ELSEVIER

Thermochimica Acta 397 (2003) 31–35

thermochimica acta

www.elsevier.com/locate/tca

Productivity and thermodynamics of marine bacterioplankton: an inter-ecosystem comparison☆

V. Mukhanov[a], O. Rylkova[a], O. Lopukhina[a], R.B. Kemp[b,*]

[a] *Institute of Biology of the Southern Seas, 2 Nakhimov Avenue, Sevastopol 99011, Ukraine*
[b] *Institute of Biological Sciences, University of Wales, Aberystwyth, Wales SY23 3DA, UK*

Received 11 September 2001; received in revised form 25 April 2002; accepted 29 April 2002

Abstract

A comparative study of the bacterioplankton abundance and functional activity was carried out in July and August 1999 in Sevastopol Bay (SB; Black Sea, Ukraine), which is warm-temperate and under considerable anthropogenic influence, and in the coastal water of the shoreline near Aberystwyth (Ab; Cardigan Bay, Wales, UK) that is cold-temperate and relatively clean. The chosen index for the investigation was the cell-specific, instantaneous rate of heat production (scalar heat flux) because it reflects the kinetics and thermodynamics of metabolism. The measurement of the native samples to secure this index was the extensive heat flow rate using an improved microcalorimetric method.

It was found that in the SB ecosystem, the average in situ bacterial abundance (*A*), biomass turnover rate (*K*), production (*P*) and cell-specific heat flux (*H*) were significantly higher than at Ab, with a tendency for values to be more variable ($2.13 \times 10^6 \pm 1.30 \times 10^6$ cells cm^{-3} (*A*), $0.05 \pm 0.02\,h^{-1}$ (*K*), 1.48 ± 0.53 mg C $m^{-3}\,h^{-1}$ (*P*), 34.51 ± 23.5 fW per cell (*H*) in SB versus $0.96 \times 10^6 \pm 0.15 \times 10^6$ (*A*), 0.015 (*K*), 0.25 (*P*), 22.31 ± 5.84 (*H*) in Ab, in the same units). The enhanced bacterial activity was partly due to the higher temperature conditions in SB (24 °C versus 17.7 °C in Ab). With the exception of the mean heat flux (19.3 fW per cell in SB versus 22.3 fW per cell in Ab), however, the SB data corrected to the average in situ temperature in Ab remained higher. The daily entropy production of the bacterioplankton communities, calculated on a volume-specific basis, was greater in the more eutrophic and polluted waters (16.0 J $m^{-3}\,K^{-1}$ per day in SB versus 6.6 J $m^{-3}\,K^{-1}$ per day in Ab and 17.0 J $m^{-3}\,K^{-1}$ per day at the polluted versus 15.0 J $m^{-3}\,K^{-1}$ per day at the unpolluted stations in SB, respectively).

Keywords: Bacterioplankton; Metabolic activity; Entropy production; Calorimetry; Heat flux

1. Introduction

It was not until relatively recent times that microcalorimetry has been recognized as a powerful tool in studies of natural microbial communities, but even now, only a few ecological studies have been carried out using this approach ([1–3]; reviews in [4,5]). The break-through is based mainly on the discovery that this non-specific method can measure the integrated metabolism, including both anaerobic and aerobic respiratory pathways of a mixed cell assemblage. It is principally important for studying cell bioenergetics and energy flows through microbial food webs under adverse natural conditions that are characterized

☆ Presented at the 12th Conference of the International Society for Biological Calorimetry, Santiago de Compostela, Spain, 7–11 September 2001.

* Corresponding author. Tel.: +44-1970-622-333; fax: +44-1970-622-350.
E-mail address: rbk@aber.ac.uk (R.B. Kemp).

0040-6031/02/$ – see front matter
PII: S0040-6031(02)00313-1

by low substrate concentrations, non-optimal temperatures, non-ideal osmotic/redox potentials, significant spatio-temporal variability in chemical and physical factors and anthropogenic impacts. In addition, by placing the whole aquatic, microbial community into a measuring ampoule, an excellent opportunity has been created to gain insight into the irreversible thermodynamics of complex biological systems.

