UNIVERSIDADE DE LISBOA

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DEPARTAMENTO DE BIOLOGIA ANIMAL

EFEITOS DA OCUPAÇÃO DA PAISAGEM, DO USO DA ÁGUA E DA IDADE DAS ALBUFEIRAS NO ESTADO TRÓFICO E NA ESTRUTURA DAS COMUNIDADES ZOOPLANCTÓNICAS

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NOTA PRÉVIA

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(...) A característica mais saliente é, decerto, a da dureza. Dureza do solo áspero, seja ele granítico ou xistoso; a dureza do clima rudemente contrastante, nos seus excessos de calor e de frio, de humidade e de secura, de ventos e de calmarias; a dureza com que a crusta terrestre se levanta nas enormes pregas das serranias e com que as rochas se opõem ao ímpeto das torrentes sem conseguirem impedir que elas as despedacem, cavando tenazmente os seus leitos atormentados. Mas ao mesmo tempo, a esta dureza, resultante da violência com que os elementos lutam entre si, corresponde também um traço comum e igualmente contraditório pela sua evidente doçura e serenidade; é o que de alguma maneira se exprime fisicamente nos resultados de uma milenária erosão: a penedia abrupta das serras e os rasgões traçados pelos rios desaparecem por completo aos olhos de quem os contempla de longe e do alto das montanhas, e daí só vê extensas planícies limitadas no horizonte por uma imensa sucessão de montanhas arredondadas (...). Trás-os-Montes é, pois, simultaneamente a terra da natureza intacta, das grandes violências, da energia acumulada e do "tempo longo" (...).

José Mattoso & Suzanne Daveau, 1998

Water is essential to life, accounting for between 60 and 99 per cent of the body weight of all plants and animals. (...) Water still supports a much greater diversity of life than land, and living organisms are to be found in virtually all flowing and standing waters on Earth, from the deepest seas and largest lakes and rivers to smallest pools and highest mountain streams. Even the ice of glaciers and the water between soil particles supports single-celled algae (...).

Field guide to water life of Britain, Reader's Digest, 1984

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RESUMO

Este trabalho decorreu em três albufeiras da Bacia Hidrográfica do Rio Douro com idades distintas e sujeitas a diferentes tipos de perturbações antropogénicas. Como consequência, estes sistemas deveriam apresentar graus de trofia muito diferentes. No entanto, as albufeiras da Serra Serrada e do Azibo foram classificadas como meso-eutróficas e a do Peneireiro como eutrófica antes do seu esvaziamento, e como meso-eutrófica após o reenchimento. As flutuações de grande amplitude no nível da água, e consequente exposição dos sedimentos litorais a ciclos de dessecação/reinundação, a decomposição da vegetação de origem terrestre, a pastorícia e a ocorrência de fogos frequentes explicam o estado trófico da Serra Serrada. Os valores mais elevados do fósforo total, do fósforo solúvel reactivo, dos nitratos, da cor da água e da clorofila *a* obtiveram-se quando o nível da água estava na cota mínima. Neste período, taxa típicos de ambientes eutroficados são predominantes no fitoplancton. Os Rotifera e os Cladocera de pequena dimensão dominam o zooplancton, reflectindo o elevado grau de perturbação e a idade desta albufeira, bem como a provável ocorrência de densidades apreciáveis de predadores. No Azibo a agricultura, a pastorícia e as actividades recreativas são as principais fontes externas de nutrientes e de matéria orgânica. A comunidade zooplanctónica é dominada por Cladocera e por Copepoda, evidenciando os baixos níveis de perturbação existentes e a provável quase ausência de predadores. As elevadas concentrações de fósforo total, de fósforo solúvel reactivo e de nitratos registadas no Peneireiro estão provavelmente relacionadas com as cargas provenientes da agricultura existente na bacia de drenagem. O impacto desta actividade é potenciado pela reduzida área desta albufeira e pelo tempo médio de residência da água. A redução das concentrações de nutrientes após o reenchimento é uma consequência da remoção da camada superior dos sedimentos.

A hidrólise dos fosfomonoésteres pela fosfatase alcalina não é importante para a regeneração do ortofosfato. Provavelmente este nutriente não é limitante. Não obstante, verificou-se que a actividade desta enzima estava significativamente correlacionada com as densidades de alguns géneros do fitoplancton e de alguns crustáceos do zooplancton. Porém, o mais plausível é que a fosfatase detectada seja essencialmente produzida pelo bacterioplancton.

Palavras chave: Albufeiras, estado trófico, perturbações antropogénicas, idade das albufeiras, comunidades fito e zooplanctónicas, actividade da fosfatase alcalina.

ABSTRACT

The present study was undertaken on three reservoirs of distinct ages and subjected to different degrees of human disturbance, located in River Douro catchment. Generally, reservoirs of different ages and subjected to distinct kinds of disturbance have different degrees of trophy. However, in what concerns to trophic state the studied reservoirs were similar. Both Serra Serrada and Azibo were classified as meso-eutrophic and Peneireiro was considered as eutrophic before the emptying and as meso-eutrophic after the refilling. Water level fluctuations, the consequent exposure of littoral sediments to cycles of drying and wetting, the decomposition of flooded terrestrial vegetation, grazing and frequent fires explain Serra Serrada trophic state. The highest values of total phosphorus, soluble reactive phosphorus, nitrate, water colour and chlorophyll *a* were recorded during the minimum water level phase. During the same period phytoplankton assemblage was dominated by taxa typical of eutrophic environments. Rotifera and small Cladocera species dominated always zooplankton community, reflecting the unstable ecological conditions of this reservoir, it age and probably the presence of high densities of predators. Agriculture, grazing and recreational activities are probably the main allochthonous sources of nutrients in Azibo. Cladocera and Copepoda dominated zooplankton community. This community is typical of systems subjected to low levels of internal disturbance. Besides, predation seems to be almost nonexistent. The high concentrations of total phosphorus, soluble reactive phosphorus and nitrates recorded in Peneireiro might be related to loads originated from agricultural practices. As a consequence of reservoir small area and mean water residence time, the negative impact of those activities on water quality can be stronger. The decrease of nutrient concentrations recorded after the refilling was a consequence of sediment upper layer removal.

The hydrolysis of phosphomonoesters by alkaline phosphatase was not important for orthophosphate regeneration. Probably phosphorus was not a limiting nutrient. Nevertheless, phosphatase activity was significantly correlated with the densities of some phytoplankton genera and with some crustacean zooplankton densities. Despite of these results it is plausible to think that the main source of phosphatase might be bacterioplankton.

Key words: Reservoirs, trophic state, human disturbance, reservoir age, phytoplankton and zooplankton communities, alkaline phosphatase activity.

1. INTRODUÇÃO GERAL

Em regiões influenciadas pelo clima mediterrânico, como é o caso de Portugal Continental, o regime de precipitação é sazonal e irregular, ocorrendo em alternância anos com invernos extremamente secos e anos com invernos muito chuvosos. Por seu turno, os verões são quentes e secos. Em consequência, as reservas de água doce existentes no nosso país são escassas. Este facto, aliado à quase inexistência de lagos naturais, levou à criação de numerosas albufeiras nos principais cursos de água. Só a parte portuguesa da Bacia Hidrográfica do Rio Douro conta com 36 albufeiras construídas, encontrando-se mais 22 projectadas (Figura 1).

Figura 1 – Localização das albufeiras construídas e projectadas na parte portuguesa da Bacia Hidrográfica do Rio Douro (Adaptado de INAG, 2003). As elipses a vermelho assinalam as albufeiras que foram objecto do presente estudo.

Em Portugal as albufeiras constituem, assim, a principal fonte de água doce para os mais variados fins – abastecimento urbano, produção de energia hidroeléctrica e irrigação.

Dada a sua localização privilegiada, muitas destas albufeiras funcionam ainda como espaços de recreio e de lazer, principalmente durante os meses de Verão.

A qualidade da água e o estado trófico das albufeiras são o resultado da interacção de factores intrínsecos e extrínsecos. Entre os primeiros, salientam-se a idade e a morfologia das albufeiras. Em relação aos segundos são de realçar as características climáticas, geológicas, edáficas e morfológicas da bacia de drenagem (Schmid-Araya & Zúñinga 1992; Wetzel, 1990) e as perturbações de natureza antropogénica (Harper, 1992; Johnes *et al.*, 1996; Rybak, 2000; Hallissey *et al.*, 2001 Szyper & Gołdyn, 2002). Ambos os tipos de factores vão regular directa ou indirectamente as características ambientais na coluna de água, tais como a temperatura, a transparência, a condutividade e a disponibilidade de nutrientes (nomeadamente fósforo e azoto). Na região de Trás-os-Montes, onde se localizam as albufeiras que são objecto do presente estudo, as perturbações causadas pelas actividades humanas, quer na vizinhança destes sistemas, quer na bacia de drenagem, estão geralmente relacionadas com más práticas agrícolas e florestais que levam à redução da heterogeneidade da paisagem, tendo como efeito final o aumento da erosão (PBHD, 1998). O agravamento dos fenómenos de erosão nas bacias de drenagem e no litoral das albufeiras leva, em especial durante períodos de intensa precipitação, à entrada de elevadas cargas de nutrientes e de matéria orgânica provenientes dos ecossistemas terrestres circundantes (Harper, 1992; McGarrigle *et al.*, 2000). Por outro lado, o consumo intenso de água, em especial nos meses de Verão, é a causa das flutuações de grande amplitude nos níveis da água que se observam na maior parte das albufeiras localizadas em regiões influenciadas pelo clima mediterrânico. Em consequência do rápido decréscimo do nível da água ocorre, repentinamente, a quebra da estratificação térmica e um aumento dos fenómenos de turbulência, causando a resuspensão de sedimentos depositados no fundo da albufeira. Este facto traduz-se no aumento do número de partículas em suspensão, verificando-se, assim, um incremento das quantidades de matéria orgânica e das concentrações de nutrientes, nomeadamente de fósforo, na coluna de água (Marques & Boavida, 1993; Barone & Naselli-Flores, 1994; Naselli-Flores, 1999; Geraldes & Boavida 1999). Outra consequência das flutuações de grande amplitude no nível da água é a exposição dos sedimentos litorais a ciclos de dessecação/reinundação, o que provoca uma diminuição da sua capacidade de retenção do fósforo e de outras partículas, que assim permanecem na coluna de água (Watts, 2000).

Como foi mencionado acima a qualidade da água resulta da interacção das características da albufeira com as da bacia de drenagem. A qualidade da água vai, por seu turno, condicionar directamente a composição e a biomassa das comunidades fitoplanctónicas (Harper, 1992; Catalan & Fee, 1994; Reynolds, 1998) e, consequentemente, as comunidades zooplanctónicas (Bays & Crisman, 1983; Pejler, 1983; Chapman *et al.*, 1985; Radwan & Popiolek, 1989; Lampert & Sommer, 1997). As interacções que se estabelecem entre: (1) as diferentes espécies do fitoplancton; (2) as espécies que constituem o zooplancton; (3) o fito e o zooplancton; (4) estas duas comunidades e outras componentes bióticas destes ecossistemas (*e.g.* bacterioplancton, macroinvertebrados e peixes), também contribuem para a regulação das diferentes populações planctónicas (Carrillo *et al.*, 1995; Lampert & Sommer, 1997 Medina-Sanchéz *et al.*, 1999, Wetzel, 2001). Por outro lado, as espécies fito e zooplanctónicas também regulam as condições ambientais na coluna de água. Por exemplo, o fitoplancton é considerado como sendo uma das principais fontes de fosfatases nos ecossistemas aquáticos (Pettersson, 1980; Olsson, 1983; Boavida & Heath, 1986; Wynne *et al.*, 1991; Spijkerman & Coesel, 1998), podendo assim, influenciar as concentrações de ortofosfato na zona eufótica da coluna de água. Os herbívoros que fazem parte do zooplancton também condicionam as concentrações dos nutrientes e da matéria orgânica, influenciando as suas taxas de sedimentação (Bossard & Uehlinger, 1993; Lampert & Sommer, 1997; Tessier *et al.*, 2001; Sommer *et al.*, 2003).

O estudo destes aspectos funcionais é fundamental para a obtenção de dados que conduzam à criação de programas de monitorização e de gestão das albufeiras de modo a ser possível prevenir a degradação da qualidade da água e assim, evitar os problemas ecológicos e de saúde pública que se levantam em consequência deste processo. A criação destes programas encontra-se prevista no Artigo 8º da Directiva 2000/60/CE de 23 de Outubro, cuja transposição para a legislação interna de cada Estado-Membro deverá ocorrer até 22 de Dezembro de 2003. No entanto, apesar destas novas exigências, muitas das albufeiras existentes em Portugal, e a região de Trás-os-Montes não é excepção à regra, ou não foram alvo de qualquer estudo ou foram-no de estudos fragmentados sem qualquer continuidade. Assim, torna-se urgente a realização de estudos continuados, integrando os vários aspectos ambientais e ecológicos, visando a obtenção de séries longas de dados.

Objectivos e estrutura da tese

Este estudo pretende ser um contributo para a caracterização de alguns processos limnológicos nas albufeiras assinaladas na Figura 1. A albufeira da Serra Serrada foi criada em 1995, e anteriormente nunca tinha sido alvo de qualquer estudo, apesar de ser a principal fonte de abastecimento de água à cidade de Bragança (34.750 habitantes, INE 2001). Não existem actividades agrícolas na sua bacia de drenagem. No entanto, a pastorícia é uma prática importante nos meses de Verão. Como consequência, nas áreas localizadas na vizinhança desta albufeira ocorrem fogos frequentes que são induzidos por pastores com o objectivo de obterem melhores pastagens para o gado. Por outro lado, durante o mesmo período, devido ao consumo intenso de água ocorrem flutuações de grande amplitude no seu nível. A albufeira do Azibo foi criada em 1982 e foi objecto de um estudo prévio entre 1987/1988 (Vasconcelos, 1990a; 1990b; 1994). O objectivo inicial subjacente à criação desta albufeira era a irrigação, contudo este projecto nunca foi concluído e actualmente esta é essencialmente utilizada para fins recreativos. A paisagem circundante é muito heterogénea, sendo constituída por manchas de vegetação autóctone, ripícola, campos agrícolas e prados. Devido ao facto de a água não ser praticamente utilizada para fins de irrigação e de abastecimento urbano, as flutuações no seu nível são praticamente inexistentes. A albufeira do Peneireiro foi criada em 1973 e destina-se essencialmente ao abastecimento urbano de Vila Flor (7.760 habitantes, INE, 2001). Esta albufeira é circundada por terrenos agrícolas e as flutuações do nível de água são de média amplitude. No presente estudo apenas foram realizadas amostras sazonais nesta albufeira, devido ao facto de no período em que decorreram as amostragens para a presente dissertação, esta ter sido completamente esvaziada devido a um “bloom” de cianobactérias e assim ter permanecido durante vários meses.

Como foi mencionado, este estudo pretende ser um contributo para a caracterização da limnologia destas albufeiras. A concretização deste objectivo implicou o desenvolvimento de várias abordagens orientadas para as seguintes questões:

1. Como variam os valores de vários parâmetros ambientais na coluna de água (várias formas de fósforo e azoto, transparência, cor da água, condutividade, temperatura, pH, e clorofila *a*) e o estado trófico em albufeiras com diferentes idades, sujeitas a flutuações no nível de água com amplitudes diferenciais e localizadas em regiões em que as formas de ocupação da paisagem são distintas?

2. Quais são os efeitos das flutuações de grande amplitude no nível de água nos valores dos parâmetros ambientais mencionados no ponto anterior e na composição das comunidades fito e zooplanctónicas?

3. De que modo é que o regime de precipitação pode influenciar os valores dos parâmetros ambientais na coluna de água e as características das comunidades fito e zooplanctónicas?

4. Que factores influenciam a estrutura das comunidades zooplanctónicas nas diferentes albufeiras?

5. Será que a actividade da fosfatase alcalina na zona eufótica da coluna de água difere em albufeiras com diferentes idades, sujeitas a flutuações no nível de água com amplitudes diferenciais e localizadas em áreas em que as formas de ocupação da paisagem são distintas?

A presente dissertação é constituída por sete capítulos, cinco dos quais (Capítulos 2 a 6) são compostos por artigos, publicados, aceites ou submetidos para publicação em revistas científicas da especialidade, e nos quais são apresentados os resultados das diferentes abordagens acima mencionadas.

O Capítulo 1 corresponde à presente introdução na qual é apresentada a problemática do estudo, bem como os seus objectivos.

O Capítulo 2 é constituído por dois artigos em que se analisa a questão levantada no ponto 1. Foram ainda determinadas e quantificadas para as albufeiras objecto do presente estudo as potenciais fontes externas de fósforo e de azoto, propondo-se medidas para a mitigação dos seus possíveis impactos negativos na qualidade da água. Na albufeira do Peneireiro foram adicionalmente analisados os efeitos da medida de gestão levada a cabo para eliminar o "bloom" de cianobactérias que ocorreu no final do Verão de 2000.

O Capítulo 3 é composto por um artigo onde são investigados os efeitos das flutuações extremas no nível de água sobre os parâmetros ambientais e sobre a composição do fito e do zooplancton na albufeira da Serra Serrada. Para atingir este objectivo foram realizadas amostragens nas diferentes fases do ciclo hidrológico da albufeira: (1) Fase de nível máximo (Janeiro a inícios de Junho); (2) Fase de esvaziamento (meados de Junho a inícios de Setembro); (3) Fase de nível mínimo (meados de Setembro a início das primeiras chuvas de

Outono/Inverno). Finalmente, são sugeridas medidas de gestão específicas para esta albufeira, de modo a minimizar os efeitos negativos destas flutuações.

O Capítulo 4 é composto por um artigo que incide sobre a questão levantada no ponto 3. Esta análise foi apenas realizada na albufeira do Azibo. Deste modo, os efeitos potenciais do regime de precipitação puderam ser avaliados sem a interferência das perturbações internas causadas pelas flutuações extremas no nível de água. Assim, foram comparados os valores das variáveis ambientais e as densidades das espécies mais abundantes do fito e do zooplancton obtidos num Inverno chuvoso e noutro considerado seco. Os verões subsequentes a estes invernos foram também comparados.

O Capítulo 5 integra três artigos onde se tenta responder à questão levantada no ponto 4. No primeiro artigo pretende-se encontrar uma explicação para as diferenças encontradas na composição específica das comunidades zooplanctónicas das albufeiras da Serra Serrada e do Azibo, relacionado-as com as diferentes características dos sistemas em questão. No segundo artigo são determinados os factores que influenciam as populações das espécies mais abundantes de Cladocera na albufeira do Azibo. Este taxon foi escolhido por ser um dos que apresenta maior diversidade específica e por ser um dos grupos dominantes na comunidade zooplanctónica desta albufeira. E finalmente, no terceiro artigo averigua-se a importância potencial dos bancos de macrófitas emergentes, existentes na albufeira do Azibo, para os Cladocera e Copepoda que aí ocorrem.

No Capítulo 6 procura-se dar resposta à questão levantada no ponto 5. São também elaboradas hipóteses relativamente às origens da fosfatase alcalina, cuja actividade foi detectada nas albufeiras da Serra Serrada e do Azibo.

Por último, o Capítulo 7 inclui uma discussão abrangente dos principais resultados obtidos em cada um dos artigos, sendo realçados os pontos mais relevantes deste estudo e levantadas novas questões, cujo esclarecimento poderá contribuir não só para uma melhor compreensão da ecologia destes ecossistemas, mas também para a sua gestão sustentável.

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2. DIFERENTES FORMAS DE OCUPAÇÃO DA PAISAGEM: ESTADO TRÓFICO E QUALIDADE DA ÁGUA

Artigo 1: Distinct age and landscape influence on two reservoirs under the same climate.

Aceite em *Hydrobiologia*.

Artigo 2: Factors affecting water quality in three reservoirs subjected to different degrees of human influence.

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Distinct age and landscape influence on two reservoirs under the same climate

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Key words: trophic state, nutrient sources, land use, reservoir age, water quality, socio-economic influence

Abstract

With the purpose of finding out whether different landscape occupation could affect water quality in two reservoirs of distinct age and subjected to the same climatic influence, several factors were investigated in a study lasting from January 2000 till December 2001. Total phosphorus, orthophosphate, chlorophyll *a* concentrations and water colour were determined monthly in winter and biweekly in summer, in two reservoirs located in the Portuguese part of River Douro catchment. Complementarily, variables such as nitrate, ammonium ion and ammonia gas, as well as water temperature, dissolved oxygen, conductivity, and transparency were measured. Trophic state of both reservoirs was assessed by computation of Carlson's Trophic State Index. The potential allochthonous sources of phosphorus and nitrogen to both reservoirs were identified and estimated. Differences between reservoirs were found for conductivity, water temperature, transparency and water colour. According to Carlson's Trophic State Index both reservoirs were classified as meso-eutrophic from winter to the beginning of summer, and as eutrophic from this period onwards. Intrinsic factors such as age of reservoir, organic matter inputs from decomposition of flooded terrestrial vegetation and exposure of littoral sediments to cycles of drying and wetting, as well as extrinsic factors such as grazing and frequent land fires, can explain the trophic state of S. Serrada Reservoir. Allochthonous sources of nutrients originated from agriculture and grazing in the catchment area, and recreational activities in the reservoir probably are the factors with greatest influence on Azibo Reservoir trophic state. Based upon the obtained data, management measures are suggested to prevent further eutrophication and water quality degradation in both reservoirs.

Introduction

In climates characterised by irregular precipitation regime such as Portugal, freshwater resources are scarce. Therefore, reservoirs can provide a large amount of water for agricultural and urban supply, as well as energy generation, fishing and recreational purposes. Watershed geology, climate, soil, land slope and intrinsic factors such as age of the reservoirs, water level fluctuations, morphometry and water residence time may have a significant effect on water quality and trophic state of those freshwater systems (Harper, 1992; Wetzel, 2001). Pressure caused by human activities in the catchment area and reservoir vicinity generally leads to an intensification of surface runoff, causing an increase in eutrophication, thus threatening water quality. Runoff rates depend mainly on land use, vegetation cover and landscape mosaic (Johnes et al., 1996; Rybak, 2000; Hallissey et al., 2001; Szyper & Gołdyn, 2002).

The present study was carried out for two years on two reservoirs located at the Portuguese part of the international Douro catchment basin, in Trás-os-Montes region (NE Portugal). Serra Serrada Reservoir was filled for the first time in 1995 and, in spite of its importance for urban water supply, it has never

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been studied. Azibo Reservoir was created in 1982. A preliminary study on main nutrient concentrations and on trophic state was undertaken in 1987/1988 (Vasconcelos, 1990); so far, this was the only limnological study on Azibo. Therefore, the purpose of the present research was to find out whether age and different landscape occupation and use of the water (causing distinct water level fluctuations) could affect water quality in the reservoirs. This was achieved through: (1) determing total phosphorus, orthophosphate and chlorophyll *a* concentrations and water colour; complementarily, measuring nitrate, ammonium ion and ammonia gas, as well as water temperature, dissolved oxygen, conductivity and transparency; (2) assessing reservoir trophic state by calculating Carlson's Trophic State Index (Carlson, 1977); (3) identifying and estimating the potential allochthonous sources of phosphorus and nitrogen which might contribute to explain reservoirs trophic state.

Study area

Location, as well as morphological and hydrological characteristics of both reservoirs, are shown in Figure 1 and Table 1, respectively. The climate in this region is continental, with warm, dry summers and long, cold winters. However, because of the influence of Mediterranean climate in the Iberian Peninsula, precipitation and temperature vary greatly over the year. Summer is the dry season and can last for 3–5 months. Precipitation regime is also very irregular, with wet winters followed by dry ones (Fig. 2).

Serra Serrada (S. Serrada) Reservoir is located in the mountainous system of Montesinho Natural Park. It was built to supply water to the city of Bragança (34 750 inhabitants, INE 2001) and to generate hydroelectric power. Consequently, pronounced water level fluctuations occur, ranging between 8 and 10 m. Thermal stratification was observed from June to August in 2000 and from June to September in 2001. Disruption of stratification was coincidental with the lowest water level. The surrounding vegetation is composed of scrubs of *Erica* spp., *Genista* spp., *Chamaespartium* sp. and *Salix* spp..

Azibo Reservoir was built for water supply and irrigation, but those uses are not significant and the reservoir is utilised mainly for recreation. In spite of being fed by three small streams, which can dry in summer, water level fluctuations in this reservoir are not very accentuated, varying between 1.5 and 2 m. Thermal stratification occurred from June to October in both years of study. The surrounding vegetation is composed of *Quercus suber*, *Quercus pyrenaica*, *Quercus faginea*, *Quercus rotundifolia*, *Genista* spp., *Cistus ladanifer*, *Salix* spp., *Fraxinus angustifolia* and *Populus* spp..

Direct human influence on S. Serrada impoundment is considered negligible. There are no villages, there has been no agricultural activity for approximately 20 years, and recreational activities are not significant. However, grazing can be very intense in the catchment basin during summer months. For most of the year there are only about 200 sheep grazing in the S. Serrada catchment, yet from May to August about 5000 sheep from lowlands are transported from the surrounding lowlands to graze in the catchment and reservoir surroundings. Consequently, this area is very often subjected to wild fires that are mainly induced by shepherds to obtain better graze. In Azibo direct influence of human activities is greater during summer when about 10 000 people use reservoir and surroundings for recreation such as swimming, camping and boating. Angling is also an important activity. According to Sampaio (pers. com.) this activity is performed by about 1000 people. The watershed area is occupied by meadows (1286 ha), woodland and scrub (935 ha) and extensive agriculture (2235 ha). The latter is found all over the year and the main crops are olives (657 ha), chestnuts (650 ha), cereals (546 ha), vineyards (144 ha) and potato (85 ha). In the reservoir shore there are intensive crops, which are less than 1% of all crops (INE, 1999). Extensive grazing also occurs in this drainage basin. According to INE (1999), there are 5766 sheep and goats, 494 cattle and 531 pigs. In Azibo catchment area there are several small villages. The total of inhabitants is about 1500 and most of them are more than 50 years old (INE, 2001). Passing nearby and over the streams that feed this reservoir there is a highway, IP4. In this highway average daily traffic volume is 6000 vehicles (Barbosa & Hvitved-Jacobsen, 1999). There is no industrial activity in both reservoir catchments.

Methods

Water parameters

Water samples were collected monthly in winter and biweekly in summer, from January 2000 to December 2001 in both reservoirs. In both cases there was one

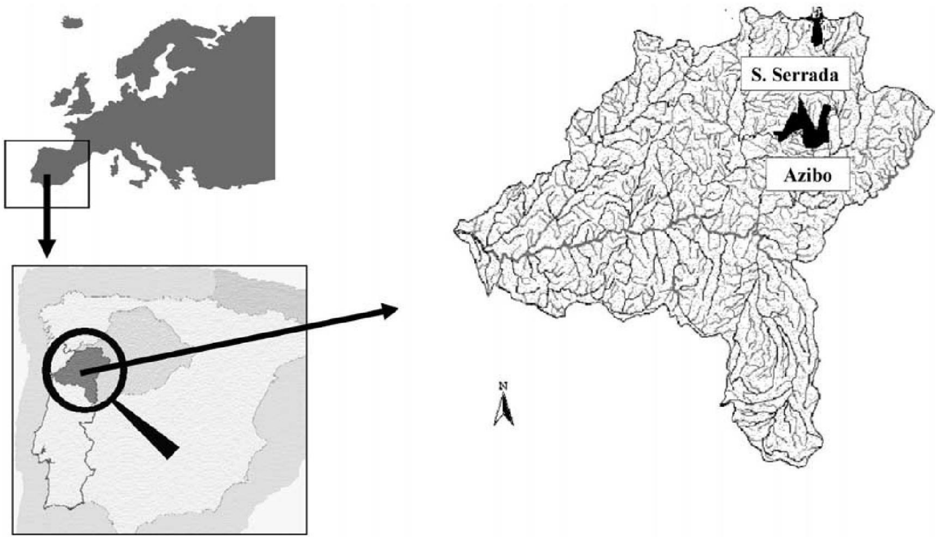

Figure 1. Location of S. Serrada and Azibo reservoirs.

Table 1. Main general features of S. Serrada and Azibo reservoirs

	S. Serrada	Azibo
Location	Latitude: 41° 57′ 12″(N)	Latitude: 41° 32′ 50″(N)
	Longitude: 6° 46′ 44″ (W)	Longitude: 6° 53′ 38″ (W)
Altitude (m)	1300	500
Geology	Granitic bedrock	Schistic bedrock
Mean annual precipitation (mm)	1300	800–1000
Mean annual air temperature (°C)	< 8.0	12.5–14.0
Watershed area (km^2)	6.7	89.0
Reservoir area (km^2)	0.25	4.10
Total capacity (m^3)	1680×10^3	54470×10^3
Maximum depth (m)	18	30
Mean depth (m)	6.72	13.2
Water residence time (years)	0.36	2.22
Year of filling	1995	1982

single sampling station, located at maximum depth. Water samples were obtained from the upper 30–40-cm stratum directly into acid-rinsed bottles and were transported to the laboratory in a cold container. Soluble reactive phosphorus (SRP) was estimated by the method of Murphy & Riley (1962) and total phosphorus (TP) concentrations were assessed after acid hydrolysis with persulfate for 60 min under high temperature and pressure. Chlorophyll *a* (CHL *a*) was obtained from a sample of 500–1000 ml of water filtered through a Whatman GF/A filter no more than 2 h after collection. Concentrations were determined spectrophotometrically after overnight extraction in 90% acetone. Water colour was determined according to the Cuthbert & Giorgio (1992) method. Physical factors such as water temperature, dissolved oxygen,

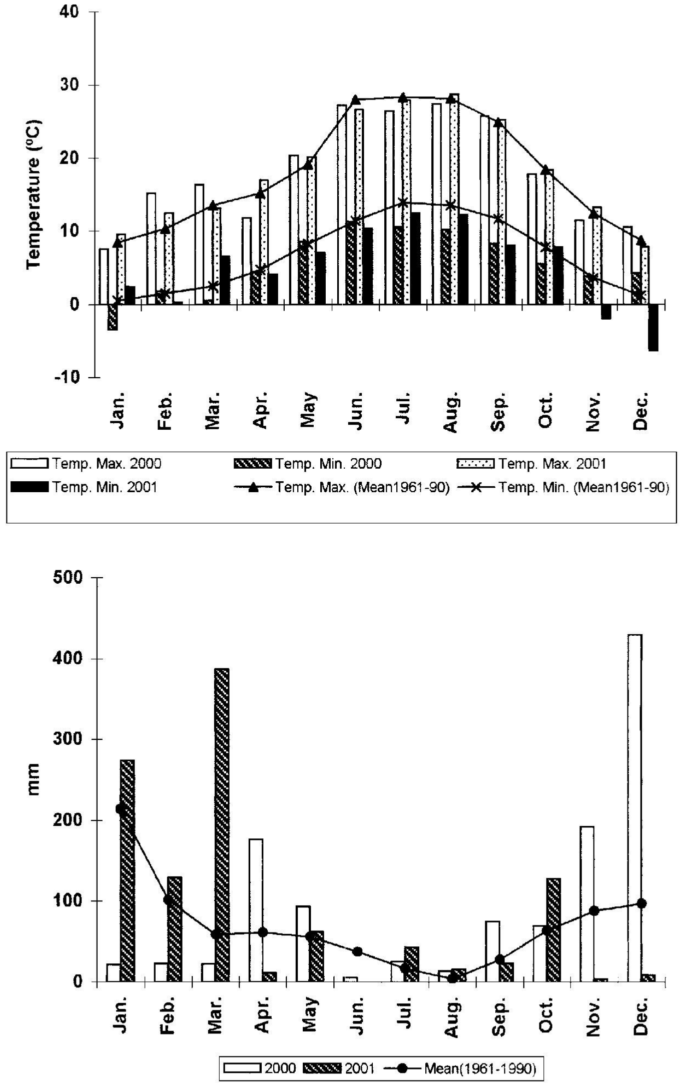

Figure 2. Temperature (A) and precipitation (B) during the period of study in Bragança, the main city closest to both reservoirs (source: Agroclima Lab- ESAB) and mean values of those variables observed between 1961 and 1990 (source: IM, Institute of Meteorology).

conductivity, pH, as well as nitrate (N-NO_3), ammonium ion (N-NH_4) and ammonia gas (N-NH_3) were measured *in situ* with a 6820 YSI Multiparameter Water Quality Monitor. Water transparency was determined as Secchi disk depth (20 cm diameter black and white disk). Carlson's Trophic State Index (TSI) (Carlson, 1977) was computed from Secchi disk transparency (TSI (SD)), TP concentrations (TSI (TP)) and CHL *a* concentrations (TSI (CHL)). All three components of the index were taken into account for better interpretation, since in reservoirs inorganic seston is in general much more abundant than organic particles.

Multidimensional Scaling (MDS) was used to identify the differences between studied reservoirs. In this method samples are arranged in a continuum in such a way that those close together are similar and those which are far apart are dissimilar. Euclidean distance was used to measure the dissimilarity between samples. A 'z-scores' transformation was used to standardize the data (Ter Braak, 1995). A Kolmogorov–Smirnov test was performed as a complement to the multivariate analysis mentioned above to assess differences in studied variables between both reservoirs (Sokal & Rohlf, 1981). All analyses were performed using SPSS 8.0.

Potential allochthonous sources of phosphorus and nitrogen

Agriculture

The main kinds of crops cultivated in the watershed and the area occupied by each one were determined from Agricultural Census database (INE, 1999). Data on quantities of N and P applied to crops, as well as on mean annual productivity of each crop, were obtained from PBHD (1998). The mean values concerning N or P removed from soil by each single species and the mean quantities of those nutrients contained in its residuals, which are returned to soil, are data mentioned in Ministry of Agriculture (Ministério da Agricultura, 1997). The N and P loads resulting from agricultural activity and potentially transferred every year into Azibo were determined by calculating the following balance adapted from PBHD (1998) for each crop:

$$PL = (A-R+L) \times C$$

where: PL – potential loading (kg/year); A – quantity of N or P application (kg/ha per year per crop); *R* – quantity of N or P removed from the soil (kg/ha per year per crop). *R* was obtained by multiplying mean productivity of each crop by the mean value of N or P removed from soil by each plant. Finally, this result was multiplied by crop's area; *L* – quantity of N and P left in the soil (kg/ha per year pre crop residuals). *L* was obtained by multiplying mean productivity of each crop by the mean value of N or P contained in the residuals. At the end, this result was multiplied by crop's area; *C* – area of the crop in the catchment basin (ha).

Livestock

The numbers of cattle, sheep and goat grazing in the watershed were taken from the Agricultural Census database (INE, 1999). Annual amounts of N and P excreted by cattle were calculated by multiplying the mean annual quantity of N and P excreted by one animal (105 kg N and 35 kg P per year) by total number of animals grazing in the catchment basin. In the same way, the total amounts of N and P excreted by sheep and goat per year were obtained by multiplying the average quantity of N and P excreted per year by one animal (21 kg N and 9 kg P) by sheep and goat all together (Ministério da Agricultura (Ministry of Agriculture) 1997). As grazing in this region is extensive and consequently animals spend most of the day in pastures and meadows, in this paper it was assumed that livestock waste was not used in agricultural fields. Conversely, pig and poultry waste was not considered because those animals are kept in stocking and their waste can be used for fertilisation.

Sewage

Sewage from small villages located at Azibo catchment basin is discharged in the streams without a previous treatment. Loads originated from sewage were calculated according to coefficients (3285 g N and 1168 g P per person and year) mentioned in PBHD (1998).

Bathing and angling

Loads originated from those activities were calculated according to coefficients adapted from Szyper & Gołdyn (2002). Therefore, amounts of N and P originated from bathing were calculated by multiplying the load coefficient from one person (1.0 g N and 0.046 g P per day) by the mean number of summer days in the region (90) and the total number of people bathing in the reservoir. The load from anglers was estimated by multiplying their total number by the mean number of angling days per year (180) and by the mean amount

Table 2. Physical factors recorded for S. Serrada and Azibo reservoirs. Minimum-maximum range, with mean and standard deviation in brackets are shown

S. Serrada	2000	2001
Water temperature (°C)	1.46-21.39 (12.51/6.59)	2.70-20.19 (12.90/6.46)
Dissolved oxygen (mg/l)	7.38-12.42 (8.55/1.45)	6.20-10.72 (8.55/1.49)
Conductivity (μS/cm)	4-10 (6.94/1.84)	3-8 (5.95/1.61)
Water transparency (m)	4.50-1.00 (2.66/1.09)	1-5 (2.85/1.14)
pH	5.77-6.56	5.95-8.34
Water colour (Pt units)	0.44-46.35 (17.39/14.27)	5.24-35.45 (18.55/9.36)
Azibo		
Temperature (°C)	5.58-24.7 (16.52/6.13)	8.06-23.83 (17.16/5.73)
Dissolved oxygen (mg/l)	7.54-11.53 (9.09/1.06)	7.21-10.78 (8.99/1.33)
Conductivity (μS/cm)	51-81 (69.87/11.09)	43-66 (56.23/7.64)
Water transparency (m)	1.5-6.0 (4.72/1.25)	1.5-5.5 (3.50/1.14)
pH	6.71-8.05	6.64-8.36
Water colour (Pt units)	0.0-9.02 (2.82/3.02)	0.0-21.81 (6.21/5.66)

of N and P produced by angler per day (156 g N and 28.8 g P).

Results

Maximum and minimum ranges, as well as mean and standard deviation values for water temperature, dissolved oxygen, conductivity, pH and water colour observed in S. Serrada are presented on Table 2. Trends in TP, SRP, N-NO_3 and CHL *a* for S. Serrada in both years are shown in Figure 3. In 2000, TP concentrations ranged between 21.01 and 113.22 μg l^{-1}, while in 2001 the variation was between 41.52 and 132.74 μg l^{-1}. The highest values of TP were reported together with the maximal values of precipitation and with the decrease of water levels occurring from July to October. Similar trends were observed for SRP. N-NO_3 concentrations varied between 0.3 and 8.0 mg/l in 2000 and between 0.1 and 17.8 mg/l in 2001 and followed a similar pattern to TP and SRP concentrations, except in summer 2001. N-NH_4 concentrations were always very low and varied between 0 and 0.3 mg/l in 2000, and were not detectable in 2001. N-NH_3 concentrations were always bellow the detection limits. CHL *a* concentrations varied between 0 and 7.97 μg l^{-1} in 2000 and between 0.1 and 10.45 μg l^{-1} in 2001. Trends in concentrations were similar in both years with a smaller peak in the beginning of spring and the highest one from the end of summer till the end of autumn.

Variation in water temperature, dissolved oxygen, conductivity, pH and water colour obtained in Azibo is shown on Table 2. TP, SRP, N-NO_3 and CHL *a* recorded in Azibo are presented in Figure 4. TP concentrations ranged between 34.28 and 89.69 μg l^{-1} in 2000, while in 2001 those ranged between 41.70 and 102.84 μg l^{-1}. During 2000 the highest values of TP concentrations were obtained from July to October, when direct influence of human activities were more intense. In 2001 the highest TP concentrations were reported together with the maximal values of precipitation and again from July to October. SRP concentrations varied from 0 to 23.86 μg l^{-1} in 2000 and from 1.53 to 15.30 μg l^{-1} in 2001, and followed a similar pattern to TP concentrations, except in summer 2001 when values varied little till the end of summer. N-NO_3 varied from 0.5 to 14.80 mg/l in 2000, while in 2001 ranged between 0.2 and 18.80 mg/l and showed similar trends to TP concentrations. However, in 2001 the largest increase in concentrations only occurred at the end of summer. N-NH_4 concentrations ranged between 0 and 0.3 mg/l in 2000, whereas in 2001 concentrations were between 0 and 0.1 mg/l. N-NH_3 concentrations were always bellow the detection limits. CHL *a* concentrations ranged between 0 and 2.73 μg l^{-1} while in 2001 varied between 0.53 and 6.57 μg l^{-1}. In both years chlorophyll concentrations peaked twice. In 2000 the peaks were observed from March to June and in autumn. In 2001 the first peak was obtained from January to March and the second one from September to December.

According to Carlson's Trophic State Index (the three components together) both reservoirs were classified as meso-eutrophic from winter to the beginning of summer and from this period onwards as eutrophic. In S. Serrada TSI (TP) varied between 48 and 72 in 2000 and between 58 and 75 in 2001. TSI (SD) ranged between 38 and 60 in 2000 and between 37 and 60 in 2001. TSI (CHL) varied between 14 and 51 in 2000 and between 8 and 54 in 2001. In Azibo TSI (TP) ranged between 55 and 69 in 2000 and between 58 and 71 in 2001. TSI (SD) varied between 34 and 54 in 2000 and in 2001 between 35 and 54. TSI (CHL) ranged between 13 and 40 in 2000 and between 17 and 49 in 2001. Considering the separate components of the Index, according to the phosphorus component both reservoirs are classified as meso- to eutrophic, mostly eutrophic. However, it is important to consider, when regarding TSI (CHL), that phytoplankton communities, in both reservoirs, were dominated by species of small dimensions (less than 50 μm). Because of their small size, phytoplankton cells are edible to zooplankters and might be object

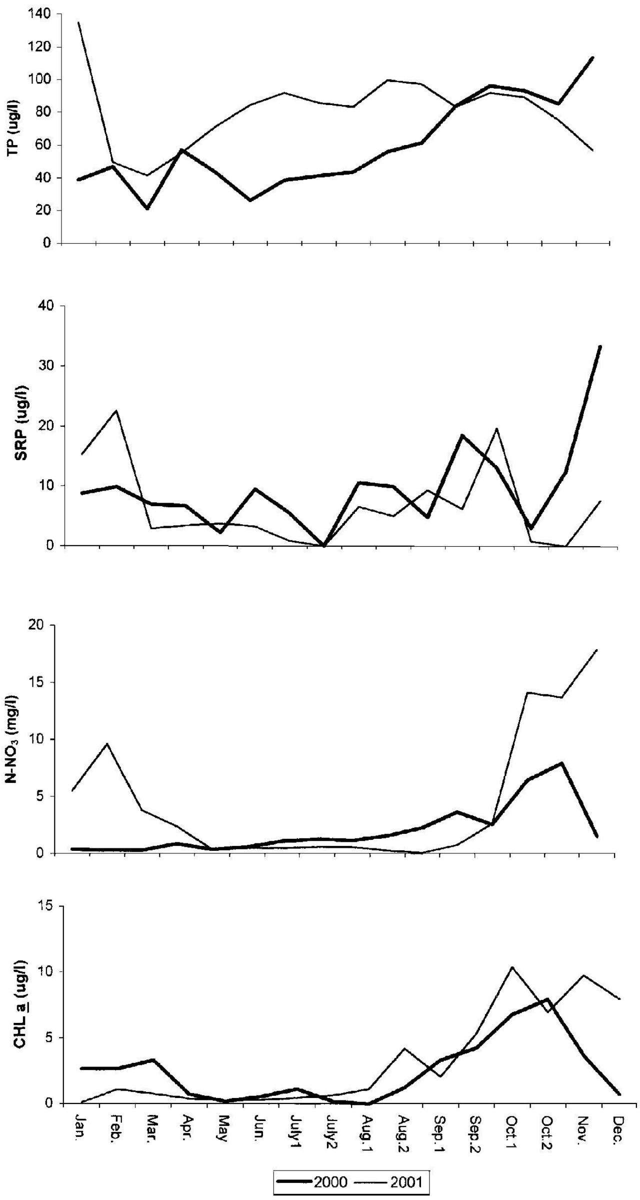

Figure 3. Variation of TP, SRP, N-NO_3 and CHL *a* concentrations in S. Serrada Reservoir for the period of study.

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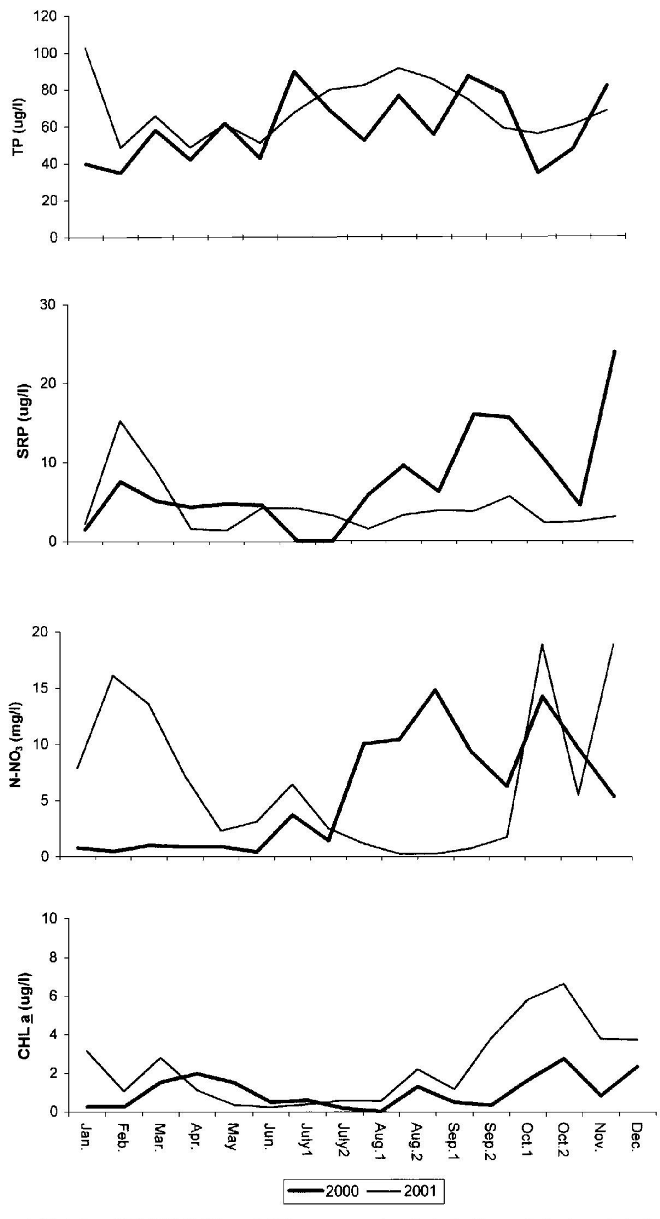

Figure 4. Variation of TP, SRP, N-NO_3 and CHL *a* concentrations in Azibo Reservoir for the period of study.

