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Partitioning of respiratory energy and environmental tolerance in the copepods *Calanipeda aquaedulcis* and *Arctodiaptomus salinus*

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ABSTRACT

Total and basal metabolism was studied in the widely distributed copepod species *Calanipeda aquaedulcis* and *Arctodiaptomus salinus* of both genders in order to estimate respiratory energy partitioning. Specific oxygen consumption was found to double in *C. aquaedulcis* than in *A. salinus*, and double in males than in females both in terms of total and basal metabolism. Respiration rates in females carrying ovisacs were 1.49 and 1.43 times higher than those in females without ovisacs for *C. aquaedulcis* and *A. salinus*, respectively. Extra energy expenditures are due to carrying ovisacs and egg respiration. There was no significant effect of salinity (0.1–40), oxygen concentration (1–8 mg O_2 l^{-1}) or crowding on oxygen consumption confirming the hypothesis that *C. aquaedulcis* and *A. salinus* are the animals with a type of respiratory metabolism independent of salinity and oxygen concentration. At critical oxygen concentrations less than 1 mg O_2 l^{-1} respiration rate fell notably by approximately an order of magnitude in both species and in both genders.

1. Introduction

Calanipeda aquaedulcis Kritschagin 1873, euryhaline Copepoda species, inhabits the coastal lagoons, salt marshes and saline wetlands (mainly transition zones, estuaries) from the Atlantic coast of Morocco (Ramdani et al., 2001) and Spain (Frisch et al., 2006) across the Mediterranean coastal areas of Europe (Brucet et al., 2008; Lucena-Moya et al., 2010; Alfonso and Belmonte, 2011), North Africa (Ramdani et al., 2001), North-west Asia (Ustaoğlu, 2004) to the Black and Azov Seas (Siokou-Frangou et al., 2004; Kovalev et al., 2006), Caspian Sea (Garber, 1951) and up to the Aral Sea (Andreev et al., 1992). This species can also penetrate to inner reservoirs of the Mediterranean basin (Samchyshyna, 2008) where it competes with euryhaline Paleoarctic Copepoda species *Arctodiaptomus salinus* Daday 1885 inhabiting mainly lentic water bodies, from small rock pools (Marrone et al., 2006) to large saline lakes (Andreev et al., 1992; Tolomeev et al., 2010). In the Crimean area of the Black Sea *C. aquaedulcis* was found in Sevastopol Bay (Gubanova et al., 2001) while *A. salinus* was abundant mainly in salt coastal lakes of the Eastern Crimea, Kerch peninsula (Shadrin et al., 2008).

Calanipeda aquaedulcis and *Arctodiaptomus salinus* are calanoid copepods spawning their eggs into ovisacs that normally remain attached to the female until emergence of nauplii. *C. aquaedulcis* produces only subitaneous eggs and carries them in ovisacs attached to females until hatching. Thereby, this year-round species inhabits only permanent water bodies (Marrone et al., 2006; Alfonso and Belmonte, 2011). In contrast, *A. salinus* produces both subitaneous and resting eggs and thus are capable of colonizing the temporary reservoirs (Jiménez-Melero et al., 2007).

Semi-permanent estuarine and ephemeral coastal pool ecosystems can undergo frequent and rapid temperature, salinity and oxygen regime changes owing to the strong local meteorological influence. Therefore, the organisms living in such ecosystems have to develop rapid adaptive reactions to environmental changes in order to survive at low dissolved oxygen concentration and crowding, variable salinity or even total desiccation.

The study of physiological responses of these species to rapid changes of environmental parameters can reveal the interspecies differences in copepod adaptability. However, the data on the physiological features of *Calanipeda aquaedulcis* and *Arctodiaptomus salinus* are noticeably lacking, despite a rather long history of ecological studies of these species.

Some ecological aspects of feeding and development of *Calanipeda aquaedulcis* were studied in their natural habitat in the Caspian and Aral Seas (Koudelina, 1950; Garber, 1951; Kortunova et al., 1972), Sicilian inland water (Marrone et al., 2006). Brucet et al. (2008)

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studied feeding of different stages of *C. aquaedulcis* in the Mediterranean salt marshes. The effect of temperature on stage duration and stage-specific mortality of *Arctodiaptomus salinus* was examined by Jiménez-Melero et al. (2007) and salinity tolerance of this species was studied by Rokneddine and Chentoufi (2004). Lapesa et al. (2004) described feeding behavior of *A. salinus* from the coastal ponds of Spain. Tolomeev et al. (2010) compared adaptive changes of feeding spectra and chemical composition of *A. salinus* from the salt lakes of South Siberia with different physico-chemical conditions. Parra et al. (2005) carried out the experiments with populations of *A. salinus* to study the negative effect of pesticides generated by intensive olive tree cultivation on aquatic ecosystem.

Our aim was to study under laboratory conditions the effect of salinity (0.1–40), dissolved oxygen concentration (0.2–8.0 mg l^{-1}), crowding (2–27 ind ml^{-1}), gender and fecundity status (egg-carrying and non-egg-carrying females) on the respiration rate of *Calanipeda aquaedulcis* and *Arctodiaptomus salinus.* Metabolic patterns as the indicators of the type of acclimation response (regulation or conformality) to changes in salinity and dissolved oxygen concentration were analyzed. Fecundity status was considered in order to estimate the cost of egg-carrying strategy. Crowding was considered in the methodological aspect in order to correct oxygen consumption measurements.

2. Materials and methods

2.1. Laboratory experiments

Calanipeda aquaedulcis and *Arctodiaptomus salinus* used in laboratory experiments were initially collected from the salt lakes located near the sea coast of Kerch peninsula (Crimea) in 2007. Copepods of these species were cultivated successfully during 2007–2011 in semi-continuous monospecific batch cultures in the Department of Mariculture of the Institute of Biology of the Southern Seas (IBSS, Sevastopol, Ukraine) through numerous generations and were used as models for various ecological and physiological investigations. The experiments were conducted on specimens of *C. aquaedulcis* and *A. salinus* separated from continuous cultures and maintained before the experiments in the filtered Black Sea water (FSW) of salinity 18–19. Mixture of microalgae *Isochrysis galbana*, *Prorocentrum minimum* and *Prorocentrum micans* was added to filtered water to feed copepods *ad libitum*.

