Seasonal shifts in phytoplankton ingestion by *Daphnia galeata*, assessed by analysis of marker pigments

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To determine food selectivity in *Daphnia galeata*, a common freshwater cladoceran, the carotenoid pigments in the guts of animals collected in a meso-eutrophic reservoir were analysed. Using the ratios of carotenoid to chlorophyll a for the main marker pigments enabled an investigation of the algal diet of this herbivorous zooplankton at the class level in natural conditions. To allow a comparison with data from pigments, food-removal experiments were carried out using *Daphnia* spp. isolated from the lake, fed with the algal assemblages present in the lake at different times. For both methods, based on gut-pigment analysis and food removal, the total and class-specific grazing rates were calculated, and food selection over time was assessed from the mean of selectivity indexes. Except for three dates, both methods agreed on the qualitative composition of the diet of *D. galeata*. This herbivorous species fed preferentially on the most available algae in the edible size range, and showed positive selection for cyanobacteria and cryptophytes during most of the growing season. The gut-pigment method seems to be an interesting tool for following the algal selectivity of freshwater cladocerans in natural conditions.

INTRODUCTION

The most severe oscillations in phytoplankton abundance and composition are often observed in mesotrophic and eutrophic temperate lakes. In these lakes, seasonal changes in phytoplankton composition result in significant changes in the size distribution and nutritional value of potential food for herbivorous zooplankton. Therefore, food selectivity in grazers may be considered as a response to phytoplankton fluctuations. Conversely, variation in the abundance of herbivorous zooplankton can often be explained by interspecific differences in resource use (DeMott and Kerfoot, 1982).

In the past 20 years, numerous studies of zooplankton grazing have focused on the feeding behaviour of various species [for a review, see DeMott (DeMott, 1994)]. These studies have addressed the effect of algal size (Bogdan and Gilbert, 1984; Hessen, 1985; McCauley, 1985; Berggreen et al., 1988; DeMott and Watson, 1991; Sommer et al., 2000), shape (Horn, 1985), nutritional quality (Cowles et al., 1988; Sierszen and Frost, 1995) and taste (DeMott, 1986). Generally, studies have evaluated diet and selectivity by comparing clearance of artificial algal suspensions or of natural assemblages. These investigations are usually based on algal counts by microscopy to assess food-removal rates in bottles containing zooplankton, with incubations without zooplankton as controls. High-performance liquid chromatography (HPLC) analysis of chlorophyll (Chl) and carotenoid pigments in the algal suspensions represents an alternative approach to algal counts in controls and incubations (Head and Harris, 1994; Meyer-Harms and von Bodungen, 1997; Descy et al., 1999; Pandolfini et al., 2000). This technique can provide precise estimates of grazing rates of zooplankton on different algal groups, and is widely applicable, provided that high taxonomic resolution is not required.

However, the long incubations needed for the food-removal technique suffer from so-called ‘bottle effects’ (Roman and Rublee, 1980). Moreover, zooplankters are often collected, isolated and incubated at densities higher than in their natural environment, and have to be acclimated to their new conditions. Such manipulations are carried out in variable conditions involving stress for the animals and may suppress the diel feeding pattern observed in nature. All these factors place the grazers in artificial conditions and cast uncertainty on the
measured clearance rates. Flowing systems could minimize some of these shortcomings (Gladyshhev et al., 1999) but problems linked to the incubation persist. Therefore, alternative techniques for measuring grazing rates and selectivity closer to natural conditions have been favoured. For instance, biomarkers, such as algal pigments and fatty acids, have allowed the study of trophic relationships in aquatic food chains (Quiblier-Lloberas et al., 1996; Leveillé et al., 1997; Gladyshhev et al., 2000).

