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ECOLOGY, BEHAVIOR AND BIONOMICS

Associations Between Macroinvertebrates and *Paralemanea mexicana*, an Endemic Freshwater Red Alga from a Mountain River in Central Mexico

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Abstract
Macrophytes are common inhabitants of lotic environments and, depending on their morphological traits, possess adaptations that provide shelter to aquatic invertebrates against strong river flow and predators. They may also be used as a food source by macroinvertebrates. The main goal of this study was to determine the relationship between the red alga *Paralemanea mexicana* and its role as a shelter and/or food source for lotic macroinvertebrates. We also conducted research on the role of microhabitat and morphological variations of the alga in determining macroinvertebrate taxon abundance, diversity, and functional group composition in a high-current velocity river. Results showed that changes in cover and morphology of *P. mexicana* were mostly correlated with river current velocity, irradiance, and seasonal variation. In turn, these were related to changes in abundance and diversity of the associated macroinvertebrate community. In addition, six macroinvertebrate functional feeding groups were evaluated for associations with the red alga: filtering and gathering collectors, piercers, scrapers, herbivore shredders, and predators. The results showed that the Trichoptera Hydroptilidae genera *Ochrotrichia* and *Metrichia* use *P. mexicana* as a food source and case-building material. The Trichoptera Glossosomatidae *Mortoniella* uses the alga as a substrate. The biotic interactions between *P. mexicana* and associated macroinvertebrates reveal the importance of macrophytes as purveyors of substrate, as food and shelter for macroinvertebrates, and also as promoters of macroinvertebrate community diversity. In addition, it was shown that macroinvertebrate herbivory likely facilitates vegetative propagation of the red alga through increased release and germination of carpospores and new gametophytes.

Introduction

The phenotypic expression of many benthic organisms inhabiting lotic environments prevents their dislodgement by the current, either holding a specific spatial position or allowing for movement in the water current (Merritt *et al* 2008). As such, several adaptations can be noted like the morphological traits of lotic macrophytes (Cattaneo *et al* 1998, Cheruvelil *et al* 2002, Declerck *et al* 2011). Likewise, macroinvertebrates that inhabit this environment possess a set of adaptations to deal with it, such as a hydrodynamic body shape, the presence of anchoring structures, as well as life cycles capable to cope with daily or seasonal environmental changes (Voshell 2010). Frequently, lotic benthic macroinvertebrates make use of macrophytes as providers of refuge from the water current, reducing the risk of dislodgement, and as a food source. The latter can be associated with habitat selection patterns of macroinvertebrates. Other factors, such as water temperature, solar radiation, and substrate composition, also have a direct effect on this behavior (Hershey *et al* 2010, Voshell 2010). The relationship between substrate type and the structure and diversity of benthic macroinvertebrate communities is determined in part by the mineral or organic substrate composition or both (Dewalt *et al* 2010, Reid *et al* 2010). The organic substrate interacts physically and biologically with aquatic macroinvertebrates, while the inorganic substrate interacts physically and chemically with them (Reid *et al* 2010). In general, population dynamics of macroinvertebrate communities are related to local environmental factors (Cattaneo *et al* 1998, Cheruvelil *et al* 2002). Interactions between macrophytic algae populations and macroinvertebrates may be of great importance to promote habitat heterogeneity, resulting in complex relationships between them since macroinvertebrates spend most of their lives attached to the substrate (Dewalt *et al* 2010).

Macrophyte communities represent one of the most widely distributed and structurally complex organic substrates in rivers. These communities can increase habitat heterogeneity by modifying some of the physical traits of lotic ecosystems, such as current velocity, substrate composition, and detritus accumulation (Dudley *et al* 1986, Alonso & Camargo 2010). Macrophytes can support filtering and gathering collector functional groups of macroinvertebrates by slowing the movement of and trapping fine organic and inorganic particles transported in the water current (Dudley *et al* 1986, Walker *et al* 2013). Some benefits to the algae can be perceived as a result of these interactions: greater growth and uptake of more nutrients in response to moderate macroinvertebrate herbivory, reduction in self-shading from sunlight as a consequence of macroinvertebrates scraping off the top layer of algae cells (Dewalt *et al* 2010, Voshell 2014), and the herbivory by grazers that facilitate nutrient regeneration and removal of older, less productive algae tissue (Lodge 1991). However, sometimes these interactions can have negative effects; for example, Dudley *et al* (1986) observed that algae and sedentary macroinvertebrates can compete for space.

Symbiotic relationships between algae and macroinvertebrates have been well documented (Hershey *et al* 2010). For example, *Nostoc parmelioides* Kützing (Cyanobacteria) and *Cricotopus nostocicola* Wirth (Chironomidae) share a relationship in which the feeding process of the chironomid midge alters the alga colony's morphology, increasing the photosynthetic rate (Ward & Cummins 1978, Dewalt *et al* 2010). Another example is the symbiosis between *Lemanea australis* Atkinson (Rhodophyceae) and *Dibusa angata* Ross (Trichoptera), in which the caddisfly uses the red alga as a food and case-building source (Resh & Houp 1986).

Lotic freshwater red algae grow under very specific conditions, and in general, they lack adaptations such as resting or dispersal cells that would allow them to spread over large ranges (Sheath & Hambrook 1990). This can limit their role as abundant and frequent taxa in a region (*op. cit.*). Taking into account the empirical evidence described in the literature, we assume that when the local environmental conditions allow the establishment of any given algae species, it usually forms conspicuous and abundant growths and significantly contributes to habitat heterogeneity, serving also as a macroinvertebrate food source and/or shelter.

The main goal of this study was to determine if a positive relationship exists between the morphological traits of the endemic red alga *Paralemanea mexicana* (Kützing) Vis *et* Sheath and the macroinvertebrates that use it for food and/or shelter, considering the influence of seasonal variation. This study was carried out using two measurement procedures in a fast-flowing river: (a) microhabitat characterization and morphological variation of the alga and (b) assessment of richness, diversity, and functional groups of macroinvertebrates associated with the alga.

