The invasion ecology of *Didymosphenia geminata* in New Zealand

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Abstract

*Didymosphenia geminata* (Lyngbye) M. Schmidt is a freshwater benthic diatom infamous for its unique ability to bloom in oligotrophic conditions. Two decades ago, there was an abrupt increase in the frequency of these blooms accompanied by rapid range expansion in the Northern Hemisphere. Hypotheses presented to explain this phenomenon included climate change and the existence of a new ‘blooming’ ecotype. In 2004, *D. geminata* was first recognised in New Zealand. It was considered a threat to biodiversity and recreational, cultural and economic values. The invasion of the Southern Hemisphere renewed research efforts towards understanding this unsightly species. Because the New Zealand occurrence represented an invasion, Biosecurity New Zealand, a government agency responsible for New Zealand’s biosecurity system, lead the response. However, they had very little information about the alga that could be used for strategic planning. Few studies outline the invasion ecology of *D. geminata*. Meanwhile, it has spread to 37 catchments of the South Island.

Managing *D. geminata’s* invasion process in New Zealand has relied on reports of the alga’s habitat profile in the Northern Hemisphere. Based on these, an early assessment of New Zealand’s suitability for *D. geminata* suggested that >70% of New Zealand’s river sections (stream order > 3) were highly suitable for *D. geminata* establishment. Yet *D. geminata* is invasive in New Zealand, and invasive species often do not follow traditional habitat preferences when in new ranges. An assessment of *D. geminata’s* invasion pathway and habitat window in New Zealand was paramount for successful species management.

My original contribution to knowledge is a description of *D. geminata’s* invasion ecology in New Zealand. I describe the species’ preferences, tolerances, constraints and limiting factors in terms of invasion, colonisation, growth, removal and recovery. My analysis is based on three studies of *D. geminata’s* invasion ecology in New Zealand. The first study, based on presence / absence data from national delimiting surveys, determined that overland spread occurred at the same rate as spread between connected bodies of water. The parameters defining *D. geminata’s* habitat window for colonisation were related to substrata, temperature, geological calcium, geological
phosphorus and source of flow. The second study used data from a series of region-scale monitoring surveys to examine *D. geminata*’s preferences, tolerances and constraints regarding biomass accumulation. The development of high biomass was constrained by water velocity at a threshold of 0.61 m s\(^{-1}\). Within this constraint, biomass accumulation correlated positively with stream temperature. The third study used data from a two-year biomass monitoring study conducted in three reaches of a hill-fed coastal river. It assessed the roles of water flow and temperature in *D. geminata*’s accumulation, removal and recovery. Temperature and the size of the standing crop were associated with all three phases. Accumulation was additionally influenced by flow variables, while removal was also related to the average size of the substrate particles and depth. The latter also influenced accumulation rates after physical removal.

My results can be used to support *D. geminata* long-term management decisions and responses. Insights gained about *D. geminata*’s invasion pathway can be applied in the spread of other aquatic pests in New Zealand. The defined habitat parameters can be used to identify high-risk streams in the North Island and potentially mitigate the impacts of *D. geminata* in infected catchments.
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1 Introduction

1.1 Aquatic invasions

Biotic invasions threaten the integrity of aquatic ecosystems worldwide. In some instances, the introduction of invasive species has caused native biodiversity loss and changes in ecosystem structure and function (Mack, Simberloff et al. 2000). Biotic invasions are often facilitated by anthropogenic disturbance to natural ecosystems (Lonsdale 1999). However, most biotic invasion theory focuses on the biological characteristics of the invading species and of the ecosystems being invaded, assuming that competition and predation are the major processes limiting the invasion outcome (Moyle and Light 1996). Indeed, New Zealand’s isolation and unique evolutionary history are often cited as primary reasons for the successful establishment of non-native plants and animals (Lee, Allen et al. 2006). According to Darwin (1845), in Lee, Allen et al. (2006), New Zealand is defenceless against alien flora from the Northern Hemisphere, which he considered competitively superior. Nevertheless, some species endemic to New Zealand are invasive in other countries. For example, Potamopyrgus antipodarum (New Zealand mudsnail) has spread widely and become a problematic invasive species in many areas, including Australia, Europe, Japan and North America (Loo, Mac Nally et al. 2007).

In New Zealand, alien species introductions are primarily dependent on human-mediated transport and trade, which has increased by orders of magnitude in the past two centuries. Over 200 aquatic plant and animal species have been introduced to New Zealand (Closs, Dean et al. 2004) and in some water bodies they dominate the biota (Wells, de Winton et al. 1997). Introduced freshwater pest plants include Hydrilla verticilata, Salvinia molesta, Ceratophyllum demersum (hornwort), Eichornia crassipes (water hyacinth) and Lagarosiphon major. H. verticilata is now established in New Zealand, while C. demersum and S. molesta are controlled and E. crassipes has been eradicated. L. major continues to invade lakes of New Zealand (Biosecurity New Zealand 2012).

In 2004, it was during a routine survey of Southland’s lower Waiau River that the freshwater stalked diatom, Didymosphenia geminata (Lyngbye) M. Schmidt was first
identified in New Zealand. Given its overseas reputation for rapid spread and massive blooms, its arrival in New Zealand was of great concern to New Zealand’s government and water users. The media also took a strong interest in *D. geminata*, with 338 articles on the species printed by one of the country’s two main publishing houses since its arrival (see Figure 1 for a few examples). The Ministry for Primary Industries (then Ministry of Agriculture and Forestry) promptly declared the species an “unwanted organism.” Under the Biosecurity Act 1993, it is an offence to knowingly spread an unwanted organism with penalties of up to five years imprisonment, and/or a fine of up to $100 000. Biosecurity New Zealand’s Import Health Standard for Equipment associated with animals or water was updated in October 2007 to require biosecurity officers to treat all freshwater fishing equipment they determine or suspect is not completely dry. The Department of Conservation introduced controls on public access to highly-valued waters. Fish and Game New Zealand, a public entity responsible for managing freshwater angling, closed popular fishing locations, placed decontamination stations at many angler access points, and in October 2008, banned the use of felt soled waders following research demonstrating the ability of *D. geminata* cells to survive for long periods out of a river in damp felts (Kilroy, Lagerstedt et al. 2007).

Soon after *D. geminata*’s discovery, Environment Southland, the statutory agency responsible for managing the Region’s natural and physical resources, commissioned a review of the biology and distribution of *D. geminata* and an assessment of the risks to New Zealand’s freshwaters. The review concluded that many New Zealand waterways and lakes may be susceptible to invasion (Kilroy 2004). A later predictive model of *D. geminata*’s range in New Zealand suggested that more than 70% of river sections (Strahler [1957] Stream Order > 3) were suitable for *D. geminata*, including multiple streams in the North Island (which are currently *D. geminata* free). However, both assessments were based on overseas observations and limited by a dearth of information with which to make any firm statement of the suitability of New Zealand’s freshwater habitat for *D. geminata*. It was completely unknown whether some other parameters (such as geology or temperature) may limit the ability of *D. geminata* to colonise (Kilroy 2004). Using occurrence data from a species’ native range to predict

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its potential distribution in new areas is problematic because historical, geographical and ecological constraints can limit a species’ native distribution (Loo, Mac Nally et al. 2007). There are some infamous New Zealand examples of introduced species extending their native niche. For example, *Trichosurus vulpecula* (the Australian brushtail possum), thought to be a herbivore, has been observed eating New Zealand native bird eggs and young (Lee, Allen et al. 2006). Another example is *L. major*, with a natural habitat of upland streams in its native South Africa, but proliferations along the shorelines of large lakes in New Zealand.

Although *D. geminata* is now established on the South Island, the North Island remains *D. geminata* free. It is not known whether this is due to a lack of suitable habitat or the success of spread management campaigns. A new assessment of *D. geminata*’s range in New Zealand is timely.

| MAF cracks down on ‘killer’ didymo algae around Te Anau rivers | Didymo threat to fisheries, power |
| Southland Times 27/12/2004 | Dominion Post 01/11/2008 |
| **Fight against didymo to be stepped up** | More funding for war on didymo |
| Didymo found in North Island | Didymo on march across South Island |
| *Dominion Post* 31/10/2007 | *Timaru Herald* 11/03/2009 |
| Māori close river to halt didymo | Didymo scare caused by tainted samples |
| Didymo hits trophy river | Toilet paper plant a nasty weed |
| *Dominion Post* 26/03/2008 | *Dominion Post* 23/03/2010 |

Figure 1. Selected New Zealand newspaper headlines about *D. geminata* (2004–2010). Note that the "Didymo scare" headline is referring to the earlier report of *D. geminata* in the North Island.
1.2 Description of *Didymosphenia geminata*

1.2.1 Distribution and biology

*Didymosphenia geminata*, a stalked Bacillariophyta (diatom), was first described from the Faroe Islands, which are located north of Scotland (Whitton, Ellwood et al. 2009). It is assumed to be indigenous to Norway, Scotland, Ireland, Sweden, Finland, France, Spain, Switzerland, Vancouver Island and other Northern Hemisphere boreal or montane regions (Kilroy 2004). The species is distinguished by its large, triundulate (having valve margins with three undulations) frustule (often > 100 µm long > 30 µm wide, Figure 2) from which polysaccharide stalks are secreted. These stalks provide a strong attachment to the substrate, and their length and thickness in comparison with the cell body is considered remarkable (Kilroy 2004). It has been suggested that the stalks’ function is to elevate cells above the substrate to avoid competition and have better exposure to current speed, which would enhance gas exchange and nutrient uptake (Aboal, Marco et al. 2012). Stalks also exhibit strong phosphomonoesterase activity, potentially conferring the alga with an ability to utilise organic phosphorous (Ellwood and Whitton 2007). Over time, the stalks lengthen and branch when the cell divides and can result in the formation of spectacular blooms. These comprise thick whitish-brown mats that can almost completely cover the substrate and be over 3 cm thick. These mats have a very tough, fibrous consistency. Sloughed mat material is often mistaken for toilet paper by the public (see Figure 1).

Although periodic blooms have historically been observed in parts of Northern Europe (Blanco and Ector 2009), their frequency appears to be increasing across the globe, primarily over the past two decades (Kawecka and Sanecki 2003; Spaulding and Elwell 2007; Bhatt, Bhaskar et al. 2008; Bothwell, Lynch et al. 2009). Concerns about blooms were first raised in Vancouver Island in 1989 (Bothwell, Lynch et al. 2009) and were followed by reports of an increased frequency of blooms in Europe (Kawecka and Sanecki 2003), Asia (Bhatt, Bhaskar et al. 2008) the United States (Miller, McKnight et al. 2009) and Canada (Kirkwood, Jackson et al. 2008). Although the species has been observed in Chile from as early as 1964 (Blanco and Ector 2009), blooms were not identified until 2011 (Segura 2011). Concurrent with increasing bloom events was range expansion in North America, Europe and New Zealand (Blanco and Ector 2009). Whitton et al., (2009) point out that these apparent increases in range and intensity may
actually reflect a response of an existing population to environmental changes rather than new invasions. However, the situation in New Zealand is a clear example of range expansion. In the eight years since 2004, *D. geminata* has spread to 37 catchments of New Zealand’s South Island.

*D. geminata* is unusual among algae in that it has the ability to bloom under oligotrophic conditions. Sundareshwar, Upadhayay et al. (2011) consider iron to be behind *D. geminata’s* ability to proliferate in low nutrient conditions; in particular, that ferric-ferrous redox shifts within mats result in phosphorus accumulation and recycling. Bothwell, Kilroy et al. (2012) have recently refuted this hypothesis based on a lack of association of iron enrichment with phosphorus uptake and the observation that blooms mainly occur in iron-poor rivers. Instead, they argue that photosynthetically-driven stalk production occurs when cell division rates are nutrient limited and light levels are high (Kilroy and Bothwell 2011). There are good experimental foundations for this argument. Kilroy and Bothwell (2011) showed that under low nutrient conditions, stalk length increases with light (except at low ambient light and temperatures) as part of an overflow mechanism to transfer fixed carbon. Under elevated nutrient conditions, cell division increases while stalk length decreases. A final hypothesis for *D. geminata’s* ability to bloom in oligotrophic conditions is the presence of phosphomonoesterase in the stalks, which is thought to indicate an ability to utilise organic phosphorus when overall phosphorous conditions are low (Ellwood and Whitton 2007). The hypotheses of Kilroy and Bothwell (2011) and Ellwood and Whitton (2007) are not mutually exclusive. Blooms could be triggered by photosynthetic overload, as per the hypothesis of Kilroy and Bothwell (2011), but sustained by organic phosphorous hydrolysed in stalks, as per the hypothesis of Ellwood and Whitton (2007).
1.2.2 Effects
Within tightly defined conditions, mass growths of *D. geminata* can extend and cover almost all available hard substrate. Mats of several centimetres thickness and up to 20 km in length have been reported (Spaulding and Elwell 2007). Correlations between the presence of extensive *D. geminata* mats, a decrease in the abundance of invertebrate species with a preference for hard substrates, and an increase in chironomids and other smaller taxa with a preference for soft sediments have been observed (Kilroy, Larned et al. 2009; Gillis and Chalifour 2010; Rost, Fritsen et al. 2011). In severely affected streams, fish populations can be impacted through decreased invertebrate populations and the elimination of macrophytes (Jonsson, Jonsson et al. 2000), a lack of suitable areas for spawning (Bickel and Closs 2008) and significant diurnal dissolved oxygen fluctuations associated with *D. geminata* mats (Larned, Arscott et al. 2007). Furthermore, *D. geminata* is known to block water intakes (Blanco and Ector 2009), reduce fishing values (Beville, Kerr et al. 2012) and decrease the aesthetic appeal of rivers and streams (Spaulding and Elwell 2007).

1.3 *D. geminata* invasion, colonisation, growth and removal

1.3.1 Conceptual model
Recently, a conceptual model of the blooming behaviour and persistence of *D. geminata* in oligotrophic streams was developed to guide research and mitigation measures (Cullis, Gillis et al. 2012). The model distinguishes dynamic phases of *D. geminata* and proposes that threshold values of controlling parameters determine movement between them (Figure 3).
Figure 3. Representation of the conceptual model for the blooming behaviour and persistence of *D. geminata* in oligotrophic waters (Cullis, Gillis et al. 2012). Each coloured box represents a proposed dynamic phase of *D. geminata* growth.

The four dynamic phases comprising the model are the initial invasion, colonisation, growth or invasive response, removal due to some disturbance event and subsequent recovery and recolonisation. Successful invasion into new streams depends on both an appropriate propagule supply and a suitable habitat window for survival (Mack, Simberloff et al. 2000; Cullis, Gillis et al. 2012). The habitat window is defined by a set of parameters and thresholds that describe the potential for *D. geminata* to survive and grow in a river. Currently, these parameters are unknown for *D. geminata* (Cullis, Gillis et al. 2012). Determining these parameters is vital for identifying streams at risk of invasion and the accumulation of nuisance growths. Removal due to disturbance is assumed to be the primary regulator of periphyton biomass (Biggs, Tuchman et al. 1999). For *D. geminata*, which is known for its great attachment strength (Kilroy 2004) and ability to change the hydrodynamic environment (Larned, Packman et al. 2011), the parameters defining biomass loss are currently unknown. They are thought to include a critical threshold of shear stress related to the potential for the disturbance of the substrate as well as the influence of the mats themselves (Cullis, Gillis et al. 2012). Also unknown, are the factors influencing biomass recovery after disturbance. This thesis examines each of the model’s four dynamic phases.

### 1.3.2 Invasion and colonisation

It is not clear whether *D. geminata* behaves differently within a recently invaded region. According to invasion theory, the invasibility of New Zealand streams is determined by their level of disturbance, the biological resistance of the native communities, and the way in which the native community is assembled (Lonsdale
One study has examined biological resistance to *D. geminata* in New Zealand, in which only late successional stages of New Zealand’s periphyton community showed partial resistance to *D. geminata* invasion (Flöder and Kilroy 2009). If *D. geminata*’s invasive success in New Zealand is driven by New Zealand’s vulnerability, rather than some extraordinary characteristic of *D. geminata*, it is expected that *D. geminata* would establish and persist within the same range of thresholds in New Zealand streams as those in native ranges. However, the organism has also become a nuisance in parts of its native range (Spaulding and Elwell 2007), so an increased biogeographic vulnerability does not necessarily explain *D. geminata*’s proliferation in New Zealand. Alternatively, *D. geminata*’s invasive success in New Zealand may be attributed to anthropogenic changes in the riverine environment, both in terms of hydrological stability with more flow regulation and water abstraction, and a high rate of transfer of the organism between waterways by humans. 

Hypotheses for *D. geminata*’s apparent expansion at the global scale include the development of an aggressive strain with particularly invasive phenotypic attributes (this could be the variant that colonised New Zealand), or that *D. geminata* was present but rare in most habitats, but recent environmental changes have made blooms more likely to occur (Spaulding and Elwell 2007). Based on correspondence analyses of the differences in morphological variation between Lake Superior (USA) populations Pillsbury et al. (2013) demonstrated evidence for the latter hypothesis. Because New Zealand experiments clearly demonstrated that blooms only form under very low phosphorus conditions (Kilroy and Bothwell 2012), it is thought that reduced phosphorus conditions in rivers is the key environmental change behind *D. geminata*’s increased nuisance behaviour and spread (Bothwell 2013). 

The rapid spread of *D. geminata* across catchments of the South Island of New Zealand has been largely due to its ability to survive for long periods out of a river. Experiments have shown that in cool, damp conditions with a little light exposure, *D. geminata* cells can remain viable for as long as 250 days (Kilroy, Lagerstedt et al. 2007). Within catchments, the species can spread downstream via connected bodies of water. This natural mechanism of dispersal should be capable of spreading *D. geminata* rapidly throughout a system. Although the cells are motile, upstream spread is likely to be facilitated by water users, including humans, birds, fish (Bhatt, Bhaskar et al. 2008), and livestock.
Dispersal is not the sole mechanism determining the geographic trajectory of *D. geminata*’s invasion. Whilst there was an initial rapid spread, *D. geminata* has not spread ubiquitously, and the rate of spread has slowed. Clearly, *D. geminata*’s range in New Zealand is influenced by a combination of dispersal and the availability of suitable habitat conditions for colonisation. So far, research in New Zealand has been directed at the dispersal stage of the invasion pathway and identified that anglers are the likely vectors of spread (Kilroy and Unwin 2011). Previous work has established their probable movements (Unwin 2009). However, better predictions of the invasion pathway and outcomes will be possible only when we also understand the factors that influence the species’ ability to establish in a new location. Streams that overlap these factors with angler pressure are at the greatest risk of invasion. The factors influencing *D. geminata*’s ability to establish in a new location are unknown. At a catchment scale, they probably include calcium (Rost, Fritsen et al. 2011) and peat (Whitton, Ellwood et al. 2009). At a reach scale, they probably include velocity (Cullis, Gillis et al. 2012) and substrate (Blanco and Ector 2009).

