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RESEARCH ARTICLE

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Diurnal gut pigment rhythm and metabolic rate of *Calanus euxinus* in the Black Sea

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Abstract The vertical distribution, diel gut pigment content and oxygen consumption of *Calanus euxinus* were studied in April and September 1995 in the Black Sea. Gut pigment content of *C. euxinus* females was associated with diel vertical migration of the individuals, and it varied with depth and time. Highest gut pigment content was observed during the nighttime, when females were in the chlorophyll *a* (chl *a*) rich surface waters, but significant feeding also occurred in the deep layer. Gut pigment content throughout the water column varied from 0.8 to 22.0 ng pigment female^{-1} in April and from 0.2 to 21 ng pigment female^{-1} in September 1995. From the diel vertical migration pattern, it was estimated that female *C. euxinus* spend 7.5 h day^{-1} in April and 10.5 h day^{-1} in September in the chl *a* rich surface waters. Daily consumption by female *C. euxinus* in chl *a* rich surface waters was estimated by taking into account the feeding duration and gut pigment concentrations. Daily carbon rations of female *C. euxinus*, derived from herbivorous feeding in the euphotic zone, ranged from 6% to 11% of their body carbon weight in April and from 15% to 35% in September. Oxygen consumption rates of female and copepodite stage V (CV) *C. euxinus* were measured at different temperatures and at different oxygen concentrations. Oxygen consumption rates at oxygen-saturated concentration ranged from an average of 0.67 μg O_2 mg^{-1} dry weight (DW) h^{-1} at 5°C to 2.1 μg O_2 mg^{-1} DW h^{-1} at 23°C for females, and ranged from 0.48 μg O_2 mg^{-1} DW h^{-1} at 5°C to 1.5 μg O_2 mg^{-1} DW h^{-1} at 23°C for CVs. The rate of oxygen consumption at 16°C varied from 0.62 μg O_2 mg^{-1} DW h^{-1} at 0.65 mg O_2 l^{-1} to 1.57 μg O_2 mg^{-1} DW h^{-1} at 4.35 mg O_2 l^{-1} for CVs, and from 0.74 μg O_2 mg^{-1} DW h^{-1} at 0.57 mg O_2 l^{-1} to 2.24 μg O_2 mg^{-1} DW h^{-1} at 4.37 mg O_2 l^{-1} for females. From the oxygen consumption rates, daily requirements for the routine metabolism of females were estimated, and our results indicate that the herbivorous daily ration was sufficient to meet the routine metabolic requirements of female *C. euxinus* in April and September in the Black Sea.

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Introduction

Copepods constitute the bulk of Black Sea mesozooplankton; of these copepods, *Calanus euxinus* is the largest species (adult prosome length ~2.7 mm) and accounts for over one-third of the total zooplankton biomass (Vinogradov et al. 1992). *C. euxinus* has been observed throughout the year, and population structure results have shown that the population is youngest (metanauplii, CI, CII, CIII) in April and December, whereas the oldest stages (CIV, CV, adults) make up >60% of the population in May, June and September (Besiktepe 2001). Nutritional conditions of female *C. euxinus* show regional differences. Yuneva et al. (1999) reported that the lipid content of *C. euxinus* from cyclonic regions was higher than that of individuals from anticyclonic regions.

Previous studies have revealed that *C. euxinus* performs a strong normal diel vertical migration, from the main pycnocline to the surface (Vinogradov et al. 1985; Besiktepe et al. 1998; Besiktepe 2001). The main pycnocline corresponds to the oxygen minimum zone (OMZ, represented by <0.6 mg O_2 l^{-1}) in the water column. *C. euxinus* are exposed to a wide range of temperature and oxygen concentration variations during their diel vertical migration. During the nighttime,

they are in the oxygen-saturated upper surface waters, with temperatures of about 24°C (in the summer months), and they stay in the oxygen-deficient lower layer (<0.6 mg O_2 l^{-1}), with temperatures of about 8°C during daytime. Lipid-rich stage V copepodites (CV) descend to the depth of the OMZ and remain in diapause there from late spring to autumn. The whole CV population does not enter diapause at the same time; around 50% of the CV population was observed in the diapausing phase in June, while only 13% of the population was in diapause in September (Besiktepe 2001). Vinogradov et al. (1990) reported that 60–75% of the CVs remained at the lower limit of the oxygenated layer at night in August. Several previous studies have shown decreases in the respiration rates of migrating and diapausing CVs (Vinogradov et al. 1992) and females of *C. euxinus* (Svetlichny et al. 2000) in decreasing oxygen concentrations in the Black Sea. Moreover, Svetlichny et al. (2000) showed that the escape locomotion (thrusts) of females become very rare under hypoxic conditions.

The present study concentrated on the diel feeding behaviour of *C. euxinus* in the Black Sea, by means of the gut fluorescence method (Mackas and Bohrer 1976). Samples were collected over short time intervals from discrete depth strata, including the OMZ, in April and September 1995, to determine the variations in feeding rates associated with depth, time of day and season. Additionally, we measured the metabolic rates of *C. euxinus* at different temperatures and oxygen concentrations. Finally, on the basis of these measurements, we discuss whether herbivorous daily rations meet the basic metabolic requirements of female *C. euxinus* in the Black Sea.

Materials and methods

Collection

Seawater samples, for measurements of chl *a* and oxygen concentrations, and zooplankton samples were collected at a daily drifting station located in the south-western part of the Black Sea (41°32′18 N; 29°29′E, 1200 m depth) between 26 and 28 April 1995 and between 27 and 28 September 1995.

