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Effects of macrophyte architecture and leaf shape complexity on structural parameters of the epiphytic algal community in a Pampean stream

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Abstract Habitat heterogeneity is one of the main factors determining distribution of organisms, and vegetation is of primary importance in shaping the structural environment in aquatic systems. The effect of macrophyte complexity on macroinvertebrates has been well researched; however, much remains to be revealed about the influence of complexity on epiphytic algae. Here, we used fractal dimension to study the effect of complexity at two scales, macrophyte architecture and leaf shape, on several parameters of the epiphytic algal community (number of individuals, biomass, taxon richness and diversity) in a Pampean stream. Four submerged macrophyte species with different complexities and associated algae were sampled in late spring, summer and autumn. Important differences were found in fractal dimension of the whole plant and leaves among macrophyte species. The particulate organic matter and chlorophyll *a* associated positively to leaf fractal dimension, but not to plant fractal dimension, partially supporting the hypothesis of a positive effect of macrophyte complexity on periphyton biomass. No association was found in fractal dimension with algal abundance, taxon richness or diversity. Complementary, a mesocosm experiment was performed with plastic imitations of different plant fractal dimensions. After four weeks, there were differences in chlorophyll *a* and autotrophy index between treatments that suggested a positive effect of complexity on autotrophic periphyton biomass. These results indicate that the well-known positive effect of macrophyte complexity on macroinvertebrates might be partially explained by a positive effect of complexity on periphyton biomass.

Keywords Periphyton · Fractal dimension · Heterogeneity · Abundance · Biomass · Diversity

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Introduction

The study of the distribution and abundance of organisms, in space and time, is one of the most

classical issues in ecology (Andrewartha 1961), and the search of explanations to such differences still constitutes the objective of great part of ecological research (Krebs 1986). Nowadays, the question of how biological diversity is maintained is central to community ecology because increasing climatic and anthropogenic threats to environments means that biodiversity is lost at a pace faster than the speed at which we are gaining knowledge (Tokeshi and Arakaki 2012). According to the habitat heterogeneity hypothesis (MacArthur and Wilson 1963), structurally complex habitats provide a high number of niches and variety of resources exploitation that maintains a high species diversity (Bazzaz 1975). In addition, complex habitats have been found to support higher number and biomass of organisms than the simple ones and influence biotic interactions and body size distributions (Stewart et al. 2003; MacAbendroth et al. 2005).

The term 'habitat heterogeneity' is usually considered interchangeable with 'habitat complexity' and 'habitat diversity,' indicating that concept under study is related to the existence of different 'kinds' of elements constituting the habitat (Tews et al. 2004; Tokeshi and Arakaki 2012). Habitat complexity has been defined as the vertical patch heterogeneity (Kolasa and Rollo 1991), a qualitative descriptor of heterogeneity (Li and Reynolds 1995), the number and distribution of patches of different kinds of habitat (Dodson 2000) and the absolute abundance of habitat structural components (MacCoy and Bell 1991). While MacCoy and Bell (1991) assigned the same status to habitat complexity and habitat heterogeneity (that defined as the relative abundance of structural components), the other authors considered habitat complexity as a component of habitat heterogeneity. Finally, Tokeshi and Arakaki (2012) proposed that habitat heterogeneity is considered a component of the structural complexity of habitats that referring to the diversity of complexity-generating elements. In addition, Tews et al. (2004) reviewed 103 studies on effects of habitat on species diversity reporting that 'habitat heterogeneity' (22 %) and 'habitat diversity' (20 %) were the most common terms, while 'habitat complexity' only registered an intermediate use (<10 %). Considering the lack of consensus on these terms and the study by Tews et al. (2004), we have decided to refer to 'habitat heterogeneity' when studying the effects of different 'kinds' of elements constituting a habitat on species diversity. However, the term 'complexity' is a property of any object 'as a whole made up of complicated or interrelated parts' (Merriam Webster 2013), and then in this study, we will refer to the macrophyte architecture and leaf shape complexity as general terms to cover all aspects of design that refer to the complicated and interrelated parts of plant and leaf. We consider that effects of complexity on the associated community diversity may be explained by the habitat heterogeneity hypothesis, but the effects on abundance, biomass and size require alternative explanations. So, it is convenient to separate what is understood by complexity (plant architecture complexity, leaf shape complexity, structural complexity) and by habitat heterogeneity (sometimes referred as habitat diversity or habitat complexity).

In freshwater ecosystems, habitat heterogeneity has often been studied by analyzing the invertebrate community associated with macrophyte beds of contrasting architecture. Authors have usually reported that complex macrophytes have more abundant and richer communities of invertebrates (Taniguchi et al. 2003; Thomaz et al. 2008; Lucena-Moya and Duggan 2011). A positive effect of plant complexity on richness is explainable by the habitat heterogeneity hypothesis, that is to say the presence of a higher number of niches (MacArthur and MacArthur 1961; Stewart et al. 2003), while a positive effect on abundance has received several explanations. The microhabitat hypothesis suggests that plants with more complex architecture present more space available for the establishment of small individuals (Morse et al. 1985; Williamson and Lawton 1991). The refugia hypothesis postulates that complex architecture has a negative effect on fish predation (Russo 1987; Coull and Wells 1983; Beukers and Jones 1998; Warfe and Barmuta 2004) and/or reduces the impact of physical stress factors such as current (Gregg and Rose 1982; Dodds and Biggs 2002). Finally, the food availability hypothesis suggests that complex architectures favor the presence of epiphytic algae and detritus (Cattaneo and Kalff 1980; Taniguchi et al. 2003; Gosselain et al. 2005; Warfe and Barmuta 2006) and the abundance of herbivorous and detritivorous invertebrates.

