Journal of Plankton Research Vol.16 no.10 pp.1441–1447, 1994

SHORT COMMUNICATION

Influence of phytoplankton composition and stratification degree on gut pigment content of *Ceriodaphnia* sp. at dawn

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Abstract. Very short-term feeding activity of the cladoceran *Ceriodaphnia* sp. was investigated *in situ* in a eutrophic reservoir in the south of Spain, using fluorimetric analysis of the gut pigment content in periods when the water column was relatively mixed or strongly stratified. The results obtained in the mixed water column showed a clear increase in gut pigment content at dawn, a period sampled with high frequency. The accumulation of the cladoceran at the depth of maximum concentration of phytoplankton, and the high gut pigment concentration in cladocerans at that depth just after dawn, suggested active feeding of *Ceriodaphnia* on phytoplankton at that time. During stratification, the abundance of *Ceriodaphnia* was higher, but the gut pigment contents were very low and they did not reflect any clear feeding patterns, with either time or depth. Changes in phytoplankton concentration and composition between the relatively mixed and the stratified water column suggest a shift in feeding activity from herbivorous to detritivorous.

The starting point for this work was our previous results analysing seasonal variability in the diurnal rhythm of *Ceriodaphnia* sp. feeding activity (Rodríguez *et al.*, 1991). There was a clear difference in gut pigment content between destratification and stratification periods, with high and low values of gut pigment content, respectively. A clear diurnal rhythm, with higher values of gut pigment contents at dawn, was also found in the destratified period (Rodríguez *et al.*, 1991). The maximum detected at dawn was also obtained in laboratory experiments (F.Echevarría, unpublished data). In this paper, we present the results of a study on the very short temporal variation of gut pigment content during the pre- to post-dawn period, sampling the upper epilimnetic layer 20 times within ~2 h, a period which covered the transition between night and day. This experiment was repeated twice, in periods characterized as 'highly stratified' and 'relatively less stratified', to test our previous hypothesis of a higher gut pigment content during the relatively less stratified period.

The experiments were carried out in La Concepción reservoir (Málaga, southern Spain), a monomictic water mass with a surface area of 2.41 km^2 and maximum depth of 60 m. Two campaigns were performed on 25 March 1986 (end of the well-mixed period) and 16 June 1986 (highly stratified period). Temperature and dissolved oxygen were measured with a YSI model 57 at 1 m depth intervals in the upper 15 m of the water column. Water samples were taken

every metre in the first 10 m (March) and 15 m (June), following an up-and-down sequence that allowed every depth to be sampled twice with a delay of ~1 h. Sampling began 1 h before dawn, so the first set of samples were taken in a relatively dark environment, while the second set of samples was taken at dawn. The sampling sequence is indicated in Figures 1 (March) and 2 (June).

Water from the selected depths was taken using a Van Dorn bottle (12 l) and subdivided in the following way. (i) 250 ml of water were placed in a dark glass bottle and fixed with Lugol's solution for a further analysis of phytoplankton by Utermohl's method. (ii) Eleven litres of water were filtered through a Nytex mesh of 45 μm pore size to study the retained zooplanktonic organisms, which were washed and fixed with formalin to a final concentration of 5%. (iii) Sampling of zooplankton for gut content analysis required a second bottle cast to

