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Growth form analysis of epiphytic diatom communities of Terra Nova Bay (Ross Sea, Antarctica)

Roksana Majewska · Maria Cristina Gambi ·
Cecilia Maria Totti · Chiara Pennesi ·
Mario De Stefano

Abstract Diatoms have been long collected from the Southern Ocean but almost no data exist for epiphytic communities, despite their high ecological significance as an important food source in Antarctic coastal food chains. Here, we present a first growth form analysis of diatoms associated with rhodophyte hosts from Terra Nova Bay, Ross Sea, Antarctica. We performed this study to gather baseline information on the species composition of epiphytic diatom communities, determine the influence of some environmental variables on the diatom distribution patterns, and assess the caveats that must be taken into account in terms of sampling design. Macroalgal material was collected during the Italian Antarctic expeditions between 1990 and 2004. Epiphytic diatoms were studied by means of scanning electron microscopy. In terms of growth forms, there were no significant differences between the diatom communities on the different macroalgal host species. Motile (mainly small-celled *Navicula perminuta* and other *Navicula* spp.) and adnate (*Cocconeis* spp.) diatoms dominated the community throughout the study period. Many of the macroalgal blades examined were also covered by epiphytic animals (calcareous bryozoans, hydroids) over most of their surface, with a significant effect on the associated diatom community structure. Our findings suggest that the bio-physicochemical characteristics of each sampling site affected the epiphytic diatom communities more than the substrate type provided by the macroalgal host or the sampling depth.

Keywords Marine epiphytic diatoms · Antarctica · Growth form · Macroalgae · SEM

Introduction

Diatoms are key biotic elements of Antarctic aquatic ecosystems, significantly influencing the dynamics of primary production, nutrient cycling, carbon transport, oxygen flow, and its activity, and all trophic processes (e.g., Fry and Wainright 1991; Nelson and Treguer 1992; Bargagli 2005). Despite their fundamental ecological role in the food webs, the biodiversity of Antarctic marine diatoms is still poorly studied. Since the first report on Antarctic microalgae by Ehrenberg (1843), there have been a number of expeditions that have sampled Antarctic diatoms, with the majority of these focusing on specimens recorded either from plankton and bottom sediments or those within general floristic surveys (Thomas and Jiang 1986; Al-Handal and Wulff 2008). Very little is known about the biodiversity and distribution of marine diatoms that live as epiphytes on the thalli of macroalgae. According to Wulff et al. (2011), there are only three investigations that deal with this subject: Thomas and Jiang (1986), Ligowki (2002), and Al-Handal and Wulff (2008). However, studies on marine epiphytic diatom communities from Antarctic waters were carried out also by Sutherland (2008), who included data on epiphytic diatoms from Cape Evans (Ross Sea) in her work on surface-associated diatoms. Quantitative investigations providing estimations of densities or biomass are just as scarce (Dayton et al. 1986).

R. Majewska (✉) · M. De Stefano
Second University of Naples, 81100 Caserta, Italy
e-mail: roksana.majewska@unina2.it

M. C. Gambi
Stazione Zoologica A. Dohrn, 80077 Ischia, Naples, Italy

C. M. Totti · C. Pennesi
Marche Polytechnic University, 60121 Ancona, Italy

It is known that in shallow waters off the Antarctic coasts, epiphytic diatoms flushed off their substrate surfaces may often represent a major constituent of the water column microalgae, being the fundamental source of primary production for the benthic communities (Everitt and Thomas 1986; Ahn et al. 1997). The nearshore zone that provides an optimal habitat for many of the Antarctic key organisms, a transition from land to sea, is particularly sensitive to climatic changes or anthropogenic pollution and, therefore, requires special attention and protection. Since vulnerable diatom assemblages are accurate proxies for environmental conditions worldwide and may also be used for the assessment of the damaging effects of climate change in Antarctic regions (Spaulding et al. 2010), it is crucial to bridge the gaps in our knowledge of living diatom communities in the Southern Ocean (De Moreno et al. 1997; Cunningham and Leventer 1998).

There is an urgent need for attention toward this issue and further research to collect accurate data that will allow a better understanding of the relationships and interactions among different diatom growth forms and their substrate. Potential diatom host specificity is still an open question. In addition, even at a global scale, strictly taxonomic and detailed ecological studies on epiphytic marine diatom communities are relatively rare due to the limited access to specialized research facilities, inadequate techniques, and the minute size of the organisms (Broady 1996; Vettorato et al. 2010). Many diatom species exhibit dimensions less than 20 μm with taxonomic features often below the resolving power of the light microscope. A floristic and quantitative analysis of these communities needs scanning electron microscopy (SEM) as a routine approach, allowing for investigating epiphytic diatom microalgae in undisturbed and almost unchanged conditions.

The first studies on the phytobenthos of the Ross Sea were carried out at the beginning of the twentieth century by Barton (1902), Foslie (1905, 1907), and Gepp and Gepp (1905, 1907). According to the recent literature, the Ross Sea macroalgal flora consists of about 40 taxa, which is equivalent to about 30 % of the Antarctic macroalgal flora (Cormaci et al. 2000). The benthic flora of Terra Nova Bay seems to be rather poor (among the macroalgae inhabiting coastal waters, nine taxa of Rhodophyceae, four of Fucophyceae and four of Chlorophyceae were recorded by the end of the last century), although it appears to be more diverse than that known from adjacent regions (Cormaci et al. 2000).

In the present paper, we report on the diatom communities associated with three Antarctic red macroalgal species: *Iridaea cordata* (Turner) Bory, *Phyllophora antarctica* Gepp & Gepp, and *Plocamium cartilagineum* (Linnaeus) Dixon, that are known to be very common in the Ross Sea (Gambi et al. 1994; Cormaci et al. 2000).

Growth form analysis is essential to better evaluate the function of diatoms with regard to their tolerance to grazing pressure and accessibility for herbivores. A few preliminary data on diatom genera from the region, including taxa associated with macroalgae, were reported by Gambi and Mazzella (1991) and Gambi et al. (1994). Other observations on diatoms associated with sponges at Terra Nova Bay are available in Cattaneo-Vietti et al. (2000). However, to our knowledge, our study is the first detailed SEM-based analysis of marine epiphytic diatoms from Terra Nova Bay (Ross Sea)—and for the whole Antarctic region in general.

Materials and methods

For the purpose of this study, thalli of three Antarctic macroalgal species *Iridaea cordata* (Gigartinales, Gigartinaceae), *Phyllophora antarctica* (Gigartinales, Phyllophoraceae), and *Plocamium cartilagineum* (Plocamiales, Plocamiaceae) were collected during the austral summer of 1989/90 (Gambi and Mazzella 1991; Gambi et al. 1994), 1993/94, 1994/95 (Gambi et al. 2000), 1997/98, 1999/00, 2000/01, 2002/03, and 2003/04. The macroalgal species used in this study are the most abundant seaweeds in the area, covering locally the hard bottoms of the Bay in great densities (Cormaci et al. 2000). They are characterized by different depth distributions and different morphological features (Gambi et al. 1994). While broad bladed *Iridaea cordata* generally dominates shallower parts of the coast (from the lower limit of fast ice formation to about 10–15 m water depth), foliose, branched *Phyllophora antarctica* is a dominant species in waters deeper than 10 m, co-occurring here with highly branched, much narrower, smaller, and less frequent *Plocamium cartilagineum* (Cormaci et al. 2000), often found to grow epiphytically on *Phyllophora* thalli (Majewska, personal observations). Material was sampled at various sites in the vicinity of the main Italian Antarctic research station, "Mario Zucchelli," located at Terra Nova Bay (74°41′S, 164°07′E). The five sampling sites localized along the coast, from Tethys Bay to Cape Russell (Fig. 1, Table 1), were selected based on their relatively easy accessibility and for the different coastal features they represent. We were aiming to find an exposed coast (Faraglione and Cape Russell), a rather sheltered area (Molo), a site covered by fast ice often throughout the entire summer period (Tethys Bay), and a site possibly influenced by a nearby penguin rookery (Adelie Cove). Sampling was performed by SCUBA diving. In most cases, the sampling procedure consisted of haphazardly positioning a quadrat (40 × 40 cm) on the bottom and scraping the algae and associated organisms off the rocky substrate with a knife (quantitative samples,

Gambi et al. 1994). The material was fixed immediately in 4 % formaldehyde solution in sea water. All samples considered here were collected between depths of 0.5 and 17 m (Table 1).

