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Exploring the use of nuclear alterations, motility and ecological guilds in epipelic diatoms as biomonitoring tools for water quality improvement in urban impacted lowland streams

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ABSTRACT

In this study we explored the use of less conventional diatom metrics (motility, nuclear abnormalities and ecological guilds) to measure changes in diatom assemblages related to the transfer of epipelic communities from urban impacted streams to less impacted sites. Three lowland streams in the Argentine Pampean plain were selected, and two sites were established per stream for a total of six sites. Three sites were referenced as low urban impact (LI), as they run through peri-urban areas, and three downstream sites were referenced as high impact sites (HI), since they run through a dense urban area. Six germination trays filled with stream sediment were placed at each HI site, and three at each LI site, and left to be colonized for 30 days before transferring three trays from the HI sites to their respective LI site upstream. Samples were collected at days 0, 15, 30 and 45 days from each tray, and diatom species were identified and classified into ecological guilds. Motility variables were measure per individual of the genera *Nitzschia* (path length, velocity and maximum velocity) through video recording. Also, the frequency of abnormal nuclear locations and nuclear membrane breakage were determined. Results showed that there were no significant differences in any motility metrics, while the nuclei variables exhibited higher proportion of abnormally positioned nuclei and nuclear membrane breakage at the HI sites. The proportion of nuclear membrane breakage decreased over time in the translocated assemblage, indicating that it could be a sensitive indicator of water quality improvement, while the ecological guilds showed significant differences on the proportion of high profile and motile diatoms. The improvement in water quality produced a significative increase in the proportion of the high profile guild in the translocated biofilm. Despite the motile forms being the dominant group, the water quality improvement allowed the high profile forms, to colonize the substrate. These results emphasize the possible use of the ecological guilds as a suitable indicator of water quality improvement in Pampean streams, especially in nutrient rich environment. These tools can provide a rapid assessment of diatom condition and could be considered supplementary to biomonitoring protocols in lowland streams.

1. Introduction

Diatoms are commonly used as biological indicators to assess water quality in rivers and streams, due to their sensitivity to a wide range of environmental stresses (Van Dam et al., 1994; Kelly, 2003; Morin et al., 2016). Their use in water quality assessments is supported in their rapid response to changes in environmental conditions of both pollution increases and habitat restoration success (Stevenson et al., 1999), their great abundance in most lotic ecosystems (Blinn et al., 1980), and their short generational times (Rott, 1991). A major advantage of using diatoms in environmental studies is that diatoms can be used to investigate the effects of toxic pollution at different levels of ecological organization (community, population, and individual levels) (McCormick and Cairns, 1994; Debenest et al., 2013; Stevenson, 2014).

Traditional metrics such as biovolume, cell density, relative abundance, indices (with special reference to indicator species), provide sensitive and powerful tools for evaluating water quality changes (e.g. Rimet et al., 2009; Morin et al., 2010; Proia et al., 2011; Arini et al., 2012; Corcoll et al., 2012; Morin et al., 2012; Cochero et al., 2015). Less conventional parameters, including behavioral, physiological and

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functional metrics are not employed as often for assessing water quality changes, although these metrics have advantages in understanding the dynamics of biological communities (Giddings et al., 2002).

Diatom motility, for example, has been measured in relation to light intensity (Cohn, 2001; Cohn et al., 2004), to metolachlor toxicity (Coquillé et al., 2015), to the toxicity in sediment elutriates (Cohn and McGuire, 2000), and it has been suggested as a useful biomonitoring tool for chronically metal-polluted waterbodies (Pandey and Bergey, 2016). Nuclear anomalies (abnormal nucleus location, micronucleus, multinuclear cell or disruption of the nuclear membrane), another non-taxonomical endpoint, have been related to the effect of exposure to the herbicide, maleic hydrazide, on a freshwater benthic diatom community in the laboratory experiment (Debenest et al., 2008). Similarly, alterations in the nucleus (abnormal nucleus location and nuclear membrane damage) in the diatoms *Fallacia pygmaea* and *Navicula novaesiberica* were reported after 7 days of exposure to Cr (IV) (chromium) treatment (Licursi and Gómez, 2013).