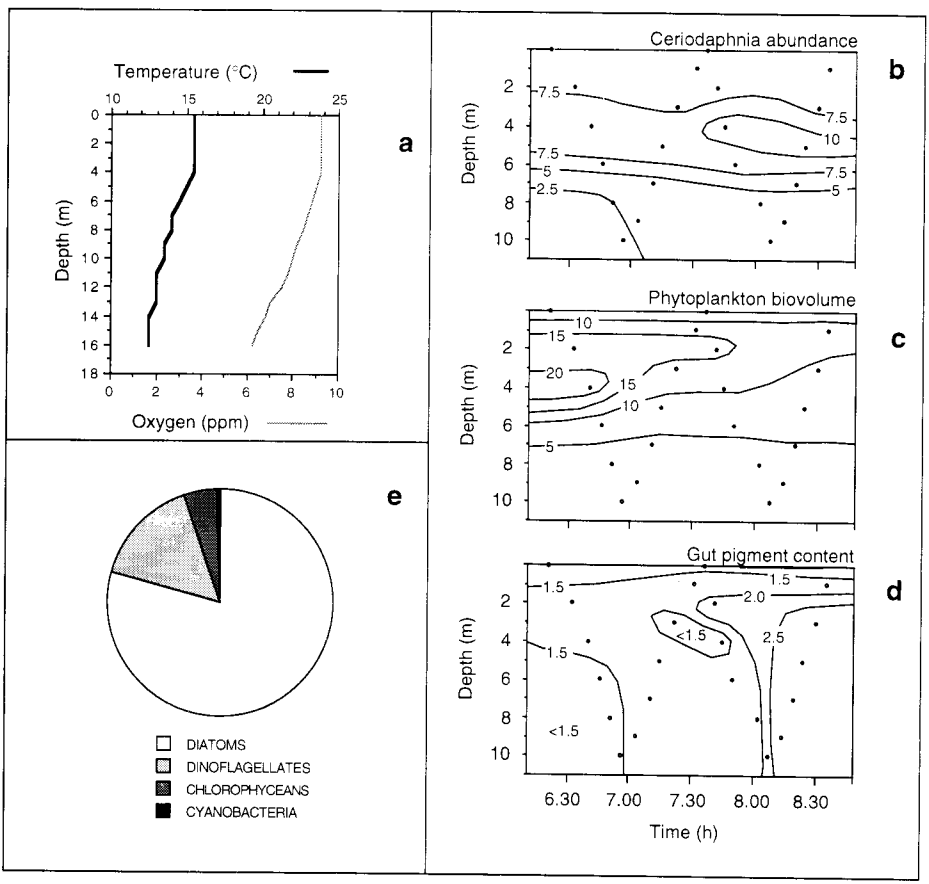

Fig. 1. Results from the sampling period in March. (**a**) Physical structure of the water column defined by temperature (°C) and oxygen concentration (mg l^{-1}) in the upper 16 m of the water column. (**b**) *Ceriodaphnia* abundance (ind. l^{-1}). (**c**) Total phytoplankton biovolume (μm^3 ml^{-1}) ($\times 10^6$). (**d**) *Ceriodaphnia* gut pigment content (ng chlorophyll + phaeopigments ind.$^{-1}$). (**e**) Average contribution of the main phytoplanktonic groups in terms of abundance.

the same depths. The zooplankton were gently filtered through a 100 μm Nytex mesh, which was immediately frozen on dry ice.

Samples for fluorimetric measurements of *Ceriodaphnia* sp. gut pigments were processed in the laboratory within the following 2 h. A total of 40–50 individuals were thoroughly washed to eliminate attached phytoplankton cells. Washed organisms were placed in acetone and left for 24 h in dark and cold conditions (4°C). After this time, specimens were homogenized and filtered through a glass-fibre filter (Whatmann GF/F). The fluorescence of the extract was measured before and after acidification (10% HCl) using a Perkin–Elmer MPF-43A spectrofluorometer with excitation and emission spectra of 428 and 667 nm, respectively. The equations given in Boyd *et al.* (1980) were used to calculate the concentrations of gut chlorophyll and phaeopigments. Total gut pigment content was expressed as chlorophyll + phaeopigment, the ratio chl/phaeo being on average ~0.1.

