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Experimental Analysis of Grazing by the Mayfly *Meridialaris chiloeensis* on Different Successional Stages of Stream Periphyton

key words: Periphyton, grazing, mayflies, diatoms, Andean streams

Abstract

In this study we determined grazing effects of the South Andean endemic mayfly *Meridialaris chiloeensis* on periphyton at different stages of successional development. Grazing effects were studied through a two-factor experimental design (colonization stages X grazer density) in a stream-side channel in spring and winter. Our results showed an absence of proportionality between grazer density and periphyton decline in response to grazers at low and intermediate levels of periphytic biomass; however, when periphyton biomass was high a direct inverse relationship was observed between post-grazing biomass and grazer density. The relationship between periphytic algae (chlorophyll *a* concentration) and periphyton (total periphytic ash-free dry mass) (C/OM index) was used as an estimation of the autotrophic fraction in the total periphyton matrix. Grazing did not alter the C/OM index indicating that both autotrophic and heterotrophic fractions of the periphyton components were reduced in the same proportion. Ordination of samples using the relative abundance of diatom species showed that herbivore effect was less evident at intermediate and late stage of colonization than at early one. These results support the statement that the outcome of the herbivore-periphyton interaction may depend on the successional stage of the periphyton community. In spring *Fragilaria pinnata* relative abundance, on the basis of cell counts, was reduced by grazing and *Nitzschia palea* was enhanced. In the winter experiment, grazing decreased *Achnanthes minutissima* relative abundance.

1. Introduction

Periphyton in natural environments exists as patches of different successional stages, which may or may not follow similar successional trajectories (FISHER, 1983; STEVENSON, 1996). After primary succession occurs, disturbances may disrupt established communities without completely removing all attached biomass, beginning secondary succession (MORIN, 1999). Disturbances by herbivores are of this type and contribute to the characteristic heterogeneity of streams (COLLETTI *et al*., 1987; POFF and NELSON-BAKER, 1997). The outcome of the herbivore-periphyton interaction depends both on the herbivore type (DENICOLA *et al.*, 1990; KAROUNA and FULLER, 1992) and the successional stage of the periphyton community (STEINMAN, 1996). Grazer-induced changes vary with mouthparts morphologies, motility and foraging behaviour of herbivores, as some are better able to access different algal growth habits than others (LAMBERTI *et al.*, 1995; WELLNITZ and WARD, 1998). The influence that a particular herbivore will have on periphytic community structure depends also on total herbivore biomass and density (HILL and KNIGHT, 1987).

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Periphyton biomass is generally reduced by grazing (FEMINELLA and HAWKINS, 1995; STEINMAN, 1996). The amount of periphyton biomass available has a positive effect on grazers since herbivores normally consume more when food limitation is reduced (CATTANEO and MOUSSEAU, 1995). Consequently, negative grazer effects become less severe in nutrient-enriched communities as algal biomass increases (HILLEBRAND, 2002; HILLEBRAND *et al.*, 2002). Nevertheless, periphyton having a large amount of biomass dominated by filamentous algae or having a large proportion of mucilage may be more difficult to remove for some grazers than periphytic mats having low biomass (WELNITZ and WARD, 1998).

Besides algae, periphyton consists of bacteria, protozoa, small metazoa, detritus and inorganic material. If enough light is available, the periphyton is generally dominated by photosynthetic organisms (HILLEBRAND *et al.*, 2002). Macroconsumers that graze on periphyton are capable of ingesting the complete assemblage, including bacteria, protozoa and small metazoa (MORALES and WARD, 2000; SCHMID-ARAYA and SCHMID, 2000). The relationship between periphytic algae (measured as chlorophyll *a* concentration or algal biovolume) and periphyton (measured as total periphytic ash-free dry mass) was suggested as an estimation of the autotrophic fraction in the total periphyton matrix (HILL and KNIGHT, 1987; WELLNITZ *et al.*, 1996).

In Andean-Patagonian streams, periphyton is typically dominated by diatoms, both in terms of biomass and abundance (GAGLIOTI, 1992; 1995). In these low order streams, the herbivorous mayfly *Meridialaris chiloeensis* (DEMOULLIN) is widely distributed and develops dense populations (PESCADOR and PETERS, 1987). This species belongs to the scraper functional feeding group and is able to effectively reduce biomass of one-week old periphyton (DÍAZ VILLANUEVA, 2002). Considering the abundance of *M. chiloeensis* in Andean streams, this species could strongly influence the development of the periphytic community. In this study, we present data from two experiments (spring and winter) designed to assess the influence of grazing by the mayfly *M. chiloeensis* on periphyton assemblages at different stages of successional development.

We aimed to test the following hypotheses: 1) the reduction of periphytic biomass will be greater when initial periphytic biomass is higher and 2) this reduction will be positively correlated with grazer density; 3) considering the scraper function of *M. chiloeensis* mouthparts, capable of removing nearly all the periphyton from substrata, its grazing impact will reduce both autotrophic and heterotrophic periphytic components equally and cause no variation in the C/OM index; and 4) the algal species response to grazing would differ in relation to periphyton successional stage (different pre-colonization times).

2. Methods

2.1. Study Site

Two field experiments were carried out in a streamside channel at the Centro de Salmonicultura (Universidad Nacional del Comahue) beside Gutiérrez stream (41°07′ S and 71°25′ W, Patagonia, Argentina). Gutiérrez stream flows through an open valley of fluvioglacial origin, along 6 km from Gutiérrez lake (785 m a.s.l.) into Nahuel Huapi lake (764 m a.s.l) with a mean slope of 4 m km^{-1}. The flow velocity varied between 23 and 96 cm s^{-1} in spring and winter respectively. The maximum discharge is in fall (rain and snow) and spring (snow-melt) and minimum in late summer. Gutiérrez stream water is extremely clear, with a pH of 7, low conductivity values (56 μS cm^{-1}) and very low nutrient concentrations (4 μg l^{-1} of P-TDP in winter and 5 μg l^{-1} of P-TDP in spring) (DÍAZ VILLANUEVA *et al.*, 2000).

