*J. Mar. Biol. Ass. U.K.* (2005), **85**, 795–800
Printed in the United Kingdom

# Diatom assemblages associated with *Sphaerotylus antarcticus* (Porifera: Demospongiae)

Cecilia Totti*[‡], Barbara Calcinai*, Carlo Cerrano[†], Cristina Di Camillo*,
Tiziana Romagnoli* and Giorgio Bavestrello*

*Dipartimento di Scienze del Mare, Università Politecnica delle Marche, Via Brecce Bianche, I-60131 Ancona, Italy.
[†]DIP.TE.RIS, Università di Genova, Corso Europa 26, I-16100 Genova, Italy.
[‡]Corresponding author, e-mail: cecilia@univpm.it

*Sphaerotylus antarcticus* is a common Antarctic sponge characterized by a dense spicule fur that covers its surface. This sponge has been sampled at Terra Nova Bay from 22 November 2001 to 29 January 2002 at weekly intervals. On its spicules, a rich assemblage of benthic diatoms has been observed, mainly composed of *Hyalodiscus* sp., a centric sessile diatom, forming short colonies. The temporal trend of the abundances of these diatoms showed a maximum of $115 \times 10^6$ cells $g^{-1}$ dry weight (dw) at the end of December. Almost all the diatom frustules observed in the sponge choanosome belonged to the planktonic species *Fragilariopsis curta*, which displayed a peak ($50 \times 10^6$ cells $g^{-1}$ dw) in the second half of January. Diatoms that were observed in the choanosome were of a size comparable to that of the inhalant pores, suggesting that sponges actively engulf diatoms, due to the pumping activity of their choanocyte chambers. Sponges, as active filter feeders, therefore represent one important vector in the transfer of energy from planktonic to benthic trophic chains in Antarctic habitats.

## INTRODUCTION

Sponges represent one of the most important components of the Antarctic benthos, having a high specific richness (more than 350 species) and very high values of biomass (about 2–4 kg $m^{-2}$) (Sarà et al., 1992). It is difficult to explain the success of this group in Antarctic habitats, which are characterized by a food supply whose quantity fluctuates during the year. Oligotrophic conditions in the water column in winter could represent a metabolic constraint for filter-feeding organisms (Matsuda et al., 1990; Cockell et al., 2000).

Recent studies (Cerrano et al., 2004a,b) indicate that Antarctic sponges can actively exploit the summer phytoplanktonic bloom, engulfing planktonic diatoms that are quickly digested. Sponges as active filter feeders therefore represent one important vector for the transfer of energy from planktonic to benthic trophic chains, a role that, in temperate and tropical habitats, is mainly played by colonial cnidarians (Gili & Coma, 1998). In Antarctic waters, on the other hand, sponges are themselves exploited as trophic resources by several other benthic organisms such as echinoderms, nemerteans and gastropods (Cerrano et al., 2000b).

Although species-specific relationships between diatoms and sponges were sometimes described, such as *Melosira* sp. in *Scolymastra joubini* Topsent, 1916 (Cerrano et al., 2000a) and *Porannulus contentus* in *Mycale acerata* Kirkpatrick, 1907 (Hamilton et al., 1997; Cerrano et al., 2004b), the presence of diatom species in Antarctic sponges has been mainly related to the filtration of phytoplankton cells from the water column. In fact, some of the most abundant diatom species of the Antarctic plankton communities, i.e. *Fragilariopsis curta*, *Thalassiosira* cf. *gracilis*, *T. perpusilla* and *Thalassiosira* spp. reached densities of several millions of cells $g^{-1}$ dw in the tissues of several sponge species (Cerrano et al., 2004a,b).

An open question concerns the way in which diatom cells penetrate inside the sponge body. Recent studies suggest that diatoms may be incorporated by the exopinacocytes of the sponge surface or by the endopinacocytes of the inhalant canals (Cerrano et al., 2004b).

In this study we investigated the temporal trend of diatom biodiversity and abundance on the surface and inside the tissues of the Antarctic sponge *Sphaerotylus antarcticus* Kirkpatrick, 1908. The results offer new evidence about the selectivity and the method of incorporation of diatoms by the sponges, together with some new details on the organization of polymastid sponges.