Materials and Methods

Paralemanea mexicana

The genus *Paralemanea* is characterized by a pseudoparenchymatous bambusiform thallus morphology that has a multiseriate filament medulla and a pattern of false and true branches (Sheath *et al* 1996a). It has a heteromorphic life cycle that includes a visible gametomorphic phase. When fertilization occurs, the gametes form a diploid cell that multiplies to produce carpospores. These carpospores germinate into a diploid *Chantransia* stage, which produces a new gametophyte through somatic meiosis (Carmona & Necchi 2002). In *P. mexicana*, the gametophyte may be present year around and, as such, have abundant populations during winter. The gametophyte also shows structural and reproductive changes associated with seasonal variation (Carmona *et al* 2014). These traits suggest that the gametophyte is a potential substrate and food source for benthic macroinvertebrates (Hambrook & Sheath 1987, Hershey *et al* 2010, Walker *et al* 2013). *Paralemanea mexicana* is restricted to mountain rivers in Central Mexico with certain microhabitat conditions (Carmona & Necchi 2002, Bojorge *et al* 2010).

Sample collection

Samples were collected from a segment of the mountainous (1890 m altitude) fifth-order Amanalco River in Central

Mexico (19°13′N, 100°07′W). Fieldwork was conducted from Nov. 2007 to Nov. 2008, which included the most contrasting seasonal environmental cycles (García 2004): the rainy, cool dry, and dry seasons. Three sampling stints were conducted in the dry season: cold early (Dec.), middle (Feb.), and warmer end (May). Three more sampling stints were conducted in the middle (Oct. and Nov.) of the rainy season. Five samples were collected during each season, gathered through an ecological transect. Water temperature and specific conductance were measured using a Conductronic™ PC-18 conductivity meter (Puebla, Mexico). Discharge (Q_3, $m^3\ s^{-1}$) was calculated according to Gore (1996). Dissolved nutrients were measured by in situ filtering using 0.45- and 0.22-μm-pore diameter membranes, and later the sample was preserved in chloroform and frozen, in order to avoid bacteriological activity in the water sample. Nutrients were measured in the laboratory with a multichannel analyzer, following standard titration. Water samples for dissolved inorganic nitrogen (DIN) and soluble reactive phosphorous (SRP) were kept cold until they were analyzed (APHA 2005).

Microhabitat characterization

Observations were made on natural boulder substrates directly on the river bed. Variations over time were monitored using a quadrat technique (Necchi *et al* 1995), which evaluates the influence of current velocity, depth, and underwater irradiance at the microhabitat level on the vegetative and reproductive characteristics of the algal population. The sampling site consisted of a 58-m^2 river segment. Each sample unit covered a 10-cm radius circle (area = 314.16 cm^2). The type and size of the sampling units were chosen based on preliminary tests and previous research (Carmona *et al* 2006). Sampling consisted of five quadrats, each 2 m apart from the other. Quadrats were positioned within each site on areas with over 5% of alga cover. Their direction and localization was chosen randomly in an interval between 0° and 180°. This procedure was repeated along the sampling quadrats (in an upstream direction). Microhabitat characteristics were recorded on all sampling dates. The microhabitat variables were measured in situ, approximately in the middle of each sampling unit center with five replicates of each sampling unit. Current velocity and irradiance were measured as close as possible to the algae using 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. The variation coefficient (VC) was used to determine whether environmental parameters were consistent (VC = 5–10%) or variable. Variability in time and space usually results from dilution/evaporation processes and biological activity (Magurran 2004). A descriptor for each sample site was calculated as $VC = SE / a \times 100$ (SE = standard error and a = mean).

Paralemanea morphological traits and macroinvertebrate assemblages

The number of thallus (gametophytes) within each sampling unit was recorded by visual estimation using a 175 cm^2 viewfinder. Twenty-five thalli were randomly selected to measure thallus height, number of primary branches, number of branches by node, number of true and false branches, number of *Chantransia*-stage associates to the gametophyte surface, and number of associated algae species. These characteristics were chosen based on preliminary tests and previous research (Carmona & Necchi 2002). The numbers and taxa of macroinvertebrates were measured for each thallus. Macroinvertebrate taxonomy was based on Merritt *et al* (2008), Bueno-Soria (2010), and Thorp & Covich (2010). Macroinvertebrate functional groups were assigned according to Cummins *et al* (2005) classification, taking into account the characterization of morphological traits that determine the food source chosen by macroinvertebrates. Microscopic observations were carried out using an Olympus™ BX51 and SZX7 microscopes with DP12 and E-330 microphotography systems.

Data analysis

Species diversity was assessed using the Shannon–Wiener diversity test ($H\ \log_{10}$). The Kruskal–Wallis tests were performed according to the data distribution to assess significant differences in environmental and morphological measurements among the six sampling dates. When these tests were significant, the Mann–Whitney test was conducted among all dates to detect which differed significantly ($P \leq 0.05$). Associations between morphometric and reproductive data, microhabitat variables, and total abundance of macroinvertebrate taxa during the six sampling dates were assessed using Spearman correlation coefficient (Gotelli & Elison 2004). The analyses were conducted using SPSS 18 software (Levesque 2006). We selected the microhabitat parameters and the algae's morphological traits based on Spearman correlation coefficient and the principal component analyses (PCA) ($P \leq 0.05$) in order to choose the most significant variables. Relationships between temporal morphological traits of *P. mexicana* and macroinvertebrate-associated taxa and physical parameters were explored through a canonical correspondence analysis (CCA), followed by Monte Carlo tests (999 permutations, $P \leq 0.05$). These analyses were performed using XLSTAT software (Addinsoft 2013).

