

# Salinity tolerance of *Calanus euxinus* in the Black and Marmara Seas

Leonid Svetlichny<sup>1,\*</sup>, Elena Hubareva<sup>1</sup>, Melek Isinibilir<sup>2</sup>, Ahmet Kideys<sup>3</sup>,  
Genuario Belmonte<sup>4</sup>, Elisa Giangrande<sup>4</sup>

<sup>1</sup>Institute of Biology of the Southern Seas, National Academy of Sciences of Ukraine, Sevastopol, 99011, Crimea, Ukraine

<sup>2</sup>Istanbul University, Faculty of Fisheries, Laleli, Istanbul, Turkey

<sup>3</sup>Institute of Marine Sciences, Middle East Technical University, Erdemli, Turkey

<sup>4</sup>CoNISMa, Stazione di Biologia Marina, Dipartimento di Biologia, Università degli Studi di Lecce, Italy

**ABSTRACT:** We investigated how stenohalinic Black Sea *Calanus euxinus*, when migrating to the north-eastern Marmara Sea, can adapt to the pronounced salinity gradient there. During field observations (2005 to 2008) females of *Calanus euxinus* with significantly smaller body length (compared with those inhabiting the brackish Black Sea at 17 to 18 psu) were found in the deep, high-saline (~38.5 psu) layers of the Marmara Sea. Similar diameters of eggs laid by small- and large-sized females and similar respiration rates in nauplii hatched from these eggs indicated the Black Sea origin of the *C. euxinus* population found in the Marmara Sea. To understand the morphological and physiological changes in *C. euxinus* during acclimation to high salinity, we studied the effect of salinity on behaviour, food consumption, growth patterns, hatching success and respiration rate of *C. euxinus* individuals collected in the Black and Marmara Seas. It was shown that the range of salinity tolerance for adult *C. euxinus* (17 to 30 psu) from the Black Sea was extended gradually to 40 psu after 1 d of acclimation to the high salinity of the Marmara Sea. Long-term parental salinity acclimation facilitates survival of eggs descending through the salinity gradient of the Marmara Sea, from brackish upper layers to deep, high-saline strata, or eggs laid in deep layers. While weight-specific respiration rates at the same temperature did not differ for *C. euxinus* juveniles from the Black and Marmara Seas, they were ~2 times higher in preadults and adults from the Marmara Sea than from the Black Sea. This may be the reason for the decrease in length and weight increments of these stages probably leading to the small-sized individuals of the seasonal *C. euxinus* population inhabiting the Marmara Sea.

**KEY WORDS:** *Calanus euxinus* · Salinity tolerance · Behaviour · Hatching success · Respiration · Black Sea · Marmara Sea

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## INTRODUCTION

*Calanus euxinus* is considered to be a phenotypic variation of *C. helgolandicus* (Papadopoulos et al. 2005, Unal et al. 2006) widely distributed in the seas of the North Atlantic. The individuals of *C. euxinus* from the Black Sea population are considerably larger than *C. helgolandicus* specimens from the Mediterranean Sea (Fleminger & Hulsemann 1987) and can accumulate more reserved lipids (up to 30–40% of body wet weight) in the oil sac (Svetlichny et al. 2009). *C. euxinus* is an important component of the diet of pelagic planktivorous fish in the Black Sea, including anchovy and sprat (Svetovidov 1964).

Although morphological differences between *Calanus euxinus* and *C. helgolandicus* are subtle, some authors (Tarkan & Erguven 1988, Benli et al. 2001, Tarkan et al. 2005) identified both species in the Marmara Sea zooplankton, probably attributing larger and smaller individuals to *C. euxinus* and *C. helgolandicus*, respectively. However, Isinibilir et al. (2009) suggested that small-sized males and females in the Marmara Sea are actually not *C. helgolandicus* but Black Sea *C. euxinus* after accelerated development in an environment with higher temperature and salinity.

Due to a positive water balance in the Black Sea, its water masses are transferred into the Marmara Sea through the Bosphorus, forming a brackish surface

\*Email: lsvetlichny@mail.ru

layer (15 to 20 m) with a salinity of 18 to 22 psu and a temperature ranging from 20 to 24°C in summer and from 8 to 9°C in winter. Below this layer, there is more saline (~38.5 psu) Mediterranean Sea water, with a constant temperature of ~15°C throughout the year (Besiktepe et al. 1994).

In the Black Sea, late copepodite stages of *Calanus euxinus* perform daily vertical migrations with an amplitude of approximately 100 m (Vinogradov et al. 1992a, Besiktepe 2001). At night they aggregate in the oxygenated surface layer with temperatures of 6 to 8°C and from 22 to 25°C during the periods of winter to spring homothermy and late spring to autumn stratification, respectively. In the morning *C. euxinus* descend to a deep (80 to 180 m), oxygen-deficient layer, with a constant temperature of 7 to 8°C all year round. During vertical migrations *C. euxinus* undergo slight salinity changes, because the salinity in the Black Sea increases with depth from 18 to 22 psu.

On the contrary, in the Marmara Sea, *Calanus euxinus* migrating vertically will experience pronounced salinity changes from 18 to 38.5 psu. Therefore, *C. euxinus* either adapted to living in a salinity gradient or is confined within a narrow brackish subsurface layer.

To understand the mechanism of acclimation of Black Sea *Calanus euxinus* to the specific conditions in the Marmara Sea, we conducted field observations of the population structure and vertical distribution of this species in the Marmara Sea and performed laboratory experiments to study the physiological and behavioural responses of *C. euxinus* from the Black and Marmara Seas to salinity changes.

## MATERIALS AND METHODS

**Copepod sampling.** Field observations of *Calanus euxinus* living in the Marmara Sea were made in April and October 2005, November 2006, February, June and November 2007, and April 2008. Zooplankton samples were collected during daytime with a closing Nansen net (opening diameter: 50 cm, mesh size: 200 µm) during the cruises of Hedef-1 at the permanent station in the north-eastern part of the Marmara Sea (40° 51' 53" N, 28° 57' 44" E). Sampling was carried out separately from the upper layer (0 to 25 m) formed by Black Sea waters (salinity between 18.7 and 23.2 psu), the intermediate layer (25 to 50 m), and the strata below the halocline (50 to 200 m) consisting of Mediterranean Sea waters (with a salinity of ~38 psu and a temperature of 15°C). In the Black Sea, *C. euxinus* were collected seasonally (from 2002 to 2007) near Sevastopol (40° 39' 52" N, 33° 24' 30" E) in the 0 to 80 m layer (salinity of 18 psu). The samples were preserved

with 4% borax-buffered formaldehyde. In the laboratory, each sample was completely transferred to a Bogorov chamber, numbers of individuals were determined for each developmental stage, and prosome lengths were measured under a dissecting microscope.

**Laboratory experiments.** Laboratory studies concerning the effect of salinity changes on the physiological and behavioural characteristics (egg survival, development time, respiration rate, gut fullness, frequency and duration of locomotor patterns in preadults and adults) of *Calanus euxinus* from the Black and Marmara Seas were conducted in autumn to spring from 2002 to 2008 and in every sampling period from 2005 to 2008, respectively. For comparison, the respiration rate of the closely related *C. helgolandicus* collected in the 0 to 30 m layer of the Ionian Sea near Taranto was examined in March 2009 (~13°C surface temperature).

