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CHANGES IN THE EPIPELIC DIATOM ASSEMBLAGE IN NUTRIENT RICH STREAMS TO THE VARIATIONS OF SIMULTANEOUS STRESSORS

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ABSTRACT

Benthic diatoms are often used for assessing environmental conditions, such as water quality and habitat conditions in stream and river systems. Although laboratory experiments have shown that each diatom species have different levels of tolerance to different stressors, few studies have been conducted in laboratory settings that analyze the responses of the diatom assemblage to the effects of multiple simultaneous variables. The aim of this study was to evaluate some structural responses (such as species composition and diversity) of the diatom assemblage on a short time scale to the effects of the simultaneous increase in four variables that are directly linked to the environmental changes affecting the Pampean streams: turbidity, nutrients (phosphorous and nitrogen), water velocity and temperature. To this end we conducted a five-week laboratory experiment using artificial channels where we simulated two environmental conditions (LOW and HIGH) employing epipelic biofilm from a mesotrophic stream. The results obtained in the experiment show that the structure of the diatom assemblage in the epipelic biofilm is affected by the simultaneous modification of temperature, water velocity, nutrient concentration and turbidity. These modifications in the assemblage included moderate decreases in diversity, small decreases in the proportion of species sensitive to eutrophication and saprobity, moderate increases in the IDP (Pampean Diatom Index) values and moderate changes in the percentages of the stalked growth-forms. The relative abundance of species such as *Luticola mutica, Navicula cryptocephala* and *Navicula lanceolata* were negatively affected by both treatments; other species such as *Planothidium lanceolatum, Caloneis bacillum, Encyonema minutum, Humidophila contenta, Luticola kotschyi, Nitzschia amphibia, Navicula veneta, Pinnularia subcapitata* var. *subcapitata* were positively affected by the HIGH treatment; and *Nitzschia fonticola* was positively affected by both treatments. The

results suggest that, in the very short term of the bioassay conducted, the diatom assemblage can modify its structure to respond in a sensitive manner to the abrupt changes in multiple physical-chemical variables.

Keywords: epipelic diatom assemblage; turbidity; nutrients; water velocity; temperature; artificial channels; Argentina Pampean plain

1. INTRODUCTION

Rising human pressure on water resources, combined with the effects of global climate changes, affect the hydrological and geomorphological state of river systems, favoring increases in variables such as nutrients and temperature, which consequently modify the structure and functioning of aquatic ecosystems (Nilsson & Renofalt, 2008; Sabater, 2008). Each of these perturbations occurs worldwide, but often in relatively discrete locales (Malmqvist & Rundle, 2002).

The Pampean plain in Argentina contains 21 million inhabitants and concentrates the majority of industrial and farming activities, and it is exposed to the most intense use of fertilizers in the country (INDEC, 2010). Furthermore, the increase of urbanization that progressively occupied cultivatable land and caused the displacement of livestock from their traditional areas to marginal lands situated in floodplains has increased the incidence of erosion and the input of particulate material into waterways. These changes in land use associate with changes in climatic patterns are having effects on the physical, chemical and hydrologic properties of the lotic systems that run through the Pampean plains (Rodrigues Capitulo et al., 2010). The climatic models for the region predict higher rainfalls (Hulme & Sheard, 1999) which might increase erosion and generate flooding, increasing the transport of sediments, nutrients, and contaminants into the water. These changes in the discharge and in the turbidity of the water can affect the residence time and the light penetration in the water column (Davies-Colley et al., 1992; Davies-Colley & Smith, 2001).

The streambed of the Pampean streams is composed of fine sediments (clay and silt) that are colonized by an epipelic biofilm in which diatoms are the dominant group,

constituting the basal trophic levels for extensive food webs (López van Oosterom et al., 2013; Ocón et al., 2013). These streams show a relatively high concentration of nutrients when they are compared with other lotic systems of the world (Bauer et al., 2002, Giorgi et al., 2005), and can be classified as eutrophic when phosphorus is considered, or as mesotrophic when nitrogen is taken into account (Feijó & Lombardo, 2007).

Benthic diatoms are often used for assessing environmental conditions, such as water quality and habitat conditions in stream and river systems and nutrient enrichment (e.g. Pan et al., 1999; Soininen et al., 2004; Kelly et al., 1995; Rott et al., 1997, 1999; Coring, 1999) and have been widely related to specific environmental conditions in different geographical regions (Soininen, 2002; Martinez de Fabricius et al., 2003; Ndiritu et al., 2006, Gomez & Licursi, 2001, Lobo et al., 2002; 2004a,b). Consequently, several studies have addressed the tolerances and preferences of diatoms along a number of environmental gradients, such as gradients in salinity, pH, trophy, saprobity and current preference (e.g. van Dam et al., 1994; Rott et al., 1997; Hering et al., 2006).

Although laboratory experiments have shown that different diatom species have different levels of tolerance to different stressors (Admiraal & Peletier, 1980; Admiraal, 1984; Licursi & Gómez, 2013), patterns of species distribution observed from field studies rarely relate directly to a single variable (Oppenheim, 1991). In a broad analysis in ecological studies in marine ecosystems, Crain (2008) found that the interaction between individual variables effect across most studies was synergistic, although additive and antagonistic effects were also common. They also concluded that as the number of stressors in a system increase, stressor pair interactions become increasingly complex and more commonly synergistic. In laboratory settings, few studies have been conducted that analyze the responses of the diatom assemblage to the effects of multiple

variables combined (such as Navarro Rodriguez, 1998; Rier & Stevenson, 2002; Lange et al., 2011), a situation that is more likely to occur in a natural stream since environmental changes rarely affect a single variable.

