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ECOLOGICAL STUDIES OF EPIKARST COMMUNITIES IN ALPINE AND PRE-ALPINE CAVES

DISSERTATION

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UNIVERZA V NOVI GORICI
FAKULTETA ZA PODIPLOMSKI ŠTUDIJ

EKOLOŠKE RAZISKAVE EPIKRAŠKIH ZDRUŽB V JAMAH ALPSKEGA IN PREDALPSKEGA SVETA

DISERTACIJA

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I declare that this thesis is exclusively my own work.

Izjavljam, da je doktorsko delo v celoti moje avtorsko delo.

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ABSTRACT

The karst ecosystem shows heterogeneity and variability of geology, hydrology, morphology and ecology in space and time. Chemical composition of water in karst plays a crucial role not only in dissolution of karst rocks and deposition but also in ecological processes. The heterogeneous nature of karst aquifers leads to difficulties in predicting groundwater flow and contaminant transport direction and travel times. For its position at the top of karst, epikarst represents the interface between surface and underground. Epikarst ecology, biodiversity and fauna have rarely been systematically studied. The typical absence of enterable passages makes necessary an indirect approach.

In this research, epikarst biodiversity in relation to environmental conditions was studied in seven Alpine and Pre-Alpine caves, located at different altitudes, in Slovenia and in Italy: Snežna jama na planini Arto (1556 m a.s.l.), Jama pod Babjim zobom (860 m a.s.l.), Zadlaška jama (300 m a.s.l.) and Pološka jama (500 m a.s.l.), in Slovenian Alps and Pre-alps, and Grotta A del Ponte di Veja (600 m a.s.l.), Covolo della Croce (875 m a.s.l.) and Grotta di Roverè Mille (1005 m a.s.l.) in Lessinian Massif. In these caves, percolating water was monthly monitored for fauna in the period of one year. Temperature, discharge, conductivity and pH were measured at the same time, and water samples for the laboratory analyses of total hardness, concentrations of various ions (calcium, chlorine, nitrate, sulphate and phosphate) and dissolved organic carbon were taken.

66 aquatic and terrestrial, mostly hypogean, species were found. Aquatic fauna was dominant in all caves, with the exception of Covolo della Croce. Caves located at higher elevations harbour less diverse communities. No animals were found when discharge was very low and in correspondence of moonmilk formations. The most abundant and diverse group was Copepoda, with thirteen different species and 776 individuals at different maturity stadia, totally representing 61% of the community (between two and three copepod species per cave in Slovenia and between one and six per cave in Italy).

The 62% of copepod species were stygobionts. Some copepod species (e.g.

Speocyclops infernus) have wide ecological tolerance to environmental variables, other have more restricted tolerances (e.g. *Lessinocamptus insoletus*). With regard of copepods, there was a good agreement between the number of observed species and the total number of predicted species, confirming that the sampling method was appropriate and sampling complete. Correlations between the copepod community structure and measured parameters, obtained with Pearson correlation coefficient and principal component analysis (PCA) show different patterns. Presence of copepods was positively correlated with conductivity and dissolved organic carbon in Grotta di Roverè Mille, and with carbonate and nitrate in Pološka jama. In other caves not so high correlations were found between copepod species and measured parameters and between pairs of species.

Investigations of the Alpine and Pre-alpine epikarst fauna can help to understand better the ecology of the epikarst fauna and its roles within the large range of different shallow subterranean habitats.

Keywords: epikarst, Alpine and Pre-Alpine caves, percolating water, speleobiology, fauna.

IZVLEČEK

Kraški ekosistem je glede na geološke, hidrološke, morfološke in ekološke značilnosti heterogen in variabilen v prostoru in času. Kemizem vode odigra pomembno vlogo pri raztapljanju in odlaganju kamnine, kot tudi pri ekoloških procesih. Heterogenost kraškega vodonosnika otežuje natančno poznavanje smeri podzemnega toka, prenosa onesnažil in njihovega časa potovanja. Epikras, ki je plitvo pod kraškim površjem, predstavlja vmesno plast med površjem in podzemljem. Ekologija, biodiverziteta in favna epikrasa so bile redko sistematično proučevane. Zaradi neposredne nedostopnosti je potreben indirekten pristop pri njegovem proučevanju.

Epikraška biodiverziteta je bila proučevana v jamah Alpskega in Predalpskega sveta v povezavi z okoljskimi dejavniki. Vzorčenje je potekalo v sedmih jamah na različnih nadmorskih višinah v Sloveniji in Italiji: Snežna jama na planini Arto (1556 m nad. v.), Jama pod Babjim zobom (860 m nad. v.), Zadlaška jama (300 m nad. v.) in Pološka jama (500 m nad. v.), vse v Alpskem in Predalpskem svetu Slovenije, ter v italijanskih Predalpah Lessinia v jamah Grotta A del Ponte di Veja (600 m nad. v.), Covolo della Croce (875 m nad. v.) in Grotta di Roverè Mille (1005 m nad. v.). V jamah smo eno leto z mesečnimi vzorčenji spremljali favno v prenikli vodi. Istočasno so bile opravljene meritve temperature, pretoka, prevodnosti, pH ter odvzeti vzorci za laboratorijske analize celokupne trdote, koncentracij različnih ionov (kalcij, klorid, nitrat, sulfat in fosfat) in raztopljenega organskega ogljika.

Najdenih je bilo 66 vodnih in terestičnih vrst, večina hipogeičnih. Prevladovala je vodna favna, z izjemo favne v jami Covolo della Croce. Jame na višjih nadmorskih višinah imajo v splošnem nižjo diverziteto. Brez živali so bili vzorci pri zelo nizkih pretokih in v povezavi vzorčnega mesta z jamskim mlekom. Najštevilčnejša in najpestrejša skupina so bili ceponožni raki, z ugotovljenimi trinajstimi vrstami in 776 osebki v različnih razvojnih stopnjah, ki so celokupno predstavljali 61% delež (ugotovljene dve do tri vrste v posamezni jami v Sloveniji ter ena do šest vrst v posamezni jami v Italiji). Med ceponožnimi raki je bilo 62% stigobiontskih vrst. Nekatere vrste (npr. Speocyclops infernus) imajo širok ekološki razpon pojavljanja v različnih okoljskih pogojih, med tem ko druge zahtevajo bolj specifične pogoje (npr. *Lessinocamptus insoletus*). Ugotovljena je bila visoka skladnost med številom najdenih ceponožnih vrst in številom predvidenih vrst, kar potrjuje, da je bila metoda vzorčenja primerno izbrana in vzorčenje popolno. Glede soodvisnosti posameznih vrst ceponožnih rakov in okoljskimi parametri, analiziranimi s pomočjo Pearsonovega koeficienta korelacije in metode glavnih komponent (PCA), obstajajo razlike. Združba ceponožnih rakov je bila v značilni pozitivni soodvisnosti s prevodnostjo in raztopljenim organskim ogljikom v jami Grotta di Roverè Mille, ter s koncentracijami karbonatov in nitratov v Pološki jami. V drugih jamah soodvisnosti med vrstami ceponožnih rakov in ekološkimi parametri niso bile v statistično značilni korelaciji.

Raziskave visokogorske epikraške favne lahko pripomorejo k celovitejšemu razumevanju ekologije epikraške favne in njene vloge v različnih plitvih podzemeljskih habitatih.

Ključne besede: epikras, jame Alpskega in Predalpskega krasa, prenikajoča voda, speleobiologija, favna.

ABBREVIATIONS USED IN THE TEXT

CC = Covolo della Croce
CN = Cadaster Number
CV = Coefficient of Variation
DOC = Dissolved Organic Carbon
JPBZ = Jama pod Babjim zobom
PCA = Principal Component Analysis
r = Pearson Correlation Coefficient
PJ = Pološka jama
PV = Grotta A del Ponte di Veja
RM = Grotta di Roveré Mille
SD = Standard Deviation
SJ = Snežna jama
p = Student’s t-test
ZJ = Zadlaška jama

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1. INTRODUCTION

1.1. Epikarst function in karst aquifers

Over 94% of the world unfrozen freshwater is stored underground (Heath 1982). In Europe, where carbonate rocks cover 35% of the surface, groundwater from karst aquifers is an especially important water resource (Ravbar 2007).

Karst aquifers can be divided into several units with different hydrological properties and flow regimes (Fig. 1.1). Precipitation represents the basis of the formation of the karst system's input function (Petrič 2002). Some precipitation enters lakes and streams, some quickly evaporates, but some also infiltrates into the soil. In turn, some of this infiltrating water moves vertically into groundwater or caves. The word *recharge* is used to describe this process. Water enters the subterranean karst system at the rock-soil interface, which typically has many small solution pockets and cavities with complex horizontal and vertical pathways - the *epikarst* (Ford and Williams 2007, Williams 2008).

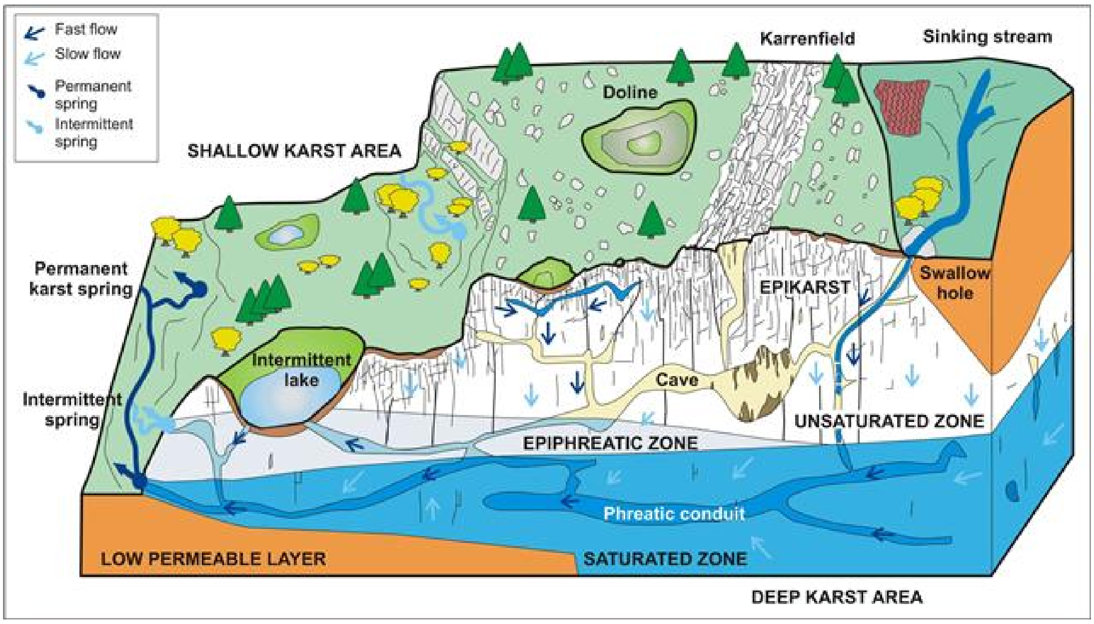

Figure 1.1: *Conceptual model of water flow in a karst aquifer system. Arrows indicate direction of water flow. From Ravbar (2007).*

The metaphor of Bakalowicz (2004) of epikarst as the skin of karst captures the essence of this boundary zone. According to a recent definition, epikarst is: »located within the vadose zone and is defined as the heterogeneous interface between unconsolidated material including soil, regolith, sediment, and vegetative debris, and solutionally altered carbonate rock that is partially saturated with water and capable of delaying or storing and locally rerouting vertical infiltration to the deeper regional phreatic zone of the underlying karst aquifer« (Jones *et al*. 2004). Epikarst retains water considerably above the water table. An important characteristic of epikarst is its heterogeneity, with many semi-isolated solution pockets whose water chemistry is also quite variable (Musgrove and Banner 2004, Pipan 2005, Williams 2008). Water dripping in caves from the narrow fissures has a lower discharge and more uniform chemistry (Musgrove and Banner 2004, Zambo 2004) and waters emerging from larger conduits can have highly variable chemistry (Raeisi *et al*. 2007). It is related to permanence time in conduits that is higher in small conduits and lower in large ones, where residence time (as short as hours or days), is in general insufficient for chemical equilibrium (Langmuir 1971). The highly heterogeneous nature of karst aquifers leads to difficulties in predicting groundwater flow and contaminant transport direction and travel times.

1.2. Importance of ecohydrological studies in karst areas

Ecohydrology can be defined as the science of integrating hydrological processes with biota dynamics over varied spatial and temporal scales (Bonacci *et al.* 2009). Smart and Worthington (2004) stress that in karst environments, water tracing finds particular value in defining the path followed by inaccessible underground streams, which could be the dispersal paths of organisms as well. For development of karst ecohydrology, experience obtained with the long history of use of subterranean fauna as groundwater tracers can be very useful (Kranjc 1997, Käss 1998, Pipan and Culver 2007a).

The karst system shows extreme heterogeneity and variability of geologic, morphologic, hydrogeologic, hydrologic, hydraulic, ecological and other parameters in time and space. The effects of living organisms on karst geomorphology are profound at an ecosystem scale, and they are widespread, intense, diverse and of fundamental importance (Tabaroši 2002, Danielopol *et al.* 2003). The evolution of entire karst landscapes is thought to be biologically controlled through the interrelationship of vegetative cover, erosion and dissolution rates (Hupp *et al.* 1995). On the other hand, chemical composition of water in karst plays a crucial role not only in dissolution and deposition but also in ecological processes (Pipan 2003, 2005, Bonacci *et al.* 2009).

Such a complex system needs interdisciplinary approach. It is highly important to understand the interaction of groundwater and surface water in karst and their influence on surface and underground biological processes. For understanding changes of underground faunas, simultaneous monitoring of many factors (e.g. temperature, pH, dissolved oxygen, nutrient inputs, quality and quantity of water recharge and flow etc.) is needed (Bonacci *et al.* 2009).

Knowledge of basic concepts about surface and underground karst landforms and ecology in karst terrains is fundamental to an integrated sustainable management of very valuable and vulnerable karst biological and water resources. Cooperation between hydrology and ecology (ecohydrology) could help in solving many critical problems dealing with sustainable development and ecosystem management.

1.3. Subsurface habitats and epikarst communities

The evolution of particular karst areas can be, beside their geological, geomorphological and hydrological characteristics, reflected also in specific distributional patterns and diversity of the fauna.

The subterranean domain includes both, air- and water-filled underground habitats that share several key features: permanent absence of light, and consequent absence of primary productivity (except for rare cases of prevalent chemoautotrophy) and reduced environmental variability relative to surface conditions (Culver and Pipan 2007).

Subsurface habitats can be divided into large cavities (caves), small cavities (interstitial habitats) and superficial (or shallow) subterranean habitats (SSHs) (Culver and Pipan 2009a). Epikarst is included among SSHs. Ford and Williams (2007) indicate that the epikarst, where the karst rock features (fissure, conduits, cracks etc.) are best developed, is generally 3-10 m deep but it can reach up to 30 m.

It is an aerated and partially saturated habitat, with a considerable storage capacity (Williams 1983), where the water generally flows vertically to the epiphreatic. Even though epikarst water is only a few meters below the surface, its residence time may be months or even more than a year (Williams 2008, Hu *et al*. 2015), buffering variations in temperature and chemical composition of underground water relative to surface water (Kogovšek 1990, Culver and Pipan 2009b). Epikarst performs hydrological functions, acting as a sponge, soaking up water during wet periods and releasing it during dry periods. This zone represents a large reservoir where the water can be stored for a certain period of time, which may be important for survival of many species living in it. It is possible to indirectly explore this habitat with sampling of dripping water in caves (Pipan 2005). Drip rates of percolation water in caves are seasonally variable dependent on rainfall but displaying a lagged response and do not respond to all types of precipitation. After long-lasting dry periods, depending on air temperature, soil moisture and vegetation, the water will manage to sink from the surface to the cave only if the rainfall is of the order of 50 mm or more. In wet periods, even the water resulting from rainfall of less than 10 mm manages to penetrate (Kogovšek and Habič 1980, Kogovšek 1990, Baker and Brundson 2003, McDonald and Drysdale 2007). In this conditions water flow within this zone very often displays a significant lateral component.

Karst ecosystem analysis should be focused on the flow of energy and the cycling of nutrients through biotic and abiotic components of the system. All of the energy is transferred from surface habitats to underground. This reliance on external (allochthonous) energy sources generally means that there are fewer energy resources available in subterranean habitats and diversity of energy resources are low (Culver and Pipan 2009a). External energy sources enter subterranean habitats in a variety of ways: wind and gravity, active movement of animals, roots, flowing water (transporting not only dissolved, but also particulate organic material), percolating water. Despite many deep subterranean aquatic habitats are energy-poor, many superficial subterranean habitats have significant amounts of carbon (Simon *et al.* 2007a). Dripping water (the most important source of organic matter in many caves) carries dissolved organic matter, some suspended particles of organic matter, and a variety of microbes, meiofauna, small particles of soil and invertebrates (Gerič *et al.* 2004, Pipan 2005, Culver and Pipan 2009a). The amount of particulate organic carbon from these sources is usually much less than that of DOC in percolating water (Gibert 1986, Simon *et al.* 2007a). DOC is important in forming biofilms (microorganisms, extracellular polysaccharides, and particles, both organic and inorganic which cover the surface of rocks and sediments in subterranean habitats), the base of the aquatic invertebrate food web in subsurface water (Simon *et al.* 2003, Boston 2004). The overall flux of organic carbon via percolating water is both spatially and temporally variable perhaps as the result of different residence times of the water in soil and epikarst (Ban *et al.* 2006, Simon *et al.* 2007b).

The distribution and the development of the present subterranean fauna depend on geomorphologic and hydrologic conditions since the Pleistocene (La Greca 1955), when climate and environmental changes occurred, affecting the colonization of subterranean environment by some species and reshaping the distribution of the species that colonized this environment before the Pleistocene. A wide variety of organisms that are found in different hypogean habitats have developed special convergent adaptations (e.g. reduced or absent eyes and pigment, appendage lengthening, and an elaboration of extra-optic sensory structures) (Culver and Pipan 2009a). Recent studies on cryptic diversity have revealed high local diversity and endemism in groundwater, and showed that species with large ranges are extremely rare (Zakšek *et al.* 2009). It seems that more habitats and the greater food supply give more opportunities for niche separation and coexistence between species.

Fauna from epikarst can be considered island-like from an evolutionary and ecological point of view (Culver and Pipan 2008); much more if the karst area is located in mountains, where horizontal migration of individuals (and genetic material) is limited. Each hydrological unit within a certain geological block (an equivalent to watershed) thus functions as an island with its own fauna (Brancelj and Culver 2005, Culver and Pipan 2008). Epikarst is both an exceptionally diverse and environmentally heterogeneous habitat where both aquatic and terrestrial species can be routinely collected. Animals collected in dripping water in caves are the ones that have been swept out of their primary habitat above the cave passage, i.e. the epikarst (Pipan 2005), although some active vertical movements through the unsaturated zone are probable.

1.4. Short history of biological studies of epikarst

Until the 20th century the epikarst zone was completely unknown to biologists and was not identified as a separate habitat (Culver and Pipan 2014). By the mid-20th century, several biologists, such as Petkovski (1959), became aware that there were stygobiotic copepods in caves with only percolating water. He recognized that the accumulation of water from above depended on fractured rock, and that there was water in tiny fissures and cracks which slowly flowed down from the ceiling. He believed that this habitat was the realm of some copepods like *Speocyclops* and many harpacticoids. Thus he didn't recognize the infiltration zone as a habitat per se, but a source of water that filled small depressions in walls.

Holsinger (1971) came to similar view with respect to a population of the amphipod *Crangonyx antennatus* living in Molly Waggle Cave in Virginia. Part of the population was in an old trough used for saltpeter mining during the American Civil War. He concluded that the only way the individuals could have got there was via what we would now call epikarst, but he reviewed it more as dispersal corridor than a habitat with a sustainably reproducing population.

Rouch (1968) recognized that the small number of individuals occurring in pools in Grotte de Sainte-Catherine (France) was much too small to constitute a viable population and concluded that there were populations in perched, i.e. epikarst, aquifers. Rouch had an advantage of collaborating with Mangin, one of the first discovers of epikarst (Mangin 1973).

Brancelj (2002) discovered a rich copepod fauna in drip pools in the shallow Slovenian cave Velika Pasica, but with relatively few reproducing individuals. Although he did not use the phrase epikarst, the cave is so shallow that most of the ceiling is epikarst. He held that reproduction was not occurring in the cave but in crevices in the cave ceiling and walls.

Pipan (2003, 2005) championed the idea that there was an epikarst habitat and fauna distinct from other subterranean habitats. Epikarst biology has been most thoroughly studied in central Slovenia, especially the Postojna Planina Cave system (Pipan 2005, Pipan and Culver 2007b, c, Kogovšek 2010). Culver and Pipan (2011) argued that epikarst was one of several aquatic shallow subterranean habitats each of which harbors a unique, troglomorphic, stygobiotic fauna. The diversity of organisms in epikarst is remarkable. Numerous specimens, belonging to diverse taxonomic groups, mostly but not exclusively aquatic, have been collected from percolating water in many karst caves. The most common and most abundant (in term of species richness and numerically) metazoans in the epikarst are copepods (Pipan 2005).

1.5. Copepods

The subclass Copepoda belongs to the crustacean class Maxillipoda. It is the most diversified group of crustaceans, comprising around 14.000 species, 2.280 genera, 210 families and 10 orders. The number of copepods is surely underestimated and new species are being discovered continually. During their long evolutionary history, starting in the lower Cretaceous, copepods spread over all the continents, as well as they successfully colonized all the available water habitats of the planet (Galassi 2001, Mori and Brancelj 2008, Rouch 1994). Copepods are a major component of the fauna of nearly every freshwater habitat, both surface and subterranean. Four orders of copepods live in fresh groundwater: Calanoida, Cyclopoida, Gelyelloida and Harpacticoida (Galassi *et al.* 2009). In Europe, where subterranean copepods are best studied, approximately half of the freshwater species are stygobiotic copepods (Galassi *et al.* 2009). Up to seven species of stygobiotic copepods have been found in a single drip in Pivka jama section of the Postojna Planina Cave system. Overall in Pvka jama, eight stygobiotic and three generalist copepod species have been found (Pipan 2005). Stygobiotic copepods are tiny aquatic dwelling species (ranging in size from 0.3 to 2.0 mm) that share morphological modifications for subterranean life as reduced or absent eyes and pigment and larger eggs (Rouch 1968). For interstitial species, miniaturization and reduction of segmentation, and even number of appendages, are common themes (Brancelj 2009). Copepods, particularly harpacticoids, with their worm-like body structure and short antennule, are adapted to interstitial and epikarst life. Apart from the reduction of the visual apparatus and body depigmentation, subterranean cyclopoids exibit weak other troglomorphic features. Pesce and Galassi (1986) emphasized the importance of reduction of spinulation on proximal segments of body.Many harpacticoids are grazers of biofilms, while cyclopoids are usually predators, often of harpacticoids (Culver and Pipan 2009a). Harpacticoids tend to be more common in interstitial habitats while cyclopoids tend to be more common in cave streams (Rouch 1994). In Europe, some genera belonging to the order Harpacticoida are frequent in the epikarst zone: *Bryocamptus*, *Morariopsis*, *Parastenocaris*and *Elaphoidella* (Pipan 2005, Brancelj 2009). Culver *et al.* (2012) list the genera *Speocyclops* and *Diacyclops* (Cyclopoida) and *Elaphoidella* and *Parastenocaris* (Harpacticoida) as having more than ten species known from epikarst, but many other genera and species have been collected.

Some copepod genera found in subterranean waters are marine in origin ("*thalassiod*" species) such as *Parastenocaris*, while some others (*Diacyclops, Eucyclops, Graeteriella, Speocyclops, Bryocamptus, Elaphoidella, Moraria*) colonized a groundwater system from superficial fresh waters, during different geologic epochs (Ward and Palmer 1994, Pesce 1985). Some copepods are cosmopolitan (*Eucyclops, Paracyclops, Diacyclops, Phyllognathopus, Attheyella, Epatophanes, Canthocamptus*), while others are endemic to a particular biogeographic region. From a biogeographical point of view the distribution of the Canthocamptidae indicates a Pangean origin, and a vicariant biogeography, with indications that the most primitive groups are Holarctic-Laurasian (Wells 2007).

As for other systematic groups, a higher degree of endemism occurs among groundwater taxa, at the ordinal, familial, generic, and more frequently species taxonomic levels (Dole-Oliver *et al.* 2000, Williamson and Reid 2001).

Frequent occurrence of copepods in the epikarst water, their distribution as well as their small size make them potential tracers of water movement within small karst areas (Pipan and Culver 2007a).

1.6. Main project goals and research hypotesis

This research is an extension of the study on epikarst fauna and ecology started by Pipan in Dinaric karst in Slovenia (Pipan 2003, 2005). Sampling of dripping water and pools in six caves in Central Slovenia she deepened knowledge about epikarst ecology and copepod species richness, distribution and diversity. She found 107 copepod species, about one third of them were stygobionts and 15 endemic for Slovenia. From a geographical point of view, she found no correlation between the distances between caves and the similarity of fauna. From canonical correspondence analysis (CCA) emerged that copepods from epikarst zone show high levels of ecological specialization and separation.

This work is based upon the ecological, hydrogeological and chemical exploration of epikarst, by means of systematic and long-term sampling of percolation water in caves using similar investigation methods as described by Pipan (2003, 2005). The research is focused in particular on copepods from percolation water in Alpine and Pre-Alpine regions in Slovenia and in Lessinian Massiff in Italian Pre-Alps.

Hypothesizing a lower biodiversity, a higher level of endemism and higher specialization to underground habitats located at higher elevation, due to major isolation, the first main goal of the present study was to make an inventory of fauna, and in particular copepod species, present in selected areas, to compare Alpine and Pre-Alpine species richness and diversity, with finding from other previously studied areas as Dinaric and Isolated karst in Slovenia (Pipan 2005, Pipan *et al.* 2008), Romania (Meleg *et al.* 2011) and USA (Pipan *et al.* 2006b).

The second goal was to measure and monitor in an extended period of time (one year) selected chemical and physical parameters of dripping water to investigate the spatial and temporal variation of water in Alpine and Pre-Alpine epikarst. In other previously studied areas, spatial and temporal variability was high.

The third main goal was to deepen the knowledge about epikarst copepods preference for different niches and their ecological limits, supposing that altitude and low temperature, together with lower food supply could limit copepod dispersal.

Drip water fauna and environmental findings from this investigation provide useful information regarding epikarst characteristics, its biodiversity, its hydrology, chemistry and saturation.

2. STUDY AREA

2.1. The Alps

Investigation was carried out in seven horizontal caves located at different altitudes (from 310 m to 1,556 m above sea level), in two karst areas of Alps and Pre-Alps in Slovenia and in Italy (Fig. 2.1).

Figure 2.1: *Geographical location of Alps and position of sampling areas (blue ovals) (www.britannica.com).*

The Alps are one of the great mountain ranges of Europe, stretching from Austria and Slovenia in the east, through Italy, Switzerland, Liechtenstein and Germany, to France in the west (Fig. 2.1). This mountain system, with many peaks higher than 4,000 m a.s.l., still provides by far the largest part of natural or semi-natural environments in central Europe, in spite of the long tradition of high mountain agriculture and the increasing pressure from tourism.

The Alps offer a high variety of environments and biodiversity, mainly due to present and past climatic conditions and the particular orographic structure of the mountain system (Benniston 2005). In the Alps, the last glacial cycle covers the time from the end of the last Interglacial to the beginning of the Holocene. In the coldest period of the Würm the permanent snow line was between 1,300-1,500 m above the present see level and long valley glaciers descended down to 500 m a.s.l. (Kranjc 1984) (Fig. 2.2). Some small remnants of the glacier are still present about above 2,500 m a.s.l. (Zupan Hajna *et al.* 2008).

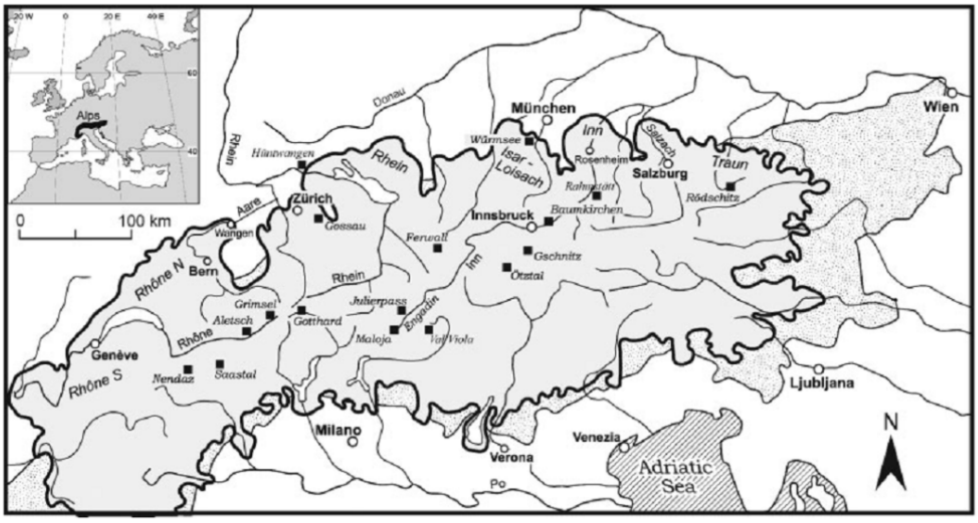

Figure 2.2: *Ice cover during last glacial period (based on Ehlers and Gibbard 2004). The extent of ice during the Last Glacial Maximum is indicated by the shaded area inside the bold line. The Alps are shown as a stippled pattern.*

The present heterogeneous climate of the Alps reflects the influence of three macroclimates: moderate continental, Mediterranean and extreme continental. The orographic barrier formed by the Alps heavily influences the climate of the southern sector of the Alps themselves, easily defended from the cold masses coming from the north. Moreover, it is clear that the masses that come from the Mediterranean Sea (wetter and mild) tend to affect more directly the southern side of the Alps smoothing out the excesses (Ehlers and Gibbard 2004). The Alps have a rainfall pattern (on average) with maximum precipitation in summer and minimum in winter (almost always snow). Spring and autumn are highly variable, with frequent thunderstorms. Climate can be locally very diverse (mainly due to heterogeneuos landform) and is influenced by the altitude, for which the temperature decreases on average 1° C every 170 m in height (www.ansa.meteo.it).