Hydrographic data

Temperature in the water column was measured with a Seabird-SBE9 CTD profiler with fluorometer. Dissolved oxygen (DO) concentrations in the seawater from different depths of the water column were determined by using a modified conventional Winkler titration method (Konovalov et al. 1994). For chl *a* measurements, seawater samples (1–2 l) from different depths were filtered through GF/F filters and stored at −20°C until analysis. Filters were ground in 90% acetone with a grinder and kept overnight in the dark at 4°C for complete extraction. Samples were then centrifuged, and their fluorescence was measured before and after acidification with two drops of 10% HCl (IOC 1994) with a Hitachi F-3000 spectrofluorometer. Calibration was performed using a commercially available chl *a* standard from Sigma.

Sampling for diel vertical migration and feeding behaviour

The zooplankton samples were collected from five different depth intervals by a Nansen closing net of 112-μm mesh size (70-cm mouth diameter). Sampling depth intervals and their characteristics are given in Table 1. Samples were collected at 3- to 5-h intervals over a 30-h period in April 1995 and over a 21-h period in September 1995. Before each tow, the location of the station was fixed. Zooplankton from the first tow were passed through 2000-μm-mesh filters to remove gelatinous zooplankton (mostly *Aurelia*, *Mnemiopsis* and *Pleurobrachia*); this filtrate was preserved with 4% borax-buffered formaldehyde for species identification and enumeration. Zooplankton from the second tow were used for the study of diel feeding behaviour. After towing the net, the cod end content was taken immediately and sieved through a 2000-μm nylon mesh and then subsequently sieved through a 1000-μm nylon mesh to remove smaller organisms; most of the adult *Calanus euxinus* were collected on this mesh. Individuals on the mesh were washed a couple of times by rinsing with GF/C-filtered seawater. Females were identified under a stereo-microscope, and groups of 10–15 females were put on GF/F filters using forceps. Filters were frozen on

Table 1 Sampling depth layers used in the present study and their characteristics (*OMZ* oxygen minimum zone)

Sampling depth stratum	Depth stratum no.	Characteristics of depth stratum
From the base of the seasonal thermocline to the surface	1	Chlorophyll *a*–rich surface water
From the depth of $\sigma_\theta = 14.6$ to the depth of seasonal thermocline	2	$\sigma_\theta = 14.6$ refers to the beginning of nitrification (Lipp and Kempe 1993)
From the depth of $\sigma_\theta = 15.4$ to the depth of $\sigma_\theta = 14.6$	3	The majority of nitrification and remineralisation of organic matter take place (Lipp and Kempe 1993)
From the depth of $\sigma_\theta = 15.8$ to the depth of $\sigma_\theta = 15.4$	4	Denitrification processes begin to occur at the depth of $\sigma_\theta = 15.4$ (Bastürk et al. 1994)
From the depth of $\sigma_\theta = 16.2$ to the depth of $\sigma_\theta = 15.8$	5	$\sigma_\theta = 16.2$ corresponds to the bottom of the OMZ. This is the daytime aggregation layer for late copepodite stages and the adults of *Calanus euxinus* (Vinogradov et al. 1992)

dry-ice within 10–15 min after collection. However, because of gut pigment destruction and pigment losses during the time spent collecting the organisms on the filters, it has recently been suggested that copepod samples should be frozen as soon as they reach the deck (Bamstedt et al. 2000). Gut pigment analyses from each net tow were made in duplicate or triplicate, depending on the abundance of females. Diel feeding behaviour of female *C. euxinus* was investigated using the gut fluorescence method of Mackas and Bohrer (1976). Filters with copepods were homogenised in 10 ml of 90% acetone, and the fluorescence of the filtrate was measured before and after acidification with 10% HCl. The gut pigment content of copepods in each depth stratum was calculated by summing the chl *a* and phaeopigment concentrations and was expressed in nanograms of pigment per female.

Respiration rates of *C. euxinus*

For measurement of respiration rates of *C. euxinus* females and migrating CVs, the zooplankton samples were collected with a Hensen net (mouth opening 70 cm, mesh size 300 μm) and experiments were conducted during cruises in April, September and November 1996. Zooplankton samples were collected from 50 m up to the surface during nighttime to separate the migrating from the diapausing CV population, because part of the CV population was in diapause in the OMZ.

Respiration rates of females and CVs were determined in seawater filtered through GF/C filters. Respiration rates were measured on board at five different oxygen concentrations (ranged from 0.4 to 3.8 mg O_2 l^{-1}) at 16°C and at five different temperatures (5°C, 12°C, 16°C, 18°C and 23°C) at oxygen-saturated concentration. These experimental temperatures are within the annual range of surface temperatures and within the range of the vertical temperature profile; the experimental oxygen concentrations are within the range of the vertical oxygen concentrations of the water column in the Black Sea. To change experimental oxygen concentrations, 5-l of filtered seawater from the surface, in an air-tight container, was sparged on board for several minutes with high-purity nitrogen.

Individuals were acclimated at selected experimental temperatures and oxygen concentrations for 4–5 h. Respiration rate experiments were run using 150- and 30-ml Winkler bottles to determine the effects of temperature and oxygen concentration, respectively. We used two to five bottles as serial replicates, each containing 8–24 individuals that had been introduced into the bottles by a wide-mouth pipette; duplicate or triplicate controls consisted of bottles without copepods. Individuals were incubated for 12–19 h at different temperatures and for 5–7 h at different oxygen concentrations while they were kept in the dark. At the end of incubation, the activity of copepods in the experimental bottles was checked. Dissolved oxygen concentration in the bottles was determined by using Winkler titration methods (Konovalov et al. 1994). Respiration rate was calculated as micrograms O_2 per milligram dry weight per hour (average dry weight was assumed to be 0.26 mg for females and 0.21 mg for CVs; Besiktepe, unpublished data) using the equation described by Omori and Ikeda (1992).

