Universidade dos Açores

Departamento de Ciências Agrárias

Tese de Mestrado em Gestão e Conservação da Natureza

Environmental conditions that constrain invertebrate communities and the performance of benthic indices to assess ecological status in Mediterranean streams

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Tese orientada por:

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Dissertação apresentada na Universidade dos Açores para obtenção do grau de Mestre em Gestão e Conservação da Natureza

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Resumo

Com a publicação da Directiva Quadro da Água em 2000, Portugal assumiu, assim como os restantes Estados Membros da Comunidade Europeia, o compromisso de alcançar o bom estado ecológico das suas massas de água superficiais até 2015. Contudo, para tal é necessário primeiramente averiguar o estado actual destas mesmas massas de água. Tendo por base as metodologias propostas para os rios do Sul de Portugal, procedeu-se ao estudo das comunidades de macroinvertebrados bentónicos existentes em 13 locais nas bacias hidrográficas de Santo André e Melides. Verificou-se que, tal como em outras ribeiras mediterrânicas, os insectos são o grupo predominante, com uma elevada densidade de *taxa* generalistas. A forma como os macroinvertebrados bentónicos respondem a diferentes variáveis ambientais permitiu averiguar que a concentração de oxigénio dissolvido, a granulometria e a concentração de matéria orgânica são factores estruturantes destas comunidades, sendo fundamentais para a posterior avaliação do estado ecológico das ribeiras.

O Índice Português de Invertebrados do Sul ($IPtI_s$) proposto para a avaliação do estado ecológico da maioria dos rios do sul de Portugal foi aplicado aos locais em estudo. Os resultados deste índice sugerem que destes treze locais apenas três se encontram num estado ecológico considerado bom ou excelente. Para uma avaliação ecológica integrada e abrangente aplicaram-se também índices de qualidade do habitat fluvial (IHF) e da galeria ripícola (QBR). Uma vez que a criação do índice $IPtI_s$ é relativamente recente, não foi ainda possível uma ampla aplicação do mesmo de modo a verificar a sua eficiência em diferentes tipos de sistemas aquáticos. Como tal, foi estudada a responsividade deste índice às pressões identificadas nas bacias em estudo. Chegou-se à conclusão que, apesar de este índice ter uma boa responsividade em ambientes lóticos, o mesmo não se verifica quando aplicado em ambientes lênticos ou com zonas de interface com águas subterrâneas, subestimando nestes a qualidade ecológica.

Palavras-chave: Directiva Quadro da Água, ribeiras, macroinvertebrados bentónicos, insectos, $IPtI_s$, estado ecológico.

Summary

With the publication of the Water Framework Directive in 2000, Portugal and all other Member States of the European Community, assumed the commitment to achieve a good ecological status of all water bodies by the year of 2015. The accomplishment of this major objective requires the assessment of the current status of all water bodies. Benthic macroinvertebrate communities of 13 locations of the Santo André and Melides river basins were assessed based on the methods proposed for the Portuguese Southern rivers. As in other Mediterranean streams, insects were the predominant group with a high density of generalist taxa. Dissolved oxygen concentration, sediment grain-size and organic matter concentration were the major environmental variables structuring these benthic macroinvertebrate communities.

The Portuguese multimetric index of the South ($IPtI_S$), proposed for the assessment of the ecological status of southern Portuguese rivers was determined for the studied locations. The obtained results suggest that only three of these thirteen sites are in an ecological status considered good or excellent. Riparian vegetation quality (QBR index) and the habitat diversity (IHF index) were also assessed for a broader and integrated ecological assessment. The recent proposal of the $IPtI_S$ index as an assessment method is relatively recent and for that reason a wider use in order to verify its responsiveness and, therefore, improve its accuracy was not possible. The responsiveness of this index to previously identified pressures in the Melides and Santo André river basins was a major objective of this study. Although this index showed a predictable response in lotic environments, it did not show a good performance when applied to lentic environments and groundwater/surface water interfaces, underestimating ecological quality.

Key-words: Water Framework Directive, streams, benthic macroinvertebrates, insects, $IPtI_S$, ecological status.

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List of abbreviations

Acronims

ARH, I.P.: Administração de Região Hidrográfica, Instituto Público

ASPT: Average Score per Taxa

BBI: Belgium Biotic Index

BMWP: Biological Monitoring Working Party

EBI: Extended Biotic Index

DSFI: Danish Stream Fauna Index

H': Shannon-Weiner index

IASPT: Iberian Average Score per Taxa

IBMWP: Iberian Biological Monitoring Working Party

ICNB: Instituto de Conservação da Natureza e da Biodiversidade

INAG, I.P.: Instituto da Água, Instituto Público

INSAAR: Inventário Nacional de Sistemas de Abastecimento de Água e de Águas Residuais

$IPtI_S$: Índice Português de Invertebrados do Sul

J: Eveness

WFD: Water Framework Directive

WWTP: Wastewater Treatment Plants

Environmental variables (unit)

Chl *a*: Chlorophyll *a* (µg/L)

Con: Conductivity (mS/cm)

CS: Current Speed (m/s)

Dep: Depth (m)

DO: Dissolved oxygen (mg/L)

GS: Grain size (Φ)

NH_4: Ammonia (mg/L)

NO_2: Nitrites (mg/L)

NO_3: Nitrates (mg/L)

P: Phosphorous (mg/L)

TDS: Total dissolved solids (g/L)

TOC: Total Organic Content (%)

WT: Water temperature (°C)

Statistics

ANOSIM: Analysis of Similarities

MDS: Multi-Dimensional Scaling

PRIMER: Plymouth Routines In Multivariate Ecological Research

SIMPER: Similarity Percentage Breakdown Procedure routine

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Chapter I

General Introduction

General Introduction

The importance of freshwater resources

In Southern Europe there are growing pressures that are a result of an intense water demand, especially in the summer months, that in association with an irregular distribution of precipitation leads to a prediction of occurrence of shortages in water supply at the medium and long term. Although freshwater ecosystems only occupy less than 1% of the Earth's surface, they can be considered hotspots since they support approximately 10% of all known species and provide goods and services with great value to human societies (Strayer and Dudgeon, 2010).

Stream Management and the Water Framework Directive (WFD)

The WFD (2000/60/EC) establishes basic principles of a sustainable water policy in the European Union with the purpose of protection, improvement and restoration of surface and groundwaters. The main objective of this framework is to achieve a good chemical and ecological status by the year 2015 (European Commission, 2000). Ecological status is an expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters, requiring the use of biological elements to assess it, namely phytoplankton, aquatic flora, benthic invertebrate fauna, fish fauna.

The ecological status of stream ecosystems is a result of natural environmental conditions and anthropogenic pressures. The greater the intensity of the pressure agents in the ecosystem, the greater the difference from its natural state (reference condition) (Ippolito *et al*, 2009).These pressures lead to an elevated loss of biodiversity, having Ricciardi *et al*. (1999) concluded that the extinctions rate in freshwater are much greater than in the terrestrial fauna. Over-exploitation, pollution, habitat fragmentation, habitat degradation or destruction, introduction of non-indigenous species, changes in current flow regime due to damn and water supply withdrawal systems, and land use changes are some of the major stressors (Geist, 2011). An increase in scale of threats implies an increase in the management effort. Although in the past few years there has been much improvement in physico-chemical, biological and ecological assessment, there has been little progress in the integrated management of water resources in a way that failed to stop deterioration (Verdonschot, 2000). A successful management can only be achieved by filling the gap between scientific quality information with collaborative involvement of the stakeholders and managers (Barmuta *et al*., 2011).

The WFD establishes five different quality classes, namely high, good, moderate, poor and bad and requires that Member States classify all water bodies. The implementation of monitoring programs will provide a coherent assessment between countries aiming at supporting the elaboration of programs of measures to restore water bodies below good status.

The ecological status is obtained by the Ecological Quality Ratio (EQR), which represents the deviation of the water body from the reference condition (undisturbed or minimally disturbed). The EQR is expressed by a numerical scale between 0 and a value slightly above 1, where values near zero stand for bad ecological status and values near one for high ecological status (Bernardo *et al*., 2009).

The major challenge in Portugal was the lack of historical data for most aquatic systems and biomonitoring programs. Adequate ecological quality assessment tools, including sampling methods, metrics and biotic indices were not available (Bernardo *et al*., 2009). Therefore, the Portuguese Water Institute (INAG) defined reference conditions for each type of river, carried out the selection of metrics responsive to stressors for each element of biological quality and established criteria for the classification of ecological status for all biological, chemical, physicochemical and hydromorphological components (INAG, 2009).

The WFD brought a change in the monitoring approach of rivers and streams, replacing an anthropocentric perspective by an ecocentric perspective, focused on ecosystem structure instead of considering water merely as a resource.

Benthic macroinvertebrates as important ecologic indicators

The WFD requires that the assessment of ecological water quality is integrated by a set of biological elements such as fish, aquatic flora and benthic invertebrates to assess the ecological water quality.

In stream systems, benthic macroinvertebrates are one of the biological elements most frequently used to assess ecological status. These animals with no backbones inhabit the streambed substrate are visible to the naked eye and able to be caught in a 500 μm screen (Duan *et al*., 2009), although this mesh size is not consensual among all authors (*e.g.,* De Pauw *et al*., 2006). This group comprises a wide diversity of species belonging to different taxonomic groups, mainly aquatic larval stages of insects, but it also includes arthropods, worms and leaches, bivalves and snails, among others.

Benthic macroinvertebrate communities play a key-role in the food web of stream ecosystems, linking producers and top predators (Song *et al*., 2007). Thus, they are an important source of food and energy to higher levels on the food web, mainly ichthyofauna but also other vertebrates. A variety of species with different feeding strategies, including the consumption of algae and bacteria, or leaves and organic matter are considered in this group (Duan *et al*., 2009). When benthic invertebrates die, they decay, leaving behind nutrients that are reused by aquatic plants and other animals in the food chain (Duan *et al*., 2009).

Macroinvertebrate communities are a key tool for assessing ecological quality because disturbances in the ecosystem change the community structure (Pires *et al.*, 2000). Some major characteristics of these organisms make them good bioindicators: a) they are ubiquitous and relatively sedentary; b) they have a great range of taxonomic and functional feeding groups (Rosado *et al*., 2011); c) they have a life cycle long enough to provide information about the stress conditions to which they were exposed (Fano *et al*., 2003; Duan *et al*., 2009); d) their high abundance; e) they are easy to sample; f) they quickly recolonize the streambed (Lüderitz *et al*., 2004); g) they show a wide range of tolerance levels, because they include a great variety of species; h) they differ in their sensitivity to water pollution and can integrate environmental changes in physical, chemical, and ecological characteristics of their habitat over time and space (Milbrink, 1983). Accordingly, these diverse communities are essential to evaluate and help to maintain the ecological integrity of streams.

Streams of the Santo André and Melides River Basins, a case study

Santo André and Melides River basins are exposed to a Mediterranean climate. This climate can be defined as oceanic temperate, humid and moderately rainy (Cancela da Fonseca *et al.,* 1993). Winter is characterized by relatively abundant rainfall, with average temperatures relatively low. Summer is dry with low rainfall and high temperatures. This characteristics fall into Koppen classification of a humid mesothermic climate (Csb) with a warm season not too long and warm (Cancela da Fonseca *et al.,* 1993).

Mediterranean streams are physically, chemically, and biologically shaped by sequential, predictable, seasonal events of flooding and drying over an annual cycle (Gasith and Resh, 1999). The duration of these events affect drought intensity, which may range from declines in discharge below average base flow levels, to intermittency, to sections drying and, ultimately to stream drought (Boix *et al*., 2010). These River basins are located in Alentejo (Southern Portugal), where the average annual temperature is approximately 15,8ºC, with the lowest temperatures registered in December and January and the highest values in July, August and September (ARH Alentejo, 2011).The average annual precipitation is 523 mm and irregular throughout the year (ERENA, 2005). The rainiest period occurs in December and January, accounting for about 40% of annual rainfall, and the less rainy season in July and August where the rain fall can be null (ARH Alentejo, 2011). Hence, the streams of these basins have a torrential regime with abundant flow in winter and very low flow in summer.

The Santo André River basin has a 60,2 km perimeter and drains a 145 km^2 area into the Santo André Lagoon (DSRH, 2004), while Melides River basin has a draining area of 65 km^2 (Freitas *et al*., 2002; Costa *et al*., 2003). These lagoons are separated from the Atlantic Ocean by sand barriers but occasionally sea water flows over the dunes and once a year (March/April) – St. André, or several times Melides – the lagoons are artificially opened. This procedure assures colonization by marine species during regular communication periods between the lagoons and the sea.

Socio-economic context

Historically, fishing and agriculture are the most important activities that took place in the surrounding areas of the Santo André and Melides watersheds, held for more than two centuries (Silveira *et al*., 2006). Nowadays, these two activities are still of high social and economic importance. During the last years fishing is the main economic activity in Santo André Lagoon; meanwhile, and in spite of some fishing activity locally important, rice production is by far the most profitable activity in the Melides area. At the Melides lagoon, man-made sea openings are established whenever it is necessary to prevent the rising of brackish water and its overwash into the rice fields. Both lagoonal environments are also used for recreational purposes. Several economically important fish species occur in these lagoons. 10-100 tons of fish are harvested annually in Santo André and the eel, *Anguilla anguilla*, was the only important catch during the last years (Bernardo, 1990; Costa *et al*., 2003).

