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Estimation of periphytic microalgae gross primary production with DCMU-fluorescence method in Yenisei River (Siberia, Russia)

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Abstract Periphyton (epilithon) gross primary production (GPP) was estimated using the DCMU-fluorescence method in the Yenisei River. In the unshaded littoral zone, chlorophyll *a* concentration (Chl *a*) and GPP value varied from 0.83 to 973.74 mg $m^{-2}$and 2–304,425 O_2 m^{-2} day^{-1} (0.64–95 133 mg C m^{-2} day^{-1}), respectively. Positive significant correlation (r=0.8) between daily GPP and periphyton Chl *a* was found. Average ratio GPP:Chl *a* for periphyton was 36.36 mg C mg Chl *a* m^{-2} day^{-1}. The obtained GPP values for the Yenisei River have a high significant correlation with values predicted by a conventional empirical model for stream periphyton. We concluded that the DCMU-fluorescence method can be successfully used for measuring of gross primary production of stream phytoperiphyton at least as another useful tool for such studies.

Keywords Chlorophyll fluorescence · Periphytic microalgae · Gross primary production

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Abbreviations

DCMU	3-(3,4-dichlorophenyl)-1, 1-dimethylurea
GPP	gross primary production

Introduction

Estimation of algal primary production (PP) is important for studying energy flow in aquatic ecosystems. Periphytic algae are believed to be the major primary producers in headwater and midregion areas of river systems, but there have been relatively few studies of algal PP in streams on an annual basis (Fuller and Bucher 1991; Uehlenger 2006). Primary productivity in flowing water systems is estimated from oxygen change or carbon dioxide change in open stream (Uehlenger 2006; Bott et al. 2006), or is measured in closed chambers placed in laboratory or *in situ* (Munn and Brusven 2004; Uehlinger and Brock 2005; Reid et al. 2006) by ^{14}C uptake (Ostrofsky et al. 1998), or by using O_2 microelectrode (Dodds et al. 1999). PP can also be calculated from chlorophyll *a* (algae biomass) estimations (Fellows et al. 2006). All the methods have some disadvantages. Methods, based on oxygen change in open stream, have difficulties in estimating the gas exchange coefficient (Bott et al. 1978). Chamber methods suffer from a poor simulation of flow patterns in the chambers (Fuller and Bucher 1991). Moreover, both the chamber and open stream methods are time consuming. Consequently, only indirect measurements, based on chlorophyll *a* concentrations, are widely and routinely used in many studies and monitoring programs. However, the destructive sampling of Chl *a*, such as extraction and subsequent quantification using high-performance liquid chromatography (HLPC) or spectrophotometry, has some disadvantages, and recently

alternative non-destructive techniques, based the measurements of fluorescence from chlorophyll, have been developed (e.g., Honeywell et al. 2002). Pulse amplitude-modulated (PAM) fluorescence methods are used for estimation of biomass and photosynthetic activity of microalgae in oligotrophic lakes and oceans (Sakshaug et al. 1997; Serodio 2004), but these methods have limitations under high algae biomass and Cyanophyta predominance (Ting and Owens 1992; Kromkamp et al. 1998). Recently, it was shown that the DCMU-fluorescence method could be used successfully for determination of chlorophyll *a* concentration and primary production of plankton microalgae in mesotrophic and eutrophic water bodies (Gaevsky et al. 2000, 2002, 2005).

The aim of present work was to study the possibility of using the DCMU-fluorescence method for estimation of periphytic microalgae gross primary production (GPP) in the Yenisei River, the largest river of Russia. Our tasks were: (1) to calculate GPP on the basis of periphytic microalgae chlorophyll *a* content and potential photosynthetic activity, measured with DCMU-fluorescence in Yenisei River; and (2) to compare GPP values calculated using DCMU-fluorescence method with worldwide literature data obtained by other techniques.

Materials and methods

Study site and sampling

This study was carried out in the Yenisei River. The main ecological features of the river are given elsewhere (Telang et al. 1991; Sushchik et al. 2007). Sampling was carried out at a site in the middle stream of the river, situated downstream of the dam of the Krasnoyarsk Hydroelectric Power Station (55°58′N, 92°43′E) and in the vicinity (upstream) of Krasnoyarsk City. The character of the river in this region is mountain-like; the bottom is pebbly and the surface of the river is ice-free during the whole winter because of the discharge of deep warm waters from the upstream reservoir. Water temperature ranged within 5–10°C and 0–5°C in spring–summer and autumn–winter, respectively. The study site was not shaded by riparian vegetation. Periphyton was sampled from 0.1–0.5 m depth monthly from March 2003 to September 2006. Periphytic microalgae were brushed in a definite volume from pebbles, collected by means of a metal frame (S=0.01 m^{-2}).

