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The spring energy budget of the algal mat community in a Crimean hypersaline lake determined by microcalorimetry

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Abstract

The energy contents (standing stock) of the floating mat formed by the green alga *Cladophora sivaschensis* and the energy transfers through it were quantified for a shallow hypersaline lake (at Cape Khersones, Crimea, Ukraine) during the spring months. Appropriate direct calorimetric techniques were applied to: (i) measure the heat energy dissipated by the mat community and by the free bacterioplankton in the water column below it; and (ii) differentiate between the heat flows by the heterotrophic and the phototrophic components of the community. It was shown that *Cladophora* biomass reached a peak of 579.5 g C m^{-2}, contributing more than 99.6% of the total mat community. Throughout the spring, the total bacterial energy transfer (6 to 23 mW m^{-2}) was as little as 1.1 to 2.6% of the total heat dissipated by the microplankton community. The rest of the estimated heat energy (584 to 1488 mW m^{-2}) was associated with *Cladophora* metabolism. In the spring community: (i) the rate of biomass accumulation in the lake photic layer significantly exceeded its heterotrophic mineralisation; (ii) the efficiency of the microbial loop was too low to process even a minor part of the accumulated organic matter. The microcalorimetric technique was shown to be a highly promising approach for further studies of natural microbial mats and biofilms, biological systems with complex metabolism that involves not only aerobic processes but also anaerobic catabolism under local hypoxic/microxic conditions.

Introduction

Hypersaline lakes are amongst the ecosystems whose biogeochemical cycling is closely coupled with and, in fact, formed by microbial processes, including primary production, heterotrophic uptake, digestion and mineralisation of the accumulated organic matter. In the Crimea at least, their greatly enhanced primary productivity is due, in particular, to the high production potential of floating mats formed by the filamentous green alga, *Cladophora sivaschensis*, C. Meyer, 1922 (Chlorophyta: Cladophoraceae). *Cladophora* spp. play a major role in lake ecosystems (Dodds and Gudder 1992) and *C. sivaschensis* is typical in this respect, forming a complex community in the Cape Khersones lake that: (i) includes anoxygenic phototrophs and aerobic/anaerobic heterotrophs; and (ii) demonstrates a pronounced imbalance in the processes of organic matter production and mineralisation (Ivanova et al. 1994).

Shadrin et al. (2001) described the annual changes in this lake. From the late winter to the autumn, the *Cladophora* mat undergoes successive structural and functional changes accompanied by a marked increase in the filament concentration and, consequently, in the biomass. From early June onwards, the

so-called ‘sandwich‘ structure of the mat forms, with a thin, dense biofilm of cyanobacteria and diatoms developing on the upper surface of the mat to change its colour from green to fulvous. At this stage in the succession, the role of the unicellular microorganisms (mainly cyanobacteria and diatoms) inhabiting the mat becomes more prominent and the deepest layer of the mat may contain local pockets that have hypoxic/microxic conditions (Peckol and Rivers 1995).

In this exploratory study, we examined an intermediate phase of the mat development from late March to late May, which was characterized by an abrupt decrease in the rate of algal metabolism against a background of extremely high biomass and biosurface concentrations inside the mat. The first aim of the work was to calculate the energy standing stocks and transfers in the phototrophic algae and the heterotrophic bacteria. According to a reasonable conceptual scheme, the first group, the primary producers, supplies the system with the flow of organic matter while the second one, forming the detrital food chain, controls the rate of mineralisation of the organic matter accumulated in the system. The kinetics of these processes and, additionally, the ratio between them can serve as the key for calculating the energy balance of the system and estimating its biological effectiveness.

The fact that all processes, including biogenic ones, are accompanied by changes in heat energy means that direct calorimetry is a powerful analytical tool (Spink and Wadsö 1976). A key property, non-specificity, makes its use compelling in studies of complex biological systems like the natural microbial communities, especially in the years since the microwatt calorimeters first became available. The type of investigation that is concerned with the complete microbial community rather than the individual species within it has been practiced in soil research from the pioneering studies in the 1930s of Hesselink van Suchtelen (1931) to the present day (see for instance Núñez-Regueira et al. 2002). In the light of this, biofilms and mats that also exhibit high biomass concentrations and enhanced microbial activity are promising systems for the similar-type environmental and analytical studies. A most persuasive reason for exploring the potential of direct calorimetry in mat studies is the stagnant hypoxic nature of the conditions common in this kind of biotope (e.g., Peckol and Rivers 1995). In contrast to respirometry, calorimetry provides the essential kinetic information on *integral* (aerobic + anaerobic) metabolism, serving as a powerful tool for constructing ecosystem network models of material and/or energy flows. At the same time, in combination with respirometry, it differentiates between the aerobic and anaerobic processes that occur in the system through the calorimetric/respirometric (CR) ratio calculated from the two measurements (Gnaiger and Kemp 1990). Thus, the second, more methodological aim of this work was to adopt a commercial microcalorimeter, known mainly as an analytical tool for environmental studies and, in particular, for microbial ecology in an approach similar to that of Pamatmat et al. (1981) for heat flow measurements of sediments with a bespoke instrument. We applied the standard microcalorimetric practice to the mat and an original technique using the same instrument first employed in a study of marine bacterioplankton (Mukhanov et al. 2003) for measuring the heat flow and thus the metabolic rate of the lake bacteria after concentrating them.

