Spatio temporal population dynamics of the invasive diatom *Didymosphenia geminata* in central-southern Chilean rivers

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HIGHLIGHTS

- Between 2010 and 2015 *D. geminata* has expanded across central-southern suitable river habitats in Chile. However, some rivers do not appear suitable for invasion by *D. geminata*.
- Climatic variables and phosphorus are among the primary determinants of suitable river habitats in Chile.
- Once established in a suitable river habitat, *D. geminata* remains present, while non-invaded rivers tend to remain free of *D. geminata*.

GRAPHICAL ABSTRACT
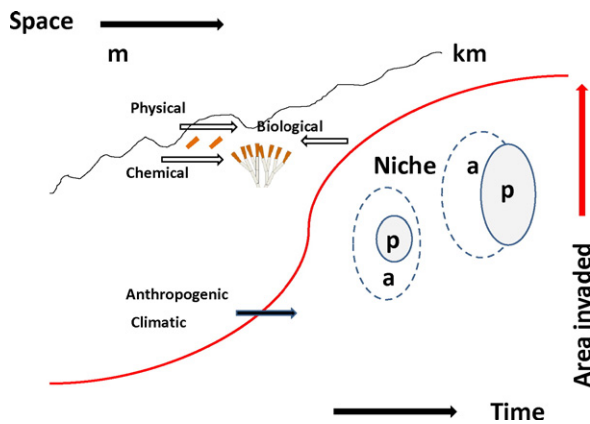
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ABSTRACT

We document the distribution of *Didymosphenia geminata* in central-southern Chilean rivers and identify the chemical and physical factors associated with its presence/absence (p/a). Repeated surveys in five successive years provided evidence that *D. geminata* could be nearing a biogeographic equilibrium in the region. *D. geminata* databases from extensive biological and environmental surveys in 187 rivers, within ten catchments, south of 38°S commenced in November 2010 and ran through May 2013. In addition, data from two other field surveys were used. The sites evenly distributed latitudinally were climatically characterized. The recent sampling program, following a published species distribution model, was designed to explore *D. geminata* distribution within thirteen catchments (34°S–48°S). An extensive river survey in 2014 (spring-summer) and in 2015 (autumn) included the p/a, and relative abundance of *D. geminata* cells in phytobenthos and in the drift. These p/a results showed that the probability of *re-encountering D. geminata* cells at sites where the species was previously found was significantly high while the probability of finding *D. geminata* cells at sites previously without the species was significantly low. This suggests that the distribution of *D. geminata* cells among suitable habitats was nearing completion. The relative abundance of *D. geminata* cells in the phytobenthos versus in the drift indicates seasonality with higher proportion of cells in the phytobenthos during the spring-summer than during the

autumn. During the final surveys, principal component analysis of chemical and physical characteristics of rivers showed significant differences between rivers with and without *D. geminata*. Based on our observations of the distribution of *D. geminata* cells among rivers with suitable habitat conditions and the fluctuating rate of spread between rivers, we conclude that *D. geminata* is probably in the ending stage of its spatial demographic expansion in Chile surmounting the different barriers of the invasive process.

1. Introduction

The recent appearance of *Didymosphenia geminata* blooms in many regions of the world in the last two decades has been the cause of ecological, scientific and public concern. An enigma regarding these blooms has been that they always occur in phosphorus (P) poor waters (James et al., 2015; Bothwell and Spaulding, 2008; Spaulding and Elwell, 2007). Recently, the appearance of *D. geminata* blooms has been tied to climatic warming (Lavery et al., 2014; Taylor and Bothwell, 2014; Watson et al., 2015). Bothwell et al. (2014) established that blooms occur when *D. geminata* does not have access to sufficient amounts of P. Besides the association of *D. geminata* with P concentration, there are many physical parameters that are also important in determining habitat suitability for this species. Among these are exposure to adequate light (James et al., 2014) and a stable substratum/flow regime that have been shown to be critical for *D. geminata* (Sastre et al. 2013; Kirkwood et al., 2009).

Recent authors have argued that because low dissolved P is the only demonstrated proximate cause of bloom formation in regions where *D. geminata* is known to be a native species, environmental changes favoring freshwater oligotrophication might be responsible for the increasing prevalence of blooms (Taylor and Bothwell, 2014, Bothwell et al., 2014). However, in the Southern Hemisphere, including the southern tip of South America, where *D. geminata* is not believed to be native, recent introductions would seem to be an important component of range expansion along with changes such as climatic warming that are occurring globally and creating more favorable conditions for *D. geminata*.

While the ecology of invasions was first explored by Elton (1958), Sandlund et al. (1999) emphasized that our understanding of invasion ecology is still very limited. However, there is agreement that one of the characteristics of an invasive species is its geographical spread over large areas within a new range (Simberloff and Rejmaneck, 2011). We also know that unlike native species, invasive species may be far from a geographical equilibrium i.e. they do not necessarily colonize all suitable habitats expected by climatic requirements (Araujo and Pearson, 2005), due to dispersal limitation, negative biotic interactions or simply because establishment time to the new range is quite recent (Le Maitre et al., 2008; Theoharides and Dukes, 2007; Wilson et al., 2007; Bridle and Vines, 2007; Geber and Eckhart, 2005).

In fact, exotic species can exhibit long time lags, sometimes decades, between initial introduction to a new region and the sudden spreading across invaded ranges (Vaclavik and Meentemeyer, 2012; Mack et al., 2000). If there are no biotic/abiotic constraints, exotic species will eventually reach this equilibrium, thus covering the entirety of a given geographic extent that best suits their ecological requirements (Araujo and Pearson, 2005). The condition of biogeographic equilibrium is a hypothesis that must to be tested empirically case by case; species distribution models have been particularly useful for this (Peña-Gomez et al., 2014).

For southern South America we have a limited knowledge of the invasive species (Nuñez and Pauchard, 2010), especially in aquatic ecosystems which might be more sensitive to the introduction of non-native species because of low biotic resistance or the proximity to human populated areas (Richardson, 2011, Richardson and Pysek, 2008).

D. geminata possesses all of the traits of a potentially successful invasive species with the ability to spread across large areas with a high dispersal capacity, large numbers of propagules and a rapid rate of vegetative reproduction. Further, Bus Leone et al. (2014) have demonstrated that natural dispersal of *D. geminata* by wildlife vectors can play an important role in the spread of *D. geminata* and other algal species in southern South America, potentially limiting or negating the efficacy of biosecurity containment measures. Even considering all these traits, it might be possible that this species only attains a geographical equilibrium in the long term.

