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PRIMARY RESEARCH PAPER

Effects of macrophyte heterogeneity and food availability on structural parameters of the macroinvertebrate community in a Pampean stream

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Abstract Environmental heterogeneity in natural ecosystems influences several parameters at the population and community levels. In freshwater ecosystems, habitat heterogeneity can be provided by macrophyte species with different structural shapes. Previous studies suggest that aquatic plants with more complex architectures will support higher number, biomass, and taxon richness of macroinvertebrates than plants with simpler shape. We investigated the influence of macrophyte structural heterogeneity (quantified by fractal dimension) and food availability (represented by epiphytic biomass) on several parameters (number of individuals, biomass, body size distribution, taxon richness, and diversity) of the macroinvertebrate community in a Pampean stream. Four submerged macrophyte species (*Egeria densa*, *Elodea ernstae*, *Ceratophyllum demersum*, and *Stuckenia striata*) and associated macroinvertebrates were sampled in late spring, summer, and autumn. Plants were photographed and fractal dimension was estimated from the images by the box-counting method. Fractal dimension was independent of plant surface area per unit of macrophyte biomass and differed significantly among species. Mean fractal dimension varied between 1.29 and 1.62, and increased following the sequence *E. densa* → *S. striata* → *E. ernstae* → *C. demersum*. Macrophyte species with higher fractal dimension supported a greater abundance of macroinvertebrates, especially those of small body size (500–1,000 μm); but fractal dimension was unrelated to macroinvertebrate biomass, richness, and diversity. However, overall animal biomass was significantly associated to the epiphytic abundance. Consequently, macrophyte heterogeneity influences macroinvertebrate density and body size distribution, while animal biomass depends on epiphytic food resources provided by plants.

Keywords Macrophytes · Heterogeneity · Macroinvertebrates · Food availability

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Introduction

Quantification of the environmental heterogeneity in natural ecosystems is of paramount importance because it affects population dynamics, community structure, and the functioning of ecosystems (Cooper et al., 1997). Complex habitats may provide more niches and greater abundance and diversity of environmental resources, thus increasing the number of

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individuals, biomass, and diversity of organisms and influencing biotic interactions and body size distributions (MacArthur & MacArthur, 1961; Stewart et al., 2003; McAbendroth et al., 2005). In freshwater systems, the complex architecture of different aquatic macrophyte species increases the physical structural heterogeneity, creates new microhabitats that can be occupied by invertebrates (Hutchens et al., 2004; Giorgi et al., 2005), and provides more refuge from predation thus reducing predator foraging success (Dionne & Folt, 1991; Warfe & Barmuta, 2004; Padial et al., 2009). It has been suggested that plants with complex shapes may provide a higher surface area than simple plants, and, therefore, may support greater macroinvertebrate biomass and density (Cyr & Downing, 1988; Cheruvelil et al., 2002) and may offer the best protection for prey species (Heck & Crowder, 1991). For this reason, macrophyte surface area, either alone or related to plant biomass, has been used as an index of macrophyte structural heterogeneity (Brown et al., 1988; Russo, 1990).

Fractal models are being increasingly used to describe many ecological phenomena including habitat heterogeneity, as they can deal with the scale-dependence associated to ecological patterns and processes in a relatively simple manner (Gee & Warwick, 1994; Halley et al., 2004). The fractal dimension describes the complexity of a shape or, in other words, the object's ability to fill the Euclidean space in which it is embedded (Sugihara & May, 1990; Halley et al., 2004). The shape of some living organisms can be described by the fractal dimension, even though they are not truly fractal objects. Nevertheless, the fractal model may be seen as a simplifying frame that helps to understand multiscale ecological phenomena (Halley et al., 2004).

A characteristic of fractal objects is that their areas become disproportionately large as the unit of measurement is decreased (Morse et al., 1985). As animals should perceive and use the habitat proportionally to their own body size (Gee & Warwick, 1994; McAbendroth et al., 2005; Robson et al., 2005), in a same fractal habitat the area perceived (and available) for smaller organisms will be greater than the area perceived by larger organisms (Morse et al., 1985; Williamson & Lawton, 1991). Then, habitat structure will influence body size spectra, and more complex habitats will harbor a greater number of individuals, especially those of smaller body size (Jeffries, 1993; McAbendroth et al., 2005). In addition, some studies reported that macrophyte species with highly dissected leaves show higher detritus trapping and epiphytic biomass and production (Cattaneo & Kalff, 1980; Gregg & Rose, 1982). Thus, the combined effects of abundant food supply, lower predation risk, and higher number of available microhabitats provided by dissected macrophytes may increase the biomass, richness, and diversity of the associated macroinvertebrates.

The aims of this study were (i) to explore different measures of fractal dimension at different scales as estimator of macrophyte habitat heterogeneity, and (ii) to investigate the influence of macrophyte structural heterogeneity on several parameters (number of individuals, biomass, body size distribution, taxon richness, and diversity) of the macroinvertebrate community associated with aquatic plants in a Pampean stream.