In addition, functional approaches such as the classification of diatoms in ecological guilds (Passy, 2007) can provide sensitive descriptors to assess the ecological health of waterbodies (Rimet and Bouchez, 2012; Stenger-Kovács et al., 2013; B-Béres et al., 2014). Three ecological guilds are defined based on their potential to utilize resources and avoid disturbance (low profile, high profile and motile). The 'low-profile' guild, containing species of short stature including prostrate, adnate, and erect diatoms, is resistant to physical disturbances (water turbulence) and have a low tolerance to nutrient enrichment. The 'high-profile' guild includes larger species that tend to form colonies, and do not resist turbulence well but are enhanced by nutrient enrichment. The 'motile' guild consists of fast-moving species. These taxa are adapted to turbulent environments and to high nutrients concentrations (Rimet and Bouchez, 2012). The advantages of working with ecological guilds is that the analysis of this metric is quick, easy, requires less human expertise, has good reproducibility, and can be adopted world-wide (Pandey et al., 2017).

The application of these parameters (diatom ecological guilds, motility and nuclear abnormalities) have been explored in epilithic assemblages (Pandey and Bergey, 2016, B-Béres et al., 2014) episamic (Licursi and Gómez, 2013), epiphytic diatom assemblages (Riato et al., 2017), artificial substrates (Stenger-Kovács et al., 2013) and diatom cultures (Cohn et al., 2004; Cohn and McGuire, 2000; Debenest et al., 2008; Coquillé et al., 2015). However, motility, nuclear anomalies and diatom ecological guilds have not yet been explored as indicators of water quality changes in the epipelic biofilm.

The epipelic biofilm in pampean streams (Argentina) is dominated by benthic diatoms (Gómez et al., 2009). These lowland streams are exposed mainly to urbanization and agricultural activities and their effects influence the response of benthic diatoms as a result of water quality changes (Rodrigues et al., 2010; Gómez et al., 2010). Teratological forms, changes in species composition and in species' tolerance to pollution have been explored to assess the water quality in lowland streams (Gómez and Licursi, 2001; Tolcach and Gómez, 2002; Gómez and Licursi, 2003; Gómez et al., 2009; Cochero et al., 2013; Licursi et al., 2016) but motility, nuclear abnormalities and the proportion of ecological guilds are parameters that have not been examined as possible water quality indicators.

In this study we explored these characteristics (motility, nuclear abnormalities, ecological guilds) in diatom assemblages induced by the transfer of epipelic communities from urban impacted sites to less impacted sites.

We hypothesized that with an improvement in water quality, epipelic diatoms would increase their motility (higher velocities and acceleration, longer distances per time unit), and the proportion of nuclear abnormalities would decrease. We also hypothesized that, in relation to their ecological guilds, a water quality improvement would favor the high profile guild while limiting the proportion of motile diatoms, whereas the proportion of low profile diatoms would remain unchanged.

2. Materials and methods

2.1. Study area

Three lowland streams in the Argentine Pampean plain were selected. Sampling sites shared similar water height, flow rate, transparency, light exposure, and the streambeds mostly composed of clay with a low proportion of gravel and sand. At each stream, two sites were established for a total of six sites. The streams and sampling sites were selected to include urban land uses with inputs of both treated and untreated effluents, based on previous research (Licursi and Gómez, 2002; Tolcach and Gómez, 2002; Cochero et al., 2015). Three sites (34°58′47.66″S/58° 3′7.81″W, 34°53′40.52″S/58° 1′22.69″W, 34°55′22.12″S/58° 4′58.91″W) were referenced as low urban impact (LI), as they run through peri-urban areas with a low agricultural impact mainly associated with runoff from greenhouses. The other three sites, located downstream from the LI sites (34°57′52.37″S/58° 0′16.96″W, 34°54′8.31″S/58° 1′35.39″W, 34°53′24.85″S/58° 2′56.07″W) were referenced as high urban impact sites (HI), since they run through a dense urban area, receiving point source discharges of untreated domestic effluents.

2.2. Experimental setup

Six germination trays (200 wells, each 2.5 cm diameter × 3 cm deep) filled with stream sediment were placed at each HI site and three at each LI site. The trays were left to be colonized for 30 days. After the colonization period, three trays were transferred from the HI sites to their respective LI site upstream (Fig. 1). This procedure was conducted simultaneously in all three streams. Reference trays were left in all HI sites to serve as controls. Statistical replicates (trays) were composed of three subsamples from each tray (wells) and were collected at days 0 (translocation day), 15, 30 and 45 days after the translocation was conducted. The translocation experiments were conducted in winter (July-August) 2016.

