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Temporal changes in biological traits of diatom communities in response to an oil spill in a subtropical river

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Abstract: Diatom growth strategies, which are used as a proxy to analyze disturbances and environmental impacts, can also provide reliable information about environmental gradients. In this study, we used diatom communities and biological traits to assess the structure of epilithic diatoms after an oil spill in a large tributary of the Iguaçu River (Paraná, Brazil). Epilithon and water samples were taken on July 12th, 2004, and after an unexpected oil spill, on the following days: 09/19, 10/03, and 10/13/2004. We observed that the spill caused a distinct shift in both diatom composition and guild group. Diversity decreased and pollution-tolerant diatoms of the motile group became dominant in response to the elevated nutrient levels and reduced light. Tube-forming diatoms, which are strong competitors for light, dominated the epilithon 24 days after the oil spill. Both diatom guilds and growth forms were successfully used to predict the environmental conditions. While diatom guilds responded to disturbances along a temporal gradient, changes inside the guilds were the main factor for understanding the environmental gradient.

Key words: diatom guilds, disturbance, diversity, epilithon, growth forms.

INTRODUCTION

Diatoms are a very diverse group of unicellular photosynthetic microorganisms characterized by a silicified cell wall known as frustule whose ornamentation is used for taxonomy purposes (Round et al. 1990). Diatoms may live free-floating in the water column (phytoplankton) or attached to submerged substrates (periphyton), lying in a polysaccharide matrix with other microorganisms (Wetzel 1983). Specialized structures in the mucilage extruding from the apical pore field, the rimoportulae and the raphe enable cells to adhere to surfaces or to slide across them (Round et al. 1990), thus acting as a competitive strategy for resources in the biofilm (Passy 2007). Diatom growth depends on light, water chemistry, temperature (Patrick and Reimer 1966), resource availability, grazing (Lange et al. 2011), and water disturbances (Peterson and Stevenson 1992).

Due to limited mobility, the attached community is exposed to adverse conditions and is thus well-suited to be used worldwide for assessing environmental impacts (Stevenson et al. 1996). Since diatom taxonomy is usually rather complex and time consuming, the use of biological traits in ecological status assessments can provide a more

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efficient alternative. The use of biological traits in predicting environmental-ecological aspects is suitable when species-level identification may be complicated or even impracticable. The application of diatom-based indices requires accurate species identification and is constrained by geographical limitations. In addition, diatom-based indices are calibrated to local information, which prevents their general use (Lobo et al. 2004a, b, Stenger-Kovács et al. 2007). Ecological guilds group species that exploit the same resources within the same ecological preferences, however, showing different adaptations (Simberloff and Dayan 1991). Diatom ecological guilds are strongly related to environmental gradients and respond to disturbance effects and resource availability (Passy 2007, Lange et al. 2011, Berthon et al. 2011, DeNicola and Kelly 2014). Studies have shown that biological traits can be successfully used for the assessment of ecological conditions in rivers (Passy 2007, Rimet and Bouchez 2011, Lange et al. 2011, Schneck and Mello 2012, Stenger-Kovács et al. 2013), lakes (Faria et al. 2015), and wetlands (Riato et al. 2017) at different levels of stress or disturbance.

Accidental oil spills in marine and freshwater affect aquatic biota by disturbing structures essential for vital processes and by causing chemical intoxication (Snow and Scott 1975). Oil discharges can also raise nutrient availability in the water column, favoring eutrophication (Snow and Scott 1975) and reducing light availability for photosynthetic organisms. In addition, losses in periphytic biomass have been recorded as a result of oil exposure (Singh and Gaur 1989, Nayar et al. 2004). On the other hand, the opposite may also occur, since algae biomass can be stimulated by oil (Snow and Scott 1975), as algae grazers can be suppressed (Cubit et al. 1987) by oil toxicity (Scholten and Kuiper 1987), thereby increasing primary production (Bakke and Johnsen 1979). These antagonizing results may reflect the controlled conditions of the studies, which cannot incorporate the multistressor effects of natural environmental conditions. There is increasing interest in the impacts of oil spills on natural benthic conditions, e.g. a study on mangrove benthic communities in French Guiana (Jézéquel et al. 2017). However, the study did not investigate primary producers and the main focus was preparedness due to high risk of oil spills from the Brazilian offshore companies.

The central idea in monitoring of aquatic environments by using biological traits is the possibility of assessing environmental conditions when limited chemical analysis data are available. We assessed the responses of epilithic diatom communities to an unexpected oil spill which occurred in the subtropical freshwater Negro River (in southern Brazil). We used diatom guilds and growth forms as a proxy to estimate the environmental health in each sample. We hypothesized that a temporal gradient was formed after the oil spill and that the discharge affected diatom community.

METHODS

STUDY AREA

The Negro River belongs to Iguaçu River basin, located between the cities of Mafra (Santa Catarina State) and Rio Negro (Paraná State) in Brazil. It is the main tributary of the Iguaçu River (SUDERHSA 2007). The river rises at an elevation of 1400 m and flows 240 km before emptying into the Iguaçu River.

