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Distribution of the bloom-forming diatom *Didymosphenia geminata* in the Ebro River basin (Northeast Spain) in the period 2006-2009

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Abstract

This paper presents the distribution and the ecological parameters of *Didymosphenia geminata* in the Ebro River basin (Northeast Spain) during the period 2006-2009. The presence of blooms in some rivers in 2009, as in Cinca River in Las Pilas bridge, is reported. *D. geminata* was found in summer at 24 localities, forming blooms in three of them. It prefers mountainous calcareous rivers, regulated or not, with oligotrophic waters, low flow conditions and well illuminated stretches. In order to prevent nuisance blooms of the species, it is necessary to study its ecology and establish a monitoring network for detecting early stages of blooms in order to take control measures.

Key words: *Didymosphenia geminata*, diatom, ecological parameters, nuisance organism, Ebro basin

Introduction

The algae, *Didymosphenia geminata* (Lyngbye) Schmidt, 1899, is a freshwater stalked diatom characterized by having large triondulate frustules (Figure 1). *D. geminata* (didymo) forms fibrous masses of yellow-brown, more than 3 cm thick, like wool or cellulose aggregates, although its colour varies depending on the type of sediments retained (Kilroy 2004). It was originally described in the Faroe Islands in 1819 by Lyngbye, and its fossil record and biogeographic studies show that its original distribution only covered the Northern Hemisphere, always at latitudes above 30°N (Blanco and Ector 2009). However, different studies showed that didymo is expanding its ecological range in environmental conditions (Kawecka and Sanecki 2003; Kilroy 2004; Spaulding and Elwell 2007). In the last few decades it has occurred more and more frequently across worldwide freshwater ecosystems and it has been cited in over 50 countries.

Didymo blooms were reported in their areas of origin in the 19th century (e.g. in the British Isles) and it seemed to be somewhat common (Whitton et al. 2009). However, blooms are increasing in Eurasian and North American areas, where they had not formed before, and these have caught the attention of researchers in the last decades. There are many studies and theories about factors leading to the formation of massive growth, for instance regulation of rivers, genetic variation, climate change, changes in land use in the watershed or changes in the N: P balance (see Whitton et al. 2009). These blooms can cover the substrate of the riverbed along several kilometres, so that this diatom is likely to have significant impacts on native species and biodiversity through ecological processes (Campbell 2005; Shearer and Erickson 2006; Larned et al. 2007; Spaulding and Elwell 2007; Larson 2008; Gillis and Chalifour 2010). Didymo is considered invasive in several countries, as in New Zealand where it was declared as an invasive nuisance in 2004.

In the Iberian Peninsula this species has been found in some localities of the Duero River basin (Blanco and Bécares 2009) and there are historical records of its presence in the Pyrenees and Mallorca (Margalef 1953; URS 2006). A complete list of records can be found in Blanco and Ector (2009). The first observations of massive growth of *D. geminata* in the Ebro River basin was in 2005 in the Ara River upstream of Ainsa, but it was not confirmed that they were due to *D. geminata* until the summer of 2006, with the new appearance of the species mass, (URS 2006). In 2006, it was also recorded from the Subordán River in Hecho (Ortiz and Cambra 2006). In subsequent years it has appeared in low abundance in several samples of biological control networks of the Confederación Hidrográfica del Ebro, although it is noted that the sampling protocol applied, underestimates the presence of this diatom (Kirkwood et al. 2007).

The objective of this study is to contribute to the knowledge of the presence and formation of blooms of *D. geminata* in the Ebro River basin, providing new data on its distribution in this area.