2.3. Physicochemical characteristics of the sites

Dissolved Oxygen (DO, mgL^{-1}), temperature (°C), conductivity ($\mu S\ cm^{-1}$), pH and turbidity (NTU) were measured at each site using a multiparametric sensor (Horiba U50). Water samples (500 ml) were collected in LI and HI sites at all occasions in order to measure nutrients (ammonium, nitrites, nitrates, soluble reactive phosphorous), biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD) samples, which were analyzed according to (APHA/AWWA/WEF, 2012).

2.4. Diatom assemblages, motility and nuclear anomalies analysis

Each sample for diatom assemblages analysis was integrated by 3 subsamples (5–10 mm; 3 wells per tray) collected by pipetting and fixed in 4% formaldehyde (Stevenson, 1984; Lowe and Laliberte, 1996). The samples were oxidized with hydrogen peroxide, washed thoroughly using distilled water and mounted on microscope slides with Naphrax®. Four hundred valves from each sample were identified using an Olympus BX 51 microscope with interference phase contrast under 1000X magnification, and standard floras by Krammer (1992); Metzeltin and Lange-Bertalot (1998); Patrick and Reimer (1966, 1975); Siver and Hamilton (2011); Krammer (2000); Krammer and Lange-Bertalot (1986); Krammer and Lange-Bertalot (1988); Krammer and Lange-Bertalot (1991a); Krammer and Lange-Bertalot (1991b); Lange-Bertalot (2000); Metzeltin and Lange-Bertalot (2005); Metzeltin and Lange-Bertalot (2007). Species were classified according to Passy (2007) into "low profile", "high profile" and "motile".

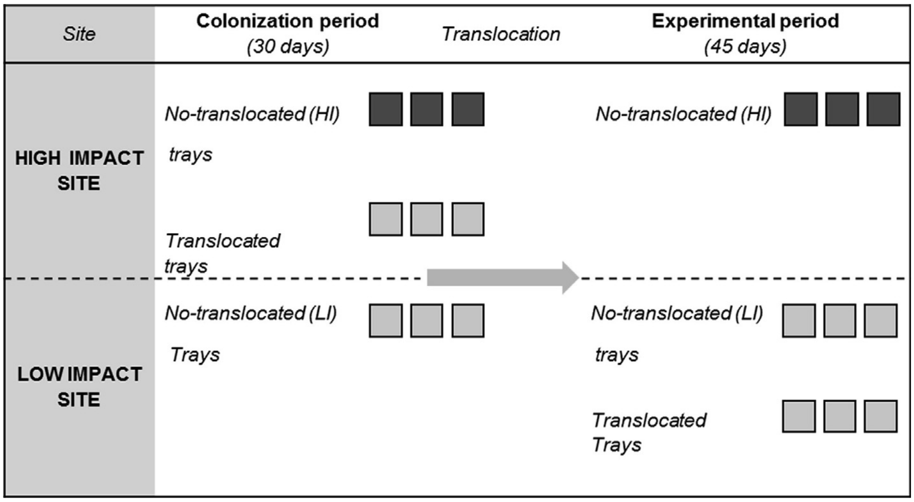

Fig. 1. Experimental design involving a translocation of germination trays with sediment from a high polluted (downstream) to a less polluted (upstream) site within a stream. Translocated trays were colonized in the high impact site and transferred to the low impact site.

For cell motility analysis, samples were collected and conserved in cool until arrival to the laboratory without fixer. Cell motility was measured using the plug-in M-track (Motion tracking and analysis) for Image J (Meijering et al., 2012) Only individuals of the genera *Nitzschia* were considered. The variables measured per individual were: velocity (µm s^{-1}), path length (µm), and maximum velocity (µm s^{-1}). A 40 µl drop of fresh sample with 100 µl of filtered water of the stream was deposited onto a microscope slide, where a 30 s video was filmed (5 fps). Ten individuals per sample were filmed.

For diatom nuclei analysis samples were collected, fixed and stained with 2% (v/v) Hoechst 33,342 (CAS No. 23491–52-3, Sigma Chemical Co.) solution. Nuclear alterations were counted under 600 × magnification with an epifluorescence microscope (Olympus BX50) with a specific filter for DAPI [4, 6-diamidino-2-phenylindole] (U-MWU2, Ex. filter, BP 330–385; Em. filter, BA 420; dicromatic filter, DM 400). A total of 400 cells for each sample were counted to determine the frequency of abnormal nuclear locations and nuclear-membrane breakage. For this evaluation, we first considered the different nuclear locations resulting from normal movements during the cell cycle, as reported by Round et al. (2007) for different diatoms, in order to establish whether or not the positions of the nuclei observed were abnormal.