### 1.3.3 Growth

Cullis, Gillis et al. (2012) postulated that bloom events are triggered by a critical threshold response to changing environmental conditions. Together, these conditions form a ‘hot spot’ of biological activity. Currently, the only known parameters determining these hotspots are high light availability (Sherbot and Bothwell 1993; Whitton, Ellwood et al. 2009), low ambient phosphorus concentrations (Kilroy and Bothwell 2012) and low flows (Kirkwood, Shea et al. 2007). However, a synthesis of these conditions do not exclusively trigger blooms, so it is likely that some other factor(s) also influence bloom development.

The importance of distinguishing between the growth of *D. geminata* cells and the growth of *D. geminata* stalks has been emphasized because they are controlled by different biophysical mechanisms (Cullis, Gillis et al. 2012). Controlled experiments have shown that under high light levels, low nutrients are associated with stalk growth while higher nutrients are associated with cell division (Kilroy and Bothwell 2011). It is believed that under low nutrient conditions, the available energy goes into stalk production (Cullis, Gillis et al. 2012). In addition to light, temperature is thought to be an important controlling variable for *D. geminata*, with an assumed preference for cooler temperatures (Kumar, Spaulding et al. 2009; Whitton, Ellwood et al. 2009;
However, the exact nature of the relationship between *D. geminata* and temperature is unknown, and may even be due to an association of temperature with some other growth-limiting factor (Cullis, Gillis et al. 2012).

Finally, it is assumed that calcium and silicate are also important for *D. geminata* growth because of its stalk requirements (cross-bridging) (Gretz, Riccio et al. 2006; Rost, Fritsen et al. 2011). Rost, Fritsen et al. (2011) observed a positive correlation between calcium and the presence of *D. geminata*. No published studies have examined the potential role of silicate in *D. geminata* survival or growth.

### 1.3.4 Removal

High flow events are considered to be the primary mechanism behind the removal of benthic periphyton (Biggs, Tuchman et al. 1999). In particular, Cullis, Gillis et al. (2012) proposed that a site-specific critical value of shear stress is responsible for the bulk of biomass removal. Above that critical value, shear removal of dead cells and aged mats is likely to occur. Cullis, Gillis et al. (2012) noted that the magnitude of biomass removal will also be influenced by the amount of removable material (the standing crop). In general, the amount lost in a flood depends on the flood intensity and the resistance of the periphyton communities (Biggs and Close 1989). No studies have specifically addressed temporal *D. geminata* biomass–flow dynamics, although negative associations between flow and standing crop have been observed (Kirkwood, Jackson et al. 2007; Kirkwood, Jackson et al. 2009). The flood event magnitude required to scour *D. geminata* is unknown, but expected to be high, given the alga’s strong attachment capabilities (Kilroy 2004).

### 1.4 Overview of the thesis

So far, the management of *D. geminata* in New Zealand has been based on limited overseas information. To provide more specific management directions, I seek to describe the invasion ecology of *D. geminata*. The following chapters represent individual research projects. They are presented in thematic order, from larger to smaller scale studies, and follow the invasion pathway from the initial invasion through to colonisation, growth, removal and recovery.

Chapter 2 is aligned with the invasion and colonisation stages of the conceptual model by Cullis, Gillis et al. (2012) and describes the spatial and temporal dynamics of the
South Island *D. geminata* invasion. It is expected that spread within connected bodies of water will occur more rapidly than human-mediated spread across catchments. Johnson and Padilla (1996) describe two potential benefits of understanding spread dynamics: First, is a better ability to predict the rates and directions of spread. By determining the habitat requirements for *D. geminata* establishment and growth, we can focus management efforts on preventing spread to any remaining catchments that are susceptible to invasion and on regulating the parameters that trigger excessive growth. This information would be crucial should *D. geminata* invade the North Island. Second, exotic species can act as ‘biological tracers’ from which we can extract information on the dispersal of future invaders. Although it has different habitat requirements, another of the world’s most aggressive invaders, *Dreissena polymorpha* (Zebra mussel), can be dispersed by similar means to *D. geminata* (Ricciardi, Serrouya et al. 1995). Should *D. polymorpha* successfully naturalize in New Zealand, its invasion potential may be stemmed from the lessons learned from studying *D. geminata*. The spatial dynamics of spread are examined to identify a potential habitat window for colonisation. It is expected that there are specific parameters distinguishing sites a) with *D. geminata*, from b) those without *D. geminata* despite a supply of propagules, and c) without *D. geminata* anywhere in the catchment. This distinction between sites that are likely receiving propagules from those that are not was made to study the characteristics of *D. geminata*–free sites within infected catchments. Unique insights could be gained from such an approach.

Chapter 3 addresses the factors that limit *D. geminata* biomass accumulation at a regional scale and is aligned with the growth phase of the conceptual model. It is expected that an interaction of limiting factors should influence biomass and production among rivers over annual climate cycles. For example, nutrient limitation will primarily only occur within certain temperature or light thresholds. While Chapter 2 provided a description of *D. geminata*’s range in New Zealand, Chapter 3 offers more detail about the actual impact of *D. geminata* (in terms of biomass) in a region and how it relates to stream variables such as nutrient concentrations and temperature. It highlights the types of streams that are susceptible to large proliferations as well as important seasonal differences in the mat structure.

Chapter 4 examines the temporal variability of *D. geminata* biomass in a hill-fed coastal river in relation to flow and temperature. It encompasses the colonisation,
growth and removal stages of the conceptual model. Specifically, this chapter seeks to
describe the factors controlling biomass accumulation and loss, and the habit window
for nuisance blooms. It is expected that flow is the primary driver of *D. geminata*
biomass and that within suitable flow conditions, seasonal differences in temperature
control biomass accrual. Knowledge of the interaction between biomass, flow and
temperature will help in the prediction of blooms in unregulated systems and
potentially prevent their occurrence.

In the final chapter, I discuss the research in terms of the dynamic phases proposed by
Cullis, Gillis et al. (2012) and offer suggestions for managing this nuisance species in
New Zealand.
2 The spread and distribution of *D. geminata* in New Zealand

Abstract

The spatial and temporal dynamics of the *Didymosphenia geminata* invasion in New Zealand are reviewed with a focus on the habitat conditions necessary to establish self-sustaining populations. The alga’s survival ability outside of a river, the prominence of human-mediated dispersal, and the natural linkages among lakes and streams have promoted rapid spread of this aquatic pest to 37 catchments of the South Island since 2004. Overland spread appears to have occurred at the same rate as spread within connected bodies of water. Although there are many potential vectors of overland spread, anglers are suspected of being the primary means of dispersal. Here, I show that the habitat window for establishment is defined by temperature, distance to the coast, geological calcium, geological phosphorus and source of flow. It is the overlap of these habitat windows with angler activity that should be the focus of efforts to prevent or slow the spread of *D. geminata*. 
2.1 Introduction

With its alarming rates of range expansion in the early 1990s (Jonsson, Jonsson et al. 2007; Bothwell, Lynch et al. 2009) and invasion into New Zealand in the 2000s, the freshwater alga Didymosphenia geminata drew the attention of a growing number of ecologists (Sherbot and Bothwell 1993; Kirkwood, Shea et al. 2007; Blanco and Ector 2009; Kilroy, Larned et al. 2009; Whitton, Ellwood et al. 2009; Bothwell and Kilroy 2011). Although *D. geminata* has not been detected in the North Island, it is now firmly established in the South Island of New Zealand. DNA testing has revealed that the founding propagules were likely imported from North America (Cary, Hicks et al. 2007).

Since its arrival, *D. geminata* has proven adept at spreading across catchments, demonstrating the importance of overland vectors. Because the distribution of *D. geminata*–affected rivers correlates with angler usage, anglers have been indirectly implicated as cross-catchment dispersers (Kilroy and Unwin 2011). Anglers have also been linked to *D. geminata* blooms in North America during the early 1990s (Bothwell, Lynch et al. 2009). In particular, felt soled waders have been identified as the probable vector (Bothwell, Lynch et al. 2009) because *D. geminata* cells can remain viable in moist felts for days after leaving a river (Kilroy, Lagerstedt et al. 2007). Accordingly, they have been banned from use in New Zealand since October 2008. Other important human-mediated vectors in New Zealand include kayaks, with 13 new incursions being observed just downstream of well-known angling locations or kayak entry sites (Kilroy and Unwin 2011). Power boats, hydroelectric or irrigation canals and 4WD all-terrain vehicles were considered potential vectors in a further 19 sites, picnicking and swimming in a further two and field staff in one. Nevertheless, it is anglers, particularly those from overseas or who were visiting from another catchment, who are considered the most important *D. geminata* transmission vectors in New Zealand (Kilroy and Unwin 2011).

Although the pattern of *D. geminata* spread in New Zealand is consistent with human vectors, the actual invasion pathways should also be limited by the availability of acceptable habitat patches (Johnson and Padilla 1996). Kilroy, Snelder et al. (2008) made a preliminary assessment of *D. geminata*’s possible range in New Zealand based on surrogate variables for temperature, light, pH and hydrological and substrate
stability. Their analysis suggested that > 70% of New Zealand’s river sections (stream order > 3; North Island inclusive) fell into the two highest habitat suitability categories for *D. geminata*. Fortunately, eight years after its initial incursion the alga does not appear to have spread to that extent. Few data were available when Kilroy, Snelder et al. (2008) made their analysis, so a more precise understanding of the contribution of habitat in regulating the distributional pattern of *D. geminata* was unable to be made. This information is vital for the development of management strategies should *D. geminata* invade New Zealand’s North Island, where minimisation of its range expansion may be a viable goal.

In their review paper, Whitton, Ellwood et al. (2009) note that the catchment as whole should be considered when assessing factors that influence *D. geminata*, because the catchment influences not only the physical and chemical conditions within streams, but may also provide an upstream source of propagules. In this study, I use survey records of 1028 reaches of 369 rivers within 106 catchments of the South Island of New Zealand and a database of catchment variables for each reach. The survey records spanned 25 January 2005 to 17 May 2012. My objectives were to: 1) document the current distribution of *D. geminata* in the South Island and 2) identify habitat parameters related to the presence and absence of *D. geminata*.

I predicted that *D. geminata* positive sites would be concentrated further from the coast, because coastal sites tend to have a fine sediment bed (Biggs and Kilroy 2000). I also expected that flow source would have a role in *D. geminata*’s distribution, in particular, that positive sites would be less likely to occur in streams with a low-elevation or hill source of flow, because of their moderate frequency of high-flow events (Biggs et al. 2008). Finally, I predicted that positive sites would be less likely to occur with increasing proportionate areas of glacier in the upstream catchment, because of their increasing suspended sediment load.
2.2 Methods

2.2.1 Study region

The South Island is the southernmost of New Zealand’s two main islands, which together span latitudes 34–47°S. The South Island landforms are predominantly uplifted sedimentary mountain ranges and extensive glacial and alluvial outwash plains. Mountain ranges oriented in a northeast-southwest direction occur down the length of the South Island. Regions to the west of these mountains experience higher rainfall than regions to the east. Kilroy, Snelder et al. (2008) describe New Zealand’s rivers as being dilute, soft and low in alkalinity with high water quality (generally) by international standards. Exceptions are rivers whose catchment geology comprises mainly soft-sedimentary rock. These, as well as rivers with glaciers in their catchments, tend to be naturally turbid at base flow (Kilroy, Snelder et al. 2008).

2.2.2 Data sources and analysis

Biosecurity New Zealand (MPI-BNZ) maintains an internet-based registry of all *D. geminata* surveys in New Zealand. It is called the Didymo Samples Database (http://www.biosecurity.govt.nz/pests/didymo/partners). The registry includes positive (visually observed) and negative records. For this analysis, I downloaded all South Island data from the database, comprising 3106 records up to 31 October 2012 (Figure 4). Of these records, 540 represent positive observations, 2253 represent negative observations at exposed sites (*D. geminata* is elsewhere in the catchment) and 333 represent negative observations at sites within a catchment in which *D. geminata* has never been recorded. To allow each site to be mapped in relation to upstream and downstream river reaches in the same catchment, the unique reach number for each site was linked with the Freshwater Environments of New Zealand (FWENZ) Database on the River Environments of New Zealand Network (Leathwick, Julian et al. 2008). The network is described by Kilroy, Snelder et al. (2008), but briefly, it was derived from a 30-m digital elevation model and contains approximately 575 000 uniquely identified river sections defined by confluences with tributaries. Each section of the river network is combined with various grids of environmental data to derive environmental variables such as climate and geology. The network data are stored as a geographical information system layer. For details of the variables contained in the FWENZ, please see Table 1.
From the MPI-BNZ database, I independently classified all 1028 unique reaches into one of the following three status categories, where status describes the reach’s status with respect to *D. geminata* at the time of writing:

1. **Negative** (n = 148): a negative reach where *D. geminata* had not been previously recorded anywhere else in the catchment

2. **Negative-exposed** (n = 543): a negative reach within a catchment with a previous record of *D. geminata*. Any catchment placement relative to a *D. geminata* positive site (upstream or downstream) was used because the data indicate that upstream spread occurred rapidly and often.

3. **Positive** (n = 337): a reach with a previous or current record of *D. geminata*.

The Wilcoxon signed rank-test (Wilcoxon 1945) was used to test for a significant difference in the rates of cross-catchment versus within-catchment spread. Multinomial logistic regression (Hosmer and Lemeshow 2000) was used to conduct a multivariate analysis of habitat characteristics that predict reach membership to the three status groups (negative, negative-exposed, positive). First, multiple records were removed, so that only the most recent record per reach remained. I initially considered 29 spatially explicit environmental variables representing climate, topography, land cover types, bedrock geology and hydrology that were available for each reach from the FWENZ database (Table 1). Dummy variables were created for categorical variables, while quasi-quantitative variables (for example, bed-rock calcium, which has a maximum value of five) were treated as continuous variables. Multicollinearity was evaluated by examining cross-correlations among all the variables. Flow and low flow, upstream catchment area and flow, and elevation and average minimum July (winter) temperature were highly correlated (0.73, 0.90 and -0.79, respectively). Based on their potential relevance for the distribution of *D. geminata*, I included low flow and July temperature and excluded flow, catchment area and elevation. Thus, the final number of variables considered for modelling was reduced to 26.

Relative risk ratios (RRR) are presented as model outcomes. The RRR of a coefficient indicates how the risk of the outcome falling in the comparison group relative to the risk of the outcome falling in the referent group changes with the variable in question. An RRR > 1 indicates that the risk of the outcome falling in the comparison group
relative to the risk of the outcome falling in the referent group increases as the variable increases. In other words, the comparison outcome is more likely. The Hosmer-Lemeshow test (Hosmer and Lemeshow 2000) was used to examine model fit. The test is computed as a Pearson $\chi^2$ from a contingency table of expected versus observed frequencies. A large p value indicates a good fit.

Figure 4. Histogram of number of MPI-BNZ Didymo Samples Database observations for the South Island, by year (2005–2012). The histogram includes all observations in the database and shows that survey effort has declined dramatically over the last three years.
Table 1: Brief description of the FWENZ and REC variables used in the multinomial logistic regression analysis of factors associated with *D. geminata* status group membership (positive, negative-exposed, negative)

<table>
<thead>
<tr>
<th>Parameter name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upstream calcium</td>
<td>Catchment average geological calcium (1 ‘very low’ to 5 ‘very high’)</td>
</tr>
<tr>
<td>Upstream hardness</td>
<td>Catchment average geological hardness (induration) (1 ‘very low’ to 5 ‘very high’)</td>
</tr>
<tr>
<td>Upstream phosphorus</td>
<td>Catchment average geological phosphorus (1 ‘very low’ to 5 ‘very high’)</td>
</tr>
<tr>
<td>Upstream alluvium</td>
<td>Area of alluvium in catchment (proportion)</td>
</tr>
<tr>
<td>Upstream bare land</td>
<td>Area of bare land in catchment (proportion)</td>
</tr>
<tr>
<td>Upstream exotic forest</td>
<td>Area of exotic forest in catchment (proportion)</td>
</tr>
<tr>
<td>Upstream glacier</td>
<td>Area of glacier in catchment (proportion)</td>
</tr>
<tr>
<td>Upstream indigenous forest</td>
<td>Area of indigenous forest in catchment (proportion)</td>
</tr>
<tr>
<td>Upstream pastoral farming</td>
<td>Proportion of annual runoff from pastoral farming</td>
</tr>
<tr>
<td>Upstream peat</td>
<td>Area of peat in catchment (proportion)</td>
</tr>
<tr>
<td>Upstream scrub</td>
<td>Area of scrub in catchment (proportion)</td>
</tr>
<tr>
<td>Upstream tussock</td>
<td>Area of tussock in catchment (proportion)</td>
</tr>
<tr>
<td>Upstream urban</td>
<td>Area of urban development in catchment (proportion)</td>
</tr>
<tr>
<td>Upstream rainfall variability</td>
<td>Coefficient of variation of annual catchment rainfall</td>
</tr>
<tr>
<td>Distance to coast</td>
<td>Distance from reach to the coast (m)</td>
</tr>
<tr>
<td>Flow</td>
<td>Total annual runoff volume (mm m⁻¹)</td>
</tr>
<tr>
<td>Low flow</td>
<td>Mean annual low flow (1 s⁻¹)</td>
</tr>
<tr>
<td># of catchment rain days &gt; 25 mm</td>
<td>Catchment rain days (&gt; 25 mm / month)</td>
</tr>
<tr>
<td>Order</td>
<td>Strahler stream order</td>
</tr>
<tr>
<td>Climate</td>
<td>Warm-extremely-wet, warm-wet, warm-dry, cool-extremely-wet, cool-wet, cool-dry</td>
</tr>
<tr>
<td>Geology</td>
<td>Alluvium, hard-sedimentary, soft-sediment, volcanic-basic, volcanic-acidic, plutonic, miscellaneous</td>
</tr>
<tr>
<td>Valley-landform</td>
<td>High-gradient, medium-gradient, low-gradient</td>
</tr>
<tr>
<td>Land cover</td>
<td>Bare, indigenous, pastoral, tussock, scrub, exotic forest, wetland, urban</td>
</tr>
<tr>
<td>Source of flow</td>
<td>Glacial-mountain, mountain, hill, low-elevation, lake</td>
</tr>
<tr>
<td>Elevation</td>
<td>Elevation at highest point on reach</td>
</tr>
<tr>
<td>Sinuosity</td>
<td>The reach length (as the fish swims) divided by the Euclidian reach length</td>
</tr>
<tr>
<td></td>
<td>(as the crow flies)</td>
</tr>
<tr>
<td>Slope</td>
<td>Ratio of the difference between the top and bottom of reach / reach length</td>
</tr>
<tr>
<td>Summer temperature</td>
<td>Mean January air temperature</td>
</tr>
<tr>
<td>Winter temperature</td>
<td>Mean minimum July temperature</td>
</tr>
</tbody>
</table>
2.3 Results

2.3.1 The spread of *D. geminata* in the South Island, 2004–2012

As of 31 October 2012, *D. geminata* had spread to 142 rivers in 37 catchments of the South Island (Figure 5, Table 2) with a median cross-catchment serial interval of 19 days (Interquartile range, [IQR, the difference between the upper and lower quartiles] 7–70) and a median within-catchment serial interval of 21 days (IQR 1–137). There was no significant difference in the rate of cross-catchment versus within-catchment spread (z = 1.095, p = 0.27). *D. geminata*’s range continues to expand, albeit at a slower rate (Figure 6). The period 2007 through 2008 saw a rapid increase in the number of invaded catchments, while rapid increases in the number of invaded rivers was sustained until late 2009.

