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Zooplankton variation in relation to hydrology in an enclosed hypoxic bay (Amvrakikos Gulf, Greece)

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Abstract

This study investigated the temporal and spatial variation of the zooplankton community of a hypoxic coastal embayment (Amvrakikos Gulf, Western Greece) in relation to hydrological characteristics during an annual cycle. The main hydrological feature was the prolonged water stratification, which determined hypoxic conditions in the deeper layers that became anoxic close to the bottom in September, while vertical mixing occurred for a very short period (October-November). The total zooplankton abundance fluctuated between 44.6 and 159.7 ind l^{-1}. Fourteen groups were recorded, among which copepods dominated accounting on average for 86.4 %. Most of the groups presented higher abundance values in winter and spring when increased chlorophyll-α concentrations were found. Oxygen depletion affects the vertical distribution of most zooplankton groups and the vertical habitat partitioning between copepod orders and their ontogenetic stages. Several taxa were recorded even in the deep, anoxic layers, but only the polychaete larvae increased in abundance with depth. Calanoids, appendicularians and bivalve larvae presented an eastward decrease of abundance in the deepest layers following the same pattern of oxygen decrease. Notwithstanding hypoxic conditions in its deepest layers, the Amvrakikos Gulf was considered a mesotrophic ecosystem, with the nutrient concentration being lower than in the past. Several biotic elements indicate that the gulf is in a transitional phase towards a better quality state and these results reinforce the need for consistent monitoring of this ecosystem.

Keywords: Zooplankton, hydrology, hypoxia, copepods, vertical distribution, Mediterranean, Amvrakikos.

Introduction

Coastal embayments are highly diverse ecosystems with a varying spectrum of physical isolation from the open sea and water circulation patterns (Ferentinos *et al.*, 2010). From an ecological viewpoint, these basins are usually characterized by high productivity and, being important nursery grounds for several commercial fish species, they are subjected to intense aquaculture and fishing. Due to their vicinity to land, they are often the receivers of urban, industrial and agricultural pollution, which historically has led to their protection by international conventions (Valiela, 1991). The high productivity of these areas, along with a combination of their morphology, circulation and stratification patterns, water retention times and freshwater inflows, has lead to the existence of hypoxic (dissolved oxygen <2 mg l^{-1}) or anoxic (dissolved oxygen <0.2 mg l^{-1}) conditions mainly in their deeper sections. During the last few decades, the number of areas characterized by hypoxic and anoxic conditions has spread exponentially around the world, having serious consequences on ecosystem functioning (Diaz & Rosenberg, 2008).

The Amvrakikos Gulf is the largest embayment in Greece and is considered one of the most important wetlands in the Mediterranean. The area is protected under the international Ramsar Convention, it has been designated as a Special Protection Area (SPA) according to the European Union Directive 79/409/EU, and is included in the Natura 2000 Network. Economically, the gulf is an important fish and shrimp fishing area, and an extensive aquaculture industry has developed there over the last 20 years (Naeher *et al.*, 2012). Until recently, only sparse oceanographic information referring to the Amvrakikos Gulf existed in the literature. During recent years, the studies of Ferentinos *et al.* (2010), Kountoura & Zacharias (2011; 2013), Naeher *et al.* (2012) and Kountoura (2014), have revealed that the gulf suffers from seasonal hypoxia (oxygen concentrations <2 mg l^{-1}) in its western part, and seasonal anoxia (oxygen concentrations <0.2 mg l^{-1}) in its eastern part. This has been caused by the excessive use of agricultural fertilizers in the surrounding area along with the increase in animal stocks, intensive fish farming and domestic effluents in the last 20 to 30 years (Ferentinos *et al.*, 2010; Kountoura & Zacharias, 2011). Kountoura & Zacharias (2013) concluded that the Amvrakikos Gulf is a mesotrophic and seasonally eutrophic gulf, but if the concentration of total phosphorus in the entire water column is taken into account, the gulf could be characterized as eutrophic. However, all these recent oceanographic studies provide data concerning specific periods (e.g. summer), or originate from sampling during certain seasons, while none of the studies have monitored the gulf on a monthly basis to provide more data on the temporal trends of the abiotic environment.

Zooplanktonic organisms are a very important biotic element in the food webs of coastal environments not only as consumers of primary production, but also as food for juvenile fish species of high commercial value (Collos *et al.*, 2005). Moreover, its sensitivity to both man-made and natural changes makes zooplankton quite suitable for assessing alterations in trophic dynamics and the ecological state of aquatic ecosystems related to changes in nutrient loading and climate. Despite the great ecological importance of zooplankton, only two studies concern this element in the Amvrakikos Gulf. The first was conducted in just one month in 1980 (Nicolaidou *et al.*, 1983), and the second for several months during 1987 (Panayotidis *et al.*, 1994). Both of these studies provided data on zooplankton species composition and abundance but did not couple these data with the area's physico-chemical parameters or oceanographic regime.

Consequently, considering the lack of information on zooplankton in the Amvrakikos Gulf, as well as the need for a long-term survey of its hydrological regime, this study aims to: a) provide the most recent data on the spatial and temporal variation of zooplankton through a 12-month survey, b) monitor the physico-chemical parameters and estimate the trophic state of the gulf during the study period, c) combine the abiotic and biotic data to assess the influence of hydrology on the zooplankton community, and d) provide a more accurate picture of the present and possible future environmental quality of this ecosystem.

Materials and Methods

Site description

The Amvrakikos Gulf is a large (405 km^2), semi-enclosed embayment, located in Western Greece (Fig. 1). Its only connection to the open Ionian Sea is a narrow strait (600 m wide, 3 km long, with a mean depth of 8.5 m). The mean depth of the gulf is 26 m, while the maximum depth of 63 m has been recorded in its eastern part. The Amvrakikos Gulf receives freshwater from two rivers in the northern area, Arachthos River and Louros River, which have drainage basins of 1894 and 785 km^2, respectively (Kountoura & Zacharias, 2011). Due to high freshwater input, the gulf has a positive water balance and is considered a brackish environment with salinity ranging from 17 to 38 (Panayotidis *et al.*, 1994; Kountoura & Zacharias, 2011).

Sample collection and analysis

Zooplankton sampling was carried out in the gulf on a monthly basis during a 12-month period (September 2008 - August 2009) at three stations (A, B and C) with depths of 32, 33 and 32 m, respectively (Fig. 1). The samples were collected using a vertically towed conical net (40 cm diameter, 1 m length, 100 μm mesh-size) equipped with a closing mechanism. The hauls were conducted in six discrete layers from the surface to 30 m depth (0-5, 5-10, 10-15, 15-20, 20-25 and 25-30 m). The total number of zooplankton samples was 210; no samples were collected at station B in September 2008 due to technical problems. All samplings were carried out during daylight hours. The filtered water volume was estimated with a Hydro-Bios flowmeter attached to the mouth of the net. Samples were fixed immediately after

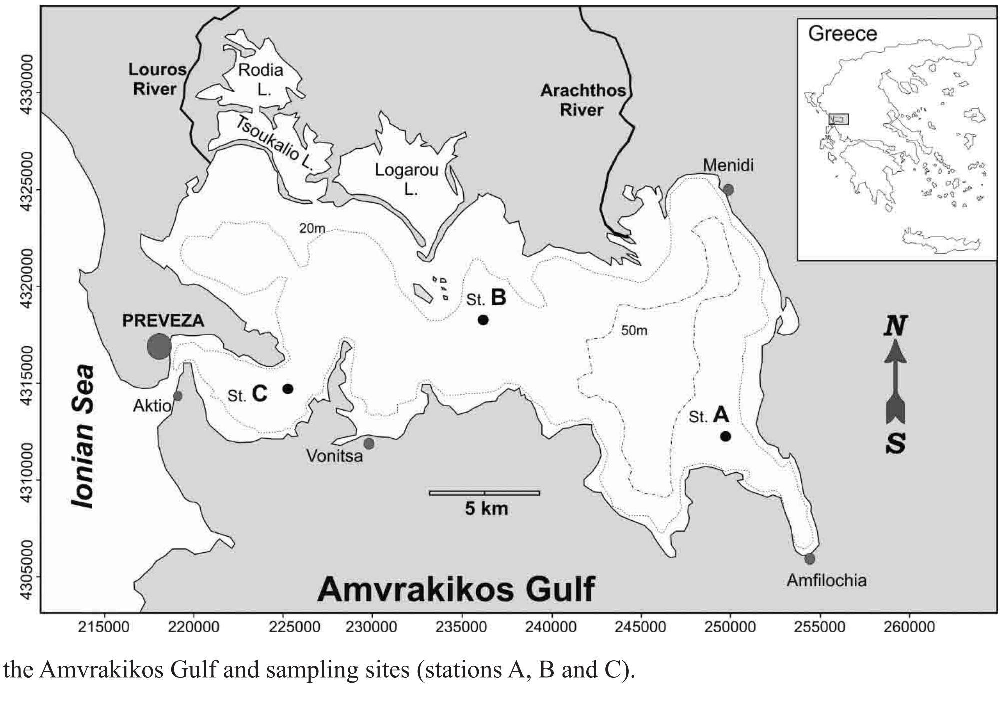

Fig. 1: Map of the Amvrakikos Gulf and sampling sites (stations A, B and C).

collection and preserved in a 4 % borax-buffered formaldehyde-sea water solution.