Results

Environmental characteristics and morphological traits of P. mexicana

During the six sampling periods carried out in a year, the river water showed relatively low ion content, near-neutral pH, mild temperatures, and high dissolved oxygen content (Table 1). These physical characteristics were consistent during all sampling periods (VC < 10%). The rest of the physical and chemical variables, such as specific conductance, discharge, SRP, and DIN, exhibited larger variations (VC = 51–65%) with increased values observed during cool and warm dry seasons. Gametophytes occurred predominantly under microhabitat conditions of fast current velocity (minimum and maximum = 69–156 cm s^{-1}), low-to-medium irradiance (minimum and maximum = 13–744 μmol photons m^{-2} s^{-1}), and shallow depth (minimum and maximum = 11–20 cm) and on boulders as a substrate (Table 1). The Kruskal–Wallis test showed significant differences between current velocity ($H = 12$; $P = 0.04$) and irradiance ($H = 24$; $P = 0.00$) among sampling dates.

Gametophytes were present throughout the seasons and covered a mean of 7.4–34% of the thallus. Significant differences in abundance were observed ($H = 15$; $P = 0.01$). The highest percentage coverage of gametophytes on boulders occurred in the cool dry season, and the lowest was in the rainy season (Table 2). There were significant differences among sampling dates for all *P. mexicana*-evaluated morphological traits (Fig 1). Thallus height changed significantly throughout the study ($U = 92$–210, $P = 0.000$–0.045). The greatest thallus heights were recorded during the cool dry season, and the shortest were observed in the rainy season. Significant differences were observed in the true and false branches between seasons ($U = 122$–202; $P = 0.001$–0.024). The number of first-order and true branches was significantly greater during two of the three rainy seasons than in the dry season ($U = 146$–203; $P = 0.001$–0.028). The dry season exhibited more false and whorled branches compared to the rainy and cool dry seasons ($U = 122$–198; $P = 0.000$–0.021).

Macroinvertebrate richness, diversity, and functional groups

Paralemanea mexicana was associated with abundant aquatic larvae and pupae. Eleven taxa of macroinvertebrates found in the sampling sites showed low diversity values during all seasons (Table 2). In terms of total abundance, the largest density of macroinvertebrates was recorded in the dry season. The Orthocladiinae chironomid subfamily was found almost exclusively in the dry season. In contrast, the Tanypodinae chironomid subfamily was at peak abundance just prior to and throughout the rainy season. Two micro-caddisfly genera of the family Hydroptilidae, *Ochrotrichia* and *Metrichia*, were found, but they were less abundant than the chironomid midges. These caddisfly larvae

Table 1 Physical and chemical characteristics of the Amanalco River.

	Rainy (27 Oct. 2007)	Cool dry (5 Dec. 2007)	Cool dry (8 Feb. 2008)	Dry (8 May 2008)	Rainy (3 Sep. 2008)	Rainy (20 Nov. 2008)	Variation coefficient (%)[a]
Temperature (°C)	16	14	15	18	17	14	10
pH	6.8	7.5	8	7	6.7	7.3	6
K_{25} (μs cm^{-1})	175	197	248	244	165	168	19
Q_3 (m^3 s^{-1})	3.5	2.9	2.3	2.6	2.9	2.3	16
Dissolved oxygen saturation (%)	100	97	94	91	92	93	4
SRP (mg l-1)	0.82	0.84	0.58	0.66	0.1	0.1	65
DIN (mg l-1)	2.10	0.53	2.76	1.0	1.27	1.7	51
CV (cm s^{-1})	89 ± 21	94 ± 65	70 ± 38	69 ± .33	132 ± 39	156 ± 69	
Irradiance (μmol photons m^{-2} s^{-1})	14 ± 5.4	40 ± 6.5	347 ± 428	744 ± 125	256 ± 86	13 ± 2.3	
Depth (cm)	11 ± 10.5	17 ± 5.5	19 ± 11.5	20 ± 4	15 ± 8	19 ± 7.5	
Number and type[b] of associated algae species	0.6 ± 0.5 Pl, Sc	0.8 ± 0.5 Pl, Oe, St	1.0 ± 0.3 Pl, Sc, Ch, St, M	2.3 ± 0.9 Pl, Sc, Ch, Oe, Cl, Va, M,	0.3 ± 0.6 Pl, Sc	1.3 ± 0.5 Pl, Sc, Oe, Cl,	

Values are given in milligrams per liter except where indicated. K_{25} = specific conductivity, Q_3 = discharge.

SRP soluble reactive phosphorous, *DIN* dissolved inorganic nitrogen, *CV* current velocity.

[a] Magurran (2004): conservative (VC < 10%) and not conservative (VC > 10%) variables (mean ± 1 SE; $n = 25$).

[b] Cyanobacteria: *Placoma* sp. (Pl); Rhodophyta: *Sirodotia suecica* Kylin (Ss) and *Chantransia* stage (Ch); Chlorophyta: *Oedogonium* sp. (Oe), *Stigeoclonium* sp. (St), and *Cladophora* sp. (Cl); Heterokontophyta: *Vaucheria* sp. (Va) and mosses (M).

Table 2 Total abundance, richness, diversity, and functional feeding groups of macroinvertebrate taxa associated to *Paralemanea mexicana* in the Amanalco River.

