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Effect of starvation on the biochemical compositions and respiration rates of ctenophores *Mnemiopsis leidyi* and *Beroe ovata* in the Black Sea

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The proximate biochemical composition and metabolic rates of ctenophores *Mnemiopsis leidyi* and *Beroe ovata* from the Black Sea were examined with respect to starvation conditions. Although organic matter content in *B. ovata* was two times higher than that of *M. leidyi* (2.51 ±0.53 and 1.14 ±0.17 mg g^{-1} of wet weight, respectively), these species did not significantly differ in their biochemical composition. In both species protein formed about 80% of the total organic matter, lipids amounted to about 10%. Carbohydrate and amino acids measured separately made up less than 6.5% of the total organic matter. Under experimental starvation (18 days at 16–18°C for *B. ovata* and 8 days at 12.4°C for *M. leidyi*), wet weights of both ctenophore species were reduced by 9.4% and 9.3% d^{-1}, respectively. The rate of organic matter decrease was nearly two times lower than that of wet weight being on average 5.9% d^{-1} in *M. leidyi* and 5.5% d^{-1} in *B. ovata*. There was no trend in percentage of the four major biochemical categories with starvation time. The glycogen content in polysaccharides reached maximum values in freshly collected ctenophores (76.0 ±7.9% in *B. ovata*, and 86.6% in *M. leidyi*), but it was reduced substantially (34.4 ±2.7% in *B. ovata* and 18.3–28.8% in *M. leidyi*) with starvation. Monosaccharide content, expressed as a percentage of total carbohydrate, decreased from 39.9% to 13.5% in *B. ovata*, and from 45.8% to 14.3–23.2% in *M. leidyi*. The relationship between respiration rate (R) and wet weight (W) of individuals during the starvation can be expressed by power function $R = R_1 W^k$ (r^2=0.85–0.94; P<0.001) for both ctenophore species. On average, k values were 0.95 and 0.83 in *B. ovata* and in *M. leidyi*, respectively. By the end of the starvation, metabolic rate per unit wet weight decreased by 33% in *B. ovata* and 46% in *M. leidyi*. Organic matter utilization was almost totally explained by respiration of ctenophores in the experiments and exceeded metabolic requirements of studied species by 11% and 15%, correspondingly. As compared with *Mnemiopsis*, *Beroe* has better tolerance to starvation which explains to some extent the success of the species survival during prolonged periods of food shortage in the Black Sea conditions.

INTRODUCTION

The ctenophores *Mnemiopsis leidyi* Agassiz A., 1865 and *Beroe ovata* Mayer, 1912 are both new species for the Black Sea ecosystem. The mass appearance of *M. leidyi* occurred in the late 1980s, giving rise to drastic changes in the plankton, such as the disappearance of some species and a huge decrease in the quantity of prey zooplankton. *Beroe ovata* in the Black Sea was initially noticed in 1997, and by 1999 the mass appearance of this species in different regions of the sea was observed (Konsulov & Kamburska, 1998; Finenko et al., 2001; Shiganova et al., 2001). The outbreak of *B. ovata*, that feeds exclusively on other ctenophore species, decreased the population of *M. leidyi*, resulting in the gradual recovery of the whole planktonic community.

The reproductive potential of these ctenophores is exceptionally high (Finenko et al., 1995; Arashkevitch et al., 2001). This, along with a high feeding and growth rates enables *Mnemiopsis* to consume prey zooplankton, and similarly renders *Beroe* capable of con-

trolling the development of *Mnemiopsis* (Finenko et al., 2001; Shiganova et al., 2001). As a result of the ctenophores predation and changing temperature, the next manifestations can be distinguished in the current seasonal succession of the Black Sea zooplankton (Finenko et al., 2003): (1) intensive spring–summer development of mesozooplankton; (2) decline in mesozooplankton abundance due to development of high *Mnemiopsis* biomass in summer and early autumn; and (3) development of *Beroe* population and decrease of *Mnemiopsis* population in autumn.

Seasonal or spatial outbreaks in ctenophore populations can strongly depress populations of lower food web level. The changes in the food zooplankton are reflected in biomass, numbers, biochemical compositions and physiological states of ctenophores themselves. Before the *Beroe* appearance, these phenomena were especially typical for the *Mnemiopsis* population. For example, in autumn 1996, the daily metabolic demand of this species in offshore waters of the Black Sea was at least two times higher than consumed zooplankton could provide (Anninsky et al., 1998). Probably this would be an explanation of ctenophore biomass decrease, which was usually observed during the autumn months. The similar phenomenon is typical for *Beroe*. Its rapidly developing population can consume daily 25% (Finnenko et al., 2000) or even to 30–80% (Shushkina et al., 2000) of the *Mnemiopsis* stock, causing reduction in available food amount to minimum in a few days (Finenko et al., 2003). This event is followed by a steady decrease and later disappearance of *Beroe* population due to lack of its food. Apparently, there are significant periods of low food availability for these ctenophore species in their life histories. The same periods of food shortage or absence were observed for other ctenophore species (Falkenhaug, 1996).

Monitoring the condition of ctenophores in the sea coupled with studying their physiological features is needed to understand the ecological role of these species in ecosystems. Information about biochemical composition during starvation may be useful in the evaluation of nutritional state of ctenophores in the field. Metabolic parameters appropriate to the nutritional state could then be used to monitor the field populations.

For these gelatinous organisms such investigations are scarce and defined mostly by studying the respiration rate (Gyllenberg & Greeve, 1979; Reeve, 1980; Kremer, 1982; Finenko et al., 1995). Only two species of ctenophores, *Pleurobrachia pileus* and *B. ovata*, (and one of jellyfish, *Aequorea victoria*) were analysed before in terms of biochemical composition (Hoeger, 1983; Arai et al., 1989; Dudkin et al., 2002). Although total organic matter content in fasting specimens usually decreased during starvation, their specific organic content decreased only in *P. pileus* (Hoeger, 1983), but increased in *B. ovata* (Dudkin et al., 2002). The experiments discovered no significant differences between the proximate biochemical composition of freshly collected and starved gelatinous animals. So, it is still unclear, if predominant organic compounds could be characteristics of the ctenophores' condition (Schneider, 1989). In this paper, we report how starvation affects the biochemical composition and respiration rate of ctenophores *M. leidyi* and *B. ovata*, which have been investigated insufficiently in this respect.

MATERIALS AND METHODS

Sampling

Investigations on the biochemical composition and respiration of *Mnemiopsis leidyi* were carried out during summer–autumn 1991–1995. Similar studies of *Beroe ovata* were fulfilled between 1999 and 2001. Ctenophores were mainly caught in the near-shore waters of the Black Sea (<16 km, salinity of 16–18 psu) on the Sevastopol coasts of the Crimea, Ukraine. Samples from the offshore region of the sea (35 samples, during the 33rd cruise of the RV 'Professor Vodyanitsky' in June 1991) were also used for determining the biochemical composition of *M. leidyi*. Additionally, some of the material (20 samples of *B. ovata*) was collected from the Black Sea coast of Turkey near Sinop during September 2001.

