Development of *Calanus euxinus* during spring cold homothermy in the Black Sea

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ABSTRACT: In copepodites and adults of *Calanus euxinus* abundance, body length and weight, chemical composition and respiration rate in relation to age were studied during the cruise of R/V 'Knorr' in April 2003 in the southwestern Black Sea and in laboratory experiments. Data on morphology and physiology of *C. euxinus* collected during other seasons were also used in comparative analyses. At high concentrations of the diatom *Proboscia alata* during spring homothermy (6.8 to 8.5°C) *C. euxinus* did not undertake diel vertical migrations to the oxygen minimum zone (OMZ). The majority of the population was located above the OMZ, feeding on phytoplankton during nighttime and daytime whilst nearly 10% of copepodite stages IV and V (CIV and CV) and adults remained constantly in the OMZ. Although a diapausing stock of the population began to form in the OMZ, the majority of CV developed without diapause. The development times of copepodite stages at 8°C were determined on the basis of field observations of ontogenetic changes in carbon content and calculated absolute growth rates. Development time from CI to CV amounted to 22 d. In CV the time for lipid deposition from 0.008 ± 0.007 mm^3 in postmolts up to a maximum volume of 0.165 ± 0.054 mm^3 in intermolts constituted 26 d. The total generation time from eggs to adults is not less than about 66 d.

KEY WORDS: *Calanus euxinus* · Lipid storage · Respiration · Growth · Black Sea · Spring cold · Homothermy

INTRODUCTION

The study of morphological and physiological diversity among populations of one species is necessary for understanding the processes of marine speciation and intraspecific adaptation strategies of the genus *Calanus*. *Calanus* in the Black Sea was first identified by Karavaev (1894) and named *Calanus finmarchicus* var. *pontica*. Jaschnov (1955) pointed out the separate status of *C. finmarchicus* and *C. helgolandicus*, and distinguished the Black Sea population as a subspecies, namely *Calanus helgolandicus* var. *ponticus*. Later, Fleminger & Hulsemann (1987) proposed giving the Black Sea population the rank of separate species and the name of *Calanus ponticus* Karavaev. Finally, a new name *Calanus euxinus* was given to this species as a re-

placement name for *C. ponticus* (Hulsemann 1991). However, Papadopoulos et al. (2005) and Unal et al. (2006) showed that genetic differences between *C. euxinus* and *C. helgolandicus* are extremely subtle and typical for conspecific populations.

Calanus helgolandicus inhabit mainly neritic waters and have an extremely wide geographical distribution range from the eastern North Atlantic and the North Sea southwards to the Mediterranean basin (Fleminger & Hulsemann 1987).

Fleminger & Hulsemann (1987) consider the Black Sea *Calanus* to be a phenetic population of *C. helgolandicus* which penetrated into the Black Sea from the Mediterranean Sea 7000 yr ago after the Dardanelles and the Bosphorus straits formed. According to an-

other hypothesis, *Calanus*, like other 'boreal relicts', entered the Black Sea when it was connected with the North Sea across Europe during the period of retreating glaciers (Zaitsev 2006). This is in agreement with the results of Papadopoulos et al. (2005) suggesting that the divergences between the northeastern Atlantic, Mediterranean and Black Sea *C. helgolandicus* populations are much older than the estimated dates of colonization of the Mediterranean and Black Seas. However, according to Polischuk (1984) the last connection between the North and Black Seas could have been in the Late Holocene (2800 to 2700 BP).

Calanus euxinus is a dominant component of the zooplankton biomass in the Rim Current system of the Black Sea. The ecology of this species differs significantly from that of *C. helgolandicus* in other European seas due to the characteristics of the basin.

Calanus helgolandicus typically inhabits saline waters (32 to 39‰) with temperatures of 9 to 20°C. This wide range is mainly due to seasonal variation; the temperature does not range widely between populations. Seasonal changes in abundance of *C. helgolandicus* are positively correlated with temperature (Bonnet et al. 2005) and food availability (Rey-Rassat et al. 2004). Overwintering *C. helgolandicus* descend to deep water and are separated spatially from the part of the population that develops in surface layers.

In contrast, *Calanus euxinus* has adapted to the low salinity of the Black Sea waters (18 to 20‰). Preadults and adults performing diel vertical migrations within the aerobic layer (0 to 150 m) to the oxygen minimum zone (OMZ) undergo temperature changes within the range 6.5 to 22°C during warm seasons (Besiktepe et al. 1998, Besiktepe et al. 2005) but in winter and spring live at 5 to 8.5°C. In deep waters the abundance of *C. euxinus* does not differ significantly between warm and cold seasons (Sazhina 1987). During the daytime, the migrating part of the population ascends to depths close to the layers where diapausing *Calanus* live, probably mixing with them. Prosome length of females from the Black Sea population is similar to (or may even exceed) the maximum prosome length of *C. helgolandicus* (2.6 mm) in the North Sea (Bonnet et al. 2005).

During summer and autumn, temperature stratification and low phytoplankton concentration in the water column cause late development stages of *C. euxinus* to reduce energy expenditure 7.2-fold through diel vertical migration from warm upper layers to the cold OMZ, thus using the energy of food consumed at night near the surface more efficiently (Svetlichny et al. 1998, 2006, Yuneva et al. 1999). Under hypoxic conditions during the daytime the catabolism of lipids is depressed whilst the synthesis of fatty alcohols from non-lipid components in the oil sac may be facilitated (Sargent & McIntosh 1974).

Previous studies have focused on the life cycle, feeding and migration behavior (Petipa 1981, Vinogradov et al. 1992a,b), duration of development (Sazhina 1987), gonad maturation, lipid deposition and molting patterns (Arashkevich et al. 1998), growth, grazing and egg production rates (Sazhina 1987, Besiktepe & Telli 2004, Besiktepe 2001), and lipid content and composition as indicators of nutritional condition of *C. euxinus* (Yuneva et al. 1997, 1999). However, this research has mostly been conducted in warm seasons and very few studies (e.g. Sazhina 1987, Vinogradov et al. 1992c) have investigated the development of *C. euxinus* during cold seasons. Among these studies, the research of Sazhina (1987) was a laboratory study and Vinogradov et al. (1992c) did not present sufficient details of the species development.

The present study analyzed the *Calanus euxinus* population age structure, abundance, dynamics of prosome length (L_p), body (V_b) and oil sac (V_s) volumes, molt phase patterns (in copepodite stages III to V [CIII to CV] only), defatted dry weight (DFDW), total lipid (TL) and wax ester (WE) concentrations, and changes in respiration rate during the cold period of early spring homothermy. These results were compared to morphological and physiological data for *C. euxinus* collected in the summer to autumn water stratification period. On the basis of data obtained in the present study and data from previous studies, we estimated growth rate and development time for copepodite stages of *C. euxinus* at a constant temperature of 8°C and tested the consistency of calculated absolute growth rates with metabolic rates.

MATERIALS AND METHODS

Zooplankton samples were collected with a Nansen closing net (opening diameter 71 cm, mesh size 112 µm) during a cruise of the R/V 'Knorr' between 17 and 23 April 2003 at 3 deep stations in the southwestern Black Sea (Fig. 1). One station was located in the cyclonic zone with a depth of 2160 m (Stn 5, 17 April, 01:30 h) and 2 stations were in the anticyclonic region with depths of 845 m (Stn 7, 18 April, 18:50 h) and 900 m (Stn 17, 22 April, 22:15 h). At all stations zooplankton was sampled by vertical hauls from the depth of the upper border of the anoxic zone with σ_t = 16.2 (86, 191 and 185 m for Stns 5, 7 and 17, respectively) to the surface. At Stn 7 (18:30 h) and Stn 17 (22 April, 22:30 h, and 23 April, 11:30 h, respectively) additional tows were made in the OMZ with $15.4 < \sigma_t < 16.2$. Further tows were made in the upper horizon of 0 to 60 m at Stns 5 and 17 during the daytime. The filtered volume was calculated from the wire length and the opening area of the net. Water temperature, salinity
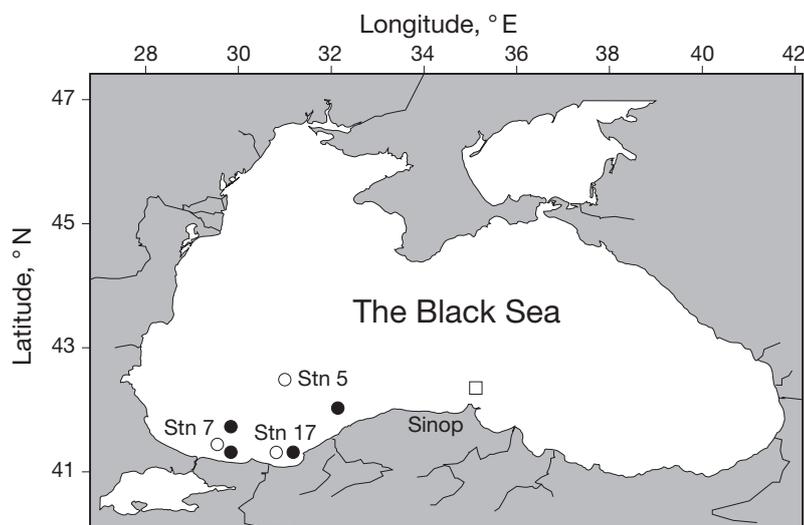
Fig. 1. Location of sampling stations in the Black Sea during the cruise (16 to 22 April 2003) of the R/V 'Knorr' (○), during the cruises (July 2000 and June 2001) of R/V 'Bilim' (●), and the monitoring station near Sinop in 1999 (□)

and *in situ* fluorescence were measured using a Sea Bird CTD probe with attached Chelsea™ fluorometers.

