Development and lipid storage in *Calanus euxinus* from the Black and Marmara seas: Variabilities due to habitat conditions

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Abstract

Oil sac volume, gonad size and moulting patterns were investigated in the copepod *Calanus euxinus* inhabiting deep and shallow zones of the Black Sea and penetrating into the Marmara Sea. In summer the *C. euxinus* population in deep layers of the Black Sea was dominated by pre-diapause and diapausing postmoult copepodite stage V (CV) with small sexually undifferentiated gonads and mean lipid content of $14.1 \pm 6.0\%$ of body volume. The lipid content of deep-living females was $7.2 \pm 4.2\%$ of body volume. At the same time, intermoult and premoult CV with enlarged gonads and low lipid content ($7.7 \pm 5.1\%$ of body volume) and females with oil sac volume of $1.4 \pm 1.0\%$ were found at shallow stations. Premoult CV with oil volume of $0.6 \pm 0.8\%$ and mature females with little visual evidence of substantial lipid storage dominated in the Marmara Sea. The differences in moulting patterns and oil sac volumes of *C. euxinus* from deep zones and shallow regions suggest that vertical migrations to the oxygen minimum zone (OMZ) are necessary for formation of large lipid reserves providing high reproductive potential of this species. On the basis of an energy balance model it was shown that under low phytoplankton concentration of about $30 \ \mu g \ C \ l^{-1}$ preadults and adults migrating to the OMZ could accumulate lipids (up to 5% of body energy content daily), in contrast to copepods constrained to shallow oxic water columns of the Black Sea and from the Marmara Sea.
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1. Introduction

Calanus euxinus (Hulsemann) is a key component of the Black Sea pelagic ecosystem. This endemic species originated from the North Atlantic *Calanus helgolandicus* which had penetrated to the Black Sea from the Mediterranean Sea (Fleminger and Hulsemann, 1987). *C. euxinus* dominates the mesozooplankton in deep-sea regions of the Black Sea and achieves adult body size exceeding that of *C. helgolandicus* occurring in the eastern Mediterranean Sea. Environmental factors are known to play an important role in determining the body size range of planktonic copepods. According to Kovalev (1968), the body size of some copepod species decreased after invasion and acclimatization to Black Sea conditions. The body length of adult pelagic copepods in the Black Sea hardly exceeds 1 mm except *C. euxinus* (with the total length of 3.5–3.7 mm). Fleminger and Hulsemann (1987) reported that adult body size of the Black Sea *C. euxinus* exceeds that of *C. helgolandicus* from the West, Mid-North and East–North Atlantic as well. Only in the Celtic Sea (Williams and

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Robins, 1982) and North Sea (Hirche, 1983) does body length of *C. helgolandicus* approximate that of the Black Sea *C. euxinus*.

Whilst *C. helgolandicus* show 5 generations in the Western English Channel (Rey-Rassat et al., 2004), in the Black Sea *C. euxinus* have 6–8 generations (Sazhina, 1987). Therefore, all stages are usually present in the samples, indicating an almost continuous recruitment in the field.

During all seasons *C. euxinus* is able to accumulate large amounts of lipids (mainly wax ester) in the body (Yuneva et al., 1997, 1999). Oil sac content in *C. euxinus* may reach 30% of body volume (Svetlichny et al., 1998) which is close to lipid content of *Calanus* from the seas of the North-East Atlantic (Miller et al., 2000). Trying to explain extremely large body size of adult *C. euxinus*, Fleminger and Hulsemann (1987) suggested that low temperature and hypoxia are the main factors.

The Black Sea pelagic upper aerobic zone is separated from lower anaerobic layers (containing hydrogen sulfide) by the main pycnocline lying at depth of 70–200 m (Vinogradov et al., 1992b). In the Black Sea definite values of oxygen concentration corresponds to precise density levels. Therefore, the pycnocline is accompanied by the oxycline where oxygen concentration decreases from 7.9–9.8 mg l^{-1} with specific density (σ_t) of 14.0–14.7 to 0.47–0.84 mg l^{-1} ($\sigma_t = 15.3$–15.9). The width of the oxycline varies from 50 to 93 m (Poyarkov, 1989). Below this, oxygen concentration decreases slowly to <0.16 mg O_2 l^{-1} at $\sigma_t = 15.9$–16.0, and can no longer be detected at $\sigma_t = 16.15$–16.20 where sulfide concentration amounts to 0.03–0.1 mg H_2S l^{-1}. Varying from 7 to 25 °C in the surface layers (0–30 m) due to seasonal fluctuations, the temperature of the deeper zone is constant throughout the year amounting to 6–8 °C.

During the period of seasonal temperature stratification in the deep regions of the Black Sea preadult and adult *C. euxinus* form two ecological groups: migrating and non-migrating individuals. One part of copepodites V (CV), all females and males perform diel vertical migration from oxygen-saturated surface layers with the temperature of 20–24 °C to cold (8 °C) oxygen minimum zone (OMZ, 0.5–0.8 mg O_2 l^{-1}). The another part of CV constantly inhabits underlying water layers with more pronounced (as low as 0.3 mg O_2 l^{-1}) oxygen deficiency (Vinogradov et al., 1992a,b). In comparison with migrating copepods, non-migrating *C. euxinus* have lower values of frequency of locomotion, respiration and ammonia excretion rates at identical temperature and oxygen concentration which indicates that they are in the stage of diapause (Svetlichny et al., 1998).

Diapausing CV appear in a few number in the sea in April–May. Up to August–September they constituted 30%, sometimes even 70% of the number of CV, last autumn their number may increase (Vinogradov et al., 1992b).

