 Methods

3.2.1 Sites

NDS deployments were conducted during July-October 2009 and June-August 2010. Seven sites within the Bow River watershed were selected for the deployments (Figure 3.1). Streams have circum-neutral to slightly alkaline pH, moderate to high slope and during the fall, are typically clear (water turbidity < 20 NTU). Streams are ice covered during November to April and open during the summer. Sites were selected based on previous observations of Didymo blooms (Table 3.1). In 2009, NDS racks were placed in areas where previous blooms had been reported (Upper Bow River, Lower Bow River, Kananaskis River) and in areas where no previous blooms had been reported (Upper Jumpingpound Creek, Jumpingpound). In 2010, the selected sites were areas where large Didymo blooms had previously been observed (Baker Creek, Lower Bow River, Louise Creek).

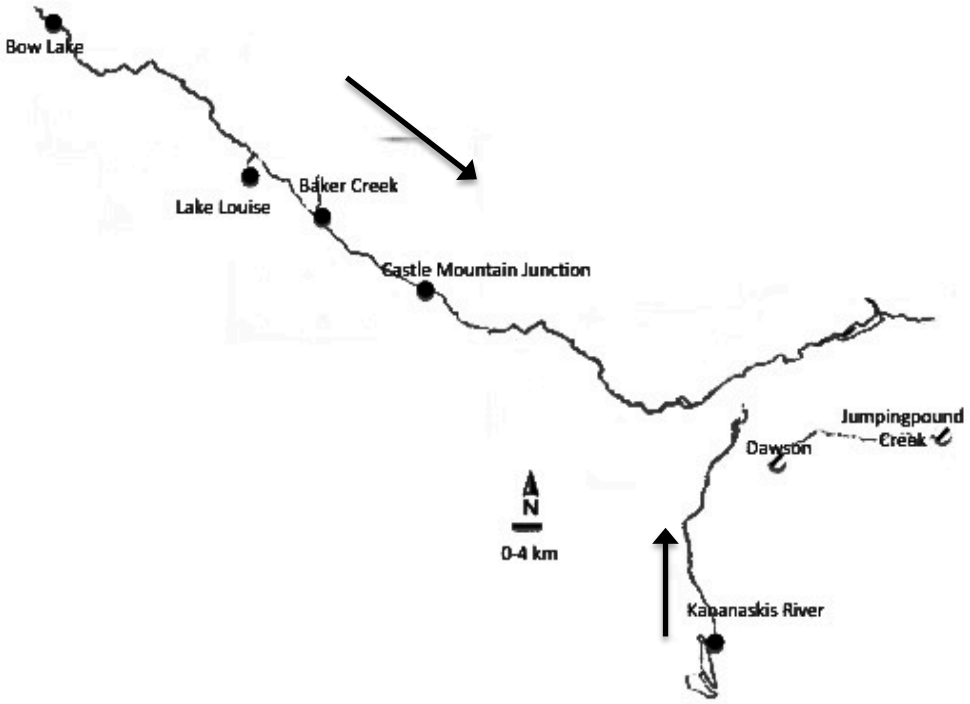


Figure 3.1. NDS deployment sites along the Bow River watershed. Bloom sites are represented by the solid circles, and non-bloom sites are the circles with lines. The Upper Bow River, Lower Bow River, Kananaskis River, Upper Jumpingpound Creek and Lower Jumpingpound Creek were sampled in 2009. Baker Creek, Louise Creek and Lower Bow River were sampled in 2010. Arrows indicate direction of water flow.

Table 3.1. Environmental stream characteristics of each NDS deployment site in 2009 or 2010. The code indicates the name of the site in subsequent figures and tables.

Site	Date Deployed	Deployment Period (Days)	Treatments*	Elevation (m)	Average Depth (cm)	Average Water Temperature (°C)	Average Water Velocity (m/s)
Baker Creek	July 27 2010	21	Control, Inorg-P, Org-P, N, N+Inorg-P, N+Org-P	1507	36.19	5.14	1.15
Upper Bow River	Aug 24 2009, Sept 14 2009	21, 21	Control, Inorg-P, Org-P	2273	31.57	8.00	0.51
Lower Bow River	Aug 24 and Sept 14 2009, July 27 2010	21, 21, 21	Control, Inorg-P, Org-P, N**, N+Inorg-P**, N+Org-P**	1449	51.42	7.34	0.82
Upper Jumpingpound Creek	July 31 and Sept18 2009	34, 21	Control, Inorg-P, Org-P	1841	39.25	7.83	0.38
Lower Jumpingpound Creek	July 31 and Sept18 2009	34, 21	Control, Inorg-P, Org-P	1266	35.34	13.35	0.65
Kananaskis	July 27	38, 21	Control, Inorg-	1699	30.97	8.76	0.17

River	and Sept15 2009		P, Org-P				
Louise Creek	June 1 and July 27 2010	21, 21	Control, Inorg-P, Org-P, N, N+Inorg-P, N+Org-P	1735	35.62	6.10	0.97

* C=control (no added nutrients), Org-P =organic phosphorus, Inorg-P = inorganic phosphorus, N = nitrogen, N + Inorg-P=nitrogen and inorganic phosphorus combination and N + Org-P nitrogen and organic phosphorus combination
**Treatments conducted only in 2010 at the Lower Bow River site.

3.2.2 NDS design and set-up

In 2009, I evaluated spatial and temporal patterns in phosphorus limitation by deploying nutrient diffusing substrata in five streams in the foothills of the Rock Mountains. Racks of NDS were deployed in three streams where *D. geminata* blooms had been previously reported (Upper Bow River, Lower Bow River, Kananaskis River) and in two streams where they had not (Upper Jumpingpound Creek and Lower Jumpingpound Creek) (Figure 3.1). I deployed NDS in late summer (Late July and August) and early fall (September). NDS devices consisted of silica disk, heated and pressed into the top of 37 mL polystyrene vials (Dynalab Corp., Rochester, NY, USA, #2636-0010) that contained agar with one of the following three nutrient treatments: a control (agar only), organic phosphorus (0.5 M $C_3H_7O_6PNa_2 \cdot 5H_2O$) or inorganic (0.5 M KH_2PO_4) phosphorus. I used sodium glycerophosphate ($C_3H_7O_6PNa_2 \cdot 5H_2O$) as a source of organic phosphorus (Hong et al. 1995). These treatments are designed to test for p-limitation (replete versus deplete) and also to determine if algal communities are capable of using organic phosphorus. While 10 replicates of each NDS treatment were deployed at each site, retrieval of between eight and 10 replicates of each treatment resulted in variable numbers of treatment replicates.