On September 12th, 2004, approximately 120,000 liters of vegetable oil and 60,000 liters of diesel oil spilled into the Negro River after a train accident. The oil leak was controlled only four days after the accident, on September 16th, 2004. The public water supply in the region was shut off to prevent the spread of oils and greases in the water distribution network (Military Police Environmental Protection Company 2004). The water supply was fully normalized on September 20th, 2004, a week after the accident.

DATA SAMPLING

Water and epilithon samples were taken between two extraction points for the public water supply (26°06’46.6’’ S and 49°47’22.1’’ W). The first sampling was done on July 12th, 2004, before the oil spill. Diatoms were initially being sampled for taxonomic investigation. Since the oil spill occurred about 22 km upstream from the sampling site (26º17’16 “S and 49º72’68” W), we chose to sample the same location with the aim of understanding the environmental and community changes after the oil spill. Sampling was performed one week after the accident (after1, September 19th) and then two weeks later (after2, October 3th). Another sample was taken 24 days after the oil spill (after3, October 13th).

Water samples were collected for physical and chemical analyses. Nitrate (NO_3^-), nitrite (NO_2^-), phosphate (PO_4^{3-}), and total solids (TS) were analyzed at “Núcleo de Controle de Qualidade (Universidade Positivo)”; chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5) were analyzed at “Centro de Pesquisa e Processamento de Alimentos (Universidade Federal do Paraná)” following the methodologies described in APHA (2012). Secchi depth and temperature were measured *in situ*. Conductivity (cond) was measured with MWA-300 and pH with a QUIMIS Q400 Aequipments (Universidade Positivo). The rainfall data (rain) for the sampling period were provided by Instituto das Águas do Paraná.

Epilithon samples were collected from cobbles (n = 3 in each sampling data; cobble size ≈ 8 to 10 cm diameter). Biofilm was removed from the substrates by scraping with toothbrushes. After washing, samples were cleaned with 10% solution of $KMnO_4$ and HCl (Simonsen 1974, modified by Moreira-Filho and Valente-Moreira 1981). For the preparation of permanent slides Naphrax® (Brunel Microscopes Ltd. U.K.; IR = 1.73) was used as a mounting medium. For the qualitative and quantitative analyses 600 valves were counted (Kobayasi and Mayama 1982). Diatoms were identified according to specific literature, for example, Patrick and Reimer (1966), Krammer and Lange-Bertalot (1986, 1991a, b), Krammer (1997a, b), Metzeltin and Lange-Bertalot (1998, 2007), Metzeltin et al. (2005), Hofmann et al. (2013), Levkov et al. (2013, 2016), and herein we provide the list of diatom taxa of the Negro River and the accompanying metrics. Diatoms were classified into three guilds: motile, high-profile, and low-profile (adapted from Passy 2007 and Berthon et al. 2011) and into six growth forms: motile, erect, colonial, tube-forming, pioneer, and planktonic (adapted from Rimet and Bouchez 2011, Berthon et al. 2011 and Faria et al. 2015).

DATA ANALYSIS

Dendrogram of similarity (two-way dendrogram, Euclidean distance) based on the environmental data was used in PC-ORD v6.0 (McCune and Mefford 2011) to investigate nutrient gradients after the spill. Bartlett’s test was applied to check homogeneity of variances across samples and Kolmogorov-Smirnov test to check normality. To describe the diatom community, we used ranking of abundance and calculated diversity indices (Shannon-Weaver) followed by a *t*-test between diversities using PAST software (Hammer et al. 2001). To investigate richness, one-way ANOVA was used followed by the Tukey test in Statistica 7.1 (StatSoft Inc. 2005). We also provide Spearman’s correlation between biotic and abiotic data (excluding the sample before the oil spill). To investigate temporal changes in guild and growth form composition in response to oil spill disturbances, Principal Component Analysis (PCA) was performed using data from all the three sampling events after the spill. Data was normalized by log x+1 transformation.

RESULTS

Dendrogram of similarity using environmental data showed that the primary clustering separated the sample taken one week after the oil spill (after1) with 100% dissimilarity (Fig.1). Sample after1 showed reduced pH (6.9) and conductivity (29 µS cm^{-1}) and increased TS (100 mg L^{-1}) values; higher BOD$_5$ (6.29 mg O$_2$ L^{-1}), COD (13.4 O$_2$ L^{-1}), NO$_3^-$ (1.9 mg L^{-1}), and NO$_2^-$(0.03 mg L^{-1}) values were also registered. The subsequent samples (after2 and after3) have been grouped together due to high similarity; both showed increases in pH (7.2) and conductivity (39 µS cm^{-1}), as well as high rainfall (200 mm), which kept the TS values high (±95 mg L^{-1}). PO$_4^{3-}$ showed a temporal gradient decreasing from after1 (0.47 mg L^{-1}) to after3 (0.14 mg L^{-1}). After3 showed some increases in water column light penetration (5 cm) as well as reduced BOD$_5$ (3.7 mg O$_2$ L^{-1}) and COD (5.5 O$_2$ L^{-1}) values; after3 was grouped with the sample before the oil spill, with ~60% similarity.