Materials and methods

A description of the Ebro River basin can be found in Oscoz et al. (2010). During summer biological sampling of the CEMAS network to assess the water quality of the Ebro River Basin in the period 2006-2009, 2006 (n= 211), 2007 (n= 323), 2008 (n= 343), 2009 (n= 362), a total of 1239 stations, some of them were visited annually. In each station samples of macroinvertebrates, diatoms and macrophytes were taken. The sampling and the analysis of the diatom samples were done according to CHE protocols (2005). At least five cobbles or small boulders were collected from unshaded areas within the main flow and free from obvious filamentous algae or siltation, and the upper surface of the stones was brushed with a toothbrush to remove the diatom film. The toothbrush was rinsed in a container with water. The sample was fixed with 4% formalin. In the literature (Spaulding and Elwell 2007) it is stated that this sampling and analytical method is inadequate to pick it up, therefore the valves detected in the samples of macrophytes were also recorded. In the laboratory the samples were oxidized with hydrogen peroxide and mounted on a slide with naphrax, for more details see the protocol. At least 400 valves were counted.

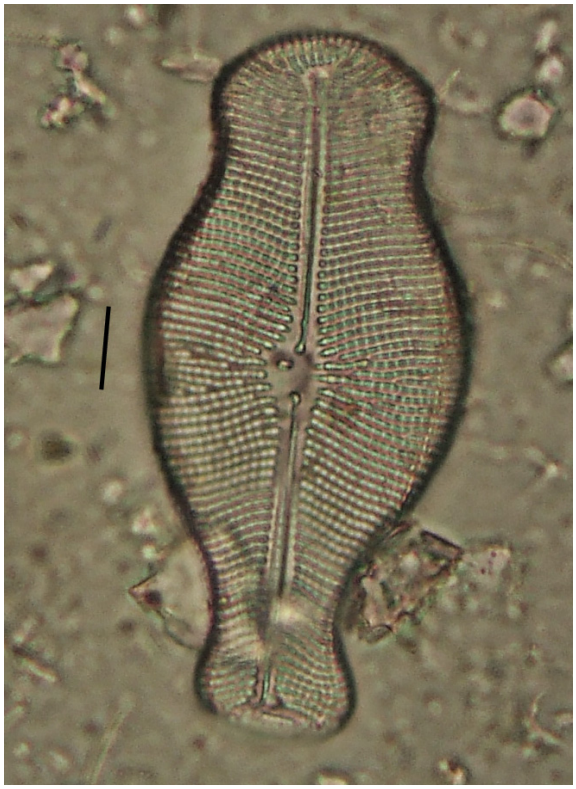

Figure 1. *Didymosphenia. geminata* from Gállego River (code 0808 in Sta. Eulália) (Scale Bar: 10µm, photograph by JPM).

Physico-chemical parameters (pH, temperature, conductivity and dissolved oxygen) were obtained in situ with a multiparametric sonde YSI 556MPS. Water samples were taken and, according to the methodologies established in the ITC-MMA.EECC-1/06 (Ministry of the Environment 2006), they were conserved at 4ºC in order to analyse the following parameters in the laboratory: nitrates, nitrites, phosphates and silica. Other hydromorphological features such as regulation by dams, flow and turbulence, type of substrata and illumination of the riverbed were assessed qualitatively, according to the ranges established in Pardo et al. (2002). After sampling all the used material was disinfected according to the protocols of disinfection of the Ebro River basin to prevent the spread of the zebra mussel (*Dreissena polymorpha* (Pallas, 1771)) and other organisms (CHE 2007). All sampling was carried out following a stated period of steady flows (four weeks).

