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Primary Research Paper

Colonisation patterns and vertical movements of stream invertebrates in the interstitial zone: a case study in the Apennines, NW Italy

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Abstract

We examined vertical migration and colonisation patterns of stream macroinvertebrates within the substratum of an Apennine creek in NW Italy. Macrobenthos was sampled at three depths in the streambed (0–5, 5–10, 10–15 cm) by means of artificial baskets filled with natural substratum. We placed 42 traps (5×5×15 cm), i.e. 21 top-opened (T-traps) and 21 bottom-opened (B-traps), each composed of three overlapping baskets (high-H, medium-M and low-L), to evaluate differences in the vertical movements. We also collected Surber samples to compare interstitial assemblages with streambed communities. The multilevel traps yielded 42 taxa, compared with 60 taxa in the natural riverbed. Interstitial traps were rapidly colonised; both taxa richness and organism number increased during the 42-day study period. We found active migration in both vertical directions, but there were more invertebrates in the top-opened traps than in the bottom-opened traps. In the T-traps the most colonised baskets were those placed at the H level, while in the B-traps the L level baskets were more rapidly colonised. The interstitial assemblages differed markedly from the streambed communities in both composition and functional organisation, with more collector-gatherers and predators in the interstitial zone and more filterers and scrapers in the natural riverbed. In Apennine lotic systems, the interstitial zone is an important habitat for stream macrobenthos, although it may not be used by all species.

Introduction

There is growing interest in the vertical dimension of stream systems. Traditionally, aquatic ecologists have perceived streams and rivers as bounded systems, consisting of riverbeds and overlying water; the exchanges of water, detritus, nutrients and organisms between groundwater and stream channels have only recently become a central element in freshwater ecology (e.g. Hynes, 1974; Bretschko, 1979, 1981, 1985, 1990, 1994, 1995; Vallet et al., 1993). Orghidan (1959) considered the hyporheic zone as the sub-surface region of lotic systems that exchanges water with the surface. In a more recent perspective, White (1993) considered the hyporheic zone as the saturated interstitial areas beneath the riverbed and in the stream banks that contain some proportion of channel water or that have been altered by channel water infiltration: this is a functional interface between groundwater and surface-water ecosystems. The riverbed represents the upper part of this hyporheic zone and it plays an important role in the functioning of lotic ecosystems (Bretschko, 1992, 1994, 1995; Weigelhofer & Waringer, 2003). Following Dole-Olivier & Marmonier (1992), we will use the term ‘interstitial habitat’ instead of hyporheic zone to indicate the permeable area of the substratum.

It is well known that the interstitial zone acts as a refuge for benthic macroinvertebrates, increasing both the resistance and resilience of stream macroinvertebrate communities (Dole-Olivier et al., 1997). Benthic macroinvertebrates are focal elements of lotic environments, playing a fundamental role in the functioning of the river and in maintenance of its self-purifying capacity (Vannote et al., 1980). The water flowing in the riverbed pore system constitutes an essential and peculiar habitat that may support rich and diversified macroinvertebrate assemblages (Giller & Malmqvist, 1998). Many invertebrate taxa use the interstitial area as a nursery zone, for the deposition and incubation of eggs and the growth of small instars (Jacobi & Cary, 1996), or as refuge, suggesting that macroinvertebrates migrate into the hyporheic zone to survive catastrophic hydrological events (Dole-Olivier et al., 1997) and to escape high surface water temperatures (Boulton et al., 1998) or droughts (Boulton, 1989). For these reasons, estimates of secondary production in streams are substantially greater when the interstitial fauna is included in the calculations: for example, Huryn (1996) demonstrated that the total benthos production was sufficient to explain the observed fish production in a New Zealand stream only when the sub-substratum production was taken into account.

