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Physiological and behavioral aspects of *Calanus euxinus* females (Copepoda: Calanoida) during vertical migration across temperature and oxygen gradients

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Abstract In the Black Sea, during summer stratification, *Calanus euxinus* (Hulsemann) undertakes diel vertical migrations with an amplitude of about 117 m from oxygenated, warm (18 °C) surface layers to hypoxic (~0.8 mg O_2 l^{-1}) zones with lower temperature (7.9 °C). When such changes in temperature and oxygen concentration are reproduced in the laboratory, total metabolism, basal metabolism and scope of activity of copepods decrease 7.2, 7.8 and 6.7 times, respectively, while the frequency of locomotory acts and mechanical power decline 3.4- and 9.5-fold, respectively. These changes allowed the copepods to conserve a significant portion of food consumed near the surface for transformation to lipid reserves. Diel respiratory oxygen consumption of migrating individuals, calculated so as to include actual duration of residence in layers with different temperature and oxygen concentrations, is estimated at 17.87 μg O_2 ind^{-1}. The net energy cost of vertical migration made up only 11.6% of the total. Copepods expend 78.6% of diel energy losses during approximately 10 h in the surface layers, while about 5.4% is required during about 9 h at depth. Hypoxia is shown to have a significant metabolic advantage during diel vertical migrations of *C. euxinus* in the Black Sea.

Key words Black Sea · Copepod · *Calanus euxinus* · Vertical migration · Energy expenditure · Temperature · Hypoxia

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Introduction

The Black Sea pelagic, upper aerobic zone is separated from lower anaerobic layers (containing hydrogen sulfide) by the main pycnocline, which lies at depth of 70 to 200 m due to the hydrology of the region (Vinogradov et al. 1992a). The pycnocline is accompanied by the oxycline, where oxygen concentration decreases from 7.9–9.8 mg l^{-1} at σ_t = 14.0–14.7 to 0.47–0.84 mg l^{-1} at σ_t = 15.3 to 15.9. The width of the oxycline varies from 50–93 m (Pojarkov 1989). Below this, oxygen concentration declines slowly to <0.16 mg O_2 l^{-1} at σ_t = 15.9 to 16.0, and can no longer be detected at σ_t = 16.15 to 16.20, where sulphide concentrations are 0.03 to 0.1 mg H_2S l^{-1}. This oxygen-deficient water, formed within the oxic/anoxic transition layer with oxygen concentration <0.64 mg O_2 l^{-1} and H_2S <0.03 mg H_2S l^{-1}, is called the suboxic zone (Yilmaz et al. 1998).

While in the surface layers (~0 to 30 m) temperature varies from 7 to 25 °C due to seasonal fluctuations, the temperature of the deeper waters below the cold intermediate water, which is characterized by core temperatures of ~6 °C, remains constant throughout the year (8 °C) (Balkas et al. 1990; Ozsoy and Unluata 1997; Yilmaz et al. 1998).

Calanus euxinus is the dominant species in the mesozooplankton of the Black Sea. During periods of seasonal temperature stratification *C. euxinus* specimens ascend at night to warm, aerated surface layers with high phytoplankton concentration, and in the morning migrate to the cold, hypoxic zone (0.35 to 1.15 mg O_2 l^{-1}, σ_t = 15.4 to 15.7), where they form aggregations just below the oxycline (Vinogradov et al. 1985; Flint 1989).

Metabolism in copepods is known to be directly affected by temperature (Marshall and Orr 1972; Vidal 1980; Ivleva 1981) and oxygen concentration (Omori and Ikeda 1984; Vinogradov et al. 1992a; Arashkevich et al. 1998). Corkett and McLaren (1978) say that *Pseudocalanus* migrating across a gradient of water layers might conserve energy of food consumed near the surface. Nevertheless, this strategy would be useful only in the case of low energy expenditure for migration. The results on the energetic costs of vertical migrations of copepods are still controversial (Klyashtorin 1984; Rudyakov 1986; Pavlova 1987; Svetlichny 1996). Moreover, data are hardly comparable, because different approaches have been used to estimate the speed and energy required for locomotion.

The aim of the present study was to analyze the cumulative effect of temperature and oxygen concentration on total and basal metabolism, and the locomotory activity of *Calanus euxinus* females performing diel migrations during summer stratification in the Black Sea. An attempt to estimate daily energy losses of copepods was made, taking into account maximal speed of locomotion, the mechanical energy expenditure and the time spent in layers with different physical and chemical characteristics.

Materials and methods

Laboratory experiments were carried out on *Calanus euxinus* females captured in summer/autumn 1996. The copepods were collected with a closing Nansen net (71 cm mouth opening, 112 μm mesh size) in offshore regions of the Black Sea, from the surface layers at night or from a depth of approximately 100 m in the daytime. Prior to experiments, copepods were acclimatized for about 0.5 h in 1 μm filtered sea water with the appropriate temperature and oxygen concentration. Controlled temperatures were maintained by refrigeration, and the oxygen concentration was decreased by bubbling nitrogen through the water. The effect of these parameters on metabolism and behavior of *C. euxinus* was investigated in separate experiments: (1) the influence of temperature in the range from 24 to 7.5 °C in oxygen-saturated water; and (2) the influence of oxygen concentration in the range from 10.0 to 0.4–0.5 mg O_2 l^{-1} at a constant temperature of about 8 °C.

