



## Conservation and management

DIANA MARIA PAOLA GALASSI · MARCO PETITTA · FABIO STOCH

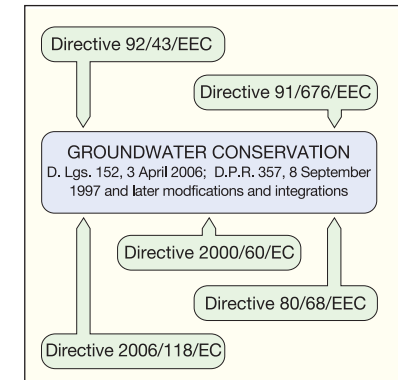
115

### ■ Current laws

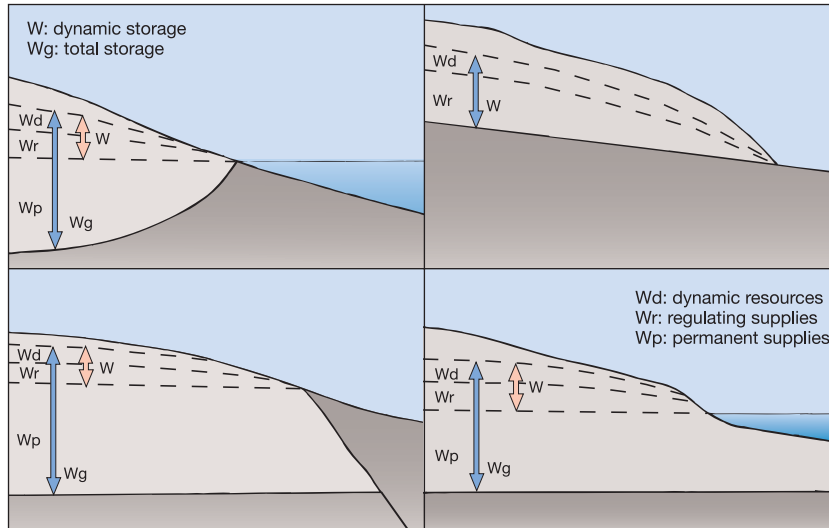
In terms of European legislation, the most important laws regarding groundwater are the Water Framework Directive (WFD) (2000/60/EC), the Groundwater Directive (2006/118/EC) and the Habitats Directive (92/43/EEC). Article 4 of the Water Framework Directive states that European Member States must protect, improve and restore underground water basins *“to reach at least good status by 2015”*. To assess the environmental

condition of such basins, Annex V of the WFD establishes that *“good groundwater status”* is that achieved by a body of groundwater when both its quantitative and chemical statuses are good. *“Quantitative status”* is an expression of the degree to which a groundwater body is affected by direct and indirect withdrawal. If this complies with Directive requirements, status is good. *“Good chemical status”* is ascribed to a groundwater body when it meets Directive requirements for the maximum levels of defined pollutants, which must not contain inputs of seawater or other types of infiltration, must not exceed the standard limit values applied by other European laws, and must not affect the ecological and chemical quality of surface water and terrestrial ecosystems associated with that body. However, only in 2006 did the WFD provide a list of pollutants for which Member States were required to establish threshold limits.

The European Community, through the WFD, acknowledges that groundwater is the most important source of freshwater and drinking water in several regions. However, of all the definitions the two directives give to groundwater, none ranks it at the same level as surface water: groundwater is an ecosystem in itself.



EU Directives and Italian laws regarding groundwater quality



Ratio between renewable resources, regulating supplies and non-renewable permanent supplies in different aquifers

As long ago as 1991, the Dutch Environmental Agency National Institute of Public Health and the Environment (RIVM) and the National Institute of Inland Water Management and Waste Water Treatment (RIZA) had produced for the EU a report on the sustainable use of groundwater resources, clearly specifying that sustainability was closely associated with the maintenance of the potential functions of groundwater bodies, among which was their capacity for hosting local, diversified fauna.

Although the ecological importance of groundwater environments has been acknowledged for years, the two directives do not emphasise the need for direct and indirect information on them.

The opposite occurs for surface waters, for which biomonitoring has become essential for assessment of water quality. This gap in the law is even more evident, as the Habitats Directive concerning the protection of habitats and species of EU interest considers groundwater systems only marginally. The list of habitats of EU interest (Annex 1) does not specify groundwater clearly, and includes only caves explored by man and not exploited for tourist purposes, ignoring aquifer areas which are not directly accessible.

The Interpretation Manual of European Union Habitats, drawn up by the European Commission in 2003, does not include groundwater associated with cave environments in the definition "*Caves not open to the public*". Nor does it

mention alluvial aquifers, or those found in non-karstified rocks, in ecotonal environments - like the hyporheic zone - all of which have great biological value. The underground species in Annexes II and IV of the Habitats Directive up to 2003 just listed the olm (*Proteus anguinus*), and only later were two other species introduced - although, sadly, they do not belong to Italian fauna (the gastropod *Paladilhia hungarica*, and the bivalve *Congeria kusceri*).

The ecology of groundwater was first considered precisely as such by Italy, with Decree no. 152/99, later abrogated and incorporated in Decree 152/06 and its modifications, which adopts the Water Framework Directive. Hypogean biocoenoses are quoted in Annex 3, covering monitoring of the characteristics of catchment basins and analysis of the impact due to human activity, although their information content is not emphasised at all. The section on the acquisition of available knowledge about groundwater expressly refers to the acquisition of data, if available, provided by analyses of hypogean communities.

Although those data are available, they have always been considered as addressed to specialists, and have never reached decision-makers. In addition, Annex 3 establishes that the geometry of the main aquifers in the catchment basin must be reconstructed, emphasising their possible intercommunication pathways, including those with surface water, hydrochemical parameters and, when present, biological characteristics.

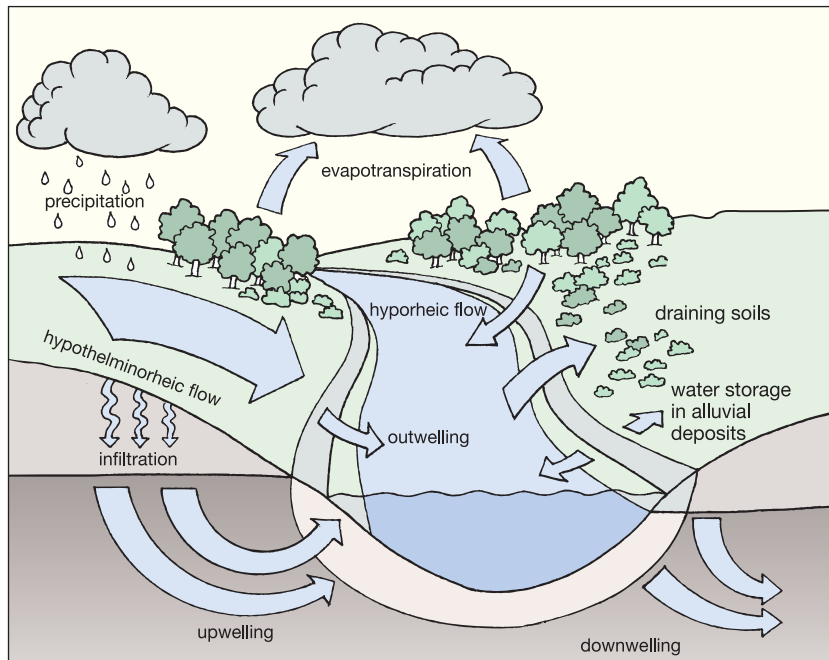


Diversion of the spring of the Peschiera (Latium)

## ■ Exploitation and over-exploitation of groundwater resources

The availability of groundwater depends on the capacity of aquifers for storing and recharging water. That is, according to the type of aquifer and its springs, we can calculate the volume of water they contain, also including the stored reserve.

Renewable water resources are made up of the water that, each year, reaches the aquifer through infiltration due to meteoric events. As this quantity tends to renew itself each year, although with some variations, these resources are considered available for human use and for the conservation of natural environments. More specifically, this is the portion of the water-table nearest to the surface, which is naturally discharged over the year. Further down the layer between the lowest water-table level reached during drought and the level of springs, there are regulating supplies, i.e., those which, although not normally discharged by aquifers, may issue according to variations in rainfall over the years and in the water-table itself. It is advisable not to use these supplies which, however, may become available in periods



Sketch illustrating interactions between water cycle, surface drainage and aquifers

of drought. At last, underneath the ground level of springs, there are permanent supplies, seldom used, which are made up of the water stored in aquifers, and whose withdrawal should be avoided to prevent exhaustion of springs and aquifer overexploitation. Incorrect aquifer management - withdrawing more water than can be provided by renewable resources - causes regulating and permanent supplies to decrease and even exhaust themselves.