A total of ninety-nine diatom taxa were identified, classified into 35 genera and 22 families. The most representative families were Naviculaceae and Cymbelaceae (Table I). Rank abundance revealed that the sample taken before the oil spill had a greater uniformity, while the sample taken after had less. Greater richness and diversity were recorded before the oil spill (S' = 77 and H' = ±3.09, respectively); the motile guild had the highest richness (S' = 49), followed by the high-profile (S' = 23) and low-profile guilds (S' = 5).

We investigated the temporal gradient after the oil spill. Diversity *t*-test showed a negative diversity gradient reducing over time ($t = 9.93$, $p < 0.001$), suggesting changes in diatom community composition as a result of a temporal environmental gradient. We found a richness temporal gradient which declined from after1 (S' = 53) to after3 (S' = 34; F = 16.80, $p < 0.001$). In after1, the motile guild presented higher richness (S' = 28) than the high-profile (S' = 21) and the low-profile (S' = 4) guilds; the three guilds decreased over time and in after3, richness was as follows: motile guilds (S' = 16), high profile guilds (S' = 15), and low-profile guilds (S' = 3). In after3 we found that 43% of diatom community relative abundance was comprised of the tube-forming diatom *Encyonema perpusillum* (Cleve-Euler) and 26% of the motile diatom *Navigeia aikenensis* (R.M. Patrick) L.N. Bukhtiyarova.

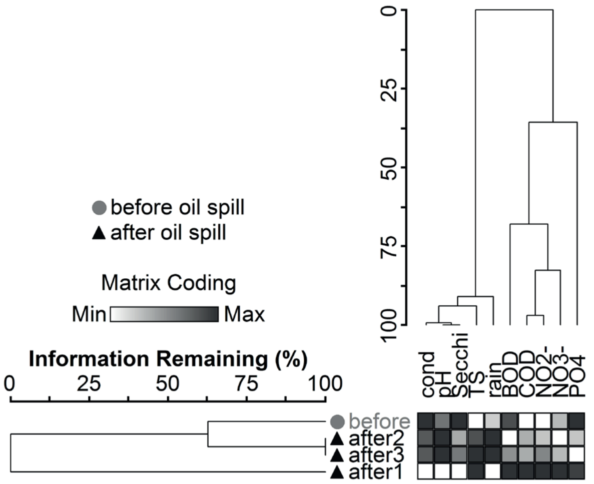

Figure 1 - Dendrogram of similarity showing environmental data before and after the oil spill in Negro River, Brazil.

Higher motile guild diversity was registered before the oil spill (H' = 3.06). Diversity *t*-tests showed that the guild diversity was lower in after1 (H' = 1.9; $t = 9.70$, $p < 0.001$) due to the abundance of *Navigeia aikenensis* and *Navicula veneta* Kützing, as well as *Mayamaea permitis* (Hustedt) K. Bruder & Medlin and *Sellaphora nigri* (De Notaris) C.E. Wetzel & L. Ector. The motile guild, which dominated the diatom community in after1 (70%) was positively correlated with phosphate concentration in water ($r = 0.99$, $p = 0.03$). The proportion of motile guild abundance decreased over time (F = 17.65, $p < 0.001$) due to replacement by the low-profile guild. Moreover, the motile diatoms *Luticula goeppertiana* (Bleisch) D.G. Mann and *N. aikenensis* persisted in the periphytic matrix after the oil spill, whereas *L. goeppertiana* grew between after1 and after2, while *N. aikenensis* was observed in the periphytic matrix until after3.

TABLE I
Diatoms from Negro River, classification and metrics.

Taxa	Growth form and ecological guild	Metric				
		Lenght (µm)	Width (µm)	Striae (10 µm)	Areolae (10 µm)	Fibulae/ Costae/Alar canals (10 µm)
Stephanodiscaceae						
Discostella stelligera (Cleve & Grunow) Houk & Klee	Planktonic; high-profile	....	6-9	15-16	....	
Melosiraceae						
Melosira varians C. Agardh	Planktonic; high-profile	12-23.2	16.8-25.3	....	....	
Aulacoseiraceae						
Aulacoseira ambigua (Grunow) Simonsen	Planktonic; high-profile	16.3-25	5-8.4	14-18	16-18	
Aulacoseira pusilla (F. Meister) A. Tuji & A. Houki	Planktonic; high-profile	6.3-8	6-7	18-20	20	
Aulacoseira tenella (Nygaard) Simonsen	Planktonic; high-profile	3.1-3.5	5.8-9	18-22	15-16	
Orthoseiraceae						
Orthoseira dentroteres (Ehrenberg) Crawford	Planktonic; high-profile	....	9-14.7	17-18	18	
Fragilariaceae						
Fragilaria fragilarioides (Grunow) Cholnoky	Erect; high-profile	26.8-46.8	3.7-4.2	13-15	....	
Fragilaria rumpens (Kützing) G.W.F. Carlson	Erect; high-profile	22-35	3-4	18-20	....	
Staurosirella leptostauron (Ehrenberg) D.G. Williams & Round var. *leptostauron*	Colonial; high-profile	14-20	5-7	6-8	....	
Staurosirella pinnata (Ehrenberg) D.G. Williams & Round	Colonial; high-profile	4.2-9.4	3-5	13-16	....	
Eunotiaceae						
Eunotia intermedia (Krasske *ex* Hustedt) Nörpel & Lange-Bertalot	Colonial; high-profile	11-15	3-4	13-14	....	
Eunotia minor (Kützing) Grunow	Colonial; high-profile	22.6-38	4-6	15-16	....	

