

# Nutritional condition of female *Calanus euxinus* from cyclonic and anticyclonic regions of the Black Sea

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**ABSTRACT** The content and composition of lipids in *Calanus euxinus* females were studied in cyclonic and anticyclonic regions in the southern Black Sea during the period of persistent density stratification (September 1996). The lipid content of *C. euxinus* from cyclonic regions was greater than of individuals from anticyclonic regions (average 101.9 and 58.8  $\mu\text{g ind.}^{-1}$ , respectively). Total lipid content (mainly wax esters, 70 to 72%, and triacylglycerols, 8 to 13%) of female *C. euxinus* was correlated with chlorophyll *a* (chl *a*) (mean integrated concentration) ( $r = 0.92$ ,  $p < 0.005$ ). The high correlation with chl *a* was shown also for wax ester content, used as a long-term index of food supply ( $r = 0.89$ ,  $p < 0.05$ ). The correlation between triacylglycerol content (a short-term index) and chl *a* was not significant ( $r = 0.66$ ,  $p < 0.2$ ). In cyclonic regions the mean integrated chl *a* concentration ( $0.38 \text{ mg m}^{-3}$ ) (as an indicator of phytoplankton food biomass) was 1.7-fold higher than in anticyclonic areas. The same trend occurred for mean integrated concentrations of nitrate and phosphate ( $0.996$  and  $0.299 \mu\text{M}$  in cyclonic and  $0.438$  and  $0.061 \mu\text{M}$  in anticyclonic regions, respectively) in the chl *a* containing layer. Thus, conditions for total primary productivity in cyclonic regions were more favorable than in anticyclonic ones. This was consistent with lipid levels in female *C. euxinus*.

**KEY WORDS:** *Calanus euxinus* · Black Sea · Food supply · Lipids · Wax esters · Triacylglycerols · Chlorophyll · Cyclonic regions · Anticyclonic regions

## INTRODUCTION

*Calanus euxinus* is one of the most important copepods of the offshore pelagic food chain of the Black Sea (Vinogradov et al. 1992b); it is a link between phytoplankton and many species of fish, including commercially important ones. Over the last decade, the abundance of *C. euxinus* has declined appreciably (Vinogradov et al. 1992b, Kovalev et al. 1998). This may be associated with the predation impact of the recently introduced ctenophore *Mnemiopsis leidyi* (Anninsky et al. 1998) and/or with changes in food (i.e. phytoplankton) composition due to pollution (Vinogradov et al. 1992b) or simply with climatic changes (Kovalev et al. 1998, Niermann et al. 1999).

In the estimation of the nutritional condition (or food provision) of marine animals in the natural environment, physiological and biochemical indices are widely used. They involve, for example, determinations of *in situ* feeding rates (measured by means of gut fullness and the degree of fluorescence in the gut from ingested phytoplankton pigments; Hayward 1980, Cox et al. 1983, Båmstedt et al. 1990, Bautista et al. 1992, Besiktepe et al. 1998), of digestive enzyme activity (Cox et al. 1983, Båmstedt & Ervik 1984), of growth rate using the ratio between RNA and DNA content (Ota & Landry 1984), and of egg production rate and fecundity (Marshall & Orr 1972, Hirche et al. 1991, Mullin 1991, Peterson et al. 1991). However, most of these methods are often difficult to use in field investigations and they are not always free from the effects of animal capture and other experimental procedures.

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A very reliable and less cumbersome indicator of the nutritional condition of *Calanus* spp. is body lipid content (Håkanson 1984, 1987, Sargent & Henderson 1986, Bamstedt et al. 1990). The level of lipid accumulation of *Calanus* has been shown under laboratory conditions to depend on the concentration and composition of the phytoplankton consumed (Lee et al. 1970, 1971, Sargent et al. 1977, Håkanson 1984). The class of storage lipid is important in characterising the copepods' nutritional state. Wax ester content is a measure of the animal's long-term nutritional history, whereas the triacylglycerol content may reflect its recent feeding history (Sargent et al. 1977, Håkanson 1984). The turnover of triacylglycerol occurs faster than wax esters. For example, the triacylglycerol content of late copepodites of *C. pacificus* reached a maximum over a few hours from the onset of feeding in the laboratory (Håkanson 1984). Much more time (weeks at high food concentrations) was required for maximum wax ester accumulation. Similar temporary ratios were shown for mobilisation of these substances in conditions of food shortage (Lee & Barnes 1975, Sargent et al. 1977, Håkanson 1984).

Temporal variability in feeding conditions (e.g. biomass of phytoplankton) influence lipid storage in *Calanus* spp. For example, the highest lipid (mainly wax ester) content in higher copepodite stages of *C. fin-*

*marchicus* and *C. helgolandicus* are known to coincide with the spring phytoplankton bloom at high latitudes (Marshall & Orr 1972, Gatten et al. 1979, 1980). Wax esters formed in periods of abundant food (i.e. spring and summer) are then utilised for energy expenditure and subsequently gonad formation during the diapausing period in winter, when these copepods are not feeding (Sargent & Falk-Peterson 1988).