Microalgae identification and counting

For microalgae counting, 5 mL of the samples were fixed with Lugol's iodine solution. Microalgae were counted and identified under an inverted microscope. To calculate the biovolume (wet weight) of algae, they were equated to appropriate geometrical shapes (or their combinations), and relevant sizes were measured using an ocular micrometer. At least 20 individuals of each species were measured in each sample. The median cell volume for each species was used for conversion of cell numbers to volumes (Hillebrand et al. 1999). Wet weight biomass was then calculated assuming a specific density of 1 g sm^{-3}.

Chlorophyll *a* spectrophotometry

For chlorophyll extraction, water samples of 100–200 mL volume were concentrated under vacuum on 0.95–1.05 μm-pore-size filters (Vladipor, Russia) covered with a thin layer (1–2 mm) of powdered $MgCO_3$. Before extraction, filters were dried at room temperature. Chlorophyll was extracted with 90% ethanol according to Nusch (1980), as follows. Layers of $MgCO_3$ containing chlorophyll were removed from the filters, placed in ethanol and heated for ~1 min until boiling and then extracted for 24 h at room temperature. Then $MgCO_3$ was removed by centrifuging. Extinction of extracts was measured in a UVIKON 943 double beam UV/VIS spectrophotometer (Kontron, France).

Extraction spectrophotometry has shortcomings (Clesceri 1989), so to test the routine spectrophotometry data, a thin-layer chromatography (Jeffrey 1981; Wiltshire et al. 2000) was simultaneously used in 2006. Coefficient of Pearson correlation between chlorophyll *a* concentrations measured by these two methods was equal to 0.95 (n=5, p<0.05). Thus, the extraction spectrophotometry appeared to be the suitable method for the estimation of chlorophyll *a* content in the phytoperiphyton in the studied site.

Fluorescence analysis

Polychromatic diuron (DCMU)-induced fluorescence method was used for estimation of total chlorophyll *a* and potential photochemical activity of photosystem 2 (PS2) of periphytic microalgae. Fluorescence (λ=685 nm) was measured with a special induction fluorometer (Fl-303) fabricated at Krasnoyarsk State University. Halogen lamp (70W) was a source of excitation light. The fluorometer was equipped with a system of replaceable filters (410±20, 510±20 and 540±10 nm) for differentiation of three groups of dominant algal taxons: (1) Chlorophyta and Euglenophyta, (2) Bacillariophyta and Dinophyta, and (3) Cyanophyta. Calculation of Chl *a* was carried out using the visualization method described in (Gaevsky et al. 2005). Total Chl *a* concentration was calculated as the sum of Chl *a* of all three groups.

Gross primary production ($gO_2\ m^{-2}\ h^{-1}$) was calculated by the empirical equation (Gaevsky et al. 2000) for phytoplankton:

$$GPP = b\ F_V/F_m\ \text{Chl}\ a\ I \quad (1)$$

where b = empirical coefficient, 0.00042, F_v/F_m is the relative variable fluorescence (arb.units), Chl *a* = chlorophyll *a* concentration of periphytic algae (mg m^{-2}), and I = average intensity of photosynthetically active radiation (PAR, Wm^{-2}). Relative variable fluorescence was calculated by the formula:

$$F_V/F_m=(F_m/F_0)/F_m, \quad (2)$$

Where F_0 = steady-state level of fluorescence and F_m = maximum level after addition of 10 μM DCMU (3-(3,4-dichlorophenil)-1,1-dimethylurea). F_0 and F_m were registered under excitation by wide beam (400–620 nm) and intensity of 120 Wm^{-2}. Gross primary production per hour was multiplying by daylight hours for daily GPP calculation. Daylight hours for Krasnoyarsk City in sampling days were determined using algorithm provided by J. Lammi (http://www.geocities.com/jjlammi) and varied from 7 to more than 17 h.

Incident radiation data were kindly given by Krasnoyarsk State Hydrometeorological Service. Coefficient 0.46 was used for recalculation these data in photosynthetically active radiation values (Rao 1984). In situ attenuation of solar radiation (W m^{-2}) was measured with a portable underwater irradiance meter with a light sensor included a silicon diode, fabricated in Krasnoyarsk State University. The irradiance meter was intercalibrated with Licor radiation sensor LI-193SA Spectral Quantum sensor (USA). The average attenuation coefficient (*k*) was 0.4 m^{-1}, and about 80% of incident surface light intensity was at the mean death of periphyton sampling.

