Size-related shifts in the physical habitat of two mayfly species (Ephemeroptera)

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SUMMARY

1. The spatial niche shifts of the nymphs of two mayfly species *(Rhithrogena semicolorata* and *Ecdyonurus* sp. gr. *venosus)* in relation to their growth were investigated in two lowland springs of northern Italy where they are dominant species.
2. Ordination of the different size classes of the two species revealed a canonical trend related to season and bottom roughness.
3. Larger nymphs of both species colonized rougher substrata. Differences between species in the roughness of areas colonized by small nymphs, which were very numerous, may have been due to competition for space.

Introduction

At a microhabitat scale the close relationship between the benthos and the stream bottom suggests the necessity not only of specifying habitat requirements at community or at species level, but also distinguishing between the different developmental stages of any single species.

During growth and development the spatial niche of an organism can change, because it requires hydraulic and topographic conditions more suitable to its new size (Osborne & Herricks, 1987; Poff & Ward, 1992) and/or modifications of its dietary needs (i.e. Palmer *et al.,* 1993). Referring to topographies, Sheldon (1969, 1980) pointed out how different sized nymphs can occupy different substratum types and crevices of different dimensions.

This study examined the spatial niche shift of two mayfly species in relation to their growth. The measurements of some hydraulic parameters and characteristics of the stream bottom, made during biological samplings, allowed the evaluation of the roles of such variables in explaining spatial niche changes.

Materials and methods

A total of sixty-two Surber samples (area 0.05 m², mesh size 0.45 mm) were taken every 45 days, from January to December 1989, in two lowland springs of artificial origin: Fontanile Borretta and F.le Grande (for further details see Buffagni, 1992). Their limited width and length made it necessary to combine samples from more than one stream. The two springs are located few hundred metres one from the other and they show very similar chemical, physical and hydrogeological features (Bertuletti, 1992; Cavagnis & Orsini, 1992) which are relatively constant and undisturbed (Cotta-Ramusino, Crosa & Buffagni, 1991). The same very rich mayfly community is present in both streams (Buffagni, 1994). Mayflies collected were sorted in the field and then fixed and preserved in 80% ethanol.

For each sample, the following variables were measured: flow velocity (with a digital Armfield velocity-type flow meter, model H33–7), depth, water temperature, substratum composition and bed roughness. Substratum composition was evaluated as $S_iP_iC_i$, where C_i is the code of each inorganic size class ranging from 0 (silt 0.0039-0.0625 mm) to 8 (large cobble 128-256 mm) and P_i is the percentage presence of each class (Crosa, Buffagni & Cotta-Ramusino, 1991). The particle size classes and their percentage presence were estimated visually (Hamilton & Bergersen, 1985).

Bed roughness was measured as the standard deviation of the heights of thirty-six graduated rods placed according to the roughness of the bottom (Gore, 1978; Cotta-Ramusino *et al.*, 1991). Moreover, for each sampled area the Froude number, a dimensionless index of turbulence, was calculated according to the formula: $U/(gD)^{0,5}$ where U = mean flow velocity, g = acceleration due to gravity and D = mean depth of water.

Two mayfly species, *Rhithrogena semicolorata* (Curtis, 1834) and *Ecdyonurus* sp. gr. *venosus*, were selected because, although they are not congeneric, they both belong to the family Heptageniidae and, during their nymphal stages, they show similar morphological and functional characteristics. In particular, the nymphs have a flattened body and are lithophilous (Grandi, 1960). Furthermore, they belong to the same functional feeding group (scraper) and in the studied sites they are dominant species and reach very high densities: up to 4700 nymphs m^2 for *R. semicolorata* and up to 2400 nymphs m^2 for *E.* gr. *venosus*:
Rhithrogena semicolorata is widespread all over Europe (Puthz, 1978); a correct specific determination of the *Ecdyonurus* taxon is not possible, because of the very complex taxonomy of the Italian species of the *Ecdyonurus venosus* group.

Specimens of the two species were counted and total length, not including cerci, was measured to the nearest 0.25 mm using a graduated eyepiece. A total of 1646 nymphs of *R. semicolorata* and 1532 nymphs of *E.* gr. *venosus* was collected and grouped in four [(I-IV) 0-2.5, 2.5-4.5, 4.5-6.5, > 6.5 mm] and five length classes [(I-V) 0-2.5, 2.5–4.5, 4.5-6.5, 6.5-10.5, > 10.5 mm], respectively.

