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## Acoustical identification of the concentration layer of a copepod species, *Calanus euxinus*

Received: 25 June 2002 / Accepted: 30 October 2002 / Published online: 19 December 2002  
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**Abstract** Swimming trajectories of *Calanus euxinus* Hulsemann in the Black Sea were studied using an echosounder at 120 and 200 kHz. *C. euxinus* were acoustically discriminated with respect to vertical migration and swimming speed, according to dissolved oxygen (DO) concentration and the timing of migrations. Species became torpid in water with DO values  $<0.5 \text{ mg l}^{-1}$ . The time spent swimming under DO conditions between 2 and  $5 \text{ mg l}^{-1}$  was insignificant, and varied greatly from the 10% to 25% of total time spent swimming under normoxic conditions ( $5\text{--}10 \text{ mg l}^{-1}$ ). *C. euxinus* formed a concentration layer in the water of 1–3 m thickness. Upward migration was completed in about 3.5 h, starting 2.5 h before and ending 1 h after sunset (average rate:  $0.95 \text{ cm s}^{-1}$ ) in summer. Species ascended discretely from the suboxic to the lower boundary of the cold intermediate layer (CIL) at  $0.82 \text{ cm s}^{-1}$ , and passed up the CIL and thermocline fast ( $2.3 \text{ cm s}^{-1}$ ). Downward migration took less time (2 h), starting  $\sim 1$  h before and ending  $\sim 1$  h after sunrise. Swimming speed within the thermocline and CIL was  $2.7 \text{ cm s}^{-1}$ ; copepods subsequently returned to daylight depth at a sinking speed of  $0.57 \text{ cm s}^{-1}$ . Total time for *C. euxinus* to settle to their nocturnal depth layer was about 5 h.

impractical with present acoustical knowledge and techniques, even though bioacoustics have functioned well in visualizing movement under water (Wiebe et al. 1990; Mutlu 1996; Stanton et al. 1996; Monger et al. 1998; David et al. 1999; Benoit-Bird and Au 2001; Brierley et al. 2001). Integrating such techniques with previously obtained background knowledge on characteristics specific to certain organisms may, therefore, be the most fruitful strategy for species identification. For instance, *Calanus euxinus* have distinct patterns of vertical migration and time spent swimming, depending on the DO concentration of the water column, as described by Arashkevich et al. (1998) and Svetlichny et al. (2000) for Black Sea copepods. Svetlichny et al. (2000) found that time spent swimming ( $T$ , %) did not depend on water temperature. Under normoxic conditions  $T$  varied widely from 15–20% to 90–95%. However, when oxygen concentration declined to the values characterizing *C. euxinus*' daytime habitat at depth ( $0.8\text{--}1.15 \text{ mg O}_2 \text{ l}^{-1}$ ),  $T$  of all investigated animals increased to 80–100% (Fig. 1).

The aim of the present work was to be able to identify concentration layers of *C. euxinus* in the Black Sea from acoustical records, based on swimming behavior in response to dissolved oxygen during vertical migration. This ability would allow in situ monitoring of the species; estimation of biomass and spatio-temporal distributional patterns during the day could then be considered in future work.

### Introduction

Acoustical studies have been used to record the progress of mesozooplankton and gelatinous macrozooplankton. Direct identification of organisms, however, appears