In habitat heterogeneity studies, the algal community is usually only mentioned as a factor that may differ among samples and is necessary to control to study complexity properly. However, a priori, some of the effects of complexity proposed for the macroinvertebrate community may be affecting algal richness and abundance at the same time. In fact, the food

availability hypothesis proposed for invertebrates implies the existence of a positive effect of complexity on periphyton biomass, which may be explained by algal microhabitat and refugia hypotheses, or by the effects of complexity on the light transmitted (Dibble et al. 1996). Among authors who studied periphyton on macrophytes of contrasting architecture, several have found a positive effect of complexity on periphyton biomass (Cattaneo and Kalff 1980; Gregg and Rose 1982; Jones et al. 2000; Gosselain et al. 2005; Warfe and Barmuta 2006; Hinojosa-Garro et al. 2010), although Taniguchi and Tokeshi (2004) reported a weakly but significant negative effect of fractal dimension on chlorophyll *a*. Finally, only a few studies have performed taxonomic identification and counting of algae, reporting a positive effect of complexity on abundance for a couple of algae species (Cattaneo and Kalff 1980; Jones et al. 2000; Hinojosa-Garro et al. 2010).

During the last years, fractal dimension has begun to be used for studying the heterogeneity generated by macrophyte beds in aquatic ecosystems (Jeffries 1993; MacAbendroth et al. 2005; Thomaz et al. 2008; Ferreiro et al. 2011). Empirical quantification of the self-similarity fractal dimension allows comparing the degree of irregularity among different objects (Mandelbrot 1967), so that it constitutes an estimator of complexity. In fractal objects, fractal dimension is independent of scale; however, this may not be the case for real objects, because they are usually multifractals (Halley et al. 2004). Then, macrophyte fractal dimensions calculated from photographs taken with different magnifications have been suggested as estimators of complexity at different scales (MacAbendroth et al. 2005; Ferreiro et al. 2011). Several authors have attributed the differences in communities associated with different macrophyte species to leaf shape differences (broad *versus* dissected leaves) that would cause a very different macrophyte architecture (Taniguchi et al. 2003; Gosselain et al. 2005; Warfe and Barmuta 2006; Lucena-Moya and Duggan 2011). However, in these studies, the effects of leaf shape and plant architecture could not be separated. The estimation of fractal dimensions at two very different magnifications may allow us to separate properly the effects of leaf shape and macrophyte architecture on aquatic organisms.

The aim of this study was to investigate the influence of macrophyte complexity on several parameters of the epiphytic algae community (number of individuals, biomass, taxon richness and diversity) associated with aquatic plants in a Pampean stream. First, we present the results of a field study where macrophyte species of different fractal dimensions and the accompanying algal communities were sampled. Then, we report a mesocosm experiment where plastic imitations of different fractal dimensions were colonized by epiphytic algae. Our hypothesis is that macrophyte complexity is positively linked to abundance and diversity of algae.

Materials and 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. The lack of a riparian forest, low current velocities and widespread high nutrient concentrations in Pampean streams allow the development of dense and diverse macrophyte communities (Feijoó and Lombardo 2007). 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). Macrophyte fragments and associated algae were collected with plastic containers (500 ml), which were gently moved until 15 cm of the plant were introduced inside and then were closed cutting the plant stems off. The 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, surface area and biomass

The macrophytes were 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. In order to quantify fractal dimension at different scales, samples were photographed with a digital camera at 7× magnification (fractal dimension D7X) and three randomly selected leaves from each sample were photographed under magnifying glass at 220× (fractal dimension D220X). 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 shades and reflections to improve quality using image analysis software. Then, they were converted into black and white, and boundary line images of the plants were obtained. The fractal dimension was estimated by the box-counting method (Sugihara and May 1990) using the ImageJ software (Rasband 1997–2008). 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 obtained from the slope of the relationship between log N (number of occupied boxes) and log $1/S$ (being S the side length of boxes). The 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. Then, leaves and branches from each sample were separated, put into a plastic bag and scanned. The surface area (A) was estimated as the double of the scanned area and calculated using the ImageJ software. Macrophyte samples were dried at 60 °C until constant weight to determine dry weight (DW). Both DW and A were used to refer macroinvertebrate and periphyton parameters to plant biomass. Finally, randomly selected leaves from each sample were preserved in freezer to perform a qualitative comparison of surface among macrophyte species by photographs obtained under scanning electron microscope (26, 1,000 and 2,500×). The D220X of each sample was determined as the mean of three macrophyte leaves. A partial analysis of D7X data has previously been published in a study about the effect of macrophyte heterogeneity on the macroinvertebrate community (Ferreiro et al. 2011), reporting significant differences in fractal dimension among macrophytes (*Egeria* < *Stuckenia* < *Elodea* < *Ceratophyllum*).

Periphyton biomass, abundance and taxonomic determination

The macrophyte fragments were introduced in glass beakers and sonicated during three sessions of 3 min, separated by intervals of 1 min. We have previously determined that 90 % of the algae are removed by this process and that sonication does not break plant cells, by observing sonicated leaves under a microscope. A 200 ml subsample was taken from the final suspension and filtered through a preweighed Whatman GF/F glass-fiber filter to determine the particulate organic matter content (POM). The 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 the photosynthetic pigments were extracted in 90 % acetone at 4 °C for 24 h. The extract was then measured using a spectrophotometer, and the chlorophyll *a* content (Chl-*a*) was estimated following APHA (1995). Finally, a 100 ml subsample was fixed with formaldehyde 2 % to perform the counting and taxonomic identification. Algae were counted under inverted microscope at 40× magnification, from 40 ml subsamples with 36-h sedimentation. The counting was performed along two perpendicular transects, counting and identifying algae so that main group abundance estimations had a standard error lower than 30 % (about 80 fields per sample). Taxa identifications were performed with taxonomic keys of epiphytic algae from Argentine Republic (Lopretto and Tell 1995). We refer to the number of taxa and not the number of species because of the difficulty in identifying microalgae under inverted microscope in our system and the fact that taxonomic resolution varied among groups. Then, richness and diversity were calculated with the number of taxa (genera + species). We are aware that different levels of taxonomic resolution may influence estimates of richness, but we argue that this is a way of considering the important morphological differences among organisms within genera as *Fragilaria*, *Gomphonema* and *Nitzschia*, which allowed us identifying species despite the low magnification level of inverted microscope. The algal diversity was estimated by the Shannon–Wiener index. The autotrophic index (AI) was calculated as the relation between POM (mg/m^2) and Chl-*a* (mg/m^2); high AI values (>200) indicate

that the community is dominated by detritus or heterotrophic organisms (APHA 1995). Epiphytic biomass (POM and Chl-*a*) and abundance were expressed per unit A and DW of macrophyte.