Eel fisheries at Santo André lagoon involve 30-50 fishermen with small rowboats, being the fyke net the main used fishing gear (Silveira *et al*., 2009). Eels' fishing in the lagoon is of remarkable importance, not only regionally but nationally, with local discharges of this species representing more than half the national discharges in some years (Silveira *et al*., 2006). The capture of other species like *Dicentrachus labrax* or *Sparus aurata* also occurs, but to a lesser extent (Bernardo, 1990).

Agriculture is currently essentially of subsistence dominated by small family farms, with only a small number of farmers producing extensive crops and legumes for sale (ICN, 2000). This activity is mostly limited to the surrounding area of the Santo André lagoon, especially in the floodplains of Cascalheira and Badoca streams. Cereals crops (oat) are the major agriculture production outside the floodplains, complemented with pasture. Livestock has some importance in the region with extensive exploitation of cattle, goats and sheep (ICN, 2000). A significant number of pig farms are also present in the Santo André river basin.

Tourism is an important economic activity, with greater importance during summer, although this activity occurs throughout the year. The lagoon is used as a bathing area and also for nautical sports. However this area is most sought after by its natural surroundings, especially for hiking and visitation throughout the year (CEZH / RNLSAS, 2004).

In Melides, the rice production has a high expression, and these crops occupy the entire area surrounding the downstream area of the Melides stream, using its flow during the floodplain flooding period. In 2008, 12 rice producers were registered in the floodplain of the Melides lagoon, with a production of 1050 ton, which corresponds to about 7000 kg / ha, most of the production being sent to 3 milling factories in Alcácer do Sal (Cecílio, SA), Figueira da Foz (Ernesto Morgado) and Oliveira de Azeméis (Valente Marques) (Freitas *et al*., 2008). Apart from this activity, agriculture is mainly for subsistence, being mainly constituted by small family farms. In this watershed, livestock is reduced, with 2 pig farms, 4 cattle farms and 6 sheep farms reported as extensive to semi-intensive systems, in 2006 (Brigada de Fiscalização do Litoral, 2006).

As in Santo André lagoon, tourism is a major economic activity during summer months, mainly because of the beaches, however rural tourism is an additional source of income all year round.

With all these economic and leisure activities, both studied basins have a high importance for the region, not only for local residents but also for tourists who visit this destinations all year. Different type and intensity of human activities affect biological communities in different ways and magnitude. Since the streams drain into the Santo André and Melides lagoons it is also important to know the socio-economic activities directly attached to these latter.

Aims and importance of this study

The growing pressure on our water ecosystems, not only by direct human activities, but also by climate change as led to an increasing concern about water resource and the associated biodiversity loss that is at stake. Studies on freshwater ecosystems have significantly grown in the past few years, and consequently scientific literature focusing on this issue (Oertli *et al.*, 2009). However, in Portugal, scientific data on macroinvertebrate communities and how they respond to anthropogenic and natural stressors is still scarce. Before the WFD implementation process only a few studies on benthic macroinvertebrates communities and their responses to stressors were available (*e.g*., Coimbra *et al.*, 1996). Although a great effort was conducted since 2000, knowledge on aquatic ecosystems and the achievement of good ecological status of Portuguese water bodies until 2015 is still not likely to be accomplished. This thesis was developed in the aim of the project GroundScene (PTDC/AAC-AMB/104639/2008), with the purpose of contributing to the implementation of the WFD.

Thus, the main objectives of this study in the Santo André and Melides River basins are:

1. To understand what are the major environmental variables structuring the benthic macroinvertebrate communities in these streams ;
2. To assess the ecological status of these river basins and evaluate the adequacy of the available assessment tools.

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Chapter II

Environmental conditions that structure benthic macroinvertebrate communities in the streams of Santo André and Melides River basins, Portugal

Environmental conditions that structure benthic macroinvertebrate communities in the streams of Santo André and Melides River basins, Portugal

Abstract: Benthic macroinvertebrate communities and environmental variables of 13 sites of two nearby small Mediterranean river basins, Santo André and Melides, were assessed in one sample occasion in spring using a standardized methodology. A total of 94 taxa, mostly identified to family level, were recorded. The insects predominate in all samples that were mainly characterized by the presence of euribiont groups, such as Chironomidae, Amphipoda and Oligochaeta. Non-insecta and eurihaline taxa increase at downstream locations. Principal Coordinate analysis (PCO) was used to emphasise variation of benthic macroinvertebrate communities and their relationship with environmental variables. These variables, mainly dissolved oxygen, grain-size and organic matter, greatly determine the structure of benthic macroinvertebrate communities. Changes in these variables may favour a higher density of organisms or a higher richness of taxa. These are the first results to the knowledge of these poorly understood stream ecosystems.

Key words: stream, River basin, Mediterranean, benthic macroinvertebrates, insecta.

Introduction

Stream ecology and, in particular, benthic invertebrate communities are mainly influenced by substrate quality and heterogeneity (*e.g.,* Beisel *et al*., 2000), pH, nutrients, oxygen and organic matter concentration (*e.g.,* Alba-Tercedor and Sánchez-Ortega, 1988), but also by hydrological conditions such as water permanence (Bonada *et al.*, 2006) and geomorphology characteristics such as altitude and slope (Feio *et al.*, 2005). Interactions between these multiple factors determine the spatial gradients established in freshwater systems. Nevertheless, anthropogenic pressures can modify these spatial patterns, since human activities might cause significant changes on stream hydrology and physical-chemical characteristics.

Benthic macroinvertebrate are one of the most studied biological communities in running waters, due to their qualities as bioindicators (Rada and Puljas, 2008). The structure of these communities react to a variety of chemical and physical variations, and because benthic macroinvertebrates have an intermediate position on the food web, natural or manmade shifts have consequences on them, and consequently in ecosystem processes.

The high seasonal variability of stream invertebrates occurring in Mediterranean areas is strongly influenced by climatic events. Mediterranean streams have annual and inter-annual flow variability with the occurrence of frequent floods and droughts. This high variability implies a reorganization of macroinvertebrate communities, as the habitat is highly modified, first by the loss of riffles and secondly by the loss of pools (Bonada, 2003), leading to a reduction in the number of taxa (Graça *et al.,* 2004). Knowledge of Mediterranean streams has been improving in recent years (*e.g.,* Bonada *et al*., 2000; Vivas *et al.*, 2002). In Portugal, the study of Mediterranean streams (located in the South), led to the conclusion that the communities of benthic macroinvertebrates contain a lower taxonomic richness than those found in streams of North and Centre (Graça *et al*., 2004). In Southwest Portugal, few studies have been developed which leads to a lack of knowledge of their communities, how they respond to environmental variables and if they correspond to those found in other Mediterranean streams.

Mediterranean streams are physically, chemically, and biologically shaped by sequential, predictable, seasonal events of flooding and drying over an annual cycle (Gasith and Resh, 1999). This annual cycle leads to abundant flow in winter and very low flow in summer, which ultimately can lead to stream drought (Boix *et al*., 2010). The variability of flow is enhanced in small river basins such as Santo André and Melides, since the small length of its streams lead to a rapid response to rainfall (Spruill *et al.*, 2000).

This study aims to present and examine collected data on the benthic macroinvertebrate community's structure of Santo André and Melides River basin streams by determining a) their taxa composition and richness and determine whether they correspond those found in other Mediterranean river; b) if the benthic macroinvertebrate communities of both River basins are similar; and c) investigate the relative contribution of several environmental variables in explaining the observed structure variation.

Methods

Study area

This study took place in two small river basins, Santo André and Melides River basins, included in the *Sado and Mira basins Hydrographic Region* and under the administration of ARH Alentejo (Administration of Alentejo Hydrographic Region). The hydrographic regions were defined in the aim of the Water Framework Directive, which determines that each Member-State must aggregate river basins into specific hydrographic region and assign it to a competent authority (European Commission, 2000). This normative was implemented by the publication of the Portuguese Water Law (Law 58/2005) that establishes hydrographic regions as the planning and water management divisions. Part of the study area is also included in a protected area, *Lagoas de Santo André e da Sancha* Natural Reserve.

A total of 13 locations were selected for this study, 7 located at the Melides river basin and 6 at the Santo André river basin. Four different locations were selected at the Melides stream (RML 1, RML 2, RML 3 and RML 4) and two additional stations were located in the tributaries Samoucal (SAM) and Cabo d'Água (CAG) (Figure 2.1.). In the Santo André river basin, sampling stations were located at Poço dos Caniços, Badoca, Cascalheira and Chaparros streams, these last two with an upstream and a downstream station.The Cascalheira and Badoca streams and Poço dos Caniços are included in the protected area *Lagoas de Santo André e da Sancha* Natural Reserve. The studied streams depend on the top phreatic aquifer, except for Fonte dos Olhos that drains water from the deep carbonate aquifer (Tibor Stigter, *pers. comm.*).

This region is influenced by an atmospheric circulation regime that affects all the coastline of southern Portugal, associated with the proximity to the Atlantic and the Golf Current, and local topographic factors. This is an area characterized by a Mediterranean climate with dry to sub-humid typical weather (Cancela da Fonseca *et al.*, 1993).

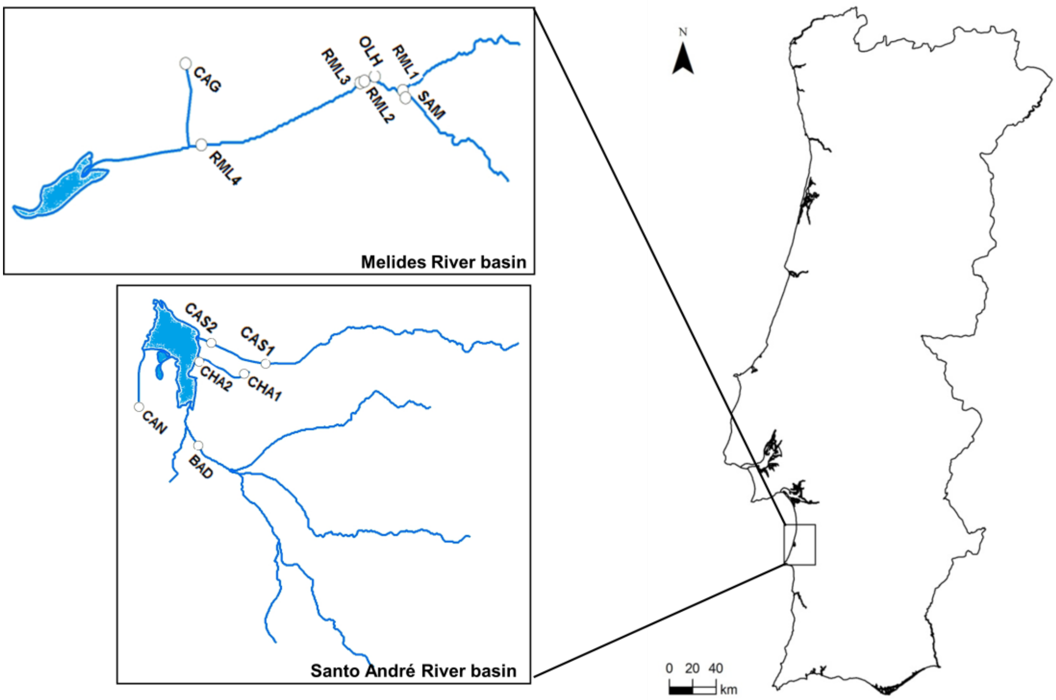

Figure 2.1- Sampling sites selected in the Melides and Santo André river basins, Southwest Portugal: Badoca stream (BAD), Cabo d'Água (CAG), Poço dos Caniços (CANS 1 and CAS 2), Serradinha stream (CHA 1 and CHA 2) Melides stream (RML1, RML 2, RML 3 and RML 4), Fonte dos Olhos stream (OLH) and Samoucal stream (SAM).

Sampling

Sampling took place in spring, May of 2011, as recommended by INAG (2008). Several environmental variables were measured *in situ* with a portable probe (YSI 600XLM): water temperature (WT – °C), conductivity (Cond – mS/cm), dissolved oxygen (DO – mg/L) and total dissolved solids (TDS – g/L).

Current speed (CS – m/s) was measured with a model 105 Valeport current meter. Depth (Dep – m) was also measured. Water samples were collected prior to the macroinvertebrate and sediment sampling to avoid bias the results. Five litres of water were collected at each site, refrigerated and transported to the certified laboratory of ARH Alentejo, for nitrates (NO_3 – mg/L), nitrites (NO_2 – mg/L), phosphorous (P – mg/L), ammonia (NH_4 – mg/L) and chlorophyll *a* (Chl a – µg/L) analysis.

Sediment samples were collected to estimate total organic content (TOC – %) and grain size. TOC was obtained by loss on ignition (480°C during, at least, 12 hours in a muffle) after drying samples in the stove at 60°C for 24 hours (Pereira *et al*., 1997). For sediment grain size, samples dried during 48 hours at 60°C, were sieved through different mesh sizes (2.00 mm, 0.50 mm, 0.25 mm and 0.063 mm) and each resultant fraction weighted.

Macroinvertebrates were collected with a 30x30 cm kick-net, with a 250 µm mesh size, used to kick/sweep representatively major habitat types identified at each sampling site during 60 seconds. The overture of the net was placed in the upstream direction to ensure that the organisms displaced by the river flow when removing sediments were drawn into the net. The same operator collected all macroinvertebrate samples, in order to minimize the sampling variability. Samples were fixed in 70% ethanol, transported to the laboratory, where they were rinsed using a sieve of 500 µm mesh size. Each sample was sorted in order to separate all individuals; these were counted and identified to the family level using a binocular stereomicroscope, with some exceptions (*eg*., Oligochaeta, Nematoda, Ostracoda), according to Tachet *et al*. (2000).