2.2. Study area in Slovenia

The Slovenian sampling area is located in Alpine areas of Gorenjska and Primorska regions, in the Eastern part of Alps. In this part of Alps precipitation is abundant, varying between 1,500 to more than 3,000 mm annually, and snow cover lasts up to 200 days (Kunaver 1983) (Tab. 2.1).

Table 2.1: *Mean maximum and minimum temperature and mean precipitation measured at Kredarica meterological station (based on thirty years mesurements: 1986-2016) (www.arso.gov.it)*

	Mean minimum temperature (°C)	**Mean maximum temperature (°C)**	**Mean precipitation (mm)**
January	-28.3	7.6	104
February	-22.8	9.4	98
March	-28.1	7.9	124
April	-17.3	9.9	152
May	-13.9	14.0	169
June	-9.6	16.3	213
July	-6.1	21.6	202
August	-6.0	18.1	228
September	-9.8	18.4	197
October	-15.6	15.0	187
November	-21.2	11.8	199
December	-25.8	9.8	120

2.2.1. Geology

The Slovenian calcareous Alps are 100 km long (from west to east) and 40 km wide (from north to south) (Kranjc 1984). Dominant rocks are Triassic limestones and dolomites, up to 1,000 m thick (Audra *et al.* 2007). The Slovenian Alps consist of two large formations: the Central Alps (Karavanke) and the Southern Limestone Alps (Julian and Kamnik-Savinja Alps). In the Limestone Alps the great majority of the terrain is built of carbonate rocks and there are a few patches of other rocks. In the Karavanke is the opposite – most of the terrain is impermeable rocks and among them there are some carbonate rocks, nearly all of Mesozoic age (Kranjc 1984).

2.2.2. Study caves

The study area includes four caves: Snežna jama, Jama pod Babjim zobom, Pološka jama and Zadlaška jama. In the short description of caves below, data are referred to the Cave Cadastre IZRK ZRC SAZU and the Speleological Association of Slovenia.

1. **Snežna jama na Planini Arto** or simply **Snežna jama** (CN: 1254) is a tourist cave located on Raduha Massif in north eastern part of the Kamnik-Savinja Alps, in the north central part of Slovenia. Snežna jama, which means "snow cave", is the largest horizontal cave (1,327 m long) on Mt. Raduha. The entrance is a shaft, opening at 1,556 m a.s.l. and leading into the first cave section – a large gallery. Permanent ice is formed in the entrance part of the cave due to cold air which flows through the shafts into the cave (Zupan Hajna *et al.* 2008).

2. **Jama pod Babjim zobom** (CN: 129) is a 359 m long tourist cave. The entrance is located at 860 m a.s.l. above the valley of the Sava river, in the west slope of the Jelovica plateau. There are no traces of Pleistocene glacial sediments or inflow into the cave, although the entire Sava valley was filled with the Bohinj glacier (Zupan Hajna *et al.* 2008). Several types of calcite crystals cover walls, wall niches and ceiling pockets.

3. **Zadlaška jama** or **Dantejeva jama** (CN: 804) is a 1,140 m long cave. The two entrances to the cave are located around 300 m a.s.l. in the south slope of Kalec hill, above the Zadlaščica River and near its confluence with the Tolminska stream, in the Julian Alps.

4. **Pološka jama** (CN: 3000) is a 10,800 m long cave. The two entrances to the cave are located around 500 m a.s.l., about 300 m south from Ravne fault on Mt. Osonica, in the Julian Alps.

2.3. Study area in Italy

The three investigated Italian caves (Grotta di Roverè Mille, Grotta A del Ponte di Veja and Covolo della Croce) are located in the southern part of the Venetian Pre-Alps, on the Lessinian Massif, in the hydrological basin of Adige river (Fig. 2.1). In the area the climate is mostly affected by Mediterranean Sea influence. Temperature in the area, in mean, ranges between -2 °C and 29 °C and annual mean precipitation is 900 mm (www.arpa.veneto.it) (Tab.2.2).

Table 2.2: *Mean maximum and minimum temperature and mean precipitation measured at Verona-Villafranca meterological station, based on thirty years mesurements; (1986-2016) (www.ilmeteo.it)*

	Mean minimum temperature (°C)	**Mean maximum temperature (°C)**	**Mean precipitation (mm)**
January	-2	5	55
February	0	9	48
March	3	13	58
April	7	17	69
May	11	22	85
June	15	26	86
July	18	29	62
August	17	28	88
September	14	24	63
October	8	18	82
November	3	11	75
December	-1	6	51

2.3.1. Geology

The Lessinian Massif comprises 691 km^2 (Boccaletti *et al.* 1990). The geological structure of the massif is dominated by carbonate rock of Cretaceous and Jurassic age in the northern part. The basement is represented by a 1,000 m deep dolomite stratum, covered by several limestone banks of different origin, reaching a thickness of about 550 m. Eocene limestone is primarily located in the southern part of the massif.

2.3.2. Study caves

In the short description of caves below, data are referred to the Cave Register of Veneto region (Federazione Speleologica Veneta).

1. **Grotta A del Ponte di Veja** or **Grotta dell'Orso** (CN: 117 V Vr) is a 180 m long cave. The entrance to the cave is located 600 m a.s.l., on the Marano Sant'Anna ridge. The cave is developed in various types of limestone: San Virgilio, Rosso Ammonitico Veronese and Biancone (Mietto and Sauro 2000). Bat colonies inhabit the cave.

2. **Covolo della Croce** or **Tana delle Sponde** (CN: 85 V Vr) is a 456 m long cave. The entrance to the cave is located 875 m a.s.l., on the ridges San Rocco – San Mauro di Saline on plateau of Velo. The cave is a part of the cave complex of Covoli di Velo.

3. **Grotta di Roverè Mille or Grotta di Monte Capriolo** or **Grotta del Sogno** (CN: 1071 V Vr) is a 240 m long tourist cave. The entrance to the cave is located 1,005 m a.s.l., in the south part of Mt. Capriolo.

2.4. Karst areas in Slovenia and in Italy and previous epikarst fauna researches in the study areas

Around 43% of the land area of Slovenia consists of carbonate rocks, mostly limestones and dolomite (Gams 2004). According to geological, hydrological and speleological characteristics the karst in Slovenia is divided into three groups (Habič 1969): (1) alpine karst (with high mountain karst, representing 17% of Slovenian karst), (2) Dinaric karst (divided in high and low karst), and (3) intermediate Dinaric−Alpine and isolated karst.

The particularly rich subterranean biodiversity in Slovenia is very well studied in Dinaric karst (Sket 1999), also with regard to epikarst fauna (e.g. Pipan 2005), some studies have been performed on percolation water fauna in a cave in isolated intermediate karst (Pipan *et al.* 2008). Some data on epikarst fauna collected in pools in caves and streams from Italian portion of Julian Alps have been published (e.g. Colla and Stoch 2002, Berera *et al.* 2005, Stoch 2008, 2011) but no data exist about stygobiont epikarst fauna of alpine karst in Slovenia.

In Italy, the main karst areas are located in Alpine regions in the north, in the Apennines Mountains, in south-eastern Italy (Puglia), in Sardinia and south Sicily (Dinelli *et al.* 2010).

Different researches on cave and epikarst fauna have been done in many karst areas along Italian territory. Many data exist about stygobiont fauna from pools and flowing water in caves in Lessinian Mountains (e.g. Caoduro *et al.* 1995, Galassi *et al.* 2009) but no long term ecological investigations were attempted until now.

This study, with a systematic investigation focused on fauna from dripping water in caves, integrates previous knowledge of Lessinian Massif epikarst fauna and provides new data from Alpine karst in Slovenia.

3. MATERIAL AND METHODS

3.1. Sampling of epikarst fauna

Epikarst zone, because of small sizes of cavities, is inaccessible using standard research methods (Pipan 2003). Epikarst fauna can only be sampled indirectly in caves by sampling pools filled with drip water or filtering percolating water. Drips, especially if sampled over extended periods of time, provide a more complete sample of communities than pools (Pipan *et al.* 2010).

Each investigated cave was monthly sampled for epikarst fauna using the continuous sampling device developed by Pipan (2003, 2005) (Fig. 3.1).

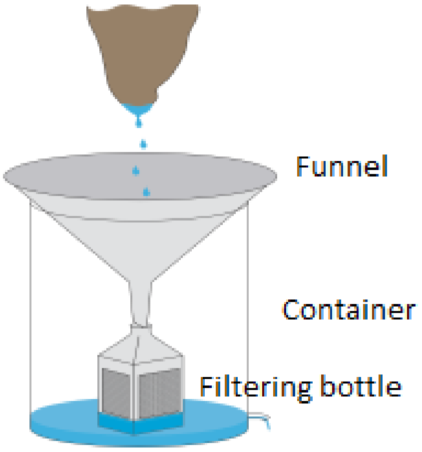

Figure 3.1: *Sketch of the sampling device. From Pipan (2003, 2005).*

Water from ceiling drips was directed via a funnel into a filtering bottle fitted on two sides with plankton netting of 60 µm mesh size. The filtering bottle was placed within a sampling container. Each sampling container had a drain 3 cm from its base such that a small amount of water remained in the container while the most of the water passed through the filtration unit and flushed out. In the filtering bottle collected animals are preserved alive in the small amount of water to the next sampling date.

Research for epikarst fauna in Slovenia started in autumn 2006 and was finished in autumn 2008. In Table 3.1 sampling periods for fauna of each Slovenian cave are shown.

Table 3.1: *Study periods of epikarst fauna sampling in caves in Slovenia.*

Cave name	**Devices placed**	**Devices removed**
Snežna jama na planini Arto	September 2006 (SJ3 was set in December 2006)	November 2007
Zadlaška jama	October 2006	December 2007
Pološka jama	December 2006	July 2008
Jama pod Babjim Zobom	August 2007	November 2008

Sampling for fauna in Italian caves started in autumn 2007 and last samples were collected in winter 2008. Sampling periods for fauna of Italian caves are shown in the Table 3.2. Grotta A del Ponte di Veja was investigated for one year, Grotta di Roverè Mille and Covolo della Croce only six months.

Table 3.2: *Study periods of epikarst fauna sampling in caves in Italy.*

Cave name	**Devices placed**	**Devices removed**
Grotta A del Ponte di Veja	October 2007	December 2008
Covolo della Croce	April 2008	December 2008
Grotta di Roverè Mille	April 2008	October 2008

3.1.1. Location of sampling sites in caves

In each of the four caves inthe sampling area in Slovenia (Fig. 2.1) five sampling devices were placed (Figs. 3.2-5) and monitored for fauna and physio-chemical characteristics of dripping water.

- In **Snežna jama na planini Arto** devices were located in first 500 m of the cave as shown in figure 3.2. To test the presence of fauna in water near ice, the first device (SJ1) was placed close to the permanent ice pillar, and SJ2 and SJ3 were placed in the seasonally icy part of the cave. One sampling device (SJ4) was positioned under deposits of moonmilk (a precipitate from limestone comprising aggregates of fine crystals of varying composition, probable result of bacterial action, Hill and Forti 1997) to test its possible effect on fauna.

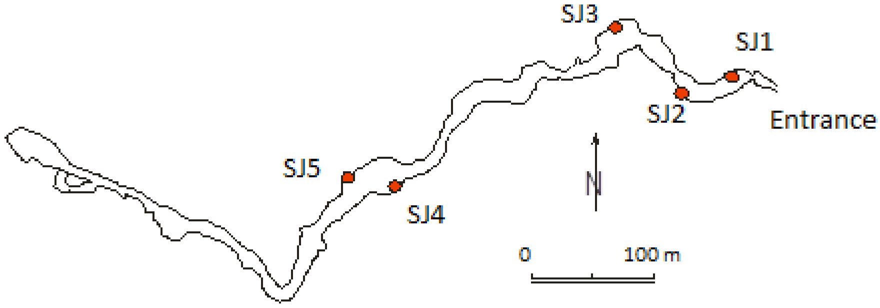

Figure 3.2: *Map of the cave Snežna jama na planini Arto, with marked locations of sampling sites.*

- In **Jama pod Babjim zobom** (Fig. 3.3) sampling devices were placed all along the cave, from 70 m to 240 m from the entrance.

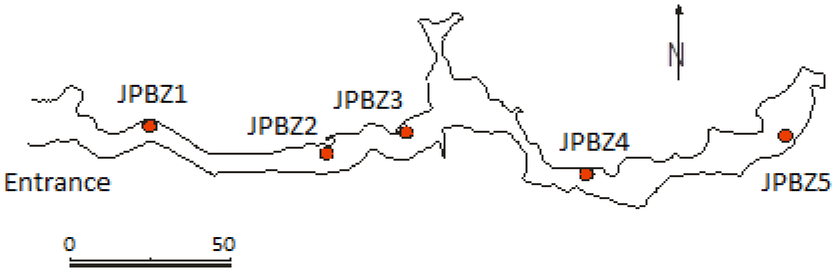

Figure 3.3: *Map of the cave Jama pod Babjim zobom, with marked locations of sampling sites.*

- In **Zadlaška jama** sampling devices (ZJ1, ZJ2, ZJ3, ZJ4 and ZJ5) were located in the cave, as illustrated in Figure 3.4, from 40 m to 200 m to the entrance. The cave is generally very dry and sampling devices were placed in sites where drips were found to be more abundant.

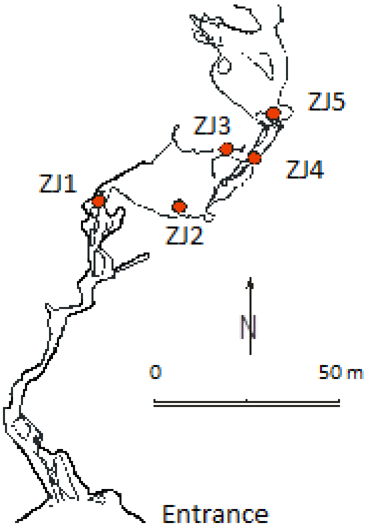

Figure 3.4: *Map of the cave Zadlaška jama, with marked locations of sampling sites.*

- In **Pološka jama** sampling devices (PJ1, PJ2, PJ3, PJ4 and PJ5) were located in the first part of the cave (up to around 100 m inside the upper entrance to the cave) as illustrated in the map of the cave below (Fig. 3.5). Sampling sites were chosen for heterogeneous water discharge.

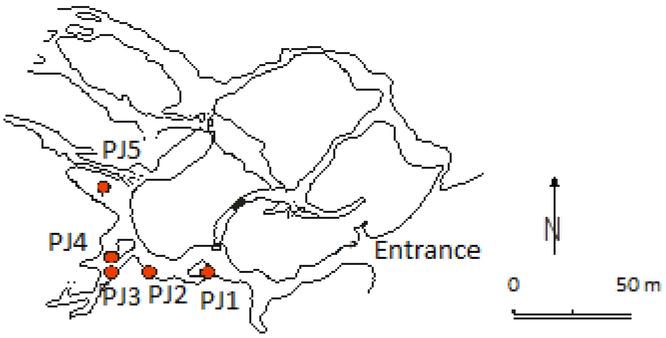

Figure 3.5: *Map of part of the cave Pološka jama, with marked locations of sampling sites.*

Sampling in caves in Italy was performed in collaboration with colleagues from the Museum of Natural History of Verona. In each cave from three to five sampling devices were placed and monitored for fauna and physico-chemical characteristic of dripping water.

- In **Grotta A del Ponte di Veja** five sampling devices were located all along the cave (Fig. 3.6), from 50 to 130 m from the entrance. Because of very low discharge sampling was not always possible.

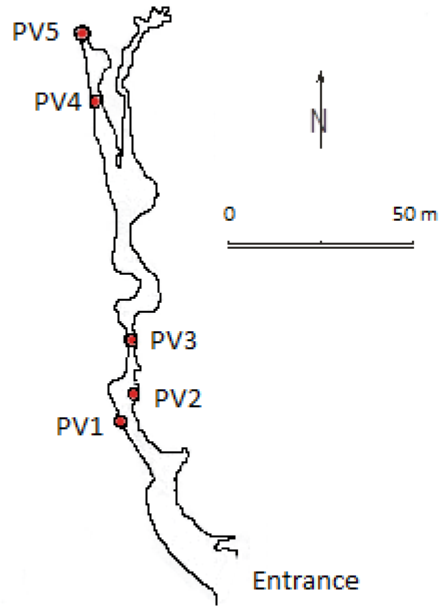

Figure 3.6: *Map of Grotta A del Ponte di Veja, with marked locations of sampling sites.*

- In **Covolo della Croce** due to difficolt accessibility of the other part of the cave, only three sampling devices were located in the first hall of the cave, at less than 50 m from the entrance (Fig. 3.7).

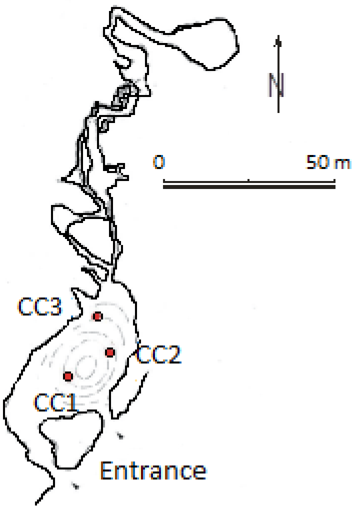

Figure 3.7: *Map of the cave Covolo della Croce, with marked locations of sampling sites.*

- In **Grotta di Roverè Mille** five sampling devices were located along the cave, from 25 m to 240 m from the entrance (Fig. 3.8).

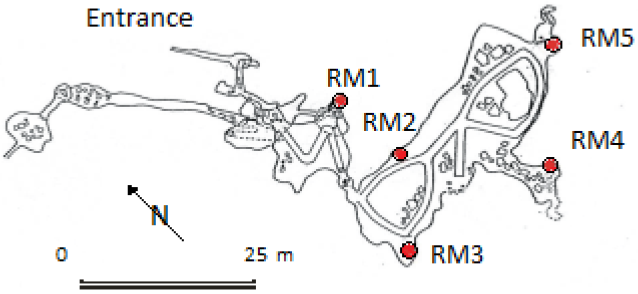

Figure 3.8: *Map of the cave Roverè Mille, with marked locations of sampling sites.*

3.2. Preservation and identification of collections

Samples of fauna were *in situ* fixed and stored in 70% alcohol. Collected organisms were sorted in the laboratory of the Karst Research Institute Scientific Research Centre of the Slovenian Academy of Sciences and Arts (IZRK ZRC SAZU) in Postojna (Slovenia), using a microscope (Nikon Eclipse 600) and identified in the laboratory of the Civic Natural History Museum of Trieste (Italy) using microscopes Leica MZ 16 and Leica DMLB in laboratories of Natural History Museum of Verona (Italy). Finally, some specimens were prepared and stored in the Civic Natural History Museum of Trieste using an experimental new method where the biological sample is preserved in a drop of glycerol on a plastic label, covered by Canada balsam and fastened with a pin in an entomological box. The method, shortly described below, is useful to preserve small size (< 1 mm) biological samples.

STORAGE METHOD:

The sample, preserved in a small drop of glycerol, was set on a transparent 5x10 mm plastic label (Fig. 3.9 a). A second plastic label 5x7 mm, with a 3 mm hole (Fig. 3.9 b) was plugged in a solution of Canada balsam and xylene. In this way it is possible to obtain a lens of balsam in the hole (Fig. 3.9 c). The label with the lens was used to cover the sample (Fig. 3.9 d). When the balsam dried (xylene evaporates), the two plastic labels adhere and the sample, preserved in glycerol, is protected by a lens of balsam.

The label with the sample can be fasten with a pin in an entomological box, together with a paper colored label (for the contrast at the microscope) and its data labels (Fig. 3.9 e). To manipulate the sample, the balsam lens can be dissolved with xilene.

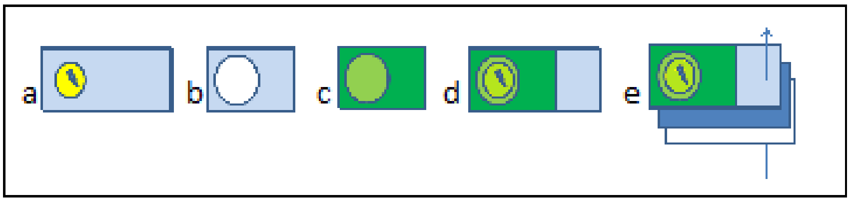

Figure 3.9: *Schematic representation of the storage method (light blue = transparent plastic labels; blue = coloured label used for contrast; yellow = glycerol; green = Canada balsam; the pin is represented as a vertical arrow).*

3.3. Measurement of environmental parameters

Measurements (temperature, conductivity, pH, discharge, and chemical analyses) were performed monthly during each monthly sampling of fauna in sampling periods (illustrated in Tables 3.1 and 3.2) and periodically from 2010 to 2013.

Temperature (°C), conductivity (μScm^{-1}) and pH of percolation water were measured *in situ* using a conductivity meter (LF 91, WTW), pH meter (323, WTW), and Combo multi-parameter Hanna Instruments. Drip rates (discharge) were measured by collecting water under drips in a graduated cylinder for timed intervals or, in case of low discharge, counting drops (1 drip = 0.2 mL) for timed intervals.

Samples of water for chemical analyses were collected directly under drips or, in case of low discharge, from the container of the sampling device for fauna. Concentration of cations (calcium) and anions (chloride, nitrate, sulphate and phosphate) as well as total hardness were determined according to Standard Methods for the Examination of Water and Wastewater (1989) in the laboratory of IZRK ZRC SAZU. The water samples were stored in plastic containers *in situ* and kept at 4° C prior to analysis.

To measure dissolved organic carbon (DOC) concentration, water was collected directly under the drips (over the course of at most one hour, to minimize losses of DOC). Than collected water was filtered through Glass Microfiber filters (25 μm) (Whatman), preserved with sulfuric acid (to $pH < 2$) and analyzed for DOC using an OI Analytical Total Organic Carbon Analyzer at the American University of Washington D.C., USA.

While considering the significant approximation of data in Alpine regions, to quantify precipitation, we used data (monthly precipitation sum) from Archive National Meteorological Service of Slovenia (Kredarica meteoroloical station monitored by Ministry of the environment and spatial planning, Slovenian Environment Agency), for Slovenian study area, and from ARPAV (Regional Agency for Environmental Prevention and Protection of Veneto) for Italian study area (www.arpa.veneto.it/temi.ambientali/climatologia/dati/commenti-meteoclimatici).

3.4. Statistical analysis

Descriptive statistics (mean, standard deviation, coefficient of variation) was used to describe physical and chemical characteristics of the drips. To show variation of each parameter the coefficient of variation (CV = SD/mean) was used.

Pearson correlation coefficient (r) was applied to compare two independent random variables (i.e. to evaluate copepod abundances with respect to chemical and physical parameters). The Student's t–test was used to calculate the statistical significance of results ($p < 0.05$ = significant correlation; $p < 0.01$ = high significant correlation). Calculations were performed using PAST (Paleontological Statistics Software Package for Education and Data Analysis, version 2.10) (Hammer *et al.* 2001).

Relation between copepod population and environmental parameters was shown using principal component analysis (PCA). PCA is a way of identifying patterns in data, and expressing the data in such a way as to highlight their similarities and differences. To interpret the data in a more meaningful form, it is necessary to reduce the number of variables to a few, interpretable linear combinations of the data. The resulting linear combinations (eigenvectors) are orthagonal to each other and correspond to a principal component. In graphs samples are represented by points and variables by vectors with common origin. A sample that is in the opposite site of the direction of the variable is scarcely influenced from that variable. The more the angle between two vectors is close to zero, much more they are positively correlated. If the angle is close to 180° they are negatively correlated.

Estimating species richness: The number of species is an index of community structure. Species richness in epikarst can not be directly measured. The use of richness estimators can be useful to estimate the real number of species present in epikarst and sampling efficiency, on the base of collected samples. The thoroughness of sampling can be gained from the species accumulation curves based on Mao-Tau procedure of Colwell (2013). From sampling epikarst fauna we obtain both incidence data (presence or absence of a species) and abundance data. An alternative approach to sampling completeness is to use estimates of total species number based on the internal structure of species abundance, especially the number of singleton and doubleton species (Chao 1987).

Three estimators were used: Chao1 and Chao2 and Jackknife1.

1. Chao1 richness estimator (for abundance data):

$$S_{Chao1} = S_{obs} + ((n - 1) / n) F_1^2 / 2F_2 \qquad (1)$$

where S_{obs} = total number of species observed in all samples; n = abundance; F_1 = frequency of singletons; F_2 = frequency of doubletons.

2. Chao2 richness estimator (for replicated incidence data):

$$S_{Chao2} = S_{obs} + ((m – 1) / m) Q_1^2 / 2Q_2 \qquad (2)$$

where S_{obs} = total number of species observed in all samples; Q_1 = frequency of unique sample occurring species; Q_2 = frequency of duplicates; m = total number of samples.

3. Jackknife1: incidence-based first-order jackknife estimator of species richness.

$$S_{jack1} = S_{obs} + Q_1 (m – 1)/m) \qquad (3)$$

where S_{obs} = total number of species observed in all samples; Q_1 = frequency of unique sample occurring species; m = total number of samples.

All computations were performed using EstimateS, version 9.1.0 (Colwell 2013).

4. RESULTS

4.1. Chemical and physical parameters

4.1.1. Temperature

In Table 4.1 mean temperature of dripping water measured at each sampling site in caves is shown, together with number of measurements in the sampling period and coefficient of variation. Temperature of dripping water was in general more temporal variable at sampling sites closer to the entrances of caves (SJ1, JPBZ1, PJ1, PV1, CC1, RM1; Figs. 3.2-3.8). In Zadlaška jama temperature was more stable than in other caves: the coefficient of variation was low (never rising over 0.05) in all sampling sites.

Table 4.1: *Temperature variability in the sampling periods (Tabs. 3.1-2) in monitored drips in caves.*
SS = sampling site;
N = number of measurements at each sampling site;
Mean = mean temperature measured at the sampling site (expressed in °C);
CV = coefficient of variation (representing temperature variation in time at each sampling site).

	CAVES IN SLOVENIA								**CAVES IN ITALY**					
	Snežna jama		**Jama pod Babjim zobom**		**Zadlaška jama**		**Pološka jama**		**Grotta A del Ponte di Veja**		**Covolo della Croce**		**Grotta di Roverè Mille**	
SS - Mean	SJ1	0.1	JPBZ1	4.4	ZJ1	10.6	PJ1	5.4	PV1	11.7	CC1	11.9	RM1	11.0
N - CV	11	2.00	11	0.36	8	0.02	9	0.31	9	0.15	5	0.15	5	0.15
SS - Mean	SJ2	1.1	JPBZ2	4.6	ZJ2	10.3	PJ2	5.2	PV2	11.8	CC2	12.0	RM2	10.0
N - CV	11	0.73	11	0.28	8	0.04	9	0.31	9	0.10	5	0.08	5	0.05
SS - Mean	SJ3	1.7	JPBZ3	5.0	ZJ3	10.4	PJ3	5.8	PV3	11.9	CC3	14.0	RM3	10.1
N - CV	9	0.29	11	0.12	8	0.05	9	0.17	9	0.04	5	0.14	5	0.07
SS - Mean	SJ4	3.1	JPBZ4	5.6	ZJ4	10.4	PJ4	5.1	PV4	11.6			RM4	9.9
N - CV	11	0.10	11	0.03	8	0.04	9	0.20	9	0.03			5	0.04
SS - Mean	SJ5	4.2	JPBZ5	6.2	ZJ5	10.1	PJ5	5.1	PV5	11.5			RM5	10.1
N - CV	11	0.07	11	0.03	8	0.03	9	0.25	9	0.04			5	0.05

Mean monthly temperature variation of dripping water in caves is shown in figure 4.1. The lowest temperatures were measured in Slovenia in Snežna jama

(1,556 m a.s.l.). In this cave the minimum value (-3 °C) was registered at SJ1, where water was periodically frozen.

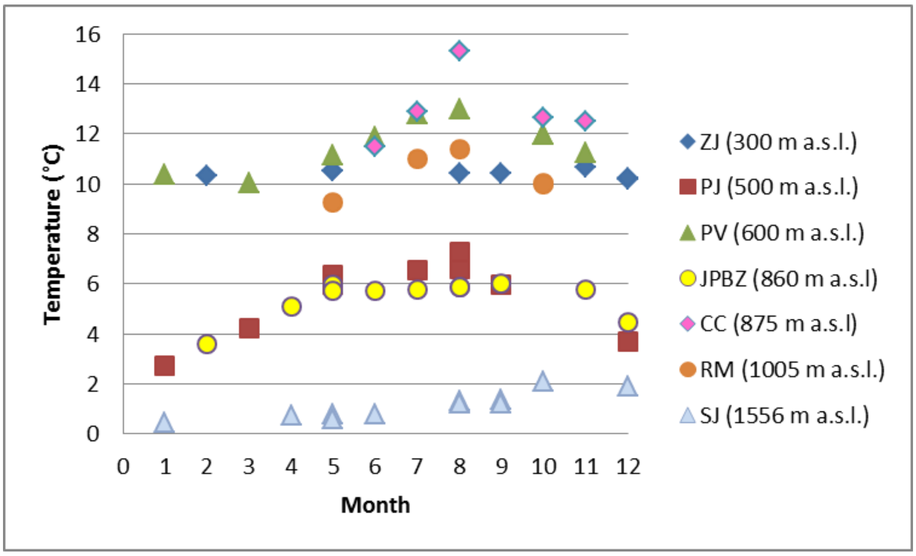

Figure 4.1: *Mean monthly variation of temperatures of dripping water in caves.*

Temperature variation did not always follow elevation gradient. In the cave Roverè Mille (1,500 m a.s.l.), for example, the mean temperature of water was always higher than temperature in Pološka jama (500 m a.s.l.) and in Jama pod Babjim zobom (860 m a.s.l.). Maximum temperature value (16.9 °C) was measured in August 2008 at Covolo della Croce (875 m a.s.l.) in Italy. In this cave temperature was higher than in Jama pod Babjim zobom despite the two caves are located at comparable altitudes but in different geographical regions.

