UNIVERSIDADE DO ALGARVE
FACULDADE DE CIÊNCIAS E TECNOLOGIA

Biological variation in temporary streams: understanding river patches at different scales for monitoring and management applications

Katarzyna Krystyna Sroczyńska

Ph.D in Marine, Earth and Environmental Sciences
Speciality in Freshwater Science

Thesis supervised by:

Dr. Luís Chícharo Faculty of Science and Technology, Campus of Gambelas, University of Algarve 8005-139, Faro, Portugal

Dr. Adrianna Wojtal-Frankiewicz Department of Applied Ecology, Faculty of Biology and Environmental Protection, University of Łódź, 90-237 Łódź, 12/16 Banacha Str., Poland

Faro

2017

Biological variation in temporary streams: understanding river patches at different scales for monitoring and management applications

Katarzyna Krystyna Sroczyńska

Ph.D in Marine, Earth and Environmental Sciences
Speciality in Freshwater Science

Thesis supervised by:

Dr. Luís Chícharo Faculty of Science and Technology, Campus of Gambelas, University of Algarve 8005-139, Faro, Portugal

Dr. Adrianna Wojtal-Frankiewicz Department of Applied Ecology, Faculty of Biology and Environmental Protection, University of Łódź, 90-237 Łódź, 12/16 Banacha Str., Poland

Biological variation in temporary streams: understanding river patches at different scales for monitoring and management applications

Declaração de autoria de trabalho

Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluído “A Universidade do Algarve tem o direito, perpétuo e sem limites geográficos, de arquivar e publicitar este trabalho através de exemplares impressos reproduzidos em papel ou de forma digital, ou por qualquer outro meio conhecido ou que venha a ser inventado, de o divulger através de repositórios científicos e de admitir a sua cópia e distribuição com objeticos educacionais ou de investigação, não comerciais, desde que seja dado crédito ao autor e editor.”

Acknowledgements

Following work would not be possible without the help of wonderful persons that I was lucky to meet during this PhD project. I would like to thank my supervisors Luís Chícharo and Adrianna Wojtal-Frankiewicz. Luís Chícharo was always supporting me in technical issues, taking care that I make a right progress and providing me with the best solutions. Adrianna Wojtal-Frankiewicz was inspiring me by giving me great ideas and advices, but also a lot of mental support. Here I would also like to thank to Pedro Range, who was co-author of all the publications, which make the large part of thesis. Pedro was always available to help me and provided me a great guidance through the duration of the project. The whole experimental work and sampling would not be possible without great dedication of my friend Marilia Claro. At the University of Algarve I also wanted to thank you people who directly worked with me: Joana Cruz, Alexandra Chícharo, Francisco Leitão, Renata Gonçalves and Radhouan Ben-Hamadou.

My great thanks also go to Professor Tomasz Boski, who inspired me to work with isotopes and provided me a contact to José Antonio González-Pérez from IRNAS-CSIC, in Seville. José Antonio supported me with all the technical issues related to isotopes and her technician Alba Carmona Navarro greatly helped me with the sample analysis.

I would also like to mention Maria Schröder, Samantha Hughes and Simone, Varandas who were the first people to teach me how to identify macroinvertebrates.

Essential role in designing the metabolic chambers had Kasia Wasiak who shared a lot of knowledge and experiences with me related to benthic community metabolism. My gratitude also goes to Victor García López, Carlos Sanchez Ureña and Almudena López Fernández who gave me hand in the field, spending many hours measuring benthic metabolism.

I‘m also thankful to my friends from Laboratório de Análises Quimicas (LAQ): Paulo Zaragosa Pedro, Marta Ribeiro e Bruno García who dedicated their time to teach me and greatly help me in sample analysis.

I also wanted to thank you people from U.S. especially Michael Vanni and his wife - Maria González who received me so well in their lab at Miami University and made my time in U.S. unforgettable. Here my deepest thanks also go to Tanner Williamson, who provided me with a lot of guidance and support during my stay in U.S.

Finally I would like to acknowledge the financial support from the IMPACT project funded by Foundation for Science and Technology (FCT) of Portugal (ERA-IWRM/0003/2009).

At the end I wanted to thank you my precious friends whose mental support was priceless: Asia Włodarczyk, my cousin - Zuza Piechocka, my family, Vitor, Necas, Jennifer Taylor and Bogna Jacniacka among others.

Summary

Biota and ecological processes are highly complex and vary at every scale. This underscores the importance of employing a multi-scale design to adequately understand these processes and complex relationships in riverine ecosystems. In addition, there is a strong need to develop appropriately scaled indicators of river ecosystem health that include this biotic complexity in a manageable fashion. Unfortunately, currently available indicators are either too complex or do not adequately capture the highly variable changes to the ecosystem. Patches are good templates for various ecological processes and because they are considered to be stable over the spatial and temporal scales, they can be used as functional filters of important processes in streams.

The aim of this thesis is to employ patch theory and multi-scale approach to develop structural and functional indicators of the ecosystem health at the patch level and evaluate in which of the scales these indicators are of the highest relevance for the patch. The system at which these indicators were tested consists of headwater intermittent streams within a Mediterranean catchment. Three scales were considered: reach scale, stream scale and catchment scale.

According to the results patch as a source of variation was not well explained by the structural measures of benthic communities at catchment scale. This was related to the effect of occurrence of a strong environmental filter (mainly altitude and its association with conductivity and temperature), which limited distribution of biota and constrained the occurrences of certain species at the smaller scales. Also, these filters were demonstrated to act indirectly through patterns in habitat formation and availability. Patch investigated at the reach scale provided slightly more predictable unit of species organization, nonetheless, still benthic communities of some of the patch types overlapped. Instead, the most consistent measures of ecosystem health that could be applied to studying patches were the metabolism measurements at the reach scale and the isotopic signatures at the stream scale. Next step forward would be to establish reference values for these two approaches for undisturbed systems, and subsequently to incorporate these measures into biomonitoring guidelines.

Following disturbance, patches have been shown to be the most appropriate unit used when evaluating biotic recovery. As such, this study represents an important step

towards development of better biomonitoring tools as well as evaluation of the restoration effort.

Keywords: spatial scale; macroinvertebrate assemblages; intermittent streams; functional indicators

Resumo

Os processos biológicos e ecológicos nas ribeiras são altamente complexos e variam em diferentes escalas espaciais e temporais. Assim, a análise dos processos e relações ecológicas ribeirinhos requer uma abordagem multi-escalas que permita capturar a variabilidade efetiva do sistema. Para tal, é necessário desenvolver indicadores que sejam representativos dessa variabilidade e complexidade. Os indicadores disponíveis são demasiado complexos ou não capturam de forma adequada as alterações, altamente variáveis, no ecossistema. Assim, o objectivo deste trabalho foi propor vários indicadores (estruturais e funcionais), nas três escalas espaciais (secção do rio; ribeira e bacia hidrográfica) num sistema de ribeiras intermitentes da bacia Mediterrânica do Algarve. Estes indicadores foram avaliados em áreas de consideradas unidades representativas (habitats) dos processos que ocorrem a nível geral, da ribeira.

Analisaram-se indicadores estruturais: variação das comunidades bentônicas de macroinvertebrados e indicadores funcionais: metabolismo do ecossistema, assinaturas isotópicas e avaliação da reciclagem dos nutrientes. Para analisar os dados foram usadas várias metodologias estatísticas como analises univariadas e multivariadas, ”Indicator value analysis” e “self organizing maps”.

Os resultados evidenciaram que a avaliação do metabolismo do ecossistema pode ser usado para caracterizar habitats na escala da secção do rio enquanto que as assinaturas isotópicas caracterizaram melhor os habitats à escala da ribeira. O terceiro indicador: reciclagem dos nutrientes foi altamente correlacionado com tipo de espécie analisado. Sendo assim, este indicador pode ser utilizado sobretudo em sistemas onde existam variações significativas nas comunidades bentônicas. Por outro lado, os indicadores estruturais revelaram não ser bons indicadores da variação entre os habitats, já que as comunidades foram estruturadas sobretudo devido à influência da altitude e a sua associação com a condutividade e temperatura.

Este estudo contribuiu para a caracterização dos ecossistemas das ribeiras do Algarve e para o desenvolvimento de metodologias mais representativas a utilizar em programas de biomonitorização em ribeiras intermitentes mediterrânicas.

Palavras chaves: Escala espacial; Comunidades de macroinvertebrados; Ribeiras temporárias; Indicadores funcionais

Table of contents

Chapter 1

1.1. General introduction

Streams and rivers are heterogeneous environments shaped by geological and hydrological processes acting at both spatial and temporal scales (Elosegi et al. 2010; Poff and Ward 1989; Vinson and Hawkins 1998). These processes directly contribute to changes in channel morphology and formation of visually distinct patches within the stream channel (Armitage et al. 1995; Gasith and Resh 1999). Patch is considered an area of the stream visually distinct from its surroundings (Forman and Godron 1986). Individual patches can be characterized by their hydrological gradient (such as rapid riffles or lentic, depositional pools) or type of substrate such as mineral (ranging from bedrock and large boulders to fine sediments); organic (i.e. woody debris or macrophytes) or a mixture of both (i.e. epilithic algae growing on mineral substrates). Such patches, commonly defined as habitats (and alternately referred either as patches or habitats, in the current work) have physical uniformity, are influenced by the similar set of environmental conditions, and constitute "unique functional processes zones" (Vannote et al. 1980). In classical ecological studies, patch is considered as a template, which is meaningful for the species patterns and ecological processes acting at spatial and temporal scales (Southwood 1977). Therefore, patches can be viewed as integrated (by space and time) units of river channel, at which important processes occur. This is why habitat is considered by many authors as the most appropriate scale to study heterogeneity of lotic ecosystems and key biological processes in streams and rivers (Stanford et al. 2005; Whited et al. 2007). Pringle et al. (1980) in his patch dynamics concept emphasize the importance of studying patches in lotic ecology for better understating of the overall spatial complexity of the ecosystem function. Viewing rivers as a mosaic of patches reduces intricacy of processes and patterns to a single dimension, which allows for better insight into their ecology, factors which regulate them, as well as allows for the link to broader range of ecosystems processes and application in management, restoration efforts and biomonitoring. For example, partitioning of stream into separate units allows validating major ecological theories such as River Continuum Concept (RCC), or Nutrient Spiralling (NS). Additionally, understanding of the processes occurring at the patch scale allows to better predict the consequences of disturbances to the ecosystems on broader scales (according to shifting habitat mosaic concept, introduced by Stanford et al. 2005).

Water Framework Directive (WFD) obligates the members of the European Community to preserve and improve the status of aquatic ecosystems by monitoring and assessing water quality using biological indicators of the ecosystem health. Based on the previously introduced patch approach, human-induced disturbances to the ecosystems as well as measures of restoration success are often assessed by changes to benthic habitats, thereby considered as different patches (Armitage and Pardo 1995). However, the ecological relevance of a patch depends on the processes considered and the scale of the observation (Cortes et al. 2009). Scales that are the most relevant to a group of studied organisms or processes are those with the highest ecological importance.

As noted by Naura et al. (2011): ”the identification of causal relationships between habitat features, processes and communities, and the scale at which those should be assessed are two major issues in designing monitoring and management programmes”. And therefore as reported by Cortes et al. (2009): “selection of appropriate scales for habitat characterization and associated metrics to describe biologically relevant responses to stressors are fundamental considerations” in the framework of WFD and other management and river restoration programs. Then, the general aim of the current work is to apply multi-scale approach to investigate patches as templates for various processes, which are of the ecological focus from the river management and the biomonitoring perspective.

The following section presents the important ecological processes and indicators of ecosystem health most commonly used in the investigation of patches as a source of variation.

1.1.1. Macroinvertebrate assemblage structure

Majority of studies which assess the river health use the response of benthic fauna, at different habitats, as a proxy to detect changes in riverine ecosystems. This is based on the assumption that abiotic factors which influence the habitat template, will determine macroinvertebrate communities found there. However, the effectiveness of macroinvertebrates as descriptors of habitats was demonstrated to differ, depending on the scale of the observation. In general, studies on structural dynamics of biota demonstrated strong influence of habitat characteristic on abundance, diversity and the trophic structure of macroinvertebrate assemblages (Brown 2003; Beisel et al. 2000;

Pardo and Armitage 1997). This is not surprising, as most of the taxa are sensitive to a wide array of habitat conditions such as: type of food available, current velocity, substrate type and stability, range of dissolved oxygen, among others (Downes et al. 1993; Douglas and Lake 1994). These habitat specific preferences impel them to be confined to particular habitats within a river reach. Habitat type and heterogeneity are regarded as good predictors of species assemblages, abundance and diversity at the reach scale (Pardo and Armitage 1997; Boyero et al. 2003a; Richards et al. 1996, 1997). However, at the larger scale habitat and heterogeneity are not good predictors of species assemblages (Boyero, 2003b; Heino et al. 2003). Weak concordance found among macroinvertebrate assemblages and their habitats at larger scales is because of the hierarchical scale dependence, where larger scale variables control habitats and processes at local scales (Feminella 2000; Rabeni and Doisy 2000; Frissell et al. 1986). For example, geological characteristics of the river basin, such as altitude, slope, and river channel morphology exert control on the grain size distribution and hydrological regime, which, in turn affect patch structure and arrangement within a river channel. In addition local variables such as predation or competition can vary over few meters, having direct or indirect control on small scale variation among species assemblages (Menge and Olson, 1990). From the biomonitoring perspective, this has large implications because it demonstrates that catchment-scale factors might obscure important effects of local disturbance on the structure of biota (Richards et al. 1997).

Hence, catchment scale properties will have direct or indirect influence on patch properties expressed at smaller scales (Richards et al.1997) and further, can mask important features of habitat in organizing stream assemblages. Consequently, in order to explain at what scale the stratification by patch is useful to understand dynamics of macroinvertebrate assemblages, one should a priori identify main drivers of patch structuration and species occurrences at the catchment scale (Hawkins et al. 2000; Heino et al. 2002, 2003). However, even then, patches might not guarantee to be sensitive descriptors of species assemblages. Knowledge about macroinvertebrate arrangements in the context of patches is undeniably important component of the ecosystem health. Nonetheless, high spatial variability in patterns of macroinvertebrate distribution and weak correlation with local factors may limit their role of structural indicators of how the system works.

1.1.2. Functional measures (rate of production and respiration)

Recently, several studies have drawn attention that using only a structural organization of biota, as indicators of ecosystem health (without considering also its functional role) contribute little to ecosystem functioning and therefore should not be used as the only indicator in assessment of the ecological status of the water bodies (Brooks et al. 2002; Bunn and Davis, 2000). As a response to these issues, the 5th European Water Framework Directive requires additional incorporation of the ecosystem processes in stream assessment protocols. Recently, functional measures have received considerable attention due to their sensitivity in response to environmental change (Bunn et al.1999; Fellows et al. 2006; Young et al. 2008). One of the most conspicuous descriptors of the ecosystem-level processes is the measure of community primary production and respiration. Few studies that measured patch specific benthic metabolism demonstrated differences in community production and respiration among different stream habitats. Gonzales et al. (2014) demonstrated high spatial variation in metabolism within a stream, related to presence of different geomorphic units, bed materials and type of transient storage. Based on these recent findings, patch-specific benthic metabolism can represent more accurate alternative indicator of changes in water quality than structural measures of benthic communities. In addition, patches viewed as distinct metabolic entities allow validating important concepts of ecosystem processes, such as river continuum concept (Bott et al. 1985). Further, by upscaling patch specific benthic metabolism to the entire reach or stream it would be possible to predict whole stream carbon budgets. However, contradictory studies also demonstrated that the information about the autotrophy or heterotrophy of the ecosystem solely is not a reliable indicator of the ecosystem health under different type of disturbances (Bunn et al. 1999; Death et al. 2009). Hence, it remains unclear wherever primary production is an efficient indicator of an ecosystem health.

1.1.3. Functional measures (Stable isotopes)

Another functional measure for assessing the ecosystem health is the use of stable isotopes, mainly C and N. The use of stable isotopes is based on the assumption that habitats and consumers of anthropogenically-disturbed ecosystems will have more distinct isotopic signatures than their counterparts in reference sites. Stable isotopes were used as functional indicators of riparian and catchment degradation (Bunn et al.

1999); sewage impacts (Di Lascio et al. 2013), agricultural land use; anthropogenic nitrogen deposition (Holtgrieve et al. 2011); wastewater effects (Morrisey et al. 2013); nutrient enrichment (Bergfur et al. 2009), among others. Additionally, measurements of carbon and nitrogen isotopic signatures at sites, with variable human impact might provide an early warning of potential disturbance problems. Only recently, isotopes were also used as an excellent tool for retesting the River Continuum Concept (Pingram et al. 2012). This was achieved by measuring isotopic signatures of invertebrates along the river longitudinal gradient (Rosi-Marshall et al. 2016). However, many studies on stable isotopes demonstrated high overlap among basal food sources and consumers, obscuring the clear effect of the disturbance on consumers and their habitats (Bergfur et al. 2009). The main source of variability among isotopic signatures is associated with high interspecific variability in consumers, as well as in their patchy food resources among and within the same rivers (Dodds et al. 2014; Lorrain et al. 2002).

1.1.4. Stoichiometry of benthic animals and their food sources

Nutrient excretion and elemental composition of consumers and their resources are not a direct indicator of the ecosystem health. However, such knowledge, especially in the context of producer/consumer interactions, is important to identify the consequences of nutrient enrichment to the ecosystem on the ecological patterns and processes in streams (Bowman et al. 2005). The extent to which benthic macroinvertebrates supply nutrients to the aquatic ecosystem depends on their own body stoichiometry and their food composition (Elser and Urabe 1999). This relationship was modelled by Sterner (1990) based on mass-balance equations under the assumption that animals are homeostatic in maintaining their internal nutrient composition. Therefore any producer-consumer nutrient imbalance might alter the availability of nutrients to primary producers. Studies investigating the overall supply and availability of elements to the stream biota are influenced by the heterogeneity of the in-stream habitats (Meyer et al. 1988). For example, exclusion of essential habitats, such as leaf litter, from the stream might have enormous ecosystem-level consequences because physical nature of substratum affects resource availability to consumers (Wallace et al. 1997). Because excretion rate is taxa and feeding group specific, from stoichiometry approach we would expect differences in excretion rates between communities with different species assemblages (taxa and feeding groups) and/or between communities that colonize habitats with distinct quality and quantity of resources. Further, we will expect that patchy distribution and abundance of benthic species in the river bottom will create spatial variation in nutrient dynamics within the same stream. Such knowledge of how patches are important to the ecosystem from the nutrient supply perspective might greatly enhance the restoration programs targeted to maintain the biotic integrity in the stream. However, the magnitude of this variation will depend on how patches differ as descriptors of the community assemblages and at what scale of observation these differences in nutrient supply variation among patches are the most apparent. Although such knowledge should be a prerequisite in designing restoration programs, only few studies investigated the role of patch (in this case riffle/pool) in spatial variability of nutrients supply (McIntyre et al. 2008) and few more investigate the variability in macroinvertebrate biomass in total nutrient availability (Benstead et al. 2010; McManamay et al. 2011).

1.2. Specificity of Temporary streams

Temporary streams are channels, which maintain water flow only seasonally and become dry when the flow ceases during dry periods (Acuña et al. 2014). Temporary streams are classified as intermittent, when the flow is maintained over some sections in the stream forming a series of disconnected pools. Disconnected pools maintain during the dry periods because of elevated water tables or directly groundwater recharge. Temporary streams are extremely dynamic ecosystems located at the interface of terrestrial and aquatic habitats (Steward et al. 2012). They are important habitats for plants and animals, spots for nutrients and carbon recycling and linking corridors to other perennial water bodies (Arthington et al. 2014; Datry et al. 2014a; Gasith and Resh 1999; Kerezsy et al. 2013; Steward et al. 2012; Williams, 1996). Although temporary streams are widely distributed and ecologically valuable, for long time they were neglected by scientists and their ecology, geography and hydrology represent an understudied area of research in comparison to perennial water courses (Datry et al. 2014). Understanding of most ecosystem processes, including validation of major ecological theories in temporary Mediterranean streams are poor, in comparison to perennial watercourses (Leigh et al. 2015). For example, RCC theory assumes longitudinal change of production, from heterotrophic headwaters which rely on prevalence of terrestrial food sources and corresponding macroinverterate assemblages towards downstream located, higher order streams and large rivers, where autotrophy dominate and main carbon subsidies are of autochthonous origin (Vannote et al. 1980). However, RCC was developed over large scale ranging from first order streams to large rivers, whereas Mediterranean basins in Portugal, commonly constitute streams from first to third order, all classified as headwater streams. Additionally, terrestrial inputs to Mediterranean streams are less pronounced than in more humid regions and autochthons benthic production is believe to be the primary energy source, even at well-shaded forested streams (Bunn et al. 1999; Douglas et al. 2005; Gasith and Resh, 1999). Therefore, RCC theory might not be so obviously manifested in these streams (Pingram et al. 2012). Further, some authors demonstrated that preferences of macroinvertebrates for specific type of habitat are less exhibited and dominated by the generalist traits in streams with higher frequency and magnitude of disturbance, such as temporary streams (Death and Winterbourn 1995). Such tactic employs lesser selectivity in terms of resource partitioning (Vannucchi et al. 2013). An evidence for such pattern, inferred from stable isotope studies, was demonstrated for higher fish consumers from intermittent sites, where resource availability is seasonally variable, and thus omnivory was promoted as an adaptive strategy to use their resources more efficiently, than their counterparts from perennial sites (Pusey et al. 2010; Douglas et al. 2005). Such generalist tactic may hamper to define the ecological niches for macroinvertebrates in temporary streams and question the use of habitat as an efficient descriptor of taxa assemblages. Additionally, Mediterranean temporary streams are subjected to highly variable hydrological regime, which has large consequences for biota dynamics and patterns. This has further implications for river management and biomonitoring. For this reason, several authors emphasized that temporary streams should be considered separately in biomonitoring (Beche et al. 2006; Mas-Mari et al. 2010; Chakona et al. 2008; Clarke et al. 2010; Argyroudi et al. 2009 Grubbs 2011 Gasith and Resh, 1999; Bonada et al. 2007). Although, large progress has been made in recent years to develop bioassessment tools in Mediterranean basin to address the specificity of this type of ecosystems to biomonitoring protocols and river basin management plans (Acuña et al. 2014; Arthington et al. 2014; Datry et al. 2014b; Feio et al. 2014; Hughes and Malmqvist 2005; Nikolaidis et al. 2013; Prat et al. 2014), still many unresolved issues exist. Mediterranean type of stream has been questioned to not adequately reflect the water quality, based on macroinvertebrate metrics. In Portugal main river typologies were established (INAG, 2008) for macroinvertebrate sampling, however there is a further need for testing whenever these typologies reflect and cover the highest spectrum of heterogeneity of the Mediterranean temporary streams. Further, little effort is dedicated into investigation of patches in intermittent streams and validating if the patches relevant for biota in perennial streams are of the same importance in temporary watercourses. This has implications on rapid bioassessment programs because techniques and methodologies required by WFD and developed for perennial streams might not be of the same importance in Mediterranean watercourses.

In summary, lack of sufficient knowledge about temporary stream ecology makes their management and protection challenging (Arthington et al. 2014; Leigh et al. 2015). Mediterranean Intermittent streams are particularly vulnerable to any kind of water diversions, impediments and flow regulations, which in turn affect the mechanism of habitat structuration (Gasith & Resh, 1999) and consequently changes in community composition (Datry et al. 2014b). For example, reduction in flow can favour lentic and more tolerant taxa (i.e. Diptera) with decrease abundance of rheophilic (i.e. *Heptagenidae*) species (Boulton, 2003). Also, some feeding strategies, such as filter feeders can be more affected than others (Death, Dewson, & James, 2009). Furthermore, global climate change predicts the increase of the temporality of streams, making them even more vulnerable to degradation in the nearest future. For all these reasons, increasing our knowledge about the ecology of temporary streams and recognize their essential role in the ecosystem function should be a prerequisite for better assessment, management and conservation of these water bodies.

Presented work should contribute to general understanding of the benthic assemblage structures and habitat-related ecological-level processes at temporary streams, at various scales and should lead to the improvement of bio-assessment methods and river management plans for better characterization of patterns and processes as well as protection and restoration of this type of ecosystems.

1.3. Objective of the thesis

The main objectives of the thesis are therefore as following:

1. Test the hypothesis of "landscape filter" and determine major drivers, which shape patches and biota occurrences at catchment scale;

2. Determine patch and stream type as a source of variation in macroinvertebrate distribution at the catchment scale;

3. Determine patch as a source of variation in macroinvertebrate distribution at the reach scale;

4. Determine patch as a source of variation in community production and respiration rates at the reach scale. Examine if patch be considered as a unique metabolic entities and if factors influencing metabolism are patch specific;

5. Determine the importance of different habitat patches in the context of food resources for consumers at the reach and stream scale;

6. Examine patch in the context of potential spatial gradients in nutrient dynamics at the reach and stream scale;

7.Summarize findings in the context of its utility in river management and bioassessemnt programs, and. identify indicators and the appropriate scale at which these indicators should be used for successful river management plans and biomonitoring.

1.4 Structure of the thesis and specific objectives of each chapter

The main body of the thesis is comprised of 6 chapters. Each chapter should respond the above-listed objectives. The second chapter tries to respond the question if catchment scale should be treated as a homogenous unit (scale) or should be subdivided into classes, according to the larger-scale factors, which may limit biota occurrences. The third and fourth chapter introduces the concept of a patch and tries to identify patterns in macroinvertebrates community structure among habitats and family-specific associations to studied habitats. Third chapter investigates the patch-macroinvertebrate relationship at the catchment scale and fourth chapter addresses similar issue, but at the reach scale. These two chapters aim to address the question of what are the factors that shape biota occurrences at these two scales and are these factors work independently on each other, or is there an interaction among them? Fifth chapter investigates the patch in a context of ecosystem-level process, at the reach scale. The main objective of this chapter is to provide an information if patch can be considered as unique metabolic entity. Challenges associated to extrapolation of the metabolism measurements from patches to catchment scale are mainly constrained by the methodologies applied, which are not able to capture the heterogeneity of the reach. For this reason, I also emphasize the methodological part of flow-through chamber to measure metabolism at different spatial units within a stream. Sixth chapter examines if patches can be viewed as distinct food source entities and how do they influence consumer's signatures and trophic food web. Isotopic signatures of patches and consumers are tested at the same reach and at different streams. The last chapter raises the similar issue as previous chapter, but it considers a patch as a unique entity which might be responsible for variation in nutrients supply to the system. At the end of the thesis the results are summarized and discussed and a separate section is dedicated to the implications that obtained results have on river management and biomonitoring.

Chapter 2

Indicator macroinvertebrate species in a temporary Mediterranean river: recognition of patterns in binary assemblage data with a Kohonen artificial neural network

K. Sroczyńska[1], M. Claro[2] , A. Kruk[3], A. Wojtal-Frankiewicz[4], P. Range[5] and L. Chícharo[1]

[1] Faculty of Science and Technology, Campus of Gambelas, University of Algarve 8005-139, Faro, Portugal

[2] Centro de Ciências do Mar, Universidade do Algarve, Campus de Gambelas 8005-139 Faro, Portugal

[3] Department of Ecology and Vertebrate Zoology, Faculty of Biology and Environmental Protection, University of Łódź, 90-237 Łódź, 12/16 Banacha Str., Poland

[4] Department of Applied Ecology, Faculty of Biology and Environmental Protection, University of Łódź, 90-237 Łódź, 12/16 Banacha Str., Poland

[5] Department of Biological and Environmental Sciences, College of Arts and Sciences, Qatar University, Doha, Qatar

This chapter has been previously published as:

Sroczyńska K, Claro M, Kruk A, Wojtal-Frankiewicz A, Range P and Chícharo L (2017) Indicator macroinvertebrate species in a temporary Mediterranean river: Recognition of patterns in binary assemblage data with a Kohonen artificial neural network.*Ecological Indicators*, *73*: 319-330.

Abstract

Current classifications used in bioassessment programs, as defined by the Water Framework Directive (WFD), do not sufficiently capture the variability present in temporary Mediterranean streams. This may result in inaccurate evaluation of the water quality biological metrics and difficulties in setting reference conditions. The aim of the study was to examine if aquatic invertebrate data of increased taxonomical resolution but expressed on a binary abundance (frequent/rare) scale and referring to good bioindicator species only suffice to indicate clear gradients in water courses with high natural variability such as intermittent Mediterranean streams. Invertebrate samples were collected from 74 sites in the Quarteira River basin, located in southern Portugal. Their classification with the use of a Kohonen artificial neural network (i.e., self-organising map, SOM) resulted in five categories. The variables that drove this categorization were associated primarily with altitude, temperature and conductivity, but also types of substrate, riparian cover and percentage of riffles present. According to the indicator species analysis (ISA), almost all the studied taxa were significantly associated with certain SOM categories except for the category that included sites with disrupted flow regime. The SOM and ISA allowed us to effectively recognize biotic and abiotic patterns. Combined application of both methods may thus greatly enhance the effectiveness and precision of biological surveillance and establish reference sites for specific channel units in streams with high natural variability such as intermittent Mediterranean streams.

2.1. Introduction

Understanding how biotic communities respond to changes in their environment, across various spatial and temporal scales is a principal focus in ecological studies (Poff, 1997). Within the last decade, this knowledge became a prerequisite, as the establishment of the water regulatory acts such as Water Framework Directive (WFD) in Europe, fostered extensive biomonitoring programs, which use aquatic biota, in particular aquatic insects, for the monitoring of human impact. However, most biomonitoring studies are constrained by the natural complexity in macroinvertebrate community patterns, resulting from their diverse traits (Bonada et al. 2007), specific preferences for different type and granulation of substrate, hydrological regimes, ranges of oxygen concentration and type of food (Beisel et al., 1998; Chaves et al., 2005;

Collier et al., 1998; Cummins and Lauff, 1967; Merrit and Cummins, 1996; Mirra et al., 2014; Pardo and Armitage, 1997; Schröder et al., 2013; Townsend and Hildrew, 1994). In order to address these natural variability in biomonitoring assessment programs several recent river classification systems based on macroinvertebrates were developed (Heino et al. 2003; Johnson et al. 2004; Munne and Prat 2011; Sanchez-Montoya et al. 2007; Verdonschot and Nijboer, 2004).

Classification systems established for Mediterranean regions highlight temporary rivers as the most heterogeneous of all of the Mediterranean river types (Munne and Prat, 2004; Robson et al. 2005; Sanchez-Montoya et al. 2007). Differences in the timing of drying and rewetting in these streams result in sites with contrasting hydrological regimes, i.e. perennial, intermittent (which dry to a series of disconnected pools), or ephemeral (which dry completely) (Gasith and Resh, 1999; Bonada et al. 2007; Gallart et al. 2012). This high diversity of hydrological states and its concomitant influence on habitat structuration results in macroinvertebrate community differences and hamper bioassessment methods and quality metrics to be comparable across various streams (Argyroudi et al. 2009; Beche et al. 2006; Chakona et al. 2008; Mas-Marti et al. 2010; Grubbs 2011; Robson et al. 2005; Watson and Dallas, 2013). High variability obtained in temporary streams also constrains the use of indicator species for defining ecological class boundaries (Sanchez-Montoya 2007). This underscores that the current classification used in WFD bioassessment programs does not sufficiently capture the variability present in temporary streams influenced by Mediterranean climate (Morais et al. 2004; Munne and Prat, 2004).

Furthermore, biomonitoring programs, including rapid bioassessment methods, require cost and time effective strategies. This is the reason why most biomonitoring approaches identify invertebrates up to the family level, instead of the genus or species levels so as to save sample processing time. However, aggregation of species data results in lower taxonomic resolution and consequently a loss of information regarding species responses to environmental factors (Dolédec et al. 2000; Parsons et al. 2003). This is because families contain many species with diverse traits. Imprecise information on the response of macroinvertebrates to environmental filters in a dataset may impede the identification of even the main gradients that influence species occurrences at the catchment scale. Therefore, on the one hand, the importance of gradient delineation for more adequate water bioassessment entails a need for lower-level taxonomic identification. On the other hand, identification of all of the taxa in an assemblage to a low taxonomic level usually greatly increases sample-processing time and may be unfeasible for the majority of bioassessment programs.

For this reason, we reduced our community data to the most representative indicator species for the entire stream system. Additionally, we used a simplified, binary (frequent/rare) scale for species abundance in order to shorten sample processing time. Assessing the abundance in a ratio scale of measurement would be extremely time consuming especially in cases of dominant genera consisting of many different species (i.e. *Baetis* spp.). Another limitation results from the fact that macroinvertebrates in temporary ecosystems respond to environmental conditions in a complex and non-linear fashion. Non-linearity and binary scale of measurement limit the possibilities of using conventional multivariate ordination methods (Brosse et al. 2001). To overcome these common drawbacks we used a Kohonen (unsupervised) artificial neural network (i.e., self-organising map algorithm, SOM; Kohonen, 2001) and the indicator species analysis by Tichý and Chytrý (2006). The advantage of both methods is particularly related to a lack of linearity assumptions and possibility of application for a binary matrix (Giraudel and Lek 2001; Tichý and Chytrý, 2006). Moreover, Kohonen artificial neural networks have already been validated for a wide range of ecological issues, including those relating to benthic macroinvertebrates (Bae et al. 2014; Chon 2011; Park et al. 2004, 2006, 2007; Penczak et al. 2006; Tszydel et al. 2009).

In order to improve the precision of biological surveillance and considering time efficiency reasons we examined if aquatic invertebrate data of increased taxonomical resolution but expressed on a binary abundance (frequent/rare) scale and referring to good bioindicator species only suffice to indicate clear gradients in water courses with high natural variability such as intermittent Mediterranean streams. We additionally tested the usefulness of a Kohonen artificial neural network and the indicator species analysis by Tichý and Chytrý (2006) for such a specific purpose.

2.2. Methods

2.2.1. Study area

The Quarteira stream system is located in a lowland coastal area in southern Portugal (37°11’20’’ N, 8°5’33’’W, Fig. 2.1). Its catchment area occupies 324 km^2 with an elevation range of 14-515 m. The stream is characterized by a Mediterranean-type climate, where most of the biological, chemical and physical processes are shaped by sequential events of annual flooding and drying (Gasith and Resh, 1999). Wet periods start in late October and last until March, with high discharge peaks, while from late June till September, the dry season proceeds, leaving temporarily disconnected pools or completely dry channels. The average annual air temperatures vary from 8 to 29 °C and average rainfall is 625 mm. Land use is mainly arable land accompanied by shrub and herbaceous vegetation, and mixed forests. Woody vegetation in the catchment consists of olive trees and other cultivation trees, such as almond, cork oak and citrus. While lower reaches are particularly associated with the giant reed (*Arundo donax*) that in some places forms impenetrable thickets, which are the dominant type of riparian vegetation. Such land use characteristics, along with scant urban development, makes the catchment relatively undisturbed. Catchment topography is characterized by a coastal plain, with a more pronounced relief in the north characterized by limestone and some karstic features. Most of the rock units in the basin are of a calcareous type with a dash of calcite-rich clays.

2.2.2. Macroinvertebrate sampling and criteria for candidate indicator species determination

Macroinvertebrates were sampled over 74 sites distributed along the Quarteira stream system from the middle of April until the beginning of July 2013 (Fig. 2.1).

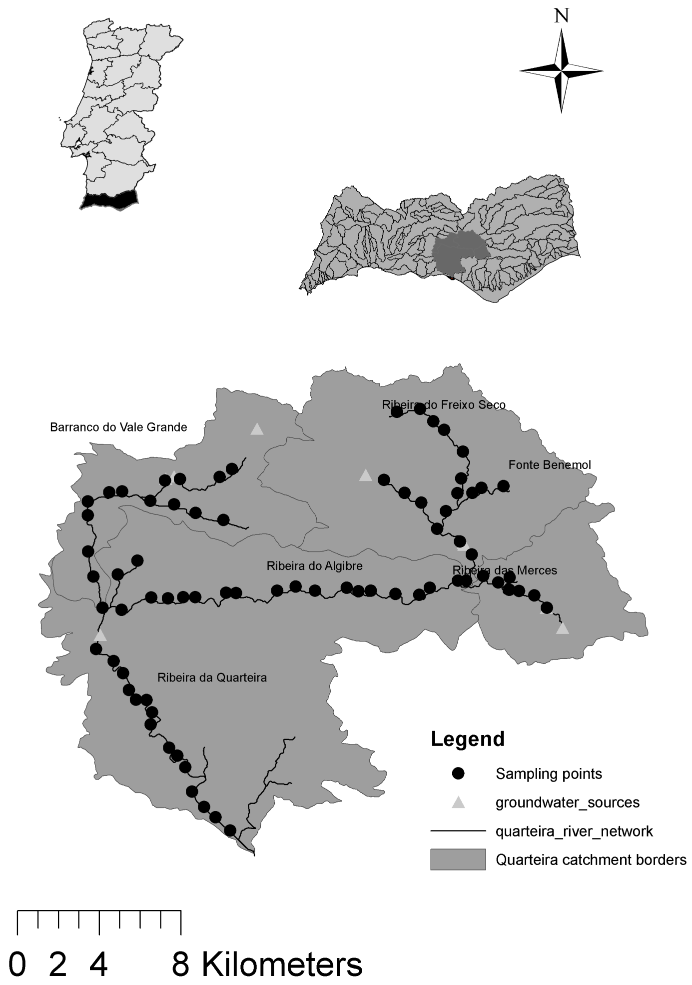


Fig. 2.1 Map of the 74 sampling sites in the Quarteira river catchment, South Portugal.

Sampled streams included: (1) perennial sites, fed by groundwater sources, which result in constant annual flow, (2) intermittent sites, which dry to a series of disconnected pools, and (3) ephemeral sites, which dry completely. Because the cessation of flow imposes a loss of connectivity between mesohabitats (riffles and pools), it is important to perform sampling during the period of steady flow when all the mesohabitats are connected. Because the process of flow cessation is very rapid, especially at the upstream parts of the catchment, some upstream sites (with a tendency to dry faster) had to be sampled earlier than sites located downstream. Such an approach was necessary to ensure sampling of all of the sites, before some of the sites turn into disconnected pools or completely dry channels. For this reason sites belonging to Freixo Seco and Barranco de Vale Grande were sampled from the middle of April to the beginning of May; Fonte Benemola and Ribeira das Merces streams were sampled from the beginning to middle of May and more downstream located in Algibre and Quarteira streams were sampled from the middle of May, through June until the beginning of July. Nevertheless, regardless the effort undertaken to sample during a continuous hydrological regime, at the time of sampling, some sites had already lost the hydrological connectivity and the water in these streams was present in the sequence of disconnected pools. Therefore these sites were classified as “sites with disconnected pools present”.

The protocol applied to sample macroinvertebrates was based on ‘Multi-habitat sampling’ (Hering et al. 2003) in accordance to monitoring techniques implemented by the EU Water Framework Directive (Directive 2000/60/EC). At each sampling site the most representative 300 m stretch was chosen. Next visually distinct areas of habitat cover were identified and respective proportion of the occurrence of each habitat in the stretch was recorded. The following habitats were sampled along the entire catchment: 1) mineral ones: megalithal (>40 cm), makrolithal (>20-40 cm), mesolithal (>6-20 cm), mikrolithal (>2-6 cm), akal (0.2-2 cm), psamal (from 6 μm to 2 mm), argyllal (<6 μm), and 2) organic ones: submerged macrophytes, emergent macrophytes, living parts of terrestrial plants, xylal (tree trunks, dead wood, branches), CPOM (deposits of coarse particular organic matter). According to the proportion of habitats present at each sampling site, 20 trawls (1 m long and 0.25 m wide) were sampled using standardized kick sampling technique with a hand-net (0.5 mm mesh, 25 cm width). Furthermore, the bulk of all of the subsamples collected at a given sampling site were treated as one sample, placed in a plastic container and preserved, for further identification, using 96% ethanol.

2.2.3. Criteria for candidate indicator species

The list of candidate species was chosen based on previous habitat-specific studies conducted in the Algibre Stream (pers. comm. Sroczynska et al. 2014). This stream was considered the most representative for the entire Quarteira Stream basin. Therefore, the selection of candidate indicator species was based on macroinvertebrate data from a 2-year (from February until August 2013 and 2014) sampling of that reach. Macroinvertebrate data included 180 habitat stratified samples (90 samples per year). The samples were identified to a lowest taxonomic level possible (mostly genus level, and, in the case of Ephemeroptera and Plecoptera, species level). From a database created in this way 17 candidate indicator species were chosen based on the following criteria: 1) species that were present in more than 10% but not more than 90% of the total number of samples; 2) indicators for good ecological status of water quality (based on species scores used in “Iberian Biomonitoring Working Party” – IBMWP) (Alba-Tercedor et al. 2002) so that the defined gradients would have ecological importance in terms of water quality assessment; 3) species with a high percentage of contribution to similarity within a given habitat resulted from SIMPER routine analysis (Similarity Percentage Contribution of PRIMER-E, Clarke & Warwick 2001). These criteria aimed to select the species that best represent the variety of habitats and reflect the variability of macroinvertebrate assemblages. Next, the selected 17 species were identified in samples from 74 sites from the Quarteira stream system. A species was considered “frequent” at a given sapling site when its frequency was higher than 5; otherwise when the frequency was in the range of 0-5 the species was considered as “rare”. The data set consisted of indicator species scores (frequent/rare) at each 74 sites was used for further analysis using SOM.

2.2.4. Environmental variables

Along with macroinvertebrate sampling, environmental variables such as water temperature [°C], air temperature [°C], [S m^{-1}], pH and dissolved oxygen concentration [mg dm^{-3}] were recorded using a multiparametric probe (YSI, Professional Plus model). Current water velocity [m s^{-1}] was measured using a two-dimensional flow tracker acoustic-Doppler velocimeter (ADV, Sontek YSI Inc., San Diego, California, United States). Water velocity was measured at two locations at riffle habitats and two locations in pool zones. Water samples for nutrient determinations of nitrite (NO_2-N), nitrate (NO_3-N), ammonium (NH_4^+-N) and phosphate (PO_4-P) were taken. Determinations for nutrients were done on a MERCK Spectroquant Nova 60, using Spectroquant®Test kits. Channel width and water depth was measured at 5 locations along the channel cross-section separate for riffle and pool zones and, for later analysis, the average water depth from each cross section was used. The percentage of shading was reported, along with other environmental variables (Fig. 2.3, Table 2.1, Appendix).

2.2.5. Data analysis

Homogenous groups of invertebrate samples were distinguished with a Kohonen unsupervised artificial neural network, i.e. a self-organising map (Kohonen 1982, 2001).

Artificial neural networks (ANNs) do not require *a priori* specification of the model underlying the analyzed phenomenon (Brosse et al. 2001). They recognize patterns in variables that are (1) expressed in any (including binary) scale of measurement, (2) exhibit normal or skewed distributions, and/or (3) are related in a complex way. ANNs are built of neurons (data-processing units), which are grouped into layers. The Kohonen ANN in this study was trained with the use of the SOM Toolbox (Vesanto et al. 2000), which was developed by the Laboratory of Information and Computer Science at the Helsinki University of Technology (http://www.cis.hut.fi/projects/somtoolbox) (Vesanto et al. 2000). The dataset used for the training consisted of the binary (0 – rare, 1 – abundant) data on 17 taxa in 74 invertebrate samples (i.e. one per sampling site). During the SOM training, the input layer of neurons served only as a flow-through layer receiving the data (Lek and Guégan, 1999). Because each input neuron received data related to one taxon, the number of input neurons was equal to the number of variables in the dataset (i.e. 17). Each input neuron transmitted repeatedly signals to each of the output neurons, which were arranged on a two-dimensional lattice. The intensity (weight) of each connection was strengthened or weakened. With this, a virtual invertebrate sample was created in each output neuron. The dissimilarity of virtual invertebrate samples was increasing along with the distance between the neurons they were created in. Moreover, the virtual invertebrate samples (and thus the respective output neurons) were clustered with use of the hierarchical cluster analysis (Ward linkage method with Euclidean distance measure) (Ward, 1963; Vesanto and Alhoniemi, 2000). Finally, each real invertebrate sample became assigned to the best matching virtual invertebrate sample (and the respective output neuron) (for details see subchapter 2.1 in Vesanto and Alhoniemi, 2000). Consequently, similar real invertebrate samples were located in the same neuron or in adjoining neurons, while those considerably different were grouped in distant regions of the SOM (Penczak et al., 2004; Bedoya et al., 2009; Penczak, 2011; Li et al., 2013; Bae et al., 2014). In the above-described way, the output neurons served for data structuring and output of results (Lek et al., 2005; Cheng et al., 2012; Stojković et al., 2013; Park et al., 2014).

Additionally, the SOM Toolbox allowed for the visualisation of the occurrence of each taxon in virtual invertebrate samples (and the respective output neurons), in the form of a greyness gradient. Because SOM does not provide any statistical verification of associations of invertebrate taxa with the SOM regions (and respective environmental conditions), we applied the indicator species analysis (ISA) by Tichý and Chytrý (2006) for binary variables. The ISA is based on fidelity expressed with the Φ coefficient of association, which was calculated from 2 × 2 contingency tables for each taxon and each SOM sub-cluster of invertebrate samples, and corrected for differently sized SOM sub-clusters. The Φ coefficient ranges from –1 (perfect negative indication) to 1 (perfect positive indication). Positive Φ values indicate that taxon occurrences are concentrated in a given SOM sub-cluster of invertebrate samples, and negative Φ values indicate that taxon occurrences are under-represented in a given SOM sub-cluster of invertebrate samples. The Monte Carlo randomization test additionally allows for assessment if the maximum Φ coefficient is significantly higher than others observed for a given taxon. If it is, the taxon is considered an indicator for the SOM sub-cluster of invertebrate samples for which the maximum Φ coefficient was observed (Tichý and Chytrý, 2006; Peck, 2011). The ISA analysis was carried out with PC-ORD statistical software (McCune and Mefford, 2011).

The significance of differences between the SOM sub-clusters in abiotic variables was assessed with the Kruskal-Wallis test and the *post-hoc* Dunn test.

2.3. Results

2.3.1. SOM partitioning

The SOM quantisation and topographic errors were 1.174 and 0.000, respectively. Two main clusters were distinguished in the output layer of SOM: X and Y (Fig. 2.2). Cluster X contained sub-clusters X_1 (with neurons A1-A3, B1, B2) and X_2 (A4, B3, B4, C3, C4), while cluster Y contained sub-clusters Y_1 (C1, C2, D1, D2), Y_2 (E1, E2, F1, F2) and Y_3 (D3, D4, E3, E4, F3, F4) (Fig. 2).

Sub-cluster X_1 encompassed the largest number of samples (21), which came from sites almost equally distributed among streams located in the upstream part of the streams system such as: Baranco de Vale Grande, Fonte Benemola, Freixo Seco and Ribeira das Merces (Fig. 2.2). Sub-cluster X_2 contained 17 samples, of which most (11 out of 17) came from the Barranco de Vale Grande Stream, four from Fonte Benemola, and remaining two from Freixo Seco and Ribeira de Algibre. Sub-cluster Y_1 contained the smallest number of samples (six), including two from Fonte Benemola and four from the Algibre Stream. Sub-clusters Y_2 and Y_3 encompassed only samples from downstream parts of the stream system, i.e. Ribeira do Algibre and Ribeira de Quarteira (Fig. 2.2).

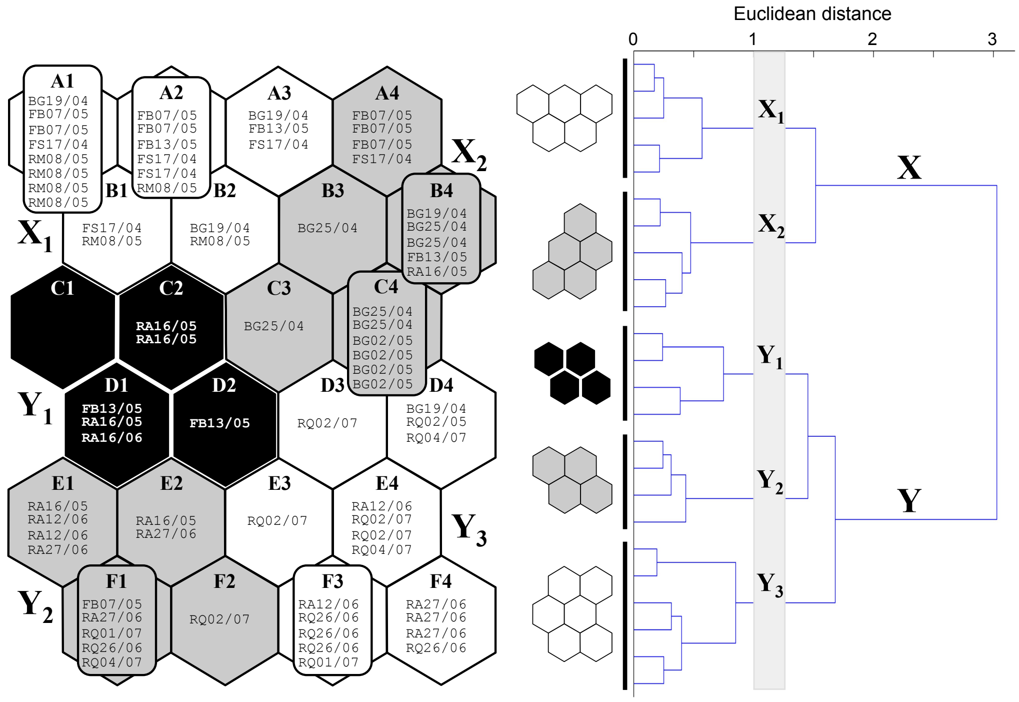


Fig. 2.2 The 24 SOM output neurons (A1-F4) arranged into a two dimensional lattice (6 × 4) with real invertebrate samples assigned. Two clusters (X, Y) and five sub-clusters (X_1, X_2, Y_1, Y_2 and Y_3) were distinguished with the hierarchical cluster analysis. The code of a sample consists of: first two initials of the river sampled (BG – Barranco de Vale Grande; FB – Fonte Benemola; FS – Freixo Seco; RA – Ribeira do Algibre; RM – Ribeira das Merces; RQ – Ribeira da Quarteira), followed by two pairs of digits (separated with a slash) for the day and the month of sampling.

There was a clear temporal pattern in sample assignation to the sub-clusters. Samples in sub-clusters X_1 and X_2 were collected at the beginning of the spring period, from the end of April until the middle of May. Sub-cluster Y_1 contain only samples from the middle of May, while the sub-clusters Y_2 and Y_3 cover the period of early summer, starting from late May until the beginning of July (Fig. 2.2). Overall, the highest resemblance in this respect was between the sub-clusters Y_1 and Y_2 (Fig. 2.2).

2.3.2. Environmental variables

Despite the fact that the SOM sub-clusters were distinguished on the basis of biotic data, they significantly differed also in the abiotic conditions at the sites that the samples were collected in. For some variables a clear trend was recorded for the sequence of sub-clusters X_1-Y_3. This trend was downward for elevation and upward for water temperature and conductivity (Fig. 2.3).

Therefore, samples assigned to sub-cluster X_1, which came from streams located at the highest elevations, recorded the lowest water temperature and conductivity. As the elevation was declining, temperature and conductivity were continually increasing across the rest of the sub-clusters eventually reaching their maximum in sub-cluster Y_3 (Fig. 3). Correspondingly, Kruskall–Wallis and *post-hoc* tests revealed significant differences ($p<0.05$) in water temperature and conductivity between the pairs of the most marginal sub-clusters X_1, X_2 and Y_2, Y_3. Elevation significantly differed between sub-cluster X_1 and Y_2, X_1 and Y_3, and X_2 and Y_3 (Fig. 3). Median concentration of dissolved oxygen slightly increased from sub-cluster X_1 to Y_1, and then more rapidly decreased from sub-cluster Y_1 to Y_3 (Fig. 3). A significant difference in dissolved oxygen was only reported between sub-cluster Y_1 and Y_3, and the highest variation in dissolved oxygen was reported for X_2. There were no differences in pH except for the one between sub-clusters X_1 and X_2. Channel width, measured at pool section, was significantly lower for sub-clusters X_1 and X_2 relative to Y_2 and Y_3. The percentage of shading decreased downstream, displaying significant differences between higher riparian cover (X_1) and more open sites (Y_2, Y_3) (Table 1). Significant differences between sub-clusters were detected for ammonia, phosphates and channel width at riffle section. Nitrites, although statistically different among habitats were mostly below the detection limit. The remaining variables, such as current velocity and depth at riffle and pool section were not significantly different ($p>0.05$) among any of the sub-clusters (Table 1).

