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MASSIVE GROWTH OF THE INVASIVE ALGAE *DIDYMOSPHENIA GEMINATA* ASSOCIATED WITH DISCHARGES FROM A MOUNTAIN RESERVOIR ALTERS THE TAXONOMIC AND FUNCTIONAL STRUCTURE OF MACROINVERTEBRATE COMMUNITY

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ABSTRACT

The objective of this paper is to determine the alteration of the taxonomic composition and functional structure of macroinvertebrate community associated with a massive growth of the invasive algae *Didymosphenia geminata* downstream of a mountain reservoir (Pajares Reservoir, La Rioja, Northern Spain). As the massive growth of the alga disappears a few kilometres downstream of the reservoir associated with the input of nutrients from a nearby village sewage, we may compare the community composition between nine stations in three different conditions: three stations heavily affected by the presence of *D. geminata*, three further downstream stations without the algal massive growth but affected by river regulation and three control stations (unregulated and without the algae). Results show a significant disturbance of the composition and structure of macroinvertebrate community in sites affected by the stream flow regulation downstream of the dam compared with unregulated streams, but the alterations are more dramatic in the area where the growth of *D. geminata* is massive because of the total substrate occupation by the algal filaments. Scrapers and others invertebrates living on the coarse substrate are especially affected at such sites. Moreover, an important increase in the relative abundance of chironomids is associated with the algal massive growth, especially in case of *Eukiefferiella devonica* and *Cricotopus spp*., reducing the assemblage diversity and leading to the taxonomic and functional homogenization of the community. Changes in the reservoir management (such as releasing the water from surface rather than from the hypolimnion) may be useful to control the massive growth of *D. geminata* and thus reducing the effects of river regulation on macroinvertebrate assemblage composition. Copyright © 2014 John Wiley & Sons, Ltd.

KEY WORDS: *Didymosphenia geminata*; massive growth; reservoir; phosphorous; temperature; macroinvertebrate community; biological traits; substrate occupancy

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INTRODUCTION

The impact of reservoirs on the ecology of river ecosystems has been well studied. Bunn and Arthington (2002) include the following: (i) alterations in physical properties of rivers, such as temperature or habitat; (ii) changes in the hydrologic regime, influencing the aquatic species, which life history primarily evolved in direct response to the natural flow regimes; (iii) loss of longitudinal and lateral connectivity, since the viability of populations of many aquatic species depends on their ability to move freely through the stream network, and the lateral expansion of floodplain habitats during flooding creates important spawning and foraging areas for many species; and finally, (iv) the invasion and success of exotic species, favoured by the alteration of flow regime, which is the main objective of this paper.

Among the various exotic species enhanced by hydrological regulation of rivers, we find the invasive alga *Didymosphenia geminata* (Lyngbye) M. Schmidt (Kirkwood *et al*., 2009), a unicellular freshwater diatom (Bacillarophycea), capable to produce large amounts of filaments, developing massive growths (Blanco and Ector, 2009). *D. geminata* has been found naturally in alpine and boreal lakes and streams in North of Europe and North America, and their massive growths have been part of the natural cycle in northern latitudes (Pite *et al*., 2009). However, in recent years, massive growths of *D. geminata* have been described in rivers worldwide (Blanco and Ector, 2009; Cullis *et al*., 2012), including in the Iberian Peninsula (Blanco and Bécares, 2009; Blanco and Ector, 2009; Tomás *et al*., 2010).

The rapid expansion of *D. geminata*, along with its ability to fully cover the river bed for several kilometres, has made this alga one of the most harmful invasive organisms in lotic systems worldwide (Blanco and Ector, 2009). Accordingly, alteration of the macroinvertebrate community has been shown in areas affected by the invasion of *D. geminata* (Kilroy *et al*., 2009; Gillis and Chalifour, 2010). However, studies on the interaction between *D. geminata* and macroinvertebrate communities are not common, and the impact that nuisance massive growths have on habitat

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structure and function of stream ecosystems is not totally understood (Kilroy *et al*., 2009; Cullis *et al*., 2012). The effects of *D. geminata* on river ecosystems mainly affects headwater streams, as in the present study, and these ecosystems play an important role in sustaining biodiversity across river network (Finn *et al*., 2011).

Macroinvertebrate communities have often been used as an indicator of the ecological status of rivers (Bonada *et al*., 2006). Usually, the taxonomic composition of the macroinvertebrate community of rivers has been studied to determine the effects of different anthropogenic pressures on the ecosystem. More recently, the use of several biological invertebrate traits (e.g. body size, reproduction, and dispersal) has given new insights of the effects of disturbances on community functional attributes (e.g. Statzner and Bêche, 2010; Monaghan and Soares, 2012; Pascal *et al*., 2012). Moreover, compared with the taxonomic approach, the study of traits presents a higher temporal and spatial stability across regions because the variability attributed to species composition of different areas is reduced using the functional traits (Statzner *et al*., 2004; Bonada *et al*., 2007).

The main objective of the present work is to elucidate the possible alteration of the taxonomic composition and the biological traits selection of the benthic macroinvertebrates associated with a massive growth of *D. geminata* located downstream of a mountain, headwater reservoir. Our first hypothesis is that the alteration associated with massive growth of *D. geminata* will affect both the composition and structure of the macroinvertebrate assemblages (reducing the stream biodiversity and changing the species dominant taxa) selecting taxa with traits that favour survival in a new architectural environment such as the dense mats of filaments of this algae. We also hypothesize that the macroinvertebrate community will recover along the stream depending on the evolution of different physico-chemical parameters that influence the algal development, mainly the temperature [low temperatures increase the algal fitness (Kumar *et al*., 2009)] and nutrients [*D. geminata* growths are better in low phosphorous content water (Kilroy and Bothwell, 2012)].

