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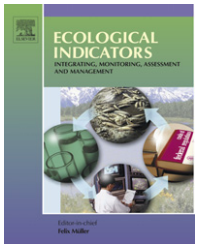

Multi-scale approach using phytoplankton as a first step towards the definition of the ecological status of reservoirs

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ARTICLE INFO

Article history:
Received 10 October 2007
Received in revised form
12 April 2008
Accepted 19 April 2008

Keywords:
Phytoplankton
Ecological status
Reservoirs
Water Framework Directive
Multivariate analysis
Ecological indicators

ABSTRACT

The growing need to analyse the present state of ecosystems and predict their rate of change has triggered a demand to explore species environment relationships for assessing alterations under anthropogenic influence. The Water Framework Directive (WFD) requires the definition of different types of water bodies which are of relevance when assessing their ecological status. The main aim of this study was to define of the types of Portuguese reservoirs located in the North and Centre of Portugal and to assess their ecological status using phytoplankton as water quality indicators. In this study, sampling was carried out in 34 reservoirs during four seasons (spring, summer, autumn and winter), through a period of 8 years (1996–2004).

Two groups of reservoirs could be distinguished, from the multivariate statistical analysis based on environmental variables and on phytoplankton assemblages: G1, lowland reservoirs located in the main rivers (Douro and Tagus), with a very low residence time, characterized by higher water mineral content (hardness and conductivity), higher concentrations of nutrients (namely, nitrates), dominated by Bacillariophyta and Chlorophyta and characterized by the presence of tolerant of poor environmental conditions species, mainly associated with meso and eutrophic states of water bodies; G2, deeper high altitude reservoirs, largely located in tributaries, with high residence time, presenting a specific species composition under reference conditions, with higher species richness. The transition from deeper and colder reservoirs (reference sites) to shallow and warmer reservoirs (impaired sites), was evident in G2, contrarily to G1, and was mostly positively correlated to organic pollution and mineral gradients. The results presented here are fundamental for the development of a routine for monitoring ecological status according to the WFD.

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doi:10.1016/j.ecolind.2008.04.006

1. Introduction

Human activity has altered the landscape over centuries, resulting in substantial loss of habitat and aquatic diversity (Young et al., 2005). Broad-scale environmental pressures such as agriculture, point and non point-source pollution, climate change, and land-use change overlap in space and time, requiring that stress measures incorporate assessments of cumulative impacts across multiple stressors (Dziock et al., 2006; Brazner et al., 2007; Danz et al., 2007). Biological assemblages are important sentinels of environmental conditions, since they can be more sensitive to the combined effects of stressors than to a single stressor (Karr, 1995; Niemi and McDonald, 2004). Therefore, they integrate cumulative impacts that would not be detected in another way or that would be otherwise underestimated (e.g. habitat degradation, highly variable pollution levels due to point and non-point pollution).

Worldwide aquatic ecosystems have been impacted by the excessive release of pollutants, leading to phytoplankton blooms and to the disruption of the structure and functioning of these systems (Robarts, 1985; Reynolds, 1992; Vasconcelos, 2001). The growing need to analyse the present state of ecosystems and to monitor and predict their rate of change, has triggered a demand for studies that explore species environment relationships and use these relationships for assessing and predicting changes under anthropogenic influence (Statzner et al., 2001; Simboura et al., 2005; Ekdahl et al., 2007). The development of indicator systems based on species environment relationships has become a widely used approach for these tasks (Statzner et al., 2001; Dziock et al., 2006). Building on this long tradition of using organisms in monitoring and assessment programs, the European Commission issued a directive mandating the use of different organism groups to monitor the integrity of inland waters and coastal regions. The Water Framework Directive (WFD-2000/60/EC) requires the use of different organism groups such as fish, invertebrates, macrophytes and phytoplankton, either singly or together, in assessing the ecological status of aquatic ecosystems. The WFD takes into account natural variation by proposing that relevant types of surface waters have to be defined first. Their characteristic species composition under reference conditions has to be described. Later on, the assessment of the ecological status shall be achieved by comparing the actual species composition to the one which would be present under undisturbed conditions (so-called reference conditions). However, such claim cannot easily be met, and several restrictions have to be taken into account when defining reference conditions, namely in artificial water bodies. Reservoirs are permanent and artificial lentic water bodies which have been consider as an integrated part of Iberic landscape. In Portugal, these structures are relatively recent and, generally, associated to multiple objectives for human benefits such as supply, irrigation, hydroelectric power and recreation. These water bodies and its biological communities are submitted to enormous spatial–temporal variations, caused by hydric resource use regime. Although Portuguese studies concerning phytoplankton for monitoring water quality are scarce and quite recent (Vasconcelos, 1991, 2001; Boavida and Gliwicz, 1996; Domingues and Galvão, 2007), currently, a larger project is underway, led by Portuguese Water Institute (INAG) to establish ecological status of all Portuguese aquatic systems, involving biological communities including fishes, macroinvertebrates, phytoplankton and macrophytes. Accordingly, the objective of this study was the definition of the different types of all Portuguese reservoirs with hydroelectric power, located in the North and Centre of Portugal. In the present paper, it is discussed the definition of the ecological status and types of reservoirs using phytoplankton as water quality ecological indicators. Based on this, several questions were addressed: what types of reservoirs were identified in the North and Centre of Portugal? How do phytoplankton assemblages and environmental variables differ among studied sites? Are landscape scale descriptors related to natural and stressor environment good for defining reference sites? Is phytoplankton community a reliable indicator of the ecological status of artificial water bodies, such as dammed reservoirs?

2. Materials and methods

2.1. Study area

This study was carried out in the North and Centre of Portugal in 34 reservoirs from six catchments: Ave (1 reservoir), Cávado (6 reservoirs), Mondego (5 reservoirs), and the Portuguese part of the international basins of Lima (2 reservoirs), Douro (11 reservoirs) and Tagus (9 reservoirs) (Fig. 1). The main purpose of all these reservoirs is hydroelectric power, although some secondary uses are also common, such as navigation, irrigation, water supply and recreation. Narrow and steep valleys of granite bedrocks morphologically characterize the Northwest of the study area. This region presents a relatively high rainfall average (more than 2200 mm/year) when compared to Mondego and Tagus catchments, with a yearly average rainfall of approximately 800 mm/year. The Douro catchment has more than 1400 mm/year in the mountainous northern areas and less than 500 mm/year in the semi-arid central part of this region. In Portugal, although the rainfall presents a high monthly variation, 70% of precipitation occurred between October and April. This extensive geographic area represents a wide range in physical and chemical characteristics, soil use and anthropogenic pressure, including both good and poor water quality conditions. Most of the population lives in the coastal area, and Ave and Cávado basins have the largest human population density in Portugal (378 and 265 hab/km^2, respectively). Therefore, many impacts associated with urbanization are present there, namely water quality problems associated with nutrient enrichment and high biochemical oxygen demand (BOD) due to industrial effluent discharges, urban development and intensive agriculture. In contrast, the eastern area of these basins is distinguished by steeper valleys and covered by remnants of native vegetation (the only national park is situated on the upper parts of the Lima and Cávado basins). Land use is dominated by agricultural activities in the more western areas. Nevertheless, the Ave basin presents the highest concentration of industry (mainly textile factories), followed by Tagus and Douro basins (mainly transformation industries and mines).

From the initial 38 reservoirs considered in our data set, 4 were removed because of missing environmental data. The

Please cite this article in press as: Cabecinha, E. et al., Multi-scale approach using phytoplankton as a first step towards the definition of the ecological status of reservoirs, Ecol. Indicat. (2008), doi:10.1016/j.ecolind.2008.04.006