Measurements of temperature (Temp), salinity (Sal), pH and dissolved oxygen (DO) were taken in the whole water column on each sampling date with a Troll 9500 (In-Situ Inc.) water quality multi-parameter instrument. Water transparency (Trans) was determined at each station using a Secchi disk. The concentration of chlorophyll-a (Chl-*a*), total phosphorus (TP), phosphates (PO_4), nitrates (NO_3) and nitrites (NO_2) was measured in water samples collected from 0, 10, 20 and 30 m depths at all three stations, using a 5 l Hydro-Bios free flow sampling bottle. All samples were transported to the laboratory in a portable refrigerator at 4 °C. Analyses of all chemical parameters were performed according to APHA, AWWA & WPCF (1998). To determine Chl-*a* concentration, 500 ml of each water sample were filtered through a Whatman GF/F glass fibre filter shortly after collection. Pigment extraction was made in 90 % acetone and concentrations were determined spectrophotometrically according to APHA, AWWA & WPCF (1998) using a BOECO S-20 spectrophotometer.

Data analysis

Identification and enumeration of the zooplanktonic groups was performed in the laboratory under a Leica stereomicroscope using the keys of Trégouboff & Rose (1978). Copepods were identified to the order level (calanoids, cyclopoids and harpacticoids). Although tintinnids, large phytoplankton and protists were often present in the samples, they were not counted because they were not efficiently collected by our sampling gear. Zooplankton abundance was calculated as ind l^{-1} from aliquots taken with a Folsom splitter from each sample. Aliquot size ranged from 1/1 (whole sample) to 1/32, depending on the abundance of zooplankton organisms in the sample.

To compare the vertical distributions of the zooplankton community, the weighted median depth was calculated for each species as follows:

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

where WMD = weighted median depth, N_{Ti} = abundance at depth *i*, and T*i* = depth (m). Although the median depth cannot represent the actual vertical distribution of a species, it is a good numerical base for the application of statistics. Thus, temporal and spatial differences between the median depths of zooplankton species were tested using either the non-parametric Mann-Whitney (U) test or the Kruskal-Wallis test.

Carlson's trophic state index (TSI) (Carlson, 1977) was used to estimate the trophic state of the Amvrakikos Gulf. It is among the most widely used indices and was selected to allow the comparison of our results with those of Kountoura & Zacharias (2013). The TSI index is based on measurements of Secchi disk transparency (SD), chlorophyll-*a* (Chl-*a*) and total phosphorus (TP) concentrations. The index ranges from 0 to 100, although theoretically there are no lower or upper boundaries. TSI values of less than 40 correspond to oligotrophic conditions, values between 40 and 50 indicate mesotrophic conditions, and values of 50 to 70 indicate eutrophic environments. Finally, index values greater than 70 are associated with hypertrophic conditions. For each of the three quality variables, i.e. Secchi depth TSI_{SD}, chlorophyll-*a* $TSI_{Chl\text{-}a}$ and total phosphorus TSI_{TP}, the TSI index was calculated according to the equations:

$TSI_{SD} = 60 - 14.41 \ln(SD)$

$TSI_{Chl\text{-}a} = 30.6 + 9.81 \ln(Chl\text{-}a)$

$TSI_{TP} = 4.15 + 14.42 \ln(TP)$

For $TSI_{Chl\text{-}a}$ and TSI_{TP}, the average epilimnetic concentrations of Chl-*a* and TP (both as μg l^{-1}) were used, while for the TSI_{SD}, the average value of the Secchi disk (m) of the three sampling stations was used. The overall Carlson's TSI index was calculated as the average value of TSI_{SD}, $TSI_{Chl\text{-}a}$ and TSI_{TP} at the three stations, as follows:

$$TSI = \frac{TSI_{SD} + TSI_{Chl\text{-}a} + TSI_{TP}}{3}$$

To investigate the dependence of zooplankton dynamics on environmental variables, the Redundancy Analysis (RDA) was employed, using the CANOCO 4.0 (TerBraak & Šmilauer, 2002) program. The analysis was based on the abundance of taxa in relation to environmental variables from each depth layer. Data values were log transformed and centred prior to the analysis. The significance of the variability explained by individual environmental variables was tested using the Monte Carlo permutation test (restricted for time series) with 999 random permutations. The results are presented as a biplot where taxa and environmental variables are plotted together.

Differences in hydrographical properties and abundance of zooplanktonic taxa between seasons or sampling stations were evaluated with the Mann-Whitney (U) test or the Kruskal-Wallis test. Correlations in the monthly variation between environmental parameters were made using Pearson's *r* correlation coefficient. All data analyses were performed using the SPSS 17.0 (SPSS Inc., 2008) statistical package.

Results

Hydrological characteristics

Temperature presented strong seasonal variation and fluctuated between 9.01 and 29.69 °C in February and July 2009, respectively (Fig. 2A). Differences in temperature profiles between stations were recorded only in December, March and April with station A presenting the highest values (Kruskal-Wallis test, $p<0.01$). Concerning the deeper part of the gulf (20-30 m), station C had higher temperatures in the autumn months. Salinity fluctuated between 21.58 at the surface of station B in February, and 42.93 at 10 m depth of station A in August (Fig. 2B). A halocline occurred between 5 and 10 m depth from February to August 2009 and in September 2008 (Fig. 2B). Differences in salinity profiles between stations were recorded only from April to August with station C presenting the highest values (Kruskal-Wallis test, $p<0.01$), especially in the upper 0-15 m layer. In the deeper parts of the gulf (20-30 m), higher salinity values were recorded at station C in the autumn. An almost permanent stratification of the water column was the main hydrological characteristic of the Amvrakikos Gulf. Specifically, the density profiles revealed that the water column was stratified throughout the year except in October and November (Fig. 2C). No significant differences were recorded in the density profiles except in August with station A presenting the highest values (Kruskal-Wallis test, $p<0.01$). In winter and early spring, the water column was not thermally homogenous as expected; rather, there was an increase in temperature from the surface down to 20 m. Specifically, the stratification started in December probably due to the accumulation of freshwater (from river runoff and increased precipitation) in the surface layer, which resulted in salinity as low as 24. In February, a sharp halocline established in the layer between 4 and 8 m at all stations, resulting in an increase of salinity, from about 22 to 32. The temperature increase during spring and especially in summer further supported water stratification, which lasted until September.

The upper part of the water column in the Amvrakikos Gulf was always sufficiently oxygenated, whereas an oxycline was observed from March to November (Fig. 2D). Dissolved oxygen presented statistically significant differences between the three sampling locations, with station A showing the lowest concentrations at depths below 20 m in all months (Kruskal-Wallis test, $p<0.01$). In particular, hypoxic conditions (DO <2 mg l^{-1}) prevailed year-round in the deeper layers of station A (deeper than 17-20 m in autumn and spring, and deeper than 25 m in winter and summer), while hypoxic conditions were recorded on only eight sampling dates at station B, and three at station C. Anoxia (DO <0.2 mg l^{-1}) was recorded only in September at depths below 25 m at all stations.

The pH values showed little variation between 7.4 (February, station A, 30 m) and 8.3 (March, station B, 4 m), with the highest values recorded in the upper layers. In September 2008 and February to August 2009, an abrupt decrease of pH was observed at depths greater than 5 m (Fig. 2E). This vertical pattern was more pronounced in spring (March to May). Differences between stations were recorded from November to May (Kruskal-Wallis test, $p< 0.01$), with the highest pH values being recorded at station C and the lowest at station A.

Water transparency, as indicated by the Secchi disc, ranged from 2.5 to 7.0 m. The lowest values were re-

