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Cell specific primary production and phagotrophy of phytoplankton in acidified lakes of Bohemian Forest

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Abstract
Grain density autoradiography was used to study primary production of individual species or genera in mixed phytoplankton assemblage of two acidified Bohemian Forest lakes. Chrysophytes and dinoflagellates were present in the phytoplankton of both Čertovo and Plešné Lakes during the studied season. In addition, large number of green algae and filamentous cyanobacteria was observed especially in Plešné Lake. Generally, higher primary production but lower algae abundance was observed in the surface layer than in the metalimnion. Due to its high abundance in Plešné Lake, *Monoraphidium* contributed far more to the total primary production than *Peridinium* in both surface and metalimnion layer. The mixotrophic flagellate *Dinobryon pediforme* showed lower volume specific activity than other autotrophic species as a possible consequence of its feeding on bacteria. The role of phagotrophy of *D. pediforme* was also studied in Čertovo Lake, where this species was dominant in the epilimnion. The experiment was based on short-term in situ incubations. The highest uptake was observed in the late spring (almost 9 bacteria per *Dinobryon* cell per hour). A proportion of bacterial production grazed hourly by a population of *Dinobryon* ranged between 11 and 261 %. A positive correlation of dissolved phosphorus and ingestion rate was observed. The light regime (dark × light) during incubation had no impact on the intensity of ingestion. The phagotrophic *Dinobryon* population may control both growth and abundance of bacterial population significantly. The significant correlation between consumption of bacteria by *Dinobryon* and dissolved phosphorus concentration suggests that bacteria may represent an alternative phosphorus source for the P-limited phagotrophic phytoplankton.

Keywords: autoradiography, *Dinobryon pediforme*, *Peridinium*, *Monoraphidium*, mixotrophy

Introduction

Autoradiography is a potentially powerful tool for measuring individual species production in mixed phytoplankton assemblages. There are two basic techniques. Track autoradiography uses thick emulsion layers to record the paths of particles in their entirety as a string of silver grains while grain density autoradiography uses a thin layer emulsion to record only the initial portions of tracks and then relates the density of grains produced to the amount of radioactivity present (Knoechel & Kalff 1976). The flow of energy and materials through an ecosystem is dependent upon the relative participation of its component species. Grain density autoradiography was used as a particularly useful tool for providing an estimate of the intraspecific distribution of primary production. In conjunction with the common used ^{14}C method of estimating community production useful for examining general patterns and trends, this method permits increased understanding of the functional interrelationships between the primary producers (Maguire & Neill 1971, Davenport & Maguire 1984).

Table 1. – Limnological and hydrochemical characteristics of Plešné and Čertovo Lakes in Bohemian Forest.

Lake	Čertovo	Plešné
Altitude [m a. s. l.]	1028	1090
Lake area [ha]	10.3	7.5
Max. depth [m]	36	18
Volume [10^6 m^3]	1.85	0.62
Euphotic layer (> 1% surface irradiation) [m]*	9.8	6.2
Transparency [m]*	4.6	1.7
pH*	4.49	4.78
Dissolved inorganic carbon (DIC) – surface / metalimnion – [mg l^{-1}]*	0.20 / 0.39	0.18 / 0.11
*Total phosphorus (surface), TP [µg l^{-1}]**	4.5	8.6
Dissolved phosphorus (surface), DP [µg l^{-1}]*	1.5	1.5
Soluble reactive phosphorus (surface), SRP [µg l^{-1}]*	0.3	0.5
Total primary production surface / metalimnion – [µg ^{14}C l^{-1} h^{-1}]*	1.56 / 0.64	3.42 / 2.29
Chlorophyll-a surface / metalimnion – [µg l^{-1}]*	2.5 / 2.6	6.7 / 17.1
Dominant algal groups*	Chrysophytes Dinoflagellates	Green algae Dinoflagellates

