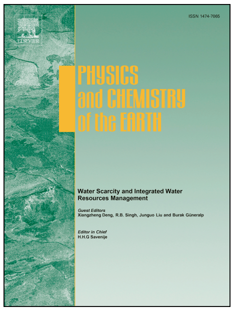
Accepted Manuscript



Water quality monitoring in the Paul do Boquilobo Biosphere Reserve

C. Baptista, L. Santos

PII: S1474-7065(15)00178-3
DOI: 10.1016/j.pce.2015.11.008
Reference: JPCE 2453

To appear in: *Physics and Chemistry of the Earth*

Received Date: 20 March 2015
Revised Date: 6 October 2015
Accepted Date: 12 November 2015

Please cite this article as: Baptista, C., Santos, L., Water quality monitoring in the Paul do Boquilobo Biosphere Reserve, *Physics and Chemistry of the Earth* (2015), doi: 10.1016/j.pce.2015.11.008.

Water quality monitoring in the Paul do Boquilobo Biosphere Reserve

Baptista, C.[a]; Santos, L.[a]

Instituto Politécnico de Tomar, Estrada da Serra, Quinta do Contador,

2300-313 Tomar, Portugal

cecilia@ipt.pt

lsantos@ipt.pt

[a] – Polytechnic Institute of Tomar

Abstract

The Paul do Boquilobo is an important wetland ecosystem classified by Unesco as a MAB Biosphere reserve also awarded Ramsar site status, representing one of the most important habitats for the resident nesting colony of Cattle Egret (*Bulbucus ibis*). Yet owing to its location, it suffers from human induced impacts which include industrial and domestic effluent discharges as well as agricultural land use which have negatively impacted water quality. The current study reports the results obtained from the introductory monitoring programme of surface water quality in the Nature Reserve to emphasize the detrimental impact of the anthropogenic activities in the water quality of such an important ecosystem. The study involved physicochemical and biotic variables, microbial parameters and biological indicators. Results after 3 years of monitoring bring to evidence a poor water quality further impaired by seasonal patterns. Statistical analysis of data attributed water quality variation to 3 main parameters - pH, dissolved oxygen and nitrates, indicating heavy contamination loads from both organic and agricultural sources. Seasonality plays a role in water flow and climatic conditions, where sampling sites presented variable water quality data, suggesting a depurative function of the wetland.

Introduction

During the last decades natural integrity of river basins and low levels of anthropogenic impact within protected areas rose in society awareness to the social-economical value of freshwater resources (Nichols et al., 2006; Verdonschot et al., 2013). Nevertheless activities, such as irrigation for agriculture and catchments for energy production pose various threats to water quality, flow reduction and ecosystem integrity (Ward and Stanford, 1995; Poff et al., 1997; Richter et al., 2003; Chester and Norris, 2006). Many natural aspects of rivers ecosystem are threatened, for example floods and water temperature changes cue fish to spawn (James et al., 2009; Pankhurst and Munday, 2011), trigger insects to begin a new phase of their life cycle, while very low flows may be critical to riparian vegetation, cause eutrophication and consequently death of aquatic vertebrates.

Monitoring plays a key role in the understanding of anthropogenic impacts on natural ecosystems, thus offering an important tool in the management of natural areas. The European Union Water Framework Directive (EC, 2000, 2011, 2013) establishes a framework for Community action in the field of water policy to implement water resources and ecological assessment as main contributors of water analysis, thus providing the basis for management and restoration of hydrological networks (Hering et al., 2010). The USA, show a long use of biological surveys to regulate water quality which become a widespread approach since 1987 (Mebane, 2001). Australia followed the trend, using nationwide water quality analysis through biological indicators since the mid 1990s, to guide water management agencies as well as the National Water Initiative in 2004. In South Africa a national monitoring program has also been developed (Roux et al., 1999) using ecological assessment, and further developing their knowledge of the biological indicators.

Common biomonitoring approaches and bioindicators used for river ecosystems involve periphyton, benthic macroinvertebrates and fish. However, because macroinvertebrate communities in rivers and streams respond to various disturbances and identification is becoming evermore generalised, offering a solution to immediate biomonitoring needs (Friberg et al., 2011). The most understood relationships are the responses of macroinvertebrates to organic pollution (Rosenberg and Resh, 1993), however, macroinvertebrates also respond to other stressors including flows and sediment (Extence et al., 2011), acidification (Murphy et al., 2013), toxins in sediments and siltation (Colas et al., 2011) and morphological degradation (Friberg et al., 2011). Biological measurements provide direct information on the condition of biota resident in the water resource, and therefore on the overall ecosystem condition. The obvious importance to management issues, with direct analytical information, can provide a more sensitive time-integrated assessment of river condition, whereas, physical or chemical variables have in many studies been determined as an insufficient methodological approach in certain environmental conditions. Repeatedly studies emphasize the impracticability to undertake chemical monitoring over short timescales without considering biota which can provide diagnostics of water quality problems beyond that necessary for ecological status assessment in its own (Oberdorff et al., 2002; Davis et al., 2006; Nichols et al., 2006; Feld et al., 2014).

The Paul do Boquilobo marsh achieved the protected area status, as decreed by the Portuguese government on the 24th of June 1980. By virtue of its uniqueness raised international interest, therefore classified as a Biosphere Reserve on 1981, the first protected area in Portugal to receive such distinction (MaB Programm, by UNESCO). In 1996, it was considered a wetland of International Importance, under the Ramsar Convention, and in 1999, further classified as a Special Protection Area under the Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and wild fauna and flora. This marsh shelters the largest colony of herons of the Iberian Peninsula and functions as a prime stop for nesting and wintering concentration of endangered waterfowl.

Wetlands are amongst the most threatened habitats (MEA, 2005). For that matter, in its current condition, Paul do Boquilobo marsh makes emphatic the evidence of heavy anthropogenic pressures and fragmented landscape.

The Paul do Boquilobo Biosphere Reserve and the Polytechnic Institute of Tomar, considered stakeholder for education and research under the new Biosphere Reserve Management Committee, guarantee the monitoring efforts in terms of surface water quality analysis, soil analysis, land cover analysis and landuse evolution.

The underlying objective is the search for the most adequate monitoring strategy, notwithstanding the assessment of the water quality in the different aquatic environments of the Nature Reserve, as a contribution to the current management and future strategies. The present monitoring programme evaluates physical, chemical and microbiological parameters, as well as benthic macroinvertebrates as first stage bioindicators.

2. Methodology

2.1 Study area

The study area, Paul do Boquilobo Nature Reserve (PBNR) located 30º23’6” N - 8º, 31’59” W (Figure 1) administratively involving the municipalities of Torres Novas and Golegã, supported by a tributary of the right margin of the Tagus river, the Almonda River. The privileged geographic location in the centre of Portugal awards special characteristics making this reserve one wetland of excellence, also unparalleled by the scarcity of such ecosystems in Portugal.