Results

Hydrography

Vertical profiles of temperature, chl *a* and dissolved oxygen in April and September 1995 are presented in

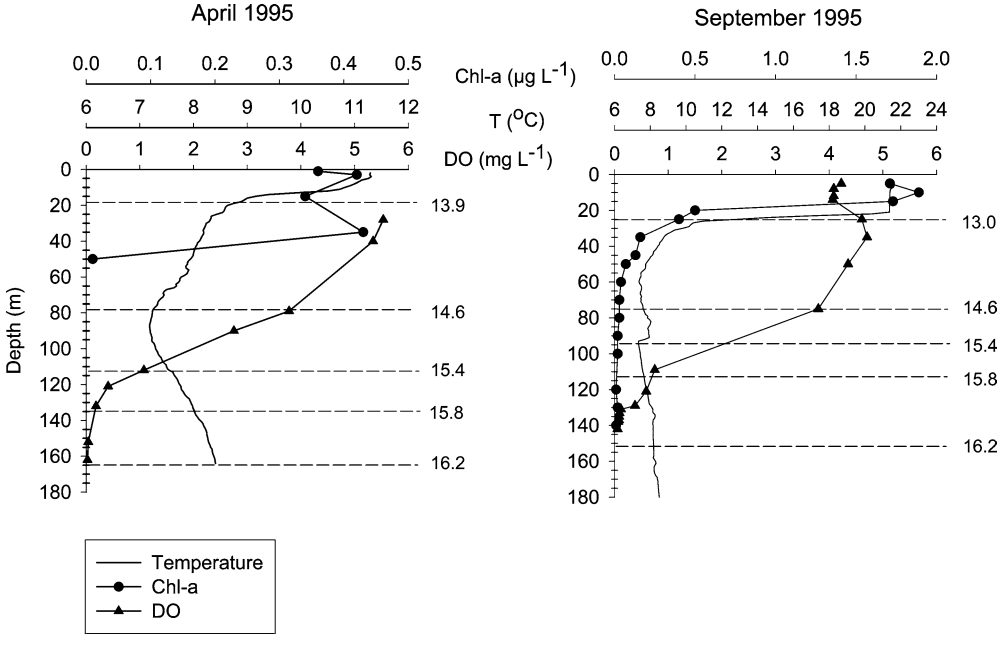

Fig. 1 Potential temperature (*T*), chlorophyll *a* (*chl a*) and dissolved oxygen (*DO*) concentrations are plotted against depth for April and September 1995. *Horizontal dashed lines* indicate the zooplankton sampling depths and the corresponding density (σ_θ) values

Fig. 1. An OMZ was observed within the depths of $\sigma_\theta = 15.8$ and 16.2 (around 140–160 m depth) throughout the sampling periods. In September, the thermocline was more pronounced, but, in April, as a result of spring seasonal mixing, the thermocline was weak. The sea surface temperature was around 23°C in September and 11°C in April. During both sampling periods, the chl *a* concentration was high in the upper surface waters and declined with depth. Depth-integrated chl *a* values for the upper 50 m were 16.3 and 30.6 mg m^{-2}, in April and September, respectively.

Diel vertical migration and diel feeding behaviour

Diel vertical migration of females and CVs in April and September 1995 is shown in Fig. 2. Females and CVs were most abundant in the upper layers at night, and, during daytime, they were most abundant in the deeper waters. The variabilities in abundance between night and day samples probably resulted from lateral intrusion of the water masses.

Diurnal feeding behaviour of females of *Calanus euxinus* throughout the oxic water column is shown in Fig. 3. There appeared to be a temporal pattern in the gut pigment content (GPC) of *C. euxinus* females in April and September, synchronised with diel vertical migration of the individuals (see Fig. 2A, B). In April, the GPC was analysed over 30 h. The highest amount of GPC was observed during the nighttime (at 2200, 0330, 2000 and 2400 hours), when females were at the two uppermost layers (Fig. 3). The GPC ranged from 4 to 22 ng pigment $female^{-1}$ in two uppermost layers. During the daytime, *C. euxinus* females were absent from the two upper layers (Fig. 2A). At 1800 hours, females were captured in the third layer, and, at 1700 and 1800 hours, they were captured in the fourth layer, indicating that they began their upward migration around 2–3 h before sunset (sunset = 1952 hours). During the daytime (at 0700, 1130, 1700 and 1800 hours), they were at the depth