In this context, the aim of this study was to evaluate structural responses (such as species composition and diversity) of the diatom assemblage on a short time scale to the effects of the simultaneous increase in four variables that are linked to the environmental changes affecting the Pampean streams: turbidity, nutrients (phosphorous and nitrogen), water velocity and temperature. To this end we conducted a laboratory experiment simulating two environmental conditions (LOW treatment and HIGH treatment) employing epipelic biofilm from a mesotrophic Pampean stream (the "Martin" stream). The manipulated temperatures were selected to represent the both the maximum increment predicted by the climatic models of the region (Hulme & Sheard, 1999) in the HIGH treatment and an intermediate situation in the LOW treatment; the nutrients (phosphorous and nitrogen), turbidity and water velocity increments in the HIGH treatment are among the largest values measured in different sites throughout the Pampean streams (Gómez & Licursi, 2001; Gómez et al., 2008; Bauer et al., 2002; Giorgi et al., 2005; Licursi, 2005; Licursi & Gómez, 2009), and in the LOW treatment these variables were increased in an intermediate level between the controls and the HIGH treatment.

There has been reported that increments in nutrients and temperature, and slower water current velocities increase algal biomass (e.g. Dodds et al., 2002; Blanchard et al., 1996; Lamb & Lowe, 1987), while turbidity usually has a negative effect on the algal development due to its shading and erosive properties (Horner et al., 1990). We then hypothesized that the simultaneous changes in these environmental variables would produce rapid but small structural changes in the diatom assemblage that inhabits the

epipelic biofilm, which would include increased total density and loss of diversity. Also, the abundance of sensitive species to organic matter and nutrients would decrease when exposed to the treatments while favoring the more tolerant species.

2. MATERIALS AND METHODS

A laboratory experiment was conducted using epipelic biofilms from a site in the "Martin" stream (34° 54'51" S - 58° 04' 39" W, Figure 1). The land use of the catchment was previously quantified using a geographical information system (Quantum GIS 2.0) employing 1:50000 land use maps (Hurtado et al., 2006). This shows that the land use in the catchment is mainly agricultural (72%) with the remainder percentage classified as suburban.

The concentrations of Soluble Reactive Phosphorous (SRP), Dissolved Inorganic Nitrogen (DIN, calculated as the sum of the concentrations of ammonia, nitrites and nitrates), Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD_5) and Chemical Oxygen Demand (COD) in the water were assessed previous to the experiment by standard methods (APHA, 1998).

2.1 Experimental setup

Nine indoor artificial channels measuring 1m (length) x 0.15m (height) x 0.10m (width) were used, each with an access ramp that ensures a laminar input flow (water depth was 0.10m). Water exiting the channel flowed past a slit, and fell into a holding tank before being pumped back to the access ramp (Figure 2). All artificial channels were exposed to a photoperiod of 14h light-10h dark. Light was provided by GE® E-biax Helical lights (6500°K, IRC82%) with an intensity of 110-115 μEinsteins of photosynthetically active radiation.

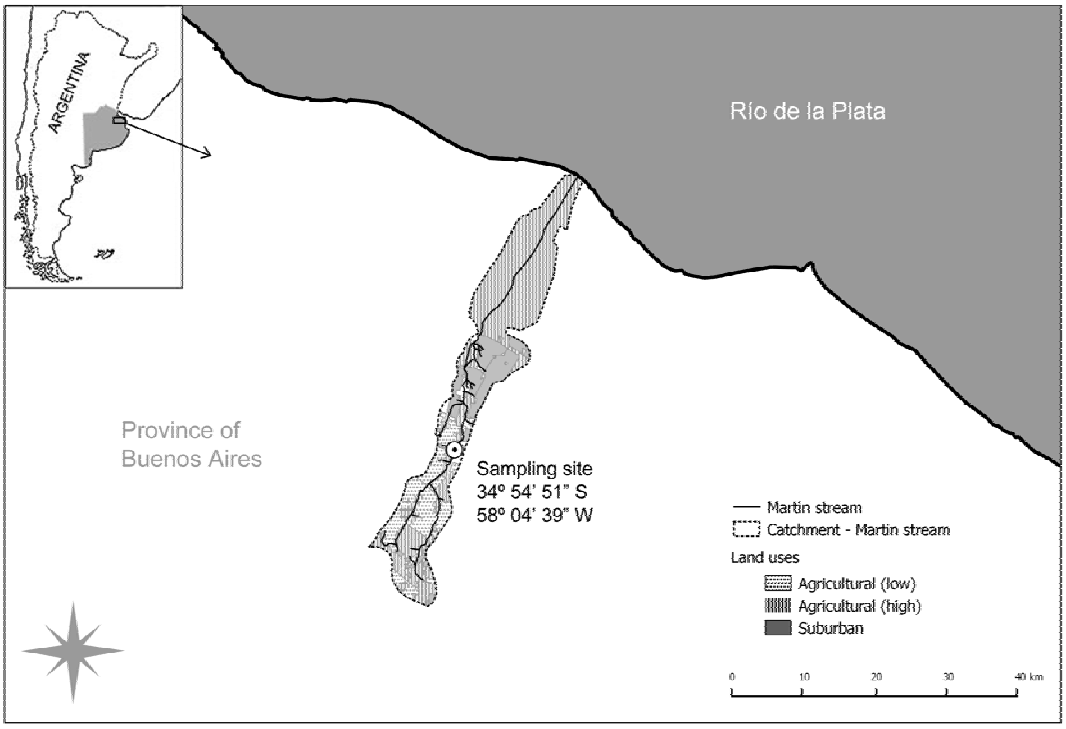

Figure 1. Map of the study area that includes the main land uses, the location of the sampling site and the limits of the catchment (modified from Hurtado et al., 2006).