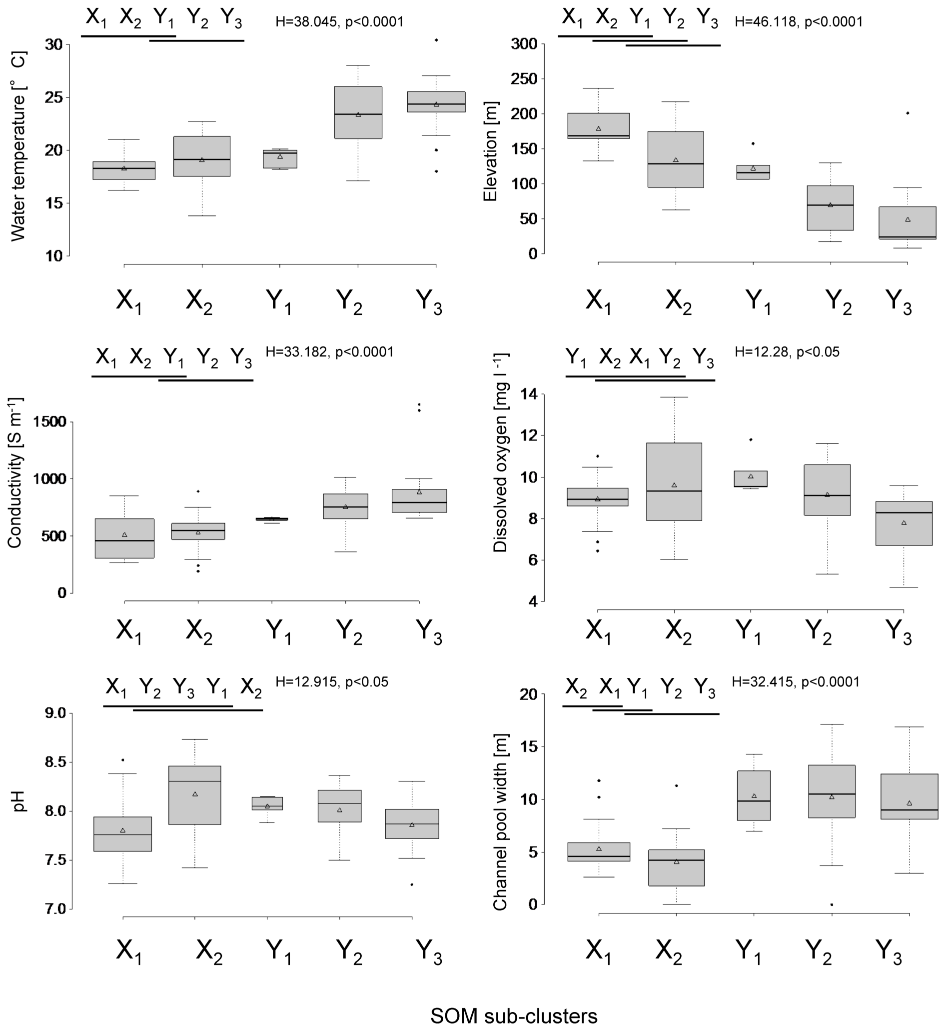


Figure 2.3 Differences between the SOM sub-clusters in abiotic variables, which were not presented directly to the Kohonen artificial neural network. Triangle – mean, horizontal line – median, black points– outliers, box – interquartile range, whiskers extend from minimum to maximum values. The width of each box is scaled in proportion to the number of replicates for each habitat. H – statistics of the Kruskal-Wallis test (df = 4, $N_{X1} = 21$, $N_{X2} = 17$, $N_{Y1} = 6$, $N_{Y2} = 12$, $N_{Y3} = 18$) applied in testing the differences between SOM sub-clusters. For SOM sub-clusters that are underlined with the same line no difference at $p \leq 0.05$ was recorded in *post-hoc* tests. For more abiotic variables see Table 2.1.

Table 2.1 Summary of environmental variables not included in boxplot presentation (based on measurements at 74 sampling points located in Quarteira River Basin in 2013). Medians (first and third quartile) are given for each SOM sub-cluster X_1-Y_3.

Environmental Variable	X_1	X_2	Y_1	Y_2	Y_3	Chi-squared	P
ammonia [mg dm^{-3}]	0.13 (0.08-0.44)	0.55 (0.14-0.72)	0.06 (0.05-0.07)	0.08 (0.05-0.08)	0.08 (0.06-0.10)	24.83	<0.001
nitrite [mg dm^{-3}]	0.01 (0.00-0.01)	0.01 (0.01-0.01)	0.00 (0.00-0.01)	0.01 (0.00-0.01)	0.01 (0.01-0.01)	14.77	0.005
nitrate [mg dm^{-3}]	1.30 (0.18-2.70)	0.34 (0.26-0.44)	1.30 (0.34-3.17)	0.50 (0.28-2.12)	0.50 (0.10-1.45)	3.32	0.506
phosphorus [mg dm^{-3}]	0.14 (0.10-0.99)	0.08 (0.06-0.15)	0.12 (0.06-0.12)	0.09 (0.02-0.19)	0.04 (0.00-0.08)	15.32	<0.05
current velocity (riffle) [m s^{-1}]	0.48 (0.41-0.57)	0.49 (0.40-0.52)	0.73 (0.60-0.86)	0.50 (0.39-0.72)	0.38 (0.28-0.50)	7.57	0.109
current velocity (pool) [m s^{-1}]	0.06 (0.04-0.12)	0.12 (0.11-0.13)	0.14 (0.09-0.20)	0.06 (0.02-0.12)	0.05 (0.03-0.06)	3.53	0.473
depth (riffle) [m]	0.11 (0.10-0.13)	0.11 (0.10-0.14)	0.16 (0.15-0.17)	0.11 (0.09-0.25)	0.10 (0.08-0.11)	5.41	0.248
depth (pool) [m]	0.26 (0.20-0.38)	0.27 (0.23-0.34)	0.45 (0.29-0.67)	0.23 (0.13-0.30)	0.41 (0.30-0.47)	6.39	0.172
width (riffle) [m]	3.70 (2.90-4.40)	4.20 (3.00-4.60)	7.20 (6.30-7.60)	6.20 (5.30-8.10)	6.50 (5.60-9.00)	11.9	<0.05
shading [%]	25 (20-70)	30 (6.00-50)	21 (10-30)	4 (2.00-6.00)	5 (2-10)	20.87	<0.001

2.3.3. Indicator species analysis

All the taxa, except one (*Ferrisia wautieri*), were significantly associated with particular SOM sub-clusters (Table 2.2 and Fig. 2.4). The highest numbers of indicator species were identified for sub-clusters Y_2 (seven) and X_1 (five, Table 2.3). Three taxa were significantly associated with Y_1, one with Y_3 and none with X_2. Typical rheophilic species such as *Habrophlebia fusca, Isoperla moselyi, Tyrrhenoleuctra minuta* were significantly more common in sub-cluster X_1, linked to colder water and more abundant riffle sections, in comparison with cluster Y characterized by higher water temperatures and prevalence of pool sections (Fig. 2.4, Table 2.2, 2.3). X_2 was the only sub-cluster that not only did not have any species significantly associated with it, but additionally was avoided by almost all of the taxa (Fig. 2.4, Table 2.2). Characteristic taxa for the Y_1 sub-cluster included: rheophilic and predator *Oulimnius* sp. (adult) and *Melanochelia riparia*, and more associated with deep, lentic habitats *Centroptiulm luteolum*. Sub-cluster Y_2 was favorable for all the taxa belonging to Trichoptera group: *Hydropsyche lobata*, *Chimarra marginata*, *Hydroptila vectis*, as well as *Oulimnius* sp. (larvae), *Caenis luctuosa, Baetis atrebatin*us and *Physella acuta* (Fig. 2.4, Table 2.2). Conditions associated with Y_3 (the lowest elevation, abundant aquatic vegetation) were strongly preferred by *Atyaephyra desmarestii*, which is tolerant to wide salinity and temperature ranges (Fig. 2.4, Table 2.2, 2.3).

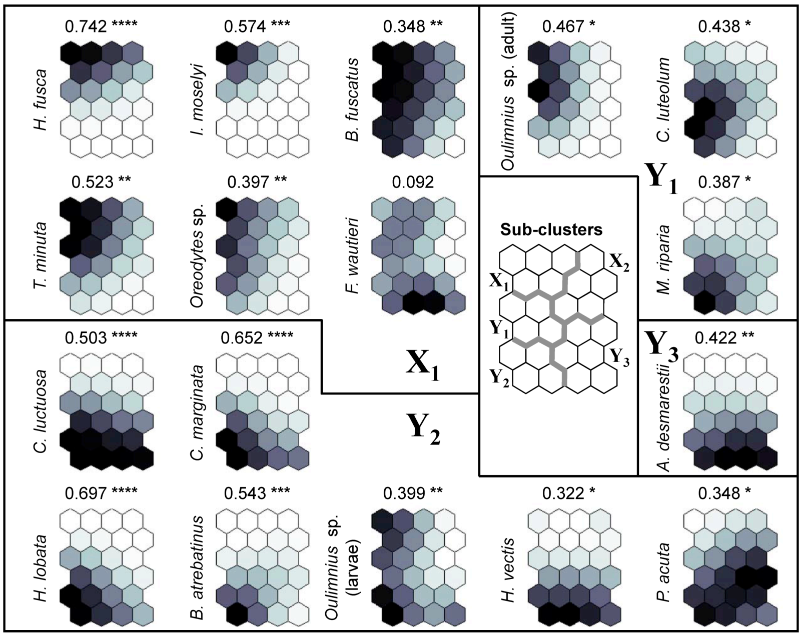


Fig. 2.4 Associations of aquatic invertebrate taxa with sub-clusters of neurons X_1-Y_3. The intensity of the greyness, higher for stronger associations, is based on virtual aquatic invertebrate samples and scaled independently for each taxon. Taxa with the similar patterns of greyness occurred in similar environmental conditions. The Φ coefficient and the significance level (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$) are calculated on the basis of real invertebrate samples, and presented above each taxon plane. No species was associated with X_2. For more details see Table 2.

Table 2 Indicator species analysis: Φ coefficients (range from -1 to 1, i.e. from maximum positive association to maximum negative association, respectively) for particular taxa in SOM sub-clusters X_1-Y_2 (compare with Fig. 2.4).

Genus/Species	SOM sub-cluster				
	X_1	X_2	Y_1	Y_2	Y_3
Atyaephyra desmarestii	-0.294	-0.280	-0.066	0.301	0.422
Baetis atrebatinus	-0.158	-0.152	-0.138	0.543	-0.049
Baetis fuscatus	0.348	-0.130	0.332	0.270	-0.609
Caenis luctuosa	-0.529	-0.491	0.460	0.503	0.501
Centroptiulm luteolum	-0.123	-0.099	0.438	0.299	-0.192
Chimarra marginata	-0.341	-0.323	0.203	0.652	0.124
Ferrisia wautieri	0.092	-0.202	0.040	0.044	0.049
Habrophlebia eldae	0.742	-0.098	-0.149	-0.335	-0.361
Hydropsyche lobata	-0.317	-0.302	0.234	0.697	0.025
Hydroptila vectis	-0.222	-0.197	-0.053	0.322	0.226
Isoperla moselyi	0.574	-0.178	-0.161	-0.170	-0.180
Melanochelia riparia	-0.223	-0.130	0.387	0.348	-0.055
Oreodytes sp.	0.397	-0.245	0.369	-0.022	-0.316
Oulimnius sp. adult	0.250	-0.178	0.467	-0.170	-0.180
Oulimnius sp. larvae	0.229	-0.377	-0.226	0.399	-0.092
Physella acuta	-0.388	0.021	-0.052	0.348	0.178
Tyrrhenoleuctra minuta	0.523	-0.176	0.440	-0.259	-0.373

2.3.4. Conceptual classes

Based on the distinguished sub-clusters, with associated abiotic variables, we defined conceptual classes, summarized in Table 2.3, to which we additionally assign qualitative information collected at each site.

Headwaters with riffles - Samples assigned to sub-cluster X_1 were collected during spring time, at higher elevation range. They were distinguished by moderate current velocity, narrow channel width and high number of riffles present. Biological habitat characteristic for them included inorganic habitats with a predominance of cobbles covered by filamentous algae and relatively high percentages of organic substrate, mainly of terrestrial origin, such as xylal, coarse particulate organic matter (CPOM) and macrophytes. Nearly half of the samples belonging to this sub-cluster came from perennial sites, with water flowing over the entire year. Indicator species for this sub-cluster were exclusively rheophilic with a capacity to remain within the substrate, at stronger currents.

Stagnant channels - Samples in sub-cluster X_2 were collected in spring time at generally lower elevations than the samples from X_1. Nearly half of the samples grouped in this sub-cluster came from first order channels, characterized by low current velocity, small depth and width, and containing a high percentage of very fine inorganic substrates with extensive cover of aquatic vegetation. This sub-cluster also contained frequent (41%) sites where only disconnected pools were present. Such conditions were not significantly preferred by any of the studied species. Physical and chemical variables (e.g. dissolved oxygen, temperature and pH) exhibited the highest variability in this sub-cluster in comparison with remaining sub-clusters.

Deep run channels - Sub-cluster Y_1 comprised mostly of samples from May. It was characterized by the highest average water velocity measured at riffles and the greatest depth in comparison to other sub-clusters, and also by wide channels and the presence of coarse mineral substrates (nearly 90% of the substrate cover). Although it contained only six samples, three species exhibited a significant preference to the above-mentioned conditions.

Deep channels with riffles - Sub-cluster Y_2 included the samples collected during summer at sites with significantly higher average temperature, in comparison with sub-clusters X_1 and X_2. This sub-cluster was restricted to second and third order, wide streams with fast currents and an abundance of riffles (except for one sample). The main type of substrate for this sub-cluster included coarse, mineral fractions, usually covered by filamentous algae, with only low percentage of aquatic vegetation. This sub-cluster exhibited the highest number of indicator species, mainly rheophilic ones, which belong to the Trichoptera group and one ephemeropteran – *B. atrebatinus*. However, a species characteristic for lotic environments, *C. luctuosa,* was also significantly associated with this sub-cluster.

Overvegetated downstream channels - Y_3 sub-cluster contained samples collected only during summer at downstream sites. The last were characterized by large channel width, slow current (riffles were present in only one third of sites), the highest water temperatures and the lowest median concentrations of dissolved oxygen. Coarse mineral substrates on their beds were densely covered by filamentous algae and aquatic vegetation. There was only one indicator species for this sub-cluster, i.e. the freshwater shrimp *A. desmarestii*.

Table 2.3 Summary of characteristics of each SOM sub-cluster. Samples and associated environmental variables were collected from 74 sampling points located in Quarteira River Basin in 2013.

Conceptual class	Headwaters with riffles	Stagnant channels	Deep run channels	Deep channels with riffles	Overvegetated downstream channels
SOM subcluster	**X_1**	**X_2**	**Y_1**	**Y_2**	**Y_3**
Time of the year	End of April, beginning of May	End of April, middle of May	Middle of May	Late May – middle July	End of June – beginning of July
Temperature range [°C]	16.20-21.00	13.80-22.70	18.20-20.10	17.10-28.00	18.00-30.40
Elevation range [m]	132.35-236.18	62.96-217.59	106.46-157.52	17.21-129.53	7.80-94.04
Average [%] cover of each habitat type (only present habitats are listed)	megalithal (≈5) macrolithal (≈7) mesolithal (≈35.5) mikrolithal (≈29) akal (≈6) psammal (≈1.7) filamentous algae (≈4.6) sumbmerged macrophytes (≈ 1.3) emerged macrophytes (≈7) living parts of terrestrial plants (≈0.2) xylal(≈ 1.2) CPOM (≈1.5)	megalithal(≈3) macrolithal (≈16) mesolithal (≈20) mikrolithal (≈29) akal (≈8) psammal (≈1) argyllal (≈4) filamentous algae (≈6.5) sumbmerged macrophytes (≈ 2) emerged macrophytes (≈9) living parts of terrestrial plants (≈0.3) xylal(≈ 1)	macrolithal (≈21) mesolithal (≈31) mikrolithal (≈36) akal (≈10) psammal (≈1) xylal (≈1)	megalithal (≈8) macrolithal (≈14) mesolithal (≈37) mikrolithal (≈30) akal (≈8) psammal (≈0.8) emerged macrophytes (≈0.8) CPOM (≈0.8)	megalithal (≈5) macrolithal (≈12) mesolithal (≈23) mikrolithal (≈25) akal (≈20) psammal (≈5) argyllal (≈1) sumbmerged macrophytes (≈ 7.4) emerged macrophytes (≈1) living parts of terrestrial plants (≈0.8)
Average [%] biofilm cover	28	12	50	50	39
Inorganic (mineral) habitats [%]	84	81	99	98	91
Organic habitats [%]	16	19	1	2	9
Sites with riffles [%]	86	44	67	100	34
Sites with pools [%]	100	94	100	92	100
Sites with intermittent regime [%]	57	59	83	100	100
Perennial sites [%]	38	—	17	—	—
Sites with disconnected pools only [%]	5	41	—	—	—
Stream order	Second to third order	Half of the sampling sites were first order streams	Second to third order	Second to third order	Second to third order
Indicator species	*Habrophlebia eldae, Isoperla moselyi,Tyrrhenoleuctra minuta, Baetis fuscatus, Oreodytes*	–	*Oulimnius* adult, *Centroptiulm luteolum, Melanochelia riparia*	*Caenis luctuosa, Hydropsyche lobata,Oulimnius larvae, Chimarra marginata, Hydroptila vectis, Baetis atrebatinus*	*Atyaephyra desmarestii*
Summarized channel characteristics	First order headwater streams with moderate current velocity, abundance of riffles, small channel width, dominated by cobble substrate and organic habitats. Substantial amount of riparian cover. Most of the perennial sites are included in this cluster	Mostly first order small channels with very slow current velocity, narrow channel width, small percentage of riffles present and high variation in dissolved oxygen and pH. Almost half of the sites are dominated by disconnected pools. Abundant aquatic vegetation and dominance of very fine mineral substrates, i.e. clays.	Second and third order streams with moderate current velocity, characterized by deep and wide channel, almost exclusively dominated by mineral substrates, influenced by intermittent regime	Second and third order streams, with abundance of riffles, dominated by mineral substrates, characterized by wide channel width and open canopy cover, with exclusively intermittent regime	Second and third order of lowland streams with slow current velocity, wide channel width, dominance of pool zones, with lower levels of dissolved oxygen. Dominated by mineral substrates, however with some abundance of macrophytes and algae. Open canopy cover. Exclusively intermittent regime.

2.4. Discussion

SOM analysis identified five stream categories. The most evident variable that drives this categorization, is related to a longitudinal (upstream-downstream) gradient additionally reinforced by altitude. Such longitudinal zonation was broadly reported earlier in the literature for aquatic insects (Allan 1975; Perry and Schaeffer, 1987; Statzner and Higler 1986). Consequently, the main physico-chemical differences between distinguished (sub)clusters are mainly observed between the upstream and downstream sites. The differences are particularly related to water temperature and conductivity, however percentage of riparian cover, channel width and occurrences of in-stream habitats additionally contributed to the observed pattern.

The classification also revealed a seasonal (spring/summer) gradient, which overlaps spatial zonation pattern. Seasonality has previously been reported for intermittent streams based on entire community quantitative characteristics by Beche et al. (2006) and Bonada et al. (2006a). These studies highlight changes in hydrological regimes among spring (wet) and summer (dry) seasons, which in turn leads to a progressive replacement of rheophilic taxa with lentic ones, i.e. adapted to slow current velocity and/or stagnant waters. In our study, this gradient is related to temperature rather than to flow cessation. This is demonstrated by the fact that we have not found any significant differences between the sub-clusters in current velocity. The increase in temperature in downstream sites is associated with lower riparian cover and wider channel width recorded downstream, but also with temporal delay resulting from the sampling strategy. It can be argued that, if all of the samples were collected simultaneously, the spatial pattern would be so evident. However, considering our results and also previous studies it appears that spatial zonation associated with altitude and conductivity are the main determinants of macroinvertebrate communities in temporary streams (Aguiar et al. 2002; Chaves et al. 2005; Graça et al. 1989; Pires et al. 2000). Chaves et al. (2005) found that sites located at higher altitudes with lower conductivity values had different macroinvertebrate assemblages compared with sites located downstream and with higher conductivity. These spatial patterns in macroinvertebrate distribution were also observed irrespectively of temporal variability (Chaves et al. 2005). Furthermore, Marchant et al. (1994) identified altitude, substratum and conductivity as the most obvious patterns in community composition in Australian rivers. We observed an increasing conductivity gradient downstream, which coincides with contribution of the groundwater and karst conduits carrying capacity along the longitudinal gradient as demonstrated by Salvador et al. (2012) in the same river basin. Moreover other studies showed only weak differences in macroinvertebrate assemblages between spring and summer for Portuguese streams (Chaves et al. 2006, Cortes et al. 1998). This is probably related to the fact that most of the macroinvertebrate traits are linked to specific flow regime and dissolved oxygen concentration and the community transition from lotic to lentic taxa only occurs when the flow starts to cease starting to form disconnected pools (Boulton 2003). Therefore, since the connectivity between mesohabitats is still maintained over spring/summer months (as demonstrated by the hydrological data), seasonal effect related to temperature is of secondary importance. Therefore, the sampling strategy probably influenced the current classification by highlighting the temporal variation, but it seems unlikely that temporal patterns overrode longitudinal zonation related to conductivity and altitude (Chaves et al. 2005; Marchant et al. 1994). Separation of the temporal effects from longitudinal influence requires synchronous sampling at high and low elevations in each season, which is logistically very challenging in this type of streams.

The longitudinal gradient in the sequence of sub-clusters X_1-Y_3 also reflects changes in percentage and type of substrate cover. Although all the streams were dominated by mineral, coarse substrate, there are evident differences in amounts of organic matter between the sub-clusters.

Additionally, SOM grouped sites of similar hydrological regimes. While sites in perennial streams were mostly grouped in sub-cluster X_1, those in intermittent and ephemeral streams primary were assigned to sub-clusters Y_1, Y_2 and Y_3, and the sites where only disconnected pools remained were almost exclusively enclosed in sub-cluster X_2, for which no indicator taxa were identified (Fig. 2.2, 2.4). Taxa with similar characteristics (rheophilic, sensitive) were significantly associated with both perennial and intermittent streams. Furthermore streams of both types were present together at sub-cluster X_1 and Y_1, which demonstrates that there was no separate category for only perennial or intermittent streams. Sub-cluster X_2, with abundant disconnected pools, was the only one, to which almost all of the studied species had negative associations (Table 2.2). The unsuitability of this sub-cluster for majority of species was also demonstrated by low current and relatively low percentage of coarse inorganic substrate which are known to positively influence taxa occurrences (Duan et al. 2008). Stream temporality is difficult to predict because it depends on many factors operating at local, catchment and regional scales. Nevertheless, accurate assessments of hydrological states (especially in reference sites) across temporary water bodies are pivotal to decide whether the ecological status of this stream can be assessed using the same methods as in permanent streams. Recent approaches to differentiate water bodies according to their aquatic state are based on hydrological data of the stream (Gallart et al. 2012). However, such data are usually absent for Mediterranean intermittent streams. The fact that SOM was efficient in grouping streams with distinct flow regimes (based only on data on certain macroinvertebrate indicators) shows that it has potential to classify temporary streams according to flow connectivity when hydrological data are missing. Additionally, classification based on direct field observations can be biased. For example sub-cluster X_2 included sites with disconnected pools, but also sites with continuous flow regime. If the ecological status evaluation was based on field observation half of these sites were classified as sites with continuous flow regime and would be treated in further ecological evaluation as permanent sites. However, as demonstrated by SOM, these sites were ecologically closer to disconnected pools than to flowing waters. This was also demonstrated by the lack of any indicator species associated to that sub-cluster. Methods for aquatic state classifications in temporary streams using biological metrics have just recently started to be developed (i.e. Bio-AS Tool by Cid et al. 2015). In this context our study may contribute to current development of new methodological frameworks that permit to predict the aquatic state of temporary streams for more adequate application of bioassessment methods.

Interestingly, current velocity measured at riffles and also at pools connected to riffles did not differ significantly between sub-clusters. However, the percentage of sites where riffles and pools were present differed between sub-clusters. Pools were present at all of the sites, but riffles were more abundant at sites belonging to sub-cluster X_1 (86%) and Y_2 (100%), while in Y_3 the frequency of sites with riffles was only 34%. This demonstrates that geomorphology and groundwater delivery responsible for the percentage of riffles and pools present, determined the classification to a greater extent than local variables, related to specific flow regime.

The complexity of species response to environmental variables usually causes distortions in analyses of abiotic-biotic relations, which often are non-linear and burdened with the horseshoe effect due to unimodal species response curves. Therefore, robust (i.e. capable of tackling the non-linearity problem) ordination techniques should be a prerequisite in studies focused on aquatic ecosystem integrity assessment. In this study the SOM analysis, based on binary abundance data of selected indicators species (i.e. not the full community structure) was proved useful for identifying gradients of altitude and water temperature, which also overlap with the smaller scale erosional-depositional substratum gradients. Moreover, SOM was efficient in detecting streams with distinct flow regime by allocating all disconnected pools to one sub-cluster. Therefore, SOM can be alternatively used to assess the flow connectivity gradient for better establishment of reference sites.

2.4.1. Indicator species

Abiotic factors that exhibited some clear trends associated with our biotic-data-based classification of sites greatly coincide with the large body of literature describing patterns in macroinvertebrate community distribution (Prenda and Gallardo-Mayenco 1999; Brittain, 1990; González et al. 2001; Cummins 1967; García and Ferreras-Romero 2008). This fact demonstrates that the set of selected indicators sufficiently covers the variety of traits and preferences of macroinvertebrates that define large-area variations in community compositions. It additionally validates the criteria used for species selection.

Family level taxonomy can be more appropriate in the analysis of streams belonging to diverse ecotypes (Sanchez-Montoya et al. 2007), or with a large variation in environmental variables (Johnson et al. 2004), but such low-resolution taxonomical identification can obscure detection gradients in streams belonging to the same ecotype with a narrow range of physiochemical variability. In this study, for example, two closely related species belonging to the same *Baetis* genus: *B. fuscatus* and *B. atrebatinus* exhibited distinct preferences (for X_1 and Y_2, respectively). This demonstrates that individual traits operate at the species level and it highlights the importance of species level identification when investigating large-scale variables that drive macroinvertebrate distributions in temporary streams. Moreover, we found differences even in occurrences between different developmental stages of the same genera (*Oulimnius* sp.). A distributional overlap between larval and adult stage of water beetles are commonly observed, however our ISA demonstrates a distinct niche usage in larval and adult phases (significant associations with Y_2 and Y_1, respectively). In our study 16 out of 17 taxa were significantly associated with the SOM sub-clusters, thus strongly influencing the stream classification and highlighting their importance as indicators.

Sites in perennial streams were not distinguished as a separate (sub)cluster, which indicates that both perennial and intermittent streams have similar characteristics, which allows common indicator species to exist in both environments. However, this was only valid for sub-cluster X_1, in which the majority of sites in perennial streams were embraced including those with groundwater effluents. This result is consistent with community studies by Grubbs et al. (2011) who found a taxonomic similarity in macroinvertebrate assemblages in perennial and temporary forested headwater streams. Additionally, for some species used in our classification there are records which demonstrate that these species are equally found in perennial as well as intermittent rivers (García and Ferreras-Romero 2008; Prenda and Gallardo-Mayenco 1999). Therefore, an ecological quality assessment can be done considering perennial and intermittent streams together, however, only at similar altitude, and the calendar of sampling must allow capturing similar water flow regimes in both kinds of habitats. Sampling perennial and temporal reaches at different flow regimes would likely result in either high stream-to-stream variation in macroinvertebrate composition (Robson et al. 2005) or strong differences in assemblages among perennial and intermittent streams (Garcia-Roger et al. 2011).

Our set of studied species was distinguished on the basis of extensive sampling of diverse habitats, and one of the selection criteria was the occurrence in less than 90% of samples. This was to avoid species that were too tolerant. Therefore the fact that sub-cluster X_2 had no indicator taxon (among those studied more stenotopic ones) is consistent with recent studies on temporary streams by Sheldon et al. (2010) and Bonada et al. (2007), which demonstrated that invertebrate assemblages in pools during the disconnection phase are impoverished, dominated by generalist taxa and exhibit overall low diversity. Although disconnected pools can serve as temporary refuges for some taxa trapped in drying-up habitats, in the end an increased nutrient level, siltation and high temperature as well as variation in oxygen concentration may become lethal for more sensitive species (Lake, 2003). For that reason, we agree that disconnected pools should be either addressed separately in river quality assessment programs or should be excluded from the biomonitoring of ecological status (Watson and Dallas 2013).

2.5. Summary and implications for biomonitoring

The current categorization by using the binary data on abundance of selected indicator species sufficed to determine five clusters of sites along the altitude and seasonal gradients. Additionally we recognized the type of substrate, riparian cover and frequency of riffles as factors that matched the recorded longitudinal and seasonal differences in stream characteristics and helped to define the site classes. Because the temporal pattern was difficult to unravel from altitude influence, future studies should sample high and low elevation sites during the same period.

Ecological studies of stream biota in Mediterranean regions are scarce (Filipe et al. 2012) and those from Portugal are mainly restricted to mid-north regions (Chaves et al. 2005, 2006, 2008), and thus the ecology of temporary streams is poorly understood (Aguiar et al. 2002, Hughes et al. 2009, Leitão et al. 2014). Although the WFD protocol applied in Portugal was adapted to Southern Portuguese rivers it is applied uniformly for the entire region, characterized by a calcareous type of geology. Our study demonstrates that this generalization will likely result in inaccurate ecological quality metrics derived from streams, due to the influence of major geomorphological and climatological variables that limit the occurrences of indicator species at finer scales, such as in our case the catchment scale within the Mediterranean ecoregion.

The Kohonen artificial neural network was effective in recognizing patterns in the binary data referring to only a set of indicator species. This effectiveness was reflected not only by significant preferences revealed with the ISA of almost all the studied taxa to certain states of abiotic variables, but also by several distinguished simultaneous gradients in abiotic data (despite the fact that they were not presented to the artificial neural network). As there is a strong need for cost-effective and rapid methods for classification of river systems, we recommend the combined use of SOM and ISA in similar type of heterogeneous Mediterranean catchments.

Acknowledgements

The authors specially acknowledge Samantha Hughes, Simone Varandas and Maria Schröder for their help in taxonomic identification of macroinvertebrates and species selection. The following work was a part of the IMPACT project funded by Foundation for Science and Technology (FCT) of Portugal (ERA-IWRM/0003/2009). Comments by two anonymous referees substantially improved the original manuscript.

Chapter 3

Independent effects of habitat and stream typology on macroinvertebrate communities and water quality index in Mediterranean-type Intermittent streams

K. Sroczyńska[1], F. Leitão[2], I. Máximo[2], P. Range[3], A. Furtado[4] , M. Claro[2] and L. Chícharo[1]

[1] Faculty of Science and Technology, Campus of Gambelas, University of Algarve 8005-139, Faro, Portugal. Corresponding author: kasiasroczynska@gmail.com
[2]Centro de Ciências do Mar, Universidade do Algarve, Campus de Gambelas 8005-139 Faro, Portugal
[3]Department of Biological and Environmental Sciences, College of Arts and Sciences, Qatar University, Doha, Qatar
[4]APA-ARH, Algarve—Administração da região Hidrográfica do Algarve, I.P., Faro, Algarve, Portugal

This chapter was submitted for the publication as:

Sroczyńska K, Leitão F, Máximo I, Range P, Furtado A, Claro M and Chícharo L. in review. Independent effects of habitat and stream typology on macroinvertebrate communities and water quality index in Mediterranean-type Intermittent streams.

Abstract

Macroinvertebrate-based water quality assessment in temporary streams is an important yet still understudied issue. Investigating different aspects of macroinvertebrate ecology in these streams is, therefore, highly necessary for the successful implementation of bio-assessment programs. We investigated the variability in macroinvertebrate community metrics and water quality index among different habitat types and stream typologies in intermittent Mediterranean streams.

The structure of benthic communities showed differences among both habitats and typologies, but there was no interaction among these two factors, indicating that the effect of stream typology does not depend on the habitats that are present in the given stream type. Overall community structure was similar among mineral substrates and macrophytes, which was also reflected in low number of taxa with significant indicator values suggesting the prevalence of generalist tactic and low selectivity in terms of habitat partitioning at these temporary streams. Much higher number of indicator taxa was found for different stream typologies providing evidence that stream types are better predictor for species occurrences than a habitat unit at this scale. Further, we reported significant effects of Habitat and Typology for water quality index. Differences were particularly between gravel and organic or depositional habitats (macrophytes/sand/POM). For the typology differences were observed between calcareous and non-calcareous stream types.

Although the Water Framework Directive (WFD) protocol applied in Portugal was adapted to Southern Portuguese rivers, we propose an additional adjustment particularly differentiating calcareous from non-calcareous stream types with special acknowledge of small mountainous streams. Additionally, due to observed differences in water quality index among organic and non-organic habitats, sampling at the reach scale should include both types of substrate.

Key words: Algarve streams, *IPtIs* index, intermittent rivers, species assemblages, spatial scale

3.1. Introduction

The ecological assessment of intermittent rivers and streams using benthic macroinvertebrates is an unresolved issue, in particular in Mediterranean countries and, therefore, studies that approach different aspects of the implementation of macroinvertebrate based water quality indexes are necessary. The main constrains of these indexes are related to complex, multi-scale way that benthic macroinvertebrates respond to their environment (Johnson and Hering, 2010). Macroinvertebrates possess very diverse traits and morphological adaptations that allow them to live in certain range of environmental conditions (Aguiar et al. 2002; Chaves et al. 2005; Graça et al. 1989; Pires et al. 2000). This is why they often display strong affinities for different substrates, oxygen concentrations and food resources (Beisel et al. 1998, Chaves et al. 2005, Cummins and Lauff 1967, Merrit and Cummins 1996, Pardo and Armitage 1997, Schröder et al. 2013, Townsend and Hildrew 1994). Consequently, previous studies on structural dynamics of benthic fauna demonstrated strong influence of habitat characteristic on abundance, diversity and the trophic structure of macroinvertebrate assemblages (Bonada et al. 2006a, Beisel et al. 2000, Brown 2003, Kubosova et al. 2010, Pardo and Armitage 1997). The affinities displayed by certain groups of macroinvertebrates to particular areas of substrate prompted the use of habitat for better managing of stream biota (Armitage and Pardo 1995, Harper and Everard 1998). For example, various studies found greater similarity in assemblages of species within the same habitat types, among different sites, whereas, much lesser resemblance was found between fauna from different habitats, within one site (Angradi 1996, Bonada et al. 2008, Parsons and Norris 1996, Rabeni et al. 2002). This multi-scale heterogeneity of macroinvertebrate distribution patterns causes the variation in biological metrics and water quality indexes, which limits their comparability across the streams (Sánchez-Montoya et al. 2007, Sánchez-Montoya et al. 2009a, Sánchez-Montoya et al. 2010). In addition to habitat characteristics, stream typology are also an important determinant for adequate biological quality assessment (Verdonschot and Nijboer 2004). High variability in biotic metrics has been observed between mountainous and lowland streams with a strong influence of stream size and bottom substrate (Lorenz and Hering 2004). Although these studies were conducted on a larger scale, a recent study provided evidence that macroinverebrate distribution patterns are also apparent at the very fine scale of the size of small headwater catchment (Sroczynska et al. 2016).

Patterns in macroinvertebrate communities and habitat specific associations are well described for temperate, perennial rivers (Kubosova et al. 2010, Schroder et al. 2013). However, little attention on this topic has been paid to intermittent Mediterranean streams (however see García-Roger et al. 2013 and Leitão et al. 2014). In general the dynamics of macroinvertebrate assemblages in temporary streams are not completely understood and sometimes seem contradictory. While few studies demonstrated some tendencies in macroinvertebrate preferences to inhabit certain habitats (Chakona et al. 2008), others found no consistent patterns (Winterbourn et al. 1981). A recent study by Garcia-Roger et al. 2013 demonstrated the importance of habitat type on macroinvertebrates communities of intermittent rivers in pools during wet season. On the other hand some authors have demonstrated that preferences of macroinvertebrates for specific types of habitat are less pronounced and dominated by the generalist traits, in streams with higher frequency and magnitude of disturbance, such as temporary streams (Death and Winterbourn 1995, Sánchez-Carmona et al. 2012). Therefore, it is unclear if previously knowledge about patterns in community structure and taxa-specific associations, established for perennial streams, are of the same relevance in temporary streams. In a similar manner, there are no explicit studies that would explain the effect of stream typology on macroinvertebrate occurrences in intermittent, Mediterranean-type catchments. Based on macroinvertebrate distribution in Europe, differences between Mediterranean stream types were much smaller than in other areas (Verdonschot and Nijboer 2004). The reason for that can be associated to extreme hydrological conditions prevailing at most of Mediterranean streams. High variability in macroinvertebrate communities and biotic metrics has been observed in dry and wet periods even in reference streams (Sánchez-Montoya et al. 2009b; Munné and Prat, 2011) with a strong influence of flow connectivity (Prat et al., 2014; Cid et al., 2016; Datry 2011; Datry et al., 2013). These extreme environmental conditions can override stream type differences. As such, a proper understanding of the effect of typology and habitat on macroinvertebrate occurrences in these types of streams is still lacking.

This knowledge is of great importance as the scale (habitat/stream) at which the variation in macroinvertebrate communities is the highest will likely cause discrepancies in water quality index and consequently will limit its comparability with other water bodies. Therefore, an identification of the scale at which such variability occurs will greatly improve protocols for water quality assessment.

Habitat unit (considered as an area of the stream visually distinct from its surrounding) is often used to evaluate heterogeneity and success of the restoration efforts (Armitage and Pardo 1995; Lepori et al., 2005). When applying rapid bioassessment protocols, some authors suggested stratified sampling through the habitat types encountered at the sampling sites to decrease the variation among samples and improve comparisons among sites (Resh and Jackson 1993; Armitage et al. 1995). On other hand, according to hierarchical scale dynamics, environmental filter acting at regional scale is stronger in determining macroinvertebrate assemblages than habitat filter (Poff 1997).

Drawing from hierarchical scale dynamics theory and based on studies from perennial streams and on few existing studies of intermittent streams (García-Roger *et al.,* 2013; Leitão *et al.,* 2014) we expect habitat type (H1) and stream typology (H2) to influence macroinvertebrate assemblages, resulting in differences in community metrics and water quality indices. Additionally we examined whenever these variations will be greater among habitat types or among streams of different typologies. Further, we expect that habitat will also interact with typology of the stream. Therefore, our last hypothesis (H3) predicts that community metrics and water quality index will depend on the habitats that are present at a given stream type.

To test these hypotheses we investigated macroinvertebrate assemblage structure at distinct habitat types within different types of streams. Additionally to community characteristics we tested the strength of associations of macroinvertebrates to habitat structure as well typology using indicator values (Dufrene and Legendre 1997) to identify the potential taxa responsible for the expected differences among habitats/typologies. Further we measured the interaction effect of habitat and stream type on community characteristics (number of families, abundance, species richness) and biotic index (IPtIs -Índice Português de Invertebrados Sul- INAG IP 2009). Iptis is a multimetric index specifically developed for Portuguese streams and rivers as part of the European inter-calibration freshwater group exercise (INAG, 2009).

3.2. Methods

3.2.1. Study area

The study area covered the Hydrographical Administrative Region for the Algarve (ARH-Algarve), located in southern Portugal (Fig. 3.1). The region is characterized by Mediterranean-type climate, where habitat structuration processes are shaped by sequential events of annual flooding and drying (Bonada and Resh, 2013), which directly affects substrate characteristics, the development of algae and macrophytes as well as accumulation of organic debris (Gasith and Resh 1999; Sabater et al. 2006).

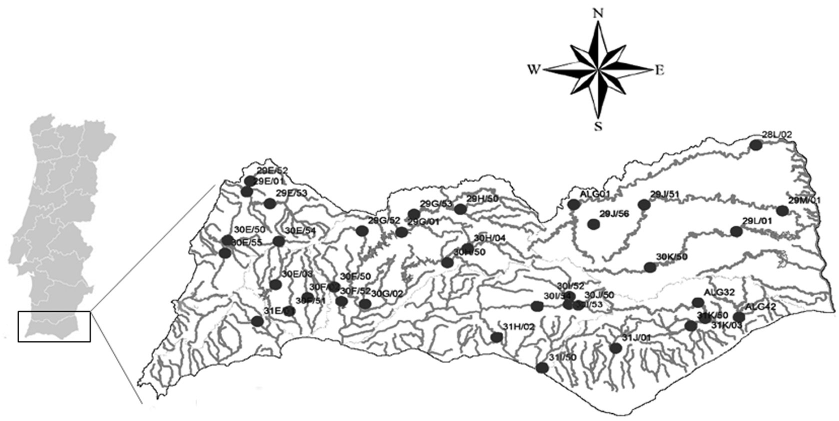


Fig. 3.1 Map indicating the Algarve region of southern Portugal with the 36 sampling sites, which are part of the monitoring grid defined by the Water Institute I. P. (INAG).

Wet periods begin in late October and generally last until April, with maximum discharge peaks occurring during winter months (November-March). During the dry season (~June - September) the stream fragments dry into temporarily disconnected pools or completely dry channels. Sampling was conducted by APA-ARH following the mandatory WFD inter calibration panel guidelines. Sampling period took place in early-middle Spring (April), during the period of moderate flow and considering a lag time of 15 days after the last intensive rain occurrence (necessary time for macroinvertebrate re-colonization defined by EU inter-calibration panel).

All of the sampled sites except for four are intermittent. In remaining four sites the water flows all over the year due to the existence of groundwater discharge.

The sampling points were selected from the monitoring grid defined by the Portuguese Water Institute I.P. (INAG) for the Algarve Water District (ARH Algarve), which included 46 obligatory sampling sites. Those sampling points were validated in the field considering the influence of source pollution and seawater intrusion and taking into account these criteria a total of 40 sites were sampled. From those 40 sampling points only 36, with the highest water quality, were selected for the purpose of this study. Selection was based on Chícharo et al. (2009) that evaluated water quality at given sites taking into consideration biological elements (macroinvertebrates, diatomaceous, fish and vegetation), physical-chemical elements as well river habitat survey following the mandatory WFD guidelines. These 36 sites were classified according to four main typologies (INAG IP, 2008): southern rivers from medium to large dimensions (M-L) – 6 sites; southern mountainous rivers (M-S) – 5 sites; southern small rivers (S-S) - 13 sites; calcareous rivers of Algarve (C) -12 sites.

3.2.2. Sampling methods

Sampling of benthic macroinvertebrates was conducted in 2009 following the WFD compliant INAG benthic macroinvertebrate sampling protocol. In accordance with mentioned protocol, sampling was conducted during the month of April when stable hydraulic conditions prevail, in order to ensure, that all the temporary habitats were present at the moment of sampling. Within each site, a representative 50 m section was defined considering the riffle zone and the adjacent sedimentation zones, in a way to best represent the diversity of the habitats present. Sampled habitats included 4 inorganic substrate types (according to their granulometry Schroder et al., 2013): boulder (> 25.6 cm), cobble (6.4 – 25.6 cm), gravel (0.2 – 6.4 cm) and sand (< 0.2 cm); and 2 organic substrate types: macrophytes (algae and aquatic plants) and POM (particulate organic matter) as defined by INAG (2009).

Benthic macroinvertebrate samples were collected using a hand-net (of 0.5 mm mesh and 25 cm width) and a standardized kick sampling method (each "sampling unit" was 2 m long and 0.25 m wide) in all habitats that were present in a section, independently on their percentage cover. Sample contents were placed in plastic containers and preserved using 96% ethanol.

3.2.3. Laboratory methods

In the laboratory, the samples were washed in order to remove the fixative and placed in a tray. Subsequently they were sorted and examined using a stereomicroscope and identified to family level, with the exception of 2 taxa that were identified to a higher level: class Oligochaeta and order Araneae Family level identification is the required to estimate the biotic index used to access water quality, according to the objectives of the WFD. No sub-sampling was used regardless of the number of individuals.

3.2.4. Data Analysis

PERMANOVA (permutational multivariate analysis of variance) was used to test for significant differences in macroinvertebrate community composition and structure, using a Bray-Curtis similarity matrix of presence/absence data, with stream Typology and Habitat as orthogonal fixed factors. Ordination by non-metric multidimensional scaling (MDS) was used to visualise patterns. MDS allows converting similarity in distance, which is represented spatially, using the Unweighted Pair Group Method (UPGMA). In order to facilitate visualization, the MDS plots were built on a reduced presence/absence matrix, by averaging the replicates in each combination of Habitat (6 types) and Typology (4 types) subtracted by three types of habitats that were not present at the typology M-S, resulting in total of 21 points visible on the MDS plot. The similarity percentages routine (SIMPER) was used to examine the contribution of each macroinvertebrate family to average resemblances between sample groups. All multivariate analyses were done using the PRIMER 6 statistical package with the PERMANOVA+ add-on (PRIMER-e, Plymouth Marine Laboratory).

For each taxon the IndVal – indicator value of association (Dufrene and Legendre 1997) was calculated. Indicator value determines the most representative taxa for a given Habitat or Typology. The values of IndVal (0-1) were based on the average relative abundance and frequency of occurrence of taxa within a given group of samples in relation to all of the other samples. The index is 1 when a taxon is present in all of the replicates of a given group and is absent in all of the other replicates. IndVal was calculated for selected families using function “strassoc” and “IndVal.g” as the association index. Group combinations of stream habitats and typologies were tested in order to evaluate if some taxa can display a more generalist distribution and be associated with more than one group. Indicator value for group combinations was done using function multipatt. All the IndVal analysis were done using “indicspecies” package in R software (De Caceres and Legendre 2009). Taxa with a significant association to one group of group combinations were crossed with the list of families, which contributed the most to the dissimilarities among steam habitats or typologies, calculated using SIMPER analyses (Clarke and Warwick 2001). Vectors representing the correlations between the frequency of occurrence of these selected taxa and the dissimilarity matrix were superimposed on the MDS ordination.

Diversity (total number of families, total number of individuals and Simpson diversity) and water quality indices (*IPtIs*) were calculated for each macroinvertebrate sample. The *IPtIs* index used for this analysis was calculated based on presence/absence of particular macroinvertebrate families, their species richness, abundance and sensibility to pollution: $IPtIs = (N^o\ of\ families \times 0{,}4) + (EPT \times 0{,}2) + ((IASPT - 2) \times 0{,}2) + [Log\ (Sel.\ EPTCD + 1) \times 0{,}2]$

Where:

EPT = number of families, which belong to the orders Ephemeroptera, Plecoptera and Trichoptera.

IASPT (Iberiam Average Score per Taxa) = this index corresponds to IBMWP index (Iberian Biological Monitoring Working Party index, Alba-Tercedor et al., 2002) divided by the number of families

Log (Sel. EPTCD) = Log10 de 1 + sum of individuals, which belongs to individuals of the following families: *Chloroperlidae, Nemouridae, Leptophlebiidae, Ephemerellidae, Philipotamidae, Elmidae, Leuctridae, Limnephilidae, Sericostomatidae, Dryopidae, Athericidae.*

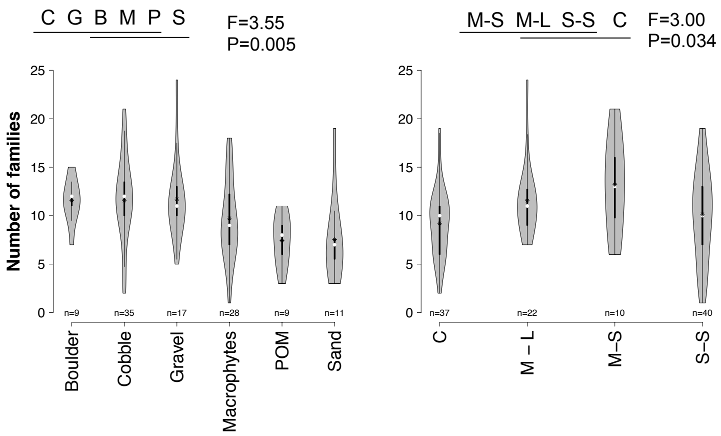
Two-way analysis of variance (ANOVA) was performed on community metrics (total number of families, total number of individuals and Simpson diversity) and water quality index (*IPtIs*) with Typology and Habitat as fixed orthogonal factors. Pairwise Multiple Comparisons among habitats and typologies were done using Student-Newman-Keuls procedure. All the univariate analyses were done using SigmaPlot software (Version 11.0, Systat Software, Inc.).

3.3. Results

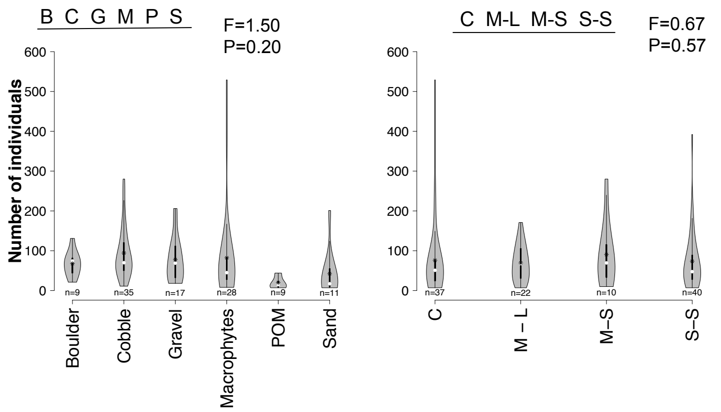
3.3.1. Community structure

A total of 9448 individuals were sampled in total from all of the habitats, with minimum density of 7 individuals/m^2 (on the habitat sand and POM belonging to C and M-L typologies respectively) and maximum 529 individuals/m^2 (on habitat macrophytes at the C type), with a total of 75 families identified. The highest mean abundance as well as number of families was found on habitat cobble, while the lowest were on habitats sand and POM (Fig. 3.2A and B). For stream typology, the highest mean abundance and number of families was on M-S streams. The lowest mean abundance was on M-L streams and the lowest number of families was found on C streams (Fig. 3.2A and B).

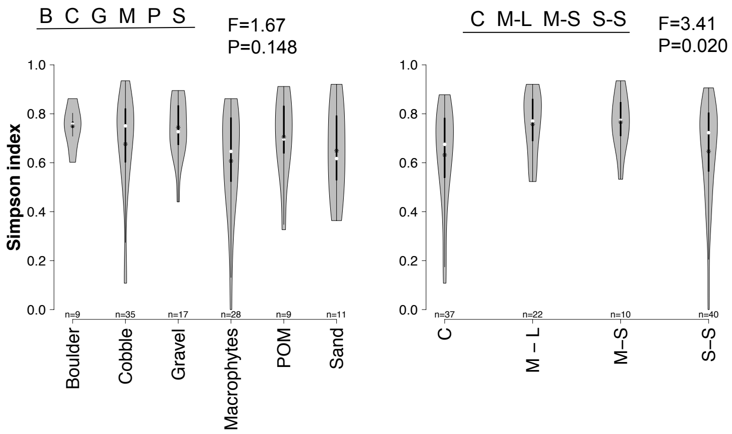
A



B



C



D

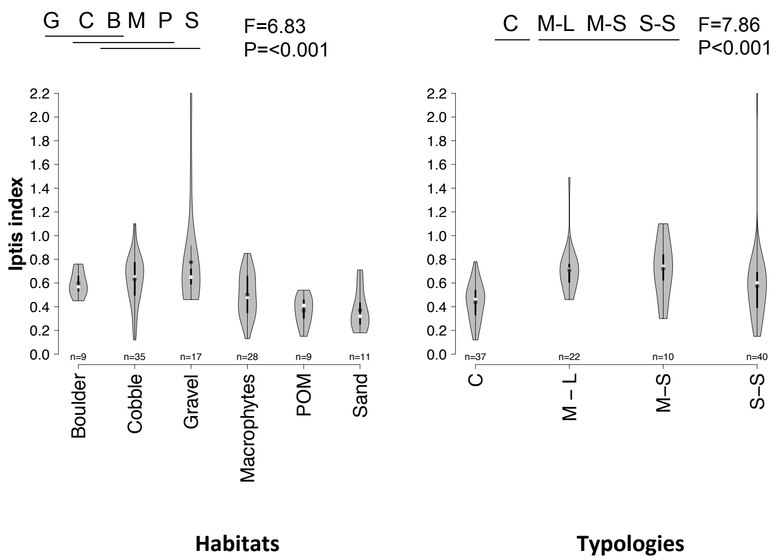


Fig. 3.2 Violin plots with number of individuals (A); number of families (B) per m^{-2};; Simpson diversity index (C) and *IPtIs* index (D) for Habitat and Typology. Grey-shaded area represents the sample distribution where: asterisk represents a mean, white circle is a median with the thick vertical lines at the first and third quartiles and thin vertical lines that extends to maximum and minimum value. F – statistics of the Two-way analysis of variance, considering two fixed, orthogonal factors: Typology and Habitat. For Habitat/Typologies that are underlined with the same line no difference at $p \leq 0.05$ was recorded in multiple comparison (Student-Newman-Keuls) tests.

