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Primary and bacterial production in three acidified lakes in the Bohemian Forest

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Abstract
Primary production (^{14}C method) and bacterial production (^{3}H-thymidine method) were measured in three acidified mountain lakes in the Bohemian Forest during the 1998 summer season. Plešné Lake (seasonal average of concentration chlorophyll *a*, Chl*a* = 14 μg.l^{-1}) was most productive; seasonal average of total primary production was 3.0 μgC.l^{-1}.h^{-1} or, on the water column basis, 290 mgC.m^{-2}.day^{-1}. In Prášilské Lake (Chl*a* = 5.0 μg.l^{-1}), total primary production was in average 1.8 μgC.l^{-1}.h^{-1} or 210 mgC.m^{-2}.day^{-1}. Čertovo Lake (Chl*a* = 2.9 μg.l^{-1}) had average total primary production 1.4 μgC.l^{-1}.h^{-1} or 150 mgC.m^{-2}.day^{-1}. Seasonal average of bacterial production expressed in terms of thymidine incorporation was highest in Plešné Lake, 14.0 pmol.l^{-1}.h^{-1}, while in lakes Prášilské and Čertovo it was lower: 3.1 pmol.l^{-1}.h^{-1} and 6.3 pmol.l^{-1}.h^{-1}, respectively. In the depth profiles, maxima of both primary and bacterial production were always found in the epilimnion: at 0.5 m or 2 m. Exudation (extracellularly released photosynthetic carbon; dissolved + in particles < 2 μm) accounted in seasonal average for 11%, 17%, and 18% of total primary production in Plešné, Prášilské, and Čertovo Lake, respectively. In seasonal average, 100%, 55%, and 19% of the carbon demand of non-filamentous bacteria could be covered from algal exudation in Prášilské, Plešné, and Čertovo Lake, respectively.

Key words: algae, photosynthesis, assimilation number, exudation

Introduction

Forest mountain lakes in the Bohemian Forest represent unique lake ecosystems which are in certain aspects different from any other lakes (Straškrabová et al. 2000). The lakes are of glacial origin, located at the altitude of about 1000 m a.s.l. During the second half of the 20th century, the lakes were subjected to massive anthropogenic acidification, which peaked in the 1980s (Kopáček et al. 1998, 2001, 2002). Zooplankton have been extinct or drastically reduced, fish are absent completely (Vrba et al. 2000, 2003a). Under these conditions, the main carbon fluxes occur at the level of microbial food webs which consist of phytoplankton, both autotrophic and mixotrophic (phagotrophic), and bacterioplankton, which is dominated by heterotrophic filaments (Vrba et al. 1996, 2000, 2003a,b).

The principal limiting nutrient is phosphorus in all the three lakes throughout the year, as indicated by low concentrations of dissolved inorganic phosphorus which are near the detection limit or undetectable, by seston stoichiometry (high C:P and N:P molar ratia), and by extremely high extracellular phosphatase activity (Vrba et al. 1996, 2000, Bittl et al. 2001). Input of aluminium from tributaries has been recognised as an important factor controlling

(impairing) phosphorus availability for microorganisms due to formation of aluminium-phosphorus co-precipitates which vanish from water column by sedimentation (Kopáček et al. 2000). Besides phosphorus, another nutrient that may potentially be in shortage and thus limit primary production is dissolved inorganic carbon (DIC). Its low concentration in lake epilimnia results from low pH (4–5). The most important phytoplankton genera include *Gymnodinium*, *Peridinium*, *Monoraphidium*, *Dinobryon*, *Limnothrix*, *Pseudanabaena* (Nedbalová & Vrtiška 2000).

Despite a long tradition of limnological research of the Bohemian Forest lakes (Vrba et al. 2000, 2003a), only few data on primary and bacterial production are available (Vrba et al. 1996).

The aim of this study was to bring a comprehensive information about seasonal and depth patterns of primary and bacterial productions in the three selected Bohemian Forest mountain lakes differing in water chemistry and plankton composition: Čertovo Lake, Plešné Lake, and Prášilské Lake, as a basis for comparison with other freshwater systems as well as with future measurements to be made on the same lakes during the ongoing recovery of the lakes from acidification.

Material and Methods

Sampling

Three acidified glacial lakes, Čertovo, Plešné, and Prášilské Lake, located in Bohemian Forest, Czech Republic (see Vrba et al. 1996 for detailed description), were sampled during the ice-free season in 1998. Samples were taken with a Friedinger sampler at the site of maximum depth from different depths representing surface (0.5 m), epilimnion (2 m), metalimnion (variable depth determined on the basis of water-column profiling), and hypolimnion (8–10 m).

Primary production

Generally, the methods used in this study were identical or compatible with the methods used during the MOuntain LAkes Research program "MOLAR" (Straškrabová et al. 1999).

