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Research Article

Regional distribution and temporal changes in density and biomass of *Didymosphenia geminata* in two Mediterranean river basins

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Abstract

This study aimed to strengthen the knowledge of *Didymosphenia geminata* distribution in Spain, and to determine which environmental variables were related to its regional distribution and temporal changes in growth and production in two Ebro River tributary basins (Iregua and Najerilla, La Rioja Region). Living cells of *D. geminata* were present in 40% of the studied sites of both basins but only four of the sites developed massive growths. The diatom is widely distributed only in mountain areas of both basins (above 690 masl), which have low average annual air temperature (8.27 ± 0.28 ºC) and low water Soluble Reactive Phosphorus (SRP) concentration (average value 0.024 ± 0.007 ppm). Massive growths occur in sites with high light intensity, hydrological regulation of river flow and the lowest measured SRP concentrations (below 0.018 ppm SRP). We carried out an intensive spatio-temporal survey in the Lumbreras River (Iregua basin), where the first massive growth was detected in La Rioja Region. The highest cell densities (1.6×10^5 cells cm^{-2}) and biomass values (480 $gDWm^{-2}$) were recorded in late summer. An increase in solar radiation and slight rise in water temperature were associated with an increase in biomass over time, but always in waters with low SRP concentration, the key factor that enhances massive growth development. The alteration of the natural streamflow in the Lumbreras River by the Pajares Reservoir and the fact that water is discharged from the hypolimnion (which decreases the summer river water temperature) are important factors in understanding why *D. geminata* shows such an increase in biomass in a Mediterranean river. This paper provides new insights into the importance of different factors controlling *D. geminata* massive proliferations in the Mediterranean climate zone, where it is assumed that the species should not develop such massive growths, compared to temperate zones where the majority of previous studies have been carried out. We suggest that changing the reservoir management strategy would be a very important contribution to the control of such significant developments.

Key words: Didymo, Ebro basin, seasonal evolution, massive growths, soluble reactive phosphorus, hydrological regulation

Introduction

Didymosphenia geminata may exist as single cells in the benthic substrates of cold streams and, under certain circumstances, may produce copious amounts of extracellular stalk material, generating massive growths and forming thick benthic algal mats that can significantly alter river ecosystems (Kilroy et al. 2009; Gillis and Chalifour 2010; Ladrera et al. 2015). Although *D. geminata* has historically been associated with cold and oligotrophic waters in northern Europe and North America (Blanco and Ector 2009), in the last few decades it has been identified at sites with no previous records in lower latitude regions in both hemispheres (Blanco and Ector 2009).

In Spain, records of *D. geminata* occurrences were scarce before the XXI century (Blanco and Ector 2008, 2009; Blanco and Bécares 2009). Reports show a distribution mostly in the Pyrenees, including in the Ebro basin, in areas such as Andorra (Margalef 1952), Bohí Valley (Dosset 1888; Margalef 1956), Aigüestortes (Margalef 1956; Vilaseca 1978) and Ordesa Valley (Cambra 1987; Cambra 1991a). However, some historical *D. geminata* records exist for other parts of Spain, including Mallorca Island in the Mediterranean Sea (Margalef 1953; Cambra 1991b). In recent years, the number of *D. geminata* records in Spain has significantly increased, particularly in the Ebro and Duero basins (Blanco and Bécares 2009; Tomás et al. 2010; Ladrera and Prat 2013). Otherwise records are relatively scarce from elsewhere in the Iberian Peninsula, and lower than in other geographical areas of Europe, probably due to the less favourable environmental conditions associated with its Mediterranean climate (Whitton et al. 2009).

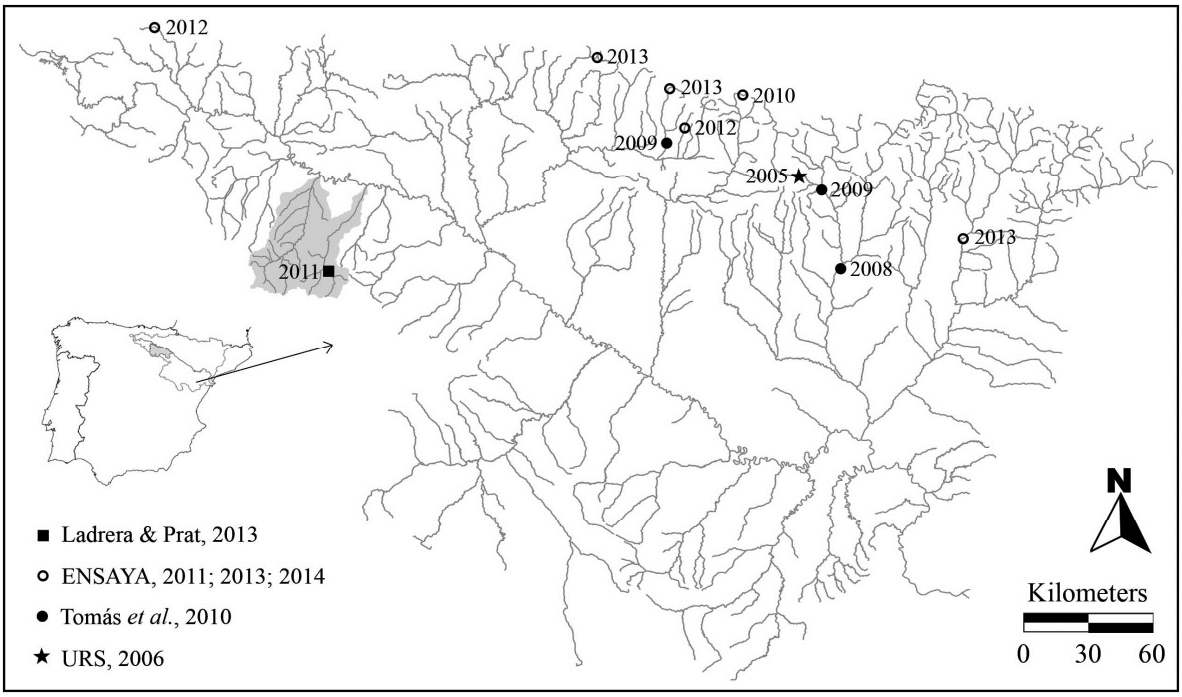

Figure 1. Reports of *D. geminata* filamentous mats in the Ebro basin before the present study (URS 2006; Tomás et al. 2010; ENSAYA 2011, 2013, 2014; Ladrera and Prat 2013). Grey marked area corresponds to the studied basins.

The first record of a massive growth of *D. geminata* in Spain was in the Ara River (Pyrenees; Ebro basin) in 2005 (URS 2006). Since then other large biomass developments of this diatom have been registered in the Ebro and Duero basins (Blanco and Ector 2008; Blanco and Bécares 2009; Tomás et al. 2010; Ladrera and Prat 2013). In Figure 1, locations of filamentous mats in the Ebro basin, where our study area is located, are shown. Most records are constrained to the Pyrenees, apart from the Nela River in the headwater of the Ebro basin, and the headwaters of the Iregua basin (Ladrera and Prat 2013), where we have focused our study. *D. geminata* cells were originally detected in 2009 in the middle Iregua (Tomás et al. 2010) and the first massive growth was detected in 2011 at the Lumbreras River (Ladrera and Prat 2013), in the upper part of the Iregua basin.

