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# Mechanisms of microalgae selection during the assembly of a planktonic community

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**Abstract** We attempt to ascertain the selection mechanisms that affect algal species' abilities to thrive in a given environment, and how the variability of the response is reflected in the community structure (total biomass, diversity) and also in community function (photosynthesis and respiration). For that, we formed algal communities, assembling eight species of freshwater cosmopolite algae with distinct morphologies (*Cosmarium contractum, Cryptomonas ovata, Euglena gracilis, Limnothrix redekei, Monoraphidium contortum, Pediastrum tetras, Planktothrix agardhii and Scenedesmus acutus*) and performed four treatments combining low and high nutrient concentrations (N and P) in the culture media and a presence or absence of herbivores (a mixture of *Daphnia magna, Keratella cochlearis* and *Brachionus calicyflorus*). Competition between pairs of algae was also studied, and the viability of the species as inocula after a senescence period. The presence of herbivores (predation) and nutrient concentration (competition) are the mechanisms affecting (individually or synergistically) microalgae assemblages, originating different alternative states from the same pool of species. However, the effect of these mechanisms may vary, depending on algal properties such as size, growth rate and viability as an inoculum. The presence of herbivores and oligotrophic conditions reduce the primary producer biomass but increase diversity due to relaxation of competition and increase the uncertainty of final states. The variations in the community structure directly reflect on community function, affecting primary production and respiration.

**Keywords** Assembly rules · Competition · Function · Herbivorism · Photosynthesis-respiration · Structure

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## Introduction

Ecological studies concerning planktonic communities have shown that the composition of an observed community in a water body can be controlled by predation (top-down processes, Carpenter and Kitchell 1993; Ortega-Mayagoitia et al. 2002), by competition for resources (bottom-up control, Cottingham 1999) or by the interaction of both factors (Ortega-Mayagoitia et al. 2003; Cottingham et al. 2004). Moreover, other factors to consider, which would explain part of the observed variance are: the community formation history (Padisák 1992; Drake 1991; Drake et al. 1999; Rojo et al. 2006) and the frequency and intensity of disturbances (Reynolds et al. 1993), and many other factors such as light,

temperature, pH, salinity, micronutrients. The complexity of this panorama and the relative lack of experimentation with multi-specific plankton systems has led to newly emerging hypotheses due to the fact that repeated field observations were not totally determinant (Rojo and Álvarez-Cobelas 2000).

An empiric pattern regarding the presence of some groups of algae and not others in certain environments has been described as succession or seasonal phytoplanktonic succession by Margalef (1958), Reynolds (1984), plankton ecologists (Harris 1986; Sommer et al. 1986), for wetlands (Chow-Fraser 1998; Rojo et al. 2000) and in a more general manner, based on a functional classification, by Olrik (1994) and Reynolds (1997). The described patterns are based on numerous field observations and are explained by different algal growth strategies, different morphologies (sizes, shapes, etc.), motility and the metabolism (photosynthesis, respiration, resource use, etc.).

In this study, we carried out an experimental design to observe the assembly dynamics of algal communities all formed from the same initial pool of species. Algae were selected by different sizes and shapes (large or small, colonial or single-celled, filamentous or not, motile or not), and their different growth strategies (sensu Olrik 1994). This assay has the following main goals: (i) to determine, under laboratory controlled conditions, the establishment of stable microalgal assemblages as a response of their functional group features to different environments (low/high nutrient concentration and presence/absence of herbivores) and (ii) to analyse the underlying mechanisms that generate the results (competition, inoculum history). We have also evaluated the influence of these two factors, nutrient concentration and herbivory, and their interaction on structural (total biomass, final composition, diversity) aspects of the community. Since it has been described that after a situation that promoted a senescent state in populations (such as a long stability period and lack of resources, Sommer et al. 1986), the study of the inoculum viability of each declining population can result of interest, we have undertaken such study. Finally, since changes in the community structures can affect functional aspects of ecosystems (Eriksson et al. 2006), we have tested whether the different algal community structures reached in the assembly process show different functional traits (photosynthesis and respiration).

## Material and methods

Eight cosmopolitan freshwater algal species have been used in the experiments of community assemblage: *Cryptomonas ovata* (unicellular, motile and small: $16 \pm 1 \times 10 \pm 1$ μm, $870 \pm 134$ μm$^3$), *Euglena gracilis* (unicellular, motile and large: $51 \pm 4$, $8 \pm 1$ μm, $1890 \pm 494$ μm$^3$), *Limnothrix redekei* (filamentous and thin: $1.7 \pm 0.5$ μm, $11.6 \pm 0.7$ μm$^3$ cell$^{-1}$), *Planktothrix agardhii* (filamentous and thick: $7.8 \pm 0.4$ μm, $148 \pm 12$ μm$^3$ cell$^{-1}$), *Scenedesmus acutus* (4-celled colonies, non-motile and small: $11.7 \pm 2.0 \times 4.0 \pm 0.5$ μm, $98 \pm 10$ μm$^3$ cell$^{-1}$), *Pediastrum tetras* (8, 16-celled colonies, non-motile and large: $9 \pm 0.4 \times 2 \pm 0.2$ μm; $127 \pm 11$ μm$^3$ cell$^{-1}$), *Monoraphidium contortum* (unicellular, non-motile and small: $32.1 \pm 0.4 \times 2.6 \pm 0.7$ μm; $57 \pm 2$ μm$^3$) and *Cosmarium contractum* (unicellular, non-motile and large: $25 \pm 1 \times 32 \pm 4$ μm, $6833 \pm 1321$ μm$^3$). The algal species were purchased from algal collections (CCAP: Collection of Algae and Protozoa at the Centre for Ecology and Hydrology, UK and the Culture Colection of Algae at the Departament of Botany, University of Coimbra, Portugal). The stock algal cultures were maintained in glass flasks with 50 ml Jaworski medium (it contained biotin, thiamin and cyanocobalamin, CCAP) and were reinoculated fortnightly in fresh medium. The algal cultures were kept in a culture room at 20°C, with 12 h light: 12 h darkness photoperiod at 30 μE m$^{-2}$ s$^{-1}$ PAR (photosynthetically active radiation). These were the physical conditions in all experiments described below, and we used the same culture room as described previously. The cultures were stirred periodically to decrease boundary layer effects. Algal cultures were axenic and all manipulations were made under sterile conditions to minimize bacterial growth. Aliquots of the stock algal cultures in exponential growth phase were the inocula for the different experiments.

