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Habitat preferences in freshwater benthic macroinvertebrates: Algae as substratum and food resource in high mountain rivers from Mexico

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ABSTRACT

The diversity of benthic macroinvertebrates in lotic systems is closely related to the availability and heterogeneity of habitats. These habitats may be of inorganic origin, such as the rocky substratum associated with the river bed, or organic such as macroscopic algae. The objective of this study was to determinate the importance of five species of algae that differ in their morphological type as a substratum and food resource regarding the associated establishment of macroinvertebrate assemblages taking account the climatic seasonality (warm dry, cool dry and rainy). We then evaluate the differences in macroinvertebrates assemblages with respect to the inorganic substratum by sampling high mountain rivers in central Mexico. The mucilaginous colonies of *Nostoc parmelioides* and *Placoma regulare,* the pseudoparenchymatous bambusiform thallus of *Paralemanea mexicana* and the laminate thallus of *Prasiola mexicana* had the highest densities of macroinvertebrates, represented by the genera *Cricotopus*, *Paramerina*, *Simulium* and Tanytarsini tribe. The relationship between algal morphological type and the richness and diversity of macroinvertebrates was positively related to specific conductivity, total dissolved solids and discharge variables. The dominant taxa associated with the inorganic substratum belonged mainly to the Trichoptera, Diptera and Ephemeroptera orders. Water temperature, discharge and concentration of orthophosphates were the main environmental variables able to explain the diversity of macroinvertebrates on this substratum. The dominance of detritivorous macroinvertebrates in these mountain rivers suggests the contribution of allochthonous organic matter possibly of anthropogenic origin. The assemblages of macroinvertebrates on inorganic substratum did not significantly differ among sites or climatic seasons.

1. Introduction

The diversity of benthic macroinvertebrates inhabiting lotic environments is directly associated with substratum diversity and habitat type (Dewalt et al., 2010). Macroscopic algae, because of the diversity of their physical structures, can increase habitat complexity and heterogeneity (Wellnitz and Ward, 2000; Liston and Trexler, 2005; Walker et al., 2013), can modify the oxygen concentration and availability of nutrients, and can provide refuge against predation (Beauger et al., 2006; Bakker et al., 2016). Studies of interactions between heterotrophic organisms and algae in rivers are scarce, because the impact of herbivory is difficult to quantify, and has been considered of lower magnitude than in terrestrial ecosystems (Wellnitz and Ward, 2000). Nevertheless, these interactions modify the relationships between aquatic communities through changes in their structure and function (Lodge, 1991; Bakker et al., 2016). For example, many species of herbivorous macroinvertebrates that are associated with algae may facilitate algal growth and dispersion of reproductive structures (Caro-Borrero and Carmona, 2016). Other macroinvertebrates, such as shredders and burrowers, promote recycling and nutrient retention by incorporating organic matter into the food chain (Grubaugh et al., 1996). In oligotrophic systems such as high mountain rivers, the heterotrophic community depends largely on algae as a food source.

The diversity of algal morphology (*e.g.* gelatinous, crusty, filamentous, laminar.) can provide substratum for the development of macroinvertebrate larvae that as adults will be an important component of the terrestrial ecosystem (Wellnitz and Ward, 2000; Di Sabatino et al., 2014). Algal structures may alleviate the physical challenges faced by aquatic larvae, such as the mechanical dragging force of current, and difficulty in capturing oxygen and/or food (Grubaugh et al., 1996; Walker et al., 2013; Liston and Trexler, 2005). These features enable algae to support a macroinvertebrate community that differs from the assemblage associated with inorganic substrata.

The composition and stability of inorganic substrata (mainly rocky) depends on hydrogeomorphological and climatological characteristics, which define ecosystems locally and change sporadically. Inorganic

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substrata tend to be more stable that organic mats, since those are subject to diverse pressures of physico-chemical and biological origin that limit their growth and dispersion (Grubaugh et al., 1996). The heterogeneity of inorganic substratum (mainly defined by grain size), also influences the diversity and availability of habitats for benthic organisms and therefore the community structure (Walker et al., 2013; Liston and Trexler, 2015).

Information about the influence of algal mats on the structure of benthic macroinvertebrate communities and their interactions is limited defined by number of published research. This paper presents empirical information on habitat preferences of benthic macroinvertebrates in high mountain rivers. Considering the diversity of algal substrata and their dependence on conditions in the river channel, we hypothesize that throughout the year we will find modifications in percentage cover and morphological types of algae, and that the macroinvertebrate assemblages will respond with structural changes. In contrast, the inorganic substratum will have greater stability, so we expect to find that macroinvertebrate assemblages are more diverse than on algae, and that they are similar in composition among the inorganic sample sites. The main objective of this study was to establish the differences among habitats in high mountain rivers, central Mexico, and to establish the preferences shown by the composition of macroinvertebrate assemblages associated with five species of algae of diverse morphological type and associated with the inorganic substratum.

2. Materials and methods

2.1. Study area

Samples were collected from a segment of the one-to–three-order mountain rivers in the *trans*-Mexican Volcanic Belt, central Mexico (19° 09′- 19° 16′N and 98° 43′- 100° 09′W, Fig. 1). It is an area of intense volcanic activity and marked altitudinal changes, diverse geological composition and abundant streams that originate from a system of mountains (altitude 1800–3200 m) and drain into the coastal plain. In general, these mountainous regions have a temperate sub-humid climate, coniferous forest vegetation and andesitic to basaltic substratum (Ferrusquía, 1993).

2.2. Physical and chemical analysis

Samples were collected on one to three occasions in each of the seven rivers between April 2015 and March 2016, during the rainy (R; June-November), cool dry (DC; December-February) and warm dry (WD; March-May) seasons (see Table 1). Water temperature, specific conductivity and pH were recorded *in situ* with a Hanna multi-sensor (HI 991300, California, USA). Oxygen saturation was recorded with an oxygen meter (YSI-85, YSI, Ohio, USA). Sampling locations located at the upstream were selected according to the ecological status, between good and excellent to avoid erroneous results dependent on environmental degradation and not on the type of substratum. samples of water (500 ml) were filtered *in situ* through 0.45 μm and 0.22 μm filter membranes (Millipore, Massachusetts, USA) and collected in sterile polypropylene bottles for the physicochemical analysis, according to the criteria established in APHA (2005). Samples were stored at 4 °C and two replicates were analyzed in the laboratory within 24 h of collection. The nutrient analyses were adapted from Standard Methods for the Examination of Water and Wastewater (APHA, 1999) and used a DR 3900 laboratory Spectrophotometer (Hach, Loveland, Colorado). Dissolved inorganic nitrogen (DIN) was calculated as the sum of the three inorganic nitrogen forms in water. NH_4–N was measured colorimetrically by the Nessler method (detection limit 0.1 mg l^{-1}), reading the absorbance at 425 nm. When values were close to the detection limits, the salicylate method was used (detection limit 0.01 mg l^{-1}), read at 655 nm. NO_3–N was measured colorimetrically by a modification of the cadmium reduction method, using gentisic acid instead of 1-naphthylamine (detection limit 0.5 mg l^{-1}) and reading absorbance at 500 nm. The low-range method (up to 0.5 mg l^{-1}) is an expanded modification of the former that employs a chromotropic acid indicator (detection limit 0.05 mg l^{-1}) reading at the same wavelength. NO_2–N was determined colorimetrically with chromotropic and sulfanilic acids as indicators (detection limit 0.01 mg l^{-1}) and reading absorbance at 500 nm. PO_4–P was estimated colorimetrically with a modification of the molybdenum blue procedure, provided by Phos Ver 3 (detection limit 0.01 mg l^{-1}), reading absorbance at 890 nm.