2.5. Statistical analyses

Differences in physical–chemical indicators were analyzed by a two factor Multivariate Analysis of Variance (MANOVA; Time: Day 0, Day 15, Day 30, Day 45; Site: HI, LI), following the model [$X = \mu + \text{Site} + \text{Time} + \text{Time} * \text{Site} + \text{Residuals}$] (Dytham, 2011). Variations in biological variables throughout the experiment and between treatments were analyzed following a crossed two-way analysis of variance (ANOVA) (Dytham, 2011). To compare the measurements obtained from the diatom assemblage, the analyses followed the model [$X = \mu + \text{Treatment} + \text{Time} + \text{Time} * \text{Treatment} + \text{error}$], with the Treatment factor having three levels (H = No translocated trays from the HI site; L = No translocated trays from the LI site; T = Trays translocated from the HI to the LI site), and the Time factor four levels (Day 0, Day 15, Day 30 and Day 45).

Normality was previously assessed by the Shapiro–Wilk test (Shapiro and Wilk, 1965); homogeneity of variance was tested by Cochran's C test (Cochran, 1951). Eta^2 (η^2) was computed as a measure of the effect size, and the Holm-Sidak (Holm, 1979) test was conducted as a post-hoc test (Cohen, 2013).

3. Results

3.1. Physical-chemical data

Results from the multivariate analyses show significant differences between LI and HI sites (Site factor: $F = 6.40$, $p < 0.05$), with a large effect size (Partial $\eta^2 = 0.84$), regardless of the sampling time (Time factor: $F = 1.8$, $p = 0.09$), and despite it having a large effect size (Partial $\eta^2 = 0.60$).The univariate analyses show that differences between sites is due to differences in nitrates, nitrites, PRS, BOD5, conductivity, pH, temperature, TSS (Table 1).

3.2. Diatom assemblage and ecological guilds

A total of 160 species were found in the epipelon during the experiment. The most abundant species in all sites were *Nitzschia palea* (Kützing) W. Smith (LI = 36%, HI = 37.3%, T = 42.6%), *Fallacia monoculata* (Hustedt) D.G. Mann (LI = 15.2%, HI = 20.3%, T = 14.5%), *Gomphonema parvulum* (Kützing) Kützing (LI = 4.01%, HI = 6.5%, T = 5.4%), and *Melosira varians* Agardh (LI = 8.8%, HI = 0.72%, T = 3.5%), *Nitzschia umbonata* (Ehrenberg) Lange-Bertalot (LI = 0.5%, HI = 6.4%, T = 4.9%), *Sellaphora pupula* (Kutzing) Mereschkowksy (LI = 2.5%, HI = 4.9%, T = 4.1%) and *Navicula schroeteri* Meister (LI = 2.8%, HI = 2.35%, T = 1.3%). When classified into their ecological guilds, the proportion of high profile diatoms were significantly higher at the LI treatment (Table 2, $p = 0.046$), while the proportion of motile diatoms was significantly higher at the HI treatment (Table 2, $p = 0.012$). Yet, these significant effects had a small effect size (Partial $\eta^2 < 0.25$). The low profile guild did not show significant differences between treatments. In addition, there were an increase in the proportion of high profile diatoms and a significant decrease of the proportion of motile diatoms at the translocated treatments (Fig. 2.; $p < 0.05$).

3.3. Diatom motility

Path length (µm), maximum velocity (µm sec^{-1}) and mean velocity (µm sec^{-1}) were not significantly different between treatments during the experiment (Table 2). Path length ranged from 74.5 to 159.2 µm, mean velocity from 0.61 to 1.34 µm s^{-1} and maximum velocity from 16.25 to 32.08 µm s^{-1}.

Path length and maximum velocity showed significant differences only at day 15 were the low impact treatments presented lower values than the high impact and the translocated treatments. (Fig. 2)

Table 1
Mean (± SD) values of the physicochemical variables measured at the LI and HI sites during the experiment, along with the significance values from the univariate analyses from the MANOVA (time, site, time*site), post-hoc comparisons for those factors and μ^2 as a measure of the effect size.

