Didymosphenia geminata in the Kootenai River in Libby, Montana: Nuisance Mat Characteristics and Management Strategies for Suppression

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Authorization to Submit Dissertation

This dissertation of Mary K. Coyle, submitted for the degree of Doctorate of Philosophy with a Major in Natural Resources and titled "*Didymosphenia geminata* in the Kootenai River in Libby, Montana: Nuisance Mat Characteristics and Management Strategies for Suppression," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

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Abstract

A native diatom of the Pacific Northwest (PNW), *Didymosphenia geminata* has progressed into a nuisance species in rivers and streams across the PNW region including the Kootenai River of Libby, Montana. Forming benthic mats that resemble sewage material, nuisance *D. geminata* alters macroinvertebrate communities, degrades aesthetic value, and affects recreational activities. Mats of *D. geminata* were first noticed in the Kootenai River in the early 2000s and have since remained a ubiquitous year-round nuisance. To research the ecology of *D. geminata* and to develop potential management strategies for nuisance mats, a flume-based mesocosm system was built below the Libby Dam near the Kootenai River.

An overview of the geographic patterns of *D. geminata* throughout northern Idaho and northwestern Montana and the environmental variables attributed to varying degrees of mat presence were analyzed for background to the development of management strategies (Appendix 1 and 2). Based on previous research, phosphorus enrichment as a suppression tool was analyzed in two studies in (2013 and 2014). These studies demonstrated that a small amount of dissolved phosphorus (P) suppressed *D. geminata* stalk production and the subsequent nuisance mat formation (Chapter 3). An in-river study of a dissolved P treatment further defined the potential of P enrichment as a management strategy for nuisance mats (Chapter 5).

Mechanisms behind nuisance mat formation of this native species were investigated to explain its seemingly random persistence and prevalence. Manipulated ratios of nitrogen to phosphorus were tested and increased nitrogen concentrations were identified to result in increased stalk growth (Chapter 4). Based on these findings, management recommendations for nuisance mats of *Didymosphenia geminata* are presented in the final chapter.

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Dedication

To my Mom and Dad

For everything

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Chapter 1: Introduction

Background

Challenging conventional knowledge of preferred habitat for nuisance growth of algal species, the diatom *Didymosphenia geminata* (Fig. 1-1) is an enigma among algae. Thriving in cold, fast flowing, oligotrophic systems, *D. geminata* dominates in systems where nutrient-limited conditions would typically result in low algal growth (Tilman et al. 1982). This diatom has recently gained notoriety for its rapid "invasion" of streams and rivers across the globe and its un-characteristic persistence. Producing gelatinous mats composed of mucopolysaccharide stalks (Ellwood and Whitton 2007), this benthic species is a concern because of its threat to the function and aesthetics of river ecosystems.

The diatom *D. geminata* was first classified by Lyngbye as *Echinell geminata* 1819, but was renamed *Gomphonema geminatum* (Lyngbye) in 1824 (Whitton et al. 2009). Several earlier publications reference *D. geminata* as *G. geminatum*, but it is now classified as *D. geminata* within the Class of Bacillariophyceae (Spaulding 2010). This asymmetrical biaphrid diatom (Fig. 1-1) is often found as a single large cell or attached to an elongated mucopolysaccharide stalk (Spaulding 2010). The diatom is one of the largest benthic diatom species ranging in size from 65-161 µm long and 36-41 µm wide. It has a "distinct, capitate headpole" and a "less capitate to bluntly rounded footpole" (Spaulding 2010) (Fig. 1-1).

Each cell has a raphe and an apical pore field located at the basal pole of the frustules (Whitton et al. 2009) that produces the mucopolysaccharide stalk. This stalk secures the cell to substrate and bifurcates with each sequential cell division, eventually leading to a dense

mat composed of >90% extracelluar polymeric material (Kirkwood et al. 2007a). However, it is not the morphometrics of the cell that make this species unique, but its paradoxical success in nutrient-limited waters throughout the world (Kilroy 2004).

Didymosphenia geminata is not classified as an introduced species in the United States because it has been historically recorded as part of the periphyton community throughout the northern latitudes. The term "nuisance" is applied to designate the existence and potential degradation to affected ecosystems by this species when it is in "nuisance" form. Nuisance mats are defined as those that extend for greater than 1 km for several months of the year (Spaulding and Elwell 2007).

The processes that allow *D. geminata* to form elongated stalks and produce excessive mass in nutrient-limited water are under debate in the scientific community (Bothwell et al. 2012). The two foremost hypotheses are: 1) stalk growth is driven by phosphorus (P) limitation (Bothwell and Kilroy 2011; Kilroy and Bothwell 2011, 2012), and 2) that within the mat matrix, an anoxic environment exists that facilitates the typical interaction between iron (Fe) and P (Wetzel 2001), which allows particle-bound phosphorus to become available in soluble form (Sundareshwar et al. 2011).

The P hypothesis suggests that in nutrient-limited conditions, the diatom diverts excess energy from photosynthesis to the production of a nutrient-poor mucopolysaccharide stalk that accesses more nutrients or light availability in the water column. Cell division is minimized and stalk production is maximized to create a large surface area for maximum P uptake via the excretion of alkaline phosphatase (Whitton et al. 2009). The excessive production of carbohydrates is seen in numerous other diatom species and has been attributed

as a stress response to nutrient-limited water (Mykylestad and Haug 1972; Myklestad 1995; Smith and Underwood 1998). This response to P-limitation is supported by studies that show *D. geminata* mats rarely form when phosphorus concentrations are >2 ug/L (Kilroy and Bothwell 2012). Furthermore, in several mesocosm studies, an increase of ambient concentrations of phosphorus suppressed stalk length and increased frequency of dividing cells (FDC) (Bothwell and Kilroy 2011; Kilroy and Bothwell 2011), suggesting that increased nutrients shift the diatom from a stalk production phase into a cell reproduction phase. Therefore, research should focus on P enrichment and determining a concentration that suppresses mat formation without causing excessive productivity, as P is often the nutrient limiting primary productivity in freshwaters (Schindler 1977). It would be counterproductive to stimulate excessive productivity in the broader algal community trying to solve one problem.

The iron redox model purports that the *D. geminata* in mats accesses previously unavailable phosphorus in the water column through a unique iron-redox gradient within the mat biofilm (Sundareshwar et al. 2011). It is this positive feedback loop that is hypothesized to lead to the development of nuisance mats. However, the mechanisms underlying the iron redox model have been repeatedly disputed by a number of researchers (e.g., Bothwell et al. 2012). Because the iron redox hypothesis lacks support and connectivity to the prevalence of *D. geminata* in the Kootenai River, this dissertation will not address the iron redox hypothesis but will investigate aspects of the phosphorus limitation model.

Distribution and occurrence of nuisance mats of *Didymosphenia geminata*

First noted by Lyngbye in 1819 in the Faroe Islands of Scotland, *D. geminata* has historically been part of the periphyton community in circumboreal regions such as Finland, France, Ireland, Italy, Norway, Scotland, Spain, Sweden, Switzerland, Vancouver Island (Canada) and parts of the Kanchou region of China (Kilroy 2004; Spaulding and Elwell 2007). The formation of mats has been documented throughout Europe since the mid-19th century with reports in the Coquet River in Northumberland, UK since the late 1950s (Bothwell and Spaulding 2008) and in 1975 in the Faroe Islands north of Scotland (Kilroy 2004). However, aspects of the ecology, hydrology, biogeochemistry, or geomorphology kept the species below nuisance levels and allowed macroinvertebrate density and fisheries to remain stable. The appearance of persistent nuisance mats throughout central Vancouver Island, British Columbia was first noted in 1989 by Bothwell and coworkers (Bothwell et al. 2009). Mat occurrence was noted near popular fishing areas, leading to the hypothesis that anglers were vehicles for the apparent spread of *D. geminata*. During that time, increasing nuisance blooms were also observed in Europe (Kawecka and Sanecki 2003), Canada (Kirkwood et al. 2007b) and Asia (Bhatt et al. 2008). Thus the transfer of the species by anglers was a logical hypothesis, as cells can remain viable for at least 50 days in damp conditions (13.6 °C or less) such as those found in felt soles of wading boots (Kilroy et al. 2007). Felt soles, leather boot tops, and neoprene waders all may act as vehicles if not decontaminated after being exposed (Kilroy et al. 2007), allowing the diatom to establish itself once re-exposed to water.

In October 2004, the presence of *D. geminata* was confirmed on the South Island of New Zealand in the lower Waiau River (Kilroy et al. 2009). This was the first documented occurrence of a nuisance mat in the southern hemisphere and it highlighted the wide flexibility of environmental conditions the species could tolerate. Not only did nuisance mats rapidly colonize the Waiau River, but within 18 months it appeared in 12 other rivers on the South Island (Spaulding and Elwell 2007). Mats are now in at least 21 rivers on the South Island (Kumar et al. 2009) but it has yet to appear on the North Island. Preventing the spread to the North Island has been attributed to i) the quick response of New Zealand's Ministry of Agriculture and Forestry (MAF) which is responsible for controlling invasive species via New Zealand's Biosecurity Act of 1993 and which immediately listed the species as an unwanted organism, and ii) less favorable environmental conditions (Bothwell and Spaulding 2008). It was estimated that a two-year delay of colonization to the North Island would reduce negative economic impacts of between $5 million to $62 million over 10 years (Vieglais 2008). Public education regarding control of the species has been improved with the "Check, clean and dry" campaign as well as fines of up to $100,000 and five years in prison if caught transporting it (MPI 2012). However, recent studies have suggested that in areas where *D. geminata* is native to the periphyton community, decreasing phosphorus concentrations may be contributing to the formation of mats by *D. geminata* (Bothwell et al. 2014; Taylor and Bothwell 2014).

Human alterations to the deposition and mobility of nitrogen on the landscape have increased the concentration of nitrogen in rivers, which also could contribute to the presence of *D. geminata* (Vitousek et al. 1997; Ellwood and Whitton 2007). In southern hemisphere locations where *D. geminata* has not been historically present, it is believed that its introduction (most likely by world-traveling anglers) to habitats where the limiting factors for

excessive growth are not present (e.g., phosphorus concentrations < 2 µg/L) it is able to "bloom" at unprecedented rates creating nuisance mats (C. Kilroy, National Institute of Water and Atmospheric Research, Christchurch, New Zealand, personal communication, 2013). Recent data suggest that the formation of nuisance mats occur not simply because of cell introductions but because of phosphorus limitation (Bothwell et al. 2014). The historic presence of cells in the context of current nuisance mat presence has rarely been investigated, probably contributing to escalated reports and perceptions that *D. geminata* is an introduced species.

Recent research provides evidence to dispute the commonality of mats that have an origin of *D. geminata* cell introduction and suggests that nuisance mats are a result of appropriate water conditions (low phosphorus) and the presence of cells (Bothwell et al. 2014). Currently, nuisance mats of *D. geminata* occur throughout North America (at least fifteen states in the U.S. and three provinces in Canada), New Zealand, South America, Europe, and Asia (Bothwell and Spaulding 2008; Cullis et al. 2012). As of 2009, the species was recognized as one of the most problematic nuisance species in lotic systems (Cullis et al. 2012). While *D. geminata* is not a new species to periphyton communities in circumboreal regions (Spaulding and Elwell 2007; Bothwell and Spaulding 2008), the formation of unprecedented nuisance mats in such varied environments is cause for concern. Nuisance mats can be 20 cm thick (Spaulding and Elwell 2007) and alter the physical and biological conditions of a lotic ecosystems (e.g., nutrient availability, species composition, and biodiversity of benthic invertebrates) (Kilroy et al. 2009; Gillis and Chalifour 2010; James et al. 2010). Mats also detract from recreational activities and aesthetic value, potentially decreasing economic revenue associated with particular lotic systems.

Research history of *Didymosphenia geminata*

Primary research focused on environmental factors affecting *D. geminata* is relatively recent (Fig. 1-2), and has predominantly focused on the processes that allow *D. geminata* to thrive under oligotrophic conditions (Ellwood and Whitton 2007; Bothwell and Kilroy 2011; Cullis et al. 2011; Kilroy and Bothwell 2011; Kilroy and Unwin 2011; Sundareshwar et al. 2011; Aboal et al. 2012; Bothwell et al. 2012). Its distribution around the world has been studied and described (Noga 2003; Kilroy 2004; Spaulding and Elwell 2007; Jonsson et al. 2007; Bhatt et al. 2008; Beltrami et al. 2008; Kilroy and Flöder 2008; Blanco and Ector 2009; Kumar et al. 2009; Rost et al. 2011; Segura 2011) but location and attributes of the current and historical presence of nuisance mats is limited. Although its ecology has been studied (Kilroy et al. 2005; Larned et al. 2007), little research has been dedicated to examine management techniques for the long-term suppression of nuisance mats (James 2011; Jellyman et al. 2011; Kilroy and Bothwell 2011). How to effectively suppress mats at the river scale is currently unknown and there is a significant need for efficient and applicable approaches for managers of systems negatively affected by the species.

The occurrence of *D. geminata* related to the history of the Kootenai River ecosystem

The closure of Libby Dam in 1972 on the Kootenai River in Montana created Koocanusa Reservoir, a 145 km long impoundment that straddles the US/Canada border between Montana and British Columbia (Fig. 1-3). The Kootenay River headwaters originate in the Kootenay Ranges of Canada flowing south into Koocanusa Reservoir and then into Montana, USA, where the spelling changes from Kootenay to Kootenai. From Montana, the Kootenai River flows northwest through Idaho, then north to re-enter Canada via Kootenay

Lake near Creston, British Columbia (Fig. 1-3). The West Arm of Kootenay Lake eventually discharges into the Columbia River system. The extreme length of Koocanusa Reservoir allows solids and associated nutrients to settle out of the water column, meaning that water which is released via the reservoir dam outlet is devoid of the sediment and nutrient loads carried by the river historically. Hoyle et al. (2010) report that Koocanusa Reservoir is a nutrient sink that removes 63% of total phosphorus, 24% total nitrogen, and 95% of suspended sediments from the Kootenay River. As a result, after closure of Libby Dam, the trophic status of the river downstream of the dam changed from eutrophic to ultraoligotrophic (Hoyle et al. 2010). Now the soluble reactive phosphorus (SRP) in the tailwater is below detection limits, while nitrogen concentrations are above 200 µg/L; thus the water leaving the reservoir is oligotrophic to ultra-oligotrophic (KTOI 2014). Historically, mining activities within the Kootenai River watershed contributed significant loads of metals and nutrients. For example, between the 1930's and 1980, the Sullivan mine near Kimberly, British Columbia was one of the world's largest zinc producers (Daley et al. 1981) and while between 1953 and 1974, a fertilizer plant near Kimberly contributed significant loads of increased orthophosphate (Bonde and Bush 1975). However, by the 1980s, pollution control measures returned the river system to oligotrophic-mesotrophic status (Ruppel and Lopez 1984).

Aesthetically unappealing and bothersome to recreationists, the occurrence of nuisance mats of *Didymosphenia geminata* within the Kootenai River has not gone unnoticed. Although first noted by John Keast Lord in 1866, the Kootenai River has one of the oldest records of *D. geminata* cells as part of periphyton in North America (as cited in Bothwell et al. 2014), it was not until the early 2000s when the presence of *D. geminata* nuisance mats in the Kootenai River were first noticed after appearing on sampling gear (Holderman and Hardy

2004). Nuisance mats quickly appeared throughout the Kootenai River and have remained ubiquitous and prevalent since then.

As of 2016, extreme nuisance mat growth of *D. geminata* has been observed below Libby Dam and continues downriver at decreasing intensity into Idaho (Fig. 1-4) with approximately 46.5 river km (29 river miles) of nuisance mats. At the Idaho/Montana border, the Kootenai Tribe of Idaho has implemented a nutrient restoration program for the Kootenai River to increase algal and macroinvertebrate productivity to sustain fish populations important to the tribe (Hoyle et al. 2010) which has been negatively affected by Libby Dam. Starting in 2005, liquid phosphate fertilizer (10 units of nitrogen-34 units of phosphorus-0 of potassium) was added at 0.31 L/min to reach a total dissolved phosphorus (TDP) concentration of 1.5 µg/L. This was increased to 3.0 µg/L TDP in 2006 (Hardy 2008). Annually, phosphorus addition begins in early June, after spring discharge has decreased to ≤ 623 klps (22 kcfs) and continues to the end of September. During this time, the amount of 10-34-0 is adjusted to match river discharge to maintain the target concentration of 3.0 µg P/L. Though *D. geminata* is still present throughout the downstream treatment section, mats are negligible (tufts only) and productivity of other algae and macroinvertebrates has greatly increased providing more forage for fish within the system than before additions started (Holderman et al. 2009; Hoyle et al. 2010).

In a regional study of *D. geminata* presence (Appendix 1), one hundred and thirty-nine locations were surveyed in Idaho and Montana (Fig. 1-5). The objective of this study was to collect and analyze the presence of *D. geminata* in cell and mat form throughout western Montana and northern Idaho and to develop a network for continued monitoring of nuisance

mat occurrence. Of the locations sampled, 51% had *D. geminata* as part of the algal community while 49% did not have any *D. geminata* cells in the scrapings. Forty-four percent of the locations with *D. geminata* cells also had significant stalk production and some level of mat formation, while 56% of the sampled locations did not, and instead had *D. geminata* as a part of the algal community with no excessive or noticeable stalk elongation. Only 14% of locations surveyed had nuisance level mat growth, suggesting that the formation of nuisance mats is rare among rivers with *D. geminata* as part of the periphyton community.

While some rivers in the United States experienced severe nuisance mat accrual, the lack of a database to track *D. geminata* mat presence may have led to historic (non-nuisance) mat presence becoming categorized as a new occurrence. This Baader-Meinhof phenomenon (Newell et al. 2005) and the rapid spread of internet news articles or blogs may have contributed to the assumption of this species as an introduced species throughout the United States and emphasizes the important need of a database of *D. geminata's* historical and current presence across the landscape.

For northwestern Montana and northern Idaho, *D. geminata* is a common and well distributed species (Fig. 1-5 and 1-6) (Appendix 1). The formation of nuisance mats is rare relative to the pervasiveness of *D. geminata* within periphyton communities. As *D. geminata* nuisance mats have appeared seemingly at random, landscape-wide changes in water quality parameters (e.g., concentrations of phosphorus, magnesium, nitrogen etc.) should be evaluated, because the presence of nuisance mats can no longer be attributed to the introduction of *D. geminata* cells.

Without analysis of nutrient concentrations and microscopic examination of the periphyton community, the ecology of river and algal communities is difficult to ascertain by visual observation. Therefore, the seemingly rapid and unrelenting appearance of nuisance mats in previously nuisance algae-free river systems has imitated the behavior of an invasive species accrual. This misperception has led many *D. geminata* nuisance mats to be labeled and addressed as introduced species in areas where historical or paleolimnological samples suggest otherwise. While this label can have positive consequences for the health of aquatic ecosystems by encouraging and reminding recreationists to employ proper stream health etiquette such as the "Check, Clean, Dry" campaign, it has severely hindered research investigating changing water quality and subsequent *D. geminata* nuisance mat formation (Taylor and Bothwell 2014). As scientists continue to simplify the presence of *D. geminata* nuisance mats as an aquatic invasive species to be stopped, the driving mechanism behind changing environmental parameters will be ignored. When confronted with new occurrences of nuisance mat growth, researchers should be asking first, "is this species historic within this stream" and secondly, "what water quality parameters have changed to drive this species into a stressed state that involves excessive carbohydrate production in the form of stalks".

Future of the Kootenai River ecosystem

The Kootenai River system in Montana has a blue ribbon rainbow trout (*Oncorhynchus mykiss*) fishery and also contains the federally listed (US Endangered Species Act of 1973) white sturgeon (*Acipenser transmontanus* - endangered) and bull trout (*Salvelinus confluentus* - threatened). Fisheries biologists from the Libby office of Montana Fish, Wildlife and Parks are concerned that extremely prodigious mats of *D. geminata* in the

Kootenai River are negatively affecting the trout fishery and overall aesthetics, while also reducing the ability to recover endangered and threatened fish species. Their concerns are based on several lines of evidence. For example, brown trout (*Salmo trutta*) populations were reduced by 50% after *D. geminata* became established in Rapid Creek, South Dakota (James 2011). The reduction was most likely due to a feeding bottleneck that occurred from the shift in the invertebrate community, with age-1 fish increasing and larger Ephemeroptera, Plecoptera, and Trichoptera feeding-dependent fish decreasing (Bothwell and Spaulding 2008).

Electroshocking surveys by Montana Fish, Wildlife, and Parks in the Kootenai River are currently being conducted in areas of high *D. geminata* mat coverage to determine if decreases in fish condition occur in those areas (R. Sylvester, Montana Fish, Wildlife and Parks, Libby, Montana, personal communication, 2014). Marshall (2007) suggested that the extent of coverage of *D. geminata* in the Kootenai River could significantly alter the food web and increase the risk of whirling disease (*Myxobolus cerebralis)* as habitat for and abundance of *Tubifex tubifex* increased with the coverage of *D. geminata*. A follow-up study by EcoAnalysts (Marshall et al. 2008) supports this conclusion showing that the density of large taxa of macroinvertebrates within the Ephemeroptera, Plecoptera, and Trichoptera orders were significantly reduced, while the density of small invertebrates such as Chironomidae had increased.

Continuing in its current proliferation, *D. geminata* could significantly degrade desirable and native fisheries in the Kootenai watershed, reduce the aesthetic appeal, and negatively affect the hydrological functions of lotic ecosystems. Given that fly fishing is a one

billion dollar/year industry in the U.S. (Root and O'Reilly 2012), declines in fishing opportunities could negatively affect the income related to recreation and tourism in the Libby area (MDLI 2012).

Acknowledging that *D. geminata* is a native species to the Kootenai River, my study seeks to determine potential mechanisms to suppress the frequency and/or occurrence of nuisance mats of by manipulating abiotic environmental variables such as dissolved phosphorus (Chapter 3), dissolved nitrogen, and the ratio between the two (Chapter 4). Specific hypotheses will be elaborated in each chapter, but the overall aim is to contribute to the understanding of *D. geminata* nuisance mats in the Kootenai River below the Libby Dam in Montana, and to examine and recommend practical methods of remediation (reduction in severity or frequency of occurrence).

This dissertation has been organized with each study broken into chapters. The second chapter is an analysis of the *Didymosphenia geminata* growth within the mobile experimental flume system (MEFS) located at the Libby Dam in Libby, Montana, USA. This study investigates how seasonality may affect nuisance mat growth within the MEFS and whether the MEFS is representative of Kootenai River *D. geminata* attachment and mat formation. Chapter 3 examines the *Didymosphenia geminata* response to the addition of phosphorus within a mesocosm environment. Specifically, I determine at what concentration of P enrichment stalk growth is suppressed. Chapter 4 expands upon Chapter 3 and examines *D. geminata* nuisance mat response to manipulated nitrogen and phosphorus concentrations and ratios. Chapter 5 is an analysis of *D. geminata* nuisance mat response to the application of a phosphorus enrichment within the Kootenai River. This chapter extrapolates the findings from

Chapter 3 from a mesocosm to a river system. The final chapter is the summary of management strategy recommendations for Kootenai River natural resource agencies and suggested future studies.

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Figures

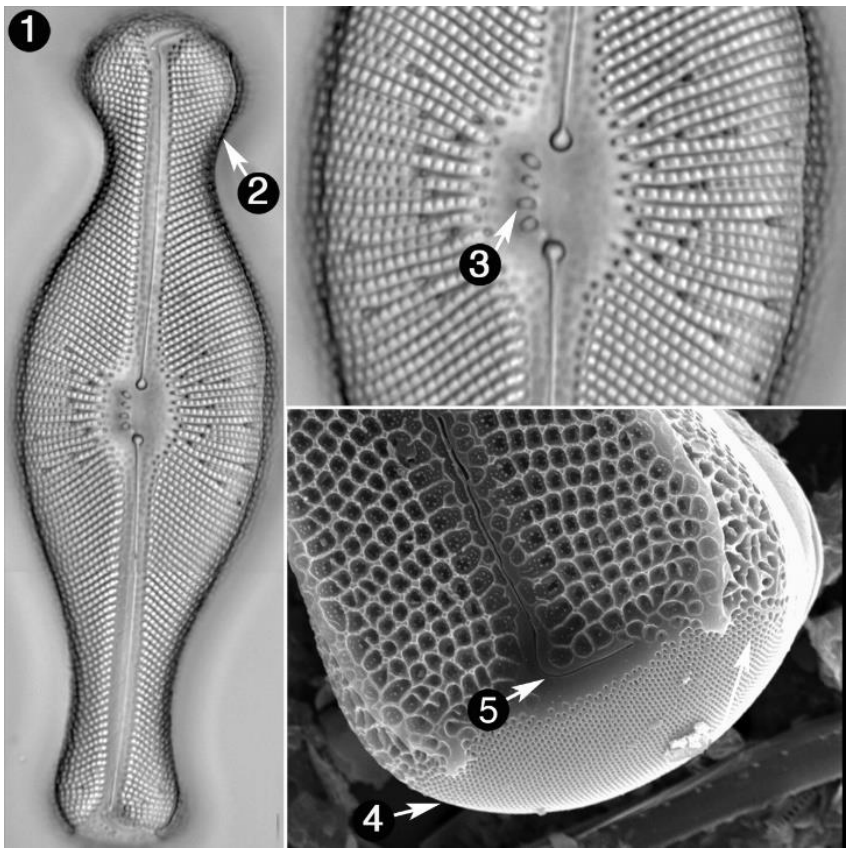

Figure 1-1. *Didymosphenia geminata* morphology and distinguishing characteristics. The defining taxonomic features as defined by Spaulding (2010) are: (1) valves large, (2) headpole capitates, (3) stigmata 2-5, (4) apical porefield present and (5) distal raphe deflected. Image Credit: Sarah Spaulding accessed from Spaulding et al. (2010).

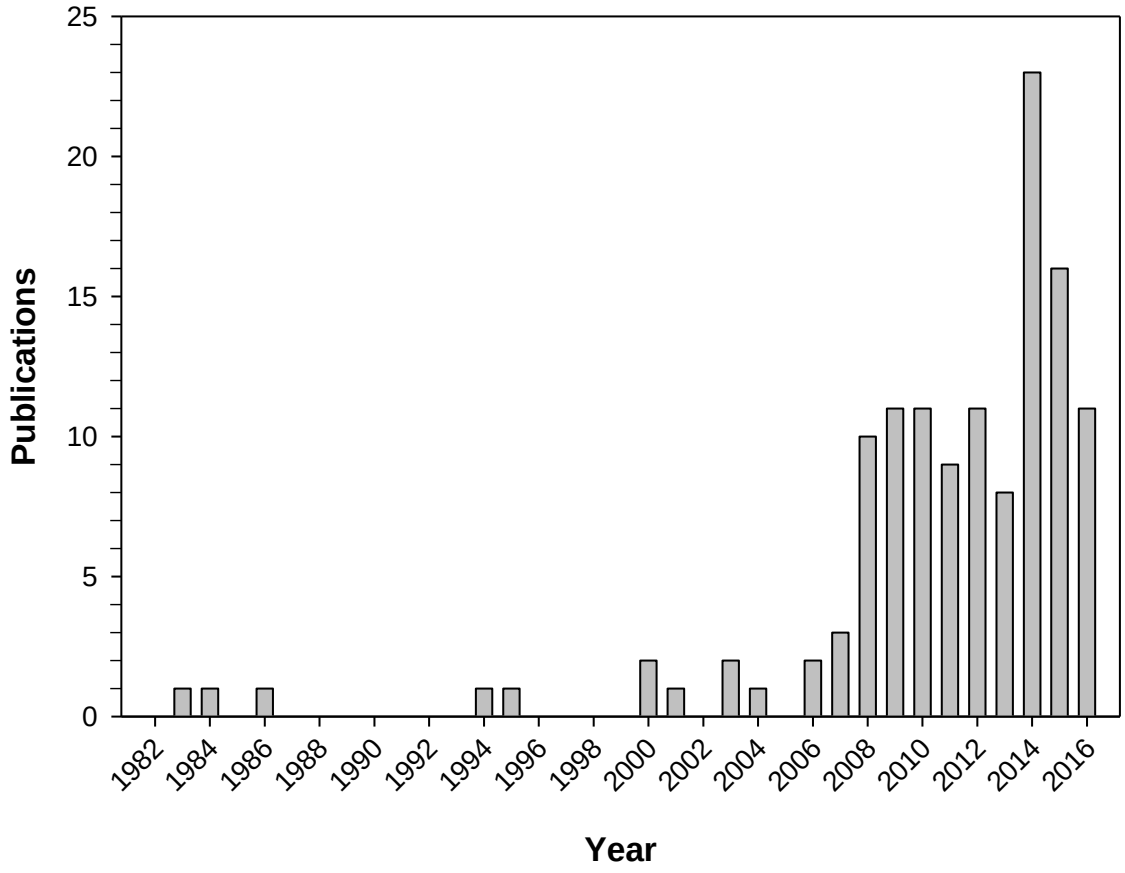

Figure 1-2. Frequency of published articles per year focused on *Didymosphenia geminata* from 1983 to 2016 sourced from Web of Science Core Collection. The majority of peer-reviewed journal articles on *Didymosphenia geminata* have been published within the last 10 years reflective of the proliferation of nuisance mats across the world.

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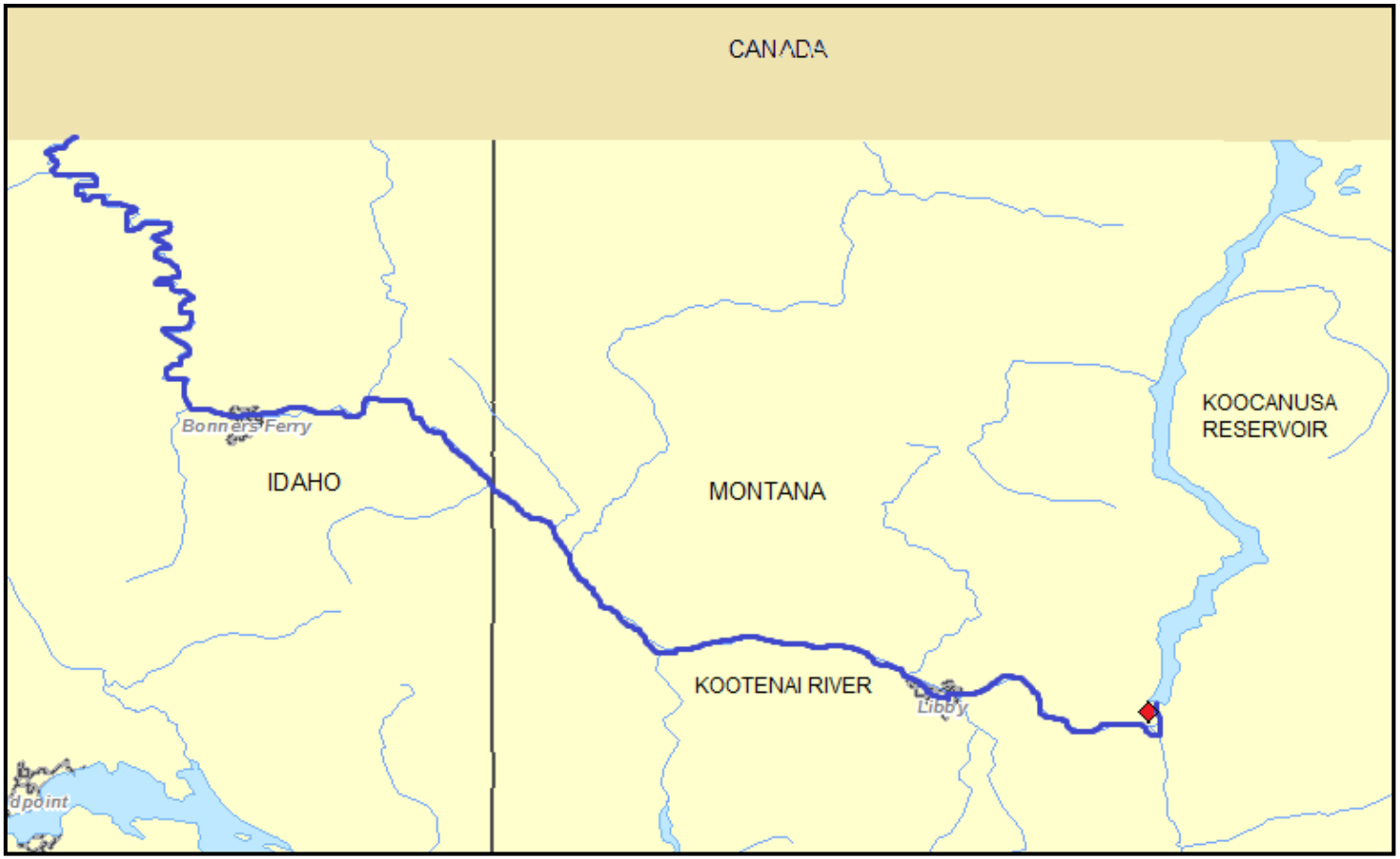

Figure 1-3. Map of study region located within Lincoln County, Montana with the Kootenai River outlined and the Kootenai River Mobile Experimental Flume Station (MEFS) marked with a red triangle.

Figure 1-4. Typical *Didymosphenia geminata* nuisance mat located below the Libby Dam in Libby, Montana.

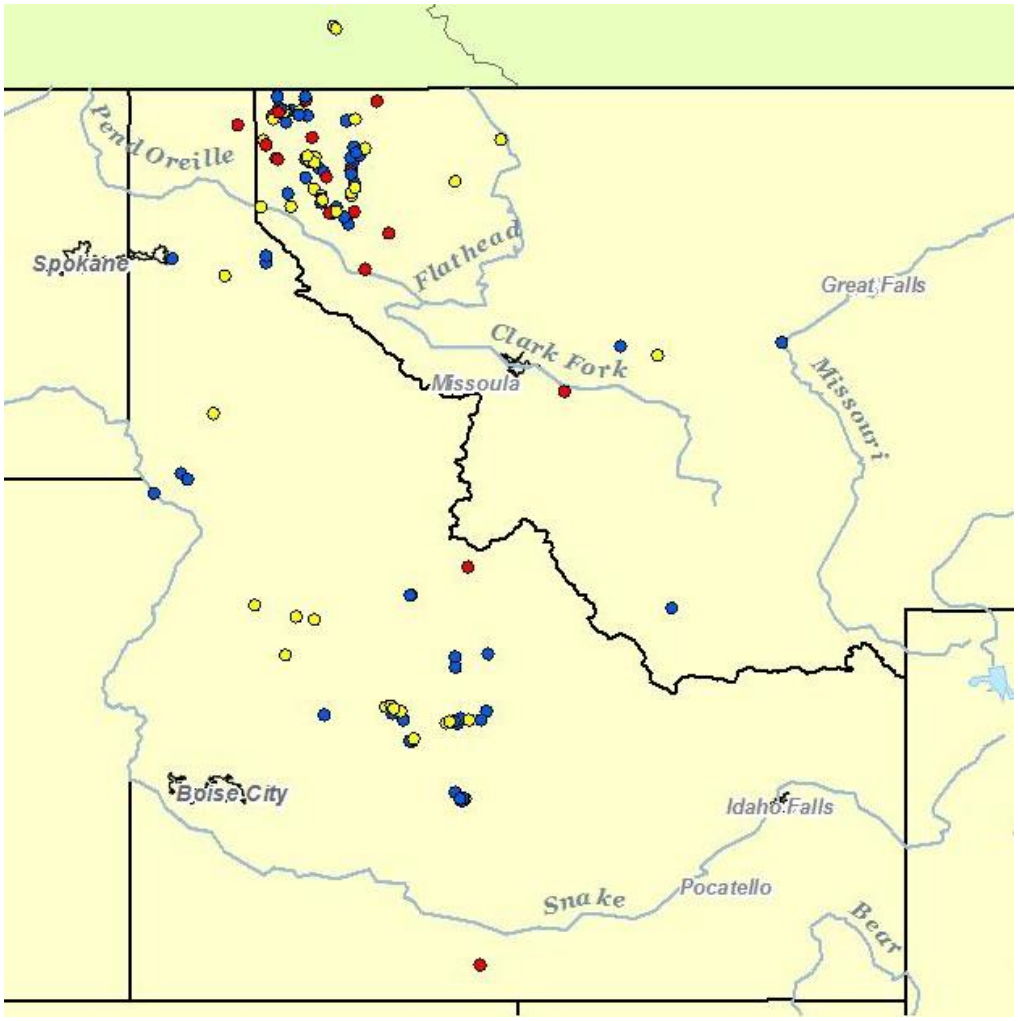

Figure 1-5. Location of *Didymosphenia geminata* samples throughout Idaho and Montana. Yellow dots are confirmed presence of *D. geminata* cells; mat presence not determined. Red dots are confirmed presence of cells and mats of *D. geminata* and blue dots are algal scrapings that were negative for *D. geminata*.

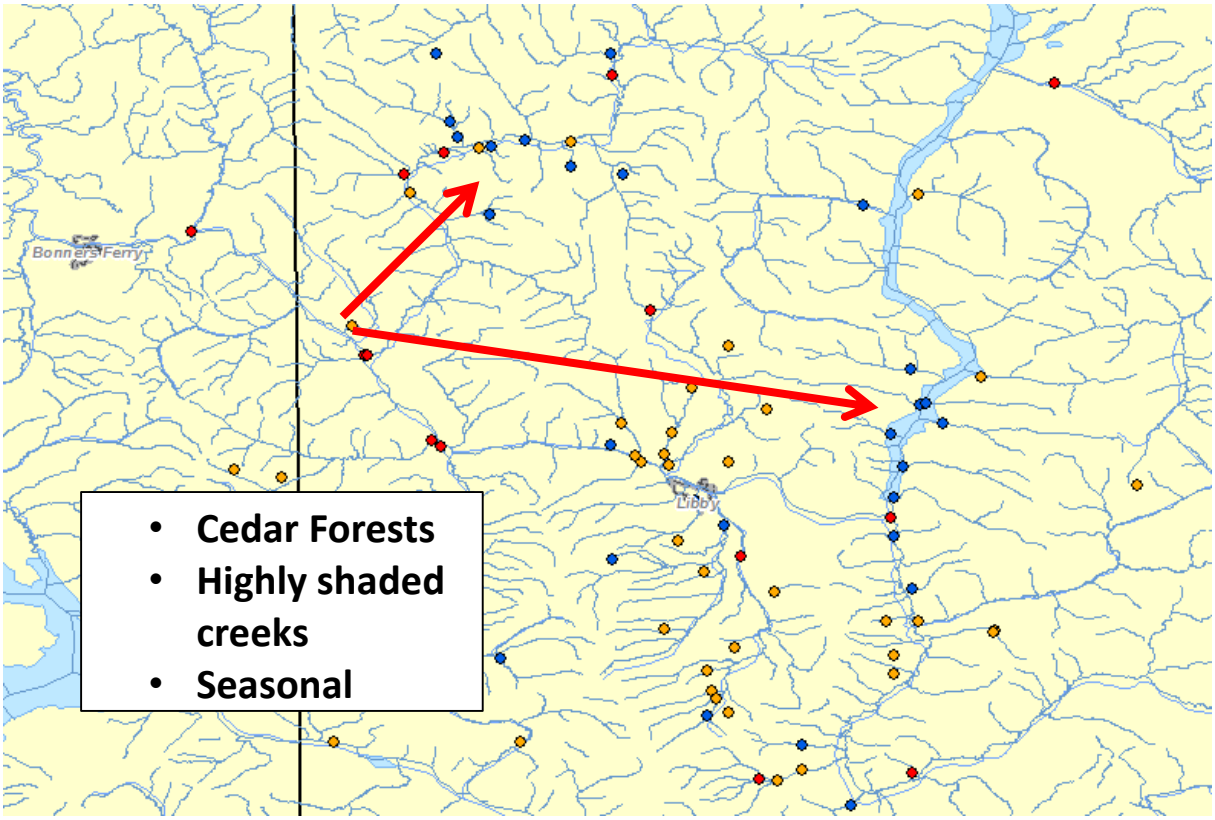

Figure 1-6. Location of samples distributed throughout the Kootenai National Forest showing the occurrence of *Didymosphenia geminata*; yellow dots are confirmed presence of *D. geminata* cells but, mat presence not determined; red dots are confirmed presence of cells and mats of *D. geminata*, while blue dots are algal scrapings that were negative for *D. geminata*.

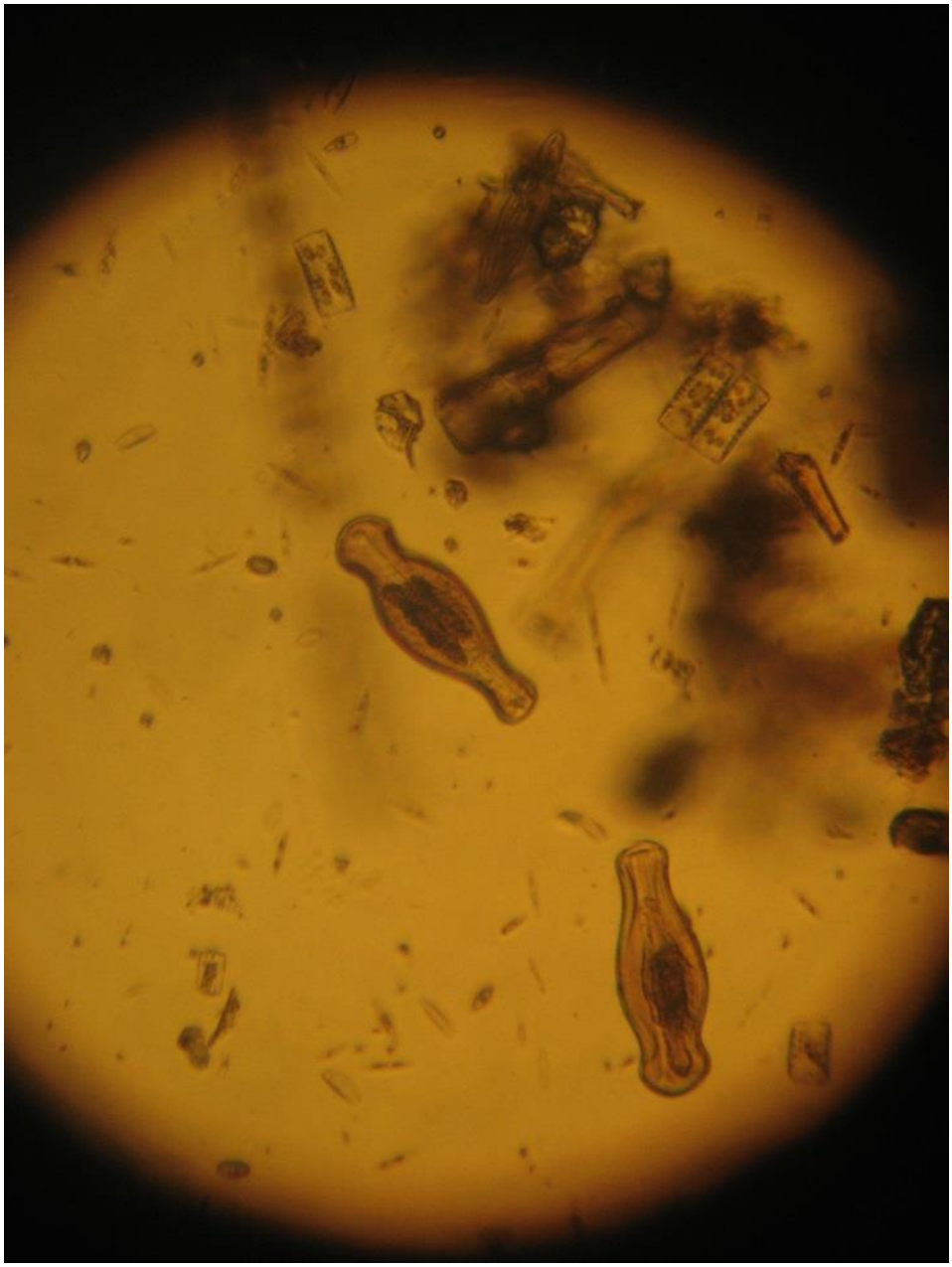

Figure 1-7. Close-up of *Didymosphenia geminata* beneath a compound microscope.

Detection of *D. geminata* cells within algal scrapings was recorded and a voucher was created with location name, GPS location, mat presence, and habitat characteristics recorded on the voucher.

Chapter 2: Seasonality of *Didymosphenia geminata* Kootenai River Libby, MT, USA

Abstract

Didymosphenia geminata has garnered significant attention from the media and scientists in the last decade due to the seemingly invasive pattern of the appearance of nuisance mats in oligotrophic rivers throughout the world. To improve understanding of the species and what environmental conditions favor the formation of these mats, scientists have used experimental flumes to pioneer much of what is known about this species. Although it is well-known that the species has cyclic patterns of growth in natural systems, this seasonality and its significance on conclusions reached from flume experiments have not been well studied. I examined the accrual and described the characteristics of the *D. geminata* mat formed on new substrate in experimental flumes during seven months (March and December in 2013, May, June, July, September in 2014, and January 2015) at the mobile experimental flume system (MEFS) below the Libby Dam, Libby Montana, USA. Cell counts and stalk lengths after 37 days of conditioning the flumes were consistently higher when conditioning started in early fall (July and September) and winter (December, January and March), while the frequency of dividing cell (FDC) was highest in December. Compared to trends in the Kootenai River, the source of the water for the flumes, cell attachment and the number of algal species in the flumes in 2015 were lower during the first 6 days of seeding, but were similar by the 7th day. Overall, these results indicate that flume studies should be undertaken in winter when *D. geminata* recruitment and growth is at a maximum, and that a prolonged condition time of 20 days or longer should be used to establish a community reflective of that in the river. Only then should results obtained from mesocosm studies be considered reflective of *in situ* conditions.

Introduction

Historically present in circumboreal regions of the world, the relatively unnoticed diatom *Didymosphenia geminata* has begun to produce large nuisance mats on the substrate of many lotic systems (Kilroy 2004; Bothwell 2009; Kirkwood et al. 2008, 2009; Reid et al. 2012; Reid and Torres 2014) composed primarily of mucopolysaccharide stalk material (Kilroy et al. 2005; Aboal et al. 2012). Much of what is known about this shift from the 'normal' to 'nuisance' state has been garnered from mesocosm experiments in flumes with foam substrate (Bothwell and Kilroy 2011; Kilroy and Bothwell 2011, 2012; Bothwell et al. 2014). While a few detailed studies have described the seasonality of growth, mat development, and morphology in natural systems (see for example Sherbot and Bothwell 1993), this cyclic nature has not been examined in mesocosms. For results from experiments to be useful to understand in-river processes, researchers must be able to put results from mesocosm experiments in context. For example, in-river research of mats typically occurs during the warm summer months near base flow conditions when benthic mats are accessible. However, this may not be the optimal time to study mats, especially if a dormant period coincides with such a summer research effort. In many rivers, peak nuisance mat growth occurs during the winter months (Beltrami et al. 2008; Whitton et al. 2009), suggesting that winter/early spring is the optimal time to conduct mesocosm research on *D. geminata* to reflect systems at their peak growth.

The objective of this study was to examine the seasonality of *D. geminata* accrual in flumes of a Mobile Experimental Flume System (MEFS) conditioned with unfiltered river water to determine if the growth of *D. geminata* in the MEFS represented the *in situ* patterns in the Kootenai River. I examined the accrual by *D. geminata* on the foam substrate in the

MEFS in each of seven months (December, January, March, May, June, July, and September) between 2013 and 2015. I was also interested to know if conditioning in the experimental flumes was representative of patterns observed in the river. To examine this pattern, I initiated a short trial in early 2015 by concurrently sampling foam substrate set out in the river and the foam in the flumes.

Methods

A mobile experimental flume system (MEFS) was built based on designs similar to that used by Bothwell (1988) and Bothwell and Kilroy (2011). The MEFS was located just below Libby Dam (Figs. 2-1 and 2-2) and supplied with unfiltered river water directly from the Kootenai River. Thirty-two flumes, each 2 m long × 0.2 m wide × 0.05 m tall constructed of clear plexiglass with an open cell 6 mm thick Styrofoam (Floracraft, Michigan, USA) substrate served as the experimental units. Water at a flow rate of 30 L/min was supplied to each flume from two large (~ 1000 L) header tanks. Because the dam outlet water originates from the 145-km long Koocanusa Reservoir, soluble reactive phosphorus concentrations were low (0.5 to 1 µg/L) year-round, while nitrate+nitrite-N exceeds 200 µg/L (KTOI 2014).

The unfiltered river water was passed over the 6 mm thick open-cell foam substrate in three flumes for 37 days after which samples were taken from each flume. To prepare flumes for the next experiment, they were washed and scrubbed, and the foam substrate was replaced with new foam. I examined flume accrual seven times in separate independent experiments starting in March and December in 2013, May, June, July, September in 2014, and January in 2015.

On day 37 after seeding started, triplicate 19 mm diam. cores were removed from random locations in each of the three flumes. Each core was gently washed in the flume to remove any unattached material before placing it into a 50 ml centrifuge tube with 10 ml of deionized (DI) water and 0.25 ml of Lugol's iodine.

In January 2015, I concurrently examined colonization in the flumes and the river. Flume substrates were set up as above, while for deployment in the river, the foam substrate was carefully fastened to cement blocks with zip ties (Fig. 2-3). The blocks with foam substrate were placed in the river in shallow water near the experimental flume system. Sampling occurred daily at 1030 UTC between 3 and 9 Jan 2015, as described below. The experiment was terminated early by increased river flows that made the in-river substrate unavailable for additional sampling.

Individual cores were placed onto a glass microscope slide, topped with a cover slip and examined with the aid of a compound microscope (Wild M40) at 120×. For each core, all visible *D. geminata* cells were counted, classified to stage of division (frequency of dividing cells, FDC), and all stalks that were attached to the foam were measured (stalk length measurement) as described by Kilroy and Bothwell (2012). Dividing cells were defined as cells that had "completed cytokinesis with a valve wall separating the adjoined daughter cell" (M. Bothwell, Environment Canada, Nanaimo, British Columbia, personal communication, 2013). These cells were considered "doublets" and FDC was calculated by dividing the number of doublets by the total number of cells counted (Bothwell and Kilroy 2011). Algal diversity was analyzed by identifying, on each core, as many unique algal taxonomic groups (species) as possible.

Statistical analyses

Data were analyzed using analysis of variance procedures, assuming a completely randomized design, followed by the Tukey's *post-hoc* HSD mean separation technique. The frequency of dividing cells, stalk length and total cell count data were averaged for the triplicate cores from three flumes. The FDC, stalk length, and total cell count were the response variable in each separate analysis and the month sampled was the dependent variable. All statistical analyses were carried out using procedure ANOVA in SAS version 9.4 (SAS Institute, Cary, North Carolina). Data for Kootenai River colonization versus flume colonization was plotted to observe trends in total cell count and algal community growth over 7 days and examined for similar patterns.

Results

Seven experiments completed over two years demonstrate the seasonality of accrual of *D. geminata* in the MEFS (Table 2-1). All experiments (by month) were independent of each other; algal growth in one month did not depend on growth in any other month.

Two days after starting unfiltered water flow through the flumes on 22 March 2013, for the first experiment, cell attachment of *D. geminata* cells was observed (from microscopic examination of cores for another study) and biofilm visible to the naked eye was observed after day three. After 23 days, *D. geminata* mats were present at the macroscopic level and increased to a depth of approximately 2-3 mm by day 37. In the second trial, started on 30 December 2013, macroscopic mat formation was clearly visible after 3 days and by day 37 all flumes were covered with dense growths of algae including several filamentous green species. Over most of the substrate, mat depth of *D. geminata* was ~5 mm thick, while in several areas

the mat depth was ~8 - 12 mm. In the third trial started on 7 May 2014, very few live cells attached to the substrate over the 37 days of seeding and no mat formed. The trial started in June 2014 had some cell attachment but negligible stalk growth, while the trial started in July 2014 had cell attachment, and mats were visible by day 37. The trial started in September 2014 had very low cell attachment and stalk growth or mat formation compared to the July and December trials. The last trial started in January 2015 had similar results to December 2014; noticeable mat growth was present by day 14 and by day 37 it was ~5-7 mm deep.

Total cell counts across months of seeding differed (ANOVA, $F_{6,21} = 15.02$, $P < 0.001$) (Table 2-2). Stalk length across months of seeding also differed (ANOVA, $F_{6,21} = 7.83$, $P < 0.001$) (Table 2-4). Overall, stalk length and total cell numbers were consistently higher in the fall and winter months (Figs. 2-4 and 2-5).

After 37 days, the experiment started in December had an average stalk length of 940 μm while that started in January had an average of 719 μm. Seeding started in July and September had slightly shorter average stalk lengths compared to the winter months at 516 and 596 μm, respectively. Stalk lengths for experiments started in the summer months (May and June) were significantly shorter ($P < 0.001$) than those started in the winter months (December and January) (Fig. 2-4; Table 2-4), with May having no detectable stalk growth (see Fig. 2-4, letters distinguish months that were similar or different from each other).

Total cell count was higher in experiments started in March and September than the winter or summer months (Fig. 2-5; Table 2-2), even though mat coverage and stalk length were higher in the winter months. FDC values were highest in experiments started in winter

(December) (Table 2-3) but overall did not differ among months except for May (Fig. 2-6). Similar to total cell count, FDC in May was nearly zero.

When comparing results of the MEFS and the Kootenai River, trends in cell attachment and FDC values were similar during the first seven days of seeding (3 Jan to 9 Jan) (Fig. 2-7 and 2-8). While the absolute numbers in *D. geminata* cell attachment, FDC, and algal species count on the foam substrate in the Kootenai River were higher than on the MEFS substrate, the patterns in all variables were similar between the river and the MEFS (Figs. 2-7, 2-8, and 2-9). At the end of the 7 days, the number of algal species in the flumes was nearly the same as in the Kootenai River (Fig. 2-9).

Discussion

The attachment of *D. geminata* cells and the growth of stalks had a pronounced seasonality in the Kootenai River below the Libby Dam, with highest cell attachment and growth during the winter months (December through March). My results also suggest that while mesocosm experiments started in summer will have some *D. geminata* growth, it is questionable if results from such experiments will be predictive of the higher growths observed in winter in the Kootenai River. I am unaware of any experiments or studies reported in the literature that have examined if the response of *D. geminata* to different treatments is comparable between experiments initiated in different seasons (see Kilroy and Bothwell 2014 for 3 day experiments between 2009 and 2010).