TABLE I (continuation)

Taxa	Growth form and ecological guild	Metric				
		Lenght (µm)	Width (µm)	Striae (10 µm)	Areolae (10 µm)	Fibulae/ Costae/Alar canals (10 µm)
Eunotia rabenhorstiana (Grunow) Hustedt	Colonial; high-profile	84-133	6-8	14-17	....	
Eunotia rabenhorstii Cleve & Grunow	Colonial; high-profile	16-27	6-8	12-14	....	
Eunotia siolii Hustedt	Colonial; high-profile	19-22	4-5	8-10	....	
Eunotia sudetica O. Müller	Colonial; high-profile	35-39	6-7	10-12	....	
Cymbellaceae						
Adlafia drouetiana (R.M. Patrick) Metzeltin & Lange-Bertalot	Motile; motile guild	15.3-15.7	3.6-4.2	24-26	....	
Adlafia muscora (Kociolek & Reviers) G. Moser, Lange-Bertalot & Metzeltin	Motile; motile guild	14-15	3-4	26-28	....	
Encyonema minutum (Hilse) Mann	Tube-forming; high-profile	12-20	4-5.2	12-14	....	
Encyonema perpusillum (Cleve) D.G. Mann	Tube-forming; high-profile	11-20	3.6-4.7	11-12	....	
Encyonema exuberans Tremarin, C.E. Wetzel, T. Ludwig	Tube-forming; high-profile	18.4-36	5.7-6.3	8-12	....	
Encyonema silesiacum (Bleisch) D.G. Mann	Tube-forming; high-profile	25-31	6-7.8	12-14	....	
Placoneis clementis (Grunow) E.J. Cox	Motile; motile guild	24-35	10-13	12-15	....	
Placoneis disparilis (Hustedt) Metzeltin & Lange-Bertalot	Motile; motile guild	36.3-56	14-15.7	12-13	18-20	
Placoneis itamoemae Straube, Tremarin & T. Ludwig	Motile; motile guild	26.3-35	13.6-14	14-18		
Placoneis porifera var. *opportuna* (Hustedt) E. Novelo, R. Tavera & C. Ibarra	Motile; motile guild	13.1-16.3	6.8-7.3	12-14	....	
Gomphonemataceae						
Gomphonema costei Metzeltin & Lange-Bertalot	Erect; high-profile	23.6-45	6.8-7.8	7-9	....	
Gomphonema exilissimum (Grunow) Lange-Bertlot & E. Reichardt	Erect; high-profile	15.2-28.4	4.2-4.7	14-16	....	

TABLE I (continuation)

Taxa	Growth form and ecological guild	Metric				
		Lenght (µm)	Width (µm)	Striae (10 µm)	Areolae (10 µm)	Fibulae/ Costae/Alar canals (10 µm)
Gomphonema gracile Ehrenberg	Erect; high-profile	55-63	8-9	10-12	....	
Gomphonema lagenula Kützing	Erect; high-profile	16-22	4-5	11-12	....	
Gomphonema mexicanum Grunow	Erect; high-profile	26.3-58.4	8.4-12	10-12	20	
Gomphonema parvulum (Kützing) Kützing	Erect; high-profile	14-20.5	3.6-4.7	13-15	....	
Gomphonema pseudoaugur Lange-Bertalot	Erect; high-profile	23.1-32.6	7.8	11-14	....	
Gomphonema pumilum (Grunow) E. Reichardt & Lange-Bertalot	Erect; high-profile	10-23.1	3.1-4.2	12-13	....	
Cocconeidaceae						
Cocconeis fluviatilis J.H. Wallace	Adnate; low-profile	20-23	10-12	18-20 c/r; 12-13 s/r	20c/r;10-11s/r	
Cocconeis placentula var. *lineata* (Ehrenberg) Van Heurck	Adnate; low-profile	30-38	23-29	18 c/r; 18-20 s/r	18 c/r; 13-15 s/r	
Achnanthidiaceae						
Achnanthidium eutrophilum (Lange-Bertalot) Lange-Bertalot	Adnate; low-profile	10-16	4-5	20 c/r; 24-26 s/r	....	
Achnanthidium exiguum (Grunow) Czarnecki	Adnate; low-profile	9-13	5-6	26 c/r; 20-22 s/r	....	
Achnanthidium minutissimum (Kützing) Czarnecki	Erect; low-profile	9-19	3-4	....	....	
Karayevia oblongella (Oestrup) Aboal	Motile; motile guild	8-16	5-6	12-4 s/r	....	
Planothidium bagualensis C.E. Wetzel & L. Ector	Erect; high-profile	12-23	6-9	16-17 c/r; 11-14 s/r	....	
Planothidium biporomum (M.H. Hohn & Hellermann) Lange-Bertalot	Erect; high-profile	16-22	6-8	12-14	....	
Planothidium dubium (Grunow) Round & L.N. Bukhtiyarova	Erect; high-profile	16-18	6-7	12-14	....	
Planothidium heteromorphum (Grunow) Lange-Bertalot	Erect; high-profile	26-31	13-14	10-11c/r; 9-10 s/r	....	