These classes were ordinated in the canonical space using correspondence analysis, an indirect technique of analysis, performed by the computer program CANOCO, version 3.10 (Ter Braak, 1988), on the log-transformed abundance of the nymphs classified in the different size classes.

Ordination axes were subsequently correlated to physical variables in order to reach an ecological interpretation (Gauch, 1982). We tested whether the variable influences were statistically significant by means of a Monte Carlo permutation test.

Differences in abundances of nymphs between areas with different bed roughness were tested by the non-parametric Mann-Whitney *U*-test (Helsel & Hirsch, 1992).

Results

The values of the measured physical variables are summarized in Table 1, while in Table 2 their correlation coefficients are shown.

In the correspondence analysis the first two axes account for 60% of the variance of the body length classes in the ordination space: 34.4% of the variance is related to the first axis, the remaining 25.6% to the second one. Fig. 1 shows the rise of the ordination scores on the two axes as nymphs increase in size. The correlation coefficient of the first ordination axis with the photoperiod (Table 2) suggests that this axis represents a seasonal gradient, expressing the size increments of the nymphs with the time; the second one is related mainly to the bed roughness. The largest percentage (17%) of the total variance of the size classes in the canonical space is related, as expected, to the photoperiod, 8% to the bed roughness and water temperature and, lastly, 4% to the turbulence. Depth, substratum composition and flow velocity do not contribute significantly to the different size classes position in the canonical space.

Because the second axis of the correspondence analysis is correlated with the bed roughness, the abundances of the different size classes of the nymphs of the two species have been examined in relation to this variable (Fig. 2).

Table 3 shows the results of the comparison between different bed roughnesses colonized by each size class, obtained by applying the Mann-Whitney *U*-test to the data of Fig. 2.

With regard to *E.* gr. *venosus*, there are no significant differences (P < 0.05) between roughness values of substrata colonized by specimens of the first three size classes. They usually colonized areas with roughness values between 0.35 and 1.80, with mean values approximately equal to 1. However, nymphs of IV and V size classes colonized areas with roughness values higher than those preferred by smaller nymphs.

For *R. semicolorata*, nymphs less than 4.5 mm in length (classes I and II) colonized areas with similar roughness (0.25 < x < 1.2, means ~ 0.6). The two remaining size classes colonized areas with progressively greater mean roughness (P < 0.001); 0.79 in the case of class III, 1.01 in class IV.

Discussion

Canonical analysis of the abundance of the nymphs classified in the different size classes revealed two

Table 1 Summary statistics of the physical variables measured in the two lowland springs

	Velocity (cm s⁻¹)	Depth (cm)	Froude number	Temperature (°C)	Substratum composition	Bed roughness
Min.	0.1	3	0	8.5	0.1	0.05
Max.	84	49	9.57	19.4	8	2.98
Mean	26	20.7	2.16	14.2	4.62	1.08
SD	18.4	11.8	1.73	3.2	2.2	0.67