*seasonal means

Primary production of individual algal species in glacial lakes in the Czech part of the Bohemian Forest was studied in 1998. Oligotrophic Čertovo Lake and mesotrophic Plešné Lake have been chosen for detailed analyses of vertical distribution and composition of the phytoplankton assemblages. All lakes are strongly acidified, with sulphate and nitrate being the dominant anions, and with elevated aluminium concentrations (Veselý 1996, Hejzlar & al. 1998). An ecologically unique type of aquatic ecosystems has developed in these lakes where higher trophic levels disappeared due to acidification and microorganisms became dominating the pelagic food webs (Vrba & al. 1996). The dissolved phosphorus (DP) concentration is very low under these conditions, which is an obstacle for a successful development of most phytoplanktonic species. On the contrary, this is a good opportunity for mixotrophic species that are capable to gain phosphorus by ingesting organic particles including bacteria. It is plausible that mixotrophic organisms have a significant impact on the whole community. The ability to feed phagotrophically and influence of the other constituents of an ecosystem, such as light intensity, temperature and relation to a bacterial population dynamics, were studied chiefly on the chrysophytes of the genus *Dinobryon* (Bird & Kalff 1986, 1987), on the species *D. divergens* (Jones & Rees 1994 a, b) and *D. cylindricum* (Caron & al. 1993, Jones & Rees 1994 a, b).

Mixotrophic forms were observed to be the most abundant at low nutrient concentration (especially dissolved phosphorus), i.e. in clear oligotrophic lakes (Jones 1997, Isaksson 1998). In the lakes with higher trophic level mixotrophs occurred mainly in winter, early spring and late summer (Sanders & al. 1989). Simultaneously, a relation between ingestion rate and light intensity was determined at some species, both positive and negative (Jones & Rees 1994 a, b, Rothhaupt 1997). After a study of microbial communities in particular locations, a significant proportion of mixotrophic flagellates on total grazing on bacteria was observed. This proportion made up 40 % in the Bohemian Forest lakes (Vrba & al. 1996). In the lake Mekkojärvi (Finland) mixotrophic flagellates were accountable for 22% of total grazing per day (Salonen & Jokinen 1988). Values of 2% to 45% were recorded by Sanders & al. (1989) in the lake Oglethorpe (USA), the highest ones in winter and spring.

Material and Methods

Sampling

Samples for autoradiography and total primary production measured with the ^{14}C-method were taken monthly (May – September 1998) from the surface layer and from the metalimnion at the oxygen maximum (3 – 6.5 m). For each depth a mixed sample of 10 l was used for the chemical measurements and the biological experiments. Each glass bottle (volume of about 125 ml) was inoculated with [^{14}C] $NaHCO_3$ (0.1 MBq) and incubated in situ (two light and two dark bottles at each depth) for 1 hour at midday.

Samples for experiments with *Dinobryon* were taken from 0.5 m and 2 m depths, because *Dinobryon* was most abundant in the epilimnion. Lake water (200 ml) from each depth was incubated in 0.5 l flasks with a tracer FLB (Fluorescently Labelled Bacteria from culture stained with DTAF – 5-(4,6-dichlorotriazin–2-yl aminofluorescein, prepared after SHERR & al. 1987) addition ($3{,}71\times10^8$ bact. per ml). Mean volume of FLB was 0,1 – 0,2 mm^3. Samples were incubated in situ, at the depth from which they had been collected. After 15, 30 and 60 minutes of incubation, 50 ml of water from each incubation flask were taken and fixed with alkaline Lugol's solution followed by formalin (2% final concentration) and decolorized by sodium thiosulfate solution. Incubation temperature was always about 15°C.

