

Microalgal communities epibiotic on the marine hydroid *Eudendrium racemosum* in the Ligurian Sea during an annual cycle

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Abstract The microalgal community associated with *Eudendrium racemosum*, a marine hydroid widely distributed in the Mediterranean Sea, was studied during an annual cycle, at monthly frequency, in a coastal station of the Ligurian Sea. Microalgae were represented mainly by diatoms, which exhibited higher abundance and biomass values between autumn and spring (max 46,752 cells mm⁻² and 1.94 µg C mm⁻², respectively), while during summer a significant decrease was observed (min 917 cells mm⁻² and 0.013 µg C mm⁻²). High levels of abundance of filamentous cyanobacteria were observed in summer. Spatial distribution of epibiotic microalgae showed a markedly increasing gradient from the basal to the apical part of hydroid colonies. Considering the growth forms of diatom communities, motile diatoms (mainly small naviculoid taxa) were the most abundant in all the periods. Adnate (*Amphora* and *Cocconeis* spp.) were distributed mainly in the basal and central part of hydroid colonies and showed two peaks (autumn and summer). Erect forms (mainly *Tabularia tabulata*, *Licmophora* spp., *Cyclophora tenuis*) were mainly distributed in the apical part of the colonies and showed their maximum densities in spring–summer. Tube-dwelling (*Berkeleya rutilans*, *Parlibellus* sp.) were observed at low densities throughout the study period, without any significant

temporal or spatial variability. Comparing the microalgal communities on marine hydroid to those grown on mimic substrata placed in the sampling station during summer, significantly higher values of abundance were observed in the hydroid, suggesting that microalgae may benefit from the polyp catabolites. This fact was particularly evident for the adnate diatoms, whose temporal trend paralleled the cycle of hydroid host.

Introduction

Microphytobenthos represent an important component in the ecology of aquatic ecosystems, both for their contribution to primary production and for their role in mediating oxygen and nutrient fluxes between substrate and water column, as well as for their role in the trophic chain (Mac Intyre et al. 1996). Among benthic microalgal communities sensu Round (1971, 1981), the most closely studied are those associated with soft bottom sediments (epipelton), for which abundance, composition, biomass and productivity values have been reported for many areas of both intertidal and subtidal habitats (de Jonge and Colijn 1994; Barranguet et al. 1997; Sundbäck et al. 2000; Welker et al. 2002; Totti 2003). On the other hand, microalgae associated with hard substrata, such as rocks (epilithon), macrophytes (epiphyton) and animals (epizoon) are poorly known, and most data refer to freshwater habitats. In the specific case of epizoic communities, knowledge is largely descriptive (Bodeanu 1987–1988; Cerrano et al. 2000, 2004a, b; Gillan and Cadée 2000; Patil and Anil 2000; Di Camillo et al. 2005; Totti et al. 2005).

Sessile invertebrates offer an excellent substratum for benthic microalgal growth because (1) they provide additional substrata to be colonized, beside the hard

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rocks, or (2) microalgae benefit from a raised position avoiding covering by sediment, or (3) microalgae may take advantage of the host's metabolic products, or (4) they are better protected against grazing, which occurs more rarely on live animals (Round 1981). The first reports on the colonization of animals by benthic microalgae date from 1949, when a rich diatom flora was reported on the bivalve *Pinna nobilis* (Round 1971 and references therein). Today, several animal phyla are known to host diatoms as epibionts: ciliates, sponges, hydrozoans, molluscs, crustaceans and also vertebrates (Round 1981; Round et al. 1990).

Sedentary animals with hard shells are the best hosts for epibiontic microalgae, while more vagile animals are less frequently colonized (Round 1981). It has been noted that the epizotic microflora often reflect those of the substrata where the animal host lives (Bodeanu 1987–1988). However, a number of associations between algae and animals show a high degree of specificity: e.g. the diatom genus *Pseudohimantidium* is known to occur only in association with a few marine Crustaceans, whereas *Synedra cyclopus* lives in association with freshwater ones. A specialized diatom microflora (*Bennettella* and *Epipellis*) grow over the whale skin, and marine birds bear dense populations of diatoms on their ventral body (Round et al. 1990). The diatom *Porannulus contentus* has been reported only in association with a few Antarctic sponges (Hamilton et al. 1997; Cerrano et al. 2004b), where it forms dense mats over the sponge surface. The relationships between algae and animals are not restricted to the epibiosis only. A number of Antarctic sponges have been found to host rich diatom communities, due to their ability to incorporate planktonic diatoms through the exopinacocytes of the sponge surface or the endopinacocytes of the inhalant canals. Diatoms remain alive for a long time inside the sponge tissues before being utilized as a food source (Cerrano et al. 2004a; Totti et al. 2005).

It has long been known that marine hydrozoans represent a highly suitable substratum for diatom colonization. Round et al. (1961) described diatom species grown on the hydroid *Amphisbetia* (formerly *Sertularia operculata*) and reported that different communities developed, depending on the current and sedimentation regimes. In several cases, a specificity for the host parts was also reported: Siqueiros-Beltrones et al. (2001), describing diatom colonization on the hydroid *Campanularia integra* (formerly *Eucopeella caliculata*), reported that monospecific communities of *Cocconeis notata* occurred inside the hydrotheca, while small naviculoids diatoms lived inside the gonophores. Recently, Di Camillo et al. (2005), investigating the diatom communities associated to several species of marine hydroids,

highlighted the existence of specific associations: *Clytia linearis* hosted *Cylindrotheca* sp. in the internal side of the theca, while *Cocconeis pseudonotata* in the external side and in the pedicels. *Campanularia hincksii* was colonized by the diatom *Cocconeis notata* exclusively inside the theca. *Syntheceium evansi* hosted *Navicula* sp. inside the theca, and several *Cocconeis* spp. outside. The disposition of diatoms on hydroids shows that, even at an extremely reduced spatial scale, a marked selection for the microenvironment occurs: diatoms living in the intrathecal space seem to gain some specific advantages, such as protection and availability of metabolic products from the host, while the disposition of diatoms in the grooves of theca and pedicels seems to be related to an “edge” effect reported for epilithic communities as well (DeNicola and McIntire 1990).

Eudendrium racemosum is a common Mediterranean hydroid characterized by large colonies and widely found in both natural environments and polluted ones. While this species appears in natural environments only during the summer period, in harbours it occurs year-round, with a series of shortened life cycles (Bavestrello et al. 2006). Previous observations pointed out that they were richly epibionted by diatoms (Di Camillo et al. 2005). In this study, our aim is to describe the diatom communities associated with *E. racemosum* throughout an annual cycle in the Ligurian Sea, considering their seasonal and spatial variability (i.e. distribution along hydroid colonies) in terms of abundance, biomass and community structure. We also set out to test the existence of host specificity by comparing communities observed on *Eudendrium* with those grown on artificial mimic substrata.

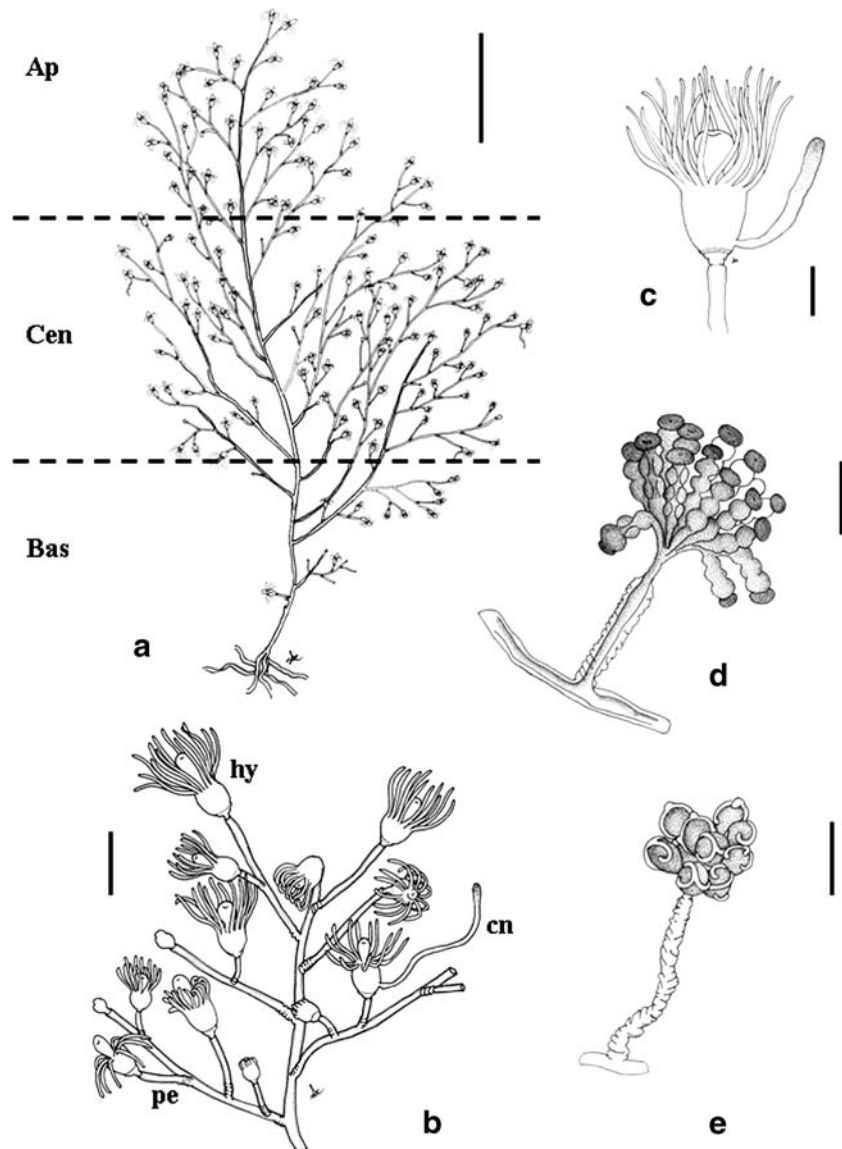
Materials and methods

Sampling

Eudendrium racemosum is an athecate hydroid (Fig. 1a) characterized by polyps bearing a long cnidophore (Fig. 1b, c). The gonophores are styloid, composed by several series of spherical chambers in the male (Fig. 1d) and by groups of eggs enveloped in bifid spadix in the females (Fig. 1e). Hydroid samples were collected on a concrete quay of the Santa Margherita Ligure harbour (Ligurian Sea), at a depth of 0.5 m, from October 2002 to October 2003, at monthly frequency. For each sampling, ten colonies of hydroid were collected and preserved in filtered seawater (on 0.45 µm porosity GFF filters) and formalin 4%.