Primary production was measured with the ^{14}C-method. Water samples were incubated *in-situ* (two light and two dark bottles at each depth) for 4 hours. The incubations were started between 9:00–10:00 Central Europe Summer Time. Each bottle (volume of about 120 ml) received 0.1–0.2 MBq of carrier-free ^{14}C-bicarbonate (final total concentration <10 $\mu gC.l^{-1}$). The assimilated ^{14}C was fractionated using a combination of filtration and acidification method (for details see Straškrabova et al. 1999) into the following fractions: (A) >2 µm, algae + bacterial filaments, (B) 0.2–2 µm, non-filamentous bacteria, (C) <0.2 µm, dissolved organic carbon. The gross primary production was calculated as A+B+C, the net primary production was assumed to be equal to A (neglecting the ^{14}C in heterotrophic filaments recovered in the same fraction). To obtain carbon fluxes ($\mu gC.l^{-1}.h^{-1}$), the rates of ^{14}C incorporation into each fraction (in $\%.h^{-1}$ of the added inorganic ^{14}C) were multiplied by the DIC concentration measured with a carbon analyser (LiquiTOC, Foss/Heraeus). To roughly estimate integral values of primary production ($mgC.m^{-2}.day^{-1}$), the per-hour values were multiplied by 12 (hours) and integrated over depth using the trapezoidal method.

Bacterial biomass and production

Samples for bacterial biomass determination were preserved in 2% formaldehyde. Single non-filamentous bacterial cells were enumerated with epifluorescence microscopy (Porter & Feig

1980) and sized with image analysis (Lucia, Laboratory Imaging; Psenner 1993). The carbon content of non-filamentous individual cells was calculated according to Norland (1993), the bacterial carbon was calculated by multiplying the bacterial abundance and average cell-carbon content. Filamentous bacteria (>5 µm) were quantified by the line-intercept method (Nedoma et al. 2001), their carbon biomass was calcualted as described in Vrba et al. (2003b). They were abundant in the lakes sampled, but they could not be separated from phytoplankton by size-fractionation and therefore their importance in carbon fluxes could not be determined in this study. Bacterial production was estimated with the ^{3}H-thymidine (^{3}H-dTr) method as described in Straškrabová et al. (1999). Briefly, subsamples (10 ml) of water sample were incubated with ^{3}H-dTr (20 nmol.l^{-1}; final concentration) at *in-situ* temperature for 60 min. The ^{3}H-dTr incorporation rate was saturated at concentrations >10 nmol.l^{-1}. Incubations were terminated by the addition of 2% formaldehyde (final concentration, the same was used for blanks), samples were then filtered through polycarbonate filters of 0.2-µm porosity, and extracted on filters 10× with 1 ml of ice-cold 5% TCA. The production rate of non-filamentous bacteria (cells.l^{-1}.day^{-1}) was calculated assuming that 50% of the total thymidine incorporation was by non-filamentous cells (Vrba et al. 2003b) and using the theoretical conversion factor of 2×10^{18} cells per mol of thymidine (Bell 1990). The carbon production of non-filamentous bacteria (µgC.l^{-1}.day^{-1}) was calculated multiplying the cell production rate by the average cell-carbon content. The incorporation of exuded photosynthetic carbon by non-filamentous bacteria (µgC.l^{-1}.day^{-1}) was estimated as the incorporation of photosynthetic carbon (µgC.l^{-1}.h^{-1}) into the B fraction (0.2–2 µm) converted to per-day values assuming a 12-hour daily production period. The share of the carbon requirements of non-filamentous bacteria potentially covered by algal exudation was calculated as a ratio of the incorporation of photosynthetic carbon to the carbon production by non-filamentous bacteria. No correction for respiration was made because both variables refer directly to changes in cellular carbon.

Other parameters

Concentration of chlorophyll *a* (Chl*a*) was measured according to Lorenzen (1967), the values were not corrected for phaeopigments. The depth profiles of the PAR (photosynthetically active radiation) intensity (I) were measured about noon with the Li-Cor model LI-250 Light Meter with the PAR spherical sensor. The depth of the euphotic layer (Z_{eu}, the depth of 1% of the surface irradiation) and the light levels at different depths, expressed as per cent surface irradiance, were calculated from the light extinction coefficients (k) derived from linear regression as the slopes of plots of log (I) versus depth (Fig. 1).

Results

Light environment

The lakes studied differed both in light transparency and in the seasonal variability of light conditions. Except for one case (Plešné Lake: 3 September 1998), plots of log (I) versus depth were linear, without breaks, indicating a roughly homogeneous light environment over the whole measured depth in all the lakes (Fig. 1). The highest depth and seasonal stability of the euphotic layer (Z_{eu}; the depth of 1% of the surface irradiation) was found in Čertovo Lake (9.5–10.0 m). In the two other lakes, Z_{eu} was lower and varied from 4.9 to 7.6 m in Plešné Lake and from 6.3 to 7.6 m in Prášilské Lake. There was a significant positive linear relationship between average Chl*a* concentration in the euphotic layer and the extinction coefficient, k (pooled data from all sampling dates; n = 14, $p < 0.0001$, $r^2 = 0.72$).

