1. Introduction

Calanus euxinus is the most dominant species in mesozooplankton of the Black Sea. It accounts for over one-third of the total zooplankton biomass [1,2], and its abundance in the deep aggregation layer can reach 2000-3000 ind. m^{-3} [3,4].

C. euxinus is present in the aerobiotic zone of the Black Sea during all seasons. It reproduces throughout the year, with maximum reproduction occurring in early spring [5]. Like most temporal and polar *Calanus* species, the life cycle of *C. euxinus* includes a diapause phase. After the winter-spring phytoplankton bloom, a part of the population enters the diapause.

The older copepodite stages of *C. euxinus* concentrate in the narrow layer near the lower limit of the oxygen zone during daytime[4,6]. Whilst some of these specimens ascends to the surface during nighttime, the remaining fraction, consisting mainly of the fifth copepodite stages (CVs), permanently stays at depth. These non-migrating copepods are assumed to be diapausing [7].

It is well known that the diapausing calanoid copepods differ in respiration and excretion rates, lipid content, mobility and feeding behaviour from the active copepods [8-10]. In contrast to oceanic *Calanus* species which have been studied very intensively, the life cycle of *C. euxinus* including its diapause phase as well as physiological peculiarities connected with the transition from active state to diapause have been poorly investigated. There are at least two reasons of that: The first, in spite of the long history of the investigation of the Black sea zooplankton, the diapause phase in *C.euxinus* was reported only in the eighties in the course of observations using submersibles and 150 L water-bottles [4,6,7]. And the second, this is due to the fact that during daytime the older copepodites concentrate in the narrow layer near the lower boundary of the oxygen zone and it is often impossible to separate diapausing and active copepods in the net samples. During nighttime the separation of diapausing part of the population from active one is much easier, because the former stays at depth while the latter ascends to the surface.

Physiological and ecological changes, connected with the initiation and termination of diapause, is of interest in two aspects. On the one hand, these changes, especially when occurring in the dominant species, determine the ecological parameters of the whole community: such as characteristics of trophic interactions, the impact on phytoplankton standing stock, energy flux and so on. On the other hand, investigation of the physiology of the diapausing copepod is important from the point of view of population and evolutional ecology as a diapause appears to be a main adaptation towards allowing herbivorous zooplankters to survive during unfavourable environmental conditions.

The present study was aimed at the analysis of the main ecological and physiological characteristics of *Calanus euxinus*: 1) vertical distribution, age structure and egg production; 2) the stage of maturity of adult females in relation to their feeding and lipid content; 3) feeding and locomotor activities, respiration and excretion rates, gonad development state, lipid content, and size structure in active and diapausing CVs.

2. Materials & methods

The study was carried out during cruise R/V Bilim in September-October 1996 (Table 1). Zooplankton was collected with a closing Nansen net (mouth opening 70 cm, mesh size 112 μm). Two layers were sampled: the surface layer (from 0 to 50 m) and the deep layer (50 m water depth above lower border of the oxygen zone). Samples for the analyses of gonad maturity, fatness and gut content as well as age and size structure of the population, were preserved with 4% buffered formaldehyde. Copepods to be used for experimental purposes were sorted and transferred into jars filled with filtered cooled sea water.

TABLE 1. Sampling stations during September-October 1996 Black Sea cruise.

Stations	Latitute (N)	Longitute (E)
M10L15	42.10	29.15
M50T15	42.50	36.15
M50V15	42.50	37.15
L37K40	41.37	28.40
M50Q30	42.50	32.30
M10T15	42.10	36.15
L50X45	41.50	39.45
M10K45	42.10	28.45
L30X45	41.30	39.45

Feeding rates of *C. euxinus* females and CVs was studied in incubation experiments. 30-50 copepods were placed in 3 litres ambient sea water from the depth of maximum fluorescence. They were kept in a refrigerator at 8°C for 24 h, and the difference between the initial and final chlorophyll-a concentration was estimated. After each experiment, released faecal pellets and, in the case of females, eggs were counted. The faecal pellets contents were studied under the light microscope at x600 magnification.

Two indices were used for the description of feeding activity in the sea: percentage of copepods with food in the gut, and food pellet size in the posterior section of the mid-gut.