Ctenophores were mainly caught by a plankton net (mouth diameter 0.5 m^2, mesh size 500 μm), but sometimes, when they were at the surface, were obtained by dipping a small bucket. Within 1–2 hours after catching, animals were taken to the laboratory and placed in aquaria. Only freshly collected animals without any damage, kept in the laboratory up to one day, were used for biochemical and respiration tests.

Starvation experiments

Three starvation experiments were conducted during September–October 1995 (*Mnemiopsis leidyi*) and October–November 2000 (*Beroe ovata*). All the ctenophores were collected near the Sevastopol coasts of the Crimea.

In the first experiment with *B. ovata* (31 specimens with wet weight ranging from 2.6 to 5.0 g), ctenophores were kept with no food in a 20-l aquarium, at a temperature of 16–18°C. Seawater in the aquarium was renewed daily. The experiment was run for 18 days. Sub-samples of ten randomly selected specimens were measured and weighed every 4–6

days, after that they were returned back. *Beroe* has a rather robust body consistence and we have not noticed any damage of the ctenophores due to procedure of reweighing. With the same periodicity, we also estimated respiration rates and biochemical composition of 4–7 ctenophores. Each animal was individually homogenized and later analysed.

In the course of another experiment, where we studied starvation of seven *B.* ovata (with initial wet weight ranging from 1.8 to 18.4 g), specimens contained in separate 5-l jars at the same temperature (16–18°C). Seawater in the jars was also renewed daily. Respiration rate and weight of ctenophores were determined with 7-day periodicity. Biochemical compositions of these animals were not analysed and length parameters were not measured. Duration of the latter experiment was 21days.

In the case of *M. leidyi* (105 specimens with wet weights ranging from 0.7 to 1.3 g), ctenophores contained in a 20-l aquarium for up to one day were first fed with zooplankton, predominantly copepods. Then, 15 individuals of similar size (11–14 mm) were placed as a group in seven separate 1.2-l jars, filled with filtered seawater. *A priori* we supposed that the ctenophores placed into each jar did not differ in their wet weights or their physiological and biochemical parameters. Over the ten days of the experiment, the water temperature was kept at 12–13°C, the ctenophores were not fed and the water in the jars was not changed. Every 1–3 days we estimated respiration of the animals from one randomly sampled jar with the subsequent utilization of these 15 specimens for biochemical analysis. The initial sample of ctenophores, accepted as the starting point of the fasting, was also collected. Specimens, after their measuring were pooled, weighed and homogenized together.

Body length measuring and weighing

The ctenophores during the starvation experiments were measured to the nearest 1 mm in oral-aboral direction, including lobes for *Mnemiopsis*. For the smallest individuals the measurements were done under a dissecting microscope on the specimens placed in plastic culture dishes almost without water. After careful blotting to remove excess water, the dishes with the specimens were weighed on an analytical balance (accuracy to 0.01 mg) and wet weight of the ctenophores was determined by difference with empty dishes.

Biochemical determinations

Beroe specimens were always analysed individually while most of the *Mnemiopsis* samples contained preliminary pooled animals. Homogenized samples of ctenophores from the sea and from the starvation experiments were stored at -20°C, in tightly closed vials. All the biochemical determinations were completed over the next 1–2 months, usually in no less than three replicates. The subsamples taken from freshly thawed homogenate were in the range from 0.1 to 0.5 g of wet weight. All the major organic components of ctenophore tissue (protein, lipids, carbohydrates and free amino acids) were quantitatively assayed by routine colorimetric techniques as described earlier (Finenko et al., 2000).

Protein was measured by the Lowry method as modified by Hartree, using HSA as a standard (Hartree, 1972). Amino acids (ninhydrin positive substances) were measured by the Pochinok method with D, L-α-alanine as a standard (Kuzmenko, 1975). Carbohydrates were determined by the Dubois method with D-glucose as a standard (Dubois et al., 1956). Fraction of mono- and polysaccharides in the total carbohydrates were separated by 80% ethanol (Zaslavsky, 1980). Structural polysaccharides were calculated by difference between the total polysaccharide and glycogen content. Glycogen was first separated in 30% KOH solution, and then estimated as well by the Dubois method (Milman et al., 1974). Total lipids were extracted using 2:1 chloroform/methanol (Folch et al., 1957) and later analysed by the method of Amenta (Amenta, 1964; Clarke et al., 1992). The standard was triolein/cholesterol (1/1). These methods were in the most part critically reviewed before (Clarke et al., 1992).

Organic matter content in ctenophores body was determined as the sum of all aforementioned organic constituents. Calorific value (1 cal = 4.186 J) of the ctenophore tissue was calculated, using well known energy equivalents of each constituent, which are 5.65 cal mg^{-1} for proteins, 9.45 cal mg^{-1} for lipids, and 4.10 cal mg^{-1} for carbohydrates (Omori & Ikeda, 1984).

Respiration rate

With the exception of 18-days *Beroe* starvation, respiration rates for both *B. ovata* and *M. leidyi* were measured using the Winkler technique (Omori & Ikeda, 1984). Ctenophores without any food were contained for 10–24 h in closed respiration chambers filled with fresh 112 μm filtered seawater. Volumes of the respirometers (0.1–2.0 l) were chosen according to body weight of the specimens (3.6×10^{-2} – 18.4 g). When starved *M. leidyi* was examined, 15 ctenophores with the length range from initial 11–14 mm to 8–10 mm at 10-days starvation were placed into the respirometer of 1.2 l. Oxygen concentration decrease during the experiment was never more than 20% of initial content.

For *B. ovata* of 15–28 mm starving during the 18-day experimental course, respiration rate was determined

by the method of closed respirometers using all-glass syringes of 20 ml. Ctenophores were placed individually in experimental respirometers containing 1 μm filtered seawater and incubated for 2–3 hours. Control syringes containing only seawater were incubated during the same period. Then oxygen concentration in the water from the syringes was measured by the polarographic method (Svetlichny et al., 2000).

RESULTS

Proximal biochemical composition of ctenophores in the sea

The composition of the body organic matter in *Beroe ovata* and *Mnemiopsis leidyi* freshly collected from the Black Sea is given in Table 1.

The content of organic matter per unit weight and the biochemical components in the body of *B. ovata* were two times higher than for *M. leidyi*. Besides, freshly collected *Beroe* had more variable content of organic matter in wet weight (SD = 21%) than *Mnemiopsis* (SD = 15%). Despite this, the two species did not significantly differ in their relative biochemical composition. Protein, the predominant organic component formed up to 80% of the total organic matter. The percentage of lipids in both species were approximately 10%. Both carbohydrates and amino acids, measured separately, did not exceed 6.5% of the total organic matter. In *B. ovata* carbohydrates and amino acids were the same (5.6–5.3%), while *M. leidyi* had 1.5 times greater carbohydrates, than free amino acids. If expressed as per cent organic matter lipids, carbohydrates and free amino acids had a coefficient variation of about 25% for both species.