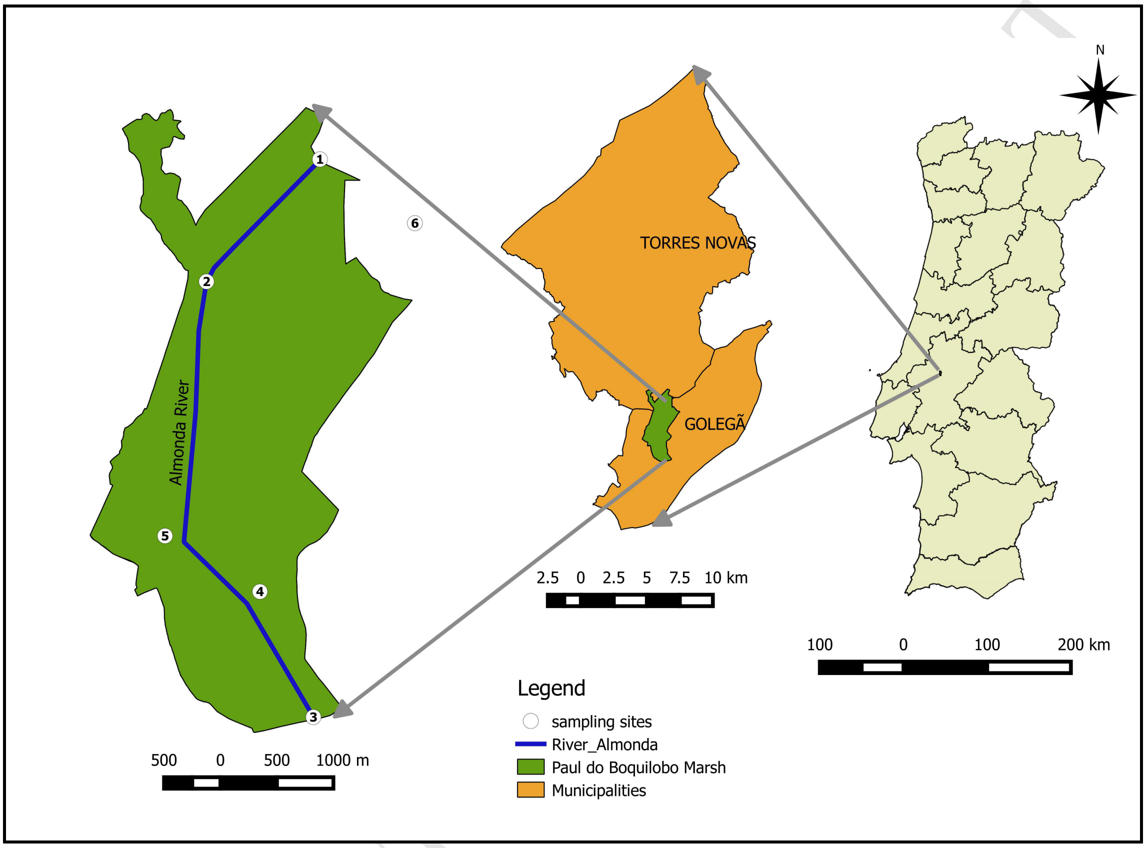


Figure 1 – Geographic location of Paul do Boquilobo Nature Reserve in the center of Portugal, with the indication of the Almonda River that crosses the study area and the sampling sites (from 1 to 6).

The area comprises the alluvial plain, cut by riparian corridors with the predominance of *Fraxinus* and *Salix* species following a complex network of temporary streams and drainage ditches beyond the Almonda River, which runs through the reservation area. Paul do Boquilobo marsh is subjected to large seasonal fluctuations of water level, keeping certain areas permanently or seasonally flooded. The marsh plays an important role in the region's water regulation - retention area during floods and recharge of groundwater in dry period.

Regarding the Reserve, the 817 ha of Paul do Boquilobo are stratified in a conventional tripartite zonation: area of total protection (core area), area of partial protection, area of complementary protection, under the management of ICNF - Institute for Nature Conservation and Forests.

2.2 Sampling Sites

Sampling sites were chosen in function of their hydrological relevance in order to allow an overview of the Reserve's water quality (Figure 1). Almonda River stands for the most important contribution to this wetland, hence the preferential choice of site selection (sites 1, 2 and 3). Furthermore collecting stations reflect sequentially entrance, midpoint and exit of the Nature Reserve, granting an overview of the water quality status. Sites were partially chosen by accessibility characteristics, considering the flooded period (site 4). With the intent of covering most habitats water quality of the natural lake, lentic environment, which under flooding conditions contributes to the water quality of the Almonda River (site 5). Besides the Almonda River, another important hydrological contribution to the marsh exhibiting evidence of pollution problems demanded a new sampling location, outside of the Reserve area within Vala das Cordas drainage ditch (site 6).

Geographical location of sites (Figure 1) is differentiated by:

Site 1- Almonda River, at the entrance of the Reserve, lotic environment with high flow, and severe seasonal fluctuations;

Site 2- Almonda River, as an intermediate point between sites 1 and 3, coincidently the beginning of the core reservation zone;

Site 3- Almonda River, the lowermost point and exit of the reserve, lotic environment observing high seasonal variation between winter and summer, aggravated during the summer due to irrigation water demand;

Site 4- One of the main tributaries of the Almonda River within the Reserve, characterized by 2 artificial drainage ditches, regularly flooded which conditioned the sampling in this site;

Site 5- Natural lake, lentic environment on the right margin of the Almonda River;

Site 6- This is the only site outside the Reserve area, however one of the main drainage ditches built to gain agricultural land, and characterized by the severe apparent pollution.

2.3 Sampling frequency and analysed parameters

Sampling took place on a monthly basis, between April 2011 and April 2012, followed by a new sampling sequence from March to May 2013, accounting for an overall amount of 16 sampling sessions and 96 individual samples.

2.3.1 *Physical, chemical and microbiological parameters*

During the current study 13 parameters were analysed and summarised in table 1. Methodologically water sampling analysis followed real time "in situ" analysis, and sampling removal, followed by laboratorial analysis (Artiola et al., 2004).

Table 1 – Methods and equipments used in environmental essays.

Parameter (unit)	**Method/ Equipment**
pH	"In situ" – Hanna Instruments HI 98103
Temperature (ºC)	"In situ" – Hanna Instruments HI 98103
Conductivity (µS/cm)	"In situ" – Hanna Instruments HI 98129
Solids: Total Dissolved Solids – TDS; Total Suspended Solids – TSS; (mg/L)	Filtration (0,45µm filter); Standard Method 2540
Dissolved Oxygen, DO (mg/L)	Winkler iodometric method with sodium azide modification; Standard Method 4500-O (C)
Chemical Oxygen Demand, COD (mg/L)	Potassium dichromate open reflux method, 2h digestion, 150ºC; Standard Method 5220
Biochemical Oxygen Demand, BOD_5 (mg/L)	5-day BOD test; Standard Method 5210
Phosphates (mg/L)	Standard Method 4500-P (E); Ascorbic acid method
Nitrates (mg/L)	Portuguese Standard NP 4338-1/96; 2,6-dimethylphenol colorimetric method
Culturable microorganisms, 22°C and 36°C (CFU/mL)	ISO 6222:1999; pour-plate method; yeast extract agar (YEA)
Coliform bacteria and *E. coli*	ISO 9308-1:2000; membrane filtration

(CFU/100mL)	method; Membrane Lauryl Sulfate Agar plate (MLSA); Oxidase and indol confirmative tests

The sampling, transport, preservation, storage and analysis followed the methods described in Standard Methods for the Examination of Water and Wastewater, section 1060 A, B, C and Table 1060: I for physical and chemical analysis, and section 9060 A and B for microbiological analysis (Greenberg et al., 1992). Manual single samples were collected in the middle of the stream at mid-depth, using 1,5L plastic containers for all parameters, except OD and phosphates (Winkler bottles and Schott flasks, respectively).

2.3.2 Bioindicators of water quality

With the primary target of sampling lotic environments, benthic macroinvertebrates sampling was carried out using a 25cm D frame hand net 500µm (35 mesh) (Barbour et al., 1996). Sampling was performed mostly in riffle habitats to allow result comparison, following Water Framework Directive (WFD), also reflected in the National Water Institute protocol's (INAG, 2008).

Macroinvertebrates were collected to individual transport containers, and preserved with 70% ethylic alcohol until laboratory sorting and identification. Macroinvertebrates were identified to family level (Tachet et al., 2002). Specimens were analyzed and classified using a BMS stereo-optical microscope (magnification X140) and an Olympus optical microscope (magnification X400), up to family level for all taxa.

Sampling underwent the period April 2011 and April 2012 collected monthly except for the month of October where weather conditions and flash floods conditioned sampling. After the analysis of this period it was decided that biomonitoring results were most significant for the period between March and May where macroinvertebrate specimens were most abundant (Nichols et al., 2006).

Immediate calculations considered the usage of the AMIIB@ software from Portuguese Environmental Protection Agency (APA) and retrieved: total number of individuals, total number of taxa, BMWP, ASPT, IBMWP, IASPT, Shannon-Wienner diversity, Evenness, and the Portuguese Index IPtIN (Alba Tercedor et al., 2002; INAG, 2009). Both IBMWP and IPtIN class IV rivers were calculated for water status classification.

Software restrictions and problems were overcome by sorting month data into seasonal data. For that matter the months of December, January and February correspond to winter; the months of March, April and May – spring; the months of June, July and August – summer and the months of September and November to autumn.

2.4 **Statistical analysis**

Statistical analysis, involved the individualisation of variables in Excel followed by explanatory study of variables, multivariate analysis using Minitab 14. Stepwise cluster analysis enabled the identification of patterns dividing core variables into large groups, where the Euclidean distance revealed similarity between every two samples. The Clustering of Variables was used to classify variables into groups when the groups were initially not known, thus reducing their number. This technique is an agglomerative hierarchical method that begins with all variables separately, each forming its own cluster. In the first step, the two variables closest together are joined. In the next step, either a third variable joins the first two, or two other variables join together into a different cluster. This process will continue until all clusters are joined into one, enabling self decision on groups that are logical for the data. The procedure allowed identification of new variables that are more intuitively understood than those found using Principal Component Analysis (PCA).