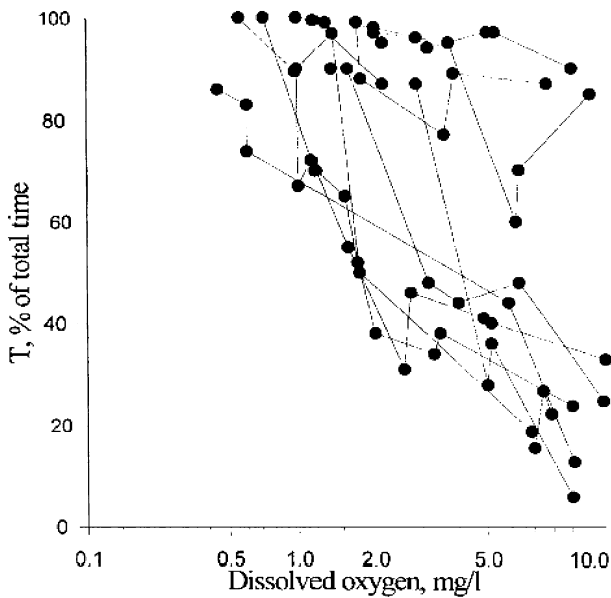
### Materials and methods

Acoustical data collected from the Black Sea in June 1991, October 1999 and July 2000 (Fig. 2) were evaluated to discriminate and identify concentration layers of the copepod *Calanus euxinus* Hulsemann during their vertical migratory loops in response to DO concentration in the water column. Acoustic data were collected with a scientific echosounder (BioSonic, model 102) at 120 kHz (October 1999, July 2000) and 200 kHz (June 1991, October 1999 and July 2000). The echosounder parameters were calibrated with a spherical ball of tungsten, and the transmitter of the echosounder was disabled to estimate the background noise that would be used to set the signal-to-noise threshold during post-processing of the

Communicated by O. Kinne, Oldendorf/Luhe

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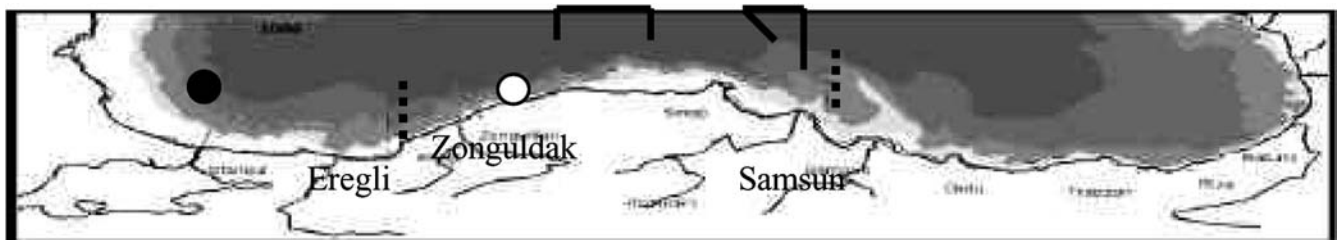
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**Fig. 1** *Calanus euxinus*. Relation of time spent swimming (% of total time) to oxygen concentration (Svetlichny et al. 2000)

data. During acoustical recording, Nansen Rosette and CTD probes (SeaBird) were cast to measure physical parameters and to take water samples for measuring dissolved oxygen with the Winkler titration method. Layered samples of meso- and macrozooplankton were collected at the stations with a Nansen opening and closing net (112  $\mu\text{m}$  mesh size and 0.7 m diameter mouth opening) in October 1999 and July 2000, during acoustical recording (Fig. 1). The depth ranges for the layered samples in October 1999 were 0–30, 30–60, 60–90, 90–120 and 120–150 m (see Table 1 for the depth ranges in July 2000). Furthermore, data from a study (Mutlu, unpublished data) on vertical distribution and on daily migration of the copepodites, females and males of *C. euxinus* were considered. In the above study, a series of discrete vertical hauls was made at a speed of 1  $\text{cm s}^{-1}$ , from the top of the anoxic layer to the surface (0–150 m) at 15-m intervals over a time series (0030–0130, 0130–0230, 0930–1030, 1730–1830, 2030–2130 and 2130–2230 hours). Recognition of the *C. euxinus* layer was based on the percent total time spent swimming against the DO concentration (Fig. 1; Svetlichny et al. 2000). Percent total time spent swimming, relative to total time (time needed to complete the migration), represents the time that *C. euxinus* requires to pass through a water layer with a specific concentration of dissolved oxygen. Svetlichny et al. (2000) estimated the speed of routine (active) swimming of *C. euxinus* by considering the movements of their mouthpart appendages; their experiments were repeated under conditions at oxygen concentrations within a range of about

**Fig. 2** Locations (thick black line June 1991; black circle October 1999; open circle and discrete lines July 2000) of acoustic studies in the Black Sea (circle fixed stations)



0.5–10  $\text{mg O}_2 \text{ l}^{-1}$ . Accordingly, their results showed the relation of the time the species spent swimming (percent of total time) relative to oxygen concentration.

