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ORIGINAL ARTICLE

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

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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-limnocrenic 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 limnocrenic and rheocrenic 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.

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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 (Botosaneau, 1998), springs are usually approached only under an epigean 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 limnocrenes (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-limnocrenic 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 boudaries around a 700 km^2 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^2 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^3 s^{-1}. Minor variations in discharge (±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 dominated 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×10 points×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-

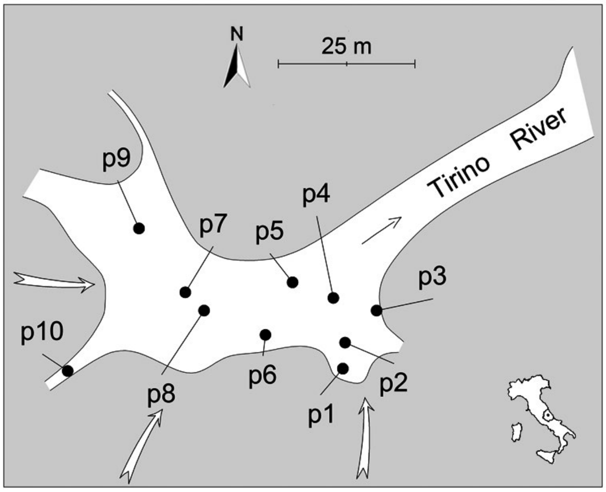

Fig. 1. Distribution of sampling sites in the Presciano spring system. Sites are coded from p1 to p10. Arrows indicate the prevailing direction of the groundwater flowpath.

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 μm). 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 (β-diversity) was calculated using Whittaker's formula: $\beta=\gamma/\alpha-1$, where γ is the total number of species collected in the spring area, and α is the mean point-site diversity (Whittaker, 1960). Point-site diversity was obtained 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 Ŝmilauer, 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 Ŝmilauer, 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® Windows version 4.51 (ter Braak and Ŝmilauer, 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 varicans*) were rarely collected (frequency ≤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 ± standard deviation: 4.3±1.1) than for non-stygobiotic species (8.2±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±11.3) and non-stygobiotic species (15.6 ± 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, β-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 hydrometric 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 limnocrenic 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										
Eucyclops serrulatus (Fisher, 1851)	nSB	3	0	34	2	4	13	2	13	1	0
Paracyclops imminutus Kiefer, 1929	nSB	1	0	5	1	0	6	2	7	0	20
Microcyclops varicans (Sars G.O., 1863)	nSB	0	1	0	1	0	1	0	0	0	0
Nitokra hibernica hibernica (Brady, 1880)	nSB	0	1	5	1	0	5	0	0	0	0
Attheyella (Attheyella) crassa (Sars G.O., 1863)	nSB	1	0	10	13	1	10	2	26	1	8
Bryocamptus (Bryocamptus) minutus (Claus, 1863)	nSB	0	0	8	0	0	0	0	0	0	4
Bryocamptus (Rheocamptus) zschokkei tatrensis (Minkiewicz, 1916)	nSB	0	6	10	4	1	12	0	1	5	1
Bryocamptus (Rheocamptus) pygmaeus (Sars G.O., 1863)	nSB	4	0	44	18	0	12	17	2	1	1
Moraria (Moraria) poppei (Mrázek, 1893)	nSB	1	0	0	3	6	18	0	461	0	0
Pesceus schmeili (Mrázek, 1893)	nSB	2	7	0	17	56	295	1	371	5	0
Bryocamptus (Rheocamptus) typhlops (Mrázek, 1893)	nSB	1	3	4	140	6	118	0	22	0	0
Bryocamptus (Echinocamptus) echinatus (Mrázek, 1893)	nSB	7	72	62	33	25	56	3	21	25	75
Epactophanes richardi Mrázek, 1893	nSB	0	0	0	0	1	0	0	3	1	0
Diacyclops italianus (Kiefer, 1931)	SB	0	0	1	0	0	0	0	0	0	0
Diacyclops paolae Pesce & Galassi, 1987	SB	3	0	117	48	11	10	2	58	111	34
Pseudectinosoma reductum Galassi & De Laurentiis, 1997	SB	0	0	0	0	2	0	0	0	0	0
Nitocrella kunzi Galassi & De Laurentiis, 1997	SB	0	1	1	1	0	0	0	0	0	0
Nitocrella pescei Galassi & De Laurentiis, 1997	SB	121	237	11	142	135	3	173	7	1	605
Parapseudoleptomesochra italica Pesce & Petkovski, 1980	SB	4	0	15	2	0	0	0	0	2	0
Elaphoidella mabelae Galassi & Pesce, 1991	SB	0	0	3	4	0	5	0	41	4	0
Parastenocaris lorenzae Pesce, Galassi & Cottarelli, 1995	SB	1	15	0	6	18	17	24	157	249	1
Simplicaris lethaea 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