	Rainy (27 Oct. 2007)	Cool dry (5 Dec. 2007)	Cool dry (8 Feb. 2008)	Dry (8 May 2008)	Rainy (3 Sep. 2008)	Rainy (20 Nov. 2008)
Taxa and functional groups in the sample (total abundance of individuals per group)	Tanypodinae-P(26) *Simulium*-FC(16) *Ochrotrichia*-PC(2) *Metrichia*-PC(2) *Lara*-DSH(2) Orthocladiinae-GC(1) *Baetis*-GC(1) *Mortoniella*-SC(1)	Orthocladiinae-GC(179) *Metrichia*-PC(19) *Simulium*-FC(16) Acari-GC/PC(1) *Ochrotrichia*-PC(1)	Orthocladiinae-GC(187) *Mortoniella*-SC(9) *Metrichia*-PC(7) *Ochrotrichia*-PC(6) *Lara*-DSH(3) Acari-GC/PC(1)	Orthocladiinae-GC(340) Tanypodinae-P(111) *Ochrotrichia*-PC(96) *Mortoniella*-SC(89) *Metrichia*-PC(42) Acari-GC/PC(5) *Simulium*-FC(5) *Lara*-DSH(3) *Baetis*-GC(2) *Cleptelmis*-HSH(1) *Phanocerus*-HSH(1)	Tanypodinae-P(93) *Metrichia*-PC(12) Orthocladiinae-GC(4) *Mortoniella*-SC(2) *Simulium*-FC(2) *Phanocerus*-HSH(1)	Tanypodinae-P(293) Orthocladiinae-GC(20) *Simulium*-FC(20) *Ochrotrichia*-PC(4) *Metrichia*-PC(1) Acari-GC/PC(1) *Baetis*-GC(1)
Dominance of macroinvertebrate taxa and functional feeding group	Tanypodinae (P)	Orthocladiinae (GC)	Orthocladiinae (GC)	Orthocladiinae (GC)	Tanypodinae (P)	Tanypodinae (P)
Total abundance	51	216	213	695	114	340
Taxa diversity (Shannon–Wiener Index)	0.44	0.23	0.16	0.43	0.24	0.2

Functional feeding groups follows the Cummins *et al* (2005) classification: gatherers–collectors (GC), piercer (PC), scrapers (SC), filterers–collectors (FC), predators (P), and herbivores–shredders (HSH).

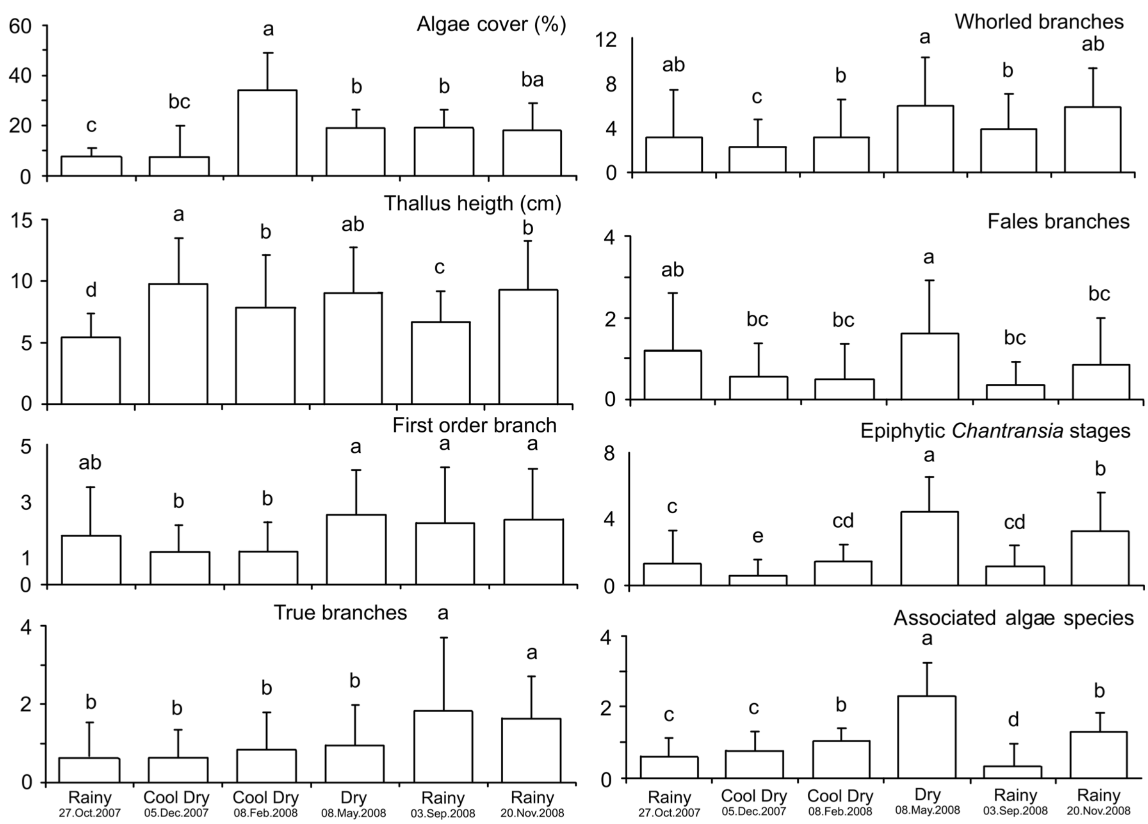

Fig 1 Morphological traits measured for seasonal samples of *Paralemanea mexicana* in the Amanalco River. Sampling dates indicated with the *same letter* do not significantly differ (Mann–Whitney test, mean ± 1 SE; $n = 25$).

are specialized to feed by piercing algal cells and sucking out the contents. The greatest micro-caddisfly abundance was during the dry season. All the taxa identified were found at every sampling station with the exception of the riffle beetle *Phanocerus* (Elmidae) for which only one individual was collected.

The diversity index values, in general, were low, between 0.44 and 0.16, and do not show a strong relationship with seasonal variation. The highest values correspond to the Oct. 2007 rainy season with a total abundance of 51 organisms belonging to eight taxa and to the May 2008 dry season with a total abundance of 695 organisms belonging to 11 taxa. Conversely, the lowest values correspond to the Feb. 2008 cool dry season represented by six taxa, but most of the samples was dominated by organisms belonging to Orthocladiinae subfamily. The other two rainy seasons (Sep. and Nov. 2008) were represented by five and six taxa, respectively, and dominated by the Orthocladiinae subfamily as well (Table 2).