To better analyse the competitive characteristics of the two fastest-growing species (*Scenedesmus acutus* -*Sc*- and *Monoraphidium contortum* -*Mn*-, known from previous results) between each other, we performed a mixed culture of both algae in oligotrophic conditions. The inocula were 250 cells ml$^{-1}$ final density for each algal species in this experiment and in all the following. The 250-ml glass flasks were used and the culture medium was composed of mineral water enriched with 6.2 μg P l$^{-1}$ and 152 μg

N $l^{-1}$ final concentration (N/P = 25). This medium has a low nutrient concentration but above the growth thresholds (Reynolds 1984). This culture medium will be named "oligotrophic medium" hereafter. In order to study competition between algae, two-species mixed cultures were performed facing the two fastest growth algae (*Sc* and *Mn*) with each one of the remaining species.

Two types of culture media were prepared for the experiments to study the effect of nutrients on the assembly of algal community (all algae together and absence of herbivores): (1) oligotrophic medium and (2) another with a tenfold nitrogen and phosphorus concentration (62 μg P $l^{-1}$ and 1520 μg N $l^{-1}$ final concentration) named "eutrophic medium". In order to check the viability of the algal populations in the final state or senescence, an aliquot of an old (after 40 days of incubation) eight-species mixed culture in oligotrophic medium was inoculated in fresh oligotrophic medium and the algal growth was followed for 20 days. In order to ascertain the effect of the herbivory on the structure, assembly, photosynthesis and respiration of the microalgae community, three species of herbivores were used: the cladoceran, *Daphnia magna* and the rotifers, *Keratella cochlearis* and *Brachionus calicyflorus. Keratella, Brachionus* and *Daphnia* individuals were extracted from stock cultures (fed with an algal mixture), choosing randomly non egg-bearing *Keratella* and *Brachionus* individuals and adult non-gravid *Daphnia* females. *Daphnia* individuals were introduced with a final density of 0.5 ind $l^{-1}$ and 10 ind $l^{-1}$ (final concentration) in the case of rotifers. Cladocerans as rotifers were added (when required) together with the algae, on the first day of experimentation. The same kind of culture media (oligotrophic and eutrophic), algal inocula from the same stock culture and the same culture conditions were used for the herbivory treatments. The culture volumes were 2 l for the treatments without herbivores and 19 l for the treatments with herbivores. In summary, the treatments to analyse the top-down and bottom-up effects and their interaction on the final algal community structure and function were: (1) oligotrophic medium without herbivores (O–H), (2) eutrophic medium without herbivores (E–H), (3) oligotrophic medium with herbivores (O+H) and (4) eutrophic medium with herbivores (E+H). All treatments were carried out in triplicate.

Samples were taken every 3–5 days during a minimum of 36 days and a maximum of 57 days. In all cases, 2 ml samples for algal counting were taken and immediately fixed with Lugol's solution and kept in the dark until counting occurred. The algal samples were sedimented in Utermöhl chambers and observed by means of an inverted microscope. Algal density was obtained from the counting of at least 400 cells of each population. Algal density was transformed in biovolume, multiplying cell densities by the cellular biovolume of each alga. Cellular biovolume was calculated from the dimensions of each species, measuring 100 cells and approximating their shapes to the closest geometrical shape (Hillebrand et al. 1999). To obtain the biomass in fresh weight, we considered the density equal to 1 g $cm^{-3}$ (Rojo et al. 2000). Algal intrinsic growth rates ($r$) were calculated assuming an exponential growth model with the data from the first 10 days of culture, that is, when the cultures reinoculated in new medium reached the exponential growth stage. Algal diversity was calculated using the Shannon index (Shannon and Weaver 1963) on density and biomass data.

For counting the small size herbivores, 50 ml samples were filtered through a 37 μm pore diameter nytal mesh every 5–10 days. The large *Daphnia* individuals were counted through direct observation.

Photosynthetic and respiratory rates of the algal community were determined in the cultures of the different treatments using a Clark electrode (Hansatech, Dubinsky et al. 1987), which detects voltage changes due to differences of dissolved oxygen concentrations in water. A calibration curve allowed us to transform voltage into $mgO_2$ $l^{-1}$ $h^{-1}$ produced and/or consumed. Between 20 and 200 ml of culture samples (depending on the algal cell density) were filtered through Millipore nitrocellulose filters (0.45 μm pore size, 47 mm filter diameter). The cells retained on the filter were resuspended in 2 ml of filtrate. In the case of herbivore treatments, when *Daphnia* individuals were detected in the sample, they were removed from the filter previously to the settled material resuspension; the rotifer respiration was not detectable under these conditions (unpublished data). Concentrated culture samples were irradiated with 100 μE $m^{-2}$ $s^{-1}$ PAR in order to measure the oxygen production by photosynthesis.