Results

Correlation between chlorophyll *a* concentrations estimated using fluorescence analysis and spectrophotometry was determined in periphyton samples in 2006 (in April, June and September) (Fig. 1). Pearson correlation coefficient r=0.90 (number of pairs n=10, p<0.05).

Periphyton average chlorophyll *a* concentration during the study period was 73 mg m^{-2} and ranged from 0.83 to 973.74 mg m^{-2} (Fig. 2a). Peaks of Chl *a* concentrations (17 June 2003, 25 May 2004, 20 April 2005 and 13 April 2006) coincided with peaks of periphyton wet biomass. A statistically significant relationship occurred for Chl *a* concentration and biomass: r=0.65 (n=42, p<0.05). Diatoms and green filamentous algae dominated in the periphyton (Table 1).

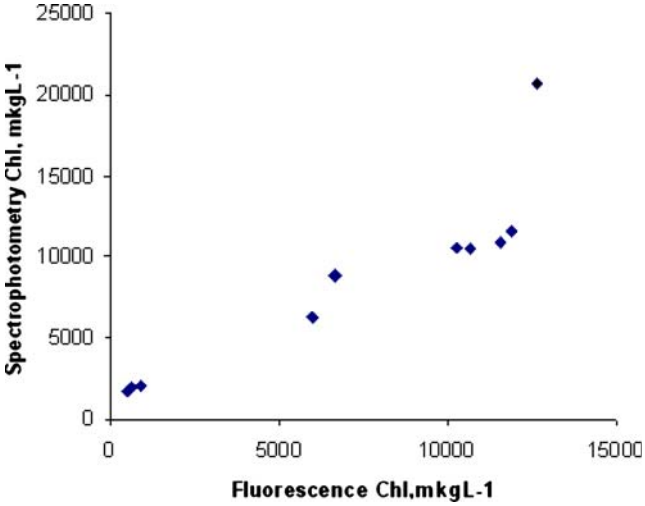

Fig. 1 Relation between Chl *a* concentration measured using fluorescence method (x-axis) and spectrophotometry (y-axis) in the Yenisei River phytoperiphyton from April to September 2006

Periphyton GPP in the study site of the Yenisei River varied from 0.294 mg $O_2\ m^{-2}\ h^{-1}$ to 20,851 mg $O_2\ m^{-2}\ h^{-1}$ (on average 1 232.5 mg $O_2\ m^{-2}\ h^{-1}$ or 385.2 mg C $m^{-2}\ h^{-1}$) or from 2 mg $O_2\ m^{-2}\ day^{-1}$ to 304 425 mg $O_2\ m^{-2}\ day^{-1}$ during the study period (Fig. 2b). GPP maximum was observed in spring, when microalgae biomass and Chl *a* peaked (Fig. 2a,b). Relationship between daily GPP and phytoperiphyton Chl *a* concentration was fitted by a regression equation (Fig. 3):

$$\log_{10} GPP = 1.6105\ \log_{10} \text{Chl} + 0.2162\ (r = 0.83, n = 42,\ p < 0.05, R^2 = 0.696) \quad (3)$$

Phytoperiphyton Chl-specific GPP (GPP:Chl *a*, mg C mg Chl *a* $m^{-2}\ day^{-1}$) varied from 0.009 to 153.59 (mean value is 36.36 mg C mg Chl *a* $m^{-2}\ day^{-1}$ or 2.42 mg C mg Chl *a* $m^{-2}\ h^{-1}$). Relation between values Chl-specific GPP and Chl *a* concentration was described by regression equation (Fig. 4):

$$\log_{10} GPP : \text{Chl}\ \alpha = 0.6105\ \log_{10} \text{Chl}\ \alpha + 0.2162\ (r = 0.50,\ n = 42,\ p < 0.05, R^2 = 0.247) \quad (4)$$

We also calculated GPP values in the Yenisei River using an empirical model for stream periphyton from temperate regions of the northern hemisphere in spring, summer and fall conditions (Morin et al. 1999):

$$\log_{10} GPP = 0.79\ \log_{10}\ \text{Chl}\ a + 1.23 \quad (5)$$

The relationship between GPP values, obtained by our method and values, predicted by the model is shown in

