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The consortium of the sponge *Ephydatia fluviatilis* (L.) living on the common reed *Phragmites australis* in Lake Piediluco (central Italy)

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Abstract

Lake Piediluco (Central Italy) displays a discontinuous reed-belt along its shore, the dominant macrophyte being *Phragmites australis* (Cav.) Trin ex Steud., the reeds of which support the growth of the sponge *Ephydatia fluviatilis* (L.). Samplings were carried out at ten stations located along the reed-belt with the aim of investigating the 'consortium', which is the result of the association between the edificatory *E. fluviatilis* and the related organisms. These last were considered at three different levels: (a) spatial localisation (epibionts, endobionts); (b) kind of association (occasional, strictly linked); (c) model of organisation (unicellular, multicellular). The associated fauna was investigated by considering both sponge biomass and life-cycle. The faunal composition of associated organisms and their quantitative rates were determined, and statistical differences among stations were tested by using 1-way ANOVA ($p < 0.05$). It emerged that there were significant differences among stations and that the dipteran *Chironomus* sp. was the most common taxon, its presence being independent of the spatial gradient. By contrast, the mean density (total number of hosted macroinvertebrates/sponge wet-weight) did not vary significantly from station to station. A positive relationship between sponge biomass (wet-weight) and the total number of taxa was found, but a sponge weight of 2000 g seemed to provide a better habitat for macroinvertebrates than higher values. This finding emerged from the non-linear relationship between sponge biomass and the number of hosted organisms. Histological analysis of the gut content of some associated organisms enhanced knowledge of their ability to utilize sponge tissues as food.

Introduction

The body of a sponge is permeated by water, which enters through inhalant pores and leaves through oscules, after flowing through the aquiferous system. Sponge organisation offers good opportunities for many invertebrates to find fairly stable habitats in which to live and develop. Sponge associated organisms encompass endobionts and epibionts. It is well known that the presence and density of the associated fauna can reflect not only the sponge body architecture, but also the ability of the sponges to protect themselves by means of bioactive products derived from associated microorganisms or from the sponge itself (Armstrong et al., 1999; Osinga et al., 2001).

Most reports dealing with the fauna associated with marine sponges concern cnidarians (Uriz et al., 1992; Meroz & Ilan, 1995; Bavestrello et al., 2002), molluscs (Klitgaard, 1995; Pansini et al., 1999), polychaetes (Pansini & Daglio, 1980; Martin et al., 1992; Magnino & Gaino, 1998; Martin & Britayev, 1998; Magnino et al., 1999b; Gherardi et al., 2001; Giangrande et al., 2002), copepods (Gotto, 1979; Malt, 1991; Humes, 1996; Mariani & Uriz, 2001), barnacles (Uriz, 1983; Magnino et al., 1999a), amphipods (Rützler, 1976; Uriz, 1983), isopods (Rützler, 1976; Dounas & Koukouras, 1986; Klitgaard, 1991), decapods (Castro, 1971; Saito et al., 2001) and echinoderms (Turon et al., 2000). The nature of the relationship between the partners is still questionable and only

in a few cases some invertebrates inhabiting marine sponges are reported to feed on their hosts (Pawlik, 1983; Tsurumi & Reiswig, 1997; Martin & Britayev, 1998; Magnino & Gaino, 1998). Presumably, sponges constitute a suitable food source for many associated organisms, a feature as yet poorly investigated or underestimated (Mariani & Uriz, 2001).

With regard to freshwater sponges, a spatial distribution of the associated organisms has been studied in the branched species *Lubomirskia baicalensis*; in this case, the diversity of the fauna was related to the presence and size of the sponge cavities (Kamaltynov et al., 1993). The same results were obtained by studying other species of Baikalian sponges, in which quantitative characteristics tend to increase with sponge body size, irrespective of the sponge species identity (Weinberg et al., 2002).

The life-cycle of *Ephydatia fluviatilis* (L.) growing on the common reed *Phragmites australis* (Cav.) Trin ex Steud. in Lake Piediluco has been previously described on the basis of an annual survey of the sponge populations in this environment (Gaino et al., 2003). That study proved that sponge morphology markedly changes according to the season and that the most relevant development of the sponge takes place in summer, when *E. fluviatilis* acquires the typical sleeve-shaped configuration and tends to expand into laminar extensions. This morphology greatly contrasts with the small over-wintering representatives that adhere to the reed rootlets and whose wandering cells present a peculiar feeding strategy, in that they are able to ingest and digest diatoms (Gaino & Rebora, 2003).

Like many marine sponges, *E. fluviatilis* offers a refuge to many organisms; these inhabit the sponge and can establish various levels of interactions with their host. A case in point is represented by the close relationship established between sponge tissues and the immature phases of the caddisfly *Ceraclea fulva.* Indeed, the larval stages of *C. fulva* exclusively feed on sponge tissues (Corallini & Gaino, 2001) and utilize the sponge spicule skeletal network to build both larval and pupal cases (Corallini & Gaino, 2003).

The aim of the present study was to investigate *E. fluviatilis* as an 'edificator' eligible for supporting epibionts and endobionts. Association between edificator and related organisms (a number of epibionts and endobionts) has been defined by Beklemishev (1951) as 'consortium'.

Materials and methods

Study area

Lake Piediluco (42° 30′ 54″–42° 32′28″ N, 12° 44′ 27″–12° 46′ 25″ E) is situated at about 20 km SE of Terni (Umbria Region, Central Italy). It is an artificially regulated lake exploited to generate hydroelectric power. It is a hydraulic complex, constituted by two waterways: the Velino River and the Triponzo Canal, which is derived from the Nera River.

The Nera River flows into the lake as its main tributary, while the Velino River is intermittently used as an effluent and tributary. The catchment basin surface is about 74.2 km^2, situated between its lowest altitude of 367.5 m and its highest of 1775 m above sea level.

The lake surface is currently around 1.5 km^2 and its perimeter is about 13 Km; the depth reaches 19.2 m. The lake has a central body running in a WE oriented, and some ramifications (Fig. 1).

The riparian vegetation mainly consists of *Phragmites australis*, which forms a reed belt intercalated with willow and poplar woods. Sampling sites (Fig. 1) were chosen along the reed belt in order to investigate the organisms associated with the sponge population, by distinguishing the marsh area (distal branches of the basin) (stations 6, 8) from the rest of the lake shore (stations 1–5, 7, 9, 10). Samplings were performed monthly from June 2000 to August 2001.

