

# *In situ* filtration responses of *Daphnia galeata* to changes in food quality

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*In the stoichiometric study of phyto–zooplankton interactions, a controversy exists about how Daphnia species regulate their feeding rate when submitted to low-quality food (i.e. high dietary carbon:phosphorus [C:P] ratio). In this study, we gathered data over 3 years on in situ clearance rates of a Daphnia galeata population, by conducting grazing experiments from April 1998 to October 2000 in the Esch-sur-Sûre reservoir (Grand-Duchy of Luxembourg). Observed clearance rates (2.5–13.5 mL individual<sup>-1</sup> day<sup>-1</sup>, mean 7.0 mL individual<sup>-1</sup> day<sup>-1</sup>) were correlated with population and environmental variables. Mean body size of Daphnia individuals was the best predictor of clearance rate ( $r^2 = 0.639$ ), followed by water temperature ( $r^2 = 0.262$ ) and P concentration in the seston ( $r^2 \leq 0.241$ ). When combined with body size, seston C:P or nitrogen:P ratios provided the best predictive regression model of clearance rate ( $r^2 = 0.852–0.897$ ). The clearance rate was always correlated negatively with P concentration in the food. Thus, Daphnia reacts to a decrease of food quality (i.e. higher C:P ratio) by an increase of its feeding rate. However, the filtration response to food quality was inhibited by high population density. We suggest that, when food quality is low and population density high, this inhibition may favour fitness of future conspecific generations. In conclusion, our results emphasize the effect of P availability on transfer rate of organic matter between lacustrine autotrophs and herbivores and pose questions about consequent effects on nutrient biogeochemical cycles.*

## INTRODUCTION

Eating plants is a key process transferring organic matter and energy at the base of the food web. The feeding process is mainly controlled by the herbivore, and its ingestion rate results from an optimisation between costs and benefits (Taghon, 1981; Darchambeau, in press). Beyond this simplistic assertion, the definition of benefits hides. By example, in the planktonic world, many factors control the nutrition of herbivorous zooplankton (Sterner and Schulz, 1998). When food levels are not sufficient, the collection of food particles is increased as long as new benefits exceed the supplementary costs. Thus, the dependence of zooplankton clearance rate versus food concentration fits a unimodal curve (Lampert, 1987). Less straightforward is the feeding response of consumers to food quality. When food quality is defined in terms of the equilibrium between two resources, one resource may be deficient while the

other is ingested in excess. In this instance, what is the best way to vary ingestion rate in response to resources with unsuitable proportions of necessary dietary components? If the collection of food particles is increased to compensate for the nutritional elemental deficiency, the excess element also is ingested with a higher rate, accentuating the problem of its disposal. By contrast, if the collection is decreased, fewer resources are ingested in excess but the consumer has still less of the deficient element.

This discussion was the hidden core of numerous papers on effects of food quality on zooplankton ingestion (from Libourel Houde and Roman, 1987 to Darchambeau *et al.* 2003). Food quality is there defined as the ratio between two useful elements, often carbon (C) and phosphorus (P) because these two important elemental resources may be encountered in inappropriate proportions in the food of some zooplanktonic species, such as *Daphnia* (Hessen and

Lyche, 1991; Hessen, 1992). Attempts were made to predict the feeding response to food quality. Physiological models, dealing with the balance between benefits and costs, predict in *Daphnia* the increase of food ingestion with the rise of dietary C:P ratio (Plath and Boersma, 2001; Darchambeau, in press). These predictions are contradicted by numerous experiments on *Daphnia* species in which a decrease (Sterner *et al.*, 1993; Van Donk and Hessen, 1993) or a stability (Rothhaupt, 1995; Lürling and Van Donk, 1997; Van Donk *et al.*, 1997; DeMott *et al.*, 1998; Hessen *et al.*, 2002; Darchambeau *et al.*, 2003) of the clearance rate is observed if food quality is lowered. To our knowledge, only the study of Plath and Boersma (Plath and Boersma, 2001) demonstrated a positive response of feeding-appendage beat rate of *Daphnia* to dietary nutrient deficiency. These *a priori* contradictory results need to be reconciled.

Some arguments may be found in the fact that all experiments did not follow the same experimental design. Several authors observed disappearance rates of algal cells in the presence of consumers and translated the observed decrease in the number of cells into clearance rates (Sterner and Smith, 1993; Sterner *et al.*, 1993; Van Donk and Hessen, 1993, 1995; Lürling and Van Donk, 1997; Van Donk *et al.*, 1997; Hessen *et al.*, 2002). This method may lead to inconclusive results about grazing rates, as it was demonstrated that the passage through the consumer gut of intact viable cells is increased when algae are nutrient-deficient (Van Donk and Hessen, 1993). Other studies have used labelled algae or beads, stopping ingestion of animals before egestion of the markers occurs (Butler *et al.*, 1989; Lürling and Van Donk, 1997; Van Donk *et al.*, 1997; DeMott *et al.*, 1998; Darchambeau *et al.*, 2003). In these experiments, except in DeMott *et al.* (DeMott *et al.*, 1998), animals were never acclimated to food quality before grazing measurements. We must note here that the response of clearance rate may be behavioural (modification of the feeding-appendage beat rate; Plath and Boersma, 2001) but also morphological (modification of the size of the filter screens and/or meshes; Lampert, 1994). Moreover higher secretion of digestive enzymes may facilitate both responses (Darchambeau, in press). So it seems more appropriate to consider that these responses may occur only at mid- (some hours for the behavioural one) to long-term scales (some days for the morphological one) (Rothhaupt and Lampert, 1992). Note also that all these responses might be phenotypic, genotypic or both, and, in natural populations, the success of different clones in the seasonal succession may result from their ability to adapt to nutrient deficiency.