## MATERIALS AND METHODS

Specimens of *Sphaerotylus antarcticus* were collected by SCUBA divers at Terra Nova Bay, at depths of 25–35 m, from 22 November 2001 to 29 January 2002, at weekly intervals. This sponge species has been studied because it is common in the study area. Both diatom content and phytopigment (chlorophyll-*a* and phaeopigments) concentration were analysed in the sponge tissues. To assess the different distributions in the sponge body, we studied the sponge choanosome separately by the spicule fur, which completely covers the sponge surface. This stratum was separated from the sponge body using a lancet. Samples for both diatom and phytopigment content were analysed in three replicates.

### *Diatoms*

Samples for diatom analysis were preserved in 4% neutralized formalin. To allow the separation of diatoms from sponge tissues, the Von Stosch cleaning procedure (Hasle & Syvertsen, 1996) was applied to sponge samples, to remove the organic component: for each sample, an aliquot of sponge material (~0.1 g) was weighed and then equal amounts of $HNO_3$ and 3× sample amounts of $H_2SO_4$ were added, after which the samples were boiled for approximately 3 min, cooled and rinsed with distilled water until free of acid. Samples were adjusted to a final volume of 1 ml by adding distilled water. The cleaning procedure resulted in the separation of diatom frustules into two valves. Counting of diatom valves was performed with an inverted microscope (ZEISS Axiovert 135) equipped with phase contrast, following the Utermöhl method (Hasle, 1978). A known volume of cleaned sample was poured with a micropipette into a cylinder/chamber complex previously filled with filtered seawater, and allowed to settle. Counting was carried out along 2–4 transects at a 400× magnification, to count a minimum of about 150 valves. Results were expressed as number of frustules per gram of sponge tissue (dry weight [dw]).

Sponge samples were also processed for observation with a scanning electron microscope (SEM), in order to investigate the diatom distribution pattern *in situ*. Fixed samples were washed and then dehydrated in a graded ethanol series. Dried samples were coated with gold-palladium in a Balzers Union evaporator. Samples were analysed with a Philips EM 515.

### *Chlorophyll-*a *and phaeopigment analysis*

Samples for chlorophyll-*a* (chl-*a*) and phaeopigment (phaeo) analysis were frozen in liquid nitrogen and stored at −80°C until the time of analysis. A weighted aliquot of sponge material (~1 g) was treated with 10 ml of acetone, homogenized and left overnight in the dark at +4°C for the extraction of pigments. Then samples were centrifuged (2500×10 min), and the surnatant was read at the spectrophotometer at wavelengths of 665 and 750 nm before and after acidification. Chlorophyll-*a* and phaeo concentrations ($\mu g\ g^{-1}$ dw) were calculated according to Lorenzen (1967).

## RESULTS

### *The sponge*

*Sphaerotylus antarcticus* (Figure 1A) is a hadromerid sponge belonging to the Polymastiidae family. The gross morphology of the sponge is hemispherical, slightly flattened. The specimens of Terra Nova Bay reached 8–10 cm in diameter and were 3–4 cm thick. The sponge body is covered by a dense spicule fur, composed of long styles