Sample collection and preparation

Specimens of *E. fluviatilis* growing on the reeds were collected from 3 stalks/m^2 per station (5 stalks were collected at station 6 in July, owing to the deep sediment covering the immersed region of the stalks) and fixed in 70% ethanol in order to measure the sponge weight and to examine the associated organisms. Several pieces were resected from the sponges and processed in the field for histological observations by fixing in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2). After 4 hours of fixation, this material was processed in the laboratory according to the routine procedures used for obtaining paraffin-embedded samples. Histological sections varying from 5 to 10 μm in thickness were stained with haematoxylin-eosin and observed by means of a Leica DMLB microscope. In particular, in order to gain an ultrastructural insight into the presence of associated unicellular organisms, some fragments were fixed in the above-described medium, post-fixed in 1% osmium tetroxide in cacodylate buffer for one hour,

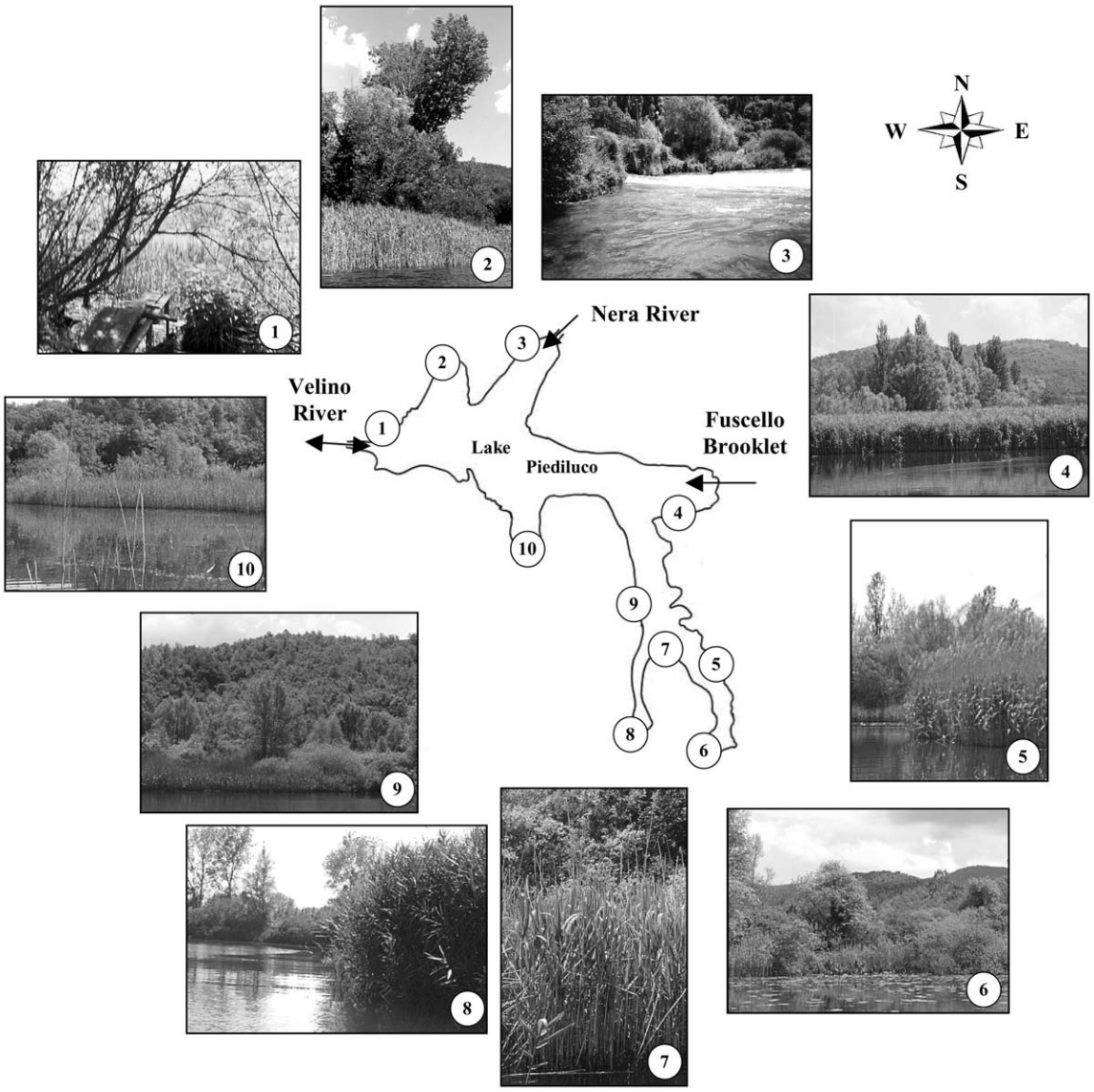

Figure 1. Location of the stations along the reed belt of Lake Piediluco.

dehydrated in a graded series of alcohol, immersed in propylene oxide and embedded in an Epon-Araldite resin mixture. Silver-to-gold sections were stained with uranyl acetate and lead citrate and observed under a Philips TEM. Sponges *in toto* were weighed as wet tissue and carefully dissected under stereomicroscopy in order to individuate the associated organisms qualitatively (biodiversity) and quantitatively (density expressed as number of individuals per sponge weight). Macroinvertebrates were removed with forceps and sorted under a stereomicroscope. For taxonomic attribution, the individuals were preserved in ethanol and utilized to reconstruct the identification keys to the lowest possible taxon.

The specific attribution of the sponges from Lake Piediluco was performed by boiling sponge fragments in 65% nitric acid, washing repeatedly in distilled water and alcohol, and gently agitating to suspend spicules. Thereafter, the spicule suspension was smeared onto a microscope slide and evaporated to dryness. A xilolo-Eukitt mixture was dropped onto the slide before the coverslip was fitted.