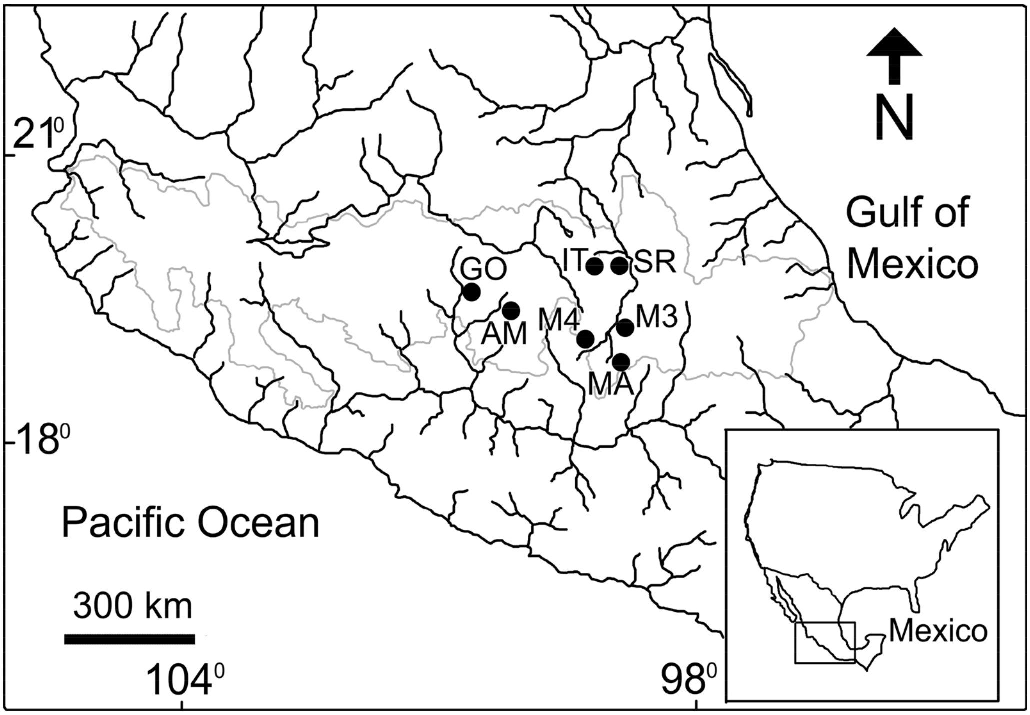

Fig. 1. Location of sampling sites in the Mexican Volcanic Belt (grey line): Amanalco (AM), Gonzalez spring (GO), La Magdalena 3rd dinamo (M3), La Magdalena 4th dinamo (M4), Monte Alegre (MA), San Rafael (SR) and Presa Iturbide spring (IT).

Table 1
Physical and chemical characteristics of mountain rivers in Central Mexico. Specific Conductivity K_{25}, DO, Dissolved Oxygen; PAR, Photosynthetic Active Radiation; Q_3, Discharge; SRP, Soluble Reactive Phosphorous. Seasons: CD, Cool Dry, D, Dry, R, Rainy.

Rivers	Season/Date	T°C	pH	K_{25} (μS cm^{-1})	DO (mg l^{-1})	Current velocity (m s^{-1})	PAR (μmol photons cm^2 s^{-1})	Q_3 (m^3 s^{-1})	SRP (mg l^{-1})	Nitrite (mg l^{-1})	Nitrate (mg l^{-1})	Ammonium (mg l^{-1})
Amanalco (AM)	WD 12.v.2015	17.7	5.8	168	7.2	0.68	744	1.10	0.61	0.043	0.39	0.26
	R 22.x.2015	17.5	6.3	168	6.5	0.83	177	2.20	0.01	0.0095	0.1825	0.02
	CD 29.i.2016	15.6	7	184	8	0.9	116	1.72	0.84	0.1	1.1	0.095
Gonzalez spring (GO)	R 22.x.2015	17.4	6.8	65	7	0.63	118	0.6	0.01	0.0045	0.007	0.015
	CD 19.i.2016	12.2	6.9	86.2	8.4	0.73	67	0.24	1.21	0.024	1.1	0.09
La Magdalena 3rd Dinamo (M3)	WD 01.iv.2015	10	7	80	9	0.29	1715	0.25	1.03	0.006	0.75	0.025
	R 15.x.2015	11.5	6.2	83	8.1	0.33	70	0.07	0.8	0.008	0.025	0.01
La Magdalena 4th Dinamo (M4)	R 13.x.2015	10.5	7	75	8.4	0.55	202	0.4	0.28	0.09	0.03	0.12
Monte Alegre (MA)	R 07.x.2015	11	6.2	47	10	0.8	1245	0.08	0.9	0.007	0.02	0.04
	CD 09.ii.2016	9.2	6.7	50	9.5	0.46	614	0.6	0.33	0.005	1.25	0.015
San Rafael (SR)	WD 05.v.2015	11.3	6.8	136	9.2	0.81	1516	0.16	0.51	0.005	0.95	0
Presa Iturbide spring (IT)	R 28.ix.2015	10.3	6.1	53	9.5	0.3	19	0.06	0.54	0.005	0.9	0
	WD 04.iii.2016	11.5	7.3	54	10.3	0.43	200	0.45	1.19	0.005	0.95	0.19

2.3. *Sampling of algae*

Natural boulder substrata were observed directly on the river bed. Variations over time were monitored by a quadrat technique (Necchi et al., 1995), which evaluates the influence of current velocity, depth, and underwater irradiance at the microhabitat level on the percentage algal cover. The sampling site consisted of a 58 m^2 river segment. Each sample unit (five units per segment) covered a 10 cm radius (area = 314.16 cm^2). Choice of type and size of the sampling units was based on preliminary tests and previous research about distribution, morphology and ecology of algae and ecological status of the rivers and sampling locations selection (Carmona et al., 2006). Sampling consisted of five quadrats 2 m apart. Quadrats were positioned within each site on areas with ≫ > 5% of alga cover. Their direction and location was chosen randomly in an interval between 0° and 180°. This procedure was repeated along the sampling quadrats (in an upstream direction in each sampling sites). Microhabitat characteristics were recorded on all sampling dates. The microhabitat variables were measured *in situ*, approximately at the center of each sampling unit with five replicates of each sampling unit. Current velocity and irradiance were measured as close as possible to the algae with a Swoffer™ 3100 current velocity meter (Seattle, WA, USA) and a Li-Cor™ Li-1000 quantum meter (Lincoln, NB USA), with a flat subaquatic sensor of photosynthetically active radiation (PAR) respectively.