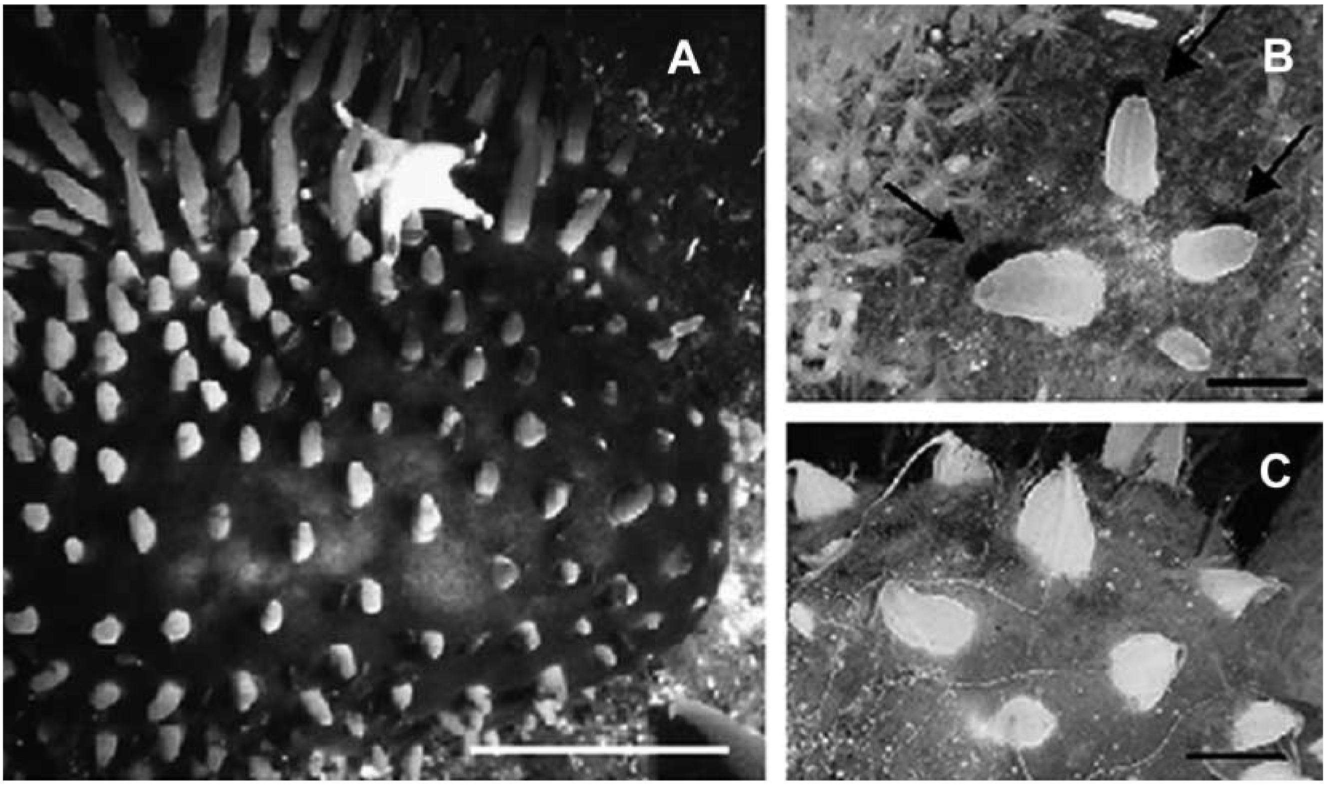

**Figure 1.** *Sphaerotylus antarcticus*: (A) large specimen: spicule fur and papillae of the aquiferus system are evident; (B) papillae of a specimen showing the apical oscular openings (arrows); (C) specimen with the tips of the papillae forming long filaments. Scale bars: A, 2 cm; B & C, 1 cm.

ispidating the surface with their sharp tips pointed towards the outside.

From the sponge surface, conical, smooth papillae arise (Figure 1B), with their number ranging from ten in small specimens to more than one hundred in large ones. While the sponge surface is intensely green-brown, the papillae range from pale yellow to green-yellow. The green-brown colour of the spicule fur was due to a rich diatom community living on the sponge spicules. The openings of the aquiferous system are found on the papillae. The papillae have a mixed function: the oscula open on the tip, while the inhalant pores are on the surface (Figures 1B & 2A).