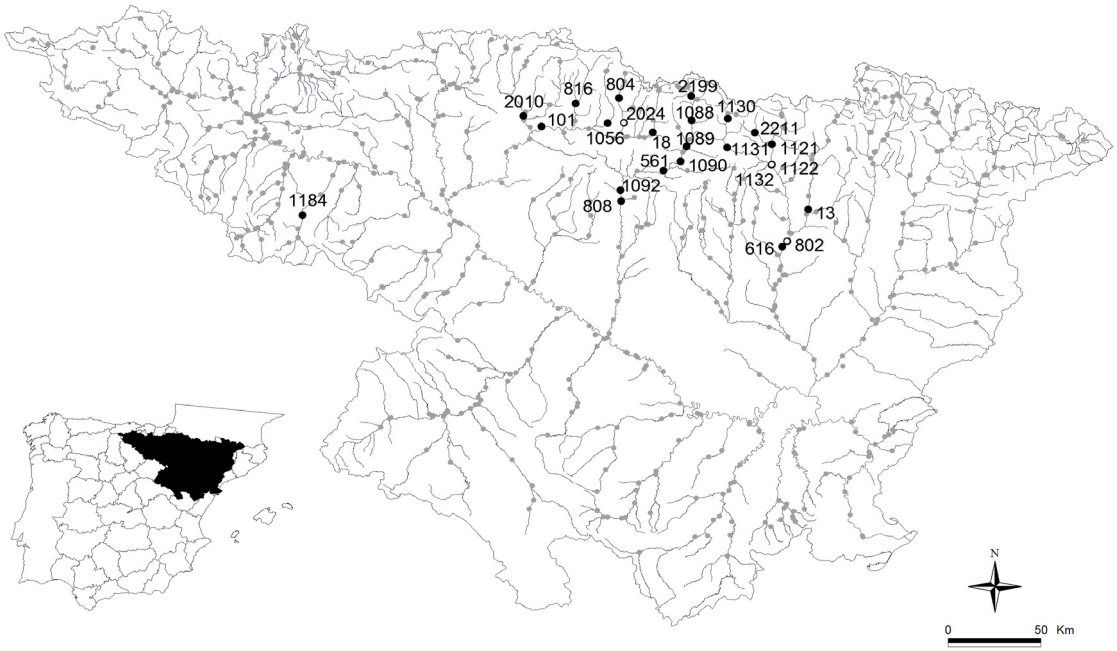

Figure 2. Distribution of *Didymosphenia geminata* in the Ebro River basin, 2006-2009. black points: presence, grey points: absence, white points: bloom.

Results and discussion

Didymosphenia geminata was recorded in 24 sampling stations in the studied years (Figure 2, Annex 1). The number of localities where didymo was presented varied in the different years: Two stations in 2006, nine stations in 2007, eight localities in 2008 and 16 sample stations in 2009. It was recorded preferentially in calcareous mountainous rivers, and only in three localities did this species form blooms (Cinca River in Puente de las Pilas, Cinca River in Ainsa and Subordan River in Embún). The distribution of the diatom in the Ebro River basin is presented in the Figure 2. Most of the localities where didymo was recorded were located in Pyrennean rivers. Only in the locality of Almarza, Iregua River (code 1184) was not located in this area. The presence of didymo in the headwaters of the Iregua River could be due to the accidental introduction from the nearby Vinuesa River (Duero River Basin) where the species is present forming blooms of varying length, both as clumps of didymo as well as single microscopic cells, which can be moved around by boaters, anglers, hunters and other aquatic recreationists.

In eight localities didymo was present for at least two years; and it can be noted that at the Ara River station in Ainsa it was found in all years. In general, the abundance of the species in the samples was low (Annex 1). This low abundance could seem contradictory in the case of the localities where blooms were recorded, but it was due to the fact that the diatom samples were taken in boulders and cobbles free of didymo blooms.

According to our data on ecological parameters didymo in Ebro River basin was found in well oxygenated waters (8.2-12.0 mg/l dissolved O_2), with a pH range of 7.3 to 9.0, and water temperature ranging from 11.6-24.7ºC. Conductivity was relatively medium-low (117-511 µs/cm) and the concentration of nutrients was low, $N0_3$ (<0.63-3.67 mg/l), $N0_2$ (0.01-0.04 mg/l), NH_4 (<0.01-0.28 mg/l), PO_4 (<0.05-0.73 mg/l); probably because didymo was recorded in mountain streams, mainly in upper oligotrophic

river stretches where these parameters are usually lower. These rivers were located in an altitude range between 337 and 1186 meters.

