ANOXIA AND ITS IMPACTS ON THE VERTICAL DISTRIBUTION OF COPEPODS IN A DEEP MEDITERRANEAN COASTAL LAKE

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ABSTRACT

The present study investigates the vertical distribution of the copepods found in a deep and anoxic Mediterranean coastal lake during a two-year period. The numerically most important adult copepods were the calanoids *Paracartia latisetosa* (66.9 %) and *Calanipeda aquaedulcis* (27.4 %), while the harpacticoids *Euterpina acutifrons*, *Harpacticus gracilis*, *Tisbe* sp. and *Microsetella* sp. along with the cyclopoid *Oithona nana* accounted on average for 5.7 %. The copepod nauplii (not distinguished to species) were found year-round and outnumbered the community. For all species, greater abundance values were found in late spring and in summer while lowest in winter. Along the vertical axis, over 90 % of the abundance of the two most important species and nauplii was recorded in the surface 0-5 m layer, except in winter and early spring when considerable proportions were also recorded in deeper layers. The percentage contribution of the harpacticoids and cyclopoid species within the copepod's community showed an increase with depth, from 5.4 % in the surface to 39.5 % in the deepest layer. An exponential increase in the average percentage of copepod carcasses with depth was also noticed. In the well oxygenated surface layer, the most important parameter influencing the seasonal cycles of all species was temperature. The depletion of oxygen and the increase of hydrogen sulphide with depth were restricting factors for the vertical distribution of calanoid copepods. However, they seemed to have affected less the presence of harpacticoid and cyclopoid species which seemed to be more tolerant to anoxia.

KEYWORDS: copepods, anoxia, hypoxia, sulphide, vertical distribution, brackishwater

1 INTRODUCTION

Anoxia constitutes an important environmental problem, affecting coastal ecosystems around the world [1]. It can be a natural phenomenon caused by vertical water stratification in water bodies with high nutrient loadings and organic matter, while it is usually enhanced by human activities that result in the strengthening of this stratification [2]. Anoxic incidents are common phenomena in productive coastal lagoons or seawater lakes, where they can result to extensive mortality of aquatic organisms from phytoplankton to fishes [3].

Copepods are the most common and widely distributed group of small aquatic crustaceans with a very important role in the aquatic food web as they constitute a linkage between primary producers and higher consumers [4]. Copepod behavioral responses to anoxia and hypoxia have increasingly attracted the attention of the scientific community during the last years due to the key position of these organisms in the ecosystem functioning [5-7]. However, there are only a few field studies dealing with the effects of anoxic/hypoxic environments on the distribution of copepods in the vertical axis in oceanic and especially in deep coastal areas [7, 8].

Aitoliko Lagoon is a deep, brackish, coastal Mediterranean lake characterized by the presence of a persistent anoxic/hypoxic layer rich in sulphide, while periodically suffers from anoxic incidents expressed as massive fish deaths [9]. Previous investigation on the zooplankton of this area (unpublished data) revealed that copepods comprise the most important element in the productivity and energy transfer of this ecosystem. Considering the particular character of this basin and the key role of copepods, the present study focuses on the impact of the anoxic conditions in the vertical distribution of these organisms and, consequently, the energy transfer in the water column.

2 MATERIAL AND METHODS

2.1 The study area

Aitoliko Lagoon is a semi-enclosed brackish basin situated in western Greece, covering an area of 1700 ha. The great depth of this basin (Z_{max} = 29 m) as well as its morphology (a bottom deeper than the threshold along the communication with the sea) and the absence of important tide excursions which rhythmically flood and drain a coastal plane, are typical characteristics of other Mediterranean coastal or seawater lakes [3, 10, 11]. Southerly, Aitoliko Lagoon is connected through narrow openings of ap-

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proximately 1 m depth to the extended and shallow Messolonghi lagoon (Fig. 1). In the surrounding agricultural area, there is a network of canals which constitutes an irritation and/or drainage system. Water from this system flows into the Aitoliko Lagoon through a pumping station in the western bank (Fig. 1). The area has a historical record of anoxic incidents since 1881 [2]. During these events, the anoxic deep layers emerge to the surface, releasing hydrogen sulphide and resulting to massive fish deaths [9].

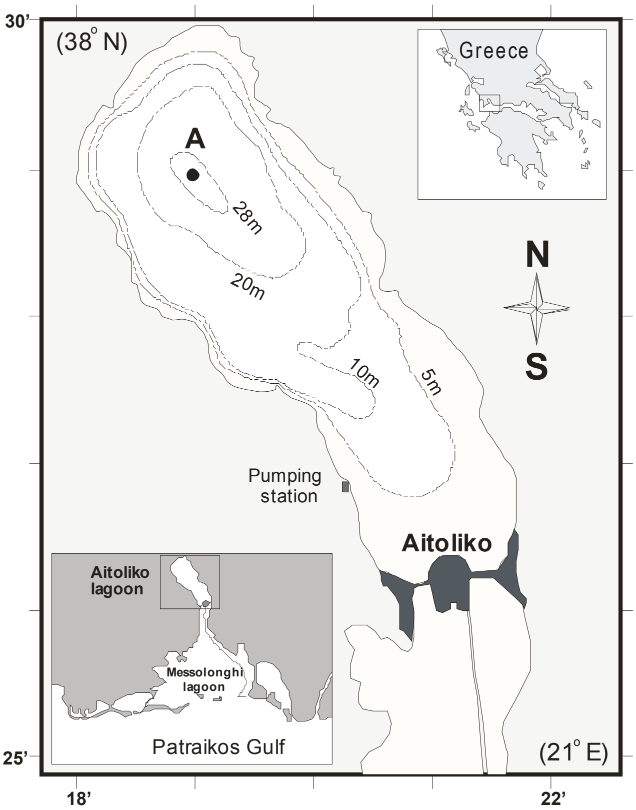

FIGURE 1 - Aitoliko Lagoon with the sampling site (station A).

2.2 Field and laboratory operations

Zooplankton sampling was carried out on a monthly basis during a two year period (April 2006 - March 2008) in station A, situated in the deeper area of Aitoliko Lagoon (Fig. 1). The samples were collected by vertical hauls of a conical plankton net (40 cm diameter, 1 m length, 50 μm mesh-size) equipped with a closing mechanism. The hauls were conducted in five depth intervals: 0-5, 5-10, 10-15, 15-20 and 20-25 m for a total of 5x24 = 120 samples. All samplings were carried out during day light hours. Filtered water volume was estimated with a Hydro-Bios flow meter attached to the mouth of the net. Sample fixation took place immediately after collection in a 4 % borax-buffered formaldehyde solution. At each sampling occasion vertical profiles of temperature, salinity, pH and dissolved oxygen (DO) were taken for the entire water column, using a Troll 9500 water quality multi-parameter instrument. Water transparency was measured with a Secchi disc. The estimation of chlorophyll-a (Chl-*a*) and hydrogen sulphide (H_2S) concentrations was made on water samples collected from 0, 5, 10, 15, 20 and 25 m, using a 5 l sampling bottle. For the determination of Chl-*a* concentration, 500 ml of the water samples taken from the above depths were filtered through a Whatman GF/A glass fiber filter shortly after collection. Pigment extraction was made in 90 % acetone and concentrations were determined spectrophotometrically [12]. Water samples for hydrogen sulphide measurements were always the first taken from the sampling bottle immediately after it was back on board. The samples were placed in plastic bottles, always completely filled. Zinc acetate and sodium hydroxide solutions were put into the bottles before filling them to preserve the samples until measurement. Four drops of 2N zinc acetate solution per 100 ml sample were used, while the final pH was always at least 9. All samples were analyzed unfiltered within 24 h using the iodometric method [12].

