Spatial patterns of surface water quality in the Cértima River basin, central Portugal†‡

Raquel Vasconcelos Ferreira,*[a] Mário Azevedo Cerqueira,[a] Maria Teresa Condesso de Melo,[b] Daniela Rebelo de Figueiredo[c] and Jan Jacob Keizer[a]

Received 17th July 2009, Accepted 28th August 2009
First published as an Advance Article on the web 30th September 2009
DOI: 10.1039/b914409a

The Cértima River is the principal source of water flowing into the Pateira de Fermentelos, which is one of the largest natural lakes of the Iberian Peninsula and has elevated conservation value. This study aims at a more comprehensive understanding of the spatial pattern in water quality and, thus, pollution problems in and especially upstream of the Pateira, including a comparison with a prior study in 2003. To this end, surface water samples were collected, in May 2007, at 29 sites covering the basin's four main types of water bodies, and analysed for electrical conductivity, dissolved oxygen, biochemical oxygen demand, total suspended solids, various nitrogen species, orthophosphate and chlorophyll a. The results confirmed the existence of marked pollution along the middle section of the Cértima's main course, which can be attributed to wastewater discharges of urban and animal husbandry origin in particular. This represents an important eutrophication risk to the Pateira. Current legislation and water management does not appear to tackle this risk in an entirely satisfactory manner, since the spatial patterns as well as actual values of key physic-chemical parameters do not appear to have changed markedly between 2003 and 2007. Amongst the various parameters, biochemical oxygen demand stands out for frequently exceeding the legal water quality standards. The type of water body proved helpful to explain part of the variation in some of the parameters. This includes clear differences in electrical conductivity between the right- and left-bank tributaries, illustrating well the heterogeneous and complex character of the Cértima basin.

Introduction

Surface water quality has in recent years become one of the most pressing environmental concerns in many parts of the world. Pollution of surface water may negatively affect aquatic ecosystems and human health, and may also result in important constraints in the utilization of water for, for example, recreation, commercial fish farming, industrial applications, and animal or human consumption. The continuing expansion of human activities worldwide has been producing an even faster growth in pollution stress on aquatic environments. Amongst the most evident anthropogenic pollution sources are the discharges of agricultural, industrial and urban wastewaters as well as the diffuse run-off of agri-chemicals from agricultural lands.[1–4] As a result, eutrophication (the biological response to the excess input of nutrients into a water-body) which was originally a natural slow process have been greatly enhanced by human activities.[5] Eutrophic aquatic ecosystems are typically characterised by, besides high nutrients loads, the recurrent occurrence of algal blooms and the associated strong depletion of dissolved

[a]*CESAM – Centre for Environmental and Marine Studies, Department of Environment and Planning, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal. E-mail: raquelferreira@ua.pt; cerqueira@ua.pt; jjkeizer@ua.pt; Fax: +00351 234 370 309; Tel: +00351 234 340 349*
[b]*CVRM/Geo-Systems Centre - Instituto Superior Técnico, Av. Rovisco Pais, 1049-001 Lisboa, Portugal. E-mail: tmelo@ua.pt; Fax: +00351 21 841 74 42; Tel: +00351 21 841 72 47*
[c]*CESAM – Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal. E-mail: dfigueiredo@ua.pt; Fax: +00351 234 372 587; Tel: +00351 234 370 350*

† Part of a themed issue dealing with water and water related issues.
‡ Electronic supplementary information (ESI) available: Raw data. See DOI: 10.1039/b914409a

Environmental impact

This study is both a follow-up and an extension of a surface water quality assessment carried out in 2003 in a Mediterranean-type basin in Portugal. It confirms the continued presence, in 2007, of marked pollution along the river's main course, especially due to pollution by urban and agro-industrial point sources at one specific urban centre. Apparently, the improvements of the public wastewater networks between 2003 and 2007 have produced no major changes in water quality and, thus, in eutrophication risk of one of the Iberian Peninsula's largest natural lakes. The densification of the 2003 sampling network has provided new insight into the importance of the catchment's four types of water bodies and, thereby, of the associated physical-environmental and land-cover/use settings.

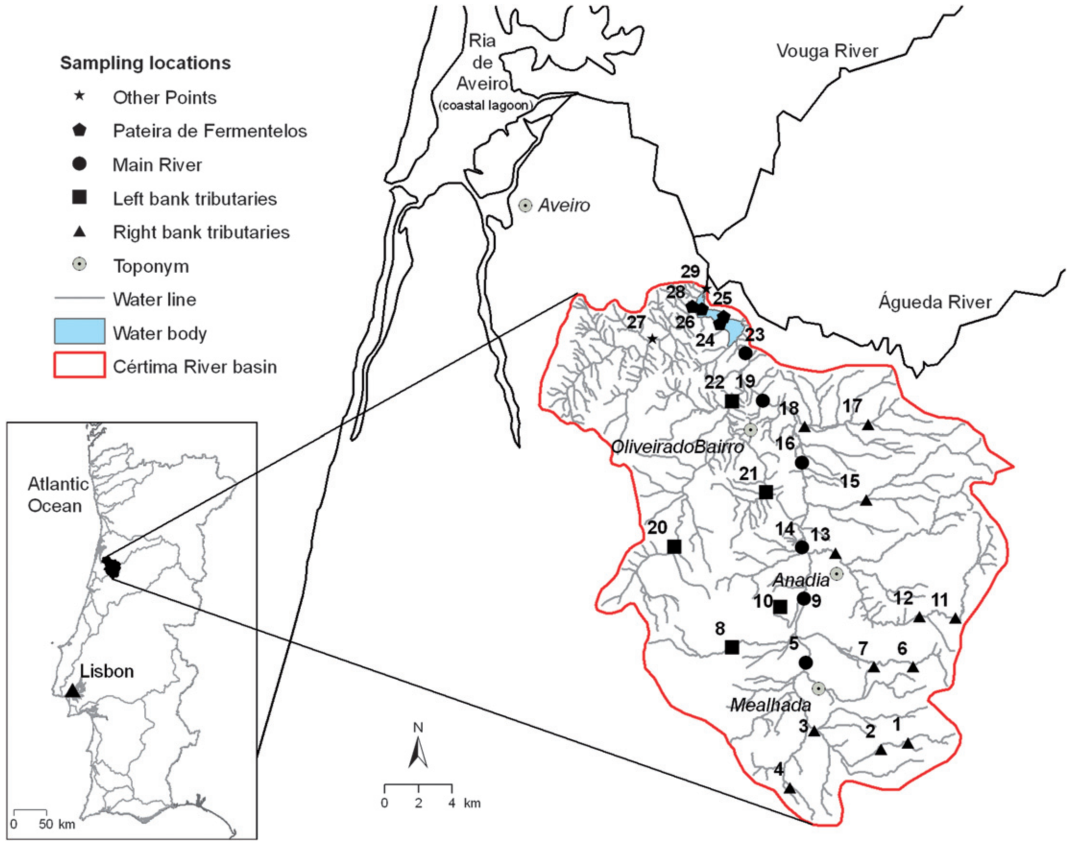

Fig. 1 Location and map of the Cértima River basin. The numbers and names of the 29 sampling sites are as follows, with the numbers in italic indicating the sites of the prior study in 2003[7]: 1, Santa Cristina; 2 (*1*), Póvoa do Loureiro; 3 (*2*), Viadores; 4, Pereiras; 5 (*3*), Lagoa Seca; 6, Várzeas; 7, Barrô; 8, Ponte dos Carros; 9, Curia; 10, Várzea; 11, Parada; 12, Algeriz; 13 (*4*), Famalicão; 14 (*5*), Malaposta; 15, Avelãs de Cima; 16 (*6*), São João da Azenha; 17, Pisão da Forcada; 18 (*7*), Landiosa; 19 (*8*), Repolão; 20, Levira; 21, Paraimo; 22, Amoreira do Repolão; 23 (*10*), Perrães; 24, Fermentelos (lake shore); 25, Óis da Ribeira (lake shore); 26, Lake – North; 27, Mamodeiro; 28, Lake – São Paio and 29, Requeixo.

