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Short Communication

Spectral characterization of *Didymosphenia geminata* under laboratory conditions: bases for a monitoring and early warning system in river systems of south central Chile

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Abstract

The effects of climate change and the severe anthropization of local river systems have contributed to the alteration of ecological processes, affecting the water quality in these systems and thus generating conditions for the emergence of algal species. In this context, the object of the present study was to evaluate the potential of remote detection techniques to obtain a spectral characterization of *D. geminata* under controlled laboratory conditions. This would provide the basic information for the design and implementation of a monitoring and early warning system. *D. geminata* cells obtained from two southern Chilean river systems were cultivated in the laboratory and analysed using hyperspectral techniques to construct their spectral signatures. The results showed the feasibility of distinguishing between the presence and absence of *D. geminata* when it occurs in association with other diatom present in the environmental. The results could be the first step towards the design and implementation of a monitoring and early warning system to facilitate existing inspection activities.

Key words: diatom, invasive algae, hyperspectral analysis, spectrometer, remote sensing

Introduction

Didymosphenia geminata (Lyngbye) M. Schmidt is a benthic freshwater diatom originally from the northern hemisphere (Reid et al. 2012; Jaramillo et al. 2015). This diatom is capable under certain conditions of aggressive invasion of oligotrophic river systems (Whitton et al. 2009; Spaulding et al. 2010; Jaramillo et al. 2015). The river invasion capability of this species translates into rapid proliferation and the formation of very large mucilaginous colonies, constituting a threat to the conservation of freshwater ecosystems worldwide (Spaulding and Elwell 2007). In the southern hemisphere it was detected in New Zealand in 2004 (Kilroy et al. 2009; Jaramillo et al. 2015) and to date it has colonised high latitudes, for example river systems in the Patagonia region of southern Chile and Argentina (Reid et al. 2012; Reid and Torres 2014; Jaramillo et al. 2015).

In Chile, *D. geminata*—commonly known as *Didymo*—was first detected in the 1960s in the Aysén and Magallanes Regions, specifically Cisnes River and Sarmiento Lake (Asprey et al. 1964). In 2010 it was detected in the Futaleufú River and since then it has spread into numerous rivers in the south central part of the country (Segura 2011). This situation has caused uncertainty among the population as to its potential effects on human health and on the various ecosystem services associated with river basins (Reid and Torres 2014). The most sensitive goods and services include tourism, water sports, sport fishing and the aquaculture industry. In view of this situation,

the Chilean State has promoted a monitoring plan to detect the presence of *D. geminata* (Díaz et al. 2012), based on standardised methodologies for analysing phytoplankton and periphyton. However these efforts require high investment in man-hours, both in the field and in the laboratory, reducing the efficiency of the plan. This has led to a search for viable monitoring alternatives, for example methods based on remote detection by satellite (Jensen 1996; Adams and Gillespie 2006; Gao 2009; Chuvieco 2010).

Remote detection techniques give access to remote areas, on different spatial scales, allowing information to be collected quickly and efficiently at a reasonable cost for the producers and the monitoring bodies (Adams and Gillespie 2006; Gao 2009; Chuvieco 2010). Today, since the launch of the Chilean satellite Fasat-Charlie in 2011, there is growing demand in Chile for the use of technologies based on multispectral remote sensors (Jensen 1996; Gao 2009). The basic principle of these systems is the fact that all bodies reflect or emit energy flows in the form of radiation (Gao 2009). Thus the physiological, phenological and structural condition of plants in general, and algae in particular, can be characterised by identifying and analysing the signals produced by their structures through the unique, characteristic spectral responses of each body (Jensen 1996; Kirill and Filatov 1999; Adams and Gillespie 2006; Gao 2009; Chuvieco 2010). Using this phenomenon, the object of the present study was to evaluate the potential of remote detection techniques to obtain a spectral characterization of *D. geminata* under controlled laboratory conditions. This will provide the basis for the design and implementation of a monitoring and early warning system for this diatom which will support decision-making on appropriate means for limiting its spread.