Additional zooplankton samples were collected by vertical hauls from 180 m to the surface at the monitoring station near Sinop in January, February, March, May, September and November 1999. Other samples obtained in the southwestern Black Sea during the cruises of the R/V 'Bilim' in July 2000 and June 2001, using the same methodology, were also utilized for comparative analyses.

The samples were immediately preserved with 4% borax-buffered formaldehyde. In the laboratory each sample was divided into several portions and then the specimens of each copepodite stage of *Calanus euxinus* were counted using a Bogorov chamber under a dissecting microscope. When possible, 30 to 40 (up to 187 from Stn 7) individuals of each copepodite stage or adults were selected and measured for body size and oil sac volume, and for jaw phase identification (in CIII to CV only) according to tooth formation inside the gnathobases of mandibles.

After measuring body size and oil sac volume, the mandibles were dissected from the whole copepodite in a drop of 1:1 glycerol:water solution and 5 phases of moulting cycle (postmolt, PO; late postmolt, LPO; intermolt, IN; early premolt, EPR; and premolt, PR) were determined according to tooth development (Marker et al. 2003, Johnson 2004). In the case of insufficient numbers of representatives of particular jaw phases the data were combined for phases PO and LPO and for EPR and PR.

Length and width of prosome (L_p and D_p [mm] and oil sack (L_s and D_s [mm]) were measured to the nearest 10 μm under a dissecting microscope fitted with an eyepiece micrometer.

V_b (mm^3) was calculated as $V_b = kL_p D_p^2$, where k is the empiric coefficient of 0.64 in males and 0.58 in females and copepodites (Svetlichny 1983). V_s (mm^3) was determined as the ellipsoid volume: $V_s = \pi/6 L_s D_s^2$.

Wet weight (WW) of *Calanus euxinus* copepodites and females was calculated as WW = V_b ρ, where ρ (g cm^{-3}) is the average body density determined using the equation ρ = 1.06 − 0.0016SV$_s$ (Svetlichny et al. 1998), where SV$_s$ (%) is the proportion of the oil sac in body volume.

To determine lipid content, copepodites and adults were selected and sorted on board the ship. Batches of individuals (50 to 100 ind. for CI to CIII, 30 to 50 for CIV, 15 to 25 for CV, and 5 to 10 for adults) were transferred into glass vials with screw caps containing 5 ml of chloroform/methanol (2:1 by volume) where 0.01% butylated hydroxytoluene was added as antioxidant (Folch et al. 1957) and extracted twice for 24 h without preliminary homogenization (Ohman 1988, Håkanson 1989). Between analytical procedures the resulting extracts were stored for about 2 wk at −20ºC and then used for further qualitative and quantitative lipid content determination in the laboratory. The sulfovanillin technique (Barnes & Blackstock 1973) was applied to define the content of TL in extracts. Lipids extracted from *Calanus euxinus* CV, dried under vacuum and weighed on a Cahn electrobalance according to Kates (1975), were used as a standard for calibration.

The lipids were fractionated by unidimensional thin-layer chromatography on 5 × 15 cm Silufoll UW254 plates (Kavalier) using solvents of different polarity. A chromatography chamber was used in which 3 cylindrical chambers of different sizes were nested (Yuneva et al. 1999). The outer chamber (diameter 15 cm, height 21 cm) was saturated with hexane for 2 h prior to fractionation, the intermediate chamber (diameter 7 cm, height 13.5 cm) was saturated with hexane/diethyl ether (9:1 v/v) mixture, while the innermost chamber (diameter 5.5 cm, height 1.0 cm) contained chloroform. The plates were activated at 105°C for 30 min and treated with a 10% solution of phosphomolybdenic acid in ethanol before the chromatography procedure. A 20 to 50 μg sample of lipid was applied to each plate under nitrogen. The chambers were quickly assembled concentrically each other and a plate was immediately put into the innermost chamber. Thus, the plate was developed in solvents with decreasing polarity for 10 min. Chromatograms were visualized by heating at 110°C for 7 min and quantified densitometrically using an ERS densitometer (Karl Zeiss). Lipid classes (phos-

pholipids, sterol, free fatty acids and triacylglycerols; the results concerning these lipid classes are not included in the present paper) and WE were identified against a suite of lipid standards (Sigma Chemical). Contents of TL and WE were given as µg ind.$^{-1}$.

After completion of lipid extraction the copepods were removed from the chloroform/methanol mixture, dried at 60°C to constant value and weighed on a balance to determine defatted dry weight (DFDW). Of the defatted individuals, 20 to 30 from stages CI to CIII and 3 to 5 (up to 10) ind. of each later stage were used for carbon content measuring with an EA 1110 CHNS analyser (CE Instruments).

Respiration rate was determined at 20°C in CI, CIV and adults of *Calanus euxinus* from Stn 7 (after 1 d acclimation to 20°C), and at 8°C in CV and female adults sampled near Sevastopol in April 2003. The data on respiration rates at 20°C of *C. euxinus* from CII to adult females captured near Sevastopol in October 2002, September and October 2003 were used for the comparative analysis.

Respiration rate (R, µg O$_2$ ind.$^{-1}$ h^{-1}) of copepods was determined using experimental and control syringes filled with 2.0 ml of natural seawater. One adult female or CV (or 4 to 10 ind. CI to IV) was transferred by a pipette into each experimental syringe, which had a protective sieve disc (mesh size 200 µm) covering the outlet. To obtain identical initial oxygen and seston content in control and experimental syringes, we connected one of each with a plastic tube and pumped the water through them both simultaneously several times. Then the syringes were separated, closed by stoppers and placed in dark chambers at 20 ± 0.5°C or 8 ± 0.5°C. Incubation periods were 1 h for adults and preadults and up to 2 h for CI to CII.

Oxygen concentration was measured using a polarographic membrane oxygen sensor OCE-057 (Marine Hydrophysical Institute, National Academy of Sciences of Ukraine) joined to a chamber (all-glass syringe), 0.5 ml in volume and containing a magnetic stirrer (Svetlichny & Hubareva 2005). The water sample from the experimental or control syringe was transferred through the needle to the chamber in 6 portions. A further 4 portions were used to calculate the average oxygen concentration.

Statistical evaluation of the data was conducted by 1-way ANOVAs. Values presented in figures and tables are means ± SD. Relationships between any 2 variables were examined using least squares linear regression with Grapher® for Windows (v.2.00).

RESULTS

Field observations

In April 2003 the temperature of the aerobic layer was 6.8 to 8.5°C indicating a state of early spring homothermy in the Black Sea (Fig. 2). The lower boundary of the aerobic layer was located at 87 m in the cyclonic region (Stn 5) and 176 to 185 m in the anticyclonic regions (Stns 7 and 17). Oxygen-deficient waters with <1 ml O$_2$ l^{-1} were deeper than 60 and 140 to 150 m in the cyclonic and anticyclonic regions, respectively. Fluorescence maxima were found at depths of 38 m (Stn 5) and 60 to 70 m (Stns 7 and 17). In these samples and within the OMZ we found a large number of alga *Proboscia alata*.