METHODOLOGY

Study area and sampling strategy

The Lumbreras Stream is a mountain headwater stream located in the Sierra Cebollera Natural Park (La Rioja, Northern Spain), in the Ebro basin (Figure 1). It is the principal tributary of the Iregua Stream in the upper stretch of its catchment, and it is regulated by the Pajares Reservoir, constructed in 1995 and with a capacity of 35 hm^3. It is primarily used for water storage from September to June, while water is released during the irrigation period, mainly in July and August, changing completely the natural hydrograph (Figure 2).

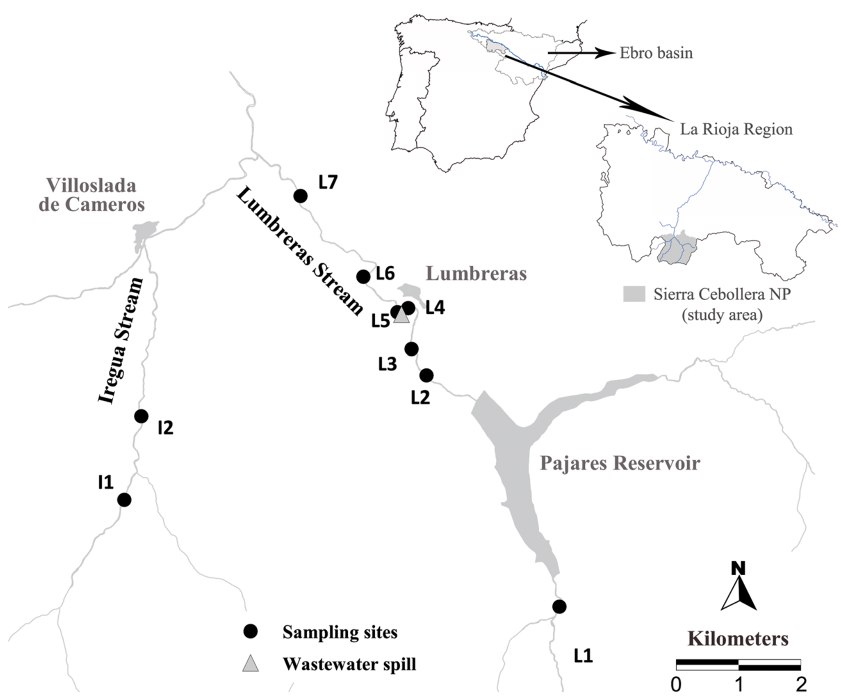

Figure 1. Sampling sites in the study area

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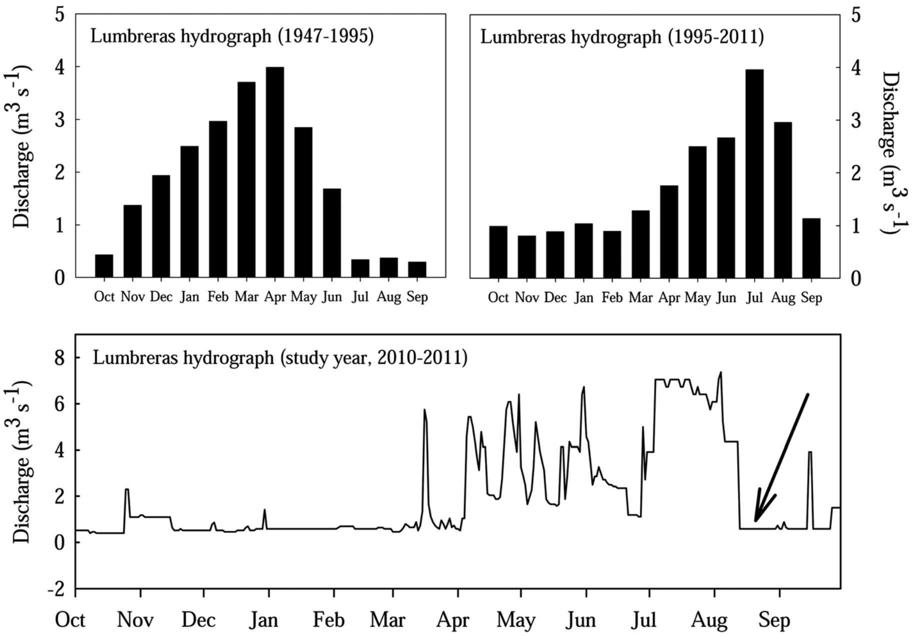

Figure 2. Lumbreras Stream discharge downstream of the Pajares Reservoir for the period 1947–1995 (before the building of the Pajares Reservoir); the period 1995–2011 (after the building of the Pajares Reservoir); and for the hydrological year of the study, 2010–2011. Arrow shows the date of the sampling period

Samples were taken in late august, 5 days after the end of the period of high flow in the Lumbreras Stream downstream of Pajares Reservoir, in nine sites (Figure 1). From these sites, three localities were selected as control, (not affected by the dam), while six sites were below the dam. The three more closely to the dam were heavily affected by the massive growth of *D. geminata*, whereas in the other three, further downstream, the massive growth of *D. geminata* was not apparent.