oxygen which, in turn, strongly affects the other biological communities (aquatic ecosystems presenting low nutrients loads and low algal biomass are classified as oligotrophic, while mesotrophic aquatic ecosystems have intermediate characteristics).[6]

The present study concerns the Cértima River basin in central Portugal (Fig. 1). In addition to the conservational importance of its lake and surroundings wetlands, it is a typical example of a complex, spatially heterogeneous catchment with distinct agricultural and forestry land uses as well as pollution pressures from urban areas, cattle raising activities and various types of industrial activities, with emphasis on the ceramics and wine-growing sectors.[7] It also provides clear examples of advanced degradation of aquatic habitats, especially in the case of the main course of the Cértima River where pollution problems has been documented for several decades.[8–10]

The Cértima River basin is a medium-sized catchment with a shallow, natural lake at its final section. This "Pateira de Fermentelos" lake is one of the largest natural freshwater lakes of the Iberian Peninsula (approximately 5.3 km^2) and, together with the surrounding lands, constitutes one of Portugal's principal wetland areas.[11] Whilst subject to severe pressures from anthropogenic activities, the Pateira de Fermentelos is protected under Portuguese legislation as an integral part of the so-called Special Protection Area of the Ria de Aveiro coastal lagoon (ref. 12, which establishes the conservation regime of several special protection areas). The Pateira also has a special legal status as a sensitive area with respect to discharges of urban wastewater (ref. 13, which transposes the Urban Wastewater Treatment Directive).[14]

The first catchment-wide, quantitative assessment of surface water quality in the Cértima River basin was carried out in 2003.[7] Focusing mainly on the river's main course, it revealed significant nutrient load as well as high biochemical oxygen demand values, in particular at Lagoa Seca (site 5) just downstream of the town of Mealhada. At this site, pollution levels were much more pronounced during the July sampling period than the preceding March and May periods. More recently, Manecas[15] reported contamination of heavy metals in bottom sediments of the Cértima River (at Anadia) and Sena[16] observed eutrophication problems in Pateira de Fermentelos that she attributed to upstream river water quality. In general, the Pateira de Fermentelos has received much more research attention than the rest of the Cértima basin, pointing out to a eutrophic to hyper-eutrophic lake.[17–21] Even in the case of the Pateira, however, there has been no regular monitoring of its ecological and chemical status as is required for an adequate implementation of the European Union's Water Framework Directive.[22]

The present research is intended as a follow-up of the above-mentioned study carried out in 2003. It aims to expand the prior catchment-wide water quality assessment or, in other words, at furthering the knowledge and understanding of the spatial patterns in selected physic-chemical parameters. In addition, a biological parameter - chlorophyll a - is included as a first step towards a more integrated approach. A key element in the assessment is the comparison of the obtained results with the Portuguese legal water quality standards (ref. 23, which transposes the corresponding European Directive on this matter).

The surface water quality parameters studied here are: electrical conductivity (EC), dissolved oxygen (DO), biochemical oxygen demand (BOD), suspended solids (TSS), phosphorus (PO_4–P) and various nitrogen forms (NH_4–N, NO_3–N, Org–N), and chlorophyll a (Chl a). EC reflects the status of inorganic pollution, and measures the total dissolved solids and ionised forms. DO represents the quantity of oxygen that is present in the water and is essential for almost all aquatic life forms. BOD estimates the degree of organic pollution. High levels of suspended solids can increase BOD and lead to depletion of DO levels. Excessive nitrogen and phosphorus levels result in eutrophication, causing proliferation of algal masses that can induce a deficit of dissolved oxygen. Chl a provides a measure of algal abundance that is often used to quantify algal blooms caused by eutrophication.[5]

For logistic reasons, the larger number of sites and parameters studied here had to be off-set against sampling frequency, *i.e.* here at a single occasion as opposed to the three occasions of the 2003 study.[7] Arguably, pollution levels can be expected to be worse during summer than spring. The 2003 data clearly show this for the heavily polluted Lagoa Seca (site 5) but less clearly for the other sites. The summer-2003 values were significantly higher than the spring-2003 values for two of the seven parameters (EC and Org–N; Wilcoxon signed rank test; $p < 0.05$). At the same time, however, the summer- and spring-2003 values of these two parameters (plus BOD, NH_4–N, TSS and DO) were strongly and significantly correlated (Spearman rank correlation coefficients; range: 0.70–0.88; $p < 0.05$). May was finally selected as a sampling period for this study, since the Cértima headwaters tend to become dry during the summer season.

Study area

The Cértima River basin is a sub-basin of the Vouga River basin (Fig. 1). The Cértima River stretches over a total length of about 43 km, from its headwaters on the west flank of the Buçaco mountain range to its outlet at Requeixo. It drains an area basin of about 540 km^2 that ranges from 4 to 563 m in elevation.[24] The Pateira de Fermentelos is, as mentioned in the introduction part, an important wetland area that results from the widening of the Cértima River in its final section. The surface area and depth of this lake vary seasonally, within ranges of 3–5 km^2 and 1–5 m, respectively.[19]

The Cértima catchment is characterized by distinct topographic, climatic, geologic, and hydrologic settings that are reflected in its rather heterogeneous land-use patterns. The climate is Mediterranean-type, although with oceanic influences. Summers are warm and dry, whereas winters are mild and wet. During dry years, the upper stretch of the Cértima river, along the Mealhada municipality, has very low flows and even falls dry. The middle stretch has symmetrical and sheer down banks, bottom sediments consisting of fine sands and abundant aquatic vegetation. The flow rates in this section increase markedly due to the contribution of several tributaries draining the eastern, forested hillslopes (right-bank tributaries). The plains of the lower part of the basin, roughly up to the municipality of Oliveira do Bairro, are subject to flood risk, especially when intense winter rain events coincide with high tides (reflecting the connectivity with the Ria de Aveiro costal lagoon).

The geology comprises Ordovician schists in the eastern, upper part of the catchment, and modern-age alluvial sands and clays in its western, middle and lower parts. The land cover consists predominantly of *Pinus pinaster* and *Eucalyptus globulus* forests (48%), located in the upper part, and agricultural lands (44%; of which 27% are vineyards); the urban areas amount to 7% of the territory.[25] The major urban centers (Mealhada, Anadia, Oliveira do Bairro) are located close to the main course of the Cértima River. The total resident population is about 85 000 inhabitants, with an average population density of 160 inhabitants per km^2.[26] In 2005, about 97% of the resident population were served by the public water supply systems, but only 67% and 64% were connected to the public sewage systems and wastewater treatment plants, respectively.[27]

Methods

Sampling and analytical procedures

In total, twenty nine sampling sites were selected to provide an adequate spatial coverage of the catchment's main water types: six sites were located along the Cértima's main course; thirteen along its right-bank tributaries; six along its left-bank tributaries; and four at the Pateira de Fermentelos (Fig. 1). These sites were sampled during a three-day field campaign from 8 to 10 May 2007.