Svetlichny et al. (2000) showed experimentally that *C. euxinus* occupying a deep daytime habitat decreased their total metabolism nearly 8-fold. Therefore, we suggest that the best advantage for *C. euxinus* in the Black Sea to utilize the energy of consumed food for growth and lipid accumulation is to descent during the day to the cold oxygen-deficient zone. The ability to decrease energy expenditure is of great importance especially during summer season when chlorophyll-*a* concentration reduces and averages 0.22 mg m^{-3} (Yunev et al., 2002). This might explain why in summer preadult and adult *C. euxinus* occupy mainly the circular Black Sea current over deep zones where they can migrate to OMZ. However, due to periodic gyres branching off from the main Black Sea Current, the part of *Calanus* population may be brought to shallow water (the depth less than 100 m) where oxygen concentration is high even near the bottom. Moreover, *C. euxinus* are carried into the Marmara Sea through the Bosporus with the Black Sea water which forms the surface layers (up to 20–40 m) with the salinity of about 20‰. Deeper strata with the salinity of 37‰ are affected by the Mediterranean Sea (Kocatas et al., 1993; Besiktepe et al., 1994). Due to salinity gradient, the ability of *C. euxinus* to perform vertical migrations in the Marmara Sea seems to be limited, and the copepods aggregated in the oxygenated upper layer with the temperature of 13–22 °C. Our laboratory studies showed that *C. euxinus* respond to sharp salinity increase by escape reaction and can survive at 37‰ only after prolonged gradual acclimation (unpublished data).

The aim of the present study was to compare lipid content, gonad size, moulting patterns and population stage structure of *C. euxinus* from deep (with oxygen-deficient layers) and shallow (oxygenated up to the bottom) regions of the Black Sea and Marmara Sea. The balance energy model was also used to test the energy-bonus hypothesis of diel vertical migrations of *C. euxinus* to OMZ.

2. Materials and methods

Zooplankton samples were collected with a closing Nansen net (opening diameter 71 cm, mesh size 112 μm) during the cruises of the R/V "Bilim" at 3 night-time deep stations in the south region of the Black Sea
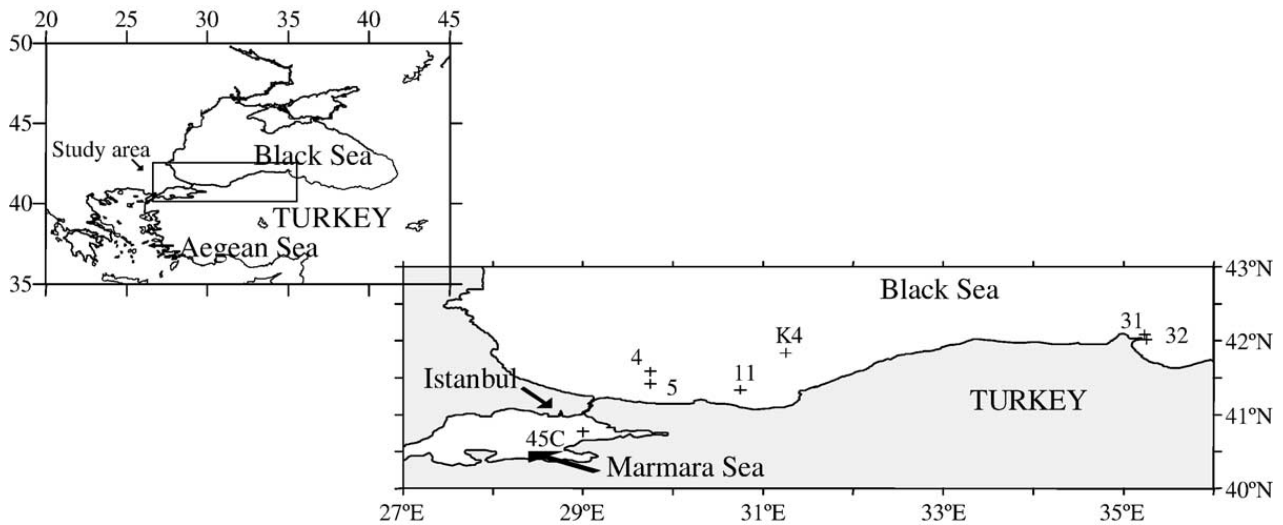
Fig. 1. Location of sampling stations in the Black and Marmara seas.

and two daytime (one deep, 300 m and one shallow, 75 m) stations off Sinop (southern Black Sea coast of Turkey) in July 2000, and also a single daytime station in the Marmara Sea (st. 45 C) in October 2000 and June 2001, and in the Black Sea (st. K4) in June 2001 (Fig. 1, Table 1). In the Black Sea during daytime zooplankton was sampled by vertical hauls from the depth with $\sigma_t=16.2$ to the surface. At night depth-stratified sampling was performed from the depth below oxycline with $15.8 < \sigma_t < 16.2$ to collect diapausing (i.e., nonmigrating) CV and in the surface layer to collect migrating *C. euxinus*. In the Marmara Sea in October 2000 zooplankton was sampled separately from the surface layers (25–0 m) formed by the Black Sea water and the strata lying under the halocline (75–25 m) consisting of the Aegean Sea water. The samples were preserved with 4% borax-buffered formaldehyde.

In the laboratory the whole sample was transferred to a Bogorov's chamber. Around 30–40 (up to 80) *C. euxinus* individuals of copepodite stages III–VI (CIII–CVI) were sorted at random from each sample. Then the copepods were stained for 24 h in a 1% Borax carmine

solution in 40 μm-filtered seawater with a concentration of 4% formaldehyde (Tande and Hopkins, 1981). This approach allowed us to examine each specimen for the following characteristics: prosome length, oil sac volume, size and state of gonad development and tooth formation inside the gnathobase.

