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Article

Ephemeroptera genera as bioindicators of agricultural impact on Atlantic Forest streams

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ABSTRACT. Insects of the Ephemeroptera order have been used as bioindicators of water quality as they respond to countless anthropic impacts. In this study, we assessed the use of Ephemeroptera as bioindicators of streams impacted by agricultural activities. We collected Ephemeroptera nymphs in six streams with different uses and soil cover in their drainage areas located in the northern region of the Brazilian state of Rio Grande do Sul. Based on soil use and cover analysis, we categorized the streams as 'natural' (drainage area with >42% native arboreal vegetation) and 'agricultural' (drainage area with >72% agricultural use). We collected a total of 2,767 organisms, distributed into four families and eleven genera. We observed greater abundance (68%) and taxonomic richness (11 genera) of Ephemeroptera in the agricultural streams. The natural streams comprise 32% of the fauna collected, with eight genera. That pattern was especially determined by the Baetidae and Leptohyphidae families. The genera *Caenis*, *Cloeodes*, and *Tricorythopsis* were abundant in the agricultural streams. In this study, we observed that genera of Ephemeroptera are tolerant to environmental changes, particularly those caused by agricultural activities. Therefore, the use of the order in studies to assess water quality must be carried out with caution.

KEYWORDS. Aquatic insects; anthropogenic influence; taxonomic resolution; environmental quality.

RESUMO. Gêneros de Ephemeroptera como biodindicadores de impactos agrícolas em córregos da Mata Atlântica. Insetos da ordem Ephemeroptera tem sido utilizado como bioindicadores de qualidade de água por responderem a inúmeros impactos antrópicos. Neste estudo avaliamos o uso dos Ephemeroptera como bioindicadores de riachos influenciados por atividades agrícolas. Coletamos ninfas de Ephemeroptera em seis riachos com diferentes usos e cobertura do solo em sua área de drenagem localizados na região norte do Rio Grande do Sul. A partir da análise do uso e cobertura do solo, categorizamos os riachos em 'naturais' (área de drenagem com >42% de vegetação arbórea nativa) e 'agrícolas' (área de drenagem com >72% de uso agrícola na área de drenagem). Coletamos um total de 2767 organismos, distribuídos em quatro famílias e 11 gêneros. Observamos maior abundância (68%) e riqueza taxonômica (11 gêneros) de Ephemeroptera nos riachos agrícolas. Os riachos naturais perfazem um total de 32% da fauna coletada com oito gêneros. Este padrão foi determinado, especialmente, pelas famílias Baetidae e Leptohyphidae. Os gêneros *Cloeodes* e *Tricorytopsis* foram abundantes nos riachos agrícolas. Neste estudo, observamos que gêneros de Ephemeroptera são tolerantes a alterações ambientais, sobretudo aquelas causadas por atividades agrícolas. Assim, o uso da ordem em estudos de avaliação da qualidade da água deve ser considerado com cautela.

PALAVRAS-CHAVE. Insetos aquáticos; influência antrópica; resolução taxonômica; qualidade ambiental.

The constant expansion of livestock and agriculture has been responsible for transforming areas with natural vegetation into fields of pasture and crops (Deegan *et al*., 2011). The increased release of pollutants associated with the use of fertilizers and pesticides (Xiaojing *et al*., 2021) and the changes in landscape constitute a major environmental issue for the integrity of aquatic ecosystems (Allan, 2004). In streams, for example, riparian zones play a fundamental role in the maintenance of the ecological status of those environments (Burdon *et al*., 2020; Huiñocana *et al*., 2020). Fully or partially removing riparian vegetation has negative consequences for the functioning of limnic ecosystems and impact the quality of aquatic environments (Siegloch *et al*., 2014; Tonello *et al*., 2021), altering physical and chemical variables of the water.

Among the physical and chemical variables of water, temperature and dissolved oxygen. Sites surrounded by vegetation tend to have lower temperature (Carvalho *et al*., 2000) and higher dissolved oxygen values (Bueno *et al*., 2005), besides an impact on the concentration of solids in suspension and turbidity (Silveira, 2004; Palharini & Pagoto, 2015).