2.2. Experimental Design

The different grazing pressure on periphyton successional stages was analysed using a two-factor experimental design. To evaluate the effect of contrasting environmental conditions, the experiment was carried out twice, in spring (17 October – 12 November 1998) and in winter (1–30 July 1999).

Experiments were conducted in an experimental channel of $400 \times 40 \times 40$ cm fibreglass structure. The channel was fed with Gutiérrez stream water and water level was maintained at 14 cm depth with a discharge of 25.8 l min^{-1}, both in spring and winter. Temperature was recorded weekly during the experiment. Daily Photosynthetic Active Radiation (PAR) was recorded by a Radiometer GUV-510 Biosphaerical Instruments (San Diego, CA) located at the Centro de Salmonicultura, besides the experimental channel. This sensor recorded solar radiation every minute.

Experimental units (treatments and controls) consisted of unglazed ceramic tiles (8×8 cm) as substrate for periphyton growth and grazing. The tiles were covered by transparent Plexiglas half cylinder (4 cm radius) that formed a semicircular tunnel, with both ends closed with a net of 1 mm mesh.

We examined three levels of algal pre-colonization time (T1: one week, T2: two weeks and T3: three weeks) and three levels of grazing pressure (ungrazed, three and five grazers). The different stages of periphyton colonization were obtained by placing 16 experimental units in the channel on each of three occasions: one, two and three weeks before grazer introduction. Last instars of *Meridialaris chiloeensis* were collected from Gutiérrez stream and introduced to the enclosures at two different densities, 469 indiv. m^{-2} (three grazer per enclosure) and 780 indiv. m^{-2} (five grazers per enclosure). The experimental abundance of three grazers per unit resembles the winter abundances in the field (457 ± 183 indiv. m^{-2}) which is higher than in spring (66 ± 9 indiv. m^{-2}). Treatments were run in four replicates, randomly distributed within the channel, and for one week. To avoid screen clogging the screens were manually cleaned every three days. On the day of grazer introduction, four replicates of each colonization time were taken as initial controls. Grazer mortality was checked after four days and at the end of the experiment. Mortality was 0.03 indiv. day^{-1} both in spring and winter. After one week of grazer introduction, all sampling units were collected placed in dark thermally insulated containers and carried to the laboratory.

2.3. Laboratory Analysis

In the laboratory, grazers were dried and weighed to quantify their final biomass. It was assumed that grazer biomass did not substantially change during one week of experiment. Periphyton was scraped from each tile with a razor-blade and washed with 100 ml distilled water. The periphyton samples were homogenised and fractionated into three subsamples of 30 ml, to determine biomass as Chl *a*, ash free dry mass (AFDM), and algal species composition and cell abundances. Subsamples for chlorophyll *a* determination were filtered through Whatman GF/C filters and extracted with hot 90% ethanol following NUSCH (1980). Measurements were carried out with a spectrophotometer at 665 nm and 750 nm, and corrections for pheophytin were performed after acidification with HCl. Subsamples for periphyton organic matter determinations (ash free dry mass, AFDM) were filtered onto pre-weighed, pre-combusted Whatman GF/C filters, and dried at 80 °C for 1.5 h. The filters were weighed, combusted at 550 °C for 1 h, and re-weighed, to determine AFDM (APHA, 1989).

Samples for species composition and abundance estimations were preserved in 4% formalin. The percentage of diatoms and non-diatom algae was calculated based on direct counts of intact, protoplast-containing cells observed in a Sedgwick-Rafter chamber of 18 μl under a microscope at 400X of magnification. Diatoms were identified and counted from a 10 ml subsample, which was treated with hydrogen peroxide to oxidise organic matter, and then mounted in Naphrax® and examined at 1000X. A minimum of 800 valves were counted in each sample.

2.4. Calculations

After identification, total algal abundance, species relative abundance, diversity (Shannon's index, H'), species richness and evenness ($J = H'/H_{max}$) were calculated. Simpson's index ($C = \Sigma p_i^2$, where p_i is the proportion of each species) was applied to estimate dominance.

To estimate the relative importance of the photosynthetic fraction in the community the relationship between chlorophyll *a* and organic matter (C/OM index) was calculated (WEBER and MCFARLAND, 1969).

Statistical differences were assessed using one-way and two-way ANOVA. *A posteriori* comparisons were performed with Bonferroni's test, and normality and homocedasticity were tested using Sigma Stat 2.03.

Taxonomic structure of diatom assemblages on ungrazed and grazed communities of different successional ages was compared by detrended correspondence analysis (DCA) using the program DECORANA (HILL, 1979). The analysis was based on relative abundances of diatom species (cell counts) that comprised an average of ≥2% of all cells counted (16 and 18 diatom species in spring and winter, respectively).

3. Results

3.1. Periphyton Biomass

Water temperature and irradiance differed between spring and winter experiments. In spring, temperature was 11 ± 1 °C and 7 ± 1 °C in winter. During the spring experiment, average of daily cumulative PAR irradiance was 4909 ± 149 μmol photon cm^{-2} with maximum irradiance of 2285 ± 56 μmol photon m^{-2} s^{-1} and during the winter experiment the values decreased to 1339 ± 77 μmol photon cm^{-2} of cumulative PAR and 969 μmol photon m^{-2} s^{-1} of maximum irradiance. In addition, biomass of individual grazers changed, with higher values in spring (2.58 ± 0.80 mg $indiv^{-1}$) than in winter (1.31 ± 0.08 mg $indiv^{-1}$).