Despite the growing interest in the sub-substratum zone, few studies have investigated the direction and patterns of within-substrate movements in the first layer of the riverbed. Early studies on movements and colonisation patterns of stream organisms showed that freshwater invertebrate populations are in continuous redistribution (Townsend & Hildrew, 1976). Williams & Hynes (1976) detected four main colonisation patterns. (1) Drift, the “downstream transport of aquatic organisms in the current”, is probably the main component of the colonisation process for most taxa (Brittain & Eikeland, 1988; Mackay, 1992), also in Apennine lotic systems (Fenoglio et al., 2004). (2) Upstream movement within the water has been recorded for many benthic invertebrates (Söderström, 1987; Panek, 1991). In a recent study of a NW Italian river, Fenoglio et al. (2002) detected evident seasonal influences in this process,involving many taxa such as *Brachyptera* sp. (Plecoptera, Taeniopterygidae), *Capnia* sp. (Plecoptera, Capniidae) and others. (3) The upstream flight of lotic insects is also an important component of the colonisation cycle of many taxa. Muller’s pioneering theory (1954) of the colonisation cycle of freshwater insects in lotic systems identified the upstream flight of imagines as compensation for the downstream displacement of immature individuals via drift. This idea was confirmed by studies demonstrating a pronounced tendency of egg-bearing females to fly upstream (Madsen & Butz, 1976; Muller, 1982). (4) Vertically upward movement from within the substrate is another important colonisation mechanism. Although generally neglected for a long time, this process is now attracting growing interest. However: (a) many studies have focused on the importance of downward movement towards the hyporheic/interstitial zone, especially in temporary streams (Delucchi, 1989) in which flow occurs either seasonally (intermittent streams) or in response to irregular rain (episodic streams) (Hose et al., 2005); (b) despite the great ecological importance of the sub-substratum zone, studies on ecological and functional aspects of interstitial areas have been performed only in recent years (Marmonier et al., 1992); (c) traditionally, most investigators have studied the meiofauna, while ecological aspects of the macrofauna in the interstitial zone have only been investigated in a few studies. The long-term RITRODAT-Lunz project was one of the most important efforts in this field (Bretschko, 1981).

The aims of the present experimental study were: (a) to provide data about colonisation patterns and vertical migration of stream macroinvertebrates in the interstitial zone; (b) to analyse the composition, structure and functional organisation of interstitial assemblages; (c) to investigate the importance of the sub-substratum as a habitat for the benthic communities of an Apennine river, a system not studied thus far.

Methods

This study was conducted in the Caramagna Creek, a small tributary of the Bormida River, NW Italy (44°36′ N–8°32′ E; altitude 280 m asl; Fig. 1). Dense woodlands, with small scattered urban areas, cover the catchment. This lotic system is of good environmental quality, reaching First Class in the Extended Biotic Index system

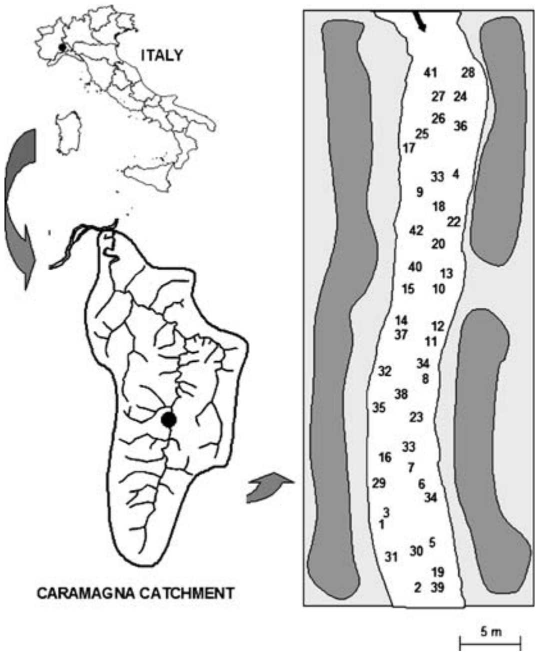

Figure 1. Location of Caramagna Creek (NW Italy) and interstitial traps position in the riffle.

(Ghetti, 1997), corresponding to an environment without human impact. Table 1 reports some abiotic parameters, measured with Eijkelkamp 13.14 and 18.28 portable instruments (parameters were measured at each sampling date).

On December 29, 2004, we placed 42 vertical stratification samplers in a large and uniform gravel riffle of the Caramagna Creek riverbed, using a random distribution. The traps consisted of a parallelepiped metal net (mesh size 1 cm), measuring 5 cm long, 5 cm wide and 15 cm high (Fig. 2). Two 5×5 cm frames were inserted to separate three overlapping 5×5×5 cm baskets (H, M, L, i.e. High, Medium and Low level). The traps were filled with clean, sterile substratum according to the granulometry of the riffle (Md approximately 9.5). Traps were individually numbered: traps 1–21 (T-traps = top-opened traps) were covered with nylon net (mesh size 265 μm) except for the apex, allowing access exclusively from the top. Traps 22–42 (B-traps = bottom-opened traps) were covered with the same net, but in this case the only access was from the bottom. The traps were left in place for a maximum of 42 days. This period was long enough to allow macroinvertebrates to reach maximum abundance, as indicated by the literature (Hauer & Lamberti, 1996) and a previous experiment in a nearby area (Fenoglio et al., 2002), and short enough to avoid floods. After 14, 28 and 42 days, we randomly selected 14 traps per sampling date (seven top-opened and seven bottom-opened) from the stream in order to investigate the colonisation process. The macroinvertebrates dislodged from each trap when it was moved were collected with a 250 μm mesh net and added to the sample.

