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### BIOCHEMICAL SUBSTRATES EXPENSES AND RESPIRATION AT SHORT-TERM STARVATION OF MARINE PLANKTONIC ANIMALS

#### UTILISATION DE SUBSTRATS BIOLOGIQUES ET RESPIRATION D'ESPECES ZOOPLANCTONIQUES EN JEUNE BREF

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One of the most important indices at estimation of productivity characteristics of hydrobionts is oxygen consumed in the process of respiration. Usually respiration (standard metabolism) is measured in small-volume respirometers (30-50 ml) where animals' activity is considerably restricted. It may underestimate respiration in comparison to natural one. Therefore respirometers of larger volume were used (600-1400 ml) (Pavlova, Melnik, 1982). Experimental containers were filled with filtered water with antibiotics (25 mg/l). Animals' state throughout 6 hours at such conditions may be named "a short-term starvation".

Simultaneously under same conditions we tried to estimate energy requirements by expense of main biochemical components of organic matter (lipids, protein, glycogen). Results were used to calculate energy budget and to compare energy expenditures, measured according to consumed oxygen and tissue substrates expense.

Three species of copepods' adults : *Scolecithrix danae* (Lubb.), *Pleuromamma abdominalis* (Lubb.), *Euchirella curticauda* Giersbr. and mature ostracods' individuals *Cypridina serrata* Müller were used. Collections were performed in tropical regions of the Indian ocean on board of R/V "Professor Vodyanitsky" in June-August. Carefully capturing crustaceans we tried not to disturb them. Velocity of planktonic net lifting was not over 0,4 m/sec. From several tows experimental animals were transformed to 10 l glass containers with filtered sea water. Individuals of the same species, sex and dimensions were divided in 2 groups. One of them was used for estimation of initial lipids, glycogen, nitrogen and carbon contents and total body mass, another one was transferred into experimental containers. Temperature was equal to 22- 24° C which corresponds to seawater temperature. Density of animals in respirometers was kept as low as possible (10-15 for *Pleuromamma* and *Euchirella* ; 30-40 for *Scolecithrix* ; 60-70 for *Cypridina*). Experiments were run in the darkness at night and at diffused light during the day. In 6 hours measuring of ammonia excretion, glycogen and lipids content and quantity of oxygen consumption was performed. Ammonia concentrations were determined by Koroleff's method (1969), lipids were extracted by Folch (Folch *et al.* 1957), glycogen was

measured by enzymatic method (Murat, Serfaly, 1974), oxygen changes were estimated by Winckler micromethod. Nitrogen and carbon content in samples, dried at 50°C were then analysed with CHN-analyser. The following calorific coefficients were used : protein - 4,3 (Schmidt-Nielsen, 1982), lipids - 9,3, glycogen - 4,2, oxygen - 3,5.

Figure 1 shows substrates' content in animals tissues immediately after capture. Quantity of lipids of 4 species fluctuated from 72 to 137, glycogen -3-27 µg.mg dry weight, nitrogen -7,3-11,5, carbon -30,0-43,0 % dry weight, respectively. Protein level was very stable in comparison to carbohydrates' and lipids' level. According to figure 1 data, there were not reliable difference in substrates content in the morning and evening, except for glycogen in ostracods. In the last case glycogen quantity in the morning was 6 times higher than in the evening. At the same time, it is necessary to note species' variability of studied components.

After short-term starvation (6 hrs) some changes in contents of protein, lipids and glycogen were recorded. Substrates expense in per cent to main expenditures is illustrated by a diagram (Fig.2). The data showed that copepod spent mostly lipids as energetic resources throughout day and night : 44-69 % dry weight (oval diagrams). Protein expenditures were considerably lower and equal to 30-40 %. Glycogen expenditures were not more than 13,5 % but its part in total expenditures for all species at night is relatively higher. Energy was got mainly under lipids oxidation : 57-72 % for ostracods, 63-83 % for copepods. Part of glycogen is small : up to 8,6 % for copepods and 13,3 % for ostracods (columns in figure 2).

