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CLADOCERA

# Low filtering rates of *Daphnia magna* in a hypertrophic lake: laboratory and in situ experiments using synthetic microspheres

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**Abstract** Zooplankton grazing was measured in Albufera of València (Spain), a shallow turbid hypertrophic lagoon dominated by filamentous blue-greens, during the period of *Daphnia magna* growth, to evaluate the role of this cladoceran in maintaining a clear water phase which takes place after flushing for rice cultivation practices. We found extremely low ingestion and clearance rates (CR) on latex beads in situ, using a Haney trap suggesting potentially strong inhibition of grazing by the filamentous cyanobacteria, still flourishing in the lagoon. To test the effect of filaments, we undertook laboratory feeding experiments using six different dilutions of the lagoon water to vary filament concentrations, and four different bead concentrations. A highly significant power function between CR and water dilution associated with filament concentrations was found, indicating that the ability of *Daphnia* to exploit smaller edible algae and thereby control phytoplankton growth would depend on filament concentration levels in the lagoon. From our results only the two more diluted treatments, $9 \times 10^3$ and $18 \times 10^3$ filament ml$^{-1}$ showed CRs not far from the range of what would be normally expected on the basis of the general relationship of feeding rate as a function of total food concentration. This means that at lower food concentrations, filaments reduce *D. magna* CR by increasing total food concentration beyond the incipient limiting level, as well as by moderate mechanical interference with the animal's feeding. However, at lagoon water concentrations above 25% (corresponding to filament concentrations of $75 \times 10^5$ filaments ml$^{-1}$), extremely low CR's, which did not respond to any food addition, were obtained for *D. magna*. From these results we can infer that at concentrations beyond the above-mentioned critical level, feeding inhibition by filamentous cyanobacteria is most probably due entirely to mechanical interference.

**Keywords** Cladocera · Eutrophication · Grazing · Cyanobacteria · Microspheres

## Introduction

Relationships between filamentous cyanophytes and zooplankton have attracted much attention in freshwater ecosystems studies due to increasing deterioration of ecological water quality. Extreme examples

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are hypertrophic warm shallow lakes, where the once clear water and macrophyte dominated lakes shift to a turbid state, in which filamentous cyanobacteria blooms are the common feature (Scheffer, 1998), and zooplankton abundance and diversity are relatively small. A striking factor is the low abundance of Cladocera in these environments when in a turbid state, although the situation can change when water is clarified by flushing; then large *Daphnia* may dominate. This occurs in the Albufera of Valencia (Spain), a hypertrophic shallow warm lagoon, which acts as a regulation reservoir for traditional rice cultivation practices (Oltra & Miracle, 1992; Miracle & Sahuquillo, 2002). The lagoon is dominated all year-round by filamentous Cyanobacteria, such as *Pseudoanabaena galeata* and *Planktothrix aghardii* (Romo & Miracle, 1993; 1994, 1995; Villena & Romo, 2003). It switches to a brief clear water phase, when rice fields (without cultivation) flooded in autumn, are drained by mid winter through the lagoon, to have the fields dry for farming activities, by means of the opening of the sluice gates that control the lagoon outflow (Vicente & Miracle, 1992). This produces a period of high flushing, in which dominant cyanobacteria are washed out to the sea with flagrant scum formation on the beach. This water renewal, besides decreasing phytoplankton density, induces a shift in phytoplankton species composition with increasing proportions of edible alga. Consequently a high development of a *Daphnia magna* population occurs, which could reinforce the process of lake clearing. The duration and the conspicuousness of this clear water phase depends on the year climatology and water resources management, but even in the better years is very short and *D. magna* disappears by the end of March (Miracle & Sahuquillo, 2002). Nowadays, in the almost permanent turbid state of the Albufera lagoon, filamentous cyanobacteria dominance seems to be one of the most important limiting factors for *Daphnia* growth, although other factors as fish predation could also influence the restricted occurrence of *D. magna*.

Since lagoon outflow is largely under human control, water quality of the lagoon, an important component of the protected area of the Natural Park of the Albufera of Valencia, could be improved with a good management of winter flushing. Restoration measures by flushing the lake with oligotrophic water have been successfully applied in other filamentous blue green dominated lakes (Jagtman et al., 1992). Moss et al. (2004) concluded from studies in an European gradient, that in Mediterranean warm shallow lakes (such as the Albufera) the influence of nutrient loading was greater than in colder latitudes and nutrient reduction should be a greater priority than biomanipulation (zooplanktivorous fish reduction) in the restoration of eutrophicated shallow waters in warm temperate regions.

In the framework of the wide number of studies that show clear evidence that large Cladocera are eliminated during blooms of filamentous cyanobacteria (Infante & Abella, 1985; Gliwicz, 1990a; Gliwicz & Lampert, 1990), we wanted to provide some insights into the role played by the reduction of filamentous cyanobacteria and *D. magna* in the Albufera clear water phases, by examining the following questions: What are the filtering rates of *D. magna* in filamentous blue green dominated lakes? Which is the maximum concentration of filaments at which *Daphnia* is able to have reasonable clearance rates? Could *Daphnia* help to maintain the clear water phase once it is started by external forcing? In order to answer these and other questions, we performed in situ and laboratory short term experiments of *D. magna* ingestion rates of edible particles and examine their dependency upon filament concentrations, not ingested by them.