In 2010, I evaluated spatial and temporal patterns in nutrient limitation (not limited [replete], phosphorus limitation, nitrogen limitation, and co-limitation) by deploying nutrient diffusing substrata in three streams of Louise Creek, Baker Creek and Lower Bow River in late summer (Late July and August). Louise Creek was also evaluated in early summer (June). In contrast to 2009, NDS contained agar with one the following six nutrient treatments: i) control

(agar only), ii) nitrogen (0.5 M $NaNO_3$), iii) inorganic (0.5 M KH_2PO_4) phosphorus, iv) organic phosphorus (0.5 M $C_3H_7O_6PNa_2 \cdot 5H_2O$), v) nitrogen and inorganic phosphorus (0.5M KH_2PO_4 and 0.5M $NaNO_3$), and nitrogen and organic phosphorus (0.5 $C_3H_7O_6PNa_2 \cdot 5H_2O$ and 0.5 M $NaNO_3$). These treatments are designed to test for nutrient limitation (replete versus deplete), the limiting nutrient and also if algal communities were capable of using organic phosphorus. Individual treatments were replicated between 8 to 10 times.

I compared patterns in nutrient limitation using NDS with those suggested from alkaline phosphatase activity and water column concentrations of total phosphorus when NDS were deployed and retrieved. Predicting patterns in nutrient limitation based on concentrations of total phosphorus can be difficult because only a portion of the total fraction is biologically available. Nevertheless, I identified three ranges in concentrations of total phosphorus that may be suggestive of phosphorus limitation of: i) high probability (< 10 µg /L total phosphorus), ii) moderate probability (11 to 30 µg /L total phosphorus) and low probability (> 30 µg /L total phosphorus). The alkaline phosphatase results were compared to a standard put forth by Healey and Hendzel (1979) where a value above five indicates severe P-limitation, between two and five is slight P-limitation and below two is no P-limitation.

1.2.3 Natural periphyton and environmental data

Once a week during sampling seasons (July 13-October 9th 2009 and June 1st-September 14 2010) each NDS site was visited and environmental (chemical, physical and biological) measures were taken. Start dates of the environmental samples varied between each site based on site accessibility. In 2009, Kananaskis River, Upper Jumpingpound Creek and Lower Jumpingpound Creek were accessed beginning July 13th. The other sites began the week of August 17th 2009. Sampling continued until the first week of October 2009. In 2010, Louise Creek was accessed at the beginning of June 2010 but all other sites were not accessed until the end of July 2010. Mean water velocity was calculated at 60% water depth using a flow metre (Global Water Flow Probe (FP111)). Water temperature was measured using a glass thermometer. Turbidity was measured on site using an Orbeco-Hellige Model 966 Turbidimeter (Orbeco-Hellige Corp. Famingdale, NY, USA). Triplicate water samples were taken at each site in acid-washed, double-distilled water rinsed Nalgene bottles with as little air as possible then stored on ice, until return to the laboratory.

At each site, three cobbles (0.05-0.1 m in diameter) were chosen randomly within 0.5-2.5 m from shore and 0.2-0.7 m water depth. Three 14.3 cm^2 circles were traced on each cobble following a PVC template and the algal material within the traced circle was mechanically dislodged from the cobble with a combination of scalpels, tweezers and toothbrushes to ensure all material was removed from the traced area. The algal material from one circle was placed in a 125 mL borosilicate bottle with distilled water and

Lugol's acid-iodine fixative and placed in the dark for later *D. geminata* identification and enumeration. The material from a second circle was wrapped in aluminum foil, kept on ice during transport to the laboratory and then stored at -20°C prior to chlorophyll a analyses. The material from the final circle was placed in a small plastic bottle and stored on ice until it was used for alkaline-phosphatase analysis, which was performed within 24 hours of sample collection.

3.2.3 Laboratory Analyses

All chlorophyll a samples were extracted into methanol and processed following the methods of Thompson et al. (1999), without correction for phaeophytin content. Chlorophyll a was analyzed with a Spectromax Gemini XS dual-scanning microplate spectroflurometer (Molecular Devices, Sunnyvale, California USA) at an excitation wavelength of 440 nm. Alkaline-phosphatase activity (APA) was determined following the methods described by Johnson et al. (1997), Steinman and Mulholland (1996), Jansson et al. (1988), Petersson (1980), Healey and Hendzel (1979) and Perry (1972). Periphyton was incubated with a fluorescent substrate and compared with chlorophyll *a* samples. Fluorescent samples were analyzed with a Turner Quantech Digital Filter Fluorometer (FM109515).

All material removed from within an entire 14.3 cm^2 was resuspended in 100 mL of water, then 10 mL sub-samples, from the well mixed 100 mL samples, were poured into setting chambers and processed using a Leica DM IRB inverted microscope (Leica

Microsystems, Wetzlar, Germany) for cell identification and enumeration. Cells were counted at a 100X magnification. *D. geminata* cells were identified by the diacritical feature of 2-5 stigmata and the conspicuous size of 60-150 μm. Water samples were analyzed within 24 hours of collection for total phosphorus (TP) following standard protocols (American Water Works Association, 1995).

3.2.4 Statistical Analyses

Descriptive statistics were calculated using Microsoft Excel (2011). Statistical analyses were conducted using the statistics package R (v2.8.1) (R Development Core Team, 2008). Maps (1:200,000) were traced by hand from maps located in the Canadian Rockies Backroad Mapbook (2011). Any chemistry data that were at detection limits were removed from analysis and all analyses used a critical $\alpha = 0.05$.

3.2.4.1 NDS statistical analyses

I tested for differences in algal biomass, measured as chlorophyll a (μg/ cm^2) and cell densities of *D. geminata* (numbers of cells/ cm^2) among treatments using a single factor analysis of variance. When the analysis of variance tests were statistically significant ($p < 0.05$), I tested for differences among treatments using Bonferroni adjusted pairwise comparisons. I identified patterns in P-limitation typically based on the statistical significance of the analysis of variance tests and differences in treatments using revealed by pairwise comparisons.