Blooms of *D. geminata* are not new phenomena; they occurred hundreds of years ago within its native range and were transient events (e.g., in Europe; Blanco and Ector, 2009, Lindstrøm and Skulberg, 2008), covering stream benthos (Blanco and Ector, 2009; Bothwell and Spaulding, 2008), which is not consistent with a novel genotype causing the recent blooms having greater spatial coverage and temporal persistence (Spaulding and Elwell, 2007; Beamud et al., 2013) and especially for the hard-to-detect invasive microorganisms (Taylor and Bothwell, 2014). An ecological niche model developed by McNyset and Julius (Spaulding and Elwell, 2007) predicted suitable habitat for *D. geminata* in the southern tip of South America and notwithstanding the implementation of biosecurity protocols in the region, eventual dispersal was likely (Blanco and Ector, 2009). For management and control of freshwater ecosystems, evidence relating to whether or not *D. geminata* has already occupied all available spaces in southern South America is important.

Although a single report of this species in Chile was made in the 1960's (Asprey et al., 1964), there has been no confirmation of the native status of *D. geminata* in South America nor has it been part of freshwater algal collections on the continent since. For this reason when surprisingly a contemporaneous bloom was first detected in the Futaleufú River in 2010 the species was assumed to have been introduced from overseas (Montecino et al., 2014; Beamud et al., 2013; Segura, 2011). This view is consistent with researchers in New Zealand dealing with recent *D. geminata* blooms. In spite of genomic studies, the phylogeographic relationships between *D. geminata* populations in the southern and northern hemispheres remain ambiguous and the origin of *D. geminata* in the southern hemisphere, including Chile, is currently unknown (Kelly, 2009, Jaramillo et al., 2015). In the present study we have assumed that even if the original identification of *D. geminata* in Chile in the 1960's was correct, the species exhibited a long time lag between then and the active spread of blooms across the Chilean Patagonia commencing in 2010. From 2010 we have been assessing the presence and absence (p/a) of *D. geminata* across central-south Chilean rivers between latitudes 36°S to 48°S. Using a comprehensive data base, developed over a period of five years of biological sampling and measurements of environmental variables, we have been evaluating the ecological niche for *D. geminata* as well as its potential distribution.

In this study, we describe the spatio-temporal population dynamics of this species. To do that, we determined and compared the important physico-chemical variables that define river basins with and without *D. geminata*. If there are no distinguishing characteristics between rivers with and without *D. geminata*, then the species is spreading across invaded ranges, i.e. there would be more habitats available for future colonization. We also assessed the probability that rivers with or without *D. geminata* would remain in that state in the near future. If these probabilities are significantly high, then there exists resistance of rivers to change their invasive stage (from invaded to non-invaded or viceversa). Using a species distribution model (SDM) we compared the

potential distribution predicted from climatic niche between four time intervals (between 2010 and 2015). If the potential distribution increases significantly between years, then the species is far from a full biogeographic equilibrium. Lastly, we analyzed whether the relative abundance of *D. geminata* at different sites changed seasonally by comparing their frequency in spring-summer and autumn periods during 2014 and 2015 in phytobenthos and in drift samples.

2. Methods and materials

2.1. Study area

The sites are from rivers located in Central and Southern Chile, Northern to Southern Patagonia and the Magellan administrative Region (36°46′ 22.08″, Bio-Bio Region; 38°23′41″–39°30′50″, Araucanía Region; 39°34′41″–40° 23′05.5″, Los Ríos Region; 41°11′42″–43°56′22″, Los Lagos Region; 44°00′54″–47°28′47″, Aysén Region; 52°12′21″–51° 03′ 36″ Magallanes Region). The sampling effort varied between periods with the exception of Magellan Region that we only sampled in spring 2010. The chronology from 10 to 13 monitored basins and number of localities for algal cells in the drift, phytobenthos and water sampling are summarized in Fig. 1 and Table 1. Many rivers in the Itata, Imperial and Bueno watersheds were new sites and only sampled in 2014–2015. Data from multiple studies, result in a total more than 300 river sites that were sampled between spring 2010 and autumn 2012 (for references see http://www.aquaticinvasions.net/2014/Supplements/AI_2014_Montecino_etal_Supplement.xls).

Between spring-summer 2014 and autumn 2015 (Appendix A), sampling points involved follow up rivers and prospective new rivers (Same rivers sampled at two sites are denoted as A and B or with numbers in Appendix A). Some background information of Central Chile to Patagonia and the Magellan Region indicates that there are very heterogeneous habitats including mixed regime of pampas and cordilleran hydrology, transition from braided channels of medium gravel, to more constrained reaches with steeper valley walls, larger substratum and increased riparian forest cover (Bus Leone et al., 2014). Also there are changes in nutrients, at the basin scale, where there are more river basins exhibiting increasing trends NO_3–N than in PO_4–P concentration (Pizarro et al., 2010). Also Patagonian lakes trophic state are nitrogen regulated (Díaz et al. 2007). Moreover, land use and 500 years of native forest deforestation has altered the NP ratios in the aquifers (Yévenes et al., 2015). In an earlier time frame (1980–2004) Lara et al. (2015) found a decreasing trend in the observed and reconstructed streamflow of the Baker River that is consistent with a precipitation decrease. However, in later years (1994–2008) other studies have reported an increase of summer streamflow for a portion of the Baker River associated with climatic warming increases in ice melt and glacier retreat and thinning (Lara et al. (2015)). It is envisaged that climatic changes such as light hours and timing of spring might alter phosphate export to rivers (Taylor and Bothwell, 2014).

2.2. Sampling programs

For most years the site selection protocol included: i. Hydraulic, physical and chemical characteristics that were potentially favorable for *D. geminata* mats (Spaulding and Elwell, 2007; Kilroy and Dale, 2006); ii. Localities with potential vectors for human transport of *D. geminata* such as recreational and touristic activities were present (Kilroy and Unwin, 2011); iii. Disturbed environments such as those affected by organic waste discharges; and iv. Easy access to riverbeds, riverbanks and safe climatic conditions among other security field considerations. During 2014–2015 the sampling program was designed according to the published SDM of Montecino et al. (2014).

At each stream a 25–50 m reach was selected and broadly inspected for hydromorphological characteristics, habitat types, general characteristics of the riverbed and riverbanks and evaluated visually for *D. geminata* mat coverage on rocks and stones or vegetation, and qualitative notations of anthropic activities and photographic records were made. Sampling for *D. geminata* cells suspended in the water column was done with a 40 μm mesh net anchored with a 1 m stick and drifting horizontally below the surface for 10 min (Díaz et al., 2012; Duncan et al., 2007). The collected material was fixed with 2% Lugol's solution (Throndsen, 1978). In each stream reach phytobenthos was sampled along three perpendicular transects from 3 randomly collected stones at each transect. An area of 4 cm^2 from each stone was scrapped with a disposable brush and all scrapings from all the 3 transect were composited together in one flask, and fixed with 4% Formalin. In cases where visible *D. geminata* growth was apparent, extra 1 cm^3 samples were taken with a syringe piston.