Data analysis

Mean abundance by sampling time (individuals/60’) was calculated for each sampling site. A Hierarchical Cluster Analysis was conducted to identify groups of stations, based on the macroinvertebrate abundance data. Data was previously log(x + 1) transformed to reduce the influence of the most abundant species over the less represented. Resemblance between samples was based on the Bray-Curtis similarity coefficient and the group-average linkage method was used to group observations. A similarity level of 40% was used to identify groups of stations. A non-parametric test, ANOSIM (Analysis of Similarities) was performed to determine if groups of stations identified with the Cluster analysis were significantly different ($p<0,01$). A SIMPER (Similarity Percentage Breakdown Procedure) analysis was used to determine the similarity level within and between groups and identify which taxa contributed most to those similarities/dissimilarities. An n-MDS (non-metric Multi-Dimensional Scaling - MDS) ordination technique was also conducted on presence/absence data of invertebrate communities, to identify spatial taxonomic patterns based on the Bray–Curtis similarity coefficient. Different symbols were used to identify stations located at Melides and Santo André River basins and an ANOSIM test was conducted to determine if there were significant taxonomic differences between river basins ($p<0,01$).

A Principal Coordinates analysis (PCO was conducted on $\log(x + 1)$ transformed abundance data of macroinvertebrate communities), using Bray-Curtis similarity as a resemblance measure. Spearman correlations of the environmental variables with the PCO axes were calculated, to understand the major environmental variables structuring the benthic macroinvertebrate communities. Those variables were represented in the PCO ordination as vectors. A similar procedure was conducted on presence/absence data, to understand the relation between environmental variables and the taxonomic patterns. Add-on). All procedures were performed using the PERMANOVA + PRIMER 6 software package (Clarke and Gorley, 2006).

Prior to this analysis, grain-size data was processed in the program GRADISTAT (Blott and Pye, 2001), a grain size distribution and statistics package for the analysis of unconsolidated sediments. Mean grain-sizes were logarithmically calculated in phi (Φ) units, where $\Phi=-\log_2 d$, and *d* the grain diameter in millimetres (mm), using the Folk and Ward graphical method (1957). The phi scale is widely used instead of the millimetre scale, since it follows a normal distribution and is more appropriate to statistic analyses (Blott and Pye, 2001). The use of this notation implies the use of both positive and negative values, since particles larger than 2mm have negative phi units. Thus, more negative phi values are associated to coarser sediment.

Results

In the present study a total of 29959 macroinvertebrate specimens were collected, with a density of 1352,513 ind/60’ (804,905 ind/60’ in Melides River basin and 1991,389 ind/60’ in Santo André River basin). A total of 94 different taxa were identified (see appendix), including 11 major taxonomical groups (Mollusca: Bivalvia, Gastropoda; Annelida: Oligochaeta; Arthropoda: Crustacea – Amphipoda, Insecta – Coleoptera, Diptera, Ephemeroptera, Hemiptera-Heteroptera, Odonata, Plecoptera, Tricoptera – Figure 2.2), 52 found in Santo André and 86 in Melides. Diptera was the most represented group, due to a predominant occurrence of chironomids through all sampling sites, particularly in CAN (Figure 2.3), followed by Amphipoda and Oligochaeta. Insects are dominant in these streams, even though non-insect organisms, such as Amphipoda, Oligochaeta or Gastropoda, are predominant in several sites such as RML 4, CAS 2, OLH and CHA 2. The class Gastropoda has a greater relevance in the Melides river basin (with 112,75 ind/60') when compared to the density found in Santo André (0,33 ind/60').

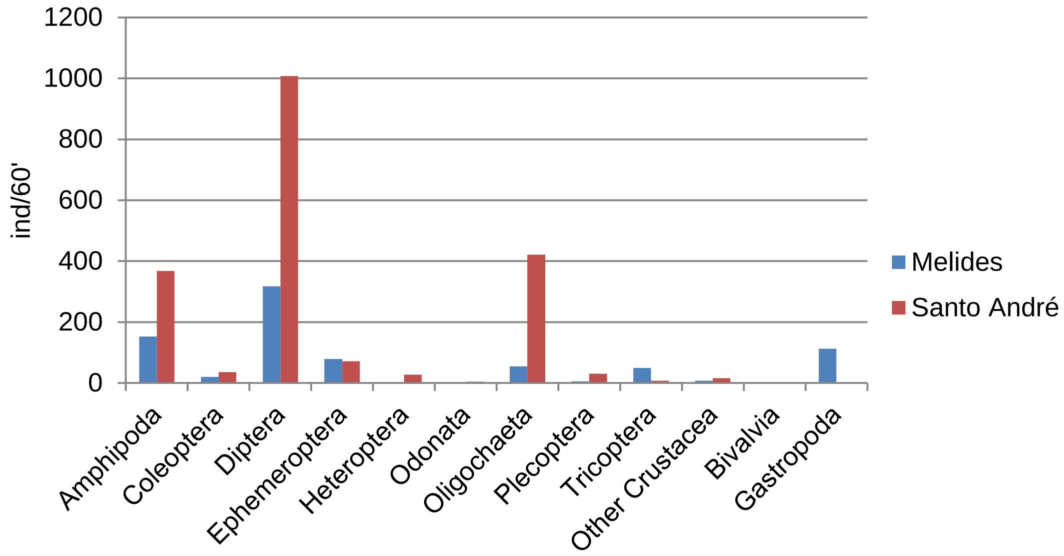

Figure 2.2 – Total amount of specimens of the different major groups in Santo André and Melides River basins.

The cluster analysis of the benthic macroinvertebrates indicated two major groups of stations, at a similarity level of 40%, namely group A (OLH, RML 2, CHA 1, CHA 2) and group B (CAS 2, BAD, CAG, RML 1, SAM, CAS 1, RML 3, RML 4), with location CAN clearly separated from all other stations (Figure 2.4). The ANOSIM test showed that groups A and B were significantly different (R=0,469; p<0,01). The SIMPER analysis showed an average similarity of 46,94% within group A and indicated that Gammaridae (24,55%), Oligochaeta (22,25%) and Chironomidae (21,59%) gave the highest contributions). Chironomidae (19,91%) also plays an important role in the resemblance within group B, followed by families Baetidae (16,62%) and Simuliidae (10,89%) (with an average similarity of 44,65%) (Figure 2.5). These groups exhibit a level of dissimilarity of 62,91%, with Baetidae (6,13%), Gammaridae (5,55%) and Simuliidae (5,10%) as the taxa that contributed more for this dissimilarity.

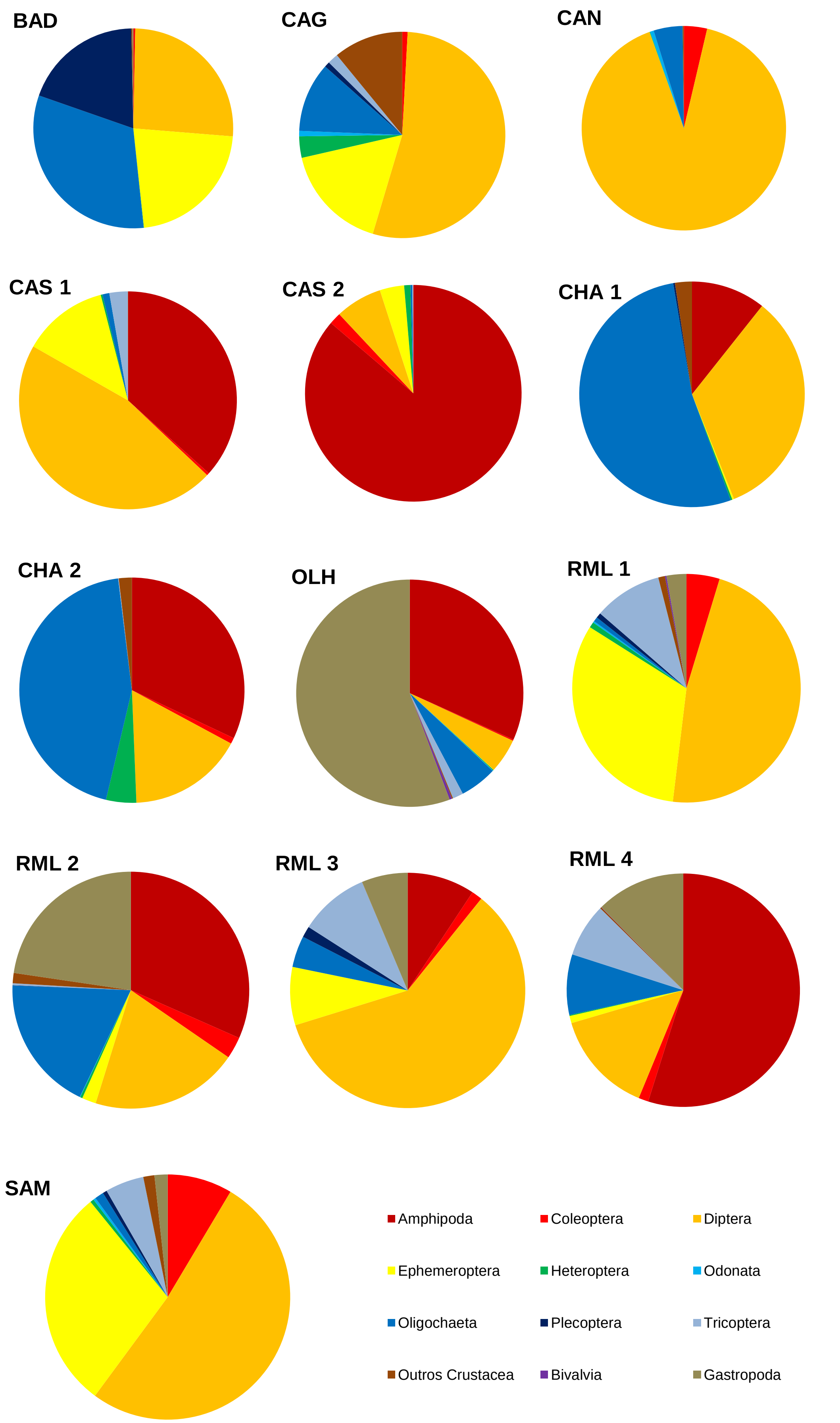

Figure 2.3 – Abundance of the major faunistic groups for each of the 13 sampling stations.

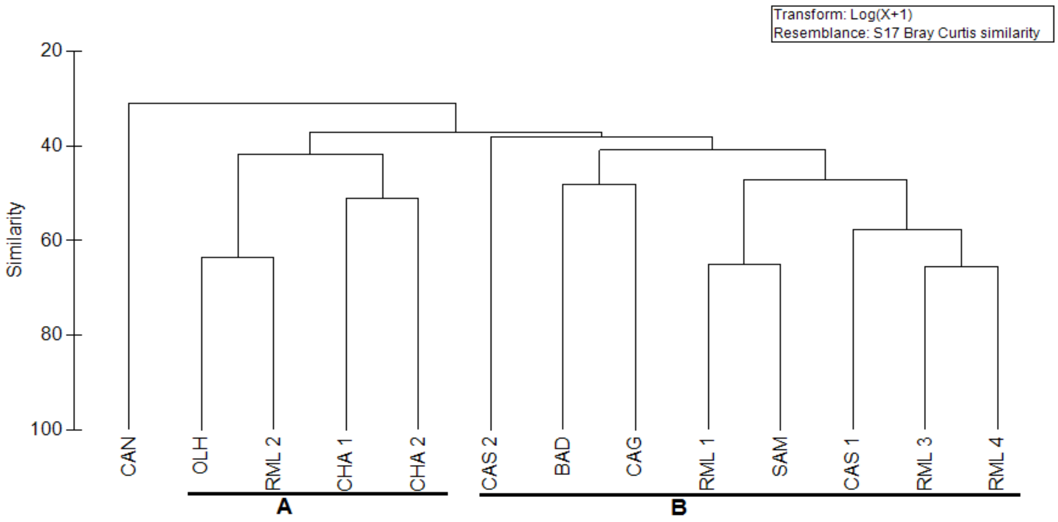

Figure 2.4 – Cluster analysis of the abundance of benthic macroinvertebrate communities of the Melides and Santo André river basins. Two groups of stations A and B, are indicated.

