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BRYOPHYTES AND VASCULAR PLANTS IN SPRINGS OF ITALIAN ALPS: BIODIVERSITY ANALYSIS AT LARGE SPATIAL SCALE AND MECHANISMS OF DISTRIBUTION AT FINE SPATIAL SCALE

Daniel Spitale

COORDINATORE DOTTORATO: Prof. Giulio De Leo

RELATORE: Prof. Giampaolo Rossetti
CORRELATORE: Dr Marco Cantonati
TUTOR: Prof. Marcello Tomaselli
CONTRORELATORI: Prof. Ireneo Ferrari
Dr Piero Guillizzoni
Dr Rosario Mosello

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Preface

This is a manuscript about plant ecology in spring habitats. Work on this habitat has been motivated by my conviction that a better knowledge of springs is urgently necessary to manage and protect them. Understanding biodiversity and its patterns of distribution at large spatial scale has a fundamental significance for conservation purpose of springs. In recent years, a general attention to biodiversity issues has been growing, however, too few efforts have been made regarding spring habitats. Fortunately, in 2005, the Museo Tridentino di Scienze Naturali prompted an innovative multidisciplinary project, called CRENODAT, and financed by a farsighted Autonomous Province of Trento. The project leader, Marco Cantonati, with the aid of its Limnology and Phycology Section, organized an expertise group with the intention of fill this knowledge gap. The project began with a workshop about methods to study spring habitats and on which is based a special Monograph of MTSN. Professor Marcello Tomaselli and Dr Alessandro Petraglia of Parma University had the mission of tutoring me in undertaking this difficult topic of plant ecology in spring habitats. During the first year of sampling activity, it was becoming clear that plant distribution in springs was shaped by mutual and interacting mechanisms acting both at large and at local spatial scale. In particular, bryophyte species, usually abundant in all springs, seem to play an important rule in structuring the entire spring community. Thus, in 2006, beside the second sampling campaign, I planned the first field experiment aiming at studing spatial distribution of bryophytes in relation to their hydrological niche. In 2007 this first experiment was followed by a second, in which I studied the mechanisms of interaction among bryophyte species. These experiments would not have been accomplished without the field assistance of my father Calogero and my mother Erina.

The final manuscript that here is presented, is organised exactly in chronological order as these different parts have been studied. In the first chapter there is a methodological contribute that is published on a Monograph of MTSN. The second chapter can be considered the bulk of the manuscript on which the third and fourth chapters are based. In particular, the third chapter is about richness and species density and it is now in print on behalf of the International Society of Limnology (SIL) which published its proceedings *International Association of Theoretical and Applied Limnology*. The fourth chapter is about bryophyte and vascular plant richness, approached with a statistical model evaluating multivariate relationships: this chapter is presently submitted on an international scientific journal. The last two chapters deal with the experimental works and are now submitted to international scientific journals too.

I dedicate this manuscript to my family that in different ways actively participated to this work. I am particularly grateful to Laura Tamburello who read and corrected the entire manuscript and gave me constructive suggestions for its improvement. She read and reread draft after draft, and the manuscript is greatly improved because of her intellectual capacity to cut right to the essential.
I also thank Marco Cantonati for a final lecture of several chapters of this manuscript.

1

INTRODUCTION

A survey on the diversity of spring habitats

Springs are usually small, but complex systems: they have a mosaic structure, a high degree of individuality and an azonal character, due to their peculiar physicochemical stability (Cantonati et al 2006). Springs are ecotones linking an aquifer to the uppermost section of a surface running water system. From the hydrogeological point of view, a spring is defined as a well delimited and defined place where groundwater comes up to the surface. Thienemann (1922) first distinguished the following types of spring based on the way in which water emerges: rheocrenes (flowing springs) in which the water spurts out of horizontal or downward sloping strata, and immediately races down into the valley as a rivulet or stream; limnocrenes (pool springs) in which the water wells up from below and current velocity is almost absent; helocrenes (seepage springs) where the water seeps up through the ground forming a swamp. Although intermediate types exist, this classification has been widely employed by many workers. Heterogeneity (as a mosaic structure) exerts a strong influence on the distribution and abundance of species, on species interactions and on the trophic structure of biological communities (Levin 1976). Because different situations may coexist in a few metres, very often these habitats host a concentrate of biodiversity (Cantonati et al 2006).

A characteristic feature of spring areas is that chemical conditions and rate of discharge of water are relatively constant. The temperature of the water emerging at the surface tends to be relatively constant. Seasonal variations up to several degrees are common, although the annual mean temperature of the water is very close to the mean annual temperature of the hydrographic basin deeding the spring. One of the most distinctive ecological feature of springs, compared to similar semiaquatic systems, such as mires or fens which are characterized by stagnating waters (Nadig 1942), is the high oxygen saturation value. Coming from underground, where respiration and decomposition are the dominant metabolic processes, spring-waters are usually deficient in oxygen and enriched in carbon dioxide. Mountain springs are, however, usually fed by small close-to-surface aquifers, and the zone immediately uphill of the spring is often made up by large boulders which determine high porosity and easy contact between groundwater and atmosphere. Mountain spring waters, and rheocrenes, in particular, are therefore often well oxygenated (Cantonati et al. 1998). In natural springs conductivity is mainly determined by the lithological characteristics of the substrate and, in particular, by its solubility. Furthermore, temperature and conductivity are frequently correlated with altitude, since both decrease with higher elevations. Lower temperatures reflect lower mean values in the drainage basin and lower conductivity occurs because the residence time of water in the aquifer is shorter, limiting interactions between water and the lithological substrate (Cantonati et al. 1998). pH is determined by lithology, by carbon dioxide content, by acid contaminants of airborne origin (nitrates, sulphates) and, in springs with slow-flowing water, by the photosynthetic activity of algae, mosses, and higher plants. In helo- and rheohelocrene springs, organic acids may also be important. While springs on carbonate substrate are well buffered and have a fairly stable pH, seasonal changes can be more pronounced in springs with weakly mineralised water. In weakly buffered springs, particularly at high elevations, pH might decrease during periods of rain (Brehm, 1986) and this is often more pronounced in areas with conifer plantations (Puhe and Ulrich, 1985).

The need of standardized methods to sample springs habitats

In the previous chapter, I introduced the great diversity of habitats that we can potentially encounter sampling springs. It turns out that before beginning a research project focused on spring biodiversity, we need to define a standardized sampling protocol wich allows a consistent site comparison, and ensures at the same time to assess the most important ecological variables. Research projects should begin with a general revision of existing sampling strategies according to the objectives of the study. It has long been recognized that sampling procedures play an important role in population and community studies in ecology (e.g. Greig-Smith 1983). Over the past few decades, however, the clarification of sampling objectives and the elucidation of sampling problems in ecology have received little attention. Sampling procedures aiming at describing spring ecomorphology should contain all the relevant information about, for example, substrates grain-size, lithology, water-flow velocity, shape on the spring bed, illumination, and eventual anthropogenic disturbances (Howein and Schroeder 2006). Qualitative variables such as spring shape could be distinguished in a rank order of complexity while others, such as lithology, only using categorical variables. Quantitative variables as water flow velocity should be expressed in quantitative values or, when discharge is too low, ordinal scale would be appropriate. As suggested by Howein and Schroeder (2006), it is important to elaborate a detailed sampling protocol, general but specific at the same time, to survey a given area. General because it should cover all the most important ecological variables but also specific where necessary. For example, rheocrene springs are the most abundant spring type on mountain habitats like the Alps, whereas helocrene springs are abundant in central Europe. Therefore, a sampling protocol improved to specifically describe rheocrene springs would better fit alpine springs.

Plant associations in spring habitats: definition and limits

Spring plant communities differ from other plant communities by their dependence on permanent, relatively cold water rich in oxygen because of the frequent turbulence on the water surface. The constancy of habitat conditions has led to the establishment of stenothermic plants, many of them relicts of past climatic periods (Willmans 1989). The character of these communities is given by the chemistry of water, by water reaction, by water temperature and variations, by the insulation, and also by the type of substrates. Other important variables able to structure spring communities are current velocity, altitude, and snow-cover duration. The mineral content of water is determined by the chemical composition of the rocks from which it emerges. As water flows through the ground before emerging as a spring or flush, it becomes enriched with mineral salts dissolved out from the rocks and soils. The composition of these dissolved salts, influences which plants will grow in the area where water emerges. Where rocks are alkaline or rich in lime and other plant nutrients, plants which grow in the springs and flushes are very different from those that are able to grow where water is acid and deficient in lime.

Ecologists are interested in associations of species as a conceptual framework to synthesise environmental characteristics. When associations have been found, one can concentrate on finding the ecological requirements common to most or all the species of an association instead of having to describe the biology and habitat of each species individually.

In an inverse approach, species associations may be used to predict environmental characteristics. Associations may be better predictors of environmental conditions because they are less subject to sampling error than individual species. The ecological interpretation of species associations is a subject open to discussion. Research examining the quantitative relationships between species associations and their environment, in a multidimensional framework such as Hutchinson's (1957) fundamental niche, should enrich this discussion with appropriate data, and provide some idea of the kind of variability to expect in species responses. In this framework, the following definition of species association was adopted (Legendre and Legendre 1998): a species association is a recurrent group of co-occurring species that have similar reactions to properties of the environment. In this way, associations are characterized by their internal stability along the sampling axes. Associated species are thus responding in a related fashion to environmental change, which implies that variations in abundance of associated species are under similar environmental control. According to this model, the recurrence of associations is an indication that the abstraction, called association, corresponds to some fundamental properties of the interaction between species and their environment. This analysis of the species-environment relationships would be incomplete if it did not include the recognition that the environment of a species is not solely composed of physical variables, but also of the other species with which it interacts in a positive or negative fashion (see also Chapter 6). This is in agreement with the notion that species frequently found together may have evolved mechanisms of biological accommodation to one another, in addition to adaptations to their common environment.

To date species associations of spring habitats have been described mainly with the phytosociological approach (Hinterlang 1992) obtaining a number of different associations. However, in that framework there are very specific rules to identify associations in agreement with the Braun-Blanquet's school. Since it is not one of the aims of this work to critically discuss this approach, I prefer to quote a representative paper where this information is available (e.g. Jörg 2003). It will be sufficient to observe that in the present work other methods were preferred to define species associations, such as those described in Legendre and Legendre (1998). Moreover, according to objectives and sampling strategy, statistical data analysis (Chapter 3) was performed using abundance data on the whole spring area, and not on a selected portion (as is usually done for phytosociological investigations). In this way one can obtain a vegetation description which is in agreement with the ecomorphological sampling done for the entire spring area. Unfortunately, I could not find any work dealing with spring associations in a strictly quantitative way. Therefore, with this study, I will try to provide a spring classification of species and habitats to fill this gap of knowledge.

The problem of plant species richness in spring habitats

It has been proposed that spring habitats may be compared to real islands because of their peculiar and well differentiated biota in contrast to the surrounding habitats (Werum 2001). The constant presence of water distinguishes, more or less sharply, the extent of a spring area. Similarly to other habitat islands, richness should be strongly related to the extension of area following the relation proposed by MacArthur and Wilson (1967). Recently, however, an entire family of different species-area relations has been suggested (Tjørve, 2003). The increase in number of species comes about for two reasons. First, as more individuals are sampled, the chance of encountering additional species increases, especially if species are not randomly distributed. Second, larger areas are likely to be more environmentally heterogeneous, thus containing additional species that differ in their niches. This increase of species number with area has been called one of the few laws of ecology, making species-area curves a prime measure of ecological patterns (Lomolino and Weiser 2001). In addition, the same Authors stated that confounding variation in area with variation in other environmental factors could lead to biased results. One way to control the area assessed, a fundamental step that is very often ignored, is to use sample units of equal dimensions, and hence to calculate species density instead of species richness.

A great deal has been written about biological diversity in recent years. Concerns about the loss of species have driven efforts to bring to the public awareness both the values of biological diversity and the threats to its continued existence (Margules and Pressey, 2000). Understanding species diversity remains one of the cornerstones of community ecology, because of the degree to which it summaries the effects of so many processes of interest. For example, ecosystem multifunctionality does require a greater number of species. Meta-analysis of the results of the first generation of experimental research on biodiversity and ecosystem functioning has revealed that individual ecosystem processes generally show a positive but saturating relationship with increasing diversity, although the mechanisms underlying these relationships are still under debate (Hector and Bagchi 2007). Although a great deal of attention has been paid to the various theories proposed to explain patterns of small scale diversity, the large number of competitive hypothesis and models suggests that an adequate synthetic understanding has not been achieved yet. Multivariate studies, that are those including more than one variable to predict species richness, are not so diffuse. Recent studies, though, demonstrated that more variance was generally explained by multivariate approaches than by the analogous univariate. Here the problem was mainly methodological, because we have to deal with interactive effects, for example among disturbance, biomass, stress etc, that together influence spring species richness. In this framework, Grace and Pugesek (1997) introduced in ecology, quite successfully, a particular analysis (SEM) in which each variable is modelled to test an a priori set of hypotheses. This analysis is not really new, because in sociology it is widely used, but in ecology it has not had many adepts yet. As a matter of fact, ten years later, Austin (2007) affirms that it is a powerful analysis that has not yet been exploited by ecologists, because its proper understanding is rather time consuming. However, it remains a powerful tool that can provide useful insight, in particular in those situations where concomitant explanations exist about a supposed mechanisms.

Bryophytes, water, and interaction strategy at population level

In spring habitats bryophytes usually cover large areas, and sometimes are able to modify significantly the water flow. Especially where few species are dominant, they can modulate the environmental forces, directly by slowing down and deviating water flow, and indirectly by transporting water among capillary space. Therefore, as suggested by Jones et al (1994), bryophytes in spring habitat can be considered as ecosystem engineers. Bryophytes create habitat patches where environmental conditions and resource availability substantially differ from the surrounding unmodified environment (Jones et al. 1994). Then, the presence of such habitat patches may affect species diversity in two ways: (1) by providing suitable habitats for species that cannot survive in the unmodified habitat, and hence increase species richness by adding new species into communities or (2) affecting the abundance of species already present within communities and hence changing the evenness of species assemblages (Badano & Cavieres, 2006). Given that ecosystem engineering results in patches where the availability of resources differs from the surrounding habitat that remains unmodified by the engineer, and that the distribution of species tends to be affected by the availability of resources, ecosystem engineering clearly has the potential to affect the distribution and abundance of species (Wright and Jones 2004). Spring bryophytes, modulating the water directly and indirectly, can therefore affect the distribution of other species regulating the water availability. Resulting spatial patterns of bryophytes distribution are the consequence of embedded and confounding processes acting at the same time. Nevertheless, these components can be organized into three main categories: (1) the water gradient across the spring, (2) the patchiness of water at micro-scale resulting from (a) substrate heterogeneity and (b) plant interactions, (3) temporal variation that modulate the water evaporation. In this frame bryophyte colonies have an active rule both spatially (because of their capacity to transport water) and temporally because of their capacity to hold water, slowing evaporation processes.

Depending on the species, these properties are more or less evident in agreement with the colony architectures of a species. These different capacities of water transport and storage are thought to be the key traits of a species when interacting with a neighbour. Previous works using bryophytes as model species have shown that the mechanism by which interactions occur is mainly through moisture availability (Mulder et al 2001; Rixen and Mulder 2005). Although an extensive literature exists about the relation between bryophytes and water (e.g. Proctor and Tuba 2002), the idea of an explicit interaction among colonies has hardly been emphasized yet (with a limited exception for *Sphagnum*-dominated habitats, Rydin 1985; 1993; 1997).

As Spicer and Gaston (1999) suggest, an interaction between an abiotic gradient and biotic interactions between species can determine the limits to the interacting species' ranges. There are many different types of biotic interaction, and in many cases a single species will exhibit a diverse range of interactions with different components of its community. Some empirical studies have sought to identify the role of biotic interactions in setting the limits to species' ranges. Importantly, interactions do not simply constrain a species' distribution and reduce the size of its realized niche. Facilitative interactions may lead to a species' realized niche being larger than its fundamental niche (Bruno et al., 2003), and might promote the expansion of a species' range margin into more severe environmental conditions than would otherwise be tolerable. For instance, the limits of a bryophyte species in relation to the distance from water could be extended in the presence of a neighbour species. Intensity and direction of plant-plant interaction is thought to be dependent on the stress degree. The stress gradient hypothesis (SGH) suggests that positive interaction (facilitation) between plants tends to be more important under stress conditions (Bertness and Callaway 1994), whereas competition should dominate in ameliorate situations. However, a complex balance between positive and negative interactions may be present in natural environment because stress level can dramatically change through time.

2

GOALS AND WORKING HYPOTHESIS

In this section the general goals and overall working hypothesis of this thesis will be provided. Specific hypotheses are reported more in detail in each chapter. The objectives of this research were twofold in relation to the spatial scale investigated.

At large spatial scale the goals were to proceed with a rigorous observation, description, and analysis of bryophyte and vascular plant diversity in spring habitats, both to propose explanations for the observed patterns and to provide the first exhaustive check list of spring plants in Trentino (that is also so far unique in the Italian Alps). Here the goal was to use a strong observational approach to thoroughly document bryophyte and vascular plants communities to fill a still existing gap of knowledge due to the fact that springs as habitats were usually considered only rather marginally until now. A second objective was to classify spring habitats and species associations to provide a useful tool for conservation management. In fact, it was supposed that the sampling of a large number of springs would allow highlighting the most endangered spring typologies. This was necessary because one goal of this part of the work was also to generate a database suitable to explore (in later studies) the potential of bryophytes and vascular plants of indicators of integrity and quality, in particular in integrated systems considering also other aspects (e.g. morphology, physicochemistry) and other components of the biota (e.g. algae, zoobenthos).

A further aim was to elaborate a model trying to explain plant diversity distribution at large spatial scale. This model was constructed with a specific statistical tool that requires the a priori formulation of the working hypothesis about the relationships among the variables. Then, these hypotheses were tested on the real data, providing the statistical probability of model correctness.

At fine spatial scale the study was characterized by an experimental approach focused on bryophytes because of their important role in structuring the entire spring community. With the first field experiment, spatial distribution of bryophytes was studied considering the different stress levels experienced by various species in relation to their distance from water. Different species are supposed to be distributed at different distance from water level, according to their hydrological niche. From this observation, and on the base of preliminary surveys, a first experiment was planned in which these different capacities in transporting water were tested. General hypothesis was that different species should have different hydrological niche to coexist. The experiment generated a water gradient along which the optimal distance from water was established using photosynthetic pigments as stress indicators. In fact, knowing the amount of pigments in condition of no stress, I was able to estimate the corresponding optimum distance from water at which species do not experience stress. This theoretical value should correspond to the ideal range of distance from water for a given species.

The second field experiment at fine spatial scale was based on several results gained during the first experiment, i.e. that different colonies were able to transport different quantities of water. Since these colonies have different water holding and transporting capacity, it was hypothesized that some form of interaction should occur amongst neighbour species. In particular, the experiment was planned to study positive and negative interactions between a target species (*Warnstorfia exannulata*) and two different neighbour species (*Scapania undulata* and *Sphagnum warnstorfii*) at different level of water stress. It was supposed that changes in stress degree could shift the balance between positive and negative interaction. An additional hypothesis was that, since in natural habitats the degree of stress fluctuates through the time, also plant interactions should change in time, shifting from positive to negative according to climatic variations.

3

METHODS

Springs selection

Informations about springs of Trentino are collected in the “Catasto sorgenti” database, developed by the Geological Survey of Autonomous Province of Trento. Since the database collects information on about 10,000 springs, a specific work was performed by Laveder (2007) in order to select the most suitable sites. General requirement were: perennial flow (or no data); no water exploitation; widespread distribution; no information about water catchments (free water outflowing); recorded flow values from 0 to 20 $L \cdot s^{-1}$. First selection allowed the reduction from more than 10,000 to 3385 springs. Further choice of springs, suitable for the hydrobiological research, considers only those studied for PUP (Provincial town-planning), which were sources with a discharge higher than 0.5 $L\ s^{-1}$ or designed for potable use, monitored in a recent survey. In this phase, the number of springs was further reduced by selecting the most suitable ones, according to the following characteristics: a) free flow without any tanks or tapping system; b) minimum assured outflow to avoid source drying (generally $0.5<Q<10\ L\ s^{-1}$); c) different spring typologies. On the base of this information, 588 springs were selected, spread over 480 locations. Among these 480 sites a further selection was performed in the field on the base of accessibility and the good match between expected and observed condition of the springs. Finally, 108 springs were sampled in the CRENODAT Project among which 86 were available for vegetation analysis (Fig 1).

Sampling strategies of plants

Research projects should begin with a general revision of sampling strategies according to the objectives of the study. It has long been recognized that sampling procedures play an important role in population and community studies in ecology (Greig-Smith 1983). Spring habitats present some peculiar characteristics that should be kept in mind before being sample. The most important is that, in comparison to other habitats, springs have an area that is limited in space. Small area is an advantage because the researcher (at least in the most frequently cases), has at first sight a complete picture of species and abundance. The problem is that spring area generally is not sharply defined and more or less visible gradients are present. Therefore, since sampling decisions must be consistent with the objectives of the study (Kenkel et al 1989), I adopted different strategies.

To accomplish the first aims of the CRENODAT project, that is to obtain a through acknowledge of spring plants, all the species in the spring area were recorded. Then, on this area, abundances were attributed to each species according to the following classes: + (cover less than 1 % but with many individuals), r (cover less than 1 % but with few individuals), 1 (cover between 1 and 5 %), 2 (5 – 25%), 3 (25 – 50 %), 4 (50 – 75 %), 5 (75 – 100 %). When spring area presented an extension not delimited, a representative portion of it was sampled. This apparent simple dichotomy, whole area and representative area, lead directly to the problem of positioning the sampling plot. Here two main approaches are in order, random and subjective. The first is called probabilistic sampling and the second non-probabilistic sampling. In this study both the approaches were used.

The aim of the non-probabilistic sampling is to select the portion (or more than one portion sometimes) of the whole area where plant assemblages are better structured than elsewhere in the spring area. This approach is very flexible and powerful because it can underline even the microhabitat differences within the spring. The problem is that the result of this description falls to researcher skills. Abundances estimated with this method are suitable to phytosociological investigation (did, but not presented in this work).

Probabilistic sampling is performed replicating randomly and independently (that is neither overlapping nor adjacent) three plots of 1 m^2 for a total of 3 m^2. For each plot, separated species collection and cover estimation are performed. Aim of the probabilistic sampling is twofold, both to provide more objective results and also to collect comparable extension of area among different springs. Chytrý and Otýpkovà (2003) proposed standard plot size depending on the type of vegetation surveyed, and for the class Montio-Cardaminetea (that is the syntaxa of spring vegetation), they advised to use plots of $4m^2$. Therefore, to satisfy this general statement allowing at the same time the sampling of smaller springs, three replicated plot of 1 m^2 were used instead of unique plot of 4 m^2. In the case of spring area smaller than 3 m^2, a minor number of independent replicates were taken. The main disadvantage of this method is essentially that small or less accessible microhabitats are not sampled, with an underestimate of the less abundant species.

A sub-class of probabilistic samples is a very special case employing in spring habitats. This sub-class can be correctly classified as probabilistic because spring selection was effectively performed random (a designed random-stratified sampling). Once a spring was selected, all the species in the spring area were recorded proportionally to the entire area. That is, species cover was not evaluated only on a selected area of springs (in this way avoiding the subjective choice) but rather on the entire area. The problem is not silly because the error introduced in estimating the extent (or the edge) of the spring area is not so determinant on the estimate of the species abundances. Delimiting subjectively a specific portion of spring area (thought to be representative) and evaluating species abundance on this portion, give more different results than take the whole spring area, even though estimating the extent of the whole area can be only approximate. Species covers on the entire area were extensively used in the statistical analysis of this work.

Besides sampling of bryophytes and vascular plants, a detailed survey of ecomorphological variables was made following the sampling protocol described in chapter 2. Water temperature, oxygen, pH and conductivity were measured by means of a field multiprobe. Water for chemical analysis, performed by the Unità Operativa Analisi delle Acque of the Istituto Agrario S. Michele all'Adige, was collected on the spring mouth.

Nomenclature of species follows Paton (1999) for liverworts, Cortini-Pedrotti (2001-2005) for mosses and Aeschimann et al. (2004) for vascular plants.

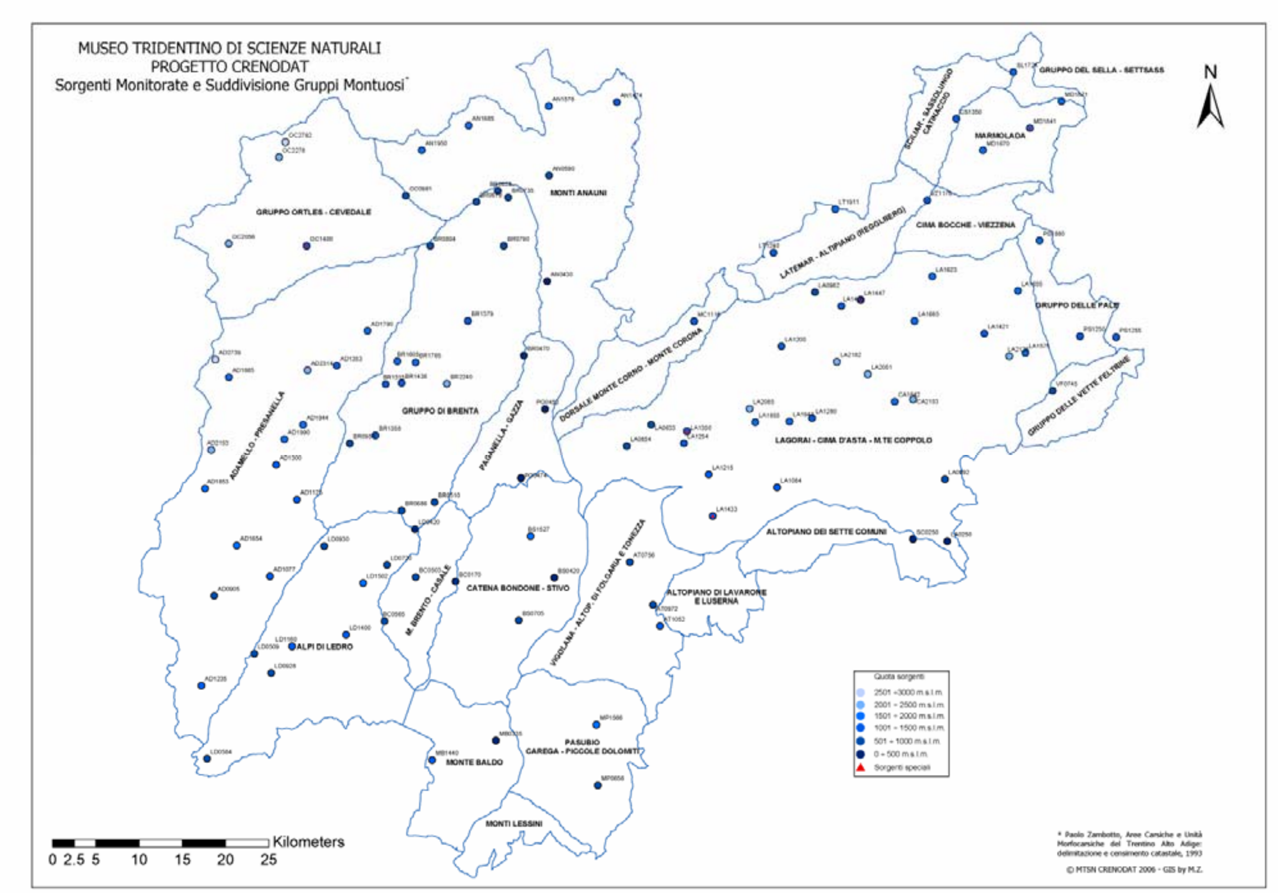

Fig 1. Map of the springs investigated in Trentino Province (Italy).

4

ECOMORPHOLOGY OF SPRINGS[1]

Given the high ecomorphological diversity existing in mountain springs, in this chapter this topic was further improved with a detailed description of sampling strategies adopted to deal with. The main ecological factors influencing distribution, abundance and structure of the crenocoenosis are presented. Aspect, delimitation of the spring area, types of shape, substrates, flow, buffer zone and disturbances are reviewed keeping in mind their potential ecological relevance. Since for this work was required to sample more than one hundred springs, a standardized field protocol was developed also on the basis of existing spring-assessment forms. The main aim of this work was to offer the basic elements to elaborate a standardized sampling procedure for a quali-quantitative description of the ecomorphologic characteristics of mountain springs. After examining morphological factors relevant for ecological research and nature conservation, a field protocol to assess spring morphology are proposed.

This chapter was part of a special issue of the Monografie del Museo Tridentino di Scienze Naturali devoted to discuss specific methods to sample crenocoenosis.

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Introduction

Springs are usually small, but complex systems: they have a mosaic structure, a high degree of individuality and an azonal character, due to their peculiar physicochemical stability (Cantonati et al., 2006). Springs are ecotones linking an aquifer to the uppermost section of a surface running water system. The traditional ecomorphological classification of springs by Steinmann (1915) and Thienemann (1922) is mainly based on current velocity conditions in the spring boil: almost absent in depressions filled by water (limnocrene or pool spring), diffuse emergence with weak currents and development of a swampy zone (helocrene or seepage spring) and rapid currents like those typical of streams (rheocrene or flowing spring). Although intermediate types exist, this classification has been widely employed by many workers. Di Sabatino et al. (1997) stated that rheocrenes are the most widespread type of springs in central-south Europe, while helocrene are frequent in Scandinavia; limnocrene can be recurrent in calcareous substrates, like karstic mountains, instead.

Even though the main types of springs have already been described in literature on the ground of a limited number of characteristics (for example current flow’s speed), specific synthetic works about ecomorphologic survey of the whole spring are hitherto rare (Cantonati and Bonettini, 1995; Howein 1998, Projektgruppe Aktionsprogramm Quellen 2004). As a matter of fact, even if it summarizes the main types of springs in three categories, the traditional Steinmann-Thienemann’s classification doesn’t provide a sufficiently detailed representation of the remarkable complexity of these environments. Heterogeneity (as a mosaic structure) exerts a strong influence on the distribution and abundance of species, on species interactions and on the trophic structure of biological communities (Levin 1976). Because different situations may coexist in a few metres, very often these habitats represent a concentrate of biodiversity (Cantonati et al. 2006). The ecological study of these environments requires specific sampling procedures to be able to survey the morphologic variability (habitats) over which the assemblages of the crenocoenosis inhabit. This is a perspective similar to the one suggested by Ilmonen and Paasivirta (2005) which stressed the necessity of a careful survey of all distinct habitat types present in a spring in order to deepen autecological studies.

The main aim of this chapter was to provide the basic elements to elaborate a standardized sampling procedure for a quali-quantitative description of ecomorphological characteristics of springs. After examining morphological factors relevant for ecological research and nature conservation, a field protocol to assess spring morphology will be proposed. Because of the peculiarity of the studied environments, this work will be mainly useful in mountain regions, from the bottom valley areas to high elevations.