On way of assessing aquatic environments is using biological communities. Benthonic macroinvertebrates exhibit broad diversity and are formed by countless taxonomic groups (Serna-López *et al*., 2020; Kumari & Maiti, 2020; Restello & Hepp, 2020), especially insects belonging to Ephemeroptera. Overall, this order is considered sensitive to anthropic changes, however, studies on Ephemeroptera assemblages show they are sensitive mainly to point-source impacts such as urbanization (Hepp *et al*., 2013, Buss & Salles, 2007, Rojas-Peña *et al*., 2021). A study carried out in Costa Rica reported that Ephemeroptera abundance is greater in impacted areas, however, diversity is higher in forest areas (Duschek *et al.*, 2019).

Buss & Salles (2007) pointed out that the order must be used with caution to assess environmental quality since some genera are tolerant to perturbation. Those authors found that not all genera of the Baetidae family are sensitive to anthropic impacts, such as *Americabaetis*, which was considered tolerant. Hepp *et al.* (2013) found that *Americabaetis*, *Baetodes*, *Apobaetis*, and *Cloeodes* were tolerant to limnologic characteristics related to urban impact, such as high pH and low values of dissolved oxygen. *Hagenulopsis*, *Farrodes*, and *Miroculis* occur in streams whose riparian vegetation has been removed for the establishment of agricultural practices, therefore, they are considered indicators of impacted sites (Brasil *et al*., 2014; Selvakumar *et al*., 2014). On the other hand, the genera of Leptophlebiidae (Shimano *et al*., 2010; Firmiano *et al*., 2017) and Leptohyphidae (Domínguez *et al*., 2001; Oliveira *et al*., 2013) are considered sensitive to environmental impact.

The northern region of the Brazilian state of Rio Grande do Sul is intensively explored for agricultural activities, resulting in significant fragmentation of remaining forests (Rovani *et al*., 2020). Thus, environmental evaluation and monitoring must be carried out often and, especially, employing safe approaches (Hepp *et al*., 2010; Restello *et al*., 2020). The use of insects as bioindicators has been traditionally reported in the literature, however, the responses presented by organisms may vary according to their tolerance to environmental changes. Order Ephemeroptera has ecological peculiarities that are yet to be elucidated (Buss & Salles, 2007; Flowers, 2009), particularly regarding diffuse impacts such as agriculture. In the present study, we sought to assess the responses of Ephemeroptera assemblies in streams impacted by agricultural activities taking into account taxonomic resolution.

Our hypothesis is that some genera are more representative of sites with agricultural impact and are considered tolerant to pollution. Therefore, when a more refined taxonomic resolution is employed, the response of Ephemeroptera is different than what the literature reports for the order, i.e., it is not linear and there are sensitive genera and tolerant genera.

MATERIAL AND METHODS

Area of study. The study was carried out in <3rd order streams located in the northern region of the Brazilian state of Rio Grande do Sul (27°12'59" and 28°00'47"S; 52°48'12" and 51°49'34"W; Fig. 1). The region has humid temperate subtropical climate that belongs to types Cfa and Cfb according to the Köpen-Geiger classification (Alvares *et al*., 2013). Rainfall is regular and well distributed along the year, with mean annual precipitation of 1500 mm (Alvares *et al*., 2013). The region is located within the Atlantic Forest biome with vegetation characterized by a mix of perennifolia stationary forest with araucaria and seasonal semideciduous forest (Oliveira-Filho *et al*., 2015).

Six streams whose land use and cover classes have been quantified by geoprocessing techniques were selected. The quantification of 'arboreal vegetation' and 'agricultural use' was carried out in a 30 m buffer in both banks of the streams, considered the riparian zone, and in the drainage area of each stream studied, following the topographic patterns of the area. After quantification, the streams were classified into natural (R1, R2, and R3) and agricultural (R4, R5, and R6) (Fig. 1; Tab. I) according to the criteria proposed by Huñocana *et al.* (2020).

