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ABSTRACT

In a meso-eutrophic reservoir, cladoceran and copepod assemblages were characterised in two sampling sites: One located in the pelagic zone (site 1) and the other in the shallow littoral zone (site 2), the latter colonised by emergent macrophytes. Samples were collected biweekly from June to July 2001 and from May to July 2002 at the two sites. At site 1, crustacean zooplankton samples were obtained by vertical hauls using a Wisconsin type net of 64 mm mesh size. At site 2 several random samples were obtained using a van Dorn bottle. Those samples were pooled together and the total sample was sieved through a 64 mm mesh size. Macrophyte relative abundance for each species was visually estimated. Macrophyte community was composed of *Glyceria declinata*, *Eleocharis palustris* and *Carex* sp. A Mann-Whitney U-test was carried out to test for statistically significant differences between sites, for environmental parameters and crustacean zooplankton species densities. *Alona rectangula*, *Alona costata*, *Alona quadrangularis* and *Chydorus sphaericus* were only found in littoral samples. *Daphnia longispina, Daphnia pulex*, *Ceriodaphnia pulchella*, *Bosmina longirostris*, *Diaphanosoma brachyurum, Copidodiaptomus numidicus,* and *Acanthocyclops robustus* were found at both sites. However, *Daphnia* and *Bosmina* densities did not differ significantly between the pelagic and littoral sites. *Ceriodaphnia*, *Diaphanosoma,* and *C. numidicus* densities were significantly higher in the pelagic site, whereas *A. robustus* densities showed the opposite pattern.

Key-words: Zooplankton, Cladocera, Copepoda, reservoir, macrophytes, pelagic zone, littoral zone

RESUMEN

En un embalse meso-eutrofico las comunidades de cladóceros y copépodos fueron caracterizadas en dos estaciones de muestreo. Una localizada en la zona pelágica (estación 1) y la otra en la zona litoral (estación 2). Esta última está colonizada por macrofitos emergidos. Los muestreos fueron efectuados quincenalmente de Junio a Julio de 2001 y de Mayo a Julio de 2002 en las dos estaciones. En la estación 1 los muestreos de crustáceos del zooplancton se obtuvieron mediante arrastre vertical utilizando una red tipo Wisconsin de 64 mm de poro. En la estación 2 se realizaron varias muestras aleatorias con una botella tipo van Dorn. Las muestras obtenidas en la estación 2 se juntaron y la muestra total resultante fue filtrada por una red de 64 mm de poro. La abundancia relativa de cada una de las especies de macrófitos fue estimada visualmente. La comunidad de macrófitos está constituida por Glyceria declinata*,* Eleocharis palustris *y* Carex *sp. Fue realizado el test de U de Mann-Whitney para detectar las diferencias estadísticamente significativas entre las dos estaciones, para los parámetros ambientales y para las densidades de los crustáceos del zooplancton.* Alona rectangula, Alona costata, Alona quadrangularis *y* Chydorus sphaericus *se han observado únicamente en los muestreos obtenidos en el litoral.* Daphnia longispina, Daphnia pulex, Ceriodaphnia pulchella, Bosmina longirostris, Diaphanosoma brachyurum, Copidodiaptomus numidicus *y* Acanthocyclops robustus *se han encontrado en ambas estaciones, no obstante las densidades de* Daphnia *y* Bosmina *no presentan diferencias estadísticamente significativas entre la zona pelágica y la litoral. Las densidades de* Ceriodaphnia, Diaphanosoma *y* C. numidicus *fueran significativamente más elevadas en la zona pelágica, mientras que la densidad de* A. robustus *presentó un patrón opuesto.*

Palabras clave*: Zooplancton, Cladocera, Copepoda, embalse, macrófitos, zona pelágica, zona litoral*

INTRODUCTION

In the littoral zone of lakes, macrophytes provide a diverse array of surfaces for colonisation, feeding and refuge, not only for plant-associated microcrustaceans but also for pelagic species (e.g. Beklioglu & Moss, 1996; Gasith & Hoyer, 1998; Jeppesen *et al.*, 1998; Kairesalo *et al.*, 1998; Bergström *et al.*, 2000; Nurminen & Horppila, 2002). According to Jeppesen *et al.*(1998) if plant beds are present, cladocerans such as *Ceriodaphnia* spp., *Diaphanosoma brachyurum* and cyclopoid copepods are often more abundant in the littoral zone than in the open waters, whereas calanoid copepod densities show the opposite pattern. Despite the abundance of literature concerning natural lakes, studies investigating the importance of macrophyte communities in reservoirs seem to be non-existent. In fact, the littoral zone of the reservoirs is strongly conditioned by water level fluctuations of large amplitude. As a consequence of those variations, macrophyte communities do not exist or are restricted in space and time (e.g. Wetzel, 1990; 2001).

