

## The dark side of springs: what drives small-scale spatial patterns of subsurface meiofaunal assemblages?

Barbara FIASCA,<sup>1</sup> Fabio STOCH,<sup>1</sup> Marie-Josè OLIVIER,<sup>2</sup> Chafik MAAZOUZI,<sup>2</sup> Marco PETITTA,<sup>3</sup> Alessia DI CIOCCIO,<sup>1</sup> Diana M.P. GALASSI<sup>1\*</sup>

<sup>1</sup>University of L'Aquila, Department of Life, Health & Environmental Sciences, Via Vetoio, Coppito, 67100 L'Aquila, Italy;

<sup>2</sup>Laboratoire d'Ecologie des Hydrosystèmes Fluviaux, Université Lyon 1, Bât. FOREL, 43 Bd du 11 Nov 1918, 69622 Villeurbanne cedex, France; <sup>3</sup>University "La Sapienza", Department of Earth Sciences, P.le A. Moro 5, 00185 Rome, Italy

\*Corresponding author: dianamariapaola.galassi@univaq.it

### ABSTRACT

Springs are amongst the most relevant Groundwater Dependent Ecosystems (GDEs) and are key research environments in freshwater ecology and biology. The strict dependency on ground water of surface spring biodiversity is widely recognized, whereas the biodiversity occurring below the spring is very poorly known. This study analyzes copepod assemblages in the subsurface habitat of a karstic rheo-limnocratic spring in relation to seventeen environmental parameters. Subsurface copepod assemblages were sensitive to microspatial variation in habitat structure, and species distributions were mostly driven by groundwater flowpath and substratum type, resulting in biologically distinct limnocratic and rheocratic sectors at the spring system scale. Habitat patchiness was reflected in differences in the microdistribution of subsurface copepods, stygobiotic assemblages being more sensitive to the measured environmental gradients than non-stygobiotic ones. In spite of the apparent stability of spring environments, copepods, as a target group, performed well as descriptors of sediment texture and hydrodynamics, and may offer relevant information for a better understanding of the potential changes generated by anthropogenic disturbance on these ecosystems.

**Key words:** Copepoda, small-scale ecology, groundwater, springs, GDE.

*Received: July 2013. Accepted: October 2013.*

### INTRODUCTION

Springs have long been considered natural laboratories, due to the well-known temporal stability of many physico-chemical parameters (Hoffsten and Malmqvist, 2000; Zollhöfer *et al.*, 2000; Di Sabatino *et al.*, 2003; Smith *et al.*, 2003; Scarsbrook *et al.*, 2007; Barquín and Death, 2006, 2009). This condition has led some authors to consider springs as environments that are relatively easy to approach and analyse under an ecological perspective. However, a growing body of evidence suggests that ecological analyses of spring environments are much more difficult than expected (Gathmann and Williams, 2006; Barquín and Scarsbrook, 2008; Cantonati *et al.*, 2011, 2012), considering that even small springs are patchy environments (Gerecke *et al.*, 1998; Stoch *et al.*, 2011). Springs are currently classified as Groundwater Dependent Ecosystems (GDEs) (Eamus and Froend, 2006), and are widely distributed worldwide. Despite being long recognised as ecotones between surface and ground waters (Botosaneanu, 1998), springs are usually approached only under an epigeal perspective, and subsurface spring habitats have been almost completely neglected (Galassi *et al.*, 2011). Moreover, most studies focused on algae, macrophytes and invertebrates living in

the spring head above the bottom substratum (Cantonati *et al.*, 2011), whilst few studies were devoted to the ecology and distribution of spring subsurface meiofauna (Stoch *et al.*, 2011).

Much previous research demonstrated that variation in physical factors may explain broad-scale patterns of abundance and distribution of meiofauna in streams (Swan and Palmer, 2000; Silver *et al.*, 2002), whilst these patterns in spring meiofauna are correlated with physico-chemical parameters, mainly related to bedrock lithology (Bottazzi *et al.*, 2011; Stoch *et al.*, 2011). However, groundwater flowpath, water current velocity, and substratum texture alone were used for the traditional classification of springs in rheocrenes, helocrenes, and limnocratic (Martin and Brunke, 2012). Unfortunately, scant information is currently available on how environmental parameters, such as water chemistry, food availability, groundwater flowpath, and substratum type may affect small-scale meiofauna assemblages in lotic ecosystems (Swan and Palmer, 2000).

Rheo-limnocratic springs may be suitable GDEs to test the contribution of the different physical and chemical descriptors in structuring meiofaunal communities on a small spatial scale. In these springs, groundwater reaches the surface by point-discontinuities of the bedrock, which

define the rheocrenic outwelling sectors of the spring, and by vertical upwelling through the sediment matrix, the limnocrenic part of the spring (Czachorowski, 1990; Galas, 2005; Dumnicka *et al.*, 2007; Bottazzi *et al.*, 2008). A mosaic-habitat structure is thus likely to occur in rheo-limnocrenic springs.

Using copepod crustaceans as a target group (Galassi *et al.*, 2009; Stoch and Galassi, 2010; Caschetto *et al.*, 2014; Di Lorenzo and Galassi, 2013; Di Lorenzo *et al.*, 2013), we carried out a small-scale analysis of subsurface copepod assemblages in a rheo-limnocrenic spring addressing the following questions: i) what is the role of groundwater flowpath, sediment texture, hydrochemistry, and organic matter availability as descriptors of subsurface spring meiofaunal assemblages?; ii) are copepods suitable indicators of habitat patchiness occurring at the spring-system scale?

## METHODS

### Study area

The Presciano spring system is located in the Gran Sasso karstic massif in the Abruzzi region, central Italy, and is one of the main outlets of the Gran Sasso fractured karstic aquifer. This aquifer is represented by an outcrop of Meso-Cenozoic carbonate rocks, with well defined boundaries around a 700 km<sup>2</sup> area. Karst morphology, such as conduits and caves, is dominant in the recharge areas, while it is less common in spring areas, due to the presence of clastic deposits of different grain size and mineral composition (sands and fluvio-lacustrine clays) (Petitta and Tallini, 2003).

The Presciano spring system is a 2000 m<sup>2</sup> seepage area, forming a large limnocrenic spring, while the rheocrenic facies occurs at the southern and western spring banks. The spring system is located at 330-335 m asl at the contact between the limestone aquifer and the aquitard (represented by Quaternary lacustrine deposits) and is characterised by a mean annual discharge of 1.9 m<sup>3</sup> s<sup>-1</sup>. Minor variations in discharge ( $\pm 10\%$ ) have been observed during the hydrogeological year. The lowest discharge period is observed in May-July, and recharge normally starts in August-September (Boni *et al.*, 2002).