Total abundance of copepodites and adults of *Calanus euxinus* in the cyclonic zone region (Stn 5) was 14 292 ind. m^{-2} and the biomass constituted 3.99 g m^{-2}. In the anticyclonic regions their abundance amounted to 12 202 and 13 088 ind. m^{-2}, or 5.7 and 7.3 g m^{-2} for Stns 7 and 17, respectively.

The age structures of *Calanus euxinus* populations in these 2 regions were different (Table 1). At Stn 5 early copepodite stages (CI to CIII) amounted to 76.1% of the total abundance (excluding nauplii). However, at Stns 7 and 17 preadults and adults (67.6 and 73.3% of the total abundance, respectively) predominated, with CV constituting 50 and 63%, respectively. All early copepodite stages and the majority of preadults and adults were found above the OMZ at the anticyclonic Stn 17 both during the day- and nighttime. Within the OMZ at 11:30 h only 3% of all CVs, 8% of all adult females and 63% of all adult males were found with their guts filled with green paste and 9.3% of all CVs had empty guts. All copepodites and adults collected at Stn 17 during the day-
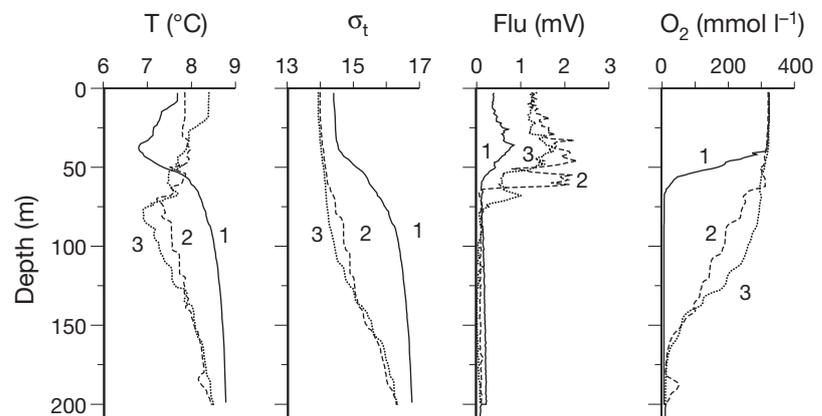
Fig. 2. Vertical profiles of temperature (T,°C), density (σ$_t$), chl *a in situ* fluorescence (Flu, mV) and oxygen concentration (O$_2$, mmol l^{-1}) from 1: cyclonic Stn 5 (17 April 2003), 2: anticyclonic Stns 7 (18 April 2003), and 3: 17 (22 April 2003)

Table 1. *Calanus euxinus*. Abundance (No.; ind. m^{-2}) and proportion (% of total abundance) of copepodite stages (CI to CV), adult females (CVIF) and adult males (CVIM) in April 2003

Stage	Stn 5 90–0 m, 01:30 h		Stn 7 200–140 m, 18:30 h		200–0 m, 8:50 h		Stn 17 190–0 m, 22:15 h		190–120 m, 22:30 h		190–120 m, 11:30 h	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
CI	3684	25.8	0	0	660	5.4	486	3.7	0	0	0	0
CII	4048	28.3	0	0	1204	9.9	712	5.4	0	0	0	0
CIII	3151	22.0	0	0	2096	17.2	2291	17.5	5	0.4	21	1.8
CIV	1427	10.0	0	0	1934	15.9	1781	13.6	57	4.6	166	14.2
CV	684	4.8	650	86.2	4157	34.1	6081	46.5	861	68.8	712	60.8
CVIF	1113	7.8	57	7.6	1318	10.7	1482	11.3	166	13.3	112	9.5
CVIM	185	1.3	47	6.2	832	6.8	255	2.0	161	12.9	161	13.7

time between depths 0 to 60 m were found to have food in their guts.

For CIV all postmolts (PO + LPO), intermolts and all premolts (EPR + PR) were found in similar proportions (39, 25 and 36%, respectively). However, among CIVs collected within the OMZ, all premolts dominated (more than 90% of total number) (Fig. 3A). In contrast, POs and INs predominated among CVs found above and within the OMZ (Fig. 3B).

Size–weight characteristics of *Calanus euxinus*

In April 2003 L_p ranged from 0.69 ± 0.03 mm in CI to 2.65 ± 0.09 mm in adult females and 2.53 ± 0.1 mm in adult males. We did not find significant variation in L_p within the molting cycle of each copepodite stage at Stn 7 (Table 2). However, V_b increased by 28% (p < 0.05) in CIII and CIV from POs to PRs and by 25% in CV from POs to INs (Fig. 4); increases were due to multiple (9.4 times in CIII, 50 times in CIV and close to 16 times in CV) increments of oil sac volume (V_s) during the molting period of these stages. In CV we found significant (p < 0.001) increases in DFDW from 63 ± 3.5 mg for POs to 127 ± 3.2 and 112 ± 5.7 mg for INs and PRs, respectively.

At Stn 7, the frequency distribution of V_s in CV was bimodal above and within the OMZ, with maxima at 0 to 4% and 18 to 26% of V_b (Fig. 5). CVs aggregating above the OMZ (85% of CV total number) were mainly POs with small lipid sacs (<8% of V_b) whilst about half of LPOs and the majority of INs and PRs contained oil sacs larger than 16% of V_b.

CVs from the OMZ (15% of CV total number) predominantly possessed large oil sacs. Among them the POs with empty guts (probably, diapausing individuals) were extremely lipid-rich (up to 30% of V_b). Mean V_s in these CV POs (0.151 ± 0.039 mm^3) did not differ significantly from V_s in CV LPOs and INs (Table 2).
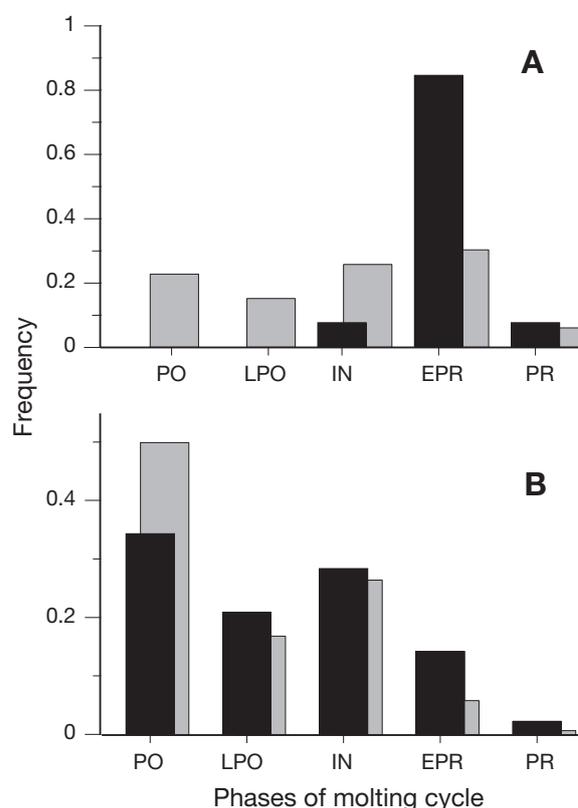
Fig. 3. *Calanus euxinus*. Jaw phase frequency distribution of copepodite stages: (A) CIV and (B) CV in total (▨) and within OMZ (■) hauls at Stns 7 (18 April 2003) and 17 (22 April 2003). PO: postmolt; LPO: late postmolt; IN: intermolt; EPR: early premolt; PR: premolt

Mean values of V_b, DFDW and dry weight (DW, mg) determined as a sum of DFDW and TL in *Calanus euxinus* (Table 2), significantly (p < 0.001) correlated with L_p following the power equations $V_b = 0.057L_p^{2.83}$ ($r^2 = 0.98$), DFDW = $0.0057L_p^{3.42}$ ($r^2 = 0.99$) and DW = $0.0082L_p^{3.45}$ ($r^2 = 0.99$), respectively. DFDW in CIs to adults increased proportionally with V_b as DFDW =

Table 2. *Calanus euxinus.* Mean ± SD values of prosome length (L_p, mm), body volume (V_b, mm³), defatted dry weight (DFDW, µg), carbon content as a percentage of defatted dry weight (C, %), oil sac volume (V_s, mm³), total lipid content (TL, µg), wax ester content (WE, µg) and dry weight (DW, µg) in April 2003. F: females; M: males; PO: postmolt; POD: diapausing postmolt: LPO: late postmolt; IN: intermoult; EPR: early premolt; PR: premolt, n: number of individuals used for measuring L_p, V_b and V_s. Blank cells: no data