SEM observations

To observe diatom communities originally settled on the host macroalgae, three replicates of each macroalgal thalli were cut, dehydrated by immersion in alcohol solution at increasing gradation, and treated in a Critical Point Dryer. After the drying procedure, the macroalgal pieces were placed on a stub and sputtered with a thin layer of gold.

Diatom cells were identified to the lowest taxonomic level possible (species or genus) and counted at a magnification of 400× (or higher, if necessary) on the surface of at least 1 mm^2 of each replicate using a JEOL JSM 60/60 LW scanning electron microscope. Each species identified was then assigned to one of the groups according to its growth form as follows: erect (forms attached to the substrate by mucilage stalks, pads, or peduncles), adnate (forms growing with the valve face adherent to the substrate and having a limited motility), motile (biraphid diatoms endowed with the ability to move), tube-dwelling (raphid forms living in mucilage tubes produced by themselves), planktonic (settled true plankton species), and plocon (cells loosely associated with substrate surface) (terminology by Round 1981).

Statistical analyses

Diatom community structure and abundance differences among the studied samples were tested by analysis of variance (ANOVA) with Statistica v.5.5 software (StatSoft, Tulsa, OK, USA). A Tukey's pairwise comparison test was run when the differences for the main effect were significant ($p < 0.05$).

Results

Well developed, complex epiphyte communities were observed on almost every macroalgal thallus examined (Figs. 2, 3). A total of 73 diatom taxa were recorded in the counts. However, in all cases, individuals of the 20 most abundant species (which exceeded the level of 3 % of relative abundance in at least one sample) contributed from 87.2 to 100 % of the diatom community (Table 2). Most of the surface of some macroalgal thalli (especially blades of *Phyllophora antarctica*) was densely covered by epiphytic sessile fauna (mainly calcareous bryozoans and hydroids; Rosso and Sanfilippo 2000) (Fig. 3).

On average, motile diatoms were the most abundant fraction of the diatom communities found on macroalgal thalli (51 %; from 6 to 77 %), as well as on the surface of the sessile fauna epiphytic on seaweeds (52 %; from 13 to 89 %). Adnate forms represented 36 % (from 6 to 86 %) of

Fig. 1 Location of sampling sites (*arrows*): *1* Cape Russell; *2* Adelie Cove; *3* Faraglione; *4* Molo; *5* Tethys Bay. The *black triangle* indicates Italian base location; the *star* indicates location of Adelie penguin rookery
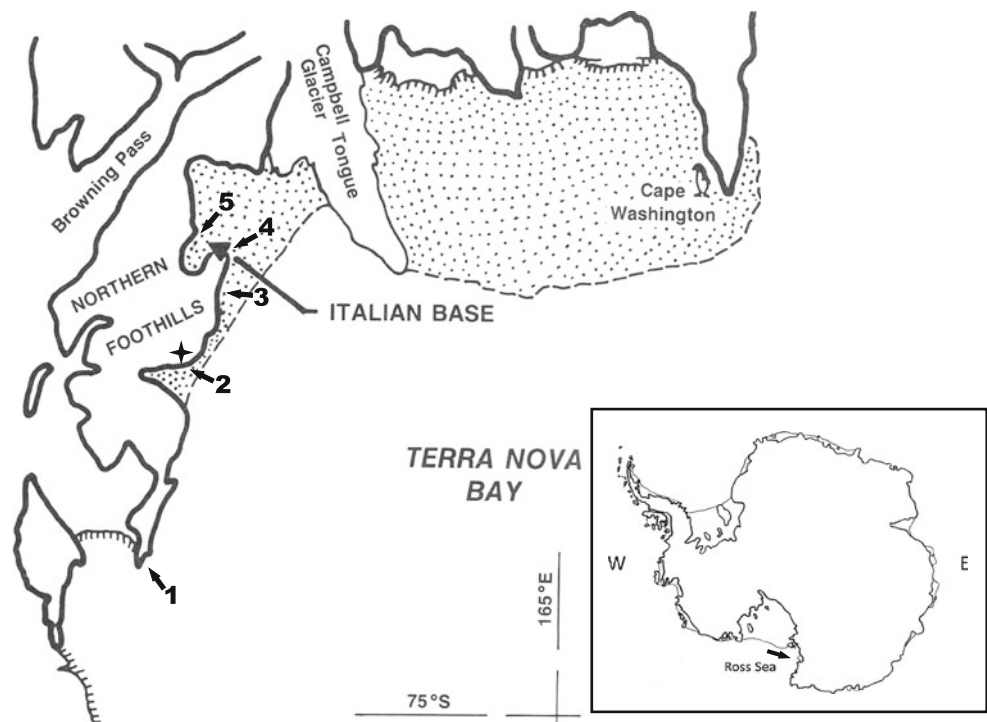
Table 1 List of samples and total diatom abundance values

Sampling station	Macroalgal host	Depth (m)	Sampling date	Total diatom abundance (cells mm^{-2}) $\pm$ SD	Total diatom abundance (cells mm^{-2}) $\pm$ SD (epizootic diatoms)
Cape Russell	*I. cordata*	17	Feb 2004	1,101 $\pm$ 86	
Cape Russell	*P. antarctica*	17	Feb 2004	322 $\pm$ 127	701 $\pm$ 345
Cape Russell	*P. cartilagineum*	15	Feb 2004	471 $\pm$ 424	494 $\pm$ 435
Adelie Cove	*I. cordata*	8	Feb 1990	1,267 $\pm$ 622	3,073 $\pm$ 461
Adelie Cove	*P. antarctica*	8	Feb 1990	2,562 $\pm$ 1,098	5,665 $\pm$ 742
Adelie Cove	*P. cartilagineum*	8	Feb 1990	974 $\pm$ 516	2,215 $\pm$ 379
Adelie Cove	*I. cordata*	0.5	Jan 1994	551 $\pm$ 274	
Adelie Cove	*P. cartilagineum*	0.5	Jan 1994	2,005 $\pm$ 211	
Faraglione	*I. cordata*	2	Jan 1990	670 $\pm$ 110	1,457 $\pm$ 219
Faraglione	*P. antarctica*	12	Jan 1990	867 $\pm$ 342	3,428 $\pm$ 962
Faraglione	*P. antarctica*	16	Jan 1994	348 $\pm$ 176	760 $\pm$ 141
Faraglione	*I. cordata*	3	Jan 1995	619 $\pm$ 947	
Faraglione	*I. cordata*	5	Jan 1998	591 $\pm$ 277	
Faraglione	*P. antarctica*	5	Jan 1998	1,724 $\pm$ 442	4,836 $\pm$ 725
Faraglione	*P. antarctica*	15	Jan 1998	1,120 $\pm$ 434	987 $\pm$ 148
Faraglione	*I. cordata*	5	Feb 2000	6,718 $\pm$ 2,470	
Faraglione	*P. antarctica*	15	Feb 2000	460 $\pm$ 163	1,299 $\pm$ 370
Faraglione	*I. cordata*	6	Feb 2001	116 $\pm$ 62	
Faraglione	*I. cordata*	8	Feb 2001	108 $\pm$ 107	
Faraglione	*P. antarctica*	8	Feb 2001	918 $\pm$ 348	1,641 $\pm$ 715
Faraglione	*P. antarctica*	13	Feb 2001	4,089 $\pm$ 1,713	8,050 $\pm$ 4,825
Faraglione	*P. antarctica*	15	Jan 2001	1,615 $\pm$ 1,228	3,062 $\pm$ 2,555
Molo	*I. cordata*	5	Feb 1994	667 $\pm$ 268	1,253 $\pm$ 188
Molo	*I. cordata*	5	Feb 1998	576 $\pm$ 229	
Molo	*I. cordata*	5	Jan 2000	404 $\pm$ 406	
Molo	*I. cordata*	6	Feb 2001	39 $\pm$ 7	
Molo	*P. antarctica*	6	Feb 2001	959 $\pm$ 152	3,364 $\pm$ 1,712
Molo	*P. antarctica*	8	Feb 2001	830 $\pm$ 225	1,207 $\pm$ 926
Molo	*P. antarctica*	12	Jan 2001	264 $\pm$ 106	572 $\pm$ 237
Molo	*P. antarctica*	15	Feb 2001	733 $\pm$ 313	1,359 $\pm$ 1,087
Molo	*P. antarctica*	12	Dec 2002	725 $\pm$ 215	464 $\pm$ 379
Molo	*P. antarctica*	17	Dec 2002	476 $\pm$ 345	
Tethys Bay	*P. antarctica*	6	Jan 1990	452 $\pm$ 191	2,936 $\pm$ 1,524
Tethys Bay	*I. cordata*	7	Dec 2002	21 $\pm$ 13	
Tethys Bay	*P. antarctica*	7	Dec 2002	550 $\pm$ 221	649 $\pm$ 97
Tethys Bay	*P. antarctica*	10.5	Dec 2002	887 $\pm$ 473	1,251 $\pm$ 767
Tethys Bay	*P. antarctica*	13.5	Dec 2002	424 $\pm$ 452	1,566 $\pm$ 399