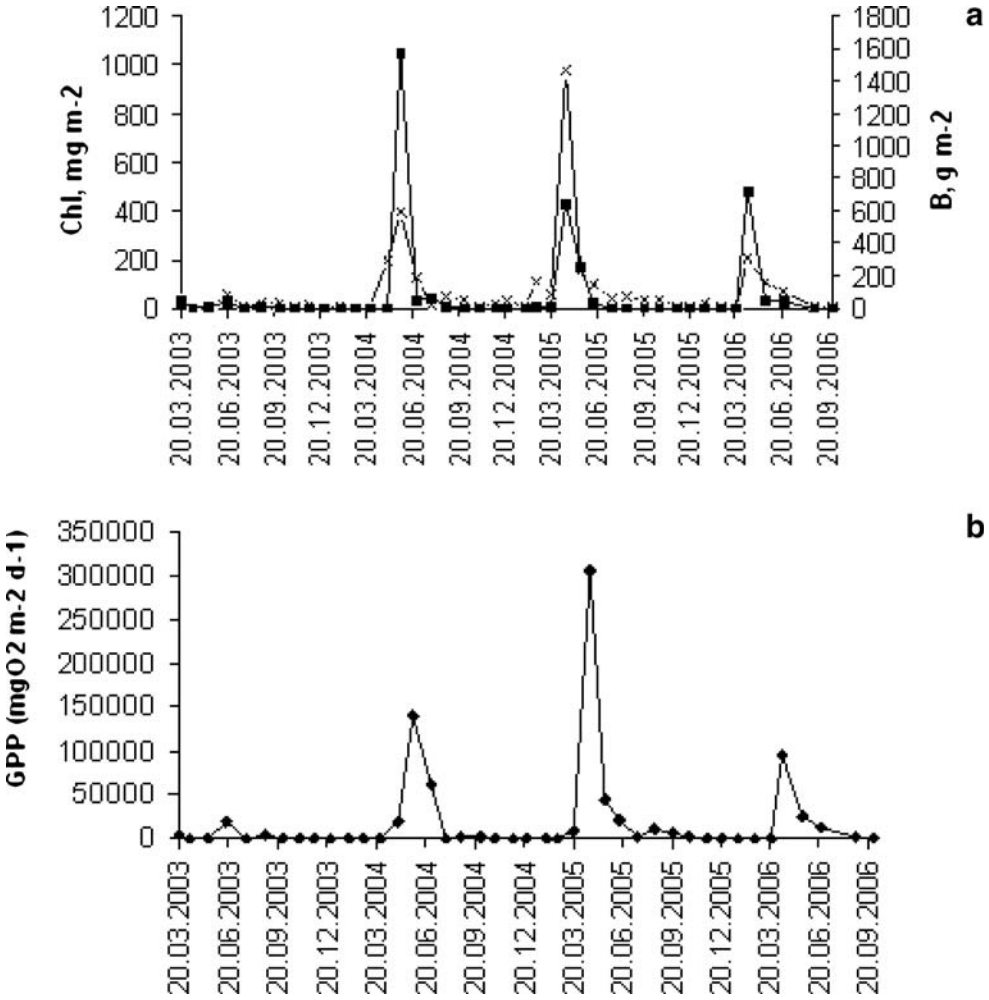

Fig. 2 Seasonal dynamics of chlorophyll *a* concentration (*squares*)and biomass (*crosses*) (**a**) and gross primary production (**b**) of phytoperiphyton in the Yenisei River (March 2003 to September 2006)

Fig. 5. Our data for the Yenisei River strongly correlated with the model values:

$$\log_{10}\text{GPP model} = 0.3413\ \log_{10}\text{Chl } a\,\text{measured} + 1.4768\ (r = 0.84, \text{n} = 42, \text{p} < 0.05) \quad (6)$$

Discussion

The upper limit of chlorophyll *a* concentration and GPP values in the unshaded littoral zone of the Yenisei River was higher than those in many other rivers (Table 2). According to Morin et al. (1999), mean areal Chl *a* and daily gross primary production for stream periphyton from temperate regions of the northern hemisphere were 27.9 mg Chl *a* m^{-2} or 223 mg C m^{-2} day^{-1}.

The species composition and seasonal dynamics of biomass of periphyton in the Yenisei River were similar to those in many other lotic ecosystems. Diatoms and green filamentous algae dominated in periphyton of the Yenisei River (Table 1) as in some other rivers (Dodds et al. 1999; King et al. 2006). Maximum of periphyton Chl *a* and GPP in the Yenisei River were observed in spring (Fig. 2a,b), as was reported for some other locations (Ostrofsky et al. 1998).