PERMANOVA analysis for abundance data and for presence/absence data was not significant for the interaction between Typology and Habitats. Nonetheless, significant main effects of Typology and Habitat were detected, indicating that these two factors independently influence community assemblage structure (Table 3.1). Average similarity between/within the groups are summarized in Table 3.2. Given that the response was similar in terms of abundance and presence/absence data, only the latter is presented. Main differences were observed among depositional habitats (sand and POM) and coarse mineral habitats (boulder, cobble, gravel), but differences were also recorded among macrophytes and gravel and macrophytes and cobble. The most similar habitats were boulder, cobble and gravel. Stream typologies also differed significantly among the S-S typology and the remaining three typologies, as well as among M-S and M-L and M-L and C. The most similar typologies were M-S and C.

Table 3.1 PERMANOVA analysis with two fixed factors (Typology and Habitat) based on presence/absence similarity matrix.

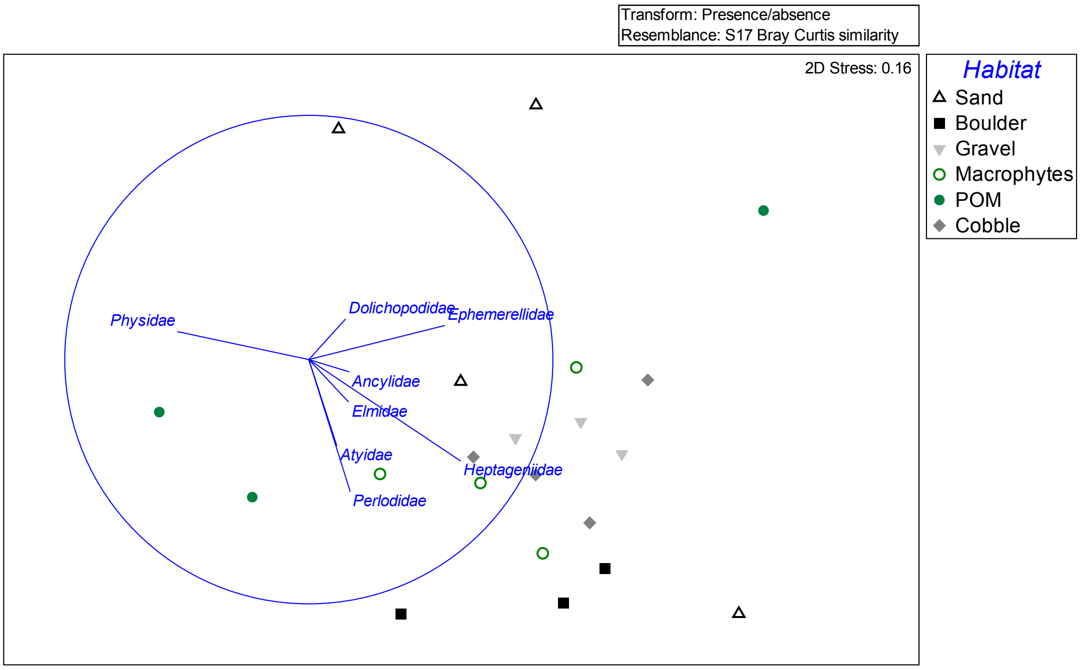
Source	df	SS	MS	Pseudo-F	P	Perms
Typology	3	12565	4188	2.517	0.001	997
Habitat	5	17947	3589	2.157	0.001	998
Typ×Hab.	12	18150	1513	0.909	0.725	997
Res	90	149740	1664			
Total	110	2051				

Table 3.2 Table with average similarity between/within groups for habitats (A) and Typologies (B). In bold are depicted habitat/typologies pairs with significant differences according to PERMANOVA pair-wise tests .

	Sand	Boulder	Gravel	Macrophytes	POM	Cobble
Sand	37.33					
Boulder	**34.67**	47.99				
Gravel	40.17	**43.98**	49.25			
Macrophytes	**36.32**	42.93	**43.20**	41.10		
POM	31.48	**31.11**	**31.72**	34.27	31.83	
Cobble	**37.09**	44.68	46.90	**41.52**	**30.86**	44.60

	S-S	M-S	M-L	C
S-S	41.62			
M-S	**38.29**	41.59		
M-L	**45.95**	**43.58**	55.04	
C	**37.62**	35.61	**40.95**	37.50

A



B

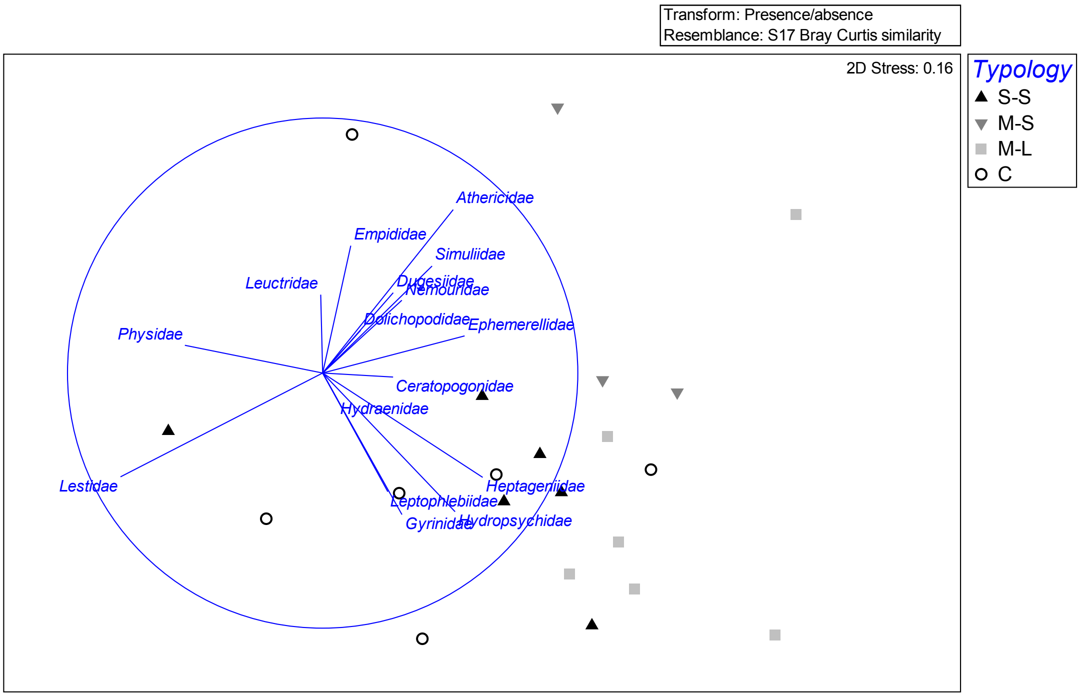


Fig. 3.3 Non-metric multi-dimensional scaling (MDS) for Habitat (A) and Typology (B); vectors represent the correlations between the MDS ordination and correlations between the frequency of ocurrence of taxa selected for each factor and the similarity matrix.

nMDS analyses showed high similarity among boulder, cobble, gravel and macrophytes habitats (Fig. 3.3A). POM and sand were detached from the rest of the habitats and showed the smallest levels of similarity within the same habitat (Table 3.2). The common taxa: *Ancylidae* and *Heptageniidae* shown similar associations with the inorganic habitats: cobble, gravel and boulder, whereas *Atyidae Elmidae* and *Perlodidae* were also strongly associated with macrophytes (Fig. 3.3A). This is also corroborated by the IndVal values where *Ancylidae* has significant association to boulder (Table 3.3 and 3.4), whereas, *Heptageniidae* has strong association to the combination of three course mineral substrates (boulder, cobble and gravel, Table 3.4). *Elmidae* and *Perlodidae* have also high IndVal for macrophytes and respective significant IndVal value for the group combinations among coarse mineral and macrophytes substrate. *Physidae* were associated both with POM and less strongly with Sand. According to nMDS *Dolichopodidae* and *Ephemerelidae* were clearly associated with sand (Fig 3.3A). Nonetheless, Table 3.3 and Table 3.4 also demonstrates high for these species for the cobble and gravel and for *Ephemerelidae* also for boulder and macrophytes.

Different set of species were related with different Typologies (Fig. 3.3B). Common rheophilic taxa: *Heptagenidae*, *Leptophlebiidae*, *Hydropsychidae* and *Gyrinidae* were associated with M-L typology. Although *Leptophlebiidae* was also strongly associated with S-S typology (Tables 3.3 and 3.4). Few common species such as: *Simuliidae, Nemouridae, Athericidae, Dugesiidae and Dolichopodidae* were evidently affiliated to M-S Typology. M-S typology is also the one with the highest (8) species with significant IndVal (Table 3.4).

Only *Heptageniidae*, *Physidae* and *Dolichopodidae* had common patterns in their associations to both Habitat and Typology. *Heptagenidae* family was associated to coarse mineral substrates and M-L typology. *Physidae* was associated to both C and S-S typologies and previously mentioned POM and Sand habitats. *Dolichopodidae* was closely related to gravel/sand and M-S typology. Remaining species rather display affiliations to either Habitat or Typology.

Table 3.3. Values of IndVal for chosen taxa in each habitat and typology; values in bold represent taxa whose indVal significantly differ among groups; the highest IndVal for each habitat/typology is represented on grey.

HABITAT	Boulder	Cobble	Gravel	Macrophytes	POM	Sand	P
Ancylidae	**0.54**	**0.26**	**0.21**	**0.07**	**0.00**	**0.08**	**0.04**
Atyidae	**0.13**	**0.13**	**0.09**	**0.56**	**0.24**	**0.00**	**0.04**
Dolichopodidae	**0.00**	**0.32**	**0.42**	**0.03**	**0.09**	**0.26**	**0.02**
Elmidae	**0.37**	**0.59**	**0.35**	**0.32**	**0.08**	**0.09**	**0.05**
Ephemereliidae	0.22	0.40	0.29	0.36	0.05	0.07	0.14
Heptageniidae	**0.40**	**0.43**	**0.38**	**0.07**	**0.00**	**0.04**	**0.01**
Perlodidae	**0.39**	**0.56**	**0.23**	**0.20**	**0.12**	**0.02**	**0.03**
Physidae	**0.06**	**0.04**	**0.00**	**0.18**	**0.50**	**0.22**	**0.05**
TYPOLOGY	C	M-L	M-S	S-S			P
Athericidae	**0.11**	**0.21**	**0.53**	**0.14**			**0.030**
Ceratopogonidae	**0.17**	**0.44**	**0.20**	**0.49**			**0.020**
Dolichopodidae	**0.05**	**0.39**	**0.45**	**0.21**			**0.005**
Dugesiidae	**0.00**	**0.14**	**0.39**	**0.02**			**0.025**
Empididae	**0.00**	**0.09**	**0.39**	**0.03**			**0.045**
Ephemerellidae	**0.09**	**0.27**	**0.28**	**0.61**			**0.005**
Gyrinidae	**0.00**	**0.36**	**0.00**	**0.04**			**0.035**
Heptageniidae	**0.04**	**0.68**	**0.18**	**0.28**			**0.005**
Hydraenidae	**0.05**	**0.07**	**0.51**	**0.08**			**0.010**
Hydropsychidae	0.23	0.43	0.45	0.22			0.065
Leptophlebiidae	**0.14**	**0.59**	**0.19**	**0.50**			**0.005**
Lestidae	**0.45**	**0.02**	**0.00**	**0.08**			**0.020**
Leuctridae	**0.09**	**0.22**	**0.54**	**0.13**			**0.010**
Nemouridae	**0.00**	**0.05**	**0.59**	**0.04**			**0.005**
Physidae	**0.48**	**0.04**	**0.16**	**0.07**			**0.020**
Simuliidae	**0.30**	**0.09**	**0.68**	**0.12**			**0.020**

Table 3.4. Groups combination for habitat types and stream typologies, with A and B values and stat = test statistic 'IndVal.g'; only taxa with significant IndVal are listed.

Groups and groups combinations	Taxa	A	B	stat	P
	HABITATS				
Boulder	*Ancylidae*	0.45	0.67	0.55	0.04
Boulder + Cobble + Gravel	*Heptageniidae*	0.97	0.50	0.70	0.00
Boulder + Macrophytes + POM	*Atyidae*	0.87	0.43	0.61	0.03
Cobble + Gravel + Sand	*Dolichopodidae*	0.92	0.41	0.61	0.02
Macrophytes + POM + Sand	*Physidae*	0.95	0.27	0.50	0.04
Boulder + Cobble + Gravel + Macrophytes	*Elmidae*	0.92	0.78	0.85	0.03
Boulder + Cobble + Gravel + Macrophytes + POM	*Leptophlebiidae*	0.99	0.57	0.75	0.03
	Perlodidae	0.99	0.50	0.71	0.04
	TYPOLOGIES				
C	*Lestidae*	0.93	0.22	0.45	0.03
M-L	*Heptageniidae*	0.67	0.68	0.68	0.01
	Gyrinidae	0.93	0.14	0.36	0.03
M-S	*Simuliidae*	0.57	0.80	0.68	0.01
	Nemouridae	0.86	0.40	0.59	0.01
	Leuctridae	0.58	0.50	0.54	0.01
	Athericidae	0.55	0.50	0.53	0.01
	Hydraenidae	0.87	0.30	0.51	0.03
	Dugesiidae	0.77	0.20	0.39	0.04
	Empididae	0.76	0.20	0.39	0.04
	Calopterygidae	0.67	0.20	0.37	0.05
C + M-S	*Physidae*	0.90	0.28	0.51	0.04
M-L + M-S	*Dolichopodidae*	0.83	0.44	0.60	0.01
	Philopotamidae	0.97	0.16	0.39	0.04
M- L+ M-S + S-S	*Leptophlebiidae*	0.94	0.65	0.78	0.01
	Ephemerellidae	0.96	0.53	0.72	0.01
	Ceratopogonidae	0.90	0.55	0.70	0.03

Families with the highest contribution to dissimilarity among habitats and typologies greatly coincide with families with significant IndVal (Table 3.3 and 3.4). Only *Leptophlebiidae* for habitat and *Philipotamidae* and *Calopterygidae* families for Typology have significant IndVal, but does not contribute to overall dissimilarity.

Habitat had seven families, which IndVal was significantly different among habitats (Table 3.3). However, from these seven families only one taxa had significant IndVal for only one habitat type and remaining taxa had only significant associations to habitat combinations (Table 3.4). For Typology from 15 taxa with significant IndVal 11 taxa were associated to one type of stream, whereas 6 families were associated to the stream types combinations.

3.3.2. Diversity and water quality indices

As previously demonstrated by the multivariate analysis Habitat vs Typology interaction was not significant for community data (neither for abundance data, nor for the presence/absence data) and for this reason we decide not to include the interaction analysis for the community descriptors. However 2 way analysis of variance detected significant main effect of Typology and Habitat on *IPtIs* water quality index and number of families (Fig. 2B and D). Simpson diversity index was only significant for Typology (Fig. 2C). In terms of water quality index the differences were between C typology, which had the lowest *IPtIs* value (Fig. 2D) and the remaining typologies. For the habitat types the differences were found among: Gravel, with the highest *IPtIs* value, and Sand, POM and Macrophytes with the respective lowest *IPtIs* values. Number of families differed only among M-S typology, which hosted the highest mean number of families (Fig. 2B) and between C typology with respective the lowest mean number of families. For the habitats, multiple comparisons test detected only significant differences among Sand (with the lowest number of families) and gravel land cobble with the respective highest number of families for all of the habitats.

3.4. Discussion

According to our first hypothesis (H1) the structure of macroinvertebrate community differered among certain habitat types, particularly between marginal habitats: sand and POM and the rest of the habitats. This finding supports the concept that macroinverterates follow the erosional-depositional gradient in their distribution patterns among habitats (Barmuta 1989, Chakona et al. 2005, García-Roger et al. 2011; Sheldon and Haick 1981).

Previous authors demonstrated that the degree of bed movement (critical force needed to move median particle diameter) and availability of interstitial space are critical factors for the distribution of invertebrates (Duan et al. 2008; Cobb et al. 1992, Townsend et al. 1997). Therefore, boulder, cobble and gravel supported higher number of families presumably by providing greater stability in terms of resistance to disturbance during flood events as well as by serving as refuge (Rice et al. 2001).). It is then expected to find greater similarities in macroinvertebrate communities among these three mineral substrates that among organic ones. Such patterns is likely related to the fact that these habitats are located at more erosional section of the channel, supporting fauna adapted to higher current velocities. An overlap among macroinvertebrate families, particularly within mineral substrata was also observed for temperate rivers (Rabeni and Gibbs 1980, Barmuta 1989) and in general is associated with high mobility of most invertebrate taxa within a reach (Mackay et al. 1992). Furthermore, MDS analysis showed that macrophytes tended to be more similar in terms of taxonomic composition to the latter three habitats. One of the possible explanation for such pattern is that macrophytes provide internal microclimate for stream biota and therefore majority of mobile taxa will more likely broaden their distribution from central-channel mineral substrata to the macrophyte areas, where the probability of finding food resources, as well as shelter for spawning and nursery space, will be higher (Pardo and Armitage 1997). An adaptive generalist response of benthic fauna to relocate among habitats potentially explains why some taxa had only significant associations to group combinations between mineral substrates and macrophytes rather than solely mineral or organic. Alternatively, predator avoidance or competition can also explain this pattern (Menge and Olson, 1990). Only one taxa had significant IndVal in relation to certain type of habitat (*Ancylidae*), while most of the remaining taxa displayed more generalistic distribution among coarse organic substrates and macrophytes. Similar pattern was observed for temperate permanent streams (Armitage and Cannan 2000) ,but also for intermittent (Datry *et al.,* 2013).

Low number of taxa with significant indicator values impels us to identify habitats with the highest ecological importance for macroinvertebrate distribution. Previous studies conducted on temporary rivers demonstrated that cobble and macrophytes habitats constituted distinct communities and were strongly preferable by macroinvertebrates in comparison to gravel and sand (Chakona et al. 2008). We demonstrated that indeed cobble and macrophytes were important habitats for some families, but they do not support distinct communities. Intermittent streams, with the prevalence of floods and droughts might have favoured the evolution of generalist traits (Hershkovitz and Gasith 2013) in order to enhance the resilience of biota to withstand conditions of frequent disturbance (Williams 1996). Such tactic employs lesser selectivity in terms of resource partitioning (Mihuc 1997; Rosi-Marshall et al. 2016; Vannucchi et al. 2013) what in consequence hampers defining ecological niches for majority of taxa at intermittent streams.

Our second hypothesis (H2) assumed that Typology will influence macroinvertebrate assemblages and so it was supported. Observed differences in macroinvertebrate communities among almost all of the stream typologies validates the general belief that the intermittent river systems are extremely heterogenous group in comparison to other stream types (Sanchez-Montoya et al. 2007). Differences among stream typologies are particularly worth attention taking into consideration the gross taxonomic resolution used in a present study. In general, the finer taxonomic resolution, the clearer separation of stream classes (Lorenz and Hering, 2004). The fact that the differences in typologies were evident at such gross resolution provides an additional argument that refinement of such intermittent system is necessary to adequately capture species patterns and better reflect water quality measures. In addition, high number of significantly associated taxa indicates that at this scale taxa specific preferences are displayed on the stream level and less habitat level.

Number of individuals and Simpson diversity index were the least informative in terms of differences among habitats and typologies. Differences in communities were reflected in differences in water quality index for both Habitat and Typology. Habitat with the highest *IPtIs* index was gravel, following by cobble and boulder-three habitats, which mainly differed from the rest of the habitat in terms of community assemblages.

Nonetheless, only gravel significantly differed from other habitats in terms of *IPtIs*. For Typology, *IPtIs* index only differed for calcareous streams. The results demonstrate that although remaining typologies support different communities, this fact is not automatically reflected in the differences in *IPtIs* index among these stream types.

We were not able to define which of two factors (Habitat or Typology) is responsible for higher variations in macroinvertebrate communities. In present study both factors are important determinants for macroinvertebrate distributions and it clearly emphasizes how patterns in macroinvertebrate occurrences are scale dependent. It is also in agreement with very small (3 out of 24 taxa) number of taxa, which exhibited common patterns in their distribution to both Typology and Habitat. Remaining taxa displayed only association to one of these factors.

It is also possible, that habitat to be a predictable unit of species assemblages needs to be considered at smaller scale such as one stream type or reach, where the environmental parameters are homogenous. Low concordance between species assemblages and substrates were also found at higher scales elsewhere (Boyero, 2003b).

Macroinvertebrate communities are influenced by reach scale factors such as type of substrate, food availability or current velocity (Beisel et al. 1998; Chaves et al. 2005; Collier at al. 1998). However, they are also influenced by larger scale variables mainly driven by changes in altitude, conductivity and temperature (Aguiar et al. 2002; Chaves et al. 2005; Graça et al. 1989; Pires et al. 2000, Sroczynska et al. 2017). Such regional filters can influence species distributions by direct control on species biological traits, or indirectly by affecting the patch structure and size. In our study, lack of interaction among habitat and stream type demonstrates that regional factors affect macroinvertebrate communities by directly controlling their traits and therefore limit their occurrence at a given stream type. Furthermore, smaller scale habitat characteristics affect macroinvertebrates independently on stream type. Such result provides a clear evidence that the effect of stream typology does not depend on the habitats that are present in the given stream type and vice versa. This finding also supports previous study on these streams indicating that larger (regional) scale factors structure benthic communities independently on the habitat scale (Sroczynska et al. 2017).

3.4.1. Implications for the biomonitoring in the intermittent streams

The inorganic, mineral substrates that compose the streambed are the most commonly sampled habitat types to assess ecological quality of waters. Previous studies indicated that sampling only the inorganic substrate of the streambed would account for a limited portion of the organisms, ignoring some of the sensitive taxa that could inhabit organic habitats such as wood, leaf litter or macrophytes (Chakona et al. 2008, Kay et al. 1999, Leitão et al. 2014, Reid et al. 2010). On the other hand, some authors suggest restricting sampling to only mineral substrates, which shelter the most pollution sensitive taxa (Beauger et al. 2006).

In general the same sampling effort at more heterogenous sites results in higher taxa richness and abundance than at sites with less number of habitats (Kay et al. 1999, Humphries et al. 1996). Our results demonstrated differences among habitats in water quality index, particularly between gravel and organic, depositional habitats (macrophytes/sand/POM). Due to these differences sampling at the reach scale should include previously mentioned habitats. However, sampling protocols should also acknowledge different stream typologies.

IPtIs index is composed of various components (please, see method section) from which EPT, IASPT and EPTCD indexes have the highest contribution to an overall *IPtIs* value. Habitat with the highest *IPtIs* index was gravel, following by cobble and boulder, whereas for stream types M-S and M-L typologies had the highest *IPtIs* score. It demonstrates that these habitats and these typologies host the most sensitive taxa. Nevertheless, highly scored taxa that appeared in M-S or M-L typologies are not because these typologies are overrepresented by gravel habitat. Instead their occurrence is independent on the habitat type and vice versa. Our study shows that these two scales shape species occurrences independently on each other what is reflected in water quality index and therefore the next step forward would be to address both scales simultaneously in development of effective biomonitoring programs.

Although the WFD protocol applied in Portugal was adapted to Southern Portuguese rivers, within this region, an additional adjustment should be made especially differentiating calcareous from non-calcareous stream types. M-S typology hosted the highest number of families with significant IndVal suggesting that this typology is important for many families with less generalistic set of traits. As such this typology must be carefully addressed in monitoring programs for water quality assessment even at such fine scale.

There is a need for rapid and cost effective strategies for biological assessment of streams and therefore this knowledge helps to understand at which scale sampling protocols should be improved to accurately represent the actual water quality status.

Acknowledgements

K. Sroczyńska holds a PhD scholarship of the IMPACT project, founded by the Foundation for Science and Technology (FCT) of Portugal (ERA-IWRM/0003/2009). F. Leitão and P. Range were also funded by grants from Fundação para a Ciência e Tecnologia (Reference SFRH/BPD/63935/2009 and SFRH/BPD/69959/2010).

Author Contributions: KS analyzed data and wrote the manuscript, FL participated in sampling and contributed to data analysis, IM, AF and MC participated in sampling and macroinvertebrate identification, PR provided editorial advice and contributed to data analysis, LC provided editorial advice.

Chapter 4

Habitat specific associations of benthic macroinvertebrates at the reach scale in Intermittent Mediterranean River

This chapter is not presented in form of an article, but constitutes an integrative part of the thesis

4.1. Introduction

Benthic invertebrates are patchily distributed in the river bed according to their substrate preferences, distinct hydrological regimes, ranges of oxygen concentrations, type of food available or species interactions (Beisel et al. 1998, Chaves et al. 2005, Cummins and Lauff 1967, Merrit and Cummins 1996, Pardo and Armitage 1997, Schröder et al. 2013, Townsend and Hildrew 1994). Consequently, habitat characteristic exert strong influence on abundance, diversity and the trophic structure of macroinvertebrate assemblages (Bonada et al. 2006a, Beisel et al. 2000, Brown 2003, Kubosova et al. 2010, Pardo and Armitage 1997). Lesser abundance of macroinvertebrates is generally found on sandy substrata than on cobble, macrophytes, wood or leaf litter (Chakona et al. 2008, Collier et al. 1998, Pardo and Armitage 1997). Smaller organisms with burrowing forms are more commonly found on finer substrates, where small interstitial spaces provide them a suitable shelter. On the other hand, coarse substrates are more stable and attract bigger and more diverse taxa (Duan et al. 2008). Smaller size substratum (1.0-3.5 cm) has higher potential to trap organic matter, which also favours the colonization of these substrates by macroinvertebrates, in comparison to substrates composed of larger or smaller particle sizes (Rabeni and Minshall 1977). In turn, macrophytes support high macroinvertebrate biomass and diversity by providing refuge from predators and surface for attachment (Collier et al. 1998, Harrison and Harris 2002). Most of the patterns in macroinvertebrate assemblage structure are related to their physiological and morphological adaptations that are closely related to the conditions of the habitat they occupy (Southwood 1977). Therefore most invertebrate species are particularly associated with different substratum types, although generalists are also common (Mihuc 1997).

Previous works demonstrated that larger scale factors influence species occurrences at the smaller scales. Factors that shaped species occurrences in studied area were related to altitude, conductivity and temperature (Sroczyńska et al. 2017). However, hydrological regimes (disconnected pools vs flowing streams) had also a considerable effect on species occurrences. Therefore, different species assemblages are supposed to be found at upstream and downstream reaches and/or at the reaches with disrupted flow regime and flowing sites (Sroczyńska et al. 2017). This finding already implies that certain species will be absent at smaller scales.

Previous works confirmed that habitats considered at the catchment scale do not provide a predictable unit for species organization (Chapter 3 *in* Sroczyńska, 2018). Therefore, the same habitat type, but encountered at different stream type will host different macroinvertebrates communities.

Some authors demonstrated that reach scale is the threshold in geomorphological hierarchy where physical habitats become homogenous, so that the environmental filter operating at the larger scale will weaken on its importance (Parsons et al. 2003). This is confirmed by many studies, which demonstrated that samples collected within a reach are more similar to samples collected among reaches (Rabeni et al. 1999; Hawkins and Vinson 2000). Therefore, studies, which investigated a variation in macroinvertebrates assemblages, within a reach, demonstrated strong influence of a patch on the macroinvertebrates distribution (Downes et al. 1993, 1995). For this reason, the aim of the current chapter is to analyse habitat specific community characteristics and species-specific associations to habitat types at the reach scale. I predict that species assemblages under similar set of environmental conditions will display stronger affinities to their habitats than it was demonstrated for the catchment scale. This will result in different communities among distinct habitats and in high number of taxa with significant IndVal. Based on previous works on this topic (Chapter 3 *in* Sroczyńska, 2018) I hypothesize to find high number of species with the preferences for the combination of course mineral+algae substrate. In addition, there was examined if current velocity influences species spatial arrangement at the reach scale.

4.2. Methods

The collection of macroinvertebrates was conducted at 300 m reach belonging to Algibre stream (Fig. 4.1). The reach is dominated by mineral substrates mainly gravel that during summer season is densely covered by filamentous algae (Fig. 4.2).

9 habitat types naturally encountered in the study reach were chosen for sampling. 5 of these habitats were classified as mineral habitats and 4 as organic (Tab. 4.1). Macroinvertebrates were sampled during wet season (April-June) on ten occasions.

Each time 9 samples (1 m long and 0.25 m wide) were taken using standardized kick sampling technique with a hand-net (0.5 mm mesh, 25 cm width) from each habitat type. It resulted in total of 90 samples (10 replicates for each habitat type). Samples were preserved, for further identification, using 96% ethanol All specimens were identified to the lowest taxonomic level and taxonomic adjustment was made for further data analysis (AQUEM 2002).

Differences in structural composition of benthic macroinvertebrates among habitats were assessed using the analysis of similarities routine (ANOSIM).

Normality assumptions and equal variance were tested using Shapiro-Wilk test and Levene's test (library "car" in R software). Tukey HSD test (library "multcomp") was performed in order to determine which treatment levels were significantly different. Whenever ANOVA assumptions were not met, the multiple comparisons Kruskall–Wallis test (library "pgirmess") was used to assess differences in univariate ecological indexes between habitats. All the multivariate and univariate analyses were done using R software (R Development Core Team 2012).

4.2.1. Indicator Value

For each taxa the IndVal – indicator value of association (Dufrene and Legendre 1997) was calculated. Indicator value determines the most representative taxa for a given habitat. The values of IndVal (0-100) were based on the average relative frequency of occurrence of taxa within a given habitat in relation to all of the habitats. The index is 100 when a taxa is present in all of the replicates of a given habitat (in all samples of a given habitat) and is absent in all of the other habitat types. IndVal consists of two components: A, positive predictive value of the species to be an indicator of the target habitat and B, which is the probability of finding the indicator species in the target habitat (De Caceres and Legendre 2009). As an example, when A=1.0 and B=0.5, means that species is present only at the sites, which belong to target habitat, but only half of the sites belonging to this habitat include the indicator species. Group habitat combinations were tested in order to evaluate if some taxa can display a more generalist distribution and be associated with more than one habitat type. Indicator value for site group combinations was done using function multipatt in "indicspecies" package in R software (De Caceres and Legendre 2009).

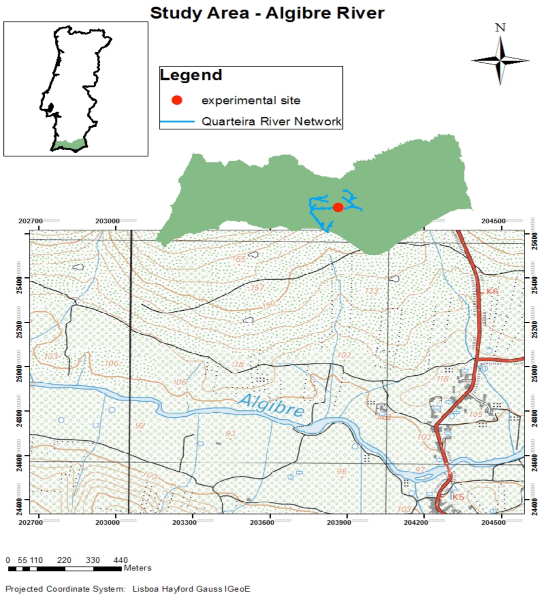


Fig. 4.1 Location of sampling reach

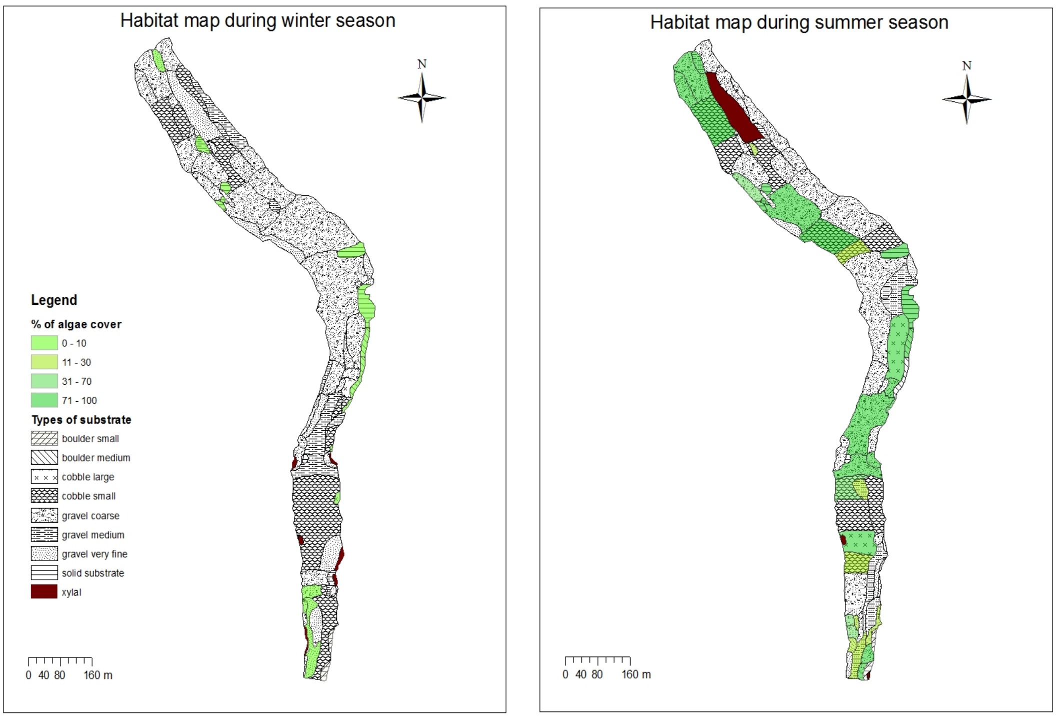


Fig. 4.2 Sampling reach during winter and summer season with respective differences in substrate types

Tab. 4.1 Mineral and organic habitats sampled in the reach

Substratum abbrevation	Substratum type	Description
FA	filamentous algae	cobbles or coarse gravel covered by the mats of filamentous algae
MIL	microlithal	medium gravel (2-6 cm)
FR	fine roots	living parts of terrestrial plant roots
XYL	xylal	large wood,trunks,branches
CPOM	coarse particular organic matter	deposits of coarse organic particulate matter (leaf litter)
MAL	macrolithal	cobbles (20-40 cm)
MES	mesolithal	coarse gravel (6-20 cm)
AKAL	akal	fine gravel (0.2-2 cm)
SAND	sand	psammal (6μm-2 mm)

4.3. Results

4.3.1. Physico-chemical parameters

Physico-chemical parameters collected at each day of sampling are summarized in Table 2. Only current velocity measures were taken separately for each habitat type and the results are presented on Figure 4.3.

Table 4.2 Physico-chemical parameters collected at each day of sampling

Day of samplig	pH	Air Temp [°C]	Water Temp [°C]	O_2 mg $^{l-1}$	Conductivity S m^{-1}	Nitrite [mg dm^{-3}]	Ammonia [mg dm^{-3}]	Phosphorus [mg dm^{-3}]	Chlorine [mg dm^{-3}]
2014-04-07	6.26	19.90	19.40	8.21	522.50	0.05	0.03	0.02	28.50
2014-04-30	5.14	26.20	20.40	9.19	753.20	0.04	0.00	0.02	-
2014-05-08	6.50	26.10	23.30	9.34	660.00	0.05	0.26	0.01	-
2014-05-15	6.73	21.60	23.30	9.66	662.00	0.04	0.05	0.01	-
2014-05-23	6.53	17.40	17.80	11.58	676.00	0.06	0.03	0.03	34.50
2014-06-02	6.27	24.70	22.50	8.79	676.00	0.03	0.02	0.03	37.00
2014-06-09	6.79	-	23.10	12.62	672.00	0.06	0.02	0.04	37.00
2014-06-20	6.36	25.50	24.40	9.24	1320.00	0.05	0.05	0.04	47.00
2014-07-03		21.69	22.54	8.74	996.00	0.05	0.02	0.04	56.00
2014-07-10	7.58	23.38	24.90	6.55	1165.00	0.05	0.07	0.04	53.20

The highest current velocity was reported at Mesolithal, Microlithal and Filamentous algae. The lowest was reported at depositional habitats such as Sand, Xylal and CPOM. Significant differences in current velocity were only reported among Mesolithal and CPOM (P=0.014), Mesolithal-Fine roots (P=0.023), Mesolithal-Macrolithal (p=0.046), Mesolithal-Sand (P=0.037) and Mesolithal-Xylal (0.036).

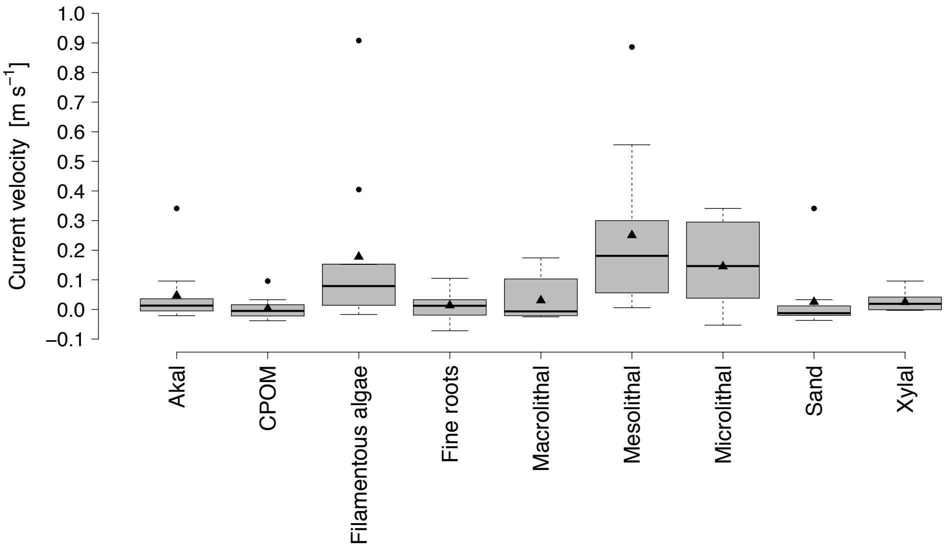


Fig. 4.3 Current velocity measured at each habitat. Where triangles represent a mean; black circles represent outliers; horizontal segment is a median; horizontal lines marking the box are first and third quartiles with "whiskers" that extends to minimum and maximum value.

4.3.2. Community descriptors

Analysis of ANOSIM detected significant differences in communities among habitats (ANOSIM statistic R: 0.1653, P= 0.001). Detailed pairwise comparisons among habitat types are presented in Table 4.3. Differences are mainly apparent among habitats such as Sand, CPOM and Akal and courser mineral substrates such as Mesolithal, Microlithal and Macrolithal. However, differences among course mineral substrates also exists especially among macrolithal and remaining micro and mesolithal.

Habitats with the lowest total abundances were Akal and Sand whereas Mesolithal and Filamentous algae were habitats with the highest total macroinvertebrate community abundances (Fig. 4.4). Significant differences in total abundance were reported between Sand and Filamentous algae, Mesolithal and Microlithal; as well as among Akal and Mesolithal (Table 4.4).

Average species richness was the highest in Mesolithal followed by Microlithal, Filamentous algae and Fine roots. Similarly as in case of total abundance, the lowest species richness was reported on habitats: Akal and Sand, which clearly stand out from the rest of the habitats (Fig. 4.4). Significant differences were reported mainly among Sand and Akal and remaining habitats (Table 4.4).

Table 4.3 PERMANOVA pairwise comparisons among Habitat types

Habitat pairs	R^2	P-value
Akal vs Filamentous algae	0.146	0.013
Akal vs Mesolithal	0.222	0.003
Akal vs Microlithal	0.132	0.022
CPOM vs Filamentous algae	0.129	0.036
CPOM vs Fine roots	0.126	0.029
CPOM vs Macrolithal	0.125	0.047
CPOM vs Mesolithal	0.253	0.002
CPOM vs Microlithal	0.153	0.009
CPOM vs Sand	0.077	0.302
Filamentous algae vs Microlithal	0.044	0.044
Filamentous algae vs Sand	0.180	0.004
Fine roots vs Mesolithal	0.206	0.003
Fine roots vs Microlithal	0.133	0.013
Macrolithal vs Mesolithal	0.229	0.001
Macrolithal vs Microlithal	0.131	0.010
Macrolithal vs Sand	0.162	0.011
Mesolithal vs Sand	0.245	0.001
Microlithal vs Sand	0.179	0.001
Sand vs Xylal	0.135	0.016

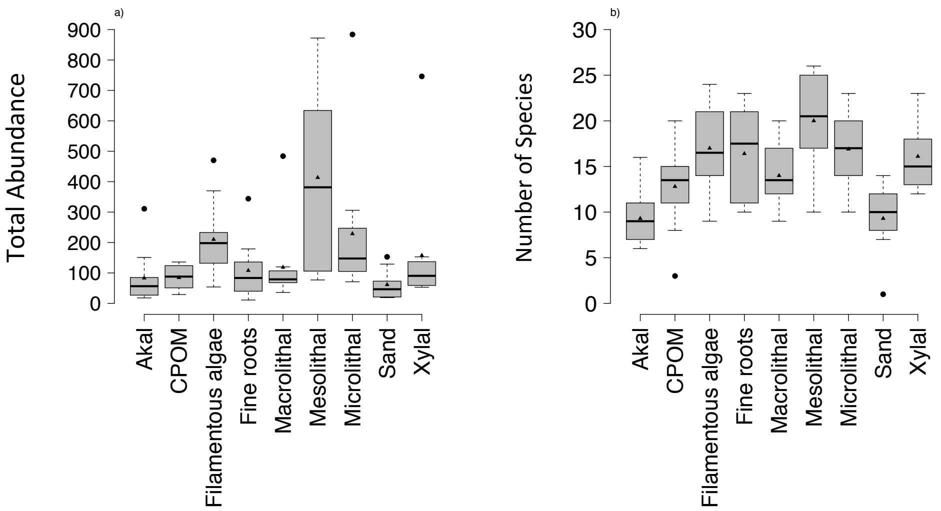


Fig. 4.4 Total Abundance and Number of Species for each Habitat. Triangles represent a mean; black circles represent outliers; horizontal segment is a median; horizontal lines marking the box are first and third quartiles with "whiskers" that extends to minimum and maximum value.

Table 4.4 Post-hoc comparisons (Kruskal-Wallis multiple comparison for Total Abundance and Tukey HSD for Number of Species) among Habitats.

	Chi-squared/F-statistic	P value	Kruskal-Wallis test/Tukey HSD
Total Abundace	32.498	<0.001	MES-AKAL; FA-SAND; MES-SAND; MIL-SAND
Number of Species	7.215	<0.001	FA-AKAL; FR-AKAL; MES-AKAL; MIL-AKAL; XYL-AKAL; MES-CPOM; SAND-FA; SAND-FR; SAND-MES; SAND-MIL; XYL-SAND

Looking at Rényi diversity plot at alpha 0 the highest total species richness was at habitat Filamentous algae, with slightly lower total species richness at Mesolithal, Xylal, Microlithal and Fine roots (Fig. 4.5). Macrolithal and CPOM have intermediate species richness, whereas, Akal and Sand have the lowest total species richness. In terms of Simpson and Shannon diversity indexes the highest value for these indexes was reported for Filamentous algae and the habitat with lowest diversity and evenness was Akal. Xylal, although had a high initial species richness, had less evenly distributed species, indicated by sharper decline of the profile line. Opposite situation was found on Sand, which had the lowest total species richness, but had lower proportions of dominant species indicated by the more horizontal shape of the profile. Instead Microlithal, although had similar total species richness as Mesolithal, Xylal and Fine roots, had less proportions of dominant species (higher evenness) and in total rank of evenness at alpha=Inf was located just after Filamentous algae (Fig. 4.5).

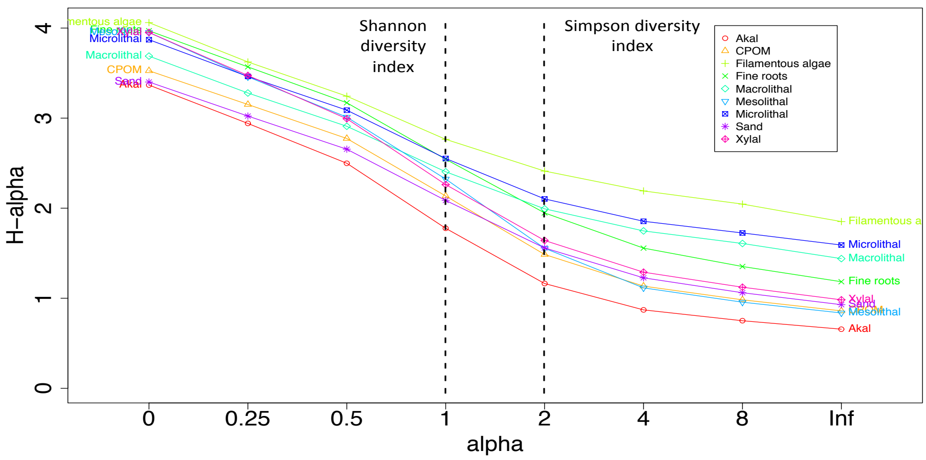


Fig. 4.5 Rényi diversity plot for macroinvertebrates at 9 habitats. Graph drawn using function renyicomp in R statistical package. The profile value for alpha=0 provide information on species richness, α = 1 and α = 2 is the Shannon and Simpson diversity index respectively, the profile value for α = infinity provide information about the proportion of the most abundant species (evenness).

4.3.3. Indicator Species analysis

When using the original indicator value method, without site group combinations, 15 species out of 96 had significant IndVal for one of the habitats (Table 4.5). Only 4 habitats out of 9 had species with significant IndVal. The habitat with the highest number of indicator species (11) was Mesolithal (Table 4.5). Macrolithal and Microlithal had only one indicator species. The only organic habitat with indicator species (2) was Fine roots.

Table 4.5 Indicator Value for individual habitat types, with A and B values and stat = test statistic 'IndVal.g'; only taxa with significant IndVal are listed.

Habitats	Taxa	A	B	stat	P
Fine roots	*Dixa*	0.53	0.50	0.51	0.01
	Physella.acuta	0.26	0.90	0.49	0.01
Macrolithal	*Procambarus.clarkii*	0.50	0.60	0.55	0.01
Mesolithal	*Baetis*	0.57	0.90	0.72	0.01
	Hydropsyche.lobata	0.69	0.70	0.69	0.01
	Hydropsyche.incognita	0.86	0.50	0.66	0.01
	Hydroptila.vectis	0.65	0.60	0.63	0.03
	Isoperla	0.78	0.50	0.63	0.01
	Chimarra.marginata	0.63	0.60	0.62	0.01
	Hydraena	0.52	0.60	0.56	0.01
	Oulimnius	0.29	1.00	0.54	0.01
	Oxyethira	0.34	0.80	0.52	0.03
	Hydropsyche.siltalai	0.70	0.30	0.46	0.04
	Plectrocnemia.laetabilis	0.50	0.40	0.45	0.03
Microlithal	*Elmidae*	0.35	0.70	0.49	0.03

When analysing group combinations with maximum three group combinations, the number of species significantly associated to one, two or three groups were 20. 7 species were significantly associated to only one group, while 13 remaining species were significantly associated to group combinations. 12 taxa were significantly associated to group combinations between mineral and organic substrates (Table 4.6).

Some taxa such as i.e. *Oulimnius* sp., *Baetis* sp., Elmidae, which were initially only associated to mineral habitats become significantly associated to group combinations between organic and mineral substrates. Similarly some taxa which were initially not associated to any of the habitats, became significantly associated to group combinations of organic and inorganic habitats (i.e. *Atyaephyra desmarestii, Simulidae, Orthocladinae, Tanypodinae*).

Table 4.6 Indicator Values for habitats and habitats combination, with A and B values and stat = test statistic 'IndVal.g'; only taxa with significant IndVal are listed.

Habitats and Habitats combinations	Taxa	A	B	stat	P
Fine roots	*Dixa sp.*	0.53	0.50	0.51	0.04
Macrolithal	*Procambarus clarkii*	0.50	0.60	0.55	0.01
Mesolithal	*Hydropsyche lobata*	0.69	0.70	0.69	0.05
	Hydropsyche incognita	0.86	0.50	0.66	0.05
	Chimarra marginata	0.63	0.60	0.62	0.05
	Hydraena sp.	0.52	0.60	0.56	0.01
Mesolithal+Microlithal	*Isoperla sp.*	0.88	0.45	0.63	0.01
CPOM+Filamentous algae+Mesolithal	*Ostracoda*	0.56	0.97	0.73	0.05
CPOM+Mesolithal+Microlithal	*Ferrisia wautieri*	0.68	0.83	0.75	0.01
Filamentous algae+Fine roots+Macrolithal	*Atyaephyra desmarestii*	0.83	0.60	0.71	0.05
Filamentous algae+Macrolithal+Xylal	*Cloen gr.Simile*	0.64	0.77	0.70	0.01
Filamentous algae+Mesolithal+Microlithal	*Oulimnius sp.*	0.65	0.90	0.77	0.01
	Tanypodinae	0.59	0.97	0.75	0.01
	Hydroptila vectis	0.90	0.57	0.71	0.02
	Elmidae	0.91	0.47	0.65	0.01
	Simuliini	0.98	0.43	0.65	0.01
	Orthocladiinae	0.91	0.43	0.63	0.04
Filamentous algae+Mesolithal+Xylal	*Baetis sp.*	0.77	0.90	0.83	0.01
Fine roots+Mesolithal+Sand	*Physella acuta*	0.61	0.70	0.66	0.03
Macrolithal+Mesolithal+Microlithal	*Plectrocnemia laetabilis*	0.86	0.30	0.51	0.03

4.4. Discussion

Consistent with the initial hypothesis reach scale provided a more detailed description of macroinvertebrates assemblage structure than it was previously demonstrated at the catchment scale. Observed differences in community were not only apparent among marginal/depositional habitats vs course mineral substrates, but also among organic vs non organic substrates as well as among different substrate granulation (Macrolithal-Mesolithal; Macrolithal-Microlithal). Main patterns observed at the catchment scale differentiated marginally located habitats (Sand and POM=here CPOM) from the rest of the habitats (although considering also a Typology factor Macrophytes differed from Gravel and Cobble). Similar pattern was also observed at the reach scale with communities inhabiting marginal habitats such as Akal, Sand, CPOM and fine roots being significantly different than communities inhabiting Microlithal, Mesolithal or Filamentous algae. However, differences were also observed among habitats which would be expected to be similar in nature such as Macrolithal and Mesolithal.

Likewise as in catchment scale the habitats with the lowest diversity and abundance were Akal, Sand and CPOM, whereas Microlithal and Mesolithal (corresponding to gravel and cobble respectively at the catchment scale) and filamentous algae had the highest species richness. Xylal and fine roots habitats- although located at depositional pool zones had high species richness, which confirms that these organic habitats with the ability to trap and filter detrital and plant material have high trophic potentiality supporting high number of taxa (Beisel et al. 1998).

Weak pattern in terms of community differences among habitat types for the catchment scale entailed concomitant low number of species with significant indVal. At the catchment scale only one taxa out of 75 families identified was significantly associated to one habitat type, while only 7 taxa was associated to habitat combinations (Chapter 3 *in* Sroczyńska, 2018). By contrast at the reach scale, the number of taxa significantly associated to only one habitat was 15 whereas when habitats combinations were included this number increased to 20 out of 96 taxa identified.

Highly significant associations of the studied taxa with certain habitats clearly coincide with their traits. Species with significant indVal associated to Mesolithal are all rheophilic species exhibiting preferences for well-oxygenated faster current waters. In this habitat the majority are Trichoptera taxonomic group (*C. marginata*, *H.incognita, H. lobata* and *H. vectis, H.siltalai*). *C. marginata, H. vectis*, *H. lobata* and *H.siltalai* are classified as collector-filterers and prefer lotic habitats (Cummins 1967). Thus, their overrepresentation in this habitat is clearly related to high current velocity measured in Mesolithal. In turn, *H. vectis* is specialized at feeding on filamentous algae and this species became significantly associated to group combination between Microlithal, Mesolithal and Filamentous algae (Barnard and Ross 2012). Another Trichoptera species: *Plectrocnemia laetabilis* is only found on stony substrates (Mendoze et al. 2015) and correspondingly was associated to the combination of only mineral substrates: Marolithal, Mesolithal and Microlithal. Collector-gatherers such as *Cloeon simile*, *Baetis* sp. were significantly associated to group combinations between mineral usually two organic substrates. *Atyaephyra desmarestii* normally feeds on suspended particulates accumulated at the surface of algae or leaves and was also respectively significantly associated to these habitats. Diptera- *Dixa* sp. is typically found on the river banks, in half submerged habitats and therefore its association to fine roots of riparian vegetation confirms its habitat preferences.

