

Tolerance of benthic macroinvertebrates to organic enrichment in highland streams of northeastern Rio Grande do Sul, Brazil

Tolerância de macroinvertebrados bentônicos ao enriquecimento orgânico em rios de montanha da Região Nordeste do Rio Grande do Sul, Brasil

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Abstract: Aim: the aim of this study was to determine the ecological valence of benthic macroinvertebrates at different pollution levels in highland rivers and streams of Rio Grande do Sul; **Methods:** the dataset proceeds from samplings performed between 2002-2011 in 35 lotic ecosystems. The Chemical Index was used to determine pollution levels. Indices of richness and Shannon diversity were applied to characterize the structure of benthic communities. The descriptors used to determine taxa's ecological valence were selected according to Coefficient of Variation and regression analyses. Groups of tolerance were identified using Interquartile range and cluster analysis; **Results:** Conductivity and Chemical Index were the descriptors best related with diversity of benthic macroinvertebrate community. These metrics were used to determine the tolerance range of 38 taxa. Interquartile range and cluster analysis revealed three groups of taxa, according to their occurrence in different levels of pollution: taxa with narrow amplitudes, present at sites with very low or very high load of organic enrichment; taxa with moderate amplitude, found until moderately polluted sites; and taxa with occurrence in widespread environmental conditions. The results, when compared to other studies in Brazil, showed differences in some taxa's tolerance. This observation indicates the need to assess the bioindication potential of these taxa in genus and species level; **Conclusion:** the present study contributes to increase knowledge about the bioindicator potential of benthic macroinvertebrates. Therefore, the study supports an advanced biomonitoring of ecological quality in mountain streams of southern Brazil.

Keywords: macroinvertebrates, bioindicators, lotic ecosystems, pollution level, southern Brazil.

Resumo: Objetivo: o objetivo do estudo foi determinar a valência ecológica de macroinvertebrados bentônicos em diferentes níveis de poluição em rios e arroios na região nordeste do Rio Grande do Sul. **Métodos:** o conjunto de dados provém de coletas realizadas entre 2002-2011, em 35 ecossistemas lóticos. O Índice Químico foi utilizado para verificar os níveis de poluição. Os índices de riqueza e diversidade de Shannon foram utilizados para caracterizar a estrutura das comunidades bentônicas. Os descritores utilizados para determinar a valência ecológica dos taxa foram selecionados de acordo com os resultados de análises do Coeficiente de Variação e de regressão. Grupos de tolerância foram identificados por meio de análises da amplitude interquartil e de agrupamento. **Resultados:** A condutividade e o Índice Químico foram os descritores que mostraram a maior relação com as comunidades de macroinvertebrados bentônicos. Estas métricas foram utilizadas para determinar a amplitude de tolerância de 38 taxa. As análises da amplitude interquartil e de agrupamento evidenciaram três grupos de taxa, conforme sua ocorrência em diferentes níveis de poluição: taxa com amplitudes estreitas, presentes em locais com enriquecimento orgânico muito alto ou muito baixo; taxa com amplitudes moderadas, encontrados até locais com poluição moderada; e taxa de ampla ocorrência em diferentes condições ambientais. **Conclusão:** o presente estudo contribui para aumentar o conhecimento sobre o potencial bioindicador de macroinvertebrados bentônicos, fornecendo bases para um biomonitoramento avançado da qualidade ecológica em águas correntes do sul do Brasil.

Palavras-chave: macroinvertebrados, bioindicadores, ecossistemas lóticos, níveis de poluição, sul do Brasil.

1. Introduction

The use of biological methods in monitoring lotic ecosystems has been increasing over the last three decades, and becoming a valuable complementary tool to chemical and bacteriological analysis (Segnini, 2003). In United States, Australia and European countries, biomonitoring is regulated by federal legislation and currently used by environmental protection agencies to evaluate watersheds ecological quality (Parsons et al., 2002; Hering et al., 2004; Baptista, 2008; EPA, 2012).