The analysis of gut pigments has become a popular tool for investigating the feeding activity of planktonic herbivores (Mackas and Bohrer, 1976; Dagg and Walser, 1987; Kleppel et al., 1988; Bautista and Harris, 1992; Landry et al., 1994; Quiblier-Lloberas, 1994; Quiblier-Lloberas et al., 1996a). The principle underlying the method is that the pigments from the ingested algal cells can be either measured directly by the gut fluorescence (for chlorophylls and derivatives), or extracted in an organic solvent and determined by HPLC analysis (chlorophylls and specific carotenoids). If the gut evacuation rate of the animals is estimated, the ingestion rate of algae, expressed as amount of pigment per unit time, can easily be calculated. This technique provides data directly from the field without the need to conduct incubations, and the determination of marker pigments provides a good basis for evaluating grazer selectivity among phytoplankton groups. However, the gut-pigment method can always be suspected of underestimating grazing rates because of the varying degree of pigment transformation and destruction, particularly in copepods (Conover et al., 1986; Lopez et al., 1988; Penry and Frost, 1991; Head and Harris, 1992, 1994, 1996; Mayzaud and Razouls, 1992; Fundel et al., 1998; Kleppel, 1998; Strom et al., 1998; Tirelli and Mayzaud, 1998; Descy et al., 1999; Pandolfini et al., 2000). By contrast, studies on freshwater cladocerans suggested that this method could be useful to determine algal selectivity by Daphnia spp. in the field (Quiblier-Lloberas, 1994; Quiblier-Lloberas et al., 1996a; Fundel et al., 1998; Pandolfini et al., 2000).

In this study, we applied the gut-pigment method to determine in situ the algal diet of D. galeata in a mesotrophic reservoir and to track the temporal changes in the diet of this species. To validate the method, we conducted food-removal experiments as a comparison with the pigment approach. We used HPLC analysis of the main class-specific marker pigments to quantify the biomass of different algae, both in the gut contents and in the algal suspensions. Although the food-removal method presents some well-known disadvantages included in the term ‘bottle effects’, this method is widely used for quantifying ingestion rates of different algae: therefore, we used it for comparison with the gut-pigment method.

**METHOD**

The study was undertaken in the Esch-sur-Šure reservoir (Grand-Duchy of Luxembourg). According to the spatial characterization of the reservoir made by Thys et al. (Thys et al., 1998), samples were taken at a central point of the lake (depth 30 m), from the epilimnion. The survey of phytoplankton composition was performed weekly throughout the study period. Algal selectivity by D. galeata was investigated by the gut-pigment method from April to October, biweekly in 1998 and 1999, and monthly in 2000. In 2000, the food-removal method was used on six occasions as a comparison with the data from pigments.

**Phytoplankton biomass and composition**

Phytoplankton were sampled weekly throughout the growing season, using a 3 l Ruttner bottle. In the stratified period, a pooled sample was constituted for the epilimnion and the metalimnion from discrete samples collected every metre. Stratification layers were delimited according to the limnological profiles established with a Hydrolab DS4 multiprobe. When the lake was not stratified, the pooled sample was constituted from discrete samples collected between 0 and 10 m. Biomass and composition were assessed through pigment analysis of the particulate fraction retained on 47 mm GF/C filters by filtration under a moderate vacuum (400 mmHg). To assess the algal biomass potentially edible for cladocerans (Gliwicz, 1977; Gliwicz and Siellar, 1980; Kasprzak and Lathrop, 1997), a portion of the water samples from the mixed layer was sieved on a 28 µm Nitex screen prior to GF/C filtration. Extraction of the algal material retained on the filters was carried out by placing the filters in 10 ml of 90% HPLC-grade acetone in a 20 ml glass scintillation vial. The vials were placed in ice in a sonication bath and sonicated for 15 min; after overnight storage in the dark at 4°C, they were sonicated again for 15 min. The final extract was filtered through 0.22 µm filters and stored in 3 ml amber vials in a freezer, prior to analysis. The HPLC system used comprises a Waters multisolvant delivery system, a Waters autosampler and two detectors: a Waters 996 diode array detector and a Waters 470 fluorescence detector [set up for detection of Chl a and derivatives λ_{ex} (excitation wavelength) 420 nm, λ_{em} (emission wavelength) >650 nm]. Separation was achieved using a ternary 30-min gradient, slightly modified from Wright et al. (Wright et al., 1991). A 25 cm Waters Nova-Pak C18 column was used. The standard procedure consisted of the injection of 50 µl of extract. Calibration was achieved using three- or four-point calibration curves, established with standards from DHI, Denmark. Carotenoids not present in the standard were quantified against fucoxanthin, using as relative response the ratio of the
specific absorbance coefficients at 440 nm in methanol. When necessary, identification of pigments was checked against a library of pigment spectra, obtained by diode array acquisition of chromatograms from pure pigment solutions and from acetone extracts of pure cultures of algae.