2.4. *Macroinvertebrates and algal structure*

The number of thalli within each sampling unit was recorded by visual estimation. The algal morphological type was recognized *in situ* according to Sheath and Cole (1992) and Komárek and Anagnostidis (2005). Taxonomic analysis used Carmona and Necchi (2002), Komárek (2013), Ramírez-Rodríguez and Carmona (2005) and Carmona and Vilaclara (2007). The numbers and taxa of macroinvertebrates were recorded for each thallus. Macroinvertebrate taxonomy was based on Merritt et al. (2008), Bueno-Soria (2010), and Thorp and Covich (2010). The genus was the taxonomic resolution used in general for this research. In some cases, for example Chironomidae family, we could identify up to subfamily and tribe level, mainly due to the lack of taxonomic keys at regional level. For organic substratum only five taxa out of 18 (28%) we are not identified to genus. In order to complete taxonomic information, macroinvertebrate functional feeding groups were assigned according to Cummins et al. (2005), considering the characterization of morphological traits that determine the food source chosen by macroinvertebrates. Olympus™ BX51 and SZX7 microscopes were used with DP12 and E-330 microphotography systems.

2.5. *Collection of benthic macroinvertebrates: inorganic substratum*

Collection points were selected at each sampling location according to multihabitat criteria to obtain a representative sample and cover all possible habitats where the benthic macroinvertebrates might be found. Sampling was performed along a 50 m transect using an aquatic D-net, mesh size 150 μm, width 30 cm, sediments were removed by kicking over three minutes, and organisms caught with the net were placed in a tray for sorting. Sampling was also conducted by manual examination and removal from the submerged faces of large rocks, branches, and leaves. A minimum of 100 individuals was collected from each location as a representative sample, deposited in plastic flasks and preserved in 70% ethyl alcohol. The individuals were sorted under an Olympus SZX7 stereoscopic microscope (Olympus Corporation, Tokyo, Japan) and were identified down to genus level when possible using the same sources as used for the algal substratum. When genus could not be determined, individuals were identified to tribe, subfamily, family, or class, in this case six taxa out of 35 were are not identified to genus (18.75%).

2.6. *Data analysis*

To evaluate habitat preferences and differences in richness and composition of macroinvertebrate assemblages, abundances were estimated on algal and inorganic substratum. To assess the effect of algal morphological types on macroinvertebrate assemblages, we analyzed the differences in percentage cover of algae among sampling sites and climatic seasons.

Algal and macroinvertebrate abundances on the two substratum types were analyzed by multidimensional scaling (MDS) to evaluate the relative similarities between samples. These analyses were based on the Bray-Curtis similarity calculated from the square-root-transformed density. Subsequently, ANOSIM (Analysis of similarity) one-way testing evaluated the significance of the MDS groups observed. A SIMPER (Similarity Percentage) procedure analyzed the macroinvertebrate taxa assemblage by climatic season, sampled site and algal substratum type; for this, each algal species was tested as a different organic substratum type. We chose the taxa that contributed with an accumulated 90%.

BEST analysis using the BIO-ENV algorithm explored the link between the environmental variables and the diversity and composition of macroinvertebrate assemblages. The BIO-ENV algorithm uses Euclidean distances of scaled environmental variables to find a subset of environmental variables that have the maximum correlation with the community dissimilarity. The biological data matrices were transformed using a square-root algorithm and the environmental data matrices were normalized and only those parameters that had a biased distribution in the Draftsman plots were transformed with the same algorithm. All tests were performed in the PRIMER V statistical program. 1.4. (Clarke and Gorley, 2006). Independently the taxonomic resolutions, this statistical analyses were used to established a pattern of taxa assembly composition and not to make ecological inferences of the species.

3. Results

3.1. Richness and composition

Five species of algae were recognized: *Nostoc parmelioides* Kützing ex Bornet et Flahault, *Placoma regulare* Broady et Ingerfeld (Cyanobacteria), *Paralemanea mexicana* (Kützing) Vis et Sheath, *Sirodotia suecica* Kylin (Rhodophyceae) and *Prasiola mexicana* J. Agardh (Chlorophyceae). The populations of *Nostoc, Paralemanea* and *Prasiola* were present in more than one site and season. The 3439 benthic macroinvertebrates collected represented 53 taxa. Taxa recorded with absolute abundance greater than 100 individuals were: *Cricotopus* (892), *Baetis* (528), Tanytarsini (396), *Simulium* (306), *Glossosoma* (165) and *Paramerina* (121).

Of the 3439 macroinvertebrates, 1699 were from the inorganic substratum and 1740 (50.6%) from the algal substratum. In general, macroinvertebrate taxa diversity was higher on the inorganic substratum than on the algal substratum at a ratio of 3:1 (Fig. 2).

3.2. The spatial and temporal cover by the algal substratum

MDS determined two groups relating algal percentage cover and sampling sites (Fig. 3): populations of *Paralemanea, Nostoc* and *Prasiola* had the highest percentage cover in Amanalco and González rivers; and populations of *Nostoc, Placoma, Prasiola* and *Sirodotia* with the highest percentage cover in Magdalena, San Rafael, Amanalco, Monte Alegre and Presa Iturbide rivers. ANOSIM analyses confirmed significant differences in algal assemblages between sampling sites, but not between climatic seasons.

3.3. Benthic macroinvertebrates associated with algal substratum

The relation between the algal morphological type and the richness and diversity of macroinvertebrates varied significantly. The mucilaginous colonies of *Nostoc* and *Placoma,* the pseudoparenchymatous bambusiform thallus of *Paralemanea* and the laminate thallus of *Prasiola* had the highest number of associated macroinvertebrates ($\gg > 100$ individuals), represented by *Cricotopus*, *Paramerina*, *Simulium* and Tanytarsini (order Diptera). MDS showed that the macroinvertebrate assemblages could be separated by the morphological type of algae, seasonally and site (Fig. 4). However, ANOSIM only confirmed the significance of differences between the assemblages by site. The SIMPER analysis showed that members of the order Diptera, Chironomidae, preferred algal substrata throughout the year.

