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INGESTION RATES OF *DAPHNIA MAGNA* STRAUS (CRUSTACEA : BRANCHIOPODA : ANOMOPODA) ON BACTERIOPLANKTON AND PHYTOPLANKTON IN AN AERATED WASTE STABILISATION POND

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Abstract. The impact of zooplankton on waste stabilisation pond (WSP) performance has been poorly studied until now. Zooplankton grazing activity is, however, worth considering as it can control the bacterioplankton and phytoplankton, which are the cornerstone organisms of the WSP treatment technology. The aim of the present study was to determine whether the grazing activity of the dominant zooplankter, *Daphnia magna*, can significantly control phytoplankton and bacterioplankton in a WSP (Differdange, Grand-duchy of Luxembourg). The biomass of phytoplankton (B_{phyto}), bacterioplankton (B_{bact}) and *Daphnia magna* ($B_{Daphnia}$) were estimated fortnightly from January to July 1998. At four key moments during this period, the ingestion rates of phytoplankton (IR_{phyto}) and bacterioplankton (IR_{bact}) by *D. magna* were determined and compared to B_{Phyto} and primary production (P_{prim}), and to Bbact and bacterial production (P_{bact}), respectively. From January to June, IR_{Phyto} varied between 66 and 92 % of B_{phyto}.day^{-1} and between 2 and 90 % of P_{prim}. In July, the grazing impact on phytoplankton increased and reached 171 % of B_{phyto}.day^{-1} and 310 % of P_{prim}, causing a significant drop in B_{phyto}. The grazing impact of *D. magna* on bacterioplankton varied between 0.1 and 18 % of B_{bact}.day^{-1} and between 0.8 and 226 % of P_{bact}. Even when higher than 100% of P_{bact}, the grazing impact did not lead to a significant decrease of B_{bact}, because of a significant input of allochtonous bacterial biomass with the influent.

Key words: *Daphnia magna*, waste stabilisation ponds, ingestion rate, fluorescently-labelled bacteria, fluorescently-labelled algae, gut passage time

INTRODUCTION

Waste stabilisation ponds (WSP) are shallow, man-made basins used for the treatment of organically polluted waste waters. Bacterioplankton and phytoplankton are traditionally pointed out as the cornerstone organisms of this type of water treatment (OSWALD *et al.*, 1957; MARAIS & SHAW, 1961). Bacteria degrade organic matter and provide algae with carbon dioxide, while algae provide bacteria with oxygen produced during photosynthesis. Zooplankton often develops in high densities in these hypertrophic water bodies, and planktonic crustaceans often dominate the zooplankton community in WSP that have relatively long water residence times (~ 20 days) (CANOVAS *et al.*, 1996).

There is growing interest in a commercial use for zooplankton living in WSP, for example as food for fish in aquaculture or as a source of chitin (SEVRIN-REYSSAC *et al.*, 1994; CAUCHIE *et al.*, 1995). From the viewpoint of commercial applications, the available quantities of zooplankton (*i.e.* its biomass) and its renewal rate (*i.e.* its production) have to be determined. Surprisingly, the production dynamics in WSP have rarely been studied (DABORN *et al.*, 1978; MITCHELL & WILLIAMS, 1982; JANA & PAL, 1983). As part of a research program dealing with the potential for using WSP planktonic crustaceans as a commercial source of chitin, the production dynamics of the dominant zooplankter *Daphnia magna* have been studied during three years in an aerated WSP located at Differdange (Grand-Duchy of Luxembourg) (CAUCHIE *et al.*, unpubl.data). *Daphnia magna* biomass ($B_{Daphnia}$) and daily production ($P_{Daphnia}$) were found to vary seasonally. It was demonstrated that temperature significantly affected the growth rate and the daily production of *D. magna* (CAUCHIE *et al.*, unpubl.data). However, it proved difficult to demonstrate the effect of food on the production dynamics of the zooplankter.

In the present study, we examined the magnitude of trophic transfers from bacterioplankton and phytoplankton to *D. magna* under four different combinations of bacterioplankton biomass (B_{bact}), bacterial production (P_{bact}), phytoplankton biomass (B_{phyto}), and primary production (P_{prim}), as observed in the WSP of Differdange from January to July 1998. These transfers are discussed in terms of their relative contributions to the daily production of *D. magna*, as well as in terms of the impact of *D. magna* feeding activity on the bacterioplankton and phytoplankton dynamics.

MATERIAL AND METHODS

Study site and sampling strategy

The study site was an aerated WSP located in the Grand-duchy of Luxembourg (49°32'N-5°55'E). It collects the domestic waste water of a small town (15,000 inhabitant-equivalents) after primary treatment (screening and coarsing). Two mechanical aerators continuously oxygenate the pond, which is roughly rectangular. Its length is about 400 metres and its width is 150 metres. Its mean and maximum depths are 2.3 and 4.0 metres, respectively. The sampling site was located along the long axis of the pond, at about 100 metres from the inlet of the pond. At this place, the water column reaches 340 cm.

From January to July 1998, the pond water was sampled twice a month around noon in order to monitor B_{bact}, B_{phyto}, $B_{Daphnia}$, water temperature (TEMP) and dissolved oxygen concentration (DO). The ingestion rates of bacterioplankton (IR_{bact}) and phytoplankton (IR_{phyto}) by *D. magna* were determined on four occasions (January 29, May 03, June 15 and July 09). On these dates, we also determined bacterial production (P_{bact}) and primary production (P_{prim}).

Biomass and production of bacterioplankton

Bacterioplankton was sampled every 40 cm, from the surface to the bottom of the pond, with a 2 l Ruttner bottle and stored in 100-ml autoclaved and acid-washed glass bottles. In the laboratory, bacteria were stained with acridine orange according to HOBBIE *et*

al. (1977) and filtered onto black membranes (pore size = 0.2 µm). They were enumerated and measured to the nearest 0.5 µm using a Leica DMRB epifluorescence microscope equipped with a blue excitation filter block (Leica I3, BP 450-490). Volumetric B_{bact} (fg dry weight (DW) l^{-1}) was determined on the basis of the size class specific density of bacteria, N_i (cells.l^{-1}) and the mean size class specific cell volume of the bacteria, V_i (µm³) (LOFERER-KRÖSSBACHER *et al.*, 1998):

$$B_{bact} = \sum_{i=1}^{n} 435.N_i.(V_i)^{0.86}$$

Vi was estimated as (LOFERER-KRÖßBACHER *et al.*, 1998):

$$V_i = \left(\left(w_i^2.\frac{\pi}{4}\right).(\ell_i - w_i)\right) + \left(w_i^3.\frac{\pi}{3}\right)$$

where w_i and ℓ_i are the mean width (µm) and mean length (µm) of the bacteria in the size class i. Areal B_{bact} was calculated by integrating volumetric B_{bact} over depth throughout the water column.