Identification and enumeration of copepod species was performed in the laboratory under a stereomicroscope. For the most abundant species, copepodites and adults were counted separately, and gender was recorded, too. Copepod nauplii were not distinguished into species. The abundance of each species was calculated as ind l^{-1} from aliquots taken with a modified Folsom splitter. Aliquot size ranged from 1/1 (whole sample) to 1/64, depending on the density of zooplankton organisms in the sample. Carcasses of dead copepods were also counted separately in all samples.

In order to have comparable data of the vertical distributions of the copepods, the weighted mean depth (WMD) was calculated for each species as follows:

$$WMD = \frac{\sum (NTi \times Ti)}{\sum NTi}$$

where WMD = weighted mean depth, NT*i* = the abundance in the depth *i*, and T_i = depth (m). Although the weighted mean depth cannot represent the actual vertical distribution of a species, it is a good numerical base for the application of statistics. Thus, differences between the WMDs of the species and their ontogenetic stages (copepodites, nauplii) and gender were tested using either a t-test or one way ANOVA on log(x+1) transformed data and were further expressed by the LSD (Least Significant Difference) test. In cases when datasets were not normally distributed and the variances were heterogeneous (Levene statistic, $p<0.05$), the non-parametric Kruskal-Wallis and post-hoc Mann-Whitney (U) tests ($p<0.05$) were applied to compare sample distributions.

Inter-annual differences of the copepod species and the physicochemical parameters were tested using the non-parametric Kruskal-Wallis and U-test. Multiple regression analysis was used to ascertain the influence of the environmental factors on each copepod species. All data analyses were performed with SPSS 17.0 (SPSS, Inc. 2008) statistical package.

3 RESULTS

3.1 Physicochemical parameters

The water temperature in Aitoliko Lagoon fluctuated between 9.44 and 31.16 °C (February 2008 and June 2007, respectively). The development of the seasonal thermocline started during spring months (March-April) and lasted until late autumn, when the progressive decrease of the surface temperature resulted to its breakdown (Fig. 2). Between November and February, the water temperature was nearly homogenous in the water column until the return of the thermal stratification in next spring.

The salinity measurements showed the brackish character of this basin. Salinity fluctuated between 18.75 in the surface layer in May 2006 to 27.65 near to the bottom in December 2007. A permanent halocline was observed in the water column throughout the study period (Fig. 3). From May to September, in both sampling years, the seasonal thermocline was combined with the permanent halocline, resulting in an extensive metalimnetic layer from 6 to 15 m (Fig. 2 and 3). Though, the stratification in winter was controlled exclusively by salinity gradient. There were statistically significant differences (U-test, $p<0.05$) for salinity between the two sampling periods

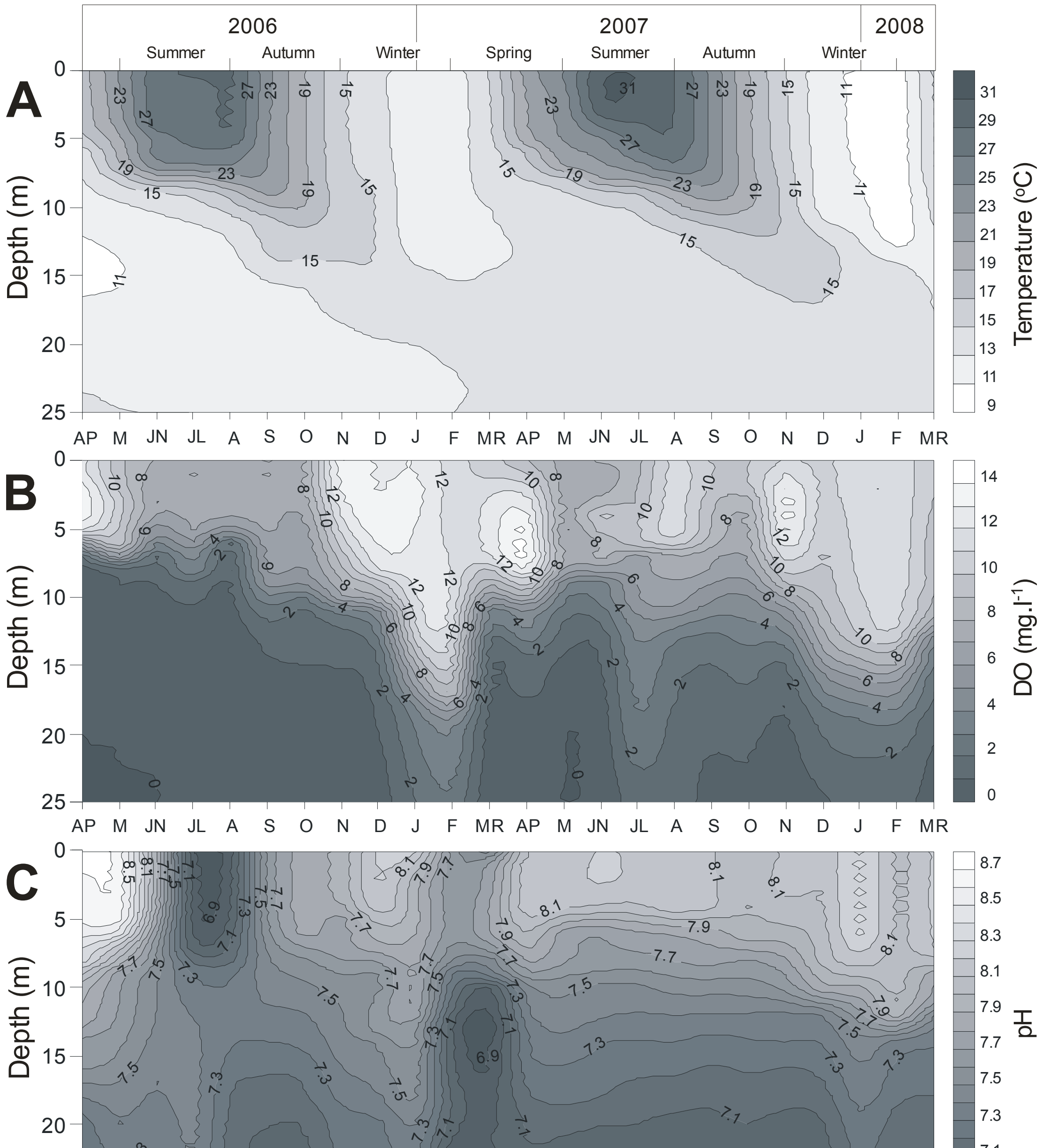

FIGURE 2 - The monthly variation of (A) temperature, (B) dissolved oxygen (DO) and (C) pH during the sampling period (April 2006 – March 2008).

