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Zooplankton diversity and distribution in a deep and anoxic Mediterranean coastal lake

G. KEHAYIAS[1], A. RAMFOS[2], P. NTZIALAS[1], S. IOANNOU[1], P. BISOUKI[1], E. KYRTZOGLOU[1], A. GIANNI[1] and I. ZACHARIAS[1]

[1] Department of Environmental and Natural Resources Management, University of Western Greece, Seferi 2, 30 100, Agrinio, Greece
[2] Department of Aquaculture and Fisheries Management, Technological Educational Institute of Messolonghi, 30200 Messolonghi, Greece

Corresponding author: gkechagi@cc.uoi.gr

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Abstract

The variation of the smaller size fraction of zooplankton was investigated during a two-year period in a brackish deep and anoxic coastal lake of western Greece (Aitoliko), along with the specific environmental characteristics of this ecosystem. The zooplanktonic community comprised a relatively small number of taxa and it was dominated by brackish-water calanoid copepods (*Paracartia latisetosa, Calanipeda aquaedulcis*) and in certain periods by rotifers and tintinnids. The zooplankton abundance showed an increase in the warmer period starting from late spring and reached maximum values in July. In the well oxygenated surface layer, temperature was the most important parameter influencing the seasonal cycles of all groups. In contrast, the oxygen depletion a few meters under the surface affected the vertical distribution of most of the zooplankton groups, which were found restricted in the surface layer, especially from spring until autumn. Only the meroplanktonic larvae of polychaetes presented increased proportions in the deeper layers. Salinity has not significantly influenced the zooplanktonic assemblages. The results reveal the degraded status of the Aitoliko basin, where the hypoxic/anoxic layers resulted in a high portion of dead organic material identified as copepod carcasses; they also underline the necessity of monitoring this ecosystem.

Keywords: Zooplankton, coastal lake, anoxia, temporal, vertical distribution.

Introduction

According to the EU Water Framework Directive (EU-WFD 2000/60) transitional waters include many different habitat types such as estuaries, deltas, lagoons, coastal lakes and ponds, wetlands and salt-marshes (Basset *et al*., 2008). All these habitats, due to their origin as ecotones, their topographical characteristics and their position on the coastline, present strong daily, seasonal and inter-annual fluctuations in their hydrological and biotic components (Nicolaidou *et al*., 2006). This is accentuated in the Mediterranean region where temporal climate variability is very intense (Lucena-Moya *et al*., 2012). Transitional waters are among the most productive marine habitats, playing an important role as nursery and feeding grounds for fishes and providing different habitat types for many species (Perez-Ruzafa *et al*., 2011). The aforementioned features make these ecosystems rather unstable and unpredictable and thus more vulnerable to the various anthropogenic pressures they are subjected to, such as extensive agricultural land use in the surrounding areas, fresh water management, mariculture, chemical pollution, technical interventions and fisheries (Basset *et al*., 2008). Yet, in these naturally or artificially stressed environments, the inhabiting biota seem to be well-adapted to the constantly varying environment, absorbing the stress without adverse effects, thus making it difficult for scientists to detect the source of stress (Elliot & Quintino, 2007).

Zooplanktonic organisms comprise a very important element in the food webs of lagoon environments not only as consumers of primary production (Collos *et al*., 2005), but also as food items for juvenile fish species of high commercial value (Ferrari & Chieregato, 1981). In brackish coastal lakes and lagoons, zooplanktonic communities are characterized by seasonal outbursts of rotifers, small-sized calanoid copepods (usually of the family Acartiidae) and meroplanktonic larvae populations, mainly during the summer-autumn period (Belmonte *et al*., 2011 and references therein). In these environments, where the benthic-pelagic coupling for zooplankton is very strong as the most abundant holoplanktonic taxa produce benthic resting eggs (Marcus, 1990; Belmonte & Pati, 2007) and most benthic organisms produce planktonic eggs, the environmental conditions close to the sediment have a crucial role in the evolution of zooplanktonic communities. However, in such environments benthos is often found under severely adverse conditions due to oxygen depletion at the deeper layers (Sagasti *et al*., 2003; Belmonte *et al*., 2011). Such hypoxic or anoxic events, although occurring naturally in transitional waters, have increased significantly in frequency, duration and range during the last decades due to anthropogenic activities that lead to eutrophication (Vaquer-Sunyer & Duarte, 2008) mainly through mismanagement of fresh water inputs and/or nutrient enrichment of the various fresh water inputs in transitional waters (Perez-Ruzafa *et al*., 2011; Lucena-Moya *et al*., 2012).

In cases of severely degraded ecosystems, oxygen-depleted layers may expand vertically in the water column and persist throughout the year. Anoxic as well as hypoxic conditions in the water column, have serious negative effects on zooplanktonic organisms because, except for causing direct mortality, they also affect the metabolism, fecundity, growth, vertical migration patterns and distribution of planktonic organisms (Marcus *et al.*, 2004; Auel & Verheye, 2007).

In the context of the EU Water Framework Directive (WFD) 2000/60/EC, the need to improve quality in transitional waters is imperative. Yet, prior to any bio-economic models and action plans a good knowledge of the components, structure and functioning mechanisms of the systems are required (Perez-Ruzafa *et al*., 2011). This study aims at the description of the temporal variation and vertical distribution of the zooplanktonic community, as well as the identification of the main environmental driving forces, in a coastal lake of Greece with a particular combination of topographical and physico-chemical features, based on a two-year investigation.

Materials and Methods

Site description

The Messolonghi-Aitoliko lagoon complex is one of the most important Mediterranean lagoon systems in terms of size, fish production and ecological value (Katselis *et al*., 2003). It is located on the coastline of Central Greece and covers a total area of 15000 ha (Fig. 1). The study area (Aitoliko basin) is located at the northern part of this complex and is a meromictic brackish coastal lake of 1700 ha. It is connected, through narrow openings of approximately 1 m depth, to the extended and shallow Messolonghi lagoon in the south. In the SW part of the area there is a freshwater inflow (pumping station D6) which transports the irrigation and rainfall drainage waters of the surrounding agricultural areas (Fig. 1).

Aitoliko basin has a historical record of numerous anoxic incidents expressed as massive fish deaths since 1881 (Leonardos & Sinis, 1997). During the last decades the frequency of such incidents has increased possibly due to the concurrent increase of nutrient loads in the basin via the wastewater treatment plant of the nearby Aitoliko town and the freshwater discharges due to the operation of the irrigation drainage pumping station (D6).

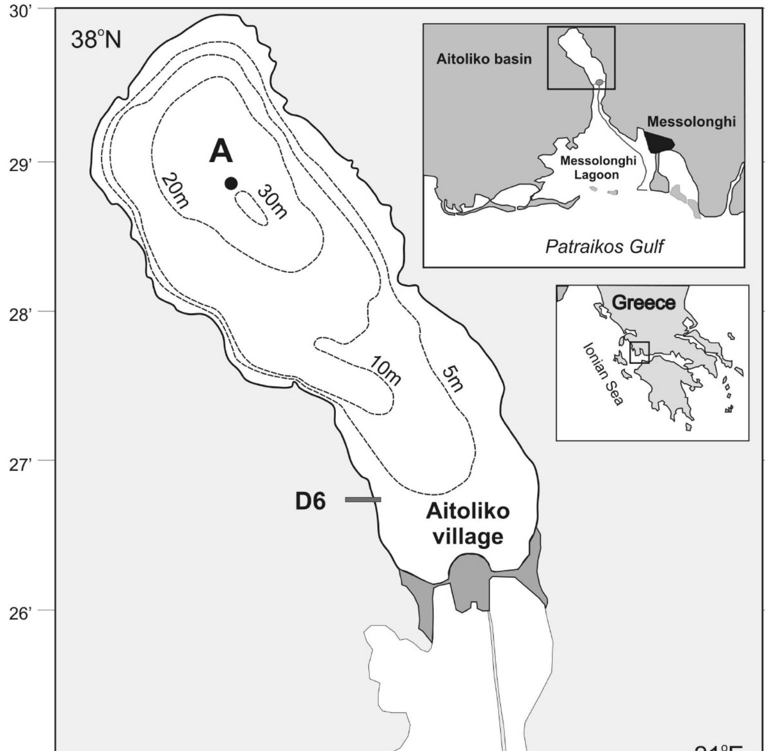

Fig. 1: Topography and sampling site (station A) in the study area. D6: freshwater inflow from irrigation and rainfall drainage waters of the surrounding agricultural areas.