Hourly volumetric P_{bact}(µg DW.l^{-1}.h^{-1}) was determined *in situ* on the basis of the incorporation rate of (4,5-³H)-l-leucine according to KIRCHMAN *et al.* (1985). For each sample, triplicate aliquots of 5 ml were incubated with the addition of 83 nM of leucine (10 % (4,5-³H)-l-leucine - 90 % non radioactive leucine). The incorporation rate of leucine was corrected for isotopic dilution using the kinetic approach described by KIRCHMAN *et al.* (1986). Supplementary triplicate aliquots of 5 ml were taken from one sample and incubated in the presence of four concentrations of added leucine in the range of 1-75 nM (10 % (4,5-³H)-l-leucine - 90 % non radioactive leucine). The leucine incorporation rates were plotted against leucine concentration, and the maximum incorporation rate (V_{max}) was determined by fitting a hyperbolic function to the data. The ratio between V_{max} and the incorporation rate for 83 nM of added leucine was used to correct the isotopic dilution, which was supposed to be similar at all depths. Two blanks were made for each triplicate by adding ice-cold TCA (5 % final concentration) immediately after the beginning of the incubation. Incubations were conducted *in situ* during one hour. Incubations were terminated by the addition of ice-cold TCA (5 % final concentration). Samples were then extracted at 85°C during 30 minutes (SERVAIS, 1995) and filtered onto 0.22 µm pore size acetate cellulose membranes. One ml ethylacetate was added to dissolve the filters. After 48 hours of storage, radioactivity associated with the filter was measured using a Beckman LS 6500 scintillation counter and the Beckman Ready Organic scintillation cocktail. Quenching was corrected with the external standard in the Compton edge shift mode (H#). Hourly volumetric P_{bact} (µg DW.l^{-1}.h^{-1}) was calculated from the leucine incorporation rate, assuming a conversion factor of 1,080 gC.mole leucine incorporated^{-1} (SERVAIS & LAVANDIER, 1995) and a carbon-dry weight ratio of 0.54 (SIMON & AZAM, 1989). Areal daily P_{bact} was calculated by integrating volumetric hourly P_{bact} over depth and by multiplying the integrated value by the 24 hours of a day.

Biomass of phytoplankton and primary production

For the determination of B_{phyto}, water was sampled every 40 cm with a 2 l Ruttner bottle and stored in 250-ml autoclaved and acid-washed glass bottles. In the laboratory, water was filtered onto a black polycarbonate membrane (pore size = 0.2 µm) and the filters were observed using a Leica DMRB epifluorescence microscope equipped as noted above. Phytoplankton was distinguished from heterotrophic protists on the basis of its red autofluorescence, and was enumerated and measured to the nearest 1 µm. Considering a carbon to dry weight ratio of 0.524 (OSWALD, 1988), volumetric B_{phyto} (pg DW.l^{-1}) was determined on the basis of the size class specific density of phytoplankton, N_i (cells.l^{-1}) and the mean size class specific cell volume of phytoplankton, V_i (µm^3) as (ROCHA & DUNCAN, 1985):

$$B_{phyto} = \sum_{i=1}^{n} 0.2298.N_i.(V_i)^{1.051}$$

Vi was estimated assuming the cells to be ellipsoids:

$$V_i = \frac{\pi}{6} a_i.b_i.c_i$$

where a_i, b_i and c_i are the mean diameter (µm), the smallest diameter (µm) and the greatest diameter (µm) of the cell in the size class i. Areal B_{phyto} was calculated by integrating volumetric B_{phyto} over depth throughout the water column.

The dominant species in the phytoplankton were determined during the algal bloom observed in June and July. Phytoplankton samples were taken at a depth of 60 cm and preserved in lugol. Determinations were made using a Leitz Laborlux microscope.

Volumetric P_{prim} was determined by the «oxygen light-dark bottle» technique (VOLLENWEIDER, 1969). Phytoplankton samples for P_{prim} were taken every 20 cm with a 2 l Ruttner bottle. Two light and two dark, acid-washed Winkler bottles (volume = 250 ml) were incubated *in situ* for 4 hours around noon. Dissolved oxygen concentration was determined in each bottle before and after the incubation, using a WTW Oxi539 oxymeter equipped with a Trioximatic300 oxygen probe and a magnetic stirrer. The gross photosynthetic rate (mg O_2.l^{-1}.h^{-1}) was estimated by summing the oxygen production rate measured in the light bottles and the oxygen respiration rate measured in the dark bottles. The gross photosynthetic rate was converted into hourly volumetric P_{prim} (mg C.l^{-1}.h^{-1}), assuming that 1.55 g of molecular oxygen are released in the water when 1 g of algal cell material is synthesised (OSWALD, 1988). Areal daily P_{prim}(mg DW.m^{-2}.day^{-1}) was determined by integrating the volumetric hourly P_{prim} over depth and by multiplying the integrated value by the ratio between the total solar irradiance during the day and the solar irradiance during the incubation, both expressed in Einstein units per square metre (MEFFERT & OVERBECK, 1985). Solar irradiance data were obtained from the nearby meteorological station of Belvaux (49°31' N - 5°56' E).

Biomass and production of *D. magna*

Zooplankton samples were taken every 60 cm with a 5 l Van Dorn bottle. They were concentrated using a Nylon net (mesh size = 80 µm) and preserved in 4 % sugar formalin solution (PREPAS, 1978). In the laboratory, *D. magna* specimens were enumerated, measured to the nearest 50 µm using a Leica dissecting microscope equipped with a micrometer, and sorted in 250 µm-wide size classes. Volumetric $B_{Daphnia}$ (mg DW.l^{-1}) was estimated as (RIGLER & DOWNING, 1984):

$$B_{Daphnia} = \sum_{i=1}^{n} N_i.(\overline{w}_i)$$

where N_i is the density of *D. magna* in the size class i, expressed in individuals.l^{-1} and $\overline{w}_i$ is the mean weight of the animals in the size class i deduced from the length-weight regressions established by CAUCHIE *et al.* (unpubl.data). The daily net production of *D. magna* ($P_{Daphnia}$) was calculated as (RIGLER & DOWNING, 1984):

$$P_{Daphnia} = \sum_{i=1}^{n} g_i.B_i$$

where g_i is the growth rate of the animals in the size class i, expressed in day^{-1} and B_i is the biomass of animals in the size class i, expressed in mg DW.l^{-1}.

