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Size spectra of heat production of microplankton of Sevastopol Bay ☆

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Abstract

The object of this study is the heat production size spectra, i.e. the integral value of heat production over the size fractions (standard increments of logarithm of the particle size) of microplankton (organisms < 120 μm). The particles of the sea water samples analyzed were separated and concentrated into fractions of different sizes by nylon nets, Sartorius membrane filters and a specially constructed fractionation–concentration unit.

The heat production of the fractions was registered by a Thermal Activity Monitor during incubation times of 40–50 h at 20°C in the dependence on the sample volume, the fraction composition, and the reference charge. 20 experiments were conducted between December 1993 and April 1994 in the Sevastopol Bay, a Black Sea harbour under anthropogenic influence.

The results obtained reflect the good metabolic state of the concentrated organisms. The heat production size spectra were quite comparable with formerly obtained size spectra of the intracellular adenosine triphosphate content. The microcalorimetric signal level (10–30 μW) evidenced the applicability of this method for studying organisms of the femto- and picoplankton range (<0.2 and 0.2–2.0 μm, respectively) and for monitoring microbial communities of oligotrophic and deep sea waters.

Keywords: Aquatic ecology; Heat production; Microplankton; Size spectra

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1. Introduction

Environmental changes are among the most acute and important problems of our time. There are numerous studies and extensive scientific programs (e.g. International Geosphere Biosphere Program, Global Ocean Flux, etc.) aimed at understanding the interactive physical, chemical and biological processes that regulate the unique environment for our life on earth and investigating the reciprocal influence of human actions [1]. Today there is an ever growing understanding of the necessity for new general models of ecological systems, taking into account more modern and realistic considerations of matter and energy cycling in the terrestrial biosphere [2–4].

However, there is still another important and complicated component, the biota, the totality of living organisms inhabiting the environment. Of all kinds of matter, just "living matter" [5,6]—living organisms and their exometabolites—is notable for the greatest activity. A complete description and a good understanding of its main structural and functional parameters are essential for the rational use and protection of the environment. Steady progress of biophysical and biochemical sciences and techniques contributes to the solution of these global problems. However, operational description schemes and new methods are required.

It seems that size spectra (SS), i.e. distribution functions of the different parameters studied (e.g. biomass, energy dissipation) over the size fractions (Fig. 1), may serve as a prospective method for studying the biota [7–9]. As is well known [10,11] and can be seen from Fig. 1, the smallest organisms (< 100 μm, i.e. $\log D < 2$ in Fig. 1) play a leading role in sea water concerning matter and energy turnover.

SS analysis reveals the "left side problem" [4], i.e. misrepresentations because of insufficiently detailed information on the small-sized part of the community and omission of some groups of organisms. Small organisms are extremely complex for being investigated by traditional methods, but fortunately they are well suited to study by modern optics, electronics and molecular biochemistry [12]. Application of such instruments as fluorescent flow cytometers, cell sorters or microcalorimeters allows a continuous registration of parameters of the size fractions, resulting in typical patterns [9]. Differences between the patterns may be used for diagnostics and quantitative estimations of changes.

Our studies of 1984–1991 in various regions of the world ocean, including the Antarctic and the Black Sea, show a rather good correlation of metabolic processes with the cell surface and intracellular ATP content of the size fractions of microplankton [13–18]. These studies reveal complicated dynamics of the microplankton (i.e. organisms < 120 μm) size fractions and show the necessity for an automatic and continuous registration of the parameters to be analyzed [14].

Because of its high precision, sensitivity and level of automation, microcalorimetry seems to be a good complement to the studies of ATP size spectra. Considering the living matter as an integral dissipative structure governed by the energy throughflow [19], the SS analysis can lead to a development of thermodynamic criteria of the stability of living matter of ecosystems on earth [20]. Studies of the