Autoradiography

The phytoplankton was filtered alive through the 1.2 μm RA Millipore filters, then rinsed with distilled water. The filter was subsequently cleared with acetone fumes before autoradiographic processing (WATT 1971, STULL & al 1973). Kodak AR–10 stripping film was applied into the microscopic slide covered with gelatine layer. Three different exposures (12, 24 and 48 hours) were required to provide favorable grain densities over a wide primary production range. Emulsion preparation and development followed standard autoradiographic procedures. Kodak D–19 developer produced satisfactory results with AR–10 emulsion (RODGERS 1969). Grains were counted in at 1000 × with a fluorescence microscope (OLYMPUS AX 70) using image analysis (LUCIA 4.20, Laboratory Imaging Prague, CZ). All grains within a 10 μm were counted to prevent underestimation of small size cells (KNOECHEL & KALLF 1976). 25 individuals per species were counted. Background grain formation was estimated by counting the grains in 25 scattered areas where no cells were presented. Background was then subtracted from each grain count in accordance with the area over which grains were counted for each individual of that species. Grain counts were corrected for self-absorption assuming cellular specific gravity of 1.2 (RODGERS 1969). Because the total amount of DIC (dissolved inorganic carbon) available in lake water was variable within both time and space, a correction for actual DIC concentration was used. Considering used exposure intervals no correction for latent image erasure was used. The possibility of spurious grain formation due to chemographic effects, adsorbed (unassimilated) ^{14}C on the algal cells, or dark fixation was checked by making autoradiographic preparation of dark-bottle ^{14}C incubation but all were so low that corrections for them were unnecessary. Data on abundance of dominant species were kindly provided by L. Nedbalová (NEDBALOVÁ & VRTIŠKA 2000).

FLB uptake

Before being enumerated in the microscope, 15 ml subsamples were filtered through polycarbonate membrane of 1mm pore size (Poretics) and stained with DAPI fluorochrome (4',6'-diamidino–2-fenylindol). Ingested FLB in each *Dinobryon* cell were enumerated in a fluorescent microscope (OLYMPUS BX 60, at 1000×). At least one hundred ingested FLBs

per sample were counted, corresponding usually to 100 – 200 *Dinobryon* individuals inspected.

Results and Discussion

Plešné Lake

Dinoflagellates (*Peridinium*) and green algae (*Monoraphidium* cf. *dybowski*) dominated the phytoplankton of Plešné Lake during the whole period investigated (Nedbalová & Vrtiška, 2000). Higher activities of both species were measured in the surface layer (Fig. 1). In the metalimnetic layer the primary production of both species was lower as a result of lower irradiation (see Table 1). Because of its size (1468 μm^3), *Peridinium* was far more productive per cell than *Monoraphidium* (34 μm^3, Fig. 1A,B). Large autotrophic cells, with a higher to-