TABLE I (continuation)

Taxa	Growth form and ecological guild	Metric				
		Lenght (µm)	Width (µm)	Striae (10 µm)	Areolae (10 µm)	Fibulae/ Costae/Alar canals (10 µm)
Planothidium rostratum (Oestrup) Round & Bukhtyiarova	Erect; high-profile	9.4-17	4.2-5.8	11-12	....	
Diadesmidaceae						
Humidophila contenta (Grunow) Lowe, Kociolek, J.R. Johansen, Van de Vijver, Lange-Bertalot & Kopalová	Colonial; high-profile	7-11	3	....	....	
Luticola goeppertiana (Bleisch) D.G. Mann *ex* J. Rarick, S. Wu, S.S. Lee & Edlund	Motile; motile guild	22-31.5	5.7-8.4	20-22	24	
Luticola kotschyi (Grunow) D.G. Mann	Motile; motile guild	15-23	4.7-7	20-25	26	
Luticola mutica (Kützing) D.G. Mann	Motile; motile guild	12.6-20	5.2-6.3	18-20	22	
Luticola saxophila (W. Bock *ex* Hustedt) D.G. Mann	Motile; motile guild	8.9-10	5.7	18-22	18-22	
Brachysiraceae						
Brachysira brebissonii R. Ross	Motile; motile guild	17-23	5-7	20	....	
Brachysira vitrea (Grunow) R. Ross	Motile; motile guild	18-26	5-6	....	....	
Nupela praecipuoides Tremarin & T. Ludwig	Motile; motile guild	9-14.7	4.2-4.7	....	....	
Amphipleuraceae						
Frustulia crassinervia (Brébisson *ex* W. Smith) Lange-Bertalot & Krammer	Motile; motile guild	48.4	10	....	....	
Frustulia neomundana Lange-Bertalot & U. Rumrich	Motile; motile guild	37-39	8-9	....	....	
Frustulia pumilio Lange-Bertalot & U. Rumrich	Motile; motile guild	14.7-17.3	4.2-5	....		
Frustulia saxonica Rabenhorst	Motile; motile guild	42-66	11-14	....	....	
Frustulia vulgaris (Thwaites) De Toni	Motile; motile guild	51-54	10-11	....	....	

TABLE I (continuation)

Taxa	Growth form and ecological guild	Metric				
		Lenght (µm)	Width (µm)	Striae (10 µm)	Areolae (10 µm)	Fibulae/ Costae/Alar canals (10 µm)
Neidiaceae						
Neidium alpinum Hustedt	Motile; motile guild	13.1-35	3.7-4.7	....	....	
Neidium affine (Ehrenberg) Pfitzer var. *affine*	Motile; motile guild	26.8-50	7.3-11	20	....	
Neidium ampliatum (Ehrenberg) Krammer	Motile; motile guild	62-71	15-16	20-22	18	
Sellaphoraceae						
Sellaphora manguinii C.E. Wetzel	Motile; motile guild	10-11	4	18	....	
Sellaphora nigri (De Notaris) C.E. Wetzel & L. Ector	Motile; motile guild	6.8-10.5	2.6-3.1	24-26	....	
Sellaphora pseudoarvensis (Hustedt) C.E. Wetzel & L. Ector	Motile; motile guild	7.3-8.9	2.1	....	....	
Sellaphora saugerresii (Desmazières) C.E. Wetzel & D.G. Mann	Motile; motile guild	8.4-13.1	3.1-3.6	20-22	....	
Sellaphora tridentula (Krasske) C.E. Wetzel	Motile; motile guild	12-14	3	....	....	
Sellaphora ventralochilensis C.E. Wetzel & L. Ector	Motile; motile guild	12-18	4.7-6	24	....	
Pinnulariaceae						
Caloneis hyalina Hustedt	Motile; motile guild	13.1-20	3.6-4.7	....	....	
Chamaepinnularia submuscicola (Krasske) Lange-Bertalot	Motile; motile guild	6-12.1	2.6-3.1	18	....	
Pinnularia brauniana (Grunow) Studnicka	Motile; motile guild	32-39	5.2-8	11-12	....	
Pinnularia microstauron var. *rostrata* Krammer	Motile; motile guild	20.5-25.2	3.4-4.2	16	....	
Naviculaceae						
Hippodonta capitata subsp. *iberoamericana* Metzeltin, Lange-Bertalot & García-Rodríguez	Motile; motile guild	17.8-20.5	4.7	5-6	....	

