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# The effect of distinct hydrologic conditions on the zooplankton community in an estuary under Mediterranean climate influence

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## Abstract

Several studies have documented effects of hydrological conditions influencing fish and benthonic communities in estuaries and coastal areas, but only few evidences of freshwater discharge on zooplankton assemblages are found. The major finding of our study in an estuary under climate variability with regulated flow by dams is that increased annual flow leads to an increase in abundance and diversity of zooplankton and decrease of jellyfish blooms. This offers suitable nursery conditions with positive consequences on the food-web functioning. The ecohydrological approach of dual regulation could be useful with controlling the timing, frequency and volume of freshwater inflow by altering dams' operational efficiency, leading to healthy functional environment and optimize adaptability to climatic changes.

**Key words:** ecohydrology, ecological indicators, dam, Northern Atlantic Oscillation, jellyfish, global climate change.

## 1. Introduction

Zooplankton is lately used as a bioindicator of anthropogenic impact, adaptability and ecosystem integrity (Ferdous, Muktadir 2009; Chícharo *et al.* 2006a). The zooplankton is specially adapted to live in estuarine environments but changes in evolutionary establish water discharge patterns can be very detrimental to sensible species, whilst increasing the chances of propagation for more resilient species. Climatic change can have a significant impact on the present patterns of Mediterranean climate, where we can expect an increased number of extreme storm events, resulting in major floods, and prolonged periods of draught (IPCC 2007). Seasonality and variability in rainfall is the principal attribute of the Mediterranean-type climate (Gasith, Resh 1999). At least 65% (and often 80% or more) of rain in the Mediterranean falls in the three months of winter, with most of the precipitation often falling during a few major storm events (Gasith, Resh 1999). The artificial modification of river beds by dams does not only result in changes of hydromorphodynamics but also have a strong impact on biota. Dams constitute obstacles for longitudinal exchanges along fluvial systems and so result in “discontinuities” in the river continuum (Ward, Stanford 1995). The building of the largest dam structure in Europe, the Alqueva dam in 2002, caused a number of modifications in the river flow that had an impact on the abiotic components and on the estuarine ecosystems (Morais 2008). The most common attribute of flow regulation is a decrease in the magnitude of flood peaks and an increase in low flows (McCartney *et al.* 2005). As a consequence higher temperatures can occur and cause stratification, higher retention time of organic matter and shifts in the water chemistry off coastal zones (Benstead *et al.* 1999). Different nutrient input ratio in N:P:Si can cause eutrophication and toxic algae blooms (Rocha *et al.* 2002). Change in flow to an estuary also limit overall freshwater habitat availability due to impacts on the periodic rejuvenation of emergent, bank and floodplain vegetation, on the Estuary Turbidity Maximum (ETM) zone (Sklar *et al.* 1998; Chícharo *et al.* 2006c). All these environmental changes on estuaries affect reproduction of coastal species (Power *et al.* 1996; Pringle 1997) and favors invasions leading to changes in native biodiversity composition (Bunn, Arthington 2002).

Recent studies have shown that by regulating hydrological processes, the biological dynamics of systems (and consequently the quality of water resources) can be controlled, and vice versa (Zalewski *et al.* 1990; Jorgensen 1996; Zalewski 2002). The ecohydrological approach can be used to preserve estuarine ecosystem health, by improving freshwater flow management in upstream areas (Chícharo *et al.* 2009a). The consequences of changes in freshwater flow related to anthropogenic impacts and climate change can be measured through the evaluation of bioindicators, as in this case, abundance and biodiversity of zooplankton, and then translate to efficient management solutions.

The hypothesis to be tested stated that with high winter freshwater inflow into the estuary under Mediterranean climate influence, a rich and diverse zooplankton community would be expected during summer. The present work aims to analyze the effect of distinct hydrologic years (2002, 2009 and 2011) through freshwater discharge changes and related variables, on zooplankton assemblages in the Guadiana estuary. A secondary aim is to evaluate changes in zooplankton assemblages after the construction in upstream areas of the Guadiana estuary of the Alqueva dam in 2002.