Flow velocity explained well the ordination of the communities what was additionally confirmed by the indicator values. Rheophilic species with the preferences for higher flow regimes were always associated to habitats where measured water velocity was the highest. For this reason in regard to rheophilic species current velocity plays important role in determining their occurrences (Graça et al. 2004).

However, since the hydraulic conditions are related to substrate type (fine gravel, sand, sand with accumulated organic matter) it is difficult to separate the effect of current velocity from type of substrate on macroinvertebrate communities. In terms of species that are filter feeders, current velocity is a deciduous factor, but other taxa that have more flexible feeding modes (collector/gatherers, scrapers) usually opt for the habitat combinations between both mineral and organic substrates where they can easily obtain the food, whose supply is not dependent on the water flow (Dewson et al. 2007; Mérigoux and Dolédec 2004).

It confirms initial hypothesis that when the group combination is considered more species became associated to the mix of organic and non-organic habitats. Such groups combinations allow them to live at preferable flow regime and substrate granulation (to obtain necessary stability, fixation and refugee in interstitial space) and at the same time take advantage of the organic resources present.

Larger scale factors were mainly related to altitude, interrelated temperature and conductivity and so the species traits. Instead, indicator values measured at the reach scale demonstrated species preferences for specific flow regime, substrate type and granulation and abundance of preferable food. These patterns were not that obvious at the larger scale. Therefore, each scale adds a new dimension of factors which determine species occurrences. This also confirms the hierarchical scale functioning concept (Feminella, 2000; Menge and Olson 1990). It is also in accordance with previous chapter (Chapter 3) which demonstrated that typology and habitat influenced species communities independently. One common pattern observed from the catchment scale and reach scale perspective was that habitats located near the margins or in pools - characterized by similar flow patterns (low current favour accumulation of organic matter) supported different communities than habitats usually encountered in the main channel.

Reach scale turned to be more preferable scale for investigating patches as a source of variation in terms of community structure, than the catchment scale (see Chapter 3 *in* Sroczyńska, 2018). Patterns in species communities were stronger and more evident as well as more significant associations were found. Nonetheless, still some of the patch types overlapped and overall only one fifth of taxa had significant indVal for certain habitat types, which is much less than was found for perennial streams in similar type of studies (Kubosova et al. 2010; Schröder 2013). In general, it confirms that most taxa in intermittent streams display generalist distribution for better use of resources.

Another possible factor explaining higher number of species with significant indVal was taxonomy. At the catchment scale taxonomic identification was undertaken at the family level, commonly used for stream biomonitoring. Instead, taxa at the reach scale were identified mostly to genus, and sometimes to species level. Family-level taxonomic identification was found to be robust in differentiating streams at catchment or ecoregion scale (Feminella et al. 2000). As the scale decreases, species become more specialized and species aggregation to higher taxonomic units may mask important patterns in their distribution (Monk et al. 2012). Thus, some specific associations and its respective impact can be obscured by the coarse taxonomy that was used at the catchment scale.

Chapter 5

Habitat-specific benthic metabolism in a Mediterranean-type intermittent stream

Katarzyna Sroczyńska[1], Marilia Claro[2], Pedro Range[3], Katarzyna Wasiak[4], Adrianna Wojtal-Frankiewicz[5], Radhouan Ben-Hamadou[3], Francisco Leitão[2] and Luís Chícharo[1,6]

[1]Faculty of Science and Technology, Campus of Gambelas, University of Algarve 8005-139, Faro, Portugal. e-mail: kasiasroczynska@gmail.com, tel: (00351)963138888

[2]Centro de Ciências do Mar, Campus of Gambelas, University of Algarve 8005-139, Faro, Portugal

[3]Department of Biological and Environmental Sciences, College of Arts and Sciences, Qatar University, Doha, Qatar

[4]Bieszczadzki Park Narodowy, ul Belzka 7, Ustrzyki Dolne 38-700

[5]Department of Applied Ecology, University of Lodz, Banacha 12/16 Str., 90-237 Lodz, Poland

[6]Centro de Investigação Marinha e Ambiental (CIMA) Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.

This chapter has been previously published as:

Sroczyńska K, Claro M, Range P, Wasiak K, Wojtal-Frankiewicz A, Ben-Hamadou R and Chícharo L (2016) Habitat-specific benthic metabolism in a Mediterranean-type intermittent stream. In *Annales de Limnologie-International Journal of Limnology*. 52: 243-252. EDP Sciences.

Abstract

A modified flow-through chamber method was used to measure gross primary production (GPP), net primary production (NPP), community respiration (CR) and associated environmental variables in an intermittent Mediterranean-type stream in southern Portugal. Three common types of in stream habitats were targeted: cobble (C), cobble covered with filamentous algae (C+A) and leaf litter (LL). NPP, GPP and CR differed significantly among all three habitats. GPP increased with chlorophyll *a* and, less strongly, with photosynthetic active radiation and, therefore, was highest in C+A habitat. The highest CR was in LL and its variation was best determined by ash-free dry mass (AFDM) of plant litter. Higher respiration in LL was related to heterotrophic activity and, to a lesser extent, to autotrophic respiration associated with periphyton. We observed a decrease of production efficiency of primary producers with AFDM in C+A and C habitats. Our results demonstrate that each habitat type should be considered as a discrete metabolic entity and that particular sets of environmental factors are responsible for habitat specific metabolic responses. Scaling up measurements from discrete habitat patches to the entire reach or stream should not be done by extrapolating the results of a single habitat type and will require quantification of habitat coverage, at the appropriate scale.

5.1. Introduction

Streams are heterogeneous environments, with fluctuating channel structure shaped by geological and hydrological processes across broad spatial and temporal scales (Elosegi et al. 2010; Poff and Ward 1989). These dynamics produce patchy distributions of autotrophic and heterotrophic organisms, influencing resource use and metabolic processes (Pringle et al. 1988; Southwood 1977; Warnaars et al. 2007). A rich body of literature has been built around the influence of habitat characteristics and habitat heterogeneity on organization of community structure (Armitage and Cannan 2000; Beisel et al. 1998; Beisel et al. 2000; Brown 2003; Palmer and Poff 1997; Rabeni and Minshall 1977; Townsend and Hildrew 1994; Wallace et al. 1997). We know that habitat heterogeneity contributes directly and indirectly to biodiversity and ecosystem functions such as primary production, decomposition and nutrient cycling (Gessner et al. 2010; Lepori et al. 2005; Wallace et al. 1997). However, relatively few studies directly investigated how habitat patchiness modulates ecosystem-level processes

(Gustafson 1998, Pringle et al. 1988). This is of increasing concern, as the rising global threat of habitat loss and fragmentation impels us to better understanding how habitat specific dynamics influence overall ecosystem processes. Benthic community metabolism is a conspicuous biological process and it integrates how whole communities are influenced by environmental variables across spatial and temporal scales (Fellows et al. 2006).

Few studies dedicated to benthic metabolism have demonstrated important differences in gross primary production (GPP), net primary production (NPP) and community respiration (CR), among different stream habitats (Clapcott and Barmuta 2010; Rier and King 1996). Gonzales-Pinzon et al. (2014) demonstrated large spatial variation in metabolism within a stream, related to presence of different geomorphic units, bed materials and type of transient storage. Additionally, Fellows et al. (2006) demonstrated that separating experimental sites according to habitat type, improved the ability to explain variation in GPP and CR along an agricultural land-use disturbance gradient. Considerable differences in GPP and CR have also been reported along gradients of biofilm structural complexity (Sabater and Romani 1996) and community composition (Busch and Fisher 1981; Velasco et al. 2003). These studies suggest that factors driving ecosystem metabolism are habitat-specific and different habitats act as separate metabolic components. Considering the functional role of individual habitats separately is, therefore, paramount to understanding how spatial heterogeneity and habitat patchiness influence ecosystem processes. This will greatly enhance our ability to predict how shifts relative habitat proportions can affect scaling estimates of stream metabolism.

The natural complexity of streams presents many challenges in measuring metabolism and numerous approaches have been developed to address these (Bott et al. 1997; Marzolf et al. 1994; Odum 1956). By far, chamber-based measurements offer the most straightforward way to investigate the influence of separate river units on the metabolism (Clapcott and Barmuta 2010; Fellows et al. 2006; Fuss and Smock 1996; Rier and King 1996; Whitledge and Rabeni 2000). One of the main advantages of metabolic chambers is the capacity to separate specific habitats and isolate factors affecting metabolism within those habitats (Bott et al. 1978).

However, metabolic chambers can alter environmental conditions in ways that influence ecosystem metabolism (Bott et al. 1997, Dodds 1991). For example, metabolic chambers have been shown to induce nutrient limitations and alter temperature, dissolved oxygen and water velocity conditions during the incubation. It is therefore important to improve the design of metabolic chambers, in order to obtain reliable data and facilitate direct comparison between studies.

Most chamber-based measurements focus on a single substrate unit, usually cobbles or gravel. This approach often fails to account for spatial changes in habitat structure, which can result in localized autotrophy in some habitats and heterotrophy in others (Whitledge and Rabeni 2000). This oversimplification can lead to erroneous conclusions when extrapolating results from the habitat to the reach scale, especially in streams subjected to frequent changes in habitat structure, such as Mediterranean-type intermittent streams

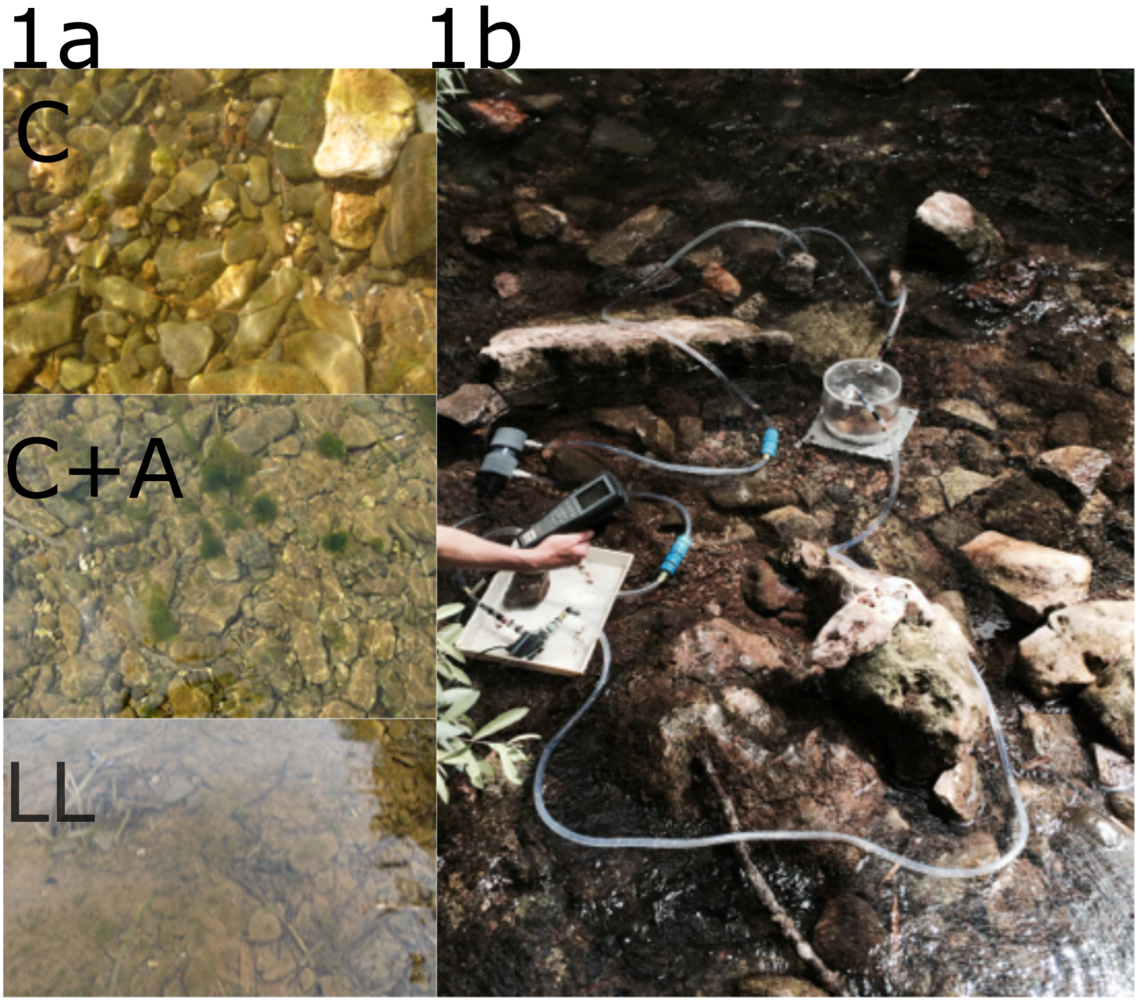
To this end, we quantified ecosystem metabolism rates (GPP, NPP, CR), in the three most common substrates in Mediterranean streams: cobbles (C), cobbles covered with filamentous algae (C+A), and leaf litter (LL). We used closed metabolic chambers modified from Wasiak (2009) where we applied an improved flow-through approach to overcome common problems associated with closed chamber techniques. In addition, we quantified water temperature, photosythetically active radiation –PAR, Chlorophyll *a*, Ash-free dry mass – AFDM, volume rate of water flow passing through the chamber -VR and nutrients (nitrite, phosphate and ammonium), allowing us to assess which factors contributed most to variation in stream metabolism among habitats. We predicted that habitats rich in biofilm and algae (C and C+A) would be net autotrophic while the metabolism of LL would be strongly heterotrophic.

5.2. Methods

5.2.1. Study site

We conducted our study in the Algibre, a first order intermittent stream in the Quarteira River Basin, southern Portugal (37° 11’ 20’’ N, 8° 5’ 33’’ W). The catchment area is ~324 km^2 with an elevation range from 14 to 515 m. The average monthly temperature varies from 8 to 29 °C and average annual rainfall is 625 mm. Catchment land use consists of arable land with shrub and herbaceous vegetation with occasional olive tree and citrus plantations. Catchment geology is predominantly limestone and non-calcareous clay (Trindade et al. 2013). The studied reach was 400 m long and, on average, 8 m wide, with natural morphodynamics, uniform channel morphology and steady flow conditions. The average depth ranged from 20-50 cm in riffles and 50-100 cm in pools. At steady flow conditions (March-May) average discharge was 1.3 $m^3 s^{-1}$ and it gradually decreased towards warmer months, being as low as 0.026 $m^3 s^{-1}$ (July-August) just before the channel completely dries. Channel substrate was predominantly gravel and cobble that during the summer season was densely covered by filamentous algae, chiefly *Cladophora agg*. and *Vaucheria* sp(p.). Riparian vegetation was dominated by *Arundo donax*, herbaceous vegetation and carob and olive trees. It was moderately developed, with a mean width of 3 m and occasional spots of very sparse canopy cover.

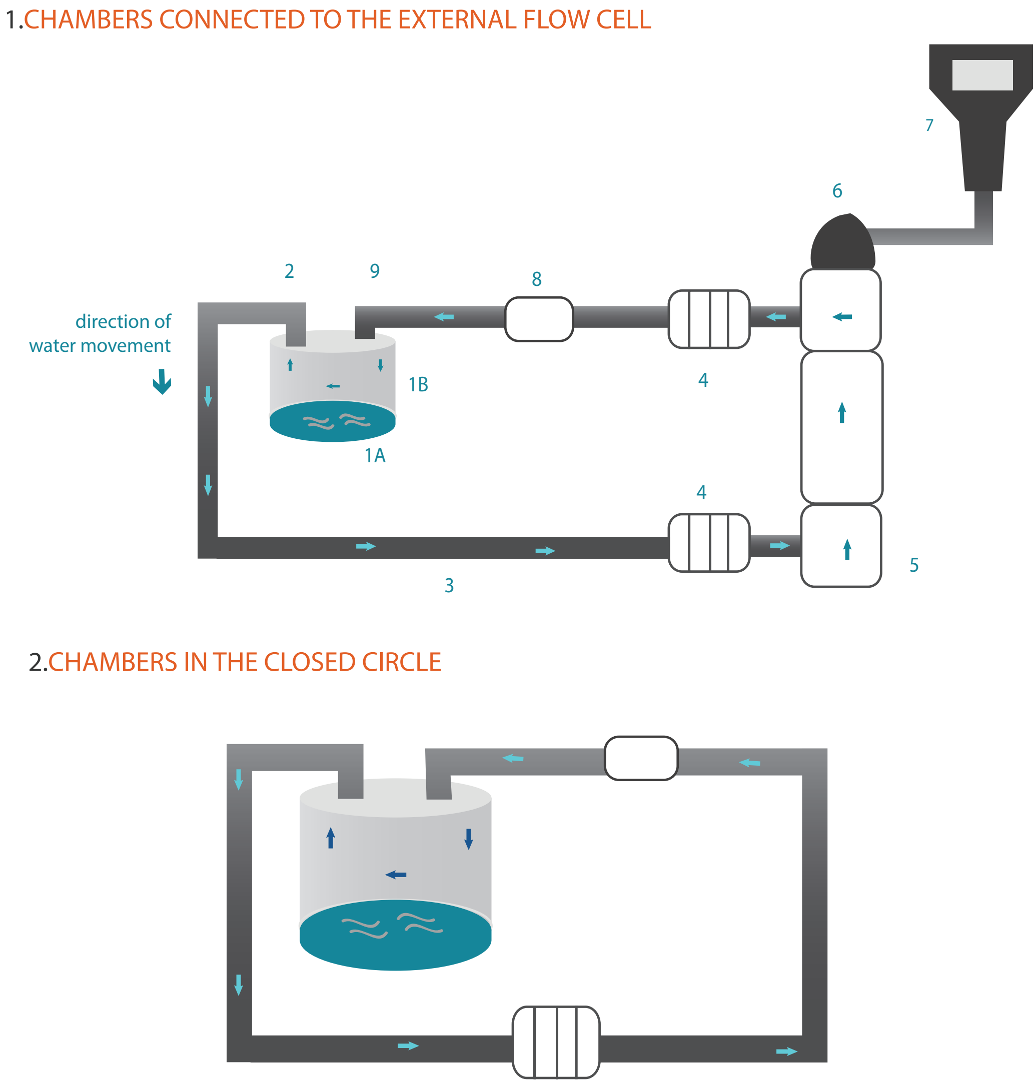
Annual variability in stream discharge directly affects substrate characteristics, the development of algae and macrophytes and accumulation of organic debris. Along the margins, depositional pool zones were filled with accumulated leaf litter from adjacent riparian vegetation, underlain with clay. We focused on the three most common in stream habitats: cobble (C), cobble covered with filamentous algae (C+A) and leaf litter (LL - Online Resource 5.1a). These habitats represent distinct biological units that emerge through interactions between hydrological and geomorphological processes. Water velocity in the study reach was, on average, 0.09 m s^{-1}, ranging from an average of 0.016 m s^{-1} in the leaf litter deposition zones to 0.035 m s^{-1} in the cobble habitat and 0.178 m s^{-1} in cobble covered with algae.



Online Resource 5.1A Three most common habitats characteristic for the Quarteira River Basin: Cobble (C), Cobble covered with filamentous algae (C+A) and Leaf litter (LL); **Online Resource 5.1B** Picture of the experimental design.

5.2.2. Experimental procedure

Metabolic measurements were done *in situ*, from 11th April through June 27th 2014, a period characterized by stable flow conditions. Thirty-nine measurements of NPP, and CR were taken in haphazardly selected patches of each habitat type (C 12; C+A 15; LL 12) within the same 400 m reach. NPP and CR were measured using enclosed acrylic metabolic chambers modified from Wasiak (2009), with several improvements to study habitat-specific metabolism (Online Resources 5.1b and 5.2).



Online Resource 5.2 Experimental design where: 1. Experimental chamber consisted of 1a Stainless steel base, 180 mm diameter and 40 mm height; 1b Acrylic cover, 160 mm height, 4.53 l volume; 2. Outflow port; 3. ⅜“ PVC plastic tube; 4. Quick release unions; 5. Flow cell (YSI 3059); 6. Polarographic DO sensor (YSI 605203) encased in the flow cell ; 7. ProPlus meter (YSI 6050000); 8. Submersible pump (NEW-JET NJ600); 9. Inflow port

We buried a metal chamber base ~[180 mm diameter] in the stream sediments (two per habitat – randomly placed). A sample of intact substrate was removed from the river bottom and gently placed on the base, where it was left submerged in the stream for 3-5 days to allow macroinvertebrates recolonization (assuming that previous studies at perennial streams of Boyero 2003a; O’Connor 1991 and Oliveira et al. 2014 will be similarly applicable to our system). In most benthic community studies, the bases were left for longer periods (Bott et al. 1978). Unfortunately this was not possible in our study, due to the risk of theft or vandalism, which did occurred, with several bases disappearing during these periods of recolonization. Chamber bases with leaf litter were prepared by filling the base unit with naturally fallen leaves of *Arundo donax*. Litter material was composed of senescent leaves, consisting of entire and partially degraded specimens (≈30-50 % decomposed – personal observation) as well as remains of stems. During each 2 h incubation the base was sealed with an acrylic chamber (of 4.53 l volume), without disturbing the previously established community. The chambers were submerged to ensure they were free of air bubbles and to equilibrate the temperature in the chambers with the stream water (Bott et al. 1978). Each chamber was connected to a submersible pump by the inflow and outflow ports, so that the water flow inside the chamber was continuously homogenized, to allow stable dissolved oxygen readings. The inflow tube was deep seated inside the chamber in a manner to not resuspend the bottom sediment, but maximizing water homogenization. Submersible pumps were powered by a portable generator (Online Resource 5.2).

NPP was measured as changes in dissolved oxygen inside each chamber, by using an oxygen sensor (YSI, Professional Plus), encased in an external flow cell (YSI, model 3059), which was sequentially connected to each of the chambers. When one measurement was finished, the flow cell was disconnected and connected to the next chamber. The tube connectors were always submerged during these operations, in order to avoid any air bubbles entering the line. This design allowed simultaneous incubation of several chambers, using only one oxygen probe. When incubations in light were finished, the water inside the chamber was completely exchanged. This was achieved by disconnecting the tubes from the circulation system and allowing the fresh water from the stream to enter the line. Considering that the flow rate of water passing through the chamber was, on average, 1.66×10^{-5} [m^3 s^{-1}] the volume of the system was totally exchanged in 5 minutes. Including this time interval we allowed approximately 20 minutes to half an hour time of acclimation before starting to measure community respiration. CR measures were done after covering the chamber with a black plastic wrapper to inhibit the light. Light and dark incubations lasted 2 hours (DO concentration recorded at 12 min intervals). Incubations were initially paired with "blank" chambers, filled only with stream water (no substrate added to the bases), to assess the metabolism of the water column. T-tests ($P>0.05$) showed that differences between corrected and uncorrected metabolic rates were negligible, so benthic metabolism rates were ultimately not corrected for water column metabolism. NPP and CR (mg DO m^{-2} h^{-1}) were calculated as follows:

$(NPP, CR)=(\Delta\ O_2)\times V\times(S^{-1})$, where:

$\Delta\ O_2$ is the change of oxygen concentration between beginning and end of the experiment per unit volume and time (mg DO l^{-1} h^{-1}), V-remaining volume (l) of the chamber (subtracted by the volume of substrate), S-stone surface area (m^2) and in case of chamber with leaf litter is the area occupied by leaf litter, which was approximately the area of the base.

GPP was estimated as the sum of rates in light and dark incubations (GPP = NPP + CR; Bott 2006). Production to Respiration ratio (P/R) describes the balance of metabolic processes during 24 h period and was calculated as GPP converted to daily metabolism divided by the CR during 24 h period following the equation: GPP/CR_{24} (Bott 2006).

Pre- and post-incubation water samples were collected to assess nutrient depletion within chambers during incubations [nitrite (NO_2^--N), ammonium (NH_4^+-N) and phosphate (PO_4-P)]. Nutrient analyses were done on a MERCK Spectroquant Nova 60, using Spectroquant®Test kits for NO_2^-, NH_4^+ and PO_4^{2-}. To validate oxygen readings taken by the DO probe, we simultaneously collected water samples for oxygen analysis, using the spectrophotometric Winkler method (Labasque et al. 2004).

Simultaneously with oxygen measurements, temperature inside the chamber was monitored (using the YSI probe model) as well as photosythetically active radiation (PAR) in µmol quanta m^{-2} s^{-1} (LI-250A Light Meter). The light sensor was placed in the water in the proximity of chambers to ensure that the amount of light that reach the sensor was the same that reach the chambers. Current velocity [m s^{-1}] in each habitat type used for the experiment was measured using a two-dimensional acoustic-Doppler velocimeter (FlowTracker Handheld ADV, Sontek YSI Inc.). After the experiment finished, the content of each chamber was taken to the laboratory and processed for determination of periphyton biomass and macroinvertebrate identification.

We scrubbed periphyton from stones and leaves into a known volume of water using a toothbrush (Biggs and Kilroy, 2000). In case of leaf litter, each piece of leave material was gently placed in the tray and superficial biofilm layer was scrubbed from both sites. The resultant slurry was thoroughly homogenised, subsampled and filtered on glass fiber filters (GF/C, 47mm Whatman) for determinations of chlorophyll *a* (Chl *a* mg m^{-2})

and ash free dry mass (AFDM mg m^{-2}). AFDM of leaf litter was measured separately including all the leaves biomass. Chl *a* was extracted in 90 % boiling ethanol and kept in the freezer for 24 h. The absorbance was read on spectrophotometer (Thermospectronic GENESYS 10UV). AFDM filters were dried at 60 °C to constant weight and AFDM represents the weight difference before and after 4h at 450 °C (Biggs and Kilroy 2000).

For AFDM analyses in the chambers containing leaf litter, all the biomass was collected, placed on the tray, dried and ashed as described above. For the chambers containing cobbles and cobbles covered with algae, Chl *a* and AFDM were calculated per stone surface area assuming that metabolically active area of stones is 60 % (Biggs and Close 1989).

5.2.3. Data Analysis

We compared ecosystem metabolism among habitats with analysis of variance (ANOVA) when assumptions of normality (Shapiro-Wilk test for small sample size) and homogeneity of variance (Leven's test) were met. When the homogeneity of variance assumption was not met, we applied the non-parametric Kruskal-Wallis Rank Sum Test using the "kruskalmc" function in the R package "pgirmess" (R Development Core Team 2012; Giraudoux 2013). We performed Tukey's post-hoc analyses and generated 95% confidence intervals using the "TukeyHSD" function in the R package "multcomp" (Hothorn et al. 2008; R Development Core Team 2012). Univariate analyses and graphs were done using R package (R Development Core Team 2012). When ANOVA demonstrated significant differences between habitats, correlation analysis were done, treating each habitat separately. Spearman product moment correlation was used to assess the relationship between explanatory and response variables as well as correlation between DO measurements and Winkler spectrophotometry oxygen determination.

Multivariate analyses were performed on normalized (Euclidean distances) metabolic responses (NPP, GPP and CR) and explanatory variables (Chl *a*, AFDM, VR, PAR, temp, nitrite, phosphate and ammonium), with habitat as a fixed factor. PERMANOVA (permutational multivariate analysis of variance) was used to test for significant differences in metabolism between the habitats. Distance-based linear models (DistLM) were used to examine the relationship between response variables and explanatory variables. Firstly, the significance of the relationship was assessed for individual environmental variables with marginal tests (999 permutations). Significant variables ($p < 0.01$) were subsequently included in model selection using the BEST procedure, which tests all possible combinations between explanatory variables and the response matrix. This procedure helps eliminate the effect of covariance between the variables, as it considers all the response variables together. Distance-based redundancy analysis (dbRDA) was used for the ordination and visualisation of the best overall DistLM solution, according to the Akaike information criterion (AIC). All multivariate analyses were done using the PRIMER 6 statistical package with the PERMANOVA+ add-on (Clarke and Warwick 2001).

5.3. Results

5.3.1. Benthic metabolism

The highest significant average GPP was recorded for the habitat C+A and the lowest for the habitat C (Fig.5.1, Table 5.1).

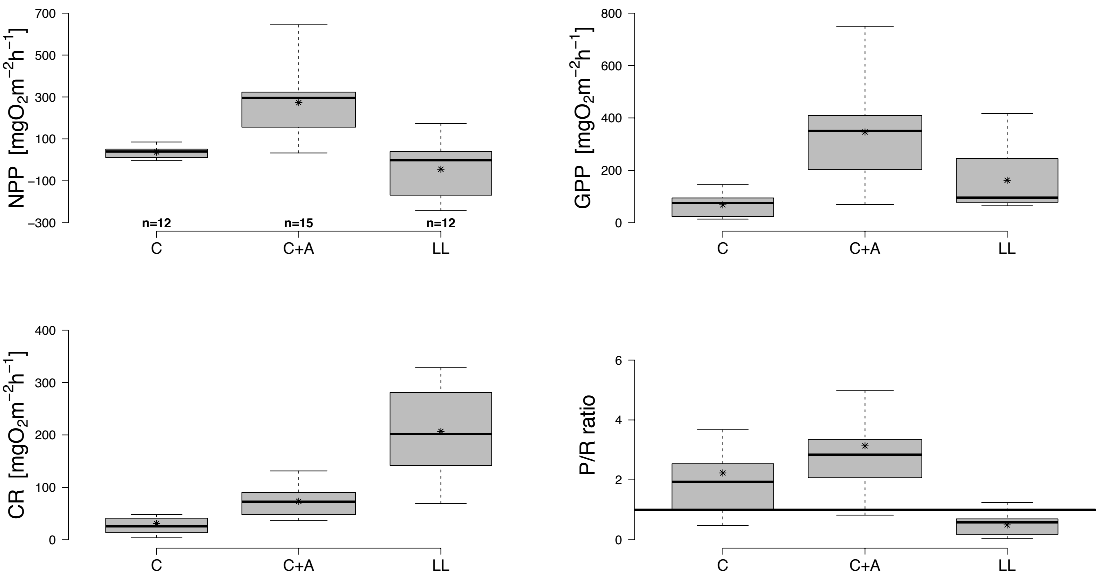


Fig. 5.1. Boxplots with NPP, GPP, CR and P/R ratio for each habitat; Where black asterisk is a mean; horizontal segment is a median; horizontal lines marking the box are first and third quartiles with “whiskers” that extends to minimum and maximum value. Horizontal line in P/R ratio boxplot marks the benchmark for autotrophy (>1) and heterotrophy (<1)

Table 5.1 Range for net primary production (NPP), gross primary production (GPP), community respiration CR [g O_2 m^2 day^{-1}] and P/R ratio for each habitat (C-cobble, C+A-cobble covered by algae, LL-leaf litter).

Habitat	NPP	GPP	CR	P/R ratio
C	-0.03-1.22	0.17-1.93	0.086-1.94	0.48-7.72
C+A	0.47-8.66	0.99-9.84	0.64-2.55	0.82-9.14
LL	-3.86-2.47	0.23-5.99	1.65-7.87	0.03-1.25

Average oxygen consumption (CR) during dark phase was 3-fold and 7-fold higher in habitat LL, relative to C+A and C, respectively (Fig.5.1). The variability in oxygen consumption during dark phase in C and C+A was lower than during light hours, while LL habitat demonstrated high variation in both respiration rate and oxygen production. All three habitats show significant differences in terms of GPP, NPP and CR (Table 5.2).

Table 5.2 Analysis of variance for Net Primary Production (NPP), Gross Primary Production (GPP) and Community Respiration (CR) for the differences between habitats

Statistical test		ANOVA			ANOVA			Kruskal-Wallis Rank Sum Test	
		GPP [mg O_2 m^{-1} h^{-1}]			NPP [mg O_2 m^{-1} h^{-1}]			CR [mg O2 m-1 h-1]	
Source of variation	*df*	Mean Sq	*F*-ratio	Pr(>F)	Mean Sq	*F*-ratio	Pr(>F)	χ^2	P
Habitat	2	272841.00	13.71	3.75E-05	373635	19.96	1.47E-06	27.01	1.37E-06
Residual	36	199.03			18719				

For NPP and GPP variables, Tukey multi pairwise comparisons showed significant differences between C+A - C (P<0.001) and C+A - LL (P<0.001), but not for LL - C (P=0.32 and P=0.25 respectively). Multiple comparisons after Kruskal-Wallis for CR showed significant differences (P<0.05) among all the groups.

Autotrophy dominated in C+A and C habitats, with positive P/R ratios (>1), while LL was entirely heterotrophic (P/R < 1; Fig. 5.1, Table 5.2). The highest mean production relatively to respiration was recorded for the C+A habitat (3.13) and the lowest for LL (0.49).

5.3.2. Environmental variables

Chl *a* ranged from 1 to 33 mg m^{-2} on C habitat, 21 to 146 mg m^{-2} on C+A habitat and 9-64 mg m^{-2} on LL (Table 5.3). The highest average Chl *a*, as well as the highest variability, was measured in habitat C+A and the lowest in habitat C. LL had twice the average Chl *a* than C habitat. AFDM ranged from 1 on C to 36 g m^{-2} on C+A, while values in LL habitat were 2 orders of magnitude higher than in the other habitats and showed a broad variation (900-1921 g m^{-2}). Temperature varied little between habitats, with average of 21-22 °C. Water flow passing through the chambers varied on a narrow interval (0.06±0.005 m^3 h^{-1}) and it did not differ among habitats.

Table 5.3 Mean ±SE for environmental variables (Chlorophyll *a*, Ash-free dry mass, Photosynthetic active radiation, Temperature, PO_4^--P, NO_2-N, NH_4^+-N) and GPP/Chl *a* measured during incubation experiments.

Measured variables	Habitat type		
	C	C+A	LL
Chlorophyll a [mg m^{-2}]	9.95±3.09	76.13±10.45	22.55±4.47
AFDM [g m^{-2}]	5.02±1.15	17.00±2.10	1382±94.99
PAR [µE m^{-2} s^{-1}]	1799±110	1495±181	1554±186
Temperature [°C]	21.51±0.55	22.57±0.37	21.68±0.46
PO_4-P [mg l^{-1}]	0.046±0.003	0.048±0.005	0.041±0.003
NO_2-N [mg l^{-1}]	0.042±0.009	0.035±0.006	0.036±0.007
NH_4^+-N [mg l^{-1}]	0.30±0.27	0.31±0.21	0.33±0.26
GPP/Chl a ratio	13.70±2.97	5.51±1.03	8.15±1.34

Nutrients varied little between measurements and paired t-test showed no significant differences in nitrites and phosphates between pre- and post- water samples for both light and dark incubations (P>0.05). Ammonium levels were mostly below the detection limits of our instrumentation (< 0.05 mg l^{-1}).

Correlation between the DO probe and Winkler spectrophotometric determination was very high (r=0.86, P<0.001), with consistently lower values obtained by Winkler spectrophotometry (3.2-18.0 mg O_2 l^{-1}) than measured by the oxygen probe (4.2-22.0 mg O_2 $l\text{-}^{1}$).

5.3.3. Relationship between benthic metabolism and habitat characteristics

The best DistLM model provided by BEST procedure included 3 vector overlays: Chl *a*, AFDM and PAR, which significantly contributed to the ordination axes (Fig. 5.2). The model explained over 60.0 % of the total variability in data. From these 3 vectors, Chl *a* and AFDM were the strongest contributors to the dbRDA analysis, having higher explanatory potential in comparison to PAR. The variation in Chl *a* and PAR were associated with the variation in NPP and GPP in C+A habitat, while AFDM was strongly associated with LL habitat and strongly correlated with CR.

PERMANOVA showed statistically significant differences in explanatory variables among all the habitats (F=21.6, P=0.001, based on 999 permutations) and pair-wise tests showed that LL habitat differed the most from the other two habitats, with higher dissimilarity between LL and C+A than LL and C. Due to differences existing between all three habitats, correlation analyses were performed separately for each habitat.

Both C and C+A habitats displayed decrease of production efficiency (expressed as GPP/Chl *a* ratio) with increasing standing stock (AFDM, Table 5.4).

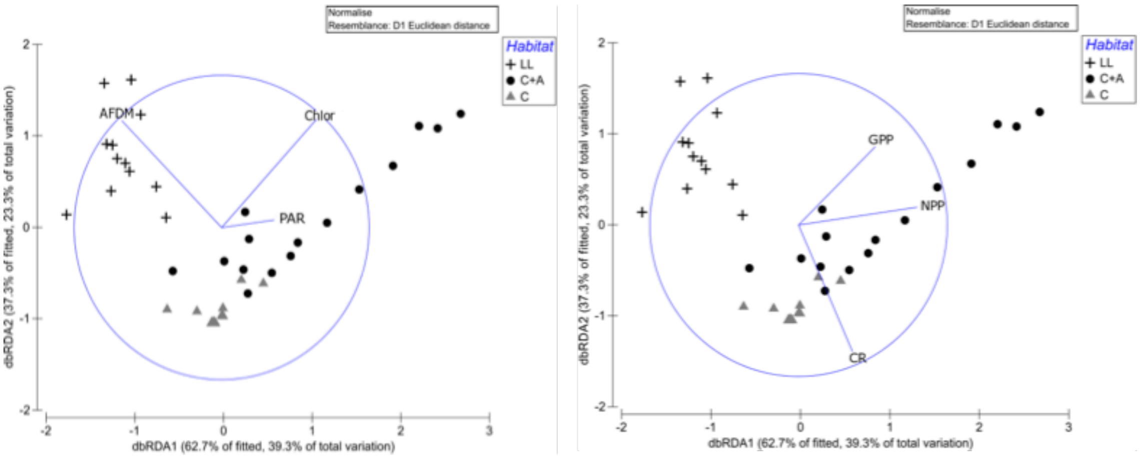


Fig. 5.2 Diagram of distance-based redundancy analysis (dbRDA) for the best distance-based linear models (DistLM) solution. Vector overlays represent significant variables included in the model, selected with the BEST procedure based on Akaike's information criterion. Left panel represents explanatory variables and right panel the response variables. Lengths of vectors indicate the relative influence of each variable for the ordination

Table 4. Spearman's rank correlation for the response and independent variables. Significant codes are as follow: <0.01 ***, <0.05 **

Habitat	Variable	NPP	GPP	CR	Chl a	AFDM	PAR	Temp
C	NPP		0.82***	0.31	0.73 ***	0.55	-0.36	0.62 **
	GPP			0.76 ***	0.60 **	0.52	-0.24	0.73 **
	CR				0.45	0.48	-0.20	0.66 **
	GPP/Chl a	-0.41	-0.09	-0.06	-0.81 ***	-0.62 **	0.39	-0.31
C+A	NPP		0.95 ***	0.44	0.31	-0.38	0.78 ***	0.10
	GPP			0.65 ***	0.51**	0.28	0.73 ***	0.16
	CR				0.75 ***	0.16	0.37	0.28
	GPP/Chl a	0.73*	0.60 **	0.08	0.35	-0.70***	0.65 ***	-0.16
LL	NPP		0.89 ***	-0.38	0.13	0.10	0.21	-0.08
	GPP			-0.14	0.22	0.16	0.06	0.15
	CR				0.59 **	0.29	-0.53	0.48
	GPP/Chl a	0.68 **	0.76 ***	0.31	-0.36	0.09	0.43	0.03

Chlorophyll *a* concentration was positively correlated with oxygen consumption (CR) in C+A and LL habitats (Fig. 5.2), although not in C habitat. High correlation existed between primary production and oxygen consumption in C and C+A habitats. By contrast it was not the case in LL habitat, where no correlation existed between GPP and CR. GPP, NPP and production efficiency (GPP/Chl *a* ratio) significantly increased with PAR in the C+A habitat. In C habitat, temperature was positively correlated with GPP, NPP and CR, regardless of PAR, which was not significantly correlated with any of these variables.

5.4. Discussion

Results were consistent with our predictions, in that all 3 habitats showed positive gross primary production, but with significantly higher net oxygen production in cobble and cobble covered with algae. Half of the leaf litter habitat patches exhibited negative NPP, indicating that oxygen was consumed more rapidly than it was produced. The leaf litter habitat was an important site for high microbial activity, however, it constituted less than 10 % of overall benthic substrate and thus its contribution at the reach-scale is probably limited. Accordingly, during stabilized flow conditions, benthic metabolism was dominated by net autotrophy, in cobble and cobble covered by algae habitats, with localized heterotrophy in leaf litter.

Physical substrate heterogeneity and biological characteristics of substrata were demonstrated to influence both GPP and CR ratio (Cardinale et al. 2002; Clapcott and Barmuta 2010; Guash et al. 1995; Sabater and Romani 1996; Sabater et al. 1998). Similar range of GPP and CR values, measured using open-channel and metabolic chamber methods were obtained in other Mediterranean ecosystems (Aristegi et al. 2010, Molla et al. 1994, 1996, Suarez and Vidal-Abarca 2001) and in desert streams (Busch and Fisher 1981; Grimm and Fisher 1984; Mulholland et al. 2001). Molla *et al.* (1994) obtained similar values of GPP (3.24 g O_2 m^2 day $^{-1}$), but higher average values of respiration (2.9 g O_2 m^2 day $^{-1}$) for periphyton communities, using dial oxygen curve method. Our estimates of GPP and CR are most similar to the results obtained by Suarez and Vidal-Abarca (2001) for whole periphyton communities by using diurnal oxygen change method (range 0.24 -10.7 for GPP and 0.26-7.29 g O_2 m^2 day $^{-1}$ for CR). Aristegi et al. (2010) reported wide ranges of GPP (0-35.3 g O_2 m^2 day $^{-1}$) and CR (1.1-17.2 g O_2 m^2 day $^{-1}$) using recirculatory chambers. However, their study encompassed streams with large variability in environmental conditions, whereas the small ranges of GPP and CR in our study were measured in only one reach. Similar values were also reported for a desert stream by Mulholland et al. (2001) (3.0 for GPP and 8.3 for CR g O_2 m^2 day $^{-1}$), using two-station diurnal oxygen change method. Accordingly, our results with metabolic chambers on C and C+A habitats are consistent with previous studies in intermittent streams in regions of Mediterranean and semiarid climate.

5.4.1. Primary Production

Previous studies found that algae production increases with the Chl *a* standing crop (Bernot et al. 2010; Morin et al. 1999). Similarly, we found that the most productive cobble covered with algae had the highest Chl *a* concentration compared to other habitats. A positive effect of algae biomass on GPP has been reported across different regions, as measured by open channel methods (Bernot et al. 2010; Bott et al. 1985; Morin et al. 1999). However, high periphyton biomass does not necessarily translate into higher production efficiency (as seen from the results of GPP/Chl-a ratio) and also reported by Velasco et al. (2003). Decrease of GPP/Chl *a* ratio with increasing standing stock of periphyton is common for stream ecosystems (Guasch et al. 1995; Morin et al. 1999; Velasco et al. 2003). This pattern can be related to different composition of the algal assemblages and biofilm thickness in cobble and cobble covered by algae habitats. Shifts in algae community along the gradient of biofilm development are well documented (Hudon and Bourget 1983; Sabater and Romani 1996). In our study, the biofilm in C+A habitat was dominated by green filamentous algae, while the biofilm in the cobble habitat was scarce and nearly invisible. Phytoplankton photosynthetic rates are known to decrease with the increasing cell wall thickness (Enríquez et al. 1996) and therefore, thicker walls of filamentous algae in C+A habitat were potentially responsible for the negative trend between GPP/Chla and periphyton biomass. Another contributory mechanism that could explain this pattern is self-shading, which is a common process associated with periphyton of well-developed and complex biofilm structures (Guash et al. 1995).

PAR was found to be positively correlated with GPP among different reaches, in open channel measurements (Acuna et al. 2004; Bernot et al. 2010; Bott et al. 1985; Mulholland et al. 2001) as well as incubation chambers, considering only epilithic assemblages (Velasco et al. 2003) and whole communities (Rosenfeld and Roff 1991). By contrast, in our study PAR explained very little variation in metabolic parameters and had distinct influence on primary production for different habitats (Table 5.4). Lack of correlation between PAR and cobble is corroborated by the low levels of chlorophyll *a* on this habitat. Interestingly, temperature independently of PAR was positively correlated with GPP on cobble, but not on cobble covered with algae. This additionally confirms that biofilm structure has important role in regulating GPP response in this temporary stream.

Primary Production in leaf litter was greater than in cobble. These differences appear to be driven by distinct substrate characteristics of C and LL. Organic conditioning of litter enhances algae GPP by allowing the use of the underlying substratum as a nutrient source (Sabater et al. 1998). Additionally, the oligotrophic nature of our study stream could exacerbate the nutrient limitation effect on algal colonization of cobbles. It is important to mention, however, that GPP in LL habitat could have been overestimated, as the total area of leaves and remaining litter material probably exceeded the area of the chamber base.

5.4.2. Community Respiration

The highest respiration was in the leaf litter habitat. Leaf litter and woody substrata host greater amount of heterotrophic organisms, such as bacteria, fungi and macroinvertebrates, which utilize the underlying substratum to acquire nutrients and eventually contribute to organic matter decomposition (Graça et al. 2001; Gulis and Suberkropp 2003; Romani and Sabater 2001). In our study, AFDM (detrital standing crop) was responsible for variation in CR and differentiated the heterotrophic leaf litter habitat, with large amounts of AFDM, from other two habitats, with smaller values of AFDM (RDA analyses). When we examined only the leaf litter habitat, however, litter biomass did not contribute to the variation in community respiration. This lack of correlation is presumably because CR in LL is fuelled by a combination of heterotrophic utilization of the allochthonous organic matter itself, but also some contribution of microbial and algal respiration associated with periphyton. Leaves used for the experiment were senescent and the long period in the water allowed microorganisms to be associated with leaf mesophyll as well as create a layer of biofilm on their surface. Additionally, the main groups of macroinvertebrates were collector-gatherers and scrapers, with only few shredders, which confirm the trophic potential of the biofilm. However, the short acclimation time of base units in the stream did not allow proper colonization of macroinvertebrates, in comparison to benthic community encountered in this habitat during conventional sampling with hand net. Base units had more mobile and drifting taxa and less burrowing organisms. Accordingly, some important burrowing shredders, such as Diptera family, may have been under represented.

In contrast, the cobble habitat had the lowest CR from all the habitats studied and, although the average Chl *a* concentration in LL was only twice higher than in C habitat, CR in LL was almost 7-fold higher than in cobble. Therefore, higher respiration in LL is likely a result of heterotrophic activity and microbial respiration associated with biofilm layer, rather than autotrophic respiration. The organic nature of leaves promotes settlement of fungi and bacteria, which obtain nutrients via leaf litter degradation. In contrast, the major part of respiration in cobble and cobble covered by algae habitats came from autotrophic respiration, which is also reflected in high correlation between GPP and CR in these habitats.

5.5. Conclusions

Most metabolism studies with benthic chambers focus only on the dominant habitat type in a river reach (Aristegi et al. 2010; Rees et al. 2005; Whitledge and Rabeni 2000). Our study clearly emphasizes that extrapolating from a single habitat to the entire reach or stream will result in significant under or over estimation of the metabolic rates, depending on the proportional dominance of habitat types. For example, benthic habitat mapping of the reach used in our study, done in previous years, indicated striking differences in habitat coverage between winter (5 % of algae cover) and summer (50 % of algae cover, Wasiak et al. 2013). Considering that, during summer, at least half of the substrate in intermittent Mediterranean streams is covered by filamentous algae, using only the cobble substrate for incubations would underestimate GPP by 67%. Accordingly, scaling up measurements from discrete habitats to the entire reach or stream requires quantification of habitat coverage. This can be achieved by the use of various techniques such as GIS-based analysis, based on visual benthic habitat mapping, for small reaches, or side scan sonar imagery to map larger areas of streams to whole catchments.

Employment of flow cell into chamber design successfully overcame two main drawbacks related to chamber metabolism measurements: bubble formation and inadequate water circulation within chambers (Bott et al. 1997; Dodds and Brock 1998; Uzarski et al. 2001). Furthermore, measurements taken by DO oxygen probe are linearly related to the oxygen concentrations measured using the Winkler method. This validates the use of *in situ* DO probes to monitor oxygen concentration during the incubation experiments and the accurateness of the metabolic rates obtained by this improved chamber design.

Acknowledgements

The authors acknowledge David Harper, Victor Garcia Lopez, Carlos Sanchez Ureña, Almudena López Fernández and Bogna Jacniacka for their contribution to experimental design and field work. We are also grateful to Tanner Williamson and Jennifer Taylor for their comments on the manuscript and English review. The authors also acknowledge anonymous referee for their valuable comments, which contributed to the manuscript improvement. This work was a part of the IMPACT project, funded by Foundation for Science and Technology (FCT) of Portugal (ERA-IWRM/0003/2009). F. Leitão was also funded by a scholarship from FCT (Reference SFRH/BPD/63935/2009).

Chapter 6

Food sources and local dietary specialization of benthic consumers in temporary Mediterranean-type streams determined from stable N and C isotopes

Katarzyna[1], Williamson Tanner[2], Claro Marilia[3], González-Pérez José Antonio[4], Range Pedro[5] Boski Tomasz[6] and Chícharo Luis[1]

[1] Faculty of Science and Technology, Campus of Gambelas, University of Algarve 8005-139, Faro, Portugal

[2] Department of Biology, Miami University, Oxford, OH, U.S.A

[3] Centre for Marine Sciences, Universidade do Algarve, Campus de Gambelas 8005-139 Faro, Portugal

[4] Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC). Avda. Reina Mecedes 10, 41012 Seville, Spain

[5] Department of Biological and Environmental Sciences, College of Arts and Sciences, Qatar University, Doha, Qatar

[6]CIMA – Centro de Investigação Marinha e Ambiental, Campus of Gambelas, University of Algarve 8005-139, Faro, Portugal

This chapter was submitted for the publication as:

Sroczyńska K, Williamson T, Claro M, González-Pérez JA, Range P, Boski T and Chicharo L. in review. Food sources and local dietary specialization of benthic consumers in temporary Mediterranean-type streams determined from stable N and C isotopes.

Abstract

We investigated $\delta^{13}C$ and $\delta^{15}N$ of basal food resources and benthic consumers at three sites in a temporary Mediterranean-type stream. From both C and N isotopic signatures, the inferred food sources for the majority of aquatic invertebrates were of autochthonous origin at all of the sites. Most of the variance in $\delta^{13}C$ and $\delta^{15}N$ of consumers was attributable to site-specific $\delta^{13}C$ and $\delta^{15}N$ values of basal food sources. The presence of moss (with conspicuous low values of $\delta^{13}C$) at one site with perennial regime was responsible for extremely negative $\delta^{13}C$ values of consumers suggesting that, at this site, moss is a potential food source for some of the macroinvertebrates. Strong dietary specialization was only exhibited by grazers, which feed primarily on epilithic algae. Conversely, the other consumers used distinct dietary resources at different sites. We found large variability among consumer species nested within the same functional feeding groups (FFGs), indicating that FFGs classification poorly reflects trophic structure and resource assimilation in this type of streams. Additionally, higher overlap in isotopic fractionation between consumers of different FFGs at intermittent, less shaded sites indicates the existence of omnivory as an adaptive strategy for consumers who live in environments were food resources are seasonally variable.

6.1. Introduction

Investigating carbon sources for lotic consumers is a principal focus in ecology. Using stable carbon isotope ratios (specifically the $^{13}C/^{12}C$ expressed as $\delta\,^{13}C$‰) allows tracing of allochthonous (e.g., terrestrial litter) and autochthonous (e.g., algae) organic matter sources through aquatic food webs. The ability to differentiate between these compartments is largely based on the assumption that algae have distinct ^{13}C signatures relative to terrestrial litter (Bunn et al. 1989; France 1996a; France 1996b; Rosenfeld and Roff 1992). Additionally, the nitrogen $^{15}N/^{14}N$ stable isotopes ratio expressed as $\delta\,^{15}N$‰ is useful in identifying trophic relationships due to constant fractionation against the heavier isotope with increasing trophic level (Minagawa and Wada 1984).

