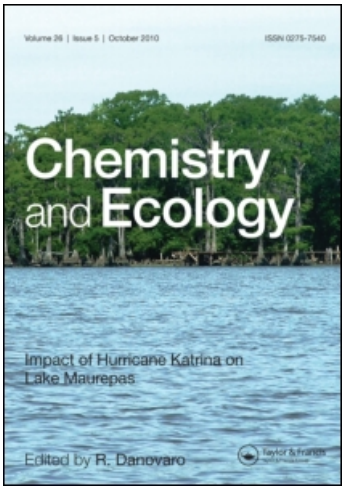

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ARE DIATOMS A FOOD SOURCE FOR ANTARCTIC SPONGES?

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Living diatoms are commonly found within Antarctic Porifera, and generally interpreted as additional food source, or as mutualists or parasites of sponge tissues. However, no data are available about temporal variations of the abundance of diatoms inside sponges especially during the winter period. In this paper we analysed the amount of diatom frustules and chlorophaeopigment concentration in six species of Antarctic sponges (*Dendrilla antarctica*, *Homaxinella flagelliformis*, *Kirkpatrickia variolosa*, *Suberites montiniger*, *Haliclona dancoi*, *Haliclona penicillata*) sampled weekly from November 2001 – before the ice melting – to February 2002. Frustule concentration in the sponge tissues was very low at the beginning of November in all the sponge species, and increased between 9 and 22 January, to reach maximum values between 29 January and 7 February. Diatom abundances were significantly higher in *H. dancoi* tissues, comparing to the other sponge species, reaching values up to 1217 ± 178 cells $*10^6\,g^{-1}$ dw at the end of January. Chlorophaeopigments, very low at the beginning of November, increased between 5 and 19 December, before the peak of diatoms. Maximum chlphaeo values ($650.5 \pm 5.9\,\mu g\,g^{-1}$ dw) were observed in *D. antarctica.* The planktonic *Fragilariopsis curta* was the most common diatom species recorded inside sponges.

These data clearly indicate that diatom concentration inside the sponge tissues is related to the summer phytoplankton bloom. The shift between the pigment and frustule peaks strongly suggests that diatoms are used as a food source by sponges and that their frustules are accumulated inside the sponge body. The lack of frustules at the beginning of summer indicates that diatom frustules are expelled or dissolved during the cold season.

Keywords: Diatoms; Sponges; Chlorophaeopigments; Endobionthic relationships; Trophic resources; Antarctica

1 INTRODUCTION

Over the last few decades several papers described the relationships between sponges and diatoms (Cox and Larkum, 1983; Gaino *et al.*, 1994; Hamilton *et al.*, 1997; Cattaneo-Vietti *et al.*, 1999; Bavestrello *et al.*, 2000; Cerrano *et al.*, 2000). A case of symbiotic association has been proposed by Cox and Larkum (1983), in which they observed a diatom species living in great abundance into a coral reef sponge from different areas, suggesting that stable associations may occur.

In Antarctic coastal waters, the evidence that large mono- or paucispecific diatom assemblages are present in the tissues of several sponges was interpreted in different ways (Gaino

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et al., 1994; Cattaneo-Vietti *et al.*, 1999). The first hypothesis considered the diatoms living inside sponge tissues as a food reserve during the austral winter (Gaino *et al.*, 1994). Later Hamilton *et al.* (1997) described *Porannulus contentus*, a diatom species living only inside the tissues of some Antarctic sponges that exhibited morpho-functional adaptation to the endosymbiotic life. Bavestrello *et al.* (2000) highlighted an inverse relationship between the chlorophyll content in sponge tissues and the amount of total sugars. This result was interpreted as a symbiosis that shifted towards a parasitism related to the mixotrophic capability of diatoms which are able to use sugars stored in sponge tissues, when light is insufficient. A typical parasitic behaviour was observed in the hexactinellid sponge *Scolymastra joubini* (Topsent, 1910) where portions of the body were invaded by monospecific assemblages of *Melosira* sp. that lived on the spicule tracts completely destroying the surrounding tissues (Cerrano *et al.*, 2000).

This bulk of data suggests a complex pattern of relationships between diatoms and Antarctic sponges, ranging from mutualism to parasitism. Nevertheless at the actual state of the art a number of important questions about such associations still remain unsolved. The seasonal dynamics of this phenomenon are not described and it is not known if diatom populations are maintained during the dark winter season inside the sponge tissues or if their presence is related to the summer phytoplankton bloom.