The answers to these studies would be very important to interpret the inter-annual variations of *D. magna* population growth and water clarification in the winter period, mainly related to reductions of blue-green filamentous, and ultimately by flushing.

## Methods

### Field

Integral water samples of the upper 1 m of the water column were taken every 3 days with a transparent tube (5 cm diameter, 1 m long, approx.2 1) during winter 2005 covering the period when *D. magna* is present (January–March). Zooplankton was concentrated in situ by filtering 3–4 replicate samples through a 35-μm nytal mesh and fixed in 4% formalin. Zooplankton species were counted using an inverted microscope at 100× and 200× magnifications. The lengths of all *D. magna* individuals collected were measured from the eye to the base of the tail spine. Males, parthenogenetic eggs and ephipia were counted separately. Water samples, taken in the same way, were used for the evaluation of phytoplankton (Utermöhl method) and chlorophyll *a* concentrations were determined with the spectrophotometric method (Strickland & Parsons, 1972).

Grazing experiments using a Haney grazing trap (Haney, 1971) were carried out in situ at 0.5 m depth on 7th February 2005; 18:00–19:00 h. When the trap was activated 3 l lake water were enclosed to which a mixture of latex microspheres (Bangs Laboratories Inc.) was automatically delivered. The microsphere mixture was adjusted to yield final bead concentrations in the trap of 600 and 240 beads ml$^{-1}$ of 5 and 9 μm diameter respectively. Bead sizes were chosen according to studies on filtering efficiency of different particle size and references of similar feeding experiments (McMahon & Rigler, 1965; Lampert, 1987; DeMott et al., 2001). Three short term, 10 min incubation time, experiments were carried out at the centre of the lake (where total depth = 1.1 m; Secchi disc depth = 0.4 m; conductivity = 1.97 mS cm$^{-1}$; temperature = 11°C). After incubation, a 10 ml sample was analysed for phytoplankton, and the rest of the trap content (2.9 l) was filtered through a 35 μm mesh size net to retain zooplankton, which was immediately anaesthetized with carbonate water and fixed with 4% formaldehyde. All *D. magna* individuals from each experiment were thereafter clarified with 8% lactic acid and placed in pure glycerine on excavated microscope slides to count the beads in their guts. Characteristics like animal length, number of eggs, sex and presence of epibionts were noted. All copepods and at least 50 *Brachionus variabilis*, (the most abundant rotifer) from each experiment were also cleared and analysed.

### Laboratory experiments

Experiments were conducted in a controlled environment chamber at 14°C with a photoperiod of 8/16 h to mimic lagoon conditions. *D. magna* collected from the lagoon with a net on 15 January 2005 were isolated and kept for a week suspended in lagoon water in the mentioned chamber to acclimatize to experimental conditions. Lagoon water was periodically renewed but no supplementary food was added. Water was collected from the lagoon at the same time than *Daphnia* and after zooplankton removal kept for use in the experiments.

A preliminary experiment was designed to determinate gut passage time and thus the incubation period for further experiments. For this experiment, groups of five *Daphnia* were transferred to 50 ml vessels filled with GF/F-filtered lagoon water and held for 4 h to allow gut clearance. Beads of two sizes, in the same final concentration as in field experiment, were added, and *D. magna* were allowed to feed for 5, 8, 12 and 18 min. After these exposure times, animals were anaesthetized immediately with carbonated water, and then fixed in formalin. This procedure was repeated again (in triplicate) but using vessels filled with whole lagoon water. Thereafter, *D. magna* were prepared and examined in the same way as the in situ experiment. Based on these experiments, an incubation exposure time of 10 min was selected for all other experiments.

The second experiment followed a factorial design with two factors, lagoon seston concentration and bead concentration, with replication (two vessels). Five adult *D. magna* were transferred to each 50 ml experimental vessel filled with lagoon seston at different dilutions and acclimatized in this feeding suspension for 1 h before the beginning of the experiment, which started with the addition of 5 and 9 μm diameter beads. We tested 6 percentages of lagoon seston (100, 50, 25, 12.5, 6.3 and 3.1%), they were made by diluting whole lagoon water with Whatman GF/F filtered lagoon water. Synthetic microspheres of two sizes were then added at four different concentrations (1, 2, 4 and 8 times the concentration used in the field experiments). After 10 min of incubation *D. magna* individuals were anaesthetized, fixed and analysed, as described before. A few animals (12 out of the 240 used) which seemed to have an anomalous general status and did not feed (i.e., some cases of high *Colacium* sp. infestation), were excluded from the analysis.

For both in situ and laboratory experiments, the following variables were determined: (1) *D. magna* dry weight (μg) calculated from length–weight relationships (Bottrell et al., 1976) using the equation $W = 0.53\,L^{2.7}$, where $W$ is the weight in μg and $L$ is the length in mm (measured from eye to spine base); (2) Ingestion Rate (IR, beads ind$^{-1}$ h$^{-1}$); (3) Clearance rate (CR, ml ind$^{-1}$ h$^{-1}$) = IR/bead concentration; (4) Mass specific clearance rate (MSCR) = CR/W and (5) phytoplankton densities in Haney trap and experimental vessel contents.