Weight-specific respiration rate was determined using experimental and control chambers (identical all-glass syringes of 2.5 ml). One or two active females or five to seven narcotized females were placed in an experimental syringe filled with filtered water with known oxygen content. Each pair of experimental and control syringes was connected with a fine plastic tube, and then the water was pumped several times through each pair of syringes to achieve equal initial oxygen concentration in experimental and control chambers. Afterwards, the syringes were separated, closed by the stoppers and incubated in a refrigerator at stable temperatures of 8.0 ± 0.5, 11 ± 0.5, 16 ± 0.5 and 21 ± 0.5 °C. The experiments lasted up to 2 h.

The oxygen concentration was determined by a polarographic membrane oxygen sensor, joined with a measuring chamber (all-glass syringe) of 0.5 ml volume, with a magnetic stirrer inside (Svetlichny and Umanskaya 1991). The water sample from the experimental syringe was transferred to the measuring chamber in six portions through a needle, without contact to the surrounding air (each portion was approximately equal to 0.4 ml). Two of the portions were used to rinse out the chamber, and the remaining four portions were used for analysis of oxygen concentration. The accuracy of the polarographic method used was equal to 0.05%. Such accuracy allowed us to determine the amount of oxygen consumed by a *Calanus euxinus* female in a volume of ~1.0 ml during 2 h at 8 °C under hypoxic conditions.

Weight-specific respiration (mg O_2 wet wt h^{-1}) rate was calculated from the equation:

$$y = (x_c - x_e)V/n_s tW \ ,$$

where x_c is the oxygen concentration in the control chamber at the end of the experiment (mg l^{-1}); x_e is the oxygen concentration in the experimental chamber at the end of the experiment (mg l^{-1}); V is the volume of the chamber (ml); n_s was the number of the specimens; t is the duration of the experiment (h); W is the mean wet weight of the copepod (mg), which was determined as $W = 0.58\,L\,d^2$, where L is the length of the metasome and d is the maximum body width in millimeters (Stepanov and Svetlichny 1981).

The mean oxygen concentration in the experiment was determined as:

$$x_m = (x_i + x_e)/2 \ ,$$

where x_i is the initial oxygen concentration, being identical in the experimental and control chambers. During the incubation period the total oxygen consumed was never more than 30% of the initial value.

To measure the oxygen consumption during basal metabolism, the copepods were narcotized by the depolarizing muscle relaxant dithylinium, which was added to the water containing the animals. Dithylinium (10 mg ml^{-1} seawater) caused complete immobility of the copepods, except heart contractions. We could not successfully narcotize copepods using the non-depolarizing muscle relaxants tubocurarini chloridium or uretanum. The latter has been frequently applied for immobilization of invertebrates and fishes (Pavlova 1987; Philippova and Postnov 1988; Belokopytin 1993). These narcotics caused the paralysis of *Calanus euxinus* thoracic muscles, which are used for escape locomotion, but did not stop motions of mouthpart appendages. Next, the copepods were rinsed out and incubated. After the experiment they were transferred to the aquarium to observe their behavior. We analyzed the results of only those experiments in which the copepods did not awaken during incubation but gradually recovered their activity after incubation.

The effects of temperature and oxygen concentration on the motion behavior were investigated in separate experiments on individuals attached to a cantilever, semiconductor, force sensor (Svetlichny 1987) in a hermetic chamber (syringe of 2.5 ml). The animals were glued by the dorsal part of the cephalon to a sensor using a drop of cyanoacrylate adhesive. Under these conditions copepods can feed, lay eggs (Sykes and Huntley 1987) and maintain their ordinary motion activity for many days (Svetlichny and Yarkina 1989). The temperature and oxygen concentration in the chamber were changed gradually (~2 °C min^{-1} and ~0.5 ml O_2 min^{-1}). The individuals were kept at each temperature and oxygen concentration for 10 min while determining drag force R (dyne), frequency F (Hz), time spent swimming T (% of total time of observation) in relation to all mouth appendages and drag force, duration P (s) and number (n) of jerks. The mean frequency of acts in a motion pattern was calculated as:

$$F = n_a/t_a \ ,$$

where n_a is the number of locomotor acts, t_a is the total duration of locomotory patterns. Behavioral actogrammes were registered as digital electric signals on a computer disk and analyzed by custom-made software.

Weight-specific mechanical energy of motion by mouthpart appendages (E_m, μg O_2 mg^{-1} wet wt h^{-1}) for individuals of about 0.35 cm was calculated from the empirical equation (Svetlichny and Umanskaya 1991) using the calorific equivalent of consumed oxygen equal to 3.6 cal mg^{-1} O_2 (Omori and Ikeda 1984):

$$E_m = 0.000146\,R\,F\,T \ ,$$

where R is the mean value of resultant drag force (dyne) and F is the mean frequency (Hz). Weight-specific mechanical energy of locomotion by the thoracic legs and abdomen (E_j, μg O_2 mg^{-1} wet wt h^{-1}) was found from the equation:

$$E_j = 5.5 \times 10^{-6} R\,P^{-0.12}\,n \ ,$$

based on the empirical equation $E_j = 1.1\,R\,F^{-0.12}$ (erg s^{-1}) for the power of jerk locomotion (Svetlichny and Umanskaya 1991).

The speed of routine swimming of copepods (V_m, cm s^{-1}) was estimated as $V_m = Fh$, where h is the mean distance covered during a single locomotor act by mouth appendages, which is equal to 0.081 cm in females with body lengths of 0.35 cm (Svetlichny 1994).