Theory

It is necessary to use a heat conduction calorimeter in the present study because it measures the instantaneous *rate* of heat flow ($\Phi = \mathrm{d}Q/\mathrm{d}t$, Watts, W) (Kemp 1998). This means that the measurement gives the kinetics of the process as well as providing thermodynamic information relating to the state functions such as the enthalpy change, ΔH. The heat flow is properly regarded as the rate of thermal (th) advancement, $\mathrm{d}_{\mathrm{th}}\xi/\mathrm{d}t$, in the energy transformations (Gnaiger 1993). Advancement of a reaction is sometimes known as the extent of reaction. It is an important concept in energy transformation because it is expressed explicitly in terms of the stoichiometric coefficients, ν_i, of the i-th species in the reaction. In other words, thermal advancement is directly related to the stoichiometry of the reaction. It must be stressed that obviously this is true irrespective of the complexity of the reaction, i.e., one as complex as the biotic growth reaction – the metabolic reaction (see Kemp and Guan 1999).

The rate of the growth reaction, the so-called metabolic rate or ‘activity‘, is simply a convenient phrase to denote the rate of advancement of the aggregated biochemical reactions in the growth reaction (Kemp 2000) of living matter, $\mathrm{d}\xi_{\mathrm{B}}/\mathrm{d}t$, or the scalar flux when the rate is expressed as specific to mass, $(1/X)(\mathrm{d}\xi_{\mathrm{B}}/\mathrm{d}t)$ where X represents the amount of biomass or cell volume. The vector flux was also

expressed for some results in this paper, i.e., the heat flow per unit surface area of the organism(s). The thermal advancement of energy transformation, $d_{th}\xi$, is related to $d\xi_B$ by the expression (Kemp, 2000),

$$d_{th}\xi = \upsilon_i \Delta H_{B,i} d\xi_B \quad (1)$$

where $\Delta H_{B,i}$ is the molar enthalpy of the reaction in terms of species i (Kemp and Guan, 1999). The change in thermal advancement, $d_{th}\xi$, is exactly equivalent to the change in heat, dQ. The conclusion of this theoretical treatment is that the calorimetrically measured heat flow is a function of the metabolic rate of the living matter under investigation.

Materials and methods

The surface water samples were collected in March-May 2002 in a shallow hypersaline lake at Cape Khersones near Sevastopol in the South-West Crimea, Ukraine (44°35′09′ N, 33°23′39′ E). It is a small (0.02 km^2) lake of marine origin separated from the Black Sea by the narrow, stony isthmus of about 15 m width, through which is a permanent infiltration of sea water. There is also a small freshwater inflow. The lake depth does not exceed 85 cm in winter and 60 cm in summer. Seasonal water temperature and salinity fluctuations occur within the ranges of 3.3 to 34 ºC and 60 to 106 g l^{-1} NaCl, respectively.

The early season formation of the floating mats of *Cladophora siwaschensis* is a characteristic of the lake. By late March when the work started, the mat at the exact sampling site was well formed and stable. The samples were carefully taken: (i) from inside the mat with a broad-mouth plastic sampler to collect *Cladophora* filaments and inter-filament water; and (ii) in the water column below the mat with a 2 L container analogous to the Niskin bottle. For the approximations of energy transfers, the *in situ* temperatures were recorded and the depth of the water column and the thickness of the mat were averaged at 30 cm and 4 cm, respectively.

Microcalorimetric measurements were carried out with an LKB BioActivity Monitor (BAM), Model 2277 (the successor is the Thermal Activity Monitor (TAM), Thermometric AB, Järfälla, Sweden) equipped with three independent sets of twin differential channels, one of each twin for the test material and the other as the control. Accordingly, three energy budget components, namely (i) the total heat flow by the mat community; (ii) the heat flow by the bacterial fraction from the mat; and (iii) the heat flow by the bacterial fraction from the water column below the mat, were measured simultaneously in each calorimetric experiment. All the experiments were conducted at 20 ºC, and the energy transfers under *in situ* temperatures were calculated from the measured heat flow assuming $Q_{10} = 2$. In the batch mode measurements, the baseline noise was less than ± 0.1 μW while the samples produced from about 5 to 63 μW per ampoule.