TABLE I (continuation)

Taxa	Growth form and ecological guild	Metric				
		Lenght (µm)	Width (µm)	Striae (10 µm)	Areolae (10 µm)	Fibulae/ Costae/Alar canals (10 µm)
Mayamaea permitis (Hustedt) K. Bruder & Medlin	Motile; motile guild	6.8-8	2.6-4	22-24	....	
Navicula cryptocephala Kützing	Motile; motile guild	26-39	4.7-5.7	14-15	....	
Navicula cryptotenella Lange-Bertalot	Motile; motile guild	15.2-23	4.7-5.2	16-18	....	
Navicula erifuga Lange-Bertalot	Motile; motile guild	22-29	4.7-6	14-15	....	
Navicula insulsa Metzeltin & Lange-Bertalot	Motile; motile guild	52.6-54.7	7.4-7.9	14-16	28	
Navicula longicephala Hustedt	Motile; motile guild	16-21	3.1-3.6	18-20	....	
Navicula rostellata Kützing	Motile; motile guild	28-34	7.8-9	11-12	....	
Navicula salinicola Hustedt	Motile; motile guild	14.7-20	2.6-3.6	16-18	....	
Navicula symmetrica R.M. Patrick	Motile; motile guild	26.8-42	5.7-7	12-13	22	
Navicula veneta Kützing	Motile; motile guild	29-33	6-7	14-15	....	
Navicula vilaplanii (Lange-Bertalot & Sabater) Lange-Bertalot & Sabater	Motile; motile guild	12-15	3	22-24	....	
Navigeia aikenensis (R.M. Patrick) L.N. Bukhtiyarova	Motile; motile guild	10.5-28	5.2-5.7	12-13	....	
Navigeia lateropunctata (J.H. Wallace) L.N. Bukhtiyarova	Motile; motile guild	18.4-26	7.3-9	15-16	....	
Pleurosigmataceae						
Gyrosiga acuminatum (Kützing) Rabenhorst	Motile; motile guild	61-98	9-11	16-19	19-21	
Gyrosigma scalproides (Rabenhorst) Cleve	Motile; motile guild	87-89	13-14	22 a 24	....	
Stauroneidaceae						
Stauroneis anceps Ehrenberg	Motile; motile guild	77-84	14-16	16-18	18	
Stauroneis thermicola (J.B. Petersen) J.W.G. Lund	Motile; motile guild	11-14	2-3	20-24	....	

TABLE I (continuation)

Taxa	Growth form and ecological guild	Metric				
		Lenght (µm)	Width (µm)	Striae (10 µm)	Areolae (10 µm)	Fibulae/ Costae/Alar canals (10 µm)
Catenulaceae						
Halamphora montana (Krasske) Levkov	Motile; motile guild	14-15	3-4			
Nitzschiaceae						
Nitzschia acicularis (Kützing) W. Smith	Motile; motile guild	30-42	3-4	14-16		
Nitzschia amphibia Grunow	Motile; motile guild	20-23	4	13-14		6-7
Nitzschia clausii Hantzsch	Motile; motile guild	32-39	3-4	....	....	8-10
Nitzschia frustulum (Kützing) Grunow	Motile; motile guild	22-28	4-5	20-24	....	9-11
Nitzschia linearis (Agardh) W. Smith	Motile; motile guild	62-66	5			10-11
Nitzschia palea (Kützing) W. Smith	Motile; motile guild	36-48	3-5	....	....	12-14
Nitzchia terrestris (J.B. Petersen) Hustedt	Motile; motile guild	47-63	4	....	....	5-7
Tryblionella debilis Arnott *ex* O´Meara	Motile; motile guild	16-27	8-11	17-19	....	7-8
Surirellaceae						
Surirella stalagma M.H. Hohn & J. Hellermann	Motile; motile guild	14-16	6-7	....	....	6-7

The low-profile guild was composed of three species of the *Achnanthidium* Kützing complex. The low-profile guild abundance increased considerably (65%) between after1 and after2 as a result of high rainfall ($r = 0.99$; $p = 0.02$). The high-profile guild diversity declined between after1 (H' = 2.44) and after2 (H' = 2.18) with a persistence of tube-forming diatoms. After1 and after2 were marked by low Secchi depth (80-83 cm), high TS (100-90 mg.L^{1}), and increases in rainfall (85.2 to 200 mm). The high-profile guild diversity decreased substantially in after3 (H' = 1.13; $t = 13.64$; $p < 0.001$) and was dominated by tube-forming diatoms.