3.2.4.2 Natural periphyton and stream statistical analyses

Nutrient limitation within streams was also assessed by the amount of alkaline phosphatase produced/hr/μg of chlorophyll a.

Linear models were used to test for any significant differences between water column characteristics (temperature, TP and water velocity) and site and season. Linear relationships between water column characteristics and the amount of AFDM or the abundance of *D. geminata* cells was determined using an analysis of covariance (ANCOVA). If no co-variations existed, the linear model was used to reveal any linear relationships.

3.3 Results

3.3.1 Patterns in Nutrient Limitation

In the Bow River basin, the majority of sites sampled had in stream phosphorus levels that suggested a high probability of phosphorus limitation (Table 3.1 and 3.2). The exceptions were the Upper and Lower Bow River sites that had only a moderate probability of being phosphorus limited. APA from natural periphyton within the stream also suggested a high probability of phosphorus limitation (with the exception of Lower Jumpingpound Creek, and Louise Creek) (Table 3.1 and 3.2). Of those sites identified as phosphorus limited, the NDS data indicate that the Lower Bow River was limited by both

inorganic and organic sources of phosphorus, Upper Jumpingpound Creek was limited by inorganic phosphorus sources in the late summer but by early fall was not limited by nutrients, the Upper Bow River and Lower Jumpingpound Creek were not limited in the late summer but by early fall were limited by organic phosphorus sources. In 2010, the observed nutrient limitation status of the sites based on NDS is inorg-P limited. However, observed nutrient limitation based on NDS did not always reflect the other methods of predicting phosphorus limitation and chlorophyll a accrual (Table 3.1) and *D. geminata* cell colonization on NDS did not reflect the same patterns of nutrient limitation. The nutrient limitation status based on NDS results differed between site and season. In late summer of 2009, the chlorophyll a results indicated that the Upper Bow River, Lower Jumpingpound Creek and Kananaskis River were considered to be not limited by phosphorus, although the in stream phosphorus concentration indicated otherwise. In the early fall, Upper Jumpingpound Creek and the Kananaskis River did not indicate phosphorus limitation. The *D. geminata* cell colonization on NDS indicated colonies that were not limited by nutrients.

3.3.2 *D. geminata* response to nutrient addition

Chlorophyll a accrual did not reflect *D. geminata* cell colonization. There was a slight positive relationship, but it was not strong (R^2 =0.27, $F_{1, 496}$ =187.6, p <0.001). The response to nutrient additions differed between accrual of chlorophyll a and accrual of *D. geminata* cells (Figures 3.2 and 3.3). Bonferroni adjusted pairwise comparisons indicate

many significant differences between treatments in the amount of chlorophyll a accrued but not in the amount of *D. geminata* cells colonizing.

Chlorophyll a accrual was equal on all treatments in the Upper and Lower Bow River sites in the late summer, but by early fall there was more accrual on organic phosphorus and inorganic phosphorus respectively (Figure 3.2). Upper and Lower Jumpingpound Creek had equal accrual on all treatments in the late summer but by early fall there was significantly more accrual on the inorganic phosphorus treatment at Lower Jumpingpound Creek (Figure 3.2). The Kananaskis River had equal accrual on all treatments throughout the entire sampling season. Baker Creek and Lower Bow River had higher accrual on the inorg-P treatments in 2010 (Figure 3.2). Louise Creek had significantly more accrual on the inorg-P treatment during both sampling efforts in 2010 and the N + inorg-P treatment during the summer sampling set 2010 (Figure 3.2).

The *D. geminata* cells colonized significantly more on the org-P NDS than the N + org-P NDS in Louise Creek summer 2010 (Figure 3.3). Otherwise, there were no significant differences between the amount of *D. geminata* cells colonizing any treatment at any site or during any season.

Table 3.2. Nutrient limitation status at each of the seven sites at each sampling period. Nutrient limitation is assessed using total phosphorus levels within the stream, APA activity within the stream periphyton and ANOVA results from chlorophyll a accrued on the NDS devices.

Year	Season deployed	Site	Total phosphorus (µg/L) when NDS were deployed	Total phosphorus (µg/L) when NDS were retrieved	Predicted phosphorus or nutrient limitation based on concentrations of TP in stream water	APA activity	Predicted phosphorus limitation based on APA	*Analysis of variance*		*Observed Nutrient Limited Status (Based on NDS)*
								F (d.f)	*P*	
2009	Late Summer	Upper Bow River	12.04	15.88	Moderate probability of P-limitation	-		$F_{(2,27)} = 2.287$	0.121	P-replete
		Lower Bow River	13.59	19.57	Moderate probability of P-limitation	-		$F_{(2,26)} = 3.503$	0.0450	P-limited
		Upper Jumpingpound Creek	6.94	5.72	High probability of P limitation	-		$F_{(2,26)} = 3.545$	0.044	Inorg-P-limited
		Lower Jumpingpound Creek	6.14	4.95	High probability of P limitation	-		$F_{(2,27)} = 0.549$	0.584	P-replete
		Kananaskis River	4.76	2.45	High probability of P limitation	-		$F_{(2,26)} = 1.127$	0.339	P-replete
	Early fall	Upper Bow River	15.88	10.95	High probability of P limitation	15.70	High probability of P limitation	$F_{(2,26)} = 4.457$	0.022	Org-P-limited
		Lower Bow River	19.57	9.13	High probability of P limitation	15.86	High probability of P limitation	$F_{(2,27)} = 21.570$	<0.001	P-limited
		Upper Jumpingpound Creek	9.34	7.98	High probability of P limitation	1.44	High probability of P-Replete	$F_{(2,27)} = 2.918$	0.071	P-replete
		Lower Jumpingpound Creek	8.62	7.50	High probability of P limitation	4.04	Low probability of P-limitation	$F_{(2,27)} = 3.646$	0.040	Inorg-P-limited
		Kananaskis River	22.86	8.43	High probability of P limitation	6.28	Moderate probability of P-limitation	$F_{(2,27)} = 0.617$	0.547	P-replete
2010	Early Summer	Louise Creek	8.68	6.50	High probability of P limitation	0.87	Predicted to be P-replete	$F_{(5,52)} = 5.656$	<0.001	Inorg-P-limited
	Summer	Baker Creek	5.24	2.14	High probability of P limitation	22.68	High probability of P limitation	$F_{(5,54)} = 6.860$	<0.001	Inorg-P-limited
		Lower Bow River	7.09	5.04	High probability of P limitation	36.45	High probability of P limitation	$F_{(5,54)} = 10.490$	<0.001	Inorg-P-limited
		Louise Creek	9.66	7.62	High probability of P	16.34	High probability of P	$F_{(5,53)} = 28.470$	<0.001	Inorg-P-limited