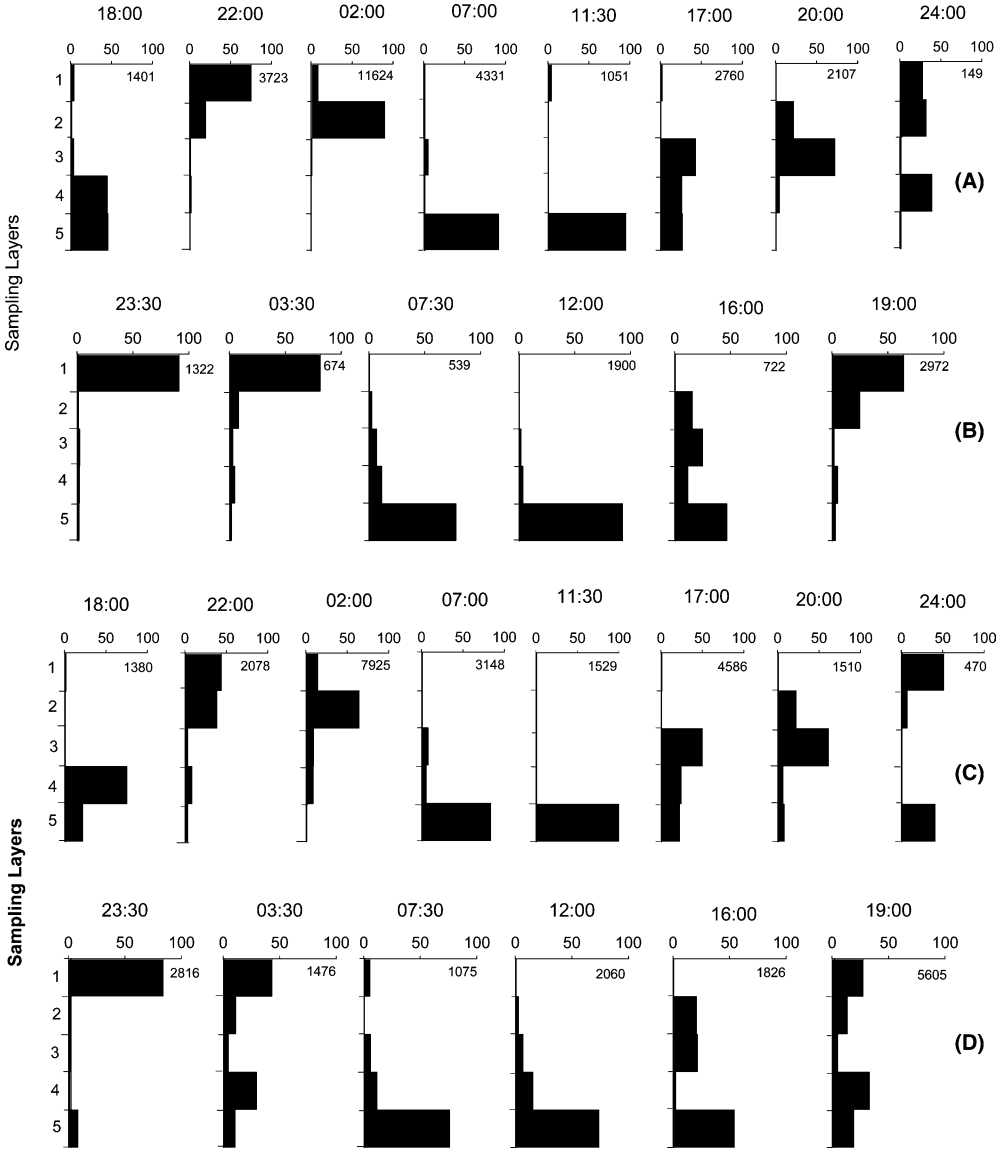

Fig. 2A–D *Calanus euxinus*. Vertical distribution of females (**A**, **B**) and stage V copepodites (**C**, **D**) at each sampling time during 26–28 April (**A**, **C**) (sunset = 1743 hours, sunrise = 0606 hours) and 27–28 September (**B**, **D**) (sunset = 1743 hours, sunrise = 0547 hours) 1995. Abundance is expressed as the percent of individuals per cubic metre for the entire profile at five depth strata (for more details about depth strata see Table 1). Numbers under the axis represent the total number of individuals (ind. m^{-2}) in the five hauls

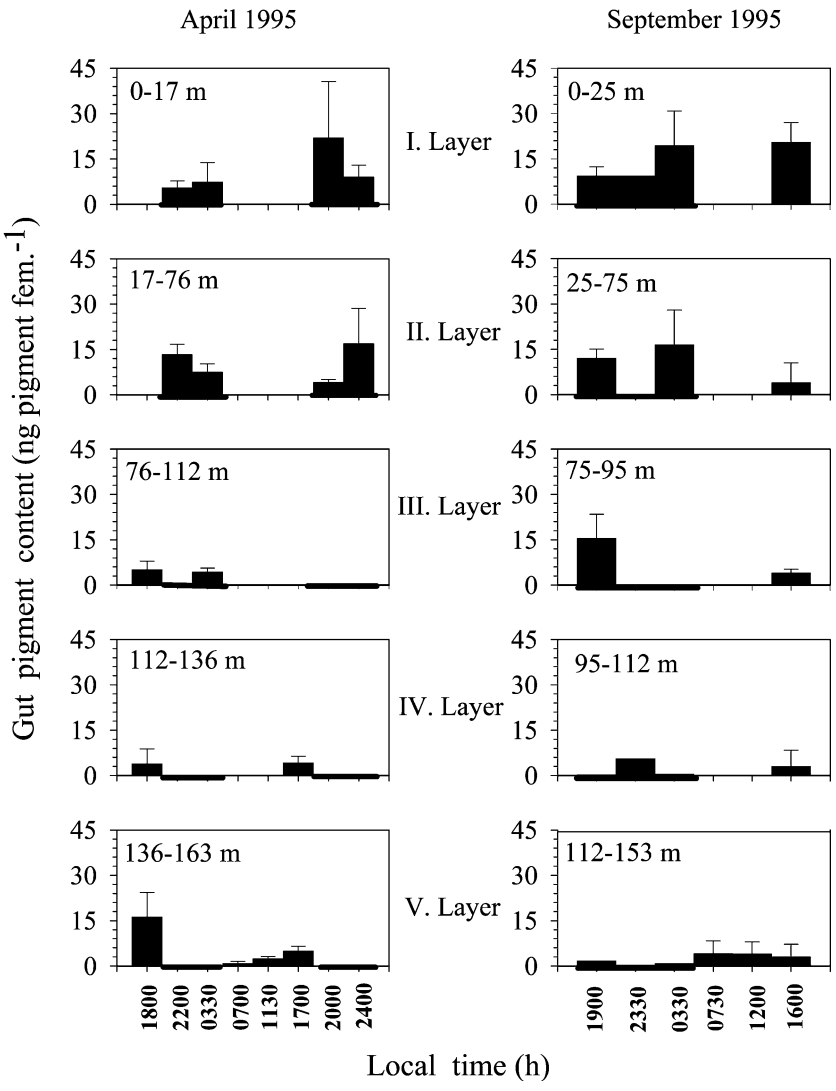

Fig. 3 *Calanus euxinus*. Gut pigment contents of females from different depth layers collected during 26–28 April 1995 (sunset = 1743 hours, sunrise = 0606 hours) and 27–28 September 1995 (sunset = 1743 hours, sunrise = 0547 hours). The depth interval of each layer is indicated in each figure. Thick lines on axis indicate night time sampling

