MONOGRAFIE DEL MUSEO TRIDENTINO DI SCIENZE NATURALI - 4

The spring habitat: biota and sampling methods

edited by
Marco Cantonati, Ermanno Bertuzzi and Daniel Spitale

MUSEO TRIDENTINO DI SCIENZE NATURALI
TRENTO - 2007

Direttore Responsabile: Michele Lanzinger
Redazione: Valeria Lencioni
Assistente di Redazione: Maddalena Nesler

In copertina: *Flowing spring in Brenta Valley* (*Adamello-Brenta Natural Park, Italian Alps*) (photo by arch. MTSN/E. Bertuzzi)

www.mtsn.tn.it
ISSN: 1970-2094
ISBN: 978-88-531-0008-5

Dedicated to Gino Tomasi,
Emeritus Director of the Museo Tridentino di Scienze Naturali,
who pioneered crenobiological research in Trentino and Italy,
on the occasion of his 80th birthday

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FOREWORD OF THE MINISTER FOR PLANNING, RESEARCH, AND INNOVATION OF THE AUTONOMOUS PROVINCE OF TRENTO

The main threat to springs, as natural environments, is their exploitation to obtain potable water or for other human uses. This anthropogenic pressure on springs is likely to increase in the near future due to the decrease in precipitation caused by climate change, which is predicted for several regions including the south-eastern Alps. In spite of this situation, spring habitats (in contrast to larger watercourses and lakes) are still poorly or not at all considered by European Directives, and national and local environmental legislation. The CRENODAT Project, funded by the Autonomous Province of Trento, has among its main objectives to explore and thoroughly document the biological relevance of these environments, and to produce the fundamental knowledge needed to develop evaluation tools and systems useful to minimise damages (e.g. decrease in ecological value of springs), related to the unavoidable increasing necessity of using springs as a drinking water resource. The Museo Tridentino di Scienze Naturali, soon to become MUSE (Trentino Science and Nature Museum), is a component of the articulated and innovative network of the Trentino research system. With reference to the research on the ecology of inland waters the Museum, given the peculiar traits that characterise its research, can provide refined taxonomic and collection-based research for accurate descriptions of new taxa (species and genera), that have been found in the Trentino springs in the framework of CRENODAT. Moreover, the Museum can make available the collections to document the quality of the aquifers of the Trento Province with items that can be examined after decades (sometimes even centuries). A process that was undertaken for lakes and for rivers many years ago, must be initiated also for springs to reach the goals of having these environments evaluated and protected by legislation, regulating their confinement and exploitation, and, when possible, favouring more sustainable ways for their use. In this context, the present volume discusses methodologies to study springs, which can be seen as an important and necessary premise. It also represents work that can be further used (e.g. by means of a more close interaction with the Public Administration) and which adds value to the Project, in the sense of the recently proposed Project-evaluation system VALGO.

Trento, December 2007

Gianluca Salvatori

FOREWORD OF THE VICE-PRESIDENT AND MINISTER FOR CULTURAL AFFAIRS OF THE AUTONOMOUS PROVINCE OF TRENTO

Springs have been exploited by man for drinking water since ancient times, but they are still not fully appreciated as special and relevant natural habitats. The CRENODAT Project, funded by the Autonomous Province of Trento, has among its main objectives to document the biological relevance of these ecosystems and to initiate different types of actions to spread knowledge and awareness on the importance of springs as natural habitats. The Museo Tridentino di Scienze Naturali, soon to become MUSE ("Trentino Science and Nature Museum"), is primarily an Institution involved in cultural mediation. What is maybe less obvious and less well known is that it is also a relevant research Institution within the articulated and innovative network of the Trentino research community. One important element characterising the research of this Museum is that its Projects are accompanied, since their formation, by efforts aimed at spreading knowledge, so as to bring environmental and scientific problems to the attention of society and to foster discussion. This is true also for the CRENODAT Project, which was publicised to the public, e.g. as follows: contribution to an international website (sorgentidellealpi.com and alpenquellen.com), publication of a popular book (text and images) inviting people to visit the most beautiful and impressive springs of Trentino, workshops including lectures for the general public, and a description of spring ecosystems in the Italian Edition of an important Ecology University textbook edited by the Co-ordinator of the Project. There is still a cultural gap that hinders full appreciation of these special environments by the general public, decision makers, and even some specialists. It is however important and urgent that spring habitats come to the attention of the general public to foster their inclusion in European Directives and in national and local legislation. These actions would help to ensure the conservation of the most valuable spring environments. This volume discusses methodologies to study spring habitats, which should be regarded as significant and relevant.