the total diatom abundance on macroalgae, but only 10 % (from 2 to 40 %) on epiphytic sessile fauna, whereas planktonic diatom abundance was significantly greater on the latter: 25 % (from 2 to 57 %) in comparison with 7 % (from 0 to 30 %) on seaweed blades. Erect forms (on average 4 and 6 %), tube-dwelling forms (1 and 2 %), and plocon (1 and 5 %, on macroalgal thalli and animal component, respectively) were less abundant (Figs. 4, 5, 6, 7, 8).

Epiphytic diatom abundance values were highly variable between the months of sampling, collection sites as well as with water depth at each site, ranging from 21 $\pm$ 13 cells mm^{-2} on *Iridaea cordata* thalli collected from Tethys Bay in December 2002 to 6,718 $\pm$ 2,470 cells mm^{-2} on blades of the same macroalgal species collected from the Faraglione sampling site in February 2000. However, even higher values were noted for the animal component as a substratum, with a maximum of 8,050 $\pm$ 4,825 cells mm^{-2} on the sessile fauna associated with *Phyllophora antarctica* found at Faraglione in February 2001 (Table 1).
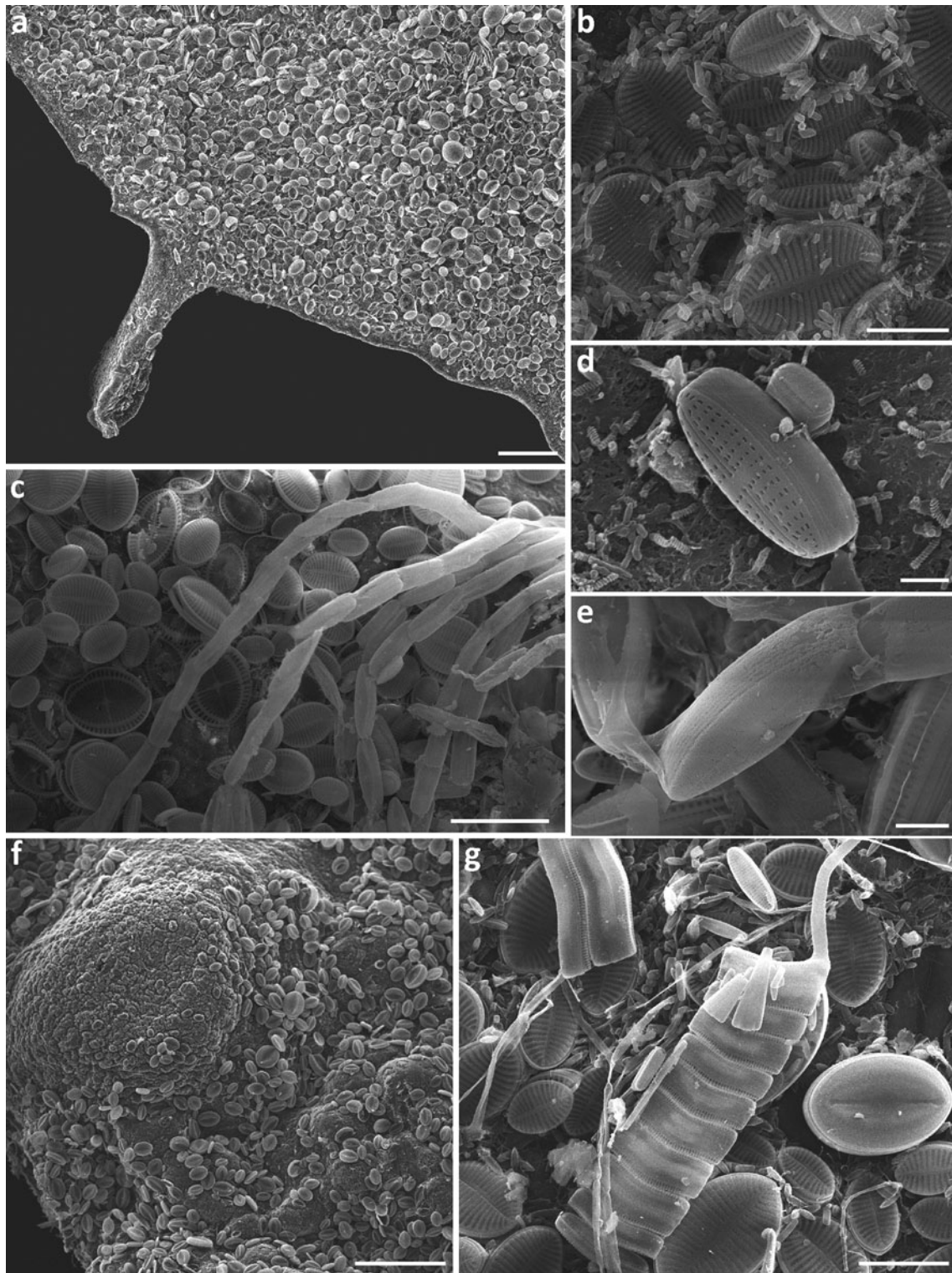
Fig. 2 Epiphytic diatom communities associated with *Phyllophora antarctica* (**a**–**e**) and *Iridaea cordata* (**f**–**g**). **a** *P. antarctica* thallus with associated microflora; **b** *Cocconeis fasciolata* and *Navicula perminuta*; **c** chains of tube-dwelling *Parlibellus* cf. *crucicula*; **d** *Navicula perminuta* and *Fragilariopsis nana*; **e** *Parlibellus* cf. *crucicula*; **f** *I. cordata* thallus with associated microflora (*Cocconeis schuettii*); **g** chain of *Achnanthes brevipes* attached to the substrate through mucilage peduncle. *Scale bars* (μm): 2 **d**; 10 **e**; 50 **b**, **g**; 100 **c**; 200 **a**; 500 **f**
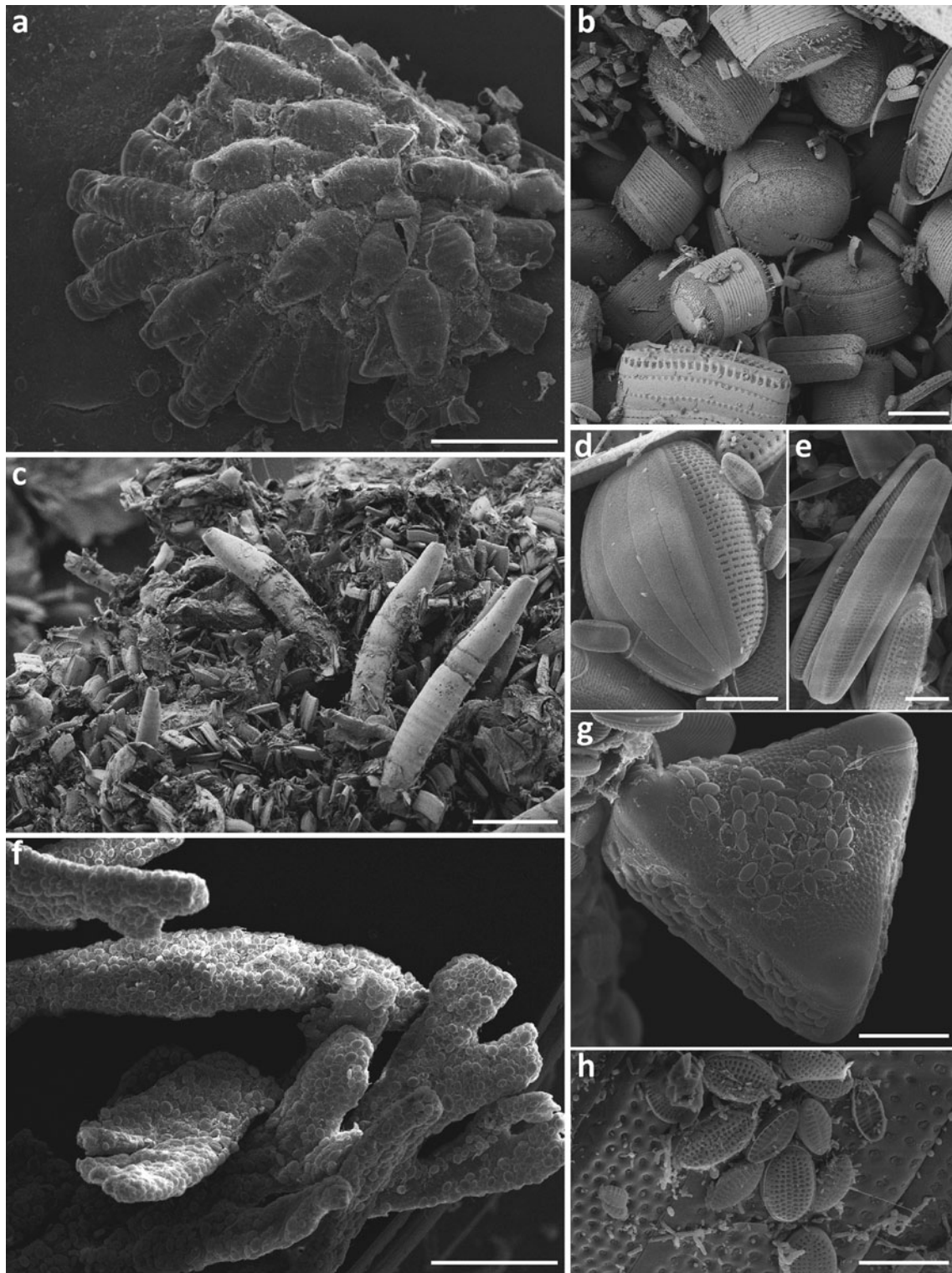
Fig. 3 Epibiotic diatoms. **a**, **c** epiphytic microfauna and its associated (epizootic) diatoms; **b** *Melosira adeliae*, *Achnanthes brevipes*, *Fragilariopsis* spp., *Achnanthes vincentii*, etc.; **d** adnate *Amphora racovitzae*; **e** motile *Trachyneis aspera*; **f** *Plocamium cartilagineum* thallus covered by diatoms; **g**, **h** *Cocconeis* cf. *californica* and *Achnanthes vincentii* epiphytic on *Trigonium arcticum. Scale bars* (μm): 10 **b**, **d**, **e**, **h**; 50 **g**; 100 **c**; 500 **a**, **f**