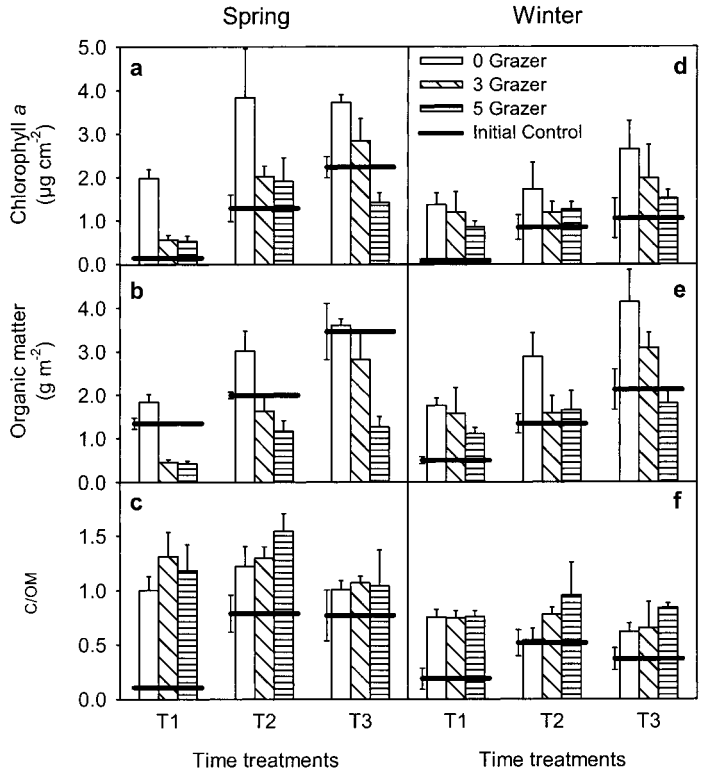

Figure 1. Periphyton chlorophyll *a* concentration, organic matter as AFDM and the C/OM index in spring and winter experiments. Values are given as an average; vertical bars indicate SE. Initial control

Table 1. Results from two-way ANOVA of Chlorophyll *a* concentration, AFDM and the C/OM ratio in both experiments. References: 0G: Ungrazed; 3G: 3 grazers; 5G: 5 grazers. T1, T2 and T3: 1, 2 and 3 weeks of pre-colonization time. S: significant, $P < 0.05$ NS: non-significant $P > 0.05$. Underlined treatments were not significantly different when assessed with *a posteriori* test (BONFERRONI) at $P > 0.05$.

Experiment	Source of variation		*P*		Significant differences (Bonferroni's test)
Spring	Chlorophyll *a*				
		G	0.0001	S	0G 3G 5G
		T	0.0001	S	T1 T2 T3
		G × T	0.3673	NS	
	AFDM				
		G	< 0.0001	S	0G 3G 5G
		T	< 0.0001	S	T1 T2 T3
		G × T	0.1886	NS	
	C/OM index				
		G	0.4085	NS	0G 3G 5G
		T	0.1031	NS	T1 T2 T3
		G × T	0.8265	NS	
Winter	Chlorophyll *a*				
		G	0.1237	NS	0G 3G 5G
		T	0.0328	S	T1 T2 T3
		G × T	0.8861	NS	
	AFDM				
		G	0.0023	S	0G 3G 5G
		T	0.0009	S	T1 T2 T3
		G × T	0.2950	NS	
	C/OM index				
		G	0.1603	NS	0G 3G 5G
		T	0.8521	NS	T1 T2 T3
		G × T	0.6236	NS	

By contrast, nutrient concentrations did not vary during our experiments (~4–5 μg l^{-1} of P-TDP).

In the first experiment (spring), initial periphyton biomass was higher in T3 (Fig. 1a–b). Chl *a* concentration in ungrazed treatment (0 grazers) reached a maximum in periphyton having had two weeks of pre-colonization (T2), and remained constant after one week (Fig. 1a). Organic matter (AFDM) accumulation increased from T1 to T2 while non significant statistical differences were observed between T2 and T3 (Fig. 1b). The C/OM index indicated that in the young periphyton (T1) the photosynthetic fraction increased compared with the initial controls (one-way ANOVA, $P < 0.05$), suggesting an increase in autotrophic biomass (Fig. 1c). Periphyton biomass decreased significantly in the presence of grazers, both in terms of Chl *a* and AFDM (Table 1, Fig. 1a and b). The lowest periphytic biomass (0.4 g AFDM m^{-2}) was reached in the treatment T1, without differences between the two grazers densities (Fig. 1a and b). The greatest reduction comparing grazed with ungrazed treatments was found in T3 with five grazers (Table 1, Fig. 1b). Grazing did not alter C/OM

index (Table 1, Fig. 1c), indicating that autotrophic-heterotrophic relationships did not change as a consequence of herbivory.

In the winter experiment, ungrazed periphyton biomass (Chl *a* and AFDM) consistently increased with community age, although Chl *a* values were comparatively lower than in spring (Fig. 1d and e). On the other hand, the AFDM values were similar to those of spring yielding significant lower C/OM index values (one-way ANOVA; $P < 0.05$) (Fig. 1f). Grazing reduced periphyton AFDM but not Chl *a* concentration (Table 1, Fig. 1e). Comparing with ungrazed periphyton biomass, the highest reduction was found in periphyton having had three weeks of pre-colonization, exposed to five grazers (Fig. 1e). Grazed periphyton biomass was similar regardless of pre-colonization time, ranging from 1.1 to 1.8 g AFDM m^{-2}, except in the treatment with three grazers in T3 (Fig. 1e). As observed in the spring experiment, grazing did not significantly alter C/OM index (Table 1, Fig. 1f); although OM was reduced by grazers, values of Chl *a* compensated the losses of OM when calculating the index (Fig. 1d–f).