For investigation of gonads we used the staining technique [11]. The copepods were stained in a 2% Borax carmine solution in filtered sea water, dehydrated in ethanol and cleared in cedar oil. Before staining, the prosome length and oil sac size were measured with a dissecting microscope at x32 magnification. The oil sac volume (V) was estimated as; $V = 1/6 \times L_o \times 3.14 \times d_o^2$, where, L_o is the oil sac length (mm), d_o is the oil sac width (mm). The copepod body volume (W, mm^3) was determined as; $W = 0.46 \times L_c \times d_c^2$, where, L_c is the body length (mm), d_c is the body width (mm). The wet weight (P,mg) was defined from $P = Wq$, where q is specific weight [12].

Respiration and ammonia excretion rates were measured under different oxygen concentrations across the range of 0.25-10 mg O_2 l^{-1} at 8°C. Decreased oxygen concentrations were obtained by bubbling nitrogen through the filtered sea water. 5 individuals were placed in a 5 ml glass syringe. Prior to a 2-3 h incubation period, each experimental syringe was connected to a control syringe with a fine tube. Then the water was pumped several times through a pair of syringes to achieve equal initial oxygen concentration in both syringes. Oxygen concentration was defined using a polarographic oxygen sensor, joined with the measuring chamber of 0.5 ml volume. The ammonia concentration was determined using the phenolhypochlorite method [13].

The parameters of locomotor activity were measured in animals attached to a semiconductor force sensor in a hermetic chamber [14]. The copepods were glued with cyacrin to a sensor by the dorsal part of the cephalon. An oxygen concentration range of 0.25-10 mg O_2 l^{-1} was obtained by circulating oxygen-deficient sea water through the chamber. One individual was maintained at each oxygen concentration for 10 min with simultaneous recording of locomotor parameters. The experiments lasted between 90-120 min. The mechanical efforts of the copepod's appendages were registered as digital electric signals on the computer floppy-disk by the cantilever semiconductor force sensor. The mean frequency (F, Hz) of impulses in locomotor patterns was estimated. The mean active locomotor period (T, %) was defined as a percentage of total time.

3. Results

3.1. AGE STRUCTURE AND VERTICAL DISTRIBUTION

During the survey *Calanus euximus* population was dominated by females, copepodid stage Vs and nauplii (Fig.1). A large amount of eggs were found in the upper layer. Nauplii and younger juvenile stages (CIs-CIIIs) were located in the upper 50 m both in the day and night time. Adults and CIVs showed vertical migrations from the deep layer by day to to the upper layer by night. Copepodid stage V consisted of two groups: one of them was concentrated in the deeper layer both day and night, the other underwent dial vertical migrations and its distribution was similar to that of adults. Sometimes a few adults were found in the deeper layer at night. The state of their gonads, however, suggested that they were spent or seemed to be dying specimens.

3.2. FEEDING AND EGG PRODUCTION

Shipboard experiments assessed feeding activity of *C. euximus* on natural sea water (Table 2). Experiments were conducted only with females and CVs collected from surface waters in the night time. All of the experiments showed extremely low rate of both ingestion of chlorophyll and faecal pellet production. No relation between above mentioned indices was noticed. The largest amount of faecal pellets was detected in the

