Polyphenols extracts from *Didymosphenia geminata* (Lyngbye) Schmidt altered the motility and viability of *Daphnia magna*

Pamela Olivares-Ferretti · Viviana Chavez · Klaudia Hernandez · Matías Peredo-Parada · Jorge Parodi

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Abstract The invasive diatom *Didymosphenia geminata* (Lyngbye) Schmidt, *D. geminata* has invaded the austral zone of Chile, causing significant ecological, scientific and societal concerns. We aimed to evaluate the viability and motility of *Daphnia magna* (*D. magna*), as a biosensor for effects of *D. geminata*. Toxicity assays were performed in dilutions of river water alone (V/V dilution) and in river water contaminated with *D. geminata* (V/V dilution) or polyphenols extracted from *D. geminata* under controlled conditions and different time (acute 30 min and 7 h). Our results indicated that *D. magna* was sensitive to increasing concentrations of *D. geminata* extracts. We observed a 50% (IC$_{50}$) viability reduction after 24 h of exposure to a 0.023 V/V dilution and the same value when using polyphenols from *D. geminata*; additionally, this treatment further reduced the motility capacity by 50% after 72 h. The *D. magna* organisms were acutely responsive, showing a 50% reduction in frequency at 15 min. We conclude that *D. magna* is sensitive to polyphenols produced by *D. geminata* in rivers, suggesting potential chronic toxic consequences on several aquatic species following exposure to these diatom substances.

Keywords Didymo · Daphnia · Viability

Introduction

Biological invasions of non-native species are a significant threat to biodiversity (Firn et al. 2015; Gurevitch and Padilla 2004) and socio-ecological systems (Taylor and Bothwell 2014). In Austral native freshwater ecosystems, the recent proliferation of *Didymosphenia geminata* (Lyngbye) Schmidt (*D. geminata*) has become a significant concern because of the effect on the ecosystem services they provide (Reid and Torres 2014; Strayer 2010; Taylor and Bothwell 2014). *D. geminata* blooms beat oligotrophic
aquatic systems for several hundred kilometres (Montecino et al. 2014; Pinto Torres et al. 2016). It altered the physicochemical conditions of the river and the benthic fauna distribution, in particular macroinvertebrate communities (Brand and Grech 2020). It produced negative ecological and economic consequences (Alpert et al. 2000; Beville et al. 2012; Taylor and Bothwell 2014). Considerable attention has been paid to this D. geminata problem, as it has invaded more than 187 rivers in many other countries (Blanco and Ector 2009; Gretz et al. 2007). This benthic diatom has been declaring an invasive species in Southern Hemisphere countries such as New Zealand (Kilroy and Unwin 2011), Argentina (Bothwell et al. 2014) and Chile (Reid and Torres 2014) since 2010 (Segura 2011). Under favourable environmental conditions, it is capable of producing blooms with large amounts of extracellular stalks in a short period and colonises far from its geographical range (Bishop and Spaulding 2017; Cullis et al. 2012). In the Southern Hemisphere, D. geminata has shown more aggressive performance, with a considerable impact due to the extensive formation of biomass (Kilroy et al. 2009), which is likely caused by the favourable climate and physicochemical water conditions (Kunza et al. 2018), allowing it to bloom over the river rocks or be present but not invading in the planktonic phase (Montecino et al. 2016).

The most commonly described D. geminata impacts are physicochemical changes in the watercourses in oligotrophic locations, including substantial increases in algal biomass, the retention of fine sediment, and benthic hydrodynamic alterations, which consequently affect biogeochemical states and processes such as redox conditions, pH and nutrient cycling in the benthic layer (Reid et al. 2012). Other impacts are described in the periphyton biomass and benthic communities, showing higher tolerant invertebrate groups densities such as Oligochaeta Chironomidae, Cladocera and Nematoda, Orthocladiinae and also non-insect taxa (Brand and Grech 2020; Kilroy et al. 2009).

The toxic impacts of this microalga start with the alteration of microenvironments and microalgal communities according to the seasonal variations of D. geminata (Chester and Norris 2006; Figueroa et al. 2018) and the possibility of these microalgae spreading to new bodies of water (Montecino et al. 2014; Reid et al. 2012) through different vectors (Leone et al. 2014). More observational and experimental work suggests that D. geminata is causing direct and indirect effects through the whole food web (Olivares et al. 2015; Parodi et al. 2015), during periphyton blooms (Chester and Norris 2006; Suren et al. 2003) with D. geminata (Kilroy et al. 2009) as well as after its removal (Larned and Kilroy 2014).