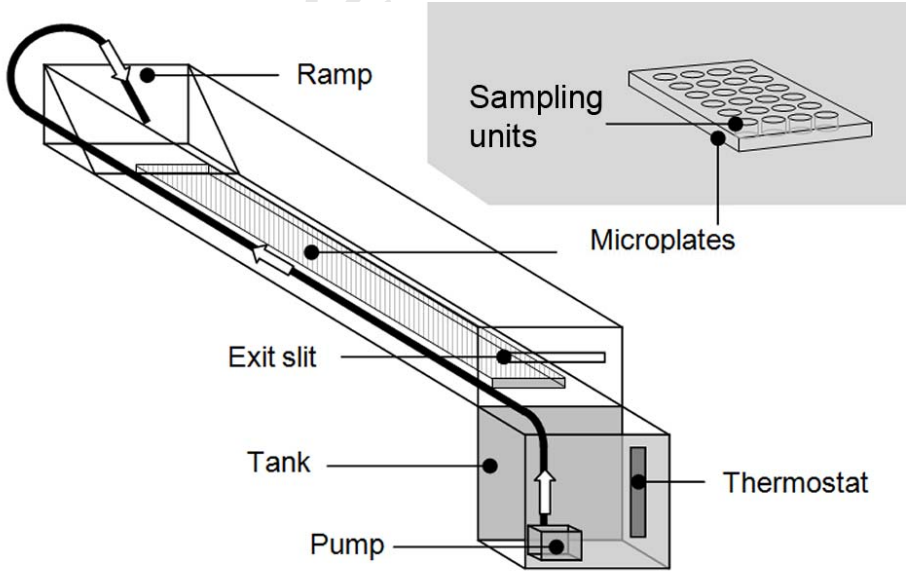

Figure 2. Design of one of the nine artificial channels used in the experiments.

Out of the nine channels, three were used as controls (C) and their physical-chemical variables were kept similar to the values measured at site from the "Martin" stream. Another three channels (HIGH treatment) were exposed to a 4°C increase in temperature, 300% increase in nutrients (SRP and DIN), 50% increase in suspended solids and 20% increase in water velocity. The temperature increase was selected to represent the maximum increment predicted by the climatic models of the region (Hulme & Sheard, 1999), while the nutrients, turbidity and water velocity increments are among the largest values measured in different sites throughout the Pampean streams (Gómez & Licursi, 2001; Gómez et al., 2008; Bauer et al., 2002; Giorgi et al., 2005; Licursi, 2005; Licursi & Gómez, 2009). The final three channels (LOW treatment) were exposed to intermediate levels of the manipulated variables: a 1°C increase in temperature, 50% increase in nutrients (SRP and DIN), 15% increase in suspended solids and 5% increase water velocity. These values were selected to be a minor increase in the modified variables from the controls, but lower than those in the HIGH treatment.

Water temperature increments were achieved using regulated Atman 70W water heaters placed in the individual tanks of each artificial channel. The increments in water velocity were achieved by setting the Chosen® Champion CX-500 water pumps, also placed in the individual tanks of each channel, at different settings. Increases in turbidity were achieved by adding sterilized suspended solids to each channel from the Martin stream where the biofilm was collected from. Nutrient increments (SRP and DIN) were achieved by adding dissolved Nitrofoska® fertilizer (use frequently in the Pampean plain for agricultural purposes, 12%N-12%P-17%K) in the established concentration for each channel.

For the biofilms to develop, each channel contained Falcon® multiwell polystyrene microplates that were first filled with sterilized sediment from the site in the "Martin" stream, for a volume in each well of 3.4cm^3. Each channel was filled with river water, and a suspension of sediment biofilms collected at the stream was later added. The biofilms were allowed to settle and colonize the sterilized sediment for four weeks previous to the experiments, and water from all channels was partially renewed with filtered stream water twice a week to prevent metabolite accumulation.

The colonization process was determined through optical inspection with an Olympus BX-50 microscope, and, when no significant changes in the diatom density were observed for three consecutive sampling dates, the colonization stage was considered to have finished. Afterwards, the different treatment manipulations were started and maintained for 5 weeks. Physical-chemical variables in each channel were measured every two days and the diatom assemblage was sampled every seven days.

2.2 Physical-chemical variables

Dissolved Oxygen (DO, mg l^{-1}), temperature (°C), conductivity (µS cm^{-1}) and pH were measured using a CONSORT C933 sensor. Turbidity (NTU) was measured using an HORIBA U10 sensor, and water velocity (m sec^{-1}) using a Schiltknecht MiniAir20. Nutrient samples were filtered through glass fiber filters (Whatman GF/F, Whatman International); ammonia (N-NH_4^+, mg l^{-1}), nitrites (N-NO_2^-, mg l^{-1}), nitrates (N-NO_3^-, mg l^{-1}) and soluble reactive phosphorous (P-PO_4^{3-}, mg P l^{-1}) were analyzed according to standard methods (APHA, 1998). Total dissolved inorganic nitrogen (DIN, mg N l^{-1}) was calculated as the sum of nitrate, nitrite and ammonia. All physical-chemical variables were determined using three subreplicates from each channel.

2.3 Epipelic biofilm sampling and analysis of the diatom assemblage

The epipelic biofilm samples were collected by pipetting the first 10 mm (equivalent to 1 cm^{-2}) of the superficial layer from wells selected at random in each channel (Gómez et al., 2009). For each sample two subreplicates (two wells) were collected per channel, and their average value was used for all statistical analyses. The samples were observed in a Sedgewick-Rafter chamber (1mL) to compute the cell density of the algal groups. To identify the diatom species, an optical microscope Olympus BX-50 with phase and interference contrast at 1000x was used. Diatoms were previously cleaned with H_2O_2, washed thoroughly using distilled water and mounted on microscope slides with Naphrax®. Four hundred valves per sample were identified to the lowest possible taxonomical level, according to standard floras by Patrick & Reimer (1966; 1975), Krammer & Lange-Bertalot (1986; 1988; 1991a; 1991b), Lange-Bertalot (1993), Krammer (1992; 2000), Lange-Bertalot & Moser (1994). Taxonomy of each taxon is given from division to infra-specific level according to Algaebase (Guiry & Guiry, 2012)

Once the diatom species were identified and quantified, their saprobic and trophic preferences were assigned based on Sládecèk (1973), van Dam et al. (1994) and Gómez & Licursi (2001). The total diatom density and Shannon's diversity index (H', Shannon & Weaver, 1949) were calculated for each sample, along with the Pampean Diatom Index (IDP, Gómez & Licursi, 2001), a diatom index developed specifically for the Pampean streams due to their particular characteristics, that integrates information about organic pollution and eutrophication. The diatoms were also classified by their growth-form according to Molloy (1992).