Methods

The radiometric laboratory experiment was carried out with fresh material of *D. geminata,* obtained from two river systems in south central Chile: the upper Bío-Bío (38º30′S; 71º13′W) and the Fuy-Llanquihue River (39º49′S; 72º05′W) (Figure 1). This was done by extracting periphyton from rocks colonised by *D. geminata* in its natural state, following the method proposed by Díaz et al. (2012). The periphyton was isolated from the accompanying benthic fauna in the laboratory; then the algal species were separated by Battarbee's method (1986) and identified under the microscope using specialized keys. The *D. geminata* cells were isolated from the periphyton by a combination of two methods: (1) successive dilutions and (2) isolation on plates (González et al. 1995; Alfonso and Leal 2000). The pure *D. geminata* material was placed on 12-well plates (6 mL) in triplicate, using Guillard's f/2 medium for diatom (Guillard 1975), creating a battery with a medium which was very low in phosphorus in order to simulate the nutrient concentration described in the literature for *D. geminata* growth (Kilroy and Bothwell 2012). *Gomphonema minuta* (Stone) Kociolek & Stoermer, was also isolated; this species grows in association with *D. geminata* and for the effects of this study could generate false positives, hindering spectral separation of the two species.

The spectral response was determined using an Ocean Optics p/n FLAME-S-VIS-NIR spectrometer (range 350 to 1000 nm) with spectral resolution 0.32 nm. The spectral response was recorded using relative reflectance in 150 measurements of samples of *D. geminata* and *G. minuta*. This experiment also included analysis of two structural components which are characteristic in *D. geminata*, cells and stalk masses, which represents the stages of latency and fruiting respectively in its phenological behaviour.

The spectrometer acquired radiometric response data through an optical fibre QR400-7-VIS-BX with 90° angle of view, connected to an artificial light source. The reflectance values were calculated from the scheme proposed by Xing and Baerdemaeker (2005), in which $P_\lambda = (S_\lambda^{body} - D_\lambda^{dark}) / (P_\lambda^{ref} - D_\lambda^{dark})*100$; where P_λ is the spectral reflectance (%), S_λ^{body} is the intensity of the energy reflected by the radiated biological body (adimensional), D_λ^{dark} is the intensity of the energy reflected taking into account the absence of light (adimensional), and P_λ^{ref} is measured from a standard reflectance (p/n WS-1-SL). The noise associated with the signal was considered on the basis of the standard deviation calculated for each wavelength in order to discard wavelengths with a high noise level. Zones with low levels of standard deviation, associated with low noise levels, were used to define zones with high reflectance or absorption in order to calibrate candidate zones for analytic domain (Saavedra et al. 2011; Saavedra et al. 2013). These measurements were averaged to obtain the representative spectral variability, eliminating spectral noise during measurement (possible outliers). The mean values for each measurement were standardised by subtracting the mean value of the spectral signature divided by the standard deviation. In this way the morphology of the spectral signature was reconstructed, creating a normal centred on 0 with dispersion 1. To identify the differences in the form of the signatures obtained, the Kolmogorov-Smirnov test was applied in order to compare the reflectance distributions of *D. geminata* and *G. minuta*,