Relationships between variables were explored with standard linear regressions with log transformed data. CR was transformed to $\log_2$ to have the same scale than treatment levels. Statistical comparisons were made using several ANOVA's (SPSS, MLG univariated and repeated measures, sums of squares model III, vessel nested within each treatment combination) with log transformed data to meet the assumptions of normality and homogeneity of variances. Tukey and Games-Howell post-hoc tests were run to find out which effects were significantly different.

## Results

### Lagoon population and "in situ" experiments

In winter 2005, after the emptying of rice fields, the chlorophyll *a* concentration did not decrease as much as in other years. It varied from 150 µg $l^{-1}$ at 12th January to a minimum of 100 µg $l^{-1}$ at the beginning of March (Fig. 1). *D. magna* in the Albufera lagoon was present from the beginning of January to the end of March reaching a peak density of 34 ind $l^{-1}$. The number of eggs per adult female was around 2 during the entire study period (Fig. 1).

During the grazing experiments filamentous cyanobacteria dominated numerically the phytoplankton community, with densities of about $2 \times 10^5$ filaments $ml^{-1}$, although other algae (especially colonial Chroococcales with minute cells) were also abundant (Table 1). In these conditions we found extremely low *D. magna* clearance rates (0.07–0.021 ml *Daphnia*$^{-1}$ $h^{-1}$) on beads (Table 2).

The presence of all size classes of *D. magna* in the lagoon during this experiment enables an evaluation of filtering rates in relation to body size/biomass. This showed (Table 2) only a slight increase in IR or CR with body length in *D. magna*: ($CR = 0.004\ L^{0.275}$, $r^2 = 0.09$), which translates into a significant inverse biomass-specific rate (Fig. 2) with smaller individuals feeding more effectively. *D. magna* clearance rates calculated from two bead sizes did not differ significantly between the three experiments (one-way ANOVA: $P = 0.226$ and $P = 0.221$ respectively for 5 and 9 mm beads). The differences between tendencies observed in Fig. 2 may be due to possible differential bead distribution in the Haney trap.

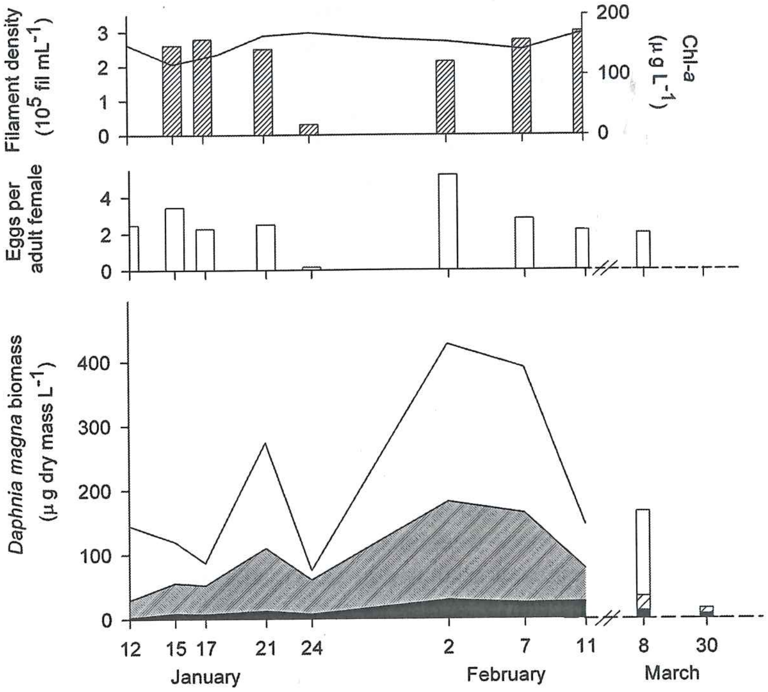

**Fig 1** Temporal changes in chlorophyll-*a* (line), filamentous cyanobacteria densities (bars) and *Daphnia magna* biomass in the Albufera lagoon. White area represents larger individuals (>1.8 mm), striped area, medium sized (0.9–1.8 mm) and black area, smaller ones (<0.9 mm). Size at maturity was estimated, for the whole population as 0.9 mm. Number of eggs per adult female (>0.9 mm) are also indicated. Males were detected at very low densities only twice at the beginning and the end of the period. (Lake conditions: temperature 11°C, conductivity 1.97 mS $cm^{-1}$, Secchi disc 0.40 m.)