The contribution of the main groups of algae to epilimnetic Chl \( a \) was assessed using CHEMTAX (Mackey et al., 1996), with an initial pigment ratio matrix derived from Descy et al. (Descy et al., 2000). CHEMTAX runs were carried out on separate bins: samples from the epilimnion, from the metalimnion, and from the 0–10 m layer when the lake was not stratified. Several algal classes, some of them sharing one or more marker pigments, were considered. Data from microscope examinations were available for comparison with the pigment data and for identifying the main taxa (R. Willame, personal communication).

**Determination of gut pigment**

Zooplankton were collected from the reservoir at the end of the morning, using a 50 cm diameter, 250 μm mesh, closing net. Zooplankton were anaesthetized in carbon-dioxide and frozen in liquid nitrogen until sorting. In the laboratory, 100–300 individuals of \( D. galeata \) were sorted and collected on GF/C filters for pigment extraction. As far as possible, depending on densities of animals, replicates were performed. The techniques for gut-pigment extraction and processing of the extract followed those of Descy et al. and Pandolfini et al. (Descy et al., 1999; Pandolfini et al., 2000). The HPLC analysis of zooplankton extracts was carried out as described for phytoplankton.

To quantify the different types of algae present in the digestive tracts of \( D. galeata \), only the main taxonomic marker of each algal class and the marker to Chl \( a \) ratio determined with CHEMTAX on the pigment content of the lake phytoplankton were used. These ratios were, respectively, 0.606 for fucoxanthin in diatoms, 0.281 for alloxanthin in cryptophytes, 0.149 for lutein in chlorophytes, 0.022 for zeaxanthin in chlorophytes and 0.183 for zeaxanthin in cyanobacteria. Multiplying the average gut content of each marker [in ng per \( D. galeata \) individual (ind.)] by the corresponding ratio gave an estimate of the Chl \( a \) biomass of the corresponding algal class. When chlorophytes were detected in \( D. galeata \) extracts by the presence of lutein, zeaxanthin was corrected by subtracting the amount corresponding to chlorophyte biomass, before being used for estimating the biomass of cyanobacteria. We assumed carotenoid conservation in \( D. galeata \) (Quiblier-Lloberas, 1994; Quiblier-Lloberas et al., 1996a; Pandolfini et al., 2000).

Class-specific ingestion rates \( (I; \text{ng Chl } a \text{ ind.}^{-1} \text{h}^{-1}) \) were calculated according to Mackas and Bohrer (Mackas and Bohrer, 1976):

\[
I = GP_s \times k
\]

where \( GP_s \) is the average gut pigment as Chl \( a \), for each algal class (ng Chl \( a \) ind.\(^{-1}\)) and \( k \) is specific excretion rate (h\(^{-1}\)).

The total ingestion rate, i.e. of total phytoplankton (all classes considered), was calculated as:

\[
I = \sum I_h
\]

The total filtration rate \( (F; \text{ml ind.}^{-1} \text{h}^{-1}) \), i.e. of total phytoplankton (all classes considered), was calculated as:

\[
F = I/C
\]

where \( C = \text{Chl } a \) concentration in the epilimnion at the corresponding sampling date.

The specific excretion rate has been estimated from direct observation of the food transiting the animals’ digestive tracts using algae labelled with a fluorescent dye ([Cauchie et al., 2000]; I. Thys, unpublished results). It varied between 8.6 h\(^{-1}\) in food-limiting conditions (0.4 mg algal C l\(^{-1}\)) and 15.1 h\(^{-1}\) in food-saturated conditions (3 mg algal C l\(^{-1}\)). In most samplings for gut content analysis, the algal biomass was low, varying between 0.06 and 0.9 mg C l\(^{-1}\) (mean algal biomass = 0.4 mg C l\(^{-1}\)). Therefore, it was estimated that \( D. galeata \) were food-limited most of the time and a unique specific excretion rate of 8.6 h\(^{-1}\) was used to calculate ingestion rates.