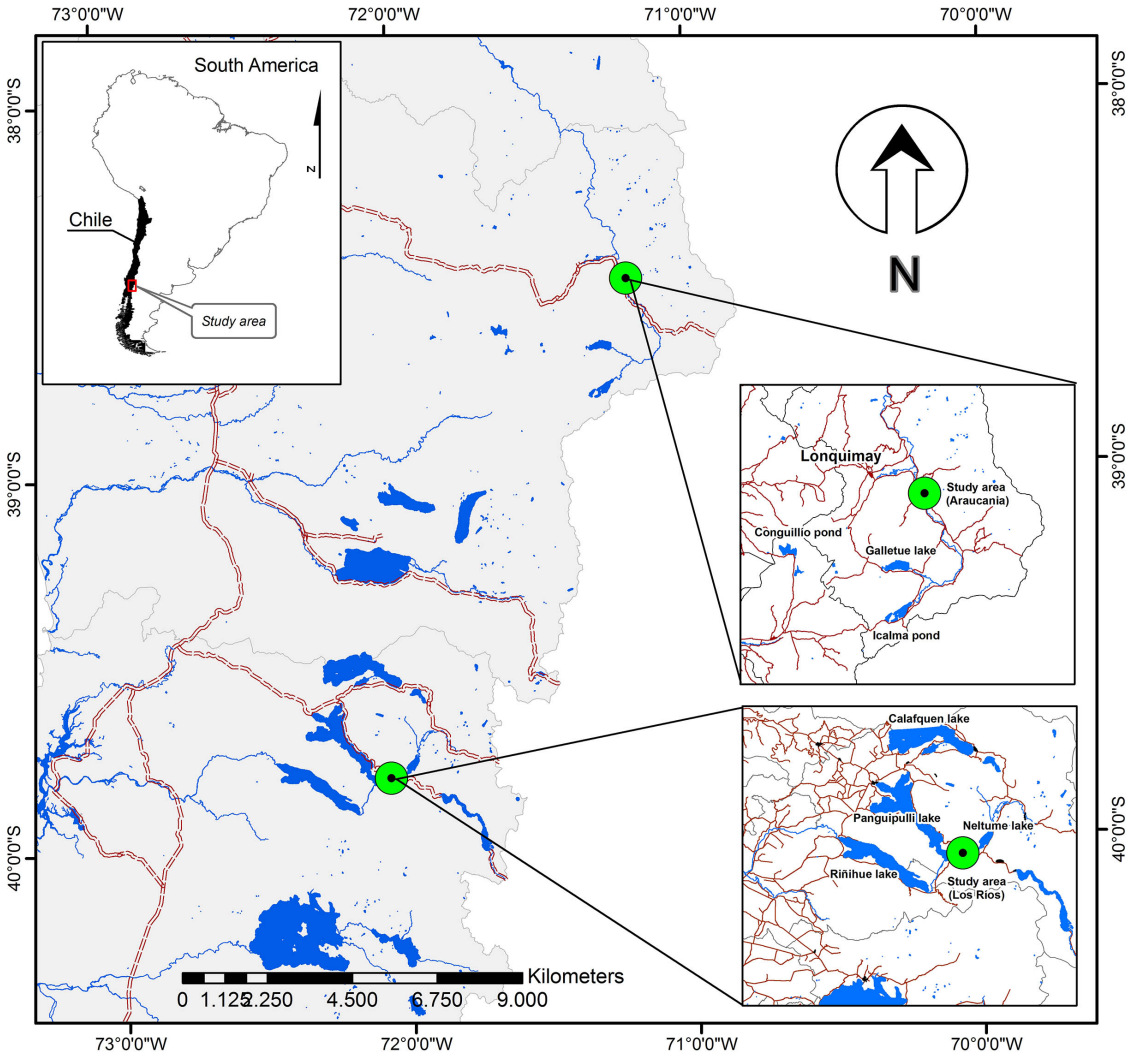

Figure 1. Study area located in the pre Andean zone of the Araucania region, Chile (Datum WGS-84).

and of the stalk and cells masses ($p < 0.05$). This test enabled us to calculate the maximum distance between the accumulated distributions of the two samples (Zar 2010).

Results

The spectral results allowed us to distinguish between cells masses of *D. geminata* and *G. minuta,* which have very similar morphologies (Figure 2A). Differences were found in spectral zones located at 600 nm, and in the near infra-red range 700 to 950 nm. The normalised spectrum presented local saddle points centred on 425, 600, 672 and 807 nm (Figure 2A). In the absorption bands associated with 600 and 672 nm, an inverse ratio was observed in the spectral response, showing lower reflectance with increasing concentration.