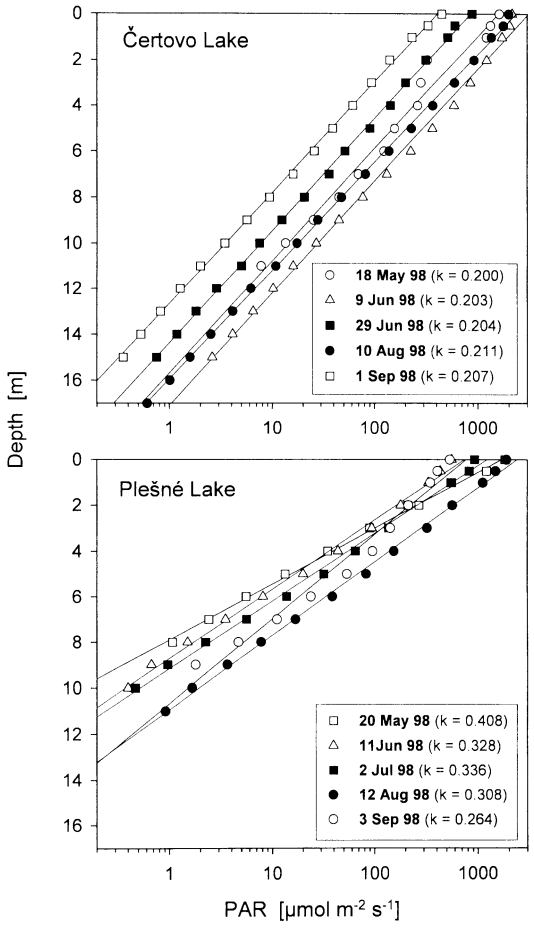

Fig. 1. Depth profiles of photosynthetically active radiation (PAR) in the Čertovo and Plešné Lakes at different sampling dates in 1998; k – light extinction coefficient (m^{-1}).

Primary production

Primary production in the lakes studied was in relation to their trophic status. The detailed data are available in Table 1 (Čertovo Lake), Table 2 (Plešné Lake), and Table 3 (Prášilské Lake). Lake Čertovo, the most oligotrophic of the lakes studied (seasonal average of Chl*a* concentration = 2.9 μg.l^{-1}; range 2.0–4.1 μg.l^{-1}), was the least productive: seasonal average of total primary production was 1.4 μgC.l^{-1}.h^{-1} (range 0.1–3.0 μgC.l^{-1}.h^{-1}), or, on the water column basis, 150 mgC.m^{-2}.day^{-1} (range 39–200 mgC.m^{-2}.day^{-1}). In Prášilské Lake (seasonal average of Chl*a* concentration = 5.0 μg.l^{-1}; range 1.9–9.7 μg.l^{-1}), average total primary production was 1.8 μgC.l^{-1}.h^{-1} (range 0.1–5.2 μgC.l^{-1}.h^{-1}) or 210 mgC.m^{-2}.day^{-1} (range 55–330 mgC.m^{-2}.day^{-1}). Plešné Lake, the most eutrophic (seasonal average of Chl*a* concentration = 14 μg.l^{-1}; range 4.8–33 μg.l^{-1}), was also the most productive: seasonal average of total primary production was 3.0 μgC.l^{-1}.h^{-1} (range 0.12–7.8 μgC.l^{-1}.h^{-1}) or 290 mgC.m^{-2}.day^{-1} (range 58–510 mgC.m^{-2}.day^{-1}). The differences among lakes were statistically significant on the per litre and hour basis (μgC.l^{-1}.h^{-1}), while the integrated values (mgC.m^{-2}.day^{-1}) did not differ significantly (ANOVA; $p = 0.022$ and $p = 0.30$, respectively) due to high seasonal variability.

In vertical profiles, the maximum of primary production was always found in the epilimnion: at depths of 0.5 or 2 m. Metalimnetic maxima of primary production were not recorded and the total primary production rates in metalimnion were in average 3–4 times lower compared to the corresponding epilimnetic values, despite of higher Chl*a* concentrations in metalimnion, compared to epilimnion (in Prášilské Lake in average 1.4 times, in Plešné Lake 2.6 times).

The chlorophyll-specific values of primary production were remarkably similar in all the three lakes (Fig. 2). In the epilimnion, at depths of 0.5 or 2 m (light penetration ≥15% of surface irradiance), the specific primary production was around 0.8 μgC.μgChl*a* $^{-1}$.h^{-1} in seasonal average, while it was significantly reduced in lower layers (0.13–0.30 μgC.μgChl*a* $^{-1}$.h^{-1} in

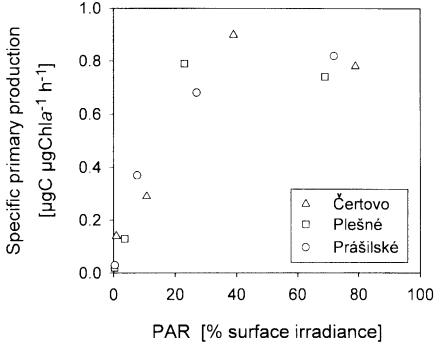

Fig. 2. The relationships between seasonal averages of chlorophyll-specific primary production and seasonal averages of percentage of surface irradiance reaching different sampling depths of three Bohemian Forest lakes in 1998.

Table 1. Primary production (PP) and selected limnological parameters in Certovo Lake in 1998.