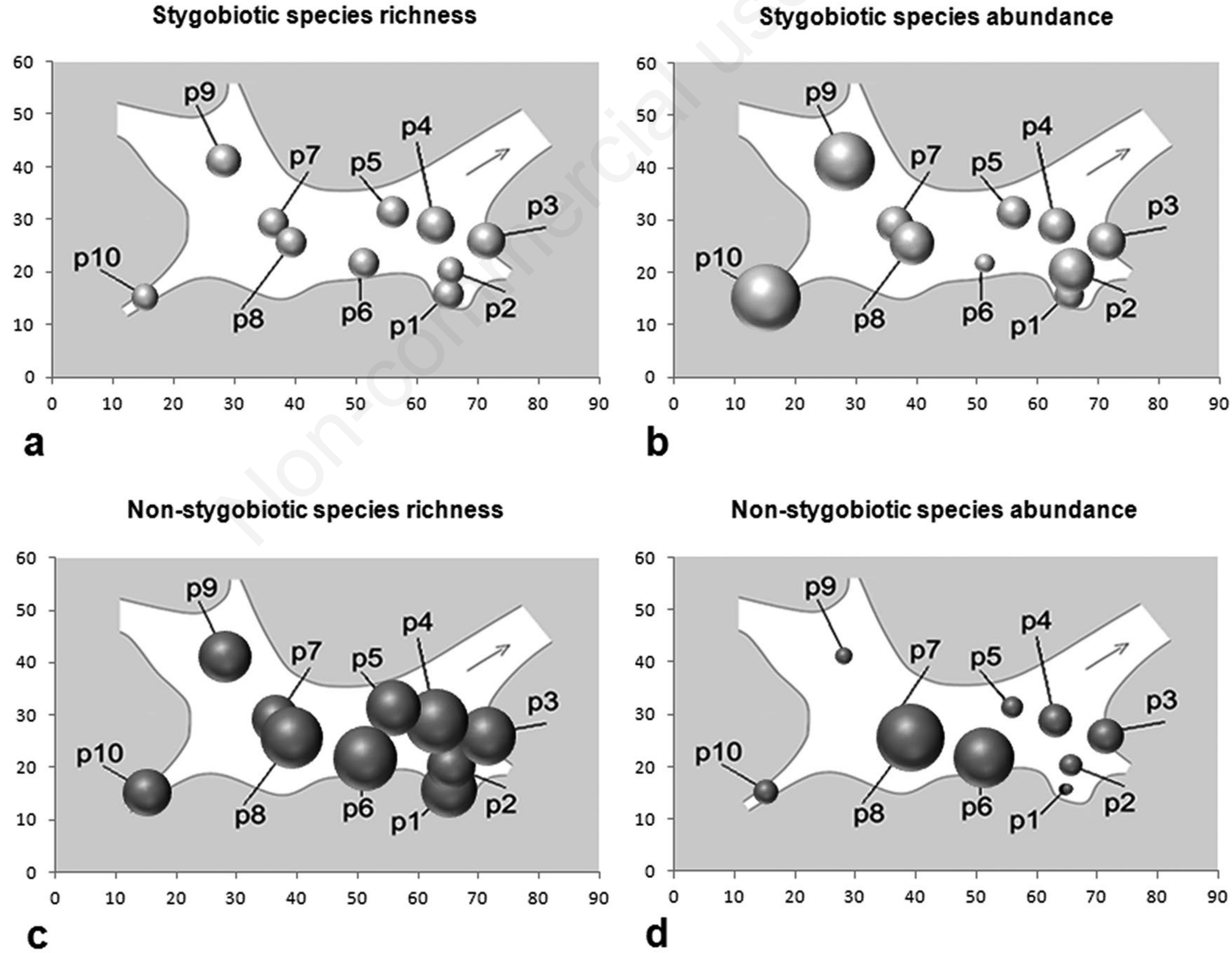

Fig. 2. Distribution of species richness and abundance (mean value per sample) in the Presciano spring. a) Mean stygobiotic species richness across sites. b) Mean stygobiotic species abundance across sites. c) Mean non-stygobiotic species richness across sites. d) Mean non-stygobiotic species abundance 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 (µS cm^{-1})	Eh (mV)	O_2 (mg L^{-1})	Mg^{2+} (mg L^{-1})	Ca^{2+} (mg L^{-1})	NO_3^- (mg L^{-1})	SO_4^{2-} (mg L^{-1})	PO_4^{3-} (mg L^{-1})	POM (mg L^{-1})	Hydrometric level (cm)	Piezometric head
p1	mean±SD	10.61±0.31	7.54±0.25	484±9	134±68	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	241	9.07	24	79	7.92	23	0.56	3.15	28	4
	min	10.28	6.99	465	43	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	131±64	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	274	9	22	78	8.4	23	0.94	7.18	61	4
	min	10.00	6.91	461	50	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	165±76	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	305	8.52	23	88	7.04	25	0.06	24.75	45	1
	min	9.37	6.95	451	21	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	190±76	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	318	9.5	22	78	9.24	24	0.11	72.41	61	3
	min	10.00	6.93	465	85	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	155±77	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	305	9.05	28	89	8.39	26	0.08	9.32	72	3