## 2.Materials and methods

### *2.1. Study area*

The Guadiana estuary is situated on the southern border between Portugal and Spain and its river basin is the fourth largest in the Iberian Peninsula, approximately 67 500 km$^2$ (Fig. 1). The estuary is approximately 70 km long, encompassing a total area of 22 km$^2$ and averaging 6.5 m in depth. It is a mesotidal estuary, with tidal amplitudes ranging from 1.3 to 3.5 m. The climate of the area is classified as semi-arid Mediterranean, with the driest months in July and August when the river flow is the lowest. It is characterized by severe draughts and heavy floods. Climate variability imposes a similar trend to river flow; thus, the average river inflows are as follows: dry years, 8-63 m$^3$ s$^{-1}$; average years, 170-190 m$^3$ s$^{-1}$; humid years, 412-463 m$^3$ s$^{-1}$ (Bettencourt *et al.* 2003; Morais *et al.* 2009). There are 1824 dams in the Guadiana river basin. The Alqueva dam increased flow regulation to up to 81% of the total catchment area (55 000 km$^2$) (Galvao *et al.* 2008) and the total volume of water retained is estimated to be 13 000 hm$^3$ year$^{-1}$ (Dias *et al.* 2004). The duration of the flood events has diminished and the water level downstream usually rises abruptly, because dam floodgates are opened when water in the storages reaches critical levels (Chícharo *et al.* 2006b).

In this study, the Guadiana estuary was divided into three sub-areas: upper with stations Alcoutim and Guerreiros do Rio; middle with stations at Foz de Odeleite and Almada de Ouro; and lower with sample taken at Esteiro Carrasqueira and Barra station. This classification is commonly used to subdivide estuaries (Olausson, Cato 1980; Chícharo *et al.* 2001). The most upper station Alcoutim is 38 km upstream from the river mouth. The upper area is characterized by water mass with salinity close to zero. The middle area is the mixing zone (salinity: 0.5-25) and the lower part is characterized by the seawater dominance where salinity is above 25 (Chícharo *et al.* 2006b). The samples were taken at ebb tide in July and August of 2002, 2009 and 2011.

### *2.2. Sampling strategy and field methodology*

At each station, vertical profiles of temperature and salinity in the water column were recorded with a YSI 6600 probe. Turbidity was determined by Secchi disk depth. Zooplankton samples were collected with a conical net (0.37 m × 1.60 m, 0.2-0.3 mm mesh-size) equipped with a flow meter (General Oceanics) towed horizontally, at a depth of 1 meter for 10 minutes, at a constant speed of 2 knots. Water samples were collected at the same depth as the zooplankton trawls, for the determination of chlorophyll *a*. The organisms collected were immediately preserved in buffered formaldehyde (4%) until laboratory taxonomic counts. Freshwater inflow data, obtained from the “Instituto Nacional da Agua” (www.inag.pt), was measured at the Pulo do Lobo hydrometric station (37°48′N, 7°38′W), which is located a few kilometers above the last point of tidal influence (Mertola) and from the uppermost sampling station (Alcoutim) (Chícharo *et al.* 2006c). The Northern Atlantic Oscillation (NAO) defined as the pressure difference between Lisbon and Reykjavik was taken into consideration since there is a significant impact on the precipitation in Southern Europe and data were obtained from the National Centre for Atmospheric Research (NCAR, USA) database (http://www.cgd.ucar.edu/cas/jhurrell/indices.html).

## 2.3. Laboratory analyses

Water samples for analysis of chlorophyll *a* were filtered through 0.7 µm pore filters (Whatman GF/F). Care was taken not to exceed 100 mmHg of vacuum pressure during filtration. The filters were then frozen (-20°C) until spectrofluorimetric analyses (Knap *et al.* 1996) were carried out using acetone as the extraction solvent (Welschemeyer 1994). The samples from each station were sub-sampled with a Folsom Splitter. The organisms were counted using a binocular microscope, and identified at least to the level of class. Mesozooplankton species abundance (ind. m$^{-3}$) was determined.

## 2.4. Data analysis

Seasonal and spatial patterns in zooplankton assemblages were analyzed by non-metric multidimensional scaling (MDS) based on triangular matrices of Bray-Curtis measures of log-transformed data of dissimilarity. The identified groups of stations were further analyzed by nonparametric ANOSIM to determine whether they were significantly different (Clarke, Warwick 2001). When appropriate, R-statistic values for pair-wise comparisons provided by ANOSIM were used to determine the dissimilarities between groups. R-statistic values were also used to test the null hypothesis that within a sampling group there has been no changes in community structure. The diversity of the samples was measured through the Shannon-Weaver index. All these statistical analyses used the PRIMER 5 Software "Plymouth Routines in Multivariate Ecological Research".