Cricotopus, *Paramerina* and Tanypodinae were associated with the mucilaginous colonies of *Nostoc* and *Placoma*. Within each *Nostoc* colony from the Monte Alegre and Presa Iturbide sites, there was one *Cricotopus* larva, and this seemed to have modified the structure of the colony, giving it an auriform appearance (Fig. 5a, b). A similar structural transformation occurred in *Placoma* colonies (Fig. 5c, d). The *Paralemanea* and *Sirodotia* populations were associated with *Simulium*, Tanytarsini and *Cricotopus* larvae (Fig. 5e-f).

3.4. Benthic macroinvertebrates associated with inorganic substratum

The taxa associated with the inorganic substratum were mainly Trichoptera, Diptera and Ephemeroptera, however with exceptions such as Amphipoda and Platyhelminthes. MDS analysis showed that the Gonzalez and Amanalco rivers tended to form a separate group, and ANOSIM confirmed a significant difference between the assemblages by site, but not by climatic season (Fig. 6).

According to the SIMPER analyses, the differences in macroinvertebrates between sites are basically determined by the abundances/dominance of certain taxa. The sampling sites shared a diversity related to Diptera (Chironomini, Tanytarsini, *Simulium*), Ephemeroptera (*Baetis, Epeorus* and *Callibaetis*), Trichoptera (*Atopsyche*, *Orthotrichia, Helicopsyche* and *Policentropus*) and Dugesiidae (Planaridae). The inorganic substratum in the Amanalco had numerous Amphipoda and Odonata with the genus *Hetaerina*, whereas even though the Gonzalez river belongs to the same drainage basin it differed in that it included *Lara* and several Trichoptera genera. Taxa such as *Glossosoma* (Trichoptera), Chironomini, Tanytarsini and *Baetis* were abundant in the rainy season. In the warm dry season *Orthothrichia*, *Helicopsyche* (Trichoptera), *Simulium* (Diptera) and *Nixe* (Ephemeroptera) were recorded. Finally, the cold dry season was represented by Amphipoda, *Hesperophylax* (Trichoptera), *Lara* (Coleoptera) *Epeorus* (Ephemeroptera) and *Hetaerina* (Odonata).

3.5. Relation between environmental and/or biological variables and macroinvertebrate assemblages

The BIO-ENV analyses showed temperature, discharge and concentration of orthophosphates to be the main environmental drivers that explained the diversity of macroinvertebrates associated with inorganic substrata ($\rho = 0.8$; $p = 0.002$). In contrast, the assemblages associated with the algal substratum were influenced by specific conductivity, total dissolved solids and discharge ($\rho = 0.3$; $p = 0.002$).

Regarding macroinvertebrate feeding preferences, the algal substrata were occupied by filterers and herbivores, and to a lesser extent by predators possibly seeking prey. By contrast, on inorganic substratum the diversity of functional feeding groups was higher and detritivores were dominant, possibly because of the greater range of substratum sizes and consequently increased habitat diversity. On both substrata, the highest density was concentrated in very few taxa. The dominant functional groups at all sites were gathering collectors, filtering collectors and scrapers, possibly related to the abundance of fine particulate organic matter (FPOM).

4. Discussion

In our study the results shows that the highest richness of macroinvertebrate taxa was recorded on inorganic substrata; however, an algal substratum promoted richness and coexistence with some taxa. Although macroinvertebrates can follow several paths to the selection of organic substrata, the selection in the present study, particularly by the chironomids, was guided mainly by the need for shelter and possibly food sources; this behaviour has been reported from other studies (Grimm and Fisher, 1989; Di Sabatino et al., 2014). Because the algal communities have a seasonal pattern determined by the rainfall pattern and the availability of nutrients, the dominant macroinvertebrate taxa may be using diverse algal morphological types to compensate for this seasonality. In general, the dominance of detritivores in these mountain rivers suggests contributions of FPOM, as expected for higher-order rivers (Vannote et al., 1980), which perhaps reflects anthropogenic action in the riverside ecosystem.

Algal substrata represent a smaller proportion of the available surface area on the river bed for macroinvertebrates than the inorganic