In this paper we analysed the trend of diatom concentration inside the tissues of some sponges species, collected during the XVII Antarctic Italian Campaign at Terra Nova (2001–2002) from November 2001 (when the pack ice is still present) to February 2002 (when the open sea starts to freeze again).

2 MATERIALS AND METHODS

Sampling was carried out by scuba diving on weekly schedule from 2 November 2001 to 7 February 2002. Sponge species (*Dendrilla antarctica* Topsent 1905, *Homaxinella flagelliformis*) (Ridley and Dendy, 1886), *Haliclona penicillata* (Topsent, 1908), *Haliclona dancoi* (Topsent, 1901), *Kirkpatrickia variolosa* (Kirkpatrick, 1907), *Suberites montiniger* (Carter, 1880) were collected at the northern cape of Tethys Bay (Terra Nova Bay, Ross Sea) between 25 and 35 m depth.

Samples for diatom determination and counting were preserved in 4% neutralised formalin, while those for chlorophaeopigment analysis were frozen in liquid nitrogen and stored at $-80\,^{\circ}C$ until the analyses. For each sponge sample, diatoms and chlorophaeopigments were measured in three replicates.

2.1 Squashing Preparation

Before the quantitative analysis of diatoms, a squashing preparation of tissues was carried out for each sponge sample. The observation of the squashed samples at the light and epifluorescence microscope indicated that diatoms represented the great majority of the autotrophic organisms into the sponge tissues, except for a minor presence of silicoflagellates (*Dictyocha speculum* Ehremberg).

2.2 Counting of Diatoms

An aliquot of sponge tissue was dried, weighted and cleaned to remove the organic component. The cleaning procedure follows von Stosch's method as outlined in Hasle and Syvertsen (1996). Sponge samples were treated by adding an equal amount of HNO_3 and $3\times$ sample amount of H_2SO_4, boiled for *ca.* 3 min, cooled and rinsed with distilled water

until free of acid. Finally, samples were adjusted to a final volume of 1 ml by adding absolute ethanol. The cleaning procedure resulted in the total digestion of the organic component and in the separation of diatom frustules in two valves. Counting of valves was performed with an inverted microscope ZEISS Axiovert 135, following the Utermöhl method (Hasle, 1978). Results were finally expressed as the number of frustules per gram of sponge tissue (dry weight).

2.3 Chlorophaeopigment Analysis

The extraction of pigments was performed by adding 10 ml of acetone 90% (v/v) to a weighted aliquot of sponge tissue (*ca.* 1 g), homogenising and leaving overnight in the dark at +4 °C. Then samples were centrifuged (2500 × 10 min) and the supernatant spectrophotometrically analysed at the wavelength of 665 and 750 nm. Chlorophaeopigment concentration ($\mu g\, g^{-1}$ was calculated using Jeffrey and Humprey (1975) coefficient for pure chlorophyll-*a*.

3 RESULTS

The list of diatom *taxa* associated to the sponges is reported in Table I. The temporal variations of diatoms and chlorophaeopigments (chlphaeo) in sponge tissues are showed in Figure 1. Owing to the cleaning procedure, the frustule concentration in the sponge tissues represents not only the living fraction but also the diatoms dead inside the sponge tissues.

In *Homaxinella flagelliformis* [Fig. 1(a)] diatoms showed a rapid increase of density between 5 and 9 January, reaching the highest value on 29 January (331 ± 14 cells $*10^6\, g^{-1}$ dw). Diatom population was generally dominated by the planktonic *Fragilariopsis curta* (95%) while centric diatoms (*Stellarima microtrias*, *Coscinodiscus* sp.) prevailed on 12 December [Fig. 2(a)]. Chlphaeo concentration showed maximum values between 19 and 28 December ($491.1 \pm 127.9\, \mu g\, g^{-1}$ dw) and decreased on 5 January, in correspondence to the increase of diatoms [Fig. 1(a)].

In *Dendrilla antarctica* tissues a first small peak in diatom frustule concentration appeared on 5 December, then values increased again from 9 January, reaching maximum levels (80 ± 36 cells $*10^6\, g^{-1}$ dw) on 7 February [Fig. 1(b)]. *Fragilariopsis curta* was the dominant species during all the season, although *Thalassiosira* spp. (*T.* cf. *gracilis* and *T. perpusilla*) increased their importance from 22 January to the end of the sampling period [Fig. 2(b)]. Chlorophaeopigment concentration showed maximum values on 19 December ($650.5 \pm 5.9\, \mu g\, g^{-1}$ dw), slightly decreasing in February [Fig. 1(b)].