The main environmental conditions responsible for massive *D. geminata* growths are high light intensity (Whitton et al. 2009; Kilroy and Bothwell 2011, 2014; James et al. 2014), low inorganic phosphate concentration (Bothwell and Kilroy 2011; Bothwell et al. 2012, 2014; Ellwood and Whitton 2007; Miller et al. 2009), high ratio of organic to inorganic phosphate (Ellwood and Whitton 2007; Whitton et al. 2009) and cold water temperature (Kilroy et al. 2008; Kumar et al. 2009; Whitton et al. 2009; Cullis et al. 2012), combined with hydrological regulation (Kirkwood et al. 2009). However, these studies have been carried out in temperate regions (mainly in North America, northern Europe and New Zealand), but the topic of *D. geminata* ecology has only recently been addressed in the Mediterranean region of southern Europe (Ladrera et al. 2015), even though several massive growths have been discovered in this climatic area (e.g. Figure 1 for Spain and Beltrami et al. (2008a, b) for Italy). Therefore, our first question was whether we could extrapolate the threshold values of the environmental variables associated with the development of massive *D. geminata* growths in these Mediterranean basins.

In a previous study (Ladrera et al. 2015), the effects of the massive *D. geminata* growth on the macroinvertebrate community in the Lumbreras River were examined. This study showed that the key environmental variables explaining the presence of *D. geminata* massive growths are the same as those in temperate regions, especially the Soluble Reactive Phosphorus (SRP) concentration, but data were only available from one river section and one sampling period. The extent to which these conditions are important in the Mediterranean climate zones and

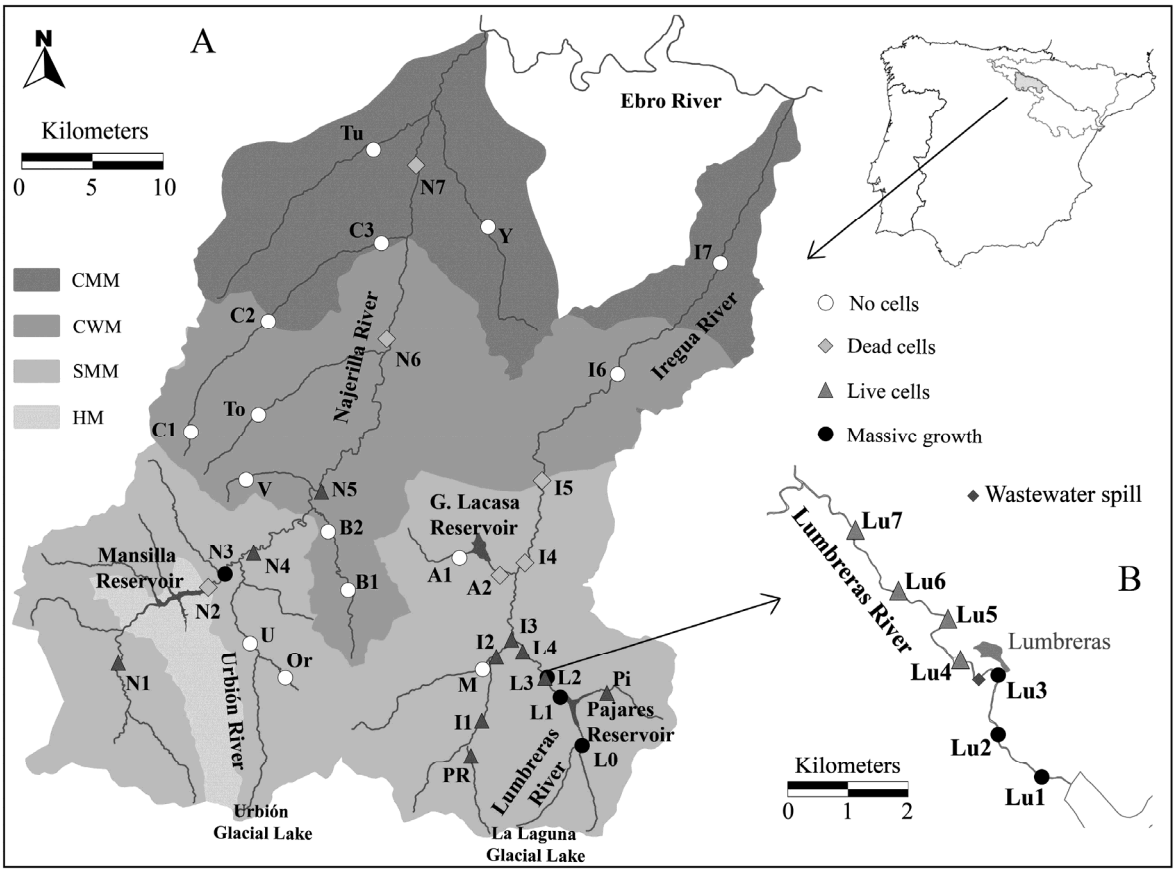

Figure 2. Study area. Different grey intensities are used to show the different river typologies (MMARM 2008) in the study area of the regional study (A) (CMM, Calcareous Mediterranean Mountain; CWM, Calcareous Wet Mountain; SMM, Siliceous Mediterranean Mountain; HM, High Mountain). Sampling sites of the spatio-temporal study are shown separately (B). For the two maps, absence or presence of *D. geminata* dead cells, living cells and massive growths are shown. Note that name of sites located at Lumbreras River in the regional study begin with "L", while sites of this river in the spatio-temporal study begin with "Lu" since they are not situated in the same locations. For details see Supplementary material Table S1.

the importance of temporal changes in algal growth is still to be discerned. Therefore, the aims of the present study are: i) to understand the distribution of *D. geminata* in two basins located within the Mediterranean climate zone; ii) to determine the environmental variables related to the presence of *D. geminata* and to its massive growths; and iii) to study the annual temporal changes of *D. geminata* massive growths. In order to reach these objectives we designed two sampling strategies: 1) an extensive spatial survey over the length of the two selected basins and 2) an intensive temporal survey in one area, the Lumbreras River, where the alga was known to be abundant in preceding years (Ladrera and Prat 2013).

Our working hypotheses are: i) *D. geminata* living cells will appear in the study area in river stretches with climatic conditions similar to those already associated with its natural distribution in other areas; in particular, streams poor in SRP and with low temperatures; ii) massive growths will occur in river stretches with the lowest SRP concentrations and relatively low temperatures; and iii) temporal changes will not only depend on the changes of temperature and SRP, but also on other factors pointed out in other geographical areas, such as light intensity.

Methods

Study area

The study was carried out in the Najerilla (100 km in length) and Iregua (62 km in length) River basins (1100 and 660 km^2 respectively; see location in Figure 2), which are tributaries of the Ebro River in La Rioja Region (11.9 and 5.7 m^3s^{-1} average discharge, respectively). The climate in this area is clearly influenced by the Mediterranean, as stated in the

river classification survey produced for the implementation of the European Union Water Framework Directive (MMARM 2008; Figure 2A). This holds true even in mountainous areas, where precipitation in July and August is scarce (36 mm month^{-1}) and summer temperatures are relatively high with mean values of 18.5 ºC and maximum values that can be higher than 30ºC (http://www.saihebro.com). In addition, both basins can be divided into two distinct areas depending on human pressure. Upper reaches are mainly affected by hydrological regulation, due to the presence of three reservoirs: Mansilla Reservoir (69.9 hm^3) in the Najerilla River, and Pajares (35.2 hm^3) and González Lacasa Reservoirs (32.9 hm^3) in the Iregua River basin. The Pajares Reservoir completely alters the natural hydrograph of the Lumbreras River (Figure 3B), decreasing flows in winter until the reservoir reaches its maximum storage capacity (Figure 3) and increasing flows and lowering temperatures below the dam in summer (Ladrera and Prat 2013). In the lower reaches, the main anthropogenic pressures are associated with agricultural (fertilizer and pesticide runoff, riverbank occupation and water abstraction) and industrial activities (mainly chemical discharges).