To study the effect of gender and fecundity status on the respiration rate of *Calanipeda aquaedulcis* and *Arctodiaptomus salinus,* total and basal (or "standard", i.e. oxygen consumption rate for maintenance only, see Ikeda et al., 2001) metabolism of ovigerous egg-carrying and non-egg-carrying females and males from both species, and oxygen consumption of subitaneous eggs of *A. salinus* were investigated in spring 2010. The effect of dissolved oxygen concentration and crowding on total metabolism of adults from both species was studied also in spring 2010 while the effect of salinity on their total metabolism was analyzed in autumn 2010. The respiration rate of diapausing eggs of *A. salinus* was examined in winter 2011. For comparison, the respiration rate of eggs of *Calanus helgolandicus* collected by plankton net in the Black Sea (near Sevastopol) was studied in March 2011.

Throughout this paper, the ovigerous (with visible black gonads in the body), egg-carrying females hereinafter are referred to as (OF) whereas ovigerous, non-egg-carrying females are referred to as (NOF) and adult males are referred to as (M).

2.2. Measurements of adult respiration rate

Total and basal metabolism (as oxygen consumption) of egg-carrying and non-egg-carrying females and adult males were studied in separate experiments. Prior to the experiments, about 50 individuals of females or males of each species were placed in 100-ml beakers containing 0.45 μm FSW. The copepods were allowed to empty guts as they were deprived of food for approximately 2 h prior the experiment. After this procedure only data from actively swimming copepods, both at the beginning and end of each incubation period were selected.

Respiration rate (R, μg O_2 ind^{-1} h^{-1}) of copepods was determined using sealed chamber method with experimental and control syringes of 2.0 ml as the respirometers. 5–10 females or males of *Calanipeda aquaedulcis* and 3–6 females or males of *Arctodiaptomus salinus* per 1 ml were gently transferred by a pipette into filled with filtered seawater experimental syringe supplied by protective sieve disc (mesh size 200 μm) at the confluent outlet.

In order to obtain identical oxygen, salinity and seston content, we connected the control and experimental syringes with a plastic tube and pumped the water through it back and forth several times. Then the syringes were separated, closed by the stoppers and placed to the dark chamber at 20 ± 0.5 °C. Incubation periods amounted to about 2–3 h. During the incubation the total oxygen consumed was never more than 20% of the initial value. There were ≥10 replicates for each experiment.

In order to investigate basal metabolism, the copepods in our experiments were narcotized until complete immobilization (not more than 2 min) with 1: 5000 MS-222 Sandoz according to common experimental practice (Gill, 1987; Knutsen et al., 2001; Van Duren et al., 2003).

Just after immobilization, double number of narcotized copepods (in comparison with the number of intact actively swimming individuals used in the experiments on total metabolism) was transferred to the experimental syringes filled by the FSW with three times lower concentration of MS-222 enough for the copepods to be immobile during 3 h exposure. During the incubation the syringes with narcotized animals were rotated every 10 min to avoid the development of O_2 gradients within the respirometer.

After incubation, the individuals were gently transferred to the fresh seawater free of MS-222 where they rapidly recovered full activity within 10–20 min. Only the results of the experiments in which the copepods did not awaken during incubation but recovered their activity after incubation were analyzed.

At the end of the exposure, the water sample from the experimental or control syringe was transferred to the measuring flow chamber with a variable volume (up to 0.3 ml) (created from a truncated all-glass syringe with truncated hub) joined to a luminescent dissolved oxygen sensor Hach LDO™. Other details of respiration rate measurements were described in Svetlichny and Hubareva (2005) and Svetlichny et al. (2010).

2.3. Measurements of adult respiration rate under different salinity and dissolved oxygen concentrations

The high tolerance of two studied species to both fresh and hyper-saline (up to 40–50) water has been established in preliminary experiments (Hubareva and Svetlichny, 2011). To study the effect of low and high salinity on copepod respiration rate, two treatments were used in the experiments with *Calanipeda aquaedulcis* and *Arctodiaptomus salinus*: (1) salinity was decreased gradually (within 6 h interval) from 18–19 to 1–1.5 at a rate of 3 per h. Copepods were kept at the lowest salinity (1–1.5) for about 20 h. After that salinity was gradually (within 6 h interval) decreased to 0.2 at a rate of 0.2 per h; (2) salinity was gradually increased from 18–19 to 30 during 6 h at a rate of about 2 per h. The following day, salinity was gradually increased from 30 to 40 at the same rate. Respiration rates at each salinity level were measured in copepods only after acclimation period not less than 1 week.

Throughout acclimation periods to low-saline and high-saline water copepods were fed *ad libitum* with the suspension of microalgae *Haemotococcus pluvialis* and mixture of microalgae *Isochrysis galbana*, *Prorocentrum minimum* and *Prorocentrum micans*, respectively.

We prepared water of low and high salinity by adding distilled water or artificial sea salt to the filtered Black Sea water of 18 ± 0.5 °C. Water salinity was measured by a Hach conductivity meter "SensIon 5" using the Practical Salinity Scale.

To examine the effect of oxygen concentration on the total metabolism, copepods were transferred into an experimental syringe with filtered seawater with different initial dissolved oxygen concentration ranged from 2.0 to 8.0 mg O_2 l^{-1}.

In order to decrease the oxygen concentration from 2.0 to 0.2 mg O_2 l^{-1}, the seawater in the syringes with the same individuals was changed gradually in 3 steps (each about 2 h) by substituting for more oxygen-depleted water after every incubation. Oxygen-deficient seawater was prepared by bubbling nitrogen through it in separate 0.5 l flask.