The results of this study also suggest that any research on *D. geminata* in a particular geographic location first should examine and report the underlying seasonality of *D. geminata* growth so that experiments can be timed to appropriately test hypotheses. For example, if

experiments are designed to examine the addition of a substance to reduce mat severity, it should be done in December-March in the Kootenai River, to capture the response at the height of growth. Few studies have examined *D. geminata* mat characteristics within a river system year round (Kirkwood et al. 2009; James et al. 2014). Most studies of *D. geminata* mats have been limited to summer when the rivers are near base flow (Flöder and Kilroy 2009; Bergey et al. 2010; Rost et al. 2011; Sivarajah et al. 2015). Whitton et al. (2009) observed that at two sites in northern England, mats were at peak during summer while the Brusago stream in Italy had mats throughout the winter (Beltrami et al. 2008). Thus, researchers must understand the seasonality of *D. geminata* at their location to match the timing of experiments, both experimental flume-type and *in-situ*, to coincide with what they wish to examine with regard to *D. geminata*. Otherwise, the applicability of results must be questioned. I feel confident that my flume studies, designed to examine if *D. geminata* growth can be reduced by the addition of various elements (Chapters 5 and 6), was timed appropriately to reflect conditions in the river because I initiated seeding in December through March when mat growth in the river was at a maximum. I assume that responses observed under maximum growth should be applicable across all seasons, and any treatment effects seen at maximum growth should also be present when growth of *D. geminata* is less vigorous due to seasonality.

The similarity of patterns in total cell count, frequency of dividing cells and algae species in January 2015 between the MEFS and the Kootenai River suggests that growth in the MEFS is representative of trends that occur in the river. The lower overall abundance of *D. geminata* in the flumes can be explained by the lower flow rate through the flumes compared to the river, meaning the substrate in the flumes does not have the same exposure to

potential settling propagules as the substrate in the river. This is a mechanical limitation of the flume/pump system and the ability to have multiple replicates in which to perform simultaneous experiments. The similar species abundance at the end of 7 days suggests that community dynamics in the flumes should be similar to those in the river. Within my seasonality study, 37 days of seeding in the winter months resulted in ~5 mm thick mats of *D. geminata* over most of the substrate, with some patches ~8 - 12 mm thick. These mats were similar to those observed in the Kootenai River and had similar characteristics of the "typical" *D. geminata* mat. Given that I performed all experiments (Chapter 5 and 6) with an initial seeding period of 37 days, this should have ensured that the flume community adequately represented the in-river community and that conclusions derived from the flume experiments should be applicable at the river scale.

Flöder and Kilroy (2009) described *D. geminata* as a secondary succession species requiring the presence of a biofilm for cell adhesion, stalk growth and mat formation (Cullis et al. 2012). While later studies have shown that *D. geminata* can accrue on substrate without prior species colonization (Kilroy and Bothwell 2011), I found that in my seven seeding experiments other diatom genera (e.g., *Encyonema*, *Cymbella*, *Achnanthes, Tabellaria*, and *Fragilaria*) first colonized the substrate (Chapter 3) before *D. geminata* cell attachment occurred. However, this may be due to the higher prevalence of these species in the water relative to *D. geminata.* Kirkwood et al. (2008) and Larned et al. (2011) also reported that the presence of a stable substrate similar to that which occurs downstream of dams promoted nuisance mats of *D. geminata* compared to environments with turbulent flows and unstable substrate. Thus, my flume setup with the immovable open-celled Styrofoam as substrate should have been appropriate for the accrual of *D. geminata*. From the total cell counts, stalk

length and FDC metrics, it is clear that *D. geminata* accrual rates and mat growth are inhibited during the late spring and summer months which may have implications for the interpretation of research conducted on *D. geminata* during the summer if that is not its peak growth season. The annual solar maximum may have reduced the success of seeding trials started in June and July. Kilroy and Bothwell (2014) reported that high incident UVR negatively affected the establishment and persistence of *D. geminata* in New Zealand, and probably elsewhere. However, it is unclear if this is a direct (UVR → *D. geminata*), or indirect (UVR → biofilm → *D. geminata*) response. Evidence supporting a direct effect was suggested by Kilroy and Bothwell (2014), however, Bothwell et al. (1993) also showed deleterious effects of UVR on shallow stream ecosystems, especially the periphyton community. The direct vs indirect interactions resulting in low *D. geminata* attachment have not been examined thoroughly. I attribute the slight increased mat presence in July compared to June to the reduced UVR incidence in August compared to June and July.

While water temperature typically increases with air temperature and light availability, the water below Libby Dam only fluctuates between 4°C and 14-17°C annually (Fig. 2-10) with low temperatures in winter, and highs in late-July, August, September, and the beginning of October. This temperature range is less than that in other unregulated northwestern streams because of the dam and the selective withdrawal to meet downstream target temperature requirements for threatened and endangered fish. I did not expect that the water pumped from the Kootenai River warmed significantly during the transition through the flumes given the water renewal was 1.3× per flume per minute and the header tanks were white plastic which did not heat appreciably in sunshine. The elevated temperatures in summer were not lethal to stream diatoms, and should not have affected accrual to the degree

observed given that metabolic rates and growth rates typically increase with temperature. Kilroy and Bothwell (2014) also observed non-typical patterns in their short-term growth experiments with *D. geminata* in flumes in New Zealand. The mechanism giving rise to these observations deserves further investigation.

The lack of significant cell attachment or stalk growth beyond a certain date in spring in the Kootenai River may be exploitable to manage *D. geminata*. Bothwell (Environment Canada, Nanaimo, British Columbia, personal communication, 2013) has observed that if a well-established mat (far along in its growth) is removed from its substrate, little or no accrual occurs during the remainder of the year, suggesting distinct periods of accrual. The observed lack of accrual during the May 2014 seeding experiment and the low responses for the experiment started in July suggests that a similar accrual pause occurs in the Kootenai River. If *D. geminata* could be prevented from forming mats early, treatment (in the form of adding P – Chapter 3) may only have to be applied for a short period, ideally in late winter to suppress mats during the peak season so that the suppression of stalk growth would hold over until the next season. Such an approach could reduce overall treatment costs and avoid excessive growth of co-occurring algae that typically result when the availability of dissolved phosphorus is increased (as seen by the high AFDM in my flumes with high P addition - Chapter 3), especially if P is added year-round. I expect that mats of other species periodically would slough off as in the flumes, but this would need confirmation with an *in situ* study.

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Tables

Table 2-1. Dates of seeding and sampling of the Mobile Experimental Flume System (MEFS) for analysis of seasonality of growth of *Didymosphenia geminata.*

Seeding Date	37 Days Later
22 March 2013	28 April 2013
30 December 2013	6 February 2014
7 May 2014	12 June 2014
17 June 2014	23 July 2014
23 July 2014	29 August
12 September 2014	18 October 2014
2 January 2014	8 February 2015

Table 2-2. *Post-hoc* ANOVA using Tukey's HSD of *Didymosphenia geminata* total cell count over multiple seasons in 2013-2015 in the Kootenai River of Libby, MT, USA.

Month	Dec	Jan	Mar	May	Jun	Jul
Jan	0.198					
Mar	0.001	0.002				
May	0.015	0.001	0.001			
Jun	0.141	0.001	0.001	0.280		
Jul	0.375	0.036	0.001	0.098	0.540	
Sep	0.003	0.051	0.131	0.001	0.001	0.001

Table 2-3. *Post-hoc* ANOVA using Tukey's HSD of *Didymosphenia geminata* frequency of *Didymosphenia geminata* frequency of dividing cells (FDC) over multiple seasons at the Kootenai River Research Station in Libby, MT, USA.

Month	Dec	Jan	Mar	May	Jun	Jul
Jan	0.600					
Mar	0.413	0.766				
May	0.003	0.010	0.020			
Jun	0.125	0.299	0.454	0.093		
Jul	0.316	0.626	0.849	0.030	0.575	
Sep	0.023	0.069	0.121	0.375	0.403	0.169

Table 2-4. *Post-hoc* ANOVA using Tukey's HSD of *Didymosphenia geminata* stalk length over multiple season at the Kootenai River Research Station in Libby, MT, USA.

Month	Dec	Jan	Mar	May	Jun	Jul
Jan	0.230					
Mar	0.002	0.027				
May	0.001	0.001	0.115			
Jun	0.001	0.001	0.136	0.926		
Jul	0.028	0.270	0.225	0.009	0.011	
Sep	0.061	0.467	0.116	0.004	0.004	0.699

Figures

Figure 2-1. Mobile experimental flume system (MEFS) at the Kootenai River Research Station downstream of Libby Dam in Libby, MT, USA.

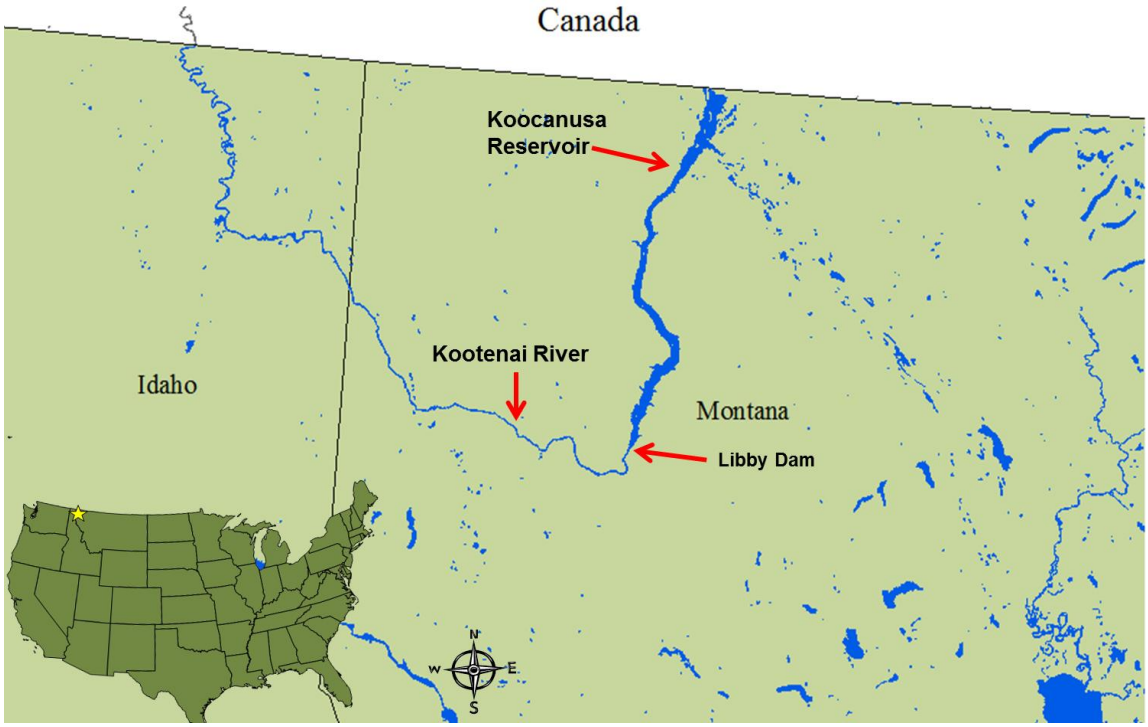

Figure 2-2. Map of the Kootenai River in northwest Montana. The mobile experimental flume system (MEFS) was located below the Libby Dam on the Kootenai River, MT. Map created with ArcMap v. 10.2.2 (ESRI, Redlands, CA).

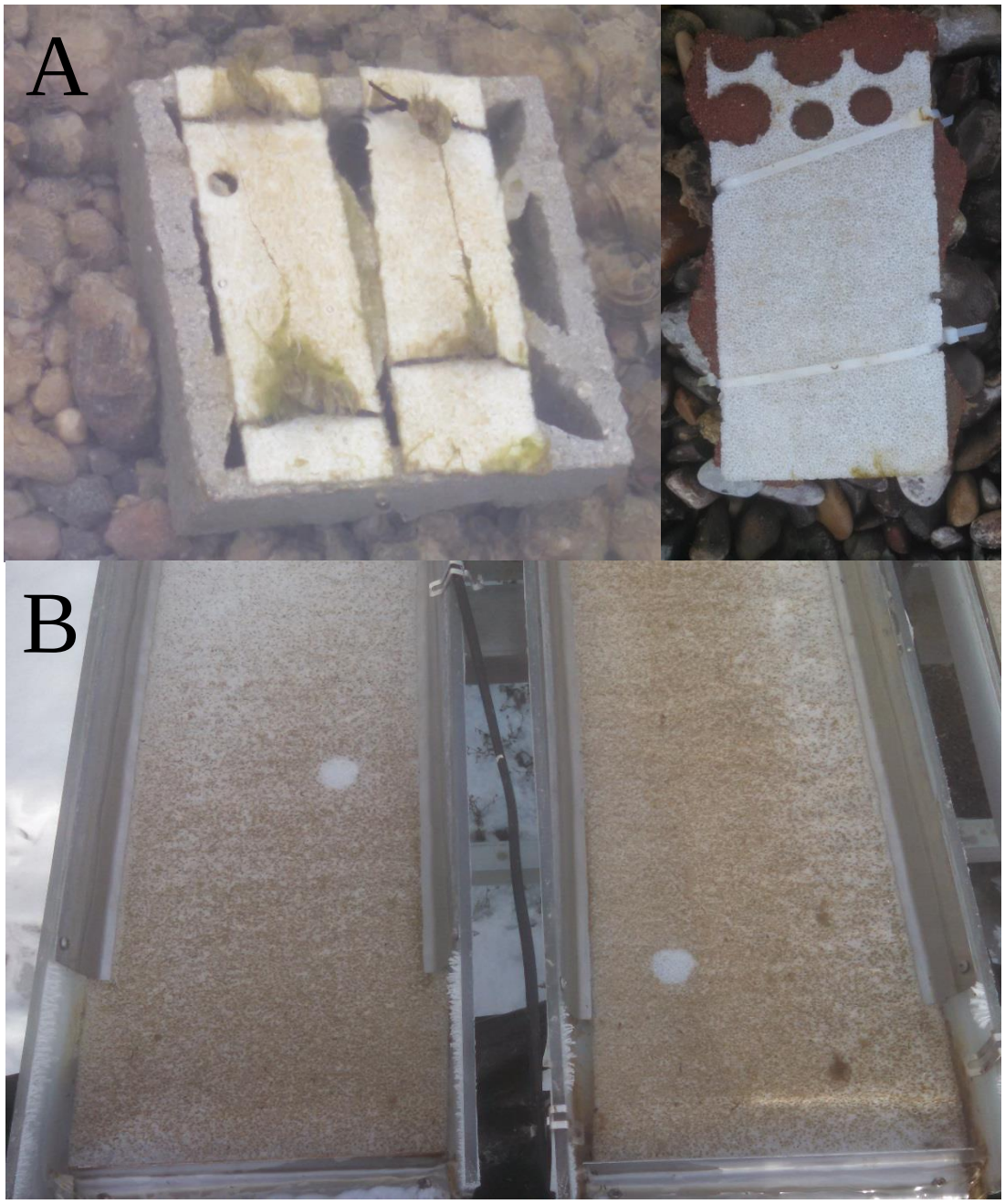

Figure 2-3. Open-celled 6 mm thick styrofoam attached to (A) bricks in the Kootenai River and (B) in the flumes of the Mobile Experimental Flume System (MEFS) to evaluate colonization of *D. geminata* at the Kootenai River Experimental Station in Libby, MT, USA.

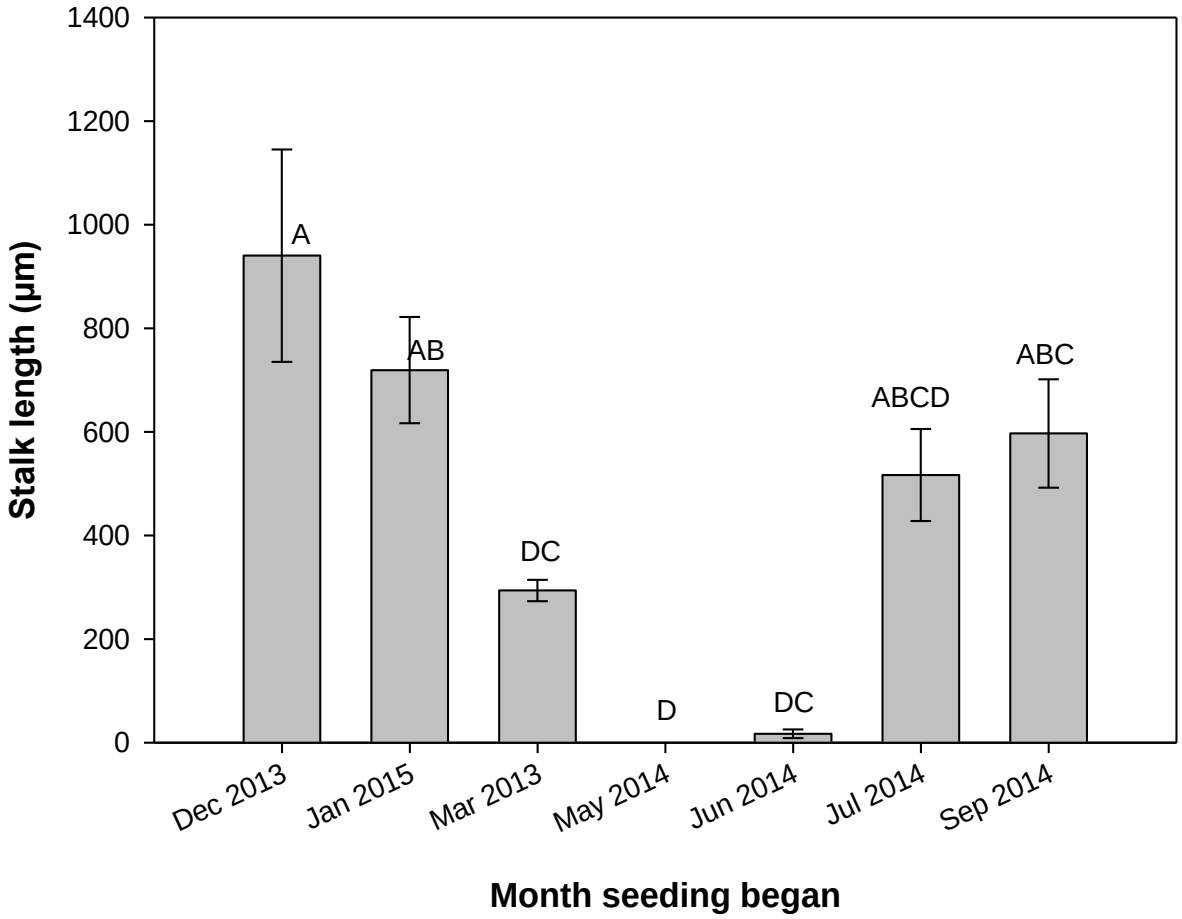

Figure 2-4. Mean stalk length (±SE) of *Didymosphenia geminata* after 37 days of seeding at different times of the year in the University of Idaho mobile experimental flume system (MEFS) located at the Kootenai River Research Station below Libby Dam in Libby, MT, USA. Means designated with different letters are statistically significant ($P<0.05$) based on Tukey's HSD mean separation procedure.

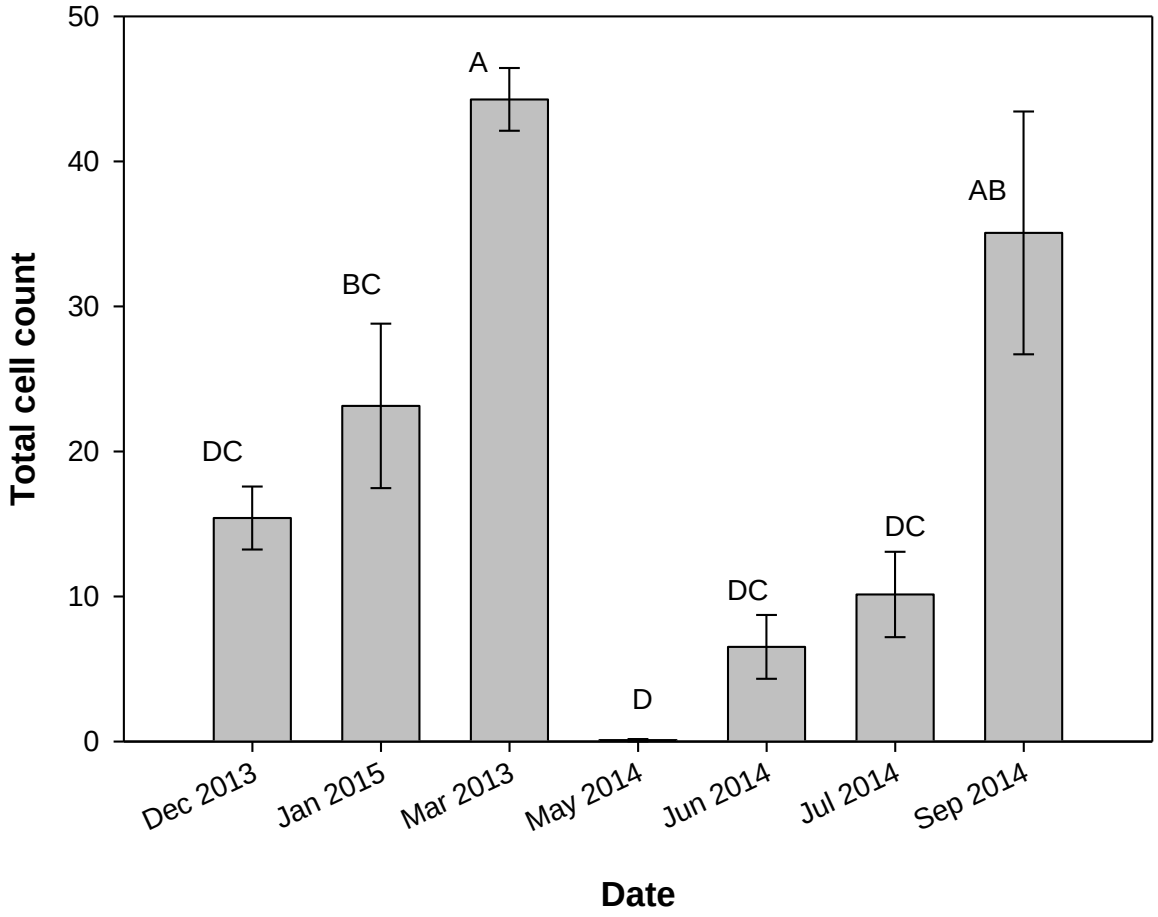

Figure 2-5. Total cells per cm^2 (mean± S.E.) of *Didymosphenia geminata* after 37 days of seeding at different times of the year in the University of Idaho mobile experimental flume system (MEFS) located at the Kootenai River Research Station below Libby Dam in Libby, MT, USA. Means designated with different letters are statistically significant ($P<0.05$) based on Tukey's HSD mean separation procedure.

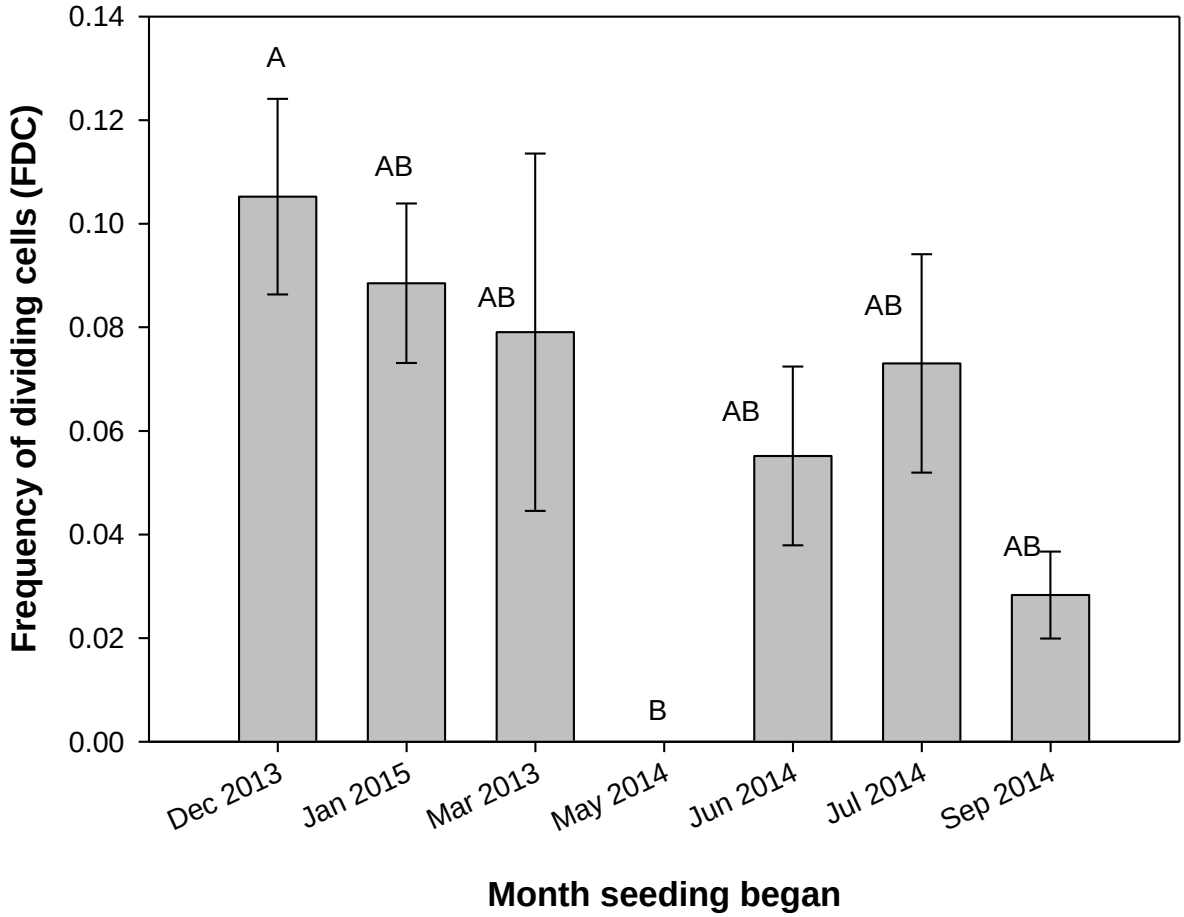

Figure 2-6. Frequency of dividing cells (mean±S.E.) of *Didymosphenia geminata* after 37 days of seeding at different times of the year in the University of Idaho mobile experimental flume system (MEFS) located at the Kootenai River Research Station below Libby Dam in Libby, MT, USA. Means designated with different letters are statistically significant ($P<0.05$) based on Tukey's HSD mean separation procedure.

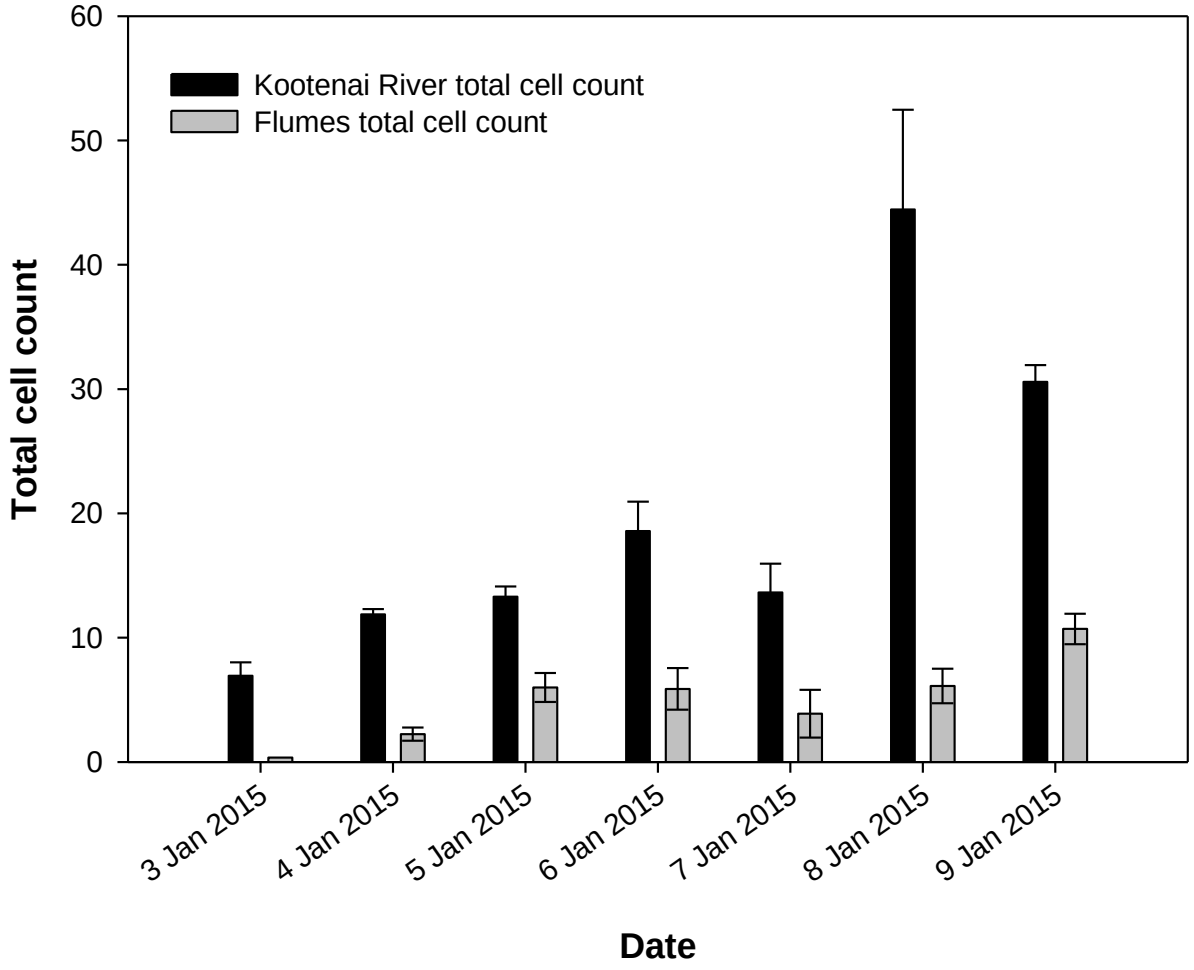

Figure 2-7. Total cell count per cm^2 (mean±S.E.) of *Didymosphenia geminata* in the University of Idaho mobile experimental flume systems (MEFS) and the Kootenai River at the Kootenai River Research Station in Libby, MT, USA.

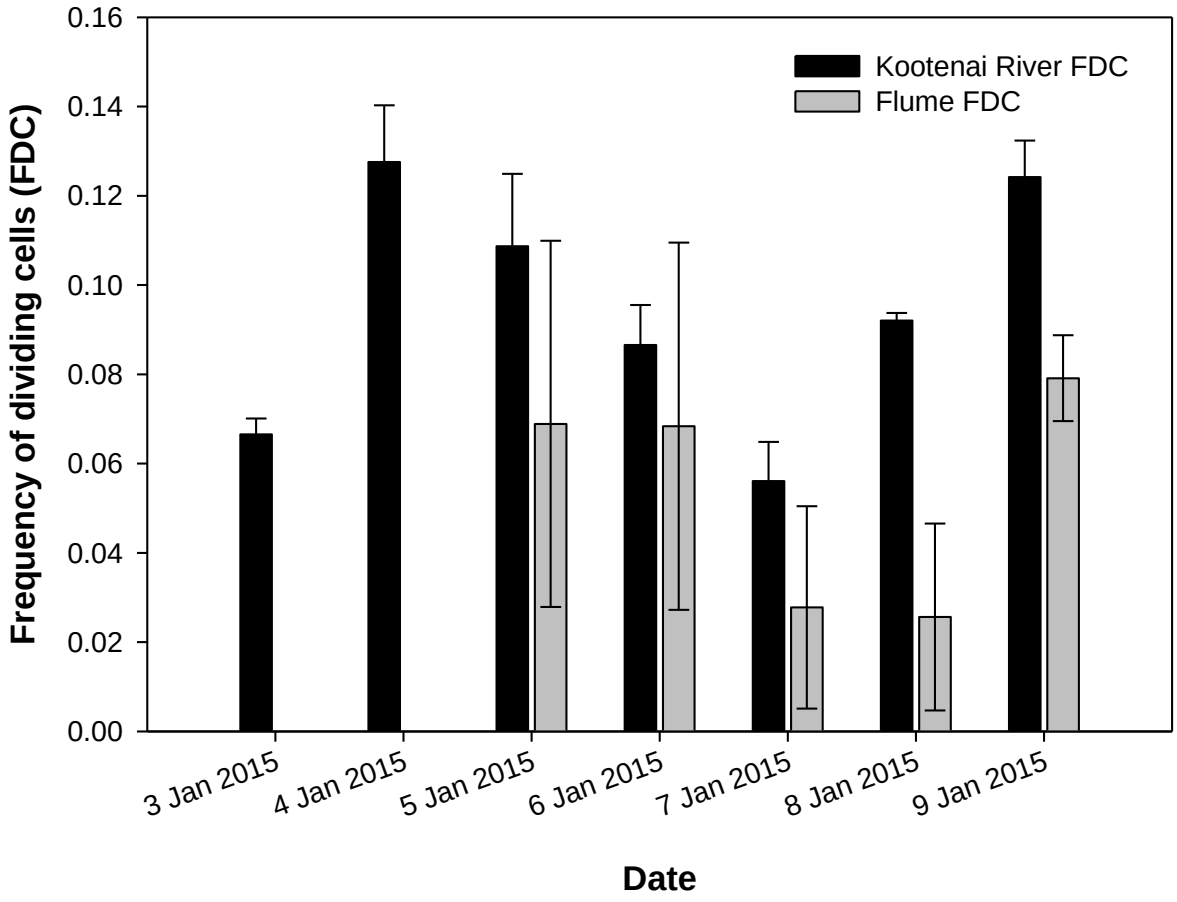

Figure 2-8. Frequency of dividing cells (mean±S.E) of *Didymosphenia geminata* in the University of Idaho mobile experimental flume systems (MEFS) and the Kootenai River at the Kootenai River Research Station in Libby, MT, USA.

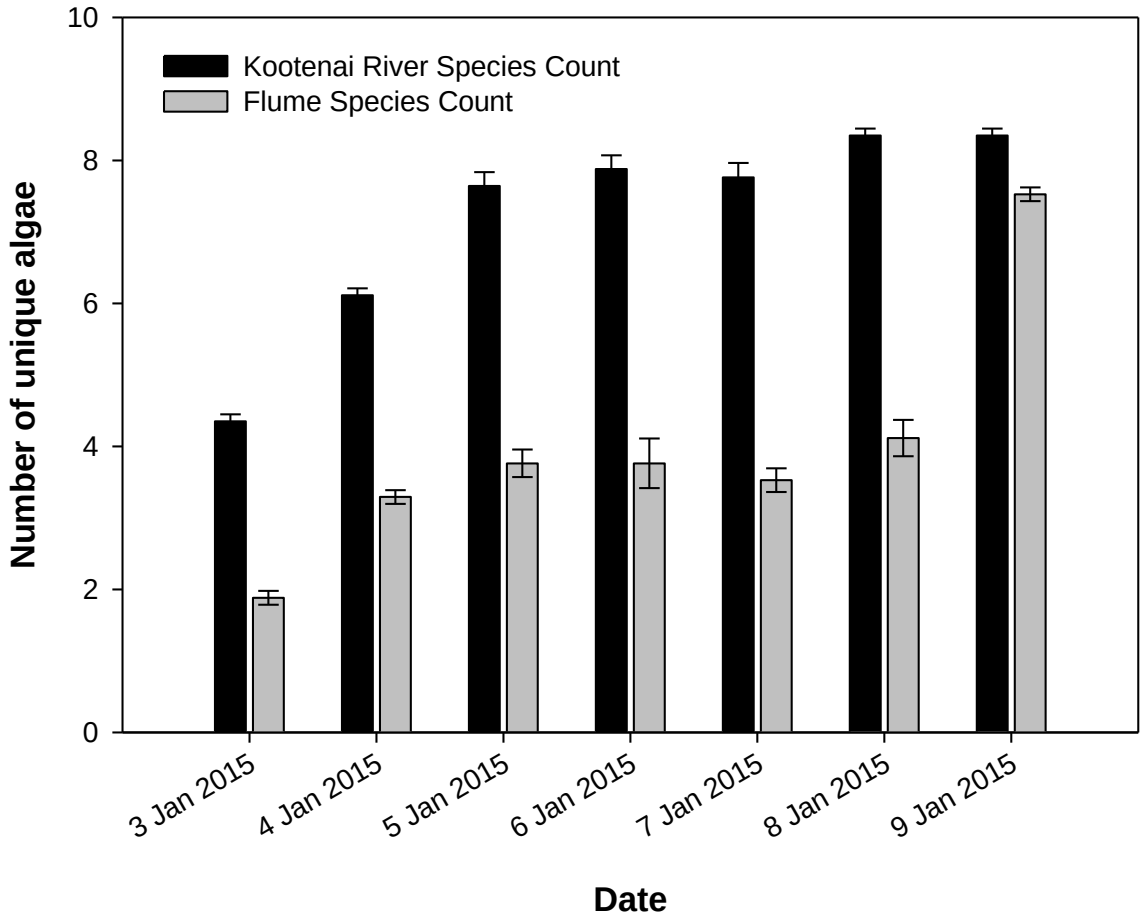

Figure 2-9. Number of unique algae per cm^2 (mean±S.E.) in the mobile experimental flume system (MEFS) and the Kootenai River at the Kootenai River Research Station in Libby, MT, USA. Algae identified were large diatoms and filamentous algae viewable with the aid of a compound microscope.

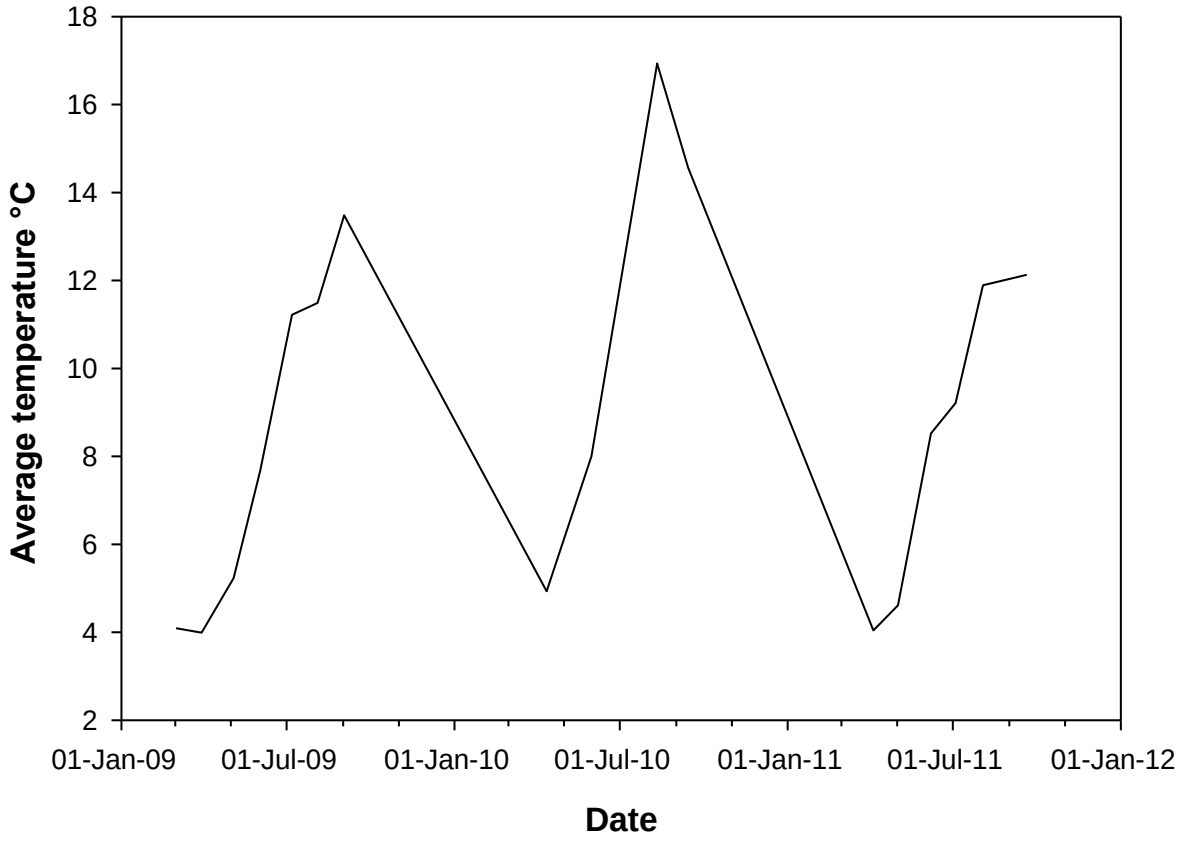

Figure 2-10. Average water temperature below Libby Dam of Libby, MT, USA from 2 March 2009 to 20 September 2011. Data provided by KTOI and used with permission. Permission to reuse must be obtained from the rightsholder.

Chapter 3: Phosphorus enrichment as a management strategy for *Didymosphenia geminata* nuisance mats in the Kootenai River, Libby MT

Abstract

Thick mats of *Didymosphenia geminata* resembling sewage or wet toilet paper smothers the substrate displacing benthic invertebrates, thereby, reducing the aesthetics of rivers and streams and overall ecosystem health and productivity. The objective of this study was to experimentally examine the addition of phosphorus for its potential as a management strategy to ameliorate the nuisance mat of *D. geminata* in the Kootenai River near Libby, MT, USA. The addition of phosphorus at all experimental concentrations significantly suppressed the stalk length of *D. geminata* relative to controls, which only received river water. Seasonality patterns were observed in the frequency of dividing cell (FDC). In 2013, FDC was higher in experimental flumes relative to the controls but only for the initial 6 weeks, while in 2014, FDC in the controls was significantly higher than all experimental additions for the entire 3-month study. The addition of dissolved phosphorus stimulated the growth of dozens of other algal species, especially at the higher concentrations, which increased competition and shading to the *D. geminata* mats. While the longitudinal downstream extent of phosphorus additions remains to be elucidated, the significant reduction of stalk length at very low P concentrations, 0.5 µg/L above ambient, suggests that it could be a viable strategy for managers to suppress nuisance mat growth of *D. geminata*.

Introduction

Thriving in cold, fast flowing, oligotrophic systems, *Didymosphenia geminata* (Lyngbye) M. Schmidt dominates the systems where nutrient-limited conditions would typically result in low algal growth (Tilman et al. 1982). This diatom has recently gained notoriety due to excessive stalk production resulting in the occurrence of nuisance mats in streams and rivers across the globe, where such mats were previously absent. Producing gelatinous mats composed of mucopolysaccharide stalks (Ellwood and Whitton 2007); this benthic diatom is a concern for its threat to the function and aesthetics of river ecosystems.

The mats produced by *D. geminata* cover the substrate, displacing large-bodied benthic invertebrates such as Ephemeroptera, Plecoptera, and Trichoptera, the preferred prey of many salmonid species (Marshall et al. 2008). The presence of *D. geminata* mats in the Kootenai River was noticed in the early 2000s, when growths appeared on sampling gear (Holderman and Hardy 2004), though previous work indicates *D. geminata* was part of the periphyton community in 1972 (Perry and Huston 1983). These nuisance mats have rapidly appeared throughout the Kootenai River, and have remained a ubiquitous annoyance. Currently, the densest mat coverage of *D. geminata* occurs near Libby Dam (up to 8 mm deep with 100% coverage) and continues downriver into Idaho, albeit with decreasing intensity.

Recent research (Bothwell et al. 2014) has indicated that phosphorus limitation in rivers due to the increase of nitrogen deposition at the landscape scale may be an underlying mechanism for the sudden appearance of mats. Several studies have shown that if total phosphorus is >2 µg/L, *D. geminata* rarely forms the nuisance mats (Kilroy and Bothwell 2012 and references therein). However, in phosphorus-limited conditions, *D. geminata* increases stalk production while the frequency of dividing cells (FDC) is reduced (Bothwell

and Kilroy 2011; Kilroy and Bothwell 2012). These findings, as well as experimental results from Kilroy and Bothwell (2011 and 2012), suggest that restoring dissolved P concentrations may be a method by which to control excessive mat growth of *D. geminata*, and hence, was the impetus for this study. While research on the basic ecology of *D. geminata* is ongoing and still maturing (Chapter 1), methods to control nuisance mats and restore river ecosystems are rare (but see, James 2010 and James et al. 2015 for examples). The objective of this study was to experimentally examine the addition of phosphorus as a management strategy to reduce *D. geminata* nuisance mat infestation of the Kootenai River near Libby, MT, USA. An experimental flume system similar in design to that used by Bothwell (1988) and Bothwell and Kilroy (2011) was built to test applications of dissolved phosphorus at different concentrations. The first experiment began on 29 April 2013 and lasted until 29 September 2013. It involved the continuous addition of phosphorus at three concentrations (3, 5, and 8 µg/L) above background to examine the response of *D. geminata*. The second experiment ran from 6 February 2014 to 17 April 2014 to coincide with the peak *D. geminata* growth period and tested six concentrations (0.5, 1.5, 2, 3, 5, and 8 µg/L) of dissolved phosphorus and a control (river water only). These studies were designed to test if the growth of *D. geminata* was related to the concentration of dissolved phosphorus i.e., masses of stalk (mat) will decrease when dissolved phosphorus concentration reaches a threshold concentration.

Methods

To address the above objectives, a mobile experimental flume system (MEFS) was built by the University of Idaho (UI) based on designs similar to that used by Kilroy and Bothwell (2011, 2012), Bothwell and Kilroy (2011), and Bothwell (1988). The MEFS was

located just downstream of Libby Dam (Fig. 3-1) and supplied with unfiltered river water directly from the Kootenai River.

Thirty-two flumes, each 2 m long × 0.2 m wide × 0.05 m tall constructed of clear plexiglass with an open cell 6 mm thick Styrofoam (Floracraft, Michigan, USA) substrate, served as the individual experimental units. River water was supplied at a flow rate of 30 L/min to each flume from two large header tanks to individual mixing tanks at the head of each flume which received injections of the assigned P treatment concentration from 120-L stock tanks via individual Stenner 45M3 single-head adjustable peristaltic pumps. Water from the Kootenai River was pumped to the header tanks and any P added for treatment was in addition to the ambient raw water P concentration. Therefore, the nutrient concentration to which *D. geminata* in the MEFS was exposed to was slightly higher than those listed, but for simplicity I reported the concentrations at which they were added. Near the MEFS, the in-river soluble reactive phosphorus (SRP) ranged from <0.5 to 1 µg/L with a detection limit of 1 µg/L, and nitrate+nitrite-N ranged from 150 to 270 µg/L (KTOI 2014).

Dissolved phosphorus was added in the form of potassium phosphate monobasic (Fisher Scientific) at three concentrations (3, 5, and 8 µg/L) in 2013 and at six concentrations (0.5, 1.5, 2, 3, 5, and 8 µg/L) in 2014. Pump dosing rates and flume discharge were checked once a week. All flumes were seeded (conditioned) with unfiltered Kootenai River water for 37 days before treatments commenced to provide a bio-film and allow accrual of *D. geminata*, as it is a secondary succession species (Flöder and Kilroy 2009). After approximately two days of water running through the system, free-floating diatoms present in the water attached to the foam substrate and began to colonize the flumes. This seeding process created *D. geminata* communities in the flumes and ensured consistent environmental conditions at the

start of the experiments. Optimum accrual and mat growth within the MEFS was evaluated in 2014 (see Chapter 2) which showed that January and February were the ideal months in which to initiate seeding. In 2013, seeding of flumes began on 22 March due to some equipment delays, while in 2014, seeding started on 30 December. During each individual experiment, all treatments, including controls, were replicated four times and flumes were randomly assigned to each of the P treatment concentrations.

Flumes were sampled biweekly by taking three randomly placed 19 mm diam. cores from the foam substrate of each flume. Each core was gently washed in the flume to remove any unattached material before placing it into a 50 ml centrifuge tube with 10 ml of DI water and 0.25 ml of Lugol's iodine. A replacement core of 22 mm diam. was used to plug the hole in the substrate to minimize hydraulic disturbances within the flume. To analyze each core, individual cores were placed onto a glass microscope slide right side up, topped with a cover slip and examined with the aid of a compound microscope (Wild M40) at 120×. For each core, all visible *D. geminata* cells were counted (quantitative counts), classified to stage of division (frequency of dividing cells, FDC), and all stalks that were attached to the foam core were measured (stalk length measurement) as described by Kilroy and Bothwell (2012). Dividing cells were defined as cells that had "completed cytokinesis with a valve wall separating the adjoined daughter cell" (M. Bothwell, personal communication, 2013). These cells were considered "doublets" and FDC was calculated by dividing the number of doublets by the total number of cells counted (Bothwell and Kilroy 2011). Any detached mat material that remained in the centrifuge tube also was placed on a glass slide and analyzed in its entirety. Post-treatment sampling occurred in both experiments (10 weeks in 2013 and 2 weeks in 2014) to detect any potential lag effects after nutrient additions ceased.

To quantify ash free dry mass (ADFM) and chlorophyll-*a* (Chl-*a*) in 2014, two additional cores were taken from each flume on each sampling occasion, and frozen individually until analysis at the University of Idaho College of Natural Resources (UI CNR) core laboratory. Standard methods (Eaton et al. 2005; USEPA 1995) and UI CNR lab protocols were followed to extract and measure Chl-*a* and AFDM. Measures of AFDM and Chl-*a* were used to calculate the autotrophic index (Collins and Weber 1978; Eaton et al. 2005) which describes the community composition. High values (>400) indicate a heterotrophic community typically composed of bacteria, fungi, and protists, while low values (50-100) indicate a primarily autotrophic community (Eaton et al. 2005).

Photosynthetically active radiation (PAR) was recorded hourly with a Li-Cor (Lincoln, NE) LI-190 terrestrial radiation sensor and a Li-Cor (Lincoln, NE) LI-1400 datalogger. Mean daily PAR for 17 July to 28 September 2013 was 36.75 mol/m^2/d, while for the period of 8 February to 17 May 2015, it was 26.87 mol/m^2/d.

Statistical analyses

Data were analyzed for the period of the nutrient addition. The frequency of dividing cells was averaged for the triplicate cores from each flume on each sampling occasion and analyzed over time with completely randomized repeated measures ANOVA with FDC as the response variable, nutrient concentration as the treatment, and time as the repeated factor. Mean stalk length was calculated from the triplicate samples taken on each sampling occasion and analyzed using the same procedures, in which stalk length was the response variable, nutrient concentration the treatment, and time was the repeated factor. For all flume studies, data were first evaluated to examine normality, and homogeneity of variance using univariate

procedures and diagnostic plots. All statistical analyses were completed with procedure MIXED in SAS software™ v. 9.4 (SAS Institute, Cary, North Carolina). In the repeated measures ANOVA, the fixed effect was the phosphorus treatment while the random effect was flumes.

In addition to these analyses, the stalk length and FDC in the first 6 weeks of phosphorus treatment in 2013 were analyzed with a completely randomized repeated measures ANOVA with procedure MIXED in SAS v. 9.4 for comparison to studies of *D. geminata* that occurred for 4-6 days (Bothwell and Kilroy 2010; Kilroy and Bothwell 2011). Because sampling occurred at bi-weekly intervals, 6 weeks was the shortest time period to analyze changes of the *D. geminata* community in response to the phosphorus treatment.

A repeated measures ANOVA was the chosen statistical analysis over a pulse-dose response model because the objective of the study was to identify the minimum phosphorus concentration that saturated *D. geminata* cells and suppressed stalk growth. While modeling the response to the 6 treatments in the 2014 study would provide insight to the growth of this diatom, the repeated measures ANOVA was more appropriate to directly test the hypothesis under investigation.

Results

Response of D. geminata *to the addition of phosphorus in 2013*

The most visible result of increasing the soluble P by 3, 5, and 8 µg P/L above ambient in the 2013 experiment was the increase of other algal species including filamentous green species such as *Spirogyra, Zygnema, Ulothrix* and diatoms such as *Encyonema, Cymbella, Achnanthes, Tabellaria, Fragilaria,* which dominated the flumes during much of

the experimental period (Fig. 3-2; Table 3-1). Algal biomass that built up over the experiment sloughed off after the addition of P ceased (Fig. 3-3).

The total number of *D. geminata* cells increased over time during the P addition, peaking on 21 July 2013 on the last day of P addition (Fig. 3-4). Although the total number of cells was slightly lower in the controls compared to the treatments, this was not statistically significant (P=0.595) (Table 3-2, 3-3). However, the Time×Treatment interaction was significant (Table 3-2). It was interesting to note that after the P additions stopped, the density of *D. geminata* cells in the treatment flumes returned to pre-treatment counts, while the counts in the control flumes remained higher than in all treatment flumes (Fig. 3-4).

Overall, the addition of P increased the frequency of dividing cells (FDC), which declined to pre-treatment counts after the addition of P stopped (Fig. 3-5; Table 3-4). While FDC was statistically higher for 3 and 8 µg/L over the first 6 weeks of P addition relative to the controls (P= 0.016 and P=0.051), there was no difference among the treatment concentrations (Table 3-5). However, over the entire period of P addition, FDC did not differ among any of the treatments or controls (P>0.05) (Table 3-4). Similar to the total cell counts, after the addition of P stopped, FDC returned to pre-treatment rates (Fig. 3-5).

For stalk length, the P concentration was significant, while the interaction of Time×Treatment and Time were not (Fig. 3-6; Table 3-6), indicating that each concentration had a significant effect on stalk length (Table 3-7). During the first six weeks of treatment, stalk length was significantly shorter in only the 5 µg/L addition compared to the controls. However, for the entire phosphorus enrichment period, stalk length was suppressed in all P treatments relative to the controls (Fig. 3-6). After the addition of P stopped, stalk length was

no longer suppressed by P enrichment and increased to comparable lengths of the control (Fig. 3-6).

During the experiment some macroinvertebrates including Chironomidae, Simuliidae and Ephemeroptera were noticed in the flumes (Table 3-8), but in samples examined under the microscope, Chironmidae occurred most frequently at 1 to 3 individuals per 3 core samples (0.12-0.35 ind./cm^2) (Table 3-8). Ephemeroptera were not observed after the sloughing event post phosphorus treatment. There was a large bloom of Simuliidae around 10 August 2013 that lasted for approximately 5 weeks. Attachment of simuliid individuals was noticed along the sides and ends of the flumes as well as inside the mixing buckets in the trailers.

Response of* D. geminata *to the addition of phosphorus in 2014

Compared to 2013, starting the experiments in early February 2014 resulted in higher growth of *D. geminata* and coverage of mats in the flumes after 37 days of seeding (Fig. 3-7). While these experiments coincided with the natural peak growth of *D. geminata*, some patchiness of mats resulted in the flumes which contributed to high variability for average stalk length and frequency of dividing cells. Similar to 2013, algal growth of species other than *D. geminata* was higher in P treatments than the controls (Table 3-1), and this biomass sloughed off (less than ¼ of each flume, but only in the <3µg P/L concentrations) approximately 12 days after the addition of P ended. This sloughing was much lower than in 2013 when over 60% of the growth detached.