4.1.2. pH

In Table 4.2 mean pH values, and variation in time of pH in sampling sites are shown.

The lowest mean pH was measured in Zadlaška jama (pH = 7.9); highest in Covolo della Croce (pH = 8.6).

Table 4.2: *pH variability in the sampling periods (Tabs. 3.1-2) in monitored drips in caves.*
SS = sampling site;
N = number of measurements at each sampling site;
Mean = mean pH measured at the sampling site;
CV = coefficient of variation.

	CAVES IN SLOVENIA								**CAVES IN ITALY**					
	Snežna jama		**Jama pod Babjim zobom**		**Zadlaška jama**		**Pološka jama**		**Grotta A del Ponte di Veja**		**Covolo della Croce**		**Grotta di Roverè Mille**	
SS – Mean	SJ1	8.4	JPBZ1	8.4	ZJ1	7.9	PJ1	8.3	PV1	8.5	CC1	8.6	RM1	8.3
N - CV	11	0.03	11	0.01	8	0.02	9	0.04	9	0.07	5	0.08	5	0.04
SS – Mean	SJ2	8.3	JPBZ2	8.3	ZJ2	8.0	PJ2	8.3	PV2	8.5	CC2	8.7	RM2	8.2
N - CV	11	0.04	11	0.03	8	0.02	9	0.02	9	0.07	5	0.04	5	0.05
SS – Mean	SJ3	8.2	JPBZ3	8.5	ZJ3	7.9	PJ3	8.3	PV3	8.5	CC3	8.5	RM3	7.9
N - CV	9	0.04	11	0.03	8	0.02	9	0.04	9	0.06	5	0.07	5	0.09
SS – Mean	SJ4	8.4	JPBZ4	8.1	ZJ4	8.0	PJ4	8.3	PV4	8.6			RM4	8.1
N - CV	11	0.02	11	0.12	8	0.02	9	0.04	9	0.01			5	0.05
SS – Mean	SJ5	8.2	JPBZ5	8.4	ZJ5	7.9	PJ5	8.2	PV5	8.5			RM5	8.0
N - CV	11	0.02	11	0.03	8	0.04	9	0.04	9	0.06			5	0.07

4.1.3. Discharge

Mean discharge at each sampling site in investigated caves is shown in Table 4.3. In the table number of sampling at each site and coefficient of variation are shown as well. Discharge was heterogeneous from cave to cave, varying temporally and spatially (among sampling sites). The caves with the lowest discharge were Grotta di Roverè Mille (always below 1 mL/min) and Zadlaška jama, where water flow rarely reached 12 mL/min. Due to low discharge at many sampling sites, sampling of water for chemical analyses was not always possible. Maximum discharge was observed in Snežna jama at the last sampling site (SJ5) where discharge was never below 35 mL/min and rised to over 800 mL/min in December and May.

Table 4.3: *Dripping rate variability in the sampling periods (Tabs. 3.1-2) in investigated drips.*
SS = sampling site;
Mean = mean discharge at the sampling site (expressed in mL/min);
CV = coefficient of variation (representing the discharge variation in the sampling period at each sampling site);
N = number of measurements done at each sampling site in the sampling period.

	CAVES IN SLOVENIA								**CAVES IN ITALY**					
	Snežna jama		**Jama pod Babjim zobom**		**Zadlaška jama**		**Pološka jama**		**Grotta A del Ponte di Veja**		**Covolo della Croce**		**Grotta di Roverè Mille**	
SS – Mean N - CV	SJ1 11	4.5 1.42	JPBZ1 11	2.8 0.71	ZJ1 8	0.1 1.00	PJ1 9	2.2 0.10	PV1 9	7.8 0.72	CC1 5	0.5 1.00	RM1 5	0.1 1.00
SS – Mean N - CV	SJ2 11	3.4 1.47	JPBZ2 11	5.1 1.25	ZJ2 8	1.0 1.00	PJ2 9	9.1 0.43	PV2 9	2.4 0.50	CC2 5	0.1 1.00	RM2 5	0.2 0.50
SS – Mean N - CV	SJ3 9	81.6 1.37	JPBZ3 11	18.8 0.26	ZJ3 8	0.9 1.35	PJ3 9	114.4 1.00	PV3 9	20.0 1.03	CC3 5	1.5 1.80	RM3 5	0.4 0.75
SS – Mean N - CV	SJ4 11	18.8 1.20	JPBZ4 11	15.4 0.60	ZJ4 8	3.7 1.30	PJ4 9	129.7 1.00	PV4 9	0.3 1.00			RM4 5	0.4 1.00
SS – Mean N - CV	SJ5 11	287.0 1.20	JPBZ5 11	13.8 0.43	ZJ5 8	0.5 1.40	PJ5 9	4.8 0.17	PV5 9	0.5 1.00			RM5 5	0.3 0.33

4.1.4. Conductivity, total hardness, $CaCO_3$, Ca^{2+} and other ions concentrations

Results from measurements of conductivity, total hardness, Ca^{2+} and $CaCO_3$ concentration in epikarst water in all seven investigated caves are briefly summarized in Table 4.4. Conductivity, total hardness, Ca^{2+} and $CaCO_3$ concentrations in dripping water were usually higher in caves located in Lessinian Massif than in Slovenian caves.

Results from measurements of other ions concentrations in caves are summarized in Table 4.5. Concentrations of ions were low. Ions with the most variable concentration were phosphates and nitrates ($0.3 < CV < 1.5$ and $0.03 < CV < 1.1$, respectively). Amount of nitrates was lower in Grotta di Roverè Mille and in Covolo della Croce, higher in other caves (up to 28 mg/L in Grotta A del Ponte di Veja). Phosphate concentration was higher in Pološka jama and in Grotta A del Ponte di Veja rising to 0.8 mg/L and 1.2 mg/L, respectively.

Table 4.4: *Ranges of physical and chemical measurements of drips in studied caves from measurements of conductivity, total hardness Ca^{2+} and $CaCO_3$ concentration.*
Mean = Mean values of each parameter in each cave;
SD = standard deviation;
(min-max) = minimum and maximum value;
N = total number of measurements of each parameter;
- = no data.

Parameter / Cave **Mean ±SD** **(min−max)** **N**	**CAVES IN SLOVENIA**				**CAVES IN ITALY**		
	Snežna jama	**Jama pod Babjim zobom**	**Zadlaška jama**	**Pološka jama**	**Grotta A del Ponte di Veja**	**Covolo della Croce**	**Grotta di Roverè Mille**
Conductivity (µS/cm)	297±26 (223 – 346) 53	252±21 (191 – 296) 55	288±37 (228 – 369) 40	230±33 (196 – 309) 45	358±78 (140 – 2870) 45	314±50 (240 – 380) 15	355±54 (290 – 470) 25
Total hardness (mg/L)	142±13 (118 – 185) 12	135±10 (115 – 156) 15	142±11 (124 – 163) 5	121±5 (109 – 152) 9	150 (127 – 175) 4	197 (182 – 237) 3	219 (201 – 255) 3
Ca^{2+} (mg/L)	54±5 (47 – 67) 12	53±4 (46 – 62) 15	51±5 (44 – 60) 5	42±7 (35 – 60) 9	60 (50 – 71) 4	74 (89 – 62) 3	83 (80 – 91) 3
$CaCO_3$ (mg/L))	138±11 (123 – 172) 12	129±20 (110 – 245) 15	-	105 (100 – 110) 4	136 (121 – 162) 4	187 (161 – 226) 3	245 1

Table 4.5: *Ranges of chemical measurements of drips in studied caves.*
Mean = Mean values of measured parameters;
SD = standard deviation (SD);
(max-min) = maximum and minimum values;
CV = coefficients of variation (representing variations between all the sampling sites within each cave).

Parameter / Cave **Mean±SD** **(min−max)** CV	**CAVES IN SLOVENIA**				**CAVES IN ITALY**		
	Snežna jama	**Jama pod Babjim zobom**	**Zadlaška jama**	**Pološka jama**	**Grotta A del Ponte di Veja**	**Covolo della Croce**	**Grotta di Roverè Mille**
Cl^- (mg/L)	2.7 ± 1.4 (1.5 – 5.1) 0.53	3.0 ± 1.8 (1.5 – 9.0) 0.70	2.0 ± 0.3 (1.5 – 2.5) 0.16	2.4 ± 0.2 (2.0 – 2.5) 0.10	2.2 ± 0.5 (2.5 – 1.5) 0.22	2.9 ± 0.6 (2.5 – 3.6) 0.20	2.5
NO_3^- (mg/L)	1.8 ± 0.5 (1.5 – 3.9) 0.25	2.9 ± 2.7 (0.4 – 9.1) 0.92	2.3 ± 0.1 (2.1 – 2.4) 0.05	5.5 ± 5.4 (1.7 – 16.0) 0.98	10.7 ± 11.7 (3.2 – 28.1) 1.10	0.4 ± 0.4 (0.2 – 0.9) 1.00	0.4
SO_4^{2-} (mg/L)	2.0 ± 1.0 (0.4 – 5.7) 0.47	3.5 ± 2.0 (0.0 – 6.4) 0.60	4.0 ± 0.2 (3.8 – 4.5) 0.06	3.1 ± 0.8 (2.0 – 4.4) 0.26	4.6 ± 0.6 (3.9 – 5.1) 0.13	4.8 ± 0.4 (4.6 – 5.3) 0.08	2.8
PO_4^{3-} (mg/L)	0.01 ± 0.01 (0.00 – 0.02) 1.50	0.02 ± 0.03 (0.00 – 0.14) 1.50	0.05 ± 0.03 (0.01 – 0.09) 0.52	0.23 ± 0.27 (0.05 – 0.81) 0.87	0.23 ± 0.07 (0.31 – 1.17) 0.30	0.01 ± 0.01 (0.00 – 0.01) 1.00	0.00

4.1.5. Dissolved organic carbon (DOC) concentration

Measurements for DOC concentration have been performed only one to three times for each sampling site (where possible). Results from DOC measurements in caves are summarized in Table 4.6. Due to low discharge, no data from Zadlaška jama were registered. In the other caves DOC vary between 0.9 mg/L in Grotta A del Ponte di Veja, and 5.7 mg/L at Covolo della Croce.

Table 4.6: *Ranges of DOC measurements of drips in the studied caves.*
Mean = Mean values of measured parameters;
SD = standard deviation;
(min-max) = maximum and minimum values;
CV = coefficient of variation (represents variation of DOC values between sampling sites in each cave);
- = no data.

Parameter / Cave **Mean±SD** **(min−max)** CV	**CAVES IN SLOVENIA**				**CAVES IN ITALY**		
	Snežna jama	**Jama pod Babjim zobom**	**Zadlaška jama**	**Pološka jama**	**Grotta A del Ponte di Veja**	**Covolo della Croce**	**Grotta di Roverè Mille**
DOC (mg/L)	3.9 ± 0.9 (2.6 – 4.7) 0.24	2.7 ± 0.6 (2.0 – 3.3) 0.24	-	3.0 ± 0.5 (2.6 – 3.8) 0.17	1.7 ± 0.5 (0.9 – 2.2) 0.31	3.7 ± 1.8 (2.3 – 5.7) 0.47	2.9 ± 1.4 (1.9 – 4.4) 0.47

4.1.6. Correlations between measured parameters

Calculation of Pearson correlation coefficient between different measured parameters, demonstrates many significant correlations between parameters (Tab. 4.7). High positive and significant correlations ($r > 0.5$, $p < 0.01$) were found for the following pairs of parameters (Tab. 4.7, column TSA): carbonate *versus* conductivity, total hardness of water *versus* conductivity and *versus* carbonate, and Ca^{2+} *versus* conductivity, carbonate and total hardness of water (shown in bold in Tab. 4.7).

Table 4.7: *Statistically significant correlations (based on calculation of Pearson correlation coefficient) between measured parameters. (* indicates $0.01 < p < 0.05$; ** indicates $p < 0.01$; TSA = total sampling area; SLO = caves in Slovenia; IT = caves in Italy). Higher correlations ($r > 0.5$ or < -0.5) are shown in bold.*

Correlated parameters	**Pearson correlation coefficient (*r*)**									
	TSA	**SLO**	SJ	JPBZ	ZJ	PJ	**IT**	PV	CC	RM
Precip. vs Temp.	0.16*	0.16*		__0.57**__	__0.52**__	__0.58**__				
Disch. vs Temp.	-0.18**		0.41**	0.28*						
pH vs Temp.		-0.44**						-0.42**		
pH vs Precip.	-0.20**	-0.20**			-0.32*	-0.33*				
pH vs Disch.			-0.28**							
Cond. vs Precip.	0.21**	0.21**		0.43**	0.41**					
Cond. vs Temp.	0.22**				0.33*					
Cond. vs Disch.						-0.37*				
Cond. vs pH		-0.20**								__0.65**__
CaCO$_3$ vs Temp.	0.34**		0.34**				-0.39**			
CaCO$_3$ vs Disch.				-0.29*			-0.33**			
CaCO$_3$ vs pH	-0.20**	0.24**					-0.40**			
CaCO$_3$ vs Cond.	__0.53**__	0.42**	__0.61**__				0.31**			
Tot. Hard. vs Temp.	0.25**	-0.31**								
Tot. Hard. vs Disch.			-0.31**				-0.33**			
Tot. Hard. vs pH							-0.31**			
Tot. Hard. vs Cond.	__0.54**__	__0.80**__	__0.73**__	0.35**	__0.51**__	__0.93**__	0.24*			
Tot. Hard. vs CaCO$_3$	__0.89**__	__0.61**__	__0.81**__				__0.92**__	__0.91**__	__1**__	
Ca^{2+} vs Temp.	0.37**	-0.23**	-0.37**				-0.25**			
Ca^{2+} vs Disch.	-0.13*		-0.32*	0.36**			-0.33**			
Ca^{2+} vs pH							-0.32**			
Ca^{2+} vs Cond.	__0.51**__	__0.60**__	0.33*		0.49**	__0.55**__	0.25*		__-0.55*__	
Ca^{2+} vs CaCO$_3$	__0.89**__	__0.57**__	0.30*				__0.94**__	__0.94**__	__0.99**__	
Ca^{2+} vs Tot. Hard.	__0.94**__	__0.82**__	__0.63**__	__0.64**__	__0.99**__	__0.68**__	__0.98**__	__0.99**__	__0.99**__	__1**__
Cl$^-$ vs Temp.	-0.20**	-0.22**	-0.28*	-0.33*						
Cl$^-$ vs Disch.				-0.37**		-0.37*				
Cl$^-$ vs Cond.			-0.28*	__0.51**__		0.47**	0.30**		__0.65**__	
Cl$^-$ vs CaCO$_3$			-0.48**			0.46**	0.40**	__0.70**__	__-0.62*__	
Cl$^-$ vs pH		0.24**								
Cl$^-$ vs Tot. Hard.			-0.42**		__0.52**__	0.43**	0.49**	__0.92**__	__-0.63*__	
Cl$^-$ vs Ca^{2+}				-0.32**	__0.65**__	__0.63**__	0.46**	__0.91**__	__-0.73**__	
NO$_3^-$ vs Temp.	0.16**			-0.31*						
NO$_3^-$ vs Disch.						-0.38**	0.28**			
NO$_3^-$ vs pH	0.20**									
NO$_3^-$ vs Cond.			__0.54**__			__0.91**__				
NO$_3^-$ vs CaCO$_3$	-0.28**		__0.66**__	__0.60**__			__-0.57**__			
NO$_3^-$ vs Ca^{2+}						0.43**	-0.45**			
NO$_3^-$ vs Cl$^-$			__-0.61**__			0.44**		__0.50**__		
SO$_4^{2-}$ vs Temp.	0.28**	0.21**	0.39**				__0.60**__			
SO$_4^{2-}$ vs pH	0.13*						0.41**			
SO$_4^{2-}$ vs Disch.				0.27*				-0.43**		

Correlated parameters	**Pearson correlation coefficient (r)**									
	TSA	**SLO**	SJ	JPBZ	ZJ	PJ	**IT**	PV	CC	RM
SO_4^{2-} vs Cond.						0.40**	-0.28**			
SO_4^{2-} vs $CaCO_3$			0.40**				__-0.76**__		__0.99**__	
SO_4^{2-} vs Tot. Hard.					__-0.74**__	0.41**	__-0.55**__		__0.99**__	
SO_4^{2-} vs Ca^{2+}					__-0.68**__		__-0.58**__		__0.96**__	
SO_4^{2-} vs NO_3^-	0.17**		0.35*		__-0.75**__	0.41**	0.48**	__0.52**__		
SO_4^{2-} vs Cl^-				-0.27*		-0.34*				
PO_4^{3-} vs Temp.	0.22**		0.28*				0.25*			
PO_4^{3-} vs pH	0.18**		0.30**				0.31**			
PO_4^{3-} vs Disch.		0.15*		-0.29*		0.48**	0.47**	__0.52**__		
PO_4^{3-} vs Cond.	-0.25**	-0.38**	0.42**	0.40**		-0.31*	-0.31**			
PO_4^{3-} vs $CaCO_3$	-0.40**		0.36**				__-0.81**__		__0.99**__	
PO_4^{3-} vs Tot. Hard.	-0.36**	-0.43**				-0.29*	__-0.77**__		__0.99**__	
PO_4^{3-} vs Ca^{2+}	-0.31**	__-0.54**__		-0.39**			__-0.72**__		__0.96**__	
PO_4^{3-} vs Cl^-	-0.17**		-0.35**	__0.57**__	__0.61**__	__-0.63**__	__-0.51**__			
PO_4^{3-} vs SO_4^{2-}	0.13**		0.25**		__0.52**__	__0.64**__	0.47**	__-0.88**__		
PO_4^{3-} vs NO^{3-}	0.38*	0.18*	__0.64**__		__-0.77**__		__0.56**__			
DOC vs Temp.	-0.34**	-0.33**	__-0.76**__							
DOC vs Disch.		-0.05**	-0.33*				-0.25**	0.32*		
DOC vs Cond.	0.20**	0.39**				__0.94**__	0.33**		__0.66**__	
DOC vs $CaCO_3$		0.25**	__-0.51**__	0.38**			0.30**	0.47**	__-0.77**__	
DOC vs Tot. Hard.		0.46**				__0.99**__	0.34**	__0.51**__	__-0.77**__	
DOC vs Ca^{2+}		0.38**	0.40**	__0.55**__		__0.71**__		__0.57**__	__-0.85**__	
DOC vs NO_3^-	-0.21*	0.21**	-0.34*			__0.84**__	-0.32**			
DOC vs Cl^-	0.16**					__0.51**__	__0.77**__	__0.62**__	__0.98**__	
DOC vs PO_4^{3-}	-0.34**	-0.16*	-0.31**			-0.33*	-0.48**	__0.87**__	__-0.67**__	*
DOC vs SO_4^{2-}	-0.34**	-0.47**	__-0.62**__	__-0.51**__		0.39**		__-0.59**__	__-0.67**__	*

4.2. Fauna

4.2.1. Total faunal composition of investigated drips

In investigated caves, both aquatic and terrestrial invertebrate taxa were found in sampled drips. The most abundant groups (in term of number of individuals) were copepods (61% of the total) and insects (19% of the total) (Fig. 4.2).

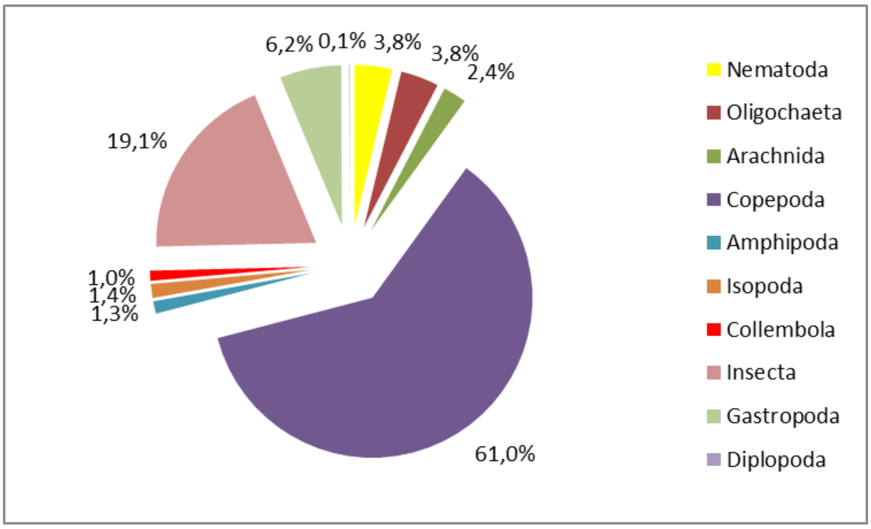

Figure 4.2: *Total faunal composition of investigated drips (abundance-based percentages).*

In Table 4.8, a list of collected taxa is reported, together with numbers of species from each group and their habitat.

Aquatic species were most abundant in all caves, with the exception of Covolo della Croce and Zadlaška jama, where aquatic species represented 40% and 38% of the total, respectively. With eighteen species, Grotta A del Ponte di Veja was the richest cave (with respect to species diversity). Caves located at higher elevation (Snežna jama and Grotta di Roverè Mille) harbor less diverse communities, with 6 and 5 species, respectively (Tab. 4.8). No animals were found when discharge was very low (< 1 mL/min). Drips investigated in Zadlaška jama had the least abundant fauna among the sampled caves. Copepods were the most diverse (Tab. 4.8) and the most abundant group representing 61% of the community (Fig. 4.2) with 776 individuals.

Table 4.8: *Number of species in samples collected in the investigated caves. Habitat of each species is shown in brackets (T = terrestrial; A = aquatic).*

	Number of species						
Taxa / Cave	**Snežna jama**	**Jama pod Babjim zobom**	**Zadlaška jama**	**Pološka jama**	**Grotta A del Ponte di Veja**	**Covolo della Croce**	**Grotta di Roverè Mille**
Nematoda	0	1 (A)	0	1 (A)	1 (A)	1 (A)	0
Oligochaeta	1(T)	1 (T)	0	0	1 (A)	1 (T)	0
Arachnida	1 (A)	1 (A)	0	0	1 (A)	1 (T)	2 (A-T)
Copepoda	2 (A)	3 (A)	3 (A)	3 (A)	8 (A)	3 (A)	1 (A)
Amphipoda	1 (A)	1 (A)	0	1 (A)	1 (A)	0	0
Isopoda	0	0	1	1 (A)	1 (A)	0	0
Collembola	0	1 (T)	2 (T)	0	1 (T)	0	1 (T)
Coleoptera	1 (T)	1 (T)	1 (T)	0	1 (T)	1 (T)	0
Diptera	0	1 (A)	1 (T)	0	1 (A)	1 (T)	1 (A)
Orthoptera	0	0	0	1 (T)	1 (T)	1 (T)	0
Dipolopoda	0	0	0	0	0	1 (T)	0
Gastropoda	0	1 (?)	0	0	1 (?)	0	0
Total species	6	11	8	7	18	10	5
Percent of aquatic species	67%	64%	38%	86%	78%	40%	60%

4.2.2. Copepoda

Totally thirteen copepod species were collected: two belonging to the order of Cyclopoida and eleven to Harpacticoida. Observed copepod species richness is different from cave to cave, varying from one species (in Grotta di Roverè Mille) to eight species (in Grotta A del Ponte di Veja) (Tabs. 4.8, 4.9).

Total abundance of cyclopoids was usually lower than total abundance of harpacticoids; only in Zadlaška jama, where copepod abundance was very low, 1 harpacticoid and 4 cyclopoids were collected (Tab. 4.9, Fig. 4.3).

Table 4.9: *List of copepod species and corresponding abundances in investigated caves (species richness is expressed as a number of different copepod species in each cave).*

Order	Species	Abundance						
		SJ	**JPBZ**	**ZJ**	**PJ**	**PV**	**CC**	**RM**
Cyclopopida	*Paracyclops imminutus* (Kiefer, 1929)	0	0	0	0	7	0	0
	Speocyclops infernus (Kiefer, 1930)	69	35	4	6	13	4	0
Harpacticoida	*Bryocamptus (Rheocamptus) zschokkei tatrensis* (Minkiewicz, 1916)	0	0	0	0	36	0	0
	Bryocamptus sp.	112	0	1	18	0	0	0
	Elaphoidella sp.1	0	176	0	0	0	0	0
	Elaphoidella sp.2	0	100	0	0	0	0	0
	Lessinocamptus insoletus (Chappuis, 1928)	0	0	0	0	25	1	0
	Lessinocamptus sp.	0	0	0	20	0	0	0
	Maraenobiotus brucei (Richard, 1898)	0	0	0	0	44	0	0
	Moraria poppei (Mràzek, 1983)	0	0	0	0	1	0	0
	Moraria stankovitchi (Chappuis, 1924)	0	0	0	0	1	0	0
	Moraria alpina (Stoch, 1998)	0	0	1	0	0	0	0
	Moraria sp.A	0	0	0	0	1	3	10
	Species richness	**2**	**3**	**3**	**3**	**8**	**3**	**1**

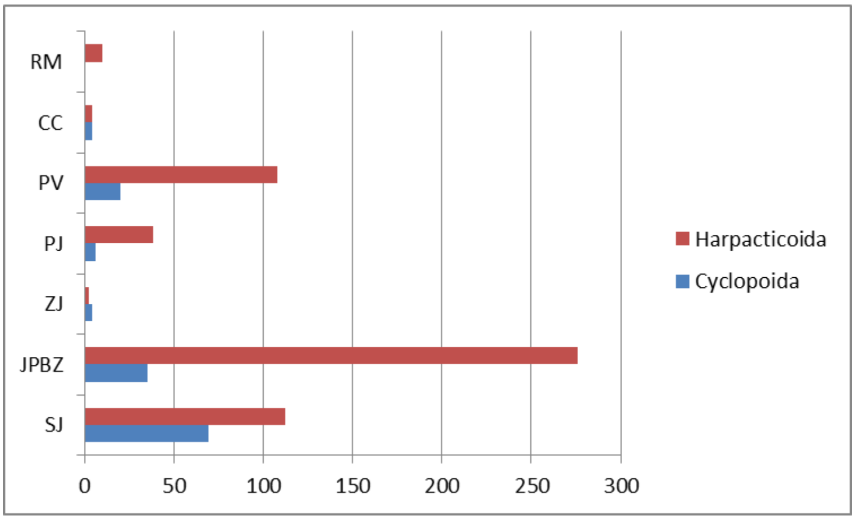

Figure 4.3: *Abundance of Cyclopoida and Harpacticoida in investigated caves. In horizontal axe the total number of individuals is presented.*

As summarized in Table 4.10 (column TSA), low but significant correlation was found between abundance of copepods and sulphate ions (r = 0.2, $p < 0.01$). Negative correlation was found between copepod abundance and dissolved organic carbon (r = - 0.17, $p < 0.05$).

Table 4.10: *Statistically significant correlations (based on Pearson correlation coefficient)(* indicates $0.01 < p < 0.05$; ** indicates $p < 0.01$) between copepod abundance (expressed in copepod/day) and measured parameters. Higher correlations ($r > 0.5$ or $r < -0.5$) are shown in bold.*

Correlations	**Pearson correlation coefficient (*r*)**									
	TSA	**SLO**	SJ	JPBZ	PJ	ZJ	**IT**	PV	CC	RM
Cop. Abund. vs SO_4^{2-}	0.20**	0.21**								
Cop. Abund. vs Cond.										**0.57****
Cop. Abund. vs pH									**0.59***	
Cop. Abund. vs DOC	-0.17*									0.49*
Cop. Abund. vs Disch.				0.28*						
Cop. Abund. vs $CaCO_3$			-0.32*		**0.66****		-0.22			
Cop. Abund. vs Tot. Hard.							-0.25			
Cop. Abund. vs Ca^{2+}							-0.26			
Cop. Abund. vs Cl^-						-0.36*	-0.30			
Cop. Abund. vs NO_3^-					0.36*					
Copepod abund. vs PO_4^{3-}						-0.39				
L. insoletus vs Disch.								**0.68****		
L. insoletus vs SO_4^{2-}								**-0.58****		
L. insoletus vs PO_4^{3-}								**0.59****		
M. brucei vs *B. zschokkei tatrensis*								**0.75****		
M. stankovitchi vs *L.insoletus*								**0.57****		
S. infernus vs *Moraria* sp.A									**0.64***	
S. infernus vs *L. insoletus*								0.36*		
S. infernus vs $CaCO_3$						**0.50****				
Moraria sp.A vs Cond.										**0.57****
Moraria sp.A vs DOC										0.49**
Moraria sp.A vs pH									**0.54****	
B. zschokkei tatrensis vs $CaCO_3$								-0.35*		
B. zschokkei tatrensis vs Tot. Hard.								0.37*		
B. zschokkei tatrensis vs Ca^{2+}								-0.39**		
B. zschokkei tatrensis vs Cl^-								-0.38*		
B. zschokkei tatrensis vs DOC								-0.43**		
M. brucei vs DOC								-0.34*		

4.2.2.1. Observed copepod species

A list of collected copepod species with their distributions and ecological status is shown in Table 4.11. Some species show typical morphological adaptation for subterranean life, including reduced or absent eyes and pigment and larger eggs. Of thirteen species collected in total, nine are considered stygobiotic, the others stygophilic (Tab. 4.11).