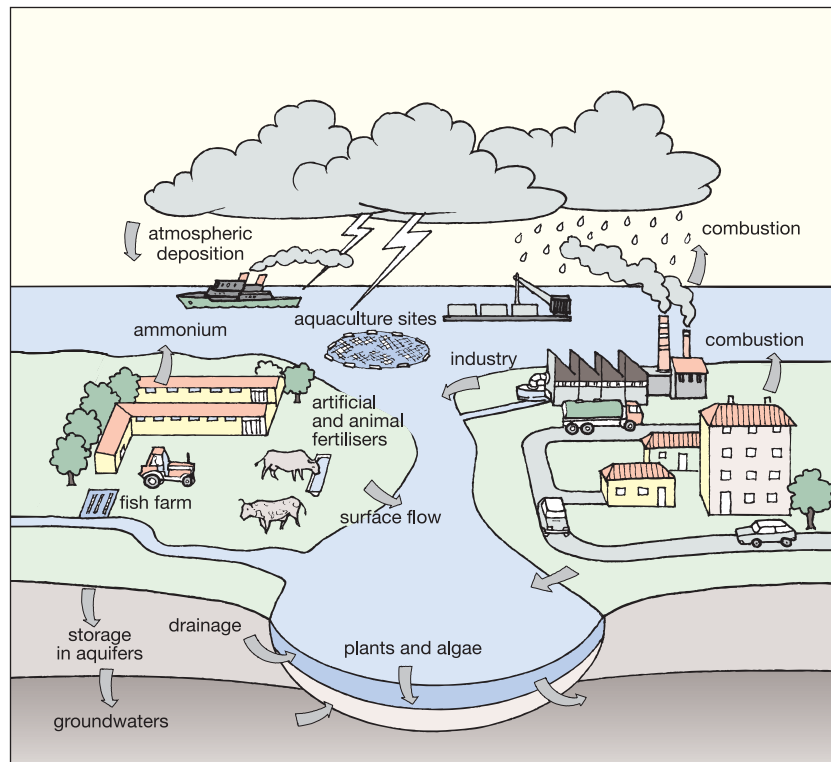
Depending on the availability of renewable resources, man uses water for all kinds of activities, collecting it from springs or by means of wells, or altering the flow and discharge of various water bodies. It is essential for exploitation within the same catchment basin, i.e., in the same aquifer or system of interconnecting aquifers, to comply with the restrictions imposed by recharge, and therefore the volume of renewable resources. However, since man is not the only user of water, and plants and animals also need it, in recent years, the definition of *maximum withdrawal limit* has been replaced by one of *sustainable withdrawal*. With regard to the total availability of renewable water resources, only a certain amount may be used for human needs, as essential



Water gushing from a spring (Casaletto Spartano, Campania)

volumes of water must be granted for the correct functioning of water-dependent ecosystems. This leads to the *minimum vital flow*, i.e., the minimum flow which must be maintained to enable flora and fauna to survive. The true value of the minimum vital flow is still being investigated by means of ecological, hydrological and hydrogeological approaches.

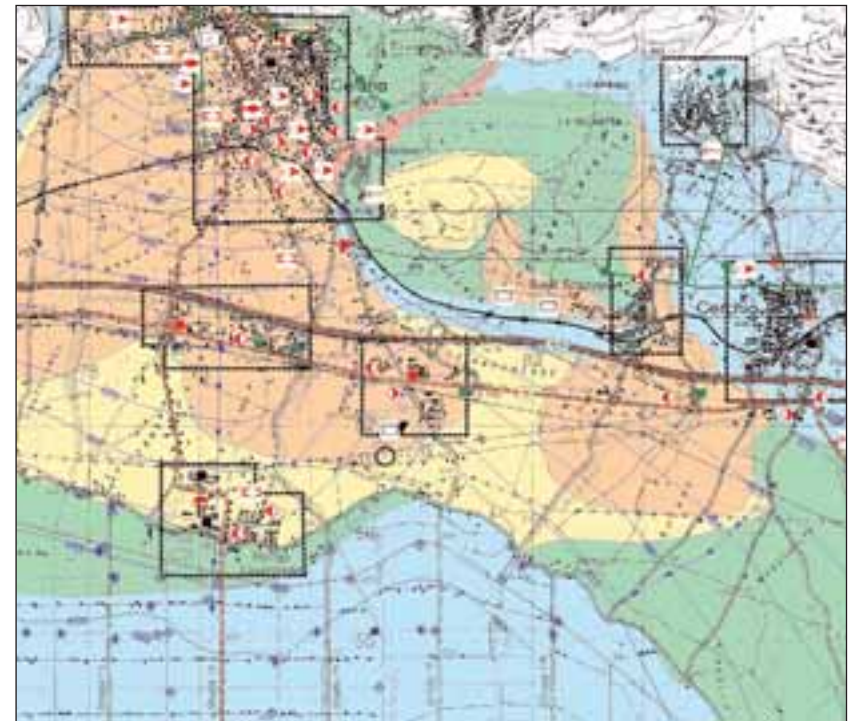
The actual application of the formulas proposed depends on the controlling institutions, like the Basin Authorities, which must also take into account the authorisations already implemented for the exploitation of water for human use. This is not limited to drinking water, which has priority, but includes civil and public consumption, water for industrial use and for animal rearing and irrigation. The last quota, at global and national levels, is extremely high, being 65% of all human uses. About 20-25% is used by industry and for the production of energy, and only 10% is used as drinking water. The hydrological balance must therefore take into account human requirements



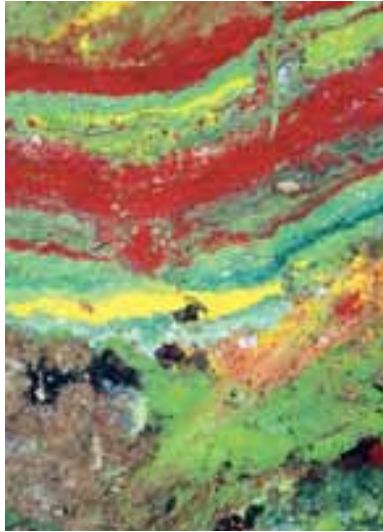
Potential anthropogenic sources of groundwater pollution

and withdrawals in a specific area, and not only the natural components of the water cycle. An integrated hydrological balance should therefore be drawn up to include surface and underground natural components as well as human ones. Monitoring the actual withdrawal is insufficient, as we must also consider which items of the natural hydrological balance are directly and indirectly influenced by various human uses.

Each withdrawal influences the availability of other components of the cycle. For instance, pumping from wells causes a decrease in spring discharge linked to the aquifer in question, whereas withdrawals from river beds may influence the water-table/river ratio and give rise to drainage of part of the water-table that does not normally contribute to surface flow. Moreover, human activities, like irrigation, may cause infiltrations, as additional water flows into the soil/subsoil system, triggering the integrating recharge of aquifers below.



Section of a vulnerability map (Piana del Fucino, Abruzzi): colours identify varying degrees of vulnerability (blue: low vulnerability, orange: high), red identifies "hazard centres", i.e., human activities that can introduce pollutants underground



Example of soil contaminated by paints

## ■ Groundwater pollution

Human activities do not only affect the natural hydrological cycle quantitatively. In fact, the most important changes to the natural hydrological environment are due to the pollution of surface- and groundwater.

The ways in which the water-table may be polluted depend on the type of pollutant, and are therefore associated with specific human activities.

In addition, pollution may be distinguished according to its spatial and temporal dynamics. It may derive from agricultural sources (mainly fertilisers and pesticides), may be associated with particular periods and

areas (in specific seasons and spreaded over large areas), or due to animal and human sewage, associated with leaking sewers and uncontrolled animal rearing (microbic and organic-chemical pollution). Pollution may also be divided into point-source and linear, continuous or discontinuous. Civil sewage systems and businesses cause chemical pollution due to the use of detergents, solvents and other liquids, through leaking pipes, either along the surface or underground.

Pollutants deriving from industrial use are more complex and varied, and contain a high percentage of hydrocarbons and chloride compounds. These produce complex pollution, depending on whether they are dissolved or in separate liquid form in the water-table and, if they float, on whether they are lighter than water (petrol and oil). They may even filter through the water-table if they are heavier than water (e.g., trichloroethylene). Potential sources of pollution are oil refineries and industrial sites, where chemical products and hydrocarbons are processed, but also warehouses and points of sale, like the underground tanks of petrol stations.

Other polluting sources are dumps from which, especially when they are illegal or unchecked, organic and inorganic pollutants may seep into the groundwater.

Despite potentially polluting human activities, groundwater pollution can be tackled by preventing and controlling it. As regards prevention, particularly

widespread and useful tools are aquifer vulnerability pollution maps, which allow activities to be planned in advance in specific areas. These maps consider vulnerability, intended as the intrinsic capacity of aquifers for storing and emitting types of pollutants from within. The assessment of vulnerability of single aquifers, which can be carried out in various ways, is based on the permeability of aquifers, the distance between the water-table and the surface, the protection provided by the overlying unsaturated medium, and the characteristics of the ground surface. Each aquifer is therefore identified by classes of permeability, colour-coded.

Another way of representing vulnerability is by means of isopiestic maps, which show the direction and way in which groundwater flows, and therefore the origin and direction of pollutants that might affect the water-table. The concept of vulnerability is then integrated with that of risk, by assessing potential producers of pollution, divided according to the activities involved (industry, animal rearing, drainage and sewage systems, etc.) and classified as "hazard centres".

In terms of protection, Italian legislation takes into account the need to guarantee good water-table quality, especially when water is collected for drinking. Protected zones are identified within these areas where - according to the distance from the offtake point and the hydrogeological characteristics of the aquifer - several potentially hazardous activities, which could introduce pollutants into the groundwater, are forbidden. Protection is also provided by monitoring the groundwater both in areas subjected to pumping and in potentially polluted areas (agricultural areas using fertilisers and pesticides, industry, towns).

In particular, in most industrial areas where several polluting activities have developed over the last century, a specific law states that the pollution they create must not extend to the nearby groundwater, and their pollutants must fall below the threshold limit values established for the wellbeing of both man and nature.