2.4 Data analysis

Differences in the physical-chemical variables between treatments were analyzed using a one-way ANOVA, and the same analysis was used to monitor that the manipulated variables fell within the planned values. Normality was previously assessed by the Shapiro–Wilk test (Shapiro & Wilk, 1965) and homogeneity of variance was tested by Cochran's C test (Cochran, 1951).

The overall differences between the controls and treatments in the species composition were analyzed conducting an Analysis of Similarity (ANOSIM), and the variations in the individual densities of the species were analyzed by means of a two-way repeated measures analysis of variance (RM ANOVA) to test for the differences among treatments and dates (Winer, 1971). This latter analysis was also used to check for significant differences in the abundance of diatoms as classified by their saprobic and trophic preferences and in the indices (H' and IDP). Probabilities within groups were corrected for sphericity using the Greenhouse–Geisser correction. All post-hoc comparisons were made with the Student–Newman–Keuls test (SNK).

Generalized eta^2 (η_G^2) was computed as a measure of the effect size (Olejnik & Algina, 2003). This statistic has two major advantages over the traditional eta^2 and partial-eta^2: first, it provides measures of effect size that are comparable across a wide variety of research designs; and second, these effect-size measures provide indices of effect that are consistent with Cohen's (1998) guidelines for defining the magnitude of the effect (Olejnik & Algina, 2003). These guidelines state that an effect size ≤ 0.20 is considered small, around 0.50 is considered a medium effect, and ≥ 0.80 is a large effect. η_G^2 provides comparability across between-subjects and within-subjects in repeated measures designs (Bakeman, 2005), and is estimated as:

$$\eta G^2 = SS_A/(SS_A + SS_{S/A} + SS_{BS/A})$$

Where *SS* represents a Sums of Squares, *A* represents a between-subjects factor (Treatment), *P* represents a within-subjects factor (Time) and *s* represents the subjects factor.

3. RESULTS

3.1 Physical-chemical parameters

The manipulated variables (SRP, DIN, temperature, water velocity and turbidity) were significantly increased in both LOW and HIGH treatments when compared to the controls ($p<0.05$, Table 1). Results from the analysis of variance show that these increments were in accordance with the planned increments for the experiment ($p> 0.05$).

	CONTROL	LOW	HIGH	p
pH	8.7 (± 0.1)	8.6 (± 0.1)	8.5 (± 0.2)	0.58
Conductivity μS cm^{-1}	633.6 (± 256.7)	723.5 (± 266.8)	1075.4 (± 292.4)	**0.01** C = LOW < HIGH
DO mg l^{-1}	7.7 (± 0.02)	7.7 (± 0.02)	7.6 (± 0.02)	0.36
Temperature °C	24.7 (± 1.3)	25.8 (± 1.2)	28.9 (± 1.4)	**0.01** C < LOW < HIGH
Turbidity NTU	32.3 (± 7.5)	36.9 (± 8.1)	48.3 (± 10.8)	**0.01** C = LOW < HIGH
Water Velocity m sec^{-1}	0.35 (± 0.01)	0.37 (± 0.01)	0.43 (± 0.01)	**0.01** C < LOW < HIGH
SRP mg l^{-1}	0.20 (± 0.12)	0.327 (± 0.19)	0.891 (± 0.53)	**0.01** C < LOW < HIGH
DIN mg l^{-1}	0.43 (± 0.40)	0.75 (± 0.65)	1.39 (± 1.30)	**0.01** C < LOW < HIGH

Table 1. Mean (± standard deviation) of the physical-chemical variables measured in the artificial channels throughout the experiment. Significant differences between the treatments are highlighted in bold (One-way ANOVA, $p<0.05$) along with a posteriori test Student-Neuman-Keuls (SNK) when significant differences were found (C=Control, LOW=Low treatment, HIGH= High treatment).

3.2 Diatom assemblage

The algal groups in the biofilm were represented mainly by diatoms. Other groups included cyanophytes, represented by *Oscillatoria tenuis*, and chlorophytes, represented by Coe*lastrum microporum, Scenedesmus opoliensis, Pediastrum duplex* and *Closterium intermedium*. Euglenophytes, although scarce, were represented by *Euglena acus* and species of the genus *Phacus.* The detailed analysis of the diatom assemblage showed that the most abundant diatoms in all channels before the experimental manipulation had started were *Diadesmis confervacea* (19.0%)*, Ulnaria ulna* (16.8%)*, Nitzschia palea* (12.6%) and *Placoneis placentula* (6.0%).

Throughout the experiment, the mean total density was slightly higher in the treated channels when compared to the controls (Figure 3), although no significant differences were found (Table 2). The diversity index, on the other hand, was significantly higher in the controls (3.5±0.3 bits ind^{-1}) than in the treatments (LOW= 3.1±0.2 bits ind^{-1}, HIGH= 3.3±0.3 bits ind^{-1}) (Figure 3). The effect size in the diversity index suggests a medium effect (η_G^2 ~0.5) due to the treatments.

The ANOSIM evidenced significant differences due to the composition of the assemblage between the HIGH treatment and the controls ($p< 0.05$), while no differences were found between the LOW treatment and either the controls or the HIGH treatment. The RM ANOVA analysis of their densities shows that the treatments caused moderate to large effects ($\eta_G^2 >0.65$; $p<0.05$) on 12 species. Species such as *Planothidium lanceolatum*, *Caloneis bacillum*, *Encyonema minutum*, *Humidophila contenta*, *Luticola kotschyi*, *Nitzschia amphibia*, *Navicula veneta*, and *Pinnularia subcapitata* var. *subcapitata* increased their densities in the channels only when exposed to the HIGH treatment. Other species such as *Luticola mutica*, *Navicula*

cryptocephala and *Navicula lanceolata* were negatively affected by both treatments, while *Nitzschia fonticola* was positively affected by both treatments. The variations throughout the experiment in the most abundant species that were affected by the treatments (over $5x10^2$ cells cm^{-2}) are shown in Figure 4, and the complete list of species identified throughout the experiment is shown in Table 3.