There was no trend with size for *B. ovata* (17–43 mm) in any of the biochemical measurements, but protein, carbohydrates and amino acids varied 2–3 times and lipids 3–4 times for similarly sized ctenophores (Figure 1). For *M. leidyi* there was less variability overall. The smallest animals (<10 mm) had the highest organic matter content, and these of moderate size (from 13 to 25 mm) were characterized by minimal values ($P<0.001$; *t*-test). In this case, 1.5–2-fold differences in the specific organic matter content and its biochemical components could be observed for the same size specimens. As the data for *Mnemiopsis* were partly obtained for pooled individuals, true dispersion for this species in fact might be stronger than that was observed.

Dynamics of body weight and size under starvation

Because a metabolically inert skeleton is absent in gelatinous animals, their body weight can fall considerably during starvation. The wet body weight in *M. leidyi* decreased on average from 0.79 to 0.32 g during ten days, and that of *B. ovata* from 3.9 ±1.5 to 0.7 ±0.2 g over an 18 day period (Figure 2). During another experiment, when seven more large specimens of *B. ovata* starved, their wet weight decreased from 8.6 ±6.5 to 2.3 ±1.8 g in 21 days. At exponential form of the dependence, the specific rate of wet weight decrease was equalled to 9.4% d^{-1} for *M. leidyi*. For *B. ovata* these values were measured to be 9.3 and 6.8 ±1.8 % d^{-1} at 18- and 21-days starvation, respectively. There was no trend in specific rate of shrinkage for *B. ovata* depending on initial wet weight of individuals.

Ctenophores' size was reduced simultaneously with the wet weight decrease, but the length–weight relationship did not significantly deviate from the equations, which were calculated earlier for ctenophores in the sea (Figure 3). All the former measurements were done by the same manner, and this relationship between length (L, mm) and wet weight (W, mg) was as follows: $W=1.31L^{2.49}$ ($r=0.99$) for *M. leidyi*; and $W=0.85L^{2.47}$ ($r=0.96$) for *B. ovata* (Finenko et al., 2000).

Along with the wet weight, the decrease in the absolute organic matter content was also calculated. In both species the shrinkage rates for organic matter were about half the rates of wet weight decrease being 5.5% d^{-1} for *B. ovata* and to 5.9% d^{-1} for *M. leidyi* on average (Figures 4A & 5A). As the ctenophores shrank in wet weight faster than organic content, at 18-days starvation, the total concentration of the organic components in *B. ovata* had increased almost twice (from 2.5 ±0.5 to 4.5 ±0.8 mg g^{-1}). At 10-days starvation, the concentration of the organic components in *M. leidyi* had increased from 0.81 mg g^{-1} to 1.23 mg g^{-1}.

Table 1. *Organic matter content and proximate biochemical composition of the ctenophores* Beroe ovata *and* Mnemiopsis leidyi *from the Black Sea.*

Species	Organic compounds	Content: mg g^{-1} wet weight (±SD)	Content: % organic matter (±SD)	N
B. ovata	Organic matter	2.51 ±0.53	100	51
	Protein	1.93 ±0.42	76.9 ±3.4	51
	Lipids	0.31 ±0.13	12.2 ±3.6	51
	Carbohydrates	0.14 ±0.03	5.6 ±1.3	51
	Amino acids	0.13 ±0.04	5.3 ±1.3	51
M. leidyi	Organic matter	1.14 ±0.17	100	59
	Protein	0.92 ±0.14	80.6 ±3.3	59
	Lipids	0.10 ±0.03	9.0 ±2.3	59
	Carbohydrates	0.07 ±0.02	6.5 ±1.4	59
	Amino acids	0.04 ±0.02	3.9 ±1.2	59

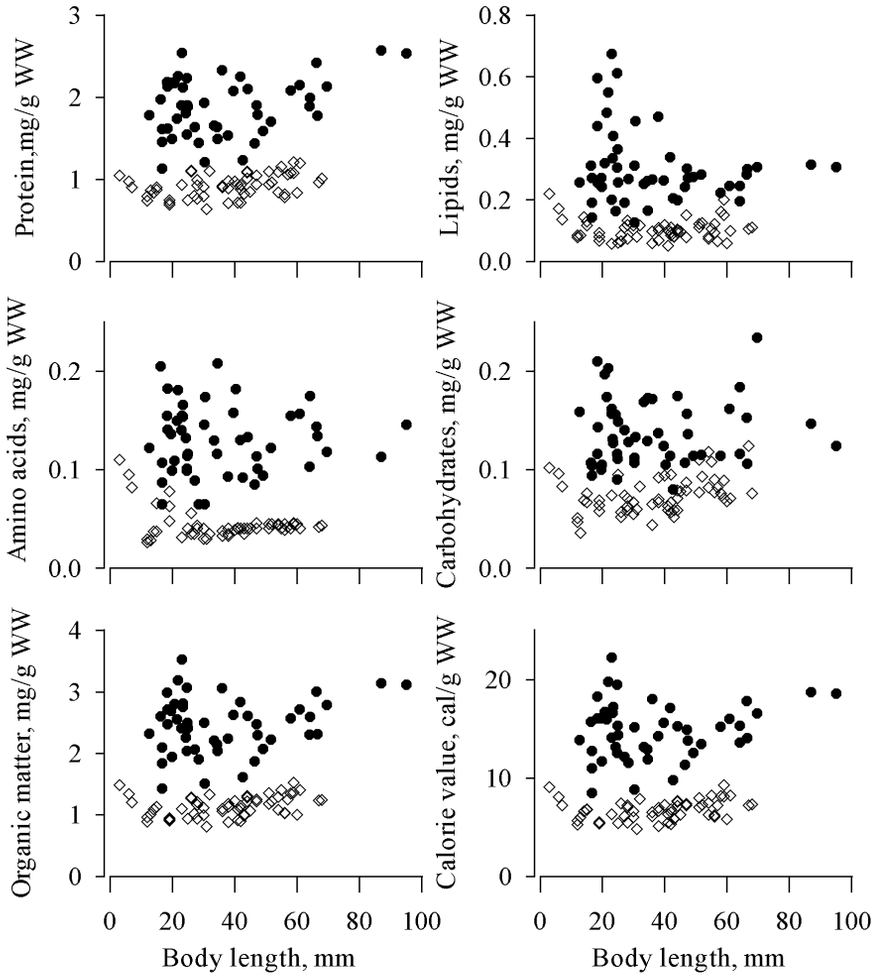

Figure 1. Biochemical composition of freshly collected *Beroe ovata* (black circles) and *Mnemiopsis leidyi* (open diamonds) in the Black Sea.