Some macroinvertebrates that have specific feeding habits can find an optimal habitat in *P. mexicana*. For example, the piercer caddisfly genera *Ochrotrichia* and *Metrichia* as well as the Orthocladiinae chironomid subfamily larvae that were found adhered to thallus surface of the alga. The greatest abundance of the caddisflies was associated with the highest density of associated moss. The presence of moss correlated with silk cases built by the caddisflies by weaving it together with *P. mexicana* and diatoms. *Metrichia*'s cases were found attached to the alga thallus in a dorsal-ventral position, similar to that observed for the Glossosomatidae caddisfly genus *Mortoniella*. The cases of *Orchrotrichia* were found adhered to the algal thallus hanging from a peduncle. When observed under a microscope, the cases had more than one peduncle even if they were only attached to the thallus by a single one. The formation of refuges did not represent the predominant form of the alga or plant tissue. The Orthocladiinae chironomids built cases adhered to *P. mexicana* with some also found within *Sirodotia suecica* filaments. Black flies in the genus *Simulium* were frequently found in the samples but were few in numbers. Genera of the Elmidae beetle family were rare, mostly represented by the xylophage (wood-eating) genus *Lara*.

The specific traits observed for the alga substrate were related to the different macroinvertebrate functional feeding groups (FFGs) identified in this study. Four dominant FFGs were found in the samples. In the order of the highest abundance, these were gatherers–collectors, predators, piercers, and filterers–collectors. Piercers, represented by two microtrichopteran genera, were abundant during the dry season and absent during the rainy season.

Red alga morphological traits and macroinvertebrate assemblages

Some significant correlations were found between alga morphological traits, microhabitat conditions, and macroinvertebrate taxa (Table 3). During the rainy season, the taxa positively correlated with *P. mexicana* morphological traits were *Metrichia* with alga cover ($\rho = 0.894$), *Simulium* with thallus height ($\rho = 0.894$), and Tanypodinae with associated algae species ($\rho = 0.90$). Negative correlations were found between *Simulium* and first-order and false branches of the alga as well as associated algae species ($\rho = -0.894$) and with Tanypodinae ($\rho = -0.90$). During the dry season, the positive correlations between macroinvertebrate taxa and alga morphological traits were first-order branches with *Metrichia* ($\rho = 0.947$) and thallus height with *Mortoniella* ($\rho = 0.894$). A negative correlation was found between algal whorled branch pattern and the genera *Mortoniella* and *Ochrotrichia* ($\rho = -0.90$ for both correlations). During the rainy season, Orthocladiinae and *Ochrotrichia* were found to be positively correlated ($\rho = 1.00$), but Tanypodinae and *Simulium* were negatively correlated ($\rho = -0.889$).

The total variances in the PCA explained the 90% of total variation in the first two axes. The first axis (78%) was corresponded to true branches, associated algae species, and the number of whorled branches. The second axis (12%) was represented by the thallus height, false branches, and the number of *Chantransia* stages. According to the CCA test (Fig 2), irradiance and current velocity had a significant influence on the abundance of the associated macroinvertebrates, where 99% of the total variation was explained by the first two axes ($P \leq 0.05$). The first axis (88%) shows a positive relationship between irradiance (0.916), total abundance of macroinvertebrates (0.314), and the number of associated algae species (0.257), in the cool dry (0.636) and dry (1.389) seasons. The second axis (11%) was positively associated with current velocity (0.407) in the two rainy seasons (0.826 and 0.677, respectively).

Discussion

We found that the thallus of *P. mexicana* favors microhabitat conditions typical of mountain rivers with low incident radiation, firm substrates, and high-speed current velocities. These traits are related to a series of morphological adaptations that are specific of algae species that thrive in rivers with the highest current velocities an alga can tolerate (Sheath & Hambrook 1990). A ridged thallus, multiple aggregated gametophytes that develop from a rhizoidal system, and a wide-branching pattern throughout the thallus (Carmona *et al* 2014) make *P. mexicana* a stable habitat for

Table 3 Spearman correlation coefficient between the morphological traits of *Paralemanea mexicana*, microhabitat variables, and macroinvertebrate taxa in the Amanalco River.

Variable	Taxa	Rainy (27 Oct. 2007)	Cool dry (5 Dec. 2007)	Cool dry (8 Feb. 2008)	Dry (8 May 2008)	Rainy (3 Sep. 2008)	Rainy (20 Nov. 2008)
Current velocity (cm s^{-1})	Orthocladiinae			−0.9			
	Tanypodinae						−0.975[a]
Irradiance (μmol photons m^{-2} s^{-1})	Orthocladiinae		0.9[a]				
	Simulium						0.894[a]
	Tanypodinae						−0.9[a]
Algal cover (%)	Tanypodinae	−0.918[a]					
	Metrichia					0.894[a]	
Total abundance of macroinvertebrates	Orthocladiinae			0.9[a]			
	Metrichia					−0.918[a]	
	Tanypodinae						1[b]
Seasonally	Orthocladiinae			0.9[a]			
	Mortoniella				0.9[a]		
	Tanypodinae						−1[b]
Thallus height	*Mortoniella*			0.894[a]			
	Simulium						0.894[a]
First-order branches	*Metrichia*				0.947[a]		
	Simulium						−0.894[a]
Whorled branches	*Mortoniella*				−0.9[a]		
	Ochrotrichia				−0.9[a]		
	Tanypodinae						0.975[a]
False branches	*Simulium*						−0.894[a]
	Tanypodinae						−0.9[a]
Associated algae species	*Simulium*				1[b]	−0.889[a]	
	Orthocladiinae					−0.892[a]	
	Tanypodinae						0.9[a]
Orthocladiinae	*Ochrotrichia*	1[b]					
Tanypodinae	*Simulium*					−0.889[a]	

[a] Correlation is significant at the 0.05 level.