In April 2003, 15 mimic substrata (Teflon) having a similar shape and height of hydroid colonies were

Fig. 1 **a** Colony of *Eudendrium racemosum* showing the apical (*Ap*), central (*Cen*) and basal (*Bas*) portions; **b** particular of a branch: *hy* hydranth, *cn* cnidophore, *pe* pedicel; **c** hydranth with cnidophore; **d** male gonophore; **e** female gonophore. Scale bars: **a** 1 cm; **b, d, e** 500 μ m; **c** 200 μ m



placed in the same quay after sterilization by boiling. They were left there and collected every month from May to October 2003.

After collection, both natural and artificial samples were treated for the following purposes: (1) taxonomic determination and counting of epibiontic microalgae for an estimate of their abundance and biomass; (2) observation of the most relevant diatom species under the Scanning Electron Microscope (SEM) after cleaning procedure; (3) SEM study of hydroid samples for in situ observation of the diatom community.

Density and biomass of epibiontic microalgae

Hydroid colonies (three replicates) were cut in three parts (basal, central and apical), using a lancet (Fig. 1a). Each part was measured using a stereomicroscope, in

terms of length and width of main stem and of all primary and secondary branches. Then, each colony part was placed in glass tubes filled with filtered seawater and treated in an ultrasonic bath to allow detachment of epizoic diatoms: samples were sonicated for 10' a number of times, with 5' intervals between successive treatments to avoid excessive heating. A maximum of 2 h were required for complete detachment of algal cells from the hydroid colony.

The suspension containing algal cells was adjusted to a final volume of 20 ml with filtered seawater and 2% neutralized formalin in Falcon tubes. These samples were then analysed for the microalgae count with the Utermöhl method, modified in accordance with Totti et al. (2004). Counting has been effected at the inverted microscope at 400 \times magnification, on 1–2 transects or on 30 casual fields, to count a minimum of

300 cells. This count was integrated with the count on the whole sedimentation chamber at $200\times$ magnification, with the aim of obtaining a more correct estimate of less frequent taxa with higher biovolume. Finally, microalgae abundance were expressed as number of cells per mm^2 of hydroid. During counting, cells were measured to estimate cell biovolumes. Biomass was calculated following Strathmann (1967) and expressed as $\mu\text{g C mm}^{-2}$ of hydroid.

Epibiontic diatoms were subdivided into the growth forms identified by Round (1981): erect (i.e. species attached to the substrate through mucus pads or peduncles), adnate (species lying on the substratum through the valve face and having a limited motility), motile (biraphid diatoms that can move freely on the substrata), tube-dwelling (i.e. naviculoid and nitzschioid diatoms living in mucilage tubes of their own making) and planktonic (i.e. true plankton species that settle on the substratum and remain healthy).

Cleaning procedure

The taxonomic determination of diatoms at species level is generally possible only after observation of the ultrastructural details of their frustules by SEM analysis, previous removal of organic matter. For each sampling, one hydroid colony, previously cut in three parts (basal, central and apical), was treated with the cleaning method of von Stosch (Hasle and Syvertsen 1997). Before SEM observation, one or more drops of cleaned material were poured on a Nucleopore polycarbonate filter fixed on a stub and left to air dry completely. Then, the stub was sputter coated with a thin layer of gold-palladium.

SEM observation of hydroid samples

Both the counting and the cleaning procedures result in a complete detachment of diatoms from hydroid colonies. To observe the diatom communities as they were originally settled on the substratum, one colony for each sampling was treated as follows: small pieces (~ 10 mm) were cut from basal, central and apical part of hydroid colony, dehydrated by immersion in alcohol at increasing gradations, and treated in a Critical Point Dryer. Then, each hydroid piece was placed on a stub and sputtered with gold-palladium for observation under the SEM (Philips EM 515, 25 kV)

Statistical analysis

Differences in terms of microalgae abundance and biomass among different hydroid parts, seasons and

between natural and artificial substrata were tested by analysis-of-variance (MANOVA), using Statistica (StatSoft, Tulsa, OK, USA) software. When significant differences for the main effect were observed ($p < 0.05$), a Tukey's pairwise comparison test was also performed.

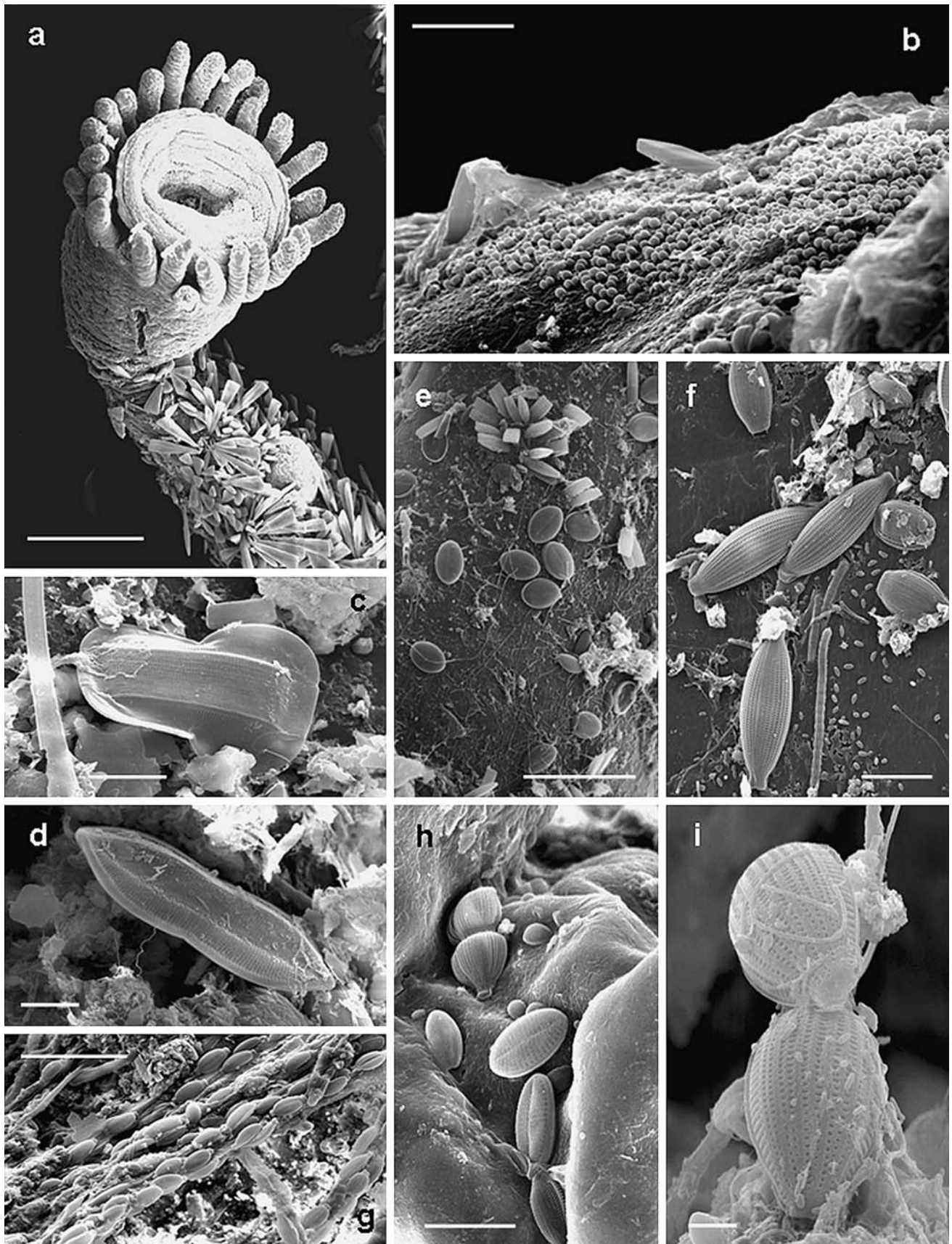
Results

Microalgal communities grown on *Eudendrium racemosum* mainly consisted of diatoms throughout the period studied. On the hydroids, the diatoms colonized only the portion covered by perisarc, while they were never recorded on the hydranth (Fig. 2a). Among diatoms, also a rich bacterial mat covered the perisarc (Fig. 2b). Recorded taxa are listed in Table 1. Higher values of diatom abundance and biomass were observed between October 2002 and April 2003, with two peaks in winter and in spring, whereas there was a sharp decrease in late spring–summer (Fig. 3a, b). Diatom abundance values ranged from $46,752 \pm 24,684$ cells mm^{-2} in February 2003 to 917 ± 331 cells mm^{-2} in October 2003, while biomass ranged from 1.94 ± 1.94 to 0.013 ± 0.003 $\mu\text{g C mm}^{-2}$ for the same periods. In contrast, filamentous cyanobacteria appeared with high densities between late spring and summer (Fig. 4a, b), with maximum abundance of $29,872 \pm 4,482$ cells mm^{-2} and biomass of 0.32 ± 0.18 $\mu\text{g C mm}^{-2}$. Algae belonging to other groups (dinoflagellates, silicoflagellates, unicellular chlorophytes and cryptophytes) occurred only occasionally. On average, abundance of cyanobacteria was lower than that of diatoms by 1–2 orders of magnitude, except in summer, when diatoms decreased markedly and cyanobacteria reached their annual maximum. Regarding the spatial distribution along the hydroid colony, diatom abundance and biomass values were significantly higher in the apical part of hydroid colonies.