Main ecomorphological factors in springs

Section 1: Georeferentiation, slope and aspect

As usual in all ecological studies, coordinates of the sampled area are required, not only to univocally establish the earth position, but also to enable the exact place to be located again in the future. Moreover, a comprehensive approach to conserving species diversity requires data on the geographic distribution, habitat, and abundance of species (Hunter and Webb, 2002). In this context, geographical information systems (GIS) are an increasingly used tool that integrates the complex information from different data sets at different geographical scales. The reference system of coordinates, as well as error measures, should always be defined. If the spring lies in an area where signals from satellites are not intercepted, it is advisable to take the measure in a more visible area recording distance and direction from the true point. The same instrument generally provides also altitude above the sea level through triangulation of satellites. Altitude is an ecological variable that influences each organism. The slope of the site is a useful characteristic for description purpose, as it gives indications about the current velocity or about the kind of vegetation. Measures are taken with an inclinometer or with a modified goniometer. Aspect is a feature that gives information on the quantity of intercepted light: autotrophic organisms can be very sensitive to the light (heliophilous-scyaphilous, i.e. sun/shade loving species).

Section 2: Delimitation of the spring area

The first problem, once the position of the spring-mouth has been located, is to delimitate the sampling area. The spring can be longitudinally subdivided in eucrenon (the spring mouth and the immediate vicinity) and hypocrenon (between the lower eucrenon to the spring-fed stream). Although theoretically the two areas are distinguished in practice it is not so straightforward. Some investigators (e.g. Illies and Botosaneanu, 1963) considered only the zone in which the annual temperature amplitude did not exceed a defined limit. A definition more applicable (Crema et al., 1996; Gerecke et al., 1998) is based on differences in the coverage by mosses (particularly abundant in the eucrenal), or the shape of the uppermost flow channel(s) (numerous separate rivulets in the eucrenal). For our experience a length minor of about 15 m could be considered as sufficient if there are not detectable morphologic variations. On the contrary, a noticeable increase in current velocity (jump of slope), or a difference in water temperature or in vegetation structure would be a clear evidence of the lower boundary of the crenon. Looking at the width of spring areas, a clear definition for delimitation is again not easy to find. The crenon is not only the submerged area, but in some cases even the bank area. For example, sometimes vegetation is not able to growth submerged, so the bank area should be considered as well. Unfortunately, the boundary of the spring area is a open question (further developed in the framework of species richness in chapter 3) that mostly can be evaluated from subjective point of view only.

Section 3: Shape, size and area

From the point of view of shape, springs can be distinguished in single (with only one emergence), systems (more than one emergence of the same kind) or complexes (more than one emergence of different type) (bavarian spring form, download from www.alpenquellen.com/quellkartierung.htm #download). Moreover the spring-bed can be straight, wavy, with bends, expanded, branched etc. and it can have different dimensions. The whole of these characteristics can give an idea of habitat complexity and it can explain the existence of a high biodiversity. For example, a spring with different emergencies and bends represents in the whole a very heterogeneous habitat and therefore it could shelter a great richness of species. In the proposed field protocol five categories of area are distinguished: < 5 m^2; 5-50 m^2; 50-100 m^2 and 100-500 m^2. Especially for plant sampling purpose, the extent of the spring area can give information about the probability to find a well structured vegetational unit. For example, when the spring area is too small, the minimal area cannot be correctly recognised and the first proviso for the phytosociological approach can fails.

Section 4: Substrates

Numerous example can be found in literature about the importance of lithology and heterogeneity of substrates in determining the type of coenosis for cyanobacteria (Cantonati et al., 1998), diatoms (Cantonati and Pipp, 2000), other algae (Cantonati 1996), zoobenthos (Bonettini and Cantonati, 1996) and bryophytes (Vitt et al., 1986). Chemical characteristics of water are mainly determined by aquifer lithology and, for this reason, it is important to ascertain the nature of rocks with geological maps or eventually by direct assessment and sampling.

In addition, it could be useful to specify the inorganic fraction, assessing percentages of: clay-silt (<0,63 mm), sand (0,63-2 mm), gravel (2-63 mm), cobbles (63-240 mm), stone (>24 cm) and rocks. The fine inorganic fraction can be roughly estimated *in situ* with the idrometer method, which is based on the different sedimentation velocity of the ground particles into water. For this purpose, it would be sufficient a graduated transparent cylinder into which insert the material. The coarser inorganic fraction that cannot be evaluated with this method, and the organic fraction (which is mainly represented by submerged or emerged cushions of mosses, algae, leaves, roots and branches), can be visually estimated. The ratio among those different substrates can provide a useful panorama of the real habitat: this is very informative considering for example that the invertebrate density can be 20 times higher on mosses than on rocks (Chanta et al., 2000). Generally, a measure of substrate heterogeneity (intended as shape) can be taken by means of the metal chain method. In practice the chain should be laid down in such a way to adhere to the substrate. Afterwards it should be taken the distance between the beginning and the end of chain: the ratio distance - length of the chain gives information on the level of heterogeneity of the substrate (Bianchi et al., 2003). If the series of measures is done through regular transects the estimate will be more informative.

Section 5: Flow

In literature it has been widely recognised that current velocity is able to influence not only the benthic assemblages (p.e. Biggs et al, 1998; Swan and Palmer, 2000), but also vegetation (e.g. Sand-Jensen and Mebus, 1996). The flow condition can be permanent during the year and through years, periodic, if the spring is active only part of the year (for example some large karstic springs) - or episodic. Different coenosis are hosted on springs with constant flow or subject to seasonal fluctuations. For example, temperature fluctuations in a permanent spring are less than in a periodic spring. Depending on the season, rainfall and aquifer size, discharge condition can be distinguished in low (minimum level of discharge), medium level or flood (maximum discharge).

The type of current flow can also be distinguished in hygropetric (thin water veil that flows on the rocks), laminar (a quiet flow without rippling), stagnant (standing water), dripping, lively (evident flow but not turbulent), turbulent (rough flow often producing foam) and waterfall. Since different classes can coexist it turns out that can be useful to attribute percentage values on them. Discharge is an important variable to record because of its relevance in structuring the coenosis. In small springs it could be suitable a graduated pail by means of what perform replicated measures in different points of the spring. In larger springs, to obtain a reliable estimate, a flowmeter could be better. To estimate discharge with flowmeter one should record measures along a transect and noting for each point also the water depth.

Finally, besides discharge it could be useful to record at least a dozen of measures of velocities scattered throughout the spring area to give information about the flow heterogeneity.

Section 6: Light conditions

Illumination is another ecological key-factor able to selects the kind of coenosis in streams and springs (Suren, 1992; Hill, 1996). There are many types of photometer on sale which provide reliable measures, especially if not exposed to direct light. But the instrument provides comparable values among sites only if measures are taken in the same weather condition, at the same day-time and in the same period of the year. If these assumptions cannot be satisfied a visual estimate of percentage canopy (perhaps attributing classes of canopy) and paying attention to distinguish deciduous and evergreen, should be sufficient. A careful measure can be taken with a camera and fisheye lens direct upwards. From the picture the percentage of leaching light can be estimated also with specific software. The value of illumination, together with the aspect, provides a realistic description of the light which is available for the site.

Section 7: Buffer zone and disturbance

In regions where agricultural, forest-management or industrial activities are widespread, springs can be severely threatened. Unfortunately, the degree to which springs are influenced by anthropogenic disturbances remains largely unknown, yet such information is urgently needed given the conservation value of the spring biota (Fensham and Price, 2004). From this point of view quali-quantitative information as for example water abstraction, spring-bed alteration, roads, buildings, farms, pastures and their distance from the spring, can give useful indications for nature conservation. A useful advice could be to record, beside the type of impact, also its distance to the spring. In this way one can estimate the strength of that impact.

The field protocol used to sampling springs is presented in the appendix.

5

DESCRIPTION OF SPRINGS AND SPECIES ASSEMBLAGES

Introduction

A major challenge to conservation biologists is to estimate the degree to which natural ecosystems and their biota have been altered by landscape-scale anthropogenic disturbances. Such disturbances may be of severe concern if they threaten the key biotopes, i.e., habitats that harbour valuable components of biodiversity and have far-reaching effects on the surrounding ecosystems. Among aquatic habitats, springs are one of most important key biotopes, because they provide suitable habitat for many rare and threatened aquatic species (Cantonati 2006). Unfortunately, works specifically devoted to spring plants in Italy are hitherto lacking (but see Ortler 1998).

A bibliographic search via ISI Science (performed on the 11th Sept 2007), using the main database like Current Contents and Web of Science, produced only 52 results (with the key words springs and vegetation, springs and flora, springs and bryophytes, springs and plants). The scarcity of works devoted to spring vegetation was only partially revealed by this search however. Spring vegetation was investigated since the fifteenth especially in the Central Europe by German speaking botanists. In the extensive work of Hinterlang (1992) on spring vegetation for example, he quoted 152 works among which only one was in English, several in

French and Italian and the remainder were all in German and none of them was scanned by ISI Science (considering all the available years). Authors who produced these extensive grey literatures, dealing with spring vegetation, discussed plant communities mainly using phytosociological approach. Moreover, these works were particularly focusing on pure classification of plant associations, giving less weight to the most explicative environmental variables able to discriminate those associations.

Vegetation diversity in springs is primarily determined by variations in physical factors such as altitude, solar radiation, water temperature and velocity. Also chemical composition of water plays a major role in determining floristic diversity and structuring spring vegetation. The most important hydrochemical factors are pH, water hardness, oxygen saturation and nutrient content. Besides, duration of snow cover, which in turn affects duration of the growing season, can play an important role in conditioning spring vegetation diversity at high altitudes. Nutrient contents, in areas heavily impacted by human activities, can explain to large extent the vegetation patters. Springs located at low altitude are often endangered habitats, because of agriculture impacts, water caption, eutrophication and pollution. These impacts can result in a rapid decrease of rare and ecologically specialized species and lead to fragmentation of their populations or to local extinction of species (Zechmeister 1988).

Aims of this chapter were to provide the first detailed study of spring vegetation in the Trentino Province (that is also the first extensive study on the Italian Alps). Filling this knowledge gap means even to provide the baseline for future comparison for conservation purpose. Besides the important check list of species for this type of habitat, the second goal was to provide a new spring classification on the base of a joint classification of environmental variables and species. It was suggested that in this way a more objective species associations and spring classification were found because, differently from usual cluster analysis used for species classification, this method allow to describe springs as habitats and spring as species association at the same time. Quite recently, new statistical approaches as LINKTREE (Clarke and Gorley 2006) have been proposed and are nowadays available in statistical package to perform this type of statistical analysis.

Methods: data analysis

Sampling of bryophytes and vascular plants was performed following the procedure explained in Chapter 3. Statistical analysis of plant assemblages were performed using species cover evaluated on the base of the whole spring area (cfr Chapter 3).

Statistical analysis

Cluster analysis on chemical data was accomplished to find spring groups within homogenous and use these groups to explore if they distinguish also significant difference in plant assemblages. Cluster analysis was performed on a resemblance matrix based on Euclidean distances. Homogeneity within these groups was evaluated with SIMPROF test available in Primer statistical package (Clarke and Gorley 2006). This test provide, by means of randomizations, which level of cluster were not significantly different from others. To evaluate if there were different plant assemblages matching with the chemical water groups, I used ANOSIM analysis. ANOSIM (ANalysis Of SIMilarities) is a multivariate technique that compares different matrices and evaluates the differences among them by permutation test. These different matrices were the clusters identified on the chemical data. ANOSIM is performed on the base of a similarity matrix calculated with Bray and Curtis coefficient.

To find species association maintaining at the same time the most uniform as possible the environmental variables, a special statistical technique was used, that is LINKTREE (Clarke and Ainsworth 1993). It compares simultaneously the environmental matrix and the species matrix to find the most homogeneous group considering both the matrices. In that sense it was deeply different from performing the cluster analysis only on species data as is routinely did in phytosociological analysis. The most explicative environmental variables for the species data were selected by BIO-ENV procedure (Clarke & Ainsworth 1993). Since many chemical variables were highly collinear, before running BIO-ENV a PcoA (analysis of the principal coordinates, Legendre and Legendre, 1998) was performed to extract the mayor variance among the following variables: pH, conductivity, Ca, Mg and Na. Then, only those principal coordinates explaining most of the variance, were retained for further analysis in LINKTREE.

To relate directly species matrix to environmental variables a constrained analysis as the Canonical Correspondence Analysis (CCA) was used (Ter Braak 1987). To minimise the influence of rare species, those observed in fewer than 5% of samples were excluded from the analysis of species abundance. Environmental data, before their inclusion in ordination analysis, were transformed by log10(x+1) and percentages by arcsine. Transformation reduced skewed distributions of environmental data. Environmental variables most effective in explaining the species distributions were identified using forward selection tested using Monte Carlo permutation tests (9999 permutations). Multiple tests were accounted for with a Bonferroni correction. Only significant variables were retained for the further canonical analysis. The significance of the first three axes and the sum of all canonical axes were tested by 9999 permutations. Collinearity among environmental variables was detected evaluating VIF (Variance Inflation Factor) in agreement with Teer Braak (1987). Since CCA was performed mainly to find how much variance of species matrix was accounted for by significant environmental variables, a variance partitioning was calculated following Bocard and Legendre (1992).

Results

In this study 86 springs were sampled and a total of 321 species were found, among which 41 liverworts, 126 mosses and 201 vascular plants. The most abundant species were *Cratoneuron filicinum*, *Saxifraga stellaris*, *Viola biflora*, *Chaerophyllum hirsutum*, *Deschampsia caespitose*, *Cardamine amara*, *Palustriella commutata*, *Bryum pseudotriquetrum*, *Brachythecium rivulare*, *Philonotis fontana*, *Rhynchostegium riparioides*, *Sanionia uncinata*, *Cirsium montanum*, *Conocephalum conicum*, *Palustriella decipiens*, *Scapania undulata*, *Palustriella falcata*, *Stellaria nemorum*, *Saxifraga rotundifolia*, *Epilobium alsinifolium*, *Rhizomnium punctatum*, *Plagiomnium undulatum* and *Crepis paludosa*. Less frequent but quite common were *Saxifraga aizoides*, *Stellaria alsine*, *Straminergon stramineum*, *Hygrohypnum luridum*, *Philonotis calcarea*, *Carex frigida*, *Carex nigra*, *Chyloscyphus pallescens*, *Dicranella palustris*, *Plagiomnium affine*, *Plagiomnium medium*, *Cephalozia bicuspidata*, *Jungermannia atrovirens*, *Pellia endiviifolia*, *Plagiochila porelloides*, *Rhizomnium magnifolium*, *Warnstorfia exannulata*.

Water chemistry of selected springs

Water chemistry, with its 26 variables investigated, showed a lot of the lithological complexity of the Trentino. Laveder (2007) stated that the mineral content of the spring water depend on the lithological composition and on the residence time of the water into the aquifer. This residence time into the aquifer depend also by own extension of the aquifers and to some extent also by the altitude where the aquifer is. In fact, at high altitude, aquifers are generally smaller and superficially. All these variables contribute to define the mineral content of water. However, two main water typology could be distinguished, sedimentary and crystalline. In the first group there are three main classes, water at Ca-HCO_3 dominance, water at Ca-Mg-HCO_3 and at Ca-(Mg)-HCO_3-SO_4 dominance. The first class groups the water from limestone, marls and argillite; the second are water from dolomites and the third from evaporite.

Also crystalline water could be distinguished into three main categories: Ca-HCO_3-(SO_4), Ca-Na-HCO_3-(SO_4) and Na-(Ca)-Cl-(SO_4) in agreement to the residence time of water into the aquifers and the consequent mineral dominance.

In addition, in the CRENODAT project several mineral springs were considered as well, like sulphur springs (PS1249 Tonadico, PS1254 Fontane negre, MD1841 Val Contrin-solforosa), iron springs (CV1447 Stol de fer, CV1350 Ferruginosa Miola, OC1399 Miniera

Celentino), iron-magnesium spring (CV1433 Acqua minerale leggera Vetriolo). Mineral springs usually were not so suitable for plants and only in some of those I found species immersed in water.

Lowland springs, near agriculture cultivations, sometimes presented high values of nutrients (especially nitrates and phosphates), as for example AN0430 (Pozza), AN0590 (Dambel), AT0972 (Masere), BR0510 (Gamberi), CV0854 (Giardini Bassa), LD0420 (Spiazzi), LD0720 (Fiavè), AT1052 (Vecchia segheria), CV0633 (Lago Lases) and CV1254 (Grave del Fiorè). Some of those "enriched springs" were ecomorphological impacted too and not rarely we found some curious devices to abstract water for agricultures uses.

Among the trace elements (that is elements occurring in concentrations lower than 0.05 mg L^{-1}) only few were above the detecting limits. Sodium and potassium - alkali metals- were present with median of 1.05 and 0.4 mg L^{-1} respectively. Alkali earth metals, such as strontium, was present with median values of 57 mg L^{-1}, and transition metals like cadmium, cupper and lead were generally under the detecting limits (0.05 μg L^{-1}) while zinc and nickel were detected more frequently (30 and 86 times) with median values of 0.23 and 28.8 μg L^{-1}. Among non-metallic elements, selenium was present above the detecting limit (0.05 μg L^{-1}) in 31 springs, reaching median concentrations of 0.4 μg L^{-1}. Finally, among radioactive elements, uranium was present in 87 springs with median of 0.39 μg L^{-1}.

ANOSIM test on water chemical groups

A cluster analysis performed on a selection of chemical variables and restricting the analysis only to the sites where vegetational data were collected, identified the following main groups (with the exclusion of the mineral springs):

1) lowland springs with high concentration of nutrients: group A
2) limestone springs: two groups differentiated by sodium, potassium, sulphates and potassium in this order: groups B, D.
3) Siliceous springs: two groups differentiated by sulphates, silica, potassium and phosphorous in this order: groups F, G.

ANOSIM analysis resulted significant (R 0.225; $P<0.001$) suggesting that significant assemblages differences exists among those groups. In particular, significant differences were detected between the assemblages found in disturbed springs (A) and all the other groups with the exception of limestone springs with high concentration of sodium, potassium, sulphates and phosphates (group D). There were significant differences also between the groups of limestone springs (C, D) and siliceous springs (F, G). Interestingly, there were not significant differences within the limestone springs (poor limestone B vs rich limestone D) and siliceous springs (F vs G). Disturbed springs (A) presented abundant species of moisture area but not characteristic of springs such as *Eupatorium cannabinum*, *Menta longifolia*, *Petasites hybridus*, *Eurhynchium speciosum* and *Fissidens adianthoides*. The species differentiating the other main groups (C, D) vs (F, G) were various, some of which exclusively found on limestone as *Palustriella commutata* and *Hymenostilium recurvirostre*, several showed only preference towards one or other substrates, like *Adenostyles glabra* and *Philonotis calcarea* predominantly on calcareous substrates, while other species were adapted only to acid springs like *Warnsorfia exannulata* and *Scapania undulata*.

Canonical Correspondence Analysis

To reduce the large data set of environmental variables forward selection was used, with a Bonferroni correction for multiple testing. Only three environmental variables explained an amount of variance statistically significant, these were: 1 Pco (F-ratio 3.97; P = 0.002), altitude (F-ratio 2.62; P = 0.002) and shading (F-ratio 1.70; P = 0.002). Other environmental variables explained an amount of variance that was not statistically significant, therefore they were not used. The canonical axes explain 57 %, 31 % and 21 % of the response table's variance, respectively; they were all significant (P<0.05) and displayed strong species-environment correlations (r = 0.944, 0.892, 0.747, respectively). It should be underlined however, that the amount of variance of species matrix was very high (Total inertia = 9.326), and consequently the sum of all the canonical enginvalues (1.089) explained only a limited portion of the total variance (12 %). The 1Pco explained a variance of 0.352 (3.7 %), altitude 0.260 (2.7 %), shading 0.219 (2.3 %) and the portion shared among them was 0.258 (3.3 %).

Plant assemblages and relative habitats

Before performing LINKTREE principal coordinates were calculated on a selection of chemical variables found to be highly collinear. The first principal coordinates explained more than 99 % of variance thus only the first was retained and actually used in BIO-ENV and LINKTREE. The final set of environmental variables used to classify the springs was: discharge, shading, substrate typology, altitude, N tot, P tot, SO_4, 1PCo. Species data was transformed by log + 1 to low the influence of dominant species and Bray Curtis similarity was used to construct the similarity matrix. Finally, LINKTREE produced the following spring classification: (species were ordered in agreement of their frequency and abundance in that group, where the ratio means the number of time the species was found on a total of springs of that group). Discharge L s^{-1}; shading classes, 1 = 0 % ÷ 4 = 100 %; type = substrate grain (see field protocol); altitude (m a.s.l.); N tot = total nitrogen, μg L^{-1}; P tot = total phosphorous, μg L^{-1}; SO_4 = sulphates, mg L^{-1}; 1 Pco = first principal coordinate, see statistical methods. For each group environmental variables are presented within their range of maximum and minimum and in brackets are median values.

- GROUP 1

Habitat: discharge 0.15÷30 (3.5); shading 3÷4 (4); type 10÷40 (40); altitude 335÷586; N tot 430÷1757 (1080); P tot 2.4÷14.0 (4.1); SO_4 1.27÷18.9 (4.8); 1 PCo -2.30÷ -0.35 (-1.74);

Species: *Rhynchostegium riparioides* 5/6, *Palustriella commutata* 6/6, *Jungermannia atrovirens* 3/6.

Spring codes: LD0509 Fontana del Cioria, PG0474 Pradi, LD0584 Fontanone, MB0335 Diaol, PG0453 Trementina, BR0470 Maso Gori, SC0250 Fontanazzo

This group is characterized by a good median discharge (confirmed by the presence of *R. riparioides*) in shading springs located at low altitude and on coarse substrate (mainly stony). The first Pco rather negative explain that this group was mainly on limestone. Typical combination was *R. riparioides* immersed and *P. commutata* on the edges of springs. Vascular plants were less important in describing these springs, however usually they were not so abundant.

- GROUP 2

Habitat: discharge 0.05÷7 (1.0); shading 2÷4 (4); type 10÷50 (30); altitude 656÷1077; N tot 235÷3038 (710); P tot 3.2÷20.3 (7.8); SO_4 1.7÷35.5 (7.4); 1 PCo -4.00÷ -1.10 (-1.28).

Species: *Palustriella commutata* 9/9, *Plagiomnium undulatum* 6/9, *Petasites albus* 5/9, *Crepis paludosa* 5/9, *Geranium robertianum* 6/9, *Eupatorium cannabinum* 4/9, *Chaerophyllum hirsutum* 4/9, *Palustriella decipiens* 4/9, *Cirsium montanum* 3/9,

Spring codes: BR0658 Faè, BR0679 Tovare, MP0656 Vallarsa, CV0854 Giardini Bassa, BR0790 Sass Ross, BS0705 Coel, AD1077 Frana Eden, AN1000 Vergnana, BR0950 Acqua Fredda.

This group was located above the first group but under the 1077 m a.s.l on limestone. Discharge was decidedly less than in the first group but it was likewise located in woody habitats; there were not a real immersed flora and *P. commutata*, always abundantly present, offer a good nursery for vascular plants.

- GROUP 3

Habitat: discharge 0.05÷35 (0.5); shading 1÷4 (4); type 10÷40 (40); altitude 745÷1235; N tot 375÷1134 (760); P tot 1.9÷11.1 (6.3); SO_4 2.1÷9.9 (3.7); 1 PCo -2.22÷ -0.15 (-0.40).

Species: *Palustriella commutata* 6/7, *Cirsium montanum* 6/6, *Brachythecium rivulare* 4/7, *Petasites albus* 3/6, *Cardamine amara* 3/6, *Rhynchostegium riparioides* 3/6, *Conocephalum conicum* 3/6, *Geranium robertianum* 3/6.

Spring codes: LD0930 Tof glera alta, AT1052 Vecchia segheria, AD0905 Vermongo bassa, BR0804 Pissidrina Sud, LD1160 Croina tre stalle, AD1235 Ponte Prese, VF0745 Madonna del Sas.

Springs falling in this group were characterized by water decidedly less calcareous (the 1Pco was less negative than on the previous groups), generally with low species richness (with the exception of Vermongo Bassa) but with the constant presence of *P. commutata* together with *C. montanum*.

- GROUP 4

Habitat: discharge 0.04÷9 (2.0); shading 2÷3 (2); type 10÷50 (40); altitude 1358÷1880; N tot 259÷1505 (676); P tot 1.8÷36.6 (6.0); SO_4 1.2÷17.5 (3.1); 1 PCo -2.28÷ -0.13 (-0.54).

Species:

Palustriella falcata 8/9, *Palustriella commutata* 8/9, *Bryum pseudotriquetrum* 9/9, *Epilobium alsinifolium* 5/8, *Chaerophyllum hirsutum* 5/9, *Petasites albus* 5/9, *Viola bifora* 6/9.

Spring codes: BR1379 Rislà, BR1436 Scala di Brenta, BR1765 Corna Rosse, LD1502 Tormendos, BR1358 Nambi, BR1605 Rivularia, AD1654 Arnò, AN1474 Fondo, PS1880 Salto Busa dei Laibi.

This group was well differentiated by the previously because of species combination that confirm the shift in altitudinal belt. *P. falcata* tend to replace in abundance (but not in frequency) *P. commutata* which were found always in combination to *B. pseudotriquetrum*. An immersed carpet of *P. falcata* generally covered these springs with at the edge, emerged, *P. commutata*. *E. alsinifolium* was typically found not just in the middle of the flow but rather on small hummock of *P. falcata* or at border flow. *V. biflora* were usually present too but always at low abundances.

- GROUP 5

Habitat: discharge 0.05÷0.8 (0.15), shading 4÷4 (4), type 10÷50 (20), altitude 430÷972, N tot 979÷7400 (2913), P tot 7.5÷73.4 (28.5), SO_4 8.4÷33.0 (14.8), 1 PCo -3.25÷-7.64 (-3.51).

Species: *Eupatorium cannabineum* 3/5, *Palustriella commutata* 3/5, *Cardamine amara* 2/5, *Cratoneuron filicinum* 2/5, *Bryum pseudotriquetrum* 2/5, *Mentha longifolia* 2/5

Spring codes: AN0430 Pozza, AN0590 Dambel, AT0972 Masere, LD0720 Fiavè, BR0510 I Gamberi.

This group of springs was characterized by anthropogenic disturbances (revealed also by the highest Ntot and Ptot) but the species did not seem to suffer this eutrophy. The presence of *E. cannabinum*, analogously to the group 2, could be linked with the high presence of nutrients. Species richness in this and in the following group was very low.

- GROUP 6

Habitat: discharge 0.15÷3 (0.75); shading 2÷4 (3); type 10÷40 (30); altitude 1350÷1670; N tot 575÷1940 (811); P tot 2.0÷19.3 (10.9); SO_4 3.8÷7.3 (6.7); 1 PCo -1.87÷ -0.50 (-0.72).

Species: *Cardamine amara* 3/4, *Menta longifolia* 2/4, *Cratoneuron filicinum* 3/4, *Palustriella decipiens.*

Spring codes: CS1350 Monzon, MB1440 Tolghe, SL1724 Antermont, MD1670 I Ciei.

This small group was characterized by high nutrient values but with a dominance of vascular plants; species richness was low.

- GROUP 7

Habitat: discharge 0.05÷12 (1), shading 2÷4 (4), type 30÷40 (30), altitude 962÷1575, N tot 255÷1272 (422), P tot 5.4÷27.5 (12.6), SO_4 2.1÷25.2 (4.3), 1 PCo 0.58÷2.51 (1.68).

Species: *Brachythecium rivulare* 9/8, *Oxalis acetosella* 7/9, *Chaerophyllum hirsutum* 6/9, *Rhizomnium punctatum* 7/9, *Cardamine amara* 5/9, *Crepis paludosa* 4/8, *Geranium robertianum* 6/9, *Petasites albus* 5/9, *Plagiomnium undulatum* 6/9, *Stellaria nemorum* 5/9, *Saxifraga rotundifolia* 4/9.

Spring codes: AD1300 Borzago, CV0962 Pian Gran, CV1200 Perengola, CV1215 Peterlazet, CV1254 Grave del Fiorè, CV1280 Val Calamento, MC1115 Poloni, OC 0981 Lago Caprioli, CV1575 Torbiera Grugola Bassa.

The springs of this group were located on porphyry or on siliceous but with relatively high water pH. Sulphates concentration can be sometimes high; springs were on woody habitats (*O. acetosella*, *R. punctatum*, *P. undulatum* and *S. nemorum* are typical species of underwood). Immersed flora was generally constituted by *B. rivulare* alone or together with *C. amara*.

- GROUP 8

Habitat: discharge 0.015÷4 (0.7); shading 1÷2 (1); type 20÷50 (40); altitude 1623÷2126; N tot 103÷1157 (337); P tot 2.0÷8.9 (6.2); SO4 1.2÷10.7 (3.3); 1 PCo 1.48÷3.15 (2.20).

Species: *Saxifraga stellaris* 11/11, *Deschampsia caespitosa* 11/11, *Bryum pseudotriquetrum* 9/11, *Cardamine amara* 7/11, *Scapania undulata* 7/11, *Brachythecium rivulare* 7/11, *Warnstorfia exannulata* 6/11, *Philonotis seriata* 6/11, *Eriophorum angustifolium* 5/11, *Epilobium alsinifolium* 5/11, *Dicranella palustris* 5/11.

Spring codes: AD1853 Val di Fumo, CV2051(2) Aia dei Sorgati, AD1790 Lago di Nambino, AD1944 S. Giuliano, CV2126 Lago Giarine, CV1626 Valmaggiore, CV1655 Busa delle Rane, AN1685 Malga Lavazzi, AN1950 Malga Bordolona, CA1642 Teleferica Brusà, CV1940 Val Trigona.

Helocrene springs were dominant in this group; it was confirmed by the presence of *W. exannulata*, *D. palustris* and *E. angustifolium*. These springs were always sunny, on siliceous substrates and generally with high species richness.

- GROUP 9

Habitat: discharge 1÷20 (5); shading 2÷4 (3); type 40÷50 (40); altitude 1421÷1435; N tot 326÷978 (779); P tot 2.3÷10.4 (3.3); SO_4 1.8÷3.5 (2.5); 1 PCo 2.31÷2.53 (2.21).

Species: *Fontinalis antipyretica* 3/3, *Brachythecium rivulare* 3/3, *Scapania undulata* 2/3

Spring codes: CV1435 Le Mandre, AD1353 Nambrone, CV1421 Tornante Slavarè.

This group was characterized by the presence of a good discharge and a well developed immersed bryoflora on siliceous substrates. The special presence of *F. antypiretica*, rather rare in Trentino springs, accounted for the presence of this group.

- GROUP 10

Habitat: discharge 0.15÷4 (2.5); shading 1÷3 (2); type 10÷50 (30); altitude 1855÷2056; N tot 119÷637 (310); P tot 5.0÷17.0 (12.0); SO_4 1.4÷11.5 (8.2); 1 PCo 1.84÷2.72 (2.21).