According to habitat characteristics this species was mainly found under low-medium flow conditions and low-high water turbulence, both in high and low turbidity waters, although it was found mainly in well illuminated river stretches. The substrate of the rivers where didymo was present was composed mainly of cobbles and boulders. Some authors suggested a relationship between the presence of the diatom blooms and regulated or lake fed streams (Kilroy et al. 2006; Kawecka and Sanecki, 2003; Kirkwood et al. 2007), but according to our data the regulation of the river could not affect the presence of *D. geminata*, as it has been recorded forming blooms both in regulated rivers (as Cinca River) and in unregulated rivers (such as the Ara River). All of the measured ecological and physico-chemical parameters measured in our study were in accordance with the ranges found in the literature according to Campbell (2005) and Whitton et al. (2009).

Conclusions

It seems that *Didymosphenia geminata* is expanding its distribution in the Ebro River basin in the last few years or, according to the literature (Whitton et al. 2009), some years the habitat conditions or other unknown parameters favoured the growth of this diatom, however the presence of blooms is not usually frequent. According to our data this species prefers mountainous calcareous rivers, regulated or not, with oligotrophic waters, relatively low flow conditions and well illuminated stretches. From our data it is difficult to relate the presence or absence of didymo only to physical and chemical characteristics of the water, as it appears to be present in a wide range of environmental conditions, this means that the potential for spread is great because the environment is not a limiting factor. The presence of *D. geminata* in the Iregua River is probably due to traslocation from the Vinuesa River may indicate the need for increasing public information and awareness about disinfection protocols (as in zebra mussel (*Dreissena polymorpha*) it is important to check, clean and dry). In order to prevent the nuisance blooms of the species, it is also necessary to study the ecology and to establish a monitoring network for detect early stages of the blooms in order to take measures of control when these early stages are detected.

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Annex 1. Records of *Didymosphenia geminata* in the Ebro River basin in the period 2006-2009. Abundance in nº of valves in the diatom samples (* found in the sample outside the count area).

Code	River/Locality	Geographic coordinates		Abundance			
		Latitude, N	Longitude, W	2006	2007	2008	2009
0013	Ésera / Graus	42º12'24"	0º20'49"	-	3	-	-
0018	Aragón / Jaca	42º34'45"	0º33'10"	-	*	-	-
0101	Aragón / Yesa	42º37'02"	1º12'34"	-	-	*	*
0561	Gállego / Jabarrella	42º24'12"	0º29'51"	-	-	-	*
0616	Cinca / Derivación Acequia Paules	42º02'29"	0º11'10"	-	*	-	-
0802	Cinca / Puente de las Pilas, Estada-Estadilla	42º03'53"	0º12'58"	-	-	*	*
0808	Gállego/Santa Eulália	42º16'12"	0º45'03"	-	-	-	*
0804	Subordán / La Peñeta	42º44'17"	0º44'46"	*	4	-	-
0816	Esca/Burgui	42º43'04"	1º00'15"	-	-	*	-
1056	Veral / Biniés	42º37'37"	0º49'08"	-	12	-	-
1088	Gállego/Biescas	42º37'41"	0º19'15"	-	-	*	*
1089	Gállego / Sabiñánigo	42º30'41"	0º21'20"	-	-	*	-
1090	Gállego / Hostal de Ipiés	42º26'35"	0º23'39"	-	*	-	*
1092	Gállego / Murillo de Gállego	42º19'13"	0º45'14"	-	-	-	*
1121	Cinca / Laspuña	42º30'25"	0º08'54"	-	*	1	*
1122	Cinca / Aínsa	42º24'57"	0º08'39"	-	-	-	*
1130	Ara / Torla E.A. 196	42º37'52"	0º06'25"	-	2	-	*
1131	Ara / Fiscal	42º30'00"	0º06'56"	-	-	-	*
1132	Ara / Aínsa	42º24'52"	0º08'29"	*	3	1	*
1184	Iregua/Almarza	42º13'39"	2º37'33"	-	-	-	*
2010	Irati/Lumbier	42º40'03"	1º19'00"	-	-	*	-
2024	Subordán / Embún	42º37'37"	0º43'14"	-	-	-	*
2199	Escarra/Escarrilla	42º44'17"	0º19'03"	-	-	-	*
2211	Bellós / Puyarruego	42º33'44"	0º03'05"	-	-	-	*