Trento, December 2007

Margherita Cogo

FOREWORD OF THE PRESIDENT OF THE MUSEO TRIDENTINO DI SCIENZE NATURALI OF TRENTO

The management of aquatic ecosystems is crucial in determining the type and the rhythm of the development of society; the awareness of this problem is recent, but widespread.

Historically the approach to the theme, "water", focussed on the management of quantities, which was considered as a subdivision of the water resource among multiple competing uses. It is only in relatively recent times that the politics of water resources has also considered the conservation of quality and the relationship between quantity and quality. A consequence of this is that a modern and comprehensive management of water resources must begin with the awareness about the intimate nature of flux of the water resources, which has, as a consequence, that their economic value depends upon several variables: quantity, quality, time and space.

A typical goal or decision of the allocation of water resources consists of delivering a certain amount of water, in a certain point in space, in a determinate moment and with a certain quality; this has unavoidable effects on uses that are successive in time and space with respect to that decision and it inevitably implies the sacrifice of incompatible targets.

Thus, an efficient management system can be defined as a consistent group of institutions and operative criteria, which allows one to take rational target decisions, that is, to know the real implications of the different alternatives and to estimate their value in relation to the goals. Therefore, it is necessary to choose those targets, which have a benefit that is superior to the opportunity cost, including also the zero option.

A series of economic, social and cultural motivations are highlighting, frequently in a dramatic way, the question of the conservation of the quality of water resources, especially with reference to the protection from pollution, in order to achieve the social goal of a better environment, a higher quality of lifestyle and finding an immediate fulfilment in the search for the solutions. The latter tends to warrant the use of water resources also for "superior" activities such as recreational uses and eventually for environmental conservation.

On the other hand, the reduction of water resources from the quantitative point of view has sharpened the problem of quality preservation, since the possibilities of using uncontaminated water are disappearing due to new sources of industrial activity. In this respect, it should be remembered that, frequently, the quantitative shortage is in reality the scarce availability of water resources with specific quality characteristics, and to preserve water quality, the until now widely adopted principle of "who pollutes, pays", is likely to be more ineffective.

The global situation is compromised to an extent, because pollution affects the entire water cycle, from glaciers to oceans. The sole principle to apply is not to pollute and to conserve, protect and investigate at least the most precious and fragile water resources, e.g. the high mountain springs, which are the real biological laboratories of biodiversity. Thus, the ultimate mission is to instil scientists to deliver to political decision makers the tools for a more effective and protective water governance, which would be carried out in parallel with the politics of landscape planning and development. This approach would foster a positive outcome, with the widest possible temporal and spatial horizons, since it is now evident that the problems of the distribution of water among the different and concurrent users and the conservation of determinate quality levels are of such extent to influence directly both the basic decision making about economic development and land use.

Giuliano Castelli

FOREWORD OF THE EMERITUS DIRECTOR OF THE MUSEO TRIDENTINO DI SCIENZE NATURALI OF TRENTO

The varied categories of aquatic habitats of the Alps-glaciers, running waters, lakes, wetlands and springs- are subjected to rather different lithological, geomorphological and climatic situations, which creates distinct problems and methodologies for the investigation of their physiography and biology. Beyond this, comparing their characteristics, it must be recognised that some spring types (e.g. very small springs on carbonate substrate) undergo changes in time. Indeed, they are not only subject to a natural evolution, correlated to the surface and deep dynamics of rock masses, but they are also affected by human impacts, which, beyond the normal use of water, cause other alterations, often unperceived and unforeseeable. It is evident that any consideration on these changing habitats must be based on rigorous and long-term research, both on their previous physical aspect and biological characteristics and on their present day physiology.

The Museo Tridentino di Scienze Naturali of Trento can boast about its rather long tradition in the study of spring habitats, which initially gave rise to explorative and specialised investigations and, in more recent times, fostered a spring inventory of the Autonomous Province of Trento, which started and was carried out by the Museum under the coordination of Enzo Vuillermin in the years between 1967 and 1974. Successively this branch of hydrobiology has been revitalised in the Museum with different purposes by Gino Tomasi, Alvise Vittori, and, at present, with renewed energy, by Marco Cantonati, Keeper of the Limnology and Phycology Section of the Museum, and his co-workers.

It is essential to remember that any advancement of knowledge, especially on spring habitats, is of fundamental importance for a correct interpretation of its biology, and for possible interventions, in the face of the actual worrying predictions on climate changes.