of the OMZ. At this time, they had considerable amounts of GPC, which increased towards evening to a range of 0.8–16.2 ng pigment female^{-1}. This indicates that feeding occurred in the OMZ.

In September, females were captured from the two upper layers during nighttime (1900, 2330 and 0330 hours). Around 2 h before sunset (1600 hours, sunset was at 1743 hours), they appeared in the second layer. In the first layer, the average GPC ranged from 9.4 to 20.5 ng pigment female^{-1}. During the daytime (0730 and 1200 hours), females were absent from the upper layers (Fig. 2B). At all sampling times during the day, sufficient females were found in the OMZ for analysis. In none of the hauls made at nighttime (1900, 2330 and 0330 hours) were enough individuals captured for replicate analyses. In these samples, individuals contained only small amounts of gut pigment. During the daytime, females from the fifth layer (OMZ) contained considerable amounts of gut pigment (ranging from 0.9 ng pigment female^{-1} at 0330 hours to 4.2 ng pigment female^{-1} at 0730 hours).

Respiration rates

Oxygen consumption rates of *C. euxinus* females and CVs versus temperature are shown in Fig. 4. When temperature increased from 5°C to 23°C in oxygen-saturated filtered seawater, the weight-specific respiration rate increased significantly for both females and CVs. The rate of oxygen consumption of females ranged from a minimum of 0.67 µg O_2 mg^{-1} DW h^{-1} at 5°C to a maximum of 2.1 µg O_2 mg^{-1} DW h^{-1} at 23°C. The oxygen consumption rate of CVs ranged from a minimum of 0.48 µg O_2 mg^{-1} DW h^{-1} at 5°C to a maximum of 1.5 µg O_2 mg^{-1} DW h^{-1} at 23°C. The results of regression analyses indicated that temperature explained 64% and 91% of the variance in oxygen consumption rates of females and CVs, respectively (see Fig. 4). Q_{10} values of CVs varied from 2.1, for the increase in temperature from 5°C to 16°C, to 1.5, for the increase in temperature from 12°C to 23°C; the Q_{10} value was 1.9 over the temperature range investigated (from 5°C to 23°C). Q_{10} values of females varied from 2.2, for the

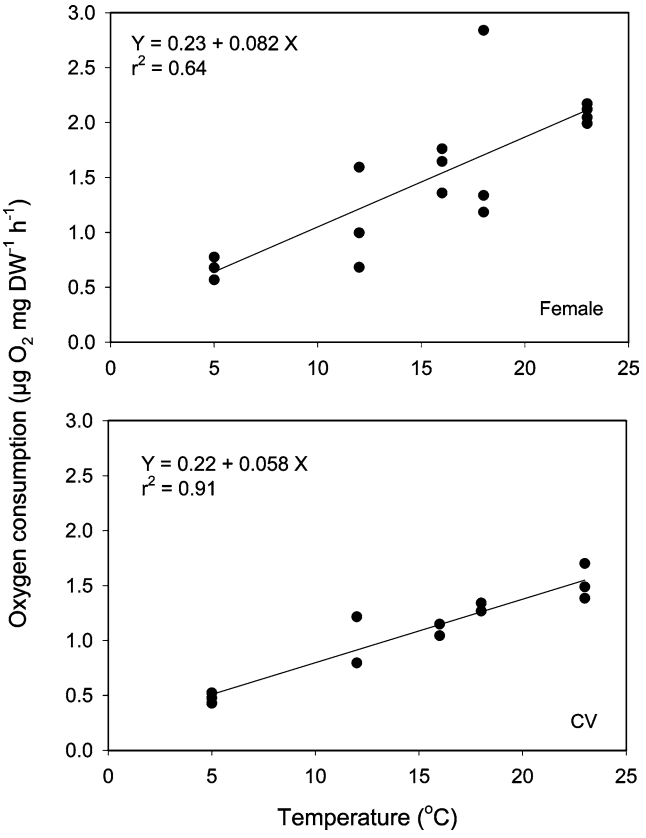

Fig. 4 *Calanus euxinus*. Respiration rates of females and CVs at different temperatures, with saturated oxygen concentration