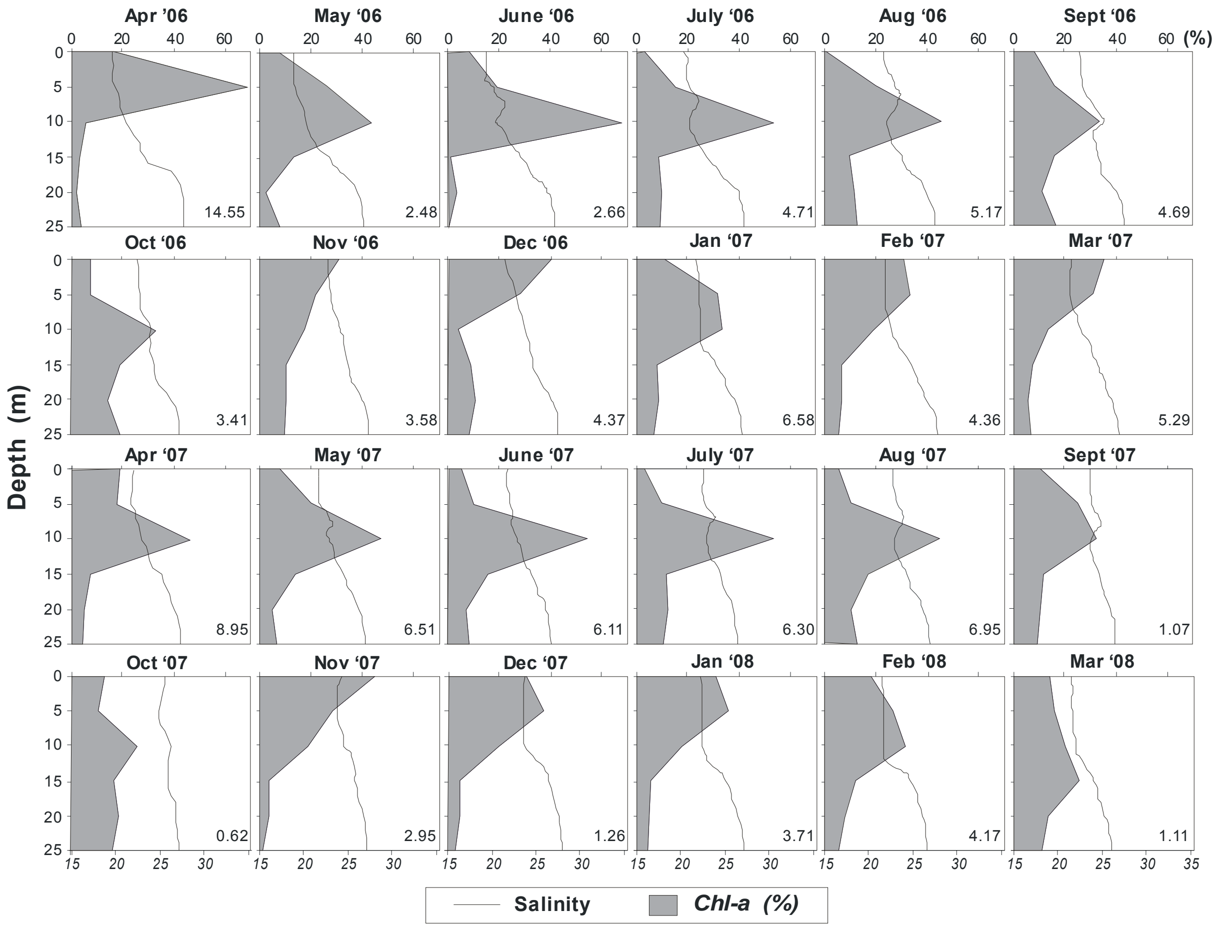

FIGURE 3 - Vertical profiles of salinity and chlorophyll-a (Chl-*a*) during the sampling period (April 2006 – March 2008). (The Chl-*a* concentration is expressed as percentage (%) in the water column sampled, while the integrated concentration values (μg l^{-1}) in the 0-25 m are shown in numbers)

(April 2006 – March 2007 and April 2007 – March 2008), with higher values being recorded during the second one. Such inter-annual differences of salinity in the water column were recorded in May, July, October and January.

Considering the concentration of dissolved oxygen (DO), the surface layer of 0-5 m was always sufficiently oxygenated with the percent saturation frequently being higher than 100 %, due to eutrophication and high primary productivity of the ecosystem. The highest DO reached up to 13.72 mg l^{-1} in April 2006 whereas, below this layer, a strong oxycline was observed (Fig. 2). During the stratification period, the DO in the meta- and hypolimnetic layers decreased leading to hypoxic (DO < 2 mg l^{-1}) or even anoxic (DO < 0.2 mg l^{-1}) conditions. Particularly, in the 15-20 m layer anoxic conditions were recorded from April to August 2006, and in May and June 2007. In the deepest layer (20-25 m) anoxia prevailed from April to August 2006, in November 2006 and from April to June 2007. At this layer, concentrations of DO greater than 2 mg l^{-1} were recorded only in January and February of both sampling years (Fig. 2). The concentration of DO was higher during the second sampling period (U-test, $p<0.05$), with inter-annual differences found in April, May, July and August in the whole water column, while such differences were recorded for most of the months in the depth layers under 10 m.

The hypoxic and anoxic conditions in Aitoliko Lagoon were coupled with the existence of high amounts of hydrogen sulphide (H_2S), being always present in depths greater than 10 m and having higher concentration near the bottom (Fig. 4). No differences in the H_2S concentration between the two sampling periods were recorded (U-test, $p>0.05$). The average concentrations values in the 10-15, 15-20 and 20-25 m were 8.77, 25.11 and 41.04 mg l^{-1}, respectively. The maximum value of 56.8 mg l^{-1} was recorded at the 25 m depth in March 2008.

The values of pH ranged from 6.80 within the metalimnetic layer in March 2007, to 8.76 in the surface waters in April 2006. In the vertical axis, higher pH values were recorded in the surface 0-5 m layer and decreasing with depth, presenting the lower values near to the bottom in all sampling occasions (Fig. 2).

The Chlorophyll-a concentration (Chl-*a*) fluctuated between 0.22 μg l^{-1} at the depth of 25 m in December 2007 to

60.1 μg l^{-1} at 5 m in April 2006. Generally, the concentration of Chl-*a* was higher during the winter and early spring months and lower in summer and autumn (Fig. 2). During most of the sampling occasions (except in November and December in both years), the greatest Chl-*a* values were recorded in the depth of 5 or 10 m, forming a deep chlorophyll maximum (Fig. 3).