The morphology of the Aitoliko basin (mean depth: 12 m, max. depth: 30 m, small length), combined with the freshwater inflows and the limited connection with the adjacent more saline Messolonghi lagoon, permits the development of a permanent pycnocline in the water column (Gianni *et al.*, 2011). This persistent stratification isolates the surface layers from the deeper ones thus creating a deep hypoxic (dissolved oxygen <2 mg l^{-1}) or even anoxic (dissolved oxygen <0.2 mg l^{-1}) layer, rich in H_2S, which is commonly released after strong wind events, resulting in mass fish deaths (Dassenakis *et al.*, 1994).

Sample collection and analysis

Zooplankton sampling was carried out on a monthly basis during a two-year period (April 2006 - March 2008) in the deepest part of the basin (station A) having a depth of 29 m (Fig. 1). The samples were collected using a vertically towed conical net (40 cm diameter, 1 m length, 50 μm mesh-size) equipped with a closing mechanism. The hauls were conducted in 5 m depth intervals (0-5 m, 5-10 m, 10-15 m, 15-20 m, 20-25 m). All sampling was carried out during daylight hours. Filtered water volume was estimated using a Hydro-Bios flowmeter attached to the mouth of the net. Sample fixation took place immediately after collection in a 4% borax-buffered formaldehyde solution.

Measurements of temperature (T), salinity (S) and dissolved oxygen (DO) were taken in the whole water column on each sampling date using a Troll 9500 water quality multi-parameter instrument. The estimation of chlorophyll-a (Chl-*a*), total phosphorus (TP), phosphates (PO_4), nitrates (NO_3), nitrites (NO_2) and ammonia (NH_4) concentrations was made on water samples collected from 0, 5, 10, 15, 20 and 25 m, using a 5l sampling bottle. Analyses of all chemical parameters were performed according to A.P.H.A., A.W.W.A. & W.P.C.F. (1998). For the determination of the Chl-*a* concentration, 500 ml of the water samples taken from the above depths were filtered through a Whatman GF/A glass fibre filter shortly after collection. Pigment extraction was made in 90% acetone and concentrations were determined spectrophotometrically.

Data analysis

Identification and enumeration of zooplanktonic taxa was performed at the laboratory under a Leica stereomicroscope for the larger specimens, whereas species determination for rotifers, tintinnids and small copepods was performed using an inverted Leica microscope under up to 400x magnification. Species identification was based on the keys of Carli & Crisafi (1983) for copepods, Ruttner–Kolisko (1974) for rotifers and Balech (1959) and Trégouboff & Rose (1978) for tintinnids. Zooplankton abundance was calculated in terms of density (ind l^{-1}) from aliquots taken with a Folsom splitter. Aliquot size ranged from 1/1 (whole sample) to 1/64, depending on the density of zooplankton organisms in the sample. In addition, for the estimation of the density of the smaller groups, the whole sample (total volume of 100 ml) was investigated under the microscope in modified Sedwick-Rafter cells. In the case of the dense surface (0-5 m) samples, four 2.5 ml aliquots (10 ml) were taken from each sample using a Hensen Stempel Pipette and examined as above. Copepod exuvia and dead copepod carcasses (e.g. copepods in advanced decayed condition) were identified and counted for each sub-sample, but they were not included in zooplankton abundance.

In order to investigate zooplankton dynamics dependent on environmental variables, the Redundancy Analysis (RDA) was employed, using the CANOCO 4.0 programme (Ter Braak & Šmilauer, 2002). The analysis was based on species abundance in relation to data for the environmental variables from all depth intervals. Data values were log transformed and centred prior to the analysis. The significance of the variability explained by individual environmental variables was tested by the Monte Carlo permutation test (restricted for time series) with 999 random permutations. The results are presented as a biplot where species and environmental variables are plotted together.

Differences in the hydrographical properties as well as in the abundance of zooplanktonic taxa among seasons or sampling years were evaluated with the non-parametric Mann-Whitney (U) test or the Kruskal-Wallis test. Correlations in the monthly variation among 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

Surface temperature in the study area was mainly characterised by temporal variability with the lowest value in February 2008 (9.87°C) and the highest in July 2007 (30.65°C). The development of the seasonal thermocline started during spring months (March–May) until its establishment at approximately 7m depth in June (Fig. 2). The thermocline was persistent during the entire summer, whereas during autumn the progressive decrease in temperature of the surface layer was accompanied by its breakdown. After this period the water column was thermally homogenous until the following spring. Below 10m, temperature fluctuated less during the course of the year, while in the deepest layers it was rather constant during the entire sampling period ranging between 12.5ºC and 13.8ºC in the 20–25 m layer (Fig. 2).