To calculate the speed of passive sinking by gravity V_p (cm s^{-1}) of copepods with the same body length, we used the empirical equation:

$V_p = {}^{1.25}\sqrt{0.47(\rho_b - \rho_w)/\rho_w v^{0.75}}$(Svetlichny 1983) ,

where ρ_b and ρ_w are the mean densities (g cm^{-3}) of *Calanus* body and sea water, respectively, v is the kinematic coefficient of viscosity (cm^2 s^{-1}). The lipid content of *C. euxinus* females usually does not exceed 10% of body wet weight (Yuneva et al. 1997), and ρ_b is equal to 1.06 g cm^{-3} (Stepanov and Svetlichny 1981).

Field observations of *Calanus euxinus* diel vertical migrations were conducted in the southern Black Sea during September 1996 and July 1997 cruises of the R.V. "Bilim", from the Middle East Technical University Institute of Marine Sciences. The zooplankton scattering layer was monitored using a shipborne acoustic doppler current profiler with a 150 kHz transducer (Erkan 1998; Erkan and Gucu 1998). Acoustic data were represented in the form of vertical profiles of mean volume backscattering strength (MVBS) values. The MVBS values (the total acoustic cross-section area of the targets within a unit volume, expressed in decibels, relative to an acoustic cross-section of 1 m^2) were calculated according to the equation given by R. D. Instruments (Erkan 1998).

The zooplankton were sampled along with the acoustic records. The samples were taken by Nansen closing net (71 cm mouth opening, 112 μm mesh size) in such a way to cover the daily cycle of vertical migration. The sampling depth ranges were chosen in terms of density levels according to major biochemical characteristics of the water column which affect the distribution of mesozooplankton in the Black Sea. Temperature, water density and oxygen concentration were also recorded by a Sea-Bird CTD probe.

Results

Total and basal metabolism and scope of activity

When temperature decreased from 22.1 ± 0.71 to 8.0 ± 0.34 °C in oxygen-saturated water, the mean values of weight-specific respiration rate of *Calanus euxinus* females decreased significantly ($r^2 = 0.74$, $p < 0.001$, $n = 48$) from 1.38 ± 0.41 to 0.5 ± 0.18 μg O_2 mg^{-1} wet wt h^{-1}, according to the equation:

$Q_t = \exp(0.072t)0.276$.

Changes in mean oxygen concentration from 7.6 ± 0.68 to 0.78 ± 0.16 mg O_2 l^{-1} at constant low temperature (8.0 ± 0.5 °C) cause an additional significant ($p < 0.001$) decrease of total metabolism to 0.193 ± 0.087 μg O_2 mg^{-1}wet wt h^{-1}, following the equation: $Q_t = 0.139 \log O_2 + 0.237$ ($r^2 = 0.44$, $n = 105$). The decrease in metabolism and locomotory activity is more pronounced when oxygen concentration becomes lower than 2 to 3 mg l^{-1}, which is consistent with the results of Marshall and Orr (1972). Considering a sequential decrease of temperature and oxygen concentrations, the level of *C. euxinus* total metabolism was reduced 7.2-fold (Fig. 1A, B). Weight-specific respiration rate of narcotized copepods decreased from 0.446 ± 0.026 to 0.197 ± 0.015 μg O_2 mg^{-1} wet wt h^{-1} with temperature change from 22.1 to 8 °C, according to the equation: $Q_b = \exp(0.061t)0.121$ ($r^2 = 0.98$, $n = 8$), and then declined significantly to 0.057 ± 0.02 μg O_2 mg^{-1} wet wt h^{-1} within the range of oxygen concentrations from 7.6 to 0.78 mg O_2 l^{-1} ($Q_b = 0.058 \log O_2 + 0.071$, $r^2 = 0.8$, $n = 32$). Considering these temperature and oxygen concentration changes, the level of the basal metabolism declined 7.82-fold (Fig. 1C, D).

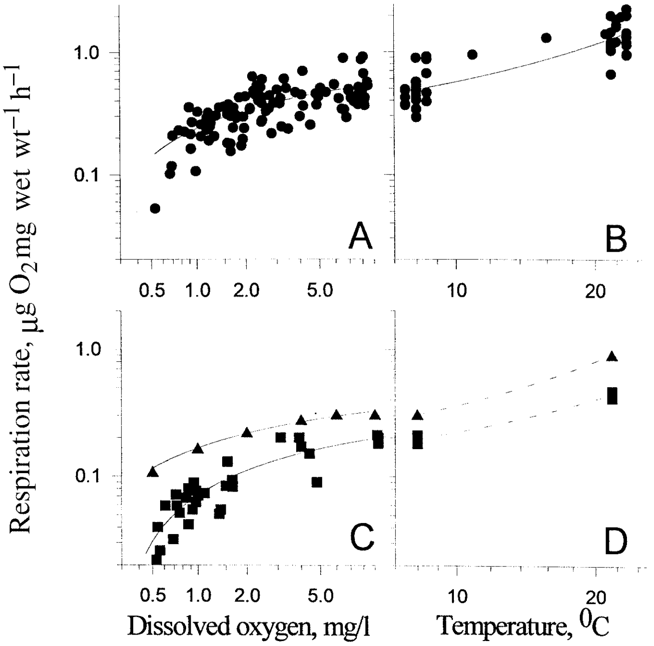

Fig. 1 *Calanus euxinus*. Effect of variations in oxygen concentration (**A, C**) and temperature (**B, D**) on total (●) and basal metabolism (■) and scope of activity (▲) of females

The scope of activity (calculated from the difference between total and basal metabolism) declined from 0.914 to 0.306 μg O_2 mg^{-1} wet wt h^{-1}, with temperature change from 22.1 ± 0.7 to 8.0 ± 0.3 °C, according to the equation: $Q_a = \exp(0.081\ t)0.16$, and decreased to 0.136 μg O_2 mg^{-1} wet wt h^{-1} ($Q_a = 0.073 \log O_2 + 0.166$) when oxygen concentration declined. In summary, the scope of activity dropped 6.72-fold, with its share in total metabolism varying between 60.8 and 70.5% (Fig. 1C, D).