Biosecurity procedures were observed to avoid *D. geminata* contamination between sites. Cleaning, washing (5% NaCl solution) and drying of all the used materials was completed including sampling net, boots, vehicle, tires. Non-reusable material was disposed in plastic garbage bags (Díaz et al., 2012; Duncan et al., 2007).

2.3. Measurements of environmental variables and water chemistry on each date and at each sampling site

For physico-chemical variables, we measured dissolved oxygen; conductivity; T°C; and pH in the field with portable multiparameter equipment YSI Professional Plus (Appendix B). During 2010–2011 current velocity in the sampled reaches was estimated with a timed float and in 2012–2013 using a fluxometer FP111 Global Water Flow Probe (Appendix B).

Data were registered with a GPS Trimble JUNO and downloaded to a computer. Water samples for chemical analysis (calcium, nitrite + nitrate, TKN, phosphate PO_4^{-3} total phosphorus, silicate, turbidity) were collected at each site and sampling date according to Chilean Norms (411/2, 3, 6 96–98) and analyzed by certified ANAM labs (www.anam.cl) using APHA Standard Methods (2005). (see Appendix B for specifications). Grab water samples were kept cool during <1 day transportation until filtering in the laboratory.

2.4. Biological analysis and cell counts

2.4.1. Phytobenthos

A1-mL subsample from each site was processed following Patrick and Reimer (1966) and Battarbee (1986) methodology, and permanent slides were prepared using Naphrax® (Battarbee et al., 2001). Standard floras were used for references (Krammer, 2009; Lange-Bertalot, 2001; Krammer and Lange-Bertalot, 1997a, 1997b; 1991, 1986). The nomenclature status of diatom species was verified using the Catalogue of Fourtanier and Kociolek (2011).

Presence or absence (p/a) of *D. geminata* was first determined qualitatively by scanning the entire permanent slides under an optical microscope (Olympus BX40) at 200–400×. Then a minimum of 200 (less when diatoms were scarce) diatoms valves were identified and quantified in random transects per permanent slide, under the same microscope at 400–1000×. Afterwards the relative abundance of *D. geminata* to other diatoms was calculated.

To check if there were live cells of *D. geminata* present after one month of field collection in samples from a basin with blooms of *D. geminata*, an analysis was done following a procedure modified from Root and O'Reilly (2012). One cubic centimeter pieces of *D. geminata* mats were stained with 0.5% neutral red (NR) solution for 30 min (live cells accumulate NR in vacuoles (Lagerstedt, 2007). Then, pieces were transferred to a Sedgewick Rafter chamber and live *D. geminata* cells attached and/or unattached to stalks were enumerated at 400× (Wetzel and Likens, 1991).

Table 1
Chronology and summary of the monitored basins and sub basins for sampling of suspended algal communities, phytobenthos and water physico-chemical measurements. Latitudinal reference includes administrative regions: Bío Bío, Araucanía, Los Ríos, Los Lagos, Aysén and Magallanes (Technical reports available at www.subpesca.cl and www.sernapesca.cl). (Sampling date S = spring SU = summer F = fall).

Basin	SUB BASIN	Sampling date (year and season)					Latitudes
		2010	2011	2012	2013	2014–2015	
Río Itata	Ñuble bajo					S–F′	36°33′27″
	Ñuble alto					S–F	36°33′37″
	Itata medio					S–F	36°47′20″
	Itata alto					S–F	37°30′27″
Río Bío-Bío	Rio Laja Alto (hasta bajo junta Rio Rucue)					S–F	37°19′47″
	Rio Bio-Bio entre Rio Vergara y Rio Laja					S–F	37°21′53″
	Rio Duqueco					S–F	37°29′36″
	Rio Bío Bio entre Duqueco y río Vergara					S–F	37°39′58″
	Rio Renaico					S–F	37°47′25″
	Rio Bio-Bio entre Rio Ranquil y Rio Duqueco					S–F	37°46′57″
	Rios Malleco y Vergara					S–F	37°52′50″
	Rio Bio-Bio entre Rio Ranquil y Rio Duqueco					S–F	37°53′46″
	Rio Bio-Bio Alto (Hasta después junta Rio Lamin)					S–F	38°40′02″
	Rio Malleco y Vergara					S–F	38°12′50″
	Río Bio-Bío Alto		F			S–F	38°42′27″
Río Imperial	Lumaco		F			S–F	38°23′41″
	Río Chol Chol					S–F	38°19′54″
	Cautín Alto		F			S–F	38°26′34″
	Río Quepe						38°40′12″
	Río Imperial		F			S–F	38°41′56″
Río Toltén	Río Allipén		F			S–F	39°00′01″
	Toltén Bajo		F			S–F	39°00′44″
	Lago Villarica y Tolten Alto					S–F	39°19′27″
	Río Pucón		F			S–F	39°30′50″
Río Valdivia	Río Valdivia Alto		F			S–F	39°34′41″
	Rio Calle Calle					S–F	39°48′47″
	Río San Pedro		F			S–F	39°42′46″
	Río San Pedro (Entre desagüe lago Panguipulli y Bajo Río Quinchilca)					S–F	50°26′34″
	Río Valdivia Bajo	S				S–F	39°57′01″
	Río Cruces	S				S–F	39°33′06″
Río Bueno	Afluentes Lago Ranco		F			S–F	40°07′13″
	Río Bueno entre Lago Ranco y Río Pilmaiquén	S				S–F	40°19′33″
	Pilmaiquén	S	S			S–F	40°39′36″
	Río Rahue hasta antes junta Río Negro	S	S			S–F	40°45′50″
Cuencas e Islas entre Río Bueno y Puelo	Petrohué	S	S			S–F	41°11′42″
	Costeras entre río Petrohue y río Puelo					S–F	41°23′03″
	Costeras entre Rio Chamiza y Rio Petrohue					S–F	41°26′46″
	Maullín		F-S			S–F	41°16′17″
	Chamiza		F-S				41°26′41″
	Cochamó		F-S				41°27′15″
	Río Blanco			F			41°24′19″
	Rio Petrohue entre Desague lago Todos Los Santos y Rio Hueñu-Hueñu			F			41°23′02″
	Río Hueñu Hueñu			F			41°15′33″
	Río Negro			F		S–F	41°02′43″
	Río Peulla			F			41°02′30″
	Costeras entre Río Petrohue y Río Puelo				F		41°27′31″
Puelo	Puelo Bajo	S	S			S–F	41°38′44″
	Rio Puelo entre desague Laguna Tagua Tagua y desembocadura			F			41°51′24″
	Rio Puelo Chico			F			41°41′59″
	Rio Traidor		S	F			41°47′05″
	Rio Ventisquero			F			41°59′55″
	Manso		F-S			S–F	41°43′44″
	Puelo Alto		F			S–F	41°44′35″
Costeras entre RíoPuelo y RíoYelcho	Costeras entre Rio Puelo y Punta Trentelhue				F		41°39′31″
	Costeras entre Río Negro y Río Vodudahue				F		41°29′53″
Islas Chiloé y circundantes	Isla Chiloe	S					42°14′04″
Yelcho	Futaleufú	S	S			S–F	43°11′35″
	Yelcho		S			S–F	42°55′00″
Río Palena y costeras límite X Región	Costeras entre río Corcovado y límite X región		S				43°30′59″
	Río Palena entre frontera y río Rosselot		S			S–F	43°38′52″
	Costeras entre limite X región y rio Palena					S–F	43°45′04″
	Río Palena Bajo		S			S–F	43°56′22″
	Río Rosselot		S			S–F	43°56′60″
Costeras e Islas entre Río Palena y Río Aisen	Río Cisnes	S	S			S–F	44°43′36″
	Costeras entre seno ventisquero y Río Cisnes		F-S			S–F	44°28′04″
Río Aysén	Río Mañiguales	S	S			S–F	45°09′41″
	Río Simpson	S	S			S–F	45°25′29″
	Río Riesco entre Desague Laguna Riesco y Río Aisen (Río Guaquer)	S					45°24′40″
	Río Aisén entre Río Riesco y desembocadura		F-S			S–F	45°21′24″
	Río Riesco		F-S			S–F	45°29′17″
Río Baker	Río Baker entre Desague lago J. M. Carrera y Río de La Colonia					S–F	47°03′04″
	Vertiente Sur Lago José Miguel Carrera					S–F	46°44′44″