Table 2 List of the most abundant diatom taxa recorded during the study period

Achnanthes brevipes Agardh	*Fragilariopsis nana* (Steemann Nielsen) Paasche
Achnanthes vincentii Manguin	*Melosira adeliae* Manguin
Amphora sp.	*Navicula* cf. *incertata* Lange-Bertalot & Krammer
Cocconeis costata Greg. var. *antarctica* Manguin	*Navicula glaciei* Van Heurck
Cocconeis fasciolata (Ehrenberg) Brown	*Navicula jejunoides* Van Heurck
Cocconeis cf. *californica* Grunow	*Navicula perminuta* Grunow
Cocconeis schuettii Van Heurck	*Parlibellus* cf. *crucicula* (W Smith) Witkowski et al.
Cocconeis sp.	*Pseudogomphonema kamtschaticum* (Grunow) Medlin
Fragilaria sp.	*Synedropsis recta* Hasle, Syvertsen & Medlin
Fragilariopsis curta (Van Heurck) Hustedt	*Trigonium arcticum* (Brightwell) Cleve
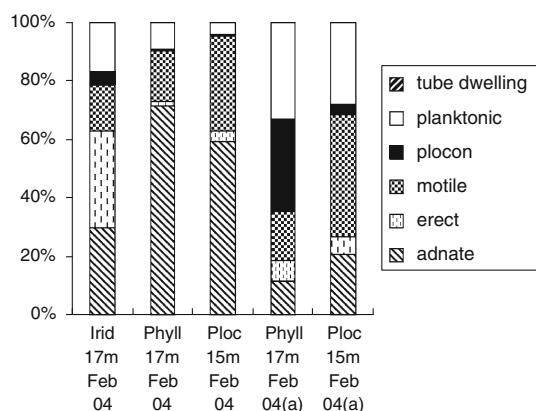
Fig. 4 Growth form percent contribution to total diatom abundance (Cape Russell). *a* indicates samples of diatom flora associated with animal component found epiphytically on macroalgae
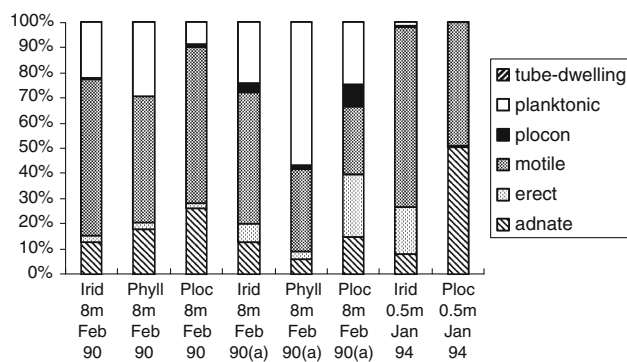
Fig. 5 Growth form percent contribution to total diatom abundance (Adelie Cove). *a* indicates samples of diatom flora associated with animal component found epiphytically on macroalgae

Distributional patterns

Table 3 presents the mean abundance values calculated for each host organism type. Comparing diatom cell density among the 3 macroalgal species and the biotic substratum represented by sessile epiphytic fauna, the abundance

values noted for the latter ($2,226 \pm 2,414$ cells mm^{-2}) were significantly higher than those calculated for *Iridaea cordata* ($961 \pm 1,698$ cells mm^{-2}, $p < 0.01$), *Phyllophora antarctica* (991 ± 916 cells mm^{-2}, $p < 0.01$), and *Plocamium cartilagineum* ($1,150 \pm 782$ cells mm^{-2}, $p < 0.01$).

In terms of growth form, adnate diatoms, mainly belonging to the genera *Cocconeis* (*C. fasciolata*, *C. schuettii*, *C. costata* var. *antarctica*, *C.* cf. *californica,* and other *Cocconeis* spp.), were remarkably more abundant on *P. antarctica* (438 ± 208 cells mm^{-2}, $p < 0.01$) than on *I. cordata* (223 ± 423 cells mm^{-2}, $p < 0.01$) or on the sessile epiphytic fauna (232 ± 251 cells mm^{-2}, $p < 0.01$). Individuals of *Achnanthes vincentii* and the genus *Amphora* were more frequent among the epizootic adnate diatom fraction.