The general foundational framework of trophic ecology in forested streams is that temperate headwater streams are primary driven by allochthonous energy sources, with the relative importance of autochthonous sources increasing in the downstream direction (Hall et al. 2001; Vannote et al. 1980). However, a growing body of research, using stable isotopes analysis (SIA), has recently demonstrated that algal production is a dominant energy source for consumers, across a diverse array of aquatic ecosystems, (Araujo-Lima et al. 1986; Douglas et al. 2005; Lau et al. 2008; Lau et al. 2009a, Lau et al. 2009b; McCutchan and Lewis 2002; Thorp and Delong 2002). Despite this rich body of research, our knowledge about how the relative contribution of allochthonous and autochthonous subsidies influence consumers and trophic food webs in intermittent Mediterranean streams is still limited. Intermittent Mediterranean streams are very heterogeneous with respect to their hydrology, composed of sites with permanent annual flow, that are interwoven with sites that have episodic flow (Gasith and Resh 1999). It is often thought that terrestrial subsidies to Mediterranean streams are less pronounced than in more humid regions and that autochthonous benthic production is the primary energy source, even in well-shaded forested streams (Bunn et al. 1999; Douglas et al. 2005; Gasith and Resh 1999). However, scant and often contradictory results of food web studies in intermittent Mediterranean streams have done little to support this general belief (Alvarez and Pardo 2009; Dieterich et al. 1997). Moreover, these studies are based solely on analysis of shifts in abundance of benthic macroinvertebrates based on functional feeding groups (FFGs) classification (Alvarez and Pardo, 2009).

To address this we explored resource origin for consumers at three reaches in a temporary Mediterranean stream system. Algae were present at all sites, with minimal allochthonous detrital accumulations. Accordingly, we expected that autochthonous subsidies would be the dominant energy source for macroinvertebrate consumers at all sites. This hypothesis (H1) should correspond to C and N values of consumers being more similar to C and N values of autochthonous food sources (considering isotopic fractionation) than to N and C values of the allochthonous detritus.

Studies about the trophic structure and resource assimilation in Mediterranean intermittent streams so far have been based on quantitative estimates of basal food resources and structure of benthic macroinvertebrates derived from functional feeding groups (FFGs) classification. According to this classification, taxa belonging to different FFGs should rely on different basal resources and should have distinct isotopic signatures. Therefore, we hypothesized (H2) that variations in isotopic composition of consumers would be larger for taxa belonging to different FFG than for the taxa within the same FFG. Additionally, we propose to test this variability across all the sites considered.

Conceptual models developed for temporary floodplain tropical rivers suggest that hydrology, via changes in habitat structure and resource availability, strongly affects consumers and their adaptive response to changes in the availability of food resources (Lewis et al. 2001; Arcagni et al. 2015; Blanchette et al. 2014). For example, food webs in Australian intermittent tropical streams dominated by omnivory are shorter and more diffuse, than food webs in other temperate streams (Bunn et al. 1999; Douglas et al. 2005; Pusey et al. 2010). This suggests that omnivory and generalistic feeding are used, as adaptive strategies for consumers who live in environments were food resources are seasonally variable. Based on this hypothesis (H3), we expected to find a greater overlap in isotopic fractionation among consumers of different FFGs at intermittent sites, relative to sites with perennial regime.

6.2. Methods

6.2.1. Study site

Three sites; Fonte Benemola (F.Benemola), Quinta da Ombria (Q. da Ombria) and Monte Seco (M.Seco), within the Quarteira River Basin, were sampled in the Algarve region of southern Portugal (Fig. 6.1). The catchment area is ~324 km^2 and the elevation ranges from 14 to 515 m. The average monthly air temperature varies from 8-29 °C and average annual rainfall is 625 mm. Land use in the catchment consists of olive tree plantations and other cultivated tree crops including almond, carob, cork oak and citrus. Non-agricultural land cover includes shrub and herbaceous vegetation, and mixed forest. Along the sites margins, depositional, low hydrodynamic energy pools are filled with accumulated leaf litter from adjacent riparian vegetation underlain by clay, while channel substrate is predominantly gravel and cobble that during summer season is densely covered by filamentous algae, mainly *Cladophora agg*. and *Vaucheria sp(p.).*

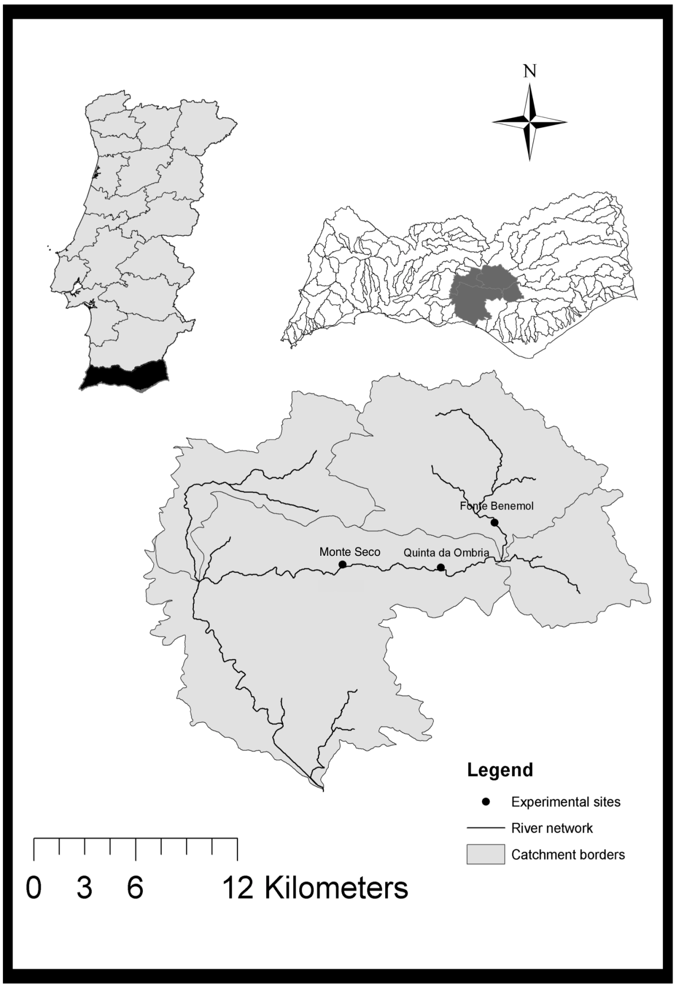


Fig. 6.1. Map showing the study reaches within the Quarteira River Basin in the Algarve region of southern Portugal.

F. Benemola (37.209708, -8.010794) is our most upstream site, located within a second order stream (the Menalva). The Menalva is primarily fed by three-groundwater sources (~60% total discharge), which results in permanent annual flow, with the lowest discharge of 0.02 m^3 s^{-1} m recorded in September and the highest of 2.75 m^3 s^{-1} in January (SNIRH). F. Benemola riparian vegetation consists of tree and shrub species such as willows, ashes, oleanders cane and African tamarisk. Estimated riparian cover for F. Benemola is approximately 65 %. Land use is mostly agroforestry consisting of typical Mediterranean vegetation such as rosemary, strawberry tree, wild olive, cork oak, carob tree, and almond tree plantations. These land use characteristics together with scant urban development makes the F. Benémola a relatively undisturbed catchment, which has been classified as Local Protected Landscape by the Law-Decree no. 142/2008, dated 24th of July. Benthic habitats consist mainly of boulders and coarse gravel of schist and limestone origin, covered by aquatic algae and moss. Dense canopy cover and narrow channel width with relatively steep river banks impedes light penetration and makes F. Benemola less productive than other two sites.

The M. Seco (37.188502, -8.090645) and Q. da Ombria (37.187473, -8.081307) sites are located within the same stream (The Algibre) which is a junction of Menalva stream and Ribeira das Merces stream. Contrary to the F. Benemola, both sites have intermittent flow regimes. Wet periods begin in late October and generally last until March, with high discharge peaks. However, during the dry season (~June - September), the stream fragments into temporarily disconnected pools or completely dry channels. At steady flow conditions (~March-May) average discharge is 1.3 m^{3} s^{-1} and it gradually decreases towards warmer months, being as low as 0.026 m^3 s^{-1} at the end of the dry season (~July-August). The period of steady flow conditions was determined based on weekly measurements of stream discharge at the most representative cross section of the stream. Annual variability in stream discharge directly affects substrate characteristics, algae and macrophytes development and accumulation of organic debris (Gasith and Resh 1999; Sabater et al. 2006). Riparian vegetation in M. Seco is dominated by wild cane (*Arundo donax*), herbaceous vegetation and carob and olive trees and is moderately developed with a 3 m wide lateral zone at both sites of the stream and occasional spots of more scattered canopy cover. Estimated riparian cover for M. Seco is 25 %. Q. da Ombria has less densely developed riparian vegetation than M. Seco (20 % of riparian cover) and it consist only of shrubs and herbaceous vegetation with wider and less steep riverbanks. Catchment land use at both sites, except for forest, also consists of olive tree plantations and orange groves, which probably reflect slightly lower water quality status than is reported for F. Benemola (Chicharo et al. 2010). The dominant substrate type at M. Seco and Q. da Ombria is cobble and gravel.

6.2.2. Macroinvertebrates and habitat sampling

At each site the most representative stream section (100 m), including riffle and pool zones, was selected. Benthic macroinvertebrates and their habitats were sampled on ten days (March 16th to May 13th) during a period of steady flow (Sroczynska et al. 2017). The taxa, sampled were chosen to cover a broad range of functional groups (Table 6.1).

Table 6.1. Feeding information about each experimental taxa based on functional feeding groups (FFG) classification (Merritt and Cummins 1996)

MAIN CLASSES	TAXON	FUNCTIONAL FEEDING GROUP	TROPHIC LEVEL	PRESUMED TYPE OF FOOD
INSECTS	*Baetis fuscatus*	collector/depositer	herbivore	detritus, diatoms
	Chimarra marginata	collector/filterer	herbivore	fine particulate organic matter (FPOM)
	Ecdyonurus sp.	collector/depositer	herbivore	detritus, diatoms
	Isoperla moselyi	predator	carnivore	Chironomidae/Simuliidae/Ephemeroptera
	Oreodytes sp.	predator	carnivore	various insects
	Pyrrhosoma nymphula	predator	carnivore	Cladocera/Chironomidae
MOLLUSK	*Ferrisia wautieri*	grazer	herbivore	periphyton attached to algae
	Physella acuta	grazer	herbivore	periphyton attached to algae
CRUSTACEAN	*Atyaephyra desmarestii*	omnivore	omnivore	organic matter
	Procambus clarkii	omnivore	omnivore	detritus/algae/other invertebrates

Macroinvertebrates were removed from stones with forceps or elutriated from soft substrates and preserved in liquid nitrogen. A minimum of three replicates of 10 taxa were collected at each site (Tab 6.1). This resulted in a total of 100 samples. Several taxa were absent at some sites: *Chimarra marginata* was absent in M. Seco, *Procambus clarkii* and *Pyrrhosoma nymphula* were absent in F. Benemola, whereas *Atyaephyra desmarestii* was only present in F. Benemola and *Oreodytes sp.* was only present in M. Seco. Consequently, taxa which were only present at one site are not used for the comparisons among sites. In total 45 samples were taken in M. Seco, 29 in F. Benemola and 26 in Q. da Ombria. Each replicate sample consisted of at least 100 mg, which corresponded to 30-40 individuals for smaller taxa such as *Baetis sp.* and *Oreodytes sp.*, 20-30 individuals for *C. marginata* and 10-15 individuals for the remaining taxa. The exception was crayfish (*P. clarkii)*, for which one replicate sample consisted of a single individual. All the individuals were the same size and belonged to the same taxa. The exception was mayflies of *Baetis sp.,* for which some individuals were too small to distinguish between separate species. However, macroinvertebrate composition demonstrated that majority (90%) of genus *Baetis sp.* were composed of *Baetis fuscatus*. Along with macroinvertebrate sampling, temperature [°C] was recorded using a multiparametric handheld probe (YSI, Professional Plus).

Samples of periphyton (2-4) were collected from the top of randomly chosen stones and allochthonous material in a form of conditioned leaves was sampled from detrital deposits from the stream margins. Fine particulate organic matter (FPOM) was collected from the surface of the stream bottom. Filamentous algae of genus *Cladophora sp.* and macrophytes were handpicked when present. All habitat samples were subsequently frozen. In F. Benemola the majority of rocks were covered by periphyton with considerable amount of aquatic moss. Therefore, for further isotope analysis we separated bulk periphyton samples into those containing periphyton scrubbed (without abundant moss) and those where moss was present. Additionally, 2-4 periphyton samples (each consisted of 1 or 2 stones) were collected at each site for biomass (chlorophyll a and Ash free dry mass -AFDM) determination. Water samples for nutrient analysis (NO_3^-, NH_4^+ and PO_4^{2-}) were also taken at each site and stored in a cooler for posterior analysis.

6.2.3. Sample processing

In the laboratory bulk periphyton from stones and leaves was scrubbed into a known volume of water using a toothbrush and thoroughly homogenized (Biggs and Kilroy 2000). The resultant slurry was subsampled and filtered on glass fiber filters (GF/C, 47mm Whatman) for chlorophyll *a* (Chl *a* mg m^{-2}) and ash free dry mass (AFDM mg m^{-2}) analysis. Chl *a* was extracted in 90% boiling ethanol and kept in freezer for 24 h. The absorbance was read on a spectrophotometer (Thermospectronic GENESYS 10UV) at 665 nm. AFDM filters were dried to constant weight at 60°C (dry weight) then burnt for 4 hours at 450 ºC (Ash Weight). AFDM represents the weight difference Dry weight and Ash Weight . Chl *a* and AFDM were calculated per stone surface area assuming that metabolically active area of stones is 60 % (Biggs and Close 1989).

Macroinvertebrates and habitat samples were liophilized, while frozen and subsequently homogenized to fine powder using an agate mortar and pestle. Stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) were determined by isotope ratio mass spectrometry (IRMS). The stable carbon and nitrogen isotopic signatures were analyzed as described in González-Pérez et al. (2015). The instrumental set up consisted of the Flash 2000 HT/IRMS system (Thermo Scientific, Bremen, Germany) micro-analyzer coupled via a ConFlo IV interface unit to a continuous flow (IRMS) Delta V Advantage from Thermo Scientific, Bremen, Germany. Sample (0.5 mg), wrapped in tin foil, were combusted in instrument furnace at 1020 °C. Isotopic ratios were expressed using δ notation where values are reported as parts per thousand (‰) deviations from Pee Dee Belemnite (PDB) for carbon and air N_2 for nitrogen standards. The standard deviation of bulk $\delta^{13}C$ and $\delta^{15}N$ was typically less than ±0.1‰.

6.2.4. Nutrient Analysis

Water samples were analysed for soluble reactive phosphorus (SRP) and ammonium spectrophotometrically (APHA 2012). Nitrate and nitrite concentrations were analysed on a Skalar autoanalyzer (Skalar SAN Plus System, SKALAR) using the cadmium reduction method (APHA 2012). All the water samples were filtered in the laboratory prior to analyses.

6.2.5. Statistical analysis

The differences in $\delta^{15}N$ and $\delta^{13}C$ of bulk periphyton between sites were examined using one-way analysis of variance (ANOVA) when assumptions of normality (Shapiro-Wilk test for small sample size) and homogeneity of variance (Levene's test) were accomplished. Tukey's post-hoc comparisons, using the "TukeyHSD" function in the R package "multcomp" (Hothorn et al. 2008; R Development Core Team 2012) were performed to distinguish differences among individual sites, when ANOVA detected significant differences.

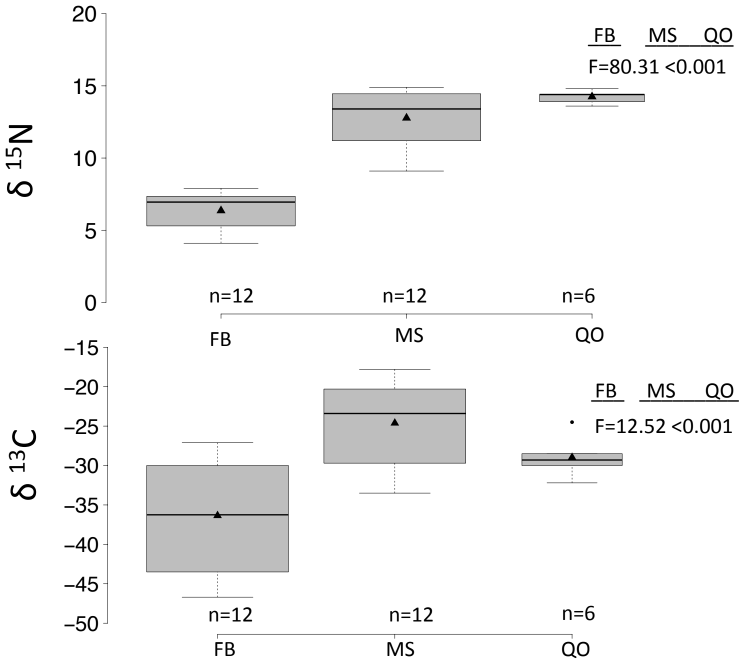


Fig. 6.2 Boxplots with $\delta^{15}N$ (A) and $\delta^{13}C$ (B) of bulk periphyton for 3 sites: FB- Fonte Benemola; MS- Monte Seco; QO- Quinta da Ombria. Triangles represent a mean; horizontal segment is a median; horizontal lines marking the box are first and third quartiles with "whiskers" that extends to minimum and maximum value. F – statistics of the analysis of variance (ANOVA) (df = 2, FB = 12, MS = 12, QO = 6) applied in testing the differences between Sites. For Sites that are underlined with the same line no difference at $p \leq 0.05$ was recorded in *post-hoc* tests.

H1 and H3 were tested using permutational analysis of variance (PERMANOVA) performed on the similarity data matrix (Euclidean distances) of $\delta^{15}N$ and $\delta^{13}C$ of consumers and their food sources with two fixed and orthogonal factors (Site x FFG). With the exception of "periphyton", the basal resource groups did not had enough replication to assure a reasonable number of permutations (<100), so we relied on the tables with the Average Distance between/within groups, instead of the statistical test.

H2 was tested using PERMANOVA with two fixed and orthogonal factors (Site x FFG) and one random factor (Species) nested in FFG. This design was only applied to consumers data. All the multivariate tests were performed using PRIMER 6 statistical package with the PERMANOVA+ add-on (Clarke and Warwick 2001). We examined differences in environmental parameters using non-parametric Kruskal-Wallis Rank Sum Test using the "kruskalmc" function in the R package "pgirmess", when the homogeneity of variance assumptions was not met (R Development Core Team 2012; Giraudoux 2013).

6.3. Results

6.3.1. Environmental parameters

Environmental parameters such as temperature, chlorophyll *a*, AFDM and chl *a*/AFDM ratio varied largely (Table 6.2), but did not differ among sites or sampling days ($P>0.05$). Nutrients (NO_3, PO_4, NH_4) had generally low values and only NO_3 was significantly higher in F. Benemola than in Q. da Ombria (Table 6.2).

Table 6.2 Minimum and Maximum value of main environmental variables recorded at each studied site. Mean values are in brackets. Statistics of Kruskal–Wallis test.

ENVIRONMENTAL PARAMETERS	Monte Seco	Quinta da Ombria	F.Benemola	Chi-squared	P
Chlorophyll a [mg m^{-2}]	75.3-610.6 (250.7)	29.0-441.0 (145)	35.1-532.9 (205.3)	2.40	0.30
AFDM [g m^{-2}]	12.49-59.75 (37.32)	2.62-53.46 (28.94)	5.98-93.40 (45.03)	1.29	0.53
Chlorophyll a/AFDM	0.003-0.030 (0.008)	0.001-0.030 (0.009)	0.003-0.009 (0.005)	0.54	0.76
Temperature [°C]	15.00-20.05 (16.83)	16.00-20.15 (17.88)	15.40-18.00 (16.24)	2.74	0.25
N dry mass [g m-2]	39.17-219.87 (104.58)	58.86-69.80 (64.33)	40.29- 389.24 (162.91)	0.37	0.83
P dry mass [g m-2]	5.66-11.45 (9.20)	7.99-11.52 (9.75)	3.53-27.00 (17.17)	1.27	0.53
C dry mass [g m-2]	1722.16-3832.75 (2530.29)	1016.40-1447.53 (1231.96)	494.03-5920.33(1904.25)	2.77	0.25
NH_4^+-N [µM]	0.00-0.26 (0.65)	0.00-0.53 (1.91)	0.62-1.32 (1.05)	1.41	0.49
NO_3-N [µM	1.93-6.82 (5.71)	2.03-4.64 (3.39)	3.46-30.60 (18.80)	12.10	0.00
PO_4-P [µM^1]	0.00-0.13 (0.05)	0.00-0.14 (0.03)	0.00-0.28 (0.15)	4.67	0.10

6.3.2. Stable isotope signatures of basal resources ($\delta^{15}N$ and $\delta^{13}C$)

We found large variability in periphyton $\delta^{15}N$ and $\delta^{13}C$ at all sites (Fig. 6.2). Q. da Ombria had the lowest variability and the highest average $\delta^{15}N$. Periphyton samples taken from M. Seco were slightly more depleted in the heavy N isotope and the most ^{15}N depleted periphyton was found in F. Benemola. Similar variation patterns were observed for $\delta^{13}C$ (Fig. 6.2). However, variability was higher between sites than within sites. The analysis of variance (ANOVA) detected significant differences in means of $\delta^{15}N$ ($F=80.31$, $P<0.0001$) and $\delta^{13}C$ ($F=12.52$, $P<0.001$) among sites (Fig. 6.2). Post hoc comparisons revealed differences only between F. Benemola and remaining two sites (Fig. 6.2), but no significant difference, in any of the isotopes, was found between M. Seco and Q. da Ombria ($P=0.30$).

The isotopic C and N signature found in detritus collected from M. Seco and Q. da Ombria varied little with mean values of 10.5±0.57 and -27.1±0.58 ‰ for $\delta^{15}N$ and δ

^{13}C respectively (Fig. 6.3, Appendix 6.1). FPOM collected only from Q. da Ombria had the highest values reported for detritus (δ^{15}N 11.2 ‰ and δ^{13}C of 33.1 ‰). Macrophytes were collected only from F. Benemola and had values 6.63 for δ^{15}N and -28.32 for δ^{13}C (Fig. 6.3, Appendix 6.1).

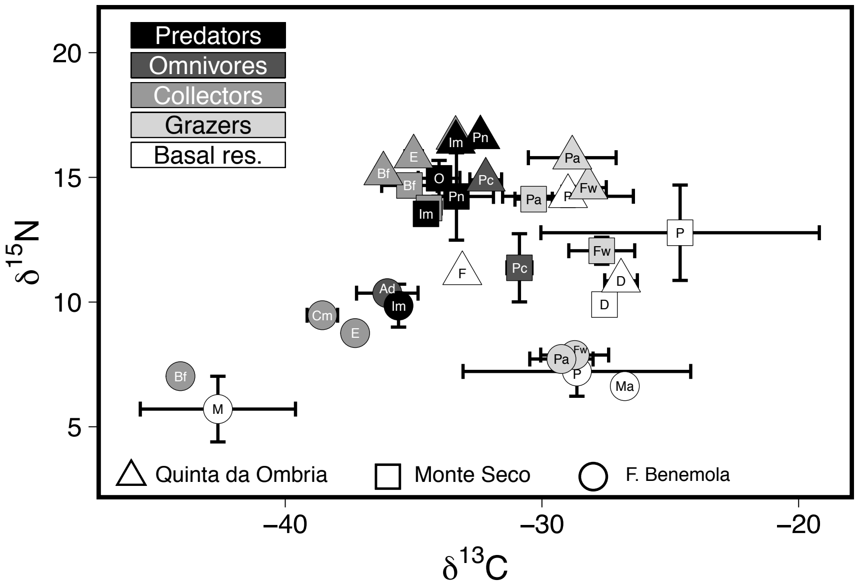


Fig. 6.3. Biplots of carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopic signatures (representing mean and standard deviation) for each taxa with colors depicting different FFGs assignment, at three studied sites. Basal food resources were: D-detritus ; F-fine particulate organic matter; Ma-macrophytes; M-moss; P-Periphyton. Taxa were: Ad- *Atyaephyra desmarestii*, Bf- *Baetis fuscatus,* Cm- *Chimarra marginata*, E – *Ecdyonurus sp.*, Fw- *Ferrisia wautieri*, Im- *Isoperla moselyi*, O- *Oreodytes sp.*, Pa- *Physella acuta*, Pc- *Procambus clarkii*, Pn- *Pyrrhosoma nymphula*

6.3.3. Stable isotope signatures of consumers (^{15}N and δ^{13}C)

F. Benemola had strikingly lower δ^{15}N values than these found for the other sites (Fig. 6.4B). The lowest average δ^{15}N was reported for collector-gatherer - *Baetis fuscatus* (7.03‰), which in other sites had values of δ^{15}N twice as high. The largest δ^{15}N value was observed for the freshwater shrimp *Atyaephyra desmarestii* (10.36‰). Consumers in M. Seco and Q. da Ombria had similar values of δ^{15}N with constantly higher values in Q. da Ombria. In M. Seco the lowest mean value of δ^{15}N was reported for omnivorous crayfish (11.37‰) and the highest value of 14.97‰ was found for the predator water beetle (*Oreodytes sp.)*. Q. da Ombria shows the smallest within site variation ranging from an average of 14.59‰ for *Ferrisia wautieri* and peaking a 16.60‰ for *Pyrrhosoma nymphula*. F. Benemola has a similar variation range of δ^{15}N values as in M. Seco ranging from 7.03 ‰ for *B. fuscatus* and peaking 10.36 ‰ for *A. desmarestii.* Taxa with largest δ^{15}N variability were the predator *Pyrrhosoma nymphula* (12.48-15.98‰) and the omnivore *Procambus clarkii* (10.0-12.74‰).

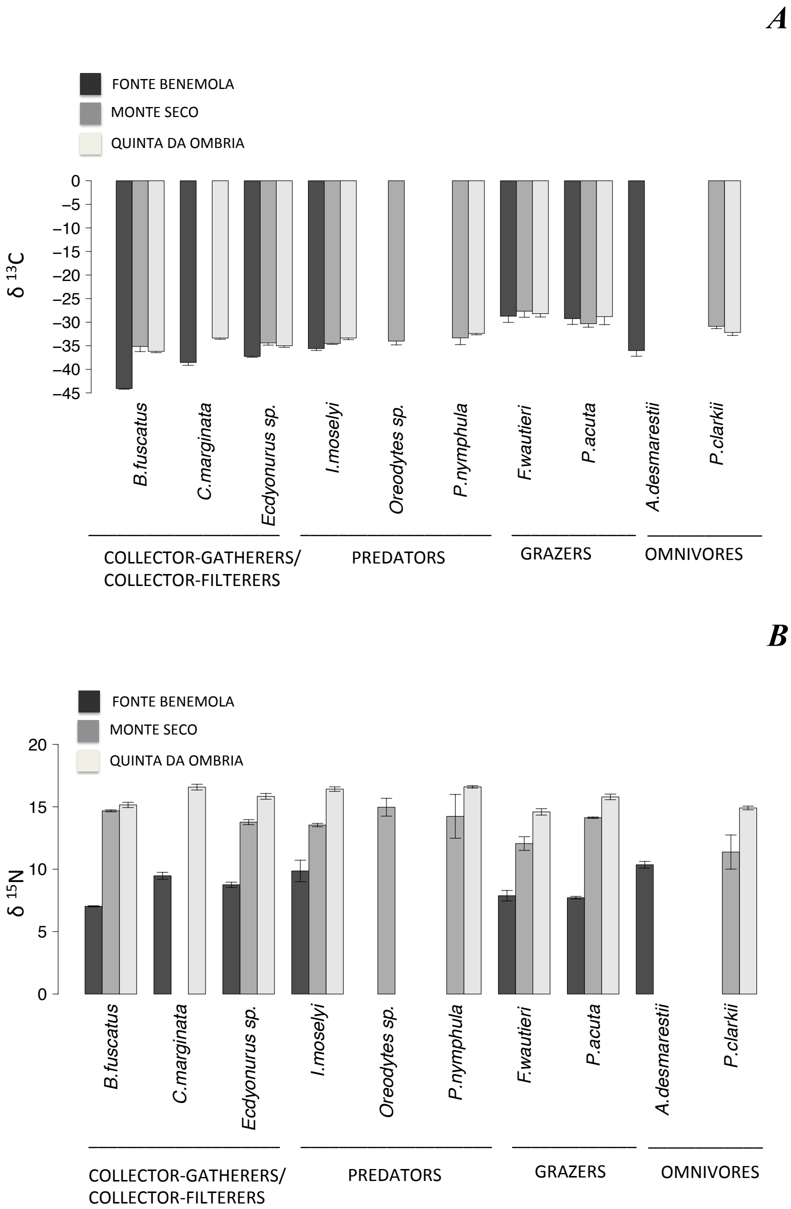


Fig. 6.4. Barplots (Mean ± SE) with $\delta^{13}C$ (A) and $\delta^{15}N$ (B) for each taxa and its trophic designation at each site. Missing bars indicate that given taxa was not collected at these sites.

Among site variation in $\delta^{13}C$ among taxa was less apparent than in case of $\delta^{15}N$ (Fig. 6.4A). However, taxa inhabiting F. Benemola also showed the most depleted ^{13}C signature, whereas M. Seco and Q. da Ombria hosted taxa more enriched in $\delta^{13}C$. The most contrasting taxa, among all of the sites, in terms of source of carbon were the most depleted

B. fuscatus (-36.18‰ in Q. da Ombria and -44.08‰ in F. Benemola) and the most enriched *F. wautieri* (-27.6‰ in M. Seco and -28.73‰ in F. Benemola).

Consequently, in terms of $\delta^{13}C$ values F. Benemola had the highest within site variation in comparison to Q. da Ombria and M. Seco.

For both elements, the variation among species nested within FFGs was always found to be lower within sites than among sites (Fig. 6.3, Table 6.3).

Table 6.3 PERMANOVA on the similarity matrix (Euclidean distance) of consumer isotopic composition $\delta^{13}C$ (A) and $\delta^{15}N$ (B) and combined $\delta^{15}N$ and $\delta^{13}C$ matrix (C). Where: Si-Site (fixed factor), FFG-Functional feeding group (fixed factor) and Sp (FFG) -Species (random factor) nested within FFG.

A)

$\delta^{13}C$						
Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Si	1	0.22	0.22	0.03	0.857	996
FFG	2	643.22	321.61	23.37	0.004	996
Sp(FFG)	5	81.86	16.37	21.84	0.001	999
Si×FFG	5	85.25	17.05	2.50	0.151	999
Si×Sp(FFG)	6	44.66	7.44	9.93	0.001	999
Res	73	54.72	0.75			
Total	95	1148.10				

B)

$\delta^{15}N$						
Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Si	1	60.39	60.39	31.81	0.002	997
FFG	2	22.83	11.41	3.29	0.143	999
Sp(FFG)	5	20.45	4.09	10.21	0.001	997
Si×FFG	5	12.48	2.50	1.32	0.382	999
Si×Sp(FFG)	6	12.22	2.04	5.09	0.001	998
Res	73	29.21	0.40			
Total	95	778.87				

C)

Combined $\delta^{13}C$ and $\delta 15N$						
Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Si	1	60.61	60.61	6.91	0.014	997
FFG	2	666.05	333.02	19.33	0.005	998
Sp(FFG)	5	102.31	20.46	17.80	0.001	999
Si×FFG	5	97.74	19.55	2.24	0.092	996
Si×Sp(FFG)	6	56.88	9.48	8.24	0.001	999
Res	73	83.94	1.15			
Total	95	1926.90				

6.3.4. $\delta^{15}N$ and $\delta^{13}C$ of consumers and their food

Biplots show the general tendency of a higher variation in $\delta^{13}C$ and $\delta^{15}N$ in basal food sources (predominantly periphyton) than in consumers (Fig. 6.3). In general, most of the studied taxa tracked their food sources and consequently the depletion in $\delta^{13}C$ and $\delta^{15}N$ of periphyton follows the depletion in consumers (Fig. 6.3). Q. da Ombria was the most $\delta^{15}N$ enriched site for both basal resources and consumers, slightly smaller, but consistent among all of the taxa decrease in $\delta^{15}N$ was observed in M. Seco and the most depleted in both: basal resources and consumers was F. Benemola (Fig. 6.3). A similarly decreasing pattern was observed for $\delta^{13}C$, however, $\delta^{13}C$ in grazers and periphyton overlapped in all of the 3 sites (Fig. 6.3). The majority of sampled taxa were significantly more depleted in ^{13}C that their presumed food sources and this observation was consistent among the three sites studied. Even considering a C fractionation of 1 ‰ per trophic level (according to Minagawa and Wada, 1984), most of the consumers had $\delta^{13}C$ 7‰ to 9 ‰ lower than sampled algae as well as detritus (Fig. 6.3).

The most evident mismatch between invertebrates and their food sources was in M. Seco, where almost no overlap was found between any of the examined functional feeding groups and their food sources. Similarly, the average Euclidean distance between basal resources and FFGs of consumers are high, in comparison to $\delta^{15}N$ demonstrating less overlap between consumers and resources for this isotope (Table 6.4A). The exception were the mollusks (*P. acuta and F. wuatieri*) with $\delta^{13}C$ values close to that in the periphyton. This suggests that large part of the consumer's diet was unaccounted for in the sampled habitats. Complementary use of $\delta^{15}N$ demonstrated that $\delta^{15}N$ of consumers never overlapped with detritus, but instead matched/resembled that in periphyton (Fig. 6.3). This is also corroborated by the results of the average Euclidean distance between basal resources and FFGs of consumers (Table 6.4B). Detritus owns the highest average distance values between all of the trophic groups (except for omnivores in M. Seco). Instead periphyton demonstrates much higher resemblance in $\delta^{15}N$ among trophic groups. This indicates taxa dependence on the autochthonous, rather than terrestrial food source.

In F. Benemola moss was found as a possible main food source for some of the macroinvertebrates, most likely *B. fuscatus*, which low $\delta^{15}N$ and $\delta^{13}C$ values matches those found in moss (Appendix 6.1). Macrophytes were sampled only in one site, however, they were not a food source for any of invertebrates. Filter feeders (*C. marginata*), in Q. da Ombria have the same C isotopic signature, but are found significantly enriched in ^{15}N relatively to sampled FPOM (Appendix 6.1). Contrary to $\delta^{13}C$, $\delta^{15}N$ values measured in consumers were more within a range of sampled basal resources. Nevertheless, high overlap in $\delta^{15}N$ values among taxa impeded identification of trophic differences, specifically between situated at the higher trophic position predators and rest of functional feeding groups (Fig. 6.3). For example, in M. Seco predators are almost completely overlapping with collectors (Fig. 6.3).

There were, however, more inconsistences between sites. In the M. Seco an omnivore crayfish (*P. clarkii)* had the lowest $\delta^{15}N$ of all sampled taxa, indicating that its diet may consists of algae with some detritus, but not other invertebrates. However, in Q. da Ombria, its tissue was more enriched in ^{15}N, suggesting some dietary shift towards algae or small invertebrates (Fig. 6. 3). Although $\delta^{13}C$ was more out of the range for macroinvertebrate diets than $\delta^{15}N$, both isotopes clearly demonstrate that the bulk of periphyton sampled did not reflected well macroinvertbrates food sources.

Table 6.4 Average distance between/within functional feeding groups and basal resources for $\delta^{13}C$ (A) and $\delta^{15}N$ (B) at three sites

A)

Site	FFG	omnivore	predator	collector	grazer	periphyton	biofilm	detritus	FPOM	moss	macrophytes
	omnivore	0.58									
	predator	3.10	0.98								
	collector	3.87	1.07	0.96							
Monte Seco	grazer	1.99	4.98	5.75	2.11						
	periphyton	5.64	8.07	8.78	4.95	5.79					
	biofilm	13.02	16.12	16.89	11.14	8.11	0.08				
	detritus	3.31	6.42	7.19	1.86	4.46	9.71	0.00			
	omnivore	0.87									
	predator	0.82	0.74								
	collector	2.67	2.03	1.42							
Q. Da Ombria	grazer	3.68	4.37	6.35	1.36						
	periphyton	2.41	3.00	4.99	1.78	1.73					
	biofilm	7.66	8.35	10.33	3.98	5.35	0.00				
	detritus	5.27	5.96	7.94	1.59	2.96	2.39	0.90			
	FPOM	0.91	0.53	1.76	4.60	3.23	8.57	6.18	0.00		
	omnivore	1.32									
	predator	1.19	0.58								
	collector	3.59	4.03	2.96							
F. Benemola	grazer	7.04	6.60	10.63	1.42						
	periphyton	5.94	5.50	9.53	2.23	2.98					
	biofilm	16.08	15.64	19.67	9.03	10.14	0.00				
	moss	6.60	7.04	3.97	13.64	12.54	22.68			3.58	
	macrophytes	9.25	8.82	12.85	2.21	3.32	6.82			15.85	0.00

B)

Site	FFG	omnivore	predator	collector	grazer	periphyton	detritus	FPOM	moss	macrophytes
	omnivore	1.60								
	predator	2.86	1.35							
Monte Seco	collector	2.81	1.01	0.57						
	grazer	2.04	1.57	1.29	1.39					
	periphyton	2.18	2.11	1.85	1.80	2.34				
	detritus	1.47	4.31	4.29	3.19	2.90	0.00			
	omnivore	0.21								
	predator	1.61	0.20							
	collector	0.96	0.74	0.74						
Q. Da Ombria	grazer	0.60	1.32	0.89	0.84					
	periphyton	0.69	2.29	1.63	1.02	0.55				
	detritus	4.05	5.65	5.00	4.33	3.36	0.27			
	FPOM	3.75	5.36	4.70	4.04	3.07	0.30	0.00		
	omnivore	0.30								
	predator	0.66	1.22							
	collector	1.68	1.31	1.19						
F. Benemola	grazer	2.56	2.06	1.27	0.35					
	periphyton	3.34	2.84	1.82	0.85	1.04				
	moss	4.65	4.15	3.01	2.09	1.61			1.60	
	macrophytes	3.73	3.23	2.05	1.17	0.82			1.35	0.00

6.3.5. FFG and Site interaction

A significant interaction in $\delta^{13}C$ and $\delta^{15}N$ between consumer taxa, nested within trophic groups, and sites was found (Table 6.3) suggesting local diet specialization among different taxa. The variation was higher among taxa nested within one trophic group, than among trophic groups.

One of the most evident example of the differences in isotopic values among taxa within trophic group was seen in *B. fuscatus* and *Ecdyonurus sp.*, - collectors known to feed on attached algae. In M. Seco, the diet of *B. fuscatus* is more enriched in $\delta^{15}N$, in comparison to *Ecdyonurus sp.* as well as to other taxa, whereas in F.Benemola *Ecdyonurus sp.* was 1.75 ‰ more enriched in ^{15}N and almost 7‰ more enriched in ^{13}C, relatively to *B. fuscatus* (Fig. 6.3, Appendix 6.1).

There was no significant interaction in Si×FFG in any of the three factor analysis (Table 6.3A,B and C). This pattern was evident among collectors and predators; grazers and omnivores and grazers and predators (Table 6.3A,B and C). In regard to predators-omnivores there was an opposite pattern with higher overlap existing between these trophic groups in perennial F. Benemola than in intermittent M. Seco and Q. da Ombria (Table 6.3B and C).

6.4. Discussion

6.4.1. Food source for aquatic consumers

Consistent with our initial predictions (H1), we found that autochthony supplied the majority of invertebrate basal resources, at all of the sites. In the intermittent M. Seco and Q. da Ombria, periphyton values often enveloped those reported for leaf litter, however complementary use of N stable isotope analysis demonstrated that consumer $\delta^{15}N$ values were closer to algal values. Interestingly, autochthonous resources contributed to consumers diet across all sites (even in shaded F. Benemola) and regardless FFG assignment. The reason for this is probably related to the identity of the studied streams. High summer temperatures, low discharge amplitude and long days with clear sky in Mediterranean regions favour the development of algae, which, contrary to detritus, is always available for consumers (Gasith and Resh 1999). The peak of the allochthonous input to Mediterranean streams normally occurs during high discharge and low temperatures. As a result, the amount of detritus entering the stream is retained for a short period of time and limited contact time between presence of shredders and detritus causes that majority of terrestrial input, which enters the stream to be quickly transported downstream and probably leaving the system before being processed. Prevalence of scrapers and collector-gatherers with scarce number of shredders at our study sites additionally highlights the importance of autochthonous material in this system.

Most of our consumers were highly depleted in ^{13}C relative to the habitats sampled, having isotopic values close to neither leaf litter, nor periphyton (Fig. 6.3). This suggests that most macroinvertebrates selectively feed or assimilate only a portion of the actively cycling (easily absorbable) fraction of periphyton or FPOM, with depleted in $\delta^{13}C$ and enriched in $\delta^{15}N$ values.

Food selectivity is common among stream macroinvertebrates (McNelly et al. 2006; Mulholland et al. 2000a; Rezanka and Hershey 2003; Rosenfeld and Roff 1992). This is because bulk periphyton samples often contain a mixture of slowly and actively cycling N, the latter being preferentially assimilated by consumers (Dodds et al. 2014; Hamilton et al. 2001; Peipoch et al. 2012; Tank et al. 2000). Moss, which comprised the bulk of periphyton sampled in F. Benemola, showed conspicuous negative $\delta^{13}C$ values (≈-40‰). It is therefore speculative if herbivore taxa in F. Benemola fed on moss or fed on a $\delta^{13}C$ depleted portion of epilithon, or on a combination.

Interestingly, the filter feeder *C. marginata*, which feeds on particulate organic matter, was highly enriched in ^{15}N at both sites. Further, their $\delta^{13}C$ and $\delta^{15}N$ values did not match the $\delta^{13}C$ and $\delta^{15}N$ of FPOM sampled from the stream bottom. This suggests benthic FPOM is not a relevant food source for filter feeders, which is congruent with previous findings (Mulholland et al. 2000a). Similarly, freshwater shrimp diet at F. Benemola is highly enriched in ^{15}N (even exceeding $\delta^{15}N$ values reported for predators, being close to value reported for filter feeder *Chimarra marginata*), which is unexpected for this N-depleted site. Shrimps are omnivorous decapods known to feed on a variety of different foods, commonly aquatic plants and FPOM (Burns and Walker 2000). In this stream, *Atyidae desmarestii* feeds on suspended particles accumulated at the surface of aquatic macrophytes and filamentous algae (personal observation). High $\delta^{15}N$ values found for *A. desmarestii* and *C. marginata* suggest that it is unlikely that their food originates from our sampling locations, rather their diet consists of highly $\delta^{15}N$ enriched FPOM material processed upstream and brought to this site by the current. However, we cannot exclude the possibility that some taxa feed on a small proportion of the detritus, originated from either microbial reworking or which was incidentally present in the bulk of periphyton sampled.

6.4.2. Site differences in periphyton

Large variability was observed within and among sites in the periphyton $\delta^{13}C$, whereas $\delta^{15}N$ was less variable within sites, but highly variable among sites. Large within site $\delta^{13}C$ variability is commonly observed for aquatic algae in stream ecosystems (France 1995; Hamilton and Luis 1992; Rosenfeld and Roff 1992; Winterbourn et al. 1986). Factors that regulate carbon uptake by aquatic producers, such as water velocity, light and temperature (Finlay et al. 1999; Ishikawa et al. 2012; Osmond et al. 1981; Sackett et al. 1965; Wienke and Fischer 1990) are rarely constant within a reach and their variation in turn affects algal $\delta^{13}C$ values (Rosenfeld and Roff 1992).

However, among site variation in $\delta^{13}C$ was higher than within site variation. This suggests that other factors, presumably site-specific differences in geo-chemical properties of the water and density of riparian cover, are responsible for among-site variability in isotopic C of periphyton as observed elsewhere (Finlay et al. 1999; Finlay et al. 2004; Palmer et al. 2001).

Interestingly, the large spatial variability in $\delta^{15}N$ observed among periphyton samples is more challenging to explain. We reported an almost two-fold $\delta^{15}N$ isotopic enrichment in the more open M. Seco and Q. da Ombria (25% and 20 % canopy cover respectively) in comparison to the highly ^{15}N depleted signature found in periphyton at F. Benemola, a site with much higher canopy cover (65%). Generally, periphyton $\delta^{15}N$ should reflect the available nitrogen pool (Macko et al. 1987), which suggests that M. Seco and Q. da Ombria receive higher nutrient inputs. In contrast, F. Benemola is characterized by more forested landscape with organic-poor alluvial soils, where soil-stream nutrient exchange is restricted. We remain unable to resolve the paradoxically higher NO_3 concentration in F. Benemola relative to Q. da Ombria (Table 6.1), despite its limited nutrient input and depleted $\delta^{15}N$ signature. Previous studies on this stream (Sroczynska et al. 2017) did not record such high NO_3 concentration and therefore it deserves further investigation into potential nitrogen sources and transformations in this ecosystem.

6.4.3. FFGs and Site interaction

We found large variability among consumers nested within the same FFG – opposed to our initial hypothesis (H2). It therefore confirms that FFG classification poorly reflect trophic structure and resource assimilation as has been showed elsewhere (Lauridsen et al. 2014; Mihuc and Minshall 1995; Rossi-Marshall et al. 2016).

Species are known for their capacity to switch resources in a fate of disturbance and environmental perturbation (Mihuc 1997). Alterations in hydrological period in the intermittent streams follow rapid changes in resource availability and their quality and we believe that it triggers the opportunistic response of animals (at individualistic level) to adapt to these new resources. One of the mechanisms, by which consumers adapt to this variability in basal resources, is selective ingestion (Dodds et al. 2014). In M. Seco and F. Benemola primary consumers exhibited higher selectivity than primary consumers in Q. da Ombria. For example, *B. fuscatus* had highly positive $\delta^{15}N$ in M. Seco indicating selective ingestion of a portion of periphyton richer in actively cycling N relatively to other collectors such as *Ecdyonurus sp*. Nonetheless, in F. Benemola, *B. fuscatus* was the least enriched in heavy N isotope of $\delta^{15}N$ for *B. fuscatus* among M. Seco and F. Benemola (Fig. 6.3). Similarly, grazing mollusks also exhibited higher selectivity in M. Seco than in other sites. This demonstrates some degree of augmentation of dietary selectivity and resource exploration by these taxa in M. Seco. The fact that mollusks shows $\delta^{13}C$ values similar to that of periphyton in Q. da Ombria and F. Benemola suggests strong specialization in a biofilm diet (Arcagni et al. 2013). Site-specific selectivity among consumers is likely related to variability in the actively cycling N pool (preferentially assimilated by consumers), which can vary greatly between basal food resources (Hamilton et al. 2001) and biofilm structure (Rezanka and Hershey 2003).

Local specialization of some taxa can often reflect the quality of food resources (McNelly et al. 2006; Mulholland et al. 2000a). However, little difference was observed in epilithon chl *a*/AFDM and C/N between sites (Table 6.2). This suggests that differences may reflect variation in epilithic structure, such as unequal distribution of actively cycling N among bulk of epilithon material (Peipoch et al. 2012; Rezanka and Hershey 2003; Wollheim et al. 1999). Further, the highly depleted isotopic signatures of *B. fuscatus*, in F. Benemola, indicate moss as a potential food source for this species. Moss can contain high phenolic content and is often considered an unpalatable and unimportant food source (Bunn et al. 1989). However, herbivores in unproductive streams can switch from epilithic algae to marginal food sources such as bryophytes in times of scarcity (McWilliam-Hughes et al. 2009). Therefore, moss should not be neglected in future analysis of food webs in temporary streams, especially at oligotrophic sites.

However, differences in resource exploration were also apparent in non-herbivore species. For example, an omnivorous crayfish revealed diet shift (as inferred from $\delta^{15}N$ value) between M. Seco and Q. da Ombria (Fig. 6.3, Appendix 6.1). This was probably due to changes in foraging locations (habitat) of this species, however feeding idiosyncrasies (also reported in France 1996b) or ontogenetic diet shifts could also explain these patterns.

Our results clearly demonstrate that $\delta^{15}N$ variation observed among consumers is more influenced by $\delta^{15}N$ variation in their basal food resources than by consumer fractionation. High among-site variation in $\delta^{15}N$ and $\delta^{13}C$ of basal food sources has consequences for $\delta^{15}N$ and $\delta^{13}C$ fractionation among and within consumer taxa, thereby puzzling the determination of the trophic position (France 1995; Lau et al. 2009; Vanderklift and Ponsard 2003; Zanden and Rasmussen 2001). Alternatively, the concept of the “trophic position” might not be applicable to this ecosystem, due to resource use by consumers being more flexible than previously believed. This implies that models based on isotopic fractionation have to account for variable site-specific food sources in order to explain trophic food web relationships in this specific ecosystem (Zanden and Rasmussen 2001).

Our initial predictions (H3) assumed that consumers from sites with seasonally variable resource availability (intermittent) adopt omnivore habits as perhaps an adaptive strategy to use resources more efficiently (Bunn et al. 1999; Douglas et al. 2005; Pusey et al. 2010). This would result in shorter food chains and greater isotopic overlap between consumers at different trophic levels (Minagawa and Wada 1984). We found this pattern for grazers and omnivores; grazers and predators as well as collectors and predators. It was particularly noticeable in collector mayflies (*B. fuscatus* and *Ecdyonuru sp.)* whose isotopic N signature was close to, and even exceeded, that of predators in the intermittent M. Seco and Q. da Ombria sites. In contrast, in F. Benemola, the differences in the isotopic N signature between collector *B. fuscatus* and predator *I. moselyi* were more evident. These results match our predictions that most predators at these two intermittent sites might feed across species coming from broad range of feeding links.

Another explanation for higher overlap in $\delta^{15}N$ in M. Seco and Q. da Ombria between referred FFGs could be associated to more autotrophic nature of M. Seco and Q. da Ombria relatively to more shaded and less productive F. Benemola. High ^{15}N and ^{13}C enrichment of periphyton and algae in autotrophic streams causes higher trophic enrichment of primary consumers relative to consumers residing in more heterotrophic streams (Jaarsma et al. 1998; Lau et al. 2009). Nevertheless, Site and FFG interaction was not significant in any of the three-factor analysis and therefore most of the variation among sites exists at the species level, but not FFG level what proves that FFGs should not be used to infer about the trophic food web relations. Large variability of species response to basal food resources needs to be addressed in biomonitoring and management programs. Choosing the most representative taxa for biomonitoring in addition to acknowledging site-specific responses should be carefully considered when using macroinvertebrate isotopic signatures for monitoring of human impacts.

Finally, our study complements existing experimental data on isotopic ratio variability among consumers (belonging to different taxa and feeding groups) in Mediterranean streams. The recent increasing use of stable isotopes as indicators of ecosystem health demands a better understanding of biota complexity to be aware of possible drawbacks in monitoring human impact on aquatic ecosystems. This is particularly important for the temporary Mediterranean-type streams, where the responses of biota are less predictable due to variation in hydrological regimes (Argyroudi et al. 2009; Beche et al. 2006; Chakona et al. 2008; Grubbs 2011; Mas-Marti et al. 2010).

In summary, our study demonstrates that omnivory, environmentally induced spatial diet heterogeneity and preferences for nutritionally richer food prevent the assignment of species to discrete trophic levels. This implies that classifications based on FFGs poorly reflect trophic structure and resource assimilation in temporary Mediterranean-type streams.

Acknowledgements

The authors specially acknowledge Paulo Zaragosa Pedro, Marta Ribeiro e Bruno Garcia from Laboratório de Análises Quimicas (LAQ) from University of Algarve for their technical support in chemical analysis of nutrients. We also acknowledge Alba Carmona Navarro from IRNAS-CSIC for her assistance in the isotopic analysis. The following work was a part of the IMPACT project funded by Foundation for Science and Technology (FCT) of Portugal (ERA558IWRM/0003/2009).

Chapter 7

Taxa identity drives nutrient excretion by benthic macroinvertebrates in temporary Mediterranean streams

Katarzyna Sroczyńska[1], Michael Vanni[2], Adrianna Wojtal-Frankiewicz[3], Marilia Claro[4], Pedro Range[5] and Luís Chícharo[1]

[1]Faculty of Science and Technology, Campus of Gambelas, University of Algarve 8005-139, Faro, Portugal. e-mail: kasiasroczynska@gmail.com, tel: (00351)963138888

[2]Department of Biology, Miami University, Oxford, OH, U.S.A

[3]Department of Applied Ecology, University of Lodz, Banacha 12/16 Str., 90-237 Lodz, Poland

[4]Centro de Ciências do Mar, Campus of Gambelas, University of Algarve 8005-139, Faro, Portugal

[5]Department of Biological and Environmental Sciences, College of Arts and Sciences, Qatar University, Doha, Qatar

Manuscript in preparation

Abstract

Ecological stoichiometry is a key concept to understand energy transfer among consumer-basal resource interface. Nutrient excretion is the most direct way by which animals support nutrients to the ecosystem. The role of stream macroinvertebrates in nutrient recycling is largely recognized, however factors that drive nutrient excretion in nature remain poorly studied.