	min	10.06	6.95	466	48	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	172±76	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	340	8.55	23	88	9.24	25	0.11	9.40	116	3
	min	9.72	6.9	475	84	4.8	13	71	4.8	20	0.01	2.31	73	2
p7	mean±SD	10.65±0.47	7.47±0.25	489±11	163±79	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	319	9.14	23	79	8.36	25	0.07	8.75	58	4
	min	10.03	6.95	468	83	5.23	16	68	4.8	16	0.01	0.84	29	2
p8	mean±SD	10.89±0.73	7.47±0.29	492±14	192±86	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	338	8.49	22	85	8.8	24	0.06	26.30	73	3
	min	9.46	7	468	59	4.94	10	68	4.4	10	0.01	0.48	45	2
p9	mean±SD	10.84±0.46	7.35±0.26	495±13	196±66	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	308	8.63	23	2	8.8	25	0.10	18.15	122	4
	min	10.33	6.9	469	101	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	203±89	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	344	8.43	28	88±16	7.92	31	0.18	5.25	15	3
	min	9.46	6.76	504	28	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-limnocrenic 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 β-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 hydrometric 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 epigean 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 epigean 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).

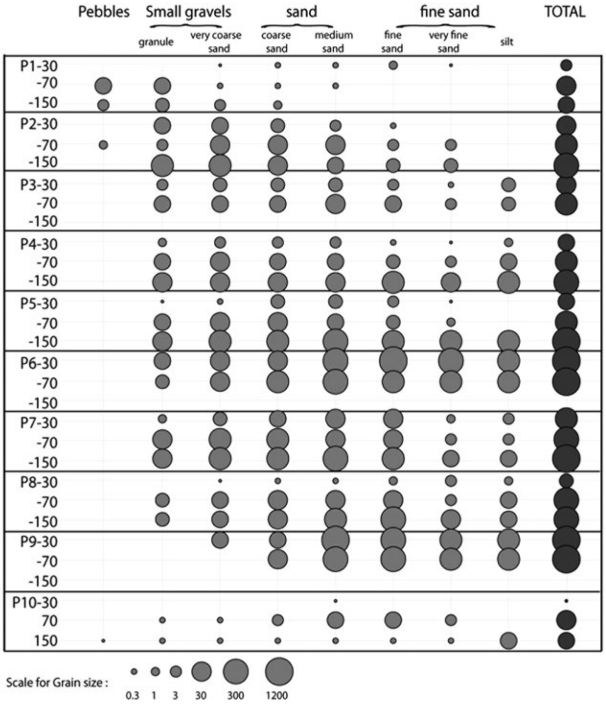

Fig. 3. Granulometric composition of the 10 sampling points at three depths below the spring bed. The total amount of sediment is given in g/10 L-sample.