Table 4.11: *Distribution and ecological status of collected copepod species.*

Copepoda	**Distribution**	**Ecological status**
Cyclopoida		
Speocyclops infernus (Kiefer, 1930)	Alpine	Stygobiont
Paracyclops imminutus (Kiefer, 1929).	European	Stygophile
Harpacticoida		
Bryocamptus (Rheocamptus) zschokkei tatrensis (Minkiewicz, 1916)	East-European	Stygophile
Bryocamptus sp.	Alpine - Endemic	Stygobiont
Elaphoidella sp.1	Alpine - Endemic	Stygobiont
Elapohoidella sp.2	Alpine - Endemic	Stygobiont
Lessinocamptus insoletus (Chappuis, 1928)	Alpine - Endemic	Stygobiont
Lessinocamptus sp.	Alpine - Endemic	Stygobiont
Maraenobiotus brucei (Richard, 1898)	East-European	Stygophile
Moraria poppei (Mràzek, 1983)	European	Stygophile
Moraria stankovitchi (Chappuis, 1924)	Alpine- Dinaric	Stygophile
Moraria alpina (Stoch, 1998)	Alpine	Stygobiont
Moraria sp.A	Alpine	Stygobiont

- **Cyclopoida**

In investigated caves only two species, belonging to the family Cyclopidae (Rafinesque, 1815), were collected: *Speocyclops infernus* (Kiefer, 1930) and *Paracyclops imminutus* (Kiefer, 1929).

Paracyclops imminutus (Kiefer, 1929): It is a European species with a wide distribution. It is considered a troglophile species and usually lives in lotic waters (Ruffo and Stoch 2006). Seven adult individuals were collected in the cave Grotta A del Ponte di Veja (Tab. 4.9). In the cave the cyclopoid *Speocyclops infernus* and six other harpacticoid species were found.

Speocyclops infernus (Kiefer, 1930): It is an alpine stygobiont species. In Europe it was found in Bulgaria, northern Italy and Slovenia (Ruffo and Stoch 2006). Individuals belonging to this species were collected in almost all investigated caves, with the exception of Grotta di Roverè Mille, where no cyclopoids at all were found (Tab. 4.9). Females with eggs were collected only in Snežna jama. Eggs (three on each side) were large and not in eggs sacs. As shown in Table 4.10, abundance of *S. infernus* was positively correlated with carbonates in Zadlaška jama ($r = 0.50$, $p < 0.01$) and with abundance of the species *M. alpina* ($r = 0.64$, $p < 0.05$), in Covolo della Croce, and *L. insoletus* ($r = 0.36$, $p < 0.05$), in Grotta A del Ponte di Veja.

- **Harpacticoida**

All collected harpacticoids belong to the family Canthocamptidae (Brady, 1980), with a great amount of subterranean species. These are harpacticoids living in continental waters, from the Arctic to the Antarctic, in about all types of water bodies and wetlands, most commonly in lake meiobenthos. The family contains more than 700 species, 500 of which are ascribed to the complex *Attheyella-Elaphoidella-Bryocamptus* (Wells 2007). In investigated caves 11 species were collected (Tab. 4.9).

Bryocamptus (Rheocamptus) zschokkei tatrensis (Minkiewicz, 1916): This subspecies was found in Bulgaria, Croatia, France, Germany, Romania and North-Italy. Usually is found in lotic environments, but frequent in springs and subterranean habitats too (Wells 2007). In total, 36 individuals belonging to this species were collected in Grotta A del Ponte di Veja together with cyclopoids (*S. infernus* and *P. imminutus*) and five other harpacticoid species. As shown in Table 4.10, the presence of this species was positively correlated with the presence of *M. brucei* ($r = 0.75$, $p < 0.01$) and total hardness of water ($r = 0.37$, $p < 0.05$), and negatively correlated with DOC, carbonate, Ca^{2+} and Cl^- ($r > 0.35$, p < 0.05).

***Bryocamptus* sp.**: This unidentified species was found in three of the four Slovenian caves (Tab. 4.9). Collected specimens show typical adaptations to cave life, like total depigmentation, eyes absence and body and appendage elongation. The species was the most abundant in Snežna jama, where 112 individuals were collected, together with *S. infernus*. In Zadlaška jama and Pološka jama the species was represented by one and 18 individuals, respectively. In both caves it was collected together with the cyclopoid *S. infernus* and another harpacticoid species (*Moraria alpina* and *Lessinocamptus* sp.). Collected individuals probably belong to the complex of *B. zschokkei* with a large number of subspecies, varieties and forms that have been described.

***Elaphoidella* sp.1** and ***Elaphoidella* sp.2**: In the cave Jama pod Babjim zobom two unidentified species belonging to the genus *Elaphoidella* were collected. The genus is widely distributed. Limited variability within populations is reported in features of setation and ornamentation and in absolute and relative lengths of various structures. Studies that reveal the existence of a complex mosaic of variability within a homogeneous geographic locality are becoming more common and are impacting on the taxonomy of the genus (Wells 2007). Collected animals show typical adaptations to subterranean life such as absence of eyes and pigmentation. Body length of the two species is different: the first is worm-like, the other one is less elongated. Both species were collected, together with *S. infernus* (Tab. 4.9), in Jama pod Babjim zobom with 176 and 100 individuals, respectively. *Elaphoidella* sp.1 looks quite similar to *E. phreatica*, common in alpine regions from France and Italy, through the Balkans and Hungary to Romania. It is considered a highly variable species. Variability in the characters of setation used in the taxonomy is complex and the identity may best be confirmed by pattern of ornamentation of the urosome and by the details of shape and setation of the caudal ramus.

Lessinocamptus insoletus (Chappuis, 1928): It is an endemic species of the Lessinian Mountains. Before this research it was known only from the cave Buso della Rana (Stoch 1997), a cave located few kilometers from the investigated caves. Specimens show adaptation to cave life, including body elongation, depigmentation and absence of eyes. Many individuals were collected in Grotta a del Ponte di Veja together with *S. infernus* and *P. imminutus* and other five harpacticoid species (Tab. 4.9). As shown in Table 4.10, presence of this species was positively correlated with *S. infernus* and *M. stankovitchi* abundance and with discharge (r = 0.68, $p < 0.01$) and PO_4^{3-} (r = 0.59, $p < 0.01$), and negatively correlated with SO_4^{2-} (r = -0.58, $p < 0.01$).

***Lessinocamptus* sp.**: Twenty unidentified individuals (cf. *L. insoletus*) belonging to the genus *Lessinocamptus* were collected in Pološka jama. Specimens show adaptation to cave life similar to the ones in *L. insoletus*. In the cave another unidentified species of the genus *Bryocamptus* and the cyclopoid *S. infernus* were collected.

Maraenobiotus brucei (Richard, 1898): It is a species present in northern Italy, Austria, England, France, Germany, Greece, Macedonia, Romania, Switzerland, and on Svalbard. It usually lives in moss but some times it was found in caves too (Dussart 1967). During the research 44 individuals were collected in Grotta A del Ponte di Veja together with cyclopoids (*S. infernus* and *P. imminutus*) and five other harpacticoid species. As shown in Table 4.10, presence of *M. brucei* was positively correlated with *B. zschokkei tatrensis* (r = 0.75, $p < 0.01$) and a low negative correlation was found with DOC (r = -0.34, $p < 0.05$).

Moraria poppei (Mràzek, 1983): This species was found in Grotta A del Ponte di Veja at sampling site PV2. It is a surface-dwelling species usually living in moss or in lentic water or some times in caves (Ruffo and Stoch 2006, Bottazzi *et al.* 2008). Distribution of this species is vast: Austria, Belgium, England, Bulgaria, Corsica, Czeck Republic, France, Germany, Greece, Ireland, Poland, Romania, Slovenia, Spain, Sweden and Switzerland. In Italy it is known from central and northern parts and from Sardinia.

Moraria stankovitchi (Chappuis, 1924): Only one individual belonging to this species was collected in Grotta A del Ponte di Veja, at sampling site PV3, where species *S. infernus* and *L. insoletus* were collected too. In Europe this species is known from NE Italy, Slovenia, and Dinaric karst. It is considered a stygophile species (Stoch and Tomasin 2002).

Moraria alpina (Stoch, 1998): It is an Alpine stygobiotic species (Stoch 2008). Only one individual was collected in Zadlaška jama together with *S. infernus* and *Bryocamptus* sp.

***Moraria* sp.A**: this species is reported in the checklist and distribution map of Italian fauna (Ckmap) as *Moraria* sp.A (Ruffo and Stoch 2006) and is waiting to be described. Probably endemic from Lessini Mountains, where before this research, it was collected only in the cave Buso della Rana (Stoch 1997). Individuals belonging to this species were collected in all three investigated Italian caves: one in Grotta A del Ponte di Veja, six in Covolo della Croce and twenty in Grotta di Roverè Mille. As shown in Table 4.10, presence of this species was positively correlated with conductivity and DOC in Grotta di Roverè Mille and with pH in Covolo della Croce.

4.3. Results from Slovenia

In all twenty investigated drips in Slovenia we found invertebrates belonging to both aquatic and terrestrial taxa, copepods (at different maturity stadia) being the most abundant (Figs. 4.2, 4.3, Tab. 4.8). The average rate of copepods collected in sampling sites was 0.04 copepodsa per drip per day. Diversity of copepod populations in Slovene Alpine epikarst was low (two or three copepod species per cave; Tabs. 4.8, 4.9). One species of Cyclopoida (*Speocyclops infernus*) was found in all four caves, while Harpacticoida (*Bryocamptus* sp., *Lessinocamptus* sp., and two species of *Elaphoidella*) had local distribution (Tab. 4.9). The richest copepod assemblage among sampled caves, in terms of total number of individuals, was observed in Jama pod Babjim zobom, where over 300 specimens were collected.

4.3.1. Snežna jama na planini Arto

Snežna jama is a cold cave (maximum temperature of the air inside the cave is 4 °C). At the entrance hall, due to cold air that flows through the entrance shaft into the cave, temperature frequently drops below zero, periodically freezing percolating water. Low temperature at the entrance to the cave caused measurements and sampling of fauna at the first sampling points (SJ1 and SJ2) not always possible (Fig. 4.4).

Figure 4.4: *Sampling site SJ1 covered by ice.*

Characteristics of the measured physical and chemical parameters of dripping water at five sampling sites in the cave are shown in Table 4.12.

Temperature of the water varied among sites from -0.3 °C (frozen water at SJ1 in October 2007) to 4.7 °C (at SJ5 in December 2006).

The most variable parameter was discharge, with data spread over a large range of values (e.g., between 0 mL/min and 880 mL/min, measured in December 2006 at SJ5) ($1.20 < CV > 1.42$). Dry periods, with low discharge, were in summer, reflecting no or low amount of precipitation, and in winter because of the snow cover.

Table 4.12: *Ranges of physical and chemical measurements of five drips in Snežna jama (2006-2007, 2010-2013). CV = variation of parameters during the sampling period. Drips with copepods are marked with asterisks (*).*

Parameter / Drip **Mean±SD** **(min–max)** CV	**SJ1**	**SJ2***	**SJ3***	**SJ4**	**SJ5***
Temperature (°C)	0.1 ± 0.2 (-0.3 – 0.3) 2.00	1.1 ± 0.8 (0.0 – 2.0) 0.73	1.7 ± 0.5 (0.9 – 2.4) 0.29	3.1 ± 0.3 (3.1 – 4.2) 0.10	4.2 ± 0.3 (3.6 – 4.7) 0.07
Conductivity (µS/cm)	311 ± 6 (306 – 317) 0.02	307 ± 32 (243 – 330) 0.10	260 ± 32 (223 – 302) 0.12	321 ± 11 (309 – 346) 0.03	294 ±19 (266 – 329) 0.06
pH	8.4 ± 0.3 (8.2 – 8.8) 0.03	8.3 ± 0.3 (7.9 – 8.8) 0.04	8.2 ± 0.3 (7.2 – 8.6) 0.04	8.4 ± 0.2 (8.0 – 8.8) 0.02	8.2 ± 0.2 (8.0 – 8.8) 0.02
Discharge (mL/min)	4.5 ± 6.4 (0.0 – 14.8) 1.42	3.4 ± 5.0 (0.0 – 14.4) 1.47	81.6 ± 111.7 (12.4 – 300.0) 1.37	18.8 ± 22.6 (5.2 – 60.0) 1.20	287.0 ± 343.7 (32.0 – 880.0) 1.20
Total hardness (mg/L)	169 ± 16 (171 – 185) 0.09	166 ± 10 (155 – 174) 0.01	142 ± 17 (162 – 118) 0.12	174 ± 11 (155 – 179) 0.06	162 ± 13 (140 – 178) 0.08
Ca^{2+} (mg/L)	59 ± 8 (51.6 – 67.3) 0.13	60 ± 7 (51.6 – 65.7) 0.12	54 ± 5. (47.3 – 60.4) 0.09	56± 3 (53.0 – 60.2) 0.05	56 ± 3 (52.4 – 58.5) 0.05
Cl^- (mg/L)	2.52 ± 0.90 (2.00 – 3.57) 0.36	2.35 ± 0.57 (2.00 – 3.00) 0.24	2.72 ± 1.44 (1.50 – 5.10) 0.51	2.01 ± 0.37 (1.50 – 2.55) 0.40	2.21 ± 0.29 (2.00 – 2.55) 0.13
NO_3^- (mg/L)	2.42 ± 0.33 (2.19 – 2.80) 0.14	2.47 ± 0.69 (1.86 – 3.22) 0.28	1.82 ± 0.36 (1.46 – 2.19) 0.20	2.79 ± 0.62 (2.41 – 3.88) 0.22	2.40 ± 0.41 (1.94 – 3.12) 0.17
SO_4^{2-} (mg/L)	3.58±1.73 (2.69 – 5.57) 0.48	1.04±1.00 (0.36 – 2.19) 0.96	2.02±0.96 (0.44 – 3.04) 0.47	3.54±1.79 (0.73 – 4.84) 0.50	4.02±1.31 (2.04 – 5.66) 0.32
PO_4^{3-} (mg/L)	0.007 ± 0.004 (0.003 – 0.010) 0.57	0.009 ± 0.006 (0.004 – 0.016) 0.66	0.006 ± 0.009 (0.000 – 0.023) 1.50	0.057 ± 0.109 (0.000 – 0.220) 1.91	0.009 ± 0.010 (0.000 – 0.028) 1.11
$CaCO_3$ (mg/L)	155 ± 18 (142 – 167) 0.12	145 ± 13 (137 – 154) 0.09	130 ± 10 (123 – 142) 0.08	162 ± 9 (156 – 172) 0.05	148 ± 5 (143 – 154) 0.03
DOC (mg/L)	3.72 ± 0.16 (3.61 – 3.84) 0.04	4.68	3.89 ± 0.92 (3.24 – 4.55) 0.24	2.95 ± 0.25 (2.78 – 3.13) 0.08	2.92 ± 0.39 (2.65 – 3.20) 0.13

As shown in Table 4.7, there are many significant correlations between measured parameters: high positive correlations ($r > 0.5$, $p < 0.01$) between $CaCO_3$ and conductivity, total hardness and conductivity, total hardness and $CaCO_3$, Ca^{2+} and total hardness, NO_3^- and conductivity, $CaCO_3$ and total hardness, and PO_4^{3-} and NO_3^- ; high negative correlations ($r < -0.5$, $p < 0.01$) between DOC and temperature, between $CaCO_3$ and SO_4^{2-}.

In total, 217 individuals belonging to different taxonomic groups were collected in the sampling period (Tabs. 4.8, 4.13).

Table 4.13: *List of taxa collected from drips in Snežna jama in 2006−2007 and their abundances (N). Troglomorphic species are shown in bold.*

Phylum/ Subphylum	***Class/ Subclass***	***Order***	***Family***	***Species***	***N***
Annelida	Oligochaeta				2
Arthropoda/ Chelicerata	Arachnida/ Micrura	Acarina/ Hydracarina			14
Arthropoda/ Crustacea	Maxillipoda/ Copepoda	Cyclopoida	Cyclopidae	***Speocyclops infernus***	69
		Harpacticoida	Canthocamptidae	***Bryocamptus* sp.**	112
		Nauplia			8
	Malacostraca/ Eumalacostraca	Amphipoda	**Niphargidae**	***Niphargus* cf. *scopicauda***	3
Arthropoda/ Hexapoda	Entognatha	Collembola	Isotomidae		3
	Insecta	Coleoptera	cf. Lathriidae		1
		Diptera			1
		Psocoptera			3
		Thysanoptera			1

Copepods were the most abundant group, representing 87% of all collected animals, and about 90% of all troglomorphic animals. In addition to copepods, 28 individuals of at least eight other invertebrate species of terrestrial and aquatic taxonomic groups were found (Oligochaeta, Amphipoda, Hydracarina, Insecta and Collembola). Amphipods were found at only one site, SJ5, where the highest temperature, discharge and sulphate ions concentration were measured (Tab. 4.12). The highest abundance of Copepoda and the other two troglomorphic taxa (Hydracarina and Collembola) were found at the sampling site SJ3 with relatively low temperatures (up to 2.4 °C), low conductivity (up to 302 µS/cm) and low values of carbonates (up to 142 mg/L) in comparison with the other sites (Tab. 4.12).

A total of 189 copepods, including nauplia, belonging to two species (*Speocyclops infernus* and *Bryocamptus* sp.) were collected (Tabs. 4.9, 4.13).

As shown in Figure 4.5, probably all present copepod species were collected in the sampling period with first samples: all accumulation curves reach the asymptote at two species.

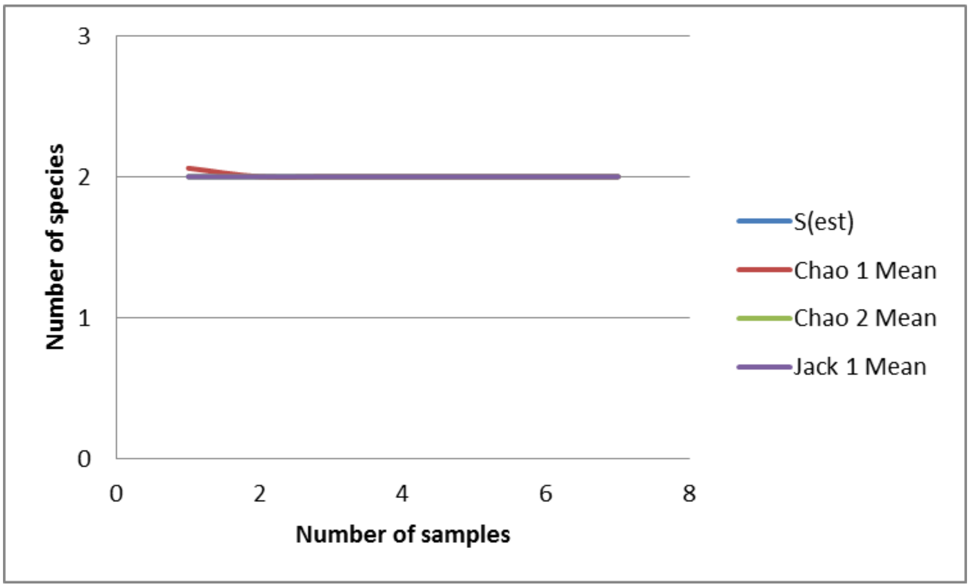

Figure 4.5: *Species accumulation curves (estimates of species richness, Chao1, Chao2, Jackknife1) for monthly samples in Snežna jama.*

Copepods were found in three of five drips (Tabs. 4.12, 4.14), in 17 of 28 samples. Overall, the total flux of copepods in the cave was 0.68 copepods per day. Most cyclopoids (*S. infernus*) were recorded at the beginning of summer (in June), while harpacticoids (*Bryocamptus* sp.) were most abundant in June and in September.

Table 4.14: *Copepod abundance at each sampling site in Snežna jama (- = no sample; * = sampling impossible due to the ice).On the last line the mean number of copepods collected each day (flux per day) in sampling sites and in the cave is expressed.*

Date/Sampling site	**SJ1**	**SJ2**	**SJ3**	**SJ4**	**SJ5**	**Total**
27/10/2006	0	13	-	0	5	**18**
08/12/2006	*	1	-	0	1	**2**
06/01/2007	*	*	2	0	8	**10**
28/04/2007	*	0	18	0	8	**26**
14/06/2007	*	24	12	0	11	**47**
05/08/2007	0	7	17	0	4	**28**
23/09/2007	0	24	25	0	9	**58**
Total	0	69	74	0	46	**189**
Flux per day	0	0.24	0.26	0	0.18	0.68

Both mature and immature individuals were found; first represented by males with mature spermatophore and females with attached spermatophores; immature individuals were represented by many juveniles (38% of the total copepod abundance) and nauplia (4% of the total copepod abundance). No pre-copulating pairs and only one ovigerous female of *Speocyclops infernus* were found. The abundance of males and females with mature attached spermatophores was highly correlated with temperature ($r = 0.8$, $p < 0.05$).

Copepods were found nearly throughout the entire range of pH, discharge and measured chemical properties of the drips, but not in the drips with high conductivity (over 300 μS/cm; SJ4) and high values of $CaCO_3$ concentration (over 160 mg/L; SJ1 and SJ4), and, of course, in frozen drips.

The PCA in Figure 4.6 shows the relation between copepod abundance and measured environmental parameters. High positive correlations between copepods and DOC and Cl^-, and negative correlations between copepod abundance and SO_4^{2-}, PO_4^{3-}, carbonate and temperature were found (Fig. 4.6, Tab. 4.13). Sampling sites SJ2 and SJ3 were influenced by concentration of Cl^- and values of DOC. In these sampling sites most copepods were collected. Higher sulphate, phosphate and nitrate concentrations characterize sampling sites SJ4 and SJ5. Besides, the sampling site SJ4 is characterized by higher temperatures.

Low but significant correlation was found between copepod abundance and $CaCO_3$ concentration ($r = -0.32$, $p < 0.05$, Tab. 4.10, Fig. 4.6). No significant correlation was found between copepod abundance and the measured ion concentrations, despite larger amounts of copepods were registered in sampling sites were concentration of ions NO_3^-, SO_4^{2-} and PO_4^{3-} were higher. In this cave, DOC concentration (Tabs. 4.6, 4.12) was generally high (from 2.6 mg/L to 4.7 mg/L).

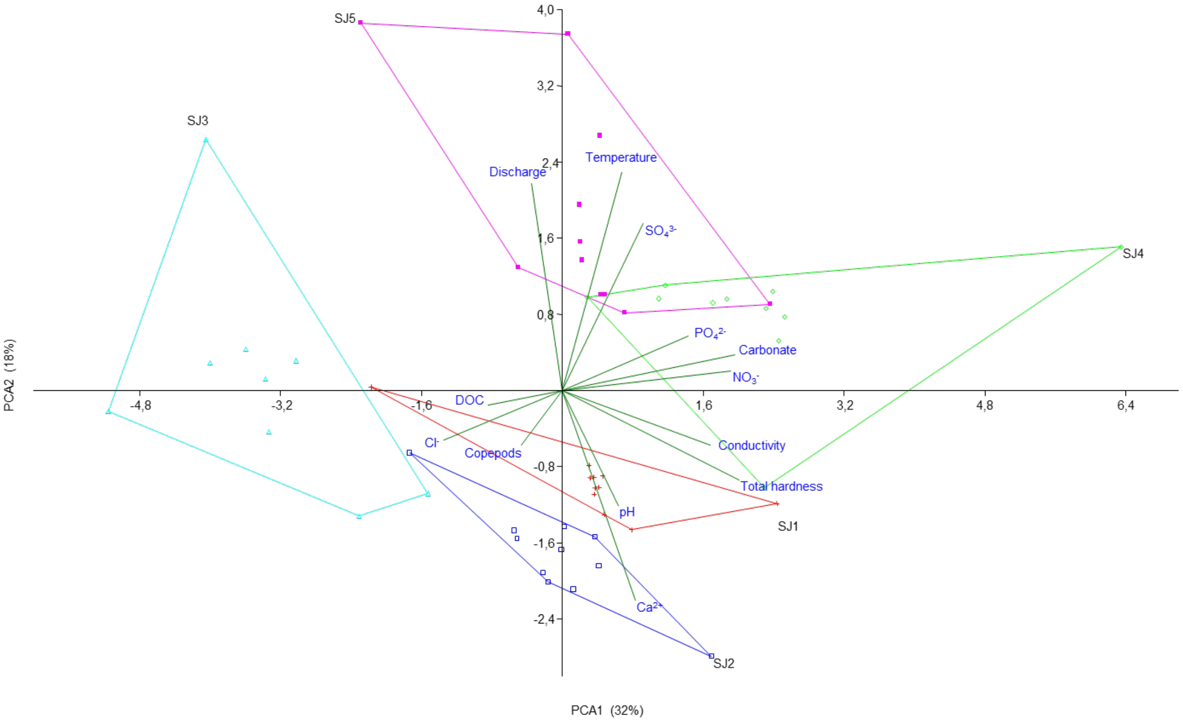

Figure 4.6: P*rincipal component analysis (PCA) showing relation between copepod population and physico-chemical parameters in Snežna jama. The PCA explains the 50% of the variance (32% with the first axis, PCA1, and 18% with the second, PCA2).*
Green vectors with common origin represent measured parameters;
Points represent samples; polygons include samples from each sampling site:
Copepod population of each sampling site is specified below:
Red = SJ1 = no copepods;
Blue = SJ2 = Bryocamptus sp. + Speocyclops infernus;
Light blue = SJ3= Bryocamptus sp. + Speocyclops infernus;
Green = SJ4 = no copepods;
Pink = SJ5 = Bryocamptus sp. + Speocyclops infernus.

4.3.2. Jama pod Babjim zobom

Characteristics of the measured physical and chemical parameters of dripping water at five sampling sites in Jama pod Babjim zobom are shown in Table 4.15. The minimum temperature (1.2 °C) was found at the sampling site close to the entrance to the cave (JPBZ1) in January 2007. At this sampling site, temperature was the most variable in comparison with other sampling sites (CV = 0.36). The highest (6.6 °C) and more constant (CV = 0.03) temperature value was measured in May 2010 at JPBZ5, the sapmling site located in the deeper position in the cave.

Table 4.15: *Ranges of physical and chemical measurements of five drips in Jama pod Babjim zobom (2007-2008, 2010-2013). CV = variation of parameters in the time at sampling sites. Drips with copepods are marked with asterisks (*).*

Parameter / Drip **Mean±SD** **(min−max)** CV	**JPBZ1**	**JPBZ2***	**JPBZ3***	**JPBZ4***	**JPBZ5***
Temperature (°C)	4.4 ± 1.6 (1.2 – 5.5) 0.36	4.6 ± 1.3 (2.0 – 5.7) 0.28	5.0 ± 0.6 (3.7 – 5.6) 0.12	5.6 ± 0.2 (5.3 – 5.9) 0.03	6.2 ± 0.2 (6.0 – 6.6) 0.03
Conductivity (µS/cm)	273 ± 10 (259 – 292) 0.04	260 ± 18 (238 – 296) 0.07	250 ± 22 (222 – 281) 0.09	250 ± 17 (227 – 276) 0.07	227 ± 23 (191 – 257) 0.10
pH	8.4 ± 0.1 (8.2 – 8.6) 0.01	8.3 ± 0.3 (7.9 − 8.8) 0.03	8.5 ± 0.3 (8.1 – 8.8) 0.03	8.1 ± 1.0 (5.6 – 8.9) 0.12	8.4 ± 0.3 (7.9 − 8.8) 0.03
Discharge (mL/min)	2.8 ± 2.0 (0.6 – 6.0) 0.71	5.1 ± 6.4 (0.0 – 16.2) 1.25	18.8 ± 4.9 (7.8 – 26.0) 0.26	15.4 ± 9.2 (1.8 – 29.4) 0.60	13.8 ± 5.9 (5.8 – 26.2) 0.43
Total hardness (mg/L)	136 ± 9 (130 – 146) 0.07	134 ± 7 (126 − 140) 0.05	138 ± 16 (128 − 156) 0.11	139 ± 7 (134 − 147) 0.05	129 ± 13 (115 – 142) 0.10
Ca^{2+} (mg/L)	48 ± 2 (47.0 – 51.0) 0.05	53 ± 3 (50.0 − 55.0) 0.05	55 ± 6 (50.0 − 62.0) 0.12	56 ± 3 (54.0 − 59.0) 0.05	52 ± 6 (46.0 − 58.0) 0.11
Cl$^-$ (mg/L)	4.00 ± 2.00 (2.00 − 6.00) 0.50	4.17 ± 4.19 (1.50 − 9.00) 1.00	2.37 ± 0.25 (2.00 − 2.50) 0.10	2.65 ± 0.24 (2.50 − 3.00) 0.09	2.13 ± 0.48 (1.50 − 2.50) 0.22
NO$_3^-$ (mg/L)	1.53 ± 0.11 (1.40 – 1.60) 0.07	6.33 ± 4.28 (1.40 − 9.10) 0.68	4.85 ± 1.00 (3.60 − 5.80) 0.21	0.70 ± 0.22 (0.40 − 0.90) 0.31	1.65 ± 0.44 (1.20 − 2.20) 0.27
SO$_4^{2-}$ (mg/L)	5.63 ± 0.68 (5.10 – 6.40) 0.12	2.20 ± 3.39 (0.00 − 6.10) 1.54	2.77 ± 1.42 (1.90 − 4.40) 0.51	2.88 ± 1.60 (0.90 − 4.70) 0.55	3.88 ± 1.61 (1.60 − 5.20) 0.41
PO$_4^{3-}$ (mg/L)	0.050 ± 0.078 (0.000 − 0.140) 1.56	0.020 ± 0.017 (0.010 − 0.040) 0.05	0.018 ± 0.029 (0.000 − 0.060) 1.61	0.018 ± 0.015 (0.010 − 0.040) 0.83	0.010 ± 0.008 (0.000 − 0.020) 0.80
CaCO$_3$ (mg/L)	124 ± 7 (115 – 130) 0.06	160 ± 70 (110 − 245) 0.44	124 ± 9 (115 − 135) 0.07	125 ± 6 (120 – 130) 0.05	118 ± 7 (115 − 125) 0.06
DOC (mg/L)	1.99	3.10	2.28	3.37	-

Conductivity was varying between 191 µS/cm (at JBPZ5) and 296 µS/cm (at JPBZ2). Values of pH varied between 8.2 and 8.4. More variable was discharge ($0.26 < CV < 1.25$): generally low, varied from 0 mL/min to a maximum of 29.4 mL/min (measured in March 2006 at JPBZ4). DOC concentration varied between 1.99 mg/L and 3.37 mg/L.