First, we compared different measurements of fractal dimension at different magnifications and analyzed its relationship with plant surface area. Then, we present the results of a field study where macrophyte species of different heterogeneity and the accompanying invertebrate communities were sampled. As food availability seems to be an important factor influencing macroinvertebrate abundance, the epiphytic community attached to macrophytes was also sampled. Our hypothesis is that macrophyte heterogeneity is directly linked to the number and diversity of macroinvertebrates, and indirectly linked to macroinvertebrate biomass through food availability provided by the epiphytic community. Consequently, our predictions are (i) that macrophyte species with greater fractal dimension will support a higher number of individuals (especially of smaller body size) and diversity of macroinvertebrates, and (ii) that macrophyte species with greater fractal dimension will support a higher epiphytic biomass that, in turn, will determine a higher macroinvertebrate biomass.

Methods

Field sampling

The study was conducted in the Las Flores stream, a second-order stream that is a tributary of the Luján

River (34°27′25″S, 59°03′56″W). The stream is situated in the Pampean region, a vast grassy plain that covers central Argentina. A lack of riparian forest, low current velocities, and high nutrient levels in Pampean streams allow the development of dense and diverse macrophyte communities (Feijoó & Lombardo, 2007). The studied reach (~300 m long) was very well preserved as neither crop production nor cattle breeding, which are the main economical activities in the region, are developed in the surrounding areas. The physicochemical and biological characteristics of the Las Flores stream are described elsewhere (Giorgi et al., 2005).

The most common submerged macrophyte species in the stream (*Egeria densa* Planch., *Elodea ernstae* St. John, *Ceratophyllum demersum* L., and *Stuckenia striata* (Ruiz et Pav.) Holub, referred to hereafter by their genus names) were sampled in December 2007 (late spring), February 2008 (summer), and April 2008 (autumn), because within these seasons most macroinvertebrate species develop their life cycles and reach the adult stage. Selected plant species encompass the great variety of structural shapes found in submerged macrophytes from Pampean streams, and they are distributed in a mosaic of small monospecific patches within the stream. A power analysis ($\alpha = 0.05$) was performed using data from a previous sampling at the same stream to determine the number of samples necessary to detect differences in macroinvertebrate abundance between macrophyte species (Sokal & Rohlf, 1995). According to the results of this test, six samples of each macrophyte species were taken at each sampling occasion, except for *Ceratophyllum*, which was not present in December 2007.

Macrophytes and associated macroinvertebrates were collected with a cylindrical mesh bag sampler (460 μm mesh) with external plastic rings to provide structure (Cheruvelil et al., 2000). The sampler was 69 cm long and 20 cm diameter and had a drawstring at the bottom to close the sampler and prevent the escape of actively swimming organisms. The sampler was gently moved until 30 cm of the plant was introduced inside and then it was closed cutting the plant stems off. Once the sample was collected, the sampler was inverted and rinsed with filtered streamwater, and contents were stored in 500 ml plastic containers. Close to each sample, an apical shoot (15 cm long) of the same species was also collected into a plastic bottle to determine epiphytic biomass. Macrophyte samples were taken close to the stream surface, avoiding senescent shoots. Samples were transported to the laboratory for subsequent analyses.

Determination of macrophyte fractal dimension

Macrophyte samples were rinsed off with filtered streamwater to remove macroinvertebrates. Then, each sample was put into a white plastic tray filled with tap water and arranged to represent its natural disposition in the stream, where plants are patterned in the direction of flow. Samples were photographed with a digital camera at two magnifications (7× and 2×). All photographs had the same format, size, and resolution (JPEG—3,456 × 2,304 pixels, and 28,346 pixels/cm, respectively).

The images were modified, eliminating shade and reflections to improve resolution using an image analysis software. Then, they were converted into black and white, and fractal dimension was estimated by the box-counting method (Sugihara & May, 1990) using the ImageJ software (Rasband, 1997–2008). It should be noted that the box-counting method is very sensitive to number of cells occupied in a grid at the finest scale, because it determines the slope of the scale–area relationship and thus fractal dimension (Halley et al., 2004).

Twenty different grids with box side-length ranging from 10 to 110 pixels were placed on each image and the occupied boxes were counted. Fractal dimension was estimated as the slope of the relationship between log N (number of occupied boxes) and log $1/S$ (being S the side-length of boxes). Parameters of the box-counting method were selected following the recommendations of Halley et al. (2004), including those related with the percentage of picture area covered by plant image, selection of box size range according to picture size and resolution, and the use of multiple grid positions. Fractal dimension was calculated on the basis of plant image area (D_A or "bulk" fractal) and of plant image perimeter (D_P or "boundary" fractal), and the obtained values were later correlated to macroinvertebrate parameters. Both estimators of fractal dimension may represent different properties of the objects: D_A would describe how plant areas are divided up in space while D_P would indicate the degree of convolution of macrophyte edge (Halley et al., 2004; McAbendroth et al., 2005).

Apical shoots collected for the determination of epiphytic biomass were also photographed at 7× magnification after sonication (see next section) and fractal dimension was determined for each shoot following the protocol indicated above. Then, leaves and branches from each shoot were separated, put into a plastic bag and scanned, and surface area was estimated using the ImageJ software. These data were used to analyze the relationship box-counting fractal dimension versus macrophyte surface area and epiphytic biomass.