Mesocosm experiment

Commercial plastic imitations of macrophytes of contrasting architecture (Fig. 1) were cut to obtain fragments with identical surface area.

The plastic imitation D7X was obtained from photographs as described for macrophytes. The total surface area was estimated multiplying the 'leaf' area by the number of leaves per whorl and the number of whorls per fragment, for each type of imitation. Plastic imitations were separated at random in six groups of each type that were tied together (3 fragments per group) to plastic baskets (1.3 × 1.3 cm^2 mesh size), with polystyrene floats and an identification mark. On November 2010, pairs of baskets were randomly assigned to plastic trays of 50 l (20 l filtered stream water + 10 l tap water) following a block design (simple, complex).

The water level was controlled so that imitations remained covered with water. After 41 days, plastic imitations were collected from the trays. At the laboratory, epiphytic algae were obtained from imitations by sonication and epiphytic biomass (POM and Chl-*a*) was estimated as described above for macrophytes. The epiphytic biomass was expressed per cm^2, and the AI was calculated as described above.

Data analyses

The differences among macrophyte species in fractal dimension (D7X and D220X), algal abundance and AI were tested by two-way ANOVAs, considering the macrophyte species and the sampling date as factors. Tukey's post hoc comparisons were applied to determine the significance of differences between groups of means. The relationships of both fractal dimensions with the different periphyton parameters (biomass, abundance, taxon richness and diversity) and AI were explored by calculating the product–moment correlations. Most of the conclusions were similar when analyzing variables referred to area or dry weight, so that we only report data of periphyton biomass and algal abundance per square centimeter of macrophyte. When differences between both kinds of variables were observed, results for both variables are reported.

In the experiment with plastic imitations, data were analyzed with one-way ANOVA (factor = level of complexity, 6 replicates) for variable mean D7X per basket, POM, Chl-*a* and AI.

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

Results

Field sampling

The algal community associated with macrophytes included 40 taxa, being dominated by *Nitzschia amphibia* (20 % of total individuals in three sampling occasions) and *Cocconeis* sp. (18 %). Other algae such as *Melosira varians* (14 %), *Gomphonema* sp. (12 %), *Navicula* spp. (12 %), *Fragilaria* spp. (8 %) and *Gomphonema parvulum* (8 %) were also well represented. The algal density differed among macrophytes ($F_{3,54} = 9.982$ and $p < 0.001$) and sampling dates ($F_{2,54} = 13.651$ and $p < 0.001$), being higher in

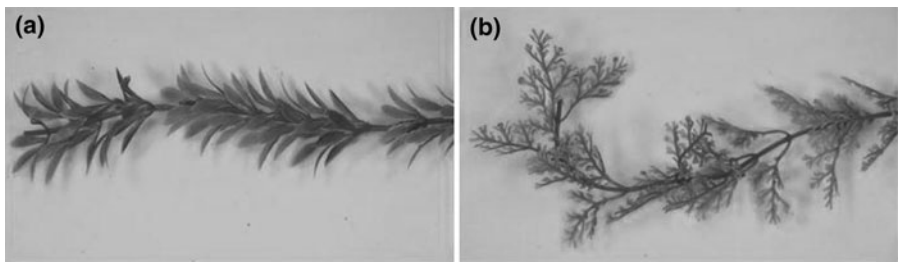


Fig. 1 Photographs of plastic imitations, **a** simple = low fractal dimension and **b** complex = high fractal dimension

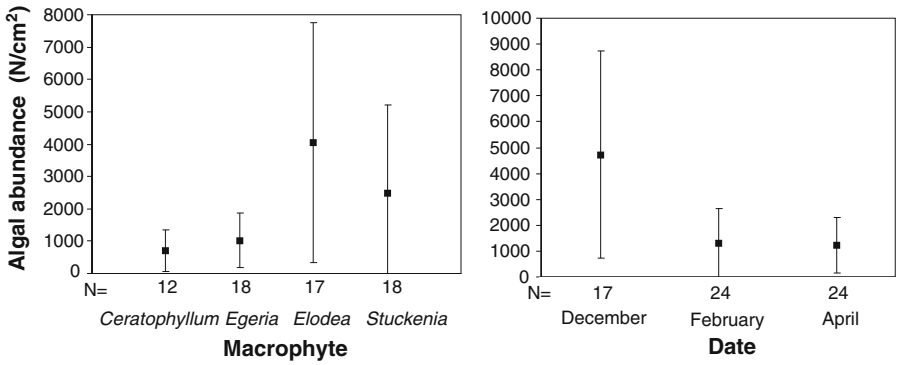


Fig. 2 Mean algal density per macrophyte species and sampling date. *Bars* indicate standard deviation. *N* number of replicates. (*Ceratophyllum* = *Egeria* < *Elodea* = *Stuckenia*: $p < 0.05$) (December > February = April: $p < 0.05$)

Elodea and *Stuckenia* than in the other macrophytes, and in December than in the other sampling dates (Tukey's post hoc comparisons, $p < 0.05$) (Fig. 2).