The water in the streams of both classifications were well oxygenated (>10 mg L^{-1}) and pH values were near neutral (~7). Turbidity varied from 2.03 to 9.06 NTUs and electric conductivity was between 0.11 to 0.62 mS cm^{-1} in the agricultural and natural streams, respectively.

Collection and identification of Ephemeroptera. The organisms were collected in the spring of 2019 and summer of 2020 using a Surber sampler (250 µm mesh and 0.09 m^2 area). Three subsamples were collected from each stream, all in rocky substrate to prevent possible effects of the types of substrates in the sampling. The material was fixated *in situ* with 70% ethanol and stored in plastic flasks. At the laboratory, Ephemeroptera were identified down to the genus taxonomic level using the keys proposed by Salles *et al.* (2004) and Mugnai *et al*. (2010). The organisms identified were recorded and deposited at the Benthonic Invertebrate Collection of the Regional Museum of Upper Uruguay (*Museu Regional do Alto Uruguai* – MuRAU) of the *Universidade Regional Integrada do Alto Uruguai e das Missões* (URI).

Data analysis. The structure of the Ephemeroptera assembly was determined based on abundance, given by the total number of organisms collected, and richness, estimated by the number of genera identified. Data normality was verified using Shapiro-Wilk test. For each family, we carried out *t* tests aiming at verifying the variation between abundance and richness in the natural and agricultural streams. Richness could not be assessed for the Caenidae and Leptophlebiidae families as they exhibited only a single