The spatial variability in the lipid content of *Calanus* spp. is also related to the differences in food supply. Previously, we showed such variability for total lipid, wax ester and triacylglycerol contents of migrating Stage V copepodites and females of *C. euxinus*, sampled from different regions of the northern Black Sea (Yuneva et al. 1997). The lipid content of similar-sized females varied by 3- to 4-fold, and correlated with both the biomass of phytoplankton (10 to 50  $\mu\text{m}$ ) ( $r = 0.82$ ,  $p < 0.005$ ) and chlorophyll *a* (chl *a*) concentration (integrated over 75 m) ( $r = 0.84$ ,  $p < 0.005$ ). Similar relationships were obtained for *C. pacificus* in the California Current and off San Diego (Håkanson 1987).

In some instances there is no strong correlation between the lipid content of *Calanus* spp. and indicators of phytoplankton biomass (Ohman 1988, Bamstedt et al. 1990, Willason et al. 1993). It has been argued that other potential food organisms such as bacteria, ciliates and detritus may have been underrated in these cases (Petipa 1981, Fessenden & Cowles 1994, Ohman & Runge 1994). The correlation may also be low where copepods feed on small phytoplankton patches, which could often be overlooked during routine measurements (Dagg 1977). Additionally, competition may decrease the amount of food accessible to copepods and therefore may influence their lipid accumulation (Raymont 1976, Parsons et al. 1984).

The Black Sea, due to its very limited water exchange with neighbouring seas, is the largest anoxic water body of the world. Only about 13% of its waters contain oxygen, and aerobic life is absent below 100 or 200 m. The main circulation characteristics of the Black Sea are the presence of a Rim Current and 2 separate cyclonic gyres occupying the western and eastern halves of the basin (Oguz et al. 1993; Fig. 1A). There are also a few permanent anticyclonic eddies (e.g. Batumi and Kizilirmak) in the southern Black Sea. The centres of the cyclonic gyres are upwelling regions, whilst those of the anticyclonic eddies show downwelling characteristics. The relatively limited number of species (Vinogradov et al. 1992b) and well-defined hydrological regime of the Black Sea seem to provide a convenient system for the study of its inhabitants' food supply. In this investigation we measured concentrations of total lipid and of the main storage lipid classes as long- and short-term indicators of the nutritional condition of *Calanus euxinus* females in different regions of the southern Black Sea.

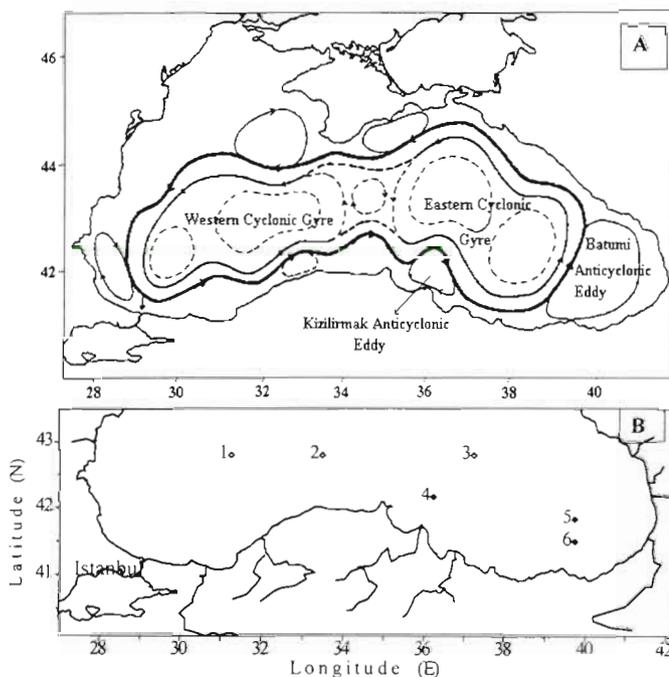


Fig. 1. (A) General circulation of surface currents in the Black Sea (modified from Oguz et al. 1993). (B) Location of sampling stations. (○) Stations in cyclonic zone, (◆) stations in anticyclonic zones

## METHODS

One survey was performed in both cyclonic and anticyclonic regions, towards the end of the summer period of stratification. At this time, mixing is minimal, the euphotic zone is depleted of nutrients, and the coupling between phytoplankton and its consumers becomes tighter (Goldman 1988).

Sampling was carried out at 6 stations in the southern Black Sea between 25 September and 4 October 1996 (on board the RV 'Bilim', Institute of Marine Sciences, Middle East Technical University, Turkey) (see Table 1, Fig. 1B). According to the research objective, 3 stations each were chosen in cyclonic and anticyclonic regions identified from the depth of  $\sigma_t = 16.2$  density value. It is known that this value corresponds to the lower boundary of the oxic layer and changes with respect to circulation direction (Vinogradov et al. 1992b, Saydam et al. 1993, Murray et al. 1995). A  $\sigma_t = 16.2$  value at a depth of 100 to 120 m is indicative of the cyclonic circulation, and at a depth of 160 to 200 m indicates the anticyclonic circulation. It should be noted that Stn 4 is not in the centre but in the periphery of the anticyclonic region.

Basic oceanographic parameters, such as temperature ( $^{\circ}\text{C}$ ), salinity ( $\text{‰}$ ), and chlorophyll *in situ* fluorescence, of the water column were measured using a Sea Bird CTD probe. Water samples for nutrient analyses were collected with a Rosette sampler attached to the CTD. Nitrate ( $\text{NO}_3$ ) and phosphate ( $\text{PO}_4$ ) concentrations were measured using standard methods (Graßhoff et al. 1983). Chl *a* concentrations were measured from 5 to 7 depths (chosen according to *in situ* fluorescence profiles) in the 0 to 75 m layer, using the fluorometric method on acetone extracts (JGOFS Protocols 1994). The lower boundary of the chl *a* containing layer was estimated from the chlorophyll fluorescence *in situ* minimum. Profiles of chl *a*,  $\text{NO}_3$  and  $\text{PO}_4$  were drawn, and mean integrated concentrations for this layer evaluated.