Tolerance values based on benthic macroinvertebrates are one of the most widely used tools for monitoring biological impacts of water pollution, particularly in streams and rivers (Chang et al., 2014). In Brazil, benthic macroinvertebrate communities have emerged as an important component to assess freshwater ecosystems biological integrity (Baptista, 2008; König et al., 2008; Copatti et al., 2010; Hepp et al., 2010). The development or adaptation of biological indices using these invertebrates is recent in our country (Monteiro et al., 2008; Mugnai et al., 2008; Junqueira et al., 2010; Couceiro et al., 2012). The efficient application of indices demands an expansion of knowledge about macroinvertebrate communities' distribution patterns on different habitat conditions (Bagatini et al., 2012), in order to modify or assign tolerance values to indicator species.

Knowledge about macroinvertebrates biodiversity in highland streams from the northeast Rio Grande do Sul is still scarce (Bueno et al., 2003; Campello et al., 2005; Buckup et al., 2007; Winckler-Sosinski et al., 2008), especially in relation to their tolerance to organic pollution, and demands detailed studies on the communities composition related to regional ecological characteristics. Surveys conducted by the authors since 2002, stored in a database, give information about biodiversity and water quality of running waters in the area. This information provides subsidies for analysis of benthic macroinvertebrate communities' environmental preferences and development of methodologies for biological evaluation. In this context, the aim of the study was to determine the amplitude of occurrence of benthic macroinvertebrate taxa, in different pollution levels, in northeastern highland rivers and streams of Rio Grande do Sul, contributing to the improvement of biomonitoring methods.

2. Material and Methods

2.1. Study area

The study area is located in the physiographic regions Encosta Superior Nordeste and Campos de Cima da Serra, in the northeast region of the State of Rio Grande do Sul, Brazil. The climate is "Cfb" type, with annual average temperature around 14°C-16°C, and pluviosity ranges from 1500-2100 mm (Pereira et al., 2009). The area has an average height of 880 m, with many lotic ecosystems, forming narrow meander valleys, with a predominance of ritral zone in watercourses. Geological substrate is of basaltic origin (Hasenack et al., 2009) and vegetation comprises Mixed Rain Forest, interspersed with Deciduous Forest and grassland. Currently, the landscape is altered due to the expansion of farming and forestry of exotic species *Pinus eliotii* Engelm, *Pinus taeda* L. and *Eucalyptus* spp. (Buckup et al., 2007).

All data used in this study comes from a database including results of different researches performed by the authors between 2002 and 2011. A total of 88 samples were selected in 35 lotic ecosystems of seven cities: Cambará do Sul (9), São Francisco de Paula (6), Caxias do Sul (3), Antônio Prado (6), São Marcos (6) and five sites along the river Três Forquilhas, between the cities Três Forquilhas and Itati (Figure 1).

2.2. Environmental variables

The streams are classified from first to third order (Strahler, 1957), characterized by fast, clean or slightly turbid waters, with depth ranging from 10 to 50 cm, substrate mainly composed of rocks, pebbles and flagstones and absent or scarce aquatic vegetation.

In every site, the following physicochemical parameters were measured: electric conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$), water temperature ($^{\circ}\text{C}$), pH (in situ, potentiometric method), oxygen saturation (%) (in situ, amperometric method), biochemical oxygen demand (BOD-5) ($\text{mg}\cdot\text{L}^{-1}$) (incubation of 20°C per 5 days), nitrate ($\text{N}\cdot\text{NO}_3$ $\text{mg}\cdot\text{L}^{-1}$) (cadmium reduction method), ammonia ($\text{N}\cdot\text{NH}_3$ $\text{mg}\cdot\text{L}^{-1}$) (salicylate method) and soluble reactive phosphorous ($\text{P}\cdot\text{PO}_4^{-3}$ $\text{mg}\cdot\text{L}^{-1}$) (ascorbic acid method). Water analyses were performed according to methods described by APHA (1998). To determine levels of organic pollution, the Chemical Index (CI) developed by Bach (1986) was applied. This index is calculated based on the eight parameters cited above and ranges from 100 (best) to 0 (worst). Water