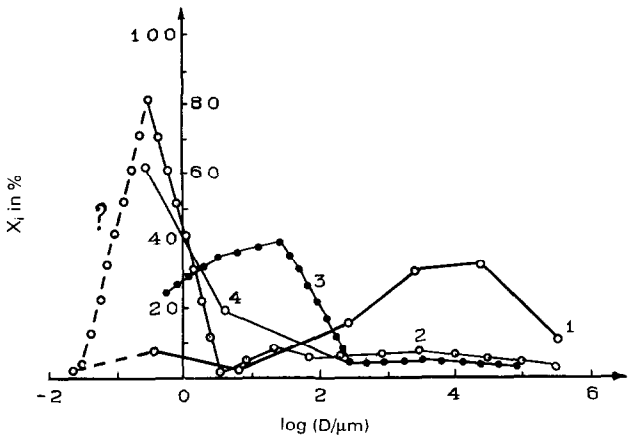

Fig. 1. Different size spectra (SS), i.e. distribution of different parameters X_i of the living matter of the world ocean over size classes, which are given as standard intervals of the logarithm of the body size D of living organisms. 1, Biomass; 2, respiration of organisms; 3, production of organic matter; 4, change of 3 resulting from data on the synthesis of organic matter by phototrophic picoplankton (cells of size 0.2–2.0 μm).

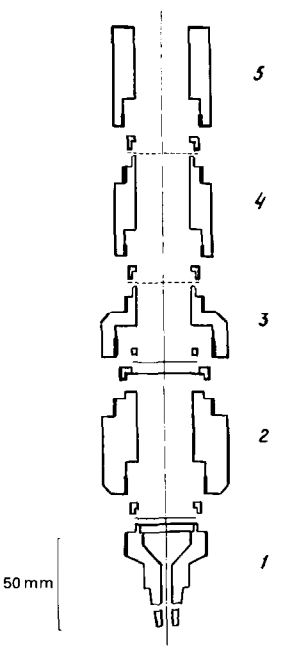

Fig. 2. Size fractionation–concentration unit: 1, base, 2–5; sections supporting membrane filters of diminishing pore size [15].

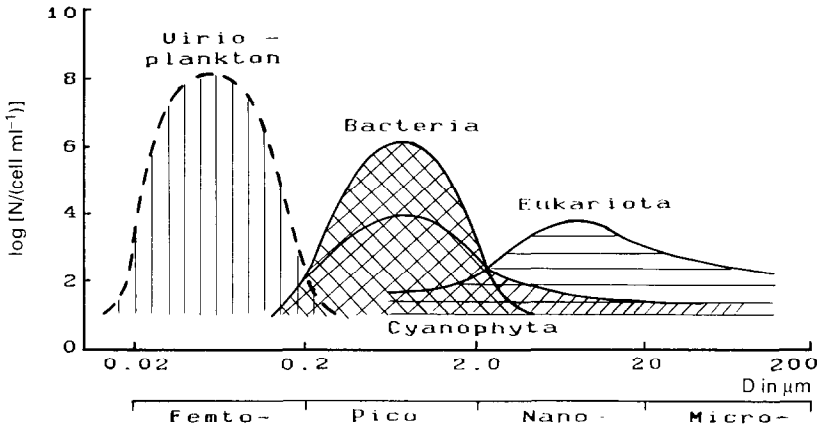

Fig. 3. Distribution of the cell density N (cells ml^{-1}) of the major taxonomic groups of microplankton of the world ocean by size fraction.

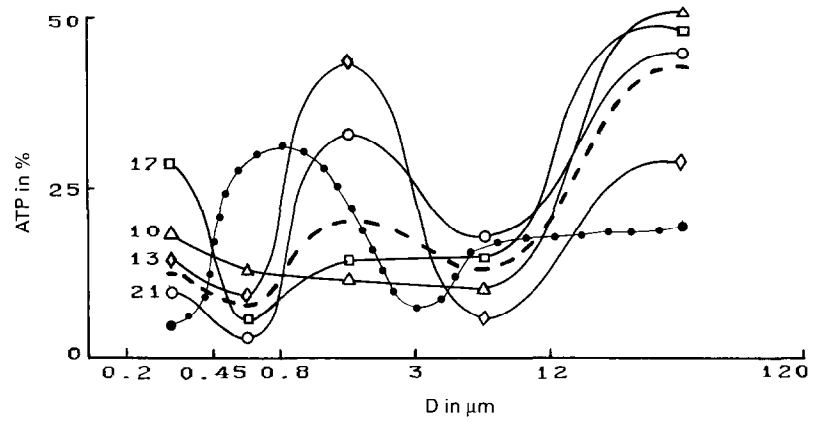

Fig. 4. ATP size spectra of microplankton from Sevastopol Bay. Numbers 10–21 correspond to the time of day (h). The broken line is the mean daily spectrum; the dotted line shows the mean ATP size spectra for oligotrophic waters of the Atlantic Ocean.

heat dissipation size spectra can be regarded as a unique and very important method.