Table 1 (*continued*)

Basin	SUB BASIN	Sampling date (year and season)					Latitudes
		2010	2011	2012	2013	2014–2015	
Río Baker	Río Baker entre arriba Río de La Colonia y Desembocadura	S		Su		S–F	46°36′37″
Vertiente del Atlántico	Río Penitente	S					52°12′21″
	Vertiente del Atlántico	S					52°05′40″
Costeras entre seno Andrew y Río Hollenberg e Islas	Río Serrano	S					50°56′24″
		S					51°14′26″
		S					51°03′36″

2.4.2. Algal cells in the drift

In the lab, a 10-mL subsample from the plankton net haul was transferred to a Falcon® tube. P/a of *D. geminata* was noted in each sample by observing an aliquot of fixed sample between a coverslip and a slide with an Olympus BX40 microscope at 400×, under phase-contrast. Then, 1 mL of fixed sample was put in a Sedgewick Rafter chamber for cell (diatom and non-diatom taxa) enumeration at 400×. At least 200 cells of the most abundant taxa were recorded. The relative abundances of *D. geminata* in the drift community were calculated. Standard floras were used for references (i.e. Round et al., 1990, Parra et al., 1982-1983, Hustedt, 1977, Patrick and Reimer, 1975, 1966; Bourrelly, 1968).

P/a data for *D. geminata* from the phytobenthos as well as its presence in the drift samples from each site were used to determine the relationship between climatic and environmental variables and the spatial-temporal distribution of the species. The relative abundances of *D. geminata* in the phytobenthos and in the drift samples were used to assess the seasonality of *D. geminata* presence on the bottom versus movement of cells in the drift.

2.5. Data analysis

The sampling data set includes interannual (2010 to 2013) and seasonal campaigns in 2014–2015 (Table 1). Water chemistry results along with climatic information were used for characterization of sites. The analysis of global bioclimatic layers was obtained from the database WorldClim (Hijmans et al., 2005) freely available online (with 19 bioclimatic variables). Bioclimatic variables define latitudinal climatic trends among basins and are relevant to the eco-physiological tolerances of the species (Kumar et al., 2009, Nix, 1986). With this data, a Principal Component Analysis (PCA) was performed to identify the climatic factors that best explain the variability between watersheds.

A second PCA was performed locally using the physical and chemical variables to examine the best combination of variables to characterize the rivers with and without *D. geminata*. This was done only for the spring summer 2014–2015 campaign (C1) and autumn 2015 campaign (C2) based on more complete data including phosphate. Also the sinuosity index was considered, i.e. the ratio between curvilinear length between the beginning to the end of a curve and the straight line between the two points of a curve (Leopold et al., 1964). To evaluate the PCA the Mahalanobis distance was used to compute the distance between two centroids, each one defined as the mean response of two sets of points in a multidimensional space (Legendre and Legendre, 1998).

The PCA's analyses were run using Statistica Statssoft 7 software and Mahalanobis distance was performed using the Morpho package available in the R environment (R Development Core Team, 2014).

Further, we assessed the likelihood that a river with *D. geminata* during the spring-summer of 2014 (t1) would also have *D. geminata* during the autumn 2015 (t2). Conversely, we also determined the probability that sites without *D. geminata* during the summer of 2014 would remain free of *D. geminata* in autumn 2015. This information is important as it gives clues about colonization/extinction events that occur across rivers.

If these probabilities do not change over time, then it is feasible to suggest that the p/a of this species in an average river does not change over time.

To estimate to what extent *D. geminata* is expanding (or not) across Chilean rivers we used all available occurrence points (ACCESS format) gathered during five years, using the same data studied by Montecino et al. (2014) and data from 2014 to 2015 surveys. We constructed Species Distribution Models (SDMs) using MAXENT (Phillips et al., 2006) to get an estimation of the distribution area of the species. The background for the models ranged from 36.5°S–47.7°S.

The first set of data were from *D. geminata* surveys between November 2010 and May 2013 in 187 rivers, located within ten catchments, south of 38°S (Montecino et al., 2014). In addition, the more recent sampling program, designed following the distribution model of Montecino et al. (op cit) spans more than 10° latitude comprising 13 basins (Fig. 1, Table 1, Appendix A). This database included extensive sampling in spring-summer 2014–2015 and autumn 2015. (Appendix A also records *D. geminata* absences (0) and presences (1)).

We constructed a series of distribution models over a period of 5 years. For each year, we considered the totality of the information obtained until that date, thus obtaining a model that represents the distributional areal extent. Then, the spread rate of the species was estimated using the areal extent predicted by the model in two successive time intervals, using the equation $r = \ln(Nx + 1/Nx)$ where Nx is the area of the model at year x.

The geographical presences and absences obtained in the different surveys were converted into a "shape" with Arc GIS. We also used the "Extract Multi Value to Points" tool "Spatial Analyst Tools' existing in ArcMap. To test the precision of the models i.e. the capacity to predict true presences and absences, we used the AUC index (obtained from the ROC curve) and the Boyce index (Schroeder, 2015; Peterson et al., 2011; Fielding and Bell, 1997).