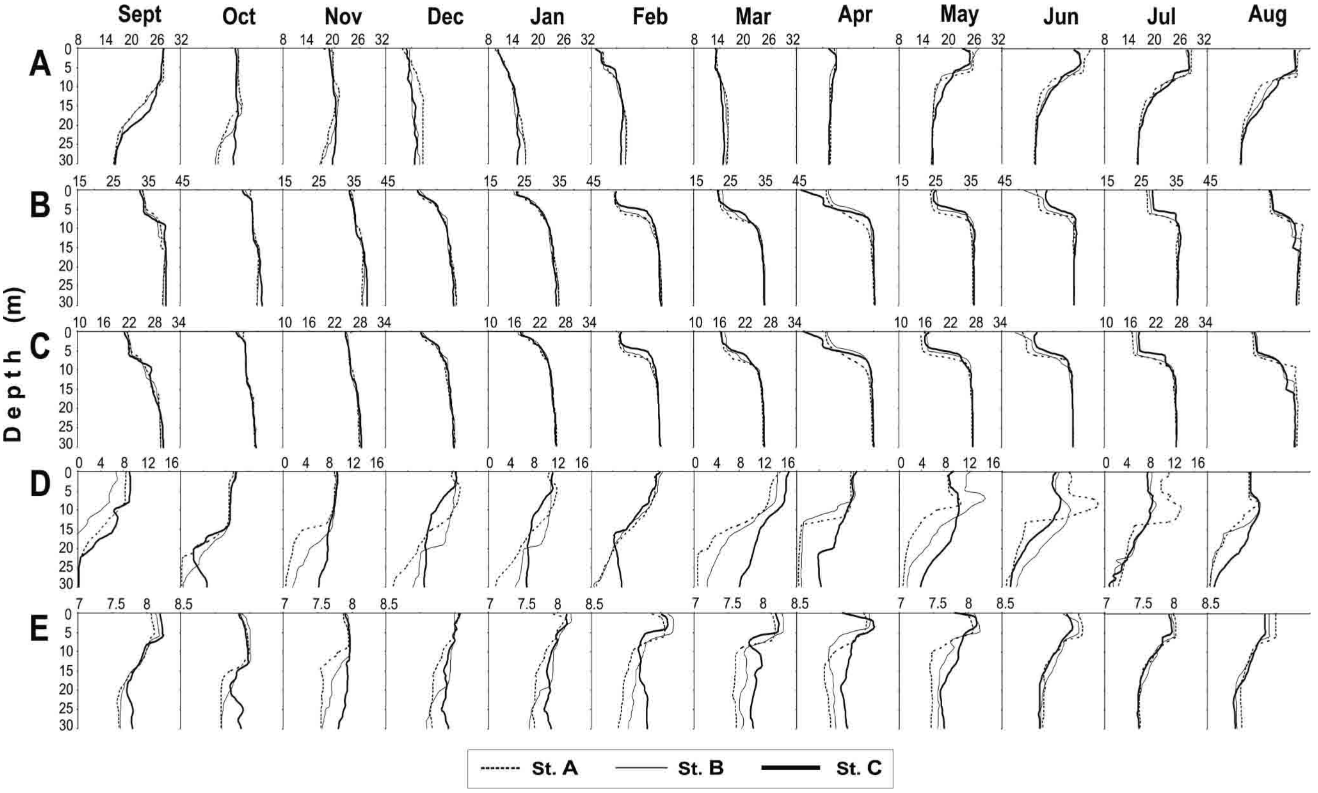

Fig. 2: Vertical profiles of **A**: temperature (°C), **B**: salinity, **C**: density (σ-t), **D**: dissolved oxygen (mg l^{-1}), and **E**: pH, at each of the three sampling stations (A, B, C) during the sampling period (September 2008 – August 2009).

corded in winter until early spring and the highest ones in October 2008. No significant differences were found between stations (Kruskal-Wallis test, $p>0.01$).

The highest Chl-*a* concentrations were recorded in December (8.92 μg l^{-1}, station C, 10 m) with a secondary peak in April (7.65 μg l^{-1}, station C, 0 m), while the lowest values were recorded in March (Fig. 3A). Differences between stations were observed only in November and January with station B presenting the highest values (Kruskal-Wallis test, $p<0.01$). The highest values were recorded either at the surface or at 10 m depth during the entire sampling period, while the lowest values were measured between the 20 m and 30 m depth layers.

The concentration of phosphates (PO_4) in the water was highest in summer (Fig. 3B) when it reached 0.167 μg l^{-1} in June near the bottom of station A. Undetectable concentrations were recorded in December and January in the upper 0-10 m layer of all stations. Generally, concentrations increased with depth at most of the sampling stations. Differences between stations were recorded only in November and April with station A presenting the highest values (Kruskal-Wallis test, $p<0.01$). The concentration of total phosphorus (TP) reached 0.29 μg l^{-1} (at 30 m depth in July) and followed the same general pattern of seasonal and vertical distribution of PO_4.

Nitrates (NO_3) showed intense seasonal variation with several peaks in November, February and July (Fig. 3C). Concentrations fluctuated between undetected values (in several sampling dates at 0-10 m) to 0.134 μg l^{-1} in February at the depth of 30 m at station A. Finally, the concentration of silicates (SiO_2) increased with depth for all sampling occasions and stations, and peaks were observed in September, January and March (Fig. 3D). No statistically significant differences between stations were recorded in the case of nitrates, while for silicates differences were found from January to April with station C presenting the lowest values (Kruskal-Wallis test, $p<0.01$).

Application of Carlson's index (TSI) for the concentration of total phosphorus TSI_{TP}, transparency TSI_{SD} and chlorophyll-*a* TSI_{Chl-a} produced the results presented in Figure 4. TSI_{TP} showed the highest variation (between 23.6 and 75.9) and fluctuated from oligotrophic to even hypertrophic boundaries. In contrast, TSI_{SD} showed low variation (between 34.2 and 46.8) and fluctuated mainly within mesotrophic conditions, while TSI_{Chl-a} variation was between 20.1 and 47.2, which indicates oligo- to mesotrophic conditions. Finally, the overall Carlson's TSI index varied between 35.8 and 50.1 with a mean of 41.3 and therefore classifies the Amvrakikos Gulf as on the boundary between the oligo- and mesotrophic levels. However, using TP measurements from the entire water

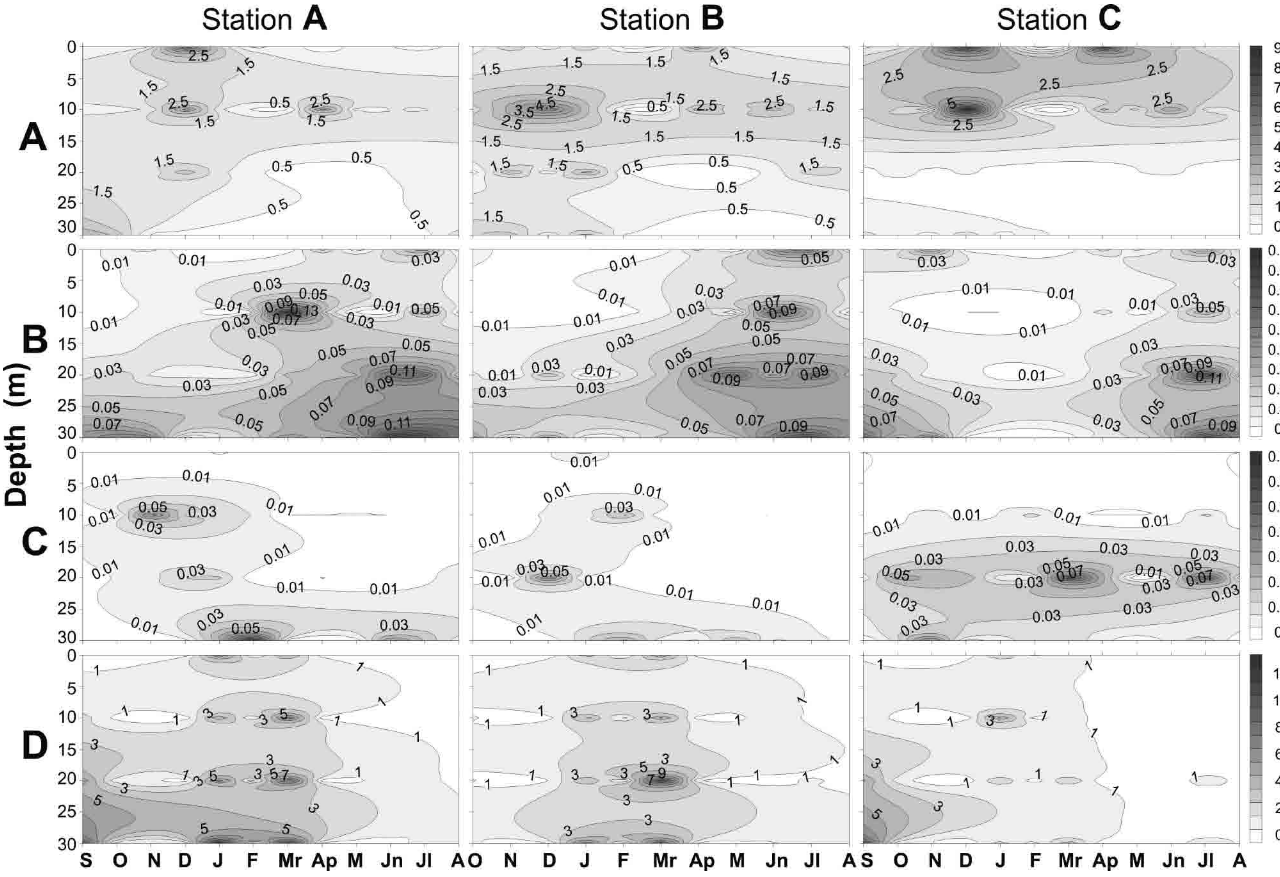

Fig. 3: Vertical profiles of the concentrations (μg l^{-1}) of **A**: chlorophyll-a (Chl-*a*), **B**: phosphates (PO_4), **C**: nitrates (NO_3), and **D**: silicates (SiO_2) at each of the three sampling stations (A, B, C) during the sampling period (September 2008 – August 2009).