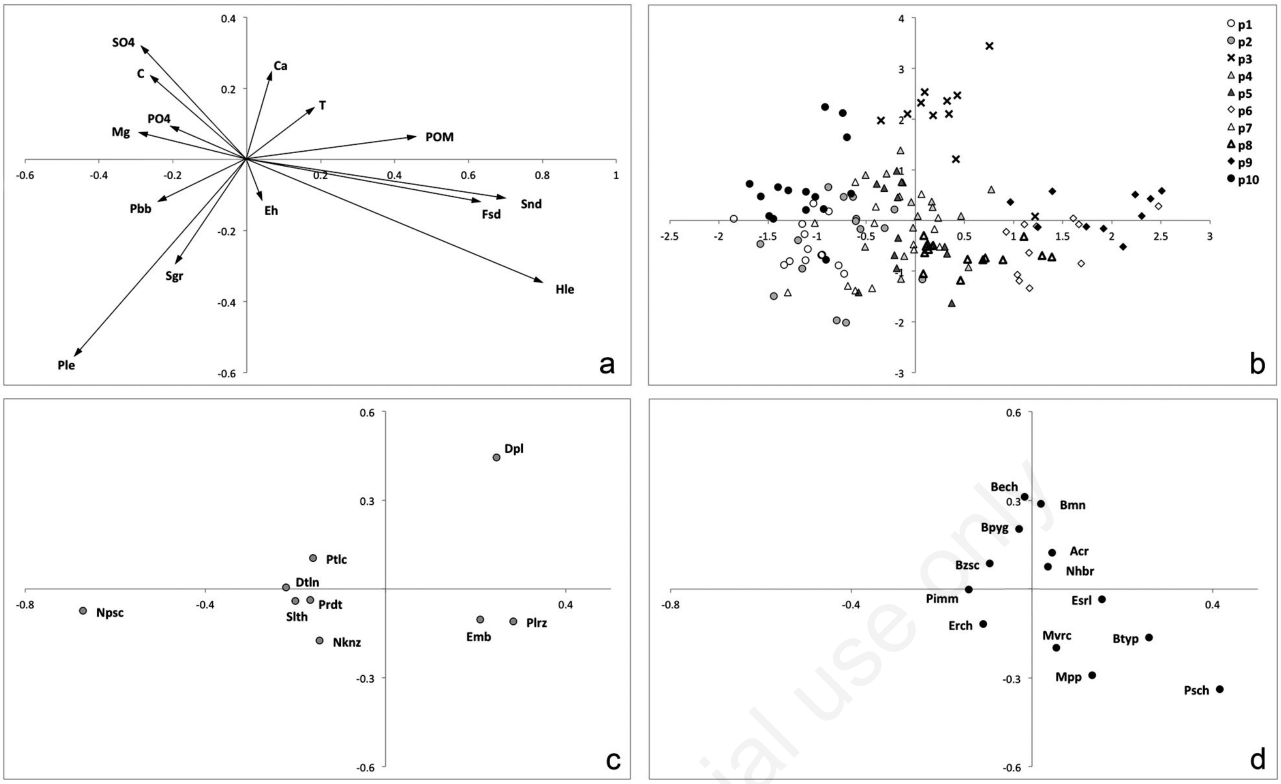

Fig. 4. Constrained ordination diagram of the Presciano spring data using the first two axes of RDA analysis. a) Ordination of environmental variables represented by arrows (T, temperature; C, specific conductivity; O_2, dissolved oxygen; NO_3, nitrate; PO_4, orthophosphate; SO_4, sulphate; Ca, calcium; 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,* Dtln; *Diacyclops paolae,* Dpl; *Pseudectinosoma reductum,* Prdt; *Nitocrella kunzi,* Nknz; *Nitocrella pescei,* Npsc; *Parapseudoleptomesochra italica*, Ptlc; *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 epigean 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 distributions 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.

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