Feeding experiments

Feeding experiments were conducted in the laboratory using fluorescently-labelled bacteria (FLB) and algae (FLA). FLB and FLA were prepared the day before the experiment using 5-(4,6-dichlorotriazin-2-yl) amino-fluorescein (DTAF) according to SHERR *et al.* (1987) and TELESH *et al.* (1995), respectively. Bacteria used for labelling were isolated from pond water by filtration. The alga used for labelling was the chlorophycean *Dictyosphaerium ehrenbergianum* NAEG., cultured in the laboratory. After P_{bact} and P_{prim} had been determined *in situ*, daphnids were collected every 80 cm with a 5 l Van Dorn bottle and brought back to the laboratory in carboys that were kept in incubators at *in situ* temperatures. In the laboratory, B_{bact} and B_{phyto} were determined and pond water was filtered over a polycarbonate membrane (pore size = 0.2 µm) in order to obtain sterile pond water. Feeding medium was constituted by adding FLB and FLA to 50 ml of filtered pond water to obatin B_{bact} and B_{phyto} concentrations similar to those *in situ*. Each feeding experiment was conducted in an incubator at *in situ* temperature and photoperiod, in triplicate glass bottles containing 50 ml of feeding medium.

In order to avoid loss of FLB or FLA through defecation, the incubation time used for the feeding experiments was set lower than gut passage time (GPT). GPT experiments were, therefore, conducted prior to each feeding experiment. GPT was determined by direct observation of the labelled food in the gut. The animals were incubated for an increasing incubation time in the feeding medium. At the end of each incubation, the daph-

nids were narcotised with carbonated water, killed with formalin and rinsed with sterile water. The animals were then individually observed under a Leica DMRB epifluorescence microscope equipped with a blue excitation filter block (Leica I3, BP 450-490). The fullness of the gut of each animal was noted, using the following scores: (1) there were no FLB nor FLA in the gut of the animal; (2) there were FLB or FLA in the first half of the gut; (3) there were FLB or FLA in the second part of the gut but not up to the distal part of the gut; (4) there were FLB or FLA up to the distal part of the gut. GPT was reached when the animal began to defecate fluorescent food.

When gut passage time had been determined, daphnids were incubated in the feeding medium during a time that was approximately 20 % shorter than the gut passage time. The feeding experiments were always conducted around 19:00 on the day of sampling. The incubation was ended by adding carbonated water to narcotise the animals. The animals were killed with formalin and rinsed with sterile water, then grouped according to size and transferred into microvials. One ml of sterile water was added to each microvial which was then shaken vigorously and sonicated until the bodies of the daphnids were destroyed. The suspension was then filtered onto a black membrane, and FLB and FLA were enumerated under a Leica DMRB epifluorescence microscope equipped with a blue excitation filter block (Leica I3, BP 450-490). The hourly IR_{bact} and IR_{phyto} (ng DW.ind^{-1}.h^{-1}) were calculated as:

$$\text{Hourly } IR_{bact/phyto} = \frac{N_{FLB/FLA} \cdot 60.\overline{w}_{FLB/FLA}}{N_{Daphnia} \cdot IT}$$

where $N_{FLB/FLA}$ is the number of FLB or FLA in the microvial (number of FLB or FLA); $\overline{w}_{FLB/FLA}$ is the mean weight of FLB or FLA used in the experiment (ng DW.(FLB or FLA)$^{-1}$); $N_{Daphnia}$ is the number of *D. magna* individuals grouped in the microvial (ind.) and IT is the incubation time (min). Equations describing the relationship between *D. magna* body size and ingestion rates were obtained by fitting a power equation to the data. Daily IR_{bact} and IR_{phyto} by the whole *D. magna* population (mg DW. (g DW *Daphnia*)$^{-1}$.l^{-1}.d^{-1}) were calculated as:

$$\text{Daily } IR_{bact/phyto} = \sum_{i=1}^{n} \left(\frac{\overline{IR}_{bact/phyto\ (i)} \cdot N_i.24}{\overline{w}_i} \right)$$

where $\overline{IR}_{bact/phyto\ (i)}$ is the mean hourly IR_{bact} or IR_{phyto} of the individuals in the size class i (ng DW.ind^{-1}.h^{-1}); Ni is the density of *D. magna* individuals in the size class i (ind.l^{-1}); w_i is the mean weight of the *D. magna* individuals in size class i (µg) determined according to CAUCHIE *et al.* (unpubl.data). The areal daily IR_{bact} and IR_{phyto} were obtained by integrating the volumetric daily IR_{bact} and IR_{phyto} over the water column.

To check whether the ingestion rates measured at 19:00 were representative of the daily mean IR_{phyto}, hourly IR_{phyto} was measured every 3 hours during 24 hours under the conditions prevailing on July 09. Feeding experiments were conducted as described above, using a feeding medium composed of 1.2 µm filtered pond water (*i.e.* pond water con-

taining the natural bacterial community) supplemented with FLA at a biomass similar to the *in situ* B_{phyto} observed on July 09.

RESULTS

The dynamics of areal values of $B_{Daphnia}$, B_{bact} and B_{phyto} are shown in Fig. 1. Low to moderate $B_{Daphnia}$ values (< 2.5 g DW.m^{-2}) were observed from January to May. $B_{Daphnia}$ began to increase steeply in June and reached very high values in early July (60.4 g DW.m^{-2}), before decreasing to about 5 g DW.m^{-2} in late July. The dynamics of areal B_{phyto} showed a first peak in February-March (maximum value = 1.1 g DW.m^{-2}) and a second one in June-July (maximum value = 8.8 g DW.m^{-2}).