Azibo Reservoir is located in the Portuguese part of the Douro River watershed and is an exception to this pattern. As water use is not very intense, annual water level fluctuations are of small magnitude, ranging between 1.5 and 2 m. This fact allows for the existence of emergent macrophytes in the littoral shallow areas of the reservoir. Macrophyte growing season runs from May to July. At the end of July water level decreases, macrophyte growth areas dry and remain so until the first autumn rains. This research represents a preliminary insight into the

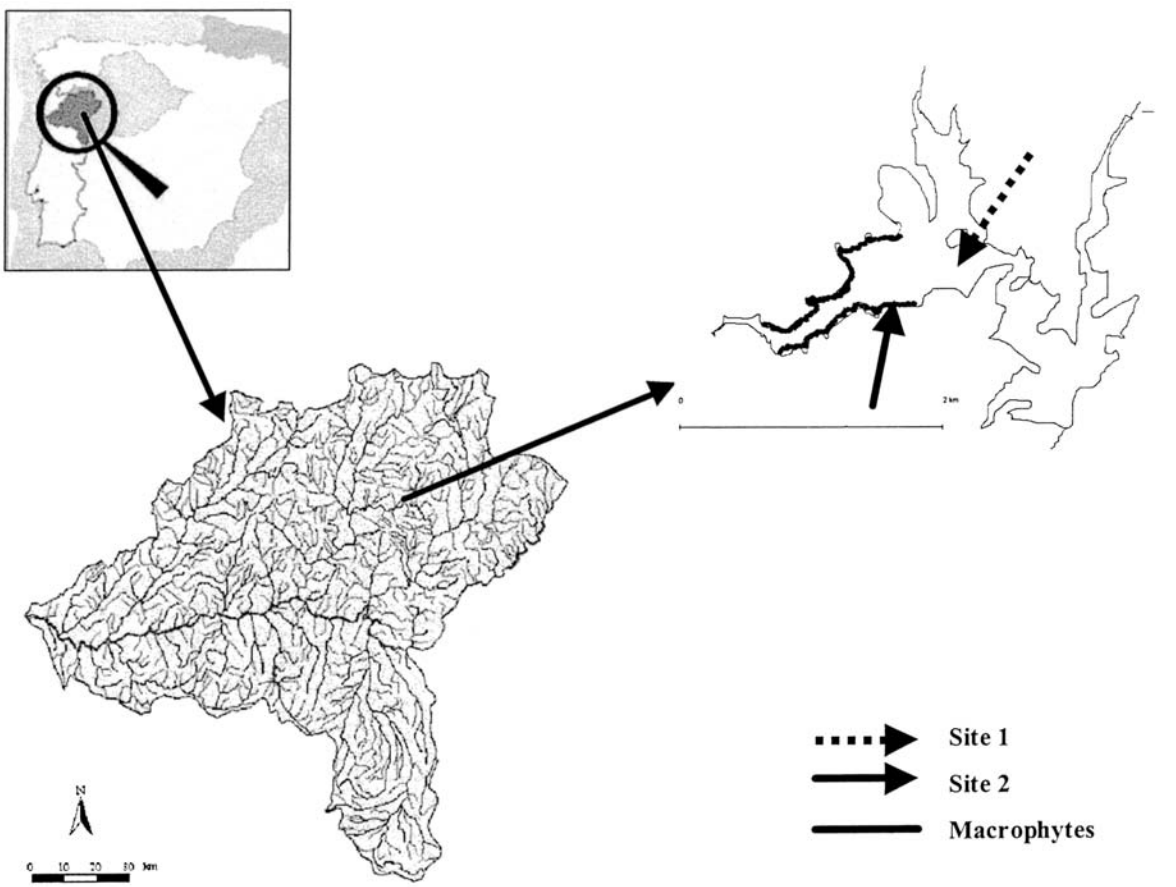

Figure 1- Azibo Reservoir with indication of sampling sites. *Embalse de Azibo y localización de las estaciones de muestreo.*

potential role of those temporary macrophyte communities in structuring microcrustacean assemblages, which dominated zooplankton in this reservoir (Geraldes & Boavida, 2004). Thus, the objective of this study was to assess whether cladocerans and copepods found in this reservoir were preferably found in shallow littoral areas with macrophytes rather than in pelagic waters. To achieve this objective crustacean zooplankton assemblages from both the pelagic zone and the littoral zone colonised by macrophytes were characterised and compared.