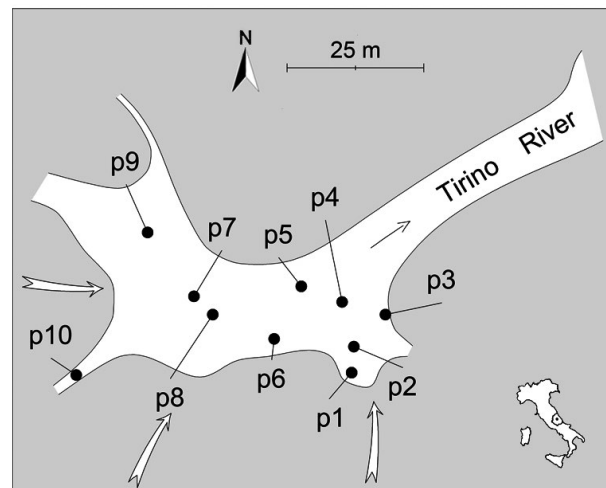
### Sampling methods

A stratified random sampling was adopted in order to capture most of the environmental heterogeneity observed over the total surface of the spring system. Ten sampling sites, coded from p1 to p10 (Fig. 1), were distributed across i) a predominantly rheocrenic, karstic sector, defined by small and medium discontinuities (up to 15 cm wide) in the carbonate bedrock, usually covered by a thin layer of large-sized sediments (p1, p2, p10); ii) a rheo-limnocrenic or predominantly limnocrenic sector domi-

nated by small-sized sediments covering the carbonate platform (p4, p5, p6, p7, p8, p9), sometimes clogged by fine sand and silt (p3).

Three samples were collected at each site at three different depths below the spring bed (-30, -70, and -150 cm) and at six dates along the hydrogeological year (January, March, May, July, October and December 2005) for a total of 180 samples. The initial design (6 dates $\times$ 10 points $\times$ 3 depths=180 samples) was reduced to 155 samples because in some sites sampling was unfeasible (*e.g.*, presence of the bedrock at 150 cm-depth for p3, p6, and p9). Samples were collected using a Bou-Rouch pump (Bou and Rouch, 1967) and mobile piezometers hammered at each sampling point-depth.

Seventeen environmental parameters were measured at each site, depth, and sampling date by taking 2-L samples before faunal sampling with the aid of a vacuum pump connected to the piezometer. Temperature, pH, specific conductivity at 25°C, redox potential, and dissolved oxygen concentration were measured in the field using a multiparametric probe (ECM Multi™; Dr. Lange GmbH, Düsseldorf, Germany); calcium and magnesium concentrations were determined by titration with EDTA; nitrate, sulphate and phosphate concentrations were measured using a HACH DR 2000 spectrophotometer (Hach Co., Loveland, CO, USA). Particulate organic matter (POM) was measured on 20-L samples, after removal of all fauna, oven-dried at 105°C, desiccated (24 h), and weighed. The dry-weighted samples were ashed at 540°C and reweighed to determine POM amount as the difference between dry and ash mass. Additional samples were taken for measuring the granulometric composition of substra-



**Fig. 1.** Distribution of sampling sites in the Presciano spring system. Sites are coded from p1 to p10. Arrows indicate the prevailing direction of the groundwater flowpath.

tum by using piezometers, with a screen with 6.0 mm-diameter holes. The granulometric composition of substratum was measured at all sites and at the three sampling depths only once, as sediment transport was negligible in the whole spring system. The weight of four granulometric classes was calculated per each site and depth on the fractional dry-sieving of desiccated samples through a stack of sieves according to the Wentworth (1922) scale: 4.0-6.0 mm (from pebble to granule; hereinafter called pebbles), 4.0-1.0 mm (from granule to very coarse sand; hereinafter called small gravel), 1.0 mm-0.125 mm (from coarse to fine sand; hereinafter called sand), <0.125 (from very fine sand to clay; hereinafter called fine sand). The hydrometric level was measured at each sampling site and date. The piezometric head was measured at each sampling date and site using the T-bar (Malard *et al.*, 2002) inserted at each site and depth, and expressed using four classes: negative (1), steady (2), positive (3), and highly positive (4).

Since both sample size and pumping rate significantly affect estimates of meiofauna density for a range of different sediment types (Hunt and Stanley, 2000; Boulton *et al.*, 2004), we followed the recommendations of Hunt and Stanley (2000) to maintain consistent pumping rates and sample volume sizes throughout the course of the study. Moreover, although Boulton *et al.* (2004) stated that 5-L samples would suffice to reveal spatial variation in meiofauna composition amongst sites with different sediment texture, a sample size of 20-L was adopted for density estimates, in order to collect rare spring subsurface copepods (Galassi *et al.*, 2011; Stoch *et al.*, 2011). Meiofauna was extracted by filtering 20-L samples collected by Bou-Rouch pump through a hand net (mesh size=60  $\mu$ m). Faunal samples were preserved in 4% formaldehyde solution. Copepods were sorted, counted, identified to species level, and assigned to two ecological categories: obligate (*i.e.*, stygobiotic), and non-obligate (*i.e.*, non-stygobiotic) groundwater dwellers, according to the definition of Galassi (2001) and Galassi *et al.* (2009).

### Data analysis

Spatial autocorrelation of total species abundance per site and total species richness per site in the spring area was assessed using Moran's I index (Moran, 1950). Correlation between frequency of occurrence and abundances was assessed with Spearman's correlation coefficient. Differences in total species richness and total abundance per site of stygobiotic and non-stygobiotic species were assessed using the non-parametric Wilcoxon's test for paired data (Wilcoxon, 1945). Finally, heterogeneity in species distribution ( $\beta$ -diversity) was calculated using Whittaker's formula:  $\beta = \gamma / \alpha - 1$ , where  $\gamma$  is the total number of species collected in the spring area, and  $\alpha$  is the mean point-site diversity (Whittaker, 1960). Point-site diversity was ob-

tained by pooling species richness per three depths and six dates at each site.

In order to assess the relationships between environmental variables and distribution patterns of subsurface copepods, a Detrended Canonical Correspondence Analysis (DCCA) was used to detect the main environmental gradients and measure gradient length (ter Braak and Šmilauer, 2002). Since gradient lengths were very short (less than two standard deviations for each axis), a Redundancy Analysis (RDA) was adopted. Despite being based on linear combinations and correlations of variables, this method performs better than other multivariate techniques in small spatial scale analyses, and with environmental gradients shorter than three standard deviations (ter Braak and Šmilauer, 2002). Environmental parameters were standardised, and copepod abundances were  $\log(x+1)$  transformed prior to statistical analysis in order to minimise differences among variances. Monte Carlo permutation tests (999 permutations) were used to assess significance of canonical axes.

DCCA and RDA were performed with CANOCO for MS<sup>®</sup> Windows version 4.51 (ter Braak and Šmilauer, 2002). All other analyses were run using the software R, version 2.15.3 (R Development Core Team, 2013).

## RESULTS

### Species diversity and assemblage structure

A total of 22 copepod species (Tab. 1) were collected, total abundance varying from 1 to 1435 individuals per species. The assemblages comprised nine stygobiotic and 13 non-stygobiotic species. The latter group mainly included surface water species, accompanied by some temporary groundwater dwellers (*Bryocamptus* (*Echinocamptus*) *echinatus*, *Bryocamptus* (*Rheocamptus*) *typhlops*, *Epactophanes richardi*, and *Moraria* (*Moraria*) *poppei*). The stygobiotic species accounted for 51.4% of the total copepod abundance in the whole spring system. A significant correlation was observed between frequency of occurrence and abundance of the 22 species (Spearman's correlation,  $r=0.76$ ,  $P<0.001$ ).