This light is not saturating (Carignan et al. 2000) and high enough to detect changes in oxygen concentration caused by photosynthesis in short periods of time (10 min). Subsequently, the same sample remained in darkness to register the decrease in oxygen concentration due to the consumption by respiration. We assumed a weight-to-weight conversion ratio of $C:O_2 = 0.312$ (Vollenweider 1969) to transform oxygen production into carbon assimilation. Photosynthetic and respiration rates were determined in 6–10 occasions for each culture (in triplicates) along the incubation time. Primary production and respiration were expressed as a unit of dry weight (DW) of algal material (biomass specific photosynthesis and respiration). DW was considered 10% of fresh weight (FW).

In order to detect differences in the growth of the two fastest growing algae (*Scenedesmus* and *Monoraphidium*) in competition with each one of the remaining seven algae, we performed two one-way analyses of variance (ANOVAs) with averaged cell density data at the stationary phase of growth. Two-way ANOVAs with Nutrient concentration and Herbivory as factors were carried out to compare total algal biomass, specific algal biomass (both time-averaged to day 36 of culture, the shortest trial), intrinsic growth rate (calculated for the first 10 days of culture), diversity (averaged from day 10 to day 36 of culture) and net primary production and respiration (also time-averaged). Variance homogeneities were checked by means of the Levene test. All statistical analyses were performed using the SPSS (version 13.0) computational software.

## Results

The algal population dynamics were different depending on treatments (nutrient concentration and presence/absence of herbivores) in 8-algal species assembly experiments (Fig. 1). The intrinsic growth rates (Fig. 2) calculated from these dynamics show that *Scenedesmus acutus* (*Sc*) and *Monoraphidium contortum* (*Mn*) exhibited the highest values practically in all situations, and the highest values were reached in the E–H treatment. *Cryptomonas ovata* (*Cr*) and *Pediastrum tetras* (*Pe*) had the highest rates in E+H treatment. Conversely, the *Limnothrix redekei* (*Li*) growth rate seems to be favoured by oligotrophic conditions and *Cosmarium contractum* (*Co*) by the situation of higher stress (oligotrophy with predation). *Euglena gracilis* (*Eu*) and *Planktothrix agardhii* (*Pl*) exhibited the lowest growth rates, even negative when the herbivores were present and absent, respectively. The two-way ANOVA (with nutrient concentration and herbivory as factors) on growth rates revealed that *Mn* and *Sc* growth rates were not affected by nutrient concentration, but by herbivory, significantly reducing the rates. *Cr* growth rates were not significantly different between treatments (Fig. 2) and *Pe* rates showed statistically significant differences due to the nutrient concentration and the interaction between this factor, and the presence/absence of predators. *Co* growth rates were different in the presence and absence of herbivores and due to the interaction of factors. *Li* growth rates were only statistically different due to the nutrient concentrations and in the case of *Eu* and *Pl*, the differences were only caused by the presence of herbivores.

In the competition trials between *Sc* and *Mn* (Fig. 3), *Sc* was the most effective competitor, reaching the highest growth rates and the highest densities. The competitive superiority of both *Sc* and *Mn* on the remaining algae was corroborated in cultures growing each one of these algae with one of the other algae, in oligotrophic medium. In all cases, *Sc* and *Mn* grew faster and reached higher cell densities than the remaining species. From these competition experiments by pairs, we observed that final densities reached by a species depended on the identity of the competitor. Thus, *Mn* reached minimum densities (Fig. 4), when competing with *Sc* and maximum densities when grown together with slow-growing algal species such as *Eu*, *Co* and *Pl*. *Mn* reached a similar development when competing with *Cr* and *Pe* despite these two species having very different morphological features. A one-way ANOVA on *Mn* averaged density reached at stationary phase of growth competing with the remaining algae separately, revealed that the density was different depending on the competing algae. The same happened with *Sc* ($P < 0.001$ for both cases).

A similar biomass pattern can be observed for *Mn* and *Sc* (Fig. 1): the highest biomass (25 and 100 mgFW $l^{-1}$, respectively) was achieved in the E–H treatment and the lowest in the O+H treatment. The two-way ANOVA (Table 1) indicates that the