Nitzschia spp., *Navicula* spp., *Gomphonema* spp. and *Fragilaria* spp. densities were significantly higher in *Elodea* than in the other macrophytes; however, *Cocconeis* sp. was more abundant in *Stuckenia* (ANOVAs and Tukey's post hoc comparisons, $p < 0.05$) (Table 1).

Significant differences were found in D220X among macrophyte species ($F_{3,54} = 13.710$ and $p < 0.001$) and sampling dates ($F_{2,54} = 6.074$ and $p = 0.004$). As for macrophyte species, D220X was higher in *Ceratophyllum* and *Elodea* than in *Egeria* and *Stuckenia*, while for temporal scale, D220X was higher in April than in February (Tukey's post hoc comparisons, $p < 0.05$) (Table 2).

The photographs obtained with the scanning electron microscope did not show thorns, hairs and callosities over the leaves of macrophytes that could have been interpreted as causing differences in leaf rugosity among macrophyte species (Fig. 3).

The biomass quantified by Chl-*a* correlated significantly with D220X ($R = 0.309$ and $p = 0.012$). As for D7X, it slightly correlated with Chl-*a* when chlorophyll was referred to macrophyte area ($R = 0.246$ and $p = 0.048$) but not when it was referred to macrophyte biomass. As for biomass quantified by POM, this was unrelated to D7X but positively related to D220X ($R = 0.432$ and $p < 0.001$) (Fig. 4). Neither D7X nor D220X was related to algal abundance.

The algal taxon richness and diversity were similar for all macrophyte species (Table 3), not being significantly related to DF7X or DF220X.

The AI was similar for all macrophyte species (*Ceratophyllum* = 212 ± 122; *Egeria* = 249 ± 171; *Elodea* = 232 ± 110; *Stuckenia* = 361 ± 442; mean ± standard deviation) and showed no significant relationship with D7X or D220X.

Mesocosm experiment

As expected, D7X was significantly higher in the complex plastic imitations (simple = 1.34 ± 0.02 and complex = 1.59 ± 0.02; mean ± standard deviation) ($F_{1,10} = 363.951$ and $p < 0.001$). Complex plastic imitations presented higher Chl-*a* ($F_{1,5} = 24.915$ and $p = 0.004$) and lower POM than simple ones ($F_{1,5} = 18.417$ and $p = 0.008$). Finally, the periphyton community was dominated either by detritus or by heterotrophic organisms (AI > 200) being AI higher in simple than in complex imitations ($F_{1,10} = 7.894$ and $p = 0.018$) (Fig. 5).

Discussion

Important differences in leaf shape among macrophyte species were found according to D220X, similarly to those previously reported for macrophyte architecture by D7X (Ferreiro et al. 2011). The Chl-*a* and POM were positively associated with D220X, but not to D7X. This partially supports the hypothesis of a positive effect of macrophyte complexity on the periphyton community (Cattaneo and Kalff 1980; Gregg and Rose 1982; Tessier et al. 2008; Hinojosa-Garro et al. 2010). The differences in Chl-*a* and AI between experiment treatments suggest a positive effect of D7X on autotrophic periphyton.

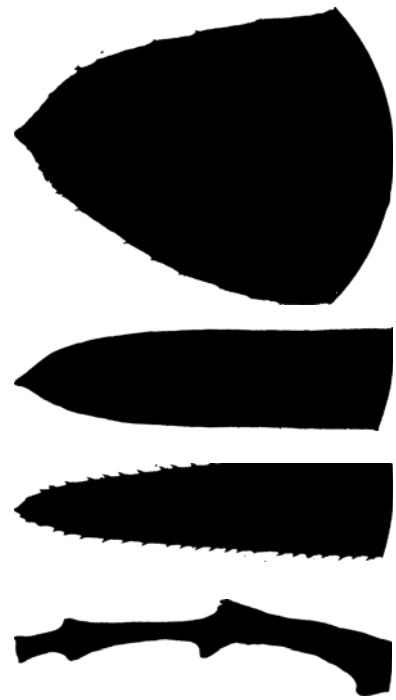
Table 1 Mean algal density (N/cm^2) per macrophyte species for the three sampling dates