	Site						Post-hoc		
	LI (mean ± SD)	HI (mean ± SD)		Time	Site	Time*Site	Time	Site	Time*Site
Temperature (°C)			**F**	10.313	12.168	0.389			
	11.6 ± 2.7	13.4 ± 2.6	**p**	< **0.001**	< **0.001**	0.761	0 > 15 = 30 = 45	HI > LI	
			η^2	0.286	0.112	0.011			
pH			**F**	0.334	5.986	1.212			
	7.6 ± 0.5	7.8 ± 0.3	**p**	0.801	**0.017**	0.313		HI > LI	
			η^2	0.013	0.080	0.049			
Conductivity (μs cm^{-1})			**F**	5.845	30.505	0.252			
	479.6 ± 270.3	781.1 ± 203.3	**p**	**0.001**	< **0.001**	0.86	45 = 30 > 0 = 15	HI > LI	
			η^2	0.155	0.270	0.007			
DO (mg L^{-1})			**F**	1.192	0.33	4.623			
	5.6 ± 2.1	5.9 ± 2.6	**p**	0.32	0.568	**0.005**			T30)HI < LI
			η^2	0.044	0.004	0.170			
DO%			**F**	1.934	1.149	5.476			
	53 ± 20.1	58.3 ± 25.7	**p**	0.133	0.288	**0.002**			T30)HI < LI
			η^2	0.066	0.013	0.188			
Turbidity (NTU)			**F**	0.242	2.252	2.707			
	91.2 ± 99.7	62.7 ± 59.4	**p**	0.867	0.138	0.053			
			η^2	0.010	0.030	0.108			
TSS (mg L^{-1})			**F**	5.843	30.233	0.257			
	0.3 ± 0.2	0.5 ± 0.1	**p**	**0.001**	< **0.001**	0.856	45 = 30 > 0 = 15	HI > LI	
			η^2	0.156	0.269	0.007			
N-NO$_3$ $^-$(mgL^{-1})			**F**	0.369	4.985	0.493			
	0.6 ± 0.3	1.1 ± 0.7	**p**	0.776	**0.04**	0.692		HI > LI	
			η^2	0.047	0.212	0.063			
N-NO$_2$ $^-$(mgL^{-1})			**F**	0.117	6.13	0.0952			
	0.1 ± 0.1	0.2 ± 0.1	**p**	0.949	**0.025**	0.962		HI > LI	
			η^2	0.015	0.269	0.013			
N-NH$_4$$^+$(mg L^{-1})			**F**	0.577	0.475	0.232			
	0.5 ± 0.7	0.7 ± 0.6	**p**	0.639	0.501	0.873			
			η^2	0.092	0.025	0.037			
DIN (mg L^{-1})			**F**	0.731	5.451	0.349			
	1.1 ± 1	2 ± 0.6	**p**	0.548	**0.033**	0.79		HI > LI	
			η^2	0.089	0.221	0.042			
P-PO$_4$$^{-3}$ (mg L^{-1})			**F**	0.062	4.679	0.051			
	0.4 ± 0.2	1.4 ± 1.3	**p**	0.979	**0.046**	0.984		HI > LI	
			η^2	0.009	0.223	0.007			
BOD$_5$ (mg L^{-1})			**F**	0.427	10.733	0.571			
	7.4 ± 4.2	14.8 ± 6	**p**	0.736	**0.005**	0.642		HI > LI	
			η^2	0.043	0.361	0.058			
COD (mg L^{-1})			**F**	1.524	3.652	0.424			
	19.4 ± 10.2	27.6 ± 10.8	**p**	0.247	0.074	0.739			
			η^2	0.179	0.143	0.050			

3.4. Nuclear abnormalities

There were more abnormally positioned nuclei at the high impact treatments (Table 2, Fig. 2, $p < 0.05$) compared to the low impact treatments although its effect size was small (Partial $\eta^2 = 0.075$). The proportion of nuclear membrane breakage was significant lower at the LI treatment than both the HI and translocated treatments. As shown in Fig. 2, at day 45 the proportion of nuclear membrane breakages were significantly higher at the HI treatment when compared to both translocated and the LI treatments.

4. Discussion

The experimental results showed that the proportion of the ecological guilds in the diatom assemblage changed, particularly affecting the high profile and motile diatoms. Also, a higher proportion of abnormally located nuclei and of nuclear membrane breakage in the diatoms' cells were measured at the impacted sites. On the other hand, there were no significant differences in endpoints associated to cell motility (velocity, acceleration or total path length) that could be attributed to the changes in water quality.