PCA explained 82.99% of the variation in the dataset in the first two axes ($p < 0.001$) revealing a temporal gradient among diatom guilds, growth forms and the samples (Fig. 2). We used replicates as independent samples and along with the heteroscedasticity, the replicates of a given sampling period were plotted together. The first sample taken after the oil spill (after1) was characterized by the motile guild ($r = -0.71$; axis 2) comprised of motile diatoms ($r = -0.98$; axis 2). The after2 sample was characterized by the low-profile guild ($r = 0.40$; axis 2) and after3, by the high-profile guild ($r = 0.48$; axis 2). The after2 sample was comprised of erect (small and ruderal) taxa ($r = 0.81$, axis 2) and planktonic ($r = 0.78$; axis 2) growth forms, whereas the after3 sample was

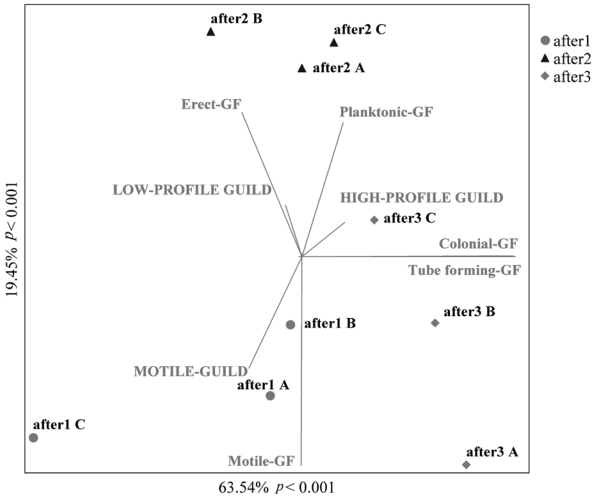

Figure 2 - Principal Component Analysis using diatom's guilds and growth forms (GF) showing temporal variation after the oil spill (A,B,C are the replicates from the same sampling date).

composed of colonial (r = 0.99; axis 1) and tube-forming (r = 0.99; axis 1) growth forms.

DISCUSSION

Vegetable oil forms a barrier on the surface water, constraining aquatic organisms by reducing dissolved oxygen and light availability. We registered high COD after the oil spill. COD in surface waters is useful for determining the degree of pollution by reflecting the total amount of hydrocarbon degradation. Thode-Filho et al. (2015) evaluated vegetable oil passive bioremediation (or natural attenuation) and revealed that the maximum biodegradation rate occurred around the thirtieth day of the experiment followed by total oxygen consumption. Reduced COD occurred in after3 and, with the high similarity among environmental variables, revealed a tendency to recover to the pre-disturbance conditions 24 days after the oil spill. Jézéquel et al. (2017) noticed high hydrocarbon degradation rates after one month under natural conditions. On the other hand, the authors also found a mortality rate of approximately 90% in the mangrove macrobenthic community exposed to hydrocarbon contamination.

The Negro River motile diatom guild was species-rich and dominated the diatom community after the oil spill. However, motile guilds in after1 showed lower richness and diversity relative to the community sampled before the oil spill. The motile guild is formed by fast-moving species and strong competitors in nutrient-enriched environments; motile guild benefits from shaded places—when these species can move into the periphyton matrix as a response to this type of physical stress (Passy 2007). After1 was marked by high TS and shading, and high nutrients which may be due to the oil discharge (Snow and Scott 1975). Negative correlation between algae abundance and suspended matter was also observed by Ács and Kiss (1993) in the Danube River. Motile diatoms may become abundant in an environment with high concentrations of nutrients and TS (Faria et al. 2015). Notwithstanding the well-established literature around diatom guilds and growth forms, locating species tolerance information was difficult. Even with an accurate taxonomical investigation, we had some difficulties in finding infra-generic ecological data. Most of the Naviculaceae, Sellaphoraceae, and Diadesmidaceae taxa are undergoing constant taxonomical reviews and consequent synonymization and reclassification (Novais et al. 2013). Additionally, new taxa are continuously being discovered. The aforementioned taxa were recorded previously in polluted rivers (Lobo et al. 2004a, b, 2016, Hermany et al. 2006, Novais et al. 2013) and in an eutrophic reservoir (Marra et al. 2016), and are considered tolerant, in particular *L. goeppertiana* which has a high tolerance to TS (Céspedes-Vargas et al. 2016). Based on the literature, therefore, we considered them a tolerant taxa. The motile guild, having low diversity and being dominated by tolerant species, could be related to the disturbance caused by nutrients and shading, which sustains our biological traits approach.