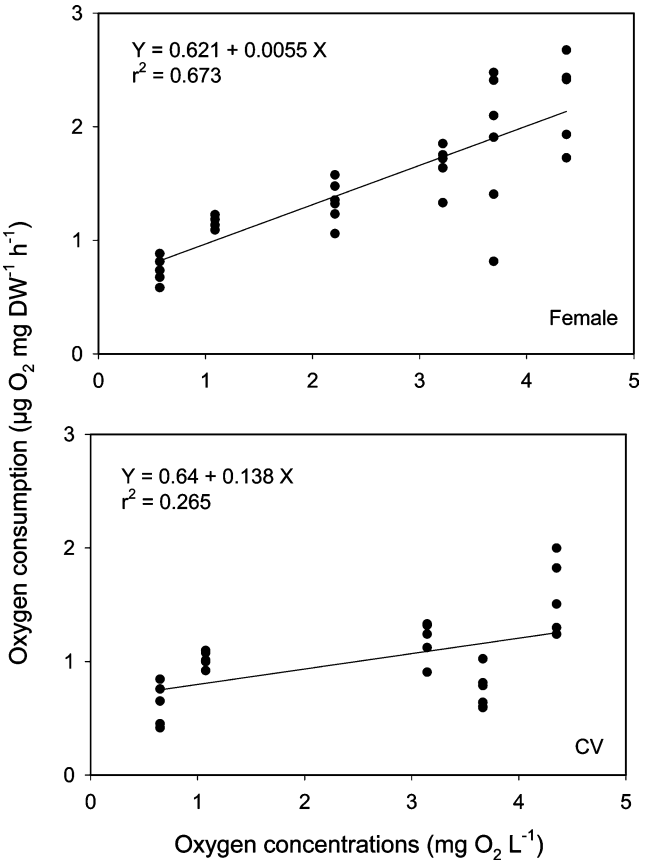

Fig. 5 *Calanus euxinus*. Respiration rate of females and CVs at different oxygen concentrations, at 16 ± 1°C

temperature rise from 5°C to 16°C, to 1.8, for the temperature rise from 12°C to 23°C; the value was 1.9 for the entire temperature range (5°C–23°C).

Oxygen consumption rates of *C. euxinus* females and CVs at different oxygen concentrations are shown in Fig. 5. Changes in oxygen concentration from 0.6 to 4.4 mg O_2 l^{-1} at constant temperature (16°C) caused increases in the metabolism of individuals. The rate of oxygen consumption ranged from a minimum of 0.62 µg O_2 mg^{-1} DW h^{-1} at 0.65 mg O_2 l^{-1} to a maximum of 1.57 µg O_2 mg^{-1} DW h^{-1} at 4.35 mg O_2 l^{-1} for CVs, and from a minimum of 0.74 µg O_2 mg^{-1} DW h^{-1} at 0.57 mg O_2 l^{-1} to a maximum of 2.24 µg O_2 mg^{-1} DW h^{-1} at 4.37 mg O_2 l^{-1} for females. The results of regression analyses indicated that 67% and 27% of the variances in oxygen consumption rates of females and CVs could be attributed to ambient oxygen concentrations, respectively (Fig. 5).

Discussion

Diel feeding behaviour

The level of gut pigment content appeared to be associated with depth. Gut pigment levels in the upper layers were high throughout the night. In September, although the depth-integrated chl *a* concentration (30.6 mg chl m^{-2}) in the upper 50 m was almost two times higher than those in April (16.3 mg chl m^{-2}), the average gut pigment level from the two upper layers was almost the same as that in April. This indicates that the increase in GPC is not always proportional to the increase in ambient chl *a* concentrations. Most of the published reports on phytoplankton–zooplankton relationships do not show the expected correlation between GPC and chl *a* concentrations in the seawater (Boyd and Smith 1980; Dagg and Wyman 1983; Wang and Conover 1986; Bautista et al. 1988; Kleppel et al. 1988; Turner et al. 1993). In the present study, probable gut evacuation during the manipulations before freezing may have caused underestimation of GPC, and thus could have resulted in the underestimation of ingestion. Such underestimation may have led to the lack of relationship between ambient chl *a* and gut pigment levels. However, phytoplankton composition might also play a role in such relationships. Phytoplankton composition in April may be better suited for *Calanus* feeding. Petipa (1964) observed that Peridinea was the dominant group and Diatomea was the second most abundant group in the gut of *Calanus*; coccolithophorids were rarely observed as a food item. While in both seasons coccolithophorids were more important than the other two groups of

phytoplankton, in April the abundance percentage of Peridinea was 19%, while that of Diatomea was 1.3% in the phytoplankton. In September, the percentages of these two groups of phytoplankton were equal (3%) (Eker et al. 1999).