[b] Correlation is significant at the 0.001 level.

macroinvertebrates and other algae. Therefore, *P. mexicana* increases the habitat heterogeneity of a river segment where it grows and can have a significant effect on the distribution of local macroinvertebrate and algae taxa.

Seasonal changes in the abundance and branching pattern of this red alga were related to habitat preferences of macroinvertebrates, predominantly caddisflies and chironomid midges, as reported in similar previous studies (Resh & Houp 1986, Sheath *et al* 1996b, Carmona *et al* 2009). The drag and damage that current velocity can cause to macroinvertebrates likely leads to evolutionary adaptations in body shape as well as feeding and movement patterns (Hershey *et al* 2010). In fact, hydrodynamic forces interact strongly with substrate type in modeling habitat conditions, and at the local scale, they represent the most important factors in determining the type and abundance of macroinvertebrates (Dewalt *et al* 2010). Therefore, the protective morphology of *P. mexicana* makes it a highly desirable substrate, offering shelter from the physical effects of high riverine current velocities.

Oligotrophic-to-eutrophic conditions were recorded year around in the Amanalco River (according to Dodds 2003: DIN 0.02–2 mg L^{-1} and SRP 0.2–0.8 mg L^{-1}). Changes in DIN were highly variable, probably due to upstream agricultural water withdrawals and releases. According to Vannote *et al* (1980) and Bowman *et al* (2005), moderate nutrient enrichment and light input can increase the abundance of algae and macroinvertebrates in a river. However, when nutrient enrichment is excessive, the opposite effect can happen and the abundance of sensitive organism decreases (Merritt *et al* 2008). The effect of moderate nutrient enrichment was confirmed in this study during different seasons. For example, the DIN showed an increase from 0.5 mg L^{-1} in the cool dry season to 2 mg L^{-1} in the rainy season, and the highest macroinvertebrate diversity (0.44) was associated with intermediate values of DIN; when nitrogen concentrations were lowest (0.5 mg L^{-1}) and highest (3 mg L^{-1}), the macroinvertebrate diversity decreased to almost half (0.23 and 0.16, respectively).

In the cool and dry seasons, gatherers–collectors and scrapers were the dominant macroinvertebrates. In the wet season, a significant portion of filterers–collectors and predators were observed, probably because an alga and prey food source became available. It is not uncommon to observe an increase in the abundance of filterer–collector macroinvertebrates in lotic environments (Merritt *et al* 2008). *Paralemanea mexicana* abundance was also related to a possible preference of micro-caddisflies for this alga when the number of its first-order and whorled branches increased. The latter could be explained due to the presence of these branches in the outer cortex which is less thick/packed than the inner cortex. Additionally, the juvenile branches in the *P. mexicana* thallus are less corticated than the older branches (Carmona & Necchi 2002), which could favor the perforation of the thallus by piercing macroinvertebrates (Lodge 1991).

The CCA results shows that a significant positive correlation exists between the branch arrangement and the number of associated algae species (including Cyanobacteria, Chlorophyceae, and other red algae) that could explain the higher occurrence and abundance of macroinvertebrates. Thallus that is more branched becomes a richer food source and diversity of microhabitats that support macroinvertebrate assemblages (Walker *et al* 2013). On the other hand, high irradiance values proved to be a unique microhabitat parameter allowing the highest growth in some algae groups during the dry and cool seasons. In fact, during these seasons, the diversity and abundance of microinvertebrates with piercing-herbivore feeding preferences was the highest, as well as for the Hydroptilidae family which is reported in association with algae nearly always (Alonso & Camargo 2010).

The presence of *P. mexicana* and particularly the differences in its morphological traits provide supporting evidence of its role as a facilitator of diverse macroinvertebrate communities and associated algae by providing refuge and nutritional sources, under stressful environmental conditions such as those of high-current velocity rivers (Alonso & Camargo 2010, Walker *et al* 2013). The CCA results were concordant with the positive Spearman correlations between the red algae and certain macroinvertebrates, mostly micro-caddisflies. In particular, thallus height and first-order branches encourage the settlement of macroinvertebrates that build portable cases. The negative correlations of red algal false branches with the presence of *Mortoniella* and *Ochrotrichia* could be related to the reduced moving capabilities of the larvae along the thallus.

The results show that the largest extensions of habitat coverage by *P. mexicana* were observed during the cool dry season (34%) and the dry season (19%). Also, the greatest richness and diversity of macroinvertebrates was recorded during the dry season (11 taxa, $H' = 0.44$). As such, these results show that excessive alga habitat cover does not necessarily increase the diversity and abundance of associated