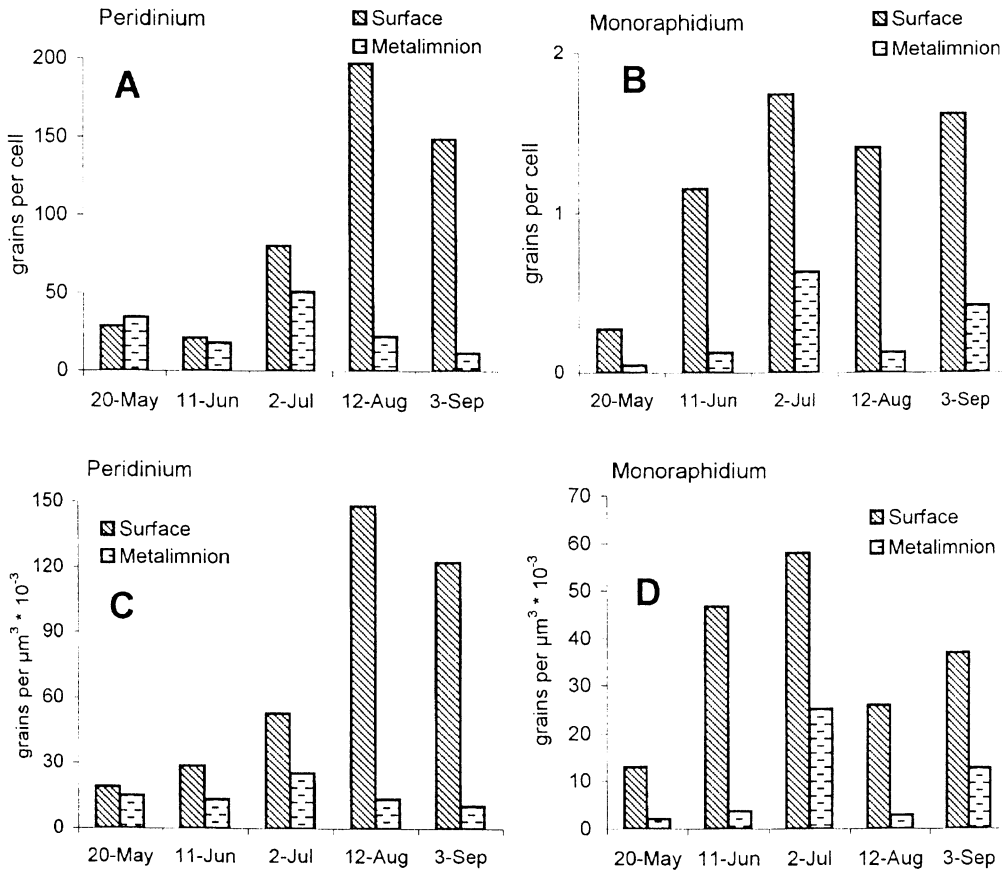

Fig. 1. – Plešné Lake – seasonal changes of per cell (A, B) and volume specific (C, D) activities of *Peridinium* and *Monoraphidium*. Notice that larger *Peridinium* cells are more active than smaller *Monoraphidium* cells (see different scales) in both surface and metalimnion layers during the whole studied season. In terms of volume specific activities the activity of *Monoraphidium* can be higher than *Peridinium* in some periods.