On average, motile diatoms represented the most abundant fraction of diatom communities (73%), followed by adnate (17%), erect (7%) and tube-dwelling growth forms (3%). Considering biomass, motile diatoms represented 48% of the total biomass value, followed by erect (25%), adnate (18%) and tube-dwelling diatoms (9%).

Temporal variability of epibiontic communities

Mean abundance and biomass values were calculated for each season (Table 2). Mean diatom abundance was higher in winter ($21,612 \pm 18,482$ cells mm^{-2}) than in any other period, while summer abundance ($3,636 \pm$



◀ **Fig. 2** Epibiotic microalgal communities on *Eudendrium racemosum* I. **a** Portion of a colony of *E. racemosum* showing a completely developed polyp: a rich assemblage of microalgae dominated by *Licmophora* spp. cover the pedicel under the polyp. **b** Bacterial mat on the perisarc of the hydroid colony. **c–d** Motile diatoms: **c** *Plagiotropis* sp. and **d** *Tryblionella constricta* lying on the surface of the colony. **e–i** Adnate diatoms: **e** specimens of *Cocconeis convexa* colonizing a clean surface of *E. racemosum*; a colony of erect diatoms (*Opephora* sp.) is also present; **f** specimens of *Amphora* spp., bacteria and filamentous cyanobacteria colonizing a clean surface of *E. racemosum*; **g** dense population of *Amphora* spp. growing on macroalgal filaments over the hydroid; **h** cells of *Amphora* spp. and *Cocconeis neothumensis* var. *marina* settled in the grooves between annuli; **i** *Amphora* sp. partially covered by bacteria. Scale bars (μm): **a** 200; **b, h** 20; **c, f** 10; **e, g** 50; **d, i** 5

3,467 cells mm^{-2}) was significantly lower than that observed in winter ($p < 0.0005$) and in the other seasons ($p < 0.005$). Diatom biomass showed a trend comparable to that of density: the values observed in summer ($0.079 \pm 0.097 \mu\text{g C mm}^{-2}$) were significantly lower than those observed in winter ($0.751 \pm 0.654 \mu\text{g C mm}^{-2}$, $p < 0.0005$) and spring ($0.654 \pm 1.003 \mu\text{g C mm}^{-2}$, $p < 0.005$).

In terms of the composition of diatom communities, a marked seasonal variability was observed, with each growth form following a different trend. Motile diatoms consisted mainly of small-undetermined naviculaid forms, followed by *Navicula* cf. *consentanea*, *Psammodictyon mediterraneum*, *Plagiotropis* sp. (Fig. 2c), *Caloneis alpestris*, *Campylodiscus* spp., *Diploneis* spp. *Tryblionella constricta* (Fig. 2d) and *Nitzschia* spp. They showed their maximum abundance between October and April, with a decrease in March (Fig. 5a). Mean seasonal abundance was highest in winter, while the minimum values were observed during the summer months. Abundance values observed in summer were significantly lower than those observed in the other seasons ($p < 0.0001$, Table 2).

Adnate diatoms mainly belonged to the genera *Amphora* (*A.* cf. *ovalis* var. *pediculus*, *A. coffeaeformis* and other *Amphora* spp.) and *Cocconeis* (*C. scutellum*, *C. stauroneiformis*, *C. neothumensis* var. *marina*, *C. convexa*) (Fig. 2e–i). They showed two maxima, in autumn–winter and in spring–early summer, while a decrease was observed in late winter (Fig. 5b). Mean seasonal abundance values were significantly higher in autumn than in summer ($p < 0.05$, Table 2).

Erect diatoms showed their maximum development between autumn and spring, with a marked decrease in summer (Fig. 5c). On average, abundance values observed in summer were significantly lower than those recorded in winter and in spring ($p < 0.0005$, Table 2). Erect diatoms showed a higher

species diversity compared to the adnate: the most important were *Licmophora* spp. (e.g. *L. abbreviata*, *L. oedipus*, *L. flabellata*), *Tabularia tabulata*, *Opephora* cf. *pacifica*, *Grammatophora marina*, *Cyclophora tenuis*, *Synedra* spp. and *Neosynedra* sp. (Fig. 6a–f). In April, when erect diatoms reached their annual maximum, *Licmophora* spp. represented 85% of this growth form. Due to their large size, erect diatoms made a significant contribution to total biomass (Fig. 7).

Tube-dwelling diatoms were observed throughout the study period (Fig. 5d), with two species: *Berkeleya rutilans* and *Parlibellus* sp. (Fig. 6g, h). Maximum values were observed in summer, with peaks occurring in June and September, but no significant differences among seasons were observed in terms of either density or biomass (Table 2). Planktonic species were observed occasionally only during winter, at very low densities.

Cyanobacteria consisted mainly of filamentous forms attributed to *Oscillatoria lutea*, followed by *Spirulina subsalsa* and cf. *Borzia* sp. (Fig. 6i, j). Cyanobacteria showed markedly seasonal behaviour (Fig. 4a, b). A rapid increase was observed in spring, and maximum abundance values were recorded during summer. On average, summer abundance ($9,610 \pm 10,266$ cells mm^{-2}) was significantly higher than that observed in autumn and winter ($p < 0.0001$). The temporal trend of biomass followed that of density, and the summer values ($0.081 \pm 0.116 \mu\text{g C mm}^{-2}$) were significantly higher than those observed in the other periods ($p < 0.0001$, Table 2).

Spatial distribution of epibiotic communities

Mean abundance and biomass values for each portion of hydroid colonies are shown on Table 3. Comparing microalgal density among the apical, central and basal parts of hydroid colonies, the abundance values observed in the basal part ($6,521 \pm 7,860$ cells mm^{-2}) were significantly lower than those of the central ($15,373 \pm 19,239$ cells mm^{-2} , $p < 0.05$) and apical parts ($17,759 \pm 17,926$ cells mm^{-2} , $p < 0.01$). A similar distribution was observed for the biomass, with the values recorded in the apical part ($0.42 \pm 0.71 \mu\text{g C mm}^{-2}$) being significantly higher than those of the basal portion ($0.16 \pm 0.2 \mu\text{g C mm}^{-2}$, $p < 0.005$).

Looking at the spatial distribution of diatom growth forms, a different pattern of distribution was observed: while motile and erect diatoms were significantly more abundant on the apical part, adnate and tube-dwelling diatoms were distributed in a uniform pattern (Table 3). On the average, the abundance values of cyanobacteria

Table 1 List of microalgal taxa recorded on *Eudendrium racemosum* during the study period**Diatoms**

Achnanthes brevipes Agardh
Achnanthes cf. *minutissima* Kützing
Achnanthes longipes Agardh
Amphora cf. *coffeaeformis* (Agardh) Kützing
Amphora cf. *ovalis* var. *pediculus* Kützing
Amphora spp.
Asterionellopsis glacialis (Castracane) Round
Bacillaria paxillifera (O.F. Müller) Hendey
Berkeleya rutilans (Trentepohl) Grunow
Biddulphia sp.
Caloneis alpestris (Grun.) Cleve
Campylodiscus cf. *clevei* Peragallo
Campylodiscus cf. *decorus* var. *eudecora* Peragallo
Campylodiscus cf. *decorus* var. *pinnata* Peragallo
Campylodiscus cf. *fastuosus* var. *fastuosus* Ehrenberg
Campylodiscus cf. *thuretii* Bréb.
Chaetoceros spp.
Climacosphenia sp.
Cocconeis convexa Giffen
Cocconeis molesta Kützing
Cocconeis neothumensis var. *marina* De Stefano, Marino et Mazzella
Cocconeis scutellum var. *parva* Grunow in Van Heurck
Cocconeis scutellum var. *posidoniae* De Stefano, Marino et Mazzella
Cocconeis scutellum var. *scutellum* Ehrenberg
Cocconeis stauroneiformis (W.Smith) Okuno
Cyclophora tenuis Castracane
Cylindrotheca closterium (Ehr.) Lewin et Reimann
Diatoma vulgare Bory
Diploneis cf. *oestrupii* Hustedt
Diploneis crabro Ehrenberg
Diploneis crabro var. *excavata* Hustedt
Diploneis oculata (Breb.) Cleve
Diploneis smithi var. *constricta* Heiden, Heiden et Kolbe
Diploneis sp.
Entomoneis ornata (J. W. Bail.) Reim.
Entomoneis sp.
Fragilariopsis sp.
Grammatophora angulosa Ehrenberg
Grammatophora marina (Lyngbye) Kützing
Gyrosigma sp.
Haslea sp.
Licmophora cf. *abbreviata* Agardh
Licmophora cf. *dalmatica* (Kützing) Grunow
Licmophora cf. *inflata* Mereschkowsky
Licmophora cf. *gracilis* (Ehrenberg) Grunow
Licmophora cf. *juergensii* Agards
Licmophora flabellata (Carm.) Agardh
Licmophora oedipus (Kützing) Grunow
Licmophora reichardtii Grunow
Licmophora sp.
Licmosphenia sp.
Mastogloia undulata Grunow
Melosira sp.
Microtabella sp.
Navicula cf. *consentanea* Hustedt
Navicula cf. *distans* (W.Smith) A.Schmidt
Navicula sp.
Neosynedra sp.
Nitzschia cf. *plana* W. Smith
Nitzschia cf. *romana* (Grunow) Grunow
Nitzschia cf. *sigma* (Kütz.) W.Smith