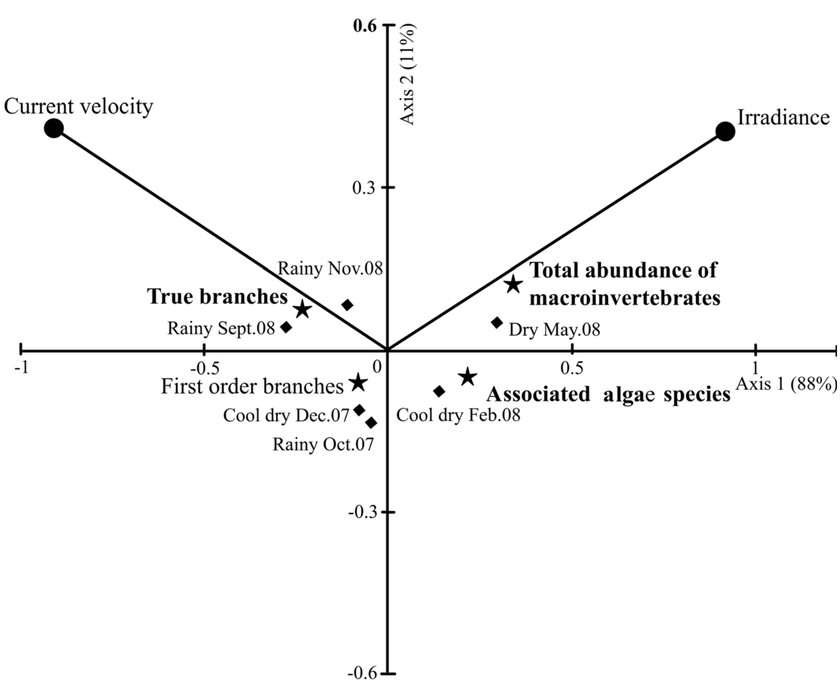

Fig 2 Canonical correspondence analysis biplot of the general distribution of morphological traits of *Paralemanea mexicana* (*star symbols*) and the total abundance of macroinvertebrates with regard to microhabitat variables (*circle symbols*) recorded in the climatic seasons (*rhombus symbols*).

macroinvertebrates, in contrast with other studies (Alonso & Camargo 2010, Walker *et al* 2013). For example, some taxa that feed by scraping rock surfaces or adhere to them, like *Simulium*, are inhibited by filamentous and mucilaginous algae, which may be an indication of competition for space (Dudley *et al* 1986). Thus, an increase in *P. mexicana* habitat cover could limit the abundance of some macroinvertebrates that do not rely on the red alga as a source of food or shelter. In contrast, the greatest abundance of the predaceous Tanypodinae midges was found during the rainy season in combination with reduced red alga habitat cover, suggesting that other interactions may occur, such as active predation, since some tanypod species are known to prey on other macroinvertebrates (Merritt *et al* 2008).

Ruptures in the thallus and a larger number of false branches and filamentous structures of the *Chantransia* stage could have been due to the presence of the *Metrichia* and *Ochrotrichia* hydroptilid caddisflies, owing to increased habitat heterogeneity (Walker *et al* 2013). These genera use fragments of the red alga for case building, similar to other caddisflies with the red alga *Lemanea* (Resh & Houp 1986). Although the caddisflies' behavior damages the gametophyte structure, it proves beneficial to the alga because it favors the release and germination of the carpospores, giving way to the next stages in the alga's life cycle (Carmona *et al* 2014). The increase in filaments of the *Chantransia* stage and thallus of the red alga *S. suecica* might also be an important energy source for macroinvertebrate herbivores (Hambrook & Sheath 1987).

The presence of more than one peduncle on *Ochrotrichia* cases is probably related to different pupation sites along the red alga thallus (Resh & Houp 1986). This trait was also observed in *Mortoniella* cases (which were mostly empty). It might be explained as the result of a search for better environmental conditions, since *Mortoniella* does not tolerate poor-water quality well (Bueno-Soria 2010), or they could represent pupal cases from which adults had emerged. *Ochrotrichia* and *Metrichia* build portable cases up until the fifth instar, which means the red alga promotes larval growth to pupation and adult stages. The greatest abundance of these genera was during the warm dry season, which is their adult emergence period (Bueno-Soria 2010).

The red alga *P. mexicana* displayed a strong structural design, well adapted to withstand the forces of strong river current. A perennial population is promoting local micro-environmental heterogeneity, providing a suitable substrate for macroinvertebrates, and supplying shelter and feeding grounds. Likewise, it was linked to the provision of shelter and materials for the construction of portable cases of the hydroptilid caddisfly genera of piercers, *Ochrotrichia* and *Metrichia*. A similar relationship between *P. mexicana* and the scraper caddisfly *Mortoniella* was observed in which the red alga serves as shelter and provides case material. These relationships were apparent because every case was found adhered to the red alga thallus attached by specialized structures such as peduncles.

The effects of herbivory on *P. mexicana*'s thallus were apparently beneficial for the alga by promoting the release and germination of carpospores. The main type of herbivory to which the alga was subject was consumption of cellular contents rather than structural tissue. However, the alga cover by *P. mexicana* plays a significant role in the establishment of the macroinvertebrate community. An excessive algal growth might promote competition for substrate by the colonization of sedentary species of macroinvertebrates that adhere to rock surfaces, but it would also favor species associated with the alga thallus. This study contributes providing evidence on interactions in aquatic ecosystems, which are rarely documented, but are important because the species or taxa groups involved could be exerting great effects on the structure and productivity of benthic organisms.

Interspecific facilitation is an ecological mechanism that can promote the efficiency of resource use and persistence in stressful conditions in *P. mexicana* and its associated macroinvertebrate assemblages. Nevertheless, these associations may not have been as common in the study area because it is restricted to an isolated geographic region with particular microhabitat conditions. The latter suggests that special morphological traits and interactions promote evolutionary adaptations to successfully compete with other lotic macrophytic species in similar tropical mountain rivers.

Conclusion

Seasonal and environmentally induced changes in habitat cover and morphological traits of *P. mexicana* were related to river current velocity, irradiance, and nutrient concentrations. These in turn were associated with richness and abundance, case building, and feeding habits of 11 benthic macroinvertebrate taxa.