Rainfall is a disturbing factor for periphytic communities which causes movement of inorganic particles and consequently shading by the drifted sediments (Ács and Kiss 1993). The high-profile guild comprises diatoms of tall stature, including stalked, filamentous, chain-forming, and tube-forming species that grow erect on substrates. These strategies provide capability to access resources as light and nutrients (Passy 2007). During their phylogenetic evolution, algae develop different strategies for attaching to surfaces or for surviving in flowing waters, after which the riverine periphyton can handle some constant disturbance level (Ács and Kiss 1993). Nevertheless, the high-profile guild becomes sensitive in light- and nutrient-rich environments (Berthon et al. 2011). Development of attached algae can be related to light penetration after the breaking of the oil barrier (Snow and Scott 1975), but we found very few changes in light penetration, maybe because the TS inputs were incited by the rainfall. However, the tube-forming *Encyonema perpusillum* (Cleve-Euler) D.G. Mann prevailed in after3 together with the low changes in light availability in the water column; their anatomic structure abets competitive ability for the use of light (Hermany et al. 2006). On the other hand, Rimet and Bouchez (2011) reported increases in tube-forming diatoms when testing pesticide-contamination effects in mesocosms. The authors hypothesized that the polysaccharide of the mucilaginous tube protected cells under chemical disturbances. We can argue, therefore, that tube-forming diatoms showed ability to compete for light and that their dominance over other growth forms could indicate chemical imbalance imposed on the diatom community, as well as reduction of available oxygen due to oil degradation.

The low-profile guild, mainly represented by *Achnanthidium minutissimum* (Kützing) Czarnecki, probably benefited from the environmental conditions that occurred in after2 due to high rainfall. The low-profile guild consists of short stature diatoms, slow-moving species, and solitary centrics (Passy 2007). The disturbance event favored pioneer growth forms and ruderal species (Peterson 1996) that are tightly attached to the substrate (Peterson and Stevenson 1992) and resistant after disturbances (Stevenson 1997, Schneck and Melo 2012) such as physical stress persisting in the periphytic matrix (Rimet and Bouchez 2012, Faria et al. 2015, Riato et al. 2017). *A. minutissimum* is largely recorded in lotic and lentic eutrophic environments (Lobo et al. 2004b, Ács et al. 2005), even in hypereutrophic conditions (Faria et al. 2013), and could be considered tolerant to strong nutrient inputs. The mean width of *A. minutissimum* valves was used as a biological trait related to nutrient levels in Swedish streams (Jarlman and Kahlert 2009, Kahlert et al. 2009). The authors proposed that mean width < 2 µm is associated with oligotrophic waters, 2.2-2.8 µm with oligo-mesotrophic waters, and > 2.8 µm with eutrophic waters. This trait applicability was tested and confirmed by Vilmi et al. (2015) who studied anthropogenic eutrophication of a large lake. The valve metrics of *A. minutissimum* from our study site showed larger widths (3-4 µm) than observed by Vilmi et al. (2015). This data may support metric answers to nutrient inputs in the water or represent a local community response to natural conditions. Therefore, we encourage further investigations of morphological variation responses to chemical contamination and anthropogenic pollution.

Both diatom guilds and growth forms were useful for predicting the environmental conditions in the Negro River. While diatom guild richness formed a temporal gradient after the oil spill, the variability of the growth forms inside the guilds gave us tools to visualize the environmental conditions without accurate chemical monitoring. Changes inside the guilds were the main factor for understanding the environment. We point out that the high-profile and the motile guild diversities decreased over time due to increases in tube-forming diatoms and the resistance and dominance of the motile diatom *N. aikenensis*, respectively. These changes inside the guilds favoring the dominance of tolerant diatoms were strongly related to the responses of diatoms to abiotic changes largely presented in the literature. The disappearance of most diatoms in after3 followed by the abovedescribed dominance may be the result of a combination of factors such as oil toxicity, low oxygen and disturbance-once the currents during raining period were able to remove dead cells. Since recolonization was not observed, we suspect that the oil density could be detrimental to the bioturbation activity, preventing natural propagule resuspension driven by the currents (Facca et al. 2002, Algarte et al. 2014).

Based on diatom functional traits, we were able to understand the environmental dynamics in a river driven by an unexpected oil spill. We were also able to find links between abiotic data, disturbances and biological traits. Therefore, we emphasize the importance of our findings in natural conditions. It is not possible to exclude the natural multistressors (such as canopy, differences in stream gradient and flow velocities, dispersal process, stochasticity, seasonality, differences in photoperiod, and/or grazers) in an uncontrolled field study. However, we highlight the importance of divulging our findings after this unexpected oil spill, as we have shown that environmental changes can be predicted by applying diatom biological tools. We believe that our results support the importance of continued investigation into diatom functional responses to physical and chemical disturbances. The use of ecological guilds is a valuable tool in monitoring environments after a disturbance because many taxa may use more than one strategy of attachment for competition or in handling physical stressors (Wang et al. 2014). Knowing diatom ecological preferences can abet their usefulness as a tool to assess environmental changes (Berthon et al. 2011) and better understand the dynamics and impacts occurring in a previously unstudied environment, as in the case of Negro River. We also support future research aimed towards applying controlled conditions to better understand diatom responses to oil contamination for possible future environmental predictions.

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AUTHOR CONTRIBUTIONS

Denise M. de Faria designed the manuscript, produced the analyzes, results and discussion. Júlio C. Costin sampled, identified and counted the diatoms during his master degree. Priscila I. Tremarin identified the diatoms and furnished diatoms metrics. Thelma A.V. Ludwig rewiewed the text and contributed to the diatoms' identification, writing and discussion.

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