Hydrometric measurements

The PASCALIS project paved the way for presenting the European Union with guidelines concerning bioquality and conservation procedures for underground aquatic ecosystems, one of which is called GEcoRA (Groundwater Ecological Risk Assessment). GEcoRA assesses the risk of functionality loss of underground aquatic ecosystems by combining three factors: the probability of a negative event (P) caused by anthropic activities on aquifer surfaces; the intrinsic vulnerability of the aquifer (V); and the potential ecological damage (D) these activities could cause. Actually, risk assessment procedures have long been used for groundwater but, until now, the D factor (damage) has been assessed as the potential loss of resource use for potable, agricultural and/or industrial

purposes, and was therefore calculated according to chemo-physical or potability criteria. GEcoRA's initial assumption is that the groundwater system is first of all an ecosystem that hosts specialised and diversified local fauna, which precociously reacts to environmental variations of its habitats and can "remember" negative event, even when there is no chemo-physical evidence. The probability of such an event (P) is associated with the magnitude of the anthropogenic pressure on the examined site, and with the type of Hazard Centre (industry, towns, landfills, cemeteries, roads, cultivated fields, animal searing and farms, quarrying, and other activities influencing aquifers negatively). In GEcoRA, probability may be P=0 (zero pressure or very low),

P=0.5 (medium pressure), and P=1 (very high pressure). Intrinsic vulnerability (V) is defined as the specific susceptibility of aquifers - and their different components, in different geometrical and hydrodynamic situations - to absorb and spread, perhaps reducing their effects, liquid or water-transmitted pollutants to such an extent that they can influence groundwater quality in space and time. GEcoRA requires dividing intrinsic vulnerability into three classes with the following scores: Low = 2, Medium = 4, and High = 8.

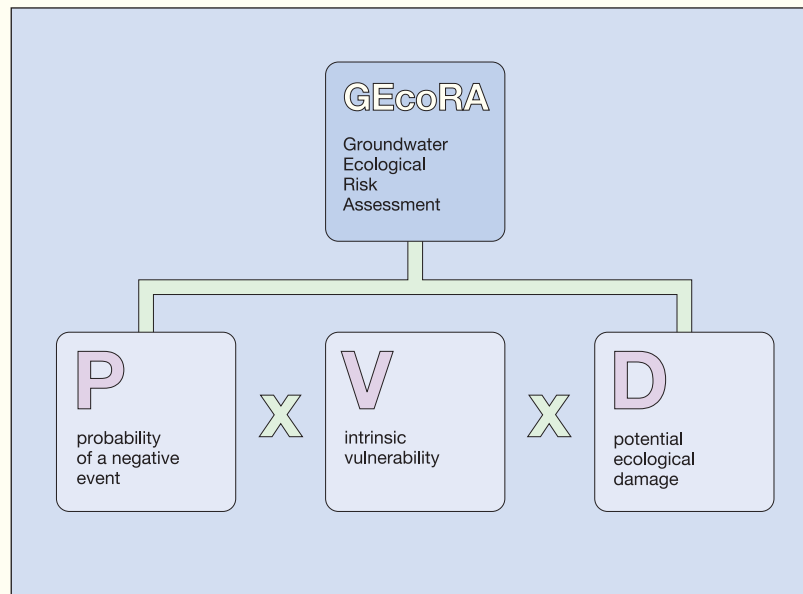
Potential ecological damage (D) is defined as biological damage, a function of the mortality rate or of physiological and metabolic alterations in stygobiont organisms.

As regards the values of D, GEcoRA defines 1 for minimal damage, 2 for

moderate damage, and 3 for extensive damage. The ecological risk (GecoR) affecting an underground aquatic system is given by the sum of products ( $P \times V \times D$ ) of each Hazard Centre in the examined area. The GecoR values are then normalised in the interval 0-1 and compared with the following tabulated values that, for five intervals of GecoR variation, identify five distinct classes of risk:

Value	Risk class	Color
0-0.20	Low	Blue
0.21-0.40	Medium	Green
0.41-0.60	High	Yellow
0.61-0.80	Very high	Orange
0.81-1	Alarming	Red

The GEcoRA procedure has so far been applied to several aquifers in Italy.



Well for agricultural irrigation (Lessini, Veneto)



An old "pumping-house" (Viarolo, Emilia Romagna)

## ■ Stygodiversity and human disturbance

Underground and ecotonal biocoenoses are very sensitive to human activities, which may sometimes exceed the sustainability threshold of the underground ecosystem.

Pollutants represent stress factors for underground biota, because they alter the ecological and physiological behaviour of stygobionts. Concentrations of pollutants in the water-table and their permanence are two fundamental variables which, combined, may alter the life-cycle of stygobionts.

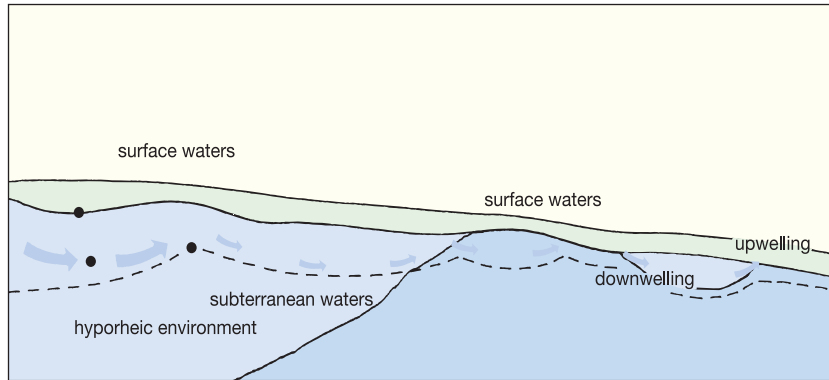
Generally speaking, when pollutants have short permanence periods (days), the observed effects regard individual stygobionts, and manifest themselves as behavioural problems (swimming and feeding).

If pollutants have longer permanence periods (days/weeks), organisms are affected by physiological and morphological changes (development of anomalies), or even alterations in their life-cycles at individual level. These may give rise to changes in the population structure and, if the pollutants persist over months/years, the structure of the polluted ecosystem may change.

Pollutants affect biota directly and indirectly: contaminants may alter the biochemical and metabolic balance of organisms through direct contact (e.g., bioaccumulation of heavy metals), and indirect contact, by altering the chemico-physical conditions of the environment, which may reach to the point of becoming unsuitable for life (e.g., producing severe phenomena of hypoxia-anoxia due to organic pollution). The contamination process influences the underground environment in all its components, both biotic and abiotic. In particular, it gives rise to changes in the structures (population density, biomass, specific diversity and trophic structure) and function (biomass production, energy, trophic dynamics, community structure, adaptive capacities) of the underground aquatic environment.

Particular conditions of groundwater organic pollution may give rise to stable, lasting settlements of stygoxenic populations. In this situation, stygoxenes may even reproduce, reach considerable population density and overcome the local stygobionts, thus altering community composition. Equally important is the damage caused to stygobiont species, which are replaced by invasive allochthonous species. These observations show how the structure of underground communities may be a short- and long-term indicator of acute and chronic pollution.

Alterations of river beds (banks, cement barriers, weirs, dams, and direct capture) may also disturb the hyporheic zone, and overpumping of water



Within the hyporheic environment, higher abundance of stygoxenes in downwelling sectors and of stygobionts in outwelling ones may be observed

from the water-table may cause the latter to fall, thus destroying stygobionts' microhabitats. Other activities, like quarrying, have considerable impact, as they accumulate sediments and clog hyporheic interstitial sediments.

Today, the quality, biological integrity and functionality of the hyporheic environment and the modifications caused to it by human activities interrupting its continuity, may be assessed by means of new integrated analyses like the recently devised HyES (Hyporheic Expert System), developed by Italian researchers.

Defining protection and management programmes for integrated hydric systems, requalification and restoration of rivers, and planning of groundwater use cannot be undertaken without detailed information on the functionality of ecotonal and underground aquatic ecosystems. This is why, today, integrated monitoring is required, i.e., an analytic approach that combines traditional methods of chemico-physical analysis with biological monitoring and the use of bioindicators.

Insights into the ecological aspect of groundwater have indicated the proper hydrogeological procedures to be applied to assess risk in these environments. Risk results from the combination of anthropogenic pressure and intrinsic vulnerability, with the additional potential damage to the ecosystem. Ecological damage may be assessed according to the mortality rate or physiological and metabolic alterations of the myriads of organisms that make up underground communities. Recently, a new procedure for groundwater ecological risk assessment (GecoRA) has been devised by Italian researchers (see pp. 124-125).

## ■ Conservation of underground ecosystems

This Habitats volume describes how groundwater is not only a natural resource to be exploited for human use, but is in particular a complex ecosystem like any other surface one. Also, compared with surface water, underground systems have an "added value", due to the great scientific and cultural importance of their fauna, often the only living testimony to Italian palaeogeographical and palaeoclimatic events. The present-day inhabitants of these environments originated from unique evolutionary events, and to destroy stygodiversity (i.e., to cause the extinction of underground species) means extinguishing the only surviving traces of those events - as if archaeologists were to lose the one and only existing find they had excavated. What would the consequences be if, instead of a single find, an entire monument were to be destroyed, or an entire city belonging to an ancient culture (in this case, the ecosystem)? The cultural loss would be irreparable.

The phylogenetic value of stygodiversity may be also analysed by means of the *evolutionarily significant units* (ESU). This definition was originally used for surface fauna, especially subspecies. However, groundwater hosts various types of ESU, at genus, family, order and class levels - that is, entire



Underground lake in the Grotta di Punta Galera (Campania)

The PASCALIS project (Protocols for the Assessment and Conservation of Aquatic Life in the Subsurface) within the 5th Framework Programme for Research and Technological Development (FP5 RTD), approved by the European Commission in 2001, aimed at defining the best strategies to assess stygodiversity, and at providing the European Commission with guidelines for the issuing of EU recommendations for more efficient protection of biodiversity in underground aquatic environments.