**Table 1** Phytoplankton composition and *Daphnia magna* lengths (mean ± SD) in the Albufera lagoon during the "in situ" experiment and in the lagoon water used for laboratory experiments

| Phytoplankton | Lagoon | Laboratory |
|---|---|---|
| Filamentous Cyanobacteria | | |
| *Pseudoanabaena galeata*, fil $ml^{-1}$ (Fil. length, µm) | 2 ± 0.5 × $10^5$ (82 ± 41) | 3 ± 0.4 × $10^5$ (76 ± 34) |
| *Planktothrix agardhii*, fil $ml^{-1}$ (Fil. length, µm) | 3 ± 0.7 × $10^3$ (201 ± 62) | 2 ± 0.3 × $10^3$ (198 ± 81) |
| Colonial Cyanophyta (cell $ml^{-1}$) (*Aphanocapsa, Merismopedia*) | $\approx 10^5$ | $\approx 10^5$ |
| Cenobial Chlorophyta (cell $ml^{-1}$) (*Ankistrodesmus, Scenedesmus, Actinastrum*) | $\approx 10^4$ | $\approx 10^4$ |
| Unicellular Chlorophytes (cell $ml^{-1}$) (*Chlorella, Oocystis, Tetraedron*, etc.) | $\approx 10^4$ | $\approx 10^4$ |
| Diatoms (cell $ml^{-1}$) (*Cyclotella, Nitzschia*, etc.) | $\approx 10^3$ | $\approx 10^3$ |
| Others (cell $ml^{-1}$) (*Cryptomonas, Pediastrum*, etc.) | $\approx 10^2$ | $\approx 10^2$ |
| *Daphnia magna* | | |
| Length (mm) (Max–min) | 1.3 ± 0.6 (2.8– 0.5) | 2.1 ± 0.4 (3.3–1.5) |

Filamentous cyanobacteria were counted exactly; only order of magnitude estimates of cells, grouped by similar forms, are given for other phytoplankton taxa

**Table 2** Filtration rates of *Daphnia magna* estimated in field and laboratory grazing experiments with beads of two sizes suspended in lagoon water (as characterized in Table 1)

| In situ | | | Laboratory | | |
|---|---|---|---|---|---|
| Daphnia size (mm) | Bead size | | Daphnia size (mm) mean ± SD (max–min) | Bead size | |
| | 9 µm | 5 µm | | 9 µm | 5 µm |
| >1.8 | 0.021 ± 0.022 | 0.020 ± 0.020 | 2.1 ± 0.4 (3.2–1.2) | 0.05 ± 0.01 | 0.04 ± 0.01 |
| 0.91–1.8 | 0.012 ± 0.019 | 0.010 ± 0.007 | 2.3 ± 0.5 empty gut (3.1–1.3) | 0.09 ± 0.06 | 0.09 ± 0.08 |
| 0.5–0.9 | 0.007 ± 0.014 | 0.012 ± 0.008 | | | |

Expressed as ml $Daphnia^{-1}$ $h^{-1}$ (mean ± SD)

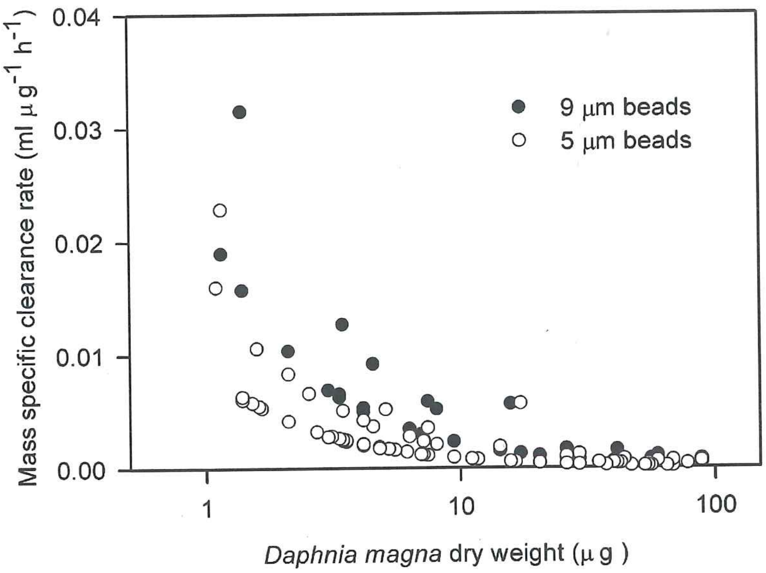

**Fig 2** *D. magna* mass specific CR on two sizes of beads in Albufera lagoon from three "in situ" experiments using a Haney trap. In this graph only individuals with beads in their gut are represented (81% from a total of 103 daphnids analysed). Smaller individuals are more efficient than larger ones. (MSCR = 0.02 × $W^{-0.90}$, $r^2$ = 0.89 for 9 µm beads and MSCR = 0.01 × $W^{-0.87}$, $r^2$ = 0.81 for 5 µm beads)

The rotifer *Brachionus variabilis* showed a mean CR of 0.003 ± 0.01 for 9 µm beads and 0.004 ± 0.01 ml $ind^{-1}$ $h^{-1}$ for 5 µm beads, from a total of 190 individuals analysed in the three field experiments. Not any ingested bead was observed in copepods.