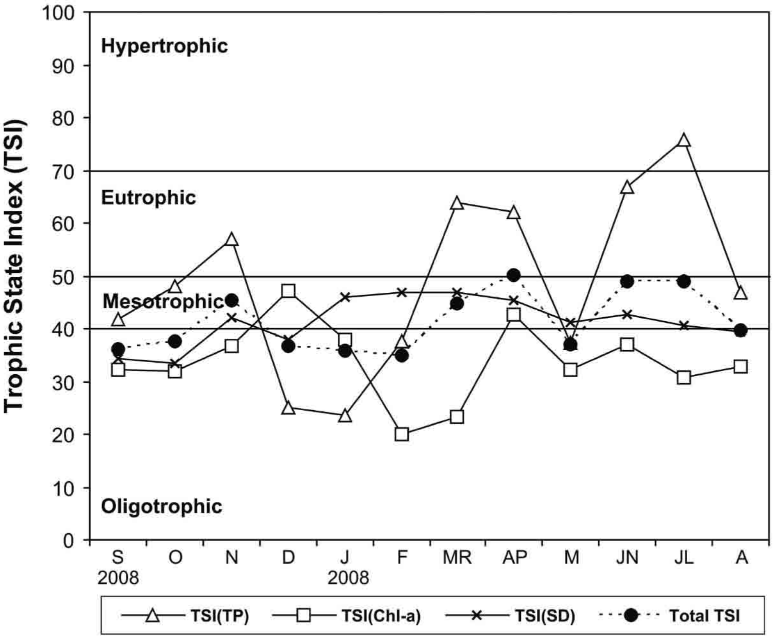

Fig. 4: Monthly variation of the average values of Carlson's TSI index for total phosphorus (TSI_{TP}), chlorophyll-a ($TSI_{Chl\text{-}a}$), transparency (TSI_{SD}), and the overall TSI index (total TSI) in Amvrakikos Gulf during the sampling period (September 2008 – August 2009).

column, the TSI values fluctuated between 38.7 and 50.8, with an average of 45.6, which clearly indicates a mesotrophic ecosystem.

Zooplankton composition and variability

Total zooplankton in the Amvrakikos Gulf presented high temporal fluctuation (Table 1), with the highest abundance in July (159.7 ind l^{-1}) and the lowest in May (44.6 ind l^{-1}) when considering the spatially averaged and depth-integrated values. The patterns of vertical distribution showed the highest abundance in the upper 0-5 m layer and a sharp decline in the deeper layers (Fig. 5). No differences between the three stations were found for any sampling date (Kruskal-Wallis test, p>0.01) when taking the abundance values in the six depth intervals into account. However, when considering only the two deepest sampling layers (20-25 and 25-30 m) where hypoxic/anoxic conditions prevailed, an east to west increase in abundance was found (Kruskal-Wallis test, p<0.01), with station C presenting the highest values (Fig. 5).

The zooplankton community comprised various taxa as reported in Table 1. Copepods dominated accounting on average for 86.4 % of total zooplankton abundance. Of these, cyclopoids (adults and copepodites) contributed with 35.6 %, calanoids with 23.2 %, and harpacticoids with 18.9 %. Cyclopoids and harpacticoids presented their maximum abundance in summer (July and August, respectively) with a secondary peak in November. Cyclopoids were found in very low abundance from January to April (Fig. 6B, C). The maximum abundance of calanoids was recorded in September with a secondary peak in February (Fig. 6A). There were no statistically significant differences in the abundance of copepod orders between the three stations in the six depth intervals (Kruskal-Wallis test, p>0.01). However, considering the deepest sampling layers (20-25 and 25-30 m), differences between stations were found for the calanoids (Kruskal-Wallis test, p<0.01), with station C always presenting the highest abundance. Moreover, calanoids was the only zooplankton group for which differences in the median depths between stations were recorded (Kruskal-Wallis test, p< 0.01), with station C always presenting the deepest distribution.

Copepod nauplii were present in low abundance year-round, except from December to April when they reached their highest numbers in February and prevailed in the zooplankton community (Fig. 6D). No significant horizontal differences were observed between stations. Station C presented the highest abundance values among the three sampling sites in the 20-30 m layer on most sampling occasions, although this was not verified statistically (Kruskal-Wallis test, p>0.01).

The vertical distribution of adults and copepodites of calanoids, cyclopoids and harpacticoids was characterized by highest abundance in the surface layer (0-5 m) and a decrease with depth in summer and autumn, while more homogenous vertical patterns were observed in winter and early spring (Fig. 6A, B, C). However, considering their median depths, copepods showed a clear vertical partitioning, with nauplii having the shallowest distribution, followed by calanoids, cyclopoids and harpacticoids (Kruskal-Wallis test, p< 0.01).

Table 1. Mean integrated (0-30 m) abundance variation (ind l^{-1} ± Standard Error) of the zooplankton groups/species recorded in the three sampling stations in Amvrakikos Gulf.

Taxa / months	**Sept**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**June**	**July**	**Aug**
Copepod cyclopoids	33.93 ± 3.99	32.34 ± 5.57	46.36 ± 7.96	31.75 ± 0.73	3.93 ± 1.79	2.14 ± 0.84	2.18 ± 0.98	2.21 ± 0.17	11.07 ± 1.26	36.35 ± 4.52	109.60 ± 36.6	47.53 ± 6.10
Copepod calanoids	48.12 ± 2.00	36.99 ± 5.29	19.84 ± 5.87	16.87 ± 1.71	14.39 ± 1.97	26.58 ± 5.23	18.42 ± 3.33	12.37 ± 2.70	8.24 ± 1.08	11.6 ± 2.27	8.68 ± 1.11	12.3 ± 3.40
Copepod harpacticoids	3.69 ± 0.30	2.05 ± 0.76	25.57 ± 4.48	15.13 ± 3.57	14.65 ± 5.67	15.25 ± 2.03	11.67 ± 3.44	11.54 ± 1.73	8.91 ± 1.26	19.58 ± 2.25	29.92 ± 4.69	32.49 ± 5.84
Copepod nauplii	1.98 ± 0.30	2.48 ± 0.65	2.18 ± 0.68	6.83 ± 2.29	16.3 ± 1.40	19.05 ± 2.20	5.98 ± 0.98	13.87 ± 1.94	5.37 ± 0.42	3.70 ± 1.02	3.20 ± 0.08	6.64 ± 1.88
Bivalve larvae	2.15 ± 0.20	1.80 ± 0.42	5.00 ± 1.36	6.04 ± 1.55	2.95 ± 0.16	4.16 ± 0.91	8.13 ± 2.42	6.37 ± 1.82	4.36 ± 0.16	3.41 ± 0.97	3.66 ± 1.50	4.32 ± 0.05
Appendicularians	3.49 ± 1.29	1.83 ± 0.17	1.95 ± 0.58	4.84 ± 0.92	2.78 ± 0.80	3.70 ± 0.57	3.28 ± 1.68	3.04 ± 0.41	1.09 ± 0.12	1.17 ± 0.32	2.17 ± 0.83	3.39 ± 0.66
Podon polyphemoides	0.53 ± 0.45	0.42 ± 0.03	0.27 ± 0.11	0.34 ± 0.12	1.38 ± 0.41	5.59 ± 0.64	1.79 ± 0.71	2.24 ± 0.52	2.64 ± 0.02	1.86 ± 0.61	0.93 ± 0.42	1.49 ± 0.55
Penilia avirostris	6.77 ± 3.05	0.33 ± 0.11	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Polychaete larvae	0.22 ± 0.05	0.38 ± 0.07	0.37 ± 0.10	0.41 ± 0.16	0.43 ± 0.08	0.92 ± 0.15	0.78 ± 0.06	1.29 ± 0.30	0.70 ± 0.05	0.75 ± 0.19	0.27 ± 0.01	0.38 ± 0.11
Gastropod larvae	0.44 ± 0.10	0.49 ± 0.06	0.35 ± 0.05	0.29 ± 0.06	0.28 ± 0.01	0.14 ± 0.07	0.14 ± 0.04	0.33 ± 0.08	1.03 ± 0.32	0.49 ± 0.12	0.58 ± 0.10	1.22 ± 0.80
Echinoderm larvae	0.50 ± 0.18	0.34 ± 0.09	0.45 ± 0.02	0.24 ± 0.02	0.25 ± 0.03	0.30 ± 0.08	0.37 ± 0.11	0.29 ± 0.10	0.98 ± 0.15	0.18 ± 0.05	0.34 ± 0.16	0.24 ± 0.06
Chaetognaths	0.06 ± 0.01	0.24 ± 0.01	0.14 ± 0.05	0.11 ± 0.05	0.46 ± 0.13	0.58 ± 0.13	0.93 ± 0.13	0.50 ± 0.23	0.11 ± 0.05	0.02 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
Medusae	1.29 ± 1.17	0.06 ± 0.05	0.13 ± 0.04	0.17 ± 0.12	0.00 ± 0.00	0.02 ± 0.01	0.02 ± 0.02	0.03 ± 0.01	0.05 ± 0.02	0.00 ± 0.00	0.20 ± 0.13	0.98 ± 0.45
Evadne tergestina	0.50 ± 0.24	0.19 ± 0.02	0.13 ± 0.03	0.05 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.01	0.13 ± 0.06	0.09 ± 0.05
Branchiostoma lanceolatum	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.02	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.18 ± 0.13	0.09 ± 0.02	0.09 ± 0.02	0.07 ± 0.01	0.06 ± 0.01
Barnacle larvae	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.25	0.09 ± 0.05	0.03 ± 0.01	0.03 ± 0.03	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.02 ± 0.02
Shrimp larvae	0.03 ± 0.01	0.05 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00
Anchovy larvae-eggs	0.02 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.03 ± 0.01	0.01 ± 0.01
Siphonophores	0.09 ± 0.07	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Pteropod larvae	0.03 ± 0.03	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.02	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Fish larvae-eggs	0.02 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.00
Crab larvae	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
Total zooplankton	**103.9** ± 12.7	**80.0** ± 10.9	**103.0** ± 7.20	**83.2** ± 8.30	**57.9** ± 8.30	**78.5** ± 5.20	**53.7** ± 4.70	**54.2** ± 7.00	**44.6** ± 2.70	**79.2** ± 3.10	**159.7** ± 41.1	**111.1** ± 12.3

The cladoceran group comprised the species *Evadne tergestina*, *Penilia avirostris* and *Podon polyphemoides* each with different seasonal variations (Table 1) but all distributed mainly in the surface layer (0-10 m) on all sampling dates (Fig. 6E). *P. polyphemoides* was present year-round and prevailed from December to August, while *P. avirostris* dominated the cladoceran abundance in September and was present until November. *E. tergestina* occurred in low numbers from September to December and from June to August (Table 1). No horizontal differences were found for any depth (Kruskal-Wallis test, p>0.01).