Detrended Correspondence Analysis (DCA) was used to select the appropriate response model for subsequent direct gradient analysis (ter Braak and Šmilauer, 2002; Lepš and Šmilauer, 2003). For the gradient analysis, both Redundancy Analysis (RDA) (linear method) and Canonical Correspondence Analysis (CCA) (unimodal method) were applied on the data, as DCA revealed that the dominant gradient length was between 3 and 4 (Lepš and Šmilauer, 2003). RDA and CCA showed similar results, but RDA explained more variance in the species-environment relationship.

Consequently, only RDA results are going to be presented. Separate RDAs were applied for each group of descriptor variables. Each run of RDA was made targeting one group after parcelling out the effects of the parameters in the remaining groups, which were used as co-variables (i.e. partial RDA). Partial RDA was run for each possible combination of targeted descriptor and covariables using CANOCO 4.5, based on species correlations and standardized species scores (ter Braak and Šmilauer, 2002). Significant descriptors for each group were identified using CANOCO's forward selection procedure and Monte Carlo permutation test (499 permutations) (Feld and

Hering, 2007). A variance partitioning scheme (Borcard et al., 1992; Liu, 1997) was applied for each group of variables based on the overall variance explained by the partial RDAs (sum of all canonical eigenvalues). This procedure allowed the distinction between unique effects (i.e. the variance explained by a single group of variables), joint effects (i.e. the variance jointly explained by variables of two groups) and unexplained variance.

Further analysis involved Canonical Correspondence Analysis (CCA), used to analyze Biological-Environment relationships in order to identify environmental factors potentially influencing macroinvertebrate assemblages. Monte Carlo permutation test was performed to assess the statistical significance of the environmental parameters and the full model to arrive at the significance of the first two canonical axes (Lepš and Šmilauer, 2003). The environmental factors used were pH, water temperature, dissolved oxygen, conductivity, BOD, COD, phosphates, nitrates, TDS, TSS, culturable bacteria 22ºC and 36ºC, and coliform bacteria. CCA was carried out on global abundances, meaning total number of individuals collected at a site over the sampling period. Taxa was log transformed (y'=log(a*y+b) downweighing rare species (samples with less than 10 individuals), analysis carried out using the CANOCO 4.5 program (ter Braak and Šmilauer, 2002). The results were represented graphically by Multidimensional Scaling (Aschonitis et al., 2015).

3. Results and Discussion

3.1. Environmental characterization

Physical variables considered for the current sampling period were Temperature, pH and Conductivity (Table 2). Temperature variations demonstrate the natural lentic and lotic characteristics of the habitats, sites 4, 5 and sites 1, 2 and 3, respectively. Site 6 behaves erroneously due to the artificial characteristics and low flow, also proven by minimum and maximum values fluctuation, where artificial regime exhibits closer values to atmospheric temperatures. Site 3 evidences higher temperature values, when compared to site 1 and 2, due to the effect of the water residence time inside the marsh.

pH values behave mostly in accordance with the geologic substrate matrix (karst region) therefore values suffer small variations due to the high buffer capacity of such water (Murphy et al., 2013).

Table 2 – Mean Temperature, pH and Conductivity for all sites during the study period.

Sites	Temperature (ºC)			pH			Conductivity (µS/cm)		
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
Site 1	15.9	9.0	21.4	7.70	6.62	8.90	483.3	194.4	869.0
Site 2	15.9	8.6	21.2	7.49	5.60	8.66	469.9	187.1	835.0
Site 3	17.4	9.1	23.8	7.90	6.50	8.76	508.7	189.8	949.0
Site 4	20.7	15.8	24.0	8.12	6.82	9.03	462.0	188.0	952.0
Site 5	17.3	8.4	24.3	7.59	6.35	8.48	395.1	146.2	747.0
Site 6	15.6	6.8	25.3	7.55	6.65	8.35	1222.0	322.0	2310.0

Conductivity is one of the physical parameters that may be associated to pollution, visible on average values observed in site 6, further exhibited during all the sampling months (Figure 2).

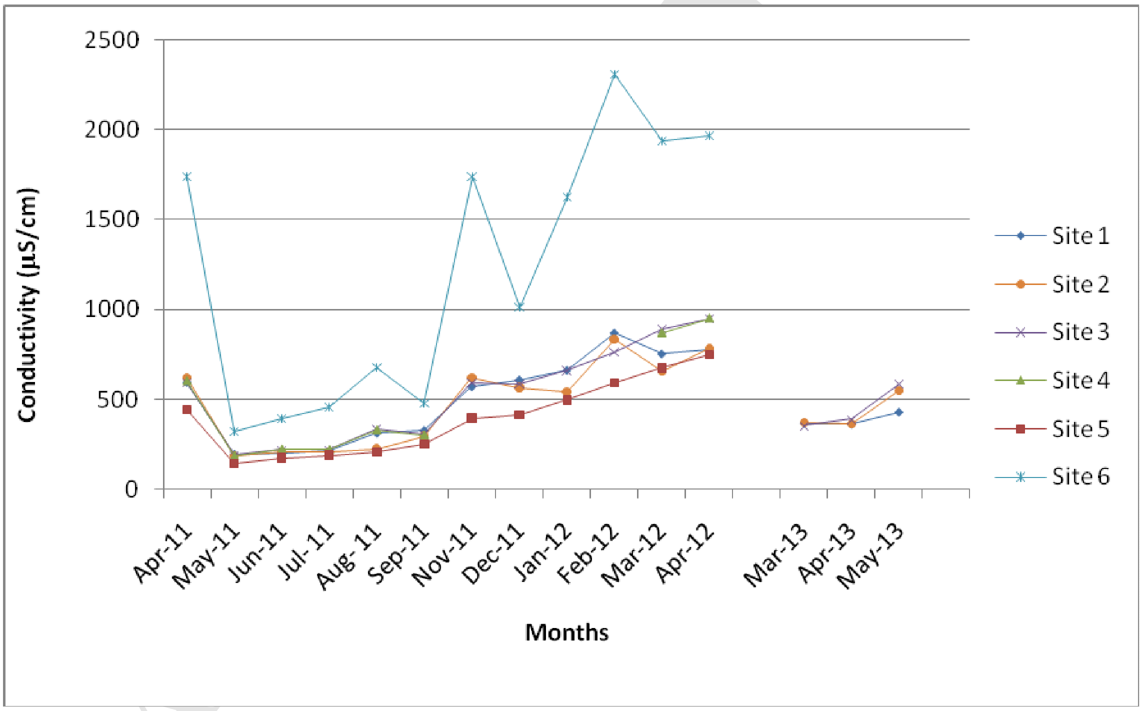


Figure 2 – Water conductivity values exhibited along the sampling period in all sampling sites.

In order to appraise results of the developed monitoring, DO and CBO_5 were chosen as demonstrative chemical parameters following the environmental objectives of minimum quality for surface waters (Portuguese legislation: DL 236/98, annex XXI).

Dissolved oxygen (Figure 3) varies along the Almonda River. Sites 1, 2 and 3, flowing from the entrance (site 1) to the exit (site 3) of the Nature Reserve, display an increasing trend in water oxygenation. Emphasizing the distinct behavior of site 6, the absence of DO strengthens the artificial characteristics and pollution problems (Extence et al., 2011).