After sampling, copepodites and adults of *Calanus euxinus* were sorted using a wide-mouth pipette and incubated during nighttime at 8°C in 1.0 l aquaria filled with 0.45 µm filtered natural seawater containing an excess of food (dinoflagellate *Prorocentrum minimum*), at salinities from 17 to 18 psu and from 18 to 22 psu for the inhabitants of the Black and Marmara Seas, respectively.

**Effect of salinity on locomotion.** To study the salinity tolerance of *C. euxinus* from the Black Sea, we analyzed changes in locomotion parameters in response to gradual changes in salinity ranging from 17 to 40 psu. Water with high salinities was obtained by adding artificial sea salt to filtered Black Sea water.

In the morning, 20 to 30 individuals were placed in 100 ml beakers and acclimated to 20°C for 1 h. After that, 10 actively moving animals were selected and put into 10 crystallizing dishes containing 20 ml of 0.45 µm filtered seawater. During the experiment, the individuals were exposed to gradual salinity changes by transfer to seawater with a salinity value differing (2 to 3 psu) from the former value.

The locomotion parameters (frequency of mouth appendage movements, Hz; time spent swimming, percent of total observation time) were examined in preadult and adult free-swimming (in 20 ml beakers) *Calanus euxinus* individuals using a photodiode in the microscope ocular combined with an impulse counter and a storage oscilloscope. The frequency of elementary acts in the locomotor pattern was calculated as  $F_L = 1/D_a$ , where  $D_a$  was the mean duration of the locomotor act calculated from 10 sequential measurements. Time spent swimming was determined as  $T = D_a n_a$ , where  $n_a$  was the number of locomotor acts. The measurements of locomotor parameters were conducted just after each salinity change and at the end of the exposure to a given salinity.

Two treatments were used in the experiments with *Calanus euxinus* from the Black Sea: (1) salinity was increased gradually from 17 to between 38 and 40 psu at a rate of 3 psu h<sup>-1</sup> for 8 h. Copepods were kept at this salinity for ~20 h; (2) salinity was lowered gradually from between 38 and 40 to 17 psu at a rate of 3 to 4 psu for 6 to 8 h. The rate of salinity change was calculated taking into account that, at a minimum gravity sinking speed of 7 m h<sup>-1</sup> (Stepanov & Svetlichny 1981), *C. euxinus* copepodites should descend from upper brackish layers to the deep, high-saline strata in 7 to 8 h, whilst they spend not less than 3 h for ascending to the surface (Svetlichny et al. 2000). During all stages of the experiments, algae (*Prorocentrum minimum*) were added to the seawater to maintain an algal concentration of ~300 µg C l<sup>-1</sup>. After long-term salinity experiments (8 h), survival of *C. euxinus* individuals was examined over 2 to 3 d.

To investigate salinity tolerance of *Calanus euxinus* from the Marmara Sea, we studied locomotory characteristics after salinity changes ranging from 22 to 52 psu. Water with high and low salinities was obtained by adding artificial sea salt to the Marmara Sea water or diluting it with de-ionized freshwater, respectively.

Two treatments were used: (1) salinity was increased gradually from 22 to 52 psu at a rate of 6 psu h<sup>-1</sup> for 5 h; (2) salinity was decreased gradually from 38.6 to 22 psu at a rate of 8 to 10 psu h<sup>-1</sup> for ~2 h. We used higher rates of salinity change in the experiments with *Calanus euxinus* from the Marmara Sea, because, during preliminary studies, such individuals survived direct transfer from 22 to 39 psu and vice versa, in contrast to Black Sea *C. euxinus*.

In total 18 and 17 experiments to study locomotory activity were conducted with *Calanus euxinus* from the Black and Marmara Seas, respectively.

To estimate the effect of salinity change on the direction of swimming, 3 to 5 females of *Calanus euxinus* kept in 0.5 l aquaria were exposed to a salinity increase from 18 to 40 psu at a rate of 2.8 psu h<sup>-1</sup> for 8 h.

**Feeding experiments.** Salinity changes (from 17 to 37 psu and from 22 to 38.5 psu for the Black and Marmara Sea *Calanus euxinus*, respectively) were performed progressing according to the feeding activity of female copepods (placed in 100 ml beakers). If the females stopped consuming the dinoflagellate *Prorocentrum minimum* after a salinity change, they were kept at this salinity until recovery of their ability to feed and, only after that, the salinity was increased again. In long-term experiments the feeding activities of Black Sea females were studied with salinity changes from 17 to 27 psu at a rate of 2 psu h<sup>-1</sup> for 5 h. Copepods were kept at 27 psu for ~20 h, and afterwards salinity was again increased gradually to up to

37 psu. Gut fullness (%) was estimated as the ratio of the amount of food consumed to the volume of the anterior part of the gut (calculated from the length and width of the gut measured using the images taken by the microscope's digital camera DSM 500). The dinoflagellates *Prorocentrum minimum* retained their mobility at salinities of 17 to 38.5 psu.

**Hatching success.** Survival of eggs produced by *Calanus euxinus* females from the Black and Marmara Seas during 7 d (in 100 ml beakers at 8 to 10°C) after a gradual long-term (over 30 h) salinity change from 17 to 37 psu and from 22 to 38.5 psu, respectively, was examined. Hatching success (%) was determined by counting the number of hatched nauplii and unhatched eggs over an incubation period of 4 d after egg laying.

**Growth experiment.** To study the effect of high salinity on growth and development of Black Sea *Calanus euxinus*, a laboratory generation of this species reared from eggs laid by females collected in the Black Sea and acclimated to 37 psu, was maintained at a salinity of 38 to 39 psu and a temperature of 17 to 19°C in a 2 l aquarium fed in excess with *Prorocentrum minimum* and *Thalassiosira weissflogii*. During the growth experiment, stage duration and prosome length of the individuals of every developmental stage were registered.

**Measurements of respiration rate.** Respiration rate ( $R$ , mg O<sub>2</sub> ind.<sup>-1</sup> h<sup>-1</sup>) of copepods was determined using closed respirometry, with experimental and control syringes of 2.0 ml as respirometers. We transferred 20 to 30 nauplii of Stages IV to VI (NIV to NVI), 4 to 10 copepodites of Stages I to IV (CI to CIV), 1 copepodite of Stage V (CV) or an adult female (CVIF) or male (CVIM) by pipette into an experimental syringe filled with filtered seawater with a protective sieve disc (mesh size: 200 µm) before the confluent outlet. In order to obtain identical initial oxygen, salinity and seston content in both the control and experimental syringes, we connected the syringes with a plastic tube and pumped the water through it several times. Then the syringes were separated, closed by the stoppers and placed into a dark chamber at either 20 ± 0.5 or 8 ± 0.5°C. Incubation periods were from 2 to 3 h for nauplii and Copepodites I to III and 1 to 2 h for Copepodites IV to VI. There were no less than 10 replicates for nauplii and for every size groups of Copepodites I to VI from the Black and Marmara Seas.

At the end of the exposure, water samples from each the experimental and control syringe were transferred to the respirometer chamber; an all-glass syringe of 0.5 ml volume joined with a polarographic membrane oxygen sensor with a magnetic stirrer inside. The oxygen concentration and respiration rate were measured according to the method described in Svetlichny & Hubareva (2005) and Svetlichny et al. (2009).