These data are good agreement with the fact that copepods in the process of short-term starvation spend mostly lipids (Lee et al., 1974 ; Mayzaud, 1976 ; Muravskaya et al., 1980 ; etc), but it differs from Ikeda opinion that lipids expense begins only after 6 days of starvation (1971). It is noted that ostracods spent the same quantity of lipids as copepod throughout experiment. Probably it was due to peculiar physiological state of crustaceans, started reproduction. In July matured ostracods' eggs were in the body and in August eggs laying in water began. In the last case the greatest amount of glycogen expenditures was also observed.

Preceding works noted low glycogen content in the bodies of marine plankters, not higher than 2,2 % of body mass (Raymont, Conover, 1961 ; Zagorodnyaya, 1979 ; Pavlovskaya, Morozova, 1981). On such basis the role of glycogen participation in energetic expenditures was not considered as a rule. Received data allow to assume more considerable role of carbohydrates in physiological processes of planktonic crustaceans. These data prove the possibility of biochemical indices uses as one of the indicators of physiological state and functional activity of small planktonic animals.

Table 1 shows respiration values of 4 zooplankton species at simultaneous registration of biochemical substrates' expense during 6 hours of starvation. It is noted that usage of experimental containers of volumes larger than it is accepted at standard metabolism estimation, was the reason for mean values increase of respiration intensity at expense of addition to active metabolism (Pavlova, Melnik, 1982).

Increase of motive crustaceans activity throughout experiments may explain rise of lipids expenditures in comparison to other substrates and, therefore, protein reduction in common spent energy (Tabl.2).

Presence of simultaneous respiration data allow to compare energy expenditure, estimated by the sum of oxidized biochemical components of organic body matter and quantity of used oxygen. For daily measurements percent of substrates' energy divergence with respiration energy varies from 8 to 49 %. For *Scolecithrix*

coincidence of compared values may be quite satisfactory. For *Pleuromamma* and *Euchirella* substrates' energy is 23 % and 49 % correspondingly higher than respiration energy (table 2). In nature, these 2 species actively migrate and negatively react to light and these may cause noted deviations. It was opposite for ostracods, started laying eggs : respiration energy was 27 % higher than energy of biochemical substrates' sum. On the one hand, increase of oxygen amount may be caused by respiration of eggs laying in water throughout exposition, on the other hand, there may not be a full registration of biochemical substrates expended for eggs formation. Ostracods eggs laying in the night time in more intensive, hence, divergence of respiration value with calculate expenditure of biochemical substrates is more. For copepods in the night time energy of oxidized protein, lipids and carbohydrates is 30-38 % higher than respiration energy.

Thus for studied species (without ostracods at the moment of eggs laying) throughout 6 hours of starvation respiration energy values were lower than sums of expended biochemical substrates. As the darkness is more approximate to natural conditions for studied species of copepods, mean divergence value may be equal 38 %.

The same comparisons made earlier for planktonic animals also revealed excess of oxidized substrates' energy above oxygen consumption for 30-70 % (Ikeda, 1971 ; Mayzaud, 1973, 1976). Use of different methods and approaches by each author stipulate for different divergence degree of compared values. However, tendency to substrates' energy excess above respiration energy is identical. As regards analysis of possible causes of such divergence in studied balance it may be noted the following. Influence of stress at collection of organisms is much less than it was earlier supposed (Ikeda, Skjoldal, 1980). Therefore, at careful collection of planktonic samples and short period of food lack influence of laboratory conditions to physiological and biochemical processes may be considered approximately identical. However, final result may be influenced by different errors of used methods. Necessity to estimate the value of initial and final content of lipids and glycogen of different groups of animals (belonging to the same cohorta) cause some errors at calculation of losses. Undoubtedly, we must have reached more precise evaluation of protein losses by measuring it in the body and not by the value of nitrogen excretion as we had to do. Quite true is the opinion that used caloric coefficients may not fit for invertebrates (Mayzaud, 1976). All mentioned gives ground to explain all divergences in the balance at expense of methodic errors at measuring and calculation of estimated values, which must be considered at further researches.

In conclusion we may state that during first 6 hours of starvation conditions, more favourable to reveal spontaneous motion of mature tropical copepods, promote the increase of oxygen spent at respiration and, mostly, lipids expenditures. For ostracods, the main importance of lipids in total losses of body matter was marked at the period of active reproduction. Glycogen participation at estimation of total expenditures may be quite considerable, especially at night.