Light is known to be one of the key factors for primary production. Increase in light availability is accompanied by changes in species composition and increase in biomass and primary production (Guash and Sabater 1995). The availability of light, as mediated by the development of riparian vegetation, appears to be the most important factor affecting primary production in southern Ontario streams, followed by substrate type and water temperature. GPP of periphyton was an order of magnitude lower at the forested site than in open site (Rosenfeld and Roff 1991). Ostrofsky et al. (1998) showed highest chlorophyll *a* concentrations were typically found in spring before the canopy filled in, and in the fall in rivers with riparian deciduous canopy. In the study site of the Yenisei River there was no riparian vegetation shading the periphyton, hence GPP in Yenisei was believed to vary, as in the other open river places GPP (Table 2), because of flood (Uehlinger 2006; Reid et al. 2006), temperature, nutrients and algae species composition (Fellows et al. 2006).

Table 1 Dominant species of periphyton microalgae and their percent of the total biomass in the littoral of the Yenisei River in vicinity of Krasnoyarsk City

Month	2003/2004	%	2004/2005	%	2005/2006	%	2006	%
Mar	*Gomphonema tenellum* Kutz.	36	*Rhoicosphenia curvata*	46.3	*Ulothrix zonata*	49.9	*Cymbella stuxbergii*	52.1
	Ulothrix zonata Kutz.	29	*Cocconeis placentula*	25	*Diatoma elongatum* var. tenue	17.4	*Chamaesiphon incrustans*	20.2
	Gomphonema ventricosum Greg.	7			*Didymosphenia geminata*	10.8		
Apr	*Rhoicosphenia curvata* (Kutz.) Grun	14	*Rhoicosphenia curvata*	43.6	*Diatoma elongatum* var. tenue	14.8	*Ulotrix zonata*	94.5
	Gomphonema ventricosum	12	*Gomphonema septum*	19.4	*Didymosphenia geminate*	18.9		
	Gomphonema tenellum	12			*Ulothrix zonata*	47.1		
May	*Didymosphenia geminata* (Lyngb.) M.Schmidt	23	*Ulothrix zonata*	90	*Ulotrix zonata*	87.6	*Stigeoclonium* sp.	21.9
	Ceratoneis arcus (Ehr.) Kutz.	14					*Ceratoneis arcus*	20.1
	Gomphonema ventricosum	14					*Fragilaria* sp.	20.8
June	*Fragilaria capucina* Desm.	23	*Diatoma elongatum* var. *tenue*	58.3	*Ceratoneis arcus*	19.3	*Ceratoneis arcus*	35
	Melosira islandica O.Mull	13	*Cymbella stuxbergii*	14	*Melosira varians*	18.8	*Fragilaria virescens*	15.6
	Fragilaria construens (Ehr.) Grun	10			*Ulothrix zonata*	15.4	*Cymbella stuxbergii*	10
July	*Synura* sp.	23	*Ulothrix zonata*	93	*Didymosphenia geminate*	36.2	No data	
	Navicula sp.	22			*Cymbella ventricosa*	19.3		
	Stigeoclomium tenue Kutz.	16			*Palmella* sp.	11.8		
Aug	*Didymosphenia geminata*	64	*Didymosphenia geminate*	40.2	*Cymbella stuxbergii*	16.6	*Cymbella ventricosa*	35.1
	Cymbella stuxbergii Cl.	19	*Cymbella stuxbergii*	14.5	*Didymosphenia geminate*	15.6	*Fragilaria crotonensis*	13.9
	Navicula sp.	3	*Rhoicosphenia curvata*	14.1	*Palmella* sp.	15.5	*Navicula* sp.	12.3
Sept	*Didymosphenia geminate*	47	*Cocconeis placentula*	37.4	*Didymosphenia geminate*	35.3	*Rhoicosphenia curvata*	28.5
	Cymbella stuxbergii	14	*Navicula* sp.	29.9	*Rhoicosphenia curvata*	14.1	*Cyanophyta* (nonidentified)	24.6
	Navikula sp.	4	*Gomphonema ventricosum*	10.5	*Ulothrix zonata*	10.2		
Oct	*Gomphonema septum* Mogh.	72	*Ulothrix tenerrima*	55.6	*Gomphonema ventricosum*	47.8		
	Melosira varians Ag.	10	*Cocconeis placentula*	13.3	*Gomphonema longiseps*	12.4		
Nov	*Rhoicosphenia curvata*	40	*Cocconeis placentula*	25.5	*Gomphonema septum*	36.1		
	Gomphonema tenellum	19	*Rhoicosphenia curvata*	17	*Cocconeis placentula*	18.2		
	Cymbella stuxbergii	6	*Chamaesiphon incrustans*	49.9	*Rhoicosphenia curvata*	17.7		
Dec	*Cocconeis placentula* Ehr.	51	*Navicula* sp.	37.7	*Gomphonema lanceolata*	23.5		
	Gomphonema ventricosum Greg.	13	Cocconeis placentula	13.5	*Cyanophyta* (nonidentified)	40.9		
	Melosira varians Ag.	9	*Chamaesiphon incrustans*	28.1				
Jan	*Cocconeis placentula*	36	*Macrospora* sp.	44.5	*Gomphonema tenellum*	42.1		
	Gomphonema olivaceum (Lyngb.) Kutz.	12	Rhoicosphenia curvata	21.8				
	Synedra acus Kutz.	8	*Cocconeis placentula*	21.4	*Rhoicosphenia curvata*	16.4		
					Chamaesiphon incrustans	21.1		
Feb	*Gomphonema tenellum*	29	*Gomphonemaventricosum*	22.1	*Gomphonema tenellum*	61.8		
	Rhoicosphenia curvata	28	*Cymbella stuxbergii*	15.8				
	Cocconeis placentula	23	*Ulotrix zonata*	25.9	*Achnanthes* sp.	12.7		