The present work, enriched by the adoption and testing of renewed modern research methodologies, represents a strong reigniting of the past activity, by combining the needs of habitat protection and of the gaining of scientific knowledge with the need of humans for high quality water resources.

Gino Tomasi

HOW TO INVESTIGATE THE ECOLOGY OF SPRING HABITATS ON THE BASIS OF EXPERIENCES GAINED FROM A MULTIDISCIPLINARY PROJECT (CRENODAT)

Daniel Spitale*, Ermanno Bertuzzi & Marco Cantonati

Limnology and Phycology Section, Museo Tridentino di Scienze Naturali, Via Calepina 14, 38100 Trento, Italy
**Corresponding author e-mail: spitale@mtsn.tn.it*

Preface

Springs represent, together with streams and rivers, one of the main running water types. As pointed out in the "sandglass" model by Cantonati & Ortler (1998), the spring can be considered the lowest point of a subterranean hydrographic system and it is, at the same time, the origin of a surface hydrographic system. From an ecological point of view the most correct approach is to recognise this dual nature defining the spring as the ecotone linking surface with underground waters. The peculiar environmental stability, due to several environmental variables (such as temperature and flow discharge), makes them a special habitat populated by several taxa relevant from a biogeographic point of view. Springs are characterised by a mosaic structure of numerous microhabitats (Illies & Botosaneanu 1963) and such richness might explain the elevated number of taxa which coexist in a few square metres (Weigand 1998). This feature alone could be sufficient in order to consider springs as a special habitat worthy of being protected. Because of the small dimensions and of the importance of the fringing semi-aquatic habitats, springs are extremely sensitive to disturbance factors, such as trampling damage by cattle, coverage by sediment, water abstractions, stripping off of the surrounding vegetation or nutrient inputs (Weigand 1998). For a survey of the spring biota as well as for a review of the actual state of knowledge see Cantonati *et al.* (2006). Although several studies on the classification of running water already exist, projects fully devoted to springs are still rare. Most knowledge derives from isolated studies comprising only few aspects

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Spitale D., Bertuzzi E. & Cantonati M., 2007 - How to investigate the ecology of spring habitats on the basis of experiences gained from a multidisciplinary project (CRENODAT). In: Cantonati M., Bertuzzi E. & Spitale D., *The spring habitat: biota and sampling methods.* Museo Tridentino di Scienze Naturali, Trento: 19-30 (Monografie del Museo Tridentino di Scienze Naturali, 4).

of spring ecology or biology, while an overall characterisation both on a regional scale and aiming at including all the biota is still lacking.

The CRENODAT Project [Biodiversity assessment and integrity evaluation of springs of Trentino (Italian Alps) and long-term ecological research]

In July 2004 the Autonomous Province of Trento (northern Italy) funded the CRENODAT Project, which involved the cooperation of several local, national and international Institutions and experts (Fig. 1).

The Autonomous Province of Trento, recognising the importance of springs, began to persue inventory projects since the 1970s. In 1993