The initial discovery of *D. geminata* was made in the lower Waiau River on 20 October 2004 but the probable point of first incursion was tracked to the Mararoa River, a tributary of the Waiau (Kilroy and Unwin 2011). *D. geminata* was not observed in another catchment until almost a year later (September 2005), when it was recorded in the Buller, Clutha and Oreti catchments, where it also became a nuisance. Year two of the invasion (2006) saw spread to one of the South Island’s most significant catchments for renewable energy, irrigation and recreation, the Waitaki. The Waitaki catchment lies predominantly in the Canterbury region, the most central and populated region of the South Island (Statistics New Zealand 2012). It is likely that the spread into this key catchment facilitated the rapid spread into a further 11 catchments in 2007. Rapid range expansion continued through 2008 and 2009, with an additional 6 catchments invaded per year, before dropping to an additional 3 catchments in 2010 and 2 in 2011. No newly invaded catchments have been observed since October 2011.
Figure 5. Map of *D. geminata* positive (yellow) and negative (green) sites in the South Island of New Zealand as of December 2012, from the MPI-BNZ Didymo Samples Database. Note that negative sites are concentrated closer to the coast.

Figure 6. Cumulative number of *D. geminata*-positive catchments. The date of the first positive observation in the centralised, high-use Waitaki Catchment is indicated. There appears to be a fairly consistent exposure time of about nine months between new catchment invasions, although this may reflect seasonal survey effort.
Table 2. D. geminata-positive catchments, in order of invasion date, and showing annual angler pressure (angler-days ± SD)

<table>
<thead>
<tr>
<th>Catchment</th>
<th>First positive</th>
<th>Angler pressure</th>
<th>Catchment</th>
<th>First positive</th>
<th>Angler pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waiau</td>
<td>20/10/2004</td>
<td>5170 ± 4100</td>
<td>Arahura</td>
<td>20/12/2007</td>
<td>1020 ± 290</td>
</tr>
<tr>
<td>Buller</td>
<td>26/09/2005</td>
<td>11160 ± 980</td>
<td>Ashburton</td>
<td>24/01/2008</td>
<td>10110 ± 1400</td>
</tr>
<tr>
<td>Clutha</td>
<td>27/09/2005</td>
<td>191480 ± 7540</td>
<td>Wairau</td>
<td>12/03/2008</td>
<td>13150 ± 1340</td>
</tr>
<tr>
<td>Oreti</td>
<td>14/10/2005</td>
<td>25510 ± 2220</td>
<td>Hokitika</td>
<td>20/05/2008</td>
<td>7010 ± 1020</td>
</tr>
<tr>
<td>Waitaki</td>
<td>21/01/2006</td>
<td>174280 ± 7540</td>
<td>Grey</td>
<td>4/06/2008</td>
<td>21220 ± 1590</td>
</tr>
<tr>
<td>Aparima</td>
<td>26/03/2006</td>
<td>8820 ± 1170</td>
<td>Mikonui</td>
<td>13/11/2008</td>
<td>440 ± 250</td>
</tr>
<tr>
<td>Hollyford</td>
<td>22/11/2006</td>
<td>660 ± 190</td>
<td>Totara</td>
<td>21/11/2008</td>
<td>370 ± 250</td>
</tr>
<tr>
<td>Takaka</td>
<td>25/01/2007</td>
<td>11110 ± 360</td>
<td>Mohikinui</td>
<td>4/01/2009</td>
<td>1010 ± 410</td>
</tr>
<tr>
<td>Wairaurahiri</td>
<td>1/02/2007</td>
<td>90 ± 90</td>
<td>Paringa</td>
<td>13/01/2009</td>
<td>360 ± 110</td>
</tr>
<tr>
<td>Motueka</td>
<td>7/02/2007</td>
<td>6190 ± 590</td>
<td>Karamea</td>
<td>15/01/2009</td>
<td>1620 ± 360</td>
</tr>
<tr>
<td>Haast</td>
<td>16/02/2007</td>
<td>910 ± 520</td>
<td>Waimea</td>
<td>20/01/2009</td>
<td>940 ± 250</td>
</tr>
<tr>
<td>Hurunui</td>
<td>27/04/2007</td>
<td>18970 ± 2020</td>
<td>Orari</td>
<td>20/01/2009</td>
<td>650 ± 530</td>
</tr>
<tr>
<td>Rangitata</td>
<td>11/05/2007</td>
<td>33500 ± 3560</td>
<td>Taramakau</td>
<td>9/02/2009</td>
<td>2890 ± 530</td>
</tr>
<tr>
<td>Ophì</td>
<td>27/04/2007</td>
<td>27490 ± 2910</td>
<td>Waimakarirí</td>
<td>26/01/2010</td>
<td>86930 ± 6250</td>
</tr>
<tr>
<td>Kakanui</td>
<td>10/05/2007</td>
<td>890 ± 380</td>
<td>Selwyn</td>
<td>5/02/2010</td>
<td>1000 ± 300</td>
</tr>
<tr>
<td>Mataura</td>
<td>23/05/2007</td>
<td>48480 ± 3770</td>
<td>Riwaka</td>
<td>11/02/2010</td>
<td>320 ± 110</td>
</tr>
<tr>
<td>Waitaha</td>
<td>25/09/2007</td>
<td>440 ± 190</td>
<td>Porari</td>
<td>21/04/2011</td>
<td>No data</td>
</tr>
<tr>
<td>Rakaia</td>
<td>5/12/2007</td>
<td>36930 ± 2300</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a Angler pressure data from (Unwin 2009).

2.3.2 River status

A multinomial logistic regression analysis was used to determine habitat characteristics that influence the probability of D. geminata status group membership (negative, negative-exposed and positive). The variables included in the most parsimonious model were mean January (summer) air temperature, distance to coast, low-elevation source of flow, hill source of flow, and the average phosphorus and calcium concentrations in the underlying geology (Table 3). There were no strong correlations among these variables (Table 4). A generalised Hosmer-Lemeshow goodness-of-fit test for multinomial logistic regression models found evidence for a good fit ($\chi^2 = 19.23(16)$, $p = 0.257$).
Table 3 Multivariate multinomial regression summary of variables associated with site *D. geminata* status. Positive sites are the reference category.

<table>
<thead>
<tr>
<th>FWENZ variable</th>
<th>Type of variable</th>
<th>RRR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>SE</th>
<th>p</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Negative</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean January (summer) air temperature (°C)</td>
<td>Temperature</td>
<td>0.78</td>
<td>0.08</td>
<td>0.015</td>
<td>0.64–0.95</td>
</tr>
<tr>
<td>Distance to coast (√km)</td>
<td>Substrate stability / nutrients</td>
<td>0.99</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
<td>0.98–0.99</td>
</tr>
<tr>
<td>Low-elevation flow source</td>
<td>Hydrology</td>
<td>2.22</td>
<td>0.71</td>
<td>0.012</td>
<td>1.19–4.14</td>
</tr>
<tr>
<td>Hill flow source</td>
<td>Hydrology</td>
<td>1.52</td>
<td>0.41</td>
<td>0.118</td>
<td>0.90–2.59</td>
</tr>
<tr>
<td>Phosphorus (scale: 1 ‘very low’ to 5 ‘very high’)</td>
<td>Nutrients</td>
<td>1.40</td>
<td>0.22</td>
<td>0.033</td>
<td>1.03–1.92</td>
</tr>
<tr>
<td>Calcium (scale: 1 ‘very low’ to 5 ‘very high’)</td>
<td>Nutrients</td>
<td>0.69</td>
<td>0.71</td>
<td>0.241</td>
<td>0.37–1.28</td>
</tr>
<tr>
<td><strong>Negative-exposed</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean January (summer) air temperature (°C)</td>
<td>Temperature</td>
<td>0.84</td>
<td>0.05</td>
<td>0.009</td>
<td>0.74–0.96</td>
</tr>
<tr>
<td>Distance to coast (km)</td>
<td>Substrate stability / nutrients</td>
<td>0.99</td>
<td>&lt;0.01</td>
<td>0.152</td>
<td>0.99–1.00</td>
</tr>
<tr>
<td>Low-elevation flow source</td>
<td>Hydrology</td>
<td>2.99</td>
<td>0.66</td>
<td>&lt;0.001</td>
<td>1.94–4.61</td>
</tr>
<tr>
<td>Hill flow source</td>
<td>Hydrology</td>
<td>1.57</td>
<td>0.21</td>
<td>0.001</td>
<td>1.20–2.04</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>Nutrients</td>
<td>0.96</td>
<td>0.09</td>
<td>0.633</td>
<td>0.79–1.15</td>
</tr>
<tr>
<td>Calcium</td>
<td>Nutrients</td>
<td>0.61</td>
<td>0.12</td>
<td>0.010</td>
<td>0.41–0.89</td>
</tr>
</tbody>
</table>

<sup>a</sup>Relative risk ratio. Negative: no *D. geminata* at site or anywhere in the catchment, negative-exposed: negative site within infected catchment, positive: *D. geminata*-positive site.
Table 4 Correlation matrix of variables included in the multivariate multinomial logistic regression model of site *D. geminata* status group membership

<table>
<thead>
<tr>
<th></th>
<th>Temperature</th>
<th>Coast</th>
<th>Low elevation</th>
<th>Hill</th>
<th>Phosphorous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coast</td>
<td>-0.30</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low elevation</td>
<td>0.14</td>
<td>-0.33</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hill</td>
<td>0.14</td>
<td>-0.09</td>
<td>-0.48</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Phosphorous</td>
<td>-0.01</td>
<td>0.23</td>
<td>0.11</td>
<td>-0.08</td>
<td>1.00</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.18</td>
<td>-0.04</td>
<td>0.29</td>
<td>-0.02</td>
<td>0.20</td>
</tr>
</tbody>
</table>

*a* mean January (summer) air temperature

*b* geological

Compared with reaches of other hydrological origin, the relative risk of reaches with a hill source of flow being negative increases by a factor of 1.52 and the relative risk of being negative-exposed rather than positive increases by a factor of 1.57. Similarly, the relative risk of reaches with a low elevation source of flow being negative increases by a factor of 2.22 and the relative risk of being negative-exposed rather than positive increases by a factor of 2.99. With each 1°C increase in mean January (summer) air temperature the relative risk that a reach is negative rather than positive decreases by a factor of 0.78 and the relative risk that a reach is negative-exposed rather than positive decreases by a factor of 0.84. Among all reaches, increasing mean January (summer) air temperature increase the probability of being *D. geminata* positive (Figure 7). The increase in probability with temperature is most marked for reaches with sources of flow other than hill or low elevation. With each 1 km increase in distance to the coast, the relative risk of a reach being negative or negative-exposed rather than positive decreases by a factor of 0.99. Distance to the coast particularly affects the probability of being negative, and has less effect on the probability of a reach being *D. geminata*–positive in streams with a low elevation flow source than in other streams (Figure 8). A unit increase in calcium decreased the relative risk of a reach being negative or negative-exposed rather than positive by a factor of 0.69 and 0.61, respectively. In all streams, geological calcium strongly influenced the probability of being positive or negative-exposed, with comparatively minor influence on the probability of being...
negative (Figure 9). Geological phosphorus had only minor influence on the probably of a reach being in each status. In all streams, increasing geological phosphorus slightly increased the probability of being negative, decreased the probability of being negative-exposed, and had almost no influence on the probability of being positive (Figure 10). Overall, unit increases in geological phosphorus increased the relative risk of being negative rather than positive by a factor of 1.40, and decreased the relative risk of being negative-exposed rather than positive by a factor of 0.96.

![Conditional effect plots of predicted probabilities for mean January (summer) air temperature (°C), by source of flow. Blue line: positive, green line: negative-exposed, red line: negative. Holding other model variables at their mean (distance to coast, calcium, phosphorus), increasing temperatures decrease the probabilities of site a being negative or negative-exposed, and increase the probability of a site being positive.](image)
Figure 8. Conditional effect plots of predicted probabilities for distance to coast (km), by source of flow. Blue line: positive, green line: negative-exposed; red line: negative. Holding other model variables at their mean (temperature, calcium, phosphorus), increasing distance from the coast decreases the probability of a site being negative and increases the probability of a site being positive. After a distance of about 300 km from the coast, the predicted probabilities of a site being negative-exposed begin to decrease.
Figure 9. Conditional effect plots of predicted probabilities for geological calcium (quasi-quantitative variable; 1–5), by source of flow. Blue line: positive, green line: negative-exposed; red line: negative. Holding other model variables at their mean (temperature, distance to coast, phosphorus), increasing calcium in the underlying geology sharply increases the probability of a site being positive and decreases the probability of a site being negative exposed. Note that the calcium threshold determining differences in the probability of being positive or negative-exposed differs between sources of flow.
Figure 10. Conditional effect plots of the predicted probabilities for geological phosphorus (quasi-quantitative variable; 1–5), by source of flow. Blue line: positive, green line: negative-exposed, red line: negative. Holding other model variables at their mean (temperature, distance to coast, calcium), increasing phosphorus in the underlying geology slightly decreases the probability of site being negative-exposed and increases the probability of a site being negative.
2.4 Discussion

Eight years after its arrival, *D. geminata* has spread to 37 catchments of the South Island. Despite initial indications that > 70% of river sections (Strahler Stream Order > 3) were highly suitable for *D. geminata*, a much smaller proportion of these reaches has actually been invaded. Within the sites included in the MPI-BNZ database, only 35% of reaches of stream order > 3 were *D. geminata* positive.

After the first sighting, there was a 12-month lag before the alga was observed elsewhere. In many catchments, particularly the smaller ones, the alga appeared to spread upstream incrementally (Kilroy and Unwin 2011), a phenomenon also observed in Iceland (Jonsson, Jonsson et al. 2007). The similar cross- and within-catchment serial intervals suggest that overland spread occurs as quickly as spread within connected bodies of water. While overland spread is almost certainly human mediated (Kilroy and Unwin 2011), within-catchment spread is a function of both human and natural vectors (Johnson and Padilla 1996). Natural vectors are thought to include the water current itself (in a downstream direction), as well as fish, invertebrates, aquatic birds and stock (up- or downstream) (Kilroy and Unwin 2011). Further indication of the critical role of human-mediated spread is the explosion in the number of newly invaded catchments one-year following the invasion of the Waitaki catchment. This fits with the findings of a 2007 National Anglers Survey, which identified that the largest contribution to cross-boundary fishing in New Zealand occurred in the lower South Island, with substantial movement of anglers between the Waitaki and its adjacent catchments (Unwin 2009). Although felt-soled waders were assumed to be important vectors, *D. geminata*’s cross-catchment range expansion continued its rapid pace for a full year after their 2008 ban in New Zealand. Other components of angling gear and other water users must also have strong transmission potential. (It is also possible that anglers ignored the ban). Alternatively, the 2009 incursions marked the end of a suspected 9–12 month lag between incursion and visible growth. Cross-catchment range expansion has slowed considerably in the past two years, possibly indicating that the alga has reached its range limits, or that the control measures and public campaigns undertaken by MPI-BNZ have finally become effective. However, it may also reflect lesser survey effort over the past three years (Figure 4). This would have significantly decreased the
likelihood of finding new incursions. Given this sampling effort issue, it is difficult to definitively describe *D. geminata*’s current distribution.

Broad scale *D. geminata* habitat patterns were evident. Negative sites were concentrated closer to the coast and at sites with lower mean summer air temperatures. Increasing geological phosphorus concentrations slightly increased the probability of a reach being negative. As predicted, sites with a low elevation or hill flow source were more likely to be negative than positive. Positive sites tended to be further from the coast and in locations with higher mean summer air temperatures and greater calcium availability in the underlying geology. Mean January (summer) air temperature and geological calcium distinguished between positive and negative-exposed sites, suggesting that adequate temperature and geological calcium supply are necessary for *D. geminata* colonisation given a suitable propagule supply. However, at any given site there are probably many combinations of variables that will restrict *D. geminata*’s colonisation window (e.g. shade, velocity).