All these considerations call for large studies on natural populations of *Daphnia* feeding on algae of varying quality. In a 3-year study, we conducted *in situ* grazing

experiments to observe seasonal variations of clearance rate of a *Daphnia galeata* population from a temperate reservoir. We analysed these variations in relation to seston elemental composition (C:N:P ratios) but also composition and biomass of phytoplankton, and other variables. As we focus on the effect of dietary nutrient deficiency, the variance of clearance rate has been carefully partitioned among all potentially explanatory variables by means of multiple regressions. Significant results were highlighted by a Bayesian analysis of the best regression model.

## METHOD

### Field data acquisition

This field study was conducted in the Esch-sur-Sûre reservoir, which lies in the northern part of the Grand-Duchy of Luxembourg. According to the OECD classification (OECD, 1982), the reservoir is considered as a meso-eutrophic waterbody (Dohet and Hoffmann, 1995). *Daphnia galeata*, *Bosmina coregoni* and *Diaphanosoma brachyurum* are the dominant cladocerans in the reservoir (Dohet and Hoffmann, 1995). The seasonal survey was conducted at a station (maximum depth 30 m) located in the middle of the lake, representative of whole lake conditions (Thys *et al.*, 1998).

Zooplankton density was measured in parallel with seston analysis from January 1998 to December 2000. Samplings were conducted weekly from April to October and monthly during winter. Stratification layers were delimited according to the limnological profiles obtained using a Hydrolab DS-4 multiprobe. The zooplankton community was sampled with a 50-cm diameter, 50- $\mu$ m mesh net towed in the epilimnion during the stratification period and in the 0–10-m layer during the mixing periods. Triplicate samples were collected and pooled to decrease heterogeneity in zooplankton horizontal distribution and sampling variability. For seston analysis, a pooled sample was constituted from discrete samples collected with a 30-L Ruttner bottle. Discrete samples were taken every meter in the same layers as those sampled for zooplankton.

Zooplankton was immediately narcotised in soda water, rinsed and preserved in 4% formalin solution (Haney and Hall, 1973). In the laboratory, the cladocerans were counted under an inverted microscope. At least 200 individuals of each species were counted, and *D. galeata* individuals measured from the upper edge of the eye to the base of the tail spine.

One hundred millilitre of the pooled seston sample was preserved with acid Lugol for microscopic analysis. Edible particles for cladocerans (Gliwicz, 1977; Gliwicz and Siedlar, 1980; Kasprzak and Lathrop, 1997) were

separated from total seston by sieving part of the pooled sample on a 28- $\mu\text{m}$  Nytex screen. Water from both edible and total fractions was divided in two parts. One was filtered *in situ* on a 47-mm GF/C (pore size 1.2  $\mu\text{m}$ ) Whatman filters and directly frozen in liquid nitrogen for pigment analysis using HPLC. The other part was filtered *in situ* on six pre-ignited 25-mm GF/C Whatman filters and directly frozen in dry ice for elemental analysis.

Pigments were extracted and analysed following Descy *et al.* (Descy *et al.*, 1999), using the HPLC protocol of Wright *et al.* (Wright *et al.*, 1991). Pigments were detected by a Waters 996 PDA detector and a Waters 470 fluorescence detector, and calibration was achieved using external standards (Pandolfini *et al.*, 2000). Chlorophyll *a* (Chl *a*) biomass of each algal class was estimated using the CHEMTAX program (Mackey *et al.*, 1996), from the concentration of selected marker pigments.

From the six filters collected by date and by fraction for the elemental analysis, three were analysed for particulate C and N contents and three for particulate P content. Particulate C and N were analysed with a Carlo-Erba CN NA1500 elemental analyser using acetonitrile as standard. Total P was analysed by spectrophotometric determination of phosphate after potassium persulphate digestion (Clesceri *et al.*, 1998). Seston elemental ratios were expressed as the ratio of the means in molar units corrected for the variance of denominator (Dagnelie, 1992).

### Feeding rates

We measured *in situ* grazing rates of *D. galeata* in parallel with variables expected to affect clearance rates: temperature, density of the zooplankton community, mean body size of the *D. galeata* population, sestonic biomass (in terms of C, N and P) and C:N:P ratios in total and edible (<28  $\mu\text{m}$ ) size fractions, algal biomass (Chl *a*) and composition in both fractions. All these factors were measured in the layer where the grazing experiment was conducted. Experiments were essentially run at mid-depth in the epilimnion but also sometimes in the metalimnion when *D. galeata* density was sufficient. In total, 89 grazing experiments were conducted from April 1998 to October 2000.