Locomotor activity

There are two main modes of *Calanus euxinus* locomotion: prolonged uniform swimming (resulting from movements of mouthpart appendages combined with irregular weak flexure of the abdomen) and short-term jerks (through of powerful metachronic thrusts of the thoracic limbs and abdomen).

The frequency of mouth appendage movements in *Calanus euxinus* is due to their individual, neurally controlled rhythms. In oxygen-saturated water at 22.1 ± 0.7 °C, the frequency of oscillations (F) of different individuals varied from 27 to 45 Hz, with a mean

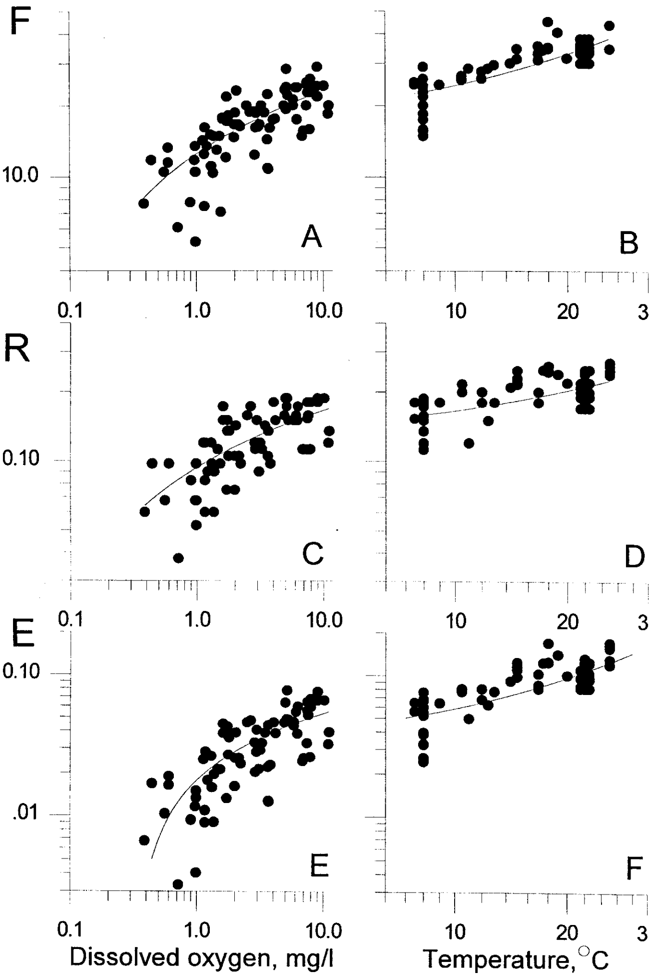

Fig. 2 *Calanus euxinus*. Relationship of frequency (*F*, Hz), drag force (*R*, dyne) and mechanical power (*E*, μg O_2 mg^{-1} wet wt h^{-1}) of the motion of mouth appendages to oxygen concentration (**A**, **C**, **E**) and water temperature (**B**, **D**, **F**)

value of 34.3 ± 3.6 Hz. When temperature decreased to 8 °C, *F*, on average, declined to 21.7 ± 3.8 Hz (Fig. 2A, B), in accordance with the formula: $F = \exp(0.031t)$ 17.8 ($r^2 = 0.65$, $n = 81$). Decreasing the oxygen concentration to 0.74 mg l^{-1} resulted in the additional reduction of *F* to 9.98 ± 2.8 Hz, following the equation: $F = 4.57 \log O_2 + 12.5$ ($r^2 = 0.55, n = 81$). Therefore, *F* declined 3.4-fold due to a combined effect of decreasing temperature and oxygen concentrations.

The integral drag force *R* (Fig. 2C, D) within the same range of temperatures under normal oxic conditions changed from 0.21 ± 0.029 to 0.152 ± 0.03 dyne, following the equation: $R = \exp(0.0207t)0.134$ ($r^2 = 0.36$, $n = 79$). Under hypoxic conditions, *R* dropped, on the average, to 0.075 ± 0.21 dyne, according to the equation: $R = 0.031 \log O_2 + 0.084$ ($r^2 = 0.49$, $n = 74$). In summary, *R* decreased 2.8-fold. It must be noted that throughout the investigated range of oxygen concentrations the drag force is significantly connected with the frequency of mouth appendage locomotion ($R = 0.0072\ F$, $r^2 = 0.75$, $n = 74$), which points to the same kinematic structure of locomotory acts under hypo- and normoxia. The frequencies of mouth appendage locomotion in *Calanus euxinus* and the congeneric species *C. helgolandicus* from the English Channel (Poulet and Gill 1988) at 15 °C are similar, i.e. 29 to 33 Hz and 28.7 to 29.9 Hz, respectively. Maximal mechanical energy ($E_{\max}$) of mouthpart locomotion (T = 100%, time spent in activity) decreased from 0.105 ± 0.023 to 0.0496 ± 0.017 μg O_2 mg^{-1} wet wt h^{-1} (Fig. 2E, F). The decrease with temperature was expressed by the equation: $E_{\max} = \exp(0.051t)0.035$ ($r^2 = 0.56$, $n = 79$). When oxygen concentration declined to 0.74 mg O_2 l^{-1}, *E* diminished to 0.011 ± 0.0052 μg O_2 mg^{-1} wet wt h^{-1} [$E_{\max} = 0.015 \log O_2 + 0.018$ ($r^2 = 0.52$, $n = 76$)]. In summary, $E_{\max}$ decreased by 9.55-fold.