The concentration of the living biomass in the mat was high enough to allow the direct measurement of its heat flow. For each calorimetric experiment, a mat sub-sample including the inter-filament water was carefully transferred directly into a sterile 3 ml glass ampoule without the need for any preparative stages. The control ampoule contained an equal volume of particle-free lake water taken from the same site and filtered using nitrocellulose membranes, 0.1 μm pore size, 47 mm diam. to give particle-free water (Sartorius #SM113 58-047). The heat flow by the mat was assumed to be associated exclusively with photoautotrophic metabolism because the *Cladophora* biomass concentration in it was several orders of magnitude higher than for other algal species. For the same reason, the budget estimates performed for the total water column ignored the heat flow by the phytoplankton.

The heat flows by free-living bacteria inhabiting the inter-filament water in the mat and by the picoplankton in the water column below the mat were measured after a two-step filtration technique (Mukhanov et al. 2003). Initially, the 100 ml sub-samples were screened with a 3 μm pore size membrane (Sartorius #SM113 02-047) to remove nano- and microplankton. The < 3 μm filtrate contained the so-called picoseston, with only the amount of heterotrophic bacteria concentrated in it being sufficient for calorimetric detection. Concentrations of cells in other taxonomic groups, including any cyanobacteria and small nanoplankton passing through the membrane, were too low (not more than 2% in terms of biomass) to affect the calorimetric measurements and, thereby, the budget calculations. For this reason, their abundance and biomass were not quantified thoroughly and the heat flow by the picoseston was assumed to be associated solely with the heterotrophic bacteria. However, all the samples were microscopically examined to check for artefacts

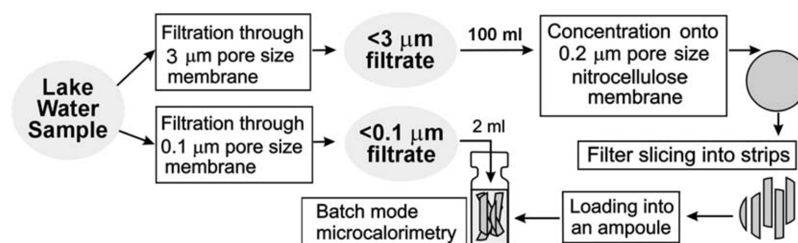

Figure 1. The experimental design.

caused, for example, by the bloom of a photoautotrophic species.

As the second stage, the 100 ml sub-samples were concentrated onto a 0.2 μm nitrocellulose membrane (Sartorius #SM113 07-047) (see Figure 1). The wet membrane (or its fragment of known area) with the concentrated cells was cut into strips to maximise exposure of the surface to the sea water and placed into a sterile measuring ampoule containing 2 ml of particle-free lake water from the same site. The ampoule was hermetically sealed and the bacterial heat flow was measured immediately after loading the glass ampoule with its filter membrane carrying the bacteria into the batch module of the microcalorimeter.

No replicate experiments were conducted because of the limited number of the independent measuring channels. However, the assessment error potentially associated with this technical problem was reduced because the analysis was conducted on relatively large sample volumes. According to Kirchman's (1993) sampling hierarchy and associated statistics, a 100 ml sample is at sampling level 2 (100 ml to >1 l) at which point the variance is equivalent to examining the spatial and temporal variation in natural microbial abundance. Thus, the concentrated picoplankton on a membrane was representative of the *in situ* assemblage, at least in its abundance. In theory, no replicates were necessary in this case.

At the same time, 100 ml was small enough to avoid the large calorimetric measurement error associated with concentrating bacteria onto the membrane. In a methodological study (V. Mukhanov and R.B. Kemp, unpubl.), it was shown that bacterial metabolism was depressed with increasing filtered volume (up to 1.5 l) and cell number (up to 10^9 cells) on/inside the nitrocellulose membrane matrix. This 'crowding effect' described a hyperbolic relationship between heat flow and the number of the concentrated bacteria. At small (up to 100 ml) filtered volumes, however, the depression-associated error was minor (below 5%).

In the algal mat experiments, the variance in heat flow by the small-volume (3 ml) replicate sub-samples of the mat is from 16 to 27% of the total, according to statistical analysis (Kirchman 1993). However, it would not greatly affect the ratio between the total algal and the bacterial heat fluxes because of the huge (up to 3 orders of magnitude) difference between them. In our view, this made a comparison of the heterotrophic and autotrophic budgets reliable.