Salinity fluctuated between 18.75 in the surface layer

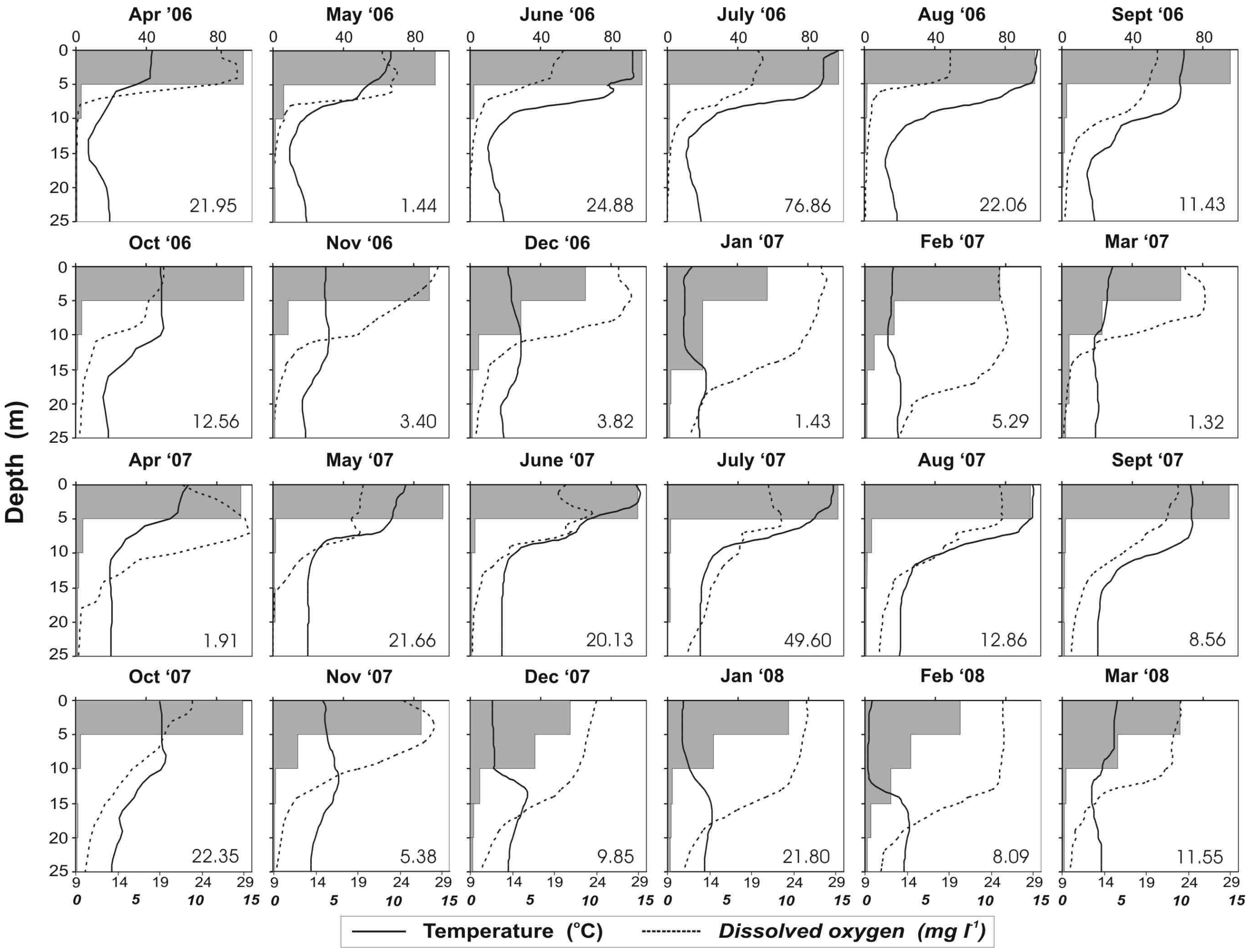

Fig. 2: Vertical distribution of temperature and dissolved oxygen at station A during the sampling period (April 2006 – March 2008). The shadowed area presents the vertical distribution of total zooplankton at station A as percentages (%) of the total caught in the water column sampled. Mean integrated abundance values (indl^{-1}) in the 0-25m are also shown.

in May 2006 and 27.65 near the bottom in December 2007. A permanent halocline was observed in the water column throughout the study period (Fig. 3A). From a seasonal point of view, lower values were recorded in spring and higher in autumn, while there was a statistically significant increase (U-test, $p<0.05$) in salinity in the total water column between the first sampling year (April 2006–March 2007) and the second one (April 2007–March 2008).

As far as dissolved oxygen is concerned, the upper part of the water column was always sufficiently oxygenated, whereas in the vertical axis a strong oxycline was observed (Fig. 2). During spring and summer months the oxygen minimum zone (OMZ) boundary (defined as the depth of 1 mg l^{-1}) was approximately at 10m depth during the first year and at 15m during the second, while in autumn and winter months OMZ exhibited a gradual deepening (Fig. 2).

The concentration of chlorophyll-a (Chl-*a*) and of nutrients (NH_4, NO_3, NO_2, PO_4 and TP) did not present statistical differences between the two sampling years (U-test, $p>0.05$). Generally, higher Chl-*a* values were recorded during winter and early spring months and lower during summer and autumn. During late spring and the summer months considerably higher Chl-*a* values were found in the sub-surface layer forming a deep chlorophyll maximum (Fig 3B). The temporal and spatial variation of nutrients is presented in Figure 4. Ammonia, phosphates and total phosphorus showed a characteristic vertical distribution with lower values in the epilimnetic layer and higher in the deeper parts of Aitoliko basin. Ammonia concentration reached 12.63mg l^{-1} in the 25m area in February 2008, while the highest values for phosphates (0.76mg l^{-1}) and TP (0.79mg l^{-1}) were recorded at the same depth (25m) in April 2008. As regards seasonality, increased concentrations of all the above nutrients were recorded in the deepest part of the hypolimnion except from December 2006 to February 2007, when decreased values were found at these depths possibly due to a vertical mixing of the water column. Nitrates presented considerable concentrations in the epilimnetic 0-15m layer, with the highest value being recorded at the surface in

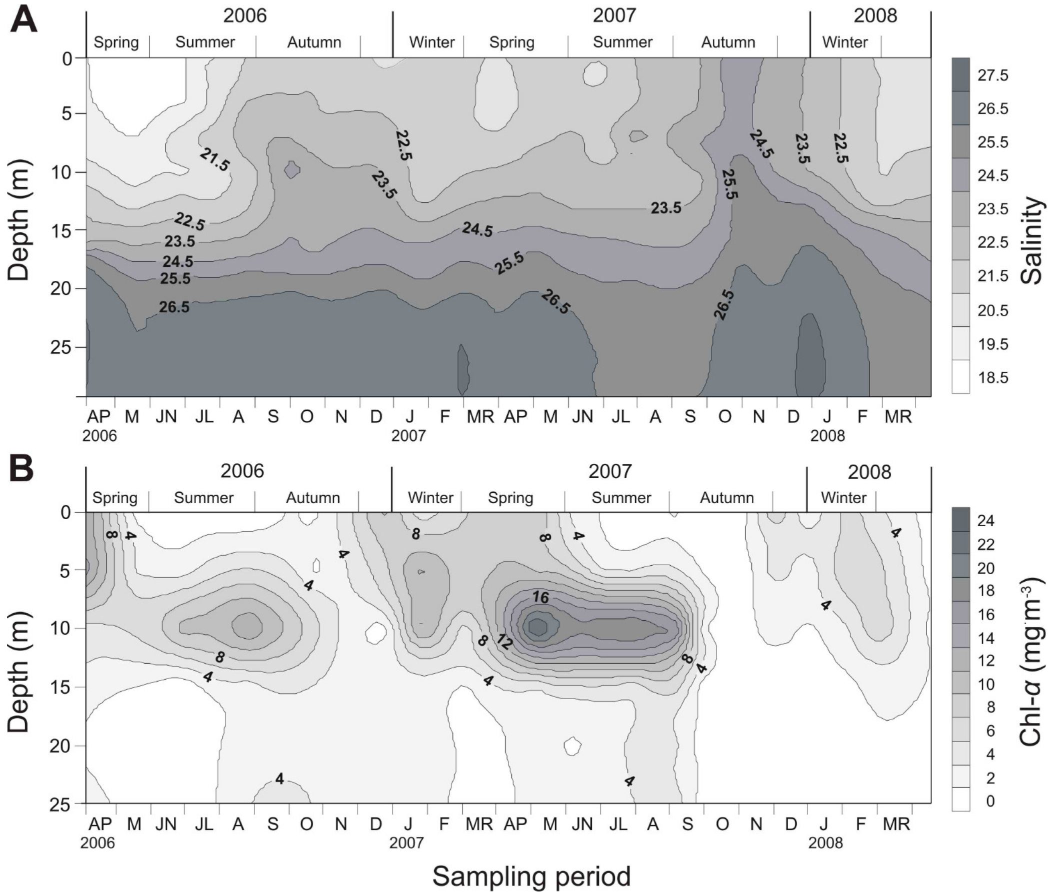

Fig. 3: Vertical profiles of salinity (A) and Chl-*a* (B) at station A during the sampling period (April 2006 – March 2008).

July 2007 (0.14mg l^{-1}). Generally, higher concentrations were found in the period from July to November for both sampling years. Two maxima were recorded for the nitrites, the first in the period between November 2006 and March 2007 and the second from December 2007 to March 2008. Higher concentrations were recorded in the 0-10m layer and the highest value (0.18mg l^{-1}) was found in January 2007 at the surface.