3.2. Periphyton Community Structure

Diatoms dominated the periphyton both in the spring and winter experiments, representing 87% and 100% of total algal abundance, respectively. In the spring experiment, diatom abundances in ungrazed periphyton reached the maximum value after two weeks of

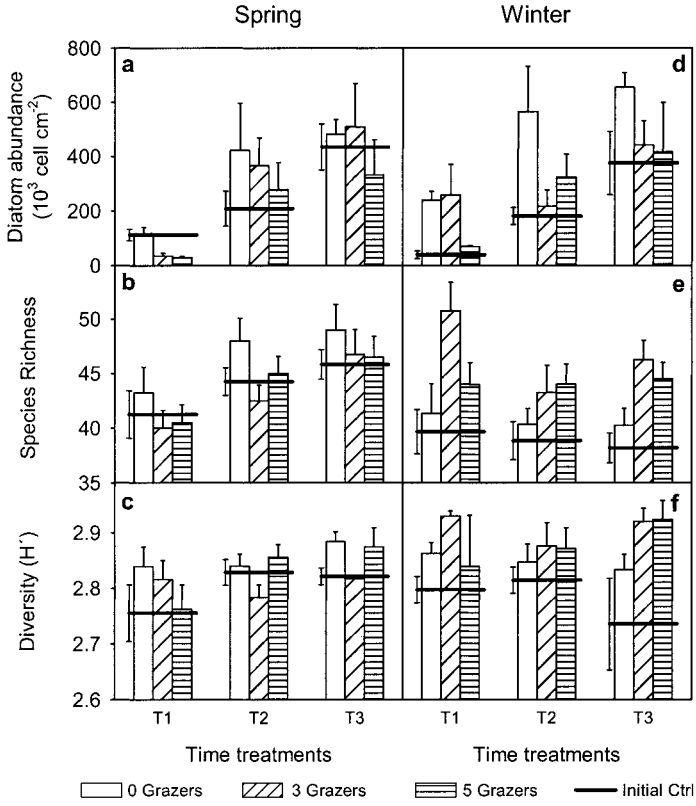

Figure 2. Periphytic diatom abundance, species richness and diversity in spring and winter experiments. Vertical bars indicate SE. Initial control error bars (± SE) are indicated at the left.

Table 2. Result from two-way ANOVA of diatom abundances, species diversity and species richness in both experiments. References: 0G: Ungrazed; 3G: 3 grazers; 5G: 5 grazers. T1, T2 and T3: 1, 2 and 3 weeks of pre-colonization time. S: significant $P < 0.05$, NS: non-significant $P > 0.05$. Underlined treatments were not significantly different when assessed with *a posteriori* test (Bonferroni) at $P > 0.05$.

Experiment	Source of variation	Factor	*p*		Significant differences (Bonferroni's test)
Spring	Abundance				
		G	0.2152	NS	0G 3G 5G
		T	0.0001	S	T1 T2 T3
		G × T	0.9185	NS	
	Diversity				
		G	0.1252	NS	0G 3G 5G
		T	0.0843	NS	T1 T2 T3
		G × T	0.2049	NS	
	Richness				
		G	0.0630	NS	0G 3G 5G
		T	0.0016	S	T1 T2 T3
		G × T	0.9207	NS	
Winter	Abundance				
		G	0.0055	S	0G 3G 5G
		T	0.0001	S	T1 T2 T3
		G × T	0.2053	NS	
	Diversity				
		G	0.0580	NS	0G 3G 5G
		T	0.6129	NS	T1 T2 T3
		G × T	0.3626	NS	
	Richness				
		G	0.0009	S	0G 3G 5G
		T	0.1639	NS	T1 T2 T3
		G × T	0.2721	NS	

pre-colonization (T2) and this value was maintained in the community of three weeks of pre-colonization (Table 2, Fig. 2a). Species richness showed the same pattern as diatom abundance; however, species diversity did not change throughout the spring experiment (Table 2, Fig. 2b, c). Grazing did not significantly affect any of these parameters (Table 2, Fig. 2a–c).

During spring, neither ungrazed nor grazed periphyton contained a clear dominant species. Thus, dominance values were low (C = 0.084 to 0.098) and evenness values were high (range 0.69–0.81). The two most abundant species were *Fragilaria capucina* DESM. and *Achnanthes minutissima* KÜTZ. (15% of total abundance) followed by *Fragilaria pinnata* EHR. and *Cyclotella stelligera* CL. & GRUN. (10% of total abundance) (Table 3).

During the winter experiment, diatom density increased steadily (Fig. 2d). As in spring, dominance values were low ($C = 0.082$ to 0.104), but substantial changes in species composition occurred during the experiment. Grazing significantly depressed diatom abundances, but without differences between grazing pressures (Table 2, Fig. 2d). In addition, grazing did not modify species diversity but species richness was significantly

Table 3. Abundances (mean 10^3 cell cm^{-2} ± s.e.) and code of diatom species used in the DCA analysis during the spring experiment. References: T1, T2 and T3: Treatments 1, 2 and 3. IC: Initial Control. 0G: ungrazed, 3G and 5 G:, 3 and 5 grazers.