Nitzschia longissima (Bréb. in Kützing) Ralfs in Pritchard
Nitzschia “*sigmoideae* group”
Odontella sp.
Opephora cf. *pacifica* (Grunow) Petit
Parlibellus sp.
Pinnularia sp.
Plagiotropis sp.
Pleurosigma sp.
Psammodictyon mediterraneum (Hustedt) Mann
Pseudo-nitzschia cf. *fraudulenta* (Cleve) Hasle
Pseudo-nitzschia sp.
Rhabdonema adriaticum Kützing
Rhabdonema cf. *minutum* Kützing
Rhabdonema cf. *hyalinum* Meunier
Rhizosolenia sp.
Skeletonema marinoi Sarno et Zingone
Stauroneis sp.
Striatella unipunctata (Lyngbye) Agardh
Surirella ovata Kützing
Surirella sp.
Synedra fulgens var. *mediterranea* Grunow
Synedra sp.
Tabellaria cf. *fenestrata* (Lyngbye) Kützing
Tabularia tabulata (Agardh) Snoeijis
Thalassionema bacillare (Heiden in Heiden et Kolbe) Kolbe
Thalassionema frauenfeldii (Grun.) Hallegraeff
Thalassionema nitzschioides (Grun.) Mereschkowsky
Thalassionema sp.
Toxarium hennedyanum (Gregory) Pelletan
Trachyneis aspera Ehb.
Trachyneis sp.
Tryblionella constricta (Kützing) Poulin
cf. *Cymbella cistula* (Hemprich) Grunow
cf. *Epithemia* sp.
cf. *Glyphodesmis* sp.
cf. *Gomphonema* sp.
cf. *Opephora gemmata* (Grun.) Hustedt
cf. *Rhabdonema* sp.
Und. pennate diatoms

Dinoflagellates

Alexandrium spp.
Oxytoxum crassum Schiller
Oxytoxum spp.
Prorocentrum compressum (Bail.) Abé
Prorocentrum lima (Ehr.) Dodge
Prorocentrum micans Ehr.
Protoperidinium diabolus (Cl.) Bal.

Chlorophyceae

Scenedesmus spp.

Silicoflagellates

Dictyocha fibula Ehr.
Dictyocha staurodon Ehr.
Octactis octonaria (Ehr.) Hovasse

Cyanobacteria

cf. *Borzia* sp.
Oscillatoria lutea C. Agardh ex Gomont
Spirulina subsalsa Oersted

Others

Coccioids

Fig. 3 Total diatoms recorded on basal, central and apical parts of *Eudendrium racemosum* colonies from October 2002 to October 2003; **a** abundance (cells mm⁻²); **b** biomass (µg C mm⁻²): mean ± SD

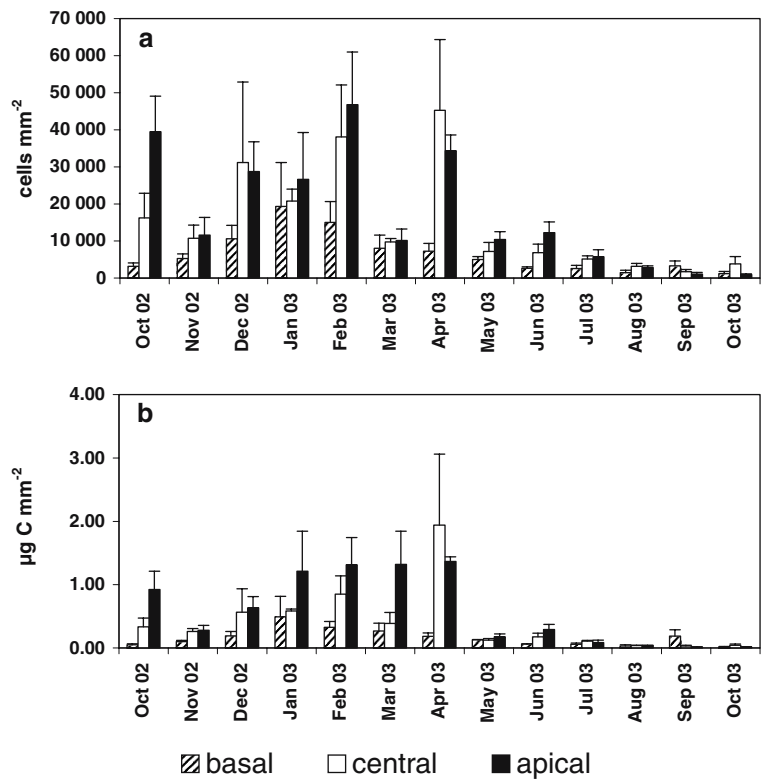
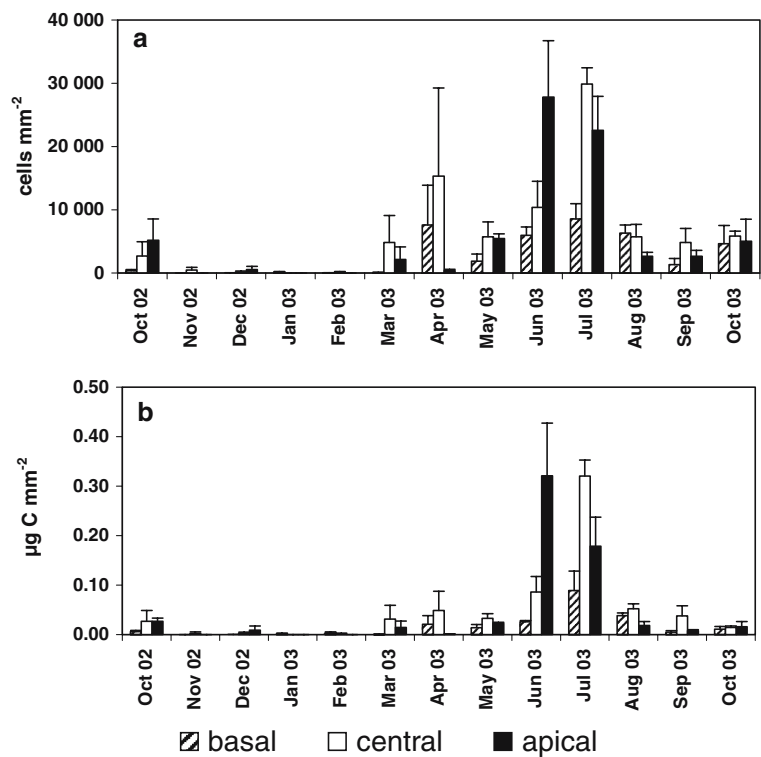


Fig. 4 Cyanobacteria recorded on basal, central and apical parts of *Eudendrium racemosum* colonies from October 2002 to October 2003; **a** abundance (cells mm⁻²); **b** biomass (µg C mm⁻²): mean ± SD



were higher at the central ($6,606 \pm 10,262$ cells mm⁻²) and apical parts ($5,726 \pm 9,873$ cells mm⁻²) than at the basis ($2,834 \pm 4,416$ cells mm⁻²), but these differences were not significant.

Epibiontic microalgae on mimic substrata

The analysis of epibiontic microalgae on the mimic substrata showed a markedly increasing gradient from

Table 2 Mean abundance (cells mm⁻²) and biomass (µg C mm⁻²) ± SD of epibiontic microalgae calculated for each season and results of the ANOVA and Tukey's tests

	Autumn	Winter	Spring	Summer	ANOVA <i>p</i> -level	Tukey's test (<i>p</i> < 0.05)
Total diatoms						
Abundance	17,441 ± 17,750	21,612 ± 18,482	18,246 ± 19,980	3,636 ± 3,467	<0.0001	Summer < (autumn, winter, spring)
Biomass	0.373 ± 0.374	0.751 ± 0.654	0.654 ± 1.003	0.079 ± 0.097	<0.0001	Summer < (winter, spring)
Motile						
Abundance	14,106 ± 15,128	17,250 ± 16,880	14,189 ± 15,934	2,292 ± 1,990	<0.0001	Summer < (autumn, winter, spring)
Biomass	0.182 ± 0.192	0.344 ± 0.344	0.181 ± 0.174	0.024 ± 0.019	<0.0001	Summer < (autumn, winter, spring) (autumn and spring) < Winter
Adnate						
Abundance	2,040 ± 1,664	1,581 ± 1,502	1,599 ± 1,127	1,006 ± 1,332	<0.05	Summer < autumn
Biomass	0.040 ± 0.029	0.033 ± 0.031	0.034 ± 0.018	0.029 ± 0.043	ns	
Erect						
Abundance	1,155 ± 1,121	2,487 ± 2,312	2,281 ± 3,674	130 ± 186	<0.0001	Summer < (winter and spring)
Biomass	0.139 ± 0.190	0.349 ± 0.491	0.421 ± 0.831	0.008 ± 0.010	<0.001	Summer < (winter and spring)
Tube-dwelling						
Abundance	133 ± 217	198 ± 310	164 ± 217	205 ± 458	ns	
Biomass	0.011 ± 0.019	0.020 ± 0.031	0.017 ± 0.025	0.019 ± 0.050	ns	
Cyanobacteria						
Abundance	1,041 ± 2,618	812 ± 2,773	6,056 ± 10,468	9,610 ± 10,266	<0.0001	(autumn and winter) < Summer
Biomass	0.008 ± 0.016	0.006 ± 0.018	0.023 ± 0.030	0.081 ± 0.116	<0.0001	(autumn, winter and spring) < Summer

the basal to the apical part. On average, the values for abundance ($4,014 \pm 1,531$ cells mm⁻²) and biomass (0.056 ± 0.030 µg C mm⁻²) on the apical part were significantly higher than those observed at the basis (respectively, $1,374 \pm 1,150$ cells mm⁻² and 0.017 ± 0.013 µg C mm⁻², $p < 0.005$). Abundance and biomass values observed in the apical part of artificial substrata were higher in May ($6,175$ cells mm⁻² and 0.1097 µg C mm⁻², respectively), while minimum values were observed in June ($2,460$ cells mm⁻² and 0.0243 µg C mm⁻²).

Epibiontic microalgae grown on artificial substrata were compared to those that settled on *E. racemosum* in the same period (May–October 2003). On average, diatom abundance values were significantly lower on artificial substrata ($2,521 \pm 1,784$ cells mm⁻²) than on the hydroid ($4,737 \pm 3,919$ cells mm⁻², $p < 0.01$). The same pattern was observed for diatom biomass, which showed significantly lower values on mimic substrata (0.033 ± 0.027 µg C mm⁻²) than on *E. racemosum* (0.093 ± 0.088 µg C mm⁻², $p < 0.005$). Cyanobacteria exhibited higher values on *E. racemosum* than on artificial substrata only in terms of biomass, due to a different species composition.