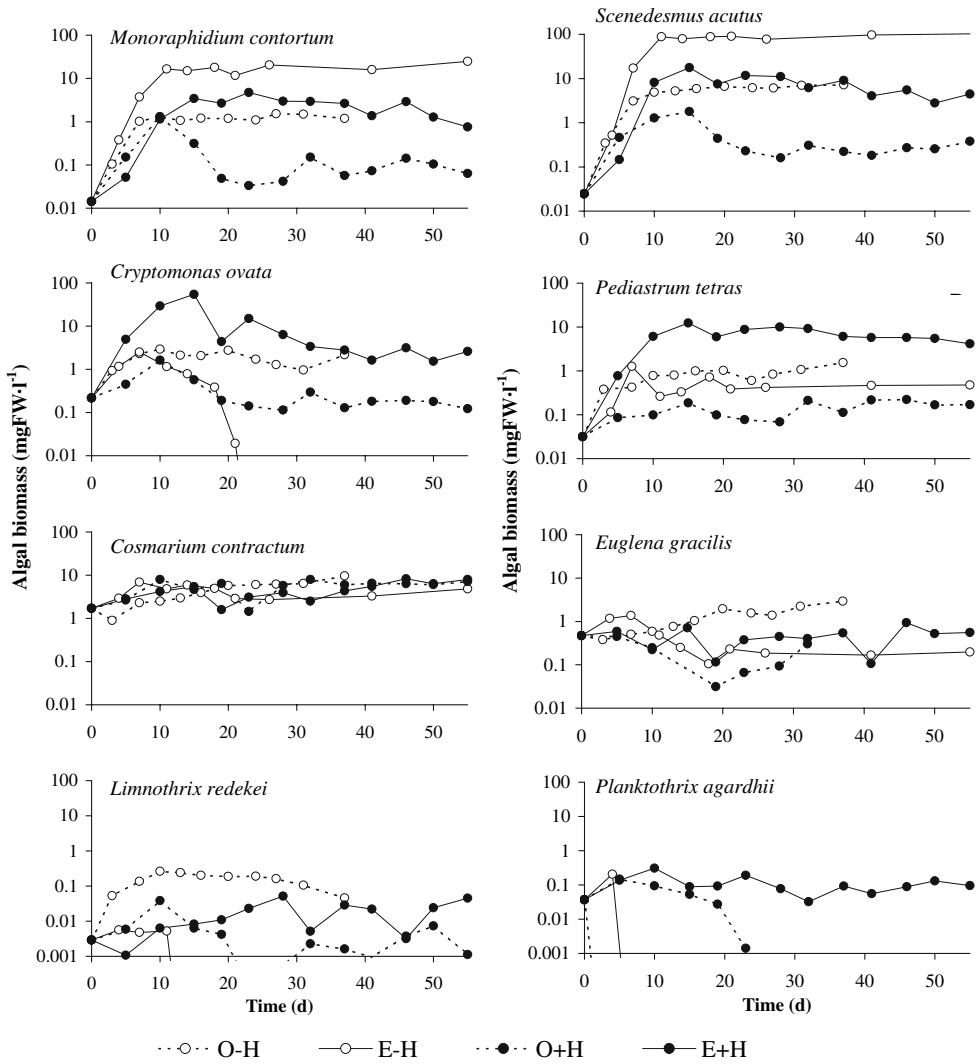


**Fig. 1** Time course of algal species biomass (fresh weight) in different culture media treatments. Broken and solid lines indicate oligotrophic and eutrophic media respectively. White and black symbols indicate absence and presence of herbivores respectively. Each point corresponds to the average of three replicates (error bars are not shown for clarity of figures)

Nutrient and Herbivory factors (and their interaction) originate statistically significant differences in the mean biomass of both species. *Cr* and *Pe* patterns were also very much alike (Fig. 1): both had the highest biomass in the E+H treatment, lower values in O–H treatment, even lower values in the E–H treatment and finally the lowest biomass was in the O+H treatment. Similar ANOVA results were obtained for these species (Table 1). *Co* (Fig. 1) did not show large biomass variations among the different treatments and there was no statistical difference due to nutrient content or herbivory presence (Table 1). *Eu* (Fig. 1), whose biomass was lower than 3 mgFW $l^{-1}$, showed statistically significant differences depending on both factors and their interaction (Table 1). *Eu* biomass was the highest in O–H treatment. The two filamentous algae (*Li* and *Pl*, Fig. 1) had a very low biomass (below 1 mgFW $l^{-1}$ in all occasions). *Pl*, similarly to *Co*, did not exhibit differences among treatments. *Li* biomass had statistically significant differences, depending on nutrient content and herbivore presence, with a higher biomass in O–H treatment, like *Eu*.

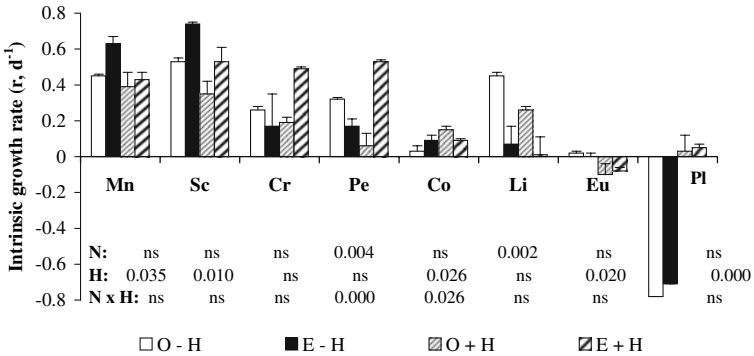


**Fig. 2** Intrinsic growth rates ($r$, day$^{-1}$) of the eight algae for the first 10 days of culture (assuming an exponential growth model) calculated on cell density data for the different treatments. Vertical bars indicate standard errors. Algae abbreviations as in Table 1. The two-way ANOVA results with nutrient concentration (N) and herbivory (H) as factors on intrinsic growth rates of each algal species (significance $P < 0.05$) are also indicated; ns, non significant