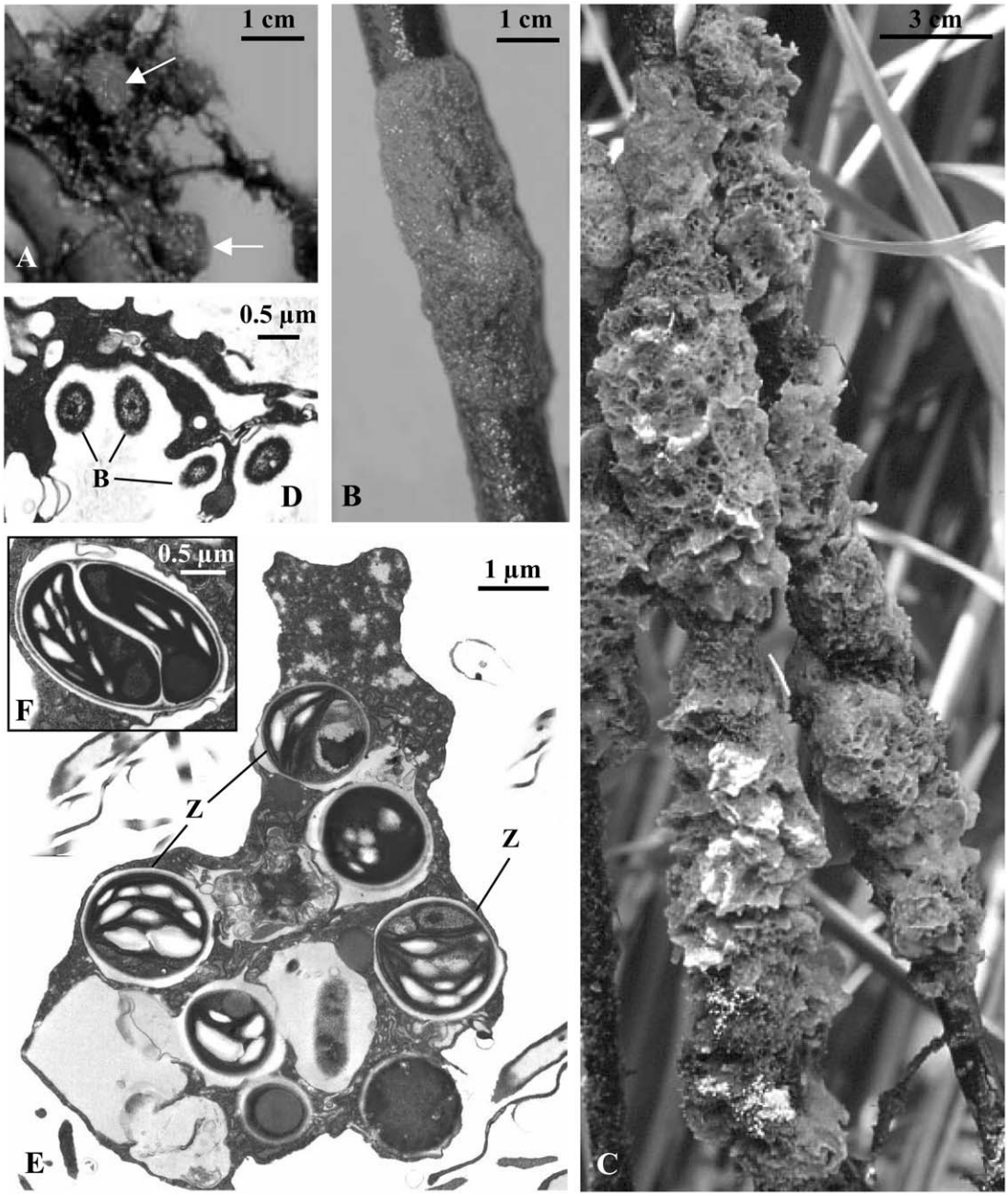

Figure 2. Variation of the morphology of *Ephydatia fluviatilis* according to the seasonal moment (A, B, C) and transmission electron microscopy of the associated unicellular organisms (D, E, F): A, small rounded specimens (arrows) enveloping the rootlets (mainly from autumn to early spring); B, encrusting specimens (mainly in late spring); C, sleeve-shaped specimens with laminar extensions (from late spring to summer); D, bacteria (B) delimited by cell cytoplasmic protrusions; E, endocellular zoochlorellae (Z) enveloped in vacuoles; F, zoochlorella in division.

Data analysis

Numbers of hosted individuals, biodiversity, evenness and dominance were tested for each sampling station, by utilizing the Shannon–Wiener index (H'), evenness index (J) and Simpson index (D). These indexes were compared by means of 1-way ANOVA, which uses stations and seasons as factors. Factors significantly detected by ANOVA were further analyzed using Tukey's test set at the 5% significance level.

The qualitative relationships among sampling stations were explored by means of cluster analysis, using the UPGMA method. Distances for constructing the cluster dendrogram were calculated by means of Sorensen's index (Sorensen, 1948). To test the relationship between macroinvertebrate abundance and the spatial position of sponges, a principal correspondence analysis (PCA) was then performed using stations as the row factor and species as the column factor.

Data dealing with fauna density and number of taxa were $\log_{10}(n + 1)$ transformed prior to analysis, in order to satisfy the assumption of normality. Mean differences among sampling stations and seasons were compared through 1-way ANOVA. Before calculating the ANOVA test, homogeneity of variances was examined.

The quantitative relationships considering the number of individuals and the sponge weight, and the richness of taxa and the sponge weight, were respectively tested by means of a linear regression model. For each regression, the linear correlation coefficient and its significance level were calculated.

For statistical analyses and graphs, R statistical software (R CRAN Project) was utilized.

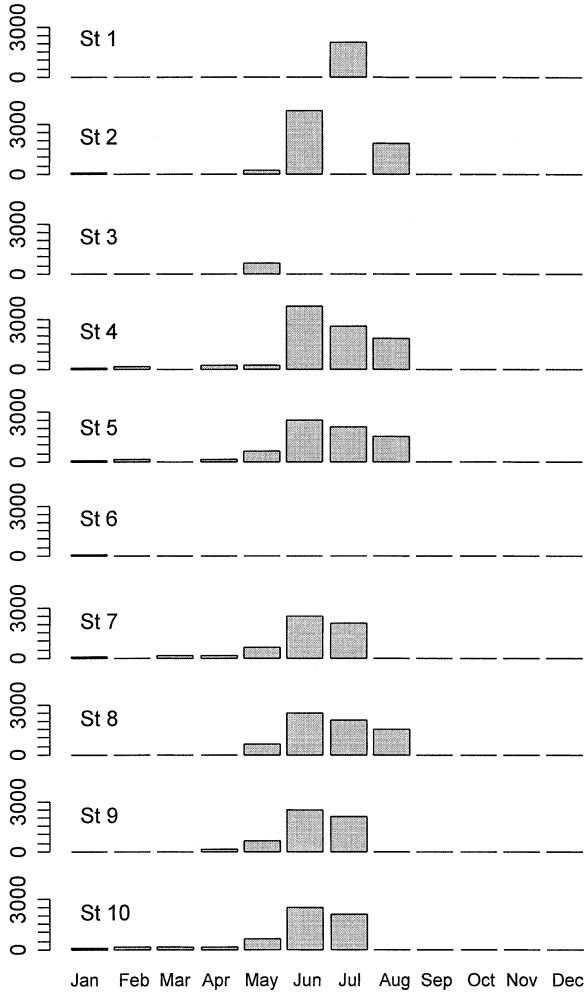

Figure 3. Bar plot showing the monthly fluctuation of the sponge weight (expressed in grams) of the specimens hosting macroinvertebrates. Values calculated for each station over one year of samplings.

Results

Spicule analysis allowed us to attribute the sponges collected in Lake Piediluco to *Ephydatia fluviatilis* (L.), the megascleres of which are exclusively smooth oxeas, ranging from 210 μm to 380 μm in length and from 8 to 16 μm in width. No gemmules have been observed during the survey on the sponge population and the attribution of the specimens to *E. fluviatilis* has been based on the sponge skeletal spicules only.

The sponge colonizes the submerged portion of *Phragmites australis* reed stalks growing along the lake shore, at depths ranging from 50 cm to 250 cm. Photographs of sponges taken in the natural environment confirm that sponge morphology varies according to the seasonal moment: small rounded specimens enveloping the rootlets (mainly from autumn to early spring) (Fig. 2A); encrusting specimens (mainly in late spring) (Fig. 2B); sleeve-shaped specimens with laminar extensions (from late spring to summer) (Fig. 2C).