Table 1. Conductivity, dissolved oxygen, pH and flow speed (mean ± SD) of the surface water in the stream reach during the study period

Parameter	Values
Conductivity (μS/cm)	359.0 ± 32.2
Dissolved oxygen (mg/l)	8.30 ± 0.18
pH	8.63 ± 0.21
Water velocity (m/s)	0.60 ± 0.20
Temperature (°C)	2.87 ± 1.42

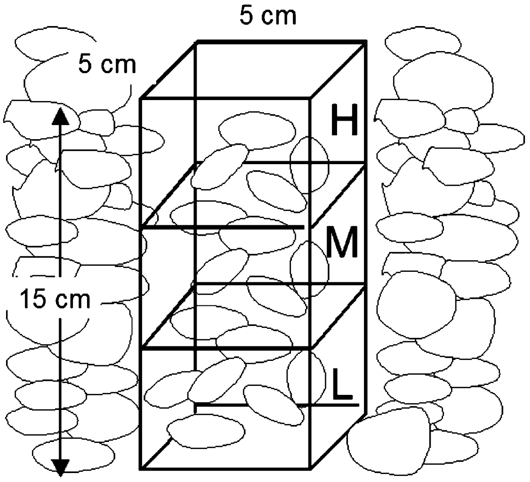

Figure 2. Trap type design.

To quantify the macroinvertebrate community composition and structure on the natural bottom of the river, we used a 0.06 m^2 sampler with a 250 μm mesh to collect 30 Surber samples in the same riffle area where we placed the traps. The Surber samples were randomly collected on three dates during the experiment (day 1, day 15 and day 35).

In the laboratory, all organisms were counted and identified to the genus level, except for

Chironomidae, Simuliidae and early instars of some Trichoptera and Diptera, which were identified to the family level. Each taxon was also assigned to a Functional Feeding Group (FFG: scrapers, shredders, collector-gatherers, filterers and predators) according to Merritt & Cummins (1996). Moreover, a classification of taxa into seven biological and seven ecological groups was conducted according to the Usseglio-Polatera et al. (2000) species traits approach.

Statistical analysis of the total number of macroinvertebrates and the taxa richness of samples collected in T-traps and B-traps was performed with ANOVA of log-transformed data. For comparison of the functional composition, we used arcsin-transformed data of FFG percentage composition.

Richness accumulation curves, generated with EstimateS 6.0 software (Colwell, 1997), were used to compare the cumulative taxa number for all samples from T- and B-traps. The preference of individual taxa to enter B- or T-traps was evaluated by indicator species analysis computed with INDVAL 2.0 software (Dufrêne, 1998). Indicator species analysis is a randomisation-based test that compares the relative abundance and relative frequency of taxa to find indicator species assemblages characterising groups of samples. A taxon's affinity for a sampling group is expressed as a percentage (Dufrêne & Legendre, 1997).

Results

Data for colonisation density and taxa richness in each trap category and in the Surber samples are reported in Table 2. The total abundance of macroinvertebrates significantly increased over time (Fig. 3) in both the T-traps ($F_{2,\ 20} = 20.15$, $p < 0.001$) and B-traps ($F_{2,\ 20} = 11.4$, $p = 0.001$). Community richness significantly increased over time in both trap types (T-traps: $F_{2,\ 20} = 15.55$, $p < 0.001$; B-traps: $F_{2,\ 20} = 4.13$, $p = 0.033$).

Top-opened traps were more rapidly colonised, with a higher number of organisms in the first period, while later there were no significant differences in macroinvertebrate abundance (Table 3). Taxa richness increased over time (Fig. 3), with a significant difference between B- and T-traps in the last sampling period (Table 3). Species accumulation curves showed that additional taxa were likely to be found with additional sampling (Fig. 4). The curves agree with the other data in that T-traps showed greater taxa richness.

The baskets closest to the trap opening (H for T-traps, L for B-traps) were the most colonised, considering both the number of individuals (Fig. 5, ANOVA $F_{2,\ 124} = 4.26$, $p = 0.016$) and the number of taxa (ANOVA $F_{2,\ 124} = 3.37$, $p = 0.038$). Hence, the most colonised baskets in the T-traps were those placed at the H level, while in the B-traps the L level baskets were most rapidly colonised. Differences between the H, M and L baskets were present in the first two sampling periods (14 and 21 days), but after 42 days the H, M and L baskets showed similar invertebrate abundance (Fig. 5) and taxa richness.