The chaetognath community was clearly dominated by one species (*Sagitta setosa*), while a few specimens of *Spadella cephaloptera* were found occasionally in the samples. Chaetognaths were more abundant from January to April, with their peak of abundance in March. They were found in very low numbers in June, July and September and were almost absent in August (Table 1). During the period of high abundance, chaetognaths were distributed mainly within the 0-15 m layer, but in November their highest numbers were recorded in the deepest 25-30 m layer at station C (Fig. 6F). The latter station always presented the lowest chaetognath abundance compared to the other two stations, although this difference was not statistically significant (Kruskal-Wallis test, p>0.01).

Appendicularians showed intense monthly variation with maximum abundance in December (Table 1). Their vertical distribution was characterized by an abrupt decrease of abundance below 15 m on most sampling dates, except in November when their distribution was more uniform (Fig. 7A). Significant differences were observed between the three stations in the 20-30 m depth layer for the entire sampling period, with station A having the lowest abundance values (Kruskal-Wallis test, p<0.01).

Bivalve larvae were numerically the most important after the copepods and presented their peak of abundance in March (Table 1). Differences between the three stations were found only in the 20-30 m depth layer with station C having the greatest abundance values (Kruskal-Wallis test, p<0.01). From December to July, their highest abundance was recorded in the surface layer (0-10 m), while in October and especially November, their vertical distribution was quite uniform (Fig. 7B).

Gastropod larvae had their maximum abundance in August with a second peak in May (Table 1), while on all sampling occasions, except in October, the larvae were found in higher numbers in the 0-5 m water layer of all sampling stations (Fig. 7C). Echinoderm larvae showed limited abundance variation throughout the year apart from a peak in May (Table 1). Generally, the 0-5 m layer was the most populated, and although the larvae were present in all layers, their abundance fell abruptly in the 20-30 m layer (Fig. 7D). Polychaete larvae (trochophores) showed an increase of abundance from Sep-

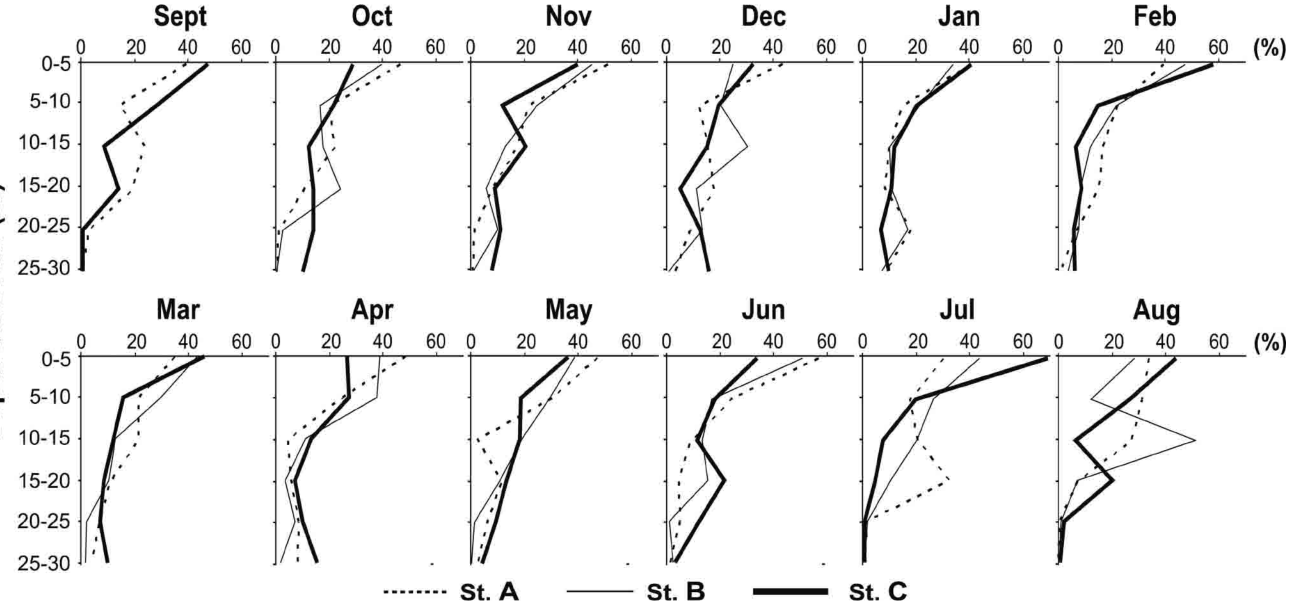

Fig. 5: Vertical distribution of total zooplankton at the three sampling stations (A, B, C) as percentages contribution (%) of each layer to the total abundance in the whole water column.

tember to April and then their numbers decreased until August (Table 1). The larvae presented a clear pattern of increasing abundance with depth, with the 20-30 m depth layer being the most populated on most sampling occasions (Fig. 7E). Decapod larvae (shrimps and crabs) were found in low abundance on most sampling dates. Their maximum density was recorded in October (Fig. 7F). No significant differences between stations of any sampling depth were recorded for the larvae of gastropods, echinoderms, polychaetes and decapods (Kruskal-Wallis test, $p>0.01$).

Concerning the ichthyoplankton of the Amvrakikos Gulf, various unidentified fish larvae and eggs were found sporadically in the samples. Anchovy larvae (*Engraulis encrasicolus*) were found on various dates and surprisingly even in winter (January), but were more abundant in September. Anchovy eggs were found on most sampling occasions except from January to March, and their maximum abundance was recorded in July. Larvae of the cephalochordate *Branchiostoma lanceolatum* presented their peak of abundance in April. They were found in almost all samples except those taken in September and February (Table 1) and generally their abundance increased in the deeper water layers.

The distribution of zooplankton taxa in relation to the environmental characteristics was analyzed using RDA. The model explained 38.2 % of the variance of the zooplankton groups and environmental data on the first two axes (Table 2). The eigenvalues of the RDA were statistically significant according to Monte Carlo permutations (sum of all axes; $p<0.001$). Temperature, dissolved oxygen, pH and salinity were the most important parameters affecting the variation of certain zooplanktonic groups. Temperature positively affected the cyclopoid copepods, medusae, cladocerans *Evadne tergestina* and *Penilia avirostris*, shrimp larvae and anchovy eggs. In addition, DO and pH positively influenced the distribution of the calanoid copepods, nauplii, appendicularians, the cladocerans *Podon polyphemoides*, and the larvae of bivalves and echinoderms (Fig. 8). In contrast, salinity seemed to affect negatively the variation of the larvae of barnacles and crabs, and chaetognaths especially. Finally, SiO_2 was positively correlated with polychaete larvae, while chlorophyll-*a*, NO_3 and PO_4 appeared to explain only minimal variation of the zooplanktonic groups.

Discussion

Environmental characteristics

The hydrology of the Amvrakikos Gulf and its hypoxic character have been recently depicted by studies conducted during summer (Ferentinos *et al.*, 2010), or other time periods (Kountoura & Zacharias, 2011; 2013; Kountoura, 2014). The present study provides the first complete annual cycle of the basic physical and chemical parameters of the Amvrakikos Gulf. The results of this investigation confirm the existence of horizontal differences in the concentration of DO with a decreasing trend eastward (Kountoura & Zacharias, 2011). This particular feature has been attributed to the specific topography and hydrography of the gulf, as well as its low physical energy (tidal, wind and currents), making this area "the only Mediterranean Sea fjord" (Ferentinos *et al.*, 2010). Conditions seem to be worse in the deeper parts of the gulf where hypoxia prevailed throughout the year in the east (station A), while in the western part (station C) hypoxia was recorded for only three months. Moreover, the present results show, for the first time, that September was the only month in the annual cycle when anoxic conditions