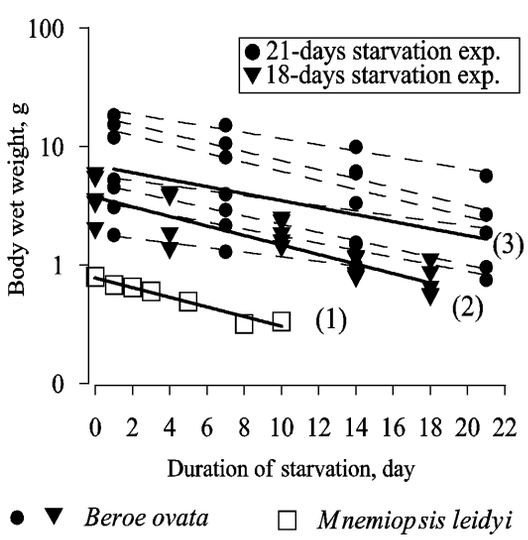

Figure 2. Dynamics of body wet weight of *Beroe ovata* (black symbols) and *Mnemiopsis leidyi* (white squares) under conditions of experimental starvation. Solid lines: (1) $W=0.77e^{-0.094t}$; $r^2=0.96$; $P<0.01$; (2) $W=3.71e^{-0.093t}$; $r^2=0.78$; $P<0.001$; (3) $W=6.87e^{-(0.068 \pm 0.018)t}$; $r^2=0.25$; $P<0.05$. Dotted lines illustrate the trends for individuals.

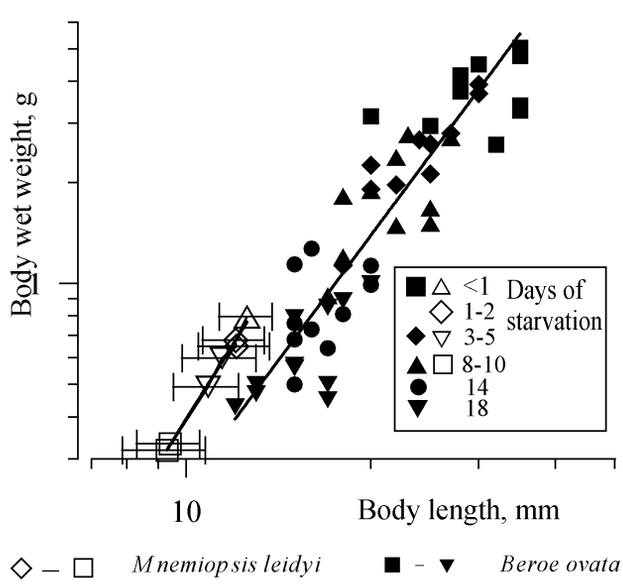

Figure 3. Relationship between length and wet weight in *Beroe ovata* (black symbols) and *Mnemiopsis leidyi* (white symbols) under conditions of experimental starvation (symbols) and in the Black Sea (lines: $W=1.31\ L^{2.49}$ for *M. leidyi*; and $W=0.85\ L^{2.47}$ for *B. ovata*) (Finenko et al., 2000). Bars are length variability (SD) on groups of 15 specimens.

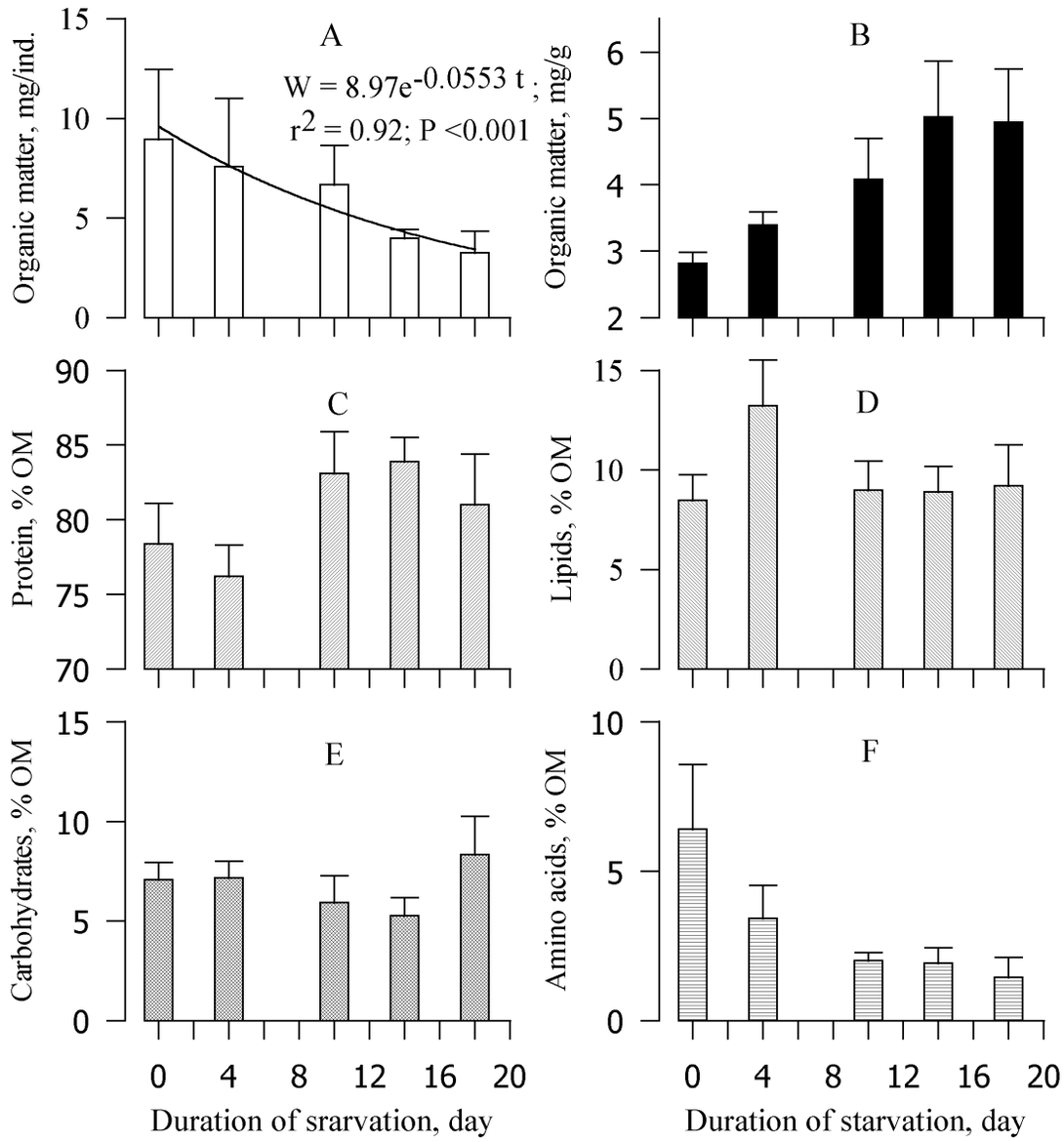

Figure 4. Dynamics of (A) organic matter and (B–F) biochemical composition of *Beroe ovata* under conditions of 18 days experimental starvation.

Individuals of each species appeared to become more compact during the starvation.