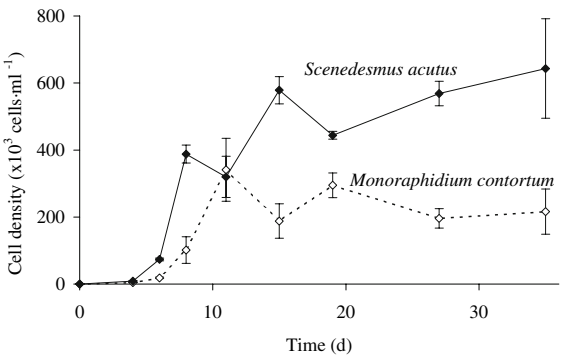


**Fig. 3** Time course of *Monoraphidium contortum* and *Scenedesmus acutus* cell density in an oligotrophic culture

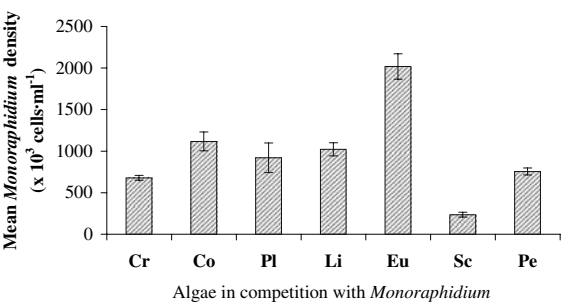


**Fig. 4** Average *Monoraphidium contortum* cell density reached at the stationary phase in oligotrophic cultures to study competition with the remaining seven algae. Abbreviations for species as in Table 1. Vertical bars indicate standard errors ($n = 3$)

Algal diversity (H) calculated from density data (Fig. 5), exhibited a decrease in all treatments especially in the E–H. Diversity, calculated on algal biomass data (Fig. 5) increased and remained higher than the initial value in the O–H and E+H treatments and decreased in the E–H and O+H treatments due to the dominance in biomass of *Sc* and *Co* respectively. The two-way ANOVA (Table 2) on algal diversity calculated from biomass data, indicated that neither nutrient nor herbivore presence as factors, independently affected final diversity (averaged from 10 to 36 days of culture); however, the interaction of both factors is highly statistically significant ($p < 0.001$).

Total biomass reached by the algal community was different in each treatment (Figs. 6 and 7). Total biomass was the highest in the E–H treatment at day 10 (111 mgFW l$^{-1}$) and it remained constant and high (about 120 mgFW l$^{-1}$). In E+H treatment, total biomass increased more slowly until day 15 (maximum value 94 mgFW l$^{-1}$) and followed by a considerable decrease in biomass due to the consumption of algae by the herbivores, resulting in a reduction of about 22 mgFW l$^{-1}$. Total biomass exhibited similar values until day 10 (13 mgFW l$^{-1}$) in the oligotrophic treatments with and without predation. The biomass remained constant in the culture without herbivores and slightly decreased in the treatment with them (Fig. 6). Total algal biomass in eutrophic medium was around 5-fold higher than in oligotrophic medium (Fig. 7), whether predators were present or not. Moreover, the presence of predators reduced to approximately half the mean algal biomass such as in the oligotrophic medium as in the eutrophic one. The algal biomass variability within each treatment was related to the controlling factors, in a way such that when predators were absent, such as in oligotrophy and eutrophy, the

**Table 1** Two-way ANOVA results (probabilities) with nutrient concentration and herbivore presence as factors on biomass for each algal species, averaged until culture day 36 (significance $P < 0.05$)

| Treatment | d.f. | Mn | Sc | Cr | Pe | Co | Li | Eu | Pl |
|---|---|---|---|---|---|---|---|---|---|
| Nutrients | 1 | <0.001 | <0.001 | 0.011 | 0.002 | ns | 0.002 | 0.001 | ns |
| Herbivory | 1 | <0.001 | <0.001 | 0.014 | 0.003 | ns | 0.002 | <0.001 | ns |
| Nutrients × herbivory | 1 | <0.001 | <0.001 | 0.005 | 0.001 | ns | 0.001 | <0.001 | ns |

d.f., degrees of freedom; Mn, Monoraphidium; Sc, Scenedesmus; Cr, Cryptomonas; Pe, Pediastrum; Co, Cosmarium; Li, Limnothrix; Eu, Euglena; Pl, Planktothrix

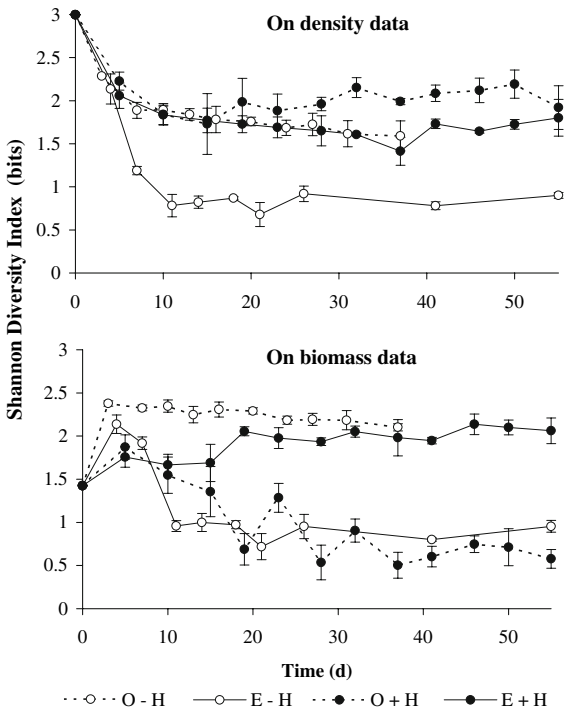


**Fig. 5** Time course of algal diversity (Shannon index) calculated on density (up) and biomass (below) data in the different treatments. Vertical bars indicate standard errors ($n = 3$)

biomass variation coefficient (CV) of the three replicates was 9%. When predators were present, the variability increased (CV = 20% for both trophic situations). The two-way ANOVA on total biomass, averaged to day 36, revealed that there were statistically significant differences due to both nutrient concentrations and herbivore presence (Table 2). Moreover, the interaction of both factors was also significant ($P < 0.001$). Time-averaged herbivore biomass was also markedly different among treatments (Table 3), reaching the highest biomass in the eutrophic treatments.