The present study illustrates the use of the calorimetric method, in combination with conventional microbiological techniques for quantifying energy flows and estimating some crucial thermodynamic variables in two bacterioplankton communities. These sites provided an appropriate contrast in environmental terms because the exposed waters at the temperate site in Cardigan Bay are comparatively unpolluted, whereas there is considerable anthropogenic influence on the relatively closed waters of the warm-temperate Sevastopol Bay (SB).

2. Experimental

Samples were collected during July and August 1999 from the surface layer of seawater at designated sites in SB (Black Sea, Ukraine), which differed in the level of pollution, and in the coastal waters of Cardigan Bay adjacent to Aberystwyth (Ab; Wales, UK; Fig. 1). For the former, the sampling sites were divided into two sets: (i) the less polluted ones, where water exchange with the open sea was reasonable (station 1 at the mouth of the bay and station 2 in the central part of the bay); (ii) the more polluted peripheral areas (station 3 in the southern bay and station 4 in the main bay, near Inkerman). Bacteria were counted using epifluorescence microscopy after staining with proflavine [6,7]. A Zeiss standard microscope equipped with an HBO-50 mercury burner was used for all observations. At least 200 cells and 20 fields were counted from each preparation.

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) by an innovative technique developed by Mukhanov et al. ([8], also a paper in preparation) that involved: (i) fractionation of the seawater samples (500–1000 cm^3) to remove zoo- and phytoplankton (using 12 μm pore size membranes); (ii) concentration of the picoplankton onto nitrocellulose membranes, 0.2 μm pore size (see the schema in Fig. 2). The wet membrane (or its fragment of known area) with the concentrated cells was placed into a calorimetric glass ampoule containing 2 cm^3 seawater, which had been taken from the same site and sterilized by microfiltration (Sartorius membranes, 0.1 μm pore size, 47 mm diameter, were used for preparing the particle-free seawater). The ampoule was hermetically sealed and the bacterial heat production rate was measured immediately after loading the glass ampoule with its filter membrane carrying the bacteria into the batch module of the microcalorimeter. At this point, all the cells were either on or inside the membrane matrix. All the microcalorimetric experiments were carried out at 20 °C. The cell-specific

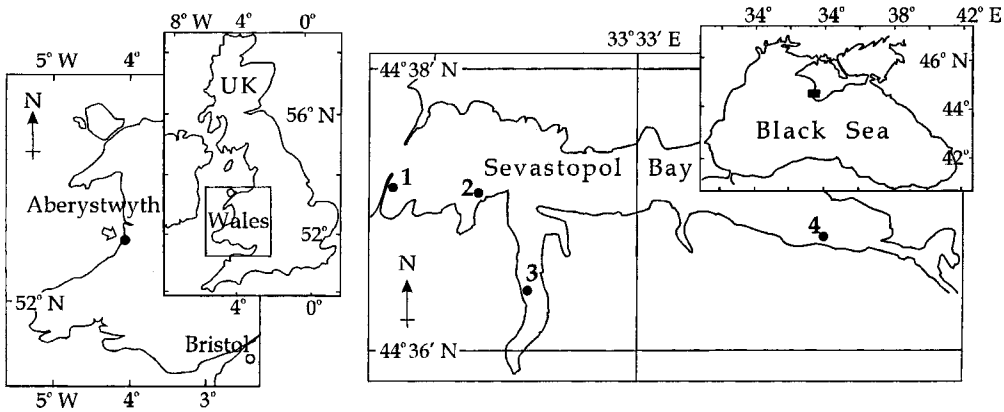

Fig. 1. Sampling sites in Crimea, Ukraine and Wales, UK.