3.2 Copepod species and vertical distribution

The copepod's community comprised of the calanoids *Paracartia latisetosa* and *Calanipeda aquaedulcis*, which dominated among the adults and copepodites accounting for 66.9 and 27.4 %, respectively, the harpacticoids *Euterpina acutifrons*, *Harpacticus gracilis*, *Tisbe* sp. and *Microsetella* sp. (accounting for 5.7 %), and the cyclopoid *Oithona nana* (0.01 %). All species presented higher abundance in late spring and summer while lowest in winter (Fig. 5). Nauplii always outnumbered the adults and copepodites, accounting on average for 68.3 % in the whole copepod's community. There were no differences between the two sampling years for any of the species abundance (U-test, $p>0.05$).

The vertical distribution of the total copepods showed a characteristic pattern with over 90 % of them being in the surface 0-5 m layer during the period of intense stratification (from April to October). In winter and early spring, there was a progressive decrease of abundance with depth. Only a few specimens were found in the deeper layers of 15-20 and 20-25 m (Fig. 4). The above pattern of vertical distribution of the total copepods was ought mainly to the dominance of the calanoids *P. latisetosa* and *C. aquaedulcis* and nauplii (Fig. 5). The two calanoid species presented similar vertical distribution (Fig. 6) and there were no significant differences in their mean depths (Kruskal-Wallis test, $p>0.05$). Furthermore, there were no differences in the mean depth between males and females, or adults and copepodites within the population of the above two species (U-test, $p>0.05$). In contrast, significant difference was recorded between the mean depth of the two calanoids combined together and the respective mean depth of the harpacticoid and cyclopoid species (U-test, $p=0.027$) which distributed deeper (Fig. 7). Indeed, the relative abundance (%) contribution of the harpacticoid and cyclopoid species within the copepod's community increased with

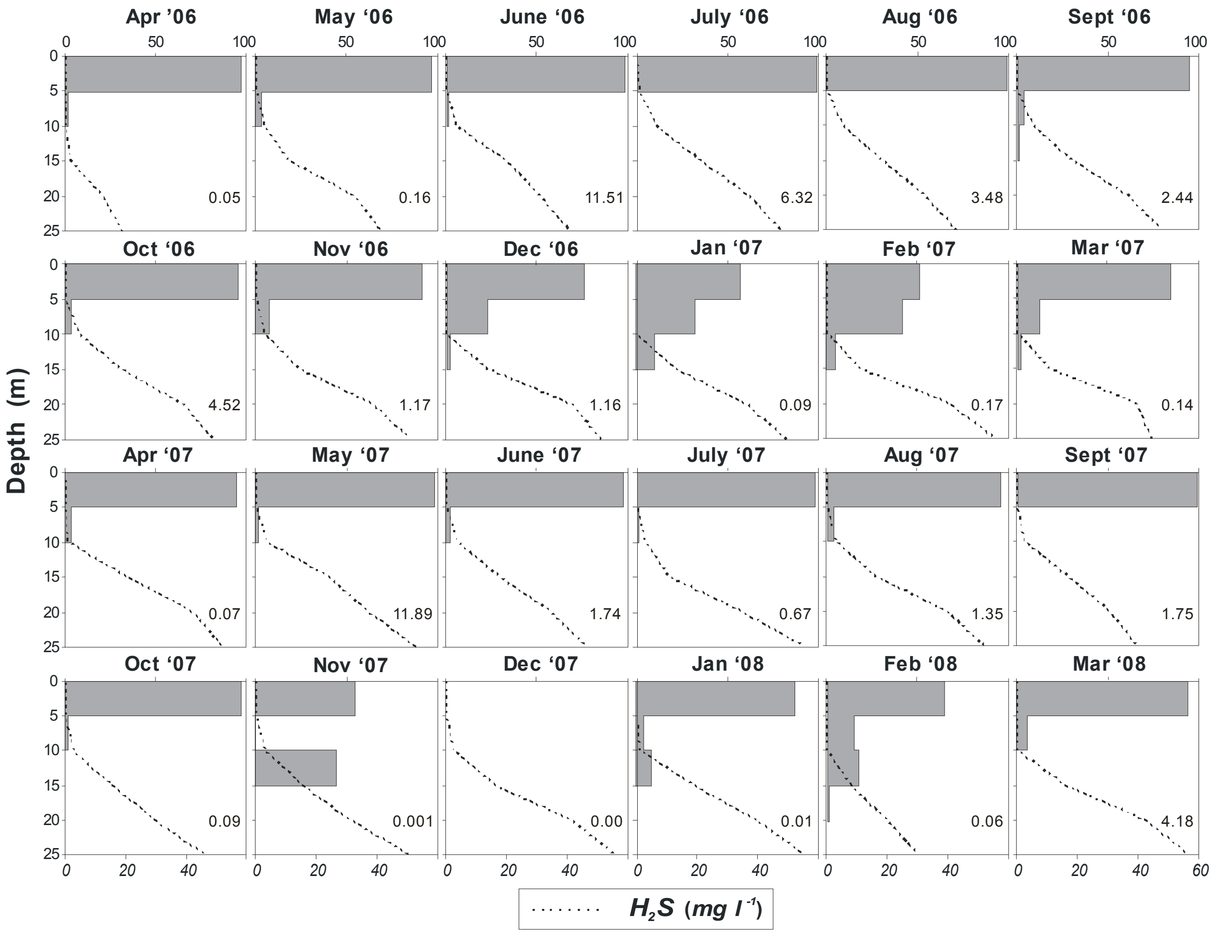

FIGURE 4 - Vertical profiles of H_2S concentration (mg l^{-1}) during the sampling period (April 2006 – March 2008). The shadowed area presents the vertical distribution of the total copepods as percentages (%) of total caught in water column sampled for the same period. Mean integrated abundance values (ind l^{-1}) in the 0-25 m are also shown.