2.4. Crowding effect

To estimate the crowding effect on respiration rate, the animals preliminary washed in the FSW were concentrated in a small flask and thereafter were quickly transferred into experimental chambers (syringes of 2 ml with effectively reduced volume to 1.5 ml) filled with aerated FSW at 20 °C and salinity of 18. The number of *Calanipeda aquaedulcis* and *Arctodiaptomus salinus* used for each measurement was in the range of 2–27 and 3–10 ind ml^{-1}, respectively. The incubation time ranged from 3 h for the lowest animal density to 1 h for the highest one. During the incubation oxygen concentration in the experimental units decreased not more than 20% from the initial level. At the end of all experiments copepod prosome length and width were measured dorsally to the nearest 10 μm under a light microscope, fitted with an eyepiece micrometer.

2.5. Measurements of egg respiration rate

To collect subitaneous eggs, about 100 females of *Arctodiaptomus salinus* carrying ovisacs during spring experiments were transferred to the flasks containing 200 ml of 0.22 μm filtered seawater and were exposed to physical stress by vigorous shaking, thus ovisacs with eggs were separated from the female bodies. Ovisacs with resting eggs were collected from winter ovigerous females kept for 2 days in separate dishes. Before the measurements all eggs were carefully washed in the FSW.

To measure egg respiration rate, either 10–20 ovisacs with subitaneous *Arctodiaptomus salinus* eggs (ca. 100–250 embryos prior hatching), or 20–40 ovisacs with ca. 200–500 resting eggs at cleavage stage freshly spawned by females were transferred by narrow-mouth pipette to experimental syringes of 1.0 ml and incubated in each syringe for 4–5 h at 20 °C and salinity of 18. For comparison, egg respiration rate was studied in *Calanus euxinus* (50 eggs per 1.0 ml). During incubation the syringes with eggs were rotated every 10 min to avoid the development of O_2 gradients within the respirometers. We controlled uniform distribution of eggs in horizontally orientated syringes.

2.6. Size-weight measurements

Prior the experiments we placed 100–200 females or males of *Calanipeda aquaedulcis* and *Arctodiaptomus salinus* into Petri dishes with small portions of filtered sea water and narcotized using MS-222. 40–80 individuals depending on species and gender were selected for measurements of dry weight (DW, mg) in 3 replications to attain a minimum total dry weight not less than ca. 300 μg. The animals were rinsed in a small amount (ca. 1 ml) of distilled water and placed on small aluminum foil dishes (ca. 8 mm in diameter). They were then dried at 60 °C for 24 h, cooled in a desiccator at room temperature, and their dry weights were measured several times using an analytical balance (Model VLM-1) at 5 μg precision. Before weighing, the prosome length (L, mm) and width (d, mm) were measured under a light microscope at a 48× magnification in 20 individuals from each batch. The length of copepod prosome was measured from anterior point of the head to the flexure joint of the thorax and abdomen. Because of methodical problems to measure individual dry weight (DW_{in}) in small sample of animals after our respiration experiment, we calculated DW_{in} of females and males of both species as $DW_{in} = CF\, L_{in}\, d_{in}^2$, where CF is gender- and species-specific condition factor calculated as $CF = DW_m/L_m\, d_m^2$, where L_m and d_m are the mean values.

Average dry weight of eggs (DW_{egg}, ng) was calculated from the equation:

$$DW_{egg} = 0.167\, k\pi D_{egg}^3,$$

where D_{egg} is the mean diameter of eggs (μm) and k is the ratio of dry weight to volume (ng μm^{-3}) equal to 0.000476 in subitaneous and 0.000623 in resting eggs (calculated from Wang et al., 2005). The diameter of copepod eggs was measured under light microscope at a 300× magnification.

Statistical evaluation of data was conducted by one-way ANOVA. Average values presented in the figures are means ± standard deviation.

3. Results

3.1. Dry weight and condition factor

During spring experiments mean dry weights of *Calanipeda aquaedulcis* females and males were equal to 0.0075 ± 0.0005 and 0.0046 ± 0.0006 mg, respectively. In *Arctodiaptomus salinus* dry weights of females and males corresponded to 0.0213 ± 0.0016 and 0.0125 ± 0.0004 mg, respectively. Mean CF values in *C. aquaedulcis* females and males were equal to 0.122 ± 0.020 and 0.139 ± 0.020, while those in *A. salinus* females and males amounted to 0.144 ± 0.011 and 0.118 ± 0.004, respectively.

3.2. Adult respiration rates

Table 1 shows that at 20 °C and salinity of 18 the mean values of respiration rate of active ovigerous females of *Calanipeda aquaedulcis* (0.082 ± 0.017 μg O_2 ind^{-1} h^{-1}) carrying on average 12.2 eggs per sac and respiration rate of *Arctodiaptomus salinus* females (0.156 ± 0.038 μg O_2 ind^{-1} h^{-1}) carrying 9.3 eggs per sac were significantly (*t*-test, $p < 0.001$) 1.49 and 1.43 times, respectively, higher than that in non-egg-carrying females.

In *Calanipeda aquaedulcis* respiration rates of active females and males were significantly (*t*-test, $p < 0.001$) 2.2 and 2.1 higher then those in narcotized individuals while in *Arctodiaptomus salinus* the ratios between respiration rates of active and narcotized females and males amounted to 2.1 and 2.6, respectively.

3.3. Effect of salinity

Non-egg-carrying females of *Arctodiaptomus salinus* showed no statistical differences (*t*-test, $p > 0.05$) of dry weight-specific respiration rates (Fig. 1) at the salinities of 0.2, 18.7 and 39.2

Table 1
Respiration rate and morphological characteristics of *Calanipeda aquaedulcis* and *Arctodiaptomus salinus* at normoxic conditions, 20 ± 1 °C and salinity of 18. Values are presented as means ± SD. Number of replicates are shown in parentheses.