Morphological examination of mandibular gnathobases was performed under a light microscope. The left mandible was dissected with needles, transferred to a drop of glycerine on a microscope slide, covered with a cover slip and examined at a magnification of 150×. The phases of moulting cycle were classified by Arashkevich et al. (2004) according to the tooth development suggested by Miller and Nielsen (1988), Miller et al. (1991). The following 5 phases were identified: postmoult, late postmoult, intermoult, early premoult and premoult.

The examination of gonads in adults females were determined according to Niehoff and Hirche (1996) and Niehoff and Runge (2003).

Body volume (V_b, mm^3) of copepodites was calculated as $V_b = 0.58 L_p\ d_p^2$, where L_p was the length of

Table 1
Sampling localities of collection of *Calanus euxinus* specimens examined in the study

| Stations | Date | Positions | | Depth strata | Total depth | H$_2$S depth |
		Lat. N	Long. E	(m)	(m)	(m)
4*	03.07.2000	41.35	29.45	160–90, 90–0	1850	132
5*	03.07.2000	41.25	29.45	170–120, 120–0, 70–0	1100	133
11*	03.07.2000	41.20	30.45	170–120, 120–0, 50–0	1400	152
31	06.07.2000	42.05	35.14	170–0	300	145
32	06.07.2000	42.01	35.15	75–0	75	No H$_2$S layer
45C	21.10.2000	40.46	29.00	100–25, 25–0	1200	No H$_2$S layer
K4	17.06.2001	41.50	31.15	135–0	1400	135
45C	24.06.2001	40.46	29.00	25–0	1200	No H$_2$S layer

*Night stations.

prosome, mm; d_p the maximal body width measured dorsally (Svetlichny et al., 1998). The oil sac volume (V_o) was determined as the ellipsoid volume: $V_o = \pi/6 L_o d_o^2$, where L_o and d_o were the length and maximal width (measured laterally) of oil sac, respectively.

Water temperature, salinity and in situ fluorescence were measured using a Sea Bird CTD probe with Chelsea™ fluorometers attached to it. Chlorophyll-*a* concentrations were determined in the layer of 0–75 m at different depths using standard fluorometric method (JGOFS Protocols, 1994).

3. Results and discussion

3.1. Characteristics of the development and lipid formation of C. euxinus in deep regions of the Black Sea

Analysis of oil sac volume of CIII–CVI in July 2000 showed that large lipid reserves were formed in CV (Fig. 2A). In comparison with CIV, the mean oil sac volume (OSV) in migrating CV increased dramatically
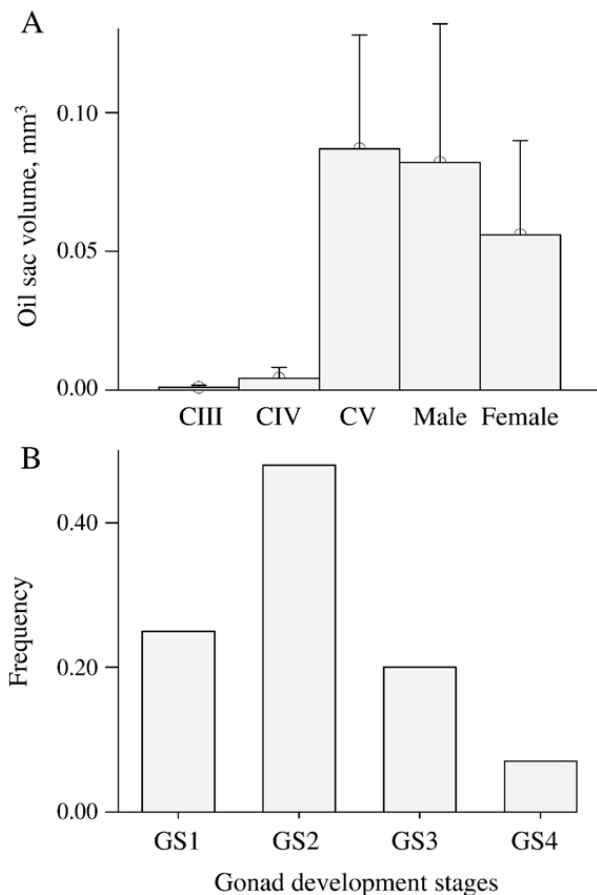

Fig. 2. Oil sac volume ($x \pm$ SD) in Stage III–V copepodites, males and females (A) and gonad development stages (GS) of females *Calanus euxinus* (B) from the deep stations in the south region of the Black Sea in July 2000.

17.4 times amounting to 0.087 ± 0.041 mm^3, or $14.1 \pm 6.0\%$ of body volume. In contrast to *C. euxinus*, in closely-related species from the North Sea total lipid proportion increased exponentially during the development of copepodites from I to V Stages (Kattner and Krause, 1987; Hygum et al., 2000). In CV of *C. euxinus* OSV varies greatly and maximum values of OSV are close to those in *C. finmarchicus* with the same body length (Miller et al., 2000). Adult lipids (0.082 ± 0.05 mm^3 in males and 0.056 ± 0.03 mm^3 in females) did not differ significantly compared to CV. Probably, these females had just moulted because most of them were with immature gonads (Fig. 2B).