The most abundant taxa in both the T- and B-trap assemblages were Chironomidae, *Dugesia* sp., Limnephilidae (small instars), *Eiseniella tetraedra*, *Capnia bifrons*, *Brachyptera* sp. and *Baetis* sp. Comparison of assemblages in the two trap types by indicator species analysis revealed two taxa with a significant preference for top–down vertical movement, *Capnia bifrons* and *Baetis* sp., and one taxon, *Brachyptera* sp., with a bottom–top preference. There were no precocious colonisers, but we detected some late colonisers, i.e. taxa particularly present in the last phase of the experiment: *Baetis* sp., *Capnia bifrons*, *Eiseniella tetraedra*, *Helicus substriatus* and Limnephilidae. Indicator values, habitat abundance and fidelity of significant taxa ($p < 0.05$) are summarised in Table 4.

Interestingly, although some groups were abundant and widespread on the river bottom, they were absent or rare in the interstitial traps: in particular, the black flies (Simuliidae) were abundant throughout the whole riffle, with a mean density of 1026 ind./m^2 in some areas, as was the mayfly *Ecdyonurus* sp. with a mean density of 52.5 ind./m^2. A similar pattern was observed for some water bugs (*Micronecta* sp.) and caddisflies (*Hydropsyche* sp.).

The most abundant FFG in the interstitial traps was collector-gatherers (50.7% in T-traps and 44.5% in B-traps), followed by predators (18.9% in T-traps and 34.1% in B-traps) and shredders (22.9% in T-traps and 16.2% in B-traps), while scrapers were not abundant (6.5% in T-traps and 4.0% in B-traps) and filterers

Table 2. Percent relative abundance for macroinvertebrates collected in the natural riverbed and from Bottom (B) and Top (T) opened interstitial traps

Taxa	Biol. traits	Ecol. traits[a]	FFG[b]	Natural riverbed	B-Traps	T-Traps
Plecoptera						
Capnia bifrons	e	B	Sh	0.48	2.79	6.37
Leuctra sp.	f	B	Sh	0.73	0.00	0.98
Nemoura sp.	f	A	Sh	0.30	0.56	1.96
Brachyptera sp.	f	B	Sh	21.07	5.03	1.96
Isoperla sp.	c	B	P	0.73	0.00	0.00
Ephemeroptera						
Ecdyonurus sp.	f	C	Sc	1.36	0.00	1.47
Baetis sp.	e	C	Cg	7.41	2.79	4.90
Caenis sp.	f	F	Cg	0.11	0.56	0.00
Habrophlebia sp.	f	C	Cg	1.23	1.12	0.98
Habroleptoides sp.	f	C	Cg	0.28	0.56	0.98
Ephemera danica	c	C	Cg	0.04	0.00	0.49
Centroptilum luteolum	f	D	Cg	0.32	1.12	0.49
Torleya major	e	B	Cg	0.02	0.00	0.00
Paraleptophlebia sp.	f	C	Cg	0.02	0.00	0.00
Trichoptera						
Limnephilidae	f	F	Sh	0.71	5.03	9.31
Potamophylax cingulatus	f	F	Sh	0.06	0.00	0.00
Psychomyidae	e	C	Cg	0.13	0.00	0.00
Glossosomatidae	e	B	Sc	0.80	1.68	0.00
Leptoceridae			Cg	0.06	0.56	0.00
Hyporhyacophila sp.	c	A	P	0.04	0.00	0.00
Beraeidae	e	A	Cg	0.02	0.00	0.00
Hydropsyche sp.	e	C	F	1.04	0.00	0.00
Wormaldia sp.	e	A	F	0.06	0.00	0.00
Goeridae	f	C	Cg	0.02	0.00	0.00
Odontocerum albicorne	c	A	Sh	0.30	0.00	0.00
Sericostoma sp.			Sh	0.06	0.00	0.00
Diptera						
Anthomidae	c	F	P	0.00	0.56	0.00
Atherix sp.	d	C	P	0.09	0.00	0.00
Chironomidae	e	F	Cg	10.78	29.6	32.3
Ceratopogonidae	c	F	P	0.78	0.56	1.47
Psychodidae	e	F	P	0.09	2.23	0.49
Anopheles sp.	e	F	Cg	0.02	0.00	0.00
Simuliidae	e	B	F	30.62	2.23	0.98
Dixidae	e	F	P	0.04	0.00	0.00
Tipulidae	c	D	Sh	1.10	0.00	0.49
Dolichopodidae	g	F	P	0.00	0.56	0.98
Tabanidae	g	F	P	0.15	0.00	0.00
Limoniidae	c	D	P	0.54	0.00	1.47
Stratiomyidae	e	D	P	0.06	2.23	1.96