In contrast to the frequency of movements, drag force and mechanical energy of locomotion, time spent swimming (*T*, %) did not depend on water temperature. Under normal oxic conditions *T* varied widely from 15–20 to 90–95%. However, when oxygen concentration declined to the values characterizing *Calanus euxinus*' daytime habitat at depth (0.8 to 1.15 mg O_2 l^{-1}), *T* of all investigated animals increased to 80–100%, independently of duration of locomotion at the beginning of the experiment (Fig. 3). The copepods can maintain such nearly continuous mouth appendage locomotion for several minutes (up to about an hour). Under oxygen concentrations below 0.5 mg O_2 l^{-1}, *C. euxinus* females became torpid.

In our experiments total duration of escape locomotion in oxygen-saturated water did not exceed 0.9% of the observation time (about 2100 thrusts h^{-1}).

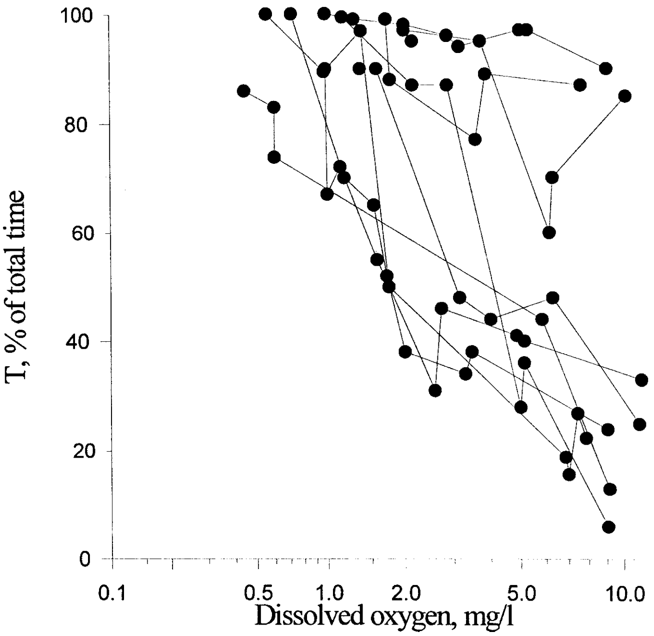

Fig. 3 *Calanus euxinus*. Relation of time spent swimming (% of total time) to oxygen concentration

Mechanical energy of this mode of locomotion is equal, on the average, to 0.154 μg O_2 mg^{-1} wet wt h^{-1}, accounting for 19.8% of the total mechanical energy expenditure for locomotion. Under hypoxia (<1.0 mg O_2 l^{-1}), thrusts become rare, never exceeding 0.05% of the observation time.

Dynamics of *Calanus euxinus* vertical distribution during migration

The vertical profiles of the MVBS values are given in Fig. 4. At night, the majority of the targets (presented by a dark shading) were observed very close to the surface. At dawn the targets descended. There were at least two separate groups of organisms undergoing distinctive vertical migration. One group reached greater depths, almost down to the onset of the anoxic layer. The other group remained at moderate layers (50 to 60 m), and spent the daytime at this layer. Before sunset, both of the groups began to ascend towards the surface. After sunset all groups were observed at the surface.

The results of zooplankton sampling also showed similar trends to the vertical movements of the zooplankton obtained by acoustics. While *Calanus euxinus* were undergoing distinctive and deep vertical movements, some other species remained at moderate layers during daytime. For instance, *Acartia* spp. preferred to remain at the near-surface layers, while *Pseudocalanus elongatus* preferred moderate layers.

The temperature changed from 21.7 to 9.1 °C in the surface layers (10 to 40 m), where the maximum density of zooplankton was observed. *Calanus euxinus* individuals started migrating downward at 5:00 hrs (local time) and reached the lower border of their habitat at 7:00 hrs (Fig. 4). Consequently, the duration of their descent was equal to 2.0 h. For about 9 h *C. euxinus* aggregations, with maximum density, remained at depths of 100 to 140 m, with a temperature of 7.4 to 7.9 °C, where the density and oxygen concentrations ranged within the limits from 15.09 to 15.75 (σ_t units) and from 4.17 to 0.6 mg O_2 l^{-1}, respectively. Animals started to ascend around 15:30 hrs and reached the surface layers at 18:30 hrs, so that their migration upward took nearly 3.0 h.

Discussion

In studies of the metabolism of marine animals, investigators prefer to focus their attention on long-term acclimation to constant environmental parameters because short-term experiments frequently lead to erratic results (Suschenya 1972; Ivleva 1981), due to the ability of organisms to under- or over-compensate for changes in their environment (Vilenkin and Vilenkina 1979). However, diel migration of *Calanus euxinus* from oxygen-saturated, warm surface water layers to cold, hypoxic zones takes only one or several morning hours (Petipa 1981; Besiktepe et al. 1998). Under these conditions complete acclimation to any environmental parameter seems unlikely.