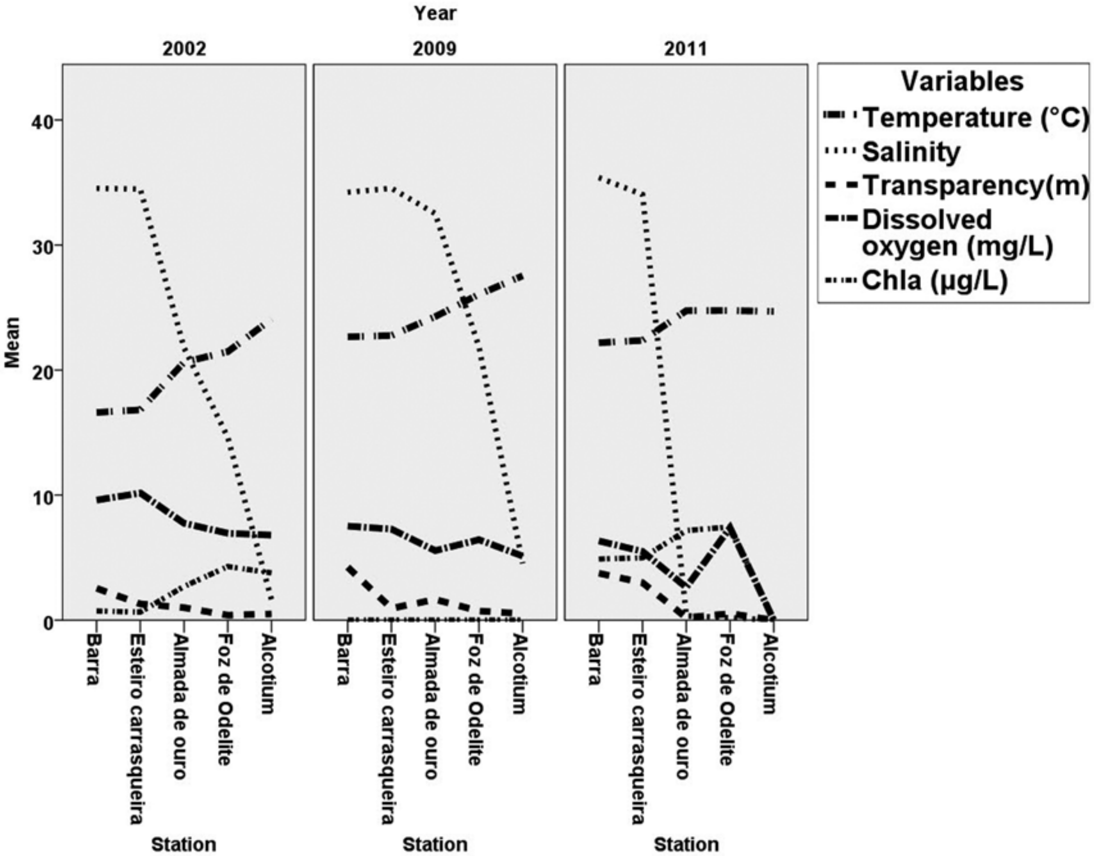

**Fig. 1.** A) Location of the Guadiana estuary in the Iberian Peninsula, with sampled stations and the division of the estuary in lower, middle and upper estuary; B) Mean values of abiotic parameters, transparency, temperature (°C), dissolved oxygen (mg L$^{-1}$), salinity and Chlorophyll *a* (µg L$^{-1}$) measured at low tides in years 2002, 2009 and 2011.