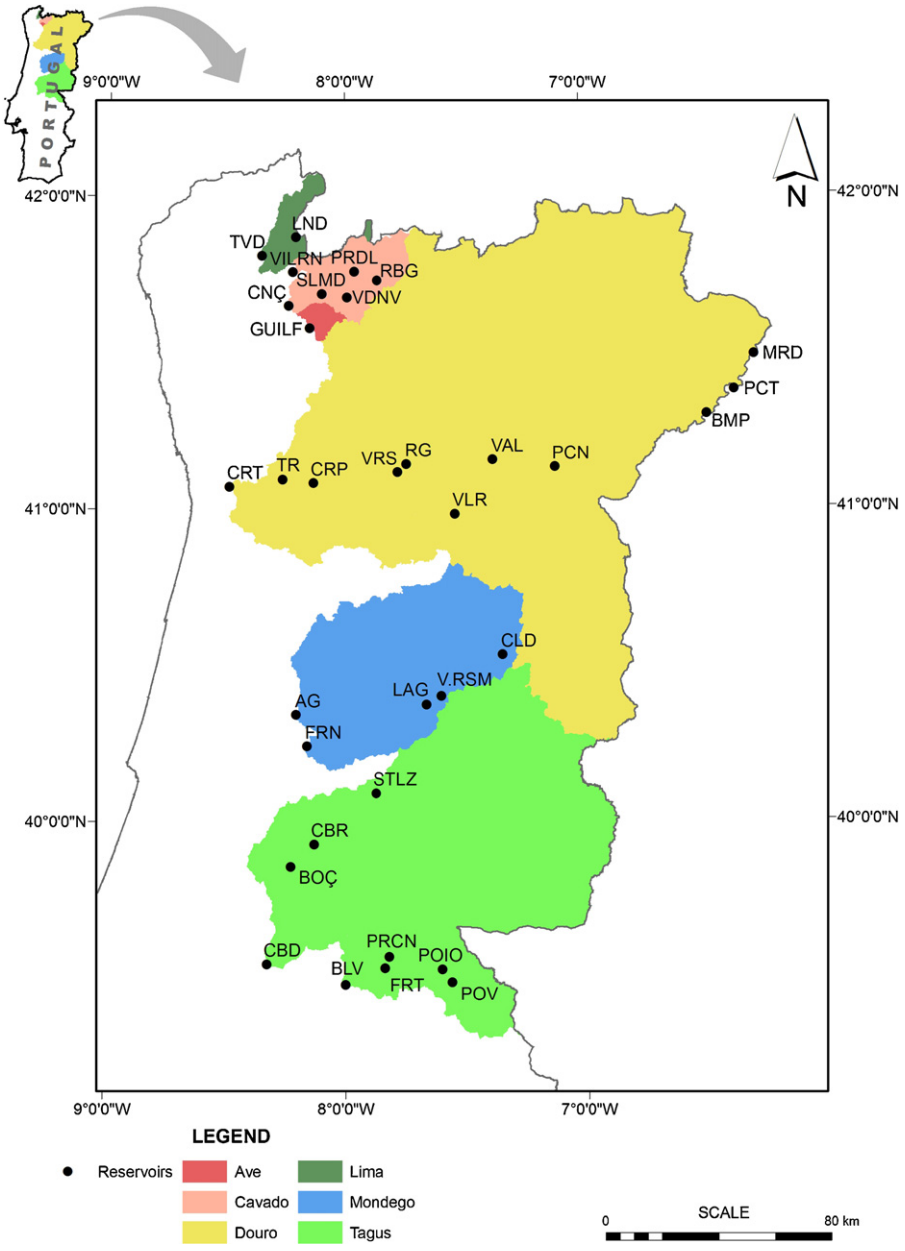

Fig. 1 – Location of the 34 reservoirs studied and their distribution through six catchments: Ave, Cávado, Mondego, and the Portuguese part of the international basins of Lima, Douro and Tagus.

majority of these reservoirs are explored as true reservoirs (24) (see Table 1), with relatively high residence time and variations along the year, mostly related to seasons. The remaining dammed water bodies (10) are ''run-of-river'' reservoirs, with very low residence time (days), presenting less stability conditioned by meteorological or hydrological conditions. The main characteristics of the reservoirs are presented in Table 1.

2.2. *Environmental parameters and chlorophyll a*

From 1996 to 2004, the environmental and biological parameters were measured by the Laboratory of Environment and Applied Chemistry (LABELEC) four times per sampling year, corresponding to spring (April/May), summer (July/August), autumn (October/November) and winter (January/February). The sampling periodicity was carried out in a yearly base on 58% of the reservoirs. The remaining reservoirs were visited biannually (26.5%) and triennially (14.7%). This sampling periodicity is also indicated in Table 1. All samples were collected at 100 m from the reservoirs's crest, at two different depths: (a) near the surface (approximately 0.5 m depth) and (b) near the bottom (2 m above bottom, only for environmental parameters).

Water temperature, turbidity, conductivity, pH and dissolved oxygen were determined *in situ* using a YSI handheld multiparameter probe (Yellow Spring Instruments). Light penetration in the water column was determined using Secchi disc method. In the laboratory, major ions, nutrient concentrations, BOD_5, total silicon, chlorophyll *a*, faecal coliforms and

Table 1 – Ranges and median values of important limnological properties of the 34 reservoirs surveyed since 1996–2004

Environmental variables	Code	Belver	Valeira	Picote	Carrapatelo	Fratel	Pocinho	Régua	Miranda	Bemposta	Crestuma-Lever	Vilarinho das Furnas	Caniçada	Lagoa Comprida	Salamonde	St[a] Luzia	Touvedo
		BLV	Val	PCT	CRP	FRT	PCN	RG	MRD	BMP	CRT	VILRN	CNÇ	LAG	SLMD	STLZ	TVD
Water column variables																	
Epilimnion																	
Surface water temperature (°C)	Temp	16.8	12.3	16.3	16.5	17.6	14.9	15.6	13.3	15.6	16.8	14.5	15.1	13.1	15.1	18.6	15.7
Turbidity (NTU)	Turb	2.96	4.85	4.06	1.69	2.50	4.97	4.26	10.8	1.67	3.13	0.44	1.01	0.84	1.01	1.07	2.68
pH (units)	pH	7.82	7.89	8.13	7.82	8.04	8.03	7.78	7.95	8.23	7.70	6.80	6.84	6.48	6.81	6.93	6.83
Dissolved oxygen (mg/L)	DO	9.76	9.53	8.61	8.29	10.1	10.8	10.3	9.20	7.98	9.30	9.42	10.1	8.81	10.1	8.63	9.58
Conductivity (μS/cm)	Cond	445	327	396	294	413	321	300	416	413	258	15.5	22.3	11.9	21.5	32.1	32.1
Ammonia-N (mg NH_4/L)	NH_4	0.18	0.17	0.13	0.11	0.20	0.15	0.14	0.27	0.18	0.10	0.08	0.09	0.12	0.06	0.12	0.09
Nitrate-N (mg NO_3/L)	NO_3	4.77	6.90	6.24	4.77	4.76	6.05	7.26	7.93	5.33	5.12	0.32	0.64	0.20	0.59	0.87	1.27
Total phosphorus (mg PO_4/L)	TotP	0.65	0.31	0.34	0.28	0.61	0.29	0.24	0.43	0.38	0.22	0.02	0.04	0.04	0.03	0.04	0.05
Chemical oxygen demand (mg O^2/L)	COD	12.8	1.40	2.17	8.73	14.1	11.4	11.8	10.7	10.5	7.26	3.01	4.43	3.94	4.79	3.85	6.73
5-day biochemical oxygen demand (mg O^2/L)	BOD_5	1.96	1.84	2.23	1.40	1.61	1.85	1.91	2.07	2.54	1.53	0.66	1.33	0.82	1.16	1.25	1.26
Total silicon (mg SiO_2/L)	SiO_2	5.56	7.28	1.69	3.14	5.92	3.29	4.34	3.15	1.26	3.57	3.59	3.02	1.12	3.80	5.52	3.70
Secchi disk depth (m)	SD	1.52	3.39	9.96	2.86	1.86	1.52	2.40	1.23	2.18	1.68	7.09	3.61	5.87	4.10	4.08	2.61
Chlorophyll *a* (mg/m^3)	Cpl_a	11.0	0.77	0.99	0.61	12.4	0.76	0.81	0.85	0.89	0.69	0.62	6.27	1.54	2.22	2.46	7.12
Faecal coliform (N/100 mL)	FColf	141	31.9	17.9	56.2	337	48.6	40.5	77.2	7.20	29.4	0.89	8.07	0.67	7.81	2.44	9.61
Hypolimnion																	
Water temperature (°C)	Temp-Hp	16.41	12.21	14.99	15.92	15.46	13.71	15.13	11.90	11.94	16.37	9.76	11.87	10.15	10.9	12.1	12.9
Turbidity (NTU)	Turb-Hp	2.73	6.58	5.79	1.99	3.38	6.36	5.50	20.98	4.60	4.07	0.75	1.72	0.59	2.64	1.98	3.24
pH (units)	pH-Hp	7.63	7.85	7.72	7.73	7.52	7.69	7.71	7.85	7.65	7.67	6.53	6.46	6.28	6.34	6.48	6.58
Disolved oxygen (mg O_2/L)	DO-Hp	8.08	8.03	4.22	6.97	5.60	7.68	8.84	6.20	2.48	8.76	8.23	7.29	8.37	6.07	5.74	6.72
Conductivity (μS/cm)	Cond-Hp	451	332	399	301	416	332	304	421	482	262	15.4	22.6	12.2	24.5	31.6	33.69
Total silicon (mg SiO_2/L)	SiO_2-Hp	5.40	3.50	2.58	3.26	6.60	3.58	4.67	3.75	1.89	3.42	3.31	3.83	1.09	3.62	5.98	4.27
Regional variables																	
Altitude (m)	Alt	46.15	105	480	71.9	74.0	125	73.5	528	402	13.2	569	162	1600	280	655	50.0
Precipitation (mm)	PP	66.49	60.6	53.4	70.2	59.6	58.9	65.5	53.8	53.45	90.4	246	183	160	173	109	204
Catchment area (km^2)	A	62802	85400	63750	92050	60000	81005	90800	63100	63850	92040	77.0	783	6	642	50.0	1700
Dam area (km^2)	Dam_A	2.86	7.95	2.44	9.52	7.50	8.29	8.50	1.22	4.05	12.9	3.46	6.89	15.7	2.42	2.46	1.72
Mean dam depth (m)	Dp	5.61	11.5	26.9	16.7	17.4	15.6	12.1	31.9	30.8	12.9	34.5	29.5	18.2	31.1	24.3	11.1
Maximum dam depth (m)	MxDp	21.00	48.0	100	59.1	43.0	49.0	42.0	80.0	87.0	65.0	94.0	76.0	29.0	75.0	76.0	43.0
Time of residence (days)	TimRes		3.39	3.27	5.76	5.24	2.50	2.10	1.45	9.52	2.24	203	38.6		21.7		3.34
Trophic state																	
Mean chlorophyll *a* (mg/m^3)		4	1	1	1	4	1	1	1	1	1	1	3	2	2	3	3
Total phosphorus (mg PO_4/m^3)	TP	5	5	5	5	5	5	5	5	5	5	3	4	4	3	4	4
Secchi disk depth (m)	SD	4	3	2	4	4	4	4	5	4	4	2	3	3	3	3	4
Mean annual energy output (GWh)		176	801	1038	870.6	347.5	534	738	1036.3	1086	366.9	225	346	48	232	55	67
Ecological status		II	II	III	IV	IV	IV	IV	V	V	V	I	I	I	I	I	I
Use type (regime)		a	a	a	a	a	a	a	a	a	a	b	b	b	b	b	b
Sampling periodicity		Annual	Trianual	Annual	Trianual	Biannual	Biannual	Trianual	Biannual	Trianual	Annual	Biannual	Annual	Annual	Annual	Annual	Annual
Principal watershead		Tagus	Douro	Douro	Douro	Tagus	Douro	Douro	Douro	Douro	Douro	Cávado	Cávado	Mondego	Cávado	Tagus	Lima