D. geminata toxicological potential to affect other organisms remains unknown. Moreover, this pennate microalga is rich in antioxidants such as polyphenols and pigments including diadinoxanthin (Lohr and Wilhelm 1999), which was also reported as D. geminata polyphenol toxicity on two salmonid species cell lines (Olivares-Ferretti et al. 2019) and Salmo salar spermatozoa activation times (Olivares et al. 2015), in this previous work the polyphenol are suggested the mechanism for generated toxic effect. In a large-scale ecological change in freshwater ecosystems, perturbations to macroscopic organisms are often documented (Ricciardi and MacIsaac 2000), suggesting the possibility that D. geminata polyphenol toxicity affected the behaviour of benthic bioindicator organisms like Daphnia magna (D. magna) and used this model to explore the toxicity (Kim et al. 2003), as an excellent tool for assessment of this problem (Jellyman et al. 2011). D. magna, due to its short life cycle, morphological characteristics and reproductive capacity, is a suitable organism for the study of toxins in aquatic environments (Baun et al. 2008; Gerhardt 2007). Our study aimed to elucidate the toxicity levels and effects of D. geminata polyphenols on D. magna, like new step in the trophic chain in aquatic ecosystem, measured viability and motility using the frequency of movement as a parameter for evidence of sublethal effects.

Methods

Sample’s collection

Rock and water samples, control without D. geminata and with 12 round flat stones and with D. geminata were collected from each of three rivers from central and south Chile during the autumn and winter of 2019 as follows: Bio-Bio River, W318358, N5718384, W304806a and Point Lamin, W307366, N5713357N5713357; Espolón River at Futaleufú Point, W2667125, N52413723; Futaleufú River at

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Yelcho Point, W269107, N5213938. The samples were provided by the Chilean National Fishery Services (SERNAPESCA). After collection, the samples were immediately transported to the LaBCEMA laboratory, Universidad Mayor Temuco. A mixture of water and rocks with *D. geminata* from each river was stored in coolers and maintained at 4 °C until arrival at the laboratory.

*D. geminata* laboratory maintenance

The samples were distributed in an implemented “artificial river” recirculation system (Parodi et al. 2015) using control water, without *D. geminata* samples and water contaminated to generate a closed system of water contaminated with *D. geminata* for six months of collection according to the SERNAPESCA biosafety protocol for laboratory assays (Authorisation No. 3500). We followed the methodology standardised by Parodi et al. (2015). Briefly, the artificial river system was prepared by mixing 50% original river water from each of the different collection points, with 50% distilled water for a total volume of 14 L, leaving a 15 cm water column above the rocks. Artificial rivers from each location were maintained under a controlled temperature of 12 °C using an expanded polystyrene insulating cover and a refrigerating gel system. The flow rates (1200 L/h) were controlled using a Plaset-Italy Model 71,009 recirculation engine, which maintained a steady flow and aeration. Macroscopic and microscopic changes in the artificial river systems were recorded daily for six months each year, as compared with initial time, like a previous report of our group. The viability of the *D. geminata* population was observed with neutral red staining, and enough material was cultured to generate a 1 g polyphenol extract from 10 ml of wet *D. geminata* for use in the subsequent procedures and experiments.

Polyphenol extraction and liquid chromatography (HPLC) peak detection

In previous reports, we used the follow protocol (Olivares-Ferretti et al. 2019). A total composed wet *D. geminata* sample (10 ml) was obtained from two to five rocks collected (*n* = 12) and maintained in a single artificial river; no cultures of the sample were used, only fresh material was used, and it was exposed to liquid nitrogen. Samples from each point (10 ml) were macerated, and the cell frustules were ruptured by sonication (Misonix XL2000 Series) in 30-min pulses with one-minute intervals until the biomass (even complex samples) was wholly homogenised. A total of 10 ml distilled water was then added to the macerated samples, and the samples were collected in 15-ml tubes. The tubes were then incubated at 30 °C under agitation for 20 min, filtered through double gauze and a Whatman No. 2 (125 mm) filter, and collected in a 20-ml glass flask (following Jofre-Fernandez et al. 2013). Finally, polyphenol detection (Total antioxidant measurement) was performed using a total of 1 g of extract. Samples were diluted with Folin-Ciocalteu reagent following the protocol described by Lowry (Lowry et al. 1951), for measured protein, but used for polyphenol detection, and the polyphenol absorbance was measured at 517 nm. Finally, total of 12 samples per year were frozen to avoid degradation until analysis by the HPLC service at the Universidad Austral de Chile to identify their profiles (Lohr and Wilhelm 1999) for detection of polyphenol in the sample. The presence of organic compounds was detected in the yellow fraction of the samples. A total of 5 different extracts from different collection points were used for the retention time measurements. A description of the antioxidant profiles of the samples and the absorbance at 440 nm with the AC18 column were determined (Macherey–Nagel, Duren, Germany).