					limitation		limitation			

Table 3.3. Nutrient limitation status using at each of the seven sites at each sampling period. Nutrient limitation is assessed using total phosphorus levels within the stream, APA activity within the stream periphyton and ANOVA results from the *D. geminata* cells colonized on the NDS devices.

Year	Season deployed	Site	Total phosphorus (µg/L) when NDS were deployed	Total phosphorus (µg/L) when NDS were retrieved	Predicted phosphorus or nutrient limitation based on concentrations of TP in stream water	APA activity	Predicted phosphorus limitation based on APA	*Analysis of variance*		*Observed Nutrient Limited Status (Based on NDS)*
								F (d.f)	*P*	
2009	Late Summer	Upper Bow River	12.04	15.88	Moderate probability of P-limitation	-		$F_{(2,27)} = 1.371$	0.271	P-replete
		Bow River	13.59	19.57	Moderate probability of P-limitation	-		$F_{(2,26)} = 1.231$	0.308	P-replete
		Upper Jumpingpound Creek	6.94	5.72	High probability of P limitation	-		$F_{(2,25)} = 2.337$	0.117	P-replete
		Lower Jumpingpound Creek	6.14	4.95	High probability of P limitation	-		$F_{(2,27)} = 0.216$	0.807	P-replete
		Kananaskis River	4.76	2.45	High probability of P limitation	-		$F_{(2,24)} = 2.349$	0.117	P-replete
	Early fall	Upper Bow River	15.88	10.95	High probability of P limitation	15.70	High probability of P limitation	$F_{(2,25)} = 1.901$	0.170	P-replete
		Lower Bow River	19.57	9.13	High probability of P limitation	15.86	High probability of P limitation	$F_{(2,20)} = 0.718$	0.500	P-replete
		Upper Jumpingpound Creek	9.34	7.98	High probability of P limitation	1.44	High probability of P-Replete	$F_{(2,13)} = 0.988$	0.399	P-replete
		Lower Jumpingpound Creek	8.62	7.50	High probability of P limitation	4.04	Low probability of P-limitation	$F_{(2,26)} = 0.072$	0.930	P-replete
		Kananaskis River	22.86	8.43	High probability of P limitation	6.28	Moderate probability of P-limitation	$F_{(2,26)} = 0.310$	0.736	P-replete
2010	Early Summer	Louise Creek	8.68	6.50	High probability of P limitation	0.87	Predicted to be P-replete	$F_{(5,54)} = 1.231$	0.307	P-replete
	Summer	Baker Creek	5.24	2.14	High probability of P limitation	22.68	High probability of P limitation	$F_{(5,54)} = 1.069$	0.388	P-replete
		Lower Bow River	7.09	5.04	High probability of P limitation	36.45	High probability of P limitation	$F_{(5,54)} = 0.924$	0.473	P-replete

	Louise Creek	9.66	7.62	High probability of P limitation	16.34	High probability of P limitation	$F_{(5,53)} = 2.693$	0.031	P-replete