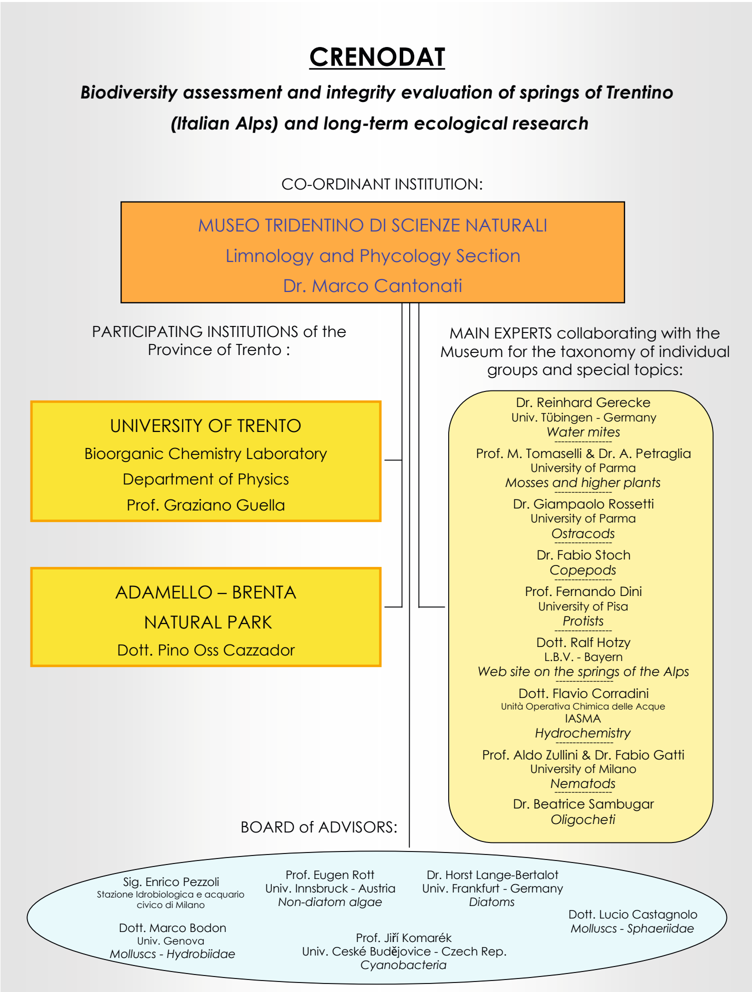

Fig. 1 - Organization and structure of the CRENODAT Project.

the springs' data set has been remarkably improved by the Geological Service; this data set was used for a first selection of 600 springs meeting the criteria for being considered within the CRENODAT Project. The most important ones were to include: (i) mainly permanent springs, (ii) habitats not affected by heavy direct impacts, (iii) all lithologies of the study area (ranging form limestones and dolomites to schists and gneisses and to different types of granites), (iv) all altitude intervals (from less than 100 to more than 2500 m a.s.l.), (v) all districts of the Province with special attention to Biotopes and Natural Parks and to territories planned to be included in protected areas.

CRENODAT aims at contributing to a thorough understanding of the structure of the biota, by obtaining a complete set of hydrochemical data covering the whole territory of the Province of Trento, by starting the investigation of the bioorganic metabolism of organisms fairly common and/or abundant in springs, by using springs for ecophysiological investigations and as ideal sites for long-term research, by selecting indicators of integrity/naturalness (community structure and typical taxa) and of environmental quality (organisms and bioorganic compounds) in relation to specific problems/parameters, and by promoting habitat-protection by means of pilot popularisation initiatives. The Project, coordinated by the Limnology and Phycology Section of the Museo Tridentino di Scienze Naturali of Trento, is structured in the following six main tasks.

I. Geomorphological and hydrochemical characterisation and biodiversity assessment

This is a comprehensive study on a representative sample of the springs of Trentino in near-nature conditions. Morphology, hydrogeology, chemical characteristics and the biota (algae, with special attention to diatoms, cyanobacteria, protozoa, water mites, microcrustaceans, crenobiontic and crenophilous snails, and other zoobenthos groups, mosses and higher plants) were investigated for about one hundred springs representative of all main lithological substrates and in areas which are relevant for nature conservation. The aim was to produce a real picture of biodiversity and its distribution in these habitats by the analysis of several groups of organisms (as well as chemical-physical variables).

II. Springs with special physical and chemical characteristics

The second task of the Project aimed at focussing on a selection of springs with special physical and chemical characteristics, such as thermal springs and/or sources with specific mineralization characteristics. Some sulphur (sulphide and sulphate) and iron springs were included as well to further investigate potential detoxification capacities of the biofilms which thrive in these environments. Unfortunately unexploited and unaffected (i.e. near-nature) springs of this type are extremely rare in Trentino.

III. Springs as "natural laboratories" (ecophysiology and bioorganic metabolism)

Since many springs are characterised by small physical and chemical fluctuations through the years, characteristic organisms can colonise these habitats and sometimes develop abundantly. The thermal constancy of these habitats should allow one to point out more easily the influence of other environmental factors. Ecophysiological characteristics and the bioorganic metabolites of spring organisms (e.g. the diatom *Diatoma mesodon* (Ehrenberg) Kützing and the chrysophyte *Hydrurus foetidus* Vill. (Trev.)) were studied. These investigations should have the goal to contribute to the identification of the factors regulating the vegetative cycle of algae very common in mountain running waters (*Hydrurus foetidus*) and to lead to the identification of secondary metabolites of particular interest for chemotaxonomical or ecological (study of the role of the compound in nature) reasons. Another object of this task was the search for bioorganic and organismal indicators of hydrological stability/instability, reflecting also the intensity of the impact of climatic changes on the territory.