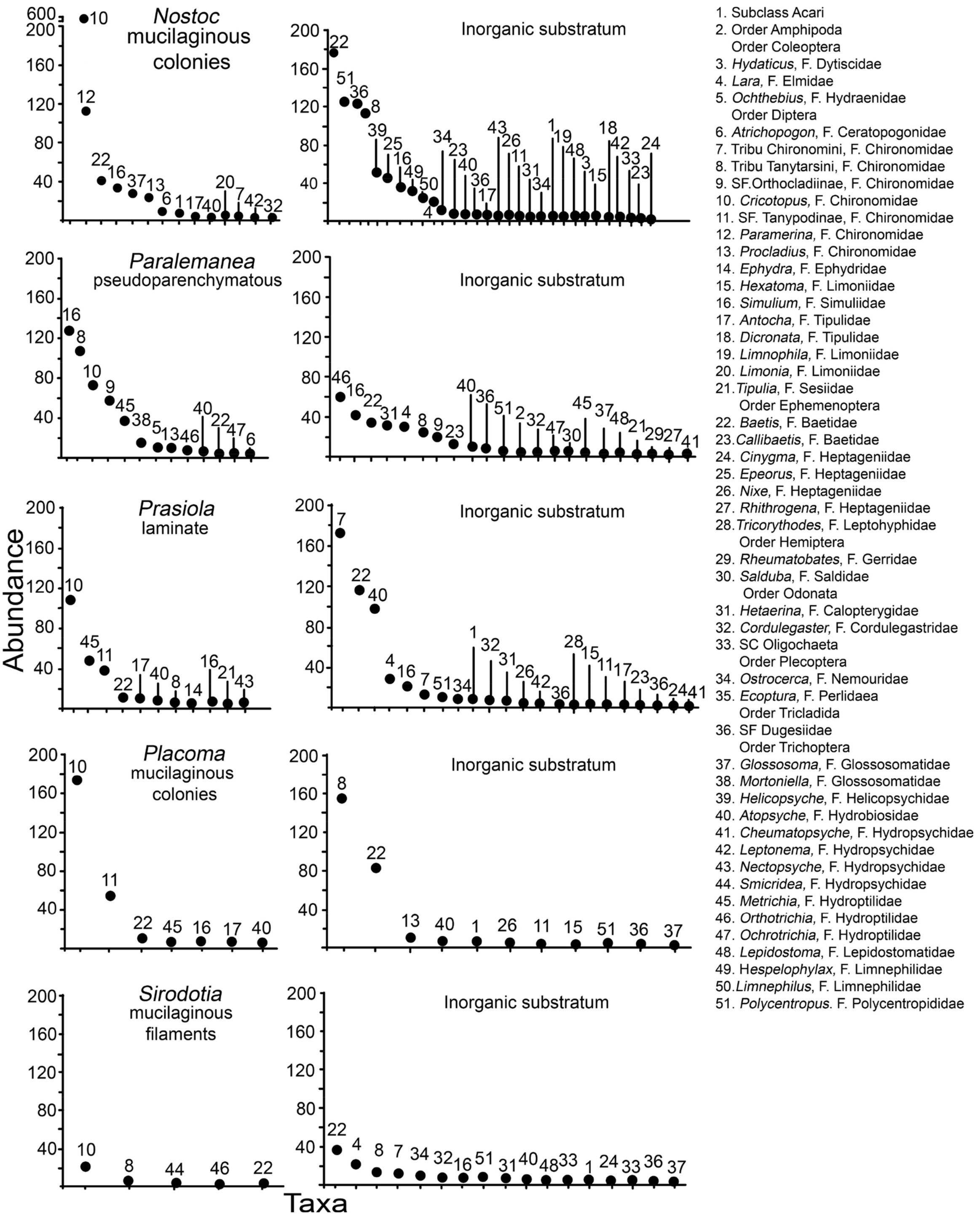

Fig. 2. Richness–diversity curves based on macroinvertebrate abundance (number of individuals) among all sites and substratum type. Left-hand panels: Total of macroinvertebrate individuals associated to macroscopic algal taxa. Right-hand panels: Total of macroinvertebrates individual associated to inorganic substratum.

substratum. However, this study shows that algal substrata support the same density of individuals, in particular Diptera. This may be related to strategic life adaptations of dipterans to colonize oligotrophic to eutrophic environments with high current velocity (Sabater and Muñoz, 2000; Ward et al., 1985). For example, *Cricotopus* larvae have been reported in a mutual interaction with *Nostoc parmelioides* (Brock, 1960; Ward et al., 1985) and recently with *Nostoc verrucosum* (Sabater and Muñoz, 2000), which is also likely to be the case in Mexican populations according our results. Likewise, the presence of *Nostoc* during rainy and dry seasons (nearly five months) may favor larval development. *Cricotopus* populations in USA rivers spend approximately nine months completing their larval development within the special cavities formed by larval mechanical action inside the *Nostoc* colonies (Brock, 1960). Mexican populations might compensate for this temporal difference by shorter larval development and/or by seeking alternative shelter in similar environmental conditions, such as other algal species with morpho-physiological strategies like those of *Nostoc* as observed for *Prasiola mexicana* and *Placoma regulare* (personal observations). Prasiolales may have different mechanisms to live in conditions of excess lighting or desiccation (Rindi and Guiry, 2004; Holzinger et al., 2017), and this may be advantageous for the associated macroinvertebrates. These mechanisms may include synthesis of

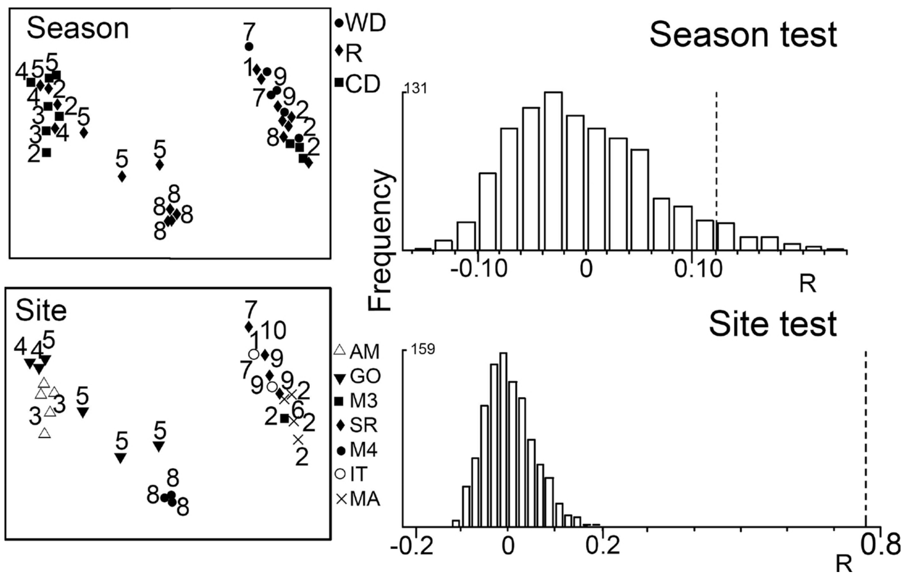

Fig. 3. Significant within-site differences in algal cover (one-way ANOSIM, global $R = 0.77$ $p \ll 0.001$), but absence of significant seasonal differences (global $R = 0.12$ $p \ll 0.05$; (12 samples, 5 algal species, 5 quadrants per sample). Bray–Curtis similarity was calculated from the square-root-transformed density. For abbreviations see Table 1.

mycosporine-like amino acids for defense against ultraviolet irradiation (Rozema et al., 2002), as well as humidity retention and nutrient uptake under procumbent sheets (Lud et al., 2001; Holzinger et al., 2017). In our study, some *Placoma* colonies, which are usually globose and hollow (León-Tejera et al., 2003), were occupied by *Cricotopus* larva that modified the shape of the colony to an elongated sac, usually adjusted to accommodate the larva.

The assemblages of macroinvertebrate taxa showed insignificant changes in relation to the diversity and percentage cover of algae during the year. However, algal morphological type is an important factor in larval development through its influence on the physical suitability of the algae as a refuge, the variety of assemblages represented by larval establishment, and the velocity of the water current to which the larvae are exposed (Watson and Rose, 1985; Grubaugh et al., 1996; Holomuski and Biggs, 2006; Walker et al., 2013). The functional feeding groups most often associated with algae were herbivorous grazers and filterers, and the presence of predators such as *Tipula* and Tanypodineae (associated with *Prasiola* and *Nostoc*) showed that algae can also function as an important area for hunting prey (Brock, 1960; Beauger et al., 2006). The piercing herbivores associated with *Paralemanea* and *Prasiola* because as a substratum can offer food and material for the construction of shelters in perennial populations (Bojorge et al., 2010; Caro-Borrero and Carmona, 2016). The high density of macroinvertebrates colonizing these algae shows that herbivory can become an important ecological mechanism in the regulation of the growth and biomass of algae mats (Caro-Borrero and Carmona, 2016), in contrast to what has previously been thought. In particular, macroinvertebrates with restricted distribution, such as those that live within or attached to algae, are the most likely to reduce algal biomass because of the specificity of the interaction (Holomuski and Biggs, 2006). Our results contribute to confirm the importance of herbivory on algae in lotic environments, whereas it has previously been considered to have low impact (Bakker et al., 2016).