Dynamics of the organic composition

The lack of substantial energy reserves and predominance of protein in the ctenophore's organic composition defines their shrinkage strategy. Protein was utilized throughout starvation in both species, and there were no large changes in their overall biochemical composition. Along with this, dynamics of the proximate biochemical composition in starved specimens had evident species features (Figures 4B–F & 5B–F). In *B. ovata*, the specific protein content (81.0–83.9%) was reliably higher (P<0.001) between 10–18 days of starvation, than at the beginning of fasting (76.2–78.4%). The lipids content (expressed also, as per cent of the total organic matter) has firstly increased (to 13.2 ±2.4%) in this species, and then returned to starting level (8.5–9.2%). The inverse pattern was demonstrated by starving *M. leidyi*: the fraction of protein in organic content gradually diminished (from 81.6–82.8% to 78.8%), and that of lipids had increased (from 8.8–10.5% to 12.9%). The changes in carbohydrate content were complicated in both cases, and ranged from 5.3% to 8.3% for *B. ovata,* and from 4.4% to 6.4% for *M. leidyi.* As for amino acids, a decrease of these compounds in starved animals was the most apparent trend, more pronounced for *B. ovata* (from 6.4 ±2.2% to 1.5 ±0.6%; P<0.001) and weak for *M. leidyi* (from 3.5 to 2.9%).

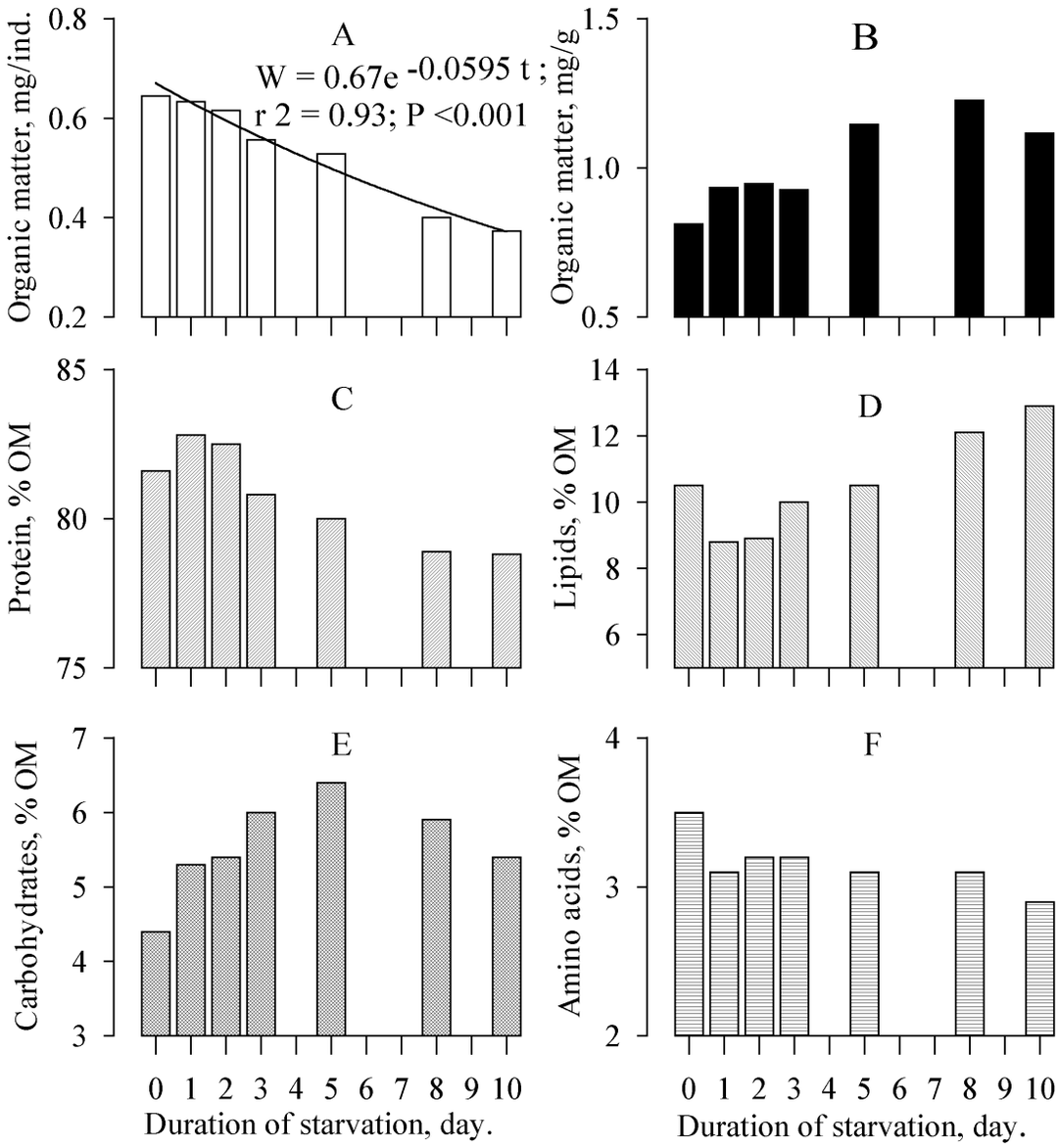

Figure 5. Dynamics of body (A) organic matter and (B–F) biochemical composition of *Mnemiopsis leidyi* under conditions of ten days experimental starvation.

Indicators of condition

According to experimental data, starved specimens would have the highest organic matter content for similar sized ctenophores. Additional indicators of the ctenophore nutritional condition were explored, looking for ratios among measured organic components (Figure 6).

The ratio between lipids and protein had different and complicated dynamics for each species in the experiments. Greater similarity between the two species was observed in dynamics of the ratio between free amino acids and protein. It decreased clearly in starved *B. ovata* and weakly for *M. leidyi*, being almost constant for the last species over the period of starvation.

Two other ratios showed more constant trends for both species. The glycogen content expressed as a per cent of polysaccharides was maximum in the freshly collected ctenophores (76.0% ±7.9 in *B. ovata*, and 86.6% in *M. leidyi*) and decreased as the duration of starvation increased (finally to 34.4 ±2.7 in *B. ovata*, and 18.3% in *M. leidyi*). Monosaccharides as a per cent of the total carbohydrates decreased from 39.9 ±9.9% to 13.5 ±6.6%, and from 45.8% to 18.7% for *Beroe* and *Mnemiopsis* respectively. These indicators reflect various aspects of ctenophore metabolism: (1) level of accumulation of reserve products (glycogen); (2) intensity of carbohydrate production (monosaccharides). Therefore, with the combined use of these indices the ctenophore conditions could be evaluated more adequately.