Nano- and picoplankton were counted by epifluorescence microscopy using proflavin hemisulphate (Haas 1982) and acridine orange (AO) (Hobbie et al. 1977). All the sub-samples were fixed in glutaraldehyde (10%, 0.1 μm prefiltered, 1% final concentration) before (AO) and after (proflavin) staining. The samples were collected onto black-stained polycarbonate membrane filters (0.2 μm pore size) at low vacuum (<80 mm Hg). In the proflavin-stained preparations, photoautotrophs were identified by the autofluorescence of the photosynthetic pigments. Phytoplankton taxa were identified and their abundance was counted by light microscopy. A Zeiss standard microscope equipped with an HBO-202 mercury lamp was used for both light and epifluorescence observations.

For the morphometric analysis, 50 bacterial cells from each sub-sample were sized according to length, diameter, cell volume and surface. Bacterial biomass was calculated assuming the volume-to-biomass conversion factor of 0.22 pg C μm^{-3} (Bratbak and Dundas 1984). This *constant ratio* model was chosen instead of the 2-factor *allometric* one (see the review by Norland, 1993) because (i) the average cell volume in the samples was relatively large (0.6 μm^3) and all the cells could be referred to one size class, the largest, according to Norland's nomenclature; (ii) the conversion factor in the allometric model is subject to essentially the same sources of errors and variabil-

Table 1. Structure, abundance and morphometry of the mat algal community.

Month	Group	Numbers, 10^3 cells ml^{-1}	Biovolume, 10^6 μm^3 ml^{-1}	Biosurface, 10^6 μm^2 ml^{-1}	Biosurface, M^2 m^{-2} (of lake surface[3])	S/V, 10^6 m^{-1}
March	*Cladophora sp.*	15.6[a]	17,305.3	3,073.8	123.0	0.18
	Flagellates	17.2	10.5	4.2	0.2	0.40
	Diatoms	194.2	128.0	93.8	3.8	0.73
	Gloeocapsa spp.	34.2	1.3	1.9	0.1	1.43
	Total	–	17,445.1	3,173.8	127.0	0.18
April	Cladophora sp.	371.2[a]	411,777.9	73,141.7	2,925.7	0.17
	Flagellates	57.0	34.8	14.0	0.6	0.40
	Diatoms	858.9	2,042.0	429.9	17.2	0.21
	Gloeocapsa spp.	28.6	8.9	6.3	0.3	0.71
	Total	–	413,863.5	73,591.9	2,943.7	0.18
May	*Cladophora sp.*	323.8[a]	359,196.3	63,801.9	2,552.1	0.18
	Flagellates	9.6	5.9	2.4	0.1	0.40
	Diatoms	82.2	184.9	39.9	1.6	0.22
	Gloeocapsa spp.	49.2	10.6	2.2	0.1	0.21
	Total	–	359,397.6	63,846.4	2,553.9	0.18

[a]10^3 filaments per ml

ity as the conversion factors for the constant ratio model (Norland 1993), and (iii) the Bratbak-Dundas factor is frequently used for the approximations at the levels of the community and the ecosystem, which makes it easier to conduct inter-study comparisons.

The microalgal carbon biomass was calculated using the regression equations given by Strathmann (1967):

$$\log C = 0.866 \log V - 0.460 \text{ for diatoms,}$$

$$\log C = 0.758 \log V - 0.422 \text{ for other groups,}$$

where C is the carbon per cell (pg) and V is the cell volume (μm^3). The biomass energy content equivalents were calculated using the conversion factors of 1 mg C = 43.45 J and 44.17 J for bacteria and algae, respectively (calculated from the average bacterial and algal enthalpies of combustion given by Duboc et al. 1999).

Results

Throughout the spring months, *C. sivaschensis* absolutely dominated the algal community in terms of biomass (from 99.2% in March to 99.9% in May) and biosurface (from 96.9% in March to 99.9% in May) (Table 1). The diatoms (up to 8.6×10^5 cells ml^{-1}), the cyanobacteria (*Gloeocapsa* spp.; up to 4.9×10^4 cells ml^{-1}) and the microflagellates (up to 5.7×10^4 cells ml^{-1}) were the representatives of the mat-dwelling phototrophs.