The oligosaprobic and oligotrophic taxa were significantly higher in the controls than in the treatments (Table 2, Figure 5), and the effect size on those categories was small ($\eta_G^2 \leq 0.20$). Throughout the experiment the IDP values (Figure 3) in all channels correspond to environments with high nutrient concentrations and organic matter (IDP>2), although these values were significantly lower in the controls (2.1±0.2) and in the LOW treatment (2.2±0.1) than in the HIGH treatment (2.5±0.2). The effect size for these modifications in the IDP suggests a medium-sized effect due to the treatments ($\eta_G^2 = 0.64$).

With regards to growth-forms, the stalked taxa were significantly more abundant in the controls (7.7±3.9 cell cm^{-2}), while decreasing moderately ($\eta_G^2 = 0.58$) in both the LOW (3.9±1.3 cell cm^{-2}) and HIGH (5.8±3.1 cell cm^{-2}) treatments. Although the other groups did not vary significantly, their mean values suggest that the stalked taxa in the treatments were replaced by motile biraphids, that increased their mean densities in the LOW (58.0±4.4 cell cm^{-2}) and HIGH (55.9±7.3 cell cm^{-2}) treatments while diminishing in the controls (38.3±12.6 cell cm^{-2}) by the end of the experiment. The same tendency was observed for the filamentous diatoms, which decreased in the controls (7.65±7.6 cell cm^{-2}) while increasing in both the LOW (22.7±20.1 cell cm^{-2}) and HIGH (21.1±12.3 cell cm^{-2}) treatments.

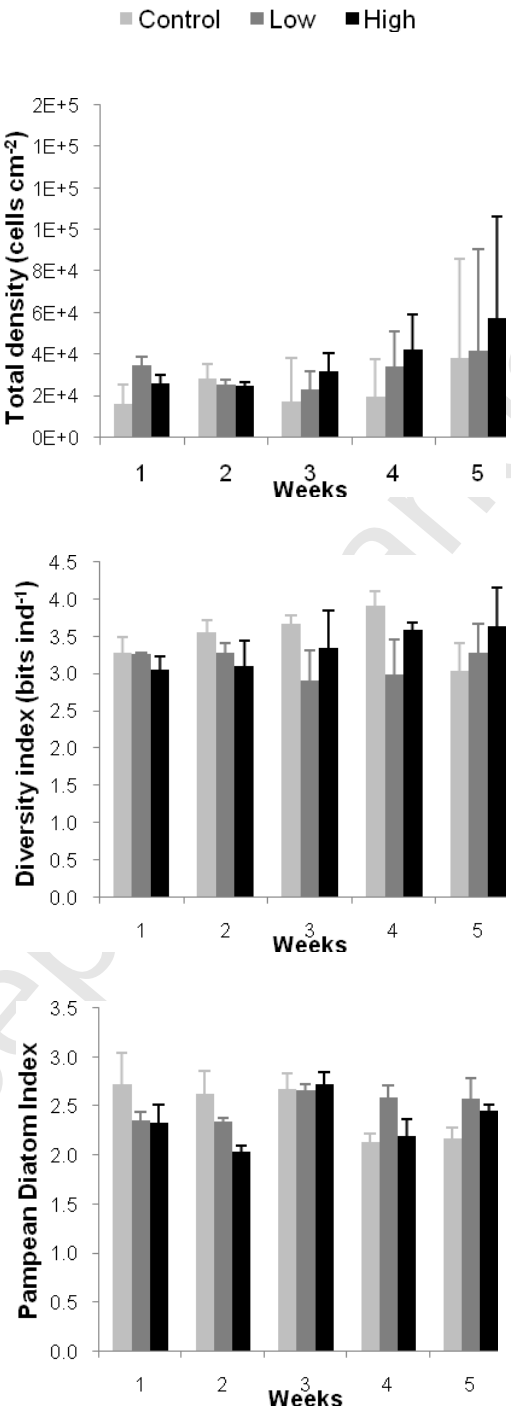

Figure 3. Total density, diversity and IDP (Pampean Diatom Index) in the diatom assemblage throughout the experiment in the controls, Low and High treatments, from weeks 1 through 5.