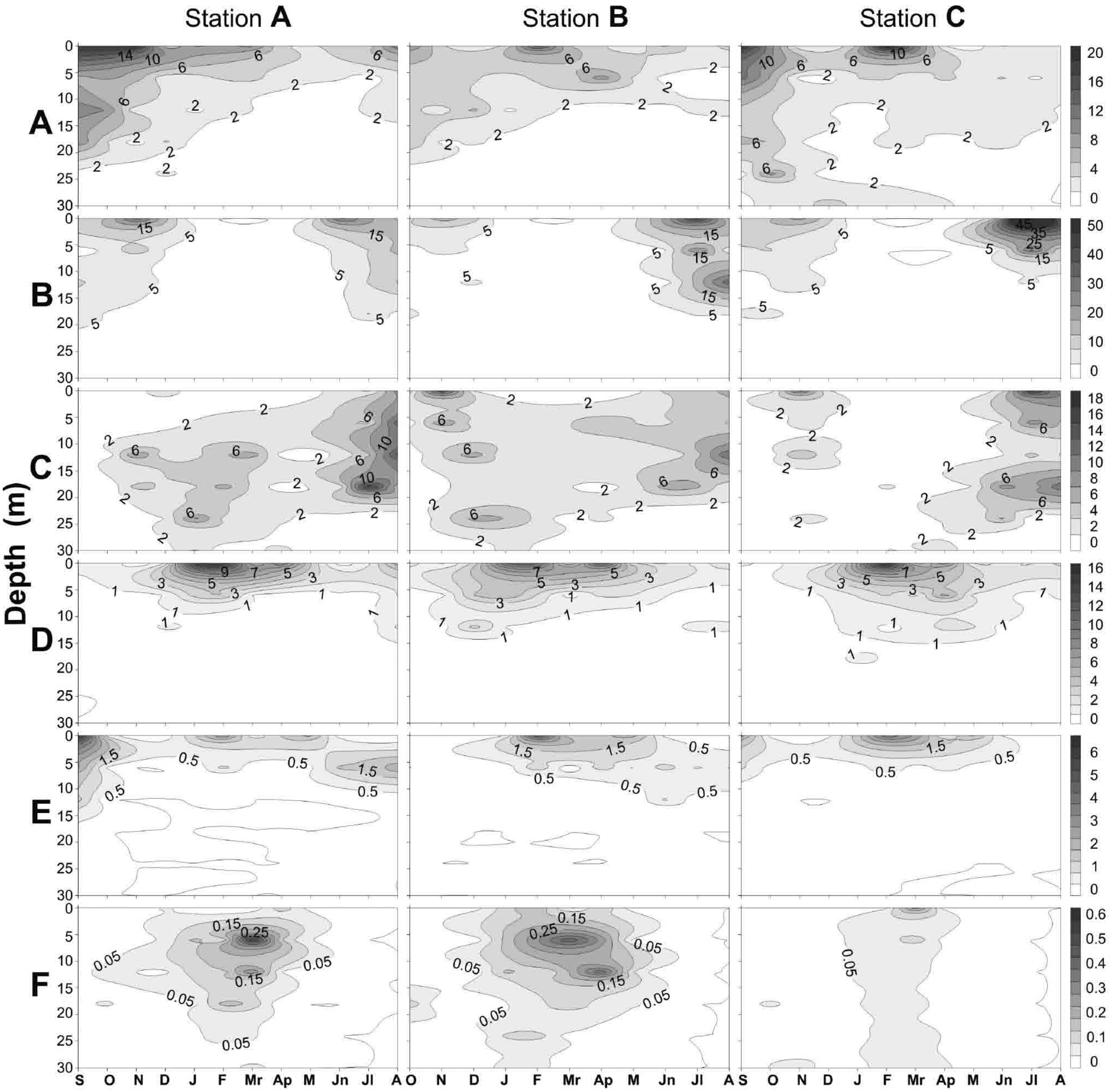

Fig. 6: Monthly variation of adults and copepodites of calanoids (**A**), cyclopoids (**B**), harpacticoids (**C**), copepod nauplii (**D**), cladocerans (**E**), and chaetognaths (**F**) at the three sampling stations (A, B, C) during the sampling period (September 2008 – August 2009).

were recorded in the entire gulf at depths below 25 m, suggesting that September may correspond to the last part of a long period of stratification, which has led to isolation of the deeper waters. Water stratification, which lasted from December to September, was likely caused in winter by the high freshwater inputs from the Rivers Arachthos and

Table 2. Summary of the results of the Redundancy analysis (RDA) between environmental parameters and zooplankton groups.

Axes	1	2	3	4	Total variance
Eigenvalues	0.287	0.095	0.030	0.021	1.000
Species-environment correlations	0.877	0.845	0.620	0.517	
Cumulative percentage variance:					
- of species data	28.7	38.2	41.1	43.2	
- of species-environment relation	64.3	85.6	92.2	96.9	
Sum of all unconstrained eigenvalues					1.000
Sum of all canonical eigenvalues					0.446

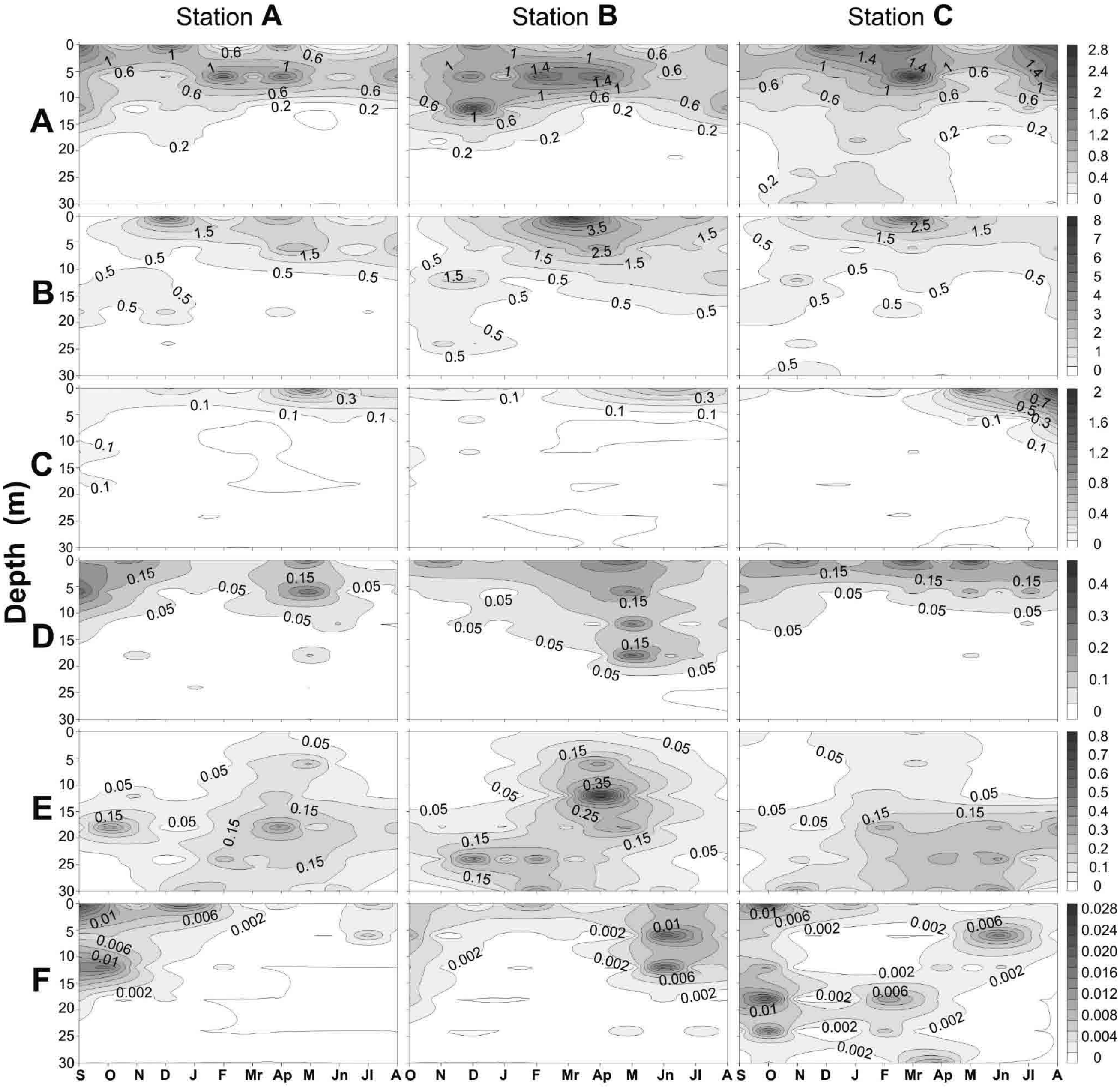

Fig. 7: Monthly variation of appendicularians (**A**) and larvae of bivalves (**B**), gastropods (**C**), echinoderms (**D**), polychaetes (**E**), and decapods (**F**) at the three sampling stations (A, B, C) during the sampling period (September 2008 – August 2009).