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Table 2. (Continued)

Taxa	Biol. traits	Ecol. traits[a]	FFG[b]	Natural riverbed	B-Traps	T-Traps
Coleoptera						
Hydrophilidae	g	G	P	0.02	0.56	0.00
Helichus substriatus	g	G	Sh	0.73	2.23	4.90
Hydraena andreinii	g	D	Sc	0.91	0.00	0.49
Hydraena assimilis	g	D	Sc	0.02	0.00	0.00
Hydraena truncata	g	D	Sc	0.13	0.00	0.00
Hydraena subimpressa	g	D	Sc	0.06	0.00	0.00
Hydraena devillei	g	D	Sc	0.04	0.00	0.00
Gyrinidae (larvae)	g	G	P	0.58	0.00	0.49
Elminthidae (larvae)	g	D	Cg	0.15	0.00	0.49
Elminthidae	g	D	Cg	0.15	0.00	0.00
Esolus sp.	g	D	Cg	0.06	0.00	0.00
Helodidae (larvae)	g	G	Sh	0.06	0.00	0.00
Dytiscidae	g	G	P	0.00	0.00	0.98
Odonata						
Onychogomphus sp.	d	D	P	0.06	0.00	0.49
Orthetrum sp.	d	F	P	0.09	0.00	0.00
Calopteryx sp.	d	D	P	0.06	0.00	0.00
Heteroptera						
Micronecta sp.	e	D	P	3.82	0.00	0.00
Megaloptera						
Sialis lutaria	e	G	P	0.02	0.00	0.00
Hymenoptera						
Agriotypus armatus	e	C	P	0.04	0.00	0.00
Planipenna						
Osmylus fulvicephalus	g	B	P	0.00	0.56	0.00
Arachnida						
Hydracarina			P	0.37	0.00	0.00
Bivalva						
Pisidium sp.	b	D	F	0.02	0.56	0.00
Gastropoda						
Lymnaea peregra	c	F	Sc	0.06	0.00	0.98
Tricladida						
Dugesia sp.	c	D	P	7.41	26.26	12.25
Anellida						
Eiseniella tetraedra	h	D	Cg	1.60	5.03	1.47
Lumbricidae	h	D	Cg	0.82	1.12	5.88
Lumbriculidae	h	D	Cg	0.26	3.35	0.98
Naididae	h	D	Cg	0.17	0.00	0.00
Tubificidae	h	E	Cg	0.13	0.00	0.49
Nematomorpha						
Gordius sp.	b	A	P	0.09	0.56	0.00
Nematoda						
Mermithidae	b	A	P	0.02	0.00	0.00

Biological and ecological traits according to Usseglio-Polatera et al. (2000). Functional Feeding Groups according to Merritt & Cummins (1996).

[a] Biological and ecological traits: see text.

[b] FFG: functional feeding groups (Cg, collectors-gatherers; F, filterers; P, predators; Sc, scrapers; Sh, shredders).