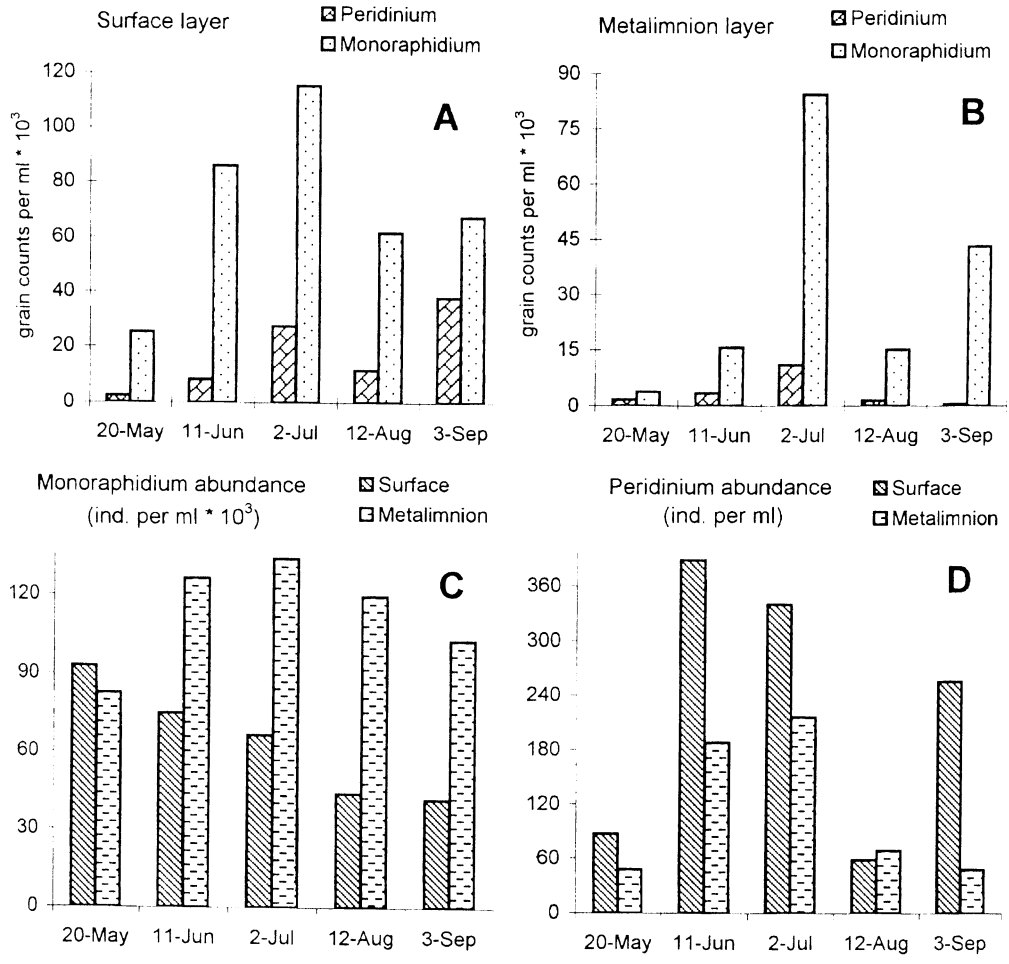

Fig. 2. – Plešné Lake – seasonal changes of total primary production per ml (A, B) and abundance (data from NEDBALOVÁ & VRTIŠKA 2000), of *Peridinium* (C) and *Monoraphidium* (D). The abundance of *Monoraphidium* cells were several orders of magnitude higher than the abundance of large *Peridinium* cells (see different scales). Due to its high abundance *Monoraphidium* contributed far more to the total primary production in Plešné Lake.

tal content of chlorophyll per cell than smaller ones, are usually more productive per cell as a result of higher photosyntetic potential (MAGUIRE & NEILL 1971).

However, in terms of primary production per biovolume, which reflects rates at which the populations are growing, in the first part of the year (until 2nd July) *Monoraphidium* was slightly more productive in surface layer than *Peridinium* (Fig 1C,D). MAGUIRE & NEILL (1971) showed that under laboratory condition smaller species of natural phytoplankton are more productive per biovolume than larger algal species. *Monoraphidium* abundance was several orders of magnitude higher than that of large *Peridinium*. Although the specific production of *Monoraphidium* was increasing until 2nd July, the total number of cell decreased (Fig. 2A). Simultaneously with this trend, the abundance of *Monoraphidium* increased in the

metalimnion probably due to sedimentation of senescent cells. On the other hand, the motile *Peridinium* was more abundant in the surface layer (Fig. 2B) where there existed more suitable light conditions (see Table 1). With the progress of summer, specific primary production of *Peridinium* increased and exceeded the growth rates of *Monoraphidium*. DESORTOVÁ (1976) described the opposite situation when the large *Peridinium* cells showed the lowest cell specific photosyntetic rate than other algal species during the whole period investigated.

In terms of total primary production, population of *Monoraphidium* contributed far more to the total primary production than that of *Peridinium* in both surface and metalimnion layer, due to its high abundance (Fig. 2C,D).

Čertovo Lake

Dinoflagellates and chrysophytes were the most important part of phytoplankton assemblage in Čertovo Lake. *Peridinium* and *Dinobryon pediforme* were almost the only important species occurring in the lake (NEDBALOVÁ & VRTIŠKA 2000). Although they are of very similar size (*Peridinium*–1321 μm³, *Dinobryon pediforme*–1084 μm³), the number of grains per cell