Treatment of biological samples

A careful analysis of each macrophyte sample was visually made and all attached macroinvertebrates were removed. Then, all collected invertebrates were passed through sieves of different pore size to separate them in six size classes (class 1: 250–500 μm; class 2: 500–1,000 μm, class 3: 1,000–2,830 μm; class 4: 2,830–5,000 μm; class 5: 5,000–10,000 μm; class 6: >10,000 μm). In December 2007, the number of sampled individuals was low; so, the whole sample was counted, identified to the lowest possible taxonomic level (mainly genus) under a binocular magnifying glass, and weighed. In February and April 2008, abundance of macroinvertebrates was higher, so each sample was separated into two subsamples using a Folsom sample splitter (McEwen et al., 1994). One subsample (representing 75% of the whole sample) was passed through sieves and dried at 60°C for biomass determination, while the other subsample (25% of the whole sample) was preserved in 70% alcohol for identification under a binocular magnifying glass. Folsom splitter performance was checked by counting the total number of individuals in both fractions. Macrophyte samples were dried at 60°C until constant weight, and macroinvertebrate biomass and number of individuals were expressed per unit dry weight (DW) of plant.

For each macrophyte sample, we counted the number of individuals of each taxon retained by each sieve, adding numbers obtained in all macrophyte samples to estimate the total number of macroinvertebrates at each sampling occasion discriminated by taxa and body size classes. We refer to the number of taxa and not the number of species because of the difficulty in identifying aquatic macroinvertebrates in our system and the fact that taxonomic resolution varied among groups. The Shannon–Wiener diversity index was also calculated using the same criteria. Functional feeding groups were determined following Barbour et al. (1999) because there are no protocols developed for pampean streams and it can be adjusted to the macroinvertebrate species found in these environments.

Apical shoots for epiphytic biomass determination were introduced in glass recipients filled with streamwater and sonicated at low speed during 3 min. Most algae were removed by this process, and sonication did not break plant cells. A 200 ml subsample was taken from the final suspension and filtered through a pre-weighed Whatman GF/F glass fiber filter to determine particulate organic matter content (POM). Filters were dried at 60°C until constant weight and combusted at 500°C for 4 h. POM was determined as the difference between dry weight and ash-free dry weight. Another 100 ml subsample was filtered through a Whatman GF/F filter and photosynthetic pigments were extracted in 90% acetone at 4°C for 24 h. The extract was then measured using a spectrophotometer, and chlorophyll-*a* content was estimated following APHA (1995). Macrophyte apical shoots were dried at 60°C until constant weight in order to express epiphytic biomass (POM and chlorophyll-*a* content) per unit DW of macrophyte. Epiphytic biomass was also estimated per macrophyte surface area.

Data analyses

The relationship between fractal dimension and plant surface area determined in individual shoots was evaluated by the Spearman's rank correlation coefficient. To allow the comparison between different macrophyte species, surface area was expressed per gram of DW (hereafter referred as A/DW). A non-parametric test was used because the variable A/DW could not be normalized.

Differences in box-counting fractal dimension among macrophyte species were tested by a two-way ANOVA, using macrophyte species and sampling date as factors. No significant differences were found among sampling dates; consequently, all data were pooled for subsequent analyses. A one-way ANOVA was performed to compare epiphytic biomass between macrophyte species. Tukey post-hoc comparisons were used to determine the significance of differences

between group means. Relationships between fractal dimension and different macroinvertebrate parameters (abundance, body size, taxon richness, and diversity) were explored by calculating product–moment correlations. Macroinvertebrate abundance was expressed as number of individuals or total biomass per gram DW of macrophyte. Macroinvertebrate body size was analyzed by estimating the relative abundance of each size class and relating them to fractal dimension. To avoid the influence of the number of individuals on the estimation of taxon richness, rarefaction curves were constructed from our macroinvertebrate sample data (Gotelli & Colwell, 2001) using the EstimateS Win 8.0 program (Colwell, 2006). Curves were adjusted according to the Clench equation (Clench, 1979), and rarefied taxon richness was estimated for each macroinvertebrate sample. Differences in functional feeding groups among macrophyte species were tested by two-way ANOVAs. For each feeding group, number of individuals per gram of DW was used as variable and macrophyte species and sampling date as factors.

All variables were checked for normality (Kolmogorov–Smirnov test, $P > 0.05$) and homogeneity of variances (Cochran C test, $P > 0.05$) before parametric tests were performed. Variables that did not meet the assumption of normality were log-transformed.