In July 2000 migrating and diapausing CV were predominantly postmoults (Fig. 3A). However, postmoults dominate in deep zones of the Black Sea during all seasons (unpublished data). Gonads of copepods in this moulting phase were extremely small (Fig. 3B) or absent (especially in diapausing animals). There is good correlation ($r = 0.78$, $n = 278$) between the state of gonads and new teeth formation phase in CV of *C. euxinus*, just as in *C. finmarchicus* from the Norwegian shelf (Arashkevich et al., 2004). Oil sac volume (Fig. 3C) reached maximum values ($18.7 \pm 5.7\%$) in diapausing CV. Consequently, vigorous lipid accumulation in the sac took place in postmoult CV before entering diapause. Whilst OSV was less than 0.08 mm^3 in about a half of postmoult CV, in diapausing CV it accounted for 0.06–0.24 mm^3 (Fig. 4).

After accumulation of large lipid amounts, postmoult CV stop migrating, enter diapause and aggregate near lower boundary of OMZ. In July the share of CV remaining at depth during night varied from 13% to 25% of the total. In September 1998 non-migrating CV were also postmoults (our unpublished data). *C. finmarchicus* from depths below 500 m during the long period of overwintering (from October till March) were still in the postmoult phase with undeveloped gonads as well (Arashkevich et al., 2004). The termination of diapause in *Calanus* may be due to the progression in moulting process and gonadogenesis. According to Tande and Hopkins (1981), from February to April the overwintering Stage V stock of *C. finmarchicus* consisted of 90–100% of individuals with large advanced gonads.

We suggested that migrating late postmoults, intermoults and premoults collected from the sea developed from postmoults which had not entered diapause because the termination of diapause in *C. euxinus* CV in the middle of summer is unlikely. Similar developmental pattern without diapause was observed in *C. finmarchicus* on the Georges Bank in summer (Durbin et al., 2000). Although the share of copepodites V with
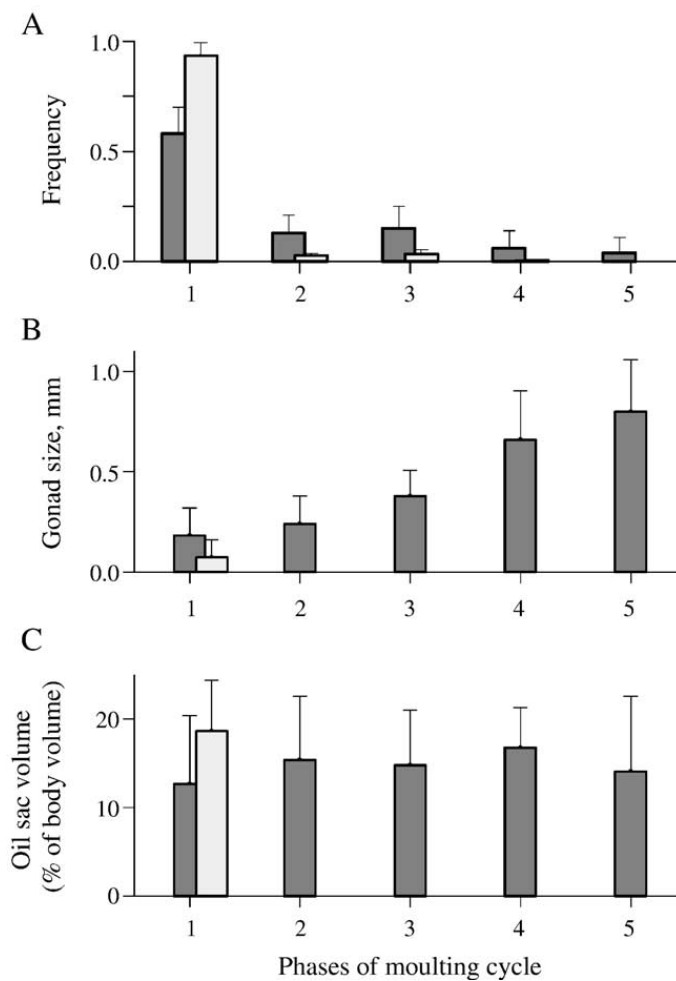
Fig. 3. Frequency distribution of moulting cycle phases (A), gonad size (B) and oil sac volume (C) in deep-living migrating (■) and diapausing (□) V copepodites of *Calanus euxinus* from the deep stations in the south region of the Black Sea ($x \pm$ SD).

small OSV increased during moulting, the main part of migrating premoults had such large lipid reserves as diapausing postmoults (Fig. 4). Probably, gonad formation in CV does not require high energy expenditure.

Irigoien (2004) suggests that lipids storage determines the overwintering depth when the buoyancy of *C. finmarchicus* is neutral. At 8 °C neutral buoyancy is likely to occur in *C. euxinus* CV with lipid reserves of 27% of body volume, or 23% of body weight (Svetlichny et al., 1998). These values are close to the results of the experiments with *C. finmarchicus* (Vesser and Jónasdóttir, 1999). However, Campbell (2004) reported that "something approximating neutral buoyancy is only attainable in a very narrow window of lipid compositions". In fact, in September–October 1996 (Svetlichny et al., 1998) and in July 2000 there was a significant number of individuals among diapausing CV of *C. euxinus* with lower (about 10%) and higher (up to 40%) lipid content and corresponding speeds of passive sinking (0.3 cm s^{-1}) and ascending (0.25 cm s^{-1}). Consequently, in order to stay at constant depth,

these copepodites have to make swimming movements. Direct observations from submersible (Flint, 1989) have shown that *C. euxinus*, being in «resting phase», really maintain significant activity. The ship-board experiments (Svetlichny et al., 1998) showed that diapausing CV at a minimal oxygen concentration (~0.2 mg O$_2$ l^{-1}) of lower boundary of their aggregation layer and temperature of 8 °C can support constant swimming by mouth appendages with the speed of 0.55 cm s^{-1}. That could be enough for choosing and maintaining the depth with optimal habitat conditions for diapausing CV of *C. euxinus*.