Taxon	*Ceratophyllum*	*Egeria*	*Elodea*	*Stuckenia*	All macrophytes
Bacillariophyceae					
Amphora minutissima W. Smith	24 (±59)	8 (±20)	11 (±23)	38 (±97)	20 (±14)
Cocconeis Ehr. sp.	114 (±109)	205 (±227)	453 (±512)	713 (±787)	371 (±269)
Cymbella Agardh sp.	8 (±12)	20 (±36)	73 (±86)	29 (±27)	32 (±28)
Eunotia Ehr. sp.	6 (±6)	12 (±36)	21 (±32)	17 (±45)	14 (±7)
Fragilaria Desm. sp. 1	61 (±100)	55 (±53)	338 (±317)	185 (±272)	160 (±133)
Fragilaria Desm. sp. 2	8 (±11)	10 (±9)	41 (±32)	41 (±41)	25 (±18)
Fragilaria Desm. sp. 3	0.3 (±0.4)	0.3 (±0.5)	1 (±1)	0.4 (±0.9)	0.4 (±0.1)
Gomphonema affine Kütz	11 (±31)	9 (±21)	34 (±38)	13 (±31)	17 (±12)
Gomphonema truncatum Ehr.	0.2 (±0.3)	0.3 (±0.7)	0.3 (±0.9)	0.2 (±0.8)	0.25 (±0.06)
Gomphonema parvulum Kütz	24 (±28)	165 (±424)	361 (±614)	109 (±135)	164 (±143)
Gomphonema Ehr. sp.	61 (±90)	94 (±96)	605 (±1024)	174 (±238)	233 (±252)
Gyrosigma Hassall sp.	0.2 (±0.6)	0.1 (±0.4)	0.2 (±0.8)	0.03 (±0.11)	0.15 (±0.09)
Melosira varians C.A.Ag.	11 (±15)	60 (±98)	408 (±1095)	610 (±1237)	275 (±293)
Navicula peregrina (Ehr.) Kütz	0.1 (±0.3)	0.4 (±0.7)	1 (±3)	1 (±3)	0.6 (±0.4)
Navicula Bory sp. 1	289 (±440)	79 (±168)	474 (±581)	133 (±137)	244 (±178)
Navicula Bory sp. 2	1 (±1)	2 (±4)	5 (±10)	8 (±17)	4 (±3)
Navicula Bory sp. 3	0.3 (±0.5)	0.2 (±0.3)	0.3 (±1.2)	5 (±16)	1 (±2)
Neidium Pfitzer sp.	0.02 (±0.08)	0.05 (±0,21)	–	6 (±24)	1 (±3)
Nitzschia amphibia Grun.	55 (±46)	247 (±330)	1059 (±1249)	281 (±308)	410 (±443)
Nitzschia lacunarum Hustedt/*Nitzschia commutata* Grun.	2 (±7)	0.4 (±0.9)	3 (±6)	4 (±9)	2 (±2)
Nitzschia paleacea (Grun.) M. Peragallo	4 (±9)	2 (±3)	18 (±29)	10 (±18)	8 (±7)
Nitzschia Hassal sp.	0.2 (±0.4)	0.2 (±0.4)	1 (±1)	0.3 (±0.8)	0.3 (±0.2)
Pinnularia Ehr. sp.	3 (±3)	2 (±2)	15 (±21)	5 (±9)	6 (±6)
Pleurosira laevis (Ehr.) Comère	1 (±2)	2 (±4)	5 (±11)	9 (±38)	4 (±4)
Rhoicosphenia abbreviata (Ag) Lange-Bertalot.	1 (±1)	1 (±2)	6 (±12)	1 (±2)	2 (±2)
Stephanocyclus meneghiniana (Kütz) Skabitschevsky	1 (±1)	3 (±4)	6 (±8)	30 (±113)	10 (±13)
Surirella Turpin sp. 1	1 (±1)	0.6 (±0.7)	1 (±1)	1 (±4)	0.8 (±0.4)
Surirella Turpin sp. 2	1 (±1)	0.2 (±0.4)	1 (±2)	–	0.4 (±0.3)
Terpsinoe Ehr. sp.	0.2 (±0.5)	2 (±5)	1 (±1)	1 (±3)	1 (±1)
Chlorophyta					
Bulbochaete C.Agardh sp.	–	0.1 (±0.5)	0.06 (±0.26)	–	0.05 (±0.06)
Chroococcus Kütz sp.	0.1 (±0.2)	0.4 (±0.6)	2 (±4)	2 (±4)	1 (±1)
Cosmarium (Corda) Ralfs sp.	0.02 (±0.08)	0.1 (±0.4)	1 (±2)	1 (±1)	0.4 (±0.4)
Cladophora glomerata (L.) Kütz	1 (±1)	15 (±25)	10 (±11)	5 (±8)	8 (±6)
Closterium (Nitzsch) Ralfs sp.	1 (±1)	1 (±2)	1 (±2)	2 (±2)	1.3 (±0.3)
Scenedesmus Meyen sp.	–	0.05 (±0.20)	0.2 (±0.7)	0.1 (±0.6)	0.09 (±0.08)
Spirogyra Link sp.	3 (±5)	1 (±2)	16 (±37)	2 (±4)	5 (±7)
Stigeoclonium Kütz sp.	9 (±20)	6 (±15)	17 (±27)	10 (±24)	10 (±5)
Zygnema C.Agardh sp.	2 (±2)	3 (±4)	41 (±55)	9 (±15)	14 (±18)
Cyanophyta					
Gomphosphaeria Kütz sp.	–	0.1 (±0.3)	0.4 (±1.8)	–	0.1 (±0.2)
Oscillatoria Vaucher ex Gomont sp.	11 (±21)	2 (±4)	12 (±22)	12 (±21)	9 (±5)
All taxa	714 (±647)	1010 (±820)	4043 (±3709)	2475 (±2742)	2154 (±2724)
N	12	18	17	18	65

±Standard deviation. *N* number of replicates

Table 2 Mean D220X per macrophyte species and sampling date

Macrophyte	Date	D220X	N
Egeria	December	1.20 (±0.02)	6
	February	1.15 (±0.04)	6
	April	1.20 (±0.05)	6
	Mean	1.19 (±0.04)	18
Stuckenia	December	1.17 (±0.03)	6
	February	1.19 (±0.04)	6
	April	1.18 (±0.02)	6
	Mean	1.18 (±0.03)	18
Elodea	December	1.26 (±0.04)	5
	February	1.20 (±0.03)	6
	April	1.28 (±0.04)	6
	Mean	1.25 (±0.05)	17
Ceratophyllum	February	1.22 (±0.02)	6
	April	1.23 (±0.03)	6
	Mean	1.22 (±0.02)	12



±Standard deviation. *N* number of replicates (*Egeria* = *Stuckenia* < *Elodea* = *Ceratophyllum*: $p < 0.05$) (April > February: $p < 0.05$)