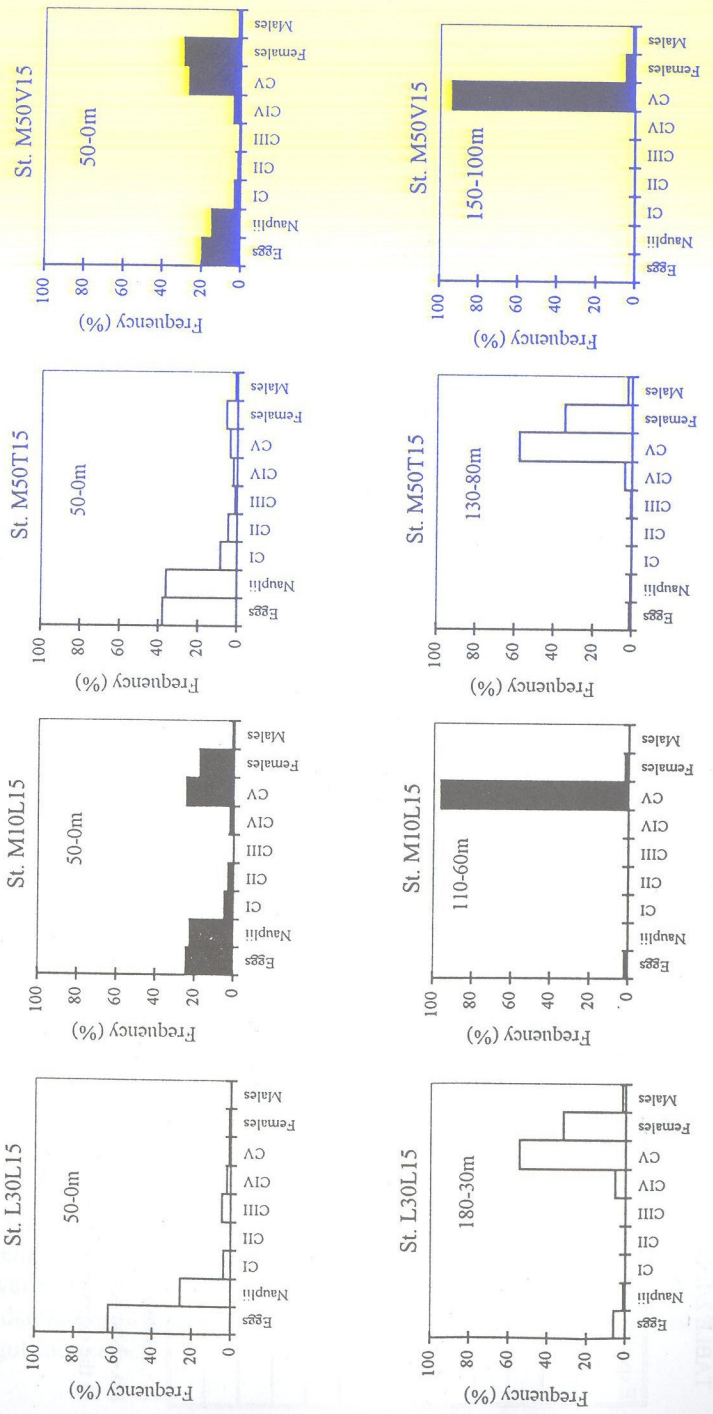

Figure 1. Age structure and vertical distribution of *Calanus euximus* at different stations in daytime (white bars) and at night (black bars).

TABLE 2. Laboratory feeding experiment with *Calanus euxinus* and natural whole-water plankton. Daily (mean+SD) ingestion of chlorophyll-a, faecal pellets production, and egg production.

Experiment	Station	Date	N	Stage	No.	Initial Chl-a (µg/L)	Final Chl-a (µg/L)	Ingestion (ng Chl/ind/d)	Faecal pellets, (no/ind/d)	Eggs, (no/fem./d)
1	L37K40	Sept., 26	Control	-	-	0.63	0.46			
			1	CVs	40		0.43	5.31	1.32	-
			3	Females	30		0.39±0.03	14.33±2.08	4.16±0.93	2.86±0.61
2	M50Q30	Sept., 28	Control	-	-	0.88	0.77			
			2	CVs	50		0.70±0.04	7.41±3.39	4.35±0.15	-
			2	Females	50		0.62±0.06	11.92±3.82	4.67±0.52	1.92±0.73
3	M10T15	Oct., 2	Control	-	-	0.21	0.25			
			1	CVs	50		0.26	0	0.42	-
			3	Females	30		0.18±0.02	5.67±2.31	18.79±3.05	11.04±6.08
4	L50X45	Oct., 4	Control	-	-	0.25	0.32			
			1	CVs	50		0.24	2.39	1.04	-
			3	Females	50		0.17±0.02	6.79±0.92	8.41±1.54	5.28±1.13

N - Number of experimental bottles
No.- Number of specimens per grazing bottles
In the experiment 1 copepods from st. M10K45 were used

experiment No 3 (Table 2). According to microscopical analysis they consisted predominantly of debris and remains of microzooplankton.

Based on the experiments the average daily rates of egg production were 2-11 eggs per females (Table 2), but really spawning females released from 5 to 25 eggs/day taking into account that from 20 to 50% of females in the experiments were immature or spent.

In the sea the number of *Calanus* containing food varied both with the location and the time (Table 3). At night the percentage of copepods with food in the gut changed from 12 to 88 among females and from 10 to 97 among CVs in the upper 50 m being less than 1 among CVs occupying the deeper layer. During daytime the descent of actively feeding CVs into the depth resulted in the increase of the percentage of CVs with food up to 32. Gut fullness assessed here as the length of food pellets in the posterior part of the midgut changed greatly also depending on the location and the time (Table 3). The values of the index were much higher at the stations where a higher percentage of copepods with food was found out.