IV. Long-term research, global change and seasonal features

Long-term ecological research, started at the beginning of the 1990s in six mountain springs located in the Adamello-Brenta Natural Park, was further developed and improved, and changes in physical, chemical and biological characteristics were used as environmental change indicators (climate change, increasing of N deposition, acid/base and trophic status variations, increasing of UV radiation). This part included the assessment of the seasonal fluctuations of the physical, chemical and biological characteristics, for a more effective separation of the long-term changes from the seasonal fluctuations. Every possible effort will be done to link this initiative in the Southern Alps to similar research, which is being carried out North of the Alps, in the Berchtesgaden National Park (Bavaria; Gerecke & Franz 2006). First attempts were conducted to apply paleolimnological techniques to sediment cores taken in limnocrene springs with sufficient sedimentation rates and undisturbed sediment accumulation zones for reconstructing recent environmental changes (last 200 years). Explorative analyses (mineralogy, isotopes etc.) on different kind of carbonate concretions, present in several spring types, were performed with the aim to explore their potentialities as environmental archives.

V. Development of implemented procedures and criteria for spring integrity/naturalness evaluation

The large amount of data collected are being reconsidered in order to develop implemented procedures and criteria for the evaluation of springs' integrity/naturalness. One goal will be the adaptation to springs of the Alps of an index developed in Germany (Fischer 1996) and based on the strength of the link of the zoobenthos taxa with the spring environment (in alternative a new index could be developed). Diatom-based indices for the assessment of trophic (e.g. Rott *et al.* 1999) and acid/base status (Coring 1993) evaluation of running waters are being applied to the springs studied and, if necessary, they will be adapted to these special environments.

VI. Educational activities to foster conservation

Acquired knowledge will be popularised thanks to the planning and carrying out of several initiatives (Internet site, popular book, nature discovery path), being aware that these environments will be preserved only if their relevance will be understood by a wider audience. The Internet site on the springs of the Alps (available in several languages, among which are German, Italian and English - www.alpenquellen.com) is a strategic tool for this part of the Project. The data produced by the Project will be published in a special volume of an international scientific journal.

A methodological framework for spring investigation

The present book is a collection of papers in which the Authors present an overview of the sampling methods and the organisms most frequently inhabiting springs. The first CRENODAT workshop (March 2005) was specifically organised to promote discussion on the sampling methods. Because of the high number of groups of organisms considered by the project, common sampling methods had to be resolved; for instance, several zoobenthos groups can be sampled with the same procedure. Useful considerations emerged during that meeting formed the core for the present volume. Each paper was reviewed by a specialist (Tab. 1). Several of these contributions contain original sampling protocols or originally modified procedures that were used for the Project, whereas others even offer an identification key of the most common spring organisms. After two works discussing the assessment of spring ecomorphology and the measurement of chemical and physical parameters respectively, the first group of papers deals with autotrophic organisms: benthic algae and cyanobacteria, lichens, bryophytes and vascular plants.

Detailed description of the springs' **morphology** is important, especially if the aim of sampling is to compare the springs. Therefore a standardised protocol is necessary. Several of the main ecological factors influencing distribution, abundance and structure of the crenocoenosis are presented in this contribution. 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 the Project required the sampling of more than one-hundred springs, a standardised field protocol was developed, also on the basis of existing spring-assessment forms. The main aim of this paper is to offer the basic elements for elaboration of a standardised sampling procedure for a quali-quantitative description of the ecomorphological characteristics of mountain springs. After examining morphological factors relevant for ecological research and nature conservation, a field protocol to assess spring morphology is proposed.

The most common **chemical and physical factors** and parameters which can be useful in understanding the ecology of springs are

Tab. 1 - List of the Reviewers of the volume The spring habitat: biota and sampling methods *(Monografie del Museo Tridentino di Scienze Naturali, 4).*