Coastal sites tend to be warmer, more turbid and slow flowing with fine sediment beds (Biggs and Kilroy 2000). Stable substrates are required to support *D. geminata* (Antoine and Benson-Evans 1986) so it is a lack of suitable substrate that is likely to drive the observed association with distance to coast, at least in part. New Zealand rivers with a low elevation source of flow are characterised by relatively high minimum flows with a moderate frequency of low intensity high flow events (Biggs, Ibbitt et al. 2008). This results in long periods of stable velocities and stable bed sediments (Biggs, Ibbitt et al. 2008). Although these sound like ideal conditions for *D. geminata*, low elevation sourced streams also tend to be very enriched and many are at least partially spring-fed (Biggs and Kilroy 2000). Rivers with a hill source of flow are characterised by moderately frequent high flows, with flood flows that can have a very high magnitude (about 3000 times the lowest flows) (Biggs, Ibbitt et al. 2008). They are the most common stream type in New Zealand (Biggs and Kilroy 2000) and encompass the full range of enrichment regimes (Biggs and Kilroy 2000). Accordingly, low elevation and hill-fed reaches had higher probabilities of being negative-exposed rather than negative or positive due to their highly variable habitat conditions.

This analysis is the first of its kind for *D. geminata*. No studies have addressed the interactive roles of nutrients, flow source, land cover, bedrock geology and topographic
characteristics on the alga’s distribution. Further, none have distinguished sites that have a propagule supply, yet are *D. geminata*–free from sites that are *D. geminata*–free with no known propagule supply. The most closely comparable studies are those of Rost, Fritsen et al. (2011) and Kumar, Spaulding et al. (2009). Rost, Fritsen et al. (2011) used survey data of 50 stream reaches in Sierra Nevada to determine if *D. geminata* distribution patterns were related to water chemistry, bedrock geology, or both. In that study, *D. geminata* was more likely to occur in streams with higher percent calcium and sulphate, higher absolute concentrations of sulphate, and lower absolute concentrations of magnesium, chloride, sodium and bicarbonate (Rost, Fritsen et al. 2011). The Sierra Nevada findings support those from Norway, where gradients in calcium and sulphate explained much of the broad scale distribution pattern (Lindstrøm and Skullberg 2008). A preference for mountainous calcareous rivers in Spain has also been reported (Tomas, Oscoz et al. 2010). I found that *D. geminata* was more likely to occur in reaches with higher geological calcium values, and that insufficient geological calcium excluded *D. geminata* from reaches with a propagule supply—further suggesting that geological calcium has a role in *D. geminata* colonisation. *D. geminata* may be unable to adhere to substrata with insufficient calcium (Geesy, Wigglesworth-Cooksey et al. 2000), thus explaining its absence. Whitton, Ellwood et al. (2009) suggest that calcium may influence *D. geminata* via its effect on another element important for its physiology, or on the suitability of *D. geminata* for grazers. Rost, Fritsen et al. (2011) hypothesized that the link between calcium and *D. geminata*’s distribution was due to the alga’s stalk requirements (cross-bridging), diatom motility, adhesion, or a combination of these factors. Because the correlation between geological and water calcium in New Zealand is unknown, it is not clear whether it is the calcium in the underlying rocks, or calcium in the water that is important for *D. geminata* colonisation. The role of calcium in *D. geminata*’s colonisation process needs to be elucidated with experimental studies.

The Sierra Nevada study also identified roles of sulphate (positive) and magnesium (negative) in *D. geminata*’s distribution. They speculated that the latter could be due to an inverse association between magnesium and calcium or “other processes yet to be determined.” While these variables were not included in my analysis, previous work in New Zealand shows that *D. geminata* is excluded from certain spring fed creeks, and that these creeks have high magnesium concentrations (Sutherland, Rodway et al.
The proportion of meta-sedimentary rock in a watershed also predicted the presence of *D. geminata* in Sierra Nevada. In Icelandic streams, the alga is less common and the occurrence less prominent in rivers with tertiary basalt bedrock (Jonsson, Jonsson et al. 2007). The bedrock geology variables (hard sedimentary, soft-sediment, volcanic-basic, volcanic-acidic and plutonic) did not distinguish between *D. geminata* positive, negative-exposed or negative sites in the South Island of New Zealand (this study). Although not included as a variable in this study, pH may also influence the alga, as it has never been found in Norway in water pH < 6.7 (Lindstrøm and Skullberg 2008). However, in their review paper, Whitton et al. (2009) state that almost all accounts of *D. geminata* mention pH values in the range from just below neutral to well above pH 8.0.

In the United States study (Kumar, Spaulding et al. 2009), mean air temperature in the warmest quarter was one of the best predictors of potential suitable habitat for *D. geminata*. In this study, mean air temperatures in January predicted *D. geminata* presence/absence— but in the opposite direction to that observed in the United States. In South Island streams, *D. geminata* presence was predicted by relatively warmer, not cooler, summer temperatures. However, (Kumar, Spaulding et al. 2009) investigated a comparatively wider range of temperatures, including much higher values. Kumar, Spaulding et al. (2009) also identified base-flow index and elevation as important predictors of *D. geminata*’s presence. However, in that model, temperature and elevation were probably collinear. In my model, there was a weak, inverse correlation between mean January (summer) air temperature and distance from coast. This suggests that substrate suitability overrides the influence of temperature. Other comparisons of water chemistry variables in the presence and absence of *D. geminata* blooms in Vancouver Island, and at *D. geminata*-positive and negative sites in Italy found no significant relationships with any of the measured environmental variables (Sherbot and Bothwell 1993; Beltrami, Cappelletti et al. 2008). However, in a North American study, *D. geminata*-affected sites were characterised by lower mean values of flow, turbidity, temperature, conductivity, pH and TP (Kirkwood, Shea et al. 2007).

Catchment proportion of peat did not distinguish between *D. geminata* status groups in this study, despite streams elsewhere with abundant *D. geminata* having peaty soils in the catchment (Whitton, Ellwood et al. 2009). Contrary to my prediction, proportionate area of glacier in the upstream catchment was also not associated with *D. geminata*’s
distribution. Peat and proportion of glacier were included as key variables in an earlier model designed to assess the suitability of areas for *D. geminata* (Kilroy, Snelder et al. 2008). My analysis of *D. geminata* occurrence data spanning eight years had only summer air temperature in common with that earlier model. This may explain the current discrepancy between the predicted range of *D. geminata* in New Zealand and the reported observations.

### 2.4.1 Limitations of this analysis

The strength of this study over other *D. geminata* presence/absence studies is the distinction made between sites with and without a propagule supply. However, the concentration of propagules was not accounted for. It is known that *D. geminata*’s invasive success depends on the density of the propagule supply (Flöder and Kilroy 2009). Sites downstream of only one positive site may have suitable habitat but few propagules. Despite this limitation, the model successfully distinguished between the three status groups.

The interpretation of this chapter’s results must be taken in the context of the MPI-BNZ Didymo Samples Database. This database does not represent systematic survey effort and it is likely that difficult-to-access sites are poorly represented. The odds of a site being positive or negative may have also depended on the season it was surveyed. A thorough read-through of the database suggested that once a positive record was obtained for a particular site, further reports for that site tended to cease or become less frequent. This meant that I was unable to investigate the possibility of range abatement or create a fourth status group of sites where *D. geminata* subsequently disappeared.

Survey effort was likely to vary seasonally and certainly did so throughout the years (Figure 4). For this reason, I made no attempt to conduct a high-resolution analysis of temporal patterns of spread. Such analysis would require systematic survey effort. However, notwithstanding the probable underrepresentation of remote sites, the database was well suited for conducting an analysis of the habitat parameters that are related to *D. geminata* status group membership.

Use of the MPI-BNZ and FWENZ databases has given us an understanding of *D. geminata* distribution at the widest scale—an entire island. However, these databases provide no insight into the unique site characteristics such as substrate, velocity and
nutrient concentrations that enable *D. geminata* to accumulate, and in some instances, bloom. These factors are addressed in the following chapters.

Although this chapter shows that *D. geminata*’s range almost completely covers the South Island, an assessment of its impact is unable to be made with presence/absence data (Kilroy and Unwin 2011). In many streams, particularly those with natural flow regimes, *D. geminata* may obtain only very low biomass. Finally, it must be kept in mind that a significant correlation does not necessarily indicate the existence of a causal relationship between factors.
2.5 Conclusions

In conclusion, *D. geminata* is a very successful invader of streams in the South Island, New Zealand. The multinomial regression analysis identified six factors that influence the probability of a reach being *D. geminata* positive, negative-exposed or negative. This information can be used to improve assessment and distribution models. At-risk sites for *D. geminata* invasion are those that are frequented by anglers (Bothwell, Lynch et al. 2009; Kilroy and Unwin 2011), have suitable substrates for colonisation, are a sufficient distance from the coast, have adequate mean January (summer) air temperatures and calcium in the underlying substrate, low phosphorous in the underlying substrate and suitable flow regimes. The pathways of invasion to these sites should be interrupted (if feasible). One management option for slowing or preventing the spread of *D. geminata* is to close rivers with an overlap of angler activity and these habitat characteristics to fishing. However, such an approach has been argued to be ‘economically inefficient’ in terms of ‘angler welfare’ (Beville, Kerr et al. 2012) and is likely to be met with fierce resistance from anglers. In large rivers, such as the Waitaki, complete closure would be very difficult to achieve. There are many users and the adjacent towns are economically dependent on the river. Furthermore, the long and sinuous nature of the river makes policing almost impossible. It’s also likely that a ban would make the river more attractive to anglers through perceptions of an ‘unfished’ habitat.

It appears that *D. geminata* will not establish at a site with insufficient geological calcium or air temperature, even if propagules are supplied from elsewhere in the catchment. Other unique site characteristics such as the presence of riparian shading, elevated nutrient concentrations, high velocity or greater depth should also influence *D. geminata*’s ability to establish colonies. Such variation in suitability at smaller spatial scales is captured in the following chapters of this thesis.
3 Didymosphenia geminata abundance thresholds in a temperate Southern Hemisphere region

Abstract

*Didymosphenia geminata* is a freshwater bloom-forming diatom capable of changing macrobenthic invertebrate community structure. Its abundance patterns in Southern Hemisphere streams with different flow regimes and during different seasons are not well understood. *D. geminata* colonies were monitored in 13 non-regulated and 8 hydrologically stable (regulated by a dam, lake or other storage) streams in Otago, New Zealand. Each stream was visited in winter and spring 2009 and in summer 2010. Scatter plots and mixed-effects regression were used to identify patterns between stream variables and *D. geminata* abundance. Its abundance was greater in hydrologically stable streams. Cool stream temperatures did not limit chlorophyll *a*, the measure used as a proxy for *D. geminata* cell biomass. Although it was also positively correlated with chlorophyll *a*, velocity limited the accumulation of high stalk biomass (measured as ash-free dry mass), at a threshold of approximately 0.61 m s\(^{-1}\). Below this threshold, stalk biomass was positively correlated with temperature. In general, *D. geminata*’s environmental preferences in this Southern Hemisphere region are aligned with those in its native Northern Hemisphere. Its success in New Zealand is therefore likely to be attributable to the suitability of New Zealand’s habitat, rather than some extraordinary characteristic of New Zealand or *D. geminata* itself.
3.1 Introduction

The freshwater benthic diatom Didymosphenia geminata (Lyngbye) M. Schmidt forms nuisance blooms in New Zealand and overseas and is thought to threaten natural ecological processes and properties in affected streams (Blanco and Ector 2009). *D. geminata* mats, which mainly comprise extracellular polysaccharide stalk material (Gretz, Riccio et al. 2006; Whitton, Ellwood et al. 2009), have been shown to change the community structure of macrobenthic invertebrates (Kilroy, Larned et al. 2009; Gillis and Chalifour 2010), increase total invertebrate abundances (James, Ranney et al. 2010), affect surface water–ground water exchange (Bickel and Closs 2008) and increase periphyton biomass (Kilroy, Larned et al. 2009). Historically found in low productivity streams in the Northern Hemisphere (Spaulding and Elwell 2007), in recent years *D. geminata* has rapidly increased its range, and is now well established in the South Island of New Zealand (Kilroy and Unwin 2011). In addition to the expansion into New Zealand, it is reported that bloom occurrences are becoming more frequent in North America, Europe and Asia (Spaulding and Elwell 2007; Blanco and Ector 2009; Cullis, Gillis et al. 2012).

Where present, *D. geminata* tends to proliferate in oligotrophic waters (Whitton, Ellwood et al. 2009), although blooms have also been reported in mesotrophic waters of Europe (Kawecka and Sanecki 2003) and eutrophic waters in the United States (Spaulding and Elwell 2007). In New Zealand, blooms primarily occur in rivers with mean dissolved reactive phosphorus (DRP) concentrations below 5 mg m$^{-3}$ (Bothwell and Kilroy 2011), yet recent survey and experimental work suggests that P availability limits cell division rates during blooms (Bothwell and Kilroy 2011; Kilroy and Bothwell 2011). *D. geminata* thrives in a wide range of hydraulic conditions (Kirkwood, Shea et al. 2007; Spaulding and Elwell 2007; Kirkwood, Jackson et al. 2009). However, studies have indicated strong links between stable river flows and increased *D. geminata* growth (Kirkwood, Shea et al. 2007; Spaulding and Elwell 2007). While a propensity for large proliferations in hydrologically stable (regulated by a dam, lake or other storage) waterways is documented (Kawecka and Sanecki 2003; Kirkwood, Jackson et al. 2009), there have been notable blooms in non-regulated rain-fed waterways in New Zealand, including the Kakanui and Lindis Rivers in Otago (V.
Hammond, pers. obs.). Blooms were also observed in the non-regulated Ara River of Spain between 2006 and 2009 (Tomas, Oscoz et al. 2010). Managing the abundance and spread of this organism would be improved with better knowledge of biomass dynamics in a variety of stream types and during different seasons.

Using data collected during a survey of streams within the Otago Region of New Zealand, I examined variables that potentially limit and facilitate *D. geminata* growth and biomass and explored their potential to predict *D. geminata* abundance. I hypothesized that *D. geminata* growth and biomass would be limited/facilitated by different variables at different times, as has been reported for other periphyton communities [e.g. Rosemond, Mulholland et al. (2000)]. For example, in the South Island’s temperate climate, seasonal temperature and light variation may determine *D. geminata* growth potential, whereas during spring and summer, when light and temperature conditions are favourable, low nutrient availability may limit its growth. In addition, due to the high biomasses that *D. geminata* mats can achieve, I hypothesized that high water velocity would prevent the accumulation of high *D. geminata* biomass in some fast flowing reaches of South Island rivers. Finally, because *D. geminata* can grow a high biomass under optimal conditions, I hypothesised that rivers with hydrologically stable flows (e.g. lakes, dams, etc.) should retain higher biomasses of mats than rivers without flow regulation.

I hypothesized that an interaction of limiting factors should influence biomass and production among rivers over annual climate cycles. For example, nutrient limitation will primarily occur only at times when temperature and light are above critical thresholds. Secondly, temperature and light limitation should occur at sites where water velocity is below a critical turbulence threshold. My analysis determines whether such thresholds can be elucidated from *D. geminata* biomass surveys among a variety of rivers and river reaches, over an annual cycle. I also interrogate the same dataset to elucidate key interactions among potential growth-limiting factors.
3.2 Materials and methods

3.2.1 River and site selection

This study focused on rivers in the Otago Region of the South Island, New Zealand (Figure 11). The region is characterised by high rainfall in the Southern Alps (> 3000 mm y\(^{-1}\)) and low rainfall in the semi-arid Central Otago valleys (< 500 mm y\(^{-1}\)). There are two principal catchments in Otago, the Clutha / Mata-Au and the Taieri. The area of the Clutha catchment totals 21 000 km\(^2\) and that of the Taieri catchment totals 5050 km\(^2\). Rivers were selected to encompass a broad and balanced range of river types [according to the New Zealand Department of Conservation’s Fresh Water Environments of New Zealand Classification, Leathwick, Julian et al. (2008)]. Of primary interest was whether rivers were hydrologically stable (regulated by a lake, dam or other storage, hereafter ‘regulated’) or not (hereafter ‘non-regulated’). Within the two contrasting flow regimes, rivers were selected based on a balanced range of mean annual flows, channel widths, stream orders and slopes. Sites were selected based on ease of access and wadeability. Each reach was 30 m long with four transects marked at 10 m intervals. Care was taken to sample only in reaches unaffected by shade. Decontamination procedures were carried out between all sites, regardless of confirmation of the presence of *D. geminata* or not.

To address seasonal persistence and abundance, monitoring was carried out in winter (July 2009), spring (October 2009) and summer (January 2010). To avoid the effects of flood scouring on *D. geminata* abundance, data were not collected within 14 days of a flood (here defined as an event with a flow $\geq$ three times the median flow). Periphyton recovery after disturbance is highly variable (Biggs and Close 1989), but can be complete after only a few days (Peterson 1996). Following a bed-mobilizing flood in a regulated stream in the United States, *D. geminata* abundance recovered to pre-flood levels within a week (Miller, McKnight et al. 2009).
3.2.2 Collecting and assessing *D. geminata*

Periphyton samples were collected at five points along each of four transects, up to a maximum depth of 0.6 m. Samples were processed for microscopic identification, determination of ash-free dry mass (AFDM) and chlorophyll *a* following the procedures of Biggs and Kilroy (2000). A 5 cm² circular area of periphyton was scraped from randomly selected rocks within the reach by mechanically dislodging biomass using a combination of dissecting tools and a toothbrush to clear the rock surface of visible periphyton. At each site, periphyton samples from each rock were pooled in a 300 ml pottle and stored on ice prior to freezing (-20°C).

I used chlorophyll *a* as a proxy for cell biomass, AFDM as a proxy for stalk biomass and the autotrophic index as a proxy for stalk:cell ratio at each site. The autotrophic index is the ratio of ash-free dry mass to chlorophyll *a*, where high values indicate large amounts of non-photosynthetic organic material compared to live plant material (Biggs
and Kilroy 2000). While these measures include the biomass of the entire periphyton community, when present, *D. geminata* was the overwhelmingly dominant species (as confirmed via microscopy). Chlorophyll *a* and AFDM data of 14 sampling occasions were dropped from the analysis because *D. geminata* was not visually observed at the site.