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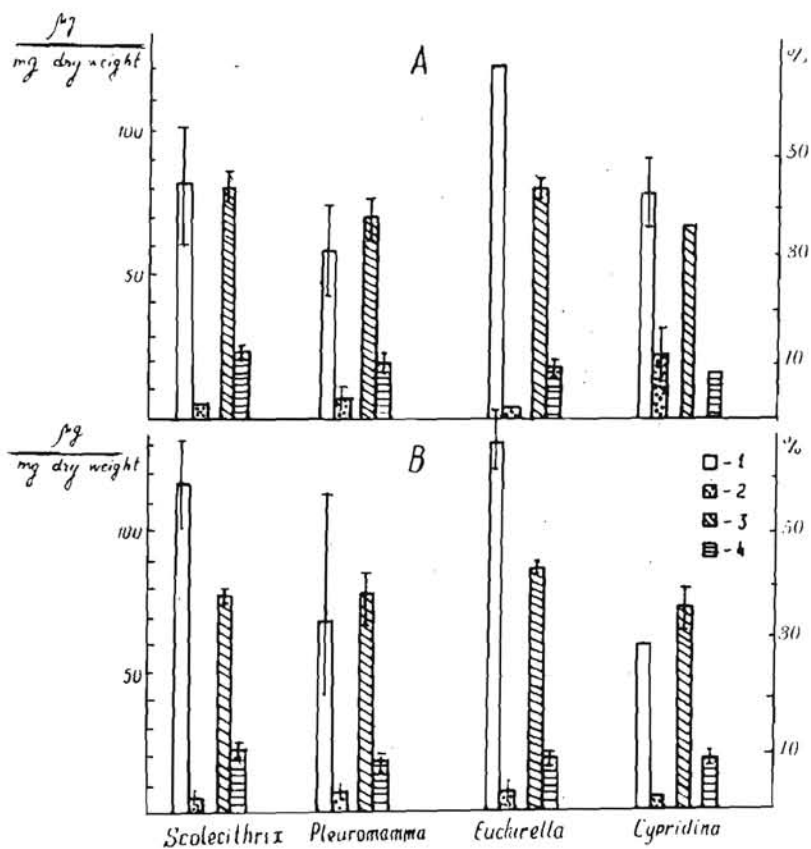


Figure 1 - Quantity of total lipids (1) and glycogen (2),  $\mu\text{g}/\text{mg}$ . dry weight, C - (3), N - (4) in % of dry weight of marine zooplankters' body, A - morning till 10 a.m., B - evening, later than 9 p.m.