	Referee	Institution - address
1	Tom Bongers	Wageningen University. Netherlands
2	Saverio Brogna	School of Biosciences, University of Birmingham. UK
3	Lucio D'Alberto	Agenzia Regionale Protezione Ambientale del Veneto. Italy
4	Elžbieta Dumnicka	Institute of Nature Conservation. Polish Academy of Sciences. Poland
5	Sergei Fokin	Università degli Studi di Pisa. Italy
6	Jacopo Gabrieli	Agenzia Regionale Protezione Ambientale del Veneto - Belluno. Italy
7	Diana Galassi	Dipartimento di Scienze Ambientali, Università degli Studi dell'Aquila. Italy
8	Renato Gerdol	Dipartimento delle Risorse Naturali e Culturali, Università di Ferrara. Italy
9	Reinhard Gerecke	Biesingerstrasse 11, Tübingen. Germany
10	Folco Giusti	Facoltà di Scienze Matematiche Fisiche e Naturali, Università degli Studi di Siena. Italy
11	Heike Howein	Sieglitzhofer Strasse 13, Erlangen. Germany
12	Ingrid Jüttner	National Museum & Galleries, Cardiff. Wales
13	Peter Martin	Christian-Albrechts-Universität zu Kiel, Zoologisches Institut. Germany
14	Claude Meisch	National Natural History Museum of Luxembourg. Luxembourg
15	Pier Luigi Nimis	Dipartimento di Biologia, Università degli Studi di Trieste. Italy
16	Claus Orendt	Hydrobiologie WaterBioAssessment, Leipzig. Germany
17	Roland Psenner	Institute of Zoology and Limnology, Universität Innsbruck. Austria
18	Sebastiano Salvidio	Facoltà di Scienze Matematiche Fisiche e Naturali, Università degli Studi di Genova. Italy

discussed in the following paper which is a synthetic and critical overview of the existing methodologies. The paper also presents a case study that relates to springs (16) and streams (5) of the Dolomiti Bellunesi National Park.

The **benthic algae** paper offers an overview of the most common methods currently used for the assessment of benthic algae (including oxygenic cyanobacteria) in shallow running waters, which are potentially suitable also for springs. It reviews the most common taxa and assemblages of algae which have been found until now in the springs of the Alps and provides information and references on their autecology. Furthermore, an identification key of the most common macroalgae of springs is presented, together with a selected iconography of macroscopic and microscopic aspects of the main species.

Lichens colonise almost every terrestrial ecosystem and, within the Italian Alps, as many as 1800 taxa, are known and only 43 of these colonise freshwater habitats. The main ecological factors influencing aquatic lichens distribution are moisture (frequency and duration of submersion), light, substrate stability and silting intensity. Lichen diversity can be used for bioindication purposes, since several taxa are very sensitive to environmental conditions. Because of the difficulty of identification in the field, it is hard to perform quantitative sampling. A list of the Alpine freshwater species known so far is presented, as well a rich bibliography.

Within spring habitats, **bryophytes** can be a dominant plant group, particularly in the montane, subalpine and alpine belts and within the underwater communities. Consequently, the study of spring communities must consider bryophyte diversity. Collecting bryophyte samples does not present particular difficulties and few precautions are sufficient to ensure correct sampling surveys. As regards the syntaxonomy of spring vegetation, this paper considers only the syntaxa dominated by bryophytes; a complete survey of this topic was in fact carried out in the vascular plants paper.

Spring vegetation is reviewed in the **vascular plants** paper. The methodologies adopted for studying both vascular flora and vegetation of springs are presented. Particular attention is devoted to describing the phytosociological method used for sampling and classifying vegetation. The most typical vascular species occurring in the spring habitat are listed and analysed in their distribution and basic ecological requirements. Finally, a survey of current syntaxonomic arrangement of the spring vegetation from the Alps is provided with details on floristic composition and synecology of five alliances.

Several papers describe zoobenthos groups, including protozoa, nematodes, molluscs, oligochaeta, water mites, copepods, ostracods, chironomids. Several of those groups can be effectively sampled in springs with the same method. The individual contributions on these groups provide further details, peculiarities and information on the most widespread species inhabiting springs.

The study of **protozoa** communities in aquifers is at an early stage; spring protozoa do not seem to have received much attention so far, with exception of thermal and mineral springs. In pristine aquifers protozoa have usually low abundance; small flagellates are by far the dominant group, although amoebae and, occasionally, ciliates may also be present in much lower numbers. Cantonati *et al.* (2006) noted that protozoa, especially ciliates, were studied to some extent in groundwater and in mosses, one of the most important substrates in springs. Ciliated protozoa are fragile and relatively small, making identification and counting very difficult. Identification of ciliates is difficult due to high species diversity in aquatic ecoystems, variability of cell sizes, damage caused by fixatives to cells and cultivation problems.

The described species may show morphological variation as well. Therefore, taxonomic errors may occur in many lists of ciliate fauna.

Nematodes (roundworms) are one of the most widespread animal group in the world, since they colonise every available habitat. Nematodes show morphological and physiological adaptation to the interstitial environment. Therefore, in the spring habitats nematodes can be found within the sediments, from where they must be separated. In some cases nematodes live on epiphytic algae covering stones on the bottom, or in the submerged mosses. Moreover, the paper deals with the most widespread extraction method and some details on the slides' preparation of nematodes.