3.2. Lipid accumulation and moulting patterns in C. euxinus from shallow zones of the Black Sea

Our previous long-term studies concerning *C. euxinus* regional distribution in the Black Sea showed that late developmental stages rarely occurred during summer in shallow zones with the depth less than 100 m. However, in July 2000, at the station with the depth of
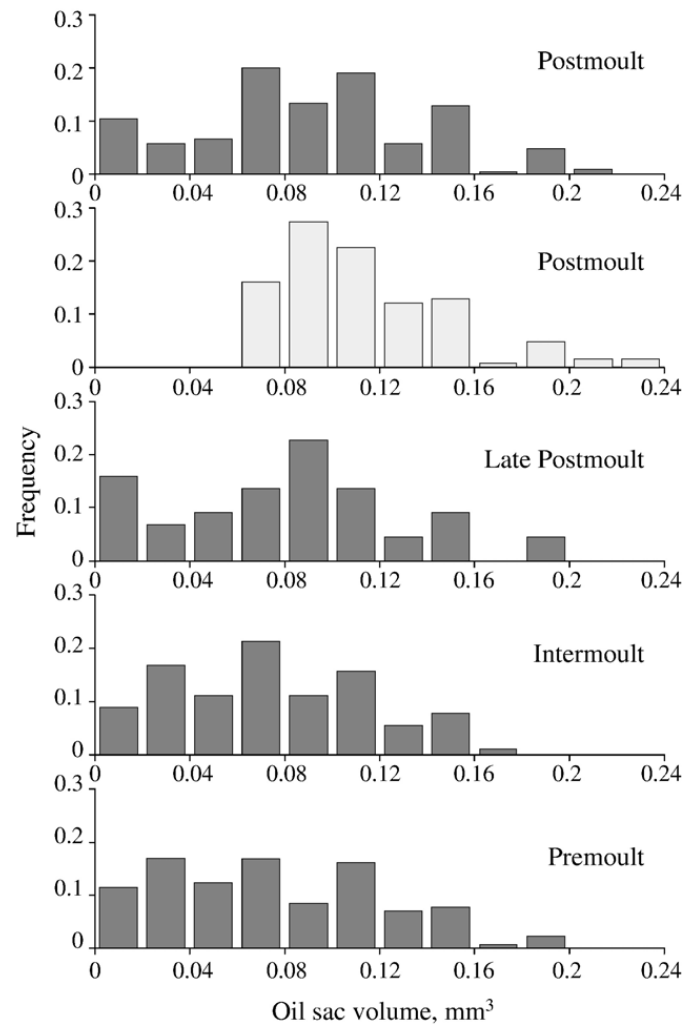
Fig. 4. Frequency distribution of oil sac volume in diapausing (□) and migrating (■) CV of *Calanus euxinus* at different moulting cycle phases.

75 m near Sinop Harbor, we found large aggregation of *C. euxinus* (Fig. 5A) where females were more abundant (2073.6 ind m^{-2}) than those of deep-sea (300 m) station (1160.5 ind m^{-2}). Probably, this *Calanus* aggregation in Sinop Harbor was formed due to an anticyclonic gyre branching off from the main Black Sea current. Vertical profiles of relative fluorescence obtained at these stations (Fig. 5B) indicated that phytoplankton concentration near the coast was very similar to that in offshore region. Nevertheless, oil sac volume of CV and females from shallow water was significantly lower than in deep water (Fig. 5C: 1.82 and 3.0 times lower, $p < 0.001$). Frequency distribution spectra of moulting phases in CIII from the two regions were similar, but intermoults and premoults dominated in CIV and especially in CV from shallow water (Fig. 5D). The same tendency, i.e., the increase in the share of intermoults and premoults of CV and reduction of their lipid content while approaching the coast, was observed near Sevastopol in September 2002 (Sve-

tlichny and Kideys, 2005). We hypothesize that hypoxia may inhibit the development of copepodites during postmoulting period and allows CV from deep regions to accumulate large amounts of lipids.