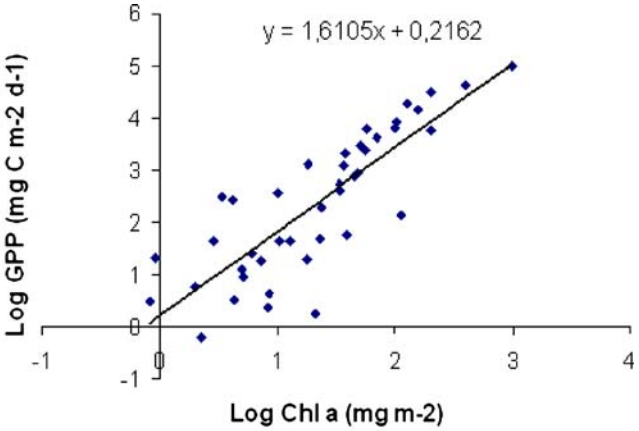

Fig. 3 Relation between log-transformed daily gross primary production and Chl *a* concentration of phytoperiphyton in the Yenisei River

Chl *a* concentration correlated with periphyton daily GPP in the Yenisei River (Fig. 3, Eq. 3). Linear positive relation between these parameters was shown earlier (Uehlinger and Brock 2005). Ostrofsky et al. (1998) proposed a regression equation for periphyton in Sundy Run: $\ln \mathrm{GPP} = 2.71 + 0.78 \ln \mathrm{Chl}\ a$. Empirical model for stream periphyton proposed an equation: $\log_{10} \mathrm{GPP} = 1.23 + 0.79 \log_{10} \mathrm{Chl}\ a$. Production of periphytic algae increased with Chl *a* standing stock (Morin et al. 1999). Data of the present study are in a good agreement with earlier findings.

Average Chl-specific GPP of periphyton in this study (36.36 mg C mg^{-1} Chl *a* day^{-1} or 116.35 mg O_2 mg^{-1} Chl *a* day^{-1}) was significantly higher than values calculated by (Morin et al. 1999) for stream periphyton (8.0 mg C mg^{-1} Chl *a* day^{-1}), but significantly lower than those in a southeast Spain river (Velasco et al. 2003), where the average value was 224.64 mg C mg^{-1} Chl *a* day^{-1}. As shown in (Ostrofsky et al. 1998), GPP:Chl *a* ratio varied from 0.4 to 32.9 mg C mg^{-1} Chl *a* day^{-1} in a seasonally shaded periphytic community. This ratio was equal 7.9 mg C mg^{-1} Chl *a* day^{-1} in summer, when light intensity was minimal and 10.9 mg C mg^{-1} Chl *a* day^{-1} in fall, 7.4 mg C mg^{-1} Chl *a* day^{-1} in spring and 12.8 mg C mg^{-1} Chl *a* day^{-1} in winter. Munn et al. (2004) reported that assimilation ratios of epilithic metabolism in the regulated Clearwater River (USA) varied from 211.9 in summer to 47.6 and 85.2 mg O_2 mg^{-1} Chl *a* day^{-1} in fall and spring, correspondingly. In the Yenisei River, maximum assimilation ratios (Chl-specific GPP) were observed in spring (March–May) and summer (June–August) (average values are 203.05 and 174.68 mg O_2 mg^{-1} Chl *a* day^{-1}), but average values in fall and winter were low (34.99 and 4.33 mg O_2 mg^{-1} Chl *a* day^{-1}).