The sponge specimens reach their maximum growth (3700 g, on average per single sponge specimen) from May to July, and start to decline slightly from August to the colder months of the year (Fig. 3).

Ephydatia fluviatilis is an important edificator for a large number of consorting organisms. This consortium can be considered at three different levels: (a) *level of spatial localization*, in which consorting units

Table 1. Biodiversity of the organisms of the consortium of the sponge *Ephydatia fluviatilis* living on the common reed *Phragmites australis* in Lake Piediluco.

Taxa	Taxa codes	Epibiont	Endobiont	Endocellular host	Occasional host	Strictly associated	Total number over one year of sampling
BACTERIA			×			×	
CHLOROPHYCEAE							
Zoochlorella sp.				×		×	
PERITRICHA							
Vorticella sp.		×			×		
HYDROZOA							
Hydra sp.		×			×		Few
TURBELLARIA							
Dendrocoelum lacteum	Dl	×			×		1
Dugesia lugubris	Dlu	×	×		×		35
OLIGOCHAETA	O	×	×		×		144
HIRUDINEA							
Erpobdella octoculata	Eo	×			×		54
Glossiphonia complanata	Gc	×			×		1
Piscicola geometra	Pg	×			×		
GYMNOLAEMATA							
Paludicella articulata		×	×		×		Few colonies
GASTROPODA							
Valvata cristata	Vc	×	×		×		2
Valvata piscinalis	Vp	×	×		×		11
Bithynia leachi	Bl	×	×		×		42
Bithynia tentaculata	Bt	×	×		×		7
Emmericia patula	Ep	×	×		×		6
Acroloxus lacustris	Al	×			×		2
BIVALVIA							
Pisidium sp.	P	×	×		×		2
ARACHNIDA (HYDRACHNELLAE)	H	×	×		×		36
OSTRACODA	Os	×	×		×		160
MALACOSTRACA							
Echinogammarus sp.	G	×	×		×		10
Asellus aquaticus	Aa	×	×		×		67
PTERYGOTA (EPHEMEROPTERA)							
Caenis horaria (MEGALOPTERA)	Ch	×			×		1
Sialis sp. (NEUROPTERA)	S	×	×		×		2
Sisyra sp. (TRICHOPTERA)	Sis	×	×			×	91
Polycentropus flavomaculatus	Pf						19
Tinodes waeneri	Tw	×	×		×	×	10
Limnephilus flavospinosus	Lf	×			×		1
Ceraclea fulva	Cf	×	×			×	20
(DIPTERA, Chironomidae)	Chir	×	×		×	×	751

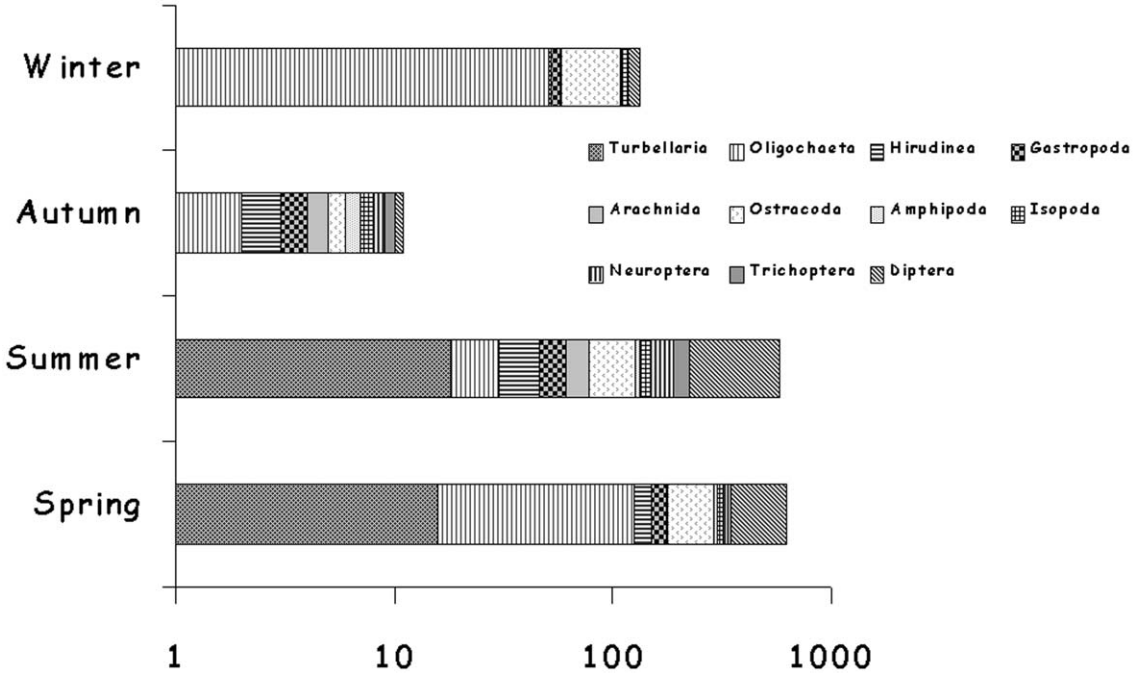

Figure 4. Total amount and seasonal distribution of organisms (logarithmic scale) constituting the consortium of the sponge *Ephydatia fluviatilis*.

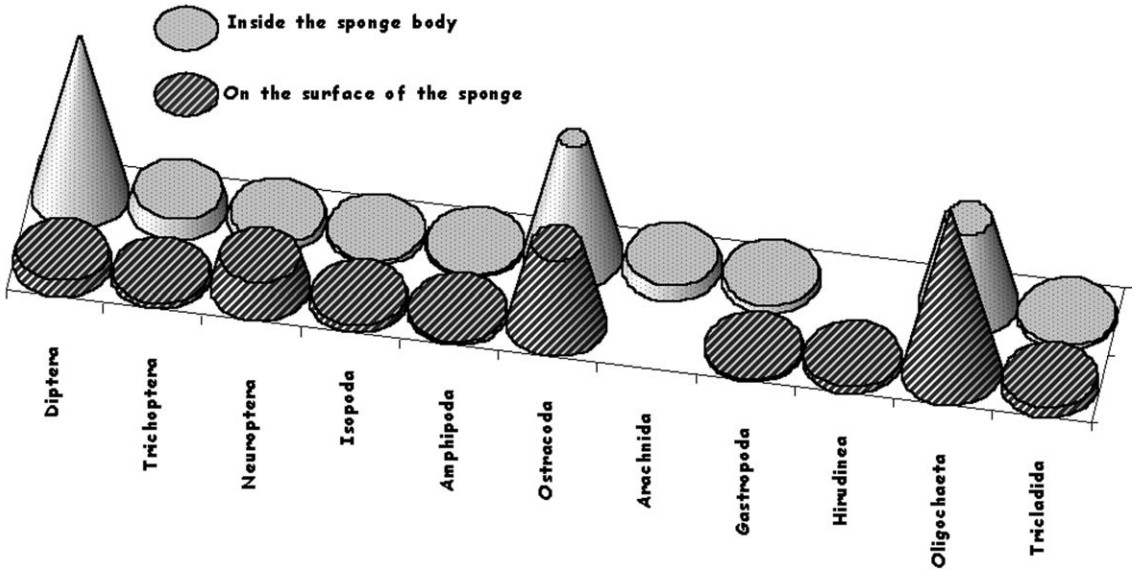

Figure 5. Spatial distribution of the multicellular organisms inside the sponge body and on the sponge surface.