Except for glaciers, groundwater is the Earth's largest freshwater reservoir (97%). Yet most research is carried out on surface water, and measures for the

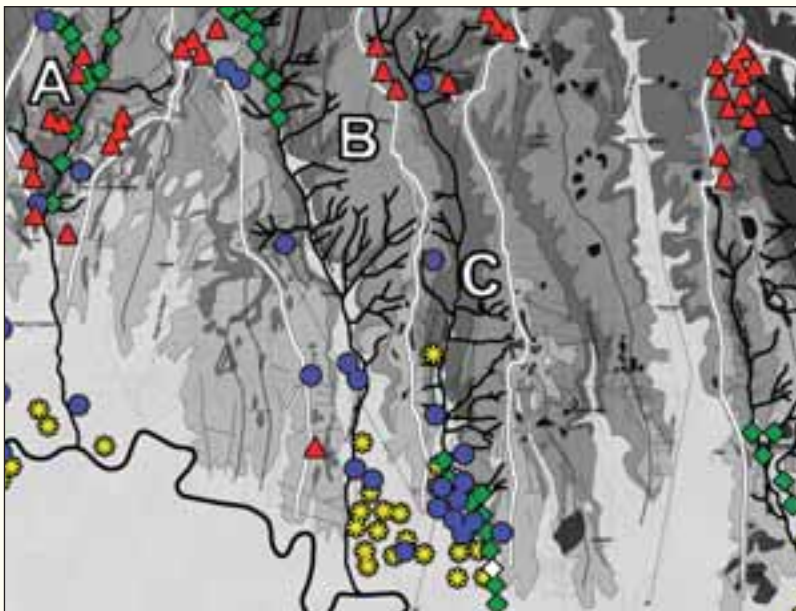
conservation of biodiversity are adopted only for it. The main obstacles to enforcing an efficient policy of stygodiversity protection are due to the present insufficient scientific knowledge about groundwater fauna, the lack of conservation strategies, and the insufficient knowledge of decision-makers about issues associated with this "dark world".

The PASCALIS project aimed at filling this gap by focusing on all three issues. First, a multi-scale integrated approach was developed to study European stygodiversity by analysing a wide range of groundwater habitats (karstic and alluvial) in five countries (Spain, France, Belgium, Italy and Slovenia). The study

included almost all taxonomic groups with stygobiont species, and applied different approaches, from morphological to molecular, to assess biodiversity at site, aquifer, basin and region levels. In Italy, this research was carried out in the area of the Lessini Hills (province of Verona). The ultimate purpose of the first phase was to identify a rigorous, detailed protocol to optimise assessment of stygodiversity on different geographical scales, a protocol that came in the form of a manual for simple use.

The second phase of the project regarded the development of operational instruments to plan conservation strategies: identifying bio-indicator groups, assessing the intrinsic value of underground fauna in different aquifers to identify conservation priorities, and identifying areas of diversity and endemism ("hotspots") in Europe.

The third phase of the project aimed at producing recommendations for the creation of a European Action Plan for the conservation of stygodiversity - a proposal for integrating the Habitats Directive with underground species and habitats of European interest, and enhancing public awareness by creating a communication channel between the project and a wide range of end-users. The final purpose of the project was to work in permanent contact with those managing water sources and natural habitats, water authorities, and the general public, in order to satisfy pressing social issues and provide common operational instruments to all EU Member States to conserve biodiversity. We hope that research may soon turn into action, and that these instruments may become European directives and national laws.



Sketch of Lessini Hills (Veneto), showing the studied area during the PASCALIS project: A - Val Fumane, B - Val Pantena, C - Valle di Squaranto: main sites in saturated karstic environments (blue), unsaturated karstic sites (red), saturated porous sites (yellow), hyporheic sites (green)



taxonomic groups of strictly stygobiont species which require even greater protection in that they have survived over geological times in underground environments and have completely disappeared from surface ones. Identifying them is of paramount importance because they are taxa that cannot be reintroduced, as they are isolated evolutionary lines not closely related to other existing taxa. They are indeed an invaluable scientific heritage for conservation.

Stygodiversity and underground ecosystems should not be preserved only for their scientific and cultural aspects. Their ecological and functional importance, as described in the chapter on the four-dimensional nature of water bodies, is closely associated with economic interests and man health, and may even influence his survival. In view of the present and potential risks that threaten the structure and integrity of groundwater ecosystems and the conservation status of their species, and considering the lack of interest displayed by current management policies (at Italian and European levels, as we emphasised when discussing the present laws), we feel that the end of this volume should list some recommendations for the conservation of stygodiversity. The word "recommendation" is drawn from the results obtained by the European project PASCALIS mentioned above (see pp. 130-131), which provides a series of practical indications for the protection of underground ecosystems.



Spring reserved for drinking-water purposes (Anversa degli Abruzzi, Abruzzi)

### ■ Integration of stygodiversity conservation and functionality of ground-water ecosystems in Italian and European policies

This integration should be made to both laws and instruments, within the fields of management policies and the sustainable use of natural resources, especially those regarding water (Water Framework Directive, and later integrations and implementations, by adding a chapter on the "satisfactory ecological status") and biodiversity (Habitats Directive, and management and conservation of the network Natura 2000). It is equally important to integrate laws and application tools regarding the use of the soil, agriculture, flood regulation of watercourses and catchment basins, assessment of environmental impact and monitoring strategies (see box on GecoRA). As regards stygodiversity, the annexes to the Habitats Directive should be amended and species requiring special attention should be added, because underground fauna is rich in endemic species at risk of extinction which are of European interest, as defined by the Directive itself.

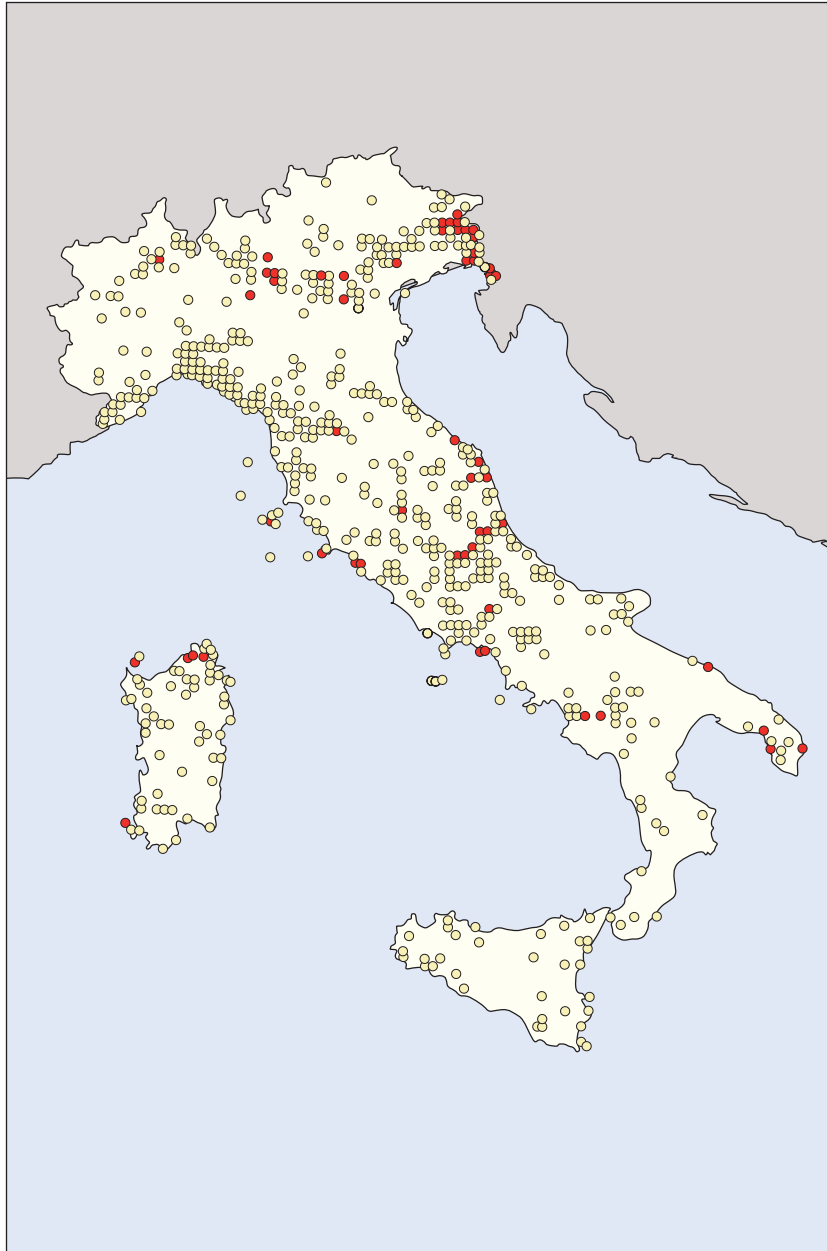


Pumping water for irrigation from a well

### ■ Compiling a list of priority species and habitats for the conservation of groundwater, identifying the most important elements of stygodiversity

As scientific knowledge is still fragmentary and laws are insufficient, a list of priority species and taxonomic groups for conservation (based on endemic, rare and phylogenetically relict species and their degree of threat) would be an operational instrument of primary importance in identifying conservation strategies. At the same time, a list of Italian groundwater habitats of great conservation importance could help to identify priority aquifers that should be preserved - not only as water resources, but also as ecosystems and related natural assets.