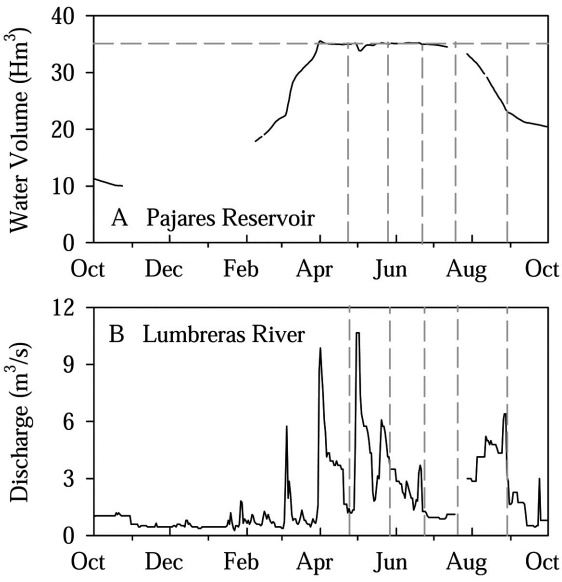

Figure 3. Pajares Reservoir water volume (A) and Lumbreras River discharge downstream of the Pajares Reservoir (B) throughout the 2012–2013 hydrological year. Reservoir capacity from November to February is not available. Grey dashed lines indicate reservoir capacity (A) and sampling dates (A, B).

Sampling strategy

Regional distribution of *Didymosphenia geminata*

To understand the distribution of *D. geminata* in the Iregua and Najerilla River basins, we collected samples from 35 sites throughout the study area (Figure 1A). Each site, covering 100 m in length, was visited once between September 13th 2012, and September 23rd 2012. During those dates flow conditions were stable in the study area, with no signs of rain. Sites were selected with the aim of covering the environmental variation of the studied basins (river order, altitude, insolation, human pressures, streamflow regulation, ambient temperature and hydromorphological conservation) and included the main rivers and tributaries. The sampling date was chosen taking into account that the only previous massive growth identified in the region reached its maximum development at the end of summer (Ladrera and Prat 2013). At each site, the presence or absence of *D. geminata* cells was determined as described in the *D. geminata* sampling section of this methodology. The presence or absence of *D. geminata* massive growths was also determined in each site. We consider massive growth to be when dense *D. geminata* mats with a thickness greater than 5 mm appear continuously along a river stretch longer than 1 km (length measured by means of a GPS device). The presence and extent of massive growths were determined by visual inspection of stream reaches where filamentous mats were detected at sampling sites.

Spatio-temporal changes

In order to determine spatio-temporal changes in *D. geminata* massive growths and to better understand the environmental variables associated with their development, a study was carried out in the Lumbreras River (Figure 1B). This is where the first *D. geminata* massive growth in the La Rioja region was detected (Ladrera and Prat 2013) and where our previous studies were carried out. Samples were taken at seven sites downstream of the Pajares Reservoir (Figure 2B) on April 21st, May 26th, June 22nd, July 17th, and August 31st, 2013. The Pajares Reservoir is primarily used to store water for summer irrigation. After intense winter rains, the reservoir reached 100% of its capacity in April 2013 (Figure 3A). The reservoir remained full until August, when the amount of water released to irrigate fields in the lower parts of the basin increased. For each site and sampling date, the density of living cells and the mat biomass were determined (methods described below). The river hydrograph and the sampling dates can be seen in Figure 3.

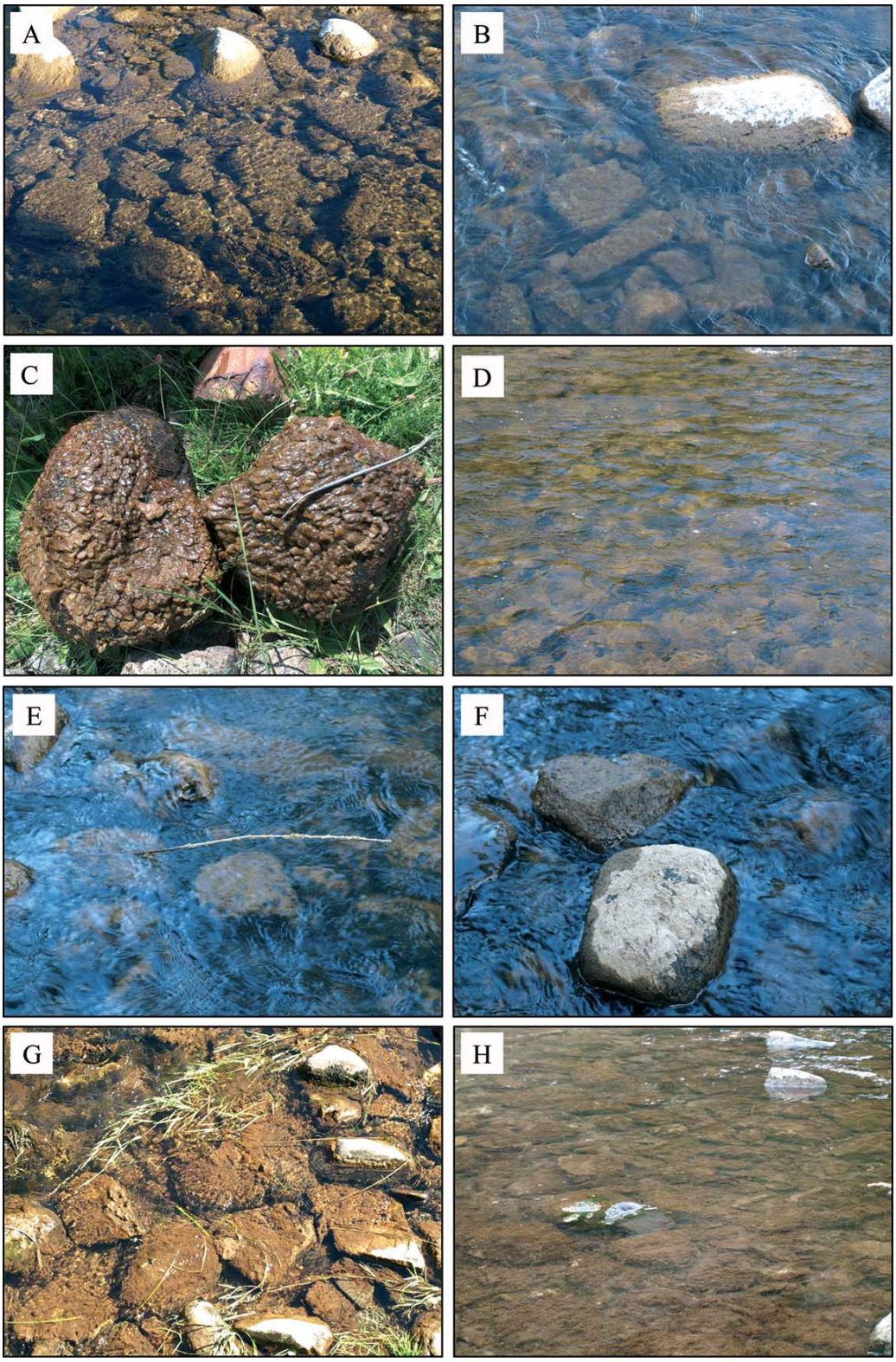

Figure 4. Images of massive growths located at sites L0 (A, B), L1 (C, D), L2 (E, F) and N3 (G, H) of the regional study.

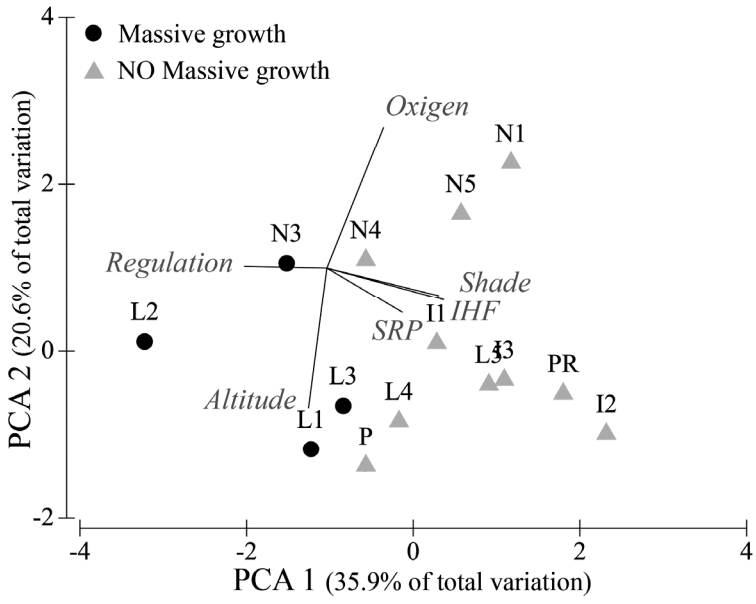

Figure 5. Principal Component Analysis (PCA) of the different sites with *D. geminata* living cells and environmental variables for the regional study.