In the present study, migration paths of the species as a function of depth and time were determined from the acoustic records at locations as close as possible to where DO concentrations were measured (profiles of water oxygen were obtained at the fixed stations in October 1999 and July 2000). Total migration time was calculated from the paths, and corresponded to 100% of the total time. Relative to total time, percent time spent at the depth where the oxygen was measured was then estimated. Accordingly, curves of time spent swimming, obtained from the enhanced echograms, were plotted against DO concentration. Passive swimming of the species was calculated from the difference between swimming speed during downward migration and speed during upward migration, as described by Svetlichny et al. (2000).

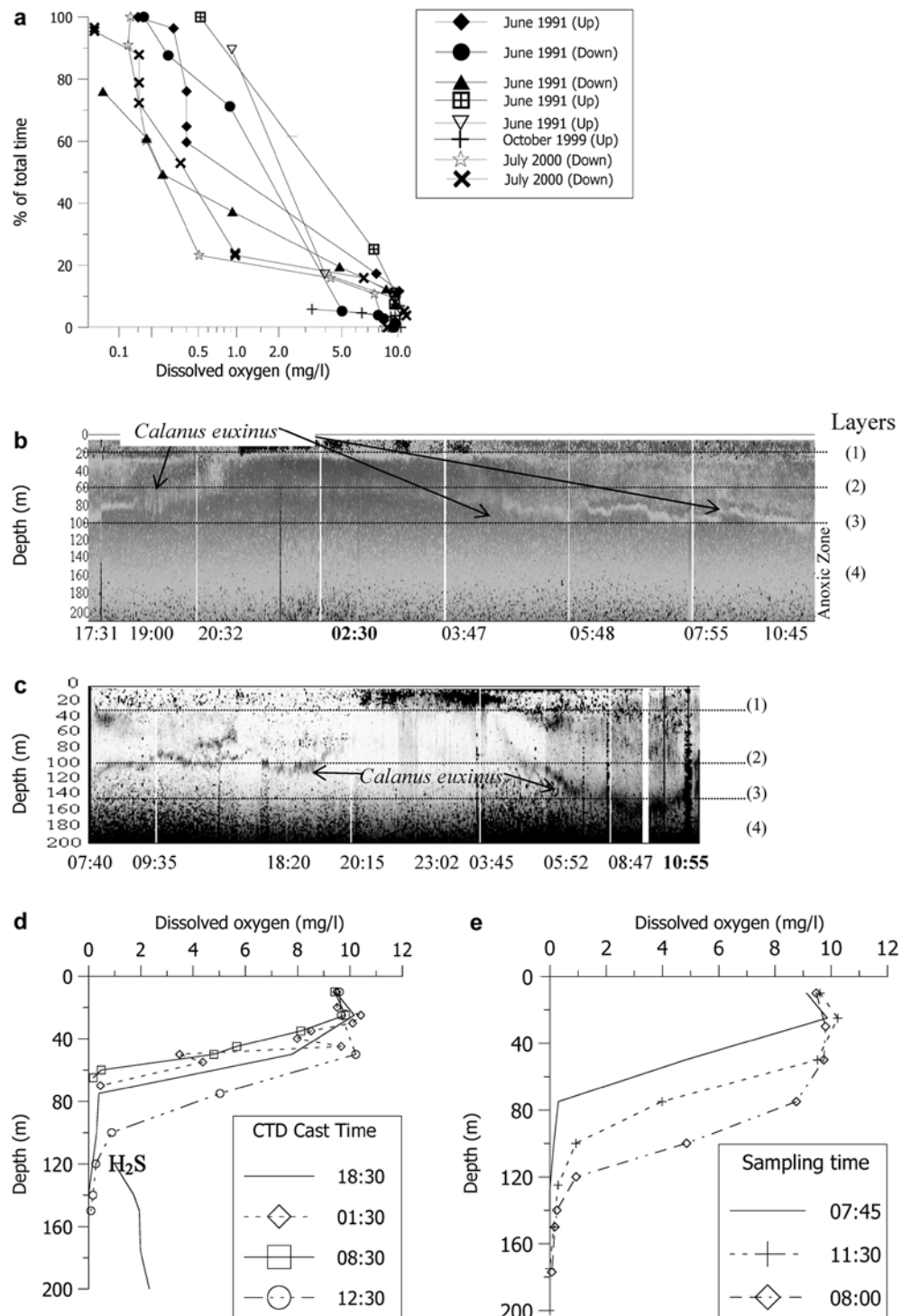
## Results

The time *Calanus euxinus* spent swimming in water with  $< 0.5 \text{ mg DO l}^{-1}$  comprised  $> 50\%$  of the total time needed to complete either upward or downward migration. *C. euxinus* appeared torpid or motionless at these concentrations; this was also occasionally true in water with DO concentrations of up to 1  $\text{mg l}^{-1}$ . The swimming time varied between 15% and 25% when the DO was between 1 and 5  $\text{mg l}^{-1}$ . When the DO reached a concentration of  $> 5 \text{ mg l}^{-1}$ , the time was  $< 10\%$ , resulting in maximum speeds of *C. euxinus* of 2.8–3.0  $\text{cm s}^{-1}$  (Fig. 3a). According to the descriptions above of the copepod's swimming behavior through water with different concentrations of DO and using the acoustic records, *C. euxinus* formed a 2–3 m thick concentration layer in