Date	Depth m	Light penetration % [a]	T °C	Chl*a* µg.l^{-1}	DIC mg.l^{-1}	^{14}C-incorporation rate %.h^{-1}	Size-fractionated primary production				Specific PP µgC.µgChla^{-1}.h^{-1}	Integral PP mgC.m^{-2}.d^{-1}
							total µgC.l^{-1}.h^{-1}	>2 µm %	0.2–2 µm %	<0.2 µm %		
18 May	0.5	79	12.7	3.3	0.075	1.23	0.97	96	2	2	0.29	44
	2	40	12.7	3.1	0.13	0.46	0.62	82	6	12	0.20	
	4	16	8.8	2.2	0.36	0.053	0.20	79	0	21	0.09	
	10	1.0	4.3	3.3	0.63	0.012	0.08	50	13	37	0.02	
29 Jun	0.5	79	16.7	2.5	0.24	1.09	2.71	87	4	9	1.09	204
	2	39	16.7	2.3	0.25	1.13	2.90	86	5	9	1.25	
	4	15	13.4	2.0	0.16	0.43	0.70	84	5	11	0.35	
	10	0.9	4.8	2.7	1.15	0.091	1.10	86	3	11	0.40	
10 Aug	0.5	78	19.6	2.1	0.34	0.80	2.82	61	5	34	1.33	166
	2	38	19.5	2.1	0.33	0.87	3.01	67	6	27	1.40	
	5.5	6.9	12.0	2.2	0.39	0.22	0.89	77	4	19	0.40	
	10	0.8	5.1	3.4	1.27	0.015	0.20	73	0	27	0.06	
1 Sep	0.5	79	13.6	3.8	0.15	0.92	1.48	84	4	12	0.39	164
	2	39	13.6	4.0	0.18	1.60	2.98	91	3	6	0.76	
	6.5	4.5	10.6	4.1	0.63	0.20	1.34	85	3	12	0.32	
	10	0.9	5.1	3.9	1.26	0.017	0.22	83	4	13	0.06	

[a] per cent of the surface PAR irradiance

Table 2. Primary production (PP) and selected limnological parameters in Plešné Lake in 1998.

Date	Depth m	Light penetration % [a]	T °C	Chl*a* µg.l^{-1}	DIC mg.l^{-1}	^{14}C-incorporation rate %.h^{-1}	Size-fractionated primary production				Specific PP µgC.µgChla^{-1}.h^{-1}	Integral PP mgC.m^{-2}.d^{-1}
							total µgC.l^{-1}.h^{-1}	>2 µm %	0.2–2 µm %	<0.2 µm %		
20 May	0.5	63	11.8	11.1	0.054	3.21	1.83	90	6	4	0.17	65
	2	15	11.7	10.5	0.065	2.10	1.42	89	6	5	0.14	
	3	6.0	10.0	14.7	0.007	1.59	0.12	93	4	3	0.01	
	8	0.1	4.7	19.3	0.48	0.040	0.20	100	0	0	0.01	
11 Jun	0.5	69	17.7	5.3	0.09	2.84	2.68	93	5	2	0.50	338
	2	22	17.6	6.0	0.21	2.98	6.67	94	4	2	1.11	
	4	4.9	8.8	16.6	0.09	3.25	2.94	91	3	6	0.18	
	8	0.2	4.8	23.7	0.38	0.077	0.31	83	10	7	0.01	
2 Jul	0.5	68	17.4	6.3	0.14	3.34	4.83	89	7	4	0.77	537
	2	21	17.4	6.3	0.21	3.57	7.81	93	4	3	1.24	
	5.3	1.7	8.0	27.1	0.13	3.95	5.26	96	2	2	0.19	
	8	0.2	4.9	29.8	0.35	0.20	0.74	86	6	8	0.02	
12 Aug	0.5	70	19.9	4.8	0.28	1.86	5.51	68	16	16	1.15	331
	2	24	19.9	5.9	0.28	2.07	6.10	83	8	9	1.03	
	5.5	2.0	8.8	17.2	0.12	1.70	2.22	95	3	2	0.13	
	8	0.3	5.3	33.0	0.95	0.11	1.09	92	4	4	0.03	
3 Sep	0.5	74	14.2	5.9	0.34	1.87	6.60	75	12	13	1.12	195
	2	30	13.5	5.3	0.18	1.22	2.26	88	6	6	0.43	
	5.5	3.5	10.8	10.0	0.21	0.74	1.64	95	3	2	0.16	
	8	0.8	5.8	25.5	0.88	0.027	0.25	81	19	0	0.01	

[a] per cent of the surface PAR irradiance

Table 3. Primary production (PP) and selected limnological parameters at Prášilské Lake in 1998.

Date	Depth m	Light penetration %[a]	T °C	Chl*a* µg.l^{-1}	DIC mg.l^{-1}	^{14}C-incorporation rate %.h^{-1}	Size-fractionated primary production				Specific PP µgC.µgChla^{-1}.h^{-1}	Integral PP mgC.m^{-2}.d^{-1}
							total µgC.l^{-1}.h^{-1}	>2 µm %	0.2–2 µm %	<0.2 µm %		
25 May	0.5	70	11.0	4.5	0.022	1.27	0.29	78	9	13	0.07	163
	3	12	9.2	5.8	0.61	0.41	2.63	69	10	21	0.45	
	10	0.1	4.2	4.4	1.09	0.011	0.13	60	0	40	0.03	
15 Jun	0.5	70	13.6	4.6	0.39	1.66	6.83	76	7	17	1.50	393
	2	23	13.6	5.2	0.41	1.37	5.87	79	9	12	1.14	
	3.5	7.8	8.6	6.2	0.61	0.55	3.50	81	5	14	0.56	
	8	0.3	4.5	4.5	1.16	0.0052	0.06	83	5	12	0.01	
7 Jul	0.5	70	14.0	5.7	0.23	1.71	4.20	95	4	1	0.73	269
	2	25	14.0	6.5	0.25	1.16	2.99	86	6	8	0.46	
	3.5	8.6	12.3	9.7	0.27	1.24	3.52	74	4	22	0.36	
	8	0.4	4.6	2.9	1.13	0.010	0.12	45	55	0	0.04	
17 Aug	0.5	74	19.7	2.6	0.33	1.08	3.76	67	11	22	1.43	226
	2	30	19.6	4.1	0.32	1.21	4.06	78	8	14	0.99	
	4.5	6.6	11.2	5.5	0.73	0.33	2.53	78	7	15	0.46	
	8	0.8	5.0	2.6	0.92	0.0073	0.07	92	0	8	0.03	
14 Sep	0.5	74	12.1	6.3	0.51	0.44	2.36	86	3	11	0.38	52
	2	30	12.1	4.7	0.51	0.12	0.65	91	5	4	0.14	
	5.5	3.5	12.1	7.5	1.39	0.0086	0.13	100	0	0	0.02	
	8	0.8	5.3	1.9	1.42	0.0043	0.06	100	0	0	0.03	