Erect forms were significantly more abundant on the sessile fauna surface than on any of the macroalgal thalli ($p < 0.0001$, Table 2). Here, the most important were *Achnanthes brevipes*, *Pseudogomphonema kamtschaticum*, and *Synedropsis recta*. Some settled planktonic species were found in the benthic diatom community: *Fragilariopsis curta* and small cells of *Fragilariopsis nana* were very common being observed in every epizootic sample. Other *Fragilariopsis* species were less frequent. Centric diatoms (e.g., *Chaetoceros* spp.) as well as *Thalassiothrix* spp. were observed occasionally and at very low densities. Generally, planktonic forms were markedly more abundant on the surface of the host animal than on any of the macroalgal thalli ($p < 0.0001$). A similar distribution was observed for the plocon forms (*Melosira adeliae*, *Trigonium arcticum*, and less frequent *Odontella litigiosa*, *Paralia sol*, *Podosira* sp.) ($p < 0.01$) and tube-dwelling fraction (*Parlibellus* cf. *crucicula*) ($p < 0.01$). On average, the abundance values of motile forms were also higher in the sessile fauna samples compared with the macroalgal ones. However, according to the results of the ANOVA test, no significant differences were found (Table 3). Motile diatoms mainly belonged to the genus *Navicula*.
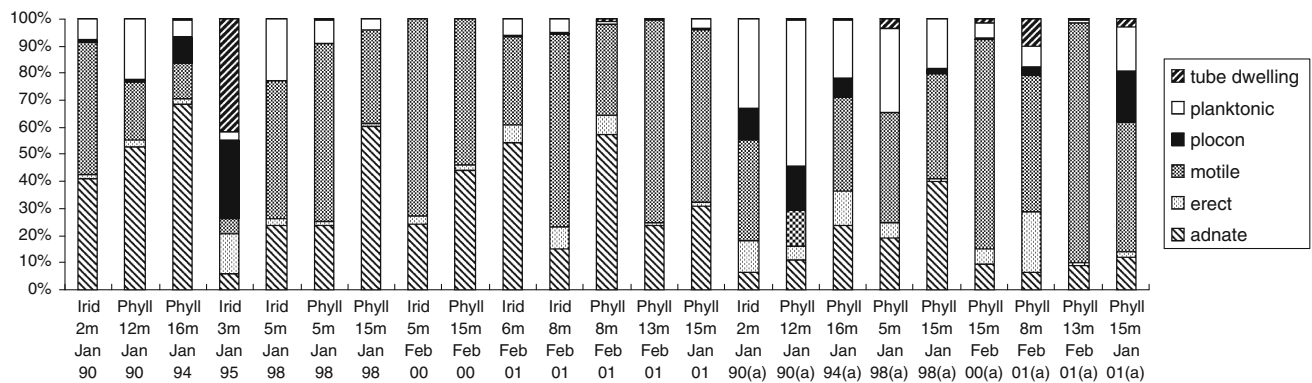
Fig. 6 Growth form percent contribution to total diatom abundance (Faraglione). *a* indicates samples of diatom flora associated with animal component found epiphytically on macroalgae

Fig. 7 Growth form percent contribution to total diatom abundance (Molo). *a* indicates samples of diatom flora associated with animal component found epiphytically on macroalgae
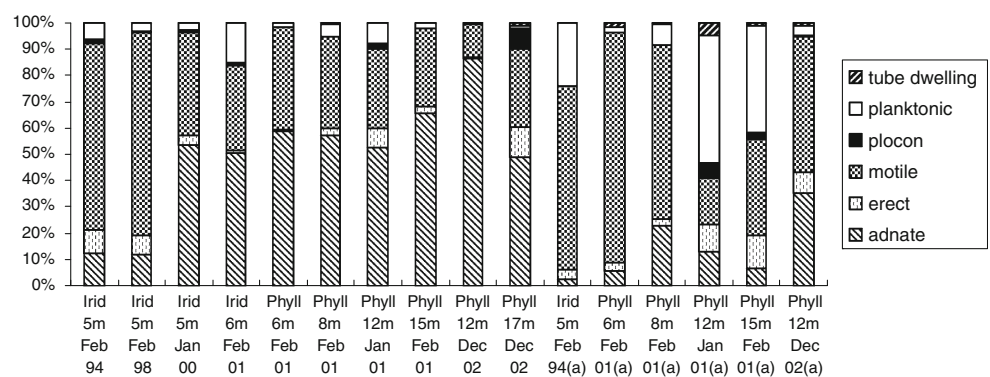
Fig. 8 Growth form percent contribution to total diatom abundance (Tethys Bay). *a* indicates samples of diatom flora associated with animal component found epiphytically on macroalgae
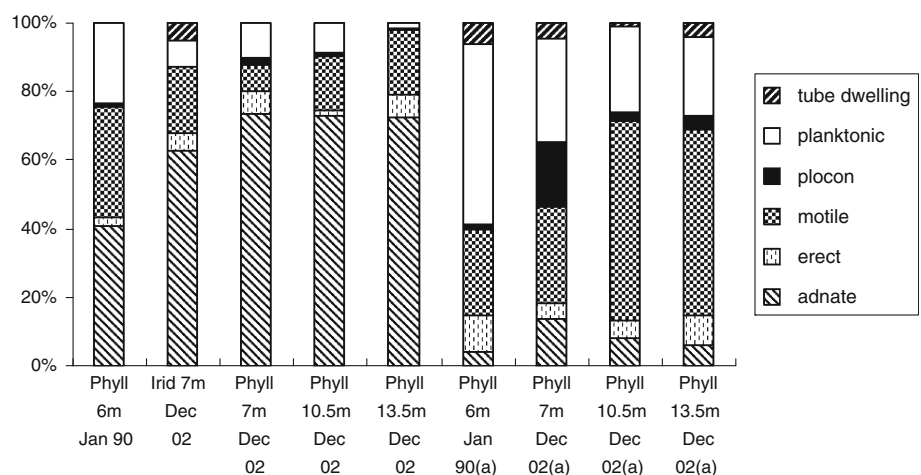
Several species were distinguished, but the most important was the small-celled *Navicula perminuta* present in great abundance in every sample regardless of the host type and species. *Trachyneis aspera* and *Nitzschia* species were less abundant and more frequent as part of the epizootic communities.

The composition of the diatom communities associated with epiphytic fauna found on examined macroalgae differed markedly from that growing directly on the macroalgal surface; therefore, the animal component has been

removed in the following statistical analyses in order to avoid a misinterpretation.

Mean abundance values for each depth range are shown in Table 4. Comparing the results, significantly higher values of total diatom number were noted for the shallowest stations (0–5 m; $1{,}438 \pm 1{,}970$ cells mm^{-2}) and lower for the deepest ones (below 15 m; 562 ± 376 cells mm^{-2}, $p < 0.05$). A similar pattern was observed for motile diatoms ($904 \pm 1{,}423$ cells mm^{-2} and 103 ± 89 cells mm^{-2} with $p < 0.05$, for the shallowest and deepest

Table 3 Mean diatom abundance (cells mm^{-2}) $\pm$ SD calculated for each host type and results of the ANOVA and Tukey's tests

	I. cordata (1)	*P. antarctica* (2)	*P. cartilagineum* (3)	Animal component (4)	ANOVA *p*-level	Tukey's test (*p* < 0.05)
Adnate	223 ± 423	438 ± 208	516 ± 429	232 ± 251	<0.0001	(1), (4) < (2)
Erect	68 ± 102	23 ± 18	15 ± 2	139 ± 161	<0.0001	(1), (2), (3) < (4)
Motile	576 ± 1,250	466 ± 714	579 ± 414	1,152 ± 2,108	ns	
Planktonic	56 ± 85	81 ± 141	35 ± 46	551 ± 810	<0.0001	(1), (2), (3) < (4)
Plocon	19 ± 48	7 ± 11	5 ± 5	112 ± 264	<0.01	(1), (2), (3) < (4)
Tube dwelling	19 ± 69	2 ± 3	0	39 ± 70	<0.01	(2), (3) < (4)
Total	961 ± 1,698	991 ± 916	1,150 ± 782	2,226 ± 2,414	<0.0001	(1), (2), (3) < (4)

sampling sites, respectively), while erect forms were most numerous below 15 m water depth ($p < 0.01$). Mean tube-dwelling diatom abundance was calculated to be highest in the shallowest zone (26 ± 137 cells mm^{-2}) in comparison with other depths (1 ± 3 cells mm^{-2} at 6–10 m, 0 ± 1 cells mm^{-2} at 11–15 m, and 2 ± 3 cells mm^{-2} below 15 m). Other growth forms were distributed in a relatively uniform fashion.

Statistical analyses indicated distinct differences among sampling stations in terms of diatom abundance. Mean total diatom number was greatest on the macroalgal samples collected from Faraglione (1,426 ± 1,944 cells mm^{-2}) and Adelie Cove (1,394 ± 846 cells mm^{-2}), and smallest on those collected from Tethys Bay (467 ± 395 cells mm^{-2}) and Molo (581 ± 348 cells mm^{-2}). However, not all of these differences were statistically important (see Table 5).

Markedly higher mean erect form number was characteristic of the communities found at Cape Russell, while settled planktonic cells were more abundant on the thalli from Adelie Cove. Notwithstanding, special attention must be paid here to the fact that site-depth-host co-variability is likely and difficult to tease apart accurately.