*Daphnia magna* laboratory maintenance

*D. magna* specimens were used according to the NCh 2083 standard: With constant temperature 16 °C, with a light/dark cycle (12 h/12 h). The culture water for *D. magna* was prepared with 25 ml saline solutions (calcium chloride: 11.76 gr/L; magnesium sulphate: 4.93 gr/L; sodium bicarbonate: 2.59 gr/L; potassium chloride: 0.23 gr/L) and filled up to 1000 ml with distilled filtered water. Finally, the water was aerated for 24 h before use. The growing culture density was from 10 to 15 *D. magna* organisms per 200 ml of water. *D. magna* were fed with 5 ml of the microalgae *Chlorella vulgaris* and *Selenastrum capricornutum* (*Raphidocelis subcapitata*) 30 ml/L per 500 ml of culture (25–35 *D. magna*) every 2 days. The approximate life cycle of *D. magna* is 3 to 4 months. The time
to mature from juveniles to adults is approximately 15 days.

**D. magna viability**

*D. magna* specimens from at least the third generation were obtained by acyclic parthenogenesis under specific growing conditions described previously. Then, neonates of *D. magna* organisms used in the test were collected by filtration through a sieve (opening size of 560-μm for *D. magna*) or separated manually within 24 h of birth. Next, ten specimens for conditioning were split into an individual glass with 50 ml of artificial river water with oxygen. The specimens were exposed to the condition for 24 to 72 h, and the mobile forms for each condition were recounted for an indication of viability after this incubation time. The control needed to show viability more significant than 80% to be considered correct for studying the viability of the different conditions. Nine independent experiments were made to describe the viability of *D. magna* in different experimental conditions.

**D. magna motility**

The motility experiment was done using fresh *D. magna*, taking the culture and depositing it in a volume of 1 ml of artificial river water; 5 adults of *D. magna* were used in 9 different experiments for all the experimental conditions, and we used 100 specimens in the total study of motility. We performed acute exposures, in mediated style with 30 min of incubation or chronic style with 72 h of incubation, to the different conditions. We measured the absorbance at 328 nm on a Peak C-7100 Series instrument spectrophotometer. The absorbance peaks were continuously recorded for two minutes, as an indirect indication of the passage of the *D. magna* versus the light emission. The number of peaks per unit of time was counted, and the frequency of events was calculated as an indication of the motility event. Under these conditions, five adults were exposed to increasing concentrations of V/V of river water contaminated with *D. geminata*, and we used a river water without *D. geminata* as control. Increasing concentrations of polyphenols extracted from *D. geminata* between 5 and 500 ppm were used, and gallic acid (1 to 1000 ppm) was added to an artificial river water solution, as positive control.

**Data analysis**

Unless otherwise indicated, the results, including image analysis, are presented as the means ± SEM. The curve is made with log (inhibitor) vs. normalized response, and we obtained the IC$_{50}$ from this equation. Statistical comparisons were performed using Student’s t-test or ANOVA, and Bonferroni post hoc test was applied with the software GraphPad prism 4. A probability level (p) of less than 0.05 was considered statistically significant.

**Results**

*D. geminata*-contaminated water effects on *D. magna* viability

The newborn and adult forms of *D. magna* were exposed to dilution by water contaminated with *D. geminata* and polyphenol extract from *D. geminata* and gallic acid. Figure 1a shows a curve of the concentration dilution V/V of the river water (Negative Control) or contaminated with *D. geminata*. The figure shows a reduction of over 50% in the percentage of life when the concentration of the river water contaminated with *D. geminata* is increased, with an IC$_{50}$ of 0.023 V/V. We explored the effect of polyphenols obtained from *D. geminata* and observed a decrease in viability (Fig. 1b) when the polyphenols were increased, with an IC$_{50}$ of 52 ppm. We compared the effect with a polyphenol standard, i.e. gallic acid (Positive control). Figure 1b shows the inhibition of viability when the concentration is increased, with an IC$_{50}$ of 4.5 ppm. Finally, we exposed *D. magna* to the IC$_{50}$ values in a chronic manner from the previous experiment, 72 h of incubation (Fig. 1c), and we observed a reduction in viability over 50% when *D. magna* was exposed to river water with *D. geminata*, a polyphenol from *D. geminata* or gallic acid.