Table 3. Percentage of feeding *Calanus* at different stations and gut fullness expressed as a length (mean±SD)of food pellets in a posterior part of the midgut

Station	Time	Depth (m)	Stage	No. of *Calanus* examined	Percent with food	Length of food pellet (µm)
L30L15	16:00	180-120	CVs	100	32	270±136
			Females	60	63.3	307±134
L37K40	00:10	50-0	CVs	25	64	549±39
			Females	50	86	617±60
M10K45	04:15	50-0	CVs	20	15	<100
			Females	50	26	132±47
M50Q30	21:30	40-0	CVs	30	10	<100
			Females	50	12	108±49
	22:00	115-70	CVs	100	1	350
M10T15	03:00	50-0	CVs	24	29.2	337±38
			Females	50	76	532±95
	03:20	150-100	CVs	100	0	-
M50V15	22:00	50-0	CVs	60	18.3	189±98
			Females	70	18.6	243±94
L50X45	20:10	50-0	CVs	20	30	267±52
			Females	50	88	607±63
	20:30	200-150	CVs	50	0	-
L30X45	19:00	25-0	CVs	18	83.3	554±53
			Females	22	81.8	569±43
	23:00	25-0	CVs	34	97	468±92
			Females	35	88.6	571±116

Up to 60% of gut contents consisted of an unidentifiable greenish or brownish debris. The recognizable remains were parts of crustacean exosceletons and small dinoflagellates. *Ceratium* and *Rhizosolenia* being in the large amount in the plankton have never been found in the guts of *Calanus*. Food remains were never observed in the guts of diapausing copepods; the walls of the copepods' guts were so compressed that the midgut looked like a narrow tube.

3.3. SIZE STRUCTURE

The size classes distribution analysis made it possible to distinct two size groups of CVs collected at night from the upper layer as well as from the deeper layer (Fig.2). Almost all of the diapausing CVs belonging to the bigger size group had small gonads and large oil sacs. In order to establish whether the bimodal size distribution reflected the size division of future males and females the prosome length of stained copepodids was sized following the sex determination (Fig.3). No difference between the prosome length of male and female CVs was found. Thus the bimodal size distribution seems to point at the age heterogeneity of this stage.

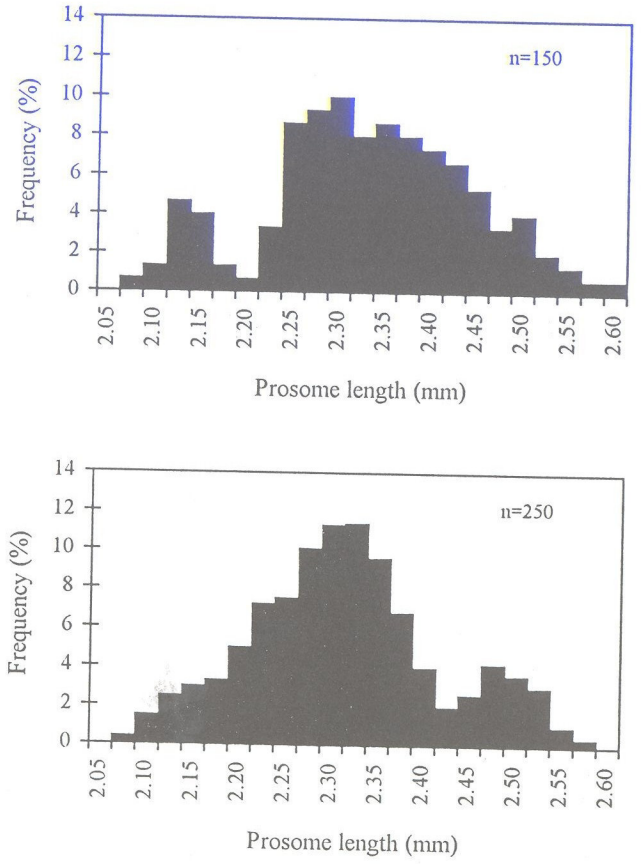

Figure 2. Size classes distribution in CVs *Calanus euxinus* collected at night from the upper layer at the St. M50V15 (the upper figure) and from the deeper layer at the St. L30X45 (the bottom figure).