Temporal analysis

Although collection was made in different months of different austral summers, thus with slightly different meteorological and local sea ice development conditions, a temporal analysis was attempted to highlight the possible general trends. Mean diatom abundance values were calculated for each month of sampling. On average, total cell abundance was lowest in December (514 ± 391 cells mm^{-2}) and highest in February (1,206 ± 1,808 cells mm^{-2}), with some intermediate values characteristic of January (949 ± 782 cells mm^{-2}, $p < 0.05$). Motile diatom abundance showed a similar trend: highest values were noted for samples collected in February (723 ± 1,371 cells mm^{-2}) and lowest for those gathered in December (83 ± 78 cells mm^{-2}, $p < 0.05$), while mean planktonic diatom abundance was highest in January (96 ± 161 cells mm^{-2}) and, again, lowest in December (24 ± 41 cells mm^{-2}, $p < 0.05$) (Table 6).

Discussion

This study is the first one presenting the epiphytic Antarctic diatom growth form analysis. This is also the first description of the epiphytic diatom flora of Terra Nova Bay. As the shallow waters adjacent to Antarctic shorelines are known to be one of the most harsh and unstable marine habitats worldwide, with various, often undetected, factors affecting living organisms (Ahn et al. 1997), any conclusion must be drawn with caution. With this investigation, we report some general trends that could be found and highlight the importance of an adequate methodology for

Table 4 Mean diatom abundance (cells mm^{-2}) $\pm$ SD calculated for each sampling depth range and results of the ANOVA and Tukey's tests

	0–5 m (1)	6–10 m (2)	11–15 m (3)	>15 m (4)	ANOVA *p*-level	Tukey's test (*p* < 0.05)
Adnate	385 ± 595	257 ± 229	482 ± 310	258 ± 117	ns	
Erect	57 ± 75	21 ± 27	18 ± 18	108 ± 205	<0.01	(2), (3) < (4)
Motile	904 ± 1,423	303 ± 411	515 ± 998	103 ± 89	<0.05	(4) < (1)
Planktonic	44 ± 62	96 ± 197	40 ± 60	60 ± 123	ns	
Plocon	21 ± 96	3 ± 6	4 ± 7	31 ± 37	ns	
Tube dwelling	26 ± 137	1 ± 3	0 ± 1	2 ± 3	ns	
Total	1,438 ± 1,970	681 ± 700	1,060 ± 1,194	562 ± 376	<0.05	(4) < (1)

Table 5 Mean diatom abundance (cells mm^{-2}) ± SD calculated for each sampling station and results of the ANOVA and Tukey's tests

	Cape Russell (1)	Adelie Cove (2)	Faraglione (3)	Molo (4)	Tethys Bay (5)	ANOVA p-level	Tukey's test ($p < 0.05$)
Adnate	281 ± 147	382 ± 378	442 ± 512	286 ± 250	312 ± 317	ns	
Erect	128 ± 236	44 ± 44	38 ± 66	24 ± 24	18 ± 24	<0.05	(3), (4), (5) < (1)
Motile	127 ± 108	775 ± 463	857 ± 1,412	245 ± 219	82 ± 87	ns	
Planktonic	77 ± 139	189 ± 288	51 ± 70	18 ± 19	49 ± 58	<0.001	(3), (4), (5) < (2)
Plocon	19 ± 29	4 ± 7	17 ± 83	7 ± 15	6 ± 9	ns	
Tube dwelling	0	0 ± 1	20 ± 101	1 ± 2	1 ± 1	ns	
Total	631 ± 423	1,394 ± 846	1,426 ± 1,944	581 ± 348	467 ± 395	<0.05	(4) < (3)

Table 6 Mean diatom abundance (cells mm^{-2}) ± SD calculated for each sampling month and results of the ANOVA and Tukey's tests

	Dec (1)	Jan (2)	Feb (3)	ANOVA p-level	Tukey's test ($p < 0.05$)
Adnate	372 ± 315	334 ± 292	378 ± 483	ns	
Erect	23 ± 29	29 ± 45	55 ± 115	ns	
Motile	83 ± 78	453 ± 490	723 ± 1,371	<0.05	(1) < (3)
Planktonic	24 ± 41	96 ± 161	43 ± 101	<0.05	(1) < (2)
Plocon	10 ± 12	18 ± 80	6 ± 15	ns	
Tube dwelling	1 ± 2	19 ± 93	1 ± 3	ns	
Total	514 ± 391	949 ± 782	1,206 ± 1,808	<0.05	(1) < (3)

the understanding of the ecological relationships between epiphytic diatoms, their algal host, and other components of the epiphytic community.

The examined thalli of three red macroalgal species were dominated by motile diatoms, followed by adnate forms. Erect, tube-dwelling, and planktonic diatoms were markedly less frequent.

Host species influence

In terms of growth form, no significant differences between the diatom communities associated with different macro-algal species were observed. The epiphytic diatom communities growing on macroalgal blades were often very uniform and homogenous. Although community complexity and diatom growth form diversity, as well as total diatom number, seemed to increase significantly in the vicinity and on the surface of sessile animals associated with their macroalgal host, a higher number of erect and tube-dwelling forms suggested that epizootic communities were in a more advanced stage of development in comparison with those growing on macroalgae (Romagnoli et al. 2006). The sessile fauna associated with macroalgae at Terra Nova Bay is mainly represented by bryozoans and serpulid polychaetes (Rosso 1991; Rosso and Sanfilippo 2000), and it is particularly abundant on the *Phyllophora* ramified thalli. According to Round (1981), sessile

invertebrates may provide a substratum conducive to the diatom community development. This is because of the weaker grazing pressure that characterizes the surfaces of the living sessile animals. Another hypothesis suggests that microalgae may benefit from the host metabolic products. In all probability, also the specific morphology and surface roughness of the animal substratum are not without importance. Thomas and Jiang (1986) in their studies on epiphytic diatoms from the vicinity of Davis Station observed that filamentous forms as well as any other topographic anomalies favoured the increase in diatom community diversity, providing a point of attachment for the higher number of diatom taxa. Our study supports this concept. We hypothesize that a vast array of structures present on the animal surface may increase the number of available microniches, where competition from other microorganisms is limited, and offer a shelter for a wider range of microalgal forms. Planktonic species, especially the small-celled ones, seemed simply to be trapped in the structural depressions and cavities of the animal surface.

Sampling site influence

According to our results, the sampling site properties seemed to affect the diatom community growth form structure more than the substratum type provided by different host macroalgal species. It is worth to noting that a

similar conclusion was drawn previously by the authors mentioned above, as well as by Snoeijs (1994), who concluded that season and salinity gradient were affecting the diatom communities epiphytic on macroalgae from the Baltic Sea to a higher degree than the substratum type. On the other hand, Al-Handal and Wulff (2008) found the distribution of epiphytic marine diatoms on different macroalgal species collected from Potter Cove to be highly variable and indicated the substratum type as the most important factor affecting the diatom community structure. Nevertheless, the latter authors in their study examined thalli of 19 macroalgal species, and it is probable that among them distinctively different morphological forms were found. Moreover, due to different techniques of samples' preparation and observation used during the study on epiphytic diatoms from Potter Cove, a direct comparison with our results might be misleading.

It seemed that the sampling site with its local hydrographic conditions and specific bio-physicochemical characteristics was affecting the diatom cell densities as well as community structure. The lowest values of mean total diatom abundance characterized Tethys Bay and Molo sampling sites located to the north of the "Zucchelli" Station, in the area, strongly subjected to the disruptive effects of fast ice and where ice breaks out later in the summer. The highest values were noted for Faraglione and Adelie Cove stations. High diatom densities recorded for the latter might be influenced by the nearby Adelie penguin (*Pygoscelis adeliae*) rookery and the higher biogenic substance inflow carried from the land to the adjacent waters (Gambi et al. 2000). It is interesting to note that during the study on planktonic microalgae from Terra Nova Bay conducted in 1990, among the 8 sampling stations (inshore and offshore), "station 2", in our study named "Faraglione", was always characterized by the highest microalgal cell densities (Andreoli et al. 1995). However, no factor was found to be related to the higher diatom abundance at Faraglione.