### 3.2.3 Measurement of physical and chemical parameters

Mean flow velocity at each site was quantified based on the average of measurements of mean column velocity taken at 15 points across each of four channel transects. A Flo-Mate 2000 (Marsh-McBirney, Maryland USA) was used to take 30-second readings at six-tenths of the water depth above the stream bed. A YSI meter (YSI Professional Pro Plus, YSI, Yellow Springs USA) was used to measure site water temperature, conductivity and dissolved oxygen. Five 50 ml water samples were taken from each site using polyethylene tubes that had been acid washed and rinsed with double distilled water. Water samples from each site were stored on ice for transport, before being frozen at -20°C. Dissolved nutrients were filtered, and dissolved organic carbon (DOC), silicate, nitrate-N plus nitrite-N (NO₃) and DRP were analysed using standard methods (Morris and Riley 1963; Murphy and Riley 1963; Wood, Armstrong et al. 1967). Nutrient concentrations were determined using a SAN-Plus segmented flow autoanalyser (SkalarAnalytical B.V., Breda, The Netherlands). The Wolman method was used to summarise substrate composition at each site (Wolman 1954). One hundred particles were measured, and their mean was used in the analysis.

### 3.2.4 Analysis

Scatter plots and matrices were used to assess potential environmental thresholds in *D. geminata* cell density (chlorophyll *a*), biomass (AFDM) and autotrophic potential (AFDM:chlorophyll *a*). Where clear thresholds limiting these variables were observed, samples where *D. geminata* was potentially limited were distinguished from the sample pool. The scatter plots then indicated whether interactions between environmental variables occurred.

Variables were transformed where necessary to achieve normal distributions and normality of the transformed variables was checked with the Skewness-Kurtosis Test (D’Agostino et al. 1990). Repeated measures ANOVA was used to assess differences in the means of measured environmental parameters between the two flow regimes and
between sites with and without *D. geminata*. As with any ANOVA, repeated measures ANOVA tests the equality of means. However, this methodology accounts for the longitudinal design, with each site being sampled on more than one occasion. The dependent variable was the environmental parameter of interest, while the independent variables were site group membership (regulated vs. non-regulated flow, and *D. geminata* positive vs. *D. geminata* negative sites. Because of winter access restrictions, floods and a river drying up, survey effort varied, with sites being visited on either two or three occasions. Therefore, mixed-effects regression was used to test for linear relationships, with a random intercept for each site allowing for automatic adjustment for unequal measurement points for each site. The site-specific random intercept can be thought of as the combined effect of omitted site-specific, time-invariant covariates that cause some sites to be more susceptible to *D. geminata* than others (e.g. substrate composition). Stream order was initially entered into the linear models as a covariate. However, as it had no effect on the models it was subsequently dropped from all analyses. Factors associated with the three *D. geminata* abundance variables (chlorophyll *a*, AFDM and autotrophic index) were examined using a subset of observations with *D. geminata* colonies present (n = 36). The factors (independent variables) of interest included season, temperature, velocity, DOC, NO3, DRP and silicate. A biplot was constructed using singular value decomposition of the data matrix to identify multivariate relationships between the biomass and stream variables and significant independent variables. Singular value decomposition is related to principle components analysis and correspondence analysis. Stata 10.1 (Stata Corp, College Station, TX) was used for all statistical analysis.
3.3 Results

3.3.1 Sites

The survey included 21 rivers (Figure 11, Table 5). Of these rivers, 13 were non-regulated and 8 were regulated (by a lake, dam or other storage). A total of 59 observations were made. The substrate compositions of regulated and non-regulated streams did not significantly differ (p > 0.05, ANOVA) and predominantly comprised cobbles and pebbles. *D. geminata* abundance was significantly higher in regulated compared with non-regulated streams (both chlorophyll *a* and AFDM, p < 0.01, Table 6).

Table 5 Upstream Catchment area, flow regime and prior *D. geminata* status of study streams

<table>
<thead>
<tr>
<th>River</th>
<th>Upstream catchment area (m²)</th>
<th>Regulated flow</th>
<th>Previous record of <em>D. geminata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Taieri (6)</td>
<td>4718655</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Dunstan (5)</td>
<td>269403</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Mototapu (5)</td>
<td>224675</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Routeburn (5)</td>
<td>79997</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Cardrona (5)</td>
<td>306935</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Manuherikia (7)</td>
<td>1947792</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Wye (4)</td>
<td>39285</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Moke west (4)</td>
<td>21564</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Lindis (6)</td>
<td>1012634</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Timaru (5)</td>
<td>145795</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Kakanui (5)</td>
<td>294302</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Dart (6)</td>
<td>581924</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Makarora (2)</td>
<td>4186</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Matukituki (4)</td>
<td>156803</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Fraser (5)</td>
<td>205264</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Greenstone (5)</td>
<td>341993</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Diamond (4)</td>
<td>73824</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Clutha (7)</td>
<td>2584108</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Moke east (4)</td>
<td>22201</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Thompsons (4)</td>
<td>67004</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Waitaki (1)</td>
<td>747</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
In New Zealand, the guideline for nuisance algal biomass is defined as an AFDM value $\geq 35 \text{ g m}^{-2}$ (Biggs and Kilroy 2000). Under this definition, nuisance levels were encountered at the regulated Moke Creek in winter 2009 (AFDM = 49.21 g m$^{-2}$), the regulated Waitaki River in spring 2009 (AFDM = 47.44 g m$^{-2}$) and the regulated Fraser River in summer 2010 (AFDM = 38.46 g m$^{-2}$).

Environmental data are presented for streams grouped by flow regime (regulated or non-regulated and by the presence or absence of $D$. geminata (Table 6). The only parameter that significantly differed between the stream types was silicate. Only NO$_3$ values significantly differed between sites with and without $D$. geminata. In the ordination of environmental variables representing the sites sampled (Figure 12), the primary axis was best represented by chlorophyll $a$ (explaining 62% of the total variance), while the concentration of DRP best represented the secondary, orthogonal axis (explaining 19% of the total variance).
Figure 12. Singular value decomposition biplot of stream variables. Circles represent non-regulated sites and triangles represent regulated sites. DRP: dissolved reactive phosphorus; AFDM: ash-free dry mass; Chl_a: chlorophyll a; Auto_index: autotrophic index; Diss_Oxy: dissolved oxygen. The lines reflect the variables, with longer lines indicating higher explained variance. The cosine of the angle between the lines approximates the correlation between the variables they represent. An angle of 0 or 180 degrees reflects a correlation of 1 or –1, respectively.
Table 6 Environmental means by flow regime and D. geminata presence / absence

| Parameter                          | Flow regime | D. geminata | |
|------------------------------------|-------------|-------------|
|                                   | Non-regulated | Regulated | Mean | Range | Mean | Range | Mean | Range | p<sub>diff</sub><sup>a</sup> | p<sub>season</sub><sup>b</sup> | Presence | Absent | p<sub>diff</sub><sup>a</sup> | Bloom<sup>c</sup> |
| Temperature (°C)                   | 7.58 (1.20–15.80) | 7.25 (0.80–14.70) | 0.77 | 0.63 | 8.28 (0.80–15.80) | 6.56 (2.00–13.50) | 0.86 | (2.50–14.70) |
| Velocity (m s<sup>-1</sup>)        | 0.67 (0.08–1.38) | 0.62 (0.14–1.39) | 0.36 | 0.15 | 0.62 (0.08–1.38) | 0.67 (0.14–1.37) | 0.39 | (0.22–0.63) |
| Dissolved organic carbon (mg l<sup>-1</sup>) | 2.09 (0.47–4.03) | 1.84 (1.02–2.89) | 0.14 | 0.71 | 2.00 (0.47–4.03) | 1.90 (1.09–2.97) | 0.70 | (1.86–2.09) |
| Nitrate-N (µg l<sup>-1</sup>)     | 26.01 (2.42–96.59) | 26.38 (2.82–122.35) | 0.37 | 0.94 | 23.23 (2.42–122.40) | 34.68 (6.28–96.59) | 0.02 | (2.82–18.77) |
| Dissolved reactive phosphorus (µg l<sup>-1</sup>) | 4.10 (0.40–25.28) | 2.45 (0.04–5.71) | 0.38 | 0.14 | 3.69 (0.40–25.28) | 2.48 (0.40–13.93) | 0.44 | (0.04–4.16) |
| Silicate (mg l<sup>-1</sup>)      | 1.91 (0.3–5.72) | 1.21 (0.10–2.50) | 0.01 | 0.34 | 1.61 (0.30–2.90) | 1.54 (0.40–3.30) | 0.78 | (1.10–2.66) |
| Chlorophyll a (mg m<sup>-2</sup>) | 16.77 (<0.10–84.08) | 6.34–250.23) | <0.01 | 0.18 | 54.16 (<0.10–4.72) | (<0.10–57.52) | <0.01 | (15.57–250.23) |
| AFDM (g m<sup>-2</sup>)           | 6.17 (<0.10–19.32) | 49.21) | <0.01 | 0.88 | 13.48 (<0.10–49.21) | 0.41 (<0.10–5.54) | <0.01 | (38.46–49.21) |

<sup>a</sup> Based on repeated measures ANOVA
<sup>b</sup> Based on season × regulation interaction term in repeated measures ANOVA
<sup>c</sup> Data from three observed blooms (AFDM ≥ 35 g m<sup>-2</sup>)
3.3.2  *D. geminata* abundance in relation to physical and chemical variables

3.3.2.1  Season

In streams with regulated flows, *D. geminata* colonies persisted in each of the three seasons. By contrast, only four of thirteen streams with non-regulated flows had visible *D. geminata* colonies each season (Routeburn, Lindis, Wye, Kakanui). In all streams with *D. geminata*, there were no seasonal differences in abundance in terms of AFDM. However, there were significant seasonal differences in chlorophyll *a* and autotrophic index values (both *p* < 0.01, ANOVA). In winter, spring and summer, mean chlorophyll *a* concentrations (mg m\(^{-2}\)) were 65.71, 90.75 and 10.61, and mean autotrophic index values were 388, 144 and 1681, respectively.
3.3.2.2 Flow regime and velocity

The singular value decomposition ordination presented in Figure 12 shows that sites on rivers with regulated flows tended to have higher chlorophyll a than non-regulated rivers. Regulated flow was associated with *D. geminata* chlorophyll a (β = 5.01, SE = 1.06, p < 0.01, R² = 0.36) and AFDM (β = 2.06, SE = 0.63, p < 0.01, R² = 0.30). Chlorophyll a was linearly associated with velocity (β = 2.01, SE = 0.76, p < 0.01, R² = 0.13, Figure 13a), but high chlorophyll a values still occurred in low-velocity sites. Mat development appeared to be limited by velocity. AFDM values above about 15 g m⁻² did not occur in velocities above 2 m s⁻¹ (with one exception, Figure 13b). Therefore, AFDM observations in sites with velocity above this level are coded separately (as solid diamonds) in subsequent figures. Further, these observations were excluded from regression analyses of AFDM. The autotrophic index values reflected the chlorophyll a and AFDM patterns (Figure 13c) with higher stalk biomass relative to pigment concentrations in low-velocity sites.
3.3.2.3 *Temperature and dissolved oxygen*

A linear relationship was observed between temperature, AFDM (p < 0.01, $R^2 = 0.18$, Figure 14b) and the autotrophic index (p < 0.01, $R^2 = 0.28$, Figure 14c). Dissolved oxygen showed no clear pattern with chlorophyll $a$ (Figure 15a), but was negatively correlated with AFDM (p < 0.01, $R^2 = 0.14$, Figure 15b), and the autotrophic index (p < 0.01, $R^2 = 0.14$, Figure 15c).

![Figure 14](image1.png)

Figure 14. Scatter plots of chlorophyll $a$ (mg m$^{-2}$), AFDM (g m$^{-2}$), autotrophic index and temperature (°C). Notes: Solid triangles indicate velocity-limited observations (velocity > 0.61 m s$^{-1}$) Solid circles indicate all other observations. The line in A was fitted by eye to indicate a constraining function. The solid lines in B and C were fitted by mixed-effects linear regression (p < 0.01, $R^2 = 0.18$; p < 0.01, $R^2 = 0.14$; respectively).
Figure 15. Scatter plots of chlorophyll $a$ (mg m$^{-2}$), AFDM (g m$^{-2}$), autotrophic index and dissolved oxygen (mg l$^{-1}$). Notes: Solid triangles indicate velocity-limited observations (velocity > 0.61 m s$^{-1}$). Solid circles indicate all other observations. The solid lines in B and C were fitted by mixed-effects linear regression ($p < 0.01, R^2 = 0.14; p < 0.01, R^2 = 0.14$; respectively).
**3.3.2.4 Dissolved organic carbon, nitrate-N, dissolved reactive phosphorus and silicate**

DOC and silicate showed no clear patterns with the *D. geminata* variables. Very high stalk biomass conditions (AFDM > 30 g cm$^{-2}$) were restricted to low DRP concentrations < 5 µg l$^{-1}$ (Figure 17b). The ordination (Figure 11) showed that DRP concentrations did not differentiate the two types of flow regimes. No clear patterns between nutrients and the autotrophic index values were evident (Figure 16c, Figure 17c).

![Figure 16. Scatter plots of chlorophyll a (mg m$^{-2}$), AFDM (g m$^{-2}$), autotrophic index and nitrate-N (µg l$^{-1}$). Notes: Solid triangles indicate velocity-limited observations (velocity > 0.61 m s$^{-1}$). Solid circles indicate all other observations.](image-url)
Figure 17. Scatter plots of chlorophyll $a$ (mg m$^{-2}$), AFDM (g m$^{-2}$), autotrophic index and dissolved reactive phosphorus ($\mu$g l$^{-1}$). Notes: Solid triangles indicate velocity-limited observations (velocity $> 0.61$ m s$^{-1}$). Solid circles indicate all other observations.
3.4 Discussion

High levels of periphyton biomass accumulate during periods of stable flows (Biggs 1996), and consistent with many observations in the literature (Kirkwood, Jackson et al. 2009; Whitton, Ellwood et al. 2009), I found greater *D. geminata* abundance in streams with regulated flows than in streams with non-regulated flows (Table 6). Seasonal patterns of *D. geminata* abundance have been reported in streams in the United Kingdom (Whitton, Ellwood et al. 2009), where stalks tend to form in spring, and Canada, where blooms typically occur in late summer to early fall (Simard and Simoneau 2007). In this study, the chlorophyll *a* and autotrophic index values showed significant seasonal variation. Chlorophyll *a* was lowest in summer and highest in spring. In contrast with the United Kingdom observation, in spring, the streams in my Southern Hemisphere sample had the lowest AFDM:chlorophyll *a* ratios. The highest AFDM:chlorophyll *a* ratio was observed in summer, suggesting that colonies were persisting as stalks without cells, a phenomenon also observed in the United Kingdom in late summer (Whitton, Ellwood et al. 2009). Stalk length, rather than density per square centimetre, may be the main driver of AFDM. Alternatively, the seasonal *D. geminata* mat dynamics that we observed may reflect the effect of conversion of chlorophyll *a* to xanthophyll (known as xanthophyll cycling (Geider, MacIntyre et al. 1998; Goericke and Montoya 1998), or other pigments in response to increasing seasonal light availability. Seasonal, light-induced xanthophyll cycling could be particularly important for *D. geminata* due to its preference for clean clear streams where light stress could be considerable during summer.

Velocity was associated with limited accumulation of high levels of stalk biomass, but was positively correlated with chlorophyll *a* (Figure 13). There are at least two possible explanations for these patterns. The first is that higher velocities are associated with increased shear stress and removal of mat material. This would explain my observations of less biomass in terms of AFDM at velocities above 0.61 m s⁻¹. Others have also identified that mat thickness of diatom communities is favoured in lower velocities (Lamb and Lowe 1987). However, an alternative explanation is that higher velocities reduce boundary layer thickness, reducing nutrient limitation and favoring cell growth rather than stalk growth. This would explain why chlorophyll *a* appeared to be promoted in higher velocities while AFDM appeared to be suppressed. *D. geminata*‘s interaction with its hydrodynamic environment was recently studied extensively by Larned et al. (2011). They identified that in low flow
conditions, *D. geminata* cells acquire nutrients through diffusion of dissolved nutrients derived from organic matter in mat matrices and the underlying substrata. At higher flows, they found increased nutrient mass transport from the overlying water to mat surfaces. It seems that *D. geminata* can acquire nutrients from different sources, depending on flow conditions. Therefore, the reduced boundary layer explanation does not fit my AFDM—velocity data as well as it does my chlorophyll *a*—velocity data. Some role of shear stress remains likely, and in the following chapter I use long-term data of the Kakanui River to show that *D. geminata* biomass is inversely correlated with near-bed velocity. Fast currents are likely to stimulate cell division by promoting transfer of nutrients to the cells at the mat surface (Arnon, Packman et al. 2007).

AFDM was negatively correlated with dissolved oxygen (Figure 15). This is likely to represent *D. geminata*’s effect on the environment. It is possible that respiration in the mats is causing reductions in dissolved oxygen. A detailed whole-reach metabolism study revealed major differences in dissolved oxygen (in terms of both diurnal variation and the timing of peaks) between river reaches that were heavily affected by *D. geminata* and reaches that were moderately affected by *D. geminata* (Larned, Arscott et al. 2007). In that study, differences in *D. geminata* biomass had greater influence on dissolved oxygen than differences in temperature or flow had on dissolved oxygen.

Stream temperature was positively associated with AFDM, my proxy for stalk biomass, as well as the autotrophic index values (Figure 14). Although temperature was not significantly correlated with DRP or nitrate-N (data not shown), it may be carrying some flow information, because flows preceding the January samples were likely to have been lower and more stable than flows preceding the July and October samples. Furthermore, stalk production is positively correlated with light (Kilroy and Bothwell 2011), which is positively correlated with temperature through seasonal differences in solar elevation. Assuming that most of the material measured was *D. geminata*, this suggests seasonal differences in stalk production relative to cell production. Three previous studies identified no temperature–abundance associations (Kirkwood, Shea et al. 2007; Kirkwood, Jackson et al. 2009; Miller, McKnight et al. 2009). Two of these studies, conducted in rivers with slightly higher temperatures than our study rivers, used cell density counts as a measure of abundance (Kirkwood, Shea et al. 2007; Kirkwood, Jackson et al. 2009), which do not include stalk material. I found no linear association between chlorophyll *a* and temperature (Figure 14). Therefore, conflicting
findings regarding temperature are probably due to differences in variables used to represent *D. geminata* abundance and differences in temperature ranges studied.