Stn	Stage	Jaw phase	n	L_p	V_b	DFDW	C	V_s	TL	WE	DW
5	CV	All	34	2.32 ± 0.10	0.626 ± 0.116	111.5		92.9 ± 74.3	98.4	76.26	209.9
	CVIF	All	24	2.62 ± 0.09	0.909 ± 0.098	153.3		65.3 ± 65.6	49.7	32.84	203.0
	CVIM	All	19	2.53 ± 0.14	0.827 ± 0.138	138.6		100.7 ± 62.6	70.8	57.44	209.4
7	CI	All	16	0.69 ± 0.03	0.025 ± 0.002	1.5		0	0.8	0	2.3
	CII	All	20	0.95 ± 0.04	0.064 ± 0.005	5.1	51.7	0	2.4	0	7.5
	CIII	PO+LPO	11	1.28 ± 0.03	0.093 ± 0.008			0.06 ± 0.05			
		IN	13	1.31 ± 0.03	0.103 ± 0.015			0.39 ± 1.00			
		EPR+PR	9	1.35 ± 0.06	0.117 ± 0.013			0.55 ± 0.41			
	CIII	Total	33	1.31 ± 0.04	0.103 ± 0.012	14.6 ± 7.1	37.9 ± 9.3	0.33 ± 0.48	3.2	0.96	17.8
	CIV	PO	11	1.75 ± 0.09	0.215 ± 0.030			0.19 ± 0.30			
		LPO	8	1.78 ± 0.08	0.219 ± 0.035			0.29 ± 0.22			
		IN	12	1.78 ± 0.05	0.262 ± 0.030			5.30 ± 3.60			
		EPR	10	1.76 ± 0.03	0.293 ± 0.030			9.76 ± 8.00			
		PR	9	1.82 ± 0,03	0.275 ± 0.013			9.50 ± 6.00			
	CIV	All	50	1.77 ± 0.07	0.245 ± 0.038	35.9 ± 2.9	34.9 ± 1.0	4.34 ± 2.24	9.8	5.40	45.7
	CV	PO	67	2.26 ± 0.06	0.529 ± 0.058	63.0 ± 3.5	31.0 ± 6.9	8.00 ± 7.00			
		POD	18	2.36 ± 0.10	0.740 ± 0.130	106.0 ± 11.4	43.3 ± 1.8	151.0 ± 39.0	172.0	129.79	278.0
		LPO	29	2.29 ± 0.08	0.641 ± 0.110			122.0 ± 64.0			
		IN	45	2.33 ± 0.11	0.714 ± 0.108	127.3 ± 3.2	42.7 ± 0.4	165.0 ± 54.0			
		EPR	19	2.28 ± 0.10	0.653 ± 0.120	112.8 ± 5.7	41.5 ± 0.7	126.0 ± 40.0			
		PR	9	2.21 ± 0.17	0.660 ± 0.160			120.0 ± 53.0			
	CV	All	187	2.30 ± 0.09	0.652 ± 0.122	90.8 ± 9.0	41.9 ± 8.1	92.0 ± 79.0	50.6	38.42	141.4
	CVIF	All	28	2.67 ± 0.06	0.953 ± 0.110	147.7	42.9 ± 0.5	65.0 ± 46.0	56.8	38.76	204.5
	CVIM	All	13	2.52 ± 0.08	0.776 ± 0.093	129.4	53.2 ± 5.9	95.0 ± 60.0	83.4	64.40	212.8
17	CV	All	44	2.29 ± 0.11	0.603 ± 0.128	102.9		81.5 ± 75.8	76.2	60.96	179.1
		POD	14	2.25 ± 0.10	0.641 ± 0.127	107.1		116.8 ± 66.6	142.9	111.90	250.0
	CVIF	All	29	2.67 ± 0.10	0.950 ± 0.152	164.1		43.5 ± 50.8	44.2	29.02	208.3
	CVIM	All	17	2.53 ± 0.08	0.820 ± 0.016	122.0		131.8 ± 95.2	91.7	77.85	213.7

−0.0063 + 0.17 V_b (r² = 0.97). In diapausing CV POs DFDW was 1.7 higher than that of CV just after ecdysis indicating that lipid accumulation in POs was accompanied by somatic growth.

Mean values of TL and WE correlated with V_s (Fig. 6) as TL = 0.00503 + 0.83 V_s (r² = 0.99) and WE = 0.00087 + 0.58 V_s (r² = 0.99) in CIII to CV with V_s < 0.065 mm³ whilst in CVs and adults with V_s > 0.065 mm³ TL = −0.0271 + 1.32 V_s (r² = 0.95) and WE = −0.0264 + 1.062 V_s (r² = 0.94).

Respiration rate

Respiration rate (R, µg O_2 ind.$^{-1}$ h^{-1}) was determined in *Calanus euxinus* from CI to adult females collected in the southeastern Black Sea in April 2003 during shipboard experiments at 20°C and in the laboratory at 8°C in CV and adult females collected near Sevastopol. Also, we studied respiration rate in *C. euxinus* copepodites and females sampled in October 2002 and in

September to October 2003 near Sevastopol at 20°C. Before the experiments individuals were kept for 1 d in seawater with a mixture of algae *Prorocentrum micans* and *Thalassiosira weisflogii* at the given temperature for acclimation.

We analyzed only the results of those experiments in which copepods performed high activity. Within the WW range of 0.03 to 1.27 mg at 20°C, respiration rate increased from 0.052 in CI to 2.13 µg O_2 ind.$^{-1}$ h^{-1} in adult females. Since the results obtained during spring and autumn experiments were similar (Fig. 7), we expressed the relationship between respiration rate and body weight at 20°C by a single power equation R = 1.61WW$^{1.01}$ (r² = 0.99). Respiration rate in CVs and adult females at 8°C was 2.7 times lower than that in individuals with similar WW at 20°C. Respiration rate at 8°C was described by the equation R = 0.69WW$^{1.05}$. The mean Q_{10} in the temperature range of 8 to 20°C for *Calanus euxinus* individuals with the same body size was equal to 2.04 ± 0.02. Similar Q_{10} values were obtained by Vidal (1980c) and Ikeda et al. (2001).
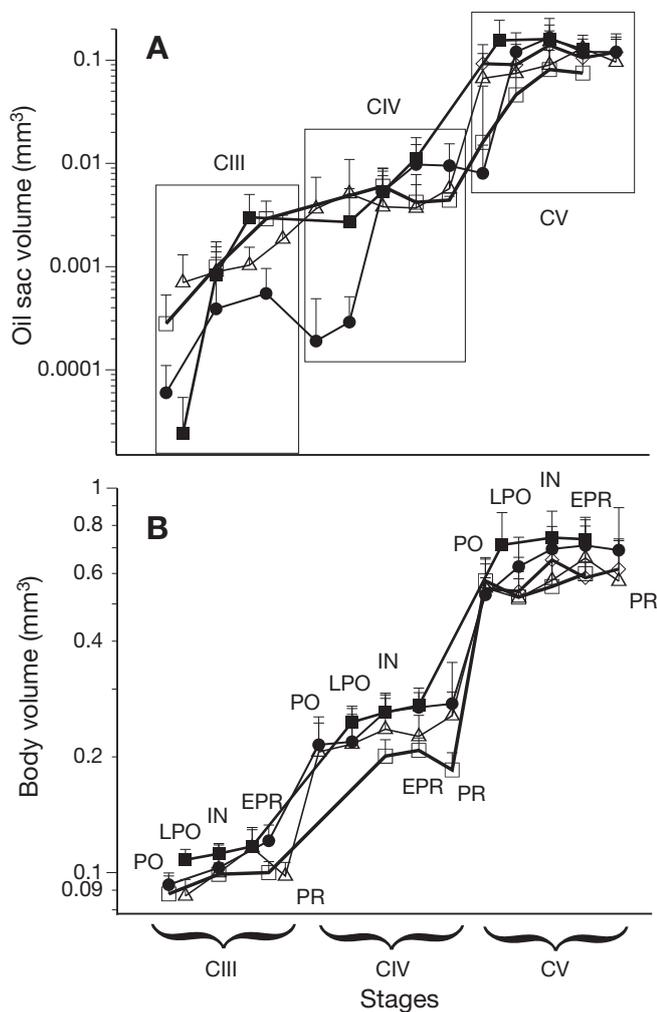
Fig. 4. *Calanus euxinus.* (A) Oil sac and (B) body volume of copepodite stages CIII to CV at different jaw phases in February (■) and September (◇) 1999, July 2000 (△), June 2001 (□) and April 2003 (●). PO: postmolt; LPO: late postmolt; IN: intermolt; EPR: early premolt; PR: premolt