Parameters	*Calanipeda aquaedulcis*			*Arctodiaptomus salinus*		
	Female with ovisac	Female without ovisac	Male	Female with ovisac	Female without ovisac	Male
Total respiration rate, μg O_2 ind^{-1} h^{-1}	0.082 ± 0.017 (24)	0.055 ± 0.011 (32)	0.064 ± 0.014 (8)	0.156 ± 0.038 (29)	0.109 ± 0.019 (30)	0.099 ± 0.016 (31)
Basal respiration rate, μg O_2 ind^{-1} h^{-1}	0.034 ± 0.008 (11)	0.025 ± 0.007 (11)	0.030 ± 0.011 (9)	0.064 ± 0.011 (12)	0.052 ± 0.012 (12)	0.038 ± 0.013 (15)
Prosome length (L), mm	0.813 ± 0.035	0.806 ± 0.037	0.692 ± 0.020	1.191 ± 0.069	1.133 ± 0.058	0.900 ± 0.046
Prosome width (d), mm	0.300 ± 0.017	0.291 ± 0.026	0.243 ± 0.013	0.456 ± 0.037	0.432 ± 0.027	0.351 ± 0.020
Dry weight (DW), mg	0.0116[*,†]	0.0083[*]	0.0057[*]	0.0373[*,†,‡] 0.0395[*,†,‡]	0.0304[*]	0.013[*]
Egg number in ovisac	12.2 ± 3.5			9.3 ± 3.2[‡] 12.3 ± 3.7[§]		

[*] Body dry weight calculated as DW = CF L d^2 (see Results).
[†] Dry weight of ovisac calculated taking into account mean number and volume of eggs and specific DW in subitaneous eggs of sacspawning copepods (Wang et al., 2005).
[‡] Mean number of eggs in ovisac in the experiment with active females.
[§] Mean number of eggs in ovisac in the experiment with narcotized females.

(3.87 ± 0.56, 3.44 ± 0.57 and 3.87 ± 0.84 μg O_2 mg^{-1} h^{-1}, respectively). Also there was no salinity dependence of specific oxygen consumption rate (SOC) in non-egg-carrying females and males of *Calanipeda aquaedulcis* kept not less than one week at the salinities of 0.1, 18 and 40 (7.75 ± 1.22, 6.43 ± 1.32 and 7.07 ± 1.20 μg O_2 $mgDW^{-1}$ h^{-1} for females and 13.1 ± 2.2, 12.6 ± 2.7 and 13.3 ± 4.2 μg O_2 $mgDW^{-1}$ h^{-1} for males, respectively).

3.4. Effect of oxygen concentration

There was no evidence of a relationship between dissolved oxygen concentration and respiration rate of *Calanipeda aquaedulcis* and *Arctodiaptomus salinus* females and males over the range of 1–8 mg O_2 l^{-1} (Fig. 2). However, when oxygen concentration decreased to sublethal (estimated as the level of dissolved oxygen which experimental copepods can tolerate only for 1–2 h) oxygen concentrations (0.2 mg O_2 l^{-1} for *C. aquaedulcis* and 0.4 mg O_2 l^{-1} for *A. salinus*), copepod respiration rates fell largely about 10 times in both species and in both genders.

3.5. Crowding effect

As experimental conditions are known to affect the respiration rate of zooplankton (Ikeda et al., 2000), the effect of crowding was examined. The respiration rates from individual measurements were expressed as a percentage of the mean respiration rate in each series of experiments (Fig. 3). Relative respiration rates of females of *Calanipeda aquaedulcis* (2–27 ind ml^{-1}, $n = 56$) and females and males of *Arctodiaptomus salinus* (3–10 ind ml^{-1}, $n = 59$) were not affected by crowding (the slopes of the regression line did not differ from zero, $p > 0.5$). In males of *C. aquaedulcis*, the correlation coefficient between relative respiration rates and population density was −0.6 and respiration rate at the density of 5–8 ind ml^{-1} was significantly ($p < 0.05$) 30%

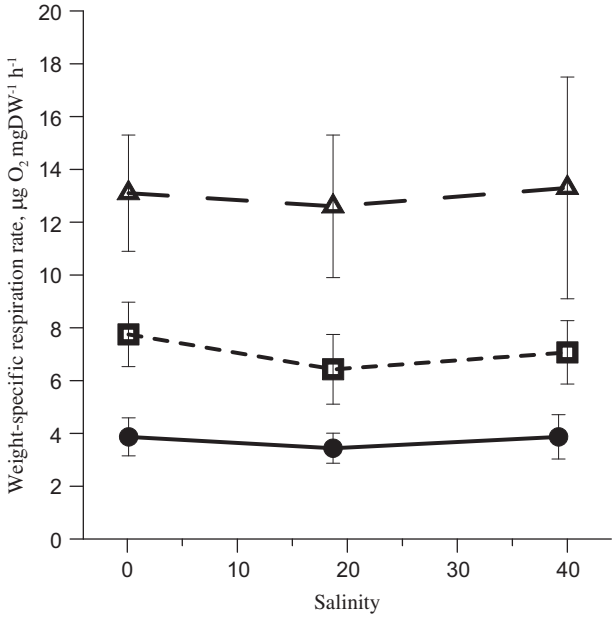

Fig. 1. Relationship between dry weight-specific respiration rate of females of *Arctodiaptomus salinus* (●, shaded circle), females (□, square) and males (△, triangle) of *Calanipeda aquaedulcis* and salinity of water.