Similar to the 2013 study, the most visible change in the flumes receiving soluble P above ambient was the increase of other algal species. Filamentous green algae and other

diatoms dominated the flumes during the entire treatment at concentrations of 3 µg P/L and above. The communities of filamentous green algae included *Spirogyra, Zygnema, Ulothrix*, while diatoms included *Encyonema, Cymbella, Achnanthes Tabellaria* and *Fragilaria* which was similar to the results observed in 2013 (Table 3-1).

Total cell counts did not differ among P treatments but increases over time were noticed (Fig. 3-8; Table 3-9). Total cell counts were similar at the beginning of the study and increased over time. However, high variability occurred once treatments began. Incorporating the peak growth season, patches of nuisance mat occurred throughout the flumes contributing to the high variance in total cell counts. Once the addition of P ended, total cell counts decreased to counts similar to the beginning of the study.

The response of the frequency of dividing cells (FDC) in this experiment was unusual in that it increased over the entire experiment in the controls, while it decreased in all P treatments (Fig. 3-9). As a result, there was a significant Time×Treatment interaction (Table 3-11) as well as a significant overall treatment effect (Table 3-11) which stemmed from the difference between treatments and controls, as none of the treatments differed from each other Table 3-12). Of interest too, was the high FDC near unity, meaning that nearly all cells observed were dividing.

Stalk length of *D. geminata* at all six phosphorus concentrations was significantly shorter than in the controls (Fig. 3-10; Tables 3-13, 3-14). After six weeks, stalk length in the P treatments was approximately 500 to 1000 µm shorter than the controls (Fig. 3-10). This was similar to the reduction observed in 2013. Interestingly, there were no statistical

differences among the P treatments (Table 3-14). After the addition of P stopped, stalk length was shorter in the experimental flumes relative to the controls (Fig. 3-10).

Ash-free dry mass increased over time, but did not differ across the P treatments (Fig. 3-11; Tables 3-15, 3-16). Similar to AFDM, the Chl-*a* concentration increased over time in all treatments and controls (Fig. 3-12), but treatments differed from the controls (Tables 3-17, 3-18) and slightly from each other on several sampling dates (Fig. 3-12; Table 3-18).

The autotrophic index ranged from 63 to 149 for the P treatments, while the controls ranged from 46 to 201. Biggs and Kilroy (2000) suggest that an AI score between 100 and 200 indicates a healthy algal community (i.e., an unpolluted system). Thus, even at the highest P concentration I used, the AI index indicated a community composed primarily of autotrophs (Fig. 3-13).

Very few macroinvertebrates were observed in the flumes in 2014, and Chironmidae was the only family present with two or fewer individuals observed on every sampling occasion.

Discussion

2013

Although there were indications that the addition of P controlled or reduced aspects of the ecology of *D. geminata* in the Kootenai River, the excessive growths of other periphyton species (Fig. 3-2; Table 3-1) makes the concentrations (3, 5, and 8 µg P/L above ambient) tested unusable as a management strategy. Creating a community composed of mats of other diatoms and filamentous periphyton resembling a eutrophic system while trying to reduce the

mats of *D. geminata* replaces one problem with another and it is doubtful that fish would be able to access large invertebrates should they inhabit the replacement mats. It is possible that the growths observed in the experimental flumes were uncharacteristically high given the higher surface area to volume ratio of the confined flumes, however, *in situ*, the nutrients would be attenuated over a longer distance, exacerbating the amount of substrate covered by replacement mats. As well, the shading created by the significant growth of the other periphyton may have had confounding effects on other measured variables (see below).

The increase of total cells observed during the experiment (Fig. 3-4) suggests that growth of *D. geminata* can be stimulated outside of its typical peak seasonal cycle. The approximate 5-fold increase in cell density (Fig. 3-4) shows that even with the onset of the growth of other periphyton, *D. geminata* density also increased. However, once the addition of P stopped, cell counts rapidly declined to pre-treatment densities. This suggests that the addition of P during a short-term period would not increase mat severity of *D. geminata* via increased cell density. What was also interesting was the sloughing of the periphyton mat after the addition of P ended which greatly decreased cell density (Fig. 3-3). It is unclear if this was a result of ending the addition of P, or a cyclic event related to the abundance of the entire periphyton community that is common in lotic communities. For example, Stelzer and Lamberti (2001) reported sloughing of the periphyton community in their experiments with high nutrient concentrations around day 17, while Bothwell (M. Bothwell, personal communication, 2013) has also observed sloughing of periphyton communities in flume studies. Whether this was an effect of physical forces on the mat, or an effect of nutrients requires further examination.

The frequency of dividing cells (FDC) showed a short-term increase (12 May to 9 June 2013) corroborating the findings of Bothwell and Kilroy (2011) and Kilroy and Bothwell (2012) who reported a significant increase of FDC in their +P experiments over three weeks. However, I did not find a significant difference in FDC between phosphorus enriched flumes and the controls when the entire experimental period was considered. This lack of a difference between short and long-term time frames is notable, as management actions would be desired for long-term duration. The lack of a long-term response may be related to interaction effects of the large amount of other periphyton that accrued over the course of the experiment and introduced high variability starting on 7-Jul-2013 (Fig. 3-5). The higher FDC in treatments relative to controls early in the experiments suggests that the addition of P warrants further examination, especially at lower concentrations that would reduce the growth of other species.

The constant stalk length over time but clear difference between the shorter stalks in the P-addition vs. controls suggests that additional P above background suppresses stalk elongation. This finding is further supported by the increase in stalk length in the experimental flumes after the addition of P stopped (Fig. 3-6). The lack of a clear treatment concentration effect suggests that cell saturation occurred at or below 3 µg P/L and that lower concentrations should be investigated. The concentration must be sufficiently high to control *D. geminata*, but low to avoid the excessive growth of other periphyton species, which made this approach unrealistic. Bothwell (1988) found P saturation of periphyton at very low concentrations (<1 µg P/L above ambient). If this is also true for *D. geminata*, the addition of P may be possible as a strategy to control nuisance mats. What becomes problematic at this point is the accurate measurement of the added P concentration because of the methodological

difficulty of determining P at concentrations <1 µg/L. I recommend that in future studies, specific conductance is used as a tracer for P concentration (e.g., Jacoby et al. 1991).

During the treatment period, phosphorus enrichment led to large blooms of other algae (Table 3-1) which created significant shading in the flumes. Thus competition for light may have suppressed stalk elongation and FDC values. This experiment also ran during the summer when mat growth is naturally lower compared to the winter when *D. geminata* growth in the Kootenai River is usually at a maximum (Chapter 2). Thus, repeating this experiment during the height of *D. geminata* growth between December and April should provide additional insight on the effectiveness of suppressing stalk growth by adding P.

The presence of chironomids in the flumes was similar to that found by others conducting flume experiments (e.g., Kelly et al. 2001; Kelly et al. 2003) and probably reflects their small size and ability to colonize new substrates. Because chironomids are active on the substrate and drift readily, but swim poorly, they are easily transferred by the in-river pump to the flume system. Although Bothwell et al. (1994) reported significant grazing effects of chironomids after 17 to 22 days in experimental flumes in the Thompson River, their densities at approximately 25-250 ind./m^2 were higher than those in the flumes and probably reflect the difference in the overall trophic status of the two systems. Thus it is unlikely that the few chironomids that were observed in the flumes had any effect on the experimental outcomes.

Overall, I conclude that the experiment provided sufficient evidence that the addition of P can reduce FDC and suppress stalk elongation, but that concentrations of soluble P above 3 µg P/L were too high, and recommend additional P experiments with a range of

concentrations to pinpoint those that will saturate *D. geminata* but avoid the excessive growth of other periphyton.

2014

In 2014, seeding and growth of the *D. geminata* mat occurred during its peak growth season (January to February) in the Kootenai River. In 2013, *D. geminata* mats were relatively uniform in all flumes, while in 2014 mat growth was thicker in all flumes, but also occurred in especially thick patches. These extra thick "tufts" within the mat matrix increased the variability of the total cell counts, stalk length, and FDC. I attribute this unequal growth pattern to differences when accrual and cell activity is most robust, as similar patterns were observed *in situ* in the Kootenai River. While undertaking FDC and stalk length measurements, I noticed qualitatively that diatoms and fewer species of green algae dominated in the 0.5, 1.5, and 2 µg P/L treatments. Because of the effort involved in counting and accurately measuring stalk length in the cores with higher overall growth, I did not quantify the other diatoms or green algae.

Total cell counts increased over time but once P additions were stopped, cell counts decreased to those at the beginning of the study (Fig. 3-8). Total cell counts did not differ (Table 3-9) across treatments (addition of P), however, time was statistically significant (Table 3-9). The observed increase in total cell count was attributed to the age of the established colony and continued growth. The tufts which occurred during peak growth inhibited me from discerning any clear patterns in total cell count related to P treatments.

In 2014, FDC was equally reduced at all P concentrations and declined over the course of the experiment, while the FDCs in the controls was approximately 5 to 10× higher, which

was unexpected compared to previous work (Bothwell and Kilroy 2011). The high FDC in 2014 was probably related to the methodology used in the analysis of the cores under the compound microscope. Because cores in 2014 had much greater biomass compared to 2013, counts were limited to cells on top of the core which likely biased the counts high, as the most active cells were expected at the mat surface (Kilroy and Bothwell 2012). This suggests I may have underestimated the total cell count, while also obtaining a higher FDC count in the control flumes where the presence of other algal species was much less than in the experimental flumes. This result highlights the need for further research in the distribution of cells within mats. When the improbably high control data was removed, FDC increased in the first two weeks and then decreased over time (Fig. 3-14).

During the spring 2014 experiment the stalk length of *D. geminata* declined over time, suggesting that additional P above background applied early in the growing season reduced long-term mat growth. I attribute suppression of stalk length to cellular saturation with P, increased competition for space, and reduced light availability from other algal species, especially *Ulothrix* and *Mougeotia*. Given the lowest P concentration (0.5 µg/L) had suppressed stalk length similar to the highest (8 µg/L) P concentration used, I conclude that cellular saturation occurred even at the lowest P concentration (Table 3-14). This uniformity in *D. geminata* mat response to all P concentrations was also reflected in the 2014 FDC response.

Much like the other community variables analyzed, Chl-*a* increased with the increase of phosphorus (Dillon and Rigler 1974). All concentrations of P resulted in significant increases of Chl-*a* compared to the control (Table 3-18), however, the response (Chl-*a*) was

not incrementally higher with increasing P concentrations, suggesting a limited response to P additions and a larger change over time.

In 2014, the greatest visible physical change observed in the flumes was an extremely large increase in the biomass of other algal species. Surprisingly, large increases in algal biomass and diversity were observed qualitatively across all phosphorus concentrations even though light availability and temperatures may have been unfavorable. However, these increases of algal biomass were not at nuisance levels as indicated by the Chl-*a* concentrations (Fig. 3-12) being less than 100 mg m^{-2} as defined by Dodds et al. 1997. These species increased competition and shading to the *D. geminata* mats and also may have influenced FDC and stalk length by reducing light and substrate availability for *D. geminata*. These reduced habitat conditions, in conjunction with the suppressed stalk growth from the increase in phosphorus (Bothwell and Kilroy 2012), is what is attributed to the treatment effects observed at all concentrations of dissolved phosphorus. However, algal growth varied significantly from 2013.

The algal species that dominates in response to the P dosage is important if P enrichment is to be considered as a management strategy for *D. geminata* nuisance mats in the Kootenai River. Similar to 2013, the algal biomass at the three highest (3, 5, and 8 µg/L) concentrations was similar and dominated by filamentous green species. In contrast, the three lowest (0.5, 1.5, and 2 µg/L) concentrations were dominated by mat forming diatoms and some filamentous green species. It was beyond the scope of this study to examine whole community responses given the slow response of invertebrates other than chironomids, but this will be an important consideration if applied *in situ*. If the transfer of energy via diatoms

stimulated by low concentrations of P is efficient, then additional growth of biomass may be tolerable in the face of suppressing *D. geminat*a in the Kootenai River.

The response of ash free dry mass did not differ across treatments (P additions), but increased significantly over time (Table 3-15). This suggests that environmental variables such as light availability and length of colony establishment played a larger role in AFDM than nutrient additions. Because Chl-*a* also increased over time, the autotrophic index decreased from 20 Feb to 6 Mar but then remained between 200 and 50 for all treatments for the rest of the study (Fig. 3-13), suggesting the system was dominated by autotrophs.

While adding a concentration of 0.5 µg/L of dissolved P above ambient would be considered an insignificant amount in most lotic systems across the Pacific northwest, the average soluble reactive phosphorus concentration in the Kootenai River near the Libby Dam ranges from <0.5 to 1 µg/L below the Libby Dam sampled by the Kootenai Tribe of Idaho (KTOI 2014). Therefore, an addition of 0.5 µg/L increases the SRP between 50 to 100%. Given Bothwell (1988) demonstrated that growth rate saturation of lotic periphyton occurs at extremely low P concentrations of 0.3 to 0.6 µg/L, I conclude that *D. geminata* cells were P saturated in the very low (0.5 µg/L) P treatment. While the 0.5 µg P/L reduced *D. geminata* mat formation, it also removed the phosphorus limitation for other algal species, as evidenced by the increase in the AFDM (Fig. 3-11). Thus it appears that any addition of P to control *D. geminata* results in an increase in the biomass of other periphyton.

Similar to 2013, few invertebrates were observed in the flumes leading to the conclusion that experimental results were not significantly influenced by grazing as has been reported in other studies (e.g., Bothwell et al. 1994). I attribute the reduction in the abundance

of chironomids in 2014 compared to 2013 to the colder water temperature and earlier part of the growing cycle during which the experiments were started.

Overall, I consider the 2014 experiment a success, showing that P saturation of *D. geminata* occurred at or below 0.5 µg P/L above ambient. While the high proportion of frequency of dividing cells in the controls was likely an artefact of the counting method given the cell density in the mats, the reduction among the P-treatments was likely influenced by the dense growths of other species. The reduction in stalk length suggests that early application of additional P in the Kootenai River should reduce the severity (depth) of the *D. geminata* mat during its typical peak growing season.

Conclusion

The long-term nature of the experiments highlights important aspects of such endeavors and the applicability of results to larger-scale systems. First, I recognize that the limited size of the flumes means that they are susceptible to influences related to uncharacteristically high surface area:volume ratios given the relatively confined space. However, *D. geminata* mats grown in the flumes never exceed those in the river and likely were somewhat shallower and shorter (see Chapter 2). Thus I am confident that the results presented here can be extrapolated to larger-scale systems. Although Bothwell and Kilroy (2011) and Kilroy and Bothwell (2011, 2012, 2014) used nearly identical flume systems, they rarely allowed significant accrual of *D. geminata* on the substrate, and experiments lasted for 3 to 21 days. I believe such short seeding and experimental times result in possible spurious conclusions. For example, when I examined the response of stalk length over the first 6 weeks in 2013, I found that only one treatment differed from the controls, while at the end of 3

months I found that all treatments differed from the controls. Thus I remain an advocate for using long-term experiments timed to occur at the peak seasonal growth of the local *D. geminata* mats to derive results most reflective of *in situ* conditions.

All studies at the MEFS showed that the stalk length of *D. geminata* was suppressed in the presence of P above the ambient in-river concentration. An addition of 0.5 µg P/L above ambient was sufficient to saturate *D. geminata* cells and resulted in reduced stalk lengths. Thus I conclude that the formation of nuisance mats is driven by P limitation. This P limitation may be related and exacerbated by the increase in N concentration observed downstream of Libby dam over the last 15-20 years (see Chapter 4). The addition of P did not result in greater nuisance mats once additions stopped, because sloughing of the mats or mat decomposition occurred in fall. The flume experiments suggest that increasing the availability of P in the Kootenai River could be a promising management strategy to reduce nuisance mat coverage below the Libby Dam. However, this must be tempered by the growths of additional species that occurred in the flumes and which may detract from such a strategy at the river-scale. The energy transfer and benefit of the diatom species observed at the lower concentrations should be investigated further, as some additional growth of biomass may be a net benefit to the ecosystem if it occurs in the form of diatoms that are easily grazed.

The response of the stalk length and FDC to the phosphorus treatments has provided further insight to the biology of *D. geminata* while supporting previous studies that investigated the relationship between phosphorus and *D. geminata* (Bothwell and Kilroy 2011; Kilroy and Bothwell 2011, 2012). Management strategies to control or suppress large-scale *Didymosphenia geminata* nuisance mats are not only rare but are currently limited to biocides (Jellyman et al. 2011), P-enrichment (James et al. 2015), chemical compounds

(Clearwater et al. 2011), and mechanical removal, all of which, except P-enrichment, are unsuitable for the Kootenai River system due to its size and the presence of highly desirable fish species, or fish species listed under the US Endangered Species Act of 1973. The results from this study have provided insight into alternative methods to reduce nuisance mats. If a phosphorus treatment was initiated at the proper time (post-peak accrual season), suppression of mat growth or a forced sloughing event could then have positive lasting effects for the rest of the year.

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Tables

Table 3-1. Algal species present among three phosphorus treatments (3, 5, and 8 µg P/L) and control at the Kootenai River Research Station in Libby, MT, USA, from 12 May 2013 to 21 July 2013. Dominant species are indicated in bold.

P Treatment	Algal Present	5/12/2013	5/26/2013	6/9/2013	6/23/2013	7/7/2013	7/21/2013
3 µg P/L							
	Achnanthidium/Achnanthes	X	X	X	X	X	X
	Asterionella	X	X	X	X	X	X
	Cymbella	X	X	X	X	X	X
	Diatoma	X					
	Encyonema		X	X	X	X	X
	Fragilaria	X	X	X	X	X	X
	Gomphonema	X		X	X	X	X
	Nitzschia	X	X				X
	Synedra	X	X	X	X	X	X
	Tabellaria	X	X	X	X	X	X
	Geminella			X	X	X	
	Microspora			X	X	X	
	Mougeotia			X	X	X	X
	Oedogonium				X	X	X
	Spirogyra			X	X		X
	Stigeoclonium						
	Ulothrix			X	X	X	X
	Zygnema	X	X	X	X	X	X
5 µg P/L							
	Achnanthidium/Achnanthes	X	X	X	X	X	X
	Asterionella	X		X	X	X	X

Table 3-1 Cont.

	Cymbella	X		X	X	X	
	Diatoma						
	Encyonema		X	X	X		X
	Fragilaria	X	X	X	X	X	X
	Gomphonema	X		X	X	X	X
	Nitzschia	X		X			X
	Synedra	X	X	X	X		X
	Tabellaria	X	X	X	X	X	X
	Geminella			X	X		
	Microspora				X		
	Mougeotia	X	X	X	X	X	X
	Oedogonium						
	Spirogyra			X			
	Stigeoclonium						
	Ulothrix		X	X	X	X	
	Zygnema	X			X	X	
8 µg P/L							
	Achnanthidium/Achnanthes	X	X	X	X	X	X
	Asterionella	X		X	X	X	X
	Cymbella	X	X	X	X		X
	Diatoma						
	Encyonema			X	X	X	X
	Fragilaria	X		X	X	X	X
	Gomphonema	X	X		X	X	X
	Nitzschia			X			X
	Synedra			X	X		X
	Tabellaria	X		X	X		X
	Geminella			X	X		

Table 3-1 Cont.

	Microspora			X	X		
	Mougeotia		X	X			X
	Oedogonium				X		
	Spirogyra			X			X
	Stigeoclonium						
	Ulothrix		X	X	X		X
	Zygnema	X	X	X	X		X
Control							
	Achnanthidium/Achnanthes	X	X	X	X	X	X
	Asterionella	X	X	X	X		X
	Cymbella	X	X	X	X	X	X
	Diatoma			X			
	Encyonema		X	X	X	X	X
	Fragilaria		X	X	X	X	X
	Gomphonema	X	X	X	X		
	Nitzschia	X	X	X			
	Synedra	X	X	X	X		X
	Tabellaria	X	X	X	X	X	X
	Geminella			X	X		
	Microspora						
	Mougeotia	X	X	X	X	X	X
	Oedogonium						
	Spirogyra					X	X
	Stigeoclonium						
	Ulothrix						
	Zygnema	X	X	X	X	X	X

Table 3-2. Repeated measures ANOVA for *Didymosphenia geminata* total cell counts in different treatment nutrient concentrations (3, 5, and 8 µg P/L) including a control (river water only) at the Kootenai River Research Station in Libby, MT, USA, from 29 April 2013 to 21 July 2013.

Effect	*F* value	*P*
Phosphorus	0.65	0.595
Time	7.72	<0.001
Time × Phosphorus	2.02	0.029

Table 3-3. *Post-hoc* pair-wise comparison for *Didymosphenia geminata* total cell counts for three phosphorus treatments (3, 5, and 8 µg P/L) and control (river water only) at the Kootenai River Research Station in Libby, MT, USA, from 29 April 2013 to 21 July 2013. Treatment was analyzed in a short-term response (first six weeks - 29 April to 9 June) and long-term response (entire duration of P addition from 29 April to 21 July) for comparison to previous *D. geminata* mesocosm studies (Bothwell and Kilroy 2011).

Date	P concentration (µg/L)	0	3	5
29 April to 9 June	3	0.243		
	5	0.411	0.715	
	8	0.647	0.116	0.211
29 April to 21 July	3	0.546		
	5	0.743	0.780	
	8	0.204	0.484	0.333

Table 3-4. Repeated measures ANOVA for *Didymosphenia geminata* frequency of dividing cells (FDC) in different treatment nutrient concentrations (3, 5, and 8 µg P/L) including a control (no nutrient addition) at the Kootenai River Research Station in Libby, MT, USA, from 29 April 2013 to 21 July 2013.

Effect	*F* value	*P*
Phosphorus	0.95	0.447
Time	4.68	0.001
Time × Phosphorus	0.74	0.731

Table 3-5. *Post-hoc* pair-wise comparison for frequency of dividing cells (FDC) for three phosphorus treatments (3, 5, and 8 µg P/L) and control (no nutrient addition) at the Kootenai River Research Station in Libby, MT, USA, from 29 April 2013 to 21 July 2013. Treatment was analyzed in a short-term response (first six weeks - 29 April to 9 June) and long-term response (entire duration of P addition from 29 April to 21 July) for comparison to previous *D. geminata* mesocosm studies (Bothwell and Kilroy 2011).

Date	P concentration (µg/L)	0	3	5
29 April to 9 June	3	0.016		
	5	0.067	0.444	
	8	0.051	0.532	0.885
29 April to 21 July	3	0.133		
	5	0.413	0.460	
	8	0.688	0.253	0.670

Table 3-6. Repeated measures ANOVA for *Didymosphenia geminata* stalk length in different treatment nutrient concentrations (3, 5, and 8 µg P /L) including a control (no nutrient addition) at the Kootenai River Research Station in Libby, MT, USA, from 29 April 2013 to 21 July 2013.

Effect	*F* value	*P*
Phosphorus	7.26	0.005
Time	1.01	0.422
Time × Phosphorus	1.67	0.083

Table 3-7. *Post-hoc* pair-wise comparison for stalk length among three phosphorus treatments (3, 5, and 8 µg P/L) and control at the Kootenai River Research Station in Libby, MT, USA, from 29 April 2013 to 21 July 2013. Treatment was analyzed in a short-term response (first six weeks - 29 April to 9 June) and long-term response (entire P addition duration 29 April to 21 July) for comparison to previous *D. geminata* mesocosm studies (Kilroy and Bothwell 2011).

Date	P concentration (µg/L)	0	3	5
29 April to 9 June	3	0.096		
	5	0.039	0.617	
	8	0.095	0.994	0.622
29 April to 21 July	3	0.019		
	5	0.002	0.267	
	8	0.001	0.168	0.768

Table 3-8. Macroinvertebrate individuals per cm^2 present among three phosphorus treatments (3, 5, and 8 µg P/L) and a control (no P addition) at the Kootenai River Research Station in Libby, MT, USA, from 12 May 2013 to 21 July 2013.

P Treatment	Species Present	5/12/2013	5/26/2013	6/9/2013	6/23/2013	7/7/2013	7/21/2013
Control							
	Chironomidae						0.12
	Oligochaeta						
	Ephemeroptera						
	Dipetera						
3 µg P/L							
	Chironomidae			0.12	0.12		0.166
	Oligochaeta						
	Ephemeroptera						
	Dipetera						
5 µg P/L							
	Chironomidae		0.06			0.35	0.35
	Oligochaeta						
	Ephemeroptera						
	Dipetera		0.06				
8 µg P/L							
	Chironomidae		0.12		0.12	0.12	0.23
	Oligochaeta		0.23				
	Ephemeroptera						0.12
	Dipetera		0.12			0.23	

Table 3-9. Repeated measures ANOVA for total cell counts of *Didymosphenia geminata* in different treatments of phosphorus concentrations (0.5, 1.5, 2, 3, 5, and 8 µg P/L) including a control (no P addition) at the Kootenai River Research Station in Libby, MT, USA, from 6 February 2014 to 17 April 2014.

Effect	*F* value	*P*
Phosphorus	1.44	0.241
Time	6.46	0.001
Time × Phosphorus	0.99	0.484

Table 3-10. *Post-hoc* pair-wise comparison for total cell counts of *Didymosphenia geminata* among six phosphorus treatments (0.5, 1.5, 2, 3, 5, and 8 µg P/L) of phosphorus treatment and a control (no P addition) at the Kootenai River Research Station in Libby, MT, USA, from 6 February 2014 to 17 April 2014.

P concentration (µg/L)	0	0.5	1.5	2	3	5
0.5	0.784					
1.5	0.248	0.207				
2	0.471	0.340	0.692			
3	0.122	0.108	0.713	0.447		
5	0.283	0.473	0.054	0.118	0.025	
8	0.337	0.277	0.857	0.829	0.584	0.078

Table 3-11. Repeated measures ANOVA for frequency of dividing cells (FDC) of *Didymosphenia geminata* in different treatments of phosphorus concentrations (0.5, 1.5, 2, 3, 5, and 8 µg P/L) including a control (no P addition) at the Kootenai River Research Station in Libby, MT, USA, from 6 February 2014 to 17 April 2014.

Effect	*F* value	*P*
Phosphorus	6.52	<0.001
Time	1.56	0.003
Time × Phosphorus	2.53	<0.001

Table 3-12. *Post-hoc* pair-wise comparison for frequency of dividing cells of *Didymosphenia geminata* among six phosphorus treatments (0.5, 1.5, 2, 3, 5, and 8 µg P/L) of phosphorus treatment and control (no P addition) at the Kootenai River Research Station in Libby, MT, USA, from 6 February 2014 to 17 April 2014.

P concentration (µg/L)	0	0.5	1.5	2	3	5
0.5	<0.001					
1.5	<0.001	0.360				
2	<0.001	0.338	0.965			
3	<0.001	0.362	0.998	0.963		
5	<0.001	0.563	0.732	0.699	0.734	
8	<0.001	0.190	0.680	0.712	0.678	0.452

Table 3-13. Repeated measures ANOVA for stalk length of *Didymosphenia geminata* in different treatments of phosphorus concentrations (0.5, 1.5, 2, 3, 5, and 8 µg P/L) including a control (no P addition) at the Kootenai River Research Station in Libby, MT, USA, from 6 February 2014 to 17 April 2014.

Effect	*F* value	*P*
Phosphorus	6.40	0.009
Time	14.31	<0.001
Time × Phosphorus	3.25	<0.001

Table 3-14. *Post-hoc* pair-wise comparison for stalk length of *Didymosphenia geminata* among six phosphorus treatments (0.5, 1.5, 2, 3, 5, and 8 µg P/L) of phosphorus treatment and a control (no P addition) located at the Kootenai River Research Station in Libby, MT, USA, from 6 February 2014 to 17 April 2014.

P concentration (µg/L)	0	0.5	1.5	2	3	5
0.5	0.004					
1.5	0.001	0.576				
2	0.001	0.543	0.960			
3	0.001	0.589	0.985	0.945		
5	0.002	0.848	0.712	0.675	0.726	
8	0.002	0.689	0.873	0.834	0.888	0.834

Table 3-15. Repeated measures ANOVA for ash-free dry mass among six phosphorus treatments (0.5, 1.5, 2, 3, 5, and 8 µg P/L) including a control (no P addition) at the Kootenai River Research Station in Libby, MT, USA, from 6 February 2014 to 17 April 2014.

Effect	*F* value	*P*
Phosphorus	0.86	0.538
Time	12.18	<0.001
Time × Phosphorus	1.15	0.316

Table 3-16. Ash free dry mass pair-wise comparison across all treatments. *Post-hoc* pair-wise comparison for ash free dry mass among six phosphorus treatments (0.5, 1.5, 2, 3, 5, and 8 µg P/L) including a control (no P addition) at the Kootenai River Research Station in Libby, MT, USA, from 6 February 2014 to 17 April 2014.

P concentration (µg/L)	0	0.5	1.5	2	3	5
0.5	0.970					
1.5	0.466	0.470				
2	0.446	0.449	0.981			
3	0.666	0.680	0.739	0.738		
5	0.070	0.064	0.317	0.269	0.156	
8	0.707	0.723	0.708	0.705	0.960	0.148

Table 3-17. Repeated measures ANOVA for chlorophyll-*a* among six phosphorus concentrations (0.5, 1.5, 2, 3, 5, and 8 µg P/L) and control (no P addition) at the Kootenai River Research Station in Libby, MT, USA, from 6 February 2014 to 17 April 2014.

Effect	*F* value	*P*
Phosphorus	10.88	<0.001
Time	42.13	<0.001
Time × Phosphorus	1.43	0.121

Table 3-18. *Post-hoc* chlorophyll-*a* pair-wise comparison among six phosphorus treatments (0.5, 1.5, 2, 3, 5, and 8 µg P/L) of phosphorus treatment and control (no P addition) at the Kootenai River Research Station in Libby, MT, USA, from 6 February 2014 to 17 April 2014.

P concentration (µg/L)	0	0.5	1.5	2	3	5
0.5	0.001					
1.5	<0.001	0.002				
2	<0.001	0.297	0.022			
3	<0.001	0.110	0.102	0.519		
5	<0.001	0.020	0.300	0.166	0.489	
8	<0.001	0.004	0.603	0.048	0.209	0.560

Figures

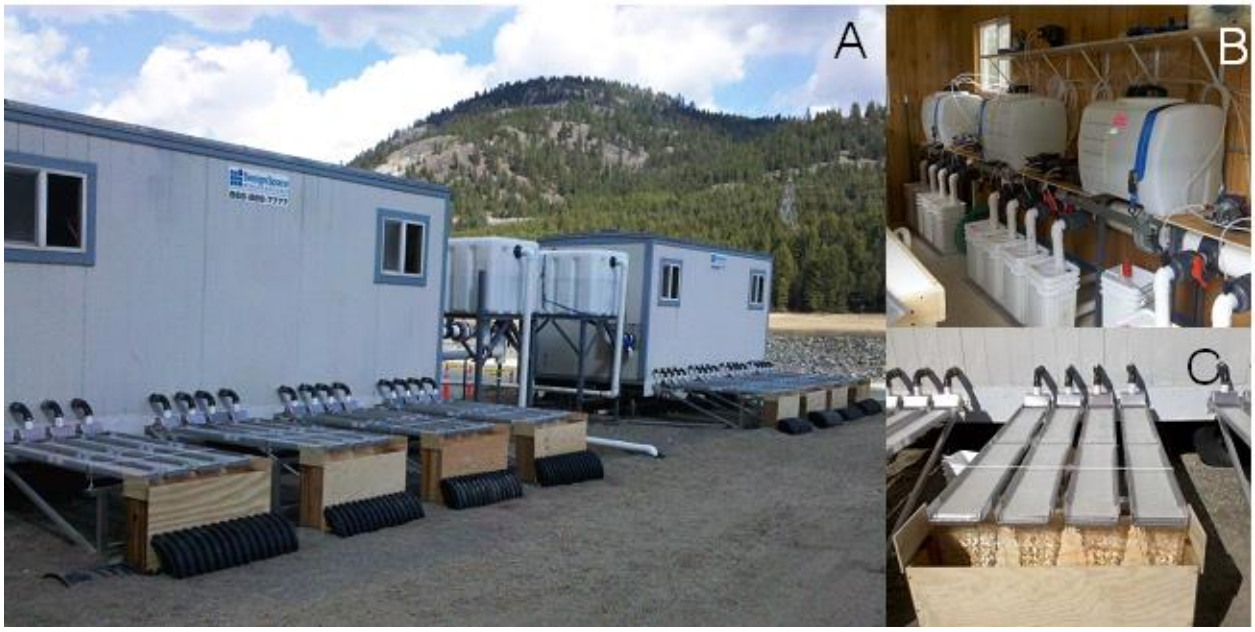

Figure 3-1. Mobile experimental flume system (MEFS) at the Kootenai River Research Station downstream of Libby Dam in Libby, MT, USA. **A**) Mobile experimental flume system (MEFS) with 32 external flumes supplied by water from the Kootenai River. **B**) Internal structure of MEFS with 120 L stock tanks on shelving and mixing header tanks for individual flumes below. **C**) External flumes of the MEFS. Flumes were lined with 6 mm open-celled Styrofoam for substrate.

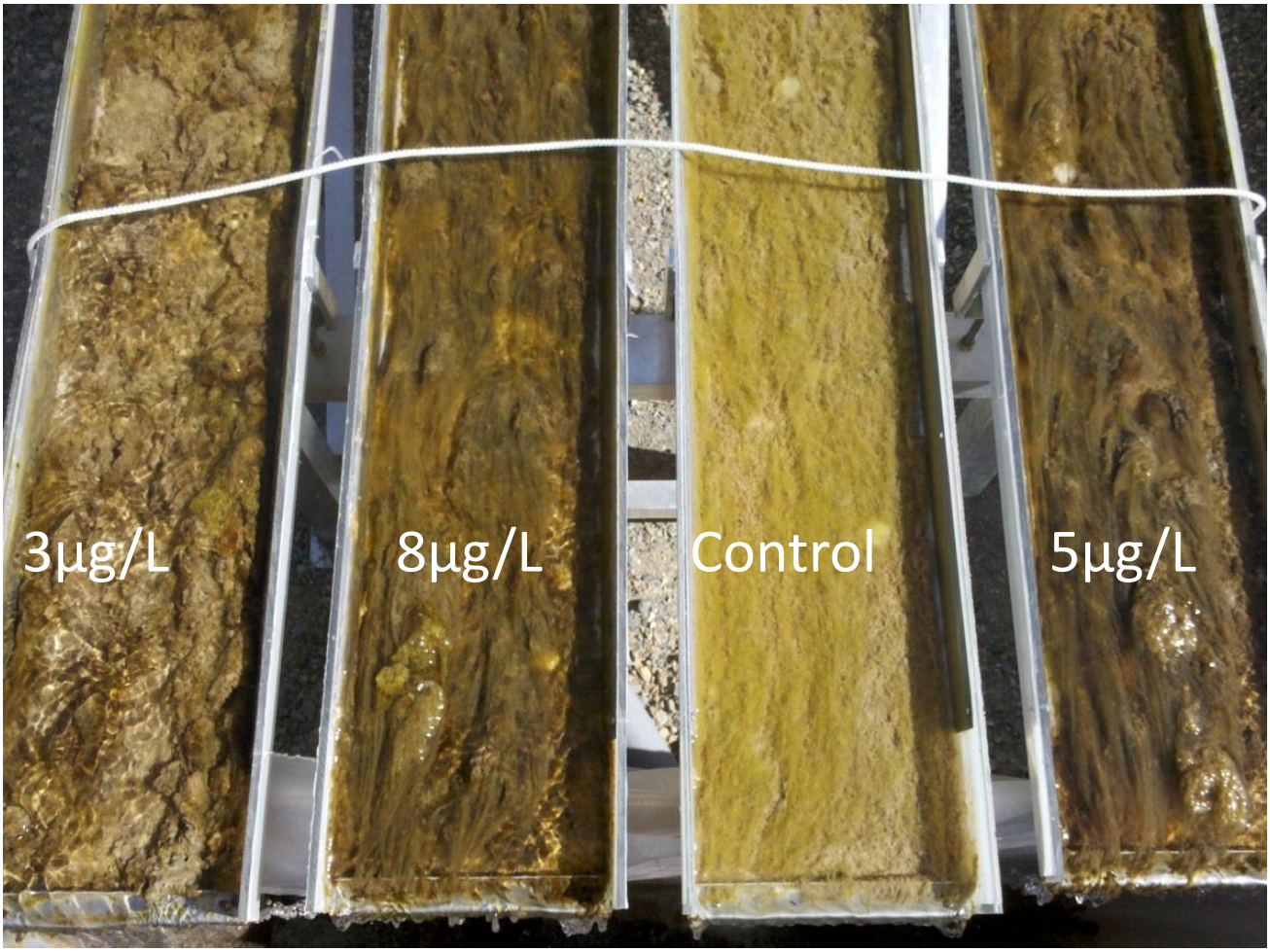

Figure 3-2. Growth of filamentous green algae and other diatoms in four experimental flumes which dominated all flumes except controls in which *Didymosphenia geminata* dominated during the 2013 P addition experiment at the Kootenai River Research Station in Libby, MT, USA. Picture taken on 16 July 2013.

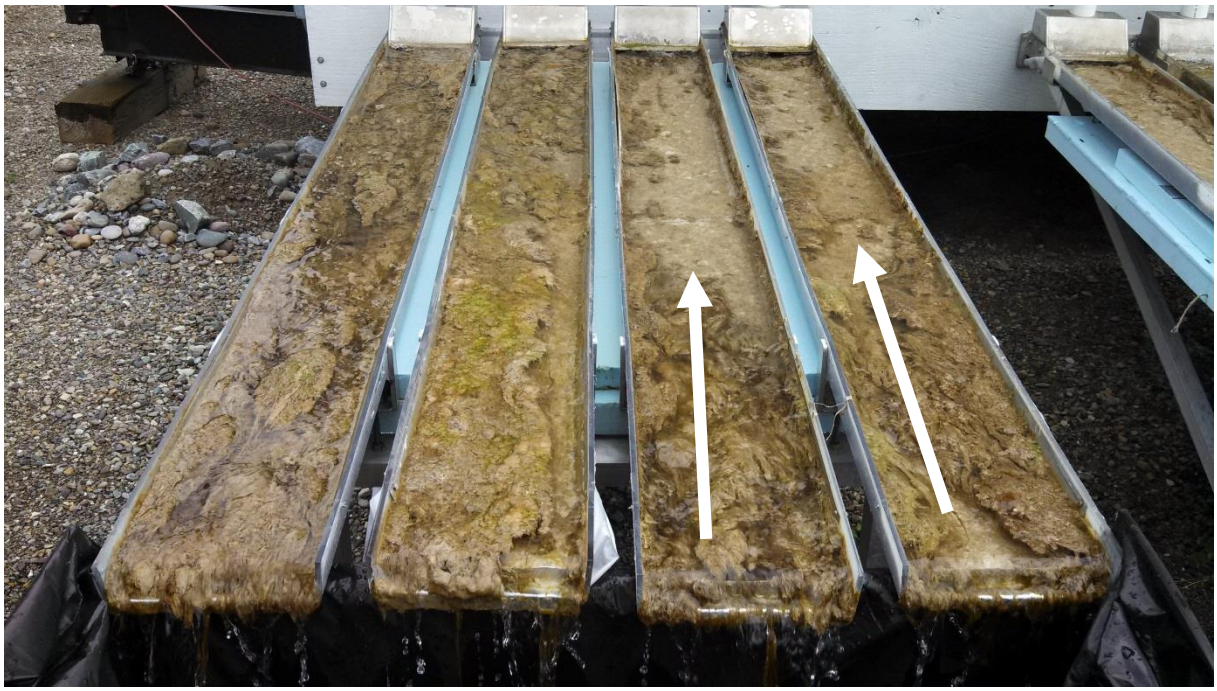

Figure 3-3. Sloughing of algae groups within the University of Idaho mobile experimental flume system (MEFS) after phosphorus addition ended. Located at the Kootenai River Research Station in Libby, MT, USA. The addition of phosphorus started on 29 April 2013 and ended on 21 July 2013.

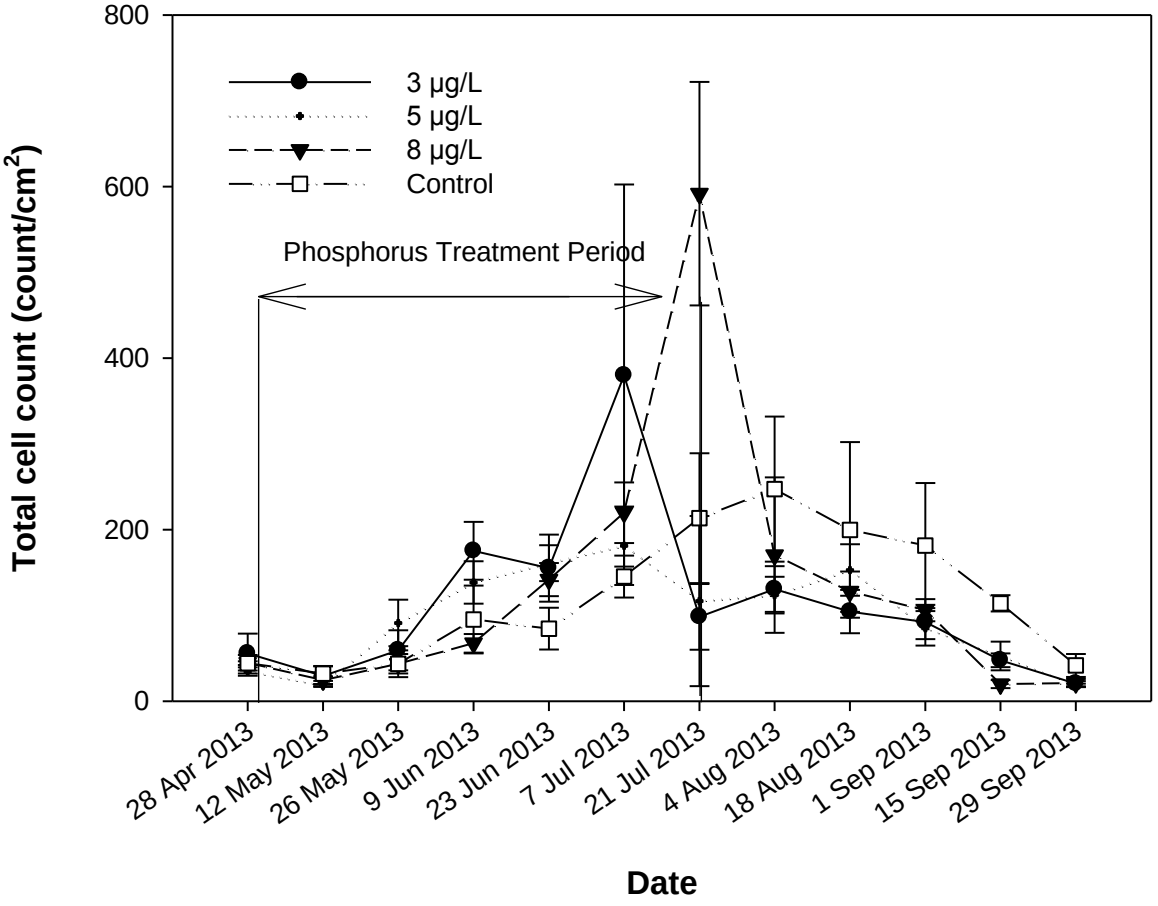

Figure 3-4. Total number of cells per cm^2 (mean±S.E.) of *Didymosphenia geminata* (means±S.E.) for the period 28 April 2013 to 29 September 2013 for three phosphorus concentrations (3, 5, and 8 µg P/L) above ambient and a control (no P addition) at the University of Idaho mobile experimental flume system (MEFS) located at the Kootenai River Research Station in Libby, MT, USA. The addition of phosphorus started on 29 April 2013 and ended on 21 July 2013.

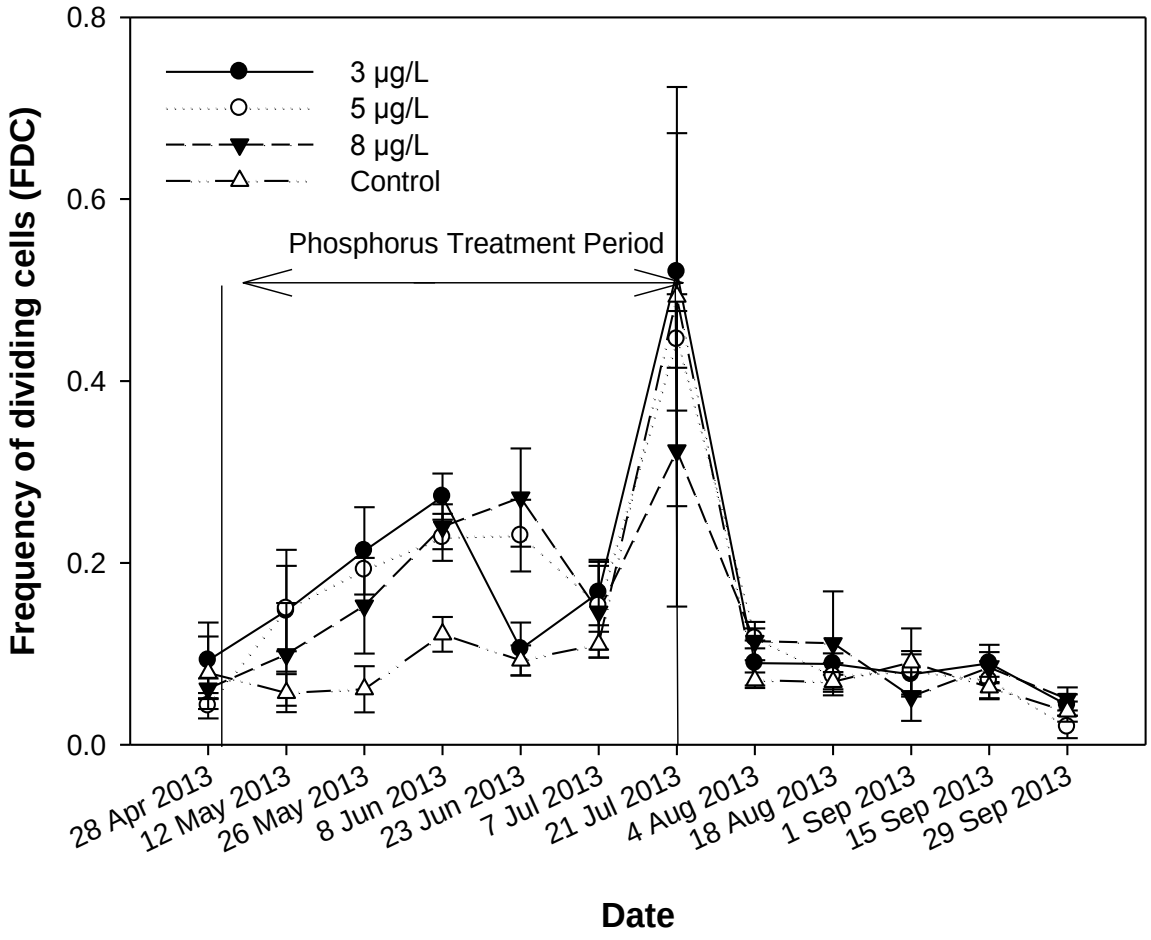

Figure 3-5. Frequency of dividing cells (mean±S.E.) of *Didymosphenia geminata* as a function of time for the period 28 April 2013 to 29 September 2013 for three phosphorus concentrations (3, 5, and 8 µg P/L) above ambient and a control (no P addition) at the University of Idaho mobile experimental flume system (MEFS) located at the Kootenai River Research Station in Libby, MT, USA. The addition of phosphorus started on 29 April 2013 and ended on 21 July 2013.

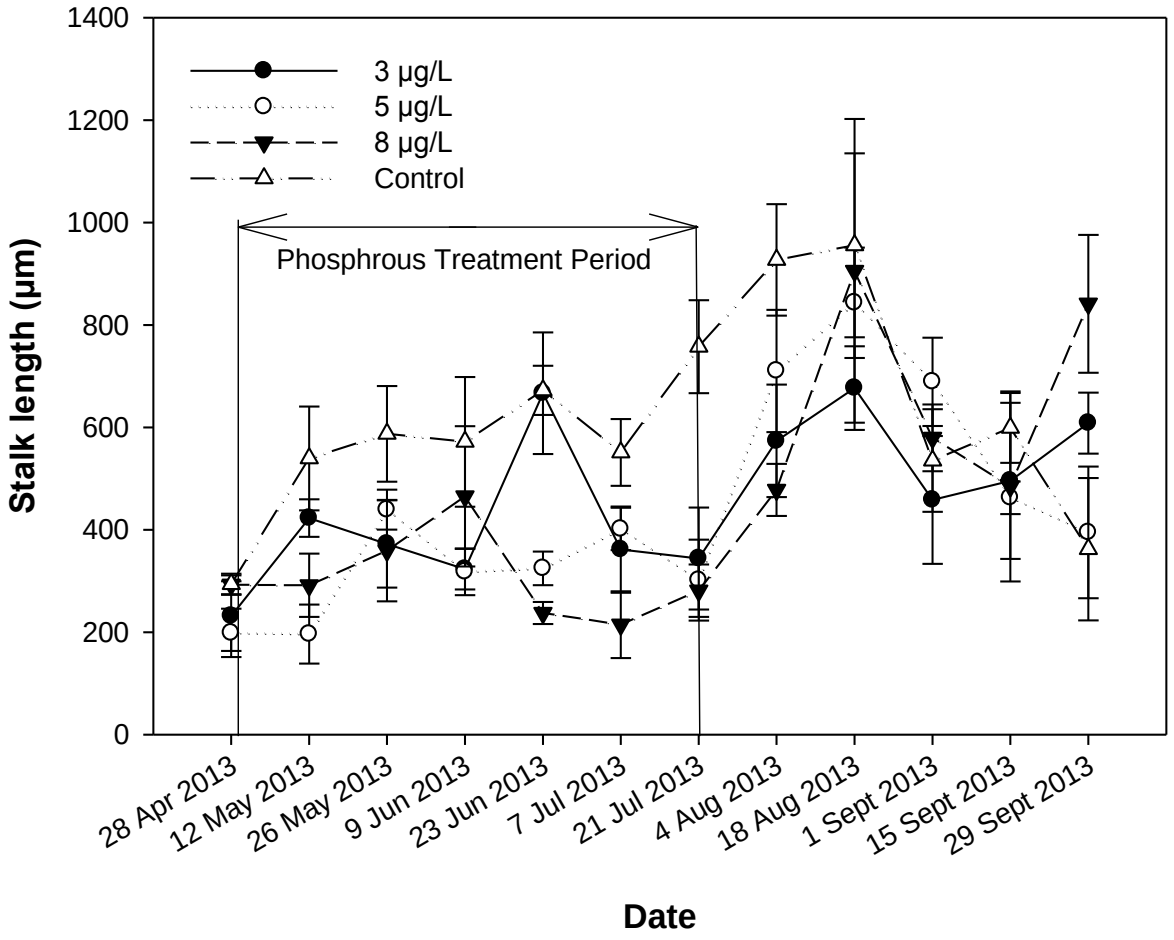

Figure 3-6. *Didymosphenia geminata* stalk length averages as a function of time in 2013 for three concentrations (3, 5, and 8 µg/L) of phosphorus addition and a control at the mobile experimental flume system (MEFS) located in Libby, MT, USA, from 28 April 2013 to 29 September 2013. Data of 28 April 2013 is pre-treatment levels with phosphorus treatment beginning 29 April 2013.

Figure 3-7. Comparison of *Didymosphenia geminata* coverage after 37 days of seeding in flumes 1 through 4 in March 2013 and January 2014 at the Kootenai River Research Station in Libby, MT, USA.

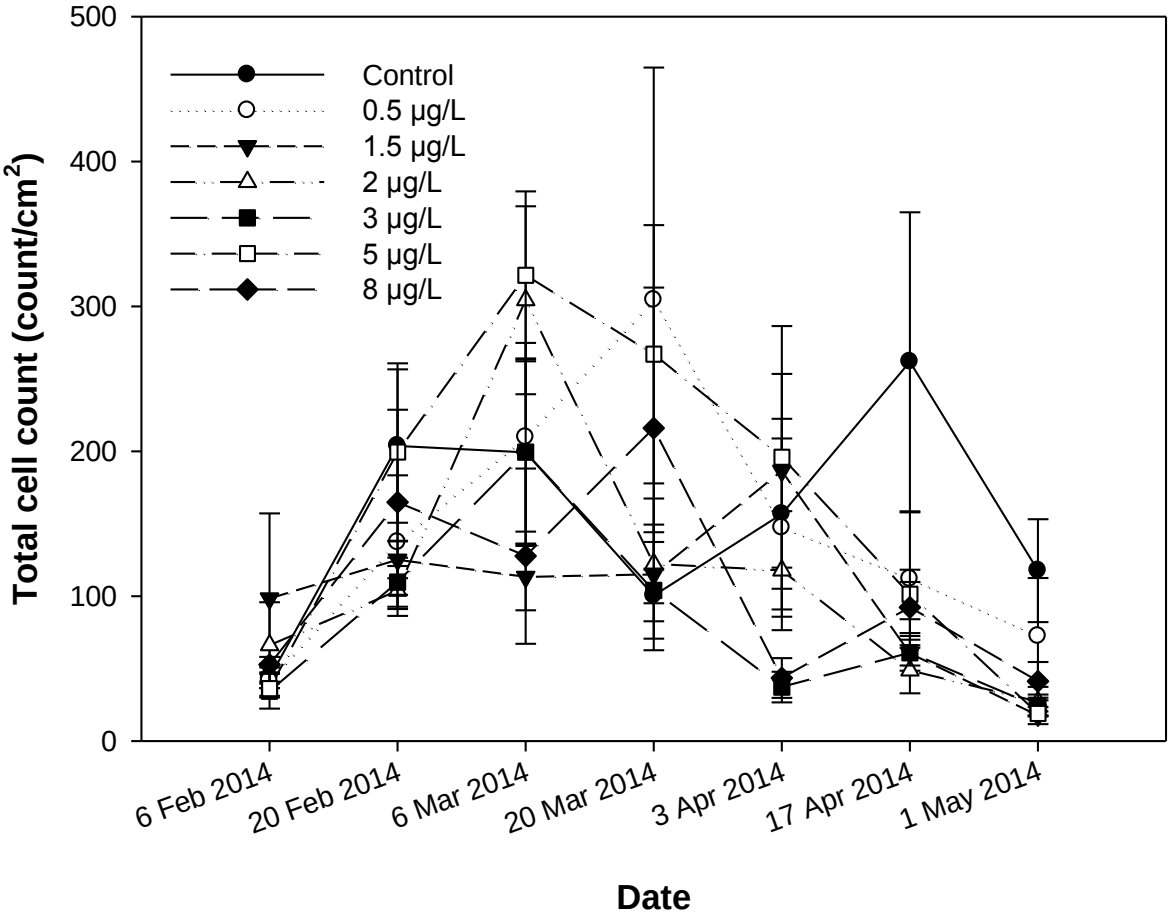

Figure 3-8. Total cell count per cm^2 (mean±S.E.) of *Didymosphenia geminata* as a function of time for the period 6 February 2014 to 1 May 2014 for six phosphorus concentrations (0.5, 1.5, 2, 3, 5, and 8 µg P/L) and a control (no P addition) at the University of Idaho mobile experimental flume system located at the Kootenai River Research Station in Libby, MT, USA. Data on 6 February 2014 and 1 May 2014 represent pre- and post-treatment samples, respectively.