Environmental variables

In both studies, water temperature (ºC), pH, conductivity (µScm^{-1}), and dissolved oxygen (ppm) were measured *in situ* using a YSI Professional Plus Multiparameter Instrument. SRP was analyzed in the laboratory following the acidic molybdate method (Murphy and Riley 1962). For SRP analysis, 125 ml water samples were collected and kept in a cool-box for transport to the laboratory, where they were kept frozen until they were analyzed. Samples were filtered (GF/F Whatmann 0.7 µm-pore size) before SRP determination, for which a spectrophotometer (Shimadzu UV-1201) (range 0.005–1.000 ppm) was used. The riparian habitat was characterized using the four components of the QBR Riparian Forest Quality Index of Munné et al. (2003): total riparian vegetation cover, cover structure, cover quality and channel alterations. The fluvial instream habitat was characterized using the IHF River Habitat Index (Pardo et al. 2002), which measures habitat heterogeneity and is composed of seven sections: substrate embeddedness, rapid frequency, substrate composition, velocity/depth conditions, % of shading, heterogeneity components and in-channel vegetation cover. Riverbed shade percentage was visually classified into five categories: 0: 0–5%; 1: 6–25%; 2: 26–50%; 3: 51–75%; 4: 76–100%.

The regional study also included site-specific measurements of the average annual atmospheric temperature, stream order (both determined from online geographical sources; http://www.iderioja.larioja.org), and intensity of hydrological regulation. The last parameter was classified into three categories, based on the influence of reservoirs and glacial lakes: 0: river sections not affected by hydrological regulation from either reservoirs or glacial lakes; 1: river sections located downstream of glacial lakes or affected by moderate hydrological regulation by reservoirs, where streamflow was not totally regulated by a reservoir; and 2: river sections affected by intense hydrological regulation, where streamflow was diverted completely from a reservoir. It should be noted that three large reservoirs and two glacial lakes (Urbión, 2.3 ha, and La Chopera, 1.5 ha) are present in the study area.

For the temporal study, the water temperature changes immediately downstream of the Pajares Reservoir (Lu1) were followed using a HOBO thermistor, which registered the temperature hourly from April to August. Insolation in the Pajares Reservoir was also determined from official geographical sources (http://www.saihebro.com).

Didymosphenia geminata sampling

At each site, in both the regional and temporal studies, 3–5 randomly chosen streambed cobbles larger than 10 cm and continuously covered by flowing water were selected. Periphyton was collected into a 125 ml plastic jar by brushing 75 cm^2 of cobble with a toothbrush, producing a single, pooled sample. Samples were fixed in the field with 4% formaldehyde and taken to the laboratory to be

identified. The samples were homogenized by intense manual agitation in the laboratory and at least 30 µl of each sample was divided between 3 covered glasses (12 mm diameter) and visualized through an optical microscope (Motic DMW B1-223ASC) at 400× magnification to detect the presence of *D. geminata* cells.

In the regional study, we determined the absence or presence of *D. geminata* cells at each site, both dead (those with deteriorated chloroplast; Kilroy and Bothwell 2012) and living (those with an intact chloroplast; Kilroy and Bothwell 2012); while in the temporal study, dead and living cells of *D. geminata* were counted to determine cell density. Periphyton, mostly composed of *D. geminata* filaments, was dried for 72 h at 70 ºC for dry weight (DW) determination. *D. geminata* dead and living cell density (cells cm^{-2}) and periphyton biomass (dry weight m^{-2}) were calculated taking into account the area of cobble surface brushed, and for cell density the volume of the microscope-visualised sample.

Data analysis

The data from the 35 sites of the regional study were grouped into four sets: i) sites without *D. geminata* cells; ii) sites with only dead cells; iii) sites with living (and dead) cells (including those with massive growths) and iv) sites with massive growths only. Nonparametric pairwise Mann-Whitney U tests were used to detect differences between groups. From sites with *D. geminata* living cells, separate Principal Component Analyses (PCAs) were carried out for the regional study and the spatio-temporal study to examine the roles of the different environmental variables in massive growth development. For the spatio-temporal study, taking into account the minimal temporal variability of the physicochemical variables, we used the values averaged throughout the study period for each sampling site, as the PCA objective was to find differences among sites affected, or not, by massive growth. For hydromorphological variables, the summer values were used, since vegetal development is maximum at this time. For both PCA analyses, environmental variables were transformed using log (x + 1) and normalized, and highly correlated variables were removed from the analysis. Finally, for the spatio-temporal study and for each environmental variable the nonparametric Kruskal-Wallis test was used to detect variables that differed significantly among sites. We then carried out a nonparametric pairwise Mann-Whitney U test for every environmental variable and pair of sites to detect significant differences between sites.

Results

Regional distribution of Didymosphenia geminata in the studied area

D. geminata cells (alive and/or dead) were found in 20 sites of the regional study (Figure 2A), which showed significantly lower IHF values and SRP concentrations than the sites without *D. geminata* cells ($P < 0.05$; Supplementary material Table S2). The majority of the 14 sites in which living cells were present were in the Siliceous Mediterranean Mountain river typology (MMARM 2008), including the four with massive growths (Figure 2A). The environmental variables characterizing sites with living cells were high altitude (all above 690 masl), lower average annual atmospheric temperatures (8.27 ± 0.28 ºC), and lower SRP concentrations (0.024 ± 0.007 ppm; Table S2). These parameters were significantly different from those of sites with only dead cells ($P < 0.05$; Table S2).

Among sites with living cells, *D. geminata* massive growths developed in four of them (L0, L1, L2 and N3; Figures 2A, 4). These four sites had significantly higher values of hydrological regulation ($P < 0.05$; Table S2), and significantly lower values of the IHF index and SRP concentration ($P < 0.05$; Table S2) compared with sites with living cells but no massive growths. *D. geminata* massive growths appeared in sites with SRP concentrations lower than 0.018 ppm (SRP = 0.012 ± 0.002 ppm; Table S2). With regard to hydrological regulation, three of the massive growths were found downstream of a large reservoir (N3, L1 and L2; Figure 2A) and the fourth one (L0) was situated in Lumbreras River upstream of the Pajares Reservoir, where streamflow is influenced by La Chopera glacial lake (Figure 2A). Sites N3, L0 and L1 had a shade percentage in the riverbed lower than 25%.

In the PCA analysis performed for sites with living cells, the first axis explained 35.9% of the total variance (Figure 5) and sites are separated out along this axis with sites having massive growths present on the left. The variables correlated with the first axis were hydrological regulation, % shade, IHF and SRP (Figure 5), in accordance with differences observed in Table S2, since these variables were significantly different (except % shade) between sites with *D. geminata* massive growths and those with living cells but no filamentous mat development. Figure 6 graphically shows the clear differences between sites with *D. geminata* massive growths and those with living cells but without filamentous mat development.