Aquifers have been specifically mentioned as essential units for conservation, because the protection of groundwater cannot be based on the protection of



"Stygo-hotspots" in Italy; red circles: main hotspots

individual sites (caves, springs, stretches of rivers), according to present conservation procedures. For instance, the fauna and ecological functionality of a particular spring depend exclusively on the conservation state of the aquifer supplying it.

In view of the interconnections existing between underground watercourses, one potentially successful strategy would be to select a network of priority aquifers as natural reserves, at regional or Italian level, thus emphasising not only their intrinsic scientific value and their vulnerability to anthropogenic pressure, but also their importance as water supplies for human use.



Drift sampling in a spring reserved for drinking-water purposes

#### ■ Improve scientific knowledge and plan monitoring strategies

As in Italy stygobiology is still in its infancy, we must improve our knowledge in order to identify Italian stygo-regions and propose suitable conservation measures. This should be implemented with a strategy for the biological monitoring of aquifers, currently almost completely absent in Italy. We hope groundwater habitats will soon be added to the list of environments to be protected. These indications could be added to the recent Action Plan in favour of Biodiversity promoted by the European Community on 22 May 2006, called "*Halting biodiversity loss by 2010 and beyond, and sustaining ecosystem services for human well-being.*" The Action Plan includes projects to halt the decline of biodiversity and the measures to be adopted to achieve these objectives (the well-known "Objective 2010"), and is based on the assessment of biodiversity loss in Europe and the world, together with the measures adopted so far by the EU to tackle the issue. The Plan emphasises that sustainable protection of biodiversity goes beyond the Natura 2000 network and threatened species. This is why the Action Plan provides for the safeguarding and restoration of biodiversity and ecosystem maintenance. Perhaps the moment has come for us to protect this dark kingdom of our world and its concealed biodiversity.

A barely known field of research analyses the response of underground biota to environmental pollutants. According to the first data obtained by international scientific research, stygobiont species may function as short- and long-term sensors of acute and chronic pollution. Although ecotoxicological monitoring in groundwater is in its experimental infancy, the first results suggest that the ecotoxicological method has operational difficulties for wide-ranging applications to groundwater. The main restrictions are: 1) the long life-cycles of stygobionts; 2) stygobionts are difficult to be reared *ex situ*; 3) the geographical distribution of species is often very restricted, which hinders identification of both species and groups of species

(targets) to be used on an extensive spatial scale. Since ecotonal environments (hyporheic environments, springs) frequently host both stygophiles and stygoxenes, assessment of the effects of pollutants - such as heavy metals, ammonia and nitrates - should also include them. The first results obtained by underground aquatic ecotoxicology reveal that stygophilic and stygobiont species are sensitive, albeit in differing ways, to short- and long-term toxicity. Preliminary data on the effects that some toxic substances found in the water-table have on stygobionts are already available. These effects are expressed in terms of lethal concentration values (LC<sub>50</sub>), i.e., the concentration of a statistically assessed

substance lethal to 50% of the animals tested during exposure or within a certain period of time following exposure. The data reported here refer to a sampling period of 96 hours, with pH and temperature (generally 10.5°C) similar to those of groundwater. As regards ammonia ions, for instance, the LC<sub>50</sub> value was observed to be 1.5 mg/l; for chlorine, measured as residual and free chlorine, LC<sub>50</sub> was almost 3 mg/l for acclimatised individual, and lower (2.25-2.7 mg/l) for those not acclimatised. The toxic effect of zinc on stygobionts was tested on several taxa, and the most sensitive species was found to be the stygobiont copepod of the genus *Parastenocaris* (LC<sub>50</sub> = 1.7 mg/l in adults and 1.27 mg/l in copepodite stages).

The only data available on pesticide toxicity on stygobionts regard Aldicarb (LC<sub>50</sub> for adult *Parastenocaris* specimens = 2.9 mg/l) and Thiram (0.003 mg/l on the same species). The test revealed that the standard dose of 0.1 mg/l allowed by law does not always ensure protection of stygobiont species, especially for pesticides such as Aldicarb, Aldicarb-sulfoxide, Cypermethrin, Ethoprophos, Fenpropathrin and Thiram. In addition, the combined effect of various pesticides (inevitable for some crops, on which sometimes more than 10 pesticides are used at a time) is synergic, i.e., it produces toxic effects at lower concentrations than those tested singly for each compound.



Differences in growth rate between control individual (left) and test one (right) of the species *Bryocamptus echinatus* exposed to Aldicarb (0.6 mg/l)

SPECIE TEST *species not belonging to Italian fauna	TOXICITY (LC <sub>50</sub> - 96 h in mg/l)	TESTED SUBSTANCE
<i>Bryocamptus echinatus</i> (stygophylic copepod crustacean)	1.26	Hexavalent chromium
	2.71	Aldicarb
	14.61	Ammonium nitrate
<i>Parastenocaris germanica</i> * (stygobiont copepod crustacean)	2.90	Aldicarb
	1.7	Zinc
	0.003	Thiram
<i>Attheyella crassa</i> (stygoxenic copepod crustacean)	3.82	Hexavalent chromium
	3.17	Aldicarb
	17.8	Ammonium nitrate
<i>Niphargus aquilex</i> (stygobiont amphipod crustacean)	180	Zinc
	4.5	Cadmium
<i>Caecidotea stygia</i> * (stygobiont isopod crustacean)	2.4	Hexavalent chromium
	2.3	Copper
<i>Trichodrilus tenuis</i> * (stygobiont oligochaete)	8.25	Zinc
	1.05	Cadmium



## Suggestions for teaching

DIANA MARIA PAOLA GALASSI

139

### ■ Ecological analysis of groundwater

Although teaching suggestions for the ecological analysis of groundwater environments may sound devoted specifically to experts and scholars with an advanced cultural background in ecology, actually the ecology of groundwater - stygo-ecology - is analysed with the same instruments and methods used for surface aquatic ecosystems.

The only obstacle is environmental access. Therefore, analysis starts from finding a hole, an opening in the ground, to reach these habitats shrouded in darkness.

A good natural laboratory is the hyporheic environment which, as we have already seen, is the interface between the surface water of a river and the phreatic environment (the saturated alluvial aquifer) underneath. The teaching experience will prove to be exciting, as students of all ages intuitively find and experimentally verify the existence of several connections between surface water, which they already know and which is part of the pastoral imagery of the human mind, and the vast underground world, which they may have only fleetingly experienced when visiting a cave.

- Objectives: to analyse the ecotones water/groundwater surface, to identify a stretch of river in which an aquifer supplies the river bed (upwelling area) and a stretch in which the river supplies the aquifer underneath (downwelling area); to study principles and methods in hyporheic environments. Field analysis of



Bou-Rouch sampling in the hyporheic environment

The springs of Clitumno (Umbria)

visual indexes to identify the various hyporheic environments sampled; to draw up a field description for the final report and to identify in the laboratory the most important biological elements for describing hyporheic communities; to classify taxa in ecological categories: stygobionts, stygophiles and stygoxenes; to assess the potential negative effects on the hyporheic environment and consequences to its biodiversity.

- Level: secondary school and university students.
- Collaboration: an environmental biologist skilled in aquatic ecology.
- Equipment: bibliography and iconography to be handed out to students to provide them with basic information on freshwater and groundwater ecology, a Bou-Rouch pump or a simple bilge pump, a piezometer (an empty metal tube with small holes at each end), a net with a 100  $\mu\text{m}$  mesh, a multiparametric probe (or several probes for each parameter, for field analysis of temperature, pH, electrical conductivity, dissolved oxygen concentration), 500-ml plastic containers, alcohol for fixing collected samples (alternatively, samples may be processed without being fixed. For equipment, see also p. 45).

The laboratory must be equipped with a stereomicroscope and an optical transmission microscope, if possible with phase-contrast enhancement,



Gravel bed of a glacial stream hosts a specific hyporheic fauna

because it shows animals more clearly and does not require their fixation and staining of specimens on slides. Students should be given a lab kit containing: 2 Petri dishes, a sorting needle, tweezers, some plastic or glass test-tubes, 70% alcohol solution, glass or plastic containers, glass cases, glycerin and dissecting needles. The laboratory must also be equipped with an iconographic atlas of freshwater invertebrates.

#### PRELIMINARY STAGE

1. Introduce the concept of groundwater ecosystems, focusing on the classification of aquifers and the description of habitats.
2. Describe hyporheic and interstitial environments. Identify the main abiotic and biotic factors regulating the hyporheic corridor.
3. Characterise and describe interstitial fauna and meiofauna in particular.
4. Describe the methods and instruments used for analysing hyporheic and other underground environments.
5. Collect information on the geology, hydrogeology and aquatic biodiversity of the basin examined.
6. Compile a field form which every student fills in during sampling and, later, in the laboratory.



Sampling in a gravel bed



Meiofauna under the stereomicroscope

#### FIRST STAGE: FIELD EXPERIENCE

7. Field experience is divided into two distinct samplings: the first, in the upwelling area, is easily identifiable, as after inserting the perforated end of the piezometer in the river bed, the water level inside the piezometer is higher than the height of the water column in the bed. The second sampling is carried out in the downwelling area, where the water level inside the piezometer is lower than, or equal to, the water column in the river bed. Both piezometers are fitted with a manual inertial pump or a Bou-Rouch pump, for collecting interstitial water together with smaller sediments, particle-sized organic matter trapped between sediments and, obviously, the fauna they contain. We suggest collecting about 10 litres per site, to enable later comparison between data collected at different sites along the same river bed. The collected samples will undergo basic chemico-physical analyses with the multiparametric probe. Students start to fill in their forms, following the indications provided in the preliminary stage. The collected samples can be fixed on the spot in 30-50% alcohol, if laboratory testing occurs after a long time (one or more weeks); otherwise, samples can be preserved for less than a week in a thermostat, at a temperature similar to that of the collection sites.