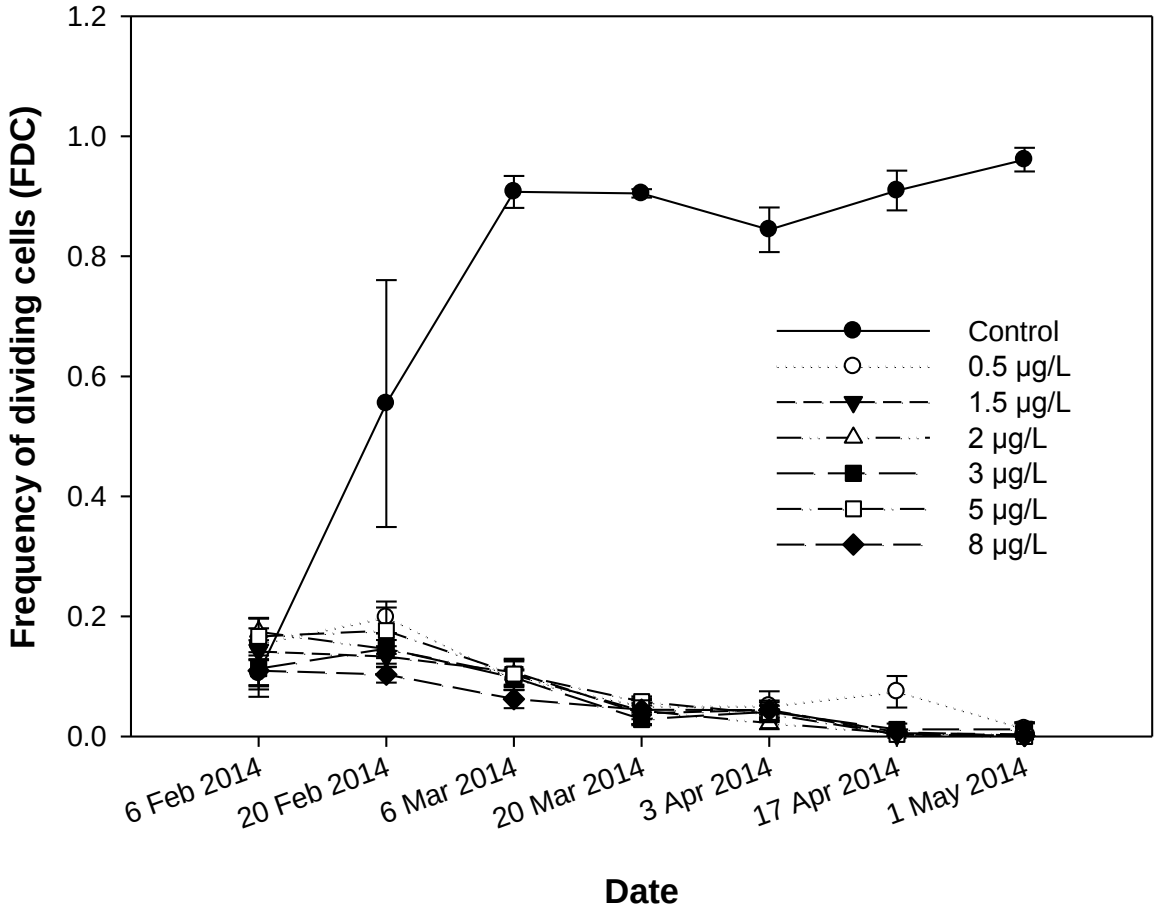

Figure 3-9. Frequency of dividing cells (mean±S.E.) of *Didymosphenia geminata* as a function of time for the period 6 February 2014 to 1 May 2014 for six phosphorus concentrations (0.5, 1.5, 2, 3, 5, and 8 µg P/L) and a control (no P addition) at the University of Idaho mobile experimental flume system located at the Kootenai River Research Station in Libby, MT, USA. Data on 6 February 2014 and 1 May 2014 represent pre- and post-treatment samples, respectively.

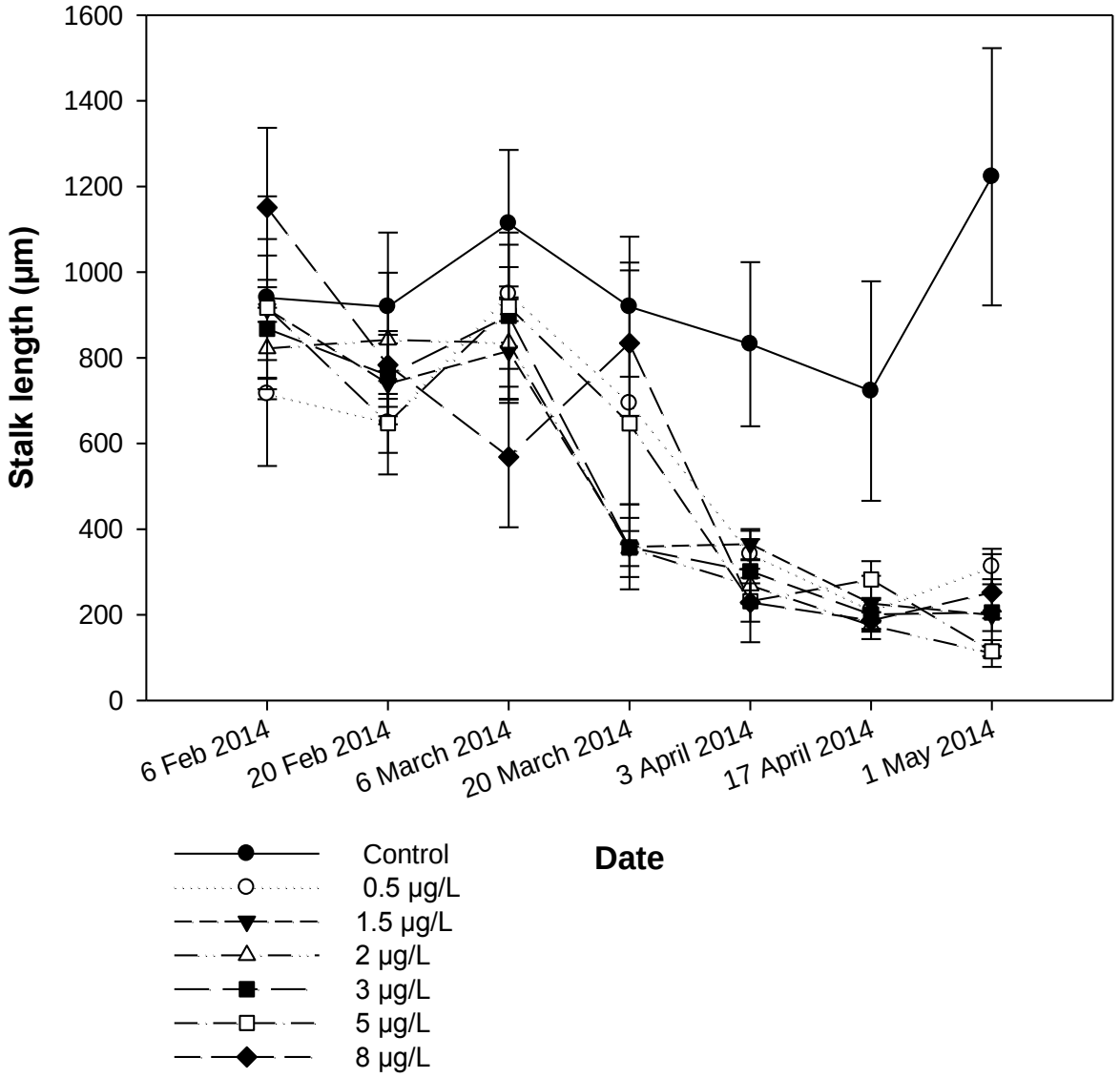

Figure 3-10. Stalk length (mean±S.E.) of *Didymosphenia geminata* as a function of time for the period 6 February 2014 to 1 May 2014 for six phosphorus concentrations (0.5, 1.5, 2, 3, 5, and 8 µg P/L) and a control (no P addition) at the University of Idaho mobile experimental flume system located at the Kootenai River Research Station in Libby, MT, USA. Data on 6 February 2014 and 1 May 2014 represent pre- and post-treatment samples, respectively.

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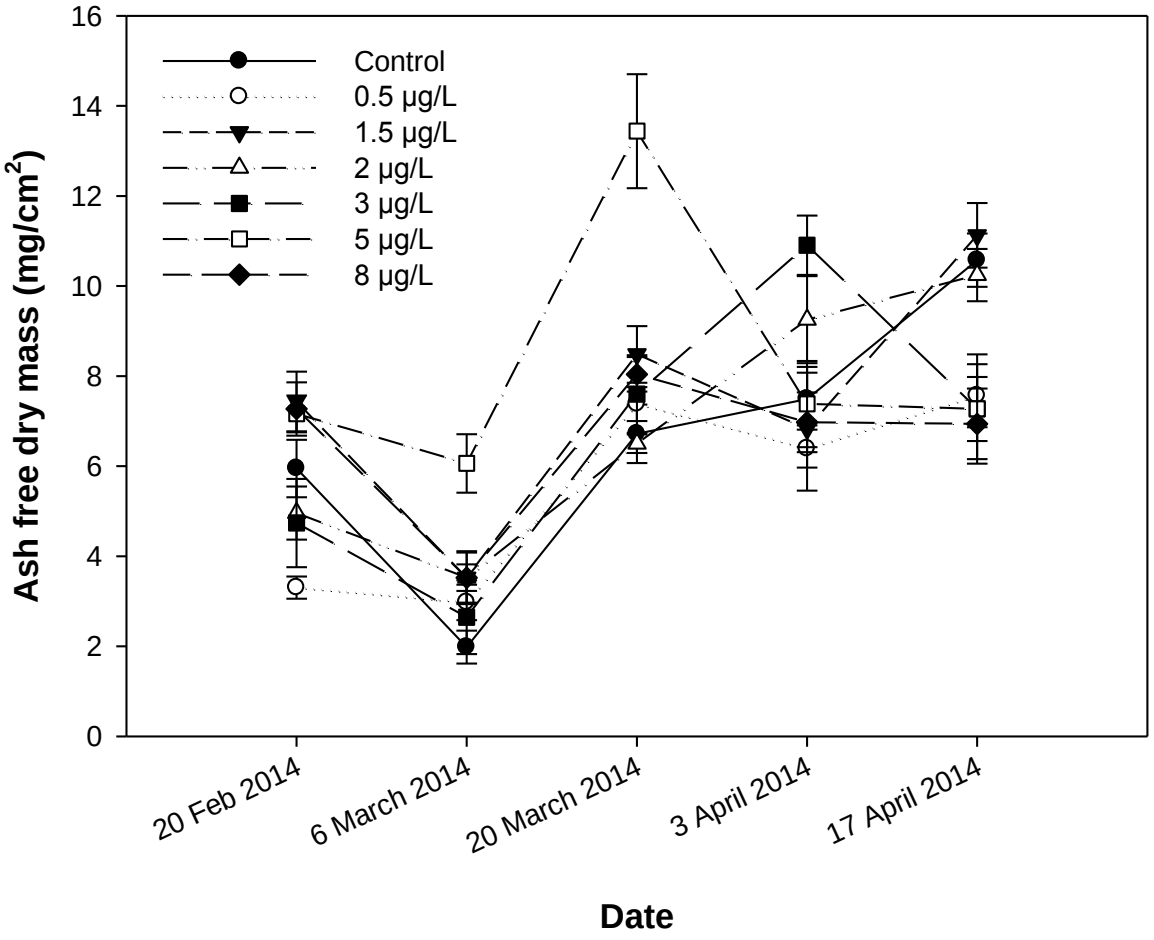

Figure 3-11. Ash free dry mass AFDM, (mean±S.E.) of *Didymosphenia geminata* as a function of time for the period 20 Feb 2014 to 17 April 2014 for six phosphorus concentrations (0.5, 1.5, 2, 3, 5, and 8 µg P/L) and a control (no P addition) at the University of Idaho mobile experimental flume system located at the Kootenai River Research Station in Libby, MT, USA.

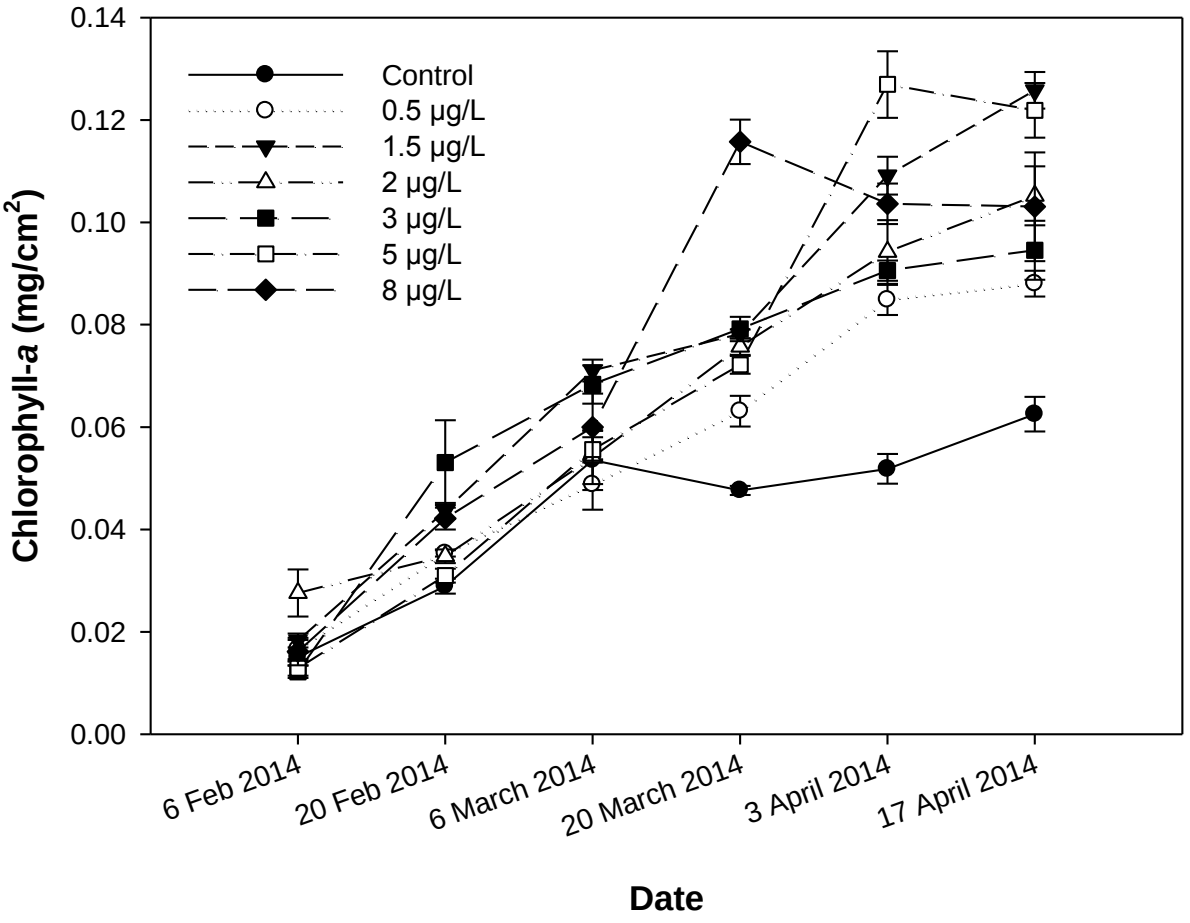

Figure 3-12. The concentration of chlorophyll-*a* (Chl-*a*, mean±S.E.) as a function of time for the period 6 February 2014 to 17 April 2014 for six phosphorus concentrations (0.5, 1.5, 2, 3, 5, and 8 µg P/L) and a control (no P addition) at the University of Idaho mobile experimental flume system located at the Kootenai River Research Station in Libby, MT, USA. Data on 6 February 2014 represent pre-treatment samples.

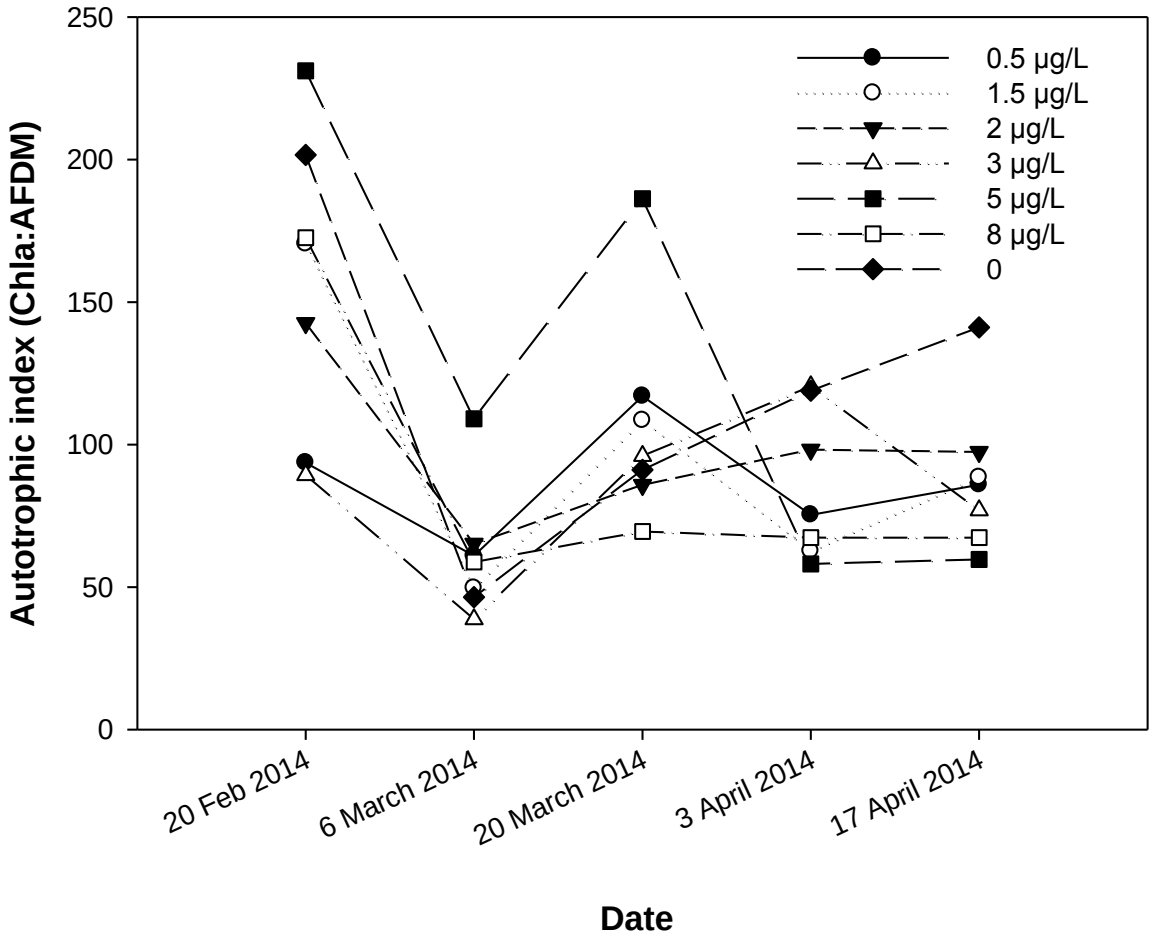

Figure 3-13. Autotrophic index (Chl-*a*:AFDM) for six phosphorus concentrations and a control as a function of time for the period 20 February 2014 to 17 April 2014.

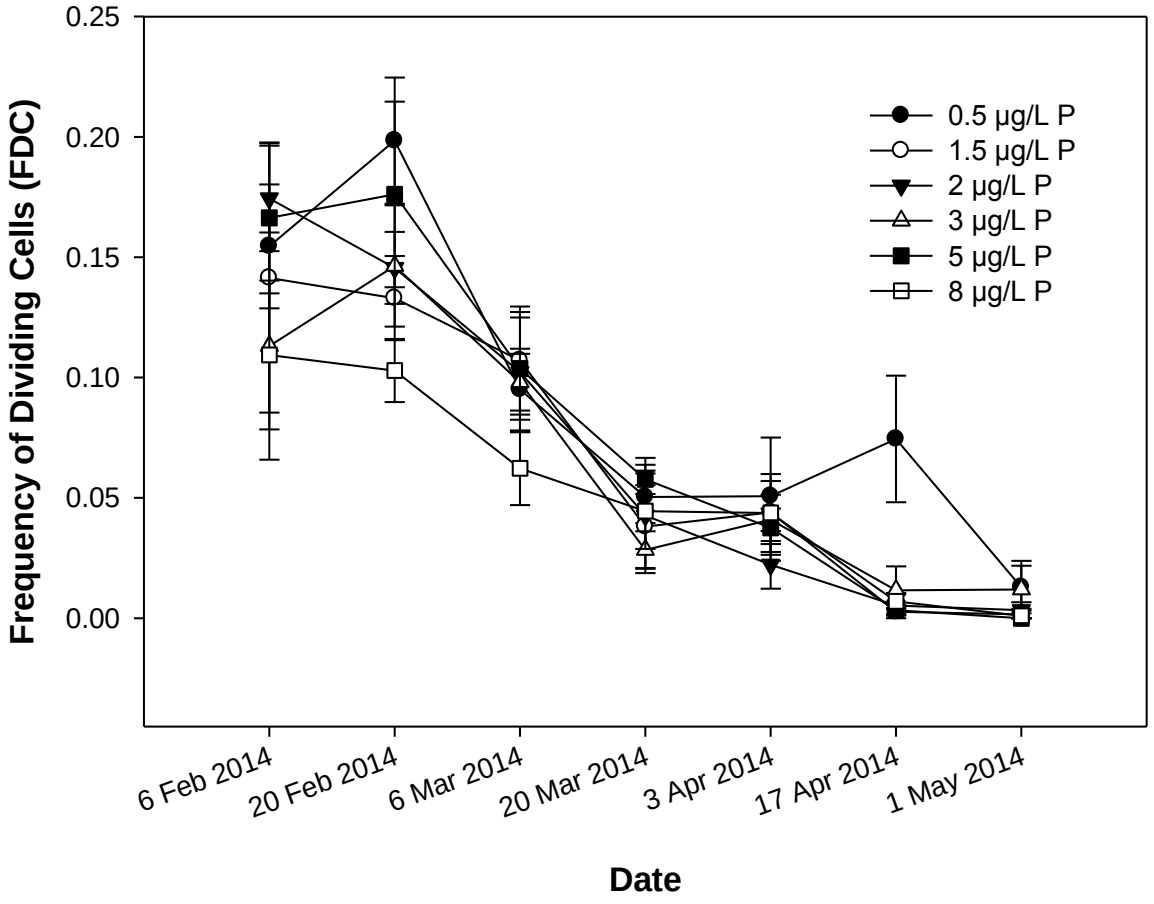

Figure 3-14. Frequency of dividing cells (mean±S.E.) of *Didymosphenia geminata* as a function of time for the period 6 February 2014 to 1 May 2014 for six phosphorus concentrations (0.5, 1.5, 2, 3, 5, and 8 µg P/L) at the University of Idaho mobile experimental flume system located at the Kootenai River Research Station in Libby, MT, USA. Data on 6 February 2014 and 1 May 2014 represent pre- and post-treatment samples, respectively. The control data has been removed to observe patterns within the six phosphorus concentrations.

Chapter 4: Response of *Didymosphenia geminata to* manipulation of dissolved nitrogen concentrations and nitrogen to phosphorus ratios

The Kootenai River in Libby, MT, USA, has had prolific nuisance mats of *Didymosphenia geminata* since 2001, though soluble reactive phosphorus (SRP) concentrations have remained stable and below detection (1 µg/L) since the mid-1990s. To evaluate if increased nitrogen (N) concentrations and higher N:P ratios have contributed to phosphorus limitation and subsequent formation of nuisance mats of *D. geminata*, a mesocosm experiment with treatments (additions) of phosphorus and nitrogen was completed from January to May in 2015. An approximately 30% increase of nitrogen (50 µg/L above ambient) resulted in longer *D. geminata* stalk, while a 60 % increase in phosphorus (0.7 µg/L above ambient) significantly decreased stalk length. However, when the N:P ratio remained high (increase of nitrogen and phosphorus), no change in stalk length relative to the control (no N or P addition) was observed, suggesting a nitrogen-driven phosphorus limitation. I conclude that within the Kootenai River, the formation of nuisance mats of *D. geminata*, may be driven by high nitrogen concentrations and phosphorus limitation. Rebalancing the soluble inorganic nitrogen: total dissolved phosphorus (SIN:TDP) through the addition of P or reducing N to pre-2000 concentrations may suppress nuisance mat growth and occurrence within the Kootenai River.

Introduction

The global production of nitrogen and its release to the environment from modern agriculture, fossil fuel consumption and other human activities has reached all-time highs within this decade (Gu et al. 2013). Aquatic ecosystems have also received increases in nitrogen, especially when detectable increases occur in such large lakes as Lake Superior

(McDonald et al. 2010). Large increases in N have also been recorded in smaller systems (Fenn et al. 2003; Musselman and Slauson 2004). This increase of nitrogen within lentic and lotic systems has the potential to affect the nitrogen to phosphorus (N:P) ratio, resulting in phosphorus limitation, which has been shown to lead to nuisance mats of *Didymosphenia geminata* (Kilroy and Bothwell 2011, 2012). The extraordinary increase of nitrogen deposition due to human alterations of the global nitrogen cycle has become a concern for scientists across the world. Between 1960 and 2001, global production of nitrogen for fertilizer increased from 1 million to 100 million tons $year^{-1}$ (Aneja et al. 2001). Human-produced nitrogen released to the atmosphere now exceeds the proportion of naturally cycling nitrogen (Vitousek et al. 1997). These changes to the nitrogen cycle have more than doubled the rate of nitrogen input to the terrestrial cycle including amplified wet and dry deposition which has far reaching consequences (Vitousek et al. 1997; Gruber and Galloway 2008).

Wolfe et al. (2006) have found that across the globe, diatom assemblages have begun to change, with the greatest changes occurring after 1950. This is partially attributed to systems that were once nitrogen limited but are now constrained by resources other than N. For the Kootenai River system, the impacts of increased atmospheric and terrestrial nitrogen deposition have yet to be evaluated, however, the landscape-wide magnitude of this increase may help explain the seemingly random explosion of stalk production and hence nuisance mat formation by the native diatom *Didymosphenia geminata* starting in the early 2000s.

The prevalence of *D. geminata* nuisance mats in low phosphorus (< 2 µg/L) conditions has been repeatedly identified (Kilroy and Bothwell 2012). Similar to other attached benthic diatom species, in nutrient stressed conditions *D. geminata* overproduces (elongates) a mucopolysaccharide stalk (Mykylestad and Haug 1972; Myklestad 1995; Kilroy and Bothwell

2011). However, neither the Kootenai River nor the Libby Dam experienced any major physical or managerial change in the early 2000s, leaving little explanation for the potential increase in phosphorus limitation. Therefore, the occurrence and continued persistence of Didymo mats may be the result of influences at the landscape or atmospheric scale. Bothwell et al. (2014) have suggested that *D. geminata* nuisance mat formation occurs not in spite of P limitation, but because of it. A suggested potential mechanism for this phosphorus limitation include, but are not limited to, atmospheric deposition of nitrogen and increased nitrogen in aquatic ecosystems resulting from terrestrial deposition (Bothwell et al. 2014). Since 2005, nitrate+nitrite-N concentrations have nearly tripled below the Libby Dam (Fig. 4-1; KTOI 2014; data reproduced in Fig. 6-1 with permission). The significant increase of nitrogen, has changed along the same timeline as the emergence and proliferation of *D. geminata* nuisance mats.

Mat coverage of the river bottom is greatest below the dam (100% benthic coverage, 3-8 cm thick) and decreases downstream into Idaho and Canada. After the Kootenai River flows through the towns of Libby and Troy, MT, USA, mat presence becomes patchy and minimal. Total nitrogen (TN) to total phosphorus (TP) ratios and soluble inorganic nitrogen (SIN) to total dissolved phosphorus (TDP) ratios similarly decrease as the Kootenai River flows through these urban areas (KTOI 2014; data reproduced with permission in Figs. 4-2 and 4-3). The highest ratios of TN:TP (Fig. 4-2) and SIN:TDP (Fig. 4-3) are observed below the dam and decrease downstream, suggesting that the high coverage of *D. geminata* nuisance mats below the dam may be potentially related to these N and P conditions. To determine if increased nitrogen and altered N:P ratios contribute to phosphorus limitation and subsequent nuisance mat formation, a mesocosm study was initiated in 2015 below the Libby Dam.

The objective of this study was to determine if growth of *D. geminata* mats (stalk length) in treatments with the addition of N or P differed from controls (no N or P enrichment). Specifically, I wanted to determine if the growth of *D. geminata* (stalk length) is negatively related to the concentration of P and positively related to the concentration of N.

Study site and mobile experimental flume system

The Kootenai River originates in the Kootenay Ranges of B.C. Canada flowing south into the trans-boundary Koocanusa Reservoir and then into Montana, USA where the spelling changes from Kootenay to Kootenai (Fig. 4-4). Built in 1972, the Libby Dam created Koocanusa Reservoir, a 145 km long impoundment that straddles the US/Canada border between Montana and British Columbia. From Montana, the Kootenai River flows northwest through Idaho, then north to re-enter Canada via Kootenay Lake near Creston, British Columbia (Fig. 4-4).

To test the aforementioned hypothesis, a mobile experimental flume station (MEFS) was built by the University of Idaho based on designs similar to those used by Bothwell (1985), Bothwell and Kilroy (2011), and Kilroy and Bothwell (2011, 2012). The MEFS was located below Libby Dam (Fig. 4-5) and supplied with river water directly from the Kootenai River. From April to September 2009-2013, the Kootenai River below the Libby Dam had an average nitrite+nitrate-N (NO_2+NO_3) concentration of 170 µg/L, a total dissolved phosphorus concentration (TDP) of 2.28 µg/L, a soluble reactive phosphorus concentration (SRP) below detection (1 µg/L), a TN:TP atomic ratio of 41, and a soluble inorganic nitrogen (SIN) (NH_4+NO_2+NO_3) to TDP atomic ratio of 100.7 (KTOI 2014). These values were used to calculate treatment concentrations for this experiment.

Sixteen flumes, each 2 m long × 0.2 m wide × 0.05 m tall constructed of clear plexiglass with an open cell 6 mm thick Styrofoam (Floracraft, Michigan, USA) substrate served as the experimental units. Water at a flow rate of 30 L/min was supplied to each flume from two large (> 1000 L) header tanks located outside the trailers to a mixing tank which received injections of treatment concentrations via individual Stenner 45M3 single head adjustable peristaltic pumps from a 120 L stock tank.

Methods

To study the influence of increased nitrogen concentrations on *D. geminata* mat growth, a series of nitrogen and phosphorus additions was tested on established *D. geminata* mats. Dissolved nitrogen in the form of sodium nitrate (Fisher Scientific) was added at 25 µg/L (~15% increase of the 3 year average of nitrate+nitrite-N concentrations below the Libby Dam) and 50 µg/L (~30% increase of the 3 year average of nitrate+nitrite-N concentrations below the Libby Dam). Dissolved phosphorus in the form of potassium phosphate (Fisher Scientific) was added at 0.35 µg/L (~30% increase) and 0.7 µg/L (~60% increase) and phosphorus and nitrogen were added together at 25N µg/L+0.35P µg/L and 50N µg/L+0.7P µg/L (Table 4-1). Water flowing from the Kootenai River through the MEFS was not treated, so all nutrient enrichments were in addition to ambient river nutrient concentrations. Therefore, the total nutrients to which the *D. geminata* mats within the MEFS were exposed was slightly greater than those listed here.

All flumes were conditioned with Kootenai River water for 37 days (2 January 2015 to 7 February 2015) before experiments started to establish robust *D. geminata* mats representative of what is observed in the Kootenai River.

All N and P treatments were replicated four times. Flumes were sampled biweekly by taking three randomly placed cores from each flume. A 19 mm diam. metal corer was used to remove the designated core from a randomly generated position in the flume and then gently washed in the flume to remove any unattached material before placing into a 50 ml centrifuge tube with a solution of 10 ml of DI water and 0.25 ml of Lugol's iodine. The removed portion of the flume substrate was replaced by pressing a 22 mm circle of new Styrofoam into the opening to minimize near substrate hydraulic disturbances within the flumes. Sampling during nutrient additions continued for 10 weeks and continued, after nutrient additions were stopped, for an additional four weeks to observe any lag effects.

To analyze each core, small sections of the core and mat growth were placed onto a glass microscope slide, topped with a cover slip and examined with the aid of a compound microscope (Wild M40) at 120×. This was repeated for the entire core until all sampled material was analyzed in its entirety.

For each core, all visible *D. geminata* cells were counted (quantitative counts) and classified to stage of division (frequency of dividing cells). All stalks that were attached to the core were measured (stalk length between node measurement) as described by (Kilroy and Bothwell 2012). Dividing cells were defined as cells that had "completed cytokinesis with a valve wall separating the adjoined daughter cell" (M. Bothwell, Environment Canada, Nanaimo, British Columbia, personal communication, 2013). These cells were considered "doublets" and FDC was calculated by dividing the number of doublets by the total number of cells counted (Bothwell and Kilroy 2011).

Both filtered (0.45 µm filter) and unfiltered water samples were taken for nutrient analysis (nitrogen and phosphorus) within the flumes for future testing. Pump dosing and flume discharge rates were checked once a week to ensure target doses were met. Photosynthetically active radiation (PAR) was recorded at hourly intervals with a Li-Cor (Lincoln, NE) LI-190 terrestrial radiation sensor and a Li-Cor (Lincoln, NE) LI-1400 data logger. To quantify the algal biomass, ash-free dry mass (AFDM) and chlorophyll-*a* (Chl-*a*) *analyses* were completed. On sampling days, one additional core was taken from each flume, placed in a 4 oz. Whirl-Pak™ bag, and frozen for analysis at the University of Idaho College of Natural Resources (UI CNR) laboratory. Standard methods (Eaton et al. 2005; U.S. EPA 1995) and UI CNR lab protocols were followed to analyze AFDM.

Statistical analyses

Data were analyzed for the period of the nutrient addition. For all flume studies, data were first evaluated to examine normality, and homogeneity of variance using univariate procedures and diagnostic plots. FDC, stalk length, and total cell counts were averaged for the triplicate cores taken on each sampling occasion from each flume and analyzed using a completely randomized repeated measures model and analysis of variance (ANOVA) procedures. Mean separations were conducted using the least squares means pair-wise comparisons. All statistical analyses were carried out using procedure MIXED in SAS software™ v. 9.4 (SAS Institute, Cary, North Carolina). Analyses of the FDC, quantitative counts, and stalk length between treatments were completed with repeated measures ANOVAs. In the analyses, FDC, stalk length, and total cells were the response variables, nutrient concentration was the treatment, and time was the repeated variable. The fixed effect was the nutrient treatments while the random effect was flumes.

Results

Seeding of flumes began on 2 January 2015, which resulted in quick accrual of *D. geminata* on the flume substrate. This initial algal community included some filamentous species of green algae and other diatoms typically associated with *D. geminata* mats. Visible nuisance mat patches appeared in late February, increasing the heterogeneity of the mats throughout the flumes. As nutrient treatments began, flumes exposed to the high dose of phosphorus (0.7 µg/L) exhibited increased algal diversity as seen in previous phosphorus treatments (Chapter 3). However, nuisance mat patches increased throughout the experiment in the high (50 µg/L) nitrogen treatment flumes, which resulted in a continuous nuisance mat by the end of the study. All other flumes exposed to the four other treatments and the controls had relatively uniform growths throughout the study with some filamentous green species and some *D. geminata* nuisance mat patches. Sloughing was observed in all flumes after nutrient additions were stopped.

The total number of cells increased in both the controls and experimental flumes over the course of the experiment (Fig. 4-6; Table 4-2), but there was no difference among treatments and the control ($P>0.05$) (Table 4-3). Time was statistically significant, but there was no treatment*time interaction ($P=0.993$). Cell counts decreased in both the controls and the treatments at the same rate after the additions of N and P ceased on 19 April 2015 (Fig. 4-6).

The frequency of dividing cells (FDC) declined over time but did not differ among treatments (Fig. 4-7; Tables 4-4 and 4-5). The FDC ranged from a high of 0.15 on 22 Feb 2015 to a low of 0.01 on 19 April 2015. Time was statistically significant, but there was no

treatment*time interaction (P=0.855). Short-term increases of FDC (within the first week) may have occurred in the flumes as seen in (Bothwell and Kilroy 2011) but no significant difference in FDC was observed over the entirety of the study.

For stalk length, the treatment*time interaction was not significant (P=0.870) but the main effects of treatment and the repeated measure of time were significant (Table 4-6). Stalk length was significantly shorter in the 0.70 µg P/L but longer in the 50 µg N/L treatments relative to the controls (Table 4-6 and 4-7). None of the other treatments differed from the controls (Fig. 4-8; Table 4-7). Similarly, stalk length in the 0.35 µg P/L was longer than the 0.70 µg P/L treatment and not significantly different from the control (Fig. 4-8; Table 4-7).

In all treatments, AFDM increased over time until it plateaued on 5 April 2015 (Fig. 4-9). There was no indication of an overall nutrient effect (Table 4-8), but several treatments differed from each other, and the 50 µg N/L was higher than the controls (Table 4-9). Samples for chlorophyll-*a* exceeded the holding time for the analysis, and were not included in the analyses. Mean daily PAR for the time period of 8 February 2015 to 17 May 2015 was 26.87 mol m^{-2} d^{-1}.

Macroinvertebrates were rare within the flumes. Chironomidae was the only family present in the sampled cores. Fewer than 1 individual per 12 cores was observed. At the end of the study, 1 – 2 Diptera larvae were observed attached to flume hardware but were not part of the sampled area.

Discussion

Total cell counts were highly variable because of the patchy tufts of mat present throughout the flumes. Cell counts increased over time until N and P additions stopped, after

which they decreased; this altered the algal community structure. Cell counts were higher than in previous *D. geminata* MEFS studies that occurred in 2013 and 2014 (see Chapter 3) which is attributed to capturing the peak growth season (unlike 2013) and the change in counting methodology to account for the higher biomass (unlike 2014). By altering the methods of analyzing the core, the entire mat was analyzed compared to 2014 in which only the top layers of the mat were analyzed due to the reduced light through the thick mat. This refinement in the method significantly increased the total cell counts in this study compared to those reported in Chapter 3. I recommend that all future analyses of *D. geminata* cores from artificial substrate use the method employed here.

The frequency of dividing cells increased for a short period of time in all treatments but then consistently decreased throughout the remainder of the experiment. Time was a significant factor, however, treatment was not, suggesting that changes in the environment (light exposure, temperature) over time may play a more important role in FDC values than the N or P treatments. My results in 2013 (Chapter 3) suggest that nutrient additions may result in a short-term effect (6 weeks) to FDC values but at a long-term scale, time or length of study (as seen in 2015) is a more crucial factor. Additional long-term studies (>2 weeks) with high sampling rates are needed to determine if FDC plateaus after time with the addition of N and P. Other studies have indicated that diatom reproduction rates can significantly fluctuate over time, even in controlled systems (Brand et al. 1981) making it difficult to analyze diatom reproduction trends at the long-term scale. Bothwell and Kilroy (2011) did identify significant relationships between nutrient additions and FDC but their study was shorter than three weeks.

The suppression of stalk length at 0.7 µg P/L but not 0.35 µg P/L, along with the 2014 P addition results, suggests that the cellular saturation of *D. geminata* occurs between 0.35 and 0.5 µg P/ L above ambient in the Kootenai River. Time and treatment were both statistically significant but there was no treatment*time interaction suggesting that the effect of treatment does not depend on time. By identifying that the cellular saturation of *D. geminata* can be achieved at such low P enrichment concentrations, management strategies to reduce nuisance mat production at the river scale become feasible. Other *D. geminata* nutrient enrichment studies have used or attempted to reach significantly higher P concentrations: 11 and 6 µg P/L (James et al. 2015) and 5 and 50 µg P/L (Bothwell and Kilroy 2011) to suppress stalk growth in *D. geminata*. However, these higher concentrations of P enrichment would be impossible to achieve in the Kootenai River which has a winter/spring discharge of ~113 to 114 m^3/s and the lower concentrations we investigated are more feasible for this river system.

While the algal community composition differed between the high nitrogen and phosphorus flumes compared to the controls (Fig. 4-10), all other flumes exposed to varying treatments were similar. As the biomass continued to increase over time, by 5 April 2016, a plateau occurred, suggesting that biomass capacity for the substrate and depth of water was reached. This increase of biomass over time is attributed to increased photosynthetically active radiation (PAR) and maturity of the mats (e.g., boundary layers). However, this physical limitation of the flumes is not considered a hindrance in the extrapolation of these results to an in-river scale. Spivak et al. (2011) in a survey of 359 mesocosm studies, found that algal response was more correlated with time than spatial scales and that conclusions about mechanisms behind algal responses to nutrient limitation obtained from mesocosm studies are applicable to large-scale ecosystems.

Previous studies at the MEFS demonstrated that a phosphorus addition over 0.5 µg/L above ambient resulted in the suppression of *D. geminata* stalk growth (Chapter 3). In this study, I have further confirmed that a minimal addition of P (0.7 µg/L) can reduce *D. geminata s*talk growth through cellular saturation with P. Bothwell (1988) demonstrated that growth rate saturation of lotic periphytic diatoms occurs at extremely low P concentrations of 0.3 to 0.6 µg/L. Given that the P addition at 0.35 ug/L did not suppress stalk growth, I conclude that *D. geminata* cellular saturation of P occurs between 0.35 and 0.5 µg/L above ambient in the Kootenai River below the Libby Dam. This suggests that a P enrichment within the Kootenai River is a realistic management strategy, if implemented using slow-release P at < 2 µg/L before peak accrual. This would not only significantly decrease the nuisance coverage of *D. geminata* but could also increase nutrient availability to other diatom species without altering the trophic status of the river system.

This study has also demonstrated that increasing nitrogen concentration eliminates the suppression of stalk growth that occurs with P enrichment. While a 0.7 µg P/L addition significantly suppressed *D. geminata* stalk growth, the combination of a 0.7 µg P/L addition and a 50 µg N/L addition resulted in stalk lengths similar to the control. Without any P enrichment, a 50 µg N/L addition resulted in significantly longer *D. geminata* stalk. The change in *D. geminata* mat presence with the addition of 50 µg N/L was visibly noticeable (Fig. 4-10) and any future management strategy must consider the importance of increased nitrogen concentrations and the N:P ratio.

While the direct atmospheric deposition of nitrogen to the Kootenai River or to the terrestrial watershed has not been quantified, diatoms can respond to N deposition as low as 3 to 8 kg per ha per year (Fenn et al. 2003). As the nitrogen concentration in the Kootenai River

continues to increase (Fig. 4-1), amplified phosphorus limitation and subsequent nuisance mat growth should be of concern to managers. Kilroy and Larned (2016) found that in N limited systems with *D. geminata* mats, the enrichment of N can increase stalk length and growth. My experiment also demonstrated that an increase of dissolved nitrogen (50 µg/L) resulted in significantly longer stalk lengths relative to the control (Fig. 4-8; Table 4-3). From 2012 to 2013 and 2013 to 2014, nitrate+nitrite-N concentrations in the Kootenai River increased 30% each year (Fig. 4-1). Increased N not only increased stalk length but also reduced the availability of P to reduce stalk growth. While the mechanisms of co-limitation between phosphorus and nitrogen are still under investigation, and there are several river systems with nuisance *D. geminata* mats without high N concentrations, recent studies have shown that excessive N can limit access to P (Harpole et al. 2011; Perini and Bracken 2014) and that nitrogen deposition shifts ecosystems to phosphorus-limited states (Elser et al. 2009; Bergström and Jansson 2006; Bergström et al. 2005). Thus, managers (Montana Fish, Wildlife and Parks or U.S. Army Corps of Engineers) should focus on reducing the input of N to the Kootenai, or increasing the availability of P to manage *D. geminata*.

While the definitive cause for the increasing nitrate+nitrite-N concentrations in the Kootenai River has yet to be determined, future management strategies to reduce *D. geminata* nuisance mats should include reducing nitrogen concentrations below those observed in 2000 or rebalancing the N:P ratio via the addition of phosphorus. As higher N concentrations continue to contribute to P limitation in the Kootenai River, increasing nitrogen concentrations will require higher P concentrations thereby limiting this management strategy because the addition of P to achieve balance will tend to increase trophic state. Further

research of nitrogen loading to the Kootenai River and the surrounding landscape should be prioritized by management agencies.

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Tables

Table 4-1. Nitrogen and phosphorus treatments for the 2015 *Didymosphenia geminata* mat ecology study at the Kootenai River Research Site Libby, MT, USA.

	Nitrogen Addition µg/L	Phosphorus Addition µg/L	Experimental Units
Control	0	0	8
N Increase	25, 50	0	8
P Increase	0	0.35, 0.7	8
Balance Ratios to Ambient	25, 50	0.35, 0.7	8

Table 4-2. Repeated measures ANOVA for total cell count of *Didymosphenia geminata* among six nutrient treatments at the Kootenai River Research Station in Libby, MT, USA, from 8 February to 17 May 2015. The treatments were phosphorus 0.35 µg P/L (P1), 0.7 µg P/L (P2), nitrogen 25 µg N/L (N1), 50 µg N/L (N2), and nitrogen and phosphorus 25 µg N/L + 0.35 µg P/L (N1/P1), 50 µg N/L + 0.7 µg P/L (N2/P2), and a control (no nutrient addition).

Effect	*F* value	*P*
Nutrients	0.71	0.649
Time	30.73	<0.001
Time × Nutrients	0.41	0.993

Table 4-3. *Post-hoc* pair-wise comparison for total cell count of *Didymosphenia geminata* among six nutrient treatments at the Kootenai River Research Station in Libby, MT, USA, from 8 February 2015 to 17 May 2015. The treatments were phosphorus 0.35 µg P/L (P1), 0.7 µg P/L (P2), nitrogen 25 µg N/L (N1), 50 µg N/L (N2), and nitrogen and phosphorus 25 µg N/L + 0.35 µg P/L (N1/P1), 50 µg N/L + 0.7 µg P/L (N2/P2), and a control (no N or P addition).

N or P concentration (µg/L)	0	N1	N2	P1	P2	N1/P1
N1 (25)	0.296					
N2 (50)	0.652	0.546				
P1 (0.35)	0.388	0.850	0.677			
P2 (0.70)	0.912	0.348	0.733	0.451		
N1/P1	0.099	0.521	0.219	0.408	0.122	
N2/P2	0.456	0.758	0.766	0.905	0.524	0.346

Table 4-4. Repeated measures ANOVA for frequency of dividing cells (FDC) of *Didymosphenia geminata* in different treatment nutrient concentrations including a control (no N or P addition) at the Kootenai River Research Station in Libby, MT, USA, from 8 February 2015 to 17 May 2015.

Effect	*F* value	*P*
Nutrients	0.38	0.882
Time	56.13	<0.001
Time × Nutrients	0.68	0.855

Table 4-5. *Post-hoc* pair-wise comparison for frequency of dividing cells of *Didymosphenia geminata* among six nutrient treatments at the Kootenai River Research Station in Libby, MT, USA, from 8 February 2015 to 17 May 2015. The treatments were phosphorus 0.35 µg P/L (P1), 0.7 µg P/L (P2), nitrogen 25 µg N/L (N1), 50 µg N/L (N2), and nitrogen and phosphorus 25 µg N/L + 0.35 µg P/L (N1/P1), 50 µg N/L + 0.7 µg P/L (N2/P2), and a control (no N or P addition).

N or P concentration (µg/L)	0	N1	N2	P1	P2	N1/P1
N1 (25)	0.570					
N2 (50)	0.865	0.462				
P1 (0.35)	0.616	0.946	0.504			
P2 (0.70)	0.410	0.795	0.323	0.743		
N1/P1	0.302	0.636	0.233	0.589	0.830	
N2/P2	0.547	0.973	0.442	0.919	0.821	0.660

Table 4-6. Repeated measures ANOVA for stalk length of *Didymosphenia geminata* among six nutrient treatments including a control at the Kootenai River Research Station in Libby, MT, USA, from 8 February 2015 to 17 May 2015. The treatments were phosphorus 0.35 µg P/L (P1), 0.7 µg P/L (P2), nitrogen 25 µg N/L (N1), 50 µg N/L (N2), and nitrogen and phosphorus 25 µg N/L + 0.35 µg P/L (N1/P1), 50 µg N/L + 0.7 µg P/L (N2/P2), and a control (no N or P addition).

Effect	*F* value	*P*
Nutrients	10.58	<0.001
Time	7.21	<0.001
Time × Nutrients	0.66	0.870

Table 4-7. *Post-hoc* pair-wise comparison for stalk length of *Didymosphenia geminata* among six nutrient treatments at the Kootenai River Research Station in Libby, MT, USA, from 8 February 2015 to 17 May 2015. The treatments were phosphorus 0.35 µg P/L (P1), 0.7 µg P/L (P2), nitrogen 25 µg N/L (N1), 50 µg N/L (N2), and nitrogen and phosphorus 25 µg N/L + 0.35 µg P/L (N1/P1), 50 µg N/L + 0.7 µg P/L (N2/P2), and a control (no N or P addition).

N or P concentration (µg/L)	0	N1	N2	P1	P2	N1/P1
N1 (25)	0.812					
N2 (50)	0.001	0.001				
P1 (0.35)	0.419	0.565	<0.001			
P2 (0.70)	0.001	0.007	<0.001	0.003		
N1/P1	0.231	0.311	<0.001	0.581	0.024	
N2/P2	0.404	0.505	0.002	0.800	0.025	0.834

Table 4-8. Repeated measures ANOVA for ash-free dry mass among six nutrient treatments at the Kootenai River Research Station in Libby, MT, USA, from 8 February 2015 to 17 May 2015. The treatments were phosphorus 0.35 µg P/L (P1), 0.7 µg P/L (P2), nitrogen 25 µg N/L (N1), 50 µg N/L (N2), and nitrogen and phosphorus 25 µg N/L + 0.35 µg P/L (N1/P1), 50 µg N/L + 0.7 µg P/L (N2/P2), and a control (no N or P addition).

Effect	*F* value	*P*
Nutrients	1.85	0.129
Time	66.36	<0.001
Time × Nutrients	0.65	0.887

Table 4-9. Ash free dry mass pair-wise comparison across all treatments. Pair-wise comparison for ash free dry mass among six nutrient treatments at the Kootenai River Research Station in Libby, MT, USA, from 8 February 2015 to 17 May 2015. The treatments were phosphorus 0.35 µg P/L (P1), 0.7 µg P/L (P2), nitrogen 25 µg N/L (N1), 50 µg N/L (N2), and nitrogen and phosphorus 25 µg N/L + 0.35 µg P/L (N1/P1), 50 µg N/L + 0.7 µg P/L (N2/P2), and a control (no N or P addition).

N or P concentration (µg/L)	0	N1	N2	P1	P2	N1/P1
N1 (25)	0.315					
N2 (50)	0.041	0.352				
P1 (0.35)	0.393	0.115	0.015			
P2 (0.70)	0.604	0.663	0.171	0.238		
N1/P1	0.122	0.637	0.638	0.042	0.361	
N2/P2	0.692	0.229	0.036	0.688	0.430	0.095

Figures

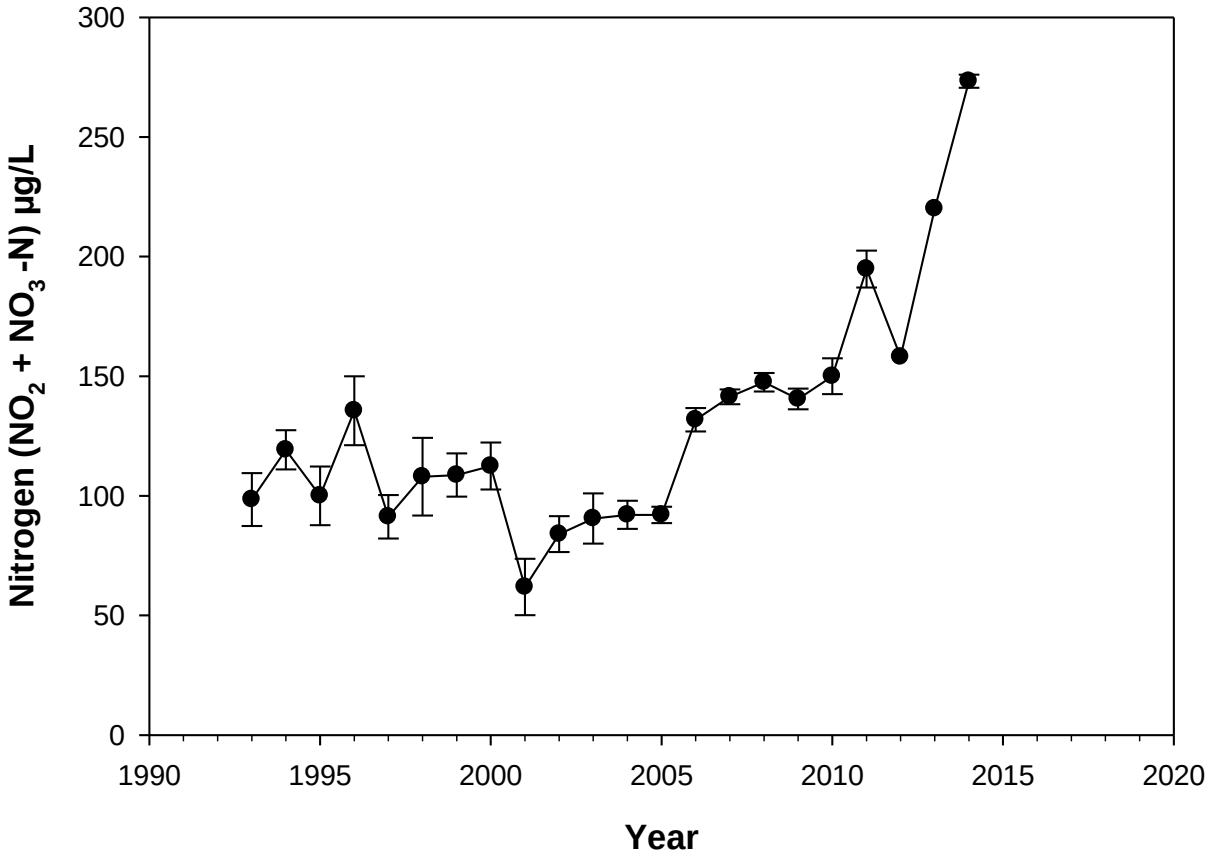

Figure 4-1. Annual average (±SE) concentrations of nitrogen (NO_2 + NO_3-N) as a function of time in the Kootenai River below Libby Dam of Libby, MT from 1993 to 2014. Data from KTOI (2014).