During the survey, *Calanus euxinus* were caught using a Nansen plankton net (112  $\mu\text{m}$  mesh size, 0.7 m diameter) at night (21:00 to 01:30 h) in surface waters (from 30–50 to 0 m). It is known that during these hours late stages of *C. euxinus* ascend from deeper waters towards the sea surface to feed (Petipa 1965, Besiktepe et al. 1998). It was easy to understand the feeding activity from microscopic observations of micro-algal remains in the guts of copepods. It turned out that almost all females caught ate well, whereas Stage V copepodites had empty (or not full) stomachs (average 80%). Therefore, only the former group was used for the evaluation of *C. euxinus* food supply.

Two replicates comprising approximately 50 *Calanus euxinus* females each were used for lipid determi-

nations. The size (prosoma length) of females used in analyses ranged from 3.3 to 3.5 mm and their calculated wet weight (Svetlichny 1983) was 1012 to 1077  $\mu\text{g ind.}^{-1}$ . Copepods were removed from the water and immediately put in a chloroform-methanol (2:1, v/v) mixture for lipid extraction (Folch et al. 1957) without preliminary homogenisation. Ohman (1988) and Håkanson (1987, 1989) showed that the extraction efficiency of whole individuals without homogenisation was good for small organisms such as copepods or fish larvae (>98% of lipids extracted). The samples were extracted twice during 24 h with the fluid transferred after each 24 h period. The resulting extracts were stored at  $-20^{\circ}\text{C}$  for no more than 2 wk. These were used for further qualitative and quantitative determinations of lipid content in the laboratory. The sulfovanillin technique (Barnes & Blackstock 1973) was applied to define the content of total lipid in extracts. Lipids, extracted from *C. euxinus*, vacuum dried and weighted on a Cahn electrobalance according to Kates (1975), were used as a standard for calibration.

The lipid was fractionated by unidimensional thin-layer chromatography on  $5 \times 15$  cm Silufoll UW254 plates (Kavalier Ltd, Czech Republic) using solvents of different polarity in special arrangement of the chromatography chamber (3 cylindrical chambers of different size were nested in each other) as described by Kopitov (1983). The outer large chamber (diameter 15 cm and height 21 cm) was preliminarily saturated for 2 h with hexane; the intermediate chamber (diameter 7 cm, height 13.5 cm) was saturated with a hexane-diethyl ether (9:1 v/v) mixture; the small innermost chamber (diameter 5.5 cm, height 1.0 cm) contained chloroform.

Plates were activated at  $105^{\circ}\text{C}$  for 30 min and treated with a 10% solution of phosphomolibdenum acid in ethanol before the chromatography procedure. A 20 to 50  $\mu\text{g}$  sample of lipid was applied to each plate under nitrogen. All chambers were quickly assembled inside in each other and the plate was immediately put into the innermost chamber. Thus, the plate was developed in solvents with decreasing polarity for 10 min. Chromatograms were visualised by heating at  $110^{\circ}\text{C}$  for 7 min and quantified densitometrically, using an ERS densitometer (Karl Zeiss, Germany). The lipid classes (phospholipids: PL, sterol: S, free fatty acids: FFA, triacylglycerols: TAG, and wax esters: WE) were identified against a suite of lipid standards (Sigma Chemical Co.). Contents of total lipid (TL) and lipid classes were given as  $\mu\text{g ind.}^{-1}$ .

Along with *Calanus euxinus* sampled from surface waters, mesozooplankton was taken from throughout the entire aerobic layer in vertical hauls with the same Nansen plankton net. Samples were treated with