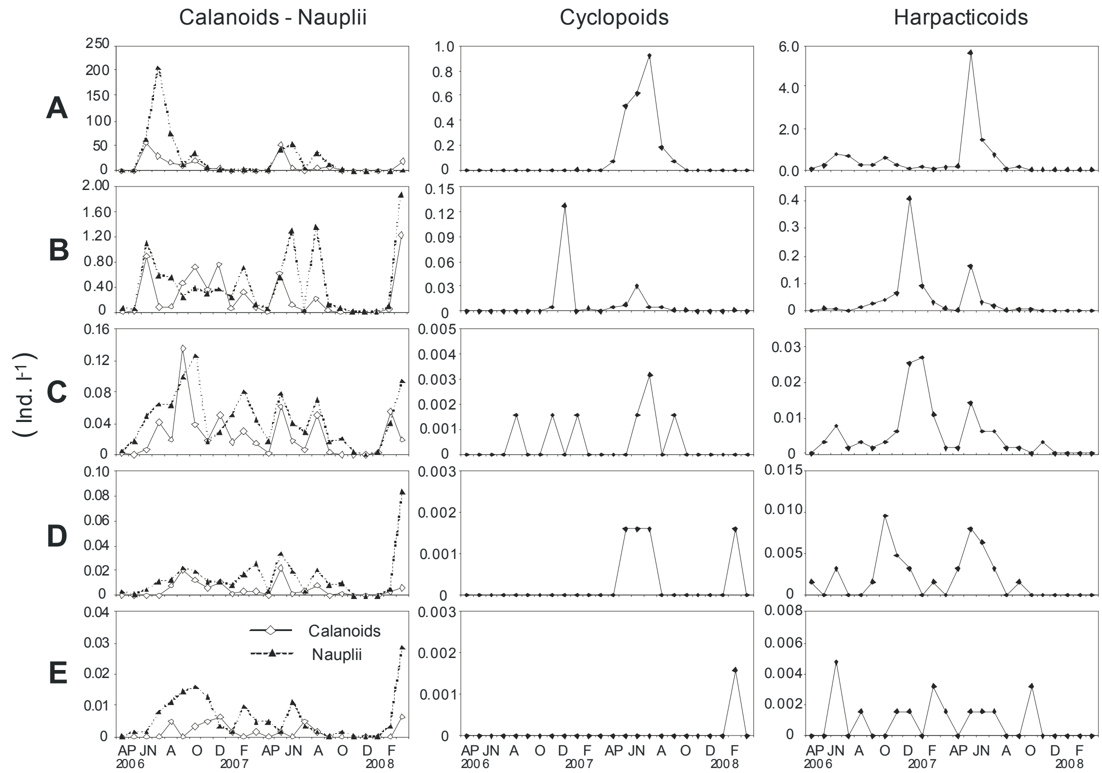

FIGURE 5 - Temporal abundance (ind l^{-1}) variation of calanoids, nauplii, cyclopoids and harpacticoids in the five depth layers A: 0-5 m, B: 5-10 m, C: 10-15 m, D: 15-20 m and E: 20-25 m, during the sampling period (April 2006 – March 2008).

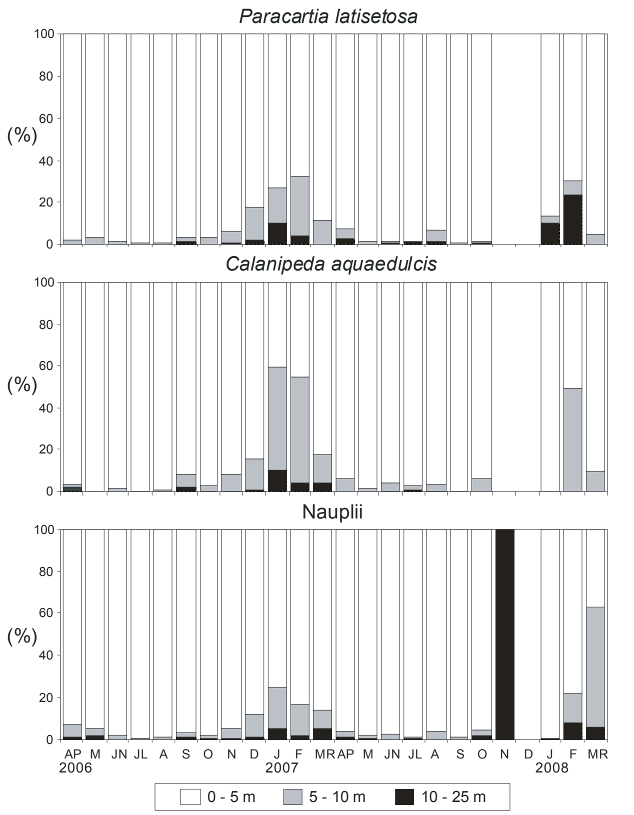

FIGURE 6 - Monthly variation of the percentages (%) of the calanoids *Paracartia latisetosa*, *Calanipeda aquaedulcis* and the copepod nauplii caught in the 0-5, 5-10 and 10-25 m depth layers during the sampling period (April 2006 – March 2008).

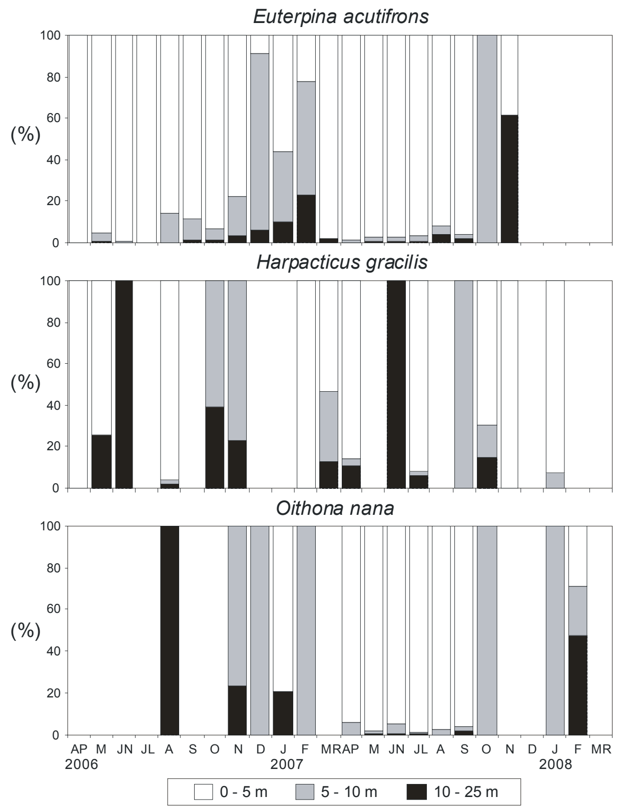

FIGURE 7 - Percentage (%) monthly variation of the harpacticoids *Euterpina acutifrons*, *Harpacticus gracilis* and the cyclopoid *Oithona nana* caught in the 0-5, 5-10 and 10-25 m depth layers during the sampling period (April 2006 – March 2008).

TABLE 1 - Multiple regression analysis between environmental factors such as temperature (Temp), salinity (Sal), H_2S concentration, Chl-*α*, pH, and the species, ontogenetic stages and gender of the copepods found in Aitoliko Lagoon.

	Environmental parameters						
Species/Groups	**Temp**	**Sal**	**H_2S**	**Chl-*α***	**pH**	(r^2)	(d.f.)
P. latisetosa (female)	0.41**	-0.28**		-0.21*		0.308	119
P. latisetosa (male)	0.44**					0.197	119
P. latisetosa (copepodites)	0.47**		-0.26*	-0.31**		0.470	119
Paracartia latisetosa	0.48**		-0.28*	-0.29**		0.479	119
C. aquaedulcis (female)	0.47**	-0.28**		-0.26**		0.367	119
C. aquaedulcis (male)	0.49**					0.238	119
C. aquaedulcis (copepodites)	0.41**		-0.41**	-0.30**		0.437	119
Calanipeda aquaedulcis	0.44**		-0.40**	-0.29**		0.452	119
Euterpina acutifrons	0.42**				0.17*	0.283	119
Harpacticus gracilis	0.35**					0.125	119
Oithona nana	0.41**				0.19*	0.241	119
Tisbe sp.		-0.32**		-0.22*		0.100	119
Nauplii	0.56**		-0.42**	-0.30**		0.630	119
Total copepods	0.49**		-0.43**	-0.30**		0.542	119
Copepod carcasses	0.61**	-0.31**		-0.13*		0.573	119

(*) = $p<0.05$, (**) = $p<0.01$)

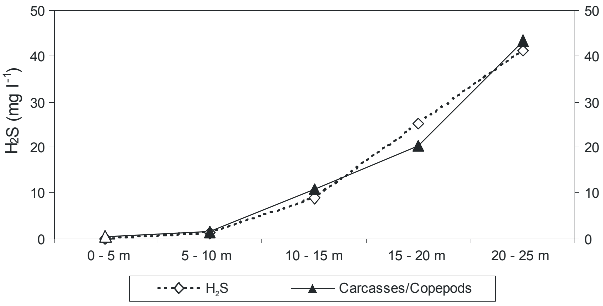

FIGURE 8 - The average ratio of carcasses/copepods and the average H_2S concentration (mg l^{-1}) in the five depth intervals (0-5, 5-10, 10-15, 15-20 and 20-25 m) in Aitoliko Lagoon.

depth, from 5.4 % in the surface 0-5 m to 39.5 % in the deepest layer (20-25 m).

