

# Near-bottom zooplankton in the continental shelf and upper slope of Heraklion Bay (Crete, Greece, Eastern Mediterranean): observations on vertical distribution patterns

P. KOULOURI<sup>1\*</sup>, C. DOUNAS<sup>1</sup>, F. RADIN<sup>1</sup> AND A. ELEFThERIOU<sup>2</sup>

<sup>1</sup>INSTITUTE OF MARINE BIOLOGY AND GENETICS, HELLENIC CENTRE FOR MARINE RESEARCH, GOURNES PEDIADOS, PO BOX 2214, 71003 HERAKLION, CRETE, GREECE AND <sup>2</sup>DEPARTMENT OF BIOLOGY, UNIVERSITY OF CRETE, VOUTES, PO BOX 2208, 71409 HERAKLION, CRETE, GREECE

\*CORRESPONDING AUTHOR: yol72@her.hcmr.gr

Received October 16, 2008; accepted in principle March 10, 2009; accepted for publication March 13, 2009; published online 9 April, 2009

Corresponding editor: Mark J. Gibbons

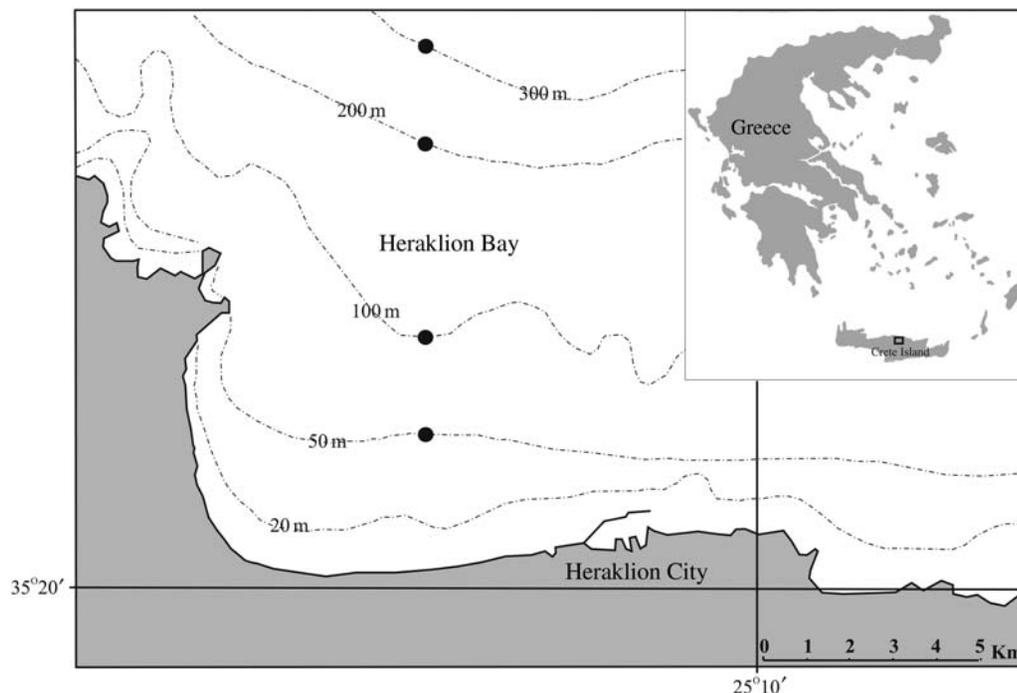
*Near-bottom zooplankton sampled by a newly developed hyperbenthic sledge (TTSS2) on the continental shelf (50–200 m) and upper slope (300 m) of Heraklion Bay (Crete, Eastern Mediterranean) consisted of both meso- and macro-zooplankton. Meso-zooplankton was composed mainly of calanoid copepods and crustacean larvae, while macro-zooplankton consisted almost exclusively of chaetognaths. Results revealed that near-bottom zooplankton is found in very high densities close to the seabed and especially on or just a few centimetres above the sediment surface. Furthermore, near-bottom zooplankton abundance was significantly higher during the night than during the day implying that the performance of “reversed” diel vertical migration by these animals is probably due to the presence of predators that use tactile stimuli, rather than vision, to locate prey.*

## INTRODUCTION

Near-bottom zooplankton associated periodically with benthic substrates has seldom been studied. Conventional zooplankton sampling methods are inefficient at sampling close to the seabed and most such samples have been obtained through the use of equipment aimed at sampling the benthic boundary layer (BBL) macrofauna (Wildish *et al.*, 1992; Wang *et al.*, 1994; Zouhiri and Dauvin, 1996; Vallet and Dauvin, 1998, 1999, 2004; Mouny *et al.*, 2000; Beyst *et al.*, 2001; Dewicke *et al.*, 2002, 2003). For the study of BBL macrofauna, specially designed samplers, generally known as hyperbenthic or suprabenthic sledges, were developed and have been frequently used over the last 30 years (Brunel *et al.*, 1978; Sorbe, 1983; Dauvin and Lorgeté, 1989; Mees and Jones, 1997; Brandt and Schnack,

1999; Cunha *et al.*, 1999; Beyst *et al.*, 2001; Dewicke *et al.*, 2002, 2003; Cartes *et al.*, 2002, 2003; San Vicente *et al.*, 2006). Nevertheless, most of these sledges do not sample the supernatant layer above the seabed in order to avoid contamination of the sample by sediment (Mees and Jones, 1997).

The existence of zooplankton close to the seabed, on many occasions, was considered accidental and episodic and as such these animals were often excluded from the study of BBL communities (e.g. Sorbe, 1989; Dauvin *et al.*, 1995; Cunha *et al.*, 1997). Recently, Dauvin and Vallet (Dauvin and Vallet, 2006) characterized the benthopelagial layer in coastal and bathyal zones as an ecocline composed of both benthic and pelagic organisms classified in eight groups according to their swimming ability: (i) permanent swimmers present



**Fig. 1.** Map of Heraklion Bay (Cretan Sea, Eastern Mediterranean) showing the location of the four sampling stations occupied during this study.

throughout the water column, (ii) temporary swimmers present throughout the water column, (iii) permanent swimmers present only in the BBL, (iv) burrowers/swimmers, (v) burrowers and temporary nuptial swimmers, (vi) benthic mobile species, (vii) benthic drift species and (viii) fish. The first three groups are typical zooplankton as well as meroplankton and ichthyoplankton.