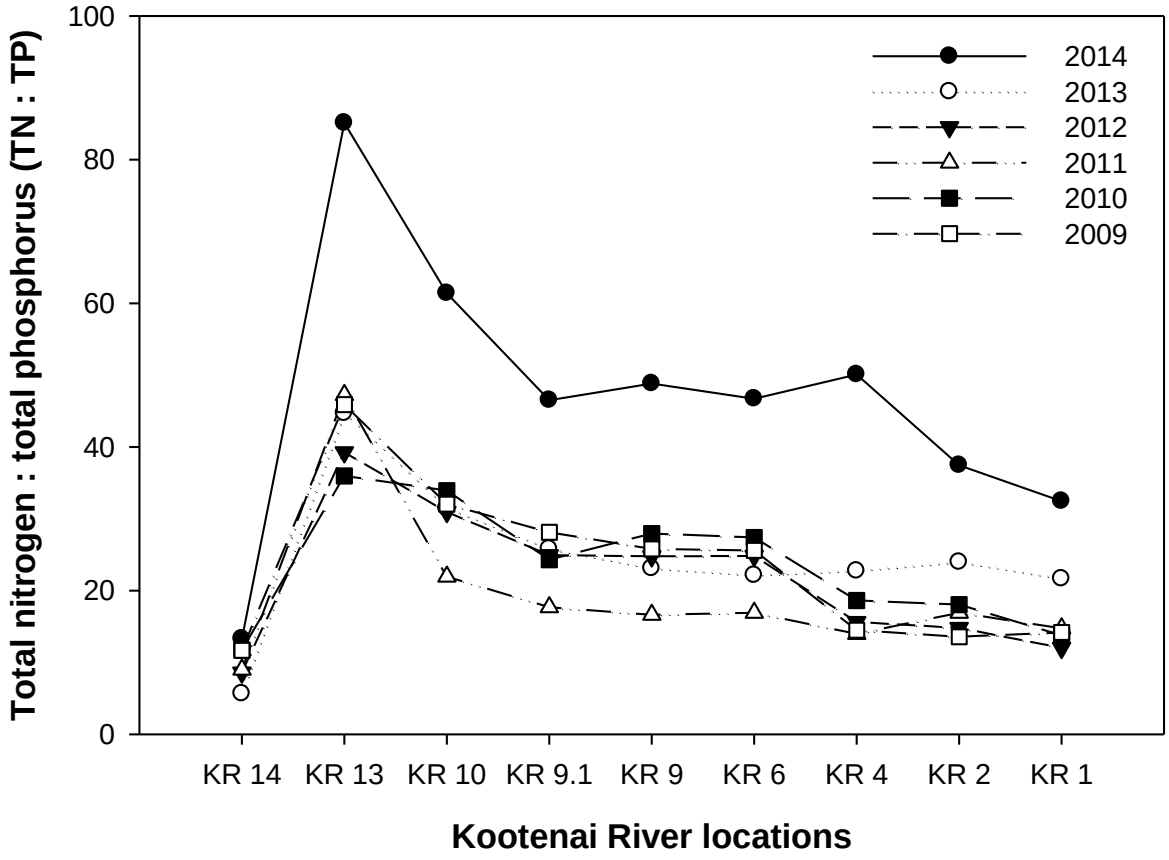

Figure 4-2. Average total nitrogen (TN) to total phosphorus (TP) atomic ratios from 2009 to 2014 throughout the Kootenai River in Montana, Idaho and Canada. KR 14 is located above Koocanusa Reservoir while KR 13 is located below the Libby Dam. KR 10 through KR 1 are progressively downstream along the Kootenai River through Montana, Idaho and back into Canada. Data provided by KTOI and used with permission. Permission to reuse must be obtained from the rightsholder.

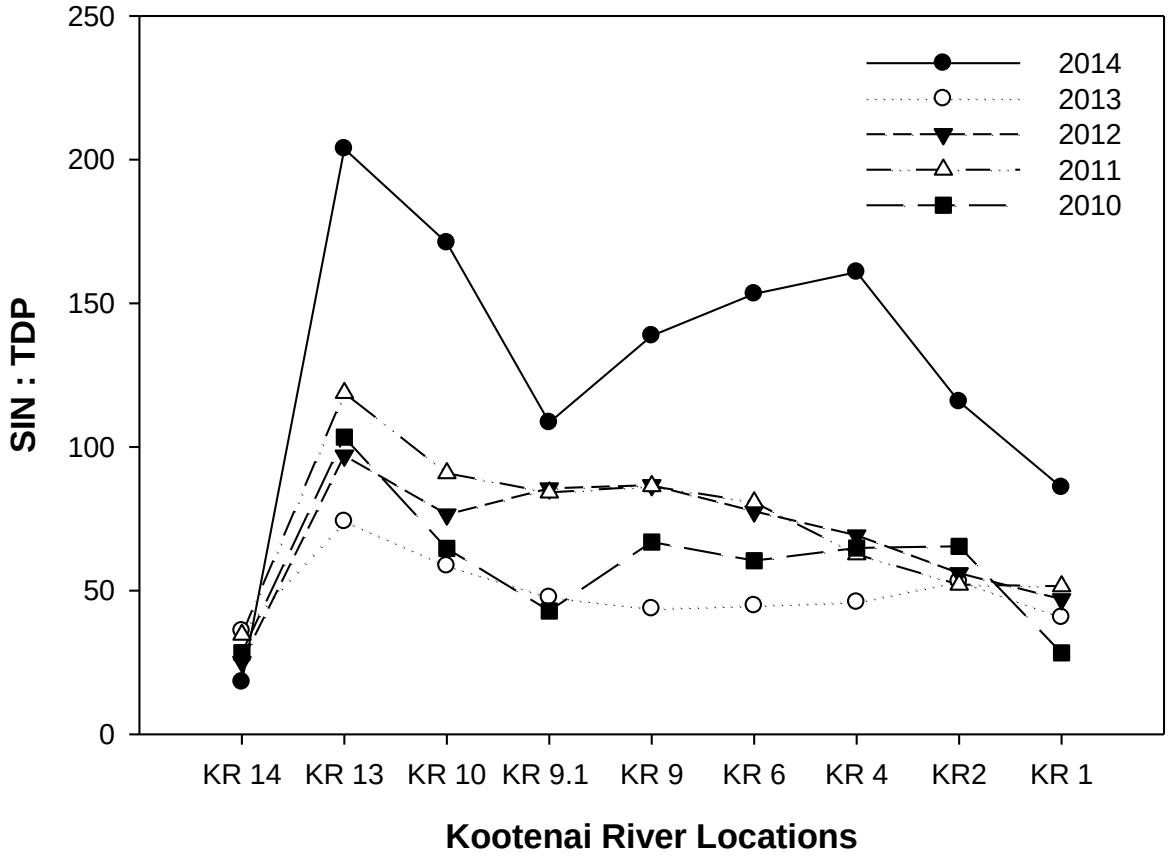

Figure 4-3. Average soluble inorganic nitrogen (SIN) to total dissolved phosphorus atomic ratios (TDP) from 2010 to 2014 throughout the Kootenai River in Montana, Idaho and Canada (KTOI 2014). KR 14 is located above Koocanusa Reservoir while KR 13 is located below the Libby Dam. KR 10 through KR 1 are progressively downstream along the Kootenai River through Montana, Idaho and back into Canada. Data provided by KTOI and used with permission. Permission to reuse must be obtained from the rightsholder.

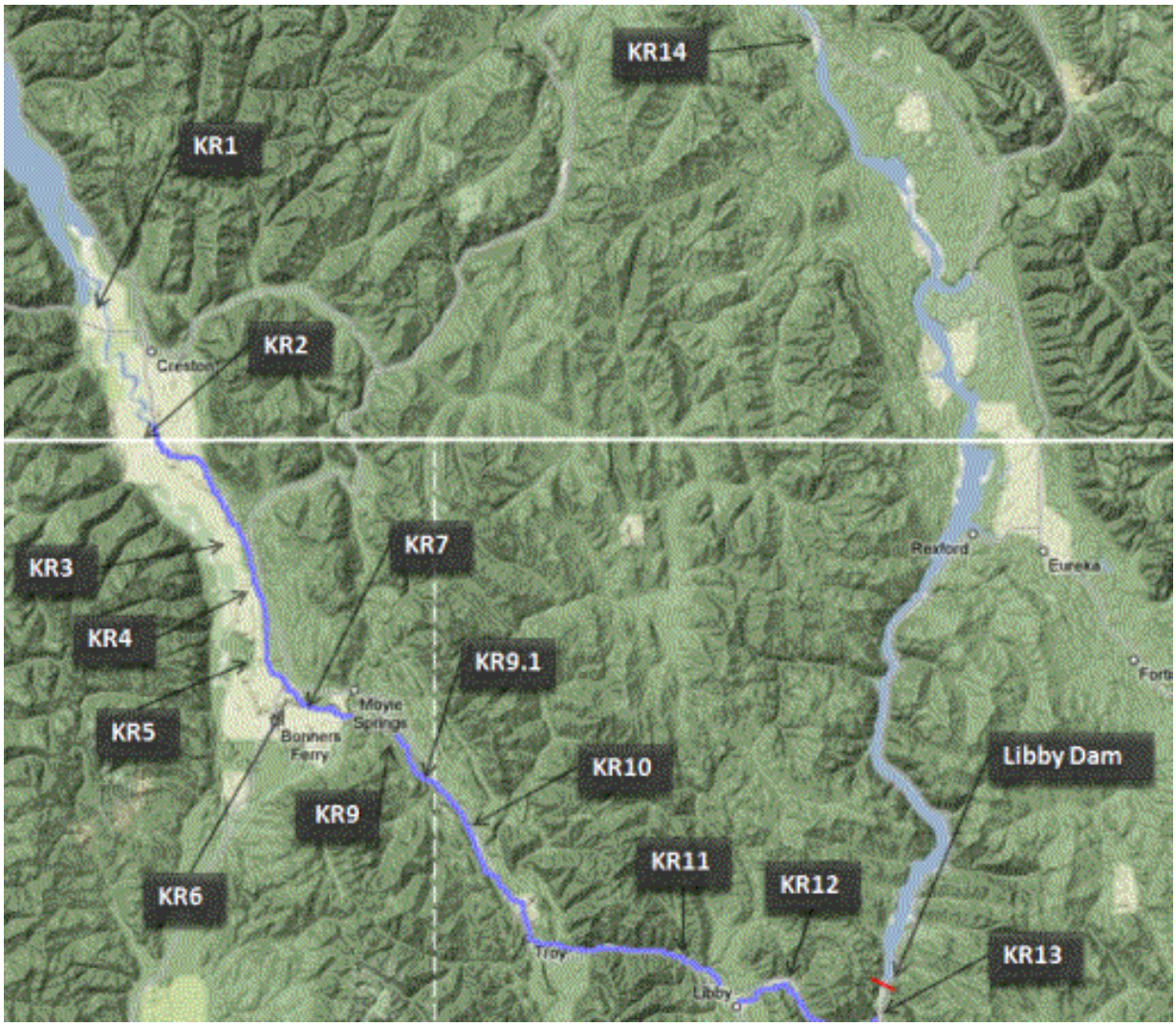

Figure 4-4. Map of the Kootenai River in Canada, Idaho and Montana. Water quality sampling locations identified in black boxes (KR14 to KR1) © [Kootenai Tribe of Idaho]. Constructed by Statistical Consulting Services, University of Idaho, Moscow, Idaho, for the Kootenai Tribe of Idaho. Reproduced by permission. Permission to reuse must be obtained from the rightsholder.

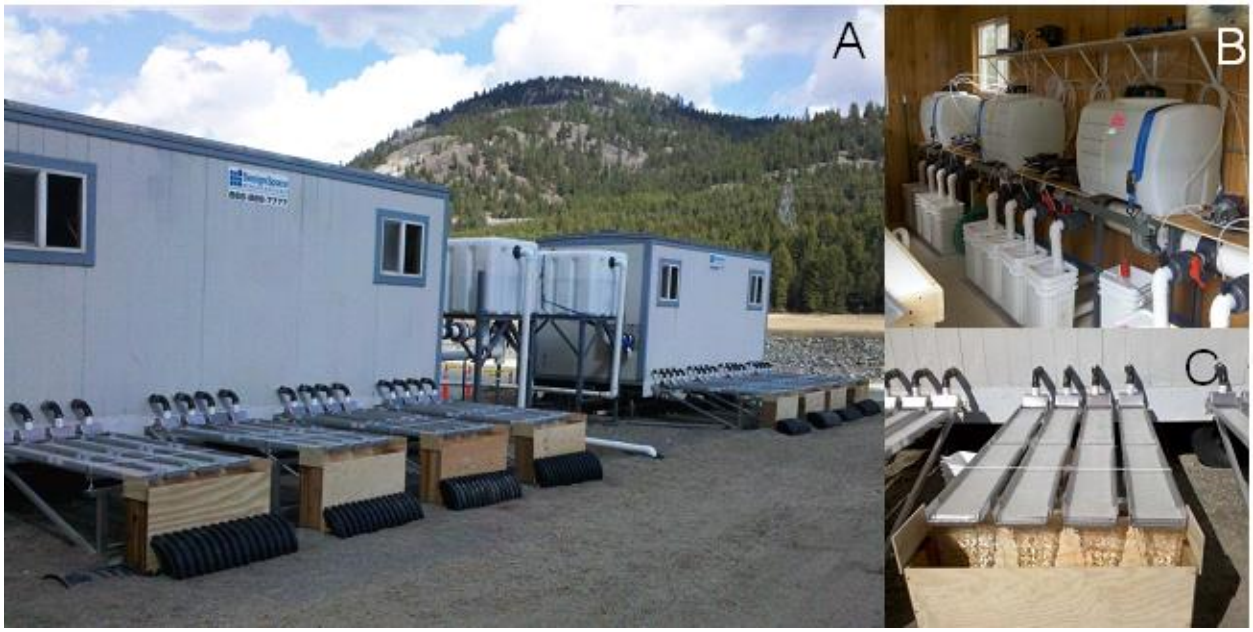

Figure 4-5. Mobile experimental flume system (MEFS) at the Kootenai River Research Station downstream of Libby Dam in Libby, MT, USA. **A**) Mobile experimental flume system (MEFS) with 32 external flumes supplied by water from the Kootenai River. **B**) Internal structure of MEFS with 120 L stock tanks on shelving and mixing header tanks for individual flumes below. **C**) External flumes of the MEFS. Flumes were lined with 6 mm open-celled Styrofoam for substrate.

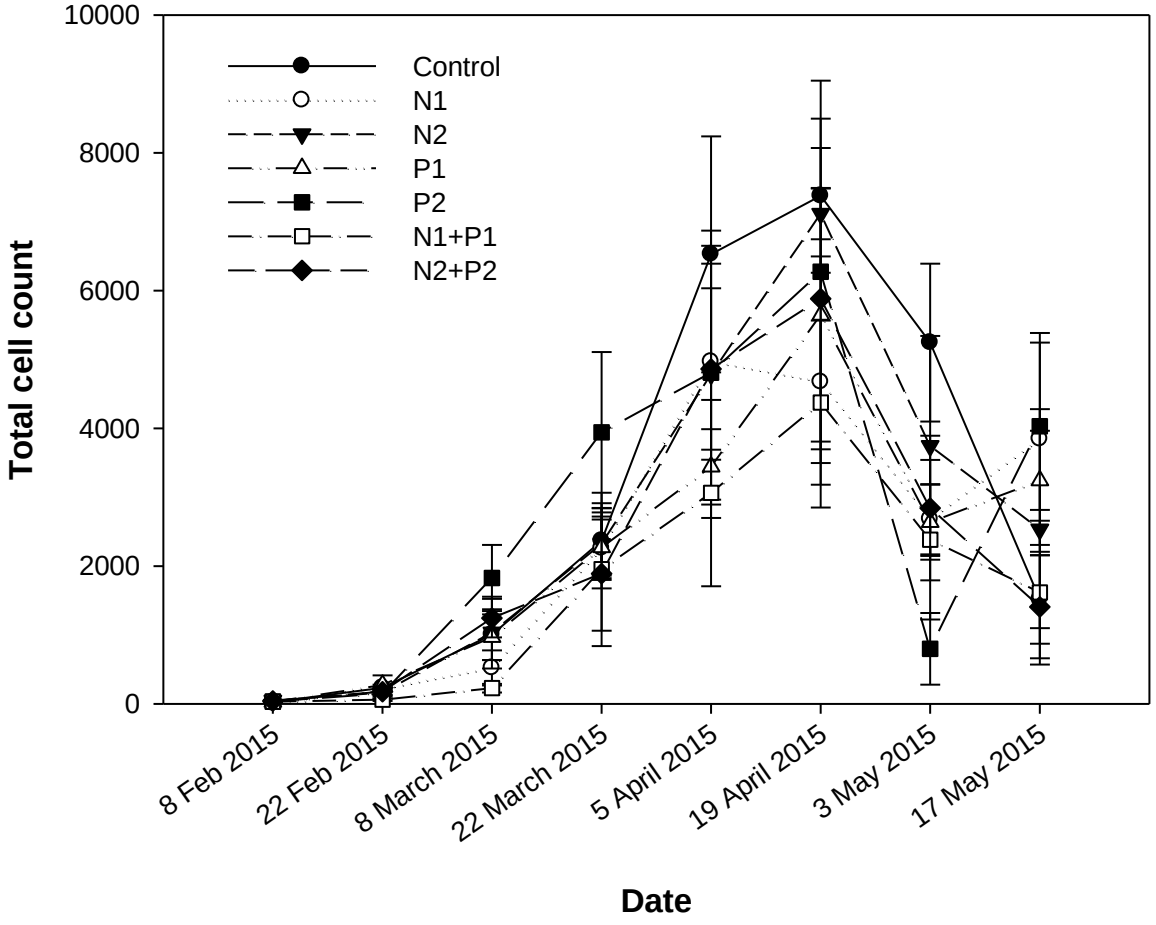

Figure 4-6. Total cell count per cm^2 (mean±S.E.) of *Didymosphenia geminata* as a function of time for the period 8 February 2014 to 19 April 2015 for six N and P concentrations (phosphorus P1-0.35 µg P/L, P2-0.7 µg P/L, N1-25 µg N/L, N2-50 µg P/L, N1/P1-25 µg N/L + 0.35 µg P/L, and N2/P2-50 µg N/L + 0.7 µg P/L) and a control (no N or P addition) at the University of Idaho mobile experimental flume system (MEFS) located below Libby Dam, Libby, MT, USA. Data on 8 February 2015 represents pre-treatment samples.

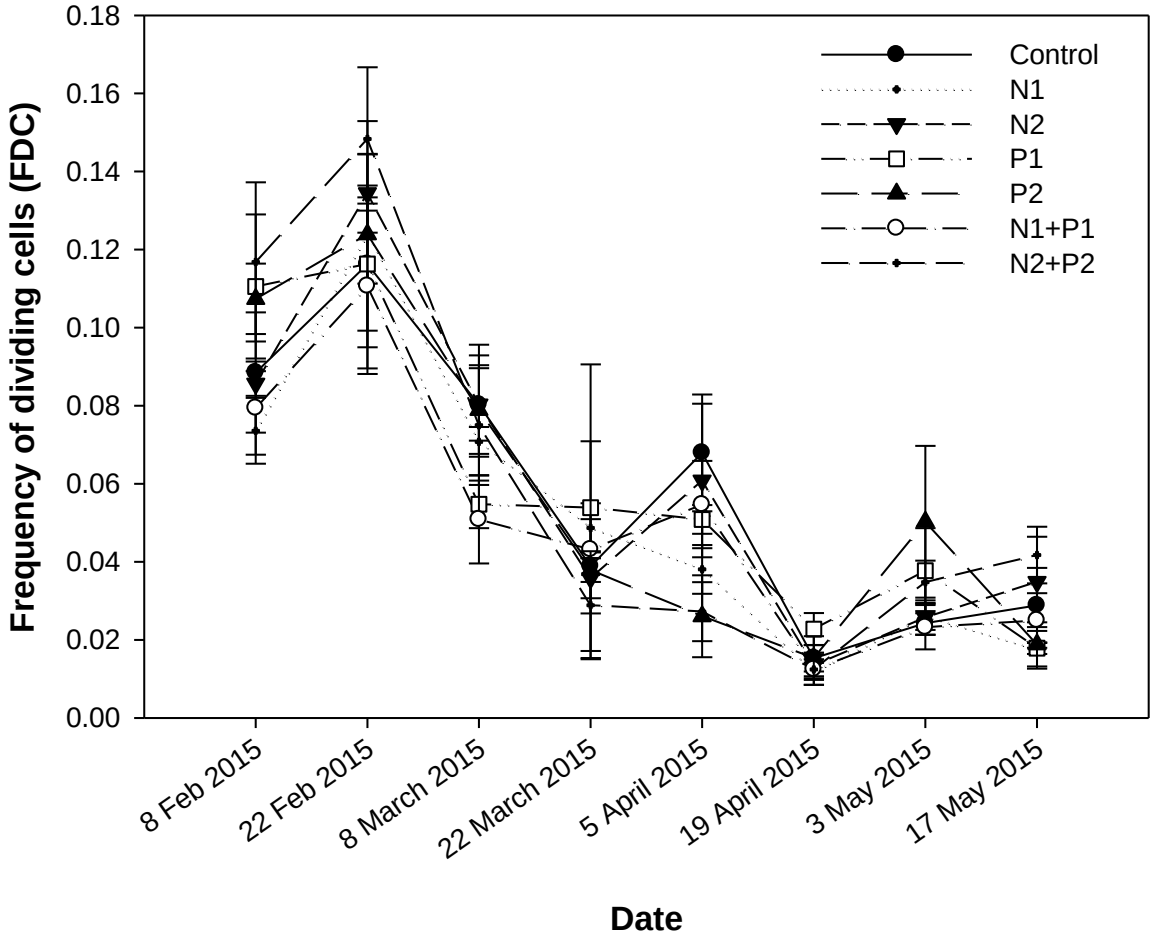

Figure 4-7. The frequency of dividing cells (mean±S.E.) of *Didymosphenia geminata* as a function of time for the period 8 February 2014 to 19 April 2015 for six N and P concentrations (phosphorus P1-0.35 µg P/L, P2-0.7 µg P/L, N1-25 µg N/L, N2-50 µg P/L, N1/P1-25 µg N/L + 0.35 µg P/L, and N2/P2-50 µg N/L+0.7 µg P/L) and a control (no N or P addition) at the University of Idaho mobile experimental flume system (MEFS) located below Libby Dam, Libby, MT, USA. Data on 8 February 2015 represents pre-treatment samples.

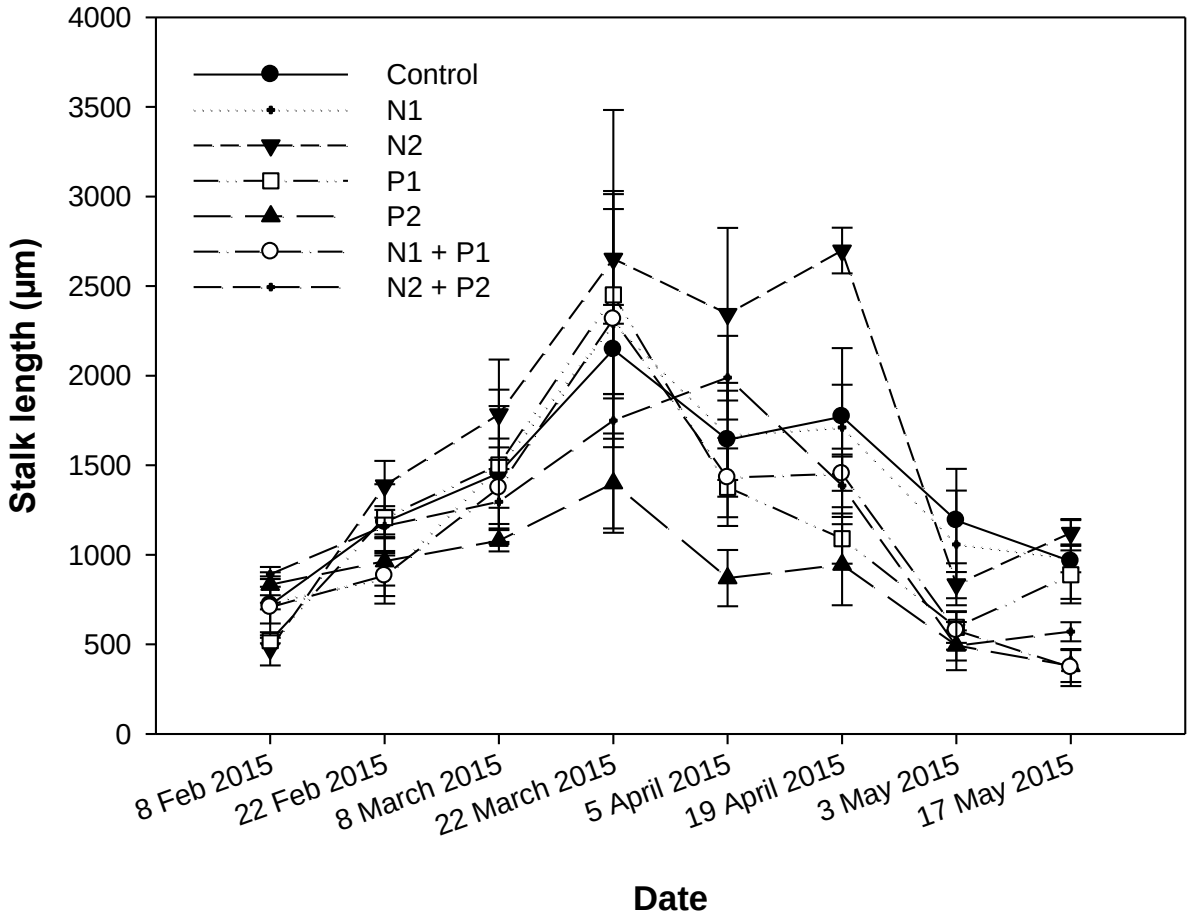

Figure 4-8. Stalk length (mean±S.E.) of *Didymosphenia geminata* as a function of time for the period 8 February 2014 to 19 April 2015 for six N and P concentrations (phosphorus P1-0.35 µg P/L, P2-0.7 µg P/L, N1-25 µg N/L, N2-50 µg P/L, N1/P1-25 µg N/L + 0.35 µg P/L, and N2/P2-50 µg N/L + 0.7 µg P/L) and a control (no N or P addition) at the University of Idaho mobile experimental flume system (MEFS) located below Libby Dam, Libby, MT, USA. Data on 8 February 2015 represents pre-treatment samples.

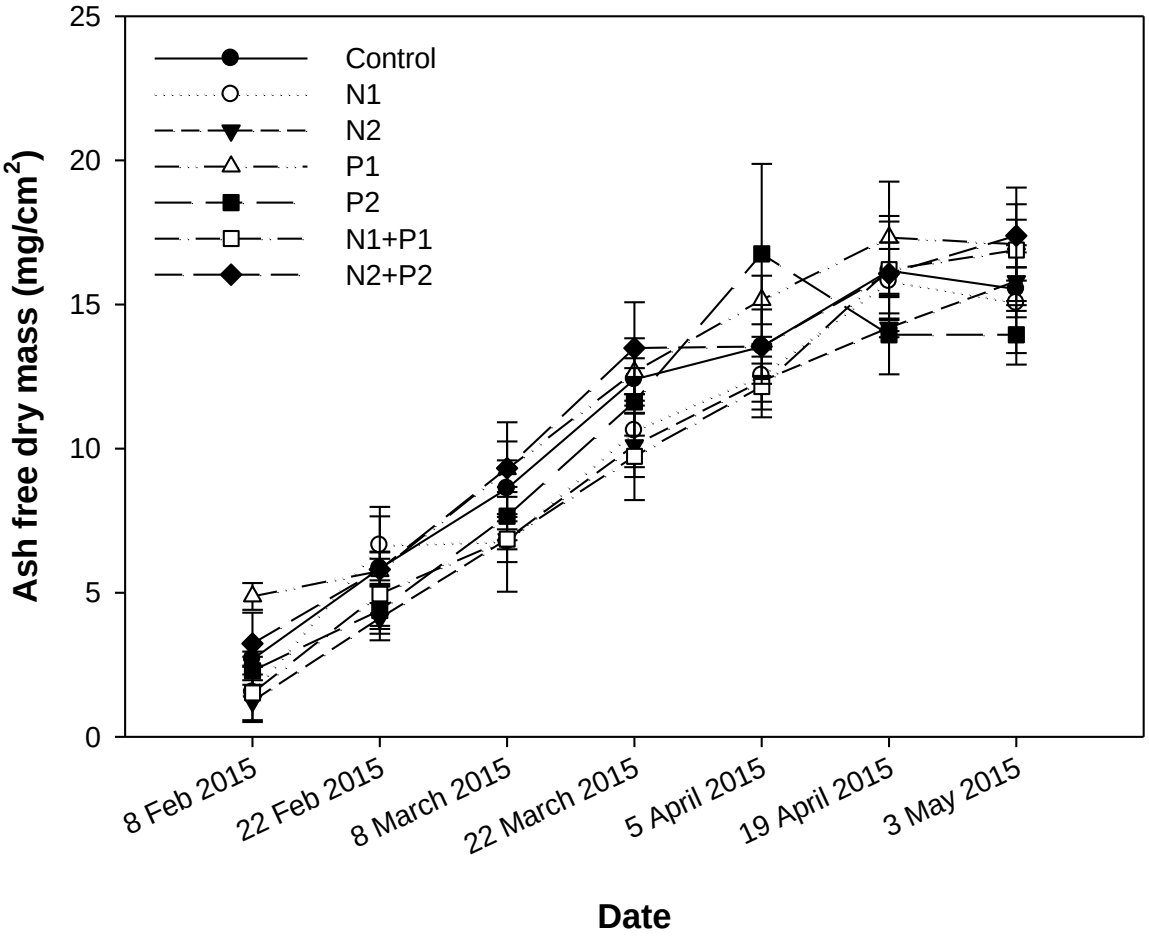

Figure 4-9. Ash free dry mass (AFDM, mean±S.E.) of *Didymosphenia geminata* as a function of time for the period 8 February 2014 to 19 April 2015 for six N and P concentrations (phosphorus P1-0.35 µg P/L, P2-0.7 µg P/L, N1-25 µg N/L, N2-50 µg P/L, N1/P1-25 µg N/L + 0.35 µg P/L, and N2/P2-50 µg N/L + 0.7 µg P/L) and a control (no N or P addition) at the University of Idaho mobile experimental flume system (MEFS) located below Libby Dam, Libby, MT, USA. Data on 8 February 2015 and 3-May 2015 represent pre- and post-treatment samples.

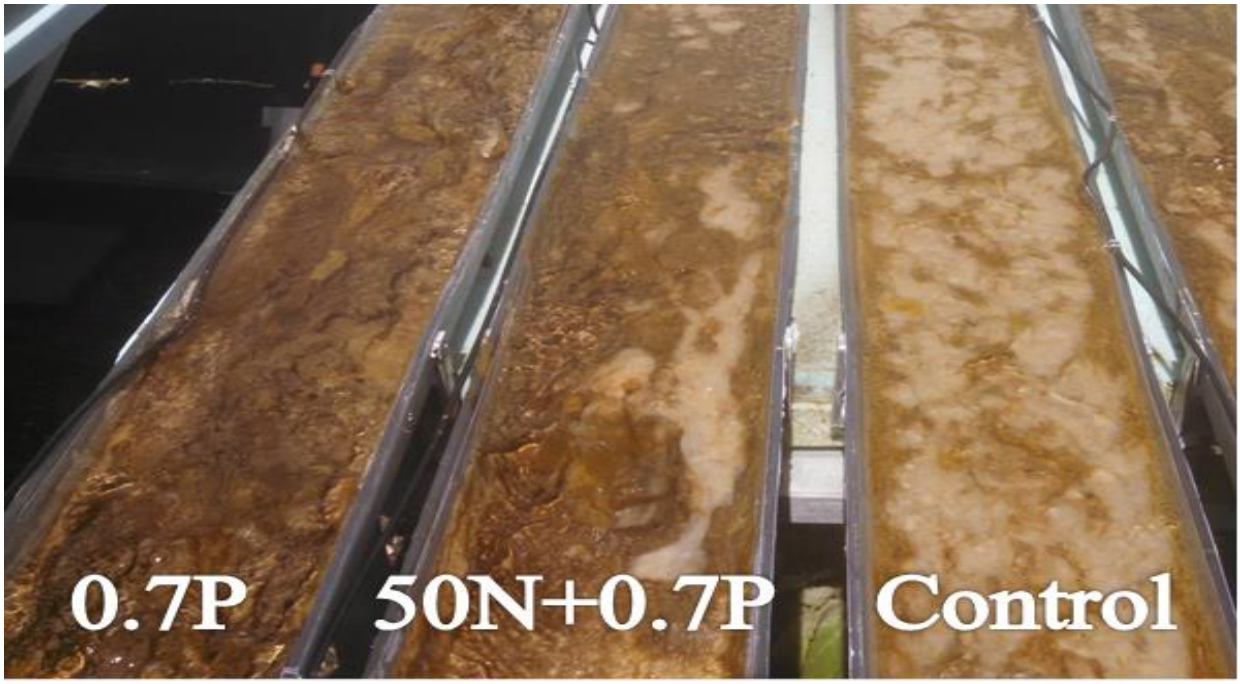

Figure 4-10. *Didymosphenia geminata* growth response to 0.7 µg/L dissolved phosphorus (left), 50 µg/L dissolved nitrogen + 0.7 µg/L dissolved phosphorus (center), and no nutrient addition (right) at the University of Idaho mobile experimental flume system (MEFS) located in Libby, MT, USA. White algal growth observed in the control (right) is *Didymosphenia geminata* excessive stalk production and mat formation.

Chapter 5: Dissolved phosphorus enrichment for the suppression of Didymo (*Didymosphenia geminata*) nuisance mats in the Kootenai River, Libby, MT.

Abstract

Nuisance mats of *Didymosphenia geminata* have occurred in the Kootenai River near Libby, Montana since the early 2000s. The diatom produces mucopolysaccharide stalks which compose the majority of the mat material which degrades the aesthetic and recreational values and ecological functions of the river in the tailwater of the Libby dam. As part of a follow-up study to a series of mesocosm experiments in which the addition of phosphorus resulted in reduced stalk lengths, an in-river dissolved phosphorus (P) enrichment was completed in the spring of 2014 to test the hypothesis that the addition of phosphorus at the river scale would reduce the nuisance mat coverage. The addition of 108.41 kg of struvite (CrystalGreen™) over 18 days increased the available phosphorus by approximately 0.8 µg/L above ambient river concentrations. After 14 days, P enrichment significantly suppressed mat depth and coverage for ~300 m downstream of the release site and resulted in nuisance mat detachment in several areas. These results suggest that P enrichment is a potential management strategy for nuisance mats in oligotrophic lotic systems. Because no whole-river management policies exist currently for *D. geminata* nuisance mats in river systems with important fisheries, this study provides a starting point to examine this potential strategy.

Introduction

Historically present in circumboreal regions of the world, *Didymosphenia geminata* has in some instances smothered the benthos of lotic systems with nuisance mats resulting from its excessive production of mucopolysaccharide stalks. The section of the Kootenai River near Libby, MT, USA, first experienced nuisance mats in the early (Spaulding and Elwell 2007) in the early 2000s, and the mats have been observed frequently since. These nuisance mats are not only visually unappealing, but also correlated with a significant shift in the macroinvertebrate community structure from large- to small-bodied species (e.g., Marshall 2007). This shift in the benthic macroinvertebrate community has caused concern that it could negatively affect salmonid fisheries and the endangered sturgeon species present in the river that have typically relied on the presence of large-bodied invertebrates. Consequently, managers are interested to find methods to reduce the severity or eliminate the occurrence of *D. geminata* mats.

Research by other investigators (Bothwell and Kilroy 2011; Kilroy and Bothwell 2011; Kilroy and Bothwell 2012) indicates that the addition of phosphorus (P), especially in oligotrophic systems, stopped the excess production of stalks and caused an increase in the frequency of dividing cells. In a series of experiments between the spring of 2013 and autumn of 2014, I experimentally examined the hypothesis that the addition of dissolved P reduced the stalk length of *D. geminata* in the Kootenai River at the mobile experimental flume system (MEFS) near the Libby Dam (Chapter 3). These experiments provided evidence that P enrichment of at least 0.5 µg/L above ambient Kootenai River background conditions removed the phosphorus limitation and subsequently suppressed *D. geminata* stalk length and mat growth suggesting that the addition of P may be one management strategy.

While James et al. (2015) reported that adding P at a calculated 6 µg/L above ambient to Rapid Creek in South Dakota decreased *D. geminata* biomass for 0.6 km downstream of the release site, results of my flume experiments demonstrated that a much lower concentration achieved the goal of reducing *D. geminata* mat growth in the MEFS at the Kootenai River. However, to examine if results from my flume experiment were applicable at the river scale, I conducted a small-scale experiment in part of the Kootenai River immediately downstream of the dam to test the hypothesis that the addition of P at a target concentration of 1.0 µg/L above ambient would reduce the abundance of *D. geminata* biomass over time. I used an approach similar to that of Ashley and Stockner (2003) and Sterling et al. (2000), whereby P was added in a solid form (struvite) that dissolved slowly to one side of the river to achieve the target concentration, while the opposite side served as a control.

Methods

Study area

The Kootenay River originates in British Columbia, Canada, and flows south to enter Koocanusa Reservoir which straddles the Canada/US border in northwestern Montana. It emerges from the reservoir as the Kootenai River, travels northwest into Idaho, and then returns north to enter Kootenay Lake near Creston, British Columbia, Canada (Fig. 5-1). Once closed, Libby Dam created the 145 km long Koocanusa Reservoir, which due to its length allows sediment and suspended material to settle, removing 63% of total phosphorus, 24% total nitrogen, and 95% of suspended sediments from the Kootenay River (Hoyle et al. 2010), which leaves the water below the reservoir ultraoligotrophic and phosphorus-limited. After closure of Libby Dam in 1972, the trophic status of the river downstream of the dam changed

from eutrophic to ultraoligotrophic (phosphorus-limited) with an in-river soluble reactive phosphorus (SRP) range from <0.5 to 1 µg/L with a detection limit of 1 µg/L (KTOI 2014; Ashley and Stockner 2003).

First noted by John Keast Lord in 1866, the Kootenai River has one of the oldest records of *D. geminata* as part of the periphyton community in North America. It was not until the early 2000s when the presence of nuisance mats of *D. geminata* in the Kootenai River were first noticed, when they began to appear on sampling gear (Holderman and Hardy 2004). The formation of nuisance mats is the result of the excessive production of mucopolysacchride stalk material (Ellwood and Whitton 2007; Chapter 1). Currently, extreme nuisance mat growth of *D. geminata* (100% benthic coverage, 2-5 cm) is observed directly below the Libby Dam and continues downriver for approximately 46.5 river km (29 river miles) at a decreasing gradient of intensity into Idaho. The study reach for the phosphorus enrichment was below the Libby Dam for approximately 1.2 km.

Phosphorus enrichment

To examine the effect of elevated in-river P on the growth of *D. geminata* in the Kootenai River, I established experimental and reference sites at 100 m intervals along both banks (Fig. 5-2). To determine flow paths and lateral mixing to estimate the amount of P needed to achieve a target of 1 µg P/L above ambient at an outflow rate from the dam of approximately 113.4 m^3/s (4000 cfs), flow cross-sections and a florescent trial were completed on 19 February and 6 March 2015, respectively (Fig. 5-3). The dye tracing showed that there was no water mixing between the left and right banks, which led me to conclude that the left bank (when facing downstream) was be a suitable reference location.

Pre-treatment sampling of all sites was completed on 23 March 2015. Starting at the most downstream site, mat depths and river coverage (Kilroy et al. 2005), and algae scrapings (Appendix 2) were taken at each sampling location. At each sampling location, mat depth was measured five times on three rocks below the water line on each sampling occasion. Percent mat coverage was estimated for each rock to calculate average mat depth and standing crop index (SCI) (Kilroy et al. 2005).

On 24 March 2015, 727 kg of slow-release CrystalGreen™ ($MgNH_4PO_4 \cdot 6H_2O$, Ostara Nutrient Recovery Technologies, Vancouver, B.C., Canada) was repacked into 49 0.35×0.66 m (14”×26”) burlap bags each weighing 13-18 kg. These bags were placed in the river attached with zip ties at 0.5 intervals along two strands of polypropylene rope; placed in parallel approximately 3 m from the shore, approximately 150 m upstream of the first sampling location on the right bank (when facing downstream). I used release estimates established by Sterling et al. (2000) who examined the release of CrystalGreen™ under a variety of velocity, temperature, and water chemistry conditions, for those that most closely corresponded to those in the Kootenai River.

The first sampling location was located below the David Thompson Bridge on the downstream right bank approximately 500 m below the dam. An additional eight sampling sites were located downstream of the release site at 100 m intervals, while two reference sites were located on the left bank opposite treatment sites 1 and 2 (Fig. 5-2). After placement of the P bags, samples (see methods above) were collected weekly on 24 and 31 March, and 6 April 2015 after which water release from Libby Dam was increased to meet downstream water requirements in the Columbia River Federal Power System, making it impossible to access the sample sites. This terminated the experiment. Before discharge increased, all bags

containing CrystalGreen™ were removed from the Kootenai River, dried and reweighed to determine the amount of P released over the 3-week period during which they were deployed.

Sites R1 (immediately downstream of in-river treatment on the right Bank of the river) and L1 (reference site 1 on the left bank of the river) were re-examined visually on November 2015, 8 months after treatment, to determine qualitatively to see if there were any carry-over effects from the treatment.

Statistical analysis

Data were analyzed for the period of the nutrient addition. The standing crop index and *D. geminata* mat depth were averaged for each of the five samples per rock on each sampling occasion and analyzed over time with a completely randomized repeated measures analysis of variance (ANOVA) with standing crop index or mat depth as the response variable, position within the river (exposure to P) as the treatment, and time as the repeated factor. These statistical analyses were completed with procedure MIXED in SAS software™ v. 9.4 (SAS Institute, Cary, North Carolina). In the repeated measures ANOVA, the fixed effect was the position within the river and time while the random effect was rock from which samples were taken.

Analyses of variance (ANOVA) was used to compare mat depth and SCI responses to P treatment at a weekly interval (one sampling event). These statistical analyses were completed with procedure MIXED in SAS software™ v. 9.4 (SAS Institute, Cary, North Carolina). A pair-wise comparison of position within the river (as in exposure to P) was completed using the least square means (LSmeans) procedures. The fixed effect was position within the river, while the random effect was the rock from which samples were taken.

Results

The dye tracing showed that no lateral mixing occurred between the left and right banks (Fig. 5-3). It also showed that the water moved in a tight "packet" down the right bank past the proposed sites. The discharge calculated from the cross-section and velocity measurements showed that approximately ¼ of the discharge from the dam's 113.2 m^3/s (4000 cfs) passed the release site and moved down the right bank of the river. Subtraction of the recovered dried CrystalGreen™ showed that 108.4 kg dissolved over the 18-day experimental period yielding an increase of ~0.8 µg P/L above ambient. This was close to my target of 1 µg P/L and above the 0.5 µg P/L saturation concentration for *D. geminata* determined in Chapter 3 above.

Between 22 January and 23 March 2015, the *D. geminata* mat coverage and depth in the Kootenai River increased (data not shown) in accordance with the seasonal cycle of *D. geminata* (see Chapter 3). Time × position, position, and time were significant (Table 5-1; Fig. 5-5), while mat depth at R1 and R2 was significantly lower compared to the control sites (Table 5-2). I used the mat depth on 23 March 2015 as depth at time zero which was consistent across all sites except at site R2 where it was slightly (5-8 mm) shallower (Tables 5-3; Fig. 5-4). Mat depth at the reference sites was consistently 25-30 mm during the three weeks of the study (Fig. 5-4).

A statistically significant ($P < 0.001$) decline in mat coverage and depth that was visually noticeable occurred after one week at the treatment sites (Fig. 5-4). The R1 treatment site had much less coverage of *D. geminata* and its mat depth was lower compared to reference site L1 (Tables 5-4). No reduced mat coverage or depth was observed at the R2 treatment site or at any of the other downstream sites (Fig. 5-4). After the second week of

treatment, large mats of *D. geminata* previously present at treatment sites R1 and R2 were absent, and the mat depth and coverage at sites R1, R2, and R3 were significantly lower compared to the reference site ($P<0.001$) (Fig. 5-4; Tables 5-5). Site R8 also had less mat coverage and depth than the reference sites (Table 5-5).

The standing crop index (SCI) exhibited a pattern similar to mat depth (Figs. 5-6 and 5-7; Tables 5-6 and 5-7). Time 0 data were collected on 23 March 2015 (Tables 5-8). After one week of treatment, the SCI at treatment site R1 was significantly lower than at the reference site L1 ($P < 0.001$; Table 5-9), while no differences were observed downstream of the R2 site (Table 5-9). After the second week of treatment, the SCI at treatment site R1, R2 and R3 was significantly lower than the reference sites ($P<0.001$; Table 5-10).

After the P addition was stopped (after 18 days of treatment), a visual assessment of the treatment sites showed that the mat was greatly reduced or gone at treatment sites R1, R2 and R3 compared to the control site (Fig. 5-8).

The in-river treatment not only reduced *D. geminata* mat coverage, but also increased diatom diversity as observed visually, but not quantified, by the increased green color throughout the beige *D. geminata* mats (Fig. 5-9). The increased species diversity as evidenced by the darker green color and the presence of filamentous green species at sites R1 and R2, not observed at the control, was similar to the trends observed in the MEFS during P enrichment (see Chapter 3; Table 3-1) which was due to an increased diversity of other periphyton species.

Eight months after the treatments stopped (November 2015), mats were present at both R1 and L1 sites, however, there were visually observable differences between the sites though these were not quantified in any way. The reference L1 site had many more pieces of mat that

extended 2-5 cm into the water column off the substrate, while at the treatment R1 site, mat thickness was much reduced (Fig. 5-10).

Discussion

Overall, I was surprised by the rapid decline of the *D. geminata* mat at sites R1 and R2 over the relatively short 18-day duration of the in-river P release experiment. The sloughing of the mats observed in the river differed from the response observed in the flumes. This may be related to the substrate type – rock vs open-celled foam – or the higher discharge in the river, but the mechanism remains to be elucidated. This response demonstrates the importance of examining the response of *D. geminata* mats across a variety of scales in the quest to find an optimal strategy to control it at the river-scale. While the pilot in-river slow release of P had an effective downstream reach of approximately 300 m after 8 days, I expect this would extend further downstream had the experiment lasted longer. I hypothesize that the *D. geminata* mat closest to the P source quickly took up the increased P available in the water and P saturation progressively moved downstream over time. It is not known if saturation with P changed the growth dynamics that resulted in the mat sloughing at sites R1 and R2 during the experiment. The loss of biomass at sites R1 and R2 would also reduce the uptake of P and allow the treatment effect to reach further downstream. The decreased mat depth and SCI at site R8 relative to the controls and R7 is attributed to previous exposure of phosphorus over the past two years as the site is located below the discharge site of the MEFS (see Chapter 3).

With a longer experimental time, I would expect that the mat would continue to saturate successively downstream and extend the downstream effectiveness. Given the dissolution rate of approximately 0.98 g/day of CrystalGreen™ (Sterling et al. 2000; Table 5-

11) or the calculated dissolution of 108.4 kg over 18 days (6.02 kg/day), the amount I added would have required ~120 days to dissolve completely after which I could have determined the effective maximum downstream distance of the treatment. Because the experiment was terminated early due to increased discharge from the dam, I recommend that it be repeated when a longer time period of low flows is available.

The appearance of other diatom and periphyton species after the *D. geminata* mats disappeared probably indicates the availability of soluble P in the ultra-oligotrophic Kootenai River below Libby Dam due to the nutrient addition. Species replacement and growth will require additional experiments to determine if it becomes problematic as seen in the flumes (see Chapter 3). It would be counterproductive if the solution to reducing mats of *D. geminata* was the excessive proliferation of other species. However, based on the flume experiments, such growth was not excessive not excessive (AFDM <100 mg/cm^2) at a P concentration of < 1 µg/L above ambient and was the reason for my target of 1 µg/L. It may be possible that the removal of *D. geminata* acts much like the replacement of cyanobacteria with small green algae which are edible by zooplankton in lentic waters and allows energy to be efficiently transferred along the food web to higher trophic levels (e.g., Harris et al. 2014). Such a cascade may also occur with the removal/replacement of *D. geminata* in the river and should be examined by also surveying the benthic invertebrate community. However, this may be difficult in the Kootenai River given the large size of the rip rap substrate that is accessible to wading at the edge of the river at and just downstream of the release site. The appearance of other periphytic algae also deserves further examination in terms of how much of the elevated dissolved P they would remove from the water column, as this could contribute to limit the effective downstream distance of the P addition.

The downstream response of 300 m during this short experiment using the slow release CrystalGreen ™ rivals the 600 m effective distance reported by James et al. (2015) in Rapid Creek using rapid release mono-ammonium phosphate (MAP 11:52, N:P) for 4 months. James et al. (2015) also used Osmocote (N:P:K 14:14:14), a pelletized slow-release fertilizer for 5 months, in a 2007 study for which they reported a decreased index of *D. geminata* biomass (IDB) with distance downstream of the release site. Although James et al. (2015) argued that the inclusion of N in their experiments was not expected to influence the response of *D. geminata*, my flume studies (Chapter 4) suggest that the presence of N does matter. Consequently, I would strongly advocate not using any materials containing N in additions in the Kootenai River.

I conclude that this pilot in-river study was successful and believe that the deployment of slow-release P to achieve a target concentration of 1 µg/L above ambient can be used to reduce the severity of the *D. geminata* mat below Libby Dam in the Kootenai River. However, given the short duration of the experiment, I recommend that additional longer-duration in-river studies be completed to more fully assess long-term response of the biotic community. I also recommend that any future studies include an examination of the macroinvertebrate community to understand its response, especially if P release increases the occurrence and/or abundance of other periphyton species.

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Tables

Table 5-1. Repeated measures ANOVA for *Didymosphenia geminata* mat depth in the Kootenai River at the Kootenai River Research Station in Libby, MT, USA, from 23 March 2015 to 6 April 2015.

Effect	*F* value	*P*
Position	39.95	<0.001
Time	5.12	0.008
Time × Position	10.33	<0.001

Table 5-2. *Post-hoc* pair-wise comparison of *Didymosphenia geminata* mat depths in the Kootenai River at the Kootenai River Research Station in Libby, MT, USA, after two weeks of a phosphorus addition from 23 March 2015 to 6 April 2015.

Position	Control	R1	R2	R3	R4	R5	R6	R7
R1	0.001							
R2	0.001	0.007						
R3	0.128	0.001	0.001					
R4	0.001	0.001	0.001	0.001				
R5	0.001	0.001	0.001	0.001	0.448			
R6	0.334	0.001	0.001	0.035	0.002	0.015		
R7	0.184	0.001	0.001	0.862	0.001	0.001	0.051	
R8	0.001	0.041	0.460	0.001	0.001	0.001	0.001	0.001

Table 5-3. *Post-hoc* pair-wise comparison of *Didymosphenia geminata* mat depths in the Kootenai River at the Kootenai River Research Station in Libby, MT, USA, treatment on 23 March 2015.

Position	Control	R1	R2	R3	R4	R5	R6	R7
R1	0.369							
R2	0.001	0.002						
R3	0.948	0.404	0.001					
R4	0.650	0.698	0.001	0.653				
R5	0.254	0.081	0.001	0.350	0.169			
R6	0.658	0.248	0.001	0.744	0.439	0.541		
R7	0.294	0.095	0.001	0.393	0.195	0.935	0.596	
R8	0.042	0.340	0.025	0.068	0.163	0.007	0.033	0.009

Table 5-4. *Post-hoc* pair-wise comparison of *Didymosphenia geminata* mat depths in the Kootenai River at the Kootenai River Research Station in Libby, MT, USA, after one week of phosphorus treatment on 30 March 2015.

Position	Control	R1	R2	R3	R4	R5	R6	R7
R1	0.001							
R2	0.902	0.001						
R3	0.002	0.001	0.009					
R4	0.001	0.001	0.001	0.007				
R5	0.002	0.001	0.010	0.964	0.006			
R6	0.354	0.001	0.364	0.001	0.001	0.001		
R7	0.311	0.001	0.326	0.001	0.001	0.001	0.939	
R8	0.001	0.017	0.003	0.001	0.001	0.001	0.027	0.032

Table 5-5. *Post-hoc* pair-wise comparison of *Didymosphenia geminata* mat depths in the Kootenai River at the Kootenai River Research Station in Libby, MT, USA, after two weeks of phosphorus treatment on 6 April 2015.

Position	Control	R1	R2	R3	R4	R5	R6	R7
R1	<0.001							
R2	<0.001	0.274						
R3	<0.001	0.048	0.360					
R4	0.268	<0.001	<0.001	<0.001				
R5	0.087	<0.001	<0.001	<0.001	0.587			
R6	0.077	<0.001	<0.001	<0.001	0.552	0.959		
R7	0.113	<0.001	0.001	0.006	0.022	0.006	0.005	
R8	<0.001	0.178	0.796	0.510	<0.001	<0.001	<0.001	0.001

Table 5-6. Repeated measures ANOVA for *Didymosphenia geminata* SCI in the Kootenai River at the Kootenai River Research Station in Libby, MT, USA, from 23 March 2015 to 6 April 2015.