DISCUSSION

Population structure of *Calanus euxinus*

Usually, late copepodite stages and adults of *Calanus euxinus* are abundant predominantly in deep shelf or slope regions in the main stream of the Black Sea Rim Current whilst towards the coast the number of early developmental stages increases (Vinogradov et al. 1992b). In contrast in April 2003 a large number of early copepodite stages of *C. euxinus* (10 883 ind. m^{-2}) were found in the central part of the Black Sea (Stn 5). At slope Stns 7 and 17, where preadults and adults predominated, total biomass amounted to 7.3 g m^{-2}. Similar and even higher values of biomass (up to 23.6 g m^{-2}) of *C. euxinus* were reported for the northeastern
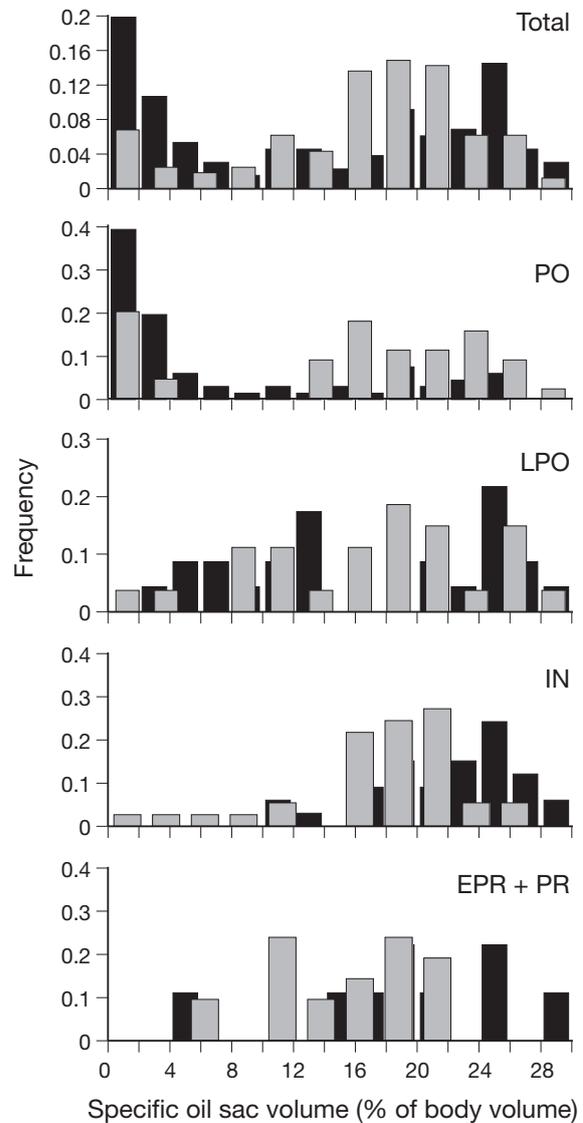
Fig. 5. *Calanus euxinus.* Oil sac volume frequency distribution in CV at different jaw phases from the total (▨) and within OMZ (■) hauls at Stn 7 (18 April 2003). PO: postmolt; LPO: late postmolt; IN: intermolt; EPR: early premolt; PR: premolt

deep region of the Black Sea in August to September 2001 to 2004 (Vinogradov et al. 2005) and June to September 2005 (Vinogradov et al. 2006) during the phytoplankton depression period. High biomass of *C. euxinus* (up to 5.5 g m^{-2}) was also found in the southwestern Black Sea in summer (Vinogradov et al. 1995), autumn (Yuneva et al. 1999, Besiktepe & Unsal 2000, Svetlinchny et al. 2006) and during the phytoplankton rich winter–spring period (Vinogradov et al. 1992c).

However, local densities of *Calanus euxinus* population in the Black Sea may be even higher, because preadults and adults usually ascend to subsurface layers at night and aggregate in the OMZ during daytime
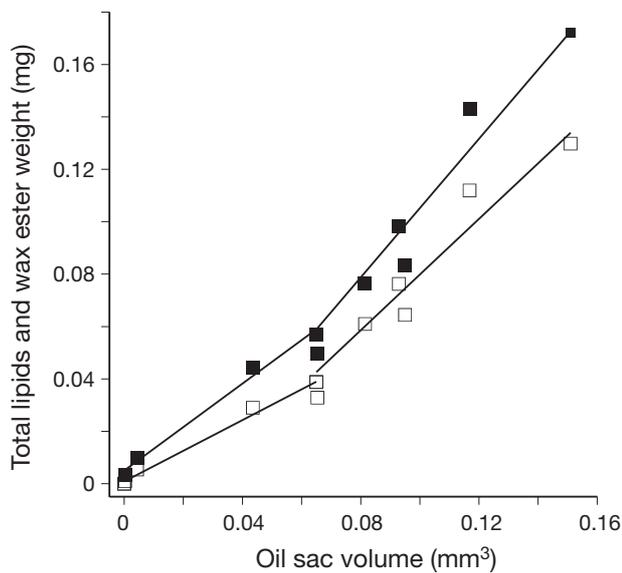
Fig. 6. *Calanus euxinus*. Total lipid (■) and wax ester (□) contents versus oil sac volume in copepodite stages
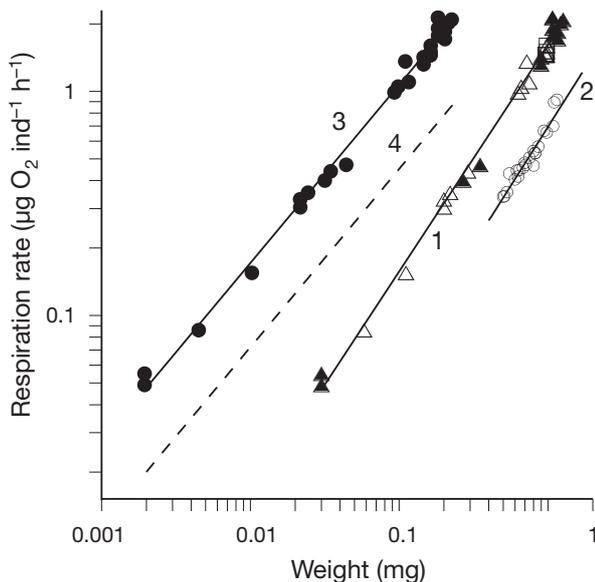
Fig. 7. *Calanus euxinus*. Relationships between respiration rate and wet weight (WW) at 20°C (1) (▲,△,□) and 8°C (2) (○), and between respiration rate and defatted dry weight at 20°C (3) (●) and 8°C (4). (△) October 2002; (▲) April 2003; (□) September to October 2003. Regression of respiration rate at 8°C against DW (4) was calculated on the basis of the relationship between respiration rate at 20°C and WW taking into account age changes in the WW:DW ratio

(Besiktepe 2001). Vinogradov et al. (1992a,c) reported on *C. euxinus* daytime aggregations with biomass 1.1 g m^{-3} in February 1991 and up to 3.5 g m^{-3} in April 1988.

Such high population densities in the closely related *Calanus helgolandicus* were found only in waters sur-

rounding the western Iberian Peninsula and in the North Adriatic (Bonnet et al. 2005). *C. euxinus* was found to be abundant during all seasons (Sazhina 1987) and over the whole open part of the Black Sea (Vinogradov et al. 1992b) whilst in the other seas of the North Atlantic *Calanus* development strongly depends upon seasonal changes in phytoplankton concentration (Ceballos et al. 2004, Rey-Rassat et al. 2004).

During all seasons late development stages of *Calanus euxinus* migrate in the morning to the OMZ where they reduce respiration rate and stop feeding (Vinogradov et al. 1992a,b). CV and adults began to feed again during the night and ascend to the upper layers (Besiktepe et al. 2005). According to our observations, in autumn 1996 and 1998 and in summer 2000 and 2001 (L. Svetlichny unpubl. data) the guts of CV and adults *C. euxinus* were empty or contained small dark lumps during daytime. But in April at the anticyclonic Stn 17 the majority of preadults and adults were above the OMZ and had food in their guts during both day- and nighttime. This may be due to a high concentration of *Proboscia alata* in deep layers which allows *C. euxinus* to feed all day long without vertical migration. Eker-Develi & Kideys (2003) also found that in March to April 1998, *P. alata* aggregated far below the euphotic zone (56 to 86 m). Probably, the huge amount of large diatoms outside the euphotic zone is due to sinking of algae after the winter–spring phytoplankton bloom.