To study basal metabolism, we narcotized *Calanus euxinus* females from the Black and Marmara Seas and *C. helgolandicus* females from the Ionian Sea by  $\text{MgCl}_2$  ( $15 \text{ g l}^{-1}$ ) diluted in seawater with a final salinity of 17, 21.8 and 39 psu, respectively. The copepods were kept under these conditions until complete immobilization. After that, *C. euxinus* individuals were transferred to experimental syringes with lower concentrations of  $\text{MgCl}_2$  ( $5 \text{ g l}^{-1}$ ) and incubated at  $20^\circ\text{C}$  for 2 to 3 h, depending on the number of individuals, while measuring the respiratory rate. This concentration of  $\text{MgCl}_2$  was sufficient to keep copepods immobile during exposure. After incubation, the individuals, transferred to seawater without  $\text{MgCl}_2$ , gradually recovered their locomotor activity after 0.5 to 1 h.

**Length–weight measurements.** To express ontogenetic changes in respiration rate of nauplii and copepodites taking into account body wet weight (WW, mg) and structural carbon body weight (SCW, mg), we calculated the wet weight of nauplii as  $\text{WW} = V_b \rho$ , where  $V_b$  was the body volume ( $\text{mm}^3$ ) and  $\rho$  was the mean density amounting to  $1.06 \text{ mg cm}^{-3}$ .  $V_b$  for Nauplii III to VI was determined using the empiric equation:  $V = 0.058L_{\text{pr}}^{2.66}$  (see 'Results: Weight–length relationships ...'), where  $L_{\text{pr}}$  was prosome length (mm). The equation was based on direct measurements of the diameter of a microscopic seawater drop (measured under a light microscope; magnification 200 $\times$ ) before and after placing a nauplius into it. To obtain a regular spherical form, the drop of seawater was put into a mixture of chloroform and castor oil with a density being equal to that of seawater. Nauplii were dried a little and then transferred into the drop of seawater. The volume of the drop was calculated using the formula for the volume of a sphere.

The body volume of copepodites of all stages was calculated as  $V_b = kL_{\text{pr}}d_{\text{pr}}^2$ , where  $d_{\text{pr}}$  is the diameter of the prosome (mm) and  $k$  is the empiric coefficient of 0.64 in males and 0.58 in copepodites and females (Svetlichny 1983). The body wet weight of *Calanus euxinus* copepodites and females was also calculated using the data of body volume and taking into account the mean body density determined as  $\rho = 1.06 - 0.0016\text{SV}_s$ , where  $\text{SV}_s$  (%) is the share of the oil sac in the body volume determined as reported by Svetlichny et al. (1998, 2009). Length and width of the prosome were measured to the nearest 10  $\mu\text{m}$  under a dissecting microscope fitted with an eyepiece micrometer.

The SCW of copepodites and adults of Black Sea *Calanus euxinus* were estimated as  $\text{SCW} = \text{WW} \times \text{CF}$ , where CF is a structural condition factor for every copepodite stage. CF was determined using the equation  $\text{CF} = \text{CW} - \text{CWE}/\text{WW}$ , where CW is the body carbon weight and CWE is the carbon weight of the wax esters (Svetlichny et al. 2009). For copepodites

and adults of Marmara Sea *C. euxinus*, SCW was calculated using the regression between the prosome length ( $L_{\text{pr}}$ ,  $\mu\text{m}$ ) and SCW ( $\mu\text{g}$ ) established for *C. helgolandicus* copepodites as  $\text{SCW} = (2.133 \times 10^{-8})L_{\text{pr}}^{2.79}$  (Rey-Rassat et al. 2004). For nauplii from the Black and Marmara Seas, SCW was determined following the equation:  $C = (3.93 \times 10^{-6})L_{\text{pr}}^{1.98}$  established by Rey-Rassat et al. (2004) for total carbon content (C) in *C. helgolandicus* nauplii because the nauplii of this species do not accumulate wax esters. In fact, the line of the regression between length and the SCW of *C. euxinus* copepodites was the continuation of the line of the regression between length and C in nauplii of *C. helgolandicus* (see Fig. 2).

Statistical evaluation of data was conducted by 1-way ANOVA and Student's *t*-test. Values presented in the figures and tables are means  $\pm$  SD.

## RESULTS

### Field observations

In the Marmara Sea near the Prince Islands, the maximum total abundance of *Calanus euxinus* in the period from 2005 to 2008 was reported in April 2005 (1260 ind.  $\text{m}^{-2}$ ). In other periods of our study the number of *C. euxinus* varied from 30 to 178 ind.  $\text{m}^{-2}$ . Although individuals of this species were found throughout the whole water column, the main portion of the *C. euxinus* population ( $58.4 \pm 17.4\%$ , mean  $\pm$  SD) aggregated in the upper layer (0 to 25 m) formed by Black Sea waters (Table 1). Proportions of  $19.7 \pm 12.1$  and  $21.9 \pm 12.3\%$  of the total number of *C. euxinus* were in the intermediate (25 to 50 m) and the deep (50 to 200 m) layers formed by more saline (up to 38.5 psu) waters from the Mediterranean Sea, respectively.

In April 2005, the age structure of the *Calanus euxinus* population of the Marmara Sea was similar in the 3 different water layers, except the absence of Stage CI in the deep zone and males (CVIM) in the mixed, inter-

Table 1. *Calanus euxinus*. Vertical distribution of individuals in the Marmara Sea near the Prince Islands, expressed as a percentage of the total population in the water

Date	Sampling layer (m)		
	0–25	25–50	50–200
Apr 2005	53.5	15.9	30.6
Oct 2005	53.6	28.5	17.9
Nov 2006	61.8	1.7	36.5
Feb 2007	80	13.3	6.7
Jun 2007	58.1	19.4	22.5
Nov 2007	26.7	40	33.3
Apr 2008	75	18.8	6.2

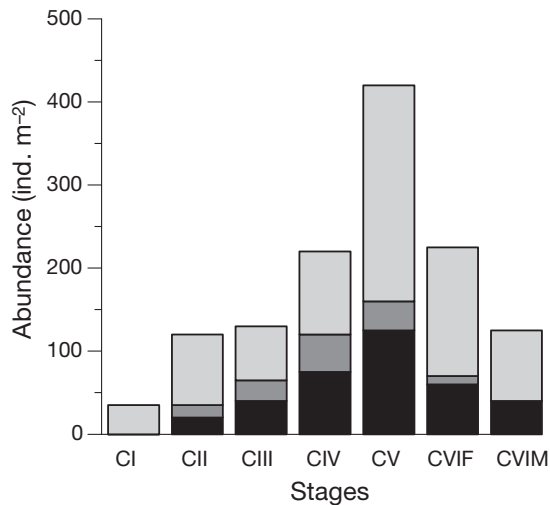


Fig. 1. *Calanus euxinus*. Abundance of different developmental stages from upper (■, 0 to 25 m), intermediate (▒, 25 to 50 m) and deep (■, 50 to 200 m) layers of the Marmara Sea during April 2005

mediate layer (Fig. 1). The CVs and females dominated the population. We distinguished 3 size groups in females constituting 38% (prosoma length of  $2.01 \pm 0.04$  mm, mean  $\pm$  SD), 48% (prosoma length of  $2.23 \pm 0.07$  mm) and 14% (prosoma length of  $2.50 \pm 0.09$  mm) of the total number.

Females from the first and second size groups occurred through all layers, whilst large individuals from the third group were only in the upper strata formed by Black Sea waters. All taxonomic characteristics for the 3 size groups of the Marmara Sea *Calanus euxinus* were identical. In April 2005, carcasses of *C. euxinus* were only found deeper than 50 m and amounted to 3.9% of the total abundance.