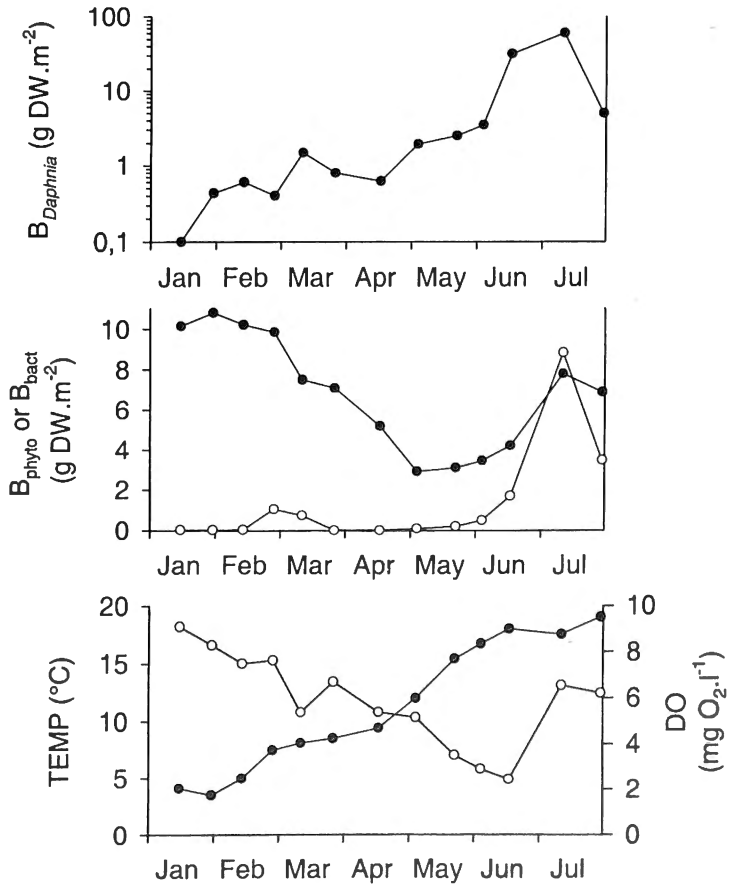

Fig. 1. – Variation of *Daphnia magna* biomass ($B_{Daphnia}$), bacterioplankton biomass (B_{bact}), phytoplankton biomass (B_{phyto}), water temperature (Temp) and dissolved oxygen concentration (DO) in the waste stabilisation pond of Differdange from January to July 1998. Middle panel: black circles = B_{bact}; open circles = B_{phyto}. Lower panel: black circles = Temp; open circles = DO.

The second, main peak of phytoplankton was dominated by the unicellular green alga *Planktosphaeria gelatinosa* SMITH. B_{bact} decreased from about 10 g DW.m^{-2} in January to 3 g DW.m^{-2} at the beginning of May. It then increased to 7.8 g DW.m^{-2} at the beginning of July. Water temperature gradually increased from 3.5°C in January to 19°C in July.

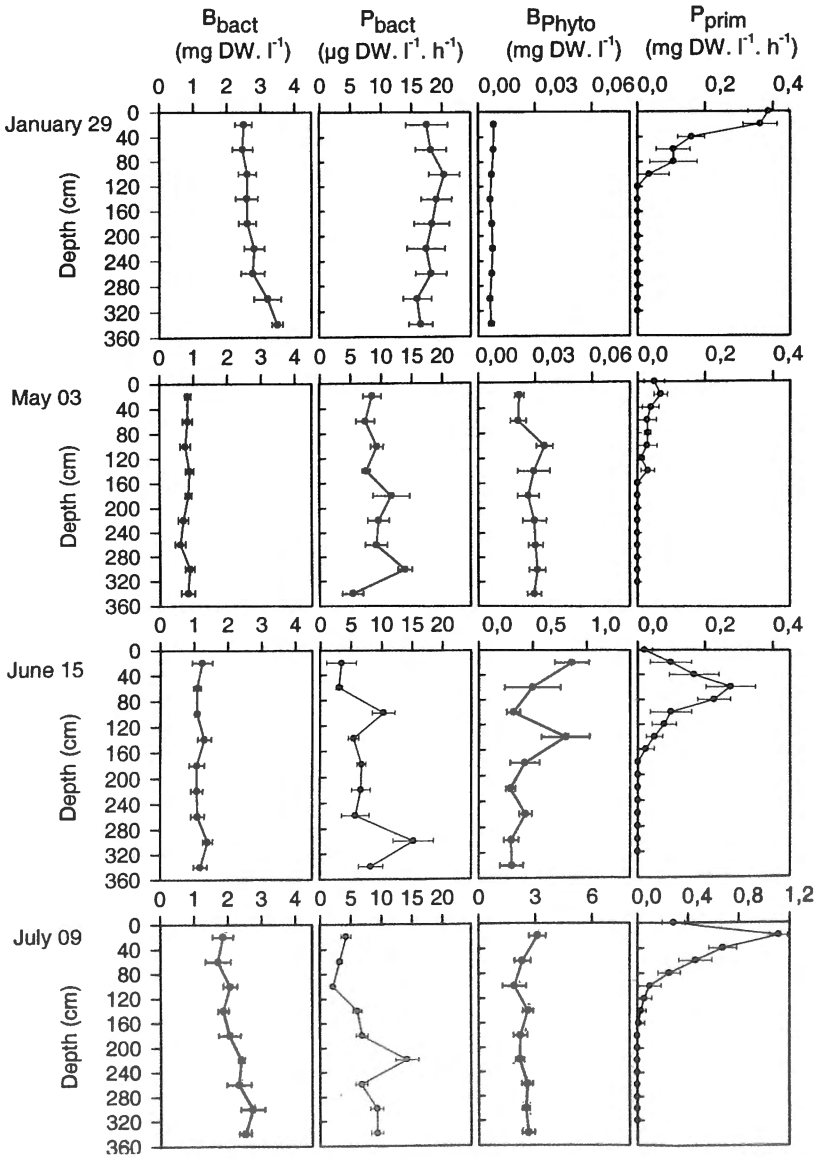

Fig. 2. – Vertical profiles of bacterioplankton biomass (B_{bact}) and production (P_{bact}), phytoplankton biomass (B_{phyto}) and primary production (P_{prim}) in the waste stabilisation pond of Differdange on the dates of the feeding experiments.

Dissolved oxygen concentration decreased from 9.1 mg $O_2.l^{-1}$ in January to 2.5 mg $O_2.l^{-1}$ in June. It increased to 6.5 mg $O_2.l^{-1}$ in July. Pearson Moment Product correlations between the variables presented in Fig. 1 are shown in Table 1. Significant positive correlations were found between $B_{Daphnia}$ and B_{phyto} and between B_{bact} and dissolved oxygen concentration. Negative correlations were found between B_{bact} and water temperature, and between water temperature and dissolved oxygen concentration.