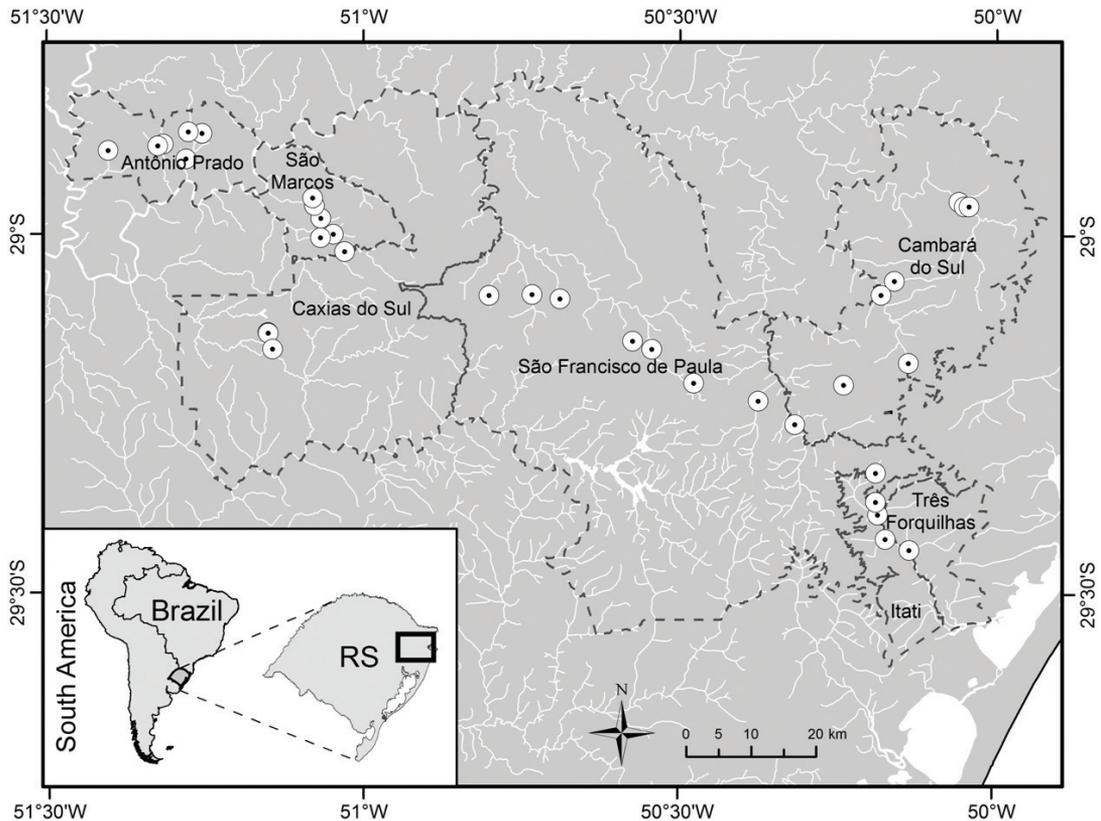


Figure 1. Sampling sites distribution in the northeast region of Rio Grande do Sul, Brazil.

quality is defined in seven classes: I (100-83, clean), I-II (82-74, slight pollution), II (73-57, moderate pollution), II-III (56-45, critical pollution), III (44-28, heavy pollution), III-IV (27-18, very heavy pollution) and IV (17-0, severe pollution).

2.3. Macroinvertebrates

Samples used in this study are stored in the Scientific Collection of the Limnology Laboratory in Universidade de Caxias do Sul (RS). In all streams, benthic macroinvertebrates were collected with a hand net (0.5 mm mesh) and manual sampling, by removing organisms from rocks (15 minutes/two collectors) and using a sieve (0.5 mm mesh) in sandy bottoms. Additionally, *basket sampler* was used in streams of Caxias do Sul. Identification was based on Lopretto and Tell (1995), Fernández and Domínguez (2001), Benetti et al. (2006), Froehlich (2007) and Mugnai et al. (2010). Macroinvertebrates were identified mainly to family level. Chironomina, due to their known tolerance to pollution, was separated from other Chironomidae.

2.4. Data analysis

To evaluate the benthic community, taxonomic richness (S) was estimated by counting the number

of identified taxa, and diversity was calculated using Shannon Index (H').