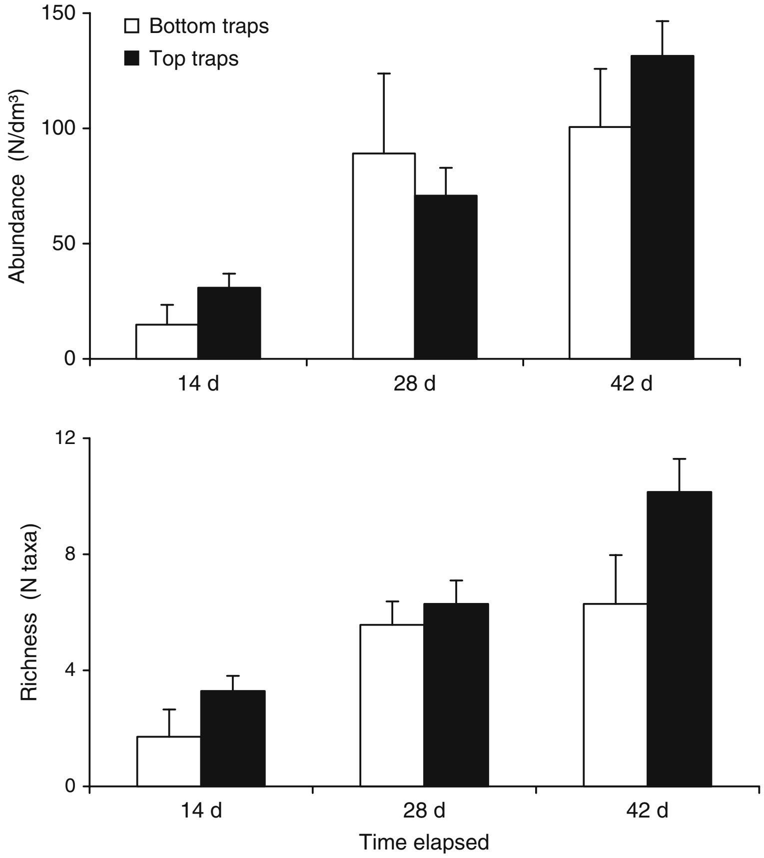

Figure 3. Time-related variation of mean abundance and community richness of macroinvertebrates found in top and bottom traps (mean ± 1SE).

almost absent (1.0% in T-traps and 1.2% in B-traps). When the functional composition of the interstitial substrata on the three sampling dates was considered, no significant differences were found between T- and B-traps (ANOVAs on arcsin-transformed percentages, all p = n.s.). Moreover, the chronological evolution of the FFG composition did not differ significantly, except for scrapers which increased over time ($F_{2,\ 41} = 6.67$, $p = 0.007$) because of the development of biofilms.

The functional composition of the macroinvertebrate community in the natural riverbed was quite different from that in the interstitial traps. In the riffle riverbed, filterers were the most

Table 3. Comparison (mean ± SD, ANOVA tests) of abundance and taxonomic richness in the T- and B-traps

Time	Parameter	Trap type		Statistics	
		Top	Bottom	*F* value	*p*
Day 14	Abundance (N/dm^3)	30.8 ± 6.3	14.8 ± 8.6	5.03	0.04*
	Richness (S)	3.29 ± 0.52	1.71 ± 0.94	2.12	0.17
Day 28	Abundance (N/dm^3)	70.9 ± 12.0	89.12 ± 34.6	0.02	0.97
	Richness (S)	6.29 ± 0.80	5.57 ± 0.81	0.389	0.54
Day 42	Abundance (N/dm^3)	131.4 ± 15.0	100.6 ± 25.3	2.26	0.16
	Richness (S)	10.1 ± 1.1	6.20 ± 1.2	3.54	0.05*

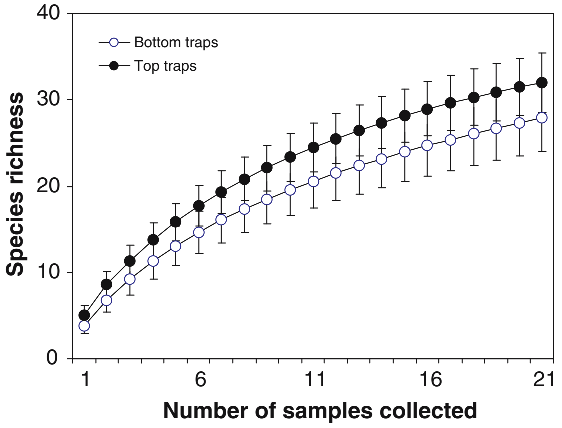

Figure 4. Taxa accumulation curves for invertebrates in interstitial traps bottom- and top-opened.

abundant FFG (31.7% of individuals), followed by shredders (24.2%) and collector-gatherers (22.3%). In comparison to the interstitial trap assemblages, a higher number of scrapers were found (10.3%) while predators were less abundant (10.6%).

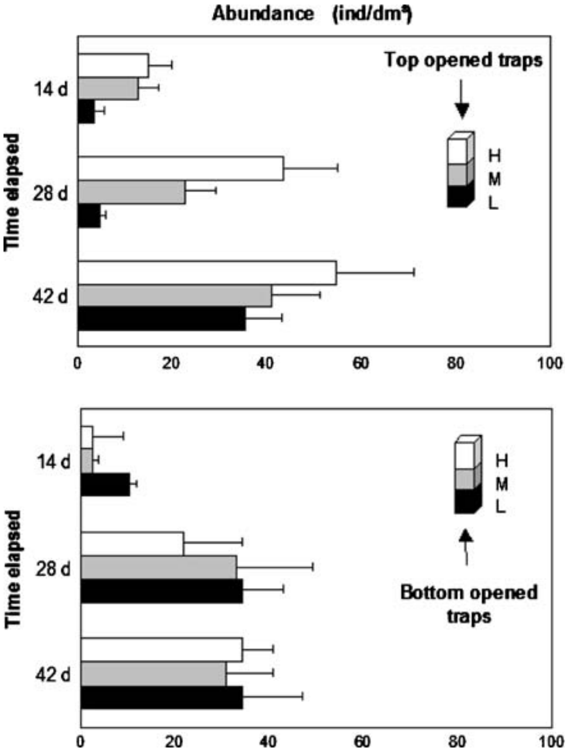

Figure 5. Number of organisms collected in the three baskets of each trap type of the different dates (mean ± SE).