Environmental variables	Paradela	Vale do Rossim	Caldeirão	Fronhas	Alto Lindoso	Alto Rabagão	Venda Nova	Guilhofrei	Bouça	Poio	Torrão	Cabril	Vilar	Póvoa Meadas	Pracana	Castelo de Bode	Aguieira	Varosa	Minimum	Maximum
	PRDL	V.RSM	CLD	FRN	LND	RBG	VDNV	GUILF	BOÇ	POIO	TR	CBR	VLR	POV	PRCN	CBD	AG	VRS		
Water column variables																				
Epilimnion																				
Temperature (°C)	17.3	14.1	17.16	18.01	15.80	16.61	14.80	17.18	16.59	17.25	19.17	18.91	15.80	18.40	20.48	19.16	18.95	16.20	12.31	20.48
Turbidity (NTU)	0.55	0.75	1.54	1.76	1.34	0.83	1.13	1.55	2.11	5.32	2.29	0.99	2.37	4.16	3.02	0.84	1.40	3.16	0.44	10.80
pH (units)	6.88	6.47	7.31	7.66	7.07	6.88	6.83	6.83	7.13	7.97	7.78	7.22	7.74	8.10	8.42	7.38	7.91	7.85	6.47	8.42
DO (mg/L)	8.73	8.76	9.17	9.69	9.43	9.35	9.18	9.57	9.87	10.17	9.29	9.20	9.57	9.49	9.52	9.02	9.21	8.88	7.98	10.76
Conductivity (μS/cm)	18.50	10.35	33.25	45.99	33.07	23.10	23.56	32.42	57.46	99.44	85.36	63.71	55.87	110.01	77.18	71.92	85.26	103.29	10.35	445.00
NH_4 (mg/L)	0.07	0.07	0.08	0.11	0.08	0.10	0.10	0.08	0.07	0.12	0.11	0.08	0.16	0.28	0.12	0.07	0.10	0.96	0.06	0.96
NO_3 (mg/L)	0.24	0.10	0.44	0.97	1.07	0.22	0.37	1.57	1.99	0.91	2.60	1.55	0.70	1.51	0.59	1.77	2.04	3.78	0.10	7.93
TotP (mg PO_4/L)	0.02	0.04	0.07	0.06	0.04	0.05	0.04	0.05	0.07	0.39	0.10	0.03	0.10	0.34	0.13	0.03	0.08	0.53	0.02	0.65
COD (mg O^2/L)	3.96	4.02	6.47	7.28	3.23	5.74	4.57	4.79	4.36	25.59	6.82	4.66	11.19	21.60	10.47	4.08	7.69	13.05	1.40	26.59
BOD5 (mg O^2/L)	0.99	0.83	1.35	1.58	1.07	1.05	1.05	1.23	1.43	5.92	1.44	1.00	1.86	2.92	1.47	0.91	1.57	3.38	0.66	5.92
SiO_2 (mg/L)	4.34	1.91	5.34	5.89	3.66	0.61	2.32	3.48	9.30	3.22	4.94	8.17	2.02	5.09	5.24	4.52	4.81	8.41	0.61	9.30
SD (m)	5.59	5.66	2.73	2.21	5.37	3.57	3.65	2.76	2.65	0.89	2.35	3.98	2.16	1.03	2.34	4.90	2.66	1.54	0.89	7.09
Cpl_a (mg/m^3)	1.10	1.80	6.19	8.39	5.79	4.16	3.27	10.42	4.36	31.35	0.77	1.96	1.06	26.97	8.79	1.63	7.84	1.11	0.61	34.58
FColf (N/100 mL)	7.07	5.45	19.10	2.00	6.33	3.69	3.73	12.34	7.91	467.00	49.52	1.20	3.55	49.96	15.57	2.48	6.10	123.00	0.67	467.00
Hypolimnion																				
Temp-Hp (°C)	9.52	11.52	15.60	10.34	11.51	11.53	10.56	14.07	12.42	15.41	15.15	10.98	12.40	14.55	13.66	12.45	14.67	9.54	9.52	16.37
Turb-Hp (NTU)	1.00	1.19	1.87	7.21	3.35	1.23	2.36	2.59	3.52	6.41	2.88	2.94	2.15	5.58	5.31	1.32	2.96	11.65	0.59	20.98
pH-Hp (units)	6.25	6.38	6.91	6.49	6.50	6.55	6.45	6.45	6.59	7.09	6.79	6.69	6.71	6.92	6.89	6.84	6.70	6.67	6.25	7.85
DO-Hp (mg O^2/L)	5.82	7.87	6.89	4.26	6.33	6.56	6.45	6.32	5.42	6.04	4.51	3.92	4.92	3.36	3.16	5.40	4.69	1.94	2.48	8.76
Cond-Hp (μS/cm)	17.11	11.80	33.97	63.23	36.41	24.40	23.33	33.34	54.14	102.57	78.40	65.83	56.00	118.07	80.92	68.70	86.19	104.63	11.80	481.90
SiO_2-Hp (mg/L)	3.77	2.02	5.90	7.61	4.27	0.72	1.93	4.81	9.58	6.22	6.54	9.19	3.68	7.55	8.22	8.26	7.72	8.45	0.72	9.58
Regional variables																				
Alt (m)	740.00	1436.46	702.00	134.00	338.00	880.00	700.00	335.63	175.00	270.00	65.00	296.00	552.00	311.45	114.00	121.50	124.70	264.00	13.20	1600.00
PP (mm)	164.85	155.14	70.60	114.46	204.26	136.68	161.02	198.88	100.11	66.63	122.72	98.91	79.41	67.27	83.37	99.03	98.27	134.04	53.41	245.97
A (km^2)	269	5	32	652	1525	101	356	122	2525	16	3252	2340	370	150	1410	1340	3100	310	4	96303
Mn. dam depth (m)	3.80	0.37	0.66	5.35	10.72	22.10	4.00	16.30	1.85		6.50	20.23	6.70	2.36	5.50	32.91	20.00	0.70	0.37	32.91
Mx. dam depth (m)	42.78	8.91	8.36	19.87	21.44	26.98	24.70	11.58	24.31	8.37	20.74	38.14	15.75	8.55	23.24	33.49	24.86	23.49	5.61	42.78
Dam area (km^2)	112.00	27.00	39.00	62.00	110.00	94.00	97.00	49.00	65.00	18.00	70.00	136.00	58.00	32.00	60.00	115.00	89.00	76.00	18.00	136.00
TimRes (days)	196.09		19.20	59.43	108.31	594.12	63.32		7.62		13.52	138.93	320.61		105.43	191.10	50.59		1.45	594.12
Trophic state																				
Mean Cpl_a (mg/m^3)	3	2	3	4	3	3	3	4	3	5	1	2	2	5	4	2	3	2	1	5
PO^4 TOT (mg PO^4/m^3)	3	4	4	4	4	4	4	4	4	5	4	3	4	5	5	3	4	5	3	5
SD (m)	3	3	4	4	3	3	3	4	4	5	4	3	4	5	5	3	4	4	2	5
AnBng (GWh)	253	28	45	–	948	97	389	11	157.2	4.8	228	301	148	1.6	61.8	390	209.6	60	1.6	1086
Ecological status	II	II	II	II	II	II	II	III	IV	IV	IV	IV	IV	IV	IV	V	V	V		
Use type	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b		
Sampling periodicity	Biannual	Annual	Annual	Biannual	Annual	Biannual	Annual	Annual	Biannual	Annual	Annual	Triannual	Annual	Annual	Biannual	Annual	Annual	Annual		
Principal watershead	Cávado	Mondego	Mondego	Mondego	Lima	Cávado	Cávado	Ave	Tagus	Tagus	Douro	Tagus	Douro	Tagus	Tagus	Tagus	Mondego	Douro		

(a) "Run-of river" reservoir. (b) Reservoir. Trophic state: 1, ultra-oligotrophic; 2, oligotrophic; 3, mesotrophic; 4, eutrophic; 5, hyper-eutrophic. Ecological status: from I, high status to V, low status.

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sulphate reduction bacteria were determined according to methodologies described by APHA (1995).

To determine the ecological status of the reservoir's watersheds, a geographic information system database was created (ESRI, ArcGIS 9.0), with 12 spatial variables. These variables were classified into 4 categories of anthropogenic stress measures that are prominent in the study area: (i) land cover, 6 land use/land cover variables derived primarily from the Corine Land Cover (CLC, 1990 and 2000) (IGEOE, 2006). Road density (km/ha basin) and proportions for the predominant CLC classes in the basin (urban areas, intensive and extensive agriculture, natural and semi-natural areas and burned areas) were determined; (ii) organic contamination load, 2 variables representing human population pressure (g BOD5/hab eq day by ha basin) and domestic animal pressure (g BOD5/animal eq day by ha basin); (iii) industrial contamination load, 3 variables representing point sources pollution, including number of quarries, mines and transformation industries in the basin (number of sources/ha basin); and (iv) hydrometric variations, yearly water level changes were determined by the differences between relative average water level and maximum theoretical water level. Points (ii) and (iii) were determined based on data from INE (2006). A five-score scale was established for all variables (from 1, high status to 5, low status). Therefore, the sum of these five-score scales reflects the final ecological status of the reservoir's watershed and was classified in the following classes: I, <18; II, 18–22; III, 22–26; IV, 26–30 and V >30. In this study, class I and II were grouped to represent reference reservoirs, and class IV and V were grouped to represent impaired sites.

Although all data characterized anthropogenic stress in some way, there was considerable variation in the types of variables used. Some variables represented the extent of non-natural land cover (e.g. percentage of land devoted to high-intensity residential uses, or to row crops), whereas others represented specific human activities (e.g. point locations of mines) and specific stressors (e.g. hydrometric alterations). All variables were expressed, when possible, on a per-unit area basis.