Results

Macrophyte heterogeneity

Given that the box-counting method is very sensitive to number of cells occupied in a grid at the finest scale, fractal dimensions based on perimeter (D_P) and on area (D_A) were compared by representing grid occupancy at the finest scale in both cases (Fig. 1). When the plant was represented as an area there was a higher degree of grid occupancy than when it was represented as a perimeter; consequently, D_A was higher than D_P for all macrophyte species. D_A also depended more on the amount of plant material included in the photograph than D_P. When a plant is photographed, as more plant material is included into the image higher is the number of occupied cells at the finest scale. But this increase in occupancy will be lower when the plant is represented as a perimeter that as an area. As it can be seen in Fig. 1, incorporating more or less plant material into the image when estimating D_A greatly changes the number of occupied cells at the finest scale, which in turn results in a higher variation of the slope of the scale-area relationship (and thus fractal dimension) than when estimating D_P. As we compared macrophyte species with different architecture sampled at the stream, which did not show similar biomasses, we consider that the use of D_P reduces the effect of how much material is photographed and allows a better comparison among species. Hence, all subsequent analyses were performed using D_P as estimator of macrophyte structural heterogeneity.

The effect of scale on fractal dimension was analyzed by calculating D_P at two magnifications (2× and 7×) (Table 1). D_P was similar at both magnifications for *Elodea* and *Ceratophyllum*, while D_P at 7× was lower than D_P at 2× for *Stuckenia* and *Egeria*. Consequently, plants with more finely dissected leaves showed similar fractal dimension at the studied scales. Box sizes in the box-counting analysis at 7× ranged from 0.05 to 0.5 cm, and macroinvertebrate body size ranged from 0.025 to 1 cm in our study. From both magnifications, we selected D_P at 7× as descriptor of macrophyte heterogeneity for subsequent analyses because it was estimated at a scale similar to that of the observed macroinvertebrates. D_P at 7× was not related to A/DW (macrophyte surface area per gram of DW of plant) (Fig. 2), and it was significantly different among species (ANOVA: $F_{3,55} = 86.35$, $P < 0.001$), while sampling date and treatment/date interaction were not significant. Therefore, D_P did not vary along the year for each macrophyte species (Table 1 and Fig. 2). Mean D_P ranged from 1.29 to 1.62, and increased significantly following this sequence: *Egeria* < *Stuckenia* < *Elodea* < *Ceratophyllum*.

Macroinvertebrate community

The macroinvertebrate community included 19 taxa, comprising 9 genera, 7 families, and 3 major groups (Table 2). The community was dominated by Amphipoda (49% of total individuals; mean of the three sampling occasions) and Gastropoda (27%). Other groups such as Odonata (8%), Hirudinea (4%), Chironomidae (4%), and Ephemeroptera (3%) were also present. The total number of individuals was higher in

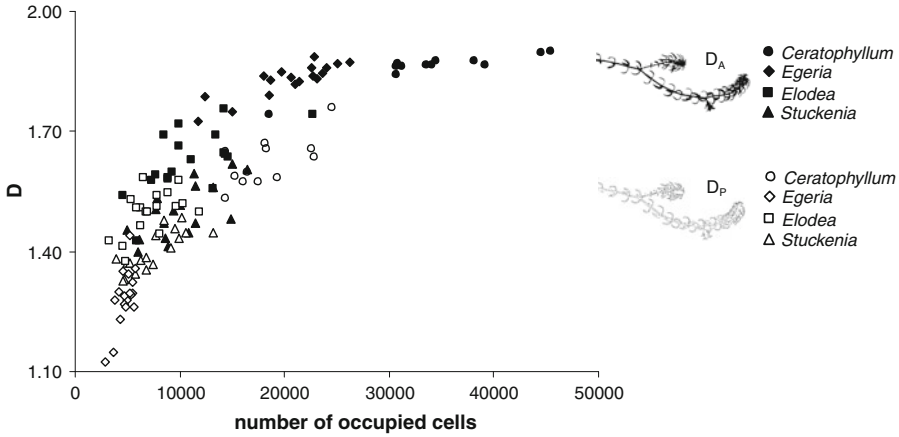

Fig. 1 Relationship between D_A or bulk fractal dimension (*closed symbols*) and D_P or boundary fractal dimension (*open symbols*) and the number of occupied cells in the grid at the finest scale (box side-length = 10 pixels), discriminated among macrophyte species. The drawing corresponds to *Elodea ernstae*

Table 1 Mean D_P (±SD) for the different macrophyte species estimated at two magnifications on the different sampling occasions

Macrophyte sp.	Month	2× Magnification	7× Magnification	
Ceratophyllum demersum	February	1.59 (±0.10)	1.60 (±0.05)	
	April	1.56 (±0.11)	1.65 (±0.06)	
	Mean	1.58 (±0.10)	1.62 (±0.06)	
Egeria densa	December	1.47 (±0.05)	1.24 (±0.08)	
	February	1.42 (±0.09)	1.31 (±0.04)	
	April	1.42 (±0.09)	1.31 (±0.07)	
	Mean	1.44 (±0.08)	1.29 (±0.07)	
Elodea ernstae	December	1.50 (±0.08)	1.52 (±0.05)	
	February	1.42 (±0.07)	1.47 (±0.06)	
	April	1.47 (±0.08)	1.51 (±0.05)	
	Mean	1.46 (±0.08)	1.50 (±0.05)	
Stuckenia striata	December	1.50 (±0.07)	1.37 (±0.04)	
	February	1.56 (±0.08)	1.41 (±0.06)	
	April	1.56 (±0.11)	1.42 (±0.05)	
	Mean	1.54 (±0.09)	1.40 (±0.05)	

Black images show the structural heterogeneity of the different plant species

size class 3 (1,000–2,830 μm) in all macrophyte species and sampling occasions, while size classes 1 (250–500 μm) and 6 (>10,000 μm) were always poorly represented. Classes 5 and 6 (5,000–10,000 and >10,000 μm) were mainly represented by Odonata, while Amphipoda and Gastropoda were more important in size classes 2 to 4 (500 to 5,000 μm) (Table 2). With respect to functional feeding groups, about 88% of individuals were scrapers or gatherer-collectors feeding on epiphytic algae. No clear differences in the macroinvertebrate community structure were observed among plant species.