Species: *Saxifraga stellaris* 5/5, *Stellaria nemorum* 4/5, *Deschampsia caespitosa* 5/5, *Bryum pseudotriquetrum* 5/5, *Rhizomnium pseudopunctatum* 4/5, *Luzula alpinopilosa* 3/5

Spring codes: CV2051 Aia dei Sorgati, OC2056 Belvedere, CV1855 Auzertol, AD1990 Siniciaga, LT1911 Costazza.

Spring of this group presented a characteristic species composition that confirm that these habitats were rather cold and or exposed at north (*L. alpinopilosa*), located at high altitude but still in woody habitats or in shaded conditions (*S. nemorum*, *R. pseudopunctatum*) and on siliceous substrates.

- GROUP 11

Habitat: discharge 0.5÷3.5; shading 1÷1; type 20÷30; altitude 2153÷2314; N tot 276÷1101; P tot 2.4÷4.0; SO_4 1.0÷1.1; 1 PCo 2.84÷3.06.

Species: *Anthelia juratzkana*, *Cephalozia bicuspidata*, *Sanionia uncinata*, *Luzula alpinopilosa.*

Spring codes: AD2153 Levade, AD2314 Amola Rock Glacier.

This was a small group of springs located well above 2000 m a.s.l., rather cold (*L. alpinopilosa*) and with species characteristic of snow bed, like *A. juratzkana*.

On a total of 86 springs this method allowed to classify 70 springs (Tab 1). The remaining sites were unique habitats not presenting analogies with any other sampled springs. For example, AD2739 Maroccaro was a rock glacier spring located at the highest altitude where the only species found were *Schistidium agassizii* and *Hygrohypnum cochlearifolium* with negligible abundances. BR0686 Rio Bianco was a large karstic spring with seasonally high discharge (about 120 Ls^{-1}) that drier in summer where dominate the aquatic moss *Cinclidotus aquaticus*. Other unclassified springs were less peculiar but for other reasons excluded, as for example CV1084 Pirga di Roncegno because it was a “beech spring” (see next paragraph) with underwood species like *Lamium galeobdolon* and *Fissidens adianthoides* and other more common mosses but all at very low abundances.

To visualize altogether the groups a further synthesis was necessary, in which only three environmental variables were selected to discriminate: altitude, 1Pco and shading (Fig 1). In this way, we can obtain a complete picture of the most common spring groups in Trentino. It should be noted however, that if at one side this result summarize almost all the sampled springs, on the other side it failed to be statistically significantly. As presented in the previous paragraphs, only the most distant groups were also statistically different. This means that, if we sampled a number of new springs, statistically we cannot exclude that those new springs would form new groups.

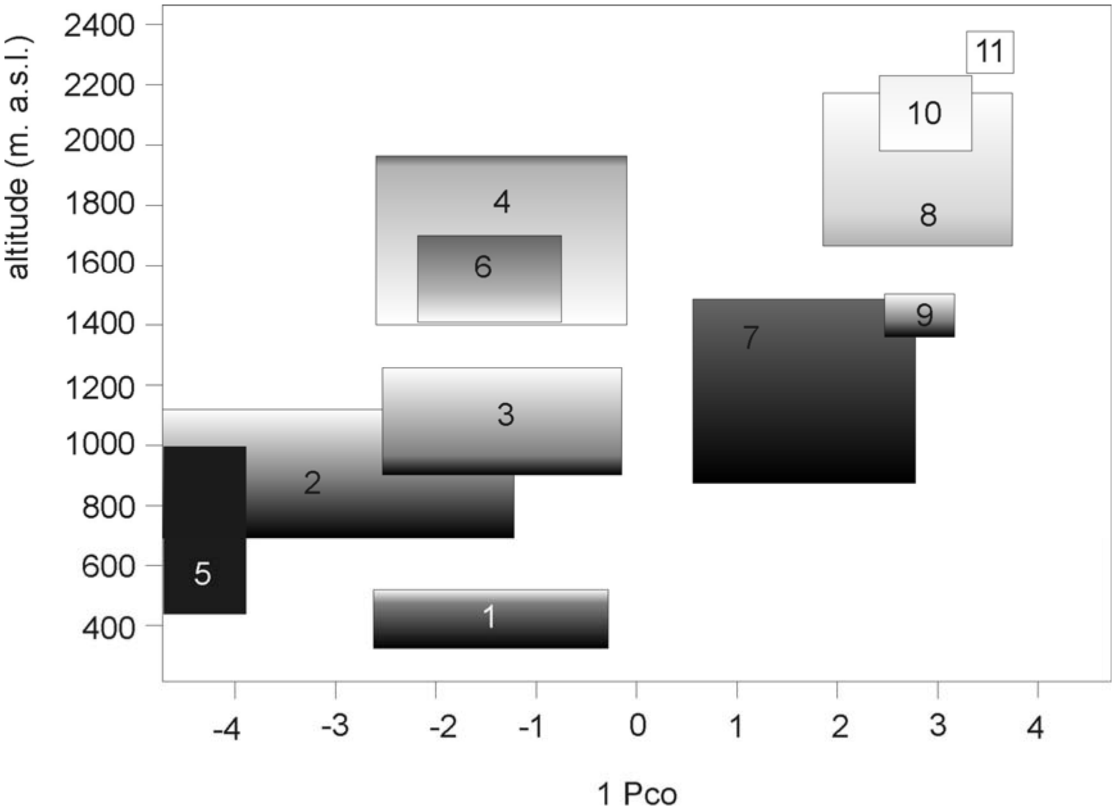

Fig 1. Schematic visualization of spring groups and species associations in a bidimensional space: numbers inside each rectangle represent identified groups by means of LINKTREE. Altitude and first Pco are chose because thought to be the best differentiating environmental variable. Black-white colour represent shading conditions of springs in that species association. Negative Pco can be interpreted as springs on limestone, positive on siliceous substrates. Rectangular sizes are proportional to association range on xy axes.

	GROUP 1 (6)	GROUP 2 (9)	GROUP 3 (7)	GROUP 4 (9)	GROUP 5 (5)	GROUP 6 (4)	GROUP 7 (9)	GROUP 8 (11)	GROUP 9 (3)	GROUP 10 (5)	GROUP 11 (2)
discharge (Ls^{-1})	0.15÷30 (3.5)	0.05÷7 (1.0)	0.05÷35 (0.5)	0.04÷9 (2.0)	0.05÷0.8 (0.15)	0.15÷3 (0.75)	0.05÷12 (1)	0.015÷4 (0.7)	1÷20 (5)	0.15÷4 (2.5)	0.5÷3.5
shading class	3÷4 (4)	2÷4 (4)	1÷4 (4)	2÷3; (2)	4÷4 (4)	2÷4 (3)	2÷4 (4)	1÷2 (1)	2÷4 (3)	1÷3 (2)	1÷1
type class	10÷40 (40)	10÷50 (30)	10÷40 (40)	10÷50 (40)	10÷50 (20)	10÷40 (30)	30÷40 (30)	20÷50 (40)	40÷50 (40)	10÷50 (30)	20÷30
altitude (m a.s.l.)	335÷586	656÷1077	745÷1235	1358÷1880	430÷972	1350÷1670	962÷1575	1623÷2126	1421÷1435	1855÷2056	2153÷2314
N tot ($\mu g\ L^{-1}$)	430÷1757 (1080)	235÷3038 (710)	375÷1134 (760)	259÷1505 (676)	979÷7400 (2913)	575÷1940 (811)	255÷1272 (422)	103÷1157 (337)	326÷978 (779)	119÷637 (310)	276÷1101
P tot ($\mu g\ L^{-1}$)	2.4÷14.0 (4.1)	3.2÷20.3 (7.8)	1.9÷11.1 (6.3)	1.8÷36.6 (6.0)	7.5÷73.4 (28.5)	2.0÷19.3 (10.9)	5.4÷27.5 (12.6)	2.0÷8.9 (6.2)	2.3÷10.4 (3.3)	5.0÷17.0 (12.0)	2.4÷4.0
SO_4 ($mg\ L^{-1}$)	1.27÷18.9 (4.8)	1.7÷35.5 (7.4)	2.1÷9.9 (3.7)	1.2÷17.5 (3.1)	8.4÷33.0 (14.8)	3.8÷7.3 (6.7)	2.1÷25.2 (4.3)	1.2÷10.7 (3.3)	1.8÷3.5 (2.5)	1.4÷11.5 (8.2)	1.0÷1.1
1 Pco	-2.30÷ -0.35 (-1.74)	-4.00÷ -1.10 (-1.28)	-2.22÷ -0.15 (-0.40)	-2.28÷ -0.13 (-0.54)	-3.25÷-7.64 (-3.51)	-1.87÷ -0.50 (-0.72)	0.58÷2.51 (1.68)	1.48÷3.15 (2.20)	2.31÷2.53 (2.21)	1.84÷2.72 (2.21)	2.84÷3.06
Rhynchostegium riparioides	5		3								
Palustriella commutata	5	5	5	5	4						
Jungermannia atrovirens	3										
Plagiomnium undulatum		4					4				
Petasites albus		3	4	3			3				
Crepis paludosa		3					3				
Geranium robertianum		4	3				4				
Eupatorium cannabinum		3			4						
Chaerophyllum hirsutum		3		3			4				
Palustriella decipiens		3				4					
Cirsium montanum		2	5								
Brachythecium rivulare			3				5	4	5		
Cardamine amara			3		3	5	3	4			
Conocephalum conicum			3								
Palustriella falcata				5							
Bryum pseudotriquetrum				5	3			5		5	
Epilobium alsinifolium				4				3			
Viola biflora				4							
Cratoneuron filicinum					3	5					
Mentha longifolia					3	3					
Oxalis acetosella							5				
Rhizomnium punctatum							4				
Stellaria nemorum							3			5	
Saxifraga rotundifolia							3				
Saxifraga stellaris								5		5	
Deschampsia caespitosa								5		5	
Scapania undulata								4	4		
Warnstorfia exannulata								3			
Philonotis seriata								3			
Eriophorum angustifolium								3			
Dicranella palustris								3			
Fontinalis antipyretica									5		
Rhizomnium pseudopunctatum										5	
Luzula alpinopilosa										4	3
Anthelia juratzkana											5
Cephalozia bicuspidata											4
Sanionia uncinata											3

Tab 1. Summary of species associations and relative springs characteristics. In rows are constancy classes: 1 = < 20%; 2 = 20 – 40 %; 3 = 40 – 60 %; 4 = 60 – 80 %; 5 = 80 – 100 %. See text for further details.

Identikit of springs with low richness

On a total of 86 springs, median value of richness (intended as the sum of vascular plants and bryophytes) was 13 species; it is defined as low richness the low quartile (less than 9 species: 17 springs). The main characteristics of these springs and the most abundant species if any are:

- High altitude and scree slope: BR2240 (Vallazza), AD2739 (Maroccaro). While in the first there was a well affirmed dominance of *Palustriella falcata* in the second the were *Schistidium agassizii* and *Hygrohypnum cochlearifolium* with negligible abundances. In both cases, the water soon disappears under the scree. Spring areas were in both cases <0.5 m^2.
- Tufa springs: LD0509 (Cioria), BR0790 (Sass Ross), AN0590 (Dambel), LD0720 (Fiavè), PG0453 (Trementina alta). Dominant species were *Palustriella commutata*, *Hymenostylium recurvirostre*, *Eucladium verticillatum* and in one case also of *Cratoneuron filicinum*. Usually those species covered entirely the spring area forming the well-known tufa rocks.
- High discharge springs: BR0470 (Maso Gori), BR0686 (Rio Bianco), LD1160 (Croina Tre Stalle), LD0584 (Fontanone). Dominant species were *Rhynchostegium riparioides*, *Cinclidotus aquaticus*, *Brachythecium rivulare*. BR0686 was a large karsitic spring (about 120 Ls^{-1}) which dried in winter.
- Limnocren spring: BC0503 (Madonnina Val Lomasona) with only vascular plants (*Lemna minor, Mentha aquatica*, *Epilobium parviflorum, Lysimachia vulgaris*, *Galium palustre*, *Equisetum palustre*). It was a spring with water that wells up from below, forming a pond with a specialized flora and without bryophyte species.
- Disturbed springs: BR0510 (Gamberi), CS1350 (Monzon), PG0474 (Pradi Terlago). These springs presented evident anthropogenic impact (neighbouring farming); here dominant species were *Mentha longifolia* and *Petasites hybridus*.

Springs under beech forests represent special cases. The common characters among these springs were the high quantity of litter that covers a large extent of area. As a consequence, species were usually present with low abundance and diversity. Characteristic species shared among the “beech springs” were difficult to suggest, because of the low number of these springs (6) and the low species shared. “Beech springs” were: AT0972 (Masere), AD1235

(Ponte Prese), BS0705 (Coel), CV1084 (Roncegno), LD0930 (Tof dela Glera Alta), LD1400 (Corteli).

Identikit of springs with high richness

Oppositely for the low diversity springs, I defined springs with high richness those falling into the upper quartile (more than 20 species: 16 springs). Springs with high plant richness did not present evident characters in most cases. However, two groups of high richness springs could be distinguished:

- Helocrene springs: AD1353 (Nambrone), AD1853 (Malga val di Fumo), CV1623 (Valmaggiore), CA1642 (Teleferica Brusà). These springs were all on siliceous substrates, exposed in full sun and with gently slope.
- Springs exposed in full sun, between 1600 and 2000 m a.s.l and near pastures: AD1654 (Val d'Arnò), AN1685 (Malga Lavazzi), AN1950 (Malga Bordolona), CV1855 (Auzertol). In all these cases, there were evident signs of cattle grazing but with low intensity.

The other springs with high richness did not present common features and the discussion on them is on the following paragraphs.

Discussion

Explorative data analysis was planned hierarchically aiming to uncover, step by step, if general patterns were present in the species and environmental matrix. Since water chemistry strictly reflect lithology heterogeneity (Laveder 2007) and since plant distribution are influenced by chemical conditions of substrate (Ellenberg 1988), the first analysis aimed to find if homogenous group of springs (from water chemical point of view) hosted also different species assemblages. This first test was statistically significant and the following considerations can be suggested: (1) plant assemblages of disturbed springs were significantly different from other non impacted springs; (2) there were significantly differences among plant assemblages, depending on the main groups of substrates; (3) there were characteristics species for each type of substrate; (4) more detailed analysis were necessary to understand within differences among groups of substrates. As regard the first point species as *Eupatorium cannabinum*, *Mentha longifolia*, *Petasites hybridus* and to some extent the mosses *Eurhynchium speciosum* and *Fissidens adianthoides* seem to be well adapted to support both water decidedly rich of nutrient and general anthropogenic impacts. This is not to say that these species were indicators of those conditions too, but rather that they can be found also in these environmental conditions.

Indeed, these species occur also in pristine habitats, but generally in springs they were more frequently found in these altered situations. The second general pattern was, as expected, that plant assemblages on siliceous substrates were generally well different from those on carbonates. However, given that the subgroups B and D (on limestone) and F and G (on metamorphic) were not significantly different considering species assemblages, it can be suggested that other environmental variables other than water chemistry were able to further shape the species assemblages.

The second analysis aimed to search, also among ecomorphological variables, which of them explained a significant portion of variance in species assemblages. CCA demonstrated that, besides chemical variables, only altitude and shading significantly contributed in shaping species assemblages. This result was rather surprisingly, because it suggests that more likely there are not general environmental variables able to explain the high diversity of plant species in spring habitats. Further attempts were made to limit the investigation only among groups of habitats within more homogenous (for example analysing only limestone springs), but only limited improvements were gained. The variance explained in different CCA (that is considering a complete analysis or only on specific groups) ranged between 5 and 15 %. This means that a very high portion of variance remains in any case unexplained. Reasons of this weakness can be interpreted as a failure of the assumptions on which CCA is supported (equal niche breath among species, equal species maxima and species maxima homogenously distributed along a gradient, Austin, 2002). A second explanation is that local rather general environmental variables were able to influence presence and abundance of species in spring habitats. In addition, and as investigated in experimental conditions (see chapter 5 and 6), bryophyte species can interact each other generating complex scenarios totally unexpected analysing only environmental variables.

The last important statistical analysis aimed to group the high number of springs in sets of sites within as uniform as possible, considering both species and environmental variables. Although this analysis had not predicting valence, the high number of springs analysed should ensure that the main groups had been identified. A number of springs did not formed groups simply because they represent special cases. Indeed, if new springs were sampled these single springs probably would form groups as well. A specific species combination and a specific values combination of environmental variables, defines the identified groups. As showed in Fig 1, groups were overlapped in different degree, and their distance underlined their difference. Six spring groups were found on limestone and five on siliceous substrates. Altitude, substrate (1Pco) and shading seem to differentiate quite well these groups. Species differences are found only comparing the opposite side of the diagram (Fig 1), whereas neighbour associations shared many species. The presence of many occasional species was particularly evident on siliceous substrates, and particularly considering the bryophyte groups. Thus, it is likely that on siliceous substrates, many other spring groups, and associations, would exist also in a limited area as the Trentino Province. The most common species (that were also usually the most abundant), presented a large tolerance of habitats. For example, *Cardamine amara* was present in 5 groups among a wide range of environmental conditions as well as *Brachythecium rivulare*. Otherwise, species like *Palustriella commutata* was found too in 5 groups but always on limestone and at low and medium altitude; at high altitude *P. commutata* become less abundant and is replaced by *P. falcata* (Hedenäs and Kooijman 2004). *P. falcata* was frequently found together with *Epilobium alsinifolium* and *Viola biflora* (cfr group 4). Shading springs at low and medium altitude are characterized by the presence of genus *Rhyzomnium* spp. and *Plagiomnium* spp. that with *Saxifraga rotundifolia*, *Stellaria nemorum* and *Oxalis acetosella* form a well distinct group on siliceous substrates. In particular, the most springs of this group were found on porphyry substrate. At higher altitude and in shiny conditions on siliceous rocks was present the association composed by *Deschampsia caespitosa*, *Saxifraga stellaris*, *Philonotis seriata* that was enriched, in helocrene springs, by species as *Warnstorfia exannulata*, *Dicranella palustris* and *Eriophorum angustifolium*. At similar altitude but in cold situations (and usually more shading) that association shifted into the next characterized by the further presence of *Luzula alpinopilosa* and *Rhyzomnium pseudopunctatum*. At higher altitude, again on siliceous rocks, liverworts typically found in snow beds become important. However, only two springs fallen in this last group.

Species richness in spring habitats can range substantially. Springs with low richness were generally those located at very high altitude where environmental conditions are too severe. In those conditions generally only few bryophyte species were present. Other peculiar habitats with low richness were tufa springs: in this sites water have high pH, conductivity, alkalinity and high concentrations of carbonates, magnesium, potassium and sodium. Only *Palustriella commutata*, *Hymenostylim recurvirostre* and *Eucladium verticillatum* were usually present with high abundance. It is likely that the dominance of these species together with the peculiar environmental conditions exclude the co-occurrence of other species. In springs with high discharge, that usually were located at low altitude, the immersed bryoflora are represented by *Rhynchostegium riparioides*, *Jungermannia atrovirens* and *Palustriella commutata*, this last one on the spring edge. *R. riparioides* are well adapted to high water flow velocity (Glime and Vitt 1987) thus it is not unexpected to find it also in springs with high discharge. In other large karstic springs, as Rio Bianco, *R. riparioides* can co-occur with *Cinclidotus aquaticus*. It is suggested that the genus *Cincliditus* spp. (Vanderpoorten, 2003) was even more typical of large karstic springs with periodic flow. Finally, other low richness springs were those disturbed by direct and indirect anthropogenic disturbance as partial water abstraction, roads, agriculture etc (more explanation on this topic are on chapter 5). Another poor understood low rich springs group was the so called “beech springs”. It was not a true group with specific species assemblages, but it was still recognizable by low richness and usually fragmented species cover. As the name suggests, these springs were located in beech forest where the substrate are covered throughout the year by a thick layer of litter. Litter is able to depress bryophyte growth and the deep shading reduces vascular plants richness (Ellenberg 1988).

A well differentiated group of springs with high plant richness were helocren springs found only on siliceous substrates. In these springs there were with high fidelity *Warnstorfia exannulata*, *Dicranella palustris* and *Eriophorum angustifolium*. The combination of high irradiance, good discharge, low flow velocity, high damped area probably contributes to high species richness.

The topic of plant diversity in spring habitats is discussed further in the next Chapters.

6

RICHNESS AND SPECIES DENSITY IN SPRINGS [1]

Introduction

Many hypotheses exist concerning specific factors that control plant species richness, but currently there is not a consensus about the mechanisms underlying these relationships (Grime 2001). As far as we know, works especially dedicated to investigate the diversity in spring habitats at the moment are completely lacking. Few works exist on spring vegetation, and those are from a phytosociological point of view (e.g. Zechmeister and Mucina 1994). Spring habitats may provide interesting contributions to individuate the relative importance of different factors controlling diversity. Springs have several characteristics which make them worth to be studied. Springs are generally distinguishable from the surrounding habitats because of a continuous presence of water throughout the year. Moreover, water has minimal temperature fluctuations during the years (Cantonati et al. 2006), and usually discharge shows only limited seasonal variations. These characteristics allow considering springs like "water islands", as proposed by Werum (2001). Accepting and recognising these unique characteristics, we may treat them also theoretically like islands. A large literature exists about diversity on islands (Whittaker et al. 2001 and references therein). One of the most important

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[1] Contents of this chapter are published on: Spitale D., Petraglia A., 2008. Springs like islands: implications on richness and species density. *International Association of Theoretical and Applied Limnology* 30. In press.

factors in determining the species richness is the area extension. As demonstrated by Lomolino and Weiser (2001), confounding variation in area with variation in other environmental factors could lead to biased results. One way to control area, a fundamental step that is very often ignored, is to use sample units of equal dimensions, and hence to calculate species density instead of species richness. We agree with Whittaker et al. (2001) in distinguishing diversity in richness (the number of species recorded in unstandardized way) and density (the number of plant species in standardized plots).

Springs and relative biota can be correctly considered as azonal (sensu Ellenberg) because of their relative independence of climate conditions. In other words, the spring communities appear in the approximately the same form because they are more determined by the presence of water rather than the overall climate as in the zonal units. Therefore, differences among spring assemblages are mainly determined by difference in water characteristics (i.e. chemistry, current flow etc.). The “azonalness” of spring assemblages can depend on the degree of “aquaticness” of the species living in a spring. Considering an altitudinal gradient for example, there will be more species variation in spring assemblages as the degree of species “aquaticness” decrease. Cantonati et al. (2006) reported that diatoms did not show relevant patterns along altitudinal gradients with reference to samples collected always from submersed substrates. However, given that in spring habitats bryophytes and vascular plants grow even outside the water, it would be interesting to explore plant richness and density in springs along altitudinal gradients.

The following hypotheses can be formulated: a) richness (intended as the summation of the bryophytes and vascular plants species per spring) depends from the spring area because of the well known species-area relationship; b) density (intended as number of species per m^2) is independent from spring area; c) since the majority of plants growing in springs are not strictly aquatic, we expected that both density and richness changing along the altitude, analogously to other habitats.

Study site and methods

See Chapter 3.

Results and discussion

Although measuring the area may be relatively easy in true islands, it could result more problematic in spring habitats. Frequently, spring boundaries are not clearly distinguishable and, therefore, the meaning of area may depend on the scale of the organisms investigated.

Obligate aquatic organisms are common in springs, but represent only part of the entire community. Bryophytes sometimes have been used as indicators of spring boundaries (Cantonati et al. 2006), but they may be informative only when species of known ecology are encountered. Vascular plants having a root system may grow farther from water than bryophytes. Notwithstanding, the equation spring = island remains valid to the extent to which the spring area can be distinguished from the surrounding environment. Since the shape of springs is usually elongate, we have first to define their dimensions. The width of a spring can be defined by means of the relationship between the moisture decrease and the distance from water. As demonstrated for bryophyte substrates (Chapter 8) a sigmoidal relation links the two variables. Supposing that this is a common trend, we might define the spring width as the point where the tangent slope reaches its maximum. In contrast, the length of springs is not so practicably definable because the longitudinal gradient is more gradual than the transversal one. Since vascular plants are the largest species in spring habitats, we used them as indicators (as a sort of umbrella species) of the minimal length. We considered the minimal length the distance from the spring source necessary to record at least one time the "relevant" vascular plants. Since there are not characteristic spring species, we consider "relevant" those species that are not present in the surrounding environment.

Once our sampling area is defined, the difference between richness and density (mean density among replicates) becomes clear (Fig. 1). Given that usually to detect the extension of spring area we used also vascular plants, richness was expected to depend on extension of spring area (R^2=0.08; t=2.78; P=0.007; df 86). As the low R^2 suggests, many other factors can contribute to the species richness other than area. Spring area was often (60% of times) so small that correct independent replicated plots were not feasible. In those cases, only one fixed plot was sampled in addition to the phytosociological relevè (which typically contains all the relevant spring species). Pooling together all the species recorded in replicated plots, we obtained a significant correlation with the number of species per relevè (r Pearson 0.894, P<0.001). A t-test for paired data (Fig. 2) revealed that, on average, phytosociological data contained more species than the sum of replicates (14±6SD vs 11±5SD, t_{84}=6.5 P<0.0001), but the high correlation demonstrates the interdependence between standardized and unstandardized methods. This result was in agreement with Chytrý (2001), who showed that generally phytosociological data overestimate richness, but we suggested that performing as many replicates as possible (in our case max = 4), values of richness provided by relevès are coherent to those provided by standardized plots. Chytrý (2001) argued that phytosociologists tend to use larger minimum areas in species-poor vegetation. This could not be the case in spring habitats following the definition of area. In fact, exactly the opposite trend was found (Fig. 3): small minimum areas were used in species-poor vegetation. Indeed, given the low variance explained (about 17 %) this trend should be considered only a weak tendency. However, this is a clear consequence of the spring borders. To overcome the problem of comparing richness of relevès of different size, Chytrý and Otýpková (2003) suggested using standardized plots. The results obtained in this study by means of replicated plots confirm the correctness of their proposal. In fact, using fixed plot of 1 m^2 and averaging the number of species among replicates, we obtained estimates of species density, which resulted independent from minimal area (R^2=0.00; t=0.14; P=0.89; df 84) and from spring area (R^2=0.02; t=1.30; P=0.20; df 84). These results suggested that (i) spring richness depended to some extent on the size of springs=islands, and (ii) that density was independent of area.

Spring richness and density clearly vary with altitude (Fig. 4), but some distinction can be suggested. Considering separately the relationship of richness with altitude for vascular plants and bryophytes (Fig. 5), the following trend was observed: vascular plants increased up to about 2000 m a.s.l. and then declined following the well-known hump-shaped curve (Grime 2001). Interestingly, bryophytes showed a change of slope at superior elevation than vascular plants (we detected a unique case at 2730 m a.s.l. where the bryoflora was reduced to only one species). Therefore, we could suggest as empirical evidence that spring richness, intended as the sum of vascular plants and bryophytes, was positively synergic up to 2000 m, opposite between 2000 and 2400, and newly but negatively synergic at higher altitude. Several explanations were suggested (reviewed by Lomolino 2001) other than sampling artefacts: altitudinal gradients in area, climate, geographical isolation of mountain communities and feedback among zonal communities. A complex inter-relationship may exist between density and richness along altitudinal gradient: at low and medium altitude, vascular plants usually dominate the spring flora. Going upwards, the size of vascular plants decreases and, therefore, many species can be recorded inside one square meter. At high altitude, bryophyte, and especially liverworts, can become dominant in richness. The summation of altitudinal effects on vascular plants and bryophytes results in a general increase of richness with altitude up to about 2400 m. At higher altitude both vascular plants and bryophytes decrease.

In the following chapter (7) species richness will be studied with a more sophisticated statistical approach (Structural Equation Modeling) able to consider together the different sources of variation and provide more detailed explanations of the diversity trends in springs.

Figure captions

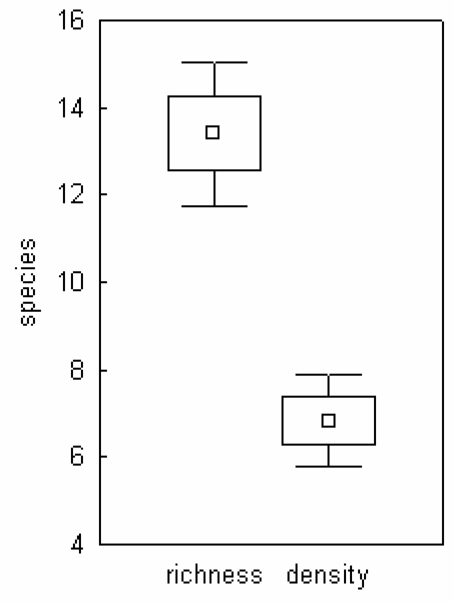

Figure 1. Difference between species richness and density. Mean; box=ES; whisker=1.96ES.

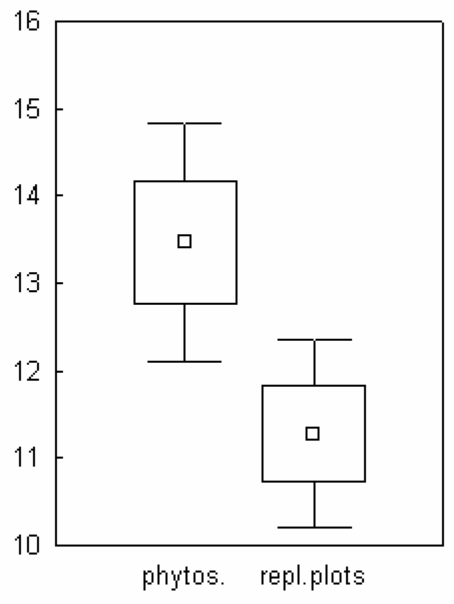

Figure 2. Difference between species richness by phytosociological method (phytos.) and by replicated plots (repl.plots). Mean; box=SE; whisker=1.96SE.

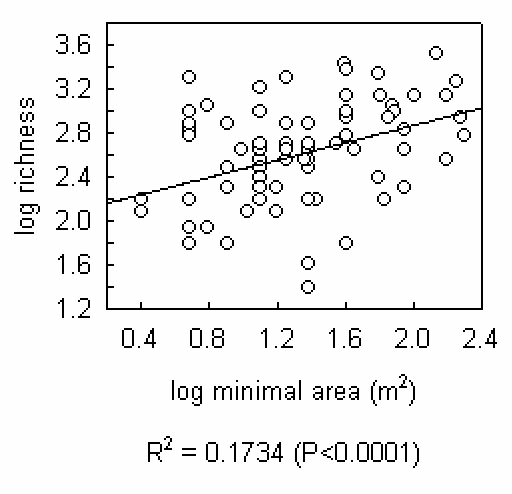

Figure 3. Linear regression between minimal area and richness.

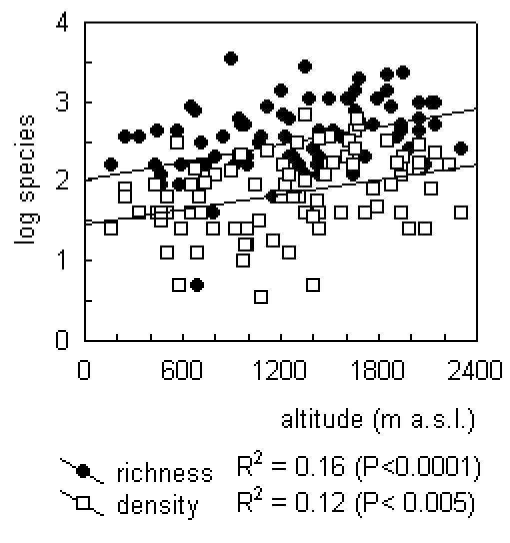

Figure 4. Relationships between richness and density (bryophytes + vascular plants) vs altitude. Linear adaptation after removing the spring located at highest altitude.