[a] per cent of the surface PAR irradiance

average in metalimnion). The individual values from all depths and dates ranged from 0.02 to 1.40 μgC.μgChl*a* $^{-1}$.h^{-1} in Čertovo Lake, from 0.01 to 1.50 μgC.μgChl*a*$^{-1}$.h^{-1} in Prášilské Lake, and from 0.01 to 1.24 μgC.μgChl*a*$^{-1}$.h^{-1} in Plešné Lake.

Bacterial production

The data on bacterial production are summarised in Tables 4–6. Bacterial production expressed in terms of thymidine incorporation was quite high in Plešné Lake: seasonal average was 14.0 pmol.l $^{-1}$.h $^{-1}$ (range 4.8–23.7 pmol.l $^{-1}$.h $^{-1}$), while in the other lakes it was lower: in Čertovo Lake in average 6.3 pmol.l $^{-1}$.h $^{-1}$ (range 0.5–15.2 pmol.l $^{-1}$.h $^{-1}$) and in Prášilské Lake 3.1 pmol.l $^{-1}$.h $^{-1}$ (0.9–6.2 pmol.l $^{-1}$.h $^{-1}$). Resembling the depth profiles of primary production, bacterial production always peaked in the epilimnion and declined towards the bottom. We found a significant positive linear relationship between primary and bacterial productions measured in individual water samples (pooled data from all lakes; n = 36, $p < 0.0001$; $r^2 = 0.43$; Fig. 3).

Importance of algal exudates as carbon source for non-filamentous bacteria

A significant portion of assimilated ^{14}C was released in the form of extracellular organic carbon production (exudation). The respective data can be found in Tables 1–3. The percentages of total primary production released as exudation (dissolved + in particles <2 μm) accounted in average (range in parentheses) for 11% (5–32%) in Plešné Lake, for 17% (0–33%) in Prášilské Lake, and for 18% (5–38%) in Čertovo Lake. The values from the lowest sampling depths located at the edge of the euphotic layer (<1% of surface irradiance) were excluded from the analysis as the photosynthetic carbon fluxes were low and scattered which made the percentage values unreliable. The amount of the carbon released as algal exudation would support doubling of bacterial carbon in the lakes Plešné, Prášilské, and Čertovo in 23, 13, and 45 days, respectively (assuming 50% growth efficiency). The average percentages of carbon demand of non-filamentous bacteria potentially covered by phytoplankton exudation were

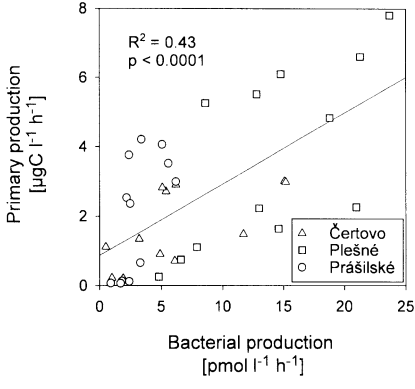

Fig. 3. The relationship between individual values of primary and bacterial production measured at different sampling depths of three Bohemian Forest lakes in 1998.

Table 4. Bacterial production and related parameters at Certovo Lake in 1998 (n.a. = not available).

Date	Depth m	Bacterial abundance[a] 10^6.l^{-1}	Bacterial carbon[a] µgC.l^{-1}	Filament carbon µgC.l^{-1}	Thymidine incorporation pmol.l^{-1}.h^{-1}	Bacterial production[a] 10^6.l^{-1}.day^{-1}	Bacterial production[a] µgC.l^{-1}.day^{-1}	Prim. prod 0.2–2 µm µgC.l^{-1}.day^{-1}	Ratio of exudate incorporation to carbon production[a]
18 May	0.5	0.30	15	119	n.a.	n.a.	n.a.	0.27	n.a.
	2	0.57	25	97	n.a.	n.a.	n.a.	0.48	n.a.
	4	0.44	16	67	n.a.	n.a.	n.a.	0	n.a.
	10	0.30	9	52	n.a.	n.a.	n.a.	0.12	n.a.
29 Jun	0.5	0.97	25	128	5.4	0.13	3.33	1.32	0.39
	2	1.30	58	96	6.2	0.15	6.67	1.78	0.27
	4	0.73	26	131	6.1	0.15	5.20	0.38	0.07
	10	0.28	8	52	0.5	0.01	0.31	0.42	1.28
10 Aug	0.5	1.41	40	123	5.1	0.12	3.51	1.58	0.45
	2	1.50	50	167	15.1	0.36	11.9	2.33	0.19
	5.5	0.85	23	128	4.9	0.12	3.15	0.39	0.12
	10	0.30	8	76	1.9	0.05	1.19	0	0.0
1 Sep	0.5	0.65	29	168	11.7	0.28	12.6	0.68	0.05
	2	0.63	34	41	15.2	0.36	20.0	1.21	0.06
	6.5	0.86	47	47	3.2	0.08	4.23	0.52	0.12
	10	0.35	12	48	1.0	0.02	0.85	0.11	0.12

[a] non-filamentous cells

Table 5. Bacterial production and related parameters at Plešné Lake in 1998 (n.a. = not available).