The Coefficient of Variation (CV) was calculated to identify, among the eight physicochemical parameters, which better differentiate the levels of pollution. Parameters that showed values near 100% are the most suitable to distinguish all environmental conditions. Values above 100% show a high standard deviation and differentiate only extreme conditions, while very low CV values do not show the variability among sites.

Data normality was verified by Kolmogorov-Smirnov test. In order to select parameters that best explain variation in community structure, two regression analyses (*Stepwise*) were performed relating environmental variables with H' : one using the eight physicochemical parameters and the other one including the Chemical Index (CI) as an additional variable, after transformation ($y = e^{a + bx}$). Parameters with the higher coefficient of determination (r^2) were selected to estimate taxa's ecological valence. Only those taxa that occurred ≥ 15 samples were included in this analysis. Ecological valence was estimated by analysis of interquartile range for the selected parameters, and is presented in Box-and-Whisker plots. Hierarchical

clustering analysis (Euclidean Distance) was also performed to verify association among taxa using the calculated values of CI. Statistical analyses were processed by the software IBM SPSS Statistics, version 21.

3. Results

According to the CI, sampling sites were classified as “clean” (41%), “slight pollution” (23%), “moderate pollution” (11%), “critical pollution” (3%), “heavy pollution” (13%), “very heavy pollution” (6%) and “severe pollution” (3%). Most “clean” and “slight pollution” sites are located in areas with riparian vegetation or grasslands, where anthropic influence is restricted to small farms and water is used for watering livestock and recreation. Sites that showed “heavy”, “very heavy” or “severe” pollution are located near or within urban areas, and receive domestic and industrial waste.

A total of 71,100 specimens were collected and 66 taxa identified. The maximum taxonomic richness was 37 taxa, recorded for São Marcos. The highest values of diversity were observed in water courses from Antônio Prado and in two rivers in São Marcos, with values among 2.5 and 2.7 (H'), respectively. Sampling sites that showed

high CI values, also had higher values of taxonomic richness and diversity, while sites considered highly polluted had lower diversity (H' =0.014 - 1.475) and taxonomic richness ($S=2 - 9$).

Considering the results of CV (Table 1) and regression analysis, conductivity proved to be useful both to differentiate ecological conditions as to explain the variation in Shannon diversity ($r^2 = 0.297$, $\rho = 0.000$) (Figure 2A). On the other hand, when the CI was included as a variable in the regression analysis, it showed a better relation with H' ($r^2 = 0.325$, $\rho = 0.000$) (Figure 2B). Therefore, conductivity and CI, were used as descriptors to estimate the ecological valence of 38 taxa.

According to the results of interquartile (Figure 3) and cluster analysis (Figure 4), three groups were evidenced: Plecoptera families, along with Calamoceratidae, Helicopsychoidea, Philopotamidae, Polycentropodidae, Chilinidae, Hydrobiidae, Aeglidae, Psephenidae, Naucoridae and Corydalidae had a restricted tolerance to low polluted environments and interquartile range around $50 \mu\text{S}\cdot\text{cm}^{-1}$ for conductivity. Hydrobiosidae shows a similar occurrence to taxa in this group, although they are found in higher conductivity values. The families Leptoceridae and

Table 1. Mean, standard deviation (\pm SD), median, minimum, maximum and Coefficient of Variation (CV) of physicochemical parameters in 88 sampling sites from 35 lotic ecosystems in northeast region of Rio Grande do Sul, Brazil.

	Temperature	BOD-5	pH	Conductivity	SaturationO ₂	N-NO ₃	N-NH ₃	P-PO ₄	CI
Mean	18.37	4.76	7.07	49.26	97.47	4.56	0.32	0.11	77.66
\pm SD	± 4.04	± 18.3	± 0.4	± 33.71	± 8.37	± 3.9	± 1.13	± 0.25	± 17.15
Median	18.8	1.4	7.1	39.3	98.9	3.3	0.04	0.051	82
Minimum	11	0	3.59	12	40	0	0	0.001	16
Maximum	31.3	172	8.5	267	118	26.14	19.2	2.66	97
CV (%)	21	294	8	87	12	92	328	295	-