According to empirical data (Morin et al. 1999), log-transformed Chl-specific daily primary production of stream periphytic community was approximately proportional to Chl *a* standing stock, but there was a systematic decline in primary production per unit Chl in sites with high standing stock. In spite of general proportional increasing of Chl-specific daily primary production with Chl *a* (Fig. 5), we observed a decrease of epilithic algae production in the Yenisei River, when Chl *a* concentrations were more than 200 mg m^{-2} (Fig. 4b) during predominance of green filamentous algae *Ulothrix zonata*. Declines in Chl-specific daily primary production with increasing standing stock appear to be common for stream periphyton.

In our study, an increase of GPP:Chl *a* values with Chl *a* concentration was observed up to ~200 mg Chl *a* m^{-2} (Fig. 4b). It is known that photoadaptation and reduction of photoinhibition can mitigate the effects of self-shading in biofilms with increasing thickness, and algal cells exposed to low light levels maintain relatively high productivity (Dodds et al. 1999). This fact can explain increasing GPP: Chl *a* with chlorophyll *a* concentration in the Yenisei River. Filamentous green algae often dominate the thickest periphyton mats but are generally rare or absent from very low standing stock assemblages. Enriquez et al. (1996) concluded that photosynthesis rate declines with increasing cell thickness of microalgae. Succession from diatom to filamentous green algae with thick cell walls would contribute to the declining trend in Chl-specific primary

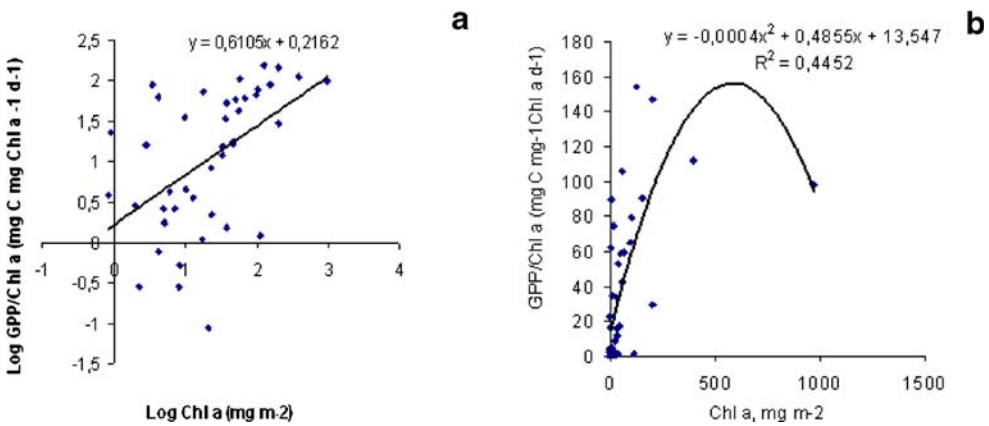

Fig. 4 Relation between daily Chl-specific GPP and Chl *a* concentration of phytoperiphyton in Yenisei River: (**a**) for log-transformed and (**b**) for non-transformed values

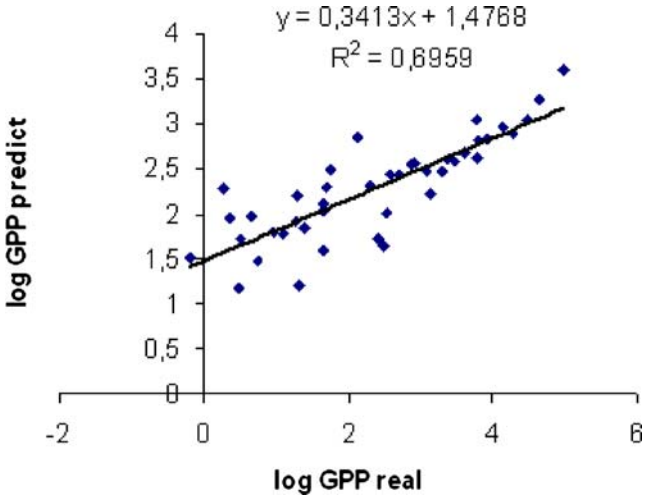

Fig. 5 Relation between observed daily GPP (mg C m^{-2} day^{-1}) data for phytoperiphyton in the Yenisei River and values predicted by Morin et al.'s (1999) empirical model for northern hemisphere phytoperiphyton

production along a biomass gradient. Nutrients deficient for algal cells, which are deeper in the matrix, may be another reason for photosynthesis efficiency declining in periphyton community. Possibly, GPP:Chl *a* values decrease, observed in the Yenisei River in the present study, was caused by changes of dominant algae species and by the increase of their biomass (Fig. 2a).