are epibionts and/or endobionts; (b) *level of association*, which includes: i. organisms able to live on the sponge, which therefore behave as on any other kind of substratum (occasional); ii. organisms strictly linked to the sponge life-cycle, which affects their occurrence and density (symbionts); (c) *level of guest organization*, which is based on the presence of unicellular or multicellular organisms. Table 1 summarizes the biodiversity of the hosted organisms, which were subdivided into the three levels of association; for each of these, the total number (unicellular excluded) over one year of samplings is reported. As far as the unicellular

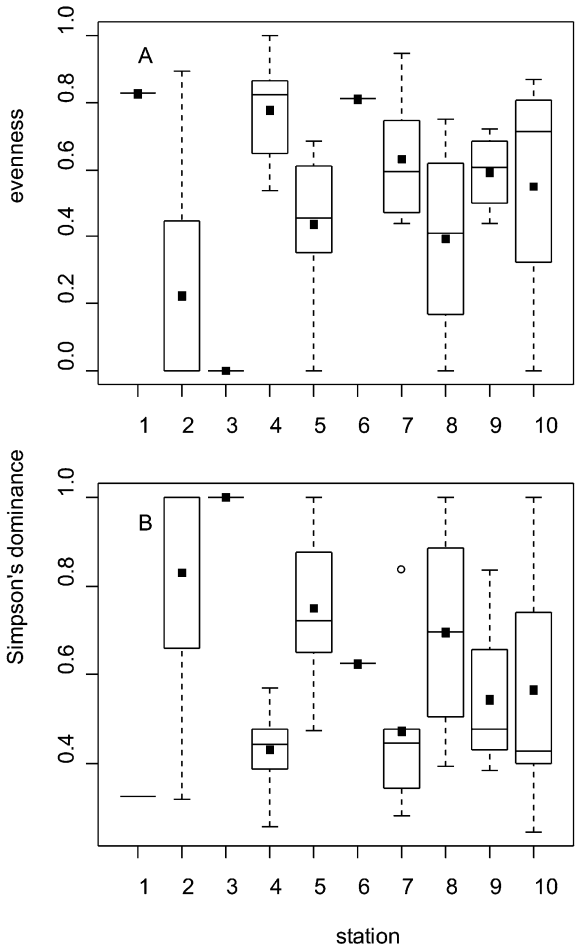

Figure 6. Box and whiskers plots of evenness (A) and Simpson's dominance (B) indexes calculated for each station. Box represents data in the 25–75% range. Dotted whiskers are minima and maxima; the line in each box is the median and the black dot is the mean.

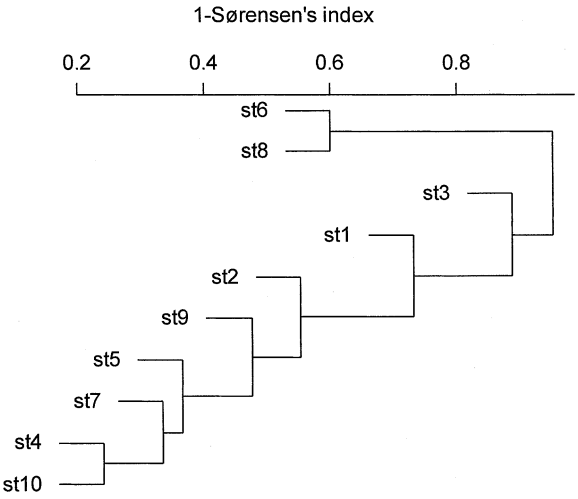

Figure 7. Cluster showing the similarity among stations, based on Sorensen's index.

organisms are concerned, TEM observations revealed the occurrence of bacteria and zoochlorellae. Bacteria are widespread in the sponge mesohyl and some images show that they are delimited by cell cytoplasmic protrusions (Fig. 2D). Zoochlorellae are endocellular and enveloped in vacuoles (Fig. 2E), where they are observed in division (Fig. 2F). The superficial (a few millimetres in thickness) green pigmentation of the sponges living on sunlit immersed culms is due to the presence of these zoochlorellae. The total numbers of organisms constituting the consortium, together with their seasonal distribution, are reported in Figure 4. The spatial distribution of the multicellular organisms on the sponge surface and inside the sponge body is synthetically illustrated in Figure 5. The discrimination between the organisms living on the sponge surface and inside its body was carried out by direct observations under a stereo-microscope after cutting the sponge with a razor blade.

Shannon, evenness and Simpson's dominance indexes calculated for each station show that the biodiversity of macroinvertebrates associated to the sponges, collected over one year of samplings, varies according to the sampling sites (Fig. 6A, B). At stations 1, 3 and 6, the sponges associated with the reed belt were collected only once. In particular, at station 3, sponges showed weak growth and scarcity of hosted fauna. Biodiversity and dominance data showed significant differences among stations (1-way ANOVA, $p < 0.05$). Station 4 presented the highest Shannon and evenness values, while station 2 exhibited the lowest ones (Tukey test $p < 0.05$). These data are confirmed by the statistically significant differences in Simpson's dominance index (1-way ANOVA, $p < 0.05$). With regard to this index, station 4 displayed the lowest levels in the July sample of 10 taxa and 86 specimens (Dominance = 0.45), whereas station 2 had the highest levels (Dominance = 1). High values of biodiversity at stations 7, 9 and 10 were recorded.