Only 8 to 9% of CV sampled within the OMZ had empty guts and large oil sacs suggesting that diapausing stock began to form just in this period. According to Vinogradov et al. (1992a) and Svetlichny et al. (1998, 2006), CV POs with empty guts and large oil sacs captured in the OMZ at night, had reduced respiration and nitrogen excretion rates, low locomotor activity and were considered as diapausing copepodites. *C. euxinus* CV in 'resting phase' with low locomotor activity have been observed in the OMZ (Flint 1989). According to Vinogradov et al. (1992b), diapausing CV appeared in low numbers in the Black Sea in April to May. In summer their numbers increased and constituted on average 30% (up to 70%) of the CV total number in autumn. However, in winter diapausing CV were absent.

Therefore, it appears that CV moulting phases LPO, IN and PR collected in April 2003 developed from POs without entering diapause.

The great number of CIV PRs (especially in the OMZ) is evidence of favorable nutritional conditions for *Calanus euxinus* in April (Fig. 3A). Crain & Miller (2001) showed that under conditions of experimental starvation the suppression of development took place in CII to CIV POs. However, *C. euxinus* CV POs dominated even at high food concentrations due to the long-

drawn process of lipid accumulation during this stage (Svetlichny et al. 2006). Therefore, the predominance of PO jaw phase could be found not only in CVs preparing to diapause (Johnson 2004), but in CVs developing without diapause. In April 2003, POs dominated CV with a small percentage of INs and PRs in the layers above and within the OMZ (Fig. 3B), in contrast to summer and autumn (Svetlichny et al. 2006) when only diapausing POs stayed in the OMZ at night.

Ontogenetic changes in size–weight characteristics of *Calanus euxinus*

According to our results, in *Calanus euxinus* L_p increased with higher increments in CIII to CV and with lower increments from CI to CIII and from CV to adults. Similar sigmoidal development has been described for *C. marshallae* (Peterson 1986). We did not find significant differences in L_p of copepodites and adults collected in April 2003, from mean annual values obtained in the southern and southwestern Black Sea during 1999 to 2003, despite the pronounced seasonal changes in surface temperature (Fig. 8). During the warm period from June to October mean L_p of *C. euxinus* females (2.63 ± 0.12 mm) did not differ significantly from that in cold months from January to April (2.66 ± 0.09 mm). This may be the result of *C. euxinus* development in the Rim Current zone of the Black Sea, in the deep layers with low and stable temperatures (6.5 to 8.5°C) throughout the year. According to Bonnet et al. (2005), in European waters the size of *C. helgolandicus* female L_p changes from 1.94 in the Aegean Sea to 2.6 mm in the North Sea. Fleminger & Hulsemann (1987) reported that within the geographical range of *C. helgolandicus* the Black Sea females have maximum body size, probably, due to the hydrochemical characteristics of the Black Sea.

In April 2003 mean DW in *Calanus euxinus* increased proportionally with L_p following the power equation DW = $0.0082L_p^{3.45}$. Besiktepe & Telli (2004) found similar age dynamics of DW in *C. euxinus* in October 2000 (DW = $0.0069L_p^{3.41}$) and May 2001 (DW = $0.0062L_p^{3.34}$).

The regression for DW against L_p has a steeper slope (3.45) than that for V_b against L_p (2.83), indicating that during *Calanus euxinus* ontogeny DW increased with lower speed than V_b resulting in reduced water content (% of V_b) in preadults and adults. DFDW per unit volume increased uniformly from 0.06 mg mm^{-3} in CI to 0.15 mg mm^{-3} in adults, DW per unit volume rose from 0.092 mg mm^{-3} in CI to 0.38 mg mm^{-3} in CV and then reduced to 0.21 mg mm^{-3} in females and 0.27 mg mm^{-3} in males. Similar DW content have been reported for CV and adults of *C. helgolandicus* from the Celtic Sea (Williams & Robins 1982).
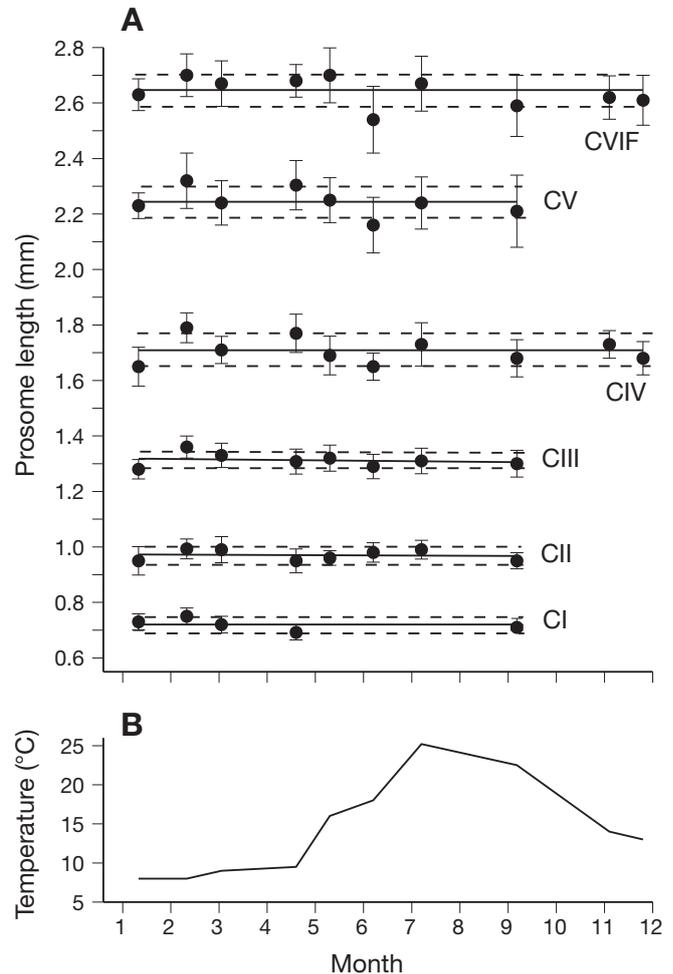
Fig. 8. *Calanus euxinus*. (A) Prosome length of copepodites (CI to CV) and adult females (CVIF) from the southwestern Black Sea (April 2003, June 2001 and July 2000) and from the southern Black Sea (off Sinop, 1999, all other months), and (B) surface water temperature during sampling. Solid line = mean annual values; dashed line = SD

Calanus euxinus accumulate lipids in the oil sac mainly in the form of WEs (Yuneva et all. 1997). However, if we transform V_s to weight of WE with the density of 0.86 g mm^{-3} (Lewis 1970), WE estimated from V_s in CV and females will be 1.23 ± 0.26 times higher than measured WE (see Table 2). According to Miller et al. (1998), in *C. finmarchicus* the ratio between estimated (from the oil sac volume) and measured WE was equal to 1.44. However, the oil sac of *C. euxinus* contains about 9% of other lipid fractions (probably, the components of oil sac cell membranes) (T. Yuneva pers. comm.). Therefore, the overestimation of lipid amount in the oil sac using the volume method will be nearly 10%. In *Calanoides carinatus* V_s in terms of weight units also was close to measured amount of reserved lipids (Arashkevich et al. 1996).

Variability in lipid reserves during the phases of CV molting cycle

In April 2003 mean V_s increased from 0.008 ± 0.007 mm^3 in POs to 0.109 ± 0.066 mm^3 in LPOs and reached 0.165 ± 0.054 mm^3 in CV INs (Table 2). Consequently, during the PO period V_s in *Calanus euxinus* increased nearly 14-fold. However, the difference between mean values in all examined LPOs and INs was found to be insignificant because V_s in both molting stages ranged from zero to the maximum (Fig. 5).

Miller et al. (2000) showed that despite high variability of V_s, its upper limit in *Calanus finmarchicus* CV ('apparent maximum' represented by an approximate upper envelope that lies above 98.5% of the data points) increased with body size. In our study we determined the upper limit of V_s (V_{ul}) as mean V_s estimated using 10 extremely lipid-rich individuals. As a result, V_{ul} in LPOs (0.173 ± 0.021 mm^3) was significantly lower than that in INs (0.226 ± 0.04 mm^3). A similar V_{ul} (0.208 ± 0.05 mm^3) was found in February 1999 also in INs. In autumn 1998 and during summer phytoplankton depression in 2000 and 2001 V_{ul} in CV INs amounted to 0.139 ± 0.016 mm^3 and 0.119 ± 0.026 mm^3, respectively, being lower than those in winter and spring.