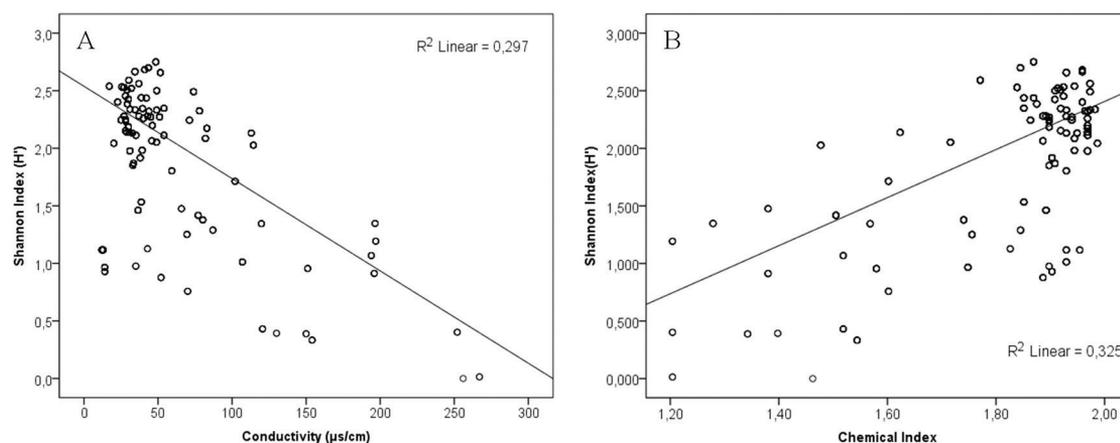


Figure 2. Regression analyses of 88 samples in 35 lotic ecosystems in the northeast region of Rio Grande do Sul, Brazil. (A) Shannon Index and conductivity (B) Shannon Index and Chemical Index.