Cluster analysis, based on Sorensen's similarity index, revealed the relationships between stations. It emerged the occurrence of two main clusters: the cluster of the stations 6 and 8, and the cluster of the remaining ones distributed along a continuous gradient (Fig. 7). By applying principal correspondence analysis (Fig. 8), it emerged that station 1 was characterized by the caddisfly *Tinodes waeneri* and station 2 by the gastropod *Emmericia patula*, whereas the

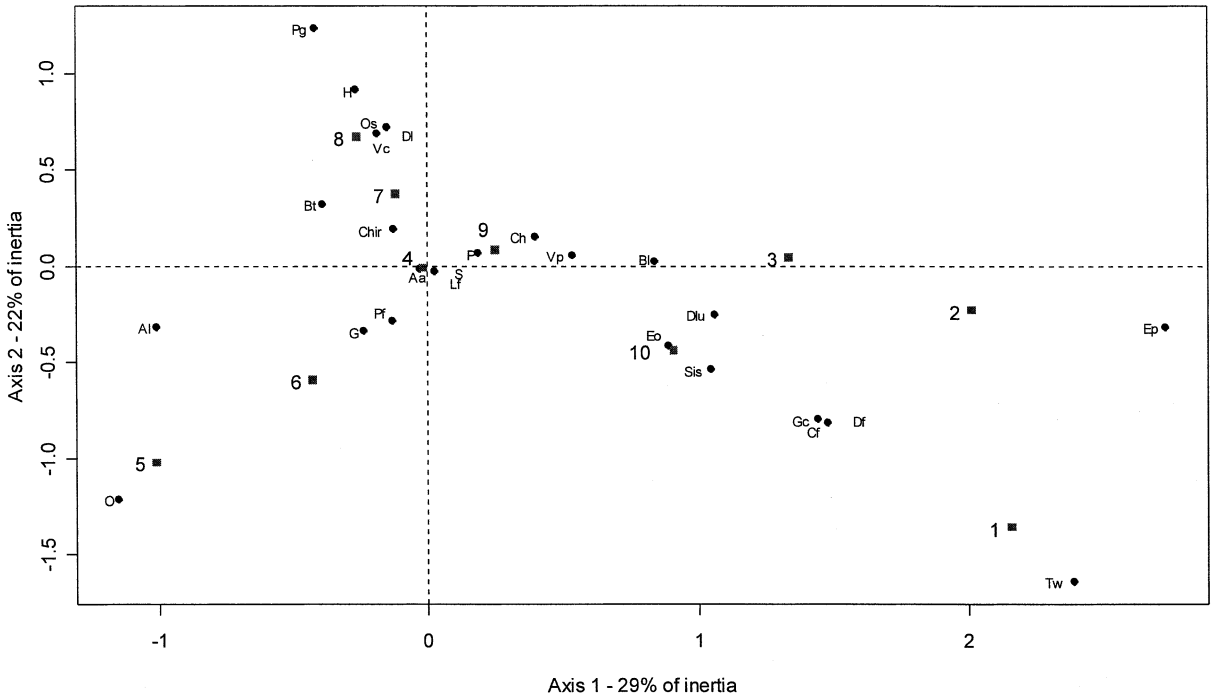

Figure 8. PCA plot of sampling sites (square dots) and abundance data of hosted macroinvertebrates (round dots). The macroinvertebrate labels are reported according to the taxa codes in Table 1.

oligochaetes dominated at station 5. By contrast, the dipteran *Chironomus* sp. was the most common taxon regardless of spatial gradient, as proved by its occurrence near to the intersection point of the axes. The first axis explained 29% of variability and the second explained an additional 22%.

Examination of the mean density (total number of macroinvertebrate specimens/wet-weight of the sponge) revealed no significant differences among stations (Fig. 9A). The same analysis, performed on the total number of taxa inhabiting sponges at each station, showed statistically significant differences (1-way ANOVA analysis: $p < 0.05$) (Fig. 9B).

Whereas the sponge weight and the total number of taxa seemed to be positively linked (linear correlation analysis, $R^2 = 0.31$, $p < 0.05$), there was no evidence of a linear correlation between sponge weight and the number of macroinvertebrate specimens (Fig. 10). Indeed, the total number of macroinvertebrate specimens and taxa hosted steadily increased concomitantly with the sponge weight up to about 2000 g. Subsequently, in contrast with the growth tendency of the sponge specimen, the number of the macroinvertebrates hosted drastically declined, while that of the taxa remained almost unchanged (Fig. 10).

Figure 11 shows a schematic representation of the temporal distribution of epi- and endobionts in the period when *E. fluviatilis* is increasing in size. In particular, specimens of *E. fluviatilis* hosting macroinvertebrates were collected at station 3 in May and at the station 6 in January only.

Histological sections and analysis of the alimentary content showed the presence of sponge spicules inside the gut of some hosted macroinvertebrates (Fig. 12), such as the gastropod *Bithynia tentaculata* (Fig. 12A), and larvae belonging to different insect orders, such as Diptera Chironomidae, *Chironomus* sp. (Fig. 12B), Trichoptera *Ceraclea fulva* (Fig. 12C) and Neuroptera *Sysira* sp. (Fig. 12D). These data allowed us to include *Chironomus* sp. and *Bithynia tentaculata* in the category of **sponge tissue feeders**. This category encompasses macroinvertebrates living either inside the sponge body or on its surface as **gatherers** and **scrapers**. Sponge tissue feeders are able to ingest sponge cells, siliceous spicules (clearly detectable both in histological sections and in the alimentary content), organic particle matter and microrganisms. Other macroinvertebrates, which belong to the category of **predators**, feed on other organisms living

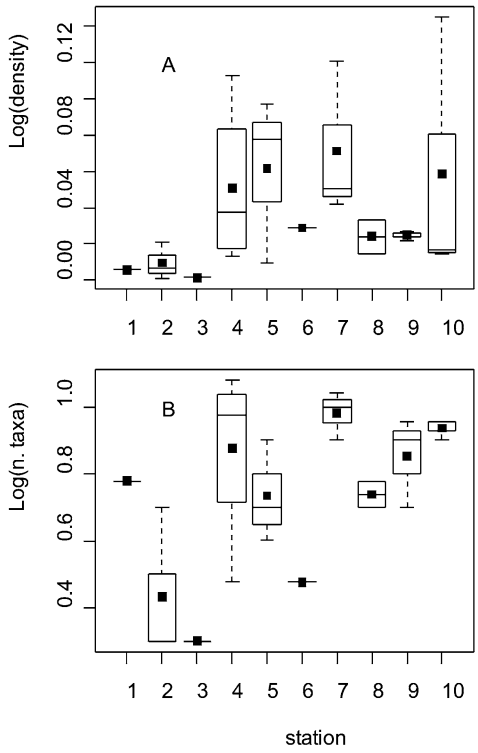

Figure 9. Box and whiskers plots of the mean density (total number of hosted macroinvertebrates/wet weight of the sponge) (A) and the total number of taxa hosted by the sponges (B) calculated for each station. Box represents data in the 25–75% range. Dotted whiskers are minima and maxima; the line in each box is the median and the black dot is the mean.

inside or on the surface of the sponge body without using sponge tissue as food.

No statistically significant seasonal differences were found in the density of epi- and endobiont macroinvertebrates colonising the sponges.

Discussion

The fauna associated with *E. fluviatilis* comprises numerous taxa, a feature confirming that this freshwater sponge provides a suitable refuge for many macroinvertebrates that live either on the surface or inside the body of the sponge. Whereas some specimens are occasional epibionts, most are epibionts and endobionts as well, thereby indicating that they are generalists and utilize the sponge as a mere substrate.