Ten water quality parameters were selected for this study: electrical conductivity (EC); dissolved oxygen (DO); biochemical oxygen demand (BOD); total suspended solids (TSS); total Kjeldahl nitrogen (TKN); ammonium (NH_4–N); chlorophyll *a* (Chl a); dissolved orthophosphate (PO_4–P); dissolved nitrite (NO_2–N); dissolved nitrate (NO_3–N). EC and DO were measured in the field using a pre-calibrated portable meter (HANNA® Instruments). Samples for the laboratory analysis of the remaining eight parameters were collected manually by direct immersion of the containers beneath the water surface to a depth of about 20 cm. All the containers used to store the samples were pre-rinsed with hydrochloric acid (pH < 2.0), distilled and deionised water, and, immediately before sampling, with water from the selected location. All samples were then stored and transported in thermal boxes to the laboratory. Water samples for BOD and TSS analysis were collected in 1.5 L polyethylene containers and processed within 6 h after collection. For Chl a determinations, samples were collected using 1 L plastic bottles that were then stored under dark and cold conditions. For TKN and NH_4–N analysis, water samples were collected into 500 mL glass containers (without any filtration step), preserved by addition of sulfuric acid (pH < 2.0), and stored in the dark at a temperature of 4 °C. Water samples of about 60 mL for PO_4–P,

NO_2–N and NO_3–N determinations were immediately filtered through a 0.45 μm pore size Milipore® HA membrane filter, stored and shipped in high density polyethylene containers for later analysis by ion chromatography, at the Activation Laboratories Ltd. (ACTLABS, Ontario, Canada). All other water quality parameters were determined at the University of Aveiro following the standard analytical methods for water quality as described in ref. 28. BOD was determined after incubation of the samples for 5 days in the dark at 20 °C, and measurement of the oxygen consumption. TSS was quantified gravimetrically after filtration of an adequate volume through a glass fibre filter and following drying to a constant weight at 105 °C. Chl a concentrations were determined by spectrophotometry (trichromatic method) after filtration and extraction in 90% acetone. TKN was determined by the Kjeldahl method (with digestion and distillation of the samples), and followed by the analysis of NH_4–N by the colorimetric indophenol blue method. Subsequently, organic nitrogen (Org–N) was computed by subtraction of the NH_4–N from the TKN values. The quality of laboratory data obtained at the University of Aveiro was guaranteed through the implementation of laboratory quality assurance and quality control methods, including the use of standard operation procedures, calibration with standards, analysis of blanks, recovery of known additions and analysis of replicates.

Data analysis

The statistical analyses were carried out using STATISTICA for Windows Release 5.5, by StatSoft Inc., except for the "runs up and down test" and the multiple comparisons following the Kruskal-Wallis test. The runs test was computed manually following ref. 29 and ref. 30, and the multiple comparisons following ref. 31. Comparison-wise type I errors 'α' were computed for multiple comparisons between the water quality parameters. This was done following the Dunn-Šidák method.[30] Rank-based descriptive statistics and non-parametric statistical tests were preferred because of the limited numbers of samples, on the one hand, and, on the other, the presence of various possible outlier values.

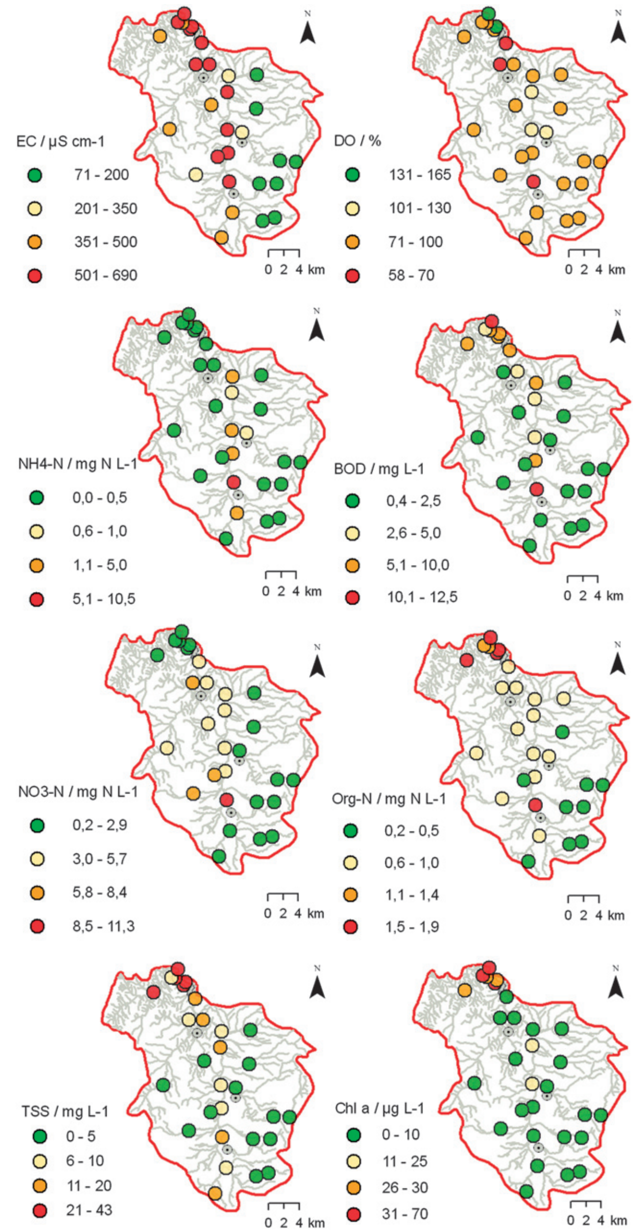

Fig. 2 Spatial patterns of the measured parameters in the Cértima River basin. Values given in legend reflect the category range (from minimum to maximum values). In the case of biochemical oxygen demand and ammonium, the circles in orange and red indicate values above the Portuguese legal water quality standards.

Results and discussion

Overview of water quality in May 2007

The spatial patterns of eight of the ten evaluated parameters are depicted in Fig. 2. The results obtained for PO_4–P and NO_2–N are not shown, since the bulk of the values were below the detection limit of the analytical method used (*i.e.* 0.02 mg P L^{-1} and 0.01 mg N L^{-1}, respectively). Table 1 provides a summary of measured values, whereas the following text highlights the most relevant results for the different parameters.

Electrical conductivity. The lowest values of EC (<250 μS cm^{-1}) were measured in the eastern part of the basin, in the mountain streams with little to no anthropogenic influence. The highest values (>600 μS cm^{-1}) were found along the middle stretch of the Cértima River. They can be attributed to discharges of untreated wastewater from domestic use and/or animal husbandry in the Mealhada urban area. The relatively high EC values of around

Table 1 Resume of the surface water quality measurements at 29 sites in the Cértima River basin in May 2007

Parameter/Units	Mean	Median	Stand. dev.	Min.	Max.
EC/μS cm⁻¹	386	441	202	71	690
DO/%	94	92	21	58	165
BOD/mg L⁻¹	3.6	2.2	3.6	0.4	12.5
TSS/mg L⁻¹	10.5	5.9	12.3	0.4	42.7
NO_3–N/mg N L^{-1}	3.0	2.2	2.6	0.2	11.3
NH_4–N/mg N L^{-1}	0.67	0.07	1.9	0.02	10.5
Org–N/mg N L^{-1}	0.73	0.6	0.5	0.1	1.9
Chl a/μg L^{-1}	11.6	4.3	15.5	2.2	70

500 μS cm^{-1} measured in the alluvial plains suggests that the pollution from the Mealhada urban area affects water quality over the entire downstream course.