The technique used for *in situ* determination of individual clearance rates involved incubation with radioactively labelled algae in a 6.5-L transparent grazing chamber (Haney, 1971) designed after Gawler and Chapuis (Gawler and Chapuis, 1987). The cultivated green alga *Kirchneriella subcapitata* (syn. *Selenastrum capricornutum*) (length 7–23  $\mu\text{m}$ , width 1.2–5  $\mu\text{m}$ ) was incubated for at least 24 h before experiments with 9.25 MBq  $\text{NaH}^{14}\text{CO}_3$  per 250 mL of culture medium. Unincorporated labelled bicarbonate was

removed by repeated centrifugation and rinsing. The algal suspension was then stored in a dark refrigerator until use in the field. The volume of labelled suspension injected in the chamber food compartment was adjusted to correspond to <10% of the algal biomass in the lake, as estimated from Chl *a* measured the preceding day. The duration of incubations was ~6 min from the closure of the chamber to the collection of zooplankton. This incubation time is normally shorter than the gut passage time of *D. galeata* (Haney, 1971; Cauchie *et al.*, 2001). At the end of incubation, the zooplankton was collected on a 100- $\mu\text{m}$  sieve, rinsed with filtered lake water, anaesthetized for 2 min in soda water, resuspended in filtered lake water and deep-frozen in liquid nitrogen. Incubations were run at least in triplicate. In the laboratory, the samples were thawed one at a time. *D. galeata* individuals were sorted by size classes (<0.5, 0.5–0.75, 0.75–1, 1–1.25, >1.25 mm), measured and placed on 5- $\mu\text{m}$  nitro-cellulose filters. When density of *D. galeata* was high, subsamples of individuals of the same size were sorted for replicating the counting. When density of *D. galeata* was low, they were not sorted by size.

For each incubation, a fraction of the suspension was collected on 0.45- $\mu\text{m}$  filters (Millipore) for determination of total suspended radioactivity. Control incubations without radioactively labelled food were also run, and all measurements were corrected for background radioactivity. All samples were placed in 20-mL scintillation vials added with liquid scintillation cocktail (FilterCount, Packard), and radioactivity (dpm) was measured with a Beckman LS 6000IC scintillation counter. Preliminary experiments revealed a constant radioactivity loss in sorted samples due to freezing and thawing (Thys, 2003). A correcting factor of 1.558 was thus applied to radioactivity measurements before calculation of clearance rates. Individual clearance rates ( $\text{mL individual}^{-1} \text{h}^{-1}$ ) were calculated according to Peters (Peters, 1984).

### Data processing

Our purpose was to determine the relative effect of P concentration in the food on *D. galeata* clearance rate. But, as P is not the only variable influencing clearance rate in natural zooplankton communities, we need to appropriately distinguish the respective effect of each potentially explanatory variable. Therefore, simple and multiple linear regressions obtained using the Statistica statistical package (StatSoft, Inc., 1996) were firstly computed. Secondly, the interacting influences of predictors including C:P or N:P ratios on clearance rate were visualized by probability distributions of predicted clearance rate. These distributions were obtained by Bayesian analysis of a multiple regression model (Gelman *et al.*, 1995).

The analysis resembles a conventional multiple regression but the interpretation is completely different (Ellison, 1996). The Bayesian analyses yield probability distributions for predicted values of clearance rate. These distributions give the probability that a particular predicted value will be actually observed. In contrast, conventional multiple regression analyses used most commonly in ecology predict confidence intervals that include the true value of the response variate in a given percentage of independent, identical studies (Ellison, 1996). In this sense, and as Bayesian analyses tell us how probable our hypotheses actually are given the available data, they allow stronger conclusions to be drawn from large-scale ecological experiments with few replicates (Ellison, 1996).

Our analysis assumed that the predicted probability distributions depend on our data alone and did not depend on any additional or external information. This assumption is termed a noninformative prior distribution (Gelman *et al.*, 1995). Probability distributions of clearance rate were calculated using the model

$$F = X + E \quad (1)$$

where  $F$  is the vector of response variate (the clearance rate).  $E$  is an independently, identically and normally distributed prediction error with mean 0 and variance  $s^2$ .  $X$  is the matrix of selected predictors and their interactions. The posterior predictive probability distribution of  $F$  for new data (or scenarios) conditional on  $X$  is a  $t$ -distribution with  $n-k$  degrees of freedom where  $n$  is the number of observations, and  $k$  is the number of parameters (one plus the predictors plus their interactions) (Gelman *et al.*, 1995). The mean of the  $t$ -distribution is

$$\text{mean}(\tilde{F}) = \hat{X}\hat{\beta} \quad (2)$$

where

$$\hat{\beta} = (X^T X)^{-1} X^T F \quad (3)$$

The variance of the  $t$ -distribution is

$$\text{var}(\tilde{F}) = (I + \tilde{X}V_{\beta}\tilde{X}^T)s^2 \quad (4)$$

where

$$V_{\beta} = (X^T X)^{-1} \quad (5)$$

$$s^2 = \frac{1}{n-k} (F - X\hat{\beta})^T (F - X\hat{\beta}) \quad (6)$$

and  $I$  is the identity matrix.