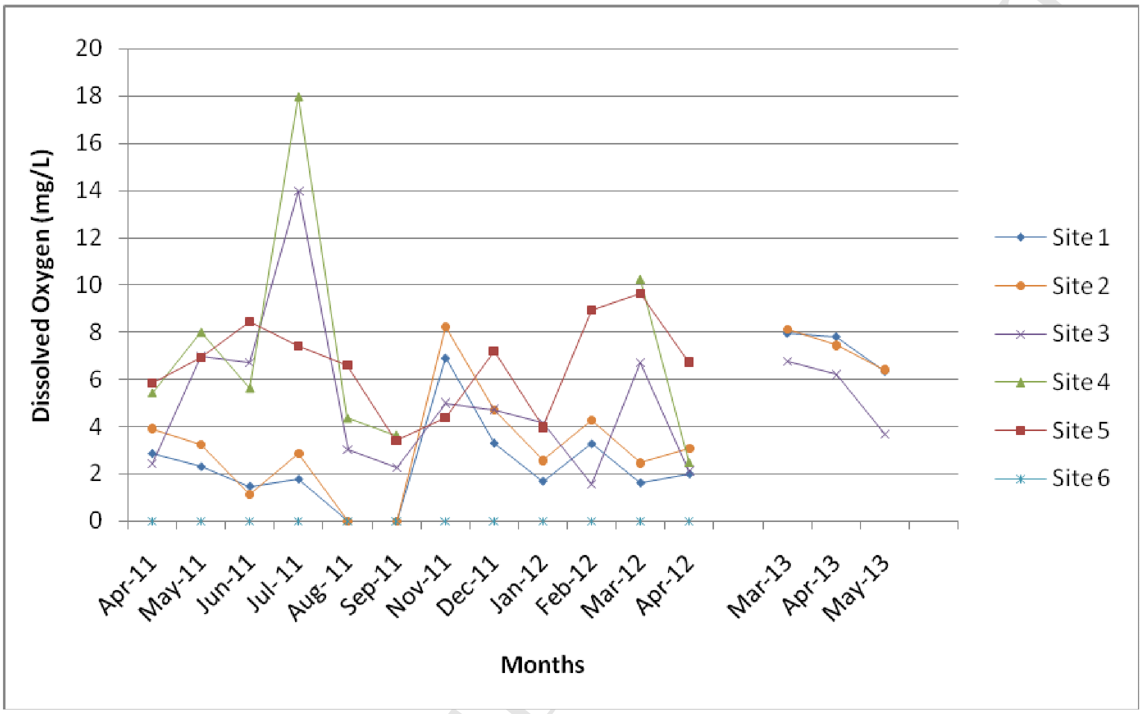


Figure 3 – Dissolved Oxygen levels exhibited along the study period in all sampling sites.

Biochemical Oxygen Demand (Figure 4) reflects the amount of oxygen needed for the microbial oxidation of organic material contamination. In general, the reached values are higher than the maximum admitted value of 5mg/L for surface waters, and most exceeding the 40 mg/L limit for the wastewater discharge allowed by the Portuguese legislation (DL 236/98, annexes XVIII and XXI). This parameter announces a detrimental situation of water quality for this protected wetland in terms of pollution. Along the Almonda River (lotic environment), the levels of BOD are slightly lower at the exit of the wetland, which sustains the intensification of dissolved oxygen. This behavior may suggest a depurative function of the Nature Reserve (Dimitriou and Zacharias, 2010; Verdonschot et al., 2013).

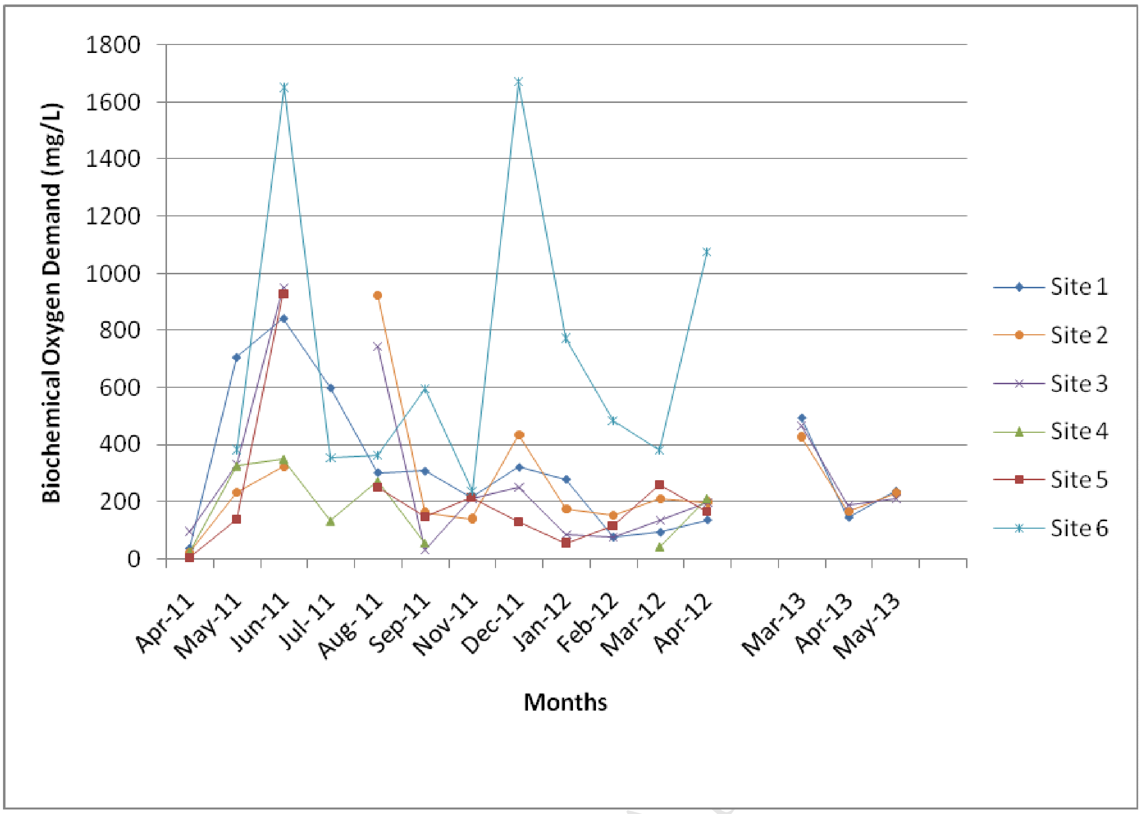


Figure 4 – Biochemical Oxygen Demand exhibited along the sampling period in all sampling sites.

3.2 Macroinvertebrates

For the first period of analysis 1695 specimens were collected and identified, while the second period returned 740 individuals, a total of 2435 specimens within 17 families. The relative abundance (Figure 5) reveals Chironomidae as the most representative family, unfolding poorer water quality.

Macroinvertebrate sampling took place in lotic environment; hence chironomid and related species were the most abundant, possibly due to close proximity of lentic marsh environment (Lepori and Malmqvist, 2007).

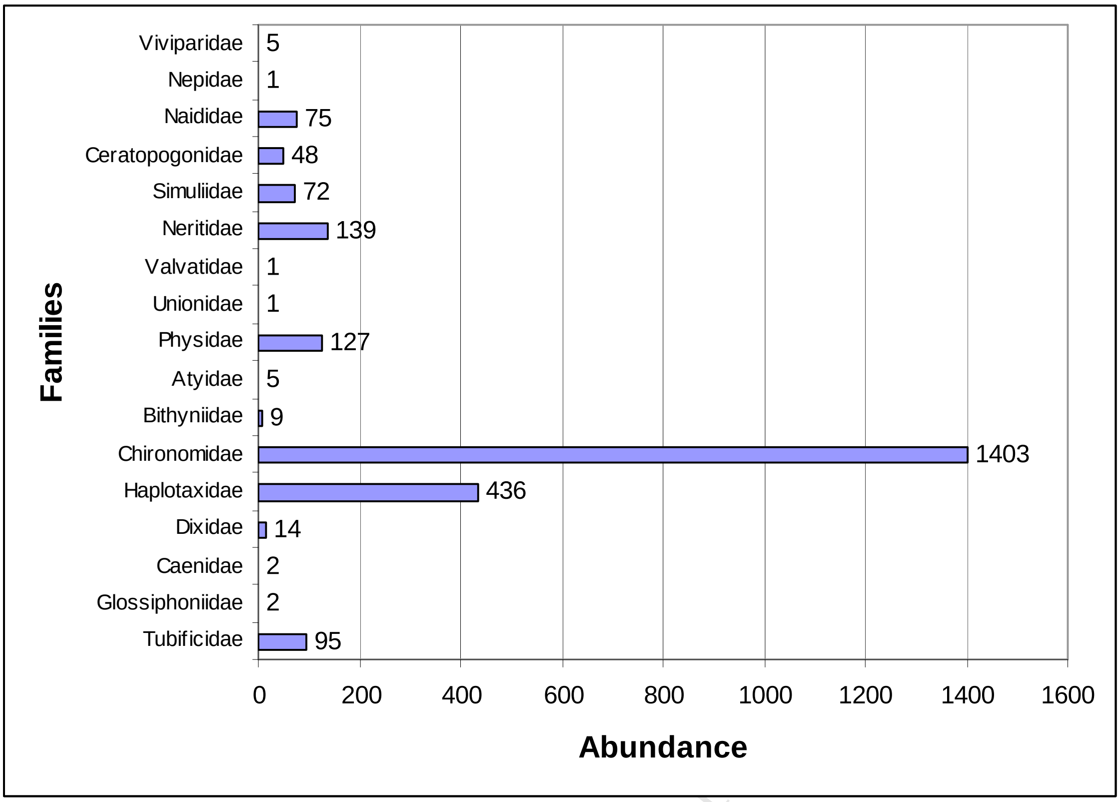


Figure 5 – Total number of macroinvertebrate individuals by family.