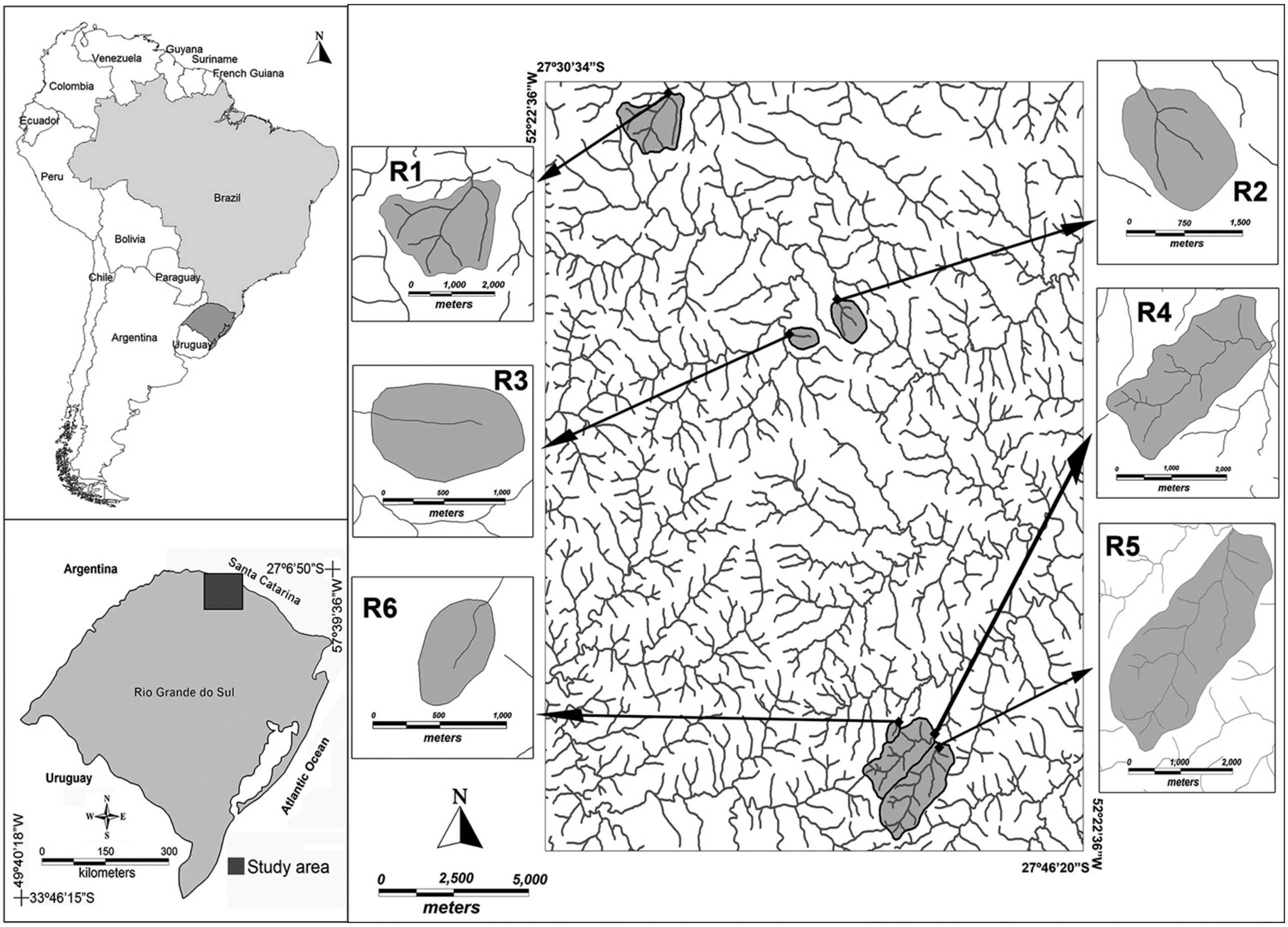

Fig. 1. Geographic location of the study area, northern region of the Brazilian state of Rio Grande do Sul, Brazil.

Tab. I. Percentage of vegetation and agriculture in the drainage area and riparian zone of the streams studied, Upper Uruguay Region (Veg, vegetation; Agr, agriculture; DA, drainage area; RZ, riparian zone).

	% vegetation		% agriculture	
Streams	VegDA	VegRZ	AgrDA	AgrRZ
Natural				
R1	47.2	72.8	33.9	26.1
R2	43.7	76.3	24.3	9.20
R3	42.2	73.0	4.08	3.98
Agricultural				
R4	5.90	7.10	87.4	83.2
R5	8.60	14.8	88.0	82.0
R6	15.6	27.2	72.6	60.3

genus. Pearson linear correlation analysis was performed to verify whether the percentages of vegetation and agriculture in the drainage area or riparian zone are related to (i) the families in the Ephemeroptera assembly and (ii) each of the genera identified.

The statistical analyses were performed using the statistical software R (R Core Team, 2022) via the 'vegan' (Oksanen *et al*., 2022) and 'ggplot2' (Wickham *et al*., 2022) data packages.

RESULTS

We collected a total of 2,767 organisms, belonging to four families and 11 genera of the Ephemeroptera order (Tab. II). In the natural streams, we collected a total of 878 specimens (32% of the total), belonging to four families and eight genera. In the agricultural streams, we collected 1,889 specimens (68% of the total), belonging to four families and 11 genera (Tab. II). The most representative family in the

Tab. II. Ephemeroptera genera identified in natural and agricultural streams, northern Rio Grande do Sul state, Brazil.

Family/Genera	Streams	
	Natural	Agricultural
Baetidae		
Americabaetis Kluge, 1992	0	23
Apobaetis Day, 1955	0	9
Baetodes Needham & Murphy, 1924	200	196
Cloeodes Traver, 1938	10	190
Paracleodes Day, 1955	34	231
Tupiara Salles, Lugo-Ortiz, Da-Silva & Francischetti, 2003	4	53
Caenidae		
Caenis Stephens, 1835	43	330
Leptophlebiidae		
Hagenulopsis Ulmer, 1920	517	465
Leptohyphidae		
Tricorythopsis Traver, 1958	2	305
Tricorythodes Ulmer, 1920	68	83
Traverhyphes Molineri, 2001	0	4
Abundance	878	1889
Richness of genera	8	11

natural streams was Leptophlebiidae at 517 specimens (59% of the fauna at those sites), while Baetidae was the most representative in agricultural streams, with 702 specimens collected (37% of the fauna samples in those streams). The genera *Americabaetis*, *Apobaetis*, and *Traverhyphes* were exclusive of agricultural streams (Tab. II).