Species	Code	T1				T2				T3			
		IC	0G	3G	5G	IC	0G	3G	5G	IC	0G	3G	5G
Achnanthes minutissima KÜTZ	Amin	18.8 ±3.4	20.8 ±4.6	5.5 ±1.7	4.3 ±0.4	45.0 ±21.1	64.2 ±27.7	68.3 ±21.9	45.9 ±20.4	58.9 ±11.9	67.5 ±7.8	91.2 ±27.6	53.2 ±20.5
A. pusilla (KÜTZ.) KÜTZ.	Apus	3.1 ±1.2	2.6 ±0.2	0.5 ±0.1	0.4 ±0.0	3.2 ±1.6	8.3 ±4.0	5.5 ±1.5	6.7 ±2.7	7.1 ±1.8	9.4 ±1.4	9.1 ±2.4	6.0 ±1.9
Cocconeis placentula EHR.	Cpla	7.5 ±1.9	8.3 ±1.7	1.8 ±0.5	1.4 ±0.2	17.0 ±6.6	21.0 ±8.3	16.4 ±3.3	13.9 ±5.0	23.5 ±4.4	25.4 ±3.1	22.9 ±6.1	12.7 ±4.1
Cyclotella stelligera CL. & GRUN.	Cste	13.5 ±2.8	12.5 ±1.5	3.5 ±1.1	2.0 ±0.2	23.5 ±8.8	36.7 ±11.7	30.8 ±5.6	23.6 ±8.5	36.9 ±5.9	51.3 ±8.8	48.3 ±14.5	28.7 ±11.1
Cymbella silesiaca BLEISCH	Csil	2.5 ±0.5	4.6 ±0.4	1.2 ±0.4	0.8 ±0.1	6.5 ±1.9	14.3 ±4.6	12.6 ±4.3	10.2 ±2.3	16.4 ±3.5	17.3 ±2.6	14.2 ±3.6	10.8 ±4.7
Fragilaria bicapitata MAYER	Fbic	4.2 ±0.6	8.0 ±0.9	1.6 ±0.3	1.5 ±0.6	12.9 ±6.1	18.0 ±11.8	14.6 ±4.3	9.4 ±2.2	13.7 ±0.4	24.1 ±2.5	10.4 ±2.6	10.9 ±3.1
F. capucina DESM.	Fcap	17.7 ±2.6	25.3 ±2.2	6.1 ±1.5	6.1 ±1.1	40.4 ±18.5	67.5 ±25.1	54.2 ±15.8	50.0 ±17.4	84.7 ±14.1	78.4 ±9.4	77.9 ±24.6	57.5 ±25.2
F. construens (EHR.) GRUN	Fcon	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.3 ±0.2	0.0 ±0.0	0.0 ±0.0	0.2 ±0.2	5.6 ±3.8	0.0 ±0.0	0.0 ±0.0	8.4 ±4.6	6.2 ±1.0
F. pinnata EHR.	Fpin	13.4 ±3.5	18.2 ±3.9	2.0 ±0.6	1.4 ±0.2	24.7 ±9.7	38.4 ±8.9	31.3 ±7.6	25.8 ±9.6	48.7 ±5.5	39.9 ±5.3	41.2 ±13.3	24.2 ±10.4
Gomphonema angustum AGARDH	Gang	2.3 ±0.2	1.4 ±0.6	0.5 ±0.1	0.7 ±0.2	4.6 ±1.6	7.3 ±2.0	8.7 ±3.2	4.4 ±1.5	9.1 ±2.6	7.8 ±1.3	7.2 ±3.0	4.6 ±1.6
Melosira varians AGARDH	Mvar	1.8 ±0.5	4.3 ±1.5	0.3 ±0.1	1.1 ±0.2	7.5 ±5.1	21.8 ±17.6	2.7 ±1.2	4.1 ±1.6	10.4 ±4.0	5.9 ±1.1	4.5 ±0.8	7.7 ±4.6
Navicula cryptocephala KÜTZ.	Nacr	1.6 ±0.2	2.1 ±0.3	0.8 ±0.3	0.4 ±0.0	3.7 ±1.6	10.0 ±4.1	7.3 ±2.0	7.3 ±3.7	5.4 ±0.5	10.4 ±1.2	9.2 ±4.5	5.4 ±2.4
Nitzschia linearis (AGARDH) W. SMITH	Nlin	5.5 ±1.2	7.1 ±1.4	2.1 ±0.6	1.3 ±0.2	11.8 ±4.9	30.1 ±15.6	24.6 ±7.5	15.3 ±5.7	32.0 ±4.9	39.3 ±3.5	41.6 ±15.6	28.9 ±12.9
N. palea (KÜTZ.) W. SMITH	Npal	2.4 ±0.6	5.9 ±0.9	3.1 ±0.2	3.4 ±0.6	7.0 ±3.2	11.8 ±6.1	25.4 ±8.9	7.1 ±1.5	23.1 ±2.6	13.1 ±4.1	33.4 ±12.2	17.6 ±7.0
N. recta HANTZSCH	Nrec	3.7 ±0.7	5.4 ±0.5	1.7 ±0.5	1.3 ±0.1	9.9 ±4.3	26.0 ±16.4	19.0 ±5.9	14.8 ±4.3	22.7 ±4.9	23.4 ±2.1	25.7 ±8.8	18.6 ±7.4
Synedra ulna (NITZ.) EHR.	Suln	2.1 ±0.7	4.3 ±1.1	1.0 ±0.3	0.7 ±0.1	4.5 ±2.2	5.1 ±0.5	4.9 ±1.2	3.2 ±0.9	6.7 ±1.9	9.8 ±1.5	6.6 ±1.6	5.7 ±2.0

Table 4. Abundances (mean 10^3 cell $cm^{-2} \pm$ s.e) and code of diatom species used in the DCA analysis during the winter experiment. References: T1, T2 and T3: Treatments 1, 2 and 3. IC: Initial Control. 0G: ungrazed, 3G and 5 G: 3 and 5 grazers.