In February and April V_s CIV INs were larger than those collected in the warm period. During the cold season we observed maximum V_b in all copepodite stages (Fig. 4B). This may be connected with better nutritional conditions for Black Sea *Calanus* during the winter–spring period. However, in summer the amount of lipid reserves was also higher in *C. euxinus* than in *C. helgolandicus* from the English Channel (Rey-Rassat et al. 2002a) but similar to that in *C. finmarchicus* (with similar body size) from the North Atlantic (Miller et al. 2000) and the North Sea (Rey-Rassat et al. 2002b).

Age dynamics of respiration rate

According of our results, WW dependent changes in *Calanus euxinus* respiration rate during the ontogeny from CI to CVI can be expressed by the equations $R = 1.61WW^{1.01}$ and $R = 0.69WW^{1.05}$ at 20°C and 8°C, respectively (Fig. 7). The slopes in these regressions are higher than the exponent values for dry body mass of about 0.8 reported on oxygen consumption rates of planktonic copepods (Vidal 1980c, Ikeda et al. 2001). However, if we change the body weight units from WW to DFDW (which reflects the amount of metabolically active matter in the body), the above equations become $R = 6.69DFDW^{0.8}$ and $R = 2.84DFDW^{0.8}$ for 20°C and 8°C respectively. This is due to a lower share

of DFDW in early copepodite stages compared with preadults and adults in *C. euxinus.*

In April 2003 weight-specific respiration rate in active females with a DW of 0.267 mg at 20°C reached 2.13 µg O_2 ind.$^{-1}$ h^{-1} or 7.9 µg O_2 mg DW^{-1} h^{-1} (18.5 µg O_2 mg C^{-1} h^{-1}), being close to maximum energy expenditure in *Calanus euxinus* females during permanent swimming at the same temperature (Pavlova & Minkina 1987, Svetlichny & Hubareva 2005). According to the equation of Ikeda et al. (2001), in epipelagic copepods with the same body weight at 20°C weight-specific respiration rate amounted to 4.96 µg O_2 mg DW^{-1} h^{-1}. After using the equation of Vidal (1980c) for *C. pacificus* at 20°C, we estimated weight-specific respiration rate in *C. euxinus* females as 8.86 µg O_2 mg DW^{-1} h^{-1}.

At 8°C, weight-specific respiration rate of *Calanus euxinus* females was 3.43 µg O_2 mg DW^{-1} h^{-1} and, according to Vidal (1980c), was 30% lower than that of *C. pacificus* (4.95 µg O_2 mg DW^{-1} h^{-1}). This difference may be due to the lower salinity of the Black Sea. For example, Anraku (1964) found more than a 2-fold decrease in respiration rate of *C. finmarchicus* during salinity reduction from 31.5 to 21.5‰. In our experiments (L. Svetlichny & E. Hubareva unpubl. data) weight-specific respiration rate of *C. euxinus* reared at 39‰ was significantly 1.3 times higher than that at the Black Sea salinity of 18 to 20‰.

Reconstruction of growth in body weight and estimation of stage duration

Although *Calanus euxinus* copepodites and adults collected in April 2003 do not represent a temporal progression of successive stages in cohort, we can reconstruct age dynamics of DW during the winter–spring period using our findings that V_s, V_b (Fig. 4) and molt increments of L_p (Fig. 8) are similar in winter and spring. The weight of body compartments may be expressed in terms of carbon taking into account the ratio of the carbon content as 0.81 for WE, 0.78 for TL containing 70 to 72% of WE, 8 to 13% of triacylglycerols, 13 to 15% of phospholipids and 3 to 5% of sterols (Yuneva et al. 1999), according to stoichiometric composition of the main lipid compounds in calanoid copepods (Ventura 2006), and as 0.42 ± 0.6 for DFDW (our data, Table 2). Our calculations were based on the results of direct measurements (Table 2). Because of absence of the data on single molting copepodite phases, we used the empiric relations of WE and TL to V_s obtained in our study (Fig. 6). Total carbon weight was determined as a sum of carbon contents in DFDW and TL. Reconstruction of age dynamics of weight compartments in CI through adult females is shown in

Fig. 9. Calculated mean total carbon content increased uniformly from 1.3 µg in CI to 28 µg in CIV PRs, changed from 44 to 147 µg during the CV PO phase, reached 205 µg in the CV interphase and then decreased to 115 µg in adult females. In CIII to CV carbon content of TL and WE tended to increase during molting periods and then reduced after ecdysis. This is connected with energy expenditure during ecdysis which may result in losses of 22% of carbon weight (Rey-Rassat et al. 2002b). In females, pronounced lipid depletion is due to gonad formation and egg production (Sargent & Henderson 1986). In contrast to TL and WE, DFDW does not vary greatly within a single development stage. Carbon content of DFDW increased nearly uniformly from 0.65 µg in CI to 67 µg in adult females.

Using our results concerning age dynamics of body weight and available literature data on growth rates in *Calanus*, we can estimate stage duration and total generation time of *C. euxinus* during winter–spring homothermy. Sazhina (1987) found that in a laboratory experiment at 18°C, development time (from egg to adult) of Black Sea *Calanus* amounted to 34 d. Stage durations amounted to 3 d for CI and CII, 4 d for CIII, 5 d for CIV and CV. However, there is no information available for growth of this species in the natural environment at 8°C. Therefore, to estimate the duration of copepodite development in *Calanus euxinus* during winter–spring homothermy at 8°C, we calculated time dependent changes in carbon body weight (CW) of CI (1.3 µg C) through CV INs (205 µg C) according to the equation: $CW_t = CW_{t-1} + P_{t-1}$, where t is time (d^{-1}) and P is absolute growth rate (mg C ind.$^{-1}$ d^{-1}).

P was calculated based on ontogenetic changes in specific growth rate (g, d^{-1}) of *Calanus finmarchicus* copepodites with similar body size and carbon content, reared at 8°C under non-limiting food concentration (Campbell et al. 2001, their Table 5). For CI to CV of this species g decreased from 0.31 to 0.042 d^{-1} (Fig. 10A). Similar dynamics of g was found in *C. sinicus* with smaller body length at 8°C (Uye 1988). Vidal (1980a) estimated g as 0.118 d^{-1} for *C. pacificus* CV. However, this value may be considered to be overesti-
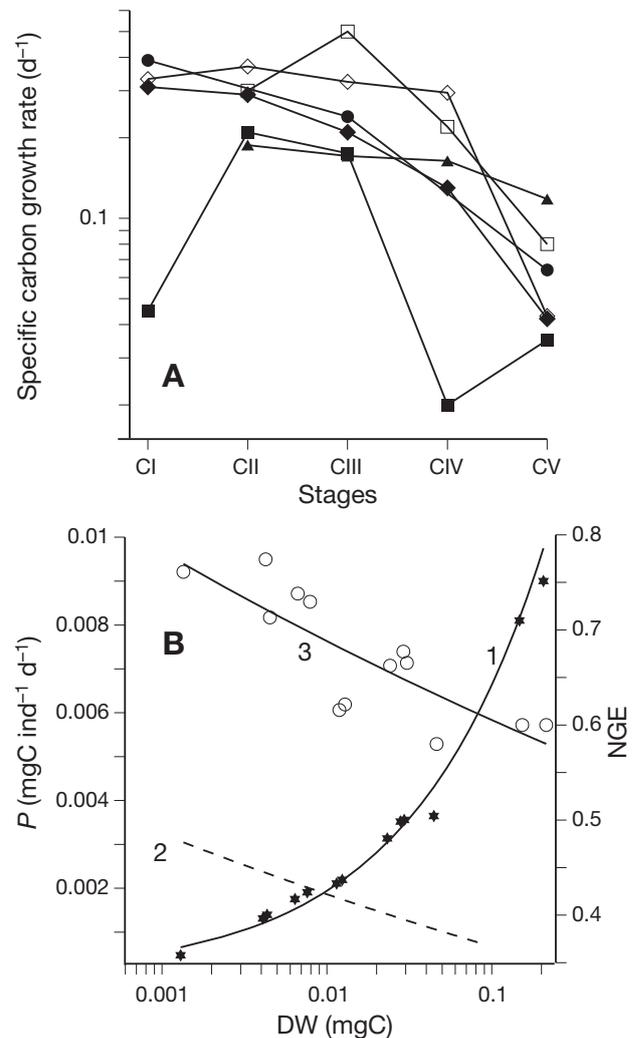
Fig. 10. Age dynamics of (A) specific carbon growth rate (d^{-1}) in *Calanus* group species, and (B) absolute growth rate (P, mgC ind.$^{-1}$ d^{-1}) and net growth efficiency (NGE) in *C. euxinus* copepodites. Growth rates in sibling *Calanus* species at high food resources: (▲) *C. pacificus*, 8°C (Vidal 1980a); (□) *C. helgolandicus*, 15°C (Harris et al. 2000); (●) *C. sinicus*, 8°C (Uye 1988); (◆) *C. finmarchicus*, 8°C (Campbell et al. 2001); (◇) *C. helgolandicus*, 15°C (Rey-Rassat et al. 2002a); (■) *C. finmarchicus*, 10°C (Harris et al. 2000). (1, ✱) P (our results); (2) NGE (Ikeda et al. 2001); (3,○) NGE (calculated as NGE = $P/P+R$) where R is respiration rate