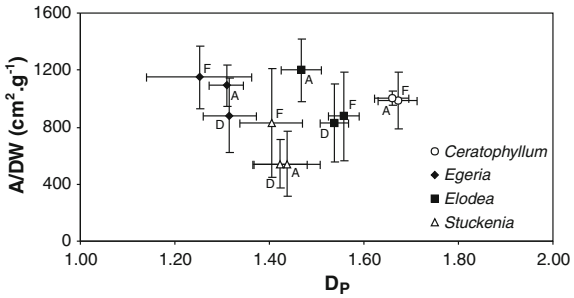

Fig. 2 Relationship between fractal dimension (D_P at 7×) and macrophyte surface area per gram of DW of plant (A/DW) determined from apical shoots of each macrophyte species on the different sampling occasions (*D* December, *F* February, *A* April). *Bars* indicate standard deviation

Macrophyte heterogeneity (estimated as D_P at 7×) was significantly related to the number of individuals but not to the overall macroinvertebrate biomass (both variables expressed per gram of DW of plant) (Table 3). In other words, in concordance with our first hypothesis, plants with more complex shapes showed a greater abundance of individuals (Fig. 3), but contrary to our second hypothesis, not a higher macroinvertebrate biomass. D_P was positively associated to body size class 2 and negatively to body size class 4, while the other classes showed no significant relationships (Table 3). Therefore, macrophytes with higher fractal dimension supported a higher abundance of macroinvertebrates of small body size (500–1,000 μm; mainly Amphipoda) but a lower number of individuals of large body size (2,830–5,000 μm; mainly Gasteropoda and Odonata). No clear differences among macrophytes were observed regarding uncorrected taxon richness (*Ceratophyllum* = 8.33 ± 2.2, *Egeria* = 6.8 ± 1.9, *Elodea* = 7.8 ± 2.5, *Stuckenia* = 6.5 ±2.8; Mean ± SD) and rarefied taxon richness (*Ceratophyllum* = 10.8 ± 3.7, *Egeria* = 9.9 ± 3.8, *Elodea* = 9.6 ± 2.9, *Stuckenia* = 8.62 ± 3.6; Mean ± SD). Neither diversity nor uncorrected and rarefied richness were significantly related to D_P (Table 3). Uncorrected taxon richness was positively associated to total macrophyte surface area ($R = 0.356$, $P < 0.01$) and biomass ($R = 0.275$, $P < 0.05$), while rarefied taxon richness ($R = 0.196$, $P = 0.114$ for surface area and $R = 0.162$, $P = 0.194$ for biomass) and the Shannon–Wiener diversity index ($R = -0.049$, $P = 0.697$ for surface area and $R = -0.004$, $P = 0.972$ for biomass) showed no relations with these variables.

Epiphytic biomass per macrophyte surface area and per gram DW of plant showed similar trends in the statistical analyses; therefore, we presented the results obtained with the latter variable. Epiphytic biomass per gram of DW of plant was higher in *Elodea* than in the other macrophyte species, either expressed as POM (ANOVA: $F_{3,61} = 11.13$, $P < 0.001$) or chlorophyll-*a* (ANOVA: $F_{3,61} = 5.806$, $P < 0.01$) (Fig. 4); however, macrophyte fractal dimension (D_P at 7×) was not significantly correlated with epiphytic biomass either as POM or chlorophyll-*a*. The number of individuals per gram of DW of plant was not significantly associated to epiphytic biomass, but overall macroinvertebrate biomass per gram of DW of plant increased with epiphytic POM ($R = 0.364$, $P < 0.001$) and chlorophyll-*a* ($R = 0.317$, $P < 0.01$) contents per gram of DW of plant.

Discussion

Fractal dimension as a measure of macrophyte structural heterogeneity

In this study, we discriminated the structural heterogeneity of different macrophyte species using box-counting fractal dimension. We found that bulk fractal dimension (D_A) was higher than boundary fractal dimension (D_P), a fact that was also observed by McAbendroth et al. (2005). Nevertheless, we consider that this difference results from the characteristics of the box-counting method and do not indicate that heterogeneity is greater when the object in considered as an "area" than when it is considered a "perimeter". Estimation of fractal dimension depends on the number of occupied boxes in a grid at the finest scale (Halley et al., 2004), which is higher when the plant is represented as a two-dimension object than when it is represented as a perimeter.