STUDY AREA

Azibo Reservoir is located in the Portuguese part of the Douro River watershed. The reservoir's total capacity is 54470 x 10^3 m^3 and it covers an area of 410 ha. Maximum depth is about 30 m, while mean depth is 13.2 m. This reservoir was filled for the first time in 1982. The direct influence of human activities on the impoundment is more accentuated during summer, when the reservoir and surroundings are used for recreational activities. Other activities found year round in the reservoir watershed are farming and grazing. Water is also used for urban supply and irrigation, but these are not significant and water level fluctuations are not very accentuated, ranging between 1.5 and 2 m. Consequently, shallow areas of the reservoir are colonised by emergent macrophytes (Fig.1). Before pike (*Esox lucius*) introduction in the 1990's, fish community was mainly composed of cyprinids. The relative abundance of cyprinid fish significantly decreased upon pike introduction (Table 1). In the region where Azibo is located, the climate is continental, with warm, dry summers and long, cold winters. However, because of the influence of Mediterranean climate in the remaining Iberian Peninsula, precipitation occurs mainly in autumn and winter with wet winters usually alternating with dry ones. Total annual precipitation varies between 800 and 1000 mm and mean annual air temperature ranges between 12.5 ºC and 14 ºC. Thermal stratification occurs from June to October. The reservoir was classified as meso-eutrophic (Geraldes & Boavida, 2003).

Table 1- Relative abundance and number of fish captured (in parenthesis) in June and August 1987/1988 before pike introduction (Formigo, 1990), and during the same period in 1994 after pike introduction (Albuquerque, unpubl. bachelor thesis)). *Abundancia relativa y número de peces muestreados (entre paréntesis) en Junio y Agosto 1987/1988 antes de la introducción de lucio (Formigo, 1990) y en lo mismo período en 1994, después de la introducción de esta especie (Albuquerque, tesis de grado)).*

	1987	1988	1994
Cyprinidae			
Chondrostoma duriensis	70.09 (164*)	80.85 (460*)	6.97 (9**)
Barbus bocagei	18.79 (44*)	15.11 (86*)	11.62 (15**)
Cyprinus carpio	10.26 (24*)	3.51 (20*)	25.60 (33**)
Carassius auratus	–	–	14.73 (19**)
Squalius carolitertii	0.43 (1*)	0.35 (2*)	***
Salmonidae			
Salmo trutta	0.43 (1*)	0.18 (1*)	–
Centrarchidae			
Micropterus salmoides	–		6.20 (8**)
Esocidae			
Esox lucius	–		34.88 (45**)

Catch effort unit * = net^{-1} Ih^{-1}; ** = net^{-1} 14 h^{-1};
***Recorded in pike guts (percentage occurrence of 7.14; n = 14 guts)

METHODS

Samples were collected biweekly from June to July 2001 and from May to July 2002 at two sampling stations (Fig. 1): One located at the pelagic zone of the reservoir (site 1) and the other located in a littoral shallow area covered by emergent macrophytes (site 2). Crustacean zooplankton samples were obtained at site 1 by taking two vertical hauls using a Wisconsin type net of 64 µm mesh size. At site 2 (water depth 55 - 60 cm) several random samples were obtained using a van Dorn bottle (length 41 cm, diameter 60 mm) that was lowered to a few centimetres above the sediment surface. Those samples were pooled together and the total sample was sieved through a 64 µm mesh size. Animals were anaesthetised with carbonated water and preserved in sugar-saturated formaldehyde (4% final concentration). Depending on density, zooplankton were counted from sub samples of 5, 10, 20 ml, or from the total sample. Animals were identified to species level, according to Scourfield & Harding (1966), and Dussart (1969). Macrophyte coverage was visually estimated at site 2. Water samples for soluble reactive phosphorus (SRP), total phosphorus (TP) and chlorophyll *a* (CHL *a*) determination were obtained from the upper 30 cm of the water column at both sampling sites. SRP concentrations were measured using the method by Murphy & Riley (1962), and TP was assessed after acid hydrolysis with persulfate for 60 min under high temperature and pressure (APHA 1989). CHL *a* was obtained from 500 to 1000 ml of sampled water filtered through a Whatman GF/A filter no more than 2 h after collection. Concentrations were determined with a spectrophotometer after overnight extraction in 90% acetone. Water temperature, dissolved oxygen, conductivity and pH were measured in situ with a 6820 YSI Multiparameter Water Quality Monitor.