The analysis of macroinvertebrate data (INAG, 2009) with the software AMIIB@ delivered several indexes (Table 3). Macroinvertebrate Average Score per Taxon (ASPT), the macroinvertebrate Iberian Average Score per Taxon (IASPT), the Biological Monitoring Working Party (BMWP), and the Iberian Biological Monitoring Working Party (IBMWP) score systems that provide a method of converting lists of macroinvertebrate taxa sampled from a stream or river on a particular occasion into two numerical indices.

Table 3 – Macroinvertebrate biological indexes, calculated with AMIIB@ software.

Site	Season	N Taxa	N Indiv.	BMWP	ASPT	IBMWP	IBMWP Class	IASPT	Shannon Wiener	Evenness	IPtIN	IPtIN Class
1	Spring	8	1271	20	3.3	28	Poor	3.50	1.02	0.49	0.300	Poor
1	Summer	3	53	3	1.5	3	Bad	1.50	0.58	0.53	0.067	Bad
1	Autumn	3	3	3	1.5	7	Bad	2.33	1.10	1.00	0.208	Bad
1	Winter	4	62	12	3.0	12	Bad	3.00	0.55	0.40	0.177	Bad
2	Spring	12	691	33	4.1	47	Acceptable	3.92	1.38	0.56	0.488	Acceptable
2	Summer	3	26	3	1.5	3	Bad	1.50	0.93	0.85	0.117	Bad
2	Autumn	6	25	3	1.5	20	Poor	3.33	1.20	0.67	0.297	Poor
2	Winter	3	36	3	1.5	6	Bad	2.00	0.63	0.57	0.114	Bad
3	Spring	7	86	16	3.2	24	Poor	3.43	1.15	0.59	0.266	Poor
3	Summer	4	79	3	1.5	7	Bad	2.33	0.66	0.48	0.190	Bad

3	Autumn	4	113	3	1.5	9	Bad	2.25	0.47	0.34	0.141	Bad
3	Winter	4	10	3	1.5	11	Bad	2.75	1.19	0.86	0.263	Poor

Score interpretation clearly classifies water quality as poor to bad excluding spring values in site 2 where acceptable water classification was obtained both in IBMWP and IPtIN scores. The reason for these scores may be explained by the temporary stream (tributary of the Almonda River) which is close to collection site 2, and therefore may contribute to a dilution factor. Surprisingly, one would expect the same impact downstream (site 3), which is not observed. The Almonda River after site 2 observes a residence time inside the marsh area, influencing water characteristics, before reaching site 3.

Classifications obtained by both IBMWP and IPtIN are very similar in results, where the only notable difference was in winter on site 3, which is clearly attributed by the evenness values registered.

Multivariate cluster analysis of variables with single linkage-distance measured by correlation coefficient distance and final partition was used to evaluate the number of groups present in the data (Figure 6). Data can be divided into 2 groups, where Group 1 can be determined by the Temperature, Phosphates, Culturable microorganisms 36ºC, Coliforms, Culturable microorganisms 22ºC, Biochemical Oxygen Demand and Total Dissolved Solids, whereas the Group 2 is constituted by the variables pH, Conductivity, Dissolved Oxygen, Nitrates, Total Suspended Solids and Chemical Oxygen Demand.

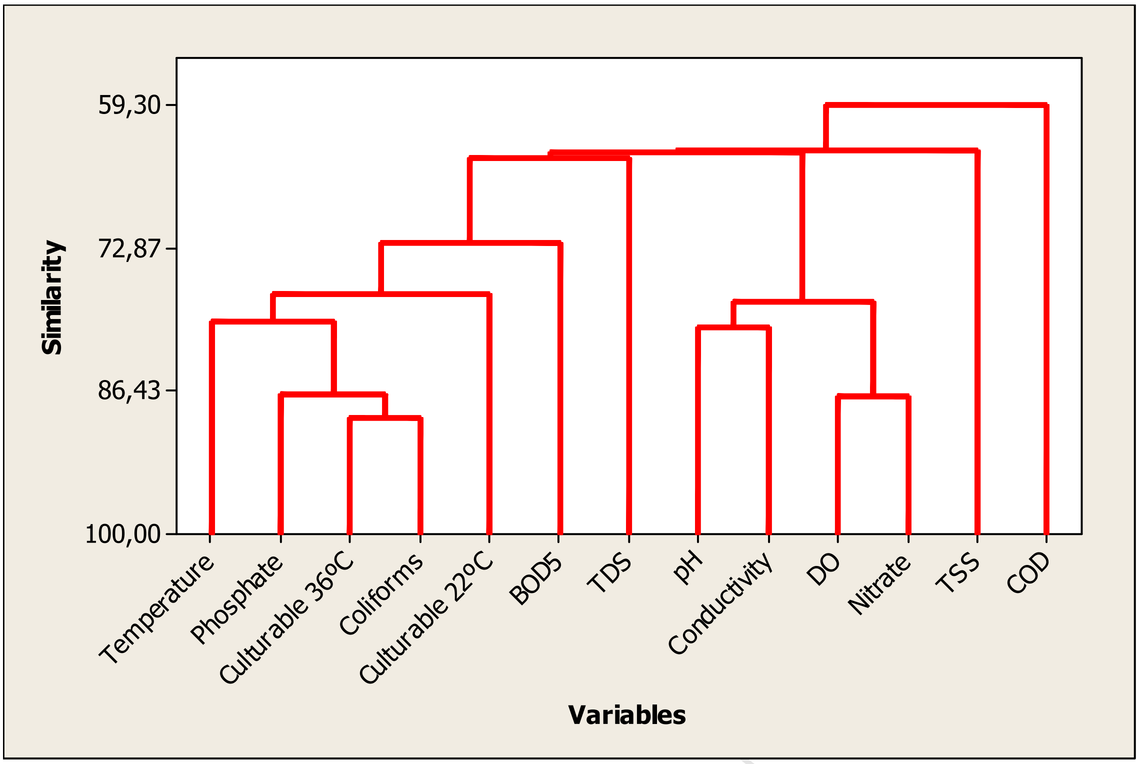


Figure 6 – Dendrogram of similarity analysis of variables with single linkage-distance measured by correlation coefficient distance and final partition

From all similarities in the analyzed variables, group 1 is easily explained by the microbial activity, nutrient (P and TDS) and temperature needs, limited by the BOD and possibly by turbidity (TDS). On the other hand, group 2 exhibits similarity of correlation coefficient between DO and N (86%), which in turn present similarity (79%) with pH and conductivity where limestone matrix contributes to alkaline values. Contaminants contribute to lower the pH and DO and to increase conductivity, hence the coefficient of correlation. Regarding the week similarity of correlation coefficient the former mentioned parameter in this group and TSS and COD (63% and 59% respectively), may be explained by the pollution events sporadically observed through discharges into the system, where COD indicates organic, non biodegradable contaminants in the system.

These observations concordantly associate poor water quality and eutrophication prone condition to this important Nature Reserve, especially when temperature rises.

Canonical analysis delivered a general RDA for all data where cumulative percentage of variance for species-environment relationships explains 47.1% of all variability, while axis 2 explains 22.2% of variability, together representing 69.3% of all variability. The result is considerable if analyzing axis 3 and 4 which represent 12.9% and 12%, respectively.

When analyzing results of the canonical correspondence analysis (RDA) plotted values of marginal effects (λ-1) and conditional effects (λ-A) it is easily understood the importance of pH, DO and N with p values of 0.016, 0.044 and 0.042 respectively (Figure 7). The importance of such variables for the complete canonical analysis is also reflected in the previously presented similarity analysis placing these variables in group 2.