Date	Depth m	Bacterial abundance[a] 10^6.l^{-1}	Bacterial carbon[a] µgC.l^{-1}	Filament carbon µgC.l^{-1}	Thymidine incorporation pmol.l^{-1}.h^{-1}	Bacterial production[a] 10^6.l^{-1}.day^{-1}	Bacterial production[a] µgC.l^{-1}.day^{-1}	Prim. prod 0.2–2 µm µgC.l^{-1}.day^{-1}	Ratio of exudate incorporation to carbon production[a]
20 May	0.5	2.45	64	47	n.a.	n.a.	n.a.	1.28	n.a.
	2	2.58	61	153	n.a.	n.a.	n.a.	1.04	n.a.
	3	1.46	34	157	n.a.	n.a.	n.a.	0.06	n.a.
	8	0.586	16	102	n.a.	n.a.	n.a.	0.00	n.a.
11 Jun	0.5	1.85	41	48	n.a.	n.a.	n.a.	1.56	n.a.
	2	1.67	40	67	n.a.	n.a.	n.a.	3.22	n.a.
	4	0.97	33	50	n.a.	n.a.	n.a.	1.20	n.a.
	8	0.71	23	59	n.a.	n.a.	n.a.	0.36	n.a.
2 Jul	0.5	1.82	32	87	18.8	0.45	8.00	4.09	0.51
	2	2.24	44	98	23.7	0.57	11.1	3.92	0.35
	5.3	0.83	23	89	8.6	0.21	5.81	1.49	0.26
	8	0.56	16	92	6.6	0.16	4.69	0.52	0.11
12 Aug	0.5	1.50	38	47	12.8	0.31	7.74	10.60	1.37
	2	1.58	38	50	14.8	0.36	8.46	5.59	0.66
	5.5	1.10	35	33	13.0	0.31	10.0	0.74	0.07
	8	0.76	24	13	7.9	0.19	6.02	0.54	0.09
3 Sep	0.5	1.32	35	88	21.3	0.51	13.5	19.14	1.42
	2	1.32	43	76	21.0	0.50	16.4	3.47	0.21
	5.5	1.34	45	67	14.6	0.35	11.7	1.24	0.11
	8	0.57	22	22	4.8	0.11	4.50	1.15	0.26

[a] non-filamentous cells

Table 6. Bacterial production and related parameters at Prášilské Lake in 1998 (n.a. = not available).

Date	Depth m	Bacterial abundance[a] 10^6.l^{-1}	Bacterial carbon[a] µgC.l^{-1}	Filament carbon µgC.l^{-1}	Thymidine incorporation pmol.l^{-1}.h^{-1}	Bacterial production[a] 10^6.l^{-1}.day^{-1}	Bacterial production[a] µgC.l^{-1}.day^{-1}	Prim. prod 0.2–2 µm µgC.l^{-1}.day^{-1}	Ratio of exudate incorporation to carbon production[a]
25 May	0.5	0.62	19	95	n.a.	n.a.	n.a.	0.32	n.a.
	3	1.15	36	108	n.a.	n.a.	n.a.	3.10	n.a.
	10	1.01	42	61	n.a.	n.a.	n.a.	0	n.a.
15 Jun	0.5	0.52	24	109	n.a.	n.a.	n.a.	5.79	n.a.
	2	0.96	56	75	n.a.	n.a.	n.a.	6.05	n.a.
	3.5	0.66	25	62	n.a.	n.a.	n.a.	2.14	n.a.
	8	0.66	33	28	n.a.	n.a.	n.a.	0.04	n.a.
7 Jul	0.5	0.34	12	91	3.4	0.08	2.84	2.21	0.78
	2	0.45	15	107	6.2	0.15	4.98	2.01	0.40
	3.5	0.58	19	83	5.6	0.13	4.40	1.66	0.38
	8	0.53	20	63	2.4	0.06	2.18	0.78	0.36
17 Aug	0.5	0.52	13	12	2.4	0.06	1.38	4.83	3.49
	2	0.58	15	17	5.1	0.12	3.30	3.76	1.14
	4.5	0.19	5	17	2.2	0.05	1.37	2.16	1.58
	8	0.30	8	11	0.9	0.02	0.60	0	0.0
14 Sep	0.5	0.41	13	22	2.5	0.06	1.91	1.63	0.85
	2	1.08	28	8	3.3	0.08	2.01	0.74	0.37
	5.5	0.31	7	14	1.8	0.04	0.99	0	0.0
	8	0.41	12	3	1.7	0.04	1.11	0	0.0

[a] non-filamentous cells

Table 7. Summary of seasonal averages (range in parentheses) of selected parameters in three Bohemian Forest lakes in 1998.