TABLE 1

Correlation matrix (Pearson Product Moment Correlation) for variables measured bimonthly in the WSP of Differdange from January to July 1998
*ns = not significant (P>0.05), * P<0.05, ** P<0.01, *** P<0.001*

	$B_{Daphnia}$	B_{bact}	B_{phyto}	Temp.
B_{bact}	– 0.08 NS			
B_{phyto}	0.88 ***	0.06 NS		
Temp.	0.56 *	– 0.70 **	0.55 NS	
DO	– 0.20 NS	0.89 ***	0.01 NS	– 0.74 **

B_{bact} and B_{phyto} values showed weak vertical variation during the day, except in June when outlier values of B_{phyto} were observed at the surface and at a depth of 120 cm (Fig. 2). By contrast, P_{bact} and P_{prim} generally varied significantly with depth. P_{prim} displayed typical profiles, with maximum values observed between 20 and 60 cm deep. Daytime $B_{Daphnia}$ vertical distribution (Fig. 3) appeared quite patchy in May and June, whereas it was homogenous in January and July. Water temperature and dissolved oxygen concentration did not vary substantially over the water column (Fig. 3). In the absence of significant variation in vertical distribution of food and physico-chemistry, *in situ per capita* IR_{bact} and IR_{phyto} were assumed to be constant throughout the water column, and only one set of feeding experiments was performed per date, using mean volumetric values of $B_{Daphnia}$, B_{bact} and B_{phyto}. The feeding experiments were performed under low (January 29), intermediate (May 03), high (June 15) and very high (July 09) $B_{Daphnia}$ values (Table 2). Moreover, the ratio of B_{bact} on B_{phyto} varied widely among these dates, decreasing from 390 in January to 29 in May, 2.5 in June and 0.9 in July.

TABLE 2

Areal values of bacterioplankton biomass (B_{bact}) and production (P_{bact}), phytoplankton biomass (B_{phyto}), primary production (P_{prim}) and Daphnia magna biomass ($B_{Daphnia}$) and production ($P_{Daphnia}$) in the aerated waste stabilisation pond of Differdange on the dates of the feeding experiments. TEMP = water temperature, DW = dry weight.

Date	*TEMP °C*	*Bbact*	B_{phyto} *g DW.m^{-2}*	$B_{Daphnia}$	P_{bact}	P_{prim} *gDW.m^{-3}.day^{-1}*	$P_{Daphnia}$
January 29	3.5	10.152	0.026	0.431	1.536	1.390	0.047
May 03	12.4	2.910	0.102	1.947	0.792	0.314	0.344
June 15	18.0	4.234	1.701	31.961	0.624	1.644	18.518
July 09	17.5	7.801	8.837	60.386	0.600	4.889	54.601

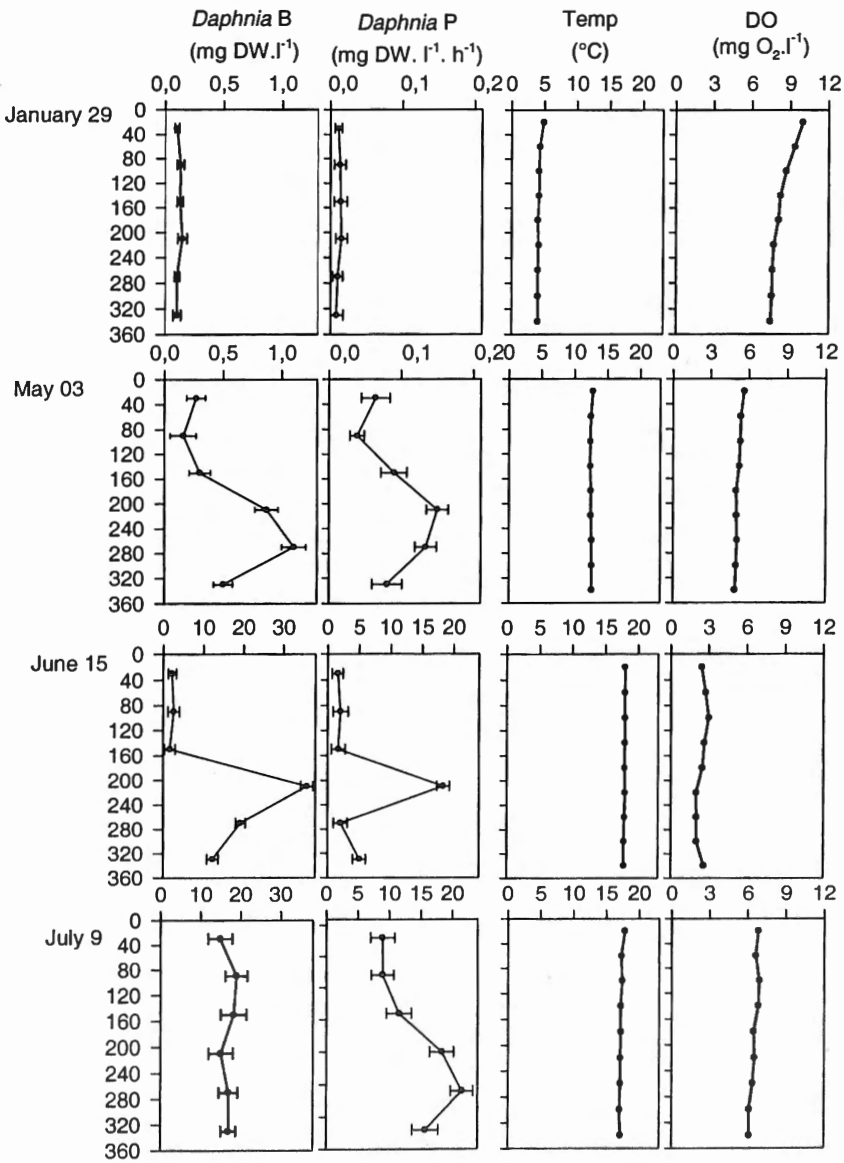

Fig. 3. – Vertical profiles of *Daphnia magna* biomass ($B_{Daphnia}$) and production ($P_{Daphnia}$), water temperature (Temp) and dissolved oxygen concentration (DO) in the waste stabilisation pond of Differdange on the date of the feeding experiments.