3. Results

3.1. Distribution and number of basin and rivers of D. geminata surveys in the central- southern- austral Chilean regions in 2014–2015

Surveys ranged in the north from the Chillán River in the Itata Basin, to the Baker River in the Baker Basin, the southerly most reach of our study (Fig. 1). Until 2015, from all the thirteen basins monitored, *D. geminata* is absent in only one: the Imperial River Basin. For all catchments the higher presence records occurred in the spring-summer season, reaching from 30% of the sites in Puelo Basin and up to 100% of the sites for the Aysén Basin. In the case of the latter basin it reached 90% presence during autumn.

In repeated surveys in later years *D. geminata* remained present in the phytobenthic samples in 96.2% of the follow-up sites, while only being found at new sites 17.8% of the time during campaign C1 (November–January). In campaign C2 (April–May) it was present at 85% and 12% of the follow-up and the new sites, respectively. The development of blooms occurred in more diverse benthic communities during C1 (not shown).

3.2. Climatic characterization of sites

Fig. 2 shows that the PCA analysis obtained with the 19 bioclimatic variables considered, clearly distributed the basins along the north to south latitudinal gradient. Positive values along the CP1 axis indicate lower temperatures; positive values along the CP2 axis indicate low rainfall conditions. Each of the sampling points is stations within the watersheds, differentiated according to its basin of origin and p/a of *D. geminata*. Filled/non-filled symbols are those sites where *D. geminata* was present/absent. Number of basin following Fig. 1.

The contribution of these variables (Table 2) set the background climatic boundaries for more suitable habitats for *D. geminata*: accordingly, and except for the Bio-Bio catchment *D. geminata* was found at lower temperatures and in the whole range of rainfall conditions (Fig. 2).
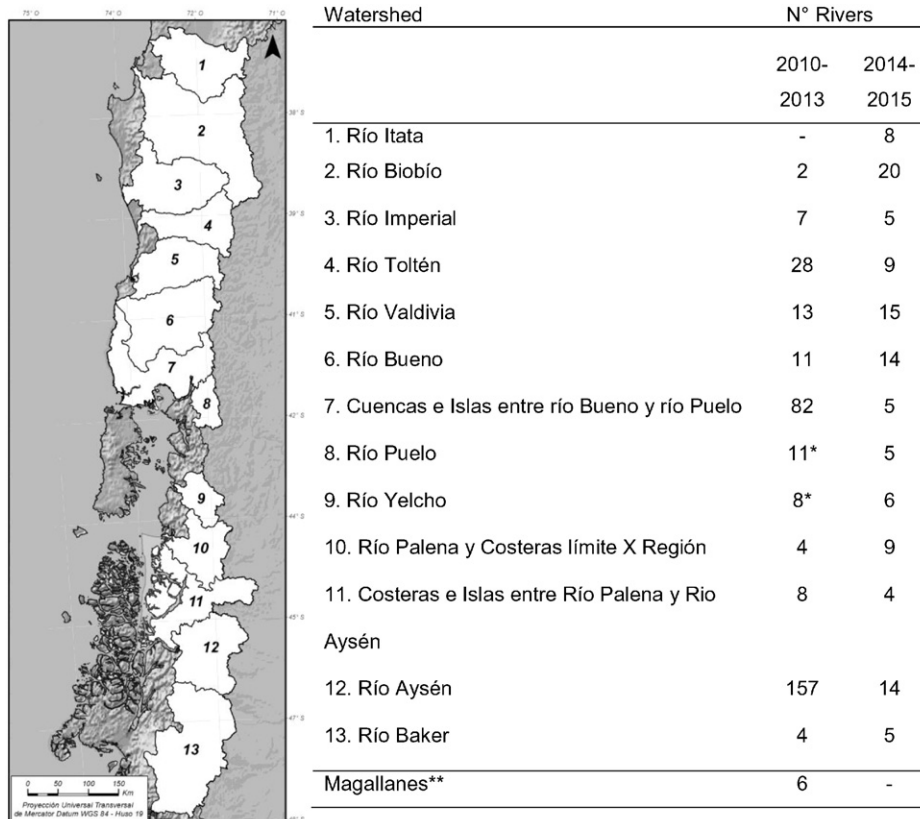
3.3. Niche analysis

In the surveys conducted in 2014 and 2015, individuals from the multivariate spaces of sites with and without *D. geminata* (Fig. 3) show a considerable overlap. When testing for differences between p/a, however, the results show significant differences between them based on permutation tests for Mahalanobis distances (1.25; P < 0.001). The first axis (PC1) was negatively correlated mainly with inorganic P and Total P (iP(′) r = −0.79, TP −0.94) and the sinuosity index while the second one (PC2) was correlated with river width, r = 0.60 (Table 3). In the first axis (PC1), the variability of absence points was higher than the points of presences, which were more aggregated around zero value. Absences were concentrated at the negative zone of the PC1 axis, which represents the highest P concentration.

3.4. D. geminata recent histories in rivers at the scale of seasons

Comparing p/a results at the scale of seasons (Table 4) we found that the probability of finding *D. geminata* in historical follow-up sites (n = 52) and the prospective sites (new sites = 175) where it has been detected in spring-summer is significantly high. This is also true for the absences in the 2014–2015 period. A significant association between the rivers was detected from the first campaign *C*1 with the rivers in the second campaign C2 for both phytobenthic as for drift algal samples (Fisher test, P < 0.001). In the phytobenthos the probability of *D. geminata* persisting in a river (P = 0.92) is not different than in the drift (proportion test Z = 1.35; P < 0.17). In the drift communities the probability of *D. geminata* persisting from spring-summer to autumn (P = 0.84) is also not significantly different than for a site to remain without *D. geminata* (P = 0.86) (proportion test Z = −0.39; P = 0.69). This indicates that the presence or absence of *D. geminata* in a particular river is highly dependent on the intrinsic physicochemical characteristics of that river. In other words, rivers are either susceptible to *D. geminata* invasion or not despite climatological and hydrological changes between spring or autumn and despite potential for new introductions.

3.5. Areal spread

The areal extents estimated for each time interval (in km^2), depicted in Fig. 4, are described in Table 5 including rate of expansion and model performance. During the first two years the spatial spread of the species decreased while the next years we observed a significant increase of the areal extent. However, during the last time interval, while the species continued to expand, it did so at a slower rate (Table 5).

Watershed	N° Rivers	
	2010-2013	2014-2015
1. Río Itata	-	8
2. Río Biobío	2	20
3. Río Imperial	7	5
4. Río Toltén	28	9
5. Río Valdivia	13	15
6. Río Bueno	11	14
7. Cuencas e Islas entre río Bueno y río Puelo	82	5
8. Río Puelo	11*	5
9. Río Yelcho	8*	6
10. Río Palena y Costeras límite X Región	4	9
11. Costeras e Islas entre Río Palena y Rio Aysén	8	4
12. Río Aysén	157	14
13. Río Baker	4	5
Magallanes**	6	-

*more than one site
**Administrative Region

Fig. 1. Map of central- southern- austral Chilean regions with watershed (13) names (excluding the Magellan Region) and total numbers of rivers including the 2010–2013 follow up and the 2014–2015 new sampled rivers. Basins are listed from north to south.