A Mann-Whitney U-test was carried out to test for statistically significant differences, between sites, for environmental parameters and species densities. Statistical analyses were performed using SPSS 8.0.

RESULTS

No significant statistical differences were found between sampling sites for environmental variables (Table 2). In site 2 the emergent macrophyte community was composed of *Glyceria declinata*, covering 40 % of the sampled area, *Eleocharis palustris* and *Carex* sp., covering each 30 % of the sampled area.

Eleven crustacean zooplankton species were observed. Since no statistically significant interannual differences were found for species densities either in site 1 or in site 2, data from both years were pooled together (Table 3). *Alona rectangula*, *Alona costata*, *Alona quadrangularis* (for statistical analyses these species were grouped together as *Alona* spp. because of their ecological similarity) and *Chydorus sphaericus* were only found in site 2. The other zooplanktonic crustaceans found in Azibo were the cladocerans *Daphnia longispina*, *Daphnia pulex* (for statistical analyses these species were grouped together as *Daphnia* spp. for the same reason stated for *Alona*), *Ceriodaphnia pulchella*, *Bosmina longirostris*, and *Diaphanosoma brachyurum*, plus the copepods *Copidodiaptomus numidicus*, and *Acanthocyclops robustus*.

Ceriodaphnia and *Diaphanosoma* densities were significantly higher in site 1. Conversely, *Daphnia* and *Bosmina* densities did not exhibit significant differences between sites. *C. numidi-*

Table 2- Mean ± SD values of the environmental variables, minimum-maximum range for pH and results of Mann-Whitney U-test. *Media ± SD de los parámetros ambientales, mínimo-máximo pH y resultados del test de U de Mann-Whitney.*

	Site 1	Site 2	P
TP (µg/l)	60.0 ± 6.3	76.7 ± 27. 1	NS
SRP (µg/l)	4.3 ± 2.0	9.9 ± 7.7	NS
CHL *a* (µlg/l)	0.8 ± 0.6	0.8 ± 0.7	NS
Water temperature (°C)	20.3 ± 3.0	21.0 ± 3.1	NS
Dissolved oxygen (mg/l)	8.8 ± 1.2	8.0 ±1.3	NS
Conductivity (µS/cm)	64.0 ± 6.4	64.0 ± 6.4	NS
pH	6.9-8.1	7.2-8. 1	NS

*P <0.05;**P < 0.01; NS: not significant

Table 3- Mean ± SD species densities (ind/m^3), and results of Mann-Whitney U-test. *Media ± SD de densidades de las especies presentes (ind/m^3) y resultados del test de U de Mann-Whitney.*

	Site 1	Site 2	P
Cladocera			
Alona spp.	0.0	4863.9 ± 8168.2	**
Bosmina longirostris	104.6 ± 184.7	44.5 ± 51.3	NS
Ceriodaphnia pulchella	1161.8 ± 1239.2	203.2 ± 300.5	*
Chydorus sphaericus	0.0	646.8 ± 959.4	**
Daphnia spp.	595.7 ± 610.8	158.7 ± 196.4	NS
Diaphanosoma brachyurum	1105.0 ± 1916.2	22.2 ± 34.0	*
Copepoda			
Acanthocyclops robustus	166.0 ± 127.3	1000.0 ± 990.2	*
Copidodiaptomus numidicus	6361.6 ± 3472.0	1847.6 ± 2210.4	*

*P <0.05;**P < 0.01; NS: not significant

cus densities were significantly higher in site 1, whereas *A. robustus* densities were significantly higher in site 2.