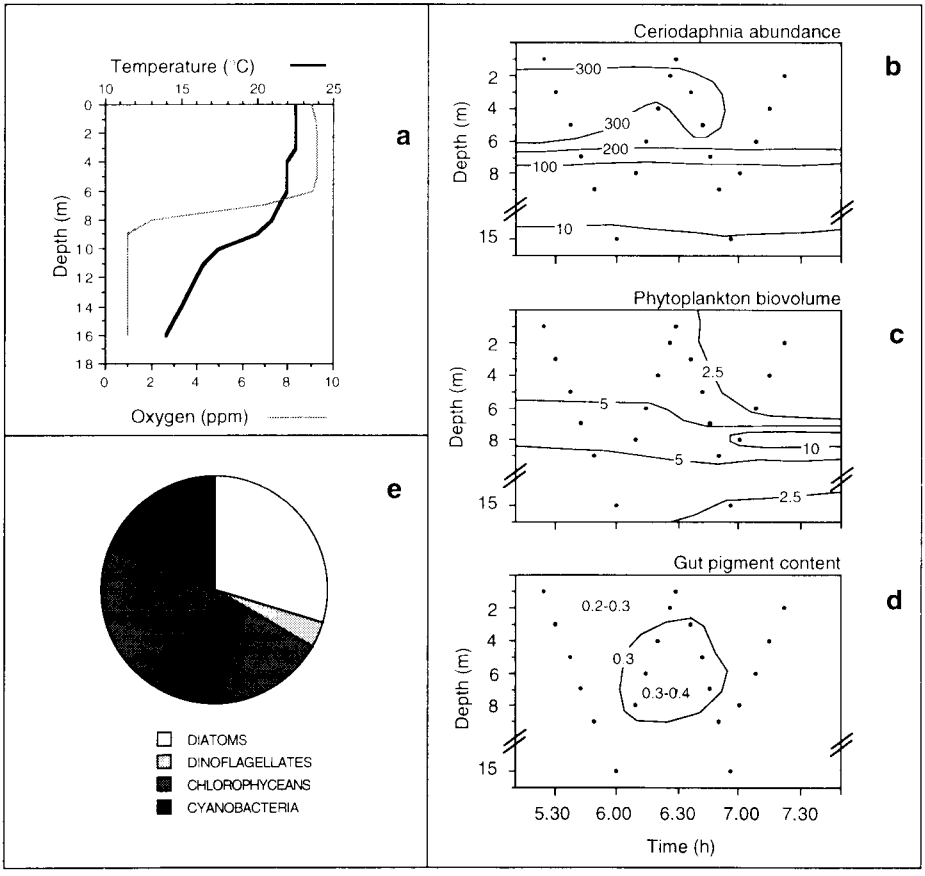

Fig. 2. Results from the sampling period in June. The sequence of graphs and units are the same as in Figure 1.

Phytoplankton cells were classified and counted by Utermohl's method using an inverted microscope. Phytoplanktonic biovolume values were calculated by multiplying each phytoplankton species abundance by its average biovolume using the data calculated by Gálvez *et al.* (1988) for the same system and period. Zooplankton were visualized and counted in a stereoscopic microscope.

Gut pigment content does not estimate the complete feeding activity of the animals, because only pigmented material in the gut is measured. If there are other non-pigmented food sources, and/or if the consumer selectively eats only some types of pigmented food, feeding estimates will be inaccurate. Both problems do occur with freshwater cladocerans (Haney, 1973; Lehman, 1976; Huntley, 1982). Hence, we used the fluorimetric method only as an estimate of zooplankton–phytoplankton interaction, and not as the basis to calculate filtration or ingestion rate (e.g. Boyd *et al.*, 1980; Dagg and Grill, 1980; Dagg and Walser, 1987; Bautista *et al.*, 1988). The advantages of the fluorimetric method are well reviewed (Kiørboe *et al.*, 1985; Head, 1986; Bautista *et al.*, 1988; Peterson *et al.*, 1990) and here we will add two more. First, it allows for *in situ* measurements, so that a variety of processes which affect the feeding behaviour of organisms can be considered (e.g. variation with depth, time of day, etc.). Secondly, it allows for measurements with a high-frequency sampling.

Figure 1 shows the results obtained in March. The surface temperature was ~15.5°C, with a smooth decrease to 12°C at 16 m. Oxygen concentration was fairly constant in the upper 6 m (9 mg l^{-1}), decreasing to 6 mg l^{-1} at 16 m. The vertical structure of the water column corresponded to the end of the mixed period (Figure 1a). *Ceriodaphnia* accounted on average for 30% (in abundance) of all zooplankton, with densities between 1 and 10 individuals (ind.) l^{-1}. Figure 1b shows the temporal and vertical variations of *Ceriodaphnia* density during the 2 h sampling period. There was a clear pattern of vertical distribution, with maximum values between 3 and 5 m depth. A progressive increase in abundance after 7:30 h was also seen. This increase occurred throughout the 0–10 m depth range, and was probably the result of vertical migration from greater depths. The spatial–temporal changes in phytoplankton biovolume are shown in Figure 1c. This variable is used as an index of pigmented food available for *Ceriodaphnia*. The pattern showed the opposite of that described for cladoceran abundance, with maximum values at the beginning of the period. The progressive decrease of phytoplankton might be related to the consumption by *Ceriodaphnia*, because the gut pigment content showed a progressive increase during the study period (Figure 1d): there were low values of gut pigment in the first part (<1.5 ng ind.$^{-1}$) and higher values from 08:10 h (>2.5 ng ind.$^{-1}$). This pattern was observed in the whole of the sampled water column, except for the upper 2 m, where no increase occurred.

In June (Figure 2), the water column was highly stratified. There was an epilimnetic layer of 7 m depth, with a temperature of ~22°C. At the thermocline, oxygen concentration decreased markedly from 9.5 mg l^{-1} in the epilimnion to ~1 mg l^{-1} at 8.5 m (Figure 2a). *Ceriodaphnia* abundance (Figure 2b) was much higher than in March, with densities of ~200–300 ind. l^{-1} in the epilimnetic layer, and this species accounted for >90% of all zooplankton.