*Nitocrella pescei* was the most abundant species (1435 individuals), showing also the highest frequency of occurrence (57%), followed by *B. (E.) echinatus* (frequency=52%, 379 individuals), *Diacyclops paolae* (frequency=38%, 394 individuals) and *Pesceus schmeili* (frequency=26%, 754 individuals). Six species (*i.e.*, the stygobiotic *Diacyclops italianus*, *Nitocrella kunzi*, *Pseudectinosoma reductum*, *Simplicaris lethaea*, and the surface water species *E. richardi* and *Microcyclops vari-cans*) were rarely collected (frequency  $\leq 2\%$ ).

Total species richness and total abundance per site did not display a significant spatial autocorrelation within the Presciano spring area (Moran's I between 0.007 and

0.433,  $P=ns$ ). Species richness did not show a significant spatial structure; values were lower (Wilcoxon's test=2.81,  $P<0.01$ ) for stygobiotic (mean species richness per site  $\pm$  standard deviation:  $4.3\pm 1.1$ ) than for non-stygobiotic species ( $8.2\pm 2.0$ ). Mean abundance per sample, on the other hand, was very similar (Wilcoxon's test=0.56,  $P=0.58$ ) for stygobiotic ( $15.8\pm 11.3$ ) and non-stygobiotic species ( $15.6 \pm 19.8$ ); however, spatial distribution was patchy (Fig. 2 b,d). Stygobiotic abundance (Fig. 2b) was higher at western sites (p10, p9), while non-stygobiotic abundance (Fig. 2d) was higher at central sites (p6 and p8), and very low elsewhere.

Even though the distribution of species richness was quite uniform in the spring system for both ecological groups,  $\beta$ -diversity was higher for stygobiotic (2.67) than for non-stygobiotic (0.90) species.

### Relationships between environmental parameters and copepod assemblages

Environmental parameters used in RDA analysis are summarised in Tab. 2 and Fig. 3, the variation in temperature and hydrochemistry at different sampling dates and depths being very small.

The first two canonical axes of the redundancy analysis (RDA) explained 73.2% of the total variance of species-environment relationship (Fig. 4), while the first

four axes explained over 87% of the total variation; Monte Carlo permutation test for all constrained eigenvalues returned a  $P$ -value lower than 0.001, indicating that species-environment correlation was highly significant. Considering that the contribution of three variables (dissolved oxygen, pH, and nitrates) was very small, a forward stepwise analysis was performed to reduce the number of variables to be represented in the analysis to 14 out of 17, leading to the first four axes of RDA explaining 88.7% of the species-environment relationship. The first canonical axis (constrained percentage of explained variance=58.3%) was positively correlated with hydro-metric level, sand, and fine sand, and to a lesser extent with particulate organic matter (Fig. 4a). This axis mainly described a grain-size gradient, from small-sized sediment particles in deeper limnocratic waters to large-sized sediment particles in the rheocrenic sectors, along with a weaker gradient in the potential for retention of organic matter, which was higher for small-sized sediments. The second axis (constrained percentage of explained variance=17.5%) was positively correlated with sulphate concentration, and to a lesser extent with calcium and specific conductivity, while there was a strong negative correlation with the piezometric head and to a lesser extent with large-sized sediments (Fig. 4a); thus, the second axis mainly described a hydraulic gradient. Samples (Fig. 4b)

**Tab. 1.** Species richness and cumulative abundances of copepod species recorded at each sampling site in the Presciano spring system.

Site		p1	p2	p3	p4	p5	p6	p7	p8	p9	p10
Number of samples		18	15	12	18	18	12	18	16	12	16
Species	EC										
<i>Eucyclops serrulatus</i> (Fisher, 1851)	nSB	3	0	34	2	4	13	2	13	1	0
<i>Paracyclops imminutus</i> Kiefer, 1929	nSB	1	0	5	1	0	6	2	7	0	20
<i>Microcyclops varicans</i> (Sars G.O., 1863)	nSB	0	1	0	1	0	1	0	0	0	0
<i>Nitokra hibernica hibernica</i> (Brady, 1880)	nSB	0	1	5	1	0	5	0	0	0	0
<i>Attheyella (Attheyella) crassa</i> (Sars G.O., 1863)	nSB	1	0	10	13	1	10	2	26	1	8
<i>Bryocamptus (Bryocamptus) minutus</i> (Claus, 1863)	nSB	0	0	8	0	0	0	0	0	0	4
<i>Bryocamptus (Rheocamptus) zschokkei tatrensis</i> (Minkiewicz, 1916)	nSB	0	6	10	4	1	12	0	1	5	1
<i>Bryocamptus (Rheocamptus) pygmaeus</i> (Sars G.O., 1863)	nSB	4	0	44	18	0	12	17	2	1	1
<i>Moraria (Moraria) poppei</i> (Mrázek, 1893)	nSB	1	0	0	3	6	18	0	461	0	0
<i>Pesceus schmeili</i> (Mrázek, 1893)	nSB	2	7	0	17	56	295	1	371	5	0
<i>Bryocamptus (Rheocamptus) typhlops</i> (Mrázek, 1893)	nSB	1	3	4	140	6	118	0	22	0	0
<i>Bryocamptus (Echinocamptus) echinatus</i> (Mrázek, 1893)	nSB	7	72	62	33	25	56	3	21	25	75
<i>Epactophanes richardi</i> Mrázek, 1893	nSB	0	0	0	0	1	0	0	3	1	0
<i>Diacyclops italianus</i> (Kiefer, 1931)	SB	0	0	1	0	0	0	0	0	0	0
<i>Diacyclops paolae</i> Pesce & Galassi, 1987	SB	3	0	117	48	11	10	2	58	111	34
<i>Pseudectinosoma reductum</i> Galassi & De Laurentiis, 1997	SB	0	0	0	0	2	0	0	0	0	0
<i>Nitocrella kunzi</i> Galassi & De Laurentiis, 1997	SB	0	1	1	1	0	0	0	0	0	0
<i>Nitocrella pescei</i> Galassi & De Laurentiis, 1997	SB	121	237	11	142	135	3	173	7	1	605
<i>Elaphrodoleptomesochra italica</i> Pesce & Petkovski, 1980	SB	4	0	15	2	0	0	0	0	2	0
<i>Elaphoidella mabelae</i> Galassi & Pesce, 1991	SB	0	0	3	4	0	5	0	41	4	0
<i>Parastenocaris lorenzae</i> Pesce, Galassi & Cottarelli, 1995	SB	1	15	0	6	18	17	24	157	249	1
<i>Simplicaris lethaea</i> Galassi & De Laurentiis, 2004	SB	0	0	0	0	0	0	1	0	0	0
Species richness		12	9	15	17	12	15	10	14	12	9

EC, ecological category; nSB, non-stygobiotic species; SB, stygobiotic species.

were arranged along a main gradient, where rheocrenic karstic sites (p1, p2, p10) were grouped together on the left side of the RDA biplot, opposite to limnocrenic sites, located on the right side of the plot. Clogged samples (p3) were located on the upper side of the plot, being characterised by a very low piezometric head. No effect of depth and sampling date on site ordination was observed; samples were clearly clustered (Fig. 4a) according to site location and following the rheocrenic-limnocrenic gradient, irrespective of depth and sampling date.