	Čertovo Lake	Plešné Lake	Prášilské Lake
Chlorophyll *a* [μg.l^{-1}]	2.9 (2.0–4.1)	14 (4.8–33)	5.0 (1.9–9.7)
Euphotic depth [m]	9.8 (9.5–10.0)	6.2 (4.9–7.6)	6.9 (6.3–7.6)
Primary production [μgC.l^{-1}.h^{-1}]	1.4 (0.1–3.0)	3.0 (0.1–7.8)	1.8 (0.1–5.2)
Areal primary production [μgC.m^{-2}.day^{-1}]	150 (39–200)	290 (58–510)	210 (55–330)
Exudation [% of primary production]	18 (5–38)	11 (5–32)	17 (0–33)
Exudation [μgC.l^{-1}.h^{-1}]	0.32 (0.04–1.08)	0.50 (0.01–1.77)	0.59 (0–1.64)
Bacterial abundance [10^{6} cells.l^{-1}]*	0.72 (0.28–1.5)	0.56 (0.56–2.58)	0.59 (0.19–1.15)
Bacterial carbon [μgC.l^{-1}]*	27 (8–58)	35 (16–64)	21 (5–56)
Thymidine incorporation [pmol.l^{-1}.h^{-1}]	6.3 (0.5–15.2)	14.0 (4.8–23.7)	3.1 (0.9–6.2)
Bacterial production [μgC.l^{-1}.h^{-1}]*	0.25 (0.01–0.83)	0.37 (0.19–0.68)	0.09 (0.03–0.21)

*non-filamentous bacteria

100%, 55%, and 19% in Prášilské, Plešné, and Čertovo Lake, respectively. Due to high variability of individual data (see Tables 4–6), the differences between lakes were not statistically significant (ANOVA: $p = 0.059$), although averages differed up to fivefold and the p value was suggestive.

Discussion

Light environment

Despite the fact that water in the lakes studied was characteristically coloured due to the presence of humic substances (Vrba et al. 2000), phytoplankton biomass was the main factor controlling light penetration, as variation in Chl*a* concentration in the euphotic layer explained 72% of the variability in water column transparency. The depth of the euphotic layer therefore depends much more on phospohorus load and primary production than on the input and fate of dissolved organic carbon in Bohemian Forest lakes.

Primary production

The accuracy of the presented estimates of primary production was influenced mainly by a limited number of sampling dates and of sampling depths, and by an accuracy of our DIC measurements, which were at the edge of the method sensitivity. Primary productivity of acidified Bohemian Forest lakes was generally similar to other lakes of comparable trophy. The calculated mean areal daily productivities of the studied lakes (150–290 mgC.m^{-2}.day^{-1},

Table 7) matched the ranges 50–300 mgC.m^{-2}.day^{-1} and 250–1000 mgC.m^{-2}.day^{-1}, common for oligotrophic and mesotrophic lakes, respectively (Wetzel 2001). The values from Bohemian Forest lakes were also comparable to the values reported for Canadian Shield Lake 223, subjected to experimental acidification (220–429 mgC.m^{-2}.day^{-1}, Shearer et al. 1987), and for mountain Castle Lake (~350 mgC.m^{-2}.day^{-1}, Goldman et al. 1989). Somewhat lower values were reported from the mesohumic lake Pääjärvi (60–160 mgC.m^{-2}.day^{-1}, Tulonen et al. 2000). A considerably lower daily areal productivity (3 mgC.m^{-2}.day^{-1}) was reported by Vrba et al. (1996) for Prášilské Lake. Such a low value may have resulted from the fact that it has been measured in late October during the autumn overturn, and, furthermore, in 1991, at an early stage of the recovery from acidification, which had begun about 1990 (Kopáček et al. 1998).

No metalimnetic maxima of primary production were found during the study period. Thus, the pronounced metalimnetic peaks of Chl*a*, observed in Plešné Lake by Hejzlar et al. (1998), and confirmed in this study (Tables 2, 3), did not result from phytoplankton growth in the metalimnion. The main factors responsible for the deep Chl*a* maxima formation were most probably accumulation of settling phytoplankton on the thermocline and an increase in the cellular content of chlorophyll *a* occurring as a consequence of low-light acclimation of the phytoplankton experiencing decreasing light intensity during sedimentation. An increase in the Chl*a*/biovolume ratio of phytoplankton in the deeper strata of all the lakes studied has consistently been observed by Nedbalová (2000). The same phenomenon has been reported by Felip & Catalan (2000) for the high mountain lake Redó.

The values of chlorophyll-specific primary production measured in the epilimnia of the lakes studied (under optimum light conditions; at depths of 0.5 and 2 m) represent estimates of phytoplankton photosynthetic capacity (assimilation number; mgC.mgChl*a*$^{-1}$.h^{-1}). The values for Bohemian Forest lakes (in average about 0.8 mgC.mgChl*a*$^{-1}$.h^{-1}; Tables 1–3, Fig. 3) are near the lower limit of the values observed commonly in lakes (Kalff 2002, Fee et al. 1987). Low values of photosynthetic capacity generally indicate that phytoplankton experience sub-optimum growth conditions (Harris & Piccinin 1977, Senft 1978). Phosphorus limitation of primary production is most probably responsible for the low assimilation numbers measured in all the lakes studied; the extremely low values observed in May may have been caused by low temperature. The permanent presence of relatively high phytoplankton biomass at low production/biomass ratio was made possible by the absence of zooplankton grazing in the Bohemian Forest lakes. Under these conditions, sedimentation was probably the only important loss process.