Many higher significant positive correlations ($r > 0.5$, $p < 0.01$; Tab. 4.7) were found (precipitation *versus* temperature, Cl$^-$ *versus* conductivity, Ca^{2+} and total hardness, NO$_3^-$ *versus* carbonate, PO$_4^{3-}$ and Cl$^-$, DOC *versus* Ca^{2+}). Negative correlation ($r = -0.51$, $p < 0.01$) was calculated between DOC and SO$_4^{2-}$.

In Jama pod Babjim zobom, in all five investigated drips, we found 361 individuals belonging to eight aquatic and terrestrial classes (Tabs. 4.8, 4.16).

Amphipoda were collected at sampling site JPBZ5 where highest temperature (between 6.0 °C and 6.6 °C) was registered (Tab. 4.15).

Table 4.16: *List of taxa collected from five drips in Jama pod Babjim zobom in 2006−2007 and their abundance (N). Troglomorphic species are shown in bold.*

Phylum/ Subphylum	**Class/ Subclass**	**Order**	**Family**	**Species**	**N**
Annelida	Oligochaeta				1
Nematoda					32
Mollusca	Gastropoda				2
Arthropoda/ Chelicerata	Arachnida/ Micrura	**Acarina/ Hydracarina**			4
Arthropoda/ Crustacea	Maxillipoda/ Copepoda	Cyclopoida	Cyclopidae	***Speocyclops infernus***	35
		Harpacticoida	Canthocamptidae	***Elaphoidella* sp.1**	176
				***Elaphoidella* sp.2**	100
	Malacostraca/ Eumalacostraca	Amphipoda	Niphargidae	***Niphargus* cf. *cornicolanus***	5
Arthropoda/ Hexapoda	Entognatha	Collembola	Isotomidae		
	Insecta	Coleoptera	Carabidae/Trechinae	***Anophthalmus micklitzi micklitzi* (Ganglbauer, 1913)**	4
		Diptera (larvae)			2

The most abundant class was Copepoda, represented by 86% of collected animals; with three species: *Speocyclops infernus* and two species of the genus *Elaphoidella* (Tab. 4.9).

Figure 4.7 shows that probably all potentially present copepod species were collected after the first sample, as all accumulation curves reached the asymptote at three species.

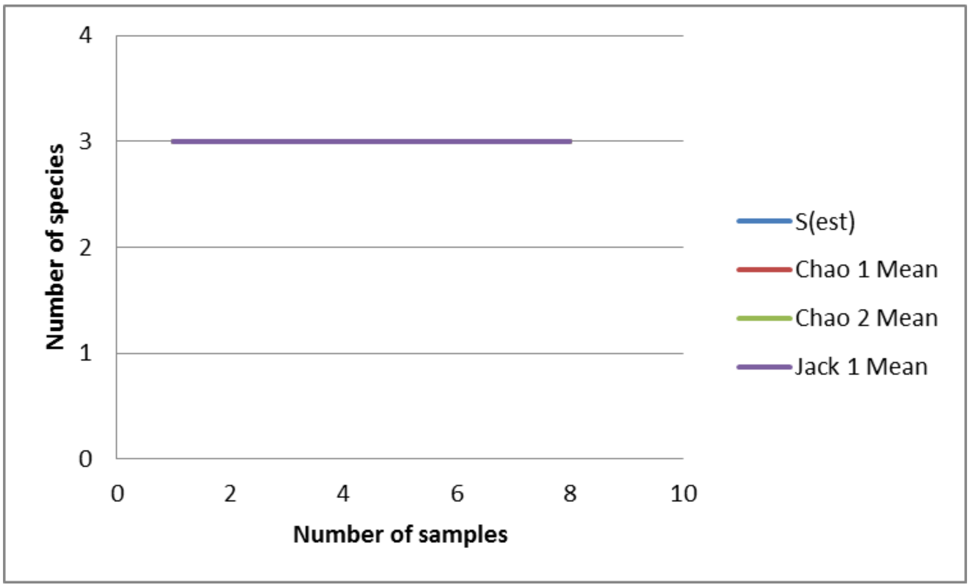

Figure 4.7: *Species accumulation curves (estimates of species richness, Chao1, Chao2, Jackknife1) for monthly samples in Jama pod Babjim zobom.*

Overall, the average rate of copepods was, approximately, 0.3 copepods per drip per day; 1.52 copepods per day throughout the cave (Tab. 4.17).

Table 4.17: *Copepod abundance and their flux per day at each sampling site in Jama pod Babjim zobom.*

Date/ Sampling site	JPBZ1	JPBZ2	JPBZ3	JPBZ4	JBPZ5	Total
30/092007	0	0	6	1	8	**15**
16/12/2007	0	1	15	0	20	**36**
17/02/ 2008	0	0	34	0	1	**35**
10/04/2008	0	0	18	0	4	**22**
01/06/2008	0	0	4	0	1	**5**
27/07/2008	0	0	112	0	5	**117**
11/10/ 2008	0	0	49	0	17	**66**
16/11/2008	0	0	8	0	7	**15**
Total	0	1	246	1	63	**311**
Flux per day	0	0.003	0.67	0.003	0.17	**1.52**

Presence of copepods was positively correlated with discharge ($r = 0.28$, $p < 0.05$; Tab. 4.10). Only low but statistically significant correlation was found between copepod abundance and discharge ($r = 0.28$, $p < 0.05$).

At the sampling site JPBZ1, where discharge was very low (between 0.6 mL/min and 6 mL/min), no copepods were found. Discharge was frequently below 3 mL/min at JPBZ2 and JPBZ4, where only one copepod was collected in all sampling period (Tab. 4.17). Sampling site JPBZ3 was the richest in copepods, with 80% of the total abundance; discharge never decreased under 7.8 mL/min (Tab. 4.15). Higher amount of copepods was found in correlation with higher temperatures at JPBZ3 and JPBZ5. At sampling site JPBZ1, where no copepods were collected, higher concentrations of PO_4^{3-} up to 0.14 mg/L were measured.

The PCA in Figure 4.8 shows the relation between measured parameters and copepod abundance.

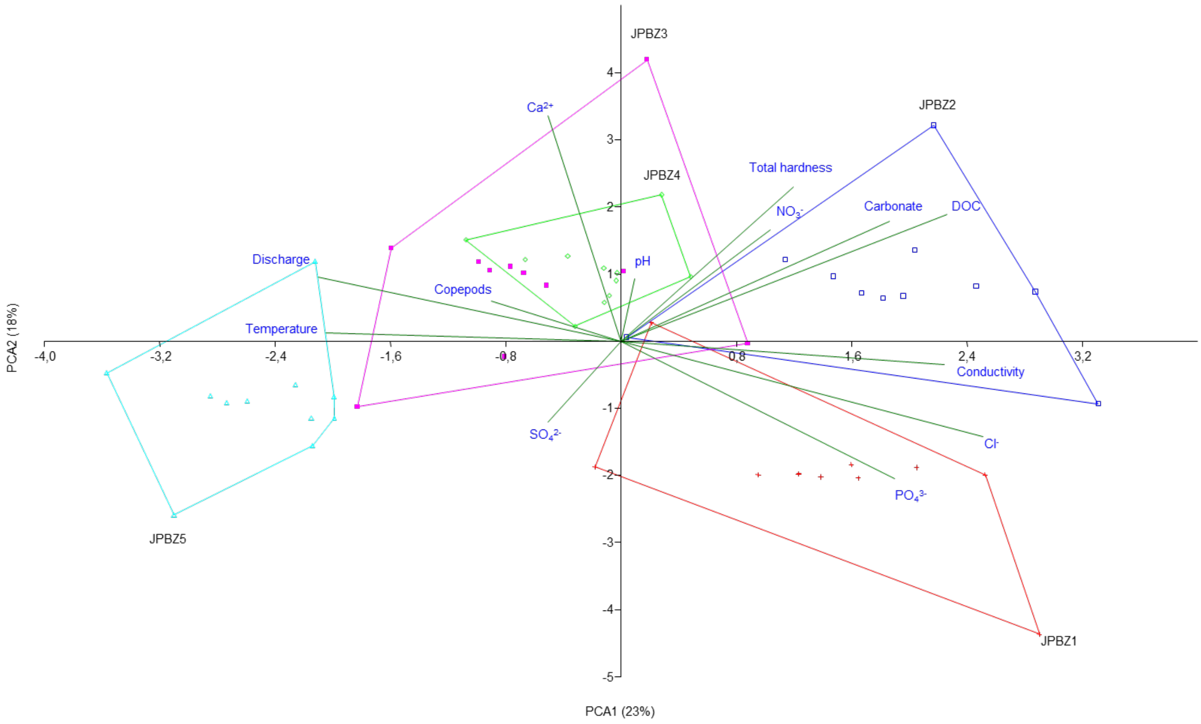

Figure 4.8: *Principal component analysis (PCA) showing relation between copepod population and physico-chemical parameters in Jama pod Babjim zobom. The PCA explains 41% of variance (23% with the first axis, PCA1, and 18% with the second, PCA2).*
Green vectors with common origin represent measured parameters;
Points represent samples; polygons include samples from each sampling site;
Copepod population of each sampling site is specified below:
Red = JPBZ1 = no fauna;
Blue = JPBZ2 = Elaphoidella sp.1*;*
Pink = JPBZ3 = Elaphoidella sp.1 + *Elaphoidella* sp.2 + *Speocyclops infernus;*
Green = JPBZ4 =Elaphoidella sp.2*;*
Light blue = JPBZ5= Elaphoidella sp.1 + *Elaphoidella* sp. + *Speocyclops infernus.*

High positive correlation is shown between copepod abundance and discharge (confirming results from calculation of Pearson correlation coefficient) and temperature. Negative correlation is shown between copepod abundance and PO_4^{3-} and Cl^-.

4.3.3. Zadlaška jama

The descriptive statistics of the physical and chemical parameters measured at five sampling sites in Zadlaška jama is shown in Table 4.18.

Table 4.18: *Ranges of physical and chemical measurements of five drips in Zadlaška jama (2006-2007). (- = no data due to low discharge). Drips with copepods are marked with asterisks (*).*

Parameter/Drip **Mean±SD** **(min−max)** CV	**ZJ1***	**ZJ2**	**ZJ3***	**ZJ4**	**ZJ5***
Temperature (°C)	10.6 ± 0.2 (10.4 − 10.8) 0.02	10.3 ± 0.4 (9.8 – 11.1) 0.04	10.4 ± 0.5 (9.8 – 11.1) 0.05	10.4 ± 0.4 (9.9 – 11.0) 0.04	10.1 ± 0.3 (9.7 – 10.5) 0.03
Conductivity (µS/cm)	298 ± 25 (276 – 334) 0.08	298 ± 50 (239 – 369) 0.17	270 ± 25 (241 – 308) 0.09	313 ± 35 (247 – 348) 0.11	258 ± 27 (228 – 293) 0.10
pH	7.9 ± 0.2 (7.6 – 8.2) 0.02	8.0 ± 0.2 (7.7 – 8.3) 0.02	7.9 ± 0.2 (7.7 – 8.2) 0.02	8.0 ± 0.2 (7.7 – 8.2) 0.02	7.9 ± 0.3 (7.4 – 8.2) 0.04
Discharge (mL/min)	0.1 ± 0.1 (0.0 – 0.2) 1.00	1.0 ± 1.0 (0.0 – 2.0) 1.00	0.9 ± 1.4 (0.0 – 3.8) 1.35	3.7 ± 4.8 (0.0 – 12.0) 1.30	0.5 ± 0.7 (0.2 – 2.0) 1.40
Total hardness (mg/L)	-	150	128	163	124
Ca hardness (mg/L)	-	57	44	60	45
Cl^- (mg/L)	-	2.50	1.50	2.00	2.00
NO_3^- (mg/L)	-	2.45	2.45	2.22	2.12
SO_4^{2-} (mg/L)	-	3.78	3.98	3.87	4.53
PO_4^{3-} (mg/L)	-	0.07	0.01	0.06	0.09

In the cave discharge was low, varying from 0 mL/min to 12 mL/min (measured in December 2006 at sampling site ZJ5). Due to low discharge, sampling for measuring DOC and $CaCO_3$ concentration was impossible, and other chemical analyses were performed only once (where possible), at the end of the sampling period (November 2007). Temperature was in mean values the higher among caves sampled in Slovenia (Tab. 4.1, Fig. 4.1), rising to 11.1 °C at ZJ2 and ZJ3.

Conductivity was varying between 228 μS/cm and 348 μS/cm. In this cave the lowest pH mean value between sampled caves was calculated (Tab. 4.2).

Negative correlations ($r < -0.5$, $p < 0.01$) were calculated between: SO_4^{3-} and total hardness, Ca^{2+} and NO_3^-, and PO_4^{3-} and NO_3^- (Tab. 4.7). Positive correlations ($r > 0.5$, $p < 0.01$) were found between: precipitation and temperature, total hardness and conductivity, Ca^{2+} and total hardness, Cl^- and Ca^{2+}, PO_4^{3-} and Cl^- and SO_4^{2-} (Tab. 4.7).

In investigated drips 19 specimens belonging to six orders were collected (Tab. 4.19).

Table 4.19: *List of taxa collected from five drips in Zadlaška jama in 2006-2007 and their abundance (N). Troglomorphic species are shown in bold.*

Phylum/ Subphylum	***Class/ Subclass***	***Order***	***Family***	***Species***	***N***
Arthropoda/ Crustacea	Maxillipoda/ Copepoda	Cyclopoida	Cyclopidae	***Speocyclops infernus***	4
		Harpacticoida	Canthocamptidae	***Bryocamptus* sp.**	1
				Moraria alpina	1
	Malacostraca	Isopoda			3
Arthropoda/ Hexapoda	Entognatha	Collembola	**cf. Sminthuridae**		2
			Isotomidae		2
	Insecta	Coleoptera	Carabidae	*Laemostenus (Antisphodrus) schreibersi* (Küster)	3
			Larvae		2
		Diptera			1

Three isopods were collected at sampling sites ZJ1, ZJ2 and ZJ4. Collected Collembola belong to two different families: Sminthuridae (in ZJ2) and Isotomidae (in ZJ5). Copepods, Isopods and Collembola showed some modifications to subterranean life, like depigmentation and absence of eyes.

As was the case in other caves the most abundant class was Copepoda (with 6 individuals) representing 34% of collected animals. Copepods belong to three species: *Speocyclops infernus* (adults and young individuals), *Bryocamptus* sp. and *Moraria alpina* (one adult individual).

Figure 4.9 shows that probably not all potentially present copepod species were collected in the sampling period, as accumulation curves do not reach an asymptote.

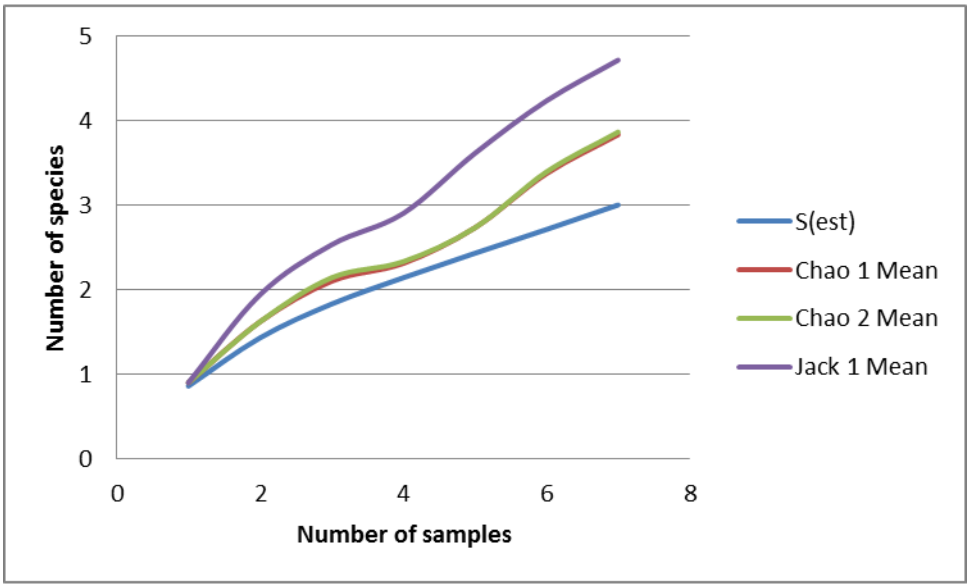

Figure 4.9: *Species accumulation curves (estimates of species richness, Chao1, Chao2, Jackknife1) for monthly samples in Zadlaška jama.*

Copepods were found in three of five drips, in 6 of 35 samples (Tab. 4.20); harpacticoids only in ZJ3 and cyclopoids in ZJ1 and ZJ5. Overall, the average ratio of copepods was under 0.1 individual per drip per day. No copepods were collected at sampling sites ZJ2 and ZJ4, where total hardness of water was higher than in other sampling sites (150 mg/L and 163.5 mg/L respectively, Tab. 4.18).

Table 4.20: *Copepod abundance and their mean flux per day at each sampling site in Zadlaška jama.*

Date/Sampling site	ZJ1	ZJ2	ZJ3	ZJ4	ZJ5	Total
05/10/2006	0	0	0	0	1	**1**
16/12/2006	1	0	0	0	0	**1**
25/02/2007	1	0	0	0	0	**1**
15/05/2007	0	0	0	0	0	**0**
11/08/2007	0	0	1	0	0	**1**
16/09/2007	0	0	0	0	1	**1**
08/12/2007	0	0	1	0	0	**1**
Total	**2**	**0**	**2**	**0**	**2**	**6**
Flux per day	0.005	0	0.005	0	0.005	**0.015**

Negative correlations between copepod abundance and Cl^- and PO_4^{3-} was found (r = -0.4 $p < 0.05$) (Tab. 4.10).

In Figure 4.10 relation between copepod population and measured environmental parameters is shown using PCA. In ZJ1 temperature was higher than in other sampling sites in the cave and higher nitrates concentration in comparison to other sampling sites was measured. In ZJ5 the highest concentration of sulphates was measured. Copepods and Cl^-, discharge and parameters involved in dissolution of $CaCO_3$ are negatively correlated (Fig. 4.10, Tab. 4.10).

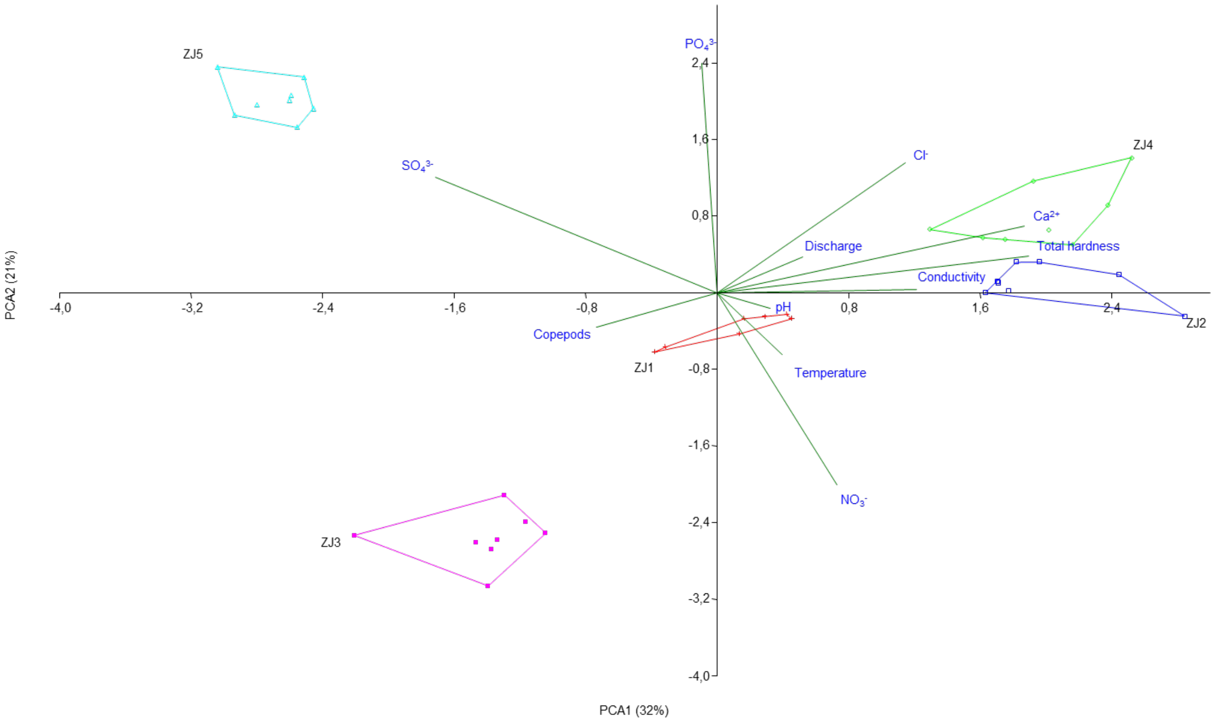

Figure 4.10: *Principal component analysis (PCA) showing relation between copepod population and physico-chemical parameters in Zadlaška jama. The PCA explains the 53% of variance (21% with the first axis, PCA1, and 32% with the second, PCA2).*
Green vectors with common origin represent measured parameters;
Points represent samples; polygons include samples from each sampling site;
Copepod population of each sampling site is specified below:
Red = ZJ1 = Speocyclops infernus;
Blue = ZJ2 = no fauna;
Pink = ZJ3 = Bryocamptus sp. + *Moraria alpina;*
Green = ZJ4 = no fauna;
Light blue = ZJ5 = Speocyclops infernus.

4.3.4. Pološka jama

The descriptive statistics of the physical and chemical parameters measured in Pološka jama is shown in Table 4.21. The minimum temperature (2.4 °C) was found at the sampling site PJ1 in January 2008, and the highest values were at the first three sampling sites during spring season. Discharge varied from 1.8 mL/min (at PJ1) to 300 mL/min (measured in May 2007 at PJ4). At this cave lower total hardness was measured in comparison with other caves (mean total hardness = 121 mg/L, Tab. 4.4).

Table 4.21: *Ranges of physical and chemical measurements of five drips in Pološka jama (2006-2008, 2011-2013) (- = no data due to low discharge).*

Parameter/Drip **Mean±SD** **(min-max)** **CV**	**PJ1**	**PJ2**	**PJ3**	**PJ4**	**PJ5**
Temperature (°C)	5.4 ± 1.7 (2.4 – 6.8) 0.31	5.2 ± 1.6 (2.5 – 6.8) 0.31	5.8 ± 1.0 (3.6 – 6.8) 0.17	5.1 ± 1.5 (2.6 – 6.6) 0.20	5.1 ± 1.3 (3.0 – 6.4) 0.25
Conductivity (µS/cm)	289 ± 14 (262 – 309) 0.05	247 ± 5 (241 – 253) 0.02	208 ± 9 (197 – 221) 0.04	204 ± 6 (196 – 212) 0.03	207 ± 5 (200 – 216) 0.02
pH	8.3 ± 0.3 (7.7 – 8.6) 0.04	8.3 ± 0.2 (8.0 – 8.6) 0.02	8.3 ± 0.3 (7.8 – 8.7) 0.04	8.3 ± 0.3 (7.9 – 8.7) 0.04	8.2 ± 0.3 (7.7 – 8.7) 0.04
Discharge (mL/min)	2.2 ± 0.2 (1.8 – 2.4) 0.10	9.1 ± 3.9 (6.8 – 17.4) 0.43	114.4 ± 114.0 (10.6 – 270.0) 1.00	129.7 ± 126.8 (10.8 – 300.0) 1.00	4.8 ± 0.8 (3.6 – 6.0) 0.17
Total hardness (mg/L)	152	124 ± 8 (118 – 130) 0.07	117 ± 2 (116 – 119) 0.02	113 ± 5 (110 – 117) 0.04	109
Ca^{2+} (mg/L)	50	42± 3 (39 – 44.5) 0.08	50 ± 14.8 (39 – 60.8) 0.30	35 ± 0.7 (34.9 – 36) 0.02	35
Cl^- (mg/L)	2.50	2.50	2.50	2.00	-
NO_3^- (mg/L)	11.58 ± 6.22 (7.2 – 15.97) 0.54	9.36 ± 7.14 (4.30 – 14.42) 0.76	1.73 ± 0.07 (1.66 – 1.80) 0.04	2.34 ± 0.49 (2.00 – 2.69) 0.21	2.44 ± 0.07 (2.40 – 2.48) 0.03
SO_4^{2-} (mg/L)	3.38	5.60 ± 0.07 (3.51 3.60) 0.01	2.70 ± 0.56 (2.10 – 2.94) 0.21	3.72 ± 0.99 (3.00 – 4.45) 0.27	2.02
PO_4^{3-} (mg/L)	0.08	0.24 ± 0.19 (0.11 – 0.38) 0.79	0.20 ± 0.20 (0.06 – 0.34) 1.00	0.43 ± 0.54 (0.05 – 0.81) 1.25	0.05
$CaCO_3$ (mg/L)	-	110	105	100	*
DOC (mg/L)	3.81	3.07	2.85	2.62	2.58

Significant negative correlation between PO_4^{3-} and Cl^-, and many positive correlations between measured parameters result (Tab. 4.7).

In Pološka jama, in all five investigated drips, we found 78 individuals belonging to six aquatic (86%) and terrestrial taxa (Tabs. 4.8, 4.9, 4.22). The most abundant were copepods (56%) and insects (24%). Amphipoda were collected at sampling sites PJ1, PJ3 and PJ5. At the sampling point PJ3 many individuals of the Orthoptera *Troglophilus neglectus* with eggs were found gathered on the collecting device during a spring season.

Table 4.22: L*ist of taxa collected from five drips in Pološka jama in 2006−2007 and their abundance (N). Troglomorphic species are shown in bold.*

Phylum/ Subphylum	***Class/ Subclass***	***Order***	***Family***	***Species***	***N***
Nematoda					2
Arthropoda/ Chelicerata	Arachnida				3
Arthropoda/ Crustacea	Maxillipoda/ Copepoda	Cyclopoida	Cyclopidae	***Speocyclops infernus***	6
		Harpacticoida	Canthocamptidae	***Bryocamptus* sp.**	18
				***Lessinocamptus* sp.**	20
	Malacostraca/ Eumalacostraca	Amphipoda	Bogidiellidae	***Bogidiella* sp.**	4
	Malacostraca	Isopoda			6
Arthropoda/ Hexapoda	Insecta	Orthoptera		*Troglophilus neglectus*	19

Three copepod species were collected in all sampling sites in the cave (Tab 4.23): *Speocyclops infernus*, and two not determinated harpacticoid species belonging to genera *Bryocamptus* and *Lessinocamptus* (Tab 4.9).

Table 4.23: *Copepod abundance and their mean flux per day at each sampling site in Pološka jama.*

Date/Sampling site	**PJ1**	**PJ2**	**PJ3**	**PJ4**	**PJ5**	**Total**
28.05.2007	2	3	0	0	0	**5**
15.08.2007	0	7	0	1	0	**8**
16.09.2007	0	2	0	0	0	**2**
8.12.2007	0	4	0	0	0	**4**
20.01.2008	0	5	1	0	2	**8**
02.03.2008	0	1	0	1	1	**3**
10.05.2008	0	7	0	0	2	**9**
20.07.2008	0	4	1	0	0	**5**
Total	**2**	**33**	**2**	**2**	**5**	**44**
Flux per day	0.003	0.06	0.003	0.003	0.008	0.07

In Figure 4.11 is shown that the sampling period was probably sufficient for collecting all present copepod species: accumulation curves reached an asymptote (at three species), with the exception of Jackknife1 species accumulation curve.