The scale of target organisms is a very important factor to be considered when selecting an index of environmental heterogeneity (Attrill et al., 2000). Dibble & Thomaz (2009) observed that fractal dimension did not vary across scales for 11 tropical and temperate macrophyte species, while McAbendroth et al. (2005) found that among 15 species, only one showed similar fractal dimension at two different scales. Plants with similar fractal dimension across different magnifications, such as *Elodea* and

Table 2 Total number of individuals of the macroinvertebrate community discriminated by taxa and size classes

Taxon	1 250–500 μm	2 500–1,000 μm	3 1,000–2,830 μm	4 2,830–5,000 μm	5 5,000–10,000 μm	6 >10,000 μm	All size classes
Turbellaria							
Planariidae	0 (±0)	11 (±11)	14 (±14)	1 (±1)	1 (±1)	0 (±0)	25 (±25)
Mollusca							
Gastropoda							
Chilinidae, *Chilina*	2 (±4)	30 (±46)	103 (±83)	25 (±27)	1 (±1)	0 (±0)	161 (±130)
Planorbidae	0 (±0)	0 (±1)	2 (±2)	3 (±5)	1 (±2)	0 (±0)	6 (±8)
Hydrobiidae, *Heleobia*	0 (±0)	2 (±4)	36 (±8)	6 (±3)	0 (±1)	0 (±0)	44 (±12)
Ampullaridae	0 (±0)	3 (±5)	70 (±75)	5 (±9)	0 (±0)	0 (±1)	79 (± 84)
Ancylidae, *Uncancylus*	0 (±0)	1 (±1)	22 (±19)	2 (±3)	0 (±1)	0 (±0)	25 (±23)
Annelida							
Hirudinea	0 (±0)	4 (±4)	28 (±18)	9 (±8)	1 (±1)	0 (±1)	42 (±24)
Hidrachnida	0 (±1)	3 (±4)	2 (±2)	0 (±0)	0 (±0)	0 (±0)	5 (±4)
Crustacea							
Amphipoda							
Hyalellidae, *Hyallela*	1 (±2)	270 (±259)	530 (±402)	12 (±4)	0 (±0)	0 (±0)	813 (±661)
Ostracoda	0 (±0)	1 (±2)	13 (±11)	0 (±0)	0 (±0)	0 (±0)	14 (±12)
Insecta							
Coleoptera							
Hydrophilidae, *Berosus*	0 (±0)	1 (±1)	5 (±4)	1 (±1)	1 (±2)	0 (±0)	8 (±5)
Diptera							
Chironomidae	1 (±1)	26 (±17)	30 (±26)	1 (±1)	0 (±0)	0 (±0)	59 (±41)
Culicidae	0 (±0)	0 (±0)	0 (±0)	1 (±1)	1 (±1)	0 (±0)	2 (±2)
Ephemeroptera							
Baetidae, *Americabaetis*	2 (±3)	7 (±7)	12 (±5)	1 (±1)	0 (±1)	0 (±0)	22 (±13)
Baetidae, *Callibaetis*	0 (±0)	2 (±2)	1 (±2)	0 (±1)	0 (±0)	0 (±0)	3 (±3)
Caenidae, *Caenis*	0 (±0)	2 (±2)	8 (±3)	1 (±1)	0 (±0)	0 (±0)	11 (±3)
Hemiptera							
Belostomatidae, *Belostoma*	0 (±0)	0 (±0)	2 (±2)	2 (±2)	0 (±1)	0 (±0)	4 (±5)
Odonata							
Coenagrionidae	0 (±0)	0 (±1)	45 (±13)	38 (±8)	14 (±5)	3 (±2)	100 (±21)
Trichoptera							
Hydroptilidae	0 (±0)	1 (±1)	7 (±10)	2 (±3)	1 (±1)	0 (±0)	9 (±14)
All taxa	7 (±5)	361 (±287)	931 (±426)	109 (±26)	21 (±9)	4 (±2)	1432 (±679)

Figures represent mean values and (between brackets) standard deviations of the three sampling occasions

Table 3 Product–moment correlations (R) between macrophyte fractal dimension (D_P) and several parameters of macroinvertebrate communities for each sample

Macroinvertebrate parameters	R	N	P
Log individuals/DW	0.464	66	<0.001
Log biomass/DW	0.197	66	ns
Log RA 2	0.308	57	<0.05
Log RA 3	−0.060	66	ns
Log RA 4	−0.394	62	<0.01
Log RA 5	−0.280	29	ns
Taxon richness	0.171	66	ns
Rarefied taxon richness	0.033	66	ns
Taxon diversity	−0.182	66	ns

Number of individuals and biomass of macroinvertebrates are expressed per gram of DW of plant. RA is the relative abundance of the different body size classes that increases from 2 to 5 (size classes 1 and 6 were not included because the number of cases was very low)