In the present study, we measured considerable amounts of GPC in females staying at the depth of the OMZ during daytime, from 0700 to 1800 hours in April and from 0730 to 1600 hours in September. The average GPC of females from the OMZ was 6.1 and 2.4 ng pigment female^{-1} in April and September, respectively. These values are higher than the background pigment fluorescence of starved females of *C. euxinus* (on average 0.68 ng pigment female^{-1}). Increases in GPC towards evening in the OMZ, particularly in April, imply that feeding occurs in the OMZ. Dagg et al. (1998) observed low, but continuous, gut pigment levels in *Metridia lucens* from deep layers (50–108 m), where low chl *a* concentrations were observed. Unlike *M. lucens*, they observed high gut pigment levels in *C. pacificus* in deep layers at the onset of the descent phase, suggesting that copepods fed on the chl *a* rich surface waters, then descended with a remarkable amount of gut pigment to deeper layers. There may be different chl *a* containing food sources for the *C. euxinus* in the lower layer. For instance, Yilmaz et al. (1998) have reported variations in particulate organic carbon (POC) concentrations with depth in the Black Sea. They observed high POC concentrations (10–15 μM) in the euphotic zone, which declined to 1–4 μM with depth, and there was a small increase in POC at the suboxic–anoxic interface. The chl *a* containing particles sinking to the deeper layers, including phytoplankton and especially diatom cells and faecal pellets, may contribute to the POC and provide a food source for *C. euxinus* staying at the depth of the OMZ during daytime. Repackaging of faecal pellets by copepods through coprophagy has been reported in the literature (Paffenhöfer and Knowles 1979; Gowing and Silver 1986; Green et al. 1992). Karl and Knauer (1991) have previously observed numerous faecal pellets in their sediment traps deployed at 80 m depth, almost the upper boundary of the sulphide zone at their station. Additionally, aggregates of some chl *a* containing small cells at the depth of the OMZ may serve as a food for *Calanus*, e.g. chrococcoid cyanobacterial cells have been reported in the deeper layers of the Black Sea, around 145 *Synechococcus* spp. cells ml^{-1} were observed at 108 m depth, where dissolved oxygen concentrations were <0.5 ml l^{-1} (Uysal 2001). Besides these, Coble et al. (1991) observed two chl *a* fluorescence maxima in the Black Sea water column during the R.V. "Knorr" cruise in 1988. A primary maximum was at the bottom of the euphotic zone, and a weak secondary peak was at the depth of the sulphide interface. This secondary peak was associated with the photosynthetic sulphur bacteria population containing bacteriochlorophyll *e* (bchl *e*), and the spectrum of bchl *e* is similar to that of chl *a* (Coble et al. 1991). However, we did not identify such a secondary peak in our in situ fluorescence profiles; rather, we observed several small fluctuations in fluorescence profiles in deep waters in April and September (Fig. 6). The sizes of both bacterial and *Synechococcus* spp. cells are <1 μm, and this size is not appropriate for copepods to remove from suspension. On the other hand, these cells might concentrate in aggregates or clusters, which could be eaten (Dagg 1993). Kosobokova et al. (2002) found 17:0 fatty acid as a biomarker for sulphate-reducing and other bacteria in the copepod *Spinocalanus antarchicus*. Thus, particle aggregates, their contents and sizes need to be studied in the Black Sea, especially in the suboxic zone, to understand the daytime feeding behaviour of *C. euxinus*.

Respiration rates

C. euxinus are exposed to a wide range of temperatures (~3-fold) and oxygen concentrations (~20-fold) during their diel vertical migration. During the nighttime, they are in the oxygen-saturated upper surface waters, with temperatures of about 24°C (in the summer months), and they stay in the oxygen-deficient lower layer, with

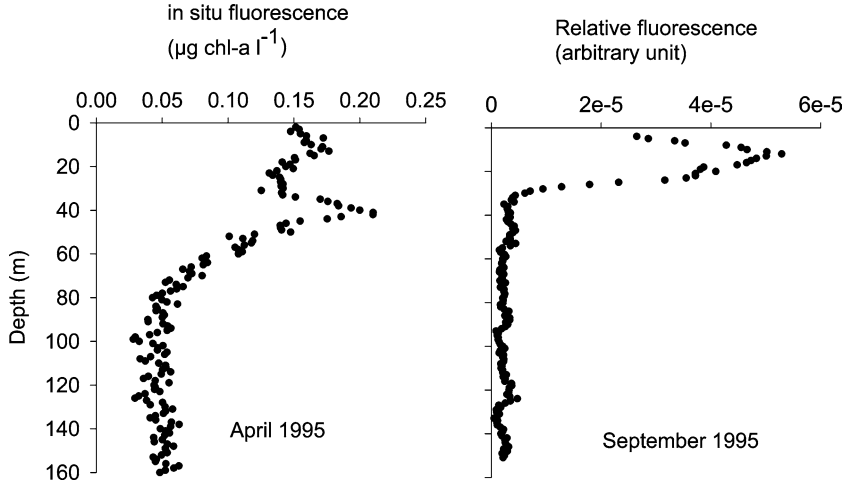

Fig. 6 Fluorescence profiles in April and September 1995

the temperatures of about 8°C, during daytime. Our results illustrate that the weight-specific respiration rate of CVs and females decreased by a factor of 3 between 23°C and 5°C in oxygen-saturated seawater and that around 7.5-fold decreases in oxygen concentration caused 2.5-fold decreases in respiration rates for CVs and 3-fold decreases for females. Respiration rates of CVs measured at different oxygen concentrations in this study are higher than those measured by Vinogradov et al. (1992) for migrating CVs using the Winkler method. These high respiration rates in our study could be due to the experimental temperature; they ran their experiments at 8°C, ours were run at 16°C. However, the respiration rates of females measured in the present study at different oxygen concentrations at 16°C are similar with the results of Svetlichny et al. (2000); they measured the respiration rates of female *C. euxinus* at different oxygen concentrations at 8°C by using a polarographic membrane oxygen sensor. Furthermore, in our study the respiration rates of females at different seawater temperatures at oxygen-saturated concentration and the respiration rates of CVs at different oxygen concentrations are lower than the respiration rates of females and CVs measured by Svetlichny et al. (1998, 2000) using the polarographic method. The discrepancies in the respiration rate measurements between Winkler and polarographic methods have been shown (Thor et al. 2002) and discussed (Le Borgne 1986) in the literature. Incubation time and crowding may influence the respiration rates of the organisms (Le Borgne 1986). It should be noted that the metabolic rates may have been underestimated in the present study, because of crowding and long incubation times. However, with this experimental set-up, it was impossible to quantify the underestimation.

The decrease in oxygen concentration from 4 to 0.5 mg O_2 l^{-1} in the ambient water caused a sharp decrease in metabolism. The coefficients of determination (r^2) for the regression analyses of oxygen concentration versus oxygen consumption indicate that the metabolic rates of females are more sensitive to changes in dissolved oxygen concentrations in ambient water than those of CVs. However, the metabolism of CVs is mostly related to temperature in the Black Sea.