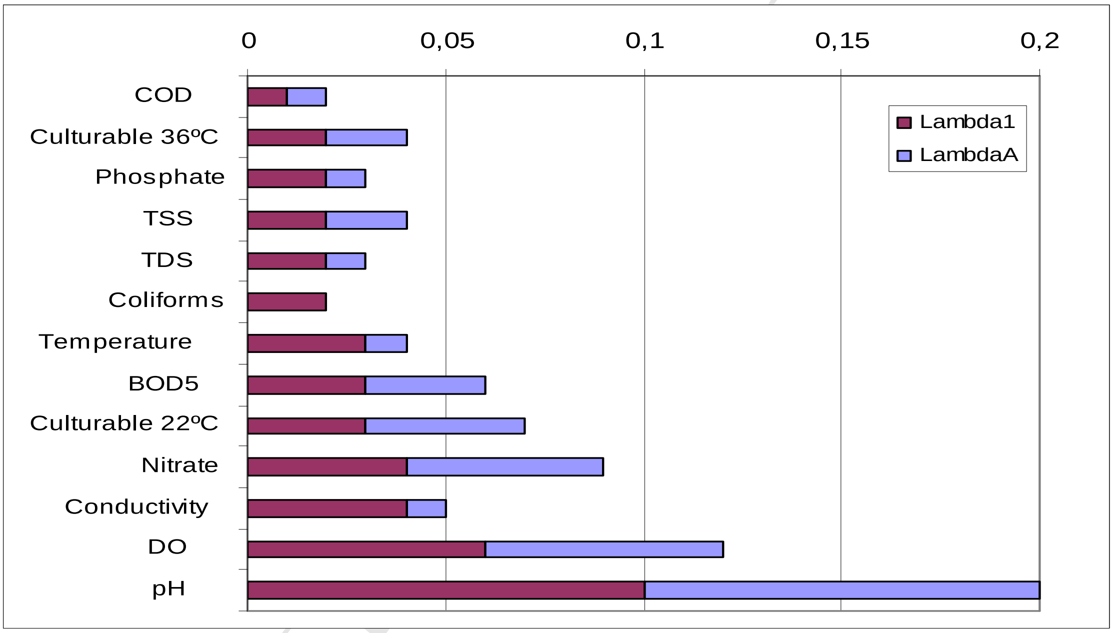


Figure 7 – Results of the canonical correspondence analysis (RDA) plotted values of Lambda 1 (marginal effects) and Lambda A (conditional effects).

Both marginal effects and conditional effects are well explained by pH and DO as in many lotic systems (Murphy et al., 2013), however both N and culturable bacteria at 22ºC present adequate explanations for conditional effects as a probable result of pollution episodes. Another variable to consider from the dataset, despite the p value of 0.842, is Conductivity where marginal effects (λ-1=0.04) also emphasize a probable pollution episode result.

Graphical biplot of species and environmental variables (RDA-MDS) enables a visual relationship between environmental variables and sampled families (Figure 8).

From the central distribution of the families, dataset common interpretation for a lotic river system (Nichols et al., 2006), it is important to emphasize the marsh connectivity and organic load. Odonata, Crustacea and Oligochaeta taxonomic groups are associated and brought to evidence by the families Nepidae, Atyidae and Tubificidae, respectively. Heavy microbiological activity promoted by temperature increase and lower flow levels do create opportunities for Oligochaeta and Crustacea groups, which in turn will provide opportunistic conditions to Odonata (Feld et al., 2014).

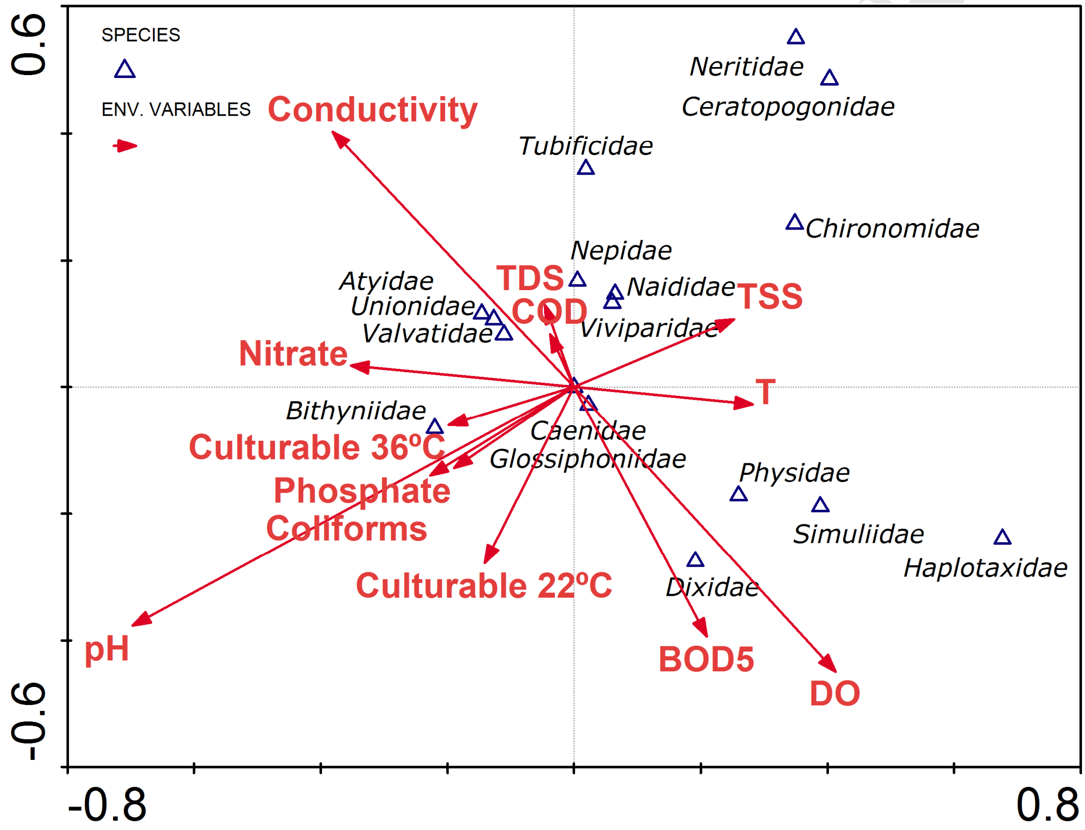


Figure 8 – Results of the canonical correspondence analysis (RDA) based on the invertebrates assemblages with respect to environmental variables. Arrows represent the environmental variables and triangles represent families.

Diptera families are clearly separated by the first axis (explaining 47% of environment-species variability) with positive correlations with TSS, T, DO and BOD_5, although negatively correlated to the remaining variables. Particular emphasis should be given to pH, the driving variable where lower values increase less sensitive families revealing a possible contamination problem.

Attempting to further explain unique effects in the variability of the system, group canonical analysis with partial RDA of both groups 1&2 with covariables set respectively as 2&1, (Figure 9) revealed that group 1 sum of axis 1 and 2 explain 68.6%, while group 2 explains 83.4% of the total variability in the system (Figure 10).