The present study was carried out as part of a multidisciplinary research programme, the main objective of which was to study BBL macrofaunal communities on the continental shelf and upper slope of Heraklion Bay (Crete, Eastern Mediterranean). For this purpose, a newly developed hyperbenthic sledge (TTSS2) (Koulouri *et al.*, 2003, 2005) was used, specifically designed to collect animals which occur on or just above the sediment surface. The specific aim of the present study was an investigation into the vertical distribution patterns of near-bottom zooplankton groups (meso- and macrozooplankton) taking special note of their presence very close to the water-sediment interface.

## METHOD

A field survey was carried out from 20 to 30 September 2001 on the continental shelf and upper slope of Heraklion Bay (Fig. 1), located on the north coast of

Crete (Eastern Mediterranean). Sampling was performed along a transect of four stations at depths of 50, 100, 200 and 300 m. At each station, the physical properties of the water column (temperature, salinity, photosynthetically active radiation (PAR) and oxygen concentration) were measured by means of a Sea-Bird Electronics (SBE-25) CTD system. Sediment samples were collected by means of a 0.0225 m<sup>2</sup> USNEL box corer at a maximum sediment depth of 15 cm. Sediment redox potential measurements were taken at 1 cm increments using calibrated combined electrodes (Russell pH, Scotland, type no. CMPT 11/280/SA1.5), as described in Pearson and Stanley (Pearson and Stanley, 1979). Sub-samples from the sediment layer of 0–1 cm depth were taken and stored at –22°C for later laboratory analysis. Grain size analysis was performed according to Buchanan (Buchanan, 1984). Chlorophyll *a* (Chl-*a*) and chloroplastic pigment equivalent (CPE) in the sediment surface (0–1 cm) were determined according to the fluorometric method of Yentsch and Menzel (Yentsch and Menzel, 1963), using a Turner 112 fluorometer. The particulate organic carbon (POC) and nitrogen (PON) concentrations in the sediment surface (0–1 cm) were determined according to the method of Hedges and Stern (Hedges and Stern, 1984), using a Perkin Elmer CHN 2400 analyzer.

*Table I: Data on sampling carried out along a bathymetric transect on the continental shelf and upper slope of Heraklion Bay (TTSS2, towed trawl simulator sledge; HS, hyperbenthic sledge; WP2, plankton net)*

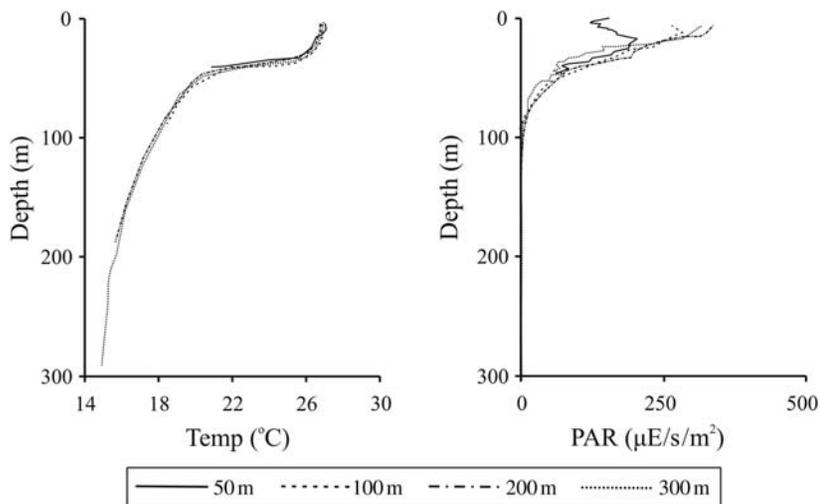
	Station 1					Station 2		Station 3		Station 4		
Coordinates	35°21.720N–25° 06.040E					35° 22.973N– 25° 05.961E		35° 25.367N– 25° 05.689E		35° 26.642N– 25° 05.623E		
Depth (m)	50					100		200		300		
Samplers	TTSS2		HS		WP2		TTSS2	HS	TTSS2	HS	TTSS2	HS
Day/night sampling period	D	N	D	N	D	N	D	D	D	D	D	
Number of tows, <i>n</i>	3	3	3	3	5	5	1	1	1	1	1	
Length of tows (m)	50	50	500	500	47	47	200	500	200	500	200	500

To study the near-bottom zooplankton, a new method was used, different in certain respects from more conventional sampling techniques. Meso- and macro-zooplankton were sampled with a Towed Trawl Simulator Sledge (TTSS2), as described by Koulouri *et al.* (Koulouri *et al.*, 2003). This sledge simulates the disturbance of the seabed caused by the passage of otter trawl groundropes over the sediment surface and simultaneously collects the suspended fauna found in the plume of sediment. The apparatus, equipped with three nets of 0.5-mm mesh size, quantitatively samples three levels (5–30, 31–56 and 57–82 cm) above the seabed. The surface area of the aperture of each net is 0.15 m<sup>2</sup> (0.58 m width × 0.25 m height). The sledge also includes an odometer for measuring the distance travelled by the gear while in contact with the seabed as well as a continuously recording video camera in order to ensure that the equipment is functioning correctly. An otter trawl groundrope, supported by two light-weight metal arms, was attached to the sides of the sledge and positioned at a distance of 1.5 m in front of the sledge. Video camera footage has shown that the groundrope causes a resuspended sediment plume up to the height of the uppermost net. Deployments were also made without the groundrope, where the apparatus performs as a standard hyperbenthic sledge (HS). Samples collected in this way with the HS were used as a reference for those collected with the TTSS2 sampler in order to estimate the zooplankton found in the layer between 0 and 5 cm above the seabed. The sledge was towed by the R/V PHILIA at a speed of  $\sim 1 \text{ m s}^{-1}$  (two knots). The ratio of the length of wire paid out to depth was 2:1.