Trophic classification of reservoirs was obtained from OECD model (Vollenweider and Kerekes, 1982) based on Total phosphorus, Secchi depth and mean chlorophyll *a* concentration.

2.3. Phytoplankton analysis

Phytoplankton samples were collected from 1996 to 2004, as described for the environmental parameters, using a Van Dorn bottle net, at a depth of approximately 0.5 m. Phytoplankton community composition was studied through inverted microscopy, following Utermohl's method (Lund et al., 1958). For the identification of phytoplankton, samples were fixed in Lugol's solution (1%, v/v) and, when possible, identified to the species level.

2.4. Statistical analysis

From an initial data set of 710 samples from 1996 to 2004, a subdata set was used for biological and environmental data expressed by means for all sampling years and for each studied reservoir ($n = 34$). Environmental data were standardized in order to obtain comparable (dimensionless) scales (Clarke and Warwick, 1994), and variables with more than 10% of data points missing were eliminated. The biological presence/absence matrix data was transformed in a probability occurrence matrix (number of presences/number of samples). In this matrix data, rare species (less then three presences in each dam, for all the samples) were omitted from statistical analyses (Forester et al., 2004; Negro and De Hoyos, 2005).

The statistical analysis of the environmental and biological matrices was performed based on multivariate methodologies: (a) for environmental data, a cluster analysis using city-block distances and a discriminant analysis based on Discriminant Canonical Analysis (DCA); (b) for biological data, a cluster analysis trough city-block distances and a comparative analysis based on non-metric multidimensional scaling analysis (n-MDS) and Similarity Percentages-species contributions analysis (SIMPER).

Multivariate analysis (Cluster and DCA) were carried out using STATISTICA® Version 7 (Stat Soft 2004) and n-MDS and other routines associated were performed using PRIMER® Version 5.2.2 (Clarke and Gorley, 2001).

Cluster analysis was used to identify natural groupings in the set of data (biological and environmental) without providing any explanation/interpretation. In this study, the similarity measures between sites were based on Ward's method and City-block (Manhattan) distances. City-block distances measure the distance as the average difference across dimensions. In most cases, this measure yields similar results to the simple Euclidean distance. However in this case, the effect of outliers is dampened (since they are not squared).

Afterwards, the matrix of environmental data was analysed by a DCA performed with a forward stepwise method of statistical significance. The DCA was used for detecting the variables that allow discrimination between different (naturally occurring) groups.

To compare phytoplankton assemblages and to classify sites along a gradient of human disturbance, a n-MDS and other routines implemented in PRIMER were used. Phytoplankton population was compared by Bray–Curtis distance calculations using untransformed population data (relative presence for each *taxa*), and the resulting distance matrix used to infer two-dimensional n-MDS plots.

Statistical differences between clusters identified in n-MDS plots were investigated by a randomization method, ANOSIM (Clarke and Warwick, 1994). This methodology employs *R* statistics to examine the existence of meaningful differences between the established groups for each considered factor (groups and differences between reference and impaired reservoirs). For each group, a Similarity Percentages-species contributions (SIMPER) was used to determine which species contributed most to the differences among the groups (Clarke and Gorley, 2001), based on the probability of occurrence.

3. Results

3.1. Environmental variables

The cluster analysis, based on city-block distances, divided the 34 reservoirs into two major groups (Fig. 2). The first cluster

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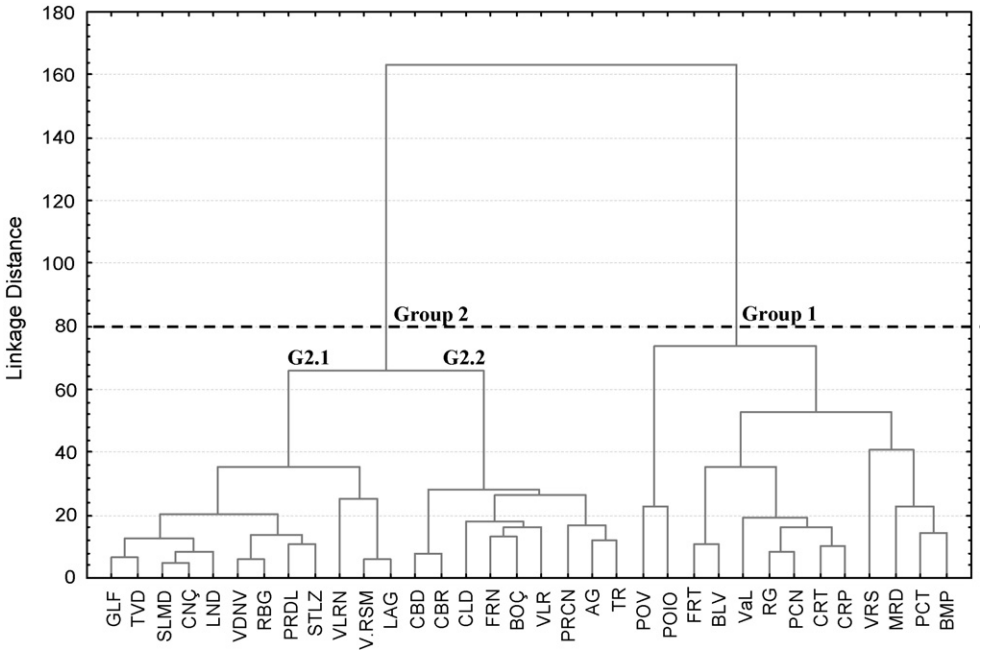

Fig. 2 – Classification of sites by Ward's method based on city-block distance, with environmental data set. The discontinuous line is the cutting line for defining two reservoirs's groups and subgroups of Group 1 (G2.1 and G2.2). See Table 1 for reservoirs's abbreviations.

(G1, Group 1) presents mainly reservoirs (77%) located in the main rivers (Douro and Tagus). These waterbodies are ''run-of-river'' reservoirs, characterized by having nearly the same inflow and outflow presenting a residence time of 1–4 days. In general, this group represents mainly reservoirs with a trophic state between eutrophic to hyper-eutrophic, mainly due to phosphorus concentration and low transparency (see Table 1).

The second cluster (G2, Group 2) represents reservoirs explored in a true reservoir regime, largely located in tributaries, with a high residence time (weeks to months), and in regions with higher altitudes and mean yearly rainfall. Additionally, these reservoir's basins present greater slopes and depths than the ones in Group 1 (Table 2). In this cluster it is possible to distinguish two sub-groups: G2.1 consisting of deep and colder water bodies located in the highest altitudes (634.43 m; range: 134–1600 m), precipitation values (yearly mean of 160.34 mm; range: 70–245 mm), slopes (13%; range: 6–22%) and depth (23 m; range: 134–1600 m). Usually, these sites are subject to low anthropogenic stress (see Tables 1 and 2); G2.2 represents shallow and warmer reservoirs subject to higher anthropogenic stress and characterized by lower altitudes (229.37 m; range: 65–552 m), slopes (9%; range: 4–13%) and precipitation values (yearly mean of 94.98 mm; range: 66–134 mm) (Table 2).

DCA presented very similar result to the cluster analysis. Consequently, the plot of the first two canonical factors (Fig. 3a) allowed to differentiate two major groups and to discriminate between reference and impaired sites within each group. Correlation coefficients among the first two factors and individual environmental variables indicated that Root 1 presented a contrast between hardness/turbidity/NO_3 and altitude/slope, whereas Root 2 displayed a contrast between OD-HP/altitude/slope and hardness/NO_3/COD. Multivariate test statistics (Wilks' lambda and corresponding F-value) indicated that there was a significant difference ($p < 0.001$) among the cluster centroids for the clusters displayed in Fig. 3a. Univariate F-tests for the individual environmental variables found significant differences among the clusters means for 14 variables from the initial 26.

The first factor explained almost all the variance (92%), and was dominated by hardness ($F = 204.06$). The second factor accounted only for 7%. Therefore, the ordination graph is consistent with the conclusion that reservoirs from Group 1, ''run-of-river'' reservoirs, tend to present higher hardness, turbidity and nutrients concentration, namely NO_3. In general, these reservoirs presented watersheds dominated by industries and agriculture that occupied about 50% of the total area (>15% of intensive agriculture).

The separation between reference and impaired sites from Group 2, results mainly from the environmental variables correlated with the second factor. Reference sites from Group 2 were characterized by watersheds with vast natural areas (more than 80%), small agriculture areas (about 16%, but only 3% of intensive agriculture) and good water quality (high DO levels in the hypolimnion. In contrast, impaired reservoirs (G2.2) were subject to higher pollution stress, probably related to watershed soil use, since they were less forested (65%) and presented more agricultural areas (>30%). In Fig. 3b it is possible to see how these reservoirs were spatially clustered.