**Food-removal experiments**

Algal suspensions were obtained from sampling the epilimnion of the Esch-sur-Sûre reservoir just before the beginning of each experiment: a pooled water sample was collected using a Ruttner bottle. Metazooplankton and large phytoplankton were excluded by filtration through a 63 μm sieve. The suspension was split into six 1 l beakers containing PVC cylinders with a 150 μm Nitex screen fitted to the bottom. The cladocerans used in all experiments were isolated from the Esch-sur-Sûre reservoir in May 2000 and maintained in the laboratory with the chlorophyte *Scenedesmus acutus* as food. In each experiment, 50 \( D. galeata \) adult females (>1 mm) were added to each of three beakers out of six, all filled with the lake water. The three suspensions without grazers served as controls. The animals were allowed to acclimate for 10–12 h in the dark in an incubator set at the lake temperature. Then, the PVC cylinders containing the animals were transferred into fresh experimental suspensions for 20–27 h. All suspensions were placed
upon a horizontal shaker to avoid food sedimentation. Afterwards, the cylinders were immersed in soda water for 2 min and the anaesthetized animals were collected under the dissecting microscope and counted. A sub-sample of the initial suspension was taken at the beginning of the experiment \((T_0)\). At the end of the experiment, the feeding suspension was fractionated on a 63 µm screen to separate faecal material from non-ingested algae. The suspensions were filtered under mild vacuum (400 mmHg) on 47 mm GF/C filters. The material retained by the filters was analysed for Chl \(a\) and derivatives, as well as for major algal carotenoids, using the same procedure as for the reservoir water samples (see above). Experiments were run monthly from May to October 2000.

The mortality of the cladocerans was negligible throughout the investigation but births were observed in some treatments. Differenced in pigment concentration between the experimental and the control series after 24 h were attributed to consumption of algae by daphnids. Chlorophyll \(a\) attributed to each algal class was estimated from chlorophylls and carotenoids using CHEMTAX and following the same procedure as for the reservoir phytoplankton samples. The one-tailed Student’s \(t\)-test was used to test for significant differences in algal class concentrations between control and experimental treatments. Class-specific ingestion rates were calculated only when differences were significant.

The calculation of class-specific filtration \((F)\) and ingestion \((I)\) rates followed the equations of Rigler and of Tackx and van de Vrie (Rigler, 1971; Tackx and van de Vrie, 1993):

\[
F = \ln\left(\frac{C_e}{C_c}\right) \times \frac{V}{N \times t}
\]

where \(F\) = clearance rate for each algal class \((\text{ml ind}^{-1} \text{h}^{-1})\), \(C_e\) = final Chl \(a\) equivalent concentration of each algal class \((\mu \text{g} \text{l}^{-1})\) in the control without grazers, \(C_c\) = final Chl \(a\) equivalent concentration of each algal class \((\mu \text{g} \text{l}^{-1})\) in the treatment, \(V\) = volume of suspension in the containers \((\text{ml})\), \(N\) = number of grazers in the treatment and \(t\) = time \((\text{h})\).

\[
I = F \times \left(\frac{C_{0\beta} + C_{0\delta}}{2}\right)
\]

where \(I\) = specific ingestion rate \((\text{ng ind}^{-1} \text{h}^{-1})\), \(C_{0\beta}\) = initial Chl \(a\) equivalent concentration of each algal class \((\mu \text{g} \text{l}^{-1})\) of the experimental suspension, \(C_{0\delta}\) = final Chl \(a\) equivalent concentration of each algal class \((\mu \text{g} \text{l}^{-1})\) in the experimental suspension.

 Clearance and ingestion rates for the whole algal community were determined from total Chl \(a\).

Comparison of methods

Specific ingestion rates obtained with gut-pigment and food-removal methods were compared date by date using Student’s \(t\)-test. A \(\chi^2\) comparison of the distribution of class-specific ingestion rates (% of total algal ingestion) obtained with each method was used to evaluate the reliability of these methods to describe the diet of \(D. galeata\).

Estimation of selectivity

The Chesson selectivity index (Chesson, 1978) was used to determine whether \(Daphnia\) spp. selects amongst the main algal classes.