The surface-to-volume (S/V) ratio averaged for the whole mat did not differ from the *Cladophora* S/V ratio of 0.18×10^6 m^{-1} owing to the absolute dominance of its biomass. In mid-spring, the total biosurface of the mat reached its maximum value of 2943.7 m^2 per m^2 of the lake surface (Table 1). The mat algal biomass grew from 24.5 g C m^{-2} (1×10^6 J m^{-2} in terms of energy) in March to its steady state value of about 550 g C m^{-2} (about 24×10^6 J m^{-2}) in April-May (Table 2). The peak value of 579.5 g C m^{-2} (25.6×10^6 J m^{-2}) was recorded in April. The algal scalar (volume-specific) and the vector (surface-specific) heat fluxes changed from the relatively high values (1.8 fW μm^{-3} and 9.8 fW μm^{-2}, respectively; at 20 °C) in March to the low and almost constant ones (about 0.03 fW μm^{-3} and 0.22 fW μm^{-2}; at 20 °C) in April-May (Table 2). Thus, there were approximately 30-fold decreases in the algal fluxes in April. At the same time, there was a steady increase in the calculated total *in situ* metabolism from March (584 mW m^{-2}; $t_{in\ situ}$ = 9.1 °C) to May (1488 mW m^{-2}; $t_{in\ situ}$ = 27 °C), as a result of the increases in

Table 2. Total algal and bacterial (in brackets) biomasses, their energy equivalents and the energy fluxes through the communities in the spring months.

Month	Biomass, g C m^{-2} (mg C m^{-2})	Standing stock, MJ m^{-2} (kJ m^{-2})	Scalar heat flux (20 ºC), fW μm^{-3}	Vector heat flux (20 ºC), fW μm^{-2}	Cell-specific heat flux (20 ºC), fW $cell^{-1}$	Metabolism (20 ºC), mW m^{-2}	In situ metabolism[1], mW m^{-2}
March	24.5	1.08	1.78	9.80	–	1,244.0	584.4
	(220.44)	(9.58)	(32.69)	(5.05)	(25.53)	(32.89)	(15.40)
April	579.5	25.60	0.03	0.22	–	560.1	600.2
	(124.72)	(5.42)	(11.99)	(1.82)	(4.73)	(6.81)	(6.41)
May	503.2	22.23	0.06	0.41	–	916.0	1,488.0
	(384.08)	(16.69)	(9.43)	(1.39)	(5.12)	(16.52)	(23.27)

[1]approximated using $Q_{10} = 2$

Table 3. Morphometry of the hypersaline bacterial community.

Month	Cell Volume, μm^3, ± 95% CI	Cell Surface, μm^2, ± 95% CI	S/V, 10^6 m^{-1}, ± 95% CI	Biovolume[1], 10^6 $\mu m^3 ml^{-1}$	Biosurface	
					10^6 $\mu m^2 ml^{-1}$	m^2 m^{-2} (of the lake surface)
March	0.78 ± 0.17	5.06 ± 1.19	6.64 ± 0.47	12.24	79.22	23.77
April	0.40 ± 0.10	2.60 ± 0.50	7.60 ± 0.66	5.01	32.98	9.89
May	0.54 ± 0.13	3.68 ± 0.50	7.31 ± 0.44	4.77	32.36	9.71

[1]inside the mat

the total *Cladophora* biomass and the lake water temperature. It is possible, however, that the heat flux values were underestimated because no viability tests were performed in this study.

The bacterial cell volume (0.4 to 0.8 μm^3 per cell; Table 3) and surface (2.6 to 5.1 μm^2 per cell) were about an order of magnitude higher than in the coastal seawater (e.g., Senicheva 1990). The total bacterial biosurface calculated for the whole water column was about 5-fold in March and 250-fold in April-May lower than those of the algae, decreasing from 23.8 to 9.7 m^2 per m^2 of the lake surface throughout the spring months (Table 3). Bacteria proved to be a minor component of the spring microplankton in terms of biomass ($<0.1\%$ in the mats and $<0.8\%$ in the total water column). Their total standing stock ranged between 5.4 and 16.7 × 10^3 J m^{-2} in terms of energy (Table 2).

Under the experimental condition of 20 ºC, the bacterial cell-specific heat flux ranged from 5 to 25 fW per cell (Table 2). For comparison, this is toward the lower end of the range for the Black Sea coastal bacterioplankton (4 to 70 fW per cell in different seasons (V. Mukhanov, O. Rylkova and R. Kemp, unpubl. data). Both the estimates were obtained for the total bacterial assemblages, without any viability tests. This means that the bacterial heat fluxes could be underestimated for both sets of data.

The scalar heat fluxes decreased with time and were about 20- (March) and 200-fold (April-May) higher than those of the algae (compare the data from Table 2) while the bacterial community had only a 40-fold higher (7.2 × 10^6 m^{-1}) S/V ratio than the algae (compare the data from Table 1, Table 3). There was not such a marked (<10-fold) difference in the bacterial and algal vector heat fluxes (Table 2). The total bacterial *in situ* metabolism (6.4 to 23.3 μW m^{-2}) was as little as 1.1 to 2.6% of the total heat dissipated by the microplankton community while the rest of the heat energy (584 to 1488 mW m^{-2}) was associated with the *Cladophora* metabolism.