There were no statistical differences between the two sampling years (U-test, p>0.05) as far as monthly precipitation in the study area is concerned (Fig. 5A). Furthermore, although the water discharged by the pumping station D6 (Fig. 5B) was 22.4% higher in the first sampling period, no statistical differences between the two sampling years were found (U-test, p>0.05). Finally, there were no significant correlations (Pearson's *r*, p>0.05) between the temporal variation of the precipitation and water discharge from D6, and nutrient (NO_3, NO_2, NH_4, PO_4, TP) and Chl-*a* variation.

Zooplankton

Total zooplankton in Aitoliko basin presented high temporal fluctuation, while in the vertical axis, abundance distribution was characterised by a sharp decline with depth and followed, in general terms, the distribution of oxygen in the water column (Fig. 2). Thus, from April to November, when the oxycline was closer to the surface, the bulk of zooplankton was found restricted to the surface layer in both sampling years while during the period of vertical mixing (December to February), when most of the water column was adequately oxygenated, zooplankton abundance presented a less steep vertical decrease (Fig. 2).

At the highly populated 0-5m surface layer, a clear summer peak in abundance was evident, especially during the first sampling year, which started in June and reached maximum values in July 2006 (Fig. 6). During the second year, the summer peak was less strong and total zooplankton abundance also showed two smaller peaks in October 2006 and January 2007, while the onset of a new peak was also observed in the final sampling (March 2008). In the sub-surface layer (5–10m) total zooplankton abundance was 15 times lower than in the 0–5m surface layer and never exceeded 28.34 ind l^{-1} presenting maximum values in January 2008 (Fig. 6). The abundance in the 10–15m layer was significantly higher than in deeper (15–20m and 20–25m) layers, while abundance never exceeded 2 ind l^{-1} below 15m in depth (Fig. 6). No significant differences in total zooplankton abundance between the two sampling years were observed in any of the layers (Kruskal-Wallis test, p>0.05).

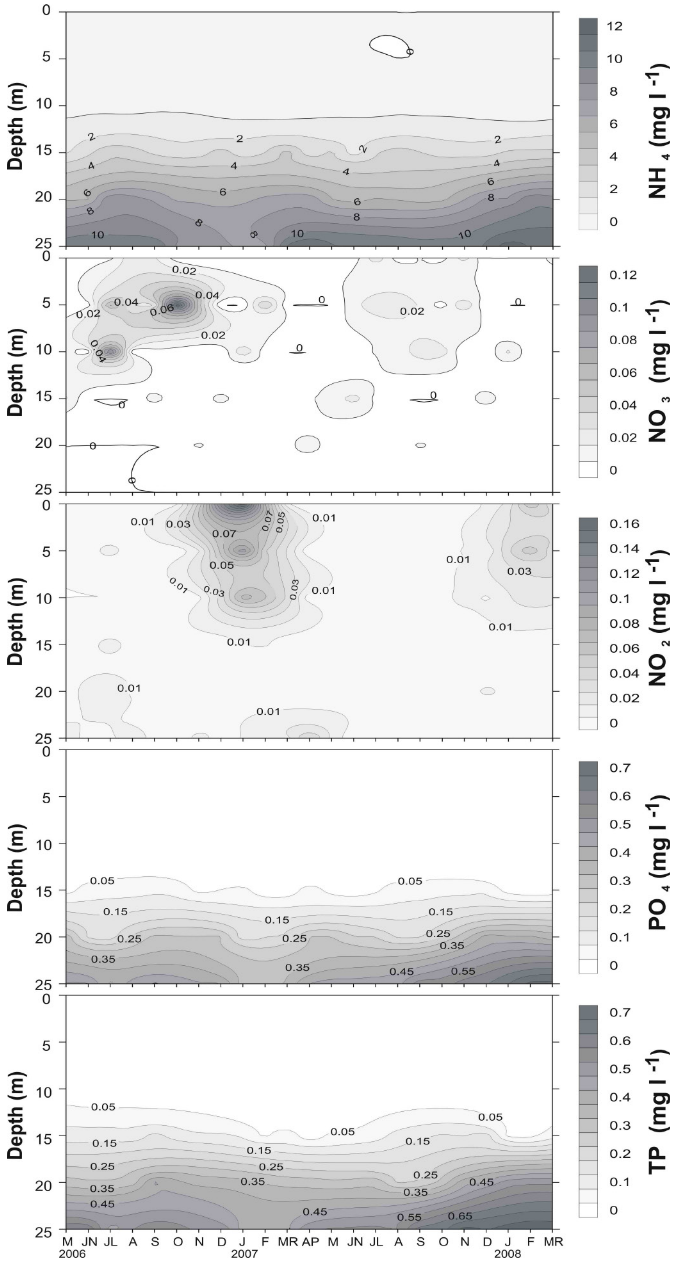

Fig. 4: Vertical profiles of ammonia (NH4), nitrates (NO3), nitrites (NO2), phosphates (PO4) and total phosphorus (TP) concentrations (mgl-1) in station A during the sampling period (April 2006 – March 2008).

The zooplankton community in Aitoliko basin is composed of copepods, rotifers, tintinnids, cladocerans, meroplanktonic larvae of polychaetes, lamellibranchia, gastropods, barnacles, echinoderms, crabs and shrimps, as well as medusae, fish larvae and eggs (Table 1). Copepods dominated in the zooplankton and accounted, on average, for 51.0%. Nauplii were the most abundant ontogenetic stage within the copepod community during most of the sampling period, with an average of 70%. Among copepods the two most abundant species were *Paracartia latisetosa* and *Calanipeda aquaedulcis* and accounted for 93.8% in the copepod community considering adults and copepodites. The cyclopoid *Oithona nana* and the harpacticoids *Harpacticus gracilis*, *Euterpina acutifrons* and *Microsetella norvegica* were found in considerably lower abundance.

Copepods presented two clear seasonal cycles during the study period showing higher abundance in late spring and summer (Fig. 7). The two most abundant species *Paracartia latisetosa* and *Calanipeda aquaedulcis* showed similar temporal and spatial variation, with a first peak of abundance in June and July 2006 respectively, while the second peak for both species was recorded in May 2007 (Fig. 8). Tintinnids were abundant in certain periods (April 2006, July 2007 and January 2008) and contributed, on average, 16.7% to the zooplankton community (Fig. 7). *Favella ehrenbergii* was by far the dominant species in all seasons and reached up to 120.9 ind l^{-1} in July 2007, while *Undella* sp. was recorded in lower abundance (19.6 ind l^{-1}) in January 2008. Rotif-

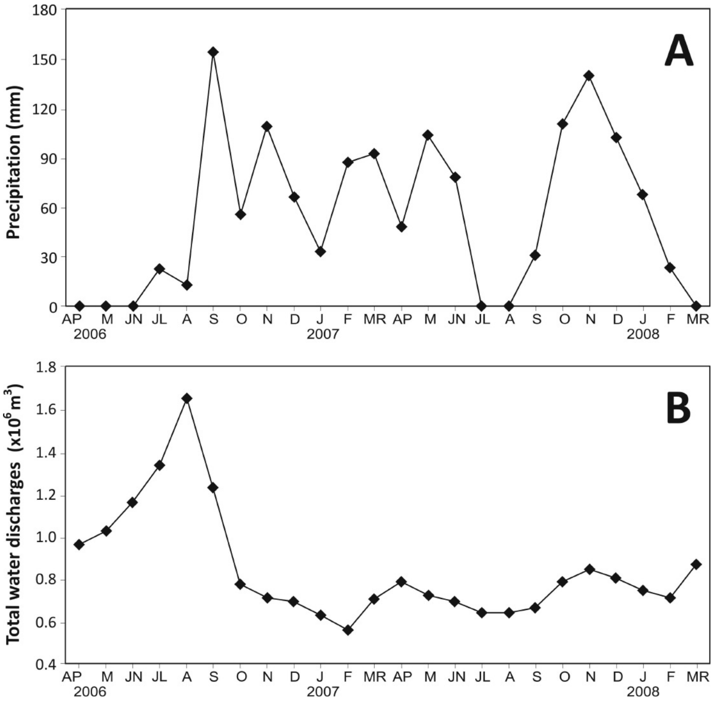

Fig. 5: (A) Monthly variation of the precipitation (mm) in the area of study. (B) Monthly variation of the water discharges of D6 pumping station.