Dissolved oxygen. The large majority of sites revealed well-oxygenated surface waters, with DO values generally above 70%. The exceptions were sites 5, 22 and 23, which are located along or nearby the main watercourse. Lagoa Seca (site 5) presented the lowest DO value, around 50%. These low values are certainly linked to the high organic matter loads' sites and the associated oxygen depletion. The type and sources of these organic matter loads, however, are likely to be distinct. Lagoa Seca (site 5) is subject to direct wastewater discharges, whereas Perrães (site 23) is an area of high biomass development. Nine sampling sites (6, 7, 10, 11, 13, 14, 16, 25 and 29) had oxygen-supersaturated waters, a consequence of the photosynthetic activity of aquatic vegetation that grows in the shallow waterbodies of the Cértima basin.

Biochemical oxygen demand. The lowest BOD values were measured at the upper eastern part and mid-western part of the basin. A comparatively high BOD value (12.2 mg L^{-1}) was found at Lagoa Seca (site 5), most likely reflecting considerable influxes of wastewater from the Mealhada urban area and/or a nearby livestock farm. Downstream of Lagoa Seca (site 5), BOD levels decrease gradually, presenting values inferior or around 3 mg L^{-1}, until Repolão (site 19). This can be attributed to oxidation of the organic matter load in combination with dilution with less polluted surface water from tributaries. Markedly high BOD values were again observed at the Pateira de Fermentelos, with a peak value of 12.5 mg L^{-1} at the Cértima's outlet (site 29). In view of the Pateira's special status in terms of wastewater discharges (see Introduction), its high BOD values are thought to reflect *in situ* net primary production biomass decomposition processes, although these processes are possibly enhanced by increased nutrient influxes from upstream areas.

Total suspended solids. Suspended matter is particularly abundant at the sites located along the Cértima's main course, with TSS measured values ranging from 0.4 to 42.7 mg L^{-1}. This can be explained by the high streamflow and, thus, sediment transport capacity of the main course compared with its tributaries, on the one hand, and, on the other, by its comparatively high wastewater discharge load. The highest TSS values (>18 mg L^{-1}), however, were recorded in the Pateira de Fermentelos. This is in close agreement with the observed pattern of BOD levels, suggesting that organic matter constitutes a significant fraction of the Pateira's suspended solids.

Nitrogen species. NO_3–N was found to be the dominant nitrogen species. Its median concentration of 2.2 mg N L^{-1} is well above those of NH_4–N and Org–N (0.07 and 0.6 mg N L^{-1}, respectively). NO_2–N concentrations, by contrast, were in general negligible, being below the detection limit at all but two sites: Amoreira do Repolão (site 22) and São Paio (site 28). The Lagoa Seca site (5) revealed the highest NH_4–N concentration (10.5 mg N L^{-1}), which agrees with the above-mentioned admixture of urban and husbandry wastewater from the Mealhada urban area. Its levels decreased gradually in downstream direction and were practically zero in the Pateira de Fermentelos. NO_3–N concentrations revealed the opposite downstream trend between Mealhada and the Pateira, thus consistent with advancing ammonia nitrification but possibly also influenced by diffuse pollution from surrounding agricultural lands. In the Pateira, Org–N concentrations exceeded those of NO_3–N. This predominance of Org–N points to an important incorporation of nitrogen by phytoplankton and possibly also to marked denitrification processes.[32]

Chlorophyll a. The highest concentrations of Chl a, with values above 27 μg L^{-1} and a peak value of 70 μg L^{-1}, were observed in the waters of Pateira de Fermentelos. These results, in conjunction with those obtained for DO, BOD, TSS and the different nitrogen species, indicate a clear link between a marked phytoplankton production in the Pateira and the influx of nutrients from the upstream Cértima basin.

Moderately high concentrations of Chl a, exceeding 10 μg L^{-1}, were found at two sites along the middle section of the Cértima river, *i.e.* Malaposta (site 14) and São João da Azenha (site 16). This could well be related to the upstream discharges in the Mealhada urban area, in combination with local conditions propitious to phytoplankton development.

Phosphate. Non-zero PO_4–P concentrations were only detected at seven sampling sites (1, 5, 6, 9, 11, 13 and 14). Most of these sites are located in the section of the Cértima River that is subject to marked discharges of domestic wastewater (known to have high phosphorus levels).[7] Amongst these sites, Lagoa Seca (site 5) clearly stands out with a much higher PO_4–P concentration than any of the other sites, *i.e.* 1.3 mg P L^{-1} *versus* 0.53 or 0.26 mg P L^{-1} found at Malaposta (site 14) and Curia (site 9), respectively.

Comparison with legal limits. Comparison of the obtained results with the minimum surface water quality levels established by Portuguese law[23] shows that these thresholds are exceeded at almost a third of the 29 sampling sites in the case of BOD (at 9 sites >5.0 mg L^{-1}). The sites that do not meet this standard include that of Lagoa Seca (site 5) - a clear pollution focus - and the next downstream site along the Cértima's main course, Curia (site 9), as well as the four Pateira' sites. In the case of NH_4–N, the legal standard of 1.0 mg N L^{-1} is not met less frequently. All six of these sites are located along the Cértima's main course, five of which downstream of the Mealhada urban area.

Spatial patterns in May 2007

Trends along the main course. The "runs up and down test" was used to assess the presence of trends of decreasing or increasing parameter values between Lagoa Seca (site 5) and Perrães (site 23), located just before Pateira de Fermentelos. The most straightforward patterns comprising two runs are those in DO, BOD and NO_3–N (Fig. 3). None of these three patterns nor those of the remaining seven parameters correspond to a statistically significant trend (Table 2). Therefore, the existence of one or more additional pollution sources downstream of Lagoa Seca (site 5) cannot be excluded. It must be noted, though, that the number of sampling points of the present series corresponds to the minimum that is required for the "runs up and down test" to

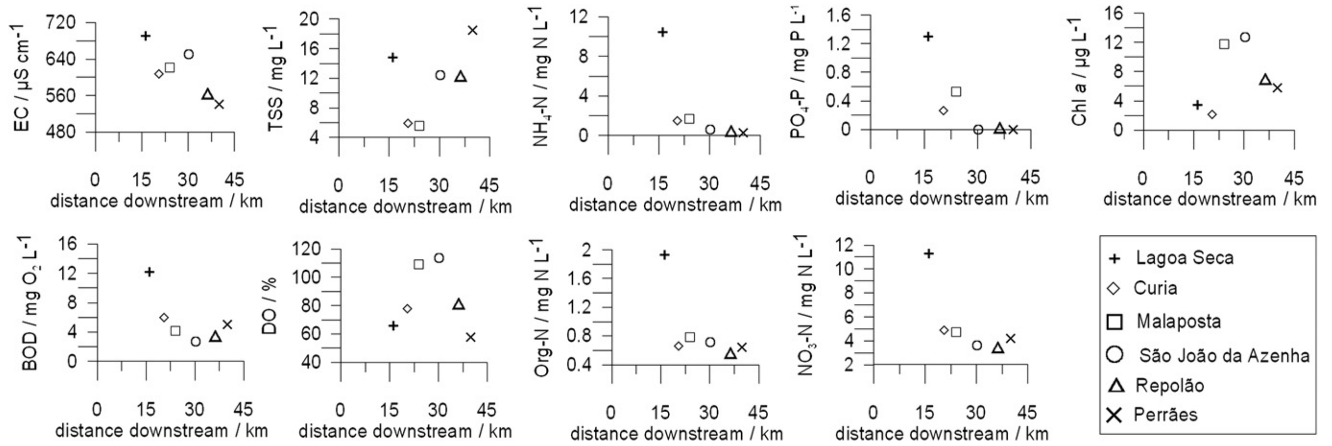

Fig. 3 Spatial patterns along the Cértima's main course between Lagoa Seca (site 5) and Perrães (site 23), before Pateira de Fermentelos, of nine of the ten measured parameters.