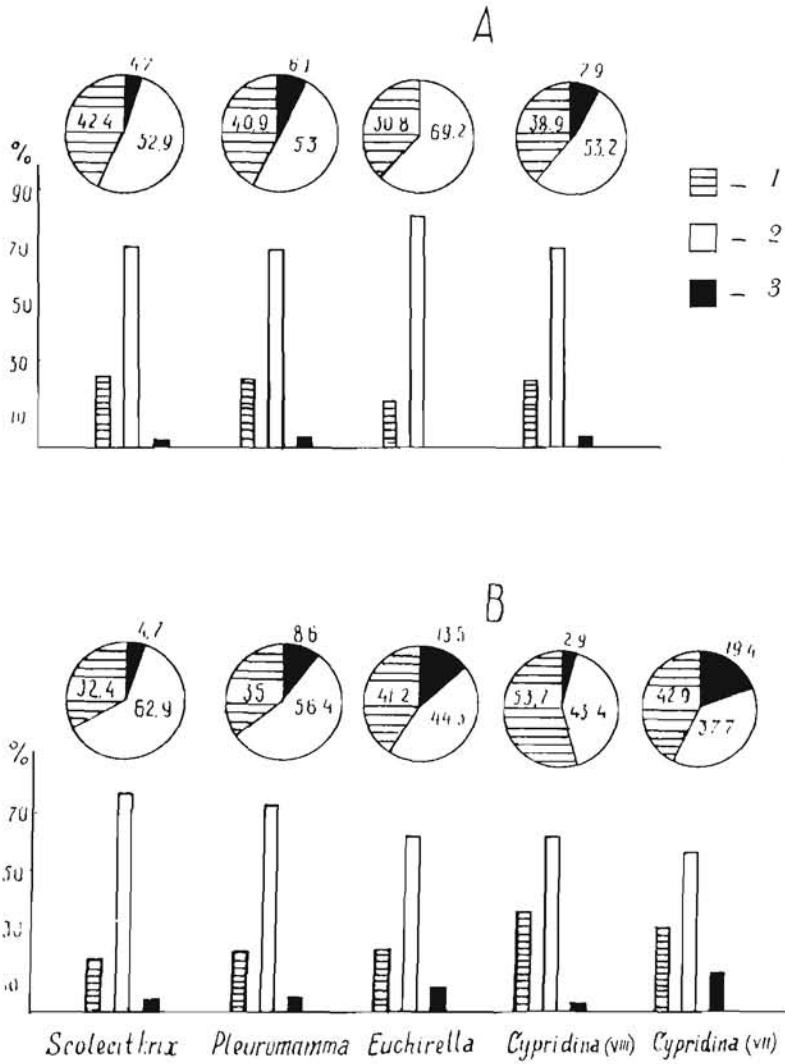


Figure 2 - Biochemical components losses during 6 hours of starvation (% of total losses). A - by the day, B - at night. 1 - protein, 2 - lipids, 3 - glycogen. Oval diagrams -  $\mu\text{g}$  of matter, columns -  $\mu\text{cal}$  of energy.

Species	Diffused light, daytime		Darkness, nighttime	
	Dry mass (1sp,mg)	$\mu\text{g. O}_2 \cdot \text{sp}^{-1} \cdot \text{hrs}^{-1}$	Dry mass (1sp,mg)	$\mu\text{g. O}_2 \cdot \text{sp}^{-1} \cdot \text{hrs}^{-1}$
<u>Scolecithrix danae</u>	0,224 $\pm$ 0,0630	3,56 $\pm$ 0,240	0,223 $\pm$ 0,0600	2,35 $\pm$ 0,54
<u>Pleuromamma abdominalis</u>	0,730 $\pm$ 0,780	7,86 $\pm$ 1,700	0,659 $\pm$ 0,0550	10,85 $\pm$ 1,770
<u>Euchirella curticauda</u>	0,972 $\pm$ 0,0080	6,53 $\pm$ 0,620	0,839 $\pm$ 0,0850	6,58 $\pm$ 0,620
<u>Cypridina serrata</u> (July, at the period of eggs formation)	-	-	0,178 $\pm$ 0,0057	1,23 $\pm$ 0,144
<u>Cypridina serrata</u> (August, at the moment of eggs laying)	0,164 $\pm$ 0,0029	1,92 $\pm$ 0,158	0,145 $\pm$ 0,0007	1,95 $\pm$ 0,309

TABLE I - OXYGEN CONSUMPTION OF 4 ZOOPLANKTON SPECIES  
OF INDIAN OCEAN DURING 6 HOURS OF STARVATION

Species	Diffused light, daytime						Darkness, nighttime					
	Biochemical substrates				Consumed O <sub>2</sub>	% of divergence	Biochemical substrates				Consumed O <sub>2</sub>	% of divergence
	Protein	Lipids	Glycogen	Sum			Protein	Lipids	Glycogen	Sum		
<u>Scolecithrix</u>	21,5	39,0	-	60,5	55,3	8	15,7	51,0	1,8	68,5	44,0	36
<u>Pleuromamma</u>	18,5	35,3	1,8	55,6	43,0	23	19,2	59,0	3,7	81,9	57,6	30
<u>Euchirella</u>	12,0	45,0	-	57,0	28,6	49	15,7	27,6	2,9	46,2	28,5	38
<u>Cypridina</u> (July)	-	-	-	-	-	-	20,3	26,6	6,6	53,5	27,4	49
<u>Cypridina</u> (August)	9,6	21,3	1,4	32,3	41,1	27	8,4	8,3	0,3	17,0	47,1	-

TABLE II - BIOCHEMICAL SUBSTRATES EXPENSE AND RESPIRATION OF 4 ZOOPLANKTON SPECIES OF THE INDIAN OCEAN DURING 6 HOURS OF STARVATION ( $\mu\text{cal.mg dry weight}^{-1}.\text{h}^{-1}$ )