Effect of *D. geminata*-contaminated water on *D. magna* motility

We explored the impact of the polyphenol obtained from *D. geminata* on the motility of *D. magna*. In
Fig. 2a, we show example traces of the motility observation of *D. magna* in the spectrophotometer; the absorbances are expressed in Y and time in X, the number of events is between 10 to 15 in the control condition are reduced to two events in the experimental condition. The quantification of the motility is shown in Fig. 2b when *D. magna* are exposed to increased concentrations of the *D. geminata* polyphenol extract; reduction form 0.1 Hrs to 0.05 Hz. The figure shows a reduction in the frequency of the peak when the concentration of polyphenol increases above 50 ppm, with an IC$_{50}$ of 52 ppm. We used this concentration to explore the time effect. We exposed the samples to 52 ppm of polyphenol extract from *D. geminata* at different times. In Fig. 2c, we show example traces of the absorbance peak, and the relation between absorbance and time. Figure 2d shows the quantification of motility at different times. We observed that 52 ppm did not induce a change in the frequency of events when used acutely; however, when *D. magna* were incubated longer than 5 min, the frequency of activity was reduced.

Effect of *D. geminata*-contaminated water on *D. magna* motility after acute incubation

We used the previous IC$_{50}$ value from water contaminated with *D. geminata* (0.023 V/V) polyphenol extract (52 ppm) and gallic acid (4.5 ppm) to observe the change in the frequency of *D. magna* after incubation for 30 min. Figure 3a shows examples of trace absorption changes in the relation between absorbance and time. Figure 3b shows motility quantification, and we observed a reduction of over 40% in the frequency when the *D. magna* samples were exposed to the different compounds. River water contaminated with *D. geminata* reduced the frequency by over 40%, and the effects of the polyphenol extract from *D. geminata* and gallic acid were similar.

Effect of *D. geminata*-contaminated water on *D. magna* motility after chronic incubation

We experimented again using the previous IC$_{50}$ value from water contaminated with *D. geminata* (0.023 V/V) polyphenol extract (52 ppm) and gallic acid (4.5 ppm) to observe the change in the frequency of *D. magna* after incubation for 72 h under different conditions, control, Negative control (River water) water contaminated (Didymo) to polyphenol extract (poly didymo) or gallic acid (Gallic). Each bar or point represents the measurements from at least 5 independent cultures and experiments (mean ± SEM). The asterisk indicates $p < 0.05$ (ANOVA).
polyphenol extract (52 ppm) and gallic acid (4.5 ppm) to observe the changes in the frequency of \textit{D. magna}; however, we increased the incubation time to 72 h to observe the chronic effects. The values were similar to those in the previous figure. Figure 4a shows examples of traces of the absorption change. The relation between absorbance and time also shows an event over 10 when the control condition is observed.

\textbf{Fig. 2} Effect of polyphenols on \textit{D. magna} motility. \textbf{a} shows the effect on the motility record of \textit{D. magna} after acute exposure to different concentrations of polyphenols (poly didymo) from \textit{D. geminata}. \textbf{b} shows the dot graph of the motility record of \textit{D. magna} after acute exposure to different concentrations of polyphenols (poly didymo) from \textit{D. geminata}. \textbf{c} shows the effect on the motility record of \textit{D. magna} after different times of exposure to polyphenols from \textit{D. geminata}. \textbf{d} shows the dot graph of the motility record of \textit{D. magna} after different times of exposure to polyphenols (poly didymo) from \textit{D. geminata}. Each point represents the measurements from at least 5 independent cultures and experiments (mean ± SEM). The asterisk indicates \( p < 0.05 \) (ANOVA).
and reduction to 5 events when polyphenols were used. Figure 4b shows the quantification of motility, and we can observe over 50% of reduction in the frequency when D. magna samples were exposed to the different compounds. River water contaminated with D. geminata decreased almost 50% of the frequency, and the effects of the polyphenol extract from D. geminata and gallic acid were similar.