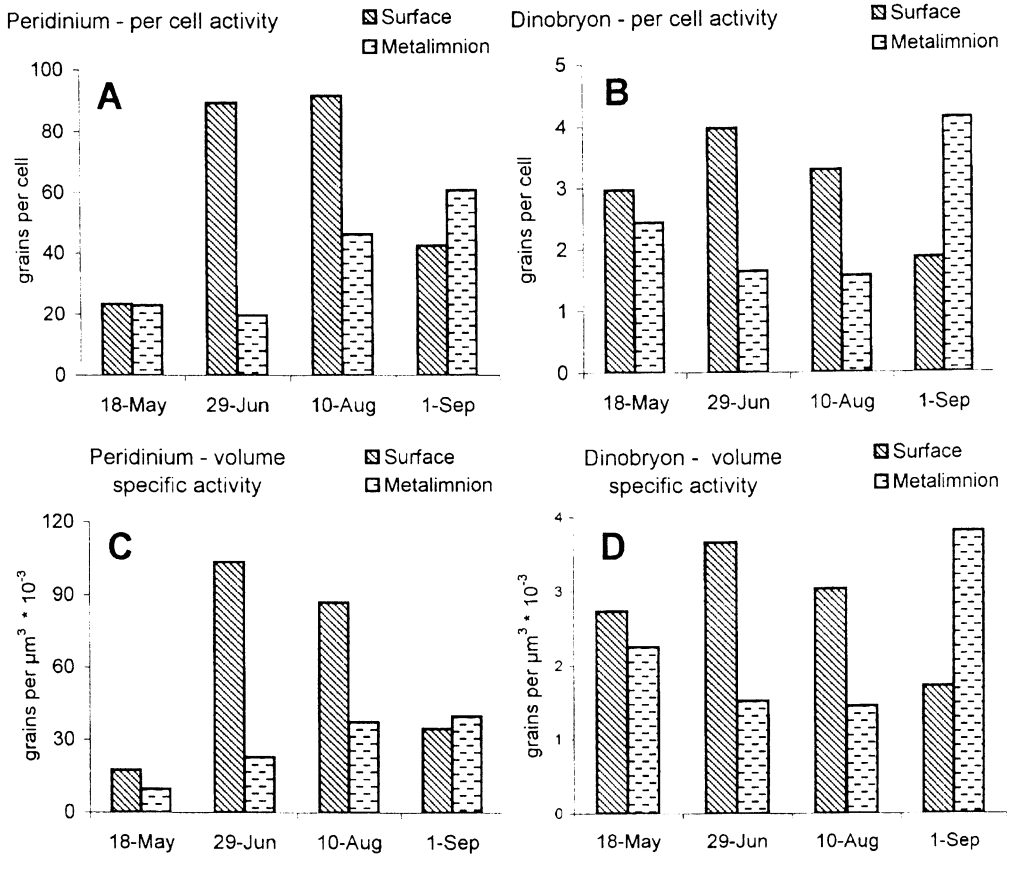

Fig. 3. – Čertovo Lake – seasonal changes of per cell (A, B) and volume specific (C, D) activities of *Peridinium* and *Dinobryon*. Both the activities of autotrophic *Peridinium* species were several order of magnitudes higher than those of mixotrophic *Dinobryon* species.

of *Peridinium* was several orders of magnitude higher than that of *Dinobryon pediforme* (Fig. 3A,B) as well as the values of specific primary production (number of grains per μm^3 of biovolume; Fig. 3C,D). This difference can be explained by feeding of *Dinobryon pediforme* on bacteria (see below). In similar nutrient limited ecosystems, the mixotrophy was often observed especially among chrysophytes (NYGAARD & TOBIESEN 1993, JONES 1997, ISAKSSON 1998).

The specific primary production was usually much higher in the surface layer than in the metalimnion during the investigated period. Only in September, the specific photosyntetic rate of both species in metalimnion exceeded the surface layer values. This could be probably due to a pronounced limitation by dissolved inorganic carbon in the surface layer (surface – DIC = 0.15 $mg \times l^{-1}$, metalimnion – DIC = 0.63 $mg \times l^{-1}$).