## RESULTS

### Resource composition

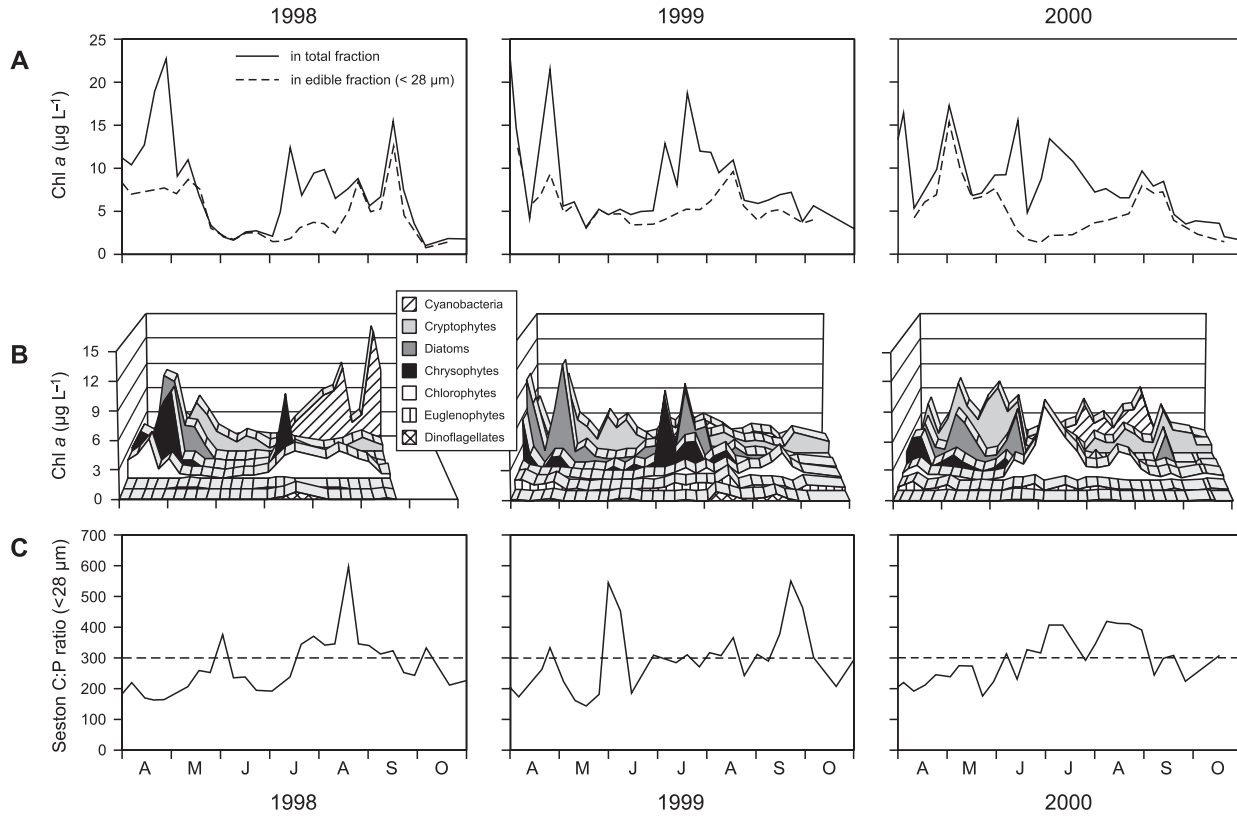
The pattern of phytoplankton development in the reservoir was relatively similar from one year to another. Chl  $a$  biomass showed a marked spring peak followed in 1998 and 1999 by a clear-water phase (Fig. 1A). The summer assemblage comprised a greater proportion of algal units  $>28 \mu\text{m}$ , while smaller algae became dominant in fall.

Phytoplankton composition varied with time (Fig. 1B). Throughout April and May, diatoms (among others, *Cyclotella* sp., *Stephanodiscus* sp., *Asterionella* sp.) and cryptophytes (*Rhodomonas* sp. and *Cryptomonas* sp.) dominated the plankton, sometimes with chrysophytes (mainly *Chrysidalis* sp.). In June 1998 and 1999, there was a marked clear-water phase during which cryptophytes were virtually the only ‘edible’ algae left. In July, large algae dominated the phytoplankton, mostly diatoms (*Fragilaria* sp.), green algae (mainly colonies of *Eutetramorus* sp.) or chrysophytes (*Chrysochococcus* sp. or *Mallomonas* sp.). Cyanobacteria were present throughout the growing season, and they developed further through August and September to become the dominant phytoplankton fraction, except in 1999, when large diatoms (*Fragilaria* sp.) prevailed. Cyanobacteria were single cells or colonies of Chroococcales or filamentous forms (mainly *Pseudanabaena*, *Planktothrix*, *Anabaena*, *Aphanizomenon* and *Leptolyngbya*).