Prosoma length of females ( $2.63 \pm 0.14$  mm) collected in the spring of 2005 in the Black Sea near Sevastopol were significantly higher ( $t$ -test,  $p < 0.001$ ) than those from the deep layers of the Marmara Sea. During this period the mean prosoma lengths in Stages N6 ( $0.64 \pm 0.04$  mm), CI ( $0.64 \pm 0.03$  mm) and CII ( $0.96 \pm 0.05$  mm) collected in the Marmara Sea near the Prince Islands did not differ significantly ( $t$ -test,  $p > 0.05$ ) from those in Stages N6 ( $0.66 \pm 0.04$  mm), CI ( $0.69 \pm 0.05$  mm) and CII ( $0.94 \pm 0.05$  mm) sampled in the Black Sea near Sevastopol.

#### Weight-length relationships in nauplii and copepodites of *Calanus euxinus*

During naupliar development, body wet weight in stages NI to NIII from the Black Sea varied between 0.0022 and 0.0039 mg, irrespective of prosoma length

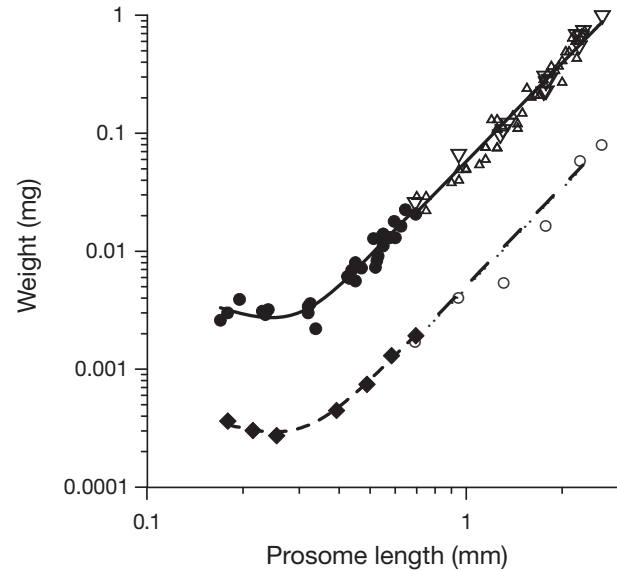


Fig. 2. *Calanus euxinus*. Wet body weight of nauplii from the Black Sea (●) and copepodites of *C. euxinus* from the Black (▽) and Marmara (△) Seas, structural carbon weight in Copepodite Stages I to VI of *C. euxinus* from the Black Sea (○) (Svetlichny et al. 2009) and carbon content in nauplii of *C. helgolandicus* (◆) (Rey et al. 2001)

which increased from 0.17 to 0.29 mm. However, changes in prosoma length from 0.425 mm for NIV to 0.695 mm for NVI resulted in an increase in body wet weight to 0.023 mg (Fig. 2), following the equation  $WW = 0.058L_{pr}^{2.66}$  ( $n = 23$ ,  $r^2 = 0.91$ ). For *Calanus euxinus* copepodites from the Black and Marmara Seas, the relationship between body wet weight and prosoma length can be described as  $WW = 0.058L_{pr}^{2.78}$  ( $r^2 = 0.97$ ,  $n = 234$ ) and  $WW = 0.053L_{pr}^{2.91}$  ( $r^2 = 0.96$ ,  $n = 184$ ), respectively.

#### Effects of salinity

##### Locomotion

In *Calanus euxinus* females and CVs sampled in deep layers of the Marmara Sea near the Prince Islands, time spent swimming did not change significantly during short-term (4 h) gradual salinity increases from 22 to 40 psu. Only in 9 experiments between 40 and 50 psu did we observe pronounced depression of locomotion, when both time spent swimming (Fig. 3A) and frequency of locomotor acts (Fig. 3B) was sharply reduced. We found no regular trends in changes in locomotor parameters of females and CVs kept for several days at 38.5 psu and then exposed to a short-term (up to 2.5 h), gradual salinity decrease to 22 psu. During gradual increases and fur-

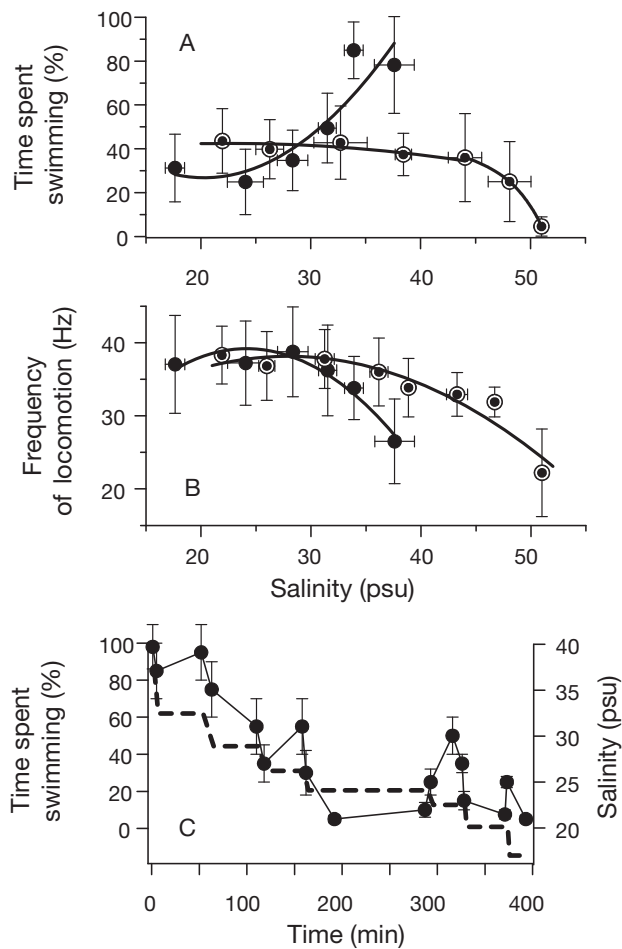


Fig. 3. *Calanus euxinus*. (A) Changes in time spent swimming and (B) frequency of locomotion in females from the Black Sea (●) during salinity increases (8 h) from 17 to 40 psu and in CVs and females from the Marmara Sea (○) during salinity increases (5 h) from 22 to 52 psu. (C) Changes in time spent swimming in females from the Black Sea during salinity decreases from 37 to 17 psu. Solid line: best fit regression curves (A,B); simple linkage curve (C). Dashed line: salinity change. Values are means  $\pm$  SD

ther decreases of salinity ranging from 22 to 38.5 psu, the copepods did not stop consuming food. *C. euxinus* even survived direct transfer from 22 to 39 psu.