In general, our data have a high significant correlation with predicted values of Morin et al.'s (1999) chlorophyll-based empirical model for stream periphyton (Fig. 5), which allows us to make a conclusion about applicability of the DCMU-fluorescence method for estimation of gross primary production of stream periphyton (epilithon). The main advantages of this method were described elsewhere (Gaevsky et al. 2005), but, briefly, in the first, in contrast to PAM-fluorescence, it is possible to determine chlorophyll *a* concentration and relative variable fluorescence when biomass of periphyton is high and Cyanophyta dominate. The second, using inhibitor of electron-transfer chain (DCMU), allows avoiding the effect of photosynthesis on the specific fluorescence yield and the obtaining of actual potential photosynthetic activity of microalgae and chlorophyll *a* concentration data. The third, method allows avoiding the disadvantages of daily incubation in closed chambers, where hydrodynamic conditions are usually far from natural. It is necessary to note that current velocity in the Yenisei River is about 2 ms^{-1} (Telang et al. 1991), and it was difficult to simulate natural turbulence in closed chambers, so this DCMU-fluorescence method is more appropriate for periphyton primary production estimation in this case. The fourth, method of GPP estimation requires incident radiation intensity data which can usually be easily obtained. And the fifth, method, the fluorometer, is suitable for monitoring of the state of the water bodies of different trophic status. It is compact and can be used in both laboratory and field studies. The analysis using the non-commercial variant of the instrument takes a little time (~5–7 min).

A disadvantage of this technique is a necessity of fluorometer calibration using alga cultures and natural alga during the period of one taxon predominance for chlorophyll *a* calculation.

Since our upper estimation of the epilithic primary production in the Yenisei River was significantly higher

Table 2 Chlorophyll concentrations and assimilation (or gross primary production, GPP) of epilithic biofilms (phytoperiphyton microalgae) in unshaded streams

Ecosystem; study period	Method	Chlorophyll *a* (mg m^{-2})	Mg O_2 m^{-2} day^{-1}	Mg C m^{-2} d^{ay-1} [a]	Reference
Mediterranean streams; all seasons	Oxygen production, laboratory incubation	59–115	28–191	8.7–60	Guasch and Sabater (1995)
River Spol, Swiss Alps; spring, summer and fall	Oxygen continuous measurements and mass-balance model	25–167	700–7,100	219–2,219	Uehlinger et al. (2003)
The Clearwater River, Idaho, USA; spring, summer and fall	Oxygen production, *in situ* chamber incubation.	5.6–28.7	800–3,200	250–1,000	Munn and Brusven (2004)
Truckee River, Nevada, USA; summer and fall	Oxygen production, *in situ* chamber incubation	50–450	3300–9,100	1,031–2,844	Uehlinger and Brock (2005)
Rivers in southeastern Australia; spring[b]	Oxygen production, *in situ* chamber incubation	1–23	0.03–7,973	0.01–2,491	Fellows et al. (2006)
The Cotter River, Australia; spring, summer and fall	Oxygen production, *in situ* chamber incubation	3.7–4.7	602–1,561	188–488	Reid et al. (2006)
Yenisei River, Russia; all seasons	DCMU-fluorescence method	0.83–973.74	2–304,425	0.64–95,133	Present study

[a] Recalculated assuming a photosynthetic quotient of 1.2 (mg C=0.3125 mg O_2) (Dodds et al. 1999; Velasco et al. 2003).
[b] September and October

than other relevant data in available literature, the DCMU-fluorescence method might overestimate the stream primary production. On the other hand, the other methods, especially chamber methods in cases of inappropriate flow simulation, might underestimate the stream primary production, and thus the DCMU method might be more realistic. The comparison of these methods should be the aim of subsequent research.

In general, the advantages of the DCMU-fluorescence method, described above, allow us to recommend it for estimation of stream periphyton primary production, at least as another useful tool for such studies.

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