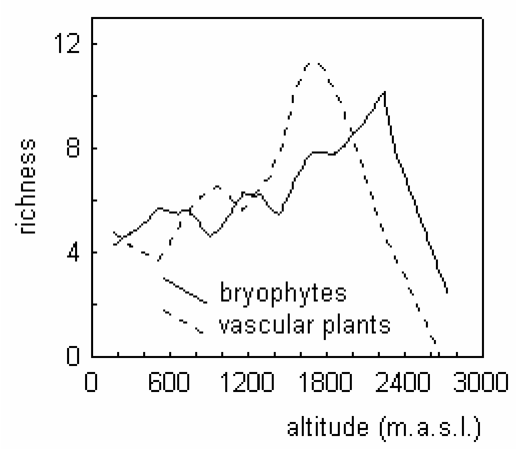

Figure 5. LOWESS diagram describing the relationship between bryophytes and vascular plants richness vs altitude.

7

HOW PLANT RICHNESS DIFFER IN MOUNTAIN SPRINGS? [1]

Introduction

Aim of this chapter was to develop and evaluate a structural equation model explaining the bryophytes and vascular plants richness along multiple environmental gradients in spring habitats. Primary interests in developing this model were: 1) to evaluate the effect of tree canopy along the altitudinal gradient on bryophyte and vascular plants richness; 2) to determine to what extent lithology was able to explain richness in the two group of plants; 3) to assess if anthropogenic disturbance lower the richness; 4) to explore, comparing competive models, which were the causal links connecting spring area, discharge and spring complexity and how these variables were related to richness.

Numerous Authors have suggested the existence of general relationships between plant diversity and different variables, such as biomass (Grime 1973), area (Gleason 1925), latitude (Currie and Paquin 1987), precipitation (Whittaker and Niering 1965), successional time (Bazzaz 1975) and disturbance (Connell 1978). Palmer (1994) listed more than 100 hypothetical explanations to describe species richness. In the last few years there has been an increasing awareness of the role of these explicative variables in the system under study (Grace

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[1] Contents of this chapter are presently submitted as: Spitale D., Petraglia A. & Tomaselli M., 2008. How bryophyte and vascular plant richness differ in mountain springs? An advance using a structural equation model.

and Pugesek, 1997). A target variable as richness, can be influenced both directly and indirectly by a supposed set of explicative variables. Failing in distinguishing these different paths and in giving them the right importance can lead to consider spurious relations (Shipley, 2000). Interrelated effects among abiotic variables, as for example canopy cover and mineral content of soil (Weiber et al 2004), fine scale spatial variations (Mancera et al 2005) or time since the last fire (Laughlin and Grace, 2006) are widespread in natural systems and they should be incorporated in the conceptual model of richness to enhance our interpretation of results. Ideally the effects of these interrelated variables should be tested experimentally, but in many cases manipulations is not possible for the high number of involved factors. One alternative to experimentation, or a first step in unexplored system, is to use the powerful statistical tool of Structural Equation Modelling. The term SEM conveys two important aspects of the procedure: (i) that the causal processes under study are represented by a series of structural (i.e. regressions) equations, and (ii) that these structural relations can be modelled pictorially to enable a clearer conceptualization of the theory under study (Loehlin 1987). The hypothesized model can then be tested statistically in a simultaneous analysis of the entire system of variables to determine the extent to which it is consistent with the data (Shipley 1997; Grace 2006). When acceptable models are obtained, the results have the potential to indicate the roles that different factors play in a system and the strengths of different pathways.

In this work we examined mountain springs because we believe that they are privileged system to investigate plant richness thanks to their characteristics. Springs are differentiated from the surrounding habitats because of a continuous presence of water throughout the year with minimal temperature fluctuations (Cantonati et al. 2006). High oxygen saturation, air humidity, hydrological stability and less hard winter conditions are some of the most important characters distinguishing springs. Plant communities in spring areas are well studied but their richness has not been specifically investigated to date. In addition, the prosperity of bryophyte and vascular plants species render the spring habitats suitable sites for richness studies. Given the unanswered question about the difference between bryophyte and vascular plant community (Steel et al 2004), the study of spring habitats could provide a singular opportunity to disentangle the importance of different environmental variables on the two groups of plants. Investigated abiotic variables in our model were altitude, tree canopy, lithology, anthropogenic disturbance, spring area, spring complexity and discharge. In particular we incorporated the indirect effect of altitude on tree canopy (Weiber et al 2004), the direct effect of lithology on richness (Virtanen et al 2003) and the direct effect of anthropogenic disturbance (Mensing et al

1998) on richness. Moreover, advances were suggested about the direct importance of spring area on richness (borrowing the traditional relationship species-area in islands, Lomolino & Weiser, 2001) and the indirect effect of discharge, both through spring area and spring complexity, in determining richness.

Methods: data analysis

THE STUDY AREA

Study area is reported in Chapter 3

MODELLING APPROACH

The modelling method used in this study is SEM (Structural Equation Model) (Bollen, 1989). SEM is a multivariate statistical methodology that encompasses factor and path analysis (Pugesek et al., 2003). Differently from multivariate regression however, SEM allows the user to test indirect effects between two explanatory variables, where effects between two variables can be mediated by another intermediary variable (Bollen, 1989). Given the interesting features of this method, it is quite surprising that the number of applications in ecology is limited (but see Johnson et al, 1991; Shipley 1997; Arhonditsis et al, 2006). SEM is a complex method and we cannot hope to explain the entire process of modelling, therefore interested reader could refer to many extensive works as for example Bollen (1989) or to a general introduction such as Loehlin (1987). Recent reviews of this method now exist also for ecology and are treated in Shipley (2000) and Grace (2006).

DATA COLLECTION AND MEASUREMENT VALIDITY

Since we were fairly confident that some of the indicators were not perfectly measured (i.e. without errors) we incorporated errors in the equations. All the measurement errors were fixed in agreement with our scientific judgement, experience and available data. Spring area can be defined as the extension where water exerts its influence. Since detecting the limit of a gradient is difficult to accomplish with low measurement error, we used an indicator in place of the total spring area. We assumed that the total area was directly proportional to the extension of the wettest area. Hence, estimating only the wettest area can be a good way to control measurement errors. To evaluate if the wettest area was a reliable indicator we calculate the Pearson correlation between total area (measured with high error) and wettest area (measured with low errors): r 0.40; $P<0.001$. Therefore in structural equation we used the wettest area as predictor of the latent total area and we fixed the measurement error at 10% of the total variance.

Within the wettest area, all the species of bryophyte and vascular plants were collected to further identification (see Chapter 3). We assumed a measurement error of 1% and 5% respectively for vascular plants and bryophytes. A quite bigger error was assigned to bryophyte richness because, in spite of the careful field survey, small liverworts could have been lost. Altitude was measured by GPS with an instrument error of 10-15 meters. We estimated this error in 1% of the total variance. Water conductivity was measured twice, in the field with portable multiprobe and in laboratory. The two measures were usually in good agreement therefore and 1% of measurement error was assigned. Canopy was estimated visually in five classes, 0, 25, 50, 75, 100 %. To estimate the error of this measure we compared the values of class canopy independently attributed by different operators. There were always good agreements, so we assigned only 5% of the total variance. Discharges in perennial springs are somewhat uniform throughout the year with only seasonal fluctuations (Cantonati et al 2007). Since we measured discharge only one time for the majority of the considered spring, we performed our survey during one season (summer 2005). Discharge was measured by graduated pail (for further detail see chapter 4) replicating the measure in different points of the spring. We attributed 10% of error at this measure.

Spring complexity was a variable that describe the spring bed. It was composed mainly by two different attributes: the shape of the spring bed (linear, wavy, bend) and the typology of spring (single source, several similar sources, several but different sources). The final scale was in six classes with increasing order of complexity. To evaluate the measurement error we followed the same strategy as for canopy but doubling the error (10%) because of the combination of different variables. The concept of disturbance consists of the processes which limit the plant biomass by causing its partial or total destruction (Grime 2001). In our framework we limit that concept omitting for example disturbances by climatic fluctuation. We considered basically direct and indirect human impacts. Examples were: water abstractions, roads (different types) building, spring bed modifications, forestry, agriculture. The intensity of these disturbances was estimated as the distance at which the disturbance took place. For example, a partial water caption just around the water source will have higher impact than an analogous situation far away downstream. The summation of the products of disturbances by their distances gave a score representing the total disturbance for that spring. The resultant was a continuous variable from 0 impacts to 16, the highest impact recorded. In analogous manner as for spring complexity we judged the amount of error in 10%.

STATISTICAL ANALYSIS

Prior to SEM analysis, data were transformed to achieve linearity with richness (Fig 1) and then transformed to normal scores with PRELIS (Jöreskog & Sörbon 1996). Several springs were removed before the analysis because they represented special cases, as mineral springs (exceptional high values of conductivity), karstic springs (highest discharge) and springs located well above the tree line (>2080 m asl). Those latter cases were removed because springs above the tree line are much more diverse than those in lower region (Philippi (1975). The final data set consider 86 springs.

SEM analysis was performed using the covariance matrix of variables and presenting a standardized solution in the output (Fig 3). To obtain a more confident solution we applied bootstrap resampling. The bootstrap method consist in extracting a random sample from original data a specified number of times (500 in this case) to generate the sample bootstrap estimates and standard errors. The bootstrap estimator and associated confidence intervals were used to determine how stable or good the sample statistic was as an estimate of the population parameters. We used a modified bootstrapping method (Bollen and Stine, 1992) because it has superior performance. For basic general statistic analysis we used STATISTICA v6 and for structural equation modelling we used EQS v6.

MODEL SPECIFICATION

Our intent was to test both just accepted theoretical explanation about richness and some new variables potentially important in spring habitats. The second general objective was to evaluate the difference in paths strength between the environmental variables and the bryophytes and vascular plants richness.

The model investigated in this paper hypothesizes that variations in richness can be explained by indirect effects of altitude through tree canopy. Patterns of altitudinal gradient may result from combined effects of many redundant or convergent processes. Because woodland communities changes according to the altitudinal gradient, the site conditions of the undergrowth changes coherently. Living inside deciduous or coniferous forest change the effects to which the light spectrum passes through the leaves, both quantitatively (depending also by the plant density) and seasonally (Messier et al 1998). Here we were not excluding that elevation per se cannot have directly influence to richness, but rather we were hypothesizing that the elevation could be less important for herbaceous species than could be the light availability. Indeed around the tree-line light ceased their effect and other climatic-related factors took place. Moreover the spring water, whose temperature show very little fluctuation both intra and inter-annually, may have a buffer altitude effects across the spring area. This latter feature was the most important characteristic allowing to consider spring like island.

The further hypothesis was that water conductivity (used as proxy of lithology) directly affects richness. The direct link between lithology and richness was suggested by several authors (reviewed in Virtanen et al 2003) who recognized the calcareous substrates richer in species than siliceous one. Explanation of this patter spans from the higher species pool hypothesis on calcareous substrate (Zobel 1997) to the evolutionary hypothesis of an older origin of calcareous species (Conti et al 1999) and to the competitive exclusion hypothesis on silicate (Gigon 1987). In addition we allowed correlating altitude and conductivity because of lithological reasons, being siliceous substrates at higher altitude than carbonates in Trentino.

The following hypothesis was that spring area contributes directly in determining richness and that spring area was determined by discharge of springs and by its morphological complexity. Whereas the first hypothesis about the relation area-species can be considered a general ecological rule (Lomolino and Weiser 2001), the second and third can be considered exploratory. The rationale of this exploratory scenario was that discharge can cause the extension of spring area because increasing discharge increases also the damped area.

Complexity of springs describe the morphology of springs bed (see data collection), therefore as the complexity increase spring area will be expected to extend because a larger area will be damped. Because complexity may, to some extent, increase the total number of available habitats, we allowed to explore this paths directly to vascular and bryophyte richness. Given that this scenario was explorative alternative models will be compared.

In our model we considered also disturbances as direct variable affecting spring richness. In this framework with disturbance we referred to anthropogenic direct and indirect disturbances (see data collection). Land uses, such as forestry and agriculture, are presumed to degrade biodiversity (Mensing et al 1998). Since the amount of anthropogenic disturbance may decrease with altitude we allow them to correlate.

Results

UNIVARIATE DESCRIPTION

Richness in spring habitat varied between from 2 to 34 species (mean 14±7SD). In total we found 167 species of bryophytes and 201 of vascular plants. Mean values per springs were, respectively, 6.9±4SD; 7.4±4SD for bryophytes and vascular plants. A t test for paired data revealed no statistical differences (t_{88} -0.80; $P>0.05$) between the groups within springs.

Species richness of bryophytes and vascular plants generally increase both with altitude and spring area and decrease with conductivity and canopy (Tab 1). To evaluate the presence of trends along the altitudinal gradient but not captured by linear models, we used LOWESS method (Locally Weighted Scatterplot Smooting) with a smoothing window of five points. In this analysis we used the entire data set, not removing any sites. The altitudinal patterns of bryophytes and vascular plants differed (Fig 2) although both were positively correlated with altitude and even each other (Tab 1). Richness increase in the two groups of plants similarly up to about 1600-1800 m a.s.l.; then vascular plants showed a rapid decreased while bryophyte reached a maximum at about 2200-2400 m a.s.l. Species accumulation curve (Fig 4), calculated only for bryophyte species, showed a higher richness on siliceous substrates than on limestone.

TESTING MODELS: MUTIVARIATE APPROACH

The results of Mardia and Bonett-Woodward-Randall test showed no significance excess kurtosis indicative of non-normality leading us to accept the hypothesis of multivariate normality, so maximum likelihood estimation technique was chosen to estimate parameters and statistical significance.

The first model hypothesized that the extension of spring area was determined by discharge and spring complexity whereas the competitive model was that area was determined directly by discharge and indirectly via spring complexity (Tab 2). Fit improvement was strongly significant ($\Delta\chi^2$ 9.05 df 1 P 0.003) indicating that the competitive model was more appropriate. Analysis of specific structural equation model found that it was consistent with the data (χ^2 = 18.01 P = 0.26 (df =15; RMSEA 0.049; CFI 1.00; GFI 0.96) [note that the non significant χ^2 in this case means that the covariance structure of the data did not significantly deviate from the covariance structure implied by the models]. Root Mean Square Error of Approximation give the discrepancy per degree of freedom: by convention there is a good model fit if it is less or equal to .05 and adequate fit if it is less than .08. Comparative Fit Index and Goodness of Fit Index range between 0 and 1 where 1 indicated a very good fit. Examination of matrix of standardized residuals and Q-Q plots did not reveal substantial discrepancies and the average was lower (0.05). Since the path from spring complexity to area was not significant we tried to eliminate it but, since the fit improvement was not significant, we did not delete this path from the final model. The variance explained by endogenous variables in the final model was fairly high for vascular plants (R^2 = 0.55) and canopy (R^2 = 0.37), less for bryophytes (R^2 = 0.24) and low for spring complexity (R^2 = 0.14) and area (R^2 = 0.06).

Standardized path coefficients for this model area shown in Fig 3. To examine the consequences of random error for this model and the sensitivity to changes in regression coefficients, we doubled the original fixed measurement errors. As showed in Tab 3 this second model fit well the data indicating that the model structure was stable. Major changes in regression coefficients occurred in the paths linking discharge-spring area-spring complexity because they shared the higher measurement error (20%). Generally the reliability of the endogenous variables increased in the model with doubled error and the major changes took place in spring complexity (from $R^2 = 0.13$ to $R^2 = 0.17$) and vascular plants richness (from $R^2 = 0.55$ to $R^2 = 0.60$). A third model with triplicate errors (this was an unlikely scenario though) failed to converge to an admissible solution but we did not further examine this problem.

Indirect effects, that are those effects mediated by other variables, were estimated simply by multiplying the standardized paths involved. Thus, for example, the indirect effect of altitude on bryophytes was 0.015 (-0.608 x -0.024) while on vascular plants was 0.31 (-0.608 x -0.502).

Finally, the Bollen-Stine's bootstrapping p-valued (on the model with original measurement error) based on 500 bootstrap samples was P=0.22, which indicates that the data did not depart significantly from the model at any conventional significance level. In Tab 3 we presented both parameter estimates from the original model and also from the bootstrap resampling procedure.

Discussion

Consideration of the relationships among variables reveals several important features of species richness in spring habitats located in Italian Alps. First, as showed by Grytnes et al (2006) and Pharo et al (1999), species richness of bryophyte and vascular plants show different patterns along environmental gradients. Previously studies have demonstrated both that bryophyte richness was higher than vascular plants (because of the role of mutualism and less efficient competitive exclusion, Slack 1990) and that no differences were detectable (Steel et al 2004). Our multivariate approach allowed comparing the two groups of plants considering at the same time the most important environmental variables in spring habitats. Richness in spring habitats resulting from a complex interaction of both direct and indirect effects that without this specific statistical approach was unlikely to be detected. The effect of altitude on vascular plants through tree canopy was positive and strong whereas the same path on bryophytes was hardly positive. By univariate approach (Fig 2), the relationship between species richness of both group of plants and altitude seem to be positive but these relations resulted from different causes. Whereas for vascular plants altitude and light were significantly related, for bryophytes the increasing with altitude was more related to conductivity (more species on siliceous substrates). Since tree canopy was an indicator of light availability, vascular plants resulted more sensitive to light competition (Bartemucci et al 2006). In contrast, bryophyte diversity can be high also at low light intensities because usually they have low light compensation point (Valanne, 1984). Moreover, bryophytes presented special features that rend them well adapted to shade conditions (Marschall and Proctor, 2004). Interestingly, this explanation seems to be valid only within the altitudinal range considered in our model. Above the tree line (out of our model range however), when canopy has no longer influence on light penetration, other explanations could be proposed. According to the lowess line (Fig 2), there was an evident hump in vascular plant richness just around the tree line (high light availability and not harsh climatic conditions). Differently, bryophytes showed a change of slope at higher altitude than vascular plants (we detected a unique case at 2730 m a.s.l. where the bryoflora was reduced to only two species). This hump at high altitude for bryophytes was concomitant with a clear change in species compositions with the predominance of small liverworts typical of snow beds (Petraglia and Tomaselli, 2007).

Water conductivity, that is an expression of substrates, played a significant role in structuring plant assemblages in springs. Previously works on other habitats, and considering only vascular plants, showed the higher richness on limestone than siliceous substrates (Virtanen et al 2003) but the opposite trend either (Wohlgemuth and Gigon, 2003). In the present study we found no significant relations from conductivity to vascular plants but, in contrast, a strong negative relation from conductivity to bryophyte richness. We explain this pattern suggesting that differently to vascular plants (for which the calcicole species are more numerous than the calcifuge, Pärtel, 2002), hydrophilous calcifuges bryophytes are more abundant than calcicoles. According to our hypothesis, the overbalance of calcifuces bryophytes lead to a more conspicuous “reservoir effects” (Pärtel, 2002) and therefore to a more richness of hydrophilous bryophytes on siliceous rocks. This large number of hydrophilous calcifuges bryophytes could be the results of two different aspects, physiological and evolutional. The first one resulted from an inefficient bicarbonate uptake as inorganic source for photosynthesis: in calcareous water (high pH) inorganic carbon exist mainly in the form of bicarbonate whereas in softwater, free CO_2 is the main carbon source (Bain and Proctor, 1980). Thus, in base-rich water there could be lesser species than on softwater because in the former the carbon source in the water is unsuitable. The second explanation is based on the assumption that if species requirement correspond to the conditions where they evolved, and if the silicate rocks are generally more damp (Michalet et al. (2002), then we should expect a larger species pool of higro-hydrophilous bryophytes on silicate. A further evidence of this explanation is gained comparing the species accumulation curve for bryophytes on siliceous and on carbonate rocks (Fig 4). The accumulation curve for limestone species was shallower than siliceous one and seems to reach an asymptote. In contrast, siliceous species showed no sign of levelling off.

Disturbances, as expected, had negatively effects on both bryophyte and vascular plants. However, results indicate that disturbance has more strongly negative effects on bryophytes than on vascular plants in all the tested model (Tab 3). This was not totally unexpected given that the most frequent disturbances were related to water availability (partial water abstraction, spring bed modifications etc). Not totally unexpected was the weak negative correlation between disturbances and altitude. This weakness was induced by pasturing disturbances occurred at altitude > 1700 m a.s.l.. Thus, while at low altitude disturbances were characterized by direct anthropogenic effects (i.e. water abstraction, bed modification, agriculture etc), at higher altitude disturbances were mainly determined by pasturing activity (livestock grazing, trampling etc). Thus, the supposed decrease of disturbances with altitude, was not confirmed.

The exploratory part of the model about the spring area-complexity-discharge paths seems fairly consistent with the expectations. Indeed, the low R^2 for area (0.07) and spring complexity (0.13) suggested that others unidentified variables played a substantial rule other than only discharge. However, to some extent discharge affects the increase of spring complexity and also of the spring area. Then, as expected, spring area influenced positively both bryophytes and vascular plants richness. In all the model tested though (low and double measurement error and bootstrapping simulations), the positive effects of area was more strongly on vascular plants than on bryophytes. This difference is a matter of scale because in species-area relation, asymptote of bryophytes richness is reached before than vascular plants.

The implied negative effect of spring complexity on vascular plant richness was perhaps the most counterintuitive result in the model. The biviariate relationship between vascular richness and spring complexity was not significant. Nevertheless, once the variables canopy, conductivity, impact and area were statistically controlled, model results implied that complexity affects negatively the vascular richness. We interpreted this negative influence as the tendency of species as *Cardamine amara* and *Saxifraga stellaris* to dominate (= to cover most of the spring area) in situations where there were a moderate discharge (= high score of spring complexity).

SPRINGS CONSERVATION

Even in mountain areas like the Trentino Province in the Southern Alps springs are severely threatened. In the last years land use and water abstraction have considerable reduced the number of springs especially at low altitude, where water are intensively exploited for agriculture use. As our results showed, spring bryophytes seem to be the most endangered because of their dependence from water. Because of human impacts, Heino et al (2005) reported significant decreases in abundance and occurrences of several bryophyte species in Finland springs. The extension of spring area, discharge and human impact are crucial variables both for vascular plants and bryophytes richness. Therefore to limit the damage of natural springs we may suggest protecting the larger springs and, in the case of water requirement for agriculture use, to capture water at some distance downward the spring source. In this way at least the spring biota will be preserved.

Figure captions

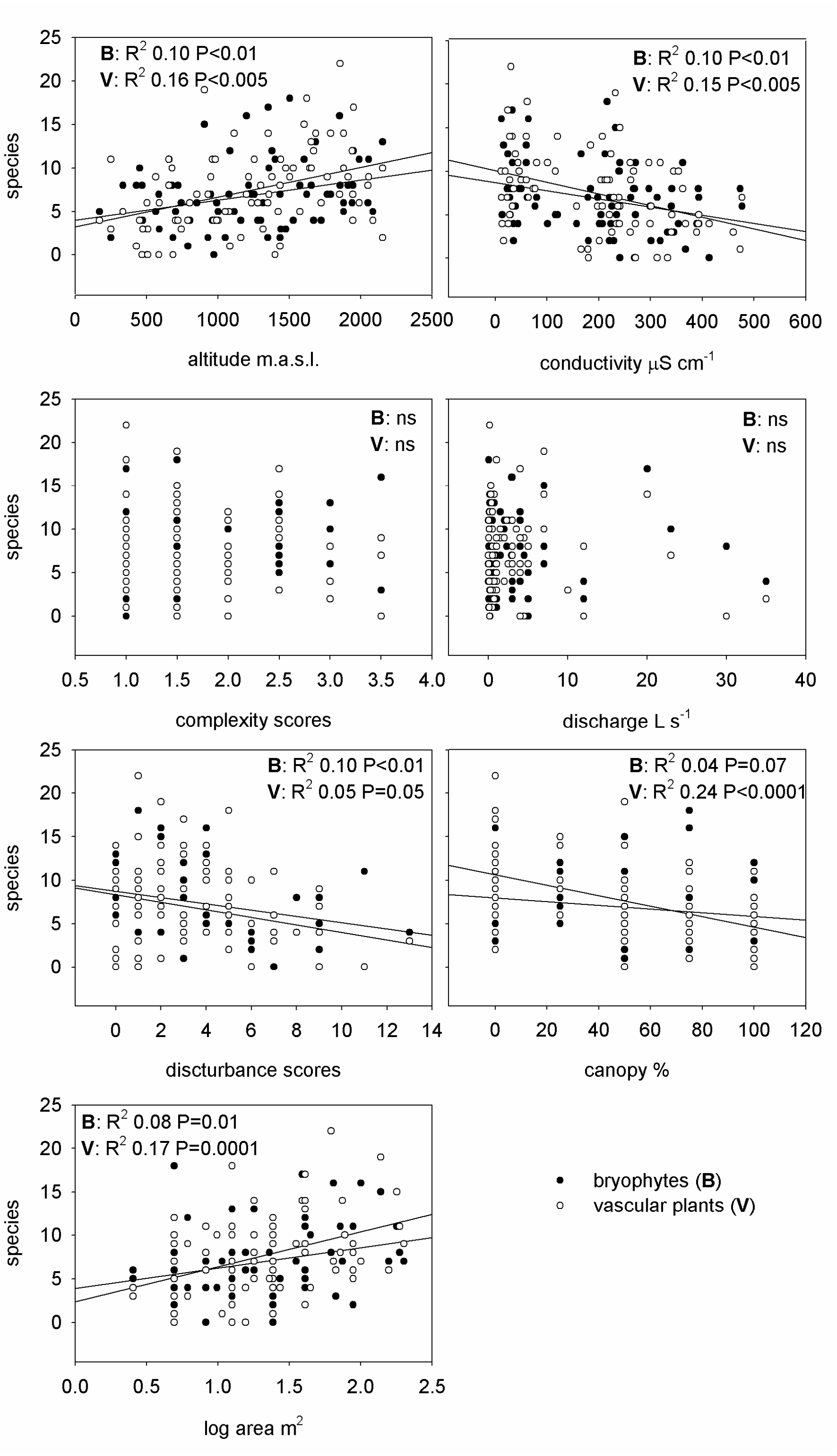


Fig. 1. Bivariate relationships between bryophytes and vascular plants richness and all other observed variables in the model. Untransformed data.

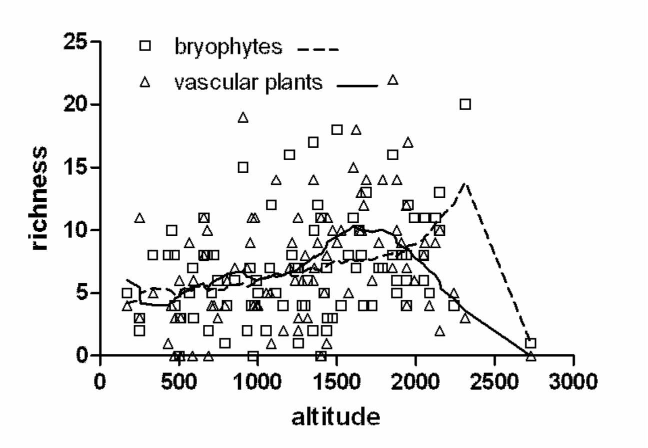


Fig. 2. LOWESS diagram describing the relationship between bryophytes and vascular plants richness vs altitude.

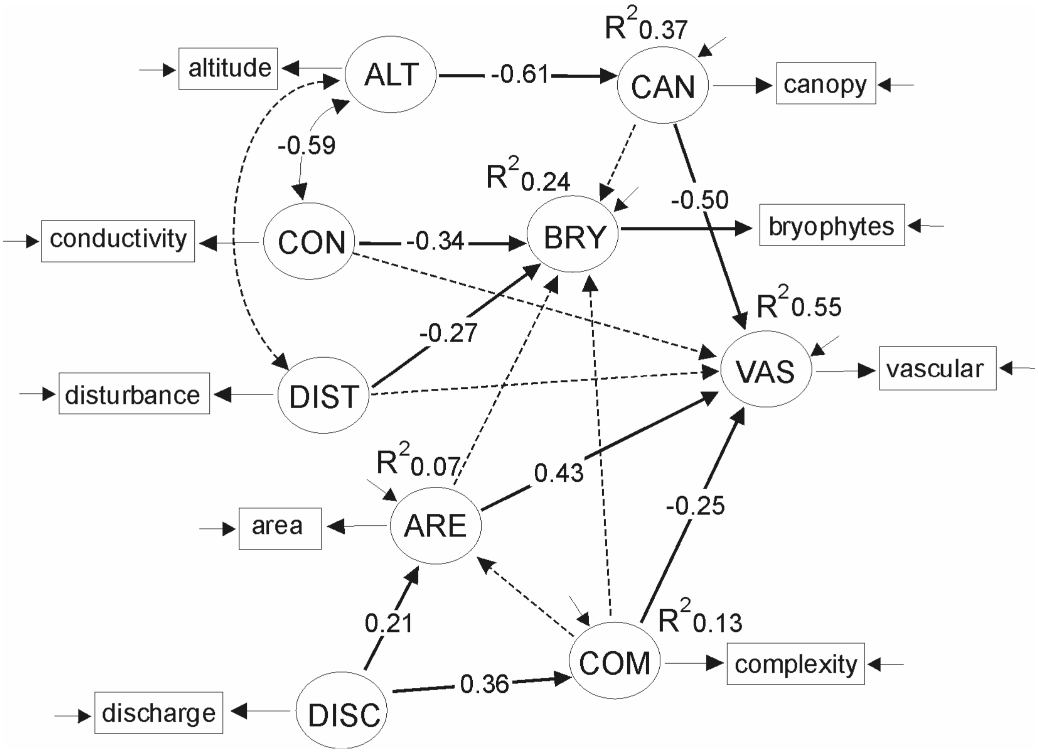


Fig. 3. The final structural equation model for bryophyte and vascular plant richness in spring habitats. Variables enclosed by ellipses are latent variables which are indicated by measured variables (in boxes). Arrows between latent variables represent completely standardized regression coefficients. The endogenous variables canopy (CAN), bryophytes (BRY), vascular plants (VAS), area (ARE) and spring complexity (COM) are shown to have 37 %, 24 %, 55 %, 7 % and 13 % of their variance explained by the model.