Species	Code	T1				T2				T3			
		IC	0G	3G	5G	IC	0G	3G	5G	IC	0G	3G	5G
Achnanthes minutissima KÜTZ.	Amin	4.0 ±1.9	35.2 ±6.9	26.0 ±10.4	5.6 ±0.9	15.7 ±3.4	100.9 ±33.5	26.2 ±6.9	44.7 ±10.4	27.4 ±5.7	122.3 ±10.6	56.0 ±8.8	62.7 ±24.2
Cocconeis placentula EHR.	Cpla	2.3 ±0.9	8.1 ±0.9	9.3 ±3.3	2.7 ±0.5	5.7 ±1.3	18.4 ±5.3	6.5 ±1.9	9.9 ±4.0	8.4 ±2.3	15.4 ±2.8	12.5 ±2.8	12.8 ±6.0
Cyclotella stelligera CL. & GRUN.	Cste	2.0 ±0.7	11.8 ±3.9	8.9 ±4.0	2.6 ±0.0	5.1 ±0.4	20.0 ±7.4	6.8 ±1.7	8.6 ±3.5	4.6 ±1.0	19.8 ±2.6	12.4 ±2.7	9.1 ±2.8
C. meneghiniana KÜTZ.	Cmen	0.9 ±0.5	3.3 ±0.8	2.5 ±1.1	1.7 ±0.4	1.7 ±0.3	3.5 ±0.9	1.5 ±0.5	3.9 ±2.1	0.9 ±0.4	8.6 ±2.8	4.1 ±0.9	3.0 ±0.9
Cymbella silesiaca BLEISCH	Csil	7.8 ±1.9	34.1 ±0.7	34.1 ±14.5	10.2 ±1.9	20.8 ±2.9	68.3 ±16.5	27.9 ±8.1	39.9 ±12.3	28.6 ±7.2	83.2 ±7.5	58.7 ±16.0	50.8 ±18.9
Fragilaria bicapitata MAYER	Fbic	1.4 ±0.4	8.3 ±1.5	9.9 ±4.5	3.4 ±0.3	5.4 ±1.6	18.8 ±6.0	15.8 ±4.2	22.4 ±5.3	7.8 ±3.6	25.1 ±3.2	19.9 ±2.9	22.7 ±10.9
F. capucina DESM.	Fcap	2.3 ±0.8	9.3 ±2.0	9.4 ±4.0	2.9 ±0.9	7.7 ±1.5	23.8 ±7.7	9.2 ±2.4	12.8 ±3.3	10.4 ±4.1	23.9 ±3.1	18.0 ±5.0	16.7 ±7.1
F. construens (EHR.) GRUN	Fcon	0.0 ±0.0	7.9 ±1.5	10.8 ±4.4	1.6 ±1.0	3.2 ±1.1	14.6 ±5.3	7.6 ±1.9	18.6 ±9.0	7.8 ±6.5	12.5 ±3.0	15.3 ±5.3	20.9 ±11.1
F. pinnata EHR.	Fpin	3.5 ±2.0	19.7 ±7.1	34.6 ±19.2	6.3 ±3.5	13.4 ±2.2	53.4 ±20.5	24.7 ±8.6	27.2 ±5.3	21.3 ±0.3	58.9 ±8.6	51.2 ±14.5	32.2 ±21.1
Gomphonema angustum AGARDH	Gang	1.7 ±0.5	4.4 ±1.4	7.9 ±4.1	2.2 ±0.3	4.1 ±1.3	14.9 ±5.3	5.3 ±0.8	7.8 ±2.0	7.2 ±1.6	18.4 ±2.4	11.1 ±2.3	11.3 ±5.1
Melosira varians AGARDH	Mvar	0.4 ±0.1	2.1 ±0.7	3.0 ±1.3	0.8 ±0.6	0.6 ±0.4	5.2 ±2.3	3.2 ±1.3	4.8 ±1.3	2.8 ±2.1	7.7 ±2.0	9.1 ±3.1	11.9 ±2.1
Navicula cryptocephala KÜTZ.	Nacr	0.1 ±0.1	1.2 ±0.3	0.7 ±0.5	0.4 ±0.0	1.0 ±0.6	6.0 ±2.4	1.3 ±0.5	0.9 ±0.3	0.7 ±0.4	2.0 ±0.7	2.3 ±1.3	1.2 ±0.8
Nitzschia linearis (AG.) W. SMITH	Nlin	0.6 ±0.0	7.8 ±1.4	2.9 ±1.0	0.9 ±0.1	2.6 ±0.8	12.0 ±3.7	3.7 ±1.3	7.2 ±2.2	0.5 ±0.2	15.9 ±2.8	8.5 ±1.2	12.6 ±9.1
N. recta HANTZSCH	Nrec	0.6 ±0.2	3.8 ±0.2	2.9 ±1.1	1.0 ±0.0	2.5 ±1.0	12.4 ±3.7	4.0 ±1.6	7.9 ±3.3	2.9 ±1.7	15.2 ±2.0	7.6 ±1.9	6.4 ±3.1
N. supralitorea LANGE-BERTALOT	Nsup	0.6 ±0.1	4.0 ±1.2	3.1 ±1.6	0.9 ±0.2	2.3 ±0.7	17.6 ±4.3	4.1 ±1.7	6.9 ±3.1	2.5 ±1.0	16.2 ±2.9	12.0 ±3.1	10.5 ±7.2
Nitzschia sp.	Nsp	0.4 ±0.1	4.7 ±0.4	3.9 ±1.4	0.5 ±0.1	2.6 ±0.5	12.8 ±4.3	2.8 ±1.3	4.2 ±1.6	7.2 ±1.4	10.7 ±4.4	7.1 ±2.0	8.1 ±4.6
Synedra pulchella (RALFS) KÜTZ.	Spul	0.0 ±0.0	7.4 ±1.1	15.5 ±7.6	7.2 ±1.9	0.0 ±0.0	20.0 ±5.7	11.3 ±2.3	15.1 ±3.4	0.0 ±0.0	22.0 ±1.8	23.8 ±4.8	17.2 ±2.1
S. ulna (NITZ.) EHR.	Suln	1.5 ±0.7	11.2 ±2.0	12.4 ±5.0	4.0 ±1.7	1.8 ±0.3	15.5 ±6.7	6.1 ±2.6	8.9 ±4.7	2.8 ±1.7	17.3 ±8.8	7.1 ±2.4	5.5 ±0.2

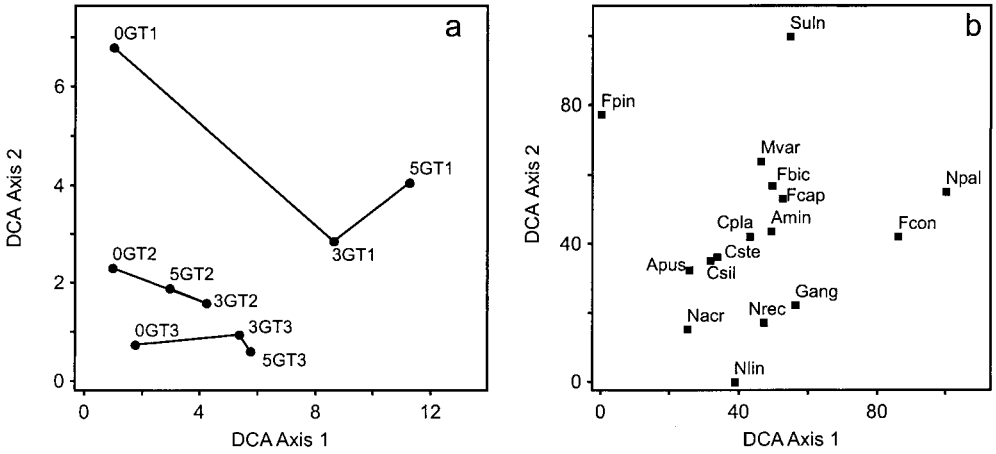

Figure 3. DCA ordination based on relative abundances of diatom species (cell counts) in spring experiment. a: samples scores for ungrazed (0G) and grazed treatment (3G and 5G) at different colonization times (T1, T2 and T3). Eigenvalues for axis 1 = 0.026; for axis 2 = 0.004. b: species scores. See Table 3 for abbreviations.