Environmental variables and macroinvertebrate sampling

Several water physico-chemical parameters, such as temperature, pH, conductivity and dissolved oxygen, were measured *in situ*, whereas total organic carbon (TOC), total sulfur content (S), NH_4^+ (NH4), NO_3^- (NO3), Soluble Reactive Phosphorus (SRP–PO4), Cl^- (Cl), SO_4^{2-} (SO4), K^+ (K), Ca^{2+} (Ca), Na^+ (Na) and Mg^{2+} (Mg) were analysed in the lab following ASTM standard methods (ASTM, 1995). At the same time, four hydromorphological variables were analysed at each site: (i) the quality of riparian habitat, which was characterized using the four components of the Qualitat del Bosc de Ribera (or Riparian Forest Quality) index of Munné *et al*. (2003); (ii) the fluvial in-stream habitat, which was characterized by the Índice de Hábitat Fluvial (or River Habitat Index; Pardo *et al*., 2002); (iii) the hydrological regime, grouping studied sites in two groups, affected or not by the hydrological regulation of the dam; and (iv) the biomass of *D. geminata*, expressed as the algal dry weight per stream bed area (g/m^2). We used the amount of *D. geminata* remaining in the Surber nets used for macroinvertebrate sampling. After sorting all macroinvertebrates, the filaments of *D. geminata* were dried for 72 h at 70 ºC for dry weight determinations.

Multi-habitat samples for the analysis of the macroinvertebrate community were collected using a 250-μm surber net according to the MacroInvertebrats QUantitatiu or MacroInvertebrates QUantitative (MIQU) sampling protocol designed by the Freshwater Ecology and Management research group of the University of Barcelona (Nuñez and Prat, 2010). This method takes eight surber (30 cm × 30 cm) samples in dominant substrates (covering each of them more than 5% of the sampling area) and four in marginal ones (less than 5%). Samples were taken proportionally to the presence of 11 different substrates classes and were stored in two buckets, one for dominant and another for marginal substrates. Samples were preserved in 4% formaldehyde and taken to the laboratory to be identified. The identification of macroinvertebrates was made generally to genus level, including the Chironomidae, except for some Diptera subfamilies, the Oligochaeta, Hydracharina and Ostracoda. If necessary, sub-sampling was performed in the sorting process and at least 300 individuals per sample were counted. Metrics indicating the effects of the two disturbances in community structure were calculated following Munné and Prat (2009).

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Biological traits

Six biological traits containing 45 categories obtained from a published database (Tachet *et al.*, 2006) were used to describe the macroinvertebrate community functional structure (Table I). The traits in this database have an affinity score assigned for each taxa ranging from 0 to 5, from null affinity to high affinity, respectively (Chevenet *et al.*, 1994). To analyse the functional structure, a dataset of relative abundance of traits per sample was built, for which the affinity of each taxon for each trait category was multiplied by the taxon abundance (Chevenet *et al.*, 1994).

Data analysis

To establish the main links between environmental variables and macroinvertebrate community, a Distance-base Linear Model (DISTLM) analysis was performed (PERMANOVA + for PRIMER) (Anderson *et al.*, 2008). The distance-based Redundancy Analysis (dbRDA) plot was used to visualize

Table I. Biological traits and categories studied according to Tachet *et al.* (2006)

Biological trait	Category	Code
Food	Microorganisms + fine sediment	microorg + sed
	Detritus < 1 mm	det < 1
	Dead plant ≥ 1 mm	dead_plant
	Living microphytes	microphyt
	Living macrophytes	macrophyt
	Dead animal ≥ 1 mm	dead_animal
	Living microinvertebrates	microinvert
	Living macroinvertebrates	macroinvert
	Vertebrates	vertebrates
Feeding habits	Absorber	absorber
	Deposit feeder	dep-feeder
	Shredder	shredder
	Scraper	scraper
	Filter-feeder	filt-feeder
	Piercer	piercer
	Predator	predator
	Parasite	parasite
Microhabitat	Flags/boulders/cobbles/pebbles	bou-cob-peb
	Gravel	gravels
	Sand	sand
	Silt	silt
	Macrophytes	macrophytes
	Microphytes	microphytes
	Twigs/roots	twigs_root
	Organic detritus/litter	detrit-litter
	Mud	mud
Flow speed	Null	null
	Slow (<25 cm/s)	slow
	Medium (25–50 cm/s)	medium
	Fast (>50 cm/s)	fast
Locomotion and substrate relation	Flier	flier
	Surface swimmer	surf-swim
	Full water swimmer	swimmer
	Crawler	crawler
	Burrower	burrower
	Interstitial	interstitial
	Temporarily attached	tem-attached
	Permanently attached	per-attached
Maximal size	≤0.25 cm	≤0.25 cm
	0.25–0.5 cm	0.25–0.5 cm
	>0.5–1 cm	>0.5–1 cm
	>1–2 cm	>1–2 cm
	>2–4 cm	>2–4 cm
	>4–8 cm	>4–8 cm
	>8 cm	>8 cm

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the final model from DISTLM analysis using CANOCO 4.5 software (Ter Braak and Smilauer, 2002). In the dbRDA plot, we show only the taxa with Spearman's correlation coefficient higher than 0.8 and 0.7 with the first and second axes of the plot, respectively. A hierarchical cluster analysis (Unweighted Pair Group Method using Arithmetic averages) based on macroinvertebrate communities (software R, package vegan) has been also performed. The resulting groups from the cluster analysis are indicated in the dbRDA plot with different symbols in the same figure (Figure 4).

Finally, for each macroinvertebrate metric and for each biological trait category, the mean and standard error of different site groups previously determined by the cluster analysis were calculated. Finally, a nonparametric pairwise U'Mann Whitney test was performed to detect differences between groups of metrics or of biological traits in the groups defined in the cluster analysis.