Our experiments, designed to reproduce the conditions experienced during vertical migration, have demonstrated the direct relationship between the metabolism and parameters of locomotory activity in *Calanus euxinus* and temperature and oxygen concentrations in the sea water. The average value of Q_{10} in females is equal to 2.05 for total metabolism, 1.87 for basal metabolism and 2.23 for scope of activity. Our results are close to the Q_{10} mean value of 2.25, which is typical for crustaceans

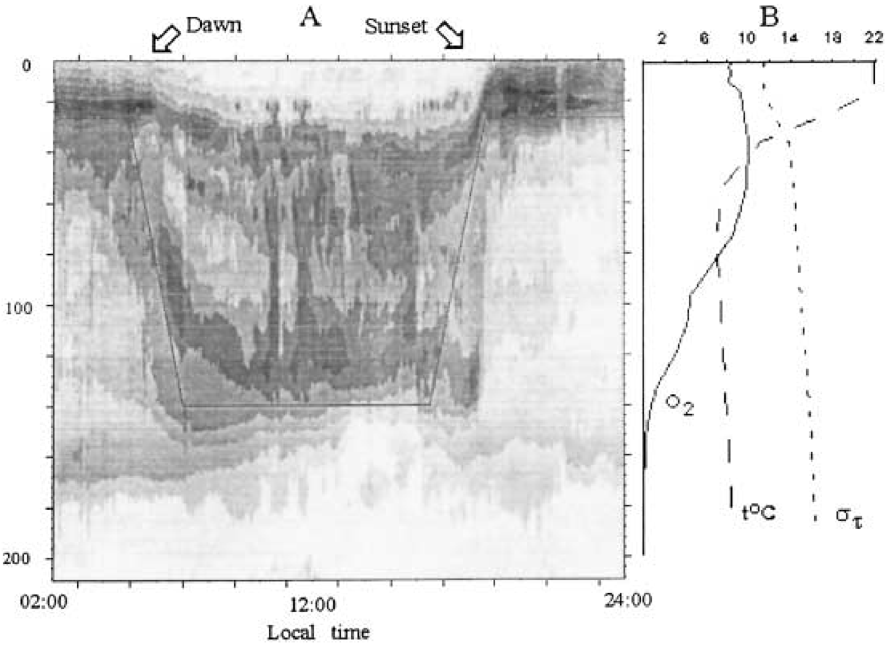

Fig. 4 Diel dynamics of the scattering layer (**A**) and vertical profiles (**B**) of temperature (°C), water density (σ_t) and oxygen concentration (O_2). The solid line in A shows the migration path of *Calanus euxinus*

acclimated to a temperature range from 10 to 25 °C (Ivleva 1981; Winberg 1983). Vidal (1980) investigated weight-specific respiration rates of *C. pacificus* at different constant temperatures (8, 12, 15.5 °C). The mean value of Q_{10} was 1.8.

The Q_{10} of temperature coefficients of frequency, drag force and mechanical energy of locomotion (1.38, 1.26 and 1.7, respectively) are close to temperature coefficients of locomotion of *Calanus euxinus*, acclimated to constant values of temperature (Svetlichny 1989). At a minimal stable temperature of about 8 °C, oxygen concentration changes from 7.6 ± 0.7 to 0.78 ± 0.2 mg l^{-1} cause the additional decrease in metabolism and locomotory activity, i.e. 2.3 to 3.5 times for respiration rate, and 2.0 to 4.5 times for mechanical parameters of locomotion. Such effects of low oxygen concentration remained after maintenance of *C. euxinus* individuals for 2 d at 1.0 mg O_2 l^{-1} (Svetlichny and Hubareva unpublished data). We did not find pronounced compensation reactions to physiologically realistic conditions. We suggest that *C. euxinus* should be considered an animal adapted to a variable environment (Galkovskaya and Suschenya 1978).

In representatives of the genus *Calanus*, feeding and locomotory functions of mouth appendages are integrated (Lowndes 1935; Vyshkvartseva 1976). Complex acts of all mouth appendages result in propulsion of body and water fluids, from which the animals extract food particles (Bundy and Paffenhofer 1996). Therefore, swimming copepods expend energy for filtration independent of whether food is present in the water. The share of energy required for filtration in the scope of activity of these animals seems to be considerable. For example, the drag force of isolated second antennae of *Calanus* is 1.5 times larger than the resultant propulsive force of the differently directed beats of all mouth appendages (Svetlichny 1991). Having analyzed the results of other investigations, Vidal (1980) concluded that the level of metabolism of feeding *C. pacificus* was about twice as high as that reported for starved, closely related species of the same genus. The respiration rates of mature animals with a dry weight of 250 mg was equal to 2.29 μg O_2 ind^{-1} h^{-1} at 21 °C (Vidal 1980), following the established relationship of total metabolism to body size and temperature. For example, according to Marshall and Orr (1972), the metabolism of starved *C. finmarchicus* was equal to 1.1–1.29 μg O_2 ind^{-1} h^{-1} at 20 °C. In our experiments, lasting upto 2 h, the respiration rate of starved *C. euxinus* females at 21 °C changes from 0.61 to 2.26 μg O_2 ind^{-1} h^{-1}, or approximately 11.3 μg O_2 mg^{-1} wet wt h^{-1}, due to the frequency and duration of mouthpart appendage locomotion. This value is close to the maximal respiration rate (12.1 μg O_2 mg^{-1} wet wt h^{-1}) of *C. euxinus* swimming freely in large respirometers of 1.4 liters at 21 °C (Pavlova 1987). At the maximal frequency of about 4500 thrusts h^{-1}, the respiration rate may reach 2.7 μg O_2 ind^{-1} h^{-1} (Svetlichny 1996). This value is 6.1 times higher than the level of basal metabolism of narcotized or completely immobile animals. Pavlova and Minkina (1987) reported that total metabolism of *C. euxinus* in respirometers of 1.4 liters at maximal locomotor activity may be two- to four-fold higher than basal metabolism. These data suggest that the level of *Calanus* locomotory activity affects the metabolic rates to a greater extent than the processes of food digestion and assimilation. Individuals placed in small respirometers of 2.5 ml might perform maximal locomotor activity during short-term experiments.