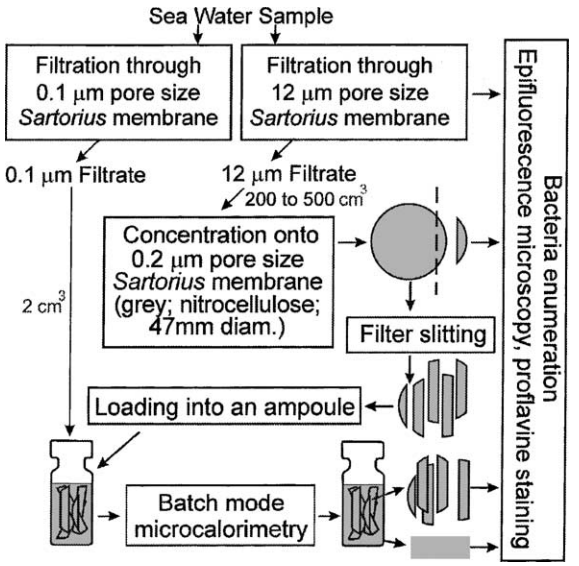

Fig. 2. The experimental design.

kinetic variables (e.g. specific growth rates and heat fluxes) were corrected to the in situ temperatures, assuming $Q_{10} = 2.5$.

The dry biomass of the bacterioplankton and the carbon equivalent of the wet biomass were calculated using the factors 0.2 and 0.1, respectively, according to [9,10]. The conversion factor of 0.22 pg C μm^{-3} [11] was used to calculate the cell volume from the carbon data. Cell-specific respiration rates were calculated from the data for the heat flux, using the oxycaloric equivalent of $-450\,kJ\,mol^{-1}$ O_2 [12]. The total number of complex experiments performed was 15, including the abundance and the production measurements together with the microcalorimetry.

3. Results and discussion

The results of the comparative experiments are summarized in Table 1 and some aspects are highlighted in Fig. 3. It was found that in the ecosystem of SB, the average bacterial abundance in situ (A), the biomass turnover rate (K), the daily production (P) and the cell-specific heat flux (H) were significantly higher than in Ab (unpaired two-tailed t-test; B: $t = 1.98$, $P = 0.07$; K: $P < 0.05$; P: $P < 0.001$; H: $P = 0.09$), with a tendency for values to be more variable ($2.13\times10^6\pm1.30\times10^6$ cells cm^{-3} (A), $0.05\pm0.02\,h^{-1}$ (K), 1.48 ± 0.53 mg C $m^{-3}\,h^{-1}$ (P), 34.51 ± 23.5 fW per cell (H) in SB versus $0.96 \times 10^6 \pm 0.15 \times 10^6$ (A), 0.015 (K), 0.25 (P), 22.31 ± 5.84 (H) in Ab, in the same units). The enhanced bacterial activity was partly due to the higher temperature conditions in SB (24 °C versus 17.7 °C in Ab).

Table 1
Biomass, activity and thermodynamics of the bacterioplankton in Sevastopol Bay and in the coastal waters of the shoreline near Aberystwyth