The analysis clearly reflects substantial differences in water chemistry among the two groups of reservoirs defined. In Fig. 4, it is possible to observe in all the graphics a pollution gradient (right to left) from Group 2 to Group 1 and from reference to impaired reservoirs based on some environmental variables, namely conductivity, hardness, NO_3, Cl, SO_4 and pH and pH of the hypolimnium (pH-HP). This gradient was again verified in depth (hypolimnion) for the first three

Table 2 – Median values and standard deviation (S.D.) of important limnological properties of the two groups of reservoirs, and within each group characteristics of reference and impaired sites

Environmental variables	Expected response to increasing perturbation	Group 1		Group 2		G 1- Reference		G 1 - Impaired		G 2- Reference		G 2 - Impaired	
		Mean (n=10)	sd	Mean (n=24)	sd	Mean (n=2)	sd	Mean (n=8)	sd	Mean (n=13)	sd	Mean (n=11)	sd
Water column variables													
Epilimnion													
Temp (ºC)	increase	15.6	1.67	16.8	1.88	14.5	3.15	15.8	1.42	15.8	1.62	18.1	1.54
pH (units)	variable	7.94	0.17	7.26	0.55	7.85	0.05	7.94	0.18	6.91	0.31	7.75	0.40
DO (mg/L)	decrease	9.39	0.90	9.36	0.43	9.64	0.16	9.43	1.04	9.30	0.48	9.42	0.39
Cond (µS/cm)	increase	358	65.2	48.6	31.3	386	83.4	345	67.3	24.9	10.1	80.9	19.1
Hardness (mg $CaCO_3$/L)	increase	141	24.6	9.92	7.15	138	1.20	138	27.4	4.53	2.60	17.4	3.67
Cl (mg/L)	increase	19.2	8.22	5.32	3.25	25.4	14.4	17.7	7.15	3.05	1.02	8.34	2.83
NH_4-N (mg/L)	increase	0.16	0.05	0.14	0.18	0.17	0.01	0.16	0.06	0.09	0.02	0.21	0.27
NO_3-N (mg/L)	increase	5.91	1.15	1.10	0.89	5.84	1.51	5.89	1.26	0.56	0.38	1.74	0.96
TotP (mg PO_4/L)	increase	0.38	0.15	0.72	3.02	0.48	0.25	0.35	0.14	1.19	4.12	0.18	0.17
SO4 (mg/L)	increase	51.4	21.6	3.50	2.67	65.2	36.7	47.6	19.8	1.43	0.66	6.40	1.27
Fe (µg/L)	increase	46.2	13.9	46.4	33.6	63.9	6.11	43.8	10.8	33.5	8.88	65.4	45.7
SiO_2 (mg/L)		3.92	1.88	4.33	2.16	6.42	1.22	3.53	1.41	3.45	1.64	5.57	2.34
SD (m)	decrease	2.86	2.58	3.47	1.62	2.46	1.32	1.96	0.56	4.32	1.48	2.45	1.24
Cpl_a (mg/m^3)	increase	2.98	4.62	6.35	7.79	5.91	7.27	2.43	4.40	3.92	2.53	8.58	11.26
FColf (N/100mL)	increase	79.0	98.0	34.0	96.0	87.0	77.0	85.0	113	6.00	5.00	73.0	144
Hypolimnion (Hp)													
Temp-Hp (°C)	increase	14.4	1.82	12.2	1.91	14.3	2.97	14.4	1.85	11.4	1.59	13.1	1.91
Turb-Hp (NTU)	increase	6.20	5.42	3.18	2.51	4.65	2.72	6.70	6.46	2.24	1.74	4.47	2.99
pH-Hp (units)	variable	7.70	0.10	6.61	0.22	7.74	0.16	7.69	0.10	6.48	0.17	6.79	0.15
DO-Hp (mg O_2/L)	decrease	6.68	2.08	5.68	1.62	8.05	0.03	6.64	2.20	6.66	1.12	4.34	1.25
Cond-Hp (µS/cm)	increase	370	73.3	49.9	31.9	392	83.9	359	80.3	26.9	13.7	81.5	21.5
Hardness (mg $CaCO_3$/L)	increase	146	27.1	10.3	7.18	142	6.31	144	31.0	5.10	3.35	17.6	3.99
NO_3-Hp (mg/L)	increase	6.33	1.19	1.42	0.93	6.08	1.59	6.37	1.30	0.78	0.55	2.20	0.73
SO_4-Hp (mg/L)	increase	52.7	21.9	3.21	2.40	66.4	38.5	49.3	19.9	1.45	0.66	5.67	1.62
PO_4-Hp (mg/L)	increase	0.32	0.19	0.11	0.23	0.39	0.25	0.31	0.20	0.02	0.01	0.23	0.32
SiO2-Hp (mg/L)		3.86	1.37	5.36	2.61	4.45	1.35	3.88	1.45	3.72	2.00	7.54	1.71
Regional variables													
Alt (m)		189	196	453	407	75.7	41.7	180	198	634	473	229	142
PP (mm)		63.2	11.2	135	50.2	63.5	4.19	64.5	12.9	160	45.8	94.9	21.8
A (Km^2)		75480	13900	881	1029	74101	15979	77549	14789	477	572	1481	1253
Mean Dam depth (m)		18.1	8.82	22.3	9.75	8.55	4.17	19.6	8.23	23.2	10.2	22.1	9.53
Max. Dam depth (m)		59.4	23.9	70.9	31.1	34.5	19.1	60.7	17.7	71.8	29.7	71.9	35.2
Dam area (Km^2)		6.53	3.73	8.38	8.67	5.41	3.60	7.44	3.81	6.13	6.43	10.7	11.1
TimRes (days)		3.94	2.54	125	149	3.39	1.15	4.12	2.90	131	177	118	111
Stress measure	increase	27.0	4.79	23.0	3.87	21.0	0.71	30.0	2.71	19.0	2.93	29	1.74
Biological variables													
Phytoplankton (%)													
Pyrrophyta (PYR)		0.77		3.45		0.18		0.94		4.27		2.39	
Cyanophyta (CN)	increase	8.47		11.4		7.45		8.75		9.41		14.2	
Chrysophyta (CRS)	decrease	0.88		5.52		1.06		0.82		8.39		2.15	
Euglenophyta (EGL)		3.73		1.81		2.40		4.09		1.22		2.52	
Bacillariophyta (DTM)		40.7		33.8		43.4		39.9		35.9		31.2	
Chlorophyta (CLP)	increase	42.1		40.1		42.7		42.0		36.7		43.9	
Cryptophyta (CRP)		3.35		3.90		2.84		3.49		4.17		3.63	

variables mention previously. In general, differences among Groups (1 and 2) and among reference versus impaired sites of Group 2, were very significant ($p < 0.001$). Contrarily these differences were less obvious between reference versus impaired sites of Group 1. Probably available data set from reference sites was not large enough to become statistically significant as to determine differences among types. There were a small number of reservoirs of this type ($n = 10$), and from this group only two sites were selected as best available ones. Only Belver and Valeira reservoirs presented "good

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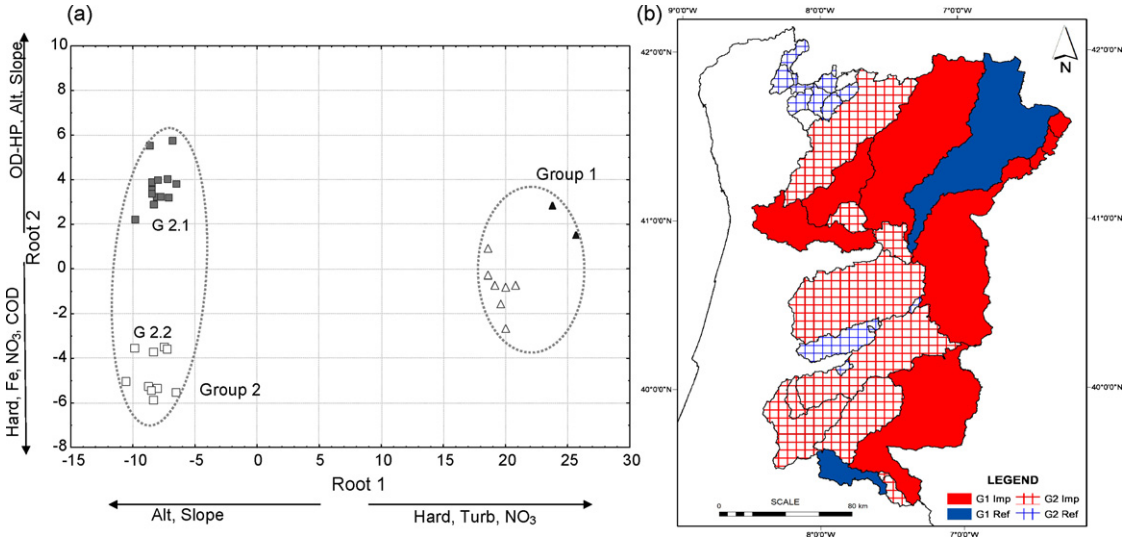

Fig. 3 – (a) Discriminant analysis of environmental data relative to cluster structure. Axis interpretation is based on correlation between each variable and the first two discriminant factors. (b) Spatial distribution of the defined reservoirs's groups. Filled (blue) and empty symbols (red) represent reference and impaired reservoirs, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

ecological status'' (Class II, see Table 1) from the score based on anthropogenic stress measures.