RESULTS

Biomass of Didymosphenia geminata

Didymosphenia geminata massive growth affected 2.3 km of the Lumbreras Stream, starting 200 m below Pajares Reservoir and was maintained until the entrance of the discharge of the untreated sewage spill coming from Lumbreras Village. In the first part of the stream, (sites L2, L3 and L4) mats of *D. geminata* covered almost all available benthic substrates, especially boulders, cobbles and pebbles, with biomass ranging between 3.5 and 6.5 g/m^2. After the wastewater spill of Lumbreras Village, *D. geminata* massive growth completely disappeared, and, as a result, 50 m below the spill no mats of *D. geminata* could be detected in the stream, being only very marginally present in L5 and L6 sampling sites (Figure 3).

Downstream of the spill, the concentration of SRP–PO4 resulted in a sevenfold increase with respect to the concentration upstream (from 0.014 to 0.099 p.p.m.). Apart from SRP–PO4, minor or no changes was detected in the other analysed parameters downstream of the wastewater discharge. Table II shows the values of those parameters for which an increase higher than 25% was detected. The concentration of the other chemical compounds was very similar before and after the sewage plant (e.g. conductivity changes from 71 to 72 μS/cm). Temperature of the studied sites has also been included in Table II to show the significant differences between sites affected or not by the streamflow regulation. The mean values ± standard error of the other physico-chemical parameters were as follows: NO3 = 0.806 ± 0.009 p.p.m.; NH4 = 0.024 ± 0.005 p.p.m.; SO4 = 5.142 ± 0.292 p.p.m.; Ca = 8.128 ± 0.844 p.p.m.; Mg = 1.116 ± 0.061 p.p.m.; pH = 8.156 ± 0.143; DO = 8.181 ± 0.280 p.p.m.; conductivity = 72.000 ± 1.286 μS/cm.

Therefore, the phosphorous (Table II) resulted the unique physico-chemical parameter in which could be detected an important change downstream of the spill coinciding with the end of the massive growth of *D. geminata*. Figure 3 shows the SRP–PO4 concentration increase and the concomitant *D. geminata* decrease in L5 site, 100 m below the spill coming from Lumbreras Village.

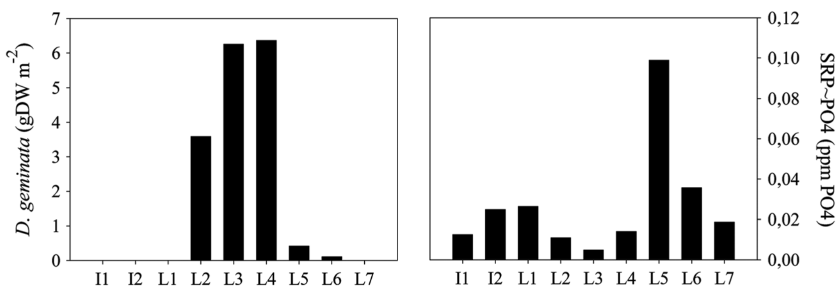

Figure 3. *D. geminata* biomass and SRP–PO4 concentration in the nine sampling sites. DW, dry weight

Table II. Values of the environmental variables that showed relevant differences among the studied sites

	I1	I2	L1	L2	L3	L4	L5	L6	L7
Tª (°C)	22.400	22.100	20.700	10.700	11.900	11.900	9.500	13.900	13.900
Cl (p.p.m.)	4.450	1.900	3.010	7.000	9.280	8.410	11.370	10.330	9.910
Na (p.p.m.)	1.950	1.910	3.380	4.360	5.160	3.990	6.560	6.650	5.670
K (p.p.m.)	0.610	0.750	0.890	0.550	0.590	0.610	1.020	0.900	0.680
S (p.p.m.)	1.650	1.790	1.780	1.120	1.270	1.100	1.400	1.540	1.330
TOC (p.p.m.)	1.530	1.350	1.100	3.650	2.640	2.950	4.090	4.580	2.920
PO4 (p.p.m.)	0.012	0.025	0.026	0.011	0.005	0.014	0.099	0.036	0.019

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Macroinvertebrate community

Stream flow differences due to regulation and the biomass of *D. geminata* resulted in the only two significant variables explaining the distribution of the invertebrate communities, according to DISTLM analysis (Figure 4). The nine sites studied were grouped by the cluster analysis in three groups according to the affection or not by the dam regulation and the presence or not of the algal massive growth: Group A includes the control sites, not affected by the reservoir nor the algal massive growth (white spots in Figure 4); Group B are the sites heavily affected by the massive growth of *D. geminata* (black spots in Figure 4); and the sites affected by dam regulation and sewage pollution (without massive growth of *D. geminata*) cluster together in Group C (grey spots in Figure 4).

The taxa with positive higher correlation with the first axis of dbRDA plot were those present in the control sites but absent or in very low relative abundance in sites affected by dam regulation, as is the case of *Gerris sp.*, *Caenis sp.*, *Synorthocladius semivirens* and *Larcasia partita* (Figure 4). On the other hand, the abundance of many taxa was significantly reduced in sites where the massive growth of *D. geminata* was present. In the sites altered by dam regulation, many reophilous taxa were present as *Epeorus sp.*, *Ancylus fluviatilis*, *Liponeura sp.*, *Drusus sp.* and the Simuliini. Finally, the abundance of some taxa increased in sites affected by the massive growth of *D. geminata*, as was the case of three midge taxa (*Eukiefferiella devonica*, *Potthastia longimana* and *Cricotopus spp.*), together with *Hydra sp.* and the dipteran empidid subfamily Clinoceriinae (Figure 4).