3.3. Lipid accumulation and moulting patterns of C. euxinus in the Marmara Sea

In the Marmara Sea two *Calanus* distinctive size groups were found in the samples collected near the Bosporus in October 2000 at the deep-sea (1200 m) station 45C (Fig. 6). In the first group (sampled from depths more than 25 m), the prosome lengths of CV and females were equal to 1.72 ± 0.22 and 2.02 ± 0.11 mm, respectively, being close to the corresponding values for *C. helgolandicus* from the Mediterranean Sea (Fleminger and Hulsemann, 1987). In the second one (collected from the near-surface layers), the prosome lengths corresponded to those in *C. euxinus* from the Black Sea (2.1 ± 0.05 mm for CV and 2.47 ± 0.09 mm for
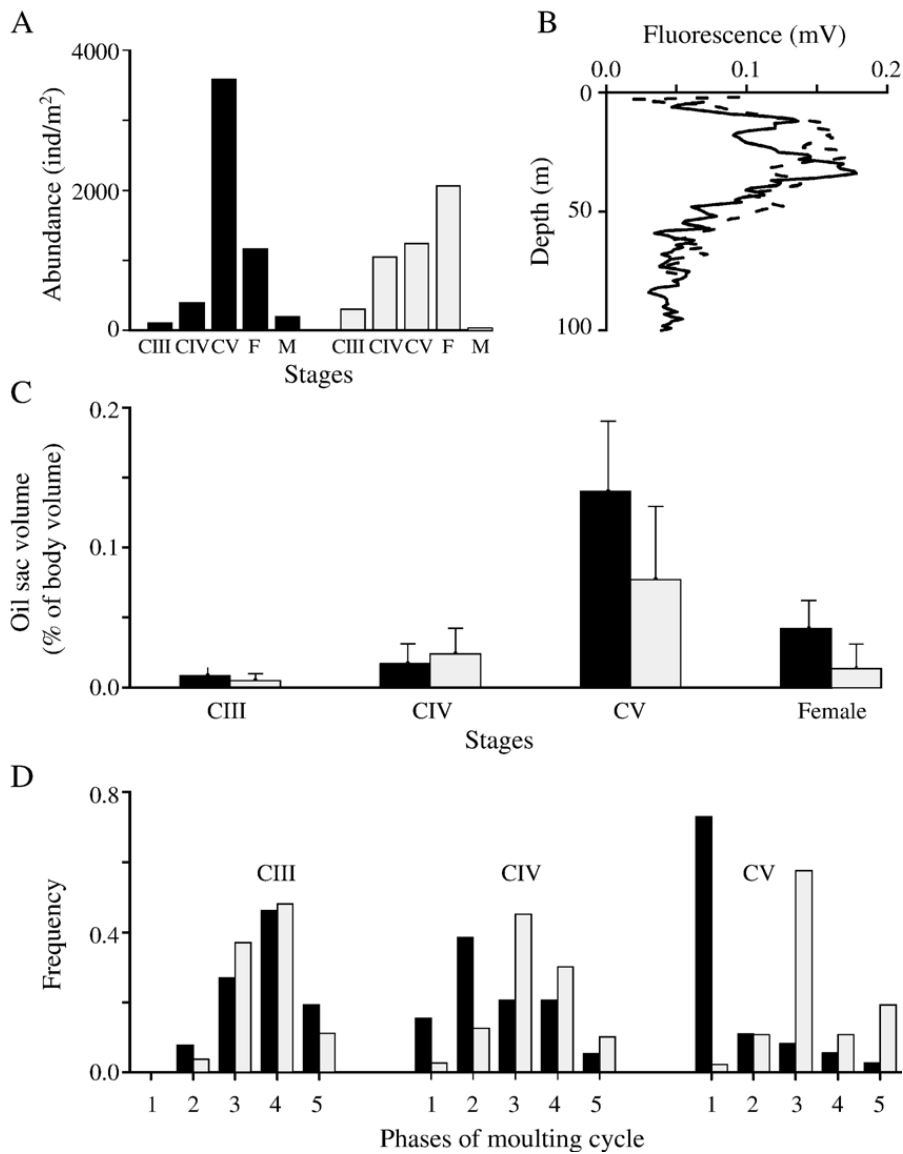
Fig. 5. Comparative characteristics of *Calanus euxinus* from deep (■) and shallow (□) zones in Sinop region. A: Abundance of III–V copepodites (CIII–CV), females (F) and males (M); B: Vertical profiles of relative fluorescence at deep (–) and shallow (---) stations; C: Oil sac volume; D: Frequency distribution of moulting cycle phases in III–V copepodites ($x \pm$ SD).
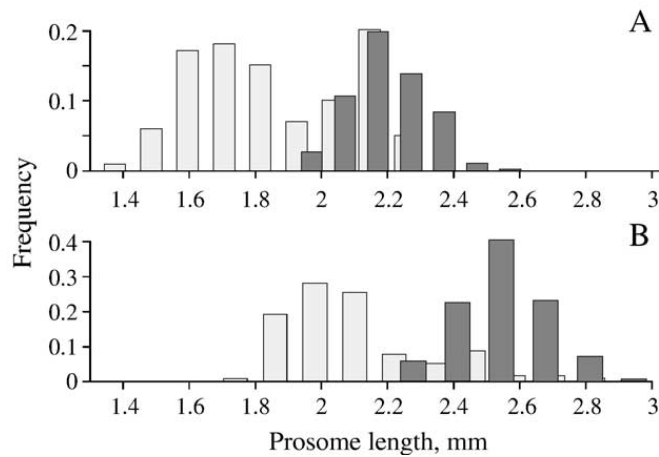

Fig. 6. Size–frequency diagrams for Stage V copepodites (A) and females (B) of *Calanus euxinus* from the Black Sea (■) and *Calanus sp.* from the Marmara Sea (□) in October 2000.

females). There were no essential morphological differences as between females and males from these two size groups in the Marmara Sea, as in comparison with Black Sea *C. euxinus*. The absence of any attributes of death (destructive changes of muscles and internal organs) in these individuals suggests successful development of *Calanus* in the Marmara Sea.

We also found two *Calanus* size groups in the samples collected in June 2001 at the station 45C in the Marmara Sea. Nevertheless, we used only big-sized individuals to be compared with *C. euxinus* near the Bosporus from the Black Sea. Our date showed that oil sac volumes of CIII developing in the surface layers are almost identical in both regions (Fig. 7A), although chl-*a* concentration in the Marmara Sea was two-fold higher (Fig. 7B). However, oil sac volumes of CIV, CV and females were 4, 17 and 7 times lower in the Marmara Sea than those in the Black Sea, respectively. In June 2001 *C. euxinus* CV subpopulation in the Marmara Sea predominantly consisted of intermoults and premoults (Fig. 7C). Consequently, our data revealed the same tendencies in moulting patterns and lipid accumulation of *C. euxinus* from the Marmara Sea and shallow zones of the Black Sea.