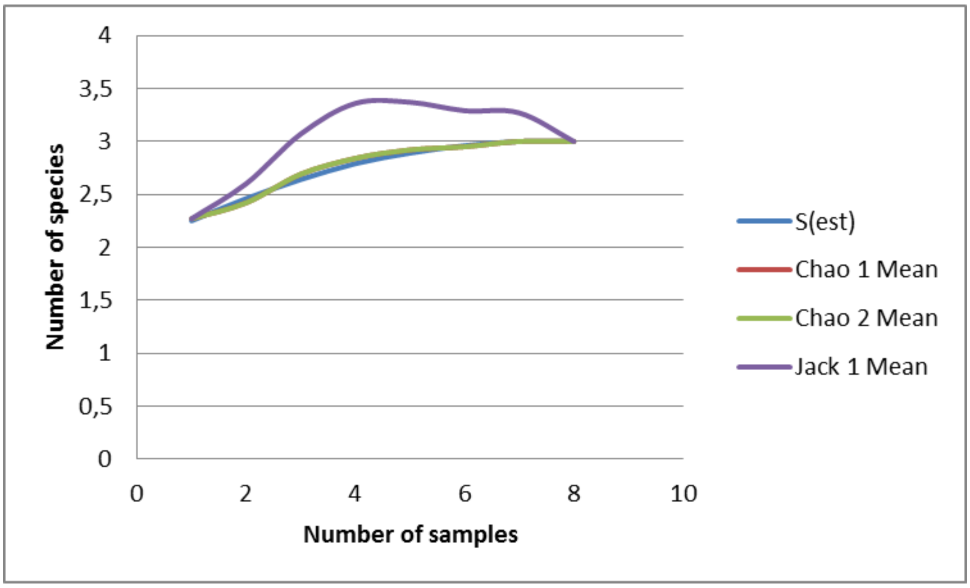

Figure 4.11: *Species accumulation curves (estimates of species richness, Chao1, Chao2, Jackknife1) for monthly samples in Pološka jama.*

Copepods were found nearly throughout the entire range of pH, discharge and chemical qualities of the drips measured in the cave. Positive correlation was calculated between copepod abundance and $CaCO_3$ ($r = 0.66$; $p < 0.01$) (Tab. 4.10) and low positive and less significant ($p < 0.05$) correlation was found between copepod abundance and NO_3^- ($r = 0.36$). Major copepod abundance was registered in correspondence of lower discharge and in sampling sites with higher concentration of NO_3^- and $CaCO_3$ (positive correlations with copepod abundance in both statistical analysis: calculation of Pearson correlation coefficient and PCA) (Tabs. 4.10, 4.21, 4.23, Fig. 4.12).

The PCA in Figure 4.12 shows correlations between measured parameters and copepod abundance. PJ1 was the sampling site in the cave with the highest total hardness (152 mg/L), higher Ca^{2+} concentration (50 mg/L) and higher conductivity (rising to 309 µS/cm). At this sampling site discharge was always below 2.4 mL/min, the maximum concentration of ion NO_3^- and DOC (15.97 mg/L and 3.81 mg/L, respectively) were measured.

The highest number of copepods was found at PJ2 (33 individuals), where the highest $CaCO_3$ concentration was measured. In other sampling sites mean copepod flux per day was below 0.01 (Tab. 4.23). At that sampling sites discharge was more variable (rising to 300 ml/min in PJ3 and PJ4) and total hardness was lower in comparison with other sampling sites (Tab. 4.21).

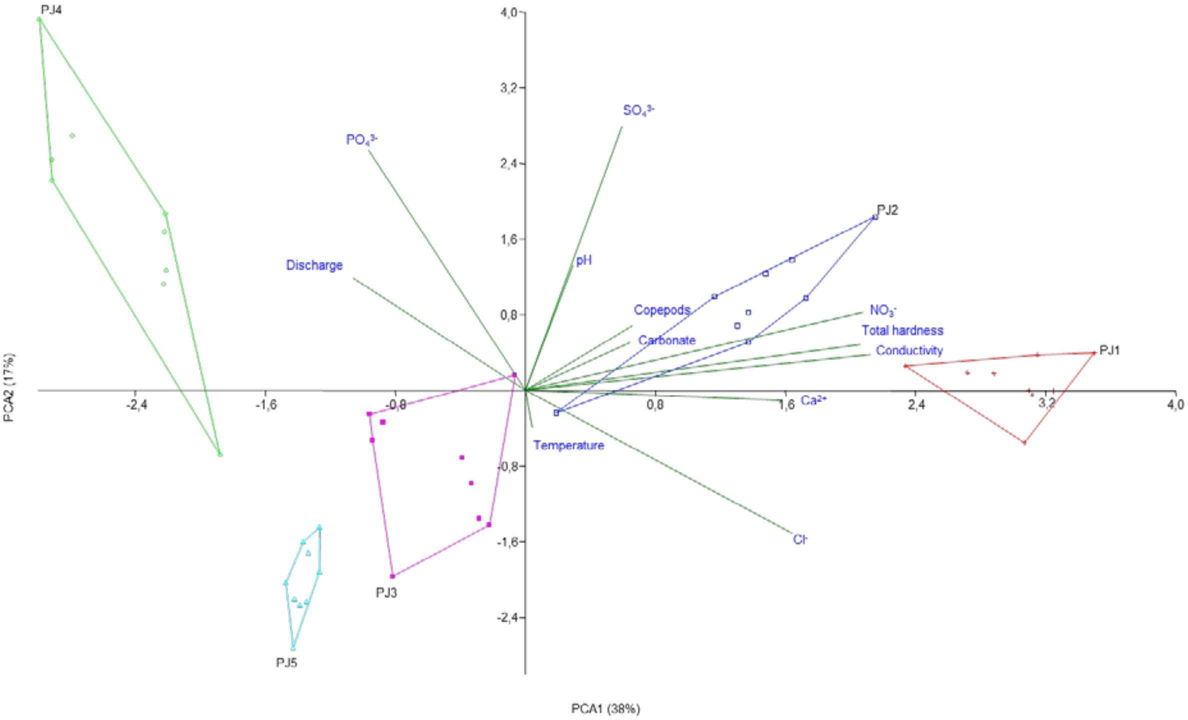

Figure 4.12: *Principal component analysis (PCA) showing relation between copepod population and physico-chemical parameters in Pološka jama. The PCA explains 55% of the variance (38% with the first axis, PCA1, and 17% with the second, PCA2).*
Green vectors with common origin represent measured parameters;
Points represent samples; polygons include samples from each sampling site;
Copepod population of each sampling site is specified below:
Red = PJ1 = Bryocamptus sp. + *Lessinocamptus* sp.*;*
Blue = PJ2 = Lessinocamptus sp. + *Speocyclops infernus;*
Pink = PJ3 = Bryocamptus sp.*;*
Green = PJ4 = Bryocamptus sp.*;*
Light blue = PJ5 = Bryocamptus sp.

4.3.5. Summary of correlations between copepod abundance and measured parameters in monitored caves in Slovenia

As shown in Figure 4.13, the majority of copepods were found in summer times.

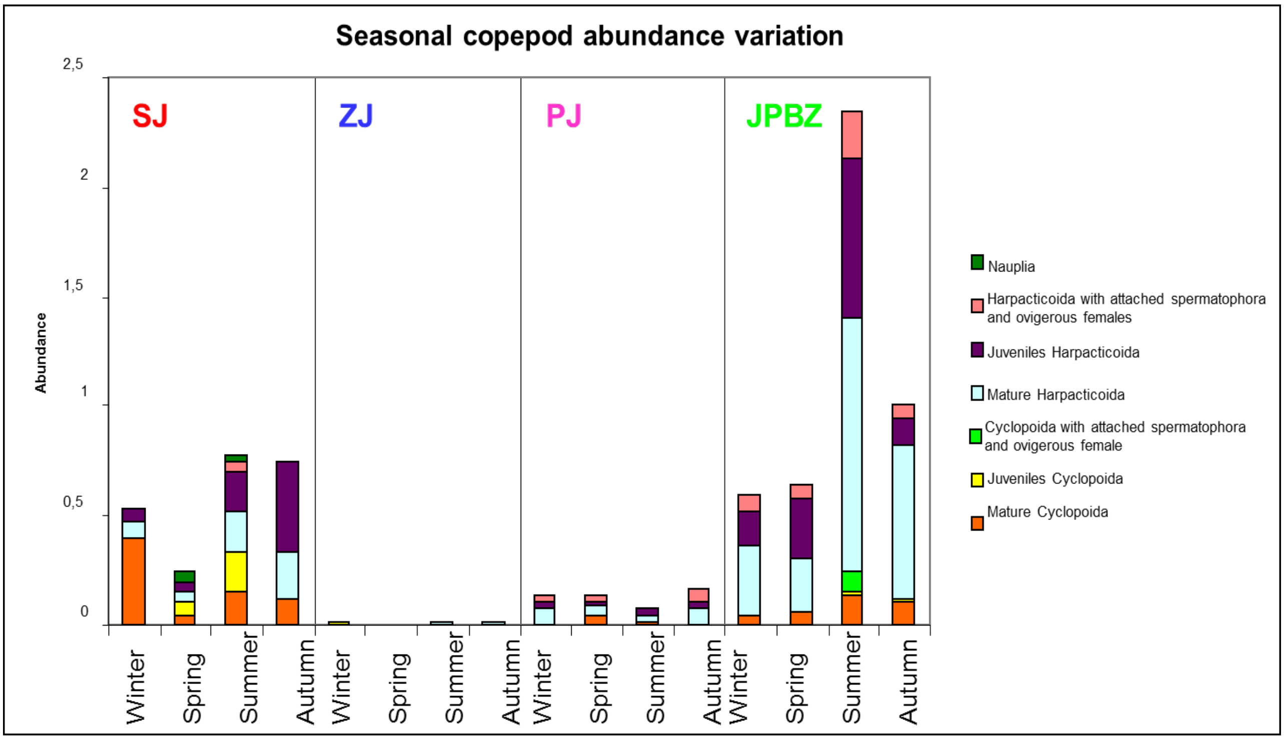

Figure 4.13: *Seasonal copepod abundance variation in Slovene caves* (*relative copepod abundances at different maturity stadia are evidenced with different colors*).

Juveniles were collected through the whole sampling year, nauplia were found only in Snežna jama and in Jama pod Babjim zobom in summer time (Fig. 4.13). Presence of juveniles and nauplia was positively correlated ($r > 0.50$; $p < 0.01$) with higher concentration of DOC and with conductivity, total hardness, Ca^{2+} and higher amounts of $CaCO_3$. Copepod abundance was positively correlated with SO_4^{2-} concentration ($r = 0.21$; $p < 0.01$) (Tab. 4.10).

The PCA in Figure 4.14 shows relations between copepod populations of each trickle in caves located in Slovenia, and measured environmental variables.

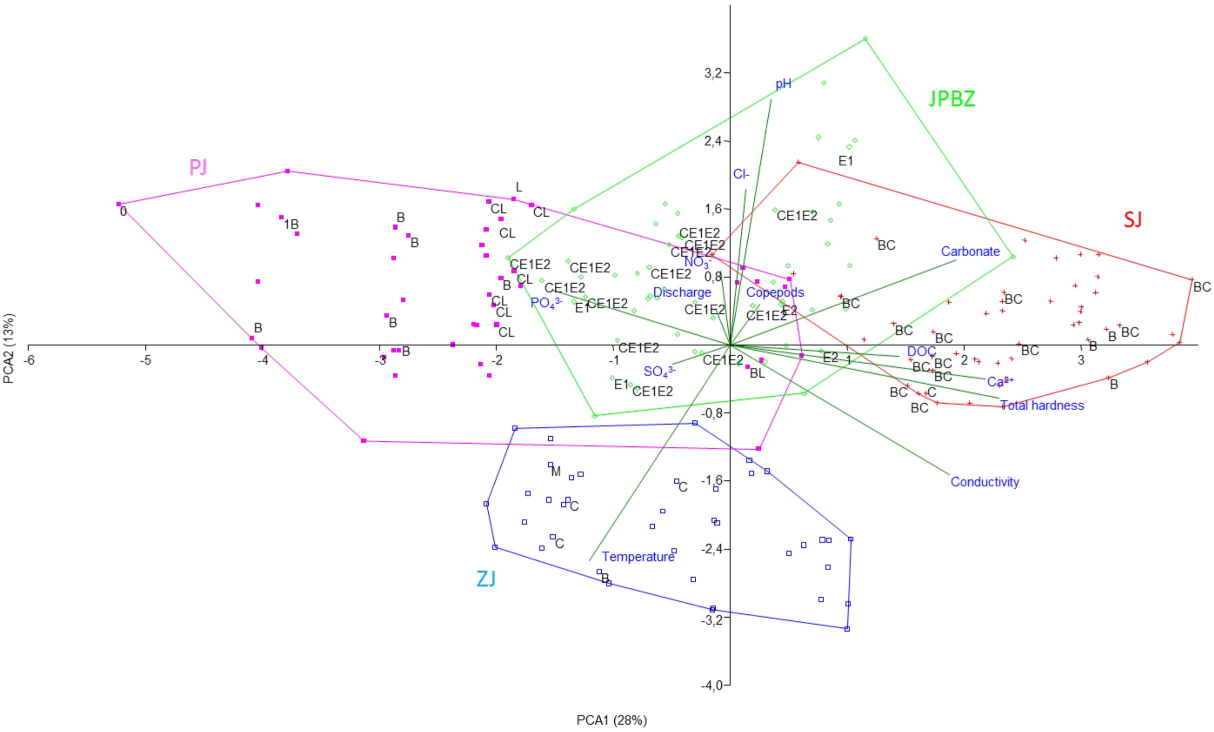

Figure 4.14: *Principal component analysis (PCA) showing relation of copepod abundance in trickles in relation to measured environmental variables. The PCA explains 41% of variance (28% with the first axis, PCA1, and 13% with the second one, PCA2).*
Green vectors with common origin represent measured parameters;
Points represent samples;
Polygons include samples from each cave:
Red = Snežna jama (SJ);
Green = Jama pod Babjim zobom (JPBZ);
Blue = Zadlaška jama (ZJ);
Pink = Pološka jama (PJ).
Copepod population of each sample is specified with letters as described below:
No label = samples without copepods;
C = Speocyclops infernus;
B = Bryocamptus sp.;
M. = *Moraria alpina;*
E1 and E2 = two species of genus Elaphoidella;
L = Lessinocamptus sp.

As shown in Table 4.24, the first axis (PC1) is mainly determined by discharge (loading = 0.999) and pH (loading = -0.285), and the second one (PC2) by conductivity (loading = 0.912) and total hardness (loading = 0.392).

Table 4.24: *Loadings from principal component analyses shown in Fig. 4.14 (Main parameters that determine the position of axes are evidenced in bold).*

Parameter	**Loading**	
	PC 1	**PC 2**
Temperature	-0.003	-0.008
Discharge	**0.999**	0.039
pH	**-0.285**	-0.001
Conductivity	-0.038	**0.912**
$CaCO_3$	-0.0002	0.006
Total hardness	-0.009	**0.392**
Ca^{2+}	-0.004	**0.115**
Cl^-	-0.001	-0.001
NO_3^-	-0.003	0.001
SO_4^{2-}	0.0002	0.0004
PO_4^{3-}	0.0002	-0.001
DOC	0.004	0.005
Copepods abundance	0.0398	0.0002

In the PCA plot (Fig 4.14) it is evident how each cave is characterized by a different environmental parameter combination. Overlapped areas represent similar environmental characteristics.

In Jama pod Babjim zobom, samples with copepods (two species of *Elaphoidella* and *S. infernus*) are not correlated with any measured parameter. In Snežna jama, total hardness, DOC concentration and $CaCO_3$ are correlated to copepod abundance. In Zadlaška jama copepod abundance was correlated with temperature. Pološka jama is characterized by higher concentration of phosphate ions than in other caves. In this cave the presence of *Bryocamptus* (B in Fig. 4.14) is correlated with phosphate concentration and *Speocyclops infernus* (C in Fig. 4.14) and *Lessinocamptus* sp. (L in Fig.4.14). The more central position in the plot is occupied by those copepod species that are more common and not dependent on either of the described parameters. Total copepod abundance was positively correlated with $CaCO_3$ concentration and pH and negatively correlated with temperature and sulphate.

A comparation between Pearson correealtion coefficient (data from Tab. 4.10, columns SLO, SJ, JPBZ, PJ, ZJ) and PCA (Figs. 4.6, 4.8,4.10, 4.12, 4.14) is shown in Table 4.25. Only a few correlations are confirmed by both analyses: the positive correlation between copepod abundance and discharge in Jama pod Babjim zobom, the negative correlation between copepod abundance and Cl^- in Zadlaška jama and the positive correlations between copapod abundance and both NO_3^- and $CaCO_3$ in Pološka jama.

Table 4.25: *Correlations between copepods abundance and measured parameters based on Pearson correlation coefficient (r) (data from Tab. 4.10) and Principal component analysis (PCA). (+ = positive correlation, - = negative correlation).*

Parameter/caves	**SLO**		**SJ**		**JPBZ**		**ZJ**		**PJ**	
Correlation	***r***	**PCA**	***r***	**PCA**	***r***	**PCA**	***r***	**PCA**	***r***	**PCA**
Temperature		-		-		+				
Conductivity						-		-		+
pH		+								+
Discharge				-	+	+		-		
Total hardness								-		+
Ca^{2+}				+		+		-		
Cl^-		+		+		-	-	-		
NO_3^-									+	+
SO_4^{2-}	+	-		-						+
PO_4^{3-}				-		-	-			
$CaCO_3$		+	-						+	+
DOC				+						

4.4. Results from Italy

In all thirteen drips investigated in Grotta A del Ponte di Veja, Grotta di Roverè Mille and Covolo della Croce, 595 individuals, belonging to both aquatic and terrestrial invertebrate taxa were found (Tab. 4.8): Nematoda, Clitellata, Arachnida, Amphipoda, Copepoda, Ostracoda, Insecta, Diplopoda and Gastropoda. The most abundant were copepods, represented by 226 individuals, 38% of total collected specimens. In explored caves eight different species of copepods were collected: two Cyclopoida (*Speocyclops infernus* and *Paracyclops imminutus*), and six Harpacticoida (*Bryocamptus (Rheocamptus) zschokkei tatrensis*, *Lessinocamptus insoletus*, *Maraenobiotus brucei*, *Moraria poppei*, *Moraria stankovitchi* and *Moraria* sp.A) (Tab. 4.9).

4.4.1. Grotta A del Ponte di Veja

The descriptive statistics of the physical and chemical parameters measured in Grotta A del Ponte di Veja is shown in Table 4.26.

Temperature, conductivity and pH were stable (CV < 0.3, Tab. 4.6). Mean temperature value was the highest among measured caves (12.8 ° C) (Tab. 4.1, Fig. 4.1). More variable was discharge, ranging from 0.2 mL/min (PV4) to 62 mL/min (measured in May 2008 at PV3) (0.5 < CV < 1.03).

Table 4.26: *Ranges of physical and chemical measurements of five drips in Grotta A del Ponte di Veja (2007−2008, 2011, 2013). (- = no data). CV = coefficient of variation (represents the time variability of the parameter at the sampling site).*

Parameter / Drip **Mean±SD** **(min−max)** CV	**PV1**	**PV2**	**PV3**	**PV4**	**PV5**
Temperature (°C)	11.7 ± 1.8 (8.9 – 14.3) 0.15	11.8 ± 1.2 (9.7 – 13.0) 0.10	11.9 ± 0.5 (11.2 – 12.8) 0.04	11.6 ± 0.4 (11.3 – 12.6) 0.03	11.5 ± 0.5 (11.2 – 12.7) 0.04
Conductivity (µS/cm)	304 ± 84 (140 – 400) 0.28	266 ± 53 (150 – 330) 0.20	594 ± 923 (150 – 2870) 1.55	295 ± 118 (170 – 440) 0.003	311 ± 98 (140 – 400) 0.31
pH	8.5 ± 0.6 (7.6 – 9.0) 0.07	8.5 ± 0.6 (7.5 – 9.0) 0.07	8.5 ± 0.5 (7.5 – 8.9) 0.06	8.6 ± 0.1 (8.4 – 8.7) 0.01	8.5 ± 0.5 (7.5 – 8.9) 0.06
Discharge (mL/min)	7.8 ± 5.6 (1.0 – 19.2) 0.72	2.4 ± 1.2 (1.1 – 4.8) 0.50	20.0 ± 20.6 (0.6 – 62.0) 1.03	0.3 ± 0.3 (0.2 – 1.0) 1.00	0.5 ± 0.5 (0.2 – 1.6) 1.00
Total hardness (mg/L)	157	127	140	175	-
Ca^{2+} (mg/L)	62	50	57	71	-
Cl^- (mg/L)	2.50	1.50	2.00	2.50	-
NO_3^- (mg/L)	28.09	3.73	7.83	3.31	-
SO_4^{2-} (mg/L)	5.10	4.80	3.90	-	-
PO_4^{3-} (mg/L)	0.20	0.16	0.31	-	-
$CaCO_3$ (mg/L)	130	120	130	160	-
DOC (mg/L)	1.72	0.95	2.19	-	1.88

In the cave 498 individuals belonging to different terrestrial and aquatic taxa (Arachnida, Amphipods, Collembola, Copepods, Diptera, Gastropods, Isopods, Nematodes, Oligochaeta, and Insects) were collected (Tabs. 4.8, 4.27).

Table 4.27: L*ist of taxa collected from five drips in Grotta A del Ponte di Veja in 2007−2008 and their abundance (N). Troglomorphic species are shown in bold.*

Phylum/ Subphylum	***Class/ Subclass***	***Order***	***Family***	***Species***	***N***
Annelida	Oligochaeta				45
Nematoda					11
Arthropoda/ Chelicerata	Arachnida/ Micrura	**Acarina/ Hydracarina**			4
Arthropoda/ Crustacea	Maxillipoda/ Copepoda	Cyclopoida	Cyclopidae	***Speocyclops infernus***	13
				Paracyclops imminutus	7
		Harpacticoida	Canthocamptidae	*Bryocamptus zschokkei (Rheocamptus) tatrensis* (Minkiewicz, 1916)	36
				Lessinocamptus insoletus	25
				Maraenobiotus brucei	44
				Moraria poppei	1
				Moraria stankovitchi	1
				Moraria **sp.A**	1
		Not identified			71
	Malacostraca/ Eumalacostraca	**Amphipoda**			5
	Malacostraca	Isopoda			9
Arthropoda/ Hexapoda	Entognatha	Collembola			67
	Insecta	Diptera			17
			Larvae		59
		Other			5
Mollusca	Gastropoda				77

The most abundant taxon was Copepoda, with 199 individuals, collected in all five sampling sites (Tab. 4.28). Collected species belonged to Cyclopoida, from family Cyclopidae (*Paracyclops imminutus* and *Speocyclops infernus*) and Harpacticoida, family Canthocamptidae (*Bryocamptus zschokkei tatrensis*, *Lessinocamptus insoletus*, *Maraenobiotus brucei*, *Moraria poppei*, *Moraria stankovitchi* and *Moraraia* sp.A) (Tabs. 4.9, 4.27).

Table 4.28: *Copepod abundance and their mean flux per day in five drips in Grotta A del Ponte di Veja.*

Date/Sampling site	PV1	PV2	PV3	PV4	PV5	Total
04/11/2007	0	0	11	1	0	**12**
15/01/2008	3	4	13	0	0	**20**
27/03/2008	0	8	0	0	1	**9**
04/05/2008	0	9	7	2	0	**18**
10/06/2008	1	0	4	4	7	**16**
22/07/2008	1	0	0	0	4	**5**
27/08/2008	2	0	2	0	1	**5**
01/10/2008	6	47	0	0	0	**53**
Total	**13**	**68**	**37**	**7**	**13**	**138**
Flux per day	0.03	0.19	0.26	0.02	0.04	0.31

As shown in Figure 4.15, accumulation curves do not reach the asymptote. It means that, in this cave, the total number of copepod species was probably underestimated and additional sampling would be necessary to collect all potentially present species.

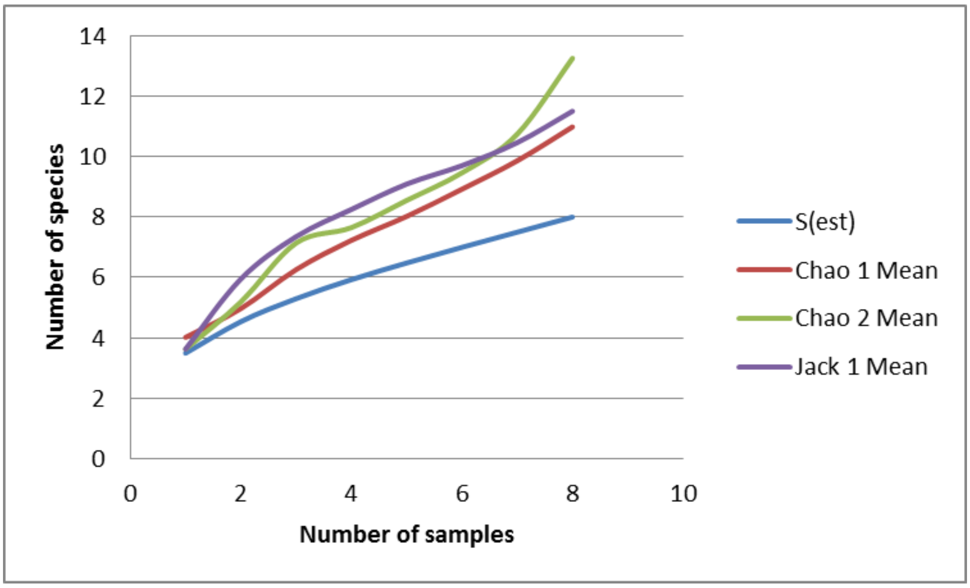

Figure 4.15: *Species accumulation curves (estimates of species richness, Chao1, Chao2, Jackknife1) for monthly samples for Grotta A del Ponte di Veja.*

With regard to copepod population, the most populated and diverse drip was PV2, with 68 copepods collected in the sampling period, belonging to four species: *Speocyclops infernus*, *Bryocamptus (Rheocamptus) zschokkei tatrensis*, *Maraenobiotus brucei* and *Moraria poppei*.

As shown in Table 4.10, higher significant positive correlations ($r > 0.5$, $p < 0.05$) were calculated between: *Lessinocamptus insoletus* and discharge ($r = 0.68$) and PO_4^{3-} ($r = 0.59$), *Maraenobiotus brucei* and *Bryocamptus (Rheocamptus) zschokkei tatrensis* ($r = 0.75$), and *M. stankovitchi* and *Lessinocamptus insoletus* ($r = 0.57$). Low positive correlation was calculated between harpacticoid abundance and DOC (Tab. 4.10). *Speocyclops infernus* abundance was correlated ($r = 0.36$, $p < 0.05$) with the number of *Lessinocamptus insoletus*. Negative correlation ($r < -0.5$, $p < 0.01$) was calculated between *Lessinocamptus insoletus* and SO_4^{2-}, and the presence of *Bryocamptus (Rheocamptus) zschokkei tatrensis* and $CaCO_3$, total hardness, Ca^{2+} and Cl^-.

The PCA in Figure 4.16 shows relation between copepod abundance and measured environmental parameters. Copepods are positively correlated with pH and negatively correlated with conductivity, total hardness, Ca^{2+}, Cl^- and $CaCO_3$ (Fig. 4.17, Tab 4.27). In sampling site PV2 low conductivity (between 150 and 330 μS/cm), low total hardness (127 mg/L), low concentration of ions (between 0.16 and 4.8 mg/L) and low DOC (0.95 mg/L) were measured. Troglobiotic copepod species (37 individuals in total) together with Amphipoda were collected at PV3 (where DOC and phosphate concentration were higher; 2.19 mg/L and 0.31 mg/L respectively) and PV5. Sampling site PV4, the less populated (0.02 copepods per day in mean), is associated with higher total hardness (175 mg/L) and Ca^{2+} concentration (71 mg/L). In PV1 the concentration of nitrate (28.09 mg/L) and sulphate (5.1 mg/L) ions was higher than in other sampling sites in the cave.

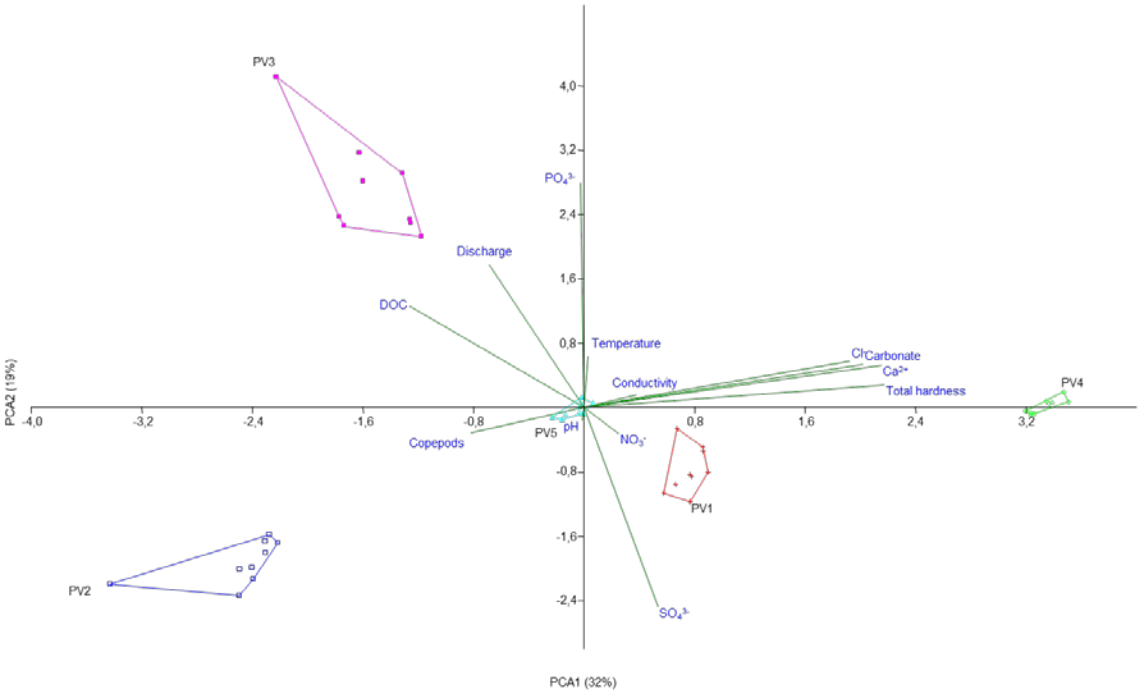

Figure 4.16: *Principal component analysis (PCA) showing relation between copepod population and physico–chemical parameters in Grotta A del Ponte di Veja. The PCA explains the 51% of the variance (32% with the first axes, PCA1, and 19% with the second, PCA2).*
Green vectors with common origin represent measured parameters;
Points represent samples; polygons include samples from each sampling site;
Copepod population of each sampling site is described below:
Red = PV1 = Bryocamptus (Rheocamptus) zschokkei tatrensis, Maraenobiotus brucei;
Blue = PV2 = Paracyclops imminutus, Bryocamptus (Rheocamptus) zschokkei tatrensis, Maraenobiotus brucei, Moraria poppei;
Pink = PV3 = Speocyclops infernus, Bryocamptus (Rheocamptus) zschokkei tatrensis, Lessinocamptus insoletus, Moraria stankovitchi;
Green = PV4 = Maraenobiotus brucei;
Light blue = PV5 = Speocyclops infernus, Bryocamptus (Rheocamptus) zschokkei tatrensis, Maraenobiotus brucei, Moraria sp.A.