Discussion

Our results presented in this work continue the results in previous studies about D. geminata toxicity and suggest the polyphenols present in the river water contaminated with the microalgae induce cell alteration, cellular death and now reduction in D. magna viability. The data present suggested a complex mechanism of the toxicity on the river, when are contaminated with D. geminata, and more future experiments are required for explain the pathway of this process. Since the introduction of D. geminata in local freshwater ecosystems, it has been a cause of concern to potential consequences on stream communities (Ladrera et al. 2018). In attempts to understand the D. geminata toxicity in a small organism, we have studied its effect on a standardised model. In our previous group research (Olivares et al. 2015), we proposed that the toxic effects are secondary to the presence of polyphenols in river water contaminated with D. geminata. Our results suggest that water contaminated with D. geminata can be mediated to reduce effects on D. magna viability and motility by the presence of polyphenols, and in recent work, we
suggested that toxic effects at the cellular level are secondary to the present of polyphenols present in the river contaminated with \textit{D. geminata} (Olivares-Ferretti et al. 2019). The experiment design in the present work utilised the \textit{D. magna} standardised model to evaluate the effects of artificial river water contaminated with \textit{D. geminata}, which was previously reported (Parodi et al. 2015). The water samples included the presence of a biological compound that has been observed in \textit{D. geminata} cells, where the pigment was measured at 440 nm identified by HPLC (Olivares-Ferretti et al. 2019) and is present in our samples. Previous studies have reported the \textit{D. geminata} effects on aquatic organisms and spermatozoa (Brand and Grech 2020; Larned and Kilroy 2014; Olivares et al. 2015). Viability observations in river water with increasing V/V concentrations resulted in typical \textit{D. magna} values; however, the level of \textit{D. magna} mortality increased when the river water was contaminated with \textit{D. geminata} (Fig. 1a). We observed the lethal doses of river water contaminated with \textit{D. geminata} and polyphenols from \textit{D. geminata} and used a standard, gallic acid (Fig. 1b). It has been reported that due to the presence of epiphytic cyanobacteria in blooms, which are microcystin promoters (Whitton et al. 2009), higher microcystin dosage on \textit{D. magna} caused chronic toxicity (Chen et al. 2005). Our results suggest that river contamination with \textit{D. geminata} may have a chronic effect on aquatic organisms (Fig. 1c).

However, we followed the idea of subtoxic effects, and in particular, we explored a mechanism to explain the deadly impact. We evaluated \textit{D. magna} motility using its endogenous fluorescence particularity by a UV light protocol, recording the absorbance of the solution, not explored the ionic change, has previous

Fig. 4 The chronic effect of different polyphenols on \textit{D. magna} motility. \textbf{a} shows the effect on the motility record of \textit{D. magna} after chronic exposure under different experimental conditions. \textbf{b} shows the bar graph of the motility record of \textit{D. magna} after chronic exposure under different experimental conditions, control, Negative control (River water) water contaminated (Didymo) to polyphenol extract (poly didymo) or gallic acid (Galic). Each bar represents measurements from at least 5 independent experiments (mean ± SEM). The asterisk indicates $p < 0.05$ (ANOVA).
report on the model, only follow the kinetic and suggest a mechanism secondary to concentration of polyphenols on the solution (Teplova et al. 2010). The traces obtained from the spectrophotometer (Fig. 2a and c) show every change registered, considering the peaks as a movement of D. magna. We observed a reduced D. magna motility when the concentration of the polyphenol extracts from D. geminata was increased in an acute manner (Fig. 2b). An increasing basic metabolism in D. magna has been reported to counteract a prolonged exposure of cytotoxins in freshwater ecosystems, and help to suggested a complex model of receptors on the model, we not explored if the polyphenols increased signalling has in previous report, but we indicated relation of the motility and the doses of polyphenols (Grzesiuk et al. 2018). Our results showed reduced motility when D. magna was incubated with polyphenol DL50 for a longer period of time (Fig. 2d). We suggest that D. geminata and the polyphenol produced by D. geminata alter motility in D. magna, and this can led to reduced viability in longer exposition, like in natural river.

The toxicity biotests were carried out by checking organism parameters (Walker et al. 2012). We recorded whether this effect occurred when river water contaminated with D. geminata. A standard sample of polyphenols and acid gallic was used (Maheshwari et al. 2017) at acute and chronic levels. Figure 3 shows a reduction on the D. magna motility in all the conditions; likewise, gallic acid had a stronger effect on motility, supporting the idea that the polyphenols had an effect on this parameter. A hypolocomotion response to water-born toxicants in D. magna has been described (Huang et al. 2017). Our data suggest an acute impact on motility mediated by the polyphenol present in the river water contaminated with D. geminata.