Analysis of the biological and ecological traits of benthic macroinvertebrates (Usseglio-Polatera et al., 2000) showed no significant differences between T- and B-traps (all ANOVA p = n.s.). Considering the biological traits, the most abundant taxa colonising interstitial substrata in both traps belonged to the '*e group*' (small or medium-sized, short-lived crawlers, with aquatic respiration and cemented eggs: 42.2%) followed by taxa belonging to the '*c group*' (medium-sized, crawlers, with egg or larval dormancy: 21.2%). Considering the ecological traits, the most abundant group was '*F*' (organisms avoiding high current velocities: 46.7%), followed by '*D*' (organisms that live near the banks or in sidearms of lowland streams: 32.8%). In the natural riverbed the most abundant biological group was the '*e group*' (55.7%) followed by the '*f group*' (medium-sized, crawlers, shredders with aquatic respiration: 26.4%), while the two most abundant ecological groups were '*B*' (organisms living in rhithronic rheophilous environments: 54.7%) and '*D*' (18.9%).

Discussion

Since the studies of Kühtreiber (1934), Schwoerbel (1961, 1964), Hynes & Coleman (1968) and Coleman & Hynes (1970), it has been known that many benthic organisms occur below the depth usually sampled by benthic samplers. In this regard, the Ritrodat-Lunz project provided a substantial amount of interesting data (Bretschko, 1981; Bretschko & Leichtfried, 1988), revealing diverse and intense interactions between the hyporheic interstitial layer and stream ecosystem.

The interstitial space of riverbed sediments is a main component of lotic systems and it is attracting growing interest (Gayraud & Philippe, 2003) because of its importance in the dynamics of physical, chemical and biological processes. This zone is populated by many macroinvertebrate species. Yet, even though several studies have shown that most invertebrates inhabiting the interstitial area are present in the top 15 cm (Collier & Scarsbrook, 2000), little is known about the movements and colonisation direction within this zone.

We detected a clear time-dependent colonisation process in our interstitial traps, with increasing taxa richness and organism density in both trap

Table 4. Indicator values, habitat abundance and fidelity for macroinvertebrates collected: (a) from T- and B-traps; (b) in the first period (early colonisers) and the last period (late colonisers)

Taxa	Indicator value	T-traps	B-traps
(a)[A]			
Baetis sp.	49.47	17/11	1/1
Capnia bifrons	28.57	24/7	4/4
Brachyptera sp.	35.37	1/1	13/8
Taxa	Indicator value	First period	Last period
(b)[B]			
Baetis sp.	42.86	0/0	11/6
Capnia bifrons	57.14	0/0	13/8
Eiseniella tetraedra	35.71	0/0	8/5
Helichus substriatum	42.86	0/0	11/6
Limnephilidae	46.15	2/2	24/7

Traps data show the total number of individuals collected and the number of traps where each single taxon was found.

[A] Only 3 out of 42 taxa with an indicator value 25 are reported.

[B] Only 5 out of 42 taxa with an indicator value >25 are reported.

types during the study period. According to Panek (1991, 1994), who recorded active movements in all directions, we detected bi-directional vertical active migrations, with no differences in the density and richness of invertebrate assemblages between the bottom–up and top–down migrations. This finding indicates that, in our study area, the first 15 cm of the substratum layer is a very rich habitat. The depth of the hyporheic interstitial layer is variable in gravel riverbeds, and it can fluctuate among different streams because of the influence of some abiotic parameters, such as oxygen, porosity and grain size distribution (Bretschko, 1994). The increase of organisms was very rapid in both trap types. Macroinvertebrate densities varied from a minimum of 0 ind./dm^3 after 14 days to a maximum to 288 ind./dm^3 after 42 days. Final mean densities of macroinvertebrates were similar to those found in other European gravel riffles (Weigelhofer & Waringer, 2003).