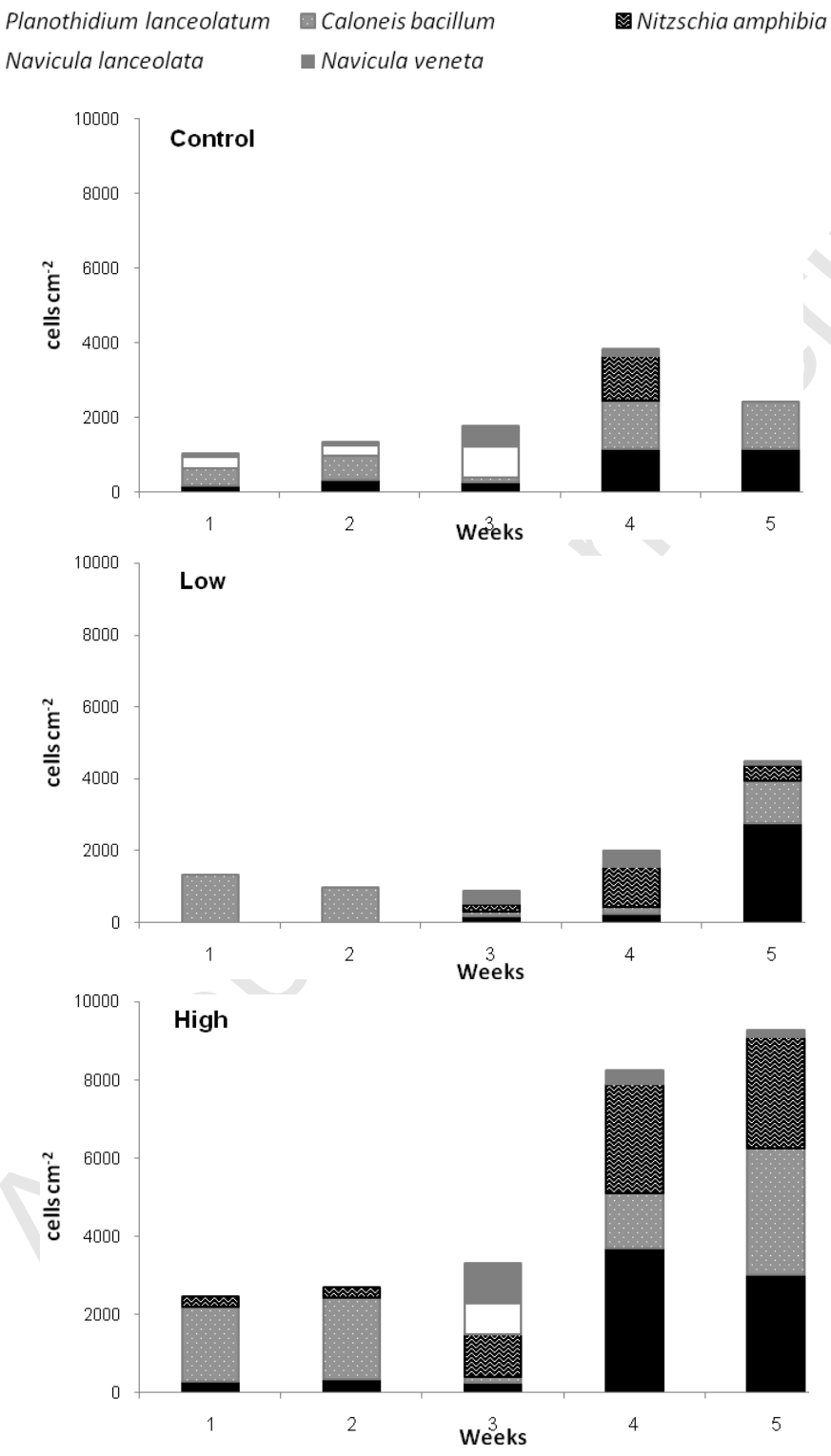

Figure 4. Mean density values of the diatoms that were affected by the treatments (LOW and HIGH) with densities over $10x5^2$ cells cm^{-2} in the five weeks of the experiment.

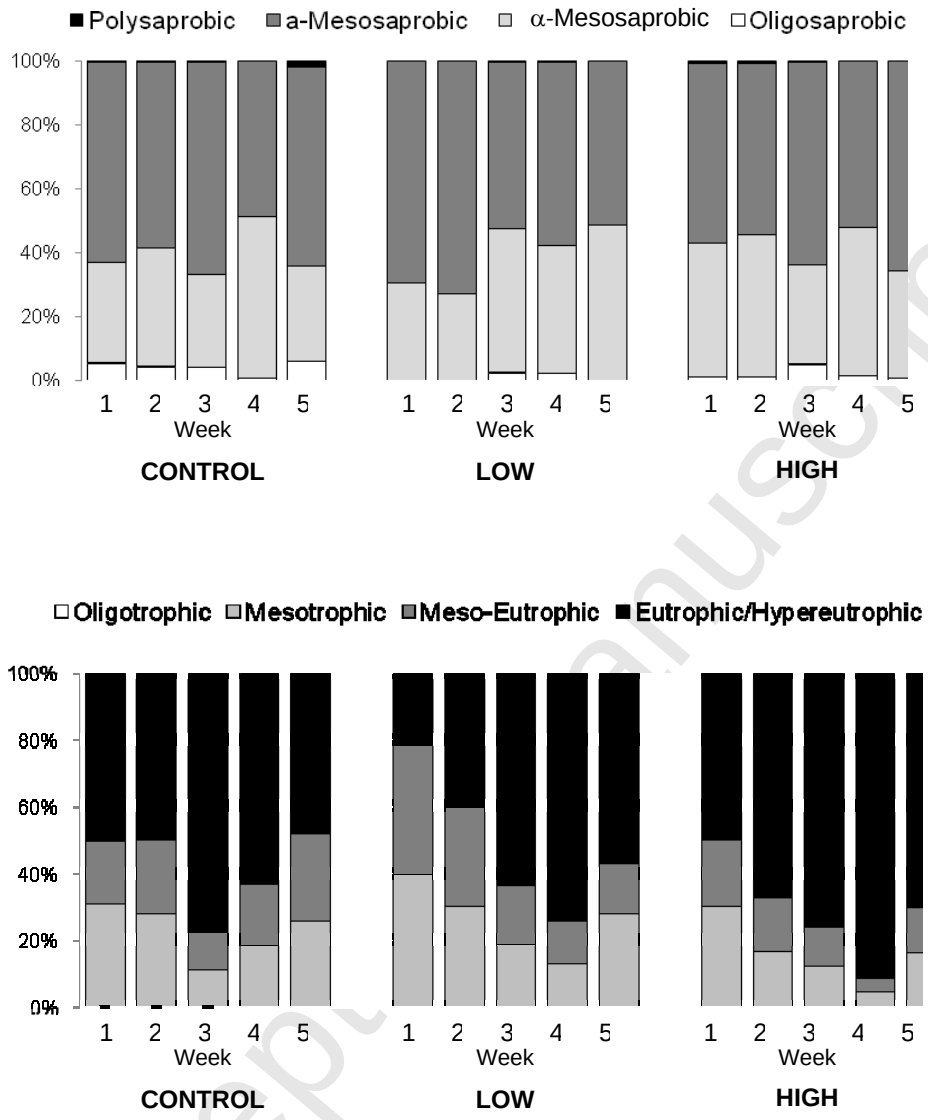

Figure 5. Variations in the mean relative abundance of diatoms as classified by their saprobic (top) and trophic (bottom) preferences in the Controls, Low and High treatments, from weeks 1 through 5.