In the 8 h locomotion activity experiments at 20°C, Black Sea *Calanus euxinus* did not show disturbance during gradual salinity changes from 17 to between 25 and 27 psu. However, when salinity increased to between 37 and 40 psu, time spent swimming rose to a maximum value of  $78 \pm 24\%$  (mean  $\pm$  SD) (Fig. 3A), while the frequency of locomotion decreased (Fig. 3B). Direction of swimming changed with increasing salinity as copepodites and adults of *C. euxinus*, usually aggregated near the bottom, ascended to the surface and constantly swam there after completion of the

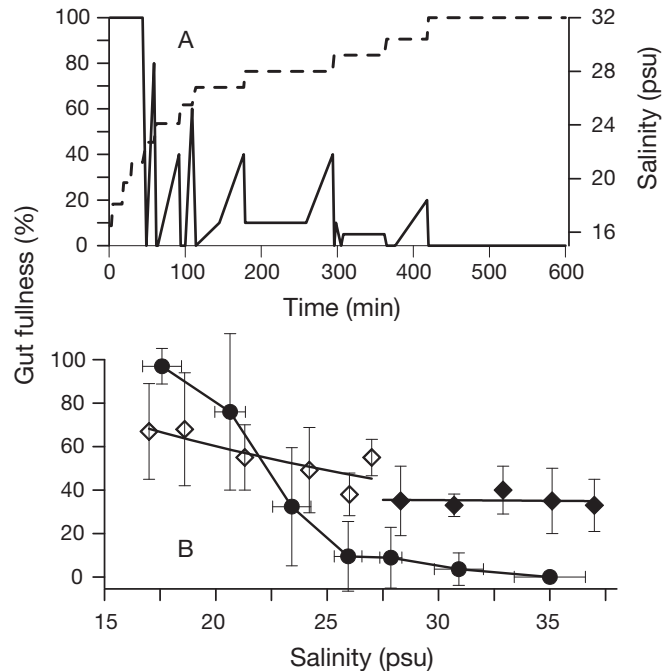


Fig. 4. *Calanus euxinus*. (A) Example of changes in gut fullness in 1 individual with gradual salinity increases from 17 to 37 psu and (B) mean values of gut fullness for 6 different individuals during short-term (●) salinity increases from 17 to 37 psu (8 to 10 h) and long-term salinity increases from 17 to 27 psu over 5 h (◇) and from 27 to 37 psu after keeping these individuals at 27 psu for 20 h (◆). The most actively feeding females were used in short-term experiments. Dashed line: salinity change. Solid lines in (B): best fit regression curves (◇, ◆) or a simple linkage curve (●). Values are means  $\pm$  SD

salinity change. Although the copepods survived a salinity increase to up to 40 psu, they stopped feeding; the muscles of some individuals became opaque, indicating irreversible tissue changes. A decrease in salinity from 37 to 17 psu caused a reduction of the time spent swimming (Fig. 3C).

#### Feeding behaviour

In the 1.5 h feeding experiments, gradual salinity increases from 17 to 25 psu did not affect food consumption in Black Sea *Calanus euxinus* females. The anterior part of the gut was constantly full of algae in all individuals. During subsequent salinity increases, every change in salinity (by 2 to 3 psu) resulted in rapid gut evacuation (Fig. 4A). The duration of the salinity acclimation period changed from 15 to 40 min at 17 to 25 psu to a period of from 80 to 120 min at 28 to 30 psu. Gut fullness decreased with increasing salinity, and, after short-term increase, *C. euxinus* females even completely stopped consuming algae at a salinity of

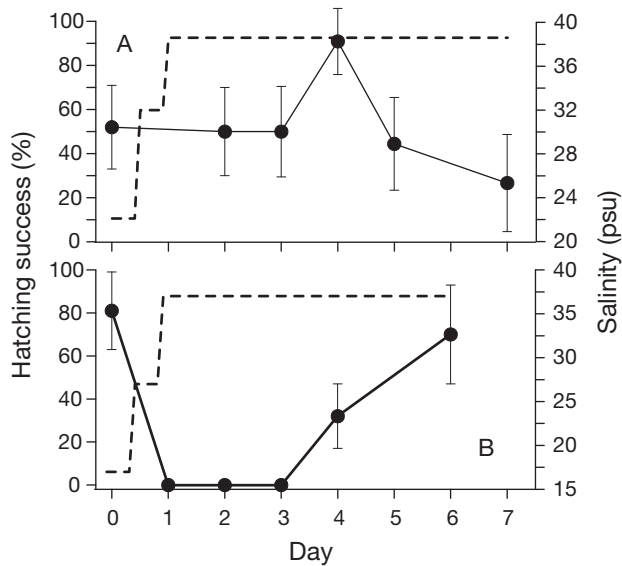


Fig. 5. *Calanus euxinus*. Hatching success of (A) eggs laid by small-sized females from the Marmara Sea after long-term salinity increases from 22 to 38.5 psu and (B) eggs laid by Black Sea females after long-term salinity increases from 17 to 37 psu. Dashed line: salinity change

>31 psu (Fig. 4B). However, after acclimation to 27 psu over ~20 h, Black Sea *C. euxinus* still had the ability to feed and fill their guts even at 37 psu.

#### Hatching success

The diameters of eggs laid by small females (prosome length of 1.9 to 2.15 mm, mean  $\pm$  SD) collected in the Marmara Sea in December 2006 and by large females (prosome length of 2.6 to 2.7 mm) collected in the same period in the Black Sea were similar ( $174.8 \pm 2.9$  and  $179.2 \pm 5.6$   $\mu\text{m}$ , respectively).

After long-term (up to 30 h) gradual salinity changes from 22 to 38.5 psu, hatching success of eggs laid by Marmara Sea *Calanus euxinus* at 38.5 psu did not vary during the 6 d after salinity increase (except on the 4th day of the experiment, with a hatching success of 91%) and amounted to between 44 and 50%. This value was similar to the hatching success of  $52 \pm 19\%$  at 22 psu (Fig. 5A).

Similar long-term experiments were conducted with Black Sea *Calanus euxinus*. After gradual salinity increases from 17 to 37 psu over 30 h, Black Sea females retained the ability to lay eggs. However, all eggs laid in the first 3 d at 37 psu were nonviable. Nauplii developed from about 40% of the eggs laid on the 4th day after a salinity increase to 37 psu; only after 6 d of parental acclimation to high salinity did the hatching success of eggs laid reach viability values

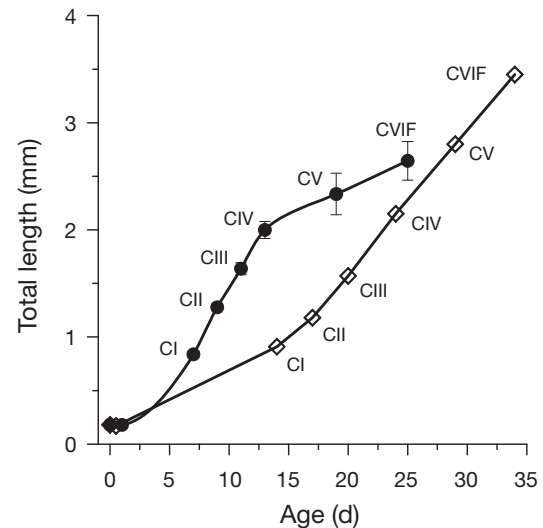


Fig. 6. *Calanus euxinus*. Increase in total body length relative to age at  $18 \pm 1^\circ\text{C}$  in our growth experiment at 38 to 39 psu (●) and in the experiments of Sazhina (1987) at 18 psu (◇)

comparable to eggs laid before the salinity increase (Fig. 5B).

In the growth experiment, development times from egg laying to CI and from CI to CIV amounted to 7 and 6 d, respectively (Fig. 6), whilst the development from CIV to adult female took 12 d. Total body length and prosome length of females were  $2.64 \pm 0.18$  mm and  $2.05 \pm 0.14$  mm, respectively.