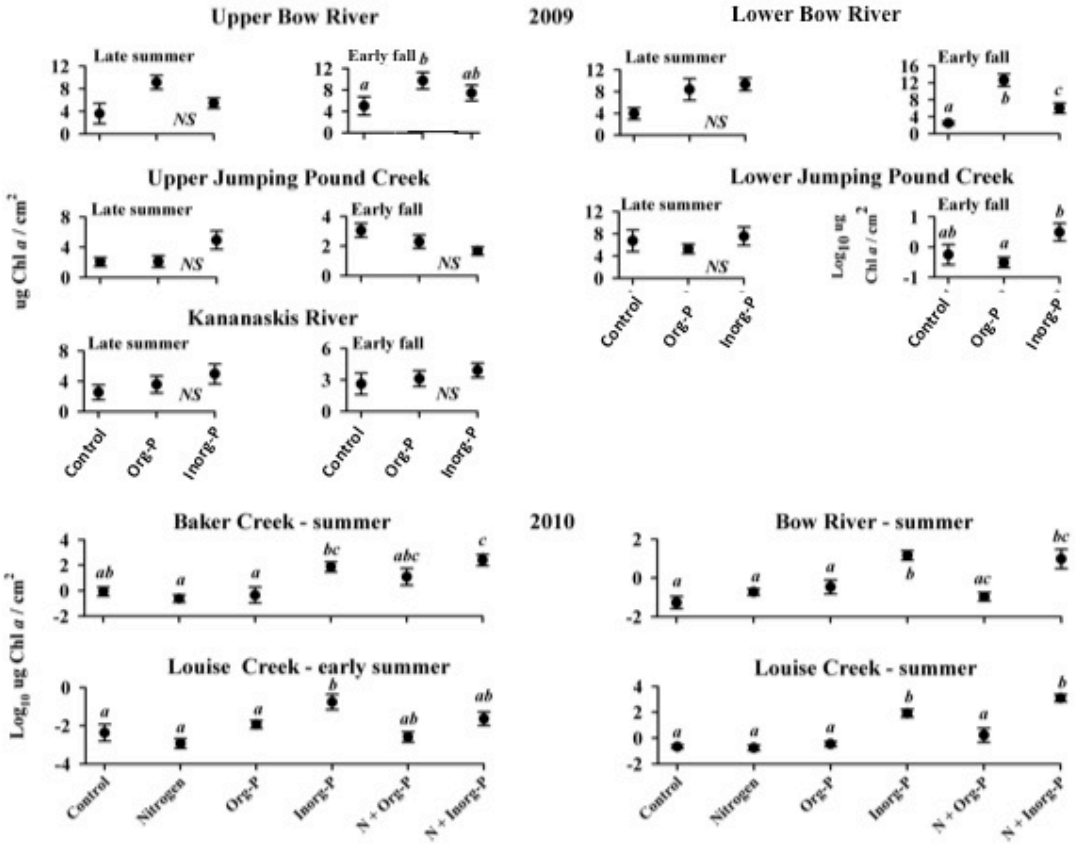


Figure 3.2. Mean µg of chlorophyll a per cm^2 on NDS devices from the six treatments; Control, Nitrogen, organic Phosphorus (org-P), inorganic phosphorus (inorg-P), Nitrogen and organic Phosphorus (N + org-P) and Nitrogen and inorganic Phosphorus (N + inorg-P) for each sampling site and time. Mean +/- standard errors (SE) are plotted and each bar represents ~N=10 for each treatment at each site. NS indicate a non-significant result, whereas similar letters also indicate no significant

difference between pairs of results. Different letters indicate a significant difference as determined by Bernoulli corrected pairwise comparisons (P<0.05).

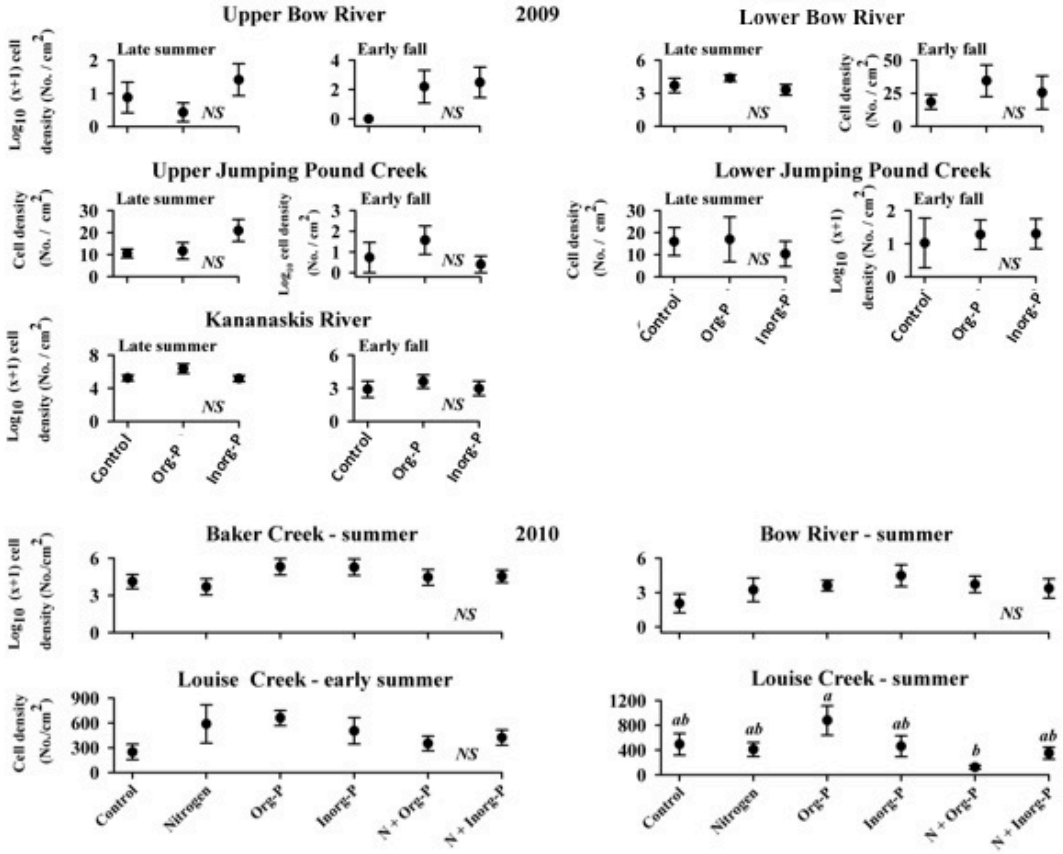


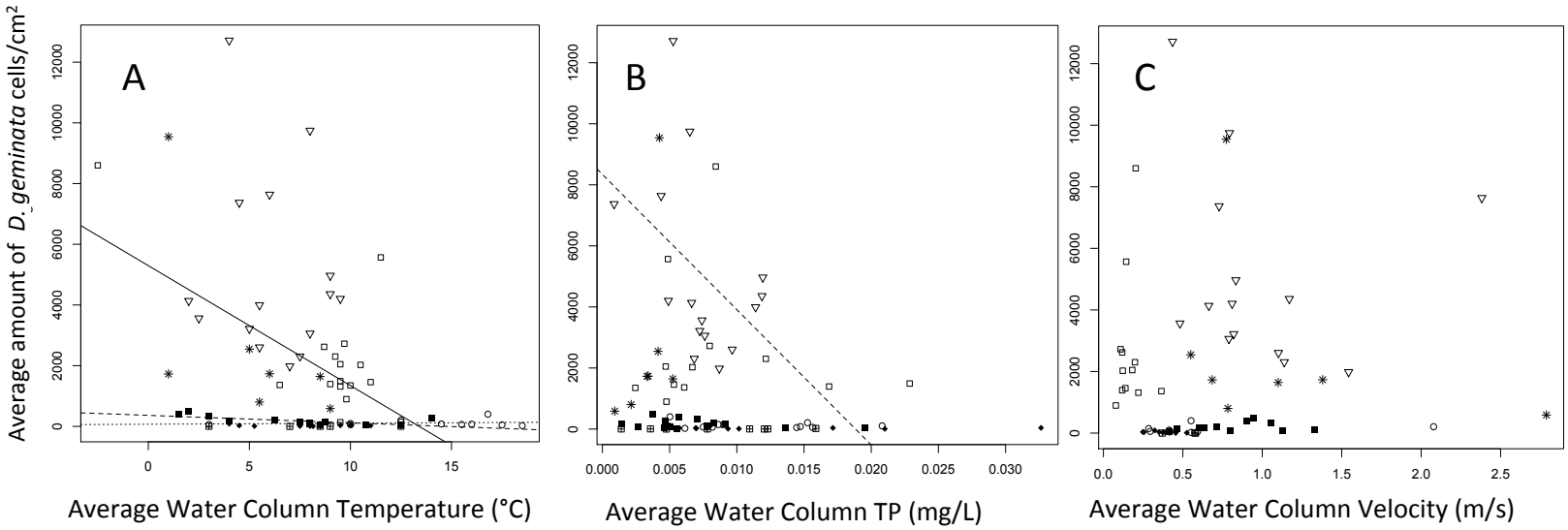
Figure 3.3. Mean *D. geminata* cell abundance per cm^2 on NDS devices from the six treatments; Control, Nitrogen, organic Phosphorus (org-P), inorganic phosphorus (inorg-P), Nitrogen and organic Phosphorus (N + org-P) and Nitrogen and inorganic Phosphorus (N + inorg-P) for each sampling site and time. Mean +/- standard errors (SE) are plotted and each bar represents ~N =10 for each treatment at each site. NS indicate a non-significant result, whereas similar letters also indicate no