		TREATMENT	TIME	TREATMENT*TIME
TOTAL DENSITY	p	0.49	0.23	0.82
(cell cm^{-2})	η_G^2	*0.77*	*0.22*	*0.09*
INDICES				
Shannon's Diversity Index	p	**0.05**	0.39	0.06
(H')	η_G^2	*0.43*	*0.12*	*0.23*
		H=L<C		
Pampean Diatom Index	p	**0.04**	**0.00**	0.07
(IDP)	η_G^2	*0.64*	*0.54*	*0.22*
		C<L<H		
SAPROBITY				
Oligosaprobic	p	**0.05**	0.54	0.33
	η_G^2	*0.15*	*0.07*	*0.28*
		C>L>H		
β-Mesosaprobic	p	0.32	0.56	0.65
	η_G^2	*0.12*	*0.06*	*0.11*
α-Mesosaprobic	p	0.50	0.19	0.61
	η_G^2	*0.12*	*0.18*	*0.07*
Polysaprobic	p	0.70	0.53	0.37
	η_G^2	*0.03*	*0.06*	*0.23*
TROPHIC STATE				
Oligotrophic	p	**0.05**	0.50	0.83
	η_G^2	*0.14*	*0.08*	*0.07*
		C>L>H		
Mesotrophic	p	0.35	0.34	0.59
	η_G^2	*0.14*	*0.12*	*0.12*
Eutrophic/Hypereutrophic	p	0.57	0.07	0.46
	η_G^2	*0.10*	*0.24*	*0.13*
GROWTH FORMS				
Adnates	p	0.21	0.06	0.42
	η_G^2	*0.32*	*0.63*	*0.21*
Solitary centrics	p	0.98	0.23	0.46
	η_G^2	*0.00*	*0.46*	*0.21*
Erects	p	0.62	0.44	**0.00**
	η_G^2	*0.11*	*0.34*	*0.48*
Filamentous	p	0.13	0.60	0.31
	η_G^2	*0.39*	*0.26*	*0.24*
Monoraphids/Prostrate	p	0.26	0.03	**0.00**
	η_G^2	*0.28*	*0.69*	*0.50*
Stalked	p	**0.04**	0.58	0.20
		C>L=H		
	η_G^2	*0.53*	*0.27*	*0.28*
Biraphid/Prostrate/Motile	p	0.31	0.53	**0.01**
	η_G^2	*0.25*	*0.29*	*0.45*
Biraphid/Prostrate/Non-motile	p	0.41	0.37	0.05
	η_G^2	*0.19*	*0.38*	*0.36*

Table 2. Effects of the treatments (TREATMENT column), sampling times (TIME column) and the interaction between the two factors (TREATMENT*TIME column) on the total density, on the diatom abundances as classified by their saprobity and trophic preferences, on the diversity, on the IDP and on their growth forms. Significant p values (RM-ANOVA, $p<0.05$) are highlighted, and η_G^2 is shown as a

measure of the effect size. Student-Neuman-Keuls results for the treatment factor are shown when significant (C=Controls, L=Low treatment, H=High treatment).

SPECIES NAME
Achnanthidium exigua (Grunow) Czarnecki
Amphora libyca Ehenberg.
Aulacoseira granulata (Ehrenberg) Simonsen
Caloneis bacillum (Grunow) Cleve
Caloneis silicula (Ehenberg.) Cleve
Caloneis ventricosa (Ehenberg.Donkin) Meister var. *minuta* (Grunow) Patrick
Cocconeis placentula Ehrenberg var. *euglypta* (Ehr.)Grunow
Cyclotella meneghiniana Kutzing
Denticula elegans Kützing
Denticula kuetzingii Grunow var. *kuetzingii*
Diadesmis confervacea Kützing
Diploneis marginestriata Hustedt
Diploneis ovalis (Hilse) Cleve
Diploneis puella (Schumann) Cleve
Encyonema minutum (Hilse in Rabh.) Mann
Encyonema silesiacum (Bleisch in Rabh.) Mann
Eolimna minima (Grunow) Lange-Bertalot
Eolimna subminuscula (Manguin) Moser Lange-Bertalot & Metzeltin
Eunotia bilunaris (Ehrenberg) Mills var. *bilunaris*
Eunotia minor (Kützing) Grunow in Van Heurck
Fallacia insociabilis (Krasske) D.G.Mann
Fragilaria capucina Desmazieres var. *capucina*
*Geissleria decussis (*Østrup) Lange-Bertalot & Metzeltin
Gomphonema affine Ktzing
Gomphonema augur Ehrenberg
Gomphonema clavatum Ehrenberg
Gomphonema minutum (Agardh) Agardh f. *minutum*
Gomphonema parvulum (Kützing) Kützing var. *parvulum* f. *parvulum*
Gyrosigma acuminatum (Kützing)Rabenhorst
Gyrosigma scalproides (Rabenhorst) Cleve
*Halamphora montana (*Krasske) Levkov
*Hantzschia amphioxys (*Ehrenberg) Grunow
Hippodonta capitata (Ehrenberg) Lange-Bertalot Metzeltin & Witkowski
Hippodonta hungarica (Grunow) Lange-Bertalot Metzeltin & Witkowski
*Humidophila contenta (*Grunow) Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová.
Luticola kotschyi (Bleisch) Mann
Luticola mutica (Kützing) D.G. Mann
Mayamea atomus (Kützing) Lange-Bertalot
Melosira varians Agardh
Navicula arvensis Hustedt
Navicula capitatoradiata Germain
Navicula clementis Grunow
Navicula cryptocephala Kützing
Navicula erifuga Lange-Bertalot
Navicula gregaria Donkin
Navicula lanceolata (Agardh) Ehrenberg
Navicula laterostrata Hustedt
Navicula pseudolanceolata Lange-Bertalot
Navicula schroeteri Meister var. *schroeteri*
Navicula subadnata Hustedt
Navicula veneta Kützing
Nitzschia amphibia Grunow f. *amphibia*
Nitzschia brevissima Grunow
Nitzschia capitellata Hustedt
Nitzschia fonticola Grunow
Nitzschia frustulum (Kützing) Grunow var. *frustulum*
Nitzschia gracilis Hantzsch
Nitzschia linearis (Agardh) W.M.Smith var*. linearis*