the water. In general, upward migration of the species was completed in about 3.5 h, starting at 1730 hours and ending at 2100 hours (0.95  $\text{cm s}^{-1}$ ) in summer. The species ascended slowly and discretely from the suboxic zone to the lower end of the CIL, at an ascending speed of 0.82  $\text{cm s}^{-1}$ , and passed up the CIL and the thermocline very fast, within 45 min at a speed of 2.3  $\text{cm s}^{-1}$ . Downward migration took less time, approximately 2 h, starting at 0415 hours and ending at 0613 hours. Swimming speed within the thermocline and CIL was 2.7  $\text{cm s}^{-1}$  (60 m for 37 min); subsequently, the copepod returned to daylight depth at a real sinking speed of 0.57  $\text{cm s}^{-1}$ . Total time for *C. euxinus* to settle to their nocturnal depth layer was about 5 h (Figs. 3b, c, 4). The case was the same in October 1999. At that time, *C. euxinus* spent the daytime preferring the well-oxygenated layer ( $> 6 \text{ mg O}_2 \text{ l}^{-1}$ ), corresponding to the lower end of the halocline, and started migrating upward from that layer to the surface by twilight (Fig. 4a, b, c).

**Table 1** Changes in abundance (ind. m<sup>-3</sup>) of meso- and macrozooplankton that can be detected at 120 and 200 kHz, off Zonguldak (Fig. 4e) and off Samsun (Fig. 4f) at different sampling times in July 2000

Location	Time	Depth (m)	Adult <i>Calanus euxinus</i>	<i>Sagitta setosa</i>	<i>Aurelia aurita</i>	<i>Pleurobrachia pileus</i>
Zonguldak	1920 hours	10–20	8.32	23.4	0	0
	2043 hours	30–45	0.87	16.8	0.35	3.12
Samsun	0143 hours	10–20	26.52	46.54	0	0
	0504 hours	10–20	4.42	6.24	0.26	0.78
	0728 hours	10–20	1.82	2.86	0.26	0
	1030 hours	10–20	6.76	5.72	0.26	0