DISCUSSION

All taxa were found in both sampling sites, except *Alona* spp. and *Chydorus sphaericus*, which were only found in the littoral zone. These species are typically associated with macrophyte communities (Scourfield & Harding, 1966). *Dapnhia* and *Bosmina* seemed to be as well widespread among macrophytes as in the pelagic zone, a similar pattern was also observed by Jeppesen et al. (1998). Conversely, *Ceriodaphnia*, *Diaphanosoma* and *C. numidicus* exhibited a clear preference for the pelagic zone; which might suggest a "shore avoidance" behaviour (Gliwicz & Rykowska, 1992). According to these authors some species tend to avoid near-shore areas because there they are more vulnerable to young fish predation. In Azibo, fish were dominated by cyprinids during the 1980's (Formigo, 1990). Although cyprinid fish are not strictly planktivorous, they can have some impact on cladoceran and copepod assemblages (Winfield & Towsend, 1992; Visman *et al.*, 1994). In fact, cyprinids start feeding on zooplankton shortly after hatching and continue feeding mainly on this food item until they are around 15 cm long (Lammens & Hoogenboezem, 1992). However, in Azibo their impact on cladoceran and copepod assemblages might have been minimised, because of the introduction of pike in the reservoir in the 1990's. This fish caused an accentuated decrease in the resident cyprinid relative abundance (Table 1). Nowadays, according to angler information, pike is the dominant fish species in Azibo. After absorbing the yolk sac, juvenile pike feed on *Daphnia* and copepods during a few weeks, before they are able to feed on macroinvertebrates (Hunt & Carbine, 1951). In fact, at the beginning of June, young fish shoals were observed near the shore in site 2 (Geraldes, pers. obs.). Thus, juvenile pike might have some temporary impact on cladoceran and copepod assemblages. *Daphnia* is one of the most important "targets" for fish predation (Gliwicz & Rykowska, 1992; Winfield & Towsend, 1992; Visman *et al*., 1994; Lampert & Sommer, 1997; Lauridsen *et al.,* 2001). However, *Daphnia* was abundant only during the winter and early spring months, being replaced afterwards by *Ceriodaphnia*, when temperature increased (Geraldes & Boavida, 2004). Therefore, in this reservoir, *Daphnia* was not an important "target" for young pike, because this cladoceran and the young pike of the year did not coexist. The most important "targets" might have been *Ceriodaphnia* and *Diaphanosoma*, whose densities were high when young fish shoals were first noticed.

Thus, the higher densities found in the pelagic zone might have been evidence of shore avoidance behaviour, as a strategy to avoid young fish predation. In fact, it is plausible to think that in the pelagic zone predation pressure was lower, since the occurrence of *Chaoborus* larvae was not detected there, and the abundance of adult cyprinids was very low. *C. numidicus* and *A. robustus* densities followed the same pattern as that described by Jeppesen *et al.* (1998). According to these authors calanoid copepods were always more abundant in the pelagic zone, whereas cyclopoid copepods densities followed the opposite pattern. According to Caramujo *et al*. (1997) *C. numidicus* youngest stages are a potential prey for *A. robustus*. Thus, higher abundance of *C. numidicus* in the pelagic zone can be regarded as a predation avoiding strategy. However, according to Wickham (1995) since cyclopoid copepods select smaller over large prey items, ciliates are potentially the preferred prey. Higher densities of ciliates and small cladocerans (e.g. *Alona* and *Chydorus*) found in littoral areas with macrophytes as compared to open water (Jeppesen *et al.*, 1998; Wetzel, 2001), and the fact that copepods are less impacted than large cladocerans by young fish predation (Visman *et al.*, 1994) might explain the clear shore preference behaviour exhibited by *A. robustus* in Azibo Reservoir. Here *A. robustus* would have the preferred prey (ciliates, smaller than young *C. numidicus*) and would be protected against predation as well (fish preferentially select cladocerans).

Except for plant-associated species and *A. robustus*, the macrophyte zone seemed not to be important either as a refuge or as a feeding habitat for crustacean zooplankton in Azibo Reservoir. However, further research is needed to elucidate: (1) daily variation on species horizontal distribution; (2) the actual impact of juvenile fish predation on crustacean zooplankton assemblages; (3) the importance of macrophytes as a refuge for young fish; and (4) the interactions between *A. robustus* and plant-associated microcrustaceans and ciliates. Clarification of the above mentioned items would constitute an important source of information to understand the ecological role of emergent macrophytes and to implement correct management practices leading to preservation of plant communities and water quality. This preliminary investigation constitutes the starting point for the research suggested above, and therefore it is worth to report it for its heuristic value.

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