### CR versus time, size of beads and filtered water

Preliminary experiments testing the feeding of large *D. magna* (Table 2) with initially empty guts on beads suspended in filtered lagoon water over different incubation times demonstrated that *D. magna* fed well on synthetic microspheres, and showed higher clearance rates on 9 µm than on 5 µm microspheres (univariate ANOVA: $P < 0.001$, two factor ANOVA, bead size repeated measures: $P < 0.001$). Bead numbers in the gut increased with time. Clearance rates reaches a maximum (3 ml $ind^{-1}$ $h^{-1}$) at 8–12 min (Fig. 3a) and egestion was observed after 18 min. Accordingly, experimental durations were standardized at 10 min.

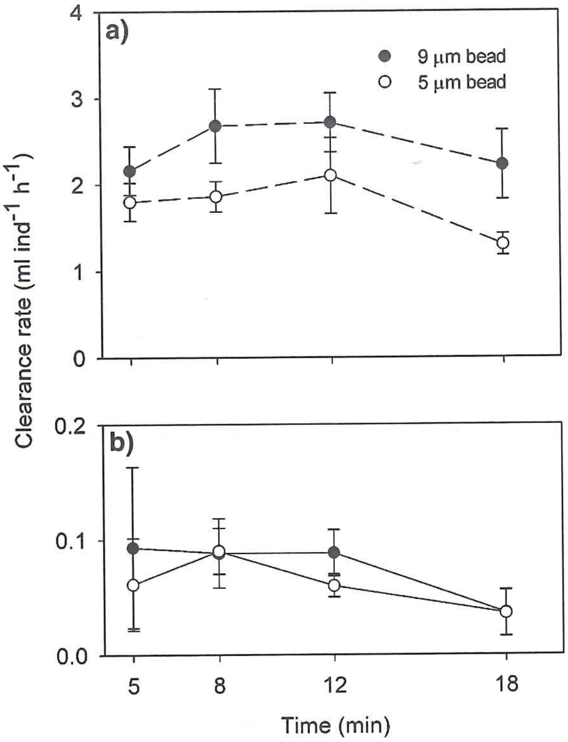

**Fig 3** Clearance rates (mean ± SE) of *Daphnia magna* at different incubation times. Results of the preliminary experiment using 9 µm beads (solid circles) and 5 µm beads (open circles) suspended in filtered lagoon water (**a**) and in lagoon water (**b**)

Repeating the experiment with lagoon water led to a severe (more than 1/30th) reduction in CR (Fig. 3b), although this value was still higher than in the in situ experiment (Table 2). Feeding inhibition by the seston, mainly filamentous cyanobacteria (Table 1) in whole lagoon water was the same for beads of both sizes, with no significant differences in their respective CRs (Fig. 3).

### IR and CR versus filament concentrations

Experiments with different dilutions of lagoon seston revealed sharp declines in *D. magna* CR's on beads of both sizes with rising seston concentration. Seston was mainly composed of filamentous bluegreens (Table 1). Other particles were smaller and are not expected to have important inhibitory effects on feeding; therefore we will consider only the filament densities. Results reflect the inhibitory effect of filamentous bluegreens on the ingestion of beads, which mimic edible spherical unicellular algae. CR can be expressed as a highly significant ($r^2 > 0.98$, $P < 0.001$) power function of lagoon filamentous bluegreen dilutions. In log scale CR declined lineally with lagoon seston concentration similarly for beads of both sizes, with a negative slope of about 0.84 for both bead sizes (Fig. 4).

To test the possible influence of bead abundances on IR we measured IR's in four bead concentration treatments. Our results (Fig 5a, Table 3) indicate no responses in ingestion rates to increasing bead concentrations above 25% lagoon seston in the feeding suspension (75,000 fil $ml^{-1}$), remaining always extremely low at the denser seston concentrations. However at lower seston concentrations, the increase in bead concentrations resulted in an asymptotic increase in IR.

To explore the possible effects of the proportion of the bead mixtures on CR, simulating the general effect of the proportion of edible algae in relation to interfering filaments, we plotted the CR variation with increasing percentage of beads: %beads/(beads + filaments). Since this variation was basically comparable for both bead sizes (reflected in the non-significant interaction effects, Table 3) both types of beads were