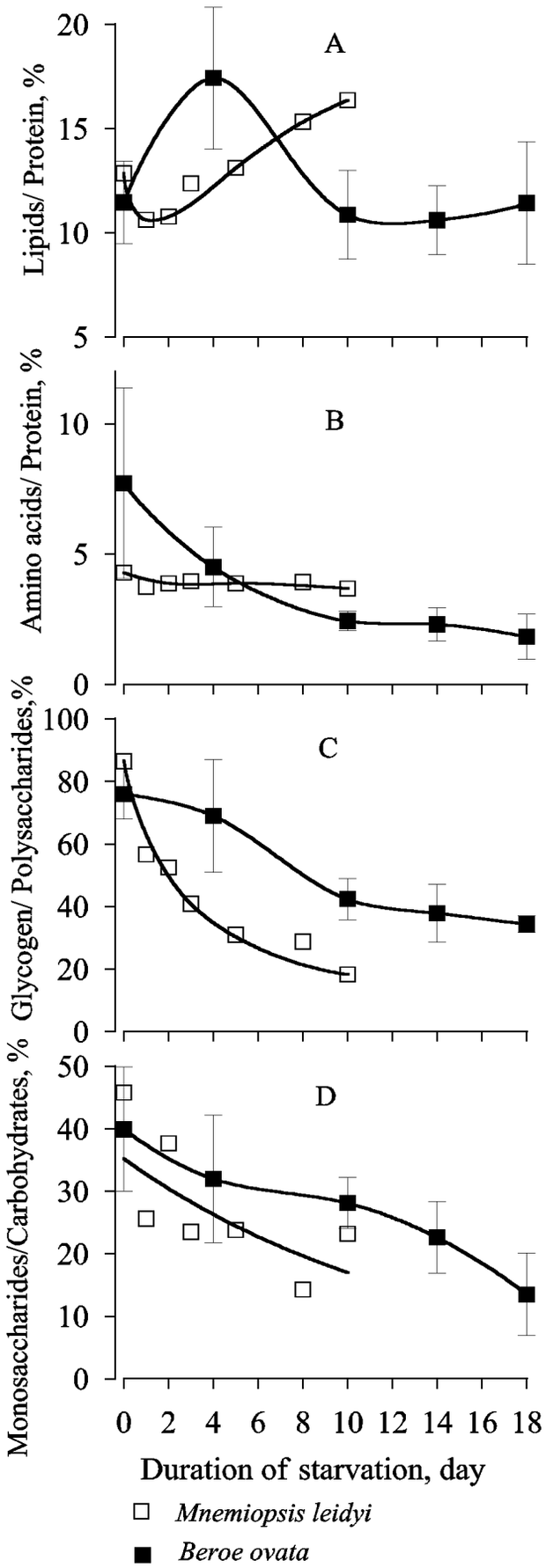

Figure 6. Ratio between biochemical fractions of body organic matter in *Beroe ovata* and *Mnemiopsis leidyi* under conditions of experimental starvation.

Table 2. *The respiration rates ($R=R_1 W^k$) of the ctenophores* Beroe ovata *and* Mnemiopsis leidyi *at different duration of starvation.*

Species	W, g	R_1(μg O_2 h^{-1})	k	N	r^2	Duration of starvation, day
B. ovata	1.25–18.38	9.65	1.04	18	0.93	≤1
	1.29–15.17	10.90	0.87	15	0.85	4–9
	0.70–9.96	7.26	1.01	11	0.94	14
	0.50–5.67	8.04	0.88	10	0.92	18–21
M. leidyi	3.64–12.09	3.30	0.83	52	0.90	<1
	0.33–0.79	1.70–4.66	(0.83)	6		1–10

W, wet weight; N, number of sample; R_1, respiration rate for 1 g specimens; k, exponent for weight.

Respiration rates

The respiration rates (R, μg O_2 ind^{-1} h^{-1}) of the different size individuals changed as a function of wet weight (W, g), with strong agreement for both ctenophore species (r^2=0.85–0.94; P<0.001) to the power equation: $R=R_1 W^k$, where R_1 = respiration rate for 1 g specimens and k = exponent for weight (Figure 7; Table 2).

All the respiration data were preliminarily grouped to evaluate the possible starvation impact on the respiration rate. The slope factor k changed for *B. ovata* in the range from 0.87 to 1.04, and varied irregularly with the duration of fasting. On the average, k values were 0.95 and 0.83 for this species and for *M. leidyi*, respectively. At such k values, the calculated rate of oxygen consumption of 1 g specimens (R_1) amounted to 6.3–12.9 μg O_2 h^{-1} for *B. ovata* and 1.7–4.7 μg O_2 h^{-1} for *M. leidyi*. Strong variability among initial *Beroe* respiration probably indicates that some individuals (the smallest by sizes) were in starving conditions already before beginning the experiment. On the whole the values for weight adjusted respiration rates generally decreased with starvation time, but there was quite a bit of scatter in the results (Figure 8). According to the equations calculated, during the whole starvation time the level of the metabolic rate diminished by 33% in *B. ovata*, and by 46% in *M. leidyi*.

Evaluation of the metabolic balance

We compared the respiration energy expenses with the ctenophores organic losses during the starvation. These calculations were performed on the following initial data using generally accepted factors.

– Wet body weight at starvation decreased exponentially from 3.7 to 0.7 g in *B. ovata* and from 0.7 to

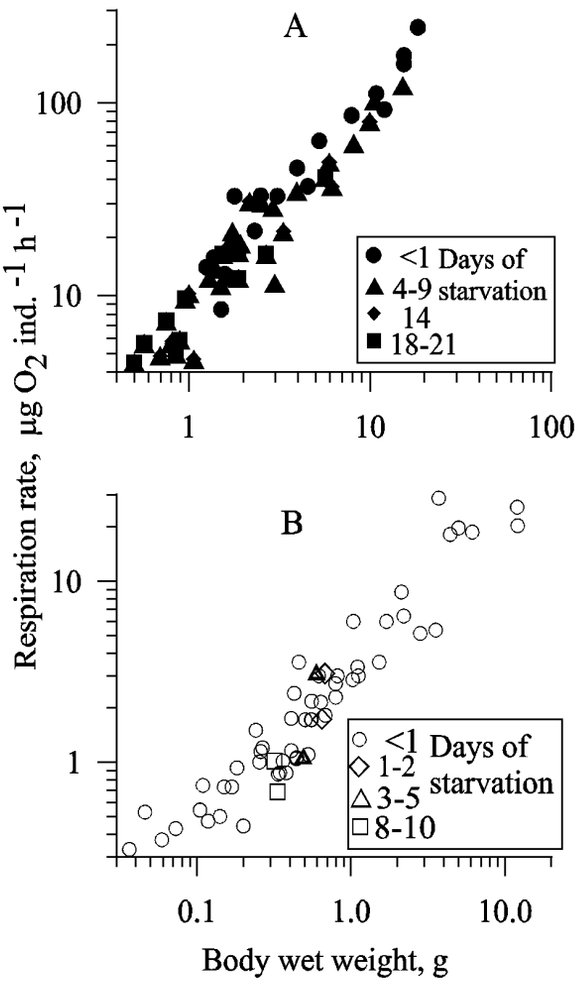

Figure 7. Relationship between respiration rate (R, µg 0_2^{-1} ind^{-1} h^{-1}) and body wet weight (W, g) in *Beroe ovata* (black symbols) and *Mnemiopsis leidyi* (white symbols) at different state of individuals.