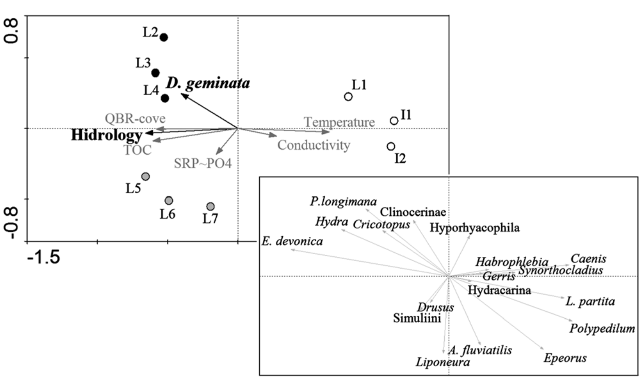

Figure 4. Distance-based Redundancy Analysis plot result from DISTLM analysis that includes also the results of the cluster analysis. Three groups of sites are indicated: the control sites (Group A, white spots), the algal affected sites (Group B, black spots) and the downstream sites affected by river regulation but without *D. geminata* massive growth (Group C, grey spots) Variables included in the final model are figured, and those significant ($p < 0.05$) are highlighted in black. Final model explained 96% of total variance (first axis 51.5% and second one 15.2%). Macroinvertebrate taxa with the highest Spearman correlation coefficient with the first two axes are represented at the lower right angle of the figure. QBR-cove, component of Qualitat del Bosc de Ribera (index of riparian quality) referring to the proportion of riparian area covered by trees and shrubs; TOC, total organic carbon (p.p.m.); SRP–PO4, concentration of soluble reactive phosphorus (p.p.m.)

From the different metrics based on macroinvertebrate community measured (Figure 5), density was not affected by the streamflow regulation, but a decrease of taxa richness, Simpson diversity index, Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa richness and EPT abundance was detected in sites affected by streamflow regulation. The massive growth of *D. geminata* did not affect either the density or the total richness of macroinvertebrates but did decrease the Simpson diversity index and increase the relative abundance of chironomids. The relative abundance of EPT taxa showed also a tendency to decrease in the presence of *D. geminata* (Figure 5). Significance of differences between the three groups of sites and for each metric are indicated in Figure 5.

Biological traits

A decrease of taxa adapted to null water velocity (Figure 6) was detected downstream of the Pajares Dam, and the locomotion mode and the substrate relation traits were different from other sites. At the same time, the percentage of burrowers and taxa adapted to live in the interstitial zone increased downstream of the dam, whereas swimmers and crawlers significantly decreased. One of the major disturbances detected in sites affected by the streamflow regulation was on the trait 'maximal size', while the percentage of organisms with body length less than 0.5 cm decreased, and the relative abundance of larger individuals increased (Figure 6).

Sites affected by the massive growth of *D. geminata* showed an important decrease of scrapers and an increase of predators (Figure 6). In these reaches, the percentage of taxa adapted to live in coarse substrate, as boulders, cobbles or pebbles, decreased, whereas the density of organisms capable of living over macrophytes or other vegetal structures as twigs or roots increased (*D. geminata* filaments in this case). The flow speed-related traits also resulted clearly altered in stream reaches affected by the massive growth of *D. geminata*, increasing the percentage of taxa adapted to slow velocity and decreasing the relative abundance of those living in high flow-velocity habitats. At the same time, organisms with the largest body length increase, whereas crawlers showed a tendency to decrease. Significance of differences between the three groups of sites and for each trait category is indicated in Table III.

DISCUSSION

Effects of Didymosphenia geminata growth

Our first hypothesis was corroborated, and, as it was expected, the development of *D. geminata* resulted in a

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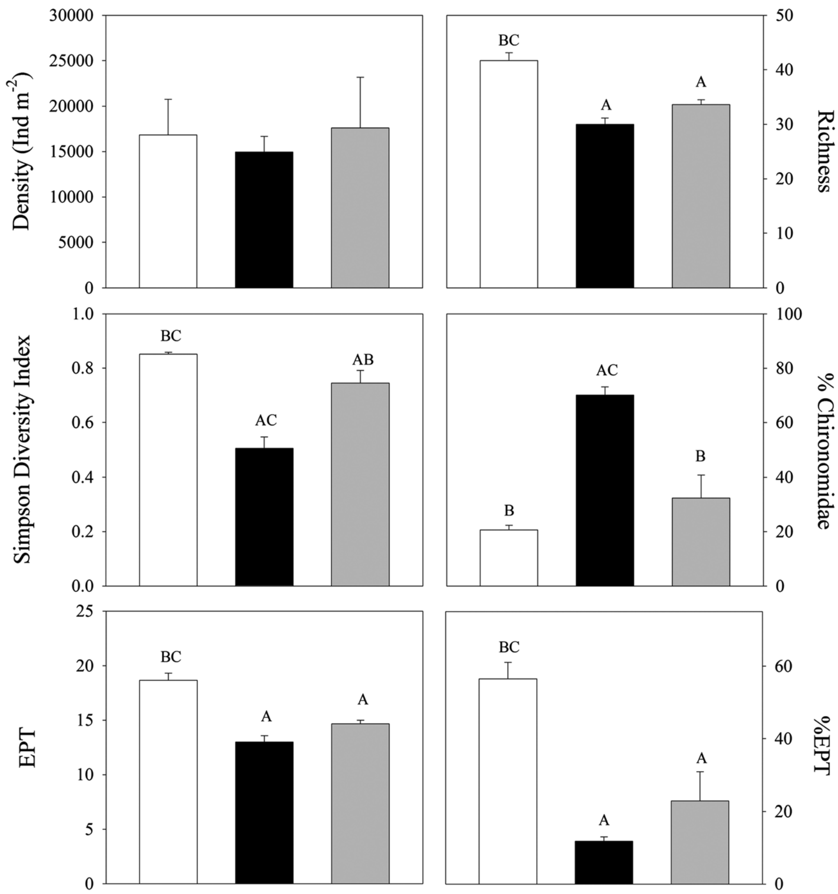