4.4.2. Covolo della Croce

The descriptive statistics of parameters measured in Covolo della Croce is shown in Table 4.29. Due to low discharge (never rising over 5.6 mL/min) in this cave, sampling of water for chemical analyses was performed only once (in October 2011). Temperature of dripping water measured in the cave (from 10.1 °C to 16.9 °C; 0.08 < CV < 0.15) was high if compared to values measured at the cave located around the same altitude as Jama pod Babjim zobom, in Slovenia (Fig. 4.1). In

Covolo della Croce higher absolute value of DOC concentration among monitored caves was measured (5.69 mg/L, at CC1).

Table 4.29: *Ranges of physical and chemical measurements of five drips in Covolo della Croce (2008, 2011). CV = coefficient of variation (represents the time variability of the parameter at the sampling site).*

Parameter / Drip **Mean±SD** **(min−max)** CV	**CC1**	**CC2**	**CC3**
Temperature (°C)	11.9 ± 1.8 (10.1 – 13.7) 0.15	12.0 ± 1.0 (11.2 – 13.1) 0.08	14.0 ± 2.0 (12.3 – 16.9) 0.14
Conductivity (µS/cm)	363 ± 11 (350 – 370) 0.03	303 ± 58 (270 – 370) 0.19	285 ± 66 (240 – 380) 0.23
pH	8.6 ± 0.7 (7.8 – 9.0) 0.08	8.7 ± 0.4 (8.2 – 9.0) 0.04	8.5 ± 0.6 (7.9 – 9.1) 0.07
Discharge (mL/min)	0.5 ± 0.5 (0.0 – 0.2) 1.00	0.1 ± 0.1 (0.0 – 0.2) 1.00	1.5 ± 2.7 (0.2 – 5.6) 1.80
Total hardness (mg/L)	171	182	237
Ca^{2+} (mg/L)	62	70	89
Cl^{-} (mg/L)	3.6	2.5	2.5
NO_3^{-} (mg/L)	0.16	0.90	0.20
SO_4^{2-} (mg/L)	4.6	4.6	5.3
PO_4^{3-} (mg/L)	0.00	0.00	0.01
$CaCO_3$ (mg/L)	160	170	225
DOC (mg/L)	5.69	3.07	2.35

A total of 62 individuals were collected in the sampling period (Tab. 4.30). Collected taxa were both aquatic (40% of the total faunal composition, represented by Nematoda and Copepoda) and terrestrial (60% of the total faunal composition, represented by Anellida, Arachnida, Diplopoda, Insecta) (Tab. 4.8). The most abundant group was Insecta (73%).

Table 4.30: *List of taxa collected from three drips in Covolo della Croce (2008) and their abundance (N). Troglomorphic animals are shown in bold.*

Phylum/ Subphylum	*Class/ Subclass*	*Order*	*Family*	*Species*	*N*
Annelida	Oligochaeta				1
Nematoda					3
Arthropoda/ Chelicerata	**Arachnida/ Micrura**				1
Arthropoda/ Crustacea	Maxillipoda/ Copepoda	Cyclopoida	Cyclopidae	***Speocyclops infernus***	4
		Harpacticoida	Canthocamptidae	***Lessinocamptus insoletus***	1
				***Moraria* sp. A**	3
		Not identified			3
Arthropoda/ Miriapoda	Diplopoda				1
Arthropoda Hexapoda	Insecta				45

Only eight individuals of Copepoda (mean flux of copepods per day = 0.04), belonging to three different troglomorphic species were collected in the sampling period (Tabs. 4.30, 4.31): *Speocyclops infernus* (at CC2 and CC3), *Lessinocamptus insoletus* (at CC2) and *Moraria* sp.A (at CC1 and CC3).

Table 4.31: *Copepod abundance and their mean flux per day in three drips in Covolo della Croce.*

Date/Sampling site	CC1	CC2	CC3	Total
10.06.2008	1	2	2	**5**
22.07.2008	0	0	3	**3**
27.08.2008	0	0	0	**0**
01.09.2008	0	0	0	**0**
Total	**1**	**2**	**5**	**8**
Flux per day	<0.01	<0.01	0.03	0.04

Because of low number of samples, statistical analyses for estimation of species richness (accumulation curves) were impossible to do. Pearson correlation coefficient shows many positive and negative correlations between measured parameters (Tab. 4.7). Presence of copepods was positively correlated with pH (r = 0.59, $p < 0.05$). Abundance of *Moraria* sp.A was correlated with pH ($r = 0.54$, $p < 0.05$) and abundance of *Speocyclops infernus* was correlated with the presence of

Moraria sp.A ($r = 0.64$, $p < 0.05$) (Tab.4.10).

The PCA in Figure 4.18 shows correlations between copepod abundance and measured parameters. In CC1 (in red) higher DOC concentration (5.69 mg/L) but lower copepod presence than in other sampling sites were observed. CC2 (in blue) was characterized by high concentration of nitrates (0.9 mg/L); CC3 (in pink) with higher $CaCO_3$ (225 mg/L), temperature (from 12.3 °C to 16.9 °C) and high total hardness (Ca^{2+} = 89 mg/L, total hardness = 237 mg/L) and higher copepod abundance (Tab. 4.31). Positive correlation between copepod abundance and pH from calculation of Pearson correlation coefficient is confirmed by the PCA too (Tab. 4.10, Fig. 4.17).

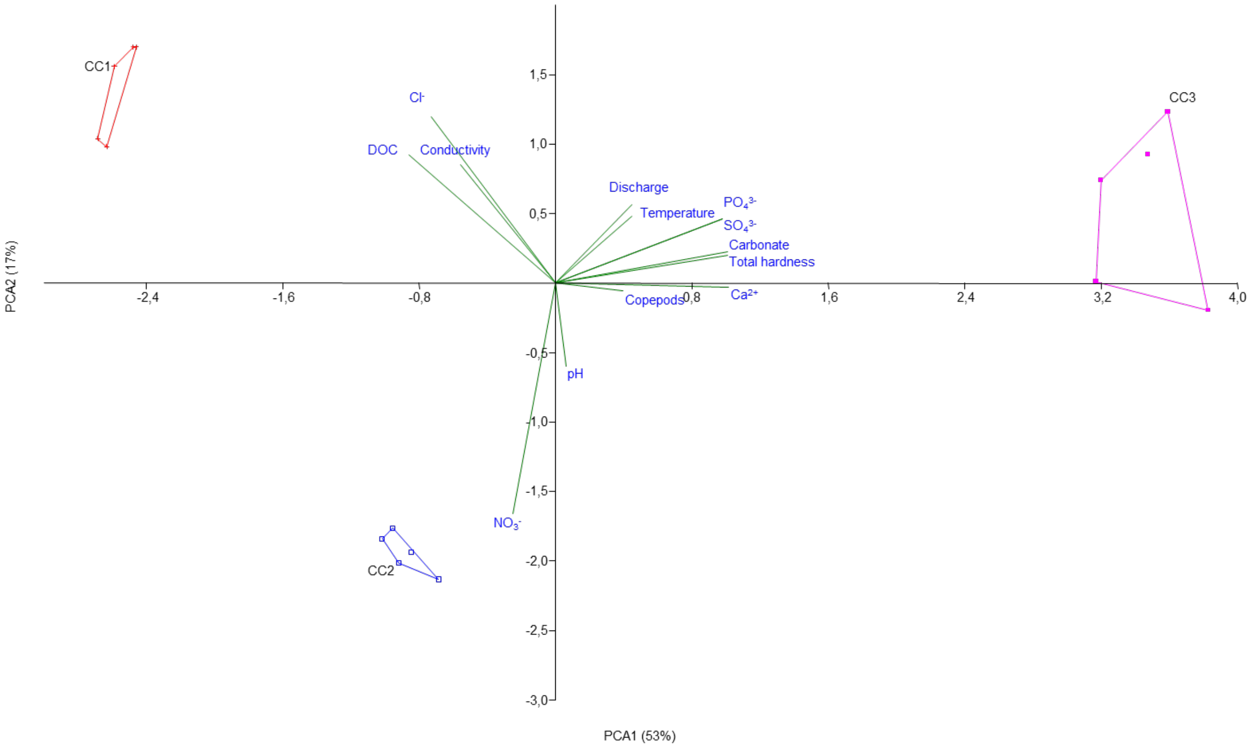

Figure 4.17: *Principal component analysis (PCA) showing relation between copepod population and physico–chemical parameters in Covolo della Croce. The PCA explains the 70% of the variance (53% with the first axes, PCA1, and 17% with the second, PCA2).*
Green vectors with common origin represent measured parameters;
Points represent samples;
Polygons include samples from each sampling site;
Copepod population of each sampling site is described below:
Red = CC1 = Moraria sp.A*;*
Blue = CC2 = Speocyclops infernus + Lessinocamptus insoletus;
Pink = CC3 = Speocyclops infernus + Moraria sp.A.

4.4.3. Grotta di Roverè Mille

The descriptive statistics of physical and chemical parameters measured at five sampling sites in Grotta di Roverè Mille is shown in Table 4.32.

Table 4.32: *Ranges of physical and chemical measurements of five drips in Grotta di Roverè Mille (2008, 2011). The drip with copepods is marked with an asterisk (*). CV = coefficient of variation (represents the time variability of the parameter at the sampling site).*

Parameter / Drip **Mean±SD** **(min−max)** CV	**RM1**	**RM2***	**RM3**	**RM4**	**RM5**
Temperature (°C)	11.0 ± 1.7 (9.1 – 12.2) 0.15	10.0 ± 0.5 (9.3 – 10.5) 0.05	10.1 ± 0.7 (9.3 – 10.7) 0.07	9.9 ± 0.4 (9.4 – 10.4) 0.04	10.1 ± 0.5 (9.4 – 10.6) 0.05
Conductivity (µS/cm)	303 ± 6 (300 – 310) 0.02	407 ± 61 (350 – 470) 0.15	352 ± 50 (300 – 400) 0.14	352 ± 51 (300 – 420) 0.14	347 ± 56 (290 – 400) 0.16
pH	8.3 ± 0.3 (7.9 – 8.5) 0.04	8.2 ± 0.4 (7.8 – 8.5) 0.05	7.9 ± 0.7 (7.3 – 8.5) 0.09	8.1 ± 0.4 (7.7 – 8.4) 0.05	8.0 ± 0.6 (7.3 – 8.5) 0.07
Discharge (mL/min)	0.1 ± 0.1 (0.0 – 0.2) 1.00	0.2 ± 0.1 (0.2 – 0.4) 0.50	0.4 ± 0.3 (0.2 – 0.8) 0.75	0.4 ± 0.4 (1.0 – 0.2) 1.00	0.3 ± 0.1 (0.2 – 0.4) 0.33
Total hardness (mg/L)	255	-	-	201	201
Ca hardness (mg/L)	91	-	-	80	80
Cl^- (mg/L)	2.50	-	-	-	-
NO_3^- (mg/L)	0.43	-	-	-	-
SO_4^{2-} (mg/L)	2.80	-	-	-	-
PO_4^{3-} (mg/L)	0.00	-	-	-	-
$CaCO_3$ (mg/L)	245	-	-	-	-
DOC (mg/L)	2.57	4.43	-	1.87	2.35

This cave is at the highest elevation (1,005 m a.s.l.) among the investigated Italian caves. Temperature was quite homogeneous in all five sampling sites ($0.04 < CV < 0.15$). The most variable temperature was observed at sampling site closer to the entrance to the cave (RM1), where the highest absolute temperature (12.2 °C) in the cave was observed too. In other sampling sites temperature varied between 9.3 °C and 10.7 °C. Temperatures measured in the cave, despite different altitudes of caves locations, are comparable with the ones measured in Zadlaška jama (300 m a.s.l.) in Slovenia (Tab. 4.1, Fig. 4.1).

Values of pH were not very variable among sampling sites and during the whole sampling period ($0.04 < CV < 0.09$), with values similar to other investigated caves (Tab.4.2). Conductivity varied between 290 µS/cm (measured at RM5 in July 2008) and 470 µS/cm (measured at RM2 in April 2008) (0.16 < CV < 0.02). Discharge was very low all along the cave and the maximum discharge (1 mL/min) was measured at the sampling site RM4 in August 2008 (0.33 < CV < 1). Due to low discharge, sampling for measuring DOC, and chemical analyses was performed only once (where possible), at the end of the sampling period (October 2008) or in October 2011.

Pearson correlation coefficient showed positive correlations between Ca^{2+} and total hardness ($r = 1$, $p < 0.01$), and between pH and conductivity ($r = 0.65$, $p < 0.01$) (Tab. 4.7).

In the cave 35 animals were collected in the sampling period. They belong to groups: Arachnida, Copepoda and Insecta (Tab. 4.33).

Table 4.33: L*ist of taxa collected from five drips in Grotta di Roverè Mille and their abundance (N). Troglomorphic animals are shown in bold.*

Phylum/ Subphylum	Class/ Subclass	Order	Family	Species	N
Arthropoda/ Chelicerata	Arachnida/ Micrura	**Acarina/ Hydracarina**			2
		Pseudoscorpionida			2
Arthropoda/ Crustacea	Maxillipoda/ Copepoda	Harpacticoida	Canthocamptidae	***Moraria* sp.A**	10
		Not identified			6
Arthropoda/ Hexapoda	Entognatha	Collembola			14
	Insecta	Diptera			1

Copepods were the most abundant (Tab. 4.33) with 0.8 copepods per day in average (Tab. 4.34).

Table 4.34: *Copepod abundance and their mean flux per day in five drips in Grotta di Roverè Mille.*

Date/Sampling site	**RM1**	**RM2**	**RM3**	**RM4**	**RM5**	**Total**
09/05/2008	0	8	0	0	0	**8**
03/07/2008	0	3	0	0	0	**3**
04/08/2008	0	3	0	0	0	**3**
01/10/2008	0	2	0	0	0	**2**
Total	**0**	**16**	**0**	**0**	**0**	**16**
Flux per day	0	0.08	0	0	0	0.08

Copepods were represented by 16 adult individuals of the species *Moraria* sp.A (the same species was collected in Covolo della Croce and Grotta di Roverè Mille, Tab 4.9) and were collected only at the sampling site RM2, where DOC concentration was higher (4.43 mg/L). Unfortunately the main part of chemical measurements was impossible at this sampling site (Tab. 4.32).

Species accumulation curves (Fig. 4.18) indicate that probably *Moraria* sp.A is the only one copepod species that populates epikarst water over Grotta di Roverè Mille, as the asymptote is reached by all curves at one species.

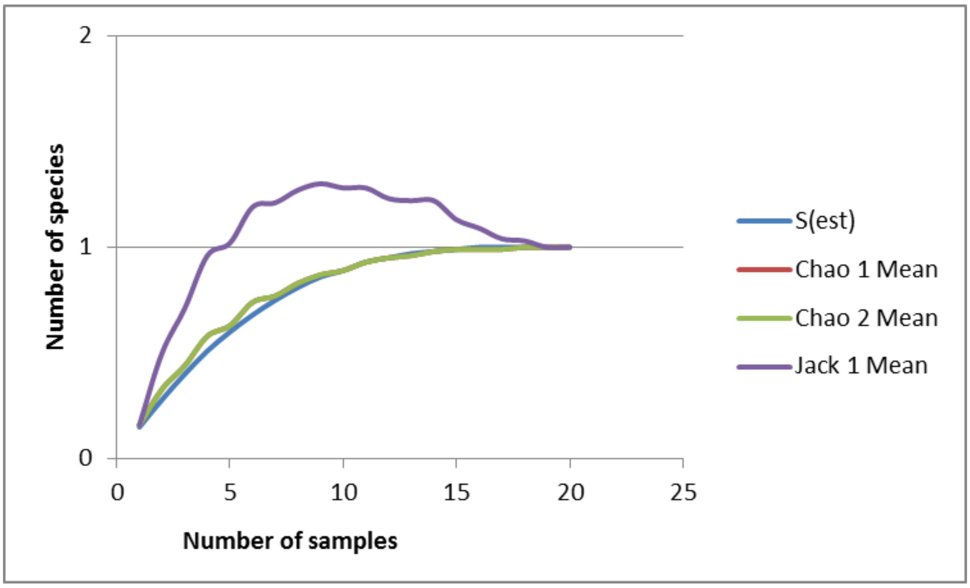

Figure 4.18: *Species accumulation curves (estimates of species richness, Chao1, Chao2, Jackknife1) for monthly samples for Grotta di Roverè Mille.*

Pearson correlation coefficient (Tab. 4.10) shows positive significant correlation between copepod abundance (the species *Moraria* sp.A) and conductivity ($r = 0.57$, $p < 0.01$) and between copepod abundance and DOC ($r = 0.49$, $p < 0.05$). Correlations are confirmed by the PCA in Figure 4.19, where the correlations between copepod abundance and physico-chemical parameters in the cave are shown.

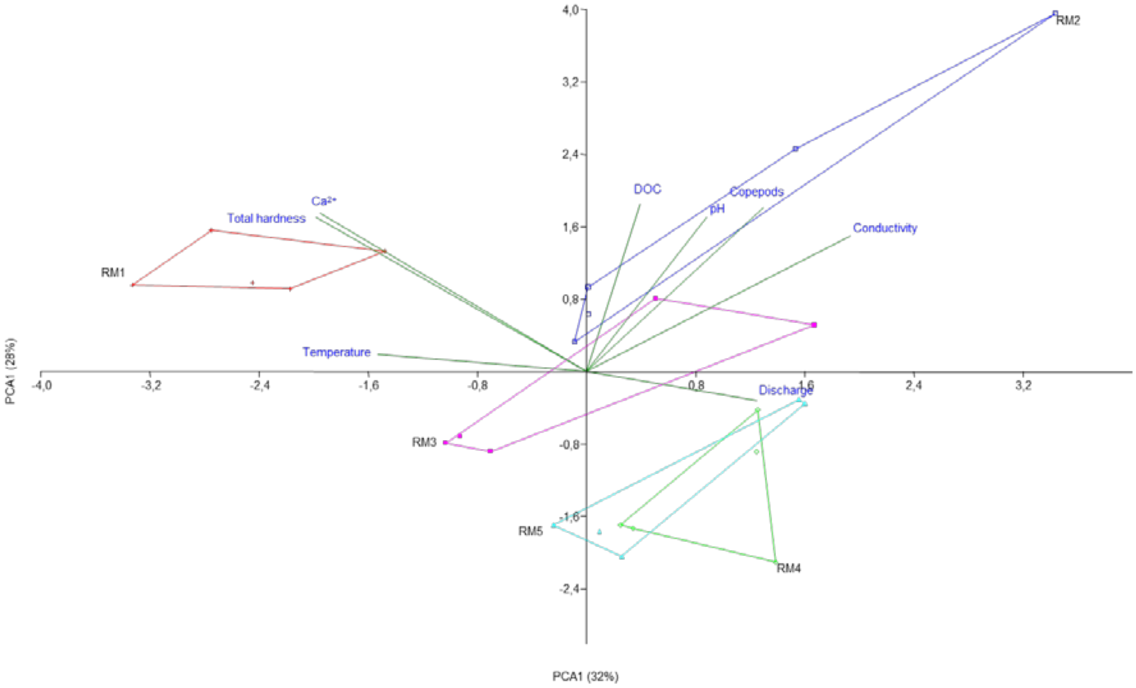

Figure 4.19: *Principal component analysis (PCA) showing relation between copepod population and physico-chemical parameters in Grotta di Roverè Mille. The PCA explains 60% of the variance (32% with the first axes, PCA1, and 28% with the second, PCA2).*
Green vectors with common origin represent measured parameters;
Points represent samples; Polygons include samples from each sampling site;
Copepod population of each sampling site is described below:
Red = RM1 = no copepods;
Blue = RM2 = Moraria sp.A*;*
Pink = RM3 = no copepods;
Green = RM4 = no copepods;
Light blue = RM5 = no copepods.

4.4.4. Summary of correlations between copepod abundance and measured parameters in monitored caves in Italy

Pearson correlation coefficient showed many positive and negative correlations between measured parameters (Tab. 4.7, column IT), but no high significant correlation was found between measured parameters and copepod abundance (Tab. 4.10). Seasonal abundance variation of copepods is shown in Figure 4.15. A higher number of animals were collected in spring and autumn samples.

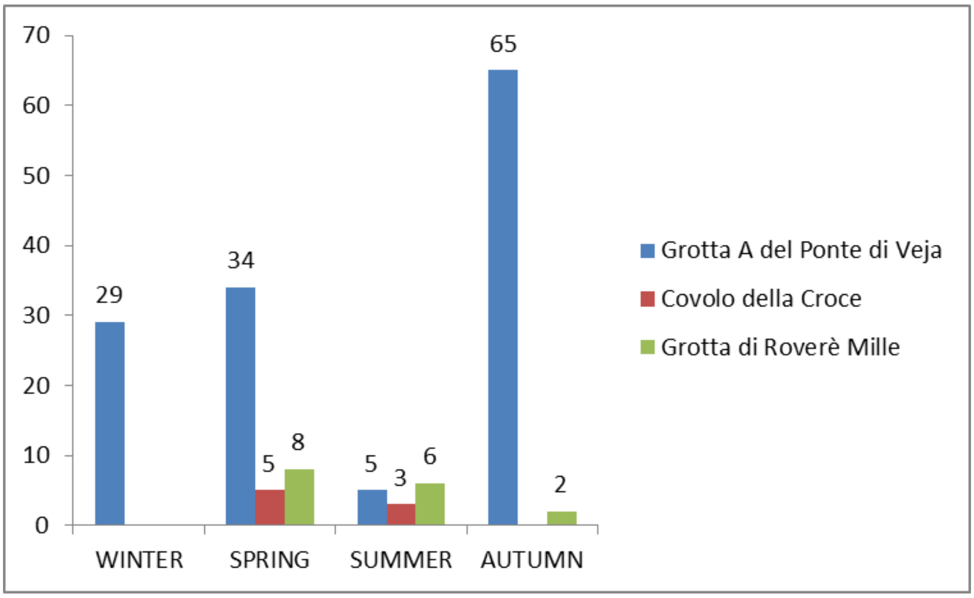

Figure 4.20: *Seasonal copepod abundance variation in Italian caves.*

The PCA in Figure 4.21 shows the correlation between copepod abundance and measured environmental parameters. As shown in Table 4.35 the position of the first axe (PC1) is mainly determinated by conductivity (loading = 0.982) and total hardness (loading = 0.176), the second (PCA2) by total hardness (loading = 0.923) and Ca^{2+} (loading = 0.313). The PCA plot shows that copepod abundance was positively correlated with discharge and PO_4^{3-}, negatively with Cl^-, DOC and conductivity (Tab. 4.36). Each cave has different ecological conditions (niches), and harbors different copepod species with different ecological needs.

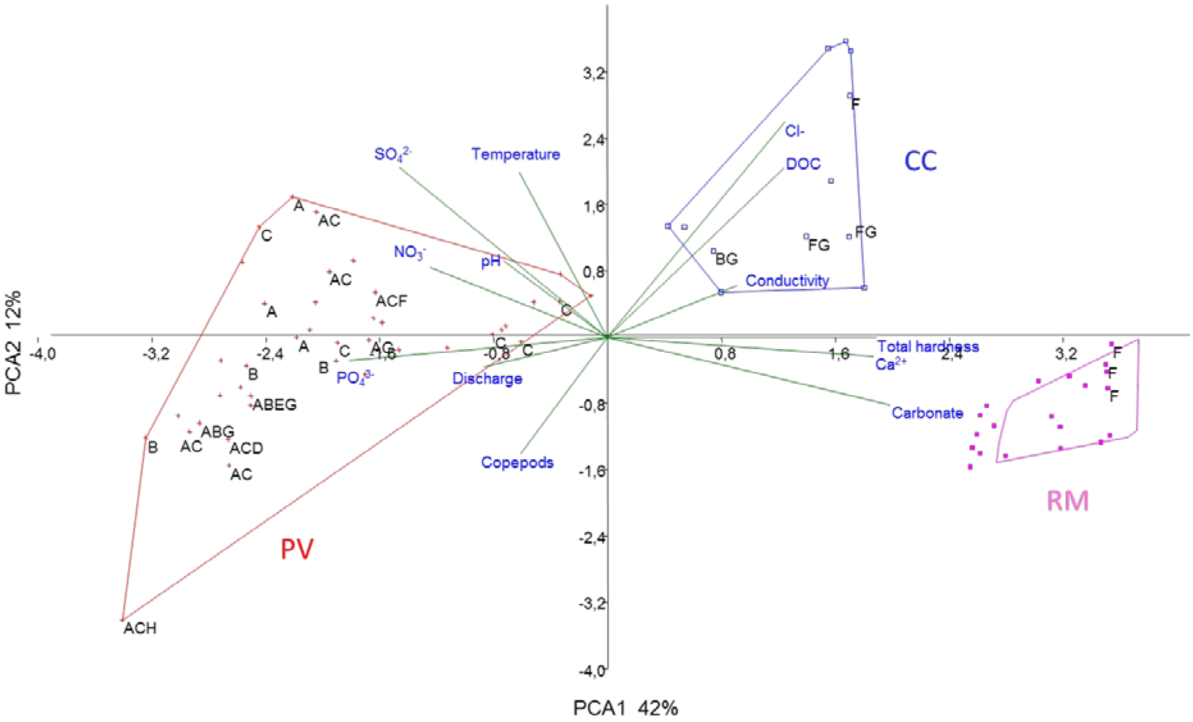

Figure 4.21: *Principal component analysis based on copepod species composition in trickles in Italian caves in relation to measured environmental variables. The PCA explains the 54% of variance (42% with the first axe, PCA1, and 12% with the second, PCA2).*
Green vectors with common origin represent measured parameters;
Points represent samples;
Polygons include saples from each cave;
Blue = Covolo della Croce;
Red = Grotta A del Ponte di Veja;
Pink = Grotta di Roverè Mille;
Copepod population of each sample is specified with letters as described below:
A = *Bryocamptus (Rheochamptus) zschokkei tatrensis;*
B = *Lessinocamptus insoletus;*
C = *Maraenobiotus brucei;*
D = *Moraria poppei;*
E = *Moraria stankovitchi;*
F = *Moraria* sp.A;
G = *Speocyclops infernus;*
H = *Paracyclops imminutus;*
No label = no copepods.

Table 4.35: *Loadings from principal component analysis shown in Fig. 4.21. (Main parameters that determine the position of axes are evidenced in bold).*

Parameter	**Loading**	
	PC 1	**PC 2**
Temperature	-0.002	-0.007
Discarge	-0.020	-0.068
pH	-0.0014	-0.004
Conductivity	**0.982**	-0.190
Carbonate	0.006	0.022
Total hardness	**0.176**	**0.923**
Ca^{2+}	0.061	**0.313**
Cl^-	0.002	0.005
NO_3^-	-0.027	-0.095
SO_4^{2-}	-0.004	-0.011
PO_4^{3-}	-0.001	-0.002
DOC	0.005	0.007
Copepod abundance	-0.0003	-0.0009

In Grotta A del ponte di Veja (in red) mean concentrations of nitrate and phosphate ions (10.7 mg/L and 0.2 mg/L, respectively) were higher than in other caves (Tab. 4.6). Copepod population in this cave was the most abundant and diverse (Tabs. 4.8, 4.9). In Covolo della Croce (in blue) mean conductivity values were lower (314 μS/cm) than in other caves (Tab. 4.4). In this cave the highest DOC between monitored caves was measured, rising to 5.69 mg/L (Tab. 4.5). The cave Roverè Mille (in pink) was characterized by higher average total hardness (219 mg/L) and Ca^{2+} (83.4 mg/L) (Tab. 4.4) than in other caves.

Table 4.36: *Correlations between copepods abundance and measured parameters based on calculation of Pearson correlation coefficient (r) and principal component analysis (PCA) in Italian sampling area (+ = positive correlation, - = negative correlation).*

Parameter/caves	**IT**		**PV**		**CC**		**RM**	
Correlation	***r***	**PCA**	***r***	**PCA**	***r***	**PCA**	***r***	**PCA**
Temperature								
Conductivity		-		-		-	+	+
pH				+	+			+
Discharge		+						
Total hardness	-			-		+		
Ca^{2+}	-			-		+		
Cl^-	-	-		-		-		
NO_3^-								
SO_4^{2-}						+		
PO_4^{3-}						+		
$CaCO_3$	-			-		+		
DOC		-				-	+	+

5. DISCUSSION

5.1. Phyisico-chemical characteristics of epikarst

5.1.1. Discharge

Among measured drips in caves in Lessinian Massif and in Slovenian Alps we can distinguish three kinds of drips: drips with constant, low discharge (below 15 mL/min); drips with constant, high discharge (over 15 mL/min); and drips with very variable discharge (from 0 to over 800 mL/min). Similar findings are described in Kogovšek (2010), who also separates drips according to the permanent flow and discharge values.