Authors who compared periphyton among macrophytes with differences in architecture have usually reported a higher biomass on the complex plants (Cattaneo and Kalff 1980; Gregg and Rose 1982; Tessier et al. 2008; Hinojosa-Garro et al. 2010). Nevertheless, Chl-*a* and POM in the Las Flores stream did not relate to macrophyte complexity for D7X (Ferreiro et al. 2011). A high periphyton biomass was found on a high D7X macrophyte species (*Elodea*); however, the periphyton biomass on other complex species (*Ceratophyllum*) was not significantly higher than that on simpler species (*Stuckenia* and *Egeria*) (Fig. 4). Scanning electron microscope photographs did not show important differences in leaf surface among macrophyte species but differences in shape and presence/number of thorns on borders (also observed in 220X photographs). These structures may serve as attachment points for the epiphytic algae and explain part of differences in the periphyton biomass among *Elodea* (lots of thorns), *Egeria* (few thorns) and *Stuckenia* (no thorns). The importance of edges for epiphytic attachment has previously been reported for diatoms, which were the dominant group in our study. Willer (1922) observed a preferential colonization of *Cocconeis placentula* on the edges and of the green alga *Protoderma viride* on the lamina of *Elodea* (in Cattaneo 1978), and a preference of diatoms for macrophyte leave edges was also reported by Düringer (1958). The epiphytic concentration on the edges and on the lamina was estimated by Cattaneo (1978) observing a preference for edges on natural as well as on plastic leaves, so this preference appears linked to some physical advantage of the margins rather than to some biological activity of the host. The positive relationship between D220X and periphyton biomass (Chl-*a* and POM) may be explained by the ability of D220X of quantifying part of complexity of edges, partly caused by kind, number and distribution of thorns. Once again, the fractal dimension showed in our study how it can easily deal with the scale dependence associated with ecological patterns and processes (Gee and Warwick 1994; Halley et al. 2004).

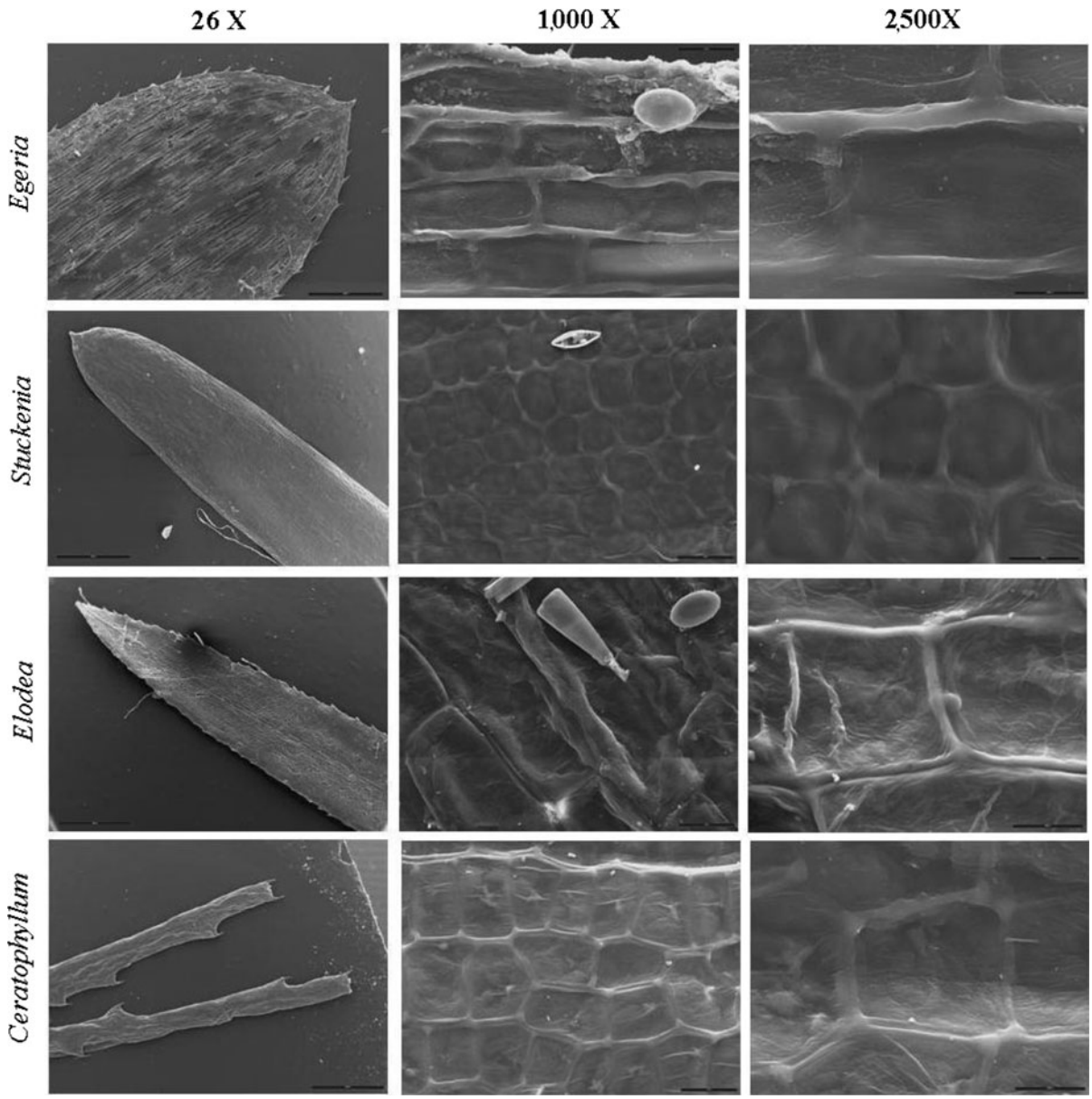


Fig. 3 Photographs from scanning electron microscope, per macrophyte species and magnification

Some algae (*Nitzschia* spp., *Navicula* spp., *Gomphonema* spp. and *Fragilaria* spp.) were more abundant on a high fractal dimension macrophyte (*Elodea*); however, we did not detect an effect of fractal dimension on taxon richness or diversity. This may be due to a lack of difference in the number of algae niches among macrophytes and/or to the taxonomic level of identification (generally genera) that may have been too low to detect differences. More research is needed to clarify this point, as to date only a couple of authors have counted and identified the epiphytic algae associated to macrophytes of contrasting architecture (Cattaneo and Kalff 1980; Jones et al. 2000; Hinojosa-Garro et al. 2010), reporting a positive association of abundance with complexity only for some algal species.