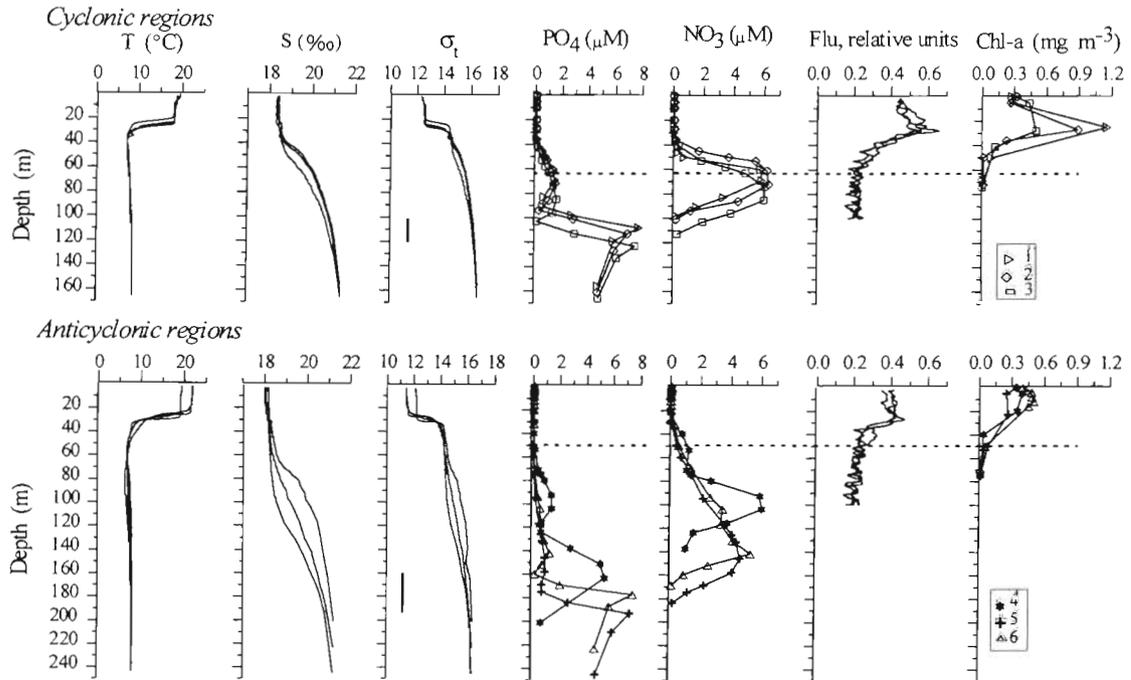


Fig. 2. Vertical profiles of temperature ( $T$ ,  $^{\circ}\text{C}$ ), salinity ( $S$ , ‰), density ( $\sigma_t$ ), phosphate ( $\text{PO}_4$ ), nitrate ( $\text{NO}_3$ ), chl *a* *in situ* fluorescence (FLU) and chl *a* concentrations from cyclonic (Stns 1, 2 and 3) and anticyclonic regions (Stns 4, 5 and 6). Bold line on  $\sigma_t$  profiles shows the lower border of the oxic zone identified as the 16.2 density value. Dashed lines indicate the lower border of the chl *a* containing layer

buffer solutions of formaldehyde, and enumeration, species identification and size measurements were carried out under a binocular microscope using a Bogorov chamber. The biomass of individual organisms was evaluated by the computation technique as described by Petipa (1957).

The hypothesis that the *in situ* lipid content of the copepod *Calanus euxinus* is a function of food supply was tested using Simple Linear Correlation analysis (Zar 1984).

## RESULTS

The physical and chemical patterns of stations in cyclonic (the eastern and the western cyclonic gyres) and anticyclonic (Batumi and quasi-permanent Kizilirmak anticyclonic eddies) regions are shown in Table 1 and Fig. 2. The temperature profiles showed the presence of a quasi-homogeneous 'mixing' layer situated 15 to 20 m above the thermocline (maximum gradient 0.6 to 1.4 $^{\circ}\text{C m}^{-1}$ ). The maximum gradient of salinity

Table 1. Hydrophysical and hydrochemical patterns and chl *a* content at the stations from the cyclonic and anticyclonic regions. Temperature and salinity are given as the mean values in the upper quasi-homogeneous (temperature) 'mixed layer'; chl *a*,  $\text{NO}_3$  and  $\text{PO}_4$  are mean integrated concentrations for the chl *a* containing layer

Stn	Coordinate	Date (d.mo.yr); time	Temperature ( $^{\circ}\text{C}$ )	Salinity (‰)	Depth (m) of $\sigma_t = 16.2$ value	$\text{NO}_3$ ( $\mu\text{M}$ )	$\text{PO}_4$ ( $\mu\text{M}$ )	Chl <i>a</i> ( $\text{mg m}^{-3}$ )
<b>Cyclonic regions</b>								
1	42 $^{\circ}$ 50 N, 31 $^{\circ}$ 15 E	26.9.96; 23:00 h	18.47	18.29	105	1.326	0.423	0.49
2	42 $^{\circ}$ 50 N, 33 $^{\circ}$ 30 E	27.9.96; 21:00 h	18.88	18.41	113	0.871	0.275	0.35
3	42 $^{\circ}$ 50 N, 37 $^{\circ}$ 15 E	2.10.96; 21:00 h	18.55	18.35	123	0.790	0.200	0.29
Mean			18.63	18.35	114	0.996	0.299	0.38
<b>Anticyclonic regions</b>								
4	42 $^{\circ}$ 10 N, 36 $^{\circ}$ 15 E	2.10.96; 01:30 h	19.20	18.24	160	0.532	0.109	0.21
5	41 $^{\circ}$ 50 N, 39 $^{\circ}$ 45 E	3.10.96; 22:00 h	21.61	18.08	193	0.371	0.032	0.19
6	41 $^{\circ}$ 30 N, 39 $^{\circ}$ 45 E	4.10.96; 22:00 h	21.72	18.01	180	0.412	0.042	0.23
Mean			20.80	18.11	178	0.438	0.061	0.21

(0.056 to 0.080‰ m<sup>-1</sup>) in cyclonic regions was observed at a depth of 40 to 50 m, and in anticyclonic regions at 60 to 120 m. In cyclonic regions the  $\sigma_t = 16.2$  value, indicating the lower boundary of the oxic layer, was located at a depth of 105 to 123 m, and in the anticyclonic regions at a depth of 160 to 193 m.