Within the analytic domain (AD), the presence or absence of *D. geminata* can be evaluated under controlled laboratory conditions. The structural components of *D. geminata* (cell and stalk masses) present separable spectral responses between 400 and 950 nm (Figure 2B). This allows separability protocols to be developed for the latent states of *D. geminata* in which only the stalk is observed.

The Kolmogorov-Smirnov test showed significant differences ($p < 0.05$) between the curves for *D. geminata* and *G. minuta*. A similar result was obtained when the morphological structures of the stalk

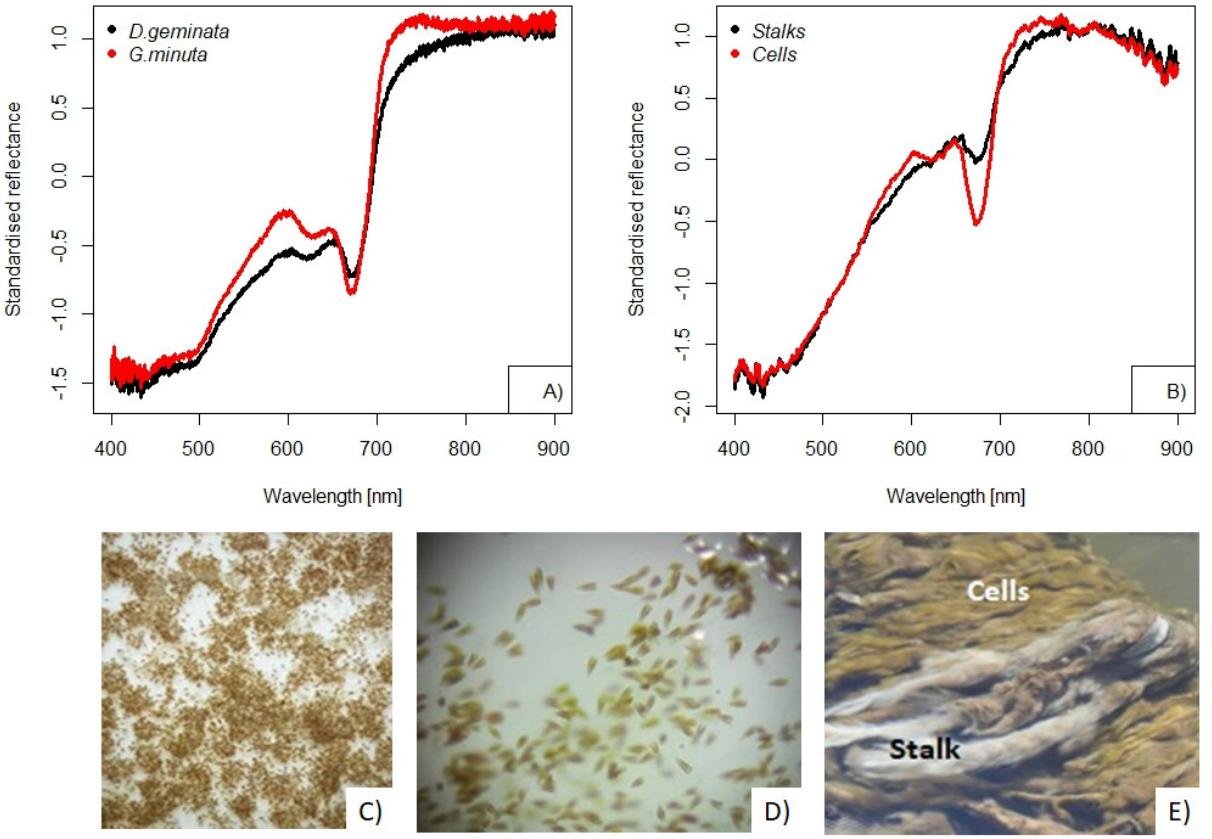

Figure 2. Spectral signatures obtained in laboratory cultures: *D. geminata* and *G. minuta* (A); Morphological structures of *D. geminata* – stalk and cells masses (B). In *D. geminata* cells masses (C); *G. minuta* cells masses (D); Cells (E); Stalk (F). Samples obtained from cultures under laboratory conditions (just as reference). Microphotographs by Katerina González.