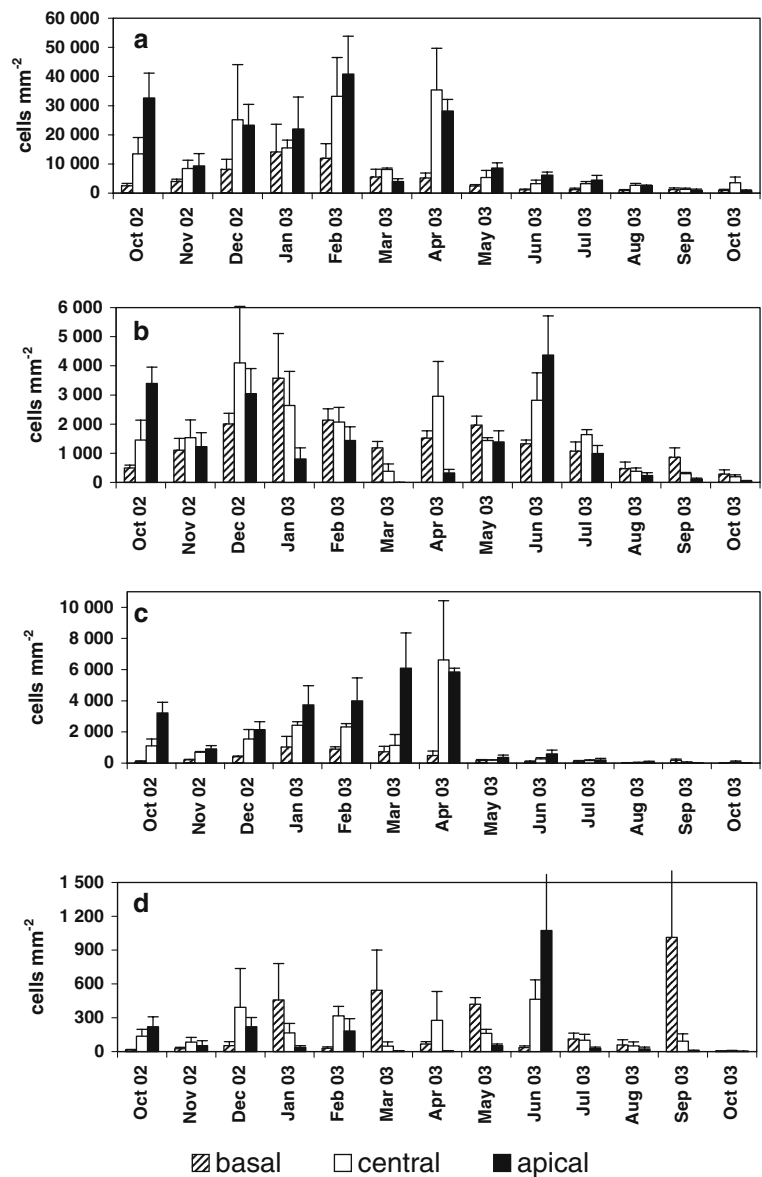
Considering the temporal trend of microalgae in both the hydroid and artificial substratum, diatom biomass values on *E. racemosum* were higher than those on mimic substrata only in the first period (May–August), while this difference was not recorded afterwards (Fig. 8a). The same trend was observed for all the diatom growth forms, as well as for cyanobacteria (Fig. 8b). It is interesting to note that, when the decrease of diatom abundance in *E. racemosum* occurred, the hydroid colonies had died (no polyps were observed).

Hydroid life cycle

On the studied substratum, colonies of *E. racemosum* were present all year around. This species shows periods with living polyps arising from the hydrocauli and periods characterized by polyp lacking; in the latter condition, the hydrocauli remain empty for a long time, continuing to serve as substrata for epibiontic microalgae.

The height of each colony of *E. racemosum* was measured, and the presence/absence of polyps and gonophores was recorded (Fig. 9). During the periods studied, the average height of the colonies decreased

Fig. 5 Trend of abundance values (cells mm⁻²) of diatom growth forms on *Eudendrium racemosum* from October 2002 to October 2003: **a** motile; **b** adnate; **c** erect; **d** tubedwelling; mean \pm SD



from 8 to 4 cm between October and January. Afterwards, values increased once again, until July, reaching 8 cm in height. In late summer and autumn the height decreased to about 4 cm. The polyp density showed two peaks, one in winter and one in summer 2003, with little or no polyp presence in spring and autumn. Unlike the hydrozoans population recorded in unpolluted waters, we observed the presence of gonophores four times during the period studied, in October 2002 and in January, May and August 2003.

Discussion

This study presents the first quantitative data set for microphytobenthos associated with a marine inverte-

brate during an annual cycle. Our results show that an abundant and diversified microalgal community lives on the surface of the marine hydroid *Eudendrium racemosum*, exhibiting markedly seasonal behaviour. In addition, a “host-effect” on the epibiotic communities was highlighted.

Most studies on microalgal communities of hard substrata (i.e. epilithic, epiphytic and epizoic), both in freshwater and marine environments, report only either relative abundance (Moncreiff et al. 1992; Sherwood and Sheath 1999; Michelutti et al. 2003; Comte et al. 2005), or biomass (DeNicola and McIntire 1990; Goldsborough and Hickman 1991; Nelson 1997; Pinckney and Micheli 1998; Ruesink 1998; Ortega-Morales et al. 2005; O’Reilly 2006) or biovolume (Müller 1999; Hillebrand et al. 2000; Busse 2002; Albay and Akcaalan

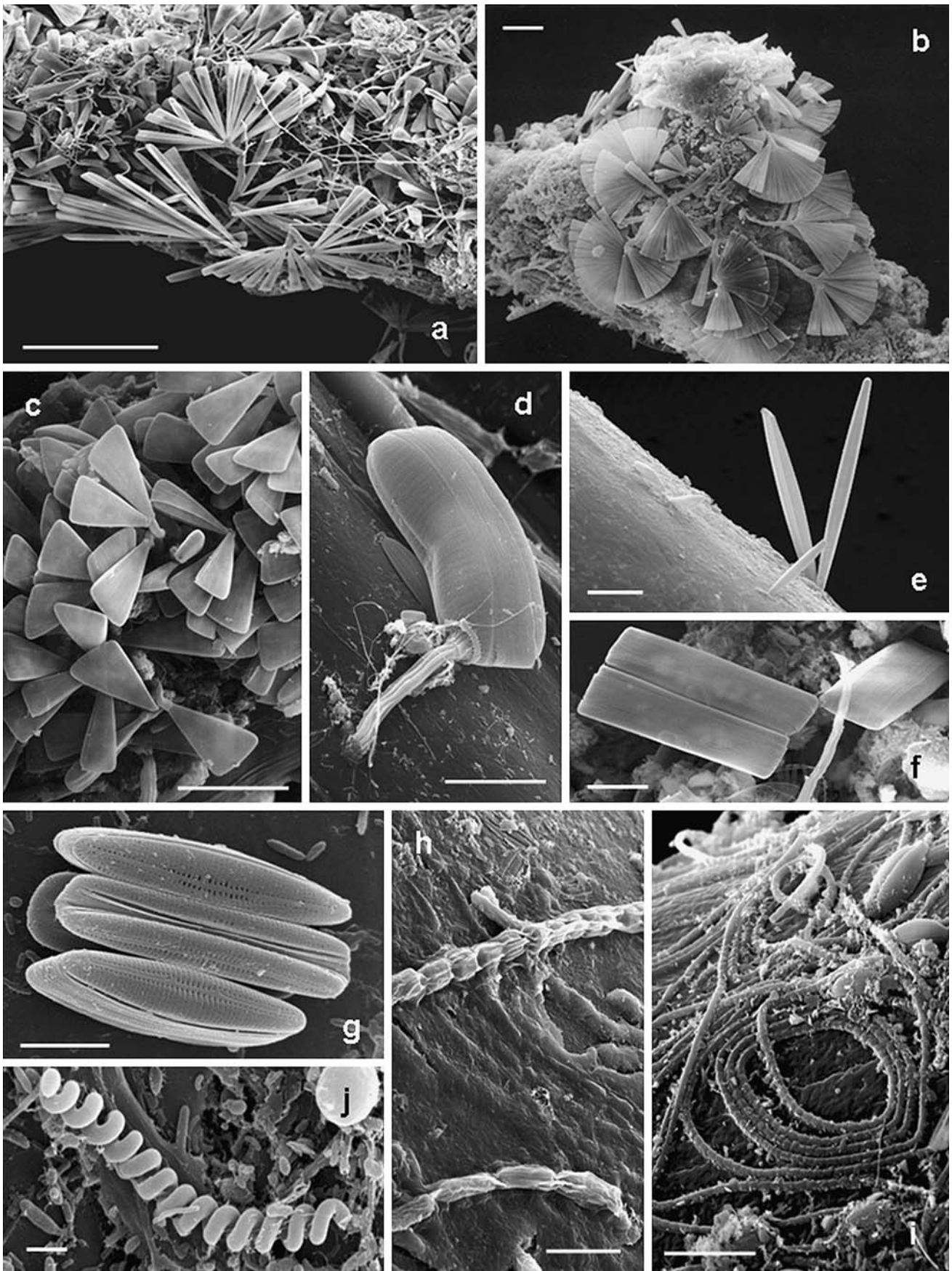


Fig. 6 Epibiontic microalgal communities on *Eudendrium racemosum* II. **a–f** Erect diatoms: **a** *Licmophora flabellata* colonies associated with a rich assemblage of other microalgae covering the stem of hydroid colony; **b** colonies of *Licmophora flabellata* rising from the organic mat covering the hydroid perisarc through mucilage stalks; **c** dense colonies of *Licmophora oedipus* and *L. abbreviata*; **d** *Achnanthes longipes* attaching to the substrate through a mucilage peduncle; **e** *Tabularia tabulata* attaching to the substrate through mucilage pads; **f** colony of *Cyclophora tenuis*. **g–h** Tube-dwelling diatoms: **g** specimens of *Berkeleya rutilans* outside their tube; **h** tube-dwelling diatoms inside their tube. **i–j** Cyanobacteria: **i** filamentous cyanobacteria (*Oscillatoria lutea*); **j** *Spirulina subsalsa* and bacteria. Scale bars (μm): **a** 200; **b** 100; **d–e**, **h–i** 20; **f** 10; **c** 50; **g**, **j** 5

2003), with only a few reporting density values. Comparing diatom densities on *E. racemosum* to those observed in other epizoic communities, values were 1–5 orders of magnitude higher than those reported on gastropod *Hydrobia ulvae* (Gillan and Cadée 2000), mussel shells (Bodeanu 1987–1988) and horseshoe crab (Patil and Anil 2000). Diatom abundance values observed in this study were even 1–2 orders of magnitude higher than those observed on hard substrata of temperate marine areas (Hudon and Bourget 1981; Brandini et al. 2001; Franco 2004), and were comparable to density values of soft sediment intertidal areas, considered to be the most productive (Admiraal et al. 1982; Delgado 1989; Delgado et al. 1991; Peletier 1996).