Sampling was carried out along the 50 m isobath during daylight hours (9:00–11:00 am), using the TTSS2 equipped with a standard otter trawl groundrope (Table I). Three tows of 50 m length were carried out. Three additional tows, each 500 m in length, were carried out over the same station with the same apparatus in HS mode (TTSS2 without the groundrope).

Finally, five vertical hauls from  $\sim 3$  m above the seabed to the water surface were made using a WP2 plankton net (0.5 mm mesh size). The same sampling procedure was repeated at night (9:00–11:00 pm). During daylight hours of the same period, additional sampling was carried out along the 100, 200 and 300 m isobaths of the study area (Table I). Along each depth, a single tow of 200 m length was carried out by the TTSS2. One additional tow of 500 m length was taken by the HS at the same depths. Sample material was fixed with 10% formalin on board immediately after collection and sorted under a dissecting microscope upon return to the laboratory. The organisms were sorted into taxonomic groups and counted.

Densities of each meso- and macrozooplankton group were standardized to the number of individuals per 100 m<sup>3</sup> for the different levels of the water column. Averaged densities of animals from the day and night samples collected by the three different sampling gears were also calculated. In order to measure densities of suspended zooplankton found in the plume of the sediment and consequently in the layer from 0 to 5 cm above the seabed, densities measured in the samples collected with the HS were subtracted from those collected with the TTSS2 in the same sampling area and then standardized to the number of individuals per 100 m<sup>3</sup>. The Mann–Whitney *U*-test was applied to assess any significant differences between daytime and night-time densities of the main taxonomic groups collected by using the three different sampling gears. In order to investigate possible similarities between different tows and vertical hauls, cluster analysis of data was performed using the Bray–Curtis similarity coefficient (Bray and Curtis, 1957). Data were standardized prior to analysis. In order to detect statistically significant differences between groups of day and night samples collected by the three different sampling gears, ANOSIM test was applied (Clarke, 1993). Environmental variables significantly correlated with the distribution pattern of the zooplankton community were examined by means of the



**Fig. 2.** Vertical profiles of temperature and photosynthetically active radiation (PAR) at the four sampling stations.

*Table II: Redox potential (Eh) values measured down to 15 cm sediment depth, silt and clay percentage and concentrations of sedimentary Chl-a, CPE, POC and PON at the four sampling stations*

	Station 1	Station 2	Station 3	Station 4
Depth (m)	50	100	200	300
Eh <sub>0–1cm</sub> (mV)	409.1	413.4	424.5	454.5
Eh <sub>1–2cm</sub> (mV)	392.4	411.2	431.7	459.7
Eh <sub>4–5cm</sub> (mV)	192.0	222.4	378.0	441.1
Eh <sub>9–10cm</sub> (mV)	77.2	126.8	119.8	353.5
Eh <sub>14–15cm</sub> (mV)	67.8	106.5	87.3	207.9
Silt and clay (%)	83.69	92.60	92.59	94.72
Chl-a (mg)	5.86	1.96	0.16	0.05
CPE (mg)	11.10	7.45	2.84	1.58
POC (g)	2.34	4.06	1.18	0.90
PON (g)	0.41	0.61	0.12	0.07

Spearman’s correlation coefficient ( $\rho$ ). The STATISTICA and PRIMER v6 statistical software packages were used for the data analyses.

## RESULTS

During the sampling period (September 2001), thermal stratification was evident and a strong thermocline was located between 30 and 50 m at all stations (Fig. 2). The 50 m station was located entirely within the euphotic zone which at that period in the continental shelf of Heraklion Bay extended down to 80 m (Fig. 2). Salinity and oxygen concentrations remained relatively stable throughout the water column with values of about 39‰ and 4.5–5.6 mL/L, respectively. Surface sediments

were classified as mud with the fraction <63 µm making up more than 80% of the sediment over the study area (Table II). The coarse-grained constituents were mainly shell fragments and debris of bivalves, gastropods, echinoderm tests, serpulid tube fragments, foraminiferan tests and sponge needles. Sediment redox potential (Eh) profiles showed values higher than 400 mV for the top 2 cm of sediment, followed by an exponential decline approaching baseline values, ranging from 60 to 200 mV. Concentrations of chlorophyll *a* (ranging between 0.05 and 5.86 mg) and CPE (ranging between 1.58 and 11.10 mg) in the sediment surface decreased in relation to depth, while POC and PON reached a peak at the 100 m depth station (4.06 and 0.61 g, respectively) and decreased at the deeper stations (Table II).

The taxonomic groups and densities (individuals per 100 m<sup>3</sup>) of zooplankton found in the samples collected by the three sampling gears used along the 50 m isobath during day and night are shown in Table III. The use of the TTSS2, the HS and the WP2 yielded totals of 3152, 8593 and 2008 individuals, respectively, identified to eight taxonomic groups of zooplankton. Crustacean larvae (mainly decapod larvae), pelagic chaetognaths and calanoid copepods were the most abundant taxonomic groups collected with the two different sledges, followed by fish larvae and cnidarians. In the plankton net samples, calanoid copepods, crustacean larvae (mostly decapod larvae) and cnidarians were the most abundant zooplankton groups followed by chaetognaths and appendicularians. Ctenophores were present only in the HS and the plankton net samples while ascidians were present only in the TTSS2 samples.