#### Respiration rates

At  $20^\circ\text{C}$  the weight-specific respiration rates ( $Q/\text{WW}$ ,  $\mu\text{g O}_2 \text{ mg}^{-1} \text{ h}^{-1}$ ) in females and males of *Calanus euxinus* from the Black Sea varied independent of the season within the range from 0.91 to  $1.34 \mu\text{g O}_2 \text{ mg}^{-1} \text{ h}^{-1}$  (Table 2). The weight-specific respiration rates of *C. euxinus* females reared in the laboratory from eggs at 39 psu ( $1.80 \pm 0.27 \mu\text{g O}_2 \text{ mg}^{-1} \text{ h}^{-1}$ ), small *C. euxinus* collected in the deep layers of the Marmara Sea (1.71 to  $1.92 \mu\text{g O}_2 \text{ mg}^{-1} \text{ h}^{-1}$  for females and 1.6 to  $2.38 \mu\text{g O}_2 \text{ mg}^{-1} \text{ h}^{-1}$  for males) and *C. helgolandicus* females from the Ionian Sea ( $1.61 \pm 0.27 \mu\text{g O}_2 \text{ mg}^{-1} \text{ h}^{-1}$ ) were significantly ( $t$ -test,  $p < 0.001$ ) higher (1.5- to 2-fold) than the weight-specific respiration rates of *C. euxinus* sampled in the Black Sea.

The ratio between total metabolism and basal metabolism amounted to 2.6, 2.4 and 1.9 for females of *Calanus euxinus* from the Black and Marmara Seas and *C. helgolandicus* from the Ionian Sea, respectively. In narcotized females from the Marmara and Ionian Seas, the weight-specific respiration rates were 2-fold higher (1-way ANOVA,  $F_{2,9} = 5$ ,  $p < 0.05$  and  $F_{7,4} =$

Table 2. *Calanus euxinus*, *C. helgolandicus*. The rate of wet-weight-specific respiration rate (Q/WW) at  $20 \pm 0.5^\circ\text{C}$  in *C. euxinus* females and males collected from the Black Sea (17 to 18.7 psu), the Black Sea *C. euxinus* females reared in the laboratory at 39 psu, the Marmara Sea *C. euxinus* females and males living at changing salinity (18.7 to 38.9 psu) near the Prince Islands, and the Ionian Sea *C. helgolandicus* females living at constant high salinity (39 psu). n: number of experiments. WW: wet weight. WW and Q/WW are given as mean  $\pm$  SD

Date	Habitat salinity (psu)	Experimental salinity (psu)	n	Wet weight (mg)	Q/WW ( $\mu\text{g O}_2 \text{ mg}^{-1} \text{ h}^{-1}$ )
<b>Black Sea, female</b>					
Mar 2007	} 17–20	18.7	38	$1.08 \pm 0.10$	$0.95 \pm 0.20$
Apr 2003		18.2	22	$1.10 \pm 0.04$	$1.34 \pm 0.36$
May 2005		17.0	10	$1.04 \pm 0.21$	$1.01 \pm 0.16$
Jul 2008		17.5	29	$1.10 \pm 0.04$	$1.25 \pm 0.36$
Sep 2003		17.5	46	$1.04 \pm 0.13$	$1.02 \pm 0.27$
Oct 2002		17.4	13	$0.91 \pm 0.14$	$0.91 \pm 0.10$
Nov 2006		17.7	10	$1.08 \pm 0.25$	$1.17 \pm 0.36$
Nov 2006 <sup>a</sup>		17.0	10	$1.07 \pm 0.06$	$0.45 \pm 0.03$
Dec 2004	39.0	39.0	20	} $0.43 \pm 0.07$	$1.80 \pm 0.27$
		17.0	9		$1.82 \pm 0.38$
<b>Black Sea, male</b>					
Apr 2003	} 17–20	18.2	15	$0.76 \pm 0.10$	$1.15 \pm 0.20$
Oct 2002		17.4	14	$0.76 \pm 0.09$	$1.19 \pm 0.27$
<b>Marmara Sea, female</b>					
Mar 2007	18.7–38.9	18.7	6	$0.39 \pm 0.09$	$1.76 \pm 0.21$
		38.9	14	$0.46 \pm 0.09$	$1.67 \pm 0.34$
Apr/May 2005	22.1–38.6	22.1	36	$0.55 \pm 0.11$	$1.71 \pm 0.29$
Oct 2005	23.2–38.7	23.2	6	$0.33 \pm 0.03$	$1.78 \pm 0.51$
Dec 2006	21.8–38.6	21.8	22	$0.59 \pm 0.13$	$1.92 \pm 0.57$
Dec 2006 <sup>a</sup>	21.8–38.6	21.8	10	$0.66 \pm 0.16$	$0.80 \pm 0.15$
<b>Marmara Sea, male</b>					
Apr/May 2005	22.1–38.6	22.1	18	$0.57 \pm 0.10$	$1.60 \pm 0.45$
Dec 2006	21.8–38.6	21.8	6	$0.47 \pm 0.02$	$2.38 \pm 0.76$
<b>Ionian Sea, female</b>					
Mar 2009	38.0–39.0	39.0	21	$0.64 \pm 0.08$	$1.61 \pm 0.27$
Mar 2009 <sup>a</sup>	38.0–39.0	39.0	7	$0.62 \pm 0.07$	$0.87 \pm 0.25$

<sup>a</sup>Respiration rate of narcotized individuals (basal metabolism)

Table 3. Temperature coefficient  $Q_{10}$  for nauplii (N), copepodite stages (CI to CV) of *Calanus euxinus* (mean  $\pm$  SD) from the Marmara and Black Seas and for other copepods. Blank cells: no data

Area	Experimental salinity (psu)	Stages					Sources
		N	CI–II	CIII–IV	CV	Female	
<b><i>Calanus euxinus</i></b>							
Marmara Sea	22	$1.97 \pm 0.33$	$1.87 \pm 0.26$	$1.69 \pm 0.19$	$2.35 \pm 0.48$	$1.96 \pm 0.28$	} Present study
Black Sea	18	$1.89 \pm 0.45$				$1.85 \pm 0.22$	
Black Sea	18					2.05	Svetlichny et al. (2000)
<b><i>Calanus pacificus</i></b>							
Pacific Ocean	30					1.8	Vidal (1980)
<b>Marine copepods</b>							
Atlantic, Indian, Pacific Oceans	34				1.8–2.1		Ikeda et al. (2001)

10.67,  $p < 0.01$ , respectively) than that in narcotized individuals from the Black Sea.

The weight-specific respiration rates in Black Sea females reared from eggs at 39 psu were  $1.82 \pm 0.38 \mu\text{g O}_2 \text{ mg}^{-1} \text{ h}^{-1}$  (mean  $\pm$  SD) 4 d after a gradual salinity decrease to 17 psu; thus, rates were similar to those at high salinity. The Marmara Sea females collected from

deep layers (38.5 psu) and kept under laboratory conditions ( $20^\circ\text{C}$ ) at a salinity of 38.5 or 22 psu did not differ significantly in weight-specific respiration rates, which varied in the range of from 1.56 to  $1.97 \mu\text{g O}_2 \text{ mg}^{-1} \text{ h}^{-1}$  over 13 d. At  $7^\circ\text{C}$ , the weight-specific respiration rate of Marmara Sea *Calanus euxinus* females was 2.6-fold lower than at  $20^\circ\text{C}$  (Fig. 7).