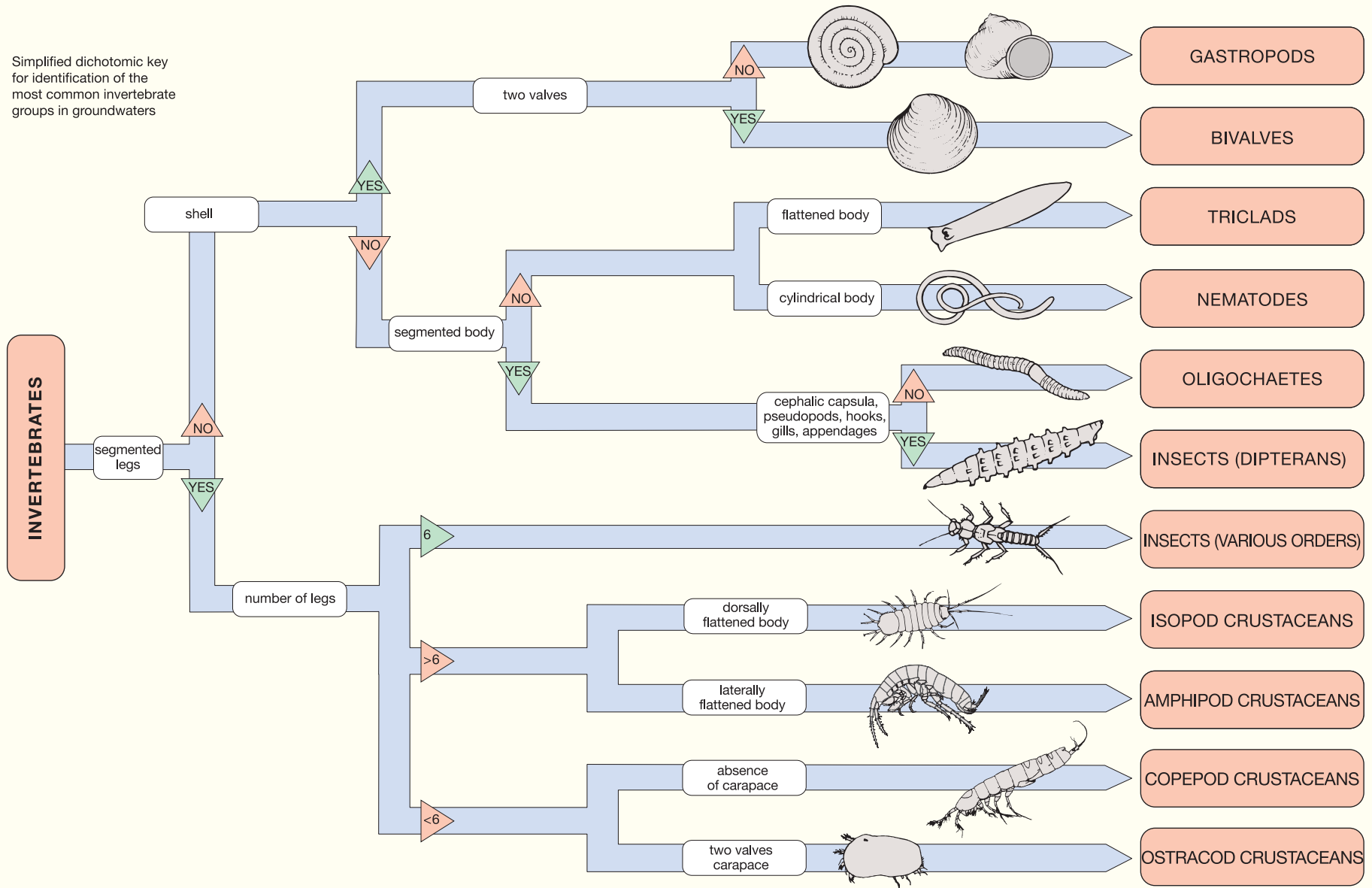
#### SECOND STAGE: LABORATORY TESTING

8. Each collection is divided into subsamples and put in Petri dishes. Students



Piezometer inserted in gravel

Simplified dichotomic key for identification of the most common invertebrate groups in groundwaters



FIELD EXCURSION TECHNICAL CHART	
NAME OF HYDROGRAPHIC BASIN	
NAME OF STREAM/RIVER	
LOCALITY village, town province region	
ALTITUDE a.s.l.	
GEOGRAPHIC COORDINATES	
DISTANCE FROM SPRING	
DISCHARGE (l/s)	
SAMPLING DATE	
NAME	
SAMPLING METHOD	
TYPE OF SAMPLING <input type="checkbox"/> quantitative <input type="checkbox"/> qualitative	
DESCRIPTION OF HABITAT notes	
<input type="checkbox"/> upwelling sector <input type="checkbox"/> downwelling sector	
GRANULOMETRY OF SUBSTRATE <input type="checkbox"/> mainly gravel - pebble <input type="checkbox"/> mainly sand - silt - clay	
HETEROGENEITY OF GRANULOMETRIC COMPOSITION <input type="checkbox"/> high: presence of various granulometric classes <input type="checkbox"/> low: dominance of two granulometric classes ( $\geq 70\%$ )	

sort material in the dishes with a stereomicroscope, and separate all the animal specimens they can identify. They collect single specimens with a Pasteur pipe or a needle with a bight angle (which inexperienced people find more difficult to handle) and place them in glass containers filled with 70% alcohol solution.

Each student is asked to identify the animal groups collected (phyla, sub-phyla and classes, see also pp. 144-145). Tiny animals can be analysed by means of microscopic preparations, under an optical transmission microscope: small crustaceans (copepods), oligochaetes and nematodes become visible.

The specimens which the teacher considers more important may then be ecologically classified by distinguishing between stygobionts and non-stygobionts. The distinction between stygophiles and stygoxenes is not always easy, as it is not necessarily associated with morphological adaptations. This is why it is better to identify stygobionts having evident morphological adaptations to life in underground environments (depigmentation, anolphthalmy, minute size, restricted number of eggs in females, etc.). A comparative analysis between the two samples may then be carried out. Students can verify that the number of stygobionts is higher in the upwelling sites than in the downwelling ones, where there are larger numbers of non-stygobionts (stygophiles and stygoxenes), like insect larvae (nymphs of ephemeropterans and plecopterans, larvae of chironomid dipterans) for which this environment provides refuge from the many predators living in benthic river habitats. Students take notes.

#### THIRD STAGE: FINAL REPORT

At this stage, students write a report, with an introduction on the general issues associated with groundwater environments, followed by an analytic description of the sampling procedure in the hyporheic environment, the laboratory results obtained, and their analysis, in view of an approach in which ecology is applied to the assessment of the potential negative effects on meiofauna caused by human activities.



Laboratory sorting

BERETTA G.P., 1992 - Idrogeologia per il disinquinamento delle acque sotterranee. Tecniche per lo studio e la progettazione degli interventi di prevenzione, controllo, Bonifica e recupero. *Hydrogeology for the purification of groundwater. Techniques for studying and designing prevention and control measures. Reclamation and recovery*. Quaderni di Tecniche di Protezione Ambientale, 18. Pitagora Editrice, Bologna.

This book focuses on soil and groundwater pollution, and provides theories on the underground movement of water and dissolved substances.

BOTOSANEANU L. (ed.), 1986 - Stygofauna Mundi. *E.J. Brill/Dr. W. Backhuys*, Leiden.

An exhaustive, rigorous description of all taxa living in groundwater throughout the world. For each animal group, there is a short description, a list of all species known up to 1985, and their worldwide distribution.

CAMACHO A.I. (ed.), 1992 - The natural history of biospeleology. *Monografías, Museo Nacional de Ciencias naturales, Consejo Superior de Investigaciones científicas*, Madrid.

An extensive collection of articles on biospeleology: several chapters are devoted to subterranean waters, and discuss general problems, such as sampling methods, the evolution and speciation of stygobiont organisms, and the proper conservation of subterranean aquatic ecosystems.

CELICO P., 1986 - Prospezioni Idrogeologiche. *Hydrogeological prospecting*. Vols. I and II. *Liguori Editore*, Naples.

A book on quantitative hydrogeology containing theoretical bases applied to real situations and data. Investigating techniques and methods are thoroughly described.

CIVITA M., 2005 - Idrogeologia applicata e ambientale. *Applied and environmental hydrogeology*. Casa Editrice Ambrosiana, Milan.

A modern book that combines theory and excellent iconography. It mostly focuses on environmental issues, and provides operational solutions for the analysed cases.

CUSTODIO E., LLAMAS M.R., 2005 - Idrologia sotterranea. *Subterranean hydrology*. Dario Flaccovio Editore, Palermo.

This book associates the quantitative theory approach with modern analysis of possible field applications for the protection and management of groundwater resources.

GIBERT J. (ed.), 2005 - World subterranean biodiversity. *PASCALIS European Research Programme, Villeurbanne*.

This book contains the proceedings of a congress at Villeurbanne (France) in 2004. The scientific articles provide a general overview of present research on the biology of groundwater, and there are also the first results of the European PASCALIS project.

GIBERT J., DANIELOPOL D.L., STANFORD J.A. (eds.), 1994 - Groundwater Ecology. *Academic Press*.

Although this monograph is rather dated, it is still the only bibliographic reference for those wishing to study underground aquatic ecosystems. The first introductory chapters are accessible to non-experts. The book becomes more complex in the following chapters, which describe particular case studies on a global scale.