Table III: Averaged densities ( $\pm$  standard deviation) of the zooplankton groups ( $N$  = number of samples) collected along the 50 m isobath by the three different sampling gears during day and night sampling periods

Zooplankton groups	TTSS2 (ind 100 m <sup>-3</sup> )			HS (ind 100 m <sup>-3</sup> )			WP2 (ind 100 m <sup>-3</sup> )		
	Day ( $N = 3$ )	Night ( $N = 3$ )	$P$	Day ( $N = 3$ )	Night ( $N = 3$ )	$P$	Day ( $N = 5$ )	Night ( $N = 5$ )	$P$
Cnidaria	10.3 $\pm$ 9.2	57.5 $\pm$ 50.2	n.s.	12.2 $\pm$ 6.5	14.6 $\pm$ 6.6	n.s.	292.1 $\pm$ 105.1	167.3 $\pm$ 66.5	n.s.
Ctenophora	–	–	–	<1	–	–	–	1.5 $\pm$ 3.4	n.s.
Crustacea (larvae)	372.8 $\pm$ 83.5	3383.4 $\pm$ 497.3	<0.05	66.2 $\pm$ 38.2	1040.4 $\pm$ 213.6	<0.05	311.8 $\pm$ 81.4	422.9 $\pm$ 163.2	n.s.
Copepoda	45.7 $\pm$ 15.5	241.7 $\pm$ 104.1	<0.05	14.0 $\pm$ 6.4	31.7 $\pm$ 5.0	<0.05	867.1 $\pm$ 268.5	409.2 $\pm$ 143.4	<0.01
Chaetognatha	97.3 $\pm$ 34.5	294.7 $\pm$ 35.7	<0.05	10.6 $\pm$ 4.1	33.8 $\pm$ 7.5	<0.05	266.2 $\pm$ 68.5	184.1 $\pm$ 99.4	n.s.
Appendicularia	8.9 $\pm$ 11.7	13.3 $\pm$ 4.4	n.s.	–	0.9 $\pm$ 0.4	<0.05	33.5 $\pm$ 25.0	54.8 $\pm$ 41.5	n.s.
Asciacea	1.5 $\pm$ 2.6	–	n.s.	–	–	–	–	–	–
Thaliacea	1.5 $\pm$ 2.6	4.4 $\pm$ 7.7	n.s.	5.2 $\pm$ 2.8	3.8 $\pm$ 1.4	n.s.	9.1 $\pm$ 16.5	–	n.s.
Fish larvae	13.3 $\pm$ 7.7	98.7 $\pm$ 49.3	<0.05	2.7 $\pm$ 3.1	30.1 $\pm$ 4.2	<0.05	12.2 $\pm$ 6.8	18.3 $\pm$ 6.8	n.s.
<b>Total</b>	<b>551.1 <math>\pm</math> 144.0</b>	<b>4093.7 <math>\pm</math> 722.2</b>	<b>&lt;0.05</b>	<b>111.0 <math>\pm</math> 56.4</b>	<b>1155.3 <math>\pm</math> 214.8</b>	<b>&lt;0.05</b>	<b>1793.5 <math>\pm</math> 472.9</b>	<b>1261.1 <math>\pm</math> 468.9</b>	<b>n.s.</b>

Statistical significance of differences in densities between day and night samples was determined with Mann–Whitney  $U$ -test ( $P$ ,  $P$ -value; n.s., not significant).

Comparison of abundances of zooplankton groups collected by the two sledges revealed that the densities of the most abundant taxonomic groups (crustacean larvae, copepods and chaetognaths) collected with the TTSS2 sampler were three to nine times higher than those measured in the samples collected with the HS (Table III), an observation which implies that most of the near-bottom zooplankton caught in the study area resides on or just a few centimetres (0–5 cm) above the seabed. On the other hand, densities of copepods, cnidarians and appendicularians collected with the plankton net were much higher than those measured in the samples collected with the TTSS2, except for chaetognaths which had similar densities and crustacean larvae which had lower densities by far (Table III). However, it should be noted that the above-mentioned densities of organisms at the water-sediment interface involve only a fraction of the total zooplankton population throughout the water column.

During the day, total zooplankton abundance recorded at the BBL using the HS was 10 times lower than that measured during the night, mostly due to the extremely high concentration of crustacean larvae (Table III). Densities of chaetognaths, copepods, fish larvae and appendicularians also increased significantly at the BBL during the same period (night versus day,  $P < 0.05$ ). During the night, there was an 8-fold increase in the total density of zooplankton recorded at the water–sediment interface with the TTSS2 sampler, mostly through increased concentrations of crustacean larvae and, to a lesser degree, chaetognaths, copepods and fish larvae (Table III,  $P < 0.05$ ). This observation indicates that a “reverse” DVM pattern occurs in certain taxonomic groups. Though most zooplankton tended to remain lower in the upper layers of the water

column at night than during the day, only densities of calanoid copepods appeared significantly decreased ( $P < 0.01$ ). Nevertheless, it should be noted that though a large part of the water column was sampled, zooplankton populations residing in the layer extending from 82 to 300 cm above the bottom were not considered.

The similarity dendrogram based on the density matrices of different zooplankton groups showed a clear separation between five groups of samples (Fig. 3). Two of these groups consisted of the day and night samples collected with the WP2 net. The day samples collected with the HS and the TTSS2 sampler comprised the third and fourth groups, respectively, while the fifth group consisted of the night samples collected from both types of sledge. The high similarity between the samples comprising the latter group (N-TTSS2, N-HS) indicates an increased efficiency of the HS during the night in collecting zooplankton residing close to the seabed. Nevertheless, the application of the ANOSIM test revealed that the groups of samples collected by the three different sampling gears during day and night sampling periods (six groups) were significantly different ( $R = 0.9$ ,  $P < 0.001$ ).

The averaged densities of the main taxonomic groups in the different layers above the seabed during both day and night along the 50 m isobath are shown in Table IV. Crustacean larvae were the most abundant group followed by pelagic chaetognaths and calanoid copepods. Other less abundant groups were fish larvae, appendicularians and cnidarians. Zooplankton densities measured in the night samples were generally higher in comparison with the day samples showing their increased nocturnal activity within this particular habitat. In particular, crustacean larvae were present in

Table IV: Averaged densities ( $\pm$  standard deviation) of the most abundant zooplankton groups collected in the different layers of the water column above the seabed (0.00–0.82 m) along the 50 m isobath during day and night sampling periods