and cell masses were compared ($p < 0.05$). These results show that the curves obtained present different shapes which are potentially distinguishable under controlled conditions.

Discussion

The results obtained allow pure cultures of *D. geminata* and *G. minuta* to be distinguished by their spectral signatures under controlled laboratory conditions. This shows that low-cost sensors (350 nm to 1000 nm) are sufficiently sensitive to be used as the basis for environmental monitoring and detection systems for diatom consisting of a single environmental. These instruments do not require much technical knowledge, facilitating access to and adoption of the technologies (Adams and Gillespie 2006; Gao 2009). The results show the potential of this technique for detecting *D. geminata* on lake and river banks; the model can easily be operated by technical staff to determine the presence or absence of the species. This potential offers us a new technology for terrestrial or aerial monitoring systems, using multispectral or hyperspectral systems (Jensen 1996; Chuvieco 2010) with which to generate information on zones with a high risk of invasion. However the application of this technique in natural environments like lake and river banks requires further study, since during certain phenological periods *D. geminata* forms part of the water column; it shares the matrix with similar species, making detection using this technology less certain (Jensen 1996).

One of the most important results is related to the potential of hyperspectral techniques for distinguishing between the phenological stages of stalk and cells masses in *D. geminata* (Rivera et al. 2013). The stalk is the mucilaginous peduncle secreted by the basal end of the cells, by which it adheres to the rocky substrate (Rivera et al. 2013; Bothwell and Kilroy 2011), while the cells is the silica cell wall which

surrounds the diatom cell (Strasburger 2004). Early identification of the stalk would allow the diatom to be detected before it generated emergent bodies which become incorporated into the water column. This would enable inspectors to restrict activities in contaminated areas, reducing the risk of cross-contamination into areas where the diatom is not present.

We recommend that the number of samples should be increased in future studies to ensure that the results are representative, allowing presence/absence curves to be drawn. It is also necessary to incorporate the factors related to different concentrations of algae under different moisture contents, such as are found in natural environments. Logistic statistical models, already implemented in other areas of knowledge, can be used in the identification of this alga (Fustos et al. 2014). With these improvements in spectral characterization, research could be extended to the relation with remote data obtained from sensors mounted in aircraft or satellites, allowing the presence/absence of *D. geminata* in time and space to be distinguished. Finally, this study suggests that geospatial technologies, based on spectral techniques, have a high potential for use in early warning systems which can detect diatom on natural environments.

Conclusions

The spectral signatures of *D. geminata* and *G. minuta*, obtained in the laboratory by remote detection techniques, allow these two algae to be distinguished. This potentially offers inspectors a rapid tool for the early detection of *D. geminata* in natural environments.

The tools and techniques of hyperspectral remote detection from space have great potential for application in real-time environmental monitoring systems, allowing rapid, efficient identification of diatom like *D. geminata* which become established in riparian areas.

The results from this study can be applied to map the geographical extent and severity of *D. geminata* across wide geographical scales (for example, all the rivers in Chile). The seasonality of mats in relation to specific watershed characteristics could be used to assess river suitability for cell survival and mat production.

This study demonstrates that we can identify *D. geminata* and *G. minuta* when they are found together in nature, by morphological characterization of their spectral signatures. However, further work is needed on analyses which would enable us to distinguish the presence of *D. geminata* under different conditions of growth and moisture, and differing compositions of environmental matrices containing other algae.

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