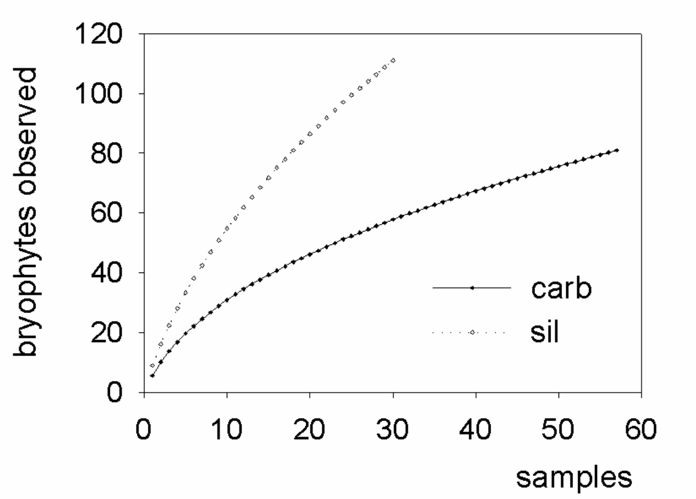
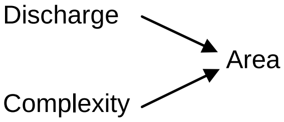
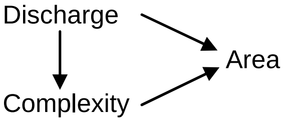


Fig. 4. Species accumulation curves of bryophytes on siliceous and carbonatous rocks. Graphs are based on species occurrence in 96 springs. Average species richness is based on 999 randomizations. Carb = carbonates; sil = siliceous.

	altitude	conduct	compl	discharge	disturb	canopy	bryophyt	vascular	area
altitude	0.271	-0.346	0.048	-0.020	-0.046	-11.581	0.526	0.943	0.015
conductivity	***-0.590***	1.270	-0.050	-0.051	0.054	16.923	-1.677	-1.818	-0.059
complexity	*0.134*	*-0.064*	0.471	0.166	-0.065	-0.520	0.548	-0.391	0.051
discharge	*-0.050*	*-0.059*	***0.315***	0.590	-0.100	0.214	0.176	-0.302	0.084
disturbance	*-0.123*	*0.066*	*-0.131*	*-0.180*	0.524	-2.180	-0.880	-0.518	-0.052
canopy	***-0.590***	***0.398***	*-0.020*	*0.007*	*-0.080*	1424.973	-21.466	-92.987	-0.971
bryophytes	***0.258***	***-0.380***	*0.204*	*0.058*	***-0.311***	*-0.145*	15.299	4.247	0.429
vascular	***0.391***	***-0.348***	*-0.123*	*-0.085*	*-0.154*	***-0.531***	*0.234*	21.529	0.935
area	*0.063*	*-0.111*	*0.156*	***0.231***	*-0.151*	*-0.054*	***0.231***	***0.425***	0.225

Tab. 1. Correlation and covariance matrices for environmental variables and richness. Values below the diagonal are correlations (italicized values), values above the diagonal are covariance and the diagonal values (underlined) are variances. As necessary, transformations were used to improve and linearize the variables (see methods).

Model 1		Model 2	
			
chi square	27.1	chi square	18.0
df	16	df	15
P	0.04	P	0.26
RMSEA	0.09	RMSEA	0.05
AGFI	0.08	AGFI	0.09

Tab. 2. Comparison of model alternatives in the exploratory part of the model (only the relevant part of the models was showed). The first hypothesis (model 1) stated that area is determined by discharge and spring complexity; the alternative hypothesis (model 2) stated that area is determined directly by discharge and indirectly through spring complexity. Fit improvement was strongly significant ($\Delta\chi^2$ 9.05 df 1 P 0.003), indicating that the competitive model (model 2) was more appropriate.

	Maximum likelihood		Bootstrap
paths	model	double errors	
altit → canop	-0.608	-0.627	-0.613
canop → bryo	0.024	0.020	-0.018
canop → vasc	-0.502	-0.520	-0.505
cond → bryo	-0.342	-0.348	-0.354
cond → vasc	-0.110	-0.102	-0.122
dist → bryo	-0.274	-0.291	-0.278
dist → vasc	-0.163	-0.171	-0.155
area → bryo	0.144	0.151	0.136
area → vasc	0.428	0.459	0.431
compl → bryo	0.132	0.131	0.123
compl → vasc	-0.254	-0.286	-0.249
disch → compl	0.359	0.408	0.351
disch → area	0.207	0.210	0.223
compl → area	0.099	0.107	0.089
altit → cond	-0.592	-0.600	-0.588
altit → dist	-0.088	-0.091	-0.080
chi-square	18.013	17.977	22.941
P	0.262	0.264	0.237
GFI	0.955	0.955	0.946
RMSEA	0.049	0.049	0.067

Tab. 3. Paths estimates in the final model, in the model with doubled measurement errors and in the simulated model with bootstrap (N=500). The non significance of the chi-square indicated that all the three model fit well the data. GFI=Goodness of Fit Index; RMSEA=Root mean square error of approximation. See text for further explanations.

8

SPATIAL DISTRIBUTION OF BRYOPHYTES [1]

Introduction

Once evaluated which were the most important environmental factors in explaining richness, the present and the following chapters deal with mechanisms of distribution at local scale. As clearly emerged in Chapter 5, environmental variables are only to a limited extend able to explain the high variance of species distribution. Consequently, mechanisms functioning at local scale should be considered to obtain a more complete scenario. In this framework, I focused the study only on bryophytes, because in spring habitats they can play a significant rule being the dominant group. Bryophytes are able to structure and modify actively the spring habitat offering and creating new possibilities to species establishment. Especially where few species are dominant, they can modulate the environmental forces, directly by slowing down and deviating the water flow, and indirectly by transporting water among capillary space. Therefore, as suggested by Jones et al (1994), bryophytes in spring habitats can be considered as ecosystem engineers. Bryophytes create habitat patches where environmental conditions and resource availability substantially differ from the surrounding unmodified environment. Then, the presence of such habitat patches may affect species diversity by providing suitable habitats

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[1] Contents of this chapter are presently submitted as: Spitale D., 2008. Spatial distribution of bryophytes along a moisture gradient: an approach using photosynthetic pigments as indicators of stress.

for species that cannot survive in the unmodified habitat (and hence increase species richness by adding new species into communities). In addition, bryophyte patches might affect the abundance of species already present within communities and hence changing the evenness of species assemblages (Badano and Cavieres, 2006).

Patterns of spatial distribution at broad and finer scale have long interested the ecologist because of the important implications for community analysis (Gaston 1996). Different explanations have been proposed to describe the spatial distribution of bryophytes, i.e. competition (Marino 1991), different establishment capacity (Li and Vitt 1994), resistance to disturbing phenomena (Suren and Ormerod 1998) and stress (Grime et al. 1990; Cleavitt 2002). Bryophytes possess a characteristic not found in vascular plants, because they are able to modify the environment via water transport within their colonies (Titus and Wagner 1984). Many mosses are ectohydric with respect to water storage and movement (Dilks and Proctor 1979; Proctor and Tuba 2002). What this means is that, especially in aquatic habitats like those of springs, bryophyte assemblages can be thought of as a complex system of species, the survival of which is strongly inter-related related by the common transport of the water. The water content of the individuals depends on their distance from a water source and also on the colony architecture (Zotz et al. 2000). Individuals close to water can be expected to have more water in their tissues than the more distant and usually each species is restricted to a specific range of moisture level according to its own requirements. A transect across the water just a few metres long could contain several whole replacements of species, from hydrophytic to xerophytic (Spitale pers. observ.). However, surprisingly few studies on this subject have been conducted so far (but see Slack and Glime 1985; Vitt et al 1986; Glime and Vitt 1987; Suren and Ormerod 1998) and never on spring habitats. The main problem for measuring the distance from water is the water table variability. Such variability can be intrinsic to the system (because of the irregular drainage) or caused by seasonal water level fluctuations. Therefore, at the edge of the colony, or in several periods of the year, individuals may show clear symptoms of drought stress. Stress has many consequences at different levels depending on the scale of observation such as cellular (Oliver et al. 2000), individual morphology (Peñuelas 1984) and patterns of distribution (Gignac et al. 1991). One way to measure the extent to which the plant experiences stress is by using photosynthetic pigments or their relative ratios (Martinez-Abaigar et al. 1994). Several pigment indices such as chlorophyll and carotenoid concentrations, chlorophyll/phaeopigments ratios and chlorophyll/carotenoid ratios can be employed as indicators of vitality or stress (Lopez and Carballeira 1989; Lopez et al. 1997).

The conceptual model employed here for explaining the spatial distribution of bryophytes in spring habitats is based on the different water contents in relation to their distance from the water surface and the consequences of water contents in determining stress. The first assumption is that each species occupies a preferred position along the water gradient (and therefore a specific distance from water surface) in accordance with its tolerance to water stress. Then, if the pigment variables act as an indicator of stress conditions, a complete wet-dry gradient will be able to reveal the optimal distance from water. Hereafter, with pigment variables, my intent is to use both pigments and ratios as stress indices. The species in Fig. 1 usually grows in a transitional zone in the spring sequence; the distance from water is described by its optimum and its tolerance interval. When the distance from water (and consequent water content) is within the tolerance interval, the species is not stressed; when the water level is higher or lower than the tolerance interval the species experiences stress (Fig. 1a: two-tailed model). The wider the δ angle, the more tolerant the species is to water level fluctuations. The stress response may also be symmetrical or asymmetrical according to the distribution around the mean or the median value. The alternative model concerns two other types of species, that is, those growing close to and those growing further from the water. This model is one-tailed and is positive or negative (Fig. 1b). In both models, stress is defined as physiological (Menge and Suntherland 1987), since it is induced by factors able to reduce the rates of photosynthetic production when their values are outside the optimal range.

Aims of the present work are: (1) to evaluate the different abilities of the selected species to transport water, (2) to evaluate whether the relations between pigment variables and water contents agree with the conceptual model, and (3) with the assumption that the median pigment variables in natural conditions correspond to the "no stress threshold" of the model, for each species to calculate the theoretical optimal distance from water.

Methods

EXPERIMENTAL DESIGN

The field experiment was conducted in a spring located in Bresimo (Trentino region, Northern Italy) at 1950 m a.s.l (N 46°25'45.37'' E 10°53'22.97''). A completely random sampling was performed in order to estimate the natural range of pigment variables in five bryophytes species. A certain number of colonies were initially located and labelled; from those ten colonies for each species were selected at random and two replicates were taken from each colony. The samples throughout this study consisted of five shoot apices 3 cm long. The random sampling across the full range of different habitats colonized by the species should warrant the average estimate of pigment variables within the no stress threshold.

To generate the experimental water gradient each species was transferred in a plastic pipe cut longitudinally (hereafter Half Pipe (HP); length=100 cm; width=10 cm). The HPs were arranged together at the same inclination (30°, to simulate the natural banks slope) with one end immersed into the water (Fig. 2). The water rises by capillarity through the bryophytes creating a natural gradient considered constant once the equilibrium state has been reached. The method was loosely inspired to that previously used by Noakes and Longton (1988), but they used an absorbent cloth to generate the gradient and along that they arranged the moss shoots. A factorial experiment with three factors was planned: two fixed factors orthogonal to one another (species with five levels and distance with ten levels) while HP was random and nested in species. Each species was replicated three times and maintained in different experimental structures for a total of fifteen HPs. In each HP the following species were inserted: *Scapania undulata* (L.) Dumort, *Warnstorfia exannulata* (Schimp.) Loeske, *Philonotis fontana* (Hedw.) Brid., *Sphagnum warnstorfii* Russow and *Pleurozium schreberi* (Willd. ex Brid.) Mitt. I expected the first three species to belong to the "type h" (Fig. 1b), because these species usually grow close to the water. *Sphagnum* was expected to follow an two-tailed model, because it generally occupies the intermediate zone (Fig. 1a). *Pleurozium* was expected to belong the "type x" because it normally colonizes a furthest zone from water. More species of "type h"were selected, because they are the widespread in spring habitats and because I wanted to test if there are differences among species which are more similar as regarding distance from water. Particular attention was paid to transfer portion of colonies into the HPs in order to preserve the natural texture (closeness). Along each HPs samples were taken at different distances from the water level: 0 cm=immersed; 15 cm= semi-emergent; 20 cm emergent; 25, 30, 35, 40, 45, 55, 70 cm (see Fig. 2 for a graphical representation). Three replicates per distance were taken for a total of 30 samples per HP (n total=3x10x15=450). Once I set up the HP, I waited 20 days before collecting the samples (Peñuelas 1984 noted strong changes in pigment contents within the first week). The HPs were covered with a transparent plastic film in order to avoid re-wetting of the treatments due to rainfall. The covers were inclined and opened on the side in such a way that the illumination was more similar to the natural conditions.

DATA COLLECTION

Five shoot apices 3 cm long were collected from the HP, suspended in air by the forceps for a few seconds to drain, cleaned if necessary, inserted in a pre-weighed plastic envelope bag and hermetically sealed to avoid loss of water. The plastic bags were stored in a cool box and transported to the lab and weighed to calculate the fresh weight. The samples were stored in the freezer until the analysis; pigment analysis was carried out within one month. Extraction was performed with cold 90 % aqueous acetone after comminuting the samples with an electric grinder. The process was optimized through 12 h refrigeration of the extracts. Afterwards, the extracted pigments were centrifuged at 3000 rpm for 15 minutes. Three ml of supernatant were read on the SAFAS UV mc^2 spectrophotometer before and after acidification with 30 μl 1M HCl. Chlorophyll *a*, *b* and phaeopigments were calculated on the basis of Lorenzen's equations (1967). The following ratios were also recorded (OD=Optical Density): OD430/OD410, OD430/OD665, OD480/OD665, and chl *a*/chl *b* (Martinez-Abaigar and Nuñuez-Olivera, 1998). In addition, the pigment extracts together with the grinded material were dried at 90°C for 12 h to obtain the dry weight and total water content percentage was calculated (TWC % d.w. = 100*(fresh weight – dry weight)/dry weight, Noakes and Longton 1988).

DATA ANALYSIS

The TWC % d.w. (hereafter only TWC) was analysed by three-way ANOVA. The factors considered were: species (five levels, fixed), distance (ten levels, fixed) and HP (three levels, random). Species and distance were crossed while HP was nested into species. Homogeneity of variances was tested by Cochran's test (Underwood 1997) and data were square root transformed (the arcsine transformation commonly used for percentages did not improve as well as square root in this data set). Tukey test was performed to check for *post hoc* comparison. Components of variation were also calculated for each level of the analysis. The percentage of variation was calculated as each component of variation divided by the sum of all components of variation multiplied by 100 (Underwood 1997). Pigment variables were log (x+1) transformed when necessary before performing regression analysis to homogenise variance (Sokal and Rohlf 1995). Linear and polynomial regression was used to evaluate the relationship between TWC and distance as well as between pigment variables and TWC.

The distance was estimated as follows: among the available significant regressions between pigment variables and TWC only the linear (because quadratic relations can give two solutions) and those with the best fit were selected. By means of the reverse of these equations, the TWC from the pigment variables in natural conditions was estimated. We may call this term: theoretical TWC (tTWC). Then, by applying the reverse experimental regression TWC–distance and utilizing the tTWC as x, I was able to estimate the theoretical distance from water. Since this method of estimating X from Y is called inverse prediction (Sokal and Rohlf 1995), in this framework I dealt with a double inverse prediction. In addition, because the second inverse regressions were not linear (the relations TWC and distance were sigmoidal) a correct way to estimate the confidence limits was problematical. I tried to solve this problem using the upper and lower quartile of pigment variables in natural conditions instead of only the median values in order to give an approximation of the confidence limits.

Results

TOTAL WATER CONTENT

As shown in Table 1 and Fig. 3, water contents were different among species and the Tukey post hoc test revealed that differences exist among all the species considered, except for *S. undulata* and *P. fontana* which were somewhat similar. Water contents were significantly different in all the ten levels (distances) (a priori comparison, Helmert Contrast *t*-values test $P<0.05$) and in each species. The significant interaction species x distance showed that the difference among species varied according to the distance considered. In addition, the significant interaction HP (species) revealed that those differences were also related to the HP. In the case of *P. schreberi* the water content had heterogeneous variance that did not improve with transformation, so the parameter of regression water-distance should be interpreted with caution. The relationship between TWC and distance, with different slopes, was sigmoidal for all the species (Fig. 3). The relationship for water-distance was the shallowest (with the halfway water content EC_{50}= 52.9 cm) in *S. warnstorfii*, while in *P. schreberi* it was the steepest (EC_{50}=21.8 cm). An intermediate situation was present for *W. exannulata*, *P. fontana* and *S. undulata* (respectively EC_{50}=33.3; 33.0; 25.7).

HOW PIGMENT VARIABLES RESPOND TO WATER GRADIENT

The relationships between pigment variables and TWC are shown in Table 2. For summary purposes, it is possible to distinguish two groups of relationships for each species, that is, those in accordance with the model and those that were not (even though that distinction is not evident). In *S. warnstorfii,* the model predicted a two-tailed response (that is a quadratic relationship, U shaped) and the order of the best response was OD430/OD410, chl *a* and phaeopigments. Unexpected relationships (linear) were OD480/OD665 and OD430/OD665. The other pigment variables showed no or a weak significant regression (considering until cubic terms). In *P. fontana* the model predicted a one-tailed positive response such as from the best was OD430/OD410 and phaeopigments. Unexpected were chl *a*, chl *b* and their ratio. In *S. undulata* almost all the pigments variable are in agreement with the model. In *P. schreberi* the model predicted a one-tailed negative response: OD430/OD665, OD480/OD665, chl *a*, chl *b*; unexpected were OD665/OD665a, OD430/OD410, chl *a*/chl *b* and phaeopigments. In *W. exannulata* the model predicted a one-tailed positive response: almost all of the pigment variables were in agreement with the model with the exception of chl *a*/chl *b*, phaeopigments and OD430/OD410.

No significant differences were detected in the mean values between the TWC in the special level 0 (immersed) and 15 (semi-emergent) (*t* test at P>0.05 level in each comparison), but there were significant differences as regards to the pigment variables (Table 3).

THEORETICAL DISTANCE FROM WATER

In order to estimate the distance from water only the linear relationships with the best fit were selected (Table 2): *S. warnstorfii* and *W. exannulata* (OD480/OD665), *P. fontana* (OD430/OD410), *S. undulata* (chlorophyll a) and *P. schreberi* (OD430/OD665). All those linear regressions were significant at P<0.001 level. The median values and the dispersion of those pigment variables in natural conditions of no stress are summarized in the Table 4.

Discussion

Spatial distribution of bryophytes in aquatic habitats is essentially governed by their, direct and indirect, relationships with water (Vitt and Glime 1994). While recording more than 90 vegetation relevès in spring habitats in Trentino (Northern Italy) during 2005-2006 within the Crenodat Project (Tomaselli M., Spitale D., Petraglia A., *work in prep*), we recognized that distance from the water surface was the main factor determining the species distribution. In spring habitats, and similarly in ecosystems like mires, fens, bogs, marshes and swamps, the zonation pattern was essentially regulated by the water level fluctuation (Kimmerer and Allen 1982). Understanding spatial distribution of the bryophyte community in relation to water availability might provide explanations about different tolerances of species to water stress.

ABOUT THE HP METHOD

In order to generate a water gradient that is as natural as possible in the field, the Half Pipe technique was used for the first time. This is useful for studying the response of bryophytes to a water gradient. Clearly the benefit of studying these semi-natural bryophyte populations is that many characteristics of the colony are maintained. In this way we can study both the effects at individual level and also at colony level. Drawbacks are essentially in the transfer step, when a portion of the colony is collected and arranged inside the HP. More careful attention must be paid in order to maintain as precisely as possible the natural texture of the colony. The HPs were independently replicated in order to estimate separately these sources of error. Components of variance (Table 1) were useful in order to assess the contribution of terms to variance. The random factor HP had the lowest weight (1.7 %) in explaining the total variance, even though it was significant. This difference could be explained by recognising the existence of some variability in natural populations and the error introduced by manipulation.

WATER CONTENT AND RELATIVE CONSEQUENCES AT COMMUNITY LEVEL

Bryophytes are able to store different quantities of water according to both the cell features (Proctor 2000) and the tri-dimensional structure of the colony (Zotz et al. 2000). These special characteristics allow them to survive at different distances from water according to their requirements. The different quantities of water along the HPs (expressed by the halfway water content, EC_{50}) were coherent with the rank of the distance from water in natural conditions. *P. schreberi* only transported a small amount of water along the HPs (Fig. 2), since growth in this species is independent from the water distance in natural conditions because atmospheric precipitations provide the water requirements. In contrast, *S. warnstorfii* was able to transport (and likely to preserve) water more efficiently than all the other species examined. *S. warnstorfii* inhabits the intermediate zone in springs, between the more aquatic species close to water (*P. fontana*, *S. undulata* and *W. exannulata*) and *P. schreberi*. Similar results were obtained with *Sphagnum* spp comparisons aiming to comprehend the observed height of each above the water table (Rydin 1993). Rank order of water contents of the capitula is the same as that of the uppermost levels reached in a hummock. Therefore, it may also apply to species and different habitats. It is certain that differences within the same assemblage influences, directly and indirectly, the species interaction in the balance between facilitation and competition for water (Callaway and Walker 1997). Even though, no experiments appear to have been conducted to explore shifts in the balance between competition and facilitation for water in bryophytes, excluding sphagna (but see Sonesson et al. 2002), it may be an important issue to explore in order to understand the spatial distribution of assemblages in aquatic environments like springs.

Another factor that may influence the transport of water, and the relative species spatial distribution, was the heterogeneous density of the gametophytes in the colony. Though not estimated directly, the significant interaction among HPs and species may be explained by the existence of different patches of density where water was stored. Water content and the relative transport from the source could not be homogenous because of varying shoot density. This characteristic may also increase the variability in the structure of spatial pattern distribution.

WHAT PIGMENT VARIABLES RESPOND TO WATER GRADIENT?

The model has been proposed only for understanding the purpose of the pigment variable trends. A more rigorous model validation was outside the scope of this paper (for the significance of validation see Rykiel 1996). A higher number of ecologically different species would be necessary in order to evaluate the model. Nevertheless, in my opinion, only having a "schematic model" of the hydrological niche like that proposed (Fig. 1), the pigment variable assessments were possible. Since the pigment variables usually did not show coherent trends, several possible explanations and consequences could be put forward. First, the pigment variables are sensitive to environmental variable other than those induced by the water gradient (Martinez-Abaigar and Nuñuez-Olivera 1998). Second, even if a pigment variable more sensitive to water stress exist, it does not mean that it would be valuable for all the species. Thus, no general solution can be proposed and the model remains rigorously untested, because the pigment variables mismatch each other.

Generally, OD430/OD665 and OD480/OD665 showed a negative trend with water content, probably because chlorophyll *a* content generally increases with water content. The most commonly employed index of chlorophyll / phaeopigments (OD665/OD665a), (Lopez et al. 1997; Martinez-Abaigar and Nuñuez-Olivera 1998) seems to be consistent only for *S. undulata* and *P. schreberi*, but less for the other species considered. Therefore, when planning a study on investigating the stress of bryophytes, it is advisable to evaluate *a priori*: (i) which type of stress will be considered (e.g. see Baxter et al. 1992 for pollution) (ii) which species respond better to that type of stress, and (iii) which pigment variables seem to be better for either species. When several pigment variables are collected for each species, a global response is difficult to extrapolate because of different pigment responses. Even though pigments usually responded to some extent to water stress, the relationships were not always easily interpretable.

With the precautions stated earlier, according to the model proposed, *S. undulata*, *W. exannulata* and *P. fontana* should belong to a "type h" (Fig. 1b) in which the stress increases with distance from water (positive relationships). For those species the best stress indicators would be chl *a*, OD480/OD665, OD430/OD410 respectively. *P. schreberi* should belong to a “type x” of the model, in which the stress decreases with distance from water (negative relationship), or, from the opposite point of view, the species is expected to experience stress as the water distance decreases. Even though this was the trend that was reasonable to expect (given its natural trait), no pigment variables were found completely in agreement. Negative relationships would be detectable looking for the opposite sign of the species “type h”, not the sign (slope) of regression. Such disagreement, which was also found by other authors (Kellomäki and Hari 1976), could be explained by admitting that such species are indifferent to experimental water treatment imposed on them or that the duration of treatment was insufficient. *S. warnstorfii* should belong to the two-tailed model (Fig. 1a), because it seems to be their particular trait in the natural environment. Also in this case the indexes were not coherent to each other. However, the ratio OD430/OD410 seems to be the better indicator for that species as regard to the water gradient. Because the trend indicates a clear change of slope and a weak asymmetry was present (Fig. 3), a predisposition to tolerate more stress induced by drought than by high water level may be proposed for *S. warnstorfii*. Analogous trends (two-tailed asymmetrical) through measuring photosynthetic rate at different water contents was obtained by Silvola and Aaltonen (1984) for *Sphagnum fuscum* and *Sphagnum angustifolium*. Visual inspection of the data reported in that work suggests that those two *Sphagnum* species seem to be more tolerant at high water content. Maximum rate of photosynthesis was between 600 and 1200 TWC and the decrease of rate was less steep with the increase of water than with the decrease under 500 %.

Since many species are not able to take up CO_2 from water when they are waterlogged, some differences were predicted between the pigment variables from distance 0 (immersed) and 15. These two treatment levels differed only as regard to the CO_2 availability (CO_2 diffuses about 10^4 times more slowly in water than in air (Glime and Vitt 1984), but not, as expected, for the TWC. Nevertheless, as showed in Tab. 3, only *S. warnstorfii* and *W. exannulata* showed significant differences. I believe that this special case occurred because of a particular pigment shift rather than by waterlogging stress. In both cases, the colour of those species at level 0 changed from red to dark green during the experiment (and the ratio of carotenoids:chlorophyll was significantly different in both species). I suppose that species like *P. schreberi* are not adapted to the immersed condition and that they are unable to take up CO_2 better than *S. warnstorfii* and *W. exannulata*. Therefore, it may be that the experiment duration was not long enough to induce waterlog stress in *P. schreberi* (but consider that phaeopigments were significantly different between 0 and 15 levels, Tab. 3). On the contrary, and as expected for the more aquatic species, *P. fontana* and *S. undulata*, pigment variables were not significantly different between level 0 and 15, because they are well adapted to grow immersed.

DISTANCE FROM WATER

There have been only a few attempts to apply the plant strategy theory to bryophytes in general (but see During 1992; Grime et al. 1990; Slack 1990) and none to spring bryophytes specifically. Muotka and Virtanen (1995) proposed a scheme of stream bryophytes life strategies in which disturbance intensity (like substratum movement) is able to select the species in those environments. Disturbance (sensu Grime: removal of biomass) usually is not an important factor regulating spatial distribution in spring habitats. In spring environments, stress, rather than disturbance, seems to be the key factor, because water flow only rarely is high such as in streams. Stress induced by water level fluctuations acts as a filter selecting only those species able to resist (stress tolerator). The wide overlap of the three aquatic species (*S. undulata, W. exannulata* and *P. fontana*) from distance point of view is coherent with the observational data. Even though wide overlapping of distance exists, *W. exannulata* presents median values rather different to the other two species. If the skewness follows the meaning in the proposed in the model, *W. exannulata* seems to be well adapted to growing at some distance from water, but can even occupy the zone close to the water. The situation appears to be different for *S. undulata* and *P. fontana*, which preferentially inhabit the zone closest to water, but they can reach the same distance as *W. exannulata*. These results may help to explain, with quantitative data, the broad impression achieved from field observations. For the other two species the estimated distance clearly reflected their habitat preference.

Figure Captions

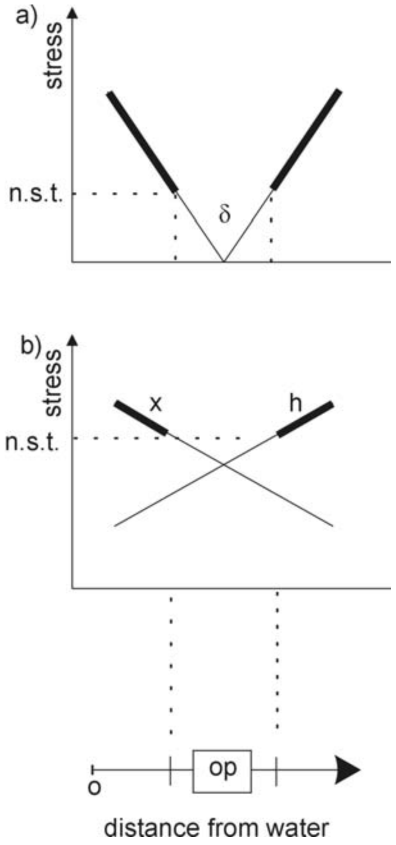

Figure 1 Conceptual model explaining the spatial distribution in relation to water stress. Arrow at bottom shows the spatial distribution of a species in relation to the distance from water. Box and vertical line across the arrow show optimal and distribution limit (tolerance). When the species is within its tolerance distance from water it is also beneath the no stress threshold (n.s.t.). a) Two-tailed model: when the water level is higher or lower than the tolerance interval the species experience stress. The δ angle and the symmetry are related to the shape (skewness) of the horizontal box plot describing the distance from water. b) One-tailed model: species h experience stress only when the water level is low and species x only when water level is high. Lines in bold show when species experience stress. (op) optimum = median value.

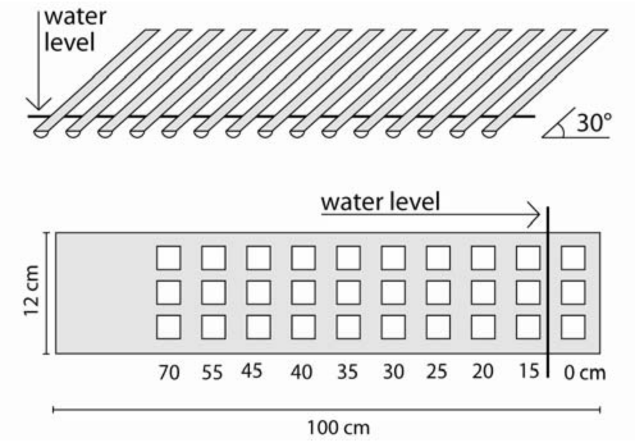

Figure 2. In the upper part, the fifteen Half Pipes used in the experiment (five species replicated three times). They were inclined at 30° and with one extremity immersed into the water. The species (one per HP) were interspersed with each other. In the lower part an example of HP: at each distance from water, three replicated samples were collected.