Table 2 Runs up & down test for spatial patterns along the Cértima's main course between Lagoa Seca (site 5) and Pateira de Fermentelos[a]

Test statistics	Water quality parameters EC/ µS cm^{-1}	DO/ %	BOD/ mg L^{-1}	TSS/ mg L^{-1}	NH_4–N/ mg N L^{-1}	Org–N/ mg N L^{-1}	NO_3–N/ mg N L^{-1}	NO_2–N/ mg N L^{-1}	PO_4–P/ mg P L^{-1}	Chl a/ µg L^{-1}
runs	3	2	2	4	4	4	2	—	3	3
n	5	5	5	5	5	5	5	0	3	5
significance	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

[a] *ns*: non significant.

be applicable. In the cases of NO_2–N and PO_4–P, this minimum number was not achieved due to the presence of ties.

Correlations in spatial patterns. The agreement between the spatial patterns of the ten parameters was assessed by means of the Spearman's rank correlation coefficient (Table 3). Comparison-wise type I errors 'α' of 0.0057, about a quarter of the correlation coefficients is significantly different from zero. Worth noting is that these coefficients all correspond to positive relationships. On the basis of these significant correlations, the parameters can be divided in three groups: i) NO_2–N, PO_4–P, Chl a and DO, all of which reveal a spatial pattern that is unrelated to those of all other nine parameters; ii) EC, BOD and Org–N, which are significantly correlated with each other as well as with all or all but one of the three parameters included in the next group; iii) TSS, NH_4–N and NO_3–N, which are correlated with two or all of the parameters of group ii. Fig. 4 illustrates the two strongest correlations (Sperman rank correlation coefficients of around 0.85), which both involve BOD.

The frequent significant relationships may indicate that the underlying pollution patterns in the Cértima River basin are rather straightforward, with the Mealhada urban area playing a predominant role as source area. The alternative explanation of strong parameter interactions does not seem to apply in the case of neither the various nitrogen species nor the two oxygen-related parameters. In the case of NO_2–N and PO_4–P, the lack of significant relationships is easily explained by the fact that the bulk of the values are not above the detection limits of the employed methods.

Table 3 Spearman's rank correlation coefficients between the ten parameters at the 29 study sites. The values in bold are statistically significant at $\alpha = 0.05$, with those in italics exceeding 0.80

r	EC	BOD	TSS	NH_4–N	Org–N	NO_3–N	NO_2–N	PO_4–P	Chl a	DO
EC	1	**0.78**	**0.68**	**0.54**	**0.66**	**0.77**	0.16	0.06	0.31	−0.13
BOD		1	***0.84***	**0.59**	***0.86***	**0.62**	0.13	0.03	0.34	−0.03
TSS			1	0.42	***0.81***	0.45	0.16	−0.20	0.43	−0.09
NH_4–N				1	0.45	0.48	−0.10	0.37	−0.07	−0.06
Org–N					1	**0.56**	0.19	0.08	0.25	0.04
NO_3–N						1	0.12	−0.01	−0.05	−0.31
NO_2–N							1	−0.16	0.17	−0.14
PO_4–P								1	−0.35	0.02
Chl a									1	0.26
DO										1

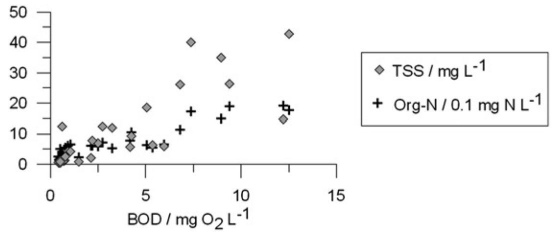

Fig. 4 Relationship of BOD with TSS as well as Org–N that correspond to the highest Spearman rank correlation coefficients in Table 3.

Variation with type of water body. To provide further insight into within-catchment spatial patterns of water quality, four types of water bodies were distinguished. This was done on the basis of the results of the prior study[7] as well as of an analysis of the present-day land-cover patterns. The four types are: i) the Pateira de Fermentelos lake, a water body subjected to reduced flow velocities and significant water residence time; ii) the main course of the Cértima River, representing locations subject to wastewater discharges from domestic, animal husbandry and industrial sources; iii) the right-bank tributaries located in a predominantly forested area; iv) the left-bank tributaries located in a predominantly agricultural area (see Fig. 1). Sites 27 (Mamodeiro, located in a left-bank tributary, flowing directly to the lake) and 29 (Requeixo, located in the mouth of the Cértima River) were excluded from this analysis for agreeing poorly with these four categories. Mamodeiro (site 27) is considered a singular sample from one of the sub-catchments, whereas Requeixo (site 29) is thought to represent a mixture of the surface water of all four types. Also, two parameters (NO_2–N and PO_4–P) were excluded from analysis, due to their large number of below-detection values. Fig. 5 resumes the values of the remaining eight parameters for each of the individual water-body types.

All of the eight parameters except DO reveal an overall contrast among the four types of water bodies that is statistically significant, indicating that type of water body can help explain the observed differences in these parameters' concentrations (Table 4). The subsequent multiple comparison tests show that between three and five of the six pair-wise comparisons correspond to statistically significant differences (Table 5). BOD and TSS involve the same statistical differences, *i.e.* a contrast between the higher values of the Pateira and the main river course, on the one hand, and, on the other, the lower values of the tributaries. This indicates that pollution in the Cértima River basin is predominantly a matter of point sources closely associated to the Cértima main course in particular, as opposed to diffuse sources due to agricultural and forestry land practices.

The other parameters all involve distinct patterns of pair-wise significant differences. A straightforward pattern is that of Chl a, with the values of the Pateira being significantly higher than those of Cértima River's main course and its tributaries. This difference can be attributed to the much longer residence time of surface water in the lake, and points to the elevated eutrophication risk of the Pateira.[33] A more complex pattern is that of EC, in which case only the values of the Pateira and of the left-bank tributaries do not differ significantly. The differences probably reflect the different lithological formations, ranging from schists in the case of the right-bank tributaries to Cretaceous sedimentary formations in the case of the left-bank tributaries and the Pateira.

Comparison with water quality in May 2003

The values of this study are compared in Fig. 6 with the May-2003 values of the preceding catchment-wide study.[7] The comparison concerns the six parameters and nine sampling points (see Fig. 1) common to both sampling campaigns. In addition, the PO_4–P values of May 2007 are compared with the total phosphorous values of May 2003.