In the Black Sea, the layers where *Calanus euxinus* aggregated during daytime, lie quite near the anoxic and sulfide zones. Therefore, considerable decreases in the ability to swim in the cold hypoxic layers are dangerous for *C. euxinus* females, especially since they have a low lipid content (not exceeding 7%, Yuneva et al. 1997), and a body density of about 1.06 g cm^{-3} (Stepanov and Svetlichny 1981). Fig. 5 shows the relationship of calculated maximal speed of routine swimming and the speed of passive gravitational sinking of animals with a body length of 0.35 cm in relation to temperature and oxygen concentration. In aerated water at 22 ± 2 °C the speed of constant swimming of females is equal to 2.8 cm s^{-1}; this is approximately 2.5 times higher than the speed of passive sinking. Close results (2.7 to 2.8 cm s^{-1}) were obtained by filming ordinary locomotion of *C. euxinus* under similar laboratory conditions (Svetlichny 1987; Minkina 1983). Under hypoxia and at low temperature, the speeds of active swimming (0.7 cm s^{-1}) and passive sinking (0.54 cm s^{-1}) are close. Consequently, the increase in time spent swimming at oxygen concentrations of 1 mg O_2 l^{-1} is the behavioral, compensative reaction which prevents the animals from descending into anoxic layers. Copepods, aggregating near the aerobic zone boundary, have to maintain lowered, but constant locomotory activity. In one experiment, lasting up to 90 min, under hypoxia (1.4 mg O_2 l^{-1}) at 8 °C, *C. euxinus* females exhibited constant oscillations of mouthpart appendages, with a frequency of 12.9 Hz (CV = 2.7%). During this time, there were only five locomotory thrusts of the thoracic legs, with a mean duration of 0.017 s. Similar behavior of *C. euxinus* aggregated at depth at daytime was observed from a submersible by Flint (1989). The energy cost of such

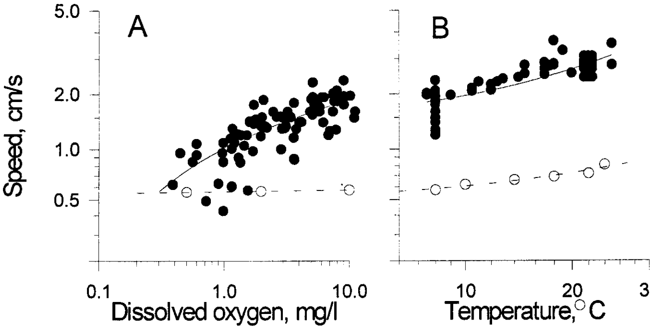

Fig. 5 *Calanus euxinus*. Effect of oxygen concentration (**A**) and temperature (**B**) on maximal speeds of routine swimming (●) and passive gravity sinking in the water (○) of females

active modes of staying beneath the oxycline is equal to half of the basal metabolism of *C. euxinus* in the surface layers. Significant reduction of locomotory activity was observed in euphausiids descending below the oxycline in the daytime at Saanich Inlet (Jaffe et al. 1999).

But does diel energy expenditure decrease? It depends on the value of energy expenditure allocated to locomotion during vertical migrations. Questions concerning the energetic cost, mechanisms and speeds of vertical migrations of copepods have been discussed widely in literature (Vlymen 1970; Enright 1977; Corkett and McLaren 1978; Winberg 1982; Raymont 1983). Petipa (1981) estimated that the cost of *Calanus euxinus* vertical migration increases the daily energy expenditure by a factor of 6 to 13. Klyashtorin (1984) calculated that mean daily total metabolism during migration was elevated by not more than 40 to 60%. Rudyakov (1986) and Geptner (1996) concluded that copepods might descend to depth passively, without any energy loss.

By our calculations, confirmed in observations of the descent of narcotized copepods (Stepanov and Svetlichny 1981), the speed of passive sinking of *Calanus* females having a body density of 1.06 g cm^{-3} decreases from 0.75 cm s^{-1} at 24 °C to 0.5 cm s^{-1} at 7.5 °C. In view of temperature and water density changes during migration (Fig. 4), the average sinking rate would be 0.55 cm s^{-1}. Therefore, during the 2.0 h of morning downward movement, animals would only passively descend 30 m. In contrast, the depth of the daytime habitat of *Calanus euxinus* is 180 or even 240 m deep (Vinogradov et al. 1992b). Consequently, animals seem to migrate actively to depth. Taking into account sharp decreases in escape locomotion, together with the decline of oxygen concentration and temperature, one can conclude that copepods reach depths mainly by use of their mouthparts for locomotion.