3.2. *Analysis based on phytoplankton assemblages*

From the 710 phytoplankton samples a total of 250 taxa were identified. From these, 55 taxa occurred less then three times in each reservoir and were excluded from the dataset (see Section 2). The 195 remaining taxa belonged to 7 divisions. Most important in terms of species number and presences were *Chlorophyta* (75 species, 40.8% of the presences), *Bacillariophyta* (58 species, 36.4% of the presences) and *Cyanophyta* (37 species, 10.2% of the presences). There were 9 taxa of *Chrysophyta* (4.0% of the presences), 5 taxa of *Pyrrophyta* (2.4% of the presences),

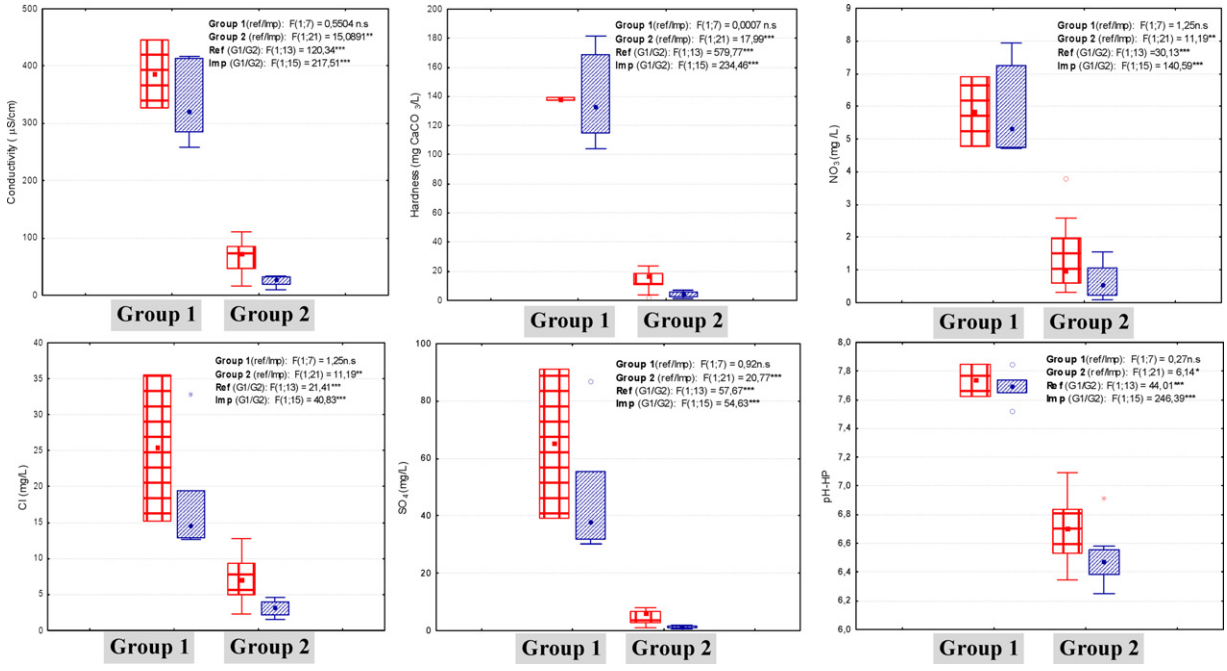

Fig. 4 – Differences in some environmental variables concentrations, from epi- and hypolimnion, in the two groups of reservoirs and within each group, in reference (blue) and impaired (red) sites. Box and Whisker diagrams show median, range and 25th and 75th percentiles of values for samples in each group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

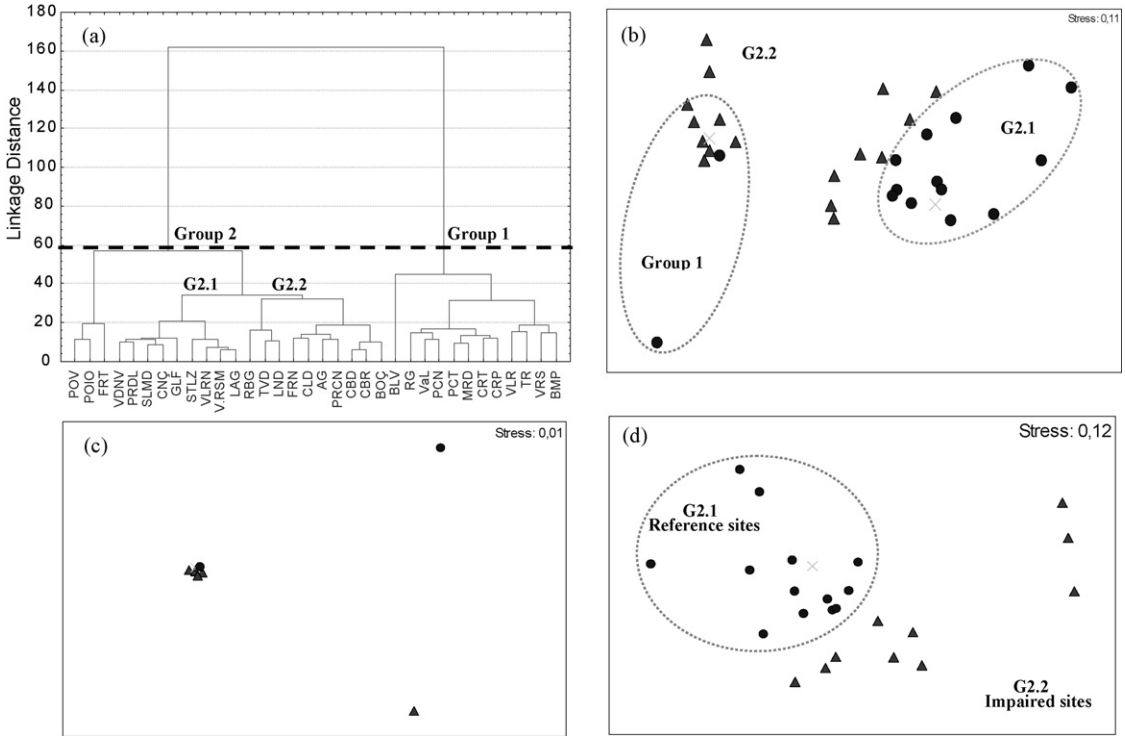

Fig. 5 – (a) Site dendrogram and (b) non-metric multidimensional scaling (n-MDS) ordination for 34 Portuguese reservoirs, based on phytoplankton assemblage data. (c) n-MDS for Group 1 and (d) Group 2, respectively. Dotted lines indicate reservoir groups produced by cluster analysis. Circles and triangles represent reference and impaired reservoirs, respectively.

and 3 taxa of *Cryptophyta* (3.8% of the presences) as well as of *Euglenophyta* (2.4% of the presences). The cluster analysis of phytoplankton assemblages data, identified in general, the same two major groups as the environmental data (Fig. 5a). The patterns revealed by cluster analysis were apparent in the Non-metric MDS ordination. The n-MDS based on species/site data for all reservoirs ($n = 34$) was able to differentiate between the two identified groups (stress value of 0.11 for 2D and 0.07 for 3D) (Fig. 5b). Additionally, this analysis was able to distinguish between undisturbed and impaired sites within Group 2 (stress value of 0.12 for 2D and 0.07 for 3D) (Fig. 5d). For Group 1, these differences are not so obvious (stress value of 0.01 for 2D and 3D) (Fig. 5c). In general, the n-MDS analysis displayed a gradient of disturbance, allowing the scattering of sites along a magnitude range of human impact.

These results were confirmed by the pairwise analysis of similarity (ANOSIM) and SIMPER tests (see Table 3). The Global ANOSIM test showed that there were significant differences ($p < 0.001$) in assemblage composition between the two groups and among reference and impaired reservoirs for all data sets ($n = 34$) and for Group 2 (see Table 3). The global *R*-value is a useful comparative measure of the degree of separation of the groups used (Clarke and Warwick, 1994). In this case, only in data from Group 1 it was not possible to distinguish between reference and impaired groups with a global R (0.351, $p = 0.194$).

Table 3 – Percentage breakdown of average dissimilarity between groups of reservoirs, and groups of impaired vs. reference sites for all reservoirs with hydroelectric power in Portugal, using SIMPER analysis

Factors	Groups	Average similarity (%)	Average disimilarity (%)	Anosim
Groups ($n = 34$)	1 2	53.14 39.83	71.30	Global $R = 0.494^{***}$
Ref/Imp ($n = 34$)	Reference Impaired	39.86 41.87	68.96	Global $R = 0.381^{***}$
Group 1 ($n = 10$)	Reference Impaired	29.66 54.44	55.30	Global $R = 0.351$ (n.s.)
Group 2 ($n = 24$)	Reference Impaired	46.80 40.47	65.35	Global $R = 0.380^{***}$

Global *R*-values for the pairwise analysis of similarity (ANOSIM) tests. Only $p < 0.001$ (***) was regarded as significant.

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The results of SIMPER analysis that compared groups and reference versus impaired sites (Table 3) are in evident agreement with the patterns observed in the previous analyses. The average dissimilarity between groups was 71.30%, making clear the existence of a strong variability among them. A total of 108 taxa (57.14% of the total *taxa*) accounted for 90% of the dissimilarity between these two groups. A value of 68.96 and 65.35% of dissimilarity between the most and least disturbed sites (for all reservoirs and within Group 2, respectively) corroborates the hypothesis that these groups are truly different. The most characteristic phytoplankton taxa of these groups are presented in Table 4 (to a cumulative percentage of 75%). From each specific *taxa* composition it is possible to see that even though both groups were visibly dominated by Bacillariophyta and Chlorophyta they only had six *taxon* in common: *Melosira ambigua*, *Trachelomonas* spp., *Scenedesmus ecornis*, *Monoraphidium* spp., *Cyclotella* spp. and *Closterium acutum*.

In Group 1, there were not obvious differences among species composition between reference and impaired sites. Indeed, from the 19 most characteristic taxa from reference sites in this group, 10 are present in impaired sites as well. This corroborates the results from Cluster and ANOSIM analysis. Both sites were clearly dominated by Bacillariophyta and Chlorophyta and characterized by the presence of tolerant species, mainly associated from meso to eutrophic states of water bodies. Therefore, meso-eutraphentic species (Van Dam et al., 1994; Tavassi et al., 2004), like *Navicula rhynchocephala*, *Melosira granulata*, *Synedra pulchella*, *Pediastrum simplex* and *Pediastrum duplex*, dominated the less disturbed sites from Group 1. Additionally, impaired sites presented eutraphentic species, namely *M. ambigua*, *Cyclotella meneghiana*, *Synedra ulna*, *S. pulchella*, *Nitzschia accicularis* and *Cocconeis placentala*, associated with blue-green algae, *Aphanizomenon flos-aquae* and *Oscillatoria planctonica*.

Reference sites from Group 2 were typified by the intolerant (oligotraphentic to oligo-mesotraphentic) species, *Melosira distans*, *Melosira italica*, *Tabellaria floculosa*, *Tabellaria fenestrata* and *Rhizosolenia eriensis* and some mesotraphentic species like *Syneda acus*. Additionally, in these reservoirs, Chrysophyta, that is known to decrease with disturbance increase, had a significant importance (11.68% versus 1.09% in the impaired sites).