Fig. 9. *Calanus euxinus*. Age dynamics of total carbon content (●) and carbon equivalents of defatted dry weight (◇), total lipids (■) and wet weight (□) in copepodites CI to adult females (CVIF)

mated, because even at 15°C and high food concentration g in *C. helgolandicus* CV amounted to only 0.08 (Harris et al. 2000) and 0.043 (Rey-Rassat et al. 2002a).

After plotting g magnitudes reported by Campbell et al. (2001) against a scale of CW in *C. euxinus* copepodite stages, we found the correlation between g and CW and calculated daily P as $P = CW(e^g – 1)$. Obtained absolute growth rates (Fig. 10B) increased from 0.00047 mg C d^{-1} in CI to 0.0075 mg C d^{-1} in CV INs following the equation $P = 0.017CW^{0.53}$.

P depends upon the respiration rate (R) and the coefficient of net growth efficiency (NGE) according to the equation NGE = $P/(P + R)$. Ikeda et al. (2001) showed that NGE in preadult and adult epipelagic copepods changed from 0.54 to 0.21 when temperature and body weight increased. According to the equations of these authors, at 8°C NGE in *Calanus euxinus* CI to CV should decrease from 0.47 to 0.37 (Fig. 10B). We estimated age dynamics of NGE in *C. euxinus* from obtained P and R after converting respiration data in µg O_2 (Fig. 9) to carbon units in µg C using the coefficient of 0.363 at RQ = 0.97 (Ikeda et al. 2000), where RQ is respiration quotient. It turned out that at nearly maximum respiration rates in our experiments NGE reduced from 0.77 in early copepodite stages to 0.6 in CV, being close to maximum values for marine copepods (Ikeda et al. 2001). Such a pattern is in accordance with the well-known conception (Winberg 1968) that in juvenile stages NGE can be close to a theoretical maximum (about 0.8), and then decreases during growth. In the experiments of Petipa (1966), NGE in *C. euxinus* diminished 10-fold from 0.55 in CI to 0.056 in CV. Consequently, P magnitudes obtained in our study using the values of g by Campbell et al. (2001) agree with maximum net growth efficiency of marine copepods.

Time dependent changes in CW of *Calanus euxinus* at a constant temperature of 8°C based on calculated P values are presented in Fig. 11A. According to our calculation, the period from CI to CV PO (when somatic growth predominated over lipid accumulation) amounted to 22 d. Similar median development time (MDT) in CI to CV of *Calanus* species at 7.6°C (26.2 d) was estimated by Thompson (1982). In the experiments of Vidal (1980b) development time for CII to CV of *C. pacificus* was equal to 16.7 d, therefore, MDT for CI to CV should not be less than 20 d. MDT of CI to CV in *C. finmarchicus* at 8°C accounted for 25 and 17 d at medium and high food concentrations, respectively (Campbell et al. 2001). Tande (1988) reported that in the same species at 6°C MDT was 31.8 d. Calculated molt weight increment (WI) as $WI_i = (DW_{i+1} – DW_i)/DW_i$ for CI to CIV in *C. euxinus* ranged from 0.9 to 2.9 in the same way as in other congeneric species (Fig. 11B).

According to our estimation, the period of intensive lipid reserve formation in CV PO to IN phrase lasted
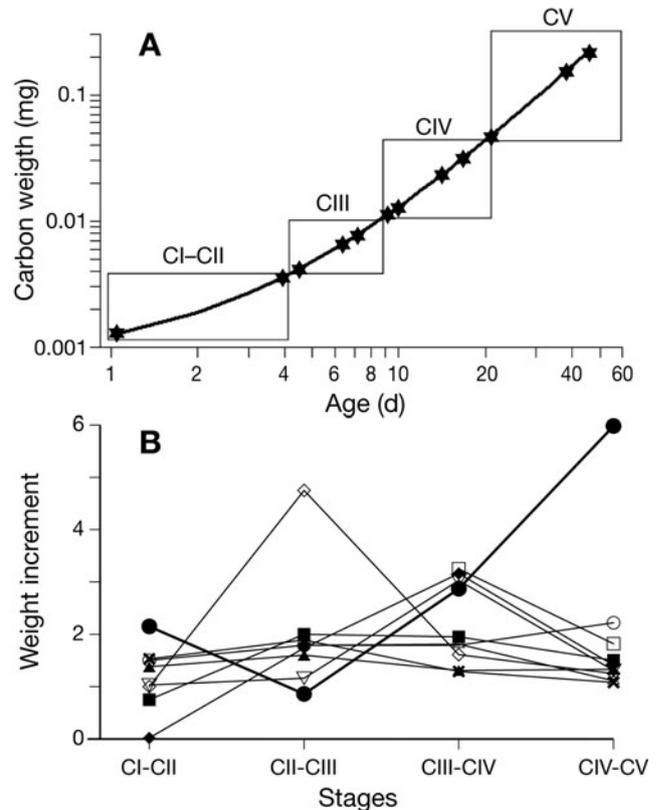
Fig. 11. *Calanus euxinus.* (A) Growth from CI to intermolt of CV and (B) mean weight increment [$WI_i = (WI_{i+1} – W_i)/W_i$] between copepodite stages for *C. euxinus* and sibling species. (△) *C. pacificus*, 8°C (Vidal 1980b); (▲) *C. finmarchicus*, 8°C (Fransz & van Arkel, 1980); (■) *C. finmarchicus*, 8°C (Carlotti et al. 1993); (□) *C. finmarchicus*, 10°C (Harris et al. 2000); (◇) *C. finmarchicus*, 5.1 to 8.3°C (Hygum et al. 2000); (○) *C. finmarchicus*, 8°C (Campbell et al. 2001); (◆) *C. helgolandicus*, April, the North Sea (Kattner & Krause, 1987); (▽) *C. helgolandicus*, 15°C (Rey-Rassat et al. 2002a); (✗) *C. sinicus*, 10.3°C (Uye 1988); (●) *C. euxinus*, 8°C (our data)

26 d. Although the duration of CV is usually increased (in comparison with other copepodite stages) in copepods accumulating large lipid amounts, we calculated this period to be significantly higher than MDT (9.6 to 12.1 d) of CV in *Calanus* studied at ~8°C (Vidal 1980a, Thompson 1982, Corkett et al. 1986, Campbell et al. 2001). However, one should take into account that in spring in *C. euxinus* CV stored lipid amount and DFDW increased 17-fold and 2-fold, respectively. Therefore, the weight increment of CIV to CV (based on field observations) is equal to 6, which is nearly 3 times higher than that calculated for *C. finmarchicus* CV reared under high food conditions (Campbell et al. 2001; see Fig. 1, Table 2 therein).

Finally, generation time from CI to adults in *Calanus euxinus* during winter–spring homothermy should not be less than 50 d. If we add to this value the duration of development of eggs and nauplii amounting to 16 d at

8°C (Corkett et al. 1986, Campbell et al. 2001), total development time for *C. euxinus* will be about 66 d, in contrast to 43.6 and 32 to 46 d in *C. finmarchicus* (Corkett et al. 1986), Campbell et al. 2001). However, the development time in *C. euxinus* may be underestimated because we have included in our calculation absolute growth rates which are close to maximum net growth efficiency for preadult and adult copepods (Ikeda et al. 2001).

Our results have shown that we can use obtained development time to estimate *Calanus euxinus* population dynamics. We found great abundance of newly developed CV POs in the second decade of April. Consequently, the generation of these CVs should appear at the beginning of March, whilst the former generation was formed early in January. Hence, during the cold homothermy in the Black Sea, *C. euxinus* cannot produce more than 2 generations.

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