Looking at the spatial distribution of microphytobenthic communities on the colonies of *E. racemosum*, diatom abundance was significantly higher in the central and apical parts of the colonies than at their basis.

This pattern of distribution was particularly evident for motile and erect diatoms, while adnate and tube-dwelling were more abundant in the basal parts. A biomass increase in the apical parts of the host has frequently been reported and interpreted as a direct response to the availability of light (Müller 1999; Albay and Akcaalan 2003). However, a host effect may also be hypothesized, considering that the microalgae in the apical part of the colony are more directly affected by the polyp presence (see below).

The microalgal community on *E. racemosum* was complex and diversified, showing the presence of all the growth forms. The presence of a well-developed assemblage of erect, long-stalked forms indicates that the communities were in a mature stage. In fact, most studies about the succession of microalgal communities on hard substrata demonstrated that a mature community, characterized by the development of erect forms, occurs after 3–5 weeks (Hoagland 1983; Hamilton and Duthie 1984; Kusakabe 1988; Tanaka and Watanabe 1990; Tuji 2000a; Hameed 2003).

Diatom communities were dominated by motile forms, followed by adnate, erect and tube-dwelling. The dominance of motile diatom taxa throughout the study period suggests that they are the most favoured of the growth forms under all environmental conditions. The selective advantage of biraphid taxa is tied to their ability to move into mature mats, making them superior competitors for nutrients and light (Hudon and Legendre 1987; DeNicola and McIntire 1990), while erect diatoms benefit from a better light exposition (Tuji 2000b; Wellnitz and Ward 2000), and several

Table 3 Mean abundance (cells mm^{-2}) and biomass ($\mu\text{g C mm}^{-2}$) \pm SD of epibiontic microalgae calculated for each hydroid part and results of the ANOVA and Tukey's tests

	Basal	Central	Apical	ANOVA <i>p</i> -level	Tukey's test (<i>p</i> < 0.05)
Total diatoms					
Abundance	6,521 \pm 7,860	15,373 \pm 19,239	17,759 \pm 17,926	<0.01	Basal < (Central and Apical)
Biomass	0.162 \pm 0.203	0.419 \pm 0.707	0.590 \pm 0.673	<0.01	Basal < Apical
Motile					
Abundance	4,557 \pm 6,339	12,208 \pm 16,101	14,136 \pm 15,662	<0.01	Basal < (Central and Apical)
Biomass	0.078 \pm 0.125	0.179 \pm 0.237	0.219 \pm 0.290	<0.05	Basal < Apical
Adnate					
Abundance	1,384 \pm 1,125	1,685 \pm 1,657	1,337 \pm 1,574	ns	
Biomass	0.031 \pm 0.024	0.037 \pm 0.038	0.031 \pm 0.040	ns	
Erect					
Abundance	346 \pm 472	1,284 \pm 2,360	2,095 \pm 2,513	<0.001	Basal < Apical
Biomass	0.028 \pm 0.045	0.187 \pm 0.549	0.326 \pm 0.484	<0.01	Basal < Apical
Tube-dwelling					
Abundance	218 \pm 426	177 \pm 241	147 \pm 351	ns	
Biomass	0.024 \pm 0.053	0.015 \pm 0.023	0.011 \pm 0.024	ns	
Cyanobacteria					
Abundance	2,834 \pm 4,416	6,606 \pm 10,262	5,726 \pm 9,873	ns	
Biomass	0.017 \pm 0.030	0.051 \pm 0.088	0.047 \pm 0.105	ns	

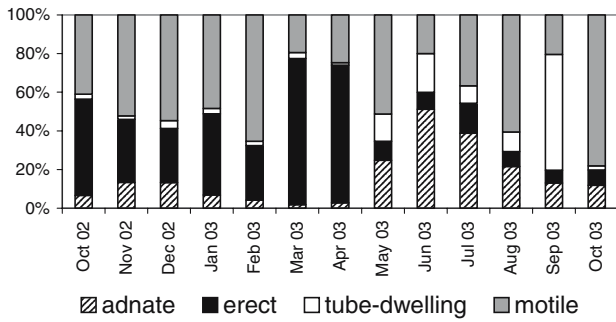


Fig. 7 Percent contribution to total diatom biomass of motile, adnate, erect, tube-dwelling diatoms

species may even regulate the length of their stalks (Lewis et al. 2002), growing over the underlying mat and becoming competitors for light in conditions of dense mat development. The growth of epiphyte microalgae is generally favoured by a nutrient increase in the water column, and the response of epiphytes to the nutrient supply may depend on their position in the matrix (Burkholder et al. 1990; Hillebrand et al. 2000). Pringle (1990) demonstrated that adnate taxa, which lie on the substratum with their valve face and have a limited motility, may be hampered, in terms of nutrient supply, by a massive development of erect taxa, being relatively isolated from the overlying medium when overgrown by a thick algal matrix. These observations are in line with our results, which show that adnate and

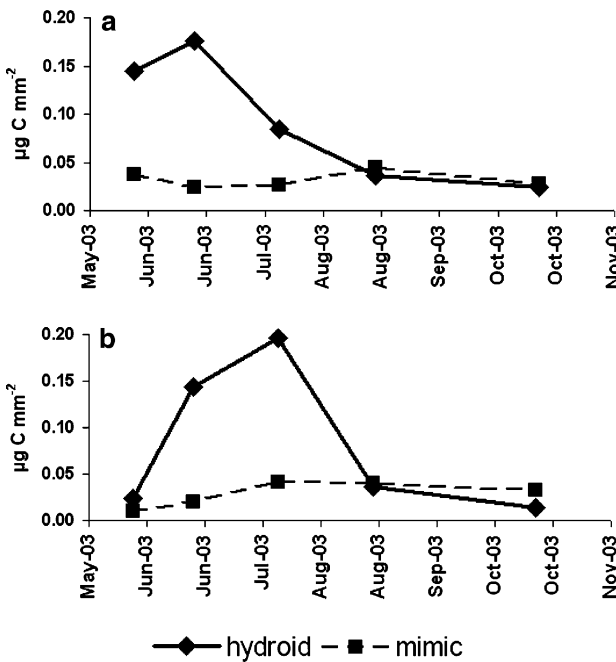


Fig. 8 Comparison of diatom (a) and cyanobacteria (b) biomass ($\mu\text{g C mm}^{-2}$) on *Eudendrium racemosum* and on mimic plastic substrata from May to October 2003

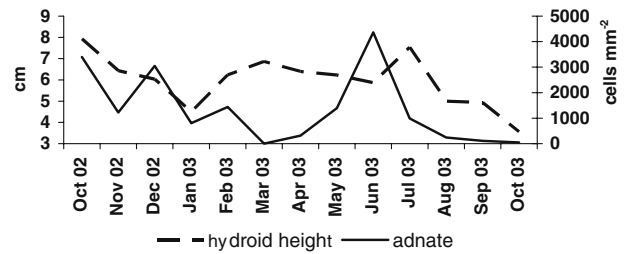


Fig. 9 Height of hydroid colonies (left axis) and abundance of adnate taxa (right axis) from October 2002 to October 2003

erect taxa develop according to an inverse pattern of distribution in terms of both space (adnate diatoms grew preferentially in the basal parts of hydroid colony, whereas erect forms colonized the apical part) and time (adnates increased their density in the spring–summer period, while erect diatoms abruptly decreased during the same period).

The temporal trend of microphytobenthic communities during the study period showed a marked seasonal variability: diatoms showed several peaks during the year, with the highest densities in winter and the lowest in summer. Among diatoms, the motile and erect growth forms reached maximum abundance values in autumn and winter, with a marked decrease in summer, while adnate diatoms exhibited two peaks, one in winter and another in summer. A seasonal pattern of behaviour by benthic microalgae has been already described in temperate waters. Microphytobenthic communities exhibit different temporal trends, depending on latitude and environmental conditions. Unfortunately, no data exist on the annual variability of epizoic communities. Generally speaking, peaks have been reported in spring–summer for the epipelagic and epilithic communities, due to the increase in the period of light for both marine (Bodeanu 1987–1988; Barranguet et al. 1997; Staats et al. 2001) and freshwater areas (Goldsborough and Hickman 1991; Sherwood and Sheath 1999; Kahlert et al. 2002). In the Adriatic Sea, Franco (2004) reported a high-seasonal variability in the abundance and biomass of epilithic communities, with maxima in spring and minimum values in winter.