Anthropogenic influence generates biodiversity loss by increasing toxins in natural freshwater (Tickner et al. 2020), allowing us to consider the possibility of toxic components from the D. geminata invasion. Our data show reduced D. magna motility without a more substantial impact, indicating that the chronic effects at sublethal doses were the same as those observed in the acute experiment, which suggests that the effects on viability are more complex and future experiment help to explain a pathway of this effect observed (Fig. 4). D. geminata changes in macroinvertebrate composition blooms have been reported to show a decreased density in determinate trophic groups (Larned and Kitroy 2014; Whitton et al. 2009). However, the D. magna enzymatic metabolism increase to chronic toxicity adaptation mechanism has also been described (Chen et al. 2005). These effects are of great interest when considering whether the same effects are noted in the cell lines of native freshwater species, other species or biological models when representing a bioindicator for water quality (Venugopal 2002) and when evaluating whether D. geminata contamination can induce changes in river biota (Cifuentes et al. 2012; Montoya et al. 2012). Furthermore, there are few records of the biota or species being affected by microalgae in Chilean rivers or other places (Ladrera et al. 2018), representing a lack of research on the effects of D. geminata in cell models or using native river species. For example, a recent study indicated a change in the microalgae composition when D. geminata was present in a river (Zamorano et al. 2019).

Conclusion

Our study aimed to assess the harmful effects of D. geminata at the macroinvertebrate level, as well as the other effects of this diatom as observed in a previous report from our group. Our data propose that at sublethal concentrations, the presence of D. geminata polyphenols affects the viability of D. magna, due to inducing a reduction of the motility clouding when long-term exposure occurs. We also suggest a mechanism to explain toxicity among macroinvertebrates present in rivers contaminated with D. geminata. Our results suggest toxic and complicated implications of D. geminata contamination; specifically, the effect of polyphenols in D. geminata had a direct impact on macroinvertebrates in rivers and finally affected all the biota of the river.

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Declarations

Conflict of interest  The authors declare that they have no conflicts of interest.

Ethical approval  This article does not include any studies with animals or human participants performed by any of the authors.

Human and animal rights  No animals or humans were used in the present work.