The abundance of the Baetidae ($t = -3.4$; $p = 0.03$) and Leptohyphidae ($t = -4.2$; $p = 0.03$) families was greater in the agricultural streams (Figs 2A and 2B, respectively). On the other hand, the abundance of the Caenidae and Leptophlebiidae did not differ among streams. We observed greater richness of Baetidae ($t = -5.0$; $p = 0.01$) and Leptohyphidae ($t = -4.2$; $p = 0.03$) in the agricultural streams (Figs 2C and 2D, respectively). *Hagenulopsis* and *Baetodes* were the most abundant genera in the natural streams, whereas *Paracleodes*, *Caenis*, and *Tricorythopsis* were the most abundant in the agricultural streams.

Baetidae exhibited a negative correlation with vegetation in the DA and RZ (Figs 3A and 3B) and a positive correlation with agriculture in the DA and RZ (Figs 3C and 3D). For Leptohyphidae, we observed a negative correlation with vegetation in the DA and RZ (Figs 3E, 3F) and a positive correlation with agriculture in the DA and RZ (Figs 3G, 3H). We did not observe correlations between land use and cover with Caenidae abundance ($p > 0.05$).

Given the relationships between land uses and cover and the genera identified, we observed that only *Cleodes* and *Tricorythopsis* exhibited significant correlations (Tab. III).