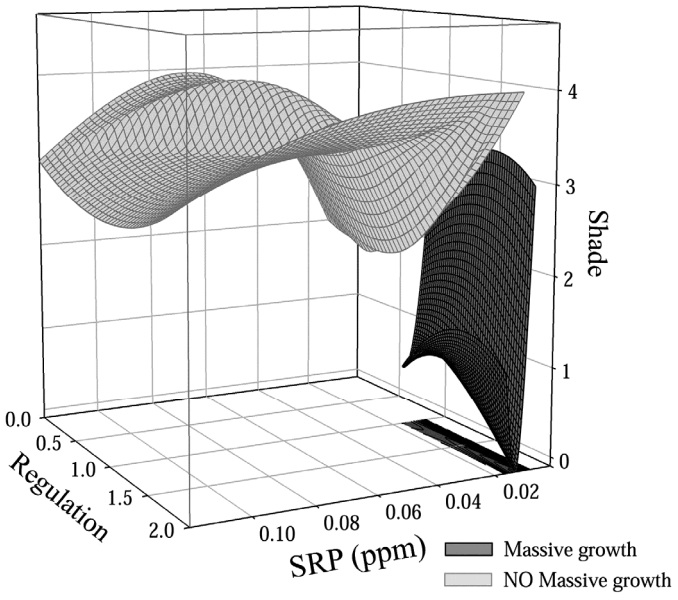

Figure 6. Mesh graph from sites of the regional study with *D. geminata* living cells, grouping sites based on the presence or absence of massive growths of the algae.

Spatio-temporal changes in Lumbreras River massive growth

In the spatio-temporal study, the living cell densities across sites were minimal at the beginning of the season (April) and increased throughout the study, reaching a maximum on August 31st (Figure 7A). Massive algal growth took place only between the Pajares Reservoir and the wastewater discharge input from Lumbreras Village (sites Lu1, Lu2 and Lu3). Density was clearly higher in those three sites (Figure 7A), with cell densities between 1.2×10^5 and 1.6×10^5 cells cm^{-2} at the end of the study period, while average cell density downstream of the Lumbreras sewage discharge was 5700 cells cm^{-2}. The largest increase in cell number occurred between July and August and was clearly higher in sites located upstream of the Lumbreras sewage discharge (Figure 7A).

As with the cell densities, the maximum increase of periphyton biomass during the study occurred between July and August (Figure 7B). At the end of the study, dry weight was almost four times higher in Lu1 (478 g m^{-2}) than in Lu2 and Lu3 (approximately 125 g m^{-2}; Figure 7B). The massive growth of *D. geminata* disappeared downstream of the Lumbreras sewage input, although isolated mats existed in Lu4 and Lu5 in August.

Temporal changes in the physicochemical variables for a given site were minimal (Supplementary material Table S3). Regarding spatial changes, temperature, conductivity, and SRP experienced a gradual increase from upstream (Lu1 site, located downstream of the reservoir), to site Lu4, located downstream of the wastewater discharge point (Table S3), although the increase in conductivity and temperature was not as pronounced as that of SRP. Only SRP was significantly different among sites ($P < 0.05$, Kruskal-Wallis test; Table S3), and when comparing pairs of sites (Mann-Whitney test), there was a significant difference ($P < 0.05$) between Lu3 and Lu4, the reach where the massive growth disappeared (Table S3).

Temporal evolution of insolation in Pajares Reservoir and temperature at site L1, immediately downstream of the reservoir, are shown in Figure 8. Insolation reached maximum values at the end of June. Temperature did not change notably downstream of the reservoir since water is released from the hypolimnion. Nevertheless, water temperature increased from April to July, maintaining values up to 10 ºC during summer (Figure 8), much lower than those found in unregulated streams of the same area (see Ladrera and Prat (2013)).

Site Lu1 was characterized by a low QBR index value and the complete absence of shade, due to the removal of the riparian forest downstream of the Pajares Reservoir (Table S3). In this river stretch, the riverbed was completely channelized, resulting in a low IHF value. These hydromorphological parameters increased substantially at Lu2, especially shade, because of the presence of a riparian forest and natural channelization.

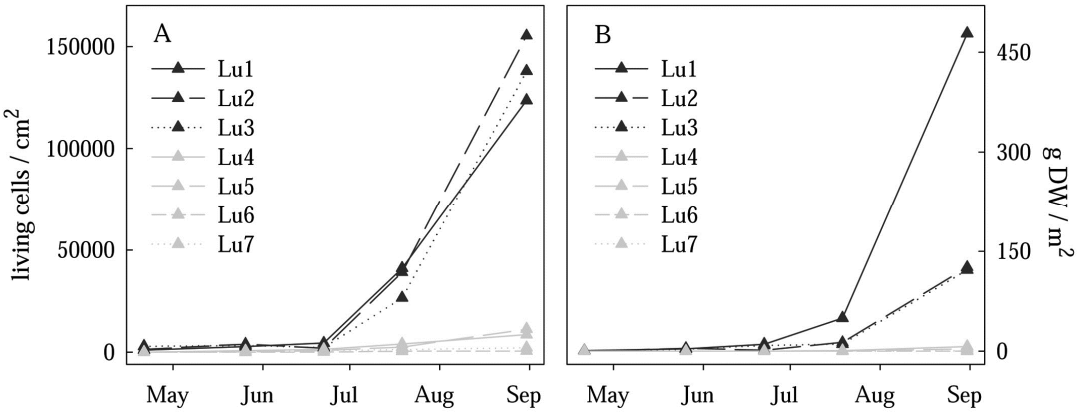

Figure 7. Spatio-temporal study. Temporal changes at each sampling site of *D. geminata* living cell density (A) and periphyton biomass (B).

The first axis of the PCA (Figure 9A) explained 68.4% of the total variance, and sites were divided along this axis, where sites with massive growth are positioned to the left. Temperature, SRP, % shade, IHF, and conductivity were most strongly correlated with the first axis, and sites with massive *D. geminata* growths were negatively correlated with these variables. SRP, IHF, and % shade were significantly lower in sites affected by massive growth (Figure 9B, sites Lu1 to Lu3), whereas temperature and conductivity were not significantly different between sites affected by massive growths versus those not affected (Figure 9B).

Discussion

The present study has focused on an area of the Ebro basin, located in the Mediterranean climate zone, where massive growths of *D. geminata* have occurred. These growths are a very recent phenomenon in the Ebro basin, the first one being spotted in 2005 (URS 2006), and are mostly restricted to the Pyrenees, where climatic conditions are more similar to those of temperate regions. Our study was developed in the only two sub-basins located on the south side of the Ebro basin that are affected by this phenomenon. The presence of massive growths in these basins is of particular importance considering that its Mediterranean climate is characterised by very low rainfalls and high temperatures in summer.

Since its first record in 2009 in the Iregua River (Tomás et al. 2010), *D. geminata* has dispersed throughout our two studied catchments—we found living cells in 14 of the 35 sampled sites—developing massive growths in both basins, the first of which was detected in 2011 (Lumbreras River; Ladrera and Prat 2013).

Our first hypothesis—that *D. geminata* will be present in rivers with low temperature waters and low SRP concentration—was validated because living cells were restricted in our study area to mountain rivers at altitudes ≥ 690 masl, average annual atmospheric temperatures ≤ 9 ºC and SRP content lower than 0.018 ppm. However, living cells were detected in several reaches with water temperatures higher than 17 ºC. The ability of *D. geminata* to live in watercourses with seasonally high water temperatures was previously reported by Kolayli and Sahin (2007), Lindstrøm and Skulberg (2008) and Kuhajek and Wood (2014). In this regard, Cullis et al. (2012) suggest that the common distribution of *D. geminata* in cold areas could be because low temperatures are usually associated with rivers with advantageous environmental conditions for this alga (especially low nutrient contents), rather than direct temperature limitation.