enhanced, especially in the young periphyton (T1) (Table 2, Fig. 2e and f). The relative abundance of *Cymbella silesiaca* BLEISCH reached 24% in the one-week initial control, but during succession it decreased. This pattern was observed both in the ungrazed and grazed treatments (Table 4). At the same time, the relative abundance of *A. minutissima* increased in the ungrazed treatment, from 17% of total abundance in T1 to 22% in T3. However, this species was substantially reduced by grazers, allowing *F. pinnata* and *Synedra pulchella* KÜTZ. to increase their abundances. In particular, the latter was enhanced in treatment T1 by three grazers (Table 4).

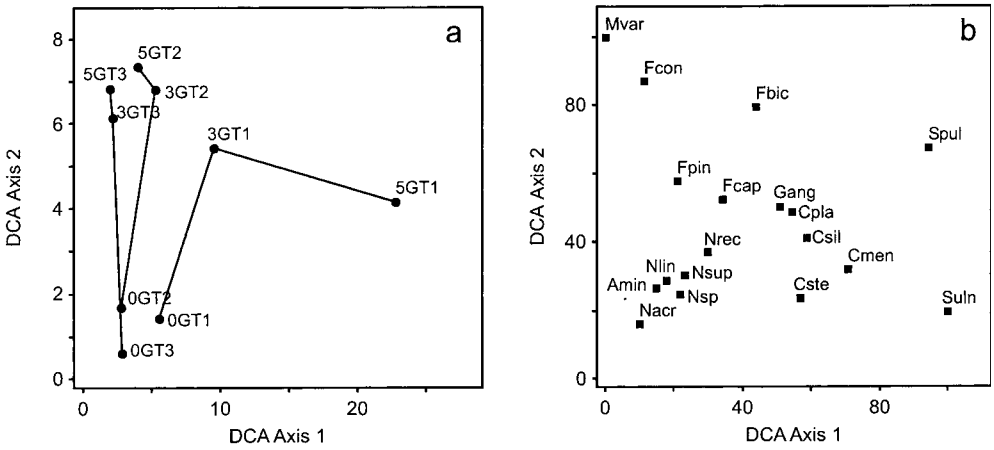

Figure 4. DCA ordination based on relative abundances of diatom species (cell counts) in winter experiment. a: samples scores for ungrazed (0G) and grazed treatment (3G and 5G) at different colonization times (T1, T2 and T3). Eigenvalues for axis 1 = 0.054; for axis 2 = 0.013. b: species scores. See Table 4 for abbreviations.

The trajectories of taxonomic change combining the three levels of algal pre-colonization time (T1, T2 and T3: one, two and three weeks of pre-colonization) and the three levels of grazing pressure (0G, 3G and 5G) showed that, in spring, *M. chiloeensis* had distinct effect on periphyton of different colonization times (Fig. 3). The influence of grazing on taxonomic change (DCA axis 1) was greater when the herbivores were introduced in the periphyton of one week of colonization (Fig. 3a). Also, the difference between grazing pressure (3G and 5G) was higher in the young community. The species ordination scores showed that *F. pinnata* (Fpin) was a species highly related to the ungrazed and young communities (Fig. 3b), while *N. palea* (Npal) was favoured by grazing (Fig. 3b).

In the winter experiment, the influence of grazing on taxonomic change was greater when five herbivores were introduced in the periphyton of one week of colonization (Fig. 4a). In the other cases, the three periphyton stages were similarly influenced by the two levels of grazing pressure (Fig. 4a). The species ordination scores showed that four *Nitzschia* species (Nlin, Nsup, Nrec, Nsp) and *A. minutissima* (Amin) were favoured in the ungrazed treatments while *Melosira varians* (Mvar) and *F. construens* (Fcon) were related to grazed communities (Fig. 4b).

4. Discussion

In spring, algal colonization in the absence of grazers showed a common pattern in which an initial accumulation phase is followed by an asymptote one (BIGGS, 1996) (Fig. 1a). In contrast, algal biomass accumulation (Chl *a*) in the winter experiment was slower, and did not reach an asymptote during the four weeks of colonization. In addition, the C/OM index was higher in spring indicating a higher proportion of the autotrophic fraction. Colonization is typically slower under low light intensity and temperature (STEVENSON, 1996) although algal cells may compensate for lower PAR irradiance by increasing cellular Chl *a* (WELLNITZ and WARD, 2000). Temperature and irradiance differed substantially between the two experiments, and probably caused the lower Chl *a* concentration values in winter. In addition, algal species representation and cell supply rates in the colonization pool would differ. Also the biomass of individual grazers changed, with higher values in spring than in winter. Similarly, WELLNITZ and WARD (2000) observed that the biomass of the mayfly *Ecdyonurus* sp. was correlated with higher reductions in periphytic abundance and lower species richness.

Grazing by *Meridialaris chiloeensis* had distinct effects on periphyton of different colonization times (Fig. 1). Nevertheless, removal of periphyton by grazers was not directly related with the available periphyton biomass. This result rejected our first hypothesis that higher initial periphytic biomass would be more reduced by grazing. WELLNITZ and WARD (1998) suggested that periphyton having a large amount of biomass may be more difficult to remove if dominated by filamentous algae or having a large proportion of mucilage. In our experiments, we did not observe the dominance of filamentous algae, but the mucilage was not quantified. Therefore changes toward a higher mucilage proportion might cause the absence of a direct relationship between removal and available biomass.