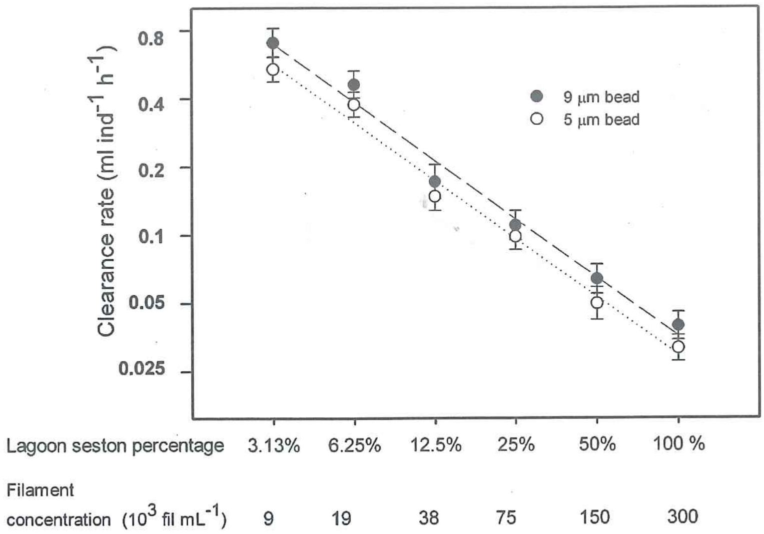

**Fig 4** CR of *Daphnia magna* in a progressive dilution range of Albufera water (displayed as lagoon seston percentage on abscissas with the corresponding filamentous cyanobacteria concentrations). CR, means and SE for 40 individuals from laboratory experiments (4 bead concentration × 2 replicate vessels × 5 daphnids each). Regression statistics for $\log_2$ CR vs. $\log_2$ filament densities are: $b = -1.5$, $r^2 = 0.52$ and $b = -1.18$, $r^2 = 0.\ 5$ for 5 μm and 9 μm beads respectively ($P < 0.001$ and $n = 228$, for both bead sizes)

combined in the analysis (Fig. 5b). CR was very low at the four higher seston concentrations (as illustrated above in terms of IR), and was essentially independent of bead proportions. However, at lower seston levels associated with greater dilution, CR increased with the initial doubling in bead concentration, but subsequent doublings reduced CR.

Comparing CR's on the two bead sizes for each individual *Daphnia*, using repeated measures ANOVA (Table 3) overall, as well as between the four higher and two lower levels separately, showed the major influence of lagoon seston level. The effect of high seston concentrations was also confirmed by ANOVA which further revealed the contrasting behaviour between lower and higher filament concentration levels. Bead concentration was significant overall and at the two lower levels of seston concentration but had no significant effect at the four higher levels. The ANOVA confirmed that most of the response variance was explained by seston concentration (67% when all data is considered), while bead concentration was only important at lower seston concentrations. The ANOVA also indicated that differences between bead size were significant (marginally less so at high seston concentrations), but interactions of bead size with treatments (water dilution level and bead concentration) were not significant, indicating that the relationships with filament densities were the same for both sizes of beads. Beads of both sizes gave very similar estimates of CR's (slightly higher for 9 μm diameter), albeit those smaller beads were proportionately more abundant. The extent of CR depression accordingly depends on the total amount of large seston, and is not affected by bead concentration per se.

## Discussion

In some years, the Albufera lagoon exhibits a brief clear water phase in mid winter, due to flushing when rice fields are drained. But during the winter of 2005, the water was not clear to the bottom and neither chlorophyll-*a*, nor filamentous cyanobacteria decreased as much as in other winters resulting in low *D. magna* densities. Moreover *Daphnia* fecundity was very low and comparable to that observed in an experimental study that used also water from a filamentous bluegreen dominated lake (Davidowicz et al., 1988). In years with a clear water phase, as in 2000 (Miracle & Sahuquillo, 2002), chlorophyll-*a* concentrations decreased much more than in 2005, to minima of one order of magnitude less (below 10 μg l$^{-1}$) and *D. magna* reach peak densities up to