Although anoxic conditions prevailed in the deeper layers of 15-20 and especially 20-25 m during certain periods, small numbers of specimens of all the copepod species occasionally recovered from these depths (Fig. 5). Moreover, nauplii were always present in these conditions. Indeed, their relative abundance (%) contribution within the total copepods did not alter with depth, even in these cases. In contrast, adult specimens of the two calanoids *P. latisetosa* and *C. aquaedulcis* were never found in the anoxic layers.

The copepod carcasses, expressed as percentages in all the copepods counted together, showed an exponential growth with increasing depth. In addition, this pattern was similar to the increase of H_2S with depth (Fig. 8), while there was a strong correlation between them (Pearson's r = 0.806, p = 0.0001).

Temperature was the most important environmental parameter which correlated positively with all species, affecting apparently their seasonal cycles (Table 1). On the other hand, Chl-*α* seemed to have exercised a negative impact in most of the copepod species, as well as in total copepods. The anoxic conditions, expressed as the H_2S concentration, affected in a negative way the vertical distribution of calanoids and nauplii, while the harpacticoid and cyclopoid species seemed to be more tolerant in the anoxic environment (Table 1). Among the other parameters, salinity negatively affected the variation of the females of both calanoid species (*P. latisetosa* and *C. aquaedulcis*) and of *Tisbe* sp., while pH showed a weak positive affection to *E. acutifrons* and *O. nana* (Table 1).

4 DISCUSSION

4.1 Physicochemical parameters

The results of the present study concerning the physicochemical and hydrological parameters of Aitoliko Lagoon are in accordance with the older reports, which describe this basin as permanently stratified with anoxic condition prevailing a few meters under the surface [2, 13, 14]. In this area anoxia is predominantly affected by morphology of the basin (large depth, small length) and the restricted connection with the sea via the Messolonghi lagoon in the south, while salt/fresh water budget and nutrient load play supplementary roles [2]. The year-round brackish character of this area came from the irrigation drainage inflow along with precipitation and land run-off. The permanent stratification in the water column was ought mainly to the vertical salinity gradient, whereas the role of temperature was secondary [2].

The main characteristic of Aitoliko Lagoon was the existence of an extensive hypoxic and anoxic environment which accounted for about 10 % of the total water volume of the whole basin [2]. Hypoxic/anoxic zones are usually formed as a result of interaction among water column stratification and factors enhancing primary production and accumulation of organic carbon, leading to increased oxygen consumption below the pycnocline [15]. Thus, the anoxic and rich in hydrogen sulphide layer in the deeper part of Aitoliko Lagoon, was created by the isolation of the water masses in these depths along with the high productivity in the surface layer [13]. According to the results on the productivity, as indicated by the Chl-*a* concentration, Aitoliko Lagoon can be considered as a mesotrophic ecosystem. The highest values of Chl-*a* recorded were similar to those measured twenty years before [13].

During the last decades, the nutrient loads in the broad area of Aitoliko and Messolonghi system were increased via primary wastewater treatment plants and freshwater discharges, through the operation of drainage pumping stations located in the cultivated agricultural areas in the vicinity of the two basins [2, 16]. These factors along with the limited water exchange between Aitoliko and Messolonghi basins enforced anoxia in Aitoliko and resulted to the increase in the frequency of holomictic anoxic incidents (expressed as massive fish deaths) during the last fifty years [2]. Recently there was an effort to reverse the degradation of the system back to its previous natural state by facilitating the water exchange between Messolonghi and Aitoliko basins. The dredging of the connecting sill between them was completed on May 2006 and resulted to the increase of the water fluxes at about 30 % [2].

The first outcome of these efforts seemed to be the oxygenation of the deeper parts of the basin in winter months of 2007 [2], while this was also found in the present study for the winter of 2008. Moreover, the increase of oxygen concentration especially in the deeper layers, along with the increase of salinity during the second sampling year, could be attributed to the entrance of greater quantities of saline waters from the Messolonghi lagoon [16]. Psilovikos (1995) [14] reported that the concentration of H_2S in Aitoliko Lagoon reached 45 mg l^{-1} which, however, is lower than the maximum values in the present study. It seems, then, that there has been an increase of the H_2S in this basin during the last 10-15 years.

Consequently, the restoration efforts established at the time of the beginning of the two-year investigation in Aitoliko Lagoon seem to have shown the first positive results as oxygen and salinity are concerned. However, the lack of significant variation in the H_2S concentrations between the two sampling years, means probably that the renewal of the deeper layers with dense and salty water coming from the Messolonghi lagoon is a slow process, considering also the great vertical extend of the H_2S rich layer.

4.2 Copepod species and vertical distribution

The copepod's community in Aitoliko Lagoon consisted of a small number of marine and brackish species. The low zooplankton biodiversity of this area could probably be ought to the harsh environmental conditions prevailing, while it was similar to other coastal lakes in the Adriatic Sea characterized by the domination of only a few species, occasionally present in very high densities [3, 10, 17]. *Paracartia latisetosa*, which was the dominant species, along with the other abundant calanoid *Calanipeda aquaedulcis* are typical members of confined environments such as lagoons, coastal lakes or wetlands [18, 19, 20]. Also, most of the harpacticoids and *O. nana* are cosmopolitan species and have been reported as residents in several brackish coastal environments of the Mediterranean [21].

The decrease of the oxygen concentration and especially the increase of the toxic H_2S with depth are undoubtedly the parameters that affected the vertical distribution of copepods in Aitoliko Lagoon. However, their influence was different among species, with the vertical distribution of the calanoid species being characterized by their accumulation in the surface 0-5 m layer, while the harpacticoid and the cyclopoid presented deeper distribution. Vertical separation patterns of copepods have been identified in several occasions and attributed to various reasons, among which is the adaptation of a specific combination of abiotic parameters (e.g. light, temperature, DO, salinity), or the diminishing of inter-specific competition for food [22, 23]. In respect to the existence of food competition among species, it should be noticed that copepods of confined coastal habitats are mainly detritivorous and omnivorous or feed on diatoms [24, 25]. Diatoms have been found to dominate the phytoplankton of Aitoliko Lagoon [13], while this group has been reported as food for *C. aquaedulcis* [26], *Euterpina acutifrons* [27] and *O. nana* [25]. However, the present data set permit only speculations on the trophic interrelations among copepod species and phytoplankton.