The highest periphyton abundance in December (late spring) is supported by previous studies, which have

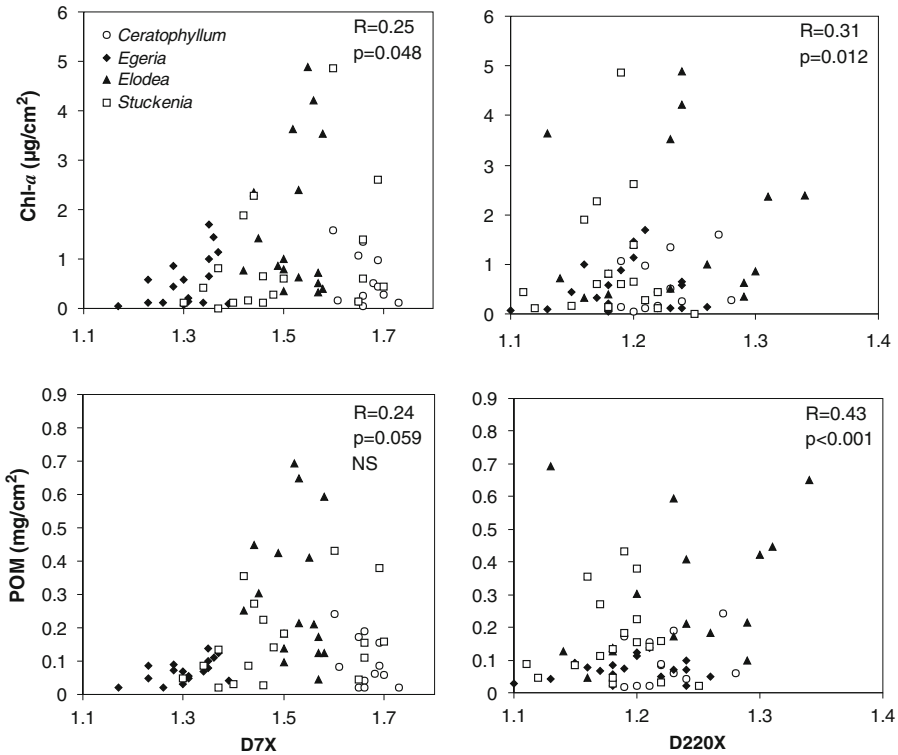


Fig. 4 Relationship of Chl-*a* and POM with D7X and D220X. *R* significant product–moment correlation coefficient, *p* *p* value, *NS* nonsignificant product–moment correlation

Table 3 Mean algal taxon richness and Shannon–Wiener diversity index per macrophyte species for the three sampling dates

	Taxon richness	Taxon diversity	*N*
Ceratophyllum	24 ± 2	0.8 ± 0.2	12
Egeria	22 ± 4	0.8 ± 0.2	18
Elodea	22 ± 4	0.9 ± 0.2	17
Stuckenia	21 ± 3	0.8 ± 0.2	18

±Standard deviation. *N* number of replicates

shown that the development of floating aquatic plant beds in summer has a negative effect on periphyton biomass by shading (Giorgi et al. 2005). Also, the diatom dominance on macrophytes and the main genera observed (*Nitzschia*, *Cocconeis*) agree with previously reported data on periphyton communities in the Las Flores stream (Giorgi and Feijoó 2010). As for habitat heterogeneity, both D7X and D220X were very different among studied macrophytes, being D220X always lower than D7X. The macrophyte species order according to D7X (*Egeria* < *Stuckenia* < *Elodea* < *Ceratophyllum*) was compatible with that supported by D220X (*Egeria* = *Stuckenia* < *Elodea* = *Ceratophyllum*); however, these results suggest that there are less structural differences among macrophytes at 220× than at 7× magnification.

The experimentation with plastic imitations allowed us to study the effect of complexity in the absence of differences in area and rugosity (identical in both treatments) and other factors such as invertebrate abundance and allelopathic substances (absent in mesocosms). The positive effect of D7X on Chl-*a* agrees with the sampling results and could be explained by an effect of architecture on shading. As far as we know, only Dibble et al. (1996) measured the light transmitted through different macrophyte species, finding that shading properties were significantly different among plants, so that transmitted light was moderately negatively correlated with spatial complexity for all plant architectures. However, this was not always dependent on spatial complexity as defined therein (i.e., interstices measurements), but also was dependent on differences in plant architecture (i.e., leaf size, arrangement and position), as the macrophyte with the lowest complexity showed the lowest light transmitted (Dibble et al. 1996). The thing is that complexity estimation performed by Dibble et al.