In the present study we quantified N and P excretion rates and elemental composition of the most abundant consumers and basal resources at three reaches: two intermittent and one perennial in the Mediterranean temporary river system. We hypothesized that excretion by consumers will be driven by both: changes in periphyton nutrient content among experiment sites and taxonomical group classification. We found large variability of periphyton nutrient content among sites what impeded to draw general conclusion of how consumer's excretion rates are mediated by the site-specific periphyton properties. Our results demonstrated that site had only significant effect for P excretion, when nutrient excretion was accounted for the dry mass. Additionally, we found no correlation between basal food resources (periphyton) and elemental composition of grazers indicating either existence of strong dietary selectivity of grazers or that food sources were not representative of actual macroinvertebrate diets.

Instead, dry mass and taxonomical group were responsible for the highest variation in excretion rates. Significant differences were also reported within the same taxonomical group, among different genera. Differences in taxa-specific excretion rates, particularly associated with extremely high P excretion of Trichoptera, were responsible for high among-site variability in aggregated nutrient excretion with considerably differences in N:P ratios among sites. Such striking taxa-specific differences in nutrient excretion will have large implication for local nutrient recycling in systems with highly dynamic macroinvertebrate communities such as temporary river system.

Keywords: macroinvertebrate excretion; nutrient dynamics; stoichiometry; periphyton elemental content

7.1. Introduction

Ecological Stoichiometry is a fundamental tool for understanding the role of biota-mediated ecosystem functions in driving nutrient cycling and vice versa (Sterner and Elser 2002). The extent to which animals supply nutrients to other members of the ecosystem depends on their own body stoichiometry and their food composition (Elser and Urabe 1999). This relationship was modeled by Sterner (1990) based on mass-balance equations under the assumption that animals are homeostatic in maintaining their internal nutrient composition. Animals achieve homeostasis by modifying the quantity and quality of their ingested food relatively to the demand of nutrients essential for their growth, metabolism and reproduction (Sterner and Elser 2002). Therefore, when the nutrient present in food exceeds the consumer's demand for this element, its excess is released via excretion (Elser and Urabe 1999).

In streams periphyton comprises the largest resource base for primary consumers. Periphyton stoichiometry is mostly influenced by nutrient and energy availability (Sterner et al. 1997). In the systems with high light:nutrient ratio, the periphyton becomes carbon rich having high C:nutrients ratio (higher carbon production per nutrient biomass). In opposite case low light:nutrients ratio produces less biomass, but higher quality periphyton (low C:nutrients ratios). Quality and quantity of periphyton was demonstrated to influence animals recycling rates (Sterner 1997; Frost et al. 2005). According to stoichiometry theory at sites with high periphyton biomass and not-limiting nutrient supply animals should recycle at higher rates (Urabe et al. 1997) relatively to sites with deficient food supplies, where consumers can easily become limited by periphyton nutrient content. However, even when the food quantity is relatively not limiting, food quality also affect consumer's performance (Frost et al. 2002; Sterner et al. 1997). Low quality food (high C:N and C:P ratios of periphyton) leads to nutrient imbalances and reduced herbivore growth rates (Urabe et al. 1997) as well as altered nutrient release (Dodds et al. 2004; Elser and Urabe 1999; Evans-White and Lamberti 2005; Frost et al. 2005; Schindler and Eby 1997; Vanni 2002). At opposite case high food quality, especially high P content of food positively affect grazer's growth rate and lead to higher P excretion (Frost and Elser 2002; Rothlisberger et al. 2008). In addition, phylogenetic constraints and body composition of consumers play also important role in governing assimilation and excretion of components (Elser et al. 1996; Evans-White et al. 2005; Frost et al. 2003; Torres and Vanni 2007). For example organisms with high P demand will excrete less P than other organisms, even at non-limited P supply (Frost et al. 2006). Although biological factors other than type of food or body stoichiometry can also influence consumer's performance (Liess and Lange 2011; Hill et al. 2010).

While the effect of food quality and quantity on growth rates of benthic consumers is relatively well studied (Stelzer and Lamberti 2002), it is not clear how periphyton quality and quantity mediates nutrient excretion in nature and under what thresholds of periphyton elemental content resource quality might outbalance the food quantity. Understanding the relationship between nutrient excretion and different quality and quantity food regimes in natural systems may help to predict how natural resource availability and consumer identity influence ecosystem function via nutrient recycling.

To study theses relationships we took advantage of natural heterogeneity of temporary streams, which results in sites with contrasting hydrological regimes, i.e. perennial vs intermittent. Intermittency influences habitat structure and resource availability. We studied two intermittent streams and one perennial reach. Perennial reach in the studied catchment is fuelled by the groundwater discharges, has relatively dense canopy cover and is of oligotrophic nature (Chícharo et al. 2010), while reaches with intermittent regimes are characterized by open canopy cover and are more vulnerable to desiccation and local nutrient enrichment (Gasith and Resh 1999). Therefore, benthic fauna at these differing streams are exposed to a large range of variation in basal resources, which are expected to differ in quality and quantity. For this reason the first objective is to examine how the recycling rates of benthic invertebrates leaving in streams with naturally variable physicochemical conditions will respond to changes in resources availability and quality among studied streams. For this experiment we used taxa that belong to various functional feeding groups (Merritt and Cummins 1996) assuming that the effect of periphyton stoichiometry will not only affect grazers, but will also extend to higher trophic levels (Tilman 1982; Vanni 1996). In addition to this site effect we also examined the effect of taxonomic variation and body stoichiometry of consumers.

We hypothesize that excretion rates (accounted for the dry mass) will vary with site and these differences will be attributable to differences in periphyton nutrient content among sites. More specifically, we hypothesize that according to light and nutrient theory intermittent open sites will have a higher food quantity than perennial sites, assuming similar nutrient supply. This will translate into higher N and P nutrient biomass of periphyton and higher recycling rates of invertebrates at open intermittent sites, relative to shaded perennial reach. Further we also expect that taxonomical group will have a significant effect on excretion rates. And this effect will be related to the body content of the studied taxa.

In order to examine if patterns in individual taxon-specific excretion rates are consistent with spatial patterns in nutrient recycling, we calculated aggregated excretion rate, for each reach, based on macroinvertebrate community composition and taxon-specific excretion rates. Quantification of spatial heterogeneity in nutrient recycling among reaches belonging to the same river basin, but with contrasting hydrological regime and productivity, constitutes an important step towards understanding of temporary streams ecology.

7.2. Methods

7.2.1. Study Site

We sampled and conducted excretion experiment at two intermittent sites and one perennial site within the Quarteira River Basin (Algarve, South Portugal) located within the Mediterranean region. The catchment is small (~324 km^2) with narrow elevation range (14-515 m). Average monthly temperatures varies from 8 to 29 °C and average annual rainfall is 625 mm. Catchment has an intermittent regime consisted of perennial sites, fed by groundwater discharges; intermittent sites, which dries to series of disconnected pools and ephemeral sites, which dries completely. Fonte Benemola is our most upstream site, located within a second order stream (the Menalva). The Menalva is primarily fed by three-groundwater sources (~60% total discharge), which results in constant annual flow. Fonte Benemola riparian vegetation consists of tree and shrub species such as willows, ashes, oleanders cane and African tamarisk. These land use characteristics together with scant urban development makes the study area a relatively undisturbed study site. Dense canopy cover and narrow channel width with relatively steep river banks impedes light penetration and makes Fonte Benemola less productive than the other two sites. The Monte Seco and Quinta da Ombria sites are located within the same first order stream (The Algibre) which is a junction of Menalva stream and Ribeira das Merces stream. Contrary to the Fonte Benemola, both sites have intermittent flow regimes. At steady flow conditions (March-May) average discharge is approximately 1.3 m^3 s^{-1} and it gradually decreases towards warmer months, being as low as 0.026 m^3 s^{-1} (July-August) just before the channel dries to a series of disconnected pools or completely dryness at some fragments. Riparian vegetation in Monte Seco is dominated by wild cane (*Arundo donax*), herbaceous vegetation and carob and olive trees and is moderately developed with a 3 m wide lateral zone at both sites of the stream and occasional spots of more scattered canopy cover. Quinta da Ombria has less densely developed riparian vegetation than Monte Seco and it consist only of shrubs and herbaceous vegetation with wider and less steeper riverbanks. Both sites reflect slightly lower water quality status than is reported for Fonte Benemola (Chicharo et al. 2010).

7.2.2. Excretion experiment

At each site the most representative stream section (100 m) was selected that included riffle and pool zones. Nutrient excretion experiments with benthic macroinvertebrates were conducted on ten days (March 16th to May 13th) during a period of steady flow. We studied ten different taxa to cover all of the taxonomical groups. Thus, we used two species of crustaceans: *Atyaephyra desmarestii* and *Procambarus clarkii*, two species of mollusks*: Ferrisia wautieri* and *Physella acuta* and six insects taxa*: Baetis fuscatus, Chimarra marginata, Ecdyonurus* sp.*, Isoperla mosely, Oreodytes* sp. and *Pyrrhosoma nymphula.* Macroinvertebrates were removed from stones with forceps or elutriated from soft substrates and placed inside experimental 50 ml bottles with pre-filtered stream water (for crayfish we used 400 ml bottles). Bottles with macroinvertebrates and controls filled only with pre-filtered water were incubated for 1 h inside a stream. Temperature was recorded along the incubation time and further correction of excretion rate for temperature was done. Initial water samples were taken from stream to quantify initial nutrient concentrations. A minimum of three replicates of 10 taxa were collected at each site. This resulted in a total of 99 samples. For smaller taxa such as *Baetis* and *Oreodytes sp.* we used 30-40 individuals for each replicate sample, *C.marginata* included about 20-30 individuals and for remaining taxa this value was between 10-15 individuals. The exception was crayfish, which replicate sample consisted of a single individual. All the individuals were the same size and belonged to the same taxa. The exception were mayflies of *Baetis* sp.*,* which individuals were too small too sometimes distinguish between separate species. However, macroinvertebrate composition demonstrated that majority of genus *Baetis* sp. were composed of *Baetis fuscatus*. After the incubation finished, the water was filtered on glass fiber filters (GF/C, 47mm Whatman) and stored in cooler for subsequent nutrient analysis. Excretion rates were determined as the difference between final nutrient concentration and initial concentrations, corrected for control. In cases where PO_4^{2-} excretion was below the detection level we used total dissolved phosphorus (TDP) to calculate the excretion rate. Macroinvertebrates were preserved in liquid nitrogen for further analysis of tissue C, N and P.

7.2.3. Macroinvertebrates and habitat sampling

At each site macroinvertebrates were sampled for community determination. Benthic macroinvertebrate samples were collected using a hand-net (0.5mm mesh, 25 cm width) and a standardized kick sampling method (each unit is 1m long and 0.25m wide). For each reach, 4-6 m trawl (0.25m width) was collected. Sample contents were placed in plastic containers and preserved using 96% ethanol.

Samples of epilithon (2-4) were collected from the top of randomly chosen stones at the same stream section and samples were subsequently frozen. Additionally at each site 2-4 epilithon samples (each consisted of 1 or 2 stones) were collected for biomass (chlorophyll *a* and Ash-free-fry-mass - AFDM) determination. Water samples for water base nutrient analysis (NO_3^-, NH_4^+ and PO_4^{2-}) were also taken at each site.

7.2.4. Sample processing

In the laboratory water samples were analysed for soluble reactive phosphorus (SRP) and ammonium spectrophotometrically (APHA 2012). Nitrate and nitrite concentrations were analysed on a Skalar autoanalyzer (Skalar SAN Plus System, SKALAR) using the cadmium reduction method (APHA 2012). We recorded the increase of NO_3^- in some treatments relatively to control. However, in contrasts in some treatments we observed a decrease of NO_3^-. This can be associated with nitrification ($NH_4^+ \rightarrow NO_3^-$) processes occurring during the incubation. Nevertheless, because the pattern of NO_3^- increase was not consistent along all of the incubation treatments, we decided to not account for NO_3^- in total NH_4^+ excretion rate calculations.

Epilithon and macroinvertebrate samples were dried to a constant weight and further macroinvertebrate samples were weighted on analytical balance to determine dry mass weight. Since the collected individuals were all the same size, the individual weight was obtained by dividing total weight per number of individuals.

Further, epilithon and macroinvertebrate samples were subsequently homogenized to fine powder using an agate mortar and pestle and subsamples were analyzed for %C and %N on a CHN elemental analyzer (Flash 2000 HT/IRMS system; Thermo Scientific, Bremen, Germany) as described in González-Pérez *et al.,* (2015). % P was analyzed with potassium persulfate digestion followed by SRP analysis using ascorbic acid method (APHA 2012). Owing to very small size, gastropods were analyzed together with their shells (Liess and Hillebrand 2005).

Periphyton from stones was scrubbed into a known volume of water using a toothbrush and thoroughly homogenized (Biggs and Kilroy, 2000). The resultant slurry was subsampled and filtered on glass fiber filters (GF/C, 47mm Whatman) for chlorophyll *a* (Chl *a* mg m^{-2}) and ash free dry mass (AFDM mg m^{-2}) analysis. Chl *a* was extracted in 90% boiling ethanol and kept in freezer for 24 h. The absorbance was read on spectrophotometer (Thermospectronic GENESYS 10UV). AFDM filters were dried at 60°C to constant weight and AFDM represents the weight difference before and after 4h at 450 °C. Chl *a* and AFDM were calculated per stone surface area assuming that metabolically active area of stones is 60 % (Biggs and Close 1989). Samples for macroinvertebrate community determination were washed in order to remove the fixative and placed in a tray. Subsequently they were sorted and examined using a stereomicroscope and identified to family level.

7.2.5. Statistical analysis

Excretion rates are highly temperature dependent and temperature during our incubations varied (14°C - 21.4°C), we corrected the excretion rates to 18°C using temperature coefficient (Q_{10}) of 2. Among site and among taxa differences in measured and tested variables were checked using the non-parametric Kruskal-Wallis Rank Sum Test using the “kruskalmc” function in the R package “pgirmess” (R Development Core Team 2012). We performed Tukey’s post-hoc analyses and generated 95% confidence intervals using the “TukeyHSD” function in the R package “multcomp” (Hothorn et al., 2008; R Development Core Team 2012).

We used the average values of environmental variables measured per day and therefore sample days represent a sample size (Algibre n=5, Quinta da Ombria n=4, F. Benemola n=4). In order to examine if body content of invertebrates correlates with periphyton nutrient content, we used only grazers (*Ferrisia wautieri, Physella acuta Baetis fuscatu*s and *Ecdyonurus* sp.), which are known to feed on periphyton in these streams (Sroczynska et al. 2017, in prep.). Correlation between the periphyton elemental content and grazer elemental content was done by pooling all the grazer samples from sites and correlating them with their periphyton analogues collected at each experimental day (thus sampling size corresponds to number of sampling days n=7).

In order to test for interaction between excretion rates (response variable) and site/taxa (factors), while controlling for the effect of body weight we performed two-way ANCOVA using body weight as the covariate. Before ANCOVA, data were tested for homogeneity of variances using Levene test. The ANCOVA was performed on $\log_{10}$ transformed data of individual excretion rate to ensure the linearity assumption of the covariate with the response variable.

Aggregated excretion rate was calculated based on individual excretion rate. The number of individuals found per square meter was multiplied by the individual excretion rate. Further, the N and P excretion rates of the entire macroinvertebrate assemblage at each site were summed. This method did not account for various individual sizes, which naturally occur in the river. Nevertheless, the individual sizes used for the excretion experiment represented the most typical sizes encountered in the reach for this taxa. Each of the measured taxa we aggregated to order therefore the excretion rates of individual species were aggregated to main orders i.e. *Oreodytes* sp. was representative for Coleoptera order etc...In the Ephemeroptera order there were some size discrepancies among main families belonging to *Baetidae*, which normally were smaller than larger in size *Heptageniidae* and *Leptophlebiidae*. For this reason the individual excretion of *B. fuscatus* was representative for all of the individuals from the family *Baetidae*, whereas, *Ecdyonurus* sp. represented the individuals from the family *Heptageniidae* and *Leptophlebiidae*. There were some orders, which were present in the community composition, but we had no excretion records for these orders (Diptera and Ostracoda). Nevertheless, their biomass constituted less than 10 % of all of the biomass so their contribution to the total aggregated excretion is probably limited (see Appendix 1). Univariate analyses and graphs were done using R package (R Development Core Team, 2012).

Experiments were performed at different dates so we additionally examined if date affect the results of excretion. However, date had no effect on the results and for this reason we dis not include date into our analysis.

7.3. Results

7.3.1. Among site differences in periphyton quality and quantity

The environmental variables measured for three sites significantly differed only for nitrate and marginally for phosphorus being both higher in F. Benemola than in remaining sites. Quantity measures such as mean and range of periphyton biomass (Chlorophyll a and AFDM) was similar among sites. Overall mean and range of N, P and C expressed in [g m^{-2}] of periphyton were the highest in F. Benemola, however we found no significant differences among sites (Tab. 7.1).

Tab. 7.1 Environmental variables RANGE (MEAN) measured at each site, where Monte Seco and Quinta da Ombria are classified as intermittent and Fonte Benemola as perennial.

ENVIRONMENTAL PARAMETERS	Monte Seco	Quinta da Ombria	F.Benemola	Chi-squared	P
Chlorophyll a [mg m^{-2}]	75.3-610.6 (250.7)	29-441 (145)	35.1-532.9 (205.3)	2.40	0.30
AFDM [g m^{-2}]	12.49-59.75 (37.32)	2.62-53.46 (28.94)	5.98-93.40 (45.03)	1.29	0.53
Chlorophyll a/AFDM	0.003-0.03 (0.008)	0.001-0.03 (0.009)	0.003-0.009 (0.005)	0.54	0.76
Temperature [°C]	15.00-20.05 (16.83)	16.00-20.15 (17.88)	15.40-18.00 (16.24)	2.74	0.25
N dry mass [g m-2]	39.17-219.87 (104.58)	58.86-69.80 (64.33)	40.29- 389.24 (162.91)	0.37	0.83
P dry mass [g m-2]	5.66-11.45 (9.20)	7.99-11.52(9.75)	3.53-27.00 (17.17)	1.27	0.53
C dry mass [g m-2]	1722.16-3832.75(2530.29)	1016.40-1447.53(1231.96)	494.03-5920.33(1904.25)	2.77	0.25
NH_4^+-N [µM]	0-0.26 (0.65)	0-0.53 (1.91)	0.62-1.32 (1.05)	1.41	0.49
NO_3-N [µM	1.93-6.82 (5.71)	2.03-4.64 (3.39)	3.46-30.6 (18.80)	12.10	0.00
PO_4-P [μM^1]	0-0.13 (0.05)	0-0.14 (0.03)	0-0.28 (0.15)	4.67	0.10

In regard to periphyton quality we only observed significant differences for % N and C:N ratio, with significantly higher % N and lower C:N measured for shaded F. Benemola in comparison to open intermittent M. Seco (Fig. 7.1).

7.3.2. Interaction effect of site and taxonomical group on excretion rates

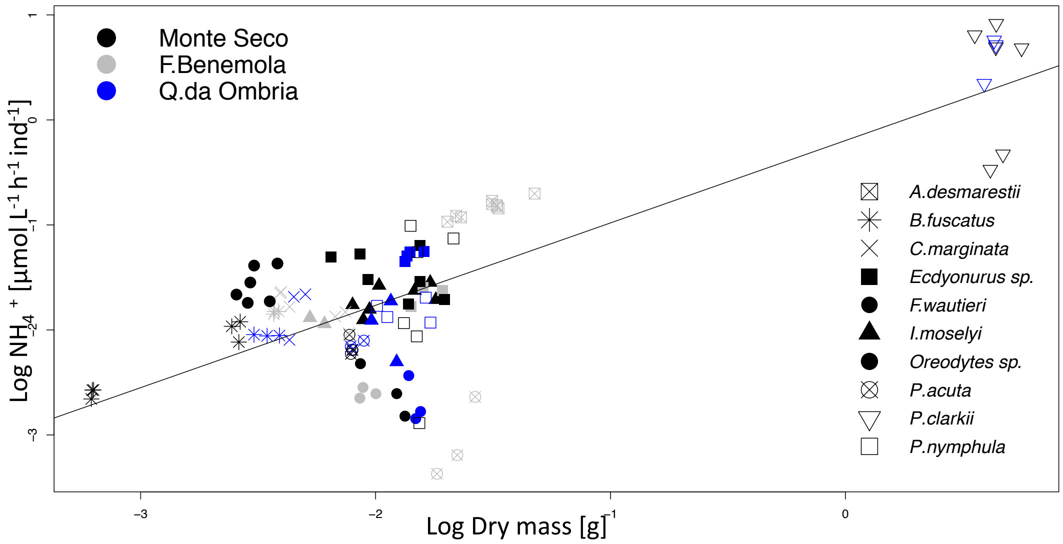
ANCOVA detected significant main effect of dry mass and taxonomical group on individual excretion rates (Tab. 7.2). However, site had only a marginal effect and this effect was stronger for PO_4^{3-} excretion than for NH_4^+. There was observed an interaction between Site and DM for PO_4^{3-}, however not for NH_4^+ indicating that PO_4^{3-}, excretion for DM is site specific.

Tab. 7.2 Two Way ANCOVA results for log NH_4^+ and log PO_4^{3-} excretion with Site and TG (taxonomical group) as factors and DM (dry mass) as covariate.

		log NH_4^+ [µmol l^{-1} h^{-1} ind^{-1}]			log PO_4^{3-} [µmol l^{-1} h^{-1} ind^{-1}]		
Source of variation	*df*	Mean Sq	*F*-ratio	Pr(>F)	Mean Sq	*F*-ratio	Pr(>F)
Dry mass	1	44.20	424.79	<0.001	27.84	182.48	<0.001
TG	2	17.41	83.67	<0.001	2.80	18.38	<0.001
Site	2	0.57	2.73	0.07	0.46	3.04	0.05
DM:TG	2	2.07	9.96	<0.001	2.81	18.43	<0.001
DM:Site	2	0.21	1.03	0.36	0.55	3.60	0.03
TG:Site	4	0.12	0.28	0.89	0.06	0.38	0.83
DM:TG:Site	4	0.39	0.95	0.44	0.08	0.54	0.71
Residuals	81	8.43			0.15		

Considering all of the species, N and P individual excretion rates were both positively correlated with log dry mass (r=0.78, P<0.001 for N and r=0.72, p<0.001 for P, Fig. 7.1A and 7.1B). The slope was similar for both P and N with distinctive higher excretion by crayfish and the lowest for mayfly - *B. fuscatus*. Caddisfly - *C. marginata* at F. Benemola excreted P at higher rates than other taxa and visually stands out from the rest of consumers (Fig. 7.1B).

A



B

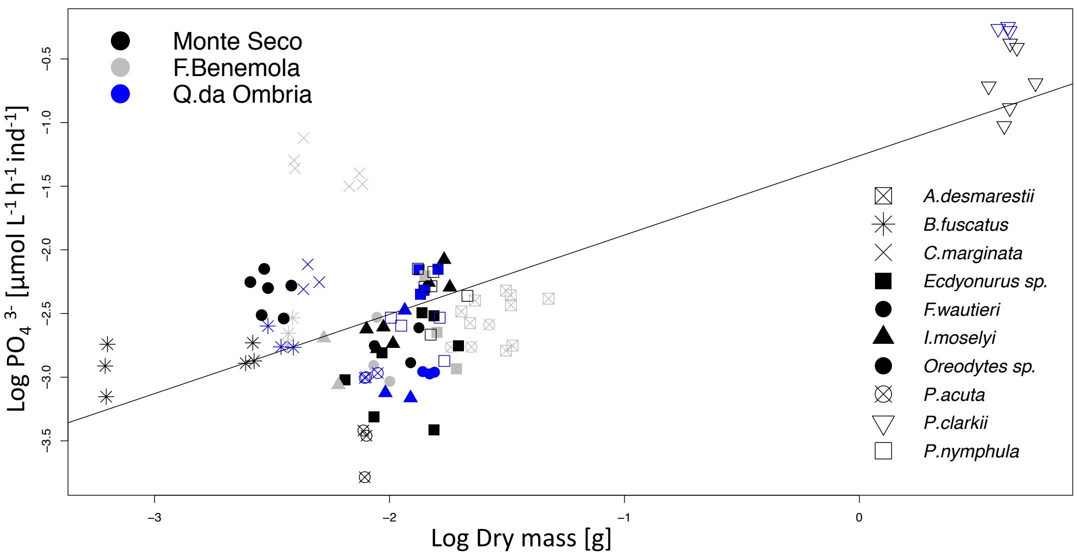


Fig. 7.1 Per individual µmol NH_4^+ (A) and µmol PO_4^{3-} (B) excretion rates by benthic invertebrates at three sites. Each point represents an individual.

Considering each species individually there were differences in PO_4^{3-} excretion rates [µmol PO_4^{3-} g^{-1} h^{-1}] of the mollusc (gastropod) *Physella acuta* (P=0.04), with significantly higher values of PO_4^{3-} excretion in intermittent Quinta da Ombria (mean=0.21) in comparison to intermittent M. Seco (0.07).

7.3.3. Relationships between food source and body content in regard to site

At all of the sites, grazer's elemental composition varied more than it varied in periphyton. The average % N, % P was higher for grazers than for periphyton what resulted in higher periphyton C:N and C:P ratios (Fig. 7.2). Average N:P ratios were nearly two fold higher in grazers than in periphyton. Grazers elemental composition and ratios were not significantly different between sites, except for C:P ratio, which differed

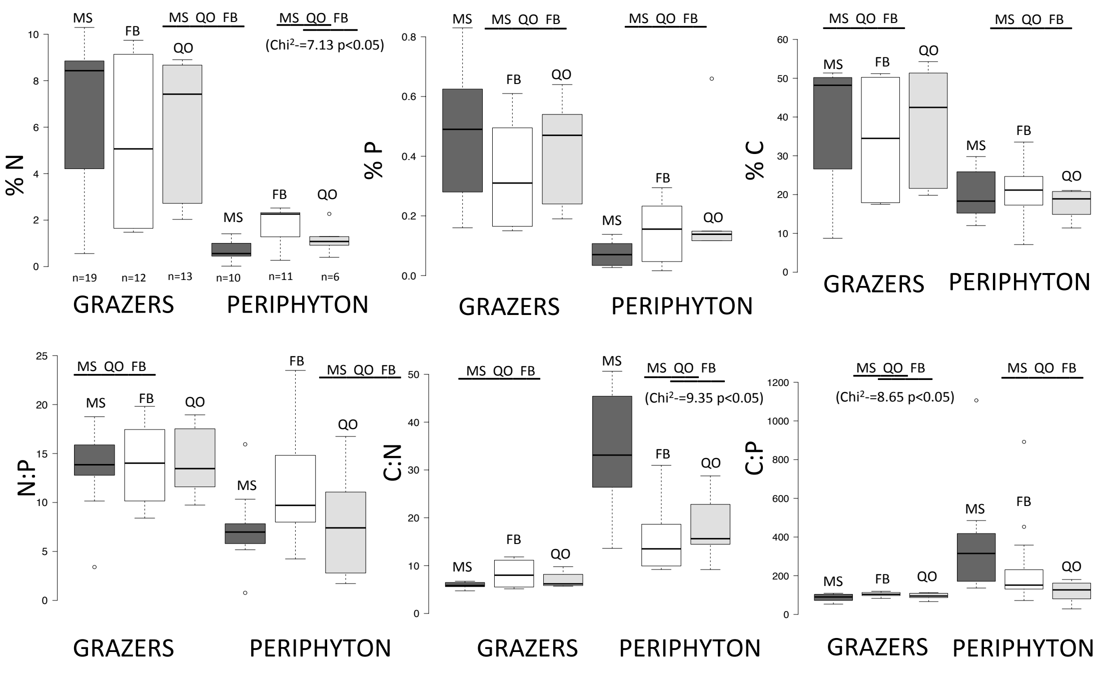


Fig. 7.2 Boxplots with elemental composition and ratios of grazers and periphyton represented for 3 sites. Horizontal segment is a median; horizontal lines marking the box are first and third quartiles with "whiskers" that extends to minimum and maximum value. Statistics of the Tukey HSD test (GRAZERS: df = 2, MS(Monte Seco) = 19, FB(F.Benemola)= 12, QO (Quinta da Ombria), PERIPHYTON: df = 2, (Monte Seco) = 10, FB(F.Benemola)= 11, QO(Quinta da Ombria)) = 6) applied in testing the differences between sites for periphyton and grazers. For sites that are underlined with the same line no difference at p ≤ 0.05 was recorded in *post-hoc* tests.

significantly with lower C:P in intermittent open M. Seco and higher C:P in perennial F. Benemola (Fig. 7.2).

Additionally, elemental composition of macroinvertebrate herbivores generally was not significantly correlated with that of their potential food sources. The exceptions were *P.acuta*, for which P body content was significantly ($P<0.001$) correlated ($r=0.96$) with P content of its potential food source, and *B. fuscatus*, for which N:P and C:N ratio were significantly positively (N:P) or negatively (C:N) correlated with N:P and C:N ratios of their basal resources ($r=0.75$, $P=0.005$, $r=-70$, $p=0.01$ respectively). Body % C was correlated with % C of basal resources only for *Ecdyonurus sp.* and *P. acuta* ($r=0.64$, $p=0.01$ and $r=0.85$, $p=0.004$ respectively).

% N content, of predator stonefly *I. moselyi* was significantly lower ($p=0.03$) in intermittent M. Seco (mean=8.63) than in shaded perennial F. Benemola (10.59). Accordingly, this pattern translated into significantly ($p=0.01$) higher C:N ratio of *I.moselyi* in M. Seco (5.663) and lower in F. Benemola (4.305).

7.3.4. Among taxa differences in elemental composition and excretion

Significant differences among taxonomical groups were reported for all of the elemental components and ratios (Fig. 7.3). Furthermore, there were significant differences for taxa belonging to the same taxonomical group (specifically among taxa belonging to insects and crustaceans).

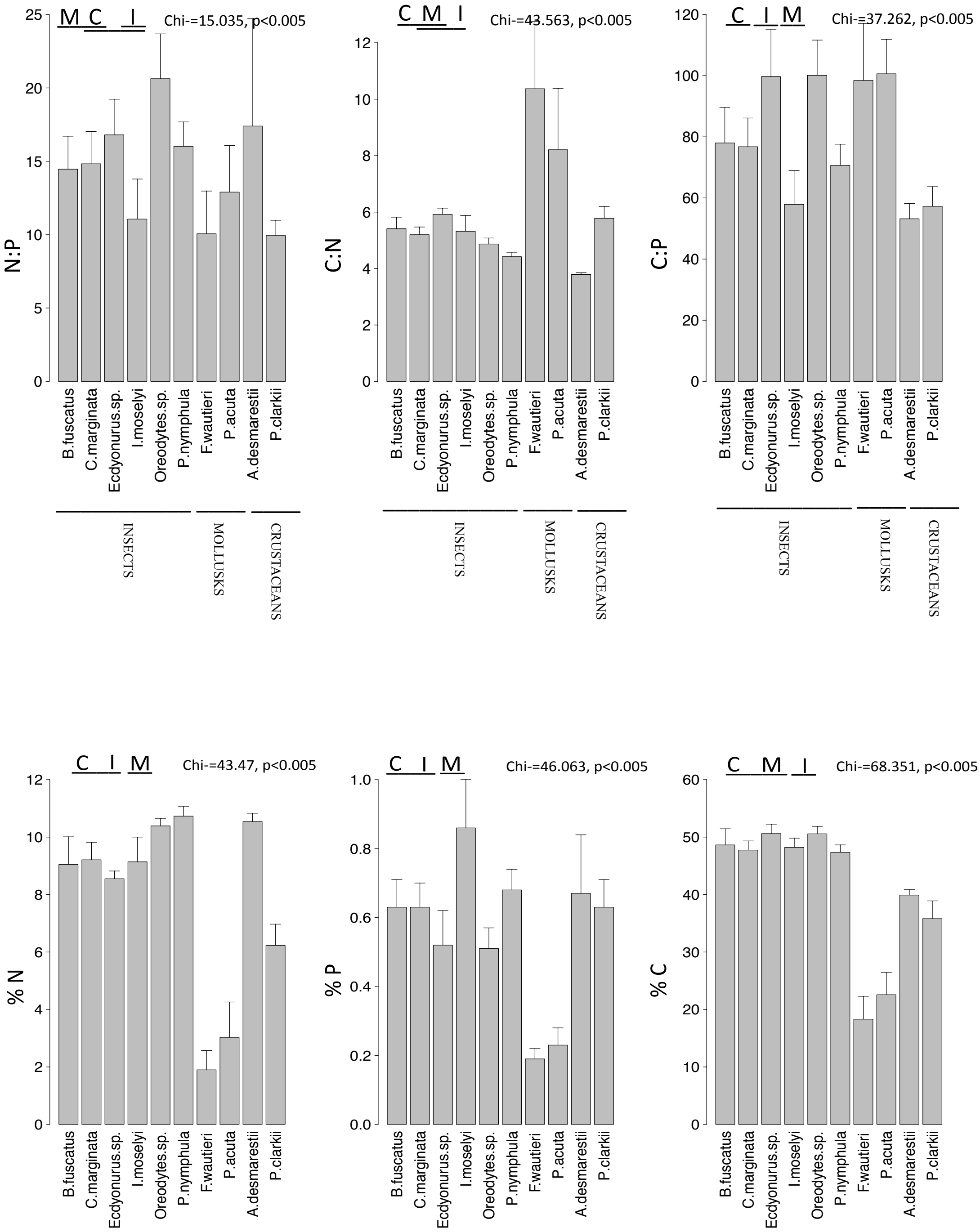


Fig. 7.3 Elemental composition and molar ratios for each taxa. Statistics of the Tukey HSD test (df = 2, I(insects) = 62, M(mollusks)= 18, C (crustaceans)=16, applied in testing the differences between taxonomical groups. For taxa that are underlined with the same line no difference at $p \leq 0.05$ was recorded in *post-hoc* tests.

N:P ratios significantly differed between mollusks (mean 11.48) and insects (mean 15.21). There were differences (Kruskal-Wallis chi-squared = 60, df = 9, p-value = $p<0.001$) among insect taxa, specifically between: *Ecdyonurus* sp. and *I.moselyi*, *I.moselyi* and *Oreodytes sp.* and *I.moselyi* and *P.nyphula*.

C:N ratio was significantly higher for mollusks (mean 9.29) in comparison to other groups (insects mean: 5.28 and crustacean mean: 4.66) (Fig. 7.3). Significant differences (Kruskal-Wallis chi-squared = 80, df = 9, $p<0.001$) were also reported within crustaceans: *A.desmarestii* and *P.clarkii* and within insects: *Ecdyonurus* sp. and *P.nympula*.

C:P ratio was distinct for all of the taxonomical groups, with the highest C:P ratio for mollusks (mean 99.53) and the lowest for crustacean (mean 54.98) (Fig. 7.3). Within insect group *I.moselyi* differed from *Ecdyonurus* sp. and *Oreodytes* sp. (Kruskal-Wallis chi-squared = 70, df = 9, p-value<0.001).

Mollusks had the lowest significant % N (mean 2.46) and % P (mean 0.21) from all of the groups, whereas % C was the highest for insects (mean 48.871) (Fig. 7.3). % N also differed (Kruskal-Wallis chi-squared = 80, df = 9, p-value< 0.001) between crustaceans: *A.desmarestii* and *P.clarkii* and insects: *Ecdyonurus* sp. and *P.nympula*. % P differed (Kruskal-Wallis chi-squared = 80, df = 9, p-value<0.001) between *I.moselyi* and *Oreodytes* sp. and *I.moselyi* and *Ecdyonurus* sp.

N excretion rate [μmol NH_4^+ g^{-1} h^{-1}] was significantly lower for mollusks (mean 0.65) in comparison to crustaceans (mean 3.27) and insects (mean 3.87) (Fig. 7.4). There also existed significant differences within the insect group, with higher NH_4^+ excretion rate for *Oreodytes* sp. relative to *I.moselyi* and *P nymphula* (post hoc tests: chi-squared = 67.743, df = 9, p-value <0.001). *Oreodytes* sp. had also the highest range (5.98-15.28) and mean (10.29) from all of the studied taxa for which the average N excretion rate was 2.69, ranging from minimum value of 0.03 measured for *P.acuta* to maximum value of 7.88 in *P.nympula*. P excretion differed only for insects in comparison to mollusks and crustaceans (Fig. 7.4). Even after excluding the strikingly higher excretion of *C.marginata* from the dataset, the differences between insects and other taxonomical groups were maintained (chi-squared = 34.509, df = 2, p-value<0.001). Within insect group *post hoc* tests (Kruskal-Wallis chi-squared = 70, df = 9, p-value< 0.001) revealed differences between *C.marginata* and *Ecdyonurus* sp.*,* and between *C. marginata* and *I.*

moselyi. N:P excretion ratio was significantly higher for crustaceans in comparison to other groups (chi-squared = 20, df = 2, p-value< 0.001); there were no differences between crustaceans and mollusks (Fig. 7.4). Within the insect group, only *C.marginata* and *Ecdyonurus* sp. differed (chi-squared = 50, df = 9, p-value<0.001).

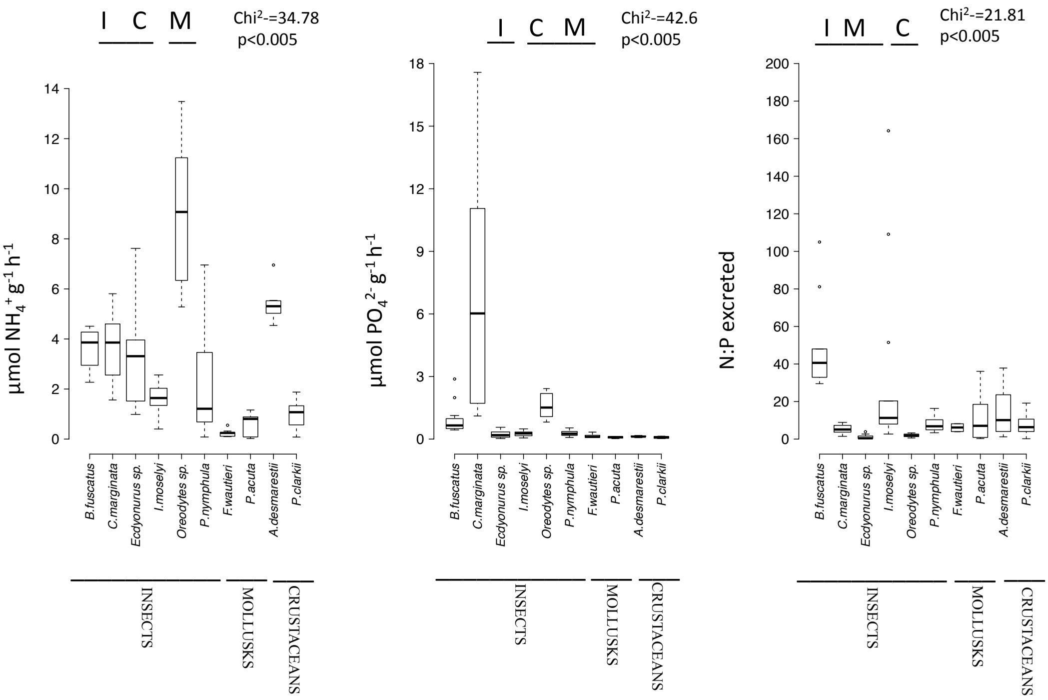


Fig. 7.4 Boxplots with NH_4^+ and PO_4^{2-}excretion rates and excreted ratio NH_4^+: PO_4^{2-} for all the taxa. Horizontal segment is a median; horizontal lines marking the box are first and third quartiles with "whiskers" that extends to minimum and maximum value. Statistics of the Tukey HSD test (df = 2, I(insects) = 62, M(mollusks)= 18, C (crustaceans)=16, applied in testing the differences between taxonomical groups. For taxa that are underlined with the same line no difference at p ≤ 0.05 was recorded in *post-hoc* tests.

7.4.5. Aggregated nutrient excretion

The highest estimated aggregated excretion rate for both NH_4^+ and PO_4^{3-} was at perennial F. Benemola (275.78 μmol N m^{-2} h^{-1} and 359.23 μmol P m^{-2} h^{-1}) (Table 7.3). Trichoptera and Ephemeroptera dominated the species composition at this site, and the high P excretion rate of Trichoptera contributed to elevated whole-reach P excretion for this reach. Intermittent Quinta da Ombria had an intermediate value of N (73.65) and P (12.23) aggregated excretion rates, and the lowest rates for both nutrients were reported at intermittent Monte Seco (22.52 and 3.25). The orders contributing most to excretion at intermittent M. Seco and Q. da Ombria were Ephemeroptera and Coleoptera. The highest N:P excretion ratio was in Monte Seco (6.91), whereas the dominance of Trichoptera at F. Benemola was responsible for higher P release, and hence low N:P excretion ratio (0.77).

Tab. 7.3 Whole-reach nutrient excretion for each order per site. N and P values of excretion rates of Ephemeroptera per individual are the average values measured for *Ecdyonurus sp.* and *B. fuscatus*; Gastropoda are the average value measured for *F.wautieri* and *P.acuta* and Decapoda are the average value measured for *A.desmarestii* and *P.clarkii*. Values of individual and aggregated excretion rates are means.

	Order	n°ind m^{-2}	% contribution	µmol NH_4^+ ind^{-1} h^{-1}	µmol NH_4^+ m^{-2} h^{-1}	µmol PO_4^{2-} ind^{-1} h^{-1}	µmol PO_4^{2-} m^{-2} h^{-1}	N:P excreted
MONTE SECO	*Coleoptera*	7.63	14.49	0.64	4.92	0.11	0.83	
	Ephemeroptera	31.30	59.42	0.53	16.58	0.06	1.83	
	Gastropoda	4.66	8.85	0.11	0.53	0.03	0.14	
	Odonata	0.67	1.27	0.83	0.56	0.11	0.07	
	Plecoptera	7.50	14.24	0.29	2.17	0.05	0.37	
	Trichoptera	0.92	1.74	0.38	0.35	0.68	0.62	
whole-reach excretion					**25.11**		**3.87**	**6.49**
QUINTA DA OMBRIA	*Coleoptera*	82.69	39.78	0.64	53.31	0.11	9.02	
	Ephemeroptera	93.78	45.11	0.53	49.69	0.06	5.49	
	Gastropoda	22.78	10.96	0.11	2.58	0.03	0.67	
	Odonata	0.56	0.27	0.83	0.46	0.11	0.06	
	Plecoptera	3.33	1.60	0.29	0.96	0.05	0.16	
	Trichoptera	4.74	2.28	0.38	1.81	0.68	3.23	
whole-reach excretion					**108.82**		**18.64**	**5.84**
FONTE BENEMOLA	*Coleoptera*	2.75	0.40	0.64	1.77	0.11	0.30	
	Decapoda	2.50	0.37	3.03	7.56	0.07	0.16	
	Ephemeroptera	123.25	18.04	0.53	65.31	0.06	7.22	
	Gastropoda	41.00	6.00	0.11	4.64	0.03	1.21	
	Odonata	0.25	0.04	0.83	0.21	0.11	0.03	
	Trichoptera	513.50	75.16	0.38	196.29	0.68	350.31	
whole-reach excretion					**275.78**		**359.23**	**0.77**

7.4. Discussion

Lack of site effect on excretion of macroinvertebrates contradicts our first hypothesis whereby site via differences in periphyton nutrient content, solely explained the macroinvertebrates excretion rates. Despite site differences in shading conditions, periphyton biomass and elemental content were similar among sites. According to our predictions based on light nutrient hypothesis intermittent open sites should produce higher quantity of periphyton, whereas periphyton at shaded, less productive F. Benemola should be present in lesser quantity, but should be richer in N and P relatively to C. Instead, natural variability in environmental parameters among sites affected periphyton grow in a manner difficult to predict basing only on the light:nutrient hypothesis. For example F. Benemola site is under groundwater influence, which alters biogeochemical properties of the water and was presumably responsible for the higher groundwater nutrient delivery to this system (showed by the high NO_3^- and PO_4^{2-} relative to other sites). Hence, this site is light limited but not nutrient limited, what likely causes that this site has different periphyton species assemblages in comparison to other sites (Marks and Lowe 1993).

This might result in similar periphyton biomass and bulk elemental content among sites despite differences in environmental and light conditions. Such presumption also matches with previous study performed on these streams, which demonstrated vast among stream variability in isotopic periphyton content with dominance of highly C depleted moss in F. Benemola relatively to less depleted filamentous algae prevailed at intermittent sites (Sroczynska et al. 2017, in prep.). Such high variability in periphyton elemental content is likely responsible for weak concordance between periphyton and grazers elemental content and impedes to draw general conclusion of how consumer's excretion rates are mediated by the site specific periphyton properties. Another explanation for such pattern can be related with the existence of dietary selectivity among invertebrates which pattern was already reported in similar study on these streams by inferring the diets of macroinvertebrates by the use of stable isotopes (Sroczynska et al. 2017, in prep). However, it must be understand that periphyton samples are bulk measures of nutrient composition and may not reflect what is actually being eaten and assimilated by specific macroinvertebrates (Dodds et al. 2014). By contrast dry mass and taxonomical group were better predictors of excretion rates than site. Allometric constraints on metabolism are widely reported from laboratory as well as from field studies for diverse groups of consumers (Vanni and McIntyre 2016). Mass specific excretion rates usually decline with the increasing body mass. Moreover, inter taxonomic differences in body elemental content and excretion rates are mainly explained by the phylogenetic constraints that result in different nutrient allocation patterns for species with different structural characteristics (Evans White et al. 2005; Liess and Hillebrand 2005). We indeed noticed differences in both elemental content and excretion rates among different taxonomical groups, but also among genera belonging to the same taxonomical group suggesting that the nutrient cycling can be strongly influenced even as low as at the genera level taxonomical resolution. Interestingly, two taxa: water beetle – *Oreodytes* sp. and caddisfly- *Chimarra marginata* had immensely high excretion for N and P respectively, but such high excretion was not reflected in their body nutrient content. For *Oreodytes sp.* such high N excretion can be related to its functional feeding group classification. As a predator, *Oreodytes sp.* feeds on diet rich in N and therefore, the excess of N is excreted at highest rates than remaining taxa. High P excretion of caddisflies was also reported by McManamay et al. 2011, who concluded that these taxa are not limited by P, allowing them to excrete P in high quantities. Also, such high P excretion can be associated to feeding mode of this group. Most of them are filter feeders and first they capture the food by their nets what allows them for better recognition of higher quality food, before its consumption.

In general our results support previous finding that phylogeny explain more variance in macroinvertebrate excretion than the spatial patterns (Frost et al. 2002).

This is mainly caused by the variability of basal food resources and difficulties in inferring macroinvertebrates diet and quantification of nutrients that are actually assimilated (Dodd et al. 2014; Fink et al. 2006). Additionally, high variability in grazers elemental content, in comparison to periphyton elemental content might indicate that consumers are not homeostatic and physiologically adapted to a wide range of C:P and C:N ratios.

7.4.1. Aggregated nutrient excretion

Aggregated excretion rates demonstrated large differences among sites, particularly perennial F. Benemola and remaining two intermittent sites. These differences were mainly associated with very high excretion rates of particular taxa, but also to high contribution of these taxa to overall species abundance. Previous studies demonstrated that differences in biomass of certain taxa can be responsible for creating biogeochemical hotspots in streams (Atkinson et al. 2013; Benstead et al. 2010; McIntyre 2008, McManamay et al. 2011; Munshaw et al. 2013). These studies usually analysed one-dominant taxa in the stream and compared differences in aggregated excretion rates of this taxa among sites where this species had high and low abundance. Present study demonstrated that spatial variability in nutrient dynamics can be associated with high excretion rates of the dominant taxa (such as in this case Trichoptera), but also spatial differences are generated by the contribution of various taxa-specific excretion rates. Stream macroinvertebrates play a large role in nutrient recycling (Wallace and Webster 1996), but detailed quantification of contributions of particular taxa to overall recycling rates remain poorly studied. Present study demonstrated that species identity with the combination with their biomass/abundance can largely influence excretion rates and ratios in streams what will have large implication for local ecosystem processes and be responsible for i.e. among-reach differences in periphyton assemblages (Cross et al. 2005). Intermittent streams are highly dynamic in terms of the frequency and duration of the hydrological period implying successive changes in community composition. For example, reduction in flow can favour lentic and more tolerant taxa (i.e. Diptera) with decrease abundance of rheophilic (i.e. *Heptagenidae*) species (Boulton 2003). Such shifts in communities will have subsequent effect on aggregated nutrient recycling rate and ratios and might completely alter nutrient dynamics in these streams within very short time scale. The effect of aggregated nutrient excretion by highly dynamic macroinvertebrate communities at spatial and temporal scales deserves further investigation.

Acknowledgements

The authors specially acknowledge Paulo Zaragosa Pedro, Marta Ribeiro e Bruno Garcia from Laboratório de Análises Quimicas (LAQ) from University of Algarve for their technical support in chemical analysis of nutrients. The following work was a part of the IMPACT project funded by Foundation for Science and Technology (FCT) of Portugal (ERA558IWRM/0003/2009).

Chapter 8

8.1 Discussion

Biota and ecological processes are intrinsically linked and vary at every single scale (Wellnitz et al. 2001; Wiley and Kolher 1997). Consequently, both structural and functional measures are inherent and complementary attributes of an ecosystem (Friberg et al. 2009) and both should be used complementary.

Community data can be analyzed by various ways, using different taxonomic resolutions and statistical packages for data analysis. For example, use of self-organizing maps (SOM) on data of increased taxonomical resolution but expressed on a binary abundance (frequent/rare) scale appeared to be an excellent tool for classifying water states and for delineation of main gradients which determine species occurrences. Traditional multivariate analysis coupled with univariate and IndVal analysis were great tools for studying the interactions among communities and their habitats as well as streams they inhabit and different scales. Finally, experimental data analysis allowed integrating the biota with the ecosystem function. Some of these techniques were used/tested for the first time in this type of ecosystem and constituted a valuable contribution to the ecology of intermittent streams. In the following section, a detailed discussion of current research findings is presented.

8.1.1. Macroinvertebrate assemblage structure

Diverse patterns in macroinvertebrate distribution occur at large and small scales as a response to abiotic influences, which operate at landscape but also at local scales (Allan et al. 1997, Richards et al. 1996). Presented results confirm the landscape filter hypothesis (Poff 1997) and the habitat based model (Kolasa 1989), where large scale factors act as filters which limit a distribution of biota and constrain the occurrences of certain species at the regional scales. These filters were demonstrated to act directly through biological patterns, but also indirectly through patterns in habitat structuration and availability. Species possess certain adaptive traits, which allow them to survive under particular set of environmental conditions. In the present study, such filters, which limited species occurrences along the catchment, were altitude and its association with conductivity and temperature (Chapter 2). However, such filters were also demonstrated to have an indirect influence through patterns in habitat structuration and availability. Habitat availability is a major mechanism (except for biological patterns) by which macroinvertebrates respond to changes in environmental conditions (Frissell et al. 1986). Therefore, another filter responsible for species occurrences within the catchment was related to structure or proportion of habitats present along the longitudinal gradient.

This study already implies that some species will be absent or less abundant at smaller scales because they did not pass the selective filter, expressed within a geomorphological hierarchy, which will allow them to be present at smaller scales. Also, it was demonstrated that at the catchment scale, environmental large-scale filters override the variables associated with different patch characteristics, which is in accordance with the "landscape filter" theory (Poff 1997). Based on these results, it is not surprising that we found a weak pattern in macroinverebrate distribution among different patches on the catchment scale. Significant overlap in taxa assemblages among main habitat types (coarse, mineral substrates and macrophytes) and weak confinement of certain family groups to given substrate types may therefore indicate 1) effect of occurrence of environmental filter on the catchment scale which limits species occurrences and is responsible for heterogeneous distribution of biota within the catchment, and 2) high mobility of stream organisms within a reach due to historical traits to be adapted to frequent disturbance and changes in availability of food resources (Williams 1996). The only distinct pattern in macroinvertebrate assemblages was found on marginal habitats characterized by low current velocity such as particulate organic matter (POM) and Sand. This demonstrates that above mentioned patterns will rather limit the use of habitat in describing variation in species assemblages at the catchment scale.