The lower boundary of the chl *a* containing layer (according to minimum values obtained from the fluorescence profiles) was located at 60 to 65 m in cyclonic regions and at 45 to 50 m in anticyclonic regions. Low phosphate (0.04 to 0.05  $\mu\text{M}$ ) and nitrate concentrations (0.02 to 0.06  $\mu\text{M}$ ) were observed down to a depth of 25 to 35 m in cyclonic regions, and down to 50 to 60 m in anticyclonic regions. Thus, mean integrated concentrations of  $\text{PO}_4$  and  $\text{NO}_3$  within the chl *a* containing layer were 4.9- and 2.3-fold higher, respectively, in cyclonic than in anticyclonic regions (Table 1). Similarly, chl *a* concentrations (as an indication of phytoplankton biomass) in cyclonic regions were on average twice as high as in anticyclonic areas. There were negative correlations between the depth of  $\sigma_t = 16.2$  value versus concentrations (mean integrated) of  $\text{PO}_4$  ( $r = -0.93$ ,  $p < 0.005$ ) and  $\text{NO}_3$  ( $r = -0.91$ ,  $p < 0.005$ ) and positive correlations between chl *a* versus  $\text{PO}_4$  ( $r = 0.94$ ,  $p < 0.005$ ) and  $\text{NO}_3$  ( $r = 0.95$ ,  $p < 0.005$ ) concentrations (Fig. 3).

The total mesozooplankton biomass did not differ notably between the 2 different regions, and on average was 2775 mg m<sup>-2</sup> (Table 2). In both regions about 20% of the total biomass was accounted for by small copepods (e.g. *Acartia clausi*, *Paracalanus parvus*, *Pseudocalanus elongatus* and *Oithona similis*) and other mesozooplankton species (e.g. *Sagitta setosa*, *Oikopleura diocica*, molluscs and polychaete larvae). The remainder (approx. 80%) of the mesozooplankton biomass consisted of 2 species—the copepod *Calanus*

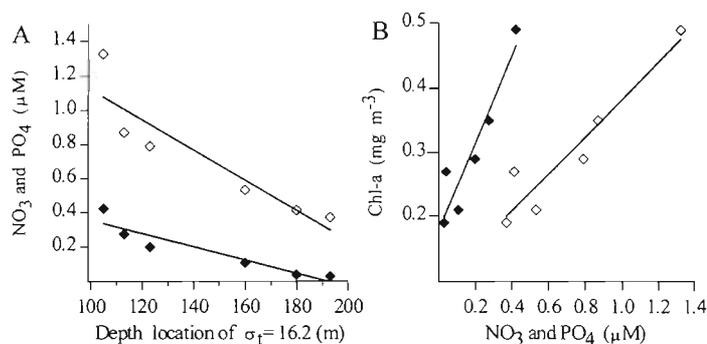


Fig. 3. Relationships of (A) depth (m) of the  $\sigma_t = 16.2$  value versus mean integrated concentrations of nitrates ( $\text{NO}_3$ ) and phosphates ( $\text{PO}_4$ ) ( $r = -0.91$ ,  $p < 0.005$  and  $r = -0.93$ ,  $p < 0.005$ , respectively), and (B) mean integrated concentrations of chl *a* versus nitrate ( $r = 0.95$ ,  $p < 0.005$ ) and phosphate ( $r = 0.94$ ,  $p < 0.005$ ). ( $\diamond$ ) Nitrates, ( $\blacklozenge$ ) phosphates

*euxinus* and the heterotrophic dinoflagellate *Noctiluca miliaris*. The biomass of *C. euxinus* in cyclonic regions was on average 3.5-fold greater than that of *N. miliaris*, but in anticyclonic regions they were present in similar proportions. The relationship between the chl *a* concentration and the *C. euxinus* and *N. miliaris* biomasses was also different: there was no relation to the biomass of *C. euxinus* ( $r = 0.4$ ,  $p > 0.2$ ), but the chl *a* concentration was negatively correlated with *N. miliaris* ( $r = -0.73$ ,  $p < 0.05$ ).

Females constituted on average 24% of total abundance of the *Calanus euxinus* population (Table 3). The number of females was significantly higher than that of males, with ratios of 30:1 and 6:1 in cyclonic and anticyclonic regions, respectively. The number of *C. euxinus* eggs in the net hauls was similar between the 2 regions and was on average 8537 eggs m<sup>-2</sup>. Stage V copepodites were always the dominating group (>50% of total *C. euxinus* abundance). The combined abun-

Table 2. Biomass (wet weight) of the main species of mesozooplankton from cyclonic and anticyclonic regions. % = as a percentage of total mesozooplankton biomass

Stn	<i>Calanus euxinus</i>		Calanoidea and other species		<i>Noctiluca miliaris</i>		Total biomass (mg m <sup>-2</sup> )
	(mg m <sup>-2</sup> )	(%)	(mg m <sup>-2</sup> )	(%)	(mg m <sup>-2</sup> )	(%)	
<b>Cyclonic regions</b>							
1	1454	68	410	19	260	12	2124
2	2283	59	788	20	768	20	3839
3	970	66	266	18	239	16	1475
Mean	1569	64	488	19	422	17	2479
<b>Anticyclonic regions</b>							
4	1430	40	459	12	1780	48	3669
5	1030	38	449	17	1188	44	2667
6	1070	37	896	31	908	32	2874
Mean	1177	38	601	20	1292	41	3070

Table 3. Abundance (ind. m<sup>-2</sup>) of different *Calanus euxinus* stages from cyclonic and anticyclonic regions. N = nauplii; C = copepodites

Stn	Eggs	NV	NVI	CI	CII	CIII	CIV	CV	Female	Male
<b>Cyclonic regions</b>										
1	4160	387.4	65	83.2	41.6	20.8	13.0	1136.2	473.2	31.2
2	9620	124.8	312	44.2	31.2	0	0	1991.6	647.4	10.4
3	12220	161.2	0	26	0	41.6	31.2	618.8	403.0	10.4
Mean	8667	224.5	125.7	51.13	24.27	20.8	14.7	1248.9	507.9	17.3
<b>Anticyclonic regions</b>										
4	7410	166.4	62.4	88.4	13.0	65	161.2	1034.8	452.4	52.0
5	8190	1690	0	0	0	10.4	10.4	410.8	543.4	83.2
6	9620	0	0	10.4	0	10.4	156	954.2	189.8	62.4
Mean	8407	619	20.8	32.9	4.33	28.6	109.2	780	395	66.0

dances of Stages I to IV copepodites was 5.5- to 12.2-fold less than Stage V copepodites.