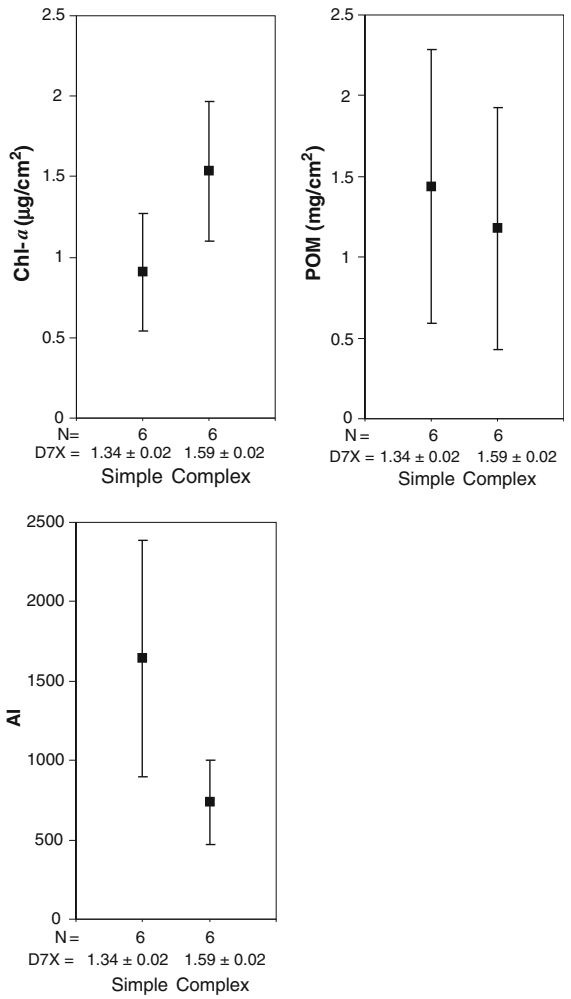


Fig. 5 Mean Chl-*a*, POM and AI per type of plastic imitation. *Bars* indicate standard deviation. *N* number of replicates and *D7X* mean fractal dimension ± standard deviation

(1996) is hardly comparable to that performed by the fractal dimension. We have previously performed estimations of D7X for *Potamogeton ferrugineus* (1.16 ± 0.03; mean ± standard deviation; $N = 5$), *S. striata* (1.33 ± 0.05) and *Myriophyllum aquaticum* (1.58 ± 0.13) (Ferreiro 2012) whose architectures are qualitatively similar to those of *Potamogeton nodosum*, *Potamogeton pectinatus* and *Myriophyllum spicatum* studied by Dibble et al. (1996), respectively. Considering this, plus this study data for *E. densa*, we could propose an order for some Dibble et al. (1996) macrophytes according to fractal dimension: *P. nodosum* < *P. pectinatus* < *E. densa* < *M. spicatum*. This ordering would suggest that complexity quantified by D7X correlates positively with light transmitted, as the percentages of light transmitted reported by Dibble et al. (1996) were as follows: *P. nodosum* = 6.8 %, *P. pectinatus* = 20.5 %, *E. densa* = 36.7 % and *M. spicatum* = 59.2 %. Much research remains to be done about this subject; however, we propose that high D7X values may associate with reduced shading effects by macrophyte leaves and favor development of autotrophic epiphyton.

The negative effect of plastic imitation complexity on POM is not supported by the field sampling results. This might be explained by differences in the species composition of the periphyton community on sampled macrophytes and plastic imitations, as AI showed that plastic imitation periphyton had a lot more of detritus or heterotrophic organisms than the one found over macrophytes. Such effect may be due to environmental differences between the lentic experimental plastic trays and the lotic Las Flores stream, and/or to some limitation of plastic imitations in supporting a regular periphyton community. For example, Cattaneo (1978) studied differences in the distribution and species of algae on the upper and underside of both natural and artificial leaves, finding a greater difference between the upper and underside of the natural leaves, probably due to preferential deposition of $CaCO_3$ mediated by photosynthesis on the upper side of plant leaves. However, a revision by Cattaneo and Amireault (1992) reported that natural diatom assemblages were usually well simulated by those on artificial substrata. Future research on periphyton autotrophs, heterotrophs and detritus in plastic imitations and macrophytes with contrasting complexity at both scales (D220X and D7X) may clarify this point.

The present study on the effects of macrophyte complexity on the periphytic community is complemented by an already published report on macrophyte complexity effect on the abundance, biomass, size, richness and diversity of macroinvertebrates, which was performed on macrophytes sampled by the analyzed here (Ferreiro et al. 2011). The main conclusions of that study were that macrophyte complexity has a positive effect on macroinvertebrate abundance (number of individuals per gram or square centimetre of macrophyte) and that food availability (quantified by POM and Chl-*a*) has a positive effect on macroinvertebrate biomass. No effect of macrophyte complexity on food availability was detected by Ferreiro et al. (2011). However, that study estimated macrophyte complexity

by fractal dimension at 7X and this may not be the ideal scale to study epiphytic algae. Our present results on D220X indicate that the positive effects of macrophyte complexity on macroinvertebrate communities (MacAbendroth et al. 2005; Thomaz et al. 2008; Lucena-Moya and Duggan 2011) may be mediated by positive effects of complexity on food availability, as has also been suggested by a recent experimental study on macroinvertebrate and algae colonization of artificial substrata of contrasting architecture (Hinojosa-Garro et al. 2010). However, several authors have reported an effect of complexity per se on invertebrates (Taniguchi et al. 2003; Hauser et al. 2006; Becerra-Muñoz and Schramm 2007). As POM and Chl-*a* are usually positively related to complexity (Warfe and Barmuta 2006; Lucena-Moya and Duggan 2011), it is often difficult to disentangle their effects and to conclude whether food availability is the main factor driving invertebrate colonization of macrophytes. In addition, our results indicate that macrophyte complexity in periphyton studies should be measured at the same and lower scales than in macroinvertebrate studies, so that estimators of complexity cover several scales relevant for the community.

In this study, both complexity estimators (D7X and D220X) were obtained at very different scales so that they would refer to quite different macrophyte properties. On the one hand, D7X would quantify macrophyte differences in architecture that are important to the associated macroinvertebrates (MacAbendroth et al. 2005; Thomaz et al. 2008) and, according to our experiment, to the autotrophic periphyton. On the other hand, D220X showed a proper quantitative measure of leaf shape differences among macrophytes that are important to the associated autotrophic and heterotrophic periphyton biomass. According to our sampling and experiment, the macrophyte complexity, specially estimated at low scale (D220X), has a positive effect on the establishment of periphyton. This suggests that the well-known positive effect of macrophyte architecture complexity on accompanying macroinvertebrate abundance and biomass may be partially explained by a positive effect of complexity on food availability.

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