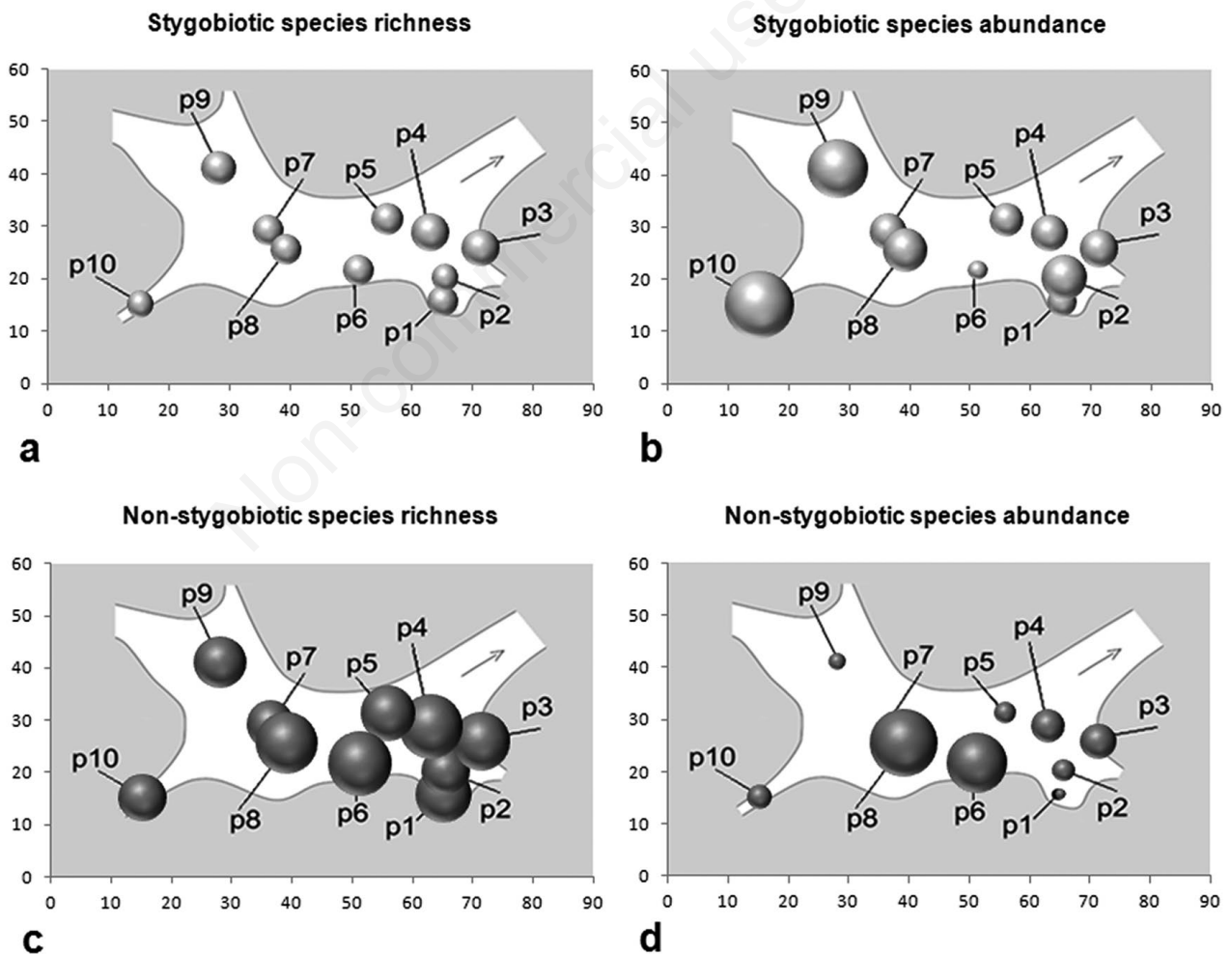
The arrangement of species on the environmental space defined by the first two RDA axes differed between stygobiotic (Fig. 4c) and non-stygobiotic (Fig. 4d) copepods. Stygobionts were scattered on the plot along an environmental gradient mainly defined by sediment texture and piezometric head. *N. pescei* was clearly associated with high piezometric head and large-sized sediment

classes. On the opposite side, *D. paolae*, *Elaphoidella mabelae*, and *Parastenocaris lorenzae* preferred limnocrenic sites, with small sized-sediment classes along with higher concentration of organic matter. Moreover, *D. paolae* was associated with sites displaying clogged sediments and very low piezometric head. Non-stygobiotic species were mainly arranged in the sectors characterised by low piezometric head, irrespective of the major environmental gradient defined by the first axis.

## DISCUSSION

### The role of environmental heterogeneity in shaping copepod assemblages

It is widely recognised that sediment texture influences local microhabitat conditions, as well as groundwater upwelling in streams (Swan and Palmer, 2000), and



**Fig. 2.** Distribution of species richness and abundance (mean value per sample) in the Presciano spring. a) Mean stygobiotic species richness across sites. b) Mean stygobiotic species abundance across sites. c) Mean non-stygobiotic species richness across sites. d) Mean non-stygobiotic species abundance across sites (circle area is proportional to the measured values, scale in meters).

**Tab. 2.** Summary of the environmental variables (mean±standard deviation; maximum and minimum value) for each sampling site.