The sestonic C:P ratio increased progressively over the year, reaching a first maximum during the clear-water phase in 1998 and 1999 and a second more marked maximum in August or September (Fig. 1C). The C:P ratio was frequently above the quality threshold for *Daphnia* (estimated  $\sim 300$  by Sterner, 1997; Urabe *et al.*, 1997; Brett *et al.*, 2000). Edible seston (i.e.  $<28 \mu\text{m}$ ) was significantly slightly poorer in N (proportionally to C) than the total fraction, whereas there was no difference in both C:P and N:P ratios between fractions (Table I). The C:P ratios were also highly significantly correlated with C:N and N:P ratios, whereas there was no significant correlation between C:N and N:P ratios.

### *Daphnia galeata* abundance and feeding response

Population dynamics of *D. galeata* were similar in all years investigated (Fig. 2). High abundances during the clear-water phase were followed by a decline in mid-June resulting in very low population densities (mid-summer decline). *Daphnia* remained scarce throughout the rest of the year except in 2000, when they developed a small autumnal peak. The lowest maximum abundance over a year occurred in 1999 ( $<5$  individuals  $\text{L}^{-1}$ ) and the highest in May 2000 ( $\pm 20$  individuals  $\text{L}^{-1}$ ).



**Fig. 1.** Seasonal variations of algal community from 1998 to 2000 in the upper layer of the Esch-sur-Sûre reservoir. **(A)** Total and edible algal biomass expressed by Chl *a*. **(B)** Class-specific phytoplankton biomass estimated through the HPLC analysis of specific marker-pigments. **(C)** C:P ratio in edible seston. The broken line represents the theoretical quality threshold ratio above which animal growth is predicted limited by food quality (Sterner, 1997; Urabe *et al.*, 1997; Brett *et al.*, 2000).

*Table I: Correlations between sestonic C:N:P ratios in the Esch-sur-Sûre reservoir from April to October in 1998, 1999 and 2000*

x	y	n	r	P <sup>a</sup>	Slope <sup>b</sup>	P <sup>c</sup>
Correlations between size fractions						
C:N total	C:N <28 µm	80	+0.808	<0.001	+1.070	0.004
C:P total	C:P < 28 µm	78	+0.832	<0.001	+0.921	0.444
N:P total	N:P < 28 µm	78	+0.768	<0.001	+0.954	0.885
Correlations between elements (all fractions)						
C:P	C:N	165	+0.440	<0.001		
C:P	N:P	165	+0.900	<0.001		
C:N	N:P	165	+0.198	0.801		

*r* are Pearson correlation coefficients.

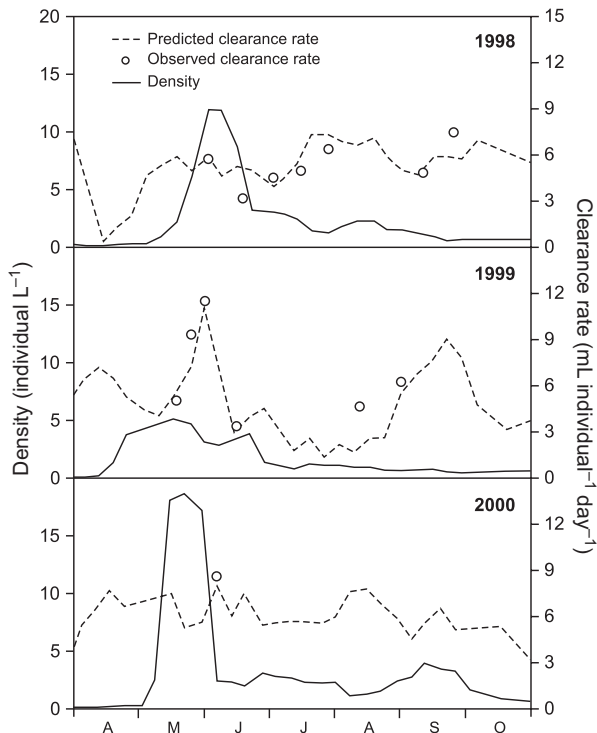
<sup>a</sup>Probability that *r* = 0.

<sup>b</sup>Slopes of the major axis regression.

<sup>c</sup>Probability that the difference between both fractions = 0 (two-tailed *t*-test for paired samples).

In total, 89 grazing experiments were run, leading to 36 observations differing in dates and/or *Daphnia* body size. Observed *D. galeata* clearance rates varied between 2.5 and 13.5 mL individual<sup>-1</sup> day<sup>-1</sup> (mean, 7.0 mL individual<sup>-1</sup> day<sup>-1</sup>).

Fourteen data out of 36 represented the clearance rate of the whole *D. galeata* population, while the resting 22 were from samples sorted by *Daphnia* size. Grazing data for the whole population are presented in Fig. 2.



**Fig. 2.** Density in the upper layer and clearance rates of *Daphnia galeata* in the Esch-sur-Sûre reservoir from 1998 to 2000. Full lines represent moving averages on three data of density. Empty circles represent observed clearance rates of the whole *D. galeata* population; samples sorted by size are not figured. Dotted lines are population clearance rates predicted from the multiple linear regression model (see Table III).