4.1. Diatom assemblage and ecological guilds

The diatom assemblage of the sites is consistent with that found in eutrophic environments with moderate to high organic pollution. *N. palea* was the dominant taxon in all the sampling sites. *F. monoculata* and *G. parvulum* were the following most abundant species in all sampling sites, both considered to be highly tolerant to organic pollution and eutrophication (Lobo et al., 2010). The moderate water quality improvement increased the abundance of *M. varians* after 45 days of exposure. Despite *M. varians* has been considered tolerant to eutrophication (Debenest et al., 2009), previous research in Pampean streams has shown that high levels of pollution can affect its density (Gómez and Licursi, 2001). The opposite response was observed in *N. umbonata*, which decreased at the translocated substrate; this is consistent with previous research that conclude that *N. umbonata* is usually found in strongly polluted sites with high concentrations of organic matter, and in the presence of industrial and urban discharges (Licursi and Gómez, 2002; Duong et al., 2007).

A higher proportion of high profile diatoms has been previously reported in resource-rich habitats (Passy, 2007). The motile guild, comprising mostly eutrophic and pollution tolerant species, can physically avoid stress by moving to resource-rich microhabitats (Johnson et al., 1997). They could control their refuge within the biofilm (Larras

Table 2

Two-way ANOVA results (Factors: Time, Treatment and Time*Treatment) for the path length (um), velocity (μm/sec), maximum velocity (μm/sec), normal nuclei, abnormal nuclear location, nuclear membrane breakage, richness and Shannon diversity index. Significant differences are highlighted in bold; post-hoc test results are also shown by Holm-Sidak test, and η^2 as a measure of the effect size.

					Post hoc		
		Time	Treatment	Time*Treatment	Time	Site	Time*Treatment
Path length (μm)	**F**	1.535	1.454	2.445			
	p	0.210	0.239	**0.03**			Day 15H = T > L
	η^2	0.039	0.025	0.124			
Velocity (μm s^{-1})	**F**	1.487	1.484	2.144			
	p	0.223	0.232	0.055			
	η^2	0.038	0.026	0.111			
Maximum velocity (μm s^{-1})	**F**	0.944	0.429	2.843			
	p	0.422	0.652	**0.014**			Day 15H = T > L
	η^2	0.024	0.007	0.146			
% Abnormal nuclear location	**F**	6.771	4.956	0.969		T = H	
	p	< **0.001**	**0.009**	0.45	45 < 0 = 15 = 30	H > L	
	η^2	0.154	0.075	0.044		T = L	
%Nuclear membrane breakage	**F**	2.285	20.294	0.782			
	p	0.084	< **0.001**	0.586		H > T > L	
	η^2	0.046	0.274	0.032			
Low profile	**F**	0.474	0.781	1.294			
	p	0.701	0.461	0.267			
	η^2	0.013	0.015	0.073			
High profile	**F**	4.483	3.174	1.708		L = T	
	p	**0.005**	**0.046**	0.127	45 = 30 > 0	L > H	
	η^2	0.107	0.050	0.081		H = T	
Motile	**F**	6.225	4.597	1.313		L = T	
	p	< **0.001**	**0.012**	0.259	0 = 15 > 45	L < H	
	η^2	0.142	0.070	0.060		T = H	

et al., 2013), and regulate the balance between access to environmental resources (light, nutrients) and exposure to stress (Fore and Grafe, 2002; Laviale et al., 2009), which explains their maximum densities at high nutrient levels. In this way, the motile growth forms increase in sewage-receiving water bodies while erect and stalked species representing the high profile guild decrease (Smucker and Vis, 2010). These characteristics of the high profile and the motile guilds are consistent with the results found in our work: the high profile diatoms were more abundant at the less impacted sites and increased with the water quality improvement, while the motile guild forms were more abundant at the highly impacted sites.

The low profile guild is limited by the disturbance caused by the instability and movement of soft substrates, favoring the establishment of the other two guild species. The low profile guild also dominates at high current velocities, whereas the high profile guild prevails in low velocities, resulting in low guild diversity at both ends of the current velocity gradient (Passy, 2007). The low current velocities in the lowland streams studied (0.07 ± 0.04 m.s^{-1}), in this research along with their high nutrient concentrations could explain why the low profile guild was similar between all treatments, representing a small proportion of the total diatoms found, whereas the motile and high profile diatoms dominated the epipelic biofilm.