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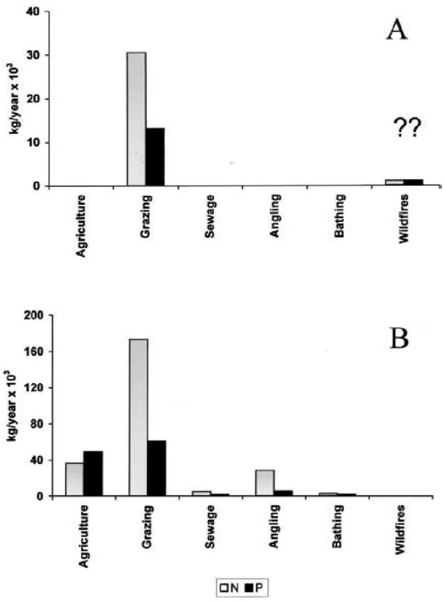

Figure 5. Potential allochthonous sources of nitrogen and phosphorus to S. Serrada (A) and Azibo (B) reservoirs (?? means 'non quantified source').

of herbivory, thus be controlled by zooplankton. Nevertheless, eutrophication is, by definition, an increase in phytoplankton biomass, reflected on chlorophyll concentrations, which reinforces interpretation of the chlorophyll component of the index: Following that, it is most correct to consider both reservoirs as oligo- to mesotrophic during the study period, instead of considring them as meso- to eutrophic.

The potential allochthonous sources of phosphorus and nitrogen are presented in Figure 5. In S. Serrada, grazing can contribute 30 450 kg of N and 13 050 kg of P per year. Wildfires can also contribute substantial loads. However, for this region there are no data allowing quantification of this source. In Azibo trophic state is possibly influenced by agriculture, grazing, sewage, as well as by angling and bathing. Agriculture can contribute 36 394 kg of N and 49 192 kg of P per year, grazing 172 956 kg of N and 60 786 kg of P per year and sewage 4927 kg of N and 1752 Kg of P per year. Angling and bathing can contribute 28 080 kg of N and 5184 kg of P and 900 kg of N and 41.5 kg of P per year, respectively.

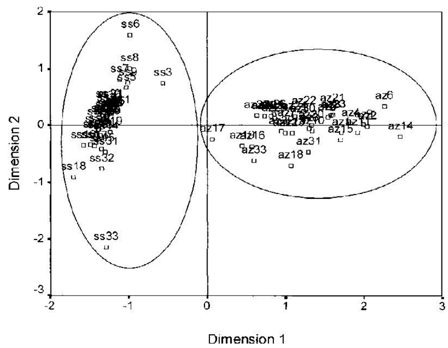

Figure 6. Results of MDS ordination (ss – S. Serrada samples; az – Azibo samples).

Results of MDS are depicted on Figure 6. In this kind of diagram, sample to sample dissimilarity is represented by the distance between points. Thus, the gradation in the spread of the points indicates the existence of two groups: One formed by samples obtained at S. Serrada and one formed by those obtained at Azibo. This means that there is an inter-reservoirs variability related to some of the studied parameters. In fact, according to Kolmogorov–Smirnov test, nutrient and CHL *a* concentrations showed no significant differences between reservoirs in spite of differences in age, landscape occupation, water use and exposure to different degrees of disturbance. However, differences between reservoirs were found for conductivity ($D_m = 1$; $P < 0.05$), water temperature ($D_m = 0.364$; $P < 0.05$), pH ($D_m = 0.758$; $P < 0.05$), transparency ($D_m = 0.455$; $P < 0.05$) and water colour ($D_m = 0.643$; $P < 0.05$).

Discussion

The values of the studied water quality parameters were similar for both reservoirs in spite of different age, landscape occupation, water use patterns and exposure to different factors of disturbance. The observed differences in water temperature, conductivity and pH might be the result of the synergistic effect of reservoir altitude and geological zone. Age may be in the origin of the high water colour obtained for S. Serrada Reservoir. According to Cuthbert & Giorgio (1992) humic compounds are products of plant decomposition and their concentration in the water can be measured by the determination of water colour. Thus, the obtained water colour values might indicate the existence of high amounts of products of terrestrial vegetation decomposition. In fact, the inundated area was previously a permanent meadow fertilised every year for potato crop production and the existing vegetation was not removed before inundation. Consequently, because of decomposition of the terrestrial vegetation, which can take several years, and because of the use of the land before creation of the reservoir, certainly large amounts of nutrients were and are being released to the water column. The almost total hypolimnetic oxygen depletion during stratification (Geraldes, unpublished data) may also be a sign of the presence of important quantities of organic matter in decomposition at the bottom of the reservoir. Similar results were obtained for other new reservoirs (Pinel-Alloul & Méthot, 1984; Kimmel et al., 1988; Robarts et al., 1992). However, it looks like that factor does not play a preponderant role in the obtained results. Actually, S. Serrrada is a highly disturbed system if compared to other lakes located in similar geological and climate regions (e.g., Negro et al., 2000). There are two sorts of disturbance: one internal, because of water level fluctuation, and the other external, originating by the combined effect of grazing and fire. Furthermore, the areas of both reservoir and catchment are small. Thus, the intensity of use of water and grazing activity which could not have had a strong impact in systems surrounded by larger catchment areas, in this reservoir could have induced a severe reduction in water quality; the observed resuspension of bottom sediments and organic matter following water runoff input after the first rains, plus the turbulence generated by water level rising and the periodical exposure of littoral sediments to cycles of drying and wetting could explain the high phosphorus concentrations and consequently the found TSI (TP) and TSI (SD) values. This assumption is supported by the results obtained by Fabre (1988) and Watts (2000a,b) in reservoirs where water level fluctuation was accentuated as well. On the other hand, grazing is not only a source of nutrients but also the main cause of wild fires in this region, since those are induced by shepherds to obtain better graze. Actually, S. Serrada catchment is one of the areas with more fires per year in the Montesinho Natural Park (Rainha & Cabral, 2001). There are no studies quantifying the nutrient inputs to this reservoir as a result of the erosive impact of rainfall on post-burned soils. However, considering the high slope and the dominant soil type in this area, which, according to Agroconsultores & Coba (1991) and to Ministério do Ambiente (1995), bear a high potential risk of erosion, then high rates of soil erosion and consequently high surface runoff are expected. In addition, some research developed in other regions has shown that the consequences of a fire can be the increase in trophic state and the subsequent decrease of water quality in the adjacent water bodies. Those effects are more accentuated in sloped areas and after intense precipitation events (Walsh et al., 1992; Shakesby et al., 1993; Townsend & Douglas, 2000; Lange, 2001; Minshall et al., 2001).

In Azibo internal disturbance caused by water level fluctuation is minimal. Moreover, the areas of reservoir and catchment are larger than the corresponding ones for S. Serrada, landscape is patchy and fires are not frequent. However, other sources of disturbance such as agriculture, mainly the intensive cultures in reservoir shore, grazing and recreational activities, can be the cause for the obtained TSI (TP) values. Those were similar to the results obtained during the study performed in1987/1988 by Vasconcelos (1990). According to estimations of the potential allochthonous sources of nutrients, agriculture and grazing seemed to be the greatest sources of N and P in the Azibo catchment. The intensity of exportation of nutrients from those activities and from sewage seems to be highly seasonal; in the beginning of the wet season the nutrient concentrations in water runoff were higher than in the water runoff generated by end of season rains. The observed seasonality is corroborated by other authors (e.g., Johnes et al., 1996; Barbosa & Hvitved-Jacobsen,1999; McGarrigle et al., 2000; Rybak, 2000). Geraldes (unpublished data) also noticed that TP concentrations in samples obtained at the terminus of one of Azibo's tributary streams were by the time of first water runoffs (November 2000/December 2001) 103 μg l^{-1}, in February 2001 decreased to 76 μg l^{-1} and in March 2001 decreased again to 16 μg l^{-1}. However, agricultural and grazing sources of nutrients are expected to decrease within few years, since most of the farmers are more than 50 years old nowadays (INE, 1999, 2001) and there is a considerable tendency for human desertification because of the low rentability of agricultural practices. Besides, the landscape in this catchment is very patchy and consequently there are numerous buffer areas such as woodlands, meadows and riparian vegetation that can minimise those potential sources of nutrients. Thus, intensive agriculture practices in the reservoir shore and recreational activities in summer are or might become in a near future the main nutrient sources to Azibo Reservoir. In fact, the values of nutrient concentrations obtained in summer can be related to those activities, which are more intensive in this period. Another possible source of pollutants that can also affect water quality in Azibo is the highway IP4. According to Barbosa & Hvitved-Jacobsen (1999) the average concentration levels of Pb, Zn and Cu in the IP4 highway runoff are 10.8, 172 and 10.7 μg l^{-1}, respectively.

Considering the possible causes for the nutrient concentrations obtained in both reservoirs and the occurrence of a bloom of *Anabaena* sp. in Azibo during the study performed by Vasconcelos (1990), plus the dominance of this cyanobacterium in the phytoplankton community more recently at the end of summer 2001 (Geraldes, unpublished data), it can be concluded that there is a need for implementation of several measures to prevent water quality degradation. In S. Serrada the following measures should be taken: (1) managing shrub communities and providing information to shepherds in order to prevent frequent fires; (2) forbidding grazing on reservoir shore; (3) allowing vegetation growth at the shores of the reservoir; (4) providing information to population of Bragança to prevent water waste and, in doing so, avoid extreme water level fluctuations in the reservoir. For Azibo the following measures are suggested: (1) restriction of fertilisers, mainly in the intensive agricultural exploitations located at reservoir shore; (2) implementation of effective sewage treatment (at least secondary treatment) in small villages; (3) establishment of well defined areas for camping and other recreational activities; (4) assessment of the actual impact of the highway IP4 on water quality and, if necessary, implementation of infiltration ponds for pollutant retention (Barbosa & Hvitved-Jacobsen, 1999); (5) implementation of environmental education projects for both residents and tourists.

Complementarily to the suggested measures, some research work should be performed. There is a lack of data concerning, e.g., soil nutrient retention capacity and erosion rates. Such data are fundamental to develop export coefficient models adapted to these areas, allowing the correct estimation of nutrient and pollutant inputs, and to make possible the development of correct management measures for these reservoirs and their watersheds. Regular programs of water quality monitoring should also be implemented.

Conclusions

The absence of industrial activity and the low rentability of agriculture are leading Trás-os-Montes region to human desertification. Thus, the implementation of new activities such as rural tourism and ecotourism could be economic alternative to local populations. Both S. Serrada and Azibo reservoirs, as well as their watersheds, have high potential for the development of activities such as boating, fishing, pedestrian walks, hunting, nature watching and rural tourism. In order to improve and maintain water quality on multiple uses perspective it is necessary to promote the sustainable development of the surroundings and of the catchment areas of both reservoirs. To achieve this goal, it is necessary to elaborate and promote management plans that specify what and where activities mentioned above can be developed without jeopardising natural and cultural values. However, their efficacy will depend on co-operation between municipalities, conservation authorities, researchers and local inhabitants.

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ERRATA

Texto

Página 1, Coluna 1, Linha 2: acrescentar antes de Portugal **in**;
Página 2, Coluna 1, Linha 10: **determing** deve ler-se como **determining**;
Página 2, Coluna 2, Linha 15: eliminar **from lowlands**;
Página 3, Coluna 2, Linha 7: **physical** deve ler-se como **environmental**;
Página 6, Coluna 1, Linha 9: **reported** deve ler-se como **recorded**;
Página 6, Coluna 2, Linha 9: **reported** deve ler-se como **recorded**;
Página 9, Coluna 1, Linha 8: **considring** deve ler-se como **considering**;
Página 10, Coluna 2, Linha 38: **2001** deve ler-se como **2000**;

Referências

Página 12, Coluna 1, Linha 43: **1e** deve ler-se como **1a**;

Tabelas

Tabela 2, Coluna 1, Linha 1: **S. Serrada** deve ser mudada para a segunda linha e os dados referentes às variáveis ambientais devem ser apresentadas na terceira coluna;
Tabela 2, Coluna 1, Linha 7: **temperature** deve ler-se como **water temperature**;

Figuras

Figura 1: falta a escala;
Figura 2: No primeiro gráfico falta a letra **A**; no segundo gráfico falta a letra **B**.

FACTORS AFFECTING WATER QUALITY IN THREE RESERVOIRS SUBJECTED TO DIFFERENT DEGREES OF HUMAN INFLUENCE

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ABSTRACT

Three reservoirs of the River Douro Basin were studied during three years with the purpose of finding out whether different landscape occupation could influence water quality and trophic state. Seasonal variation of total phosphorus, soluble reactive phosphorus, nitrate, chlorophyll *a*, ammonium ion, ammonia gas, water temperature, water colour, dissolved oxygen, pH and transparency were assessed. Seasonal abundance of phyto- and zooplankton were also followed. Furthermore, the potential allochthonous sources of nitrogen and phosphorus were identified and estimated. Despite of reservoirs being subjected to different landscape occupation and distinct amounts of nutrient inputs, differences among them were only found for conductivity, water colour and water temperature. The exception was at the beginning of the study, when soluble reactive phosphorus and chlorophyll *a* concentrations were significantly higher in Peneireiro Reservoir. However, this reservoir was totally emptied to eliminate a bloom of *Anabaena* and *Microcystis* and after the refilling both soluble reactive phosphorus and chlorophyll *a* were similar to those recorded in the other reservoirs. According to Carlson's Trophic State Index, S. Serrada and Azibo reservoirs were classified as meso-eutrophic. Peneireiro was classified as eutrophic before emptying and as meso-eutrophic after refilling. Besides climate and geology, it seems that the strongest effects on those reservoirs were water residence time and reservoir area. Very short cycles of both watering/dewatering and large water residence time can contribute to the trophic state of those reservoirs. Besides, reservoir area is susceptible of influencing the magnitude of the potential impacts of nutrient inputs on water quality.

Key words: Reservoirs, water quality, trophic state, cyanobacteria, water residence time, management measures

INTRODUCTION

Reservoir intrinsic characteristics are the result of the interactions between adjacent terrestrial and aquatic ecosystems. Catchment features such as area, geology, soil, slope, morphology, precipitation and land occupation strongly condition water level fluctuations/ water residence time and nutrient concentrations in reservoirs. Pressure caused by human activities in the catchment area and in reservoir vicinity generally changes landscape cover (e.g. deforestation, degradation of riparian vegetation), leading to an intensification of surface runoff during rain events (Harper, 1992; Johnes *et al.*, 1996; Rybak, 2000 Wetzel, 2001). The potential impacts of the external nutrient loading on trophic state and on phyto- and zooplankton abundance/community structure depend on water residence time and on the reservoir area (Schindler, 1987; Toja *et al.*, 1995; Reynolds *et al.*, 1998; Sakamoto & Okino, 2000).

The present study was carried out on three reservoirs located at the Portuguese part of the Douro catchment basin, in Trás-os-Montes region (NE Portugal). The objective of the study was to find out whether different landscape occupation could affect water quality and trophic state in these reservoirs. Total phosphorus, soluble reactive phosphorus, chlorophyll *a*, nitrate, ammonium ion and ammonia gas, as well as water colour, water temperature, dissolved oxygen, conductivity and transparency, were measured. Furthermore, reservoir trophic state was assessed according to Carlson (1977), and phyto- and zooplanktonic communities were characterised. Potential allochthonous sources of phosphorus and nitrogen were identified and estimated.

STUDY AREA

Location, plus morphological and hydrological characteristics of the studied reservoirs are shown in Table 1. S. Serrada Reservoir was filled for the first time in 1995 for water supply (34 750 inhabitants, INE 2001) and to generate hydroelectric power. As a result of these uses, accentuated water level fluctuations (between 8 and 10 m) occur. The maximum level phase is reached during winter, whereas the minimum level is attained in August or September. Water level fluctuation pattern is similar every year. Thermal stratification is in general observed from June to August/beginning of September. Disruption of stratification is coincidental with the lowest water level. Azibo Reservoir was filled for the first time in 1982 and it is used mainly for recreation. Other uses are water supply and irrigation, but those are not significant and water level fluctuations do not occur or are of very small amplitude. Thermal stratification occurs from June to October. Peneireiro Reservoir was filled for the first time in 1973 mainly for urban supply (7 760 inhabitants, INE 2001). Direct human influence is considerable all over the year. The surroundings of the reservoir are used for camping and for jogging. Annual water level fluctuations vary between 3 to 4 m. Because of frequent repairs the reservoir was always below its maximum capacity during 2000. Thermal stratification was never detected during the period of study. However, in summers 2001 and 2002 an accentuated decrease of temperature and dissolved oxygen concentrations were recorded close to the bottom of the reservoir. Cyanobacteria blooms are very frequent. During the period of study this reservoir was completely emptied after August 2000 to eliminate a bloom of *Anabaena* and *Microcystis*. During this process, the upper layer of the sediments was also removed with the objective of reducing nutrient availability and consequently algal biomass in the future. This reservoir only became fully filled in April 2001.

S. Serrada catchment area is occupied by scrubs of *Erica* spp., *Genista* spp. and *Chamaespartium* sp. There are no villages, there has been no agricultural activity in the region for approximately 20 years and recreational activities are not significant. However, in the catchment basin grazing can be very intense in summer. For most of the year there are only about 200 sheep grazing, but from May to August about 5 000 sheep are transported from the surrounding lowlands to graze in reservoir surroundings and in the catchment. As a consequence of grazing this area is often subjected to wild fires that are mainly induced by shepherds to obtain better graze. Azibo catchment area is occupied by meadows (28.9%), woodland and scrub (21.0%) and extensive agriculture (50.1%). The main crops are olives (29.4%), chestnuts (29.1%) cereals (24.4%), vineyards (6.4%) and potato (3.8%). Extensive grazing also occurs in this basin. There are 5 766 sheep and goat, 494 cattle and 531 pig (INE, 1999). In Azibo catchment there are several small villages. The total of inhabitants is around 1 500 (INE, 2001). In addition, during summer about 10 000 people use the reservoir for swimming, camping and boating. Angling is also an important activity, performed by about 1 000 people. Peneireiro catchment area is occupied by agricultural fields (80.7%) and meadows (19.3%). The main crops are olives (52.0%), orchards (20.8%) and vineyards (15.3%). Extensive grazing also occurs in this basin. There are 925 sheep and goat (INE, 1999). In the catchment area there are 914 inhabitants (INE, 2001).

In the region where reservoirs are located, the climate is continental, with warm, dry summers and long, cold winters. However, because of the differential degree of influence of Mediterranean climate and altitude, mean annual temperature and total annual precipitation differ among reservoirs. The sub-climatic region where S. Serrada is located is characterised by a total annual precipitation over 1300 mm and a mean annual air temperature below 8ºC. The sub-climatic region where Azibo is located is characterised by a total annual precipitation between 800 and 1 000 mm and mean annual air temperature between 12.5ºC and 14ºC. Peneireiro is located in a sub-climatic region where mean annual temperature is above 14 ºC and total annual precipitation is below 400 mm.

METHODS

Water samples were collected in January (winter), April (spring) and in August (summer) during 2000, 2001 and 2002. No samples were obtained in January 2002. In each reservoir there was one single sampling station, located at maximum depth. Water samples were obtained from the upper 30-40 cm stratum, from mid water column (5-10 m) and from the bottom. In Peneireiro during 2000, because of the low depth, samples were only taken at surface. Samples were placed into acid rinsed bottles and were transported to the laboratory in a cold container. Soluble reactive phosphorus (SRP) was estimated by the method of Murphy & Riley (1962) and total phosphorus (TP) concentrations were assessed after acid hydrolysis with persulfate for 60 minutes under high temperature and pressure (APHA, 1989). Average chlorophyll *a* (CHL *a*) concentrations were calculated from samples taken with a van Dorn bottle at 2 m intervals in the euphotic zone (the extension of the euphotic zone was considered to be about twice the Secchi depth). CHL *a* concentrations were determined spectrophotometrically after overnight extraction in 90% acetone. Water colour was determined according to Cuthbert & Giorgio (1992) method. Environmental variables such as water temperature, dissolved oxygen, conductivity, pH, as well as nitrate ($N\text{-}NO_3$), ammonium ion ($N\text{-}NH_4$) and ammonia gas ($N\text{-}NH_3$) were measured *in situ* with a 6820 YSI Multiparameter Water Quality Monitor in the upper 30-40 cm stratum, in mid water column and in the bottom. Water transparency was determined as Secchi disk depth (20 cm diameter black and white disk). Carlson's Trophic State Index (TSI) (Carlson, 1977) was computed from Secchi disk transparency (TSI (SD)), TP concentrations (TSI (TP)) and CHL *a* concentrations (TSI (CHL)). Potential allochthonous sources of phosphorus and nitrogen were identified and estimated according to the methodology described in Geraldes & Boavida (2003). Zooplankton samples were obtained on each sampling date by taking two vertical hauls (15 m long or maximum reservoir depth) using a Wisconsin type net of 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 and identified to species in 5, 10, 20 ml sub-samples or in total sample. For phytoplankton analysis water samples were collected with a van Dorn bottle at 2 m intervals in the euphotic zone in each sampling date. Samples were fixed *in situ* in Lugol's solution. Phytoplankton were counted according to Utermöhl (1958), using an inverted microscope at 200X/400X magnification. Algae were identified to genus.

To identify the differences among studied reservoirs, considering environmental variables, Multidimensional Scaling (MDS) ordination method was performed. According to this method, samples are arranged in a continuum in such a way that those close together are similar and those which are far apart are dissimilar. The dissimilarity between samples was measured by the Euclidean distance computation. A z-scores transformation was used to standardise the environmental data. Complementarily to the multivariate analysis a Kruskal-Wallis test was performed to assess differences in studied variables among reservoirs. Cluster analysis (UPGMA method, Euclidean distance measure) was performed to assess differences among reservoirs in what concerned phyto- and zooplankton abundance and composition. Taxa abundance was log (x+1) transformed. All analyses were performed using SPSS 11.3 and SYSTAT 8.0.

RESULTS

Environmental variables

On MDS diagram, sample to sample dissimilarity is represented by the distance between points (Fig. 1). The gradation in the spread of points concerning the present results indicates the existence of four groups formed by: (1) samples obtained in S. Serrada (s1-s8); (2) samples obtained in Azibo (a1-a8); (3) samples obtained in Peneireiro before emptying (p1 - p3); (4) samples obtained in Peneireiro during and after refilling (p4-p8). This means that there is inter-reservoir variability related to some of the studied variables. According to Kruskal-Wallis test, differences among reservoirs were found for conductivity (χ^2= 17.6; $p<0.001$), for water colour (χ^2 = 6.6; $p<0.05$) and for temperature (χ^2 = 4.8; $p<0.05$) (Table 2). Despite of differences in landscape occupation and water use TP and $N\text{-}NO_3$ concentrations were similar among all reservoirs. An exception was only recorded for SRP

(χ^2 = 8.4; P<0.05) and CHL *a* (χ^2 = 7.9; P<0.05) concentrations, which were significantly higher in Peneireiro before emptying. However, during refilling and afterwards no significant differences were obtained for both variables (Figs. 2 and 3). Data concerning $N\text{-}NH_4$ and $N\text{-}NH_3$ were not considered in statistical analysis because the first variable always ranged between 0.0 and 0.1 mg/l and the second was always below detection limits.

In S. Serrada the main potential allochthonous source of nitrogen and phosphorus was grazing, which could contribute 30 450 kg of N and 13 050 kg of P per year. Wildfires can also contribute large loads. However, this source was not quantified because for this region there are no data allowing its estimation. In Azibo potential allochthonous sources of these nutrients were agriculture, grazing, sewage, angling and bathing. The total contribution of these sources could amount to 243 257 kg of N and 116 956 kg of P per year. In Peneireiro agriculture, grazing and sewage could contribute 29 647 kg of N and 34 263 kg of P per year. Thus, considering reservoir areas, potential loads are 1218 kg of N and 522 kg of P per year/ha in S. Serrada, 593 kg of N and 285 of P per year/ha in Azibo and 1976 kg of N and 2278 of P per year/ha in Peneireiro. Concerning the three components of Carlson's Trophic State S. Serrada and Azibo were classified as meso-eutrophic, whereas Peneireiro was classified as eutrophic before emptying and as meso-eutrophic during and after refilling (Table 3).

Biotic variables

Phytoplankton communities

During winter *Tabellaria*, *Cyclotella* and *Cryptomonas* dominated the phytoplanktonic community of S. Serrada. During spring and summer densities of most of all Chlorophyceae showed an accentuated increase. In summer 2002, *Staurastrum* was dominant. *Anabaena* was also present but in low densities (Table 4). In Azibo during winter no algal dominance was recorded. Conversely, during spring and summer phytoplankton was dominated by *Chlamydomonas* like cells and *Cyclotella ocellata* (Table 5). In Peneireiro during summer before emptying, *Anabaena* and *Microcystis* were dominant and in August/September 2000, a bloom of these species was found. During this period, genera such as *Selenastrum* and *Pediastrum* were also found. Just after refilling phytoplankton density showed an accentuated decrease. From summer 2001 afterwards *Cyclotella* and *Cryptomonas* densities increased to values similar to those observed in winter and spring 2000. However, during summer 2001 and mainly during spring 2002 *Anabaena* was detected in high densities again (Table 6). Minor inter-annual variation in phytoplankton composition and abundance was found in S. Serrada. Conversely, major inter-annual differences were observed in Azibo. In fact, samples obtained were not clearly grouped although summer 2000, as well as spring and summer 2002 made a well-defined group because of the high densities of *Chlamydomonas* like cells. On the other hand, the remaining samples obtained in this reservoir were grouped with samples from Peneireiro. Samples obtained in Azibo during summer 2001 and in Peneireiro during summer 2000 were placed at a greater distance from the other samples because of the high densities of *Cyclotella* and *Anabaena*/*Microcystis*, respectively (Fig. 7A).

Zooplankton communities

In S. Serrada zooplankton was dominated by Rotifera, except during winter and spring 2000 and during summer 2002 when the copepod *Tropocyclops prasinus* and nauplii became dominant. Cladocera was the least abundant taxon and the most common species was *Ceriodaphnia quadrangula* (Fig. 4). In Azibo Cladocera (*Daphnia longispina*, *Ceriodaphnia pulchella*, *Bosmina longirostris* and *Diaphanosoma brachyurum*) and Copepoda (*Copidodiaptomus numidicus*) were the dominant taxa (Fig.5). In Peneireiro the zooplankton community before emptying was dominated by nauplii of *Cyclops vicinus* (Copepoda). Other species observed during this period were *Chydorus sphaericus*, *Daphnia hyalina*, *Bosmina longirostris, Alona* spp. (Cladocera), *Asplanchna priodonta* and *Keratella cochlearis* (Rotifera). Rotifera became progressively more abundant after refilling. *Polyarthra* spp., *Asplanchna priodonta*, *Keratella cochlearis* and *Synchaeta* spp. were the most representative zooplanktonic taxa (Fig. 6). Inter-annual variability in zooplankton community was less accentuated than in phytoplankton community. Samples were grouped in four distinct groups: (1) samples obtained in Peneireiro during summer 2000, spring 2001, spring 2002 and summer 2002 when rotifers densities were higher; (2) samples obtained in S. Serrada during summer 2000, spring and summer 2001 and during spring and summer 2002; (3) samples obtained in Azibo during summer 2000, spring and summer 2001 and during spring and summer 2002; (4) samples obtained in all reservoirs when zooplankton densities were very low (Fig. 7B).

DISCUSSION

Despite of different landscape occupation the values obtained for studied environmental parameters were similar among reservoirs. The observed differences in water temperature and conductivity are the result of the effect of both reservoir altitude and geological zone location. Considering S. Serrada location and the low water residence time, it could be expected that this reservoir be oligo- or mesotrophic such as lakes and reservoirs located in similar geological and climate regions (Boavida, 2000; Negro *et al.*, 2000). However, in this reservoir periodical severe water level fluctuations occur and the surroundings are subjected to intense grazing and to frequent wildfires. Thus, the high TSI (TP) and TSI (SD) can be explained by: (1) Resuspension of bottom sediments and exposure of littoral sediments to periodical cycles of drying and wetting (e.g. Watts, 2000); (2) Grazing, which is not only a source of nutrients but also of organic matter; (3) Mineralization of terrestrial vegetation that was not removed when reservoir was first filled (Geraldes & Boavida, 2003). There are not studies quantifying the impacts of fire on S. Serrada water quality. However, data obtained in other regions have shown that one of the consequences of a fire can be the increase in trophic state (e.g. Walsh *et al.*, 1992; Shakesby *et al.*, 1993; Minshall *et al.*, 2001). Thus, the trophic state of this reservoir can also be influenced by the very frequent fires occurring in the catchment. In Azibo the amplitude of water level fluctuations is very small when compared to those occurring in S. Serrada. Besides, fires are not frequent and slope is lower than in S. Serrada catchment. In addition, the area of the reservoir is large and landscape in this catchment is very patchy, occurring numerous buffer areas such as woodlands and riparian vegetation. Consequently, it was expected that the potential impact of runoff was lower. However, TSI (TP) values are similar to those recorded in S. Serrada. The obtained values can be explained by the higher mean water residence time in Azibo. It is well known that the larger is water residence time, the higher are nutrient concentrations (Toja, 1990; Toja *et al.*, 1995; Kennedy, 1999). Besides, shallow areas of the reservoir are colonised by macrophytes. According to Kennedy & Walker (1990) the metabolic activity of these plants can increase nitrogen and phosphorus concentrations in the reservoir during periods of constant water level. Nutrient concentrations recorded in Peneireiro also seem to be related to mean water residence time, which is similar to that of Azibo. In addition, the catchment is mainly occupied by agricultural fields and the area of this reservoir is very small. Consequently potential impact of runoff can be intensified.

Contrarily to expectations CHL *a* values in S. Serrada and in Azibo were low. In S. Serrada low phytoplankton biomass can be explained by high reservoir instability caused by water level fluctuations. In fact, when the lowest water level was reached, CHL *a* values showed a tendency to increase, decreasing again with the first rains (Geraldes, per. obs.). In Azibo zooplankton was dominated by herbivorous species (Geraldes & Boavida, submitted). Thus, it is plausible to conclude that phytoplankton biomass is controlled by zooplankton. The high CHL *a* concentrations found in Peneireiro before emptying were related to a mixed bloom of *Anabaena* and *Microcystis*, which are not edible by zooplankton. The latter species was only detected in summer when $N\text{-}NO_3$ concentrations increased. Considering that *Anabaena* is favored when nitrogen concentrations are low, it was expected that after summer 2000 until the first autumn rains *Microcystis* became dominant. However, it was impossible to verify this assumption because by September the reservoir was completely emptied. The emptying of Peneireiro was decided by reservoir managers to eliminate both algae. Furthermore, the upper layer of the sediments was removed with the objective of reducing internal nutrient availability and consequently future algal biomass. In fact, after refilling, nutrient and CHL *a* concentrations decreased to the same levels of those found in S. Serrada and in Azibo. Similar trends were also mentioned for reservoirs submitted to the same management measures (Harper, 1992) or emptied for repair procedures (Marques & Boavida, 1993; Geraldes & Boavida, 1999). It seems that neither the phytoplanktonic nor the zooplanktonic community were significantly affected by emptying. In fact, in spite of the decrease in nutrient concentrations *Anabaena* was still found after refilling. Thus, it is plausible to conclude that spores were not totally removed with the sediments and the remaining ecological conditions were still favourable to the growth of *Anabaena*. Besides, most zooplanktonic species densities found after refilling were higher or similar those observed before emptying. The only change was an increase in rotifer densities during and after the refilling. This pattern was also observed by Crispim & Boavida (1993) during the refilling of a reservoir that was emptied for repairs located in Tagus Basin.

The intensity of the influence of landscape occupation on trophic state and consequently on water quality seems to be strongly dependent on water residence time and on reservoir area. Very short cycles of watering/dewatering associated to high grazing and fire intensity can explain the high values of TSI (TP) in S. Serrada. Conversely, large water residence time favours phosphorus retention rates in reservoirs (e.g. Kennedy & Walker, 1990; Kennedy, 1999) also leading to high TSI (TP). This effect is increased in Peneireiro because of the small area of this reservoir. Thus, complementarily to the management measures in cacthments and in reservoirs surroundings (e.g. managing shrub communities to prevent frequent fires, restriction of fertilisers use, establishment of defined areas for recreational activities) it is necessary to manipulate water residence time. In S. Serrada manipulation of water residence time can be achieved by preventing water waste and consequently avoiding extreme and sudden water level variations. The manipulation of water residence time in hypolimnion in Azibo, and near the bottom during the period of low oxygen concentrations in Peneireiro could contribute to the decrease in trophic state.

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Table 1 - Location and morphometric parameters of S. Serrada, Azibo and Peneireiro reservoirs.

	S. Serrada	**Azibo**	**Peneireiro**
Location	Latitude: 41º57' (N) Longitude: 6º46' (W)	Latitude: 41º32' (N) Longitude: 6º53' (W)	Latitude: 41º16' (N) Longitude: 7º9' (W)
Altitude (m)	1300	500	400
Geology	granitic bedrock	schistic bedrock	schistic bedrock
Watershed area (km^2)	6.7	89.0	2.5
Reservoir area (km^2)	0.25	4.10	0.15
Total capacity (m^3)	1680 x 10^3	54470 x 10^3	768 x 10^3
Maximum depth (m)	18	30	11
Mean depth (m)	6.72	13.2	5.1
Water residence time (yr)	0.36	2.22	2.00
Year of filling	1995	1982	1973

Table 2 - Minimum-maximum range of physical factors recorded for S. Serrada, Azibo and Penireiro reservoirs. *Data not obtained because of the low depth of the water in the reservoir during this period.

	2000	2001	2002
S. Serrada			
Water temperature (ºC)			
Surface	1.5-18.2	2.7-19.7	10.4-20.8
Mid water column	1.5-16.6	2.7-11.4	7.3-15.6
Bottom	1.5-16.6	2.7-10.2	7.2-11.4
Dissolved oxygen (mg/l)			
Surface	8.2-9.6	8.2-9.6	7.9-9.6
Mid water column	7.5-8.2	5.6-8.2	4.4-7.7
Bottom	5.3-8.2	0.2-8.2	1.6-7.7
pH			
Surface	6.3-6.5	6.1-8.2	8.0-8.3
Mid water column	6.3-6.5	6.4-7.3	6.0-7.8
Bottom	6.1-6.5	6.5-7.1	5.8-7.2
Conductivity (µS/cm)			
Surface	4.0-7.0	4.0-8.0	6.0-9.0
Mid water column	4.0-7.0	4.0-8.0	5.0-9.0
Bottom	4.0-7.0	3.0-27.0	5.0-21.0
Surface water colour (Pt units)	8.6-36.7	8.6-19.5	12.1-34.2
Water transparency (m)	2.5-3.0	3.0-4.0	1.5-3.0
Azibo			
Water temperature (ºC)			
Surface	5.8-21.6	8.1-23.8	14.6-23.3
Mid water column	5.8-14.9	8.1-17.1	9.1-18.0
Bottom	5.8-10.8	8.1-11.9	8.1-9.9
Dissolved oxygen (mg/l)			
Surface	7.5-10.3	8.1-11.8	9.2-9.4
Mid water column	8.3-10.3	3.3-11.8	7.4-7.7
Bottom	0.4-10.3	0.1-10.2	1.6-7.1
pH			
Surface	7.2-8.1	6.6-8.3	7.7-7.9
Mid water column	8.0-8.1	7.6-7.9	7.3-7.4
Bottom	7.0-8.1	7.4-7.8	6.2-7.1
Conductivity (µS/cm)			
Surface	51-80	50-65	56-69
Mid water column	51-66	46-57	49-59
Bottom	51-64	45-55	46-58
Surface water colour (Pt units)	6.1-9.1	4.1-8.2	1.9-4.0
Water transparency (m)	3.0-6.0	2.5-5.0	2.0-7.0
Peneireiro			
Water temperature (ºC)			
Surface	4.8-24.0	8.5-23.8	18.1-21.8
Mid water column	*	8.5-19.9	15.1-21.0
Bottom	*	8.5-13.7	12.6-13.6
Dissolved oxygen (mg/l)			
Surface	8.4-11.9	7.5-9.7	8.0-10.4
Mid water column	*	7.5-7.0	8.0-8.5
Bottom	*	1.6-7.5	1.4-8.5
pH			
Surface	6.7-9.7	6.2-7.9	7.7-8.0
Mid water column	*	7.9-7.0	7.7-8.3
Bottom	*	6.3-7.1	6.3-7.1
Conductivity (µS/cm)			
Surface	29-56	29-38	36-44
Mid water column	*	28-33	36-44
Bottom	*	29-34	29-44
Surface water colour (Pt units)	6.5-17.4	5.5-12.0	6.1-10.0
Water transparency (m)	*	2.0-6.0	3.0-4.0

Table 3- Maximum and minimum ranges of the three components of the Carlson's Trophic State Index. *Data not obtained because of the low depth of the water in the reservoir during this period.

	2000	2001	2002
S. Serrada			
TSI (TP)	60-63	62-75	65-67
TSI (SD)	44-47	40-44	44-52
TSI (CHL)	28-40	8-45	44-54
Azibo			
TSI (TP)	57-63	60-71	64-66
TSI (SD)	34-44	37-45	32-50
TSI (CHL)	28-40	17-45	33-46
Peneireiro			
TSI (TP)	65-82	67-82	61-69
TSI (SD)	*	34-42	42-54
TSI (CHL)	57-70	29-36	42-54

Table 4 - Phytoplankton densities in S. Serrada reservoir. (-) Absence; (1) 1-1000 cells/l; (2) 1001-10000 cells/l; (3) 10001-100000 cells/l; (4) 100001-1000000 cells/l; (5) >1000000 cells/l. Winter, spring and summer from each year are represented as "Win", "Spr" and "Sum", respectively. Years 2000, 2001 and 2002 are represented as 00, 01 and 02, respectively.

S. Serrada	**Win00**	**Spr00**	**Sum00**	**Win01**	**Spr01**	**Sum01**	**Spr02**	**Sum02**
Chlorophyceae								
Botryococcus	-	-	2	-	-	-	-	-
Chlamydomonas	-	-	3	-	-	-	-	-
Cosmarium	1	1	3	-	3	3	1	-
Crucigenia	1	-	2	2	1	2	2	-
Dictyosphaerium	1	2	2	1	1	2	3	2
Monoraphidium	2	1	2	-	3	-	3	2
Oocystis	1	-	-	-	1	-	2	-
Paulschulzia	-	-	-	-	1	-	2	-
Scenedesmus	-	-	2	-	-	-	1	1
Staurastrum	1	1	2	1	1	1	2	5
Staurodesmus	-	-	2	-	-	1	2	2
Tetraedron	-	-	-	-	-	1	-	1
Bacillariophyceae								
Tabellaria	1	1	2	3	2	3	2	2
Other pennate diatoms	-	-	1	1	2	2	1	2
Cyclotella	2	1	2	2	1	3	2	2
Cyanophyceae								
Anabaena	1	1	2	-	2	2	2	2
Merismopedia	-	-	-	-	-	2	2	2
Dinophyceae								
Peridinium	-	-	1	-	-	4	2	3
Cryptophyceae								
Cryptomonas	2	1	3	2	1	3	2	3
Chrysophyceae								
Dinobryon	1	1	3	-	1	4	4	-

Table 5 - Phytoplankton densities in Azibo reservoir. (-) Absence; (1) 1-1000 cells/l; (2) 1001-10000 cells/l; (3) 10001-100000 cells/l; (4) 100001-1000000 cells/l; (5) >1000000 cells/l. “Win”, “Spr”, “Sum”, “00”, “01”, “02” have the same meaning as in Table 4.

Azibo	**Win00**	**Spr00**	**Sum00**	**Win01**	**Spr01**	**Sum01**	**Spr02**	**Sum02**
Chlorophyceae								
Chlamydomonas	2	-	4	-	-	-	4	3
Cosmarium	1	-	-	-	-	3	-	-
Crucigenia	1	-	1	-	-	3	1	2
Dictyosphaerium	1	-	1	-	-	-	-	-
Monoraphidium	2	-	-	1	1	-	2	-
Oocystis	-	-	-	-	-	-	2	-
Scenedesmus	1	1	-	-	-	3	2	1
Staurastrum	1	-	-	-	-	-	1	-
Staurodesmus	-	-	-	-	-	-	-	1
Tetraedron	1	-	-	-	-	2	-	1
Volvox	-	1	-	1	1	1	1	-
Bacillariophyceae								
Asterionella	-	-	-	1	1	2	3	-
Fragilaria	1	-	-	1	1	2	-	-
Other pennate diatoms	1	1	1	1	1	-	2	1
Cyclotella	2	-	3	-	1	5	4	3
Cyanophyceae								
Anabaena	1	1	1	-	-	2	-	1
Oscillatoria	1	-	-	-	-	-	-	-
Dinophyceae								
Gymnodinium	-	1	1	-	-	-	-	-
Peridinium	-	-	-	1	-	-	-	-
Ceratium	1	1	1	1	1	2	2	3
Cryptophyceae								
Cryptomonas	1	2	1	-	1	3	2	2
Chrysophyceae								
Dinobryon	1	1	1	-	-	-	2	1

Table 6 - Phytoplankton densities in Peneireiro reservoir. (-) Absence; (1) 1-1000 cells/l; (2) 1001-10000 cells/l; (3) 10001-100000 cells/l; (4) 100001-1000000 cells/l; (5) >1000000 cells/l. "Win", "Spr", "Sum", 00, 01, 02 have the same meaning as in Table 4.

Peneireiro	**Win00**	**Spr00**	**Sum00**	**Win01**	**Spr01**	**Sum01**	**Spr02**	**Sum02**
Chlorophyceae								
Botryococcus	-	1	-	-	-	-	-	1
Cosmarium	-	-	2	1	1	1	-	1
Crucigenia	2	2	-	1	-	-	2	-
Dictyosphaerium	-	-	3	-	-	1	-	-
Monoraphidium	2	4	3	2	-	-	2	-
Oocystis	1	-	-	-	-	-	-	-
Pediastrum	-	-	3	-	-	-	-	-
Scenedesmus	1	3	3	1	1	1	2	2
Selenastrum	-	-	4	-	-	-	-	-
Staurastrum	-	-	-	-	-	1	-	-
Tetraedron	1	2	2	1	-	-	1	2
Bacillariophyceae								
Asterionella	-	-	-	-	-	-	2	-
Fragilaria	-	-	-	1	-	1	-	-
Other pennate diatoms	2	2	2	1	-	1	2	2
Cyclotella	2	4	2	1	1	1	3	3
Cyanophyceae								
Anabaena	1	1	5	-	-	2	4	1
Microcystis	-	-	4	-	-	-	-	-
Dinophyceae								
Peridinium	-	-	-	-	-	-	2	-
Cryptophyceae								
Cryptomonas	1	3	3	1	-	1	2	3
Chrysophyceae								
Dinobryon	-	-	-	-	-	1	3	-

Figure captions:

Figure 1- Results of MDS ordination, considering all studied environmental variables (s - S. Serrada samples; a- Azibo samples; p- Peneireiro samples). 1, 2, 3 are samples obtained in winter, spring and summer 2000; 4, 5, 6 are samples obtained in winter, spring and summer 2001 and 7, 8 are samples taken in spring and summer 2002.

Figure 2 - Variation of TP (A), SRP (B) and $N\text{-}NO_3$ (C) concentrations in each reservoir.

Figure 3 - Variation of CHL *a* concentrations in each reservoir.

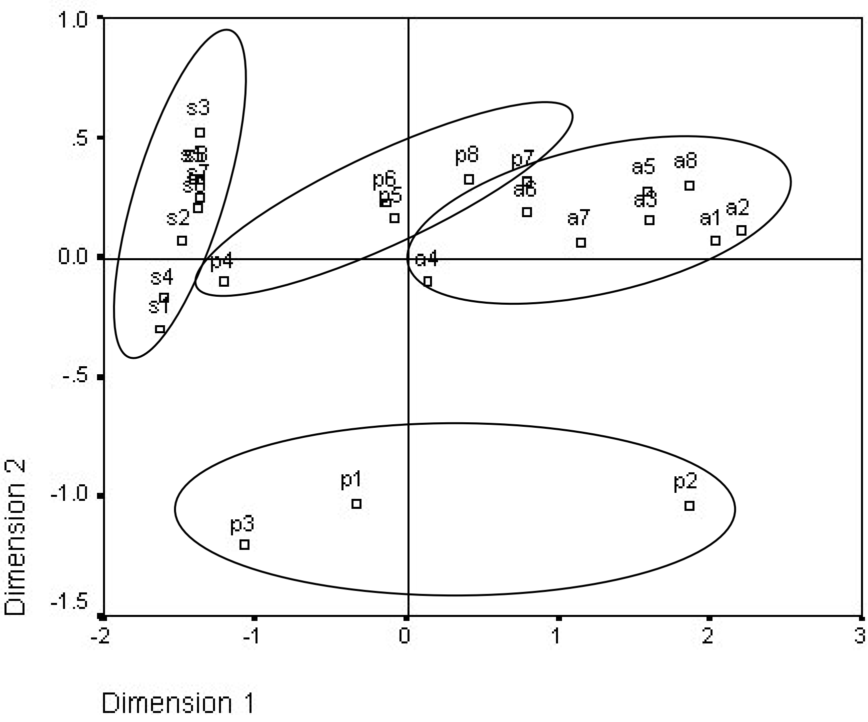
Figure 4 - Densities of the main zooplankton taxa found in S. Serrada.