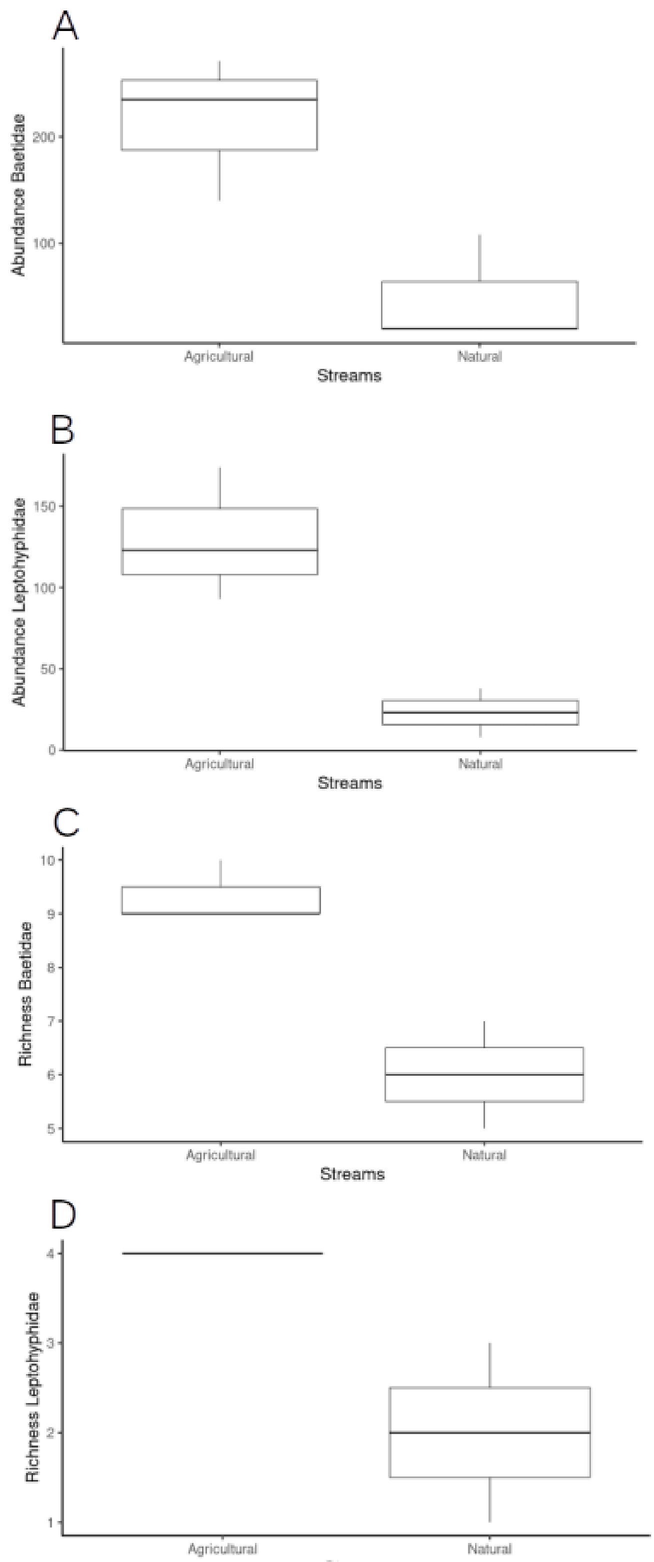

Fig. 2. Boxplot for abundance and richness of families of the Ephemeroptera order between the natural and agricultural streams: A, abundance of Baetidae; B, abundance and Leptohyphidae; C, richness of Baetidae; and D, richness of Leptohyphidae.