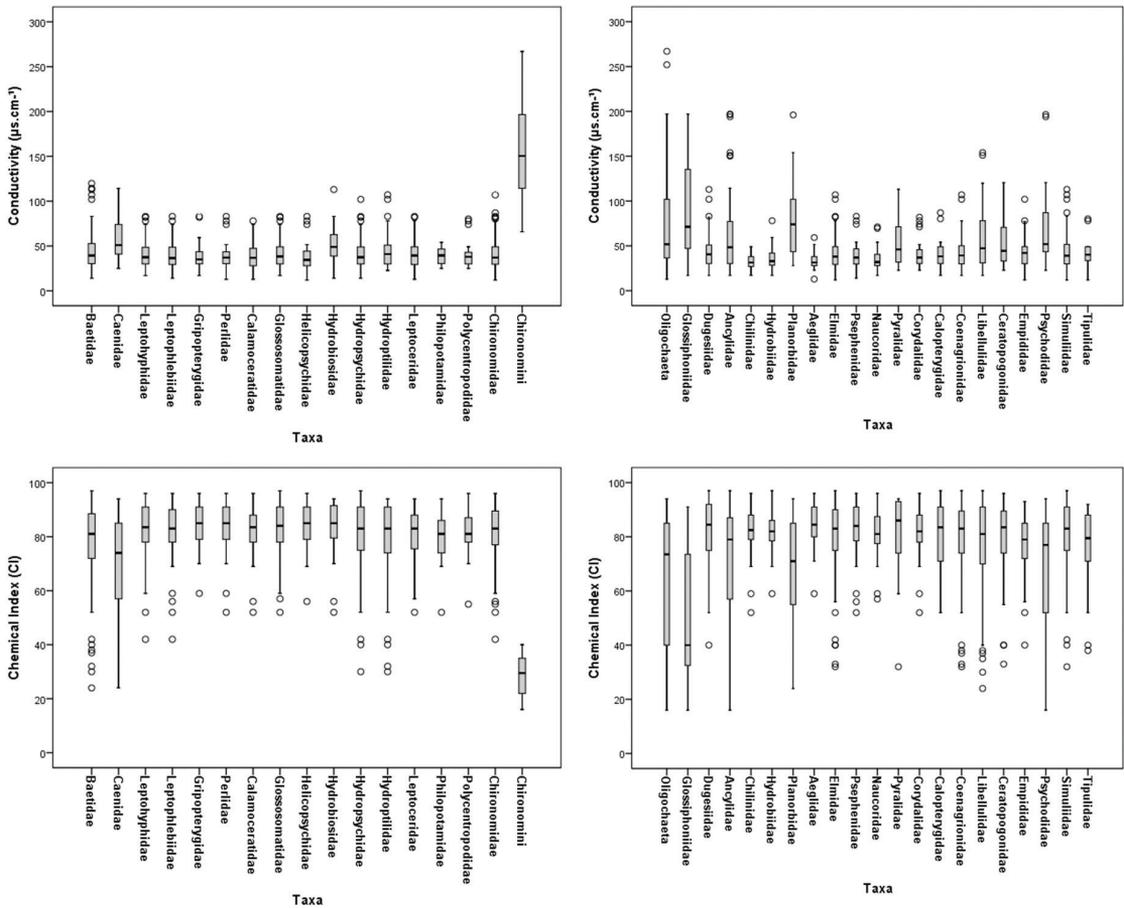


Figure 3. Occurrence of 38 macroinvertebrate taxa in the amplitude of conductivity and Chemical Index in 35 lotic ecosystems of the northeast region of Rio Grande do Sul, Brazil.

Glossosomatidae, although clustered to this group in the dendrogram, have larger interquartile ranges and were, therefore, put together with the moderate tolerance taxa.

An opposite situation is presented by Ancyliidae, Planorbidae, Baetidae, Caenidae, Psychodidae, Libellulidae, Oligochaeta and Glossiphoniidae, which were observed in a wide spectrum of conductivity and CI. These organisms can be found from unpolluted to the most polluted sites. Chironomini was the only taxa that showed a narrow occurrence in highly polluted environments.

Dugesiiidae, Leptohiphidae, Leptophlebiidae, Glossosomatidae, Hydropsychidae, Hydroptilidae, Leptoceridae, Pyralidae, Coenagrionidae, Chironomidae, Elmidae, Ceratopogonidae and Empididae showed an interquartile range broader than the first group, but were not found in the most polluted environments. Tipulidae, Simuliidae and Calopterygidae, despite having a high frequency at low conductivity values, were observed at wider ranges of CI, and are related to this group.

4. Discussion

Physicochemical measurements are routinely performed in watercourses monitoring, and these analyses are standardized and regulated (Brasil, 2005). Nevertheless, these measures are specific and represent a transient situation (Queiroz, 2008). The inclusion of biological measures is an important approach to evaluate ecosystems' integrity and long term effects of pollutants (Roque and Trivinho-Strixino, 2000). Biological methods application requires knowledge on the sensitivity and tolerance of organisms to different types of human impact, in distinct regions. The survey of Rio Grande do Sul highland streams provides information on the tolerance range to the habitat physicochemical conditions of 38 benthic macroinvertebrate taxa.

The descriptors selected to estimate taxa's ecological valence were useful in differentiating ecological conditions and the amplitude of taxa, as shown by CV and regression analyses. The CI reflects contamination of water bodies caused by