Kirkpatrickia variolosa showed a first increase of frustule concentration on 5 January followed by a peak with maximum values on 29 January (109 ± 17 cells $*10^6\, g^{-1}$ dw). Chlphaeo increased on the same dates but the maximum levels ($457.1 \pm 68.9\, \mu g\, g^{-1}$ dw) were measured on 5 January [Fig. 1(c)]. *Fragilariopsis curta* strongly dominated in the diatom pool, and a certain increase of *Thalassiosira* spp. was observed at the end of January [Fig. 2(c)].

Diatom abundance in *Suberites montiniger* showed a first peak on 5 January, dominated by centric diatoms (mainly *Stellarima microtrias*), reached the maximum values on 22 January (114 ± 11 cells $*10^6\, g^{-1}$ dw) after which a decrease was observed [Fig. 1(d)]. The main peak was due to *F. curta* and the frequency of *Thalassiosira* spp. increased at the end of sampling period [Fig. 2(d)]. Chlphaeo concentration was maximum on 12 December ($257.2 \pm 4.4\, \mu g\, g^{-1}$ dw) and decreased in correspondence to the frustule increase [Fig. 1(d)].

In *Haliclona penicillata*, frustule concentration slightly increased between 12 and 28 December, while a rapid enhancement appeared from 5 January with maximum values on

TABLE I List of diatom taxa observed into the Antarctic sponges *Dendrilla antarctica*, *Homaxinella flagelliformis*, *Kirkpatrickia variolosa*, *Suberites montiniger*, *Haliclona dancoi*, *Haliclona penicillata* from November 2001 to February 2002.

Achnantes cf. *longipes* Agardh
Actinocyclus actinochilus (Ehremberg) Simonsen
Actinocyclus sp.
Amphora sp.
Asterolampra marylandica Ehremberg
Asteromphalus hookeri Ehremberg
Asteromphalus hyalinus Karsten
Asteromphalus parvulus Karsten
Asteromphalus roperianus (Greville) Ralfs in Pritchard
Cocconeis costata Gregory
Coscinodiscus oculus iridis Ehremberg
Coscinodiscus sp.
Diploneis crabro Ehremberg
Entomoneis cf. *paludosa* (W. Smith) Reimer
Entomoneis sp.
Fragilariopsis curta (Van Heurck) Hustedt
Fragilariopsis cylindrus (Grunow) Krieger
Fragilariopsis linearis (Castracane) Frenguelli
Fragilariopsis obliquecostata (Van Heurck) Heiden
Fragilariopsis rhombica (O'Meara) Hustedt
Fragilariopsis ritscheri Hustedt
Fragilariopsis sublinearis (Van Heurck) Heiden
Fragilariopsis vanheurcki (Peragallo) Hustedt
Fragilariopsis sp.
Haslea vitrea (Cleve) Simonsen
Hyalodiscus cf. *subtilis* Bailey
Licmophora sp.
Navicula sp.
Nitzschia sp.
Plagiotropis lepidoptera (Gregory) Reimer
Plagiotropis sp.
Pleurosigma sp.
Porannulus contentus Hamilton, Poulin, Yang and Klöser
Porosira pseudodenticulata (Hustedt) Jousé
Stauroneis sp.
Stellarima microtrias (Ehremberg) Hasle and Sims
Thalassiosira cf. *gracilis* (Karsten) Hustedt
Thalassiosira gravida Cleve
Thalassiosira perpusilla Kozlova
Trachineis aspera (Ehremberg) Cleve
Trachyneis sp.
Tricotoxon rehimboldti (Van Heurck) Reid and Round
Undetermined centric diatoms
Undetermined pennate diatoms

7 February (184 ± 29 cells $*10^6\,g^{-1}$ dw). Chlorophaeopigments were highest on 28 December ($330.7 \pm 32.1\,\mu g\,g^{-1}$ dw), then values decreased [Fig. 1(e)]. *Fragilariopsis curta* was the most abundant diatom in all the sampling period (72.4% on 7 February), except on 28 December when *Plagiotropis* sp. dominated. The latter reached high percent values between 19 December and 5 January. *Thalassiosira* spp. increased at the end of sampling period [Fig. 2(e)].