Contrarily, disturbed sites were characterized mostly by tolerant species of several divisions such as Chlorophyta, Bacillariophyta, and Cyanophyta. The present blue-green algae belonged mostly to genera whose ability to produce toxins that can affect a variety of organisms, including humans is known, like *Aphanizomenon flos-aquae*, *Anabaena* spp., *Microcystis aeruginosa*, *Microcystis pulverea* and *Microcystis flos-aquae* (Vasconcelos, 1999, 2001; Dokulil and Teubner, 2000; de Figueiredo et al., 2006).

4. Discussion

Multivariate analyses based on environmental variables and phytoplankton assemblages, allowed to define the different types of surface waters from North and Centre of Portugal. From the studied 34 reservoirs, it was possible to identified and delimit two types of dammed water bodies which were characterized by different hydromorphological features, water chemistry characteristics and by a specific species composition. Group 1 had mostly ''run-of river'' reservoirs located in the main rivers (Douro and Tagus), with very small residence time; Group 2 was represented by deeper dammed water bodies with higher residence time, largely located in tributaries, in regions with higher altitudes and average yearly rainfall. In general, Group 1 represented reservoirs with a trophic state between eutrophic to hyper-eutrophic, mainly due to phosphorus concentration and low transparency. Since all these reservoirs belong to International river basins, the trophic state observed may be a consequence of the great anthropogenic pressures that characterized such basins, namely due to upstream intensive agriculture practiced in Spain. The differences in retention time and water depth have a large impact on how eutrophication manifests. According the Vollenweider models, lakes with a high retention time (generally the deeper lakes) will have a lower nutrient concentration than the lakes with a very low retention time (generally the shallower ones) (GIG, 2007).

In general, median Secchi depth, total phosphorus and chlorophyll *a* concentration were comparable with those reported in previous surveys (Boavida and Marques, 1996) and confirmed the hyper-eutrophic status of the majority of the reservoirs from G1. G2 reservoirs were quite variable, displaying a clear disturbance gradient, with deep colder sites, mainly oligotrophic, while warmer sites showed higher values, mostly eutrophic and hyper-eutrophic status (Boavida and Marques, 1996). Therefore, in this study, it was possible to identify distinct gradients of human disturbance, along which environmental variables and phytoplankton assemblages changed within both reservoirs types.

Among the 26 environmental variables used in multivariate analysis, nitrate concentration and water mineral content were mainly responsible for the dissimilarity among these two groups (Fig. 3). The transition observed in Fig. 4, from impaired reservoirs of Group 1 to reference reservoirs of Group 2, reflects substantial differences in water chemistry between the two Groups defined and within reference versus impaired sites. These chemical properties of the water body, originated from geological characteristics of the watershed, seem to assume major importance. The results presented here are consistent with several studies developed in rivers (Stevenson, 1997; Wetzel, 2001) as well as in lakes and reservoirs (Wetzel, 2001; de Figueiredo et al., 2006; Tolotti et al., 2006), who proposed geological properties as an ultimate variable that determines the composition of aquatic community assemblages on a larger spatial scale. However, at a smaller scale, physical characteristics (e.g. reservoir size, temperature/elevation) and human-influenced water quality gradients (e.g. nutrients, BOD_5, COD, turbidity) were more important. The same results were obtained along spatial and environmental gradients at a larger-scale, based on diatom assemblages (Lim et al., 2007); macroinvertebrates, macrophytes and fishes (INAG, 2006).

The typology identified in hydroelectric Portuguese reservoirs and that was based on environmental variables was also corroborated by changes in phytoplankton assemblage.

This study has identified distinct gradients along which phytoplankton assemblage structure changes within north

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Table 4 – Average contribution (Ct.%) of species mainly responsible for intra-group similarities

(a) Group 1		Group 2		Reference		Impaired	
Species	Ct.%	Species	Ct.%	Species	Ct.%	Species	Ct.%
Melosira ambigua	5.60	*Synedra* spp.	6.76	*Synedra accus*	6.93	*Melosira ambigua*	7.20
Scenedesmus opoliensis	5.29	*Melosira ambigua*	5.36	*Dinobryon* sp.	6.18	*Closterium acutum*	3.86
Cyclotella meneghiniana	4.37	*Sphaerocystis schroeteri*	5.31	*Navicula* spp.	4.68	*Cyclotella* spp.	3.39
Trachelomonas spp.	3.90	*Navicula* spp.	4.86	*Sphaerocystis schroeteri*	4.29	*Scenedesmus ecornis*	3.30
Scenedesmus ecornis	3.81	*Dinobryon* sp.	4.45	*Melosira ambigua*	4.11	*Trachelomonas* spp.	3.06
Navicula cryptocephala	3.68	*Cyclotella* spp.	3.82	*Cyclotella* spp.	3.55	*Monoraphidium* spp.	2.68
Oocystis spp.	3.64	*Scenedesmus* spp.	3.69	*Monoraphidium* spp.	3.12	*Synedra ulna*	3.02
Synedra ulna	3.51	*Closterium acutum*	3.67	*Scenedesmus* spp.	3.00	*Navicula* spp.	2.42
Navicula rhynchocephala	3.23	*Monoraphidium* spp.	2.71	Unidentified dinoflagellates	2.81	*Scenedesmus opoliensis*	2.36
Pediastrum simplex	3.02	*Asterionella formosa*	2.55	*Crucigenia tetrapedia*	2.58	*Monoraphidium* spp.	2.36
Monoraphidium spp.	2.93	*Staurastrum* spp.	2.40	*Closterium acutum*	2.48	*Melosira granulata*	2.29
Diatoma vulgaris	2.81	*Melosira granulata*	1.91	*Aphanothece* spp.	2.44	*Staurastrum messikommeri*	2.10
Pandorina morum	2.51	*Aphanothece* spp.	1.87	*Melosira granulata*	2.37	*Oocystis* spp.	2.07
Melosira granulata	2.45	*Scenedesmus quadricauda*	1.59	*Tabellaria floculosa*	2.30	*Aphanizomenon flos-aquae*	2.04
Micractinium pusillum	2.38	*Scenedesmus ecornis*	1.55	*Asterionella formosa*	2.26	*Cyclotella meneghiniana*	1.86
Synedra acus	2.29	*Microcystis pulverea*	1.45	*Stauradesmus* sp.	2.21	*Sphaerocystis schroeteri*	1.82
Staurastrum messikommeri	2.09	*Crucigenia tetrapedia*	1.42	*Peridinium* sp.	2.19	*Synedra* spp.	1.81
Ankyra spp.	1.97	*Stauradesmus* sp.	1.39	*Tabellaria fenestrata*	2.07	*Staurastrum* spp.	1.81
Cyclotella spp.	1.93	Unidentified dinoflagellates	1.34	*Rhizosolenia* sp.	2.06	*Ankyra* spp.	1.75
Pediastrum boryanum	1.76	*Fragilaria crotonensis*	1.32	*Dinobryon bavaricum*	1.89	*Scenedesmus quadricauda*	1.74
Closterium acutum	1.66	*Schroederia setigera*	1.31	*Monoraphidium komarkovae*	1.79	*Navicula cryptocephala*	1.72
Scenedesmus smithii	1.55	*Tabellaria floculosa*	1.31	*Spondylosium planum*	1.78	*Synedra acus*	1.71
Oscillatoria planctonica	1.33	*Tabellaria fenestrata*	1.25	*Elakatothrix gelatinosa*	1.47	*Diatoma vulgaris*	1.58
Pediastrum duplex	1.27	*Ankistrodesmus falcatus*	1.19	*Staurastrum* spp.	1.43	*Coelastrum reticulatum*	1.56
Nitzschia acicularis	1.23	*Trachelomonas* spp.	1.16	*Mallomonas* sp.	1.34	*Fragilaria crotonensis*	1.53
Actinastrum gracillimum	1.22	*Peridinium* sp.	1.12	*Melosira distans*	1.29	*Pediastrum simplex*	1.53
Aphanizomenon flos-aquae	1.19	*Melosira distans*	1.09	*Scenedesmus ecornis*	1.18	*Ceratium hirundinella*	1.52
Closterium spp.	0.99	*Dinobryon bavaricum*	1.09	*Dinobryon sertularia*	1.07	*Pediastrum duplex*	1.31
Scenedesmus arcuatus	0.98	*Dictyosphaerium pulchellum*	1.00			*Nitzschia acicularis*	1.29
		Microcystis pulverea	1.00			*Scenedesmus* spp.	1.28
		Cosmarium spp.	0.98			*Scenedesmus smithii*	1.24
		Aphanocapsa spp.	0.96			*Asterionella formosa*	1.24
		Monoraphidium komarkovae	0.95			*Schroederia setigera*	1.20
		Spondylosium planum	0.88			*Anabaena* spp.	1.10
						Micractinium pusillum	1.05
						Microcystis aeruginosa	0.89
						Microcystis pulverea	0.86
						Navicula rhynchocephala	0.86
						Achnanthes sp.	0.77
						Scenedesmus acutus	0.77

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(b)