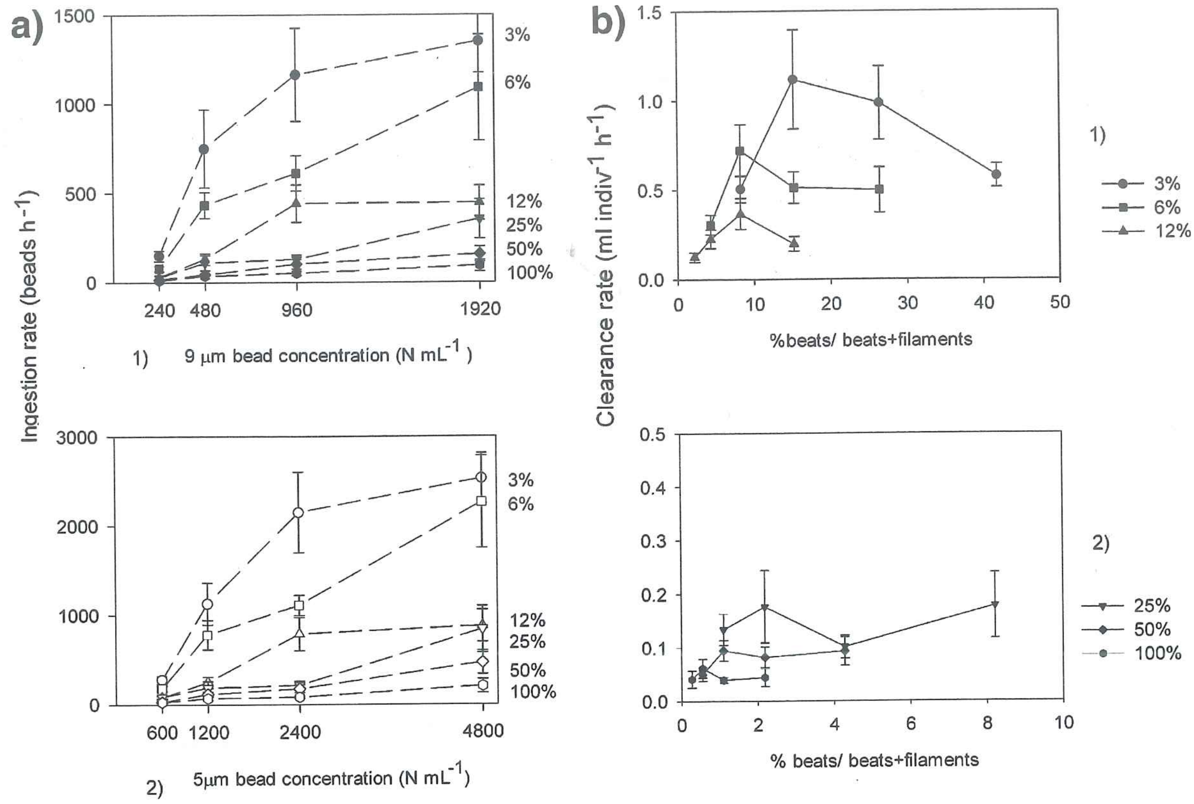

**Fig 5** (**a**) Ingestion rates of *D. magna* in relation to food (bead) concentrations at various dilution levels of lagoon seston under laboratory conditions (lagoon seston percentage is indicated on the right). Means and SE of 10 individuals for each treatment combination. (1) 9 μm beads; (2) 5 μm beads; (**b**) *D. magna* CR's in relation to varying percentages of beads in bead-filament food mixtures (%beads/beads + filaments in abscissas) in laboratory experiments at (1) low and (2) high-lagoon seston concentrations. CR values shown are means and SE for ten individuals for each treatment combination

almost an order of magnitude higher than in 2005 (300 ind $l^{-1}$), with fecundity maxima up to 15 eggs per adult female.

The possible influence of phytoplankton quality as a factor in *D. magna*'s lower density in 2005, was evidenced by the extremely low clearance rates on beads obtained in the Albufera lagoon in situ experiments in the studied year (Table 2). CR were less than 1/60th of the values measured in situ for *D. magna* in Dutch eutrophic lakes (Gulati, 1978), and far lower than the estimate of 1.3 ml animal$^{-1}$ h$^{-1}$for a mean size cladocera of 2 mm, below the incipient limiting level (ILL), given by Peters & Downing (1984). Our results indicate that the high concentration of phytoplankton in the form of filaments and also large colonies in the lagoon at that time reduces the ingestion and clearance rates on small more edible unicellular algae.

A further indicator of filaments interference in feeding was the relation between clearance rate and body size that is a main source of the variance of IR or CR and can be described by a power equation (Lampert, 1987; Hawkins & Lampert, 1989). The exponent b derived from a regression with log transformed data has been a reference in many studies. The regression slope obtained in Albufera experiments ($b = 0.275$, IR or CR vs. body length) was very low compared to that normally reported ($b = 1$–$3$, mainly below the ILL, Peters & Downing, 1984; Haney, 1985; Darchambeau & Thys, 2005), which is attributable to the high densities of filaments. Our results agree with the findings of DeMott et al. (2001) for *Daphnia* feeding on seston in a eutrophic lake. The negative effects of filaments on feeding rate are more pronounced in large than small daphniids, in view of the wider carapace gape of the

**Table 3** Effect of lagoon seston dilution (SP) and bead concentration (BC) on *Daphnia magna* clearance rates (CR) on 9 and 5 μm beads

| Explanatory variable | All data | | | | High seston (4 levels) (38 to 200 × $10^3$ fil $ml^{-1}$) | | | | Low seston (2 levels) (9 and 19 × $10^3$ fil $ml^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | df | *P* value | % variation explained | Tukey | df | *P* value | % variation explained | Tukey | df | *P* value | % variation explained | Tukey |
| Inter-subjects | | | | | | | | | | | | |
| Seston percentage (SP) | 5 | <0.001*** | 67 | All except 3–6, 12–25, 50–100 | 3 | <0.001*** | 37 | All except 12–25, 50–100 | 1 | 0.043* | 7 | |
| Bead concentration (BC) | 3 | 0.004** | 7 | 1–2 | 3 | 0.129 | 5 | – | 3 | 0.014* | 17 | 1–2 |
| SP*BC (vessel) | 38 | 0.430 | 18 | | 24 | 0.652 | 15 | – | 10 | 0.081 | 25 | |
| Intra-subjects | | | | | | | | | | | | |
| Bead size (BS) | 1 | <0.001*** | 14 | | 1 | 0.001** | 9 | | 1 | <0.001*** | 37 | |
| BS*SP | 5 | 0.822 | 1 | | 3 | 0.766 | 1 | | 1 | 0.495 | 1 | |
| BS*BC | 3 | 0.080 | 4 | | 3 | 0.132 | 5 | | 3 | 0.246 | 7 | |
| BS*SP*BC (vessel) | 15 | 0.429 | 18 | | 24 | 0.533 | 16 | | 10 | 0.070 | 25 | |

Results from repeated measures ANOVA with log2 transformed data. Three fixed factors "seston percentage" and "bead" concentrations one with repeated measures, "bead size", and one random factor "vessel", nested within each treatment combination, with five replicates former, which allows entry of a greater number of filaments into the filtration chamber that accordingly requires more frequent cleaning, and thus loss of the edible food already collected (Gliwicz & Lampert, 1990; DeMott et al., 2001). This increased rejection rate adversely affects larger animals, since it causes reduced feeding rate and greater rejection costs.