Sites	T (°C)	pH	Specific conductivity ( $\mu\text{S cm}^{-1}$ )	Eh (mV)	O <sub>2</sub> (mg L <sup>-1</sup> )	Mg <sup>2+</sup> (mg L <sup>-1</sup> )	Ca <sup>2+</sup> (mg L <sup>-1</sup> )	NO <sub>3</sub> <sup>-</sup> (mg L <sup>-1</sup> )	SO <sub>4</sub> <sup>2-</sup> (mg L <sup>-1</sup> )	PO <sub>4</sub> <sup>3-</sup> (mg L <sup>-1</sup> )	POM (mg L <sup>-1</sup> )	Hydrometric level (cm)	Piezometric head
p1	mean±SD	10.61±0.31	7.54±0.25	484±9	6.63±1.13	20±2	69±11	6.38±1.09	20±2	0.09±0.13	1.21±0.81	15±11	4±0
	max	11.23	7.86	499	9.07	24	79	7.92	23	0.56	3.15	28	4
	min	10.28	6.99	465	5.39	15	38	4.4	16	0.01	0.28	2	4
p2	mean±SD	10.59±0.30	7.49±0.31	485±9	6.77±1.01	19±2	74±2	6.08±1.12	21±2	0.16±0.32	1.64±1.79	36±17	3±1
	max	11.32	7.81	499	9	22	78	8.4	23	0.94	7.18	61	4
	min	10.00	6.91	461	5.46	14	70	4.4	17	0.01	0.24	15	2
p3	mean±SD	10.66±0.89	7.57±0.27	497±26	6.64±0.98	18±5	77±9	5.81±1.05	22±2	0.04±0.02	11.03±8.59	21±18	1±0
	max	11.61	7.84	557	8.52	23	88	7.04	25	0.06	24.75	45	1
	min	9.37	6.95	451	4.85	4	52	4.4	19	0.01	0.96	0	1
p4	mean±SD	10.85±0.60	7.52±0.23	488±7	6.69±1.09	19±2	74±2	6.33±1.39	20±3	0.04±0.03	12.60±18.97	37±15	2±0
	max	11.96	7.83	495	9.5	22	78	9.24	24	0.11	72.41	61	3
	min	10.00	6.93	465	5.14	16	71	3.5	14	0.01	1.20	22	2
p5	mean±SD	10.78±0.63	7.53±0.23	485±13	6.70±0.99	19±3	75±6	6.23±8.39	20±2	0.03±0.02	2.03±2.13	45±16	2±0
	max	12.51	7.81	506	9.05	28	89	8.39	26	0.08	9.32	72	3
	min	10.06	6.95	466	5.3	15	66	4.4	17	0.01	0.59	28	2
p6	mean±SD	10.82±0.72	7.40±0.35	498±15	6.57±1.04	18±3	78±4	6.26±1.36	22±1	0.04±0.03	5.69±2.40	91±15	2±0
	max	12.53	8.05	520	8.55	23	88	9.24	25	0.11	9.40	116	3
	min	9.72	6.9	475	8.4	13	71	4.8	20	0.01	2.31	73	2
p7	mean±SD	10.65±0.47	7.47±0.25	489±11	6.75±1.19	20±2	74±3	6.48±1.19	20±2	0.03±0.02	3.36±2.56	45±11	3±1
	max	11.87	7.8	497	9.14	23	79	8.36	25	0.07	8.75	58	4
	min	10.03	6.95	468	8.3	16	68	4.8	16	0.01	0.84	29	2
p8	mean±SD	10.89±0.73	7.47±0.29	492±14	6.43±1.08	18±4	75±4	6.26±1.12	20±3	0.03±0.01	7.66±6.84	58±12	3±0
	max	11.96	7.88	511	8.49	22	85	8.8	24	0.06	26.30	73	3
	min	9.46	7	468	5.9	10	68	4.4	10	0.01	0.48	45	2
p9	mean±SD	10.84±0.46	7.35±0.26	495±13	6.88±1.11	19±3	76±2	6.3±1.07	22±1	0.03±0.03	6.79±7.15	98±16	3±1
	max	12.15	7.75	507	8.63	23	2	8.8	25	0.10	18.15	122	4
	min	10.33	6.9	469	5.44	12	79	4.8	20	0.01	0.32	84	2
p10	mean±SD	11.05±0.56	7.48±0.34	582±23	6.51±0.94	20±5	71	6.46±0.96	27±3	0.07±0.06	3.08±1.56	5.7±2	2±1
	max	11.93	7.88	599	8.43	28	88±16	7.92	31	0.18	5.25	15	3
	min	9.46	6.76	504	5.44	11	106	5.3	19	0.01	0.83	1	0

consequently may affect the small-scale distribution of subsurface meiofauna among microhabitats. Indeed, several researchers argued that groundwater flowpath and substratum overwhelmingly affect small-scale meiofaunal patterns in streams (Dole-Olivier and Marmonier, 1992; Stanley and Boulton, 1993; Fowler and Scarsbrook, 2002; Silver *et al.*, 2002; Olsen and Townsend, 2003). This was already demonstrated specifically for copepods by Rouch (1988) and Rouch and Lescher-Moutoué (1992) for the Lachein brook in France. However, the role of the environmental parameters in shaping distribution patterns of meiofaunal copepod assemblages in springs was, up to now, virtually unknown.

The analysis of the measured environmental parameters in the Presciano spring system revealed the presence of an unexpectedly strong spatial heterogeneity at the small spatial scale. Small-scale habitat patchiness predominantly relied on differences in grain-size composition of sediments across sites and piezometric head (*i.e.*, groundwater flowpath). Habitat patchiness generated differences in the microdistribution of subsurface copepods; conversely, the variation in hydrochemistry had a minor effect in shaping patterns of subsurface copepods if compared to groundwater flowpath and substratum, contrasting with the results obtained at the broad spatial scale for spring meiofauna (Stoch *et al.*, 2011).

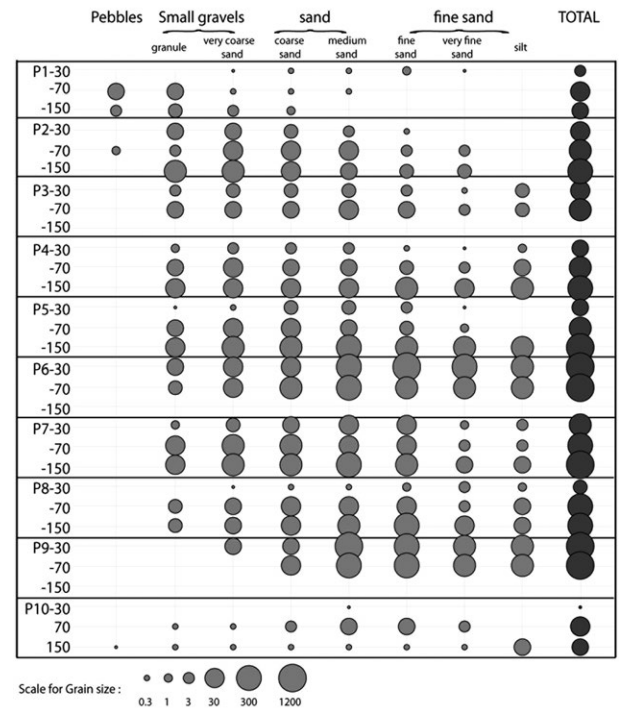
**Copepods as descriptors of hydrodynamics and sediment texture**

The hydrogeological setting of rheo-limnocratic springs may explain such small-scale spatial heterogeneity reflected in habitat patchiness and uneven copepod species distributions, stygobiotic species being more sensitive to the detected environmental gradients than non-stygobiotic ones. Indeed, the  $\beta$ -diversity of stygobiotic assemblages was higher than for non-stygobiotic ones. This may be due to the higher ecological specialisation of stygobionts. One stygobiotic species (*N. pescei*) was most frequent and abundant at the rheocrenic and fractured sectors of the spring system (Galassi and De Laurentiis, 1997a). At these sites, stygobionts may have been flushed out from the bedrock fractures and could have also colonised nearby sediments, as already hypothesised by Fiasca *et al.* (2004). On the other hand, three species (*D. paolae*, *E. mabelae* and *P. lorenzae*) were most abundant in fine-sediment spring sectors. They preferred the true interstitial habitat, as exemplified by the worm-like members of the genus *Parastenocaris* (Galassi and De Laurentiis, 2004), which show peculiar adaptations to live among the grains. Conversely, non-obligate stygobiotic species dominated the central sector of the spring system, characterised by fine sediments, low water flow, high hydro-metric level, and higher particulate organic matter content, and were poorly represented in strong upwelling sites.