The visibly higher 2007 values for BOD, NH_4–N, Org–N and PO_4–P were observed at Lagoa Seca (site 5). These differences can be caused by a stronger impact of the pollution sources upstream this site. Five of the six parameters reveal a strong agreement of the values of May 2007 with those of May 2003, with Spearman rank correlation coefficients ranging from 0.65 to

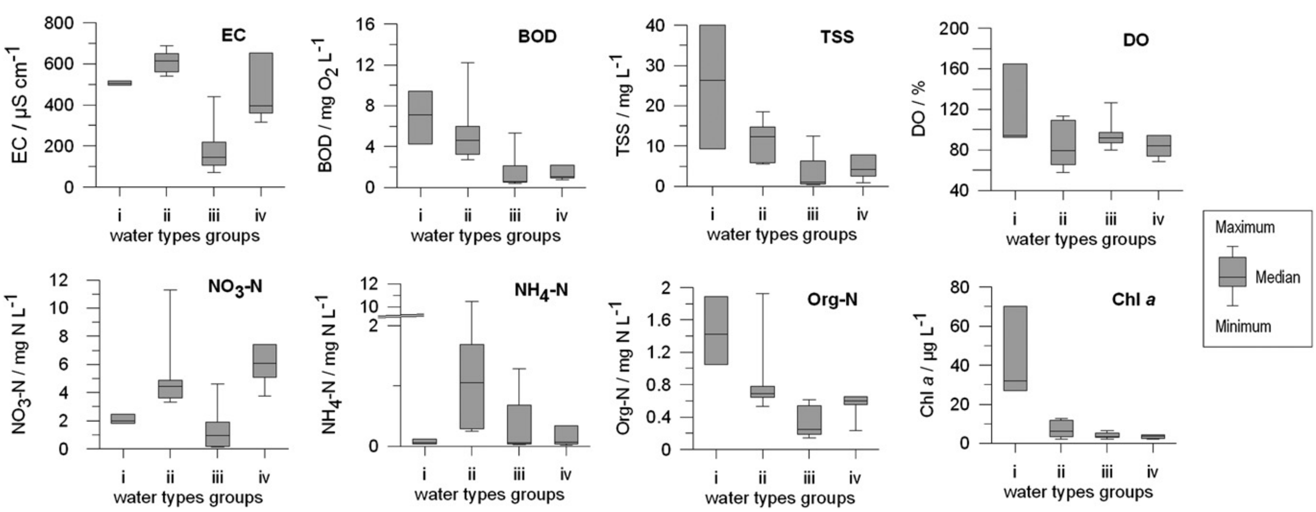

Fig. 5 Box-and-whiskers plots of electrical conductivity, biochemical oxygen demand, total suspended solids, ammonium, organic nitrogen, nitrate, chlorophyll a and dissolved oxygen, for the four types of water-bodies in the Cértima River basin.

Table 4 Kruskal-Wallis test (H's and, in brackets, corresponding p values) for overall contrasts between the four types of water-bodies in the Cértima basin (see Fig. 1; $n_1 = 4$, $n_2 = 6$, $n_3 = 12$ and $n_4 = 5$). The H-values in italics are statistically significant at $\alpha = 0.05$

	Water quality parameters							
Test statistics	EC	DO	BOD	TSS	NH_4–N	Org–N	NO_3–N	Chl a
H	*19.47* (<0.001)	5.66 (0.13)	*17.41* (0.001)	*15.38* (0.002)	*9.34* (0.025)	*17.91* (0.001)	*9.34* (<0.001)	*9.85* (0.020)

Table 5 Multiple comparison tests between the four types of water-bodies (see Fig. 1; $n_1 = 4$, $n_2 = 6$, $n_3 = 12$ and $n_4 = 5$). The test-statistics in italics correspond to differences in parameter values that are statistically significant at an overall $\alpha = 0.05$

Water type groups: pair wise comparisons	Observed difference in multiple comparisons between water type groups						
	EC	BOD	TSS	NH_4–N	Org–N	NO_3–N	Chl *a*
1 vs. 2	*0.52*	−3.56	−2.54	*2.89*	−1.63	*1.87*	*0.28*
1 vs. 3	*5.45*	*9.87*	*9.09*	−6.74	*11.20*	−0.33	*4.76*
1 vs. 4	−4.77	*4.82*	*4.96*	−9.33	*4.16*	*4.51*	*5.97*
2 vs. 3	*12.29*	*7.73*	*5.29*	*3.59*	*7.29*	*8.22*	−3.34
2 vs. 4	*1.97*	*2.56*	*1.02*	*3.40*	*0.13*	−2.72	−2.30
3 vs. 4	*4.74*	−1.23	−2.85	−6.16	*0.94*	*10.78*	−4.83

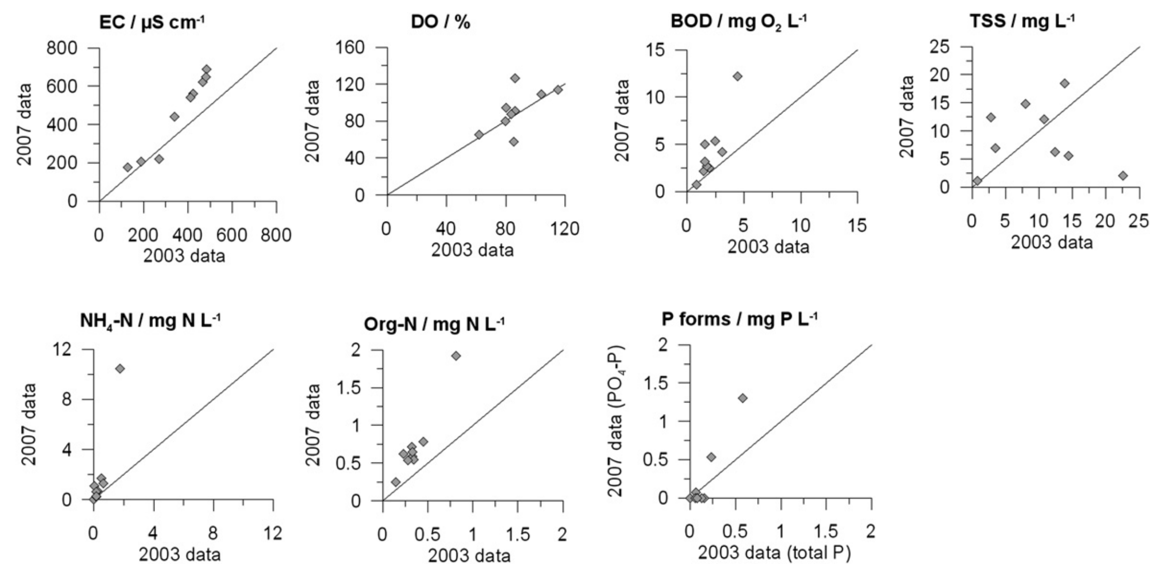

Fig. 6 Relationships between the May-2003[7] and May-2007 values of electrical conductivity, biochemical oxygen demand, total suspended solids, ammonium and organic nitrogen, dissolved oxygen and phosphorous forms, at nine sampling point in the Cértima River basin.