N number of cases, *ns* not significant

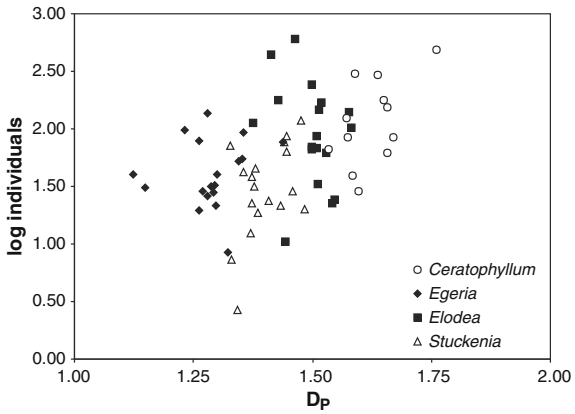

Fig. 3 Relationship between macrophyte fractal dimension (estimated by D_P at 7×) and number of individuals per gram of DW of plant (at log-scale)

Ceratophyllum in our case, may be self-similar at the studied scales. In our work, fractal dimension at 7× allowed the estimation of an environmental heterogeneity relevant to macroinvertebrates (especially to the smaller ones that are also the more abundant components of the community). Fractal dimension calculated at 2× may be relevant to quantify habitat heterogeneity for larger organisms like fishes.

It has been accepted that the more complex the habitat, the larger the surface area available for colonization for fauna and epiphytic algae (Cyr & Downing, 1988; Cheruvelil et al., 2002; Hauser et al., 2006). Nevertheless, an index of habitat heterogeneity should be independent of substrate area, as both parameters represent different environmental features (Taniguchi et al., 2003). Previous studies have reported that fractal dimension covaried with macrophyte surface area (McAbendroth et al., 2005; Thomaz et al., 2008), though we found no relationship between both variables for three independent sampling dates. This discrepancy might be attributed to the different methods used to calculate surface area. McAbendroth et al. (2005) estimated fractal dimension and area from the same photographs, and they excluded two heterogeneity indices that correlated with surface area from their analyses. Thomaz et al. (2008) calculated fractal dimension from a set of macrophyte samples, while surface area was obtained through regression between dry weight and area from independent samples. In our study, D_P and surface area were estimated from different photographs of the same plant: a photograph of the whole

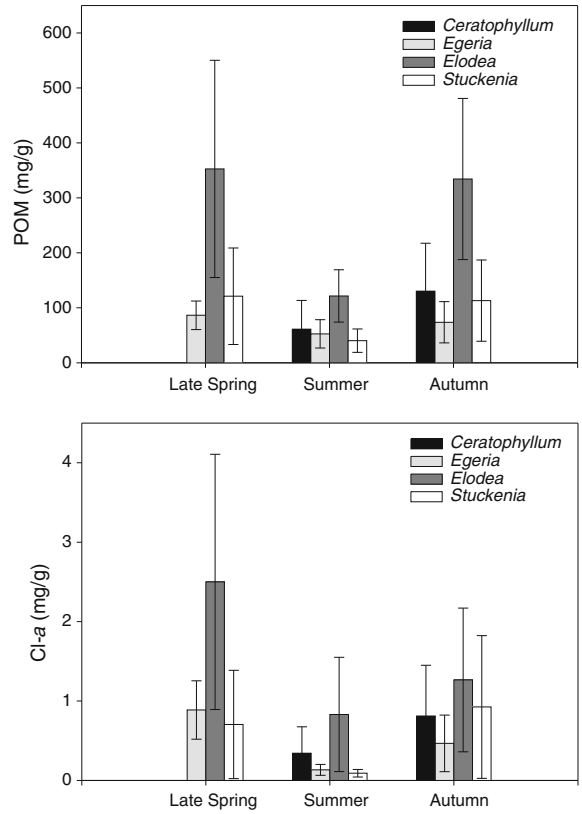

Fig. 4 Mean epiphytic biomass (expressed as POM or chlorophyll-*a* in milligram per gram of DW of plant) discriminated by macrophyte species and season (±SD)

shoot was used for D_P estimation, while a scanned image where branches and leaves were separated was used for surface area estimation. The general assumption that a more complex macrophyte provides an increased surface area is not supported by this study, as D_P was not correlated with surface area per gram of DW of plant.

Macrophyte heterogeneity and macroinvertebrate community

Our results indicate that number of individuals per gram of DW of plant was influenced by macrophyte heterogeneity, while macroinvertebrate biomass per gram of DW of plant was related to potential food availability (measured as epiphyton biomass per gram of DW of plant). Positive relationships between macrophyte structural heterogeneity and invertebrate density were reported by several authors (Cheruvelil et al., 2000; Cheruvelil et al., 2002; Thomaz et al., 2008; Dibble & Thomaz, 2009). We also observed that an increase in D_P was linked to a higher relative abundance of small body size individuals and to a lower abundance of large organisms. An important characteristic of a fractal curve or surface is that its length or area, respectively, becomes disproportionately large as the unit of measurement is decreased. Therefore, for animals living on a fractal surface, the smaller the animal the greater the surface would appear to be in absolute terms, as well as in relative ones (Morse et al., 1985; Williamson & Lawton, 1991). McAbendroth et al. (2005) found a greater number of small-bodied macroinvertebrates in more complex macrophyte stands. Our study also provided evidence of the link between macrophyte structural heterogeneity and number and body size distribution of associated macroinvertebrates.