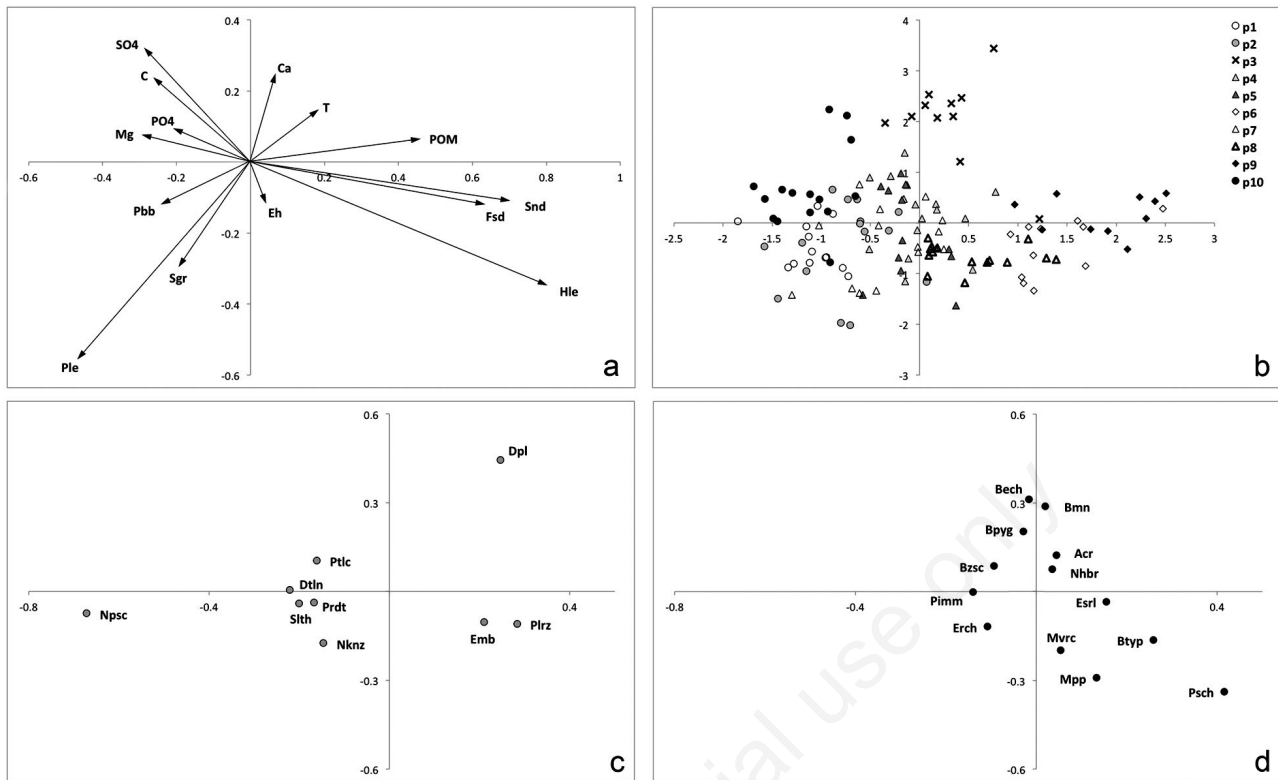
This condition is likely attributable to the absence of a surface water network in the recharge area, suitable for drift of epigeic species in ground water (Di Lorenzo *et al.*, 2005). Moreover, low-altitude springs fed by the Gran Sasso aquifer lie along no-flow and seepage limits defined by aquitards (Amoruso *et al.*, 2013), further limiting the potential for dispersal of epigeic species from the recharge areas to the Presciano spring system. Copepods thus can be considered reliable descriptors of sediment texture and groundwater flowpath in this patchy spring system (Galassi and De Laurentiis, 1997a, 1997b).

Stygobiotic species distribution was not sensitive to depth; probably the broad-scale upwelling in springs flattens the expected differences in the vertical dimension, in sharp contrast to that observed in riverine ecosystems (Gibert *et al.*, 1994 and references therein).

The results showed that the distribution of most stygobiotic species was not correlated with particulate organic matter content. Recent experimental studies highlighted the low nutrient requirements of stygobionts, suggesting that trophic availability, both in terms of input of allochthonous organic matter and microbial biomass, was not a major determinant for the survival of stygobiotic species, plausibly due to their slow metabolic rate (Foulquier *et al.*, 2010, 2011).



**Fig. 3.** Granulometric composition of the 10 sampling points at three depths below the spring bed. The total amount of sediment is given in g/10 L-sample.



**Fig. 4.** Constrained ordination diagram of the Presciano spring data using the first two axes of RDA analysis. a) Ordination of environmental variables represented by arrows (T, temperature; C, specific conductivity; O<sub>2</sub>, dissolved oxygen; NO<sub>3</sub>, nitrate; PO<sub>4</sub>, orthophosphate; SO<sub>4</sub>, sulphate; Ca, calcium; Mg, magnesium; POM, particulate organic matter; Plev, piezometric level; Hle, hydrometric level; Pbb, pebble; Sgr, small gravel; Snd, sand; Fsd, fine sand). b) Ordination of sites sampled at six different dates and three different depths. c) Ordination of stygobiotic and d) non-stygobiotic copepod species (stygobiotic species acronyms: *Diacyclops italianus*, Dtl; *Diacyclops paolae*, Dpl; *Pseudectinosoma reductum*, Prdt; *Nitocrella kunzi*, Nknz; *Nitocrella pescei*, Npsc; *Parapseudoleptomesochra italica*, Ptic; *Elaphoidella mabelae*, Emb; *Parastenocaris lorenzae*, Plrz; *Simplicaris lethaea*, Slth; non-stygobiotic species acronyms: *Eucyclops serrulatus*, Esrl; *Paracyclops imminutus*, Pimm; *Microcyclops varicans*, Mvrc; *Nitokra hibernica*, Nhbr; *Attheyella crassa*, Acr; *Bryocamptus minutus*, Bmn; *Bryocamptus pygmaeus*, Bpyg; *Bryocamptus typhlops*, Btyp; *Bryocamptus zschokkei*, Bzsc; *Bryocamptus echinatus*, Bech; *Moraria poppei*, Mpp; *Pesceus schmeili*, Psch; *Epactophanes richardi*, Erch).