(Group 1)				(Group 2)			
Reference		Impaired		Reference		Impaired	
Species	Ct.%	Species	Ct.%	Species	Ct.%	Species	Ct.%
Scenedesmus opoliensis	11.32	*Melosira ambigua*	6.08	*Synedra accus*	7.89	*Melosira ambigua*	9.76
Navicula rhynchocephala	11.32	*Cyclotella meneghiniana*	4.95	*Dinobryon* sp.	7.01	*Closterium acutum*	5.25
Pandorina morum	7.55	*Trachelomonas* spp.	4.62	*Sphaerocystis schroeteri*	4.92	*Sphaerocystis schroeteri*	4.20
Oocystis spp.	4.4	*Scenedesmus opoliensis*	4.42	*Navicula* spp.	4.88	*Synedra* spp.	3.86
Melosira granulata	4.4	*Navicula cryptocephala*	3.89	*Melosira distans*	3.74	*Navicula* spp.	3.54
Staurastrum sebaldi	3.77	*Synedra ulna*	3.66	*Stauradesmus* sp.	3.44	*Cyclotella meneghiniana*	3.44
Scenedesmus ecornis	3.77	*Scenedesmus ecornis*	3.52	Unidentified dinoflagellates	3.22	*Ceratium hirundinella*	3.24
Pediastrum boryanum	3.77	*Oocystis* spp.	3.45	*Cyclotella* spp.	3.20	*Staurastrum* spp.	3.17
Actinastrum hantzschii	3.77	*Diatoma vulgaris*	3.03	*Crucigenia tetrapedia*	2.96	*Fragilaria crotonensis*	2.91
Pediastrum simplex	3.14	*Pediastrum simplex*	3.02	*Monoraphidium* spp.	2.79	*Scenedesmus* spp.	2.49
Synedra acus	3.14	*Monoraphidium* spp.	2.97	*Tabellaria floculosa*	2.57	*Schroederia setigera*	2.44
Monoraphidium spp.	2.52	*Cyclotella* spp.	2.52	*Scenedesmus* spp.	2.54	*Scenedesmus ecornis*	2.44
Pediastrum duplex	1.89	*Micractinium pusillum*	2.46	*Asterionella formosa*	2.53	*Asterionella formosa*	2.34
Monoraphidium komarkovae	1.89	*Navicula rhynchocephala*	3.38	*Closterium acutum*	2.50	*Synedra ulna*	2.03
Closterium spp.	1.89	*Melosira granulata*	2.27	*Peridinium* sp.	2.44	*Scenedesmus quadricauda*	2.02
Synedra utermohlii	1.89	*Staurastrum messikommeri*	2.18	*Aphanothece* spp.	2.37	*Coelastrum reticulatum*	2.01
Ankistrodesmus gracilis	1.89	*Synedra pulchella*	2.05	*Dinobryon bavaricum*	2.17	*Aphanizomenon flos-aquae*	1.93
Rhizosolenia sp.	1.89	*Ankyra* spp.	1.95	*Tabellaria fenestrata*	2.08	*Trachelomonas* spp.	1.78
Synedra pulchella	1.89	*Pandorina morum*	1.92	*Melosira italica*	1.92	*Melosira granulata*	1.78
		Closterium acutum	1.78	*Spondylosium planum*	1.82	*Monoraphidium* spp.	1.69
		Nitzschia acicularis	1.77	*Rhizosolenia* sp.	1.73	*Staurastrum messikommeri*	1.65
		Aphanizomenon flos-aquae	1.70	*Elakatothrix gelatinosa*	1.60	*Ankyra* spp.	1.47
		Scenedesmus smithii	1.59	*Monoraphidium komarkovae*	1.53	*Oocystis* spp.	1.37
		Pediastrum boryanum	1.24	*Staurastrum* spp.	1.36	*Pediastrum duplex*	1.29
		Actinastrum gracillimum	1.16	*Mallomonas* sp.	1.27	*Anabaena* spp.	1.24
		Oscillatoria planctonica	1.14	*Dinobryon sertularia*	1.23	*Scenedesmus acutus*	1.22
		Scenedesmus arcuatus	1.10			*Microcystis aeruginosa*	1.20
		Scenedesmus quadricauda	1.10			*Ankistrodesmus falcatus*	1.19
		Cocconeis placentula	1.00			*Microcystis pulverea*	1.10
						Dinobryon sp.	1.09
						Microcystis flos-aquae	0.98
						Aphanothece spp.	0.91
						Nitzschia acicularis	0.90

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and centre Portuguese basins. The differences detected among reservoir phytoplankton indicated that species compositions were structured by factors related to geographic location, reservoir type and anthropogenic pressure.

Phytoplankton reacted to various environmental influences and therefore can be used as ecological indicator organisms. However, careful analysis is necessary to distinguish between effects of natural variability and anthropogenic disturbances. Some authors (Sabater and Nolla, 1991; Negro and De Hoyos, 2005) reported that phytoplankton distribution (namely diatoms) in Spanish reservoirs were influenced by both basin geology and land use. Likewise, phytoplankton assemblages in Canadian and Greek rivers were influenced by a combination of natural and anthropogenic factors (Cumming et al., 1995; Temponeras et al., 2000; respectively). Given the fact, that the studied reservoirs are mainly used for hydroelectric power, must not be disregarded the effects of fluctuations in water level or discharge on species composition, directly related to the management of reservoirs, since these water bodies and its biological communities are submitted to enormous spatial–temporal variations, caused by hydric resource use regime (GIG, 2007).

The phytoplankton of many lakes, especially those of higher trophic levels, is dominated by large, colony forming species of cyanobacteria such as the referenced above. Permanent cyanobacterial dominance is, therefore, regarded as the ultimate phase of eutrophication occurring world-wide (e.g. Robarts, 1985; Pizzolon et al., 1999; Dokulil and Teubner, 2000). Excessive abundance or 'blooming' of cyanobacteria generally has detrimental effects on the domestic, industrial and recreational uses of water bodies and is in many cases a direct motivation for restoration measures (Dokulil and Teubner, 2000).

There has been extensive theoretical and empirical work done on the characterization of stressor gradients in the freshwater ecosystem context (Barbour et al., 1999; Brown and Vivas, 2005; Danz et al., 2007). Therefore, following Bailey et al. (2007) criteria, our methodology becomes more comprehensive and objective and can allow more powerful, objective bioassessments, since: (1) quantifies all human activities (e.g. agriculture, mining, urban development) that could potentially affect the aquatic ecosystem, at multiple scales including the reservoir and its drainage basin; (2) does not include explicitly the effects of human activity on the aquatic ecosystem; (3) expresses human activity in scale-independent units (e.g. road density in m ha, % basin with intense agriculture) allowing to compare the relationships determined from reservoir water column to larger cumulative effects contexts.

Reservoirs are artificial or heavily modified water bodies (AWB or HMWB). For HMWB and AWB, the reference conditions on which status classification is based are within the range of ''Maximum Ecological Potential'' (MEP). The MEP represents the maximum ecological quality that could be achieved for these systems, once all mitigation measures that do not have significant adverse effects on its specified use or on the wider environment have been applied (GIG, 2007). Therefore, only sites showing nearly undisturbed physico-chemical, hydromorphological and biological conditions were chosen as reference sites, as explained in Section 2 (see Section 2.2). Nevertheless, for G1 with only 10 reservoirs, it was difficult to find a large quantity of reference sites. Most ''run-of river'' reservoirs in Portugal lie in densely populated regions and therefore represent rather impacted sites. So it was not easy to find many reservoirs fulfilling reference criteria. Only 2 sites (20% of all sampled G1 sites) were selected as reference sites. Therefore, it was not possible to set reliable reference conditions for the type for the moment. Additionally, this G1 sites were less diverse in terms of species richness (see Table 2). This might be seen as an indication that the G1 sites investigated here as ''best available'' ones do not represent proper reference sites. Subsequently, further work has to be undertaken. Maybe it will be possible to find a larger variety of less impacted ''run-of river'' reservoirs or flushed lakes in other European countries. It would be interesting to compare their phytoplankton assemblages with the results presented here. Nevertheless, for the chlorophyll *a* concentration our results were compared with other reservoirs from the Mediterranean region. As expected for the majority of the reservoirs indicated as reference for G2 and for Valeira (reference site for G1) the chlorophyll *a* values were in the range (0.74–3.73 mg/m^3) proposed by the European Commission in the Lake Mediterranean GIG Intercalibration Report (2007) for reference conditions in this systems.

In this paper we presented a framework that seeks to determine the types and ecological status of Portuguese reservoirs located in the North and Centre of Portugal using phytoplankton as water quality indicator. The types developed here do not contradict the proposal by INAG (2006). The abiotic types proposed were confirmed by biocoenotic types, since they were derived from the species composition. This way it is possible to assign characteristic species assemblages to these types. Such ascription is an essential prerequisite for the development of an assessment procedure according WFD where the assessment shall be done by comparing the actual species composition to the one that would be present under reference conditions. A considerable variation in the phytoplankton community could be detected among the two types of reservoirs differing significantly in terms of composition and *taxa* richness (see Table 2). The SIMPER analyses allowed defining, for both regulated systems, the *taxa* typical of non disturbed and disturbed sites (Table 4b). This aspect as obvious applications for the WFD since it may contribute to define the reference situation, which is the basis of the ecological assessment. Moreover, such *taxa* may be classed further in a quantitative scale, since it is ranked according to the probability of belonging to each group, allowing to the definition of the four levels established by the WFD.

Phytoplankton seems to be a good indicator for multi-scale and cumulative disturbance effects with a view to integrate future worldwide monitoring in reservoirs. However, we must point out that there is a lack of information for a great number of phytoplankton species, namely concerning individual autoecology. We entirely agree with various authors who state that more research is needed to improve the knowledge of ecological responses in aquatic organisms and that this should result in important biological insights and better understanding of species-environmental relations (Tavassi et al., 2004; Tolotti et al., 2006). For this reason, our future studies should focus on the documentation of clear relation-

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ships between phytoplankton communities and different human impacts on artificial water bodies.

Acknowledgements

This study was carried out within the framework of collaboration agreements between INAG (National Water Institute) and other universities, namely the UTAD (University of Trás-os-Montes e Alto Douro) for the study of Portuguese water reservoirs. We would like to thank the LABELEC staff for the environmental and phytoplankton data. The authors also thank the two anonymous reviewers who helped to improve the manuscript.

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Please cite this article in press as: Cabecinha, E. et al., Multi-scale approach using phytoplankton as a first step towards the definition of the ecological status of reservoirs, Ecol. Indicat. (2008), doi:10.1016/j.ecolind.2008.04.006