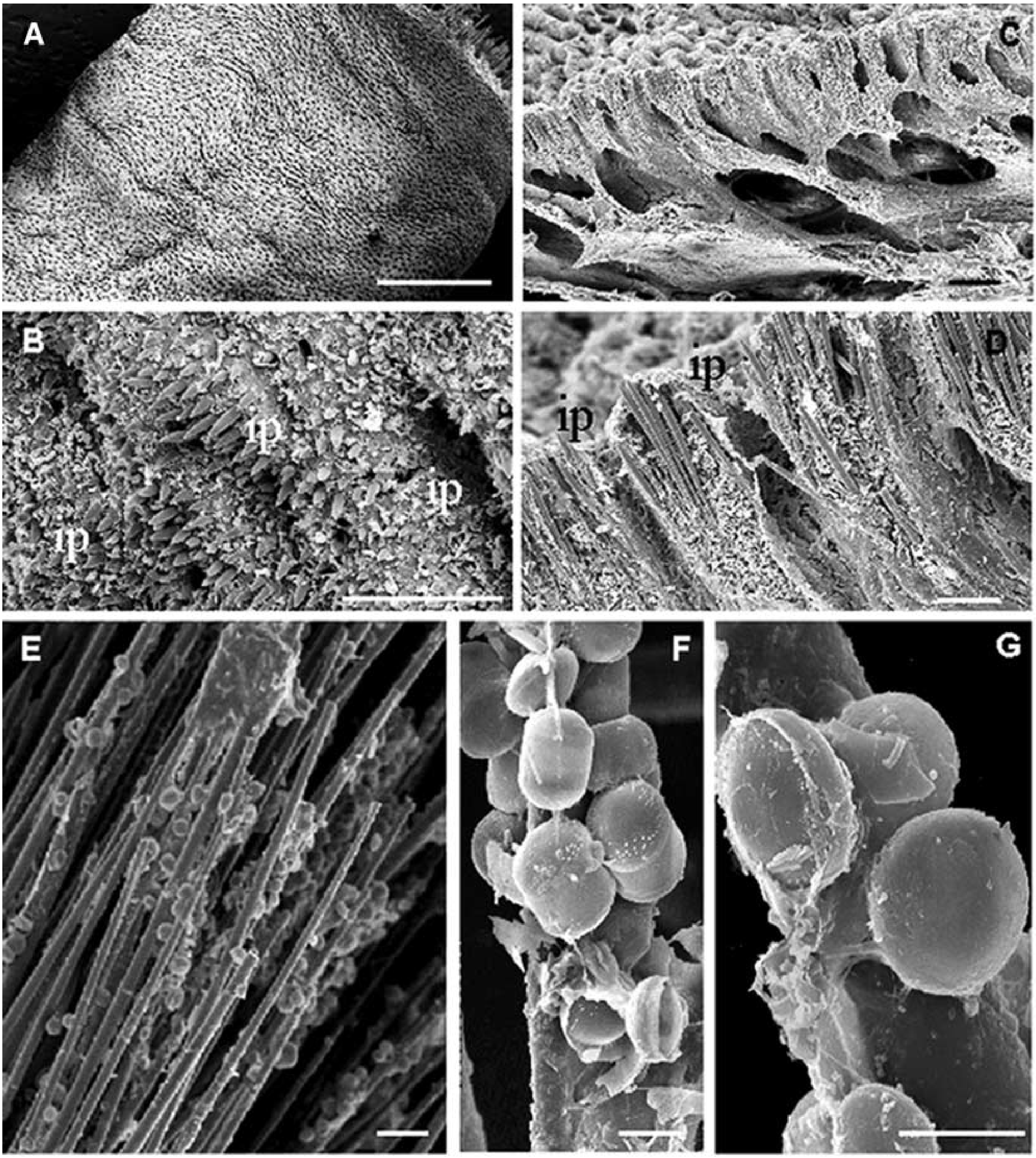

**Figure 2.** *Sphaerotylus antarcticus*: (A) surface of a papilla crossed by several incurrent pores; (B) enlargement showing the pores surrounded by a palisade of ensiform tylostyles; (C) cross section of the papilla with the incurrent canals; (D) enlargement of the incurrent canals with interposed fans of ensiform tylostyles; (E) long styles of the spicule fur widely colonized by benthic centric diatoms; (F & G) enlargements of spicules showing benthic diatom colonization. ip, inhalant pore. Scale bars: A, 1 mm; B & D, 50 $\mu$m; C & E, 100 $\mu$m; F & G, 20 $\mu$m.