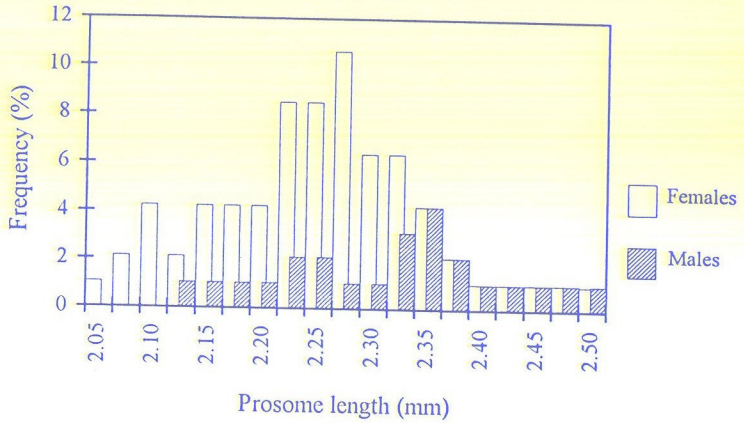

Figure 3. Size classes distribution in the future females and males at copepodite V stage of *C. euxinus*.

3.4. GONAD DEVELOPMENT

In *Calanus euxinus* visible gonads appear at the fifth copepodite stage or more seldom at the forth stage. At the earlier state of development prior to sex differentiation the genital system consists of a small gonad having flask- or pear-shape form. At that state the gonads are no longer than 100-150 μm. While developing the gonads elongate. In the future females can be seen cylindrical ovary, two oviducts and diverticula; the male CV system shows relatively well developed testis and the single left duct.

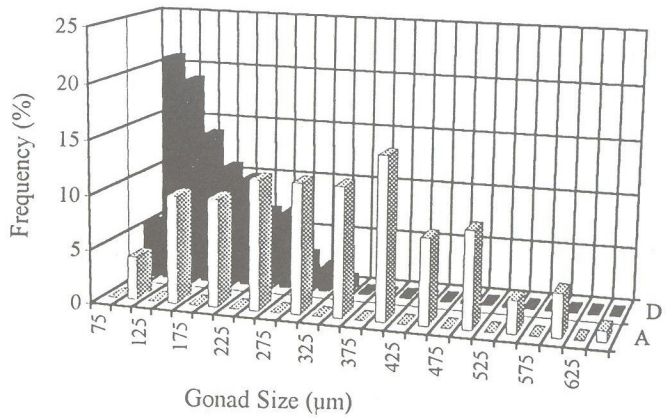

Figure 4. Gonad size classes distribution in active (A) and diapausing (D) CVs at the St. L30X45.

The size frequency distribution of gonads in surface (active) CVs and deep living (diapausing) CVs was differed (Fig.4). In the active CVs the gonad size ranged from 75 to 650 mkm. Among the diapausing CVs small gonads (100-150 mkm) before sex differentiation prevailed; the gonad length never reached more than 350 mkm.

A relationship between oil content and gonad size is shown in Fig.5. When gonads increased in size, oil content decreased in deep living CVs. Among the active (migrating to the surface) CVs we could see just moulted specimens with small gonads and almost without oil storage. While growing up, part of them showed maximum sizes in gonads and little oil content. The others reached a maximum in oil sac size but had small, sex undifferentiated gonads.