3.6. D. geminata cell counts and relative abundance

The seasonal changes in the median relative abundance of *D. geminata* between the benthic and the drift algae were highly significant (p < 0.00001) (Fig. 5). During spring-summer, *D. geminata* was more prevalent in the benthic diatom taxocenosis while during the autumn, it was much more prevalent in the drift algae.

4. Discussion

Our results indicate that *D. geminata* has increased its distribution in south-western South America since 2010 and suggests that it now occupies much of the available suitable habitat in central-southern Chile (Fig. 4). The accuracy of the models used for estimation of areal extent as shown in Table 5 is noteworthy. These values give confidence that our areal estimations are reasonably accurate. In spite of the fact that the time period of our study was a relatively short, it is to our knowledge, the only data set available showing the dynamics of an invasive aquatic species in Chile. In summary, even though the spatial dynamic of this species has experienced fluctuations, it continues expanding toward central Chile as well as southern Patagonia, all of these processes have occurred in a 5 year time span.

Similarly in New Zealand following the introduction of *D. geminata* into that country in 2004, it had spread to most suitable larger rivers on the south island within a 6 year period (Kilroy and Unwin, 2011). This expansion occurred in spite of concerted efforts to control movement of the species by human vectors.

The early stages of a species invasion are not accurate predictors of potential distribution (Vaclavik and Meentemeyer, 2012). Our SDMs from 2010 to 2012 showed that the initial occupancy of surveyed rivers was between ~3000–4000 km². However, between 2012 and 2014, a very large increase occurred with 18,000 km² being accumulated by 2014 (Fig. 3d and Table 5). A further expansion between 2014 and 2015 of up to 19,895 km² (Fig. 3e and Table 5) indicates that the expansion is still occurring but at a much lower rate. However, the SDMs are in agreement with the interannual PCA results from 2010 to 2013 (Montecino et al., 2014) showing that the *D. geminata* presences were included in the sites where *D. geminata* was absent, but that PCA results obtained in surveys conducted in 2014–2015 show significant differences between river habitats with and without *D. geminata*, clearly establishing that available spaces for *D. geminata* have already been filled. Furthermore our survey of *D. geminata* in different seasons in 2014 and 2015 (Table 4) was important in showing that the probability of being present (absent) where it has been found (not found) before were highly significant, again indicating specific catchment factors for this species (Ellwood and Whitton, 2007). While expansion to additional habitats is possible and new data would be needed to assess that, the existing data suggest that further expansion might occur at a much lower rate. In other words, the species is not currently in a full biogeographic equilibrium but might be in the near future. Here, we emphasize the necessity of large-scale temporal studies to examine the dynamic of invasive species, something that is rarely done.

In the last century ecological integrity of freshwater systems has been altered by invasive species and sometimes these changes have been attributed to aquatic recreational activities (Strayer, 2012, Ricciardi and MacIsaac, 2011, Kilroy and Unwin, 2011, Whitton et al., 2009, Strayer et al., 2006). In theory ecological resistance to invasions is related to species richness with more diverse communities being more resistant to newly introduced species (Levine et al., 2004). According to this diversity–invasiveness hypothesis (Fridley et al., 2007), it might be expected that Andean and Patagonian oligotrophic rivers would offer low resistance (biotic acceptance) to non-native species with low nutrient requirements allowing them to become easily incorporated into riverine benthic communities in an extensive latitudinal altitudinal climatic range.

In Chile, climatic variables were important large-scale indicators of river habitat suitability for *D. geminata* presence (Fig. 2). River basins in the colder and drier regions were most likely to favor *D. geminata* presence. While cool seasonal air temperatures and low seasonal precipitation favor *D. geminata*, those factors alone do not explain the distribution among some basins. In particular in adjacent basins or sub-basins (it is prevalent in the Puelo but absent in the Petrohué) with similar climatic conditions, the presence or absence of *D. geminata* must be the result of other factor(s). We suggest that there are constraints due to different levels of phosphorus and nitrogen inputs to these sub-basins possibly arising from different geologic volcanic origins or possibly from different glaciation processes.

Moreover, our results showing that the relative abundance of *D. geminata* cells in the benthos versus in the drift change with season are in agreement with the conceptual model of Cullis et al. (2012). This model based on a range of disciplines including hydrology, geomorphology, biogeochemistry, and ecology, synthesizes the relationship between biomass growth rate and nutrient concentrations for cells, mats, and total biomass of *D. geminata* compared to other potentially competing benthic algae. In Argentina, hydrological observations show that *D. geminata* appeared, colonized and invaded the bed of the Futaleufú River at low flow situations in spring and summer and its blooms declined in autumn -when the flows increase- after having been dried in summer when flows were the lowest (Sastre et al., 2013). All of this would indicate a seasonality to the benthic versus the drift phases of the *D. geminata* population in rivers.

Added to the ecophysiological attributes of unicellular species, many climatic factors can affect *D. geminata* absence in more northern regions and distinctive physicochemical habitat constraints in specific rivers or basin such as flow accumulation, geologic age or precipitation seasonality (Montecino et al., 2014). These variables explained the highest percentage of the total variance in the initial studies in southern Chile. Here the PCA analysis shows (Table 3) the highest negative relationship between *D. geminata* presence with the concentration of inorganic dissolved P and Total P. *D. geminata* was present at low phosphorus concentrations (i.e. phosphate) considering the range from ultra–oligotrophic conditions (0.005 mg/l total phosphorus, to oligotrophic conditions (0.005–0.01 mg/l total phosphorus). This confirms the findings of many other workers of the negative effect that higher P levels have on *D. geminata* but this effect is largely related the
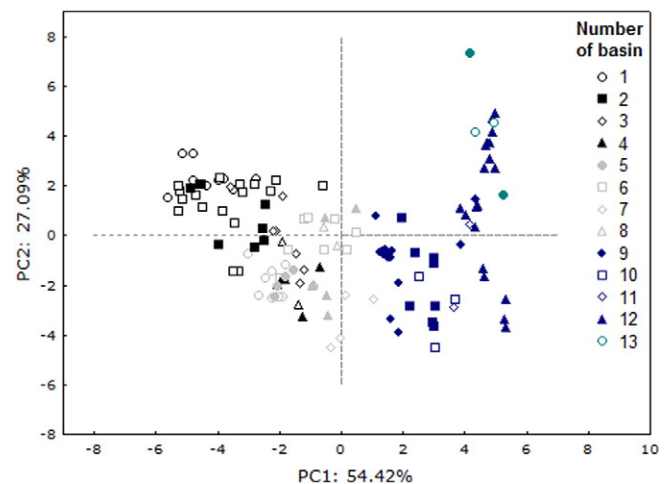

Fig. 2. Principal components analysis performed with 19 bioclimatic variables. Each of the points corresponds to sampling points in rivers within each watershed. Symbols correspond to rivers along this latitudinal gradient from the Itata River in the north to the Baker River in the south (filled symbols are those sites where *D. geminata* was present).