Contents of TL and of the main lipid classes of *Calanus euxinus* females are shown in Fig. 4. TL concentrations from the cyclonic regions were on average 1.7-fold greater than in anticyclonic regions (101.9 and 58.8  $\mu\text{g ind.}^{-1}$ , respectively). A similar difference between the 2 regions was also shown for WE which make up 70 to 72% of TL. Concentrations of TAG (8 to 13% of TL), PL (13 to 15%) and S (3 to 5%) of *C. euxinus* females sampled from cyclonic regions were higher than those from anticyclonic areas. The level of *C. euxinus* TL responded to differences in chl *a* concentration ( $r = 0.95$ ,  $p < 0.005$ ; Fig. 5). Chl *a* also correlated well with WE contents ( $r = 0.89$ ,  $p < 0.05$ ). Neither TAG nor PL correlated with chl *a*, but both displayed

positive trends ( $r = 0.71$ ,  $p < 0.2$  and  $r = 0.61$ ,  $p < 0.2$ , respectively).

## DISCUSSION

In the Black Sea, density stratification of the upper layer in summer prevents the penetration of the bottom layers, which are rich in nutrients, into the surface waters. Nutrients transported from deeper waters by conventional winter 'mixing' are completely consumed during the winter-spring phytoplankton bloom. In fact, in 1996 the spring bloom was apparently over by May, when deep chlorophyll minima and low primary production rates were observed (Stelmakh et al. 1998, Yilmaz et al. 1998). In the open waters, phytoplankton growth in the post-bloom period is fuelled mainly by means of regenerated nutrients, and 'new' production can only occur if 'new' nutrients are brought from

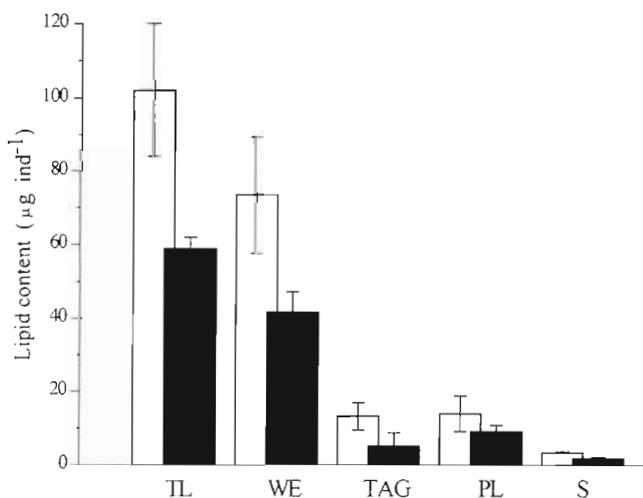


Fig. 4. Average content of total lipids (TL) and main lipid classes of *Calanus euxinus* females from cyclonic (open bars) and anticyclonic (dark bars) regions. WE = wax esters, TAG = triacylglycerols, PL = phospholipids, S = sterols. Error bars show 1 standard deviation

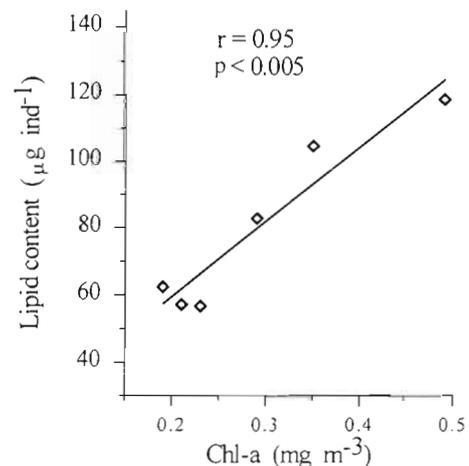


Fig. 5. Relationship of total lipid (TL) contents of *Calanus euxinus* females versus mean integrated concentrations of chl *a*

depth up into the lower strata of the euphotic zone (Goldman 1988).

The penetration of nutrients into the chl *a* containing layer of the Black Sea during summer stratification occurs in regions of cyclonic circulation, where doming is found. In this study, the increase in nutrient concentration started at 40 m, and the chl *a* containing layer extended down to 60 to 65 m within the cyclonic regions (Fig. 2). In contrast, the increase in nutrient concentration was not seen until 55 to 65 m in regions with anticyclonic circulation, i.e. only below the base of the chl *a* containing layer. In the present study, the close relationships found between depth of the  $\sigma_t = 16.2$  value and  $\text{NO}_3$ ,  $\text{PO}_4$  and chl *a* concentrations from both cyclonic and anticyclonic regions confirm the important role of hydrodynamics in ecosystem structure and productivity (Fig. 3). The higher phytoplankton biomass (deduced from both chl *a* and *in situ* fluorescence levels) in cyclonic regions (Table 1, Fig. 2) might indicate that the food of herbivorous zooplankton was more plentiful in this region.