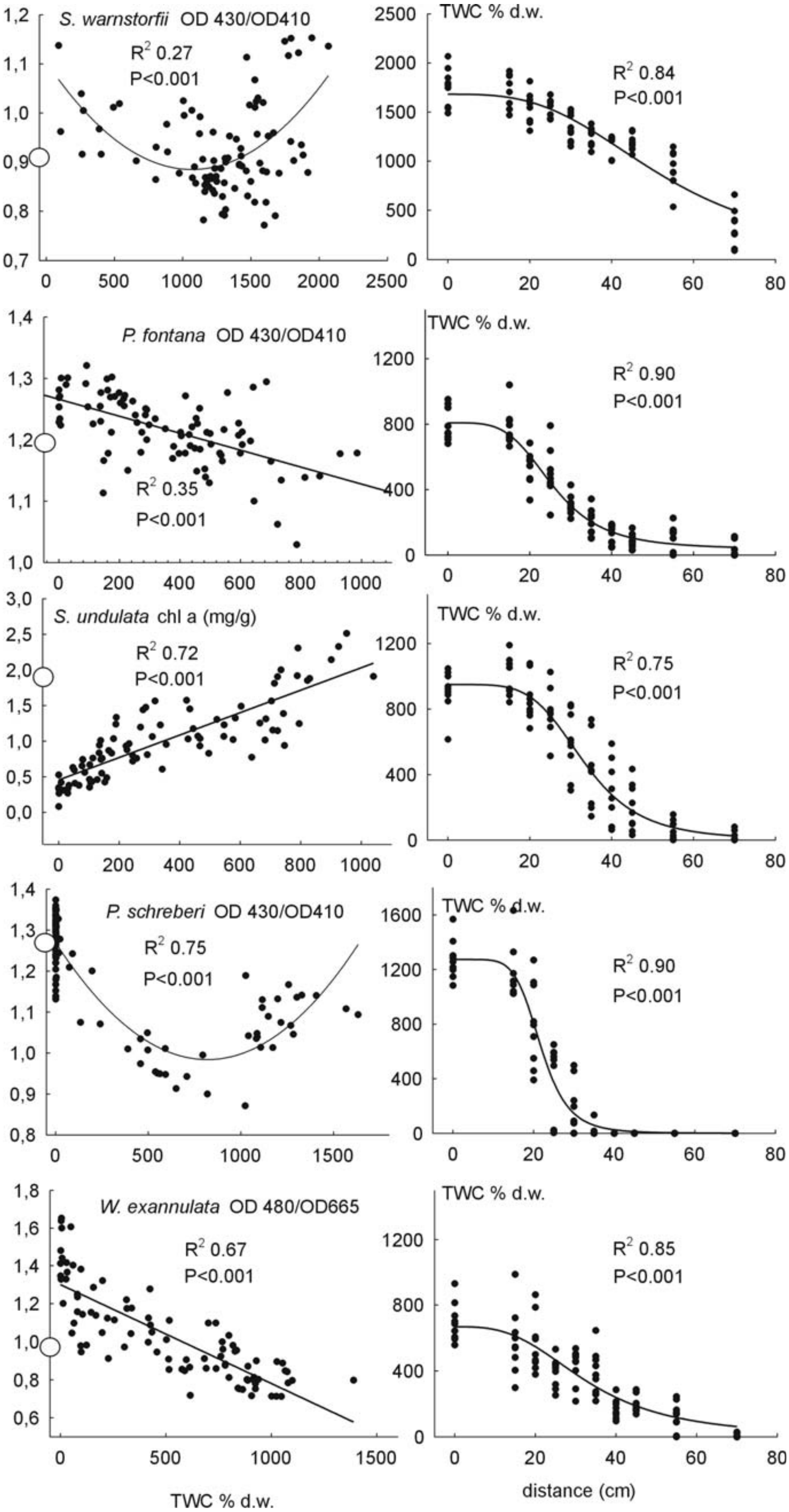

Figure 3 Left diagrams: relationship between a selection of pigment variables and TWC. Circles on the ordinate axis represents the median value of that pigment in natural conditions. Right diagrams: TWC along the distance from water source in the five species. TWC=Total Water Content % on dry weight. Untransformed data.

source	d.f	MS	F	P	var. comp. %
distance (D)	9	3749.2	119.4	<0.0001	39.3
species (S)	4	6622.3	66.8	<0.0001	44.8
D*S	36	187.8	6.0	<0.0001	9.4
HP(S)	10	99.2	3.2	0.002	1.7
D*HP(S)	90	31.4	6.6	<0.0001	4.8
Error	299	4.7			

Table 1 ANOVA model with three factors: factor distance and species were orthogonal and HP was nested in species. Distance and species were fixed and HP random. $X_{ijkl} = \mu + D_i + S_j + D_{sij} + HP(S)_{k(j)} + DHP(S)_{ik(j)} + e_{(lijk).}$ Var. comp. = variance component, expressed in percentage.

	S. warnstorfii		*P. fontana*		*S. undulata*		*P. schreberi*		*W.exannulata*	
	linear	quadratic	linear	quadratic	linear	quadratic	linear	quadratic	linear	quadratic
OD430/OD665	-3.62***	-2.23*	-3.00**	4.28***	-6.42***	ns	-5.63***	ns	-12.55***	2.61*
OD665/OD665a	ns	ns	ns	ns	8.84***	-4.17***	-7.14***	8.10***	ns	ns
OD430/OD410	ns	5.65***	-6.88***	ns	7.94***	-3.97***	-8.33***	10.24***	ns	2.76**
OD480/OD665	-7.76***	2.37*	-3.84***	4.53***	-8.77***	ns	-5.57***	ns	-13.76***	3.15**
chl a (mg/g dw)	3.20**	4.10***	ns	-6.04***	14.89***	ns	4.21***	ns	7.63***	ns
chl b (mg/g dw)	2.68**	2.37*	2.60*	-5.29***	14.64***	ns	3.80***	ns	7.82***	ns
chl a/chl b	2.22*	ns	ns	-3.42***	5.04***	ns	7.96***	-5.94***	8.02***	3.87***
feopigm (mg/g dw)	ns	3.36**	-4.19***	ns	ns	ns	2.51*	4.03***	-5.05***	3.45***

Table 2 Relationships between pigment variables and TWC. Linear and quadratic models were tested. Significant quadratic terms means that the polynomial model added significantly to the linear model. Since two tailed *t* test (linear, d.f. 88; quadratic, d.f. 87) were performed to evaluate the parameters significance, the sign indicates the type of relation: linear + (/), linear – (\), quadratic + (U) and quadratic − (∩). Parameters of the linear and quadratic regression fitted by least-squares, *P<0.05,**P<0.01, ***P<0.001. OD = Optical Density.

	S. warnstorfii	*P. fontana*	*S. undulata*	*P. schreberi*	*W. exannulata*
OD430/OD665	-3.92**	-1.22	-1.55	-0.10	-3.43**
OD665/OD665a	2.16*	-1.25	-0.26	0.33	-0.57
OD430/OD410	3.70**	1.38	-1.99	0.96	-0.38
OD480/OD665	-2.89*	-1.55	-1.54	-1.01	-3.81**
chl a (mg/g dw)	4.00**	0.72	-0.43	-0.77	1.62
chl b (mg/g dw)	1,77	0.58	-1.59	-1.39	1.54
chl a/chl b	1.00	1.18	0.03	-0.27	2.54*
feopigm (mg/g dw)	6.33***	1.28	1.79	4.73**	-0.02

Table 3 Differences in pigment variables at the special level 0 (immersed) and 15 (semi-emergent). Paired *t* test (d.f. 16); significance *P*-level: **P*<0.05,***P*<0.01, ****P*<0.001.

		natural assemblages			theoretical distance from water (cm)		
species	pigments ratio	median	l.q.	u.q.	median distance	l.q.	u.q.
P. schreberi	OD430/OD665	2.80	2.75	2.88	27.8*	27.8*	62.4*
S. warnstorfii	OD480/OD665	1.69	1.60	1.93	25.9	13.8	66.1
W. exannulata	OD480/OD665	1.07	0.95	1.23	27.3	0,0	35,4
P. fontana	OD430/OD410	1.20	1.17	1.25	0,0	0,0	46,0
S. undulata	chl a (mg/g)	1.86	1.31	2.23	0,0	0,0	32,0

Table 4 Pigment variables from natural assemblages and theoretical distance from water. Since the computations were on the basis of experimental regressions, distance from water means on inclined plane (30°). L.q and u.q. = lower and upper quartiles. * Given that *P. schreberi* is a xerotolerant species, their natural spatial distribution is not related to the distance from water.

9

INTERACTION AMONG BRYOPHYTE SPECIES [1]

Introduction

Many recent studies have shown that plant species can have strong, and often species-specific, direct and indirect positive and negative effects on other plant species (Hunter and Aarssen 1988; Brooker et al 2007). It is thought that positive interactions (facilitation) predominate in environmental stress conditions and negative interactions (competition) in ameliorated conditions (Bertness and Callaway 1994). Facilitation and competition may operate simultaneously and the overall effect of one species on another may vary in space and time (Travis et al 2006). Most of the studies performed to date have examined the effect of abiotic stress over a spatial gradient in generating intensity variations of biotic interactions (reviewed in Cheng et al 2006). Such an approach implies a relatively uniform frequency of stress in a particular place over time and does not explicitly consider temporal gradient of stress. However, the variable time may be able to change the intensity of biotic interactions, but few experimental studies have explicitly considered temporal variations (but see Kikvidze et al 2006). Previous works using bryophytes as model species have shown that the mechanism by which interactions occur is mainly through moisture availability (Mulder et al 2001; Rixen and Mulder 2005). Moisture in

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[1] Contents of this chapter are presently submitted as: Spitale D., 2008. Switch between competition and facilitation in a variable environment.

a colony is determined by its water storage capacity that is influenced by the species morphology (growth form) and the structure and density of the moss colony (Proctor 2000). Therefore, in these species interaction intensity is regulated by inherent colony characteristics and whatever climatic event influencing moisture degree.

Although an extensive literature exists about the relation between bryophytes and water (e.g. Proctor and Tuba 2002), the idea of an explicit interaction among colonies has hardly been emphasized yet (with a limited exception for sphagnum-dominated habitats, Rydin 1985; 1993; 1997). In a previous experiment, using the liverwort *Scapania undulata* (L.) Dumort. and the mosses *Warnsorfia exannulata* Russow and *Sphagnum warnstorfii* (Schimp.) Loeske as model species, Spitale (*unpublished*) demonstrated a high difference in water transport among the colonies (Fig 1). In particular, *Sphagnum* was able to adsorb and retain significantly more water than the other two species, whereas the difference between *Warstorfia* and *Scapania* was subtler. On the base of these differences and observing that in natural habitats those three species are abundant and share most of the available space, it may be suggested that the presence of a species can affect the neighbour by water transfer among colonies.

A field manipulative experiment was designed to study positive and negative interactions between a target species (*Warnstorfia*) and two different neighbour species (*Scapania* and *Sphagnum*) at different level of water stress. The variable time was experimentally evaluated simulating a random drought event in addition to the different level of water stress. The following hypotheses were tested: (1) Given that different colony architectures result in different moisture absorption and retention, it is hypothesized that a colony of a species can influence the moisture of an adjacent colony. Direction and intensity of interaction will depend on the strength of stress experienced: when stress is low, competition is expected to predominate, whereas when stress is high facilitation could dominate. Drought stress in natural environment varies through time because of climatic variability (rainy periods are likely to be less stressful than dry ones, at lest for hygrophilous species). It follows that interaction between two species could change from competitive to facilitative from time to time. (2) Therefore, the second hypothesis is that different interactions will be observed if a drought treatment occurs in different times.

Methods

STUDY AREA

This field experiment was conducted at Malga Bordolona in the Bresimo valley (N 46°25’45.37’’ E 10°53’22.97’’), Trentino Province, Italian Alps at 1980 m a.s.l. Climate is temperate semicontinental, with cold winters and cool summers (min –17°C; max 22°C) and rainfall has a markedly uneven distribution with a maximum peak at the end of spring and one in early autumn; winter is generally cold and dry.

The site is a natural reo-helocren spring with a complex system of different sources which extend over about 1 ha; substrate is metamorphic rock with water conductivity of 22.5±1.5SE μScm^{-1}, pH 6.3±0.2SE and P-PO_4 of 2.5±0.5SE $\mu g\ L^{-1}$. The most abundant bryophyte species are *Scapania undulata*, *Sphagnum warnstorfii*, *Warnstorfia exannulata* and less abundant but still common are *Philonotis fontana*, *Dicranella palustris*, *Brachythecium rivulare*, *Warnstorfia sarmentosa*, *Straminergon stramineum*, *Sphagnum magellanicum*, *Bryum pseudotriquetrum, Chyloscyphus polyanthos*. For this experiment, the most abundant species were used. The leafy liverwort *Scapania undulata* is a plant procumbent to erect, bright green, reddish green or sometimes dark purplish red in colour; it is 10 cm long or more in suitable habitats, it forms large, compact extension of monospecific stands both immerged in water and emerged. It is a species that prefers acid or circumneutral water, shaded or insolated habitats. *Sphagnum warnsorfii* has shoots up to 15 cm long, variously crimson, rose pink or green flecked with pink in colour. It forms carpets and tussocks and it is present in small hummocks or lawns in rich fens. *Warnsorfia exannulata* is a plant of moderate size up to 20-30 cm long, with stems sparsely to regularly pinnately branched, leaves from ovate-triangular to falcate or sometimes straight. This species is commonly found in habitats of intermediate mineral richness, in fens, around springs and also on lake shores.

EXPERIMENTAL DESIGN

Different levels of water stress were generated arranging the colonies in contact with water in such a manner to produce a water gradient. Levels of stress were defined according to distance from water: near water corresponded to low stress, middle distance to medium stress and high distance to high stress. The additional stress event, the intensity of which was variable depending on climatic variation, was generated simulating a drought, occurring in a random date.

Because growth form of these species can be to some extent modified in relation to water level (Vitt and Glime 1984), stands of monospecific species were chosen from visibly uniform sites (see also spatial patterns below). Intact portions of colonies were transferred in experimental units (Half Pipe, hereafter HP, Spitale *unpublished*). HPs were plastic tube longitudinally cut, 90 cm long and 12 cm wide and 10 cm height. HPs were arranged together with one side immersed into a pond of the spring and uniformly inclined at about 30°, to simulate the natural slope of the spring. Species were transferred in each HP according to the following design (Fig 2): treatment species with three levels, *Sphagnum* downward *Warnstorfia*, *Scapania* downward *Warnstorfia* and *Warnstorfia* alone (hereafter called treatment *Sphagnum*, *Scapania* and *Warnstorfia*); treatment distance from water with three levels, low (15 cm), middle (35 cm) and high (60 cm). To investigate the effects of a stress variable in time HPs were raised 7 cm up to water level for 33-36 days to simulate a drought. The extent of these droughts simulates a natural but occasional water level fluctuation. Growth measurement of *Warnstorfia* was performed by tying pieces of nylon thread around the stems of apical fragments 3 cm long (Zechmeister 1995). Individuals from which these fragments were extracted derived from the same uniform patch (40 x 40 cm wide). Branches of these fragments were detached to obtain uniform starting structure. Soon after the marking, fragments were carefully inserted into the HPs. Recently, gametophyte fragments of brown mosses (like *Warnstorfia*) have been used with success also in fen restoration (Mälson and Rydin 2007).

The experiment was fully factorial with three levels of species crossed with three levels of distance from water and two levels of water level, for a total of 72 HPs and 360 marked fragments (5 replicated fragments for each HP x 3 distance from water replicated 2 times x 3 combinations of species x 3 replicated drought + 1 control of drought). A set of 18 HPs was immersed into the water for the entire duration of the experiment (control of drought), whereas three sets of 18 + 18 +18 HPs were raised up (drought simulation) for 33-36 days, each one in a successive data randomly chosen. The control for drought was denominate "constant stress" while drought treatments replicated through time were called "variable stress". After drought treatment, the set of HPs was re-immersed at normal water level. The experiment started on 18th May 2007 with the fragment insertion into the HPs; fifty days after the first set of HPs was raised up to simulate the drought. The second set was raised on 5th August and the third on 8th September. The experiment lasted 5 months before harvesting the fragments on 25th October. Fifty day after the beginning of the experiment (and before the drought treatments) fragments of *Warnstorfia* were measured and reinserted in the same position, to check for differences among HPs. This check allowed estimating the unexplained variance among treatments induced by external factors not explicitly considered in the experiment (i.e. difference in density plus random effects).

The variable water content into HPs was measured before the first drought, during each drought and at the end of the experiment. As water content changes rapidly following weather variations, only comparisons within date will be presented. However, to maintain as uniform as possible measurement conditions, water content was sampled after at least 4-5 days of no rain.

DATA COLLECTION

Fragment growths of *Warnsorfia* were measured using millimetre paper. Branches and stems were measured with an error of ± 1 mm; total length of growth was expressed as the sum of branches and stem length. Net growth was calculated as the difference between the total length and the initial length (3 cm).

Water content in the HPs was measured collecting four individuals (5-6 cm long) near the marked fragments (4 replicates for each distance for a total of 72 samples in each date). These samples were immediately inserted into plastic bags with hermetical sealing and weighted in laboratory. Dry weight was calculated after a treatment of 48 h at 60 °C. Total Water Content was calculated as TWC % d.w. = 100*(fresh weight – dry weight)/dry weight. Air, water and HP temperature were registered every 30 minutes throughout the experiment by means of temperature data loggers, while rain was measured by a pluviometer.

SPATIAL PATTERN ANALYSIS

In addition to the experimental approach, a further set of measurements was performed to identify natural spatial patterns of density and water content in *Warnstorfia*. As Birse (1957) showed, the growth form of a species in a specific microhabitat is influenced by its position in respect to the water table. She observed that packing (density) of a colony in hygro-hydrophylous species can be related to the position (and fluctuation) of the water table. To investigate spatial pattern of density and water, an area of 1 m^2 entirely covered by a colony of *Warnstorfia* was chosen as representative of a natural distribution. On this 1 m^2 and using a regular lattice 10x10 cm, portions of moss carpet were extracted with a razor frame (2x2 cm) and the apical 3 cm (volume = 2x2x3 = 12 cm^3) were conserved and analysed. Apical portion was taken because its water content could be slightly different than the bottom one. The height above a fixed reference point was measured for each sample in the grid.

STATISTICAL ANALYSIS

Differences of growth before the drought treatments were evaluated by three separated nested ANOVA, one for each distance: each analysis with two factors, species and HP nested in species. Final growth data were analysed by a three way fully factorial ANOVA with two fixed factors (species and distance) and one random (time of drought). A priori post hoc comparisons were made with Helmert contrast in agreement with the following: for treatment species, *Warnstorfia* vs *Sphagnum* + *Scapania* and *Sphagnum* vs *Scapania*; for treatment droughts, control (0) vs drought 1+2+3; for treatment distance low vs middle, middle vs high. Differences among drought (1, 2, 3) were evaluated by a post hoc Tukey test. Necessary transformations were made before analysis to remove heterogeneity of variance. For growth data log 10 was used, whereas for percentage of total water content the Box-Cox transformation.

Interaction intensity was calculated following the index proposed by Armas et al (2004) because of its strong mathematical and statistical properties, which overcome problems shown by other frequently used indices. RII = $(B_W - B_0)/(B_W + B_0)$, where B_W is the mean growth of *Warnstorfia* with neighbour species and B_0 the growth of *Warstorfia* alone. RII, interaction intensity, has defined limits (+1, -1), is symmetrical around zero with identical absolute values for competition and facilitation (positive values indicate facilitation). Once evaluated homogeneity of variance between B_W and B_0, the variance of RII was calculated following the method proposed by Armas et al (2004).

Spatial patter of density and water content of *Warnstorfia* in natural habitat were investigated by contour maps (gridding using Kriging); in addition, spatial autocorrelation was estimated by Moran's correlograms (Fortin and Dale 2005). To model the spatial variation of density a partial regression was used, with Y density, X water, W spatial coordinates incorporating fourth degree polynomial (Legendre and Legendre 1998). Since a physical water accumulation was expected in the lowest points of the plot because of gravity, a trend-surface equation was computed to account for it, using only the Z coordinate (top-bottom axis). Then, once significant terms were retained by backward elimination, residuals of this equation were used to compute partial regression.

Results

SPATIAL PATTERN ANALYSIS

Significant linear relation was found between density and water ($R^2 = 0.26$; $P < 0.0001$). Significant positive autocorrelation was found in the first distance classes of the correlograms (Fig 3), supporting the hypothesis of patchiness of water and density. The distance at which the first maximum negative autocorrelation value is found indicates the size of patches. This occured in class 5 for water, corresponding to a distance of 25 cm; the same trend was present for density but the value of autocorrelation after Bonferroni correction for progressive comparison was not significant. To express the response variable density as a nonlinear function of the plot coordinates xy, a trend surface analysis was performed on the residuals of the trend-surface equation using the z coordinate. A significant trend surface was found, involving z^3 that accounted for $R^2 = 0.310$. To estimate how much of the variation of the response variable density can be attributed exclusively to W (spatial coordinates xy and polynomial terms x^3, x^4) and to X (water content) a partial linear regression was performed on the residuals of trend equation. The multiple regression of density on X and W together explained a $R^2 = 0.695$, while density on X alone explained a R^2 of 0.428. The fraction of density explained only by W was $R^2 = 0.124$; the fraction shared between X and W was $R^2 = 0.143$.

NET GROWTH AND TWC BEFORE DROUGHT TREATMENTS

Fifty days after the experiment started, there were not differences in growth neither among species (Tab 1) nor among HPs, with the exception of the HPs at high distance from water. Nevertheless, a further post hoc Tukey test on this term was not able to reveal any difference. According to the three ANOVAs, differences among species were not significant at any level of distance from water. The growth rate, calculated as $(G_{t1}-G_{t0})/47$ days, was unexpectedly high, 0.30, 0.20, 0.09 mm/day respectively for the low, middle and high distance (average among species).

Total Water Content (TWC) before drought showed significant differences among species (*Warnstorfia* vs *Sphagnum* + *Scapania* $P<0.0001$; *Sphagnum* vs *Scapania* $P>0.05$) and among distances (Tab 2). A priori comparison between low vs medium and medium vs high levels were both significant at $P<0.0001$).

TWC DURING DROUGHT TREATMENTS

The effect of droughts and relative differences of TWC during the experimental period should be interpreted in the light of climatic fluctuation (Fig 4). There were evident differences in total rain as well as in mean air temperature throughout the experiment. In particular, during the third drought, air temperature dropped significantly and it snowed.

In the first drought, interaction species*distance was significant and post hoc comparisons revealed significant differences between distance low vs middle and middle vs high for all the species with the exception of *Scapania* for which level middle did not differ from high (Tab 3). Contrasts between *Warnstorfia* vs *Sphagnum* + *Scapania* and *Sphagnum* vs *Scapania* were significant at all distances with the exception of level low where *Sphagnum* did not differ from *Scapania*. Post hoc comparisons of significant term species*droughts showed significant differences between control and droughts for all the planned contrasts.

In the second drought, post hoc comparisons of term species*distance revealed that there were not significant differences among species at low distance from water (Tab 3). Other planned contrasts were all significant.

In the third drought all the first-order interactions were significant (Tab 3). Post hoc comparisons of species*distance term indicated that there were not significant differences among species at low distance from water and that the contrast *Sphagnum* vs *Scapania* was significant both at middle and high distance. The term species*drought revealed interesting difference from the previous droughts because only in *Scapania* the control of drought significantly differed from drought. Post hoc comparison of term distance*drought revealed significant difference between control and drought within distance only at middle and high distance from water.

NET GROWTH AFTER DROUGHT TREATMENTS

The three-way factorial anova on final growth (Tab 4) showed significant second-order interaction, species*distance*drought. Drought treatments replicated through time on *Warnstorfia* alone were not significant at any distance from water, and comparisons among drought within species were non significant at high distance from water (Fig 5). Significant growth differences were found in the treatment *Sphagnum* and *Scapania* both at low and middle distance from water (Fig 5). In particular, post hoc Tukey test calculated to compare replicated droughts, revealed significant difference ($P = 0.0002$) between drought 1 and 3 at middle distance in *Sphagnum*. A priori comparison between control of drought vs droughts 1+2+3, were significant ($P < 0.0001$) in *Scapania* at middle distance. At low distance from water, post hoc Tukey test showed significant ($P = 0.02$) difference between drought 1 and 3.

INTERACTION INDEX

Interaction intensity (Fig 6) showed that if stress was stable and low *Sphagnum* and *Scapania* were competitive to *Warnstorfia*; if stress was stable-medium *Sphagnum* was indifferent and *Scapania* competed; if stress was stable-high *Sphagnum* facilitated and *Scapania* competed. If plants experienced a stress variable-low they generally facilitated the growth of *Warnstorfia* (with the exception of the first drought in *Scapania*). In the case of variable-medium stress, the sign of the interaction depends on the time in which the drought happened, and at variable-high stress *Sphagnum* facilitated and *Scapania* competed two out of three droughts.

Discussion

In bryophyte colonies both negative and positive effects of density on growth have been observed (Rydin 1995; Petersen et al 2001): while negative density dependence is most likely caused by increased competition for resources, positive density dependence is claimed to be due to improved microclimatic conditions (presumably moisture) for growth within the colonies. Thus, it will be expected positive or negative dependence of density and water according to the balance of these mechanisms. Since in natural habitats *Warnstorfia* showed a significant ($P < 0.0001$) positive relation between density and water content, we can argue that positive effects of improved moisture, due to density, was generally more than the negative effects of intraspecific competition. Moreover, there was also a significant spatial structure of density and water as showed in correlograms and in contour maps (Fig 3). Positive and significant spatial autocorrelation in the first distance class supported the hypothesis of patchiness of density and water. The extent of these patches (or range of influence) is probably species-dependent, as demonstrated both in semi-natural conditions (Fig 1) and in the field for *Warnstorfia*. In natural habitats, density of *Warnstorfia* was (~ 70 %) accounted for spatial coordinates and for water content. In particular, density was explained for a ~ 43 % by water content, while the pure contribute of spatial heterogeneity was a ~ 12 %. This natural heterogeneity of density is transferred inside the experimental units (HPs) modifying to some extent the water gradient. Nevertheless, the water gradient into HPs was rather predictable, with an explained variance of 84 %, 75 % and 85 % respectively for *Sphagnum*, *Scapania* and *Warnstorfia*. As a consequence, the unexplained variance of these water gradients was attributable to natural clump of density, spatial heterogeneity and manipulation errors.

A validation of the HPs method was gained from the ANOVAs performed on fragments growth before drought treatments, showing that differences among replicates were generally not significant. More interesting, there were significant differences in TWC among species, but evidently, these effects were not enough to generate significant differences in growth. Thus, a length of 47 days-treatment would be not sufficient to demonstrate facilitation or competition among these species.

Because of natural climatic variations (Fig 4), drought strength was not similar among replicated treatments, resulting in different degree of stress experienced by the species. Even though TWC was measured once per drought (after at least 4-5 days of no rain), it provided some indication about the treatment efficiency. If a drought occurred in a rainy lag time, probably the stress experienced by species was lesser than in a dry period. It results that drought 1 was probably more efficient than drought 2 and 3. The amount of water content in *Warnstorfia* depended by the downward species (and relative distance from water), but also by a complex balance between mean air temperature (influencing the evaporation process) and lag time from the last rain.

Final growth (Tab 4; Fig 5) in the levels distance from water was generally consistent with the expectations (higher growth near water and minimum at high distance). In contrast, growth in the treatment species and droughts was more variable according to the shift between competition and facilitation and the climatic fluctuation. More variability in growth occurred at middle distance from water, probably because it was the position, along the water gradient, most susceptible to variations. At high distance from water, representing the treatment most stressful, there was a general depression of growth and an absence of significant differences of growth among the treatment species (with the exception of the contrast between *Sphagnum* and *Scapania* in the control of drought). More interesting, when the colony of *Warnstorfia* was alone, drought treatments had no effects on it at any level of stress but when *Warnstorfia* was upward a species, either *Sphagnum* or *Scapania*, droughts had stronger effects on it (Fig 5). It was rather surprising, because these results suggest that since neighbour species can have a double effect, either negative or positive, a target species growing alone experiences a more uniform life condition. That is, growing alone means to run lesser risk than cohabiting with neighbours, even though the direction of these risks can yield positive or negative interactions.

Theories predict that facilitation should be higher in stressful environment (Bertness and Callaway 1994; Callaway et al 2002). Considering that a drought should be a stressful event (intensity of which depends on climatic fluctuations), comparing the control of drought and droughts within distance (Fig 5), we can infer that *Scapania* actually facilitates the growth of *Warnstorfia* at medium stress (the treatment *Sphagnum* showed the same trend but it was statistically not significant). Interestingly, if a stable stress was considered (control of drought), we expected competition at low distance from water (low stress) and facilitation at middle distance. Indeed, at low distance both *Scapania* and *Sphagnum* competed (Fig 6), but at medium stress *Scapania* still competed and *Sphagnum* was indifferent. This different response, to some extent unexpected (but see discussion on Maestre et al 2006), could be explained recurring to the length of water gradient. Since the two neighbour species, *Scapania* and *Sphagnum* differ substantially in their colony architecture, and consequently in their water transport inside the colony (Fig 1), at a given distance from water the length of the gradient is different between the two species. In fact, considering *Sphagnum* at stable stress, we observed a complete shift from negative, null and positive interaction through respectively low, medium and high stress. *Scapania* did not shift from competitive interaction (at low stress) to positive (at medium stress), because medium and high distance represent the tail of its water gradient. Thus, the expected shift from competitive to facilitative interaction would occur in a position along the gradient between low and middle distance from water. Then, as predicted by Travis et al (2006), at high stress interaction may come back again to competition.

When the mechanism of plant-plant interaction involves water as in the present study, variable stress events occurring at different times in natural environment can have very different outcomes (Fig 6). For example, drought 1 was probably the most effective, considering that air temperature reached the highest values and total rainfall was less than during other droughts (Fig 4). In that condition, *Scapania* competed both at low, middle and high distance from water. Along a gradient of increasing environmental severity, Maestre and Cortina (2004) showed as interaction shifted from negative, to positive and back to negative following a hump-backed relationship. Thus, in the case of drought 1 in *Scapania*, we observed only negative interactions probably because the level of stress shifted totally to the right in the hump-backed curve. *Sphagnum*, under the same environmental conditions, followed the second half of the hump curve, shifting from positive to negative passing respectively through low and middle distance from water. Drought 2 and 3 were more similar each other (Fig 4), because during drought 2 rainfall was more than during drought 3 but also mean air temperature was more, influencing the overall evaporation process (it should be considered that evaporation process occur at different velocity in *Sphagnum* and *Scapania*). However, interaction shifts were again well explained by the hump curve. In conclusion, it seems that interactions in these species can be interpreted in the light of gradient length of stress (as recently underlined by Maestre et al, 2006 and Lortie and Callaway, 2006) even though future studies focusing on long term dynamics will improve our ability to predict these interaction processes.

Figure captions

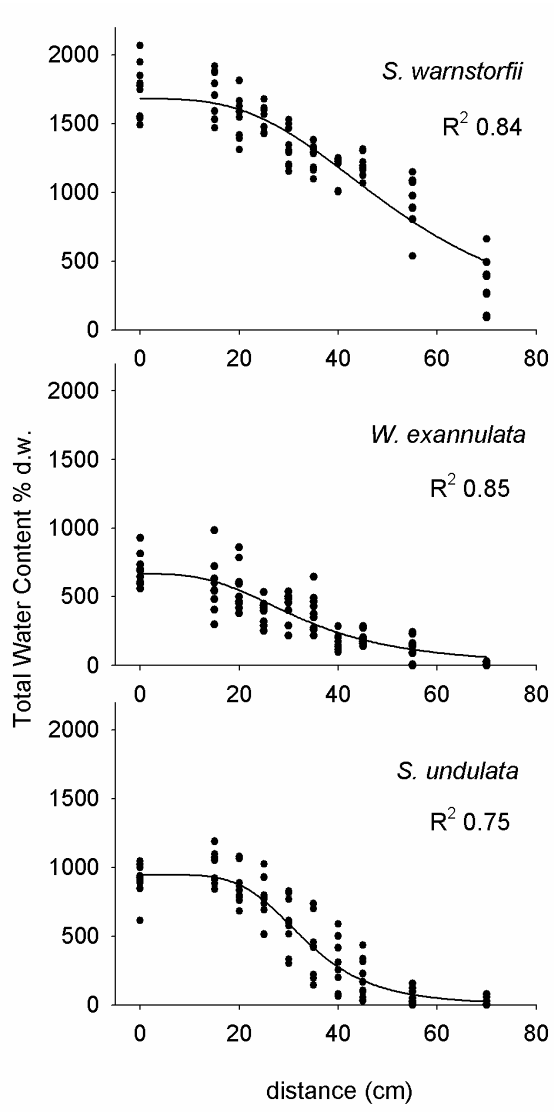


Fig 1. Differences in Total Water Content in the three bryophyte species arranged into experimental units. Species are *Sphagnum warnstorfii*, *Warnstorfia exannulata* and *Scapania undulata*.