The presence and abundance of macroinvertebrates associated with the inorganic substratum shows a relationship with mountain rivers with temperate water, high current velocity, shallow water, and sporadic phosphorus inputs; this concurs with other studies in mountain rivers (Wohl et al., 1995; Grubaugh et al., 1996). Macroinvertebrate assemblages were mainly characterized by Ephemeroptera, Trichoptera and Diptera, which are relatively constant throughout the climatic seasons. Many macroinvertebrates were associated with dry and cool dry seasons; this suggests that the life histories of these taxa could be adapted to the hydric stress conditions, as has been seen in other lotic systems (Wohl et al., 1995; Buss et al., 2014).

With respect to water quality, the main physicochemical differences between sites can be associated with the geographical characteristics of the two basins in which they are situated: i) the Basin of Mexico with lower temperature, specific conductivity, dissolved solids and nutrient concentrations, and ii) the Valle de Bravo-Amanalco Basin, with higher temperature, discharge and SRP values. These differences may explain the environmental preferences and different percentage cover of algal species among the sites, and the relationship between greater algal cover and abundance of associated macroinvertebrates.

Algae as a substratum may be a selective parameter in the establishment and resilience of some macroinvertebrate taxa; for example, filterers prefer to be associated with macrophytes where FPOM

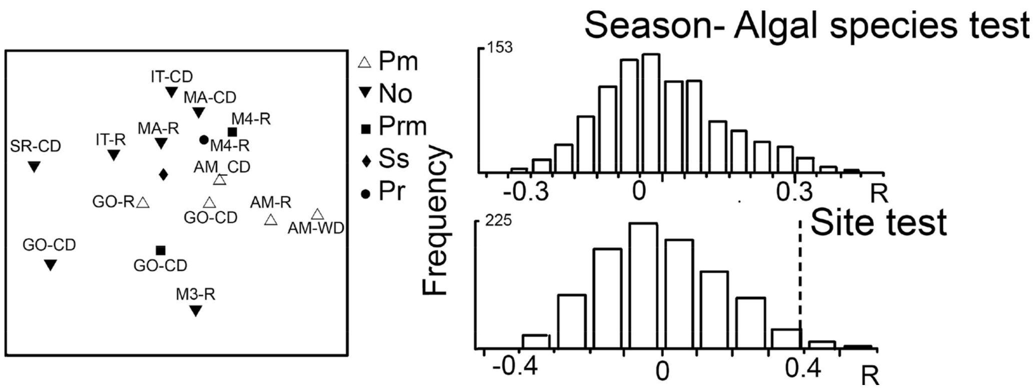

Fig. 4. Significant differences in macroinvertebrate assemblage composition by site (one-way ANOSIM, global $R = 0.39$ $p \ll 0.02$), but not by algal morphological type and seasonally (global $R = -0.006$ $p \ll 0.5$). (12 samples, 18 taxa, 1740 individuals). Bray–Curtis similarity was calculated from the square-root-transformed density. Absolute abundances of macroinvertebrates associated with organic substratum were used. *Paralemanea mexicana* (Pm), *Nostoc parmelioides* (No), *Prasiola mexicana* (Prm), *Sirodotia suecica* (Sc), *Placoma regulare* (Pr). For abbreviations see Table 1.

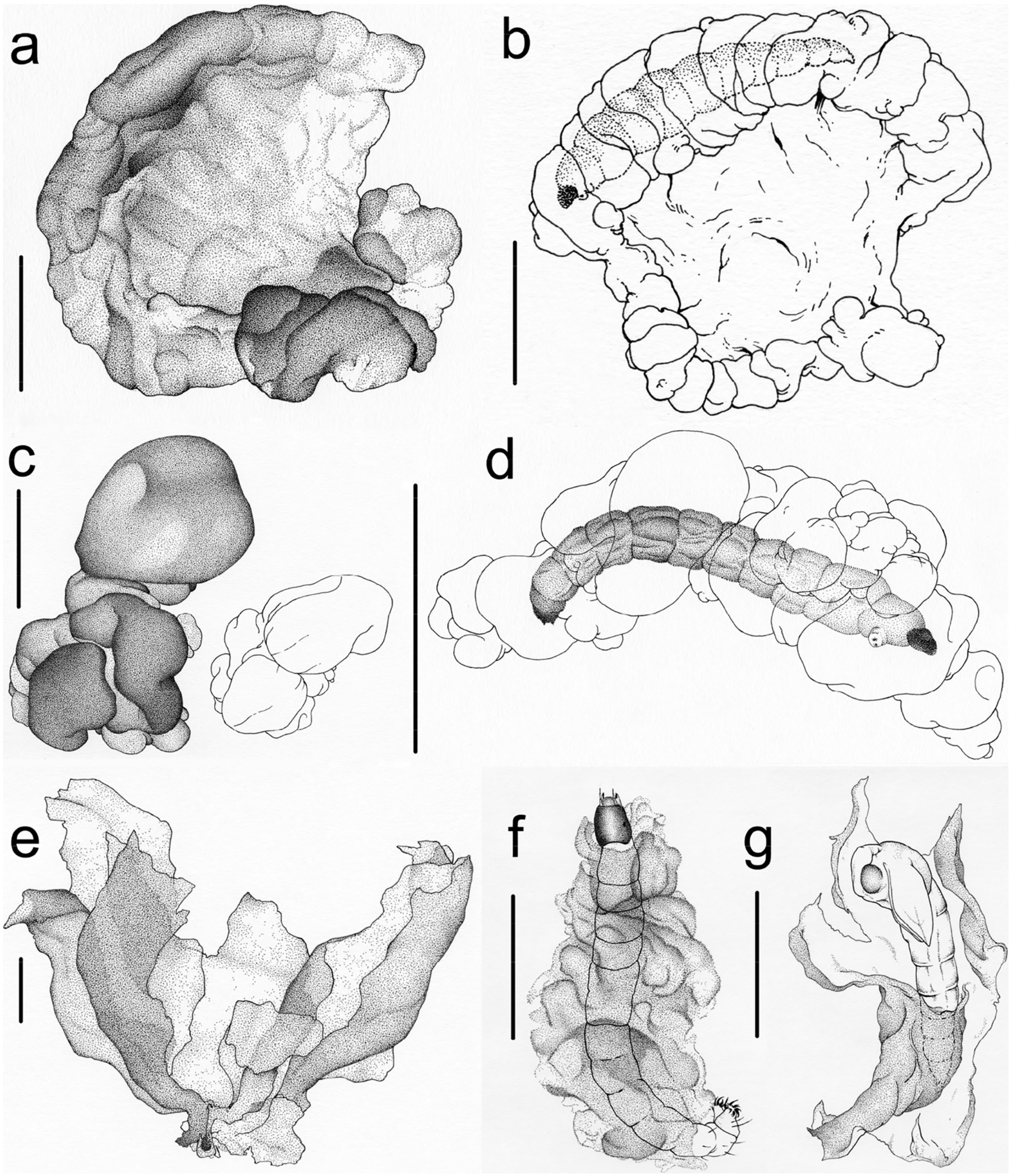

Fig. 5. Macroinvertebrates associated with algal substratum. a) Mucilaginous colony of *Nostoc* with auriform appearance. b) *Cricotopus* larvae inside *Nostoc*. c) Mucilaginous colonies of *Placoma.* d) *Cricotopus* larvae inside *Placoma*. e) Laminate thallus of *Prasiola.* f) *Cricotopus* larvae wrapped *Prasiola*. g) *Cricotopus* pupae wrapped *Prasiola*. Scale bar 5 mm.