In order to understand another factor linked to the permanence strategy of the species during the community assembly, we studied the recuperation capability of each alga (Fig. 8). We inoculated an aliquot of a 40-day-old 8-algal species culture in new oligotrophic medium. *Mn* resulted in the highest densities, even higher than that reached by *Sc*. *Pl, Li* and *Cr* did not develop in the new culture.

In all treatments, biomass specific net primary production (NPP, Fig. 9) was highest the first days of culture. It decreased later on to reach slightly negative values in some cases. The first-day's NPP was markedly higher in the E–H treatment. The two-way ANOVA showed statistically significant differences in NPP (averaged to 36 day of culture) due to

**Table 2** Two-way ANOVA results (probabilities) with nutrient concentration and herbivore presence as factors on total biomass, algal diversity calculated on density and biomass data and averaged from day 10 to day 36 of culture, net primary production (NPP) and respiration averaged until culture day 36 (significance $P < 0.05$)

| Treatment | d.f. | Total biomass | Diversity (on density) | Diversity (on biomass) | NPP | Respiration |
|---|---|---|---|---|---|---|
| Nutrients | 1 | <0.001 | <0.001 | ns | <0.001 | <0.001 |
| Herbivory | 1 | <0.001 | 0.001 | ns | 0.004 | ns |
| Nutrients × herbivory | 1 | <0.001 | 0.019 | <0.001 | ns | 0.011 |

d.f., degrees of freedom

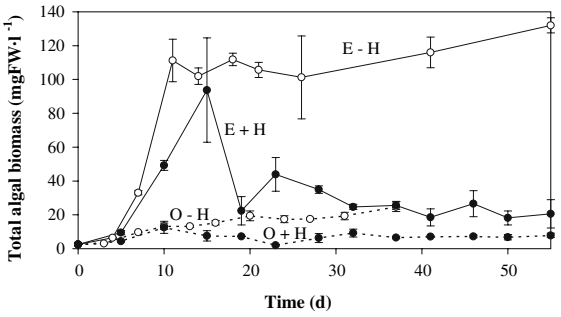


**Fig. 6** Time course of total algal biomass in the different culture media treatments. Vertical bars indicate standard errors ($n$ = 3)

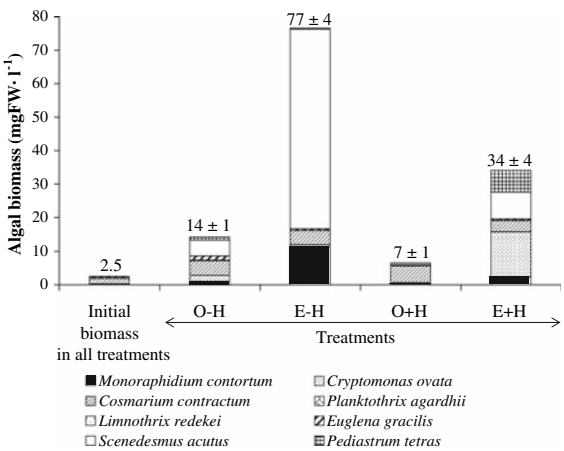


**Fig. 7** Biomass reached by each algal species in the treatments. Numbers indicate total biomass (mean of three replicates ± standard error). Total initial biomass (the same in all treatments) and species contribution are also indicated

nutrient concentration and herbivore presence (Table 2). The algal assemblage developed in the E–H treatment, which was characterized by a dominance of small-sized and fast-growing algae (*Sc* and *Mn*), exhibited biomass specific photosynthesis values significantly higher (until four times) than those reached by the algal community formed in oligotrophic medium (characterized by a dominance of

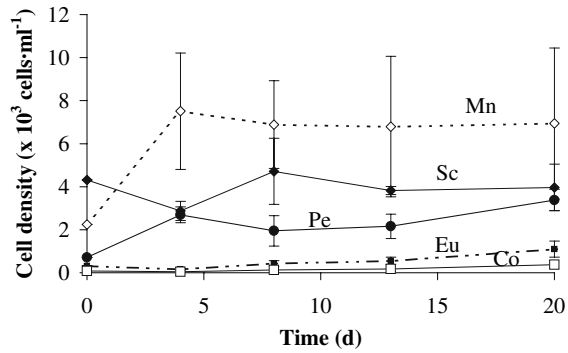


**Fig. 8** Cell densities of algal species after reinoculation of a 40-day old mixed culture in new oligotrophic medium. Vertical bars indicate standard errors ($n$ = 3)

large species of slow growth like *Co* and *Eu*). Maximum values of biomass normalized gross primary production (GPP) were 0.02 mgC mgDW$^{-1}$ h$^{-1}$ in the O+H treatment, 0.04 mgC mgDW$^{-1}$ h$^{-1}$ in the O–H and the E+H treatments, and 0.12 mgC mgDW$^{-1}$ h$^{-1}$ in the E–H treatment.

Algal community biomass normalized respiration showed sharp differences among treatments (Fig. 9). Respiration was minimal in O–H treatment and maximal in E+H treatment, although in the E+H treatment, the respiration showed dynamic fluctuation. The two-way ANOVA on time-averaged respiration showed that there were only significant differences due to the nutrient level (Table 2). The respiratory activity, which was significantly higher (from two to six times) in the eutrophic medium without herbivore presence compared to oligotrophic medium, was also related to the differences in the algal composition of the different assemblages for each treatment.