Figure 5 - Densities of the main zooplankton taxa found in Azibo.

Figure 6 - Densities of the main zooplankton taxa found in Peneireiro.

Figure 7- Cluster analysis diagram depicting samples according to phytoplankton (A) and zooplankton (B) taxa densities. S, A, P and 1, 2, 3, 4, 5, 6, 7, 8 have the same meaning as in figure 1.

Figure 1.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p5
p8
p7
a6
a5
a8
a3
a7
a1
a2
a4
p1
p2
p3

Figure 2.

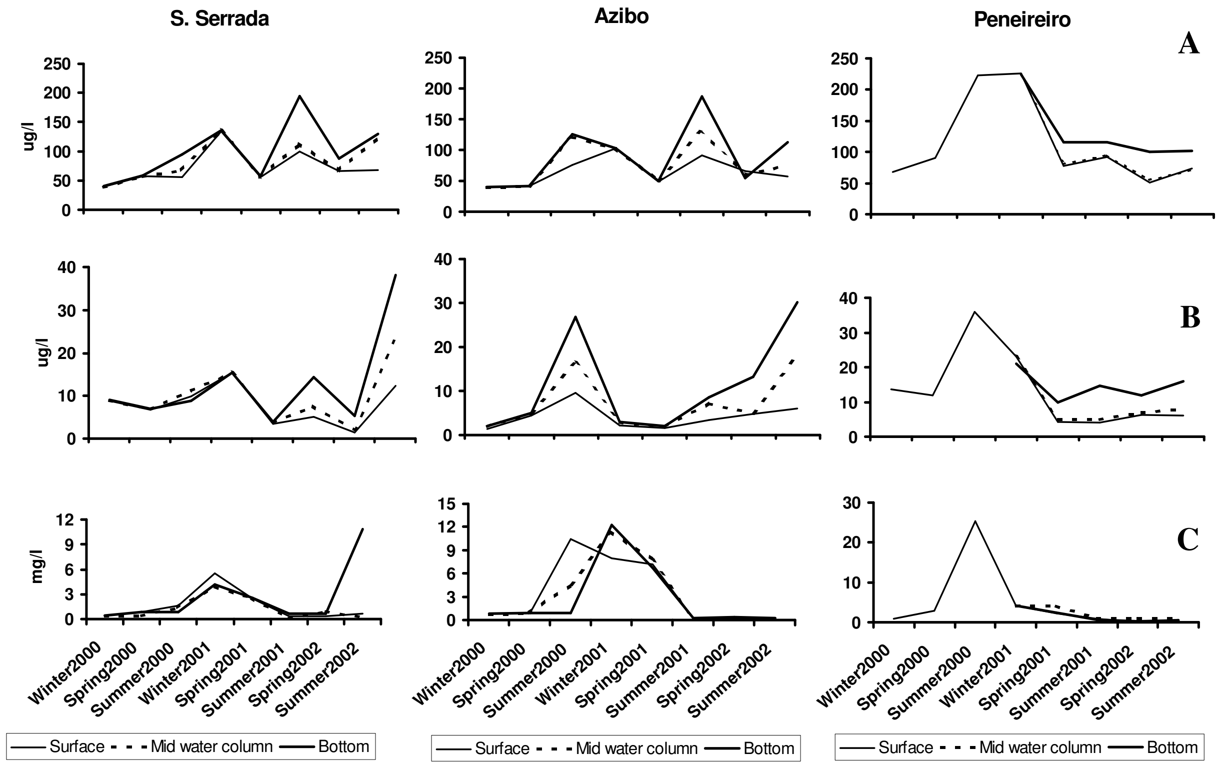

S. Serrada
Azibo
Peneireiro
A
B
C
ug/l
mg/l
250
200
150
100
50
0
40
30
20
10
15
12
9
6
3
Winter2000
Spring2000
Summer2000
Winter2001
Spring2001
Summer2001
Spring2002
Summer2002
Surface
Mid water column
Bottom

Figure 3.

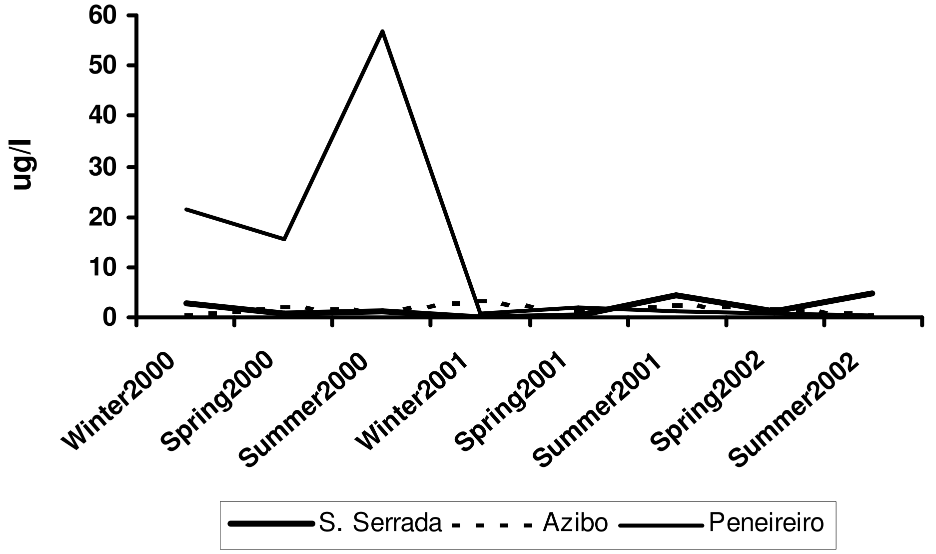

60
50
40
30
20
10
0
ug/l
Winter2000
Spring2000
Summer2000
Winter2001
Spring2001
Summer2001
Spring2002
Summer2002
S. Serrada
Azibo
Peneireiro

Figure 4.

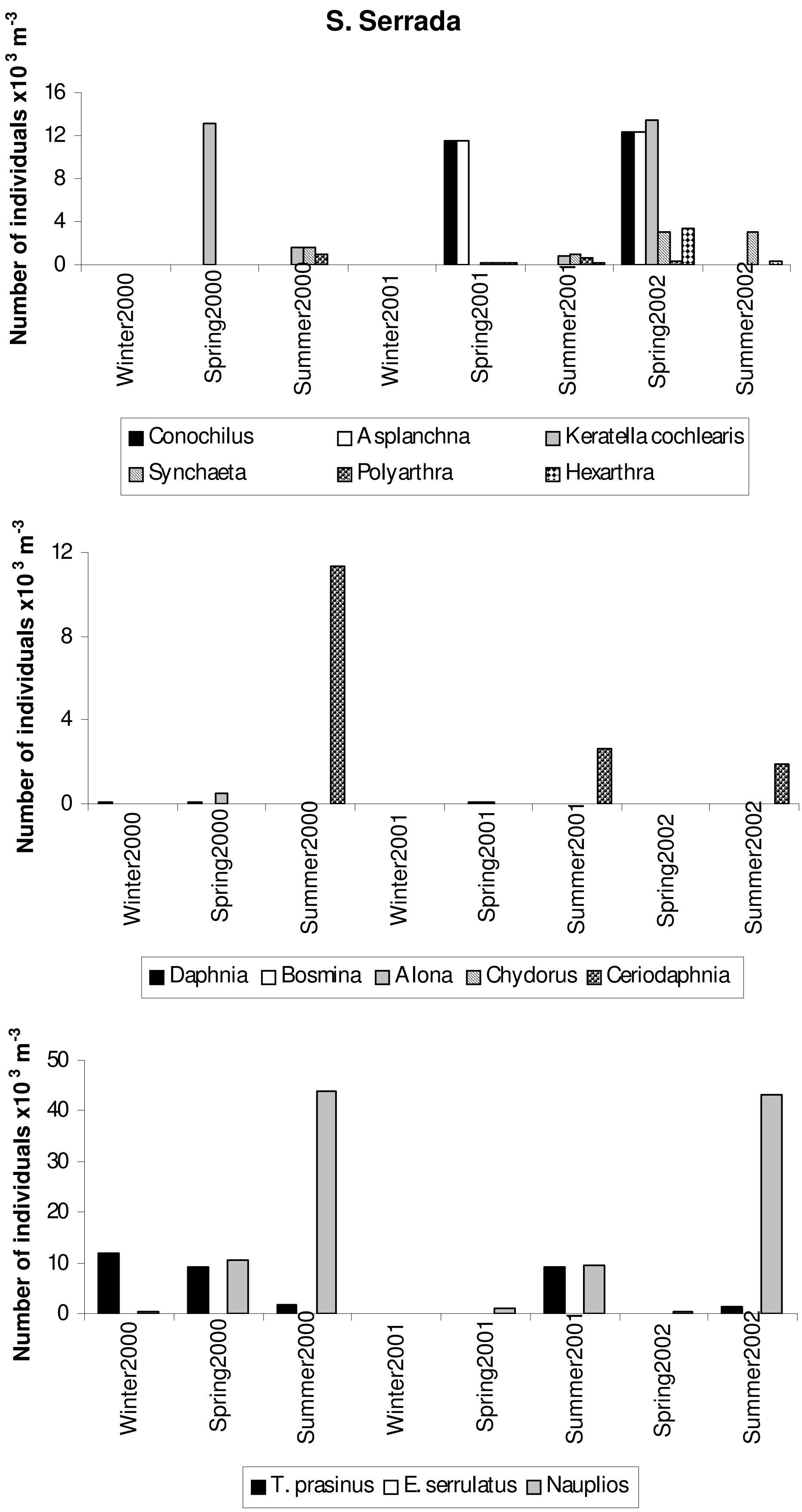
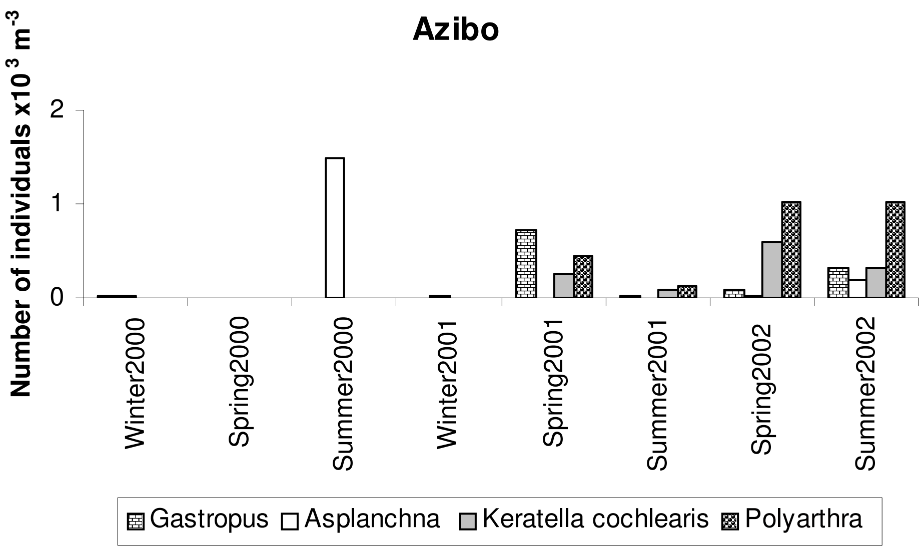
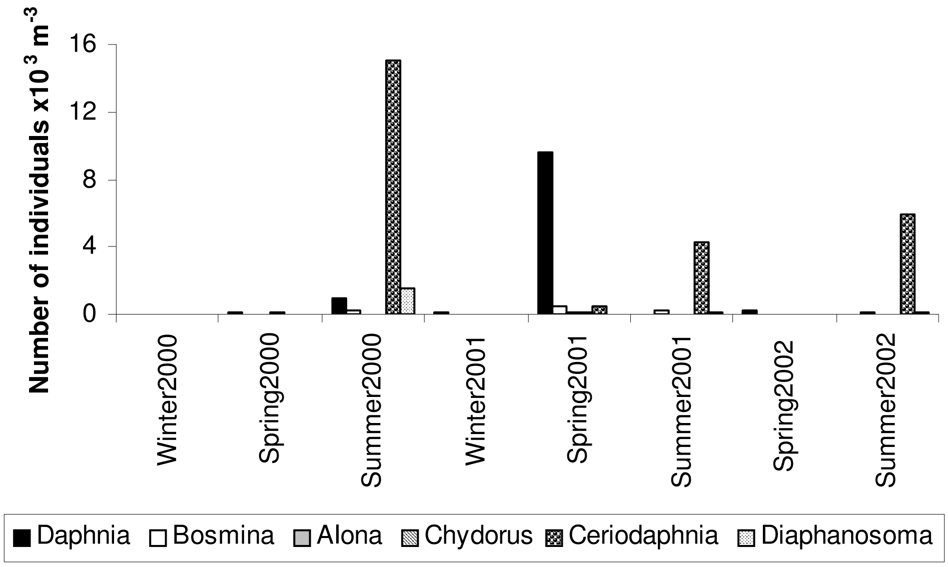

S. Serrada
Number of individuals x10³ m⁻³
16
12
8
4
0
Winter2000
Spring2000
Summer2000
Winter2001
Spring2001
Summer2001
Spring2002
Summer2002
Conochilus
Asplanchna
Keratella cochlearis
Synchaeta
Polyarthra
Hexarthra
Number of individuals x10³ m⁻³
12
8
4
0
Winter2000
Spring2000
Summer2000
Winter2001
Spring2001
Summer2001
Spring2002
Summer2002
Daphnia
Bosmina
Alona
Chydorus
Ceriodaphnia
Number of individuals x10³ m⁻³
50
40
30
20
10
0
Winter2000
Spring2000
Summer2000
Winter2001
Spring2001
Summer2001
Spring2002
Summer2002
T. prasinus
E. serrulatus
Nauplios

Figure 5.


Azibo
Number of individuals x10³ m⁻³
2
1
0
Winter2000
Spring2000
Summer2000
Winter2001
Spring2001
Summer2001
Spring2002
Summer2002
Gastropus
Asplanchna
Keratella cochlearis
Polyarthra


Number of individuals x10³ m⁻³
16
12
8
4
0
Winter2000
Spring2000
Summer2000
Winter2001
Spring2001
Summer2001
Spring2002
Summer2002
Daphnia
Bosmina
Alona
Chydorus
Ceriodaphnia
Diaphanosoma

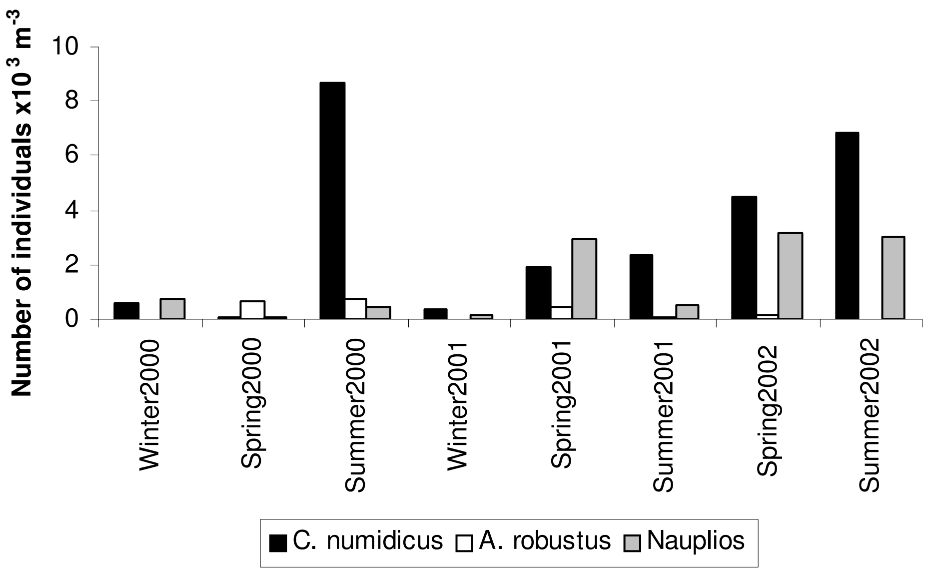
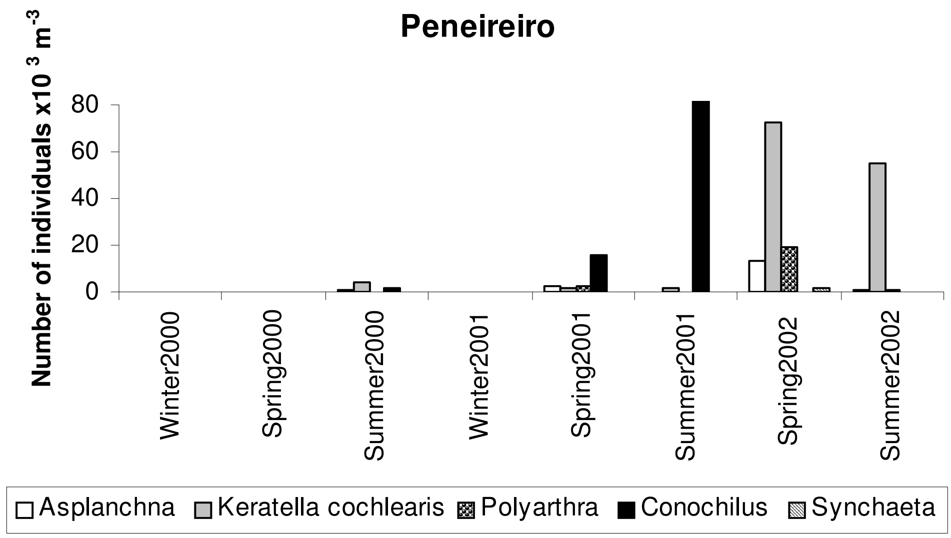
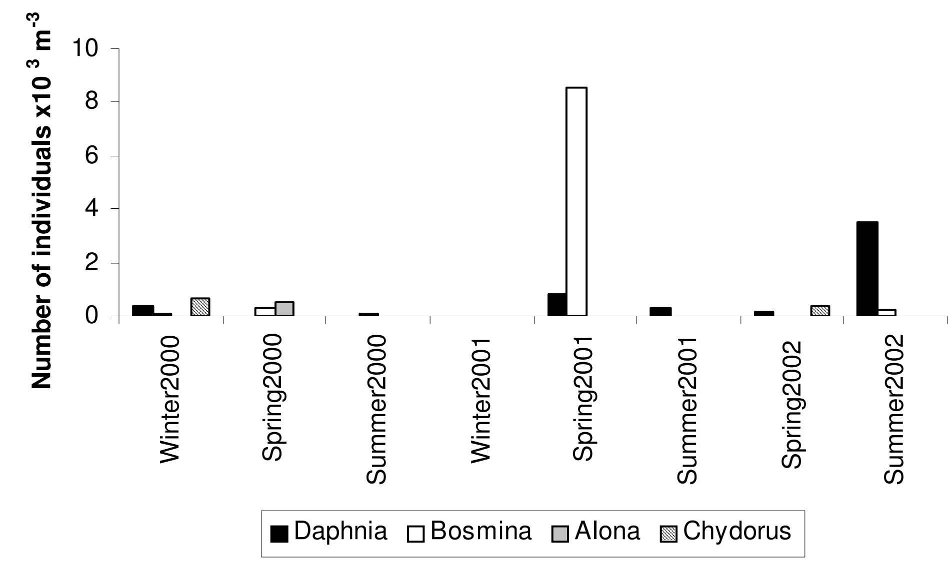

Number of individuals x10³ m⁻³
10
8
6
4
2
0
Winter2000
Spring2000
Summer2000
Winter2001
Spring2001
Summer2001
Spring2002
Summer2002
C. numidicus
A. robustus
Nauplios

Figure 6.


Peneireiro
Number of individuals x10 3 m-3
80
60
40
20
0
Winter2000
Spring2000
Summer2000
Winter2001
Spring2001
Summer2001
Spring2002
Summer2002
Asplanchna
Keratella cochlearis
Polyarthra
Conochilus
Synchaeta


Number of individuals x10 3 m-3
10
8
6
4
2
0
Winter2000
Spring2000
Summer2000
Winter2001
Spring2001
Summer2001
Spring2002
Summer2002
Daphnia
Bosmina
Alona
Chydorus

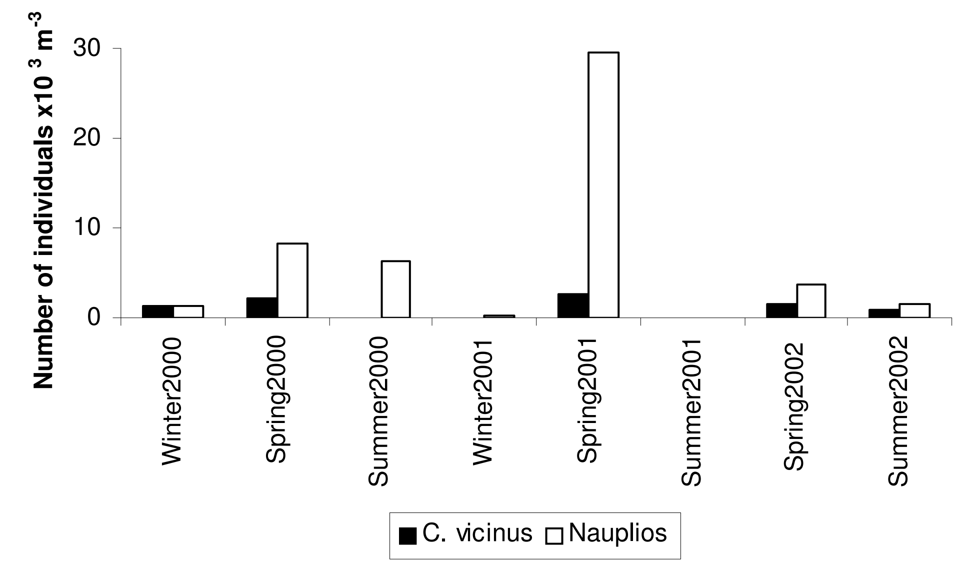
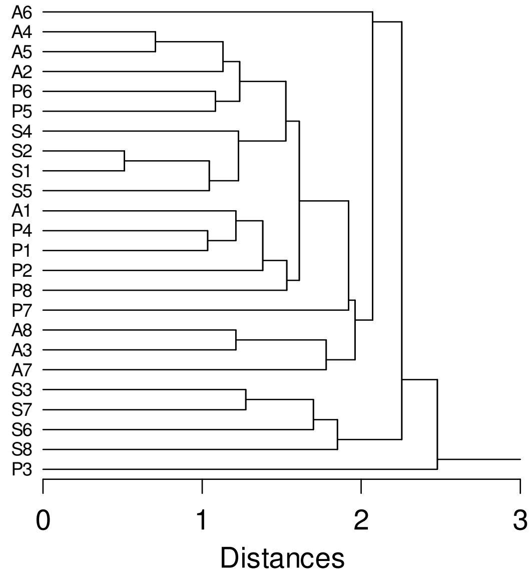
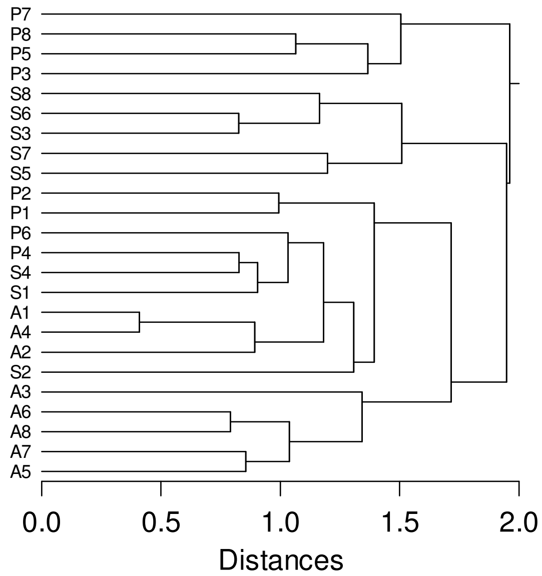

Number of individuals x10 3 m-3
30
20
10
0
Winter2000
Spring2000
Summer2000
Winter2001
Spring2001
Summer2001
Spring2002
Summer2002
C. vicinus
Nauplios

Figure 7.

A


A6
A4
A5
A2
P6
P5
S4
S2
S1
S5
A1
P4
P1
P2
P8
P7
A8
A3
A7
S3
S7
S6
S8
P3
0
1
2
3
Distances

B


P7
P8
P5
P3
S8
S6
S3
S7
S5
P2
P1
P6
P4
S4
S1
A1
A4
A2
S2
A3
A6
A8
A7
A5
0.0
0.5
1.0
1.5
2.0
Distances

.

3. EFEITOS DAS FLUTUAÇÕES DO NÍVEL DA ÁGUA NA LIMNOLOGIA DE UMA ALBUFEIRA

Artigo 3: Seasonal water level fluctuation: Implications for reservoir limnology and management.

Submetido para publicação.

SEASONAL WATER LEVEL FLUCTUATION: IMPLICATIONS FOR RESERVOIR LIMNOLOGY AND MANAGEMENT

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ABSTRACT

With the purpose of finding out whether seasonal water level fluctuations could affect water quality in a reservoir subjected to those changes, trends in environmental variables and in phyto- and zooplankton assemblages were analysed. Reservoir hydrological cycle was characterised by the following regime: (1) Maximum level phase, from January to the beginning of June; (2) Emptying phase, from mid June to the beginning of September; (3) Minimum level phase, from mid September to the beginning of the first autumn/winter rain events. The highest values of total phosphorus, soluble reactive phosphorus, nitrate, water colour and chlorophyll *a* were found during the minimum level phase. Phytoplankton assemblage was dominated by taxa typical of meso-eutrophic environments during emptying/minimum level phases. However, during the maximum level phase, taxa generally found in more oligotrophic systems were observed here also. Zooplankton assemblage was always dominated by Rotifera and small Cladocera species, which are specialists on small particle feeding. To reduce the nutrient and organic matter loading into the water column several management measures are proposed.

Key-words: Nutrients, phytoplankton and zooplankton assemblages, reservoirs, reservoir management, water level fluctuation.

INTRODUCTION

In reservoirs physical, chemical and biological features are influenced by seasonal surface-level fluctuations which are pretty much associated to anthropogenic utilization (Wetzel, 1990). Such fluctuations are more frequent in reservoirs located in regions where rain events are strongly seasonal, occurring in a very irregular regime. Besides, local human populations intensively use the water stored in most of those reservoirs for urban and hydroelectric power supply, irrigation and recreation. In spite of being currently believed that water level fluctuations exert influence on water quality, conditioning its use, remarkably few studies have been undertaken to understand the mechanisms by which those hydrological patterns may influence reservoir dynamics. The discussions one finds in the scientific literature mainly concern the effects of the filling process in brand new reservoirs (Kimmel et al., 1988; Masundire, 1992; Robarts et al., 1992; Garrido & Bozelli, 2000; Leitão & Léglize, 2000) or the effects of refilling in reservoirs that were totally emptied for repairs (Pinel-Alloul & Méthot, 1984; Boavida & Crispim, 1993; Crispim & Boavida, 1995; Marques & Boavida, 1993; Geraldes & Boavida, 1999). Studies focusing on the effects of periodical water level fluctuations are even more scarce and were mostly performed on Sicilian (e.g. Barone & Naselli-Flores, 1994; Naselli-Flores,1999) and on Australian (e.g. Watts, 2000) reservoirs. Consequently, there is a paucity of basic information required to underpin the correct management of the reservoirs subjected to periodic water level fluctuations.

S. Serrada Reservoir is located in the Iberian Peninsula, in the Portuguese part of the River Douro catchment. In this region precipitation occurs mainly in autumn and winter, but in a very irregular regime, with one wet winter usually followed by a few dry ones. Besides, this reservoir is subjected to intense water use for urban supply during the summer months. Thus, in a simplified way, the hydrological cycle of this reservoir was characterised by the following regime: (1) Maximum level phase, from January to the beginning of June; (2) Emptying phase, from mid June to the beginning of September; (3) Minimum level phase, from mid September to the beginning of the first autumn/winter rain events. Therefore, the objectives of this study were to examine how those water level fluctuations could affect: (a) total phosphorus, soluble reactive phosphorus, nitrate, and chlorophyll *a* concentrations; (b) water colour, water transparency, dissolved oxygen and conductivity; (c) phytoplankton and zooplankton assemblages. The ultimate aim of this study was to offer valid suggestions for reservoir management.

STUDY SITE

S. Serrada is located on granitic bedrock at an altitude of 1300 m a.s.l.. The total capacity of the reservoir, spreading over 25 ha, is 1680 x 10^3 m^3. Maximum depth is 17 m and mean depth is 6.7 m. In the region where this reservoir 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, but in a very irregular regime (Fig. 1). Direct human influence on S. Serrada impoundment is considered negligible. There are no villages, there has been no agricultural activity in the area for approximately 20 years and recreational activities are reduced to a minimum. However, in the catchment basin grazing can be very intense during the summer months. Consequently, this area is very often subjected to wild fires mainly induced by shepherds to obtain better graze. This reservoir was filled for the first time in 1995 for urban supply and to generate hydroelectric power. As a result of these uses, accentuated water level fluctuations occur. The annual range of water level variation is between 8 and 10 m (Fig. 2). Those variations are seasonal and related to precipitation events. Thermal stratification was observed from June to August/ beginning of September (Fig. 3) during this study period. Disruption of stratification was coincidental with the lowest water level. During the period of study this reservoir was considered meso-eutrophic. Further information concerning morphological and hydrological characteristics of this reservoir can be found in Geraldes and Boavida (2003).

METHODS

Samples were collected monthly in winter and biweekly in summer, from January 2000 to December 2002 at one single sampling station, located at the deepest point of the reservoir. Water samples for soluble reactive phosphorus (SRP) and total phosphorus (TP) determinations were obtained from the upper 30-40 cm stratum into acid rinsed bottles and were transported to the laboratory in a cold container. During the stratification period water samples were also taken from the middle water column (7 to 10 m) and from the bottom. SRP was estimated by the method of Murphy and Riley (1962) and TP was assessed after acid hydrolysis with persulfate for 60 min under high temperature and pressure (APHA, 1989). Environmental variables such as water temperature, dissolved oxygen, conductivity, pH, as well as nitrate ($N-NO_3$), ammonium ion ($N-NH_4$) and ammonia gas ($N-NH_3$) were measured in situ at 1 m intervals with a 6820 YSI Multiparameter Water Quality Monitor. Chlorophyll *a* (CHL *a*) was obtained from 500 to 1000 ml of integrated sample water taken from the euphotic zone and filtered through a Whatman GF/A filter no more than 2 h after collection. Concentrations were determined spectrophotometrically after overnight extraction in 90% acetone. Water colour was determined according to the Cuthbert and Giorgio (1992) method. Water transparency was measured with a 20 cm diameter black and white Secchi disk.

Zooplankton samples were obtained on each sampling date by taking two vertical hauls using a Wisconsin type net of 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 in 5, 10, 20 ml subsamples or in total sample. Animals were always identified to species/genus level. For phytoplankton analysis integrated water samples were collected from the euphotic zone. Samples were fixed "in situ" in Lugol's solution. Phytoplankton samples were counted according to Utermöhl (1958), using an inverted microscope at 200/400x magnification. Algae were identified to genus.

A Kruskal-Wallis test was performed for each environmental variable to determine whether mean values, obtained at maximum level phase, at emptying phase and at minimum level phase, were significantly different. This statistical analysis was performed using SYSTAT 8.0. To evaluate the association between taxa composition and environmental variables a Redundancy Analysis (RDA) was performed. A linear model of ordination was used, because a preliminary Detrended Correspondence Analysis (DCA) resulted in turnovers < 2 SD. According to Ter Braak (1995) this is the recommended criterion for choosing linear models of ordination instead of uni-modal models. Relative abundances of the most representative phytoplankton and zooplankton taxa were transformed to log (x+1). For the purpose of the statistical analyses, all pennate diatoms except *Tabellaria* were retained in a single category because all genera were present in low densities. The same procedure was adopted for *Keratella* species in the zooplankton community. Environmental variables showing skewed distributions were also log (x+1) transformed. In RDA, a forward selection procedure was used to add significant explanatory variables to the model. Those were added in the order of the greatest additional contribution to the total variance explained. Statistical significance in RDA was assessed by Monte-Carlo permutation tests (9999 permutations). The computer program CANOCO version 4.0 was used to perform both the preliminary DCA and the RDA.

RESULTS

Environmental variables

Mean TP, N-NO_3, water colour, conductivity and CHL *a* concentrations were the highest during minimum level phase. Conversely, water transparency was lowest during the same period. SRP mean concentrations were also slightly higher during this period than during the other months of the year. However, those differences were not statistically significant. TP, water colour, conductivity and CHL *a* decreased during maximum level phase. Mean N-NO_3 decreased during the emptying phase. N-NH_3 concentrations were always below detection limits (Table 1). During the period of study and depending on precipitation intensity, S. Serrada reached the maximum level phase in one or two weeks. Thus, at the beginning of maximum level phase TP, N-NO_3, water colour, and CHL *a* concentrations were high, decreasing afterwards.

Phytoplankton and zooplankton assemblages

Chlorophyceae, Bacillariophyceae, Cryptophyceae and Chrysophyceae were the most representative taxa in the phytoplankton community (Fig 4A). From all algae found in S. Serrada (Table 2), only the most abundant genera (Table 3) were considered in RDA. The forward variable selection approach to RDA revealed a strong contribution of water temperature ($P = 0.0001$), water colour ($P = 0.004$) and N-NO_3 ($P = 0.007$) to the observed significant association between phytoplankton assemblage and environmental variables (Monte-Carlo test; $P = 0.0001$). The ordination space defined by the first two RDA axes (Fig.5A) accounted for 80.5 % of species-environment relations and represented 23.6 % of the variation in species data. Distribution of sample scores in this ordination space reflected a clear distinction between maximum level phase, emptying phase and minimum level phase. *Monoraphidium*, *Cyclotella* and *Tabellaria* exhibited a tendency to increase when temperature and water colour were low and intermediate N-NO_3 concentrations were recorded. Consequently, their relative abundance was higher during the maximum water level phase. Conversely, *Dinobryon* seemed to prefer higher temperatures, low N-NO_3 concentrations and low water colour. Thus, the relative abundance of this genus was higher during the emptying phase. *Aulacoseira*, *Peridinium*, *Cosmarium* and *Anabaena* were associated to low N-NO_3 and high temperature. On the other hand, *Staurastrum*, *Staurodesmus Scenedesmus, Tetraedron* and *Crucigenia* were associated with high N-NO_3 concentrations, high water colour and intermediate temperatures. Thus, those taxa were more abundant during the minimum level phase. *Cryptomonas* occupied a central position in the ordination space, which might denote that this taxon has ecological flexibility to grow under a wide range of environmental conditions.

Rotifera was the most abundant taxon, except in summer and in autumn when Cladocera and Copepoda became dominant (Fig 4B). From all zooplankton species found in S. Serrada (Table 2) only the most representative were considered in RDA (Table 4). The forward variable selection approach to RDA revealed a strong contribution of temperature ($P = 0.005$), CHL *a* ($P = 0.007$), SRP ($P = 0.01$) and TP ($P = 0.03$) to the observed significant association between zooplankton assemblage and environmental variables (Monte-Carlo test; $P = 0.005$). The ordination space defined by the first two RDA axes (Fig.5 B) accounted for 84.2 % of species-environment relation and represented 25.4 % of the variation in species data. Distribution of sample scores in this ordination space reflected a clear distinction between maximum level phase and emptying/minimum level phases. In fact, distinction between the latter was not so clear. *Daphnia* was associated to lower temperatures and to maximum level phase. Conversely, *Ceriodaphnia* was clearly related to the emptying phase, when temperature was higher. *Conochilus* and *Asplanchna* were associated with low values of TP and CHL *a* (maximum level phase). *Polyarthra* abundance was also high during this period. However, in the ordination space, this species was associated to high concentrations of TP. In fact, the abundance of this rotifer was higher after strong rain events when TP concomitantly increased. *Gastropus*, *Keratella*, *Hexarthra*, *T. prasinus* and nauplii abundances were related to the increase in system instability and to the higher TP and CHL *a* concentrations (emptying and minimum level phases).

DISCUSSION

Environmental variables

The increase of TP, N-NO_3 and CHL *a* towards minimum level phase could have been a consequence of the increment of suspended particulate material in the water column, resulting from water turbulence generated during emptying plus the disruption of stratification at the end of this phase. Barone and Naselli-Flores (1994) and Naselli-Flores (1999) also observed an increase in phytoplankton biomass supported by the renewed availability of nutrients at the end of the emptying phase in Sicilian reservoirs subjected to severe water level fluctuations.

In S. Serrada an increase in water colour was also observed. This parameter is a measure of the concentration of humic compounds resulting from terrestrial plant decomposition (Cuthbert & Giorgio, 1992). Thus, high water colour might indicate the presence of large amounts of products of terrestrial vegetation under decomposition which were suspended into the water column. Moreover, the disturbance caused by sheep grazing activity and by the frequent fires seemed to be another source of organic matter (Geraldes & Boavida 2003). Nutrients and water colour decreased during maximum level phase. This phase was a period of water level stability. Consequently, sedimentation of particulate material could have been favoured (Böstrom et al., 1988; Wetzel, 2001). Another consequence of the disturbance caused by extreme water level fluctuation is the exposure of littoral sediments to cycles of drying and wetting, which might have implications on nutrient cycling, namely on phosphorus availability. This assumption is supported by the results obtained by Fabre (1988) and Watts (2000) in reservoirs where water level fluctuation was accentuated and where refilling took place over a short period of time. According to those authors, littoral sediments that are periodically exposed, experiencing cycles of drying and wetting, have less capacity to adsorb nutrients than those that remained inundated. Preliminary experiments based upon the experimental design developed by Watts (2000) showed that in S. Serrada the sediments that were periodically exposed released greater amounts of SRP after the addition of water than those that were permanently inundated (Geraldes unpubl. data).

Phytoplankton and zooplankton assemblages

During maximum level phase phytoplankton assemblage was composed of taxa strongly associated to low temperatures and generally found in: (1) nutrient poor environments – *Tabellaria*, *Cosmarium* and *Dinobryon* (Reynolds, 1998); (2) mesotrophic environments – *Cyclotella* (Lecointe et al. 1993); (3) enriched environments and/or transient phases of filling – *Monoraphidium* (Reynolds, 1999). However, phytoplankton assemblage structure could have been influenced by complex interactions of physical, chemical and biological factors. Thus, it is plausible to assume that *Daphnia*, which coincidentally reached the maximum abundance during this period, could have exerted some grazing control over edible algae, influencing phytoplankton assemblage (Lampert & Sommer, 1997). During both the emptying and the minimum level phase an increase in *Scenedesmus*, *Crucigenia* and *Peridinium*, together with the dominance of *Staurastrum*, were observed. Those species are typically found in meso-eutrophic environments (Reynolds, 1998) and some *Staurastrum* species are also common in reservoirs with large amounts of organic matter (Negro et al., 2000). However, the increase in TP and SRP concentrations seemed not to have had a significant influence on phytoplankton assemblage composition/structure. In fact, the concentrations of those phosphorus chemical forms were always high and preliminary experiments on phosphorus limitation seemed to indicate that this nutrient was not limiting (Geraldes unpubl. data). Changes in water temperature and the increase in N-NO_3 concentrations in the water column were probably in the origin of those shifts. Similarly to other reservoirs (Barone et al., 1991; Naselli- Flores & Barone 1994; Naselli-Flores, 1999), the reduction of light penetration in the water column caused by the increase of suspended particulate material, together with changes in zooplankton, could also have influenced the algal composition.

Specialists on small particle feeding, which food preferences are mostly detritus-bacteria and small phytoplankton, always dominated zooplankton assemblage in this reservoir. Most species, *Ceriodaphnia* and *T. prasinus* included, can only take particles smaller than 20 µm (Lampert & Sommer, 1997). Those assemblage patterns are typical of reservoirs subjected to periodical water level variations (Schmid-Araya & Zuñiga, 1992). According to those authors, the above mentioned species evidence adaptive advantage over larger Cladocera and Copepoda, not only on account of their feeding behaviour, but also because they are r-strategists, having shorter generations and a greater tolerance to large amounts of organic matter in the water. An exception was *Conochilus* which, according to Sladeček (1983), is typical of environments poor in organic matter. However, seasonal succession could have been related, not only to changes in environmental variables caused or not by water level variation, but also to biotic interactions. Examples could be the competitive interactions that might have occurred among *Ceriodaphnia* and some rotifers as well as shifts in phytoplankton assemblage (Lampert & Sommer, 1997). Since a wide range of physical and chemical complex interactions is likely to influence phytoplankton and zooplankton assemblages, it is necessary to be cautious when relating the observed changes to water level variations. In fact, some shifts in those assemblages could have been induced directly or indirectly by water fluctuations, but others could have been caused by factors varying seasonally, although independently of water level fluctuations (e.g. water temperature).

Management implications and concluding remarks

One could expect S. Serrada to be oligo- or mesotrophic because reservoirs and lakes located in similar climatic and geological regions of the Iberian Peninsula were generally found in those trophic conditions (Boavida, 2000; Negro *et al.*, 2000). However, S. Serrada is a highly disturbed system, if compared to those. The intense water use causes accentuated water level fluctuations, contributing to a great increase in nutrients and organic matter in the water column. The increase in the costs of water treatment supported by municipalities is a consequence of the degradation of water quality/ecosystem health caused by the increase in suspended particles in the water column. It is urgent to provide information to resident populations to prevent water waste. This measure would allow the maintenance of higher water levels during the summer months. Consequently, a smaller extension of littoral sediments would be exposed to cycles of drying and wetting and the amounts of suspended particulate material would be lower as well. Thus, the internal loading of nutrients and organic matter would be reduced. To reduce the external sources of nutrients and organic matter, measures to prevent frequent fires in the catchment area, to forbid sheep grazing in the reservoir shores and to allow vegetation growth at the shores of the reservoir should be implemented. Complementarily to the suggested measures, some research work should be performed. There is a lack of long term data concerning environmental variation patterns and their influence on biotic community dynamics. Furthermore, it is important to understand to what extent shifts in reservoir dynamics (mainly in biotic components) are induced by water fluctuations and/or by seasonal factors acting independently of water fluctuations. Such data are fundamental to develop predictive water quality models adapted to this particular area, rending possible the development of correct management practices in a multipurpose use perspective of the reservoir and its catchment.

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Table 1- Mean ± SD values of the environmental variables and minimum-maximum range for pH obtained for maximum level phase (1) emptying phase (2) and minimum level phase (3) and results of Kruskal-Wallis test. Variable abbreviations used in RDA are indicated between brackets.

Variables	**1**	**2**	**3**	**P**
Water colour (Pt) (Col.)	15.7 ± 7.7	18.0 ± 12.6	30.8 ± 11.5	**
Water transparency (m) (Secc.)	2.9 ± 0.9	2.9 ± 1.0	1.7 ± 0.4	**
Water temperature (ºC) (Temp.)				
Surface	9.3 ± 5.5	19.4 ± 1.3	10.2 ± 4.1	**
Middle water column	9.3 ± 5.5	16.3 ± 2.1	10.2 ± 4.1	**
Bottom	9.3 ± 5.5	11.1 ± 2.3	10.2 ± 4.1	NS
Dissolved oxygen (mg l^{-1}) (DO)				
Surface	8.6 ± 1.6	8.4 ± 0.9	8.6 ± 1.5	NS
Middle water column	8.6 ± 1.6	6.9 ± 2.0	8.6 ± 1.5	NS
Bottom	8.6 ± 1.6	3.2 ± 2.9	8.6 ± 1.5	**
Conductivity (µS cm^{-1}) (Cond.)				
Surface	6.0 ± 1.5	8.3 ± 0.8	8.1 ± 1.5	**
Middle water column	6.0 ± 1.5	8.5 ± 3.7	8.1 ± 1.5	**
Bottom	6.0 ± 1.5	14.6 ± 10.2	8.1 ± 1.5	**
pH (PH)				
Surface	6.9-7.4	5.4-8.1	7.0-8.5	NS
Middle water column	6.9-7.4	6.5-7.3	7.0-8.5	NS
Bottom	6.9-7.4	5.4-7.8	7.0-8.5	NS
N-NO_3 (mg l^{-1}) (NO_3)				
Surface	5.8 ± 8.7	0.9 ± 0.8	13.3 ± 15.2	**
Middle water column	5.8 ± 8.7	0.8 ± 1.1	13.3 ± 15.2	**
Bottom	5.8 ± 8.7	6.9 ± 10.3	13.3 ± 15.2	*
N-NH_4 (mg l^{-1}) (NH_4)				
Surface	0.2 ± 0.5	0.1± 0.2	0.4 ± 0.8	NS
Middle water column	0.2 ± 0.5	0.1± 0.2	0.4 ± 0.8	NS
Bottom	0.2 ± 0.5	1.0 ± 2.5	0.4 ± 0.8	NS
TP (µg l^{-1}) (TP)				
Surface	59.9 ± 25.3	70.1 ± 19.2	82.0 ± 11.4	**
Middle water column	59.9 ± 25.3	92.2 ± 25.7	82.0 ± 11.4	**
Bottom	59.9 ± 25.3	136.5 ± 35.1	82.0 ± 11.4	**
SRP (µg l^{-1}) (SRP)				
Surface	8.7 ± 7.5	6.6 ± 4.7	9.5 ± 7.2	NS
Middle water column	8.7 ± 7.5	9.7 ± 6.1	9.5 ± 7.2	NS
Bottom	8.7 ± 7.5	19.2 ± 13.0	9.5 ± 7.2	**
Chlorophyll *a* (µg l^{-1}) (CHL) Integrated sample	1.7 ± 1.3	1.9 ± 1.6	8.0 ± 4.7	**

* $p<0.05$; ** $p<0.01$; NS: not significant

Table 2- Composition of phytoplankton and zooplankton assemblages in S. Serrada.