Zooplankton groups	0–0.05 m	0.05–0.30 m	0.31–0.56 m	0.57–0.82 m
Day (ind 100 m <sup>-3</sup> )				
Crustacea (larvae)	6133.2 $\pm$ 1669.0	75.2 $\pm$ 47.8	42.4 $\pm$ 28.9	80.9 $\pm$ 48.8
Copepoda	633.7 $\pm$ 310.5	23.0 $\pm$ 12.0	7.5 $\pm$ 5.4	11.5 $\pm$ 7.5
Chaetognatha	1733.0 $\pm$ 690.6	18.6 $\pm$ 15.3	5.3 $\pm$ 4.8	8.0 $\pm$ 4.0
Others	461.7 $\pm$ 296.0	24.8 $\pm$ 19.3	10.2 $\pm$ 10.1	25.6 $\pm$ 17.3
Total	8961.6 $\pm$ 2798.7	141.5 $\pm$ 92.3	65.4 $\pm$ 45.4	126.0 $\pm$ 70.2
Night (ind 100 m <sup>-3</sup> )				
Crustacea (larvae)	46861.2 $\pm$ 9946.2	728.6 $\pm$ 153.2	1146.77 $\pm$ 348.44	1245.8 $\pm$ 260.8
Copepoda	4199.8 $\pm$ 2081.7	28.3 $\pm$ 6.3	38.90 $\pm$ 13.93	27.9 $\pm$ 5.8
Chaetognatha	5219.6 $\pm$ 714.7	30.5 $\pm$ 1.3	42.88 $\pm$ 10.80	27.9 $\pm$ 14.0
Others	2541.5 $\pm$ 2152.6	51.3 $\pm$ 29.4	59.7 $\pm$ 17.9	37.6 $\pm$ 6.5
Total	58822.1 $\pm$ 14403.0	838.6 $\pm$ 163.0	1288.24 $\pm$ 387.97	1339.1 $\pm$ 250.7

densities one to two orders of magnitude during the night in the different levels of the BBL. During both sampling periods, zooplankton showed high densities just above the seabed (0–0.05 m) followed by a sharp decrease in the upper levels of the BBL (0.05–0.82 m) where densities seemed to be similar (Table IV).

Indicative densities of near-bottom zooplankton measured at the 50, 100, 200 and 300 m depth stations during the day from samples collected with the TTSS2 and the HS are shown in Fig. 4. Calanoid copepods, crustacean larvae and pelagic chaetognaths accounted for more than 80% of the total near-bottom zooplankton in the study area. The densities of dominant zooplankton groups collected by using the TTSS2 seemed to increase with depth, except for crustacean larvae which were abundant only at the shallower stations of the continental shelf (50 and 100 m). A similar pattern was observed for the zooplankton groups collected with the HS. At all depths, near-bottom zooplankton densities were at least three times higher when the TTSS2 was used.

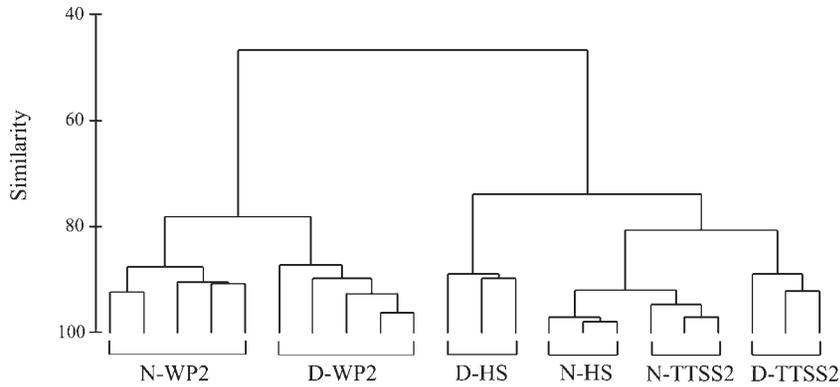
The bathymetric distribution of near-bottom zooplankton groups in the study area was found to be significantly correlated with depth, light and water temperature as well as concentrations of Chl-*a*, CPE, POC and PON in the surface sediment ( $\rho = 1$  or  $-1$ ,  $P < 0.05$ ). More specifically, total zooplankton densities measured in the samples collected with the TTSS2 were positively correlated with depth ( $\rho = 1$ ,  $P < 0.05$ ) and negatively correlated with temperature, light and concentrations of Chl-*a* and CPE in the surface sediment ( $\rho = -1$ ,  $P < 0.05$ ). Chaetognaths seemed to be negatively correlated with concentrations of POC and PON in the surface sediment ( $\rho = -1$ ,  $P < 0.05$ ). On the other hand, total zooplankton densities measured in the samples collected with the HS were negatively

correlated with sedimentary POC and PON concentrations ( $\rho = -1$ ,  $P < 0.05$ ). Chaetognaths and copepods seemed to be positively correlated with depth ( $\rho = 1$ ,  $P < 0.05$ ) and negatively correlated with water temperature and light as well as sedimentary Chl-*a* and CPE concentrations ( $\rho = -1$ ,  $P < 0.05$ ).