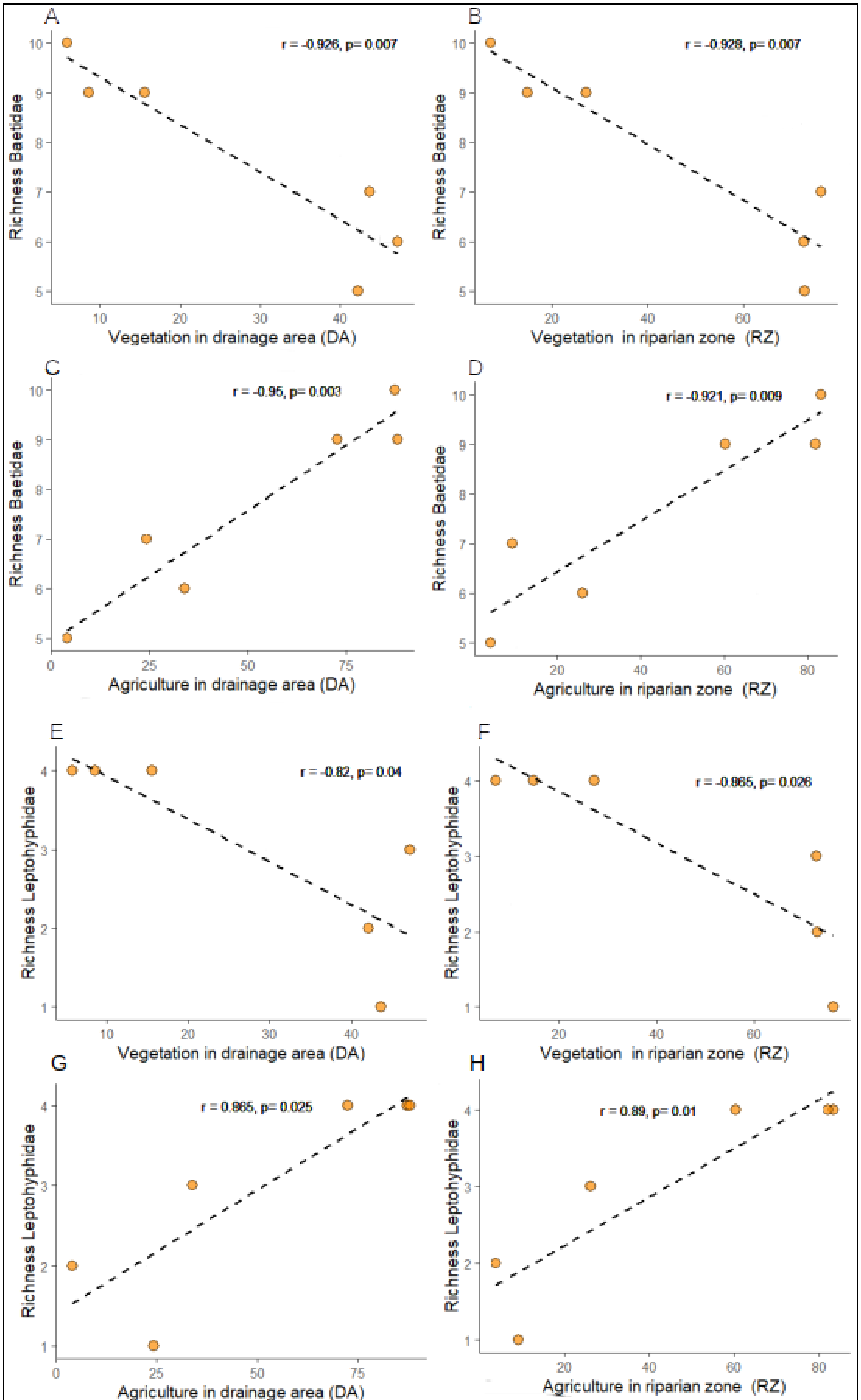

Fig. 3. Pearson linear correlation among Baetidae richness: A, percentage of vegetation in the DA; B, vegetation in the RZ; C, percentage of agriculture in the DA; and D, agriculture in the RZ, and Leptohyphidae richness: E, percentage of vegetation in the DA; F, vegetation in the RZ; G, percentage of agriculture in the DA; and H, agriculture in the RZ (DA, drainage area; RZ, riparian zone).

Tab. III. Pearson correlation values between Ephemeroptera genera and the percentage of vegetation and agriculture in the DA and RZ. *significant correlations ($p < 0.05$) (DA, drainage area; RZ, riparian zone).

Taxa	% Veg DA	% Veg RZ	% Agr DA	% Agr RZ
Baetidae				
Americabaetis	- 0.64	- 0.67	0.58	0.63
Apobaetis	- 0.63	- 0.65	0.57	0.60
Baetodes	- 0.50	- 0.46	0.30	0.32
Cloeodes	- 0.82*	- 0.84*	0.76	0.82*
Paracleodes	- 0.58	- 0.56	0.49	0.47
Tupiara	- 0.70	0.70	0.65	0.63
Caenidae				
Caenis	- 0.48	- 0.47	0.51	0.53
Leptophlebiidae				
Hagenulopsis	- 0.14	- 0.24	- 0.43	- 0.40
Leptohyphidae				
Tricorythopsis	- 0.89*	- 0.88*	0.88*	0.86*
Tricorythodes	- 0.018	- 0.05	0.09	0.07
Traverhyphes	- 0.54	- 0.57	0.49	0.53

DISCUSSION

We observed greater abundance and richness of genera in the streams with more intense agricultural uses. The richness of the Baetidae and Leptohyphidae genera was also higher in the agricultural streams, which does not corroborate studies that point to higher richness of Ephemeroptera genera in more preserved streams (Alves *et al*., 2006; Arimoro & Muller, 2010; Siegloch *et al*., 2014).

Buss *et al*. (2002) stated that Baetidae organisms are little sensitive to environmental changes and are easily found at sites with intermediate levels of degradation. On the other hand, Domínguez *et al.* (2006) and Buss & Salles (2007) reported that Baetidae species are demanding of sites with higher environmental integrity. Therefore, the lack of consensus among records in the literature is evident. The dietary habit of Baetidae must be taken into account in the analysis since they are scraping organisms. In that sense, streams surrounded by agriculture exhibit low levels of plant cover in their banks, which leads to greater sunlight incidence onto the stream and facilitates the establishment of periphyton (Huñocana *et al*., 2020), thus increasing the abundance of Baetidae.

Likewise, our results do not corroborate studies that report sensitivity by Leptohyphidae to disturbances (Peres, 1988; Callisto *et al*., 2002; Chagas *et al*., 2017). Thus, we can propose that Baetidae and Leptohyphidae adapted to the altered stretches of streams, possibly explained by the hypothesis of intermediate disturbance of anthropic origin. Some studies state that intermediate disturbances may explain the higher richness of Ephemeroptera nymphs in areas with anthropic impact (Siegloch *et al*., 2008; Souza *et al*., 2011).