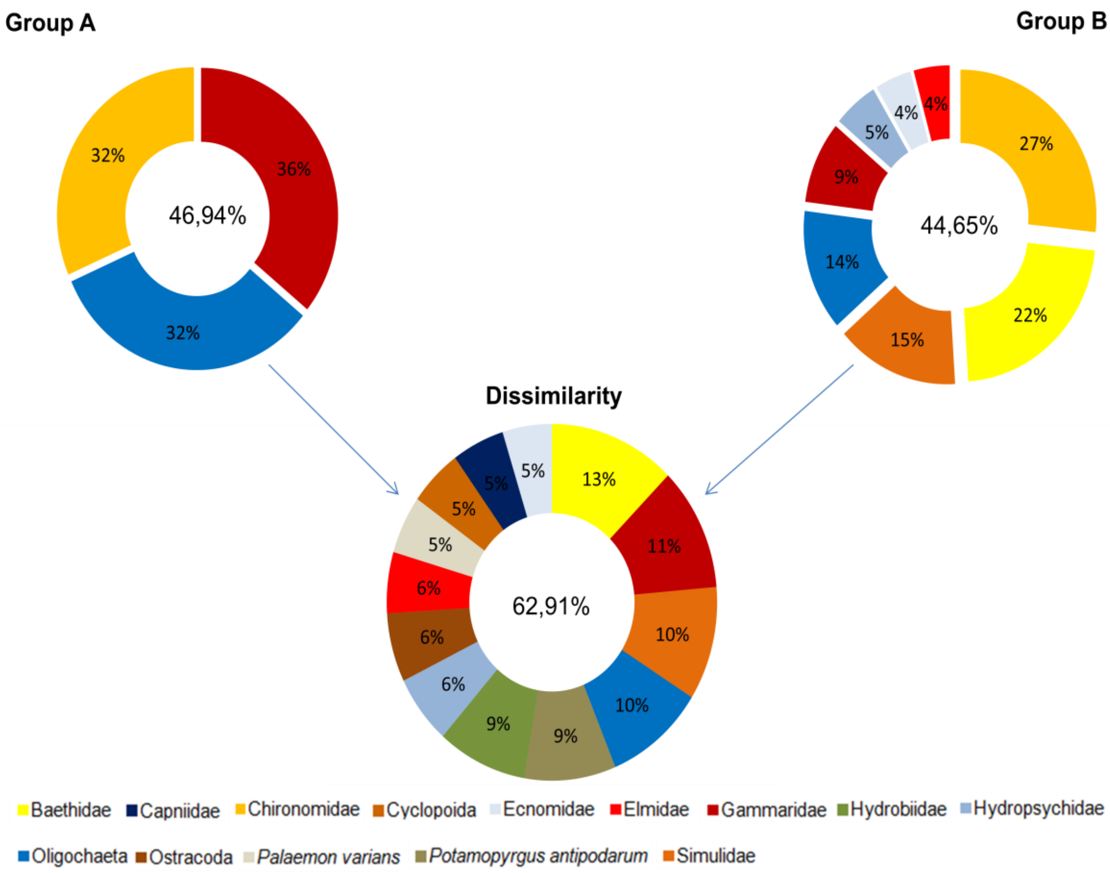

Figure 2.5 – Pie plots with the similarity within each group (A and B) and the average dissimilarity between groups indicated inside. Taxa with higher contributions to the similarity within and the dissimilarity between groups are indicated.

MDS ordination of the presence/absence dataset showed a separation of the benthic composition between river basins (Figure 2.6), with a stress of 0,18 – stress values greater than 0,2 indicate that the plotting is close to random, stress values lower than 0,2 indicate a useful two-dimensional image and less than 0,1 corresponds to an ideal ordination (Clarke, 1993). There are significant differences between river basins, as indicated by the ANOSIM test (R=0,485; p<0,01).

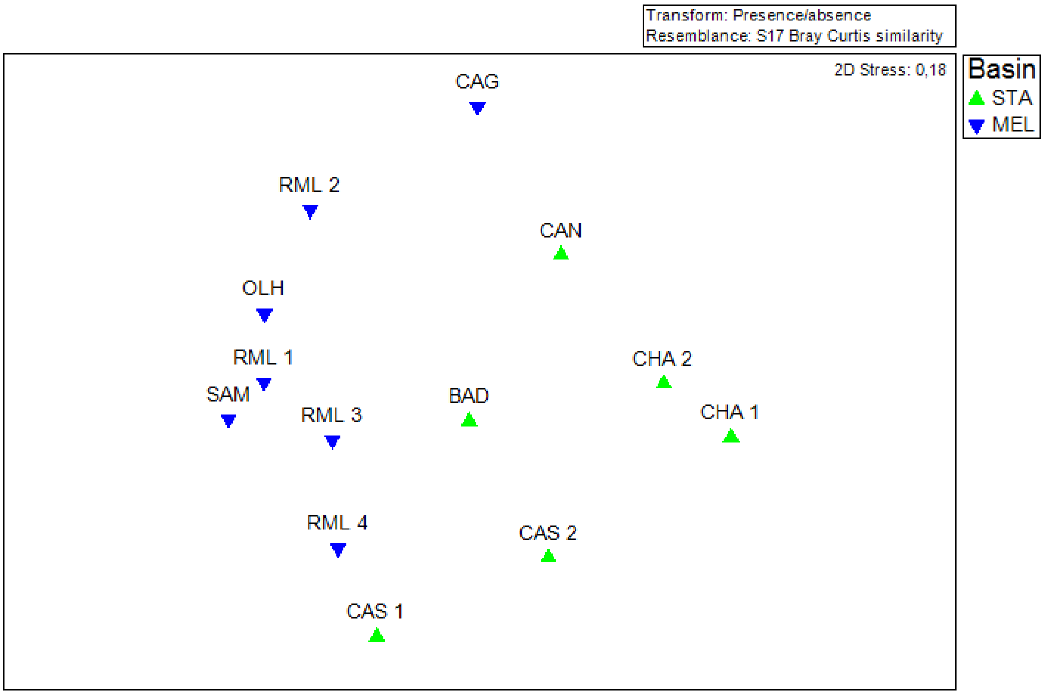

Figure 2.6 – MDS ordination of the similarity between sampling stations, based on the presence/absence dataset. Abbreviations: STA – sampling sites in Santo André River basin; MEL – sampling sites in Melides River basin.

Environmental variables are presented in Table 2.1. CAN sampling site registered higher values in six of the thirteen measured variables (TOC, WT, Con, TDS, Chl *a* and P). The same stream had the coarser and finer sediment, in the sites CAS 1 and CAS 2, respectively. Besides CAN, CHA 2 also had the higher value of total organic content. Higher concentrations of dissolved oxygen occurred in CAG and RML 1 sites. The depth of all sites is relatively low, ranging between 0,20m and 1m. Lentic environments with a flow so low that was unmeasurable were noticed in CAN, CHA 1, CHA 2 and RML 1. Greater concentrations of nitrates and nitrites were found in BAD stream.

The first two axes of the PCO conducted on density data accounted for 45,3% of variance (Figure 2.7). Correlations of environmental variables with the first axis suggest dissolved oxygen (0,59), total organic content (0,51) and sediment grain-size (-0,47) as the variables most related to the benthic macroinvertebrate community patterns. Higher concentrations of dissolved oxygen are more related with the sampling sites of group B, and higher concentration of organic matter and finer sediments with group A. The relationship between the presence/absence of taxa and the environmental variables

(Figure 2.8 - the first two axes explain 43% of the variability) emphasize sediment grain-size and chlorophyll *a* as the most correlated with the first axis (-0,49 and -0,62, respectively). In general, finer sediments characterize Santo André sampling sites while Melides sampling sites are characterized by coarser sediments.

Table 2.1 - Results of the environmental variables measured at each sampling station. Abbreviations and units: GS – grain-size (Φ); TOC – total organic content (%); WT – water temperature (°C); DO – dissolved oxygen (mg/L); Con – conductivity (mS/cm); Dep – depth (m); TDS – total dissolved solids(g/L); CS – current speed (m/s); Chl *a* – chlorophyll *a* (µg/L); NH_4 – ammonia (mg/L); NO_2 – nitrites (mg/L); NO_3 – nitrates (mg/L); P- phosphorous (mg/L).

Sites	GS	TOC	WT	DO	Con	Dep	TDS	CS	Chl *a*	NH_4	NO_2	NO_3	P
BAD	0,28	0,02	22,38	6,66	0,89	0,70	-	0,33	3,60	0,04	0,31	15,00	0,23
CAG	1,52	0,01	19,40	13,20	0,18	1,00	-	0,08	2,40	0,04	0,03	3,00	0,03
CAN	0,59	0,15	29,20	7,16	9,92	0,20	5,96	0	71,00	0,04	0,03	2,00	0,32
CAS 1	-1,00	0,02	23,43	9,67	0,70	0,40	0,47	0,07	2,40	0,04	0,03	4,50	0,05
CAS 2	2,08	0,01	21,85	8,94	0,63	0,40	0,43	0,13	-	0,04	-	-	-
CHA 1	2,00	0,09	17,54	2,36	0,37	0,40	0,28	0	11,00	0,04	0,04	2,00	0,09
CHA 2	0,72	0,15	23,09	8,14	0,72	0,20	0,49	0	12,00	0,08	0,06	9,20	0,07
OLH	1,40	0,01	19,19	6,05	0,51	1,00	0,37	0,16	0	0,04	0,03	12,00	0,08
RML 1	-0,05	0,03	24,08	10,09	0,64	0,40	0,42	0	0,40	0,04	0,03	2,00	0,15
RML 2	-0,59	0,02	20,54	5,08	0,56	1,00	0,40	0,05	0	0,04	0,11	8,00	0,24
RML 3	-0,51	0,02	20,55	6,42	0,55	0,30	-	0,17	1,90	0,04	0,14	8,10	0,23
RML 4	-0,44	0,01	25,27	7,90	0,59	0,30	0,37	0,08	0,70	0,04	0,09	6,20	0,08
SAM	0,22	0,03	22,26	9,90	0,58	0,40	0,40	0,10	0,20	0,04	0,03	2,00	0,08

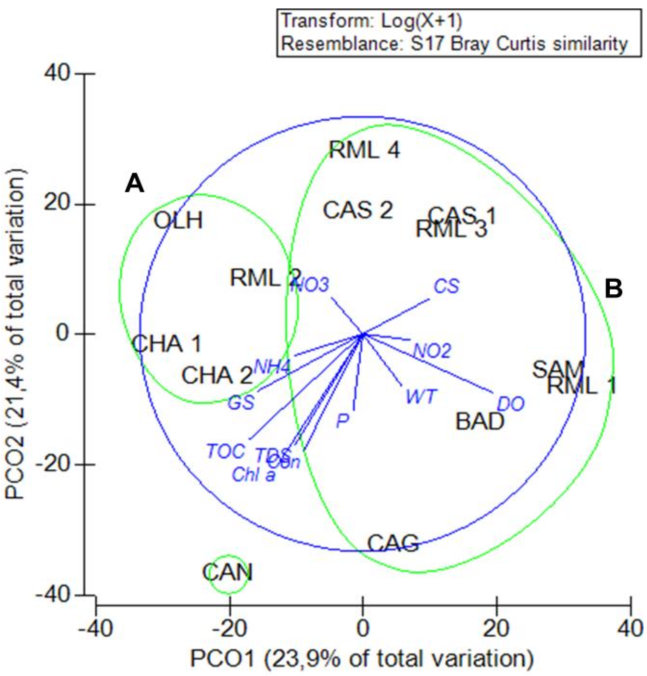

Figure 2.7 - Diagram of a Principal Coordinates analysis of the influence of environmental variables, as vectors, in the grouping of benthic communities. Abbreviations and units: GS – grain-size (Φ); TOC – total organic content (%); WT – water temperature (°C); DO – dissolved oxygen (mg/L); Con – conductivity (mS/cm); Dep – depth (m); TDS – total dissolved solids(g/L); CS – current speed (m/s); Chl *a* – chlorophyll *a* (µg/L); NH_4 – ammonia (mg/L); NO_2 – nitrites (mg/L); NO_3 – nitrates (mg/L); P- phosphorous (mg/L).

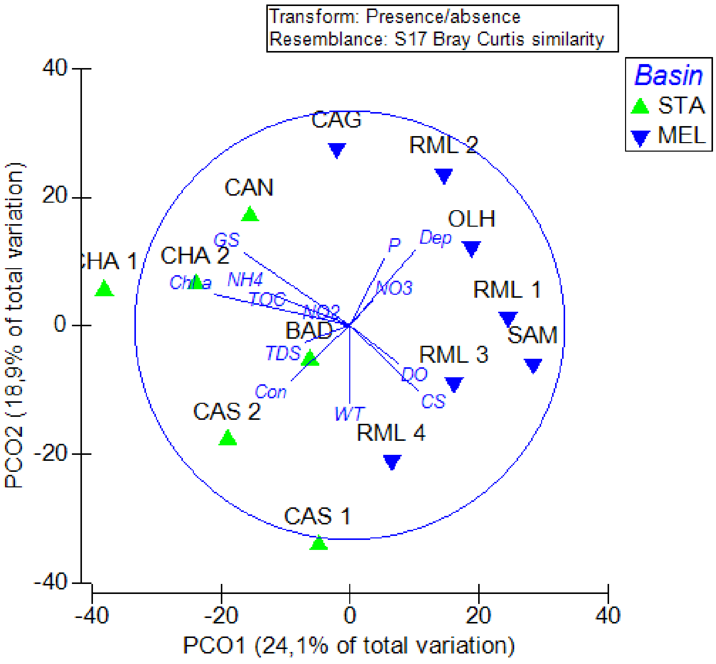

Figure 2.8 - Diagram of a Principal Coordinates analysis of the influence of environmental variables, as vectors, in the presence/absence of taxa in the grouping of each basin. Abbreviations and units: GS – grain-size (Φ); TOC – total organic content (%); WT – water temperature (°C); DO – dissolved oxygen (mg/L); Con – conductivity (mS/cm); Dep – depth (m); TDS – total dissolved solids(g/L); CS – current speed (m/s); Chl *a* – chlorophyll *a* (µg/L); NH_4 – ammonia (mg/L); NO_2 – nitrites (mg/L); NO_3 – nitrates (mg/L); P- phosphorous (mg/L).