The present study is aimed at the analysis of microplankton heat production size spectra and some difficulties of obtaining and interpreting the microcalorimetric data.

2. Experimental

Seawater samples (250–500 ml) were taken at 9 a.m. from the surface by means of a plastic bucket and filtered through a nylon sieve of 120 μm mesh size to re-

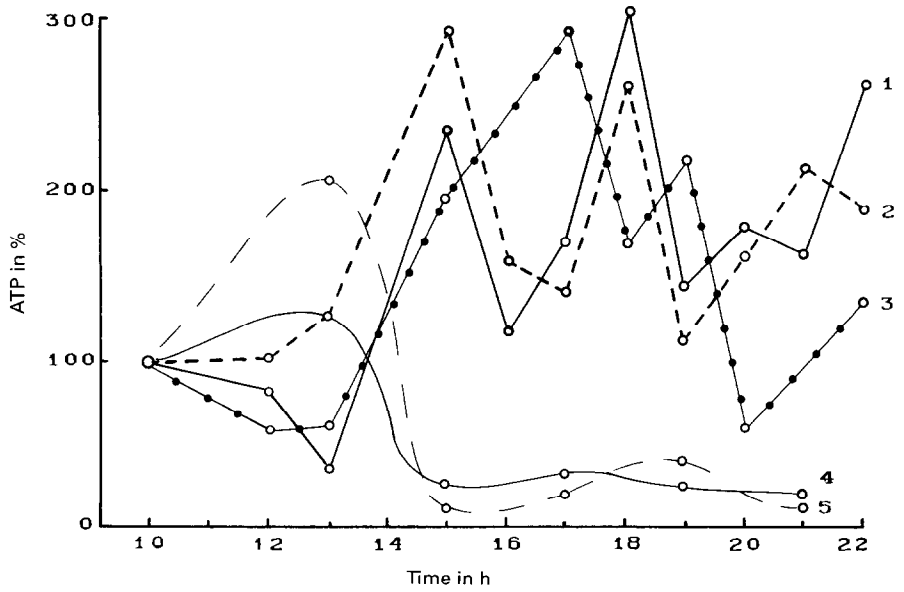

Fig. 5. Daily dynamics of the ATP content of different size fractions of microplankton of Sevastopol Bay (% of initial values). 1, ATP of the (3–120) μm fraction; 2, (0.45–3.0) μm fraction; 3, (0.2–0.45) μm fraction; 4, chlorophyll-*a* content of the (0.45–120) μm fraction; 5, (0.45–3.0) μm fraction.

move large particles. The samples were prepared for incubation in the following manner.

2.1. Series 1

Samples of native sea water were introduced by pipette into glass ampoules (2.5 ml), which were sealed for measurement. The reference ampoule was filled with distilled water.

2.2. Series 2

The microplankton organisms were concentrated on membrane filters and divided into size fractions with the help of the "microplankton fractioner" unit (i.e. size fractionation and concentration of the plankton) (Fig. 2) [15]. A nylon sieve of 15 μm diagonal, a 2.5 μm nucleopore filter, and Sartorius membrane filters of 0.2, 0.1, 0.05 μm pore size were used for filtration under a vacuum of 0.2 atm. The filters were placed into ampoules, and water which had passed through the concentrator (i.e. 0.05 μm pore size filter) was added as "sterile water" [10].