Bacterial populations inhabiting the mat and the underlying water column differed in their abundance and biomass. The ratio between the two habitats changed from 6:1 to 3:1 over the spring months (Table 4). In March, they contributed nearly equal parts to the total bacterial metabolism (7.53 vs 7.87 mW m^{-2}, respectively) but this difference increased with time up to 2-fold (7.86 vs 15.41 mW m^{-2}, respectively).

Table 4. Comparison of the bacterioplankton abundance and metabolic rates in the two biotopes.

Month	Location	Abundance, 10^6 cells $ml^{-1} \pm$ 95% CI	Biomass, μg C ml^{-1}	Metabolism (20 °C), mW m^{-2}	In situ metabolism[b], mW m^{-2}
March	Mat	15.67 ± 7.72	2.690	16.02	7.53
	Free water[a]	2.53 ± 1.02	0.434	16.87	7.87
April	Mat	12.68 ± 1.05	1.116	2.40	2.57
	Free water	3.50 ± 0.39	0.308	4.41	3.84
May	Mat	23.70 ± 3.17	2.816	4.84	7.86
	Free water	8.79 ± 1.68	1.044	11.68	15.41

[a]water column below the mat; [b]approximated using Q_{10} = 2

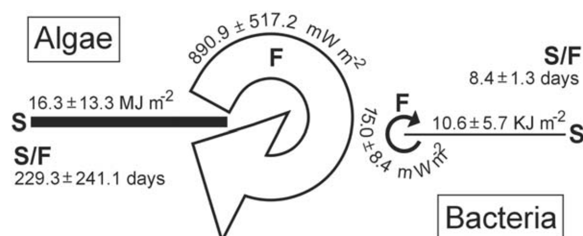

Figure 2. A simplified spring energy budget calculated for the total water column. The energy standing stock (S), the energy flux (F), the stock renewal time (S/F) and their standard deviations (n = 3) are shown.

Discussion

A simplified conceptual scheme of the aquatic food web was used for the energy budget approximations in which the fate of the primary production in the lake water column was determined by two pathways: (i) burial of organic matter into the lake bottom sediments; and (ii) recycling the organic matter in the food web, by (a) grazing of it by phytophages, and (b) mineralisation of it by heterotrophic bacteria, initially in the lake water column and later in the sediments. The *Cladophora* mat 'grew' over the spring months, 'matured' through successive structural changes in summer and, finally, sunk leaving the water column in autumn, i.e., its transport from the lake surface to the sediments was seasonal and short-term. The same succession was possible because the *Cladophora* filaments were poorly digestible and there was only a slow (if any) utilization of them by metazoans. Consequently, the mat grazing losses were neglected in the approximations. Basing on this concept, the energy budget (Figure 2) involves the two major components (algal and bacterial energy standing stocks, S in Figure 2) and the energy flows through them (algal primary production and bacterial mineralisation; F in Figure 2). The kinetics of the latter processes were assessed precisely from the heat flows by the communities.

The huge disproportions in the budget components (Figure 2), both in terms of the standing stocks (S) and fluxes (F), mean that by far the greatest part of the organic matter produced in the lake and accumulated in the form of the floating *Cladophora* mats was finally deposited to the lake sediments supplying the energy/matter budget of benthic microbial processes. It appears to be a general characteristic of the hypersaline ecosystem that the organic carbon mineralisation occurs predominantly in the bottom sediments (Matsumoto 1989) and mainly through anaerobic microbial processes (Post and Stube 1988; Cayol et al. 1995). The portion of the mat primary production recycled by the bacterioplankton to the plankton food web proved to be small (Figure 2), indicating that the microbial loop in the lake plankton was relatively inefficient.

The functional state of both the compartments of the system was well characterised by the turnover time for their standing stocks, shown in Figure 2 as the stock-to-flux (S/F) ratios. The values were extremely large, especially in the algal population, from a month to almost 1.5 years. The lake free-living bacteria, being about 10-fold more abundant than the marine bacterioplankton in the sea coastal waters (10^5 to 10^6 cells ml^{-1}, Chepurnova et al. 1993), demonstrated a prolonged turnover time for their standing stock, 7 to 10 days versus 5 to 12 h in the marine bacteria depending on the season (data for the nearby Sevastopol Bay; Mukhanov et al. 2001).