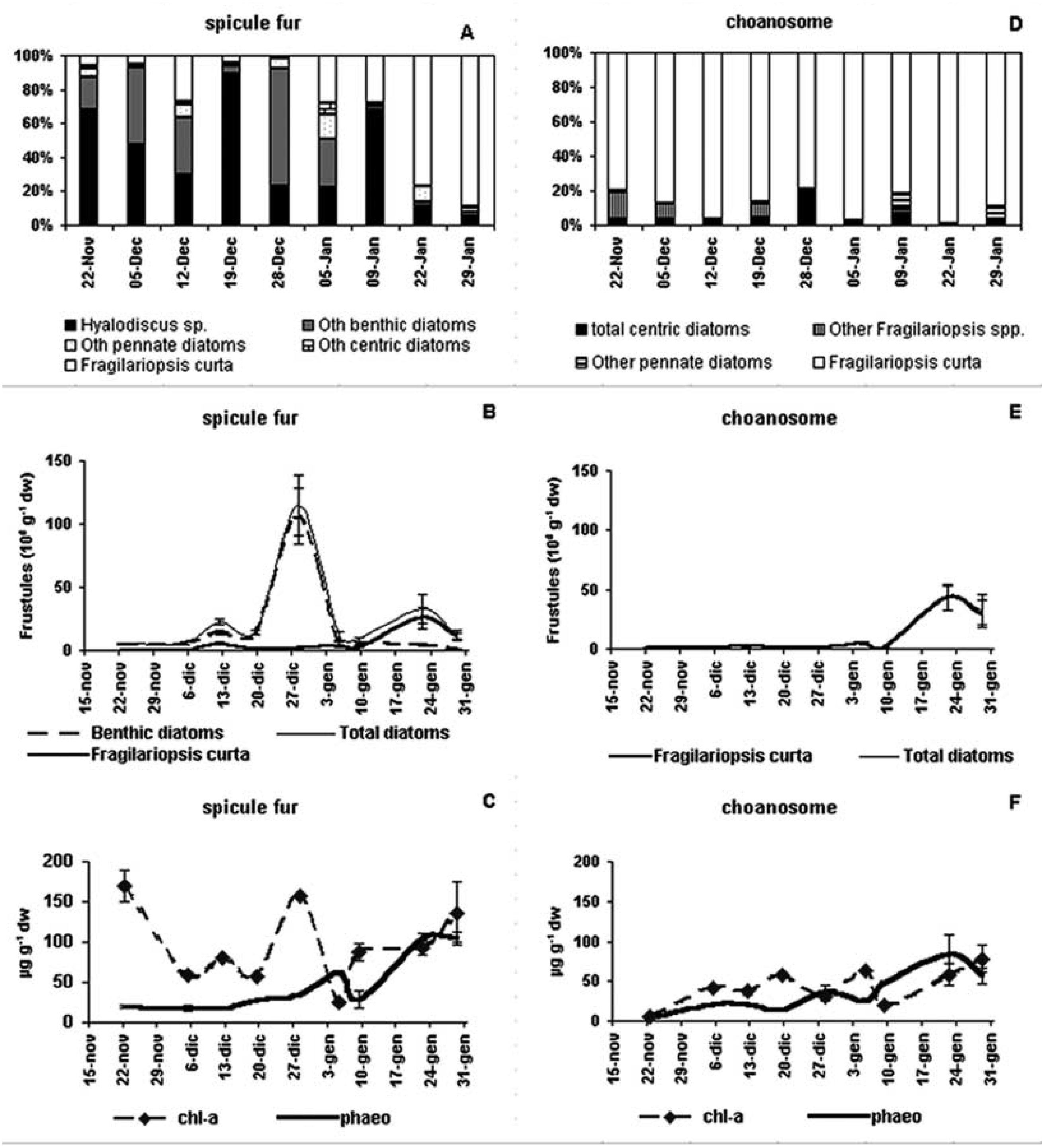

**Figure 3.** Diatom abundance, biomass and diversity in *Sphaerotylus antarcticus* from November 2001 to January 2002. Diatom per cent composition in the spicule fur (A) and inside the sponge tissues (D); main taxa are represented and less abundant species were grouped into major categories. Trend of chlorophyll-*a* and phaeopigment concentration ($\mu$g g$^{-1}$ dw) ±SE in the spicule fur (B) and in the sponge tissue (E).Trends of diatom frustule (cells×10$^6$ g$^{-1}$ dw) concentration ±SE in the spicule fur (C) and in the sponge tissue (F).

In the last period of summer, when the pack ice again covered the sea surface, several specimens showed all the papillae ending in long filaments in the place of the oscula, which are closed (Figure 1C). In this condition, the sponges are probably unable to filter water.

The SEM analysis showed that inhalant pores are uniformly distributed on the surface of the papillae (Figure 2A). In preserved material, the pores have an oval shape, 30–60×10 $\mu$m. They are lined by a palisade of ensiform tylostyles, with the tip arising from the sponge pinacoderm (Figure 2B). In cross section, the inhalant canals under the pores are clearly visible. They are 50–60 $\mu$m wide and 100–200 $\mu$m long and are divided from each other by spicule fans (Figure 2C,D). These canals are perpendicular to the main axis of the papilla and merge in canals of larger diameters parallel to the papilla axis (Figure 2C). These canals are lined with flattened pinacocytes (Figure 2D).