significant difference between pairs of results. Different letters indicate a significant difference as determined by Bernoulli corrected pairwise comparisons (P <0.05).

3.3.3 *D. geminata* response to natural stream conditions

A negative linear relationship existed between the abundance of natural stream *D. geminata* cells and the average water column temperature (R^2 =0.64, $F_{13, 65}$ =8.91, p =4.18x10^{-10}) (Figure 3.4 a). Lower Bow River and Lower Jumpingpound Creek had a significantly different relationship. Their *D. geminata* cell abundance did not have a relationship with the average temperature of the water column (p =0.02 and p =0.017 respectively). Lower Jumpingpound Creek had the highest water temperature of all the sites (Figure 3.4 a), but the average water temperature at Lower Bow River was not significantly different from other sites (Figure 3.4 a).

Figure 3.4. The mean *D. geminata* cell count over the average temperature (°C) (A), the average TP (B) and the average water velocity (C) of the stream. Each symbol represents a different site. The star is Baker Creek, the box with the cross is Upper Bow River, the closed square is Lower Bow River, the closed diamond is Upper Jumpingpound Creek, the open circle is Lower Jumpingpound Creek, the open square is Kananaskis River and the open triangle is Louise Creek. For plot A the solid line is the linear over all of the sampling areas, the dashed line is the linear regression at Lower Bow River and the dotted line is the linear regression at Lower Jumpingpound Creek. Both Lower Bow River and Lower Jumpingpound Creek had relationships that differed significantly from the significant negative relationship found at all other sites, so they were plotted individually. For plot B the dashed line is the negative linear relationship between TP and *D. geminata* cells that was present at Louise Creek. No relationship was found at any other site.



There was a significant negative relationship between the natural stream *D. geminata* cells and the average TP of the water column (R^2 =0.61, $F_{13, 67}$ =8.20, p =1.51x10^{-9}) (Figure 3.4 b) but this was driven by the strong negative relationship at Louise Creek (p =0.012). There was no relationship between TP and *D. geminata* cells at any other site (R^2 =0.26, $F_{1, 64}$ =1.60, p =0.20). There was no relationship between *D. geminata* cell abundance and average water column velocity (R^2 =0.0069, $F_{1, 62}$ =0.43, p =0.51) (Figure 3.4 c).

3.4 Discussion

3.4.1 *D. geminata* response to nutrient additions

Overall, my stream APA and TP results indicate that the sites I studied had a high probability of phosphorus limitation. In this study, I also used chlorophyll a accrual and *D. geminata* cell colonization on NDS devices to assess the nutrient limitation. The chlorophyll a accrual on the NDS supported the APA and TP results. At all sites, the chlorophyll a accrual on NDS devices increased on the phosphorus treatments at some point during the sampling season, which indicated that each site experienced a period of phosphorus limitation. When streams were considered P-limited, inorg-P limitation was the most frequent limiting nutrient, based on chlorophyll a accrual on NDS devices. In contrast, the *D. geminata* colonization was unaffected by nutrient addition and the results indicated that *D. geminata* was not limited by nutrients. This contrasts with the chlorophyll a results and indicates that, in this situation, chlorophyll a is not a good measure of *D. geminata* cell colonization.

3.4.1.1 Chlorophyll a accrual

Chlorophyll a and *D. geminata* cell abundance did not have a similar response to P-additions. This suggests that there must be multiple other algal cells beyond *D. geminata* cells within the samples that contribute to the chlorophyll a measurement. An increase in available P in a P-limited system should increase algal growth (Dodds et al. 2002, Biggs 2000, Schindler 1977). Therefore, the observed relationship in chlorophyll a accrual is what I expected for normal algal colonies. I was surprised considering the low nutrient concentration in the study streams, that at certain periods over the sampling season the chlorophyll a NDS results suggested streams were not limited by nutrients or limited by organic phosphorus sources. These periods of no nutrient limitation did not occur over the same time period for each stream (e.g. Lower Jumpingpound Creek not limited in late summer, Upper Jumpingpound Creek not limited in early fall). These results are comparable to the *D. geminata* cell NDS results and may suggest: i) a community dominated by more *D. geminata* during these time periods, ii) a change in the stream environment that I did not detect, or iii) the NDS devices were not working properly for these periods of time.

3.4.1.2 *D. geminata* colonization

In concurrence with previous studies (Kirkwood et al. 2009, Kirkwood et al. 2007 and Spaulding and Elwell 2007), *D. geminata* blooms were found in streams with a potential for high P-limitation, as inferred from alkaline phosphatase activity levels and total phosphorus levels within the stream. High levels of alkaline phosphatase are observed in times of phosphorus

limitation and where Org-P is the only form of available phosphorus (Elwood and Christmas 1998, Chrost et al. 1984, Francko 1983, Healy 1975). High levels of AP activity, and therefore P-limitation, were evident in many of the study streams and Baker Creek, Upper Bow River, Lower Bow River and Kananaskis River. The high level of alkaline phosphatase activity indicated that *D. geminata* might have been employing enzymes to utilize an org-P source (Bowman et al. 2005, Hernandez et al. 2003, Strjsova et al. 2003, Whitton et al. 1991, Healy and Hendzel 1979). If this were true, I would have expected to see an increase in *D. geminata* colonization on the Org-P NDS devices at those sites.