The distinct imbalance between the primary producers and the mineralisers in the budget is well illustrated in Figure 3. In terms of available biomass, i.e., the energy accumulated in the system, the algae exceeded the bacteria by 2 to 3 orders of magnitude, with the ratio being greater (up to 10^4) in the mat. The imbalance in the energy transfers, defined as the ratio

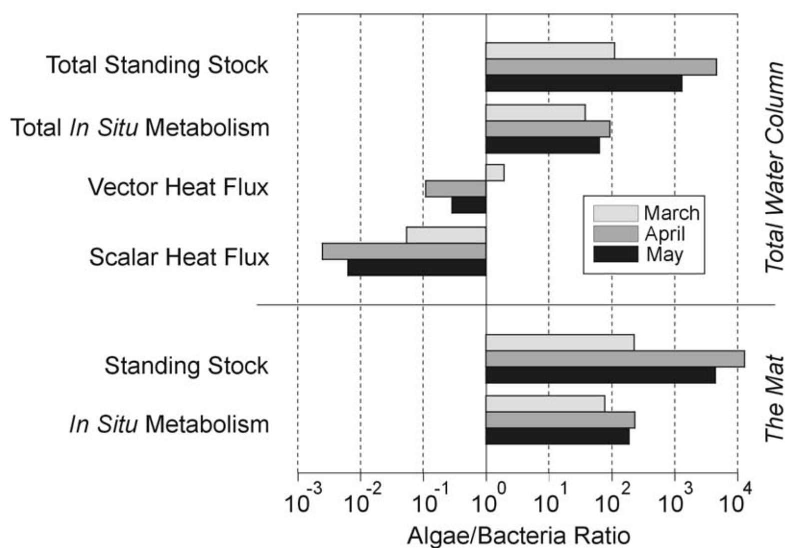

Figure 3. The algae-to-bacteria ratios calculated for the total water column and for the mat only.

between the total metabolic expenditures of the primary producers and the mineralisers, showed the same regularity but it was feebly marked at only 10^2, owing to the ~300-fold more intensive scalar metabolic flux in the bacterial cells (Table 2). Thus, there was a strong imbalance between the autotrophic and heterotrophic processes in the lake water column and especially in the mat. At least over the spring months, both the systems can be considered as net autotrophic, with an insignificant heterotrophic component (photoautotrophy $>>$ heterotrophy). It is also to be expected that the imbalance can sharply invert (photoautotrophy $<<$ heterotrophy) in the lake sediments.

Contrary to the situation for the total *in situ* metabolism, there was an inversion in the ratio between the algal and bacterial scalar heat fluxes (Figure 3), i.e., the bacterial flux exceeded by about 10^2-fold the algal one, in accordance with Kleiber's rule (1932, 1961). Intrinsic to the rule is the fact that the higher the surface-to-volume ratio of an organism, the more intensive its energy flux per cell volume (see Theory section). By generalizing published respirometric data, Caron et al. (1990) showed that the rule is applicable to natural microplankton, more precisely to the planktonic protozoans. Over a range of about 6 orders of magnitude difference in cell volume, from large sarcodines (up to 10^8 μm^3) to small flagellates (about 10^2 μm^3), and under exponential growth at 20 ºC, the protozoan volume-specific respiration rate increases by 3 orders of magnitude, from 3.4×10^{-8} in *Chaos carolinense* (Holter and Zeuthen, 1949) to 4.0×10^{-5} nl O_2 μm^{-3} h^{-1} in *Paraphysomonas imperforata* (Caron et al., 1985) that is equivalent in molecular terms between 1.52×10^{-18} and 1.79×10^{-15} mol O_2 μm^{-3} h^{-1}. However, after converting these data to the 'per cell surface' basis by assuming the cells were spherically shaped, present calculations showed that the surface-specific respiration rate was independent of the cell volume ($r^2 = 0.00017$), with the mean of 357.8 ± 72.3 (95% CI) $\times 10^{-7}$ nl O_2 μm^{-2} h^{-1} (or 1.6×10^{-15} mol O_2 μm^{-2} h^{-1}). This value may be interpreted as the highest possible oxygen flux, the 'limit' observed in actively growing cells under favourable conditions. Using the averaged oxycaloric equivalent of -450 kJ mol^{-1} O_2 (Gnaiger and Kemp, 1990) for conversion, the 'limit' is 199.7 fW μm^{-2} in terms of the heat flux. It follows from the 'limit' concept that the energy/matter flux through a unit of biosurface is independent of the cell size in actively growing cell populations, i.e., some 'limit' vector flux exists for unicellular microorganisms. This might be considered a promising concept in aquatic microbial ecology because it can potentially serve as a 'common denominator' for diverse and different-scale microplankton.