A remarkable phenomenon observed in the lakes studied was the very rapid turnover of dissolved inorganic carbon (turnover rate of the order of 0.1–1 %.h^{-1}, this means in fact a turnover time measured in days). Such a turnover rate is comparable to the turnover rate of phytoplankton biomass and thus the carbon availability could be potentially a limiting factor of primary production in the lakes. However, the turnover times of orthophosphate estimated in the epilimnia of the lakes Plešné and Čertovo using radiotracer method were much shorter (~3 min) (Nedoma – unpubl. data; Znachor et al., submitted) indicating that P is the limiting nutrient in these lakes. The P-deficiency of the lakes has been proved by other independent methods, namely seston stoichiometry (high C:P ratio, Vrba et al. 1996, Kopáček – unpubl. data) and phosphatase activity (extremely high values, Bittl et al. 2001).

Bacterial production and its relationship to algal exudation

Due to limitations imposed by the methods available, our estimates of bacterial production in the Bohemian Forest lakes are less reliable than our primary production data. Bacterioplankton of all the lakes studied comprises, besides commonly occurring small unicellular bacteria,

also a substantial portion of filamentous microorganisms (Vrba et al. 2003b). The conversion between thymidine incorporation and carbon production is based on a calculation of the number of bacterial cells produced, assuming a constant amount of thymidine incorporated per bacterial cell produced (conversion factor: Bell 1990) – a principle not applicable to the filaments. Consequently, the validity of our calculations of carbon fluxes connected to bacterioplankton depends on the validity of our rather simplifying assumptions.

The simplest and most reliable index of heterotrophic productivity of the studied lakes is thymidine incorporation (pmol.l^{-1}.h^{-1}), which was the highest in Plešné Lake, consistently with the highest primary production in this lake. In the two other lakes, however, the trends in bacterial and primary production were not consistent – in Prášilské lake the bacterial production was disproportionally low, probably as a consequence of reduced total bacterial biomass in late summer, caused by grazing of filamentous bacteria by *Daphnia* (Vrba et al. 2003b). Generally, the thymidine incorporation rates measured in the lakes studied matched the values common in comparable lakes (Petit et al. 1999, Tulonen 2000).

Maximum thymidine incorporation was always observed in the epilimnion and it decreased towards the hypolimnion roughly in concurrence with primary production. The phenomenon of similar trends of bacterial and primary production with regard to both season and depth has been generally observed in most lakes (Cole et al. 1988, Pace & Cole 1994). It is interpreted as the consequence of control of bacterial production by the availability of organic substrates originating from dying (cell lysis, sloppy feeding by zooplankton) or living (exudation) phytoplankton, or as the consequence of a parallel effect imposed by other factors (nutrient availability, temperature, grazing pressure). In the Bohemian Forest lakes, the coupling of bacterial and primary production was not very tight (statistically, the variations in primary production can explain about 50% of the variations in bacterial production). It is difficult to decide which part of the observed coupling was caused by the regulation of bacterial growth by substrate availability and which by the parallel effect of temperature or by the simultaneous effect of decreasing temperature and light intensity towards the deeper strata of the water column.

To elucidate the above question, we measured extracellular production of carbon by phytoplankton and its incorporation by bacteria. We estimated which part of bacterial carbon requirements was covered by freshly excreted algal exudates, by comparing the directly measured bacterial incorporation of exuded carbon to bacterial carbon demand estimated from the bacterial production. This approach, however, especially in the presence of filamentous bacteria, is subject to significant errors and biases – the presented data thus should be taken with caution.

Because of problems with filaments, we restricted our calculations of carbon fluxes to the size-fraction 0.2–2 μm containing nominally unicellular bacteria (but contaminated by a certain and variable portion of filaments, Nedoma et al. 2001). The results indicate the highest potential coupling of primary and bacterial production in the mesotrophic Plešné Lake, where bacterial production could be completely covered by phytoplankton exudates only. In accordance with this finding, Plešné Lake was characterised by the highest phytoplankton biomass and by the highest ratio of phytoplankton to bacterioplankton biomass. On the other hand, despite the highest percentage of exudation by phytoplankton observed, the lowest degree of coupling between bacterial and primary production was calculated for Čertovo Lake (only 20% of bacterial carbon demand could be covered by phytoplankton exudates). The lake was characterised by the highest degree of acidification, the lowest phytoplankton biomass, and the lowest ratio of phytoplankton to bacterioplankton biomass. The bacterial production in Čertovo Lake therefore probably relies mainly on allochtonous organic carbon.

The percentage of extracellular release of assimilated carbon by phytoplankton (exudation)

is known to increase with the decreasing trophy of a lake (review BAINES & PACE 1991). For some European mountain lakes, this trend has been confirmed by STRAŠKRABOVÁ et al. (2000) and it was also valid for the lakes included in our study: exudation rose with the decreasing trophy of the lakes. It should be noted that the percentage of exudation was underestimated in this study because of underestimation of exudate incorporation by filamentous bacteria. Generally, the percentage of exudation in the Bohemian Forest lakes matched the percentage observed in lakes of comparable productivity (5–50%; BAINES & PACE 1991, REČHE et al. 1997, PETIT 1999, STRAŠKRABOVÁ et al. 2000).

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