Variables	Sevastopol Bay				Aberystwyth mean in situ
	Stations 1 and 2	Stations 3 and 4	Mean in situ	Mean (17.7 °C)	
A (10^6 cells ml^{-1})	1.19 ± 0.75	3.07 ± 1.01	2.13 ± 1.30		0.96 ± 0.15
B (mg C m^{-3})	26.18 ± 16.55	67.54 ± 22.18	46.86 ± 28.56		21.12 ± 3.28
B_e (kJ m^{-3})	1.204	3.107	2.156		0.972
K ($10^{-2}\,h^{-1}$)	6.29 ± 2.21	3.75 ± 2.08	5.00 ± 2.42	2.79 ± 1.33	1.50 ± 1.13
P (mg C $m^{-3}\,h^{-1}$)	1.46 ± 0.42	1.51 ± 0.69	1.48 ± 0.53	0.83 ± 0.30	0.25 ± 0.22
H (10^{-15} W per cell)	46.51 ± 32.9	22.50 ± 2.52	34.51 ± 23.5	19.33 ± 13.2	22.31 ± 5.84
R[a] (fmol O_2 per day per cell)	8.93 ± 6.32	4.32 ± 0.48	6.63 ± 4.51	3.71 ± 2.53	4.28 ± 1.12
M (kJ $m^{-3}\,h^{-1}$)	0.19 ± 0.06	0.21 ± 0.05	0.20 ± 0.12	0.11 ± 0.06	0.08 ± 0.03
M[a] (mmol O_2 m^{-3} per day)	10.13 ± 3.22	11.20 ± 2.67	10.68 ± 6.40	5.86 ± 3.21	4.27 ± 1.60
E (J $m^{-3}\,h^{-1}\,K^{-1}$)	0.625	0.710	0.667		0.275
E/B_e ratio ($10^{-4}\,h^{-1}\,K^{-1}$)	5.188	2.283	3.092		2.829
T in situ (°C)	23.8 ± 1.40	24.00 ± 2.30	24.00 ± 2.10		17.70 ± 1.60

A: cell abundance; B: biomass; B_e: energy equivalent of the biomass calculated for the average biomass values; K: biomass turnover rate; P: production; H: cell-specific heat flux; R: respiration rate (calculated from H by using the oxycaloric equivalent of $-450\,kJ\,mol^{-1}$ O_2) [12]; M: community metabolic losses; E: entropy production; T: in situ temperature. The values are mean ± S.D.

[a] Respiration rates were calculated on a "per day" basis to allow the convenient comparison with the published hydrobiological data.

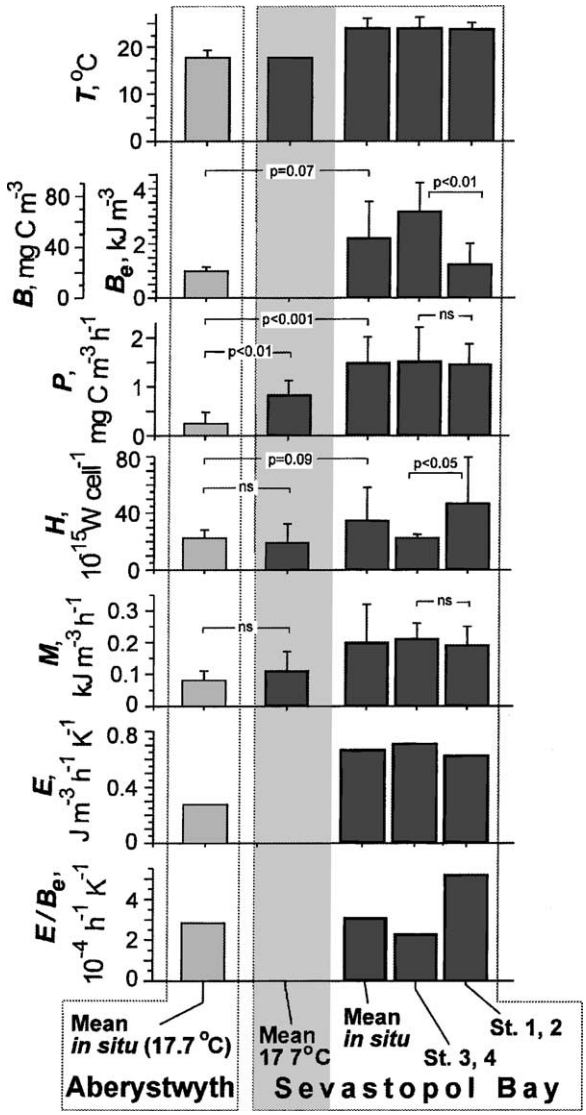

Fig. 3. Functional and thermodynamic characteristics of the bacterioplankton in the studied ecosystems. *B*: biomass; B_e: energy equivalent of the biomass; *P*: production; *H*: cell-specific heat flux; *M*: community metabolic losses; *E*: entropy production; *T*: in situ temperature. Values are mean ± S.D. Data were compared with unpaired two-tailed *t*-test; ns: not significant.