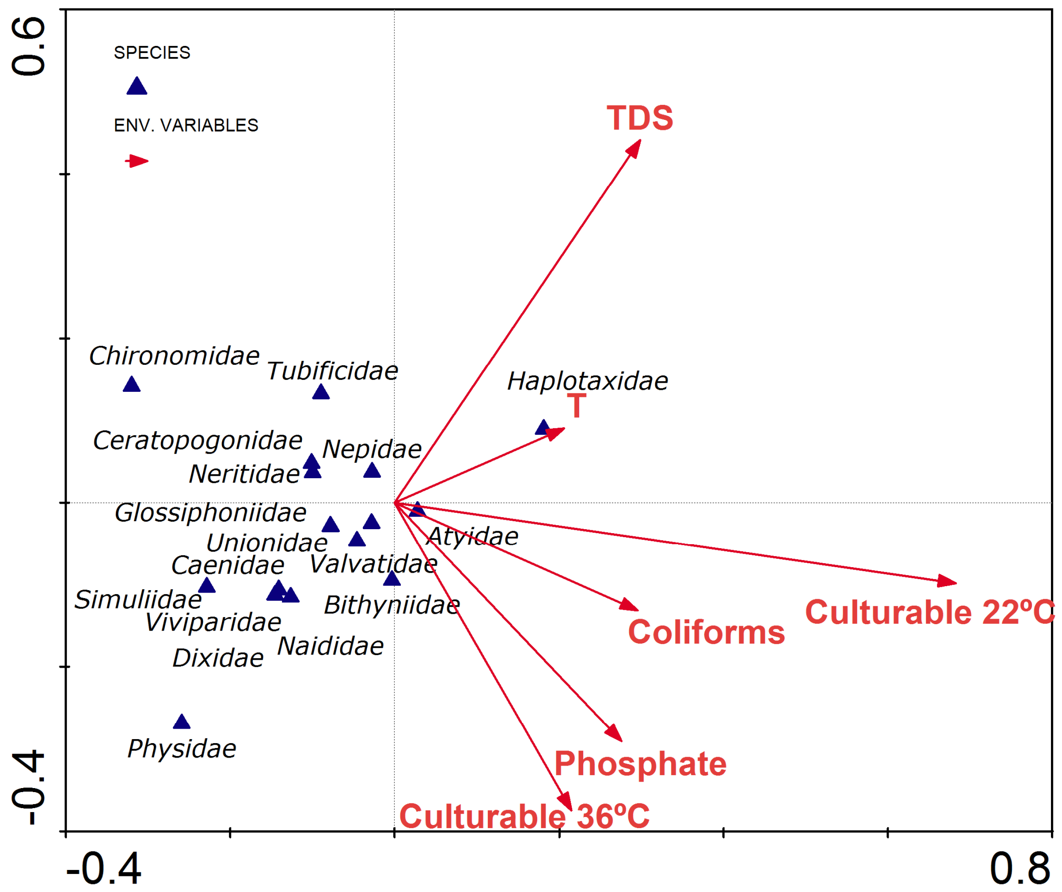


Figure 9 – Partial RDA of Group 1 with covariables as Group 2.

Group 1 establishes clear conditions of organic pollution with the development of a considerable bacterial load, therefore deteriorating conditions and depleting oxygen which globally influences the presence of biodiversity. The percentage of explanation (68.6%) is lower than the overall percentage (Figure 8) probably due to the frequency and duration of such conditions, which may be associated to organic pollution events.

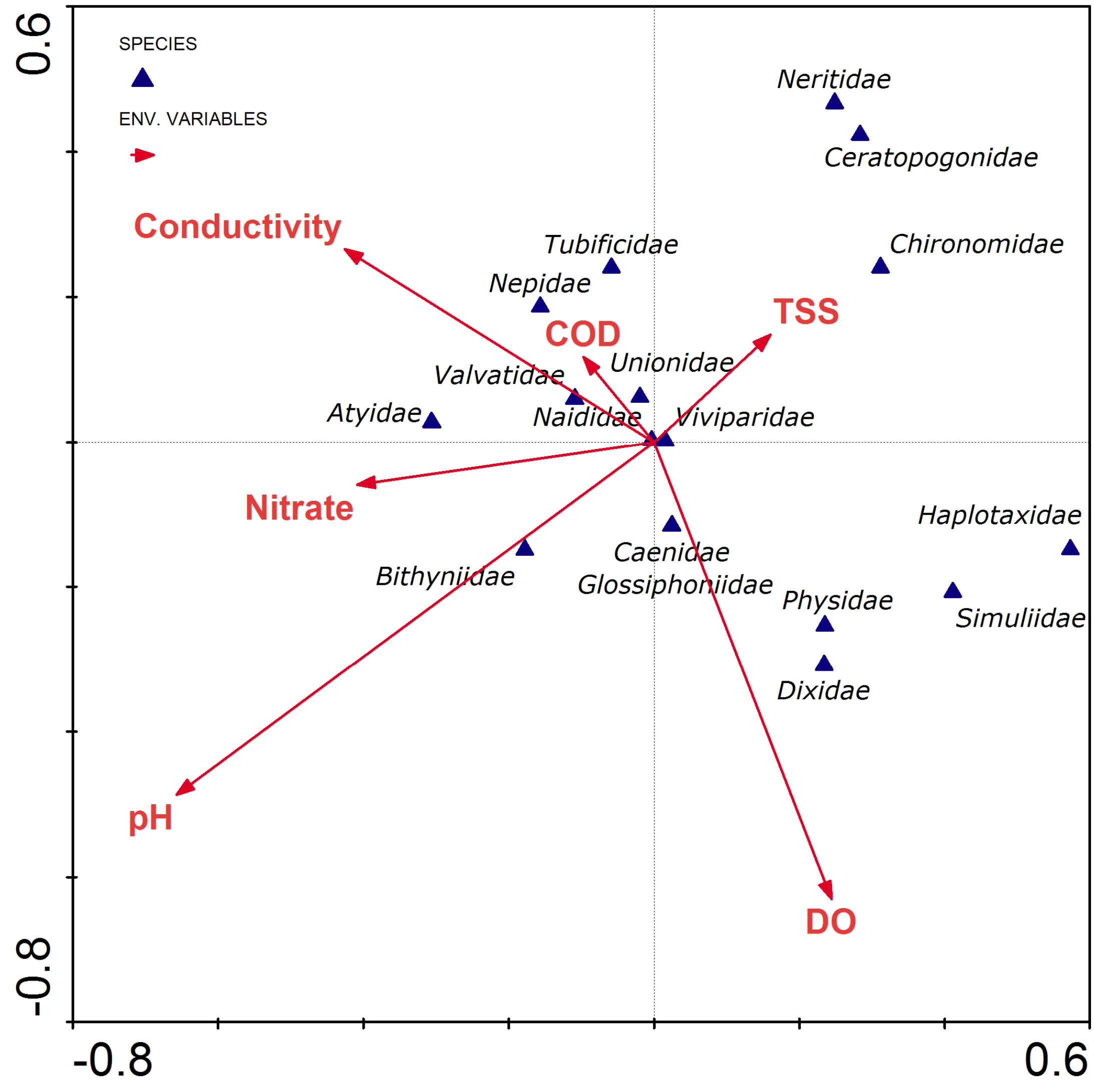


Figure 10 – Partial RDA of Group 2 with covariables as Group 1.

On the other hand group 2 comprehending the most important variables in the system pH, DO and Nitrates, in this way explaining 83.4% of the variability (Figure 10), overwhelmingly confirms the importance of these variables to the ecosystem's health, hence the conservation importance. It is a known fact that Dissolved Oxygen is one of the most important driving factors in ecosystem quality (Poff et al., 1997; Richter et al., 2003; Nichols et al., 2006; Friberg et al., 2011; Feld et al., 2014) where hydromorphologic characteristics can play an important role and may not necessarily mean contamination impact. On the other hand pH is commonly a strong environmental gradient of acid–base status, associated to impairment despite sensitivity to natural variations. For the Almonda River pH's importance stands on the strong buffer effect of the alkaline waters which withstand high levels of impact with little fluctuation. One would expect pH of alkaline rivers to be a weak indicator assuming pollution remains at average levels (Murphy et al., 2013), nonetheless in this study pH represents the main driving variable pinpointing a strong pollution impact.

4. CONCLUSIONS

Through the study of ecological indicators and chemical parameters, it was found that the surface waters have a very poor quality; high Biochemical Oxygen Demand values indicate a severe organic biodegradable contamination; statistical analysis suggests pH and dissolved oxygen as the driving variables, where higher levels reveal better biological quality.

Nitrates may bring to evidence the common landuse by agriculture where impact is evident, despite the seasonal trend of this activity.

Water quality is strongly influenced by the geographic variable of sampling sites which presents different pollution levels and also appears conditioned by seasonality.

Most critical site correspond to Vala das Cordas ditch (site 6), but the upstream contributions are globally detrimental.

Main target variables for a first effort in ecosystem conservation should focus on Nitrates, Dissolved Oxygen and pH.

The pollution level must be controlled at once in order to allow the natural behavior of the ecosystem and the preservation of that important wetland.

ACKNOWLEDGEMENTS

The authors wish to thank the following environmental engineering' students involved in the laboratorial work: Adélia Morais, Cláudia Oliveira, Sarah Oliveira, Palmira Hilário (2010/11); Ana Godinho, André Fróis, André Oliveira, José Marôco, Vasco Lopes (2011/12); Júlia Azevedo, Luísa Cabral, Priscila Galizes, Renata Sampaio, Thalissa Mesquita (2012/13).

REFERENCES

Alba-Tercedor, J., Jáimez-Cuéllar, P., Álvarez, M., Avilés, J., Bonada, N., Casas, J., Mellado, A., Ortega, M., Pardo, I., Prat, N., Rieradevall, M., Robles, S., Sáinz-Cantero, C.E., Sánchez-Ortega, A., Suárez, M.L., Toro, M., Vidalabarca, M.R., Vivas, S., ZamoraMuñoz, C., 2002. Caracterización del estado ecológico de ríos mediterráneos ibéricos mediante el índice IBMWP (antes BMWP'). Limnetica 21(3-4), 175-185.