The sharp summer decrease of diatoms observed in this study may be interpreted in several ways. The meteorological conditions of the study area greatly affect the microalgal communities: during the summer of 2003, exceptionally high-temperature values were recorded throughout the Mediterranean Sea. In the Ligurian Sea, during the summer of 2003, surface temperatures were 2–3°C higher than mean values for this area (Bozzano et al. 2004). Moreover, these anomalous conditions persisted until the end of October,

and we hypothesize that they may be involved in the observed decrease of benthic microalgae on *E. racemosum*. The summer decrease could also be interpreted as an effect of the grazing pressure. Grazing may remove a significant part of epiphyte biomass (Mazzella et al. 1992; Nelson 1997; Müller 1999; Hillebrand et al. 2000), and a summer increase in grazing pressure has frequently been reported (Galanti and Romo 1997; Müller 1999; Worm and Sommer 2000). Although the influence of grazing on microalgae populations has not been specifically addressed in this study, potential grazers of epizoic communities, such as amphipods and polychaetes, were often observed in our samples during the summer. Moreover, grazing had a selective effect on the growth forms, being especially intense on the stalked diatoms, such as *Licmophora*, which almost disappeared in summer, while those attached to the substrata through the entire valve face were less sensitive to the grazers (Underwood et al. 1992; Hillebrand et al. 2000; Jones et al. 2000).

While diatoms showed their maximum abundance values in winter, cyanobacteria presented high densities in spring–summer, with these levels not being observed during the rest of the year. The summer increase of cyanobacteria has been often reported in the literature data for epilithic, epiphytic and epipelagic communities from freshwater, seawater, and even from hypersaline lagoons, being generically related to their tolerance for high temperature (Whitton and Potts 1982; Jørgensen et al. 1983; Pinckney et al. 1995; Müller 1999; Hillebrand et al. 2000; Albay and Akcaalan 2003; Totti 2003; Ortega-Morales et al. 2005). Moreover, looking at the effect of grazing, Jones et al. (2000) reported that filamentous cyanobacteria are not easily grazed.

Diatom communities showed significantly higher abundance and biomass values on *E. racemosum* than on artificial substrata when polyps were living, while the values were very similar when the polyps were absent. These results suggest that microalgae, and diatoms in particular, obtain some benefits from the hydroid host. The relationships between epibiontic microalgae and their hosts have been interpreted in several ways. Round (1981) observed that the development of epibiontic microalgae was enhanced by waste products of host, while Cognie and Barillé (1999) pointed out that oyster pseudofaeces stimulated microalgal growth. Regarding the epiphytic communities, several authors hold that macrophytes are used by diatoms merely for the purpose of attachment, as they simply increase the available surface area (Sullivan 1979). The existence of a host specificity was suggested by Sullivan (1984), as certain epiphytic diatoms were

distributed only on particular macrophytes, but these have also been interpreted as a response to different physical conditions (Sullivan 1981). In contrast, many authors hypothesized that a nutrient exchange between the hosts and periphyton would occur (Pinckney and Micheli 1998; Ács et al. 2003; Albay and Akcaalan 2003). Microalgal communities growing on aquatic plants are often more abundant and diversified than those on experimental artificial substrata. Ács et al. (2003) reported a stimulation of epiphytic microalgae growth through the secretion of nutrients or organic compounds by macrophytes. The degree of host specificity has been related to the trophic conditions of water column, as the differences between epiphytic communities on macrophytes and artificial substrata decrease under eutrophic conditions (Eminson and Moss 1980; Burkholder et al. 1990; Jones et al. 2000).

Epibiontic microalgae on *E. racemosum* appear to be influenced by the host, suggesting that diatoms may benefit from the polyp catabolites. The hypothesis of such trophic relationships is enforced by the marked oligotrophy of the study area (Goffart et al. 2002) and by the observation that the differences in terms of diatom abundance between hydroid and mimic substrata were high in the first period, while they decreased at the end of summer, when polyps died. Further backing for the hypothesis is provided by observation of the trend of adnate diatoms in relation to the height of hydroid colonies (Fig. 9), which we can interpret as a sign of animal health. The trend of adnate diatoms ran parallel to the cycle of the hydroid host, except in spring, when the polyps decreased in number and a rich community of erect diatoms developed (see above). Adnate growth forms, which live attached to the substratum and have limited motility, whereas are hampered in terms of nutrient supply from the water column, may easily benefit from the nutrient exchange with the host because of their intimate association with the latter (Round 1981; Sullivan 1984), especially in light of the fact that they mainly consist of *Amphora* spp., for which facultative heterotrophy has been reported (Ricard 1987).

References

- Ács É, Borsodi AK, Makk J, Molnár P, Mózes A, Rusznyak A, Reskóné MN, Kiss KT (2003) Algological and bacteriological investigations on reed periphyton in Lake Velencei, Hungary. *Hydrobiologia* 506–509:549–557
- Admiraal W, Peletier H, Zomer H (1982) Observation and experiments on the population dynamics of epipelagic diatoms from an estuarine mudflat. *Estuar Coast Shelf Sci* 14:471–487
- Albay M, Akcaalan R (2003) Comparative study of periphyton colonisation on common reed (*Phragmites australis*) and

- artificial substrate in a shallow lake, Manyas, Turkey. *Hydrobiologia* 506–509:531–540
- Barranguet C, Herman PMJ, Sinke JJ (1997) Microphytobenthos biomass and community composition studied by pigment biomarkers: importance and fate in the carbon cycle of a tidal flat. *J Sea Res* 38:59–70
- Bavestrello G, Puce S, Cerrano C, Zocchi E, Boero N (2006) The problem of seasonality of benthic hydroids in temperate waters. *Chem Ecol* 22 (Suppl 1):197–205
- Bodeanu N (1987–1988) Structure et dynamique de l'algoflore unicellulaire dans les eaux du littoral romain de la mer Noire. *Cerc mar IRCM* 20–21:19–250
- Bozzano R, Sparnocchia S, Picco P, Cappelletti A, Schiano ME, Cappa C (2004) Mediterranean warming: analysis of sea temperature time series from the buoy ODAS Italia 1. *Rapport du 37e Congrès de la CIESM, Barcellona (Spain)*, p 87
- Brandini FP, da Silva ET, Pellizzari FM, Fonseca ALO, Fernandes LF (2001) Production and biomass accumulation of periphytic diatoms growing on glass slides during a 1-year cycle in a subtropical estuarine environment (Bay of Paranaguá, southern Brazil). *Mar Biol* 138:163–171
- Burkholder JM, Wetzel RG, Klomparens KL (1990) Direct comparison of phosphate uptake by adnate and loosely attached microalgae within an intact biofilm matrix. *Appl Environ Microb* 56(9):2882–2890
- Busse S (2002) Benthic diatoms in the Gulf of Bothnia. Community analysis and diversity. *Acta Universitatis Upsaliensis, Uppsala*, pp 30
- Cerrano C, Arillo A, Bavestrello G, Calcinai B, Cattaneo-Vietti R, Penna A, Sarà M, Totti C (2000) Diatom invasion in the antarctic hexactinellid sponge *Scolymastra joubini*. *Polar Biol* 23:441–444
- Cerrano C, Calcinai B, Cucchiari E, Di Camillo C, Nigro M, Regoli F, Sarà A, Schiaparelli S, Totti C, Bavestrello G (2004a) Are diatoms a food source for Antarctic sponges? *Chem Ecol* 20(1):57–64
- Cerrano C, Calcinai B, Cucchiari E, Di Camillo C, Totti C, Bavestrello G (2004b) The diversity of relationships between Antarctic sponges and diatoms: the case of *Mycale acerata* (Porifera, Demospongiae). *Polar Biol* 27(4):231–237
- Cognie B, Barillé L (1999) Does bivalve mucus favour the growth of their main food source, microalgae? *Oceanol Acta* 22(4):441–450
- Comte K, Fayolle S, Roux M (2005) Quantitative and qualitative variability of epiphytic algae on one Apiaceae (*Apium nodiflorum* L.) in a karstic river (Southeast of France). *Hydrobiologia* 543:37–53
- Delgado M (1989) Abundance and distribution of microphytobenthos in the bays of Ebro delta (Spain). *Estuar Coast Shelf Sci* 29:183–194
- Delgado M, De Jonge VN, Peletier H (1991) Experiments on resuspension of natural microphytobenthos populations. *Mar Biol* 108:321–328
- DeNicola DM, McIntire CD (1990) Effects of substrate relief on the distribution of periphyton in laboratory streams. I. *Hydrology. J Phycol* 26:624–633
- Di Camillo C, Puce S, Romagnoli T, Tazioli S, Totti C, Bavestrello G (2005) Relationships between benthic diatoms and hydrozoans (Cnidaria). *J Mar Biol Ass UK* 85:1373–1380
- Eminson DF, Moss B (1980) The composition and ecology of periphyton communities in freshwaters. I. The influence of host type and external environment on community composition. *Br Phycol J* 15:429–446
- Franco C (2004) Colonizzazione di diatomee epilithiche su differenti substrati rocciosi. Thesis, University of Ancona
- Galanti G, Romo S (1997) Epiphyton biomass on the floating leaved water chestnut (*Trapa natans*) and its importance for the carbon balance in the eutrophic Lake Candia (N. Italy). *Mem Ist Ital Idrobiol* 56:95–111
- Gillan DC, Cadée GC (2000) Iron-encrusted diatoms and bacteria epibiotic on *Hydrobia ulvae* (Gastropoda: Prosobranchia). *J Sea Res* 43:83–91
- Goffart A, Hecq JE, Legendre L (2002) Changes in the development of the winter-spring phytoplankton bloom in the Bay of Calvi (NW Mediterranean) over the last two decades: a response to changing climate? *Mar Ecol Prog Ser* 236:45–60
- Goldsborough LG, Hickman M (1991) A comparison of periphytic algal biomass and community structure on *Scirpus validus* and on a morphologically similar artificial substratum. *J Phycol* 27:196–206
- Hameed HA (2003) The colonization of periphytic diatom species on artificial substrates in the Ashar canal, Basrah, Iraq. *Limnologia* 33:54–61
- Hamilton PB, Duthie HC (1984) Periphyton colonization of rock surfaces in a boreal forest stream studied by scanning electron microscopy and track autoradiography. *J Phycol* 20:525–532
- Hamilton PB, Poulin M, Yang JR (1997) A new diatom genus *Porannulus* (Bacillariophyta), associated with marine sponges around King George Island, South Shetland Islands, Antarctica. *Diat Res* 12:229–242
- Hasle GR, Syvertsen EE (1997) Marine diatoms. In: Tomas CR (eds) Identifying marine phytoplankton. Academic Press, San Diego, CA, pp 5–385
- Hillebrand H, Worm B, Lotze HK (2000) Marine microbenthic community structure regulated by nitrogen loading and grazing pressure. *Mar Ecol Prog Ser* 204:27–38
- Hoagland KD (1983) Short-term standing crop and diversity of periphytic diatoms in a eutrophic reservoir. *J Phycol* 19:30–38
- Hudon C, Bourget E (1981) Initial colonization of artificial substrate: community development and structure studied by scanning electron microscopy. *Can J Fish Aquat Sci* 38:1371–1384
- Hudon C, Legendre P (1987) The ecological implications of growth forms in epibenthic diatoms. *J Phycol* 23:434–441
- Jones JI, Moss B, Eaton JW, Young JO (2000) Do submerged aquatic plants influence periphyton community composition for the benefit of invertebrate mutualists? *Freshw Biol* 43:591–604
- de Jonge VN, Colijn F (1994) Dynamics of microphytobenthos biomass in the Ems Estuary. *Mar Ecol Prog Ser* 104:185–196
- Jørgensen BB, Revsbech NP, Cohen Y (1983) Photosynthesis and structure of benthic microbial mats: microelectrode and SEM studies for cyanobacterial community. *Limnol Oceanogr* 28(6):1075–1093
- Kahlert M, Hasselrot AT, Hillebrand H, Kurt P (2002) Spatial and temporal variation in the biomass and nutrient status of epilithic algae in Lake Erken, Sweden. *Freshw Biol* 47:1191–1215
- Kusakabe A (1988) Ecological study on epiphytic algae in Lake Biwa. In: L Biwa Research Institute. *Lake Biwa Study Monographs* 4:1–61
- Lewis RJ, Johnson LM, Hoagland KD (2002) Effects of cell density, temperature, and light intensity on growth and stalk production in the biofouling diatom *Achnanthes longipes* (Bacillariophyceae). *J Phycol* 38:1125–1131
- Mac Intyre HL, Geider RJ, Miller DC (1996) Microphytobenthos: the ecological role of the secret garden of unvegetated, shallow-water marine habitats. I. Distribution, abundance and primary production. *Estuaries* 19(2A):186–201