Chlorophyll *a* was not associated with nitrate-N (Figure 16). In one nutrient enrichment experiment, increasing levels of nitrate-N triggered an initial but not sustained increase in the frequency of dividing *D. geminata* cells (Bothwell and Kilroy 2011). However, results regarding nitrogen have been inconsistent. In another experiment, *D. geminata* showed no clear response to ammonium nitrate enrichment (Kirkwood, Jackson et al. 2007). In the United States, a negative *D. geminata* VBI–TN association was identified in Glacier National Park (Schweiger, Ashton et al. 2011), while no association with dissolved inorganic N was identified in the Colorado Front Range (Miller, McKnight et al. 2009). On average, compared with the sites used in Miller, McKnight et al. (2009), the sites in this study had much lower nitrate-N concentrations. Overall, the weak and inconsistent relationships between N and *D. geminata* suggest a non-critical role of N in *D. geminata* growth dynamics.

Chlorophyll *a* not associated with DRP(Figure 17). Kilroy and Bothwell (2012) found that 2-year mean ambient DRP was negatively associated with standing crop index (a metric more closely related to AFDM) in 15 South Island river sites where *D. geminata* was present. Furthermore, two North American studies found a negative association of *D. geminata* abundance and cover with concurrent ambient TP and TDP (Kirkwood, Jackson et al. 2009; Miller, McKnight et al. 2009). Kilroy and Bothwell (2011) demonstrated that experimental P enrichment enhances cell division, but not stalk production, and this mechanism is likely to explain the observed negative associations.

### 3.4.1 Potential limitations of this study

The strengths of the dataset used here include its good spatial and seasonal coverage. It also distinguished between the biomasses of live plant material and non-photosynthetic organic material and identified some potential thresholds for each. However, future studies should look more closely at the potential for xanthophyll cycling to decouple the relationship with cell density/biomass. Others have used *D. geminata* cell density counts as a measure of *D. geminata* abundance (Flöder and Kilroy 2009; Kirkwood, Jackson et al. 2009; Miller, McKnight et al. 2009). Cell density counts do not take into account the biomass of the stalks, which is the feature of *D. geminata* that makes it a nuisance alga. Understanding the triggers of blooms requires an understanding of both cell and stalk biomass dynamics. Stalk length
and the frequency of dividing cells would have given more accurate measures of growth and growth dynamics. While it is possible that AFDM and chlorophyll \( a \) measures used were contaminated by other algal species, the impact of this is likely to be minimal. Every sample was checked microscopically, and interestingly, the samples seemed to fall into one of three types: no didymo cells at all, very few didymo cells, or an overwhelming abundance of cells and cells with stalks. It was only the latter group that were used in the analysis. Furthermore, if significant error through contamination did exist, I would have expected to see positive correlations between chlorophyll \( a \), DRP and NO\(_3\). No such associations were evident.

The use of nutrient data collected at the time of sampling rather than long-term, integrated nutrient concentrations is likely to have weakened the reported associations because of the variable nature of nutrient concentrations and delays in periphyton response to nutrient availability (Kilroy and Bothwell 2012). However, I was interested in the state of \( D. \) \( \text{geminata} \), rather than the growth rate, and other studies have successfully modelled \( D. \) \( \text{geminata} \) state based on ambient nutrient concentrations (Kirkwood, Jackson et al. 2009; Miller, McKnight et al. 2009). Unfortunately, the state of \( D. \text{geminata} \) will be affected by flow history. While I did not sample within 14 days of a flood greater than three times the median flow, I was unable to account for total accrual time in my analyses. This was a considerable limitation of this study.

Finally, I did not account for the top-down effect of grazers. While various macroinvertebrates are known to graze on \( D. \text{geminata} \) (Larned, Arscott et al. 2007), to my knowledge, significant effects of grazers on in situ \( D. \text{geminata} \) biomass have not been demonstrated to date.

### 3.5 Conclusions

Managing \( D. \text{geminata} \) requires information about its ecophysiology, particularly its environmental constraints. The data presented here suggest that the accumulation of high biomass is restricted to sites with velocities below 0.61 m s\(^{-1}\). There was also some suggestion of increased nutrient transport from the overlying water to mat surfaces promoting cell growth, as there was a positive correlation between chlorophyll \( a \) and velocity. This switch to conditions favourable for cell growth rather than stalk growth may also explain the observed suppression of AFDM at higher velocities.
In Otago streams, the presence and abundance of the alga are strongly associated with stable flows, making any stream with a stable flow regime susceptible to successful invasion and the accumulation of high biomass. Regulated streams pose a greater challenge for the management of *D. geminata* proliferations, but decreases in hydrological variability due to increased water abstraction in non-regulated streams may increase the potential for *D. geminata* to proliferate in these systems. Furthermore, non-regulated streams do not offer the potential to manage nuisance blooms through flushing flows.
4 Temporal variability of *Didymosphenia geminata* biomass in a hill-fed coastal river (Kakanui, New Zealand)

Abstract

*Didymosphenia geminata* biomass was monitored at three sites in a hill-fed coastal river from April 2008 to May 2010. During this period, five floods of sufficient magnitude to remove *D. geminata* from the stream bed occurred. A visual biomass index (VBI), calculated as mat thickness (mm) × percentage cover was used as a metric for *D. geminata* biomass. Maximum biomass (VBI = 284.07) was observed at the Maheno site in April 2008, about 43 days after a flood of at least three times the median flow. *D. geminata* biomass was independently associated with mean water temperature, mean substrate particle size (area, mm$^2$) and mean site velocity. *D. geminata* VBI net accumulation rates were independently associated with standing VBI and flow (albeit weakly). Controlling for flood magnitude ($Q_{\text{max}}$) and days since $Q_{\text{max}}$, removal of *D. geminata* was associated with pre-flood biomass, mean site depth, mean water temperature and mean substrate particle size. Post-flood recovery was significantly associated with the size of the standing crop at the first post-flood sample and mean site depth. Blooms were predicted as more likely to occur during periods of low flow, with increasing days of accrual, at higher water temperatures and in slower velocities. Overall, *D. geminata*–flow dynamics are heavily influenced by the size of the standing crop and water temperature.
4.1 Introduction

Diatoms are important primary producers in freshwater ecosystems. They are a rich food source for many aquatic invertebrates and can purify stream water through the assimilation of dissolved nutrients and other material (Biggs 1996). However, during periods of stable flow, periphyton can accumulate to high levels (Biggs 1996) and threaten in-stream biodiversity and water quality. *Didymosphenia geminata* is an example of a diatom species that can form large proliferations, particularly in oligotrophic rivers. Under nutrient-limited, high-light conditions, *D. geminata* produces stalks composed of extracellular polymeric substances, and this is the reason for its development of very high biomass in oligotrophic rivers (Kilroy and Bothwell 2011). The alga has a strong preference for stable channels and regulated flow regimes (Kirkwood, Jackson et al. 2009), and it is thought that the primary control on the removal of *D. geminata* mats is scour resulting from bed-mobilizing floods (Cullis, Gillis et al. 2012).

Physical disturbance by flooding is the most important factor controlling periphyton biomass in New Zealand streams (Biggs and Close 1989). The amount lost in a flood depends on the intensity of the event and the resistance of the communities (Biggs 1996). The intensity of the event interacts with the characteristics of the substrate to determine bed mobility. This is an important mechanism for biomass removal from river beds during floods, particularly in reaches with beds comprising small sediment. In reaches with bedrock substrate, shear stress is probably the dominant removal mechanism (Biggs 1996). Resistance is influenced by the degree to which the periphyton can resist being torn from the substrate. A tall-growing diatom like *D. geminata* should be highly affected by high flow events. Furthermore, the conditions shown to promote *D. geminata* stalk development (low nutrients, high light) (Kilroy and Bothwell 2012) have been associated with less resistance to scour (Biggs, Tuchman et al. 1999). However, *D. geminata* mats have been shown to reduce form-induced stresses and near-bed turbulent velocity fluctuations, which may reduce their risk of detachment (Larned, Packman et al. 2011). Thus, *D. geminata* may have stronger than expected resistance to scour. The flood event magnitude required to scour *D. geminata* colonies is unknown.
Although others have studied the effects of flow on periphyton biomass dynamics (Biggs and Close, 1989), none have specifically addressed the association for *D. geminata*, which for the reasons outlined above, may behave differently to other periphytic diatoms. The main objectives of this study were to describe the dynamics and correlates of *D. geminata* biomass in terms of standing crop, accrual rates, and resistance to and recovery from floods in an unregulated hill-fed coastal river and determine the event magnitude required for scour removal. A further objective was to define the habitat window for nuisance blooms.
4.2 Methods

4.2.1 Description of study stream

*D. geminata* was monitored fortnightly by Department of Conservation staff at three sites on the Kakanui River from April 2008 to May 2010 (Figure 18). A total of 35 observations were made at each site. The Kakanui River is a hill-fed coastal river flowing from the Kakanui Mountains to the Pacific Ocean. According to Biggs, Ibbitt et al. (2008), New Zealand streams with a hill source of flow are characterised by relatively low minimum flows compared with the high flows, with moderately frequent high flows of greater than about six times the low flow. The Kakanui River has a catchment area of 894 m² consisting of approximately 35% river valley and 40% rolling hills or downland of less than 600 m elevation. The remaining 25% of the catchment is mountainous, reaching 1640 m above sea level. The river experiences periods of extremely low flows, which are exacerbated by water abstraction for irrigation. Flow data were obtained from two flow stations, at Clifton Falls Bridge (catchment area 294 km²), and at Mill Dam (catchment area 546 km²). Clifton Falls records from 1981–2011 indicate a mean 7-day low flow of 0.479 m³ s⁻¹, a mean flow of 2.993 m³ s⁻¹ and a median flow of 1.520 m³ s⁻¹. Downstream at Mill Dam, water flow records indicate a 7-day low flow of 0.884 m³ s⁻¹, a mean flow of 5.348 m³ s⁻¹ and a median flow of 1.954 m³ s⁻¹. The Kakanui River’s water resource is heavily utilized for irrigation. There are 47 takes with a total allocation of 1.139 m³ s⁻¹. There is 0.876 m³ s⁻¹ of water consented for withdrawal from the reach between Cliften Falls and Mill Dam. A 2010 flow analysis suggests that between 0.2 m³ s⁻¹ and 0.4 m³ s⁻¹ was being taken from this reach at any time, about 50% of what is allocated. A minimum flow restriction is in place in the Kakanui River, whereby farmers must stop taking water if the river falls below a discharge of 0.25 m³ s⁻¹. However, restriction breaches are known to occur (Constantine 2010). Floods with bedload movement are generally the result of storms and may occur at any time. The Kakanui River and its main tributary, the Kauru River, have comparable nutrient concentrations (2006–2007 means, Kakanui: DRP 0.006 mg l⁻¹, NNN 0.030 mg l⁻¹; Kauru: DRP 0.007 mg l⁻¹, NNN 0.022 mg l⁻¹) (Ozanne 2012).
Figure 18. Location of Kakanui River and monitoring sites within the Kakanui Catchment. Sites are marked as grey diamonds. Inset map indicates location of Kakanui Catchment with the South Island, New Zealand.
4.2.2 Description of sampling sites

The Five Forks monitoring site (Figure 19) was predominantly run habitat, with riffle percentages ranging from 5%–25% depending on flow (higher flows generally result in less riffle habitat). There was virtually no shading of the site. Channel velocities tended to be much higher towards the left of the channel when looking downstream (often double the velocities of the right hand side). The channel averaged 16.8 m in width over the survey period, and compared with the other sites, was relatively deep (averaging 26 cm). The substrate comprised 1% sand/medium gravel (<16 mm), 74% coarse gravel/small cobble (16–128 mm) and 25% large cobble/boulder (<129 mm).

Figure 19. The Five Forks $D. \text{geminata}$ monitoring site on the Kakanui River. Photo taken looking downstream. Where visible, permanent transect markers are indicated by grey arrows.
The Robb’s Crossing monitoring site (Figure 20) varied between run and riffle habitat depending on flow (the higher the flow, the more run habitat, and vice versa). At very low flows, 5%–20% of ‘edge’ habitat is created by a lack of water. Of the three sites, Robb’s Crossing had the widest channel (averaging 25.1 m), and was the shallowest (averaging 14 cm). Velocities were typically higher towards the middle of the channel, decreasing at both sides. The substrate comprised 2% sand/medium gravel (<16 mm), 89% coarse gravel/small cobble (16–128 mm) and 9% large cobble/boulder (<129 mm).

Figure 20. The Robb’s Crossing *D. geminata* monitoring site on the Kakanui River. Photo taken looking downstream. Where visible, permanent transect markers are indicated by grey arrows.
The Maheno monitoring site (Figure 21) was predominantly riffle habitat, with run percentages ranging from 5%–30%, depending on flow. The only shading of the site occurred toward the far right of the channel when looking downstream. Velocities tended to be much higher in the middle section of the channel, decreasing toward both ends. The channel averaged 13 m in width and 20 cm in depth throughout the study period. The substrate comprised 3% sand/medium gravel (<16 mm), 95% coarse gravel/small cobble (16–128 mm) and 2% large cobble/boulder (<129 mm).

![Figure 21. The Maheno D. geminata monitoring site on the Kakanui River. Photo taken looking downstream. Where visible, permanent transect markers are indicated by grey arrows.](image)

### 4.2.3 Assessing *D. geminata*

At each of the three sites, three transects were permanently marked five metres apart. Along each transect 10 permanent stations were established equal distances apart. The same stations were assessed on each subsequent monitoring occasion. Ten rocks were selected along each transect by placing a ruler or callipers straight down to the stream bed at each permanent station. The rock touched, regardless of size, was measured for maximum length, width and depth. A visual assessment was then made of the rock for
percentage cover of *D. geminata* and mat thickness (0, < 1 mm, 1–5 mm, 6–15 mm, 16–30 mm, >30 mm). The same visual assessment was then made on each selected rock for native periphyton. For analysis, the visual assessment data were converted into a visual biomass index (VBI), calculated as mat thickness (mm) multiplied by percentage cover (Kilroy 2008). Unless indicated otherwise, mean site VBI was used in all analyses.

### 4.2.4 Measurement of physical and chemical site parameters

At the top transect of each site, conductivity readings were taken at each of the 10 permanent stations. At the same stations, near-bed velocity (0.2 of total depth) was measured using an OTT flow gauge (OTT, Germany). Revolutions were recorded over 20-second periods. From these readings, mean site conductivity and mean site velocity was calculated for each sampling occasion. One pH measurement was taken at each site using a pHtestr2 (Oakton, Illinois). Hobo temperature loggers (Onset, Massachusetts) were placed at all three study sites to record temperature data at 15-minute intervals. Average daily temperature was generated from these 15-minute interval readings. No effort was made to account for diel variability. Daily average flow data were obtained from the Clifton Falls and Mill Dam gauging stations operated by the Otago Regional Council. To analyse the effect of flow conditions on the *D. geminata* variables, the flow data were analysed in terms of the average flow for periods of 14-days leading up to any monitoring occasion. This corresponds with the average time interval between the monitoring occasions.

### 4.2.5 Analysis

Independent variables for modelling included mean site depth, site conductivity, site water temperature, site near-bed velocity, days of accrual (days since a flood ≥ three times median flow), 14-day average flow, season and mean substrate particle size (calculated as mean value of all stones measured during the *D. geminata* observation at that site [\(n = 30\]]; hereafter, mean stone area). Dependent variables included *D. geminata* standing crop (VBI), net accumulation rate and post-flood net accumulation rate (recovery), post-flood percent change in biomass (removal) and bloom presence. Variables were transformed where necessary to achieve normal distributions and normality of the transformed variables was checked with the Skewness-Kurtosis Test (D'Agostino et al. 1990). Mixed-effects regression was used for the modelling with a random intercept for each site to represent heterogeneity in the data. The site-specific
random intercept can be thought of as the combined effect of omitted site-specific (time-invariant) covariates that cause some sites to be more susceptible to *D. geminata* than others (for example, nutrient concentrations). A principal components analysis of the correlation matrix was used to identify multivariate relationships between variables. The principal components eigenvalues indicate how much variation in the dataset each principal component explains. Small eigenvalues correspond to the thinnest directions with the least explained variation. The eigenvalues are calculated by taking linear combinations of the original variables.

Using the equation presented in Uehlinger (1991), *D. geminata* net accumulation rates \( (k) \) were calculated by assuming an exponential change in VBI during the time interval \([t_1, t_2]\):

\[
k = \frac{\ln(x(t_2)) - \ln(x(t_1))}{t_2 - t_1}
\]

where \( x(t_1) \) and \( x(t_2) \) are mean VBI values of the study reach at the first and subsequent sampling dates.

Stata 10.1 (Stata Corp, College Station, TX) was used for all analysis.
4.3 Results

4.3.1 Sites

Over the study period, the maximum flow recorded at Cliften Falls was 100.91 m$^3$s$^{-1}$, and at Mill Dam was 384.18 m$^3$s$^{-1}$. The coefficient of variation of flow was 2.07, the frequency of floods greater than three times the median flow was 22, and the average high flow peak (using a threshold of three times the median) was 18.09 m$^3$s$^{-1}$ for Cliften Falls and 15.02 m$^3$s$^{-1}$ for Mill Dam. The sites significantly differed from each other in terms of mean water depth, mean water velocity, mean stone area and mean VBI (Table 7).