The results of the GPT experiments are shown in Fig. 4. In the January experiments, the proportion of non-feeding individuals, *i.e.* individuals which had not ingested fluorescent food, decreased from 100 to 35 % within the first 15 minutes of incubation. It then stayed around 35 % for incubation times up to 60 minutes. Individuals which had begun

to defecate fluorescent food were only observed in experiments lasting 60 minutes. In the three other GPT experiments, animals defecating fluorescent food were observed within 5 to 9 minutes from the beginning of the incubation. In May, June and July experiments, the proportion of the non-feeding individuals remained as high as 10-15 % for incubations longer than 15 minutes. On the basis of the results presented in Fig. 4, the incubation duration was set at 30 minutes for January experiments and 4 minutes for the three other sets of experiments.

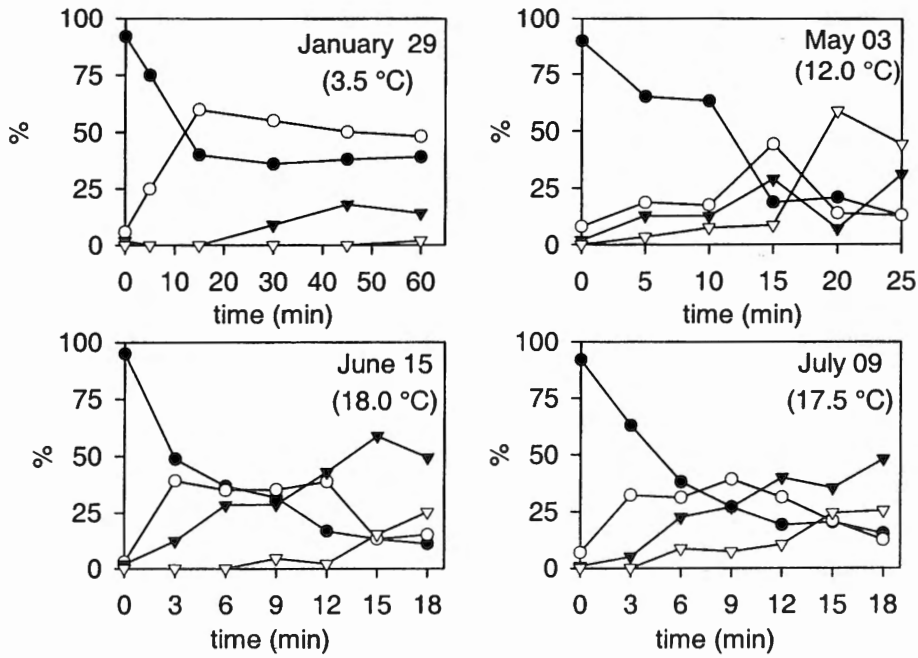

Fig. 4. – Evolution of the proportion of the individuals having no fluorescently labelled algae (FLA) in the gut (black circles), FLA in the first half part of the gut (open circles), FLA in the second part of the gut but not up to the distal part (black triangles) or FLA up to the distal part of the gut (open triangles) as a function of increasing incubation time in *Daphnia magna* kept under constant conditions in the laboratory and provided with *in situ* concentrations of FLA. Water temperature indicated in brackets.

The relationships between *D. magna* body length and IR_{bact} and IR_{phyto} (Fig. 5) could be adequately described by a power function (Table 3). The exponent b ranged from 1 to 2, except for January IR_{bact} where it was 3.12 and for May IR_{phyto} when it was 0.87. Areal daily IR_{bact} and IR_{phyto} increased exponentially from January to July (Table 4). The proportion of B_{bact} and B_{phyto} ingested per day by *D. magna* varied from 0.1 to 17.6 % and from 65.7 to 171.1 %, respectively. *D. magna* ingested daily a small percentage of P_{bact} in January (0.8 %) and May (0.9 %), and a large percentage of P_{bact} in June (119.7 %) and July (226.0 %). The percentage of P_{prim} ingested daily by *D. magna* increased from 1.7 % in January to 319.2 % in July.

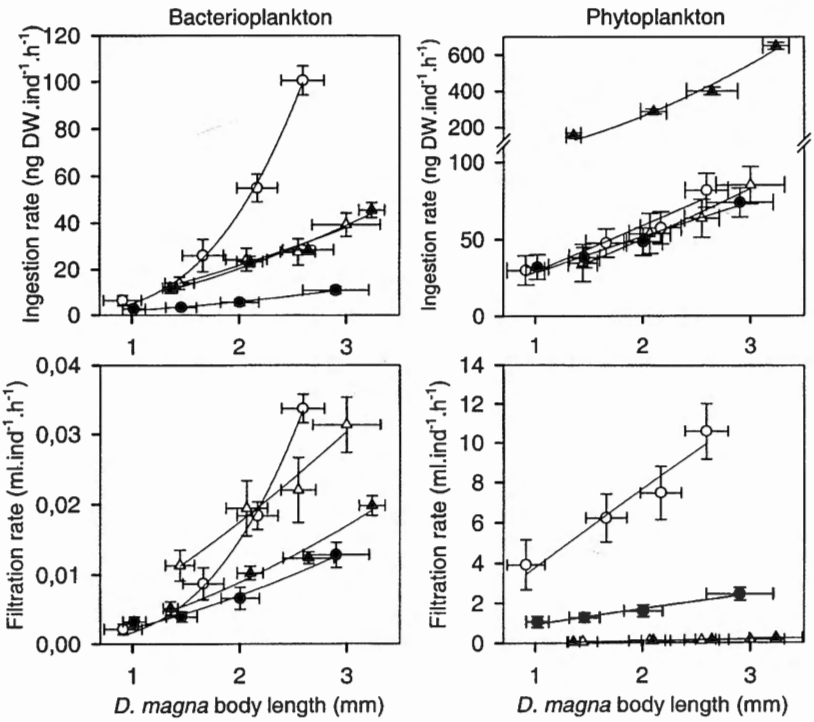

Fig. 5. – Size specific ingestion rate (upper panels) and filtration rates (lower panels) of bacterioplankton (left panels) and phytoplankton (right panels) by *Daphnia magna* on four different dates. Open circles = January 29; black circles = May 03; open triangles = June 15; black triangles = July 09.