**Fig. 3a–e** *Calanus euxinus*. Relation of time spent swimming (% of total time) to dissolved oxygen concentration (a) (up upward migration; down downward migration), acoustical observation of upward and downward migrations in June 1991 in southwestern (b) (sunrise at 0515 hours and sunset at 2010 hours) and in southeastern (c) (sunrise at 0504 hours and sunset at 2004 hours; see Fig. 2 for the locations: 1 mixed layer; 2 thermocline; 3 cold intermediate layer; 4 homogeneous deep layer). Profiles of dissolved oxygen at different times of acoustical records in panel b (d) and in panel c (e)



**Fig. 4a–g** *Calanus euxinus*. Acoustical observation of upward and downward migrations in October 1999 at 120 kHz (a) and 200 kHz (b). Profiles of dissolved oxygen (DO,  $\mu\text{M l}^{-1}$ ;  $100 \mu\text{M l}^{-1} = 3.2 \text{ mg l}^{-1}$ ) and chlorophyll *a* ( $\text{mg l}^{-1}$ ) at 1607 hours of acoustical records in panels a and b (c) and vertical distribution of meso- and macrozooplankton abundances between 1949 and 2008 hours in panels a and b (d) (sunrise at 0709 hours, mid-time between noon and dusk, at 1600 hours, and sunset at 1832 hours; see Fig. 2 for the locations). Acoustic records (200 kHz) at the stations: acoustical cross-section at a station located off Zonguldak (e) (dawn at 0447 hours and dusk at 2141 hours) and on a transect from offshore to the coast off Samsun (f) in July 2000 (dawn at 0432 hours and dusk at 2047 hours); vertical dissolved oxygen distribution off Zonguldak and Samsun (g)