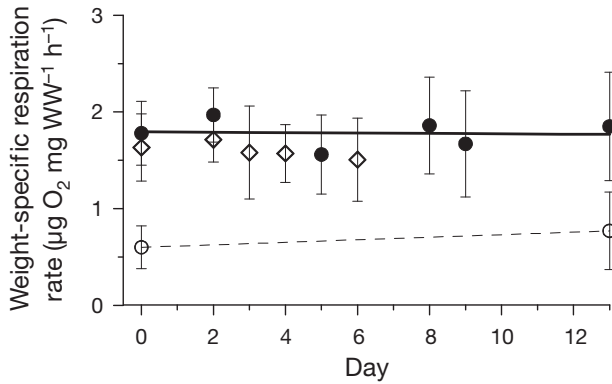


Fig. 7. *Calanus euxinus*. Wet-weight-specific respiration rate in small-sized females from deep layers of the Marmara Sea at experimental salinities of 22 psu (●) and 38.6 psu (◇) and a temperature of 20°C and at 22 psu (○) and 7°C. Solid line: linear regression curve. Dashed line: simple regression curve. Values are means  $\pm$  SD (bars). WW: wet weight

The temperature coefficient ( $Q_{10}$ ) obtained in the range from  $7.5 \pm 0.5$  to  $20 \pm 0.5^\circ\text{C}$  equalled from 1.69 to 2.35 for different developmental stages of *Calanus euxinus* from the Marmara Sea.  $Q_{10}$  of nauplii ( $1.89 \pm 0.45$ ) and females ( $1.85 \pm 0.22$ ) from the Black Sea were similar to the corresponding values for Marmara Sea individuals (Table 3).

The respiration rate of *Calanus euxinus* from the Marmara Sea increased from  $0.024 \pm 0.005 \mu\text{g O}_2 \text{ ind.}^{-1} \text{ h}^{-1}$  in nauplii (developed from eggs laid by small females) to  $1.29 \pm 0.49 \mu\text{g O}_2 \text{ ind.}^{-1} \text{ h}^{-1}$  in females (with a wet weight of  $0.679 \pm 0.028 \text{ mg}$ ), following the equation:  $R = 1.67\text{WW}^{0.90}$  (Fig. 8A). In *C. euxinus* collected in the Black Sea the respiration rate rose from  $0.033 \pm$

$0.008 \mu\text{g O}_2 \text{ ind.}^{-1} \text{ h}^{-1}$  in nauplii to  $0.088 \pm 0.036 \mu\text{g O}_2 \text{ ind.}^{-1} \text{ h}^{-1}$  in CIII and again from CIII to adult females (with a wet weight of  $1.370 \pm 0.096 \text{ mg}$  and a respiration rate of  $1.378 \pm 0.376 \mu\text{g O}_2 \text{ ind.}^{-1} \text{ h}^{-1}$ ); the slopes of the regressions are 0.4 and 1.14, respectively.

## DISCUSSION

### Field observations

Our results showed that during the period from 2005 to 2008 the total abundance of *Calanus euxinus* in the Marmara Sea near the Prince Islands varied from 30 to  $1260 \text{ ind. m}^{-2}$  ( $0.15$  to  $6.3 \text{ ind. m}^{-3}$ ), whilst in the south-western part of the Black Sea from 2000 to 2007 the total number of this species changed from 1247 to  $13790 \text{ ind. m}^{-2}$ , or 8.3 to  $76.6 \text{ ind. m}^{-3}$  (Isinibilir et al. 2009) and was, on average, 47 times higher than that in the north-eastern Marmara Sea. According to Isinibilir et al. (2008, 2009), in the Marmara Sea, maximum abundance of *C. euxinus* was observed in spring, whilst this species nearly disappeared in summer, probably due to high temperatures in the surface and deep layers. On the contrary, in the off-shore regions of the Black Sea, where a cold ( $6$  to  $8^\circ\text{C}$ ) intermediate layer is constantly present, cold-water *C. euxinus* can develop all year round (Sazhina 1987, Vinogradov et al. 1992b).

In April 2005, when the abundance of *Calanus euxinus* reached its maximum value, copepodite stages from CII to CVI occurred in brackish surface, intermediate and more saline deep layers of the Marmara Sea. The low proportion of carcasses (3.9%) found only in

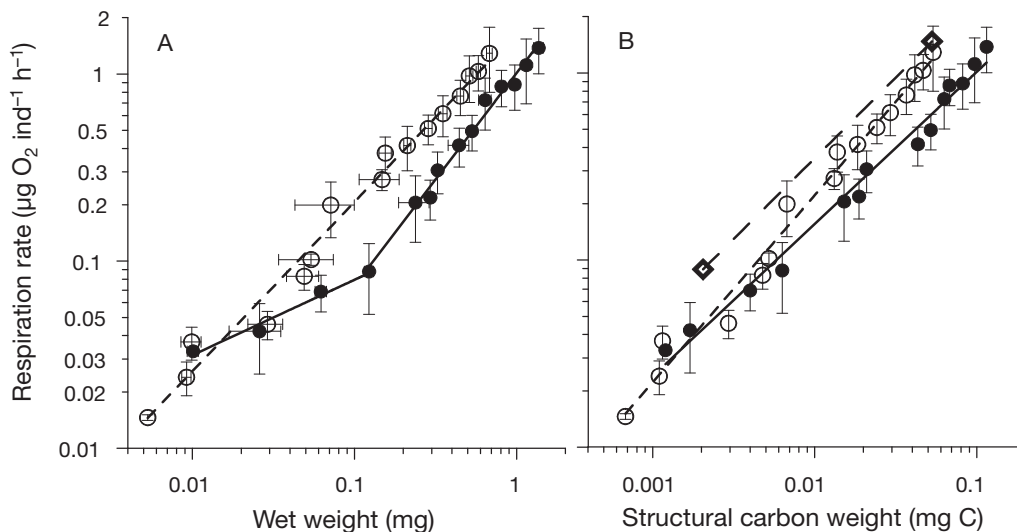


Fig. 8. *Calanus euxinus*. Respiration rate relative to (A) wet weight and (B) structural carbon weight in nauplii and copepodites from the Black (●) and Marmara (○) Seas and copepodites of *C. pacificus* (◇) from Puget Sound (Vidal 1980) at  $20 \pm 0.5^\circ\text{C}$ . Values are means  $\pm$  SD (bars). Lines: power regression curves; solid for Black Sea individuals, dashed for Marmara Sea individuals, infrequent dashed for *C. pacificus*

the 50 to 200 m layer indicates that *C. euxinus* in the Marmara Sea possess a broad salinity tolerance and can move through the salinity gradient.

### Salinity tolerance

The results of our experiments showed that a short-term (4 h) salinity change from 22 to 38.5 psu did not affect locomotor and feeding activity or hatching success in *Calanus euxinus* from the Marmara Sea. On the contrary, in *C. euxinus* from the Black Sea, during a gradual salinity increase from 17 to 37 psu over 8 h, the time spent swimming rose, while the frequency of locomotion decreased at the highest salinity values because the limbs began to move slowly (Fig. 3). Probably, the copepods tried to carry out escape behaviour but were constrained by slow-moving limbs which indicate a depressed muscular function. Similar increases in the time spent swimming and decreases in the frequency of locomotion under low oxygen concentration were reported for Black Sea *Calanus euxinus* in a previous study (Svetlichny et al. 2000). In the estuarine copepod *Eurytemora affinis* exposed to salinity changes from 0 to 35 psu, the increase and decrease of salinity, respectively, increased and decreased the overall swimming activity (Seuront 2006).

In the Black Sea, the swimming activity of *Calanus euxinus* changes in accordance with circadian rhythms. During daytime, individuals of this species descend to deep layers (Vinogradov et al. 1992a). In the Marmara Sea, *C. euxinus* exhibiting the same behavioural pattern will experience an acute increase in salinity. One may suggest that the ability to respond to high salinity by increasing locomotion activity allows *C. euxinus* to stay in brackish layers at the surface, prolonging the period of salinity acclimation. According to our results, during a gradual salinity increase from 17 to 37 psu over 30 h *C. euxinus* females retained the ability to feed and began to lay viable eggs after 5 d at high salinity. The effect of parental acclimation to salinity on egg hatching success in copepods has been poorly investigated. However, in decapods, egg-carrying females subjected to variable salinity conditions should produce larvae whose salinity tolerance is enhanced (Charmantier et al. 2002).