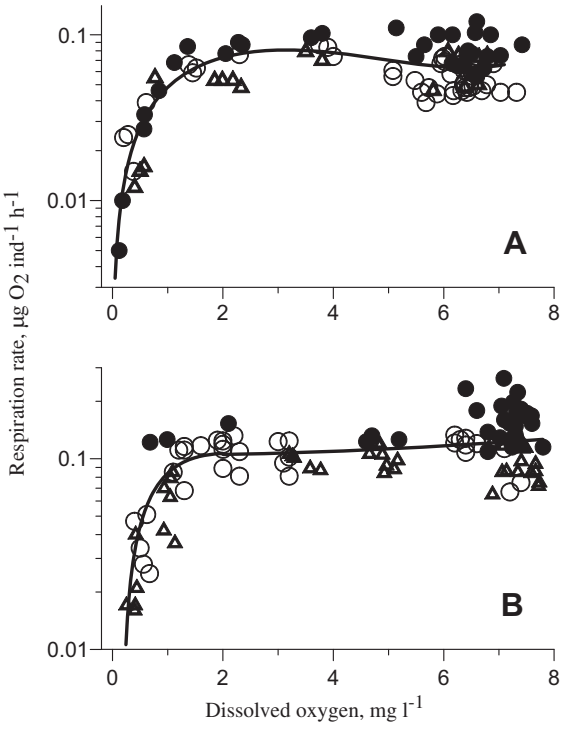

Fig. 2. Relationship between respiration rate and ambient oxygen concentration in egg-carrying (●, shaded circle) and non-egg-carrying (○, open circle) females and males (△, triangle) of *Calanipeda aquaedulcis* (A) and *Arctodiaptomus salinus* (B).

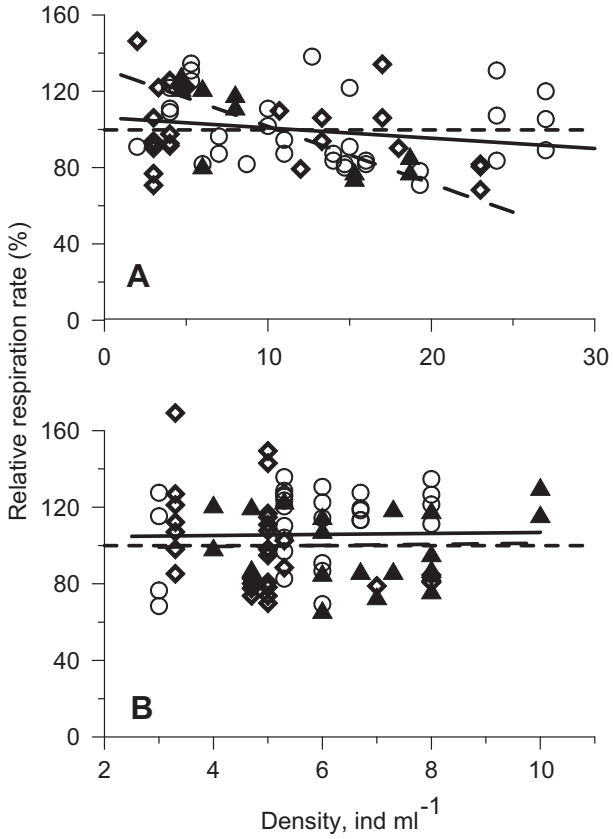

Fig. 3. Relationship between relative respiration rate (expressed as per cent of the mean values taken as 100%, dotted line) of females with ovisacs (◆, diamond), females without ovisacs (○, open circle) and males (▲, shaded triangle) of *Calanipeda aquaedulcis* (A) and *Arctodiaptomus salinus* (B) and population density (ind ml^{-1}) of animals in the respirometers. Dashed lines show the regression of relative respiration rate in females. Hatched line show the regression of relative respiration rate in males.

higher in comparison with the respiration rate at the density of 15–19 ind ml^{-1}, probably, due to low number of measurements ($n = 10$).

3.6. Egg respiration rate

The respiration rate of resting eggs freshly spawned by *Arctodiaptomus salinus* females was equal to 0.66 ± 0.22 ng O_2 $embryo^{-1}$ h^{-1} (Table 2). Those eggs were apparently delayed hatching eggs (Chen and Marcus, 1997) because a small number of nauplii had been hatching gradually from the eggs during 5 months from December till April despite the fact that the eggs remained alive. The respiration rate of subitaneous eggs of *A. salinus* at late embryonic stage prior hatching (nauplii hatched within 24 h) appeared to be 2.4 times higher (1.62 ± 0.15 ng O_2 $embryo^{-1}$ h^{-1}). In fresh subitaneous eggs of *Calanus euxinus* the oxygen consumption rate was equal to 4.75 ± 1.17 ng O_2 $embryo^{-1}$ h^{-1}.

4. Discussion

4.1. The partitioning of respiratory energy

The ratio of total to basal (standard) metabolism in copepods characterizes the level of their activity. The ratio between **s**pecific oxygen consumption rates (SOC) in non-feeding active and narcotized *Calanipeda aquaedulcis* and *Arctodiaptomus salinus* was found to be 2.1 and 2.4 in non-egg-carrying females, and 2.2 and 2.6 in males, respectively (Fig. 4), i.e. 52–62% of their respired energy is due to locomotory activity. This indicates relatively high activity level of copepods in our experiments because during continuous swimming at a rate of 5–10 body lengths s^{-1} the respiration rate of copepods was 3.1 times higher than that during rest (Morris et al., 1985; Paffenhöfer, 2006) while the maximum ratio of active to basal metabolism (both narcotized or non-moving individuals) in copepods did not exceed 6 (Svetlichny and Hubareva, 2005).

The SOC of the active males reached 12.6 and 7.6 μg O_2 mg^{-1} h^{-1} for *Calanipeda aquaedulcis* and *Arctodiaptomus salinus*, respectively, and was approximately twice that in active females, probably, due to higher swimming activity of males. Many calanoid species males showed a significantly higher moving activity than females (Van Duren and Videler, 1995; Kiørboe and Bagøien, 2005; Uttieri et al., 2007; Kiørboe, 2007; Dur et al., 2010; Michalec et al., 2010). However, in our experiments the SOC of narcotized males was also approximately twice higher than that of narcotized females indicating that males have higher metabolic status.