Phytoplankton	Zooplankton
Chlorophyceae	**Rotifera**
Botryococcus	*Asplanchna priodonta*
Chlamydomonas	*Collotheca* sp.
Closterium	*Conochilus* sp.
Cosmarium	*Euchelanis* sp.
Crucigenia	*Gastropus* sp.
Dictyosphaerium	*Hexarthra* sp.
Gonium	*Keratella cochlearis*
Micrasterias	*Keratella quadrata*
Monoraphidium	*Keratella tecta*
Oocystis	*Ploesoma* sp.
Paulschulzia	*Polyarthra* sp.
Scenedesmus	*Synchaeta* sp.
Selenastrum	*Trichocerca* sp.
Staurastrum	**Cladocera**
Staurodesmus	*Alona* sp.
Tetraedron	*Alona costata*
Bacillariophyceae	*Alona quadrangularis*
Amphora	*Alona rectangula*
Aulacoseira	*Bosmina longirostris*
Cocconeis	*Ceriodaphnia quadrangula*
Craticula	*Chydorus sphaericus*
Cyclotella	*Daphnia longispina*
Cymbella	*Simocephalus* sp.
Gomphonema	**Copepoda**
Gyrosigma	*Eucyclops serrulatus*
Navicula	*Macrocyclops albidus*
Nitzschia	*Tropocyclops prasinus*
Tabellaria	Nauplii
Cyanophyceae	
Anabaena	
Chroococcus	
Merismopedia	
Dinophyceae	
Gymnodinium	
Peridinium	
Euglenophyceae	
Trachelomonas	
Cryptophyceae	
Cryptomonas	
Chrysophyceae	
Dinobryon	
Mallomonas	
Synura	

Table 3 - The most common genera of phytoplankton assemblage quantified using the mean (± SD) of the relative abundances: (1) maximum level phase; (2) emptying phase (3) minimum level phase. Taxa abbreviations used in RDA are indicated between brackets.

	1	2	3
Chlorophyceae			
Cosmarium (Cosm.)	5.02 ± 13.50	6.57 ± 18.09	1.04 ± 1.11
Crucigenia (Cruc.)	1.67 ± 2.04	1.61 ± 2.64	2.64 ± 4.53
Dictyosphaerium (Dict.)	4.96 ± 8.97	2.78 ± 3.46	6.59 ± 8.60
Monoraphidium (Mono.)	13.39 ± 20.76	1.93 ± 3.88	5.96 ± 7.54
Oocystis (Oocy.)	0.28 ± 0.57	0.22 ± 0.65	0.04 ± 0.11
Scenedesmus (Scen.)	0.62 ± 1.89	1.90 ± 4.04	2.63 ± 2.24
Staurastrum (Stau.)	5.21 ± 13.27	26.05 ± 38.86	35.29 ± 28.80
Staurodesmus (Star.)	0.33 ± 0.71	0.49 ± 0.79	3.16 ± 6.35
Tetraedron (Tetr.)	0.04 ± 0.12	0.79 ± 1.79	1.30 ± 2.28
Bacillariophyceae			
Aulacoseira (Aulo.)	0.22 ± 0.38	0.45 ± 0.57	0.09 ± 0.23
Cyclotella (Cycl.)	12.90 ± 16.71	6.74 ± 13.10	9.61 ± 11.07
Tabellaria (Tabe.)	8.81 ± 14.83	2.97 ± 2.83	2.54 ± 2.31
Other pennate diatoms (Pen.)	2.74 ± 7.79	3.33 ± 8.26	3.79 ± 5.83
Cyanophyceae			
Anabaena (Anab.)	2.47 ± 3.31	1.11 ± 0.97	2.72 ± 3.03
Dinophyceae			
Peridinium (Peri.)	0.31 ± 0.59	2.08 ± 5.30	0.04 ± 0.13
Cryptophyceae			
Cryptomonas (Cryp.)	20.12 ± 23.84	14.67 ± 12.30	22.20 ± 18.39
Chrysophyceae			
Dinobryon (Dino.)	20.90 ± 30.20	26.26 ± 28.98	0.36 ± 0.61

Table 4 - The most common taxa of zooplankton assemblage quantified using the mean (± SD) of the relative abundances during: (1) maximum level phase; (2) emptying phase (3) minimum level phase. Taxa abbreviations used in RDA are indicated between brackets.

	1	**2**	**3**
Rotifera			
Asplanchna (Aspl.)	20.89 ± 29.48	4.37 ± 8.92	6.03 ± 12.33
Conochilus (Cono.)	26.46 ± 33.55	2.59 ± 7.46	3.67 ± 6.40
Gastropus (Gast.)	0.21 ± 0.83	0.47 ± 1.72	0.35 ± 0.63
Hexarthra (Hexa.)	0.05 ± 0.15	1.08 ± 1.72	0.19 ± 0.60
Keratella (Kera.)	7.38 ± 14.04	16.82 ± 28.21	16.06 ± 24.05
Polyarthra (Poly.)	12.15 ± 25.64	1.15 ± 2.08	6.42 ± 10.30
Synchaeta (Sync.)	2.20 ± 4.70	2.63 ± 3.84	17.83 ± 19.74
Cladocera			
Ceriodaphnia (Cqua.)	6.97 ± 18.41	22.17 ± 24.89	7.71 ± 8.19
Daphnia (Dlon.)	4.94 ± 13.37	0.42 ± 0.76	1.90 ±2.31
Copepoda			
Tropocyclops (Tpra.)	6.82 ± 15.33	17.43 ± 22.30	26.76 ± 30.55
Nauplii (Naup)	11.93 ± 19.76	30.87 ± 28.53	13.00 ± 13.42

Figure captions:

Figure 1- Precipitation during the period of study in Bragança, the main city closest to S. Serrada (source: Agroclima Lab - ESAB) and mean values of the variable observed between 1961 and 1990 (source: IM, Institute of Meteorology).

Figure 2- Variations of water level in S. Serrada during the study period.

Figure 3- Seasonal temperature and dissolved oxygen profiles in S. Serrada.

Figure 4- Relative abundance (%) of the main Phytoplankton (A) and Zooplankton (B) taxa.

Figure 5 – RDA ordination diagram depicting the effects of environmental variables on Phytoplankton (A) and Zooplankton (B) assemblages. Circles are the scatters for samples taken at the maximum level phase; triangles are the scatters for samples taken at the emptying phase and squares are the scatters for samples taken at the minimum level phase. Dashed arrows are the species and solid arrows are the environmental variables evidencing significant correlations with the canonical axes. Codes for environmental variables are given in Table 1, codes for phytoplankton and zooplankton are given in Table 4 and 5, respectively.

Figure 1.

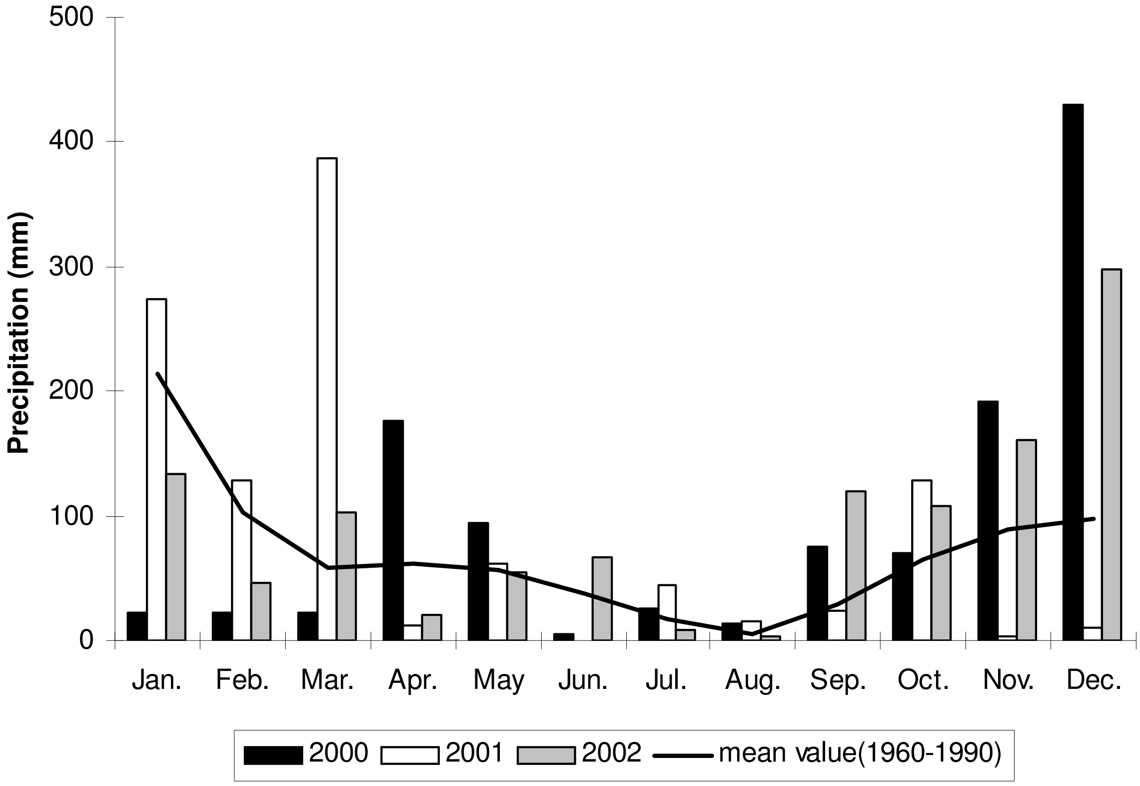

500
400
300
200
100
0
Precipitation (mm)
Jan.
Feb.
Mar.
Apr.
May
Jun.
Jul.
Aug.
Sep.
Oct.
Nov.
Dec.
2000
2001
2002
mean value(1960-1990)

Figure 2.

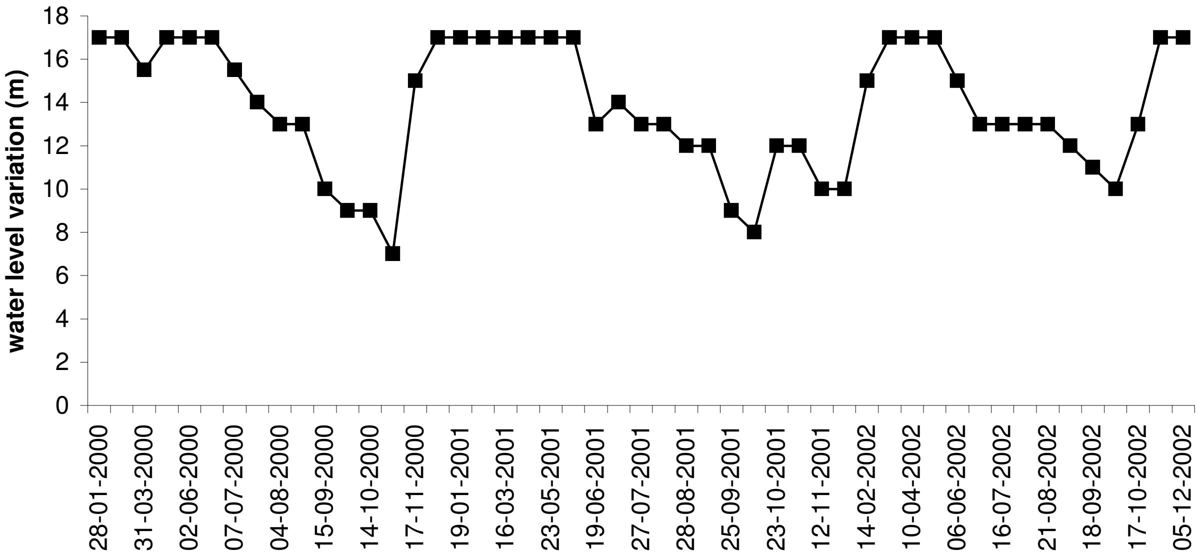
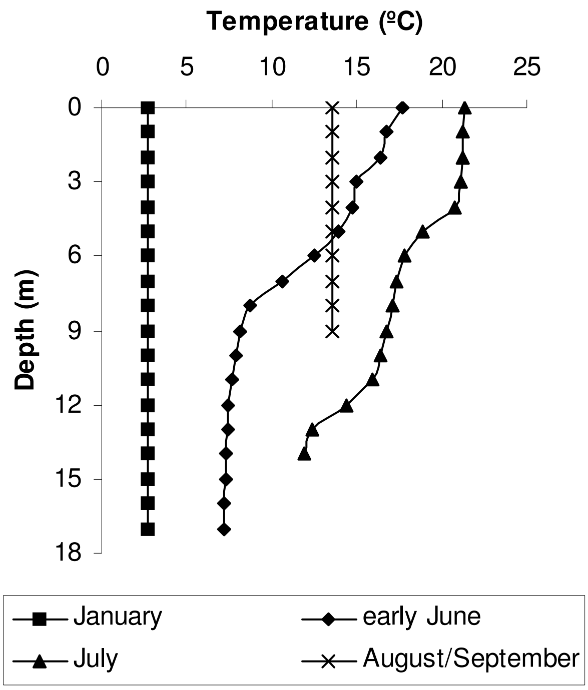

water level variation (m)
0
2
4
6
8
10
12
14
16
18
28-01-2000
31-03-2000
02-06-2000
07-07-2000
04-08-2000
15-09-2000
14-10-2000
17-11-2000
19-01-2001
16-03-2001
23-05-2001
19-06-2001
27-07-2001
28-08-2001
25-09-2001
23-10-2001
12-11-2001
14-02-2002
10-04-2002
06-06-2002
16-07-2002
21-08-2002
18-09-2002
17-10-2002
05-12-2002

Figure 3.


Temperature (ºC)
0 5 10 15 20 25
Depth (m)
0 3 6 9 12 15 18
January
early June
July
August/September

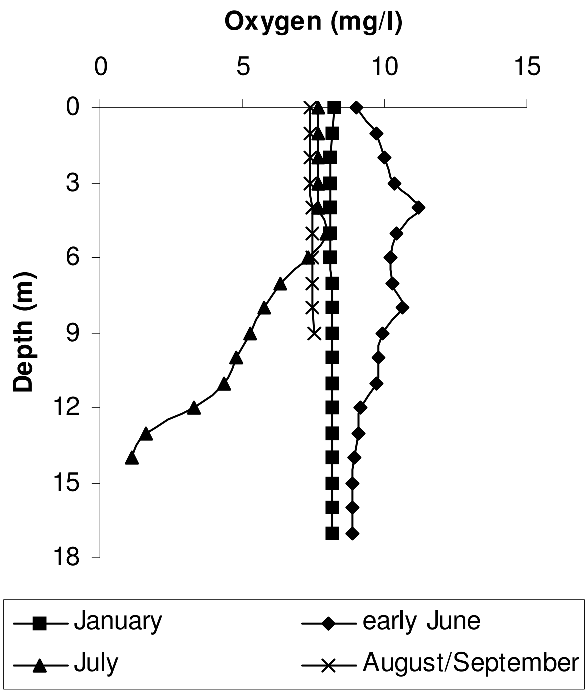
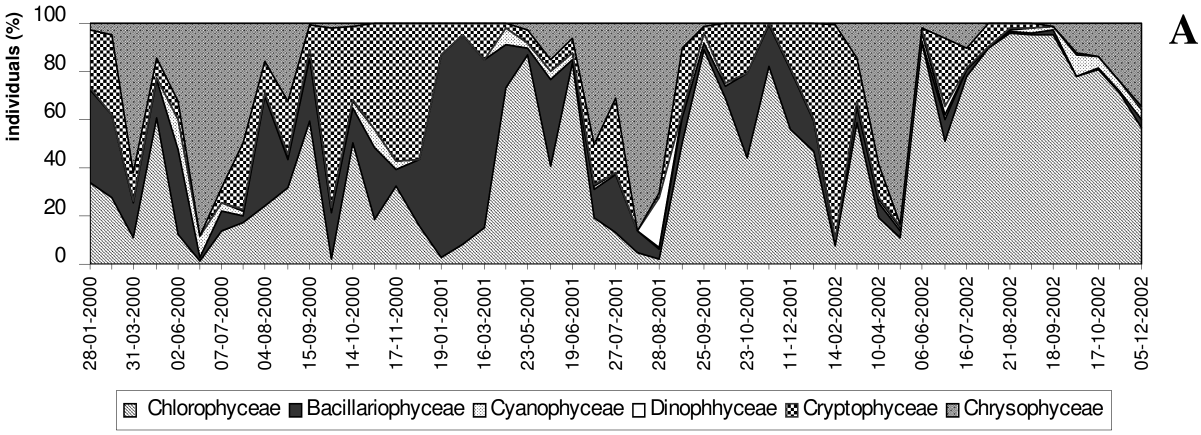

Oxygen (mg/l)
0 5 10 15
Depth (m)
0 3 6 9 12 15 18
January
early June
July
August/September

Figure 4.


A
individuals (%)
100
80
60
40
20
0
28-01-2000
31-03-2000
02-06-2000
07-07-2000
04-08-2000
15-09-2000
14-10-2000
17-11-2000
19-01-2001
16-03-2001
23-05-2001
19-06-2001
27-07-2001
28-08-2001
25-09-2001
23-10-2001
11-12-2001
14-02-2002
10-04-2002
06-06-2002
16-07-2002
21-08-2002
18-09-2002
17-10-2002
05-12-2002
Chlorophyceae
Bacillariophyceae
Cyanophyceae
Dinophhyceae
Cryptophyceae
Chrysophyceae

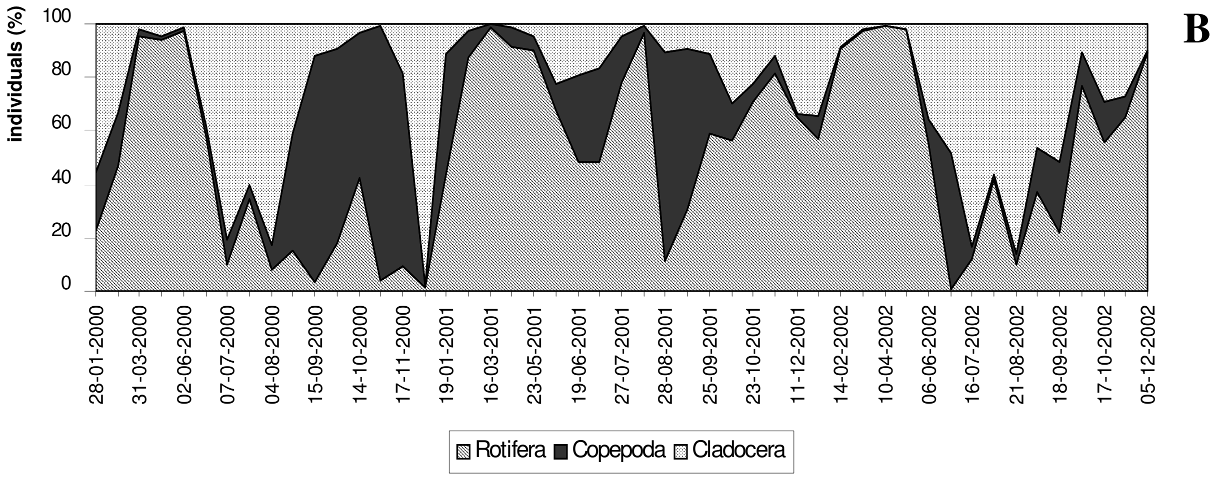
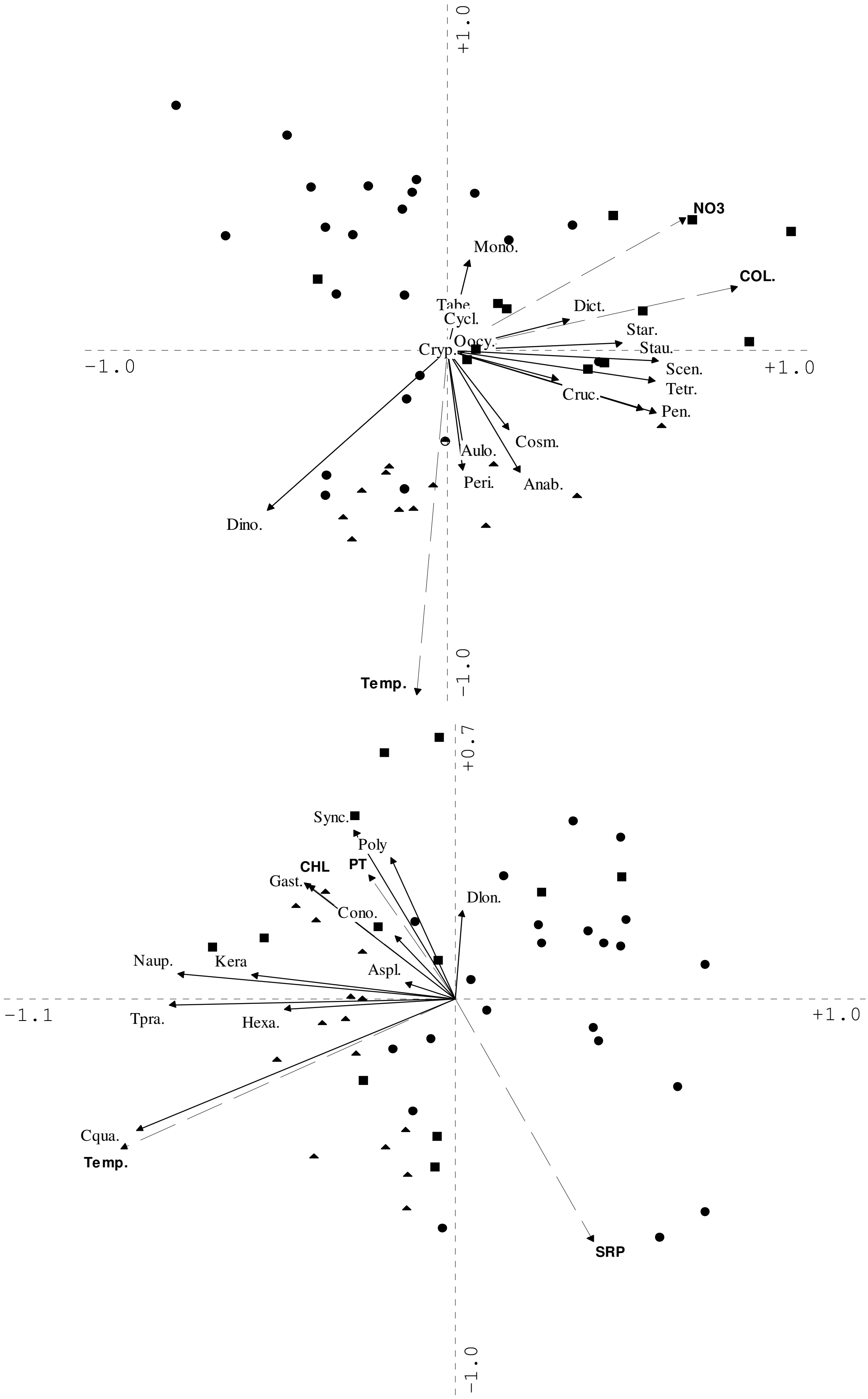

B
individuals (%)
100
80
60
40
20
0
28-01-2000
31-03-2000
02-06-2000
07-07-2000
04-08-2000
15-09-2000
14-10-2000
17-11-2000
19-01-2001
16-03-2001
23-05-2001
19-06-2001
27-07-2001
28-08-2001
25-09-2001
23-10-2001
11-12-2001
14-02-2002
10-04-2002
06-06-2002
16-07-2002
21-08-2002
18-09-2002
17-10-2002
05-12-2002
Rotifera
Copepoda
Cladocera

Figure 5.


+1.0
-1.0
+1.0
-1.0
NO3
COL.
Temp.
Mono.
Tabe.
Cycl.
Oocy.
Cryp.
Dict.
Star.
Stau.
Scen.
Tetr.
Cruc.
Pen.
Cosm.
Aulo.
Peri.
Anab.
Dino.
+0.7
-1.1
+1.0
-1.0
Sync.
Poly
CHL
PT
Gast.
Cono.
Dlon.
Naup.
Kera
Aspl.
Tpra.
Hexa.
Cqua.
Temp.
SRP

4. VARIAÇÕES NA LIMNOLOGIA DE UMA ALBUFEIRA EM DOIS ANOS CONSECUTIVOS: UM COM UM INVERNO CHUVOSO E OUTRO COM UM INVERNO SECO

Artigo 4: Limnological variations of a reservoir during two successive years: One wet, another dry

Submetido para publicação.

LIMNOLOGICAL VARIATIONS OF A RESERVOIR DURING TWO SUCCESSIVE YEARS: ONE WET, ANOTHER DRY

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ABSTRACT

Changes in environmental variables as well as in phyto and in crustacean zooplankton abundance were investigated in a meso-eutrophic reservoir during two successive years: One wet, another dry. In the wet winter, both total phosphorus and soluble reactive phosphorus reached their maximum, whereas water transparency achieved the minimum during the same period. The obtained data suggest that total phosphorus and soluble reactive phosphorus peaks observed in the water column were related to the increase in nutrient loading as a consequence of the intense rainfall occurred during this period. Phytoplankton composition was dominated by *Cyclotella* spp., except during the dry winter when *Anabaena* became dominant. The dominance of this alga seemed to be related to $N\text{-}NO_3$ depletion. The establishment of more stable environmental conditions (*e.g.* low water turbulence, larger water retention time) and the increase of irradiance during the dry period (Summer 2001 and Winter 2001/2002) also favoured growth of this cyanobacterium. Concomitantly to *Anabaena* dominance *Ceriodaphnia* abundance decreased whereas nauplii increased. Although variation between the two years of study seems to be related to the sequence of a pluvial and a dry year, further research is needed to evaluate whether variations in precipitation intensity influence reservoir ecological processes.

Key words: Nutrients, wet and dry years, reservoirs, phytoplankton, crustacean zooplankton

INTRODUCTION

Seasonal events are the most important factors influencing Mediterranean aquatic systems. However, both intensity and quantity of precipitation can vary markedly from one year to another. Temperature has a narrower range of fluctuation but, in wet winters, this parameter is generally slightly higher than in dry winters. This variability can generate different kinds of seasonal patterns, modifying water turnover time and changing the intensity of environmental and biological processes occurring in the water column (Catalan & Fee 1994; Armengol *et al*. 1999). Furthermore, it is common sense that the external loading of nutrients, organic matter and other substances such as pollutants increases when intense precipitation occurs. Besides, inputs at the beginning of rain events are higher than those generated at their end (Barbosa & Hvitved-Jacobsen 1999). However, the intensity and the magnitude of these inputs depend on land use, vegetation cover and landscape patchiness (Rybak 2000).

Azibo Reservoir is located in the Iberian Peninsula on the Portuguese part of international River Douro catchment. In this region, most precipitation occurs between October and March in a very irregular pattern from one year to another (Fig. 1). Total annual precipitation varies between 800 and 1000 mm, and in a "normal" autumn/winter season total precipitation is around 630 mm. In contrast to what happens in other reservoirs in the region, water level fluctuations caused by human activity are not very accentuated in Azibo. Thus, this reservoir provides a good environment to study the potential effects of quantity and intensity of precipitation without the interference of internal disturbances generated by extreme anthropogenic water level fluctuations.

Therefore, the objective of the present study was to assess variation of total phosphorus, orthophosphate, nitrate, ammonium ion, ammonia gas, dissolved oxygen, conductivity, pH and water transparency during two successive years: One wet, another dry. In a similar way variation of biotic attributes such as chlorophyll *a*, phytoplankton and crustacean zooplankton composition or species relative abundance were analysed.

STUDY AREA

Azibo Reservoir is located in the Portuguese part of River Douro catchment. The area of the reservoir is 410 ha and its total capacity is 54 470 x 10^3 m^3. Maximum depth is about

30 m, mean depth is 13.2 m. Mean water residence time is approximately 2.2 years. This reservoir was first filled in 1982 and it is used mainly for recreation purposes. Other uses are urban water supply and irrigation, yet these are not significant. Water level fluctuations vary between 1.5 and 2 m. In Azibo, direct influence of human activities is larger during summer, when reservoir and its surroundings are used for recreation such as swimming, camping, boating and angling. All year round activities in the catchment area are farming and grazing. The potential nutrient loads generated by those activities are around 243 275 kg of N and 116 956 kg of P per year (Geraldes & Boavida 2003). During the period of study thermal stratification occurred from June to October and the reservoir was classified as meso-eutrophic.

METHODS

From October 2000 to September 2002, samples were collected monthly in winter and biweekly in summer at one single sampling station, located at the deepest point of the reservoir. Water samples for orthophosphate (determined as soluble reactive phosphorus, SRP) and total phosphorus (TP) determinations were obtained from the upper 30-40 cm stratum, except during the stratification period, when samples were drawn from the mid water column (7 to10 m) and from the bottom as well. Samples were placed into acid rinsed bottles and transported to the laboratory in a cold container. SRP concentrations were estimated by the Murphy and Riley method (1962) and TP was assessed after acid hydrolysis with persulfate for 60 minutes under high temperature and pressure (APHA 1989). Chlorophyll *a* (CHL *a*) was obtained from 500 to 1 000 ml of integrated water samples taken at the euphotic zone and filtered through a Whatman GF/A filter no more than 2 h after collection. Concentrations were determined spectrophotometrically after overnight extraction in 90% acetone. Environmental variables such as water temperature, dissolved oxygen (DO), conductivity, pH, as well as nitrate (N-NO_3), ammonium ion (N-NH_4) and ammonia gas (N-NH_3) were measured *in situ* with a 6 820 YSI Multiparameter Water Quality Monitor. Water transparency was measured with a 20 cm diameter black and white Secchi disk.

Zooplankton samples were obtained on each sampling date by taking two vertical hauls using a Wisconsin type net of 64 µm mesh size. Animals were anaesthetised with carbonated water and preserved in sugar-saturated formaldehyde (4% final concentration). Depending on density, zooplankton was counted in 5, 10, 20 ml sub-samples or in total sample. Animals were always identified to species, according to Scourfield and Harding (1966) and Dussart (1969). For phytoplankton analysis integrated water samples were collected from the euphotic zone. Samples were fixed *in situ* in Lugol's solution. Phytoplankton was counted according to Utermöhl (1958), using an inverted microscope at 200X/400X magnification. Algae were identified to genus, according to Bourrelly (1966; 1968; 1970).

Considering that the highest precipitation occurs between October and March in the region, the obtained samples were grouped in four distinct periods: (1) October 2000 to March 2001 (Winter 2000/2001); (2) April 2001 to September 2001 (Summer 2001); (3) October 2001 to March 2002 (Winter 2001/2002); (4) April 2002 to September 2002 (Summer 2002). To test whether the mean values of total phosphorus, orthophosphate, nitrate, ammonium ion, ammonia gas, dissolved oxygen, conductivity, pH and water transparency were influenced by precipitation variation, a covariance analysis (ANCOVA) was carried out. This analysis was also used to test the influence of environmental variables mentioned above on the increase in *Anabaena* densities and to assess whether the presence of this alga could have had some impact on crustacean zooplanktonic assemblage composition. Cluster analysis (UPGMA method, Euclidean distance measure) was performed on log (x+1) transformed abundance data of the most representative phytoplankton and zooplankton taxa to assess differences between seasonal periods for species abundance. For the purpose of statistical analyses pennate diatoms except *Asterionella formosa* and *Fragilaria* sp. were retained in a single category because each taxon was present in low densities. All statistical analyses were performed using SYSTAT 11.5.

RESULTS

Environmental variables

Total precipitation reported from October 2000 to March 2001 was 1 482 mm, whereas the value reported from October 2001 to March 2002 was 424 mm (source: Agroclima-ESAB). Thus, Winter 2000/2001 was considered a wet winter, whereas Winter 2001/2002 was a dry one. Data concerning $N\text{-}NH_3$ are not shown because this variable was below detection in most samples. Significant differences among seasons when precipitation was considered as covariate were only found for conductivity ($F = 10.45$, $p < 0.001$ at the surface; $F = 7.70$, $p < 0.05$ at mid water column; $F = 15.45$; $p < 0.001$ at the bottom) and for temperature ($F = 13.49$, $p < 0.001$ at the surface; $F = 10.94$, $p < 0.001$ at mid water column). Despite the distinct amounts of precipitation fallen, mean values of the environmental variables mentioned above were in general similar in both winters and summers (Table 1). Conversely, the maxima and the minima of some environmental variables were different between winters. In fact, during Winter 2000/2001 TP and SRP reached their maxima (102.8 and 23.9 µg l^{-1}, respectively), whereas water transparency achieved the minimum (1.5 m). During Summer 2001 and Summer 2002 both minimum and maximum reached by those variables were similar (Figs. 2 and 3).

Phytoplankton composition and abundance

Among all algae observed in Azibo (Table 2) the commonest were: (1) Chlorophyceae – *Monoraphidium*, *Chlamydomonas* like cells, *Scenedesmus*, *Crucigenia*, *Tetraedron*, *Oocystis*, *Paulschulzia* and *Cosmarium*; (2) Bacillariophyceae – *Asterionella*, *Fragilaria* and *Cyclotella*; (3) Chrysophyceae – *Dinobryon*; (4) Cyanophyceae – *Anabaena*; (5) Cryptophyceae – *Cryptomonas*; (6) Dinophyceae – *Ceratium*. Cluster analysis (Fig. 4A) performed on algae relative abundance (Table 3) indicated that abundance and composition were similar in both summers, whereas dissimilarities were more marked between winters. In fact, in Winter 2000/2001 *Ceratium*, *Monoraphidium* and *Cyclotella* were dominant. On the other hand, in Winter 2001/2002 from October to December 2001 *Anabaena* became dominant. Concomitant with *Anabaena* dominance, a sudden depletion in N-NO_3 concentration occurred, while SRP values remained almost unchanged (Fig. 5). However, results of ANCOVA indicate that temperature ($F = 5.50$; $p < 0.05$), conductivity ($F = 8.78$; $p < 0.05$) and precipitation ($F = 11.79$; $p < 0.05$) contributed to the high density of this alga recorded in Winter 2001/2002. In addition, the presence of *Anabaena* influenced *Cyclotella* abundance ($F = 7.79$; $p < 0.05$). The highest CHL *a* concentrations were obtained in both winters. During these periods taxa composed of large individuals were more abundant (e.g. *Ceratium*; *Anabaena*). Conversely, in both summers, taxa composed of small individuals were dominant in the phytoplankton community.

Zooplankton composition and abundance

From all zooplanktonic species found in Azibo (Table 2) only the most abundant were considered in this approach. Those species were: The cladocerans *Daphnia longispina*, *Ceriodaphnia pulchella, Bosmina longirostris* and *Diaphanosoma brachyurum*; The copepods

Copidodiaptomus numidicus, *Acanthocyclops robustus* and their nauplii. Cluster analysis results (Fig. 4B) performed on species relative abundance (Table 4), separated Winter 2000/2001, Summer 2001 and Summer 2002 from Winter 2001/2002. In fact, during the latter winter, coincidentally with *Anabaena* dominance, nauplii abundance increased, whereas *Ceriodaphnia* decreased. According to ANCOVA, the presence of *Anabaena* influenced *Ceriodaphnia* ($F = 11.34$; $p < 0.05$) and nauplii ($F = 7.45$; $p < 0.05$) abundances. No significant differences among seasons were found for the other taxa when *Anabaena* was considered as covariate.

DISCUSSION

Except for conductivity and temperature, mean values of environmental variables were similar in both years. However, differences were found when comparing maxima and minima of both 2001 and 2002 winter ranges. In fact, TP, SRP and winter temperature maxima, as well as transparency minimum, were recorded in the wet winter (Figs. 2 and 3). Similar results were obtained by several authors in reservoirs located in regions influenced by Mediterranean climate (Armengol *et al.* 1994, 1999, Soria *et al.* 2000) or in other regions subjected to extensive dry and wet periods (Chalar & Tundisi 1999). Thus, the analysis of mean values of environmental variables does not provide a good estimation of the impacts of rainfall on reservoir limnology. In fact, the effects of precipitation (*e.g.* large amounts of particles in water column) were only detected during short periods of time (Geraldes, personal observation). This can be explained by: (1) Occurrence of sedimentation/retention in periods of less turbulence (Boström *et al.* 1988; Chalar & Tundisi 1999); (2) Reduction of the external loads at the end of the rainfall period (Johnes *et al.* 1996, Barbosa & Hvitved-Jacobsen 1999, McGarrigle *et al.* 2000, Rybak 2000, Miranda & Matvienko 2003); (3) Intensity of recycling by biotic components (Catalan & Fee 1994; Reynolds *et al*. 1998). Despite the lack of data concerning the intensity of those processes in Azibo reservoir, one could expect that, similarly to what happens in other reservoirs, those changes were related to the balance between the above mentioned processes (sedimentation, nutrient loads, recycling). Geraldes and Boavida (2003) noticed during the wet winter in samples obtained downstream of one of Azibo's tributaries that TP concentrations were 103 µg/l at the beginning of the rainfall period, decreasing subsequently to 76 µg/l and 16 µg/l, the latter at the end of the precipitation period. TP concentrations in the reservoir varied concomitantly with the trends observed downstream. Conversely, during dry winter TP concentrations were much lower (around 70 µg/l) at the beginning of the rainfall period. At the end of the rain period, TP concentrations were similar to those obtained during the wet winter, but in the reservoir TP concentrations did not change much. The estimated potential loads generated by anthropogenic activities in the catchment and in reservoir surroundings are around 243 275 kg of N and 116 956 kg of P per year (Geraldes & Boavida 2003). Despite of the capacity of the reservoir, which is large enough to dilute those loads, temporary increases in nutrient concentrations in water column occur during intense rain events. As it is evidenced by data mentioned above, nutrient inputs decreased progressively until the end of the rain period. However, these values can change from one year to another as a function of rain quantity and intensity. Furthermore, because of the existence of numerous buffer areas in the reservoir catchment (e.g. woodlands, riparian vegetation) the actual loading can be much lower than the estimated, even during wet years. During dry periods, particles transported by wind from surroundings can be an important source important source of P, influencing this nutrient concentrations in water column during both summers and also during dry winter (e.g. Cole *et al.* 1990). Those particles were often presented at the water surface of this reservoir, especially during late spring and summer months, and consisted of terrestrial insects and plant fragments (Geraldes, personal observation).

The most remarkable change in phytoplankton composition was the dominance of *Anabaena* from October to December 2001 (dry winter). Why did this alga become dominant during this period? Vasconcelos (1990) also recorded in this reservoir the dominance of *Anabaena* during an extensive dry period, which occurred subsequently to a wet winter as well. Similar patterns were described by other authors (e.g. Ahn *et al.* 2002). The conditions created by the subsequent dry periods (Summer 2001 and Winter 2001/2002) provided the ecological optimum for *Anabaena* dominance. According to Reynolds (1998) the dominance of a particular algal species is the result of a stochastic combination of environmental variables. *Anabaena* is not only favoured by N-NO_3 depletion (Sakamoto & Okino, 2000), but also by more stable environmental conditions (e.g. absence of water turbulence, larger water retention time) (Reynolds *et al.* 2002) and higher irradiance (Ahn *et al.* 2002). This ecological optimum was achieved in Azibo during the mentioned dry period and consequently *Anabaena* had adaptive advantage over *Cyclotella* and *Ceratium*, which were the dominant genera during the wet winter. Even after the increase in N-NO_3, subsequent to stratification disruption, *Anabaena* densities only decreased when water temperature dropped below 10ºC and after the precipitation events recorded in January 2002. At the beginning of Winter

2002/2003 *Anabaena* was again detected in very low densities. However, because of high water turbulence generated by heavy precipitation occurred during this period, it did not reach the high densities recorded during Winter 2000/2001. In fact, from November 2002 onwards *Anabaena* was not detected.

The influence of *Anabaena* dominance on the crustacean zooplankton abundance is well known (*e.g.* Lampert & Sommer 1997). In fact, zooplankton composition is frequently changed and copepods and rotifers often replace cladocerans. The results of covariance analysis indicate that the decrease in *Ceriodaphnia* and the increase in nauplii abundance in the dry winter are a consequence of the presence of this cyanobacterium. However, other factors, such as temperature (*Ceriodaphnia* could also have been affected by the slightly lower temperatures recorded in dry winter e.g. Lynch 1978) and other abiotic or biotic interactions beyond the scope of the present paper could also have influenced the abundance of crustacean zooplanktonic species.

The observed changes in nutrient concentrations, in *Anabaena* and crustacean zooplankton abundance during the studied years were, at least, partially related to variations in rainfall intensity. However, this study is a preliminary approach. Thus, it is necessary to obtain longer data series, enabling a simultaneous analysis of intra- and inter-annual ecosystem changes, in order to fully understand in what way variations in precipitation can influence reservoir ecological processes. These data are crucial to develop correct management measures (e.g. development and restoration of buffer areas such as woodlands and riparian woods) to minimise and predict the negative impact of extreme precipitation and nutrient loading on ecosystem health and consequently on water quality.

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Table 1- Mean ± SD of environmental variables. Minimum-maximum range is shown for pH.

	Winter 00/01	**Winter01/02**	**Summer 01**	**Summer 02**
Water transparency (m)	3.2 ± 1.7	2.9 ± 0.6	4.3 ± 0.8	4.2 ± 1.6
Water temperature (ºC)				
Surface	11.4 ± 3.1	11.0 ± 4.3	21.1 ± 2.9	19.7 ± 4.1
Mid water column	11.4 ± 3.1	11.0 ± 4.3	19.6 ± 3.7	20.6 ± 1.7
Bottom	11.4 ± 3.1	11.0 ± 4.3	11.7 ± 1.1	9.9 ±1.3
Dissolved oxygen (mg l^{-1})				
Surface	8.9 ± 1.3	8.6 ± 0.7	9.4 ± 1.4	8.4 ± 1.0
Mid water column	8.9 ± 1.3	8.6 ± 0.7	8.6 ± 1.5	8.1 ± 1.1
Bottom	8.9 ± 1.3	8.6 ± 0.7	1.1 ±1.7	2.2 ± 1.3
Conductivity (µS cm^{-1})				
Surface	57.0 ± 11.5	49.6 ± 5.5	60.6 ± 5.6	66.2 ± 8.5
Mid water column	57.0 ± 11.5	49.6 ± 5.5	60.3 ± 6.1	68.0 ± 4.7
Bottom	57.0 ± 11.5	49.6 ± 5.5	52.7 ± 3.1	60.5 ± 8.9
pH				
Surface	6.6 - 7.4	6.9 - 8.4	7.7-8.1	6.6-7.4
Mid water column	6.6 - 7.4	6.9 - 8.4	7.9-8.5	7.5-7.8
Bottom	6.6 - 7.4	6.9 - 8.4	7.0-8.1	6.2-7.4
$N\text{-}NO_3$ (mg l^{-1})				
Surface	10.4 ± 4.2	8.6 ± 7.5	2.6 ± 2.4	0.3 ± 0.2
Mid water column	10.4 ± 4.2	8.6 ± 7.5	2.3 ± 2.2	0.1 ± 0.1
Bottom	10.4 ± 4.2	8.6 ± 7.5	1.7 ± 2.4	0.5 ± 0.6
$N\text{-}NH_4$ (mg l^{-1})				
Surface	0.01 ± 0.03	0.0	0.01 ± 0.03	0.7 ±1.5
Mid water column	0.01 ± 0.03	0.0	0.0	0.1 ± 0.1
Bottom	0.01 ± 0.03	0.0	0.0	0.5 ± 0.6
TP (µg l^{-1})				
Surface	64.4 ± 23.8	59.8 ± 9.1	68.2 ±17.0	62.5 ± 7.8
Mid water column	-	-	78.9 ± 29.6	78.5 ± 17.81
Bottom	-	-	120.5 ± 33.5	93.2 ± 32.6
SRP (µg l^{-1})				
Surface	11.5 ± 7.4	4.1 ± 2.4	2.9 ± 1.2	7.0 ± 4.2
Mid water column	-	-	7.7 ± 3.2	13.4 ± 5.3
Bottom	-	-	8.8 ± 3.0	17.3 ± 7.7
Chlorophyll *a* (µg l^{-1}) Integrated sample	2.1 ± 0.9	3.7 ± 1.9	1.2 ± 0.5	1.0 ± 0.5

Table 2- Composition of phytoplankton and zooplankton communities in Azibo Reservoir between October 2000 and September 2002.