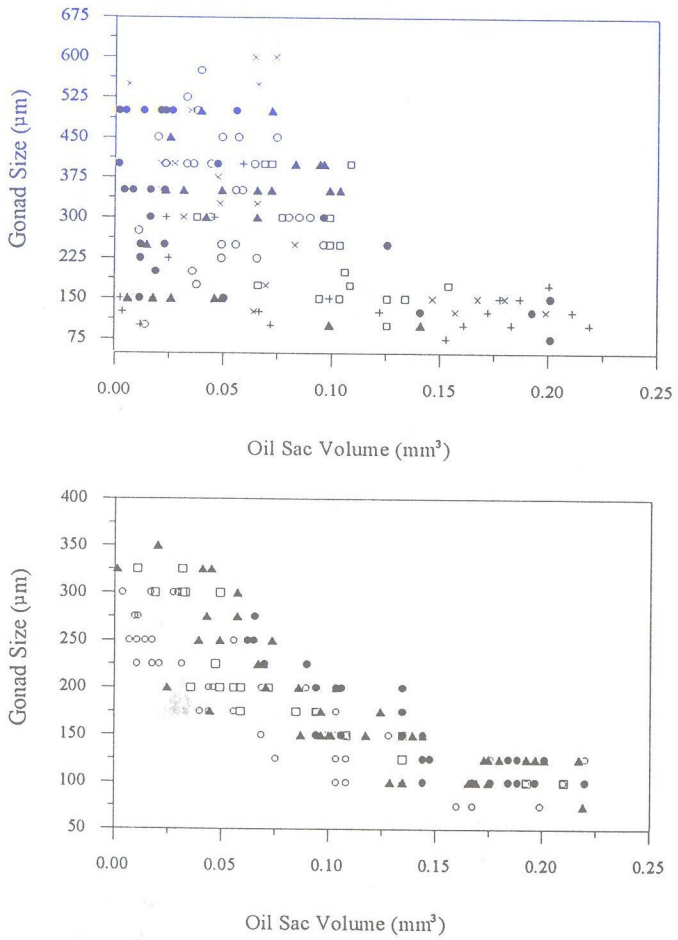

Figure 5. Relationship between gonad size and oil sac volume in CVs *C. euxinus* collected at night from the upper layer (the upper figure) and from the deeper layer (the bottom figure) at different stations.

3.5. RESPIRATION AND EXCRETION

Both active and diapausing CVs had weakly dependent type of respiration when oxygen concentrations changed from the saturation to values of approximately 3 mg/l (active CVs) and 2 mg/l (diapausing CVs) (Fig.6). At lower oxygen concentrations the respiration rate reduced sharply. Within the range of oxygen concentrations from 8.7 to 0.3 mg/l the respiration rate decreased from 0.5 to 0.14 mkg O_2/mg wet weight/h in active CVs and from 0.23 to 0.064 mkg O_2/mg wet weight/h in diapausing CVs. Diapausing copepodids became torpid at lower oxygen concentrations, 0.3 and 0.5 mg O_2/l, correspondingly.

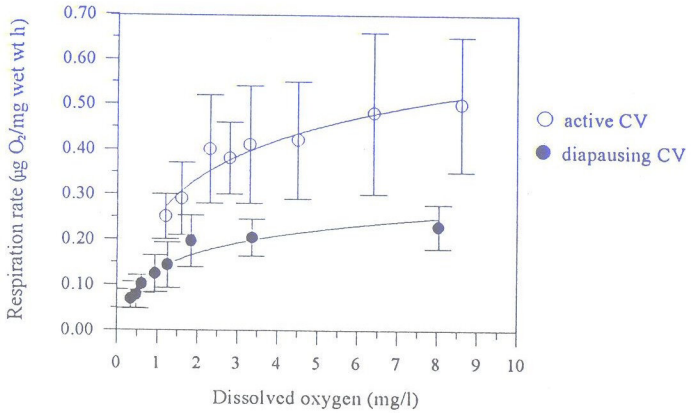

Figure 6. Respiration of CVs of *Calanus euxinus* at different ambient oxygen concentrations.

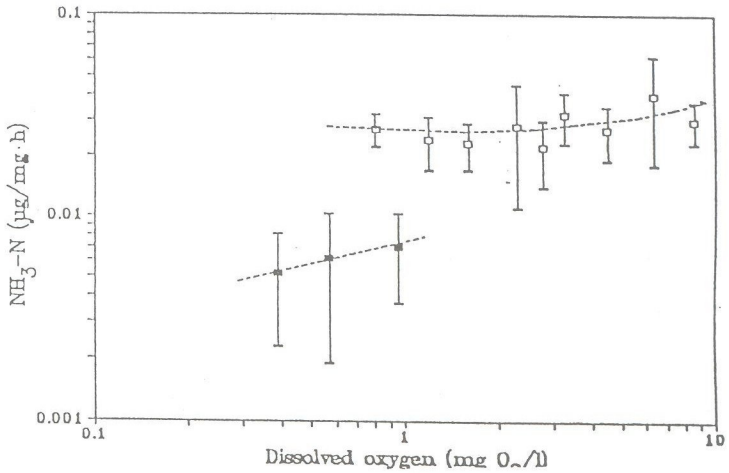

Figure 7. Ammonia excretion rate in active CVs (open squares) and diapausing CVs (filled squares) of *Calanus euxinus* at different ambient oxygen concentrations.