Depth influence

During the study period, a relatively weak influence of the sampling depth on diatom distribution and abundance could be observed, since the diatom cell number seemed to decrease with increasing depth (possibly excluding the shallowest sites, 0.5–2 m, that might be strongly subjected to various disturbances, such as waves movement, ice scouring, or freezing and thawing processes). Despite decreasing total diatom abundance, average erect form number was highest at the deepest sites of sampling (below 15 m). The most likely explanation for this pattern could be the decrease in light availability along the depth gradient, and the erect diatoms' better response to lower

irradiance conditions that occur in deeper waters. The effect of light availability on the vertical structure of benthic diatom communities has been reported in several studies. According to the results of Hudon and Bourget (1983), the upright fan-shaped (*Synedra tabulata*) and arborescent (*Gomphonema kamtschaticum*) forms occurred frequently under various light intensities, and their densities did not decrease with increasing depth within the subsurface zone, which was the case for many other diatoms observed. Gosselain et al. (2005) in their study on epiphytic algae from the St. Lawrence River (Canada) found the light to play a key role and explain up to 50 % of the variance in epiphyte biomass, which was lower and less variable for the microalgae exposed to >13 % of surface light. The authors pointed out, however, that in dense macroalgal beds, especially close to the water surface, the available light can be reduced significantly; thus, depth may not always be a good proxy for light. It is also worth noting that in our study, deeper stations were often characterized by gentler water movement and reduced ice disturbance, which might have encouraged a more stratified epiphytic community development.

Temporal variability

Although the study period included only the three summer months, marked temporal variability could be seen. Total diatom number was increasing significantly from December to February, simultaneously with motile forms increasing dominance. Biraphid taxa that can move freely into mature algal mats appeared to be the effective competitors for light and biogenic elements (Hudon and Legendre 1987). The highest planktonic diatom contribution to the epiphytic community was observed in January, generally coinciding with the fast ice and pack ice melting period. In Antarctic waters, the ice cover disappearance that starts with the short austral summer implies high light availability and permits the rapid planktonic community development. This, together with a marginal ice zone with high concentrations of nutrients and strong stratification of the upper water column caused by pack and fast ice melting, may support local diatom blooms (El-Sayed 1971; Arrigo and McClain 1994; Cunningham and Leventer 1998). It is postulated that in Antarctic marine environments, nutrients and trace elements are rarely (or never) limiting (Warnke et al. 1973) and that the most important factors that influence the primary production are light and sea ice cover (Everitt and Thomas 1986). Here must be mentioned, however, that according to the classification used in this work, all *Fragilariopsis* species have been included into the "planktonic forms" group and represent the majority within this fraction. Species belonging to the above-mentioned genus are often described to be sea ice

associated; therefore, their increasing number on the macroalgal thalli is likely to be directly related to the gradual release of diatom cells during sea ice melting (Everitt and Thomas 1986; Garrison et al. 1987; Scott et al. 1994; Cunningham and Leventer 1998; Sutherland 2008).

Diatom communities versus anthropogenic impact

As it is commonly known, a local low-level but continuous anthropogenic contamination from the research stations in Antarctic environments does exist (Lenihan and Oliver 1995; De Moreno et al. 1997; Edwards et al. 1998; Duquesne and Riddle 2002; Thompson et al. 2003; Tin et al. 2009). There are evidences showing that organic enrichment of coastal waters and sediments has a great impact on benthic community structure and abundance (Dayton and Robilliard 1971; Crockett 1997). Conlan et al. (2004) found benthic invertebrate fauna in the vicinity of McMurdo Station (Ross Sea) sewage outfall to be a third as abundant as communities at the reference uncontaminated sites, while Dayton et al. (1986), who had studied the benthic microalgal standing stock at McMurdo Sound, reported a close correlation between benthic invertebrates and benthic microalgae densities. Potential risk related to Italian Antarctic station operation and its impact on the fauna of Terra Nova Bay was discussed by Bargagli (2005), and a range of identified effects of human activities on the Antarctic environment was quoted by Tin et al. (2009). However, no relevant data on benthic microalgal communities have been published. A general lack of references prevented us from thoroughly understanding whether, or how, the epiphytic diatom communities are affected by anthropogenic contamination. Further study is, therefore, needed to evaluate the human impact on the benthic microflora in this area.

Methodological remarks

Diatom cell number found on macroalgal thalli in some cases varied greatly between the replicates indicating the patchy diatom distribution that might be due to the herbivorous fauna grazing activity (Gambi et al. 1994, 2000) or caused by a combination of physical and biological factors (Douglas 1958). With this observation, we would like to stress the importance of adequate replication as the small viewed area of examined sample may be not representative for the mature community.

It is interesting that the small-celled *Navicula perminuta* and *Fragilariopsis nana* that were present in great abundance at every sampling site throughout the whole sampling period in the time span of 15 years (1990–2004) were not observed in other microalgal studies of the adjacent

regions. Sutherland (2008) in her studies on surface-associated diatoms from Cape Evans found 31 epiphytic diatom species associated with *Phyllophora antarctica*, mostly large-celled taxa. This might be due to the different sampling period: macroalgae collected by Sutherland were taken from under the sea ice in November, which might explain the absence or lower densities of the sea ice-associated species. A general scarcity of other comparative studies and data prevent us from reaching definitive conclusions, since it is not known whether the Antarctic diatom communities epiphytic on the same macroalgal host species, growing within the similar depth range and water body (Ross Sea), may differ so greatly. However, a question about methodological correctness might arise from the comparison of these results. Acid oxidation that is a common method of diatom sample preparation may damage the siliceous structure of the diatom cell wall and sometimes completely dissolve less silicified valves (Taylor et al. 2005). The risk of valve dissolution increases while dealing with marine diatom species as their cell walls contain less silica per biovolume unit than freshwater taxa (Conley et al. 1989). In addition, brushing or shaking epiphytic diatoms off the host plant most often dramatically disturbs the proportions of the community components and destroys all the intricate relationships between them, making the technique completely inappropriate for ecological studies (Majewska, personal observation).

To better illustrate the complex structural architecture of epiphytic diatom communities, we intentionally focused upon epiphytic diatom growth forms instead of species, proposing a new approach to study this subject. Details regarding taxonomic composition of examined communities will be provided in a separate publication.

References

Ahn I-Y, Chung H, Kang J-S, Kang S-H (1997) Diatom composition and biomass variability in nearshore waters of Maxwell Bay, Antarctica, during the 1992/1993 austral summer. Polar Biol 17:123–130

Al-Handal AY, Wulff A (2008) Marine epiphytic diatoms from the shallow sublittoral zone in Potter Cove, King George Island, Antarctica. Bot Mar 51:411–435

Andreoli C, Tolomio C, Moro I, Radice M, Moschin E, Bellato S (1995) Diatoms and dinoflagellates in Terra Nova Bay (Ross Sea—Antarctica) during austral summer 1990. Polar Biol 15:465–475

Arrigo KR, McClain CR (1994) Spring phytoplankton production in the western Ross Sea. Science 266:261–263

Bargagli R (2005) Antarctic ecosystems: environmental contamination, climate change, and human impact. Ecol Stud, vol 175. Springer, Berlin

Barton ES (1902) Algae. In: Report on the collections of Natural History made in the Antarctic regions during the voyage of the "Southern Cross". London, British Museum (Natural History)

Broady PA (1996) Diversity, distribution and dispersal of Antarctic terrestrial algae. Biodivers Conserv 5:1307–1335

Cattaneo-Vietti R, Bavestrello G, Cerrano C, Gaino E, Mazzella L, Pansini M (2000) The role of sponges in the Terra Nova Bay ecosystem. In: Faranda FM, Guglielmo L, Iancora A (eds) Ross Sea ecology. Italian Antarctic expeditions (1986–1995). Springer, Berlin, pp 539–549

Conlan KE, Kim SL, Lenihan HS, Oliver JS (2004) Benthic changes during 10 years of organic enrichment by McMurdo Station, Antarctica. Mar Pollut Bull 49:43–60

Conley DJ, Kilham SS, Theriot E (1989) Differences in silica content between marine and freshwater diatoms. Limnol Oceanogr 34(1):205–213