Table 6 Spearman rank correlation coefficients (r's and, in brackets, corresponding p-values) between the May-2003 and May-2007 values at nine sites in the Cértima River basin. The values in italics are statistically significant at $\alpha = 0.05$

	Water quality parameters					
Test statistics	EC	DO	BOD	TSS	NH_4–N	Org–N
r	*1.00* (<0.001)	0.65 (0.058)	*0.73* (0.025)	−0.07 (0.86)	*0.81* (0.008)	*0.69* (0.041)

Table 7 Wilcoxon signed rank test (Z's and, in brackets, corresponding p-values) for site-specific differences in May-2003 and May-2007 parameter values. The values in italics are statistically significant at $\alpha = 0.05$

	Water quality parameters					
Test statistics	EC	DO	BOD	TSS	NH_4–N	Org–N
Z	*2.31* (0.021)	1.48 (0.14)	*2.55* (0.011)	0.18 (0.86)	*2.67* (0.008)	*2.67* (0.008)

1.00 (Table 6). In four instances, this corresponds to a statistically significant monotonic relationship. Apparently, the spatial pattern underlying the variability in BOD, NH_4–N, Org–N and possibly also DO along the main course of the Cértima river has remained unaltered over the 4-year period from 2003 to 2007 and, thus, has not been changed markedly by the extension of the

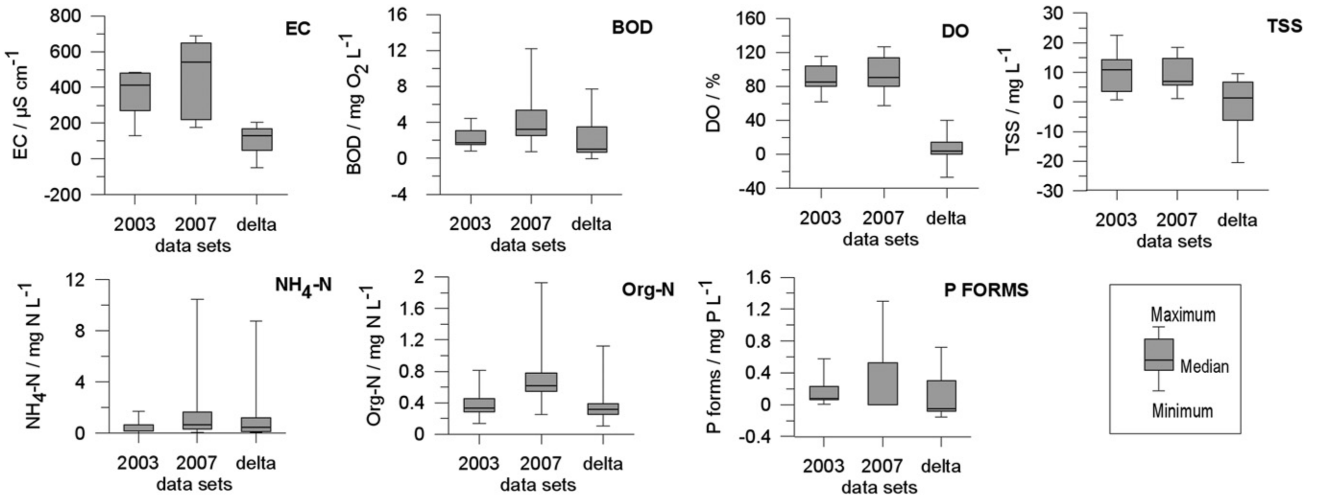

Fig. 7 Box-and-whiskers plots of the May-2003[7] and May-2007 values, and their differences, of electrical conductivity, biochemical oxygen demand, total suspended solids, ammonium and organic nitrogen, dissolved oxygen and phosphorous forms.

sewage and wastewater treatment systems that was carried out since 2003.

In the case of EC, BOD, NH_4–N and Org–N, the consistency in spatial patterns coincides with statistically significant differences in local parameter values (Table 7). All these four parameters involve higher values in May 2007 than in 2003 (Fig. 7), whereas also DO reveals some tendency for higher 2007 than 2003 values. In two instances, those of BOD and Org–N, the 2007 values are significantly higher than the 2003 values not only at the level of the individual sampling points but also on overall terms (Table 8).

Thus, the present results clearly suggest that the public works of improving urban wastewater discharges systems have not produced major changes in the spatial polution patterns along the Cértima's main course nor in the actual levels of key physic-chemical parameters like BOD and various N species. This can be due to various factors like failure to reduce nutrient and organic matter influxes from the principal urban and especially non-urban point sources, and mobilization of local nutrient stock that have accumulated on the streambed bottom over the past few decades. Admittedly, however, differences in antecedent weather and hydrometric conditions may interfere in the comparison of the present data with those obtained four years earlier. This is especially true for the actual pollution levels, since the spatial patterns in March, May and July 2003 were in general consistent (10 out of 14 parameter combinations were significantly correlated).

Unfortunately, antecedent rainfall data are only available for May 2007, whereas river discharge data are lacking for both periods.

Trophic status

This study presents clear evidence of enhanced nutrient loads and, thus, a risk of eutrophication in the Cértima River basin, in particular along the river's main course downstream of the Mealhada urban area and in the Pateira de Fermentelos. In spite of its narrow temporal window, the present data set can be used to classify the trophic status within the Cértima basin. Dodds *et al.* (1998)[6] distinguished three trophic classes for streams and lakes, based on the cumulative frequency distributions of two nutrients - total nitrogen (TN) and total phosphorus (TP) - as well as Chl a (Table 9). Since total phosphorus was not determined in this study, it will not be considered in the classification presented underneath.

The trophic status of the Cértima water bodies is highly variable, ranging from fully oligotrophic (*i.e.* with respect to TN as well as Chl a) to fully eutrophic. This variation broadly agrees with the four types of water bodies mentioned earlier. The right- and left-bank tributaries are all oligotrophic with respect to Chl a (range: 2–7 μg L^{-1}) but vary from oligo- to eutrophic with respect to TN (range: 0.4–8 mg N L^{-1}). In the case of the left-bank tributaries, TN values correspond to eutrophic conditions whereas in the case of the right-bank tributaries TN values

Table 8 Mann-Whitney U test (U's and, in brackets, corresponding p-values) for overall differences in May-2003 and May-2007 parameter values. The values in italics are statistically significant at $\alpha = 0.05$

	Water quality parameters					
Test statistics	EC	DO	BOD	TSS	NH_4–N	Org–N
U	25 (0.17)	31 (0.40)	*18* (0.047)	37.5 (0.79)	20 (0.070)	*14* (0.019)

Table 9 Threshold values for classification of the trophic status of streams and lakes (adapted from ref. 6)

	Trophic state	TN/mg N L^{-1}	TP/mg P L^{-1}	Chl a/μg L^{-1}
Streams	Oligotrophic	<0.70	<0.025	<10
	Mesotrophic	0.70–1.50	0.025–0.075	10–30
	Eutrophic	>1.50	>0.075	>30
Lakes	Oligotrophic	<0.50	<0.025	<8
	Mesotrophic	0.50–1.26	0.025–0.071	8–25
	Eutrophic	>1.26	>0.071	>25

appear to differ with the size of the upstream population centers. Trophic status is oligotrophic downstream of hamlets (sites 1, 11, 12), mesotrophic following small villages (2, 6, 7, 15), and eutrophic after medium-sized urban areas (sites 3, 4, 13, 17, 18). The main course of the Cértima river upstream of the Pateira is characterised by eutrophic TN conditions (range: 4–24 mg N L^{-1}) in combination with generally oligotrophic Chl a conditions (range: 2–13 μg L^{-1}). Chl a values are mesothropic at two sites along the Cértima's middle stretch (14 and 16), probably due to an abundant aquatic vegetation. In line with previous studies[17–21] in the Pateira euthrophic conditions prevail with respect to both parameters (range: 27–70 μg L^{-1} and 3–4 mg N L^{-1} for Chl a and TN, respectively). The same is true for the Requeixo site at the Cértima's outlet, reflecting the admixture of water coming from the Pateira.