Discussion

Patterns of macroinvertebrate communities are expected to be best explained by a combination of numerous environmental variables, although sometimes single variables might explain a major part of the observed variation (Wiberg-Larsen *et al.*, 2000). The properties of a habitat within an aquatic ecosystem are assumed to determine the types of macroinvertebrate communities (Bailey *et al.*, 2004). Insects, amphipods (Crustacea), oligochaetes (Annelida) and molluscs were the dominant groups in the study area, similarly to what was found in other stream ecosystems (*eg.* Vivas *et al*., 2002; Acuña*et al*., 2005; Maiolini *et al*., 2006). This higher proportion is due to the fact that this class is characterized as highly dispersive (Vinson and Hawkins, 1998) and in case of disturbances the recolonization from some other near-by stream, in the same catchment or from beyond that, will normally repair damage in a few insect generations' time (Zwick, 1992).

Ephemeroptera (mayflies), Plecoptera (stoneflies), Odonata (dragonflies), Trichoptera (caddisflies), Coleoptera (riffle beetles), and Diptera (true flies) were the orders of Insecta found. As documented in other Mediterranean streams, the dipterans were the most abundant group, mostly represented by chironomids (Chironomidae) that were present in all sampling sites (*e.g.,* Coimbra *et al*., 1996; Bonada *et al*., 2000; Vivas *et al*., 2002; Acuña *et al*., 2005; Chaves *et al*., 2008). In Mediterranean streams, other authors registered a tendency of Diptera predominance followed by Ephemeroptera (*eg.* Coimbra *et al.,* 1996; Pires *et al*., 2000; Vivas *et al*., 2002) which is not observed in these streams, since the Gammaridae are the second dominant group. This can be explained by the location of these two river basins in a low land area, close to the coast, with much less areas with a lotic regime, that is preferred by the majority of the Ephemeroptera (Gasith and Resh, 1999). The dominance of Gammaridae over other groups occurs mainly in RML 4, CAS 2 and CHA 2 sampling sites that are downstream locations. Here, one of the common found taxa is *Gammarus chevreuxi* a highly mobile species that can be found in brackish and freshwater (Subida *et al*., 2009). As it is well known (Maitland, 1966; Mancinelli, 2012), movements between the lagoons and the streams can be considered a possibility for this group of crustaceans. Also, Santo André and Melides lagoons are supplied by two aquifers, one shallow and other deep and calcareous, which may enhance the occurrence of Gammaridae, known to occur in calcareous waters.

Although chironomids occur throughout all locations, the density found in CAN was extremely high when compared to all other sampling sites (4069,33 ind/60’). Chironomids are pioneer species, ubiquitous, highly mobile and resistant to disturbances (Acuña *et al*., 2005). They are known to be little affected by environmental changes and promptly recolonize following disturbance (Pires *et al*., 2000). This is in agreement with the highly stressful environment noticed in CAN sampling site, with an intermittent regime, strongly affected by salinity variations due to freshwater flooding during a period of the year and brackish water flushing events related to the Santo André lagoon level rise in other periods. High values of conductivity, temperature, dissolved solids and chlorophyll *a* were registered, which are only sustained by the most tolerant (and generalist) species, leading to a low diversity.

Besides CAN, other conspicuous sampling site is OLH, an artificialized spring with a high density of *Potamopyrgus antipodarum* (Mollusca, Gastropoda). This species is found in estuaries, brackish waters and in freshwater systems (Robson, 1923; Boycott, 1936); initially exclusive of estuarine and brackish waters, it is known to be an invader in freshwater ecosystems (Boycott, 1936; Hunter and Warwick, 1957). It is extremely active, travelling at speeds up to 3 cm/min (Heywood and Edwards, 1962), prefers sandy bottoms (Dorgelo, 1988), constant temperatures and flow (Richards *et al.,* 2001) and has a wide tolerance to nutrient enrichment (Alonso and Camargo, 2003). This is consistent with the characteristics and concentration of nitrates found in this sampling site. Múrria *et al*. (2008) found a negative relation between *P. antipodarum* and Chironomidae, which may be caused by the movement of this mollusc that displaces chironomids from their tubes. This can be an explanation for the lower density of chironomids in this site (as generally occurs in Mediterranean streams).

In general, non-insect taxa were found with a large density in RML 4, CAS 2, CHA 2, and OLH. This suggests that these locations have a lower probability of extreme events, such as droughts. In fact, drought effects are linked to intrinsic characteristics of organisms, such as the ability to take refuge, high colonization rates and delayed hatching of drought-resistant eggs (Delucchi, 1988). Locations characterized by intermittency have a higher percentage of insects since colonization with flying taxa is faster and simpler, because their adult forms are not aquatic. As stated by Meyer *et al*. (2003), stream drying requires a high tolerance or specific adaptations of the aquatic fauna. Organisms with a full aquatic life-cycle are not able to colonize these locations, since the migration is more difficult and time consuming, and one drought event can put in jeopardy the whole community.

Two different groupings were identified when using macroinvertebrate densities and presence/absence, indicating that although the Melides and Santo André river basins have distinct taxonomic compositions, the dominant taxa show the influence of other major habitats characteristics. Groups identified based on the taxa density of individuals and their respective taxa, indicate a separation between lotic habitats (group A) and lentic locations and with an interface with groundwater (group B). While the first group is characterized by the dominance of Gammaridae, Oligochaeta and Chironomidae (21,59%), group B has Chironomidae, Baetidae and Simuliidae as major common taxa.

The PCO results show that higher concentration of dissolved oxygen, lower concentrations of total organic content, and coarser grain-size are associated to the stations of group B. The opposite is characteristic of group A. A lower velocity flow decreases the transport capacity of both inorganic and organic matter, which leads to deposition. As these are small streams with riparian gallery, although there is no transport of organic material in these locations due to the reduced flow and the reduced autotrophic productivity outstanding the shading caused by such gallery, they contribute with a significant amount of organic matter, considered therefore allochthonous (Vannote*, et al*.,1980; Allan and Castillo, 2007). The decomposition of that organic matter leads to an increased biochemical oxygen demand, due to direct chemical reactions and aerobic respiration, with consequent decreasing of dissolved oxygen (Brabec *et al*., 2004; Allan and Castillo, 2007). Though OLH sampling site (group A) does not have a low current speed, it has a groundwater interface. Groundwater frequently has very low concentrations of dissolved oxygen, but an enriched concentration of carbon dioxide due to microbial processing of organic matter as water passes through soil (Allan and Castillo, 2007). CAN sampling site with an elevated food availability and benthic macroinvertebrate density, but low taxa richness is an example of what was pointed out by Chaves *et al*. (2005) in streams of the Mondego River basin, in which invertebrate densities were related with food availability (organic matter), while taxa richness was highly associated with hydraulic-related parameters controlled by climatic conditions.

Santo André river basin has a higher density of benthic macroinvertebrates, but a lower taxonomic richness when compared to Melides river basin. This suggests that Santo André River basin communities are mostly based on generalist and opportunistic groups of organisms (Dauer *et al.*, 1992). Further, analyses show that Melides taxa are constrained by coarser sediments. As pointed out by several authors, less diverse stations were related to finer sediment grain size, especially sands, since size and mobility of their particles constrain benthic communities to species capable of penetrating the substrate interstices (e.g. Beisel *et al*., 1998; Pinto and Feio, 2009; Duan *et al*., 2009). Habitats characterized by fine sediment are more homogeneous, which implies less habitat diversity and removes much of its potential to accommodate a wider range of species. (Vannote *et al*., 1980; Beisel *et al*., 1998).

Conclusions

The structure of the communities found in the present study streams is different from others recorded in different mediterranean streams. The sampling locations are in general, similar in density of the dominant taxa, with a large number of specimens of euribionts, tolerant to a large variety of conditions, ubiquitous and pioneers, mostly from Chironomidae family of the Diptera order. The sampling sites located downstream had a larger percentage of non-insecta with the occurrence of eurihaline families, which migrate between the streams and the respective lagoons. Overall, the Melides River basin holds a greater taxonomic richness than the Santo André River basin.

The results obtained show that abiotic factors greatly influence the structure of benthic macroinvertebrate communities, with dissolved oxygen, grain-size and organic matter constraining these benthic macroinvertebrate communities.

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Chapter III

Adequacy of benthic indices to assess the ecological status of small Mediterranean river basins

Adequacy of benthic indices to assess the ecological status of small Mediterranean river basins

Abstract: The European Water Framework Directive (WFD) requires the achievement of a “good” ecological status of water bodies giving great importance to biological components of the ecosystems. Within this framework, a multimetric index for the evaluation of ecological quality based on benthic macroinvertebrate communities (Portuguese multimetric index of the South, $IPtI_S$) was applied in Santo André and Melides River basins. As part of an integrative approach physical-chemical, riparian and fluvial habitat qualities were also took into account. It was found that all but three of the thirteen sampling sites achieved the goal of the WFD. However, it was also found that this index has low responsivity in lentic environments or with groundwater sources what may give rise to an underestimation of the ecological quality in these ecosystems.

Key words: $IPtI_S$, macroinvertebrates, ecological quality, stressors.

Introduction

Freshwater habitats can be considered hotspots of biodiversity since they provide goods and services of great value to human societies in matters of economy, culture, leisure, aesthetics, science and education. However, they are also under several human pressures due to their use for irrigation, waste disposal, harvest of plants, industries, farming, among others. These activities have negative effects, such as loss of biodiversity, habitat degradation and fragmentation, flow modification and invasion of non-native species. Variations in environmental conditions such as fluctuations in temperature, precipitation and nutrients concentrations also cause important changes in freshwater ecosystems.

Setting environmental objectives for aquatic systems has become a worldwide concern and the Water Framework Directive was published in the European Union (2000/60/CE) with the objective of protecting and improving the waterbodies’ status, not only for human purposes but for maintaining the integrity of these ecosystems “*per se”*. The WFD sets the achievement of good ecological status and good chemical status for surface waters by 2015 as its major objectives. The ecological status is understood as the expression of the structural and functional quality of aquatic ecosystems associated to surface waters (European Commission, 2000). The WFD gives great importance to the biological components of the ecosystem, recognizing that chemical water quality alone is inadequate to predict or evaluate the overall environment quality or the potential impacts of forcing factors at the community and ecosystem levels (Fano *et al*., 2003; Mistri *et al*., 2008). Hence, a chemical approach is associated to an ecological one, which has aquatic communities such as benthic macroinvertebrates as resource. The assessment of ecological status requires the development of adequate tools, based on the identification of surface water types, the definition of type-specific reference conditions, and the classification of all water bodies within five ecological quality classes, ranging from high to bad (European Commission, 2000).

In the case of rivers, the recommended biological elements for ecological status classification are: composition, abundance and age distribution of fish fauna; composition and abundance of aquatic flora and benthic macroinvertebrates (European Comission, 2000). Water types were defined in order to define adequate reference conditions for areas with similar characteristics, so that ecological status classifications are comparable between rivers. In Portugal, the identification of river types was based on the following factors: altitude, size of catchment area, latitude, longitude, geology, runoff, slope, medium flow, average annual precipitation, coefficient of variation of precipitation, annual mean temperature and amplitude (INAG, 2008).

Benthic macroinvertebrates are widely used as ecological indicators and several metrics are commonly used in Europe, such as: eveness (J), richness, the Shannon-Weiner diversity (H'), the Belgium Biotic Index (BBI), the Extended Biotic Index (EBI), the Danish Stream Fauna Index (DSFI), the Biological Monitoring Working Party (BMWP) andthe Average Score per Taxa (ASPT) (Morais *et al*., 2004). The BMWP and ASPT, were later adapted to the Iberian Peninsula resulting in the indices IBMWP and IASPT (Alba-Tercedor *et al*., 2002). Shannon-Weiner index, eveness and richness are diversity metrics, while BBI, EBI, DSFI, (I)BMWP and (I)ASPT are based on the tolerance or sensitiveness of taxa to pollution. These later score each taxonomic group, based on the existing knowledge concerning their tolerance to pollution. No specific assessment tools were developed to assess the ecological status based on benthic macroinvertebrates in Portugal before the implementation of the WFD. Two multimetric indices were recently proposed, one applied to the majority of the river types of the Northern region and the otheradapted for rivers and streams of the Souther region, the Portuguese multimetric index of the North (*Índice Português de Invertebrados do Norte*), $IPtI_N$, and the Portuguese multimetric index of the South (*Índice Português de Invertebrados do Sul*), $IPtI_S$, respectively (INAG, 2009). Metrics that integrate both indices (widely used in Europe) allow determining the composition and abundance of benthic macroinvertebrate taxa, describe gradients of degradation and discriminate quality classes. The recent creation of these indices has not yet allowed their wide use unlike other European indices (*e.g.,* IBMWP).These indices have been improved over time to better translate ecological quality of the sampled sites. The validation of these new indices requires testing its applicability to different river and habitat types, in order to assess their accuracy to discriminate between disturbed and undisturbed systems and to improve its performance.

This study has two main objectives:

a) Assess the ecological quality of the sampling sites;
b) Evaluate the adequacy of the applied tools.

Methodology

Sampling

This study was performed in streams of Santo André and Melides River basins belonging to the type Small lowland streams of southern Portugal (S1; <=100). The sampling methods were the same as indicated in Chapter II.

Data analysis

The Portuguese multimetric index of the South ($IPtI_S$) was used to assess the ecological status at each sampling site. This index was calculated using the software AMIIB@, avalilable at the Portuguese Water Institute website. $IPtI_S$ is a multimetric index that includes other indices previously developed that integrate information on sensitive/tolerant taxa, such as the IBMWP (Iberian Bio-Monitoring Working Party) and the IASPT (Index of Average Score per Taxon). IPtIs is calculated as indicated in Table 3.1.