Cormaci M, Furnari G, Scammacca B (2000) The Macrophytobenthos of Terra Nova Bay. In: Faranda FM, Guglielmo L, Iancora A (eds) Ross Sea ecology. Italian Antarctic expeditions (1986–1995). Springer, Berlin, pp 493–502

Crockett AB (1997) Water and wastewater quality monitoring, McMurdo Station, Antarctica. Environ Monit Assess 47:39–57

Cunningham WL, Leventer A (1998) Diatom assemblages in surface sediments of the Ross Sea: relationship to present oceanographis conditions. Antarct Sci 10(2):134–146

Dayton PK, Robilliard GA (1971) Implications of pollution to the McMurdo Sound benthos. Antarct J US 6:53–56

Dayton PK, Watson D, Palmisano A, Barry JP, Oliver JS, Rivera D (1986) Distribution patterns of benthic microalgal standing stock at McMurdo Sound, Antarctica. Polar Biol 6:207–213

De Moreno JEA, Gerpe MS, Moreno VJ, Vodopivez C (1997) Heavy metals in Antarctic organisms. Polar Biol 17:131–140

Douglas B (1958) The ecology of the attached diatoms and other algae in a small stony stream. J Ecol 46(2):295–321

Duquesne S, Riddle MJ (2002) Biological monitoring of heavy metal contamination in coastal waters in the vicinity of Casey Station, Windmill Islands, East Antarctica. Polar Biol 25:206–215

Edwards DD, McFeters GA, Venkatesan MI (1998) Distribution of Clostridium perfringens and fecal sterols in benthic coastal marine environment influenced by sewage outfall from McMurdo Station, Antarctica. Appl Environ Microb 64(7):2596–2600

Ehrenberg CG (1843) Verbreitung und Einfluss des mikroskopischen Lebens in Süd- und Nord-America. Abh Königl Akad Wiss Berlin 1:291–445

El-Sayed SZ (1971) Observations on phytoplankton bloom in the Weddell Sea. In: Llano GA, Wallen IE (eds) Biology of the Antarctic Seas IV, Washington DC. Antarct Res Ser 17:301–313

Everitt DA, Thomas DP (1986) Observations of seasonal changes in diatoms at inshore localities near Davis Station, East Antarctica. Hydrobiology 139:3–12

Foslie M (1905) Den botaniske sampling. Det Kgl. Norske Vidensk. Selsk Skr Mus Aarsberet (1904):15–18

Foslie M (1907) Antarctic and subantarctic Corallinaceae. Botanik 4:1–16

Fry B, Wainright S (1991) Diatom sources of 13C-rich carbon in marine food webs. Mar Ecol Prog Ser 76(2):149–157

Gambi MC, Mazzella L (1991) Second Italian oceanographic expedition in the Ross Sea, Antarctica: preliminary report on the quantitative and functional studies of benthic communities of Terra Nova Bay. Nat Sc Comm Ant Ocean Camp 1989–90 Data Rep I:349–367

Gambi MC, Lorenti M, Russo GF, Scipione MB (1994) Benthic associations of the shallow hard bottoms off Terra Nova Bay, Ross Sea: zonation, biomass and population structure. Antarct Sci 6(4):449–462

Gambi MC, Buia MC, Mazzella L, Lorenti M, Scipione MB (2000) Spatio-temporal variability in the structure of benthic populations in a physically controlled system off Terra Nova Bay: the shallow hard bottoms. In: Faranda FM, Guiglielmo L, Ionora A (eds) Ross Sea ecology. Springer, Berlin, pp 527–538

Garrison DL, Buck KR, Fryxell GA (1987) Algal assemblages in Antarctic pack ice and in ice-edge plankton. J Phycol 23:564–572

Gepp A, Gepp ES (1905) Antarctic algae. J Bot 43:105–109

Gepp A, Gepp ES (1907) Marine algae. I. Phaeophyceae and florideae. Nat Hist 3:1–15

Gosselain V, Hudon C, Cattaneo A, Gagnon P, Planas D, Rochefort D (2005) Physical variables driving epiphytic algal biomass in a dense macrophyte bed of the St. Lawrence River (Quebec, Canada). Hydrobiology 534:11–22

Hudon C, Bourget E (1983) The effect of light on the vertical structure of epibenthic diatom communities. Bot Mar 26(7):317–330

Hudon C, Legendre P (1987) The ecological implications of growth forms in epibenthic diatoms. J Phycol 23:434–441

Lenihan HS, Oliver JS (1995) Anthropogenic and natural disturbances to marine benthic communities in Antarctica. Ecol Appl 5(2):311–326

Ligowki R (2002) Diatoms in different habitats of Admiralty Bay, Antarctica. In: John J (ed) Proceedings of the 15th international diatom symposium, Perth, Australia. ARG Gantner Verlag, Ruggell, Liechtenstein, pp 173–191

Nelson DM, Treguer P (1992) Role of silicon as a limiting nutrient to Antarctic diatoms: evidence from kinetic studies in the Ross Sea ice-edge zone. Mar Ecol Prog Ser 80(2–3):255–264

Romagnoli T, Bavestrello G, Cucchiari EM, De Stefano M, Di Camillo CG, Pennesi C, Puce S, Totti C (2006) Microalgal communities epibiontic on the marine hydroid Eudendrium racemosum in the Ligurian Sea during an annual cycle. Mar Biol 151:537–552

Rosso A (1991) Infralittoral Bryozoa associated to macroalgae from the First Italian Antarctic oceanographic expedition (Terra Nova Bay, Ross Sea). Bol Soc Biol Concept 62:179–186

Rosso A, Sanfilippo R (2000) Shallow-water bryozoans and serpuloideans from the Ross Sea (Terra Nova Bay, Antarctica). In: Faranda FM, Guiglielmo L, Ionora A (eds) Ross Sea ecology. Springer, Berlin, pp 515–526

Round FE (1981) The ecology of algae. Cambridge University Press, Cambridge

Scott P, McMinn A, Hosie G (1994) Physical parameters influencing diatom community structure in eastern Antarctic sea ice. Pol Biol 14:507–517

Snoeijs P (1994) Distribution of epiphytic diatom species composition, diversity and biomass on different macroalgal hosts along seasonal and salinity gradients in the Baltic Sea. Diatom Res 9(1):189–211

Spaulding SA, Van de Vijver B, Hodgson DA, McKnight DM, Verleyen E, Stanish L (2010) Diatoms as indicators of environmental changes in Antarctic and subantarctic freshwaters. In: Smol JP, Stoermer EF (eds) The diatoms: applications for environmental and Earth sciences. Cambridge University Press, New York, pp 267–283

Sutherland DL (2008) Surface-associated diatoms from marine habitats at Cape Evans, Antarctica, including the first record of living *Eunotogramma marginopunctatum*. Polar Biol 31:879–888

Taylor JC, de la Rey PA, van Rensburg L (2005) Recommendations for the collection, preparation and enumeration of diatoms from riverine habitats for water quality monitoring in South Africa. Afr J Aquat Sci 30(1):65–75

Thomas DP, Jiang J (1986) Epiphytic diatoms of the inshore marine area near Davis Station. Hydrobiology 140:193–198

Thompson BW, Riddle MJ, Stark JS (2003) Cost-efficient methods for marine pollution monitoring at Casey Station, East Antarctica: the choice of sieve mesh-size and taxonomic resolution. Mar Pollut Bull 46:232–243

Tin T, Fleming ZL, Hughes KA, Ainley DG, Convey P, Moreno CA, Pfeiffer S, Scott J, Snape I (2009) Review. Impacts of local human activities on the Antarctic environment. Antarct Sci 21(1):3–33

Vettorato B, Laudares-Silva R, Talgatti D, Menezes M (2010) Evaluation of the sampling methods applied to phycoperiphyton studies in the Ratones River estuary, Brazil. Acta Limnol Bras 22(3):257–266

Warnke DA, Richter J, Oppenheimer CH (1973) Characteristics of the nearshore environment off the south coast of Anvers Island, Antarctic Peninsula. Limnol Oceanogr 18:131–142

Wulff A, Iken K, Quartino ML, Al-Handal A, Wiencke C, Clayton MN (2011) Biodiversity, biogeography and zonation of marine benthic micro- and macroalgae in the Arctic and Antarctic. In: Wiencke C (ed) Biology of Polar benthic algae. Walter de Gruyter GmbH & Co. KG, Berlin, pp 23–52