## CONCLUSIONS

Groundwater dependent ecosystems (GDEs) are becoming key research topics in freshwater biology and ecology (Howard and Merrifield, 2010). Springs are included in the definition of GDE given by Hatton and Evans (1998), and in more detail, they fit two different categories of GDEs, being at the same time both surface GDEs, maintaining vegetation and epigeal fauna, and also subsurface groundwater dependent ecosystems (SGDEs), the first type being relatively well studied, the second one almost completely unknown (Tomlinson and Boulton, 2008, 2010). We have instead analysed the *dark side* of this dual-faceted GDE at a small spatial scale. Our study demonstrates that local patchiness in environmental parameters is paralleled by differences in species distri-

butions beneath the spring surface under undisturbed conditions. The results obtained may offer relevant information for a better understanding of the potential changes generated by anthropogenic disturbance on these vulnerable ecosystems.

## ACKNOWLEDGMENTS

The research project was partially granted by the European Community (Life plus project AQUALIFE) and by the Gran Sasso – Laga National Park (Italy). CM was funded by the French National Research Agency ‘InBio-Process’ project (ANR-06-BDIV-007-InBioProcess 2007-2010). The authors would like to thank the two anonymous reviewers for making useful comments on the first draft of the manuscript.



## REFERENCES

- Amoruso A, Crescentini L, Petitta M, Tallini M, 2013. Parsimonious recharge/discharge modeling in carbonate fractured aquifers: The groundwater flow in the Gran Sasso aquifer (Central Italy). *J. Hydrol.* 476:136-146.
- Barquín J, Death RG, 2006. Spatial patterns of macroinvertebrate diversity in New Zealand springbrooks and rithral streams. *J. N. Am. Benthol. Soc.* 25:768-786.
- Barquín J, Death RG, 2009. Physical and chemical differences in karst springs of Cantabria, northern Spain: do invertebrate correspond? *Aquat. Ecol.* 43:445-455.
- Barquín J, Scarsbrook M, 2008. Management and conservation strategies for coldwater springs. *Aquat. Conserv.* 18:580-591.
- Boni C, Pianelli A, Pierdominici S, Ruisi M, 2002. [Le grandi sorgenti del Fiume Tirino (Abruzzo)]. [Article in Italian]. *Boll. Soc. Geol. Ital.* 121:411-431.
- Botosaneanu L, 1998. Studies in crenobiology: the biology of springs and springbrooks. Backhuys Publ., Leiden: 261 pp.
- Bottazzi E, Bruno MC, Mazzini M, Pieri V, Rossetti G, 2008. First report on Copepoda and Ostracoda (Crustacea) from northern Apenninic springs (N. Italy): a faunal and biogeographical account. *J. Limnol.* 67:56-63.
- Bottazzi, E, Bruno MC, Pieri V, Di Sabatino A, Silveri L, Carolli M, Rossetti G, 2011. Spatial and seasonal distribution of invertebrates in Northern Apennine rheocene springs. *J. Limnol.* 70:77-92.
- Bou C, Rouch R, 1967. [Un nouveau champ de recherches sur la faune aquatique souterraine]. [Article in French]. *C. R. Acad. Sci.* 265:369-370.
- Boulton AJ, Dole-Olivier M-J, Marmonier P, 2004. Effects of sample volume and taxonomic resolution on assessment of hyporheic assemblage composition sampled using a Bou-Rouch pump. *Arch. Hydrobiol.* 159:327-355.
- Cantonati M, Füreder L, Gerecke R, Jüttner I, Cox EJ, 2012. Crenic habitats, hotspots for freshwater biodiversity conservation: toward an understanding of their ecology. *Freshwater Sci.* 31:463-480.
- Cantonati M, Gerecke R, Jüttner I, Cox EJ, 2011. Springs: neglected key habitats for biodiversity conservation. *J. Limnol.* 70(Suppl.1):1-187.
- Caschetto M, Barbieri M, Galassi DMP, Mastroiello M, Rusi S, Stoch F, Di Cioccio A, Petitta M, 2014. Human alteration of groundwater-surface water interactions (Sagittario River, Central Italy): implication for flow regime, contaminant fate and invertebrate response. *Environ. Earth Sci.* 71:1791-1807.
- Czachorowski S, 1990. Caddisflies (Trichoptera) of the springs of the Kraków-Częstochowa and the Miechów Uplands (Poland). *Acta Hydroch. Hydrob.* 32:391-405.
- Di Lorenzo T, Cipriani D, Bono P, Rossini L, De Laurentiis P, Fiasca B, Pantani C, Galassi DMP, 2005. Dynamics of groundwater copepod assemblages from the Mazzoccolo karstic spring (central Italy). *Meiofauna Marina* 14:97-103.
- Di Lorenzo T, Galassi DMP, 2013. Agricultural impact on Mediterranean alluvial aquifers: do groundwater communities respond? *Fund. Appl. Limnol.* 182:271-282.
- Di Lorenzo T, Stoch F, Galassi DMP, 2013. Incorporating the hyporheic zone within the river discontinuum: Longitudinal patterns of subsurface copepod assemblages in an Alpine stream. *Limnologia* 43:288-296.
- Di Sabatino A, Cicolani B, Gerecke R, 2003. Biodiversity and distribution of water mites (Acari, Hydrachnidia) in spring habitats. *Freshwater Biol.* 48:2163-2173.
- Dole-Olivier MJ, Marmonier P, 1992. Ecological requirements of stygofauna in an active channel of the Rhône River. *Stygologia* 7:65-75.
- Dumnicka E, Galas J, Koperski P, 2007. Benthic invertebrates in karst springs: does substratum or location define communities? *Int. Rev. Hydrobiol.* 92:452-464.
- Eamus D, Froend R, 2006. Groundwater-dependent ecosystems: the where, what and why of GDEs. *Aust. J. Bot.* 54:91-96.
- Fiasca B, Di Lorenzo T, De Laurentiis P, Pantani C, Galassi DMP, 2004. [Biodiversità acquatica sotterranea nel Parco Nazionale del Gran Sasso-Monti della Laga: analisi taxocenotica e proposte di conservazione]. [Article in Italian]. *Studi Trent. Sci. Nat. Acta Biol.* 81:157-166.
- Foulquier A, Malard F, Mermillod-Blondin F, Montuelle B, Dolédec S, Volat B, Gibert J, 2011. Surface water linkages regulate trophic interactions in a groundwater food web. *Ecosystems* 14:1339-1353.
- Foulquier A, Simon L, Gilbert F, Fourel F, Malard F, Mermillod-Blondin F, 2010. Relative influences of DOC flux and subterranean fauna on microbial abundance and activity in aquifer sediments: new insights from <sup>13</sup>C-tracer experiments. *Freshwater Biol.* 55:1560-1576.
- Fowler RT, Scarsbrook MR, 2002. Influence of hydrologic exchange patterns on water chemistry and hyporheic communities in three gravel-bed rivers. *New Zeal. J. Mar. Fresh.* 36:471-482.
- Galas J, 2005. Human impact on physical and chemical properties from Cracow-Częstochowa Upland (Southern Poland). *Pol. J. Ecol.* 53:329-341.
- Galassi DMP, 2001. Groundwater copepods (Crustacea: Copepoda): diversity patterns over ecological and evolutionary scales. *Hydrobiologia* 453/454:227-253.
- Galassi DMP, De Laurentiis P, 1997a. Two new species of *Nitocrella* from Italian groundwaters (Crustacea: Copepoda: Ameiridae). *It. J. Zool.* 64:367-376.
- Galassi DMP, De Laurentiis P, 1997b. *Pseudectinosoma reductum* sp. n., a new ectinosomatid harpacticoid from spring waters in Italy (Crustacea: Copepoda). *Hydrobiologia* 356:81-86.
- Galassi DMP, De Laurentiis P, 2004. Towards a revision of the genus *Parastenocaris* Kessler, 1913: establishment of *Simplicaris* n. gen. from groundwater in central Italy and review of the *P. brevipes*-group (Copepoda, Harpacticoida, Parastenocarididae). *Zool. J. Linn. Soc.-Lond.* 140:417-436.
- Galassi DMP, Fiasca B, Del Tosto D, 2011. Patterns of copepod diversity (Copepoda: Cyclopoida, Harpacticoida) in springs of central Italy: implications for conservation issues, p. 199-226. In: D. Defaye, E. Suárez-Morales and J.C. Vaupel Klein (eds.) *Crustaceana monographs, studies on freshwater Copepoda: a volume in honour of Bernard Dussart*. Brill NV, Leiden.
- Galassi DMP, Huys R, Reid JW, 2009. Diversity, ecology and evolution of groundwater copepods. *Freshwater Biol.* 54:691-708.
- Gathmann FO, Williams DD, 2006. Insect emergence in Canadian coldwater springs: spatial and temporal patterns, and species-environment relationships. *Ann. Limnol.-Int. J. Lim.* 42:143-156.
- Gerecke R, Meisch C, Stoch F, Aciri F, Franz H, 1998. Eu-