Louros. The homogenous vertical distribution of salinity and density, and to a lesser degree, temperature in October and November, provide indications of a mixing occurring in the water column during this period. However, the homogenous vertical distribution of the DO concentrations recorded only at station C in November and the persistence of a strong oxycline in the central and eastern parts of the gulf (stations B and C, respectively) suggest a weak vertical mixing, which probably did not reach the deeper layers in the entire gulf. This is also supported by the similar pattern of pH variation recorded at the three stations. The present results show that in this particular enclosure, vertical water mixing that is crucial for the oxidation of the deeper layers is limited not only in space but also in time with late autumn (November) being the most important period of the year for this process. This is the first report of vertical water mixing occurring in an area considered permanently stratified (Kountoura & Zacharias, 2011; Kountoura, 2014). In an investigation lasting several months of a two-year period, Kountoura (2014) concluded that the high freshwater input of the two rivers during the autumns of 2009 and 2010 did not permit the creation of the hydrological conditions required for a vertical mixing that would be expected in this season. The freshwater discharges of the two rivers are affected by the local weather conditions, especially precipitation in their watersheds, and are regulated by the management decisions of the Public Power Corporation pertaining to the upstream hydroelectric power dams built on both rivers. Consequently, this poses a major

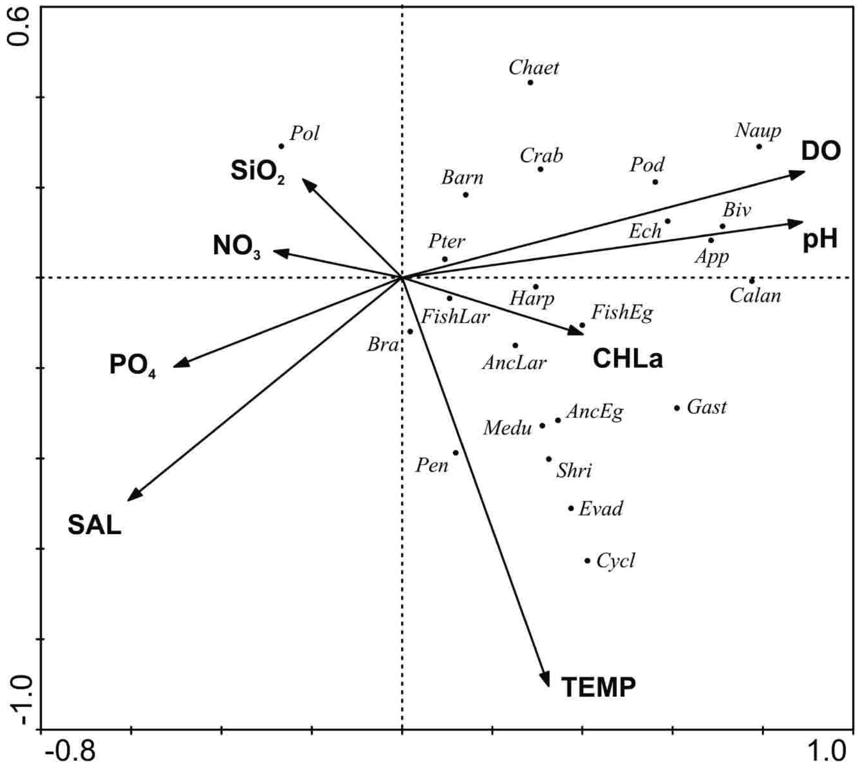

Fig. 8: Results of the Redundancy analysis (RDA) biplot of zooplankton groups/species and environmental parameters. Zooplankton groups/species are abbreviated as: *Calan*, calanoids (adults and copepodites); *Cycl*, cyclopoids (adults and copepodites); *Harp*, harpacticoids (adults and copepodites); *Naup*, copepod nauplii; *Chaet*, chaetognaths; *App*, appendicularians; *Pod*, *Podon polyphemoides*; *Evad*, *Evadne tergestina*; *Pen*, *Penilia avirostris*; *Medu*, medusae; *Biv*, bivalve larvae; *Barn*, barnacle larvae; *Ech*, echinoderm larvae; *Gast*, gastropod larvae; *Pol*, polychaete larvae; *Pter*, pteropod larvae; *FishLar*, fish larvae; *FishEg*, fish eggs; *AncLar*, anchovy larvae; *AncEg*, anchovy eggs; *Shri*, shrimps larvae; *Cra*, crabs larvae; *Bra*, *Branchiostoma lanceolatum* larvae.

management issue concerning water discharge into the gulf and could affect its hydrology.

According to the water circulation model proposed by Kountoura (2014) for the Amvrakikos Gulf, a quantity of seawater from the Ionian Sea enters the gulf and flows into its deepest section. This flow enriches the deepest water layers with oxygen. This pattern can be identified in the salinity profiles recorded during the present study in autumn, where more saline water was present in the deepest layer of station C, possibly due to its greater proximity to the gulf's sea-opening. This inflow of seawater with greater DO concentrations and pH values could have lasted for most of the sampling period and may explain the higher values of both parameters in the deeper layers of station C compared to the other two stations.

The concentrations of Chl-*a* recorded in this study were similar to the values reported by Kountoura & Zacharias (2013) and showed only slight differences between those reported in older studies of the gulf (Panayotidis *et al.*, 1994). The seasonal maximum of Chl-*a* concentration in December and the second increase in April was probably due to the presence of diatoms that are the dominant phytoplankton group in the Amvrakikos Gulf (Panayotidis *et al.*, 1994) and benefit from the inflow of nutrients provided by the increased river runoff in winter. The higher SiO_2 concentrations found in winter and early spring could have promoted the increase of diatoms in April, thus leading to the high Chl-*a* levels recorded in that month. On the other hand, the peak of Chl-*a* concentration was recorded in December and was probably due to the seasonal maxima of different phytoplankton species other than those recorded in April, although this cannot be verified by the present study. The higher concentrations of SiO_2 and Chl-*a* in the central and eastern parts of the gulf (stations B and A) can be attributed to the high nutrient inputs from the water discharge of both rivers.

According to a previous investigation conducted in 1987 (Friligos *et al.*, 1997), the Amvrakikos Gulf was thought to be a highly eutrophic basin, based on the concentration of nutrients and comparisons with other eutrophic areas, although no direct estimates using trophic indices were used in that research. In the recent study by Kountoura & Zacharias (2013), the gulf was characterized as a meso- to eutrophic ecosystem using four seasonal TSI measurements according to Carlson (1977), while TP measurements from the entire water column were used to estimate an overall TSI, which indicated a eutrophic ecosystem. In contrast, by using the same methodology, in this study we conclude that the Amvrakikos Gulf is a mesotrophic ecosystem. The differences between the two studies are probably due to the selection of different sampling locations. In this sense, deeper stations or/and sites close to river outflow or urban sewage runoff, as in the case of Kountoura & Zacharias (2013), might have resulted in higher TP concentrations that raised the TSI estimates. Furthermore, the phosphate and nitrate concentrations recorded in this study are by far lower than those reported in previous studies (Panayotidis *et al.*, 1994; Friligos *et al.*, 1997). In particular, PO_4 and NO_3 varied between 0.34 and 0.54 μg $l^{-1}$and 1.21 to 2.11 μg l^{-1}, respectively in 1987, compared to 0.006 and 0.106 μg l^{-1} and 0.003 and 0.026 μg l^{-1} in the present study. Consequently, it appears that the concentrations of the basic nutrients as well as the trophic state of the Amvrakikos Gulf have decreased significantly over the last twenty years.

Zooplankton variation

The total zooplankton abundances recorded in the Amvrakikos Gulf were significantly higher than those recorded in other neritic areas of the Mediterranean (e.g. Berline *et al.*, 2012). This could be due mainly to the smaller mesh size (100 μm) of the sampling net used during the present study (Calbet *et al.*, 2001) in comparison to the 200 μm mesh nets used in the majority of other zooplankton surveys. On the other hand, the total zooplankton abundance recorded in the Amvrakikos Gulf fell within the range of values found in eutrophic coastal ecosystems or lagoons where sampling was performed with nets of 125 μm (Vidjak *et al.*, 2007; 2009), 90 μm (Jamet *et al.*, 2001), or even 50 μm (Kehayias *et al.*, 2013).

Previous information on zooplankton in the Amvrakikos Gulf can be found in two studies conducted in June 1980 (Nicolaidou *et al.*, 1983) and in nine months of 1987 (Panayotidis *et al.*, 1994) using nets with larger mesh size (200 μm). Although the use of different mesh size (100 μm in the present study) does not allow a direct comparison with older results, the qualitative data may help identifying possible alterations in the zooplankton community through time. According to Panayotidis *et al.* (1994), the peak of total zooplankton abundance was recorded in the winter (February) of 1987 instead of in the summer (July) as observed in the present study, while in both studies minimum abundance was recorded in May. Moreover, copepods were not always the dominant zooplankton group, since cladocerans prevailed in the summer and autumn of 1987 (Panayotidis *et al.*, 1994). These differences may indicate an alteration in the zooplankton community with a decrease of the contribution of cladocerans, or may be a side effect of the different net porosity between the two studies, which could have resulted in increased abundance of the smaller copepods in contrast to cladocerans.

The seasonal variation of some of the numerically most important zooplanktonic groups was characterized by higher abundance values in winter and spring. This was likely due to the occurrence of higher phytoplankton biomass indicated by the Chl-*a* concentrations recorded in these seasons. Berline *et al.* (2012) reported similar winter peaks of Chl-*a* at various Mediterranean sites and found positive correlations of Chl-*a* with the increase of copepod, cladoceran and to a lesser degree appendicularian numbers. However, the absence of a strong interrelation between zooplankton abundance and Chl-*a* in the present study is in accordance with the general view that in brackish ecosystems few organisms strictly depend on phytoplankton (Gaudy *et al.*, 1995). Indeed, other components such as detritus or organisms of the microbial pathway can be used as alternative food sources when phytoplankton is quantitatively or qualitatively inadequate (Christou, 1998; Terbiyik Kurt & Polat, 2013).