In comparison with respiration rates of females and males in *Arctodiaptomus salinus*, respiration rates of females and males in *Calanipeda aquaedulcis* were 1.9 and 1.55 times lower (Table 1). In contrast, the values of SOC for both genders of *C. aquaedulcis* were on average 1.8 times higher (Fig. 4). This difference can only partly be explained by the general rule of the decrease of SOC with an increase in body weight of species. According to a comprehensive analysis of respiration – body mass datasets in oceanic epipelagic copepods (Ikeda et al., 2001), it is assumed that metabolic rate of copepods scales with dry body mass as $DW^{-0.2}$. Mean DW in *A. salinus* females and males was, correspondingly, 3.66 and 2.75 times higher than those in *C. aquaedulcis*. Hence, theoretically, SOC of *C. aquaedulcis* females and males should be only 1.3 and 1.22 higher than those in *A. salinus*. Whereas the SOC of narcotized specimens of both genders of *C. aquaedulcis* was found to be also 1.8–2.0 times higher than SOC of *A. salinus*, it could be assumed that the higher energetic requirements of *C. aquaedulcis* are due to a two-fold higher somatic growth rate (Aganesova, 2011) and generative production (experimental data, Aganesova, unpublished), in comparison with *A. salinus*.

Significant differences between the respiration rates of active and narcotized OF and NOF in our experiments (Table 1) allow us to calculate a contribution of transport and respiration of eggs to the total respired energy. The respiration rate of eggs in the ovisac (R_{egg}) can be expressed as a difference between respiration rates of narcotized ovigerous R_{nOF} and narcotized non-ovigerous R_{nNOF} females: $R_{egg} = R_{nOF} - R_{nOF}$. Hence, the cost of egg sac transport (R_{et}) can be determined as:

Table 2
Egg respiration rate in *Calanipeda aquaedulcis* and *Arctodiaptomus salinus* at 20 ± 1 °C and salinity of 18. Values are presented as means ± SD. Number of replicates are shown in parentheses.

Parameters	*Calanipeda aquaedulcis*	*Arctodiaptomus salinus*		*Calanus euxinus*
	Subitaneous eggs	Subitaneous eggs	Resting eggs	Subitaneous eggs
Respiration rate, ng O_2 $embryo^{-1}$ h^{-1}	0.74*	1.54* 1.62 ± 0.15 (6)	0.67 ± 0.22 (8)	4.75 ± 1.17 (5)
Egg diameter, μm	103 ± 6 (28)	143 ± 8 (36)	144 ± 9 (47)	181 ± 6 (53)

* Calculated as the difference between respiration rate of narcotized females with ovisacs and without ones taking into account mean number of eggs in ovisac.

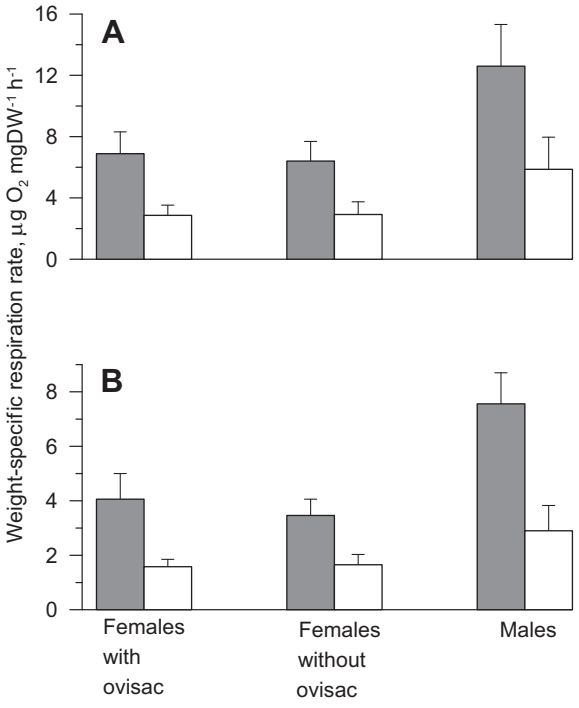

Fig. 4. Weight-specific respiration rate of active (■, shaded bar) and narcotized (□, empty bar) females with ovisacs, females without ovisacs and males of *Calanipeda aquaedulcis* (A) and *Arctodiaptomus salinus* (B).

$$R_{et} = R_{aOF} - R_{aNOF} - R_{egg},$$

where R_{aOF} and R_{aNOF} are the respiration rates of active OF and NOF, respectively.

Calculated mean values of R_{egg} and R_{et} amounted to 0.009 µg O_2 ovisac^{-1} h^{-1} and 0.018 µg O_2 ind^{-1} h^{-1}, respectively, in *Calanipeda aquaedulcis*, and 0.019 µg O_2 ind^{-1} h^{-1} and 0.028 µg O_2 ind^{-1} h^{-1}, respectively, in *Arctodiaptomus salinus*. Thereby, oxygen consumption of eggs in the ovisac constituted 16.4 and 17.4% of the total metabolism of females of *C. aquaedulcis* and *A. salinus*, respectively, and the subsidiary metabolic expenditures (e.g. dragging the ovisac taking into account water resistance to movement and gravity of the ovisacs), made up 32.7 and 25.7% of total metabolism, respectively.

Only two studies (Glazier, 1991; Parra et al., 2003) compared respiration rates of ovigerous and non-ovigerous females of planktonic crustaceans. Parra et al. (2003) carried out experiments on *Neolovenula alluaudi* (Diaptomidae) and found that oxygen consumption rates were higher in females carrying eggs than in those that did not. However, when SOC was calculated, no statistically significant differences were found between these groups. Glazier (1991) measured the respiration rates of embryos, brooding females of *Daphnia magna* and females without broods, and found that the cost of carrying a brood in *D. magna* was negligible whilst the SOC of females (5.25 µl O_2 mg^{-1} h^{-1}) whose broods were surgically removed was identical to that of brooding females. We consider that this could be accounted for the presence of a large mass of embryos in the brood pouch with low SOC in comparison with SOC of females. According to Glazier (1991), the SOC of eggs of *D. magna* averaged 47.5% of the female body DW amounting to 2.0 µl O_2 mg^{-1} h^{-1}.