The gross primary production/respiration ratio (Fig. 10) varied between 0.6 and 6.1, and only in some treatments, was maximal during the first days of culture. In O–H and E+H treatments, the ratio was maximal around days 17 and 10, respectively.

**Table 3** Herbivore time-averaged density and biomass for different treatments

| Treatment | Brachionus | | Keratella | | Daphnia | |
|---|---|---|---|---|---|---|
| | Density | Biomass | Density | Biomass | Density | Biomass |
| Oligotrophic | 2 ± 0 | 1.3 ± 0 | 2 ± 1 | 0.2 ± 0.05 | 11 ± 2 | 1943 ± 246 |
| Eutrophic | 84 ± 80 | 65 ± 61 | 10 ± 4 | 1 ± 0.3 | 18 ± 1 | 2137 ± 988 |

Means of triplicates ± standard errors. Density in ind l$^{-1}$ and biomass in µgFW l$^{-1}$

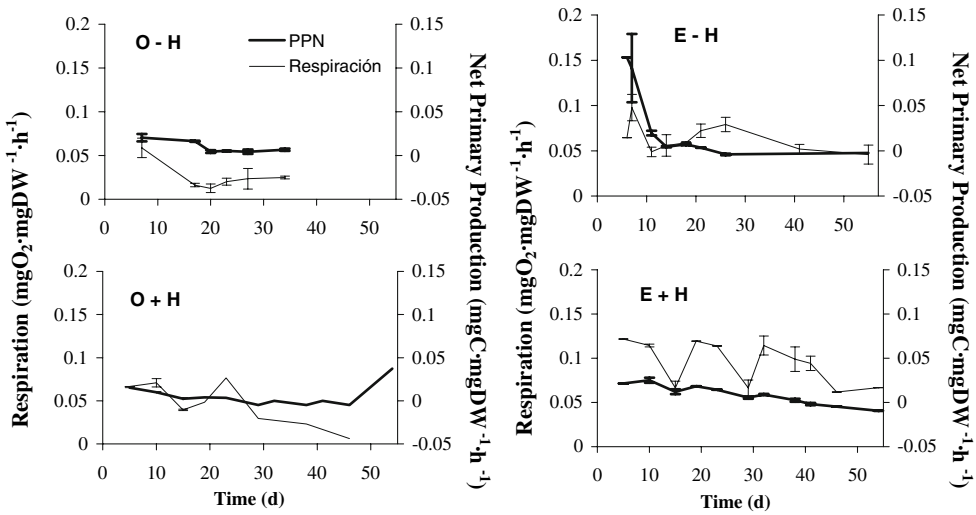


**Fig. 9** Time course of net primary production (NPP) and respiration (average of three replicates and standard error) of the algal community in the different treatments

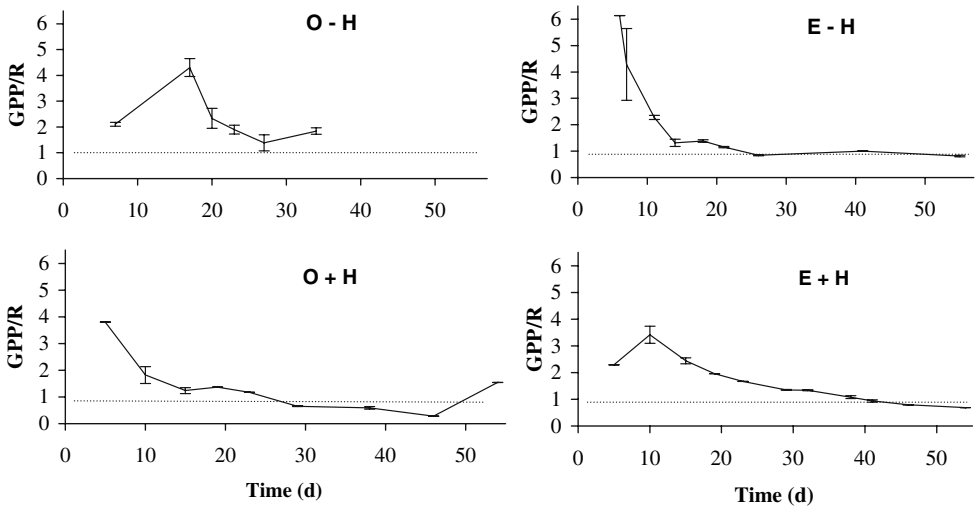


**Fig. 10** Time course of gross primary production/respiration ratio (average of three replicates and standard error) of the algal community in the different treatments. Dotted line indicates the boundary between net autotrophy and net heterotrophy

## Discussion

As it was expected (Vollenweider 1969; Cottingham et al. 2004), the total algal biomass was higher in the eutrophic medium than in oligotrophic medium and the presence of herbivores reduced the primary producers' biomass in our experiments. However, the variability in the response is remarkable; in the absence of herbivores, total biomass and assemblage composition was much more stable (Naselli-Flores et al. 2003) both in eutrophy and oligotrophy, such as with communities of practically a monoculture of opportunistic algae and communities with high diversity. However, when the presence of microalgae is controlled or reduced by the herbivores, a higher variability is observed, neither related to the diversity nor final structure. Therefore, with a species pool of different functions (Olrik 1994; Reynolds 1997), a predictability degree independent of diversity and controlled by the herbivory can be found. Thus, not

only the total biomass is altered in the presence of herbivores, as would be expected, but also the algal assemblage composition and with it, the diversity and stability or capability to originate a large variety of different communities.