## 3. Results

The abiotic parameters in the estuary measured from 1st of July to 31st of August showed always an increases during the season, in water temperature (°C), chlorophyll *a* concentration and turbidity but registered a decrease in dissolved oxygen (Fig. 1). Higher temperatures were registered in the upper estuary and decreased towards the coast. The estuarine turbidity maxima (ETM) zone has changed its position longitudinally, between Almada de Ouro and Foz de Odeleite. Chlorophyll *a* concentration peaked in July 2002 with 5.61 µg $L^{-1}$ and increased up to 10.09 µg $L^{-1}$ at the middle/upper part. There was a positive trend between zooplankton abundance and annual water discharge. In 2002 the mean (±standard deviation) annual inflow was 27.91±60.72 $m^3 s^{-1}$ and the mean of zooplankton was 229.10 ind. $m^{-3}$. In 2009 the average annual inflow as 16.69±21.32 $m^3 s^{-1}$ and 1660.17 ind. $m^{-3}$ in zooplankton were identified. During 2009 short freshwater pulses in June and August from the Alqueva dam, with a discharge on 19th June (41.52 $m^3 s^{-1}$) and in August (three days with mean 14 $m^3 s^{-1}$) were registered. After that there was an increase in zooplankton abundance and diversity after these peaks. During 2011 the mean inflow was 191.24±310.74 $m^3 s^{-1}$ and 17 652 ind. $m^{-3}$ in zooplankton samples were present (Fig. 2). The average winter inflow (from 1st of January until 31st of March) displayed also a positive trend with abundance where a 95.17% increase in inflow resulted in 86.03% increase in abundance in 2011 from 2009 and 98.71% from 2002 (Fig. 2). During the study the river inflow was generally stable and lower than the historical average, except for artificially regulated high short peaks of inflows from the Alqueva dam, in June and August of 2009, with a discharge of 40 $m^3 s^{-1}$. There was a negative trend between NAO index with zooplankton abundance (Fig. 2) where gradually decreasing values of NAO (2002 positive (0.76), 2009 slightly negative (-0.41) and in 2011 (-1.57)) correspond with a gradual increase in zooplankton abundance (ind. $m^{-3}$). The results showed that high density of jellyfish species, specially the non-native, *Blackfordia virginica*, was linked to a decreased in abundance of zooplankton (Fig. 2).

A total of 33 zooplankton taxa were identified in the Guadiana River and jointly grouped together by relevance for further analysis (Table I). The majority of the taxa were found at the downstream stations at lower part of the estuarine zone (stations Barra and Esteiro da Carrasqueira) (Fig. 3).

In 2011 the maximum overall density was registered (17 652 ind. $m^{-3}$), followed by 2009 with 1660 ind. $m^{-3}$, and 229 ind. $m^{-3}$ in 2002. In 2011, Copepoda Calanoida presented 86.68% of total abundance, in 2002 more than 75% but no such trend was found in 2009 (56%). After the Copepoda, Decapoda larvae and Cladocera were the taxa more represented in terms of abundance during all the studied years. The Shannon diversity index was the highest in 2009, namely at Barra (J’ = 0.52) and Foz de Odelite (J’ = 0.59). The Cluster analysis was done in order to define differences between the stations, years and months separately (Fig. 4) where above 80% of similarity was found between coastal stations with the same trends in all the studied years. The multi-dimensional scaling (MDS) ordination of log trans-

**Table I.** List of taxa identified during zooplankton sampling in the Guadiana estuary.

| Identified zooplankton taxa | Group taxa |
|---|---|
| Ctenophora | Ctenophora |
| Polychaeta larvae | Polychaeta larvae |
| Gastropoda larvae | Molusca larvae |
| Bivalve larvae | Molusca larvae |
| *Evadne* spp. | Cladocera |
| *Penilia* spp. | Cladocera |
| *Podon* spp. | Cladocera |
| *Bosmina longirostris* | Cladocera |
| *Daphnia* spp. | Cladocera |
| Copepoda - Calanoida | Copepoda - Calanoida |
| *Acartia clausii* | Copepoda - Calanoida |
| Caridea mysis | Decapoda larvae |
| Brachyura zoea | Decapoda larvae |
| *Oikopleura* spp. | Apendicularia |
| *Mesopodopsis slabberi* | Mysidacea |
| Cypris | Cirripedia larvae |
| *Sagitta* spp. | Chaetognata |
| Hidromedusae not identified | Cnidaria - jellyfish |
| Insect larvae | Insect larvae |
| *Paragnatha formica* | Isopoda |
| Nauplius Cirripedia | Ciiripedia |
| Amphipoda | Amphipoda |
| Brachyura (megalopa larvae) | Decapoda larvae |
| Cnidaria larvae | Cnidaria larvae |
| Ostracoda | Ostracoda |
| Doliolida | Urocordata |
| Copepoda - Cyclopoida | Copepoda - Cyclopoida |
| Cumacea | Cumacea |
| *Sphaeroma* spp. | Isopoda |
| *Idotea* spp. | Isopoda |
| *Blackfordia virginica* | Cnidaria - jellyfish |
| *Aurelia aurita* | Cnidaria - jellyfish |
| *Bougainvillia muscus* | Cnidaria - jellyfish |
| *Maeotias marginata* | Cnidaria - jellyfish |

formed abundances of zooplankton species based on Bray-Curtis similarities evidenced the existence of some distinct zooplankton assemblages between years (Fig. 5) but no distinctive pattern between stations (Fig. 6). One way ANOSIM analysis showed the dissimilarities between years with Global R values of 0.24 (significance level, p = 0.1%) and higher random distribution between months R = 0.043 (p = 13.8%) and stations R = 0.036 (p = 26.1%).