Table 7 Summary of study parameters (mean ± SD) by D. geminata monitoring site

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Five forks</th>
<th>Robbs Crossing</th>
<th>Maheno</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water depth (cm)</td>
<td>26 ± 8</td>
<td>14 ± 4</td>
<td>20 ± 7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Water temperature (°C)</td>
<td>12.24 ± 5.84</td>
<td>13.47 ± 6.19</td>
<td>12.11 ± 5.55</td>
<td>0.73</td>
</tr>
<tr>
<td>Velocity (m$^1$s$^{-1}$)</td>
<td>0.19 ± 0.11</td>
<td>0.23 ± 0.11</td>
<td>0.33 ± 0.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean stone area (mm$^2$)</td>
<td>17 806.26 ±</td>
<td>6723.29 ±</td>
<td>5863.42 ±</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VBI</td>
<td>128.37 ± 73.84</td>
<td>62.97 ± 60.11</td>
<td>112.79 ± 76.71</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Based on repeated measures ANOVA

4.3.2 D. geminata VBI

Maximum D. geminata VBI values were recorded at Five Forks in December 2009 (236.37), Robbs Crossing in June 2008 (214.33) and Maheno in April 2008 (284.07). Mean VBI over the study period differed between sites (ANOVA, p < 0.001). Mean D. geminata VBI (calculated using data of all sites with D. geminata present) also differed between season (ANOVA, p < 0.001), and was 143.76 in summer, 101.26 in autumn, 63.26 in winter and 84.58 in spring. Season accounted for 18.36% of the variation in D. geminata biomass. Overall, D. geminata biomass appears to be decreasing in the Kakanui River because it was negatively associated with Julian date ($\beta = -0.003$, SE = 0.001, $p < 0.01$, $R^2 = 0.02$).
There was considerable temporal variability of *D. geminata* biomass during the study period. The temporal fluctuations generally reflected the flow ($\beta = -2.01$, SE = 0.40, $p < 0.01$, $R^2 = 0.17$, Figure 22, Figure 23) and temperature ($\beta = 0.02$, SE = 0.003, $p < 0.01$, $R^2 = 0.26$, Figure 24, Figure 25) regimes of the river. *D. geminata* biomass increased during stable flow periods with the exception of the Five Forks site during the period January–May 2010, when biomass decreased, despite stable flow. Other significant predictors included velocity ($\beta = -14.60$, SE = 3.28, $p < 0.01$, $R^2 = 0.17$, Figure 26) and mean stone area (mm, $\beta = 2.67$, SE = 0.44, $p < 0.01$, $R^2 = 0.12$, Figure 27). Entering all significant independent variables into a multivariate random effects regression model yielded velocity, mean stone area and temperature as independent predictors of *D. geminata* VBI, with an $R^2$ of 0.50. Variance inflation factors were calculated to determine the influence of multicollinearity in the model. Although the mean variance inflation factor (1.55) was well below the threshold (10) for the presence of multicollinearity (Chatterjee, Hadi et al. 2000), it should be noted that only 55% of velocity’s variance was independent of the other variables in the model. Days of accrual exhibited a curvilinear association with VBI (Figure 28).

Removal of *D. geminata* from the stream bed occurred when a flow of more than 54 m$^3$ s$^{-1}$ was recorded, which is approximately 30 times the median value. Flow exceeded this level during floods in July 2008, and February–March, May and July 2009 at Cliften Falls, as well as in September 2009 at Mill Dam. The July 2008 flood eliminated 74–86% of *D. geminata* and 98–100% of native periphyton at the three sites. A flood of similar magnitude in February–March 2009 eliminated up to 48.5% of *D. geminata* VBI, but native periphyton VBI increased. The flood in late May 2009 eliminated 50.5–100% of *D. geminata* and 39.6–91.4% of native periphyton. The July 2009 flood eliminated up to 83.8% of *D. geminata* and 79–87.6% of native periphyton.
Figure 22. *D. geminata* VBI (spikes) and maximum daily flow (m$^3$s$^{-1}$, red line) over the Kakanui River study period. A) Five Forks, B) Robbs Crossing, C) Maheno. Note that it took flows greater than 54 m$^3$s$^{-1}$ to remove *D. geminata* biomass.
Figure 23. Scatter plot of *D. geminata* VBI and 14-day average flow (m$^3$ s$^{-1}$) with site observations coded separately.
Figure 24. *D. geminata* VBI (spikes) and temperature (dashed line) over the Kakanui River study period. A) Five Forks, B) Robbs Crossing, C) Maheno. Temperature data were not available early in the study. Note the absence of bloom at the Robbs Crossing monitoring site over summer 2009–2010.
Figure 25. Scatter plot of *D. geminata* VBI and temperature. The solid lines were fitted separately for each site by univariate linear regression (Five Forks p < 0.001, $R^2 = 0.73$; Robbs Crossing p = 0.001, $R^2 = 0.20$; Maheno p < 0.001, $R^2 = 0.42$). Site observations are coded separately in the figure.

Figure 26. Scatter plot of *D. geminata* VBI and instantaneous stream bed velocity (m s$^{-1}$). Dashed line fitted by eye to indicate a constraining function.
Figure 27. Scatter plot of *D. geminata* VBI and mean stone area (mm$^2$). The solid lines were fitted separately for each site by univariate linear regression (Five Forks $p = 0.05$, $R^2 = 0.08$; Robbs Crossing $p = 0.26$; Maheno $p < 0.001$, $R^2 = 0.32$).

Figure 28. Scatter plot of *D. geminata* VBI and days of accrual (days since flood). Lines were fitted by locally weighted scatterplot smoothing (band width 0.8). Note that after about 100 days of accrual, VBI starts to decline.
4.3.3 **Relationships between variables**

A principal components analysis was used to examine relationships between the *D. geminata* and stream variables (Figure 29). Factor axis 1 (eigenvalue 3.95) explained 56.38% of the total variation in the variables and represented a gradient related to velocity, flow, time since flood and temperature. Factor axis 2 (eigenvalue 1.44) explained 20.59% of the total variance, and represented a gradient related to mean stone area. Depth and VBI loaded close to evenly on both axes. To further examine the association between flow and temperature, the two variables were modelled using effects regression. A significant inverse relationship was observed ($\beta = -2.58$, SE = 0.55, $p < 0.01$, $R^2 = 0.55$).

![Figure 29. Principal components analysis of stream variables. Factors 1 and 2 explained 56.38% and 20.59% of the variance, respectively. TSF: time since flood; flow: 14-day average flow; dept: mean site depth; area: mean site substrate particle size; velocity: mean site velocity; temperature: mean site temperature; VBI: mean site visual biomass index. The horizontal axis shows projections onto the first principal component, the vertical axis the second component.](image)

4.3.4 **D. geminata net accumulation rates**

*D. geminata* VBI net accumulation rates (VBI per day) were significantly associated with concurrent biomass ($\beta = 0.003$, SE = 0.001, $p < 0.01$, $R^2 = 0.03$), temperature ($\beta = 0.0001$, SE = 0.00004, $p = 0.01$, $R^2 = 0.08$), flow ($\beta = 0.007$, SE = 0.003, $p = 0.04$, $R^2 = 0.02$).
0.01) and days of accrual ($\beta = -0.006$, SE = 0.002, $p = 0.02$, $R^2 = 0.01$). There were seasonal differences in accumulation rates ($p < 0.01$, repeated measures ANOVA). They were fastest in spring (0.03), followed by summer (0.01), winter (0.001) and autumn (-0.02). Concurrent biomass and flow independently predicted accumulation rate (VBI per day), with an $R^2$ of 0.05. An assessment of variance inflation factors indicated that for both variables, 91% of the variation was independent.

### 4.3.5 *D. geminata* resistance and recovery

Variables predicting *D. geminata* biomass removal ($n = 36$) were examined while controlling for $Q_{\text{max}}$ (maximum flow recorded during the flood) and days since $Q_{\text{max}}$. Surprisingly, the logarithm of the product of $Q_{\text{max}}$ times pre-flood VBI, which corresponds to the periphyton scour potential of Biggs and Close (1989), did not explain any of the variance in biomass removal. However, it was explained by pre-flood biomass ($\beta = 0.003$, SE = 0.001, $p < 0.01$, $R^2 = 0.21$), depth ($\beta = 6.33$, SE = 2.19, $p < 0.01$, $R^2 = 0.20$), temperature ($\beta = -0.01$, SE = 0.001, $p < 0.01$, $R^2 = 0.38$) and mean stone area ($\beta = 0.42$, SE = 0.13, $p < 0.01$, $R^2 = 0.29$). Extending the multivariate analysis beyond controlling for $Q_{\text{max}}$ and days since $Q_{\text{max}}$ was not possible because of the limited number of observations.

Controlling for $Q_{\text{max}}$ and days since $Q_{\text{max}}$, post-flood net accumulation rates ($n = 36$) were significantly associated with VBI at the first post-flood sample ($\beta = 0.01$, SE = 0.005, $p = 0.03$, $R^2 = 0.11$) and depth ($\beta = -15.67$, SE = 5.68, $p < 0.01$, $R^2 = 0.10$).

### 4.3.6 Blooms

In New Zealand, the guideline for nuisance algal biomass is defined as an AFDM value $\geq 35$ g m$^{-2}$ (Biggs and Kilroy, 2000), which corresponds to a VBI of 220. Under this definition, bloom conditions were observed at Five Forks during Nov–Dec 2008, after an accrual period of 58 days, Feb 2009 after 56 days, Dec 2009 after 32 days, and Jan–Mar 2010 after 78 days. During these blooms flow did not exceed 1.14 m$^3$ s$^{-1}$. At Robb’s Crossing, only one bloom was observed, which occurred during June 2008, after 45 days of flow not exceeding 1.91 m$^3$ s$^{-1}$. At Maheno, blooms were observed during Apr–Jun 2008 after 41 days of accrual, Dec 2009 after 32 days and Feb 2010 after 100 days in flows up to 2.29 m$^3$ s$^{-1}$. All blooms occurred after at least 15 days of flow under the annual median value. However, the association between stable low flow periods and bloom development was not consistent. At Robb’s Crossing, blooms did
not develop during the 2009–2010 summer despite long periods of low flow (Figure 22).

There were too few observations of a whole-site bloom (n = 8) to conduct a multivariate analysis of the factors associated with site blooms. However, univariate mixed-effects logistic regression analyses on transect-level blooms (n = 31, with transects nested in sites) identified that blooms were more likely to occur in lower flows (β = -0.73, SE = 0.27, p < 0.01), slower velocities (β = -17.52, SE = 8.12, p = 0.03), warmer temperatures (β = 0.36, SE = 0.09, p < 0.01) and with increasing days of accrual (β = 0.70, SE = 0.22, p < 0.01). Some data indicate a flow and temperature habitat window for blooms (Figure 30).

Figure 30. Scatter plot of 14-day average flow (m$^3$ s$^{-1}$) and temperature (°C) with observations recorded during bloom conditions marked (solid maroon diamonds, based on transect data). Note the low-flow high-temperature window for blooms.
4.4 Discussion

Standing *D. geminata* biomass (VBI) was positively associated with temperature and mean stone area, and negatively associated with velocity (Figure 31). A curvilinear association between days of accrual and VBI was evident. Temperature explained the most variation in VBI. Some seasonality was evident, with higher summer biomasses and faster spring and summer accumulation rates. These rates were also faster when concurrent biomass, flow and temperature were higher and they slowed with increasing days of accrual. Greater resistance to floods was associated with lower pre-flood biomass, lower temperatures, larger stone size and greater depth. Faster post-flood recovery was positively associated with post-flood standing crop and negatively associated with depth.

In general, the results are consistent with those from similar studies in the literature, with the exception of temperature, which was an influential variable in this study of standing crop, but not in others of growth rates (Kirkwood, Shea et al. 2007; Kirkwood, Jackson et al. 2009; Miller, McKnight et al. 2009). The Kakanui River experienced much higher temperatures than the rivers included in the studies of Kirkwood et al. (2007). It is possible that temperature effects could be elucidated in the Kakanui River.
because nutrients and light were unlikely to have been limiting. Kakanui River data from 2006–2011 indicate a soluble inorganic nitrogen:dissolved reactive phosphorus ratio of 30.80 (Ozanne 2012), and the monitoring sites were unshaded. It is known that when cells are not limited by nutrients (Bothwell 1988; DeNicola 1996) or light (Bothwell 1988) maximum growth rates are set by temperature. The observed VBI—temperature association supports the finding of a positive AFDM—temperature association in Otago streams (Chapter 3).

An accumulating body of evidence demonstrates that time since the last flood is a dominant factor controlling periphyton biomass (Biggs and Close 1989; Uehlinger 1991). I found a medium-strength, curvilinear association between time since the last flood and *D. geminata* biomass. Although it is assumed that scour removal is the primary control on *D. geminata* biomass (Cullis, Gillis et al. 2012), biomass decreased at the Five Forks and Robb’s Crossing sites during Autumn 2010 following bloom conditions despite the extended period of stable flow. Periodic sloughing probably occurred when the drag of sections of the developing mat exceeded the mats’ attachment strength (Biggs, Tuchman et al. 1999). The self-limiting nature of this association was probably why Kilroy and Bothwell (2012) found no association of standing crop with days of accrual in their observation of 31 sites in rivers of the South Island, New Zealand. Instead, they found that the proportion of gravels and sand at the site and flow variation explained up to 61% of *D. geminata* biomass. That study was conducted in summer, and single observations were made after at least 3 weeks of accrual—potentially masking any relationship.

In agreement with my findings, and those aforementioned (Kilroy and Bothwell 2012), stone size correlated with periphyton biomass in a prealpine river in Switzerland (Uehlinger 1991). Uehlinger (1991) explained this phenomenon by inferring that larger stones protruding from the stream bed offer a more favourable nutrient supply. Others (Ruthermann, 1990, in Uehlinger, 1991) suggest that it is the greater stability of large rocks that affords them greater resistance to flood disturbance. However, no association was found between *D. geminata* cell density and rock size after short-term (8 day) exposure of clean rocks to bloom conditions (Bergey, Cooper et al. 2010). In that study, it was the roughness of the rocks and condition of the biofilm that was found to influence biomass. There was probably insufficient variation in the study river’s
hydrological regime over the 8-day period to elucidate any influence of rock size on *D. geminata* biomass.

The negative association between *D. geminata* VBI and velocity identified in this study and for other periphyton species (Uehlinger 1991), suggest that shear forces may control the spatial distribution of periphyton biomass in the study reaches. Unlike Uehlinger (1991), I found no association between standing crop and depth. However, like others (Kirkwood, Shea et al. 2007; Kirkwood, Jackson et al. 2009; Miller, McKnight et al. 2009), I found a negative association between flow and *D. geminata* biomass. Elevated flows affect periphyton biomass by the direct effects of shear stress as well as by increasing sediment mobility, which leads to abrasion (Larned, Packman et al. 2011). In this study, up to 100% of *D. geminata* was removed following severe flooding. It should be noted that stable periods of low flow did not exclusively lead to the accumulation of high *D. geminata* biomass. For example, high biomasses did not develop at the Robbs Crossing or Maheno sites during summer 2009–2010. Compared with the other sites, few *D. geminata* colonies were present over the preceding winter at Robbs Crossing. It would seem that blooms must also be associated with high cell densities, just as Kilroy and Bothwell (2011) hypothesised. Of the three sites, Maheno is likely to have the highest nutrient concentrations because it is the furthest downstream. This may have influenced *D. geminata* mat development, because in New Zealand and overseas, high biomass tends to be restricted to low nutrient concentrations (Chapter 3)(Kilroy and Bothwell 2012).

Despite its negative association with standing *D. geminata* crop, flow was weakly, positively associated with *D. geminata* accrual rates. This could be driven by changes in nutrient concentrations, because nutrients are highly correlated with flow in New Zealand rivers (Biggs and Close 1989). However, because the VBI probably better reflects stalk than cell biomass, this seems unlikely. Accrual rates also had weak, positive associations with temperature and standing biomass—but probably only up to a certain threshold, because the rates slowed with increasing days of accrual (again, probably reflecting the age and health of the mats). Based on similar findings, Uehlinger (1991) suggested that inter- or intra-specific interactions may begin to control biomass accumulation after long periods of floods. Of the *D. geminata* variables in this chapter, accrual rates had the weakest correlations and the least amounts of explained variance.
Biggs and Close (1989) demonstrated in New Zealand streams that the efficiency of scour is more influenced by pre-flood biomass than the magnitude of the event. In agreement with this, removal of *D. geminata* in the Kakanui River was limited by the availability of removable material. It has been shown experimentally that increases in flow differentially affect periphyton according to its age (Villeneuve, Montuelle et al. 2010). The structural integrity of mats often declines with increasing age and biomass (Biggs, Tuchman et al. 1999), so thicker, most likely older, mats are probably less resistant to scour disturbance. Although resistance generally decreases with increasing intervals between disturbances (Larned, Packman et al. 2011), days of accrual was not directly associated with resistance in my study. I did find a negative association between resistance and stone size, in agreement with the findings of Uehlinger (1991), but inconsistent with those of Biggs and Close (1989), who had a very small sample size (n = 5). The negative association between stone size and resistance identified in this study is probably confounded by the positive association between stone size and standing biomass.

Although flows of more than three times the median flow are commonly used to describe flood frequencies relevant to river biota in New Zealand rivers (Clausen and Biggs 1997; Kilroy and Bothwell 2012), *D. geminata* persisted in the Kakanui river during flows of up to 30 times the median value. The event magnitude required to remove *D. geminata* from the stream bed was previously unknown. Because *D. geminata* mats reduce form-induced stresses and near-bed velocity fluctuations (Larned, Packman et al. 2011), some have suggested that the flow rate necessary to produce bed disturbance is higher in the presence of thick mats than in streams with discontinuous mat coverage (Cullis, Gillis et al. 2012).

The ultimate goal of *D. geminata* management is the prevention of blooms. Eutrophication is the most common driver of lotic algal blooms (Biggs 2000), but *D. geminata* blooms are not triggered by eutrophication events (Kawecka and Sanecki 2003; Bothwell, Lynch et al. 2009). On Vancouver Island and in New Zealand, blooms occur in clear, shallow, nutrient poor streams with ample light (Sherbot and Bothwell 1993; Kilroy and Bothwell 2012). This study identified that temperature, flow, velocity and days of accrual potentially control bloom development. Although others have linked low flows to bloom development (Kirkwood, Jackson et al. 2009), this study is the first to show that temperature is also important.
4.5 Limitations of this study

The lack of nutrient data is a limitation of this study. However, a study incorporating both nutrient and flow data identified that flow regime is more important than nutrients in determining the potential for algal proliferations (Biggs 2000). Because of the temporal distribution of the flood events my ability to model *D. geminata* response was limited. The accrual rates were calculated using VBI data, which include both cell and stalk material. Therefore, they indicate overall biomass accrual rates, not *D. geminata* growth rates *per se*. Finally, I had no measure of PAR, which would have been useful for determining the relative influences of light and temperature on biomass.