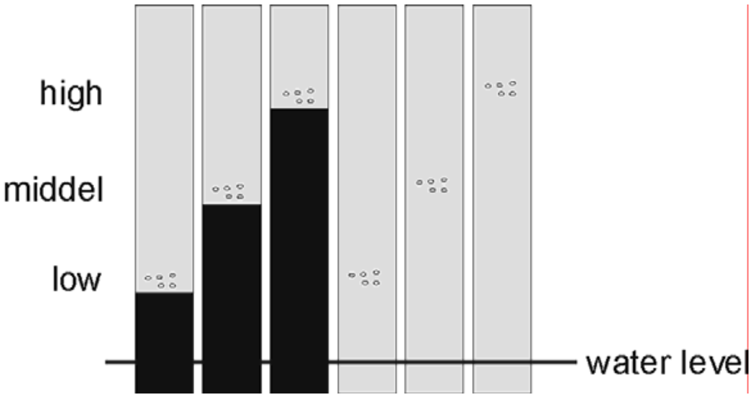


Fig 2. Experimental units (HPs) in which colonies were transferred: in black are *Scapania undulata* or *Sphagnum warnstorfii* and in grey is *Warnstorfia exannulata*. Low, middle and high are levels of treatment distance from water; dots are marked *Warnstorfia* fragments.

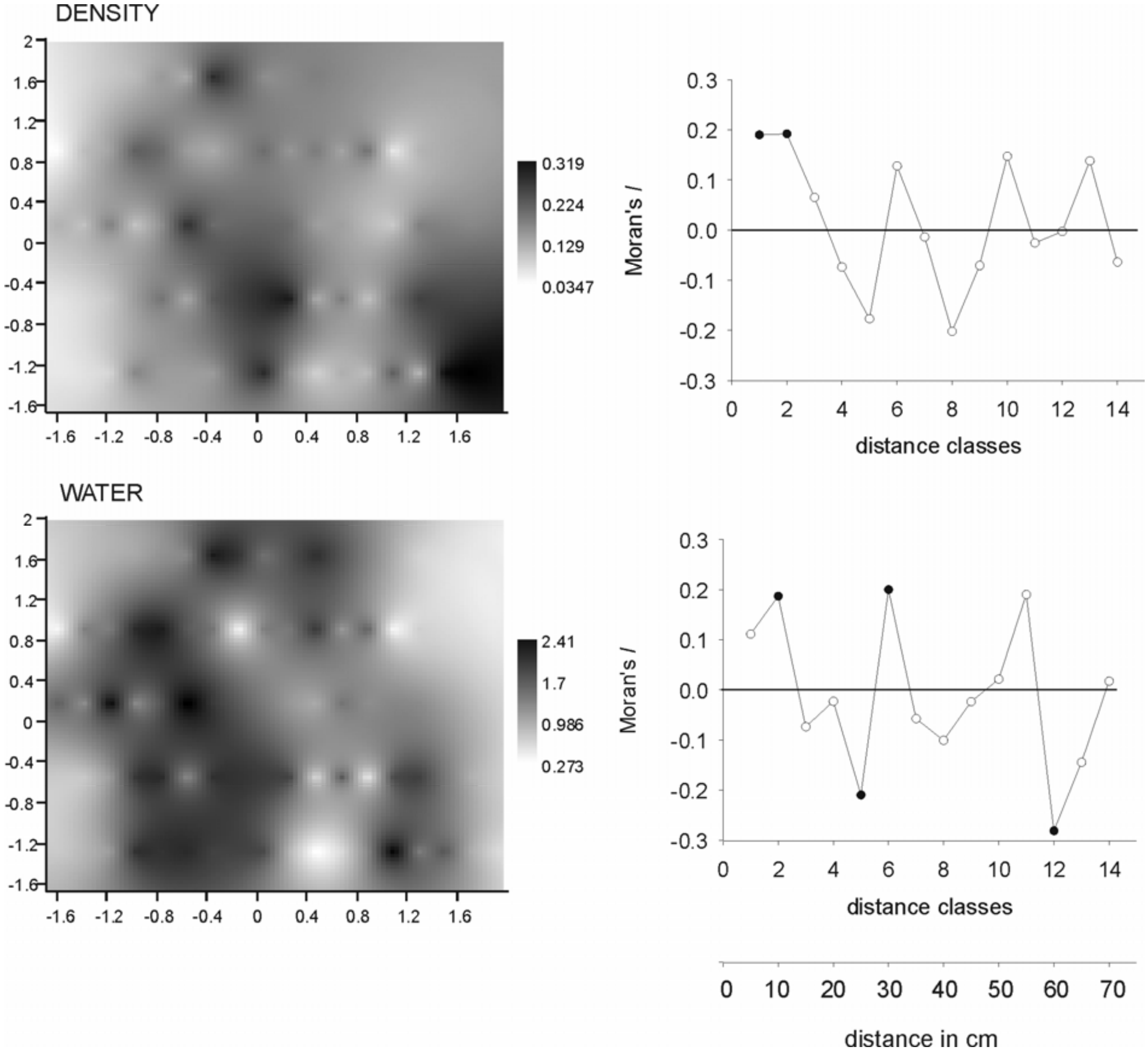
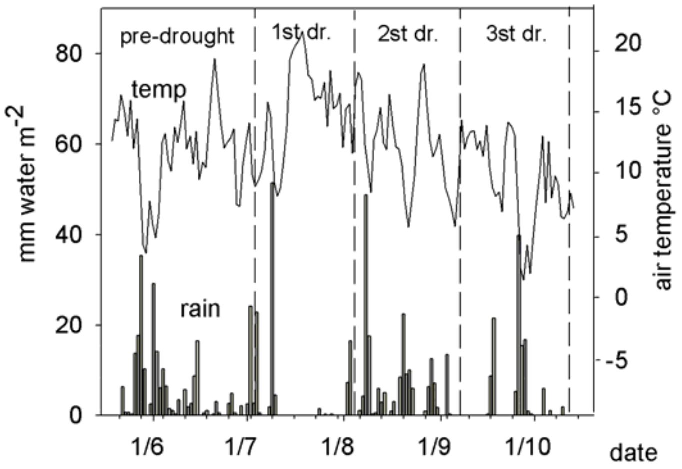


Fig 3. Density and water content of *Warnstorfia exannulata* in natural habitat. On the left interpolated maps of density and water and their corresponding correlograms on the right. Dark dots in correlograms are significant values of autocorrelation after progressive Bonferroni correction. Density and water are expressed in grams per 12 cm^3 of sample volume. Gradient from black to white colour indicates progressive variations where black is high density or high content of water.



period		mean temp	total rain	lag time
18/05-02/07	pre-drought	11.6	226	47
03/07-04/08	1st drought	14.7	90	33
05/08-07/09	2st drought	11.7	172	34
08/09-13/10	3st drought	9.3	121	36

Fig 4. Climatic variations during the field experiment. Rainfall and air temperature are distinguished in four lags of time, that is pre-drought period and the three independent drought replicates.

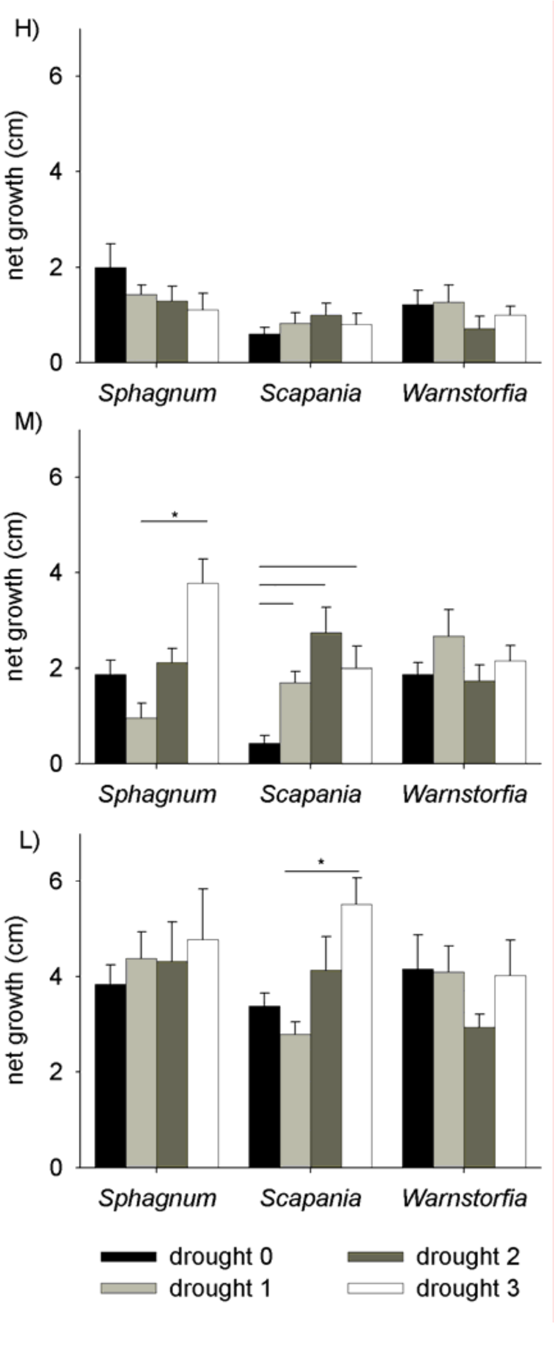


Fig 5. Final net growth of *Warnstorfia exannulata* fragments in treatments species (*Sphagnum* = *W. exannulata* upward *Sphagnum warnstorfii*, *Scapania* = *W. exannulata* upward *Scapania undulata* and *Warnstorfia* = *W. exannulata* alone) distances from water (L = Low − graph on the bottom, M = Middle − graph in the middle and H = High − graph on the top) and droughts (0 = always immersed, 1, 2, 3 independent replicates of drought). Horizontal bars show significant differences (P<0.05): with asterisk = post hoc Tukey test; without asterisk = significant Helmert contrast, see methods for further details. Vertical bars are SE.

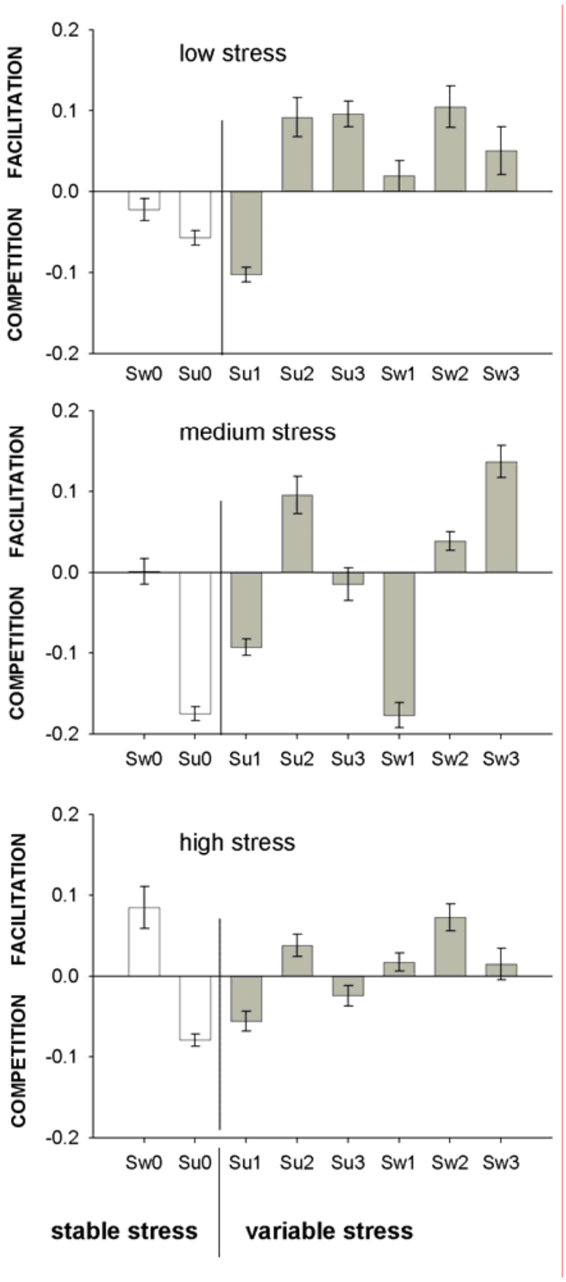


Fig 6. Interaction intensity calculated following Armas et al (2004) RII = (BW – B0)/(BW + B0), where BW is the mean growth of *Warnstorfia exannulata* with neighbour species and B0 the growth of *Warstorfia* alone. RII has +1, -1 limits, positive values indicate facilitation and negative competition. Stable stress means the treatment drought 0 (= experimental units always immersed) and variable stress means the treatment drought independently replicated at three different times. Sw0 = treatment *Sphagnum warnsorfii* in drought 0; Su0 = *Scapania undulata* in drought 0; Sw1-Sw2-Sw3 = *Sphagnum warnstorfii* in drought 1, 2, 3; Su1-Su2-Su3 = *Scapania undulata* in drought 1, 2, 3. Vertical bars are SE calculated following Armas et al (2004).

distance	treatment	SS	df	MS	F	p	species	mean	SD
low	species	0,016	2	0,008	0,946	0,392	*S.w.*	0,655	0,092
	HP(species)	0,014	3	0,005	0,558	0,644	*S.u.*	0,679	0,097
	error	0,947	114	0,008			*W.e.*	0,654	0,082
middle	species	0,011	2	0,006	0,756	0,472	*S.w.*	0,631	0,087
	HP(species)	0,006	3	0,002	0,272	0,846	*S.u.*	0,620	0,102
	error	0,848	114	0,007			*W.e.*	0,607	0,063
high	species	0,008	2	0,004	1,075	0,345	*S.w.*	0,546	0,066
	HP(species)	0,032	3	0,011	2,989	**0,034**	*S.u.*	0,528	0,058
	error	0,408	114	0,004			*W.e.*	0,543	0,060

Tab 1. Differences of growth after 47 days and before drought treatments in the three levels of distance from water. S.w. = *Sphagnum warnsorfii*, S.u. = *Scapania undulata*, W.e. = *Warnstorfia exannulata*.

	df	SS	MS	F	P
species	2	2,181	1,090	12,817	**0,000**
dist	2	67,426	33,713	396,303	**0,000**
species*dist	4	0,135	0,034	0,397	0,810
error	134	11,399	0,085		

Tab 2. Two-way ANOVA of Total Water Content between factor species (three levels) and distance (three levels) after 47 days and before drought treatments. Dist = treatment distance from water. R^2 of the model = 0.873.

		drought 1		drought 2		drought 3	
	df	F	p	F	p	F	p
sp	2	42,5	0,000	13,8	0,000	17,2	0,000
dist	2	158,6	0,000	198,5	0,000	266,5	0,000
dr	1	42,4	0,000	2,4	0,131	17,5	0,000
sp*dist	4	5,2	**0,001**	3,9	**0,007**	9,4	**0,000**
sp*dr	2	3,4	**0,041**	1,6	0,204	12,1	**0,000**
dist*dr	2	0,1	0,945	0,3	0,709	7,1	**0,002**
sp*dist*dr	4	1,3	0,283	1,0	0,439	2,3	0,071

Tab 3. Three-way ANOVA of Total Water Content during drought treatments replicated independently at three different times. Sp = treatment species; dist = treatment distance from water; dr = drought. R^2 of the models: drought 1, R^2 0.898; drought 2, R^2 0.893; drought 3, R^2 0.925.

	effect	SS	df	MS	F	p
sp	fixed	0,165	2	0,082	1,289	0,342
dist	fixed	5,018	2	2,509	53,841	**0,000**
dr	random	0,132	3	0,044	0,607	0,641
sp*dist	fixed	0,075	4	0,019	0,495	0,740
sp*dr	random	0,383	6	0,064	1,691	0,206
dist*dr	random	0,280	6	0,047	1,234	0,355
sp*dist*dr	random	0,453	12	0,038	2,501	**0,004**
error		4,892	324	0,015		

Tab 4. Three-way ANOVA of final growth of *Warnstorfia exannulata*. Sp = treatment species; dist = treatment distance from water; dr = drought. R^2: 0.571.

10

CONCLUSIONS

The uneven plant distribution in nature has a multiple origins. In literature there are two classical models that have been recently reconcilied as mutual instead of alternative: the environmental control model, where environment is deemed responsible for the observed variations in the presence or abundance of species; and the biotic control model, where interactions are considered to be the primary factors structuring communities. Biodiversity analysis at large spatial scale in springs of Trentino represented a singular opportunity to evaluate the strength of the first model, while the biotic control was experimentally investigated at fine spatial scale, with a study on the rule of plant-plant interactions and mechanisms of spatial distribution.

On the basis of 86 springs investigated, 167 bryophyte and 201 vascular plants species identified, statistical classification of spring habitats with the related species identified 11 groups. Each group was distinguished by a specific range of environmental variables (range of discharge, shading, size, substrates, altitude, concentrations (in the water) of nitrogen, phosphorus, sulphates, and a synthetic variable representing pH, conductivity, Ca, Mg and Na), and by a specific combination of species-abundances. On calcareous substrates six groups were identified mainly differentiated by altitude (with a shift from *Palustriella commutata* to *P. falcata*), by discharge (with the presence of *Rhynchostegium riparioides*), and by nutrients, which identify disturbed springs. On silicate substrates, springs were usually more heterogeneous because of a minor species fidelity. However, five groups were identified. For example, at low altitude and in shaded conditions, species like *Stellaria nemorum*, *Saxifraga rotundifolia*, and the genus *Rhyzomnium* spp. formed a distinct group, especially on porphyry. At higher altitude and in exposed sites, *Saxifraga stellaris* and *Deschampsia caespitosa* were usually present, and in helocrene springs they were found together with *Warnstorfia exannulata*, *Dicranella palustris*, and *Eriophorum angustifolium*. In the alpine belts, species adapted to cold conditions, such as *Luzula alpinopilosa* and the liverwort *Anthelia juratzkana*, were found in habitats resembling snow-beds. Bryophyte and vascular plant check lists, together with spring classification, represent the first exhaustive result of this type for the Autonomous Province of Trento, which –significantly- is also the first for the Italian Alps. This study also provided the first baseline for future comparisons for conservation purposes. In addition, this first classification provides the basis for further integrated classifications considering also other component of the biota (e.g. algae, zoobenthos). In fact, an indirect but important conclusion was that plant associations were not able to discriminate, for instance, among different types rheocrenes. It is likely that an integrated classification will be more suitable for this scope.

Canonical correspondence analysis on species and environmental variables suggested that only altitude, substrate (with its proxy called 1Pco) and shading were important in structuring plant community in springs at large spatial scale. Overall the CCA explained 12% of the variance in species abundance, but it varied depending on the species-sites selection. However, this low explained variance suggested that other important variables were apparently missing. The high species diversity and the ecological mechanisms operating at fine spatial scale are supposed to be the main reasons of this low explained variance.

Bryophyte and vascular plant richness in spring habitats depends on a complex interaction of environmental variables. In particular, altitude, tree-canopy cover, lithology, anthropogenic disturbance, spring area, spring complexity, and discharge, both directly and indirectly, were able to explain 24% and 55% of the total variance of respectively bryophyte and vascular plant richness. It was advanced a model in which tree-canopy cover, used as proxy of light availability to understory vegetation, has a strong negative effect on vascular plants but not on bryophyte richness. While for vascular plants altitude and light were significantly related, for bryophytes the increasing richness with altitude was more related to conductivity (more species on siliceous substrates). Since tree canopy was an indicator of light availability, vascular plants resulted more sensitive to light competition. In contrast, bryophyte diversity can be high also at low-light intensities, because they usually have low-light compensation point. Water conductivity, that is an expression of geological substrates in nature-near habitats, played a significant role in structuring plant assemblages in springs. Previous works on other habitats, considering only vascular plants, showed higher richness on limestone than on siliceous substrates (Virtanen et al 2003) or the opposite trend either (Wohlgemuth & Gigon, 2003). In the present study I found no significant relation between conductivity and vascular plants, but, in contrast, a strong negative relation between conductivity and bryophyte richness. Higher bryophyte richness on siliceous substrates was the reason of the low species fidelity found, and the consequent difficulty to distinguish plant associations on silicate. Disturbances had negative effects on both bryophyte and vascular plants. However, results indicate that disturbance has a more strongly negative effect on bryophytes than on vascular plants in all the tested models. This was not totally unexpected, given that the most frequent disturbances were related to water availability (partial water abstraction, spring-bed modifications etc). Given that bryophytes do not have a complex root system, it is likely that they are more susceptible to this kind of disturbance. An unexpected result was the weak negative correlation between disturbances and altitude. This weakness was induced by disturbances due to pastures occurring at altitudes > 1700 m a.s.l. Thus, while at low altitude disturbances were characterized by direct anthropogenic effects (i.e. water abstraction, substrate modification, agriculture etc), at higher altitude disturbances were mainly determined by cattle-breeding activities (livestock grazing, trampling etc). The exploratory part of the model about the spring area-complexity-discharge paths seems fairly consistent with the hypothesis. Indeed, the low variance explained for area and spring complexity suggested that other unidentified variables (different from discharge) played a substantial role. However, discharge affects the increase of spring complexity and also of the spring area to some extent. Then, spring area influenced positively both bryophytes' and vascular plants' richness. In all the model tested, though, the positive effects of area was stronger on vascular plants than on bryophytes. This difference is likely to be a matter of scale, because in species-area relationships asymptote of bryophyte richness is reached earlier than in the case of vascular plants. The links connecting discharge and spring area with richness are relations of extreme importance for conservation and management purposes. It was indirectly demonstrated that water caption can reduce the spring area, causing a reduction of plant richness. Therefore, to limit the damage of natural springs, we may suggest to protect also larger springs, and, in the case of water requirement for agriculture, to abstract water at some distance downwards from the spring source. In this way, at least the peculiar spring biota will be preserved.

Analysis at large spatial scale demonstrated as a large amount of community variability remains unexplained, supporting the hypothesis that also mechanisms operating at fine spatial scale would be important in determining species richness and abundance. Bryophytes are able to structure and modify actively the spring habitat, offering and creating new possibilities to species establishment. They can modulate the environmental forces, directly by slowing down (and deviating) the water flow and indirectly by transporting water among capillary space. Bryophytes create habitat patches where environmental conditions and resource availability substantially differ from the surrounding unmodified environment. Then, the presence of such habitat patches may affect species diversity by providing suitable habitats for species that cannot survive in the unmodified habitat (and hence increase species richness by adding new species into communities). Therefore, recognizing the crucial rule that bryophytes could have, with the first field experiment, spatial distribution of bryophytes was investigated considering their hydrological niche. It was demonstrated that different colonies had different water holding and transporting capacity; however, their calculated hydrological niches were widely overlapping. To estimate the range of their hydrological niche, photosynthetic pigments were used as stress indicators. An important point not underlined yet in literature was that the reliability of these photosynthetic pigments in indicating the stress condition experienced by a plant: indeed, unexpectedly, this was even found to be species-specific. Therefore, before using these indices one should ensure which of them are suitable for the species investigated. Optimal distance from water for each species was calculated in agreement with the range of no stress. The wide overlap of the three aquatic species (*Scapania undulata*, *Warnstorfia exannulata*, and *Philonotis fontana*) in their hydrological niche well explains their coexistence. Even though wide overlapping of distance exists, *W. exannulata* presents rather different median values. *W. exannulata* seems to be well adapted to grow at some distance from water, but it can also occupy the zone close to the water. The situation appears to be different for *S. undulata* and *P. fontana*, which preferentially inhabit the zone closer to the water, but they can reach the same distance as *W. exannulata*. *Sphagnum warnsorfii* has a predicted distance from water that was, as expected, far away from the water, followed by *Pleurozium schreberi* that is a xerotolerant species. In conclusion, even though only a limited number of species were tested, it seems that to bryophyte coexistence in spring habitats requires widely overlapping hydrological niches.

An further unexpected result stemmed from the previous experiment was that, although the hydrological niches of the species did widely overlap, they were rather differentiated to the respect of water holding capacity and water transport within the colony. These differences among colonies were supposed to generate some form of interaction. Many recent studies have shown that plant species can have strong, and often species-specific, positive and negative effects on other plant species. The balance between positive (facilitation) and negative (competition) interaction are thought to be dependent upon the degree of environmental stress. The idea was that interactions could influence the realized hydrological niche of a species enlarging or shrinking the fundamental niche according to the environmental stress experienced by a species. Therefore, a second field experiment was planned to study the complex balance between positive and negative interaction in relation to an imposed water stress. It was demonstrated that species should not be considered as competitors or facilitators in absolute terms. Rather, as predicted by some current models, facilitation occurs when environmental conditions are severe, while competition predominates in ameliorate conditions. In addition, it was demonstrated that when stress become higher, facilitation can shift again towards competition. This last result was very innovative, and for the first time ascertained in a field experiment. In natural environments, the degree of stress (in this case water availability) can change through time. The second most important result linked the natural environmental variability to the plant-plant interaction balance. Synthesising, this experiment has both a general ecological value, and also a specific value for spring habitats. Since the mechanisms and the effects of environmental fluctuations on spring bryophytes were pointed out, now specific tools are available to investigate the effects of abnormal fluctuations, like those generated by environmental changes. Thus, future studies will allow to predict these effects also in understudied habitats like springs.

Finally, the present thesis provides ecological foundations to understand occurrence and role of bryophytes and vascular plants in spring habitats of the Alps. These are a necessary prerequisite for the development of specific assessment and evaluation methods, which can take advantage also from a macroscopic and structuring component of the spring biota, such as bryophytes and vascular plants. These tools are necessary and urgently needed, since the predicted reduction of water availability in many regions due to climate change makes spring habitats conservation an increasingly difficult issue.

REFERENCES

Aeschimannn D. Lauber K., Moser D.M., Theurillat J.P., 2004. Flora alpina. Zanichelli, Bologna.

Arhonditsis G.B., Stow C.A., Steinberg L.J., Kenney M.A., Lathrop R.C., McBride S.J. , Reckhow K.H., 2006. Exploring ecological patterns with structural equation modelling and Bayesian analysis. Ecological Modelling 192: 385–409.

Armas C., Ordiales R., Pugnaire F., 2004. Measuring plant interaction: a new comparative index. Ecology 85(10): 2682–2686.

Arrhenius O., 1921. Species and area. Journal of Ecology 9: 95–99.

Austin M.P., 2002. Spatial prediction of species distribution: an interface between ecological theory and statistical modelling. Ecological Modelling, 157: 101–118.

Austin M.P., 2007. Species distribution models and ecological theory: A critical assessment and some possible new approaches. Ecol. Mod. 200: 1–19.

Badano E.I., Cavieres L., 2006. Ecosystem engineering across ecosystems: do engineer species sharing common features have generalized or idiosyncratic effects on species diversity? J. Biogeogr. 33: 304–313.

Bain J. & Proctor M.C.F., 1980. The requirement of aquatic bryophytes for free CO2 as inorganic carbon source: some experimental evidence. New Phytol., 86: 393–400.

Bartemucci P., Messier C., Canham C.D., 2006. Overstory influences on light attenuation patterns and understory plant community diversity and composition in southern boreal forest of Quebec. Canadian Journal of Forest Research 36: 2065–2079.

Baxter R, Emes M.J., Lee J.A., 1992. Effects of an experimentally applied increase in ammonium on growth and amino–acid metabolism of Sphagnum cuspidatum Ehrh. ex Hoffm. from differently polluted areas. New Phytol 120: 265–274.

Bazzaz F.A.,1975. Plant species diversity in old field successional ecosystems in southern Illinois. Ecology 56: 485–488.

Bertness M.D., Callaway R., 1994. Positive interaction in communities. Trends in Ecology and Evolution 9: 191–193.

Bianchi C.N., 2003. I fondi duri. Gambi M.C. & Dappiano M. (Eds), Manuale di metodologie di campionamento e studio del benthos marino mediterraneo. SIBM, Genova: 638 pp.

Biggs B.J.F., Goring D.G. & Nikora V.I., 1998. Subsidy and stress responses of stream periphyton to gradients in water velocity as a function of community growth form. Journal of Phycology 34: 598–607.

Birse E., 1957. Ecological studies of growth form in bryophytes. III. The relationship between the growth–form of mosses and ground water level supply. Journal of Ecology 46:9–27.

Bollen K.A., Stine R.A., 1992. Bootstrapping goodness of fit measures in structural equation models. Sociological Methods and Research, 21: 205–229.

Bollen K.A. 1989. Structural equations with latent variables. John Wiley and Sons, New York, New York, USA.

Bonettini A.M., Cantonati M., 1996. Macroinvertebrate assemblages of springs of the River Sarca catchment (Adamello-Brenta Regional Park, Trentino, Italy). *Crunoecia* 5: 71–78.

Brehm J., 1986. Der pH-Wert von Buntsandsteinquellen und Bächen. II. Der Waldquell-pH Wert nach der Höhenlage der Wasseraustritte und der Hangneigungsrichtung der Quelleneinzugsgebiete im Knüllgebirge. Beitr. Naturk. Osthessen 21: 53–66.

Brooker R.W., Maestre F.T., Callaway R.M., Lortie C.L., Cavieres L.A., Kunstler G., Liancourt P., Tielborger K., Travis J.M.J., Anthelme F., Armas C., Coll L., Corcket E., Delzon S., Forey E., Kikvidze Z., Olofsson J., Pugnaire F., Quiroz C.L., Saccone P., Schiffers K., Seifan M., Touzard B., Michalet R., 2007. Facilitation in plant communities: the past, the present, and the future. Journal of Ecology doi:10.1111/j.1365–2745.2007.01295.x.

Bruno J.F., Stachowicz J.J., Bertness M.D., 2003. Inclusion of facilitation into ecological theory. Trends Ecol. Evol. 18: 119–125.

Callaway R.M., Brooker R.W., Choler P., Kikvidze Z., Lortie C.J., Michalet R., Paolini L., Pugnaire F.I., Newingham B., Aschehoug E.T., Armas C., Kikodze D., Cook B.J., 2002. Positive interactions among alpine plants increase with stress. Nature 417: 844–848.

Callaway R.M., Walker L.R., 1997. Competition and facilitation: a synthetic approach to interactions in plant community. Ecology 78: 1958–1965.