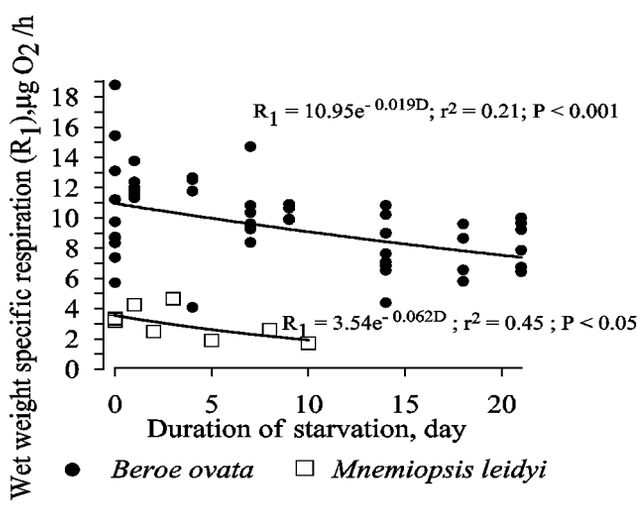

Figure 8. Dynamics of respiration rate for 1 g wet weight specimens (R_1, µg 0_2^{-1} ind^{-1} h^{-1}) in *Beroe ovata* (black circles) and *Mnemiopsis leidyi* (white squares) under experimental starvation.

0.3 g in *M. leidyi* (Figure 2).
– Organic body weight decreased exponentially from 8.97 to 3.32 mg in *B. ovata* and from 0.67 to 0.37 mg in *M. leidyi* (Figures 4A & 5A).
– The daily organic losses were 5.53% and 5.95% of body weight in *B. ovata* and in *M. leidyi* respectively (Figures 4A & 5A).
– Calorific values of these ctenophores were 5.895 cal mg^{-1} organic matter for *B. ovata*, and 5.964 cal mg^{-1} organic matter for *M. leidyi*.
– At the metabolic oxidation efficiency, calculated taking into account the ctenophore biochemical composition, along with the metabolic calorific content of protein (4.5 cal mg^{-1}), lipid (9.4 cal mg^{-1}), and carbohydrate (4.1 cal mg^{-1}) (Haskin, 1981), the correction factor for energy losses was estimated as 0.83.
– The value of the calorific equivalent of the oxygen consumed was accepted as 3.4 cal mg^{-1} O.
– The losses of organic matter expressed as energy values (R_m,) were determined as:

$$R_m = \sum_{t=0}^{18} [(W_t + W_{t+1})/2 \times 0.055 \times 5.895 \times 0.83] = 27.67 \text{ cal}$$

for *B. ovata*, and as

$$R_m = \sum_{t=0}^{10} [(W_t + W_{t+1})/2 \times 0.059 \times 5.964 \times 0.83] = 1.47 \text{ cal}$$

for *M. leidyi*, where W_t and W_{t+1} are initial and day over organic weights (mg).
– Metabolic requirements of the ctenophores starved (R, cal) were calculated as follows:

$$R = \sum_{t=0}^{18} [(W_t + W_{t+1})/2^{0.95} \times 10.95e^{-0.019D} \times 3.4 \times 10^{-3} \times 24]$$

= 24.72 cal for *B. ovata*; and

$$R = \sum_{t=0}^{10} [(W_t + W_{t+1})/2^{0.83} \times 3.54e^{-0.062D} \times 3.4 \times 10^{-3} \times 24]$$

= 1.25 cal for *M. leidyi*, where W_t and W_{t+1} are initial and day over wet weights (g); D is duration of the starvation (day).

Above data allow us to conclude, that total organic losses in starved ctenophores were very close to the respiratory expenses of these animals (R_m=27.67 and R=24.72 cal ind^{-1} for *B. ovata*; R_m=1.47 and R=1.25 cal ind^{-1} for *M. leidyi*). Organic matter utilization exceeded metabolic requirements of studied species by 11% and 15%, correspondingly.

DISCUSSION

The proximate biochemical composition of the ctenophores *Beroe ovata* and *Mnemiopsis leidyi* from the

Black Sea has been investigated before (Anninsky & Gubanova, 1998; Finenko et al., 2001). An increase in the examined samples to 51 specimens for *B. ovata* and to 59 specimens for *M. leidyi* in fact did not change the mean values of biochemical constituents of the ctenophores. Very similar values were also obtained for *B. ovata* in other investigations, especially concerning protein (2.47 mg g^{-1} wet weight) and lipid (0.28 mg g^{-1} wet weight) contents (Shiganova et al., 2001). Besides, estimated values corresponded well to the results of CHN analysis, from which organic matter content in *B. ovata* (C × 1.9) amounted to 1.33–3.99 mg g^{-1} wet weight (Kremer et al., 1986), or to 2.85 mg g^{-1} wet weight from another set of data (Vinogradov et al., 2000). Organic matter content in *M. leidyi*, estimated by the same method could be as 0.82–1.20 mg g^{-1} wet weight (Kremer et al., 1986), 1.24 mg g^{-1} wet weight (Youngbluth et al., 1988), or 0.55–4.58 mg g^{-1} wet weight (Borodkin & Korjicova, 1991). This permits us to assume that further increases in the numbers of analysed specimens will not significantly change the average values on ctenophores' proximate composition.

At the same time a wide individual variability in content of organic constituents especially for *B. ovata* is not explained only by experimental methods or possible changes in weight specific organic content with ctenophore size. Although weight specific organic content can significantly increase with decrease of ctenophores size, such dependence is mainly observed for the smallest individuals. Kremer et al. (1986) found an increase in organic content only for *Beroe* <15 mm, larger ones showed no clear trend. The same was seen for *Mnemiopsis*: although the smallest specimens (<10 mm) always had maximal weight specific organic content, trends for the larger specimens were different (Kremer et al., 1986; Reeve et al., 1989; Anninsky, 1994; Vinogradov et al., 2000). Other factors, influencing ctenophore biochemical composition more strongly than growth trends, would be possible reasons for this phenomenon and organic content variability in *B. ovata*. Indeed, the starvation experiments have shown that the absence of food in the ctenophores *B. ovata* and *M. leidyi* changed both the total and weight specific organic matter content as well as the biochemical composition in fasting specimens. Freshly collected ctenophores, could also differ, depending on their nutritional status. That is especially true for *B. ovata*, of which the population could consume the *Mnemiopsis* resources during less than a month (Finenko et al., 2003), and then undergo prolonged starvation. Therefore, the variability in the content and biochemical composition of *B. ovata* could be especially high.

Compaction of the body or increase of organic matter content per unit wet weight may be considered to be the principal feature of adaptive changes in ctenophore tissue at starvation. This phenomenon is confirmed by the calculations of the metabolic balance and some independent studies. For *Beroe* from the Black Sea the similar results (1.5-fold increase for protein content and even 3-fold increase for some carbohydrates) were also obtained in the 2-week starvation experiments (Dudkin et al., 2002). Besides, accumulation of the tissue pigments in starved specimens may also indicate dehydration of the ctenophores at the starvation (Cibulsky et al., 2002). It is difficult to confirm the same for *Mnemiopsis*, as organic content in this species has been studied before only in relation to different food regimes (Kremer, 1982; Reeve et al., 1989), but not starvation. According to Reeve at al., (1989), carbon/dry weight ratio for two populations of *Mnemiopsis* has increased after two days feeding and 4–5-day starving periods. The authors connected this to the increase of food availability, which leads to a heavy imbalance in carbon budget of starving individuals (Kremer & Reeve, 1989). In reality, the increment of the carbon/dry weight ratio could also be caused by starvation of ctenophores.