Phosphorus is considered a critical factor for the distribution of *D. geminata* (Bothwell et al. 2014). Different studies have indicated that living cells of *D. geminata* generally appear in sites with low TP and/or TDP concentration (Kirkwood et al. 2007; Miller et al. 2009), although Bothwell and Kilroy (2011) showed rapid division of *D. geminata* cells exposed to high concentrations of P for short periods. In our case, the mean SRP value of sites with living algal cells was 0.024 ± 0.007 ppm. We found living *D. geminata* cells in only one site in a river stretch with a higher SRP (0.119 ppm). In our spatio-temporal study, a 96% reduction of living *D. geminata* cells were found at the Lu4 site, following the inputs from a sewage plant (SRP average values of 0.021 ± 0.002 ppm, with an increase of 100% with respect to site Lu3). Therefore, cells of this diatom are able to survive occasionally with SRP

concentrations higher than 0.100 ppm, although living cell density clearly reduces under high SRP values, when other periphyton species could acquire a competitive advantage over *D. geminata* (Ellwood and Whitton 2007; Miller et al. 2009).

Low SRP concentration was also clearly linked to massive growth development in our study area (our second hypothesis), in accordance with other studies that highlight this factor as the major variable related to *D. geminata* massive growths (see Bothwell et al. 2014). The development of massive growths under low inorganic phosphate concentration has been attributed to different factors: i) stalk production in response to very low P may be a strategy to move cells out of the benthic boundary layer and into the water column, where there is greater delivery of growth-limiting P (Aboal et al. 2012; Bothwell et al. 2014); ii) the high content of phosphatases present in *D. geminata* stalks may permit the transformation of organic phosphorus compounds, and therefore growth in low levels of inorganic dissolved phosphorus (Ellwood and Whitton 2007; Aboal et al. 2012); and iii) high friction associated with flow at mat surfaces leads to very low velocities and predominantly diffusive transport within mats, which may in turn favour the release of solutes derived from retained organic matter within and below mats (Larned et al. 2011). The role of phosphate as a determining factor for *D. geminata* massive growths was confirmed to some degree by our results. In the spatio-temporal study in the Lumbreras River, the massive growth disappeared in site Lu4, located downstream of the sewage discharge of Lumbreras Village. The only environmental variable that significantly differed there was SRP. This aspect is confirmed in the regional study: SRP concentration in sites with *D. geminata* massive growths was significantly lower compared to sites with living cells but not affected by massive growths.

With respect to SRP threshold values for massive growth development, distinct ranges have been detected for different geographical areas, but they are generally lower than in the present study. In our spatio-temporal study, the disappearance of the massive growth in the Lumbreras River took place at Lu4, where mean SRP reached values of 0.021 ± 0.003 ppm, while in the regional study, SRP concentrations always remained below 0.018 ppm in sites affected by massive growths. These results suggest a threshold value, around 0.020 ppm of SRP, above which massive growths do not develop in our study area. Kilroy and Bothwell (2012) established 0.002 ppm of SRP as the value above which massive growths could not exist in rivers in New Zealand.

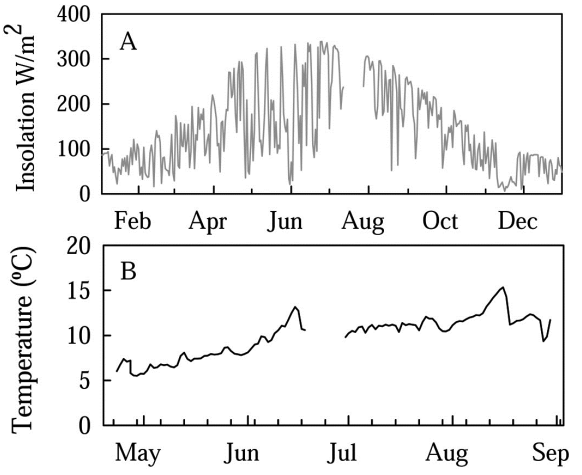

Figure 8. Temporal evolution of insolation (Wm^{-2}) in Pajares Reservoir, and water temperature changes (hourly measurements) during the spatio-temporal study period at the Lu1 site.

This threshold value is tenfold lower than that determined in our basins. However, new data are necessary to confirm our results by means of further chemical studies. Future studies should also consider new insights related to water nutrients which were not achieved in the present assessment, especially organic phosphate and its relation to inorganic components, since a high organic:inorganic phosphate ratio has been identified as one of the main factors related to massive growth development in other regions (Ellwood and Whitton 2007; Whitton et al. 2009).

Hydrological regulation was a key factor determining massive growths in the study area. Different studies have described massive growth of *D. geminata* in regulated rivers (see Kirkwood et al. 2009). In our regional study, massive growths were always located in rivers with streamflow regulated by large reservoirs discharging water from the hypolimnion or by glacial lakes. Mansilla and especially Pajares Reservoir clearly alter the hydrological regime of the Najerilla and Lumbreras Rivers respectively (Ladrera and Prat 2013), creating uniform and more stable habitats, where larger sized substrates and constant water velocity enhance *D. geminata* massive growth development (Kirkwood et al. 2007; Kilroy et al. 2008; Kumar et al. 2009). The fact that water is discharged from the hypolimnion of these reservoirs causes low summer water temperatures in the lower river stretches (Ladrera and Prat 2013), which also favour proliferation of *D. geminata* (Kilroy et al. 2008; Kumar et al. 2009; Whitton et al. 2009). La Chopera lake also regulates Lumbreras streamflow upstream of the Pajares

Reservoir. The prevalence of *D. geminata* massive growth development occurring downstream of lakes has also been related to the dissolved P depletion on lake surfaces during the summer (Bothwell et al. 2014). In the Mediterranean area, reservoirs used for irrigation purposes (e.g., Pajares Reservoir) alter natural river conditions during the irrigation season (beginning in May), increasing the flow of low temperature water in summer (Ladrera et al. 2015). As these reservoirs are oligotrophic, the water had low soluble phosphate, giving a competitive advantage to *D. geminata* over native algal species, adapted to the natural conditions that existed prior to dam construction (Bunn and Arthington 2002; Cullis et al. 2012).

The alteration of the natural streamflow in the Lumbreras River by the Pajares Reservoir and the fact that water is discharged from the hypolimnion (which decreases the summer river water temperature) are important factors in understanding why *D. geminata* shows such an increase in biomass in a Mediterranean river.

Light availability, which is favourable to autotrophic periphyton (Whitton et al. 2009; Kilroy and Bothwell 2011; James et al. 2014), was also advantageous to *D. geminata* massive growths in both our intensive and spatio-temporal studies. Increases in *D. geminata* growth under high light conditions seem to be especially effective when water nutrients are scarce (Kilroy and Bothwell 2011). These authors showed that under nutrient-limited conditions, the frequency of *D. geminata* cell division reduces. Photosynthetic production under high light conditions can generate a "fixed carbon overflow" that cells are incapable of storing and so it is exported as extracellular polymeric substances (i.e. stalks). The highest growth rates in our intensive study took place in site Lu1, where there was no shade and the lowest SRP concentrations. The importance of light availability for growth rates was reflected in site Lu2 where, under similar environmental conditions except for a substantial increase in shading (from 5% in Lu1 to 60% in Lu2), periphyton biomass decreased by 70%.