Results

Composition and biomass of the phytoplankton potentially edible by cladocerans

As the dynamics of phytoplankton were similar in the Esch-sur-Sûre reservoir from 1 year to another, only the variation of phytoplankton classes in 2000 is shown (Figure 1). The phytoplankton edible by cladocerans, estimated from pigment biomass in the \(<28\ \mu\text{m}\) fraction, varied with time. Throughout April and May, small diatoms (\(Cylindrotheca\) sp.) and cryptophytes (\(Rhodomonas\) sp. and \(Crypto- monas\) sp.) dominated the plankton, sometimes with chrysophytes (mainly \(Chrysidalis\) sp.). In June, there was a marked clear-water phase during which cryptophytes were almost the only ‘edible’ algae left. In July, large algae, mostly diatoms (\(Fragilaria\) sp.) and green algae (mainly colonies of \(Eutetramorus\) spp.), dominated the phytoplankton (Figure 2). Small cryptophytes, chlorophytes and cyanobacteria were present, however, and they developed further through August and September to become the phytoplankton fraction. Cyanobacteria were single cells or colonies of \(Chroococcales\) or filamentous forms (mainly \(Pseudanabaena, Oscillatoria, Anabaena, Aphanizomenon\) and \(Leptolyngbya\)). As the \(<28\ \mu\text{m}\) phytoplankton fraction was dominant in the late-summer phytoplankton, it is likely that some of these blue-green filaments were small enough to pass through the 28 µm sieve used for phytoplankton fractionation. The algal community varied in a similar way in the metalimnion, although diatoms were more abundant there than in the epilimnion.

Comparison of methods

Estimation of total grazing rates

A comparison of filtration rates on total algae obtained from the food-removal experiments (equation 4) and from the gut pigments (equation 3) is presented in Figure 3. Both methods provided filtration rates which were in the range of published values for \(D. galeata\) (Table I). For four dates out of six, however, filtration rates calculated from the two
methods differed significantly \( (P < 0.05) \). The greatest difference between the two methods occurred in late summer, when cyanobacteria were abundant.

Estimation of the diet and of food selection
Figure 4 details the class-specific ingestion rates obtained by both techniques. The ingestion rates obtained with the two techniques often differed in magnitude, with differences being statistically significant. On five occasions, the methods agreed and gave similar ingestion rates: for diatoms, cryptophytes and cyanobacteria on May 27, for cryptophytes on June 20 and for chlorophytes on July 10. From a qualitative point of view, algal pigments found in \( D. \text{galeata} \) guts corresponded, in most cases, to the algae ingested by the cladocerans in the food-removal experiments (Figure 4). One notable discrepancy was found for the July experiment, where zeaxanthin was found in the zooplankton extracts, indicating the presence of cyanobacteria in the animals’ guts, but cyanobacteria were apparently not consumed during the food-removal experiment. Furthermore, the composition of the algal diet of \( D. \text{galeata} \) assessed by the two methods differed significantly \( (\chi^2, P < 0.0001) \) for the June, July and October experiments. In other words, in three experiments out of six, the methods determined different diets. The food spectrum (assortment of algal classes ingested) determined by the food-removal method was generally more varied than that found with the gut-pigment technique. Only the major ingested pigments were recovered in \( D. \text{galeata} \) extracts, which indicates possible detection problems as a result of low pigment concentrations and possible pigment degradation.

Interestingly, both techniques indicated a dominant contribution of cyanobacteria and cryptophytes to the cladoceran diet. Cryptophytes, which are small single-celled flagellates that can be readily ingested by \( D. \text{galeata} \), were moderately abundant throughout the year in the reservoir. In contrast, cyanobacteria were partly composed of filamentous taxa that became dominant in mid-summer (see Figures 1 and 2). Diatoms were ingested only in spring and in small quantities. \( Daphnia \text{galeata} \) consumed relatively few chlorophytes, except in July when they constituted the main algal group in the cladoceran diet. This period, Chl \( a \) from chlorophytes accounted for 31% of the gut total pigment, while the food-removal method indicated that they represented 73% of total Chl \( a \) ingested. This discrepancy results from the fact that the food-removal experiment did not reveal ingestion of cyanobacteria at this date. Dinoflagellates and euglenophytes, present in low numbers, were not consumed by \( D. \text{galeata} \) in significant amounts.

Similar seasonal changes in the algal diet of the cladocerans were observed (Figure 5) with the exception of cyanobacteria, which were not consumed in the July food-removal experiments.

Similar shifts in selectivity pattern can be seen throughout the 3 years of survey, as shown by the selectivity index (Figure 6). In spring, \( D. \text{galeata} \) rejected diatoms despite this group forming 30–40% of total algal biomass (as judged by HPLC analysis).
from pigment analysis). When cryptophytes dominated the phytoplankton (during the clear-water phase), they were eaten rather unselectively by *Daphnia* spp. As soon as they became minor representatives of the phytoplankton (summer 1998 and 1999 or end of summer 2000), *Daphnia* spp. preferentially ingested cryptophytes. Although chlorophytes were usually abundant in summer (30–60% of algal biomass), lutein was found in *Daphnia* spp. extracts in small amounts, with the exception of July 2000. By contrast, zeaxanthin, essentially from cyanobacteria, was found in most samples. In spring and the beginning of summer, *Daphnia* spp. showed a preference for this group, which was constituted of relatively small taxa (*Oscillatoria* and *Chroococcus*). Conversely, at the end of summer, when cyanobacteria blooms comprising large taxa developed (i.e. *Anabaena* spp., *Aphanizomenon* spp.), they were randomly ingested or even avoided, especially in 1998 and 1999.