For any treatment, there was no significant treatment effect on *D. geminata* colonization of NDS devices. *D. geminata* cell abundance did not differ significantly between treatments. This contradicts the theory proposed by Elwood and Whitton (2007) that *D. geminata* creates a stalk to increase its surface area for organic phosphorus uptake. The sampled *D. geminata* populations do not seem to grow solely in response phosphorus additions even though *D. geminata* grows in streams that are considered P-limited. Considering the low nutrient concentrations in our streams, I was surprised that the *D. geminata* colonies were not affected by nutrient additions. This could be due to various possibilities including: i) their nutrient demands were being met even though the concentration of nutrients within the stream was very low ii) they were limited by another factor including light (Kilroy et al. 2012), velocity (Tomas et al. 2010, Kirkwood et al. 2009, Larned et al. 2006, Kilroy et al. 2005d), temperature (Lindstrom and Skulberg 2008, Kirkwood et al. 2007, Blanco and Ector 2002) or any other environmental factor, or iii) the NDS devices are not a suitable measure to assess the growth of *D. geminata* colonies.

3.4.2 *D. geminata* response to natural stream conditions

D. geminata cell abundance varied between site and season. Different relationships to treatments were observed at different sites and at different times of year, which lends support to the hypothesis that it is not solely the addition of phosphorus that affects the growth of *D. geminata* but other factors must interact with *D. geminata* to affect its growth. The survey of 76 sites within the Canadian Parks (Chapter 2) identified temperature, TP and water velocity as potential environmental drivers of *D. geminata* growth. I looked more closely at the relationship between those environmental drivers and *D. geminata* at these sites.

The environment within the streams at each of my NDS sites differed in their physical and chemical make-up. The temperature of the streams varied significantly between sample sites. At most sites there was a negative relationship between the abundance of *D. geminata* cells and the temperature. This supports results that have found *D. geminata* to have an affinity to bloom in cold water (Lindstrom and Skulberg 2008, Krammer and Lange-Bertalot 2000). However this relationship was not present at Lower Bow River or Lower Jumpingpound Creek, where *D. geminata* cell abundance was not affected by fluctuations in temperature. These results suggest that in some locations, temperature may be an important driver of for *D. geminata* growth, but it is not the only driver that determines increases in *D. geminata* abundance. As well, there may be other factors that are equally or more important for *D. geminata* growth and bloom development.

Water velocity was found to be significantly different between sites and season but there was no linear relationship between the amount of *D. geminata* cells and the water velocity.

Velocity was fastest (15 m/s) in Baker Creek and slowest in Kananaskis River (<12m/s). However, both of these Creeks had large amounts of *D. geminata*. Upper Jumpingpound Creek and Lower Jumpingpound Creek, the two sites where no *D. geminata* blooms were observed, had an intermediate velocity. My results support those that have found *D. geminata* thriving in a wide range of water velocities from slow and shallow, to deep and fast (Kilroy et al. 2005d, Canter-Lund and Lund 1995, Land and Lowe 1987). *D. geminata* blooms have been observed in stable flow environments (Kirkwood et al. 2009, Lindstrom and Skulberg 2008, Kirkwood et al. 2007, Larned et al. 2006), but I did not measure flow stability. Larned et al. (2006) indicate that the best predictor for *D. geminata* blooms are days since flood, because floods scour the blooms and return biomass to low levels. Other studies have found an increase in *D. geminata* blooms below impoundments or other regulated flow regimes (dams or lake outflows) (Kirkwood et al. 2007, Kilroy et al. 2005d, Kawecka and Sanecki 2003, Skulberg 1982, Dufford et al. 1987). Therefore another combination in the matrix of important conditions for successful *D. geminata* growth may be the stability of the water velocity at the time of sampling rather than how fast the water is actually moving.

TP levels were statistically significantly different at each site. The TP within the water column was significantly lower in Baker Creek than other sites (4 μg/L vs. 6-11 μg/L at other sites). This low TP level is supported by the alkaline phosphatase data that shows a very P-limited stream. However, a linear relationship between TP and *D. geminata* cell abundance did not exist at Baker Creek or any other site except Louise Creek. Louise Creek was found to be slightly P-limited, but had a strong negative relationship with the abundance of *D. geminata* cells and the amount of TP present within the stream. This relationship was not found in the amount

of AFDM within the stream. These results suggest that at Louise Creek and Baker Creek, low TP levels are important for the growth of *D. geminata*. This relationship does not exist at the other sites.

3.4.3 Conclusion

Overall, there is no clear picture of which combination of factors creates the ideal situation for *D. geminata* growth. It may be that the NDS system I used was not useful for monitoring *D. geminata* colonization because although *D. geminata* blooms in P-limited streams, it is not the nutrients that are solely responsible for its growth or *D. geminata* will not colonize NDS devices. If we assume the NDS devices worked well to assess *D. geminata* growth, it does not increase its growth on org-P treatments and does not have reduced growth on inorg-P treatments. It appears that it is a combination of many different environmental conditions that will result in a suitable environment for *D. geminata* growth. However, this combination of factors does not seem to be consistent over space and time. This lends further support to the hypothesis presented in Chapter 2, that there are multiple strains of *D. geminata* that have different environmental requirements and different blooming capacities. Therefore, to determine the combination of factors required for *D. geminata* to bloom, we must first determine if there are different strains of *D. geminata,* determine where they are located and then conduct nutrient manipulations on similar strains of *D. geminata,* at similar sites and times of year.

The lack of response to treatments and variation in space and time by *D. geminata* also suggest that I may not have identified the appropriate suite of environmental variables that are

necessary for its growth. There are many other potential variables within the stream environment that may be controlling their growth including: light (Kilroy et al. 2012), flow stability (Tomas et al 2010, Kirkwood et al. 2007, Larned et al. 2006, Kilroy et al. 2005d) and substrate type (Bergey et al. 2010, Kilroy et al. 2005d) to name a few.