Table 2 Correlations between environmental variables and intra-set correlations of environmental variables with the first two axes of correspondence analysis. The correlations between depth, velocity and Froude number are not shown because the Froude number was calculated as a function of these variables. $* = P < 0.05; ** = P < 0.01; *** = p < = P < 0.001$
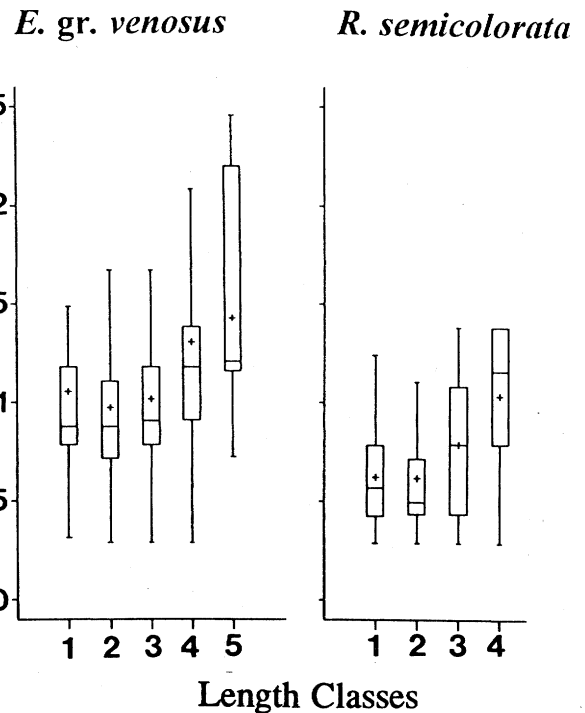
Roughness	−0.05	0.53***						
Velocity	0.45**	0.14	0.06					
Temperature	−0.06	0.09	0.12	0.30*				
Depth	0.07	0.07	0.04	0.08	0.58***			
Substratum comp.	0.08	0.11	0.29*	0.06	0.08	−0.03		
Photoperiod	0.61**	0.22	0.20	0.38**	0.49***	0.53***	0.25	
Froude number	0.43**	0.13	−0.50	–	−0.01	–	−0.04	0.10
	AX1	AX2	R	V	T	D	S	P
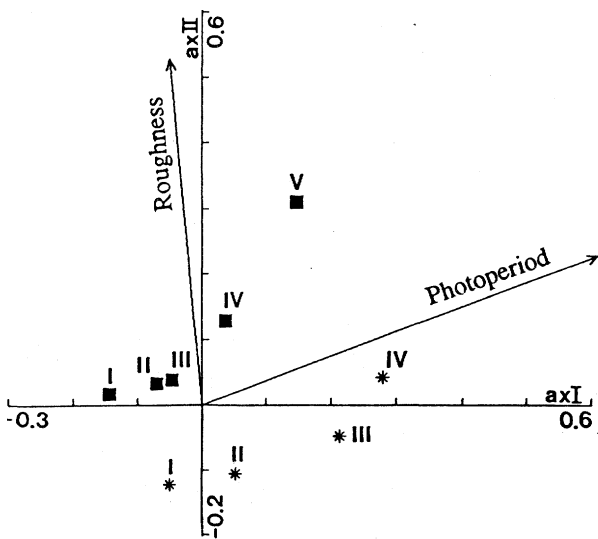
Fig. 1 Correspondence analysis ordination diagram of the different size classes of the two mayfly species with bed roughness and photoperiod represented by arrows. (•) *E. gr. venosus* (*) *R. semicolorata.*

Fig. 2 Ranges of bed roughness colonized by each size class are illustrated through Box-and-Whisker Plot. Each plot divides the data into four areas of equal frequency: the box encloses the middle 50%. The vertical lines extend to the last observations within 1.5x the interquartile range beyond either end of the box. The horizontal line and cross inside each box are, respectively, the median and the mean values. *E.* sp. gr. *venosus* and *R. semicolomta* nymphs are grouped in five [(I-V): 0-2.5, 2.5-4.5, 4.5-6.5, 6.5-10.5, > 10.5 mm] and four length classes [(I-IV): 0-2.5, 2.5-4.5, 4.5-6.5, > 6.5 mm], respectively

major axes of variations, both related to the size increase of the two species of mayfly. The seasonal trend (axis I) was the clearest, while the second (axis II) could be explained as a spatial trend.

The absence of correlation between the biological data and substratum composition, water depth and current velocity suggests that these variables do not influence the distribution of the studied mayfly nymphs in these streams. The absence of correlation

Table 3 Mann-Whitney U-test applied to the data of Fig. 2: comparison between bed roughness colonized by each size class of *R. semicolorata* and *E.* gr. *venosus* larvae. * = P < 0.05; ** = P < 0.01; *** = P < 0.001; NS, not significant

		I	II	III	IV	I	II	III	IV	V
R. semicolorata	I	–								
	II	NS	–							
	III	***	***	–						
	IV	***	***	***	–					
E. gr. *venosus*	I	***	***	***	**	–				
	II	***	***	***	**	NS	–			
	III	***	***	***	*	NS	NS	–		
	IV	***	***	***	NS	***	***	***	–	
	V	***	***	***	*	***	***	***	NS	–
		I	II	III	IV	I	II	III	IV	V
		R. semicolorata				*E.* gr. *venosus*				

with the mean current velocity does not accord with the size-related colonization of different substrata by some trichopteran larvae, which is dependent on current velocity (Osborne & Herricks, 1987; Poff & Ward, 1992), or with the results of Collier (1994) regarding *Deleatidium* (Ephemeroptera, Leptophlebiidae). At high flow and on heterogeneous substrata, *Helicopsyche borealis* (Hagen) larvae chose crevices between separate stones over exposed surfaces (Vaughn, 1987). In these condition only larger larvae occurred on upper surfaces of stones, probably because older instars are better able to maintain their position in the current. Although neither our research, nor that of Hearnden & Pearson (1991), revealed significant separation of size classes of nymphs among various types of substratum, this factor could doubtlessly determine different colonization of taxa according to individual size.