3.4. Energy model of C. euxinus growth

To estimate quantitatively the effect of hypoxia on lipid storage in *C. euxinus*, we developed a model of their diel growth (G, % of body energy content) using the equation: $G = (R - Q - F)100/W$, where R (cal mg C^{-1} day^{-1}) is carbon-specific ingestion rate according to the results of feeding experiments with *C. helgolandicus* (Huskin et al., 2000) assuming that both deep- and shallow-living copepods feed only at night during 11 h with identical ingestion rates; Q is mean carbon-specific daily energy losses (Svetlichny et al., 2000) calculated for deep-living *Calanus* ($Q_1 = 0.4$ cal mg C^{-1} day^{-1}) which pass through temperature (18–8 °C) and oxygen (10–0.8 mg O_2 l^{-1}) gradients during 2 h and spend 11 h under hypoxic conditions and for shallow-living *Calanus* ($Q_2 = 0.6$ cal mg C^{-1} day^{-1}) undergoing temperature changes only; F is the energy of undigested food being equal to 0.3 R (Conover, 1966); W is the body energy content assuming that carbon content in both V copepodites and females of *C. helgolandicus* (with prosome lengths similar to those in *C. euxinus*) constituted about 0.11 mg (Williams and Robins, 1982) and caloric value of 1 mg carbon is 9.4 cal.
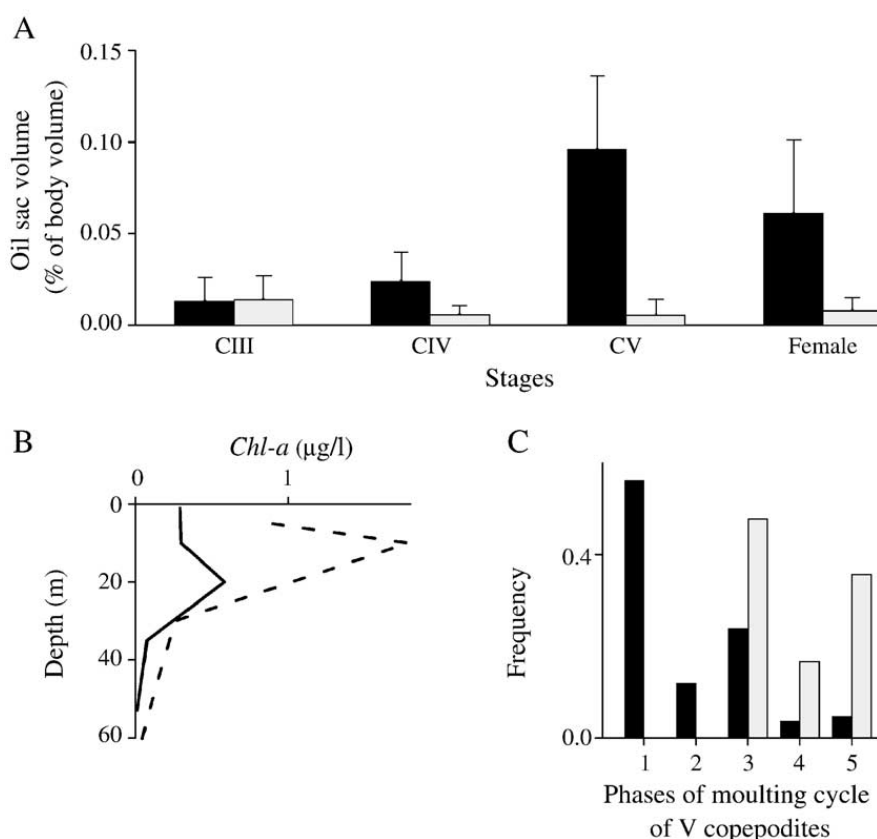

Fig. 7. Comparative characteristics of *Calanus euxinus* collected near the Bosporus from the Black Sea (■) and Marmara Sea (□) sides. A: Oil sac volume; B: Vertical profiles of chlorophyll-*a* concentration in the Black (–) and Marmara (- - -) seas; C: Frequency distribution of moulting cycle phases in V copepodites from the Black and Marmara seas ($x \pm$ SD).

Our calculation showed (Fig. 7) that the benefit of *C. euxinus* staying in hypoxic layers during daytime is insignificant when *R* exceeded 2 cal mg C^{-1} day^{-1}, or 21.3 μg C ind^{-1} day^{-1}. Such ingestion rates are determined experimentally in *C. helgolandicus* females at phytoplankton concentration more than ~300 μg C l^{-1} (Huskin et al., 2000). However, at scarce rations this advantage is more pronounced. For example, at daily ration of 1.0 cal mg C^{-1} day^{-1}, or 12 μg C ind^{-1} day^{-1} which corresponds to food concentration of about 30 μg C l^{-1} (Fig. 8), shallow-living *C. euxinus* cannot grow whilst the copepods migrating to oxygen-deficient zone are able to accumulate lipids up to 5% of body energy content per day. Similar growth rates (7.6%) were obtained for *C. helgolandicus* held in the laboratory at 15 °C and food concentration of 77.5 μg C l^{-1} (Rey-Rassat et al., 2002). Only at high food concentrations (about 300 μg C l^{-1}) growth rate of late copepodite stages may rise to 13% (Mauchline, 1998) and even to 18% (Rey-Rassat et al., 2002).