Table II gives the results of simple linear regressions of clearance rates against environmental variables. Body size emerged as the most important factor explaining *D. galeata* clearance rates (63.9% of total variance). As expected, filtration activity increased proportionally to body size. The water temperature is the second best explanatory variable (26.2%), with reduction of clearance rates when temperature increases. Next, several variables expressing seston P content were singled out: C:P ratio, N:P ratio and particulate P concentration of the edible fraction (respectively, 24.1, 21.0 and 20.0%), followed by N:P ratio, particulate P concentration and C:P ratio of the total fraction (12.2, 11.6 and 11.5%). When particulate P became deficient (lower particulate P concentrations or higher C:P or N:P ratios), filtration activities increased. Worth noticing is that Chl *a* biomass, both in total and <28 µm fractions, was not significant ( $P > 0.05$ ).

Owing to the likely high correlations of body size with some variables, it seemed more appropriate to test the significance of multiple models containing the mean body size and one of the other explanatory variables

(Table III). Once again, all N:P and C:P ratios are significant and, in linear combinations with mean body size, provide highly explanatory models of individual clearance rates ( $r^2 > 0.85$ ). We may here note that water temperature was highly correlated with body size and explained a part of the variance of clearance rate already explained by mean body size.

For exploring further the role of seston P deficiency on clearance rate, we have constructed a multiple regression model using a forward selection procedure ( $F$  to enter = 1; the  $F$  value determines how significant the contribution of a variable to the regression has to be in order for it to be added to the model). The best variable association selected 3 variables (mean *Daphnia* body size, N:P ratio in total seston and crustacean density in the upper layer) providing in linear combination a model explaining 92.0% of clearance rate. Matrix  $X$  of Equation 1–6 was filled for the 36 observations of clearance rate with an independent term, the predictors and their interactions:

$$X = [1 \ S \ NP \ D \ S \times NP \ S \times D \ NP \times D \ S \times NP \times D] \quad (7)$$

where  $S$  is the mean body size of *D. galeata*,  $NP$  is the N:P ratio in total seston, and  $D$  is the crustacean density. The predicted probability distributions of clearance rate were obtained by a Bayesian analysis of the multiple regression model. Probability distributions indicate the responses of clearance rate to selected combinations of mean size, N:P ratio and crustacean density (Fig. 3). For given values of mean *Daphnia* body size and density, an increase of seston N:P ratio causes clearance rate to increase. For example, when mean size is 1.2 mm and crustacean density is 5 individuals  $L^{-1}$ , a N:P ratio of 15 yields a slightly variable clearance rate with a mode of 9.2  $mL\ individual^{-1}\ day^{-1}$ . If N:P ratio is increased to 60, clearance rate becomes higher with a modal value of 15.1  $mL\ individual^{-1}\ day^{-1}$ . A higher crustacean density generally yields more variable clearance rates with lower modes, except for small animals feeding on P-rich seston (N:P ratio = 15) for which clearance rate is slightly increased. Interestingly, the influence of N:P ratio on clearance rate is decreased under high crustacean density.

## DISCUSSION

Our *in situ* observations are in good agreement with the prediction of our physiological model of *Daphnia* regulation (Darchambeau, in press): *Daphnia* increases its clearance rate when food becomes P-deficient. This study corroborates experimental works of Libourel Houde and Roman (Libourel Houde and Roman, 1987) on the copepod *Acartia tonsa* and of Plath and Boersma (Plath and Boersma, 2001) on the cladoceran

Table II: Simple linear regressions between clearance rates of *Daphnia galeata* and environmental explanatory variables ( $y = b_0 + b_1x$ )

Explanatory variables x	Minimum	Mean	Maximum	r	r <sup>2</sup>	P <sup>a</sup>
Body size (mm)	0.35	0.86	1.37	+0.80	0.639	<0.001
Temperature (°C)	9.9	17.2	22.1	-0.51	0.262	0.001
C:P ratio (<28 µm)	187	304	454	+0.49	0.241	0.002
N:P ratio (<28 µm)	21	38	61	+0.46	0.210	0.005
Particulate P (<28 µm) (µg L <sup>-1</sup> )	1.0	2.6	5.2	-0.45	0.200	0.006
N:P (total)	15	39	71	+0.35	0.122	0.037
Particulate P (total) (µg L <sup>-1</sup> )	1.5	4.1	7.4	-0.34	0.116	0.042
C:P (total)	142	303	549	+0.34	0.115	0.043
% of inedible algae <sup>b</sup>	0	27	77	-0.34	0.114	0.044
Chlorophytes (µg Chl a L <sup>-1</sup> )	0.0	0.3	1.7	-0.33	0.111	0.047
Euglenophytes (µg Chl a L <sup>-1</sup> )	0.0	0.1	0.4	-0.27	0.074	0.110
Particulate C (<28 µm) (µg L <sup>-1</sup> )	154	287	597	-0.27	0.073	0.110
Chl a (total) (µg L <sup>-1</sup> )	2.2	6.0	12.7	-0.27	0.073	0.092
Crustacean density (individuals L <sup>-1</sup> )	1.8	16	41	-0.27	0.072	0.114
Chrysophytes (µg Chl a L <sup>-1</sup> )	0.0	0.2	1.3	-0.26	0.067	0.127
Particulate N (<28 µm) (µg L <sup>-1</sup> )	22	43	83	-0.26	0.066	0.130
Dinoflagellates (µg Chl a L <sup>-1</sup> )	0.0	0.1	0.9	-0.24	0.056	0.165
Particulate C (total) (µg L <sup>-1</sup> )	204	450	888	-0.23	0.052	0.180
Particulate N (total) (µg L <sup>-1</sup> )	25	70	161	-0.17	0.030	0.310
C:N (total)	6.4	7.9	9.7	-0.15	0.023	0.377
Cyanobacteria (µg Chl a L <sup>-1</sup> )	0.0	0.6	3.8	-0.14	0.021	0.404
Chl a (<28 µm) (µg L <sup>-1</sup> )	1.4	4.0	8.6	-0.14	0.020	0.405
Diatoms (µg Chl a L <sup>-1</sup> )	0.0	0.2	0.9	-0.07	0.005	0.678
C:N (<28 µm)	7.0	8.0	8.9	+0.05	0.003	0.750