References

Alpert P, Bone E, Holzapfel C (2000) Invasiveness, invasibility and the role of environmental stress in the spread of non-native plants. Perspect Plant Ecol Syst 3:52–66
Baun A, Hartmann NB, Grieger K, Kusk KO (2008) Ecotoxicity of engineered nanoparticles to aquatic invertebrates: a brief review and recommendations for future toxicity testing. Ecotoxicology 17:387–395. https://doi.org/10.1007/s10646-008-0208-y
Beville ST, Kerr GN, Hughey KFD (2012) Valuing impacts of the invasive alga *Didymosphenia geminata* on recreational angling. Ecol Econ 82:1–10. https://doi.org/10.1016/j.ecolecon.2012.08.004
Bishop I, Spaulding S (2017) Life cycle size dynamics in *Didymosphenia geminata* (Bacillariophyceae). J Phycol 53:652–663
Blanco S, Ector L (2009) Distribution, ecology and nuisance effects of the freshwater invasive diatom *Didymosphenia geminata* (Lyngbya) M. Schmidt: a literature review. Nova Hedwigia 88:347–422
Bothwell M, Taylor B, Kilroy C (2014) The Didymo story: the role of low dissolved phosphorus in the formation of *Didymosphenia geminata* blooms. Diatoma Res 29:229–236
Brand C, Grech M (2020) Recent invasion of *Didymosphenia geminata* (Lyngbya) M. Schmidt in a Patagonian regulated river promotes changes in composition and density of macroinvertebrate community. Biol Invasions 22:1903–1915. https://doi.org/10.1007/s10530-020-02230-8
Chen W, Song L, Ou D, Gan N (2005) Chronic toxicity and responses of several important enzymes in *Daphnia magna* on exposure to sublethal microcystin-LR. Environ Toxicol 20:323–330. https://doi.org/10.1002/tox.20108
Chester H, Norris R (2006) Dams and flow in the Cotter River, Australia: effects on instream trophic structure and benthic metabolism. Hydrobiologia 572:275–286
Cifuentes R, González J, Montoya G, Jara A, Ortíz N, Piedra P, Habit E (2012) Relación longitud-peso y factor de condición de los peces nativos del río San Pedro (cuenca del río Valdivia, Chile). Gayana (concepción) 76:86–100
Cullis J, Gillis C, Bothwell M, Kilroy C, Packman A, Hassan M (2012) A conceptual model for the blooming behavior and persistence of the benthic mat-forming diatom *Didymosphenia geminata* in oligotrophic streams. J Geophys Res. https://doi.org/10.1029/2011JG001891
Figueroa F, Pedreros P, Cruces F, Abdala-Díaz R, Hernández V, Becerra J, Urrutia R (2018) Effect of Didymosphenia geminata coverage on the phytobenthic community in an Andean basin of Chile. Rev Chil Hist Nat. https://doi.org/10.1186/s40693-018-0080-y
Firn J et al (2015) Priority threat management of invasive animals to protect biodiversity under climate change. Glob Chang Biol 21:3917–3930
Gerhardt A (2007) Aquatic behavioral ecotoxicology—prospects and limitations. Human Ecol Risk Assess Int J 13:481–491. https://doi.org/10.1080/10807030701340839
Gretz MR, Riccio ML, Kiemle SN, Domozych DS, Spaulding SA (2007) Didymosphenia geminata as a nuisance diatom: runaway stalk production results in mats with significant environmental impact. J Phycol 43:16–16
Grzesiuk M, Mielecki D, Pilzys T, Garbizc D, Marcinkowski M, Grzesiuk E (2018) How cyclophosphamide at environmentally relevant concentration influences *Daphnia magna* life history and its proteome. PLoS ONE 13:e0195366. https://doi.org/10.1371/journal.pone.0195366
Gurevitch J, Padilla D (2004) Are invasive species a major cause of extinctions? Trends Ecol Evol 19:470–474
Huang Y, Campana O, Włodkowic D (2017) A millifluidic system for analysis of *Daphnia magna* locomotory responses to water-born toxicants. Sci Rep 7:17603. https://doi.org/10.1038/s41598-017-17892-z
Jellyman PG, Clearwater SJ, Clayton JS, Kilroy C, Blair N, Hickey CW, Biggs BJF (2011) Controlling the invasive diatom *Didymosphenia geminata*: an ecotoxicity assessment of four potential biocides. Arch Environ Contam Toxicol 61:115–127. https://doi.org/10.1007/s00244-010-9589-z
Kilroy C, Unwin M (2011) The arrival and spread of the bloom-forming, freshwater diatom, *Didymosphenia geminata*, in New Zealand. Aquat Invasions 6:249–262
Kilroy C, Larned S, Biggs B (2009) The non-indigenous diatom *Didymosphenia geminata* alters benthic communities in New Zealand rivers. Freshw Biol 54:1990–2002. https://doi.org/10.1111/j.1365-2427.2009.02247.x
Kim BC, Park KS, Kim SD, Gu MB (2003) Evaluation of a high throughput toxicity biosensor and comparison with a *Daphnia magna* bioassay. Biosens Bioelectron 18:821–826. https://doi.org/10.1016/S0956-5663(03)00027-7
Kunza LA, Gillis CA, Haueter JZ, Murdoch JN, O’Brien JCM (2018) Declining phosphorus as a potential driver for the onset of *Didymosphenia geminata* mats in North American Rivers. River Res Appl 34:1105–1110
Ladrera R, Goma J, Prat N (2018) Effects of Didymosphenia geminata massive growth on stream communities: smaller organisms and simplified food web structure. Plos ONE 13
Larned S, Kilroy C (2014) Effects of Didymosphenia geminata removal on river macroinvertebrate communities. J Freshw Ecol 29:345–362
Leone PB, Cerda J, Sala S, Reid B (2014) Mink (Neovison vison) as a natural vector in the dispersal of the diatom *Didymosphenia geminata*. Diatoma Res 29:259–266. https://doi.org/10.1080/0269249X.2014.890957
Lohr M, Wilhelm C (1999) Algae displaying the diadinoxanthin cycle also possess the violaxanthin cycle. Proc Natl Acad Sci U S A 96:8784–8789
Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Maheshwari R et al (2017) Characterization of the phenolic compound, gallic acid from Sansevieria roxburghiana Schult and Schult. f. Rhizomes and antioxidant and cytotoxic activities evaluation. Pharmacogn Mag 13:S693–S699. https://doi.org/10.4103/pm.pm_497_16