Ammonia excretion rate in active CVs was much higher than that in diapausing CVs - approximately 0.02 and 0.006 mkg NH3-N/mg wet weight/h correspondingly (Fig.7).

3.6. LOCOMOTOR ACTIVITY

Oxygen deficiency influenced upon the frequency of neurogenic rhythm of mouth appendages locomotion (Fig.8). In active CVs it decreased proportionally to oxygen concentration from 22.5 to 11.5 Hz; in diapausing CVs - from 12.3 to 7.5 Hz. Unlike the elementary motor activity, the behavioral reaction of copepods to hypoxia was manifested as the increase of the total duration of locomotor activity (Fig.9). When oxygen concentration declined to 0.1 mg O_2/l, the mouth appendages locomotion became nearly persistent. Such sharp behavioral response seems to be the compensation of reduction of locomotor speed under oxygen deficiency conditions.

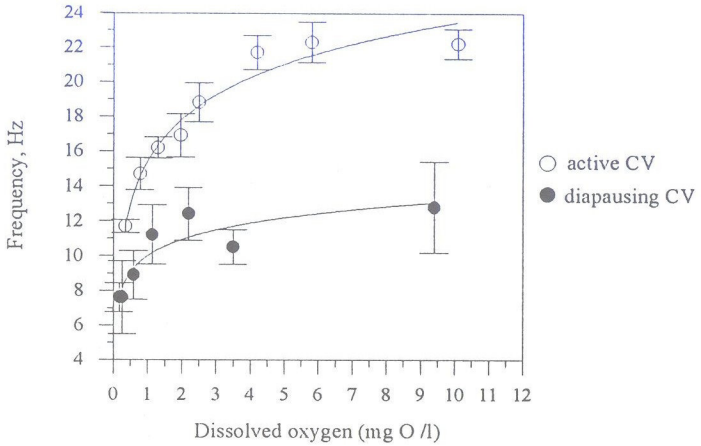

Figure 8. Rhythm frequency of mouth appendages locomotion.

4. Discussion

During our investigation the *C.euxinus* population was divided into two: active individuals, represented by all developmental stages, and diapausing CVs. The nauplii and younger copepodites inhabited the upper layer throughout the day. The older stages (CIV-CVI), being in the active phase, underwent daily vertical migrations, while the diapausing CVs stayed at depth all day long. Sometimes a few adults and CVs with food were found among the diapausing copepods staying in the deep layer at night. However, the state of their gonads suggested that these specimens were not diapausing but were either newly molted copepods, spent females or dying individuals.