In our experiments respiration rates of subitaneous eggs in *Calanipeda aquaedulcis* and *Arctodiaptomus salinus* (calculated from the values of R_{egg} taking into account the mean number of eggs per ovisac) amounted to 0.74 and 1.54 ng O_2 embryo^{-1} h^{-1}, while their SOC were calculated as 2.73 and 2.02 µg O_2 mg^{-1} h^{-1}, respectively. Measured values of respiration rate of subitaneous eggs of *A. salinus* (1.62 ± 0.15 ng O_2 embryo^{-1} h^{-1}) appeared to be very close to calculated ones (Table 2) confirming the correctness of our estimation of respiratory energy partitioning.

For comparison we present below our own and literature data on the respiration rate of eggs of other copepod species at 20 °C as a volume specific respiration rate (VSR). Mean values of VSR of subitaneous eggs in *Calanipeda aquaedulcis* and *Arctodiaptomus salinus* amounted to 1.22 and 0.99 µg O_2 mm^{-3} h^{-1} while that in *Calanus euxinus* studied in our experiments was found to be 1.56 µg O_2 mm^{-3} h^{-1}. VSR of subitaneous embryos (in pre-hatching phase) of *Centropages tenuiremis* calculated from their respiration rate (Wu et al., 2009) and egg diameter (Wang et al., 2005) was equal to 2.9 µg O_2 mm^{-3} h^{-1} taking into account the temperature coefficient of 2.05 (Wu et al., 2009). Maximum VSR of *Pontella mediterranea* subitaneous eggs with the diameters of 130–150 µm (Santella and Ianora, 1990) in 30 h after spawning at 20 °C (Romano et al., 1996) reached 4.9 µg O_2 mm^{-3} h^{-1}.

We suggest that the higher VSR of eggs of *Calanus euxinus*, *Centropages tenuiremis* and *Pontella mediterranea* can be explained by shorter period of embryonic development in these free-spawning species in comparison with egg-brooding *Calanipeda aquaedulcis* and *Arctodiaptomus salinus* (Kiørboe and Sabatini, 1994). We cannot explain the extremely high value of VSR (30.7 µg O_2 mm^{-3} h^{-1}) in subitaneous eggs of *Acartia tonsa* (Nielsen et al., 2007). According to these authors, oxygen consumption of one egg of *A. tonsa* with the diameter of 82.3 µm at 21 °C was equal to 0.28 ± 0.07 nmol O_2 h^{-1}, or 0.009 µg O_2 egg^{-1} h^{-1} whilst, according to literature data (see Hubareva et al., 2008), oxygen consumption of *A. tonsa* females at close temperature varied from 0.043 to 0.13 µg O_2 ind^{-1} h^{-1}. From these data, oxygen consumption of one egg made up 7–20% of oxygen consumption of *A. tonsa* female, whereas in our experiments oxygen consumption of one subitaneous egg constituted 1.3 and 1.4% of oxygen consumption of *C. aquaedulcis* and *A. salinus* females, respectively.

In our study, the respiration rate of resting eggs in *Arctodiaptomus salinus* was found to be 2.4 times lower than that of subitaneous eggs which is in accordance with the differences in respiration rates of subitaneous and resting eggs of other copepods (Romano et al., 1996; Wu et al., 2009).

4.2. Effect of salinity on respiration rate

Curiously, two species, named one “freshwater” (*Calanipeda aquaedulcis*), another “salty” (*Arctodiaptomus salinus*), in reality inhabit similar environments with similar range of salinity, and despite most researchers ranking *A. salinus* among freshwater species, we did not found any literature descriptions of the studied species in the natural freshwater habitat.

According to Remane (1934), the critical salinity of 5–8 could be considered as a physiological boundary between freshwater and marine copepod species. However, in our experiments the individuals reared at the salinity of 18 survived a gradual decrease of salinity down to 0.1–0.2 and gradual increase of salinity up to 40. Live nauplii hatched from the ovisacs of females of *Calanipeda aquaedulcis* and *Arctodiaptomus salinus* in fresh and hypersaline water as well. This indicates that populations of both species are able to develop and colonize the waters with wide salinity range. Such extremely wide range of salinity tolerance was indicated for intertidal harpacticoid copepods of the genera *Tigriopus*, *Tachidius* and *Tisbe* found to be active within the range of 0–60 (Finney, 1979).

The ability to tolerate low salinities in copepods is ascribed to osmotic processes requiring additional energy losses (Gyllenberg

and Lundqvist, 1978; McAllen and Taylor, 2001). The minimum thermodynamic cost of ionic regulation in brackish animals has been estimated theoretically from 1 to 5% of the total metabolic energy (Potts, 1954). Goolish and Burton (1989) estimated theoretically that the daily energy required to produce organic osmolites following hyperosmotic stress in *Tigriopus californicus* amounted to 11.6% of the total energy. Such values are close to permissible errors for the methods of respiration rate measurements.

In our experiments, the SOC of *Arctodiaptomus salinus* females and females and males of *Calanipeda aquaedulcis* was found nearly constant in salinity range of 0.1–40. Similarly, there was no evidence of salinity-associated respiratory distress in respiration experiments with *Eurytemora affinis* in the range of 0–40 (Roddie et al., 1984). The respiration rate of *Pseudodiaptomus hessei* females also showed no differences over the range of 3–31 (Isla and Perissinotto, 2004). There was no evidence of changes in respiration rate and moving activity of *Arctodiaptomus spinosus* within the salinity range of 3–66 (Newrkla, 1978).

Gross (1957) suggested that oxygen consumption rates in crustaceans do not manifest increased osmotic work, whereas muscular activity is associated with an escape reaction from unfavorable conditions.