The negative correlation of most species to Chl-*a*, was the result of the different seasonal maxima of Chl-*a* concentration and copepod abundance, as well as their diverse vertical distribution. This could probably indicate that the density of phytoplankton in the area was adequate to support the secondary production and, thus, it does not constitute a restricting factor for the copepod's abundance. Yet, the deep Chl-*α* maximum does not necessarily mean higher phytoplankton biomass [28] or higher primary production rates [29] and, consequently, the relation of this parameter with copepod's spatial variation could be more complicated, or even absent.

Most of the harpacticoids identified in the present study are benthic forms which may live in and on the bottom sediments, while their presence in plankton samples can be due to suspension processes and also to active migration [30]. The above could explain the deeper distribution of these species. Similarly, the investigation of Lučić and Kršinić (1998) [31] in the Mali Ston Bay (southern Adriatic) demonstrated significant aggregation of the harpacticoid *E. acutifrons* and the cyclopoid *O. nana* in deeper layers. Unfortunately, there are no references on the vertical distribution of the two calanoid species in deep estuarine areas or coastal lakes in order to access possible differences with the present results.

Although we can not refer to an actual vertical separation among species in Aitoliko Lagoon, the selection of the surface layer by the calanoids could be due to their lower tolerance to the hypoxic or anoxic conditions, in comparison to the harpacticoid and cyclopoid species which presented a deeper distribution. There are various references about the adaptation of higher anoxia tolerance by the benthic harpacticoids [8, 32], while there are also reports of some pelagic calanoids which are capable of surviving in such conditions of elevated sulfide concentrations [7]. Unfortunately, there are no references on this issue concerning the present species.

Paracartia latisetosa and *Calanipeda aquaedulcis* are calanoids typical of confined environments and produce resting eggs to survive harsh conditions [18, 33]. These eggs allow to the species to remain in the same environment even if in a non active stage. The anoxic layer is a problem for the hatch of resting eggs [34], but the -25 m depth is a small portion of the basin bottom. Resting eggs probably accumulate there without any re-injection of nauplii into the plankton, but all those which sink on oxygenated sediments (those until 10-15 m) will replenish the water column at the reproductive period. In contrast to calanoids, harpacticoids and cyclopoids do not produce resting eggs but can enter an inactive resting phase as copepodites [34]. Thus, the presence of harpacticoids and cyclopoids in the deep anoxic layers was probably due to their resistance (resting phase) more than to their derivation from the bottom. Unfortunately, due to the immediate preservation of the samples, it can not be demonstrated if the specimens collected in the anoxic layers were active or not.

The high tolerance of copepod nauplii in the hypoxic and the anoxic conditions has been reported in other occasions [3]. Since the nauplii were not identified to species or even to family level, it was not possible to distinguish which copepod nauplii were found within the anoxic layers. Considering the probable higher tolerance of the harpacticoid and cyclopoid copepodites and adults found under hypoxic conditions, it could be suggested that their naupliar stages possess also the ability of surviving in these environments. Thus, the nauplii found in the anoxic conditions could be only these of the above species, though this could not be verified with the present data set.

Among the environmental parameters, temperature clearly affected the seasonal variation of the numerically most important copepod species, which presented maxima in the warmer period of the year. *P. latisetosa* and *C. aquaedulcis* are considered as typical summer copepods in the Mediterranean coasts [23, 33, 35-37]. The same stands also for *E. acutifrons* and *O. nana*, both of which showed a strong correlation with temperature [25].

The proportions of copepod carcasses showed a similar pattern of increase with depth as the concentration of H_2S, which along with the sharp decrease of copepods in the vertical axis, means that this chemical parameter is responsible for the diminishing of life in the deeper parts of Aitoliko Lagoon. It must also be taken into consideration that the lethal effects of H_2S increase in the lower pH values [6], such as those recorded in the deeper layers. In this sense, the combination of the above two parameters could have been responsible for the high mortality of copepods. On the other hand, De Meester and Vyverman (1997) [38] observed migration of the copepod *Acartia tonsa* in a meromictic lake, where the animals were reported to migrate into the anoxic hypolimnion and remain there for >12 h. The hypolimnion also contained sulphide, and the authors speculate that the population may have been genetically adapted to withstand the toxic waters. The general conclusion is that more information is needed to understand the interplay of physiological, morphological and behavioural adaptations to anoxia and sulphide as well as their influence on life history events and reproductive phases. In any case, in a few meters under the surface of Aitoliko Lagoon, the conditions turn to be inhospitable and, thus, a major part of the secondary productivity of the ecosystem is transformed to dead organic load and, eventually, concentrates/precipitates to the bottom.

The present study, being in agreement with the reports of Gianni et al. (2011) [2], showed that the recent technical interventions towards the increase of the water communication between Aitoliko and Messolonghi basins have resulted to the improvement of oxygenation of the deeper layers of the former basin. However, it seems that greater time interval will be required for the vertical distribution of the zooplankton invertebrates and especially copepods to be affected. Future observation of these variations in parallel with the physicochemical and hydrological characteristics of this basin will contribute to the establishment of models explaining such biotic-abiotic interactions in anoxic environments worldwide.

REFERENCES

[1] Diaz, R.J. (2001) Overview of hypoxia around the World. Journal of Environmental Quality 30, 275–281.

[2] Gianni, A., Kehayias, G. and Zacharias, I. (2011) Geomorphology modification and its impact to anoxic lagoons. Ecological Engineering 37, 1869–1877.

[3] Barić, A., Grbec, B., Kušpilić, G., Marasović, I., Ninčević, Ž. and Grubelić, I. (2003) Mass mortality event in a small saline lake (Lake Rogoznica) caused by unusual holomictic conditions. Scientia Marina 67, 129-141.

[4] Gonçalves, A.M.M., De Troch, M., Marques, S.C., Pardal, M.A. and Azeiteiro, U.M. (2010) Spatial and temporal distribution of harpacticoid copepods in Mondego estuary. Journal of the Marine Biological Association of the United Kingdom 90, 1279–1290.

[5] Marcus NH, Lutz RV, Chanton JP (1997) Impact of anoxia and sulfide on the viability of eggs of three planktonic copepods. Marine Ecology Progress Series 146, 291–295.

[6] Invidia, M., Sei, S. and Gorbi, G. (2004) Survival of the copepod *Acartia tonsa* following egg exposure to near anoxia and to sulfide at different pH values. Marine Ecology Progress Series 276, 187–196.

[7] Ekau, W., Auel, H. Pörtner, H.-O. Gilbert, D. (2010) Impacts of hypoxia on the structure and processes in pelagic communities (zooplankton, macro-invertebrates and fish). Biogeosciences 7, 1669–1699.

[8] Vopel, K., Dehmlow, J. and Arlt, G. (1996) Vertical distribution of *Cletocamptus confluens* (Copepoda, Harpacticoida) in relation to oxygen and sulphide microprofiles of a brackish water sulphuretum. Marine Ecology Progress Series 141, 129-137.