Effect	***F* value**	***P***
Position	38.94	<0.001
Time	11.87	<0.001
Time × Position	12.99	<0.001

Table 5-7. *Post-hoc* pair-wise comparison of *Didymosphenia geminata* standing crop index (SCI) in the Kootenai River at the Kootenai River Research Station in Libby, MT, USA, after two weeks of a phosphorus addition trial from 23 March 2015 to 6 April 2015.

Position	Control	R1	R2	R3	R4	R5	R6	R7
R1	0.001							
R2	0.001	0.016						
R3	0.040	0.001	0.001					
R4	0.005	0.001	0.001	0.001				
R5	0.001	0.001	0.001	0.001	0.450			
R6	0.347	0.001	0.001	0.011	0.087	0.016		
R7	0.078	0.001	0.001	0.788	0.001	0.001	0.022	
R8	0.001	0.053	0.604	0.001	0.001	0.001	0.001	0.001

Table 5-8. *Post-hoc* pair-wise comparison of *Didymosphenia geminata* standing crop index (SCI) in the Kootenai River of Libby, MT, USA, after 0 weeks of phosphorus treatment on 23 March 2015.

Position	Control	R1	R2	R3	R4	R5	R6	R7
R1	0.369							
R2	0.001	0.002						
R3	0.948	0.404	0.001					
R4	0.650	0.698	0.001	0.653				
R5	0.254	0.081	0.001	0.350	0.169			
R6	0.658	0.248	0.001	0.744	0.439	0.541		
R7	0.294	0.095	0.001	0.393	0.195	0.935	0.596	
R8	0.042	0.310	0.025	0.068	0.163	0.007	0.033	0.009

Table 5-9. *Post-hoc* pair-wise comparison of *Didymosphenia geminata* standing crop index (SCI) in the Kootenai River of Libby, MT, USA, after one week of phosphorus treatment on 30 March 2015.

Position	Control	R1	R2	R3	R4	R5	R6	R7
R1	0.001							
R2	0.905	0.001						
R3	0.002	0.001	0.010					
R4	0.001	0.001	0.001	0.008				
R5	0.003	0.001	0.012	0.965	0.007			
R6	0.366	0.001	0.375	0.001	0.001	0.001		
R7	0.090	0.001	0.115	0.001	0.001	0.001	0.479	
R8	0.001	0.099	0.001	0.001	0.001	0.001	0.005	0.029

Table 5-10. *Post-hoc* pair-wise comparison of *Didymosphenia geminata* standing crop index (SCI) in the Kootenai River of Libby, MT, USA, after two weeks of phosphorus treatment on 6 April 2015.

Position	Control	R1	R2	R3	R4	R5	R6	R7
R1	<0.001							
R2	<0.001	0.469						
R3	<0.001	0.056	0.224					
R4	0.189	<0.001	<0.001	<0.001				
R5	0.072	<0.001	<0.001	<0.001	0.009			
R6	0.063	<0.001	<0.001	<0.001	0.008	0.957		
R7	0.096	<0.001	<0.001	<0.001	0.752	0.007	0.003	
R8	<0.001	0.053	0.215	0.977	<0.001	<0.001	<0.001	<0.001

Table 5-11. Water quality parameters of the Kootenai River below Libby Dam of Libby, Montana. Data provided by the United States of the Army Corps of Engineers, Seattle District. Permission to reuse must be obtained from the rightsholder.

	Alkalinity mgCaCO3/L	Sulfate mg/L	Chloride mg/L	Calcium mg/L	Magnesium mg/L	Potassium mg/L	Sodium mg/L	Hardness mgCaCO3/L	Dissolved Copper mg/L	Dissolved Lead mg/L	Dissolved Zinc mg/L
22-May-12	121	24.4	2.49	37.1	9.34	0.822	3.76	131	0.001	0.001	0.005
23-Jul-12	101	15.3	1.17	28.5	7.67	0.500	1.66	103	0.001	0.001	0.005
24-May-11	131	28.3	3.13	37.2	10.2	0.644	4.37	135	0.001	0.001	0.005
26-Jul-11	101	14.4	1.15	6.95	6.28	0.500	1.56	43.2	0.001	0.001	0.005
19-Oct-11	103	16.1	1.17	24.0	7.33	0.500	1.96	90.2	0.001	0.001	0.005
25-May-10	122	26.6	3.03	26.7	10.1	0.627	3.05	108	0.001	0.001	0.005
10-Aug-10	109	21.0	1.86	28.1	9.18	0.500	2.61	108	0.001	0.001	0.005
26-Oct-10	114	22.3	2.05	26.1	8.63	0.500	2.58	101	0.0002	0.001	0.005
Average	111.248	21.050	2.006	26.831	8.591	0.574	2.694	102.425	0.001	0.001	0.005

Figures

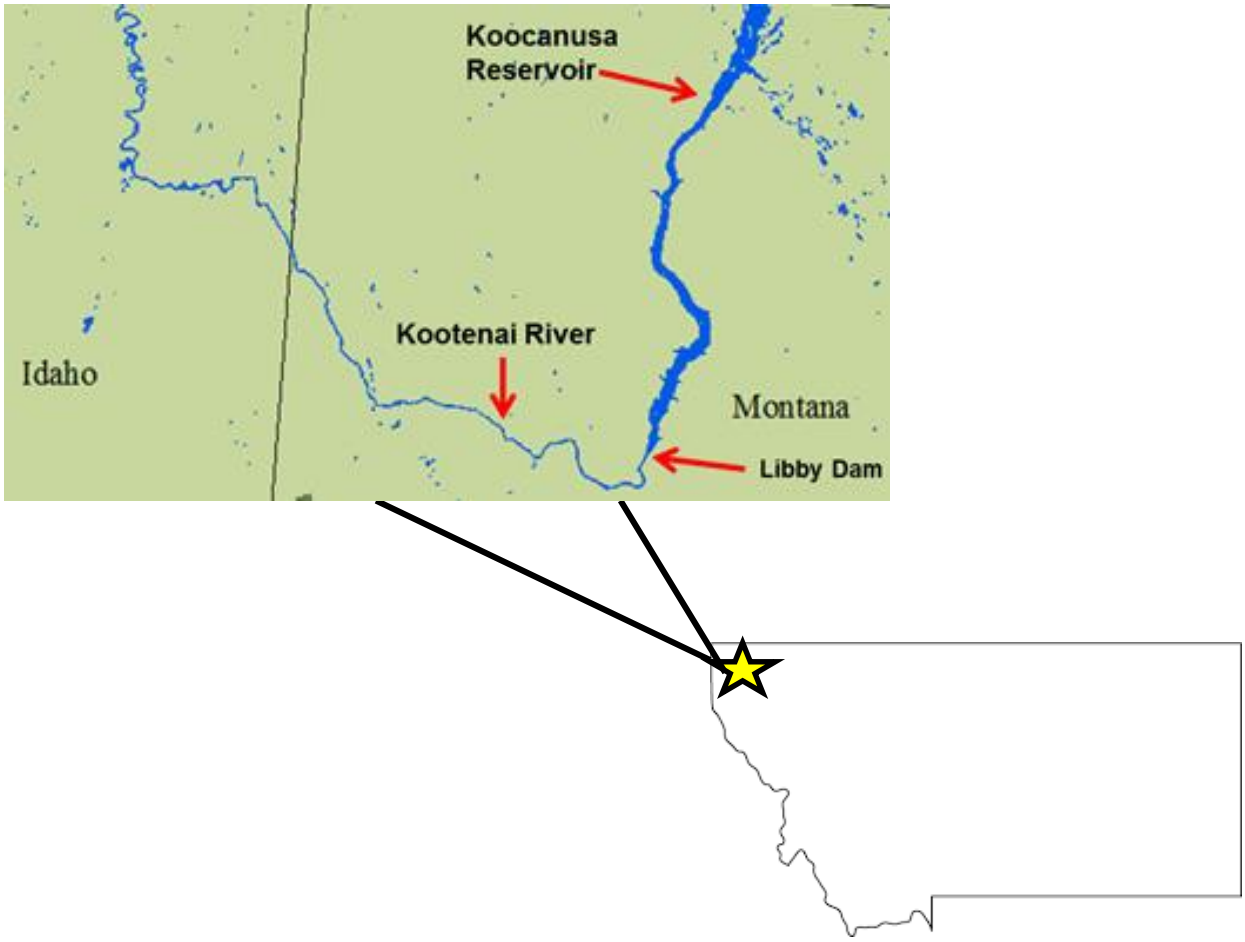

Figure 5-1. Map of the Kootenai River system in northwest Montana, USA. Study area is located immediately below the Libby Dam.

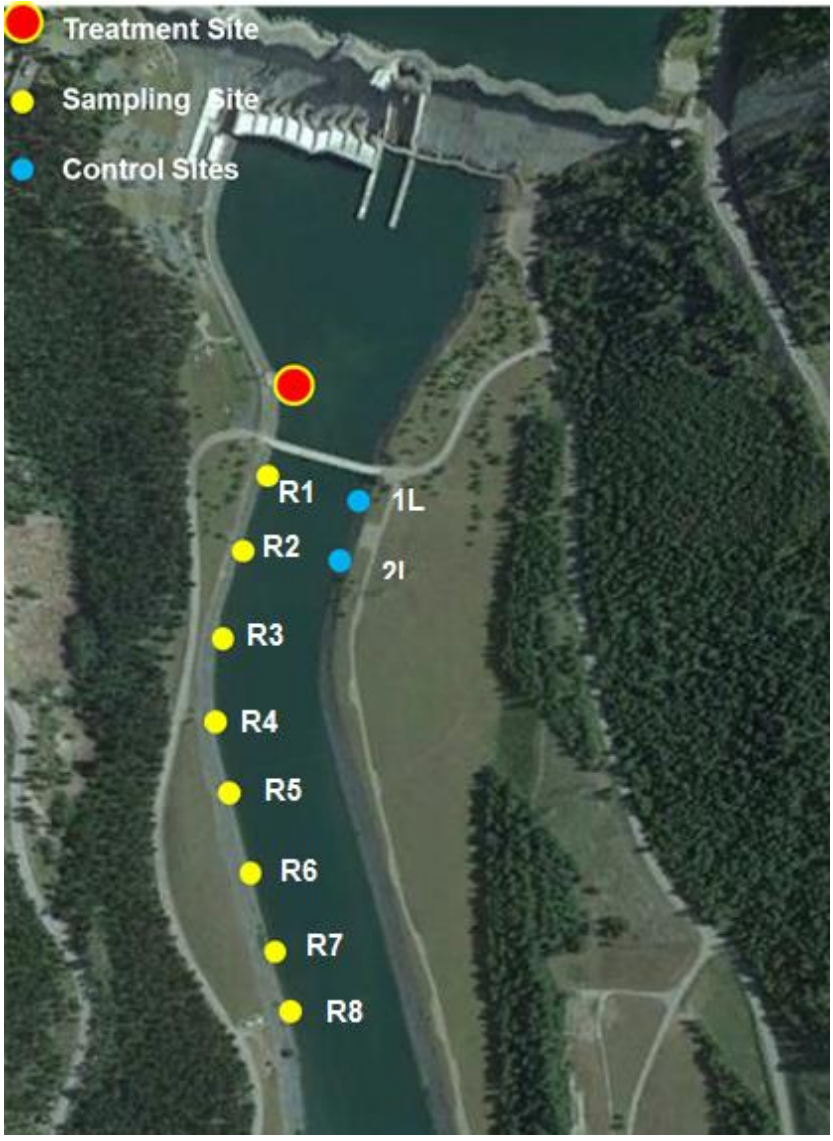

Figure 5-2. Treatment sampling locations (solid yellow dots), reference sampling locations (blue dots) and release site (red dot) for the in-river pilot experiment to reduce *Didymosphenia geminata* by adding CrystalGreen™, slow-release phosphorus crystals to the Kootenai River below Libby Dam (top of photo) in Libby, MT, USA.

Figure 5-3. A fluorescent dye trial in the Kootenai River Libby, MT, USA, on 19 February 2015 to confirm water currents, flow paths, and lateral mixing downstream of the intended in-river release site.

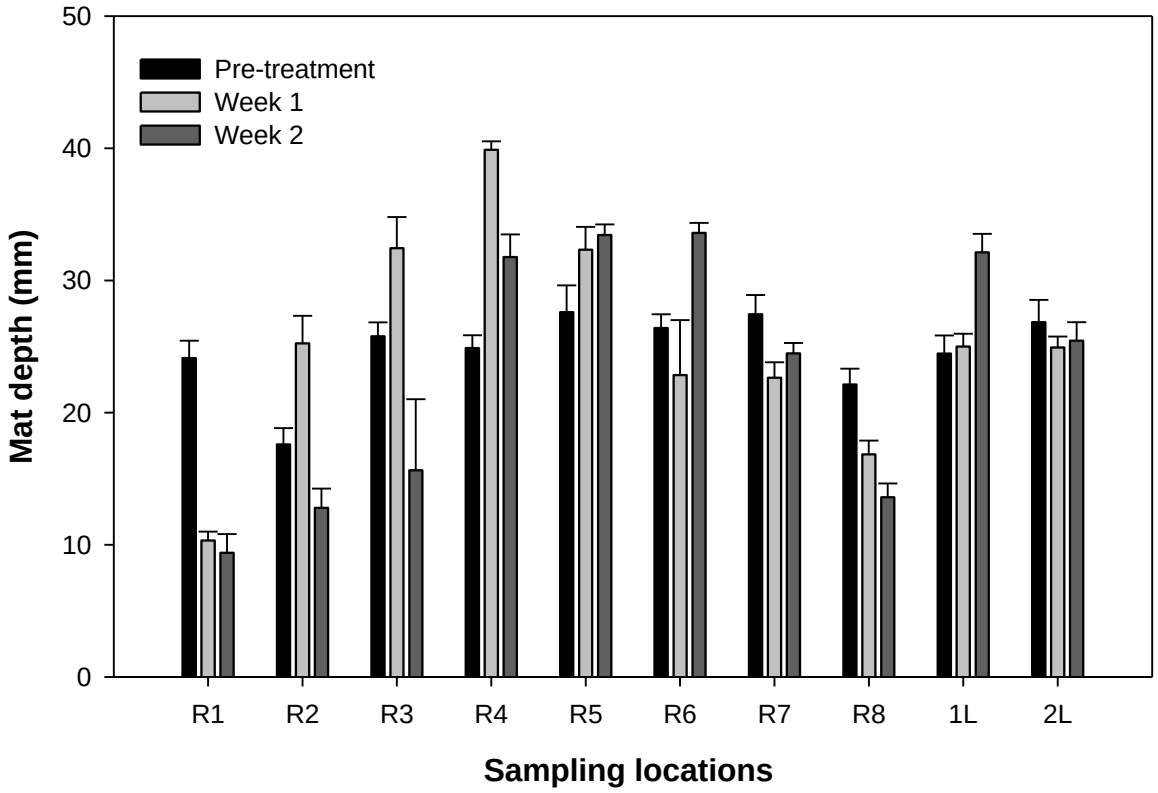

Figure 5-4. Mat depth (mean±S.E.) of *Didymosphenia geminata* at sampling locations R1 through R8 downstream of the in-river P release site and at control sites L1 and L2 on opposite side of river from release site during the slow-release phosphorus experiment in the Kootenai River, Libby, MT, USA. Pre-treatment sampling occurred on 23 March 2015 while 30 March and 6 April 2015 are after one and two weeks of P treatment, respectively.

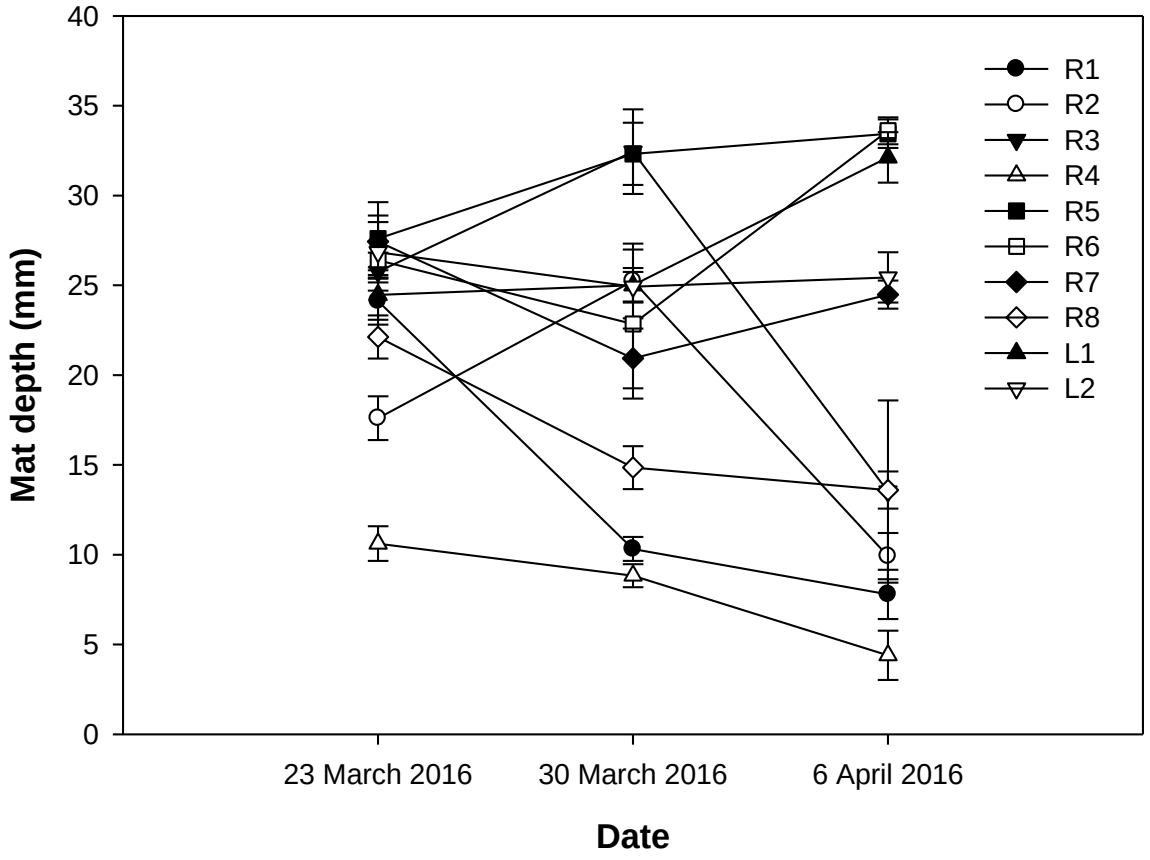

Figure 5-5. Interaction plots of mat depth (mean±S.E.) of *Didymosphenia geminata* at sampling locations R1 through R8 downstream of the in-river P release site and at control sites L1 and L2 on opposite side of river from release site during the slow-release phosphorus experiment in the Kootenai River, Libby, MT, USA. Pre-treatment sampling occurred on 23 March 2015 while 30 March and 6 April 2015 are after one and two weeks of P treatment, respectively.

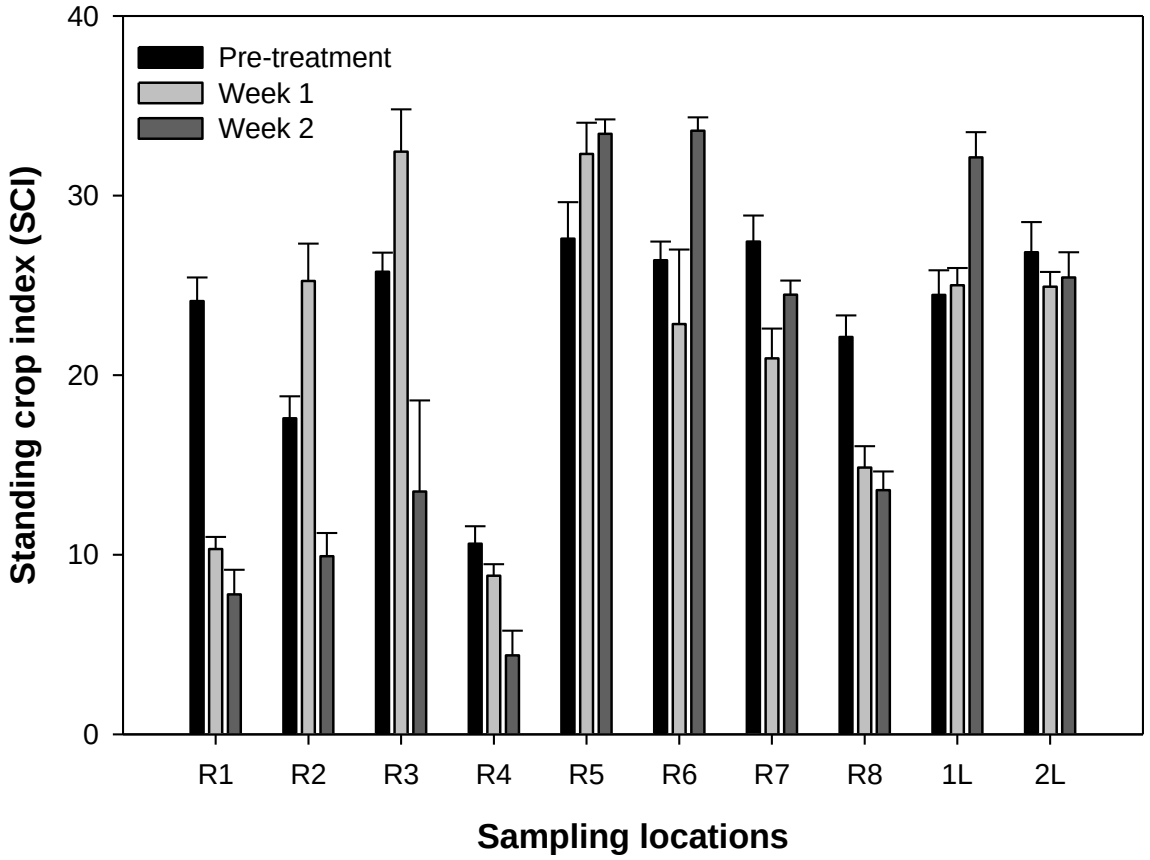

Figure 5-6. Standing crop index (SCI) (mean±S.E.) of *Didymosphenia geminata* at sampling locations R1 through R8 downstream of the in-river P release site and at control sites L1 and L2 on opposite side of river from release site during slow-release phosphorus experiment in the Kootenai River, Libby, MT, USA. Pre-treatment sampling occurred on 23 March 2015 while 30 March and 6 April 2015 are after one and two weeks of P treatment, respectively.

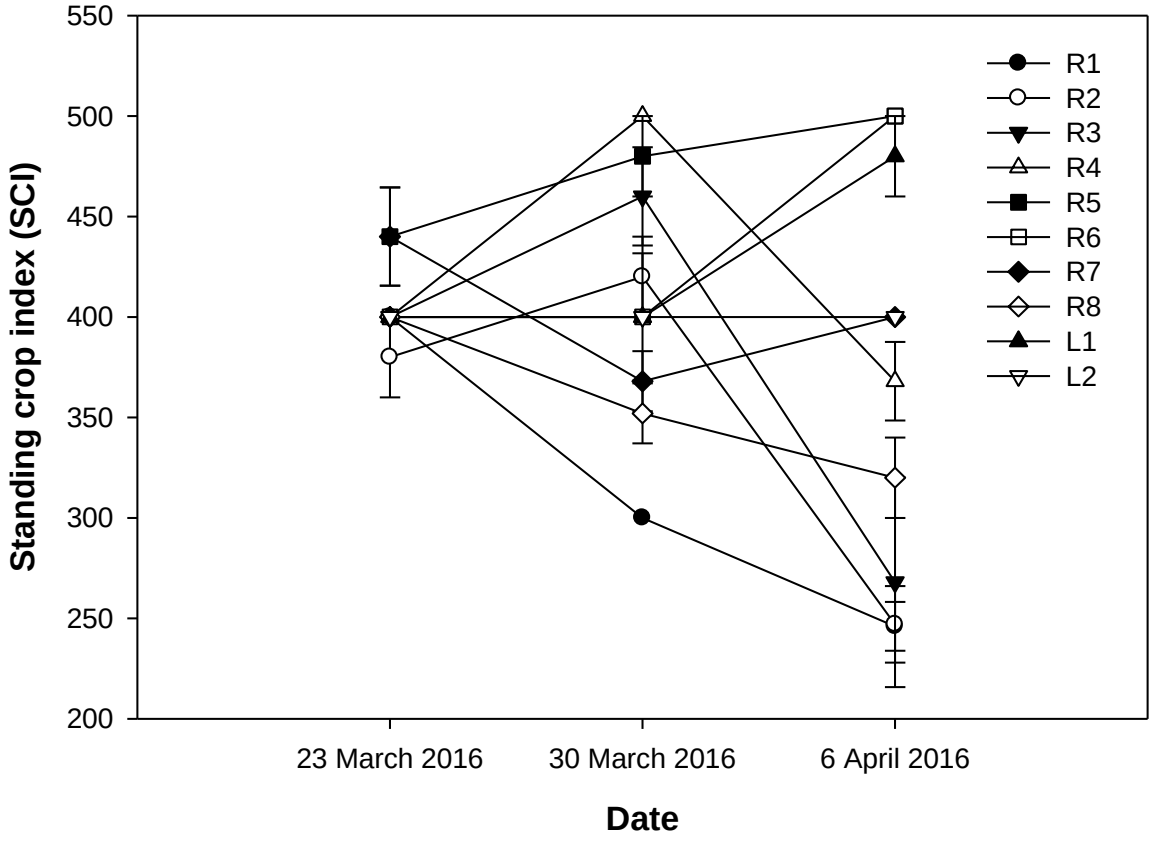

Figure 5-7. Interaction plots of standing crop index (SCI) (mean±S.E.) of *Didymosphenia geminata* at sampling locations R1 through R8 downstream of the in-river P release site and at control sites L1 and L2 on opposite side of river from release site during slow-release phosphorus experiment in the Kootenai River, Libby, MT, USA. Pre-treatment sampling occurred on 23 March 2015 while 30 March and 6 April 2015 are after one and two weeks of P treatment, respectively.

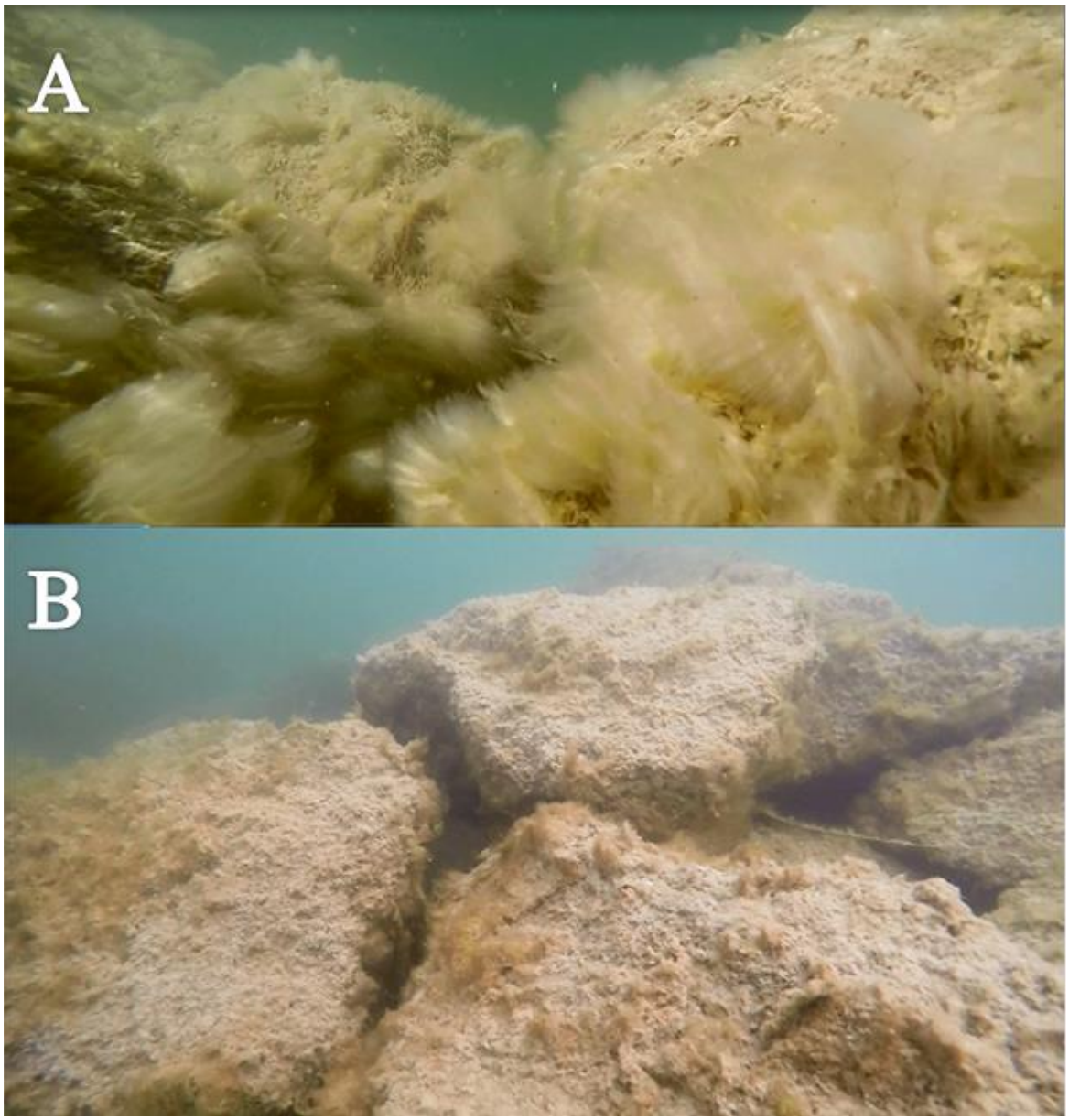

Figure 5-8. Underwater photos of the substrate and *Didymosphenia geminata* mats at the reference L1 site (A) and the treatment site R1 (B) in the Kootenai River, Libby, MT, USA after 18 days of applying a slow-release phosphorus addition at the treatment site.

Figure 5-9. Slow-release phosphorus treated section of the Kootenai River, Libby, MT, USA after 18 days. Increased algal diversity observed in the darker color of algae along the substrate indicated by white arrows.

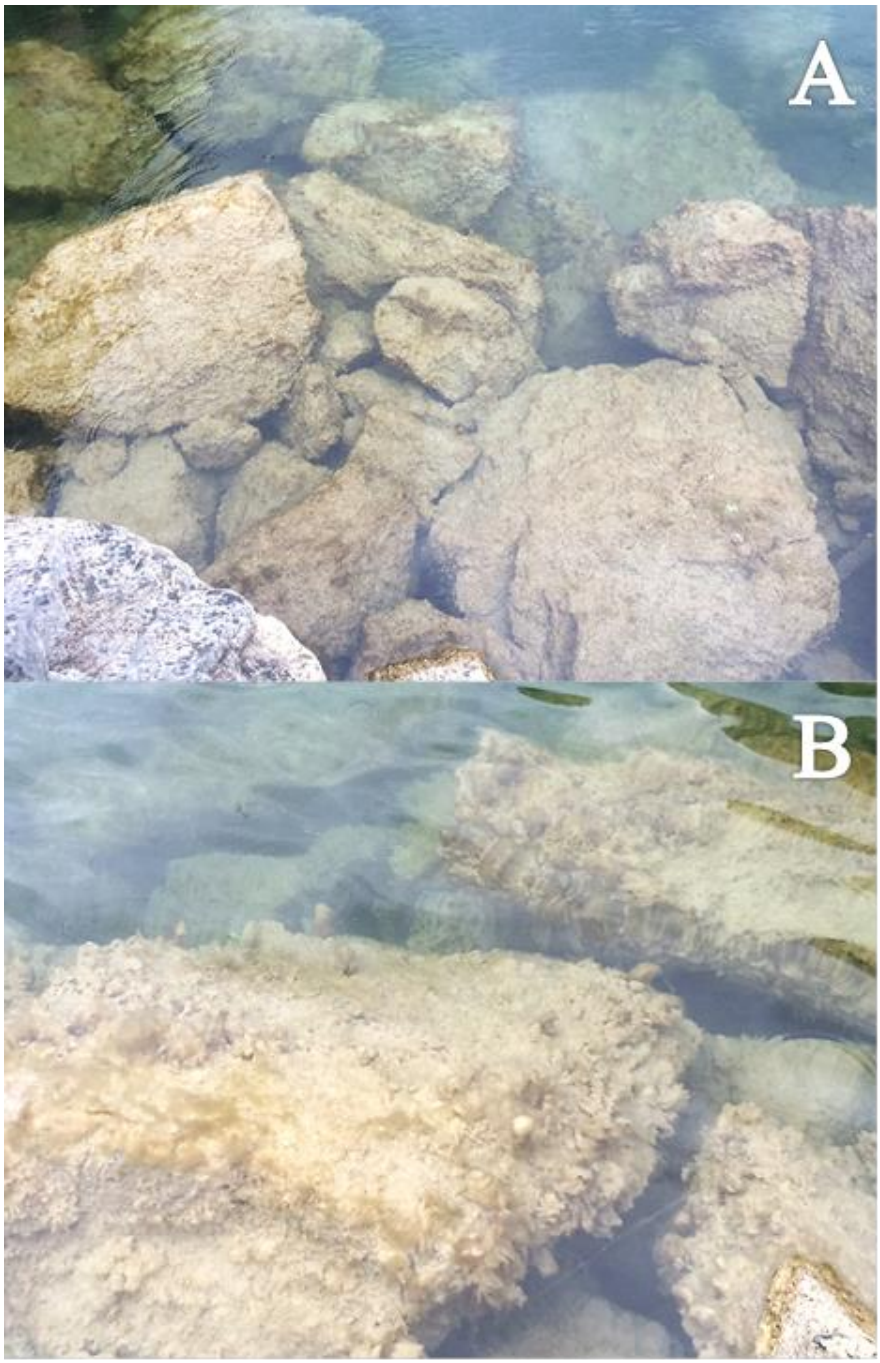

Figure 5-10. Comparison of *Didymosphenia geminata* mat coverage 8 months post in-river P addition at the treatment site R1 (A) downstream of the release site and the reference site L1 (B) in the Kootenai River, Libby, MT, USA.

Chapter 6: Management recommendations

Three years of combined mesocosm and field research summarized above have contributed to expand the understanding of the source, the ecology, and potential management strategies to reduce the severity of nuisance mats of *D. geminata* in the Kootenai River immediately downstream of Libby Dam. My surveys throughout the Kootenai National Forest and various locations in Idaho and Montana have demonstrated that *D. geminata* is a widespread, native species in the inland northwest and that it occurs most commonly in a non-mat phase (Appendix 1 and 2). After dispelling the assumption that this is an introduced species to this area, I investigated changes to the watershed that altered the growth of this native species to form nuisance mats. This research focused on changes in water quality within the Kootenai River over the past 30 years, which highlighted potential factors contributing to the formation of nuisance mats including changes in dissolved nitrogen and phosphorus and their ratio below the Libby Dam of Libby, Montana, USA (Chapter 3 and 4). The results from these studies were developed into a management strategy for state and federal management agencies of the Kootenai River.

To evaluate the presence of *D. geminata* on the Kootenai Forest and to understand the ecology and growth patterns of nuisance mats, I surveyed nuisance mat growth in tributaries to, and in the main stem of the Kootenai River (Appendix 2). Peak mat growth (at varying degrees), in unregulated tributaries to the Kootenai River occurred from late May to August. These mats began to disintegrate in September and October resulting in a “restart” of the nuisance mat community in December and January (Appendix 2). This degradation was attributed to higher flows and subsequent scouring seen during early winter as well as decreased light availability in the tributaries compared to the main stem of the Kootenai

River. Previous studies (Spaulding and Elwell 2007; Whitton et al. 2009; Cullis et al. 2012) reported that the habitat window (when visual tufts of *D. geminata* first appear) is when discharge (Q) is sufficiently low to allow cell adhesion onto substrate, when there is sufficient light availability to promote stalk growth from high photosynthesis, and when nutrient stress occurs. The requirement for high light availability paired with nutrient stress (low phosphorus or high nitrogen to phosphorus ratio) contributes to the production of stalk material as cells attempt to access portions of the water column that may have the needed nutrients to increase growth via cellular reproduction (Myklestad and Haug 1972; Myklestad 1977; Myklestad 1995; Underwood et al. 2004).

The discharge range in which light availability provides a habitat window is suggested to be 50% of the maximum discharge (Q_{max}) of spring peak flows (C.A. Gillis, personal communication, Restigouche Rivers Watershed Management Council; Cullis et al. 2012). Factors that contribute to the formation of nuisance mats may not be solely changes in discharge, but also changes in landscape processes that alter the availability of phosphorus and nitrogen and the ratio at which these occur, changes in flow regimes such as earlier ice-out dates (Lavery et al. 2014), and/or the duration and timing of spring runoff (Bothwell et al. 2014). These factors need further evaluation as my research project solely focused on the concentrations of phosphorus (P) and nitrogen (N) and their ratios.

Studies of the addition of phosphorus in 2013, 2014, and 2015 at the University of Idaho mobile experimental flume system (MEFS) suppressed *D. geminata* mat growth and saturated cells with P at very low concentrations (~ 0.5 µg/L above ambient). The 2013 experiments demonstrated that although the addition of P resulted in higher total cell counts of *D. geminata*, it did not result in increased nuisance mats, even after the addition of P was

stopped. Because nuisance mats result from the production of stalk material, management strategies should focus to suppress stalk growth regardless of total cell counts. Thus, adding small amounts of P at certain times of the year (typically when other algae are light and/or temperature limited such as in winter) may be a viable strategy for whole-river treatment without too many unintended consequences such as stimulating the prolific growth of other algae. In addition, P enrichment should target mats of *D. geminata* that exist currently and grow actively during the year – primarily those in littoral areas with high light penetration. I also suggest that future research examine pulse dosing of P during peak growth to determine if suppression effects hold through the summer season. I hypothesize that if properly timed, the addition of phosphorus will not only suppress the growth of nuisance mats, but prevent nuisance mats for the rest of the summer season. However, while the addition of P may suppress the growth of *D. geminata* stalks that results in nuisance mats, the continued increase of nitrogen at the landscape scale may increase the amount of phosphorus needed to suppress nuisance mats. This could result in the growth of other algae at nuisance levels (eutrophication). It requires further investigation.

Because P concentrations have remained consistently low before, during and after the emergence of nuisance *D. geminata* mats in the Kootenai River (pre-2000 and thereafter), the cause of mat formation could not be completely explained by environmental changes in phosphorus although the mats in the experimental flumes responded to altered P concentrations. However, when I manipulated nitrogen concentrations and the N:P ratio in 2015 (Chapter 4), I was able to demonstrate that high concentrations of nitrogen increased stalk formation, and that a higher N:P ratio negated any reduction in stalk lengths from the addition of P. These results suggest that the increasing nitrogen deposition, which is occurring

widely across the United States, may contribute to phosphorus limitation resulting in the formation of *D. geminata* nuisance mats. Alternatively, it may increase photosynthesis in N-limited ecosystems and drive stalk production (Kilroy and Larned 2016). Not only does this suggest that P-limitation is driven by increasing N concentrations, but also that any management strategy focused on P enrichment will require increasing amounts of P to achieve the suppression of *D. geminata* mats. This holds major implications for lotic systems across the world and for any future management strategies for *D. geminata* that use P enrichment, because it would require the addition of P that may cause systems to cross trophic boundaries; an often undesirable condition and impermissible from a regulatory perspective.

Because of accessibility reasons, most research of *D. geminata* mats occur when rivers are at base flow, my research on *D. geminata* in a dam-regulated river system provided an opportunity to evaluate the seasonality of mat accrual, stalk length and the frequency of dividing cells throughout the year. I documented distinct seasonality of each of for *D. geminata* in the Kootenai River, suggesting that the application of P to suppress stalk growth could be concentrated during a short period of the year, thereby minimizing the concurrent growth of other algae typically seen in response to higher ambient P concentrations. Adding P to suppress *D. geminata*, only to have the river overwhelmed by other algal species is not a desirable outcome. For the Kootenai River, I found that peak growth of *D. geminata* occurred in the winter months, and mats, while prevalent year-round, began to degrade and disintegrate in early to mid-fall (Appendix 2) The results from this study not only provide further insights into the ecology of *D. geminata* nuisance mat formation and its life cycle, but also into the timing for focusing management strategies. These results suggest that P could be applied during or slightly before peak growth for a couple of months (January to February) to inhibit

accrual thereby suppressing mats from reaching their full growth, or causing mats to completely slough off (Chapter 5). By targeting the early accrual period, one could achieve a potential hold over effect into summer, and it would also significantly reduce the length of treatment time/total management costs. This is noteworthy for all managers evaluating management strategies for nuisance mats as it impacts several criticisms of P enrichment including the bloom of other algal species, total cost, feasibility, and ecosystem changes due to the addition of P. Continued research on the seasonality of mats within systems is needed, especially in unregulated rivers. As mentioned above, most research in unregulated systems occurs during summer months when rivers are at base flow and mats are easily accessible. However, this restricts research on accrual periods and potential P enrichment timing. Further research of this topic will improve *D. geminata* management and analysis of nuisance mats.

The in-river addition of P, although shorter than the envisioned duration (e.g., Chapter 5), showed that the first response of nuisance mats of *D. geminata* to higher ambient in-river P concentrations was sloughing which progressed downstream in the two weeks. Further follow-up with a duration longer than that afforded in 2015 is recommended to fully explore this potential in-river management strategy. The manner in which the P was deployed (solid, slow dissolving granules) bodes well for a simple application process should this be expanded to the whole-river scale. A full-scale P enrichment should employ a struvite-type P source to keep N concentrations low and to allow for a slow-dissolution rate of P. Based on the results of the nitrogen study, increased environmental N requires higher P concentrations to achieve similar *D. geminata* stalk suppression, and so any P source should have minimal amounts of N. My study suggests that traditional agricultural fertilizer that contains nitrogen is not an appropriate source of phosphorus for the suppression of *D. geminata*. The slow-dissolve P

(struvite) not only minimizes N but also reduces labor costs by requiring minimal replenishment of the nutrient. For application, the P source should be placed in burlap-type bags or other type of containers that would stop the pellets from moving downstream and allow for a point source release of the treatment. Keeping the P source in one area will also allow researchers to study the downstream impacts on *D. geminata* and to regulate or manipulate this point source. These bags should be tied together or anchored to allow the retrieval and/or to replenish the P source. By keeping the nutrient confined to bags, researchers will also be able to weigh the dried material post-treatment to see how much P dissolved into the water over time. Few studies have used P enrichment as a management strategy to reduce *D. geminata* nuisance mats, but those that have (e.g., James et al. 2015), determined that P enrichment can effectively suppress nuisance mats for 0.6 km. However, because that study used an agricultural-type fertilizer, Osmocote™: 14-14-14 in 2007 and MAP: 11-52-0 in 2008, nitrogen present in the fertilizer may have suppressed the efficacy of the treatment if the *D. geminata* present in Rapid Creek, SD reacts similar to that in the Kootenai River in response to additional N. Using a struvite-based P source such as CrystalGreen™ (5-28-0-10 Mg) deployed in porous bags is the best treatment plan to suppress the formation of nuisance mats of *D. geminata.* A P enrichment management strategy is a feasible short-term solution for *D. geminata* nuisance mats.

One of the greatest hurdles to understanding this species has been the lack of coordination and cooperation among those interested in it, and a lack of historical and current data of *D. geminata* cell and mat presence. The creation of an online *D. geminata* database (Appendix 1) has allowed widely distributed stakeholders and citizen scientists to enter *D. geminata* data. By providing a forum in which the historical presence and the state of *D.*

geminata can be recorded, changes in patterns of mat formation and severity can be observed over time. These changes can help identify landscape-wide alterations, as well as changes in ecosystems such as macroinvertebrates, fisheries, and wildlife. Fishery managers, ecologists, and other interested parties should be informed about this resource to encourage the establishment of *D. geminata* datasets and observations from around the world.

Management strategies

My evaluation of changes in water quality in the Kootenai River illustrated that increases in the N concentration over the last 10 years and reduced P from impoundment have led to extremely high TN:TP ratios ranging from 35 to 85, while ratios of SIN:TDP have ranged from 74-203. I suggest that high N:P ratio contributes to the overproduction of stalk material by the diatom resulting in the subsequent formation of nuisance mats (Chapter 4). Unfortunately, the current trend in the availability of N does not bode well for the Koocanusa/Kootenai River ecosystem (Chapter 4; Fig. 4-1). The first response for Kootenai River managers (Montana Fish, Wildlife and Parks or U.S. Army Corps of Engineers), should be to lower the high nitrogen by preventing excess nitrogen from anthropogenic sources from entering the aquatic environment. Given this is a longer-term process, the immediate solution to rebalancing the N:P ratio is via the addition of P. Because the addition of P is considered a pollution event under the Clean Water Act (CWA), any long-term addition of P would require a discharge permit under EPA's National Pollutant Discharge Elimination System (NPDES). The data presented in this dissertation should facilitate such an application. It must be recognized that advocating for the addition of P is simply to rebalance the N:P ratio while N remains elevated, and to re-introduce P that has been eliminated due to closure of the dam, an event that has irrevocably altered the availability of nutrients downstream of the dam.

My recommendation for the suppression of nuisance mats of *Didymosphenia geminata* in the Kootenai River is the addition of a slow-release source of P such as struvite at a dosage equal to approximately 1 µg/L SRP above ambient (Chapter 5). As the longitudinal reach of treatment is unknown but estimated to be < 1 km, multiple treatment sites should be established and repeatedly monitored for downstream effectiveness. These sites should be prioritized to target sections of the Kootenai River with the most severe mats. Suggested areas include below the Libby Dam, below the Fisher River/Kootenai River confluence, and any other areas important to recruitment of juvenile fish. Furthermore, I recommend that any phosphorus enrichment occur before or during peak growth (Feb-Mar) and during low discharge to have the highest impact on the nuisance mats and to reduce the amount of P needed for successful cell saturation and mat suppression. This time period is different than what is observed in unregulated streams that experience high spring runoff (Appendix 2). Tributaries that experience high discharge during spring months exhibit peak growth later as light availability increases with decreasing stream depths (Fig. A2-5). The spring runoff is hypothesized to help scour nuisance mats resulting in much lower mat occurrence compared to the Kootenai River. The instability of the river bed and the fluctuating light availability most likely contribute factor of why nuisance mats are not present year-round in these tributaries compared to the Kootenai River which experiences year-round coverage. Therefore, management strategies for mat presence within tributaries will vary from what is recommended for the Kootenai River. My research suggests that a continued increase of nitrogen will negate any P enrichment management strategies and will require higher P treatments in the future if N continues to increase as it has over the past 10 years.

Future research

Water quality (N and P), changes in the spring hydrograph (timing and volume), and stability of substrate, and watershed changes (amount of water over time) are possible factors influencing mat growth at the landscape level. Several researchers have attempted to pinpoint one of these variables as the sole driving factor explaining the prevalence of nuisance mats of *D. geminata* across the globe (Rost et al. 2011; Bothwell et al. 2014) but few have scientifically investigated how these factors may interact to drive nuisance mat formation and explain why mat formation occurs in such variable conditions (Cullis et al. 2012). Changes in climate not only drive changes in the timing of spring runoff but also alter nutrient pulses during the time of peak mat formation (spring runoff) and substrate scouring, factors which relate to nuisance mats. I suggest that these variables be further evaluated and investigated. Specifically, increased N and subsequent higher dissolved inorganic (DIN) to soluble reactive phosphorus (SRP) ratios, timing or pulses of spring runoff and changes in phosphorus or nitrogen pulsing should be studied for several years throughout the Kootenai National Forest. I also strongly suggest that *D. geminata* researchers first examine the historical presence of *D. geminata* within their study area either through paleolimnological studies or historical periphyton records before any ecological studies are conducted on current nuisance mats. Understanding long-term changes of the periphyton community is imperative to draw meaningful conclusions about introduced species and/or changes in environmental variables which may be indicative of large changes in the landscape.

I recommend that managers of the Kootenai River (Montana Fish, Wildlife, and Parks or U.S. Army Corps of Engineers) use slow-release phosphorus (struvite) to reduce *D. geminata* nuisance mat prevalence. In conjunction with this, I recommend that monitoring

downstream effects for an entire year should be undertaken to understand long-term and longitudinal effects. The diversity of algal species, macroinvertebrate populations, and fish communities should also be monitored along with *D. geminata* mat depth and standing crop index to provide a comprehensive profile of changes to the river ecology and trophic levels. While the ecosystem downstream of Libby dam is far from natural, the recent appearance of *D. geminata* has resulted in another undesirable change. The research I have presented in this dissertation supports the hypothesis that the lack of phosphorus and its imbalance with nitrogen contribute to the success of *D. geminata*. While managers work to reduce the high concentration of nitrogen currently present in the system, the addition of P to reduce nuisance mats must be considered a viable alternative to restore ecosystem functions and aesthetics.

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Appendix 1: Distribution of *Didymosphenia geminata* in Idaho and Montana

Abstract

Didymosphenia geminata was historically regarded as a rare benthic diatom, generally isolated to latitudes above 30˚N. This, and the sporadic and sparse nature of historical records of periphyton composition of lotic ecosystems contributed to *D. geminata* being considered an introduced species when it appeared in mat form across the globe in seemingly random patterns. As our understanding of it has improved, it has become accepted as a native species across much of the United States, especially in the western United Sates. However, detailed analysis of large regions of the US is still limited. The objective of this chapter was to examine the presence/absence of *D. geminata* and its form (cell or mat) throughout western Montana and northern Idaho to develop basic knowledge and provide baseline information for continued future monitoring. Of 139 surveys sites in Idaho and Montana, 51% had *D. geminata* as part of the algal community. Forty-four percent of the locations with *D. geminata* had significant stalk production and mat formation, while in 56% of the samples *D. geminata* was a part of the algal community with no significant stalk growth. Only 14% of locations surveyed had nuisance level mat growth (28% of rivers) suggesting that nuisance mat formation is rare among rivers with *D. geminata* as part of the periphyton community in northern Idaho and western Montana.

In cooperation with Trout Unlimited, a web-integrated database was developed to catalog *D. geminata* locations and associated data that have been previously collected by biologists and ecologists from around the world. These efforts have helped illuminate that *D. geminata* is commonly present in freshwater lentic systems in northwestern Montana and Idaho and occurs most often as a regular member of the periphyton community.

Introduction

Thriving in cold, fast flowing, oligotrophic systems, biomass associated with the diatom *Didymosphenia geminata* (Lyngbye) M. Schmidt dominates in systems in which the nutrient-limited phosphorus (P) conditions would typically result in low algal biomass (Tilman et al. 1982). This diatom has recently gained notoriety due to its excessive production of stalk tissue resulting in the occurrence of nuisance mats in streams and rivers across the globe, where such mats were previously absent. Producing gelatinous mats composed of mucopolysaccharide stalks (Ellwood and Whitton 2007); this benthic diatom is a concern because of its threat to the function and aesthetics of river ecosystems. The mats produced by *D. geminata* covers the substrate, thereby displacing large-bodied benthic invertebrates such as Ephemeroptera, Plecoptera, and Trichoptera, the preferred prey of many salmonid species (Marshall et al. 2008). The presence of *D. geminata* mats in the Kootenai River was noticed in the early 2000s, when growths appeared on sampling gear (Holderman and Hardy 2004). However, previous work showed that *D. geminata* was part of the periphyton community in 1972 (Perry and Huston 1983), and one of the oldest records from North America also shows it has had a long history in the Kootenai River systems (as cited in Bothwell et al. 2014). These nuisance mats have rapidly appeared throughout the Kootenai River, and have remained a ubiquitous annoyance. Currently, the densest mat coverage of *D. geminata* occurs near Libby Dam (up to 8 mm deep with 100% coverage) and continues downriver into Idaho for approximately 46.5 river km (29 river miles), albeit with decreasing intensity. The first step to manage this species, as with any management plan, must be to have sufficient background information to fully understand the scale of the problem so that appropriate actions can be evaluated and selected. To successfully manage nuisance mats of

Didymosphenia geminata, requires an understanding of the relationship between the biological characteristics of the mats and abiotic factors. Thus, the first objective of this part of my research was to determine the presence/absence of *D. geminata* in the intermountain Pacific Northwest (northern Idaho and northwestern Montana) in cell and mat form using a survey of streams. For all positive cell detections, voucher samples were created, and the data set was translated into a geographic information system and mapped in ArcMap (ERSI 2010) for ease of display and comprehension by managers. A second objective was to streamline and standardize the *D. geminata* data collected among agencies, researcher, and volunteers. Too often in research projects, crucial variables are not collected as they seem irrelevant to the study objective at hand. However, this leads to missing information and hampers other research. For example, depending on the research question, researchers tend to collect unique water quality parameters such as total phosphorus (TP) and total nitrogen (TN) vs. soluble reactive phosphorus (SRP) and dissolved inorganic nitrogen (DIN). While working with government agencies it became clear that a large backlog of *D. geminata* data exists that has never been compiled or analyzed. To further the use of previously collected data and simultaneously advance the understanding of *D. geminata*, I worked to develop a web-integrated "Didymo Database" that will host data from federal and state agencies in a standardized manner.

The final objective was to create an easily accessible database for researchers across the world. To do this, a Microsoft Access database was designed to maintain the information and an online database was created through iNaturalist for the public and professionals to enter information about mats and cell detections. Collectively, the goal was to systematically capture and organize existing data on *D. geminata* to provide a foundation of knowledge that

is easily and widely accessible in a standardized format. This will allow researchers to use this information as a reference for future research and comparisons to better understand this species. In addition, I hope it will serve as a point for collaboration not only among scientists but also the public. The use of citizens in a monitoring effort should allow much more data to be collected for a relatively low investment.

Methods

Algal scrapings from lotic ecosystems were collected by traveling to sites across northwestern Montana and northern Idaho. Emphasis was placed on areas with prior observations/records of mats. To maximize the sample area, I enlisted the assistance of federal (USFS, EPA, USGS) and state agency personnel (Montana Fish, Wildlife, and Parks and Idaho Department of Fish and Game), private individuals (e.g., fly fishermen or concerned citizens), and personnel from non-governmental organizations (NGOs) such as Trout Unlimited (TU), Kelly Creek Fly Casters (KFC), and the Federation of Fly Fishers (FFF) (Table A1-1). Soliciting assistance for algae scrapings was done through presentations at monthly social meetings (TU, KCF), regional conferences (Idaho AFS, SFS), and agency training sessions (USFS). PowerPoint presentations were given to several of the larger organizations (e.g., TU Libby, KFC, and IDFG) to disseminate as much information about the ecology of *D. geminata*, the methods for collecting samples, and how to identify *D. geminata* mats. Volunteers were asked to collect a scraping from rocks from various depths at one location in the stream or river to provide a representative sample. This sample was placed in a standard 15 ml centrifuge tube filled at least ¼ to ½ full with stream water to which 1-2 ml of Lugol's Iodine was added to preserve the sample. Each vial was accompanied by a data sheet asking for a standardized set of eight variables (Figs. A1-1 and A1-2) (Kilroy et al. 2005;

Marshall et al. 2007; Cullis et al. 2012). The sample and sheet were then returned via mail to the University of Idaho for analysis.