Changes in swimming speed and oxygen consumption at hypo- and hyper-osmotic stress can be attributed to changes in body density of copepods as a result of alterations in ionic content of the body fluids (McAllen et al., 1998); these may be due to the differences in energy requirements for sustained locomotion (McAllen and Taylor, 2001). According to our data (unpublished results), after salinity changes body density of *Calanipeda aquaedulcis* and *Arctodiaptomus salinus* varied in proportion to the density of water while the speed of passive sinking was constant. Apparently, these species could be considered as osmoconformers in the studied range of salinities (0.1–40). The counterbalance between the densities of inner and surrounding water allows keeping energy requirements for sustained locomotion at changing salinity.

4.3. Effect of dissolved oxygen on respiration rates

Dissolved oxygen as well as salinity is considered to be the dominant factor affecting the inner lentic and costal shallow sea water communities. According to Prosser and Brown (1961), there are two types of respiratory response to dissolved oxygen concentration changes. In the animals from the first type, respiration decreases as the oxygen concentration diminishes. A decrease in oxygen consumption is commonly accompanied by the reduction in locomotory activity (Ikeda, 1977; Svetlichny et al., 2000; Svetlichny and Hubareva, 2002). The animals from the second type show no oxygen-dependent effects in the wide range of oxygen concentrations until to some critical value below which their respiration rates decline rapidly. The concentration of dissolved oxygen at which such a response is observed in the majority of marine animals varies in the range of 1–3 mg O_2 l^{-1} due to the level of locomotory activity, acclimatization shift, body size and metabolic features (Suschenya, 1972; Diaz and Rosenberg, 1995).

We found that the respiration rate of OF, NOF and M in *Calanipeda aquaedulcis* and *Arctodiaptomus salinus* at 20 °C and salinity of 18 showed oxygen-independent type of response in the range of 1.0–7.8 mg O_2 l^{-1} and oxygen-dependent type in the range of 0.1–0.25–1.0 mg O_2 l^{-1} when oxygen consumption sharply decreased about ten times (Fig. 2). At oxygen concentrations less than ~0.5 mg O_2 l^{-1} the animals reduced moving activity during 1–2 h of exposure and did not survive through longer incubation period.

A similar respiration–oxygen relationship was found by McAllen and Taylor (2001) in *Tigriopus brevicornis*; the respiration rate of this species started to decrease only at 5% of oxygen saturation. It is probable that the ability of the animals to maintain respiratory independence in the wide range of oxygen concentrations facilitates swimming and feeding activity at sporadic and relatively short hypoxia events. A decrease in oxygen concentration down to the critical value either promotes entering a state of dormancy, or kills them (McAllen et al., 1999). Inversely, the oxygen-dependent type of metabolism allows conserving the energy under hypoxic conditions that can be considered as an adaptation of oceanic and marine mid-water crustaceans to living in oxygen-deficient zones (Childress and Seibel, 1998; Svetlichny et al., 2000).

4.4. Crowding

The use of small containers and high densities of specimens is the main reason for obtaining a measurable difference in oxygen concentrations between experimental and control chambers. However, the perception of stimuli during contact with a container wall, interaction between individuals and accumulation of excreta could all affect the metabolic rates of studied animals.

According to Pavlova (2006), the respiration rate of marine copepods performing diel vertical migrations decreases progressively with the volume of experimental bottles because of the suppression of locomotor activity. However, during short-term experiments in small volumes both oxygen consumption and moving activity of some copepods can be close to those in large volumes because the limitation of dislocation in space does not reduce the locomotory activity of the species which move using mouth appendages (Svetlichny and Umanskaya, 1991). Dur et al. (2011) showed that variations in density and vessel size did not affect significantly the swimming behavior of estuarine copepods *Pseudodiaptomus annandalei* and *Eurytemora affinis*. Also, Buskey et al. (1996) found no significant relationship between the density and swimming speed in the mangrove cyclopoid copepod *Dioithona oculata*. There was no evidence of density-dependence of respiration in *Oithona similis* (Castellani et al., 2005), *Calanus finmarchicus* (Zeiss, 1963), *Temora stylifera* and *Centropages typicus* (Razouls, 1972).

Our results showed that in short-term experiments (1–3 h) respiration rates of OF, NOF and M of both *Calanipeda aquaedulcis* and *Arctodiaptomus salinus* were not affected by the densities of specimens in the range of 2–27 and 3–10 ind ml^{-1}, respectively. Despite the fact that no correlation was found between the density of copepods and individual respiration rates, we studied the effects of salinity and dissolved oxygen on the metabolism of active animals at the densities not higher than 10 females or males of *C. aquaedulcis* and not higher than 6 females or males of *A. salinus* per 1 ml. Such a sampling design produces measurable and significant difference in oxygen concentrations between experimental and control respirometers after 2 h incubation.

5. Conclusion

Our new data on the respiration rates and comparative analysis of species-specific patterns of metabolism in two copepod species *Calanipeda aquaedulcis* and *Arctodiaptomus salinus* showed that these euryhaline calanoid copepods with similar ecology and distribution areas under unstable conditions can also easily adapt to fresh and marine waters. Both species were found to have osmo- and oxygen-independent metabolism because their respiration rates did not change in the range of salinities from 0.1 to 40 and oxygen concentrations from 1.0 to 8.0 mg O_2 l^{-1}.

Despite the fact that SOC was found higher in *Calanipeda aquaedulcis* than in *Arctodiaptomus salinus*, both in terms of the total and basal metabolism, the partitioning of the respired energy demonstrated similar patterns. In our experiments the quota of total metabolism associated with swimming activity was 52–55% in females and 53–62% in males.

Our experimental data indicate that carrying eggs in the ovisacs (mean number of 12 eggs) increases significantly the oxygen consumption rate by 49.5 and 43.1% in females of *Calanipeda aquaedulcis* and *Arctodiaptomus salinus*, respectively. The extra energy expenditures related to ovisac gravity and hydrodynamic resistance increased oxygen respiration rate by 32.7% in *C. aquaedulcis* and 25.7% in *A. salinus* females carrying the ovisacs in comparison with non-egg-carrying females, while oxygen consumption by embryos in the ovisac contributed only 16.4 and 17.4% in *C. aquaedulcis* and *A. salinus,* respectively.

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