GRIEBLER C., DANIELOPOL D.L., GIBERT J., NACHTNEBEL H.P., NOTENBOOM J. (eds.), 2001 - Groundwater ecology. A tool for management of water resources. *Austrian Academy of Sciences, Institute of Limnology Vienna- Mondsee*.

An interesting monograph describing the ecology of groundwater in technical language. It explains issues regarding the management of water resources from both legal and applicational viewpoints. It contains interesting examples of sustainable management of groundwater resources in several European countries.

MINELLI A., CHEMINI C., ARGANO R., RUFFO S. (eds.), 2002 - La fauna in Italia. *Touring Editore Milano, Ministero dell'Ambiente e della Tutela del Territorio*, Roma.

A text easy to consult: educational but accurate, and covering all aspects of Italian fauna. A special chapter is devoted to the palaeogeographic vicissitudes undergone by Italy, with various examples regarding organisms found in subterranean waters.

NOTENBOOM J., VERSCHOOR A., VAN DER LINDEN A., VAN DER PLASSCHE E., REUTHER C., 1999 - Pesticides in groundwater: occurrence and ecological impacts. RIVM report 601506002. <http://www.rivm.nl/>. Report of the Dutch Environmental Agency which analyses, for the first time, the ecological effects of pesticides on stygobionts living in groundwater. The bibliography is particularly interesting, as it lists scientific articles on groundwater ecotoxicology.

RUFFO S., STOCH F. (eds.), 2005 - Checklist e distribuzione della fauna italiana. *Checklist and distribution of Italian fauna. Memorie del Museo Civico di Storia Naturale di Verona, 2° serie, Sezione Scienze della Vita*, 16: 307 pp. (with CD-ROM). The most updated list and distribution of animal species living in Italy; it includes almost all the taxonomic groups described in this Habitats volume.

WILKENS H., CULVER D.C., HUMPHREYS W.F. (eds.), 2000 - Subterranean Ecosystems - Ecosystems of the World 30. *Elsevier Science B.V., The Netherlands*. A fascinating monograph, both for its iconography and description of unique underground habitats in the world. Interesting articles on individual stygobionts alternate with case studies in borderline environments, like the Movile Cave. Subterranean ecosystems are described in an interesting yet simple way.

## Glossary

- > Amphi-atlantic: taxon distributed on both shores of the Atlantic: organisms living in Europe, Africa and America.
- > Anchialine: environment in brackish coastal groundwater into which seawater penetrates. This definition generally refers to caves and lava tubes.
- > Anophthalmic: congenital lack of eyes.
- > Anthropogenic: involving the influence of man on nature; induced or altered by the presence and activities of man.
- > Aquiclude: only very slightly permeable or almost impermeable portion of rock or soil. It confines water in an adjacent, more permeable aquifer.
- > Aquifer: portion of rock or soil with sufficiently good permeability to host a water-table and therefore to allow water to move within it in a relatively short time.
- > Aquitard: portion of rock or soil with little permeability, sufficient however, to host a water-table of limited potential and whose movement occurs over long periods.
- > Crenobiont: species whose complete life-cycle is totally dependent on spring environments.
- > Dispersion: general word to define the intrinsic attitude of a species for either moving away from its original location in search of similar or better conditions (dispersion, diffusion, exploration) or long-distance dispersion, which implies that populations overcome physical barriers. This is the basis for the biogeographical model of dispersion.
- > Dystrophic: environment enriched with dissolved humic matter.
- > Efficient infiltration: amount of rainfall that filters underground, reaching the water-table.
- > Epigeal: relating to the surface.
- > Epigeal (karstification): component of the karstic process affecting the Earth's surface and developing on the ground surface.
- > Eutrophic: environment rich in nutrients.
- > Evapotranspiration: loss of water from the soil both by evaporation from the surface and by transpiration from the plants growing on it.
- > Gravitational water: groundwater that does not interact with the medium around it and moves according to the conditions of permeability encountered.
- > Hazard centre: potentially hazardous anthropogenic activity which may introduce pollutants underground and therefore into the water-table.
- > Heterotroph: an organism that cannot synthesise inorganic matter autonomously; its survival depends on organic compounds pre-synthesised by other organisms called autotrophs.
- > Hydraulic conductivity: quantitative expression of permeability; extent to which gravitational water flows through rock or soil.
- > Hydraulic gradient: a line joining the points of the highest elevation of water in a series of vertical open pipes rising from a pipeline in which water flows under pressure.
- > Hydrological balance: balance between flow and recharge in catchment basins, including precipitation, evapotranspiration, run-off, efficient infiltration, and pumping.
- > Hypogean: below the surface of the ground.
- > Isoleths: isograms on a graph showing the occurrence or frequency of a phenomenon as a function of two variables; they show the direction of the water-table and enable calculation of the hydraulic gradient.
- > Linear spring: groundwater flowing directly into a river bed.
- > Meiofauna: organisms of any systematic group generally associated with interstitial benthos (meiobenthos), which are retained by a 45-µm mesh (the lower limit is still being discussed by experts).
- > Metameric: having body segments.
- > Microphthalmic: abnormal smallness of eyes or, in compound eyes, reduced number of ocelli.
- > Mineral (spring): spring with mineral contents (dissolved salts > 1 g/l) higher than usual.
- > Minimum vital flow: minimum watercourse flow ensuring the survival of ecosystems depending on the watercourse itself or on the water-table.
- > Nyctemeral: synonymous with circadian, i.e., occurring in approximately 24-hour periods or cycles.
- > Oligotrophic: an environment which is deficient in nutrients.
- > Ontogeny: the biological development or course of development of an individual organism, from zygote to adult.
- > Permanent supplies: deep, non-renewable portion of groundwater; this is the aquifer supply, whose pumping may sometimes deplete groundwater irreversibly.
- > Permeability: a measure of the ease of fluid flow through rocks.
- > Permeability (limit of): contact between two rocks or soils having different permeability, which limits or prevents the passage of groundwater.
- > Permeability (threshold): limit of permeability of an aquifer, allowing storage of permanent water supplies at depth.
- > Photic: reaction to or production of light by living organisms.
- > Phreatic: the water-table closest to the Earth's surface, from which it is separated by the unsaturated zone.
- > Plesiotypic: of, or relating to, the primitive environment of an animal group, i.e., the habitat in which the ancestors of the group presumably lived, and whose members later colonised a new habitat (secondary habitat).

> Pollutant: a chemical or biological compound that can modify the characteristics of groundwater, worsening them, and thus making the water useless for man's purposes.

> Psammon: the habitat used by an ecological community consisting of minute plants and animals that live in the water, filling the interstices of sand adjacent to a body of fresh water.

> Regulating supplies: portion of groundwater supplies which are renewed over several years, which is pumped only during periods of drought, and which allows groundwater to emerge from springs, thanks to the hydraulic gradient.

> Renewable resource: portion of groundwater which is renewed on a yearly basis, thanks to effective infiltration; the yearly flow of springs.

> Run-off: the water from rain or melted snow that flows over the surface.

> Saturated zone: portion of the underground environment, with voids totally filled with water. It corresponds to the water-table and lies below the unsaturated zone.

> Speciation: gradual evolution from and replacement of one species by another.

> Specific retention: capacity of rocks or soil to retain water.

> Storage: water storage in aquifers, at such depths as to make this water a barely usable resource due to natural causes.

> Taxon: general word to indicate any taxonomic group in zoological and biological systematics: kingdom, class, order, family, genus, species.

> Thermal spring: spring with a temperature which is higher than that of typical springs (exceeding 20°C).

> Unsaturated zone: portion of underground soil - between the ground surface and the underlying water-table, with voids which are not totally occupied by groundwater.

> Vicariance: process by means of which offspring populations separate (and possibly diverge until they become different species) through fragmentation of an area in which an ancestral population already existed.

> Vulnerability (of aquifers): intrinsic capacity of aquifers for being subjected to pollution.

> Water-table: the upper limit of the portion of the ground wholly saturated with water, which gravity enables to flow freely through underground pores and fractures.