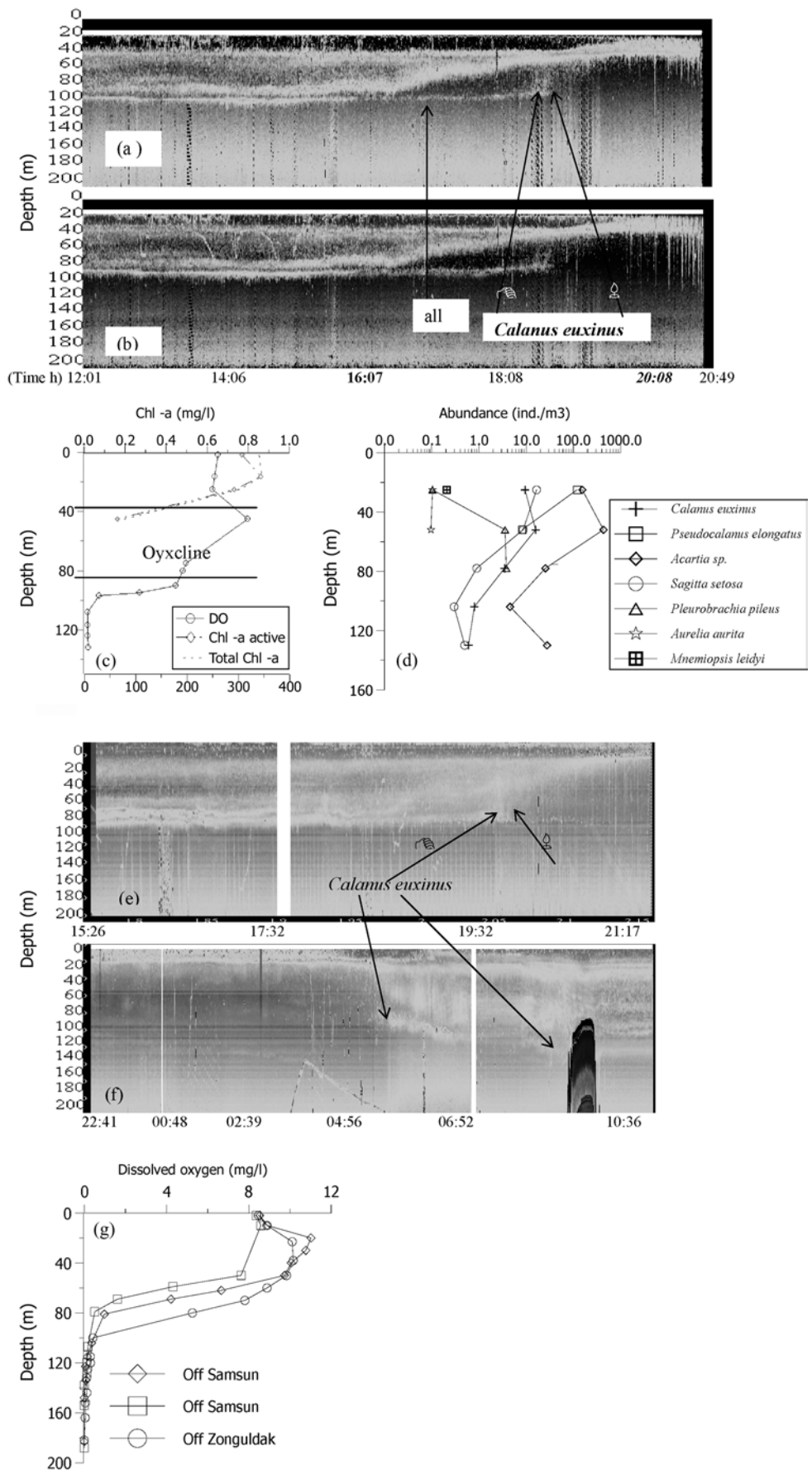


Figure 4d shows the vertical distribution of some meso- and macrozooplanktonic species from the layered samples, collected between 1949 and 2008 hours (Fig. 4a, b) in October 1999. No acoustical scattering was observed below 30 m depth at that time, whereas most of the species, especially *C. euxinus*, *Sagitta setosa*, *Pseudocalanus elongatus* and *Acartia* sp., were very abundant within the upper 25 m. At the same time, *Pleurobrachia pileus* was mostly found below 30 m, where no scattering was observed. The rest of the gelatinous organisms were found at the surface in low abundances. Another study of the species conducted in July 2000 showed similar migratory behavior to that seen in both June 1991 and October 1999. At both 120 and 200 kHz, the *C. euxinus* daylight scattering layer was observed in water with DO concentrations  $>4 \text{ mg l}^{-1}$ , which corresponded to the lower limit of the permanent halocline off Zonguldak (Fig. 4e), whereas it was within the suboxic zone off Samsun, characterized by  $\text{DO} < 1 \text{ mg l}^{-1}$  (Fig. 4f, g). *C. euxinus* started ascending at their maximum speed of  $2.3 \text{ cm s}^{-1}$ , since they were within moderately oxygenated water instead of the suboxic zone. If *C. euxinus* occasionally stay within the suboxic zone during the daytime, their upward or downward migration would take longer (Figs. 3b, c, 4f) than when they begin migration from just above the suboxic zone (Fig. 4a, b, e). If *C. euxinus* settle down to the suboxic layer, swimming speed would be high ( $2.7 \text{ cm s}^{-1}$ ) through the well-oxygenated water and decelerate to a sinking speed of  $0.57 \text{ cm s}^{-1}$  (Figs. 3b, c, 4e).