In order to negate the possible temperature effect, the SB data were corrected to the average in situ temperature in Ab ($Q_{10} = 2.5$)—these data are presented against the Grey background in Fig. 3. With the exception of the mean heat flux (19.3 fW per cell in SB versus 22.3 fW per cell in Ab), the corrected values remained higher in SB. The difference was significant only for the variable *P* ($P < 0.01$) after normalization. This may be explained by the anthropogenic eutrophication and the organic pollution sustaining a higher standing stock and, as a result, the greater productivity of the bacteria in SB. The slightly decreased heat fluxes after correction could indicate the depression of bacterial metabolism in the polluted aquatic areas. However, the statistical analysis does not support this suggestion. Similarly, the heat fluxes measured within the boundaries of SB were lower at the more polluted stations (# 3 and 4) where the bacterial biomass (*B*) was almost three times as large as that measured at stations 1 and 2 (67.54 mg C m^{-3} versus 26.18 mg C m^{-3}; *t*-test: $t = 2.99$, $P < 0.05$). In general, the highest metabolic activity was observed at low bacterial concentrations, and vice versa. This was likely to be due to an oscillatory dynamics of the summer planktonic microbial community.

Present estimates of the bacterioplankton metabolism well agree with published data on heterotrophic picoplankton respiration. The bacterial heat flux measured in the Ab community and expressed as the cell-specific respiration (4.28 ± 1.12 fmol O_2 per day per cell, Table 1) was similar to that estimated by Blight et al. [13] for the <0.8 μm planktonic fraction in North Wales (UK) waters (0.4–6.8 fmol O_2 per day per cell) and obtained by Biddanda et al. [14] for the <1 μm fraction in Louisiana (USA) shelf waters (2.4–8.7 fmol O_2 per day per cell). The same estimates for SB are in good agreement with Shumakova's data [15] on the respiration of bacterioplankton in summer at the stations 1 and 2 in SB. Blight et al. [13] noted that cell-specific respiration values may be underestimated owing to the specificity of the fluorochrome: the DAPI count may potentially overestimate the number of metabolically active bacteria [16]. The same remark is true for staining with proflavine as well.

The total community metabolism in Ab (4.27 ± 1.60 mmol O_2 m^{-3} per day in terms of the purely aerobic process) proved to be also in the range (approximately 1–17 mmol O_2 m^{-3} per day) documented by Blight et al. [13] that provides additional evidence of the reliability of the method. In this connection, the combination of the respirometric and the calorimetric

measurements [12] seem to be very promising and distinctly valuable for aquatic microbiologists, providing information on the ratio between aerobic and anaerobic processes in mixed assemblages. It is suggested that this combination of methods can be improved by using a technique that marks the metabolically active cells (e.g. CTC staining for identification of the cells with active electron transport system) [17].

One of the values of the microcalorimetric approach lies in the fact that, besides conventional hydrobiological variables, it allows the estimation of some thermodynamic properties of natural microbial communities. It was found in this study that the daily entropy production of the bacterioplankton communities in the seawater, calculated on a volume-specific basis was greater in the more eutrophic and polluted waters (16.0 $J\,m^{-3}\,K^{-1}$ in SB versus 6.6 $J\,m^{-3}\,K^{-1}$ in Ab; and 17.0 $J\,m^{-3}\,K^{-1}$ versus 15.0 $J\,m^{-3}\,K^{-1}$ at the polluted and relatively clean stations in SB, respectively; Fig. 3). In thermodynamic terms, interpreting the results depend on which of the natural processes is considered, the ecological succession from oligotrophy to eutrophy (as in lakes) or the biological self-purification of the water environment. Thus, the results corroborate either of the 'conflicting' thermodynamic concepts, the 'two-stages principle' of entropy production (in ontogenesis) or the well-known minimum entropy production principle postulated by Prigogine and Wiame [18]. Pleasant as it is to speculate, it is nevertheless likely that such generalizations are rather premature, especially for complex marine ecosystems exposed to anthropogenic stress.