The second hypothesis, highly related with the first one, stated that the reduction of periphytic biomass would be greater with higher grazer density. Our results showed an absence of proportionality between grazer density and periphyton decline in T1 and T2 in both experiments. By contrast, in T3 treatments a direct relationship was observed. Therefore, our hypothesis was not confirmed by the results, which showed that in low and intermediate periphyton biomass (T1 and T2) the food resources (algae) were limited for *M. chiloeensis*. The same pattern of a lack of a linear decrease of periphytic biomass in response to grazing was observed by HILL and KNIGHT (1987) in experiments with the mayfly *Ameletus validus* suggesting that a faster algal replacement during their experiments may have occurred.

Nevertheless, in our T3 treatment, with a higher initial periphyton biomass, we did observe the proportional decline. If the lack of this decline in T1 and T2 was due to faster algal replacement we should have observed the same in T3, therefore we suggested that food limitation was the cause of the obtained results.

LAMBERTI *et al*. (1987) suggested the existence of a threshold value in periphyton biomass, which is too low to be grazed by herbivores and can be considered as an "algal refuge" (MCINTIRE *et al.*, 1996). During our spring experiment, in very young periphyton (one week of pre-colonization time) we observed that both grazer densities reduced periphyton biomass to the same level of 0.4 g AFDM m^{-2}. These results suggest that no further decrease can be caused by *M. chiloeensis* indicating that it may have reached the threshold, although only two grazer densities were tested. If all *M. chiloeensis* density treatments reduced periphyton to the threshold level, then no relationship would be expected between grazer density and periphyton abundance.

Periphyton of different stages differed not only in biomass but also in the composition of the assemblage. The first steps of periphyton development consist in the colonization of bacteria and fungi hyphae together with organic detritus (KORTE and BLINN, 1983). As a consequence, this young stage would achieve very low Chl *a* values, causing the low C/OM values found in the initial control of T1, both in spring and winter. Afterwards, raphid diatoms are the most frequent early colonizers (WETHERBEE *et al.*, 1998), followed by rosette forming diatoms (KATOH, 1992) and finally green filamentous algae appeared (BIGGS, 1996). In our spring experiment, the small monoraphid *Achnanthes minutissima* and the araphid *Fragilaria capucina* were the most abundant early colonizers. The former has been repeatedly described as a first colonizer in many studies (KORTE and BLINN, 1983; ROEMER *et al.*, 1984; STEVENSON *et al.*, 1991) and it was also found to dominate the periphyton in previous experiments carried out in Gutiérrez stream (DÍAZ VILLANUEVA *et al.*, 2000). Grazing did not alter C/OM index indicating that both autotrophic and heterotrophic fractions of the periphyton components were reduced in the same proportion. These results confirmed our third hypothesis.

Our fourth hypothesis stated that algal species response to grazing would differ in relation to periphyton stages. In both experiments, diatom relative abundance variation was more affected by grazers in the young periphyton (T1) (Figs. 3 and 4). In particular, during the spring experiment *F. pinnata* was reduced by grazing and the species ordination scores indicated it was related with ungrazed treatments. By contrast, *N. palea* was enhanced by grazing. PETERSON *et al.* (1998) found that *F. pinnata* may be resistant to digestion and our own results obtained in *Meridialaris diguillina* DEMOULLIN, a very closely related taxon to *M. chiloeensis*, showed that 40% of *F. pinnata* cells were found alive in their faeces (DÍAZ VILLANUEVA and ALBARIÑO, 2003). *F. pinnata* is a filamentous species that grows entangled in the algal matrix, and therefore, would be more exposed to grazing despite of its resistance to digestion. On the other hand when periphyton biomass is low, *N. palea*, a motile species, may grow adjacent to the substrate (JOHNSON *et al.*, 1997) and this habit would have conferred a refuge to herbivory. Consequently, this replacement could be due to species growth habits. Herbivore effect on diatom relative abundance in T2 and T3 was less evident (Fig. 3). These results support the statement that the outcome of the herbivore-periphyton interaction depends on the successional stage of the periphyton community.

In the winter experiment, the relative abundance of *A. minutissima* increased throughout the experiment, becoming dominant in ungrazed T2 and T3 (20% and 22% of total abundance, respectively). Grazing effect on this diatom species was to decrease its relative abundance. Active selection of this small species by the herbivores is doubtful. MCCORMICK and STEVENSON (1989) found that *A. minutissima* can be resistant to grazing when it grows closely attached to substrata but it could be preferentially grazed when it grows in a mucilaginous matrix and consequently it cannot outgrow predator pressure. The trade-off between competitiveness and herbivore resistance described in terrestrial and intertidal

systems was applied to the periphyton physiognomy by GRAHAM and VINEBROOKE (1998). These authors stated that herbivore-resistant groups are firmly attached to substrata and in contrast, loosely attached taxa are suppressed by grazing. Nevertheless, many algae are epiphytic on other algae or attached to detritus, a situation that could position algal cells higher regardless to their physiognomy (WELLNITZ and WARD, 2000). *A. minutissima* can remain in the deeper layers of periphyton because it tolerates low light intensities (JOHNSON *et al.*, 1997). During winter, heterotrophic conditions (low irradiance and comparatively lower C/OM values) prevailed in the deeper layers of periphyton. Under these unfavourable conditions, *A. minutisima* may have migrated to the upper layers, thus becoming more vulnerable to grazing by *M. chiloeensis*.

In highly heterogeneous systems such as streams, periphyton is distributed in patches of different colonization times. Our results emphasise the importance of considering algal patches of different biomass and taxonomic content in assessing the effect of grazers. Therefore, the outcome of the herbivore-periphyton interaction may depend on the successional stage of the periphyton community.

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