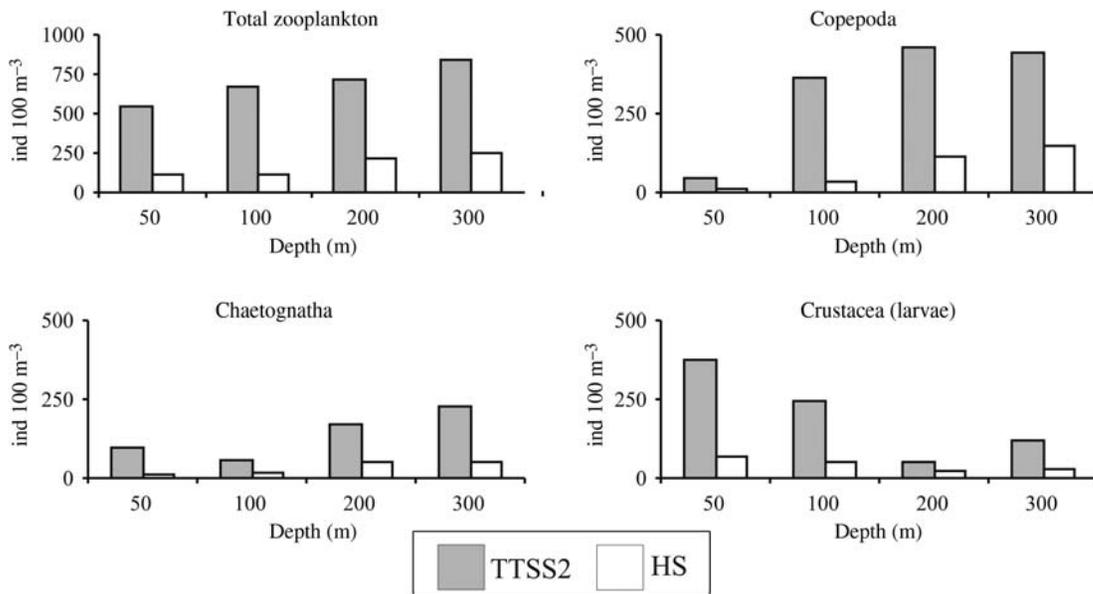
## DISCUSSION

Zooplankton studies usually underestimate near-bottom populations because of the limitations of conventional sampling equipment (Rios-Jara, 1998; Brodeur and Terazaki, 1999; Choe and Deibel, 2000). Though plankton nets may adequately sample zooplankton living in the water column, they are not suitable for collecting zooplankton occurring very close to the sediment surface (Lasenby and Sherman, 1991). Furthermore, most HSs that have been developed and used for the study of BBL macrofauna do not sample the water column just above the seabed in order to avoid contamination of the samples by sediment (Mees and Jones, 1997). However TTSS2, a recently developed sampling gear, used in the continental shelf and upper slope of Heraklion Bay (Koulouri *et al.*, 2003), collected large numbers of meso- and macrozooplankton organisms living on or a few centimetres above the sediment surface. Meso-zooplankton was composed mainly of calanoid copepods and crustacean larvae, while macrozooplankton consisted almost exclusively of chaetognaths. The gear was also effective at sampling larval fish.

Comparison of samples collected both with the TTSS2 and the same apparatus used as a HS revealed very high concentrations of zooplankton within the first few centimetres above the seabed (0.00–0.05 m) and



**Fig. 3.** Similarity dendrogram based on density matrices of the zooplankton groups collected by the three different sampling gears along the isobath of 50 m during day and night sampling periods (D: day; N: night; PN: WP2 plankton net;  $R = 0.9$ ,  $P < 0.001$ ).



**Fig. 4.** Densities of the most abundant zooplankton groups collected by using the TTSS2 sampler and the HS at the four sampling stations.

limited numbers within the upper levels of the BBL (0.05–0.82 m), indicating a close association of these animals with the sediment surface. Until now, only a few studies have revealed that certain species of calanoid copepods are concentrated close to the bottom or even attached to objects or particles of the sediment (Stearns and Forward, 1984; Fancett and Kimmerer, 1985; De Stasio, 1993). Other studies carried out on the continental shelf of Conception Bay and Gulf of Alaska showed much higher concentrations of pelagic chaetognaths (500  $\mu\text{m}$  mesh size) within 1 m of the bottom than those recorded throughout the upper layers of the water column (Brodeur and Terazaki, 1999; Choe and Deibel, 2000). Results of the present study indicate that near-bottom zooplankton may form a considerable

fraction of the zooplankton population throughout the water column.

Recently, field experiments conducted in the same area of Heraklion Bay showed that the range of sediment penetration is very small (0.5–1 mm) when the TTSS2 is equipped with different types of groundropes (Dounas *et al.*, 2002, 2005). Nevertheless, the densities of two main zooplankton groups, i.e. calanoid copepods and pelagic chaetognaths, calculated from samples collected with heavier types of groundropes were significantly higher than the ones calculated from samples collected with lighter types such as the one utilized in the present study normally used by otter trawls of Heraklion Bay (Koulouri *et al.*, 2003). This observation indicates that there are zooplankton concentrated not

Table V: Comparison of mean densities (ind 100 m<sup>-3</sup>) of macrozooplankton collected by using different sledges in various areas (*n* = number of hauls; MG: Macer-GIROQ sledge; B: Beyer's sledge; TTSS2: towed trawl simulator sledge; HS: hyperbenthic sledge)

Region	Depth (m)	<i>n</i>	ind 100 m <sup>-3</sup>	Reference
English Channel				
Trezen Vraz	75	25MG	2518	Zouhiri and Dauvin (1996)
Trezen Vraz	75	5MG	1235	Vallet and Dauvin (1998)
off Plymouth	75	5MG	513	Vallet and Dauvin (1998)
Norman-Breton Gulf	33	5MG	331	Vallet and Dauvin (1998)
Bay of Seine	29	5MG	299	Vallet and Dauvin (1998)
Dover Strait	25–29	10MG	192	Vallet and Dauvin (1998)
Bay of Saint-Brieuc	33	28MG	590	Vallet and Dauvin (1998)
Eastern Bay of Seine	8–13	38MG	488	Wang <i>et al.</i> (1994)
Western Baltic				
Kieler-Bucht	28–32	16B	1473	Hesthagen (1973)
Eastern Mediterranean				
Heraklion Bay	50	6TTSS2	300	This study
Heraklion Bay	50	6HS	57	This study
Heraklion Bay	100–300	3TTSS2	186	This study
Heraklion Bay	100–300	3HS	60	This study

only within the first few centimetres above the seabed but also probably lying on the sediment surface. This kind of demersal behaviour could be an advantage in reducing visually directed predation, because even if predators do succeed in taking prey from the bottom, they would have difficulty in perceiving organisms attached to particles of detritus or sediment (Fancett and Kimmerer, 1985).

Most studies of diel vertical migration (DVM) document a descent during the day and an ascent at night (normal DVM) (Hays, 2003). In the present study, many zooplankton taxa exhibited a “reverse DVM”, where their depth was deeper at night than during the day. This behaviour is usually associated with the presence of large numbers of invertebrate predators using tactile rather than visual stimuli to locate their prey (e.g. Ohman *et al.*, 1983). However, zooplankton recorded on the continental shelf of Heraklion Bay by using the TTSS2 sampler seem to descend not just to deeper layers of the water column at night but also to be highly concentrated on the sediment surface or within a few centimetres above it, an observation that has not been made before.