## 4. Discussion

Water discharge is considered to be one of the main parameters affecting zooplankton seasonal variations in rivers (Saunders, Lewis 1988) and estuaries (Chícharo *et al.* 2006c). Our results showed an important variability of zooplankton assemblages structure between the distinct hydrologic years studied. In previous research done, it was demonstrated that the sustainability of the Guadiana estuarine ecosystem can only be maintained by controlling the volume and timing of release of the freshwater discharge (Chícharo, Chícharo 2006). In fact, not only the mean and the total amount of freshwater released during winter justify the higher summer diversity in zooplankton in an estuary**.** During the hydrologic year of 2009 a low winter inflow was registered but small freshwater pulses occurred in summer (freshets: 40-50 $m^3 s^{-1}$), what seemed to support the important zooplanktonic diversity registered during this period. In fact, at the Guadiana estuary modelling results showed that after a freshwater input, an increase in phytoplankton diversity is expected due to reduction of nutrient competition and this favors an increase of zooplankton diversity (Chícharo *et al.* 2006c). The freshwater discharge can have different effects on the variety of species. In case of freshet the water velocity would be not too fast and the zooplankton organisms can cope with it (Sluss *et al.* 2008), and be retained inside the estuary. Zooplankton would also benefit from having a more diverse prey assemblage, since there are greater fluctuating physicochemical conditions during freshwater pulse (Droop 1983).

By the contrary the hydrologic year of 2002 was characterized by winter flow below average and no important freshet during summer. In fact, the low zooplanktonic abundance and diversity found in

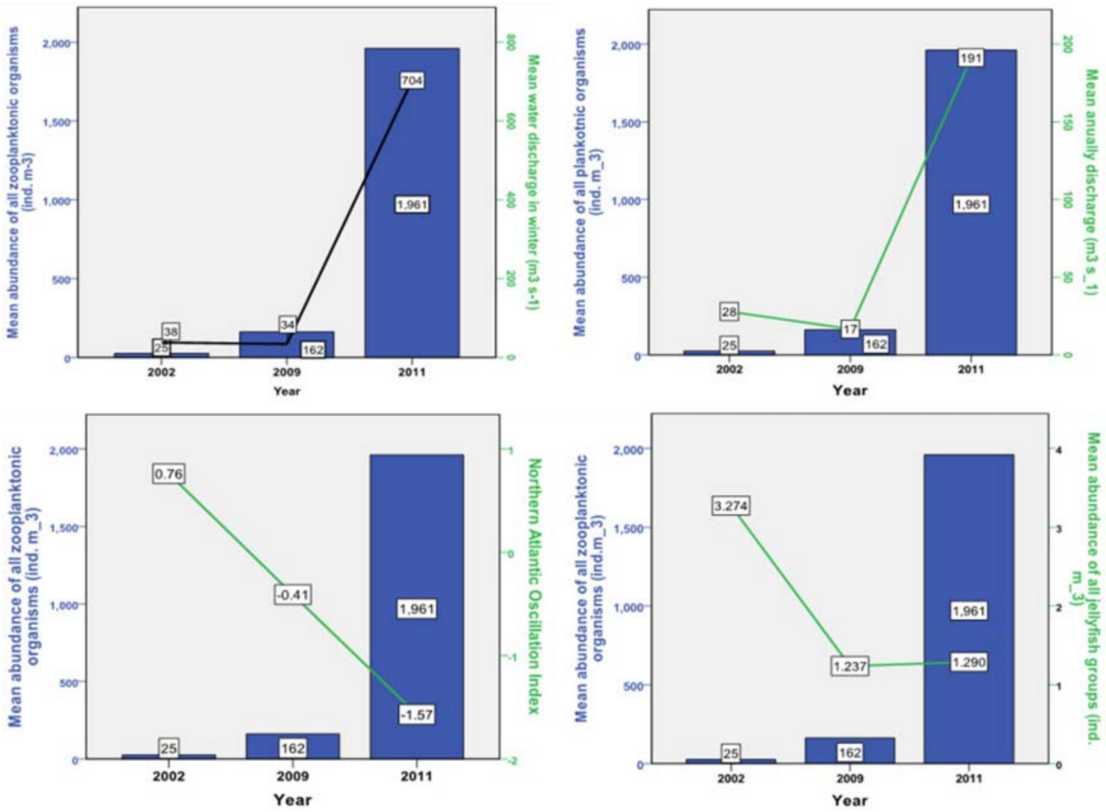

**Fig. 2.** Mean abundance of all zooplankton groups (ind. $m^{-3}$, bar chart) and their correlation with (a) mean water discharge in winter, Q ($m^3 s^{-1}$), from 1st of January until 31st of March, (b) mean annual water discharge, Q ($m^3 s^{-1}$), (c) NAO – Northern Atlantic Oscillation Index, and (d) jellyfish abundance (ind. $m^{-3}$).