Table 3.1 – Description of the calculation of $IPtI_S$ and included metrics (INAG, 2009).

Index/Metrics	**Description**
$IPtI_S$	Nº taxa x 0,2 + EPT x 0,2 + (IASPT – 2) x 0,4 + log (Sel. EPTCD + 1) x 0,2
Number of taxa	Total number of existent taxa
EPT	Number of families belonging to the orders Ephemeroptera, Plecoptera and Trichoptera
IASPT	IBMWP divided by the number of taxa punctuated by IBMWP
Log (Sel. EPTCD + 1)	Log10 of 1 plus the sum of the abundances of the families Chloroperlidae, Nemouridae, Leuctridae, Leptophlebiidae, Ephemerellidae, Philopotamidae, Limnephilidae, Psychomyiidae, Sericostomatidae, Elmidae, Dryopidae and Athericidae

The final value of the index results from the sum of the weighted metrics. Two normalization steps are performed: i) each metric is multiplied by the reference value (Tables 3.2); ii) after the quotient between the value obtained and the reference value of this type of river (median of the reference condition, 0,99) will be determined (Table 3.3). This normalization steps aim at obtaining a final value expressed as Ecological Quality Ratios (EQR). The definition of the environmental status from the value of EQR is carried out under a set of five classes as in IBMWP and have five colors to distinguish the level of disturbance of each sample site (Table 3.4). The Shannon-Weiner diversity and eveness were also calculated using the same software in order to perceive if diversity is a factor influencing the biotic index. A Hierarchical Cluster Analysis was performed to understand which metrics were more correlated to the final result of the biotic index, based on a resemblance matrix calculated using Spearman rank correlations between metrics.

Table 3.2 - Reference values of the metrics used in $IPtI_S$, according to INAG (2009).

Metrics	Reference values
EPT taxa	10.00
Number of taxa	27.00
IASPT-2	3.29
Log (Sel EPTCD+1)	2.48

Table 3.3 – Reference value of $IPtI_S$ and the thresholds between the different classes of ecological status of this index (INAG, 2009).

Reference values	
$IPtI_S$	0,99
High/Good (EQR)	0.95
Good/Moderate (EQR)	0.70
Moderate/Poor (EQR)	0.47
Poor/Bad (EQR)	0.23

Table 3.4 - Ecological quality classes of $IPtI_S$ and EQR limits, according to INAG (2009).

$IPtI_S$		
Class	**Ecological Quality**	**EQR limits**
I	High	>0,95
II	Good	0,70 < *x* < 0,95
III	Moderate	0,47 < *x* < 0,70
IV	Poor	0,23 < *x* < 0,47
V	Bad	< 0,23

For the purpose of achieving an integrated approach (as demanded by the WFD), several physical and chemical parameters were measured at each site. The riparian vegetation quality (QBR index) and the habitat diversity (IHF index) (Jáimez-Cuéllar *et al*., 2004) were also determined. The water quality based on physical-chemical parameters was achieved according to INAG (2009) for the parameters: dissolved oxygen (O_2), oxygen saturation rate, ammonium (NH_4), nitrates (NO_3) and total phosphorus (P). This ecological status is obtained by the principle "one out all out" with only two categories for the chemical status: Good and Bad (the threshold for the good status is shown in Table 3.5).

The QBR index assesses the degree of naturalness of the channel, geomorphology and the riparian vegetation cover: degree, structure and quality. This index scores highly disturbed sites with values near 0 and natural sites with 100 (Table 3.6).

The IHF index evaluates the heterogeneity of the habitat through its physical characteristics: hydrological conditions, substrate composition, shading and aquatic vegetation. As the QBR index this index scores between 0 and 100, which indicates better quality for values near 100 (Table 3.7).

Table 3.5 - Threshold for good status based on general physical-chemical parameters (adapted from INAG, 2009).

Parameter	Boundary
Dissolved oxygen	≥ 5 mg/L
Oxygen saturation rate	60% - 120%
NH_4	≤ 1 mg/L
NO_3	≤ 25 mg/L
P	≤ 0,13 mg/L

Table 3.6 - Ranges of the QBR index quality classes (Jáimez-Cuéllar *et al*., 2004).

Quality level	QBR
Undisturbed riparian forest, very good quality, natural state	≥ 95
Riparian forest with some alterations, good quality.	75-90
Beginning of significant alterations, intermediate quality	55-70
Strong alteration, poor quality	30-50
Extreme degradation, bad quality	≤ 25

Table 3.7 – Ranges of the IHF quality clases (Prat *el al*., 2012).

Quality level	**IHF**
Habitat well built. Excellent for the development of macroinvertebrate communities. Biological indices can be applied without restrictions.	> 60
Habitat that can support a good macroinvertebrate community but in which, by natural causes (*e.g.,* floods) or man-made, some elements are not well represented. Biological indices should not be lower, but it is possible an effect on them.	40-60
Habitat impoverished. Possibility of obtaining low values of biological indices for problems with habitat and water quality. The biological data interpretation should be made cautiously.	<40

A Principal Coordinates analysis (PCO) included in the Add-on PERMANOVA of the PRIMER 6 package (Clarke and Gorley, 2006), was performed using a similarity matrix based on the results of metrics calculated by the AMIIB@ program ($IPtI_s$, IASPT, IBMWP, Shannon-Wienner diversity index, Evenness index, EPT and EPTCD). Similarities were calculated using Bray-Curtis similarity coeficient. To better understand the relation betweenenvironmental conditions and the results of the metrics, vectors representing the correlations between environmental variables and the PCO axes were overlaid in the previous PCO. The parameters used were: the environmental variables total organic content, water temperature, dissolved oxygen, conductivity, depth, total dissolved solids, current speed, chlorophyll *a*, ammonia, nitrites, nitrates, phosphorous, grain-size (statistics regarding this were calculated as in Chapter II); QBR and IHF indices; and a ratio of non-insect specimens/insect specimens. This last ratio was created to understand if intermittency might be related to ecological status, since higher values of this ratio indicate a bigger percentage of non-insect specimens, and non-insect species are more related to perennial sites.

Human pressures at each location were assessed and related to the results of benthic metrics, to understand their efficiency identifying different levels of human pressure. The INSAAR (National Inventory of Water Supply Systems and Wastewaters) database (http://insaar.inag.pt/) was used to identify the location of Wastewater Treatment Plants (WWTP) and septic tanks, the treatment level, the location of the discharge and total population served at each studied stream; the reports of the Brigada de Fiscalização do Litoral, (2006) and Erena, (2005), indicated other various pressures occurring in the Melides and Santo André river basins as well as field knowledge. According to this information, the potential sources of pressure that might affect the ecological quality are described in Table 3.8. A rank of pressures was attributed: 0 –

absent, 1 – low, 2 – moderate, 3 – high and 4 – very high. In the case of the stressor "agriculture" the rank was attributed as following: 0 – absent; 1 – family farming; 2 – extensive farming; 3 – semi-intensive farming; 4 – rice cultivation/intensive farming. Livestock was ranked based on the number of units and animal species: 0 – absent; 1 – cattle grazing or sheep farming; 2 – cattle farming plus grazing, or pig farming; 3 – pig farming plus cattle grazing; 4 – pig plus cattle farming. Urban pressure was scored as: 0 – absent; 1 – discharge point at a distance which favours the dispersion and depuration of the urban sewage; 2 – effluents with secondary treatment serving less than 2000 inhabitants; 3 – effluents with secondary treatment serving more than 2000 inhabitants; 4 – effluents with primary treatment serving more than 2000 habitants and septic tanks without secondary treatment. Forestry was based only on presence or absence of monoculture tree plantations in the study area, with 0 for absence and 1 for presence.

This data was correlated to the PCO axes and plotted as vectors over the PCO analysis in order to understand how these pressures explain the ecological quality determined by benthic metrics.

Table 3.8 – Characterization of the potential pressures at each sampling station.

Sampling Station	Agriculture	Livestock	Urban	Forestry
BAD	1	3	4	0
CAG	0	0	0	0
CAN	0	0	0	0
CAS 1	1	2	2	0
CAS 2	1	1	1	0
CHA 1	1	0	0	0
CHA 2	1	1	0	0
OLH	1	0	0	0
RML 1	1	1	0	0
RML 2	1	1	2	0
RML 3	1	1	2	0
RML 4	4	2	2	0
SAM	1	1	0	0

Results

The results of the IPtI$_s$ (Table 3.9) indicate that most of the sampling stations are considered to have a poor ecological status, with only three of the 13 sites classified as good or excellent. Comparing the two river basins, the Santo André River basin does not have any sampling site at good ecological status. This means that the three sites with a good ecological condition belong to the Melides River basin. The different approaches used to estimate the quality of the sampling sites show different classification results. Sites CAS 1, CAS 2, CHA 2, OLH, RML 4 and SAM were classified as in a good physical – chemical status. Most of the sites present a poor or bad riparian gallery, and only BAD was considered to have good QBR. Most sites had a good habitat structured and some showed a moderate habitat structure. OLH and RML 3 stations showed a very low consistency of classifications obtained with different indices. A poor ecological status and a bad quality of the riparian vegetation were identified by IPtI$_s$ and QBR, respectively, while IHF indicated, a good habitats structure and a good physical-chemical status. RML 3 showed a good ecological quality, a bad riparian quality, excellent habitat structure and a bad physical-chemical status.

Table 3.9 – Ecological quality status given by the different quality measures used.

Samplig site	Physical-chemical quality	IPtI$_s$	QBR	IHF
BAD		0,594	80	55
CAG		0,441	55	42
CAN		0,39	65	48
CAS 1		0,471	5	67
CAS 2		0,371	50	68
CHA 1		0,318	70	70
CHA 2		0,328	20	70
OLH		0,44	25	59
RML 1		1,001	45	74
RML 2		0,545	5	58
RML 3		0,839	5	73
RML 4		0,598	40	70
SAM		0,957	30	70

The results obtained for the IPtIs metrics are indicated in Table 3.10, showing the same tendency for all metrics. Cluster analysis (Figure 3.1) corroborated that relationship between metrics, showing a correlation higher than 0,6 between all metrics determined by AMIIB@, and a correlation higher than 0,9 between and EPT. In the PCO analysis (Figure 3.2) these axes explain 95,7% of the variability between the calculated metrics. A tendency of all metrics to increase along the first axis is shown, towards the stations with a better ecological status.

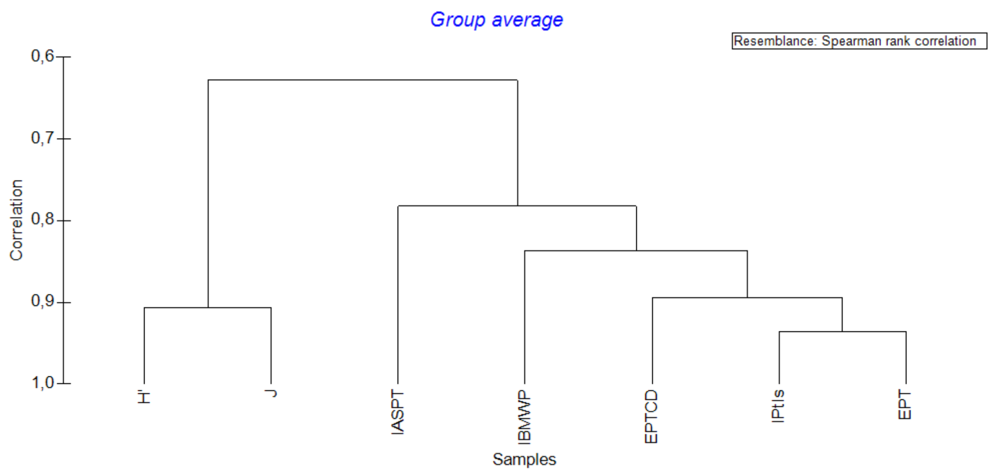

Figure 3.1 – Hierarquical Cluster analysis based on the correlations between the metrics commonly used to assess ecological status.

Table 3.10 – Results of the individual metrics used in $IPtI_S$.

Sampling site	Nº Total de Taxa	IASPT	EPT Taxa	Sel. EPTCD
BAD 1	21	4,60	4	2
CAG	14	4,46	4	0
CAN	16	4,13	1	0
CAS 1	15	4,07	4	2
CAS 2	15	3,73	2	0
CHA 1	9	4,33	2	0
CHA 2	13	3,85	1	0
OLH	14	4,43	4	0
RML 1	30	5,20	11	42
RML 2	15	4,07	4	23
RML 3	23	5,17	9	27
RML 4	16	4,93	5	8
SAM	28	5,11	10	59

The results show that finer sediments (the finer the sediment the larger the vector GS) are strongly associated with lower quality grades (correlation to the first axis of the PCO of -0,66). On the other hand, higher current speeds are associated to better quality status (correlation of 0,39). It is perceived that non-insect taxa occur in a higherpercentage in locations classified with low quality, which indicates that mostly perennial locations are considered by these metrics to have poor ecological status (correlation of -0,57 with the first axis). As indicated previously in Table 3.9, the vectors corresponding to the indices QBR (correlation of -0,26) and IHF (correlation of 0,31) do not follow the trend of the principal index used. QBR follows a trend opposite to the ecological quality given by the index based on macroinvertebrates and, although the IHF does not fully follow the trend of the latter, has a greater resemblance to these.

The vectors reflecting the human pressures existing at each sampling station (based on Table 3.8) are strongly related to stations considered to have a moderate ecological quality. When comparing the physical-chemical quality with the pressures identified in Table 3.8, a correspondence between them is not seen. As an example, RML 4 and BAD have higher human pressures when compared CAG and CAN and are classified in a better ecological status.