Marine sponges, probably owing to their richness in species and large size of the specimens, offered better opportunity to investigate sponge/whole fauna assemblage relationships in comparison with freshwater sponges. From the bulk of data, it emerged that whereas some marine sponge species do not show characteristic association (Voultsiadou-Koukouras et al., 1987), others have taxa that constitute a real 'ecological community' because of their quite constant composition (Westinga & Hoetjes, 1981; Koukouras et al., 1985).

Recent observations on polychaete assemblage inhabiting the marine sponge *Geodia cydonium* showed that this sponge is utilized as a living space without any specificity (Gherardi et al., 2001). In addition, in accordance with previous studies, most of the species found in *G. cydonium* are also common on other substrates, a feature stressing that the presence of polychaetes is related to their occurrence in the immediate area, more than to the sponge itself (Koukouras et al., 1985, 1996). Nevertheless, a monitoring carried out on the benthic fauna of the neighbouring environment of the *Phragmitetum* (unpublished data) suggested that some taxa are not associated with *E. fluviatilis*. For instance, it occurs for some representatives of Gastropoda (Planorbidae and Ly*mnaea fontinalis*), Bivalvia (*Sphaerium* sp., *Musculium* sp. and *Anodonta* sp.) and Hirudinea (*Helobdella* sp., *Batracobdella* sp. and *Placobdella* sp.).

Notwithstanding the large number of papers dealing with the fauna associated with sponges (see introduction section), it is difficult to ascertain, in terms of costs/benefits, if the hosted macroinvetrebrates have a commensal or parasitic habit. The predatory activity of the polychaete *Haplosyllis spongicola* on sponge internal tissue was proved by histological investigations that revealed the presence of terrigenous sediments, which typically reinforce the sponge canal walls, in the gut lumen of the inhabiting worms (Magnino & Gaino, 1998). In addition, the occurrence of pigmented debris inside the worm gut, similar to the pigmented cells of the host sponge *Anomoianthella lamella*, confirmed this parasitism relationship (Magnino et al., 1999a). These data corroborate previous observations that this worm feeds on sponge tissue (Pawlik, 1983; Tsurumi & Reiswig, 1997). Remnants of siliceous spicules in the digestive tract of *Chironomus* sp. and *Bithynia tentaculata* testify the ability of these animals to exploit sponge tissue as a food source, either when moving on the surface of *E. fluviatilis* or after penetrating inwards where they take advantage of the canals of the aquiferous system. The presence of Chironomidae in freshwater sponges is fairly common. According to Roback (1968), several species appear to be true 'sponge parasites', whereas others are probably ac-

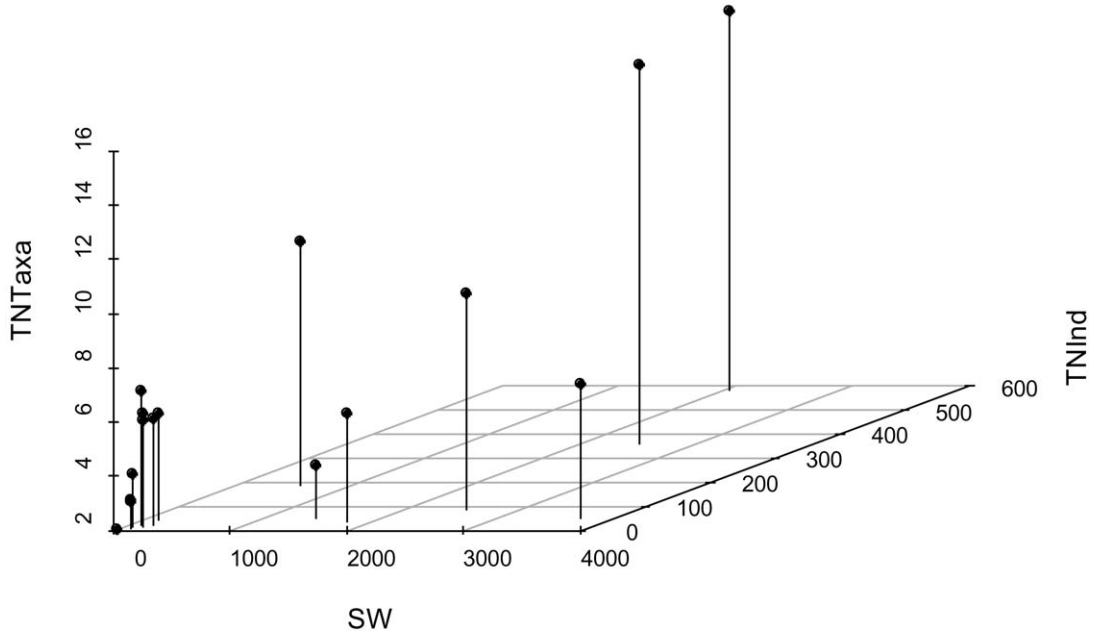

Figure 10. 3D scatter plot showing the relationships among sponge weight (SW), total number of taxa (TNTaxa) and total number of hosted organisms (TNInd).

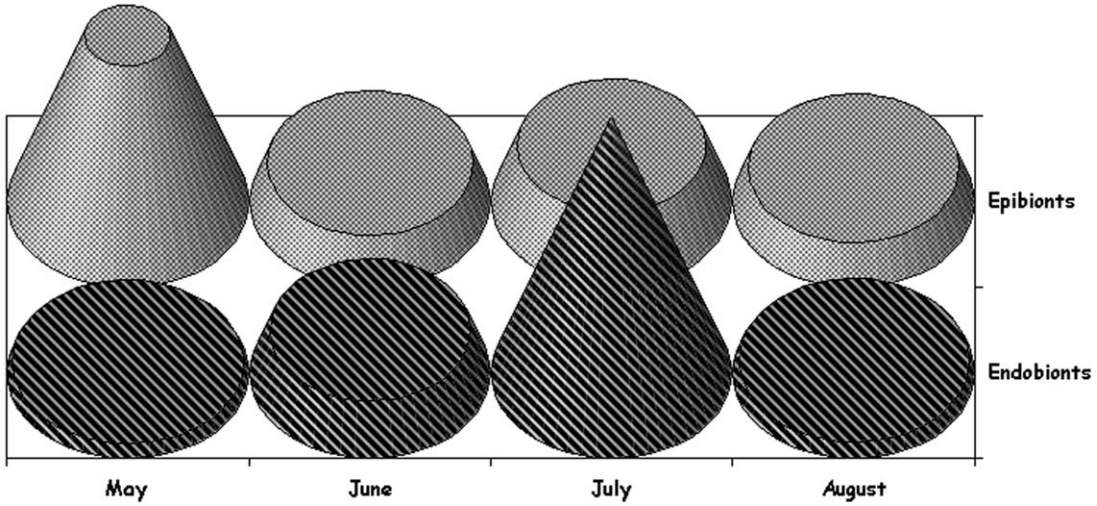

Figure 11. A schematic representation of the temporal distribution of the epi- and endobiont macroinvertebrates colonising *Ephydatia fluviatilis* from May to August.

cidental dwellers in this habitat and only rarely their digestive tract contains spicule fragments. It therefore seems reasonable to state that Chironomidae larvae living on or inside sponges display a wide range of feeding behaviour, depending on the kind of relationship, which varies from occasional association to true parasitism. On the whole, our data show that these chironomids constitute the most numerous taxon, and that they reach their highest density in spring and summer, concomitantly with the growth of the sponge body.