Variables are classified in descending order of explained variance (r<sup>2</sup>). r are Pearson correlation coefficients. n = 36.

<sup>a</sup>Probability that b<sub>1</sub> = 0.

<sup>b</sup>Units: Chl a:Chl a 100.

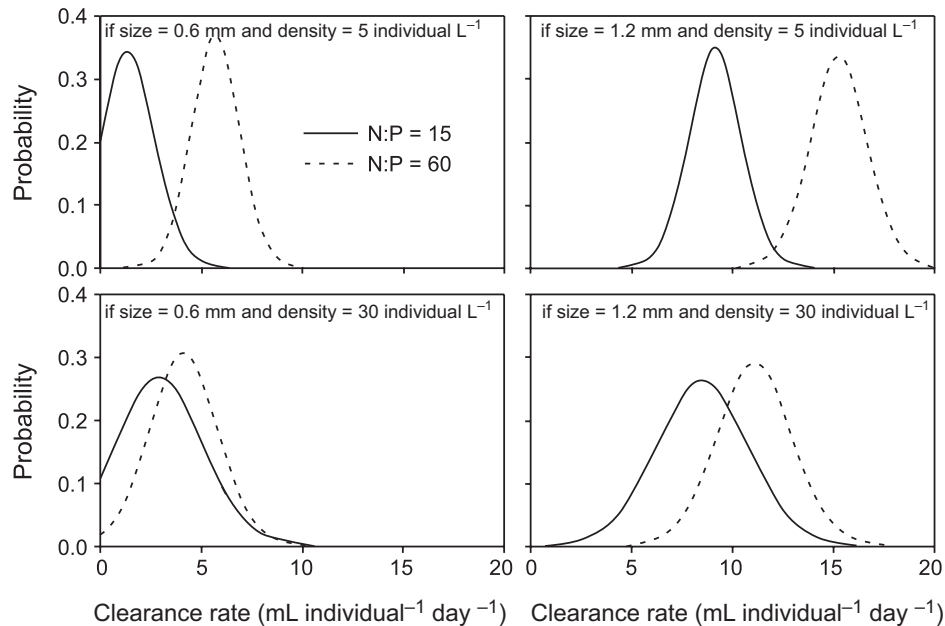
Table III: Multiple linear regressions between clearance rates in mL individual<sup>-1</sup> day<sup>-1</sup> of *D. galeata* and two environmental explanatory variables ( $y = b_0 + b_1x_1 + b_2x_2$ )

x <sub>1</sub>	x <sub>2</sub>	r <sup>2</sup>	b <sub>0</sub>	b <sub>1</sub>	b <sub>2</sub>	P for b <sub>1</sub> <sup>a</sup>	P for b <sub>2</sub> <sup>a</sup>
Body size <sup>b</sup>	N:P (total) <sup>b</sup>	0.897	-8.24 <sup>b</sup>	+12.9 <sup>b</sup>	+0.108 <sup>b</sup>	<0.001	<0.001
Body size	C:P (total)	0.890	-8.51	+12.9	+0.0148	<0.001	<0.001
Body size	N:P (<28)	0.881	-8.45	+11.8	+0.140	<0.001	<0.001
Body size	C:P (<28)	0.852	-8.02	+11.2	+0.0178	<0.001	<0.001
Body size	Cryptophytes	0.708	-4.20	+11.3	+0.595	<0.001	0.009
Body size	Crustacean density	0.696	-1.40	+11.4	-0.0837	<0.001	0.019
Body size	C:N (<28)	0.693	+7.30	+12.7	-1.40	<0.001	0.021
Body size	C:N (total)	0.690	+2.98	+11.8	-0.763	<0.001	0.026

Same units as in Table II. Models are classified in descending order of explained variance (r<sup>2</sup>). Only models with both slopes b<sub>1</sub> and b<sub>2</sub> significantly different from 0 (P < 0.05) are showed. n = 36.

<sup>a</sup>Probability that b<sub>1</sub> or b<sub>2</sub> = 0.

<sup>b</sup>They are the variables and parameter values used for predicting clearance rate during the whole study period figured in Fig. 2.