Table 2

Contribution of climatic variables incorporated in the PCA (Fig. 2). In bold the bioclimatic variables with the highest contribution to the PCA are highlighted. The eigenvalue of each component and the cumulative variance is also indicated at the bottom.

Variable	PC 1	PC 2
Average annual temperature	**− 0.89**	− 0.23
Average diurnal temperature range	**− 0.88**	0.12
Isothermality	**− 0.85**	− 0.10
Temperature seasonality	− 0.37	0.49
Minimum temperature of the warmest month	− 0.98	0.00
Minimum temperature of the coldest month	− 0.62	− 0.45
Annual range of temperature	**− 0.84**	0.22
Average temperature of the wettest month	− 0.78	− 0.33
Average temperature of the driest month	**− 0.91**	− 0.22
Average temperature of the warmest month	**− 0.94**	− 0.09
Average temperature of the coldest month	**− 0.81**	− 0.34
Annual precipitation	0.18	**− 0.95**
Precipitation of wettest month	− 0.28	**− 0.85**
Precipitation of driest month	0.75	− 0.60
Seasonality of precipitation	**− 0.94**	0.18
Precipitation of wettest quarter	− 0.33	**− 0.86**
Precipitation of driest quarter	0.74	− 0.62
Precipitation of warmest quarter	0.74	− 0.62
Precipitation of coldest quarter	− 0.31	**− 0.87**
Eigenvalue	**10.34**	**5.15**
Cumulative variance (%)	**54.42**	**81.51**

Table 3

Contribution of physicochemical variables incorporated in the PCA (Fig. 3), in bold the variables with the greater contribution to PCA (absolute value), the eigenvalue of each component and the cumulative variance are also highlighted for the physico-chemical space of *D. geminata* presences and absences.

Variable	PC 1	PC 2	PC 3	PC 4	PC 5
Altitude	− 0.14	− 0.12	0.33	− 0.47	0.32
River width	− 0.10	**0.60**	0.13	− 0.03	− 0.40
Ca	− 0.17	− 0.32	− 0.01	− 0.34	0.22
Conductivity	− 0.32	− 0.38	− 0.05	**− 0.54**	− 0.14
Distance to the origin	− 0.28	0.42	0.11	− 0.41	− 0.22
Sinuosity index	**− 0.66**	− 0.44	− 0.05	− 0.26	− 0.06
River lenght	− 0.29	0.42	0.31	**− 0.53**	− 0.15
Nitrate	− 0.10	− 0.22	**0.91**	0.28	− 0.07
Total N	− 0.11	− 0.23	**0.92**	0.27	− 0.05
Dissolved oxygen	− 0.02	0.18	0.12	− 0.39	0.26
Slope	0.16	− 0.41	− 0.06	0.09	0.49
pH	0.02	0.33	0.10	− 0.16	0.52
Inorganic P	**− 0.79**	− 0.27	− 0.14	0.06	− 0.15
Organic P	− 0.67	0.33	− 0.05	0.32	0.29
Total P	**− 0.94**	− 0.03	− 0.13	0.21	0.04
Depth	0.08	0.47	0.14	− 0.03	− 0.11
Si	− 0.66	− 0.44	− 0.05	− 0.26	− 0.06
Temperature	− 0.02	− 0.23	− 0.14	0.07	**− 0.77**
Turbidity	− 0.63	0.35	− 0.09	0.36	0.20
Velocity	0.01	− 0.04	− 0.05	− 0.01	0.05
Eigenvalue	**3.18**	**2.31**	**2.03**	**1.77**	**1.75**
Cumulative variance (%)	**15.91**	**27.47**	**37.61**	**46.46**	**55.20**

production of blooms in rivers which we did not measure in our study (Bothwell et al., 2014; Khan-Bureau et al., 2014). *D. geminata* is often present in rivers with higher P but does not form blooms (Taylor and Bothwell, 2014). For this reason the strong negative relationship in our PCA analysis with *D. geminata* p/a did not translate into an important descriptor for overall the invasive areas of the species.

5. Conclusions

Our results document the overriding importance of climatic and physical factors that facilitate the invasiveness of *D. geminata* once it is first introduced to a new geographic region. The monitoring and analysis of the spatio-temporal population dynamics of this invasive stalked diatom provided novel ecological and environmental characterization in an extensive gradient of temperate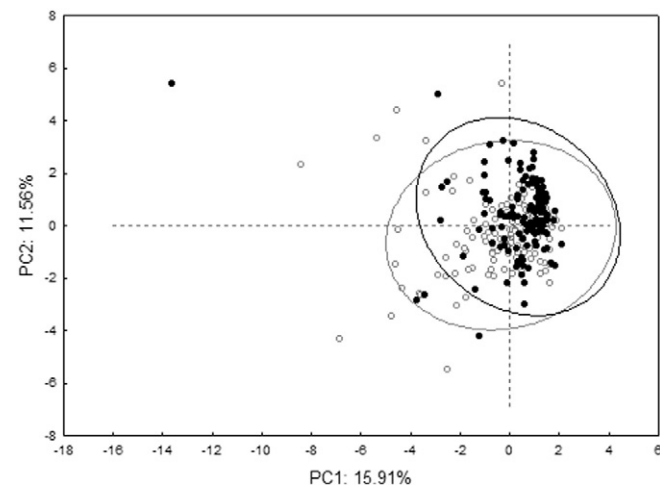 rivers demonstrates that after the first appearance of a new aquatic microbial species in a large geographical region of the world, all evidence indicates that most suitable habitats have been colonized in a 5 year period. The implications of this conclusion, about biogeographic equilibrium being reached, for attempted management of aquatic microorganisms are profound, both domestically and internationally.

The present study documents that between 2010 and 2015 *D. geminata* has been spreading among suitable river habitats in central-southern Chile but that rate of spread is showing signs of slowing. The data also suggest that rivers not presently invaded might be unsuitable and might remain free of *D. geminata* in the future.

We confirm the overarching idea that phosphorus is the main chemical variable that drives *D. geminata* abundance (bloom formation) and we identify the importance of climatic variables in defining suitable river habitats. In Chile in spring these conditions are met and *D. geminata* blooms are found. In autumn *D. geminata* suspended in water is relatively more abundant but does not form mats.

Acknowledgements

Fig. 3. Principal Component Analysis (PCA) showing the niche for *D. geminata* presence (filled dots) as well as the physico-chemical space for *D. geminata* absence (empty dots) with surveys from 36.5°S–47.7°S. Ellipsoids include 95% of total data (presence is dark line and absence is light line).