Pringle (1982) found that substratum particle size was the most important variable affecting the distribution of different sizes of *Gammarus pulex* (L.). He observed that mean *Gammarus* size was significantly correlated with median particle size in a simple regression. In the same way, Williams & Moore (1986) noted that large individuals (6-16 mm long) of *Gammarus pseudolimnaeus* Bousfield were found predominantly in microhabitats featuring large substrata. Other field and laboratory experiments on *Gammarus* indicated that selection of substratum particle size is significantly correlated to body size (Rees, 1972; Gee, 1982).

On the contrary our results showed larval distribution to be related to substratum roughness but not to particle size composition. This could be explained if roughness, in the present study, provided a better descriptor of the complex hydraulic characteristics close to the stream bottom (i.e. turbulence and force of flow; for further details see Statzner & Holm, 1982; Statzner, 1988 & Vogel, 1989). Statzner, Gore & Resh (1988) have shown that substratum particle size is not as useful in describing physical habitat characteristics as is commonly believed. This is because the size distribution of substratum often reflects past flow conditions within a stream rather than present flow conditions (Lamberti & Resh, 1979).

Bed roughness is an important ecological variable for benthic communities in that it is connected with habitat space and protection from current and predators (Hildrew & Townsend, 1977; Ward, 1992). Moreover, topographic differences between single rocks may present spatial constraints to the movement of aquatic invertebrates (Underwood & Chapman, 1989; Poff & Ward, 1992).

There were significant differences of colonization among areas with different roughness, both between size classes of the same species and between the species. Larger nymphs tend to colonize rougher areas. It is interesting to observe how the nymphs, of either species, with body length between 6.5 and 10.5 mm colonized areas with similar roughness (Table 3). This suggests a preference related to body size and not to a species-specific habitat selection. Abundances of nymphs in this size class are much lower than those of the early instars due to the usual phenomena of mortality/emigration (and emergence in the case of *R. semicolorata)* of benthic populations. At such low densities competition (intra- or interspecific) is unlikely (Peckarsky, 1979; McAuliffe, 1984; Feminella & Resh, 1990), allowing large nymphs to occupy substrata simply according to their size-related needs.

Dissimilar colonization by different sized nymphs

could also be due to the exclusion of young nymphs from the most favourable microhabitats by intraspecific competition with larger nymphs (Prince, 1975; Hart, 1983; Williams & Moore, 1986).

Rhithrogena semicolorata and *Ecdyonurus* sp. gr. *venosus* nymphs over 6.5 mm belong to the last larval instars. So, the shifting of larger nymphs to higher roughness areas could also be due to behavioural changes of nymphs (Sweeney & Vannote, 1981) which, towards the end of development, moved to large stones to find suitable sites for emergence (Elliott & Humpesch, 1983).

Furthermore, predation can affect distribution of different sizes of aquatic macroinvertebrates (Hildrew, Townsend & Henderson, 1980; Flecker & Allan, 1984; Teague, Knight & Teague, 1985; Whale, 1992). The only taxa able to prey on large nymphs of *R. semicolorata* and *E.* gr. *venosus* in the study areas are *Cottus gobio* Linnè and *Padogobius martensi* (Günter), although neither is likely to be sufficiently numerous to affect larval distribution strongly (Peckarsky & Dodson, 1980).

The different colonization of areas with distinct roughness shown by the early stages of the two species could be due to resource partitioning along a physical habitat dimension (Schoener, 1974). The importance of competition for space in determining the structure of benthic communities, although mainly for sedentary species, has been demonstrated by many authors (Peckarsky, 1979; Peckarsky & Dodson, 1980; Hart, 1983; Hemphill & Cooper, 1983). Environmental conditions in streams rarely remain constant enough to allow competitive interactions to become evident, due to the resulting low density of invertebrates (Gray, 1981). In relatively stable physical environments, like these lowland springs, competition can play an important role (McAuliffe, 1984; Feminella & Resh, 1990). The first instars of the two species were present at very high densities, except in summer, so that resource partitioning related to substratum roughness could have been apparent.

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