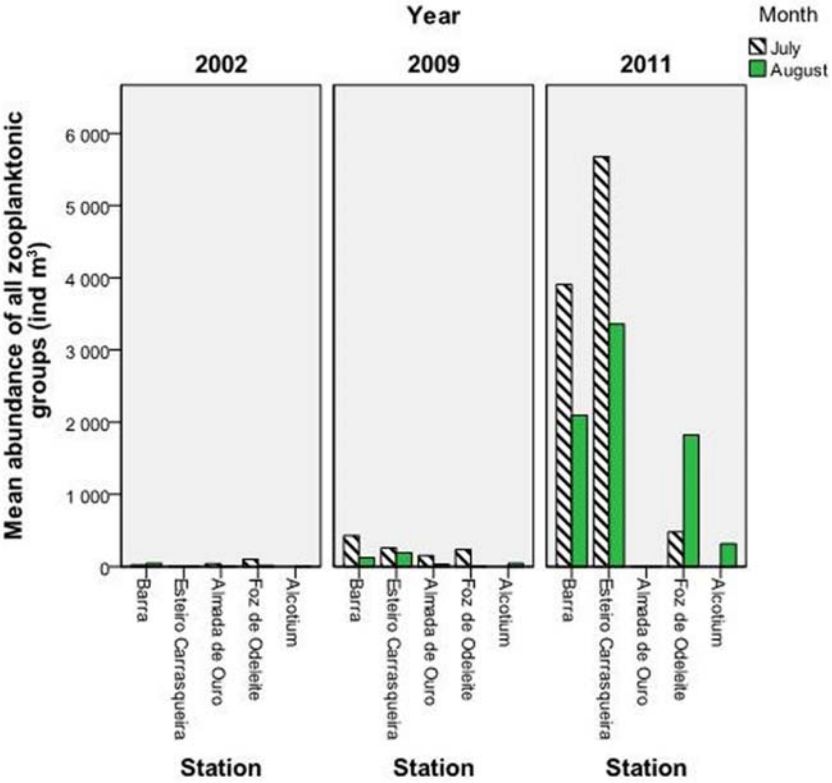

**Fig. 3.** Distribution and abundance of all zooplankton groups (ind. m$^{-3}$) at each part of the estuary in comparison between years.