Table 1 shows the abundance ( $\text{ind. m}^{-3}$ ) of some zooplankton species that can be acoustically detected. Net samples collected from two different layers off Zonguldak at night showed that abundance of *C. euxinus* was higher between 10 and 20 m than between 30 and 45 m; thus, acoustical identification of the *C. euxinus* layer was confirmed by net samples. This was especially true for the samples off Samsun. *C. euxinus* concentration was high between 10 and 20 m at 0143 hours, while it decreased after the downward migration had started. However, two successive ascendance bursts were observed during *C. euxinus*' upward migration (Fig. 4a, b, e). This pattern could be due to a time difference between when female and male specimens start migrations, as mentioned by Enright and Honegger (1977) and observed by Mutlu (unpublished data). Females started upward migration earlier at dusk, from the deep layer to the surface, and remained in the mixed layer longer at night than the male individuals did. Downward migration was reversed; males started downward migration earlier and stayed in the deep layer longer than females did. Another acoustic study in October 1999 showed a time delay in the start of upward migration between female and male individuals of *C. euxinus* of about 10–15 min, the males migrating later. A similar pattern was observed in July 2000. Daytime distribution of the species occurred in a layer from 1 m (October) to 3 m (June and July) above the bottom. The daytime concentra-

tion was, however, located either within the suboxic zone or just above that zone. It was very clear that when the suboxic zone was located shallower than 100 m, the *C. euxinus* layer was within the suboxic. If the suboxic was deeper than 100 m, *C. euxinus* stayed just above the suboxic zone.

## Discussion

Frequencies of 120 and 200 kHz were used for in situ species' identification of a copepod layer in the Black Sea. By looking at acoustical records directly and by using time spent swimming in response to distinct DO concentrations in the water column, we concluded that the species *Calanus euxinus* formed the concentration layer. Wiebe and Greene (1994) formulated relationships between minimum detectable size of the target and the acoustical frequency such that:  $\sim 10 \text{ mm}$  individuals can be detected at 120 kHz;  $4 \text{ mm}$ , at 420 kHz; and  $1.5 \text{ mm}$ , at 720 kHz. However, David et al. (1999) determined that planktonic objects detected at 120 and 38 kHz have size limits, i.e.  $3 \text{ mm}$  at 120 kHz and  $10 \text{ mm}$  at 38 kHz, corresponding to one-quarter of the wavelength. Accordingly, the minimum size would be about  $2 \text{ mm}$  at 200 kHz. "Detectable size of the target is actually a very complex subject and depends on many things. The important factors include diameter of the animal relative to the acoustic wavelength (hence wave number, 'ka' value), orientation, material properties, numbers of animals per unit volume, sensitivity of the echosounder, and distance from the transducer. Also, it is important to understand that even if an animal is detectable while isolated, once it is near another animal that has a greater target strength, the latter animal is the one that is detected" (Stanton, personal communication). There are three species (*Pleurobrachia pileus*, Ctenophora; *Sagitta setosa*, Chaetognatha; and *Calanus euxinus*, Copepoda) of zooplankton that are  $>3 \text{ mm}$  long and migrate through the water column from the deep to the surface (Vinogradov et al. 1985; Mutlu and Bingel 1999; Besiktepe 2001). However, only *C. euxinus* migrates vertically from the suboxic zone to the surface.

The highest daytime concentration of *C. euxinus* ( $104 \text{ ind. m}^{-3}$  averaged from a  $15 \text{ m}$  thick layer) occurred within the suboxic zone ( $\text{DO} < 1 \text{ mg l}^{-1}$ ) in April 1995. The thickness of the layer of highest concentration did not exceed  $2 \text{ m}$ , and in the autumn this narrowed to  $1 \text{ m}$  (Vinogradov and Shushkina 1982). This means that real abundance could be equal to  $15 \times 104 / 2 = 780 \text{ ind. m}^{-3}$ , taking the  $2 \text{ m}$  thickness (Vinogradov et al. 1985) into account. Following the same calculation, the abundance varied temporally between 570 and  $4200 \text{ ind. m}^{-3}$  (Vinogradov et al. 1985; Besiktepe 2001).