To analyze the sample scrapings, subsamples (~ 5-8 ml) of each sample were placed on a glass microscope slide, topped with a cover slip, and examined with the aid of a compound microscope (Wild M40) at 120×. This process was repeated until the algal community was deemed fully analyzed. If 5 or more cells of *D. geminata* were found, a positive detection was recorded for the sample. Digital microphotographs were taken of the cells in positive samples, and the algae scraping was placed on a standard 3" × 5" index card to create a voucher for cell presence in that sample. The index card was left to air dry completely before it was inserted into a small paper envelope labeled with photograph number, stream characteristics, and coordinates. Characteristics observed in subsamples such as presence of stalk material from the *D. geminata* cells and notable other algal species were also recorded. All data were then entered into a Microsoft Access© database.

Didymosphenia geminata detections were plotted with ArcMap v. 10.2.2 (ESRI 2010). The map was developed to not only provide a visual representation of *D. geminata* presence for trend analysis, but also to allow for the comparison of physical variables by overlaying geographic and chemical information (Fig. A1-3). This map provided a reference of mat presence in 2014.

The *D. geminata* database was designed by Megan Nissley as part of the University of Idaho Center for Research on Invasive and Small Populations (CRISSP) National Science Foundation funded Research for Undergraduates (REU) internship program in 2014. The database is based in Microsoft Access and was designed to allow researchers across the world to enter their data in a standardized format. The data entry form standardizes cell or mat

detection, water chemistry information, physical characteristics of the stream, and location coordinates.

Results

Objective 1:

Algae samples were provided by numerous individuals and organizations (Table A1-1). From the algae scrapings and mapping of *D. geminata* cells, the commonality of this algal species within the intermountain northwest region was clarified. Of the 139 locations surveyed, 51% had *D. geminata* as part of the algal community. Of those locations with *D. geminata*, 44% had significant stalk production and mat formation at some level, while in 56% of the positive samples, *D. geminata w*as a normal part of the algal community with no stalk growth (Table A1-2). Of the samples with stalk growth, only 28% were considered to have nuisance mats (14% of all locations with *D. geminata*). This survey clearly demonstrated that *D. geminata* is common throughout the region sampled and occurs most commonly in a non-nuisance mat producing phase.

The most notable habitat characteristic for *D. geminata* presence in creeks, was light availability and the related surrounding vegetation (Fig. A1-5). Creeks dominated by dense cedar forests rarely had *D. geminata*, while it was present in creeks surrounded by scattered pine forests. Further analysis of water quality characteristics in relation to *D. geminata* mat presence in Kootenai River tributaries is discussed in Appendix 2.

Objective 2:

Through the outreach effort over 400 sample kits were distributed of which only 72 (18%) were returned. I found that agency personnel with an investment in local river ecology and health (ecologists) had the greatest return rate (50-97%), while recreationists or

state/federal biologists had the lowest return rates. I found that “Size of River” and “% Shade” had the most inconsistent responses and should be accompanied with additional detailed instructions in the future. Of the eight fields, the one specifically labelled as “Mat Presence” allowed me to catalog stalk formation and potential nuisance mat presence.

Objective 3:

The Microsoft Access database housed all of the information associated with each sample. It was sent to the Montana Natural Heritage Program (MNHP) and all records from Montana were uploaded into their online native species database. This database populates a map and allows the public to access records and submit data for review. However, because the MNHP program is supported by state funds, only data from Montana could be included.

For data outside of Montana, I collaborated with researchers from Environment Canada, North Carolina State University, and Trout Unlimited to create a website to log *D. geminata* mat and cell detections across the United States through iNaturalist (https://www.inaturalist.org/ projects/trout-unlimited-didymo-sampling). This website allows anyone from around the world to submit pictures, data, and observations of *D. geminata*. This has evolved into a larger project now led by TU, and researchers are actively working to have the iNaturalist database available as an iPhone App. This spin-off project is based on the sample ‘kit’ I designed for my regional study.

Discussion

Objective 1:

As the recognition of *D. geminata* mats and subsequent publications increased (see Chapter 1), the perception of *D. geminata* mat presence by the public and/or managers may have become skewed (frequency illusion). For example, as more people became aware of

what “Didymo” was, more people began to see and report nuisance mats regardless of historical presence of mat growth. This “the more you look for something, the more you find it” illusion, may have skewed the perception that *D. geminata* was “overtaking” streams and rivers across the United States seemingly within 5 years. While some rivers in the United States experienced the occurrence of severe nuisance mats, the lack of an adequate database for the presence of *D. geminata* mats may have resulted in the classification of historic (non-nuisance) mat presence as new invasions. This frequency illusion may have contributed to the “knee-jerk” assumption that it was an introduced species throughout the United States and emphasizes the importance of having an adequate database documenting the occurrence of *D. geminata* across the landscape.

For northwestern Montana and northern Idaho, my study has demonstrated that *D. geminata* is a common and widely distributed species (Fig. A1-3). Nuisance mat formation is rare relative to the pervasiveness of *D. geminata* within periphyton communities. As *D. geminata* nuisance mats have appeared seemingly at random, landscape-wide changes in water quality should be evaluated, especially nitrogen pollution as the presence of nuisance mats cannot be attributed to the introduction of new *D. geminata* cells.

Cataloguing the distribution of *D. geminata* throughout the intermountain Pacific Northwest is necessary to understand this species and its response to changing environmental conditions. The creation of vouchers for cell presence in rivers and the record of mat presence have established a reference for *D. geminata* locations in the intermountain Northwest. By monitoring these locations, any shifts from the periphyton phase to the nuisance mat phase can be detected in the future.

As nutrient concentrations and microscopic diatoms are imperceptible, understanding what is occurring in the ecology of the river and algal community is impossible from visual observation. Therefore, the seemingly rapid and unrelenting appearance of nuisance mats in previously 'algae-free' river systems can be easily mistaken for the accrual by an invasive species. Consequently, this misperception has led many *D. geminata* nuisance mats to be labeled and addressed as the occurrence of an introduced species. While this label can have positive consequences for the health of aquatic ecosystems by encouraging and reminding recreationists to employ proper stream health etiquette such as the "Check, Clean, Dry" campaign, it has severely hindered research investigating changing water quality and subsequent *D. geminata* nuisance mat formation (Taylor and Bothwell 2014). As scientists continue to simplify the presence of *D. geminata* nuisance mats as an aquatic invasive species to be stopped, the driving mechanism behind changing environmental parameters will be ignored. My study demonstrates that *D. geminata* is present in non-mat form in much of the intermountain Northwest and that this species occurs most commonly in a microscopic periphyton form. When confronted with a new occurrence of nuisance mat growth, researchers should be asking first, "is this species historic within this stream?" and secondly, "what water quality parameters have changed to cause this species to be stressed and produce excessive carbohydrates?" These questions can only be adequately addressed if historic information is available in a database.

Objective 2:

In future citizen science efforts involving my protocol (Figs. A1-1 and A1-2), accountability (return of sample vials) should be prioritized with repeated follow-up meetings/contacts and deadlines. A suggested method is using motivated groups of the public,

such as fly fishing groups including members of Trout Unlimited. In addition, friendly competitions of most rivers sampled between clubs or members within clubs could also ensure return of vials. Maximizing competition and accountability (through club presidents) for return of vials should help increase the rate of returned vials and ensure the overall success of the program.

For all *D. geminata* records, researchers should strive to collect at minimum all eight variables outlined in Fig. A1-2, of which comments on mat presence/absence are most important. Maximizing and standardizing the data collected (observed mat characteristics and environmental conditions) will help better identify patterns related to the presence of nuisance mats to better understand the ecology behind this phenomenon. These data and the most current map of *D. geminata* detections have been provided online at www.didymo.weebly.com to encourage future collaboration between *D. geminata* researchers.

Objective 3:

If the presence of *Didymosphenia geminata* nuisance mats continues to be viewed as the result of an invasion, management strategies will continue to be focused in that direction, hindering our understanding and wasting limited resources. Establishing a record of *D. geminata* distribution and mat presence is imperative to understand how mat coverage is changing in the United States. It is also critical to derive realistic management strategies. Continued research into hypotheses that focus on causation of mat formation without first examining the presence of *D. geminata* at the regional landscape and the patterns of growth within related waters will lead to skewed conclusions (Taylor and Bothwell 2014; Bothwell et al. 2012). Development of a distribution database for *D. geminata* is far overdue for managers

and researchers across the world and should be a priority for anyone working with this species.

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Tables

Table A1-1. *Didymosphenia geminata* sampling vial distributions and returns from various recreational and professional organizations.

Samplers	**Number of Vials Distributed**	**Number of Vials Returned**	**% Returned**
Trout Unlimited (Libby Chapter)	20	0	0%
Kelly Creek Flycasters	25	2	8%
Trout Unlimited (Pennsylvania: Katy Dunlap, Jake Lemon)	15	8	53%
American Fisheries Society (Idaho Chapter)	40	0	0%
University of Idaho CNR (Frank Wilhelm, Chris Pike, Katie McBaine)	15	5	33%
Idaho Dept. of Fish and Game Regional Snorkel Crews	75	4	5.3%
USFS Canoe Gulch (Paul Hooper)	40	20	50%
Sawtooth NRA (Scott Vuono)	30	29	97%
MT Fish Wildlife and Parks, Kalispell (Amber Steed)	25	0	0
MT Fish Wildlife and Parks, Kalispell (Mike Hensler)	25	0	0
Trout Unlimited, Bozeman (Dave Kumlien)	10	0	0
Trout Unlimited (North Idaho - Bill Scudder)	5	2	40%
Clinton Begley (Kayaker)	2	2	100%
TOTAL	**327**	**72**	**22%**

Table A1-2: Algae scraping results for *Didymosphenia geminata* sampling throughout Idaho and Montana.

Algal Sample ID	Date Collected	Stream Name	Latitude	Longitude	State	Country	Descriptive Location	Date Analyzed	Cells Present	Stalk Present	Stream Mat Present
116	22-Jun-14	Alturas Lake Creek	43.982153	-114.84558	ID	USA		28-Jul-14	TRUE	TRUE	FALSE
36	17-Jul-13	Barrow Creek	48.500951	-115.28321	MT	USA	~200 upstream of the FDR	17-Jul-13	FALSE	TRUE	FALSE
22		Bear Creek	48.16982	-115.31487	MT	USA			TRUE	TRUE	FALSE
30		Beaver Creek	43.54283	-114.4354283	ID	USA		09-Sep-13	TRUE	FALSE	FALSE
5		Beaverhead Creek	45.005833	-112.846944	MT	USA	from dam tailgaters to confluence of creek	02-Jun-14	FALSE	FALSE	FALSE
47		Big Boulder Creek	44.65268	-114.261659	ID	USA		27-Aug-13	FALSE	FALSE	FALSE
16	09-Jul-13	Big Cherry Creek	48.192832	-115.314331	MT	USA		09-Jul-13	TRUE	TRUE	FALSE
99	07-Jul-14	Big Creek	45.103789	-114.850115	ID	USA	Taylor Ranch below	21-Jul-14	TRUE	TRUE	TRUE

Table A1-2 Cont'd.

Algal Sample ID	Date Collected	Stream Name	Latitude	Longitude	State	Country	Descriptive Location	Date Analyzed	Cells Present	Stalk Present	Stream Mat Present
80	01-Sep-13	Big Creek - Flathead River	48.281214	-114.51450	MT	USA		01-Sep-13	TRUE	TRUE	FALSE
66	17-Jul-13	Big Creek - Kootenai	48.747411	-115.352914	MT	USA	~200 yds downstream of FDR road/bridge	17-Jul-13	FALSE	FALSE	FALSE
128	20-Jul-14	Blackfoot River	47.018007	-113.240389	MT	USA	By Scotty Brown Bridge	28-Jul-14	FALSE	FALSE	FALSE
133	23-Mar-14	Blackfoot River	46.945803	-112.956319	MT	USA		22-Jul-14	TRUE	FALSE	FALSE
41		Bobtail Creek	48.44081	-115.600410	MT	USA		18-Jun-14	TRUE	FALSE	FALSE
28		Boundary Creek	44.91594	-115.600410	ID	USA		09-Sep-13	TRUE	FALSE	FALSE
58		Bowery Creek	44.21408	-114.273920	ID	USA		27-Aug-13	FALSE	FALSE	FALSE

Table A1-2 Cont'd.

Algal Sample ID	Date Collected	Stream Name	Latitude	Longitude	State	Country	Descriptive Location	Date Analyzed	Cells Present	Stalk Present	Stream Mat Present
38	17-Jul-13	Bristow Creek	48.544356	-115.293836	MT	USA	~200 upstream of the FDR	17-Jul-13	FALSE	FALSE	FALSE
120	16-Jul-14	Buck Creek	48.311509	-115.665704	MT	USA		22-Jul-14	FALSE	FALSE	FALSE
122	17-Jul-14	Bull River	48.085601	-115.780193	MT	USA		22-Jul-14	TRUE	FALSE	FALSE
156	07-Jul-15	Bull River - Main	48.047203	-115.834394	MT	USA	Sanders County	20-Aug-15	TRUE	FALSE	FALSE
152	23-Jun-15	Bull River - Main	48.047203	-115.834394	MT	USA	Sanders County	20-Aug-15	TRUE	FALSE	FALSE
76	15-Aug-13	Bull River-Canada	49.472792	-115.451825	BC	Canada	Near confluence of Bull and Kootenay	15-Aug-13	TRUE	FALSE	FALSE
24	15-Jul-13	Callahan Creek	48.456472	-115.890878	MT	USA	downstream of where the road crosses the creek	15-Jul-13	TRUE	TRUE	TRUE

Table A1-2 Cont'd.

Algal Sample ID	Date Collected	Stream Name	Latitude	Longitude	State	Country	Descriptive Location	Date Analyzed	Cells Present	Stalk Present	Stream Mat Present
112	16-Jul-14	Canyon Creek	48.425282	-115.303085	MT	USA		22-Jul-14	FALSE	FALSE	FALSE
2		CDA River	47.556884	-116.287879	ID	USA	24.6 mile marker NF CDA	02-Jun-14	TRUE	FALSE	FALSE
17	15-Jul-13	Cedar Creek	48.430264	-115.629214	MT	USA	upstream of hwy	15-Jul-13	TRUE	FALSE	FALSE
54		Champion Creek	44.11890	-114.50050	ID	USA		28-Aug-13	FALSE	FALSE	FALSE
45		Champion Creek	44.11859	-114.495975	ID	USA		09-Sep-13	FALSE	FALSE	FALSE
88	13-Jul-14	Cherry Creek	45.887268	-116.832826	OR	USA	Snake River, river left	22-Jul-14	FALSE	FALSE	FALSE
114	17-Jul-14	Clark Fork River	48.085425	-116.012036	MT	USA		24-Jul-14	TRUE	FALSE	FALSE
63	18-Dec-13	Clearwater River	46.499232	-116.375951	ID	USA		18-Jun-14	TRUE	FALSE	FALSE

Table A1-2 Cont'd.

Algal Sample ID	Date Collected	Stream Name	Latitude	Longitude	State	Country	Descriptive Location	Date Analyzed	Cells Present	Stalk Present	Stream Mat Present
6	19-Dec-13	Clearwater River	46.499232	-116.375951	ID	USA	North fork	11-Jun-13	TRUE	TRUE	FALSE
102	07-Jul-14	Cliff Creek	45.10425	-114.849917	ID	USA	25 m from confluence of Big Creek	21-Jul-14	FALSE	FALSE	FALSE
97	16-Jul-14	Cody Creek	48.273556	-115.292756	MT	USA		18-Jul-14	FALSE	FALSE	FALSE
104	16-Jul-14	Cool Creek	48.8261	-115.7746	MT	USA		21-Jul-14	FALSE	FALSE	FALSE
78	15-Aug-13	Cripple Horse Creek	48.477311	-115.254497	MT	USA	upstream of hwy	15-Aug-13	FALSE	FALSE	FALSE
106	16-Jul-14	Dunn Creek	48.385950	-115.314609	MT	USA		21-Jul-14	FALSE	FALSE	FALSE
105	16-Jul-14	Dutch Creek	48.452033	-115.667936	MT	USA		21-Jul-14	FALSE	FALSE	FALSE
95	16-Jul-14	East Fork Pipe Creek	48.61675	-115.617061	MT	USA		18-Jul-14	TRUE	TRUE	TRUE

Table A1-2 Cont'd.

Algal Sample ID	Date Collected	Stream Name	Latitude	Longitude	State	Country	Descriptive Location	Date Analyzed	Cells Present	Stalk Present	Stream Mat Present
157	07-Jul-15	EF Bull River East Branch	48.109406	-115.780664	MT	USA	Sanders County	20-Aug-15	FALSE	FALSE	FALSE
62		Elk Creek	44.185928	-115.52215	ID	USA		26-Aug-13	TRUE	FALSE	FALSE
39	08-Jul-14	Fernan Lake Tributary	47.688547	-116.694916	ID	USA		22-Jul-14	FALSE	FALSE	FALSE
20	09-Jul-13	Fisher River	48.361467	-115.319619	MT	USA	Upstream of bridge by DRC access, near the conflue	09-Jul-13	TRUE	FALSE	TRUE
14	23-Aug-13	Fisher River	48.048	-115.292	MT	USA	Where Raven Creek comes into the Fisher River	23-Aug-13	TRUE	FALSE	FALSE
74	15-Aug-13	Five Mile Creek	48.534742	-115.207042	MT	USA	upstream of hwy	15-Aug-13	TRUE	FALSE	FALSE

Table A1-2 Cont'd.

Algal Sample ID	Date Collected	Stream Name	Latitude	Longitude	State	Country	Descriptive Location	Date Analyzed	Cells Present	Stalk Present	Stream Mat Present
81	01-Sep-13	Flathead River	48.601437	-114.160763	MT	USA	North fork	01-Sep-13	TRUE	FALSE	FALSE
21	23-Aug-13	Flattail Creek	48.824	-115.716	MT	USA		23-Aug-13	TRUE	FALSE	FALSE
33	09-Jul-13	Flower Creek	48.382208	-115.562858	MT	USA	near intersection of Flower Creek RD	09-Jul-13	FALSE	TRUE	FALSE
61		Fourth of July Creek	44.15907	-114.473729	ID	USA		09-Sep-13	FALSE	FALSE	FALSE
92	16-Jul-14	Fourth of July Creek	48.933456	-115.884289	MT	USA		22-Jul-14	FALSE	FALSE	FALSE
90	16-Jul-14	Fowler Creek	48.785414	-115.652417	MT	USA		22-Jul-14	FALSE	FALSE	FALSE
42		Frenchman Creek	43.523962	-114.461792	ID	USA		09-Sep-13	FALSE	FALSE	FALSE
60		Goat Creek	44.64585	-115.82427	ID	USA		12-Sep-13	TRUE	FALSE	FALSE

Table A1-2 Cont'd.

Algal Sample ID	Date Collected	Stream Name	Latitude	Longitude	State	Country	Descriptive Location	Date Analyzed	Cells Present	Stalk Present	Stream Mat Present
59		Gold Creek	44.63048	-114.513708	ID	USA		09-Sep-13	FALSE	FALSE	FALSE
37	09-Jul-13	Granite Creek	48.352639	-115.526278	MT	USA	where the road crosses the creek	09-Jul-13	FALSE	TRUE	FALSE
159	07-Jul-15	Graves Creek	47.685722	-115.4047	MT	USA	Sanders County	20-Aug-15	FALSE	FALSE	FALSE
94	16-Jul-14	Grizzly Creek	48.735119	-115.817192	MT	USA		18-Jul-14	FALSE	FALSE	FALSE
108	16-Jul-14	Gus Creek	48.81901	-115.81559	MT	USA		21-Jul-14	FALSE	FALSE	FALSE
43		Hell Roaring Creek	44.14045	-114.521725	ID	USA		05-Sep-13	FALSE	FALSE	FALSE
79	23-Aug-13	Himes Creek	47.954	-115.338	MT	USA		23-Aug-13	FALSE	FALSE	FALSE
51		Holman Creek	44.145780	-114.314712	ID	USA		10-Sep-13	FALSE	FALSE	FALSE

Table A1-2 Cont'd.

Algal Sample ID	Date Collected	Stream Name	Latitude	Longitude	State	Country	Descriptive Location	Date Analyzed	Cells Present	Stalk Present	Stream Mat Present
129	22-Jun-14	Iron Creek	44.198696	-115.000978	ID	USA		28-Jul-14	TRUE	FALSE	FALSE
31		Iron Creek	44.125045	-114.583202	ID	USA		11-Sep-13	TRUE	FALSE	FALSE
34	17-Jul-13	Jackson Creek	48.465672	-115.318444	MT	USA	~200 upstream of the FDR along	17-Jul-13	FALSE	TRUE	FALSE
75	15-Aug-13	Kootenai River	49.452783	-115.430786	BC	Canada	railroad. ~ 2 miles away from Koocanusa.	15-Aug-13	TRUE	FALSE	FALSE
9	15-Jul-13	Lake Creek	48.44889	-115.879167	MT	USA	Dow upstream of hwy	15-Jul-13	TRUE	TRUE	TRUE
107	16-Jul-14	Lang Creek	48.793841	-115.715988	MT	USA		21-Jul-14	FALSE	FALSE	FALSE
19	12-Jul-13	Leigh Creek	48.224342	-115.601286	MT	USA	upstream of where the road crosses	12-Jul-13	TRUE	FALSE	FALSE

Table A1-2 Cont'd.

Algal Sample ID	Date Collected	Stream Name	Latitude	Longitude	State	Country	Descriptive Location	Date Analyzed	Cells Present	Stalk Present	Stream Mat Present
40	17-Aug-13	Libby Creek	48.11828	-115.54808	MT	USA		18-Jun-14	TRUE	FALSE	FALSE
13	09-Jul-13	Libby Creek	48.314139	-115.504767	MT	USA	upstream of bridge by the FWP office	09-Jul-13	TRUE	TRUE	TRUE
144	05-Jul-15	Little Blackfoot	46 27'54.63N	112 25'27.24	MT	USA		10-Aug-15	TRUE	TRUE	FALSE
35	07-Aug-13	Little Cherry Creek	48.174268	-115.546520	MT	USA	N48.17426 W115.54655	07-Aug-13	FALSE	TRUE	FALSE
11	10-Jul-13	Lower Quartz	48.438889	-115.636667	MT	USA	downstream of where the road crosses the creek	10-Jul-13	TRUE	FALSE	FALSE
89	11-Jul-14	Maloney Creek	46.038131	-116.626372	ID	USA		22-Jul-14	FALSE	FALSE	FALSE
111	16-Jul-14	Meadow Creek	48.78386	-115.92391	MT	USA		22-Jul-14	TRUE	TRUE	TRUE
73	07-Aug-13	Miller Creek	48.08234	-115.42863	MT	USA		07-Aug-13	FALSE	FALSE	FALSE

Table A1-2 Cont'd.

Algal Sample ID	Date Collected	Stream Name	Latitude	Longitude	State	Country	Descriptive Location	Date Analyzed	Cells Present	Stalk Present	Stream Mat Present
127	20-Jul-14	Missouri River	47.046067	-111.996387	MT	USA	5 miles from Holter Dam	28-Jul-14	FALSE	FALSE	FALSE
7	18-Jul-13	Moyie River	48.715	-116.188056	ID	USA	At Kootenai confluence (mats observed below	18-Jul-13	TRUE	TRUE	TRUE
15	09-Jul-13	Parmenter Creek	48.466364	-115.591047	MT	USA	Moyie below Deanna Bidwells property	09-Jul-13	TRUE	FALSE	FALSE
26		Payette River	45.029078	-116.059633	ID	USA	South fork	28-Aug-13	TRUE	FALSE	FALSE
123	16-Jul-14	Peoples Creek	48.338273	-115.314375	MT	USA		22-Jul-14	FALSE	FALSE	FALSE
50		Petit Lake Creek	43.59042	-114.514461	ID	USA	At outlet	09-Sep-13	FALSE	FALSE	FALSE
117	22-Jun-14	Petit Lake Creek	43.983456	-114.862703	ID	USA		28-Jul-14	FALSE	FALSE	FALSE

Table A1-2 Cont'd

Algal Sample ID	Date Collected	Stream Name	Latitude	Longitude	State	Country	Descriptive Location	Date Analyzed	Cells Present	Stalk Present	Stream Mat Present
109	16-Jul-14	Pheasant Creek	48.81758	-115.83064	MT	USA		22-Jul-14	TRUE	FALSE	FALSE
126	16-Jul-14	Pine Creek	48.597295	-115.989524	MT	USA		22-Jul-14	TRUE	TRUE	FALSE
100	07-Jul-14	Pioneer Creek	45.103528	-114.850203	ID	USA	approx 25 m from confluence with Big Creek	21-Jul-14	FALSE	FALSE	FALSE
18	10-Jul-13	Pipe Creek	48.427514	-115.595722	MT	USA	downstream of where the road crosses the creek	10-Jul-13	TRUE	FALSE	FALSE
64	07-Aug-13	Pole Creek	43.543550	-114.451343	ID	USA		28-Aug-13	FALSE	FALSE	FALSE
71		Poorman Creek	48.14921	-115.54184	MT	USA		07-Aug-13	TRUE	FALSE	FALSE
3		Prichard Creek	47.658307	-115.971612	ID	USA	Confluence of NF CDA		FALSE	FALSE	FALSE

Table A1-2 Cont'd.

Algal Sample ID	Date Collected	Stream Name	Latitude	Longitude	State	Country	Descriptive Location	Date Analyzed	Cells Present	Stalk Present	Stream Mat Present
8	23-Aug-13	Quartz Creek	48.479	-115.653	MT	USA		23-Aug-13	TRUE	FALSE	FALSE
67	07-Aug-13	Ramsey Creek	48.13921	-115.53682	MT	USA		07-Aug-13	TRUE	FALSE	FALSE
124	17-Jul-14	Red Top Creek	48.760987	-115.917488	MT	USA		22-Jul-14	TRUE	FALSE	FALSE
115	22-Jul-14	Redfish Lake Creek	44.150518	-114.91425	ID	USA		28-Jul-14	FALSE	FALSE	FALSE
48		Redfish Lake Creek	44.144815	-114.914278	ID	USA	At inlet	29-Aug-13	FALSE	FALSE	FALSE
10	13-Jul-13	Rock Creek	46.669444	-113.6725	MT	USA	Composite sample - 6 points along river	13-Jul-13	TRUE	TRUE	FALSE
154	07-Jul-15	Rock Creek - Sanders	47.975267	-115.728322	MT	USA	Sanders County July	20-Aug-15	FALSE	FALSE	FALSE

Table A1-2 Cont'd.

Algal Sample ID	Date Collected	Stream Name	Latitude	Longitude	State	Country	Descriptive Location	Date Analyzed	Cells Present	Stalk Present	Stream Mat Present
151	18-May-15	Rock Creek - Sanders County	47.975267	-115.728322	MT	USA	Sanders County	20-Aug-15	FALSE	FALSE	FALSE
93	16-Jul-14	Runt Creek	48.849401	-115.868056	MT	USA		18-Jul-14	FALSE	FALSE	FALSE
101	07-Jul-14	Rush Creek	45.104417	-114.860806	ID	USA		21-Jul-14	FALSE	FALSE	FALSE
25		Salmon River	45.321601	-114.414650	ID	USA	Near Headwaters	28-Aug-13	TRUE	FALSE	TRUE
29		Salmon River	42.265157	-114.322456	ID	USA	East fork	27-Aug-13	TRUE	FALSE	TRUE
119	22-Jun-14	Salmon River	44.209924	-114.930841	ID	USA		28-Jul-14	TRUE	TRUE	FALSE
118	22-Jun-14	Salmon River	44.000767	-114.832958	ID	USA		28-Jul-14	TRUE	TRUE	FALSE

Table A1-2 Cont'd.

Algal Sample ID	Date Collected	Stream Name	Latitude	Longitude	State	Country	Descriptive Location	Date Analyzed	Cells Present	Stalk Present	Stream Mat Present
87	11-Jul-14	Salmon River Tributary	45.993599	-116.575472	ID	USA	approx 15 meters above river	22-Jul-14	FALSE	FALSE	FALSE
1		Shoshone Creek	47.709608	-115.970885	MT	USA	2.2 miles upstream	02-Jun-14	FALSE	FALSE	FALSE
68	07-Aug-13	Silver Butte Creek	48.00758	-115.36803	MT	USA		07-Aug-13	FALSE	FALSE	FALSE
44		Smiley Creek	43.542982	-114.475647	ID	USA		09-Sep-13	FALSE	FALSE	FALSE
96	17-Jul-14	South Fork Bull River	48.187970	-115.803971	MT	USA		18-Jul-14	FALSE	FALSE	FALSE
113	16-Jul-14	Spread Creek	48.830039	-115.856756	MT	USA		22-Jul-14	FALSE	FALSE	FALSE
46		Stanley Lake Creek	44.253589	-115.005782	ID	USA		26-Aug-13	FALSE	FALSE	TRUE

Table A1-2 Cont'd.

Algal Sample ID	Date Collected	Stream Name	Latitude	Longitude	State	Country	Descriptive Location	Date Analyzed	Cells Present	Stalk Present	Stream Mat Present
131	22-Jun-14	Stanley Lake Creek	44.253274	-115.008323	ID	USA		28-Jul-14	TRUE	TRUE	FALSE
55		Stanley Lake Creek	44.246826	-115.048011	ID	USA	Near inlet	11-Sep-13	TRUE	FALSE	FALSE
32	15-Aug-13	Sutton Creek	48.759722	-115.285278	MT	USA	upstream of hwy	15-Aug-13	FALSE	TRUE	FALSE
158	07-Jul-15	Swamp Creek	47.904953	-115.626608	MT	USA	Sanders County	20-Aug-15	FALSE	FALSE	FALSE
77	15-Aug-13	Tobacco River	48.896172	-115.1158	MT	USA	access site near the hwy	15-Aug-13	TRUE	FALSE	TRUE
4		Teepee Creek	44.940510	-115.738041	ID	USA	50 mile marker NF River Rd	02-Jun-14	TRUE	TRUE	FALSE
134	06-Apr-14	Thompson River	47.607072	-115.207786	MT	USA		01-Aug-14	TRUE	FALSE	TRUE

Table A1-2 Cont'd.

Algal Sample ID	Date Collected	Stream Name	Latitude	Longitude	State	Country	Descriptive Location	Date Analyzed	Cells Present	Stalk Present	Stream Mat Present
125	05-Jul-14	Thompson River	47.883065	-115.025923	MT	USA	30 miles down Thompson Rive Rd	22-Jul-14	TRUE	TRUE	TRUE
72	07-Aug-13	Trail Creek	48.038412	-115.46047	MT	USA		07-Aug-13	TRUE	FALSE	FALSE
49		Trap Creek	44.185928	-115.52215	ID	USA		26-Aug-13	FALSE	FALSE	FALSE
155	07-Jul-15	Trout Creek	47.832056	-115.643192	MT	USA	Sanders County	20-Aug-15	FALSE	FALSE	FALSE
52		Unnamed Tributary to Salmon Ri			ID	USA		09-Sep-13	FALSE	FALSE	FALSE
56		Valley Creek			ID	USA		26-Aug-13	TRUE	FALSE	FALSE
130	22-Jun-14	Valley Creek	44.233065	-114.987393	ID	USA		28-Jun-14	TRUE	FALSE	FALSE

Table A1-2 Cont'd.

Algal Sample ID	Date Collected	Stream Name	Latitude	Longitude	State	Country	Descriptive Location	Date Analyzed	Cells Present	Stalk Present	Stream Mat Present
27		Valley Creek	44.132964	-114.554450	ID	USA		09-Sep-13	TRUE	FALSE	FALSE
150	18-May-15	Vermillion Creek	47.843853	-115.528133	MT	USA	@ Red Bridge	20-Aug-15	FALSE	FALSE	TRUE
153	07-Jul-15	Vermillion Creek	47.843853	-115.528133	MT	USA	@Red Bridge in July	20-Aug-15	FALSE	FALSE	TRUE
149	18-May-15	Vermillion Creek	47.870503	-115.320719	MT	USA	@207 Bridge	20-Aug-15	TRUE	TRUE	TRUE
121	16-Jul-14	Waper Creek	48.905988	-115.665704	MT	USA		22-Jul-14	TRUE	TRUE	TRUE
110	16-Jul-14	Warland Creek	48.503	-115.276	MT	USA		22-Jul-14	FALSE	FALSE	FALSE
57		Warm Springs Creek	44.145790	-114.404366	ID	USA		11-Sep-13	TRUE	FALSE	FALSE
69	07-Aug-13	West Fisher Creek	48.05199	-115.42874	MT	USA		07-Aug-13	TRUE	FALSE	FALSE

Table A1-2 Cont'd.

Algal Sample ID	Date Collected	Stream Name	Latitude	Longitude	State	Country	Descriptive Location	Date Analyzed	Cells Present	Stalk Present	Stream Mat Present
70	23-Aug-13	West Fisher Creek	48.04	-115.483	MT	USA		23-Aug-13	TRUE	TRUE	FALSE
91	16-Jul-14	West Fork Yaak River	48.933456	-115.667897	MT	USA		22-Jul-14	FALSE	FALSE	FALSE
53		Williams Creek	44.55076	-114.512079	ID	USA		09-Sep-13	FALSE	FALSE	FALSE
12	07-Aug-13	Wolf Creek	48.23351	-115.28452	MT	USA		07-Aug-13	TRUE	FALSE	FALSE
65		Yaak River	48.561495	-115.973718	MT	USA	East Fork		TRUE	TRUE	FALSE
103	16-Jul-14	Yaak River	48.81073	-115.87482	MT	USA		21-Jul-14	TRUE	TRUE	TRUE
23	27-Jul-13	Yaak River	48.561719	-115.970428	MT	USA	Upstream of HWY bridge - access site	27-Jul-13	TRUE	TRUE	TRUE

Algal Scraping Protocol

Thank you for helping provide algae community samples to improve our understanding of Didymo distribution throughout the Northwest.

1. Sampling location is from one spot on the river/creek. Algal samples can be taken from various depths/rocks as needed at the sampling location

2. The goal is to gather the most diverse collection of algae within the sampling location (various colors, lengths, types)

3. Edges of the river /lake should be focused on. Algae can be scraped or grabbed off of substrate.

4. With the algae in the vial, (at least ¼ -½ full).On the vial: record name of river, date and location sampled.

5. On the data sheet, record River Name, Your Name, GPS locations if possible, Didymo mat presence (yes or no), Shade level (low, med, high), Size of water body (small, med, large). Write down any other comments/details (overall mat characteristics, other algae presence, etc.).

6. Add the Lugol's (small vial) to the sample (larger vial). If possible, seal the larger vial with electrical tape.

Folders can be mailed back to

Katie Coyle
7157 HWY 37
Libby, MT 59923

OR

Frank Wilhelm
University of Idaho
Department of Fish and Wildlife Science
875 Perimeter Drive Mail Stop 1136
Moscow, Idaho 83844

Any questions please feel free to call or email me. 208-315-1348 coyl9970@vandals.uidaho.edu

Figure A1-1. Data sheet instructions for *Didymosphenia geminata* distribution sampling.

Date	River Name	Sampler Name	Location	GPS	Didymo Mats (Y/N)	Shade level (low, med, high)	Size of water body (S,M,L)	Comments

Figure A1-2. Data sheet for collecting *Didymosphenia geminata* distribution samples

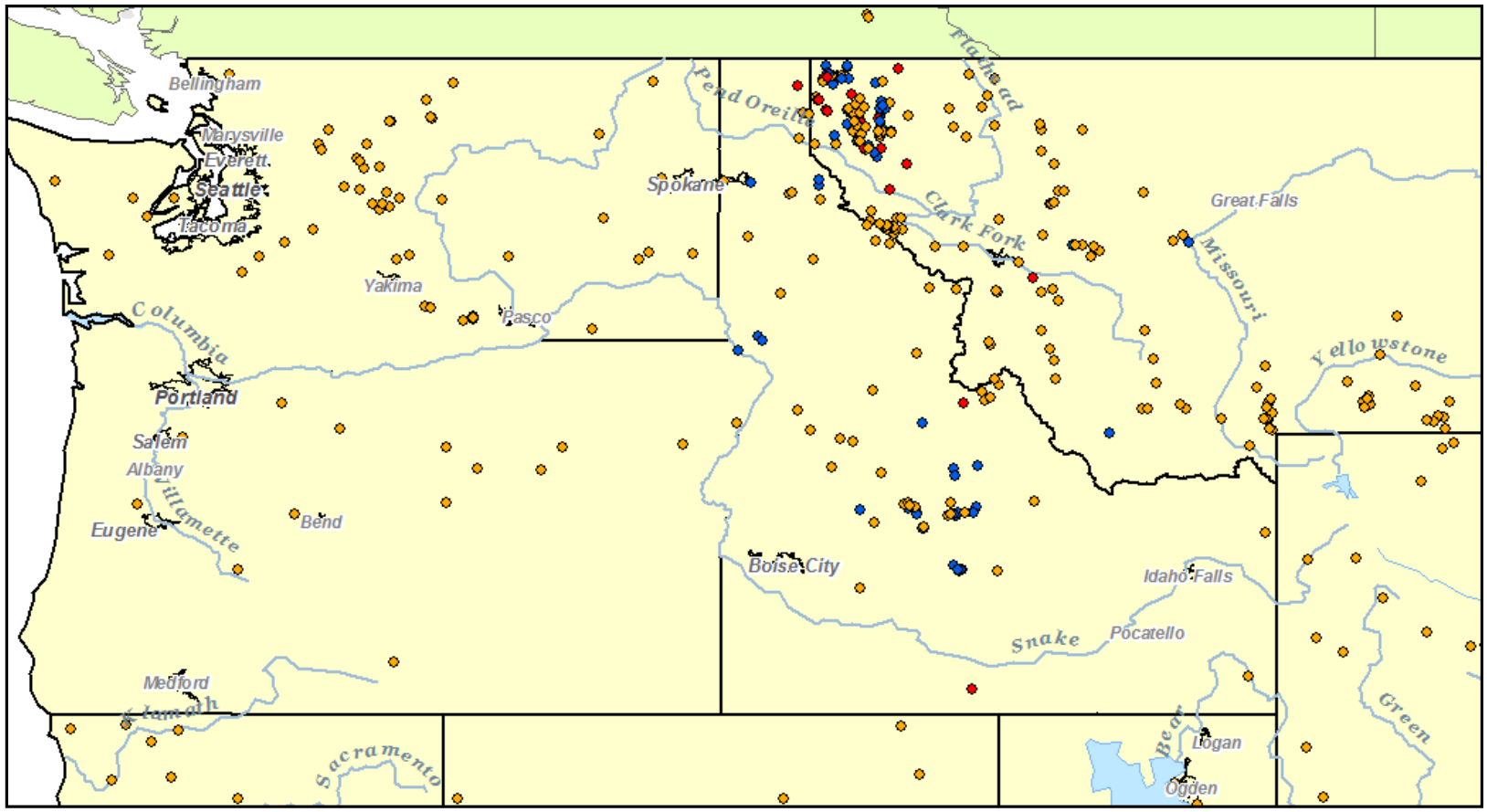

Figure A1-3. Locations of *Didymosphenia geminata* throughout the intermountain Northwest. Data compiled from algal scrapings collected in my study and that of Tait (2010). Yellow dots are confirmed presence of *D. geminata* cells; mat presence not determined. Red dots are confirmed presence of cells and mats of *D. geminata*, and blue dots are algal scrapings that were negative for *D. geminata*.

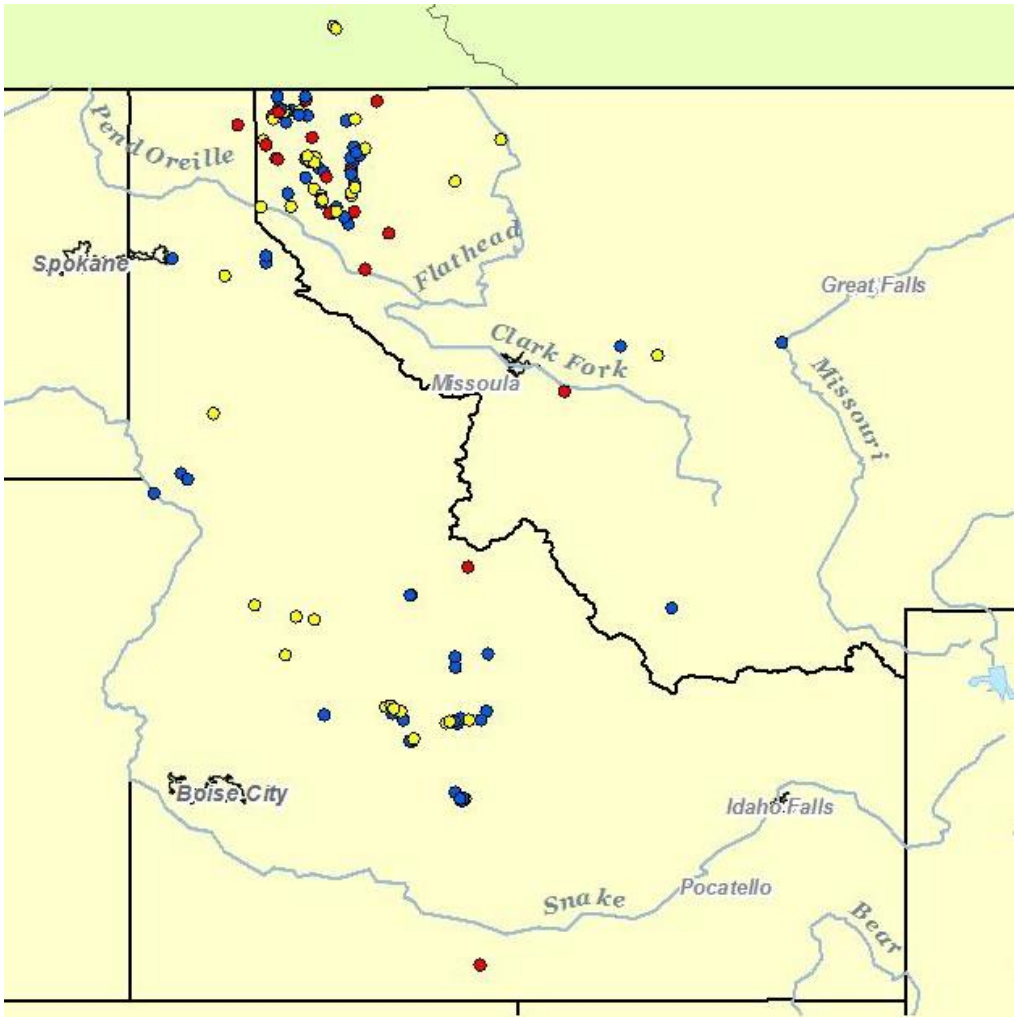

Figure A1-4. Locations of *Didymosphenia geminata* throughout Idaho and Montana. Yellow dots are confirmed presence of *D. geminata* cells; mat presence not determined. Red dots are confirmed presence of cells and mats of *D. geminata* and blue dots are algal scrapings that were negative for *D. geminata*.

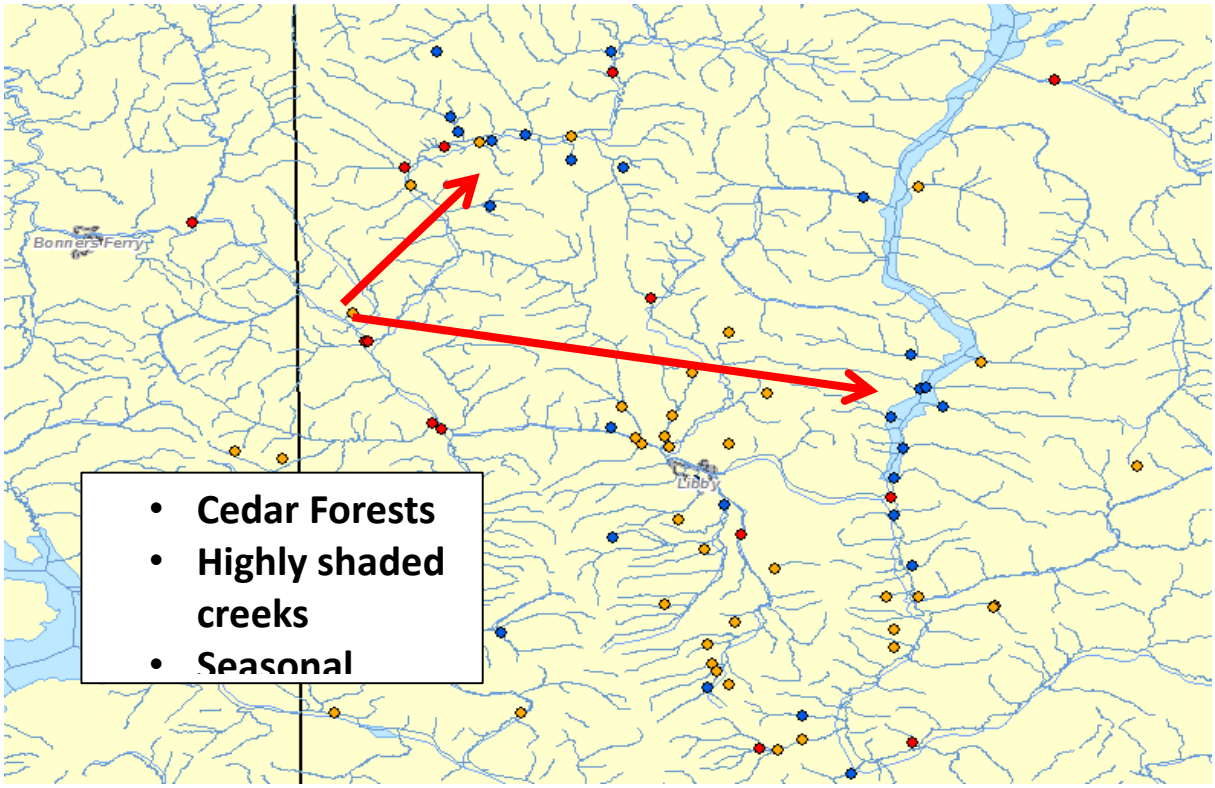

Figure A1-5. *Didymosphenia geminata* distribution on the Kootenai National Forest, Montana. Yellow dots are confirmed presence of *D. geminata* cells; mat presence not determined. Red dots are confirmed presence of cells and mats of *D. geminata*, and blue dots are algal scrapings that were negative for *D. geminata*. Red arrows are pointed towards regions that had high prevalence of negative detections of *D. geminata* and habitat characteristics outline in the box.

Appendix 2: Characteristics of *Didymosphenia geminata* abundance and coverage type in tributaries of the Kootenai River, in Idaho and Montana, USA

Abstract

Nuisance mats of *Didymosphenia geminata* have been present in the Kootenai River system since 2001. However, this species has been present in the Kootenai River ecosystem as part of the periphyton community without any significant mat growth before (record from 1866) and after it was impounded by Libby Dam. The shift of *D. geminata* from an unnoticed member of the periphyton community to one of nuisance status, given the prolific production of stalk material, in the absence of visible changes to the Kootenai River in the late 1990's is not well understood. I examined the abundance and cover type (cells, mats, and nuisance mats) of *D. geminata* in tributaries of the Kootenai River to better understand the ecology and diversity of cell coverage across the Kootenai River watershed.

In tributaries, peak mat coverage occurred from late May to August which began to degrade in September and October. Mat material detached at high flows in early winter, after which the *D. geminata* community "restarted" growth. Overall, *D. geminata* growth varied in persistence, depth and coverage across tributaries. In creeks with nuisance mat growth, light and temperature were highest. Mechanisms underlying the wide range of mat coverage are hypothesized to be changes to the watershed, specifically, the concentrations of available phosphorus and nitrogen. Water quality characteristics should be monitored and evaluated within these tributaries at a high frequency for at least an entire year to further elucidate and identify which specific factor contributes to mat growth.

Introduction

Native to the Kootenai River, *Didymosphenia geminata*, has progressed from an unseen member of the periphyton community to producing nuisance mats since 2001 (Holderman and Hardy 2004). These nuisance mats cover the benthos, altering the invertebrate community structure from large- to small-bodied individuals (Marshall et al. 2008; Marshall 2007), and degrading the aesthetics of the Kootenai River in Libby, MT, USA. In the river proper, peak growth occurs between February and April (Chapter 2), and the greatest mat coverage is directly below the Libby Dam with a decreasing gradient of coverage and mat depth progressing downstream. As the Kootenai River enters northern Idaho, mat presence is reduced to small "tufts" on rocks which does not impact the invertebrate community (Holderman and Hardy 2004).

This recent and persistent appearance of nuisance mats, not only in the Kootenai River but also in several of its tributaries, has led to the hypothesis that *D. geminata* is an introduced species because the pattern of occurrence is similar to that often reported for an exotic species (Elwell and Spaulding 2007; Root et al. 2012; Seguar 2011; Reid et al. 2012). However, historical records and current studies have shown that *D. geminata* has been present in this system since before 1866 (as cited in Bothwell et al. 2014), and it cannot be classified as an introduced species to the region (Perry and Huston 1983). This suggests that the recent proliferation of nuisance mats in the Kootenai River is due to some other ecosystem change(s) that have stimulated the species to form these nuisance mats.

Further investigation into the Kootenai River system has shown that areas with the highest *D. geminata* mat coverage (below the Libby Dam) have the highest total nitrogen concentrations, total nitrogen to total phosphorus (TN:TP) and soluble inorganic nitrogen to

total dissolved phosphorus (SIN:TDP) ratios (Figs. A2-1, A2-2, A2-3, Chapter 4). Moving downstream from Libby Dam, these ratios decrease as does the *D. geminata* mat coverage, suggesting a possible relationship. Several tributaries to the Kootenai River have varied coverage of *D. geminata* mats providing a range of coverage which could be examined in relation to the nutrient concentrations and ratios to identify potential mechanisms that may explain conditions that result in mat formation. To clarify patterns of growth within tributaries across seasons, I examined mat coverage characteristics monthly for one year in 16 tributaries of the Kootenai River.

Methods

Tributaries to the Kootenai River spanning a wide range of *D. geminata* abundance and cover types were selected based on previous observations (Figure A2-1; Table A2-1). The 16 tributaries were chosen based on similarity of gradient, riparian vegetation cover and size, and range of *D. geminata* coverage (Fig. A2-4; Table A2-1). The *D. geminata* community within these tributaries was surveyed monthly from July 2014 to July 2015. Monthly sampling did not occur in November or December of 2014, and in January 2015 because the tributaries were ice-covered. In March 2015 the streams were also inaccessible because of flooding from snowmelt runoff. At each stream, a survey reach for repeated sampling was marked and on each sampling occasion, a 19 mm diameter scraping was taken from an area representative of the mat coverage in each tributary. A 19 mm metal corer was used to cut through mats to the substrate and delineate the sample area. This sample area was then scraped off with a metal scraper and placed into a 50 ml centrifuge tube with 0.25 ml of Lugol's iodine and 10 ml of DI water. A standing crop index (SCI) and Index of Didymo Coverage (IDC) (Kilroy et al. 2005) was calculated to estimate substrate coverage of the mats.

The IDC has been shown to be a reliable indicator of *D. geminata* abundance and ash-free dry mass (AFDM). It has been consistently used to classify *D. geminata* coverage (Kilroy et al. 2005), while the SCI (average depth × percent coverage) provides insight to the mat coverage of a benthic ecosystem. IDC was determined by sampling ten rocks per reach in each tributary and estimating percent algal coverage, percent *D. geminata* coverage and by measuring five mat depths on the rocks. These values were then used with an IDC conversion table to calculate the IDC score which was then multiplied by the percent cover (Table A2-2). The frequency of dividing cells (FDC) and total cell count were calculated from the 19 mm scrapings taken at each reach to calculate growth rate over time.

Results

The presence and growth of *D. geminata* mats varied across tributaries of the Kootenai River and occurred as single microscopic stalks, tufts, small mats and nuisance mats (Table A2-3). However, the latter type was rare in the 16 tributaries (Appendix 1). Two of the 16 tributaries had nuisance level mats for most of the spring and summer, while mats were present in several tributaries throughout the entire year (Fig. A2-5).

Algal community characteristics

The tributaries with *D. geminata* as part of the algal community but with no visible mat growth were: Long Canyon Creek, Boundary Creek, South Fork of Trout Creek, and Myrtle Creek. Creeks with no *D. geminata* were Fisher Creek and Pipe Creek. Creeks with the greatest total cell count of *D. geminata* were also those that had the greatest mat depths (Figs. A2-6 and A2-7). Frequency of dividing cells for nearly all streams with *D. geminata* as part of the algal community was consistently low, ranging from 0.025 to 0.10 (Fig. A2-8). Long Canyon Creek had an abnormally high FDC at 0.33. Stalk length was longest in Lake

Creek and the Yaak River (Fig. A2-9). Many creeks with cell presence did not have any stalk growth as *D. geminata* was "well-behaved" within these creeks (Fig. A2-9). Stalk length was longest in July and declined by August. This was similar to mat depth and total cell counts, suggesting that the decline of mat and stalk growth begins in August and mat integrity began to decrease after August.

Mat characteristics

Decomposition of the mats across all tributaries was observed in September and October of 2014. Reduced integrity of the mats combined with increased flow rates resulting from precipitation events in fall reduced mat coverage. This effectively restarted the *D. geminata* mat community by removing most of the existing mat material.

Libby Creek, Lake Creek, and Yaak River had the greatest mat depth across all months (Fig. A2-6). Lake Creek and Yaak River also had the highest IDC scores over the entire growing season, while all other streams peaked in July and August (Fig. A2-10). SCI also demonstrated that Lake Creek, Libby Creek, and the Yaak River had the greatest nuisance mat presence across the growing season (Fig. A2-11). In all tributaries, mat coverage and depth peaked between late May and August. In comparison, the Kootenai River *D. geminata* mat growth peaked in February and March (Chapter 2).

Light and temperature characteristics

Due to ice cover in the tributaries during winter, dataloggers were collected in November 2014 and redeployed in March 2015. However, over the entire year and deployments, several loggers were lost due to vandalism and as such, the data set for all tributaries is only complete from 9 July to 12 November 2014. Within all creeks, temperatures ranged between 10 to 25 °C from July to September, decreasing to 4-5 °C in November (Fig.