### Growth at different salinity levels

Variations in salinity can also affect the growth and development time of crustaceans (Anger 2003). For example, salinity increases from 10 to 27 psu (Lee & Petersen 2002) and from 15 to 25 psu (Devreker et al.

2007) resulted in a reduction of development time in the copepod *Eurytemora affinis*. According to our results, the development time in *Calanus euxinus* from egg laying to CIV at 18°C and 39 psu was nearly 2-fold shorter (Fig. 6) than that in the experiments of Sazhina (1987) conducted at the same temperature and a salinity of 18 psu (13 and 24 d, respectively). However, the development from CIV to adult females in our study (12 d) took more time compared with the data of Sazhina (1987; 10 d), whilst length increment during this period tended to be lower in preadults kept at 39 psu than in those kept at 18 psu. Therefore, the pro-some length of Black Sea females ( $2.05 \pm 0.14$  mm, mean  $\pm$  SD), reared in the laboratory at 39 psu, was significantly ( $p < 0.001$ ) lower (1.3-fold) than that in the females ( $2.62 \pm 0.09$  mm) collected from the Black Sea and similar to that of females ( $2.01 \pm 0.04$  mm) inhabiting the deep layers of the Marmara Sea where the salinity was 38.5 psu and the temperature was 15°C.

### Respiration rate

Despite the fact that many copepod species are osmoconformers, their respiration rates can be influenced by salinity. For example, the respiration rate of *Acartia clausi* and *A. tonsa* increased during salinity changes from 24 to 33 psu and from 2 to 20 psu, respectively (Calliari et al. 2006). On the contrary, in *Tigriopus brevicornis*, an increase in salinity caused a decrease in the oxygen consumption rate and activity as well (McAllen & Taylor 2001). Reduction of the respiration rate in *Calanus finmarchicus* was reported for salinity changes from 34 to 21.5 psu (Anraku 1964) and from 34 to 17 psu (Marshall & Orr 1972).

In our experiments at 20°C, the weight-specific respiration rate in adult *Calanus euxinus* collected in the Black Sea (salinity of 17 to 18.7 psu) was 1.5- to 2-fold lower than in *C. euxinus* living in the Marmara Sea (with a salinity gradient of 18.7 to 38.5 psu), in *C. helgolandicus* collected in the Ionian Sea (salinity of 39 psu), as well as in Black Sea *C. euxinus* females reared in the laboratory from eggs at 39 psu (Table 2). Weight-specific respiration rates in females captured in the deep layers of the Black and Marmara Seas and maintained at high salinity were higher than those of Black Sea females living at low salinity, independent of the experimental salinity. This is probably due to a significantly higher level of basal metabolism in the females inhabiting high-saline layers, because we did not find any differences in the locomotor parameters of active individuals from the Black and Marmara Seas. The ratios between total metabolism and basal metabolism amounted to 2.6 and 2.4 in the females from the Black and Marmara Seas, respectively, which corre-

spend to time spent swimming of about 50% (Svetlichny & Hubareva 2005) or mean daily time spent swimming at 20°C (Svetlichny & Yarkina 1989). One may suggest that the difference between metabolic rates in *C. euxinus* females from the Black and Marmara Seas are due to the developmental acclimation to various salinities in their environment.

On the contrary, the weight-specific respiration rates in nauplii and CIs from these areas were similar (Fig. 8). We found a significant difference between the metabolic rates of the Black and Marmara Seas *Calanus euxinus* only in the copepodite stages from CIII through CVI. Progressive divergence in length, weight and lipid content for CIII was reported by Isinibilir et al. (2009). This is probably due to the ability of late copepodite stages of *C. euxinus* in the Black Sea to migrate daily to cold hypoxic layers, saving energy that can be used for growth (Svetlichny et al. 2009).

In Black Sea *Calanus euxinus* the carbon weight of metabolically inert wax esters accumulated in the oil sac and reached 50% of the carbon body weight (Svetlichny et al. 2009), whilst in the Marmara Sea, late copepodite stages were not able to form large amounts of wax esters (Svetlichny et al. 2006). Therefore, use of structural carbon weight instead of wet or dry body weight appears to be more appropriate when comparing the metabolic rates in *Calanus* spp. with different contents of either metabolically active or inert body compounds. After converting wet weight to structural carbon weight, ontogenetic changes in the respiration rate of Black Sea *C. euxinus* from nauplii to adults were expressed using the allometric equation:  $R = 6.6SCW^{0.81}$  ( $r^2 = 0.99$ ), whilst the respiration rate of Marmara Sea *C. euxinus* was determined with  $R = 22.1SCW^{0.999}$  ( $r^2 = 0.98$ ). Consequently, metabolic rates in the Marmara Sea *C. euxinus* females were 2.5-fold higher than those in Black Sea copepodites with similar SCW, or similar to Black Sea females with 2.2-fold higher SCW. As long as the temperature coefficients ( $Q_{10}$ ) in *C. euxinus* from the Black and Marmara Seas are close (Table 3), we consider the salinity to be the factor behind the difference in metabolic rates. In fact, in comparison with Black Sea *C. euxinus*, higher rates of respiration were found in *C. helgolandicus* females from the Ionian Sea at 39 psu (see Table 2) and in copepodites and females of the closely related species *C. pacificus* from Puget Sound at ~30 psu (Vidal 1980), with similar SCW (Fig. 8B) calculated taking into account the SCW/DW ratio by Rey-Rassat et al. (2004). In nauplii of *Eucalanus pileatus* collected from the shelf off the south-eastern United States (Köster et al. 2008), respiration rate ( $\sim 0.043 \mu\text{g O}_2 \text{ ind.}^{-1} \text{ h}^{-1}$ ) was also 1.3-fold higher than that in nauplii of *C. euxinus* ( $0.033 \pm 0.0058 \mu\text{g O}_2 \text{ ind.}^{-1} \text{ h}^{-1}$ ) with a similar body length of 0.6 mm.

## CONCLUSIONS

In summary, our results suggest that stenohalitic Black Sea *Calanus euxinus*, which penetrate the Marmara Sea, with its pronounced salinity gradient, through the Bosphorus, can form a seasonal population there. The ability to increase locomotor activity in response to salinity changes allows *C. euxinus* to periodically adapt to high salinity, ascending to surface brackish layers after sinking to a deep zone of more saline waters in the Marmara Sea. Long-term parental salinity acclimation facilitates the survival of eggs descending through the salinity gradient of the Marmara Sea, from the brackish upper layers to the high-saline strata, or eggs laid in deep waters. However, high temperature and salinity of the layers below 15–20 m accelerate the development and depress the growth rate of preadult and adult *C. euxinus* in the Marmara Sea. Length and weight increments in these stages decrease, probably due to an increase in the respiration rate during development at high salinity. Consequently, mainly small-sized females and males with or without a small oil sac (Svetlichny et al. 2006, Isinibilir et al. 2009) inhabit the Marmara Sea. Nevertheless, the body size and respiration rate in nauplii and early copepodite stages developed from eggs laid by small females from the Marmara Sea are the same as those in individuals from the Black Sea. This phenomenon confirms the Black Sea origin of the *C. euxinus* population in the north-eastern part of the Marmara Sea.

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