Montecino V, Molina X, Kumar S, Castillo M, Bustamante R (2014) Niche dynamics and potential geographic distribution of Didymosphenia geminata (Lyngbye) M. Schmidt, an invasive freshwater diatom in Southern Chile. Aquat Invasions 9:507–519

Montecino V et al (2016) Spatio temporal population dynamics of the invasive diatom Didymosphenia geminata in central-southern Chilean rivers. Sci Total Environ 568:1135–1145. https://doi.org/10.1016/j.scitotenv.2016.03.080

Montoya G, Jara A, Solís-Lufí K, Colin N, Habit E (2012) Primeros estadios del ciclo de vida de peces nativos del Río San Pedro (Cuenca del Río Valdivia, Chile). Gayana (concepción) 76:86–100

Olivares P, Orellana P, Guerra G, Peredo-Parada M, Chavez V, Ramirez A, Parodi J (2015) Water contaminated with Didymosphenia geminata generates changes in Salmo salar spermatozoa activation times. Aquat Toxicol 163:102–108

Olivares-Ferretti P, Hernandez K, Peredo-Parada M, Chavez V, Carmona E, Astuya A, Parodi J (2019) Polyphenols obtained from Didymosphenia geminata (Lyngbye) Schmidt altered the viability and proliferation of salmonids cells lines SHK-1 and CHSE-214. Aquatic Toxicol 211:141–147. https://doi.org/10.1016/j.aquatox.2019.03.022

Parodi J, Olivares P, Chavez V, Peredo-Parada M (2015) Laboratory handling of Didymosphenia geminata (Lyngbye) Schmidt and the effect of control efforts on viability. Adv Biosci Biotechnol 06:508–516. https://doi.org/10.4236/abb.2015.68053

Pinto Torres M, Frangopulos M, Ruiz S, Quilahuilque G, Mora C (2016) Relationship between viable cells transport of Didymosphenia geminata and other invasive species in Tierra del Fuego Island. Chile. https://doi.org/10.13140/RG.2.2.14529.76642

Reid B, Torres R (2014) Didymosphenia geminata invasion in South America: ecosystem impacts and potential biogeochemical state change in Patagonian rivers. Acta Oecologica 54:101–109

Reid B, Hernandez K, Frangopulos M, Bauer G, Lorca M, Kilroy C, Spaulding S (2012) The invasion of the freshwater diatom Didymosphenia geminata in Patagonia: prospects, strategies, and implications for biosecurity of invasive microorganisms in continental waters. Conserv Lett 5:432–440

Ricciardi A, MaIsaac HJ (2000) Recent mass invasion of the North American Great Lakes by Ponto-Caspian species. Trends Ecol Evol 15:62–65. https://doi.org/10.1016/S0169-5347(99)01745-0

Segura P (2011) A slimy invader blooms in the rivers of Patagonia. Science 331:18–18. https://doi.org/10.1126/science.331.6013.18

Strayer D (2010) Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. Freshwater Biol 55:152–174

Suren A, Biggs B, Duncan M, Bergey L (2003) Benthic community dynamics during summer low-flows in two rivers of contrasting enrichment 2. Invertebrates. N Z J Marine Freshwater Res 37:71–83

Taylor BW, Bothwell ML (2014) The origin of invasive microorganisms matters for science, policy, and management: the case of Didymosphenia geminata. Bioscience 64:531–538. https://doi.org/10.1093/biosci/biu060

Teplova VV, Andreeva-Kovalevskaya ZI, Sineva EV, Solomin AS (2010) Quick assessment of cytotoxins effect on Daphnia magna using in vivo fluorescence microscopy. Environ Toxicol Chem 29:1345–1348. https://doi.org/10.1002/etc.169

Tickner D et al (2020) Bending the curve of global freshwater biodiversity loss: an emergency recovery plan. Bioscience 70:330–342. https://doi.org/10.1093/biosci/biaa002

Venugopal V (2002) Biosensors in fish production and quality control. Biosens Bioelectron 17:147–157

Walker C, Sibly R, Hopkin S, Peakall D (2012) Principles of Ecotoxicology. 4th edn., Boca Raton

Whitton B, Ellwood N, Kawecka B (2009) Biology of the freshwater diatom Didymosphenia: a review. Hydrobiologia 630:1–37

Zamorano D, Peredo-Parada M, Lillo DJ, Parodi J, Diaz CA (2019) Mat thickness associated with Didymosphenia geminata and Cymbella spp. in the southern rivers of Chile. PeerJ. https://doi.org/10.7717/peerj.6481

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