PHYTOPLANKTON	ZOOPLANKTON
Chlorophyceae	**Rotifera**
Botryococus	*Asplanchna priodonta*
Chlamydomonas	*Collotheca*
Closterium	*Collotheca mutabilis*
Cosmarium	*Conochilus*
Crucigenia	*Filinia*
Dictyosphaerium	*Gastropus*
Gonium	*Hexarthra*
Micrasterias	*Keratella cochlearis*
Monoraphidium	*Keratella quadrata*
Paulschulzia	*Ploesoma*
Scenedesmus	*Polyarthra*
Selenastrum	*Pompholix sulcata*
Staurastrum	*Testudinella*
Staurodesmus	*Trichotria*
Tetraedron	**Cladocera**
Volvox	*Alona costata*
Bacillarophyceae	*Alona rectangula*
Amphora	*Alona quadrangularis*
Asterionella formosa	*Bosmina longirostris*
Aulacoseira	*Ceriodaphnia pulchella*
Cocconeis	*Chydorus sphaericus*
Craticula	*Daphnia longispina*
Cyclotella ocellata	*Daphnia pulex*
Cymbella	*Diaphanosoma brachyurum*
Fragilaria	**Copepoda**
Gomphonema	*Acanthocyclops robustus*
Gyrosigma	*Copidodiaptomus numidicus*
Navicula	Nauplii
Nitzschia	
Tabellaria	
Cyanophyceae	
Anabaena flos-aquae	
Chroococcus	
Merismopedia	
Oscillatoria	
Dinophyceae	
Ceratium hirundinella	
Gymnodinium	
Peridinium	
Euglenophyceae	
Phacus	
Trachelomonas	
Cryptophyceae	
Cryptomonas	
Chrysophyceae	
Dinobryon	
Mallomonas	

Table 3 - The most abundant genera of the phytoplankton community of Azibo Reservoir, quantified using the mean (± SD) of relative abundances.

	Winter 00/01	Winter 01/02	Summer 01	Summer 02
Chlorophyceae				
Chlamydomonas	1.4 ± 2.1	3.8 ± 6.5	13.8 ± 29.6	13.0 ± 25.9
Cosmarium	0.4 ± 0.9	0.5 ± 0.5	0.6 ± 1.7	0.1 ± 0.1
Crucigenia	2.8 ± 3.2	0.7 ± 0.4	2.7 ± 6.5	0.2 ± 0.5
Monoraphidium	16.6 ± 27.2	0.1 ± 0.2	0.7 ± 1.6	0.1 ± 0.2
Oocystis	0.1 ± 0.2	1.6 ± 2.7	0.8 ± 2.0	0.1 ± 0.1
Paulschulzia	1.5 ± 1.7	17.2 ± 23.6	0.9 ± 2.8	11.3 ± 28.4
Scenedesmus	0.4 ± 0.4	1.1 ± 1.2	0.6 ± 0.6	0.6 ± 0.4
Tetraedron spp.	0.0	0.5 ± 0.4	0.2 ± 0.3	0.2 ± 0.2
Bacillariophyceae				
Cyclotella	31.1 ± 21.5	16.6 ±19.2	59.9 ± 37.2	61.7 ± 35.4
Asterionella	9.7 ± 12.7	2.2 ± 2.0	0.3 ± 0.5	0.6 ± 1.2
Fragilaria	2.4 ± 2.7	3.2 ± 6.3	3.7 ± 9.9	0.1 ± 0.1
Other pennate diatoms	4.4 ± 6.0	1.1 ± 1.6	0.1 ± 0.1	0.4 ± 0.5
Cryptophyceae				
Cryptomonas	6.5 ± 8.1	9.6 ± 6.4	9.8 ± 16.4	8.2 ± 10.0
Cyanophyceae				
Anabaena	4.5 ± 6.0	41.4 ± 30.1	3.4 ± 7.2	0.2 ± 0.3
Dinophyceae				
Ceratium	20.2 ± 13.9	0.1 ± 0.1	4.5 ± 9.0	3.1 ± 5.1
Chrysophyceae				
Dinobryon	0.0	0.3 ± 0.4	0.0	0.2 ± 0.3

Table 4 - The most common genera of the zooplankton community of Azibo Reservoir, quantified using the mean (± SD) of relative abundances.

	Winter 00/01	Winter 01/02	Summer01	Summer02
Cladocera				
B. longirostris	1.2 ± 1.2	3.3 ± 1.4	1.8 ± 1.0	1.6 ± 1.9
C. pulchella	25.0 ± 28.5	10.1 ± 17.0	38.3 ± 33.8	25.9 ± 24.7
D. brachyurum	-	-	3.2 ± 5.1	5.3 ± 10.4
D. longispina	12.9 ± 8.7	13.2 ± 10.7	9.4 ± 19.0	5.5 ± 7.3
Copepoda				
A. robustus	0.9 ± 1.2	3.9 ± 3.7	1.7 ± 1.5	0.9 ± 0.9
C. numidicus	44.1 ±16.6	31.1 ± 19.1	31.5 ± 20.4	43.6 ± 13.9
Nauplii	15.9 ± 23.1	38.4 ± 15.3	14.1 ± 11.0	17.9 ± 13.5

Figure captions:

Figure 1- Precipitation occurred per month between 2000 and 2002 in the main city closest to Azibo reservoir (source: Agroclima Lab-ESAB) and monthly means based on rainfall data from 1961 to 1990 (source: IM, Institute of Meteorology).

Figure 2- Monthly variation of surface water temperature and water Secchi transparency with precipitation during the period of study in Azibo Reservoir.

Figure 3- Monthly variation of surface total phosphorus, soluble reactive phosphorus, nitrate, chlorophyll *a*, all compared with variation in precipitation, during the period of study in Azibo Reservoir.

Figure 4- Cluster analysis diagram depicting periods defined according to phytoplankton (A) and zooplankton (B) composition and abundance, during the period of study in Azibo Reservoir. Winter1 - Winter2000/2001 samples, Summer1 - Summer 2001 samples, Winter2 - Winter 2001/2002 samples, Summer2- Summer 2002 samples.

Figure 5- Variation of $N\text{-}NO_3$ and SRP surface water concentrations and *Anabaena* densities during the period of study in Azibo Reservoir. $N\text{-}NO_3$ is expressed in mg l^{-1} and SRP in $\mu g\ l^{-1}$.

Figure 1.

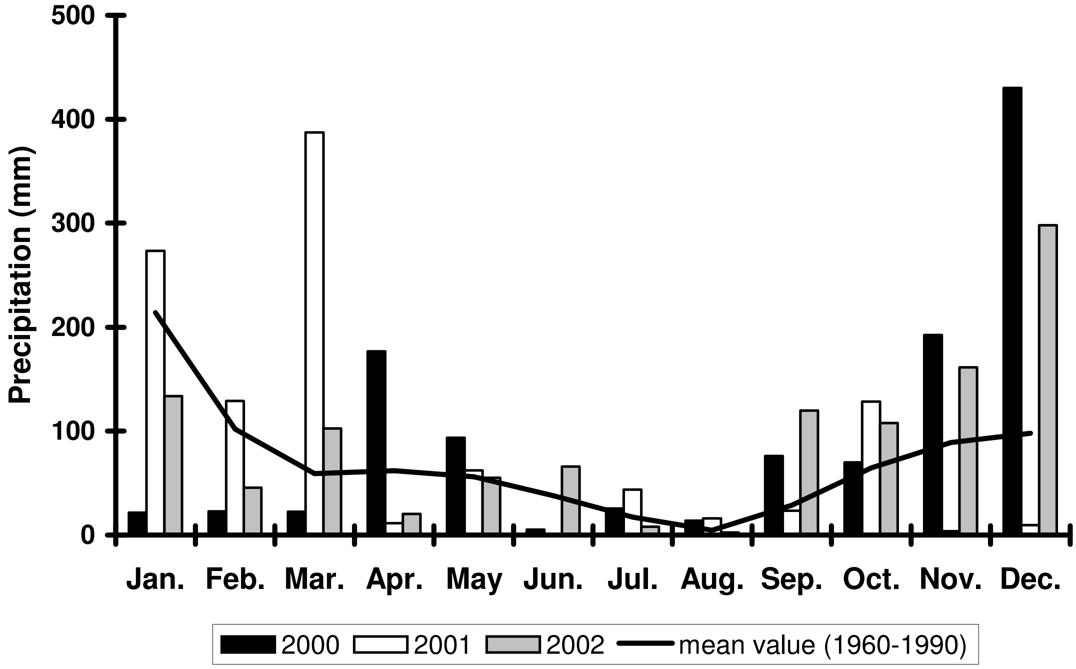

500
400
300
200
100
0
Precipitation (mm)
Jan.
Feb.
Mar.
Apr.
May
Jun.
Jul.
Aug.
Sep.
Oct.
Nov.
Dec.
2000
2001
2002
mean value (1960-1990)

Figure 2.

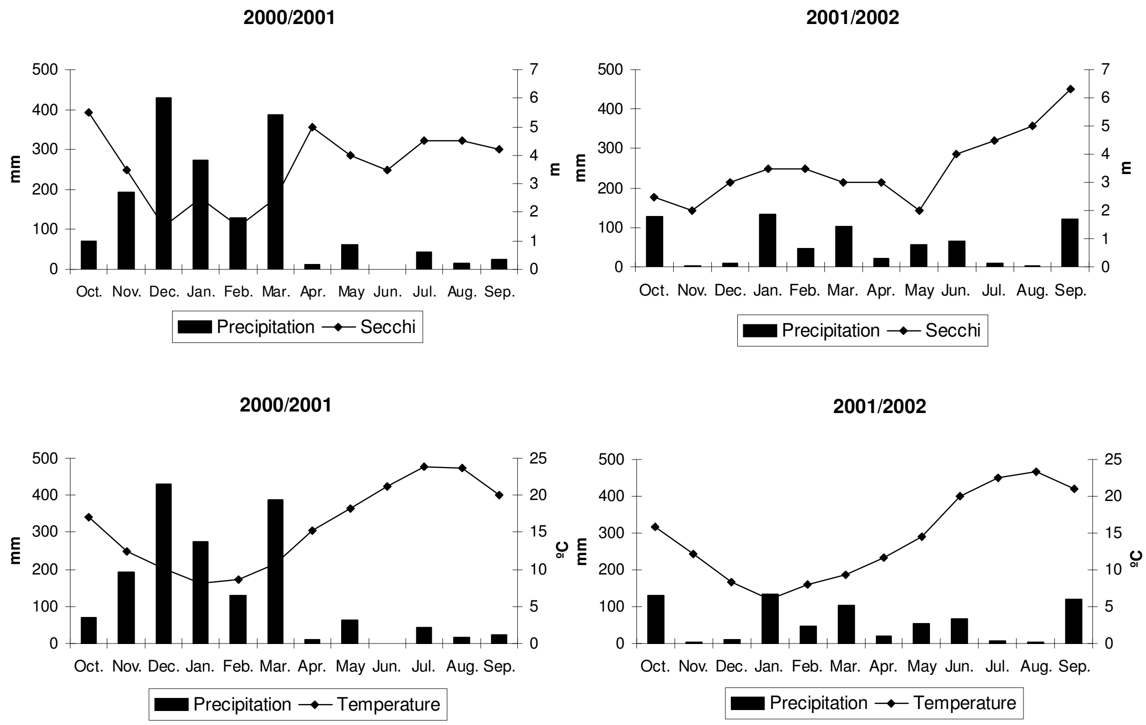

2000/2001
2001/2002
mm
m
ºC
Oct. Nov. Dec. Jan. Feb. Mar. Apr. May Jun. Jul. Aug. Sep.
Precipitation
Secchi
Temperature

Figure 3.

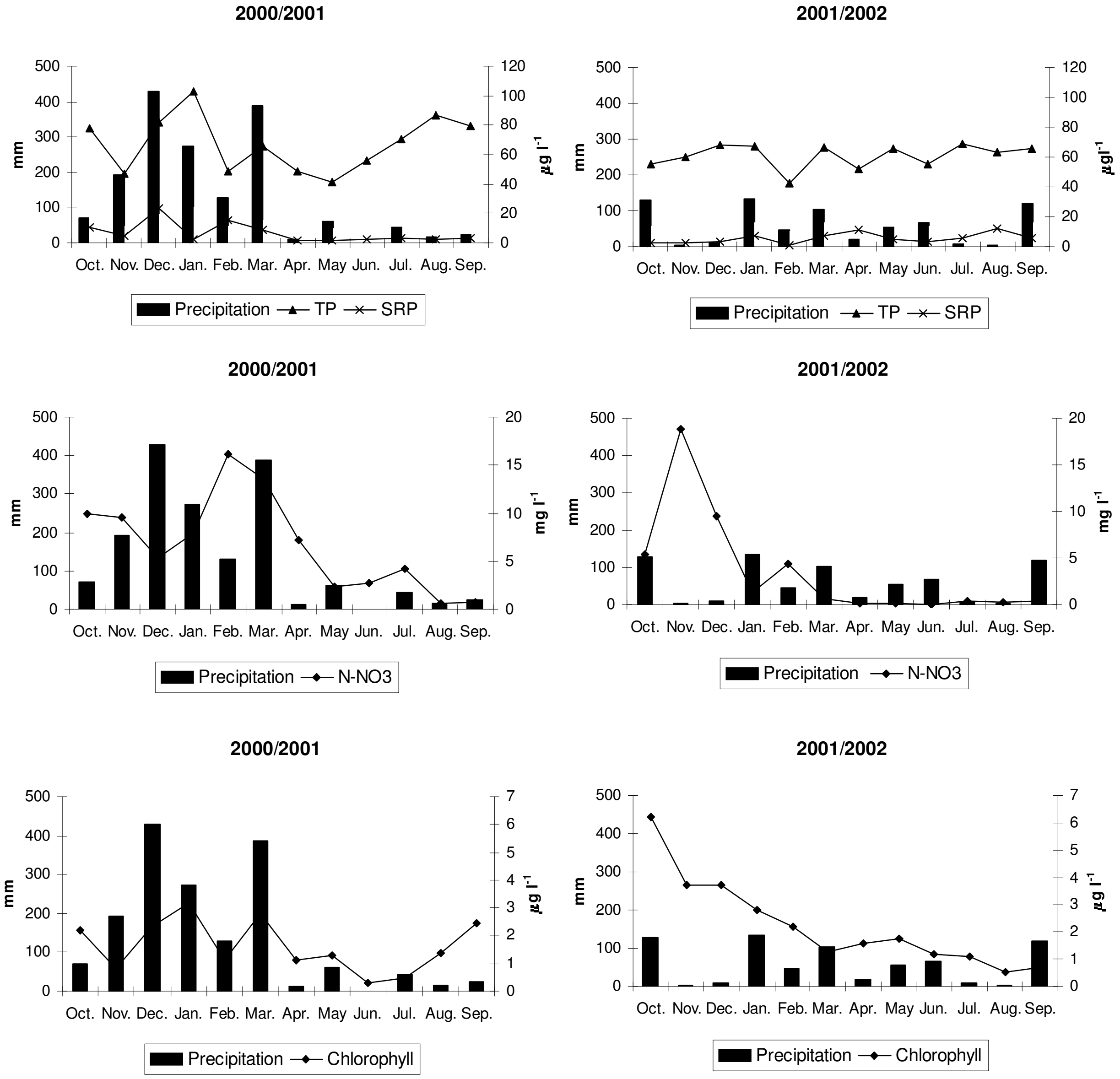

2000/2001
2001/2002
mm
µg l-1
mg l-1
Oct. Nov. Dec. Jan. Feb. Mar. Apr. May Jun. Jul. Aug. Sep.
Precipitation
TP
SRP
N-NO3
Chlorophyll

Figure 4.

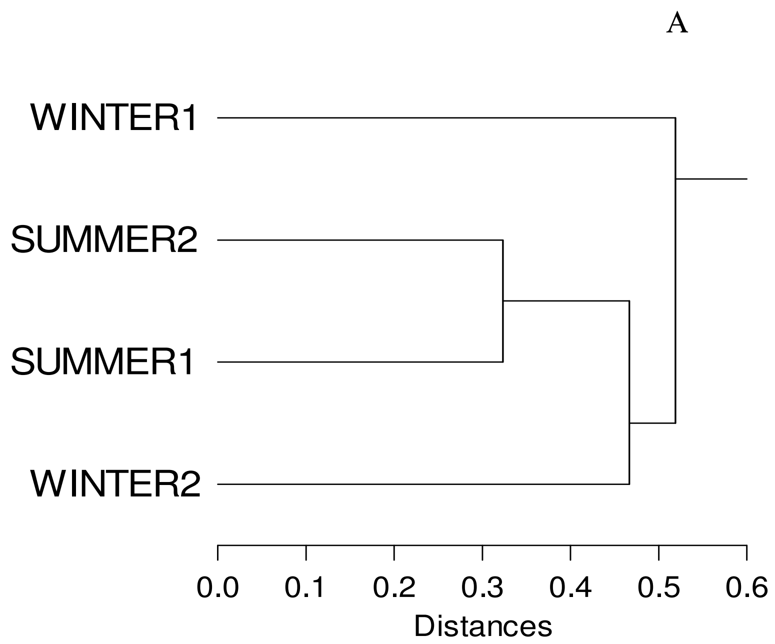
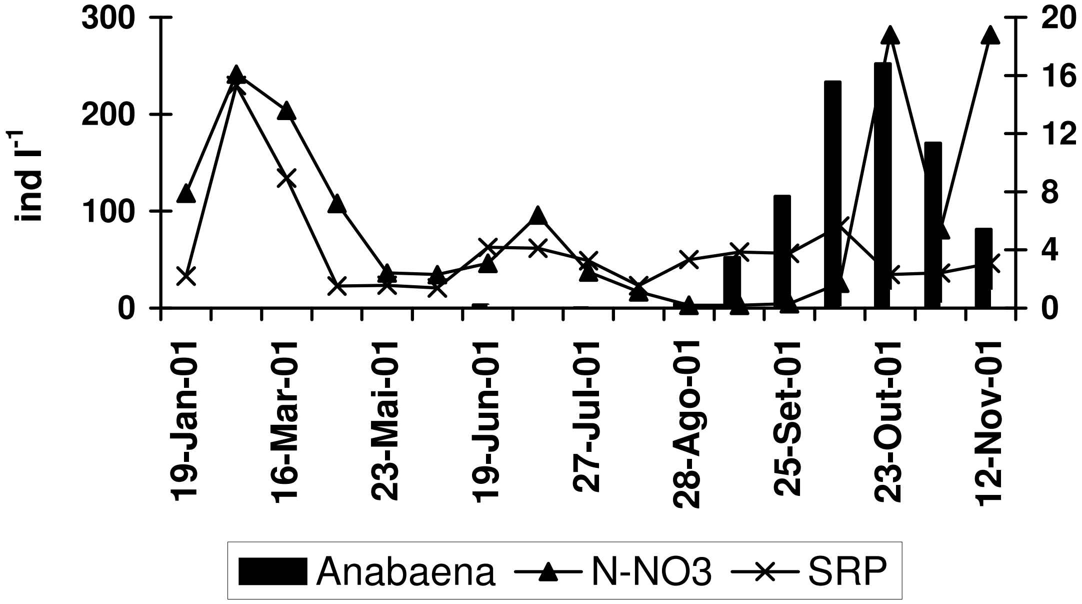

A
WINTER1
SUMMER2
SUMMER1
WINTER2
0.0 0.1 0.2 0.3 0.4 0.5 0.6
Distances
B
WINTER2
SUMMER1
WINTER1
SUMMER2
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7
Distances

Figure 5.


300
200
100
0
ind l-1
20
16
12
8
4
0
19-Jan-01
16-Mar-01
23-Mai-01
19-Jun-01
27-Jul-01
28-Ago-01
25-Set-01
23-Out-01
12-Nov-01
Anabaena
N-NO3
SRP

5. FACTORES ESTRUTURANTES E COMPOSIÇÃO DAS COMUNIDADES ZOOPLANCTÓNICAS

Artigo 5: Conductivity, temperature, pH, water level fluctuation and reservoir age: More important than phosphorus and chlorophyll in structuring zooplankton communities?

Submetido para publicação.

Artigo 6: What factors influence the cladoceran assemblage of a meso-eutrophic reservoir?

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CONDUCTIVITY, TEMPERATURE, PH, WATER LEVEL FLUCTUATION AND RESERVOIR AGE: MORE IMPORTANT THAN PHOSPHORUS AND CHLOROPHYLL IN STRUCTURING ZOOPLANKTON COMMUNITIES?

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ABSTRACT

Zooplankton abundance, composition and dynamics were followed in two reservoirs of River Douro catchment monthly in winter and biweekly in summer, from January 2000 to December 2001. Environmental variables such as temperature, dissolved oxygen, conductivity, water transparency, as well as nitrate, ammonium ion and ammonia gas were measured in situ. Total phosphorus, soluble reactive phosphorus and chlorophyll *a* were determined in the laboratory. Rotifera and small Cladocera were dominant in S. Serrada Reservoir, except in late summer and fall when the copepod *Tropocyclops prasinus* became dominant. In Azibo Reservoir Cladocera and Copepoda were dominant during the whole study period, except in July 2001. Annual variation in composition and abundance was found between the zooplankton communities of the compared reservoirs. DCA confirmed those trends. According to CCA the environmental variables significantly explaining species variance are conductivity, water temperature, TP, SRP, CHL *a*, pH, and dissolved oxygen. However, those variables only explained 42.2% of the species variance. Therefore, other factors such as the degree of disturbance (e.g. caused by anthropogenic water level fluctuations), reservoir age and biotic interactions could also have had a role in the structuring of zooplankton communities.

Key words: Reservoir age, temperature, water level fluctuation, zooplankton structure

INTRODUCTION

Zooplankton communities are a result of colonisation followed by species selection processes (Margalef, 1983; Wetzel, 2001). Both colonisation and selection depend on physical and chemical water conditions in reservoirs. Geology, climate, watershed features and the degree of human disturbance directly affect water quality. Therefore, the dynamics and nature of zooplankton communities may constitute an important source of information for correct management practice implementation (e.g. Bays & Crisman, 1983; Pejler, 1983; Sladeček, 1983; Schmid-Araya & Zuñiga, 1992; Caramujo & Boavida, 2000). In spite of that, remarkably few studies concerning environmental influence on zooplankton composition, abundance and dynamics in the reservoirs of the Portuguese part of the River Douro international catchment have been undertaken. The existing studies are preliminary, mainly concerning species composition of the plankton communities (Branco & Guimarães, 1987, 1993; Branco *et al.* 1992; Vasconcelos 1990a; 1994). Consequently, the available data are scarce, relatively old and discontinuous, and there is no integrated limnological research, which would constitute the scientific underpinnings for correct management and monitoring of these aquatic ecosystems.

The present study was carried out on two reservoirs located at the Portuguese part of the River Douro catchment in Trás-os-Montes region (NE Portugal). S. Serrada Reservoir was filled for the first time in 1995 and it has never been studied. Azibo Reservoir was created in 1982. Previous studies on this reservoir (Vasconcelos 1990a, 1990b, 1994) undertaken in 1987/1988, were qualitative and limited in time.

Therefore, the objectives of the present research were: (1) to determine zooplankton composition, abundance and dynamics during a two year cycle in both reservoirs; (2) to assess the influence of the studied environmental variables and parameters upon those communities. An attempt was made to relate different characteristics of the reservoirs such as geology, disturbance degree caused by water level fluctuations related to anthropogenic use, age of the reservoir and fish assemblages to the differences found between zooplankton communities. It was an aim of the researchers to offer a contribution for reservoir management, to be implemented in the near future.

STUDY SITES

S. Serrada is located on granitic bedrock at an altitude of 1 300 m a.s.l. in the mountainous system of Montesinho Natural Park. The total capacity of the reservoir, spreading over 25 ha, is 1 680 x 10^3 m^3. The surrounding vegetation is composed mainly of *Erica* sp., *Genista* sp., *Chamaespartium* sp. and *Salix* sp.. The fish community is composed mainly of the Iberian chub (*Leuciscus carolitertti*) and the brown trout (*Salmo trutta*). Although human influence on the impoundment seems negligible, fire in the surrounding land is often induced by shepherds to obtain better graze for livestock (sheep and goat). This reservoir was filled for the first time in 1995 to supply water to the city of Bragança and to generate hydroelectric power. As a result of these uses, accentuated water level fluctuations occur. The annual range of water level variation is 8 to 10 m. Thermal stratification was observed from June to August in 2000 and from June to September in 2001. Disruption of stratification was coincidental with the lowest water level.

Azibo is located at an altitude of 500 m a.s.l. on schistic bedrock, included in an area classified as Protected Landscape of the Azibo Reservoir. The reservoir is 410 ha and its total capacity is 54 470 x 10^3 m^3. The surrounding vegetation is composed of *Quercus suber*, *Quercus pyrenaica*, *Quercus faginea*, *Quercus rotundifolia*, *Genista* spp., *Cistus ladanifer*, *Salix* sp., *Fraxinus angustifolia* and *Populus* sp.. The fish community is mainly composed of the Iberian nase (*Chondrostoma polylepis*), the Iberian barbel (*Barbus bocagei*), the Iberian chub (*Leuciscus carolitertti*), carp (*Cyprinus carpio*), plus exotic piscivorous blackbass (*Micropterus salmoides*) and pike (*Esox lucius*). The direct influence of human activities on the impoundment is greatest during summer when the reservoir and surroundings are used for recreation such as fishing, swimming, camping and boating. Other activities found all over the year in the surroundings are farming and grazing (mainly sheep). This reservoir was filled for the first time in 1982 and is used mainly for recreation. Other uses include water supply and irrigation, yet those are not significant and water level fluctuations are not very accentuated, consequently the water level variation is 1.5 to 2 m. Thermal stratification occurred from June to October in both years of study.

The climate in Trás-os-Montes region 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, but in a very irregular regime, with wet winters usually followed by dry ones. The sub-climatic region where S. Serrada is located is characterised by a mean annual precipitation over 1 300 mm and a mean annual air temperature below 8 ºC. Whereas the sub-climatic region where Azibo is located is characterised by a mean annual precipitation between 800 and 1000 mm and mean annual air temperature between 12.5 ºC and 14 ºC.

MATERIALS AND METHODS

Zooplankton samples were collected monthly in winter and biweekly in summer, throughout 2 annual cycles, from January 2000 to December 2001. On each sampling date two vertical hauls were collected in the pelagic zone in both reservoirs using a Wisconsin type net of 64 mm mesh size. Animals were anaesthetised with carbonated water and preserved in sugar saturated formaldehyde (4% v/v final concentration). Depending on density, zooplankton were counted in subsamples of 5, 10, 20 ml or in total sample. For identification of the different taxa the following keys were used: Scourfield & Harding (1966); Dussart (1969); Ruttner-Kolisko (1974) and Pontin (1978).

Water samples for soluble reactive phosphorus (SRP), total phosphorus (TP) and chlorophyll *a* (CHL *a*) determinations were taken from the upper 30-40 cm stratum directly into acid rinsed bottles and were transported to the laboratory in a cold container. SRP concentrations were estimated by the method of Murphy and 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 1 000 ml of sample water filtered through a Whatman GF/A filter no more than 2 h after collection. Concentrations were determined spectrophotometrically after overnight extraction in 90% acetone using Lorenzen's equations (Lorenzen, 1967). Environmental variables such as water temperature, dissolved oxygen, conductivity, pH, as well as nitrate ($N\text{-}NO_3$), ammonium ion ($N\text{-}NH_4$) and ammonia gas ($N\text{-}NH_3$) were measured in situ with a 6820 YSI Multiparameter Water Quality Monitor. Water transparency was measured with a 20 cm black and white Secchi disk. Carlson's Trophic State Index (TSI) (Carlson, 1977) was computed from Secchi disk transparency values (TSI (SD)), TP concentrations (TSI (TP)) and CHL *a* concentrations (TSI (CHL)).

A Kolmogorov-Smirnov test (Sokal & Rohlf, 1981) was performed to assess differences in environmental variables between years for both reservoirs. This statistical test was performed using SYSTAT 8.0. Detrended Correspondence Analysis (DCA) was used to assess the relationships between zooplankton taxa and reservoir. To determine the influence of the studied environmental variables on the species composition a Canonical Correspondence Analysis (CCA) was performed. In both analyses taxa were included only if they reached a relative abundance larger than 1%. Absolute zooplankton counts were transformed to log (x+1) and rare species were down weighted. In CCA the automatic forward selection procedure by Monte Carlo permutation tests (9999 permutations) was used to remove the redundant environmental variables, allowing the selection of those contributing most to the explanation of the whole data set (Ter Braak, 1995). The computer program CANOCO version 3.1 was used to perform both analyses.

RESULTS

Environmental factors

Environmental variables and parameters reached similar values during both years in each reservoir (Table 1), with exception of TP, SRP and CHL *a*, which slightly increased in 2001. When comparing reservoirs, however, differences were found with respect to conductivity (D_m= 1; $p<0.001$), water temperature (D_m= 0.364; $p<0.05$), water transparency (D_m = 0.42; $p<0.05$) and pH (D_m= 0.758; $p<0.001$). TP, SRP and CHL *a* concentrations were higher in S. Serrada than in Azibo, although not statistically significant. TP concentrations increased during summer and CHL *a* concentrations were higher at the end of summer than during the remaining year in both reservoirs in both study years.

Although S. Serrada could sporadically be considered oligotrophic according to the chlorophyll component of TSI, e.g. 14 and 8 in 2/6/00 and in 19/1/01, respectively, both reservoirs were classified as meso-eutrophic according to this trophic index. Actually, in S. Serrada TSI (TP) varied between 48 and 72 in 2000 and between 58 and 75 in 2001; TSI (SD) ranged between 38 and 60 in 2000 and between 37 and 60 in 2001; TSI (CHL) varied between 14 and 51 in 2000 and between 8 and 54 in 2001. In Azibo TSI (TP) ranged between 55 and 69 in 2000 and between 58 and 71 in 2001; TSI (SD) varied between 34 and 54 in 2000 and in 2001 between 35 and 54; TSI (CHL) ranged between 13 and 40 in 2000 and between 17 and 49 in 2001. However, TSI (SD) should be regarded with caution both in fall 2000 and in winter 2001 when reservoirs received abnormally high amounts of particulate matter transported in surface runoff.

Zooplankton communities

Zooplankton species/genera found in both reservoirs (Table 2) exhibited annual variation in abundance. Species composition in both communities also changed from one year to the next. During both years of study the zooplankton community of S. Serrada was characteristically dominated by Rotifera or small Cladocera (mainly *Ceriodaphnia quadrangula*), with a seasonal shift in dominance occurring in late summer and fall, when *Tropocyclops prasinus* became the dominant species (Fig.1, A). The zooplankton community of Azibo was dominated by Cladocera or by Copepoda in both years, except in 13/7/01 when a bloom of the rotifer *Polyarthra* sp. occurred (Fig.1, B).

In S. Serrada the densities of some Rotifera such as *Keratella quadrata*, *Synchaeta* sp., *Conochilus* sp., *Polyarthra* sp. and *Hexarthra* sp. increased in 2001. Conversely, densities of *Asplanchna* sp. decreased. *K. cochlearis* was represented in very small numbers in 2000. However, in summer 2001 a bloom of this species occurred (Fig. 2). At the end of July *K. cochlearis* densities increased from the usual values (aprox. 1 500 ind. m^{-3} in summer) to 33 000 ind. m^{-3}, and in the beginning of August to 260 530 ind. m^{-3}. By the end of this month the density was 630 ind. m^{-3}. Because of problems with graphic scaling this taxon is not represented in figure 2. The representativity of the Cladocera was similar in both years. Concerning Copepoda, in 2001 nauplii became more abundant and the littoral copepods *Macrocyclops albibus* and *Eucyclops serrulatus* were not found.

In Azibo in 2001 densities of Rotifera and Copepoda slightly increased. Concomitantly, Cladocera densities decreased in 2001. From 2000 to 2001 a modification in the rotifer community occurred. In 2000 *Asplanchna* sp. was dominant, whereas in 2001 *K. cochlearis* and *Synchaeta* sp. were the dominant species. *Gastropus* sp. also became more abundant in 2001. *Polyarthra* sp., which was almost always poorly represented (0-700 ind. m^{-3}) during the two years of study, became the dominant taxon of all zooplankton community in 13/7/2001 (Fig. 3). At this time this taxon reached a density of 32 500 ind. m^{-3}. Because of problems with graphic scaling this taxon is not represented in figure 3. Concerning Cladocera, *Daphnia longispina* was represented in all samples gathered in 2000 and in summer was practically replaced by *Ceriodaphnia pulchella*, which became dominant until the end of autumn. However, in 2001 *Daphnia* was not observed from July to October and *Ceriodaphnia* was dominant most of the time. *Diaphanosoma* was observed early in 2001 and was not found in August. In the Copepoda community nauplii densities strongly increased in 2001. *Acanthocyclops robustus* densities decreased from 2000 to 2001. The results of DCA support what was stated above; there is not only evidence of the existence of seasonal and annual variations in zooplankton communities of each reservoir, but also variations between the two reservoirs are perceived (Fig. 4). In fact, species joint together such as *A. rubustus*, *Copidodiaptomus numidicus*, *Diaphamosoma* sp., *C. pulchella* and *Daphnia pulex* were only found in Azibo. *Gastropus* sp. and *Bosmina* sp. were found in both reservoirs but in higher densities in Azibo. On the other hand, *Synchaeta* sp., *Conochilus* sp., and *Hexarthra* sp. which were found in both ecosystems, were better represented in S. Serrada. Species such as *T. prasinus*, *M. albibus, E. serrulatus* and *C. quadrangula* only occurred in S. Serrada.

Influence of environmental factors on zooplankton

The ordination diagram depicted on figure 5 resulted from CCA analysis applied to the most correlated environmental factors and zooplankton species. Axes 1 and 2 together explained 42.2% of the variance in species abundance data. The species-environment correlations were strong; 0.980 for axis 1 and 0.712 for axis 2. With forward selection and Monte Carlo permutation test, a sub-set of environmental factors that significantly ($p<0.05$) explained the variation in species data was identified. In descending order of significance, the factors included in that sub-set were: Conductivity (Cond.), water temperature (Temp.), TP, SRP, CHL *a*, pH and dissolved oxygen (DO). In this study some species were found to be associated to an increase in conductivity. These are the copepods *A. robustus*, and *C. numidicus*, and the cladocerans *Bosmina longirostris* and *Daphnia longispina*. Other taxa such as *Diaphamosoma brachyurum, Ceriodaphnia pulchella, Daphnia pulex*, *Gastropus* sp. and nauplii of *A. robustus* and of *C. numidicus* were associated, not only to the increasing conductivity, but also to high temperatures, high pH and high DO concentrations (taxa found only or more abundant in Azibo). Whereas, *T. prasinus* and nauplii, *Conochilus* sp., *Keratella quadrata*, *C. quadrangula*, *M. albidus* and *E. serrulatus* were related to lower values of all those variables and parameters (taxa associated to S. Serrada Reservoir). *Synchaeta* sp., *K. cochlearis*, *Polyarthra* sp., *Hexarthra* sp. and the nauplii of *T. prasinus* seemed to be associated to high concentrations of TP and CHL *a*. *Asplanchna* sp., *Alona* sp. and *Chydorus spaericus* can be found along the SRP axis in the multivariate analysis diagram.

DISCUSSION

The observed differences in temperature, conductivity and pH of the studied reservoirs might be a consequence of reservoir location in different sub-climate and geological zones. Age and the degree of disturbance caused by anthropogenic activities also influence reservoir features such as water transparency, nutrient concentrations and consequently the trophic state. Both S. Serrada and Azibo were classified as meso-eutrophic, according to TSI, during the study period (only sporadically, S. Serrada was found oligotrophic). However, TSI (TP) and TSI (SD) were slightly higher in S. Serrada than in Azibo. One could expect S. Serrada to be oligo- or mesotrophic because reservoirs and lakes located in similar climatic and geological regions of the Iberian Peninsula and subjected to similar human influence were generally found in those trophic conditions (Boavida, 2000; Negro *et al.*, 2000). The observed resuspension of bottom sediments and organic matter following water runoff input in S. Serrada, plus the turbulence generated by the rise in water level, and the periodic exposure of littoral sediments to cycles of alternating drying and wetting could explain the high phosphorus concentrations and consequently the TSI (TP) values computed for this reservoir. This assumption is supported by the results obtained by Fabre (1988) and Watts (2000a; 2000b) in reservoirs where water level fluctuation was accentuated as well. The frequent fires induced by shepherds in the surrounding land to obtain better graze can also lead to a potential increase in nutrients such as nitrogen and phosphorus in this reservoir. In the present case there are no studies quantifying the nutrient inputs caused by soil loss subsequent to those fires, but some research developed in other regions of Portugal has shown that a consequence of fire might be the increase in trophic state of the adjacent water bodies (Shakesby *et al.*, 1993).

Differences in zooplankton abundance and composition were found between both reservoirs and years of study as documented by DCA. Specialists on small particle feeding which food preferences are mostly detritus-bacteria and small phytoplankton (Pejler, 1983) were dominant in the S. Serrada community. According to Sladeček (1983), taxa such as *Keratella*, *Hexarthra*, and *Conochilus* only take particles smaller than 10 mm. *T. prasinus* also feeds on detritus, bacteria and small phytoplankton (Dussart, 1969). In fact, in unstable condition reservoirs, Rotifera and small Cladocera evidence adaptive advantage over larger Cladocera and Copepoda, not only on account of their feeding behaviour, but also because they are r-strategists. Actually, they have shorter generations and a greater tolerance to turbidity (Schmid-Araya & Zuñiga, 1992; Seda & Devetter, 2000). According to Bays & Crisman (1983) and Pejler (1983) this kind of community is typical of eutrophic systems. In fact, considering the high TSI values obtained for this reservoir, the observed zooplankton community pattern was expected. However, TSI values were not significantly different from those obtained for Azibo. Therefore, it can be concluded that the zooplankton community in S. Serrada was more a reflex of the emptying pattern and other factors than of trophic state. It could also be a consequence of reservoir age. In fact, because this is a recently created reservoir, it is possible that a “stable” community was not established yet (Robarts *et al.*, 1992).

Zooplankton community in Azibo was dominated by Cladocera and Copepoda. According to several studies (Bays & Crisman, 1983; Pejler, 1983; Siegfried & Kopache, 1984; Schmid-Araya & Zuñiga, 1992; Seda & Devetter, 2000) this pattern suggests a low level of disturbance and a medium degree of trophy. The dominance of the calanoid copepod *C. numidicus* over the cyclopoid copepod *A. robustus* is another evidence of a medium degree of trophy (Caramujo & Boavida, 2000). However, an increase in small particle feeding rotifers was observed in 2001. It is not clear whether this was an indication of a tendency to increased trophic state or just a consequence of the atypically rainy winter.

The low zooplankton densities and the significant changes in the abundance of several species observed in 2001 could be related to the atypical, high precipitation occurred from November 2000 to March 2001. In fact, over this period the total rainfall recorded at the weather station located at Escola Superior Agrária de Bragança was 1 412 mm (AgroClima pers. comm.). After the unusual rainy events, the water residence time of the reservoirs declined, and consequently a considerable flushing effect could have occurred on both reservoirs, inducing the exportation of zooplankton, together with their immature instars and food sources. This fact could have affected the recruitment of most species. Hatching of resting eggs laying on the sediments would have permitted the habitat recolonisation (Hairston *et al.*, 2000; Crispim & Watanabe, 2001). However, according to Rey (1984), in general only 10-25% of the total zooplankton assemblages produce resting eggs. As a result, after an adverse period it is expected that zooplankton densities be lower for some time because the recolonisation processes starting from resting eggs are slow.

CCA revealed that the environmental variables and parameters conductivity, water temperature, TP, SRP, CHL *a*, pH and dissolved oxygen together only explained 42.2% of the variation in species composition. No other variables tested were statistically significant. This suggests a potentially significant role for biotic processes. Local biotic factors such as competition, predation, and food quality, among others, could be important community structuring forces (Lampert & Sommer, 1997; Wetzel, 2001). An example that might evidence biotic and abiotic factors acting together is the replacement of *D. longispina* by the small cladoceran *C. pulchella* which occurred in the summer of 2000 in Azibo. This phenomenon, also observed by Vasconcelos (1990a) in this reservoir, was possibly explained by changes in phytoplankton community (Hulsmann & Weiler, 2000), by the greater efficiency of *Ceriodaphnia* to feed at temperatures above 20 ºC (Lynch, 1978) and by the increasing predation by 0+ or planktivorous fish over *Daphnia* (Siegfried & Kopache, 1984). Another argument is that different fish assemblages can distinctly impact zooplankton assemblage patterns. Actually, in Azibo the introduction of pike in the nineteen nineties lead to a decrease in the resident cyprinid fish densities (A. J. Albuquerque, unpubl. bachelor thesis). Cyprinids are not strictly planktivorous, yet they can have some impact on *Daphnia* and on other large-bodied zooplankton in some lakes (Winfield & Townsend, 1992). The impact of those predators is less important on Copepoda because their escape power is greater than that of *Daphnia* (Visman *et al.*,1992). Conversely, S. Serrada fish assemblage seemed to be dominated by the cyprinid *Leuciscus carolitertti*. Thus, the highest densities of *Daphnia* observed in Azibo were related not only to the environmental variables and parameters mentioned above, but probably also to differences in the fish assemblages. In fact, in 1987/1988 Rotifera dominated the zooplankton community (Vasconcelos, 1990a). This can be explained by the age of Azibo at the time those previous studies were undertaken or, more plausibly, by the fish assemblage, which before pike introduction was mainly composed of cyprinids. Another difference between both systems, potentially affecting predation pressure on large-bodied Cladocera, was the occurrence in S. Serrada of Chaoboridae midge larvae (A. Geraldes unpubl. data), which also feed on *Daphnia* (Lampert & Sommer, 1997).

The results of the present study can support the following conclusion: Even though conductivity, water temperature, TP, SRP, CHL *a*, pH and dissolved oxygen explained some of the variability, abundance and composition of zooplankton communities in the reservoirs, other factors such as age of reservoirs, the anthropogenic disturbance degree, reflected in the water level fluctuation, and the biotic interactions also seemed to play an important role in the structuring of zooplankton communities. It is desirable that any further limnological studies actually contribute to fully assess the relative importance of all those different variables for the studied communities. The obtained data constitute a preliminary study and might be a basis to develop management plans to mitigate some negative impacts resulting from anthropogenic activities performed in the reservoirs and catchments. Furthermore, data obtained in this study will allow monitoring the efficiency of those plans by detecting changes in those communities, which can be related to changes in water quality and in the overall ecosystem health.

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Table 1- Environmental factors recorded for S. Serrada and Azibo reservoirs in 2000 (A) and in 2001 (B). Minimum-maximum range, with mean/standard deviation between brackets; (Mean/SD).

A

	S. Serrada	**Azibo**
Water temperature (ºC)	1.46-21.39 (12.51/6.59)	5.58-24.7 (16.52/6.13)
Dissolved oxygen (mg l^{-1})	7.38-12.42 (8.55/1.45)	7.54-11.53 (9.09/1.06)
Conductivity (µS cm^{-1})	4-10 (6.94/1.84)	51-81 (69.87/11.09)
Water transparency (m)	4.50-1.00 (2.66/1.09)	1.5-6.0 (4.72/1.25)
pH	5.77-6.56	6.71-8.05
$N\text{-}NO_3$ (mg l^{-1})	0.30-8.00 (2.05/2.25)	0.50-14.80 (5.59-5.12)
$N\text{-}NH_4$ (mg l^{-1})	0.00-0.30 (0.08/0.09)	0.00-0.30 (0.13/0.11)
$N\text{-}NH_3$ (mg l^{-1})	Not detectable	Not detectable
TP (µg l^{-1})	38.89-113.22 (59.07/27.20)	34.28-89.69 (59.23/18.80)
SRP (µg l^{-1})	0.00-33.36 (9.72/7.78)	0.00-23.86 (7.47/6.38)
Chlorophyll *a* (µg l^{-1})	0.00-7.97 (2.49/2.38)	0.00-2.73 (1.02/0.84)

B

	S. Serrada	**Azibo**
Water temperature (ºC)	2.70-20.19 (12.90/6.46)	8.06-23.83 (17.16/5.73)
Dissolved oxygen (mg l^{-1})	6.20-10.72 (8.55/1.49)	7.21-10.78 (8.99/1.33)
Conductivity (µS cm^{-1})	3-8 (5.95/1.61)	43-66 (56.23/7.64)
Water transparency (m)	1-5 (2.85/1.14)	1.5-5.5 (3.50/1.14)
pH	5.95-8.34	6.64-8.36
$N\text{-}NO_3$ (mg l^{-1})	0.10-17.8 (5.96/10.43)	0.20-18.8 (6.38/6.50)
$N\text{-}NH_4$ (mg l^{-1})	Not detectable	0.00-0.10 (0.01/0.03)
$N\text{-}NH_3$ (mg l^{-1})	Not detectable	Not detectable
TP (µg l^{-1})	41.52-134.74 (78.97/23.04)	41.70-102.84 (67.12/16.77)
SRP (µg l^{-1})	0.00-22.61 (6.47/6.75)	1.38-15.30 (4.02/3.45)
Chlorophyll a (µg l^{-1})	0.1-10.45 (3.10/3.64)	0.53-6.57 (2.26/1.95)

Table 2- Zooplankton species/genera found in both reservoirs.