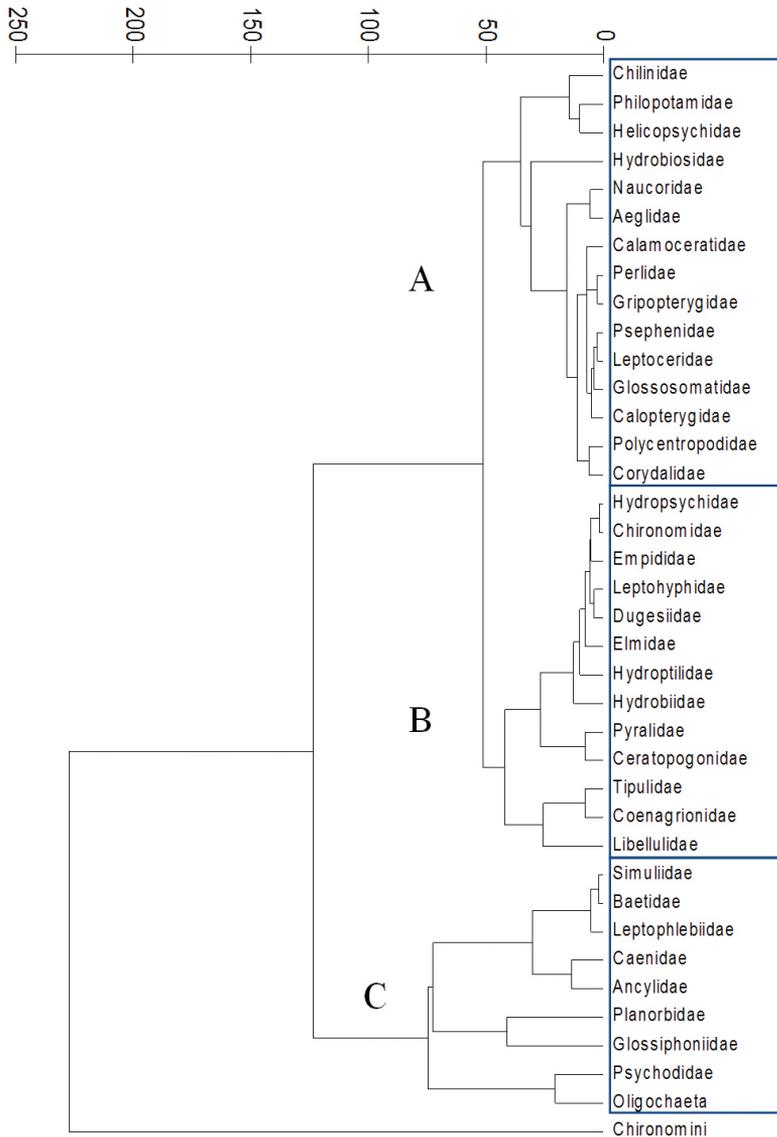


Figure 4. Similarity (Euclidean Distance) of benthic macroinvertebrate fauna according to Chemical Index in 35 highland streams at the northeast region of Rio Grande do Sul, Brazil. A) Sensitive taxa; B) Intermediate taxa; C) Tolerant taxa. Cophenetic correlation coefficient = 0,92.

release of organic waste (Bach, 1986), which has direct consequences on biological communities. Conductivity, as a single measure, has been indicated as an important variable to benthic macroinvertebrate communities' structure (Melo, 2009). The lotic ecosystems researched are primarily formed by volcanic rocks (Hasenack et al., 2009) and tend to have lower conductivity, since these rocks' inert materials are not easily dissolved in water and increases in conductivity values can indicate organic contamination (APHA, 1998; EPA, 2012).

The quartiles and cluster analysis revealed organisms with narrow amplitudes in the conductivity and CI spectrum, organisms with wide amplitudes and those with moderate amplitudes.

Among the first group, Plecoptera and Trichoptera, in temperate regions, are known to live in clean and oxygenated waters, with low amount of nutrients (Tomanova and Tedesco, 2007; Froehlich, 2007). However, Bispo et al. (2002) and Tomanova and Tedesco (2007) found the genus *Anacroneria* Klapálek, 1909 in sites with a high degree of organic contamination, in the Almas river (Goiás) and in the Amazon basin, respectively. In this study, Plecoptera naiads were not found in polluted waters, and Perlidae proved to be a good indicator of clean waters, as well as Gripopterygidae. Most Trichoptera families were sensitive to organic enrichment, information also reported in previous studies (Bueno et al., 2003; Campello et al.,

2005; Buckup et al., 2007; Monteiro et al., 2008; König et al., 2008; Winckler-Sosinski et al., 2008; Milesi et al., 2009; Hepp et al., 2010; Junqueira et al., 2010). Plecoptera, Trichoptera and Ephemeroptera are considered sensitive to environmental disturbances and anthropic influence (Bispo et al., 2006). Thus, many environmental assessment studies are based on these orders (Rosenberg and Resh, 1993; Bispo et al., 2006; Buckup et al., 2007; Crisci-Bispo et al., 2007). This sensitivity was confirmed for Plecoptera and most Trichoptera, suggesting that identification in family level is enough for bioindication analyses. Ephemeroptera showed a wide range of environmental tolerance and its use as bioindicator must be further investigated, considering genus or species. Thus, it is recommended that the use of EPT index should be taken with caution.