4.6 Conclusions

This study is the first to examine *D. geminata* biomass, resistance to floods, and accumulation and recovery rates year-round in a naturally flowing system over a long time scale. A complex of interrelated factors controlled the spatial and temporal biomass dynamics. However, it is clear that temperature and flow played key roles in biomass accumulation, which led to further increases in growth and recovery rates. In other words, the more *D. geminata* there is in a system, the greater the potential for nuisance proliferations that are difficult to manage. For non-regulated streams affected by *D. geminata*, the work in this chapter suggests that consents and minimum flow restrictions for water abstraction should be carefully reviewed in the warmest months of the year, because it appears that a combination of low flows and high temperatures can trigger blooms. This chapter also raises a number of implications for the application of flushing flows to remove *D. geminata* from regulated streams. First, because it was identified that floods of at least 30 times the median value are required to remove *D. geminata* biomass, flushing flows may simply be infeasible in many streams. Furthermore, the amounts of biomass before and after the flood influence recovery rates. But the actual potential for removal is limited by the biomass of the standing crop. This presents a conundrum for water managers. Biomass must be high enough to be successfully dislodged, but not so high that a substantial amount of propagules remains in the system after the flood. Further complicating the issue is that accumulation rates vary seasonally, which will influence the likely effectiveness of flushing flows. In sum, *D. geminata*’s response to the hydrological regime is influenced by a number of factors, all of which should be taken into account by those seeking to control its biomass.
5  Summary of findings

5.1  Introduction

My original contribution to knowledge is a description of the environmental drivers and constraints on the colonisation and growth of *Didymosphenia geminata* in New Zealand rivers. I am the first to show that cross-catchment dispersal occurs as rapidly as dispersal within connected bodies of water. Although others have observed that *D. geminata* is more common in calcareous waters, I show that geological calcium strongly influences the probability of successful colonisation. The threshold determining sufficient calcium differs between streams of different hydrological origin. This likely reflects calcium’s placement within a cascade of factors influencing *D. geminata*’s colonisation niche. Finally, I show the important roles of standing crop, temperature and flow characteristics in *D. geminata*’s habitat window for bloom and removal. In this chapter, I align the results of this thesis with phases in the conceptual model developed by Cullis, Gillis et al. (2012) (Figure 32).
Figure 32. Summary of thesis contributions to the conceptual model of Cullis, Gillis et al. (2012)

**Growth (accumulation)**
- Stalks constrained by velocity (0.61 m s\(^{-1}\)) & correlated with temperature
- Live material correlated with velocity
- Accrual potential is seasonal, and associated with substrate size and days of accrual
- Blooms occur during low flows and high temperatures
- Blooms predicted by low flows, increasing days of accrual, higher temperatures and slower velocities
- Post flood recovery occurs faster when residual biomass is higher and streams are shallower

**Colonisation**
- Associated with summer air temperature, distance to coast, source of flow, calcium & phosphorus
- Occurs in very low phosphorus environments

**Removal**
- Resistant to floods up to 30 times higher than the median flow
- Associated with pre-flood biomass, depth, temperature and substrate size

**Invasion**
- Cross-catchment dispersal occurs as rapidly as within-catchment dispersal
- Facilitated by key foci
- Has slowed considerably
5.2 Invasion
The size of individual cells, their survival ability outside of a river (Kilroy, Lagerstedt et al. 2007), the stalks’ ability to attach to substrata (Gretz, Riccio et al. 2006; Aboal, Marco et al. 2012) and the prominence of human activities as vectors for dispersal (Kilroy and Unwin 2011) have prompted rapid spread of *D. geminata* to 37 catchments of the South Island within the first eight years of its introduction. I expected that range expansion would occur more rapidly within- than across- catchments. However, the rates were similar so overland spread by suitable vectors appears to be an equally important dispersal mechanism (Chapter 2). However, this is probably highly context dependent. If high-use catchments are invaded, they may act as foci for rapid secondary spread to other catchments. For example, there was a one-year lag between the first catchment invasion (Waiau) and the subsequent invasions (Buller, Clutha, Oreti). One year after the Waitaki catchment, one of the most popular for angling and one of the most common trans-catchment angling destinations (Unwin 2009), was invaded there were nine new catchment-level invasions within a 6-month period. This suggests that the distance and frequency of travel between water bodies are critical influences on *D. geminata* spread.

5.3 Colonisation
Despite initial fears, *D. geminata* has not become an omnipresent feature of New Zealand’s waterways. Range expansion has slowed considerably over the past three years and there have been no new cross-catchment invasions over the past 12 months (Chapter 2). Unfortunately, because of the decreasing survey effort over time, it is impossible to tell whether this means the alga has reached its range limit in the South Island. In New Zealand, suitable habitat is defined by warmer summer temperatures (up to 15°C), greater distance from the coast, and less phosphorus and more calcium in the underlying geology (Chapter 2). Generally, streams with a low elevation or hill source of flow have unsuitable habitat for *D. geminata*. It is probably a combination of elevated nutrients and a lack of suitable substrata in these streams that make them unsuitable for colonisation because they have relatively benign flow regimes when not in flood (especially low-elevation streams) (Biggs, Ibbitt et al. 2008). Geological calcium had a very strong influence on a site’s probability of being *D. geminata* free in the presence of propagules. It is possible that *D. geminata* cells are unable to adhere to substrata with insufficient calcium (Geesy, Wigglesworth-Cooksey et al. 2000), thus
explaining the alga’s absence from sites with low calcium within the substrata. However, because water calcium was not measured, I am unable to differentiate the form of calcium (geological versus water) that is relevant to this niche preference. Based on the work of Rost et al. (2011), I suspect it is water calcium, because they found that percent calcium in the water was the best predictor of *D. geminata* presence in their sample of US streams. Although significant, phosphorous in the underlying geology had only minimal impact on a site’s probability of being suitable for *D. geminata*. The observed negative association between phosphorus and habitat suitability fits the general profile of *D. geminata* being restricted to low phosphorus conditions (Kilroy and Bothwell 2012).

### 5.4 Growth

As expected, there was a cascade of thresholds that limited *D. geminata* biomass accumulation. Strong seasonality was evident in *D. geminata* standing crop, accrual rates and mat structure. In Otago streams (Chapter 3), chlorophyll *a* concentrations were highest in spring and lowest in summer. In the Kakanui River, also in Otago (Chapter 4), biomass (as a visual-based metric) was highest in summer and lowest in winter, with the fastest accumulation rates in spring. These divergent results reflect seasonal differences in mat structure. In summer, the ratio of *D. geminata* stalk versus live plant material was approximately four times that of the winter ratio and 12 times that of the spring ratio. While these observations may also reflect xanthophyll cycling, they correspond with the observations of Whitton, Ellwood et al. (2009), who reported the presence of motile cells in winter that accumulated and developed into colonies in spring. By late summer, these colonies often persisted as stalks without cells. My *D. geminata*-specific findings slightly differ from the profile of other periphyton communities in New Zealand, in which biomass accrual rates were fastest in summer (Francoeur, Biggs et al. 1999).

*D. geminata* has been described as a cold water species (Kilroy, Snelder et al. 2008; Blanco and Ector 2009) and some have suggested that it is limited by high temperatures rather than favoured by low temperatures (Kilroy, Snelder et al. 2008). In the North Island, currently *D. geminata*-free, summer water temperatures in large lowland rivers regularly exceed 20°C, which may contribute to the exclusion of *D. geminata* (Kilroy, Snelder et al. 2008). The regional study (Chapter 3) showed although *D. geminata*
grows preferentially within low nutrient and velocity environments, below a velocity threshold of 0.61 m s\(^{-1}\), its stalk development appears to be maximised with increasing temperatures (up to 16°C, the maximum observed). Others have also observed that higher temperatures favour \(D. \text{geminata}\) growth and expansion (Antoine and Benson-Evans 1986).

Nutrient concentrations were not associated with stalk biomass in this thesis (Chapter 3), in agreement with the hypothesis of Cullis, Gillis et al. (2012). Kilroy and Bothwell (2011) established that stalk growth is a photosynthetic response to high-light, low-nutrient conditions. They acknowledged that “these processes must be compounded by other factors to produce the excessive biomass encountered in \(D. \text{geminata}\) blooms.” My data show that these other factors include high temperatures, low velocity, low flow and the number of days of accrual (Chapter 4).

It is difficult to measure the exact role of temperature in diatom growth because it is correlated with light, which also influences algal growth rates (DeNicola 1996). In one study, temperature was found to better correlate with algal growth rates in artificial streams than light (Bothwell 1988). In another, \(D. \text{geminata}\) stalk length increased with increasing light, but to a lesser extent at low temperatures (Kilroy and Bothwell 2011). Finally, the production of extracellular polymeric substances among benthic diatoms of intertidal mudflats was clearly affected by temperature in controlled light conditions (Wolfstein and Stal 2002). However, because Moke Creek was in bloom in winter (Chapter 3), photosynthetic overflow must also be capable of occurring at very low temperatures. Alternatively, the winter mats at this site were remnants of summer growth.

Chlorophyll \(a\) showed no correlation with DRP or NO\(_3\), but a positive correlation with velocity (Chapter 3). The lack of nutrient relationships contrasts with the series of experiments conducted by Bothwell and Kilroy (2011) examining the role of nutrients in \(D. \text{geminata}\) cell division. In a controlled environment, elevated nutrient concentrations promoted cell division. The role of velocity is probably of promoting transfer of nutrients to cells at the mat surface (Arnon, Packman et al. 2007).

I also examined the factors associated with biomass accumulation and post flood recovery rates. Accumulation rates correlated with standing crop, temperature, flow and days of accrual in the Kakanui River (Chapter 4). Temperature and flow were inversely
correlated, but in my model, temperature and days of accrual explained more of the variance in accumulation rates than flow. Faster recolonisation was associated with greater amounts of residual standing crop and depth, reiterating the importance of propagules and light in colony development.

5.5 Removal
Removal was not extensively studied in this thesis, and certainly not to the extent required to contribute to the model of Cullis, Gillis et al. (2012) (Figure 32). However, some general conclusions can be drawn. Substantially elevated flows are required to remove *D. geminata* mats. For example, in the Kakanui River, flow in excess of thirty times the median was required. The amount lost depends on the standing crop before the flood (Chapter 4). Mat coverage clearly determines the availability of material for removal, but because it alters the hydrodynamic environment (Larned, Packman et al. 2011), the extent and health of the mats themselves also influence scour resistance. Paraphrasing Cullis, Gillis et al. (2012), there is likely to be some critical threshold for mat size whereby drag potential overcomes the reduction of form induced stresses. Greater resistance to floods was associated with lower stream temperatures and greater depth (Chapter 4), which can probably be explained by their influence on mat structure. In lower temperatures and greater depths, *D. geminata* stalks are probably shorter and less influenced by drag. Larger stone size, which probably reflects the mobility of the substrata, also inferred greater resistance to floods.

5.6 Management
At present, *D. geminata* is recognised as a “pest” in New Zealand, meaning that it has serious adverse and unintended effects on economic wellbeing, conservation, ecological processes, biological diversity or the relationship of Māori with their ancestral lands, waters, sites, waahi tapu (traditional sacred places) and taonga (treasures) (Biosecurity New Zealand 2012).

It is not possible to eradicate *D. geminata* from New Zealand. However, control and/or containment programmes can prevent further spread and minimise impacts. The containment programme for *D. geminata* includes public awareness campaigns and legislative controls on the distribution of *D. geminata* (Vieglaïs 2007). Control options for invasive species include one-off actions (such as flushing flows), sustained control to restrict the target species to a threshold density (such as minimum flow restrictions),
or sporadic control (such as the use of biocides) (Closs, Dean et al. 2004). The estimated annual economic cost of *D. geminata* to New Zealand is $24 million (Giera and Bell 2009).

The work of myself and others (Kilroy and Unwin 2011) has identified the likely invasion pathway of *D. geminata* in New Zealand. Kilroy and Unwin (2011) established that anglers are the most likely vectors, while Unwin (2009) documented their likely movements. In the second chapter of this thesis, I showed that spread occurred just as quickly across- as within- catchments, and that the rate of cross-catchment spread intensified following the infection of a key catchment. I further showed that the parameters defining the habitat window for colonisation were distance from the coast, mean summer air temperature, source of flow, and calcium and phosphorus in the underlying geology. This knowledge, previously unavailable in the literature, can be used to devise a *D. geminata* management plan for the North Island. This plan should aim to interrupt the invasion pathway by focussing on key catchments. Key catchments are those frequented by cross-boundary anglers in centralised or highly populated areas that fall within the habit window for colonisation identified in this thesis.

Trying to regulate *D. geminata* biomass in an infected river is significantly more problematic. In Chapter 4, I showed that the size of the standing crop determines accrual rates, resistance and recovery from floods. Low biomasses are more resistant to floods. At the same time, higher biomasses promote faster post flood recovery. This diminishes the utility of flushing flows as a biomass management option in regulated streams. Further, accrual rates are fastest in spring, the season in which flushing flows are more likely to occur in unregulated streams. Blooms occurred in the Kakanui River during periods of low flows and high temperatures. These results suggest that water abstraction for irrigation may need to be carefully managed to prevent extended periods of low flows in the hottest months of the year triggering *D. geminata* blooms.

5.7 Limitations

The major limitation of the work carried out in this thesis is that for the most part, it was observational. Experimental studies are needed to determine the critical values of the habitat parameters that drive the dynamic states of *D. geminata* (Cullis, Gillis et al.
2012). *D. geminata* has recently been successfully cultured in the laboratory for the first time, offering great potential for future research.

Nevertheless, my observational studies offered complementary insights. For example, I did not observe higher stalk biomass in lower nutrient concentrations. According to the findings of Kilroy and Bothwell (2011), I should have seen higher stalk biomass in lower nutrient conditions, and Cullis, Gillis et al. (2012) integrated this assumption into their proposed model of *D. geminata* growth in response to increasing nutrients. Because I did not observe this in natural systems, there must be additional physical controls on stalk length. My results indicate that the key controls are velocity and temperature. Although experiments are required to precisely determine cause and effect relationships, observational studies can be useful for determining natural impact.

Another limitation of this thesis is the use of relatively crude biomass estimates. I decided to use measures of accumulation because the habitat supporting mass accumulation was my primary concern. However, the results of the regional study (Chapter 3) would have been more robust if cell density counts and stalk length measures had been used instead of chlorophyll *a* and AFDM. The former would have taken considerably longer to process, however, meaning that less sites overall could have been included in the survey. I wanted to include as many sites as possible. Similarly, a better understanding of temperature and stalk dynamics would have been achieved in the Kakanui study (Chapter 4) if more precise measures of biomass had been used. In that study, only small amounts of variance in accumulation rates were able to be explained by the significant predictors. This could be at least partially explained by a failure to differentiate cell accumulation from stalk accumulation. Furthermore, to fully contribute to a mechanistic understanding of *D. geminata* biomass removal, the channel morphology and substrate stability of the Kakanui River sites needed to be identified and integrated with the biomass and flow data.

Throughout this thesis, I have made reference to non-peer reviewed research reports. While I tried to minimise this as much as possible, it does reflect the paucity of peer-reviewed reports of *D. geminata*’s invasion ecology in New Zealand. Finally, I used data collected by external agencies. The MPI-BNZ database is biased in terms of survey effort, and I bore this in mind when deciding on an analysis approach for Chapter 2. The Kakanui River data were collected by staff of the Department of
Conservation. This dataset is comparatively robust as the sampling programme and methods were devised through expert consultation (Kilroy 2008) and a strict protocol was followed throughout the data collection period. However, observer bias is likely to be present, as more than one person made the visual biomass estimates. The influence of this bias is likely to be random and non-differential so would only have weakened the observed associations.

5.8 Future research
This thesis focused on identifying D. geminata’s ecological niche in New Zealand. I identified the parameters that define D. geminata’s colonisation window, but not their critical values. An experiment using artificial channels containing substrata with different phosphorus and calcium levels should be conducted to validate or refute the findings of Chapter 2. It also remains to be elucidated whether it is water calcium or geological calcium that is important for D. geminata colonisation success. Temperature-controlled flumes should be used to examine colonisation and growth under different temperatures. The observed seasonality in mat structure should be investigated with more precise measures (such as cell density, cell division and stalk length). A factorial experiment using temperature and light controlled channels should be conducted to determine their relative importance in stalk development. Finally, relatively little peer-reviewed research has been published from New Zealand on the multi-trophic and ecosystem level impacts of D. geminata.

5.9 Concluding remarks
Streams subject to human disturbance have been the most heavily impacted by D. geminata in New Zealand. The impoundment of water for hydroelectric generation stabilises natural flow regimes, which has facilitated the development of massive biomasses in regulated systems, and in many cases, the presence of year-round D. geminata mats (Chapter 3). There was some indication that non-regulated rivers that are heavily utilised for irrigation also support high D. geminata biomass (Chapters 3–4). These observations accord with invasion theory regarding anthropogenic disturbance and the increased success of immigrant species (Mack, Simberloff et al. 2000).

Overall, it appears that D. geminata’s invasive success in New Zealand is because New Zealand offers ideal habit conditions; namely relatively low average temperatures and
nutrient concentrations, and sufficient calcium in many of its catchments. These habitat preferences mirror those in its native range and it does not appear to have extended its niche. Not all catchments have been invaded, including those that might have been expected to based on their proximity to infected streams. The lack of an incursion into the North Island is also encouraging, suggesting that education and perhaps unsuitable conditions are limiting its spread. The data in this thesis suggest a decreasing biomass over time in the Kakanui River. The impact of *D. geminata* in other Otago streams was very low (with a few exceptions). Range expansion has slowed over the past three years. Bloom events may even become rare, as they appear to have done in Vancouver (Bothwell, Lynch et al. 2009) and Iceland (Jonsson, Jonsson et al. 2007).

The *D. geminata* invasion in New Zealand was a tragedy that affected many of the attributes we associate with healthy rivers. However, relative to the impacts of poorly managed intensive urban and agricultural land-use, the impacts of *D. geminata* are far less severe. For example, a review of the literature on the land treatment of farm-dairy effluent in New Zealand and its impact on water quality identified that in all studies, the measured concentration of N and P in drainage water was higher than the ecological limits considered likely to stimulate unwanted aquatic weed growth (Houlbrooke et al. 2004). In recent years, the impact of agricultural land use on water quality has grown as a result of increased stocking rates and use of nitrogen fertilizers and a move away from low-intensity to high-intensity land use (Ministry for the Environment 2013). Managing those impacts presents a far greater challenge for New Zealand’s waterways.

The *D. geminata* experience demonstrates that New Zealand waterways are vulnerable to invasion, and that invasive species are aided in their spread by human action. From my research, I conclude that the best management approach for new invaders would be to focus not only on the match between available habitat and the invading species’ preferences, but also on vector movements between suitable habitats. Indeed, human behaviour change campaigns have been a key outcome of the *D. geminata* invasion in New Zealand.
References