Three levels of porosity can be distinguished within karstified carbonate rocks: primary (associated with inter-granular pore space), secondary (associated with joints and fractures) and tertiary (with solution-enhanced conduits) (Ford and Williams 2007). Water movement can occur through any or all of these structures, leading to a continuum of subsurface water flow pathways. At one extreme, flow may be rapid when associated with tertiary porosity. In some cases, water can basically bypass epikarst via a conduit through the entire epikarst (Bakalowicz 2004). When permanence time of water in the rock is higher (for lower porosity or higher ceiling thickness), discharge is generally more constant (Williams 2008, Kogovšek and Urbanc 2007, Kogovšek 2010). As is shown, discharge was very variable in SJ3 and SJ5 in Snežna jama and PJ4 in Pološka jama. In many alpine areas carbonate rocks have been tectonically transformed, and later exposed to erosion; consequently, large fissures are present. It is probably the case of Pološka jama, which is located in strongly fractured carbonates.

At the other extreme is matrix flow: water movement associated with primary porosity. In this case, water movement can be very slow and can be considered to form part of an unsaturated water store. It is likely the case of Zadlaška jama, Grotta A del Ponte di Veja, Covolo della Croce and Grotta di Roverè Mille, where discharge was constantly low.

In other studied caves, water flow is likely a combination of different flow pathways. Other physico-chemical characteristics of dripping water and fauna can help in interpretation of water flow through the epikarst zone.

5.1.2. Temperature

Temperature of water at monitored caves varied following altitudinal gradient. In Italian caves (at comparable altitudes) temperature was higher than in Slovenian Alps (Fig. 4.1). It is probably due to the geographical position of the Lessinian Massif; more exposed to wetter and mild air masses coming from Mediterranean Sea (Ehlers and Gibbard 2004).

When the ceiling of the cave was high or/and discharge low, temperature data refer more to temperature variation of the air in the cave than temperature variation of water in epikarst. For these reasons we consider more representative of epikarst conditions, temperature data from drips with higher discharge (over 15 mL/min), where more immediate measurement was possible. At comparable discharge rates, in some of these drips temperature was more variable in others less. As expected, temperature of dripping water was more constant in sampling sites located in the deeper part of caves with respect of the ones closer to the entrances (more influenced by the surface variations) (Tab. 4.1). Covington and Perne (2015) describe pulses of temperature and cave atmosphere dynamics, transport within karst conduits, and models of speleogenesis which help to understand processes that occur in karst. In Snežna jama and in Jama pod Babjim zobom temperature was positively correlated with discharge (Tab. 4.7). The correlation was higher in Snežna jama than in Jama pod Babjim zobom. Comparing sampling sites SJ4 and JPBZ3 or JPBZ4, the different variability of temperature is evident (Tabs. 4.1, 4.12, 4.15). It probably depends on different flow path of water and consequently different permanence time in the epikarst. Bottrell and Atkinson (1992), found that in White Scar Cave, England, there were three epikarst flow components: 1. rapid through-flow with a residence time of 3 days; 2. short-term storage of 30-70 days; and 3. long residence time of 160 days or more, water flushed out only during periods of high flow. Where the water flow is faster and/or the flow path is shorter temperature varies more (following surface temperature variation); where is slow and/or the flow path is longer, temperature reaches the equilibrium and is more constant. Kogovšek (2010) observed that temperature of the water in drips from slow flow is relatively constant, varying only one or two degrees. Low gap between measured maximum and minimum temperature was observed in Snežna jama (Tab. 4.12), in Jama pod Babjim zobom at JPBZ3, JPBZ4 and JPBZ5 (Tab. 4.15), in Zadlaška jama (Tab. 4.18), in Grotta A del Ponte di Veja at PV3, PV4 and PV5 (Tab. 4.28) and in Grotta di Roverè Mille (Tab. 4.34) where drip flows were not constant.

Low variation of temperature is not always reflected in low discharge and laminar flow (no correlation between drip rate and coefficient of variation of temperature) as flow path of water through epikarst can be very complex. Temperature was frequently positively correlated with precipitation (Tab. 4.7), but data on precipitation used for statistical analyses were from two metereological stations close to monitored areas. Due to very variable local metereological conditions in Alps, this correlation should be confirmed with deepening the study.

Precipitation can influence water temperature in many ways. The “new” water input in epikarst disturbs the equilibrium and makes more variable temperatures probably activating some larger conduits (inactive in case of dry conditions). Snow cover may act in both response of discharge to precipitation (as snow stays on the surface and enter epikarst only when it melts) and temperature variation (under the snow, influence of surface temperature variation is buffered; Zhang 2005).

5.1.3. Conductivity, Ca^{2+}, $CaCO_3$ and total hardness

Dynamic variations in karst water routing, and their variability between individual drips and over time, have important implications, influencing, for example, solute concentrations, Mg/Ca ratios, and the dynamics of carbonate precipitation (Fairchild and Baker 2012). Conductivity of percolating water in karst caves is generally high, largely because of the high concentration of Ca^{2+} ion due to kinetics of the Ca^{2+} - CO_3^{2-} - $CaCO_3$ system. Higher conductivity implies longer residence time of water in epikarst as the water becomes saturated with $CaCO_3$ (Covington *et al*. 2012), and high concentration of calcium ions results. Different values of Ca^{2+} concentration give us information on the permanence time of water in the epikarst rock. From calculation of Pearson correlation coefficients (Tab. 4.7) as well as from multivariate analyses (PCA), conductivity, Ca^{2+} and total hardness were, as expected, positively correlated in monitored caves.

In the Slovenian Alps conductivity of dripping water was lower than in Italian caves. Comparable conductivity values (under 300 µS/cm) were found in Isolated karst in Slovenia (Pipan *et al.* 2008) and in Paştera Ungurului and Paştera Ciur Izbuc in Romania (Meleg *et al.* 2011) (Tab. 5.1). In monitored caves conductivity was generally lower than in Dinaric karst in Slovenia (Tab 5.1).

Table 5.1: *Average values of conductivity of drip water for caves located in Dinaric, Isolated and Alpine karst in Slovenia, in Lessinian Massif in Italy and in Romania (data from Pipan 2005, Pipan et al. 2008, Meleg et al. 2011).*

Sampling area	Cave	Conductivity (µS/cm)
Dinaric karst – Slovenia	Pivka jama	416
	Črna jama	377
	Županova jama	371
	Postojnska jama	342
	Dimnice	330
	Škocjanske jame	308
Lessinian Massif - Italy	Grotta A del Ponte di Veja	358
	Grotta di Roverè Mille	355
	Covolo della Croce	314
Alps – Slovenia	Snežna jama	297
	Zadlaška jama	288
	Jama pod Babjim zobom	252
	Pološka jama	230
Romania	Paştera Vadu Crişului	381
	Paştera Ungurului	256
	Paştera Ciur Izbuc	265
Isolated karst – Slovenia	Huda Luknja	228

The differences in conductivity values can be due to permanence time of water in the epikarst or to different pH of water that can accelerate the dissolution of carbonate rocks process. It could be confirmed by the positive correlation between conductivity and pH in Snežna jama and Pološka jama, in Slovenian Alps, and in Grotta di Roverè Mille, in Italy (Tab. 4.7, Figs. 4.6, 4.12, 4.19).

Composition of rocks and temperature can act on the $CaCO_3$ dissolution as well: at lower temperatures the reaction is slower (low correlation between temperature and parameters involved in dissolution processes was observed, see Tab. 4.7).

5.1.4. pH

As also typical for karst waters (Sasowsky and Dalton 2005), pH of dripping water in monitored caves was slightly basic. As shown in Table 5.2, in Slovenian Alps pH values were similar to other investigated areas in Slovenia (Dinaric karst and Isolated karst); in Italy pH values were more similar to results from Romania.

Differences in parent rock or soil composition and anthropogenic impacts could likely be the reasons for the discrepancy. Low but significant positive Pearson correlation coefficients (Tab. 4.7) between pH and temperature, precipitation and discharge mean that probably permanence time of water in the epikarst could influence pH values.

Table 5.2: *Average values of pH in epikarst water for caves located in Dinaric, Isolated and Alpine karst in Slovenia, in Lessinian Massif in Italy and in Romania (data from Pipan 2005, Meleg et al. 2011, Pipan et al. 2008).*

Sampling area	Cave	pH
Dinaric karst – Slovenia	Županova jama	7.6
	Dimnice	7.7
	Črna jama	7.8
	Postojnska jama	7.8
	Pivka jama	7.8
	Škocjanske jame	7.9
Isolated karst – Slovenia	Huda Luknja	8.0
Alps – Slovenia	Zadlaška jama	7.9
	Snežna jama	8.3
	Pološka jama	8.3
	Jama pod Babjim zobom	8.4
Lessinian Massif – Italy	Grotta di Roverè Mille	8.1
	Grotta A del Ponte di Veja	8.5
	Covolo della Croce	8.6
Romania	Paştera Vadu Crişului	8.4
	Paştera Ciur Izbuc	8.5
	Paştera Ungurului	8.7

5.1.5. Other ions

In epikarst many semi-isolated solution pockets whose water chemistry is also variable are present (Pipan 2005, Kogovšek 2010).

Variation of ions' concentration probably reflects different land use on the surface. Ions' concentration in monitored caves was generally low, especially PO_4^{3-} concentration (< 0.3 mg/L) (Tab. 4.6).

SO_4^{2-} ions are naturally present in soil and vary with soil composition. These ions are less concentrated in Alpine caves in Slovenia and Italian caves than in other caves in Slovenia (Tab. 5.3).

Low ions concentration, especially NO_3^-, is probably due to location of caves in sites where anthropogenic disturbance is low. NO_3^- concentrations were relatively high in Grotta A del Ponte di Veja, with values comparable to that measured at Pivka jama in Dinaric karst (where the major amount of nitrate ions was related with the presence of a campground and associate structure on the surface) (Pipan 2005). At Grotta A del Ponte di Veja the activity of the close farmhouse can cause higher concentration of these ions.

Table 5.3: *Average values of ions in epikarst water for caves located in Dinaric, Isolated and Alpine karst in Slovenia and in Lessinian Massif in Italy (data from Pipan 2005, Pipan et al. 2008).*

Sampling area	**Cave**	**Cl^-**	**NO_3^-**	**SO_4^{2-}**
Dinaric karst - Slovenia	Županova jama	1.4	0.3	8.7
	Dimnice	5.5	3.1	5.2
	Črna jama	1.1	0.6	4.9
	Postojnska jama	1.3	3.1	5.7
	Pivka jama	2.4	11.8	5.4
	Škocjanske jame	0.4	1.1	6.4
Isolated karst - Slovenia	Huda Luknja	1.1	8.1	14.2
Alps - Slovenia	Zadlaška jama	2.0	2.3	4.0
	Snežna jama	2.7	1.8	2.0
	Pološka jama	2.4	5.5	3.1
	Jama pod Babjim zobom	2.9	2.9	3.4
Lessinian Massif - Italy	Grotta di Roverè Mille	2.5	0.4	2.8
	Grotta A del Ponte di Veja	2.2	10.7	4.6
	Covolo della Croce	2.9	0.4	4.8

Results of Pearson correlation coefficients (Tab. 4.7) and principal component analysis (PCA) are heterogeneous and the interpretation is difficult. Insufficient data about variation of ions' concentration in time may be the cause of discrepancy of results.

5.2. Biotic characteristics of epikarst

Epikarst can be considered as a patchwork of many different micro-habitats. Each niche harbors a typical population. Culver and Pipan (2014) suggest that special attention must be paid to the following fluxes and parameters, which are likely to be important environmental factors for faunal composition: light, habitat dimension (pore size), vertical depth (as a predictor of environmental variability as well as connectivity with the surface), quantity of organic carbon and nutrients, presence of competition and predation in the habitat.

5.2.1. Organic carbon and nutrients in epikarst

Aquatic as well as terrestrial invertebrates in percolation water are important and sometimes, together with dissolved and fine particulate organic matter, the only source of organic nutrients for organisms in deeper subsurface habitats, especially so in areas of barren surface landscape such as high mountain karst (Culver and Pipan 2009, Simon *et al.* 2007a).

Quantity of organic carbon and nutrients has an impact on faunal composition. Water movement from surface to caves is the basic transport process in term of energy and mass flow. The main source of organic carbon in epikarst water is the soil (Culver and Pipan 2014). We know very little about processing in the epikarst itself but the concentration of total organic carbon (TOC) in water that comes out of epikarst in ceiling drips in caves tends to be relatively low, often around 1 mg/L (Simon *et al*. 2007, Ban *et al*. 2008). Simon *et al*. (2003, 2010) showed that the DOC in percolating water was an important carbon source and an important source of epilithic biofilms in caves. DOC concentration may be very relevant in determining the distribution of copepods, not because copepods are utilizing dissolved organic carbon directly, but because they feed on micro-organisms that are directly utilizing DOC. In temperate zone caves, the concentration of DOC in epikarst water collected from ceiling drips is typically about 1 mg/L (Simon *et al.* 2007a), but it can reach over 2000 mg/L in some circumstances (Laiz *et al.* 1999). Ban *et al*. (2008) report that DOC in epikarst drip waters varies on an annual cycle, but it can reach values of 3 mg/L. In what is a highly anomalous case, Laiz *et al*. (1999) report organic carbon values from epikarst drips of up to 2200 mg/L in Altamira Cave, Spain. In the study caves in Slovenia and in Italy DOC concentration was between 0.95 mg/L (measured in Grotta A del Ponte di Veja) and 5.69 mg/L (in Covolo della Croce) and was higher (mean = 3.2 mg/L) than in Dinaric karst (mean = 1.1 mg/L) (Simon *et al.* 2008). Ban *et al*. (2008) suggest that the differences in the relationship between drip rate and DOC concentration are either due to the differences in the length (and time) of the flow paths, with longer flow paths losing more DOC during transport, or to differences in size of the reservoir feeding the drip, with larger reservoirs being diluted during major rainfall events. In the case of epikarst, organic carbon and nutrients enter the system as dissolved or particulate organic matter in percolating water. Positive correlation results between DOC and conductivity (Tab. 4.7, Figs. 4.5, 4.16). Conductivity, in karst areas, can be an index of residence time of water in epikarst, confirming the theory of Ban *et al.* (2008).

Statistical analyses show both positive and negative correlations between copepod abundance and DOC (Tabs. 4.10, 4.25, 4.36) what means that also other parameters are important when biotic interactions are studied. Additional measurements are necessary to clear results.

5.2.2. Ecology of epikarst fauna

Different combinations of measured environmental parameters determine different niches colonized by different animal communities. Given the high levels of variation in chemical and physical parameters in the epikarst, it is interesting to investigate the extent to which epikarst animals occur throughout such range of physico-chemical conditions.

Both Slovenia and Lessinian Massif are considered as a hotspot for cave fauna (Culver and Pipan 2014, Galassi 2009 *et al.*), with high number of endemic species and stygobionts. The same trend was observed with regard of epikarst fauna in Dinaric karst in Slovenia (Pipan 2005). Presence of new (undescribed) copepod species and amphipod species in sampled caves confirm observations of Pipan (2005) that the level of single-cave endemism among the epikarst stygofauna is generally high. The historical component, mostly defined by geological age of an aquifer, appears to be important in increasing both total species richness and degree of endemism (Galassi 2009). The diversity of organisms in epikarst is remarkable, but not all species found in dripping water in investigated caves are endemic, or even troglobionts or stygobionts (Tab. 5.4).

Since epikarst is well above the water table, and the amount of water storage in epikarst varies, it also includes some air-filled habitat. Together with aquatic species (Copepoda, Amphipoda, Collembola and Hydracarina) from epikarst water, many terrestrial species were collected (Tab. 4.8). Abundance of fauna in drips in caves was frequently correlated with discharge (Papi and Pipan 2011), probably because, just as mineralized particles can be mobilized into the water column, so can be epikarst inhabitants (Culver and Pipan 2014).

Table 5.4: *Ratio of troglomorphic species in the samples from investigated caves. (N = number of species, C = number of troglomorphic species).*

Taxa	**Number of species**						
	SJ	**JPBZ**	**ZJ**	**PJ**	**PV**	**CC**	**RM**
	N - C	**N - C**	**N - C**	**N - C**	**N - C**	**N - C**	**N - C**
Nematoda		1 - 0		1 - 0	1 - 0	1 - 0	
Oligochaeta	1 - 0	1 - 0			1 - 0	1 - 0	
Aracnida	1 - 1	1 - 1			1 - 1	1 - 0	2 - 1
Copepoda	2 - 2	3 - 3	3 - 3	3 - 3	8 - 3	3 - 3	1 - 1
Amphipoda	1 - 1	1 - 1		1 - 1	1 - 1		
Isopoda			1 - 1	1 - 1	1 - 1		
Collembola		1 - 1	2 - 2		1 - 1		1 - 1
Coleoptera	1 – 0	1 – 1	1 - 0		1 - 0	1 - 0	
Diptera		1 - 0	1 - 0		1 - 0	1 - 0	1 - 0
Orthoptera				1 - 0	1 - 0	1 - 0	
Dipolopoda						1 - 0	
Gastropoda		1 (?)			1 (?)		
Total number of species	6	11	8	7	18	10	6
Percent of troglomorphic species	67%	64%	75%	80%	17%	30%	50%

The presence of stygomorphic amphipods at some drips in studied caves (SJ5 in Snežna jama, JBBZ5 in Jama pod Babjim zobom, PJ1, PJ3 and PJ5 in Pološka jama and PV3 in Grotta A del Ponte di Veja) may indicate larger fissures in the water flowpath. Sampling sites with amphipods were in general wormer, the ones with higher mean discharge and lower concentrations of $Ca^{2+}$and $CaCO_3$. *Niphargus* cf. *scopicauda* was found at SJ5 in Snežna jama (Tab. 4.13), *Niphargus* cf. *cornicolanus* at JPBZ5 in Jama pod Babjim zobom (Tab. 4.16) and *Bogidiella* sp. at PJ1, PJ3 and PJ5 in Pološka jama (Tab. 4.22). An amphipod species (new for science) was found at PV3 in Grotta A del Ponte di Veja (Tab. 4.27).

Very little is known about terrestrial epikarst habitat. Terrestrial species only dominated in percolation water in Covolo della Croce in Lessinian Massif and in Huda Luknja, a cave from Isolated karst in north eastern Slovenia (Pipan *et al.* 2008), but not in caves in Dinaric karst of Slovenia (Pipan 2005) and not even in other monitored caves in Slovenian Alps and Lessinian Massif.

5.2.3. Copepod species diversity and richness in epikarst

Copepods are frequently the most abundant in dripping water from epikarst (e.g. Pipan 2005, Galassi *et al.* 2009, Pipan *et al.* 2008, Meleg *et al.* 2011),and was confirmed by results from samples from caves in Slovenian Alps and Lessinian Massif. Migration rates (mostly passive, as they are transported by water currents) of epikarst copepods may be higher than for the most other taxa because of their small size. Copepod distribution and abundance may provide some important clues as to lateral movements of epikarst water (Pipan 2005).

A total of 13 copepod species were collected in Alpine Slovenian caves and in Lessinian Massif (Tab. 4.9). Estimation of species richness (accumulation curves) shows that probably our estimation of the number of copepod species (based on observed species in drip samples) is close to the actual number, with the exception of Zadlaška jama and Covolo della Croce where more species can be expected. Distribution of different copepod species depends on their ecological needs. Some species have wide ecological tolerance, some other more restrict. An extreme case of a single drip endemism, *Elaphoidella kieferi*, was observed in Škocjanske jame in Dinaric karst (Petrovski and Brancelj 1985, Pipan 2005). Between collected species in Slovenian Alps and in Lessinian Massif, *Speocyclops infernus*, have wide ecological tolerance to environmental variation (it is evident in principal component analysis, where samples with this cyclopoid species are spread in the plot; C in Figs. 4.6, 4.17). Ovigerous females of *S. infernus* were collected only in Snežna jama. Eggs (three on each side) were large and not in egg sacs. These are considered as typical adaptations to groundwater environments as a result of K-selection (Dürbaum 1995, Berger and Maier 2001). Large eggs ensure the food supply of nauplii in an oligotrophic environment (Brancelj 1986, Culver *et al.* 1995, Turk *et al.* 1996). The other copepod species have more restricted distribution (and are more grouped in PCA plots).

Copepod populations in Slovene Alpine epikarst are less diverse (on average two copepod species per cave) and less abundant (average rate of copepods: 0.05 copepod per drip per day) than the epikarst fauna from the Dinaric karst, where on average eight species per cave and 0.1 copepod per drip per day were found (Pipan 2005). Similar is with an epikarst fauna from Organ Cave, West Virginia, USA (Pipan *et al*. 2006), where an average of one copepod per drip per day was found. This suggests that alpine epikarst habitat, due to severe environmental conditions on the surface (i.e. lower winter and higher summer temperatures) compared to Dinaric karst, harbors smaller number of species. Populations of investigated drips are more similar to populations from Isolated karst in Slovenia, where also two copepod species per cave were found (Pipan *et al*. 2008).

The 62% of observed copepod species in Slovenian Alps and Lessinian Massif in Italy were stygobionts (Tab. 4.11). Comparison between data about frequency of stigobiotic copepods in Lessinian Massif, in Slovenia, in Romania and in USA shows, that increasing of number of different copepod species in dripping water in caves is frequently due to non stygobiotic ones (lower percent of stygobionts) (Fig. 5.1).

Slovenian Alps and Lessinian Massif (may be due to major geographic isolation and different climatic conditions) harbor a less diverse but more specialized fauna (more similar to Isolated karst in Slovenia). In Grotta A del Ponte di Veja a higher number of copepod species (6) with respect to other monitored caves was found. It was probably due to the structure of the rock above the cave. Crustacean population at different sampling sites together with physical and chemical data (minor total hardness and more variable temperature) show, for example, how, in this cave, sampling site PV2 is probably more directly connected with surface than the others (Tab. 4.28).

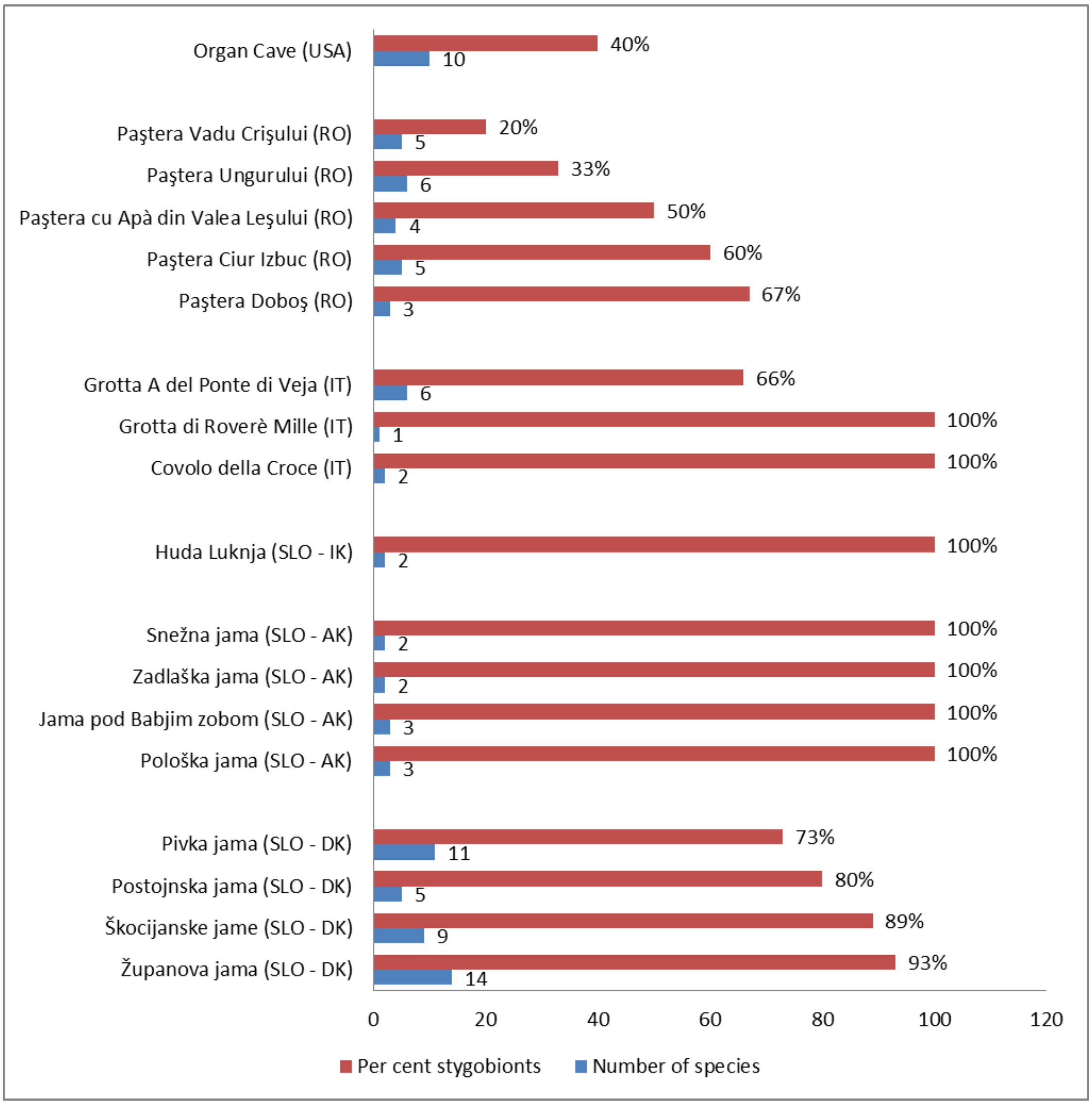

Figure 5.1: *Ratio of stygobiotic copepods taken from epikarst water in drips in Slovenian (SLO, DK = Dinaric karst; IK = Isolate karst; AK = Alpine karst), Romanian (RO), Italian (IT) and West Virginia (USA) caves. (Sources: Pipan 2005, Pipan et al. 2008, Meleg et al. 2011, Pipan et al. 2006b).*

Culver and Pipan (2014), from data of Pipan (2005) from Dinaric karst, conclude that it is unlikely, but possible, that the epikarst community itself changes seasonally, but it is more likely that copepods get washed out from epikarst in greater number after most rainy periods (winter, in that case). Results from Slovene Alps and Italy show different seasonal trends (Fig. 4.4): in most populated caves in Slovenian

Alps, the major part of copepods were collected in summer and springs times, in Italian caves in autumn and spring. A positive correlation was found between discharge and copepod abundance in Jama pod Babjim zobom and with the abundance of *Lessinocamptus insoletus* in Grotta A del Ponte di Veja (Tab. 4.10). Since the copepods collected in drips are samples of individuals washed out of their habitat, it is more likely that seasonal effects are the result of differences in washout rate rather than changes in copepod community.

While no studied caves entirely lack an epikarst fauna, some drips did. Many ecological factors could determine the absence of copepods. Pipan (2005) showed that the ceiling thickness could be correlated with copepod abundance. Data from Alpine and Pre-Alpine caves show that is likely that the habitat dimension (dimension of fractures in the rocks) and consequent permanence time of water in epikarst influence copepod abundance, and absence in the case of very low discharge (more compact rocks).

Likewise Pipan and Culver (2014) observed that copepods tend not to be found in water with greater temperature than 8.2 °C and Ca^{2+} higher concentrations than 57.5 mg/L. In monitored caves copepods were found at temperatures rising to 16°C as well. With regard to Ca^{2+} concentration, it is known that water in epikarst can be supersaturated with respect to Ca^{2+} (part of the mechanism of dissolution-deposition of $CaCO_3$) and this may cause physiological problems for animals (e.g. during molting). This could be the case of the two sampling sites ZJ2 and ZL4 in Zadlaška jama, where no animals were collected (Tab. 4.19). At Grotta A del Ponte di Veja at sampling site PV4 (the less populated and where only the stygophyle species *Maraenobiotus brucei* was collected) a high concentration of Ca^{2+} was observed as well (Tab. 4.26). High concentration of Ca^{2+} can indicate a longer permanence of water in epikarst and/or a minor renovation of water that could mean a minor nutrient supply (a positive correlation between Ca^{2+} and DOC was frequently found, Tab. 4.7).

Papi and Pipan (2011) evidenced that presence of moonmilk or ice can be other two reasons for copepods absence in dripping water. Moonmilk could act as a safety net not allowing copepods to fall down transported by water. The ice could close passages. Absence of copepods was observed at temperatures below 2 °C.

No copepods were found at sampling sites close to the entrances of the caves probably because of high variation of ecological and hydrological conditions in superficial part of the epikarst.

5.3. Conclusions

Intensive study of the epikarst fauna and epikarst water parameters confirms previous findings that the karst system shows heterogeneity and variability of morphological, hydrogeological, and ecological parameters in time and space. Data from Alps and Pre-Alps, in Italy and in Slovenia, show that the variability of measured parameters, where the human impact is very low, is mainly due to different permanence time of water in epikarst. Epikarst is the site of most of the water storage above the water table in karst, and an important shallow subterranean habitat. Epikarst fauna sampled directly from dripping water represents unbiased but represented sample of the primary habitat. Different taxa can be found, but the most common are copepods. Some copepod species are widely distributed, while some others have more restricted ecological needs.

Comparison of findings from Alps and Pre-Alps, and findings from other previously sampled areas shows that in caves located at higher elevations, less diverse fauna populates the epikarst, although still species rich. A high percent of troglomorphic and endemic species was observed as well. Many species new for science were found showing that epikarst is still undersampled and not completely understand habitat yet. Relativelly little is known about life history characteristics of non-copepod epikarst species.

An understanding of the physical, chemical and biological processes under the surface of karst is an essential element for the protection of karst waters and caves. Especially important is that flow paths of contaminants, both vertically and horizontally, are highly unpredictable in karst areas. To understand them tracer tests are performed, but it is suggested that also copepods, with their small size and frequent occurrence in the water column could be potential water tracers, as already Pipan and Culver (2007a) stated.

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