Mixotrophy of *Dinobryon pediforme*

In Čertovo Lake the highest abundance of *Dinobryon pediforme* was found in June (about 4000 cells per ml), then it decreased to 900 cells per ml in August. The second maximum

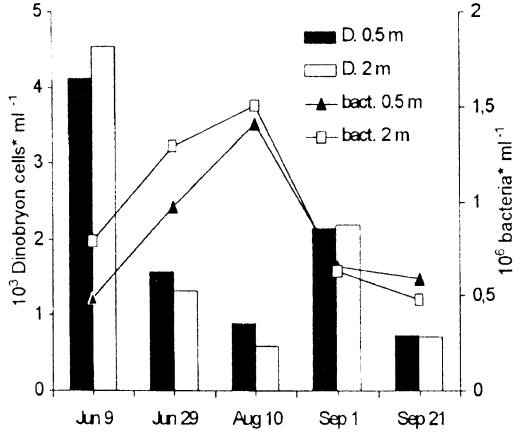

Fig. 4. – Abundance of *Dinobryon*. (D) and bacteria (bact.) in Čertovo Lake in two epilimnetic depths.

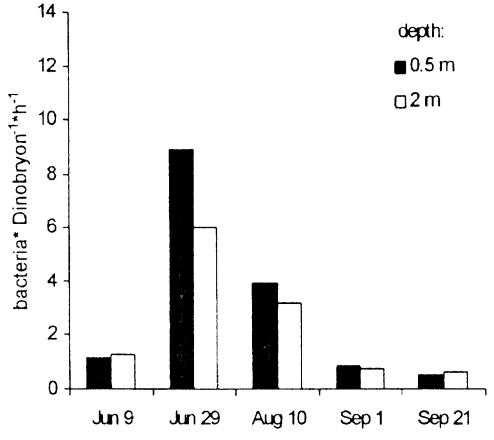

Fig. 5. – Ingestion rate of *Dinobryon* per hour in Čertovo Lake.

Table 2. – A proportion of bacterial production grazed hourly by a population of *Dinobryon* (%).

Date	Depht	
	0.5 m	2 m
June 29	261	129
August 10	68	12
September 1	16	11

appeared at the beginning of September (2000 cells per ml). The values of *Dinobryon* abundance did not differ distinctly (Fig. 4).

Bacterial abundance peaked in August (1.4×10^6 bact. per ml at 0.5 m, $1{,}5\times10^6$ bact. per ml at 2 m). Before and after this peak the abundance ranged between 0.48×10^6 bact. per ml and 0.79×10^6 bact. per ml (Fig. 4). The bacterial maximum occurred just at the moment of minor abundance of *Dinobryon*, so the curves of the abundances had an inverse pattern, which suggested a top-down controlling effect.

Ingestion rates per *Dinobryon* cell were the highest in period from June to August, with the maximum value in June (almost 9 bact. per cell$\times h^{-1}$, Fig. 5). There was also a considerable proportion of individuals in colonies at this time. We assume that population of *Dinobryon* was in an increasing growth phase and this could influence the ingestion rate positively, because *Dinobryon* perhaps compensated for a consumption of N and P by ingesting bacteria (Caron & al. 1993). This phenomenon could relate to the total organic nitrogen (TON) and total phosphorus (TP) concentration ratio, which was maximum in July and August (Kopáček, unpubl.). Abundance of the prey had no noticeable impact on ingestion rates.

Using bacterial production data, we calculated the impact of the total *D. pediforme* population on bacteria (Table 2). *Dinobryon* was able to graze even more than 260 % of bacterial standing stock hourly. In this case the values from both depths fairly differed. The intensity decreased with depth and season.