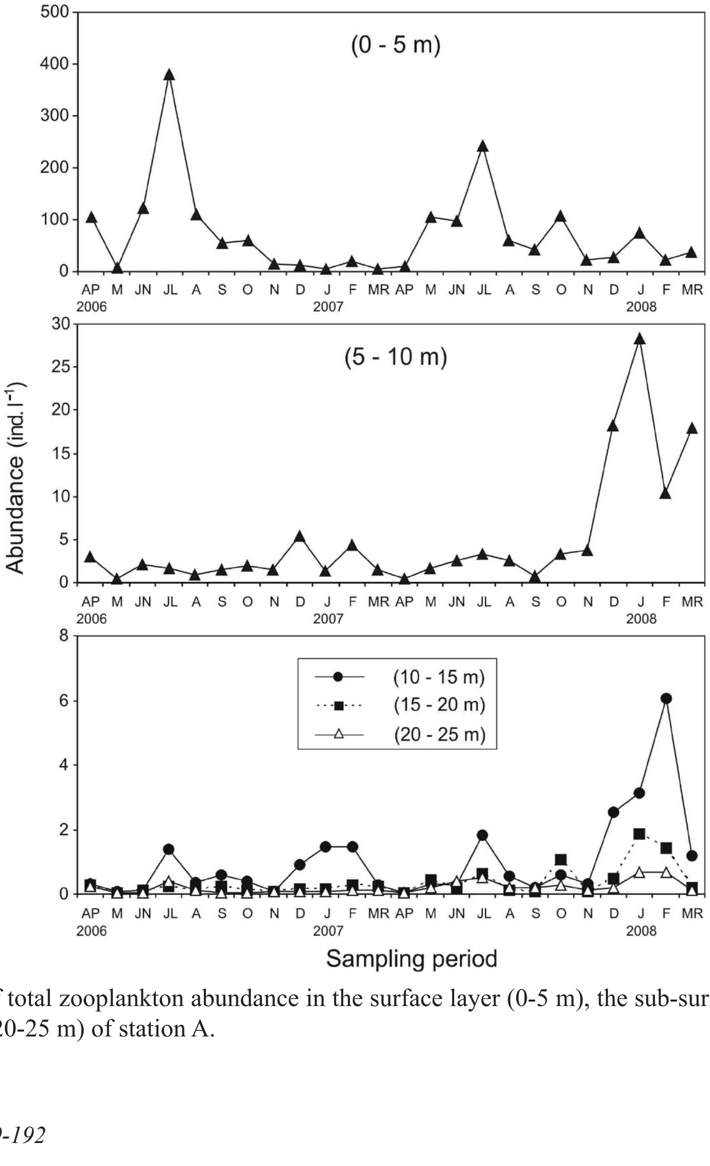

Fig. 6: Seasonal distribution of total zooplankton abundance in the surface layer (0-5 m), the sub-surface layer (5-10 m) and the deep layers (10-15, 15-20 and 20-25 m) of station A.

Table 1. List of the zooplankton taxa recorded in Aitoliko basin.

ROTIFERS
- Bdelloida
- *Brachionus calyciflorus* (Pallas, 1766)
- *Conochilus unicornis* (Rousselet, 1892)
- *Filinia longiseta* (Ehrenberg, 1834)
- *Gastropus stylifer* (Imhof, 1891)
- *Hexarthra* sp. (Schmarda, 1854)
- *Kellicottia longispina* (Kellicott, 1879)
- *Keratella quadrata* (Müller, 1786)
- *Ploesoma hudsoni* (Imhof, 1891)
- *Synchaeta* sp.

COPEPODS
- Calanoida
  - *Calanipeda aquaedulcis* (Kritchagin, 1873)
  - *Paracartia latisetosa* (Kritchagin, 1873)
- Harpacticoida
  - *Euterpina acutifrons* (Dana, 1847)
  - *Harpacticus gracilis* (Claus, 1863)
  - *Microsetella norvegica* (Boeck, 1865)
- Cyclopoida
  - *Oithona nana* (Giesbrecht, 1893)
- Nauplii

CLADOCERANS
- *Podon* sp. (Lilljeborg, 1853)

TINTINNIDS
- *Favella ehrenbergii* (Claparéde & Laachmann, 1858)
- *Undella* sp. (Daday, 1887)

MEDUSAE

DECAPOD LARVAE
- Crabs
- Shrimps

BARNACLE LARVAE

FISH LARVAE - EGGS
- *Engraulis encrasicolus* (Linnaeus, 1758)
- Others

ECHINODERM LARVAE

GASTROPOD LARVAE

LAMELLIBRANCHIA LARVAE

POLYCHAETE LARVAE

ers, which classified third in abundance in the zooplankton (14.6%), exhibited abrupt peaks during July in both years, although during the second year high abundance was also observed in October and December 2007 (Fig. 7). Ten rotifer species were recorded during the entire study period. The rotifer community was apparently represented by freshwater species, although *Synchaeta* genus also includes brackish water species. The order Bdelloida and the species *Brachionus calyciflorus* accounted for 52.3 and 41.1%, respectively, in the community. The former was dominant from July to December 2007, while the latter in June and September 2006 and in June 2007. Bdelloida also dominated the community under the 0-5m surface layer presenting an average proportion of 75%. *Synchaeta* sp. and *Polyarthra* sp. accounted for 4.1 and 1.8%, being the most abundant rotifers in December 2006 and in February 2007, respectively. *Ploesoma hudsoni*, *Keratella quadrata*, *Conochilus unicornis*, *Filinia longiseta*, *Kellicottia longispina* and *Gastropus stylifer* were found sporadically in the samples. Polychaete larvae (trochophores) followed in abundance in the zooplankton community and accounted on average for 6.9%. There was no clear seasonality in the abundance of this taxon, although higher values were recorded during winter and spring months, especially under the surface layer (Fig. 7). The presence of lamellibranchia larvae did not show any clear seasonality, in contrast to the gastropod larvae which presented maximum abundance in certain periods of the year (i.e. summer and autumn) during the study period (Fig. 7).

Among the remaining zooplankton groups, the cladoceran genus *Podon* sp. presented considerable abundance mainly during spring months in the surface layer. Larvae of barnacles were found mainly during spring to early summer and echinoderm larvae mainly in the second sampling year between June and December 2007. A few specimens of decapod larvae (crabs and shrimps), as well as medusae were found sporadically, while among the representatives of the ichthyoplankton were a few unidentified fish eggs and mostly larvae and eggs of anchovy (*Engraulis encrasicolus*), being more abundant in the summer of both years. It must be pointed that almost all the eggs found under the surface layer, and especially in the deeper strata of the Aitoliko basin, were in a decayed condition.