According to submersible observations (Vinogradov et al. 1987; Flint 1989), migrating females of *Calanus euxinus* form aggregations near the lower boundary of the oxycline, in the layers where oxygen concentration varies between 0.5 and 0.8 mg O_2 l^{-1}. The lower boundary of the oxycline is characterized by oxygen concentrations of 0.33 to 0.59 mg l^{-1} and water density of 15.29 to 15.92 (Poyarkov 1989). During our acoustic observations of *C. euxinus* migrations, the oxygen concentration of 0.6 mg l^{-1} was found at a depth of about 142 m. Only in this layer was the lower boundary of the acoustic density maximum recorded, due to the aggregations of migrating *C. euxinus*. Since at night the core of the acoustic density maximum relates to the upper boundary of the thermocline (about 25 m), the average amplitude of migration may be estimated as 117 m. According to acoustic observations, migration downwards takes 2.0 h and movement upwards lasts for 3.0 h, therefore the average speeds of migration descent and ascent are equal to 1.63 and 1.08 cm s^{-1}, respectively. Similar migration speeds (2.8 and 0.94 cm s^{-1}) for *C. euxinus* were obtained by a series of vertical tows (Besiktepe et al. 1998).

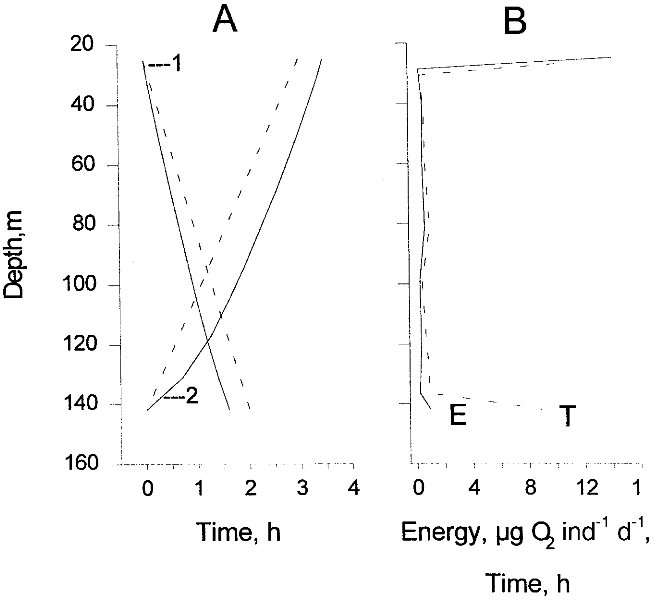

Fig. 6 *Calanus euxinus*. Trajectory of migration (**A**) and vertical distribution (**B**) of diel energy expenditure (*E*, μg O_2 ind^{-1} d^{-1}) in accordance with the residence time (*T*, h) of *C. euxinus* in 10-m layers. Descending (*1*) and ascending (*2*) paths of *C. euxinus*, calculated on the basis of physiological (–) and acoustic (- - -) data

We may also calculate the speed and energy cost of diel vertical migration of *Calanus euxinus* based on experimental relationships of metabolism and locomotory activity to water temperature and oxygen concentration in accordance with vertical profiles determined in this region together with average values of migration amplitude (Fig. 4). To simplify the calculation, we have made the following assumptions:

1. The speed of *Calanus euxinus* during descent is expressed as $V_d = V_a + V_p$, the speed of the ascent is defined as $V_u = V_a - V_p$, where V_a is the maximal swimming speed and V_p is the speed of sinking by gravity (cm s^{-1}).
2. The total duration of migration is equal to the sum of time intervals, necessary for copepods to pass through a sequence of 10-m layers with appropriate mean values of temperature, oxygen concentration and water density.
3. Total metabolism (Q_{tot}) during migration is a combination of basal metabolism (Q_b) and the energy cost of locomotion (E) in accordance with the residence time of *Calanus euxinus* in the above-mentioned 10-m layers. Total metabolism can be expressed as $Q_{tot} = Q_b + E/k$, where k is the coefficient of muscle contraction efficiency, being equal to 0.1 (Svetlichny 1996).
4. Copepods start migrating downward at 5:00 hrs and begin to ascend at 15:30 hrs. Our calculation shows that copepods could spend 1.6 h for their descent from 25 to 142 m depth, with a mean speed of 2.03 cm s^{-1}, which is close to the value of 1.67 cm s^{-1} from the acoustic data. The duration of ascent (3.47 h) and its mean speed (0.94 cm s^{-1}) are in agreement with those

values calculated from the data of acoustic observations (Fig. 6A).

Net energy cost of locomotion during vertical migration is equal to 2.07 μg O_2 ind^{-1} d^{-1}, or 11.6% of diel energy losses (17.87 μg O_2 ind^{-1} d^{-1}). *Calanus euxinus* individuals lose the main part of the budget (78.6%) in the surface, oxygen-saturated strata (18 °C) over 10.03 h, while they expend only 5.4% to stay 18.9 h in hypoxic layers (7.9 °C) (Fig. 6B). This phenomenon is due to a considerable decrease in metabolism (7.7-fold) in copepods living in deep layers. Therefore, vertical migrations of *C. euxinus* to cold, oxygen-deficient zones (even with maximal swimming speed) provide a substantial benefit, allowing the animals to use most of the food consumed in surface layers for the synthesis of wax esters (Yuneva et al. 1997), which are necessary for gonad development (Sargent and Henderson 1986).

Estimating the role of temperature and hypoxia, we can conclude that the latter factor is of major importance in the strategy of vertical migrations. Even in the period of seasonal homothermia in the Black Sea, *Calanus euxinus* metabolism diminishes 2.6-fold due to hypoxia. This might explain why late developmental stages of *C. euxinus* avoid coastal zones with depths of less than 60 to 80 m and where oxygen concentration is high throughout the water column.

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