Unlike phytoplankton, the total mesozooplankton biomass in the compared regions did not reveal striking differences. There were, however, considerable differences in the biomass ratios of the main species; i.e. the copepod *Calanus euxinus* versus the heterotrophic dinoflagellate *Noctiluca miliaris*. *C. euxinus* predominated in cyclonic regions, whereas in anticyclonic areas the biomass of *N. miliaris* was higher than that of *C. euxinus* (Table 2). The relationships between these 2 species and food supply (in terms of chl *a*) were also different: no relationship existed for the *C. euxinus* biomass, whereas a strong negative correlation was seen for *N. miliaris*.

In general, the biomass of zooplankton alone is considered to be a poor indicator of their nutritional condition and productivity (Raymont 1976, Parsons et al. 1984). In our case, one of the reasons for the lack of correlation between the *Calanus euxinus* biomass and chl *a* might be due to heavy predation on copepods by ctenophora (*Pleurobrachia rhodopsis* and *Mnemiopsis leidyi*), medusae and planktivorous fishes (Anninsky et al. 1998, Kovalev et al. 1998). However, the strong correlation between the biomass of *Noctiluca miliaris* and chl *a* may testify that, in contrast to *C. euxinus*, predators did not exert significant feeding pressure on *N. miliaris*. Indeed this dinoflagellate has no known predators in the Black Sea. At the same time it is known that the *N. miliaris* food ration may include copepod and fish eggs (Mironov 1954, Elbrächter & Qi 1998). But in our investigations we seldom observed eggs in the food vacuoles of *N. miliaris*, in which microalgal remains prevailed.

It is likely that in the Black Sea *Noctiluca miliaris* and *Calanus euxinus* can be considered as food competi-

tors. Both species appear to feed effectively on phytoplankton during night-time in surface waters, and diatoms are their preferred food items (Mironov 1954, Petipa 1965, 1981, Besiktepe et al. 1998, Elbrächter & Qi 1998). However, unlike *C. euxinus*, *N. miliaris* is able to grow and reproduce whilst feeding on smaller sizes of phytoplankton (5 to 25  $\mu\text{m}$ ) at a much lower food concentration (Mironov 1954, Petipa 1981, Elbrächter & Qi 1998). In our case such an ability might allow *N. miliaris* to avoid competitive pressure from *C. euxinus* in anticyclonic regions of low food abundance. It is worth noting that the biomass of *N. miliaris* increased from 422  $\text{mg m}^{-2}$  in cyclonic regions to 1292  $\text{mg m}^{-2}$  in anticyclonic regions (Table 2).

During the survey in September 1996 Stage V copepodites and females dominated the *Calanus euxinus* population (56 and 24 % of total numbers, respectively) in both compared regions (Table 3). Besiktepe et al. (1998) found that over 95 % of females made daily vertical migrations to feed in September. The presence of eggs in the net hauls testified that *C. euxinus* females had spawned and the relatively small numbers of nauplii and Stage I to III copepodites (Table 3) indicated, apparently, that the females had only begun to reproduce, no more than 1 wk earlier (Sazhina 1987).

Contents of TL (and their main classes, WE, PL, TAG and S) of actively feeding *Calanus euxinus* females reacted to variations in phytoplankton abundance (in terms of chl *a*) between the compared regions (Fig. 4). All lipid contents were higher in cyclonic regions. A significant correlation with chl *a* was found for TL ( $r = 0.95$ ,  $p < 0.005$ ; Fig. 5) and WE ( $r = 0.85$ ;  $p < 0.05$ ) contents, which account for most of the TL (over 70%; Fig. 4). The correlation between TAG (8 to 13 % of TL) and chl *a* was not significant ( $r = 0.71$ ,  $p < 0.2$ ) (Fig. 5). In June 1991 a close relationship between the chl *a* and lipid contents was found not only for female *C. euxinus*, but also for the feeding Stage V copepodites (Yuneva et al. 1997). Correlation coefficients between WE and chl *a* were high for both Stage V copepodites and females ( $r = 0.90$  and  $0.85$ , respectively,  $p < 0.005$ ), whereas those between TAG and chl *a* were not ( $r = 0.62$  and  $0.53$ , respectively,  $p > 0.2$ ). Similar results were obtained for WE and TAG contents of *C. pacificus* in their correlation with primary production (Håkanson 1987).

It has been shown that calanoid copepods have a marked propensity to convert their largely phytoplankton diet into WE, and WE content is the long-term indicator for the nutritional condition of many species of the genus *Calanus* (see review by Sargent & Henderson 1986). *C. euxinus* and the closely related *C. pacificus* and *C. helgolandicus* start to synthesise WE during at copepodite Stages III to IV (Lee et al. 1972, Håkanson 1984, Yuneva unpubl. data) reaching

a maximum at the Stage V copepodite (Lee et al. 1972, Håkanson 1984, Yuneva et al. 1997). In the laboratory, the duration of copepodite Stages IV and V of *C. euxinus* before becoming adult averages 2 wk (Sazhina 1987). The level of WE accumulation during these stages depends on the animal's food supply (Sargent et al. 1977, Håkanson 1984). Before moulting into females, Stage V copepodites of *C. euxinus*, as a rule, diapause for some time (exact time unknown) in the suboxic zone (Vinogradov et al. 1992a). During this period some of the energy derived from the stored WE may be invested for the development of gonads which will eventually be spent at spawning (Gatten et al. 1980, Sargent & Henderson 1986, Sargent & Falk-Peterson 1988). So, whereas the WE content of feeding Stage V copepodites reflects its feeding history, that of females may not, because of mobilization processes. In our investigation the level of WE mobilization of *C. euxinus* females was insignificant, because reproduction had only begun (deduced from the low number of early copepodite stages, Table 3), allowing us to obtain a close correlation between the chl *a* concentration and WE content. Therefore it is very likely that WE levels reflected differences in the nutritional condition of *C. euxinus* females in cyclonic and anticyclonic regions for at least several weeks prior to sampling.