**DISCUSSION**

Assessment of grazing rates by gut-pigment method and food-removal method

The main purpose of this study was to test the validity of the gut-pigment method for tracking the temporal changes in the diet of *D. galeata* in *in situ* conditions. To check whether gut pigments are reliable indicators of diet composition, we compared the diet determined from pigments with that obtained from food-removal experiments. Although the design of the food-removal experiments may involve some perturbations for the cladocerans, the excellent concordance between the filtration rates obtained with this method and both published values and those measured *in situ* with the grazing chamber (Table I versus Figure 4) proves the validity of the food-removal method to assess *Daphnia* spp. grazing. Therefore we used the grazing rates determined from food-removal experiments to check the reliability of the gut-pigment method to measure total and class-specific grazing in *D. galeata*.

The diet estimated in the food-removal experiments was generally more varied than that given by the gut-pigment method. Nevertheless, both methods agreed on the qualitative composition of the diet of *D. galeata*, which ingested mainly cryptophytes and cyanobacteria during the study period. Both methods showed similar temporal changes in selectivity towards cryptophytes and cyanobacteria.

In many cases, the estimates of class-specific filtration rates from xanthophylls in *D. galeata* guts and from food-removal experiments differed in magnitude. However,
filtration rates on total algae were in the same range, although differences were significant. In some cases in 1998 and 1999 the gut-pigment method gave much higher total filtration rates than commonly found for this species (data not shown). These overestimates were observed in animals collected during the clear-water phase, which contained much higher pigment levels than normal. Alloxanthin (from cryptophytes) was systematically responsible for these high pigment contents. An excretion experiment conducted in 2002 in the same conditions (clear-water phase) showed that after being incubated for 2 h in filtered water, cladoceran guts still contained high amounts of pigments. This suggests that at very low food levels \textit{D. galeata} may retain the algal food in its guts by decreasing its excretion rate (largely <8.6 h$^{-1}$). Some studies have shown that gut passage time in marine and freshwater zooplankton varies largely with food concentration (Murtaugh, 1985; Pasternak, 1994) and temperature (Cauchie et al., 2000). In \textit{Neocalanus}, gut passage time was constant at a food concentration >4.0 µg Chl a l$^{-1}$ but slowed dramatically at lower concentrations (Dagg and Walser, 1987). Moreover, by applying a constant $k$ value, we hypothesized that all animals fed continuously. This may also be a source of error in the estimation of grazing rates from gut pigments. By direct observation of the food in the gut during short-term grazing experiments with fluorescently labelled algae, Cauchie et al. (Cauchie et al., 2000) showed that, in a population of \textit{D. galeata}, 20–30% of individuals did not feed. This repeated observation indicates that feeding is discontinuous in this cladoceran and inter-individual variations in the feeding rhythm probably also occurred within the \textit{D. galeata} population in the Esch-sur-Sûre reservoir.

These variations in feeding rhythm obviously cannot be taken into account to correct the ingestion rates determined from gut pigments. Beside these problems occurring at very low food levels, grazing rates from gut pigments were in the range of published values for the cladoceran species studied. The largest discrepancy between methods was observed during summer when cyanobacteria were dominant. Some of these algae, ingested by \textit{D. galeata} at this period, could pass through the gut without destruction and could be excreted undamaged into the environment. If so, food-removal experiments underestimated filtration rates of cyanobacteria, as illustrated in the July and September experiments.