There is a general consensus that predation might be expected to be less within stands of plants that have complex leaves (Heck & Crowder, 1991), but evidences from studies made in freshwater systems are controversial. Some authors (Dionne & Folt, 1991; Warfe & Barmuta, 2004) reported lower predation rates in aquatic plants with more complex shapes, whereas the opposite was observed by Warfe & Barmuta (2006). Warfe et al. (2008) further addressed this question, pointing out that the potential role of vegetation as refuge may be related to the absolute value of interstitial space available to an invertebrate prey but unavailable to his fish predator. In fine-leaf macrophytes, interstitial space will be more partitioned and will be less accessible to organisms of larger body size thus reducing predation risk. There are no previous studies evaluating predation pressure in Las Flores stream, but most abundant fish species reported for this stream (*Cnesterodon desenmaculatus, Phalloceros caudimaculatus, Astyanax eigenmanniorum*, and *Bryconamericus iheringi*) (Di Marzio et al., 2003) feed on algae and invertebrates. Even though these are small fishes (1–10 cm long), their body size range is higher than that of the invertebrates they can potentially predate. Consequently, the higher number of small individuals associated to more complex macrophytes observed in this study should be explained not only by the greater habitat availability but also by the refuge to predation that they provide to invertebrates.

Remarkably, macroinvertebrate biomass per gram of DW of plant was not related to D_P but with epiphytic biomass, which represents the amount of potential food resources for macroinvertebrates. Studies analyzing the relationship between macrophyte structural heterogeneity and invertebrate biomass have shown contradictory evidence: positive relations have been reported by Cheruvelil et al. (2000), Cheruvelil et al. (2002), and McAbendroth et al. (2005), whereas lack of association has been observed by Taniguchi et al. (2003) and Cremona et al. (2008). The correlation between macroinvertebrate and epiphytic biomass in our study is not surprising as most individuals were grazers and collectors that feed on epiphytic algae. When comparing macrophytes with different architecture, some researchers have found higher epiphytic algal biomass on plants with complex shapes (Cattaneo & Kalff, 1980; Gregg & Rose, 1982; Tessier et al., 2008). However, in our study, the amount of potential food resources was unrelated to macrophyte heterogeneity as no significant relationship was found between D_P and epiphytic biomass (as POM or chlorophyll-*a* content per gram of DW of macrophyte). We observed the greatest epiphytic biomass in *Elodea*, which showed a high D_P value, however, epiphytic biomass of the other complex species (*Ceratophyllum*) was not significantly different to those of the more simple species (*Stuckenia* and *Egeria*). Nevertheless, there are factors not considered in this study like macrophyte surface texture and allelopathic activity that might be hiding a positive

effect of structural heterogeneity on epiphytes (Gross et al., 2003). The structural heterogeneity of a habitat has also been associated to a higher number of species that can potentially occupy it, as it will provide a wider range of niches (MacArthur, 1965). However, we did not find relationships between D_P and macroinvertebrate richness and diversity. Uncorrected taxon richness was positively correlated to total plant surface area and biomass, suggesting a species–area relationship produced by passive sampling (Connor & McCoy, 1979). In other words, when enlarging macrophyte area or biomass an increasing proportion of the total number of associated macroinvertebrates will be randomly sampled, thus entrapping more of the rarer species as sample size increases (Attrill et al., 2000). The lack of a significant relationship between rarefied taxon richness (which corrects the influence of density of individuals on the number of species) and plant surface area and biomass also supports this hypothesis. Although some researchers have reported a positive relationship between macrophyte heterogeneity and macroinvertebrate taxa richness, in these studies plant surface area was either not considered as a factor separated from structural heterogeneity (Cremona et al. 2008) or it correlated with structural heterogeneity (Thomaz et al., 2008). However, McAbendroth et al. (2005), who explicitly discriminated the effect of macrophyte surface area from that of heterogeneity, also found that heterogeneity was unrelated to species richness. Our study analyzes separately both aspects and gives evidence to the idea that habitat heterogeneity may control animal abundance rather than the number of species.

Final considerations

In this study, box-counting fractal dimension has proved to be a simple and useful tool to estimate and compare macrophyte structural heterogeneity. However, some methodological aspects need to be considered to properly use this heterogeneity index, including selection of parameters for the box-counting method, type of fractal dimension (bulk vs. boundary), scale of magnification, and invariance of fractal dimension with macrophyte surface area.

We assessed the effects of macrophytes on the number of individuals and body size distribution (through plant heterogeneity) and on macroinvertebrate biomass (as physical structures that support epiphytic food resources). In Pampean streams, habitat heterogeneity is mainly generated by the aquatic vegetation, a substratum that varies along the year and could easily be removed by floods. Macrophyte assemblages support dense macroinvertebrate communities that, in turn, serve as a food source to other macroinvertebrates and fishes (Giorgi et al., 2005). Seasonal or unexpected changes in the taxonomic composition and relative abundances of macrophyte species may modify the abundance and biomass of macroinvertebrates, affecting the whole trophic food web in these streams. Our work confirms the well-known influence of aquatic vegetation heterogeneity on the accompanying macroinvertebrates assemblages in freshwater systems, and suggests that in Pampean streams food supply and predator refuge affect invertebrate biomass and abundance, respectively.

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