Figure 5. Mean ($n = 3$) and standard error of different metrics calculated for the three groups previously determined by cluster analysis: Group A (Control) in white bars, Group B (Affected by the algal massive growth) in black bars and Group C (Affected by the reservoir but without *D. geminata*) in grey bars. Letters above bars indicate the groups significantly different ($p < 0.05$) according to U'Mann Whitney test and using the coding letters of each group (A, B and C). EPT, Ephemeroptera, Plecoptera and Trichoptera

significant alteration of the riverine ecosystem. According to other studies (Larson, 2007; Spaulding and Elwell, 2007; Kilroy *et al*., 2009; Gillis and Chalifour, 2010), *D. geminata* was associated in the Lumbreras Stream (Figure 5) with an important increase of the relative abundance of chironomids and with a clear decrease of macroinvertebrate diversity, leading to the homogenization of the community. Two chironomids taxa, *Eukiefferiella devonica* and *Cricotopus spp*., living usually among mats of filamentous algae (Mundie, 1957; Storey, 1987; Ferrington and Berg, 2008) seem to be especially favoured by the algal dominance. This is the first time that chironomids are identified to genus level in rivers affected by *D. geminata*.

The community disturbance of sites affected by *D. geminata* was also clearly visible in the biological traits of taxa selected in the area where the filaments of this alga dominate, resulting in an important decrease of scrapers and an increase of predators. The disadvantage of scrapers in algal affected reaches could be related with the reduction of nutritional food in the river as consequence of several reasons: (i) the rapid colonization and the structure of *D. geminata* filaments made impossible the grazing over hard substrates of many scrapers (e.g. the limpets or some Ephemeroptera); (ii) the composition of *D. geminata* filaments, containing a high percentage of polysaccharides (Gretz, 2008), which are not palatable or nutritious as other forms of algae with higher lipid and/or protein content (Blanco and Ector, 2009); and (iii) the rapid expansion of *D. geminata* mats, leading to the exclusion of other algae species from hard surfaces (Blanco and Bécares, 2009; Kilroy *et al*., 2009), which could be an important source of food for scrapers. The predators increase may be related with the sharp increase of chironomids abundance (Allan and Castillo, 2007), as midges are an important source of food for predators (Armitage, 1995). In this case, most abundant predators were not only the small-size predators (*Hydra sp*., *Dugesia sp*.) but also the Diptera Tipulidae and Clinoceriinae. The last three are capable to move in between the dense net of algal filaments and capturing the abundant chironomid fauna.

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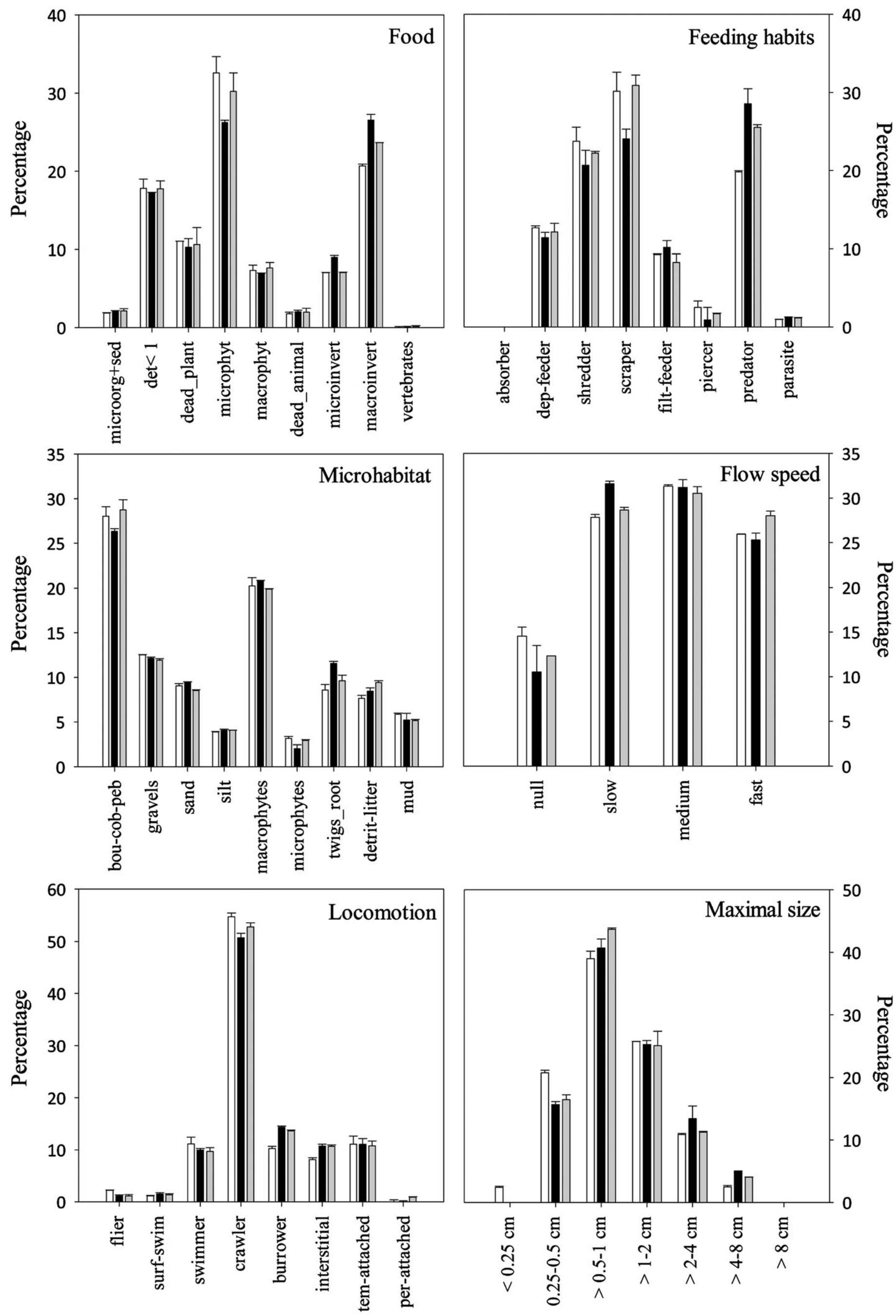