Below the 0-5m surface layer, abundance of all taxa was significantly lower compared with the overlying layer. It is also worth noting that the average proportion of copepod carcasses was 12.7% of total zooplankton in the 0-5m surface layer, while increased to 24.3% in the 5-10m layer and higher than 50% in the underlying layers. Under the 0-5m surface layer and down to 15m, polychaete larvae increased their contribution to approximately 43% of total abundance (as an overall average in the time series). Beneath this depth the smaller zooplankton specimens such as tintinnids and rotifers dominated the zooplankton community, followed by lamellibranchia and gastropod larvae.

Data on environmental variables and zooplankton were analyzed using RDA. The model explained 52.5% of the variance of zooplankton groups and environmental data by the first two axes (Table 2). The eigenvalues of the RDA were statistically significant according to the Monte Carlo permutations (sum of all axes; $p<0.001$). Temperature and dissolved oxygen (DO) were the most important parameters, which positively affected the variation of various zooplankton groups (Fig. 9). In contrast, salinity, but also NH_4, PO_4 and total phosphorus (TP), exercised negative effects and were not correlated with any of the groups. There was a clear discrimination of four zooplanktonic groups (the larvae of gastropods and barnacles and all the ontogenetic stages of copepods), which were positively related to temperature. The DO and in a

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

Axes	1	2	3	4	Total variance
Eigenvalues	0.435	0.090	0.027	0.009	1.000
Species-environment correlations	0.876	0.776	0.495	0.378	
Cumulative percentage variance:					
- of species data	43.5	52.5	55.2	56.1	
- of species-environment relation	77.0	93.0	97.7	99.3	
Sum of all unconstrained eigenvalues					1.000
Sum of all canonical eigenvalues					0.565

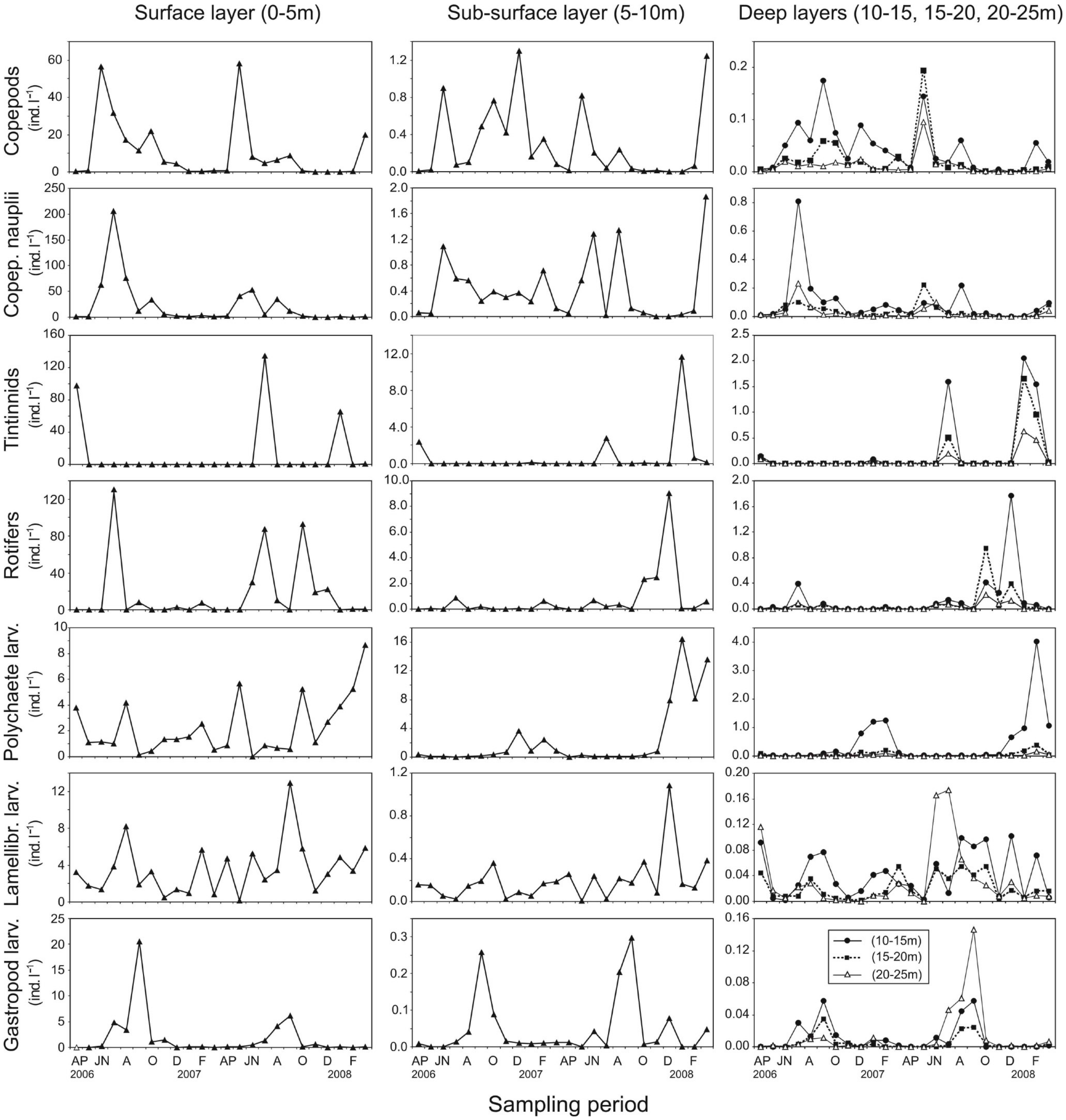

Fig. 7: Seasonal distribution of the most abundant zooplankton groups in the surface layer (0-5 m), the sub-surface layer (5-10 m) and the deep layers (10-15, 15-20 and 20-25 m) of station A during the sampling period (April 2006 – March 2008). Copepods account for the adults and copepodites of all copepod species.