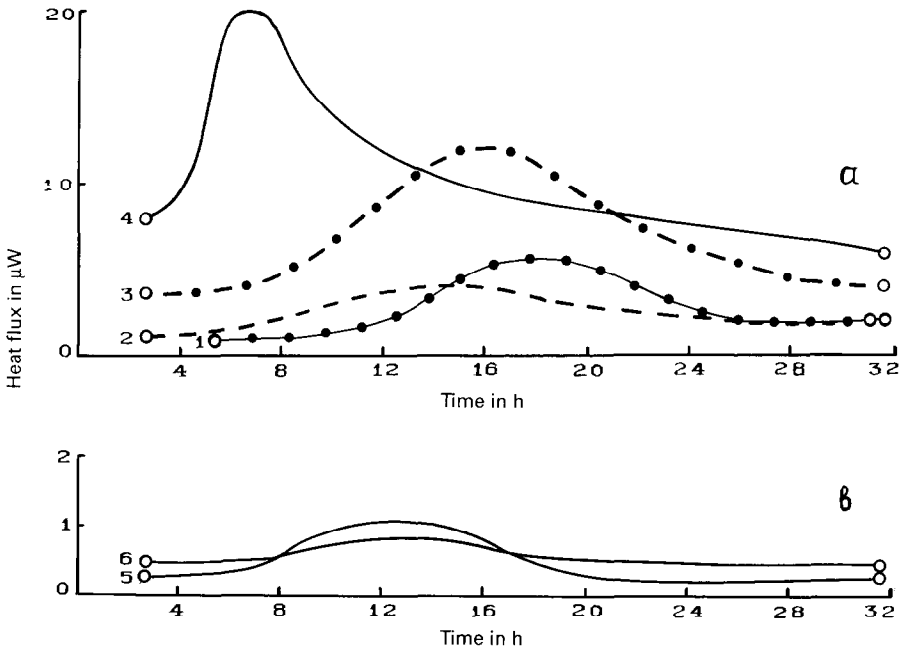

Fig. 6. Daily dynamics of the heat production of the different size fractions of microplankton of Sevastopol Bay (reference, distilled water). Size fractions (μm): 1, (0.05–0.2); 2, (0.2–2.5), concentrated in the water above the filters; 3 and 4, the same as in 1 and 2, but concentrated and incubated on the filters; 5, natural sea water; 6, "sterile sea water", i.e. the water which has passed through a 0.05 μm pore size membrane.

2.3. Series 3

Portions of "sterile water", alone or with concentrated size fractions or organ introduced into the ampoules without the filters. The reference ampoule for series 2 and 3 was filled with distilled water.

The ampoules were transferred into a Thermometrics-2277 Thermal Activity Monitor (TAM); after thermal equilibration for 60 min, the heat signals were registered during 40–50 h at 20°C.

2.4. Series 4

The same handling was performed as in series 2, but a sterile membrane filter was placed in the reference ampoule with the sterile water to take account of possible artifacts caused by chemical or physical processes.

Twenty experiments (four fractions in each) were made between October 1993 and April 1994 at Sevastopol Bay, the main Black Sea harbour which suffers from some anthropogenic influence. The surface water temperature during the period of studies was 7–10°C.

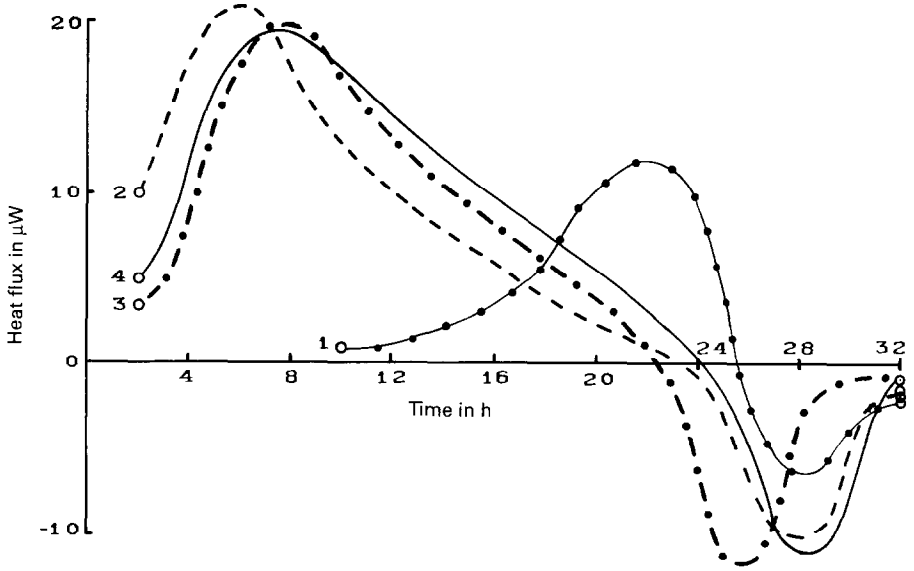

Fig. 7. Dynamics of heat production of the different size fractions of microplankton of Sevastopol Bay (reference, sea water sterilized by filtration through 0.05 μm pore size). Size fractions (μm): 1, (0.05–0.2); 2, (0.2–2.5); 3, (2.5–10); 4, (10–120).