Table 4

Comparison of the same sites according to the presence (p) or absence (a) of *D. geminata* in the spring-summer (t1) and autumn (t2) campaigns 2014–2015. Frequency of rivers remaining with *D. geminata* (pp), the frequency of rivers loosing *D. geminata* (pa), the frequency of rivers not having *D. geminata* at t1 and present in t2 (ap) and the frequency of rivers that remain without *D. geminata* (aa). The rows represent number of sites during the t1 campaign and the columns represent the number of sites during the t2 campaign for phytobenthos and drifting algae net samples.

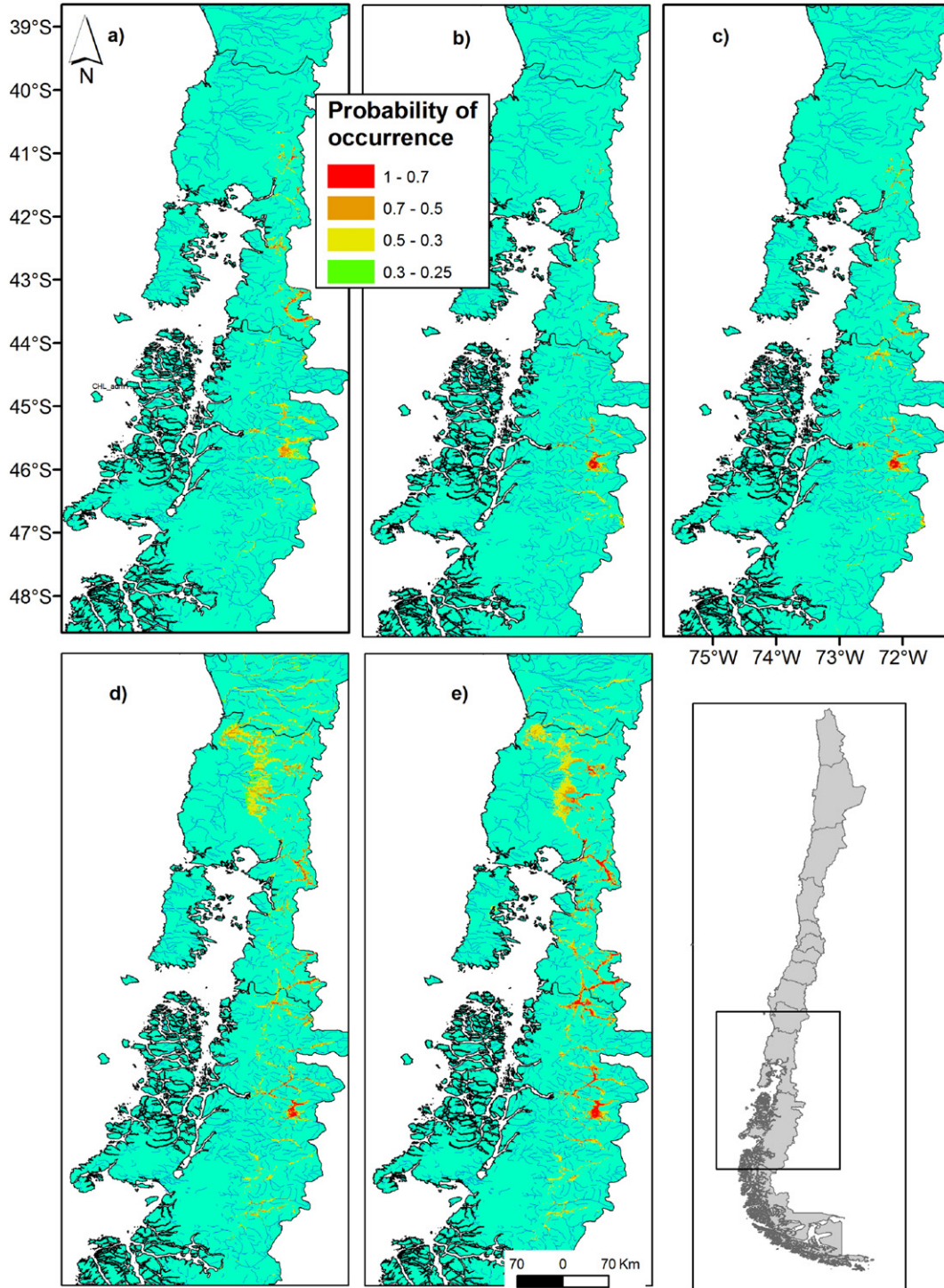| Phytobenthos				Drifting algae			
t1/t2	p	a	Total	t1/t2	p	a	Total
p	57	20	77	p	54	8	64
a	5	113	118	a	10	53	63
	62	133	195		64	61	127

Fig. 4. Probability of *D. geminata* occurrence in central- southern Chile (map location bottom right) in different periods (models are constructed with cumulative data). a,b,c,d,e are years 2010 - 2015 according to Table 5. Higher probabilities are depicted in eastward rivers from 41°S to 48°S.

Table 5
D. geminata cumulative occurrences, areas of the potential species distribution model, rate of expansion and model performance. Area under the ROC-curve (AUC) index obtained from the Receiver Operating characteristic (ROC) and the Boyce index.

Year	Occurrences	Potential model area	Spread rate	AUC	Boyce index
		km^2	km^2 y^{-1}		
2010	21	4193		0.998	0.96
2011	47	2254	−0.62	0.997	0.91
2012	55	2992	0.28	0.997	0.92
2014	149	17,935	1.79	0.985	0.98
2015	227	19,895	0.10	0.976	0.98

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jdeveco.2016.03.009.
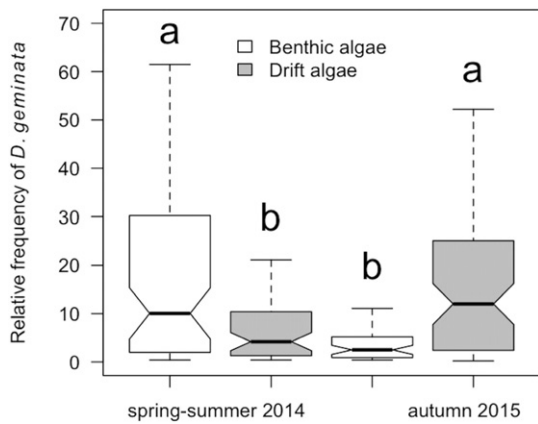
Fig. 5. *D. geminata* relative frequency (%) in phytobenthos and in drift samples from all the same sites where cells were counted in spring-summer (December 2014–January 2015) and autumn (May 2015) campaigns. Different letters show significant differences (Mann Whitney pairwise test and p values are adjusted by Bonferroni correction).

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