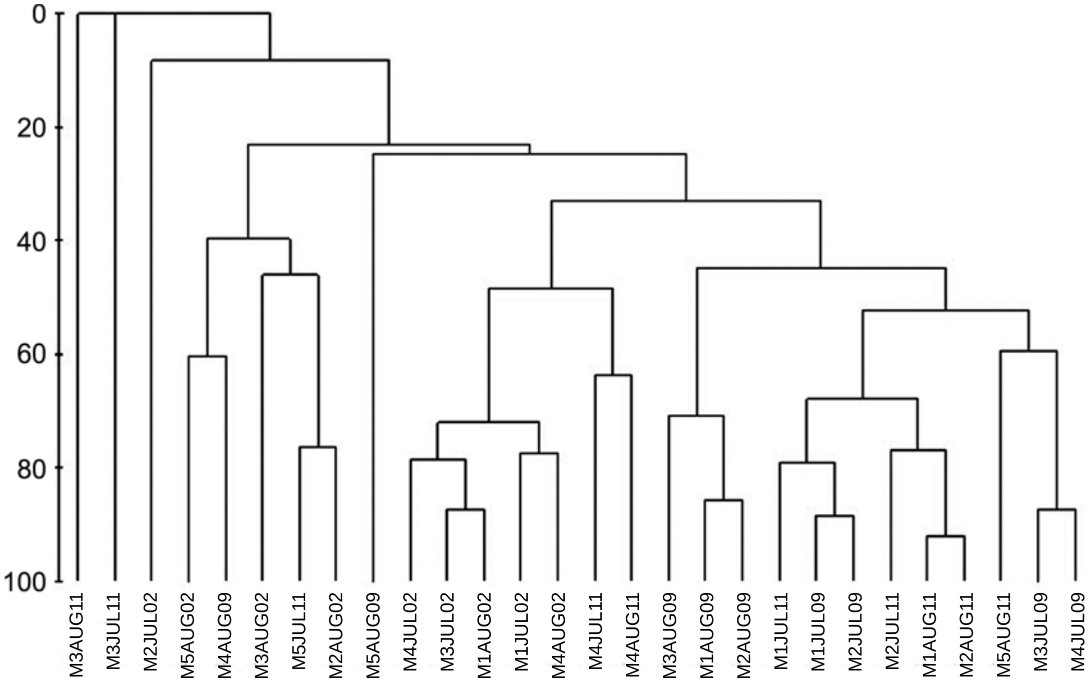

**Fig. 4.** Hierarchical clustering of species groups average by Bray-Curtis similarity index where 100 represents the highest similarity and where *M1*: coastal station Barra, *M2*: Esteiro Carrasqueira, *M3*: Almada de Ouro, *M4*: Foz de Odeleite, *M5*: Alcoutim; *Jul*: July, *Aug*: August; *02*: 2002, *09*: 2009 and *11*: year 2011.

2002, was linked a NAO index positive, during this year, which justify the reduction of precipitation and freshwater flow in regions of Southern Europe as the Guadiana estuary (Chícharo *et al.* 2006a). This was also amplified by the beginning of the water storage by the Alqueva dam, which gates closed early in 2002. The freshwater flow directly affected the abundance and diversity of zooplankton, mainly due to the fact that during winter floods, nutrients and sediments are carried into the estuary which increases primary productivity and facilitates fish and crustacean to reach the nursery areas (Morais *et al.* 2009), thus also increasing the temporary zooplankton (meroplankton) during summer. We hypothesized that in 2002, as those condition were not achieved, a reduction of general productivity occurred, what was supported by the low chlorophyll *a* concentration and low zooplankton abundance and diversity. Consequently aquatic communities undergo a yearly cycle where abiotic (environmental) controls dominate during floods but are reduced when the discharge declines and biotic controls (e.g. predation, competition) can become important (Gasith, Resh 1999). This was registered in the Guadiana estuary during the summer of 2002 when the biotic control by jellyfish blooms disrupted the energy transfer to upper levels, since jellyfish are able to consumed large densities of zooplankton (Boero *et al.* 2008).

In 2011, the hydrologic conditions were characterized by a high winter flow what, besides the positive effects on spring/summer productivity of plankton, also contributed to an absence of summer medusa blooms. This was attributed to the winter flood perturbation of the sessile phase of estuarine jellyfish (Cnidaria polips), that did not survive in freshwater salinities (Purcell 2005). This event allowed the reduction of jellyfish blooms and the development of an important community of zooplankton organisms, both in number and diversity, in the summer. In fact before the Alqueva dam construction, a low abundance of jellyfishes in 1997 was registered which can also be related to the high freshwaster flow during the winter where values of 6913 $m^3 s^{-1}$ were registered in January 1997. The growing number of jellyfish blooms is an alarming situation for general functioning of marine ecosystems and evidences of the mechanisms that control their dynamics are very useful for ecohydrological management purposes.

The increase of jellyfish blooms in the Guadiana estuary seemed to indicate a change in the community structure of this estuary after the construction of the Alqueva dam, also stressed by the climate change in the areas under Mediterranean climate influence. The increase trend in jellyfish was mainly due to species as *Aurelia aurita, Maeotias marginata* and especially *Blackfordia virginica*. This result is also supported by previous studies of Chícharo *et al.* (2009b), where even higher densities of *B. virginica* were registered, namely 37.1 ind. $m^{-3}$. In the present study the occurrence of the invasive species *M. marginata* was observed for the first time in Guadiana estuary, with only a few individuals, in August 2002. These invasions were explained by modified flow regimes that encourage alien species in estuarine areas (Bunn, Arthington 2002). Also warmer water temperatures (and subsequent water-column stability) tend to favour higher jellyfish abundance, and large climatic signals, such as the North Atlantic Oscillation (NAO), could affect the zooplankton communities and structures (Molinero *et al.* 2005).