### Laboratory experiments

Lampert (1987) reported clearance rates for *D. magna*, feeding on *Chlorella* and *Chlamydomonas*, in the same order of magnitude than our results with beads in GF/F filtered water. However when the same experiment was repeated in the same conditions but with the whole lagoon water instead of filtered water, CR led to a severe reduction with the extreme low values characteristic of hypereuthrophic environments (DeMott et al., 2001). In this experiment, CR values were slightly higher than the "in situ" measurements (Table 2) probably due to a hunger effect (Ringelberg & Royackers, 1985) because the *Daphnia* were allowed to empty their guts by holding them in filtered lagoon water for 4 h before the experiment.

The experiment with different dilutions of lagoon seston revealed sharp declines in *D. magna* CR's on beads of both sizes with rising large seston or filament densities, which could be nicely described by a significant power function (Fig. 4). This reduction of CR by large seston particles is either attributable to (1) mechanical interference of large daphniids' filtering activity by increasing densities of filamentous cyanobacteria through increasing rejection rates, as reported previously for large *Daphnia* (Burns, 1968; Webster & Peters, 1978; Gliwicz & Lampert, 1990) or (2) the depression of CR by total food concentrations above the ILL. In order to evaluate these alternative mechanisms, different bead concentrations were tested and two types of responses were obtained depending on filament densities (Fig. 5). At lower filament densities we obtained a typical functional response where ingestion increases with food availability until the ILL is achieved, where after CR decreases. This relationship described by Porter et al. (1982) for *D. magna* feeding on *Chlamydomonas reinhardi* gave an ILL value around $10^4$ cells ml$^{-1}$. However, at higher filament densities the extremely low ingestion rates found, suggest a mechanical collapse of *D. magna*'s collector apparatus by filaments that can agglutinate beads and not allow them to enter the daphniid feeding chamber (Haney, 1985). According to Gliwicz (1990b), there is a critical threshold concentration of blue-green filaments (ca. 67,000 fil ml$^{-1}$ for *Aphanizomenon flos-aquae*) above which *Daphnia* growth is not possible. At the highest seston concentrations in our experiment, *D. magna* may have reached a corresponding critical filament concentration level above which it shows a similar minimum ingestion rate regardless of bead concentration. Our experiments showed that attenuation of *D. magna* CR was more regulated by increasing food availability at concentrations below $19 \times 10^3$ fil ml$^{-1}$, and increasing more as filament concentrations get more diluted. Conversely, at filament concentrations above $75 \times 10^3$ ml$^{-1}$, CRs of *D. magna* vary mainly as a function of mechanical interference. At intermediate concentrations around $38 \times 10^3$ ml$^{-1}$ both processes are probably important.

Statistical analyses of CR highlight different limiting processes. Above a threshold concentration of interfering particles, the main source of CR depression reflects the phenomenon of particle rejection, whereas at lower concentrations, the asymptotic relationship of IR with increased bead concentrations becomes evident, confirming that *D. magna* can tolerate dilute concentrations of filamentous blue-greens. Although mechanical interference is still taking place, the general CR response to rising food availability is evident, even permitting ingestion of some filaments as demonstrated by some previous studies examining low filament concentrations (Davidowicz et al., 1988; Gilbert & Durand, 1990). Our gut content analyses of *D. magna* under a fluorescence microscope with filters for ficobilines and chlorophyles provided no evidence for utilization of blue-green filaments; we only detected their presence near the mandibles. Although *Daphnia* may be able to feed on short blue green filaments or detritus derived from them (Gulati et al., 2001) we found in our study conditions, that the disadvantages incurred by such filaments seem more important than the net food intake.

Water for the two experiments and for all treatment combinations shares the same origin, because lagoon water was diluted with the same water filtered through GF/F, so the possible presence of kairomones or toxins in the medium must be the same in all treatments. Our study was performed in the period of the year when filament concentrations have been reduced by water renewal, although there are no studies from this period, for summer months Bradt & Villena (2002) reported low concentration of microcystins in the Albufera lagoon despite the extremely high concentrations of cyanobacteria. So all results appointed to filamentous interference with food intake, although we do not discharge that chemical along with mechanical interference of these filaments could also be involved.

The development of *D. magna* populations in Albufera lagoon in winter, just after rice fields are emptied, is affected by the concentrations of inedible filamentous cyanobacteria. These findings suggest that one of the measures in the restoration of this eutrophicated lagoon to be considered is that hydrological management of the lagoon at this time of the year should dilute cyanobacterial populations in the lagoon at least to the above mentioned levels, which will ensure an unconstrained feeding response by *D. magna*, without severe inhibition and energetically costly feeding on dense filamentous plankton that returns only low ingestion rates.

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