S. Serrada	**Azibo**
Rotifera	**Rotifera**
Asplanchna priodonta (Aspl.)	*Asplanchna priodonta (Aspl.)*
*Collotheca sp.**	*Collotheca mutabilis**
Conochilus sp. (Cono.)	*Collotheca sp.**
*Euchelanis sp.**	*Conochilus sp. (Cono.)*
Gastropus sp. (Gast.)	*Filinia sp.**
Hexarthra sp. (Hexa.)	*Gastropus sp. (Gast.)*
Keratella cochlearis (Kcoc.)	*Hexarthra sp. (Hexa.)*
Keratella quadrata (Kqua.)	*Keratella cochlearis (Kcoc.)*
*Ploesoma sp.**	*Keratella quadrata (Kqua.)*
Polyarthra sp. (Poly.)	*Ploesoma sp.**
Synchaeta sp. (Sync.)	*Polyarthra sp. (Poly.)*
*Trichocerca sp.**	*Pompholix sulcata**
	Synchaeta sp. (Sync.)
	*Testudinella sp.**
	*Trichotria sp.**
Cladocera	**Cladocera**
Alona sp. (Alon.)	*Alona* sp. (Alon.)
*Alona costata**	*Alona costata**
*Alona quadrangularis**	*Alona quadrangularis**
*Alona rectangula**	*Alona rectangula* *
Bosmina longirostris (Blon.)	*Bosmina longirostris* (Blon.)
Ceriodaphnia quadrangula (Cqua.)	*Ceriodaphnia pulchella* (Cpul.)
Chydorus sphaericus (Chid.)	*Chydorus sphaericus* (Chid.)
Daphnia longispina (Dlon.)	*Daphnia longispina* (Dlon.)
Simocephalus sp.*	*Daphnia pulex* (Dpul.)
	Diaphanosoma brachyurum (Diap.)
Copepoda	**Copepoda**
Eucyclops serrulatus (Eser.)	*Acanthocyclops robustus* (Arob.)
Macrocyclops albidus (Malb.)	*Copidodiaptomus numidicus* (Cnum.)
Tropocyclops prasinus (Tpra.)	Nauplii (Naua)
Nauplii (Naus)	

* occasional species/genera; between brackets taxa abbreviation used in DCA and CCA

.

Figure captions

Figure 1- Relative abundance (%) of Rotifera, Copepoda and Cladocera in S. Serrada (A) and Azibo (B) reservoirs.

Figure 2 - Annual variation of zooplankton abundance and composition in S. Serrada Reservoir.

Figure 3 - Annual variation of zooplankton abundance and composition in Azibo Reservoir.

Figure 4 - Results of DCA. Scatters with samples: S. Serrada – black circles; Azibo – black squares (A) and with species (B). For a better understanding in Scatter A it was decided not to use complete sample date, but only the month. In summer months with biweekly samples the notation must be read according to the following example: Jun 00 means the first sample performed on June 2000 and Jun'00 means the second sample. In the same way Jun 01 means the first sample performed on June 2001 and Jun'01 the second sample.

Figure 5 - Results of CCA. Species abbreviations are the same as in table 2.

Figure 1.

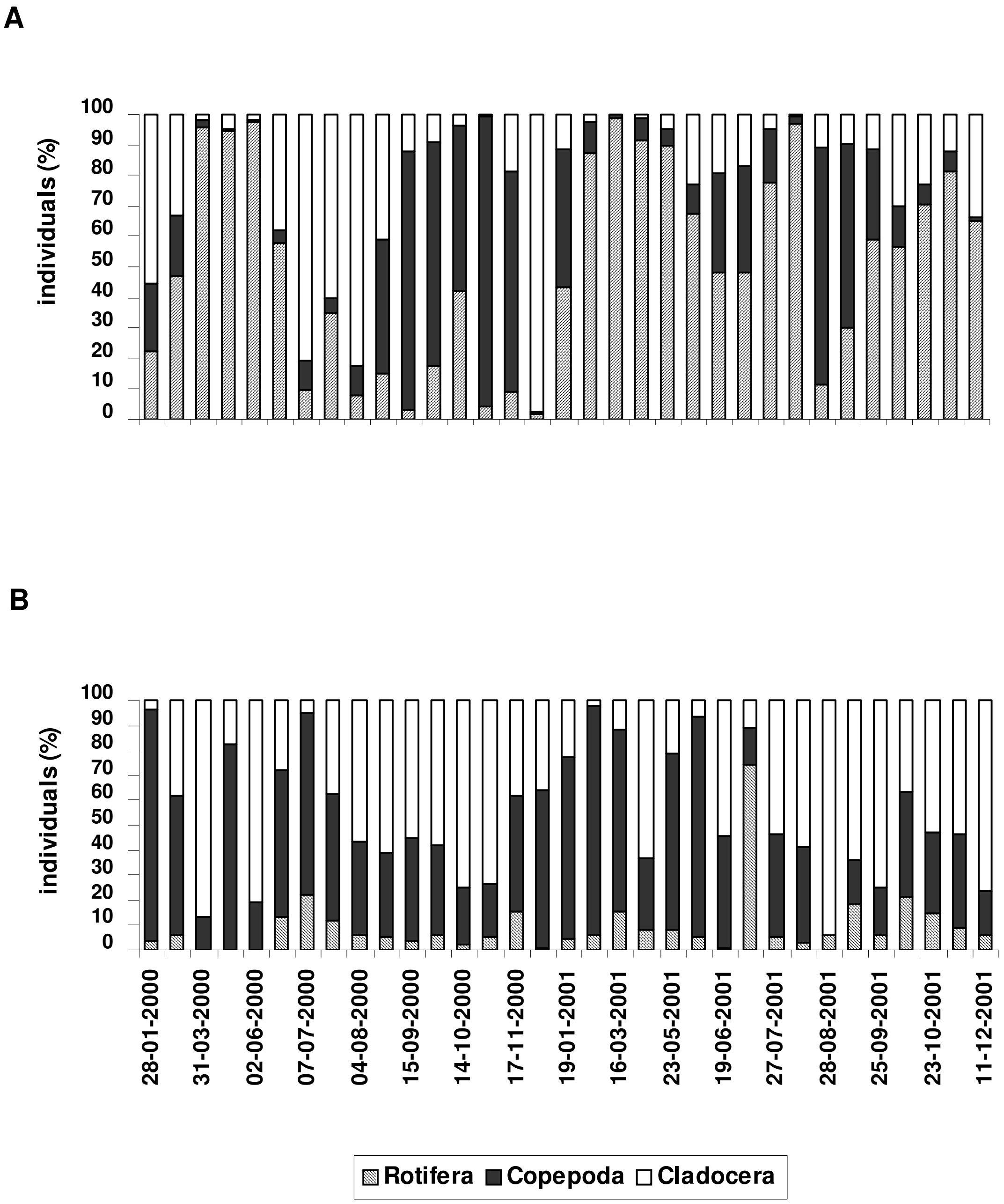

A
individuals (%)
100
90
80
70
60
50
40
30
20
10
0
B
individuals (%)
28-01-2000
31-03-2000
02-06-2000
07-07-2000
04-08-2000
15-09-2000
14-10-2000
17-11-2000
19-01-2001
16-03-2001
23-05-2001
19-06-2001
27-07-2001
28-08-2001
25-09-2001
23-10-2001
11-12-2001
Rotifera
Copepoda
Cladocera

Figure 2.

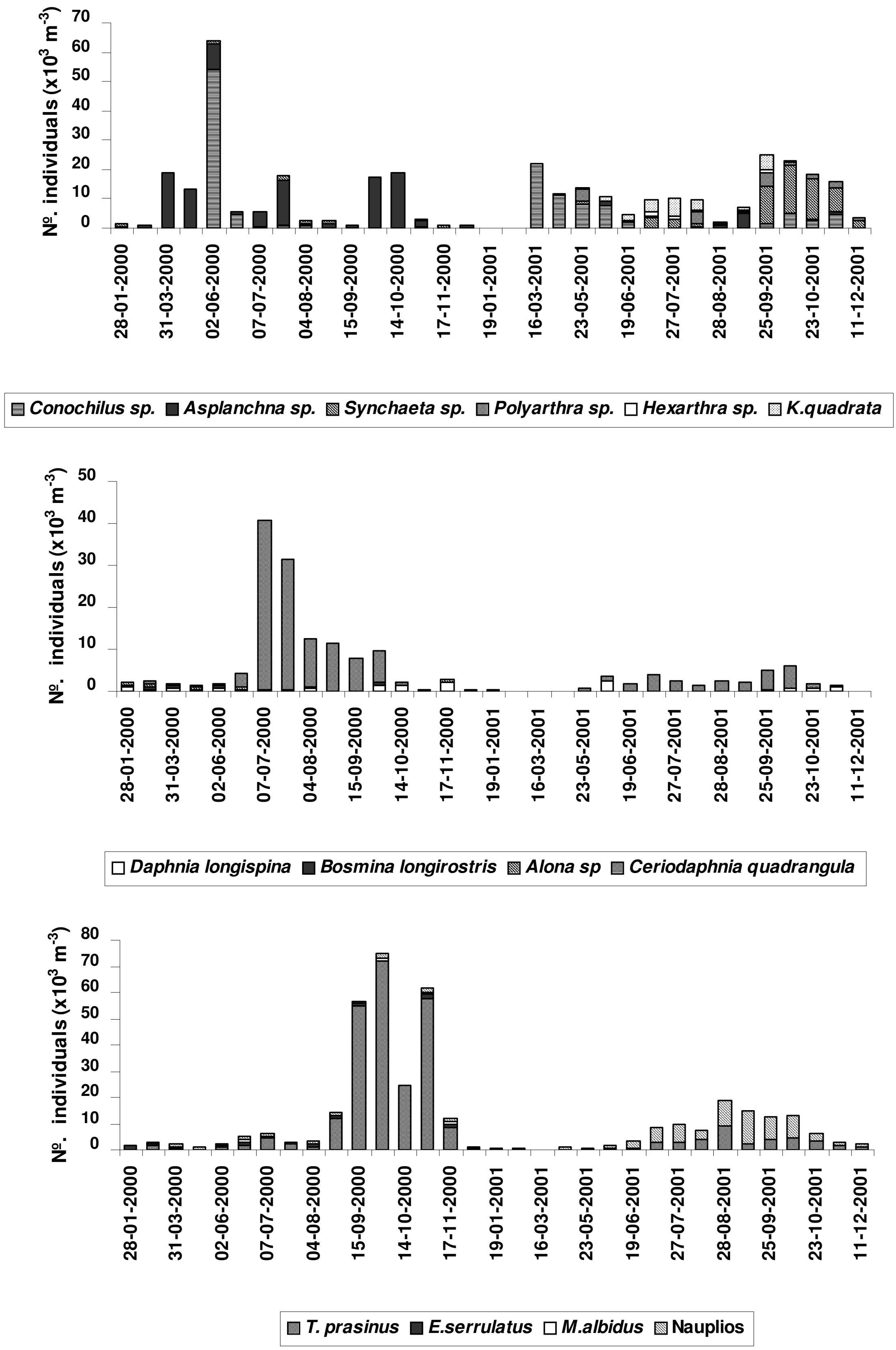
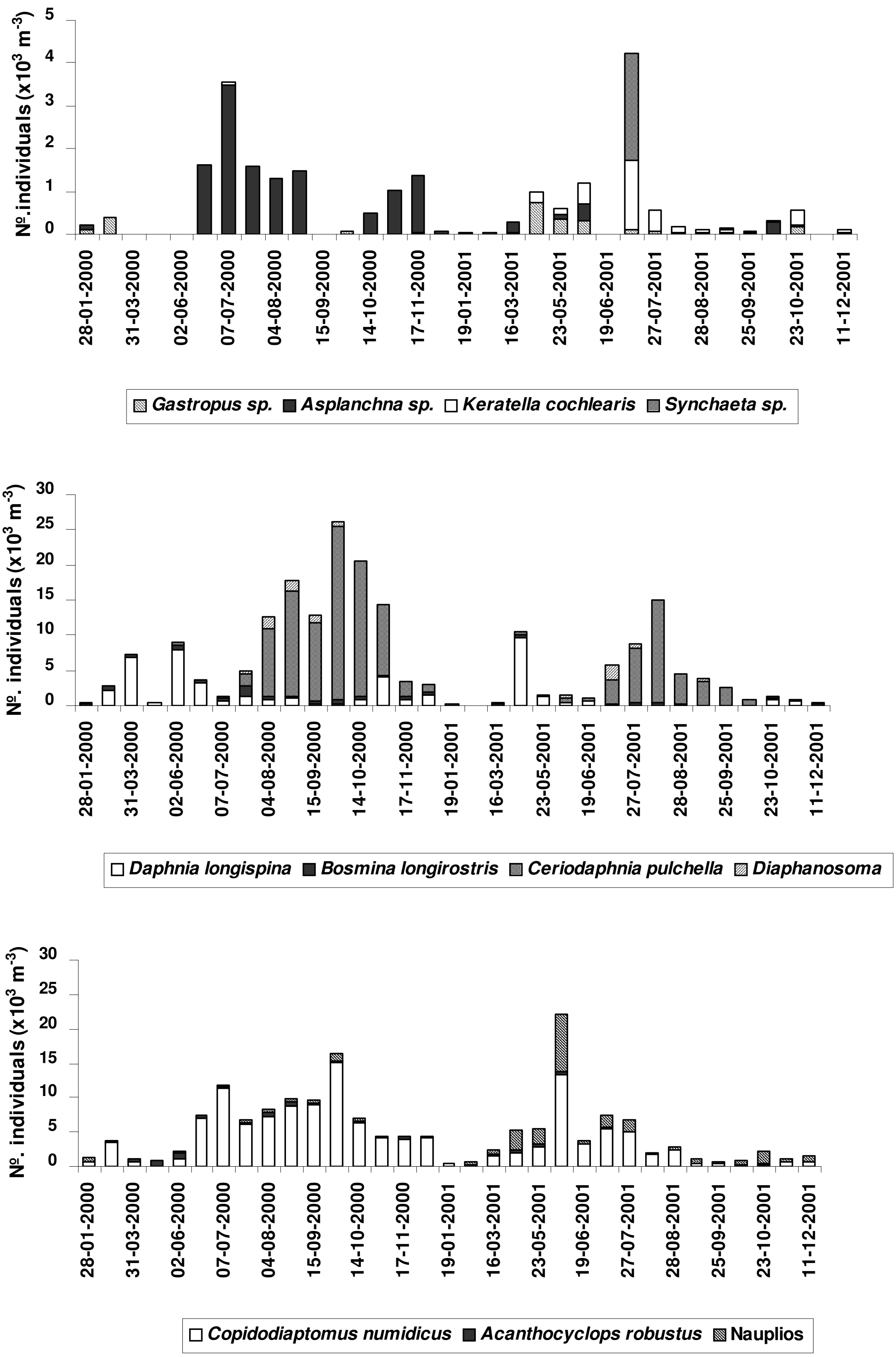

Nº. individuals (x10³ m⁻³)
70
60
50
40
30
20
10
0
28-01-2000
31-03-2000
02-06-2000
07-07-2000
04-08-2000
15-09-2000
14-10-2000
17-11-2000
19-01-2001
16-03-2001
23-05-2001
19-06-2001
27-07-2001
28-08-2001
25-09-2001
23-10-2001
11-12-2001
Conochilus sp.
Asplanchna sp.
Synchaeta sp.
Polyarthra sp.
Hexarthra sp.
K.quadrata
Nº. individuals (x10³ m⁻³)
50
40
30
20
10
0
Daphnia longispina
Bosmina longirostris
Alona sp
Ceriodaphnia quadrangula
Nº. individuals (x10³ m⁻³)
80
70
60
50
40
30
20
10
0
T. prasinus
E.serrulatus
M.albidus
Nauplios

Figure 3.


Nº.individuals (x10³ m⁻³)
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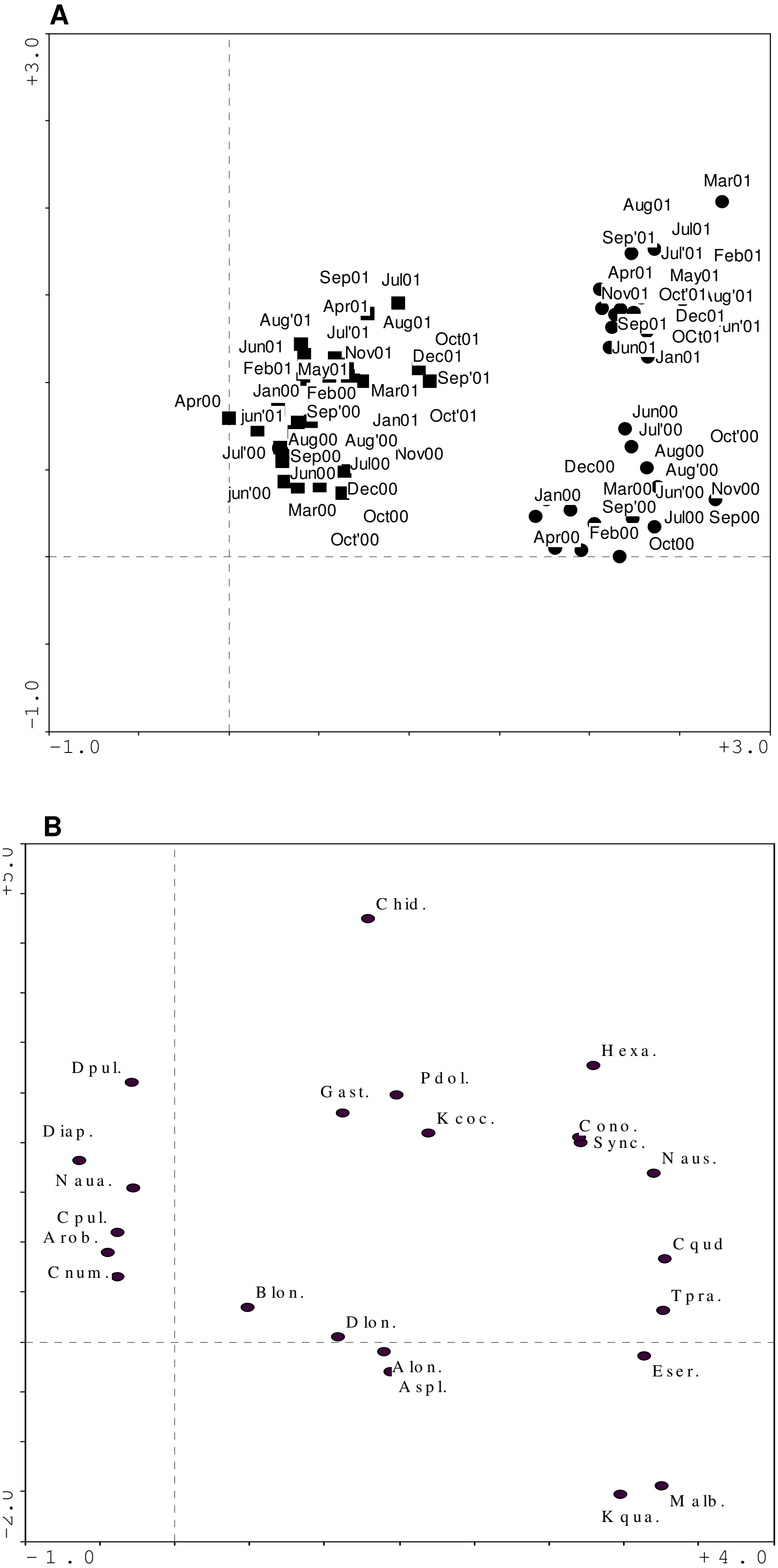
4
3
2
1
0
28-01-2000
31-03-2000
02-06-2000
07-07-2000
04-08-2000
15-09-2000
14-10-2000
17-11-2000
19-01-2001
16-03-2001
23-05-2001
19-06-2001
27-07-2001
28-08-2001
25-09-2001
23-10-2001
11-12-2001
Gastropus sp.
Asplanchna sp.
Keratella cochlearis
Synchaeta sp.
Nº. individuals (x10³ m⁻³)
30
25
20
15
10
5
0
Daphnia longispina
Bosmina longirostris
Ceriodaphnia pulchella
Diaphanosoma
Copidodiaptomus numidicus
Acanthocyclops robustus
Nauplios

Figure 4.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-1.0
+3.0
Mar01
Aug01
Jul01
Sep'01
Jul'01
Feb01
Apr01
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Oct'01
Dec01
Sep01
OCt01
Jun01
Jan01
Sep01
Jul01
Apr01
Aug'01
Aug01
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May01
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Jan00
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Mar01
Apr00
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Sep'00
Jan01
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Aug00
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Mar00
Oct00
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Aug00
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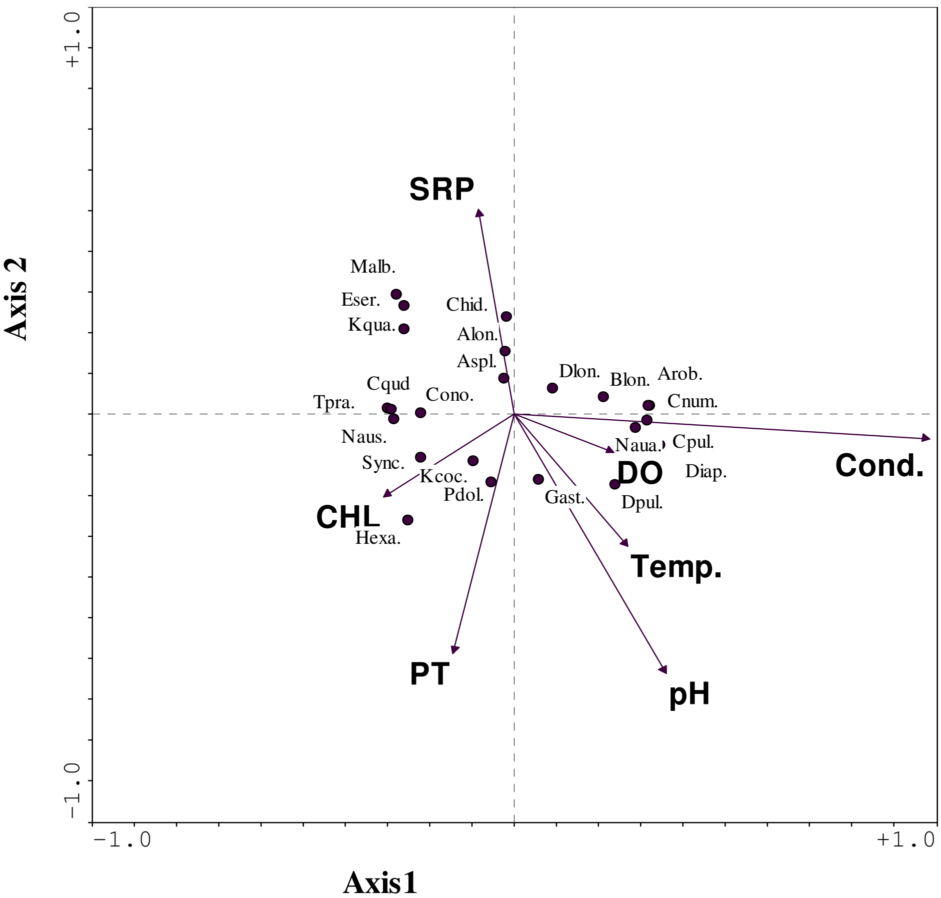
Jan00
Mar00
Jun'00
Nov00
Sep'00
Jul00
Sep00
Apr00
Feb00
Oct00
B
+5.0
-2.0
-1.0
+4.0
C hid.
H exa.
D pul.
P d o l.
G ast.
K c o c.
D iap.
C o n o.
S y n c.
N aus.
N aua.
C pul.
A ro b.
C qud
C num.
T pra.
B lo n.
D lo n.
A lo n.
E ser.
A spl.
M alb.
K qua.

Figure 5.


+1.0
-1.0
-1.0
+1.0
Axis 2
Axis1
SRP
Malb.
Eser.
Kqua.
Chid.
Alon.
Aspl.
Dlon.
Blon.
Arob.
Cnum.
Cqud
Cono.
Tpra.
Naus.
Sync.
Kcoc.
Pdol.
Gast.
Naua.
Cpul.
Diap.
DO
Dpul.
Cond.
CHL
Hexa.
Temp.
PT
pH

WHAT FACTORS INFLUENCE THE CLADOCERAN ASSEMBLAGE OF A MESO-EUTROPHIC RESERVOIR?

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ABSTRACT

Cladoceran assemblage patterns were investigated in a meso-eutrophic reservoir from January 2000 till December 2002. Trends of environmental factors (water temperature, conductivity, pH, and nutrient concentrations), as well as of biotic factors (availability of potentially edible phytoplankton and densities of herbivorous and carnivorous copepods) were assessed. Zooplankton community was dominated by Cladocera and Copepoda. Cladoceran assemblage patterns were identified by performing a cluster analysis on abundance data of the most abundant species: *Daphnia longispina*, *Ceriodaphnia pulchella, Bosmina longirostris* and *Diaphanosoma brachyurum*. In winter and spring, *Daphnia* was dominant. In early summer and end of autumn, *Ceriodaphnia* and *Daphnia* coexisted. During summer, *Ceriodaphnia* was dominant and coexisted with *Diaphanosoma*. *Bosmina* was present in low densities during the whole study period. Cladoceran populations exhibited non-synchronous peaks of abundance, evidencing different ecological optima. Temperature seemed to be the main structuring factor of this assemblage. The way biotic interactions influenced cladoceran assemblage in this reservoir was not clear and needs further research.

Key-words: Zooplankton, Cladocera, Copepoda, environmental factors, phytoplankton availability

INTRODUCTION

Zooplankton composition, abundance and dynamics are structured by both environmental factors and biotic interactions (Wetzel 2001). The understanding of the way those structural forces interact constitutes an important source of information to implement correct management practices, in order to maintain water quality of reservoirs in a perspective of multiple-use approach. Cladocerans are important components of lakes and reservoirs, because of their significant role in the functioning of those ecosystems. As grazers they may influence phytoplankton dynamics and consequently the relationships between chlorophyll and nutrients (Lampert & Sommer 1997, Tessier et al. 2001). Cladocerans also regulate bacterioplankton and protozoan populations as well as detritus availability, because under certain conditions they feed on those items (Schneirder-Olt & Adrian 2001). Consequently, they will interfere on the epilimnetic cycle of organic matter by reducing seston concentrations, thus modifying particle sedimentation rates (Bossard & Uehlinger 1993, Sommer et al. 2003). On the other hand, cladocerans are important in food web linkage. They can be an important food source for carnivorous copepods (Kerfoot 1978), for aquatic insect larvae such as those of Chaoboridae (Schum & Maly 2000), and for 0+ fish and adult planktivorous fish (Siegfried & Kopache 1984, Beklioglu & Moss 1996).

This paper focuses on the cladoceran assemblage patterns in Azibo Reservoir, an impoundment built in the Portuguese part of River Douro catchment. The main goals of the study were: (1) to characterise cladoceran assemblage patterns; (2) to assess the influence of environmental factors upon this assemblage; (3) to determine the availability of the potentially edible phytoplankton; (4) to determine the densities of the copepods *Copidodiaptomus numidicus* Gurney (a potential competitor) and *Acanthocyclops robustus* Sars (a potential predator); (5) to attempt to qualitatively evaluate the potential impact of fish predation on cladoceran assemblage.

STUDY AREA

Azibo Reservoir is located in the Portuguese part of River Douro catchment. The area of the reservoir is 410 ha and its total capacity is 54470 x 10^3 m^3. Maximum depth is about 30 m, mean depth is 13.2 m. This reservoir was filled for the first time in 1982 and it is used mainly for recreation. Minor uses are water supply and irrigation. Water level annual fluctuations are not very accentuated, varying between 1.5 and 2 m. The direct influence of human activities on the impoundment is greatest during summer, when the reservoir and surroundings are used for recreation such as fishing, swimming, camping and boating. Farming and grazing (mainly sheep) are activities found all over the year in the surroundings. In this area, annual precipitation varies between 800 and 1000 mm and mean annual air temperature ranges between 12.5 ºC and 14 ºC. During the period of study thermal stratification occurred from June to October and the reservoir was classified as meso-eutrophic. Further information concerning this reservoir can be found in Geraldes & Boavida (2003).

MATERIALS AND METHODS

Samples were collected monthly in winter and biweekly in summer, from January 2000 to December 2002 at one single sampling station, located at the deepest point of the reservoir. Zooplankton samples were obtained on each sampling date by taking two vertical hauls using a Wisconsin type net of 64 µm mesh size. Animals were anaesthetised with carbonated water and preserved in sugar-saturated formaldehyde (4% v/v final concentration). Depending on density, zooplankton were counted in 5, 10, 20 ml subsamples or in total sample. Animals were always identified to species level, according to Scourfield & Harding (1966) and Dussart (1969); for phytoplankton analysis integrated water samples were collected from the euphotic zone. Samples were fixed "in situ" in Lugol's solution. Phytoplankton were counted according to Utermöhl (1958), using an inverted microscope at 200x/400x magnification. Algae were identified to genus, according to Bourrelly (1966, 1968, 1970). Fish assemblage characterisation was based on local angler information.

Water samples for soluble reactive phosphorus (SRP) and total phosphorus (TP) determinations were obtained from the upper 30-40 cm stratum, from the middle water column (7 to 10 m) and from the bottom, into acid rinsed bottles and were transported to the laboratory in a cold container. SRP concentrations were estimated by the method of Murphy & Riley (1962) and TP was assessed after acid hydrolysis with persulfate for 60 min under high temperature and pressure (APHA 1989). Chlorophyll *a* (CHL *a*) was obtained from 500 to 1000 ml of integrated water sample filtered through a Whatman GF/A filter no more than 2 h after collection. Concentrations were determined spectrophotometrically after overnight extraction in 90% acetone. Environmental variables such as water temperature, dissolved oxygen, conductivity, pH, as well as nitrate ($N\text{-}NO_3$), ammonium ion ($N\text{-}NH_4$) and ammonia gas (N-NH_3) were measured in situ at 1m intervals with a 6820 YSI Multiparameter Water Quality Monitor. Water transparency was measured with a 20 cm diameter black and white Secchi disk.

To characterise cladoceran assemblage patterns, the general procedure recommended by Green & Vascotto (1978) was used. Patterns were identified by performing a cluster analysis (UPGMA method, Pearson distance measure). This analysis was performed on log (x+1) transformed abundance data of the dominant cladoceran species. Subsequent to this analysis, a Kruskal-Wallis test was carried out for each environmental factor, for phytoplankton and for copepod densities. This procedure was performed in order to determine whether the obtained clusters varied significantly from each other in what concerned mean value of each environmental factor and each alga and copepod mean densities. All statistical analyses were performed using SYSTAT 8.0.

RESULTS

Zooplankton community description and characterisation of cladoceran assemblage

Cladoceran species recorded in Azibo were: *Daphnia longispina* Müller, *Ceriodaphnia pulchella* Sars, *Bosmina longirostris* Müller, *Diaphanosoma brachyurum* Liéven, *Alona costata* Sars, *Alona rectangula* Sars, *Alona quadrangularis* Müller, *Chydorus sphaericus* Müller and *Daphnia pulex* De Geer. However, *Alona* spp., *Chydorus sphaericus* and *Daphnia pulex* were only found in less than 5% of total samples in very low densities and consequently they were considered as occasional and were not taken into account in the statistical analyses. Although not the main focus of investigation, Rotifera and Copepoda were recorded because these organisms are important components of the zooplankton and because of their trophic interactions with cladocerans. Rotifera were dominated by *Polyarthra* spp., *Asplanchna priodonta* Gosse, *Keratella cochlearis* Gosse, and *Conochilus* spp.. Two species of Copepoda were found; the calanoid *Copidodiaptomus numidicus* and the cyclopoid *Acanthocyclops robustus*, the former dominating over the latter. Since Rotifera was not a very representative taxon in this reservoir (Fig. 1) only interactions between Cladocera and Copepoda were focused. *Daphnia* densities ranged between 27 and 8021 ind./m^3 in 2000, between 0 and 9549 ind./m^3 in 2001 and between 0 and 1560 ind./m^3 in 2002. *Ceriodaphnia* varied between 0 and 24605 ind./m^3 in 2000, between 8 and 14610 ind./m^3 in 2001 and between 0 and 14197 ind./m^3 in

2002. *Bosmina* ranged from 0 to 1401 ind./m^3 in 2000, from 3 to 509 ind./m^3 in 2001 and from 0 to 637 ind./m^3 in 2002. Even at times in low densities, this cladoceran was almost always present in all obtained samples. *Diaphanosoma* was present only in summer months and during this season its density varied between 419 and 1783 ind./m^3 in 2000, between 96 and 2196 ind./m^3 in 2001 and between 64 and 5061ind./m^3 in 2002 (Fig. 2). Considering *Daphnia*, *Ceriodaphnia*, *Bosmina* and *Diaphanosoma* densities, three clusters were obtained (Fig. 3): (A) *Dapnhia* was dominant and coexisted with *Bosmina* (samples obtained from January to June 2000 and from January to April 2002); (B) *Daphnia*, *Ceriodaphnia* and *Bosmina* coexisted (samples obtained in 2 June 2000, July 2000, from October 2000 to May 2001, from October 2001 to December 2001, from May to June 2002 and from 17 October to December 2002); (C) *Ceriodaphnia* was dominant and coexisted with *Bosmina* and *Diaphanosoma*. *Daphnia* was not detected or was present at very low densities (samples obtained from August to September 2000, from June to September 2001 and from July to 3 October 2002).

Influence of environmental, abiotic factors upon cladocerans

Variables such as precipitation, water transparency, water temperature, conductivity, pH, TP and CHL *a* were significantly different among clusters. DO, N-NO_3 and SRP only showed significant differences in the middle water column and/or at the bottom. Those differences were related to the stratification period. In fact, part of the samples that integrated cluster B and those integrating cluster C were obtained during stratification. As N-NH_3 was not detected in most samples, data concerning this variable are not shown (Table 1). When *Daphnia* was dominant (Cluster A) mean values of water temperature, conductivity and TP were the lowest. When *Daphnia* and *Ceriodapnhnia* coexisted (Cluster B) mean values of precipitation and CHL *a* were the highest, and mean values of water transparency were the lowest. When *Ceriodapnhnia* dominated and coexisted with *Diaphanosoma* (Cluster C) mean values of water temperature, transparency, conductivity and TP were the highest. Concomitantly, mean values of precipitation and CHL *a* were the lowest.

Influence of environmental, biotic factors upon cladocerans

Availability of edible phytoplankton

Samples grouped in cluster A were dominated by *Paulschulzia* sp. (Chlorophyceae), which represented more than 50 % of all identified taxa. Samples grouped in cluster B were dominated by the centric diatoms. *Cyclotella ocellata* Pantocksek constituted about 99 % of all centric diatoms identified. *Chlamydomonas* like cells and *Anabaena* were also abundant. Samples grouped in cluster C were dominated by *Cyclotella ocellata*. In all clusters, small Chlorophyceae such as *Scenedesmus* spp., *Cosmarium* spp., *Monoraphidium* spp., *Crucigenia* spp., and *Oocystis* spp. were present in very low densities. *Cryptomonas* spp. were also always present in slightly higher densities. The densities of *Chlamydomonas* like cells, centric diatoms, *Paulschulzia* sp., *Fragilaria* spp., *Asterionella formosa* Hassal and *Ceratium hirundinella* Schrank significantly differed among clusters (Table 2).

Interactions with a potential competitor

Since *C. numidicus* is herbivorous, it was considered to be a potential competitor of cladoceran species. This copepod was always very abundant in Azibo. In addition to the high densities observed all year round, population peaks occurred at the beginning and at the end of summer (Fig.4). Mean densities obtained for each cluster were the following: (A) 2302.3 ind/m^3; (B) 3868.6 ind/m^3 and (C) 6254.5 ind/m^3. Kruskal-Wallis test evidenced significant differences among clusters (χ^2 = 7.97; $p<0.05$). Nauplii were considered together because both *C. numidicus* and *A. robustus* nauplii are herbivorous, therefore functionally alike, and could also act as potential competitors of cladocerans. For cluster A, mean nauplii densities were 916.9 ind/m^3. For cluster B, mean nauplii densities were 750.5 ind/m^3. For cluster C mean nauplii densities were 1542.9 ind/m^3. No significant differences were found among clusters (χ^2 = 4.99; $p>0.05$).

Interactions with potential predators

A. robustus is carnivorous and consequently was considered to be a potential predator of the cladocerans. Densities of *A. robustus* were always low, ranging between 0 and 796 ind/m^3 (Fig. 4). However, this copepod was present in most samples taken during the study period. For cluster A the observed mean density was 229.6 ind/m^3. For cluster B it was 134.9 ind/m^3. For cluster C mean density was 175.1 ind/m^3. No significant differences were found among clusters (χ^2 = 1.97; $p>0.05$).

During the present study, Chaoboridae larvae were not detected in samples. Concerning fish, only qualitative information provided by anglers was available. Fish assemblage was composed of: (1) endemic cyprinid fish – Iberian barbel (*Barbus bocagei* Steindachner) and Iberian nase (*Chondrostoma polylepis* Steindachner); (2) carp (*Cyprinus carpio* Linnaeus); (3) exotic piscivorous – blackbass (*Micropterus salmoides* Lacepede) and pike (*Esox lucius*

Linnaeus). According to anglers information it seems that pike became dominant over the other species. This could be a consequence of the intensity of pike predation over cyprinids.

DISCUSSION

Influence of environmental, abiotic factors

In Azibo, a seasonal succession was apparent in the cladoceran assemblage, with one or more species dominating in turn as changing conditions and resources became suitable for each. During the winter and early spring months *Daphnia* was dominant and *Ceriodaphnia* was virtually absent. After this period, *Daphnia*'s population decreased till undetectable levels. At the same time *Ceriodaphnia*'s population increased to its maximum density, becoming dominant from July to September. During this period *Daphnia* was not detected in samples, meaning that it was present in extremely low densities. Lynch (1978) also observed the replacement of *Daphnia* by C*eriodaphnia* in summer and attributed this phenomenon to the better efficiency of *Ceriodaphnia* to feed at temperatures above 20ºC. Tessier et al. (2001) found some evidence that *Ceriodaphnia* seems to have higher requirements for phosphorus than *Daphnia*. Considering that highest water temperatures and TP concentrations occurred in summer in Azibo, it can be concluded that in the winter and early spring months *Daphnia* had adaptive advantage over *Ceriodaphnia*, whereas in summer it was the other way around.

Influence of environmental, biotic factors

Composition of the phytoplankton community can both influence and be influenced by the observed trends in cladoceran assemblage, although only a small fraction of phytoplankton is edible by herbivorous zooplankton. In fact, the ability to ingest algae is largely determined by algal cell size, and edible algae are defined as those species with maximum dimension smaller than 50 µm. Inedible species are larger (Lampert & Sommer 1997, Murdoch et al. 1998). The main algal groups generally referred to as included in the diet of herbivorous zooplankton are: *Chlamydomonas* like cells, *Scenedesmus*, *Crucigenia*, *Monoraphidium*, *Cosmarium*, *Navicula*, *Nitzschia*, *Cyclotella* and *Cryptomonas*. (Canter-Lund & Lund 1995, González 1998, Gladyshev et al. 1999). All taxa mentioned above, except *Cyclotella* and *Clamydomonas* like cells, were always in very low densities in Azibo. According to Murdoch et al. (1998) herbivorous zooplankton, especially *Daphnia*, are able to suppress the biomass of edible algae far below the level set by nutrients. In fact, considering TP concentrations, Azibo was classified as meso-eutrophic. However, CHL *a* concentrations were lower than in typical meso-eutrophic reservoirs of the same region (Geraldes & Boavida 2003). Thus, results of the present study can be explained by the grazing pressure over edible algae, first by *Daphnia* and subsequently by C*eriodaphnia* and *Diaphamosoma*. *C. numidicus* and nauplii are herbivorous, too. Consequently, they could also have exerted some grazing pressure on phytoplankton. However, this interpretation should be regarded with some caution, because it can be misleading. First of all, phytoplankton assemblages are not only regulated by phytoplankton − cladoceran/copepod grazing interactions, but also by phytoplankton − protozoan and phytoplankton − fungi interactions and nutrient concentrations as well. Besides, environmental factors also play an important role in phytoplankton dynamics (Carrillo et al. 1995, Medina-Sánchez et al. 1999). Furthermore, Cladocera and *C. numidicus* are not strictly herbivorous, but also feed on bacteria, protozoans and detritus (Kerfoot & Kirk 1991, González, 1998 Gladyshev et al. 1999, Schneider-Olt & Adrian 2001). Considering the previous statements, a question arises: Do competitive interactions exist between those species in this reservoir? Data obtained in this study do not provide an objective answer to this question. In fact, several authors reported the existence of competitive interactions in experimental conditions between *Daphnia* and *Ceriodaphnia* (Lynch 1978, Smith & Cooper 1982), between *Daphnia* and *Bosmina* (Kurmayer 2001) and between *Daphnia* and *Diaphanosoma* (Matveev 1987). However, in Azibo, a lack of synchrony in population peaks of *Daphnia* and *Ceriodaphnia*, on one hand, and of *Daphnia* and *Diaphanosoma* on the other hand, was observed. Therefore, competitive interactions were probably avoided, because species have different ecological optima and consequently utilise resources differently. Nevertheless, there were periods of *Daphnia* − *Ceriodaphnia* and of *Ceriodaphnia* − *Diaphanosoma* coexistence. In addition, *Bosmina* coexisted with the other cladocerans. Furthermore, the herbivorous copepod *C. numidicus* was always present in relatively high densities and so were the nauplii of both copepod species. However, coexistence does not ensure the existence of competition because, in general, species explore different ecological niches and resources are not always limiting. In fact, those cladocerans, as well as *C. numidicus*, have different morphological mechanisms for food resource exploitation, which will be reflected on the size of particles each can ingest. According to Lampert & Sommer (1997), the upper particle size boundary for small cladocerans is about 20 µm, while for large cladocerans and copepods that limit is about 50 µm. It can then be concluded that those species might exploit different food resources as a strategy to avoid direct competition. Moreover, as stated above, cladocerans and herbivorous copepods can feed on a broad range of items unselectively: Phytoplankton, protozoans, bacteria and detritus.

Competitive interaction between cladoceran and *C. numidicus*, if actually existed, could have been attenuated by the potential predator – prey relationship that might also exist between *C. numidicus* and *A. robustus*. The evidence that *C. numidicus* was a prey for *A. robustus* was pointed out by Caramujo et al. (1997) in a reservoir located at the Portuguese part of Tagus River watershed where the two species coexisted. However, in Azibo, *A. robustus* densities were very low and the probable predation pressure over *C. numidicus* or over some of the cladocerans might have had no significant impact on those populations. According to several authors, in the cladoceran assemblage large-bodied cladocerans were especially susceptible to fish predation (Beklioglu & Moss 1996, Caramujo et al. 1997, Lampert & Sommer, 1997). Siegfried & Kopache (1984) observed that the increased predation by 0+ fish and by planktivorous fish at the beginning of summer lead to a decline in *Daphnia* population. Up to the nineteen eighties cyprinid fish were dominant in Azibo (Formigo, 1990). Consequently, they could have had some impact on the cladoceran assemblage. According to Vasconcelos (1990) at this time the zooplankton community was dominated by rotifers. This might have been an evidence of the existence of a considerable predation pressure by fish. Cyprinids are not strictly planktivorous, but they can have some impact on *Daphnia* and on other large-bodied zooplankton in some lakes and reservoirs (Winfield & Towsend 1992). However, introduction of pike (*Esox lucius*) in the nineteen nineties lead to an accentuated decrease of the resident cyprinid fish densities. Therefore, the impact of cyprinid predation on cladoceran and copepod assemblages might have been minimised. On the other hand, pike alevins feed on *Daphnia* and copepods during the first few weeks after absorbing the yolk sac and before they are able to feed on macroinvertebrates (Hunt & Carbine 1951). In this way, very young pike might have some temporary impact on cladoceran and copepod assemblages as well.

CONCLUSIONS

The present results suggest that abiotic factors such as temperature had a direct and strong influence on cladoceran assemblage patterns. However, the existing complex abiotic/biotic interactions and their structural role on the cladoceran assemblage are not fully understood. Thus, further research will be required to clarify points concerning: (1) cladoceran grazing preferences and their impact on phytoplankton community; (2) the existence of competitive relationships among cladocerans and *C. numidicus*; (3) the predation pressure by *A. robustus* upon cladocerans and *C. numidicus* (4) the intensity and seasonality of fish predation; (5) regulation of mentioned items by abiotic factors. These topics will be better clarified after complementary experimental field and laboratory work. Although further research is needed the present study can be regarded as the first evaluation of the ecological role of the cladoceran zooplankton. This reservoir is located in a region where the absence of industrial activity, concomitantly with the low rentability of agriculture, are leading to human desertification. The implementation of new activities such as boating, fishing, pedestrian walks, hunting, bird watching and rural tourism in the reservoir and in its catchment may constitute economic alternatives to local populations. However, to make those alternatives possible, it is necessary to improve and maintain water quality on a multiple use perspective. In the future, long-term data on the cladoceran assemblage will certainly allow to monitor and predict the effects of human activities on reservoir ecological processes and consequently on water quality. Furthermore, those data will add to the understanding of the limnology of reservoir ecosystems influenced by Mediterranean climate.

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Table 1- Mean values of the environmental variables calculated for clusters of samples and results of Kruskal-Wallis test. * p<0.05; ** p<0.01.