Kilroy and Bothwell (2012) have found that *D. geminata* will undergo higher frequency of cell division with increased levels of phosphorus, especially if light levels are high. *D. geminata* will also increase the growth of its stalk under high light conditions and low nutrient levels. This relationship between low nutrients and high light is also observed in other diatom species (Hill et al. 2011, Smith and Underwood 2000). As light plays a huge role in *D. geminata* growth, it may be useful to conduct the experiment again while measuring the amount of light reaching the devices, or controlling the amount of light reaching the devices. Other studies have found that in order for diatoms to colonize a substrate there should be a microbial colony first (Barranguet et al. 2005), which will then allow short statured diatoms to colonize (Hudon and Bourget 1981) allowing future colonization of larger diatoms such as *D. geminata. D. geminata* will also colonize on rougher substrates as opposed to smoother substrates (Bergey et al. 2010) potentially because rougher substrates have a greater biofilm biomass (Clifford et al. 1992, Blinn et al. 1980). Therefore a bare substrate like the NDS devices used, may not be the ideal substrate to recruit colonization by the large diatom *D. geminata.*

There were many other limitations to this study that may have affected my results. The first is that we were conducting the experiment on streams with a variable environment. Streams were only sampled weekly; therefore I was only getting a snapshot as to what the stream and

stream community looked like. I was missing variability that would occur over the course of the day. Also because I was on natural streams, they each had a unique set of environmental characteristics that could have affected the algal responses within them. In future, it would be ideal to have the experiment set up within experimental streams where the environmental conditions would be identical at all times and between all streams.

The NDS devices themselves also have numerous limitations. First of all, by setting devices of different treatments within the same rack it is possible to get cross contamination between them. This would alter the nutrient composition of each device, therefore also altering the community capable of surviving on each device. In future, I would place the devices perpendicular to the direction of water flow so that each device does not have water flowing over it that has previously flowed over another device. As previously mentioned, a bare device such as the silica disks used on my NDS devices, may not be the appropriate substrate to use to recruit the colonization of *D. geminata* (Bergey et al. 2010, Clifford et al. 1992, Blinn et al. 1980). Perhaps a rougher substrate with an intact biofilm would yield more favourable results.

Chapter Four: **General Conclusion**

The results of this thesis strongly suggest that *D. geminata* requires a specific set of environmental conditions. Each study conducted within this thesis pointed towards a population of *D. geminata* that contains multiple strains with differing environmental requirements and abilities to bloom. Chapter 2 identified a suite of environmental variables (stream order, water velocity and TP) that aid to determine the likelihood of finding *D. geminata* cells and the amount of *D. geminata* cells present at a particular site. This survey did not uncover the environmental requirements that would cause *D. geminata* cell to begin forming blooms. Chapter 3 did not identify environmental requirements for *D. geminata* either. Results supported previous studies (Bothwell and Kilroy 2011, Kirkwood et al. 2009, Lindstrom and Skulberg 2008) that found *D. geminata* blooming in P-limited streams, but we did not see an increase of *D. geminata* with P additions. There was a strong influence of site sampled and time of sampling but each site had a unique set of environmental characteristics while still harbouring *D. geminata* blooms. I hypothesize that *D. geminata* requires a combination of many different environmental characteristics to create a perfect environment. Either we did not identify the appropriate suite of environmental variables, or there are multiple combinations that could result in a bloom. This lends further support to the hypothesis that *D. geminata* exists as multiple strains, and we must first determine what these strains are before we can determine their environmental requirements.

Further work is needed before any drastic measures can be taken to eradicate *D. geminata*. Future studies should focus primarily on uncovering any genetic or morphological differences between *D. geminata* between and within sites. Then, focus should be on the

environmental requirements of any different types of *D. geminata*. It is necessary to determine if there is one strain with a very complex suite of environmental requirements yet to be identified, or multiple strains with different environmental requirements needing different management strategies.

As the mystery surrounding *D. geminata* blooms in P-limited streams remains, future studies could continue to look at nutrient sources. One management idea is an input of P that would stop bloom development because *D. geminata* blooms in oligotrophic streams. The results from chapter 2 suggest this may not work because I saw no reduced cell counts on IP substrates. However, Kilroy and Bothwell (2012) found that increased cells do not necessarily mean increased bloom material. Other studies have seen a decrease of *D. geminata* bloom development with higher IP levels (James 2012). However, as we have yet to determine the complex suite of environmental matrices that *D. geminata* requires, utilizing stream river water from blooming sites along with nutrient enrichment may help to capture some of those requirements and lead to more effective management strategies. Streamside channels were utilized to look at *D. geminata* cell division with nutrient additions (Bothwell and Kilroy 2011). They had promising results and found that nutrient additions and high light levels resulted in an increase in *D. geminata* cell division but not bloom creation. These could be used again to determine the set of environmental conditions that cause *D. geminata* blooms to form.

Experimental herbicide applications have begun in New Zealand (Clearwater et al. 2011, Jellyman et al. 2010) in order to mitigate the large bloom events. A significant effect of chelated copper solution reduced the number of live *D. geminata* cells within the affected streams. There

were no significant long-term effects within the sediments, fish or invertebrate communities however there were significant fish mortalities during the initial introduction of the copper solution. As blooms have been clearly identified as invasive, and continue to spread within the New Zealand River systems, herbicide application may be one of the only ways to get rid of *D. geminata* blooms. In the Canadian Rocky Mountains, this type of control may be a bit premature as *D. geminata* blooms have not been identified as invasive. Also, it may not be feasible as the stream areas are protected within the National Park boundaries.

Until such time as we can identify the requirements and types of *D. geminata,* management efforts should continue to focus on prevention of *D. geminata* spread. Until more information is acquired, we cannot be sure of vectors of transport. Caution should be maintained to prevent further accidental movement of *D. geminata* or a blooming strain of *D. geminata*. Management of tourism should continue to caution the public about the spread of *D. geminata* and enforce the "CHECK, CLEAN, DRY" campaign that are being used in New Zealand to promote clean travel from river to river.

As the global human population continues to expand and become more mobile, we have an increased probability of species introduction. This can shift species composition within a community, altering the communities' long-term viability. Therefore, studies such as the one we conducted on *D. geminata* are imperative for the preservation of local communities. With more information regarding the environmental requirements of potential invasive species, we are better able to protect vulnerable communities from their invasion, and preserve the ecological integrity of local communities.

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