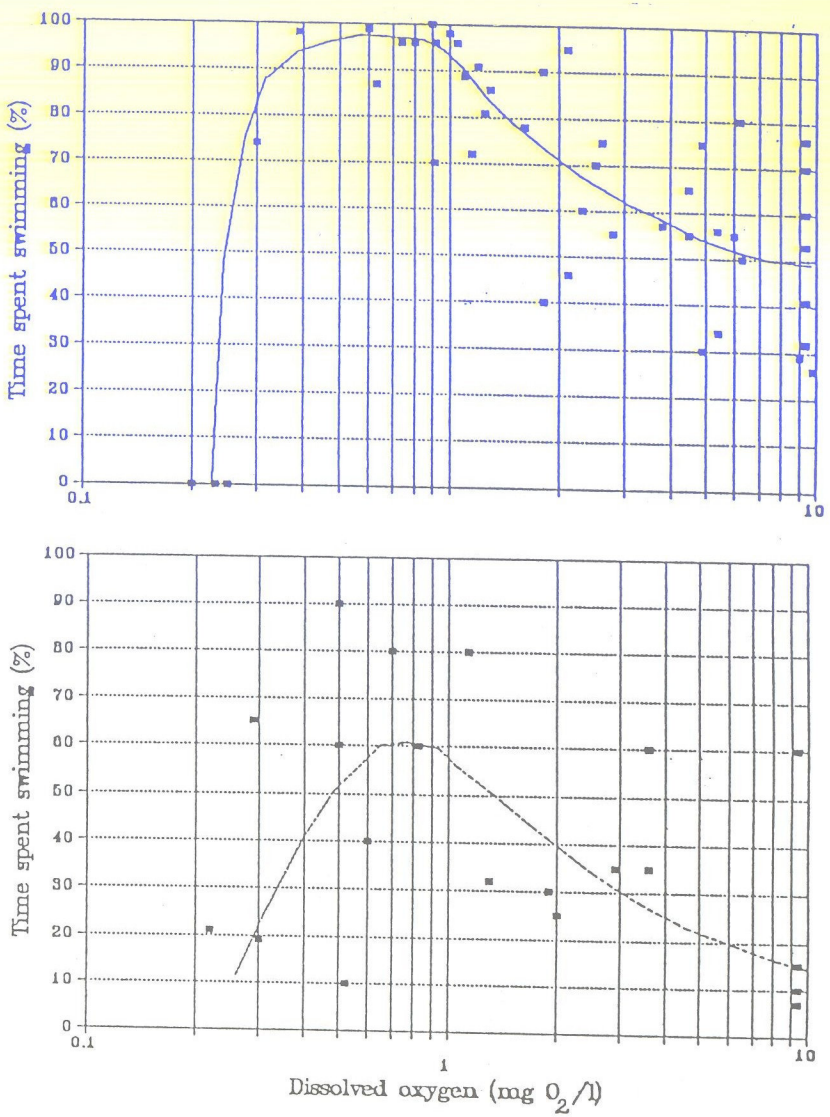

Figure 9. Time spent swimming (percentage of total time) in active (upper figure) and diapausing (bottom figure) CVs of *Calanus euxinus*.

During the survey, the population seemed to reproduce actively indicated by the large number of eggs and nauplii in the samples as well as the large proportion of mature females (50-80% of the total number of adult females). A relationship was found to be present between egg production and feeding activity of females in the field. In contrast, no such relationship was observed in experimental animals. The reason for this is explained by the fact that in the laboratory only consumed phytoplankton was determined whereas in the field the copepods' diet at that time consisted mainly of detritus, debris and small planktonic animals. Egg production rate ranged from 5 to 25 eggs/female/day. Thus, there were two possible reasons why *Calanus* sustained relatively high egg production: the heterotrophic microplankton, ciliates and dinoflagellates, formed a disproportionate fraction of the diet, or their feeding behavior was switched from herbivory to carnivory.

The analysis of size class distribution allowed the distinction of two groups among diapausing CVs, which were likely to belong to different generations. All copepods belonging to the larger group had small undifferentiated gonads and large lipid sacs. As no difference between the prosome length of CV males and females was found we assume that these larger and fatter copepods grew and developed under more favourable food conditions.

A relationship between gonad size and oil sac volume in diapausing CVs was found. An increase in gonad size was accompanied by a decrease in oil sac volume which suggests that gonad development occurs at the expense of stored lipids. Two strategies employed by the surface CVs were distinguished: 1) accumulation of lipids with no gonad development, and 2) gonad development without lipid storage. The group of CVs stage exhibiting the former strategy is likely to enter diapause whereas the group adopting the second strategy is composed of the active CV individuals.

The diapausing and active CVs differed considerably in their motor activity, respiration and excretion rates. Under all oxygen concentrations studied, these indices for active CVs were approximately twice as high as for diapausing ones.

The daytime aggregation of *C. euxinus* is known to have a two-layer structure. The lower layer formed by diapausing CVs is located where the oxygen concentration is 0.21-0.29 mg l^{-1}. The upper layer consisting of migrating CVs and adults, is located at the point where oxygen concentration is 0.26-0.76 mg l^{-1} [7]. As the diapausing copepods have a lower specific weight than the active ones [12], the mechanism allowing diapausing CVs to concentrate in the lower layer is not yet clear. Based on our experiments, a higher sensitivity to oxygen deficiency in active CVs than in diapausing ones could be suggested. The former group became torpid at oxygen concentrations below 0.35-0.40 mg l^{-1}, the latter at oxygen concentrations 0.15-0.20 mg l^{-1}. To prevent sinking into layers where oxygen conditions are unfavourable, copepods increase their motor activity which reaches a maximum at the oxygen concentration close to the lethal for them.

At present little is known about formation, duration and termination of the diapause phase in *C.euxinus*. Our data on gonad development together with analyses of body lipid content and size structure for both diapausing and active CVs suggest that the formation

of diapausal stock occurs not only in spring but additionally that each summer-autumn generation is likely to separate into two groups: active and diapausing. The development of gonads marks the termination of the diapause phase.

5. Acknowledgments. This work was supported by the Turkish Scientific and Technical Research Council (TUBITAK), and by the Scientific Affairs Division of NATO within the framework of the Science for Stability Programme.

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