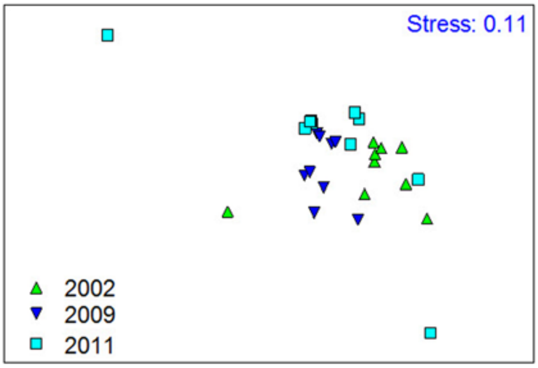

**Fig. 5.** Non-metric multidimensional scaling (MDS) of log transformed abundances of zooplankton groups based on Bray-Curtis similarities, evidencing the existence of clear distinct assemblages in zooplankton community between years 2002, 2009 and 2011.

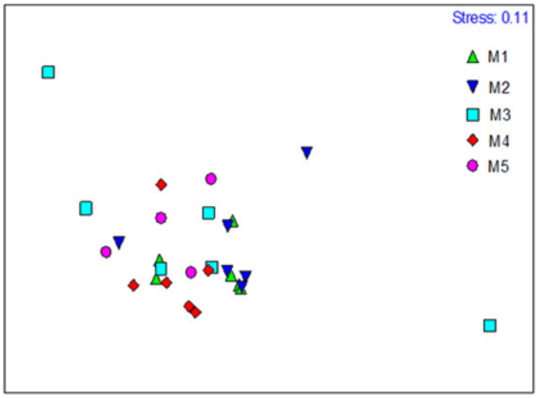

**Fig. 6.** Non-metric representation of multidimensional scaling (MDS) ordination of log transformed abundances of zooplankton groups presenting no significant pattern between different stations where *M1*: the coastal station Barra, *M2*: Esteiro Carrasqueira, *M3*: Almada do Ouro, *M4*: Foz de Odeleite and *M5*: the upper most station Alcoutim.

The comparison of our results with Esteves *et al.* (2000) (Table II), who analyzed the zooplankton community with similar methodology before Alqueva dam construction, in 1996/1997, showed the importance of taxa like Cladocera, Insecta larvae and Mysidacea, where freshwater habitat was more available. Since cladocerans and mysids are a crucial group among estuarine zooplankton and the most useful and nutritive group of crustaceans for higher members of fish in the food chain (Thorp *et al.* 1994; Ferdous, Muktadir 2009) this could affect higher levels of food web (Benstead *et al.* 1999; Marques *et al.* 2005), already threatened by the increased trend of jellyfish.

As conclusion, it can be stated that when a hydrologic year with high winter freshwater inflow into the estuary occurs, a summer rich and diverse zooplankton community develop contributing to the preservation of a healthy estuarine ecosystem. This should be complemented with the importance of freshets and with an absence of jellyfish blooms during summer. The ecohydrological approach of dual regulation proved that biotic factors strongly relate to abiotic and are crucial to identify sharp environmental changes which could help us to define the thresholds of a healthy functional environment. Further research should be conducted in the Guadiana estuary to discover the consequences of environmental modifications, not only in relation to what impact a flow discharge has on the biota, but to understand how the biological processes, namely the jellyfish blooms, might modify the environment, its abiotic parameters, and ecosystem functioning

**Table II.** Comparasion of zooplankton taxa present in 1997 (source: Esteves *et al.* 2001), before the construction of Alqueva dam, and after in 2002, 2009, 2011 (in %) (source: present study).

| | **1997** | **2002** | **2009** | **2011** |
|---|---|---|---|---|
| | | **Dam Construction** | | |
| Amphipoda | 0.01 | 0 | 0 | 0.01 |
| Apendicularia | 0 | 0 | 1.19 | 0.4 |
| Chaetognatha | 3 | 0.07 | 4.31 | 0.56 |
| Cirripedia larvae | 0.01 | 0 | 10.89 | 0.04 |
| Cladocera | 35 | 2.11 | 34.98 | 7.16 |
| Cnidaria - jellyfish | 0 | 10.42 | 0.7 | 0.06 |
| Copepoda | 50.95 | 84.73 | 23.92 | 87.25 |
| Ctenophora | 0 | 0.01 | 0 | 0 |
| Mysidacea | 2 | 0.93 | 0.39 | 4.05 |
| Decapoda larvae | 6 | 0.47 | 20.48 | 0.37 |
| Insecta | 3 | 0.1 | 0 | 0 |
| Molusca larvae | 0.02 | 0 | 0 | 0 |
| Polychaeta larvae | 0.01 | 1.16 | 3.73 | 0.04 |
| Urocordata | 0 | 0 | 0.12 | 0.04 |

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