The observed bathymetric distribution pattern of zooplankton occurring at the water-sediment interface in the study area seems to be influenced by physical conditions such as temperature and light and not by the presence of food supply as indicated by concentrations of sedimentary POC, PON, Chl-*a* and CPE. A negative correlation of near-bottom zooplankton with sedimentary organic matter was also observed along a deep-sea transect down the Catalan sea slope (northwestern Mediterranean) (Cartes *et al.*, 2002).

When Vallet and Dauvin (Vallet and Dauvin, 1998, 1999) compared the mean densities of near-bottom macrozooplankton collected in different areas in the English Channel and the Baltic Sea, they found that densities varied considerably from one area to another (Table V). The highest densities were recorded on shallow coarse sand bottoms of the western English Channel (2518 and 1235 ind. m<sup>3</sup>) (Zouhiri and Dauvin, 1996; Vallet and Dauvin, 1998) and on muddy substrates of the continental shelf of western Baltic Sea (1473 ind. m<sup>3</sup>) (Hesthagen, 1973). Nevertheless, in most areas, the mean densities of macrozooplankton ranged between 192 and 590 ind. m<sup>3</sup>. In Heraklion Bay, during the present study, much lower densities were found (57 and 60 ind. m<sup>3</sup>) by using a typical HS. However, when the TTSS2 sampler was used in the same area, the estimated macrozooplankton densities (186 and 300 ind. m<sup>3</sup>) were similar to those recorded in similar localities of the English Channel by using a Macer-GIROQ sledge (Table V). Taking into account the fact that the TTSS2 was initially constructed to simulate the disturbance of the seabed caused by the passage of an otter trawl groundrope over the sediment surface, the above-mentioned observations may suggest that typical HSs do not sample the supernatant layer just above the seabed and as a result near-bottom zooplankton densities may be under-estimated.

In conclusion, further development and application of innovative sampling equipment, such as the TTSS2, that provides stimulation to the small animals in order to swim up from the substrate, may assist in overcoming problems related to the inaccessibility of near-bottom zooplankton and thus make zooplankton studies more comprehensive.

## ACKNOWLEDGEMENTS

The authors acknowledge the technical support provided by the captain and the crew of R/V *PHILIA*. We are also grateful for comments on the manuscript made by Margaret Eleftheriou and Dr Christos Arvanitidis.

## FUNDING

This work was carried out in the framework of the project “Development of a new method for the quantitative measurement of the effects of otter trawling on benthic nutrient fluxes and sediment biogeochemistry,” which was financed by the European Commission (DG. XIV, Studies for the support of CFP).

## REFERENCES

- Beyst, B., Buysse, D., Dewicke, A. *et al.* (2001) Surf zone hyperbenthos of Belgian sandy beaches: seasonal patterns. *Estuarine Coastal Shelf Sci.*, **53**, 877–895.
- Brandt, A. and Schnack, K. (1999) Macrofaunal abundance at 79°N off East Greenland: opposing data from epibenthic-sledge and box-corer samples. *Polar Biol.*, **22**, 75–81.
- Bray, J. R. and Curtis, J. T. (1957) An ordination of the upland forest communities of southern Wisconsin. *Ecol. Monogr.*, **27**, 220–249.
- Brodeur, R. D. and Terazaki, M. (1999) Springtime abundance of chaetognaths in the shelf region of the northern Gulf of Alaska, with observations on the vertical distribution and feeding of *Sagitta elegans*. *Fish. Oceanogr.*, **8**, 93–103.
- Brunel, P., Besner, M., Messier, D. *et al.* (1978) Le traîneau suprabenthique Macer-GIROQ: appareil amélioré pour l'échantillonnage quantitatif étagé de la petite faune nageuse au voisinage du fond. *Int. Rev. Ges. Hydrobiol.*, **63**, 815–829.
- Buchanan, J. B. (1984) Sediment analysis. In Holme, N. A. and McIntyre, A. D. (eds), *Methods for the Study of Marine Benthos*. Blackwell Scientific Publishers, Boston, pp. 41–65.
- Cartes, J. E., Grémare, A., Maynou, F. *et al.* (2002) Bathymetric changes in the distributions of particulate organic matter and associated fauna along a deep-sea transect down the catalan sea slope (Northwestern Mediterranean). *Prog. Oceanogr.*, **53**, 29–56.
- Cartes, J. E., Jaume, D. and Madurell, T. (2003) Local changes in the composition and community structure of suprabenthic peracarid crustaceans on the bathyal Mediterranean: influence of environmental factors. *Mar. Biol.*, **143**, 745–758.
- Choe, N. and Deibel, D. (2000) Seasonal vertical distribution and population dynamics of the chaetognath *Parasagitta elegans* in the water column and hyperbenthic zone of Conception Bay, Newfoundland. *Mar. Biol.*, **137**, 847–856.
- Clarke, K. R. (1993) Non-parametric multivariate analyses of changes in community structure. *Aust. J. Ecol.*, **18**, 117–143.
- Cunha, M. R., Sorbe, J. C. and Bernardes, C. (1997) On the structure of the neritic suprabenthic communities from the Portuguese continental margin. *Mar. Ecol. Prog. Ser.*, **157**, 119–137.
- Cunha, M. R., Sorbe, J. C. and Moreira, M. H. (1999) Spatial and seasonal changes of brackish peracaridan assemblages and their relation to some environmental variables in two tidal channels of the Ria de Aveiro (NW Portugal). *Mar. Ecol. Prog. Ser.*, **190**, 69–87.
- Dauvin, J. C. and Lorgeré, J. C. (1989) Modifications du traîneau Macer-GIROQ pour l'amélioration de l'échantillonnage quantitatif étagé de la faune suprabenthique. *J. Res. Oceanogr.*, **14**, 65–67.
- Dauvin, J. C. and Vallet, C. (2006) The near bottom layer as an ecological boundary in marine ecosystems: diversity, taxonomic composition and community definitions. *Hydrobiologia*, **555**, 49–58.
- Dauvin, J. C., Sorbe, J. C. and Lorgeré, J. C. (1995) Benthic boundary layer macrofauna from the upper continental slope and the Cap-Ferret canyon (Bay of Biscay). *Oceanol. Acta*, **18**, 113–122.
- De Stasio, B. T., Jr (1993) Diel vertical and horizontal migration by zooplankton: population budgets and the diurnal deficit. *Bull. Mar. Sci.*, **53**, 44–64.
- Dewicke, A., Rottiers, V., Mees, J. *et al.* (2002) Evidence for an enriched hyperbenthic fauna in the Frisian front (North Sea). *J. Sea Res.*, **47**, 121–139.
- Dewicke, A., Cattrijsse, A., Mees, J. *et al.* (2003) Spatial patterns of the hyperbenthos of subtidal sandbanks in the southern North Sea. *J. Sea Res.*, **49**, 27–45.
- Dounas, C., Davies, I., Hayes, P. *et al.* (2002) Development of a new method for the quantitative measurement of the effects of otter trawling on benthic nutrient fluxes and sediment biogeochemistry, Study Project No. 99/036, Final Report, European Commission.
- Dounas, C., Davies, I., Hayes, P. *et al.* (2005) The effect of different types of otter trawl groundrope on benthic nutrient fluxes and sediment biogeochemistry. *Am. Fish. Soc. Symp.*, **41**, 539–544.
- Fancett, M. S. and Kimmerer, W. J. (1985) Vertical migration of the demersal copepod *Pseudodiaptomus* as a means of predator avoidance. *J. Exp. Mar. Biol. Ecol.*, **88**, 31–43.
- Hays, G. C. (2003) A review of the adaptive significance and ecosystem consequences of zooplankton diel vertical migrations. *Hydrobiologia*, **503**, 163–170.
- Hedges, J. I. and Stern, J. H. (1984) Carbon and nitrogen determinations of carbonate-containing solids. *Limnol. Oceanogr.*, **29**, 657–663.
- Hesthagen, I. H. (1973) Diurnal and seasonal variations in the near-bottom fauna—the hyperbenthos—in one of the deeper channels of the Kieler Bucht (Western Baltic). *Kiel. Meeresforsch.*, **26**, 1–10.
- Koulouri, P., Dounas, C. and Eleftheriou, A. (2003) A new apparatus for the direct measurement of otter trawling effects on the epibenthic and hyperbenthic macrofauna. *J. Mar. Biol. Assoc. UK*, **83**, 1363–1368.
- Koulouri, P., Dounas, C. and Eleftheriou, A. (2005) Preliminary results on the effect of otter trawling on hyperbenthic communities in Heraklion Bay, Cretan Sea, Eastern Mediterranean. *Am. Fish. Soc. Symp.*, **41**, 529–538.
- Lasenby, D. C. and Sherman, R. K. (1991) Design and evaluation of a bottom-closing net used to capture mysids and other suprabenthic fauna. *Can. J. Zool.*, **69**, 783–786.
- Mees, J. and Jones, M. B. (1997) The hyperbenthos. *Oceanogr. Mar. Biol. Annu. Rev.*, **35**, 221–255.
- Mouny, P., Dauvin, J. C. and Zouhiri, S. (2000) Benthic boundary layer fauna from the Seine estuary (eastern English Channel, France): spatial distribution and seasonal changes. *J. Mar. Biol. Assoc. UK*, **80**, 959–968.