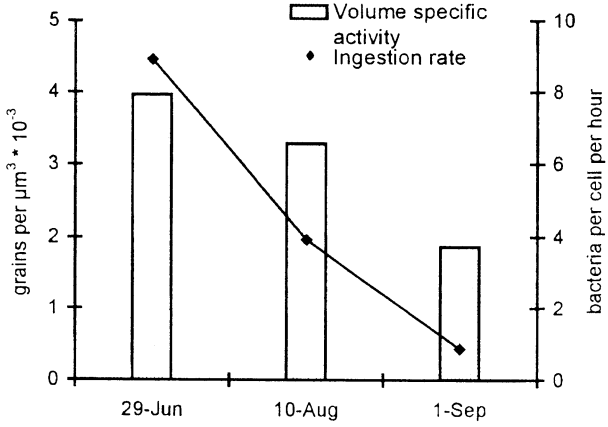

Fig. 6. – Comparison of ingestion rate and volume specific activity of *Dinobryon pediforme* in the surface layer of Čertovo Lake. Note parallel decrease of both parameters during the summer season. The data from June 9 and June 29 are omitted, as parallel measurements of volume specific activity and ingestion rate were not done.

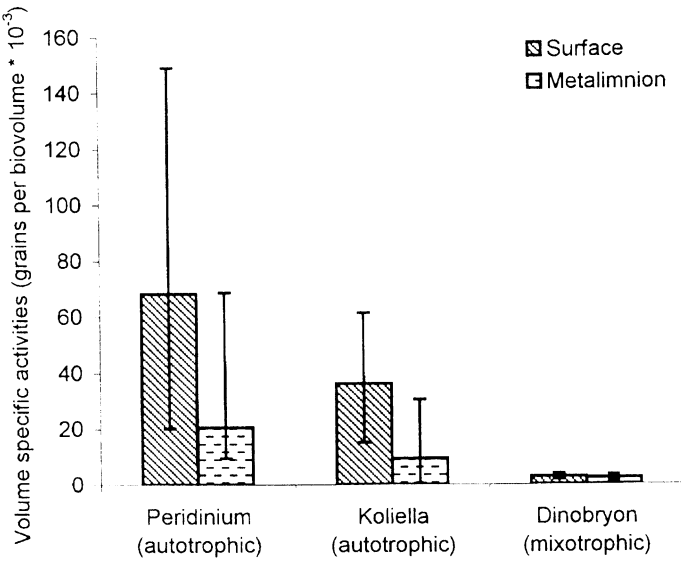

Fig. 7. – Comparison of individual volume specific primary production of autotrophic and mixotrophic species from Čertovo and Plešné Lakes (mean values with ranges). Notice that both autotrophic species had higher volume specific activities than the mixotrophic *Dinobryon* species.

The most distinctive ingestion rate values were recorded in July, when the lowest bacterial growth occurred. Regression analysis of grazing rate and some chemical parameters yielded few but important significant correlations. The negative relationship between DP concentration and grazing rate corresponds to the theory that the rate of phagotrophy increases together with phosphorus limitation. The lower was the DP concentration the higher was the total grazing rate. As bacteria have higher N:C and P:C ratios than Eucaryota, it is favourable for phagotrophs to gain these elements by their consumption (Boraas & al. 1988, Olrik 1998, Caron & al. 1993). It might suggest bacteria to be an important source of phosphorus for the mixotrophic *Dinobryon* in acidified Čertovo Lake severely limited by phosphorus. It is also possible that *Dinobryon* gains some growth factors from bacteria as the volume specific photosynthetic and ingestion rates had similar pattern (Caron & al. 1993; Fig. 6).

Conclusions

Generally, higher primary production (both per cell and volume specific activities in Čertovo and Plešné Lakes) but lower abundance of phytoplankton were observed in the surface layer than in the metalimnion. The mixotrophic flagellate *Dinobryon pediforme* has lower volume specific autotrophic activity than other autotrophic species likely as a result of its feeding on bacteria (Fig. 7). *Dinobryon* population may control abundance of bacterial population significantly. The inverse significant correlation between consumption of bacteria by *Dinobryon* and dissolved phosphorus concentration might suggest that bacteria might represent an alternative phosphorus source for the P-limited phytoplankton.

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