Callaway R.M., Walker L.R., 1997. Competition and facilitation: a synthetic approach to interactions in plant community. Ecology 78: 1958–1965.

Cantonati M., 1998. Distribution and seasonality of the phytobenthos along two mountain spring streams in catchments of contrasting lithology. Supplemento Bollettino Museo Civico Storia Naturale Venezia 49: 357–367.

Cantonati M., 1998. Le sorgenti del Parco Adamello–Brenta. Parco Documenti, 11. Publisher and distributor: Parco Adamello–Brenta, Via Nazionale 12, I–38080 Strembo (Trento). 177 pp.

Cantonati M. & Bonettini A.M., 1995. Ecomorfologia di sorgenti del Sarca nel Parco Adamello-Brenta (Trentino). Quaderni della Stazione Ecologia, Museo Civico di Storia Naturale, Ferrara 9: 345–351.

Cantonati M., Rott E., Pipp E., 1996. Ecology of cyanophythes in mountain springs of the River Sarca catchment (Adamello-Brenta Regional Park, Trentino, Northern Italy). Archiv für Hydrobiologie, Supplement Algological Studies 83: 145–162.

Cantonati M., Pipp E., 2000. Longitudinal and seasonal differentiation of epilithic diatom communities in the uppermost sections of two mountain spring-fed streams. Verhandlungen Internationalen Vereinigung für Limnologie 27: 1591–1595.

Cantonati M., Bertuzzi E., Gerecke R., Ortler K. & Spitale D., 2005. Long-term ecological research in springs of the Italian Alps: six years of standardised sampling. Verhandlungen Internationalen Vereinigung für Limnologie 29: 907–911.

Cantonati M., Gerecke R. & Bertuzzi E., 2006. Springs of the Alps–sensitive ecosystems to environmental change: from biodiversity assessments to long–term studies. Hydrobiol. 562: 59–96.

Chanta S.C., Cloutier L., Cattaneo A., 2000. Epiphytic algae and invertebrates on aquatic mosses in a Quèbec stream. Archiv für Hydrobiologie 147 (2): 143–160.

Cheng D.L., Wang G.X., Chen B.M., Wei X.P., 2006. Positive interactions: crucial organizers in a plant community. Journal of Integrative Plant Biology 48(2): 128–136.

Chytry M., 2001. Phytosociological data give biased estimates of species richness. J. Veg. Sci. 12: 439–444.

Chytry M. & Zdenka O., 2003. Plot size used for phytosociological sampling of European vegetation. J. Veg. Sci. 14: 563–570.

Clarke K.R., Gorley R.N., 2006. Primer v6: User Manual/Tutorial. PRIMER-E: Plymouth.

Cleavitt N.L., 2002. Stress tolerance of rare and common moss species in relation to their occupied environments and asexual dispersal potential. J. Ecol. 90:785–795.

Connell J.H., 1978. Diversity in tropical rain forest and coral reefs. Science 199: 1302–1309.

Conti E., Solits D.E., Hardig T.M. & Schneider J., 1999. Philogenetic relationships of the Silver Saxifrages (Saxifraga, Sect Ligulatae Haworth): implications fort he evolution of substrate specificity, life histories, and biogeography. Molec. Phylogen. Evol., 13: 536–555.

Cortini Pedrotti C., 2001–2005. Flora dei muschi d’Italia. I e II parte. Antonio Delfino Editore, Roma.

Crema S., Ferrarese U., Golo D., Modena P., Sambugar B., Gerecke R., 1996. Ricerche sulla fauna bentonica ed interstiziale di ambienti sorgentizi in area alpina e prealpina. Centro di Ecologia Alpina, Trento, Report 8: 1–104.

Currie D.J., Paquin V., 1987. Large–scale biogeographical patterns of species richness of trees. Nature, 329: 326–327.

de Candolle A., 1855. Géographie botanique raisonnée: ou l'exposition des faits principaux et des lois concernant la distribution géographique des plates de l'epoque Actuelle. Maisson, Paris.

Dilks T.J.K., Proctor M.C.F., 1979. Photosynthesis, respiration and water content in bryophytes. New Phytol 82: 97–114.

During H.J., 1992. Ecological classifications of bryophytes and lichens. In: Bates JW and Farmer AM (eds) Bryophytes and Lichens in a Changing Environment. Clarendon Press, Oxford.

Ellenberg H., 1988. Vegetation Ecology of Central Europe. Cambridge University Press.

Fensham, R.J., Price R.J., 2004. Ranking spring wetlands in the Great Artesian Basin of Australia using endemicity and isolation of plant species. *Biological Conservation* 119: 41–50.

Fortin M.J., Dale M.R.T., 2005. Spatial analysis: A guide for ecologists. Cambridge University Press, Cambridge.

Gaston K.J., 1996. Biodiversity: a biology of numbers and difference. Blackwell Science, Oxford UK.

Gerecke R., Meisch C., Stoch F., Acri F., Franz H., 1998. Eucrenon/ Hypocrenon ecotone and spring typology in the Alps of Berchtesgaden (Upper Bavaria, Germany). A study of microcrustacea (Crustacea: Copepoda, Ostracoda) and water mites (Acari: Halacaridae, Hydrachnellae). In: Botosaneanu L. (Ed.), Studies in crenobiology. Backhuys Publishers. Leiden: 167-182.

Gignac L.D., Vitt D.H., Bayley S.E., 1991. Bryophytes response surfaces along ecological and climatic gradients. Vegetatio 93: 29–45.

Gigon A., 1987. A hierarchic approach in causal ecosystem analysis: the calcifuge–calcicole problem in Alpine grasslands. Ecol. Stud. 61: 228–244.

Gleason H.A., 1925. Species and area. Ecology 6: 66–74.

Gleason, H.A., 1922. On the relation between species and area. Ecology 3: 158–162.

Glime J.M., Vitt D.H., 1984. The physiological adaptations of aquatic Musci. Lindbergia 10: 41–52

Glime J.M., Vitt D.H., 1987. A comparison of bryophytes species diversity and niche structure of montane streams and stream banks. Can J Bot 65: 1824–1837.

Grace J.B., 1999. The factors controlling species density in herbaceous plant communities: an assessment. Perspective in Plant Ecology, Evlution and Systematics 2: 1–28

Grace J.B., Pugesek, B.H., 1997. A structural equation model of plant species richness and its application to a coastal wetland. The American Naturalist, 149(3): 436–460.

Grace J.B., 2006. Structural equation modeling and the study of natural systems. Cambridge Univ. Press.

Greenlee J.T., Callaway R.M., 1996. Abiotic stress and the relative importance of interference and facilitation in montane bunchgrass communities in western Montana. American Naturalist 148: 386–396.

Greig-Smith P., 1983. Quantitative plant ecology. Blackwell Publishing Limited.

Grime J.P., Rincon E.R., Wickerson B.E., 1990. Bryophytes and plant strategy theory. Bot. J. Linn. Soc. 104: 175–186.

Grime J.P., 1973. Control of species diversity in herbaceous vegetation. Journal of Environmental Management 1: 151–167.

Grime J.P., 2001. Plant strategies, vegetation process, and ecosystem properties, 2nd. John Wiley & Sons, LTD.

Grytnes J.A., Heegaard E., Ihlen P.G., 2006. Species richness of vascular plants, bryophytes, and lichens along an altitudinal gradient in western Norway. Acta Oecologica 29: 241–246.

Hector A., Bagchi R., 2007. Biodiversity and ecosystem multifunctionality. Nature 448: 188–191.

Heino J., Virtanen, R., Vuori, K.M., Saastamoinen, J., Ohtonen, A. & Muotka, T., 2005. Spring bryophytes in forested landscapes: land use effects on bryophyte species richness, community structure and persistence. Biol. Cons. 124: 539–545.

Hill W.R., 1996 - Effects of light. In: Stevenson R.J., Bothwell M.L. & Lowe R.L. (eds), *Algal Ecology*. Academic Press, San Diego: 121-149.

Hinterlang D., 1992. Vegetationsökologie der Weichwasserquellgesellschaften zentraleuropäischer Mittelgebirge. Crunoecia 1: 5–117.

Howein H., 1998. Erfassung und strukturelle Typisierung der Quellen im Nationalpark Berchtesgaden. Institut für Geographie Universität Erlangen- Nürnberg, Diplomarbeit: 1-113 + Anhang.

Howein H., Schroeder G., 2006. Geomorphologische Untersuchungen. In: Franz H. & Gerecke R. (Eds), Quellen im Nationalpark Berchtesgaden. Lebensgemeinschaften als Indikatoren des Klimawandels. Nationalpark Berchtesgaden, Forschungsbericht, 51: 268 pp.

Hunter A.F., Aarssen L.W., 1988. Plants helping plants. BioScience 38(1): 34–40.

Hunter M.L., Webb S.L., 2002. Enlisting taxonomists to survey poorly known taxa for biodiversity conservation: a lichen case study. Conservation Biology 16: 660–665.

Hutchinson G.E., 1957. Concluding remarks. Cold Spring Harbour Symposia on Quantitative Biology 22: 415–427

Illies J., Botosaneanu L., 1963. Problèmes e méthodes de la classification écologique des eaux courantes, considerées surtout du point du vue faunistique. Mitteilungen der Internationalen Vereinigung für theoretische und angewandte Limnologie 12: 1–57.

Ilmonen J., Paasivirta L., 2005. Benthic macrocrustacean and insect assemblages in relation to spring habitat characteristic: patterns in abundance and diversity. Hydrobiologia 533: 99–113.

Jaccard P., 1901. Distribution de la flore alpine dans le Bassin des Dranes et dans quelques régions voisines. Bulletin Société Vaudoise des Sciences Naturelles, 37: 241–272.

Johnson M.L., Huggins D.G., DeNoyelles F. Jr., 1991. Ecosystem Modelling with Lisrel: A New Approach for Measuring Direct and Indirect Effects. Ecological Applications 1(4): 383–398.

Jones C.G., Lawton J.H., Shachak M., 1994. Organisms as ecosystem engineers. Oikos 69: 373–386

Jöreskog K.G. & Sörbon D., 1996. PRELIS: User’s reference guide. Scientific Software, Chicago.

Jörg E. 2003. A critique for phytosociology. J. Veg. Sci. 14: 291–296.

Kellomäki S., Hari P., 1976. Rate of photosynthesis of some forest mosses as a function of temperature and light intensity and the effect of water content of moss cushion on photosynthetic rate. Silva Fennica 10: 288–295.

Kenkel N.C., Juhàsz-Nagy P., Podani J., 1989. On sampling procedures in population and community ecology. Vegetatio, 83: 195–207.

Kimmerer R.W., Allen T.F.H., 1982. The role of disturbance in the patterns of a riparian bryophyte community. Am. Mid. Nat. 107: 370–383.

Kluge J., Kessler M., Dunn R.R., 2006. What drives elevational patterns of diversity? A test of geometric constraints, climate and species pool effects for pteridophytes on an elevational gradient in Costa Rica. Global Ecol. Biogeogr. 15: 358–371.

Laughlin D.C., Grace J.B., 2006. A multivariate model of plant species richness in forested systems: old–growth montane forests with a long history of fire. Oikos 144: 60–70

Laveder R. 2008. Sites selection within CRENODAT project. In: The spring habitat: biota and sampling methods. Cantonati M., E. Bertuzzi & D. Spitale, 2006 [eds.], Monog. Museo Trid. Sc. Nat. 4: in press.

Legendre P., Legendre L., 1998. Numerical ecology. 2nd English edn. Elsevier Science BV, Amsterdam

Levin S.A. 1976. Population dynamic models in heterogeneous environments. *Annual Review of Ecology and Systematics* 7: 287–310.

Li Y., Vitt D.H., 1994. The dynamics of moss establishment: temporal responses of nutrients gradient. Bryologist 97: 357–364.

Loehlin J.C., 1987. Latent variable models. Erlbaum, Hillsdale, N.Y.

Lomolino M.V., Weiser M.D., 2001. Towards a more general species–area relationship: diversity on all islands, great and small. Journal of Biogeography, 28: 431–445.

Lomolino M.V., 2001. Elevation gradients of species–density: historical and prospective views. Glob. Ecol. Biog. 10: 3–13.

Lopez J., Carballeira A., 1989. A comparative study of pigments contents and response to stress in five species of aquatic bryophytes. Lindbergia 15: 188–194.

Lopez J. Retuerto R. Carballeira A., 1997. D665/D665a index vs frequencies as indicators of bryophyte response to physicochemical gradients. Ecology 78: 261–271.

Lorenzen C.J., 1967. Determination of chlorophyll and pheopigments: spectrophotometric equations. Limn. Ocean. 12: 343–346.

Lortie C.J., Callaway R.M., 2006. Re–analysis of meta–analysis: support for the stress–gradient hypothesis. Journal of Ecology 94: 7–16.

MacArthur R.H., Wilson E.O., 1967. The theory of island biogeography. Princeton University Press.

Maestre F.T., Cortina J., 2004. Do positive interactions increase with abiotic stress? A test from a semi–arid steppe. Proc. R. Soc: Ser. B 271: 331–333.

Maestre F.T., Valladares F., Reynolds J.F., 2006. The stress–gradient hypothesis does not fit all relationships between plant–plant interactions and abiotic stress: further insights from arid environments. Journal of Ecology 94: 17–22.

Mälson K., Rydin H., 2007. The regeneration capabilities of bryophytes for rich fen restoration. Biological Conservation 135: 435–442.

Mancera J.E., Meche G.C., Cardona–Olarte P.P., Castañeda–Moya E., Chiasson R.L., Geddes N.A., Schile L.M., Wang H.G., Guntenspergen G.R. & Grace J.B., 2005. Fine–scale spatial variation in plant species richness and its relationship to environmental conditions in coastal marshlands. Plant Ecology 178: 39–50.

Margules C.R. & Pressey R.L., 2000. Systematic conservation planning. Nature, 405: 243–253

Marino P.C., 1991. Competition between mosses (Splachnaceae) in patchy habitats. J. Ecol. 79: 1031–1046.

Marschall M., Proctor M.C., 2004. Are bryophytes shade plants? Photosynthetic light responses and proportions of chlorophyll a, chlorophyll b and total carotenoids. Annals of Botany 94: 593–603.

Martinez–Abaigar J., Nùñez–Oliveira E., 1998. Ecophysiology of photosynthetic pigments in aquatic bryophytes. In: Bates JW, Ashton NW, Duckett JG (eds) Bryology Of the Twenty–first Century. British Bryological Society.

Martinez–Abaigar J., Nùñez–Oliveira E., Sanchez–Diaz M., 1994. Seasonal changes in photosynthetic pigment composition of aquatic bryophytes. J Bryol 18: 97–113.

McIntosh R.P., 1985. The background of ecology. Cambridge University Press, Cambridge, UK.

Menge B.A., Sutherland J.P., 1987. Community regulation: variation in disturbance, competition, and predation in relation to environmental stress and recruitment. Am. Nat. 130: 730–757.

Mensing D.M., Galatowitsch S.M., Tester J.R., 1998. Anthropogenic effects on the biodiversity of riparian wetlands of a northern temperate landscape. Journal of Environmental Management 53(4): 349–377.

Messier C., Parent S. & Bergeron Y., 1998. Effects of overstory and understory vegetation on the understory light environment in mixed boreal forests. J. Veg. Sci., 9: 511–520.

Michalet R., Gandoy C., Joud D. & Peges J.P., 2002. Plant community composition and biomass on calcareous and siliceous substrates in the Northern French Alps: comparative effects of soil chemistry and water status. Arctic, Antarctic, and Alpine Research 34: 102–113.

Mulder C.P.H., Uliassi D.D., Doak D.F., 2001. Physical stress and diversity–productivity relationships: the role of positive interactions. Proc. Nat. Acad. Sc.i USA, 98:6704–6708.

Muotka T., Virtanen R., 1995. The stream as a templet for bryophytes: species' distribution along gradients in disturbance and substratum heterogeneity. Fresh. Biol. 33: 141–160.

Nadig A., 1942. Hydrobiologische Untersuchungen in Quellen des schweizerischen Nationalparks im Engadin (unter besonderer Berücksichtigung der Insektfauna).Geographische Werkstätten H.R. Sauerländer & Co., Aarau: 432 pp.

Noakes T.D., Longton R.E., 1988. A water–content gradient for the growth of bryophytes. In: Glime JM (ed) Methods in Bryology, The Hattori Botanical Laboratory. Miyazaki, Nichinan, Japan

Oksanen J., Minchin P.R., 2002. Continuum theory revisited: what shape are species responses along ecological gradients? Ecol. Model, 157: 119–129.

Oliver M.J., Velten J., Wood A.J., 2000. Bryophytes as experimental models for the study of environmental stress tolerance: Tortula ruralis and desiccation–tolerance in mosses. Plant Ecol. 151: 73–84.

Ortler K., 1998. La vegetazione. In: Le sorgenti del Parco Adamello–Brenta. Cantonati M. (Ed) Parco Documenti 11. Parco Adamello Brenta, Strembo (TN).

Palmer M.W., 1994. Variation in species richness: towards a unification of hypotheses. Folia Geobotanica & Phytotaxonomica 29: 511–530.

Pärtel M., 2002. Local plant diversity patterns and evolutionary history at the regional scale. Ecology 83: 2361–2366.

Paton J.A., 1999. The Liverwort Flora of the British Isles. Harley Books, Martins, Colchester, Essex.

Peñuelas J., 1984. Pigments and morphological response to emersion and immersion of some aquatic and terrestrial mosses in N.E. Spain. J. Bryol. 13: 115–128.

Petersen B., Hanslin H.M., Bakken S., 2001. Testing for Positive Density–Dependent Performance in Four Bryophyte Species. Ecology 82: 70–88.

Petraglia A. & Tomaselli M., 2007. Phytosociological study of the snowbed vegetation in the Northern Appenines (Northern Italy). Phytocoenologia 37: 67–98.

Pharo E.J., Beattie A.J., Binns D., 1999. Vascular plant diversity as a surrogate for bryophyte and lichens diversity. Conserv. Biol. 13: 282–292.

Philippi G., 1975. Quellfurgesellschaften der Allgäuer Alpen. Beitr. Naturk. Forsch. Südw–Deut. 34: 259–287.

Pignatti S., 1982. Flora d’Italia. Edagricole, Bologna.

Proctor M.C.F., 2000. Mosses and alternative adaptation to life in land. New Phytologist 148: 1–6.

Proctor M.C.F, 2000. The bryophyte paradox, tolerance of desiccation, evasion of drought. Plant Ecol 151: 41–49.

Proctor M.C.F., Tuba Z., 2002. Poikilohydry and homoihydry: antithesis or spectrum of possibilities? New Phytol 156: 327–334.

Projektgruppe Aktionsprogramm Quellen, 2004. *Bayerischer Quelltypenkatalog.* Bayerisches Landesamt für Wasserwirtschaft (Hrsg.), München.

Puhe J., B. Ulrich, 1985. Chemischer Zustand von Quellen im Kaufunger Wald.. Arch. Hydrobiol. 102 (3): 331–342.

Pugesek B.H., Tomer A., Von Eye A., 2003. Structural Equation Modeling: applications in Ecological and Evolutionary Biology. Cambridge University Press, Cambridge, UK.

Rixen C., Mulder C.P.H., 2005. Improved water retention links high species richness with increased productivity in arctic tundra moss communities. Oecologia 146: 287–299.

Rydin H., 1993. Mechanisms of interactions among Sphagnum species along water–level gradients. Adv Bryol 5: 153–185.

Rydin H., 1997. Competition among bryophytes. Advances in Bryology 6: 135–168.

Rydin H., 1985. Effect of water level on desiccation of Sphagnum in relation to surrounding Sphagna. Oikos 45: 374–379.

Rykiel E.J., 1996. Testing ecological models: the meaning of validation. Ecol Mod 90: 229–244.

Sand-Jensen K., Mebus J.R., 1996. Fine-scale patterns of water velocity within macrophyte patches in streams. *Oikos* 76: 169–180.

Shipley B., 1997. Exploratory Path Analysis with Applications in Ecology and Evolution.The American Naturalist, 149: 1113–1138.

Shipley B., 2000. Cause and correlation in biology. Cambridge University Press, Cambridge, UK. Slack N.G., 1990. Bryophytes and ecological niche theory. Bot. J. Linn. Soc. 104: 187–213.

Silvola J., Aaltonen H., 1984. Water content and photosynthesis in the peat mosses Sphagnum fuscum and S. angustifolium. Ann Bot Fenn 21: 1–6.

Slack N.G., 1990. Bryophytes and ecological niche theory. Bot. J. Linn. Soc. 104: 187–213.

Slack N.G., Glime J.M., 1985. Niche relationships of mountain stream bryophytes. Bryol 88: 7–18.

Smith A.J.E., 1990. The Liverworts of Britain and Ireland. Cambridge University Press.

Sokal R.R., Rohlf F.J., 1995. Biometry: the Principles and Practice of Statistics in Biological Research. Freeman WH and Company, New York.

Sonesson M., Carlsson B., Callaghan T.V., Halling S., Olof Björn L., Bertgren M., Johanson U., 2002. Growth of two peat–forming mosses in subarctic mires: species interactions and effects of simulated climate change. Oikos 99: 151–160.

Spicer J.I., Gaston K.J., 1999. Physiological Diversity and its Ecological Implications. Blackwell Science, Oxford.

Spitale D., Petraglia A. 2008. Springs like islands: implication on density and species richness. Proceedings of the International Association of Theoretical and Applied Limnology, 30.

Spitale D., 2008. Assessing the ecomorphology of mountain springs: suggestions from a survey in the South–eastern Alps. In: The spring habitat: biota and sampling methods. Cantonati M., E. Bertuzzi & D. Spitale, 2008 [eds.], Monog. Museo Trid. Sc. Nat. 4: in press.

Steel J.B., Wilson J.B., Anderson B.J., Lodge R.H.E. & Tangney R.S., 2004. Are bryophyte communities different from higher–plant communities? Abundance relations. Oikos 104: 479–486.

Steinmann P. 1915. Praktikum der Süßwasserbiologie 1. Teil. Die Organismen des fließenden Wassers. Sammlung naturwiss. Berlin (Borntraeger). 118 Abb. Praktikum, 7: 184 pp.

Suren A.M., 1992. Meiofaunal communities associated with bryophytes and gravels in shaded and unshaded alpine streams in New Zealand. New Zealand Journal of Marine and Freshwater Research 26: 115–125.

Suren A.M., Ormerod S.J., 1998. Aquatic bryophytes in Himalayan streams: testing a distribution model in a highly heterogeneous environment. Fresh. Biol. 40: 697–716

Thienemann A., 1922. Hydrobiologische Untersuchungen an Quellen. Archive für Hydrobiologie, 14: 151–190.

Swan C.M., Palmer M.A., 2000. What drives small-scale spatial patterns in lotic meiofauna communities? Freshwater Biology 44: 109–121.

Titus J.E., Wagner D.J., 1984. Carbon balance for two Sphagnum mosses: water balance resolves a physiological paradox. Ecology 65: 1765–1774.

Tjørve E., 2003. Shapes and functions of species–area curves: review of possible models. Journal of Biogeography 30: 827–835

Travis J.M.J., Brooker R.W., Clark E.J., Dytham C., 2006. The distribution of positive and negative species interactions across environmental gradients on a dual–lattice model. Journal of Theoretical Biology 241: 896–902.

Underwood A.J., 1997. Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge University Press, Cambridge.

Valanne N., 1984. Photosynthesis and photosynthetic products in mosses. In Dyer AF, Duckett JG, eds. The experimental biology of bryophytes. London: Academic Press, 257–273.

Virtanen R., Dirnböck T., Dullinger S., Grabherr G., Pauli H., Staudinger M., Villar L., 2003. Patterns in the plant species richness of European high mountain vegetation. In: Nagy L., Grabherr G., Körner C. & Thompson D.B.A. (eds). Alpine Biodiversity in Europe, Springer, Berlin: 149–172.

Vitt D.H. and Glime J.M., 1984. Structural adaptation of aquatic Musci. Linbergia 10: 95–110.

Vitt D.H., Glime J.M., LaFarge–England K., 1986. Bryophyte vegetation and habitat gradients of montane streams in western Canada. Hikobia 9: 367–385.

Weiber E., Forbes S., Schauwecker T., Grace J.B., 2004. Multivariate control of plant species richness and community biomass in blackland prairie. Oikos 106: 151–157.

Werum M., 2001. Die Kieselalgengesellschaften in Quellen. Abhängigkeit von Geologie und anthropogener Beeinflussung in Hessen (Bundesrepublik Deutschland). Hessisches Landesamt für Umwelt und Geologie.
Westhoff V., Van der Maarel E., 1973. The Braun–Blanquet approach, p. 626–828 In: Whittaker R.H. [ed.], Classification of plant communities. Junk, the Hague.
Whittaker R.H., Niering W.A., 1965. Vegetation of the Santa Catalina Mountains, Arizona: a gradient analysis of the south slope. Ecology 46: 429–452.
Whittaker R.J. Willis K.J., Field R., 2001. Scale and species richness: towards a general, hierarchical theory of species diversity. J. Biog. 28: 453–470.
Wilmanns O., 1989. Ökologische Pflanzensoziologie. 4. Auflage. Quelle & Meyer, Heidelberg: 378 pp.
Wohlgemuth T., Gigon A., 2003. Calcicole plant diversity in Switzerland may reflect a variety of habitat templets. Folia Geobotanica 38: 443–452.
Wright J.P., Jones C.G., 2004. Predicting effects of ecosystem engineers on patch–scale species richness from primary productivity. Ecology 85(8): 2071–2081.
Zechmeister H.G, 1988. Quellmoore und Quellfl uren des Waldviertels. Thesis, Wien Universität.
Zechmeister H.G., 1995. Growth rates of five pleurocarpous moss species under various climate condition. Journal of Bryology 18: 455–468.
Zechmeister H.G., Mucina L., 1994. Vegetation of European springs: High–rank syntaxa of the Montio–Cardaminetea. J. Veg. Sci. 5: 385–402.
Zobel M., 1997. The relative role of species pools in determining plant species richness: an alternative explanation of species coexistence? TREE 12: 266–269.
Zotz G., Schweikert A., Jetz W., Westerman H., 2000. Water relations and carbon gain are closely related to cushion size in the moss Grimmia pulvinata. New Phytol 148: 59–67.

bavarian spring form, download from http://www.alpenquellen.com/quellkartierung.htm #download

Appendix

Museo Tridentino di Scienze Naturali
CRENODAT Project

FIELD PROTOCOL TO SURVEY SPRING

Spring Name	Code					Date/hour

Municipality:

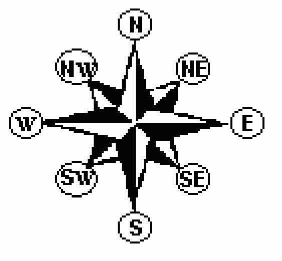
Essential information to reach the spring and useful means of transport (and time spent)

Coordinates		Tips of survey
North	East	☐ GPS ☐ Other…
Altitude m. a.s.l.		

SECTION A: slope

Slope	0-2°	2-15°	15-25°	25-35°	35-45°	60-90°
at left						
at right						
above						
outlet						

aspect



SECTION B: shape, size, area

Shape			Size
☐ single ☐ system (similar springs) ☐ complex (different springs)	☐ straight	☐ expanded	Wide m
	☐ wavy	☐ branched	
	☐ with bend	☐ twisted	Lenght m
	☐ forked		

Area m^2	☐ < 5	☐ 5 – 50	☐ 50 – 100	☐ 100 – 500	☐ > 500

SECTION C: substrate and flow

Main lithology	☐ limestone	☐ siliceous (..)

Inorganic* (%)				Organic (%)			
	Clay < 0.63 mm		Stone > 240 – 960 mm		bryophytes cushion		Branches
	Sand > 0.63 – 2.0 mm		Rocks		bryoph. submerged		Roots
	Gravel > 2 – 63 mm		Tufa		Algae		Other
	Pebble > 63 – 240 mm		Other		Leaves		
*Assessment: ☐ by sight ☐ with cylinder							

Flow type

☐ hygropetric	%	☐ laminar	%	☐ dripping	%	☐ standing	%
☐ lively	%	☐ waterfall	%	☐ turbulent	%		%

Flow condition

☐ permanent	☐ periodic	☐ episodic
☐ statement of others	☐ verified	☐ suspect
☐ continuous flow	☐ disappear after m......	

Flow velocity

☐	Apparently still water. Pool spring.
☐	Rheocrene with small discharge (≤ 0.1 Ls^{-1}). Max velocity< 30 cm s^{-1}.
☐	Rheocrene with few L s^{-1} which emerge on little slope. Rheocrene with discharge < 1L if it emerge on steep slope. Velocity < 50 cm s^{-1} except on vertical jump.
☐	Small stream and medium-high rheocrene. Rheocrene with little L discharge if it emerges on steep slope. Max velocity 50 cm s^{-1} ÷ 100 cm s^{-1}.
☐	Stream with high discharge. High spring that emerge on waterfall. Max velocity ± 100 cm s^{-1} or >

SEZIONE D: illumination

☐	Exposed spring. Possible high grass but exposure towards the S, SW or W.
☐	High grass and exposure towards the NW, N, NE o E. Covering to 25 %.
☐	Covering to about 50 % because of shrubs, plants or rocks.
☐	Shaded. Underwood. Covering to 75 % but exposure towards the SE, S, SW or W.
☐	Very shaded. Underwood. Covering to > 75 % but exposure towards the NW, N, NE or E.
☐ constant all the year	☐ higher in winter

SECTION E: buffer zone and disturbances

Disturbances	Distance m	Comments
☐ water abstraction		
☐ footpath		
☐ trampling by cattle		
☐ spring-bed alteration		
☐ building		
☐ road		
☐ farm		
☐ nothing		

a) < 1 m	b) > 1-2 m	c) > 2-5 m	d) > 5-10 m	e) > 10 m

SKETCH OF THE STUDY AREA	LEGEND	
N	P	plants
	C	shrubs
	░	grass
	o	bryophytes

SECTION F: FIELD DATA

Weather

Covering %		Precipitation	Previous days	Air temperature(°C) Atmospheric pressure (mmHg)
☐ 0-25 ☐ 50-75	☐ 25-50 ☐ 75-100	☐ Yes ☐ No		

Multiprobe:

Water temp	dissolved O_2(mg L^{-1})	O_2 saturation %	Cond. (μS cm^{-1})	pH	Redox

Discharge L s^{-1}:	☐ flow meter	☐ graduated pail	☐ other
Running	☐ low water	☐ medium	☐ flood

Transect for measuring discharge with the flow-meter

DISTANCE(cm)	0	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190
DEPTH (cm)																				
velocity (m s^{-1})																				

Flow velocity

point n°	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
DEPTH. (cm)																				
velocity (m s^{-1})																				

SECTION G: SYNTHESIS OF SPRING TYPE

☐ Type 1) Rocky spring	
☐ Type 2) Stony spring	
☐ Type 3) Pebbly spring	☐ a) Rich vegetation
	☐ b) Poor vegetation
☐ Type 4) Gravely spring	☐ a) Rich in vegetation
	☐ b) Poor vegetation
☐ Type 5) Clay-sandy spring	☐ a) Laminar outflow
	☐ b) Linear outflow