The variations in the proportions of the different ecological guilds suggest their possible use as a suitable indicator of water quality improvement in nutrient-rich streams such as the Pampean streams.

4.2. Diatom motility

The use of diatom motility as a tool for measuring water quality has been scarcely addressed, as well its use in recovery experiments. A variety of environmental (Cohn and Disparti, 1994) and anthropogenic perturbations (Svensson et al., 2014; Coquillé et al., 2015) have shown to alter the movement rate and patterns of live diatoms. Mean velocity has been shown to decrease (Pandey and Bergey, 2016; Gupta and Agrawal, 2007), or increase (Coquillé et al., 2015) due to an impairment in water quality.

One possible explanation for our varied results could relate to the fact that most measurements have been conducted at the genus level. Despite that previous research has shown that cell length and/or size did not have significant effects on diatom velocity (Drum and Hopkins, 1966; Bertrand, 1999; Cohn and Disparti, 1994; Gupta and Agrawal, 2007), other factors like temperature, pH, nutrient concentrations, and the presence of heavy metals, have been reported to cause species-specific responses (Cohn and McGuire, 2000; Cohn and Disparti, 1994; Cohn and Weitzell, 1996; Gupta and Agrawal, 2007; Pandey and Bergey, 2016).

The results show that the motility parameters of *Nitzschia* are lower than those reported for other experiments (Cohn and Disparti, 1994; Cohn and Weitzell, 1996; Pandey and Bergey, 2016), suggesting that this parameter is highly variable even within the same taxonomical genus. Pandey and Bergey (2016) recorded different velocity values, ranging from 10 ± 3 and 9 ± 3 um s-1 for *Nitzschia sigmoidea* and 12 ± 2 and 9 ± 4 um s-1 for *Nitzschia linearis* in highly polluted and unpolluted sites respectively. Considering this, the use of diatom motility endpoints as a tool for the assessment of urban impacts should be further explored at the species level.

4.3. Nuclear abnormalities

Previous studies detected herbicides and heavy metals concentrations (Ni, Cu, Pb) in some of the sites studied (Gómez et al., 2008; Sierra and Gómez, 2010, Solis et al., 2017), that are known to lead to increments in abnormal nucleus locations and in nucleus membrane breakages (Licursi and Gómez, 2013) and to the appearance of micronuclei and nuclear fragmentations (Debenest et al., 2008).

Few reports indicate how intracellular organelles, specifically the nucleus of live diatoms, react to different environmental and anthropogenic disturbances (Coombs and Volcani, 1968; Casotti et al., 2005). It has been found that the exposure to toxicants such as aldehydes, herbicides, colchicine, hexavalent chromium or ultraviolet radiation can have important intracellular consequences, including DNA dispersion, multinuclear cells, presence of micronuclei o DNA fragmentation (e.g. Coombs and Volcani, 1968; Buma et al., 1995, 1996; Casotti et al., 2005; Debenest et al., 2008; Duke and Reimann, 1977; Rijstenbil, 2001;