The maximum vector heat fluxes detected in this study for the algae and the bacteria were 9.8 fW μm^{-2} (4.9% of the 'limit' flux) and 5.1 fW μm^{-2} (2.6%), respectively (Table 2). A possible explanation of their relatively small values might be that, under the relatively adverse conditions of hypersalinity *in situ* (and in the calorimetric vessel), both the assemblages were

far from maintaining the 'limit' flux. Another possibility is that this might be a result of extremely high concentrations of their biomasses (and biosurfaces) in the lake. The more intriguing result was that the ratios between the algal and bacterial vector heat fluxes were relatively small ($< 10^1$) and, in March, even inverted, as well illustrated in Figure 3. This might also indicate the scale independence of the variable. The ecological meaning of this phenomenon has to be coupled with that of the concept of the *biosurface*. 'Biovolume' is frequently used as a term and a time-variable quantity in aquatic ecology, but the 'biosurface' is often underestimated by ecologists even though potentially it can have even more profound ecological meaning than 'biovolume'.

To illustrate the point, if it is assumed for the marine bacterioplankton in the Black Sea (Chepurnova et al. 1988) that the average cell abundance is 10^6 cells ml^{-1} and the cell volume is 0.1 μm^3 (spherical cells), then its biosurface equals 1.13 m^2 m^{-3} or about 17 m^2 m^{-2} for coastal waters at the average depth of 15 m. The total bacterial biosurface in the lake was close to this value (Table 3). However, the biosurface concentration (from 32.4 to 79.2 m^2 m^{-3}) was higher by almost two orders of magnitude. On the contrary, the lake bacteria demonstrated lower vector heat fluxes at 20 ºC, 1.4 to 5.1 fW μm^{-2} (Table 2), as opposed to about 20 to 50 fW μm^{-2} in marine bacteria based on data for Sevastopol Bay (calculated from Mukhanov et al. 2003).

Turning to the marine phytoplankton, the maximum biosurface of an individual species does not exceed about 0.4 m^2 m^{-3} (*Cerataulina sp.*) while the estimated total value for the whole phytoplankton community is usually below 40 m^2 m^{-3} according to data for the Northern coastal waters of the Black Sea (Minicheva and Zotov, 2003). In contrast, the value for the same variable for the lake pleuston was never lower than 127 m^2 m^{-2} (in March). In April, the total biosurface of the *Cladophora* population reached the enormous value of 2926 m^2 per m^2 of the lake surface, concentrated in the thin mat layer. It seems highly likely much of the mat consisted of dead algae which served only as a skeleton for the living matter. As a result, the so-called 'surplus' of non-functioning biosurface could produce underestimated fluxes. This could be true for the bacteria as well, but probably to a lesser degree. Undoubtedly, viability tests would improve the flux estimates under such conditions, and it is even possible that the values close to the 'limit' flux might be detected for individual, actively growing *Cladophora* filaments and bacterial cells. However, the averaged estimates obtained for the total cell population including dead and inactive/dormant cells make *ecological* 'sense' because they characterize the *efficiency* of energy/matter transport through the total biosurface of a natural microbial community. The same index might be a good addition to the analyses of other community levels.

Unfortunately, ecological studies applying the microcalorimetric approach are still rare (Pamatmat et al. 1981; Pamatmat 2003; Tornblom 1995; also see review by Larsson and Gustafsson 1999). In some ways, this study only begins to explore the potential of direct calorimetry for investigating the metabolic fluxes and energy flows in plankton and pleuston communities as well as aspects of their non-equilibrium thermodynamics. There are still technical problems to solve and its use is not essential for studying the energetics of living systems that deploy only aerobic catabolic processes because this can be assessed by indirect calorimetry to measure the oxygen uptake/evolution rate. Nevertheless, there is no substitute for direct calorimetry for investigating the overall process of anaerobic/anoxygenic metabolism. In addition, no other method gives the instantaneous rate of metabolism and thus ready access to the kinetics of it. This potential would be most suitably realized if the heat flow measurements could be conducted under controlled light conditions as well as in the dark. In respect of aquatic systems and, in particular, the application to floating and benthic mats, calorimetric measurements should give deeper insight into the patterns of switching between different metabolic pathways under highly fluctuating pH and Eh conditions. In particular, one of the more intriguing components of the mat metabolism, that of anoxygenic photosynthesis (Padan 1989; Krumbein and Stal 1991) by some of the organisms, can be measured by photomicrocalorimetry (Petrov 1975; Johansson and Wadsö 1997) that can provide simultaneous anaerobic and visible light experimental conditions. With this addition to the armoury of the ecological calorimetrist, it will be possible to add vital information about energy flows to that already elucidated for material flows in aquatic communities.

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