Variables	Clusters A	Clusters B	Clusters C	χ^2
Precipitation (mm)	61.2	132.5	33.3	11.18**
Water transparency (m)	3.8	3.7	5.1	8.73*
Water temperature (ºC)				
Surface	10.5	15.0	21.8	24.95**
Middle water column	11.9	14.0	19.5	19.68**
Bottom	9.6	10.4	10.9	5.16
Dissolved oxygen (mg l^{-1})				
Surface	9.2	8.7	8.7	1.22
Middle water column	9.1	8.7	7.9	6.00
Bottom	8.2	5.1	1.2	21.97**
Conductivity (µS cm^{-1})				
Surface	55	59	69	14.86**
Middle water column	56	57	65	8.32*
Bottom	55	56	60	2.68
pH				
Surface	6.9-7.4	5.4-8.1	7.0-8.5	7.43*
Middle water column	6.9-8.2	6.6-8.5	6.6-8.4	5.21
Bottom	6.7-7.7	5.2-8.0	6.2-7.6	1.25
N-NO_3 (mg l^{-1})				
Surface	2.2	6.8	3.5	5.34
Middle water column	1.4	7.2	2.6	8.00*
Bottom	1.1	6.3	1.3	11.47**
N-NH_4 (mg l^{-1})				
Surface	0.1	0.5	0.6	1.08
Middle water column	0.1	0.6	0.9	2.66
Bottom	0.1	0.9	1.7	1.57
TP (µg l^{-1})				
Surface	49.4	61.6	69.9	10.73*
Middle water column	67.2	65.4	80.8	7.74*
Bottom	55.9	75.7	119.0	23.15**
SRP (µg l^{-1})				
Surface	5.5	6.3	5.9	0.20
Middle water column	5.7	6.3	10.8	9.31*
Bottom	5.4	10.0	15.7	11.09**
Chlorophyll *a* (µg l^{-1}) Integrated sample	1.4	2.1	0.9	8.28*

Table 2- Mean densities (ind.l^{-1}x 10^3), percentage of occurrence (in brackets) of the main phytoplankton taxa in each cluster and results of Kruskal-Wallis test * p<0.05; ** p<0.01.

Phytoplankton	**Clusters**			χ^2
	A	**B**	**C**	
Dimensions: < 50 µm				
Chlorophyceae				
Scenedesmus spp.	0.25 (0.40)	1.66 (0.78)	1.49 (0.43)	2.34
Cosmarium spp.	0.21 (0.34)	0.38(0.18)	3.71 (1.07)	2.03
Monoraphidium spp.	0.32 (0.50)	1.20 (0.56)	0.11 (0.03)	4.93
Tetraedron spp.	0.13 (0.20)	0.63 (0.30)	0.46 (0.13)	0.62
Crucigenia spp.	0.30 (0.48)	0.80 (0.38)	9.38 (2.70)	0.38
Oocystis spp.	0.86 (1.36)	0.28 (0.13)	0.33(0.09)	4.56
Chlamydomonas like cells	0.26 (0.41)	49.15(23.11)	57.83 (16.65)	7.47*
Bacillariophyceae				
Small pennate diatoms	1.11 (1.76)	0.69 (0.33)	0.58 (0.17)	1.08
Centric diatoms	5.96 (9.46)	108.0 (50.78)	257.82 (74.25)	8.34*
Cryptophyceae				
Cryptomonas spp.	5.93 (9.41)	6.11 (2.87)	8.53 (2.46)	0.15
Dimensions: > 50 µm				
Chlorophyceae				
Paulschulzia sp.	36.55 (57.96)	4.50 (2.12)	0.55 (0.16)	7.08*
Bacillariophyceae				
Fragilaria spp.	1.81 (2.87)	0.38 (0.18)	0.12 (0.04)	10.68**
Asterionella formosa	2.88 (4.57)	1.77 (0.83)	0.42 (0.12)	10.84**
Cyanophyceae				
Anabaena flos-aquae	5.99 (9.51)	35.25 (16.58)	3.72 (1.07)	0.07
Dinophyceae				
Ceratium hirundinella	0.09 (0.14)	1.60 (0.75)	2.06 (0.59)	12.13**
Chrysophyceae				
Dinobryon sp.	0.40 (0.63)	0.26 (0.12)	0.12 (0.03)	4.17

Figure legends

Figure 1- Relative abundance (%) of Rotifera, Copepoda and Cladocera in Azibo Reservoir.

Figure 2 - Seasonal and inter-annual variation of cladoceran densities in Azibo Reservoir.

Figure 3- Dendrogram depicting the three clusters resulting from multivariate analysis based on cladoceran densities.

Figure 4 - Seasonal and inter-annual variation of copepod and nauplii densities in Azibo Reservoir.

Figure 1.

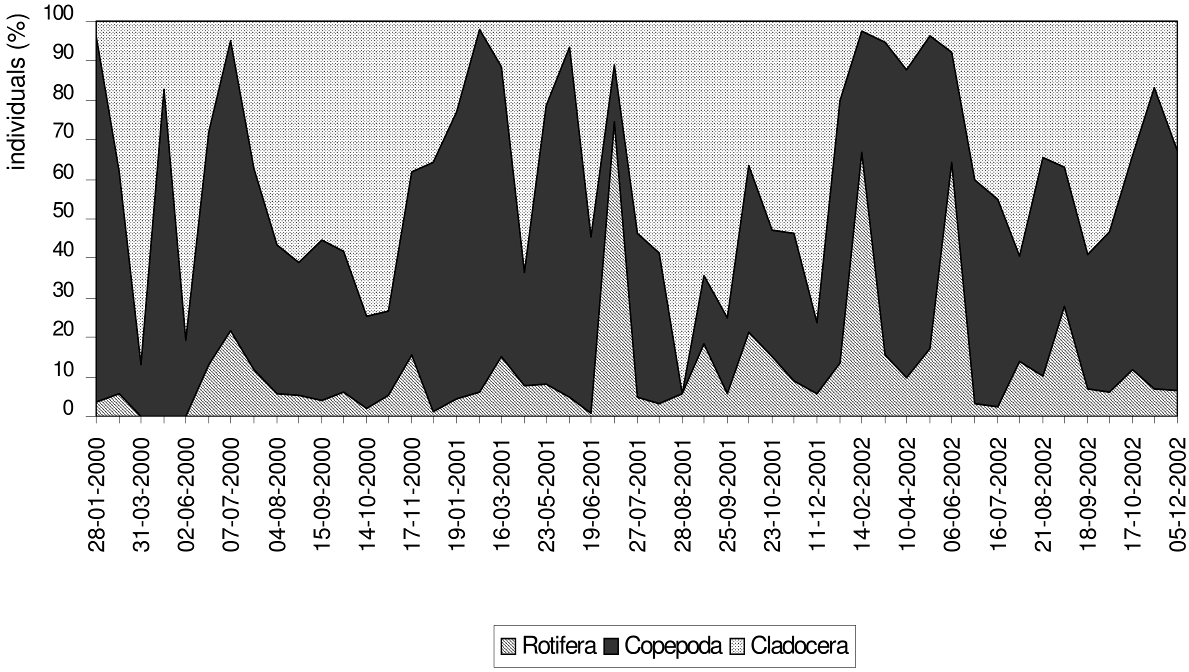

individuals (%)
100
90
80
70
60
50
40
30
20
10
0
28-01-2000
31-03-2000
02-06-2000
07-07-2000
04-08-2000
15-09-2000
14-10-2000
17-11-2000
19-01-2001
16-03-2001
23-05-2001
19-06-2001
27-07-2001
28-08-2001
25-09-2001
23-10-2001
11-12-2001
14-02-2002
10-04-2002
06-06-2002
16-07-2002
21-08-2002
18-09-2002
17-10-2002
05-12-2002
Rotifera
Copepoda
Cladocera

Figure 2.

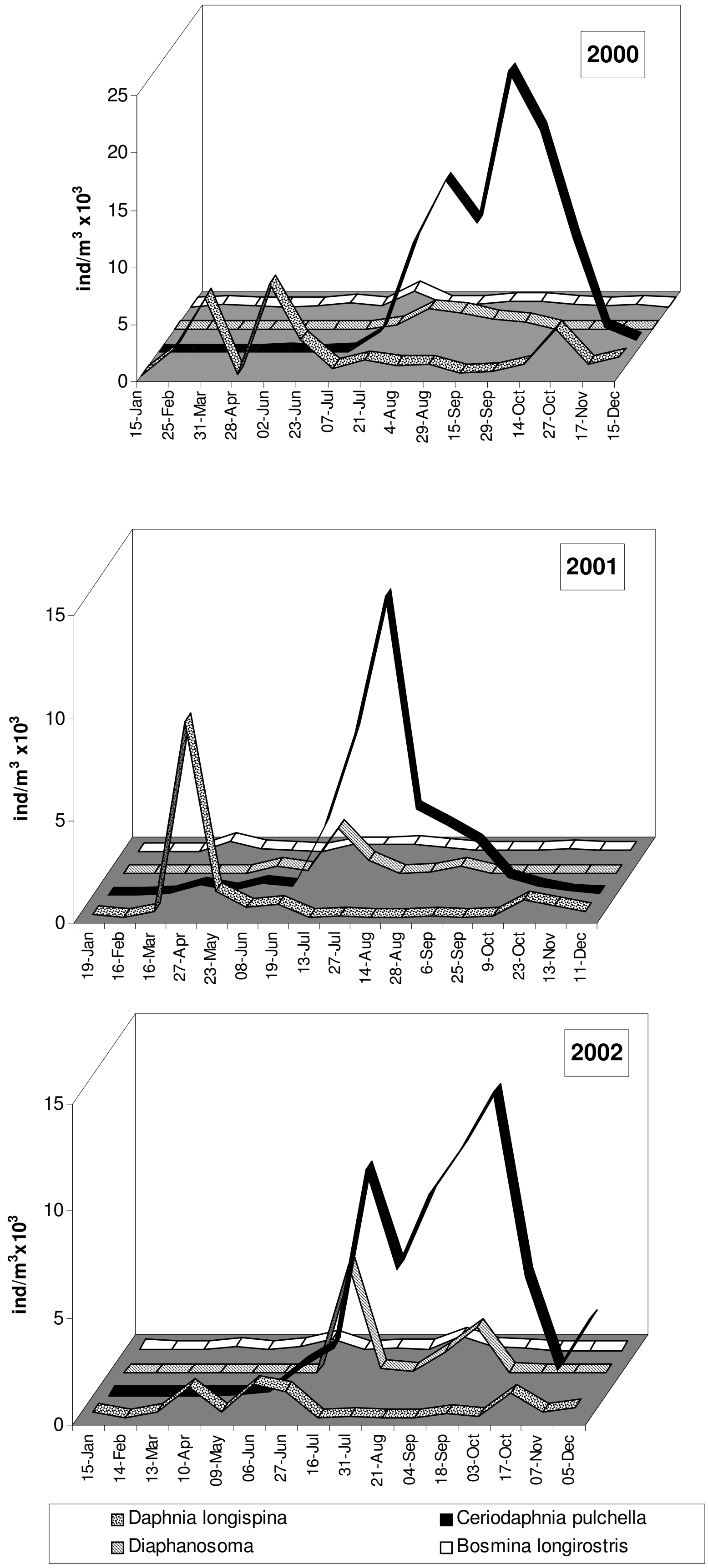

2000
ind/m3 x10^3
0
5
10
15
20
25
15-Jan
25-Feb
31-Mar
28-Apr
02-Jun
23-Jun
07-Jul
21-Jul
4-Aug
29-Aug
15-Sep
29-Sep
14-Oct
27-Oct
17-Nov
15-Dec
2001
ind/m3 x10^3
0
5
10
15
19-Jan
16-Feb
16-Mar
27-Apr
23-May
08-Jun
19-Jun
13-Jul
27-Jul
14-Aug
28-Aug
6-Sep
25-Sep
9-Oct
23-Oct
13-Nov
11-Dec
2002
ind/m3x10^3
0
5
10
15
15-Jan
14-Feb
13-Mar
10-Apr
09-May
06-Jun
27-Jun
16-Jul
31-Jul
21-Aug
04-Sep
18-Sep
03-Oct
17-Oct
07-Nov
05-Dec
Daphnia longispina
Ceriodaphnia pulchella
Diaphanosoma
Bosmina longirostris

Figure 3.

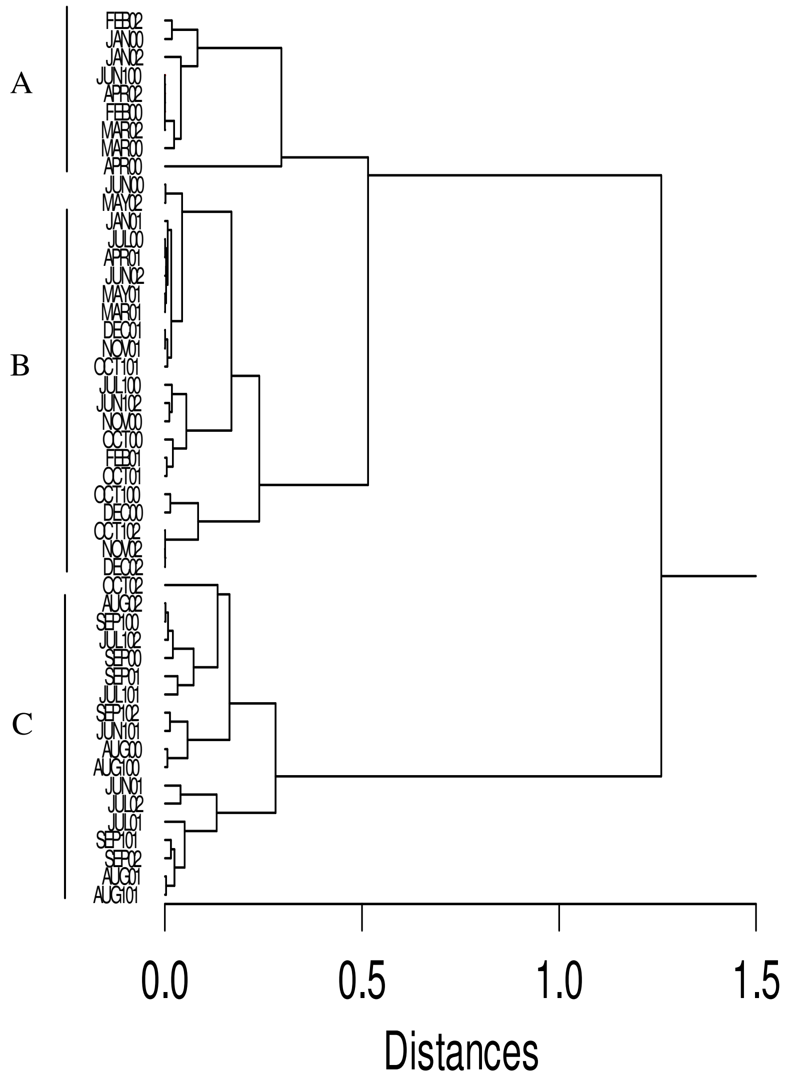
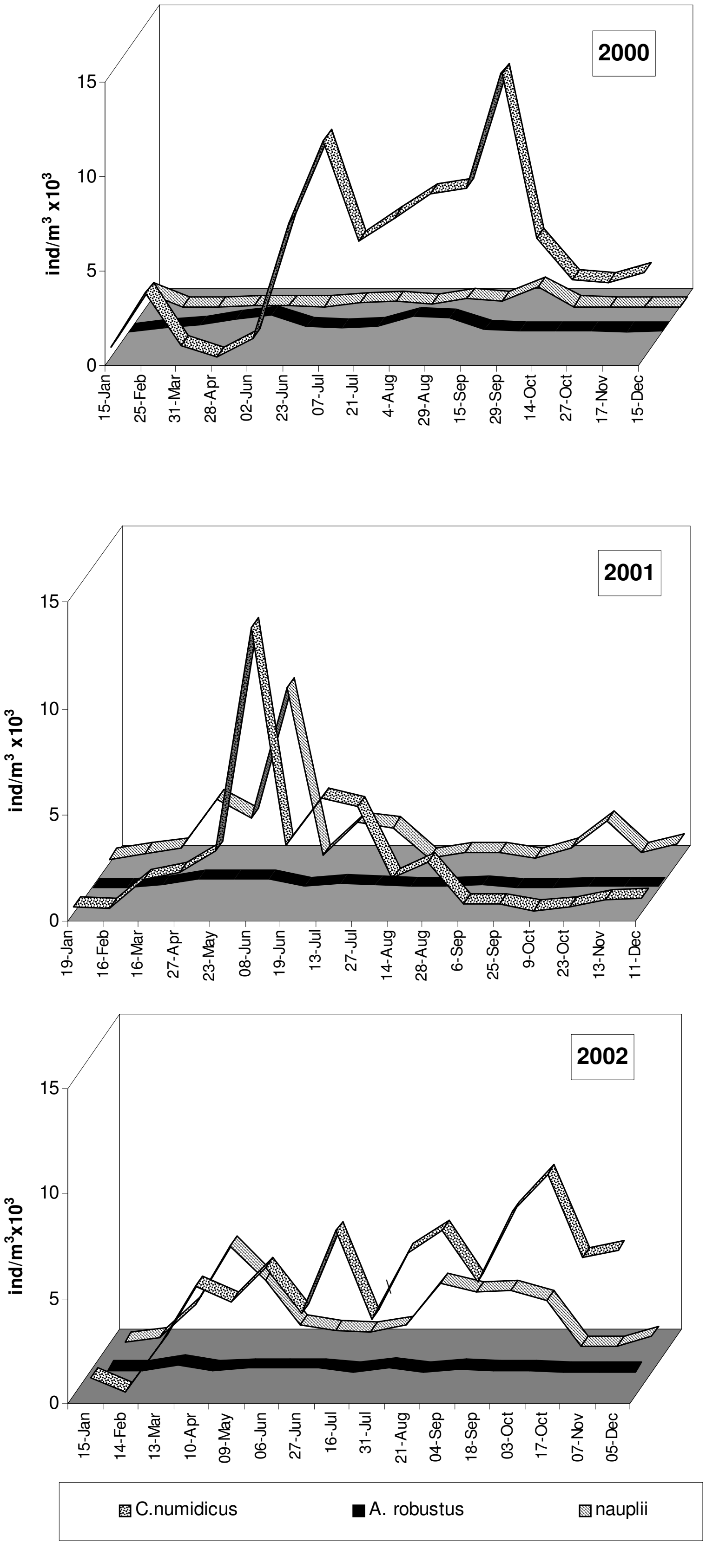

A
B
C
FEB02
JAN00
JAN02
JUN100
APR02
FEB00
MAR02
MAR00
APR00
JUN00
MAY02
JAN01
JUL00
APR01
JUN02
MAY01
MAR01
DEC01
NOV01
OCT101
JUL100
JUN102
NOV00
OCT00
FEB01
OCT01
OCT100
DEC00
OCT102
NOV02
DEC02
OCT02
AUG02
SEP100
JUL102
SEP00
SEP01
JUL101
SEP102
JUN101
AUG00
AUG100
JUN01
JUL02
JUL01
SEP101
SEP02
AUG01
AUG101
0.0
0.5
1.0
1.5
Distances

Figure 4.


2000
ind/m³ x10³
0
5
10
15
15-Jan
25-Feb
31-Mar
28-Apr
02-Jun
23-Jun
07-Jul
21-Jul
4-Aug
29-Aug
15-Sep
29-Sep
14-Oct
27-Oct
17-Nov
15-Dec
2001
ind/m³ x10³
0
5
10
15
19-Jan
16-Feb
16-Mar
27-Apr
23-May
08-Jun
19-Jun
13-Jul
27-Jul
14-Aug
28-Aug
6-Sep
25-Sep
9-Oct
23-Oct
13-Nov
11-Dec
2002
ind/m³x10³
0
5
10
15
15-Jan
14-Feb
13-Mar
10-Apr
09-May
06-Jun
27-Jun
16-Jul
31-Jul
21-Aug
04-Sep
18-Sep
03-Oct
17-Oct
07-Nov
05-Dec
C.numidicus
A. robustus
nauplii

HOW IMPORTANT ARE EMERGENT MACROPHYTES TO CRUSTACEAN ZOOPLANKTON IN A MESO-EUTROPHIC RESERVOIR?

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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 colonized by emergent macrophytes (site 2). Samples were collected biweekly from June to July 2001 and from May to July 2002 at the two sampling stations. 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 several random samples were obtained using a van Dorn bottle. Those samples were pooled together and the total sample was sieved through a 64 µm mesh size. Macrophyte coverage was visually estimated at site 2. This community was composed of *Glyceria declinata*, *Eliocharis palustris* and *Carex* sp. A Mann-Whitney U-test was carried out to test for statistically significant differences, between sites, for environmental parameters and 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 in both sites. *Daphnia* and *Bosmina* densities did not differ significantly between 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, shallow littoral zone

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 pelagic zone, whereas calanoid copepods 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 River Douro 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. Macrophytes 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 was a preliminary insight on the potential role of those temporary macrophyte communities in structuring microcrustacean assemblages, which dominated zooplankton in this reservoir (Geraldes & Boavida, submitted). Thus, the objective of this study was to assess whether cladocerans and copepods found in this reservoir preferred shallow littoral areas with macrophytes rather than pelagic waters. To achieve this objective crustacean zooplankton assemblages from the pelagic zone and from the littoral zone colonised by macrophytes were characterised and compared.

STUDY AREA

Azibo Reservoir is located in the Portuguese part of River Douro catchment. The total capacity is 54470 x 10^3 m^3 and its area is 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 all over the year in reservoir catchment 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). Macrophytes 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. 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 occurred from June to October. The reservoir was classified as meso-eutrophic (Geraldes & Boavida, 2003).

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 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 in subsamples of 5, 10, 20 ml or in 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 stratum in both sampling sites. SRP concentrations were estimated by the method of 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 spectrophotometrically 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 (Table1). In site 2 the emergent macrophyte community was composed of *Glyceria declinata*, covering 40 % of the sampled area, *Eliocharis palustris* and *Carex* sp., covering each 30 % of the sampled area.

Eleven crustacean zooplankton species were observed. Since no statistically significant inter-annual differences were found for species densities either in site 1 or in site 2, data from both years were pooled together (Table 2). *Alona rectangula*, *Alona costata*, *Alona quadrangularis* (for statistical analysis these species were grouped together as *Alona* spp.) 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 analysis these species were grouped together as *Daphnia* spp.), *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. numidicus* 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 nearshore areas because there they are more vulnerable to young fish predation. In Azibo, fish were dominated by cyprinids during the nineteen eighties (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). However, their impact on cladoceran and copepod assemblages might have been minimised, because the introduction of pike (*Esox lucius*) in the reservoir in the nineteen nineties. This fish caused an accentuated decrease of the resident cyprinid densities. Nowadays, according to angler information, pike is the dominant fish species in Azibo. Juvenile pike after absorbing the yolk sac feed on *Daphnia* and on copepods during 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" of fish predation (Gliwicz & Rykowska, 1992; Winfield & Towsend, 1992; Visman *et al*., 1994; Lampert & Sommer, 1997; Lauridsen *et al.,* 2001). However, during the period of study *Daphnia* was abundant only during the winter and early spring months, being afterwards replaced by *Ceriodaphnia*, when temperature increased (Geraldes & Boavida, submitted). Therefore, another possible interpretation is that, in this reservoir, *Daphnia* was not an important "target" for young pike. The most important "targets" might have been *Ceriodaphnia* and *Diaphanosoma*, which densities were high when young fish shoals were first noticed. Thus, the higher densities of both zooplankton genera in the pelagic zone might evidence shore avoidance behaviour and could have been 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 adult cyprinids were in very low densities. *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. The clear shore preference behaviour exhibited by *A. robustus* might have been related mainly to food availability. In fact, according to Wickham (1995), cyclopoid copepods select smaller over large prey items and consequently ciliates potentially are the preferred cyclopoid prey. In this way, considering that: (1) ciliates are more abundant in macrophyte beds (Jeppesen *et al.*, 1998); (2) *Chydorus* and *Alona* spp. are smaller than other cladocerans and were more abundant in macrophytes; (3) *Ceriodaphnia*, *Diaphanosoma* and *C. numidicus* also feed on ciliates (Schneider-Olt &

Adrian, 2001); (4) copepods are less impacted than large cladoceran by fish predation (Visman *et al.*, 1992), it is plausible to regard *A. robustus* behaviour as a response to food availability and as a strategy to avoid direct competition.

Except for plant-associated species and *A. robustus*, the macrophyte zone seemed not to be important either as refuge or as feeding habitat to crustacean zooplankton in Azibo Reservoir. However, further research is needed to elucidate: (1) diel 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; (4) the interactions between *A. robustus* and plant-associated microcrustacean/ciliates. Clarification of the above mentioned items will 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 will constitute the starting point for the above suggested research, and therefore it is worth report it for its heuristic value.

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Table 1- Mean ± SD values of the environmental variables, minimum-maximum range for pH and results of Mann – Whitney U-test.

	Site 1	Site 2	P
TP(μg l^{-1})	60.0 ± 6.3	76.7 ± 27.1	NS
SRP (μg l^{-1})	4.3 ± 2.0	9.9 ± 7.7	NS
CHL *a* (μg l^{-1})	0.8 ± 0.6	0.8 ± 0.7	NS
Water temperature (ºC)	20.3 ± 3.0	21.0 ± 3.1	NS
Dissolved oxygen (mg l^{-1})	8.8 ± 1.2	8.0 ±1.3	NS
Conductivity (μS cm^{-1})	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 2- Mean ± SD species densities (ind.m^{-3}) and results of Mann-Whitney U-test.

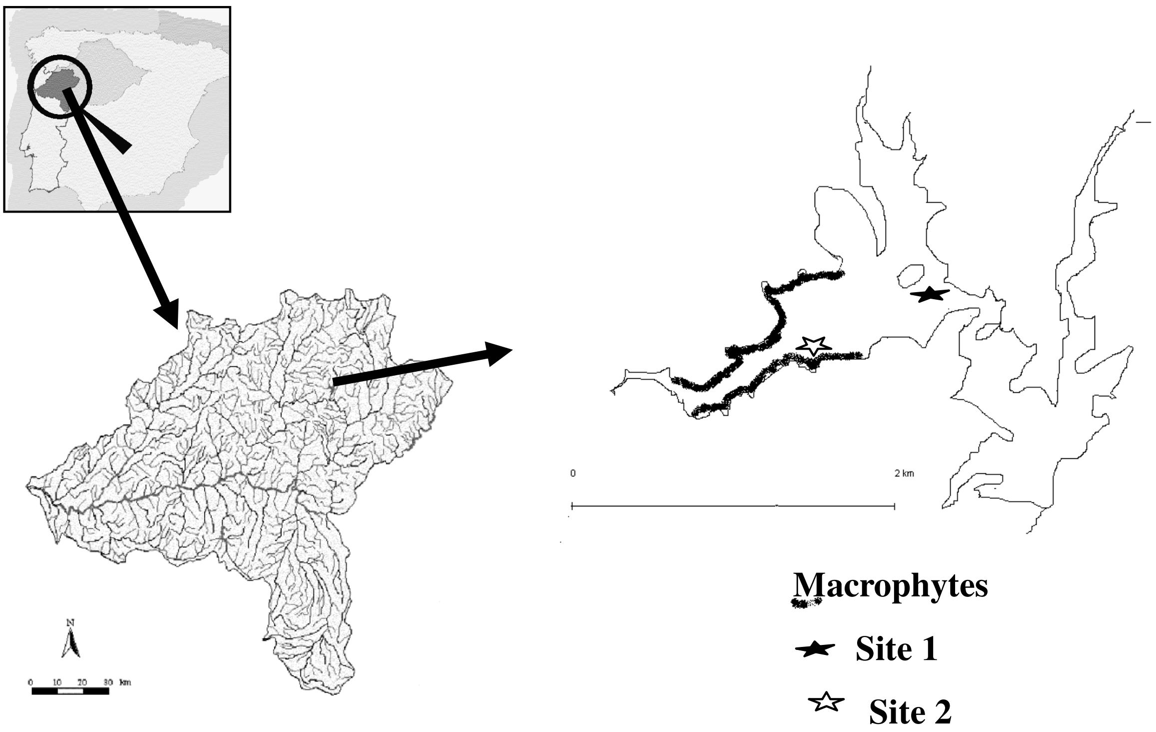
	Site 1	Site 2	P
Cladocera			
Alona spp.	0.0	4863.9 ± 8168.2	**
Bosmina longirostris	104.6 ± 184.7	44.5 ± 51.3	NS
Ceriodaphnia pulchella	1161.8 ± 1239.2	203.2 ± 300.5	*
Chydorus sphaericus	0.0	646.8 ± 959.4	**
Daphnia spp.	595.7 ± 610.8	158.7 ± 196.4	NS
Diaphanosoma brachyurum	1105.0 ± 1916.2	22.2 ± 34.0	*
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

Figure captions

Figure 1- Azibo Reservoir with indication of sampling sites.

Figure 1.



6. ACTIVIDADE DA FOSFATASE EM DUAS ALBUFEIRAS COM CARACTERÍSTICAS LIMNOLÓGICAS DISTINTAS

Artigo 8: Do distinct water chemistry, reservoir age and disturbance make any difference on phosphatase activity?

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Do distinct water chemistry, reservoir age and disturbance make any difference on phosphatase activity?

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ABSTRACT

Alkaline phosphatase activity was assessed concomitantly with total phosphorus, orthophosphate and phosphomonoester concentrations in two meso-eutrophic reservoirs with distinct age and subjected to different kinds of environmental influence. Differences in conductivity, temperature and pH were found. However, during the study period alkaline phosphatase activity was similar in both reservoirs. Water colour was higher in S. Serrada Reservoir. This fact can be related to (1) reservoir age (2) high internal disturbance (3) large imputs of allochthonous detritus, resulting from the combined effect of grazing, fire and catchment slope. Despite the high water colour recorded in S. Serrada, alkaline phosphatase activity was apparently not inactivated by humic substances. Besides, the obtained results demonstrated that hydrolysis of phosphomonoesters by alkaline phosphatase was not important for orthophosphate regeneration in these reservoirs. Probably orthophosphate was always available to biota. In fact, in the experiments based on Selenastrum capricornutum *Printz algal test, similar phytoplankton growth responses were obtained for different phosphorus concentrations. Thus, these results seem to indicate that phosphorus was not a limiting nutrient in either reservoir. Although phosphatase activity was significantly correlated with some phytoplankton genera in both reservoirs, no significant correlations were found between enzyme activity and chlorophyll-*a*. Significant correlations between phosphatase activity and crustacean zooplankton were only recorded in S. Serrada. In spite of these results there was some indication that the main source of phosphatase might have been bacteria involved in decomposition processes instead of phyto- and zooplankton taxa.*

Key-words: Alkaline phosphatase; orthophosphate regeneration; phytoplankton; zooplankton; reservoirs

1. INTRODUCTION

Traditionally (Vollenweider 1968), as well as over recent decades (Harper 1992; Wetzel 2001), phosphorus has been clearly identified as the principal nutrient underlying cultural eutrophication of lakes and reservoirs all over the world. Among the several forms of phosphorus, orthophosphate is the only one directly available to biota. Consequently, when managing reservoir trophic state it is of ultimate importance to understand the mechanisms of orthophosphate regeneration by biota and their contribution to the observed concentrations of this chemical form of phosphorus in the water column. One of the mechanisms of orthophosphate regeneration is hydrolysis of complex phosphorus compounds by extracellular alkaline phosphatases. These enzymes are often found as free dissolved enzymes or bound to cell surfaces or membranes and their main sources are bacterioplankton (Jansson *et al.* 1981; Halemejko & Chrost 1984; Wynne *et al.* 1991; Rai & Jacobsen 1993), phytoplankton (Pettersson 1980; Jansson *et al.* 1981; Olsson 1983; Boavida & Heath 1986; Wynne *et al.* 1991; Spijkerman & Coesel 1998; Štrojsová *et al.* 2002) and zooplankton (Boavida & Heath 1984; Bogé *et al.* 2002). Phosphomonoesters are the most frequent substrates of phosphatases occurring in fresh waters (Kuenzler & Perras 1965). Thus, the concentrations of those compounds are often inversely correlated with phosphatase activity (Heath & Cooke 1975; Berman 1970; Hashimoto *et al.* 1985; Boavida & Heath 1988; Wynne *et al.* 1991; Feuillade & Dorioz 1992). According to these authors the level of alkaline phosphatase activity can be taken as an indicator of phosphorus deficiency. However, although many organisms respond with production of phosphatases when orthophosphate availability is low (adaptive production), others continue to produce them independently of orthophosphate concentration in the environment (constitutive production). In addition, phosphatase activity is susceptible of being temporarily inactivated when humic compounds are present in the environment. This phenomenon is a consequence of the formation of complexes between enzyme and the mentioned compounds (Kim & Wetzel 1993; Boavida & Wetzel 1998). In this manner, phosphatase is not necessarily an indicator of orthophosphate abundance or deficiency. Jones (1972) also mentioned that phosphatase activity could be used as a measure of lake trophy, but again this is not an universal rule in many systems, since no significant correlation was always found either between enzyme activity and orthophosphate or between phosphatase activity and phosphomonoesters (e.g. Boavida 1991; Boavida & Marques 1995; Geraldes & Boavida 1999). Nevertheless, data concerning phos-

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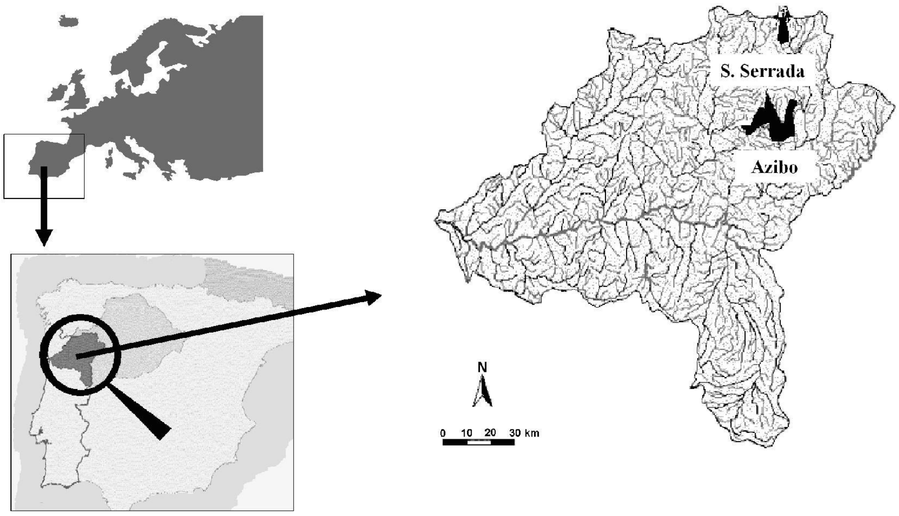

Fig. 1. Location of S. Serrada and Azibo reservoirs in the map of Portugal.

phatase activity, when used with some caution, can be useful indicators of phosphorus recycling and availability.

The present study was conducted form January 2000 to December 2002 in two reservoirs with distinct physical and chemical water features, age and anthropogenic disturbance. Because it was the first time a limnological approach was taken in the study of both reservoirs, the main objectives of this research were: (1) to assess any seasonal differences found in phosphatase activity between reservoirs and relate them to environmental variables such as conductivity, temperature, water colour and pH (2) to determine the potential importance of enzymatic hydrolysis as an orthophosphate regeneration mechanism; (3) to measure the intensity of association between phosphatase activity and the dominant phytoplankton and crustacean zooplankton taxa.

2. STUDY AREA

S. Serrada (41° 57′ N, 6° 46′ W, altitude 1300 m) and Azibo (41° 32′ N, 6° 53′ W, altitude 500 m) reservoirs are located in Portuguese part of River Douro catchment (Fig. 1).

S. Serrada Reservoir is lying on granitic bedrock and the total capacity of the reservoir, spreading over 25 ha, is 1680×10^3 m^3. Maximum depth is 17 m, mean depth is 6.7 m. Direct human influence on S. Serrada impoundment is considered negligible. There are no villages close by, there has been no agricultural activity for approximately 20 years, and recreational activities are not significant. However, grazing can be very intense in the catchment during summer months. Consequently, this area is very often subjected to wild fires often induced by shepherds to obtain better graze. This reservoir was filled for the first time in 1995 to supply water and hydroelectric power to the city of Bragança. As a result of these uses, accentuated water level fluctuations occur. The annual range of water level variation is between 8 and 10 m. Thermal stratification was observed from June to August/beginning of September. Disruption of stratification was coincidental with the lowest water level. During the period of study this reservoir was classified as meso-eutrophic.

Azibo Reservoir is located on schistic bedrock. The area of the reservoir is 410 ha and its total capacity is 54470×10^3 m^3. Maximum depth is about 30 m, while mean depth is 13.2 m. This reservoir was filled for the first time in 1982 and it is used mainly for recreation. Other uses are water supply and irrigation, yet those are not significant and water level fluctuations are not very accentuated, varying between 1.5 and 2 m. In Azibo direct influence of human activities is greater during summer, when reservoir and surroundings are used for recreation such as swimming, camping, boating and angling. Other activities found all over the year in the catchment area are farming and grazing. During the period of study thermal stratification occurred from June to October and the reservoir was classified as meso-eutrophic. In the region where both reservoirs are 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, but in a very irregular regime, with wet winters usually alternating with dry ones. Further information concerning morphological and hydrological characteristics of both reservoirs can be found in Geraldes & Boavida (2003).

Tab. 1. Environmental factors recorded for S. Serrada and Azibo reservoirs during the study period. Minimum-maximum range is shown for each factor, with mean/standard deviation below between brackets; (mean/SD).

	2000	2001	2002
S. Serrada			
Water temperature (ºC)	1.46-21.39	2.70-20.19	2.79-21.05
	(12.51/6.59)	(12.90/6.46)	(12.37/6.06)
Dissolved oxygen (mg l^{-1})	7.38-12.42	6.20-10.72	7.19-10.40
	(8.55/1.45)	(8.55/1.49)	(8.45/1.53)
Conductivity (µS cm^{-1})	4-10	3-8	7-12
	(6.94/1.84)	(5.95/1.61)	(8.06-1.53)
Water transparency (m)	1.00-4.50	1.00-5.00	1.00-3.00
	(2.66/1.09)	(2.85/1.14)	(2.88/0.63)
Water colour (T.A. units)	0.22-1.02	0.31-0.83	0.41-1.06
	(0.52/0.25)	(0.54/0.16)	(0.62/0.20)
pH	5.77-6.56	5.95-8.34	6.33-8.77
Azibo			
Water temperature (ºC)	5.58-24.70	8.06-23.83	6.08-23.70
	(16.52/6.13)	(17.16/5.73)	(13.31/5.80)
Dissolved oxygen (mg l^{-1})	7.54-11.53	7.21-10.78	7.00-10.19
	(9.09-1.06)	(8.89/1.33)	(8.47/1.00)
Conductivity (µS cm^{-1})	51-81	43-66	42-76
	(69.87/11.09)	(56.23/7.64)	(61.00/10.57)
Water transparency (m)	1.50-6.00	1.50-5.50	2.00-9.00
	(4.72/1.25)	(3.50/1.14)	(4.40/1.85)
Water colour (T.A. units)	0.21-0.37	0.21-0.59	0.21-0.54
	(0.26-0.05)	(0.31/0.11)	(0.28/0.09)
pH	6.71-8.05	6.64-8.36	6.93-8.08

2. METHODS

Samples were collected monthly in winter and bi-weekly in summer, from January 2000 to December 2002 at one single sampling station, located at the deepest point of each reservoir. Water samples for orthophosphate (determined as soluble reactive phosphorus - SRP), total phosphorus (TP), phosphomonoesters (PME), alkaline phosphatase activity (APA), chlorophyll-*a* (CHL-*a*) and water colour measurements were obtained from the upper 30-40 cm stratum directly into acid rinsed bottles and were transported to the laboratory in a cold container. SRP concentrations were estimated by the method of Murphy and Riley (1962) and TP was assessed after acid hydrolysis with persulfate for 60 min under high temperature and pressure. PME concentrations were determined according to Boavida (1991). APA was measured fluorimetrically, using 4-methylumbelliferylphosphate as substrate (Pettersson & Jansson 1978). CHL a was obtained from 500 to 1000 ml of water filtered through a Whatman GF/A filter no more than 2 h after collection. Concentrations were determined spectrophotometrically after overnight extraction in 90% acetone. Water colour was determined spectrophotometrically at 440 nm, according to Cuthbert & Del Giorgio (1992), so that it would give a measure of the amount of humic compounds in solution. Environmental factors such as water temperature, dissolved oxygen, conductivity and pH were measured *in situ* with a 6820 YSI Multiparameter Water Quality Monitor. Water transparency was measured with a black and white 20 cm diameter Secchi disk. Zooplankton samples were obtained on each sampling date by taking two vertical hauls (depth range 10 to 15 m, sometimes coincidental with maximum reservoir depth) and using a Wisconsin type net with 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 in sub-samples of 5, 10, 20 ml or in the whole sample. Animals were always identified to species. Phytoplankton samples were collected from the upper 30-40 cm stratum and were fixed *in situ* in Lugol's solution. Phytoplankton were counted according to Utermöhl (1958), using an inverted microscope at 200X/400X magnification. Algae were identified to genus.

Because of many logistic constraints preventing correct performance of the *Selenastrum capricornutum* Printz algal assay bottle test (Miller *et al.* 1978), phosphorus limitation in the reservoirs was investigated seasonally during 2002 by means of a modification of this classical experiment; a fixed volume of a solution spiked with increasing concentrations of phosphorus (0-50-100-200 µg l^{-1} KH_2PO_4) was added to equal volumes of whole reservoir water, expecting a phytoplankton growth response whenever there was P-limitation in the reservoir. CHL-*a* concentrations were measured at day-0, day-7 and day-14. Phytoplankton growth response was measured as the difference between CHL-*a* concentrations at day-14 and day-7, and day-7 and day-0. Incubation temperature was 21 ± 2 ºC. Illumination was provided by 11 W Ecotone fluorescent lighting during the whole 24 hours cycle.

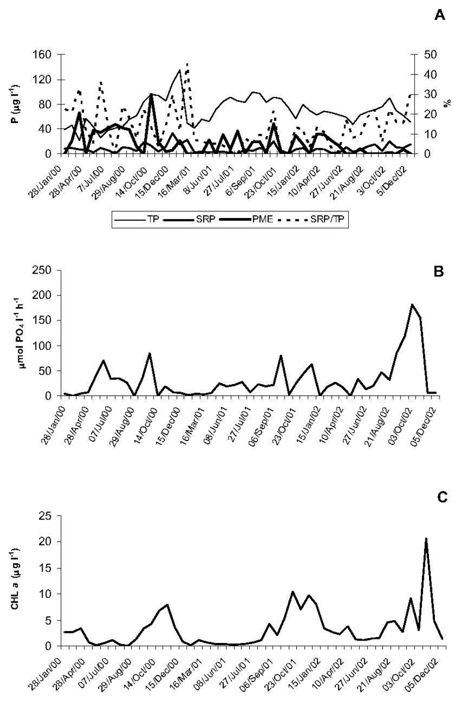

Fig. 2. Trends observed, during this study, in TP, SRP; PME and TP/SRP (A); APA (B) and in CHL-*a* (C) in S. Serrada.

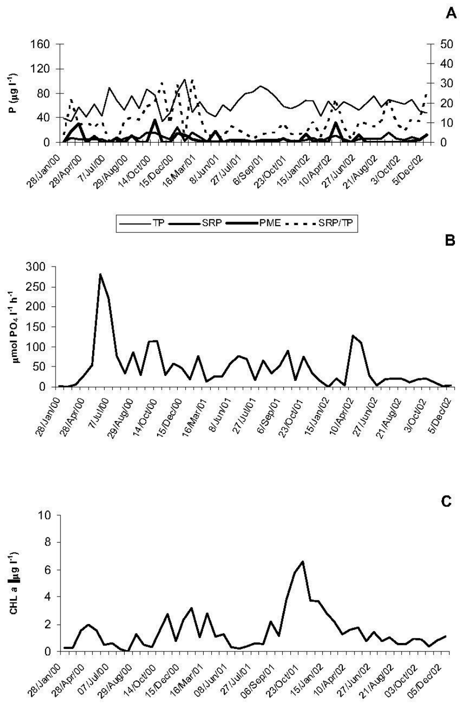

Fig. 3. Trends observed, during this study, in TP, SRP; PME and TP/SRP (A); APA (B) and in CHL-*a* (C) in Azibo.

A Kolmogorov-Smirnov test was performed to assess differences among water chemistry and physical parameters during the study period for both reservoirs. This test is based on the maximum absolute difference (D_{max}) between the observed cumulative distribution functions for both samples. When this difference is significantly large, the two distributions are considered different. Correlations among APA, water chemistry parameters, phyto- and zooplankton and CHL-*a* were determined using the non-parametric Spearman correlation test (Sokal & Rohlf 1981). All statistical tests were performed using SYSTAT 8.0.

3. RESULTS

3.1. Phosphatase activity and environmental variables

Dissimilar conductivity (D_{max} = 1; p <0.01), water temperature (D_{max} = 0.34; p <0.05), water transparency (D_{max} = 0.49; p <0.01), pH (D_{max} = 0.53; p <0.01) and water colour (D_{max} = 0.75; p <0. 01) were found between reservoirs (Tab. 1). Unlike the physical characteristics, TP and SRP concentrations were similar in both reservoirs. PME (D_{max} = 0.35; p <0.05) and CHL-*a* (D_{max} = 0.31; p <0.05) concentrations were higher in S. Serrada than in Azibo (Figs 2 and 3).

In S. Serrada, significant correlations were found between CHL-*a* and water transparency (r = -0.74; p <0.01) and between CHL-*a* and water level variation (r = -0.60; p <0.01). TP was significantly correlated with water transparency and water level variation (r = 0.32; p <0.05; r = -0.74; p <0.01, respectively). In this reservoir water transparency was inversely correlated with water colour (r = -0.54; p <0.01). In Azibo CHL a was inversely correlated with water transparency (r = -0.54; p <0.01). Water transparency was inversely correlated with water colour (r = -0.51; p <0.01).