**Table 1.** *List of identified diatom taxa associated with* Sphaerotylus antarcticus.

*Achnantes* cf. *longipes* Agardh
*Actinocyclus* sp.
*Amphora* spp.
*Asteromphalus hookeri* Ehrenberg
*Asteromphalus hyalinus* Karsten
*Asteromphalus* sp.
*Biddulphia* sp.
*Cocconeis costata* Gregory
*Cerataulus* sp.
*Thalassiosira* sp.
*Coscinodiscus* sp.
*Dimerogramma* sp.
*Diploneis crabro* Ehrenberg
*Entomoneis* cf. *paludosa* (W. Smith) Reimer
*Fragilariopsis curta* (Van Heurck) Hustedt
*Fragilariopsis linearis* (Castracane) Frenguelli
*Fragilariopsis obliquecostata* (Van Heurck) Heiden
*Fragilariopsis rhombica* (O'Meara) Hustedt
*Fragilariopsis ritscheri* Hustedt
*Fragilariopsis sublinearis* (Van Heurck) Heiden
*Fragilariopsis* spp.
*Gyrosigma* sp.
*Hyalodiscus* sp.
*Navicula* spp.
*Nitzschia sigma* (Kützing) W. Smith
*Nitzschia* spp.
*Odontella aurita* (Lyngbye) C.A. Agardh
*Plagiotropis* sp.
*Pleurosigma intermedium* Smith
*Pleurosigma* sp.
*Porosira pseudodenticulata* (Hustedt) Jousé
*Stauroneis* sp.
*Stellarima microtrias* (Ehrenberg) Hasle & Sims
*Trachineis aspera* (Ehrenberg) Cleve
*Trachineis* sp.

### *The diatom assemblages*

The diatom assemblage observed on the Antarctic sponge *Sphaerotylus antarcticus* is listed in Table 1. The two zones of the *S. antarcticus* body, the spicule fur and the choanosome, showed a very different composition in terms of the diatom assemblages.

The spicules of the external fur were colonized by a rich community of benthic diatoms, mainly represented by the sessile centric *Hyalodiscus* sp. (Figures 2E–G & 3A). Other benthic species were also observed, such as *Achnanthes* cf. *longipes*, *Biddulphia* spp., *Diploneis crabro*, *Entomoneis paludosa*, *Trachineis aspera*, *Navicula* sp., *Gyrosigma* sp., *Pleurosigma intermedium*, and *Pleurosigma* sp. In the last weeks of the observations, from the beginning of January, benthic diatom abundances decreased, while cells of the planktonic *Fragilariopsis curta* became more and more abundant (Figure 3A).

The trend of abundance of benthic diatom frustules on the spicule layer showed minimum values at the end of November ($6\times10^6$ cells $g^{-1}$ dw of sponge spicules). Then, an increase was observed in mid December ($23\times10^6$ cells $g^{-1}$ dw), and a second higher peak occurred at the end of December ($110\times10^6$ cells $g^{-1}$ dw). Until the end of December, the total diatom trend was strongly driven by the abundances of benthic diatom assemblages, which represented the main fraction. In the second half of January, a new peak was observed ($33\times10^6$ cells $g^{-1}$ dw), due to planktonic diatoms (mainly *Fragilariopsis curta*, Figure 3B).

Chlorophyll-*a* and phaeo concentration in the spicule fur ranged, respectively, from 25 to 169 and from 3 to 84 $\mu g\,g^{-1}$ dw. The trend of chl-*a* showed several peaks in correspondence with those of the abundance of benthic diatoms, except at the beginning of the sampling period, where high chl-*a* values were observed. On the other hand, the trend of phaeo concentration (Figure 3C) was strongly related to that of the abundance of *Fragilariopsis curta* ($P<0.01$; R=0.86; N=8).

The diatom assemblage of the sponge choanosome showed a completely different composition, with the major fraction being represented by *Fragilariopsis curta* (80–97%), while benthic diatoms were almost completely absent (Figure 3D). The trend of abundance (Figure 3E) showed low values until mid January, when the main peak was observed ($44\times10^6$ cells $g^{-1}$ dw). At the end of January, this peak showed the first signs of decreasing. The trend of the total frustule abundance in the sponge choanosome strongly overlapped that of *F. curta*. Moreover, the occurrence of the peak fitted with the trend of *F. curta* observed on the sponge surface, and cell densities of *F. curta* did not display significant differences between the spicule fur and the choanosome (one-way analysis of variance, not significant).

The trend of chl-*a* concentration (Figure 3F) indicated that no living diatoms were present inside the choanosome at the beginning of our sampling period. In the subsequent period, the chl-*a* concentration increased following an oscillatory trend, while the phaeo concentration was strongly correlated ($P<0.01$; R=0.94; N=8) with the abundance of diatom frustules.