- Ohman, M. D., Frost, B. W. and Cohen, E. B. (1983) Reverse diel vertical migration: an escape from invertebrate predators. *Science*, **220**, 1404–1407.
- Pearson, T. H. and Stanley, S. O. (1979) Comparative measurements of the redox potential of marine sediments as a rapid means of assessing the effect of organic pollution. *Mar. Biol.*, **53**, 371–379.
- Rios-Jara, E. (1998) Spatial and temporal variations in the zooplankton community of Phosphorescent Bay, Puerto Rico. *Estuarine Coastal Shelf Sci.*, **46**, 797–809.
- San Vicente, C., Ramos, A. and Sorbe, J. C. (2006) Suprabenthic euphausiids and mysids from the South Shetland Islands and the Bransfield Strait, Southern ocean (BENTART-95 cruise). *Polar Biol.*, **29**, 211–222.
- Sorbe, J. C. (1983) Description d' un traîneau destiné a l' échantillonnage quantitatif étagé de la faune suprabenthique néritique. *Ann. I. Oceanogr. Paris*, **59**, 117–126.
- Sorbe, J. C. (1989) Structural evolution of two suprabenthic soft-bottom communities of the South Gascogne continental shelf. *Sci. Mar.*, **53**, 335–342.
- Stearns, D. E. and Forward, R. B., Jr (1984) Photosensitivity of the calanoid copepod *Acartia tonsa*. *Mar. Biol.*, **82**, 85–89.
- Vallet, C. and Dauvin, J. C. (1998) Composition and diversity of the benthic boundary layer macrofauna from the English Channel. *J. Mar. Biol. Assoc. UK*, **78**, 387–409.
- Vallet, C. and Dauvin, J. C. (1999) Seasonal changes of macrozooplankton and benthic boundary layer macrofauna from the Bay of Saint-Brieuc (Western English Channel). *J. Plankton Res.*, **21**, 35–49.
- Vallet, C. and Dauvin, J. C. (2004) Spatio-temporal changes of the near-bottom mesozooplankton from the English Channel. *J. Mar. Biol. Assoc. UK*, **84**, 539–546.
- Wang, Z., Dauvin, J. C. and Thiébaud, E. (1994) Preliminary data on the near-bottom meso- and macro-zooplanktonic fauna from the eastern Bay of Seine: faunistic composition, vertical distribution and density variation. *Cah. Biol. Mar.*, **35**, 157–176.
- Wildish, D. J., Wilson, A. J. and Frost, B. (1992) Benthic Boundary Layer Macrofauna of Browns Bank, Northwest Atlantic, as potential prey of juvenile benthic fish. *Can. J. Fish. Aquat. Sci.*, **49**, 91–98.
- Yentsch, C. S. and Menzel, D. W. (1963) A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. *Deep-Sea Res.*, **10**, 221–231.
- Zouhiri, S. and Dauvin, J. C. (1996) Diel changes of the benthic boundary layer macrofauna over coarse sand sediment in the Western English Channel. *Oceanol. Acta*, **19**, 141–153.