\textbf{Daphnia galeata diet}

In a review of \textit{Daphnia} spp. feeding and nutrition, Lampert (Lampert, 1987) concluded that food selection is primarily determined by particle diameter and shape. Shape is especially important for thin, elongated particles such as filaments. However, a study by DeMott (DeMott, 1995) provided evidence of the importance, in freshwater cladocerans, of prey hardness, in addition to size constraints, in food selection. Few studies have attempted to follow the feeding behaviour of \textit{Daphnia} spp. throughout the seasonal changes in phytoplankton

\begin{table}
\centering
\caption{Literature review of \textit{D. galeata} filtration rates (ml ind.$^{-1}$ h$^{-1}$) measured in situ or quasi-in situ}
\begin{tabular}{lll}
\hline
Filtration rates & Method & Authors \\
\hline
0.04$^a$ & $0.04-0.05$ & $^{32}$P-labelled in laboratory 15$^\circ$C & Burns, 1969 \\
0.10$^a$ & $0.08-0.12$ & $^{32}$P-labelled in laboratory 20$^\circ$C & Burns, 1969 \\
0.15 & $0.05-0.26$ & $^{32}$P-labelled in laboratory & Burns and Rigler, 1967 \\
 & $0.07-0.39$ & $^{14}$C-labelled in laboratory & Stuchlik, 1991 \\
 & $0.15-0.98$ & $^{32}$P-labelled \textit{in situ} & Haney, 1985 \\
0.14 & $0.11-0.16$ & $^{14}$C-labelled \textit{in situ} & Bogdan and McNaught, 1975 \\
0.35 & $0.16-0.42$ & $^{14}$C-labelled \textit{in situ} & Bogdan and McNaught, 1975 \\
0.33 & $0.13-0.46$ & $^{14}$C-labelled \textit{in situ} & Bogdan and McNaught, 1975 \\
0.27 & $0.06-0.87$ & $^{32}$P-labelled \textit{in situ} & Haney, 1973 \\
 & $0.20-0.79$ & \textit{in situ} & Zankai, 1983 \\
 & $0.06-0.46$ & $^{14}$C-labelled \textit{in situ} & Zankai and Ponyi, 1986 \\
0.27 & $0.16-0.34$ & Food-removal \textit{in situ} & Kasprzak and Lathrop, 1997 \\
0.53 & $0.27-1.14$ & $^{14}$C-labelled \textit{in situ} & I. Thys, unpublished results \\
0.43 & $0.04-1.16$ & Food-removal \textit{in situ} & Present study \\
0.60 & $0.18-1.15$ & Gut-pigment \textit{in situ} & Present study \\
\hline
\end{tabular}
\footnotesize{$^a$Weight filtration rates converted considering a mean weight of 4.06 µg.}
\end{table}
composition. By means of the gut-pigment method, we attempted to provide information on the diet of *D. galeata* over several years in a reservoir undergoing large changes in phytoplankton size and composition. Taxonomic resolution was limited by the class-specific estimate of algal biomass through pigment analysis but complemented by a taxonomic determination of phytoplankton by microscopy (R. Willame, personal communication).

According to our gut-pigment data, supported by clearance experiments for the year 2000, cryptophytes and cyanobacteria were the two groups most consumed by *D. galeata*. They were also among the dominant algae in the lake from April to October. This can be partly
Fig. 5. Comparison of *D. galeata* selective ingestion measured by gut-pigment and food-removal methods, in the Esch-sur-Sûre reservoir in 2000. Relative ingestion of the main algal groups is expressed by the proportion of algal classes in the diet. Whiskers above and below the point indicate the standard deviation.
Fig. 6. *Daphnia galeata* selectivity for the main algal groups measured by the gut-pigment method, in the Esch-sur-Sûre reservoir in 1998, 1999 and 2000. Selectivity is assessed by the Chesson selectivity index. The index ranges from 0 (negative selection represented by the grey area) to 1 (positive selection characterized by the hatched area) and non-selectivity is 0.5 (broken line). Whiskers above and below the point indicate the standard deviation.
explained by the variations in the edible fraction (<28 μm) of the algal assemblage: in spring, this fraction was composed of cryptophytes with some small diatoms, while in summer, cryptophytes, green algae and cyanobacteria made up most of the edible fraction. With the notable exception of cyanobacteria, which were ingested even when in low numbers, \textit{D. galeata} grazed the phytoplankton groups according to their availability in the lake, provided that they were in their food-size spectrum [0.32–40 μm according to Geller and Müller (Geller and Müller, 1981)]. However, the calculation of Chesson’s selectivity index provides some evidence for a selectivity pattern (Figure 6) that is validated by the data from the food-removal experiments conducted in 2000. Amongst algae, flagellates have a weak and easily broken cell wall. This may explain why cryptophytes were preferred; accordingly, Knisely and Geller (Knisely and Geller, 1986) found that the flagellates \textit{Cryptomonas} and \textit{Rhodomonas} were more efficiently grazed than the coccoid algae all year round by \textit{Daphnia} spp. Other reports [e.g. (Reynolds et al., 1982)] suggested that grazing losses are an important factor in the dynamics of cryptomonads.