Landscape filter theory may therefore explain inconsistences existing in studies investigating biota associations to different patches distributed along the river continuum. For example, Palmer et al. (1991) compared associations of distinct fauna with six habitat types along the headwater, midreach and downstream sections and the consistent associations were only obtained for midreach and downstream sections, but not for headwaters. Authors attributed this inconsistency to smaller and less distinct patches in headwaters in comparison to mid and downstream reaches. Although, in our study headwaters were more heterogenous, the factors, which structure patches over the catchment scale are again an effect of geomorphological attributes acting on larger scale. Therefore, summarizing, landscape filter can influence biota distribution and associations with patches by direct control on species biological traits, or indirectly by affecting the patch structure and size.

Some authors demonstrated that reach scale is the threshold in geomorphological hierarchy where physical habitats become homogenous, so that the importance of the environmental filter operating at the larger scale will be weakened (Parsons et al. 2003). This is confirmed by many studies which demonstrated that samples collected within a reach are more similar to samples collected among reaches (Rabeni et al. 1999; Hawkins and Vinson, 2000). Therefore, studies which investigated a variation in macroinvertebrates assemblages within a reach, demonstrated strong influence of a patch on the macroinvertebrates distribution (Downes et al. 1993, 1995). Rabeni et al. (2002) found that habitat types within a river segment had biological meaning throughout the stream and they can be considered as distinct faunal units. Above studies suggest that reach scale will be a better predictor of species assemblages relatively to its patch because of the uniformity of geological and environmental factors. However, accordingly to our study on the reach scale, patterns in assemblages among habitats were similarly weak. Habitats, such as POM and Sand had less taxa than remaining habitats and were distinct, but similar among each other. These patterns again might be a result of higher similarity of patches located within slow waters, in comparison to water channels located within riffles. However, in terms of taxa associations to certain habitats, we found slightly more taxa with higher IndVal associated to certain habitat types. Habitat based model assume that there is an association between the species and patch structure at various scales (Kolasa 1989). The scale at which such association is manifested is determined by the generalist or specialist nature of individuals. Therefore, the more generalist species, the larger will be the fragment of hierarchical habitats to which it responds. Whereas, more specialist species will use smaller fragment of hierarchical habitats. Consequently, this could explain why at smaller scale, there were more associations to given habitats, than at the catchment scale.

Another explanation for higher substratum associations of species on the reach scale can be related to the lower taxonomic level of identification of samples taken on the reach than at the catchment scale. Although, in general higher taxonomic identification of community distribution patterns does not differ in comparison to lower species level (Corkum 1989; Kay et al. 1999), taxon resolution seems to have an effect on species-specific substratum associations (Schröder 2013). Therefore, it is possible that results of substratum associations from the reach scale were more distinctive due to lower taxonomic resolution used in this study. Nevertheless, both scales do not seem satisfactory in terms of detecting strong patterns in macroinvertebrate assemblages in studying patches as a source of variation. In general, concepts of environmental filters (Poff 1997; Statzner et al. 2004) and mesohabitats (Pardo and Armitage 1997) developed for reach scales in perennial water courses allowed to distinguish different patches as distinct units having ecological significance. We demonstrated that although environmental filter indeed filtered out species possessing distinctive biological traits, it was more apparent at the catchment scale, independently on habitat filter. Further, we demonstrated that habitat filter on the reach scale was slightly stronger but still not as strong as previously described for perennial streams (Phillips 2003; Kubosova et al. 2010). It appears, that studies with the use of biota assemblages at intermittent rivers do not necessarily need to be stratified through the patch type. In this case, patch is not a good descriptor of variation.

8.2. Functional measures (rate of production and respiration)

Functional measures of benthic community production and respiration were far more promising in terms of studying patches, than structural measures of benthic assemblages. Primary Production as well as Respiration were greater across habitats than within habitats. Because such consistency and similarity in function are inherent components of patch definition, our study demonstrated that benthic metabolism can be used to define in-stream patches. Furthermore, partitioning of stream into distinct metabolic patches allows for better prediction of the environmental controls which exert on each patch. Such knowledge permits for more efficient use of benthic metabolism as an indicator of an ecosystem health and also for studying functional heterogeneity. For example, Fellows et al. (2006) demonstrated that separation of in-stream habitats into cobble and sediment increased the explanatory power of linear model to predict the benthic metabolism measures as descriptors of disturbance gradient. Additionally, defining in-stream patches allows to predict changes in metabolism along the stream continuum. It is based on spatial arrangement and proportion of patches at each river segment, shaped by hierarchical large-scale factors. According to our study, environmental parameters influencing community metabolism were patch-specific, which strongly confirm the usefulness of this scale in studying variability. Clapcott and Barmuta (2010) demonstrated that benthic production and respiration at the same patch were weakly correlated with other benthic community measures, such as bacterial productivity. This indicates that metabolic entity of a patch might be dependent on functional scales of organization (individual, populations, communities). Better definition of metabolic organization in relation to this functional resolution would allow for better correlation of environmental factors, which influence the metabolism at each scale of organization. Subsequently, it would allow to more efficiently target restoration efforts, as well as measures of the ecosystem health to detect disturbance. Although, our study is limited to only one temporal scale (stable flow), it is possible that at broader spatial scales temporal variability can override in-stream variability (e.g. Clapcott and Barmuta 2010).

8.1.2. Functional measures (Stable isotopes)

It appears that patch as a unique metabolic entity will also influence the stream food web and nutrient cycling in the stream. Thus, according to our predictions, patch at the reach scale should be regarded as distinctive units of carbon subsidies to the ecosystem and should be responsible for different rates of nutrient supply. However, inferring from stable carbon isotope studies, patches at this scale were not a good predictor of variation and failed to define patches as distinct sources of organic matter.

Autochthonous and terrestrial subsidies of carbon to the ecosystem vary in its isotopic signatures. Further, isotopic fractionation is influenced by various environmental factors acting locally, but also on a larger scale (Finlay et al. 1999; Finlay, 2004; Rosenfeld and Roff 1992). Therefore, similarly as metabolism measures, isotopic signatures integrate the response of various factors over spatial and temporal scales (Lefebvre et al. 2007). Current work demonstrated that isotopic signatures among habitats and among individual consumers, at the reach scale, yielded significant overlap. Such high variation among stream habitats in isotopic signatures hampered to identify patches as distinctive units of carbon subsidies. High baseline variation in isotopic signatures among different stream compartments was already demonstrated (Cabana and Rasmussen 1996; Rosenfeld and Roff 1992; Hamilton et al. 2001). This is because factors, which decide about the isotopic fractionation vary even at very small scales and even biofilm structure or dominant type of plant or presence of microorganisms might introduce high source of variation within the same patch type (Rezanka and Hershey 2003; Mulholland et al. 2000b). Consumers associated with given patches will rarely rely on solely one patch, but more likely will be also dependent on links with surrounding patches (Closs and Lake 1994). This was demonstrated by high overlap in isotopic signatures existing among taxa belonging to the same functional feeding group (FFG). This clearly indicates that the concept of the "trophic position" might not be applicable to this ecosystem, due to resource use by consumers being more flexible than previously believed. Therefore, food web descriptors at the reach scale should not be defined by the patch under study, nor by the animals associated to that patch.

In turn, at the stream scale, other factors, operating at slightly bigger scale overrule the within reach isotopic variation, what allowed to detect significant differences in isotopic signatures among patches (especially epilithon patches). Significant differences were noticeable among patches, at streams influenced by different land use, riparian shading and groundwater effluent. Such finding is important in terms of using stable isotopes for detection of disturbance to the ecosystem. However, according to the present study, the effect of environment or disturbance to individual patch can only be detected at the level of streams. Therefore isotopic signatures of different patches, but mostly epilithon, are proposed to be studied at stream or higher scale, where abiotic factors were demonstrated to overrule the within a reach variations in isotopic signatures, which obscure the differentiation of unique patches. This also entails the response of biota, which isotopic signatures were demonstrated to be highly influenced by the site.

Concluding, patches as a source of variation were best defined by functional measures: benthic community metabolism at the reach scale and source of organic matter at the stream scale. It might appear contradictory at first that patches were defined as different metabolic units, but could not be defined in the context of distinct carbon subsidies. This is probably due to the complex process involved in isotopic fractionation, which depends not only on many environmental factors acting with different intensities at very small to large scales, but also on the biochemical pathways of photosynthesis of different plants and aquatic algae (Maberly et al. 1992).

Although environmental parameters also influence differently the rate of production and respiration of organisms living within a community, they cannot influence their autotrophic or heterotrophic character. Therefore, metabolism seems to be a more straightforward process, where the boundary at which autotrophy or heterotrophy prevails, is clearer and depends more on the dominance of particular autotrophic or heterotrophic organisms within a patch. This study also coincides with the idea of hierarchical patch dynamics (Poole et al. 2002), where processes operating at various multiple scales define, destroy or alter patch characteristics.

8.1.3. Nutrient cycling by animals

Nutrient excretion is the most direct way by which animals support nutrients to the ecosystem (Vanni, 2002). These nutrients are further taken up by primary producers or heterotrophic organisms and become a base for the trophic food webs. In aquatic systems, N and P are the most important nutrients because they can limit primary production. Therefore the ratios at which they are excreted by animals can directly decide about N or P limitation (Elser et al. 1988, Sterner and Elser, 2002) as well as can change species composition of algae (Smith, 1983). For this reason the quantification of such ratios can help to understand if animal communities, distributed in a heterogeneous way can be responsible for the hotspots in nutrient recycling. Because species-specific attributes (size, phenology, body mass) strongly influence their excretion rates, my primary hypothesis assumed that patchy distribution of species within a stream will give rise to biochemical hotspots within that stream. Unfortunately, lack of sufficient individuals within each patch impeded to quantify among patch variability in nutrient excretion. However, it was possible to quantify among stream aggregated excretion rates. Current results demonstrated that excretion is mainly driven by species identity and less so by site. Phylogenetic constraints and body composition of consumers were indeed demonstrated to play important role in governing excretion of components (Evans-White et al. 2005; Frost et al. 2003; Elser et al. 1996). Differences in species-specific excretion rates, particularly associated with extremely high P excretion of Trichoptera, were responsible for high among-site variability in aggregated nutrient excretion with considerably high differences in N:P ratios among sites. My results thereby indicate, that differences in abundance and species composition confined to different sites will influence nutrient recycling. Nutrient dynamics influenced by excretion of organisms is usually associated with dominant taxa biomass (Caraco et al. 1997). Here, Trichoptera group was also dominant at the site with the highest excreted P, however, such high P excretion at this site was also associated to immensely high Trichoptera-specific P excretion. Observed differences in N:P ratios among sites (especially F. Benemola and remaining sites) are probably a consequence of a combination of species specific excretion rates, species biomass and richness. Although, it was impossible to measure such pattern on the reach scale, I believe that weak confinement of macroinvertebrates to their habitats at the reach scale would not give a rise to significant nutrient hotspots at this scale. Nevertheless I cannot exclude the possibility of existing some local hotspots observed at the microscale within one patch (Atkinson et al. 2013; McIntyre et al. 2008).

Measuring of nutrient excretion allowed to combine what we know about structural organization of communities with their functional role and this combined knowledge contributed to the understanding of the effect that biota can exert on ecosystem functions. Given that most of the studied streams are oligotrophic such knowledge can have important consequences for the local nutrient dynamics. For example, McManamay et al. (2011) calculated that excretion from macroinvertebrate consumers supported 1.5 to 2 % of N and 12 to 119 % of P, greatly contributing to nutrient supply for autotrophs, especially during times of the year when nutrient concentrations were low.

8.2. Testing the RCC theory and food web structure on small headwater intermittent streams

River Continuum Concept (RCC) represents a holistic approach where physical habitat conditions along the river continuum will entail important responses of biota at the population, community and ecosystem level (Vannote et al. 1980). However, RCC was tested on mostly large, temperate river systems, constantly flowing over the entire course, and therefore not all streams and river systems can be accommodated within this framework (Wiley et al. 1990; Lake et al. 1986; Cushing et al. 1995). Ward and Stanford (1983) noted that basic ecological theories might not accurately represent river systems with certain level of impoundment or regulation. These authors developed discontinuum concept, which addresses the problem of streams where disruptions in ecological processes impede testing of fundamental ecological theories. One component of RCC predicts the patterns of metabolism along the river continuum and assumes that the importance of autochthonous sources of organic matter to the stream should increase in downstream direction (Minshall 1995). Therefore small headwater streams (1-3 order) with dense canopy cover restrict light penetration and will limit autotrophic production. Further, trophic food base will be mostly supported by terrestrial inputs of organic matter to these streams. It is assumed that most food sources for consumers at headwater streams will be supported by allochthonous carbon (Hicks 1997; Finlay 2001; Hall et al. 2001). However, recent development in stable isotope techniques demonstrated that at many highly shaded headwater streams autochthonous production, in fact, constituted a major carbon sources for consumers (Pingram et al. 2012; Thorp and Delong 2002). Similar finding was also showed for small tropical headwater reaches (March and Pringle 2003; Lau et al. 2009; Yam and Dudgeon 2005). Far less is known about the riparian input and the importance of basic carbon subsidies on trophic food webs in small temporary Mediterranean catchments. In general, riparian input to Mediterranean streams is less obvious as in their temporary counterparts, and majority of fauna is dependent on autochthonous production (Bunn et al. 2003). There are also some records from intermittent streams in Australia, which illustrated a contradictory pattern (Reid et al. 2008).

Our study demonstrated that headwater streams located more upstream had higher riparian cover and slightly higher percentage of in-stream habitats of terrestrial origin, in comparison to reaches located downstream. The stable isotope studies, performed on reaches with distinct percentage of riparian cover, demonstrated that autochthonous food sources predominated regardless of shading influence. The following might explain this pattern: 1) Mediterranean streams located in dry Algarve region have general sparse woody vegetation, that already limits the amount of material entering the stream. Further, limited riparian input diminishes the retentive capacity of the stream and reduces the overall amount of detritus available. Finally, the peak of the allochthonous input to Mediterranean streams normally occurs during high discharge and low temperatures (contrary to southern hemisphere where the peak of allochthonous input occurs during spring and summer) (Gasith and Resh, 1999). As a result, the amount of detritus entering the stream is limited and retained only briefly, and the limited contact time between presence of shredders and detritus causes that majority of terrestrial input which enters stream is quickly transported downstream and may even leave the system before is being processed; 2) High summer temperatures, low discharge amplitude and long days with clear sky in Mediterranean regions favour development of algae, which contrary to detritus, is always available for consumers (Gasith and Resh 1999). Finally, 3) The terrestrial inputs were demonstrated to be of general lower quality than autochthonous sources. This particularly pertains to the Mediterranean streams, where many types of the terrestrial litter which fall into the stream, constitute an unpalatable form for macroinvertebrates.

This study is consistent with other studies on temporary Mediterranean streams which demonstrated prevalence of scrapers and collector-gatherers, and autochthonous material as a principal source of organic matter for stream consumers (Alvarez and Pardo 2009; Rossi-Marshall et al. 2016; Vannucchi et al. 2013). Accordingly to the my results, small headwater intermittent streams of the studied catchment did not follow the typical RCC concept, with consequences for the food webs dynamics in this ecosystem. Contrasting findings were demonstrated for intermittent streams in Australia, where stream food web was primary dependent on detrital resources (Closs and Lake 1994). The importance of detritus in these studies was demonstrated to be an adaptive response of benthic communities to frequent disturbances such as events of flooding and drying (Boulton and Lake 1992). This is because during floods, attached algae are scoured from substrate, whereas detritus, according to these studies, was always available, even after severe floods. It constituted a non-limiting food source for consumers. Detrital-based food webs in upland streams, characterized by weak top-down interactions reflect a long-term resilience to flood disturbance. Drawing from above, benthic community at the present study may rely on autochthonous production and thus represents an example of low resilience to flooding disturbance. In the streams receiving minimal input of terrestrial detritus, such as the streams studied here, recolonization of macroinvertebrates after winter flood, needs to follow the colonization of algae (Siegfried and Knight 1977). This pattern also matches the visual observation for these streams, where macroinvertebrates emergence coincides with the first algae development. This might have further implications for the food web and predator-consumer interactions. For example, top predators in these streams will have larger impact in regulating algal biomass, a common pattern for lowland streams (Power 1992).

Intermittent streams were demonstrated to be dominated by omnivory and dietary generalism (Reid et al. 2008; Closs and Lake 1994; St Clair 1994) as an adaptation to changes in resource supply. Our study was limited to only benthic invertebrates, and therefore lack of higher consumers, such as fish impedes to conclude whether omnivory is indeed a most common tactic. However, this study demonstrates that majority of consumers follow a local abundance of a resource and the selectivity is only exhibited at the small habitat scale, reflected in differences in epilithon nutritional structure. This implies that only a portion of food, with overall better nutritional quality, will be processed and transported to higher trophic levels.

Interestingly, our study does not support the common notion that FFG classification reflect trophic structure and resource assimilation by consumers. For this reason, FFG should not be applied in this ecosystem to infer about trophic relations.

In summary, the streams studied here follow a longitudinal erosional-depositional pattern reflected in differences in temperature, conductivity, riparian cover and habitat heterogeneity between upstream and downstream reaches. Nevertheless, these streams do not follow the RCC tenet in terms of longitudinal changes in stream metabolism and food web dynamics. Autotrophy prevails even at highly shaded streams and limited allochthonous input to this system imply the reliance on autochthonous carbon subsidies. Food web based on autochthonous subsidies is less resistant to disturbance, and subject to compensating measures, such as: 1) macroinvertebrate lag in recolonization, following algae appearance, and 2) consumers follow a local abundance of a resource and exhibit local selectivity for high nutritional value of basal resource.

Although autotrophic sources for consumers prevail in this ecosystem, terrestrial subsidies can also be important on the patch scale and for some individual taxa. Also, patches of terrestrial leaf packs have higher microbial activity and can serve as an important spots of nutrient recycling in the reaches were nutrients are temporary limited.

8.3. Implications for biomonitoring

Aquatic ecosystems worldwide suffer from increasing degradation which disturb its natural ecological state. Main threats to the aquatic systems are related to hydrological regime alteration, physical habitat modifications, water pollution and variety of other human activities which directly or indirectly affect the ecological status of these systems. Such examples of water deterioration resulted in many local, regional and international initiatives to control and assess aquatic resources and restore degraded water bodies. Development of effective monitoring assessment programs for a long time has been a challenge for many ecologists and managers. It is generally agreed that an effective assessment method, which will evaluate the functional value of a given ecosystem, requires a holistic ecosystem-based approach (Verdonshot 2000; Diaz et al. 2004). Such approach should focus on individual organisms and their habitats, but also should consider factors, which structure these components at various temporal and spatial scales (Bunn and Davis 2000). It is generally assumed that the first step for developing an ecological assessment framework is to delineate ecological regimes or stream types and then deliver areas of patches, which can be quantitatively defined according to their physical structure and ecological process (Verdonshot 2000). The first pass of the present work was to deliver boundaries for stream classification. As demonstrated by Sanchez-Montoya et al. (2007), temporary streams were identified as the most heterogeneous ecotype and further refinement of this ecotype is necessary (Morais et al. 2004; Munne and Prat 2004). Current WFD classification in South Portuguese rivers is applied uniformly for the entire region characterized by calcareous type of geology. Our study clearly demonstrated, that such classification does not adequately reflect the heterogeneity of this ecosystem and it should be further refined into upstream and downstream sites with the additional reference to the period of sampling (corresponding to cluster X-upstream and cluster Y-downstream from Chapter 2). Additionally, including typology in our analysis demonstrated differences in water quality index among calcareous and non-calcareous stream types.

There is no single scale which would be appropriate for all of the ecological processes and structural organization of communities. The hierarchy of processes occurring in streams needs to be considered by the prism of the objective of the study, and therefore patterns of interests should be investigated considering spatial and temporal boundaries at which these patterns are the most perceived (Underwood and Chapman 1996).

As stated by Verdonshot (2000) in his review about the integrative ecological assessment methods: „Integrated ecological assessment is not always everything everywhere. Although termed catchment approach, it does not require knowing everything about the catchment, but rather knowing everything about the ecologically relevant interactions within the catchment in relations to the stream system functioning".

This work delivered a set of structural and functional measures related to patch, considered as a habitat unit and evaluated at which scales these indicators of ecosystem health are of the highest relevance, relatively to the patch. Patch as a source of variation was not well explained by the structural measures of benthic communities, at catchment, nor at the reach scale.

Rabeni et al. (2002) stated that channel units have distinct properties, which are not found at other spatial scales, and therefore can be used in management and restoration practices. Similarly, habitat unit was identified as the most appropriate scale to study heterogeneity and success of the restoration efforts. When applying rapid bioassessment protocols, some authors suggested stratifying sampling through the habitat type to decrease the variation among samples and improve comparisons among sites (Resh and Jackson 1993; Resh et al. 1995; Armitage et al. 1995). However, stratification is based on an assumption that biota preferences for certain type of habitat from the reach scale will be consistently translated to higher spatial scales. In this study, there was no strong pattern in macroinvertebrate structure. This only demonstrates that bioassessment programs, which collect invertebrates by stratification through the habitat type, will be only useful if the scale at which the patch is recognized as ecologically important is clearly specified. Similar conclusion can be drawn in relation to the restoration of the structural heterogeneity in streams. Lepori et al. (2005) demonstrated that diversity metrics of organisms at restored reaches were similar relatively to unrestored reach at patch and reach scale. Authors concluded that restoration efforts might be of little benefit to biodiversity if they are not targeted into appropriate scale of structural heterogeneity relevant for studied organisms. Therefore, if restoration measures are targeted for streams within Quarteira catchment, enhancing instream substrate heterogeneity might contribute little to overall diversity. However, it is also important to what type of organisms the restoration efforts are designated, as for example the response of fish can be different than macroinvertebrates (Peterson and Rabeni 2001).

Structural measures are easy to quantify and are widely accepted as the most common measures of the ecosystem health and water quality status (Bonada et al. 2006b). However, few authors recently questioned their usefulness in relation to overall ecosystem functioning (e.g. Brooks et al. 2002; Bunn and Davis, 2000). Brooks et al. (2002), pointed out that macroinvertebrate community structure have limited use in assessing ecosystem rehabilitation. These and other authors (Bunn and Davis 2000), indicated ecosystem level processes as better indicators of change at local scales. The main pitfalls of using macroinvertebrates as indicators of ecosystem health are related to high spatial and temporal variability within stream community (Heino et al. 2004) and insensitivity of community structure to detect change in some streams which followed restoration (Brooks et al. 2002). Bunn and Davis (2000) stated that changes in patterns do not always follow changes in processes and therefore solely structural measures should not be used to assess the stream integrity (Bunn et al. 1995).

As an alternative for structural measures, several authors have demonstrated the effectiveness of functional measures of ecosystem health (Bunn and Davis, 2000; Cardinale et al. 2002; Fellows et al. 2006; Fernandes et al. 2015 in Portugal). Ecosystem-level processes offer an integrated response to disturbances and are less variable over spatial and temporal scales (Bunn and Davis 2000). Bunn et al. (1999) successfully used benthic metabolism and stable isotopes measurements to develop simple predictive model of how these indicators will change with the reduction of riparian vegetation and land use activities. Morrissey et al. (2013) demonstrated that macroinvertebrates $\partial^{15}N$ were enriched at sites influenced by sewage input and these $\partial^{15}N$ values were correlated with traditional macroinvertebrate community metrics used in biomonitoring. Similar study by Di Lascio et al. (2013) showed that isotopic signals (^{15}N and ^{13}C) were efficient to detect changes in aquatic communities affected by sewage inputs, even when species abundance and community structure remained unaffected.

Large majority of studies, which evaluated restoration efforts targeted to increase habitats heterogeneity demonstrated no difference in macroinvertebrate diversity at restored streams (e.g. Palmer et al. 2010). On the other hand, ecosystem-level processes were more responsive to changes in habitat heterogeneity (Cardinale et al. 2002).

Accordingly to our study the most consistent measures of the ecosystem health, which could be applied to study patches were metabolism measurements at the reach scale and isotopic signatures at the stream scale. Next step in the investigation should focus on establishing a reference values for these two approaches, for undisturbed systems, and incorporate these measures into biomonitoring guidelines. Additionally, studies are needed to set the reference points for isotopic differentiations, at stream scale, in order to distinguish variation among isotopic signatures originated from natural occurrences (i.e. groundwater effluence) from the variation introduced due to anthropogenical activities (reduction in riparian cover, land-use change). Further investigation of the factors which control the variability of basal resource, but also among consumers, will allow us to determine how much of the variation is associated to each of the disturbance. Additionally, it is worth to note that if consumers are used as indicators of ecosystem health, one needs to be aware of large variability among species response to basal food resources. The most representative taxa should display consistent response in its feeding habitats relatively to the site in order to avoid confounding the response to the disturbance with the response of biota to site-specific factors.

Following disturbance, patches have been shown to be the most appropriate unit used when evaluating biotic recovery. As such, this study represents an important step towards development of better biomonitoring tools as well as evaluation of the restoration effort.

References

Acuña V, Datry T, Marshall J, Barceló D, Dahm CN, Ginebreda A and Palmer MA (2014) Why should we care about temporary waterways?. *Science* 343(6175): 1080-1081.

Acuna V, Giorgi A, Muñoz I, Uehlinger URA and Sabater S (2004) Flow extremes and benthic organic matter shape the metabolism of a headwater Mediterranean stream. *Freshwater Biology* 49: 960-971.

Aguiar FC, Ferreira MT and Pinto P (2002) Relative influence of environmental variables on macroinvertebrate assemblages from an Iberian basin. Journal of the North American Benthological Society 21(1): 43-53. DOI: 10.2307/1468298.

Alba Tercedor J, Jáimez-Cuéllar P, Álvarez M, Avilés J, Bonada N, Casas J, et al. (2002) Caracterización del estado ecológico de ríos mediterráneos ibéricos mediante el índice IBMWP (antes BMWP'). Limnetica 21(3-4): 175-185.

Allan D, Erickson D and Fay J (1997) The influence of catchment land use on stream integrity across multiple spatial scales. *Freshwater Biology* 37: 149–161.

Allan JD (1975) The distribution ecology and diversity of benthic insects in Cement Creek Colorado. *Ecology* 56: 1040-1053. DOI: 10.2307/1936145.

Álvarez M and Pardo I (2009) Dynamics in the trophic structure of the macroinvertebrate community in a Mediterranean, temporary stream. *Aquatic sciences* 71(2): 202-213. doi: 10.1007/s00027-009-9160-z

Angradi TR (1996) Inter-habitat variation in benthic community structure, function, and organic matter storage in 3 Appalachian headwater streams. *Journal of the North American Benthological Society* 15(1): 42-63.

APHA (2012) Standard Methods for the examination of water and wastewater. 20th ed, American Public Health Association, Washington DC

Araujo-Lima CARM, Forsberg BR, Victoria R and Martinelli L (1986) Energy sources for detritivorous fishes in the Amazon. *Science (Washington)* 234(4781): 1256-1257. doi: 10.1126/science.234.4781.1256

Arcagni M, Campbell LM, Arribére M, Kyser K, Klassen K, Casaux R, Miserendino ML and Ribeiro Guevara S (2013) Food web structure of Lake Moreno (Northwest Patagonia, Argentina) using C and N stable isotopes. *Limnologica* 43: 131–142. doi: 10.1016/j.limno.2012.08.009

Arcagni M, Rizzo A, Campbell LM, Arribére MA, Juncos R, Reissig M, Kyser K, Barriga JP, Battini M and Guevara SR (2015) Stable isotope analysis of trophic structure, energy flow and spatial variability in a large ultra oligotrophic lake in Northwest Patagonia. *Journal of Great Lakes Research* 41(3): 916-925. doi: 10.1016/j.jglr.2015.05.008

Argyroudi A, Chatzinikolaou Y, Poirazidis K and Lazaridou M (2009) Do intermittent and ephemeral Mediterranean rivers belong to the same river type? *Aquatic Ecology* 43(2): 465-476. doi: 10.1007/s10452-008-9176-9

Aristegi L, Izagirre O and Elosegi A (2010) Metabolism of Basque streams measured with incubation chambers. *Limnetica* 29: 301-310.

Armitage PD and Cannan CE (2000) Annual changes in summer patterns of mesohabitat distribution and associated macroinvertebrate assemblages. *Hydrological Processes* 14: 3161-3179.

Armitage PD and Pardo I (1995) Impact assessment of regulation at the reach level using macroinvertebrate information from mesohabitats. *Regul. Riv. Res. Managem.* 10: 147-158.

Armitage PD, Pardo I and Brown A (1995) Temporal constancy of faunal assemblages in 'mesohabitats' – application to management? *Arch. Hydrobiol*. 133: 367–383.

Arthington AH, Bernardo JM and Ilhéu M (2014) Temporary rivers: linking ecohydrology, ecological quality and reconciliation ecology. *River research and applications*. 30(10): 1209-1215.

Atkinson CL, Vaughn CC, Forshay KJ and Cooper JT (2013) Aggregated filter-feeding consumers alter nutrient limitation: consequences for ecosystem and community dynamics. *Ecology*. 94(6): 1359-1369.

Bae MJ, Chon TS and Park YS (2014) Characterizing differential responses of benthic macroinvertebrate communities to floods and droughts in three different stream types using a Self Organizing Map. *Ecohydrology* 7(1): 115-126. DOI: 10.1002/eco.1326.

Barmuta LA (1989) Habitat patchiness and macrobenthic community structure in an upland stream in temperate Victoria, Australia. *Freshwater Biology* 21(2): 223-236.

Barnard P and Ross E (2012) The Adult Trichoptera (Caddisflies) of Britain and Ireland. RES Handbook Volume 1, Part 17.

Beauger, A, Lair N, Reyes-Marchant P and Peiry JL (2006) The distribution of macroinvertebrate assemblages in a reach of the River Allier (France), in relation to riverbed characteristics. *Hydrobiologia* 571: 63–76. DOI:10.1007/s10750-006-0217-x.

Beche LA, Mcelravy EP and Resh VH (2006) Long-term seasonal variation in the biological traits of benthic-macroinvertebrates in two Mediterranean-climate streams in California, USA. *Freshwater Biology* 51(1): 56-75.

Bedoya D, Novotny V and Manolakos ES (2009) Instream and offstream environmental conditions and stream biotic integrity: importance of scale and site similarities for learning and prediction. *Ecological Modelling* 220: 2393-2406. DOI:10.1016/j.ecolmodel.2009.06.017.

Beisel J, Usseglio-polatera P, Thomas S and Moreteau J (1998) Stream community structure in relation to spatial variation : the influence of mesohabitat characteristics. *Hydrobiologia* 389: 73-88.

Beisel J, Usseglio-Polatera P, Thomas S and Moreteau J (2000) The spatial heterogeneity of a river bottom: a key factor determining macroinvertebrate communities. Springer, Netherlands, pp. 163-171.

Benstead JP, Cross WF, March JG, McDowell WH, Ramirez A and Covich AP (2010). Biotic and abiotic controls on the ecosystem significance of consumer excretion in two contrasting tropical streams. *Freshwater Biology* 55(10): 2047-2061.

Bergfur J, Johnson RK, Sandin L and Goedkoop W (2007) Assessing ecological integrity of boreal streams: a comparison of structural and functional responses. *Fundamental and Applied Limnology* 168: 113–125.

Bergfur J, Johnson RK, Sandin L and Goedkoop W (2009) Effects of nutrient enrichment on C and N stable isotope ratios of invertebrates, fish and their food resources in boreal streams. *Hydrobiologia* 628: 67–79.

Bernot MJ, Sobota DJ, Hall RO, Mulholland PJ, Dodds WK and Webster JR (2010) Inter-regional comparison of land-use effects on stream metabolism. *Freshwater Biology* 55: 1874–1890.

Biggs BJF and Close ME (1989) Periphyton biomass dynamics in gravel bed rivers: the relative effects of flows and nutrients. *Freshwater Biology* 22: 209–231.

Biggs BJF and Kilroy C (2000) Stream periphyton monitoring manual. Published for NZ Ministry for the Environment by NIWA, Christchurch: 76-91.

Blanchette ML, Davis AM, Jardine TD and Pearson RG (2014) Omnivory and opportunism characterize food webs in a large dry-tropics river system. *Freshwater Sciences* 33(1): 142-158. doi: 10.1086/674632

Bonada N, Doledec S and Statzner B (2007) Taxonomic and biological trait differences of stream macroinvertebrate communities between Mediterranean and temperate regions: implications for future climatic scenarios. *Global Change Biology* 13(8): 1658-1671. DOI: 10.1111/j.1365-2486.2007.01375.x.

Bonada N, Resh VH (2013) Mediterranean-climate streams and rivers: geographically separated but ecologically comparable freshwater systems. *Hydrobiologia* 719(1): 1-29.

Bonada N, Rieradevall M, Dallas H, Davis J, Day J and Figueroa R (2008) Multi-scale assessment of macroinvertebrate richness and composition in Mediterranean-climate rivers. *Freshwater Biology* 53: 772–788. DOI:10.1111/j.1365-2427.2007.01940.x.

Bonada N, Rieradevall M, Prat N and Resh VH (2006a) Benthic macroinvertebrate assemblages and macrohabitat connectivity in Mediterranean-climate streams of northern California Benthic macroinvertebrate assemblages and macrohabitat connectivity in Mediterranean-climate streams of northern California. *Journal of the North American Benthological Society* 25: 32–43.

Bonada NN, Prat VH, Resh B and Statzner B (2006b) Developments in aquatic insect biomonitoring: a comparative analysis of recent approaches. *Annual Review of Entomology* 51: 495–523.

Bott TL (2006) Primary production and community respiration. In: Hauer F.R. and Lamberti G.A. (eds.), Methods in Stream Ecology, 2nd edition, Academic Press, Burlington MA, 663-690.

Bott TL, Brock JT, Baattrup-Pedersen A, Chambers PA, Dodds WK, Himbeault KT, Lawrence JR, Planas D, Snyder E and Wolfaardt GM (1997) An evaluation of techniques for measuring periphyton metabolism in chambers. *Canadian Journal of Fisheries and Aquatic Sciences* 54: 715–725.

Bott TL, Brock JT, Cushing CE, Gregory SV, King D and Petersen RC (1978) A comparison of methods for measuring primary productivity in streams. *Hydrobiologia* 60: 3–12.

Bott TL, Brock JT, Dunn CS, Naiman RJ, Ovink RW and Petersen RC (1985) Benthic community metabolism in four temperate streams: an inter-biome comparison and evaluation of the river continuum concept. *Hydrobiologia* 123: 3–45.

Boulton AJ and Lake PS (1992) The ecology of two intermittent streams in Victoria, Australia. II. Comparisons of faunal composition between habitats, rivers and years. *Freshwater Biology* 27: 99–121.

Bowman MF, Chambers PA and Schindler DW (2005) Changes in stoichiometric constraints on epilithon and benthic macroinvertebrates in response to slight nutrient enrichment of mountain rivers. *Freshwater Biology* 50(11): 1836-1852.

Boyero L (2003a) The effect of substrate texture on colonization by stream macroinvertebrates. *Annales de Limnologie - International Journal of Limnology* 39: 211-218.

Boyero L (2003b) The quantification of local substrate heterogeneity in streams and its significance for macroinvertebrate assemblages. *Hydrobiologia* 499(1): 161-168.

Brittain JE (1990) Life history strategies in Ephemeroptera and Plecoptera. In: Campbell IC (ed.) Mayflies and Stoneflies: Life History and Biology. Kluwer Academic Publishers: Dordrecht, 1-12.

Brooks SS, Palmer MA, Cardinale BJ, Swan CM and Ribblett S (2002) Assessing stream ecosystem rehabilitation: limitations of community structure data. *Restoration Ecology* 10: 156–168.

Brosse S, Giraudel JL and Lek S (2001) Utilisation of non-supervised neural networks and principal component analysis to study fish assemblages. *Ecological Modelling* 146: 159-166. DOI: 10.1016/S0304-3800(01)00303-9.

Brown BL (2003) Spatial heterogeneity reduces temporal variability in stream insect communities. *Ecology Letters* 6: 316–325. doi:10.1046/j.1461-0248.2003.00431.x.

Bunn SE and Davies PM (2000). Biological processes in running waters and their implications for the assessment of ecological integrity. Springer, Netherlands, pp. 61-70.

Bunn SE, Barton DR, Hynes HN, Power G, Pope MA (1989) Stable isotope analysis of carbon flow in a tundra river system. *Canadian Journal of Fisheries and Aquatic Sciences* 46(10): 1769-1775. doi: 10.1139/f89-224

Bunn SE, Davies PM and Mosisch TD (1999) Ecosystem measures of river health and their response to riparian and catchment degradation. *Freshwater Biology* 41: 333-345.

Bunn SE, Davies PM and Winning M (2003) Sources of organic carbon supporting the food web of an arid zone floodplain river. *Freshwater Biology* 48: 619–635.

Bunn SE, Lonergan NR and Kempster MA (1995) Effects of acid washing on stable isotope ratios of C and N in penaeid shrimp and seagrass: implications for food-web studies using multiple stable isotopes. *Limnology and Oceanography* 40: 622–625

Burns A and Walker KF (2000) Biofilms as food for decapods (Atyidae, Palaemonidae) in the River Murray, South Australia. *Hydrobiologia* 437(1-3): 83-90. doi: 10.1023/A:1026555501638

Busch DE and Fisher SG (1981) Metabolism of a desert stream. *Freshwater Biology* 11: 301-307.

Cabana G and Rasmussen JB (1996) Comparison of aquatic food chains using nitrogen isotopes. *Proceedings of the National Academy of Sciences of the USA* 93: 10844–10847.

Caraco NF, Cole JJ, Raymond PA, Strayer DL, Pace ML, Findlay SEG and Fischer DT (1997) Zebra mussel invasion in a large, turbid river: phytoplankton response to increased grazing. *Ecology*. 78: 588–602.

Cardinale BJ, Palmer MA, Swan CM, Brooks S and Poff NL (2002) The influence of substrate heterogeneity on biofilm metabolism in a stream ecosystem. *Ecology* 83: 412–422.

Chakona A, Phiri C, Magadza CH and Brendonck L (2008) The influence of habitat structure and flow permanence on macroinvertebrate assemblages in temporary rivers in northwestern Zimbabwe. *Hydrobiologia* 607(1): 199-209.

Chaves ML, Chainho PM, Costa JL, Prat N and Costa MJ (2005) Regional and local environmental factors structuring undisturbed benthic macroinvertebrate communities in the Mondego River basin Portugal. *Archiv für Hydrobiologie* 163: 497–523. DOI:10.1127/0003-9136/2005/0163-0497.

Chaves ML, Costa JL, Chainho P, Costa MJ and Prat N (2006) Selection and validation of reference sites in small river basins. *Hydrobiologia* 573(1): 133-154.

Cheng L, Lek S, Lek-Ang S and Li Z (2012) Predicting fish assemblages and diversity in shallow lakes in the Yangtze River basin. *Limnologica* 42: 127-136. DOI: 10.1016/j.limno.2011.09.007.

Chicharo LMZ, Leitão F, Máximo I, Ben Hamadou R, Moreira da Silva M, Furtado A (2010) Caracterização Sazonal da Qualidade Ecológica da Água das Ribeiras do Algarve: Ribeira de Quarteira/Algibre e Ribeira da Seixe. ICCE-International Center for Coastal Ecohydrology, Faro

Chicharo LMZ, Leitão F, Máximo I, Hamadou RB, Moreira da Silva M (2009) Caracterização da Qualidade Ecológica da Água das Ribeiras do Algarve-Macroinvertebrados bentónicos como bioindicadores. *Relatório de Monitorização Relativo ao ano de 2009*.

Chon TS. 2011. Self-organizing maps applied to ecological sciences. *Ecological Informatics* 6(1): 50-61. DOI: 10.1016/j.ecoinf.2010.11.002.

Clapcott JE and Barmuta LA (2010) Metabolic patch dynamics in small headwater streams: exploring spatial and temporal variability in benthic processes. *Freshwater Biology* 55(4): 806-824.

Clarke A, Mac Nally R, Bond N and Lake PS (2010) Flow permanence affects aquatic macroinvertebrate diversity and community structure in three headwater streams in a forested catchment. *Canadian Journal of Fisheries and Aquatic Sciences* 67(10): 1649-1657.

Clarke KR, Warwick RM (2001) Change in Marine Communities: An Approach To Statistical Analysis and Interpretation, 2nd edition PRIMER-E Ltd, Plymouth, U.K.

Closs GP and Lake PS (1994) Spatial and temporal variation in the structure of an intermittent-stream food web. *Ecological Monographs* 64: 1–21.

Cobb DG, Galloway TD and Flannagan JF (1992) Effects of Discharge and Substrate Stability on Density and Species Composition of Stream Insects. *Canadian Journal of Fisheries and Aquatic Sciences* 49: 1788–1795: DOI:10.1139/f92-198.

Collier KJ, Ilcock RJ and Meredith AS (1998) Influence of substrate type and physico-chemical conditions on macroinvertebrate faunas and biotic indices of some lowland

Waikato New Zealand streams. *New Zealand Journal of Marine and Freshwater Research* 32: 1–19. DOI: 10.1080/00288330.1998.9516802.

Corkum LD (1989) Patterns of benthic inverte- brate assemblages in rivers of northwestern North America. *Freshwater Biology* 21: 191–205

Cortes RMV (1992) Seasonal pattern of benthic communities along the longitudinal axis of river systems and the influence of abiotic factors on the spatial structure of those communities. *Archiv für Hydrobiologie* 126: 85-103.

Cortes RMV, Hughes SJ, Varandas SGP, Magalhães M and Ferreira MT (2009) Habitat variation at different scales and biotic linkages in lotic systems: consequences for monitorization. *Aquatic Ecology* 43(4): 1107-1120.

Cummins KW and Lauff GH (1967) The Influence of Substrate Particle Size on the Microdistribution of Stream Macrobenthos. *Hydrobiologia* 34: 145-181. DOI: 10.1007/BF00141925.

Cushing CE, Cummins KW and Minshall GW (1995) River and Stream Ecosystems of the World. Elsevier, Amsterdam.

Datry T, Arscott DB and Sabater S (2013) Recent perspectives on temporary river ecology. *Aquatic Science* 73: 453. https://doi.org/10.1007/s00027-011-0236-1

Datry T, Corti R, Claret C et al. (2011) Flow intermittence controls leaf litter breakdown in a French temporary alluvial river: the "drying memory". *Aquatic Science* 73: 471. https://doi.org/10.1007/s00027-011-0193-8

Datry T, Larned ST and Tockner K (2014a) Intermittent rivers: a challenge for freshwater ecology. *Bioscience*. 64 (3): 229-235

Datry T, Larned ST, Fritz KM, Bogan MT, Wood PJ, Meyer EI and Santos AN (2014b) Broad-scale patterns of invertebrate richness and community composition in temporary rivers: effects of flow intermittence. *Ecography*. 37(1): 94-104.

De Caceres M, Legendre P (2009) Associations between species and groups of sites: indices and statistical inference. *Ecology* 90: 3566–3574.

Death RG and Winterbourn MJ (1995) Diversity patterns in stream benthic invertebrate communities: the influence of habitat stability. *Ecology* 76: 1446-1460.

Death RG, Dewson ZS and James AB (2009) Is structure or function a better measure of the effects of water abstraction on ecosystem integrity?. *Freshwater Biology* 54(10): 2037-2050.

Dewson ZS, James AB and Death RG (2007) A review of the consequences of decreased flow for instream habitat and macroinvertebrates. *Journal of the North American Benthological Society*. 26(3): 401-415.

di Lascio A, Rossi L, Carlino P, Calizza E, Rossi D and Costantini ML (2013) Stable isotope variation in macroinvertebrates indicates anthropogenic disturbance along an urban stretch of the river Tiber (Rome, Italy). *Ecological Indicators* 28: 107-114.

Diaz RJ, Solan M and Valente RM (2004) A review of approaches for classifying benthic habitats and evaluating habitat quality. *Journal of Environmental Management* 73(3): 165-181.

Dieterich M, Anderson NH and Anderson T (1997) Shredder-collector interactions in temporary streams of western Oregon. *Freshwater Biology* 38: 387-393. doi: 10.1046/j.1365-2427.1997.00252.x

Dodds WK (1991) Micro-environmental characteristics of filamentous algal communities in flowing freshwaters. *Freshwater Biology* 25: 199-209.

Dodds WK and Brock J (1998) A portable flow chamber for in situ determination of benthic metabolism. *Freshwater Biology* 39: 49-59.

Dodds WK, Collins SM, Hamilton SK, Tank JL, Johnson S, Webster JR, et al (2014) You are not always what we think you eat: selective assimilation across multiple whole-stream isotopic tracer studies. *Ecology* 95(10): 2757-2767.

Dolédec S, Olivier JM and Statzner B (2000) Accurate description of the abundance of taxa and their biological traits in stream invertebrate communities: effects of taxonomic and spatial resolution. *Archiv für Hydrobiologie* 148(1): 25-43.

Douglas MM and Lake PS (1994) Species richness of stream stones: an investigation of the mechanisms generating the species- area relationship. *Oikos* 69(3): 387-396.

Douglas MM, Bunn SE and Davies PM (2005) River and wetland food webs in Australia's wet-dry tropics: general principles and implications for management. *Marine and Freshwater Research* 56: 329–342. doi: 10.1071/MF04084

Downes BJ, Lake PS and Schreiber ESG (1993) Spatial variation in the distribution of stream invertebrates: Implications of patchiness for models of community organization. *Freshwater Biology* 30: 119–132.

Downes BJ, Lake PS and Schreiber ESG (1995) Habitat structure and invertebrate assemblages on stream stones: A multivariate view from the riffle. *Australian Journal of Ecology* 20: 502–514.

Duan X, Wang Z and Tian S (2008) Effect of streambed substrate on macroinvertebrate biodiversity. *Frontiers of Environmental Science and Engineering* 2: 122–128. doi:10.1007/s11783-008-0023-y.

Dufrene M, Legendre P (1997) Species Assemblages and Indicator Species: The need for a Flexible Asymmetrical Approach. *Ecological Monographs* 67: 345-366.

Elosegi A Díez J and Mutz M (2010) Effects of hydromorphological integrity on biodiversity and functioning of river ecosystems. *Hydrobiologia* 657: 199–215 doi:10.1007/s10750-009-0083-4.

Elser JJ and Urabe J (1999) The stoichiometry of consumer-driven nutrient recycling: Theory, observations, and consequences. *Ecology 80*(3): 735-751.

Elser JJ, Dobberfuhl DR, Mackay NA, Schampel JH (1996) Organism size, life history, and N: P stoichiometry toward a unified view of cellular and ecosystem processes. *BioScience*, *46*(9), 674-684. *BioScience.* 46(9): 674–684.

Enríquez S, Duarte CM, Sand-Jensen K and Nielsen SL (1996) Broad-scale comparison of photosynthetic rates across phototrophic organisms. Oecologia. 108: 197-206.

Evans-White M, Stelzer RS and Lamberti G (2005) Taxonomic and regional patterns in benthic macroinvertebrate elemental composition in streams. *Freshwater Biology*. 50(11): 1786–1799. doi:10.1111/j.1365-2427.2005.01455.x

Feio MJ, Ferreira J, Buffagni A, Erba S, Dörflinger G, Ferréol M, ... and Urbanič G (2014) Comparability of ecological quality boundaries in the Mediterranean basin using freshwater benthic invertebrates. Statistical options and implications. *Science of the Total Environment* 476: 777-784.

Fellows CS, Clapcott JE, Udy JW, Bunn SE, Harch BD, Smith MJ and Davies PM (2006) Benthic metabolism as an indicator of stream ecosystem health. *Hydrobiologia* 572(1): 71-87.

Feminella JW (2000) Correspondence between stream macroinvertebrate assemblages and 4 ecoregions of the southeastern USA. *Journal of the North American Benthological Society* 19(3): 442-461.

Fernandes I, Duarte S, Cassio F and Pascoal C (2015) Plant litter diversity affects invertebrate shredder activity and the quality of fine particulate organic matter in streams. *Marine and Freshwater Research* 66**:** 449-458.

Filipe AF, Lawrence JE and Bonada N (2013) Vulnerability of stream biota to climate change in mediterranean climate regions: a synthesis of ecological responses and conservation challenges. *Hydrobiologia* 719(1): 331-351. DOI: 10.1007/s10750-012-1244-4.

Finlay JC (2001) Stable carbon isotope ratios of river biota: Implications for carbon flow in river food webs. *Ecology* 82: 1052–1064.

Finlay JC (2004) Patterns and controls of lotic stable carbon isotope ratios. *Limnology and Oceanography* 49: 850– 861.

Finlay JC, Khandwala S and Power ME (2002) Spatial scales of carbon flow in a river food web. *Ecology* 83: 1845–1859.

Finlay JC, Power ME and Cabana G (1999) Effects of water velocity on algal carbon isotope ratios: implications for river food web studies. *Limnology and Oceanography* 44: 1198–1203. doi: 10.4319/lo.1999.44.5.1198

Forman RTT and Gordon M (1986) Landscape Ecology. John Wiley and Sons, New York. 640pp.

France RL (1995) Critical examination of stable isotope analysis as a means for tracing carbon pathways in stream ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences* 52: 651–656.

France RL (1996a) Absence or masking of metabolic fractionations of 13C in a freshwater benthic food web. *Freshwater Biology* 36(1): 1-6. doi: 10.1046/j.1365-2427.1996.00052.x

France RL (1996b) Carbon-13 conundrums: limitations and cautions in the use of stable isotope analysis in stream ecotonal research. *Canadian Journal of Fisheries and Aquatic Sciences* 53(8): 1916-1919. doi: 10.1139/f96-121

Frissell CA, Liss WJ, Warren CE and Hurley MD (1986) A hierarchical framework for stream habitat classification: viewing streams in a watershed context. *Environmental Management* 10: 199-214.

Frost PC, Tank SE, Turner MA and Elser JJ (2003) Elemental composition of littoral invertebrates from oligotrophic and eutrophic Canadian lakes. *Journal of the North American Benthological Society.* 22(1): 51–62.

Fuss C and Smock L (1996) Spatial and temporal variation of microbial respiration rates in a blackwater stream. *Freshwater Biology* 36: 339-349.

Gallart F, Prat N, Garcia-Roger EM, Latrón J, Llorens P, Barberá GG and Froebrich J (2012) A novel approach to analysing the regimes of temporary streams in relation to their controls on the composition and structure of aquatic biota. *Hydrology and Earth System Sciences* 16(9): 3165-3182. DOI: 10.5194/hess-16-3165-2012.

García RA and Ferreras-Romero M (2008) Distribution patterns of Hydropsychids and Rhyacophilids species (Trichoptera) in a not regulated Mediterranean river (SW Spain). *Limnetica* 27: 227-238.

García-Roger EM, del Mar Sánchez-Montoya M, Gómez R, Suárez ML, Vidal-Abarca MR and Latron J (2011) Do seasonal changes in habitat features influence aquatic macroinvertebrate assemblages in perennial versus temporary Mediterranean streams? *Aquatic Sciences* 73: 567–579. DOI: 10.1007/s00027-011-0218-3.

García-Roger EM, Sánchez-Montoya MDM, Cid N, Erba S, Karaouzas I, Verkaik I, Demartini D (2013) Spatial scale effects on taxonomic and biological trait diversity of aquatic macroinvertebrates in Mediterranean streams. *Fundamental and Applied Limnology/Archiv fur Hydrobiologie* 183(2): 89-105.

Gasith A and Resh VH (1999) Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. *Annual review of ecology and systematics* 17(30): 51-81.

Gessner MO, Swan CM, Dang CK, McKie BG, Bardgett RD and Wall DH (2010) Diversity meets decomposition. *Trends in Ecology and Evolution* 25: 372–80.

Giraudel JL and Lek S (2001) A comparison of self-organizing map algorithm and some conventional statistical methods for ecological community ordination. *Ecological Modelling* 146(1): 329-339. DOI: 10.1016/S0304-3800(01)00324-6.

Giraudoux P (2013) pgirmess: Data analysis in ecology R package version 1.5.8. http://CRAN.R-project.org/package=pgirmess.

González JM, Basaguren A and Pozo J (2001) Life history and production of Caenis luctuosa (Burmeister) (Ephemeroptera Caenidae) in two nearby reaches along a small stream. *Hydrobiologia* 452(1-3): 209-215. DOI: 10.1023/A:1011918708334.