Despite a considerable difference in temperature conditions and the extent of seawater pollution, the E/B_e ratio (the entropy production per the energy equivalent in the living biomass) proved to be similar in both the ecosystems (3.1×10^{-4} and $2.8 \times 10^{-4}\,h^{-1}\,K^{-1}$ in SB and Ab, respectively; Fig. 3). It is noteworthy that the estimates of the E/B_e ratio obtained at different sites in SB fluctuated about the mean value evaluated for Ab, the ecosystem of which is more healthy and safer with respect to its ecology.

Acknowledgements

The authors are grateful to Ms. Olga G. Naidanova and Dr. Vladimir P. Parchevsky for providing valuable advice in the course of this investigation. The work was supported by INTAS Grant nos. 96-1961 and 99-1390.

References

[1] M.M. Pamatmat, G. Graf, W. Bengtsson, C.S. Novak, Mar. Ecol. Progr. Ser. 4 (1981) 135.
[2] H. Vandenhove, R. Merckx, H. Wilmots, K. Vlassak, Soil Biol. Biochem. 23 (1991) 1133.
[3] E. Tornblom, Microbial Activity and Biomass in Aquatic Surface Sediments: A Community Level Approach, PhD thesis, Institute of Limnology, Uppsala University, 1995.
[4] L. Gustafsson, in: M.A. Sleigh (Ed.), Microbes in the Sea, Ellis Horwood Ltd., Chichester, 1987, p. 167.
[5] B. Larsson, L. Gustafsson, in: R.B. Kemp (Ed.), Handbook of Thermal Analysis and Calorimetry, Vol. 4, Elsevier, Amsterdam, 1999, p. 390.
[6] J.E. Hobbie, R.J. Daley, S. Jasper, Appl. Environ. Microbiol. 33 (1977) 1225.
[7] L.W. Haas, Ann. Inst. Oceanogr. (Paris) 58 (1982) 261.
[8] V.S. Mukhanov, O.A. Rylkova, O.A. Lopukhina, R.B. Kemp, in: Proceedings of the Third Interdisciplinary Symposium, Moscow State University Press, Moscow, Russia, 2000, p. 12.
[9] S.E. Luria, in: I.C. Gunsalus, R.Y. Stainer (Eds.), The Bacteria, Vol. 1, Academic Press, New York, 1960, p. 1.
[10] Y.I. Sorokin, H. Kadota (Eds.), Techniques for the Assessment of Microbial Production and Decomposition in Fresh Waters, I.B.P. Handbook, Vol. 23, Blackwell Scientific Publications, Oxford, 1972.
[11] G. Bratbak, I. Dundas, Appl. Environ. Microbiol. 48 (1984) 755.
[12] E. Gnaiger, R.B. Kemp, Biochim. Biophys. Acta 1016 (1990) 328.
[13] S.P. Blight, T.L. Bentley, D. Lefevre, C. Robinson, R. Rodrigues, J. Rowlands, P.J.leB. Williams, Mar. Ecol. Progr. Ser. 128 (1995) 61.
[14] B. Biddanda, S. Opsahl, R. Benner, Limnol. Oceanogr. 39 (1994) 1259.
[15] G.V. Shumakova, Ekologiya Morya 9 (1987) 25 (in Russian).
[16] U.L. Zweifel, Å. Hagström, Appl. Environ. Microbiol. 61 (1995) 2180.
[17] G.G. Rodriguez, D. Phipps, K. Ishiguro, H.F. Ridgway, Appl. Environ. Microbiol. 58 (1992) 1801.
[18] I. Prigogine, J.M. Wiame, Experientia 2 (1946) 451.