Nitzschia microcephala Grunow
Nitzschia palea (Kützing) W.Smith
Nitzschia paleacea (Grunow) Grunow
Nitzschia sigma (Kützing) Smith
Nitzschia subacicularis Hustedt in Schmidt
Nitzschia umbonata (Ehrenberg) Lange-Bertalot
Pinnularia gibba Ehrenberg
Pinnularia microstauron (Ehrenberg) Cleve var. *microstauron*
Pinnularia subcapitata Gregory *var. subcapitata*
*Placoneis gastrum (*Ehrenberg) Mereschkovsky
Placoneis placentula (Ehrenberg) Heinzerling
Placoneis pseudanglica (Lange-Bertalot) Cox
Planothidium lanceolatum (Brebisson ex Kützing) Lange-Bertalot
Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot
Sellaphora pupula (Kützing) Mereschkowksy
Sellaphora seminulum (Grunow) Mann
Stauroneis anceps Ehrenberg
Surirella angusta Kützing
Surirella linearis Smith
Surirella ovalis Brebisson
Surirella subsalsa Smith
Tryblionella angustata W.Smith
Tryblionella kuetzingii Álvarez-Blanco & S.Blanco
Tryblionella levidensis W.Smith
Ulnaria ulna (Nitzsch.) Compère

Table 3. Diatom species identified throughout the experiment in all the artificial channels.

4. DISCUSSION

The results obtained in the experiment show that the structure of the diatom assemblage in the epipelic biofilm is affected by the simultaneous modification of temperature, water velocity, nutrient concentration and turbidity. These modifications in the assemblage included changes in diversity, in the proportion of species sensitive to eutrophication and saprobity, and in the percentage of stalked diatoms. These effects were noticeable in the most intensive treatment (HIGH), where moderate decreases were found in the diversity index, small decreases in the proportion of oligotrophic and oligosaprobic species, moderate increases in the IDP values, and moderate decreases of stalked diatoms, which revealed a detriment in the water quality.

When we analyzed changes in the densities of the species, we found that out of the twelve species that were affected by the treatments: three were negatively affected by both treatments (*Luticola mutica, Navicula cryptocephala, Navicula lanceolata*), eight were positively affected by the HIGH treatment (*Planothidium lanceolatum, Caloneis bacillum, Encyonema minutum, Humidophila contenta, Luticola kotschyi, Nitzschia amphibia, Navicula veneta, Pinnularia subcapitata* var. *subcapitata*) and one species was positively affected by both treatments (*Nitzschia fonticola*). This latter not only responded more sensibly to the treatment but also the size of the effects was larger.

Even though the algal biomass is known to be enhanced by the increments in nutrients (e.g. Dodds et al, 2002; Dodds, 2006), temperature (Blanchard et al., 1996) and water current velocities < 40 cm s^{-1} (Lamb & Lowe, 1987; Steinaman & McIntire, 1986), in our study the combination of these variables along with increased turbidity did not translate into a significant increment in total density. It is likely that turbidity, which generally has a negative effect on the biomass of the biofilm due both to the shading it

produces and the erosive properties of the sediments (Horner et al., 1990), represented an important factor in modulating the overall development of the diatoms.

Decreases in the diversity of the assemblage when under stress have been reported (such as Patrick & Reimer, 1966; Sabater, 2000; Gold et al., 2003; Spencer et al., 2008), although it has to be considered that the diversity indices for the benthic diatom assemblages are very variable, and no authors have described a direct cause-effect relationship between chemical pollution and diversity (Ricciardi et al., 2009). Furthermore, Lobo et al. (1995) demonstrated that the distribution pattern of diatom species diversity, measured by Shannon's index in Japanese lotic systems, was consistent with the intermediate disturbance hypothesis (Connell, 1978), and indicates that highest diversity is maintained under intermediate disturbance by pollution. Our results show that moderate changes in the species' diversity when the assemblage was exposed to multiple stressors were manifested after only five weeks of exposure, but only in the most intense treatment. And variations in the tolerance of the diatom assemblage due to that treatment manifested as a decrease in the sensitive species (oligotrophic and oligosaprobic) and as an increment in the IDP values. This coincides with responses observed in other nutrient-rich Pampean streams, where the proportions of sensitive species, even when low, diminish with the increments of nutrients and organic matter (Gómez et al., 2008; Licursi & Gómez, 2009)

The results also show a decrease in stalked diatoms in the treated channels, with slight increments in motile biraphids. The latter include species from the genera *Navicula*, *Nitzschia*, and *Sellaphora*, which are classified as part of the "motile guild" by Passy (2007). This guild includes comparatively fast moving species that are superior competitors for nutrients in nutrient-rich environments (Fairchild et al., 1985;

Van der Grinten et al., 2004) and can physically avoid stress within the benthic mat by moving to resource-rich microhabitats (Johnson et al., 1997).

The results exhibited in this article showed the sensitivity of the diatom assemblage in the very short term of the bioassay conducted to changes in multiple physical-chemical variables, modifying its structural parameters even in response to a moderate stress. We had hypothesized that the manipulation of multiple physical-chemical variables would produce rapid but small structural changes in the diatom assemblage, which were evident in the very short term of the bioassay conducted. The effects of the environmental variables on the diversity and on the proportion of sensitive species were observed only in the more intensive treatment, with no significant changes in the total density, suggesting that the structure of the assemblage is resistant to lesser impacts in these descriptors, while changes in the relative abundance of some diatom species were evident in the intermediate treatment.

Although the results obtained represent a simplification of the complex interactions that occur in natural systems, they provided an approach to the effects of combined variables on the diatom assemblage. However, experiments at longer time scales that study the different interactions between the physical-chemical variables, in conjunction with field experiments, are required to be able to accurately predict the effects of global changes on these micro-communities, and to understand the possible repercussions that these changes might have on the functioning of streams.

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