**Fig. 3.** Probability distributions of clearance rate of *Daphnia galeata* for eight scenarios: all combinations of low and high values for seston N:P ratio (in total fraction), mean *Daphnia* body size and crustacean density. The area under each curve is 1 (d.f. =  $36 - 8 = 28$ ). In the lake, seston N:P = 15 and 60 are equivalent respectively to seston C:P =  $\sim 150$  and  $\sim 592$ .

*Daphnia magna*. But it invalidates the results of Van Donk *et al.* (Van Donk *et al.*, 1997), Lürling and Van Donk (Lürling and Van Donk, 1997), DeMott *et al.* (DeMott *et al.*, 1998), Hessen *et al.* (Hessen *et al.*, 2002) and Darchambeau *et al.* (Darchambeau *et al.*, 2003). All these authors failed to detect any (positive or negative) influence of food C:P ratio on *Daphnia's* clearance rate. In most experimental designs, animals were not acclimated to food quality before grazing experiments. However, in DeMott *et al.* (DeMott *et al.*, 1998), animals were acclimated for 40 h at 5 different food qualities (C:P range going from 120 to 900) before the measurement of feeding rate. Their analysis of variance (ANOVA) showed no effect of the treatment on weight-specific ingestion rate but their results were affected by high intra-group variances (see Fig. 6 in DeMott *et al.*, 1998). To reconcile these contrasting experimental results, we can hypothesize that the filtration response of herbivores to food nutrient-deficiency is not immediate, but needs some hours to develop. This response cannot be observed in studies without long-term acclimation. In this study, dealing with natural communities, we observe animals displaying long-term phenotypic and/or genotypic adaptations to low food quality.

The results of Plath and Boersma (Plath and Boersma, 2001) suggest that the increased clearance rates observed in animals feeding on P-deficient food may result from higher beat rates of feeding appendages. Morphological

adaptations may also lead to more efficient collection of particles. As already observed in low-food conditions (Lampert, 1994; Lampert and Brendelberger, 1996), we may suspect that *Daphnia* adapted to P-deficient diet have larger filter screens and/or finer meshes. It should be interesting to observe whether these morphological differences do exist among natural (monoclonal or non-monoclonal) populations of different sites with varied food conditions.

Evidences also showed an increase of the feeding activity of some insects when their food was nutrient-deficient (Slansky and Feeny, 1977; Reynolds, 1990). In these cases, clearly greater ingestion allows higher access in the same unit of time to the deficient element. But the rise of feeding activity is not a common response of animals to dietary nutrient-paucity. For example, dietary amino acid deficiency is known to decrease feed intake in fish (de la Higuera, 2001), the inverse of poultry and pigs (Boorman, 1979; Henry, 1985). A possible explanation of intake decrease in fish is that this behaviour would prevent or delay the onset of metabolic disorders (de la Higuera, 2001). We may suspect that the ingestion response to food quality is species-dependent and, above all, depends on the nature of the deficient element. Energy-lacking food may probably not induce an increase of food intake, while the deficiency of structural elements may be compensated by increased ingestion.



An *a priori* curious response of filtering rates to the density of zooplankton was detected: the clearance rate is depressed by high-zooplankton density only at high seston N:P ratio (Fig. 3). The inhibitory effect of crowding on the ingestion rate of *Daphnia* species is well-known (Hayward and Gallup, 1976; Helgen, 1987; Matveev, 1993). It is produced by released chemicals and, although still largely unclear, may provide between conspecifics an additional, density-dependent mechanism of population regulation (Burns, 2000). Crowding also induces in some species a decrease in clutch size, but with bigger egg size, and hence longer survival of juveniles without food (Burns, 1995; Cleuvers *et al.*, 1997). This mutual intraspecific influence on reproduction parameters, called life-strategy shift by intraspecific interaction (LiSSII), may be an adaptive response under unfavourable environmental conditions (Cleuvers *et al.*, 1997). As the LiSSII, the reduction of ingestion observed under high density conditions may be seen as an adaptive behaviour favouring fitness of future conspecific generations, and especially when food quality is low.

A rise of ingestion rate implies in *Daphnia* a lowered residence time of the food into the gut, and hence decreased assimilation efficiency and higher nutrient turnover (Darchambeau, in press). So indirect effects of consumer behavioural response to changes in food quality might be expected, and need further investigations. For example, it should be very interesting to investigate the interactive, indirect effects of food quality and grazing on nutrient recycling and stimulation of algal growth (Stern, 1986). Another important question is the fate of the non-assimilated elements, knowing the importance of egested materials in total sedimentation (Sarnelle, 1999). Indeed, the stoichiometry of sinking particles is largely influenced by grazing importance and egested fraction of food (Elser *et al.*, 1996; Darchambeau *et al.* in press), both parameters observed in this study varying as a function of food quality.

In conclusion, our results emphasize the importance of P availability in resources for *D. galeata* in natural conditions. The observed increase of clearance rates with the rise of seston P-deficiency is an appropriate adaptive behavioural response to nutrient-deficiency. Direct and indirect effects of this response should be further studied, with a particular focus on the biogeochemical cycle of the excess element.

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