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References

Addinsoft (2013) XLSTAT. Getting started manual. Addinsoft, Paris, p 104

Alonso A, Camargo JA (2010) Comparison of two methods for estimating the abundance, diversity and habitat preference of fluvial macroinvertebrates in contrasting habitats. Limnologica 40:23–29

APHA (American Public Health Association) (2005) Standard methods for examination of water and wastewater, 21st edn. Port City Press, Washington, DC

Bojorge MG, Carmona JJ, Cartajena AM, Beltrán MY (2010) Temporal and spatial distribution of macroalgal communities of mountain streams in Valle de Bravo Basin, Central México. Hydrobiologia 641: 159–169

Bowman MF, Chambers PA, Schindler DW (2005) Changes in stoichiometric constraints on epilithon and benthic macroinvertebrates in response to slight nutrient enrichment of mountain rivers. Freshwater Biology 50: doi: 10.1111/j.1365-2427.2005.01454.x

Bueno-Soria J (2010) Guía de identificación ilustrada de los géneros de larvas de 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 Mexico, México City, 228

Carmona JJ, Necchi O Jr (2002) Taxonomy and distribution of *Paralemanea* (Lemaneaceae, Rhodophyta) in Central Mexico. Cryptogam Algol 23:39–49

Carmona JJ, Montejano ZG, Necchi O Jr (2006) The ecology and morphological characterization of gametophyte and Chantransia stage of *Sirodotia huillensis* (Batrachospermales, Rhodophyta) from a river in Central Mexico. Phycol Res 54:108–115

Carmona JJ, Bojorge M, Beltrán Y, Ramírez R (2009) Phenology of *Sirodotia suecica* (Batrachospermaceae, Rhodophyta) in a high altitude stream in Central Mexico. Phycol Res 57:118–126

Carmona JJ, Bojorge G, Ramírez R (2014) Phenology of *Paralemanea mexicana* (Batrachospermales, Rhodophyta) in a high-altitude stream in Central Mexico. Phycol Res 62:86–93

Cattaneo A, Galanti G, Gentinetta S, Romo S (1998) Epiphytic algae and macroinvertebrates on submerged and floating-leaved macrophytes in an Italian lake. Freshw Biol 39:725–740

Cheruvelil KS, Soranno PA, Madsen JD, Robertson MJ (2002) Plant architecture and epiphytic macroinvertebrate communities: the role of an exotic dissected macrophyte. J N Am Benthol Soc 21:261–277

Cummins KW, Merritt RW, Andrade PCN (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

Declerck SAJ, Bakker ES, Lith BR, Kersbergen A, Donk EV (2011) Effects of nutrient additions and macrophyte composition on invertebrate community assembly and diversity in experimental ponds. Basic Appl Ecol 12:466–475

Dewalt RE, Resh VH, Hilsenhoff LW (2010) Diversity and classification of insects and Collembola. In: Thorp JH, Covich AP (eds) Ecology and classification of North America freshwater invertebrates, Thirdth edn. Academic, Italy, p 1021

Dodds WK (2003) Misuse of inorganic N and soluble reactive P concentrations to indicate nutrient status of surface waters. J N Am Benthol Soc 22:171–181

Dudley TL, Cooper SD, Hemphill N (1986) Effects of macroalgae on a stream invertebrate community. J N Am Benthol Soc 5(2):93–106

García E (2004) Modificaciones al sistema de clasificación climática de Koppen. Instituto de Geografía, UNAM, México, p 246

Gore JA (1996) Discharge measurements and stream flow analysis. In: Methods in streams ecology (Ed FR Hauer, GA). Academic, San Francisco, pp 51–78

Gotelli NJ, Elison AM (2004) A primer of ecological statistics. Sinauer Associates, Inc., Sunderland, p 510

Hambrook JA, Sheath RG (1987) Grazing of freshwater Rhodophyta. J Phycol 23:656–662

Hershey AE, Lamberti GA, Northington RM (2010) Chapter 17: aquatic insect ecology. In: Thorp JH, Covich AP (eds) Ecology and classification of North America freshwater invertebrates, Thirdth edn. Academic, Italy, p 1021

Levesque R (2006) SPSS programming and data management, a guide for SPSS and SAS users, 3rd edn. Raynald Levesque and SPSS Inc, Chicago, p 522

Lodge DM (1991) Herbivory on freshwater macrophytes. Aquat Bot 41: 195–224

Magurran AE (2004) Measuring biological diversity. Blackwell, Malden, p 256

Merritt RW, Cummins KW, Berg MB (2008) An introduction to the aquatic insects of North America (4th ed.). Kendall/Hant, 1158

Necchi O Jr, Branco LHZ, Branco CCZ (1995) Comparison of three techniques for estimating periphyton abundance in bedrock streams. Archiv für Hydrobiol 134:393–402

Reid HE, Brierley GJ, Boothroyd IKG (2010) Influence of bed heterogeneity and habitat type on macroinvertebrates uptake in peri-urban streams. Int J Sediment Res 25:203–220

Resh VH, Houp RE (1986) Life history of the caddisfly *Dibusa angata* and its association with the res alga *Lemanea australis*. J N Am Benthol Soc 5(1):28–40

Sheath RG, Hambrook JA (1990) Freshwater ecology. In: Cole KM, Sheath RG (eds) Biology of the red algae. Cambridge University Press, Cambridge, pp 423–453

Sheath RG, Müller KM, Vis ML, Entwisle TJ (1996a) A re-examination of the morphology, ultrastructure and classification of genera in the Lemaneaceae (Batrachospermales, Rhodophyta). Phycol Res 4:233–246

Sheath RG, Müller KM, Colbo MH, Cole KM (1996b) Incorporation of freshwater Rhodophyta into the cases of chironomid larvae (Chironomidae, Diptera) from North America. J Phycol 32:949–952

Thorp JH, Covich AP (eds) (2010) Ecology and classification of North America freshwater invertebrates, Third editionth edn. Academic, Italy, p 1021

Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE (1980) The river continuum concept. Can J Fish Aquat Sci 37(1):130–137

Voshell JR Jr (2010) A guide to common freshwater invertebrates of North America. McDonald & Woodward, Granville, p 442

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

Ward GM, Cummins KW (1978) Life history and growth pattern of *Paratendipes albimanus* in a Michigan headwater stream. Ann Entomol Soc Am 71:272–284