TABLE 3

Cell concentrations of bacterioplankton and phytoplankton used in the feeding experiments and power functions describing the dependence of the ingestion rate (IR) and the filtering rate (FR) on the Daphnia magna *body length (L). Equation: IR or FR = a . L b ; For units, see Fig. 5*

	Concentration (cells.ml^{-1})	*IR*	*FR*	r^2
Bacterioplankton				
January 29	3.3 10^7	0.0017 $L^{3.12}$	1.70 $L^{3.12}$	0.997
May 03	1.0 10^7	0.0024 $L^{1.57}$	2,35 $L^{1.57}$	0.984
June 15	1.4 10^7	0.0068 $L^{1.36}$	6.78 $L^{1.36}$	0.963
July 09	2.6 10^7	0.0030 $L^{1.58}$	2.95 $L^{1.58}$	0.972
Phytoplankton				
January 29	650	28.96 $L^{1.01}$	3.78 $L^{1.01}$	0.944
May 03	2,500	28.21 $L^{0.87}$	0.94 $L^{0.87}$	0.972
June 15	41,700	21.43 $L^{1.22}$	0.04 $L^{1.22}$	0.979
July 09	220,000	73.42 $L^{1.83}$	0.03 $L^{1.83}$	0.983

TABLE 4

Ingestion rates and filtering rates of the Daphnia magna *population on bacterioplankton and phytoplankton in the aerated waste stabilisation pond of Differdange*

	January 29	*May 03*	*June 15*	*July 09*
Bacterioplankton				
FR_{bact} ($l.m^{-2}.day^{-1}$)	4.0	8.2	599.9	591.00
IR_{bact} (g DW m^{-2} day^{-1})	0.012	0.007	0.747	1.356
IR_{bact} (% of B_{bact} day^{-1})	0.1	0.2	17.6	17.4
IR_{bact} (% of daily P_{bact})	0.8	0.9	19.7	226.0
Phytoplankton				
FR_{phyto} ($l.m^{-2}.day^{-1}$)	3137.3	2233.3	2970.2	5816.5
IR_{phyto} (g DW m^{-2} day^{-1})	0.024	0.067	1.486	15.117
IR_{phyto} (% of B_{phyto} day^{-1})	92.3	65.7	87.4	171.1
IR_{phyto} (% of daily P_{prim})	1.7	21.3	90.4	309.2

The diel pattern of variation in IR_{phyto} is shown in Fig. 6. The diel pattern was unimodal for 1.0 mm long *D. magna* and bimodal for 2.5 mm long ones. For 1 mm long animals, the mode was located at 13:00. For 2.5 mm long animals, the modes were observed between 7:00 and 10:00, and at 19:00. The relative amplitude of hourly IR_{phyto}, δ, (*i.e.* the ratio between the highest and the lowest IR_{phyto} values observed over the nycthemeron) reached 2.09 and 2.46 for 1 mm long daphnids (juveniles) and 2.5 mm long daphnids (adults), respectively (Table 5). The ratios between the IR_{phyto} calculated from all hourly measures made during the nycthemeral monitoring and the IR_{phyto} measured at 19:00 (*i.e.* when the ingestion experiments were performed on January 29, May 03, June 15 and July 09) were quite close to 1.

TABLE 5

Diel relative amplitudes of hourly IR_{phyto} over the 24 hours cycle (δ) and comparison of the daily IR_{phyto} calculated from all the hourly IR_{phyto} values measured every 3 hours and the daily IR_{phyto} extrapolated from the hourly IR_{phyto} measured at 19 00. d is the daily maximum hourly IR_{phyto} divided by the daily minimum hourly IR_{phyto}

D. magna body length (mm)	δ	Daily IR_{phyto} *(µg DW. ind-1.d-1)*		Calculated on *Extrapolated ratio*
		Calculated	*Extrapolated*	
1.0	2.09	5.88	6.08	0.97
2.5	2.46	6.32	5.85	1.08

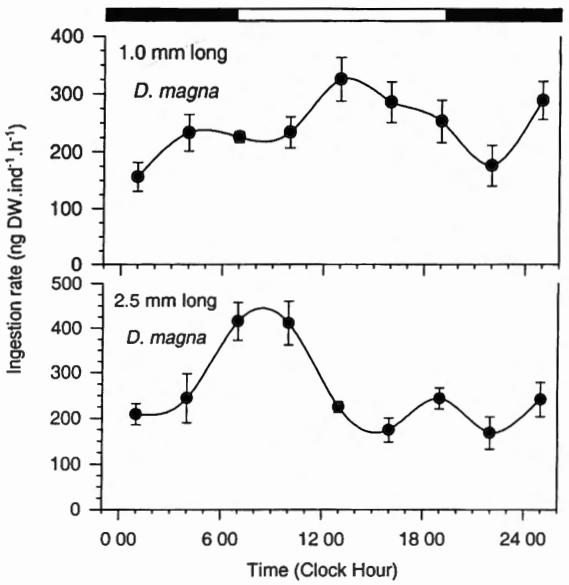

Fig. 6. – Diel variations of the hourly IR_{phyto} (mean ± standard deviation) of 1.0 and 2.5 mm long *D. magna* under the feeding conditions observed on July 09.

DISCUSSION

On the basis of the dynamics presented in Fig. 1 and the correlations shown in Table 1, the variation in $B_{Daphnia}$ in the pond of Differdange appeared tightly linked to the availability of algal food and, to a lesser extent, to water temperature. The importance of phytoplankton in the daily ration of *Daphnia magna* will be discussed below on the basis of the results of the feeding experiments. The correlation between $B_{Daphnia}$ and water temperature reflected the strong dependence of the growth rate of *D. magna* on water temperature (BOTTRELL *et al.*, 1976). An increase of water temperature from 10 to 20°C shortens the generation time from more than 20 days to less than 5 days (CAUCHIE *et al.*, unpubl.data).

B_{bact} apparently varied independently of $B_{Daphnia}$, but was correlated with dissolved oxygen concentration and water temperature. The decrease in dissolved oxygen concentration from January to June resulted from a gradual decrease in the input of well-oxygenated rain water in the sewage, as rainfall decreases from winter to spring. As a consequence, anaerobic bacteria most probably became dominant over aerobic ones. The slower metabolic rate of anaerobes compared to aerobes (DROSTE, 1997), coupled with an increase in the density of bacterivorous protozoans generally observed in spring in WSP (CANOVAS *et al.*, 1996), most probably caused the continuous decrease of B_{bact} observed from January to May. The release of organic material by phytoplankton is a major source of carbon for bacterioplankton. This may explain why the increase of B_{phyto} observed in June and July was followed by a significant increase of B_{bact}.