## DISCUSSION

These data show an increase in the variety of recorded relationships between diatoms and Antarctic sponges. Several sponge species use diatoms, particularly *Fragilariopsis curta*, as a food source (Cerrano et al., 2004a). In *Scolymastra joubini*, diatoms show parasitic behaviour towards the sponge: they actively reproduce inside the sponge tissues, which are quickly destroyed (Cerrano et al., 2000a). *Porannulus contentus* is a diatom specialized for an epibiontic life on the surface of some sponge species, and it may also be used as food (Hamilton et al., 1997; Cerrano et al., 2004b).

In this study, we show that a rich community of benthic diatoms mainly represented by the sessile centric *Hyalodiscus* sp. lived epibionthic on the spicule fur of *Sphaerotylus antarcticus*. Moreover, planktonic diatoms were incorporated inside the choanosome, where they were digested and used as a trophic source.

The extremely dense spicule fur of *Sphaerotylus antarcticus* represents a complex three-dimensional microhabitat for benthic organisms: benthic diatoms fix on the spicules, forming short moniliformis chains, while the sediment that accumulates between the spicules hosts a rich meiofauna mainly composed of harpacticoids and nematodes (unpublished). The benthic diatom abundance in this habitat, reaching more than 100 millions of cells $g^{-1}$ dw of sponge spicules, could represent a source of dissolved organic matter, which enriches the water filtered by the sponge, and represents an additional trophic resource that is exploited by *Sphaerotylus*, as described by Reiswig (1990).

The high values of chl-*a* observed at the end of November, associated with the relatively low abundance of diatoms, were already observed in other Antarctic sponges in similar conditions (Cerrano et al., 2004a) and may be interpreted as a chromatic adaptation of diatoms to low light levels at the beginning of austral summer (Falkowski & Owens, 1980).

The trend of abundance of benthic diatoms inside the spicule fur showed a main peak at the end of December. In the same period, the high chl-*a* concentration associated with low phaeo concentration would suggest that diatoms were in an active phase of development. Phaeo pigment concentration in the spicule fur increased at the end of January, when the abundance of *Fragilariopsis curta* increased, indicating that cells of this species were partially decayed when they reached the sponge surface.

On the other hand, benthic diatoms were never observed inside sponge tissues, where only planktonic ones, mainly *Fragilariopsis curta*, were present. The trend of abundance of *F. curta* inside the tissues of *Sphaerotylus antarcticus* overlaps those described in several other Antarctic sponge species that are able to exploit this diatom species as a trophic source (Cerrano et al., 2004a).

The size of planktonic diatoms and particularly that of *Fragilariopsis curta* (apical axis 10–42 $\mu$m; transapical axis 3.5–6 $\mu$m) is compatible with that of the inhalant openings of the sponge (30–60 $\times$ 10 $\mu$m), while *Hyalodiscus* sp., which dominated the benthic diatom assemblages of the spicule fur, has dimensions too large for the pores (diameter 20–30 $\mu$m; pervalvar axis up to 35 $\mu$m).

Moreover, the benthic diatoms are strongly fixed to the spicule of the fur. These facts indicate that only diatoms which can be resuspended in the water are actively incorporated through the inhalant openings of the aquiferous system, due to the pumping activity of the choanocyte chambers of the sponge.

Although it is well known that the main trophic source for sponges is plankton smaller than 10 $\mu$m (Reiswig, 1971; Pile et al., 1996; Witte et al., 1997; Ribes et al., 1999), diatoms seem to play a role in sponge-food composition that is not negligible (Gaino & Rebora, 2003). In the Mediterranean sponge *Dysidea avara*, they range from 4 to 26% of the diet, depending on the annual cycle (Ribes et al., 1999). The large amount of diatoms ingested by Antarctic sponges suggests that in the austral summer, diatoms may represent nearly the totality of the food supply.

It is generally assumed (Reiswig, 1971) that particle capture is accomplished by either pinacocytes or choanocytes, depending upon particle size. Particles in the picoplankton size-range are captured with high efficiency by the choanocytes, while particles as large as the diameter of the pores are ingested by pinacocytes lining the canal system. Although we have demonstrated that in the volcanosponge *Scolymastra joubini* diatoms are also ingested by the pinacocytes lining the external sponge surface (Cerrano et al., 2000a), the walls of the aquiferous system are probably the main method of diatom engulfing.