*S. setosa* swam at a maximal speed of  $1 \text{ cm s}^{-1}$ , and migratory movement upward took 4 h in the Black Sea; *C. euxinus* lagged behind *S. setosa*, which ascended from

deeper layers (Vinogradov et al. 1985). Individuals of *P. pileus* were distributed throughout almost the entire water column and had two concentration maxima (Vinogradov et al. 1985; Mutlu and Bingel 1999). In autumn (September 1995), *C. euxinus* was within the oxygen minimum zone (OMZ; Tugrul et al. 1992), while *S. setosa* stayed in a layer just above the OMZ (Besiktepe and Unsal 2000). Since abundance of *C. euxinus* in the concentration layer was high, the probability of detection of *C. euxinus* at 120 and 200 kHz frequencies could increase, as Svetlichny et al. (2000) detected them at 150 kHz. Although its individual response lies in the Rayleigh zone for 120 and 200 kHz, *C. euxinus* could still be detected by these frequencies. Patterns of time spent swimming as a function of DO, as determined from acoustical records in the present study, showed trends similar to the experimental results of Svetlichny et al. (2000) (Figs. 1, 3a); both showed a similar relation between the time spent swimming (5 h of total time) and oxygen concentration. Net samples, collected in April 1995 (Mutlu, unpublished data), showed that the slope of the function of time in hours versus the depth where the *C. euxinus* concentration was observed changed in the different layers with different DO concentrations. The slope increased with the DO concentration. Swimming speed of *C. euxinus* during migration varied from a passive sinking speed of  $\sim 0.57 \text{ cm s}^{-1}$  within the suboxic zone to an active speed of  $2\text{--}3 \text{ cm s}^{-1}$  (upward) and to  $2.7 \text{ cm s}^{-1}$  (downward) through well-oxygenated water. Svetlichny et al. (2000) showed that the speed of active swimming of the species was equal to  $2.8 \text{ cm s}^{-1}$  in aerated water and of passive swimming was equal to  $0.54 \text{ cm s}^{-1}$  under hypoxia. Similar migration speeds ( $2.8$  and  $0.94 \text{ cm s}^{-1}$ ) for the species were obtained by a series of vertical tows (Besiktepe 2001). Overall, the species completed its upward migration in 3 h, while its downward migration lasted less time (2 h). In some cases, downward migration could last longer (5–7 h) than usual, depending on location of the suboxic zone. In some cases, where the suboxic zone was located in a layer deeper than 100 m and where its daylight concentration layer remained just above the suboxic zone, the species' upward migration was completed in a very short time ( $\sim 1$  h), at its highest speed. Svetlichny et al. (2000) concluded that migration downwards takes 2.0 h and movement upwards lasts for 3.0 h. The present study also showed that two successive movements during the downward and upward migrations took place. This could be due to the timing of separate migrations of female and male specimens, as postulated by Marshall and Orr (1955) and Enright and Honegger (1977). Enright and Honegger (1977) determined that males returned to depth earlier than females in the May samplings and also mentioned the early upward migration of females observed by Mutlu (unpublished data) in the Black Sea in April 1995. As the present work showed, the thickness of the diurnal concentration layer of *C. euxinus* varied between 1 m in October 1999 and 3 m in June 1991 and July 2000.

Vinogradov et al. (1985) showed that hemipopulations of this species formed a narrow concentration layer that did not exceed 2 m thickness in waters with DO concentrations of  $0.4\text{--}0.5 \text{ ml l}^{-1}$  during the daytime; the thickness narrowed to 1 m in October (Vinogradov and Shushkina 1982).

**Acknowledgements** This work was carried out within the NATO TU-Fisheries and Black Sea projects; the IMS-METU was funded by the Scientific and Technical Research Council of Turkey (TUBITAK), by the Scientific Affairs Division of NATO as part of the Science for Stability program, and by a project (METU-AFP-99-06-01-01) linked with other programs of TUBITAK/Turkey and NATO-SIP. The hydrographical data were obtained from the Physical and Chemical Oceanography Dept. of the IMS-METU. I thank the crew of R.V. "Bilim" for assistance at sea.

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