- Mazzella L, Buia MC, Gambi MC, Lorenti M, Russo GF, Scipione MB, Zupo V (1992) Plant-animal trophic relationships in the *Posidonia oceanica* ecosystem of the Mediterranean Sea: a review. In: John DM, Hawkins SL, Price JH (eds) Plant-animal interactions in the Marine Benthos, Systematic Association Special vol 46, Clarendon Press, Oxford, pp165–187
- Michelutti N, Holthman AJ, Douglas MSV, Smol JP (2003) Periphytic diatom assemblages from ultra-oligotrophic and UV transparent lakes and ponds on Victoria Island and comparisons with other diatom surveys in the Canadian arctic. *J Phycol* 39:465–480
- Moncreiff CA, Sullivan MJ, Daehnick AE (1992) Primary production dynamics in seagrass beds of Mississippi Sound: the contributions of seagrass, epiphytic algae, sand microflora, and phytoplankton. *Mar Ecol Prog Ser* 87:161–171
- Müller U (1999) The vertical zonation of adressed diatoms and other epiphytic algae on *Phragmites australis*. *Eur J Phycol* 34:487–496
- Nelson TA (1997) Epiphyte-grazer interactions on *Zostera marina* (Anthophyta: Monocotyledones): effects of density on community function. *J Phycol* 33:743–752
- O'Reilly CM (2006) Seasonal dynamics of periphyton in a large tropical lake. *Hydrobiologia* 553:293–301
- Ortega-Morales BO, Santiago-Garcia JL, López-Cortes A (2005) Biomass and taxonomic richness of epilithic cyanobacteria in a tropical intertidal rocky habitat. *Bot Mar* 48:116–121
- Patil JS, Anil AC (2000) Epibiotic community on the horseshoe crab *Tachypleus gigas*. *Mar Biol* 136:699–713
- Peletier H (1996) Long-term changes in intertidal estuarine diatom assemblages related to reduced input of organic waste. *Mar Ecol Prog Ser* 137:265–271
- Pinckney JL, Micheli F (1998) Microalgae on seagrass mimics: does epiphyte community structure differ from live seagrasses? *J Exp Mar Biol Ecol* 221:59–70
- Pinckney J, Paerl HW, Fitzpatrick M (1995) Impact of seasonality and nutrients on microbial mat community structure and function. *Mar Ecol Prog Ser* 123:207–216
- Pringle CM (1990) Nutrient spatial heterogeneity: effects on community structure, physiology, and diversity of stream algae. *Ecology* 71:905–920
- Ricard M (1987) Diatomophycées. In: Sournia A (ed) Atlas du phytoplankton marin, vol 2. Editions du CNRS, Paris
- Round FE (1971) Benthic marine diatoms. *Oceanogr Mar Biol Ann Rev* 9:83–139
- Round FE (1981) The Ecology of algae. Cambridge University press, Cambridge
- Round FE, Sloane JF, Ebling FJ, Kitching JA (1961) The ecology of Lough Ine. X. The hydroid *Sertularia operculata* (L.) and its associated flora and fauna: effects of transference to sheltered water. *J Ecol* 49:617–629
- Round FE, Crawford RM, Mann DG (1990) The diatoms. Biology, morphology of the genera. Cambridge University Press, Cambridge
- Ruesink JL (1998) Diatom epiphytes on *Odonthalia floccosa*: the importance of extent and timing. *J Phycol* 34:29–38
- Sherwood AR, Sheath RG (1999) Seasonality of macroalgae and epilithic diatoms in spring-fed streams in Texas, USA. *Hydrobiologia* 390:73–82
- Siqueiros-Beltrones DA, Serviere-Zaragoza E, Argumedo Hernández U (2001) First record of the Diatom *Cocconeis notata* Petit living inside the hydrotheca of a hydrozoan epiphyte of *Macrocystis pyrifera* (L.) C. AG. *Océanides* 16(2):135–138
- Staats N, de Deckere EMGT, de Winder B, Lucas Stal LJ (2001) Spatial patterns of benthic diatoms, carbohydrates and mud on a tidal flat in the Ems-Dollard estuary. *Hydrobiologia* 448:107–115
- Strathmann RR (1967) Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. *Limnol Oceanogr* 12(3):411–418
- Sullivan MJ (1979) Epiphytic diatoms of three seagrass species in Mississippi sound. *Bull Mar Sci* 29(4):459–464
- Sullivan MJ (1981) Community structure of diatoms epiphytic on mangroves and *Thalassia* in Bimini Harbour, Bahamas. In: Ross R (eds) Proceedings of 6th Diatom Symposium 1980. Recent, Fossil diatoms Budapest. O Koeltz, Königstein pp 385–398
- Sullivan MJ (1984) Community structure of epiphytic diatoms from the Gulf Coast of Florida, USA. In: Mann DG (eds) Proceedings of 7th Diatom Symposium 1982, Philadelphia, O Koeltz, Königstein, pp 373–384
- Sundbäck K, Miles A, Göransson E (2000) Nitrogen fluxes, denitrification and the role of microphytobenthos in microtidal shallow-water sediments: an annual study. *Mar Ecol Prog Ser* 200:59–76
- Tanaka S, Watanabe T (1990) The colonization process of a typical epilithic algal community *Homoeothrix janthina-Achnanthes japonica* community in a less polluted river in Japan. *Jpn J Phycol* 38:167–177
- Totti C (2003) Influence of the plume of the River Po on the distribution of subtidal microphytobenthos in the northern Adriatic Sea. *Bot Mar* 46:161–178
- Totti C, De Stefano M, Facca C, Ghirardelli LA (2004) Microphytobenthos. In: Gambi MC, Dappiano M (eds) Mediterranean marine benthos: a manual of methods for its sampling and study. *Biol Mar Medit* 11(1):247–266
- Totti C, Calcinai B, Cerrano C, Di Camillo C, Romagnoli T, Bavecstrello G (2005) Diatom selection by the Antarctic sponge *Sphaerotylus antarcticus* Kirkpatrick, 1908 (Porifera, Demospongiae). *J Mar Biol Ass UK* 85:795–800
- Tuji A (2000a) Observation of developmental processes in loosely attached diatom (Bacillariophyceae) communities. *Phycol Res* 48:75–84
- Tuji A (2000b) The effect of irradiance on the growth of different forms of freshwater diatoms: implications for succession in attached diatom communities. *J Phycol* 36:656–661
- Underwood GJC, Thomas JD, Baker JH (1992) An experimental investigation of interactions in snail-macrophyte-epiphyte systems. *Oecologia* 91:587–595
- Welker C, Sdrigotti E, Covelli S, Faganeli J (2002) Microphytobenthos in the Gulf of Trieste (northern Adriatic Sea); relationship with labile sedimentary organic matter and nutrients. *Estuar Coastal Shelf Sci* 55:259–273
- Wellnitz TA, Ward JV (2000) Herbivory and irradiance shape periphytic architecture in a Swiss alpine stream. *Limnol Oceanogr* 45(1):64–75
- Whitton BA, Potts M (1982) The biology of Cyanobacteria. In: Carr, Whitton BA (eds) Botanical monographs, vol 19. Blackwell Scientific Publications, London, pp 515–542
- Worm B, Sommer U (2000) Rapid direct and indirect effects of a single nutrient pulse in a seaweed-epiphyte-grazer system. *Mar Ecol Prog Ser* 202:283–288