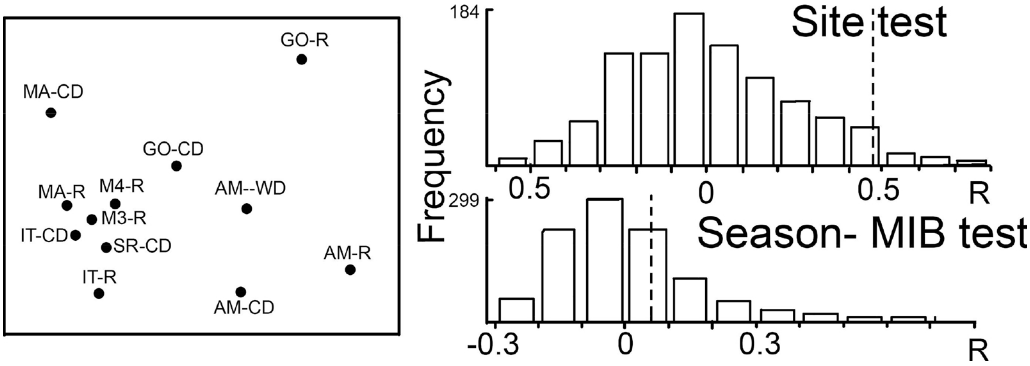

Fig. 6. Significant differences in macroinvertebrate (MIB) assemblage with inorganic substratum composition by site (one-way ANOSIM, global $R = 0.5$ $p \ll 0.04$), but not by season (global $R = 0.058$ $p \ll 0.2$). Bray–Curtis similarity was calculated from the square-root-transformed density. Absolute abundances of macroinvertebrates associated with inorganic substrata were used (7 sites, 33 taxa, 1699 individuals). For abbreviations see Table 1.

retention is greater than on a rocky substratum (Watson and Rose, 1985; Beauger et al., 2006; Di Sabatino et al., 2014). Also, algae can be a food source for macroinvertebrates where current velocity makes food uptake an arduous task. In this sense, the association of macroinvertebrate assemblages with algae may involve random processes of habitat selection (Hambrook and Sheath, 1987; Buss et al., 2014). Even a co-evolutionary adaptation process occurs (Ward et al., 1985), where a mutualist relationship can favor the establishment and colonization of algal types with diverse physiognomy and, in particular micro-environmental conditions.

5. Conclusions

Diversity of macroinvertebrate taxa was greater on the inorganic substratum; assemblages did not differ significantly between sites or climatic seasons, but there were differences between sites in the abundances/dominance of some taxa. The diversity and abundance of macroinvertebrates associated with an inorganic substratum were mainly explained by factors such as seasonality in discharge and anthropogenic influence on orthophosphates concentration. On an algal substratum, variation in abundance and diversity in macroinvertebrate assemblages was related to differences in algal percentage cover and the relationship with the spatial and seasonal requirements of each algal species. This study contributes with empirical evidence, showing how algal structural configurations may favor the macroinvertebrates that are specialized to colonize them, such as members of the Diptera on the mucilaginous algae (*Nostoc* and *Placoma*), bambusiform thallus (*Paralemanea*) and laminate thallus (*Prasiola*).

Algae as a substratum promote species diversity and coexistence with macroinvertrebrates under conditions of high current velocity and nutrient inputs. The dominance of detritivores in these mountain rivers suggests the contribution of allochthonous organic matter, a result expected for higher-order rivers than rivers in this study, a characteristic that suggests anthropogenic action in the riverine ecosystem. Changes in algal diversity and cover may associated with the occupation by the same macroinvertebrates assembly associated with different algal species throughout the year. Water temperature is relatively stable and so does not control the life cycle of macroinvertebrates as it does in temperate or cold regions.

Conflict of interest

The authors declare that they have no conflict of interest.

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References

APHA (American Public Health Association), 1999. Standard Methods for the Examination of Water and Wastewater, 20th. Washington, D.C.

APHA(American Public Health Association), 2005. Standard Methods for Examination of Water and Wastewater, 21st. Port City Press, Washington, D.C.

Bakker, E.S., Wood, K.A., Pagés, J.F., Veen, C.G.F., Christianen, M.J.A., Santamaría, L., Nolet, B.A., 2016. Herbivory on freshwater and marine macrophytes: a review and perspective. Aquat. Bot. 135, 18–36.

Beauger, A., Lair, N., Reyes-Marchant, P., Peirg, J.L., 2006. The distribution of macroinvertebrates assemblages in a reach of the river Allier (France), in relation to riverbed characteristics. Hydrobiologia 571 (63). http://dx.doi.org/10.1007/s10750-006-0217-x.

Bojorge, M.G., Carmona, J.J., Cartajena, A.M., Beltrán, M.Y., 2010. Temporal and spatial distribution of macroalgal communities of mountain streams in Valle de Bravo Basin, central México. Hydrobiologia 64, 159–169.

Brock, E.M., 1960. Mutualism between the midge *Cricotopus* and the alga *Nostoc.*. Ecology 41 (3), 474–483.

Bueno-Soria, J., 2010. Guía De Identificación Ilustrada De Los géneros De Larvas De *Nostoc.* Insectos Del Orden Trichoptera De México. [Illustrated Identification Guide to the Genera of Insect Larvae of the Order Trichoptera in Mexico]. National Autonomous University of México, México City.

Buss, D.F., Baptista, D.F., Nessimian, J.L., Egler, M., 2014. Substrate specifyenvironmental degradation and disturbance structuring macroinvertebrates assemblages in neotropical streams. Hydrobiologia 518, 179–188.

Carmona, J.J., Necchi Jr., O., 2002. Taxonomy and distribution of *Paralemanea* (Lemaneaceae, rhodophyta) in central Mexico. Cryptogam. Algol. 23, 39–49.

Carmona, J., Vilaclara, G., 2007. Survey and distribution of Batrachospermaceae (Rhodophyta) in high-altitude tropical streams from central Mexico. Cryptogam. Algol. 28 (3), 271–282.

Carmona, J., Montejano, Z.G., Necchi Jr., O., 2006. The ecology and morphological characterization of gametophyte and Chantransia stage of *Sirodotia huillensis* (Batrachospermales, Rhodophyta) from a river in central Mexico. Phycological Res. 54, 108–115.