Molluscs colonise a wide variety of substrates, from submerged stones and pebbles, to interstitial spaces in gravel and coarse sand, sandy sediment, muddy sediment, leaves and other plant debris, aquatic plants, submerged or moist moss, stocks and roots of land plants, moist rocks and the hygropetric zone. Most common taxa are presented as well as the referenced literature. An exhaustive review of the sampling method is introduced.

Oligochaetes live in soil and sediments of freshwater and sea water. Some of them swim freely in the water with serpentine-like body movements. The "limicolous" or "microdrile" oligochaetes, traditionally set apart from the "terricolous" or "megadrile", are small as opposed to the latter. Their small size, their poor looks and the lack of easily perceivable morphological peculiarities, led to a lack of attention for this group in hydrobiology. Actually, they deserve such attention because of their ecological and biogeographical interest, and their ability to colonise all aquatic environments, from subterranean to superficial ones, as well as to artificial ones such as water treatment facilities and aqueducts. Moreover, taxonomic difficulties, due mostly to laborious and complex (colouration, dissection, mounting) preparation techniques, have often discouraged their study, especially in routine ecological research.

Many studies have produced evidence that **water mites** play an outstanding role within invertebrate communities of spring habitats, not only in terms of species diversity and the evolution of particular habitat preferences, but often also with regard to their high population densities and particular significance within the food web. Water mites are still little documented in springs of several areas. This could be due to unfavourable life conditions - in fact, most Hydrachnidia are very vulnerable from modifications of substrata, water quality or discharge. However, in undisturbed natural spring habitats with year-round stable flow they are nearly obligatorily present, in general with a high diversity. Therefore, if they do not appear in the faunistic documentation of such springs, this is principally due to an inadequate sampling methodology. The paper reports on the status of knowledge of this group, together with many suggestions on how to perform an accurate sampling and a simple key to the major groups typical of spring habitats.

In the Alpine springs, microcrustaceans are represented by numerous species of copepods and ostracods, which depend both on their habitat preferences and a spectrum of different adaptations. In a previous study in the Prealpi Giulie Natural Park, **copepods** played an important role in the taxa richness (among zoobenthos): copepods represented about the 15% of diversity. The order Harpacticoida is the most abundant in the spring habitat, also in part because of the occurrence of stygobiontic species dominating in karst environments. Generally, springs where copepods diversity is high, present a small discharge and high values of moss cover. Samples should be taken in each microhabitat, as moss carpets and the interstitial habitat; in the sorting phase, special attention should be paid, because of the small size of copepods.

Ostracods are commonly found in most inland waters, where they are abundant in the benthic and periphytic communities, but also occur in marine environments, interstitial and groundwater habitats, and even in semi-terrestrial environments. In spite of their widespread occurrence, recent non-marine ostracods have so far received relatively little attention by scientists, especially because of problems related to their taxonomic classification. Also the autecology of ostracods is still largely unknown and often based on speculative assumptions, although these organisms are of particular interest as potential environmental indicators in freshwater ecosystems. The occurrence of non-marine ostracods in a wide variety of aquatic, semi-terrestrial, and interstitial habitats makes it difficult to establish standardised sampling protocols. The collection methods are similar to those commonly used in studies of freshwater benthos, but some advices may be useful to optimise the sorting phase.

As in almost all other habitats, aquatic insects are by far the most diverse animal group in springs with respect to both species richness and morphological and physiological adaptations. Without exception, the life cycles of all insect species found in springs includes at least one stage adapted to terrestrial life. The life cycles of many insects pose a methodological problem for the study of their diversity, since many species can be identified to species level only as adults. In some cases, when species can be identified at the last larval stage, these stages are often found only during a short time period of the year. This is especially true for the extremely numerous dipterans, but in some cases also for the stoneflies and some groups of caddisflies. To investigate their diversity it is necessary to adopt time-consuming methodologies. Established methods include the use of emergence or light traps or the breeding of larvae or mature pupae in the laboratory.

The aquatic insect family richest in species in springs are the **Chironomidae**, dominant even in terms of number of individuals in the zoobenthic community. Midges are also the most important group of hosts of the parasitic larvae of many crenobiontic water mite species. Considering the importance of this family on one side, but the large amount of time necessary to identify larvae to species on the other, several authors proposed a sampling method based on emergence traps, which are positioned only during periods when emergence is at a maximum.