According to our third hypothesis, temporal changes of *D. geminata* growth were linked not only to temperature—water temperature consistently showed relatively low values due to reservoir outflow—but also to high light intensity. The highest growth rate occurred in summer (from June 22nd, although additional growth may have continued past the end of the study), when light intensity was maximum and favoured higher photosynthetic rates in *D. geminata.* These results join general agreement that *D. geminata* is favoured by a combination of high light conditions and low temperature, especially

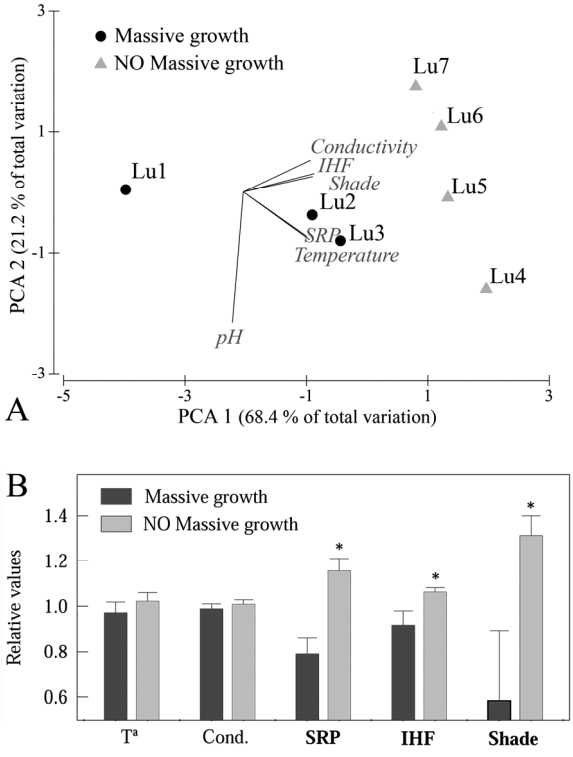

Figure 9. Spatio-temporal study. Principal Component Analysis (PCA) of data from the spatio-temporal study (A). Mean values ± standard error of the environmental variables related to the first axis of the PCA, grouping sites according to massive growth of *D. geminata* presence or absence (B). * mean significant differences ($P < 0.05$) between groups according to Mann-Whitney test.

important at the time of year when stalk formation is beginning (Whitton et al. 2009).

In conclusion, the present study provides new data on *D. geminata* distribution in two Mediterranean basins and yields information of the conditions in which the algae can develop massive growths in Mediterranean streams. However, further studies in other Mediterranean areas could be interesting in order to obtain new information about factors related to *D. geminata* distribution and massive growth development under these climatic conditions. In our study, *D. geminata* living cells were widely present in the two studied basins, especially in mountain areas with the lowest ambient temperatures and water SRP concentrations. Among areas with *D. geminata* living cells, massive growths were related to high light intensity and hydrological regulation, which decreases the temperature and increases (below reservoirs) or maintains (below natural lakes) summer flows of these Mediterranean streams. However, the key factor that enhances massive growth development is low SRP concentration. When SRP content increases in the water, the massive growth disappears since

D. geminata does not need the massive stalk production to obtain phosphate from the water. The SRP threshold value for massive growth development in our study basins is approximately 0.020 ppm, generally higher than values described for other geographical areas, especially for New Zealand Rivers.

We can also conclude that alterations of the natural river regime, which change the environmental factors characteristic of Mediterranean rivers, enhance the growth of *D. geminata*. It makes the development of massive growths possible, thus giving a competitive advantage to this alga over other primary producers. Otherwise the Mediterranean regime, especially the low summer flow and high water temperatures, will pose more difficulties for *D. geminata* massive growth development. Possible management measures for controlling massive growth below reservoirs should be related to changes in management protocols of discharge releases. Delivery of water from the epilimnion (where the temperature is higher) may be a solution to reduce stalk formation of *D. geminata*, together with the restoration of riparian forest downstream of the Pajares Reservoir to reduce light intensity. However, if inorganic phosphate concentration is very low, the control of massive growth could be limited. Experimental changes in the studied basins may provide important lessons to better understand the importance of temperature and light in controlling *D. geminata* massive growth in Mediterranean streams.

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References

Aboal M, Marco S, Chaves E, Mulero I, García-Ayala A (2012) Ultrastructure and function of stalks of the diatom *Didymosphenia geminata*. *Hydrobiologia* 695: 17–24, http://dx.doi.org/10.1007/s10750-012-1193-y

Beltrami ME, Cappelletti C, Ciutti F (2008a) *Didymosphenia geminata* (Lyngbye) M. Schmidt (Bacillariophyta) in the Danube basin: New data from the Drava river (northern Italy). *Plant Biosystems* 142: 126–129, http://dx.doi.org/10.1080/11263500701872788

Beltrami ME, Cappelletti C, Ciutti F, Hoffmann L, Ector L (2008b) The diatom *Didymosphenia geminata*: distribution and mass occurrence in the Province of Trento (Northern Italy). *Verhandlungen International Vereinigung Limnologie* 30(4): 593–597

Blanco S, Bécares E (2009) Una nueva alga invasora llega a Espa-a: *Didymosphenia geminata*. *Quercus* 283: 30–35

Blanco S, Ector L (2008) *Didymosphenia geminata* (Bacillariophyta, Gomphonemataceae), una amenaza para nuestros ríos. *Cuadernos de Biodiverssidad* 26: 3–6, http://dx.doi.org/10.14198/cdbio.2008.26.01

Blanco S, Ector L (2009) Distribution, ecology and nuisance effects of the freshwater invasive diatom *Didymosphenia geminata* (Lyngbye) M. Schmidt: a literature review. *Nova Hedwigia* 88: 347–422, http://dx.doi.org/10.1127/0029-5035/2009/0088-0347

Bothwell ML, Kilroy C (2011) Phosphorus limitation of the freshwater benthic diatom *Didymosphenia geminata* determined by the frequency of dividing cells. *Freshwater Biology* 56: 565–578, http://dx.doi.org/10.1111/j.1365-2427.2010.02524.x

Bothwell ML, Kilroy C, Taylor BW, Ellison ET, James DA, Gillis CA, Bladon KD, Silins U (2012) Iron is not responsible for *Didymosphenia geminata* bloom formation in phosphorus-poor rivers. *Canadian Journal of Fisheries and Aquatic Sciences* 69: 1723–1727, http://dx.doi.org/10.1139/f2012-112

Bothwell ML, Taylor BW, Kilroy C (2014) The Didymo story: the role of low dissolved phosphorus in the formation of *Didymosphenia geminata* blooms. *Diatom Research* 29: 229–236, http://dx.doi.org/10.1080/0269249X.2014.889041

Bunn SE, Arthington AH (2002) Basic Principles and Ecological Consequences of Altered Flow Regimes for Aquatic Biodiversity. *Environmental Management* 30: 492–507, http://dx.doi.org/10.1007/s00267-002-2737-0

Cambra J (1987) Flore et végétation algologiques des eaux épicontinentales de la Réserve Naturelle des vallées d'Ordesa (Pyrénées aragonaises). *Candollea* 42: 475–490

Cambra J (1991a) Contribució an coneixement de les algues epifitiques en estanys dels Pirineus. *Scientia Gerundensis* 17: 17–27

Cambra J (1991b) Diatom check-list from Catalonian Countries (Eastern Spain). Butlletí, Societat Catalana d'Història Natural 8: 5–39

Cullis JDS, Gillis CA, Bothwell ML, 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. *Journal of Geophysical Research* 117(G2): G00N03, http://dx.doi.org/10.1029/2011JG001891

Dosset JA (1888) Sinopsis de las diatomáceas de Aragón y de Caldas de Bohí. Imprenta de R. Miedes, Zaragoza

Ellwood NTW, Whitton BA (2007) Importance of organic phosphate hydrolyzed in stalks of the lotic diatom *Didymosphenia geminata* and the possible impact of atmospheric and climatic changes. *Hydrobiologia* 592: 121–133, http://dx.doi.org/10.1007/s10750-007-0728-0

ENSAYA (2011) Red de Control Biológico en Ríos 2010. Technical report for Ebro Hydrographic Confederation. Zaragoza, 306 pp

ENSAYA (2013) Explotación de la red de control operativo y de referencia de ríos en la Cuenca del Ebro en aplicación de la Directiva Marco del Agua, 2012. Technical report for Ebro Hydrographic Confederation. Zaragoza, 407 pp

ENSAYA (2014) Explotación de la red de control ecológico de ríos en la Cuenca del Ebro en aplicación de la Directiva Marco del Agua, 2013. Technical report for Ebro Hydrographic Confederation. Zaragoza, 523 pp