The homogeneity of the vertical profile of B_{bact} and B_{phyto} throughout the water column probably resulted from efficient mixing of the water column by the mechanical aerators. The vertical distribution of $B_{Daphnia}$ appeared patchy during May and June. Because the aerators induced an efficient mixing of the water, hydrodynamic factors did not cause this patchiness. On the other hand, the swarming of *D. magna* was not an adaptation to the presence of predators since neither fish nor other common daphnid consumers were present in the pond. In the presence of homogeneously distributed food, the vertical distribution of daphnids might also be expected to be homogeneous (PIJANOWSKA & DAWIDOWICZ, 1987). It is therefore not known why *D. magna* tended to aggregate near the bottom in May and in June.

GPT experiments revealed that feeding activity was neither synchronised nor continuous in the *D. magna* population. Such high variability in GPT has been observed in other *Daphnia* species (ZANKAI, 1983 ; MURTAUGH, 1985 ; GERRITSEN *et al.*, 1987). This variability could have a purely experimental origin. When transferred from one medium to another with different characteristics (food concentration, water temperature, etc.), animals can be stressed and may need from 15 to 30 minutes to recover constant physiological rates (BURNS, 1968 ; PORTER *et al.*, 1982). We transferred animals carefully during the feeding experiments and the animals did not present any signs of stress (rejection of food from the filtering appendages with the postabdomen, prostration or increase in swimming speed) during or after transfer. However, the animals were transferred from a medium containing unfiltered pond water to a feeding medium containing filtered pond water to which bacteria and algae were added, but which did not contain detritus and protozoans, both abundant in the pond (H.M. CAUCHIE, pers. obs.). Daphnids are indeed known to feed not only on bacteria and algae but also on detritus and protozoa (*e.g.* PETERSON *et al.*, 1978 ; PORTER *et al.*, 1983 ; JÜRGENS, 1994). Total food availability was therefore certainly lower in the feeding medium than in the pond and could have caused a variation in the feeding behaviour of the animals. Nevertheless, the persistence of a significant proportion of non-feeding animals during long experiments (> 15-30 minutes) suggests that, besides methodological biases, an actual inter-individual variation exists in the feeding activity of *D. magna*.

In *Daphnia*, the distance between the setules of the filter combs increases with body length (KORINEK *et al.*, 1986 ; LAMPERT & BRENDELBERG, 1996). As a consequence, small individuals can retain small particles such as bacteria more efficiently than large individuals, whereas large animals can handle large particles more easily than small animals. High values of the exponent *b* are therefore observed for *Daphnia* when large green algae and cyanobacteria are offered as food (HOLM *et al.*, 1983 ; BRENDELBERGER, 1985 ; STUCHLIK, 1991). By contrast, low *b* values are observed when bacteria are offered as food (PETERSON *et al.*, 1978 ; PORTER *et al.*, 1983). Except on January 29 for IR_{bact}, intermediate to low *b* values were observed as a consequence of the small size of the FLB (length = 1 µm ; width = 0.5 µm) and FLA (diameter = 5 µm). The high value of *b* observed for IR_{bact} in January probably reflects an adaptation of the filtering apparatus to low food during winter. Daphnids are indeed able to increase their filtration area and to reduce the open distance between the setules of the endites of their third and fourth thoracic limbs (KORINEK *et al.*, 1986). As a consequence, an increase of the value of *b* is observed under conditions of low food availability (LAMPERT & BRENDELBERGER, 1996).

Even when B_{bact} was significantly higher than B_{phyto}, *D. magna* predominantly fed on algae. This is in good agreement with the bulk of evidence presented earlier (PETERSON *et al.*, 1978; GELLER & MÜLLER, 1981) and reflects the low ability of this species to capture bacteria compared to algae (BRENDELBERGER, 1985). This difference in capture efficiency resulted in a lower impact of *D. magna* on the bacterial community than on the algal community. From January to June, B_{phyto} was maintained at a low level because the *D. magna* population consumed a high proportion of B_{phyto}. Despite this high grazing pressure, the green alga *Planktonsphaeria gelatinosa* was able to develop a high biomass. In early July, $B_{Daphnia}$ reached an uncommonly high value resulting in an overgrazing of the phytoplankton and a collapse of B_{phyto}. The high grazing pressure of *D. magna* is certainly a major cause of the weak phytoplankton development generally observed in waste stabilisation ponds (NAMÉCHE, 1998). Significant grazing pressure on bacterioplankton was only observed when $B_{Daphnia}$ reached high values. In June, the *D. magna* population ingested one fifth of B_{bact} daily and more than 100 % of the P_{bact} daily. However, Bbact did not decrease because of the input of allochtonous B_{bact} with sewage. The grazing pressure on P_{bact} was maximum in early July, resulting in a decrease in B_{bact}. Because, however, such high $B_{Daphnia}$ are only transient in waste stabilisation ponds, the grazing pressure of the *D. magna* population on the bacterioplankton dynamics must be considered, on the whole, as marginal.

The gross production efficiency (GPE) of *D. magna* on the combined biomass of bacterioplankton and phytoplankton ($P_{Daphnia}$ / (IR_{bact} + IR_{phyto})) reached 130, 465, 830 and 331 % in January, May, June and July, respectively. Such obviously too high values of GPE indicate, as far as $P_{Daphnia}$ was correctly estimated, that the total quantity of food ingested by *D. magna* was underestimated. This may indicate that the detritic particulate matter constitutes a significant part of the *D. magna* ration in waste stabilisation ponds. The next stage of our trophodynamic study of waste stabilisation ponds will therefore involve the labelling of detritus and the estimation of its ingestion rate by *D. magna*.

The extrapolation of daily ingestion rates from a few short-term experiments is questionable. The monitoring of diel variation of IR_{phyto} revealed, however, that a single measure of IR_{phyto} made at 19 :00 correctly reflected the mean IR_{phyto} over 24 hours. Diel patterns of IR_{phyto} in adults were similar to those observed in other *Daphnia* species (STARKWEATHER, 1975, 1983; HANEY & HALL, 1975), with the highest ingestion rates being generally observed during the night or at light-to-darkness or darkness-to-light transition. By contrast, the daytime maximum of IR_{phyto} observed in juveniles was quite uncommon. Visual predation by fish is often pointed out as a major cause of the occurrence of maximum feeding rates at night (STARKWEATHER, 1983). In the WSP of Differdange, this type of predation was non-existent and there was thus no disadvantage of feeding during the day. Under these conditions, the temporal separation of the maximum feeding activity of juveniles from that of adults can be hypothesised to be a niche shift leading to a reduction of intra-specific competition for food.

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