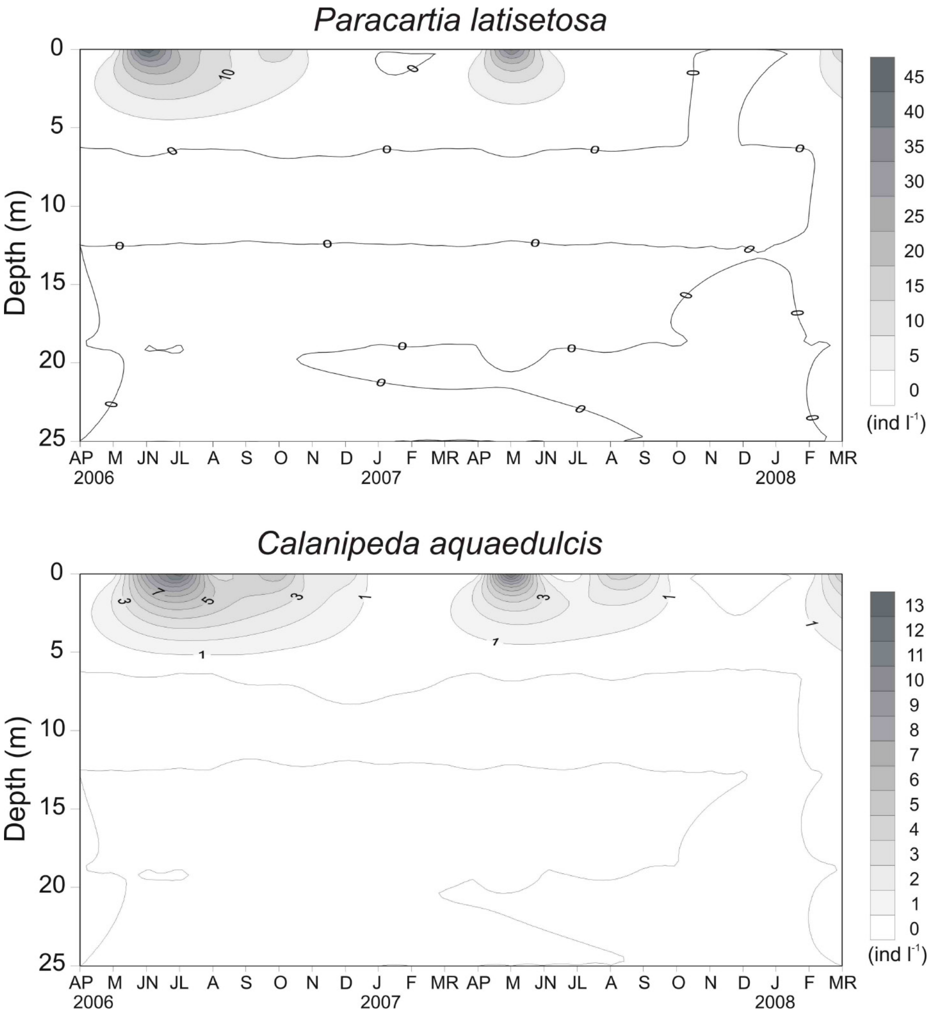

Fig. 8: Surfer plots of the variation of *Paracartia* latisetosa and *Calanipeda aquaedulcis* in station A during the sampling period (April 2006 – March 2008).

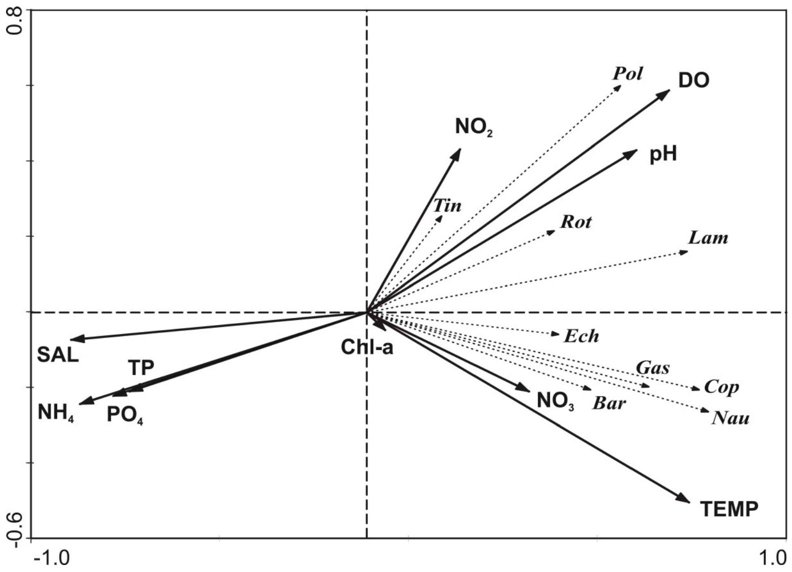

Fig. 9: Redundancy analysis (RDA) biplot of zooplankton groups and environmental parameters. Zooplankton groups are abbreviated as: Cop, adult copepods and copepodites; Nau, copepod nauplii; Rot, rotifers; Tin, tintinnids; Lam, lamellibranchia larvae; Bar, barnacle larvae; Gas, gastropod larvae; Pol, larvae of polychaetes; Ech, larvae of echinoderms.

lesser degree pH seemed to have affected the variation of rotifers and the polychaete and lamellibranchia larvae, while Chl-*a* seemed to have played a minimum role in the variation of all zooplanktonic groups.

Discussion

Hydrological characteristics

This study provides additional information on the particular hydrological characteristics of Aitoliko basin through a two-year investigation, since some of the results from the first year of investigation, which concerned the hydromorphological structure, as well as the abiotic elements and the trophic condition of the area, have been presented in the studies of Gianni *et al*. (2011, 2012). The present results verify the anoxic character of Aitoliko basin, which has been identified during the past decades. A permanent stratification in the water column is the principal feature of the Aitoliko basin and is due mainly to vertical salinity rather than temperature gradient (Gianni *et al*., 2011). The increased salinity values during the second sampling year indicate the inflow of sea water from the Messolonghi lagoon (where salinity exceeds 40‰), probably as a result of dredging activities in the connecting sill between Aitoliko and Messolonghi basins, which were completed in May 2006 and resulted in a 30%, approximately, increase in water fluxes (Gianni *et al*., 2011). The oxygenation of the deeper parts of the basin in the winter of both sampling years could be due to the above management efforts, considering that this is the first record of such a ventilation incident art these depths of the basin (Gianni *et al*., 2011). Also, the vertical distribution of ammonia, phosphates and total phosphorus in the winter months of the first sampling year, could be related to the above indicating an intense vertical mixing of the water column during this period. However, the same incident was not repeated in the following winter in the case of the above nutrients. In most of the sampling months the deeper parts of the area were characterised by increased concentrations of ammonia. This was probably related to oxygen depletion and the anaerobic decomposition of organic matter at these depths, while the same explanation could be retained for the large quantities of phosphates and total phosphorus in the greater depth layers.

The concentrations of nutrients reported in this study reflect the mesotrophic state of the basin (Gianni *et al*., 2012). Although the irrigation drainage inflow is considered the main nutrient supply in the basin, there are no measurements on the water flowing from the D6 pumping station, while these water discharges were not correlated with any of the nutrients. Moreover, there are no estimations of the quantity of water originating from runoff due to precipitation entering the basin, which means that various processes might be responsible for the seasonal variation of nutrients in this area.

Zooplankton

This study is the first study of the zooplankton community of Aitoliko basin and revealed a brackish community of invertebrates. However, the use of the specific plankton net with a porosity of 50μm might have missed a fraction of the smaller tintinnids and did not hold nonloricate ciliates in general. On the other hand, a great part of the larger zooplankton such as certain large copepod species, the larvae of decapod crustaceans and fish larvae may avoid the specific net. The investigation of Vidjak *et al*. (2009) on the sampling performances of plankton nets on zooplankton population structure indicated that the use of 125μm mesh size plankton nets in combination with Niskin bottles is the best sampling approach for the study of all zooplankton components in stratified estuaries. Consequently, the sampling technique used in this study was characterized by a degree of selectivity and, thus, it may have influenced the composition of the zooplankton community recorded.

The low number of copepod species and the dominance of only two species (i.e. *Paracartia latisetosa* and *Calanipeda aquaedulcis*) in the copepod community of Aitoliko basin reveals the very low biodiversity of this area, which is common in several coastal lakes of the Mediterranean (Benović *et al*., 2000; Belmonte *et al*., 2001). Belmonte *et al*. (2001) suggested that the potential diversity of the plankton in a confined area could be larger than the effective diversity measured using classical zooplankton sampling methods, considering the resting benthic stages of several species. Unfortunately, there was no investigation on this issue with the present data sets, while there is no such information for the area.