Gillis CA, Chalifour M (2010) Changes in the macrobenthic community structure following the introduction of the invasive algae *Didymosphenia geminata* in the Matapedia River (Québec, Canada). *Hydrobiologia* 647: 63–70, http://dx.doi.org/10.1007/s10750-009-9832-7

James DA, Mosel K, Chipps SR (2014) The influence of light, stream gradient, and iron on *Didymosphenia geminata* bloom development in the Black Hills, South Dakota. *Hydrobiologia* 721: 117–127, http://dx.doi.org/10.1007/s10750-013-1654-y

Kilroy C, Bothwell M (2011) Environmental Control of Stalk Length in the Bloom-Forming, Freshwater Benthic Diatom *Didymosphenia geminata* (Bacillariophyceae). *Journal of Phycology* 47: 981–989, http://dx.doi.org/10.1111/j.1529-8817.2011.01029.x

Kilroy C, Bothwell ML (2012) *Didymosphenia geminata* growth rates and bloom formation in relation to ambient dissolved phosphorus concentration. *Freshwater Biology* 57: 641–653, http://dx.doi.org/10.1111/j.1365-2427.2011.02727.x

Kilroy C, Bothwell ML (2014) Attachment and short-term stalk development of *Didymosphenia geminata*: effects of light, temperature and nutrients. *Diatom Research* 29: 237–248, http://dx.doi.org/10.1080/0269249X.2014.889043

Kilroy C, Larned ST, Biggs BJF (2009) The non-indigenous diatom *Didymosphenia geminata* alters benthic communities in New Zealand rivers. *Freshwater Biology* 54: 1990–2002, http://dx.doi.org/10.1111/j.1365-2427.2009.02247.x

Kilroy C, Snelder TH, Floerl O, Vieglais CC, Dey KL (2008) A rapid technique for assessing the suitability of areas for invasive species applied to New Zealand's rivers. *Diversity and Distributions* 14: 262–272, http://dx.doi.org/10.1111/j.1472-4642.2007.00406.x

Kirkwood AE, Jackson LJ, McCauley E (2009) Are dams hotspots for *Didymosphenia geminata* blooms? *Freshwater Biology* 54: 1856–1863, http://dx.doi.org/10.1111/j.1365-2427.2009.02231.x

Kirkwood AE, Shea T, Jackson LJ, McCauley E (2007) *Didymosphenia geminata* in two Alberta headwater rivers: an emerging invasive species that challenges conventional views on algal bloom development. *Canadian Journal of Fisheries and Aquatic Sciences* 64: 1703–1709, http://dx.doi.org/10.1139/f07-152

Kolayli S, Sahin BS (2007) A taxonomic study on the phytoplankton in the littoral zone of Karagöl Lake (Borçka- Artvin/Turkey). *Turkish Journal of Fisheries and Aquatic Sciences* 7: 171–175

Kuhajek JM, Wood SA (2014) Novel techniques for the short-term culture and laboratory study of *Didymosphenia geminata*. *Diatom Research* 29: 293–301, http://dx.doi.org/10.1080/0269249X.2014.889042

Kumar S, Spaulding SA, Stohlgren TJ, Hermann KA, Schmidt TS, Bahls LL (2009) Potential habitat distribution for the freshwater diatom *Didymosphenia geminata* in the continental US. *Frontiers in Ecology and the Environment* 7: 415–420, http://dx.doi.org/10.1890/080054

Ladrera R, Prat N (2013) Changes in macroinvertebrate community and biotic indices associated with stream flow regulation and wastewater inputs in Sierra Cebollera Natural Park (La Rioja, Northern Spain). *Limnetica* 32(2): 353–372

Ladrera R, Rieradevall M, Prat N (2015) Massive growth of the invasive algae *Didymosphenia geminata* associated with discharges from a mountain reservoir alters the taxonomic and functional structure of macroinvertebrate community. *River Research and Applications* 31: 216–227, http://dx.doi.org/10.1002/rra.2731

Larned ST, Packman AI, Plew DR, Vopel K (2011) Interactions between the mat-forming alga *Didymosphenia geminata* and its hydrodynamic environment. *Limnology and Oceanography: Fluids and Environment* 1: 4–22, http://dx.doi.org/10.1215/21573698-1152081

Lindstrøm E-A, Skulberg O (2008) *Didymosphenia geminata* - a native diatom species of Norwegian rivers coexisting with the Atlantic salmon. In: Bothwell ML, Spaulding SA (eds), Proceedings of the 2007 International Workshop on *Didymosphenia geminata*. Nanaimo, BC, Canada, 2008. Canadian Technical Report on Fisheries and Aquatic Sciences, pp 35–40

Margalef R (1952) La vida en las aguas dulces de Andorra. *Monografía del Instituto de Estudios Pirenaicos* 69: 1–107

Margalef R (1953) Materiales para la hidrobiología de la Isla de Mallorca. Publicaciones del Instituto de Biología Aplicada de Barcelona 11: 5–111

Margalef R (1956) Estudios hidrobiológicos en los valles de Bohí. In: Actas II Congrès international d'études pyrénéennes. Luchon-Pau, France, September 21–25, 1954. Instituto de Estudios Pirenaicos (Spain) and Centre National de la Recherche Scientifique (France). Volume 3, pp 87–109

Miller MP, McKnight DM, Cullis JD, Greene A, Vietti K, Liptzin D (2009) Factors controlling streambed coverage of *Didymosphenia geminata* in two regulated streams in the Colorado Front Range. *Hydrobiologia* 630: 207–218, http://dx.doi.org/10.1007/s10750-009-9793-x

MMARM (2008) ORDEN ARM/2656/2008, de 10 de septiembre, por la que se aprueba la Instrucción de Planificación Hidrológica. Ministerio de Medio Ambiente y Medio Rural y Marino. Boletín Oficial del Estado, 229. 22 de septiembre de 2008

Munné A, Prat N, Solá C, Bonada N, Rieradevall M (2003) A simple field method for assessing the ecological quality of riparian habitat in rivers and streams: QBR index. *Aquatic Conservation: Marine and Freshwater Ecosystems* 13: 147–163, http://dx.doi.org/10.1002/aqc.529

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural water. *Analytica Chimica Acta* 27: 31–36, http://dx.doi.org/10.1016/S0003-2670(00)88444-5

Pardo I, Álvarez M, Casas J, Moreno JL, Vivas S, Bonada N, Alba-Tercedor J, Jáimez-Cuéllar P, Moyà G, Prat N, Robles S, Suárez ML, Toro M, Vidal-Abarca MR (2002) El hábitat de los ríos mediterráneos. Dise-o de un índice de diversidad de hábitat. *Limnetica* 21(3–4): 115–133

Tomás P, Oscoz J, Durán C, Fernández D, Marín JP, Miranda R, Leunda PM (2010) Distribution of the bloom-forming diatom *Didymosphenia geminata* in the Ebro River basin (Northeast Spain) in the period 2006–2009. *Aquatic Invasions* 5: 285–289, http://dx.doi.org/10.3391/ai.2010.5.3.05

URS (2006) Investigación del origen de depósitos sobre sustratos sumergidos, en el río Ara (cuenca del Cinca). Technical report for Ebro Hydrographic Confederation. Barcelona, 30 pp

Vilaseca JM (1978) Fitoplancton de los lagos pirenaicos. Graduate Thesis, University of Barcelona, Barcelona, 102 pp

Whitton BA, Ellwood NTW, Kawecka B (2009) Biology of the freshwater diatom *Didymosphenia*: a review. *Hydrobiologia* 630: 1–37, http://dx.doi.org/10.1007/s10750-009-9753-5

Supplementary material

The following supplementary material is available for this article:

Table S1. Location of the studied sites.

Table S2. Mean values and range of environmental variables in the four groups established in the regional study.

Table S3. Environmental variables from the spatio-temporal study.

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