Our results showed that *Tricorythopsis* (Leptohyphidae), *Cloeodes* (Baetidae), and *Caenis* (Caenidae) were more abundant in the agricultural streams. Such results contradict those presented by Firmiano *et al.* (2017), who report that *Tricorythopsis* is sensitive to perturbation. Impacted environments may exhibit lower habitat availability, with less input of allochthonous material into the system and predominance of fine particulate organic matter, which favors the prevalence of collecting organisms, thus justifying the presence of *Tricorythopsis* in the agricultural streams. The presence of opercular gills facilitate the tolerance against suspended solids and, in very turbid streams and rivers, that family becomes an important component of the benthonic community (Domínguez *et al*., 2006). Such result shows that we must be careful when generalizing the tolerance of families of the order Ephemeroptera since some genera may be tolerant to changes in adjacent areas.

The presence of *Cloeodes* in the agricultural streams also differ from the results presented by several studies that state the genus is sensitive to pollution (Buss & Salles, 2007; Chacón & Segnini, 2007; Forero-Céspedes & Reinoso-Flórez, 2013). *Cloeodes* was recorded in association with algae biofilm (periphyton) or rocky substrates (Souza *et al*., 2011), while Domínguez *et al.* (2006) observed that nymphs are found in a broad range of habitats, both in well-oxygenated streams and in poorly oxygenated sites. The higher presence of *Cloeodes* in agricultural streams shows that the genus tolerates agricultural use both in the riparian zone and in the drainage area of the basin assessed.

In the natural streams, *Hagenulopsis* (Leptoplhebiidae) was the most abundant. Habitat heterogeneity is one of the main factors of distribution of that family (Goulart & Callisto, 2005). The structure of a community of aquatic insects may be influenced by the existing resources, substrate variety, and environmental heterogeneity, thus justifying the abundance of that family in the natural streams (Abelho, 2001; Boyero, 2003; Taniguchi & Tokeshi, 2004; Burdett & Watts, 2009). To Flowers & de la Rosa (2010), the family Leptoplhbiidae is widely tolerant to temperature and certain levels of pollution. However, *Hagenulopsis* tolerates different environmental conditions and may be found in waters with better conditions (Flowers & de la Rosa, 2010).

Americabaetis (Baetidae) nymphs were exclusively found in agricultural streams and are considered less sensitive to environmental impacts (Callisto *et al*., 2001; Buss & Salles, 2007; Souza *et al*., 2011). *Americabaetis* occur in several types of habitats, including sites with some level of perturbation (Siegloch *et al*., 2008), which may explain in this case the exclusivity in agricultural streams.

Caenis was another abundant genus in the agricultural streams. Those are sturdy and little demanding organisms that may tolerate a broad range of environmental conditions and may live in polluted and eutrophic waters, where many other members of the Ephemeroptera order are unable to survive, which justifies the abundance of that group in the agricultural streams (Flowers & de la Rosa, 2010; Oliveira *et al*., 2017).

Many works with aquatic insects as bioindicators analyze the fauna of Ephemeroptera, Trichoptera, and Plecoptera jointly, with Ephemeroptera responding as sensitive organisms to anthropic impact (Junqueira *et al*., 2010; Chang *et al*., 2014; Valente-Neto *et al*., 2018; Huiñocana *et al*., 2020). However, there are gaps regarding the patterns of different genera. We observed such fact in this study when analyzing Ephemeroptera genera, finding that some are tolerant to agricultural impact as they were more abundant in that type of stream.

Our results suggest the genera of the Ephemeroptera order are sensitive to agricultural impact. The so-called agricultural streams had greater abundance and richness of organisms. *Caenis*, *Cloeodes*, and *Tricorythopsis* were the most abundant in the agricultural streams, contradicting some studies as previously mentioned. Works only at the order level may not be sufficient to indicate the sensitivity of the group to agricultural impact. We observed that, in order to determine the agricultural impact on Ephemeroptera, the organisms must be identified down to the taxonomic level of genus, corroborating our hypothesis.

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