Artiola, J.F., Pepper, I.L., Brusseau, M., 2004. Environmental Monitoring and Characterization, Elsevier Academic Press, USA.

Aschonitis, V.G., Feld, C.K., Castaldelli, G., Turin, P., Visonà, E., Fano, E.A., 2015. Environmental stressor gradients hierarchically regulate macrozoobenthic community turnover in lotic systems of Northern Italy. Hydrobiologia 756 (1-2), DOI: 10.1007/s/10750-015-2407-x.

Barbour, M.T., Stribling J.B., Gerritsen, J., Karr, J.R., 1996. Biological Criteria: Technical Guidance for Streams and Small Rivers, revised ed., United States Environmental Protection Agency, Washington, D.C.

Borcard, D., Legendre, P., Drapeau, P., 1992. Partialling out the spatial component of ecological variation. Ecology 73, 1045–1055.

Chester, H., Norris, R., 2006. Dams and flow in the Cotter River, Australia: effects on instream trophic structure and benthic metabolism. Hydrobiologia 572 (1), 275–286.

Colas, F., Archaimbault, V., Devin, S., 2011. Scale-dependency of macroinvertebrate communities: responses to contaminated sediments within run-of-river dams. Science of the Total Environment 409 (7), 1336–1343.

Davis, J., Horwitz, P., Norris, R., Chessman, B., Mcguire, M., Sommer, B., 2006. Are river bioassessment methods using macroinvertebrates applicable to wetlands? Hydrobiologia 572, 115–128.

Dimitriou, E., Zacharias, I., 2010. Identifying microclimatic, hydrologic and land use impacts on a protected wetland area by using statistical models and GIS techniques. Mathematical and Computer Modelling 51, 200–205.

EC, 2000. European Union Water Framework Directive 2000/60/4d of the European Parliament and of the Council of 23 October 2000: establishing a framework for Community action in the field of water policy. Official Journal of the European Communities 222122000 L 327/1–327/72.http://ec.europa.eu/environment/ water/water-framework/index_en.html. (21.01.15)

EC, 2011. Common Implementation Strategy for the Water Framework Directive (2000/60/EC). Guidance Document 14. Guidance on the Intercalibration Process 2008–2011. Office for Official Publications of the European Communities, Luxembourg.

EC, 2013. Commission Decision of 20 September 2013 Establishing, Pursuant to Directive 2000/60/EC of the European Parliament and of the Council, the Values of the Member State Monitoring System Classifications as a Result of the Intercalibration Exercise and Repealing Decision 2008/915/EC. Official Journal of the European Union (L 266/1-47).

Extence, C.A., Chadd, R.P., England, J., Dunbar, M.J., Wood, P.J., Taylor, E.D., 2011. The assessment of fine sediment accumulation in rivers using macro-invertebrate community response. River Research and Applications 29 (1), 17–55.

Feld, C.K., de Bello, F., Dolédec, S., 2014. Biodiversity of traits and species both show weak responses to hydromorphological alteration in lowland river macroinvertebrates. Freshwater Biology 59, 233–248.

Feld, C.K., Hering, D., 2007. Community structure or function: effects of environmental stress on benthic macroinvertebrates at different spatial scales. Freshwater Biology 52, 1380–1399.

Friberg, N., Bonada, N., Bradley, D.C., Dunbar, M.J., Edwards, F.K., Grey, J., Hayes, R.B., Hildrew, A.G., Lamouroux, N., Trimmer, M., Woodward, G., 2011. Biomonitoring of human impacts in freshwater ecosystems: the good, the bad and the ugly. Advances in Ecological Research 44, 1–68.

Greenberg, A., Clersceri, L., Eaton, A., 1992. Standard Methods for the Examination of Water and Wastewater, eighteenth ed., APHA, Washington.

Hering, D., Borja, A., Carstensen, J., Carvalho, L., Elliott, M., Feld, C.K., Heiskanen, A.S., Johnson, R.K., Moe, J., Pont, D., Solheim, A.L., van de Bund, W., 2010. The European Water Framework Directive at the age of 10: a critical review of the achievements with recommendations for the future. Sci. Total Environ. 408, 4007–4019.

INAG, 2008. Manual para a avaliação biológica da qualidade da água em sistemas fluviais segundo a Directiva Quadro da Água – Protocolo de amostragem e análise para os macroinvertebrados bentónicos. Ministério do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional, Instituto da Água, I.P., Lisboa.

INAG, 2009. Critérios para a Classificação do Estado das Massas de Água Superficiais – Rios e Albufeiras. Ministério do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional, Instituto da Água, I.P., Lisboa.

James, L.A., Rathburn, S.L., Whittecar, G.R., 2009. Management and Restoration of Fluvial Systems with Broad Historical Changes and Human Impacts, The Geological Society of America, USA.

Lepori, F., Malmqvist, B., 2007. Predictable changes in trophic community structure along a special disturbance gradient in streams. Freshwater Biology 52, 2184–2195.

Lepš, J., Šmilauer, P., 2003. Multivariate Analysis of Ecological Data using CANOCO, Cambridge University Press, UK.

Liu, Q., 1997. Variation partitioning by partial redundancy analysis (RDA). Environmetrics 8, 75–85.

MEA (Millennium Ecosystem Assessment), 2005. Ecosystems and Human Well-being: Synthesis, Island Press, Washington, DC.

Mebane, E.A., 2001. Testing bioassessment metrics: macroinvertebrate, sculpin, and salmonid responses to stream habitat, sediment, and metals. Environmental Monitoring and Assessment 67, 293–322.

Murphy, J.F., Davy-Bowker, J., McFarland, B., Ormerod, S.J., 2013. A diagnostic biotic index for assessing acidity in sensitive streams in Britain. Ecological Indicators 24, 562–572.

Nichols, S.J., Robinson, W.A., Norris, R.H., 2006. Sample variability influences on the precision of predictive bioassessment. Hydrobiologia 572, 215–233.

Oberdorff, T., Pont, D., Hugueny, B., Porcher, J., 2002. Development and validation of a fish-based index for the assessment of 'river health' in France. Freshwater Biology 47, 1720–1734.

Pankhurst, N.W., Munday, P.L., 2011. Effects of climate change on fish reproduction and early life history stages. Marine and Freshwater Research 62, 1015–1026.

Poff, N.L., Allen, J.D., Bain, M.B., Karr, J.R., Prestegaard, K.L., Richter, B.D., Sparks, R.E., Stromberg, J.C., 1997. The natural flow regime: a paradigm for river conservation and restoration. BioScience 47, 769–784.

Richter, B.D., Mathews, R., Harrison, D.L., Wigington, R., 2003. Ecologically sustainable water management: managing river flows for ecological integrity. Ecological Applications 13, 206–224.

Rosenberg, D.M., Resh, V.H. (Eds.), 1993. Freshwater Biomonitoring and Benthic Macroinvertebrates, Chapman and Hall, New York.

Roux, D.J., Kleynahans, C.J., Thirion, C., 1999. Biological monitoring and assessment of rivers as a basis for identifying and prioritizing river management options. Water Science and Technology 39, 207–210.

Tachet, H., Richoux, P., Bournaud, M., Usseglio-Polatera, P., 2002. Invertebrés d'Eau Douce (second corrected impression), CNRS Editions, Paris.

ter Braak, C.J.F., Šmilauer, P., 2002. CANOCO Reference Manual and CanoDraw for Windows User's Guide Version 4.5. Biometris-Plant Research International. Wageningen and Ceske Budejovice.

Verdonschot, P.F.M., Spears, B.M., Feld, C.K., Brucet, S., Keizer-Vlek, H., Borja, A., Elliott, M., Kernan, M., Johnson, R.K., 2013. A comparative review of recovery processes in rivers, lakes, estuarine and coastal waters. Hydrobiologia 704, 453–474.

Ward, J.V., Stanford, J.A., 1995. The serial discontinuity concept: extending the model to floodplain rivers. Regulated Rivers: Research & Management 10 (3-4), 159–168.

Water quality monitoring in the Paul do Boquilobo Biosphere Reserve

Baptista, C.[1]; Santos, L.[1]

Highlights:

1. Paul do Boquilobo marsh is a classified nature reserve, Ramsar site and Biosphere reserve;
2. Water quality parameters indicate heavy organic pollution;
3. Chemical parameters such as BOD and COD are above legal values particularly in site 5;
4. Macroinvertebrates bring to evidence chironomid families indicating poor water quality;
5. Statistical analysis identifies DO and pH as driving variables.