The following contribution provides a synthetic overview of the most common **zoobenthos sampling methods** suitable for springs. Well established stream macroinvertebrate sampling methods must be modified to overcome problems generated by typical features of the spring habitat. Other characteristics of springs such as the reduced seasonal fluctuations of several environmental factors, may be an advantage.

Among vertebrates, **amphibians and reptiles** are generally relatively easy to sample, especially if compared with other terrestrial species, e.g. mammals and birds. To produce accurate inventories of the amphibian and reptile fauna in spring habitats it is usually necessary to make use of a variety of survey methods, which will sample all possible niches. In order to compile a list of species occurring in Alpine springs, the suitable methods could be acoustic sampling, opportunistic capture and pit fall trapping; whereas for collection of quantitative and qualitative data on amphibian richness one can use Visual Encounter Surveys (VES).

Since the Project required a large number of springs to be sampled, a **selection** among available **sites** was advisable. Information about the springs of Trentino are collected in the "Spring inventory" database, developed by the Geological Survey of Autonomous Province of Trento. To deal with the problem of the site selection, many criteria have been used: perennial flow, naturalness, different morphological and flowing typologies. One of the main goals was to diversify locations according to the distribution on Trentino's territory, that is composed by a wide lithological variety. Because aquifer geology affects chemical composition of spring waters, ionic composition of groundwater is influenced by the interaction with the aquifer matrix: higher concentrations of the main ions (Ca^{2+}, Mg^{2+}, HCO_3^-) are measured in groundwaters leaking calcareous and dolomitic rocks. Instead, groundwater flowing through magmatic or metamorphic rocks shows a lower content of ions, due to the weak solubility of these lithologies.

To recognise the effects of environmental change in springs, investigations carried out over a long time-span are necessary. Long-term monitoring programmes may highlight ongoing changes, but usually it is very difficult, if not impossible, to have a record starting from a "natural" and pristine situation. Remote high mountain springs are likely to be the best candidates for **paleolimnological studies**, though diffuse airborne pollution and sheep- and cattle-breeding may well have induced some changes in the last century or so. The ideal situation would be to have past "pictures" of present day springs, intending for *past* a time with no detectable human influence.

The paper on the **secondary metabolites of spring organisms**, that is compounds which are characteristic of a limited range of species, offers an overview on the chemotaxonomically or ecologically important compounds. To the best of our knowledge, untill now the secondary metabolism of spring organisms is an unexplored field. Such an outcome might derive from the scarce attention of natural products by chemists toward this habitat or to the absence in springs of freshwater organisms previously studied elsewhere. Target compounds that are being examined are mainly photosynthetic pigments such as scytonemin (photoprotective pigment in cyanobacteria), mycosporine-like amino acids (MAAs)(UV-absorbing compounds) and lipids.

Information about springs in the Bavarian biotope mapping is fragmentary and can only be found for spring-moors which cover a greater area. Even in the field of water management one can hardly find any experiences dealing with springs as habitats. The LBV (Bavarian Association for the Protection of Birds) aims at evaluating as many natural areas as possible in relation to funding opportunities available in the involved regions. Springs from Bavaria were, at first, classified following the "classical" Steimann-Thienemann morphological types; then a selection of ecologically meaningful variables was chosen. Different forms (available also on the Internet) allow the classification of springs, from basic notions to detailed ones. Thus a series of activities were planned and carried out in order to **protect spring habitats** and restore damaged springs.

In the Adamello-Brenta Natural Park a **detailed survey** on how many springs there could be in two selected areas of special interest of the Park was carried out. This study produced unexpected results: 142 springs were recorded along an altitudinal gradient between 1600 m a.s.l. and 2600 m a.s.l. Many of them have a small discharge and almost all are rheocrenes.

Since springs are heterogeneous habitats in which many organisms co-habit in a relatively small area, many specialists must be involved in order to perform a reliable biodiversity inventory. This brief presentation of papers may provide an idea of the competencies involved in the project and the work needed to obtain reliable results. We also hope that this volume may be valuable for the planning of methodologies for future ecological investigations on springs.

Acknowledgements

The Authors are sincerely grateful to the University and Scientific Research Service of the Autonomous Province of Trento for funding the CRENODAT Project by means of its Research Projects Fund, to the Reviewers of the individual papers of this *Monography* who greatly helped in improving the quality of the volume and to Prof. Elliot Shubert (The Natural History Museum, London) for conducting the linguistic editing of the manuscripts.

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