- crenon-hypocrenon ecotone and spring typology in the Alps of Berchtesgaden (Upper Bavaria, Germany). A study of microcrustacea (Crustacea: Copepoda, Ostracoda) and water mites (Acari: Halacaridae, Hydrachnellae), p. 167-182. In: L. Botosaneanu (ed.) *Studies in crenobiology. The biology of springs and springbrooks*. Backhuys Publ., Leiden.
- Gibert J, Danielopol DL, Stanford JA, 1994. *Groundwater ecology*. Academic Press: 571 pp.
- Hatton T, Evans R, 1998. Dependence of ecosystems on groundwater and its significance to Australia. Occasional Paper No. 12/98, Land and Water Resources Research and Development Corporation. CSIRO Australia: 77 pp.
- Hoffsten P-O, Malmqvist B, 2000. The macroinvertebrate fauna and hydrogeology of springs in central Sweden. *Hydrobiologia* 436:91-104.
- Howard J, Merrifield M, 2010. Mapping groundwater dependent ecosystems in California. *PLoS ONE* 5:e11249.
- Hunt GW, Stanley EH, 2000. An evaluation of alternative procedures using the Bou-Rouch method for sampling hyporheic invertebrates. *Can. J. Fish. Aquat. Sci.* 57:1545-1550.
- Malard F, Dole-Olivier MJ, Mathieu J, Stoch F, 2002. *Sampling Manual for the Assessment of Regional Groundwater Biodiversity*. Accessed on: 17 October 2008. Available from: <http://www.pascalis-project.com>
- Martin P, Brunke M, 2012. Faunal typology of lowland springs in northern Germany. *Freshwater Sci.* 31:542-562.
- Moran PAP, 1950. Notes on continuous stochastic phenomena. *Biometrika* 37:17-23.
- Olsen DA, Townsend CR, 2003. Hyporheic community composition in a gravel-bed stream: influence of vertical hydrological exchange, sediment structure and physiochemistry. *Freshwater Biol.* 48:1363-1378.
- Petitta M, Tallini M, 2003. Groundwater resources and human impacts in a Quaternary intramontane basin: the case study of the L'Aquila Plain (Central Italy). *Water Int.* 28:57-69.
- R Development Core Team, 2013. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Available from: <http://www.R-project.org>
- Rouch R, 1988. [Sur la répartition spatiale des Crustacés dans le sous-écoulement d'un ruisseau des Pyrénées]. [Article in French]. *Ann. Limnol.-Int. J. Lim.* 24:213-234.
- Rouch R, Lescher-Moutoué F, 1992. [Structure du peuplement des Cyclopidés (Crustacea: Copepoda) dans le milieu hyporhéique d'un ruisseau des Pyrénées]. [Article in French]. *Stygologia* 7:197-211.
- Scarsbrook MR, Barquín J, Gray D, 2007. New Zealand cold-water springs and their biodiversity. *Science for conservation* 278. Science & Technical Publ., New Zealand: 72 pp.
- Silver P, Palmer MA, Swan CM, Wooster D, 2002. The small scale ecology of freshwater meiofauna, p. 217-239. In: S.D. Rundle, A.L. Robertson and J.M. Schmid-Araya (eds.), *Freshwater Meiofauna: biology and ecology*. Backhuys Publ., Leiden.
- Smith H, Wood PJ, Gunn J, 2003. The influence of habitat structure and flow permanence on invertebrate communities in karst spring systems. *Hydrobiologia* 510:53-66.
- Stanley EH, Boulton AJ, 1993. Hydrology and the distribution of hyporheos: perspectives from a mesic river and a desert stream. *J. N. Am. Benthol. Soc.* 12:79-83.
- Stoch F, Galassi DMP, 2010. Stygobiotic crustacean species richness: a question of numbers, a matter of scale. *Hydrobiologia* 653:217-234.
- Stoch F, Gerecke R, Pieri V, Rossetti G, Sambugar B, 2011. Exploring species distribution of spring meiofauna (Annelida, Acari, Crustacea) in the south-eastern Alps. *J. Limnol.* 70(Suppl.1):65-76.
- Swan CM, Palmer MA, 2000. What drives small-scale patterns in lotic meiofauna communities? *Freshwater Biol.* 44:109-121.
- ter Braak CJF, Šmilauer P, 2002. *CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination (version 4.5)*. Ithaca, NY, USA.
- Tomlinson M, Boulton A, 2008. Subsurface groundwater dependent ecosystems: a review of their biodiversity, ecological processes and ecosystem services. *Waterlines Occasional Paper* No 8.
- Tomlinson M, Boulton A, 2010. Ecology and management of subsurface groundwater dependent ecosystems in Australia - a review. *Mar. Freshwater Res.* 61:936-949.
- Wentworth CK, 1922. A scale of grade and class terms for clastic sediments. *J. Geol.* 30:377-392.
- Whittaker RH, 1960. *Vegetation of the Siskiyou Mountains, Oregon and California*. *Ecol. Monogr.* 30:279-338.
- Wilcoxon F, 1945. Individual comparisons by ranking methods. *Biometrics Bull.* 1:80-83.
- Zöllhöfer JM, Brunke M, Gonser T, 2000. A typology of springs in Switzerland by integrating habitat variables and fauna. *Arch. Hydrobiol.* 121:349-376.