Thus, the results of our field observations and application of our model circumstantially support our hypothesis that diel vertical migrations to hypoxic
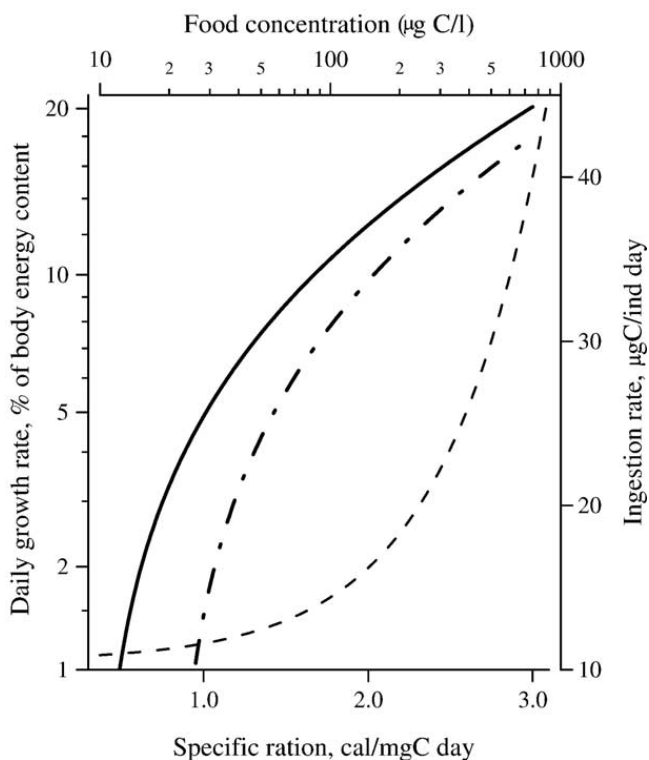

Fig. 8. Energy model of growth for preadult and adult *Calanus euxinus* from deep (—) and shallow (– • –) regions. The relationship between daily ration and food concentration for *Calanus helgolandicus* (---) is based on Huskin et al. (2000, see Table 3, ingestion rates at *Prorocentrum micans* concentrations of 854 and 220 μg C l^{-1}).

layers are essential for lipid reserve formation in *C. euxinus* in the Black Sea.

However, the effect of hypoxia results not only in the decrease of energy expenditure but in changing of metabolic pathways. According to our data (Svetlichny et al., 1998), under conditions of short-term acute hypoxia atomic O:N ratio of *C. euxinus* reduces from about 20 to 8.4 indicating the transition from mixed protein–lipid catabolism to pure protein utilization. At low oxygen concentrations lipid catabolism is limited and protein becomes the main metabolic substrate. This may suppress the somatic growth and increase the efficiency of wax esters accumulation. The biochemical aspects of wax ester formation under hypoxic conditions have been analyzed by Sargent (1978).

The question we address here is how *Calanus* species living in well-oxygenated water can accumulate lipids. Lipid reserve formation in *Calanus* species is a highly specialized adaptation for life at high latitudes with short and sporadic periods of food availability (Marshall and Orr, 1972). The four generations of *C. helgolandicus* off Plymouth described by Rey-Rassat et al. (2004) were produced either during or following a chlorophyll peak of 4–6 μg l^{-1} corresponding to food concentration of about 200–300 μg C l^{-1}. *C. helgolandicus* off NW Spain showed a strong link between spring phytoplankton blooms (with maximum chlorophyll-*a* concentration up to 7.6 μg l^{-1}) and reproduction time (Ceballos et al., 2004). According to Harris et al. (2000), during changing of food concentration from 61 to 238 μg C l^{-1} the ration of *C. helgolandicus* CV increased from 16 to 27 μg C ind^{-1} d^{-1}. At chlorophyll-*a* concentration of <3 μg l^{-1} *C. helgolandicus* and *C. finmarchicus* sharply decreased the rates of egg production. Campbell et al. (2001) observed a strong effect of food limitation on lipid storage, the critical concentrations for development and growth in *C. finmarchicus* were about 70 and 100 μg C l^{-1}, respectively. Minimum food concentration when there was little visual evidence of substantial lipid storage in *C. finmarchicus* CV was found to be 24 μg C l^{-1}, or ~0.5 μg chl *a* l^{-1}. Rey-Rassat et al. (2002) reported approximately two-fold difference in OSV in *C. helgolandicus* from mesocosms with food concentrations of 77.5 and 278 μg C l^{-1}. Hygum et al. (2000) studied the patterns of structural growth with the production of lipid for storage by *C. finmarchicus* copepodites reared in mesocosms at different natural phytoplankton concentrations in a Norwegian fjord. The total storage lipid content in CV from mesocosms with low (0.62–0.88 μg chl *a* l^{-1}) and high (1.59–2.68 μg chl *a* l^{-1}) resources varied between 28.9–60.2 and 69.9–77.0 μg, respectively. Hygum et al. (2000) considered these

maximum lipids content to be an upper threshold for the amount of lipid that can be deposited. However, the Black Sea migrating and diapausing CV of *C. euxinus* even at low algae concentrations (0.29–0.68 μg chl *a* l^{-1}) have total lipid content of 36.3–115 and 112.6–196.6 μg, respectively (Yuneva et al., 1997).

4. Conclusion

After penetrating into the Black Sea, *C. euxinus* could optimize their life cycle strategy using the unique temperature and oxygen concentration gradients in the deep zones during warm seasons.

Due to diel vertical migrations to cold hypoxic layers, the Black Sea *C. euxinus* decrease mean daily energy expenditure and accumulate lipids at low phytoplankton concentration.

In late copepodite stages affected by low temperature and oxygen concentration, the development is inhibited and the duration of growth increases resulting in upsizing and formation of large lipid reserves in *Calanus* body. Extremely intensive lipid accumulation takes place during early developmental period in post-moult CV.

Acknowledgements

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