Below the thermocline, the abundance decreased to <10 ind. l^{-1} at 15 m. On the other hand, a temporal variation with higher epilimnetic abundances was also observed during the early part of the sampling period. Absolute values of phytoplankton biovolume were lower than those found in March. The temporal variation of the phytoplankton biovolume (Figure 2c) showed an opposite trend to the distribution pattern of *Ceriodaphnia*. At this time, however, it seemed that the temporal changes in phytoplankton biovolume were not affected by *Ceriodaphnia* grazing. Two reasons led to this suggestion. (i) Total biovolume in the whole of the studied water column was similar throughout the sampling period, with an aggregation of cells at ~8 m after dawn (Figure 2). This might have happened because the phytoplankton was dominated by the dinoflagellate *Peridinium cinctum* (at least in biovolume terms), whose flagellated cells are able to swim and aggregate at the desired depth in response to light stimuli (e.g. Niell *et al.*, 1987; Gálvez *et al.*, 1988). (ii) Gut pigment contents in *Ceriodaphnia* were very low (<0.4 ng ind.$^{-1}$) and showed no variation during the sampling period (Figure 2d). The low gut pigment content would suggest a low grazing activity on phytoplankton during this period.

Phytoplankton decreased in total biovolume from March to June. In addition, an equally important change between the two periods was in the qualitative composition. In March, there was a clear dominance of diatoms (80% of total phytoplankton abundance, Figure 1e), while in June this group only accounted for 30% (Figure 2e). Moreover, the species diversity was much higher in March, with *Cyclotella meneghiniana*, *Melosira granulata*, *Asterionella formosa*, *Gyrosigma* sp., *Nitzschia acicularis* and *Fragillaria crotonensis* as the most important species. This latter species was the only quantitatively important diatom detected in June. The large size of *F.crotonesis* colonies makes its consumption by *Ceriodaphnia* difficult, taking into account that cells >25 μm are not easily consumed by small cladocerans such as *Ceriodaphnia* (Wetzel, 1975). The most important group in June were the chlorophyceans (47% in cell number; Figure 2e), mainly due to the high abundance of *Oocystis lacustris*. Cyanobacteria also showed an important increase from March to June, with the coccoid cell *Synechoccocus* sp. as the most important component. Chlorophyceans and cyanobacteria are hardly ingested by cladocera. Chlorophyceans are scarcely affected by zooplankton grazing because of their thick cellulose cell walls. Even the passage of these cells through the zooplankton gut does not destroy them. Wetzel (1975) proposed an increased renovation rate for the cells which have passed through zooplankton guts, since they might use the nutrients released into the gut lumen by digestion of other cells. Cyanobacteria may also be unsuitable as food because of toxin production, nutritional inadequacy and small size (Porter, 1973; Porter and Orcutt, 1980). The most abundant dinoflagellate in the water column was *P.cinctum*, which actually showed higher abundances in March than in June. In contrast to the cyanobacteria, the large size of this dinoflagellate (~45 μm of equivalent spherical diameter) makes its consumption by *Ceriodaphnia* difficult (Wetzel, 1975).

In conclusion, in June there was less pigmented food because there was less phytoplanktonic biomass, but there was also a taxonomic change, such that most

of the cells whose abundance increased were not easily grazed by *Ceriodaphnia*. The fact that the fluorimetric method only accounts for pigmented food, together with the high abundance and low gut pigment content of *Ceriodaphnia* in June, suggests that they may have changed from a herbivorous diet during March to a more omnivorous diet in June (Lehman, 1976), when the species must obtain energy from other sources, basically detritus, microzooplankton or bacteria (Urabe and Watanabe, 1990; Rodríguez *et al.*, 1991). Several authors have reported maximum populations of small cladocerans such as *Bosmina*, *Chydorus* and *Ceriodaphnia* during summer when they apparently graze on bacteria (Haney, 1973; Conde-Porcuna, 1993).

The seasonal differences in gut pigment contents between relatively mixed and stratified periods support our previous results (Rodríguez *et al.*, 1991): gut pigment contents were much lower in the stratified water column than in the relatively mixed one, a result previously obtained by other authors (Haney, 1988; Conde-Porcuna, 1993). The absolute values of gut pigment contents agreed well. During the mixed period, nocturnal values detected in both cases were ~1 ng ind.$^{-1}$, increasing to ~3 ng ind.$^{-1}$ at dawn. In stratification, gut pigment content values were <0.4 ng ind.$^{-1}$. The short-term variation of gut pigment content during the dawn period also confirms the punctual increase at dawn during March (Figure 1D), as shown previously in a study of a daily cycle (Rodríguez *et al.*, 1991). This punctual increase at dawn was not coincident with the highest phytoplankton concentrations (Figure 1), suggesting a discontinuous feeding behaviour in *Ceriodaphnia*, in which sunlight might act as a stimulant to feeding activity.

Acknowledgements

This research was supported by CICYT projects (MAR90-0339, MAR91-0813 and AMB92-1387-E). We thank Dr J.Rodríguez and colleagues of Grupo de Investigación en Redes Tróficas Pelágicas (GIRTP). Two anonymous referees made helpful comments and corrections.

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Received on February 10, 1994; accepted on May 23, 1994