Unlike WE, the TAG content may be considered as a measure of the immediate nutritional history of copepods over a period of several days (Sargent et al. 1977, Håkanson 1984). There are 2 main sources of TAG in *Calanus* spp. (Sargent & Henderson 1986). The first one is lipid contained in their food and, therefore, various factors, such as duration of feeding, ensembles of consumed phytoplankton, omnivorous feeding, etc., may influence TAG content. WEs, when they are modified to gonads (Gatten et al. 1980, Arashkevitch et al. 1998), are the second source of TAG which is the stor-

age lipid found in *Calanus* eggs (Lee et al. 1972, Sargent & Henderson 1986, Sargent & Falk-Peterson 1988). TAG is rapidly synthesised during *Calanus* feeding but it is also utilised quickly. Besides spawning, TAG is also spent during locomotion, especially if copepods are performing diurnal vertical migrations (Sargent & Falk-Peterson 1988). Differences in energy expenditure during locomotion and variations in egg reproduction rates under changeable environmental conditions may account for the high variability seen in the TAG content of *Calanus* species in this and previous studies (Håkanson 1987, Yuneva et al. 1997) and consequently for the absence of a correlation with phytoplankton abundance.

The daily energy expenditure of copepods may influence not only TAG, but also total lipid accumulation. If energy expenditure is high, a smaller portion of consumed food is used for lipid accumulation. For example, it has been suggested that low concentration of lipids in *Calanus* spp. in tropical regions may be linked not only with a poor food supply, but also with the energy cost of extensive vertical migration (Benson et al. 1972, Lee et al. 1972).

The depth of vertical migration of *Calanus euxinus* in the Black Sea depends on density stratification of water masses (Vinogradov et al. 1992a), and therefore it is different for cyclonic and anticyclonic regions. So during the summer stratification period *C. euxinus* females descend (after night feeding in warm and oxygen-enriched water) to deeper water with low temperature (7 to 8°C) and oxygen concentration (0.3 to 0.4 ml l<sup>-1</sup>) (Besiktepe et al. 1998). Water densities in these oxygen-depleted layers (= suboxic zone) are about  $\sigma_t = 15.6$  to 15.8 (Murray et al. 1995), corresponding to a depth of 65 to 84 m in cyclonic regions and to a depth of 120 to 158 m in anticyclonic regions (Fig. 2); thus the distance of vertical migrations of *C. euxinus* in anticyclonic areas is twice as far as that in cyclonic regions.

Using data from Besiktepe et al. (1998) on timing of ascent and descent of *Calanus euxinus* females from September 1995, we could depict diel vertical migration patterns for cyclonic and anticyclonic regions (Fig. 6). Apparently not only the distance of migration, but also the timing of descent and ascent as well as the duration spent at depth differs to a certain degree for copepods from different regions. All these variables may affect its total daily expenditure.

Measurements of basal and active metabolism, in terms of oxygen consumption rates, made under different temperature and oxygen concentrations for *Calanus euxinus* females have been reported elsewhere (Svetlichny & Gubareva 1998). Combining this data with temperature (Fig. 2) and oxygen concentration profiles typical for cyclonic and anticyclonic regions (Bastürk et al. 1994), Svetlichny et al. (unpubl. data)

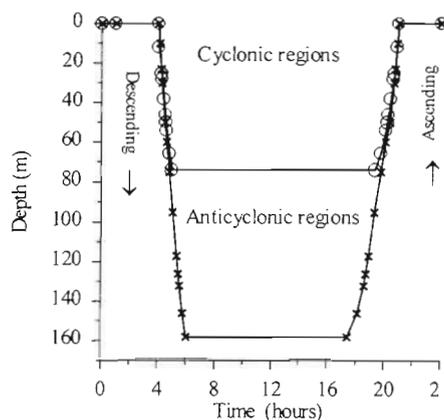


Fig. 6. Diurnal vertical migration patterns of *Calanus euxinus* females in cyclonic (O) and anticyclonic (x) zones

discovered that daily energy expenditure of *C. euxinus* females varied from 18.6 to 21.0  $\mu\text{g O}_2 \text{mg}^{-1}$  wet weight in cyclonic and anticyclonic regions, respectively. So the differences in the daily energy expenditure between the 2 regions were not large (<12%) and would not be expected to greatly affect the level of food assimilation by copepods.

Thus differences in the accumulation of lipid reserves in *Calanus euxinus* in cyclonic and anticyclonic regions of the Black Sea are largely due to variations in the levels of phytoplankton food in the 2 areas. These, in turn, can be attributed to differences in the hydrodynamics and supply of nutrients to the chl *a* containing layer.

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