Discussion

Based on the results of the methods proposed for Portuguese Southern rivers ($IPtI_s$), only three sampling stations located at the Melides river basin fulfil the objective of good status, as required by the WFD. These results also indicated that streams of Santo André have lower ecological quality, requiring stringer measures to achieve that target until 2015. When comparing these classifications with those obtained for the same streams under the Management Plan of the Sado and Mira basins Hydrographic Region (ARH Alentejo, 2011), some differences can were observed. The streams of Melides, Samocal and Badoca were classified as poor and Cascalheira as moderate in the aim of the management plan, while this study indicates a better classification, hypothesizing an improvement in ecological status along the last two years. However, there is no indication of the precise location of the sampling sites and on the biological elements used to obtain that classification.

This study shows that all the metrics included in the index used to evaluate ecological quality follow the same trends. The metric more correlated with $IPtI_s$ is the number of EPT families (cumulative number of families of Ephemeroptera, Plecoptera, Trichoptera). This metric includes sensitive taxa, which decreases with increasing amount of urban and livestock pressure (Bonada *et al*., 2006; Ippolito *et al*., 2009). However these classes also are rheophilic, with preference for fast flowing waters (Bonada, 2003), and are known to reside on cobble and gravel sediments (Duan *et al.*, 2009). Therefore, the lower number of this metric may not be associated to increased stressors but with lower current speeds and finer sediments, especially as it was observed in CAG, CAN, CHA 1 and 2, sites considered to have low pressures but with a lentic regimes.

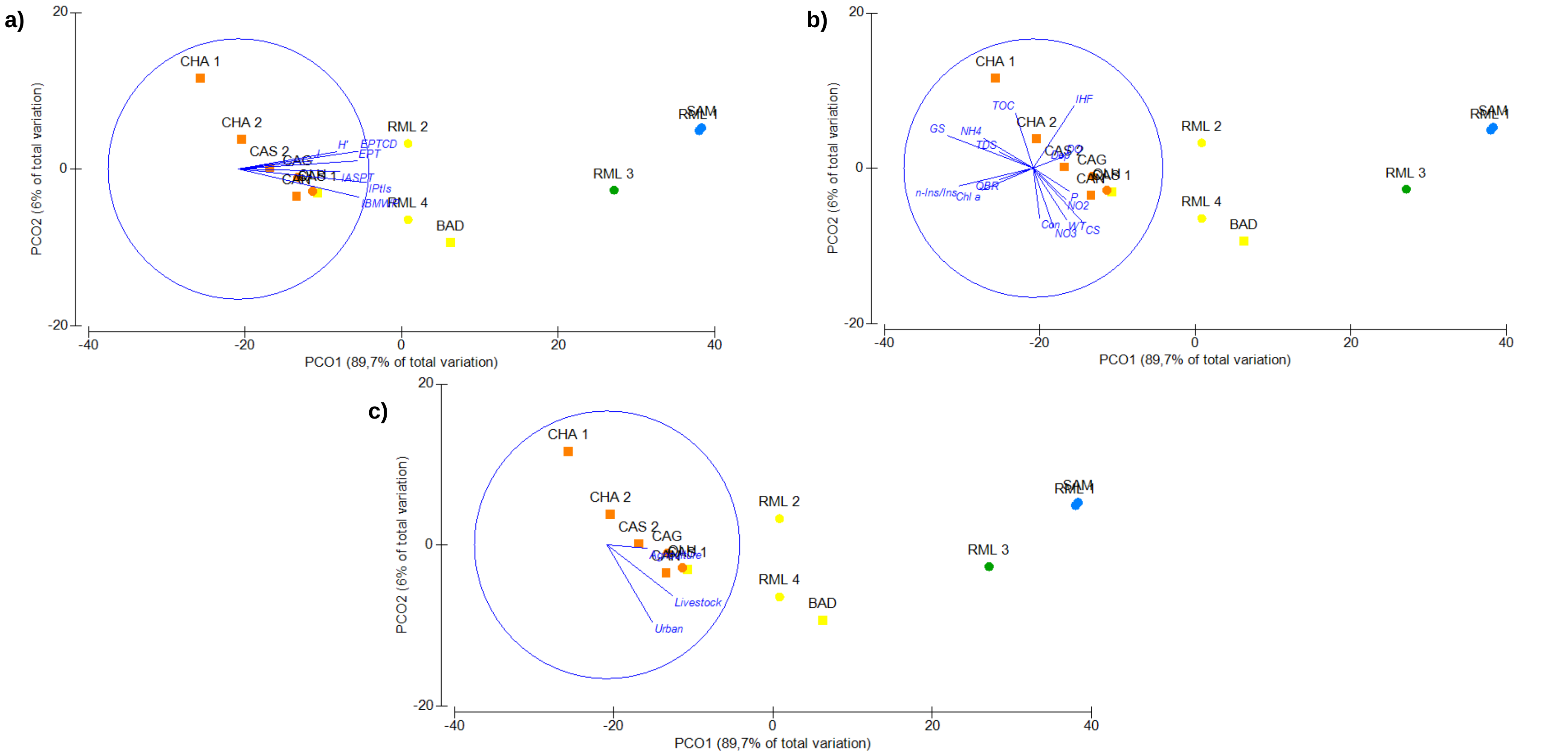

Figure 3.2 – Diagrams of a Principal Coordinates analysis of the influence of the metrics used to achieve ecological status in each sampling station based on benthic macroinvertebrates divided into: a) vectors symbolizing each metric with the length of the vectors as a measure of its importance, all vectors start from the same point, and the second end points in the direction of increasing influence; b) vectors as environmental variables; c) influence of the metrics used to achieve ecological status in each sampling station based on benthic macroinvertebrates and the relationship between these stations and the human pressures existent in each. Sampling stations are represented by symbols: □ - included in Santo André River basin, ○ - included in the Melides River basin. The color of each sampling site is attributed according to the classification of the $IPtI_S$.

Grain size and current speed are directly related to the habitat regime (lentic or lotic) and are, therefore, the most significant natural environmental-gradients affecting the classification of ecological quality in these streams, with fine-grained sediments and lentic environments associated with lower ecological quality classes. With decreasing current velocity finer material, both inorganic and organic can be deposited. Compared with homogeneous streambeds, substrata exhibiting a wide range of particle sizes create a physically more complex and heterogeneous habitat (Beisel *et al*., 1998; Voelz and McArthur, 2000; Duan *et al*., 2009). This statement is in agreement with the classification of IHF relatively to substrate, once this index gives higher scores to streams with rapids and substrate composed by stones, pebbles and gravel (Jáimez-Cuéllar *et al.*, 2004). Such environments can provide more suitable conditions for species with different requirements, and therefore, support a greater variety of benthic species. It is predictable that as sediment becomes mostly composed by sand, there is less available colonization area, especially for aquatic insects, once sandy beds are compact and the interstices between sand particles are too small for benthic macroinvertebrates to move and live within them, also they are unstable and subject to rapid erosion and deposition, leaving invertebrates insufficient time to colonize (Duan *et al.*, 2009; Pinto and Feio, 2009). Thus they prefer gravel and cobble that generally support a more diverse macroinvertebrate community than sand (Nedeau *et al*., 2003). The diversity of benthic invertebrates is directly proportional to the availability of different micro-habitats. This follows the assumption of Vannote *et al.*, (1980) which states that stable and homogeneous environments have lower diversity, whereas instable and heterogeneous environments allow a higher species richness due to a broader range of available conditions.In his way, once the $IPtI_s$ index favours higher diversity, locations classified as good by it have good scores in IHF index.

The TOC in these streams can be strongly influenced by the input of material from the riparian vegetation (leaves, fruits, wood,…) and limiting the entry of light into the channels, thus conditioning the existence of environmental gradients transition between the river and the adjacent terrestrial vegetation (Pardo *et al*., 2002). In this case, this fact is translated in not only TOC vector being negatively related to ecological quality, but also de QBR index in contrary to what was expected. The negative relation of the QBR with $IPtI_s$ results are explained by the fact that higher QBR scores are mainly encountered in lentic environments, which leads to an increasing of organic matter and for that, opportunistic species scored poorly in macroinvertebrate ecological indices, such as Chironomidae and Oligochaeta.Thus, it appears that the quality of riparian gallery does not constrain the community structure of benthic macroinvertebrates, contrary to the findings of other authors.Castela *et al*. (2008), Barquín *et al*. (2011) andCheimonopoulou*et al*. (2011) found strong relations between the benthic indices and QBR, where higher values of QBR would lead to a greater biodiversity of the biological communities.

As highlighted in previous studies, a discrepancy between chemical and biological measures of water quality was found in this study (Faulkner *et al*., 2000; Foy *et al*., 2001; Baker *et al*., 2003). Wenn (2008), explains that while chemical status recovers rapidly, ecological status does not recover so rapidly, revealing pollution past episodes and / or sporadic. That was a major reason to include an integrated ecological approach in the WFD, since chemical indicators are not always representative of the ecological status of streams. The abiotic factors evaluated (whether considered or not in the chemical quality index) do not give an explanation about the ecological status. Higher water temperatures are related with higher conductivities and lower dissolved oxygen content (Wetzel, 1993). Since these streams are small and shallow, there is a large influence of solar heating in the water temperature and for that a variation throughout the day of these parameters so that they will not reflect on the biological communities. The ratio non-insect/insect taxa is negatively related to ecological quality, which might be explained by the fact that the taxa whose abundance more contribute to this ratio, Hydrobiidae and Gammaridae, are not highly scored in the IBMWP index. The low score of Hydrobiidae is due to its tolerance to organic pollution, although Gammaridae are intolerant to organic pollution, this taxa is not as sensitive as EPT (Paisley *et al*., 2011). Therefore, these low scores in IBMWP are reflected in $IPtI_S$, and this index does not have these taxa in special consideration in the other terms of the formula (like EPT or EPTCD).

Four types of stressors where characterized in these two small river basins: agriculture, livestock, urbanization and forestry. However, the forestry occupation does not have a pattern that might cause impacts in aquatic ecosystems. The $IPtI_S$ index did not reflect the pressures gradient identified in the sampling sites. In this case study, higher pressures are associated with moderate quality, especially in BAD and RML 4 sampling sites, although these two sites are considered to have higher human pressures. The Badoca sampling site has an important human pressureresulting from urban effluents, with a great number of habitants served by the WWTP's, and from cattle grazing. RML 4 has considerable pressures from urban effluents, livestock and rice cultivation. This activity occurs in the fields nearby this location and has a seasonal impact in this location, since the rice fields drain to the streams in September to dry the fields and proceed with the harvest procedures (Freitas *et al*., 2008). Besides being a seasonal pressure, the rice fields drainage occurs downstream to RML 4, so this stressor does not have an impact on water quality in the study sites and, consequently on the benthic macroinvertebrate community. All the other stressors lead to nutrients increase, caused by the effluents discharged directly into the water by the WWTP's, nutrients leaching from nitrogen excreted by animals and nitrogenous fertilizers used in agriculture. These pressures might cause eutrophication, especially with NO_3 and NO_2, but concentrations that where measured in the Melides and Santo André streams do not exceed the limits established for a good physical-chemical status.

However, undisturbed or slightly disturbed locations are classified as having a poor ecological quality. These locations are predominantly lentic and with sediments dominated by sand, with the exception of OLH that is dominated by sand but with a lotic regime. This station is a particular case, since it has a groundwater interface and the habitat was altered in order to create a recreational area that affected the sediment characteristics (sediment was transported from the Melides lagoon) and lead to a loss of natural characteristics. The benthic macroinvertebrate community of this location was dominated by an invasive species, *Potamopyrgus antipodarum*. This is a species with high tolerance to organic pollution and nutrient enrichment (Alonso and Camargo, 2003) and it is known to change the structure of communities originally present (Múrria *et al*., 2008).

The differences between lentic and lotic habitats are also emphasized by the results obtained at sites RML 2 and 3, which have the same stressors, but the last one have a better ecological classification, because the current speed is greater and for that the substrate and fluvial habitat (IHF) is more heterogeneous. This considerable increase of current between one station and another is due to the existence of a weir between them.

Since the IPtIs index was proposed recently, its efficiency is still very poorly known. Yet, studies in other streams in southern Portugal (*e.g*., Coimbra *et al.* 1996) also indicate that the use of indices such as BMWP or IASPT in lentic conditions is not appropriate. With the arrival of the dry season, the river continuum is interrupted giving rise to the occurrence of ponds. An increase in temperature and plant detritus occurs in those conditions, leading to an increase in salinity due to evaporation and higher nutrients concentration. These harsh conditions are similar to those found in polluted sites, causing a predominance of tolerant taxa (Coimbra *et al*., 1996). Pinto *et al*. (2004), suggest the replacementof the metric EPT by EPTO (Ephemeroptera, Plecoptera, Tricoptera and Odonata) in Mediterranean streams. The addition of Odonata to the metric compensates the absence of Plecoptera in lentic habitats. These are also sensitive to pollution taxa but, unlike Plecotera are more abundant and diverse in lentic waters (Bouchard, 2004).

Generally, this index does not efficiently classifies stations with different environmental characteristics, i.e. which have groundwater sources or are lentic. Since these lentic environments have sandy bedstream substrates this fact is in agreement with Pinto and Feio (2009), which found low efficiency of this index in sandy bedstreams. This is also in agreement with the low responsiveness found in other metrics to evaluate lentic habitats, as seen above. Withdrawing of the analysis stations with these features, it appears that the index $IPtI_s$ responds relatively well to the existing pressures, when plied in lotc habitats.