[9] Leonardos, I. and Sinis, A., (1997) Fish mass mortality in the Etolikon lagoon, Greece: the role of local geology. Cybium 21, 201–206.

[10] Benović, A., Lučić, D., Onofri, V., Peharda, M., Carić, M., Jaspica, N. and Bobanović-Ćolić, S. (2000) Ecological characteristics of Mljet Island seawater lakes (South Adriatic Sea) with special reference to their resident populations of medusae. Scientia Marina 64, 197-206.

[11] Belmonte, G., Scirocco, T. and Denitto, F. (2011) Zooplankton composition in Lake Varano (Adriatic Sea coast, Italy). Italian Journal of Zoology 78, 370-378.

[12] A.P.H.A., A.W.W.A. & W.P.C.F. (1998) Standard Methods for the Examination of Water and Wastewater. American Public Health Association, American Water Works Association and Water Pollution Control Federation, Washington D.C.

[13] Danielidis, D.B. (1991) A systematic and ecological study of the diatoms in the lagoons of Messolongi, Aitoliko and Kleissova. Ph.D. Thesis, 287 pp. University of Athens (in Greek).

[14] Psilovikos, A. (1995) Evaluation and Management of the Lower Acheloos Drainage Water Budget for the Development and Environmental Enhancement of its Estuary, Lagoons and the Greater Area. Aristotel University of Thessaloniki (in Greek).

[15] Rabalais, N.N., Diaz, R.J., Levin, L.A., Turner, R.E., Gilbert, D. and Zhang, J. (2010) Dynamics and distribution of natural and human-caused hypoxia. Biogeosciences 7, 585–619.

[16] Marazioti, C. E., Flessia, G., Vlachos, P., Koutrouli, E., Ntaikou, I., Antonopoulou, G., Pakou, C., Kalfas, C., Kornaros, M. and Lyberatos, G. (2010) On the environmental state of the Mesologgi lagoon in Greece. Fresenius Environmental Bulletin 19, 1151–1164.

[17] Kršinić, F., Carić, M., Viličić, D. and Ciglenečki, I. (2000) The calanoid copepod *Acartia italica* Steuer, phenomenon in the small saline Lake Rogoznica (Eastern Adriatic coast). Journal of Plankton Research 22, 1441–1464.

[18] Belmonte, G. and Potenza, D. (2001) Biogeography of the family Acartiidae (Calanoida) in the Ponto-Mediterranean Province. Hydrobiologia 453/454, 171–176.

[19] Belmonte, G. (1992) Diapause egg production in *Acartia* (*Paracartia*) *latisetosa* (Crustacea, Copepoda, Calanoida). Bolletino di zoologia 59, 363-366.

[20] Gonçalves, A. M. M., Pardal, M. A., Marques, S. C., De Troch, M. and Azeiteiro U. M. (2010) Distribution and composition of small-size zooplankton fraction in a temperate shallow estuary (western Portugal). Fresenius Environmental Bulletin 19, 3160–3176.

[21] Carli A. and Crisafi P. (1983) Copepodi lagunari. In: Guide per il riconoscimento delle specie animali delle acque lagunari e costiere italiane. 11. AQ/1/230, CNR, Roma, 125 pp.

[22] Mauchline, J. (1998) The biology of calanoid copepods. Advances in Marine Biology 33, 1–710.

[23] Sato, K.-I., Yamaguchi, A., Ueno, H. and Ikeda, T. (2011) Vertical segregation within four grazing copepods in the Oyashio region during early spring. Journal of Plankton Research 33, 1230–1238.

[24] Kleppel, G.S. (1993) On the diets of calanoid copepods. Marine Ecology Progress Series 99,183–195.

[25] Vidjak, O., Bojanić, N., Kušpilić, G., Marasović, I., Ninčević Gladan, Ž. and Brautović, I. (2006) Annual variability and trophic relations of the mesozooplankton community in the eutrophicated coastal area (Vranjic Basin, eastern Adriatic Sea). Journal of the Marine Biological Association of the United Kingdom 86, 19-26.

[26] Brucet, S., Compte, J., Boix, D., López-Flores, R. and Quintana, X.D. (2008) Feeding of nauplii, copepodites and adults of *Calanipeda aquaedulcis* (Calanoida) in Mediterranean salt marshes. Marine Ecology Progress Series 355, 183–191.

[27] Camus, T. and Zeng, C. (2011) Reproductive performance, survival and development of nauplii and copepodites, sex ratio and adult life expectancy of the harpacticoid copepod, *Euterpina acutifrons*, fed different microalgal diets. Aquaculture Research 43, 1159–1169.

[28] Cullen, J.J. (1982) The deep chlophyll maximum: Comparing vertical profiles of Chlorophyll-α. Canadian Journal of Fisheries and Aquatic Sciences 39, 791–803.

[29] Harisson, W.G. (1990) Nitrogen utilization in chlorophyll and primary productivity maximum layers: an analysis based on the f-ratio. Marine Ecology Progress Series 60, 85–90.

[30] Uriarte, I. and Villate, F. (2005) Differences in the abundance and distribution of copepods in two estuaries of the Basque coast (Bay of Biscay) in relation to pollution. Journal of Plankton Research 27, 863–874.

[31] Lučić, D. and Kršinić, F. (1998) Annual variability of mesozooplankton assemblages in Mali Ston Bay (Southern Adriatic). Periodicum Biologorum 100, 43–52.

[32] Vopel, K., Dehmlow, J., Johansson, M. and Arlt, G. (1998) Effects of anoxia and sulphide on populations of *Cletocamptus confluens* (Copepoda, Harpacticoida). Marine Ecology Progress Series 175, 121-128.

[33] Belmonte, G. and Pati, A.C. (2007) Hatching rate and diapause duration in eggs of *Paracartia latisetosa* (Copepoda: Calanoida). Journal of Plankton Research 29, suppl.1, i39–i47.

[34] Dahms, H.-U. (1995) Dormancy in the Copepoda—an overview. Hydrobiologia 306(3), 199–211.

[35] Lakkis, S. (1994) Coexistence and competition within *Acartia* (Copepoda, Calanoida) congeners from Lebanese coastal water: niche overlap measurements. Hydrobiologia 292/293, 481-490.

[36] Quintana, X.D., Comín, F.A. and Moreno-Amich, R. (1998) Nutrient and plankton dynamics in a Mediterranean salt marsh dominated by incidents of flooding. Part 2: Response of the zooplankton community to disturbances. Journal of Plankton Research 20, 2109-2127.

[37] Annabi-Trabelsi, N., Daly-Yahia, M.N., Romdhane, M.S. and Ben Maïz, N. (2005) Seasonal variability of planktonic copepods in Tunis North Lagoon (Tunisia, North Africa). Cahiers de Biologie Marine 46, 325-333.

[38] De Meester, L. and Vyverman, W. (1997) Diurnal residence of the larger stages of the calanoid copepod *Acartia tonsa* in the anoxic monimolimnion of a tropical meromictic lake in New Guinea. Journal of Plankton Research 19, 425–434.

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