The highest abundance of *P. latisetosa* and *C. aquaedulcis* was recorded in summer and coincided with the maxima of the copepod nauplii. The thermophilous character of both species has been reported well (Lakkis, 1994; Quintana *et al*., 1998; Annabi-Trabelsi *et al*., 2005; Belmonte & Pati, 2007; Brucet *et al*., 2008), and although the copepod nauplii have not been identified to species, we assumed a summer reproductive period for these species. In the vertical axis *P. latisetosa* and *C. aquaedulcis* also showed similar distribution with the bulk of their populations concentrated in the surface layer, possibly due to their lower tolerance to the hypoxic or anoxic conditions as is true for many calanoid species (Mauchline, 1998). Unfortunately, there are no references on this issue concerning the above two species.

Tintinnids are also common inhabitants of the lagoonal zooplankton, while *Favella ehrenbergii* is among the dominant species in various lagoons of the eastern Mediterranean (El-Maghraby & Halim, 1965; Daly Yahia *et al*., 2005; Vidjak *et al*., 2009). Larvae of polychaetes, lamellibranchia and gastropods are usually among the dominating members of zooplankton in brackish environments frequently referred to as the 'autochthonous' component of lagoons (Ambrogi *et al*., 1989). *Brachionus calyciflorus* dominated the community of rotifers. Several rotifer species were abundant residents of brackish lagoons in the Mediterranean region, such as *B. calyciflorus* in Lake Manzalah in Egypt (Zakaria *et al.*, 2007), or *Synchaeta* sp. and *Keratella* sp. in the Krka river estuary in Croatia (Vidjak *et al*., 2009).

Various physicochemical (e.g. temperature, salinity, pH, nutrients, oxygen), biological (Chl-*a*) and topographical parameters have been correlated with the structure and distribution of zooplanktonic communities in lagoons (Chicharo & Chicharo, 2000). Among these, temperature seemed to be the most important for the seasonal fluctuations of zooplankton abundance, especially in the surface layer of Aitoliko basin, leading to a concise pattern of seasonal variability, which is quite typical of Mediterranean lagoons. In such areas, outbursts of zooplankton populations are observed during spring and summer months followed by generally low abundance values from late autumn to early spring (Sei *et al*., 1996; Bianchi *et al*., 2003). Copepod, tintinnid and rotifer populations were primarily responsible for the spring-summer annual peaks observed in this study, whereas meroplanktonic organisms (larvae of gastropods, polychaetes and lamellibranchia) contributed less. On the contrary, during winter months, meroplanktonic taxa constituted a significant part of the zooplankton community and in some cases the majority of total zooplankton abundance. The presence of the larvae and especially the eggs of the anchovy (*Engraulis encrasicolus*) in summer months coincide with its main reproductive period in the Mediterranean (Tsikliras *et al*., 2010). Larvae and eggs of this species have also been reported from other coastal lakes (e.g. Lake Varano) in the Adriatic (Belmonte *et al*., 2011). However, the bad condition of almost all the eggs found in the subsurface layers in Aitoliko basin, reveals the inhospitable character of the environment at these depths. Indeed, under the 0-5m surface layer, biological productivity decreases dramatically and only the larvae of polychaetes appear to be better adapted to such conditions. Thus, the results of this study show that a high proportion of zooplankton (e.g. copepods) cannot survive under the surface layers and is found in the form of decayed specimens in the deeper strata, probably constituting a valuable amount of energy for the decomposer community.

The almost permanent presence of the oxycline in the water column clearly influenced the vertical distribution of zooplankton. As the surface layer was always well oxygenated the bulk of zooplankton preferred to remain in this layer throughout the year. Kimmel *et al.* (2009) showed that the vertical extent and severity of the hypoxic conditions in the water column, are important factors influencing the vertical distribution of zooplankton. Except from causing direct mortality, hypoxia (DO < 2.0ml l^{-1}) seriously affects the metabolism, fecundity, growth and population dynamics of zooplanktonic organisms (Marcus *et al*., 2004), the vertical distribution and the vertical migration patterns of species (Auel & Verheye, 2007; Criales-Hernandez *et al*., 2008; Pierson *et al*., 2009), as well as the interaction between zooplankton and its predators (Keister *et al.*, 2000). The presence of bottom anoxic layers has been found to limit the available habitat for zooplankton as most organisms tend to avoid these layers and remain restricted to the well oxygenated surface part of the water column (Criales-Hernandez *et al*., 2008).

Furthermore, the presence of the anoxic layer might also affect the population dynamics of the most abundant taxa in the study area (i.e. copepods, rotifers and tintinnids), since they can produce resting eggs that sink to the bottom (Marcus, 1990). Considering that anoxic conditions can reduce the viability and hatching success of calanoid copepod resting eggs (Marcus, 1990), it is reasonable to expect that a significant portion of the eggs that sink to the anoxic part of the study area will be lost. Yet, the sediments along the perimeter of the basin up to a depth of at least 5m are not subject to hypoxic or anoxic conditions and the resting eggs laid in this zone may successfully hatch and sustain the new populations.

On the other hand, the presence, even in very low numbers, of almost all taxa in the anoxic (DO < 0.2ml l^{-1}) deep layers during the summer months is worth noting. Many zooplanktonic organisms have been found to tolerate or even prefer hypoxic layers in order to complete their diel or seasonal migration (Auel & Verheye, 2007), or to find refuge from predators (Ludsin *et al*., 2009). Adaptation to anoxia has also been found to exist in some cases. De Meester & Vyverman (1997) observed migration of the copepod *Acartia tonsa* to the anoxic hypolimnion where it remained for more than 12h. Sessile benthic organisms of many taxa have also been found to tolerate hypoxic or even anoxic conditions, although the response of their communities is highly dependent on the severity, persistence and temporal sequence of hypoxia (Levin *et al.*, 2009) and in some cases even successful recruitment may occur under hypoxic conditions (Sagasti *et al*., 2003). Whether any of the above can be applied in our case or whether the organisms were found accidentally in the deep anoxic layers of the Aitoliko basin is an interesting topic for further investigation.

The negative correlation of all the zooplankton groups with certain environmental parameters such as salinity, ammonia, phosphates and total phosphorus, actually reflects the effect of depth to zooplankton density, considering that all these parameters showed an increase with depth. Salinity stands out as one of the most important parameters in environments with strong zonation patterns (Gaudy *et al*., 1995; Marques *et al*., 2008), or in cases where man-made changes have altered the freshwater inputs in the ecosystem (Sei *et al*., 1996; Badosa *et al*., 2008). In this study, however, salinity presented very small seasonal variation and thus, it is not expected to influence zooplanktonic assemblages significantly. The observed increasing trend of salinity, at least between the two sampling years, cannot be considered with certainty as being the sole factor capable of strongly affecting the zooplankton community.

A strong relation between zooplankton abundance and Chl-*a* is not always expected in lagoon ecosystems as few organisms are strictly dependant on phytoplankton and especially copepods (Gaudy, 1989), the dominant group in our samples. Other components such as detritus or organisms of the microbial pathway can be exploited as alternative food sources constituting important, if not necessary, items when phytoplankton is quantitatively or qualitatively inadequate (Gaudy *et al*., 1995).

To conclude, the environmental conditions in Aitoliko basin and especially the existence of an almost permanent anoxic subsurface layer poses a constant threat for all trophic levels of the food web, which according to physical forces and anthropogenic impact (e.g. freshwater inputs, technical works) can at any time lead to a more or less severe crisis. This study is an important step towards a better evaluation of the ecological status of the basin, since deep knowledge of the ecosystem organization and functioning is necessary prior to any assessments. Considering that there were no previous reports on the zooplankton of this area, and thus comparable data, this study is substantial for the investigation of the mechanisms affecting the zooplanktonic community, and could constitute a basis for developing a monitoring process for the biotic elements of the ecosystem.

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