The higher abundance and relative contribution of nauplii recorded in winter suggest that this season is possibly the main reproductive period of copepods in the gulf. Although nauplii were not identified to species or copepod order levels, it can be assumed that the calanoids accounted for their first peak of abundance in winter and cyclopoids accounted for the second peak in spring. Additionally, the seasonal succession of the adults and copepodites of calanoids and cyclopoids could possibly indicate competitive interactions, or/and could be due to different diet preferences. The increase of phytoplankton in winter and spring seemed to have triggered the increase of *Podon polyphemoides*, which dominated the cladoceran community and presented higher abundance in this period. The seasonal succession of the three cladoceran species is in agreement with other reports from the eastern Mediterranean (Siokou-Frangou, 1996; Alvanou, 1999; Büyükateş & İnanmaz, 2007), with *P. polyphemoides* dominating the community during winter, and *Penilia avirostris* and *Evadne tergestina* showing higher abundances in summer. The seasonal abundance peaks of *P. avirostris* and *E. tergestina* in the Mediterranean are always associated with high temperature (Siokou-Frangou *et al.*, 1998; Terbiyik Kurt & Polat, 2013).

Temperature is considered one of the most important environmental parameters affecting the seasonal variation of several species of copepods, cladocerans, and in some cases appendicularians (Siokou-Frangou *et al.*, 1998; Christou, 1998; Jamet *et al.*, 2001; Calbet *et al.*, 2001; Fernandez de Puelles *et al.*, 2003; Kehayias *et al.*, 2013). The present study also reveals the importance of other environmental elements for the temporal variation of some of the most important zooplankton groups. Thus, the larvae of bivalves and echinoderms and the appendicularians seemed to be influenced by DO and pH, which presented elevated values in the upper layers of the Amvrakikos Gulf. Surprisingly, despite the brackish character of the Amvrakikos Gulf, salinity proved to be of less importance to the spatial and temporal variation of most of the zooplanktonic groups. In fact, the negative correlation of certain groups with salinity, as well as with phosphates, reflected the effect of depth on zooplankton abundance, as both these parameters increased with depth. Indeed, the characteristic feature in the vertical distribution of most of the numerically important zooplankton groups in the gulf was the decrease of abundance with depth during most of the sampling period, with the exception of October and November. The water mixing occurring at the end of autumn seems to have affected the vertical distribution of appendicularians, chaetognaths, and the larvae of bivalves and gastropods, resulting in a more uniform distribution. On the other hand, polychaete larvae, which are carnivorous and detritivorous, were the only group with increased abundance in the deeper layers and appeared to be better adapted to low oxygen and increased organic matter conditions (Kehayias *et al.*, 2013).

A characteristic feature of the distribution of copepods in the Amvrakikos Gulf was the vertical separation of their ontogenetic stages (nauplii, copepodites and adults) and orders. Similar vertical partitioning phenomena are usually reported for greater water depths ranging from tens to hundreds of meters (Fragopoulu *et al.*, 2001; Paffenhöfer & Mazzocchi, 2003; Sato *et al.*, 2011), while no such information exists for the 0-30 m depth layer. Generally, vertical habitat partitioning is expected to result from the existence of different abiotic and biotic elements in the water column. Paffenhöfer & Mazzocchi (2003) hypothesized that a combination of variables, including feeding behaviour, temperature preference and predation could determine the ranges of vertical distribution in copepods. Oxygen demand is also of great importance for all aquatic organisms, especially in areas with oxygen depletion. Thus, the vertical position of the younger ontogenetic copepod stages, such as nauplii, near the water surface may be explained by the optimal DO conditions present in these upper water layers, as also suggested by the results of the RDA analysis. Moreover, the different vertical positions of calanoids, cyclopoids and harpacticoids could be due to their oxygen demands and mainly their resistance to hypoxia, as certain cyclopoids and harpacticoids seem to display greater resistance to hypoxic concentrations than calanoids (Kehayias *et al.*, 2012). This may explain the shallower distribution of calanoids observed in the RDA diagram. However, Paffenhöfer & Mazzocchi (2003) also reported the deeper distribution of harpacticoids compared to calanoids and cyclopoids in a sufficiently oxygenated subtropical area. Therefore, oxygen depletion may not be the only determining factor. The vertical separation of copepods and other zooplankters has been frequently explained by the reduction of inter- or intraspecific competition for food (Kehayias & Ntakou, 2008; Sato *et al.*, 2011). This explanation is valid for oligotrophic ecosystems where food is a limiting factor on the vertical axis (Fragopoulu *et al.*, 2001; Paffenhöfer & Mazzocchi, 2003), but seems to be less valid for a mesotrophic environment such as the Amvrakikos Gulf. On the other hand, various orders and species of copepods probably utilize different energy sources, the vertical distribution and availability of which has not been investigated during the present study. Thus, while measures of order abundance provide general insight, those of genera, species and even ontogenetic stages are required to obtain a thorough understanding of the functioning of copepod communities (Paffenhöfer & Mazzocchi, 2003).

The unique hydrology of the Amvrakikos Gulf has resulted in the decrease of oxygen concentration and the presence of hypoxia and anoxia in the water column, and this has clearly influenced the vertical and, to a degree, the horizontal distribution of zooplankton. The higher abundance values of calanoids, appendicularians, bivalve larvae and nauplii, in the deeper water layers of station C compared to the other two stations were probably due to the greater DO concentrations resulting from the inflow of seawater from the Ionian Sea at this depth, as mentioned previously (Kountoura, 2014). Hypoxic and anoxic conditions in the water column have serious negative effects on zooplanktonic organisms as, apart from causing direct mortality, they also affect the metabolism, fecundity, growth, vertical migration patterns, and distribution of plankton (Auel & Verheye, 2007). However, many zooplanktonic organisms have been found to tolerate or even prefer hypoxic or anoxic layers in order to complete their diel or seasonal migration, or find refuge from predators (Ludsin *et al.*, 2009). Thus, the presence, even in very low numbers, of several taxa in the anoxic deep water layers in September is worth noting. Similar findings have also been reported by Kehayias *et al.* (2012) in the anoxic layers of the Aitoliko lagoon. However, it should be noted that with the analytical methodology utilized during the present study, it was not possible to discriminate between dead and live specimens found at these depths, unless they were in an advanced state of decay (Elliott & Tang, 2009).

The present study reports the first appearance of the European lancelet *Branchiostoma lanceolatum* along the coast of Western Greece. This species, once very abundant along the north-eastern Atlantic coast and the Mediterranean, become increasingly rare after the early 1900s and is also listed in the Black Sea Red Data Book. During recent decades, muddy areas have expanded as a result of increased eutrophication and organic pollution, causing a severe decline of *B. lanceolatum* populations at global scale (Rota *et al.*, 2009). The presence of *B. lanceolatum* has been associated with the good environmental quality of its habitat, which is sandy bottom sediment (Rota *et al.*, 2009), and therefore its presence in the Amvrakikos might indicate the good environmental condition of this ecosystem. Furthermore, the seasonal variation of *B. lanceolatum* larvae is in accordance with the report of Sarda *et al.* (1999) on its reproduction in the western Mediterranean, possibly suggesting the establishment of a local population of this species in the Amvrakikos Gulf.

The higher abundance of anchovy (*Engraulis encrasicolus*) larvae and eggs observed in the summer months corresponded with the main reproductive period of this species in the Mediterranean. However, the presence of larvae and eggs in winter is surprising as it is known that this species terminates its breeding in late autumn. Mandić *et al.* (2012) also reported a small number of anchovy eggs in the Adriatic Sea (Boka Kotorska Bay) and suggested that they may have originated from a delayed end of the spawning season due to elevated temperatures in the winter. The presence of anchovy larvae in the Mediterranean in winter may be considered noteworthy for studies on the influence of global climate change on the biology and ecology of aquatic organisms.

The presence of only two chaetognath species and especially the absence of *Sagitta enflata*, which is the most common chaetognath in the neritic areas of the Mediterranean (Kehayias, 1997), highlight the particular hydrological conditions and the enclosed character of the Amvrakikos Gulf. The dominance of *Sagitta setosa* is expected in a dilution basin such as the Amvrakikos, since this species is considered an indicator of low salinity marine areas affected by freshwater and is abundant in the northern Adriatic Sea (Kehayias, 1997). The seasonal variation of *S. setosa* is clearly influenced by salinity and the negligible occurrence of this species in summer was probably due to the salinity maximum observed in this period.

In conclusion, the present investigation covering a complete annual cycle revealed that the zooplankton community of the Amvrakikos Gulf was strongly affected by the hydrology of this basin. The water stratification observed for most of the year and the hypoxic conditions present in the deeper water layers affected the horizontal and especially the vertical distribution patterns of the different zooplanktonic groups. The present study reveals some general patterns of the spatial and temporal variation of the main zooplanktonic groups. However, a future detailed investigation of the copepod community that dominated zooplankton abundance will be essential to identify the temporal and spatial patterns of this important group in this particular embayment. A more in depth knowledge of the distribution of copepod species would shed light on the role of the area's hydrology as regards the organization and functioning of zooplankton in this ecosystem. Although there are strong indications of a degraded ecological state of the Amvrakikos Gulf due to hypoxic conditions, some elements indicate that the basin is in a transitional phase towards a better state. The present study may represent a promising starting point for a monitoring program aimed at following the evolution of the ecosystem's biotic compartments and assessing its trophic conditions.

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