A2-12). The Yaak and Fisher rivers had the highest temperatures over those four months. Light intensity and duration also decreased from summer to fall. The Fisher and Yaak rivers had the highest light intensity throughout the peak growing season (Fig. A2-13). By November, light intensity in all tributaries was <2,000 lux.

Discussion

For the Kootenai River, an impounded system, mat growth peaks in February and persists nearly year round (Chapter 2). This pattern of high growth in the spring or year round has been observed in other regulated systems that have stable channels (e.g., Kirkwood et al. 2009). However, for the tributaries studied, mat growth peaked between May and August. I attribute this to the spring runoff and bed disturbance resulting in shear stress, which physically removes the *D. geminata* mat (Cullis et al. 2012). This suggests that bed-moving discharge could act as a control mechanism in areas with dense mat coverage. This could be achieved via increased discharge that cause the bed to move, likely less than ideal in dam tailwater areas where bed stability is of great importance. However, it may be possible to add substrate of smaller size such as gravel that is then moved downstream in a scouring action by high release discharge. Such a mechanical mechanism should be given further consideration, especially for areas immediately downstream of dam sites.

Previous studies have found that the habitat window or when visual tufts of *D. geminata* in unregulated streams first appear on the substrate coincides with i) discharge (Q) sufficiently low to allow cell attachment onto substrate, and ii) enough light availability to promote stalk growth (Cullis et al. 2012). This has been reported as approximately 50% of the maximum discharge (Q_{max}) of peak flow in spring (C.A. Gillis, personal communication, Restigouche Rivers Watershed Management Council; Cullis et al. 2012). Changes in flow

regimes such as earlier ice-out (Lavery et al. 2014) or the duration or timing of spring runoff (Bothwell et al. 2014) may also contribute to the variation seen in the growth of mats in the tributaries.

Future studies should concentrate on frequent water quality (NO_2+NO_3, SRP, DIN:SRP) sampling to increase the potential to detect relationships between water quality parameters and the mat coverage of *D. geminata.* Based on previous studies, SRP and NO_2+NO_3, should be prioritized and monitored at weekly intervals for several months (Chapter 4). If possible, SRP detection limits should be 1 µg/L as results from Chapters 3 and 4 indicate that changes in mat growth can occur with 0.5 µg/L increases above ambient of dissolved phosphorus.

Understanding the detailed ecology of this species throughout a delineated region will help to further illuminate the perceived increase of nuisance mats across the globe. The potential factors influencing the varying levels of *D. geminata* growth within tributaries of the Kootenai River are many. Water quality, timing of spring runoff, stability of substrate, and discharge (quantity and duration) are all factors that possibly interact to influence mat growth. I suggest that these variables be further evaluated and investigated at the watershed scale. I also conclude hat *D. geminata* can be present in periphyton communities and that nuisance mats in the United States may not be an introductions of a foreign species but rather a change in environmental variables and may be indicative of large-scale landscape shifts such as those resulting from climate change or anthropogenic influences to nutrient cycles (Taylor and Bothwell 2014).

Most of the research on *D. geminata* has centered on a singular river with particularly heavy nuisance mats (e.g., Sundareshwar et al. 2012; Kirkwood et al. 2009; Gillis and

Chalifour 2010; James et al. 2014; Reid et al. 2012; Tomás et al. 2010; Richardson et al. 2014; Bergey et al. 2009; Segura 2011). Examining these individual rivers and their particular variables and then comparing them to others across the globe leaves many large gaps in the dataset that may be important to answering the question of "why" this native diatom has become a nuisance. Research at the landscape scale, in particular, nonpoint source nutrient pollution, changes in DIN:SRP, sediment loading and scouring changes and timing are important to understand the response of periphyton communities and in particular the stalk growth of *D. geminata.*

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Tables

Table A2-1. Tributaries of the Kootenai River with *Didymosphenia geminata* presence that were sampled July 2014 to July 2015. The North Fork of Trout Creek had no cells detected but previous scrapings have shown *D. geminata* presence.

Didymo Coverage	Tributary Name	State	Didymo Cell Presence	Didymo Mat Presence	Dam
No Cells Present	Pipe Creek	MT	None	None	N
	Fisher Creek	ID	None	None	N
No Mats Cells Present	Boundary Creek	ID	Yes	None	N
	Myrtle Creek	ID	Yes	None	N
	Long Canyon	ID	Yes	None	N
	SF Trout	ID	Yes	None	N
Low - Tufts	Callahan Creek	MT	Yes	Low - Tufts	Y
	Ball Creek	ID	Yes	Low - Tufts	N
	Smith	ID	Yes	Low - Tufts	Y
	NF Trout	ID	Yes	Low - Tufts	Y
Medium Mat Coverage	Fisher River	MT	Yes	Medium Mats	N
	Moyie River	ID	Yes	Medium Mats	Y
	Tobacco River	MT	Yes	Medium Mats	N
Heavy Mat Presence	Yaak River	MT	Yes	Heavy Mats	N
	Libby Creek	MT	Yes	Heavy Mats	N
	Lake Creek	MT	Yes	Heavy Mats	N

Table A2-2. Table for calculating Index of Didymo Coverage (IDC) thickness scores. These scores of mat thickness were multiplied by the percent coverage to calculate the final IDC score. Methods are based on Kilroy et al. (2005) and are correlated with ash-free dry mass (AFDM).

Mat Depth	IDC Score
0	0
<1mm	1
1-5mm	2
6-15mm	3
16-30mm	4
(>30mm	5

Table A2-3. Average characteristics (mean ± SD) of *Didymosphenia geminata* from July 2014, October 2014, February 2015 and May 2015 within 16 tributaries of the Kootenai River, Libby, Montana, USA.

Didymo Presence	Stream	Avg. Depth (mm)	SCI	IDC	Total Cell Count	FDC
No Cells	Pipe C.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Fisher C.	0 ± 0	0 ± 0	0 ± 0	0.85 ± 2.09	0 ± 0
	Boundary C.	0 ± 0	0 ± 0	0 ± 0	2.12 ± 2.84	0 ± 0
	Myrtle C.	0 ± 0	0 ± 0	0 ± 0	1.85 ± 3.04	0 ± 0
Cells, No Mats	Long Canyon C.	0 ± 0	0 ± 0	0 ± 0	1.71 ± 1.97	0 ± 0
	SF Trout C.	0 ± 0	0 ± 0	0 ± 0	1.28 ± 1.38	0 ± 0
	Callahan C.	0.10 ± 0.16	0.26 ± 0.43	0.75 ± 1.24	6.87 ± 11.08	0 ± 0
Low Mats	Ball C.	0.15 ± 0.27	1.92 ± 3.89	3.45 ±6.85	73.68 ± 104.50	0.02 ± 0.03
	Smith C.	0.11 ± 0.30	0.58 ± 1.54	0.83 ± 2.20	186.28 ± 289.04	0.02 ± 0.05
	NF Trout C.	0.12 ±0.34	3.56 ± 9.42	3.22 ± 8.54	0.57 ± 1.39	0 ± 0
	Fisher R.	0.47 ± 0.40	4.04 ± 4.31	6.26 ± 6.01	175.87 ± 203.91	0.01 ± 0.03
Medium Mats	Moyie R.	0.57 ± 0.45	8.03 ± 7.49	13.5 ± 11.74	870 ± 1998.82	0.03 ± 0.03
	Tobacco R.	0.16 ± 0.30	3.46 ± 8.25	5 ± 11.78	104.37 ± 218.26	0.02 ± 0.03
	Yaak R.	1.74 ± 1.16	116.7 ±93.81	92.37 ± 62.87	589.25 ± 1012.37	0.03 ± 0.02
Heavy Mats	Libby C.	1.91 ± 1.78	39.20 ± 49.55	20.75 ± 26.96	1776.12 ± 1964.86	0.02 ± 0.02
	Lake C.	3.88 ± 1.65	248.97 ± 178.85	115.75 ± 71.59	1708.5 ± 1978.42	0.01 ± 0.02

Figures

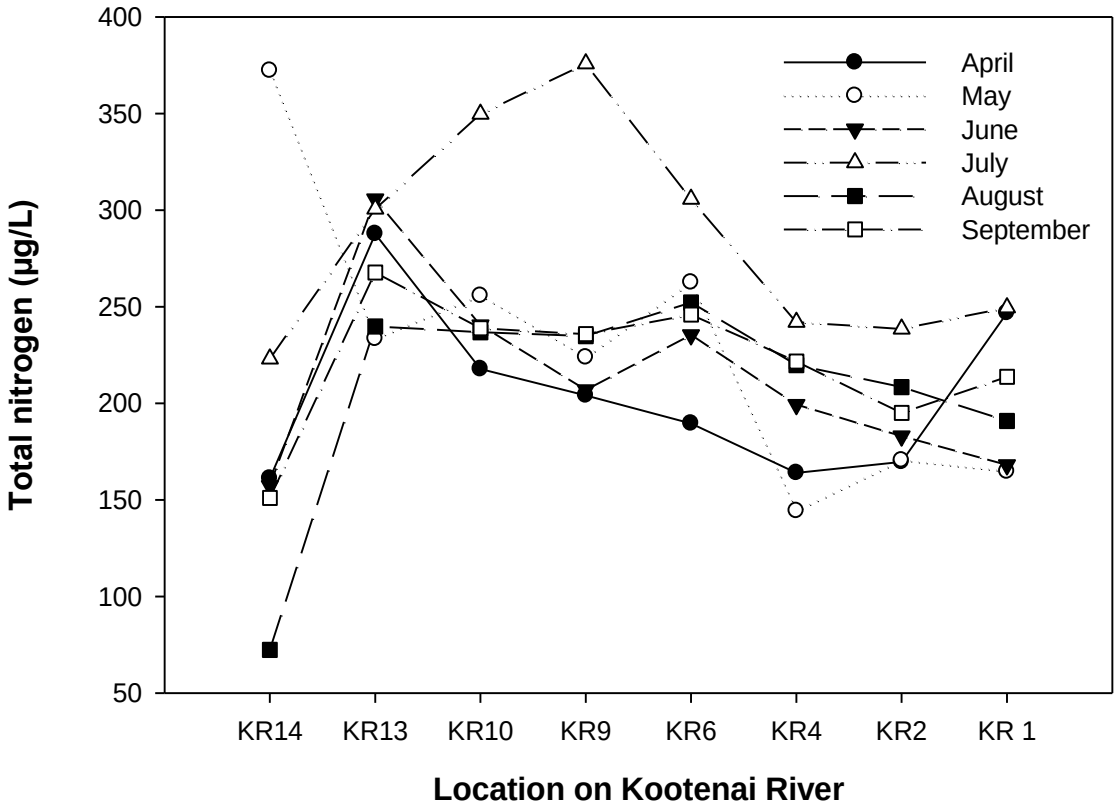

Figure A2-1. Total nitrogen concentrations (µg/L) in the Kootenai River from KR 14 (near Kootenay River, BC) to KR 13 (below Libby Dam) and KR 1 (near Kootenay Lake, BC) from April to September 2013. Data provided by KTOI and used with permission. Permission to reuse must be obtained from the rightsholder.

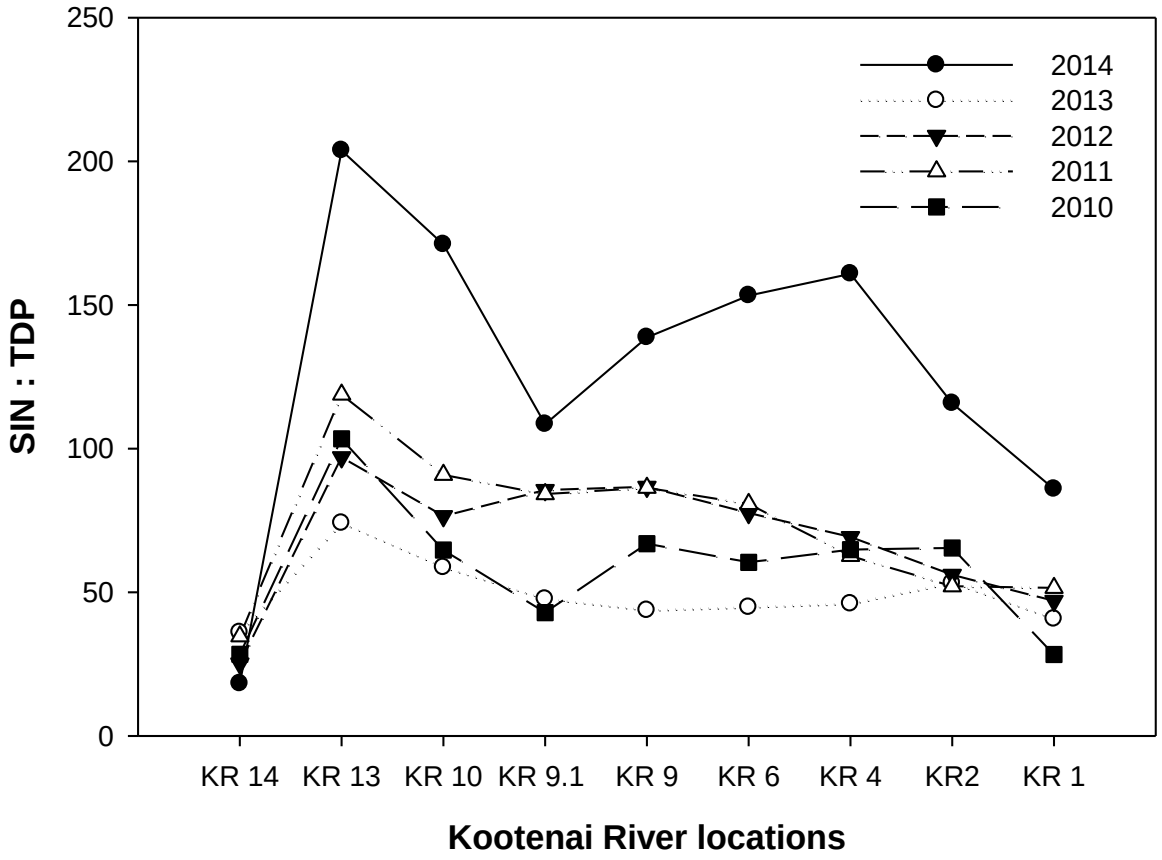

Figure A2-2. Average soluble inorganic nitrogen (SIN) to total dissolved phosphorus atomic ratios (TDP) from 2010 to 2014 throughout the Kootenai River in Montana, Idaho and Canada (KTOI 2014). KR 14 is located above Koocanusa Reservoir while KR 13 is located below the Libby Dam. KR 10 through KR 1 are progressively downstream along the Kootenai River through Montana, Idaho and back into Canada. Data provided by KTOI and used with permission. Permission to reuse must be obtained from the rightsholder.

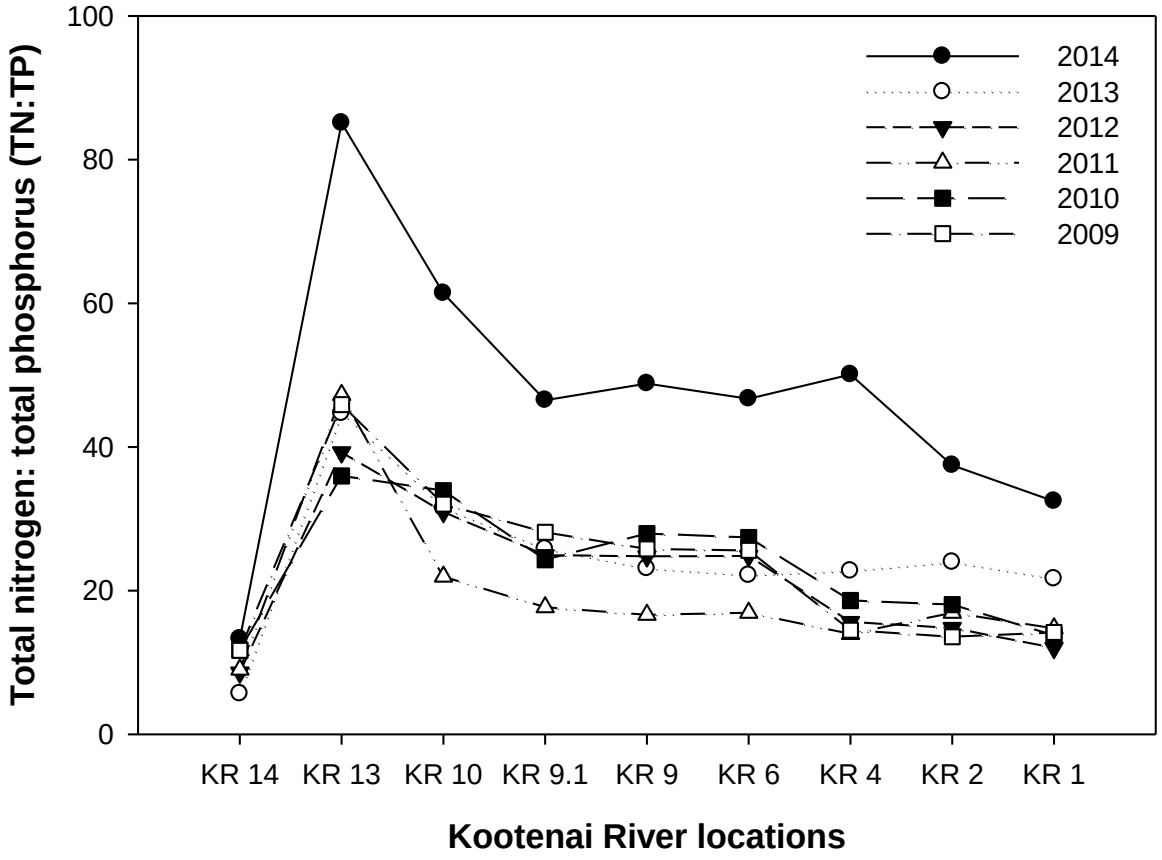

Figure A2-3. Average total nitrogen (TN) to total phosphorus (TP) atomic ratios from 2009 to 2014 throughout the Kootenai River in Montana, Idaho and Canada. KR 14 is located above Koocanusa Reservoir while KR 13 is located below the Libby Dam. KR 10 through KR 1 are progressively downstream along the Kootenai River through Montana, Idaho and back into Canada. Data provided by KTOI and used with permission. Permission to reuse must be obtained from the rightsholder.

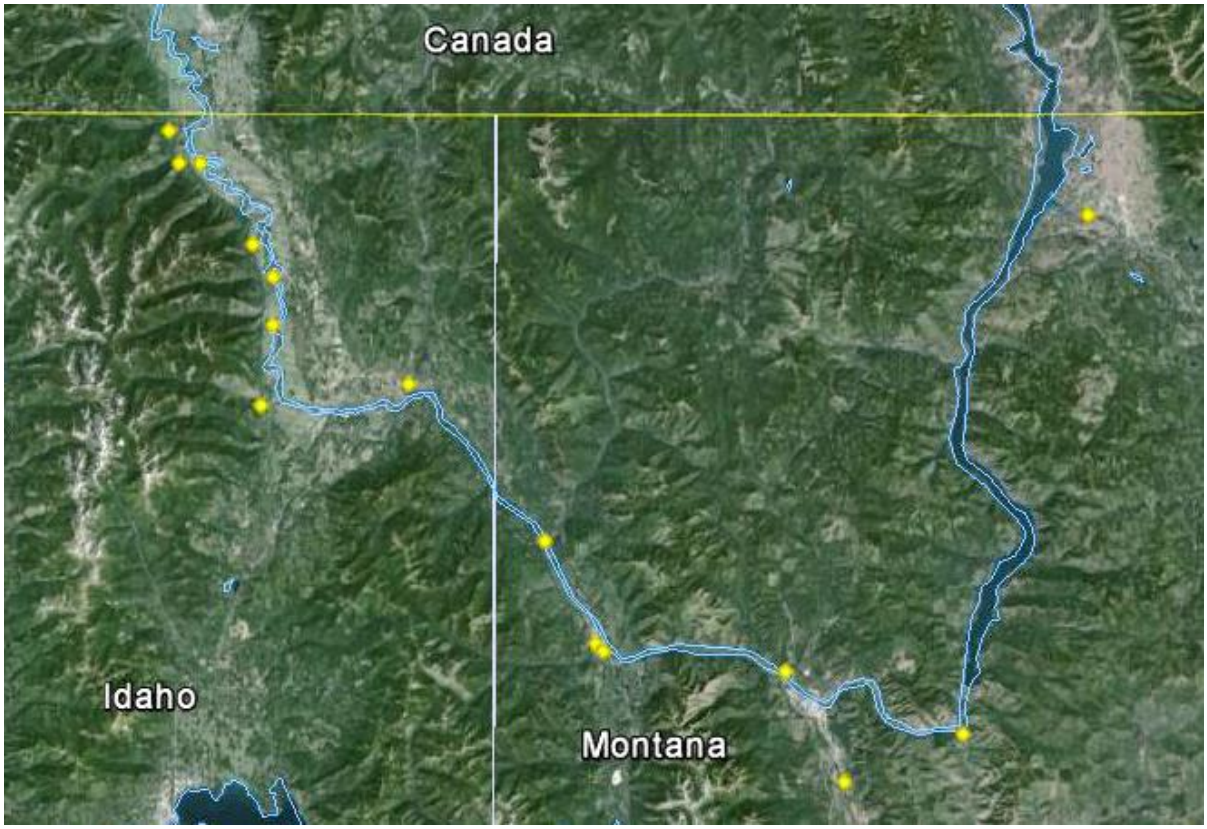

Figure A2-4. Tributaries of the Kootenai River that were sampled for *Didymosphenia geminata* mat characteristics.

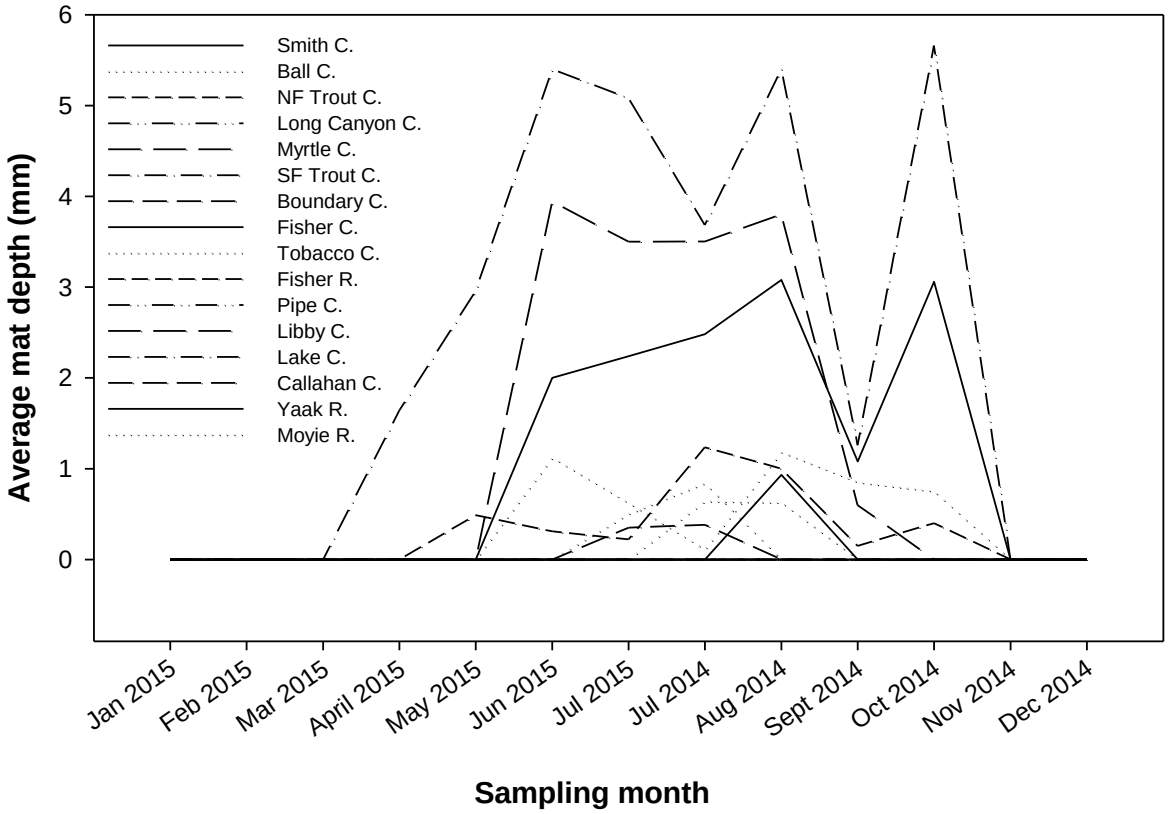

Figure A2-5. Average mat depth of *Didymosphenia geminata* from a 19 mm diameter scraping in tributaries of the Kootenai River in Montana and Idaho, USA. Sampling occurred from July 2014 to July 2015.

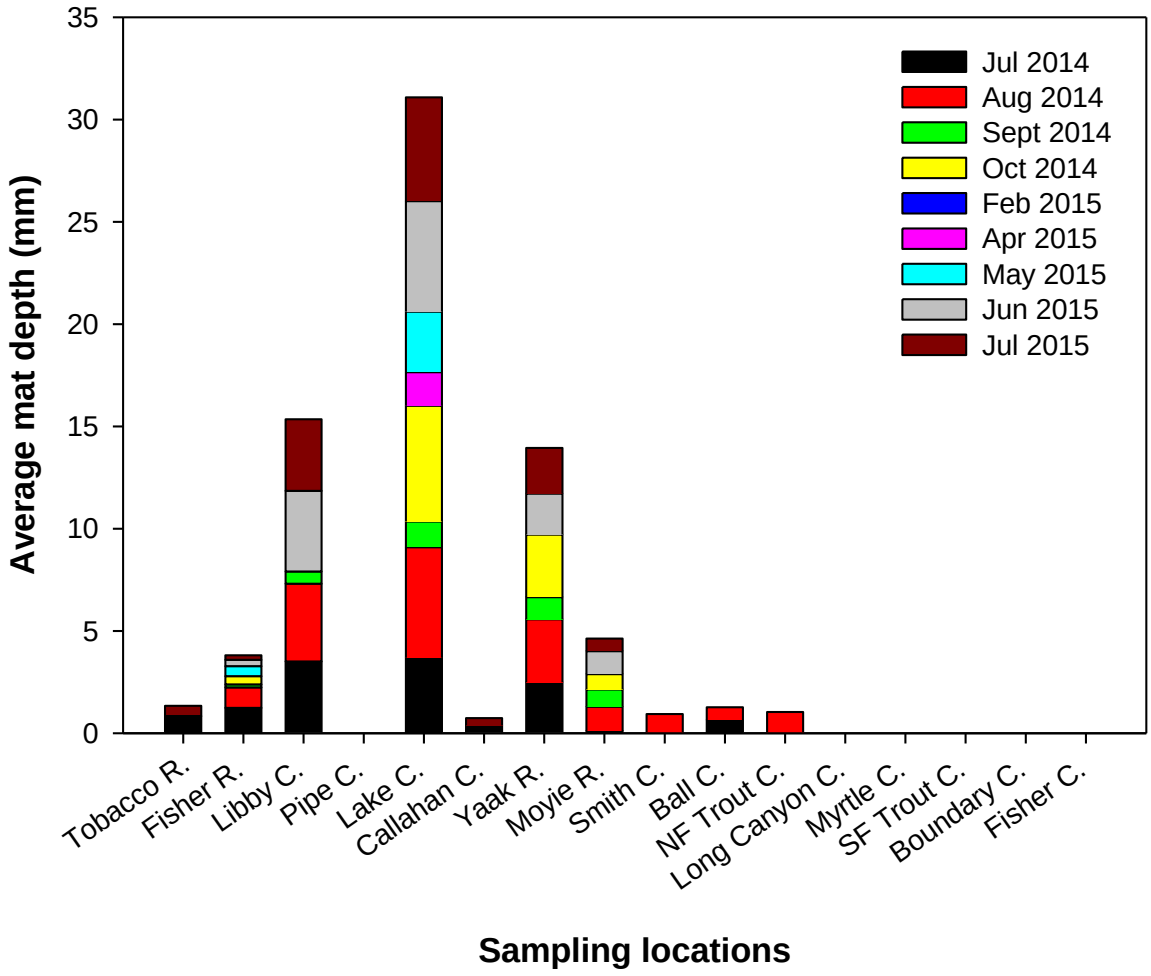

Figure A2-6. Average mat depth of *Didymosphenia geminata* from July 2014 to July 2015 across tributaries of the Kootenai River. Tributaries are in Montana and Idaho, USA.

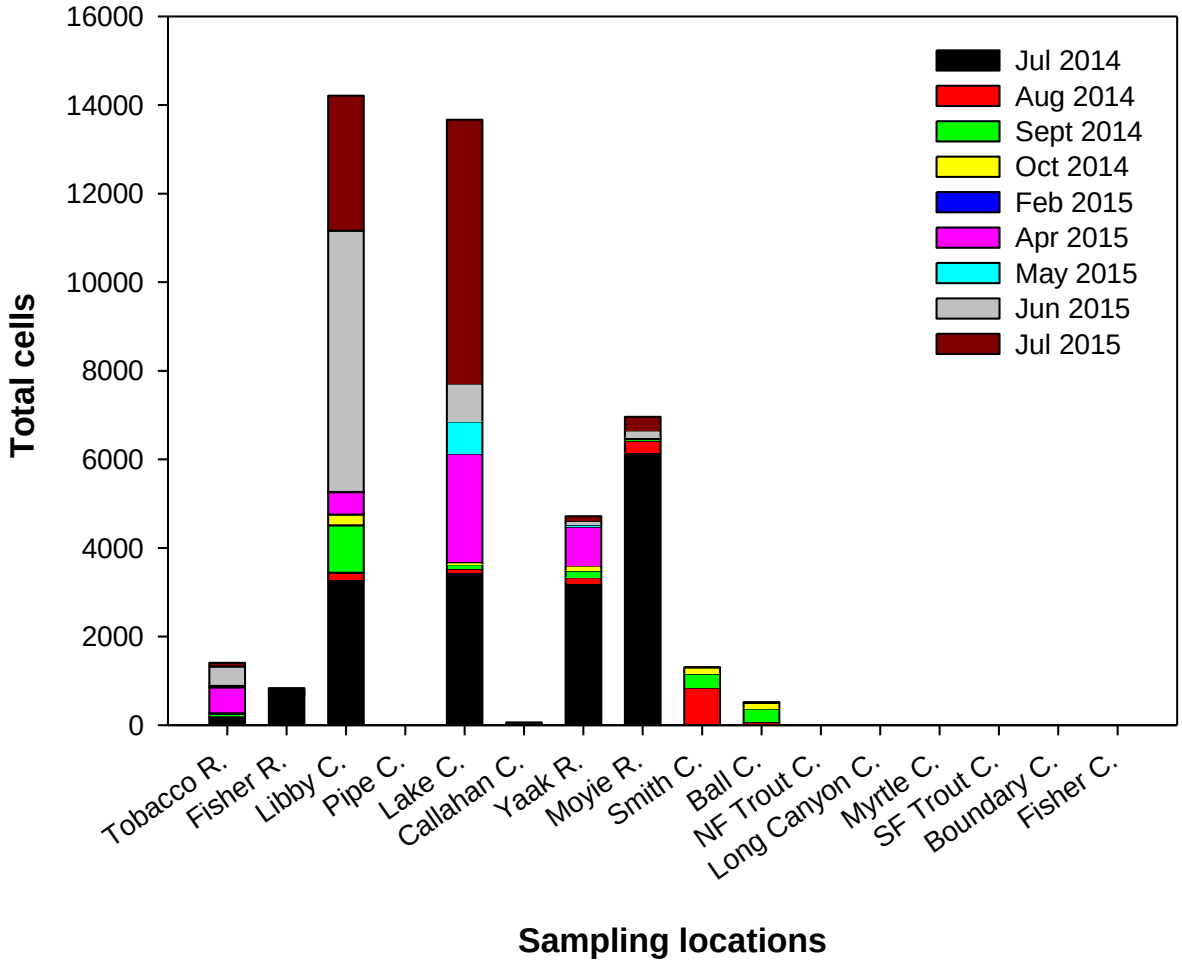

Figure A2-7. Total live cell count of *Didymosphenia geminata* from a 19mm diameter scraping in tributaries of the Kootenai River in Montana and Idaho, USA. Sampling occurred from July 2014 to July 2015.

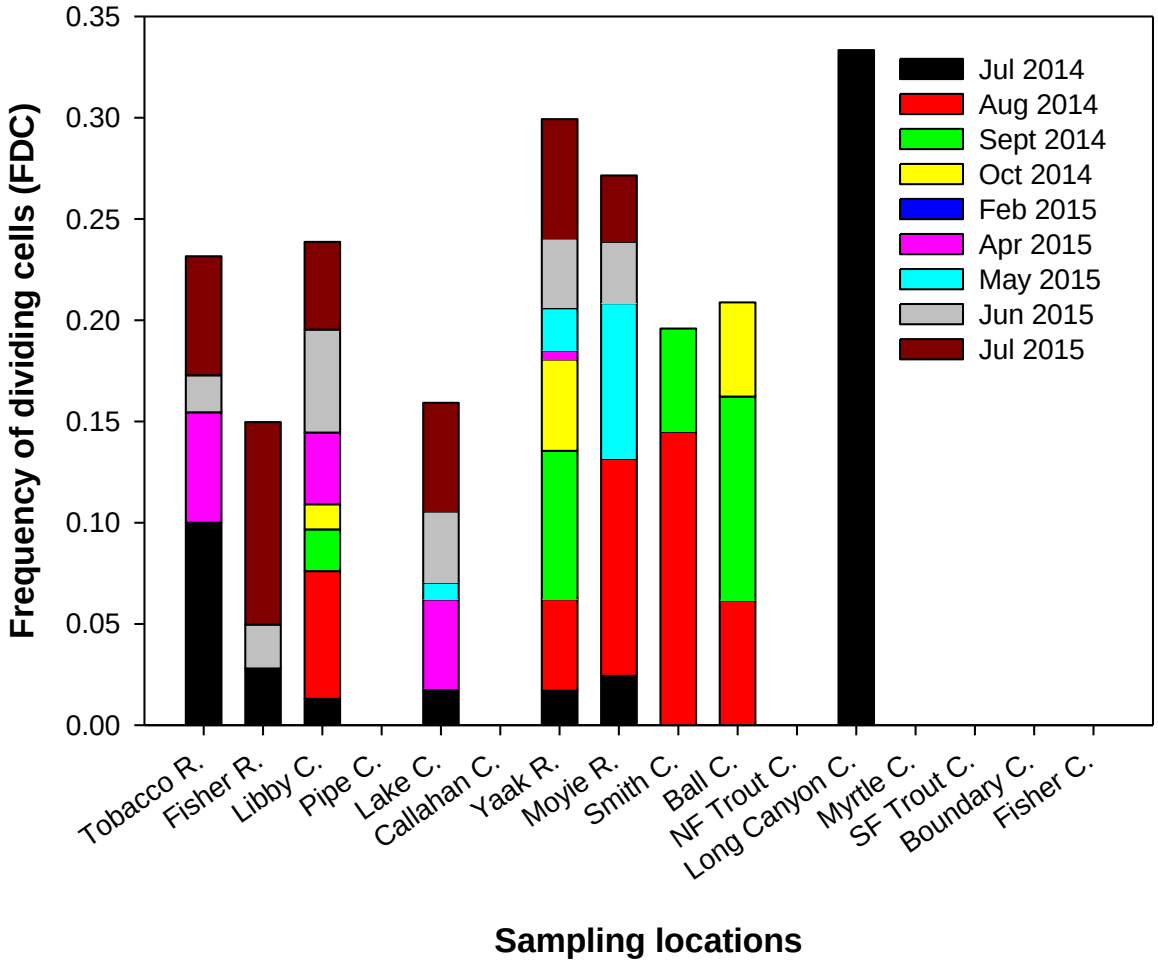

Figure A2-8. Frequency of dividing cells (FDC) of *Didymosphenia geminata* from a 19 mm diameter scraping in tributaries of the Kootenai River in Montana and Idaho, USA. Sampling occurred from July 2014 to July 2015.

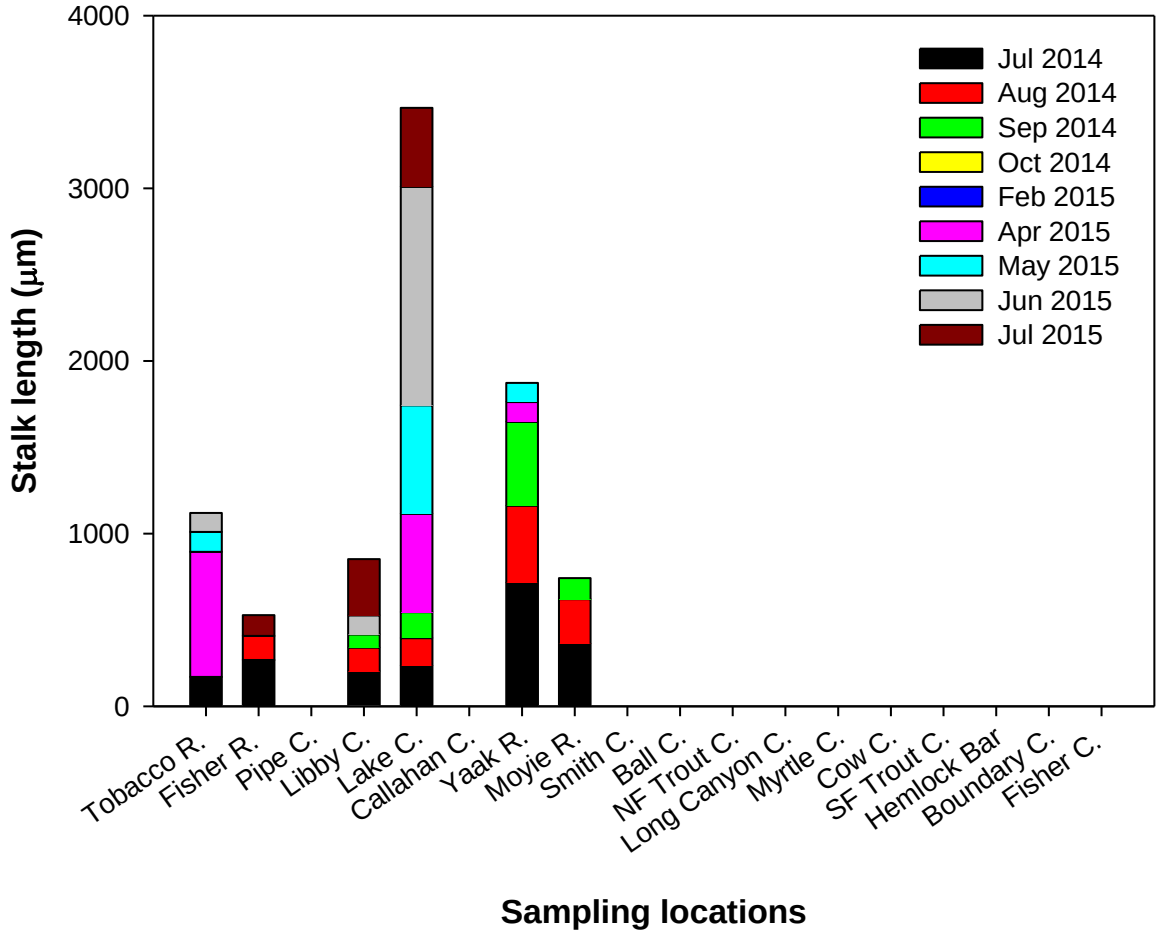

Figure A2-9. Stalk length of *Didymosphenia geminata* from a 19 mm diameter scraping in tributaries of the Kootenai River in Montana and Idaho, USA. Sampling occurred from July 2014 to July 2015.

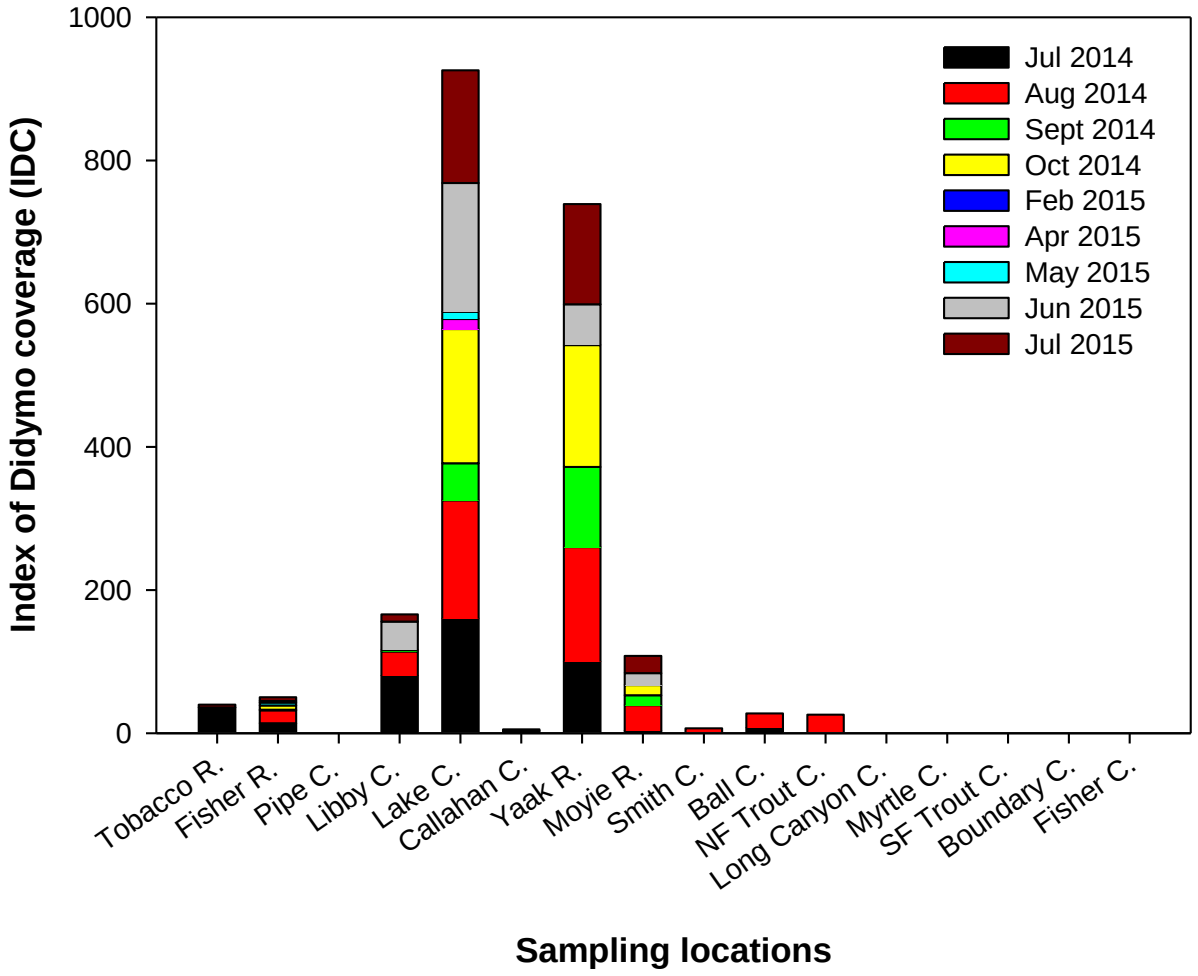

Figure A2-10. Index of Didymo Coverage (IDC) from July 2014 to July 2015 across tributaries of the Kootenai River. IDC is a value correlated with ash-free dry mass (AFDM) of *Didymosphenia geminata.* Tributaries are in Montana and Idaho, USA.

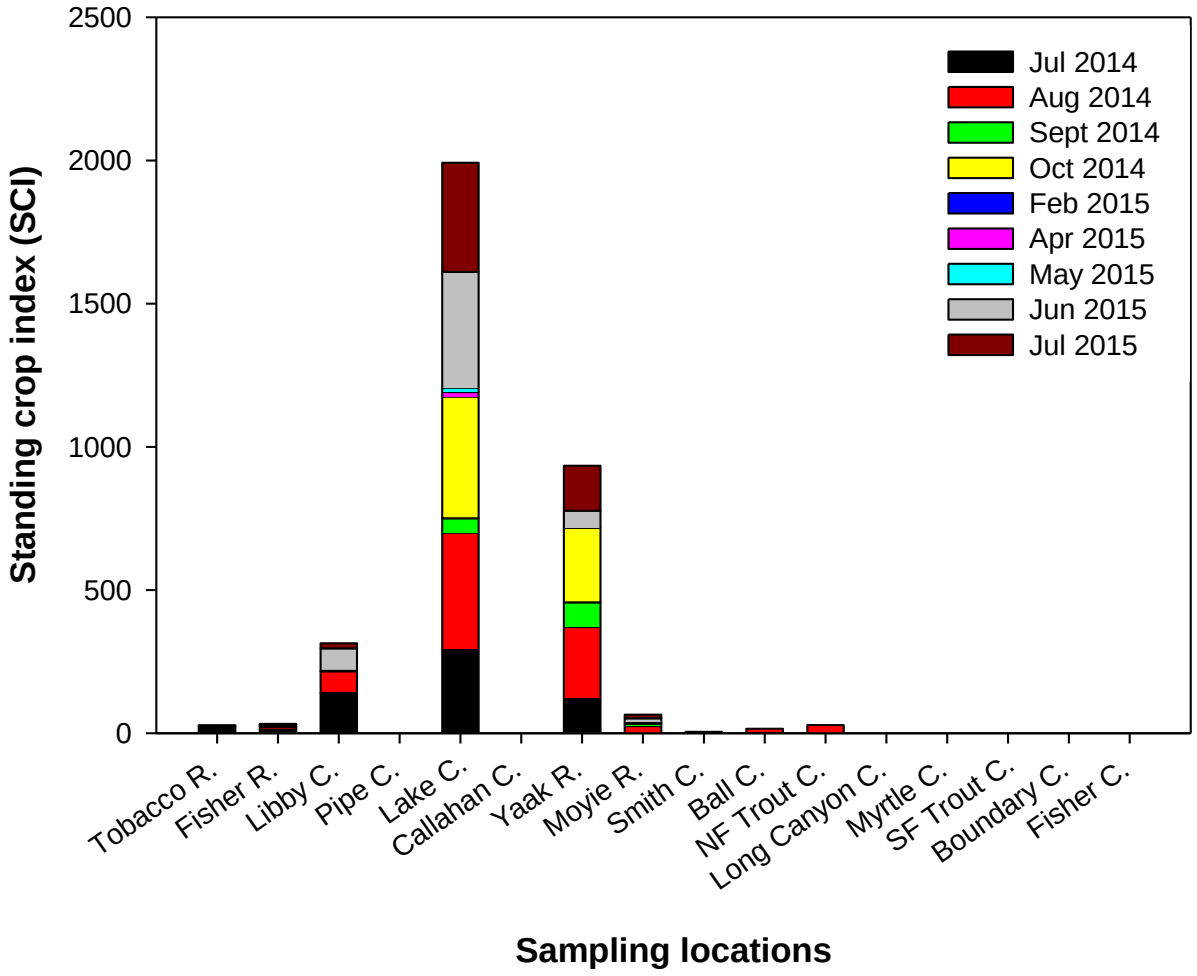

Figure A2-11. Standing crop index (SCI) from July 2014 to July 2015 across tributaries of the Kootenai River. Standing crop index of *Didymosphenia geminata* is percent coverage multiplied by average depth. Tributaries are in Montana and Idaho, USA.

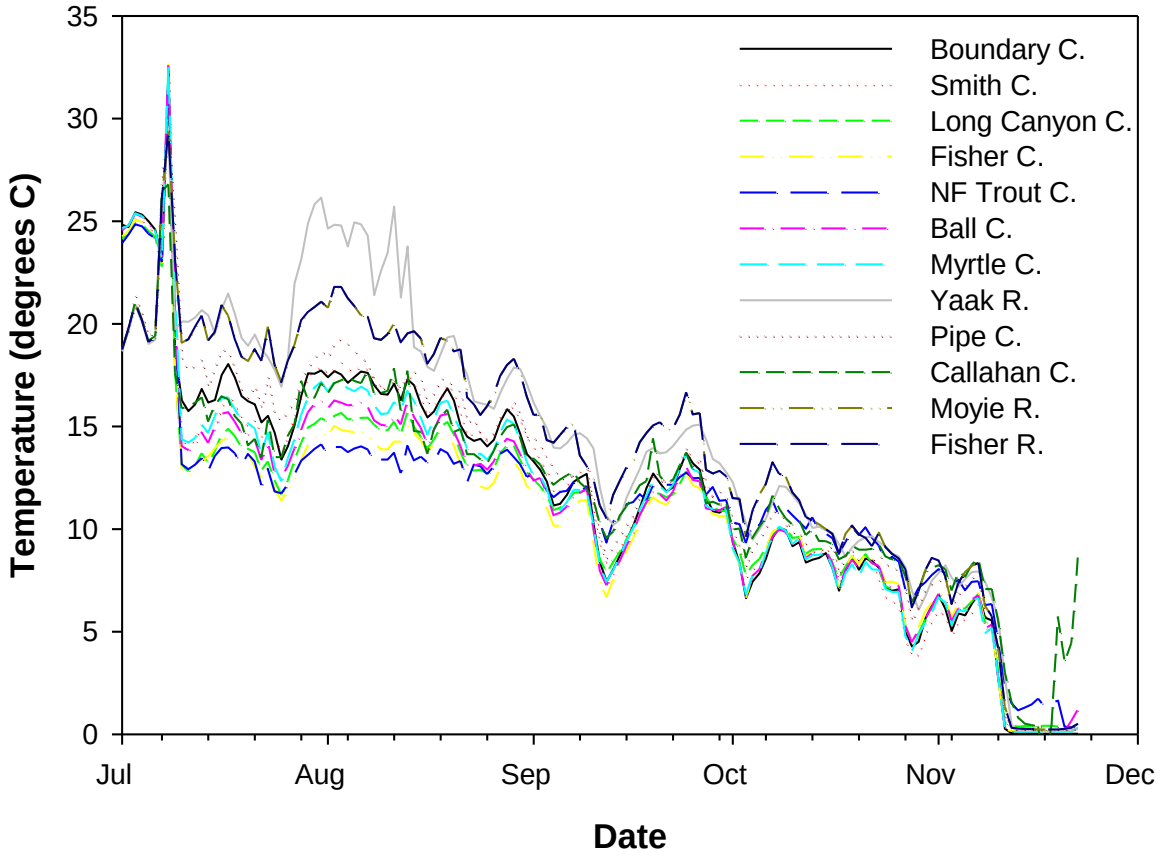

Figure A2-12. Temperature degrees Celsius of 16 tributaries of the Kootenai River from in northwestern Montana and northern Idaho, USA, from 1 July to 22 November 2014.

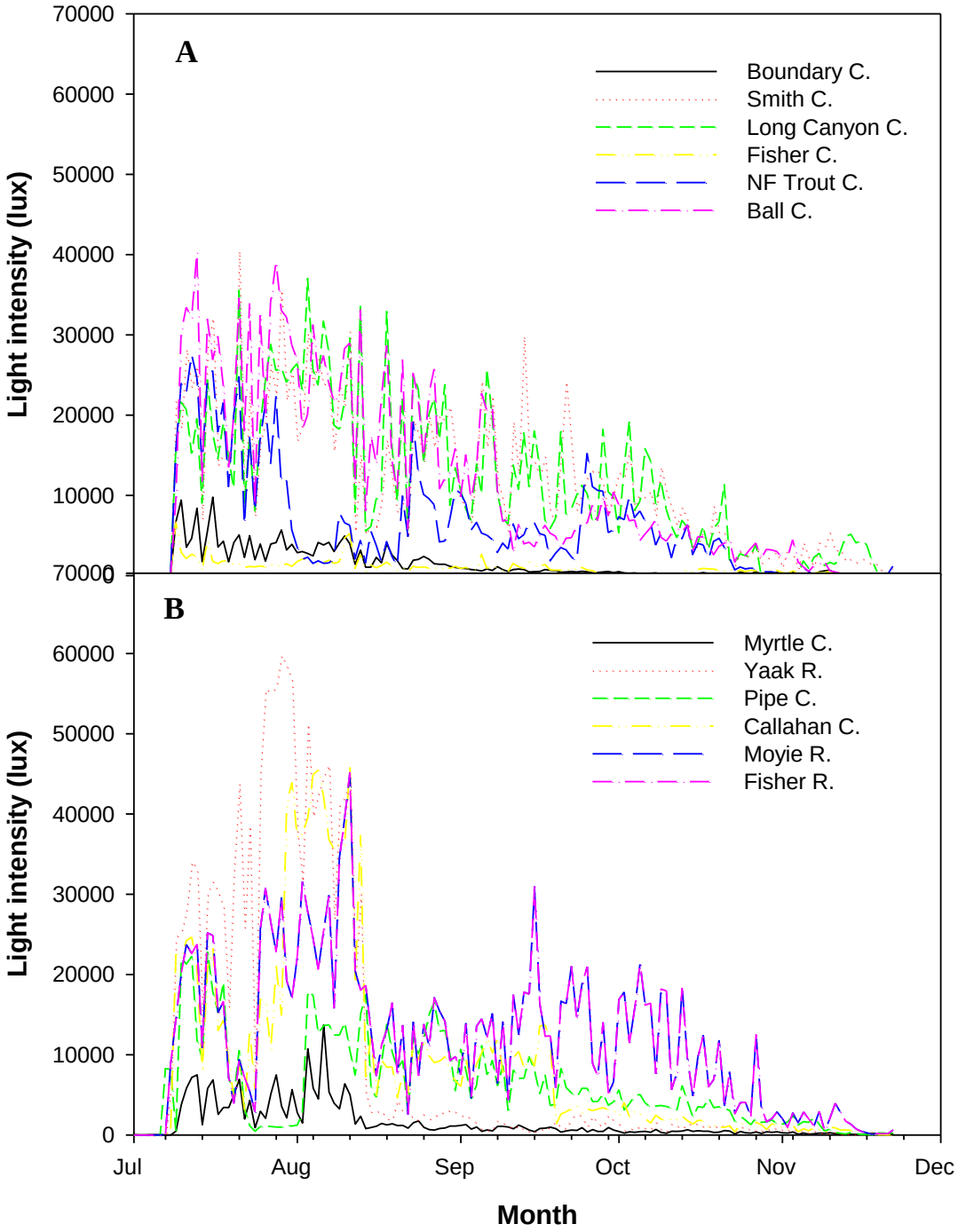

Figure A2-13. Light intensity (lux) of 16 tributaries of the Kootenai River from northwestern Montana to northern Idaho, USA, from 1 July to 22 November 2014. Tributaries are broken into two graphs for clarity of data.