Larvae belonging to Sisyridae have been observed crawling on the sponge surface or living in natural cavities of freshwater sponges (Roback, 1968). The duration of the larval stages has been seen to depend on the condition of the host species, which include *E. fluviatilis* among others (Weissmair, 1994). Fragments of siliceous spicules have also been found in the

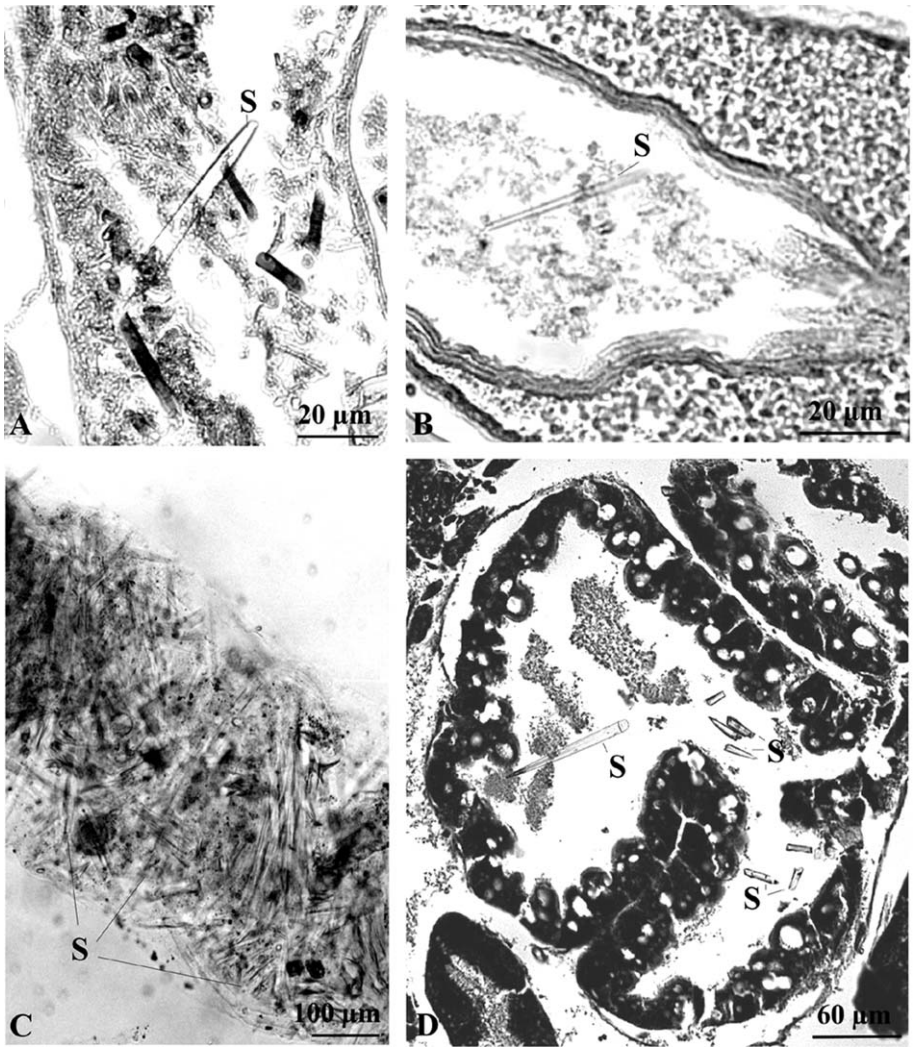

Figure 12. Sponge spicules (S) inside the gut of: A, *Bithynia tentaculata*; B, *Chironomus* sp.; C, *Ceraclea fulva*; D, *Sysira* sp. A, B, D: histological sections; C: portion of the gut *in toto*.

larval gut of *Sisyra* sp., a feature consistent with the definition of these larvae as parasites. Among the inhabitants of *E. fluviatilis*, the caddisfly *Ceraclea fulva*, exclusively associated to this sponge, is the species that derives most benefit, since it utilizes sponge tissue as food and the sponge siliceous spicules as material to build up the case (Corallini & Gaino, 2001, 2003).

The sponge architecture remarkably affects presence and distribution of its associated fauna. This feature has been recently pointed out in *G. cydonium* where a large number of species belonging to polychaetes were exclusive of the cortical layer, a result also confirmed by statistical analysis that showed a clear cut between cortical and choanosomal assemblage (Gherardi et al., 2001).

Recent research on the life-cycle of *E. fluviatilis* in Lake Piediluco showed that sponge morphology seasonally varies from thin encrusting laminae (in spring) to sleeve-shaped specimens (in summer) to small over-wintering specimens (Gaino et al., 2003). The progressive growth of *E. fluviatilis*, whose weights reach their maximum values in June and July, fosters biodiversity by offering a greater surface and larger cavities. Indeed, density analysis revealed that the highest value of associated organisms is reached in spring and summer, concomitantly with the increasing sponge size. In addition, the larval release, in

summer, causes the formation of empty cavities in the sponge body, which may facilitate inward penetration of macroinvertebrates. Nevertheless, a sponge weight of 2000 g constitutes a threshold value because further growth is not paralleled by an increasing number of macroinvertebrates. These results emphasize a non-linear correlation between sponge size and its inhabiting macroinvertebrates, thereby differing from literature reports dealing with polychaete assemblages living in marine sponges (see review in Gherardi et al., 2001).

With regard to spatial distribution, the biodiversity of the macroinvertebrates hosted by *E. fluviatilis* significantly differs among the sampling sites. For instance, some taxa predominate only in the northern sector of the lake – Gastropoda (station 3) and *Sisyra* sp. (station 2) – as shown by PCA. Cluster analysis revealed that sponge/macroinvertebrate association present at stations 6 and 8 differs markedly from that occurring at the remaining stations, thereby reflecting the strictly marshy environment, which hinders sponge growth. The environmental effects on sponge/associated taxa have been recently pointed out for the polychaete assemblage in *G. cydonium* from two study sites having different local conditions (Gherardi et al., 2001).

In conclusion, sponges constitute a complex living space for numerous taxa whose presence, arrangement and relationship with the host reflect not only the sponge organization but also the environmental and seasonal conditions of the habitat. *E. fluviatilis* mainly constitutes a shelter for most of the associated fauna, whereas, for some of them, sponge debris in the digestive tract confirms a clear trophic relationship.

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