Scarsbrook (1995) reported three distinct patterns of vertical distribution of macroinvertebrates in the sub-substratum: organisms with a pattern of reduced abundance with depth, organisms with uniform distribution and organisms that increased in density with depth. We detected a uniform distribution for all taxa at the end of the study period, with no significant preference for a particular depth.

Our study supports the hypothesis that the interstitial zone is an important habitat for stream macrobenthos (Del Rosario & Resh, 2000), although it may not be used by all taxa. Chironomidae constituted almost a third of the total number of organisms in the interstitial zone, representing the most abundant benthic group, as reported in other studies (Weigelhofer & Waringer, 2003). Flatworms *Dugesia* sp. were also abundant: they actively move in this zone, gliding within substrate elements because of their flattened body. Limnephilidae were also conspicuously present, especially with small instars; the small size of juveniles probably allows them to colonise this particular habitat, while large-cased mature individuals are almost absent in the interstitial zone. Another well represented group is Plecoptera, with two typical winter stoneflies: *Brachyptera monilicornis*, an endangered and extremely localised species (Agosta et al., 2000), and *Capnia bifrons*, known to colonise the substratum in different periods of its life cycle (Hynes, 1979). Interestingly, very few rheophilic taxa were found in the interstitial zone, even though all traps were located in a riffle: these organisms have an inherent need for current, either because they rely on it for feeding purposes or because their respiratory requirements demand it, and this need cannot be satisfied within the elements of the riverbed.

Our analysis of the functional, ecological and biological characteristics of taxa found in the subsurface zone showed that interstitial colonisers are:

(a) small or medium-sized (*sensu* Usseglio-Polatera et al., 2000); (b) with generalist trophic roles, mainly detritivores (collector-gatherers or shredders), likely feeding on detritus and biofilms, or predators; (c) mostly with flexible, cylindrical-shaped or elongated bodies; (d) with an ecological preference for low-flowing environments and lentic microhabitats; (e) crawlers or walkers. Due to the lack or paucity of primary production in the sub-substratum (Müllner & Schagerl, 2003), invertebrates probably feed on detritus and biofilms derived mainly from allochthonous inputs and on the associated microflora: fine particulate organic matter sustains a high number of collector-gatherers while coarse organic matter attracts shredders. We found that some large shredders (e.g. *Brachyptera* sp.) and collectors (e.g. *Eiseniella tetraedra*) were late colonisers, which likely colonised the traps when the amount of organic matter became more substantial and biofilms were well developed. The high number of predators in the sub-substratum, compared with their occurrence in surface streambed communities, can be related to a 'numerical response', *sensu* Giller & Malmqvist (1998): hunting predators likely aggregate in patches with high prey density, particularly when prey are poorly mobile, as in the interstitial zone. The numerical increase of scrapers during the study period was likely due to the increased presence of biofilms on the substratum elements. In bed sediments, biofilms are major organic sources and they also represent important retention mechanisms: Leichtfried (1991, 1994, 1995) demonstrated the importance of this element in the ecology of hyporheic interstitial biocoenoses. In this context, our results confirm the findings of other studies (Hose et al., 2005).

Our study demonstrated that, in Apennine lotic systems, gravel riverbeds can sustain rich and diversified invertebrate assemblages that actively move within the substratum and have a composition and structure quite different from the surface streambed communities. Interstitial habitats are an important component of the biological and ecological system of small creeks. The interstitial zone represents a unique habitat for many organisms, acting as a source of colonisation movements; this zone is highly permeable to invertebrates and can be an important medium connecting the groundwater layer with the stream surface and also connecting different stream reaches, for example during low-flow conditions and droughts. Moreover, our analysis of the functional organisation of interstitial assemblages suggested that this area plays a key role in stream metabolism, allowing a large portion of the allochthonous organic matter to enter the lotic food system.

The maintenance of natural substratum characteristics is essential for the correct functioning of the entire stream system. Most studies agree that the penetration of organisms into deeper sediment layers depends mainly on the availability of interstitial habitats, rendering the amount of fine particles in the interstices a key determinant of hyporheic colonisation (Maridet et al., 1996). In the last few decades, alteration of the morphology and hydrology of many streams and rivers has modified the natural characteristics of lotic habitats: one of the most dangerous effects of canalisation is the alteration of transport and sedimentation processes. Fine sediment accumulations in large tracts of a river may clog the interstices, restrict hyporheic water exchange and lead to a decrease in oxygen (Brunke, 1999). These elements could reduce both the density and taxa richness of macroinvertebrates living in the substratum, with serious biological and functional effects on the whole stream system and a decline of the self-purification capacity of the stream.

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