In spite of distinct reservoir age and human influence, as well as the different values found for water temperature, conductivity, pH, water colour and transparency, APA was found to be similar in both reservoirs With a few exceptions, APA was low (Figs 2 and 3) in both reservoirs.

In S. Serrada APA was significantly correlated with temperature (r = 0.43; p <0.05), water colour (r = 0.50; p <0.01), conductivity (r = 0.50; p <0.01) and water level variation (r = -0.35; p <0.05). In Azibo significant correlations were found among APA and both temperature (r = 0.35; p <0.05) and conductivity (r = 0.34; p <0.05).APA was not significantly correlated with TP, SRP, PME and CHL-*a* either in S. Serrada or in Azibo.

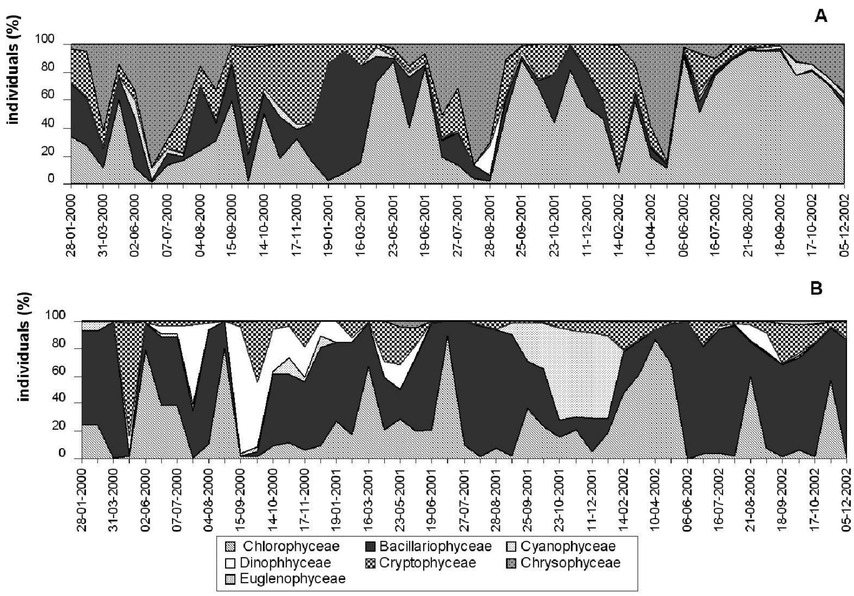

Fig. 4. Relative abundance of the main phytoplankton taxa in S. Serrada (A) and in Azibo (B).

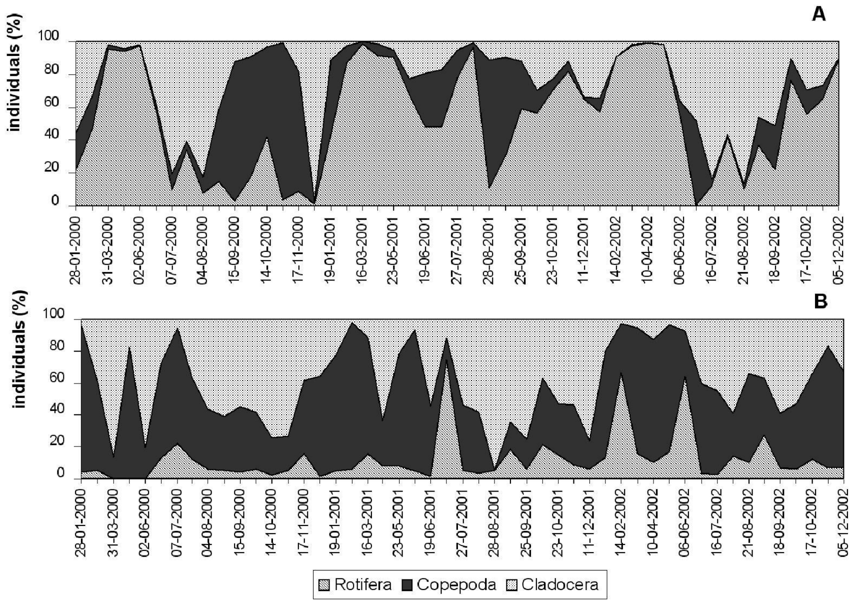

Fig. 5. Relative abundance of Rotifera, Copepoda and Cladocera in S. Serrada (A) and in Azibo (B).

3.2. Phosphatase activity and phytoplankton abundance

Chlorophyceae, Bacillariophyceae, Chrysophyceae, Cyanophyceae, Cryptophyceae and Dinophyceae were the most common taxa found in both reservoirs (Fig. 4). In S. Serrada the most representative algae were: (1) Chlorophyceae – *Monoraphidium*, *Dictyosphaerium*, *Chlamydomonas* like cells, *Scenedesmus*, *Crucigenia*, *Staurastrum* and *Cosmarium*; (2) Bacillariophyceae - *Tabellaria* and *Cyclotella*; (3) Chrysophyceae - *Dinobryon*; (4) Cyanophyceae - *Anabaena*; (5) Cryptophyceae - *Cryptomonas*; (6) Dinophyceae - *Peridinium*. In Azibo the most common algae were: (1) Chlorophyceae - *Monoraphidium*, *Chlamydomonas* like cells, *Scenedesmus*, *Crucigenia*, *Tetraedron*, *Oocystis*, *Volvox*, *Paulschulzia* and *Cosmarium*; (2) Bacillariophyceae - *Asterionella*, *Fragilaria* and *Cyclotella*; (3) Chrysophyceae - *Dinobryon*; (4) Cyanophyceae - *Anabaena*; (5) Cryptophyceae - *Cryptomonas*; (6) Dinophyceae - *Ceratium*. No significant correlations were found between APA and CHL-*a* in either reservoir. Concerning the relationship between alkaline phosphatase and algal genera, in S. Serrada significant correlations were found among APA and *Staurastrum* (r = 0.36; p <0.05), *Scenedesmus* (r = 0.23; p <0.05) and *Anabaena* (r = 0.33; p <0.05). The only significant correlation for Azibo was found between APA and *Ceratium* (r = 0.61; p <0.01).

3.3. Phosphatase activity and crustacean zooplankton abundance

In S. Serrada crustacean zooplankton was more abundant during the summer months, whereas in Azibo crustaceans were always dominant over rotifers, except in three sample dates. (Fig. 5). In S. Serrada the taxa dominating crustacean zooplankton were *Ceriodaphnia quadrangula* and *Tropocyclops prasinus*. Significant correlations were found among APA and *Ceriodaphnia* (r = 0.57; p <0.01), *T. prasinus* (r = 0.30; p <0.05) and their nauplii (r = 0.49; p <0.01). In Azibo the most abundant crustacean zooplankton were: *Daphnia longispina*, *Ceriodaphnia pulchella*, *Bosmina longirostris*, *Diaphanosoma brachyurum* and *Copidodiaptomus numidicus*. APA was significantly correlated with neither of those.

3.4. Selenastrum capricornutum *type test*

Phytoplankton responded similarly to phosphorus additions in both reservoirs (Figs 6 and 7). It was expected that phytoplankton growth response was proportional to the increasing phosphorus concentrations whenever the nutrient was limiting. However, similar phytoplankton growth responses were obtained for different phosphorus concentrations. Results of these experiments might indicate that phosphorus was not a limiting nutrient in either reservoir.

4. DISCUSSION AND CONCLUSIONS

Differences in temperature, pH, conductivity and water colour, as well as in reservoirs' age and human influence, seemed to have had a negligible effect on APA and consequently on orthophosphate regeneration. In fact, the lack of significant inverse correlations between APA and SRP on one hand, and between APA and PME on the other hand, in both reservoirs, demonstrated that the hydrolysis of PME by phosphatases was not an important process in orthophosphate regeneration. Besides, the absence of a significant correlation between SRP and CHL-*a* might indicate that orthophosphate was not a limiting nutrient for phytoplankton in both reservoirs. In spite of the fact that results of *Selenastrum capricornutum* type test were not fully conclusive, they seem to corroborate this indication.

Considering the above mentioned facts, three questions arise: (1) if reservoirs seemed not to be P limited why was APA always detected and significantly correlated with *Scenedesmus*, *Staurastrum* and *Anabaena* in S. Serrada and with *Ceratium* in Azibo? (2) why was APA correlated significantly with *Ceriodaphania* and *T. prasinus* in S. Serrada? (3) what are the possible origins of the detected phosphatase activity?

In fact, phosphatase can also be produced by algae in a constitutive way independently of orthophosphate concentrations in the water (Berman 1970; Petterson 1980; Boavida & Heath 1986, 1988). According to Wynne (1981), Wynne *et al.* (1991) and Cotner & Wetzel (1992) in many situations extracellular phosphatases may also be involved in metabolic processes not directly connected with P nutrition such as chloroplast activity and protein synthesis. Consequently, phosphatases are produced even when environmental orthophosphate concentrations are high. It is possible that *Scenedesmus*, *Staurastrum*, *Anabaena* and *Ceratium* produce phosphatases in a constitutive way. The same explanation can be applied to the significant correlations found between APA and *Ceriodaphania/T. prasinus* in S. Serrada (e.g. Boavida & Heath 1984; Bogé *et al*. 2002). In addition, herbivorous zooplankton can release phosphatases of algal origin ingested with the food (Boavida & Heath 1984). Moreover, the concomitant increase of those zooplankters densities with APA does not mean that they were the main source of phosphatase; the observed significant correlations between APA and those species could be a matter of coincidence. APA exhibited a tendency to be higher in summer and those populations also peaked in this period, because at the time they might have had better ecological conditions (e.g. temperature, resource supply). On the other hand, no significant correlations where found between APA and CHL-*a* in either reservoir. This again might indicate that phytoplankton was not the main source of phosphatase or that not all algae were producing the enzyme.

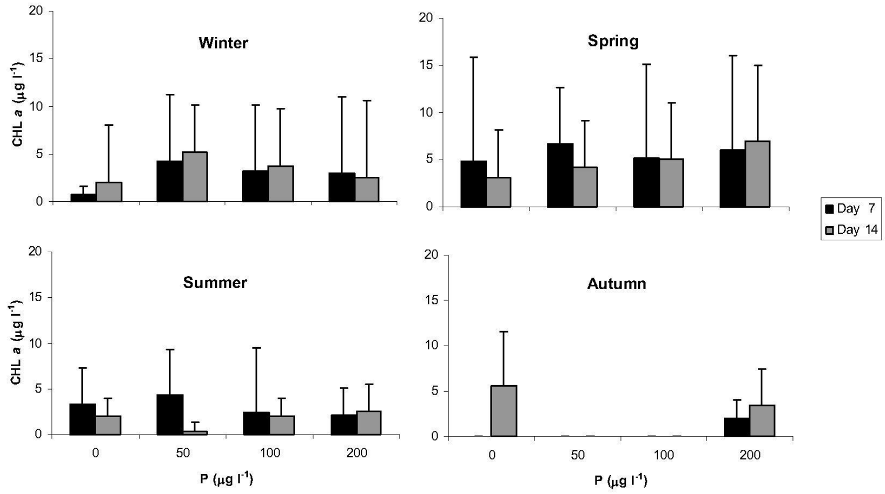

Fig. 6. Results of *Selenastrum capricornutum* type test in S. Serrada.

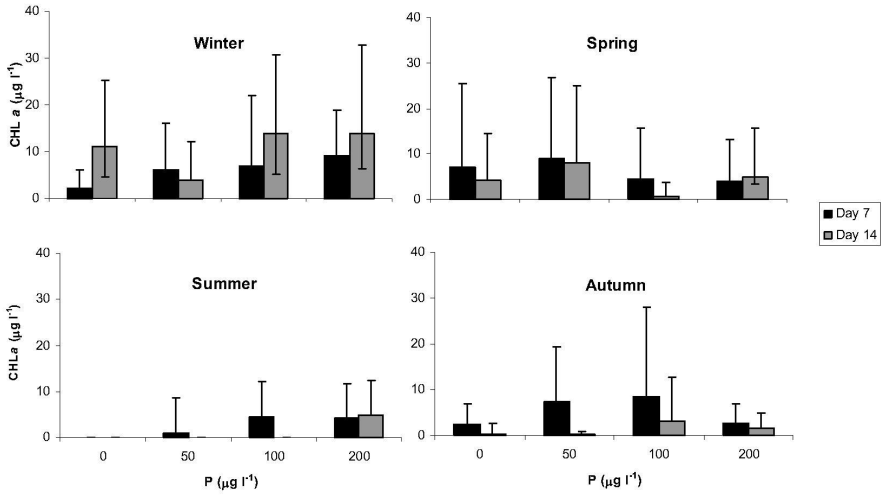

Fig. 7. Results of *Selenastrum capricornutum* type test in Azibo.

Water colour was higher in summer than in all other seasons in both reservoirs. This parameter is a measure of the concentration of humic compounds in the water (Cuthbert & Del Giorgio 1992). Humic compounds are products of plant decomposition. Thus, the higher water colour obtained in S. Serrada might indicate the existence of high amounts of products of terrestrial vegetation under decomposition. In fact, the inundated area was previously a permanent meadow and the existing vegetation was not removed before inundation. Moreover, the disturbance caused by grazing activity and the frequent fire events seemed to account for appreciable organic matter loading into this reservoir (Geraldes & Boavida, in press). The turbulence and the atypical disruption of thermal stratification during summer months, generated by water level fluctuations, lead to resuspension, into the water column, of sediments, organic matter and nutrients up until then laying at the bottom (Harper 1992; Gottgens 1994; Wetzel 2001), as well as the associated bacteria (Sherer *et al.* 1992). Consequently, phosphatases detected in the water could also have been produced by bacteria. In fact, Marxsen & Witzel (1991) and Marxen & Schmidt (1993) detected high phosphatase activity in the surface layers of sediments. Because of logistic constraints no assays on bacterial growth were performed to evaluate whether bacteria were P limited in the studied reservoirs. However, according to Wetzel (2001), bacterioplankton requirements for phosphorus are four to ten times higher than phytoplankton needs. Thus, bacterioplankton can be P limited in S. Serrada, and the significant positive correlation between APA and water colour might indicate that APA detected in water column could be related to the resuspension phenomenon. Considering those facts and the lack of correlation between APA and CHL-*a*, it is plausible to think that the main source of phosphatase in S. Serrada were bacteria involved in decomposition processes. Despite of the high water colour observed in this reservoir, it seemed that APA was not inactivated by humic substances present in the reservoir as observed by Kim & Wetzel (1993) and Boavida & Wetzel (1998). According to those authors hydrolytic reactivation is possible by exposure to UV irradiance in the upper layer of the photic zone of lakes. In Azibo water colour was lower, indicating that the decaying material existing in the water column was much less than in S. Serrada. This fact can be related to reservoir age (most of the terrestrial vegetation remains might have been already decomposed), to the low degree of internal disturbance (because of the stable water level organic matter was not suddenly resuspended from the bottom) and to the low inputs of allochthonous detritus (those inputs seemed to be higher in S. Serrada as a result of the combined effect of grazing, fire and catchment slope (Geraldes & Boavida, in press). However, as APA was not correlated either with CHL-*a* or with zooplankton in Azibo, it is probable that algae and zooplankton were not the main sources of phosphatase. There is some indication that after intense rain events there were high inputs of allochthonous phosphatases. In fact, after a strong rain event APA detected in samples obtained at one Azibo's temporary tributaries was much higher than the activity measured inside the reservoir before the rain (Geraldes, unpubl. data). Pettersson (1980) also described similar results obtained in other lakes. During dry weather APA could be related to bacteria involved in the decomposition of particulate organic matter, and these could be P limited. Those particles were often present at the water surface, especially during late spring and summer months, and consisted of terrestrial insects and plant fragments of various sizes probably transported by wind from the adjacent land. Some of the plant remains were also fragments of macrophytes existing in the shores of the reservoir. The disturbance caused by recreational activities could also be a source of organic matter influencing APA in this reservoir (Geraldes, pers.obs.).

Contrary to expectations, APA was similar in both reservoirs. Hydrolysis of PME by phosphatase was not important in orthophosphate regeneration. There was also some indication that phytoplankton and zooplankton were not the main source of phosphatase, although some taxa were correlated with APA. It seems that the main producers of phosphatases in both reservoirs might have been bacteria involved in the decomposition processes.

It is apparent from the present results that distinct water chemistry, reservoir age, and disturbance do not make any difference with respect to phosphatase activity in the water. However, the obtained data constitute a preliminary study and might be the basis for further field and experimental research to elucidate these points.

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7. DISCUSSÃO GERAL

Os resultados obtidos sugerem que a qualidade da água nas albufeiras estudadas é condicionada pela forma de ocupação da paisagem, de utilização do solo e pelos padrões de uso da água nas bacias de drenagem. A idade das albufeiras também é susceptível de condicionar a qualidade da água.

De acordo com o índice do estado trófico de Carlson (Carlson, 1977) a albufeira da Serra Serrada foi classificada como meso-eutrófica. À semelhança de outros lagos e albufeiras localizados em regiões idênticas do ponto de vista climático e geológico (*e.g.* Boavida, 2000; Negro *et al.*, 2000), seria de esperar que este sistema fosse oligotrófico ou mesotrófico. No entanto, ao contrário dos sistemas acima mencionados, a albufeira da Serra Serrada está sujeita a elevados níveis de perturbação. Uma das fontes de perturbação são as flutuações de grande amplitude no nível da água. Estas oscilam entre os 8 e os 10 metros, e são originadas pelo elevado consumo de água por parte da população de Bragança nos meses de Verão (Capítulo 2, artigo 1; Capítulo 3, artigo 3). O ciclo hidrológico desta albufeira é caracterizado por três fases: (1) fase de nível máximo (Janeiro - princípio de Junho); (2) fase de esvaziamento (meados de Junho - princípio de Setembro); (3) fase de nível mínimo (meados de Setembro - primeiras chuvas). Na fase de esvaziamento a rápida descida no nível da água causou um aumento da turbulência e a quebra repentina da estratificação térmica. Em consequência, ocorreu a ressuspensão dos sedimentos do fundo, aumentando a quantidade de nutrientes (nomeadamente fósforo) e de outro material particulado na coluna de água. Simultaneamente, deu-se o aumento das concentrações de clorofila *a*. Estes valores atingem os seus máximos na fase de nível mínimo. Um padrão semelhante foi também observado em albufeiras sujeitas ao mesmo tipo de perturbações (Barone & Naselli-Flores, 1994; Naselli-Flores, 1999) e noutras que foram esvaziadas para reparações (Marques & Boavida, 1993; Geraldes & Boavida, 1999). As concentrações de nutrientes e a quantidade de partículas em suspensão na coluna de água atingiram o seu mínimo durante a fase de nível máximo. É provável que durante este período os processos de sedimentação sejam favorecidos devido à estabilização no nível da água (Böstrom *et al.*, 1988; Wetzel, 2001). Outra das consequências das flutuações no nível da água é facto de os sedimentos litorais estarem constantemente sujeitos a ciclos de dessecação/reinundação. Este fenómeno também contribuiu para as concentrações relativamente elevadas de fósforo que foram observadas. De acordo com Fabre (1988) e Watts (2000) os sedimentos que estão periodicamente sujeitos a estes ciclos têm menos capacidade para adsorver nutrientes do que aqueles que estão permanentemente inundados. Experiências, baseadas no protocolo desenvolvido pelo segundo autor, levadas a cabo no presente estudo, também demonstraram a ocorrência do mesmo facto na albufeira da Serra Serrada (Capítulo 3, artigo 3).

Para além das perturbações causadas pelas flutuações do nível de água, há a considerar as relacionadas com a pastorícia praticada na bacia da albufeira, e que é mais intensa durante os meses de Verão. Associada a esta actividade está a ocorrência de fogos muito frequentes nos matos existentes em toda esta área. Estes fogos são, na sua maior parte, induzidos pelos pastores com o objectivo de obter melhores pastagens. Estas práticas de acordo, com os resultados obtidos no Capítulo 2 (artigos 1 e 2), poderão ser as principais fontes externas de nutrientes e de matéria orgânica. Embora as entradas de nutrientes, provenientes das zonas ardidas em redor da albufeira, não tenham sido quantificadas no presente estudo, considerando que o tipo de solo existente tem um risco de erosão potencial elevado (Agroconsultores & Coba, 1991) e os acentuados declives desta área, é de esperar que as entradas de nutrientes e de matéria orgânica, após fortes chuvadas, sejam significativas à semelhança do que sucede noutras regiões (Walsh *et al.*,1992; Shakesby *et al.*, 1993; Townsend & Douglas, 2000; Lange, 2001; Minshall *et al.*, 2001). Nesta albufeira o parâmetro ambiental cor da água atingiu valores mais altos de que no Azibo e no Peneireiro. Este facto indicia a presença de concentrações relativamente elevadas de compostos húmicos na coluna de água. De acordo com Cuthbert & Giorgio (1992) estes compostos são produtos da decomposição de plantas. Assim, os valores obtidos para a cor da água, poderão ser explicados pela entrada de matéria orgânica proveniente da bacia de drenagem. Por outro lado, há a considerar que área onde hoje existe a albufeira era um prado permanente que até há vinte anos atrás era continuamente fertilizado para a produção de produtos agrícolas, e que a vegetação existente na altura da criação da albufeira não foi previamente removida. Considerando igualmente a idade da albufeira e o tempo que a vegetação de origem terrestre demora a ser decomposta, os valores da cor da água e as concentrações de fósforo observados são também uma consequência da não remoção das plantas de origem terrestre. Todos estes factores de perturbação podem, assim, explicar os valores elevados obtidos para as componentes do índice de estado trófico de Carlson TSI (TP) calculada a partir das concentrações de fósforo total e TSI (SD) determinada a partir dos valores da transparência da água. Se, por um lado, parece não existirem dúvidas das implicações que as variações extremas no ciclo hidrológico têm nas concentrações dos nutrientes e nas quantidades de matéria orgânica observadas na coluna de água, já os seus efeitos na variação da composição e da estrutura das comunidades fito e zooplanctónicas são mais difíceis de avaliar. A composição da comunidade fitoplanctónica também sofreu algumas alterações do longo do ciclo hidrológico da albufeira. Assim, durante a fase de nível máximo coexistiram taxa típicos de ambientes pobres em nutrientes, com outros típicos de ambientes mesotróficos e eutróficos. Posteriormente, durante as fases de esvaziamento e de nível mínimo, estes grupos foram sendo substituídos por outros associados a ambientes mais eutroficados e a temperaturas mais elevadas. Por seu turno, a comunidade zooplanctónica foi quase sempre dominada por rotíferos. No Verão, o cladócero *Ceriodaphnia quadrangula* tornou-se dominante, e no princípio do Outono o copépode ciclopóide *Tropocyclops prasinus* foi por vezes a espécie mais abundante. No entanto, uma vez que estas comunidades são reguladas por um conjunto de interacções complexas entre os factores físicos, químicos e biológicos, é necessário ter uma certa cautela quando se relacionam as alterações ocorridas apenas com as flutuações no nível da água. De facto, algumas mudanças na composição destas comunidades podem ser directa ou indirectamente influenciadas pelas flutuações no nível da água, mas outras podem ser causadas por factores que variam sazonalmente mas independentemente destas variações como é, por exemplo, o caso da temperatura.

Na albufeira do Azibo as flutuações no nível da água são mínimas, e a reduzida quantidade de material particulado na coluna de água parece indicar que os fenómenos de ressuspensão dos sedimentos do fundo são pouco significativos. Tanto na vizinhança da albufeira, como sua na bacia de drenagem, os fogos são pouco frequentes. No entanto, este sistema também foi classificado como meso-eutrófico. De acordo com os resultados obtidos (Capítulo 2, artigos 1 e 2), as principais fontes externas de fósforo e de azoto parecem ser as actividades agrícolas, em especial algumas culturas intensivas que existem nas proximidades das margens da albufeira, a pastorícia e alguns esgotos sem tratamento provenientes de várias aldeias existentes na bacia de drenagem. Outras fontes de fósforo e azoto são as actividades de lazer (*e.g.* Szyper & Gołdyn, 2002). De facto, estima-se que de Julho a Setembro, esta albufeira seja frequentada por cerca de 10.000 veraneantes e durante a época de pesca por cerca de 1.000 pescadores desportivos. No presente estudo só foram estimadas as contribuições potenciais de cada uma destas fontes em azoto e fósforo. Apesar de não ter sido possível obter dados que permitam precisar as quantidades destes nutrientes que realmente entram na albufeira, poder-se-á considerar que a intensidade destas entradas depende do declive, das formas de ocupação do solo, da cobertura vegetal e da heterogeneidade da paisagem. De facto, tendo em conta os valores das contribuições potenciais de cada uma destas fontes, seria de esperar que as concentrações de nutrientes fossem mais elevadas na coluna de água. No entanto, se se considerar os declives do terreno, menos acentuados do que na bacia da Serra Serrada, a extensa área da albufeira, a elevada heterogeneidade da paisagem, onde em simultâneo com os campos agrícolas e prados subsistem matas autóctones, matos e matas ripícolas, poder-se-á supor com alguma legitimidade que grande parte das quantidades de nutrientes e de matéria orgânica que eventualmente entraria neste sistema ou fica retida nos ecossistemas circundantes, ou é diluída na enorme massa de água que constitui a albufeira. O estado trófico do Azibo também poderá ser em parte explicado pelo tempo médio de residência da água nesta albufeira ser relativamente elevado (*e.g.* Kennedy & Walker, 1990; Toja *et al.*, 1995; Reynolds *et al.*,1998)

As entradas de nutrientes, à semelhança do que foi verificado noutros sistemas por Johnes *et al.* (1996), Barbosa & Hvitved-Jacobsen (1999), McGarrigle *et al.* (2000) e Rybak (2000), parecem ocorrer com mais intensidade durante as primeiras chuvas e nos invernos mais chuvosos. No presente estudo foram determinadas as concentrações de fósforo total (TP) e de fósforo solúvel reactivo (SRP) na parte terminal de um ribeiro tributário desta albufeira, e verificou-se a ocorrência de um padrão semelhante ao descrito pelos autores acima mencionados. De facto, logo após as primeiras chuvas, as concentrações eram elevadas, decrescendo com o passar do tempo, mesmo que ocorressem outras chuvadas igualmente muito intensas. Este mesmo padrão também se verificou na coluna de água da albufeira. Na abordagem realizada no Capítulo 4 (artigo 4) verificou-se também que num Inverno chuvoso os valores de TP e de SRP atingiam valores máximos mais elevados do que num Inverno considerado seco, embora os valores médios destes parâmetros na coluna de água não tenham diferido significativamente entre os dois invernos. Porém, este estudo só se refere aos invernos de 2000/2001 e de 2001/2002, pelo que será necessário realizar mais amostragens para obter mais séries de dados de modo a ser possível averiguar qual é o real impacto das variações do regime de precipitação na limnologia das albufeiras. De momento, fica em aberto se *Anabaena* se torna sempre dominante quando um longo período seco é precedido de precipitações mais elevadas do que o habitual. Ao contrário do que foi observado por outros autores (Rey, 1984; Catalan & Fee, 1994; Reynolds *et al.*, 1998), o restante fitoplancton e a maioria dos crustáceos zooplanctónicos não foram aparentemente muito afectados pela redução do tempo de residência da água, causado pelos elevados valores de precipitação que ocorreram no Inverno de 2000/2001. Em relação aos rotíferos verificou-se, após as grandes chuvadas ocorridas no Inverno mencionado, uma tendência para um aumento das densidades de algumas espécies (Capítulo 5, artigo 5).

A albufeira do Peneireiro antes de ser esvaziada apresentava concentrações médias de TP, de SRP e de nitratos mais elevadas do que as observadas nas restantes albufeiras (Capítulo 1, artigo 2). Este resultados podem ser explicados pelo facto de esta albufeira ter uma área muito pequena, de o tempo médio de residência da água ser relativamente longo e de estar circundada por terrenos agrícolas. Dado que ocorreu um "bloom" de *Microcystis* e *Anabaena*, este sistema foi completamente esvaziado e a camada superior dos sedimentos foi removida com o objectivo de reduzir a disponibilidade de nutrientes e, consequentemente, a biomassa do fitoplancton no futuro. De um modo semelhante ao que aconteceu noutros sistemas mencionados por Harper (1992), após o reenchimento, as concentrações de nutrientes desceram, tendo atingido valores semelhantes aos observados nas albufeiras da Serra Serrada e do Azibo. Porém, a abundância de *Anabaena* continuou a ser considerável em algumas amostras, o que indica que este processo de gestão só poderá ser eficiente se o tempo médio de residência da água for manipulado e se forem tomadas medidas simultâneas a nível da bacia para reduzir as cargas externas de nutrientes.

Apesar de as albufeiras da Serra Serrada e do Azibo terem sido classificadas como meso-eutróficas, a composição das suas comunidades zooplanctónicas difere bastante, o que indicia a existência de condições ecológicas distintas (Capítulo 5, artigo 5). Estas são uma consequência das diferentes formas de perturbação a que estes sistemas estão sujeitos, bem como da sua localização em altitudes e terrenos geológicos distintos. A idade das albufeiras também é susceptível de contribuir para as diferentes condições observadas. Na Serra Serrada a comunidade zooplanctónica é dominada por rotíferos, e a partir do Verão pelo cladócero *Ceriodaphnia quadrangula*. No princípio do Outono o copépode *Tropocyclops prasinus* tornou-se, por vezes, dominante. De acordo com Pinel-Alloul & Méthot (1984), Schmid-Araya & Zuñiga (1992) e Seda & Devetter (2000) este tipo de comunidade é típico de sistemas sujeitos a situações de elevada instabilidade. De facto, as espécies que compõem esta comunidade são na sua maior parte r-estrategistas e como tal têm uma resposta rápida às variações extremas dos recursos. As suas preferências alimentares são principalmente detritos, bactérias e fitoplancton de pequenas dimensões, sugerindo assim, mais uma vez, a sua capacidade de se adaptarem a sistemas sujeitos a elevados níveis de perturbação, onde estes itens alimentares são os mais abundantes (Pejler, 1983). No entanto, e dado que estas comunidades são reguladas por interacções complexas que se estabelecem entre os factores de natureza abiótica e biótica, a única explicação para a composição do zooplancton poderá não ser só a elevada instabilidade da Serra Serrada. Por ser um sistema relativamente recente é provável que não esteja ainda estabelecida uma comunidade estável (*e.g.* Masundire, 1992; Robarts *et al.*, 1992; Garrido & Bozelli, 2000).

Na albufeira do Azibo a comunidade zooplanctónica é dominada pelos cladóceros *Daphnia longispina*, *Ceriodaphnia pulchella*, *Diaphanosoma brachyurum* e *Bosmina longirostris* e pelo copépode calanóide *Copidodiaptomus numidicus*. De acordo com vários estudos (*e.g.* Bays & Crisman, 1983; Pejler, 1983; Siegfried & Kopache, 1984; Schmid-Araya & Zuñiga, 1992; Seda & Devetter, 2000) esta comunidade apresenta um padrão típico de sistemas estáveis sujeitos a um nível baixo de perturbação interna. Outro factor que também poderá explicar a existência de comunidades zooplanctónicas distintas nas albufeiras estudadas são as diferenças na intensidade de predação. Os dados obtidos no presente estudo (Capítulo 5 artigos 5 e 6) indiciam que a intensidade da predação por parte dos peixes é praticamente insignificante no Azibo. Até aos anos 90 a comunidade piscícola desta albufeira era dominada por ciprinídeos (Formigo, 1990) e os rotíferos eram dominantes no zooplancton (Vasconcelos, 1990). De acordo com Winfield & Townsend (1992) os ciprinídeos podem ter algum impacto nas populações de *Daphnia* e de outras espécies zooplactónicas de grandes dimensões corporais. Porém, após a introdução do lúcio (*Esox lucius*) as populações de ciprinídeos existentes no Azibo foram praticamente dizimadas por este peixe, que rapidamente se tornou a espécie piscícola dominante. Hunt & Carbine (1951) afirmam que os cladóceros e copépodes só são presas dos lúcios nas primeiras duas ou três semanas após a absorção do saco vitelino. Posteriormente, esta espécie alimenta-se de macroinvertebrados e por fim de outros peixes. Assim, tendo em conta estes dados, é legítimo supor que a predação por peixes não é muito intensa nesta albufeira. Pelo contrário, na Serra Serrada, segundo informações dos pescadores, uma das espécies dominantes é o ciprinídeo *Leuciscus carolitertii*. Considerando este aspecto, e o ainda facto de nesta albufeira terem sido detectadas larvas de Chaoboridae que também se alimentam de zooplancton (Lampert & Sommer, 1997), é provável que a comunidade zooplanctónica da Serra Serrada esteja sujeita a uma maior pressão de predação.

Na albufeira do Azibo, foram determinados quais são os factores que poderão influenciar a comunidade de cladóceros. Este taxon para além de ser um dos grupos que domina o zooplancton desta albufeira, é também um dos que apresenta maior diversidade específica. Neste estudo foram consideradas as espécies *Daphnia longispina*, *Ceriodaphnia pulchella*, *Diaphanosoma brachyurum* e *Bosmina longirostris* por serem as mais abundantes (Capítulo 5, artigo 6). Durante o Inverno até ao princípio da Primavera *Daphnia* é a espécie dominante. *Ceriodaphnia* só começa a ser detectada quando a temperatura da água se aproxima dos 15ºC.

Assim, durante o Outono e a Primavera, período em que ocorrem temperaturas desta ordem de grandeza, *Daphnia* e *Ceriodaphnia* coexistem. Nos meses de Verão *Daphnia* deixa de ser observada e *Ceriodaphnia* torna-se dominante, coexistindo com *Diaphanosoma*. Esta última espécie só se encontra presente durante o Verão. *Bosmina* esteve sempre presente mas em densidades baixas. *Daphnia*, *Ceriodaphnia* e *Diaphanosoma* apresentam picos de abundância desfasados, o que deixa antever diferentes óptimos ecológicos. *Daphnia* é dominante durante o período do ano em que as temperaturas e as concentrações médias de fósforo total são mais baixas, enquanto que *Ceriodaphnia* o é quando os valores médios destas variáveis são mais elevados. Lynch (1978) também observou um padrão semelhante para estas populações. De acordo com este autor a substituição de *Daphnia* por *Ceriodaphnia* nos meses de Verão dever-se-ia ao facto de a segunda espécie ser mais eficiente a alimentar-se quando as temperaturas subiam acima dos 20º C. Os resultados obtidos no presente estudo também evidenciam que a temperatura poderá ser o principal factor estruturante desta comunidade. No entanto, as concentrações dos nutrientes e da clorofila *a* também terão certamente um papel importante neste processo. Nesta albufeira, a maior parte dos taxa fitoplanctónicos considerados edíveis estão sempre em densidades muito baixas, o que faz supor que sejam alvo de herbívoria praticada pelas espécies mencionadas. De acordo com Murdoch *et al.* (1998) os herbívoros que fazem parte do zooplancton, em especial os indivíduos do género *Daphnia*, muitas vezes fazem cair a biomassa do fitoplancton edível a níveis abaixo do limite imposto pelas concentrações de nutrientes. Constatou-se que no Azibo, onde os herbívoros são os constituintes dominantes do zooplancton, as concentrações de clorofila *a* são mais baixas do que seria de esperar numa albufeira meso-eutrófica. Este resultado pode assim, ser explicado pela herbívoria exercida por *Daphnia*, *Ceriodaphnia*, *Diaphanosoma*, pelo copépode *Copidodiaptomus numidicus* e pelos seus nauplios. Apesar de em condições experimentais terem sido descritos fenómenos de competição entre as diferentes espécies de cládoceros (*e.g.* Lynch, 1978; Smith & Cooper, 1982; Matveev, 1987; Kurmayer, 2001) no presente caso parecem existir evidências de que a competição é evitada. De facto, as diferentes populações alvo desta abordagem têm óptimos ecológicos distintos e consequentemente, ou não coexistem, ou têm picos de abundância desfasados no tempo. Para mais, as espécies que coexistem têm diferentes mecanismos morfológicos para a exploração dos recursos alimentares, o que implica que cada uma delas ingira partículas com diferentes dimensões (Lampert & Sommer, 1997). Por outro lado, estes organismos alimentam-se não só de fitoplancton mas de um conjunto vasto de itens alimentares tais como protozoários, bactérias e detritos (Kerfoot & Kirk, 1991; González, 1998; Gladyshev *et al.*, 1999;

Schneider-Olt & Adrian, 2001). Também existem evidências que os efeitos da predação sobre estes grupos não é muito acentuada. Esta ilação é baseada no facto de as densidades do copépode ciclópoide *Acanthocyclops robustus*, um potencial predador destas espécies, serem muito baixas. Por outro lado, existem evidências de que este copépode se alimenta preferencialmente de *C. numidicus* (Caramujo *et al.*, 1997) e de ciliados (Wickham, 1995). Como já foi referido, a predação por peixes e por macroinvertebrados aquáticos parece ser também praticamente inexistente nesta albufeira.

Segundo Beklioglu & Moss (1996), Gasith & Hoyer (1998), Jeppesen *et al.* (1998), Kairesalo *et al.* (1998), Bergström, *et al.* (2000) e Nurminen & Horppila (2002) as comunidades de macrófitas aquáticas constituem importantes zonas de refúgio e de alimentação para muitas espécies de crustáceos que fazem parte do zooplancton de muitos lagos. No entanto, no Azibo, à excepção de *Alona* spp. e de *Chydorus sphaericus*, que de acordo com Scourfield & Harding (1966) são espécies que se encontram sempre associadas à vegetação do litoral, e do copépode *A. robustus*, nenhuma das outras espécies mostrou qualquer preferência pelo povoamento de macrófitas existente em parte da zona litoral desta albufeira (Capítulo 5, artigo 7). Pode assim, concluir-se que este povoamento não constituirá uma área preferencial nem de refúgio, nem de alimentação para a maioria das espécies estudadas. Provavelmente este facto estará ligado à fraca pressão predatória que parece existir na zona pelágica do Azibo, ao contrário do que verifica nos trabalhos mencionados no início deste parágrafo.

Um dos mecanismos mais importantes da regeneração do ortofosfato (determinado como SRP) é a hidrólise enzimática de esteres complexos de fósforo, nomeadamente de fosfomonoesteres (PME). Este processo dá-se por acção de fosfatases que são produzidas principalmente pelo bacterioplancton (Jansson *et al.*, 1981; Halemejko & Chrost, 1984; Wynne *et al.*, 1991; Rai & Jacobsen, 1993) e pelo fitoplancton (Pettersson, 1980; Olsson, 1983; Boavida & Heath, 1986; Wynne *et al.*, 1991; Spijkerman & Coesel, 1998). No entanto, também podem ser produzidas pelo zooplancton (Boavida & Heath, 1984; Bogé *et al.*, 2002). Apesar de as diferenças existentes entra a albufeira da Serra Serrada e a do Azibo no que diz respeito (1) aos valores da condutividade, cor da água, temperatura, pH, transparência e dos PME; (2) aos níveis e tipo de perturbação de origem antropogénica a que estão sujeitas; (3) às comunidades fito e zooplanctónicas, a actividade da fosfatase alcalina (APA) é semelhante em ambos os sistemas (Capítulo 6, artigo 8). A ausência de correlações significativas, em ambas as albufeiras, entre APA e SRP e entre SRP e PME indicia que a hidrólise destes compostos pelas fosfatases não é importante no processo de regeneração do ortofosfatato. Por outro lado, a ausência de correlações significativas entre as concentrações de SRP e as de clorofila *a* parecem indicar que o fósforo não é um nutriente limitante nestes dois sistemas. Os resultados das experiências baseadas no ensaio do *Selenastrum capricornutum* Printz, desenvolvido por Miller *et al.* (1978), realizadas durante o presente estudo, apesar de não serem totalmente conclusivos, parecem também corroborar esta suposição. Porém, foram detectadas correlações significativas entre APA e alguns taxa pertencentes ao fitoplancton em ambas as albufeiras, e entre APA e *Ceriodaphnia*/*T. prasinus* somente na Serra Serrada. Uma explicação para este facto é a fosfatase poder ser produzida de forma constitutiva por estes organismos (*e.g.* Berman, 1970; Petterson, 1980; Boavida & Heath, 1986; 1988), ou que as densidades das suas populações variem do mesmo modo que a APA. Por outro lado, poder-se-á supor que as fosfatases sejam essencialmente produzidas pelo bacterioplancton. De facto, segundo Wetzel (2001) as necessidades de fósforo por parte do bacterioplacton são quatro a dez vezes mais elevadas do que as do fitoplancton. Assim, nestas albufeiras, ao contrário do que parece suceder com fitoplancton, o bacterioplancton poderá ser limitado pelo fósforo.

Embora o presente estudo constitua uma abordagem preliminar, os resultados obtidos permitem conhecer e avaliar: (a) os possíveis efeitos que as diferentes formas de ocupação da paisagem, do uso do solo e da água têm na limnologia destas albufeiras; (b) se bem que de uma forma muito incipiente, algumas consequências da variação do regime de precipitação nos parâmetros ambientais e nas comunidades fito e zooplanctónicas; (c) alguns factores de natureza abiótica e biótica que parecem ter um papel preponderante na estruturação das comunidades zooplactónicas; (d) a importância da actividade das fosfatases na regeneração do ortofosfato. No entanto, muitos outros aspectos permanecem por esclarecer. Sugerem-se, assim, futuras linhas de investigação interdisciplinares que deverão incidir sobre os seguintes pontos:

1. Obtenção de informação detalhada sobre a capacidade de retenção de nutrientes por parte dos solos localizados na vizinhança e na bacia de drenagem das albufeiras. Também é fundamental averiguar quais são as verdadeiras causas da erosão destes solos, bem como determinar a que taxas é que esta ocorre;

2. Determinar as quantidades de nutrientes (nomeadamente de fósforo e azoto) e de matéria orgânica que entram anualmente nestas albufeiras. Simultaneamente avaliar quais são as taxas de sedimentação das partículas e determinar que percentagem é que

fica retida nos sedimentos do fundo e que quantidade permanece realmente na coluna de água;

3. Caracterizar e monitorizar a composição das águas de escorrência provenientes das áreas ardidas localizadas na bacia de drenagem da albufeira da Serra Serrada;

4. Caracterizar e monitorizar a composição das águas de escorrência do IP 4 no troço da via que passa nas proximidades do Azibo. Dado que esta estrada passa por cima de ribeiros tributários desta albufeira, é lícito supor que poluentes como metais pesados possam entrar neste sistema aquático;

5. Distinguir quais são as variações nas comunidades fito e zooplactónicas da albufeira da Serra Serrada que são realmente causadas pelas flutuações no nível da água daquelas que são provocadas por fenómenos sazonais independentes destas;

6. Conhecer melhor as interacções que se estabelecem entre as comunidades fito/zooplanctónicas e as restantes componentes abióticas e bióticas destas albufeiras. Numa fase posterior da investigação será também fundamental analisar o modo como a sua composição e estrutura variam em resposta a alterações nos processos estruturantes da paisagem (*e.g.* padrões climáticos, precipitação, ocupação do solo). Só assim será possível conhecer e prever os padrões de variação destas comunidades ao longo do tempo, e detectar tendências, por vezes subtis, mas que podem ser utilizadas em programas de monitorização para avaliar como é que os sistemas aquáticos estão a evoluir. Estes dados são também importantes para permitir o ajustamento das medidas de gestão integrada sempre que necessário;

7. Esclarecer de uma forma mais aprofundada quais são e como se estabelecem, em determinados anos, as condições ambientais que no final do Verão e durante o Outono, favorecem a dominância da cianobactéria *Anabaena flos-aquae* na albufeira do Azibo. A obtenção destes conhecimentos é essencial para que seja possível prever de um modo acurado o possível aparecimento de "blooms" desta alga;

8. Investigar se o fósforo e o azoto são realmente limitantes para o fitoplancton das albufeiras estudadas. É também importante investigar qual é a principal fonte de

fosfatases, e se a sua produção é sempre constitutiva ou, se em algumas circunstâncias a sua síntese pode ser induzida.

Numa época em que os recursos hídricos são cada vez mais escassos, não só devido ao consumo crescente, mas também à deterioração da qualidade da água das albufeiras, urge tomar medidas que permitam a manutenção/recuperação do bom estado ecológico destes sistemas. As albufeiras abordadas nesta dissertação, bem como as bacias onde estão inseridas, têm potencialidades para o desenvolvimento de actividades ligadas ao turismo de natureza e rural. Numa região onde a agricultura é pouco rentável e a indústria praticamente inexistente, estes empreendimentos poderão constituir alternativas económicas para as populações. No entanto, para que estas actividades sejam implementadas, é necessário melhorar o estado ecológico destes sistemas. Por outro lado, há a considerar que o aumento dos custos do tratamento de água para abastecimento urbano é proporcional à intensidade da degradação dos ecossistemas aquáticos. Deste modo, torna-se importante a existência de um conhecimento profundo da ecologia destas albufeiras, nomeadamente dos óptimos ambientais das comunidades aquáticas e das suas respostas às perturbações, quer de origem natural quer humana. Espera-se assim, que a informação disponibilizada neste estudo, embora preliminar e incompleta, e a futura implementação das sugestões de investigação atrás mencionadas, venham a contribuir de forma decisiva para um melhor conhecimento destes ecossistemas, permitindo desenvolver medidas adequadas para a sua gestão numa perspectiva de desenvolvimento sustentável.

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