It is well-established that the trophic status of fresh water bodies may be influenced by a large number of factors, including their physic-environmental settings and the land cover and management practices of the surrounding lands. In the case of the Cértima River basin, it is likely a natural trend of nutrient enrichment exists from the uplands underlain by schists to the lowlands on unconsolidated deposits. However, a massive nutrient input of anthropogenic origin is superimposed on this trend, in particular through wastewater discharges directly into the Cértima's main course. Future plans for improving or even controlling the trophic conditions of the Pateira de Fermentelos will, therefore, have to explicitly address these upstream nutrient influxes (including those from specific point sources) and perhaps also the accumulated nutrient stocks of very heavily polluted river stretches as in Lagoa Seca (site 5). Measures like phosphate stripping at sewage treatment works and sediment trapping by vegetation buffers or mechanical removal of accumulated sediments and/or dense vegetation stands[34,35] are examples of interventions that are complementary to those foreseen in the current EU-derived legislation of the EC Urban Wastewater Treatment Directive[14] and the EC Nitrates Directive.[36]

Conclusions

The main conclusions from this first comprehensive assessment of the catchment-wide spatial patterns in surface water quality in the Cértima River basin (central Portugal) are the following:

i) the types of water bodies distinguished here are helpful to explain an important part of the variation in water quality within the catchment, reflecting complex interactions with especially geological settings, land cover/use, settlement patterns and water management;

ii) pollution is to a large extent caused by point sources of urban and non-urban wastewater discharges along the Cértima's main course and, in particular, at one of the larger urban centers (Mealhada), contributing significantly to the euthrophication risk of the Pateira de Fermentelos;

iii) in terms of accomplishment of legal water quality standards, biochemical oxygen demand (BOD) clearly is the most critical parameter;

iv) the spatial patterns as well as actual values of six key physic-chemical parameters suggest that the ongoing improvements of the sewage and wastewater networks have had no major impact on surface water quality;

v) besides Chl a, further biological parameters need to be studied to allow an integrated assessment of surface water quality as is also required for the implementation of the European Water Framework Directive.[22] To this end, the present study is currently being followed up on by a quantitative characterisation of the benthic macro-invertebrate communities at a selection of the present sampling sites.

Acknowledgements

The authors are grateful to Clara Sena for her support and help in field work. The authors also thank the anonymous reviewers for comments on an earlier draft of this paper. The laboratory analyses were partially supported by the EcOwEt project (POCI/CTE-GEX/58951/2004) financed by the Portuguese Foundation for Science and Technology (FCT) with co-funding by FEDER through the POCI2010 Programme.

References

1 M. Sondergaard and E. Jeppesen, *J. Appl. Ecol.*, 2007, **44**, 1089–1094.
2 K. Kangur and T. Mols, *Hydrobiologia*, 2008, **599**, 31–39.
3 G. Orioli, M. Sabbatini, J. Marchena and R. Vazquez, *Hydrological Sciences Journal*, 2008, **53**, 834–843.
4 P. J. A. Withers and H. P. Jarvie, *Sci. Total Environ.*, 2008, **400**, 379–395.
5 B. Moss, *Ecology of fresh waters: man and medium, past to future*, 3rd edn., Blackwell Publishing, Oxford, 2007.
6 W. K. Dodds, J. R. Jones and E. B. Welch, *Water Res.*, 1998, **32**, 1455–1462.
7 M. A. Cerqueira, F. N. Vieira, R. V. Ferreira and J. F. Silva, *Environ. Monit. Assess.*, 2005, **111**, 297–306.
8 J. M. A. Rino and M. C. P. Gil, *Revista de Biologia da Universidade de Aveiro*, 1987, **1**, 53–103.
9 M. J. Pereira, S. F. P. Almeida, J. M. Rino, M. C. Gil and R. M. Pinho, *4° Congresso da Água (4th Water Congress)*, Lisboa, Portugal, 1998.
10 S. F. P. Almeida, *Limnetica*, 2001, **20**, 205–213.
11 C. M. Laranjeira and G. Nadais, *EPPO Bulletin*, 2008, **38**, 487–495.
12 Decreto-Lei 384B/99, 23 September 1999; Diário da República, série I-A, n.° 223/99, 1999.
13 Decreto-Lei 152/97, 19 June 1997; Diário da República, série I-A, n.° 139/97, 1997.
14 European Communities, Directive 91/271/EEC of 21 May 1991 concerning urban wastewater treatment, *Official Journal of European Communities L135*, 30 May, 1991.
15 I. Manecas, MSc Thesis, Portuguese University of Aveiro, 2006.
16 C. Sena, MSc Thesis, Portuguese University of Aveiro, 2007.
17 M. C. P. Gil, PhD thesis, Portuguese University of Aveiro, 1988.
18 A. J. Calado, A. M. Freitas and V. M. Veloso, *Revista de Biologia da Universidade de Aveiro*, 1991, **4**, 55–71.
19 A. J. Calado and S. C. Craveiro, *Nordic Journal of Botany*, 1995, **15**, 641–654.
20 M. J. Pereira, PhD Thesis, Portuguese University of Aveiro, 1999.
21 D. R. de Figueiredo, M. J. Pereira, A. Moura, L. Silva, S. Barrios, F. Fonseca, I. Henriques and A. Correia, *FEMS Microbiol. Ecol.*, 2007, **59**, 638–650.
22 European Communities, Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for the Community action in the field of water policy, *Official Journal of the European Communities L327*, 22 December, 2000.
23 Decreto-Lei 236/98, 1 August 1998; Diário da República, série I-A, n.° 176/99, 1998.
24 AMBIO, CHIRON, PROFABRIL, DRENA, HLC and FBO, *Plano de Bacia Hidrográfica do Rio Vouga, 1ª Fase, Análise e Diagnóstico de Referência Anexo 1-Análise Biofísica (Revisão 1)*, Ministério do Ambiente e do Ordenamento do Território, Instituto da Água, Direcção Regional do Ambiente do Centro, Lisboa, 2002.

25 IA, ISEGI and IGP, *Corine Land Cover 2000*, 2006, http://www.apambiente.pt/Paginas/default.aspx, Accessed 8 January 2007.
26 INE, *Population census - Census 2001. Final results. Centre Region*, 2002, http://www.ine.pt, Accessed 9 September 2008.
27 INE, *Territorial statistics: environment; reference period: 2005*, 2008, http://www.ine.pt, Accessed 11 September 2008.
28 APHA, *Standard Methods for the Examination of Water and Wastewater* 19th edn., American Public Health Association, Washington, DC, 1995.
29 F. J. Rohlf and R. R. Sokal, *Statistical tables*, 2nd edn., WH Freeman and Company, New York, 1981.
30 R. R. Sokal and F. J. Rohlf, *Biometry*, 2nd edn., W.H. Freeman and Company, New York, 1981.
31 W. J. Conover, *Practical non-parametric statistics*, 3rd edn., John Wiley & Sons, Inc., New York, 1999.
32 C. Neal, H. P. Jarvie, A. Love, M. Neal, H. Wickham and S. Harman, *J. Hydrol.*, 2008, **350**, 154–165.
33 J. Hilton, M. O'Hare, M. J. Bowes and J. I. Jones, *Sci. Total Environ.*, 2006, **365**, 66–83.
34 S. R. Carpenter, N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley and V. H. Smith, *Ecol. Appl.*, 1998, **8**, 559–568.
35 V. H. Smith, G. D. Tilman and J. C. Nekola, *Environ. Pollut.*, 1999, **100**, 179–196.
36 European Communities, Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources, *Official Journal of the European Communities L375*, 31 December 1991.