Caro-Borrero, A., Carmona, J., 2016. Associations between macroinvertebrates and *Paralemanea* mexicana, an endemic freshwater red alga from a mountain river. Neotrop. Enthomol. 45 (6), 665–674.

Clarke, K., Gorley, R., 2006. User Manual/tutorial. PRIMER-E Ltd., Plymouth.

Cummins, K.W., Merritt, R.W., Andrade, P.C.N., 2005. The use of macroinvertebrate functional groups to characterize ecosystem attributes in selected streams and rivers in south Brazil. Stud. Neotrop. Fauna Environ. 40 (1), 69–89.

Dewalt, R.E., Resh, V.H., Hilsenhoff, L.W., 2010. Diversity and classification of insects and Collembola. In: Thorp, J.H., Covich, A.P. (Eds.), Ecology and Classification of North America Freshwater Invertebrates, third edition. Academic Press, Italy, pp. 849–869.

Di Sabatino, A., Cristiana, G., Pinna, M., Lombardo, P., Miccoli, F.P., Marini, G., Vignini, P., Cicolani, B., 2014. Structure, functional organization and biological traits of macroinvertebrates assemblages from leaf-bags and benthic samples in a third order stream of Central Apennines (Italy). Ecol. Indic. 46, 84–91.

Ferrusquía, I., 1993. Geology of Mexico: a synopsis. In: Ramamoorthy, T.P., Bye, R., Fa, J. (Eds.), Biological Diversity of Mexico, Origins and Distribution. Oxford University Press, New York.

Grimm, N.B., Fisher, S.G., 1989. Stability of periphyton and macroinvertebrates to disturbance by flash floods in a desert stream. J. N. Am. Benthol. Soc. 8 (4), 293–307.

Grubaugh, J.W., Wallace, J.B., Houston, E.S., 1996. Longitudinal changes of macroinvertebrates communities along an Appalachian stream continuum. Can. J. Fish. Aquat. Sci. 53, 896–909.

Hambrook, J.A., Sheath, R.G., 1987. Grazing of freshwater Rhodophyta. J. Phycol. 23, 656–662.

Holomuski, J.R., Biggs, B.J.F., 2006. Food limitation affects algivory and grazer performance for New Zeland stream macroinvertebrates. Hydrobiologia 561, 83–94.

Holzinger, A., Herburger, K., Blaas, K., Lewis, L.A., 2017. The terrestrial green macroalga *Prasiola calophylla* (Trebouxiophyceae, Chlorophyta): ecophysiological performance under water-limiting conditions. Protoplasma 254 (4), 1755–1767. http://dx.doi.org/10.1007/s00709-016-1068-6.

Komárek, J., Anagnostidis, K., 2005. Cyanoprokaryota: Teil 2: Oscillatoriales. Bd. 19/2. Elsevier, München.

Komárek, J., 2013. Cyanoprokaryota; Teil 3: Heterocytous Genera. 19/3. Springer, Berlin.

León-Tejera, H., Montejano, G., Cantoral-Uriza, E., 2003. Some little known Hydrococcaceae (Cyanoprokariota) from central Mexico. Arch. Hydrobiol. Suppl. Algol. Stud. 109, 363–374.

Liston, E.S., Trexler, J.C., 2005. Spatiotemporal patterns in community structure of macroinvertebrates inhabiting calcareous periphyton mats. J.N. Am. Benthol. Soc. 24 (4), 832–844.

Lodge, D.M., 1991. Herbivory on freshwater macrophytes. Aquat. Bot. 41, 195–224.

Lud, D., Buma, A.G., Van De Poll, W., Moerdijk, T.C., Huisjes, A., 2001. DNA damage and photosynthetic performance in the Antarctic terrestrial alga *Prasiola crispa* ssp. *antarctica* (Chlorophyta) under manipulated UVB radiation. J. Phycol. 37, 459–467.

Merritt, R.W., Cummins, K.W., Berg, M.B., 2008. An Introduction to the Aquatic Insects of North America, 4th. Kendall/Hant publishing company.

Necchi, O. Jr., Branco, L.H.Z., Branco, C.C.Z., 1995. Comparison of three techniques for estimating periphyton abundance in bedrock streams. Arch. für Hydrobiol. 134, 393–402.

Ramírez-Rodríguez, R., Carmona, J., 2005. Taxonomy and distribution of freshwater *Prasiola* (Prasiolales, Chlorophyta) in central México. Cryptogam. Algol. 26, 1–12.

Rindi, F., Guiry, M.D., 2004. Composition and spatial variability of terrestrial algae assemblages occurring at the bases of urban walls in Europe. Phycologia 43, 225–235.

Rozema, J., Björn, O., Bornman, J.F., Gaberščik, A., Häder, D.P., Trošt, T., Germ, M., Klisch, M., Gröniger, A., Sinha, R.P., Lebert, M., He, Y.Y., Buffoni-Hall, R., Bakker, N.V., Van De Staatt, J., Meijkamp, B.B., 2002. The role of UV-B radiation in aquatic and terrestrial ecosystems an experimental and functional analysis of the evolution of UV-absorbing compounds. J. Photochem. Photobiol. 66, 2–12.

Sabater, S., Muñoz, I., 2000. *Nostoc verrucosum* (Cyanobacteria) colonized by a Chironomid larva in a Mediterranean stream. J. Phycol. 36, 59–61.

Sheath, R.G., Cole, K.M., 1992. Biogeography of stream macroalgae in North America. J. Phycol. 28, 448–460.

Thorp, J.H., Covich, A.P., 2010. Ecology and Classification of North America Freshwater Invertebrates, third edition. Academic Press, Italy.

Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R., Cushing, C.E., 1980. The river continuum concept. Can. J. Fish. Aquat. Sci. 37 (1), 130–137.

Walker, P.D., Wijnhoven, S., Van der Velde, G., 2013. Macrophyte presence and growth form influence macroinvertebrate community structure. Aquat. Bot. 104, 80–87.

Ward, A.K., Dahm, C.N., Cummins, K.W., 1985. *Nostoc* (Cyanophyta) productivity in Oregon stream ecosystems: invertebrate influence and differences between morphological types. J. Phycol. 21, 223–227.

Watson, W.G., Rose, F.L., 1985. Influences of aquatic macrophytes on invertebrate community structure, guild structure, and microdistribution in streams. Hydrobiologia 128, 45–56.

Wellnitz, T.A., Ward, J.V., 2000. Herbivory and irradiance shape periphytic architecture in a Swiss alpine stream. Limnol. Oceanograph. 45 (1), 64–75.

Wohl, D.L., Wallace, B., Meyer, J.L n., 1995. Benthic macroinvertebrates community structure, function and production with respect to habitat type, reach and drainage basin in the southern Appalachians (USA). Freshw. Biol. 34 (3), 447–464.