González-Pérez JA, Jiménez-Morilloa NT, de la Rosa JM, Almendros G and González-Vila FJ (2015) Pyrolysis-gas chromatography–isotope ratio mass spectrometryof polyethylene. *Journal of Chromatography A* 1388: 236–243. doi: 10.1016/j.chroma.2015.02.039

Gonzalez-Pinzon R, Haggerty R and Argerich A (2014) Quantifying spatial differences in metabolism in headwater streams. *Freshwater Sciences* 33(3): 798-811. doi:10.1086/677555

Graça MA, Pinto P, Cortes R, Coimbra N, Oliveira S, Morais M and Malo J. (2004) Factors Affecting Macroinvertebrate Richness and Diversity in Portuguese Streams: a Two-Scale Analysis. *International Review of Hydrobiology*. 89(2): 151-164.

Graça MAS, Ferreira RCF and Coimbra CN (2001) Litter processing along a stream gradient: the role of invertebrates and decomposers. *Journal of the North American Benthological Society* 20: 408-420.

Graça MAS, Fonseca DM and Castro ST (1989) The distribution of macroinvertebrate communities in two Portuguese rivers. Freshwater Biology 22: 297-308.

Grimm NB and Fisher SG (1984) Exchange between interstitial and surface water: implications for stream metabolism and nutrient cycling. *Hydrobiologia* 111: 219–228.

Grubbs SA (2011) Influence of flow permanence on headwater macroinvertebrate communities in a Cumberland Plateau watershed, USA. *Aquatic Ecology* 45(2): 185-195.

Guash H, Martí E and Sabater S (1995) Nutrient enrichment effects on biofilm metabolism in a Mediterranean stream. *Freshwater Biology* 33: 373-383.

Gulis V and Suberkropp K (2003) Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. *Freshwater Biology* 48: 123-134.

Gustafson EJ (1998) Quantifying landscape spatial pattern: what is the state of the art? *Ecosystems* 1: 143–156.

Hall RO, Likens GE and Malcolm HM (2001) Trophic basis of invertebrate production in 2 streams at the Hubbard Brook Experimental Forest. *Journal of the North American Benthological Society* 20: 432–447.

Hamilton SK and Lewis WM (1992) Stable carbon and nitrogen isotopes in algae and detritus from the Orinoco River floodplain, Venezuela. *Geochimica et Cosmochimica Acta* 56: 4237–4246.

Hamilton SK, Tank JL, Raikow DF, Wollheim WM, Peterson BJ and Webster JR (2001) Nitrogen uptake and transformation in a midwestern US stream: a stable isotope enrichment study. *Biogeochemistry* 54(3): 297-340.

Harper D, Everard M (1998) Why should the habitat-level approach underpin holistic river survey and management? *Aquatic Conservation* 8: 395–413.

Harrison SS and Harris IT (2002) The effects of bankside management on chalk stream invertebrate communities. *Freshwater Biology*. 47(11): 2233-2245.

Hawkins CP and Vinson MR (2000) Weak correspondence between landscape classifications and stream invertebrate assemblages: Implications for bioassessment. *Journal of the North American Benthological Society* 19: 501–517.

Heino J, Louhi P and Muotka T (2004) Identifying the scales of variability in stream macroinvertebrate abundance, functional composition and assemblage structure. *Freshwater Biology* 49: 1230-1239

Heino J, Muotka T, Mykrä H, Paavola R, Hämäläinen H and Koskenniemi E (2003) Defining macroinvertebrate assemblage types of headwater streams: implications for bioassessment and conservation. *Ecological applications* 13(3): 842-852.

Heino J, Muotka T, Paavola R, Hämäläinen H and Koskenniemi E (2002) Correspondence between regional delineations and spatial patterns in macroinvertebrate assemblages of boreal headwater streams. *Journal of the North American Benthological Society* 21(3): 397-413.

Hering D, Buffagni A, Moog O, Sandin L, Sommerhäuser M, Stubauer I, ... and Verdonschot P (2003) The development of a system to assess the ecological quality of streams based on macroinvertebrates–design of the sampling programme within the AQEM project. *International Review of Hydrobiology* 88(3-4): 345-361.

Hershkovitz Y, Gasith A (2013) Resistance, resilience, and community dynamics in mediterranean-climate streams. *Hydrobiologia* 719(1): 59-75.

Hicks BJ (1997) Food webs in forest and pasture streams in the Waikato region, New Zealand: a study based on analyses of stable isotopes of carbon and nitrogen, and fish gut contents. *New Zealand Journal of Marine and Freshwater Research* 31: 651–664.

Holtgrieve GW, Schindler DE, Hobbs WO, Leavitt PR, Ward EJ, Bunting L and Lisac MJ (2011) A coherent signature of anthropogenic nitrogen deposition to remote watersheds of the northern hemisphere. *Science* 334(6062): 1545-1548.

Hothorn T, Bretz F and Westfall P (2008) Simultaneous inference in general parametric models. *Biometrical Journal*. 50(3): 346-363. doi: 10.1002/bimj.200810425

Hudon C and Bourget E (1983) The effect of light on the vertical structure of epibenthic diatom communities. *Botanica Marina* 26: 317-330.

Hughes SJ and Malmqvist B (2005) Atlantic Island freshwater ecosystems: challenges and considerations following the EU Water Framework Directive. *Hydrobiologia* 544(1): 289-297.

Hughes SJ, Santos J, Ferreira MT, Caraca R, Mendes AM. 2009. Ecological assessment of an intermittent Mediterranean river using community structure and function: evaluating the role of different organism groups. Freshwater Biology 54(11): 2383-2400. DOI: 10.1111/j.1365-2427.2009.02253.x.

Humphries P, Davies PE, Mulcahy ME (1996) Macroinvertebrate assemblages of littoral habitats in the Macquire and Mersey rivers, Tasmania: implications for the management of regulated rivers. *R&D Management* 12: 99–122.

INAG IP (2008) Tipologia de rios em Portugal Continental no âmbito da implementação da Directiva- Quadro da Água. I - Caracterização abiótica. Ministério do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional. Instituto da Água, I. P.

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

Ishikawa NF, Doi H and Finlay JC (2012) Global meta-analysis for controlling factors on carbon stable isotope ratios of lotic periphyton. *Oecologia* 170(2): 541-549 .

Jaarsma NG, De Boer SM, Townsend CR, Thompson R and Edwards ED (1998) Characterising food-webs in two New Zealand streams. *New Zealand Journal of Marine and Freshwater Research* 32: 271–286.

Johnson RK, Goedkoop W and Sandin L (2004) Spatial scale and ecological relationships between the macroinvertebrate communities of stony habitats of streams and lakes. *Freshwater Biology* 49(9): 1179-1194. DOI: 10.1111/j.1365-2427.2004.01262.x.

Johnson RK, Hering D (2010) Spatial congruency of benthic diatom, invertebrate, macrophyte, and fish assemblages in European streams. *Ecological Applications* 20(4): 978-992.

Kay WR, Smith MJ, Pinder AM, McRae JM, Davis JA and Halse SA (1999) Patterns of distribution of macroinvertebrate families in rivers of north-western Australia. *Freshwater Biology* 41: 299–316.

Kerezsy A, Balcombe SR, Tischler M and Arthington AH (2013) Fish movement strategies in an ephemeral river in the Simpson Desert, Australia. *Austral Ecology* 38(7): 798-808.

Kindt R, Coe R (2005) Tree diversity analysis: a manual and software for common statistical methods for ecological and biodiversity studies. World Agroforestry Centre.

Kohonen T (1982) Self-organized formation of topologically correct feature maps. *Biological Cybernetics* 43: 59-69. DOI: 10.1007/BF00337288.

Kohonen T. (2001) Self-Organizing Maps. 3rd ed. Springer: Berlin, 501.

Kolasa J (1989) Ecological Systems in Hierarchical Perspecitive: Breaks in Community Structure and Other Consequences. *Ecology* 70(1): 36-47.

Kubosova K, Brabec K, Jarkovsky J and Syrovatka V (2010) Selection of indicative taxa for river habitats: a case study on benthic macroinvertebrates using indicator species analysis and the random forest methods. *Hydrobiologia* 651: 101–114. DOI:10.1007/s10750-010-0280-1.

Labasque T, Chaumery C, Aminot A and Kergoat G (2004) Spectrophotometric Winkler determination of dissolved oxygen: re-examination of critical factors and reliability. *Marine Chemistry* 88: 53–60.

Lake PS (2003) Ecological effects of perturbation by drought in flowing waters. *Freshwater biology* 48(7): 1161-1172.

Lake PS, Barmuta LA, Boulton AJ, Campbell IC and St Clair RM (1986) Australian streams and northern Hemisphere stream ecology: comparisons and problems. *Proceedings of the Ecological Society of Australia* 14: 61–82.

Larned ST, Datry T, Arscott DB and Tockner K (2010) Emerging concepts in temporary-river ecology. *Freshwater Biology* 55(4): 717-738.

Lau DC, Leung KM and Dudgeon D (2008) Experimental dietary manipulations and concurrent use of assimilation-based analyses for elucidation of consumer–resource relationships in tropical streams. *Marine and Freshwater Research* 59(11): 963-970. doi: 10.1071/MF07213

Lau DC, Leung KM and Dudgeon D (2009a) Are autochthonous foods more important than allochthonous resources to benthic consumers in tropical headwater streams? *Journal of the North American Benthological Society* 28(2): 426-439. doi: 10.1899/07-079.1

Lau DC, Leung KM and Dudgeon D (2009b) What does stable isotope analysis reveal about trophic relationships and the relative importance of allochthonous and autochthonous resources in tropical streams? A synthetic study from Hong Kong. *Freshwater Biology* 54(1): 127-141. Doi: 10.1111/j.1365-2427.2008.02099.x

Lauridsen RB, Edwards FK, Cross WF, Woodward G, Hildrew AG and Jones JI (2014) Consequences of inferring diet from feeding guilds when estimating and interpreting consumer resource stoichiometry. *Freshwater Biology* 59: 1497-1508. doi: 10.1111/fwb.12361

Lefebvre S, Clement JC, Pinay G, Thenail C, Durand P and Marmonier P (2007) 15N-nitrate signature in low-order streams: effects of land cover and agricultural practices. *Ecological Applications* 17(8): 2333-2346.

Leigh C, Boulton AJ, Courtwright JL, Fritz K, May CL, Walker RH and Datry T (2015) Ecological research and management of intermittent rivers: an historical review and future directions. *Freshwater Biology* 61: 1181–1199

Leitão F, Hughes SJ, Atanasova N, Furtado A and Chicharo L (2014) Habitat-oriented sampling of macroinvertebrates affects the determination of ecological status in temporary Mediterranean River Systems. *River Research and Applications* 30: 1233–1247: DOI:10.1002/rra.2800.

Lek and Guégan JF (1999) Artificial neural networks as a tool in ecological modelling an introduction. Ecological Modelling 120: 65-73. DOI: 10.1016/S0304-3800(99)00092-7.

Lek S, Scardi M, Verdonschot PFM, Descy JP and Park YS (2005) Modelling Community Structure in Freshwater Ecosystems. Springer: Berlin, 518 pp.

Lepori F, Palm D and Malmqvist B (2005) Effects of stream restoration on ecosystem functioning: detritus retentiveness and decomposition. *Journal of Applied Ecology.* 42: 228–238.

Lepori F, Palm D, Brännäs E and Malmqvist B (2005) Does restoration of structural heterogeneity in streams enhance fish and macroinvertebrate diversity? *Ecological Applications* 15:2060-2071.

Lewis Jr WM, Hamilton SK, Rodriguez MA, Saunders III JF and Lasi MA (2001) Foodweb analysis of the Orinoco floodplain based the aquatic food web of a tropical lowland stream based on production estimates and stable isotope data. *Journal of the North American Benthological Society* 20:241-254. doi: 10.2307/1468319

Li F, Bae MJ, Kwon YS, Chung N, Hwang SJ, Park SJ, Park HK, Kong DS and Park YS (2013) Ecological exergy as an indicator of land-use impacts on functional guilds in river ecosystems. Ecological Modelling 252: 53-62. DOI: 10.1016/j.ecolmodel.2012.09.006.

Lorenz A, Feld CK and Hering (2004) Typology of streams in Germany based on benthic invertebrates: Ecoregions, zonation, geology and substrate. *Limnologica-Ecology and Management of Inland Waters*. *34*(4): 379-389.

Lorrain A, Paulet YM, Chauvaud L, Savoye N, Donval A and Saout C (2002) Differential δ13C and δ15N signatures among scallop tissues: implications for ecology and physiology. *Journal of Experimental Marine Biology and Ecology.* 275: 47–61

Maberly SC, Raven JA and Johnston AM (1992) Discrimination between 12 C and 13 C by marine plants. *Oecologia* 91: 481–492.

Mackay RJ (1992) Colonization by lotic macroinvertebrates: a review of processes and patterns. *Canadian Journal of Fisheries and Aquatic Sciences* 49(3): 617-628.

Macko SA, Fogel ML, Hare PE and Hoering TC (1987) Isotopic fractionation of nitrogen and carbon in the synthesis of amino acids by microorganisms. *Chemical Geology* 65: 79–92

March JG and Pringle CM (2003) Food web structure and basal resource utilization along a tropical island stream continuum, Puerto Rico. *Biotropica* 35: 84–93.

Marchant R, Barmuta LA, Chessman BC (1994) Preliminary study of the ordination and classification of macroinvertebrate communities from running waters in Victoria Australia. *Marine and Freshwater Research* 45(6): 945-962. DOI: 10.1071/MF9940945.

Marzolf ER, Mulholland PJ and Steinman AD (1994) Improvements to the diurnal upstream-downstream dissolved oxygen change technique for determining whole-stream metabolism in small streams. *Canadian Journal of Fisheries and Aquatic Sciences* 51: 1591-1599.

Mas-Martí E, García-Berthou E, Sabater S, Tomanova S and Muñoz I (2010) Comparing fish assemblages and trophic ecology of permanent and intermittent reaches in a Mediterranean stream. In: Global Change and River Ecosystems—Implications for Structure, Function and Ecosystem Services (Eds. Stevenson R. J. & Sabater S.). Springer, Netherlands, pp. 167-180.

McCune B and Mefford MS (2011) PcOrd multivariate analysis of ecological data version 6.06. MjM Software Design Gleneden Beach Oregon USA.

McCutchan Jr JH and Lewis Jr WM (2002) Relative importance of carbon sources for macroinvertebrates in a Rocky Mountain stream. *Limnology and Oceanography* 47(3): 742-752. doi: 10.4319/lo.2002.47.3.0742

McIntire CD (1968) Physiological-Ecological studies of benthic algae in laboratory streams. *Journal (Water Pollution Control Federation)* 40(11): 1940-1952.

McIntyre PB, Flecker AS, Vanni MJ, Hood JM, Taylor BW and Thomas SA (2008) Fish distributions and nutrient recycling in a Neotropical stream: can fish create biogeochemical hotspots? *Ecology* 89: 2335–2346.

McManamay RA, Webster JR, Valett HM and Dolloff CA (2010) Does diet influence consumer nutrient cycling? Macroinvertebrate and fish excretion in streams. *Journal of the North American Benthological Society 30*(1): 84-102.

McNeely C, Clinton SM and Erbe JM (2006) Landscape variation in C sources of scraping primary consumers in streams. *Journal of the North American Benthological Society* 25: 787–799. doi: 10.1899/0887-3593(2006)025[0787:lvicso]2.0.co;2

McWilliam-Hughes SM, Jardine TD and Cunjak RA (2009) Instream C sources for primary consumers in two temperate, oligotrophic rivers: possible evidence of bryophytes as a food source. *Journal of the North American Benthological Society* 28(3): 733-743. doi: 10.1899/08-103.1

Mendoza G, Ventura M and Catalan J (2015) Environmental factors prevail over dispersal constraints in determining the distribution and assembly of Trichoptera species in mountain lakes. *Ecology and evolution*. 5(13): 2518-2532.

Menge BA and Olson AM (1990) Role of scale and environmental factors in regulation of community structure. *Trends in Ecology & Evolution*. 5(2): 52-57.

Mérigoux S and Dolédec S (2004). Hydraulic requirements of stream communities: a case study on invertebrates. *Freshwater Biolology.* 49 (5): 600–613

Merritt RK and Cummins KW (1996) An introduction to the aquatic insects of North America. 3rd edition. Kendall/Hunt Publishers: Dubuque Iowa

Meyer JL, McDowell WH, Bott TL, Elwood JW, Ishizaki C, Melack JM and Rublee PA (1988) Elemental dynamics in streams. *Journal of the North American Benthological Society 7*(4): 410-432.

Mihuc TB (1997) The functional trophic role of lotic primary consumers: generalist versus specialist strategies. *Freshwater Biology* 37(2): 455-462. doi: 10.1046/j.1365-2427.1997.00175.x

Mihuc TB and Minshall GW (1995) Trophic generalists vs trophic specialists: implications for food web dynamics in post-fire streams. *Ecology* 76: 2361–2372. doi: 10.2307/2265813

Minagawa M and Wada E (1984) Stepwise enrichment of 15N along food chains: further evidence and the relation between d15N and animal age. *Geochimica et Cosmochimica Acta* 48: 1135–1140

Minshall GW, Cummins KW, Petersen RC, Cushing CE, Bruns DA, Sedell JR and Vannote RL (1985) Developments in stream ecosystem theory. *Canadian Journal of Fisheries and Aquatic Sciences* 42(5): 1045-1055.

Minshall GW, Robinson CT, Royer TV and Rushforth, SR (1995) Benthic community structure in two adjacent streams in Yellowstone National Park five years after the 1988 wildfires. *The Great Basin Naturalist* 55(3): 193-200.

Mirra C, Correia MJ, Félix PM, Chaves ML, Chainho P and Costa JL (2014) A diversidade de macroinvertebrados de pequenas ribeiras mediterrânicas do Sudoeste de Portugal. *Ecologia* 7: 58–69.

Molla S, Malchik L and Casado C (1994) Primeros datos sobre el metabolismo de un arroyo temporal mediterráneo de Sierra Morena (Córdoba). *Limnetica* 10: 59–67.

Molla S, Maltchik L, Casado C and Montes C (1996) Particulate organic matter and ecosystem metabolism dynamics in a temporary Mediterranean stream. *Archiv für Hydrobiologie* 137: 59–76.

Monk WA, Wood PJ, Hannah DM, Extence CA, Chadd RP and Dunbar MJ (2012). How does macroinvertebrate taxonomic resolution influence ecohydrological relationships in riverine ecosystems? *Ecohydrology.* 5 (1): 36–45.

Morais M, Pinto P, Guilherme P, Rosado J and Antunes I (2004) Assessment of temporary streams: the robustness of metric and multimetric indices under different hydrological conditions. *Hydrobiologia* 516: 229–249.

Morin A, Lamoureux W and Busnarda J (1999) Empirical models predicting primary productivity from chlorophyll a and water temperature for stream periphyton and lake and ocean phytoplankton. *Journal of the North American Benthological Society* 18: 299–307.

Morrissey CA, Boldt A, Mapstone A, Newton J and Ormerod SJ (2013) Stable isotopes as indicators of wastewater effects on the macroinvertebrates of urban rivers. *Hydrobiologia* 700(1): 231-244.

Mulholland PJ, Fellows CS, Tank JL, Grimm NB, Webster JR, Hamilton SK and Peterson BJ (2001) Inter-biome comparison of factors controlling stream metabolism. *Freshwater Biology* 46: 1503-1517.

Mulholland PJ, Tank JL, Sanzone DM, Wollheim WM, Peterson BJ, Webster JR and Meyer JL (2000a) Food resources of stream macroinvertebrates determined by natural-abundance stable C and N isotopes and a 15N tracer addition. *Journal of the North American Benthological Society* 19(1): 145-157. doi: 10.2307/1468287

Mulholland PJ, Tank JL, Sanzone DM, Wollheim WM, Peterson BJ, Webster JR and Meyer JL (2000b) Nitrogen cycling in a forest stream determined by a 15N tracer addition. *Ecological Monographs* 70(3): 471-493. doi: 10.1890/0012 9615(2000)070[0471:NCIAFS]2.0.CO;2

Munne A and Prat N (2004) Defining river types in a Mediterranean area: a methodology for the implementation of the EU Water Framework Directive. *Environmental Management* 33: 1–19. DOI: 10.1007/s00267-003-0098-y.

Munne A and Prat N (2011) Effects of Mediterranean climate annual variability on stream biological quality assessment using macroinvertebrate communities. *Ecological Indicators* 11(2): 651-662. DOI: 10.1016/j.ecolind.2010.09.004.

Naura M, Sear D, Alvarez M, Penas F, Fernández D and Barquín J (2011) Integrating monitoring, expert knowledge and habitat management within conservation organisations for the delivery of the water framework directive: a proposed approach. *Limnetica 30*(2): 0427-446.

Nikolaidis NP, Demetropoulou L, Froebrich J, Jacobs C, Gallart F, Prat N et al. (2013) Towards sustainable management of Mediterranean river basins: policy recommendations on management aspects of temporary streams. *Water Policy* 15: 830–849.

O'Connor NA (1991) The effects of habitat complexity on the macroinvertebrates colonising wood substrates in a lowland stream. *Oecologia* 85: 504-512.

Odum HT (1956) Primary production in flowing waters. *Limnology and Oceanography* 1: 102–117.

Oksanen JF, Blanchet G, Kindt R, Legendrem P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Henry MH, Wagner S, Wagner H (2015): vegan: Community Ecology Package. R package version 2.3-0. URL http://CRAN.R-project.org/package=vegan

Oliveira VC, Gonçalves EA and Alves RG (2014) Colonisation of leaf litter by aquatic invertebrates in an Atlantic Forest stream. Braz. J. Biol. 74(2): 267-273.

Osmond CB, Valaane N, Haslam SM, Uotila P, Roksandic Z (1981) Comparisons of δ13C values in leaves of aquatic macrophytes from different habitats in Britain and Finland; some implications for photosynthetic processes in aquatic plants Oecologia: 50(1): 117-124.

Palmer CG, O'Keeffe JH and Palmer AR (1991) Are macroinvertebrate assemblages in the Buffalo River, southern Africa, associated with particular biotopes?. *Journal of the North American Benthological Society* 10: 349-357.

Palmer MA and Poff NL (1997) The influence of environmental heterogeneity on patterns and processes in streams. *Journal of the North American Benthological Society* 16: 169-173.

Palmer MA, Menninger HL and Bernhardt E (2010) River restoration, habitat heterogeneity and biodiversity: a failure of theory or practice?. *Freshwater biology* 55(s1): 205-222.

Palmer SM, Hope D, Billett MF, Dawson JJ and Bryant CL (2001) Sources of organic and inorganic carbon in a headwater stream: evidence from carbon isotope studies. *Biogeochemistry* 52(3): 321-338. doi:10.1023/A:1006447706565

Pardo I and Armitage PD (1997) Species assemblages as descriptors of mesohabitats. *Hydrobiologia* 347: 111–128.

Park YS, Chon TS, Kwak IS and Lek S (2004) Hierarchical community classification and assessment of aquatic ecosystems using artificial neural networks. *Science of the Total Environment* 327(1): 105-122. DOI: 10.1016/j.scitotenv.2004.01.014.

Park YS, Kwon YS, Hwang SJ and Park S (2014) Characterizing effects of landscape and morphometric factors on water quality of reservoirs using a self-organizing map. *Environmental Modelling and Software* 55: 214-221. DOI: 10.1016/j.envsoft.2014.01.031.

Park YS, Lek S, Scardi M, Verdonschot PF and Jørgensen SE (2006) Patterning exergy of benthic macroinvertebrate communities using self-organizing maps. *Ecological Modelling* 195(1): 105-113. DOI: 10.1016/j.ecolmodel.2005.11.027.

Park YS, Song MY, Park YC, Oh KH, Cho E and Chon TS (2007) Community patterns of benthic macroinvertebrates collected on the national scale in Korea. *Ecological Modelling* 203(1): 26-33. DOI: 10.1016/j.ecolmodel.2006.04.032.

Parsons M and Norris R (1996) The effect of habitat-specific sampling on biological assessment of water quality using a predictive model. *Freshwater Biology* 36(2): 419-434.

Parsons M, Thoms MC and Norris RH (2003) Scales of macroinvertebrate distribution in relation to the hierarchical organization of river systems. *Journal of the North American Benthological Society* 22(1): 105-122.

Peck JE (2011) Multivariate analysis for community ecologists. Step-by-step using PC-ORD. MjM Software Design Gleneden Beach Oregon USA.

Peipoch M, Martí E and Gacia E (2012) Variability in δ15N natural abundance of basal resources in fluvial ecosystems: A meta-analysis. *Freshwater Sciences* 31(3): 1003-1015. doi: 10.1899/11-157.1

Penczak T (2011) Fish assemblages composition in a natural, then regulated, stream: A quantitative long-term study. *Ecological modelling* 222(13): 2103-2118.

Penczak T, Kruk A, Grzybkowska M and Dukowska M (2006) Patterning of impoundment impact on chironomid assemblages and their environment with use of the self-organizing map (SOM). *Acta Oecologica* 30(3): 312-321. DOI: 10.1016/j.actao.2006.05.007.

Penczak T, Lek S, Godinho F and Agostinho AA (2004) Patterns of fish assemblages in tropical streamlets using SOM algorithm and conventional statistical methods. *Ecohydrology and Hydrobiology* 4: 139-146.

Perry JA and Schaeffer DA (1987) The longitudinal distribution of riverine benthos: a river dis-continuum? *Hydrobiologia* 148: 257–68.

Peterson JT and Rabeni CF (2001) An evaluation of channel units as descriptors of fish habitat in a low-gradient warmwater stream. *Transactions of the American Fisheries Society* 130: 911–926.

Phillips EC (2003) Habitat Preference of Aquatic Macroinvertebrates in an East Texas Sandy Stream. *Journal of Freshwater Ecology* 18: 1–11. DOI:10.1080/02705060.2003.9663946.

Pingram MA, Collier KJ, Hamilton DP, David BO and Hicks BJ (2012) Carbon sources supporting large river food webs: a review of ecological theories and evidence from stable isotopes. *Freshwater Reviews.* 5(2): 85-103.

Pires AM, Cowx IG and Coelho MM (2000) Benthic macroinvertebrate communities of intermittent streams in the middle reaches of the Guadiana basin (Portugal). *Hydrobiologia* 435: 167-175.

Poff NL (1997) Landscape filters and species traits: towards mechanistic understanding and prediction in stream ecology. *Journal of the North American Benthological Society* 16: 391–409.

Poff NL and Ward JV (1989) Implications of streamflow variability and predictability for lotic community structure: a regional analysis of streamflow patterns. *Canadian Journal of Fisheries and Aquatic Sciences* 46: 1805-1818.

Poole GC (2002) Fluvial landscape ecology: addressing uniqueness within the river discontinuum. *Freshwater Biology* 47: 641–660.

Power ME (1992) Habitat heterogeneity and the functional- significance of fish in river food webs. *Ecology* 73: 1675– 1688.

Prat N, Rieradevall M, Barata C, Munné A (2013): The combined use of metrics of biological quality and biomarkers to detect the effects of reclaimed water on macroinvertebrate assemblages in the lower part of a polluted Mediterranean river (Llobregat River, NE Spain). *Ecological indicators* 24*:* 167-176.

Prat N, Gallart F, Von Schiller D, Polesello S, García-Roger EM, Latron J, et al. (2014) The mirage toolbox: an integrated assessment tool for temporary streams. *River Research and Applications*. http://dx.doi.org/10.1002/rra.2757.

Prenda J, Gallardo A and Mayenco A (1999) Distribution patterns species assemblages and habitat selection of the stoneflies (Plecoptera) from two Mediterranean river basins in southern Spain. *International review of Hydrobiology* 84(6): 595-608. DOI: 10.1002/iroh.199900054.

Pringle CM, Naiman RJ, Bretschko G, Karr JR, Oswood MW, Webster JR, Welcomme RL and Winterbourn MJ (1988) Patch dynamics in lotic systems: the stream as a mosaic. *Journal of the North American Benthological Society* 7: 503–524.

Pusey BJ, Arthington AH, Stewart-Koster B, Kennard MJ and Read MG (2010) Widespread omnivory and low temporal and spatial variation in the diet of fishes in a hydrologically variable northern Australian river. *Journal of Fish Biology* 7: 731–753.

R Core Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria URL http://www R-project org/.

Rabeni CF and Doisy KE (2000) Correspondence of stream benthic invertebrate assemblages to regional classification schemes in Missouri. *Journal of the North American Benthological Society* 19: 419–428.

Rabeni CF and Gibbs KE (1980) Ordination of deep river invertebrate communities in relation to environmental variables. *Hydrobiologia* 74(1): 67-76.

Rabeni CF and Minshall GW (1977) Factors affecting microdistribution of stream benthic insects. *Oikos* 29: 33-43.

Rabeni CF, Doisy KE and Galat DL (2002) Testing the biological basis of a stream habitat classification using benthic invertebrates. *Ecological Applications* 12: 782–796.

Rabeni CF, Wang N and Sarver RJ (1999) Evaluating adequacy of the representative stream reach used in invertebrate monitoring programs. *Journal of the North American Benthological Society* 18: 284-291.

Rees GN, Bowen PM and Watson GO (2005) Variability in benthic respiration in three southeastern Australian lowland rivers. *River Research and Applications* 21: 1147-1156.

Reid DJ, Quinn GP, Lake PS and Reich P (2008) Terrestrial detritus supports the food webs in lowland intermittent streams of south-eastern Australia: a stable isotope study. *Freshwater Biology* 53: 2036–2050.

Reid HE, Brierley GJ, Boothroyd IKG (2010) Influence of bed heterogeneity and habitat type on macroinvertebrate uptake in peri-urban streams. *International Journal of Sediment Research* 25: 203-220. DOI:10.1016/S1001-6279(10)60039-X.

Resh VH, Jackson JK (1993) Rapid assessment approaches to biomonitoring using benthic macroinvertebrates. In: Rosenberg DM and Resh VH (eds.), Freshwater biomonitoring and benthic macroinvertebrates, Chapman and Hall, New York, pp. 195–233.

Resh VH, Norris RH and Barbour MT (1995) Design and implementation of rapid assessment approaches for water resource monitoring using benthic macroinvertebrates. *Australian Journal of Ecology* 20(1): 108-121.

Rezanka KM and Hershey AE (2003) Examining primary producer–consumer interactions in a Lake Superior tributary using 15N-tracer, grazer-reduction, and nutrient-bioassay experiments. *Journal of the North American Benthological Society* 22: 371–387.

Rice SP, Greenwood MT, Joyce CB (2001) Macroinvertebrate community changes at coarse sediment recruitment points along two gravel bed rivers. *Water Resources Research* 37: 2793–2803. DOI:10.1029/2000WR000079.

Richards C, Haro RJ, Johnson LB and Host GE (1997) Catchmentand reachscale properties as indicators of macroinvertebrate species traits. *Freshwater Biology* 37: 219–230.

Richards C, Johnson LB and Host GE (1996) Landscape-scale influences on stream habitats and biota. *Canadian Journal of Fisheries and aquatic sciences* 53(S1): 295-311.

Rier ST and King DK (1996) Effects of inorganic sedimentation and riparian clearing on benthic community metabolism in an agriculturally-disturbed stream. *Hydrobiologia* 339: 111–121.

Robson BJ, Hogan M and Forrester T (2005) Hierarchical patterns of invertebrate assemblage structure in stony upland streams change with time and flow permanence. *Freshwater Biology* 50(6): 944-953. DOI: 10.1111/j.1365-2427.2005.01376.x.

Romaní AM and Sabater S (2001) Structure and activity of rock and sand biofilms in a Mediterranean stream. *Ecology* 82: 3232-3245.

Rosenfeld JS and Roff JC (1991) Primary production and potential availability of autochthonous carbon in southern Ontario streams. *Hydrobiologia* 224: 99–109.

Rosenfeld JS and Roff JC (1992) Examination of the carbon base in southern Ontario streams using stable isotopes. *Journal of the North American Benthological Society* 11: 1-18.

Rosi-Marshall EJ, Vallis KL, Baxter CV and Davis JM (2016) Retesting a prediction of the River Continuum Concept: autochthonous versus allochthonous resources in the diets of invertebrates. *Freshwater Science* 35(2): 534-543.

Sabater S and Romaní AM (1996) Metabolic changes associated with biofilm formation in an undisturbed Mediterranean stream. *Hydrobiologia* 335:107-113.

Sabater S, Gregory SV and Sedell JR (1998) Community dynamics and metabolism of benthic algae colonizing wood and rock substrata in a forest stream. *Journal of Phycology* 34: 561-567.

Sabater S, Guasch H, Romaní AM, Muñoz I and Cortés SS (2006) Hydrology, light and the use of organic and inorganic materials as structuring factors of biological communities in Mediterranean streams. *Limnetica* 25(1): 335-348

Sackett WM, Eckelmann WR, Bender ML and Be AW (1965) Temperature dependence of carbon isotope composition in marine plankton and sediments. *Science* (Washington, DC) 148: 235-237.

Salvador N, Monteiro JP, Hugman R, Stigter TY and Reis E (2012) Quantifying and modelling the contribution of streams that recharge the Querença-Silves aquifer in the south of Portugal. *Natural Hazards and Earth System Science* 12(11): 3217-3227.

Sánchez-Carmona R, Encina L, Rodríguez-Ruiz A, Rodríguez-Sánchez MV, Granado-Lorencio C (2012) Food web structure in Mediterranean streams: exploring stabilizing forces in these ecosystems. *Aquatic Ecology* 46(3): 311-324.

Sanchez-Montoya MDM, Punti T, Suarez ML, Vidal-Abarca MDR, Rieradevall M, Poquet J and Prat N (2007) Concordance between ecotypes and macroinvertebrate assemblages in Mediterranean streams. *Freshwater Biology* 52(11): 2240-2255. DOI: 10.1111/j.1365-2427.2007.01826.x.

Sánchez-Montoya MM, Vidal-Abarca MR, Suárez ML (2010) Comparing the sensitivity of diverse macroinvertebrate metrics to a multiple stressor gradient in Mediterranean streams and its influence on the assessment of ecological status. *Ecological indicators* 10(4): 896-904.

Schröder M, Kiesel J, Schattmann A, Jähnig SC, Lorenz AW, Kramm S (2013) Substratum associations of benthic invertebrates in lowland and mountain streams. *Ecological Indicators* 30: 178–189. DOI:10.1016/j.ecolind.2013.02.012.

Sheldon AL, Haick RA (1981) Habitat selection and association of stream insects: a multivariate analysis. *Freshwater Biology* 11(5): 395-403.

Sheldon F, Bunn SE, Hughes JM, Arthington AH, Balcombe SR and Fellows CS (2010) Ecological roles and threats to aquatic refugia in arid landscapes: dryland river waterholes. *Marine and Freshwater Research* 61:885–895. DOI: 10.1071/MF09239.

Siegfried CA, Knight AW (1977) The ef- fects ofwashout in a sierra foothill stream. *The American Midland Naturalist* 98(1): 200–207.

SNIRH Sistema Nacional de Informação de Recursos Hídricos - http://snirh pt/ Accessed 05 December 2016

Southwood TRE (1977) Habitat, the templet for ecological strategies? *Journal of Animal Ecology* 46: 337-365.

Sroczyńska K, Claro M, Kruk A, Wojtal-Frankiewicz A, Range P and Chícharo L (2017) Indicator macroinvertebrate species in a temporary Mediterranean river: Recognition of patterns in binary assemblage data with a Kohonen artificial neural network. *Ecological Indicators* 73: 319-330. doi: 10.1016/j.ecolind.2016.09.010

Sroczyńska K, Claro M, Range P, Ben-Hamadou R, Wojtal-Frankiewicz A and Chícharo L (2014) Substratum preferences of macroinvertebrates in a Mediterranean-type intermittent river: a tool to predict habitat suitability under future scenarios of climatic change, Conference on European Climate Change Adaptation – Research and Practice, Lisbon, Portugal from 10-12 March 2014.

Sroczyńska, K (2018) Biological variation in temporary streams: understanding river patches at different scales for monitoring and management applications (Doctoral dissertation).

St Clair RM (1994) Diets of some larval Leptoceridae (Trichoptera) in south-eastern Australia. *Australian Journal of Marine and Freshwater Research* 45: 1023–1032.

Stanford JA, Lorang MS and Hauer FR (2005) The shifting habitat mosaic of river ecosystems. *Internationale Vereinigung fur Theoretische und Angewandte Limnologie Verhandlungen 29*(1): 123-136.

Statzner B and Higler B (1986) Stream hydraulics as a major determinant of benthic invertebrate zonation patterns. *Freshwater Biology* 16: 127-139. DOI: 10.1111/j.1365-2427.1986.tb00954.x.

Statzner B, Doledec S and Hugueny B (2004) Biological trait composition of European stream invertebrate communities: assessing the effects of various trait filter types. *Ecography* 27: 470–488.

Sterner RW (1990) The ratio of nitrogen to phosphorus resupplied by herbivores: zooplankton and the algal competitive arena. *The American Naturalist 136*(2): 209-229.

Steward AL, Von Schiller D, Tockner K, Marshall JC and Bunn SE (2012) When the river runs dry: human and ecological values of dry riverbeds. *Frontiers in Ecology and the Environment*. 10(4): 202-209.

Stojković M, Simić V, Milošević D, Mancev D and Penczak T (2013) Visualization of fish community distribution patterns using the self-organizing map: A case study of the Great Morava River system (Serbia). *Ecological Modelling* 248: 20-29. DOI: 10.1016/j.ecolmodel.2012.09.014.

Tank JL, Meyer JL, Sanzone DM, Mulholland PJ, Webster JR, Peterson BJ and Leonard NE (2000) Analysis of nitrogen cycling in a forest stream during autumn using a 15N-tracer addition. *Limnology and Oceanography* 45(5): 1013-1029. doi: 10.4319/lo.2000.45.5.1013

Thorp JH and Delong MD (2002) Dominance of autochthonous autotrophic carbon in food webs of heterotrophic rivers. *Oikos* 96(3): 543-550. doi: 10.1034/j.1600-0706.2002.960315.x

Tichý L and Chytrý M (2006) Statistical determination of diagnostic species for site groups of unequal size. *Journal of Vegetation Science* 17: 809-818. DOI: 10.1111/j.1654-1103.2006.tb02504.x.

Townsend CR and Hildrew AG (1994) Species traits in relation to a habitat templet for river systems. *Freshwater Biology* 31: 265-275.

Townsend CR, Doledec S, Scarsbrook MR (1997) Species traits in relation to temporal and spatial heterogeneity in streams : a test of habitat templet. *Freshwater Biology* 37: 367–387.

Trindade MJ, Rocha F, Dias MI and Prudêncio MI (2013) Mineralogy and grain-size distribution of clay-rich rock units of the Algarve Basin (South Portugal). *Clay Minerals* 48: 59-83.

Tszydel M, Grzybkowska M and Kruk A (2009) Influence of dam removal on trichopteran assemblages in the lowland Drzewiczka River Poland. *Hydrobiologia* 630(1): 75-89. DOI: 10.1007/s10750-009-9781-1.

Underwood AJ and Chapman MG (1996) Scales of spatial patterns of distribution of intertidal invertebrates. *Oecologia* 107: 212–224.

Uzarski DG, Burton TM and Stricker CA (2001) A new chamber design for measuring community metabolism in a Michigan stream. *Hydrobiologia* 455: 137-155.

Vanderklift MA and Ponsard S (2003) Sources of variation in consumer-diet δ15N enrichment: a metaanalysis. *Oecologia* 136(2): 169-182

Vanni MJ and McIntyre PB (2016) Predicting nutrient excretion of aquatic animals with metabolic ecology and ecological stoichiometry: a global synthesis. *Ecology* 97(12): 3460-3471.

Vannote RL, Minshall GW, Cummins KW, Sedell JR and Cushing CE (1980) The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* 37(1): 130-137.

Vannucchi PE, López-Rodríguez MJ, de Figueroa JMT and Gaino E (2013) Structure and dynamics of a benthic trophic web in a Mediterranean seasonal stream. *Journal of Limnology* 72(3): 606-615.

Velasco J, Millan A, Vidal-Abarca MR, Suarez ML, Guerrero C and Ortega M (2003) Macrophytic, epipelic and epilithic primary production in a semiarid Mediterranean stream. Freshwat. Biol. 48: 1408–1420.

Verdonschot PF and Nijboer RC (2004) Testing the European stream typology of the Water Framework Directive for macroinvertebrates. *Hydrobiologia* 516(1-3):35-54. DOI: 10.1023/B:HYDR.0000025257.30311.b7.

Verdonschot PFM (2000) Integrated ecological assessment methods as a basis for sustainable catchment management. *Hydrobiologia* 422/423: 389–412.

Vesanto J and Alhoniemi E (2000) Clustering of the self-organizing map. IEEE Transactions on Neural Networks 11: 586-600. DOI: 10.1109/72.846731.

Vesanto J, Himberg J, Alhoniemi E and Parhankangas J (2000) SOM Toolbox for Matlab 5. Report A57. Helsinki Helsinki University of Technology. WEB site: <http://www.cis.hut.fi/somtoolbox/package/papers/techrep.pdf>. Accessed 2015 March 5.

Vinson MR and Hawkins CP (1998). Biodiversity of stream insects: variation at local, basin, and regional Scales 1. *Annual review of entomology* 43(1): 271-293.

Wallace JB, Eggert SL, Meyer JL and Webster JR (1997) Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science* 277: 102-104.

Ward Jr JH (1963) Hierarchical grouping to optimize an objective function. *Journal of the American Statistical Association* 58: 236–244.

Ward JV and Stanford JA (1983) The serial discontinuity concept of lotic ecosystems. In Fontaine TD and Bartell SM (eds), Dynamics of Lotic Ecosystems. Ann Arbor Science, Ann Arbor, pp. 29–42.

Warnaars TA, Hondzo M and Power ME (2007) Abiotic controls on periphyton accrual and metabolism in streams: Scaling by dimensionless numbers. *Water Resources Research* 43(8): 1-13.

Wasiak K, Sroczyńska K, Claro M, Range P, Ben-Hamadou R, Wojtal-Frankiewicz A, and Chícharo L (2013) Metabolic response of aquatic communities to seasonal changes in environmental variables in a Mediterranean-type intermittent river, Conference on Ecohydrology, Biotechnology&Engineering, Towards the Harmony between Biogeosphere and Society on the basis of Long Term Ecosystem Research, Łódź, Poland from 16-19 September 2013

Wasiak KA (2009) Studies on the Effect of Phosphorus upon Headwater Stream Ecosystem Processes, PhD dissertation, Department of Biology, University of Leicester, Leicester LE1 7RH, UK.

Watson M and Dallas HF (2013) Bioassessment in ephemeral rivers: constraints and challenges in applying macroinvertebrate sampling protocols. African Journal of Aquatic Science 38(1): 35-51. DOI:10.2989/16085914.2012.742419.

Wellnitz TA, Poff NL, Cosyleón G and Steury B (2001) Current velocity and spatial scale as determinants of the distribution and abundance of two rheophilic herbivorous insects. *Landscape Ecology*. 16(2): 111-120.

Whited DC, Lorang MS, Harner MJ, Hauer FR, Kimball JS and Stanford JA (2007) Climate, hydrologic disturbance, and succession: drivers of floodplain pattern. *Ecology 88*(4): 940-953.

Whitledge GW and Rabeni CF (2000) Benthic community metabolism in three habitats in an Ozark stream. *Hydrobiologia* 437: 165-170.

Wienke C and Fisher G (1990) Growth and stable carbon isotope composition of cold-water macroalgae in relation to light and temperature. *Marine Ecology Progress Series* 65: 283-292.

Wiley MJ, Kohler S and Seelbach P (1997) Reconciling landscape and local views of aquatic communities: lessons from Michigan trout streams. *Freshwater Biology 37*(1): 133-148.

Wiley MJ, Osborne LL and Larimore RW (1990) Longitudinal structure of an agricultural prairie river system and its relationship to current stream ecosystem theory. *Canadian Journal of Fisheries and Aquatic Sciences* 47: 373–384.

Williams DD (1996) Environmental constraints in temporary fresh waters and their consequences for the insect fauna. *Journal of the North American Benthological Society*. 15(4): 634-650.

Winterbourn MJ, Rounick JS, Cowie B (1981) Are New Zealand stream ecosystems really different? *New Zealand Journal of Marine and Freshwater Research* 15(3): 321-328.

Winterbourn MJ, Rounick JS, Hildrew AG (1986) Patterns of carbon resource utilization by benthic invertebrates in two river systems: a stable carbon isotope study. Arch Hydrobiol 107: 349-361.

Wollheim WM, Peterson BJ, Deegan LA, Bahr M, Hobbie JE, Jones D, Hershey AE, Kling GW, Miller MC (1999) A coupled field and modeling approach for the analysis of nitrogen cycling in streams. *Journal of the North American Benthological Society* 18: 199-221.

Yam SWR and Dudgeon D (2005) Stable isotope inves- tigation of food use by Caridina spp. (Decapoda: Atyidae) in Hong Kong streams. *Journal of the North American Benthological Society* 24: 68–81.

Young RG, Matthaei CD and Townsend CR (2008) Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health. *Journal of the North American Benthological Society* 27: 605–625.

Zanden M and Rasmussen JB (2001) Variation in δ15N and δ13C trophic fractionation: implications for aquatic food web studies. *Limnology and Oceanology* 46(8): 2061-2066. doi: 10.4319/lo.2001.46.8.2061

Zar JH (1996) Biostatistical analysis. Prentice-Hall International Inc: New Jersey.

Appendix 6.1 Stable isotope signatures ($\delta^{15}N$ and $\delta^{13}C$: mean and standard deviation) for consumers and their habitats. Value for bulk periphyton is an average of what grew on the rocks (epilithon, biofilm, filamentous algae and moss in F. Benemola).

		MONTE SECO			**QUINTA DA OMBRIA**			**F. BENEMOLA**		
		$\delta^{15}N$	$\delta^{13}C$	n	$\delta^{15}N$	$\delta^{13}C$	n	$\delta^{15}N$	$\delta^{13}C$	n
INSECTS	*Baetis fuscatus*	14.67 (0.08)	-35.16 (1.08)	6	15.15 (0.21)	-36.18 (0.3)	3	7.03 (0.05)	-44.08 (0.13)	3
	Chimarra marginata	---	---	--	16.58 (0.23)	-33.36 (0.25)	3	9.47 (0.28)	-38.55 (0.59)	6
	Ecdyonurus	13.77 (0.2)	-34.39 (0.47)	7	15.83 (0.23)	-35 (0.34)	4	8.75 (0.21)	-37.28 (0.16)	3
	Isoperla moselyi	13.53 (0.13)	-34.52 (0.81)	7	16.42 (0.18)	-33.53 (0.36)	3	9.86 (0.86)	-35.59 (0.41)	3
	Oreodytes	14.97 (0.71)	-34.01 (0.73)	6	---	---	---	---	---	---
	Pyrrhosoma nymphula	14.23 (1.75)	-33.33 (1.44)	6	16.6 (0.1)	-32.4 (0.28)	3	---	---	---
MOLLUSK	*Ferrisia wautieri*	12.06 (0.55)	-27.67 (1.28)	3	14.59 (0.26)	-28.2 (0.7)	3	7.88 (0.42)	-28.73 (1.32)	3
	Physella acuta	14.13 (0.06)	-30.32 (0.73)	3	15.79 (0.26)	-28.82 (1.71)	3	7.72 (0.27)	-29.24 (1.23)	3
CRUSTACEAN	*Atyaephyra desmarestii*	---	---	--	---	---	---	10.36 (0.27)	-36.02 (0.13)	9
	Procambarus clarkii	11.37 (1.37)	-30.87 (0.49)	5	14.9 (0.15)	-32.19 (0.62)	2	---	---	---
PERIPHYTON BULK	---	12.78 (1.91)	-24.62 (5.42)	12	14.24 (0.40)	-28.98 (2.55)	6	6.62 (1.36)	-35.62 (8.27)	12
EPILITHIC ALGAE		13.59 (1.21)	-22.38 (4.41)	8	14.32 (0.44)	-29.00 (2.85)	5	7.50 (1.63)	-27.58 (4.68)	5
BIOFILM	---	13.50 (1.70)	-17.85 (0.10)	2	11.40(4.20)---	-22.20(3.20)-	2	---	---	
FILAMENTOUS ALGAE	---	11.28 (2.41)	-31.14 (2.10)	4	---	---	---	---	---	1
MOSS	---	---	---	--	---	---	---	5.71 (1.32)	-42.62 (3.02)	6
MACROPHYTES	---	---	---	--	---	---	---	6.63 (NA)	-26.77 (NA)	1
FPOM	---	---	---	--	11.15 (NA)	-33.10 (NA)	2	---	---	---
DETRITUS	---	9.90 (NA)	-27.56 (NA)	1	10.85 (0.19)	-26.92 (0.64)	3	---	---	---

Appendix 7.1 Macroinvertebrate composition at each site. Taxa used for aggregated excretion rate calculation are in bold.

SITE	ORDER	FAMILY	IND M^{-2}
MONTE SECO	**Ephemeroptera**	***Baetidae***	**88.16**
	Ephemeroptera	***Leptophlebiidae***	**2.25**
	Ephemeroptera	***Heptageniidae***	**1.16**
	Ephemeroptera	***Caenidae***	**0.41**
	Gastropoda	***Physidae***	**8.66**
	Gastropoda	***Ferrissiidae***	**0.66**
	Trichoptera	***Hydroptilidae***	**1.83**
	Coleoptera	***Dytiscidae***	**9.07**
	Coleoptera	***Elmidae***	**12.83**
	Coleoptera	***Hydroscaphidae***	**0.17**
	Coleoptera	***Hydrophilidae***	**0.17**
	Odonata	***Gomphidae***	**0.67**
	Plecoptera	***Perlodidae***	**5.25**
	Plecoptera	***Leuctridae***	**2.25**
	Diptera	*Chironomidae*	125.00
	Diptera	*Limoniidae*	5.25
	Diptera	*Tipulidae*	0.25
	Diptera	*Culicidae*	0.33
	Oligochaeta	*Enchytraeidae*	0.50
	Ostracoda		158.50
QUINTA DA OMBRIA	**Ephemeroptera**	***Baetidae***	**269.58**
	Ephemeroptera	***Heptageniidae***	**1.58**
	Ephemeroptera	***Caenidae***	**3.42**
	Ephemeroptera	***Leptophlebiidae***	**2.25**
	Odonata	***Gomphidae***	**0.92**
	Odonata	***Coenagrionidae***	**0.25**
	Gastropoda	***Ferrissiidae***	**11.50**
	Gastropoda	***Physidae***	**56.08**
	Gastropoda	***Hydrobidae***	**0.25**
	Coleoptera	***Dytiscidae***	**11.83**
	Coleoptera	***Elmidae***	**78.75**
	Trichoptera	***Hydroptilidae***	**8.33**
	Trichoptera	***Hydropsychidae***	**1.75**
	Trichoptera	***Psychomyiidae***	**0.75**
	Trichoptera	***Polycentropodidae***	**0.25**
	Trichoptera	***Philopotamidae***	**0.42**
	Trichoptera	***Leptoceridae***	**0.25**
	Plecoptera	***Perlodidae***	**8.25**
	Plecoptera	***Leuctridae***	**1.17**
	Oligochaeta	***Enchytraeidae***	**35.00**
	Diptera	*Chironomidae*	212.50
	Diptera	*Simuliidae*	1.50
	Hydracnidae		0.25
	Ostracoda		50.00

SITE	ORDER	FAMILY	IND M^{-2}
FONTE BENEMOLA	**Ephemeroptera**	***Baetidae***	
	Ephemeroptera	***Caenidae***	
	Ephemeroptera	***Ephemerellidae***	
	Ephemeroptera	***Leptophlebiidae***	**123.25**
	Odonata	***Aeshnidae***	**0.25**
	Gastropoda	***Ferrissiidae***	
	Gastropoda	***Hydrobidae***	
	Gastropoda	***Physidae***	
	Gastropoda	***Planorbidae***	**41.00**
	Coleoptera	***Hydrophilidae***	
	Coleoptera	***Scirtidae=Helodidae***	
	Coleoptera	***Elmidae***	**2.75**
	Trichoptera	***Hydroptilidae***	
	Trichoptera	***Leptoceridae***	
	Trichoptera	***Hydropsychidae***	
	Trichoptera	***Psychomyiidae***	
	Trichoptera	***Polycentropodidae***	
	Trichoptera	***Philopotamidae***	**513.50**
	Heteroptera	***Gerridae***	
	Decapoda	***Atyidae***	**2.50**
	Diptera	*Chironomidae*	264.00
	Diptera	*Anthomyiidae*	4.75
	Diptera	*Tipulidae*	0.25
	Hirudinea	*Glossiphoniidae*	13.75
	Isopoda	*Asellidae*	33.50
	Oligochaeta	*Enchytraeidae*	8.75
	Ostracoda		78.25