Results obtained in this study showed that female and CV *C. euxinus* are very tolerant to low oxygen concentrations. Stalder and Marcus (1997) reported 100% mortality in adult *Acartia tonsa* at 0.7 mg DO l^{-1}, and they observed significant decreases in the survival of *Labidocera aestiva* and *Centrapages hamatus* at 1.4 mg DO l^{-1}. In their survival experiments, they incubated experimental bottles for 24 h. We observed mortality at 1.1 and 0.62 mg l^{-1} oxygen concentrations, and maximum mortality was only 10% for CV and 25% for females during 6.5 h incubation at 0.62 mg DO l^{-1}. The exposure time to hypoxic water is important for the survival of the individuals. However, we did not observe any mortality at 0.77 mg DO l^{-1} when the animals were incubated at 5°C for 6.5 h.

From the gut pigment content and respiration rate data produced in the present study, we attempted to estimate the cost of respiration for female *C. euxinus* in the Black Sea. For this estimation, the temperature and oxygen concentrations set in the experiments allowed us to calculate metabolic requirements of *Calanus* only from the two uppermost layers. Respiration rates under oxygen saturation at the mean temperature of the two upper layers (8.5°C for April, 12°C for September) were converted to carbon units using a respiratory quotient (RQ = 0.72, Omori and Ikeda 1992). From the diel vertical migration pattern, it was estimated that female *Calanus* spend 7.5 h day^{-1} in April and 10.5 h day^{-1} in September in the two uppermost layers. After these time durations were accounted for, daily respiration was 0.58 μg C $ind.^{-1}$ day^{-1}, representing 0.5% of the body carbon, in April and 1.5 μg C $ind.^{-1}$ day^{-1}, equal to 1.3% of the body carbon, in September. Our results illustrated that maximum feeding occurred at the two uppermost layers (in the sampling layers corresponding to the euphotic zone; euphotic zone depth was 38 m in April and 17 m in September) during nighttime. The background fluorescence for the starved individuals was measured as 0.68 ± 0.39 ng pigment $female^{-1}$. After this value was accounted for, the overall average gut pigment concentration of female *Calanus* from two uppermost layers was calculated to be 10.1 ng pigment $female^{-1}$ in April and 14.0 ng pigment $female^{-1}$ in September (Table 2). Using the linear equation of Dam and Peterson (1988), the estimated gut evacuation rate constant was 1.86 h^{-1} in April and 3.0 h^{-1} in September. Then, we calculated average ingestion rates from the two uppermost layers by multiplying GPC and the gut evacuation rate constant; average ingestion was 18.7 and 42 ng pigment $ind.^{-1}$ h^{-1} in April and in September, respectively. Daily consumption by female *C. euxinus* was estimated by taking into account the feeding duration and gut pigment concentrations in the two uppermost layers. The phytoplankton C:chl *a* ratios are 76 in April and 65 in September (Eker 1998). These phytoplankton C:chl *a* ratios were used to convert the consumed gut pigment to carbon. Based on the rough estimation, mean daily carbon rations derived from herbivorous feeding by female *C. euxinus* were 10.65 μg C $ind.^{-1}$ day^{-1}, equal to 9.3% of female body carbon weight (~115 μg C $female^{-1}$, Telli, unpublished data) in April, and to 28.7 μg C $ind.^{-1}$ day^{-1}, representing 25% of the body carbon in September (Table 2). Daily assimilated carbon was computed as 7.46 μg C $ind.^{-1}$ day^{-1} in April and 20.1 μg C $ind.^{-1}$ day^{-1} in September, assuming an assimilation efficiency of 70% (Conover 1978), and around 8% of this assimilated carbon was devoted to respiration (Table 2), the rest, around 90%, was dedicated to production.

Our estimation is limited to the euphotic zone (the two upper layers of the water column), but Svetlichny et al. (2000) determined diel respiratory oxygen consumption of female *C. euxinus* including actual durations of residence in layers with different temperatures

Table 2 *Calanus euxinus*. Gut pigment content (*GPC*, ng pigment female^{-1}), ingestion (I_1, ng pigment female^{-1} h^{-1};I_2, μg C female^{-1} day^{-1}), herbivorous daily carbon ration as percentage of body carbon (*C*), daily assimilated carbon (*A*) and percentage of daily basic metabolic carbon requirements (*R*) from the euphotic zone during the sampling times in April and September 1995

Time (hours)	GPC	I_1	I_2	C	A	R
April 1995						
1800	–	–	–	–	–	–
2230	8.7	16.18	9.22	8.02	6.45	8.96
0230	6.8	12.67	7.22	6.28	5.05	11.44
0630	–	–	–	–	–	–
1130	–	–	–	–	–	–
1600	–	–	–	–	–	–
2000	12.35	22.97	13.09	11.38	9.16	6.31
2400	12.33	22.93	13.07	11.36	9.15	6.32
Average	10.05	18.69	10.65	9.26	7.46	8.26
September 1995						
1900	8.7	26.2	17.88	15.55	12.51	12.29
2330	8.7	26.1	17.81	15.49	12.47	12.34
0330	18.8	56.3	38.42	33.41	26.89	5.72
0730	–	–	–	–	–	–
1200	–	–	–	–	–	–
1600	19.9	59.6	40.68	35.37	28.47	5.40
Average	14.03	42.05	28.7	25.0	20.1	8.94

and oxygen concentrations. They calculated the daily female respiration rate as 12.5 μl O_2 in the summer/autumn period, equal to 4.8 μg C ind.$^{-1}$ day^{-1}. Using their results, we estimated if herbivorous feeding in September (late summer period) would meet the daily requirements. This value corresponds to 28% of the assimilated carbon in September. So, our results indicate that the herbivorous daily ration in both cases would be sufficient to meet the routine metabolic requirements (metabolism with uncontrolled swimming activity, for details see Ikeda et al. 2001) of female *C. euxinus* in the Black Sea.

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