Filamentous and colonial cyanobacteria are generally rejected by cladocerans and their presence decreases the filtration rates for other food items (Gliwicz, 1977; Webster and Peters, 1978; Gliwicz and Siedlar, 1980; Richman and Dodson, 1983; Porter and McDonough, 1984; Hein et al., 1993). Our results showed that cyanobacteria were effectively ingested by \textit{D. galeata} and constituted an important part of its algal diet (up to 80% of the Chl \textit{a} ingested). Filtration rates for other algal groups were not significantly decreased when cyanobacteria were dominant, even if the lowest filtration rates had been measured in July. Although ingestion of colonial cyanobacteria by \textit{Daphnia} spp. has already been reported (Knisely and Geller, 1986; Fulton and Pael, 1987), evidence for positive selection for this group is scarce. One may suspect that the zeaxanthin (characteristic of cyanobacteria) that we found in \textit{D. galeata} extracts actually came from cyanobacteria adhering to cladocerans or from non-ingested cyanobacteria trapped in the animal’s filtration apparatus. However, disappearance of cyanobacteria was also shown in food-removal experiments, which suggests that these algae were actually ingested.

Another potential source of error was that zeaxanthin is common to both cyanobacteria and chlorophytes, but this was taken into account by subtracting from total zeaxanthin the amount corresponding to chlorophyte biomass (see Method). In agreement with our results, Quiblier-Lloberas et al. (Quiblier-Lloberas et al., 1996b) observed large amounts of zeaxanthin in extracts from cladocerans (\textit{D. longispina} and \textit{Ceriodyina quadriangularia}). \textit{Daphnia galeata} might also ingest cyanobacterial pigments indirectly through the consumption of ciliates (Ederington et al., 1995). \textit{Daphnia galeata} effectively preyed on ciliates during ‘food-removal’ experiments (Thys et al., submitted for publication) and these protozoans actually induced cyanobacterial mortality in the dilution experiments performed by Jacquet (Jacquet, 2003), but only in August, while we observed grazing on cyanobacteria by \textit{D. galeata} from May to September. Thus, although an indirect trophic transfer might have occurred, it could only partly account for the zeaxanthin found in \textit{D. galeata}.

In the Esch-sur-Sûre reservoir cyanobacteria were a significant component of the phytoplankton from spring to autumn from 1998 to 2000, and a large part of their biomass was in the <28 μm size range, according to size fractionation by filtration (in 1998, 1999 and 2000, respectively, 32, 92 and 51% of cyanobacterial biomass was in the edible fraction). This relatively large proportion of small cells or colonies was confirmed by microscopy. This substantial contribution of small cyanobacterial to the phytoplankton of the Esch-sur-Sûre reservoir may explain why they were consumed by \textit{D. galeata}. Moreover, genetic analysis currently being undertaken shows the presence of picocyanobacteria (R. Willamme, personal communication) that might be grazed by \textit{D. galeata} (although their contribution is minimal). Indeed, several authors have stressed the importance of colony size for cyanobacteria consumption by \textit{Daphnia} spp. (Lynch and Shapiro, 1981; Holm et al., 1983; Gulati et al., 2001). Small \textit{Microcystis} colonies have been reported as being readily ingested by \textit{D. hyalina} (Thompson et al., 1982) and they apparently did not interfere with filtering. \textit{Daphnia ambigua} fed unselectively in a mixture of unicellular \textit{Microcystis} and \textit{Chlamydomonas} but showed negative selection for colonial \textit{Microcystis} spp. (Fulton and Pael, 1983). Our study, based on gut-pigment analysis of natural populations of \textit{D. galeata} sampled directly from a lake, thus indicates that cyanobacteria may represent an important food source for freshwater herbivorous cladocerans.

In summary, the analysis of algal pigments in \textit{D. galeata} extracts provided direct and consistent information of \textit{D. galeata} diet and its variation over time. Our observations confirm that \textit{D. galeata} ingested the most abundant algae in the small size range but also showed a shift in selectivity over the growing season, with frequent preferential ingestion of cryptophytes and small cyanobacteria.

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