Tissue dehydration, which accompanied metabolism in the starved ctenophores, is the only explanation for the differences between wet and organic weight shrinkage rates for the specimens in the experiments. Probably, that occurred mainly due to gelatinous matrix catabolism. These tissues dominating the wet weight of ctenophores are especially watery. Matrix of *Mnemiopsis* has at least ten times lower carbon content than that observed for ctenes, lobe muscles and gut wall (Reeve et al., 1989). The same is well known for mesogloea of jellyfish which has a very watery consistence and can retain up to 96% of all the organisms' water (Shenker, 1985; Arai, 1989; Lucas, 1994). Mesogloea containing organic compounds were quite often the primary organic resource in these animals during starvation periods (Madin et al., 1981; Larson, 1986). According to the above data, the same metabolic peculiarity at low food level is characteristic of ctenophores as well.

Analysing the organic composition dynamics in starved ctenophores by present and other available data (Hoeger, 1983; Dudkin et al., 2002), two stages for this process could be distinguished. Upon initial light starvation, the energy requirements of the ctenophores were presumably satisfied using the following organic compounds: (1) small functional molecules (glucose, free amino acids, etc.); (2) storage body components (glycogen and some lipids); (3) proteins, performing the reserve function. At the latter stages of

starvation, all body components, and, especially - protein, lipids and carbohydrates, were utilized almost without any preference. Catabolism of glycogen has increased, but that of structural polysaccharides remained at a minimal level.

As the storage compounds have not been accumulated in lobate ctenophores (glycogen content in *M. leidyi* was 2–3%, and reserve lipids were 1–2% of organic weight), duration of the first stage of starvation for this species was short. In the experiment at 12.4°C it did not exceed three days. During this time, though protein remained the principal catabolic compound, lipids, monosaccharides, and amino acids were utilized especially intensively (Figures 4–6). As starvation was extended, the protein disassimilation increased which caused some increase in the body lipids, probably because of structural components (such as sterols and phospholipids) (Anninsky & Gubanova, 1998).

When compared with *M. leidyi,* dynamics of organic composition in starved *B. ovata* was more complicated. Its body storage content appears to be higher than that in the former species. Although glycogen content in *B. ovata* is only ~1% of the organic weight, the higher percentage ($P<0.001$; *t*-test) of the total lipid (~12%) indirectly points to a larger storage lipid content. Correspondingly, on the basis of the body's glycogen and amino acid decrease, duration of the first stage starvation for *Beroe* could run to 4–10 days. In fact, only after 10-day fasting, all the main organic components were utilized at equal rates. Disassimilation of the protein dominated permanently, and that of lipids was increasing up to the 18th day of starvation. The same for this ctenophore was found independently. According to Dudkin et al. (2002), *Beroe* in the course of the starvation has utilized glucose and metabolic carbohydrates most intensively especially in comparing with the utilization of protein and of structural carbohydrates. These changes in the biochemical composition of starved specimens of both *B. ovata* and *M. leidyi* in general did not differ from those that were observed in the ctenophore *Pleurobrachia pileus* from the North Sea (Hoeger, 1983; Schneider, 1989).

The main organic compounds and their ratios usually do not permit us to determine the duration of fasting and food supply in these gelatinous animals. Although, there is supposition, that for some species the total lipid could be an exception in this respect (Schneider, 1989): sometimes their large amounts testified rather to starvation than provision of feeding for ctenophores (Figures 4–6).

Ratios between structural and storage carbohydrate (or lipid) fractions are to be more important for evaluation of the ctenophores food supply in the sea. Earlier we found that under the Black Sea conditions glycogen content in *M. leidyi* strongly correlated with the storage lipids. Moreover, these body compounds changed quantitatively in dependence of seasonal and spatial mesoplankton abundance (Anninsky et al., 1998).

Visually, the investigated ctenophores during starvation had low activity. The respiration levels for starved specimens of *B. ovata* were 1.3 times and for *M. leidyi* two times less than initial values when they were calculated for wet weight of ctenophores. At a temperature of about 20°C the respiration rate in starved *M. leidyi* decreased 1.5 fold in three days (Finenko et al., 1995). However, because the specific organic content showed a trend to increase in starved specimens, the above values did not reflect the actual differences between initial and final metabolic levels. Respiration levels during ctenophore starvation in terms of organic body weight decreased more strongly and approximately equally for both species: by 2.4 times for *B. ovata* and 3-fold for *M. leidyi.*

Very close values of energy expenses determined by respiration and by weight losses separately mean that utilized body tissues in starved ctenophores were mainly metabolically dissipated. Slightly lower values of respiratory expenses could be partially due to incomplete tissue oxidation, organic losses with the excretory products, mucus secretion and spawning processes. According to our unpublished data, starvation in *B. ovata* did not prevent spawning. Their daily reproduction in starving specimens can reach 1% of wet body weight, and even more if it was calculated relative to the body organic matter. Daily mucus production for *M. leidyi* in the summer months amounted to 1% of wet weight (Korneeva & Shiganova, 1995). Furthermore, up to 50% of carbon and nitrogen excreted by this species usually were included with organic excretory products (Kremer, 1977; Kremer & Reeve, 1989). Lower values (10–40% of organic nitrogen in dissolved excretory products) are known for five other ctenophore species (Kremer et al., 1986).

If the main organic losses were due to mucus secretion, its production in terms of the total organic losses during the starvation did not exceed 11% for *B. ovata* or 15% for *M. leidyi.* Because *B. ovata* specimens starved at a higher temperature, which relaxes mucus viscosity, the difference of the above values in the same conditions could be even stronger. This circumstance and the fact that the rate of organic weight decrease for *B. ovata* was also lower than for *M. leidyi* allows us to suppose a better tolerance to starvation for the former species. Taking into account the differences in the temperature and assuming $Q_{10} = 2.25$, the rate of organic weight decrease for *Beroe* at 12.4°C would be about 3.8% d^{-1}. So, comparing with *Mnemiopsis*, *Beroe* could be deemed almost 1.5 times more tolerant to a lack of food, which might

explain to some extent the survival success of *Beroe* during prolonged starvation in the Black Sea conditions.

This species is usually present in the Black Sea plankton from the end of August to the second half of November. Our personal observations revealed that only single individuals were met later in February (Turkish waters) and in the second half of December 2002 (near Sevastopol). It is unclear how and where some *Beroe* individuals can survive the most part of the year (about nine months), in order to provide for the population recruitment during the next autumn. Our calculations have shown that in the most favourable fasting conditions (specimen with initial wet weight of 65 g, organic weight of 0.2 g, seawater temperature of 5 °C, organic shrinkage rate at $Q_{10} = 2.25$ of 2.1% d^{-1}, duration of fasting nine months) some individuals could survive during this time almost without any food. Even with complete starvation, their final organic body weight amounted to 0.7 mg, but it may be enough for survival success. *Beroe* survival rate will increase considerably if occasional food (i.e. *M. leidyi* and *P. pileus*) becomes available. Additional studies of ecology and physiology of the species at lower temperatures are needed before this part of their life history will be completely understood.

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