The authors gratefully acknowledge F. Regoli, M. Nigro and S. Schiaparelli for collecting sponge specimens. This research was supported by PNRA (the Italian Antarctic Programme) and MIUR grants.

## REFERENCES

Cerrano, C., Arillo, A., Bavestrello, G., Calcinai, B, Cattaneo-Vietti, R., Penna, A., Sarà, M. & Totti, C., 2000a. Diatom invasion in the Antarctic hexactinellid sponge *Scolymastra joubini*. *Polar Biology*, **23**, 441–444.

Cerrano, C., Bavestrello, G., Calcinai, B., Cattaneo-Vietti, R. & Sarà, A., 2000b. Asteroids eating sponges from Terra Nova Bay, East Antarctica. *Antarctic Sciences*, **12**, 425–426.

Cerrano, C. et al., 2004a. Are diatoms a food source for Antarctic sponges? *Chemistry and Ecology*, **20**, 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 Biology*, **27**, 231–237.

Cockell, C.S., Stokes, M.D. & Korsmeyer, K.E., 2000. Overwintering strategies of Antarctic organisms. *Environmental Reviews/Dossier Environnement*, **8**, 1–19.

Falkowski, P.G. & Owens T.G., 1980. Light–shade adaptation: two strategies in marine phytoplankton. *Plant Physiology*, **66**, 592–595.

Gili, J.-M. & Coma, R., 1998. Benthic suspension feeders: their paramount role in littoral marine food webs. *Trends in Ecology and Evolution*, **13**, 316–321.

Gaino, E. & Rebora, M., 2003. Ability of mobile cells of the freshwater sponge *Ephydatia fluviatilis* (Porifera, Demospongiae) to digest diatoms. *Italian Journal of Zoology*, **70**, 16.

Hamilton, P.B., Poulin, M. & Yang, J.R., 1997. A new diatom genus *Porannulus* (Bacillariophyta), associated with marine sponges around King George Island, South Shetland Islands, Antarctica. *Diatom Research*, **12**, 229–242.

Hasle, G.R., 1978. The inverted microscope method. In *Phytoplankton manual monographs on oceanographic methodology. 6* (ed. A. Sournia), pp. 88–96. Paris: UNESCO.

Hasle, G.R. & Syvertsen, E.E., 1996. Marine diatoms. In *Identifying marine diatoms and dinoflagellates* (ed. C.R. Tomas), pp. 5–385. San Diego: Academic Press.

Lorenzen, C.J., 1967. Determination of chlorophyll and phaeopigments: spectrophotometric equations. *Limnology and Oceanography*, **12**, 343–346.

Matsuda, O., Ishikawa, S. & Kawaguchi K., 1990. Seasonal variations of particulate organic matter under Antarctic fast ice and its importance to benthic life. In *Antarctic ecosystems: ecological change and conservation* (ed. K.R. Kerry and G. Hempel), pp. 145–148. Berlin: Springer.

Pile, A.J., Patterson, M.R. & Witman, J.D., 1996. *In situ* grazing on plankton $<10\,\mu$m by the boreal sponge *Mycale lingua*. *Marine Ecology Progress Series*, **141**, 95–102.

Reiswig, H.M., 1971. Particle feeding in natural populations of three marine demosponges. *Biological Bulletin. Marine Biological Laboratory, Woods Hole*, **141**, 568–591.

Reiswig, H.M., 1990. *In situ* feeding in two shallow water hexactinellid sponges. In *New perspective in sponge biology* (ed. K. Ruetzler), pp. 504–510. Washington DC: Smithsonian Institution Press.

Ribes, M., Coma, R. & Gili, J.-M., 1999. Natural diet and grazing rate of the temperate sponge *Dysidea avara* (Demospongiae, Dendroceratida) throughout an annual cycle. *Marine Ecology Progress Series*, **179**, 190–199.

Sarà, M., Balduzzi, A., Barbieri, M., Bavestrello, G. & Burlando, B., 1992. Biogeographic traits of Antarctic demosponges. *Polar Biology*, **12**, 559–585.

Witte, U., Brattegerd, T., Graf, G. & Springer, B., 1997. Particle capture and deposition by deep-sea sponges from the Norwegian–Groenland Sea. *Marine Ecology Progress Series*, **154**, 241–252.

*Submitted 31 August 2004. Accepted 14 April 2005.*