Figure 6. Mean ($n = 3$) and standard error of different biological trait categories calculated for the three groups previously determined by cluster analysis. The bars symbols are as in Figure 5

The altered composition of the macroinvertebrate community in sites affected by the massive growth of *D. geminata* is due mainly to the alteration of the stream habitat (Larson, 2007), as a consequence of the total substrate occupation. According to other authors (Kirkwood *et al*., 2007; Kilroy and Bothwell, 2012), the coarse substrates, especially boulders, cobbles and pebbles, were particularly affected by *D. geminata* growth, because this material is very stable in headwater streams (Kirkwood *et al*., 2009; Kumar *et al*., 2009; Cullis *et al*., 2012). The total substrate occupation resulted in a decrease of the relative abundance of the taxa adapted to live on the coarse material, whereas taxa living on macrophytes and other small plant structures, as roots and twigs, were enhanced by the presence of *D. geminata*

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Table III. Statistical significance of biological trait analysis between groups, graphically represented in Figure 6. Letters indicate the groups significantly different ($p < 0.05$) respect to each group according to U'Mann Whitney test and using the following coding letters: A, control group; B, sites affected by *D. geminata*; C, sites affected by river regulation but without algal massive growth. n.s., no significant differences

Biological trait	Category	Group A	Group B	Group C
Food	Microorganisms + fine sediment	BC	A	A
	Detritus < 1 mm	B	A	n.s.
	Dead plant ≥ 1 mm	n.s.	n.s.	n.s.
	Living macrophytes	B	A	n.s.
	Living microphytes	n.s.	n.s.	n.s.
	Dead animal ≥ 1 mm	n.s.	n.s.	n.s.
	Living microinvertebrates	B	AC	B
	Living macroinvertebrates	B	AC	B
	Vertebrates	n.s.	n.s.	n.s.
Feeding habits	Absorber	n.s.	n.s.	n.s.
	Deposit feeder	n.s.	n.s.	n.s.
	Shredder	C	n.s.	A
	Scraper	B	AC	B
	Filter-feeder	B	A	n.s.
	Piercer	C	n.s.	A
	Predator	BC	AC	AB
	Parasite	BC	A	A
Microhabitat	Flags/boulders/cobbles/pebbles	B	AC	B
	Gravel	BC	A	A
	Sand	C	C	AB
	Silt	n.s.	n.s.	n.s.
	Macrophytes	B	A	n.s.
	Microphytes	n.s.	n.s.	n.s.
	Twigs/roots	B	AC	B
	Organic detritus/litter	B	A	n.s.
	Mud	C	n.s.	A
Flow speed	Null	C	n.s.	A
	Slow (<25 cm/s)	B	AC	B
	Medium (25–50 cm/s)	n.s.	n.s.	n.s.
	Fast (>50 cm/s)	C	C	AB
Locomotion and substrate relation	Flier	BC	A	A
	Surface swimmer	n.s.	n.s.	n.s.
	Full water swimmer	B	A	n.s.
	Crawler	BC	A	A
	Burrower	BC	A	A
	Interstitial	BC	A	A
	Temporarily attached	n.s.	n.s.	n.s.
	Permanently attached	n.s.	n.s.	n.s.
Maximal size	≤0.25 cm	BC	A	A
	0.25–0.5 cm	BC	A	A
	>0.5–1 cm	C	n.s.	A
	>1–2 cm	n.s.	n.s.	n.s.
	>2–4 cm	BC	AC	AB
	>4–8 cm	BC	AC	AB
	>8 cm	n.s.	n.s.	n.s.

filaments, experiencing an increase in their percentage. In relation to the habitat alteration described, it has been shown that stalk production and colony coalescence in *D. geminata* reduces near-bed turbulence, which is likely to prevent dislodgement of communities under high velocity (Larned *et al*., 2011). In this sense, our results demonstrate an increase in the relative abundance of taxa adapted to live in slow-flowing areas as are sites affected by *D. geminate* growth.

Finally, it should be noted that climber taxa, as Simuliini, *Liponeura sp*. or *Ancylus fluviatilis* (Tachet *et al*., 2006), showed a significant decreased in reaches affected by the algal growth. These taxa are present mainly in the downstream sites of Lumberas stream where the algal growth resumes (Group C sites; Figure 6). Negative effects of *D. geminata* on macroinvertebrates living on rock surfaces or fixed to the substrate have been previously described

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(Larson, 2007), and it is due to the loss of suitable habitat because the nuisance algae covers the stream bottom and because the mats trap fine sediment also reduces the substrate suitable for attachment of fixed filter-feeder or climber macroinvertebrates (Larson, 2007). However, not all attached organisms were negatively affected by *D. geminata* because it resulted favourably for *Hydra sp*. because of its capacity to live attached to branches or other plant structures (Kovacevic, 2012).