The different structures of the algal community in the steady state (Naselli-Flores et al. 2003) in each treatment are the result of how the controlling factors affect in different ways, not only the different algae but also the different algae combinations. Moreover, the algal characterization as a function of growth strategy is not the only useful information (it is not determinant or concluding) to predict what the algal assemblage will be over the course of time. For instance, the higher nutrient concentration favoured the proliferation of small-sized fast-growing algae, such as *Sc* and to a lesser degree, *Mn* (colonizing species, Olrik 1994; Reynolds 1997). Both species were also the dominant algae in the laboratory microcosm experiments that Novales-Flamarique et al. (1993) designed to study the influence of fish foraging behaviour on plankton-nutrient relations. However, the situation is very different despite the eutrophic conditions when herbivores are present and predating on these species, which are very susceptible to predation pressure due to the immobility and small size (Olrik 1984; Reynolds 1984). The combined interaction of factors will originate different final alternative states. In eutrophic conditions with herbivores, the two opportunistic algae (*Sc* and *Mn*) will not only have to resolve the trade-off between higher growth and higher predation pressure but also compete for assemblage with *Pe* and *Cr*, which are good competitors. In the opposite situation, we found the slow-growing algae, less attractive to predators like *Co, Eu* and *Pl.* The presence or absence of algae in the final communities results from initial growth rates, which do not allow for an increase in population size when the colonizing species are present and because of their ability to resist predation pressure. Mainly *Co* and *Pl* are not affected by herbivore presence. In the case of *Co*, it is chiefly due to the high sedimentation rates and in the case of *Pl*, because of size and morphological features (Gliwicz 1990).

Another main aspect in the achievement of a final state or another is the inoculum history (Pádisak 1992), and as we have observed after a situation that promoted a senescent state in populations (such as a long stability period and lack of resources, Sommer et al. 1986), the inoculum viability of each declining population will be different. In this way, for example, the fastest-growth population (*Sc*) and clearly dominant in competition with the other colonizer (*Mn*), does not have the most efficient inoculum in terms of recovering high densities in new culture medium. This can cause dominance to alter among them, in similar conditions. Another clear example is *Cr* and *Pe*, which in spite of their different morphology, have behaved as similar functional units in all treatments (also similarly considered in the field empirical PEG-model, Sommer et al. 1986). These algae are effective opportunists that are favoured by the competition relaxation with *Sc* and *Mn*, because of high predation pressure by the herbivores on them. However, they differentiate in that *Pe*, due to high polymorphisms, develop large colonies, which allow survival in spite of predation stress (Rojo, unpublished). For that reason, although Olrik (1994) and Reynolds (1997) consider this *Pediastrum* species a potential colonizator (Olrik 1994, Association Reynolds 1997), this genus is also considered as S (stress-tolerant, Olrik 1994). Moreover, *Pe* shows a high viability in the senescent population, and alternates with *Cr* (of less viable senescent inoculum) in the inoculum history. In 2000, a debate among phytoplankton ecologists (Reynolds et al. 2000) culminated with ten assembly rules (in the sense of Weiher and Keddy 1999), which would explain in part, the presence of algae in the assemblages during each moment and place. Some of these rules, obtained from repeated empirical observations, were corroborated in this laboratory study. The algal assemblage formed from a species pool will depend on a series of assembly rules derived from the physiological and morphological features of the species and the complex interaction system. The prevision for the most probable assembly, based on the algal growth strategies, will be altered by the effect of predators and by the inoculum viability, which at the same time, will condition new combinations of competitors. In oligotrophic conditions, diversity is controlled by lack of a founder controller (Weiher and Keddy 1999), and different strategies for uptake and the retention of resources (Levins 1979; Tilman 1982). Conversely, in eutrophy, the presence of good competitors will add to the loss of diversity. The presence of herbivores reduces the primary producers biomass, but also increases the diversity by

relaxing competition (Harris 1986) and increases uncertainty for the final state, since variability of herbivore dynamics is included in the assembly trajectories at the same time that the diversity in diet is increased (Pimm 1982). Therefore, we can state that the diversity reached from a known pool of species not only has to be evaluated for each structure resulting from interactions or underlying mechanisms but also as the set for all possible final states (Fukami 2004).

Finally, we want to point out that changes in the community structure affect functional aspects of ecosystems, such as primary production and respiration. In those conditions favouring the proliferation of fast-growth and high respiratory rate algae (*Sc* and *Mn;* Olrik 1994), communities have higher photosynthetic and respiratory activities. Regarding the production: respiration ratio, cultures (batch cultures, without medium renewal) behave autotrophically (GPP/R $> 1$) till almost day 30 of incubation and only from this moment (in some cases after a longer time period), most showed a heterotrophic behaviour (GPP/R $< 1$). This observation is in accordance with the data on production: respiration ratio in the plankton community reported by Carignan et al. (2000) for oligotrophic lakes. These authors, using as methodology, the measurement of production and consumption of oxygen to determine photosynthesis and respiration, demonstrated that the production: respiration ratio exceeded one in almost all studied lakes. Their results are in contradiction with those previously obtained by Del Giorgio and Peters (1994) who, based on the utilization of the $^{14}C$ method, determined that GPP/R was lower than one in the lakes they studied, and concluded that in such lakes, heterotrophy dominated. Our data on photosynthesis and respiration, obtained in laboratory experiments, but with a similar methodology to that of Carignan et al. (2000) reinforce the conclusions argued by them about the net autotrophy of planktonic (epilimnetic and metalimnetic) communities.

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