Bueno et al. (2003) and Milesi et al. (2009), studying watercourses in northeastern and northern regions of Rio Grande do Sul, recorded a predominance of Corydalidae, Naucoridae and Psephenidae in low conductivity values ($\leq 20 \mu\text{S}\cdot\text{cm}^{-1}$), while Elmidae and Simuliidae were associated with higher conductivities (near $100 \mu\text{S}\cdot\text{cm}^{-1}$). These observations follow the same patterns found in the present study, where Corydalidae, Naucoridae and Psephenidae were absent in the most polluted sites, and could be considered bioindicators of low levels of organic enrichment. Elmidae and Simuliidae showed moderate amplitude in the selected descriptors.

Some macroinvertebrates prevalent in polluted waters (Chironomini, Psychodidae, Glossiphoniidae and Oligochaeta) are usually indicated as the most tolerant to environmental disturbances (König et al., 2008; Monteiro et al., 2008; Hepp et al., 2010; Junqueira et al., 2010; Ferreira et al., 2012). Psychodidae, Glossiphoniidae and Oligochaeta were found in all water quality classes, but with higher abundances in heavily polluted sites. Organic enrichment causes the disappearance of sensitive species, thus reducing competition for resources and favouring proliferation of tolerant organisms. In Chironomini, the presence of proteins similar to hemoglobin in some species allows them to survive in environments with extremely low oxygen contents, which justifies their indication to severely impacted environments (Hellawell, 1986; Rosenberg and Resh, 1993; Monteiro et al., 2008; Mugnai et al., 2008; Junqueira et al., 2010). Chironomini was found only in sites with high levels of pollution. Usually,

the taxonomic level of family, Chironomidae, is associated with impacted environments in biotic indices (Hellawell, 1986; Monteiro et al., 2008; Mugnai et al., 2008; Junqueira et al., 2010). For the study area, Chironomidae proved to be an inadequate indicator of this condition, since it occurs from clean to critically polluted sites and, apart from Chironomini, was not found in degraded environments.

Monteiro et al. (2008) in Goiás, and Junqueira et al. (2010), in Minas Gerais and Rio de Janeiro states, found the families Ancyliidae, Baetidae, Caenidae and Libellulidae in environments with low organic enrichment. In Rio Grande do Sul mountain streams, these taxa occurred from unpolluted sites to those with high loads of organic pollution. Such differences reflect the levels of tolerance seen by species in these families that occur in distinct areas, due to geographical and climatic regional conditions (Chang et al., 2014).

Similar observations were made regarding taxa with moderate amplitudes, Monteiro et al. (2008) and Junqueira et al. (2010) found most of these taxa in environments with low organic enrichment, while in the present study they were found up to critical pollution. Vasconcelos et al. (2013) recommend that biomonitoring programs on large scales may use information on family-level based, since it provides rapid identification and processing, which reduces the final cost and produces similar responses to genus level when covering large geographic extensions. On the other hand, Lenat and Resh (2001) assert that family taxonomic level often contains taxa covering a wide range of tolerance values at the genus/species level and may not detect smaller differences in water quality. Although for some taxa in this study family identification may suffice bioindication analyses, others with a widespread occurrence in different levels of organic enrichment show a weak potential as bioindicators. In these cases, analyses of tolerance at genus or species level should be performed.

The ecological valence of macroinvertebrates is used to define sensitive and tolerant organisms, constituting the basis of many biological indices used to monitor rivers and streams (Hellawell, 1986). However, prior to the use of these methods, it is necessary to determine tolerance values for bioindicator species, since the biogeographical conditions differ from one region to another. The present analysis of the taxa's extent of occurrence in different environmental conditions, defined by conductivity and CI, contributes to increase

knowledge about benthic macroinvertebrate communities and can support future biomonitoring studies.

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