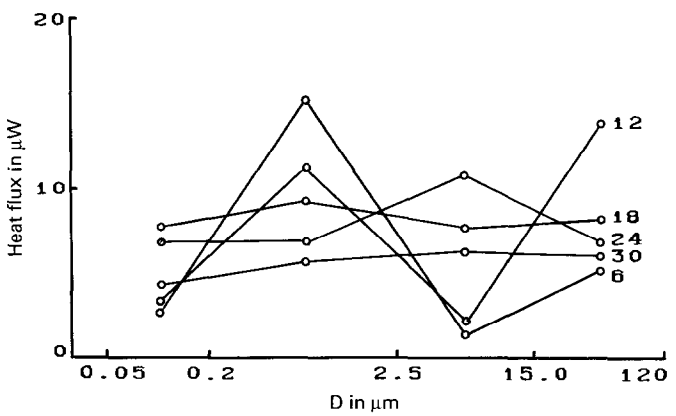

Fig. 8. Size spectra of the heat production of microplankton, derived from the dynamics of the heat production of the particular size fractions of series 2 experiments; numbers 6–30 correspond to the time (h) from the start of incubation.

3. Results

The microplankton ATP size spectra and the dynamics of ATP content in the different size fractions (Figs. 3–5, correspondingly) were obtained during earlier

studies [14,17]. From the heat evolution curves (Figs. 6 and 7) obtained during the present studies the heat production size spectra of Fig. 8 were derived.

4. Discussion

An analysis based on a theoretical model (the ideal minimum ecosystem, i.e. a hierarchical structure of living matter taken as a whole) leads to the prediction of a shift from a taxonomic description of natural aquatic communities to a formalized quantitative scheme of the biota decomposition (size spectra sets), which promotes the application of highly developed techniques and measurement of integral characteristics of the communities studied [4,19]. Operational descriptions of the structure and state of natural planktonic communities can be developed by the use of size spectra of the principal biochemical substances (ATP, DNA, RNA, proteins, photopigments) or heat dissipation by the application of a fluorescent flow cytometer, cell selectors and microcalorimeters and by mathematical processing of the data using allometric regression equations.

The living (more precisely, living and inanimate) matter of the ecosystem is investigated (in addition to this approach) simply as matter having a specific structure and composition. The study of "biological specificity" consists in investigations of basic chemical compounds rather than taxonomic units, which are not suited for on-line processing. Such systems of parameters describes the storage of genetic information and its use in the process of development of biomass, i.e. consumption and creation of organic substances.

The samples of native sea water or fractions of concentrated microorganisms (series 1 and 3) gave only low signal levels (Fig. 6). Higher signal levels (10–30 μW) were obtained from fractions concentrated and inoculated with the membrane filter technique (series 2 and 4; Figs. 6 and 7). A comparatively high signal level (10 μW) was obtained in a flow-through cylinder with concentrated microplankton kept in a 500 ml glass flask. Unfortunately, this was not the case with the particular size fractions. This line of investigations has to be developed with some modifications accounting for the results obtained in series 2–4 experiments. A registration time of 40 h proved to be sufficient for the heat production registration (Figs. 6 and 7).

The most striking result seems to be the temporary negative heat production rate obtained with the experiments of series 4 (Fig. 7), and might presumably be due to certain endothermic processes or to microbial growth in the reference ampoule. Importantly, these microorganisms are supposed to pass through 0.05 μm pores, which are even smaller than the 0.2 μm pores used for water sterilization in many experimental methods [11,21].

The separate investigations using electron microscopy and batch culture techniques, the existence of biological structures, e.g. *Treponema hyponeustonicum*, was found in the Black Sea and other seas, which have a size between 0.02 and 0.17 μm and are capable of proliferating and producing populations of heterogenous size. It was reported that other species can pass through small pores because of their soft cell structures [21]. The heat production curves (Figs. 6 and 7) are quite similar to

cell dynamics [10,22,23] describing the multiplication of bacteria in small volumes of sea water under the influence of solid surfaces as a matrix for adhesion. The roles of the surface-to-volume ratio [24] seems important for the results and methods discussed. Furthermore, the temperature of 20°C is optimal for culturing bacteria [10].

Thus the Thermal Activity Monitor seems to be the preferred tool for research on highly delicate and poorly studied small organisms of the pico- (0.2–2.0 μm) and femtoplankton (<0.2 μm) range. This point is very interesting, as such organisms are the main source for both fluxes (i.e. production and destruction) of organic matter in oligotrophic waters (about 50% of the earth's surface and all deep layers of the ocean). A study of such organisms can be of major importance for many global scale models of matter and energy budgets and nutrient cycling.

Acknowledgment

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