## List of species

*Abyssidrilus cusps* - 51  
*Acanthocyclops* - 55, 56  
*Acanthocyclops agamus* - 56, 76  
*Acanthocyclops gordani* - 56  
*Acanthocyclops kieferi* - 56  
*Acanthocyclops troglophilus* - 56, 73  
*Aktedrilus ruffoi* - 52  
*Alzoniella* - 49, 74, 76  
*Anthrobathynella stammeri* - 59, 75  
*Arganiella* - 76  
*Asellus aquaticus* - 61  
*Asellus cavernicolus* - 61  
*Atrioplanaria* - 46  
*Atrioplanaria morisii* - 46  
*Atrioplanaria racovitzai* - 46  
*Attheyella crassa* - 137  
*Attheyella paranaphthalica* - 75  
*Avenionia* - 76  
*Bathynella* - 59  
*Bathynella skopljensis* - 59, 73  
*Belgrandia* - 49  
*Belgrandia stochi* - 49, 73  
*Bogidiella* - 65, 77  
*Bogidiella albertimagni* - 65, 75  
*Bogidiella aprutina* - 65  
*Bryocamptus echinatus* - 136, 137  
*Bubalocerus* - 47  
*Bythinella* - 49  
*Bythiospeum* - 49, 74  
*Bythiospeum calepii* - 111  
*Caecidotea stygia* - 137  
*Carinurella* - 66  
*Carinurella paradoxa* - 66, 74  
*Cernosvitoviella* - 51, 52  
*Chthonasellus* - 62  
*Chthonasellus bodoni* - 62  
*Congerina kusceri* - 117  
*Cypria cavernae* - 52, 53, 73  
*Dendrocoelum collinii* - 46  
*Dendrocoelum italicum* - 46  
*Diacyclops* - 56, 75  
*Diacyclops charon* - 56  
*Diacyclops group languidoides* - 75  
*Diacyclops languidus* - 74, 75  
*Earthworm* - 50  
*Elaphoidella* - 57, 59, 74  
*Elaphoidella elaphoides* - 58  
*Elaphoidella pseudophreatica* - 57  
*Eucyclops* - 56  
*Eucyclops graeteri* - 56  
*Fissuria* - 49, 76  
*Flatworms* - 46  
*Gallasellus* - 62  
*Gammarus* - 111  
*Graeteriella* - 55, 56  
*Graziana* - 49  
*Haber* - 51  
*Haber monfalconensis* - 51  
*Hadzia* - 50, 65, 107  
*Hadzia adriatica* - 65  
*Hadzia fragilis* - 73  
*Hadzia fragilis stochi* - 65, 101  
*Hadzia minuta* - 65, 76  
*Hadziella* - 49, 74  
*Hadziella ephippiostoma* - 47  
*Halicyclops* - 56  
*Hauffenia* - 47, 74  
*Hauffenia subpiscinalis* - 48  
*Hauffenia tellinii* - 49  
*Heleobia* - 95  
*Hexabathynella* - 59  
*Higginsia ciccaresei* - 46, 76, 84  
*Hispanobathynella* - 59  
*Iglica* - 49, 74  
*Iglica vobarnensis* - 48  
*Ilvanella* - 77  
*Ilvanella inexpectata* - 65  
*Ingolfiella* - 77  
*Ingolfiella (Tyrrhenidiella) cottarellii* - 67  
*Islamia* - 49, 76  
*Islamia cianensis* - 49  
*Istriana* - 49  
*Istriana mirnae* - 73  
*Lessinocamptus* - 57, 74, 75  
*Limnosbaena finki* - 60  
*Longigammarus* - 65  
*Longigammarus planasiae* - 65  
*Marifugia* - 50  
*Marifugia cavatica* - 50, 73  
*Megacyclops* - 55  
*Meridiobathynella* - 59  
*Metacrangonyx ilvanus* - 67, 77  
*Metacyclops* - 56  
*Metacyclops gasparoi* - 56  
*Metacyclops postojnae* - 56  
*Metaingolfiella mirabilis* - 67, 76  
*Microcerberus* - 75  
*Microcerberus ruffoi* - 62  
*Microcharon* - 62, 75  
*Microcharon marinus* - 62  
*Microcharon novariensis* - 62  
*Monodella stygicola* - 60, 76, 84  
*Monolista* - 53, 63, 74, 87  
*Monolista julia* - 63  
*Monolista pavani* - 63  
*Monolista racovitzai* - 40, 63  
*Monolista schottlaenderi* - 40, 63  
*Moraria* - 57  
*Morariopsis* - 57, 73  
*Morariopsis aff. scotenophila* - 102  
*Muceddina multispinosa* - 56, 84  
*Mussel shrimp* - 52, 53  
*Niphargus* - 64, 65, 66, 83, 87, 101  
*Niphargus aquilex* - 66, 137  
*Niphargus bajuvaricus* - 66  
*Niphargus bajuvaricus grandii* - 64, 66, 75  
*Niphargus costozze* - 64  
*Niphargus dolenanensis* - 66  
*Niphargus forelli* - 73  
*Niphargus group kochianus* - 66  
*Niphargus group longicaudatus* - 66, 74, 75  
*Niphargus group orcinus* - 66  
*Niphargus group speziae* - 66  
*Niphargus italicus* - 66, 74  
*Niphargus krameri* - 73  
*Niphargus longicaudatus* - 64  
*Niphargus longidactylus* - 74  
*Niphargus pescei* - 64  
*Niphargus poianoi* - 76, 92, 93, 95  
*Niphargus pupetta* - 66, 74  
*Niphargus similis* - 73, 113  
*Niphargus spinulifemur* - 73  
*Niphargus stefanelii* - 66  
*Niphargus steueri* - 43, 66  
*Niphargus stochi* - 73  
*Niphargus strouhali* - 73  
*Niphargus stygius* - 66, 73, 74  
*Niphargus transitivus* - 64, 66, 74  
*Niphargus tridentinus* - 66  
*Nitocrella* - 57, 59  
*Nitocrella pescei* - 58, 109  
*Nitocrella psammophila* - 92, 93  
*Nitocrella stammeri* - 75  
*Nitocrella stochi* - 73  
*Olm* - 41, 69, 70, 71, 73, 83, 88, 101, 105, 107, 117  
*Opossum shrimp* - 60  
*Orientalina* - 49, 76  
*Paladilhia hungarica* - 117  
*Paladilhiosis* - 49, 74  
*Paladilhiosis robiciana* - 49  
*Paladilhiosis virei* - 48  
*Paracyclops imminutus* - 42  
*Paramorariopsis* - 57, 74, 75  
*Parapseudoleptomesochra* - 59  
*Parastenocaris* - 59, 137  
*Parastenocaris germanica* - 137  
*Parvidrilus spelaus* - 51  
*Pauluccinella* - 49, 76  
*Pezzolia* - 49  
*Phreatica* - 49  
*Phreatica bolei* - 49  
*Polycelis benazzii* - 46

- Prawn - 41  
*Proasellus* - 61, 74, 111  
*Proasellus acutianus* - 61  
*Proasellus adriaticus* - 62  
*Proasellus amiterminus* - 62  
*Proasellus cavaticus* - 61, 74  
*Proasellus diana* - 62  
*Proasellus faesolanus* - 62  
*Proasellus franciscocoli* - **61**  
*Proasellus* group *deminutus* - 61  
*Proasellus intermedius* - 74  
*Proasellus ligusticus* - 61  
*Proasellus patrizii* - 61, 77  
*Proasellus pavani* - 61  
*Proteus anguinus* - 41, 69, **70**, 73, 117  
*Proteus anguinus parkelj* - 71  
*Pseudavenionia* - 49  
*Pseudectinosoma* - 57, 76  
*Pseudectinosoma galassiae* - 57  
*Pseudectinosoma reductum* - **58**, 109  
*Pseudolimnocythere* - **53**, **92**, 93  
*Pseudolimnocythere hypogea* - 53, 76  
*Pseudoniphargus* - 77  
*Pseudoniphargus adriaticus* - 67  
*Pseudoniphargus africanus italicus* - 67  
*Pseudoniphargus duplus* - 77  
*Pseudoniphargus planasiae* - 67  
*Pseudoniphargus sodalis* - 67  
*Rhyacodrilus* - 51  
*Rhyacodrilus dolcei* - 51  
*Rhyacodrilus gasparoi* - 51  
*Salenthydrobia ferreri* - 76  
*Salentinella* - 66, 107  
*Salentinella angellieri* - 66, **67**  
*Salentinella franciscocoli* - 66  
*Salentinella gracillima* - 66, 76  
*Sardhoratia* - 49, 76  
*Sardobathynella* - 59, 76  
*Sardopaladilhia* - 49, 76  
*Scutariella* - 47  
 Shrimp - 47  
*Simplicaris* - 59  
*Sketodrilus flabellisetosus* - 52  
*Speleaeomysis* - 60, 76  
*Speleaeomysis bottazzii* - **60**, 61  
*Speocyclops* - **54**, 55, 56  
*Speocyclops infernus* - 56  
*Sphaeromicola* - 53  
*Sphaeromicola sphaeromicola* - 53  
*Sphaeromicola stammeri* - 53  
*Sphaeromides* - 50, 53  
*Sphaeromides virei* - 62, **73**  
 Spring snail - 47  
*Stenasellus* - 77  
*Stenasellus assorgiai* - 62  
*Stenasellus nuragicus* - 62  
*Stenasellus racovitzai* - 62  
*Stygiomysis* - 60, 76  
*Stygiomysis hydruntina* - 61, **76**, **84**  
*Synurella* - 111  
*Tethysbaena argentarii* - 60, 77  
*Tethysbaena siracusae* - 60, 77  
*Trapezicandona stammeri* - 53, 76  
*Trichodrilus tenuis* - 137  
*Troglocaridicola* - 47  
*Troglocaris* - 47, 68, 73  
*Troglocaris anophthalmus* - **68**  
*Troglocaris planinensis* - 68  
*Troglochaetus beranecki* - 49, **50**  
*Troglodiptomus sketi* - 55, 73  
*Troglomysis* - 60  
*Tubifex pescei* - 51  
*Typhlocaris* - 41, 68  
*Typhlocaris salentina* - **68**, 76, 84  
*Typhlocirolana* aff. *moraguesi* - 62, 77  
*Typhlocirolana moraguesi* - 62  
*Tyrrhenogammarus* - 65, 77  
*Tyrrhenogammarus catacumbae* - 65, 77  
*Tyrrhenogammarus sardous* - 65  
 Woodlice - 61

We would like to thank Tiziana Di Lorenzo, Barbara Fiasca, Dina del Tosto and Paola De Laurentiis, passionate researchers of the University of L'Aquila, who provided us with unpublished data and who are battling, with us, for enhancing awareness of the ecological importance and dignity of groundwater. Thanks are also due to Enrico Gattone and Gianfranco Tomasin, for contributing to complex biological sampling campaigns throughout Italy and whose results have enabled us to express the concepts of this book.

Some drawings have been modified from those published in the quoted volumes by Celico and Civita.

The authors assume full responsibility for any errors or omissions in the text.

This volume was produced with funds from the Italian Ministry of the Environment and Territorial Protection

Printed in July 2008  
by Graphic Linea Print Factory - Udine

Printed in Italy