Conclusions

This study indicated that few sampling sites were classified in a good ecological status by the $IPtI_s$ index, with only three locations presenting good or excellent quality and half considered poor. However, the results of the different assessment tools did not coincide, hence an ecological assessment integrating these indices should be used, in order to better interpret the results obtained with the benthic macroinvertebrate indices, since these can be influenced by habitat characteristics. Current speed, sediment grain-size and TOC seem to influence significantly the classification results since these environmental factors constrain the benthic community structure and the taxonomic composition at each site. More heterogeneous habitats support a greater diversity of organisms. On the other hand, locations with lentic environments have a more homogeneous habitat type and are, in general, poorly scored by the $IPtI_s$. Species that colonize lentic environments tolerate greater concentrations of TOC and also tolerate organic pollution, therefore these species are equally found in lentic or polluted sites. Ultimately, we concluded that this index does not respond accurately to stressors when the habitat characteristics are different from those that were used to develop the index. Under the current definition this index should only be used for lotic habitats, since lentic habitats have natural pressures similar to human pressures, confounding the interpretation of the results.

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Chapter IV

Final Remarks

Final Remarks

The WFD implementation process currently in place at the European Union led to a strong increase in the development of monitoring programs, to support the assessment of water bodies' ecological status. Some of the European countries had previously developed such programs. However, in Portugal, as in other countries, there were no monitoring programs including biological elements. The scarcity of biological data on some biological elements and the absence of sampling protocols also contributed to delay the implementation of this Directive and the fulfilment of the established dead lines. Monitoring programs are currently ongoing, but specific characteristics of the Portuguese stream systems constrain the use of the tools developed, as it was emphasized by the results of this study.

The data compiled in this thesis improves the understanding of benthic invertebrate communities in the streams of the Santo André and Melides river basins and allows to reach a set of conclusions that were discussed in chapters II and III. The key findings are summarized below.

The studied streams are characterized by communities with a dominance of insects over other taxonomical groups. The most common and widespread taxa was the Chironomidae family of the Diptera order that are known to be euribiont and tolerant to a large variety of conditions. Higher densities were found in Santo André River basin, while higher taxonomic diversity was found in Melides. Taxa richness was highly associated with hydraulic-related parameters controlled by climatic conditions (current speed), while invertebrate abundance was related with food availability (total organic content), once an important number or the streams of Santo André watershed were considered to be lentic. This is coherent with the ecological status attributed to these streams, where the streams in the Melides River basin were the best classified. More heterogeneous habitats host a greater diversity of organisms. Thereby, the locations with lentic environments, with more homogeneous characteristics were, in general, poorly scored by the $IPtI_s$. Species that colonize lentic environments tolerate greater concentrations of TOC and also tolerate organic pollution, therefore these species are equally found in lentic or polluted sites. Only three of the sampling sites were classified in a good ecological status by the $IPtI_s$ index, therefore considered to achieve the goal of the WFD. Nevertheless, this index does not respond accurately to natural stressors, such as habitat characteristics different from those for which the index was developed. Under the current definition this index should only be used for lotic habitats, since lentic habitats have natural pressures similar to human pressures, confounding the interpretation of the results.

Future directions

Biotic interactions, such as competition and predation are likely to have an important contribute regulating benthic invertebrate abundance and distribution. Thereby it would be important that further studies would take these interactions into account, not only within benthic invertebrate communities but also with other communities like fish, for instance, that may have considerable effects in benthic communities driven by predation. These effects can lead to a misinterpretation of the real ecological status existent. A multi-season approach also would be ideal to understand the ecology of these Mediterranean streams, how they change throughout the year and how they respond to extreme events, once structure of communities change seasonally.

Finally, rehabilitation measures to improve the ecological status of the lotic streams considered not to be with good ecological quality should be taken in order to understand how efficient are the assessment tools and, ultimately, to fulfil the goals of the WFD by 2015.

Appendix

List of taxa identified and respective density (ind/60’) in each sampling station

	BAD	CAG	CAN	CAS 1	CAS 2	CHA 1	CHA 2	OLH	RML 1	RML 2	RML 3	RML 4	SAM
Acroloxus	0	0	0	0	0	0	0	0	0	0	0	0,333333	0
Aeshnidae	0	0	4,666667	0	0	0	0	0	0,333333	0	0	0	0
Ancylus	1,5	0	0	0,5	0	0	0	0	12	0	10	3,333333	4,444444
Anthomyiidae	0,5	0	0	0,5	0	0	0	0	0,333333	0	0,666667	0	0
Assiminea	0	0	0	0	0	0	0	0,444444	0,333333	0	0	0,333333	0
Athericidae	0	0	0	0	0	0	0	0	0	0	0	0	0,222222
Baetidae	203	34	1	203,5	17	2	0	0	103,6667	5,333333	167,6667	7	130,6667
Brachycentridae	0	0	0	0	0	0	0	0,444444	0	0	11,33333	27	3,333333
Branchiobdellidae	0	0	0	0	0	0	0	0	0	0	0,333333	0	0
Caenidae	3	6	0	0,5	0	0	0	0,222222	11,33333	11,66667	0,333333	0,333333	29,55556
Capniidae	180,5	2	0	0	0	2	0	0	2,666667	0,333333	32	0	3,333333
Ceratopogonidae	1	0	84,33333	0	0,333333	2	6	0,888889	14,33333	0,666667	1	0	0
Chaoboridae	0	0	0	0	0	0	0	0	0	0	0	0	0,222222
Chironomidae	96	86	4069,333	107	20,33333	270	504	27,77778	111,6667	177,3333	1152,333	76,66667	290,4444
Chrysomelidae	0	0	0	0,5	0	0	3	0	0	0	0,666667	0,666667	0,222222
Coenagrionidae	0	0	21	0	0	0	0	0,444444	0,333333	0,333333	0	0	0
Corbicula	0	0	0	0	0	0	0	0	0	0	0	0,333333	0
Cordulegasteridae	0	0	0	0	0	0	0	0	0	0	0	0	0,222222
Corixidae	0	0	0	0	0	0	153	0	2	0,666667	0	0	0,888889
Culicidae	0	0	0	0	0	0	0	0	0,333333	0	0	0	0
Curculionidae	0	0	0	0	0,333333	0	0	0	0	0	0	0	0,222222
Cyclopoida	1,5	24	4	0	0	0	54	0,222222	2	2,666667	0	0	7,555556
Daphniidae	0	2	0	0	0	0	0	0	0	0	0	0	0

Decapoda	0	0	0	0	0	0	0	0	2	9,333333	0	0	1,333333
Dixidae	0,5	0	0	0	4	0	0	0	13	0	0	0	1,777778
Dolicopodidae	0	6	0	0	0	0	0	0	0	0	0	0	0
Dryopidae	0,5	0	0	0	0	0	0	0	0,333333	0	0	0	0
Dytiscidae	1	0	9	0	2	0	0	0	7,666667	3,333333	0	0,333333	2,222222
Ecnomidae	0	4	0	31,5	0	0	3	1,333333	23,33333	0	2	1	10,22222
Elmidae	0,5	0	0	1,5	0,333333	0	0	0,222222	6,666667	22,66667	27,33333	8,333333	43,55556
Empididae	4	0	0	0	0	0	0	0	0	0	0,333333	0	0,444444
Ephydridae	0	0	20,33333	0	0	0	24	0,222222	0	0	0	0	0,222222
Gammaridae	0,5	0	1	587,5	403	88	1128	194,4444	0	279,3333	195,3333	401,6667	0
Gastropoda	0	2	0	0	0	0	0	0	0	0	0	0	0
Gerridae	0	0	0	0	0	0	0	0,444444	0	0,333333	0	0	0,444444
Glossosomatidae	0	0	0	0	0	0	0	0,222222	0	0	4,333333	0	0,888889
Gomphidae	0	0	0	0	0	0	0	0	0,333333	0,333333	0	0	0
Gyrinidae	0	0	0	4	0	0	0	0	1	0	1,333333	0,666667	1,555556
Haliplidae	0	0	0	0	0	0	0	0	0	0,333333	0	0	2
Helophoridae	0	0	0	0	1	0	0	0	2	0	0	0	0
Hydracarina	0	4	5	0	0	0	0	0	2,333333	0,333333	0,666667	0	1,111111
Hydraenidae	0	0	13	0	0	0	0	0	2	0	0	0	0,222222
Hydrobiidae	0	0	0	0	0	0	0	212,2222	0	48,66667	122	86,66667	0
Hydrochidae	0	0	0	0	2,666667	0	15	0	0	0	0,333333	0	0
Hydrometridae	0	0	0	0	0	2	0	0	0,333333	0	0	0	0
Hydrophilidae	0,5	2	145,6667	0	0,666667	0	0	0,888889	1,666667	0	1,333333	0	1,333333
Hydropsychidae	0	0	0	12	0,666667	0	0	0,222222	11,33333	0	174	7	11,11111
Hydroptilidae	0	0	0	0	0	0	0	0,888889	0,333333	0	2	0	1,333333
Hydroscaphidae	0	0	0	0	0,333333	0	0	0	0	0	0	0	0

Hygrobiidae	0	0	0	0	1	0	12	0	0	0	0	0	0
Janiridae	6,5	0	0	0	0	0	0	0	0	0	0	0	0,444444
Laophonte	0	0	5	0	0	0	0	0	0	0	0	0	0
Lepidoptera	0	0	0	0	0,333333	0	0	0	0	0	0	0	0
Leptoceridae	0	0	0	0	0	0	0	3,333333	3	1,333333	0	2,666667	1,555556
Leptophlebiidae	0	0	0	0	0	0	0	0	33	0	0	0	15,33333
Lestidae	0	2	0	0	0	0	0	0	0	0	0	0	1,777778
Leuctridae	0	0	0	0	0	0	0	0	0,666667	0	0	0	0
Libellulidae	0	0	1	0	0	0	0	0	0	0	0	0	0
Limoniidae	7,5	32	0	0	1	0	3	0	1	0	1	0	0,222222
Lymnaeidae	0	0	0	0	0	0	0	0,666667	0	0,333333	0	0	0
Mesoveliidae	0,5	0	2	2,5	3,666667	0	0	0	0	0	0	1	1,111111
Naucoridae	0	8	0	0	0	0	0	0	0	1	0	0	0
Nematoda	4,5	2	1,333333	0	0	0	0	0,222222	0,333333	0	0,333333	1	0
Nevrorthidae	0	0	0	0	0	0	0	0	0,333333	0	0	0	0
Noteridae	0	0	0	0	0	0	0	0	0,333333	0	0	0	0,444444
Notonectidae	0	0	0	1	0	0	0	0	0,333333	0,666667	0	0	0,222222
Oligochaeta	299,5	26	210,6667	16,5	1,333333	436	1563	32,44444	3	163,6667	90,66667	61,33333	7,111111
Ostracoda	0,5	12	100	0	0,333333	14	6	20,88889	0,333333	4	1	0,333333	6,222222
Palaemon varians	0	0	0	0	0	20	6	0,666667	0	0,333333	0	1,333333	0
Palaemonidae	0	0	0	0	0	0	6	0	0,333333	0	0,333333	0	0
Perlodidae	0,5	0	0	0	0	0	0	0	0	0	0	0	0
Phriganeidae	0	0	0	0	0	0	0	0	0	0	0	0	0,444444
Physidae	0	0	0	0	0	0	0	1,555556	0,333333	1,666667	0,333333	0	2,222222
Planorbidae	0	0	0	0	0	0	0	0,222222	0	0	0	0	3,777778
Polycentropodidae	0	0	0	0	0	0	0	2,888889	4,666667	1	1	0,333333	0,222222

Potamopyrgus antipodarum	0	0	0	0	0	0	0	126,6667	0	149,3333	0	0	0
Psychodidae	0	2	0	0	0	0	12	1,111111	1,333333	0	0,333333	0	0,888889
Ptychopteridae	1,5	2	0	0	0	0	0	0	0	0,333333	0,333333	0	0
Pulmonata	0	0	0	0	0	0	0	0	0,333333	0,333333	0	0	0
Rhagionidae	0,5	0	0	0	0	0	0	0	0	0	0	0	0
Rhyacophilidae	0	0	0	0	0	0	0	0	1	0	0,666667	0	0
Sericostomatidae	0	0	0	0	0	0	0	0	1	0	0	0	0
Sialis	0	0	0	0	0	28	0	0	0	0	0	0	0,222222
Simulidae	131	0	0	632,5	6,333333	2	33	0	69,66667	0	92	27,33333	16,44444
Sphaeriidae	0	0	0	0	0	0	0	2,222222	1	0	0	0	0
Stratomyidae	0	0	0	0	0	0	0	0	0,333333	0	0	0	0
Tabanidae	0	0	0	0	0,333333	0	0	0	0	0	0	0	0
Thaumeleida	0	0	0	0	0	0	0	0	0	0,333333	0	0	0
Tipulidae	0,5	0	0	0	0,333333	0	0	0	6	0	1	0	0,666667
Tricoptera	0	0	0	0	0	0	0	0	0	0	6,333333	16,33333	1,777778
Turbellaria	0	0	0	0	0	0	0	0	0	0,666667	86	0	0,222222
Veliidae	0	0	2	0,5	0,666667	0	0	0,222222	1	0	0	0	0,222222
Vertigo	0	0	0	0	0	0	0	0	0	0	0,666667	0	0