Community recovery and the importance of river regulation

We should emphasize that the massive growth of *D. geminata* seems to be enhanced by the river regulation through the combination of stable flow environment, lower temperatures and low phosphorous content, which are the main factors that allow the diatom to form dense mats (Kirkwood *et al*., 2007, 2009; Kumar *et al*., 2009; Kilroy and Bothwell, 2012). The flow conditions and the temperature did not change significantly along sample sites below the reservoir. However, the phosphorous content increased sevenfold after the Lumbreras Village spill, coinciding with the disappearance of the algal mats. This results are according with other studies that suggest that this alga has a competitive advantage in areas where the concentration of phosphorous is very low (Miller *et al*., 2009; Kilroy and Bothwell, 2012) thanks to the numerous phosphatases present on the surface of its filaments (Ellwood and Whitton, 2007).

We have already discussed the effects of *D. geminata*, but does the invertebrate community recover when the algal massive growth disappears? Our second hypothesis predicts this recovery. To test this hypothesis, the effects of the river regulation should be analysed by comparing control sites (Group A) with downstream reservoir sites not affected by the algal massive growth (Group C).

Our results show clear differences in community composition and functional attributes of the macroinvertebrate community between sites of Group A and Group C. Thus, the Simpson diversity index, total and EPT richness, and the abundance of EPT taxa, decreased in the regulated stream respect the control sites, which are similar to the results obtained by other studies (Horsák *et al*., 2009; Navarro-Llácer *et al*., 2010).

The main alteration associated with the reservoir were consequence of the streamflow generated downstream of it. The reservoir is used for summer irrigation, with discharges up to 10 times higher than the streamflow when the irrigation time is finished (as was the case of our samples, taken just 5 days after the resume of the maximum flows). These kind of high flow episodes have been previously identified as one of the most important factor altering benthic biota in streams (Imbert and Perry, 2000; Lytle, 2000; Robinson *et al*., 2003; Bruno *et al*., 2010; Kimura *et al*., 2011). Accordingly, in the present study, the relative abundance of those taxa adapted to live in null or low-speed current areas declined in all six sites affected by hydrological regulation (e.g. *Gerris sp*.).

Another trait particularly affected by the hydrological regulation was the locomotion mode, clearly dependent on the existing flow (Tomanova and Usseglio-Polatera, 2007; Statzner and Bêche, 2010). The streamflow regulation led to a decline in the percentage of swimmers and crawlers, especially those exposed and sensitive to the high current speed existing 5 days before the sampling day (Snook and Milner, 2002; Horrigan and Baird, 2008; Cid, 2010). Likewise, burrowers and interstitial organisms increased their relative abundance below the reservoir, which could be related to the capacity of these organisms to avoid the high flow rates, using the substrate as an efficient refuge (Resh *et al*., 1988; Poff and Ward, 1990; Dole-Olivier *et al*., 1997).

Finally, sites affected by streamflow regulation experienced a decrease in the relative abundance of small body size taxa and an increase of large-size organisms. This trend is consistent with other studies (Mérigoux and Dolédec, 2004) and has been associated with different anatomical or behavioural strategies that allow large body-size taxa (with stronger attachment structures) to withstand high hydraulic stress. However, the association between hydraulic conditions and body size is not always clear because parameters such as morphological adaptation, substrate characteristics or lifespan can intervene (Mérigoux and Dolédec, 2004; Statzner *et al*., 2004; Statzner, 2008; Statzner and Bêche, 2010).

Therefore, clear differences between control sites and those affected by the reservoir but not by the algal massive growth existed, which show the alteration associated with the reservoir, mainly because of its hydrological regulation. However, these sites have a more diverse community respect to those reaches affected by *D. geminata*. Evidences that a clear community recovery is produced when the massive growth disappeared (after the sewage plant) include the decline in the percentage of chironomids to reach values similar to control sites. In addition, the relative abundance of scrapers and organisms adapted to live on boulders, cobbles and pebbles recovered with the disappearance of the algal massive growth, whereas the percentage of organisms adapted to live on roots and other plant structures, as *D. geminata* filaments, decreased to values similar to those in control sites. Finally, it should be note that the DISTLM analysis clearly confirmed the differences between sites below reservoir depending if they are affected or not by *D. geminata*. So according to our hypothesis 2, when the algal massive growth disappears in downstream areas with similar temperature but higher levels of nutrients, the

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macroinvertebrate community partially recovers, with some metrics similar to the control sites, while others are still significantly different because of the effects of river regime alteration (Figures 4 and 5).

In conclusion, an important alteration of the macroinvertebrate community associated with the Pajares Reservoir exists in the Lumbreras Stream. This alteration is especially related to the hydrological regime changes due to regulation that encouraged the massive growth of the invasive alga *D. geminata* (higher flows, lower temperatures and very low phosphorous concentrations). The present work represents the first study that focuses on the effects of this algal massive growth on the functional structure of the macroinvertebrate community, along with the taxonomic composition to genus level. Our results indicate that sites affected by the massive growth of *D. geminata* experienced a more intense alteration of the stream community compared not only to control but also to the reaches affected by the flow regulation. The community affection by *D. geminata* was related to the full occupancy of the streambed by algal filaments, leading to an intense change in the environmental conditions of the fluvial ecosystem. From the management point of view, we suggest that changes in the reservoir management practices (such as releasing the water from surface rather than from the hypolimnion, which will reduce the effect of temperature alteration) may be useful to control the *D. geminata* massive growth and thus reducing the effects of river regulation.

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