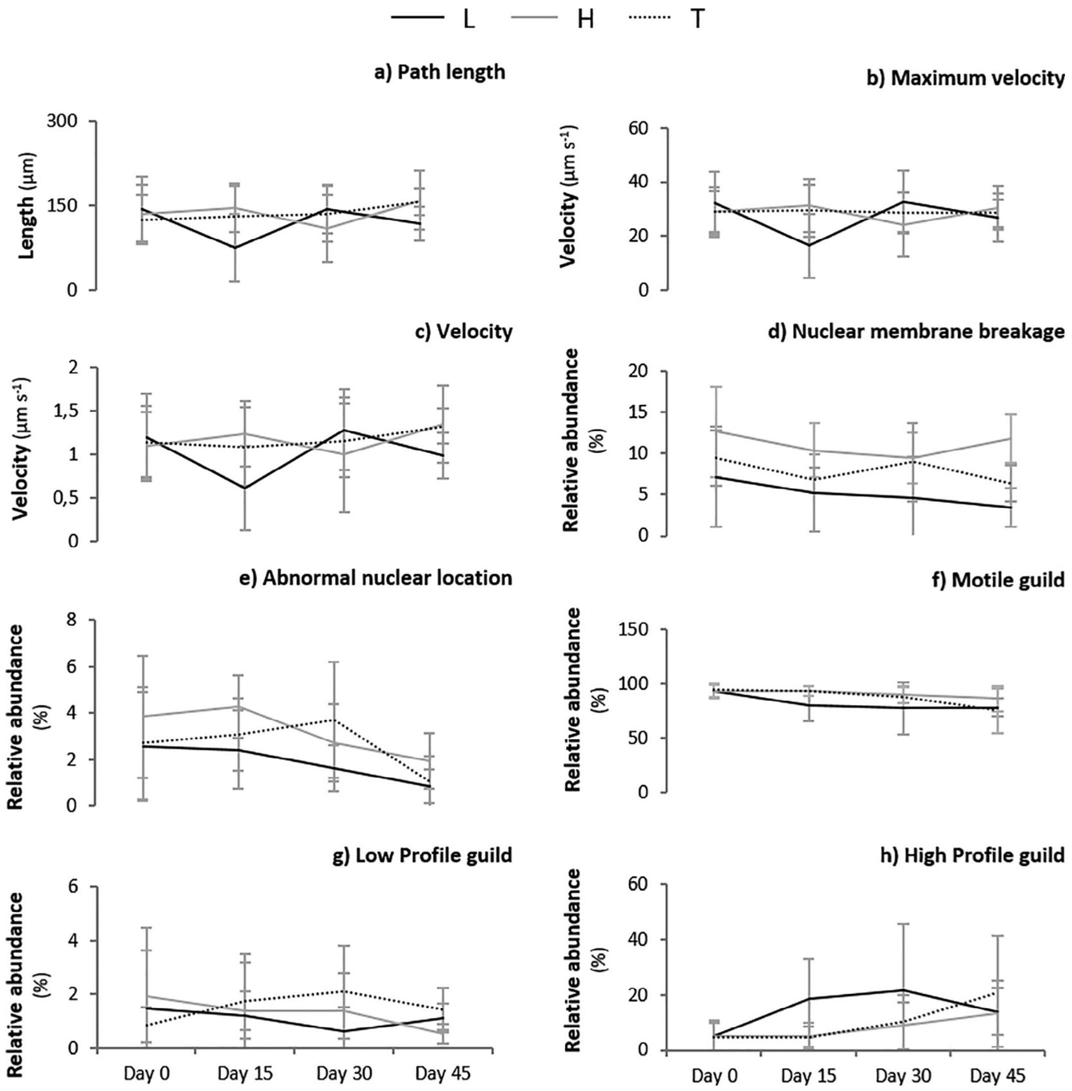

Fig. 2. Motility measures: a) path length (μm); b) maximum velocity (μm sec^{-1}), c) velocity (μm sec^{-1}). Nuclear measures: d) nuclear membrane breakage e) abnormal nuclear location. Ecological guilds: f) motile, g) Low profile, h) high profile. Lines represent the three treatments (L = Low impact, H = High impact and T = Trays translocated from the HI to the LI site) during all sampling dates.

Licursi and Gómez 2013). Most of these ecotoxicological studies were conducted using small populations of certain species or communities, and almost all of them were in laboratory conditions.

Even though the proportion of abnormal nucleus location was higher at the most impacted sites, this variable did not change over time at the translocated trays as it was expected, so its use as an indicator of the recovery of the assemblage is uncertain. On the other hand, the proportion of nucleus membrane breakage did decrease over time in the translocated assemblage, indicating that this variable could provide with a sensitive indicator of water quality improvement. Since alterations in nuclear endpoints have been associated with the presence of pesticides and heavy metals, future studies should contemplate their measurement in surface waters and sediment.

5. Conclusions

We hypothesized that the diatom assemblage could provide with sensitive indicators of water quality improvement, even in lowland streams with high concentrations of nutrients and organic matter. The proportion of nuclear abnormalities and the classification of diatoms in ecological guilds demonstrated to be suitable indicators, as they responded significantly to the translocation of the epipelic community. Endpoints related to diatom motility, on the other hand, were highly variable in the field experiments, and should be further explored in relation to specific stressors.

In distinction from other more traditional endpoints measured in the diatom assemblage to monitor urban streams, the water quality improvement proposed in these bioassays were mild, and yet significant differences were found in the monitored variables. These tools can provide a rapid assessment of diatom condition and could be considered supplementary to current biomonitoring protocols in lowland streams.

CRediT authorship contribution statement

María Mercedes Nicolosi Gelis: Conceptualization, Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Joaquín Cochero:** Conceptualization, Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Jorge Donadelli:** Methodology. **Nora Gómez:** Conceptualization, Funding acquisition, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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