Frustule abundance in *Haliclona dancoi*, very low from the beginning of November to the end of December, increased from 9 January to reach maximum values (1217 ± 178 cells $*10^6\,g^{-1}$ dw) on 29 January. The highest concentrations of chlphaeo ($328.9 \pm 16.9\,\mu g\,g^{-1}$ dw) were measured on 19 December and decreased in correspondence to the peak of diatoms

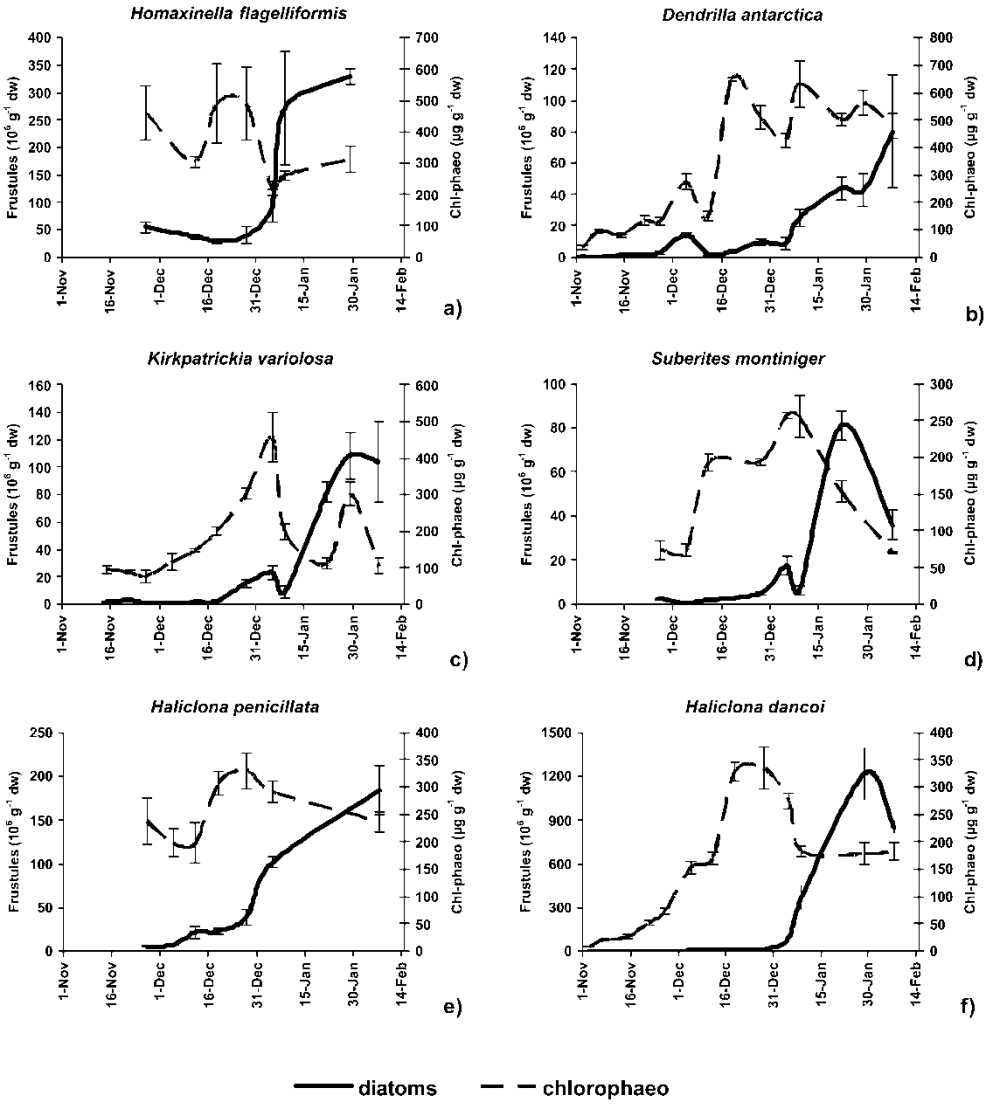

FIGURE 1 Trend of diatom frustule (cells $*10^6\,g^{-1}$ dw) and chlorophaeopigment concentration ($\mu g\,g^{-1}$ dw) in sponge tissues from November 2001 to February 2002.

[Fig. 1(f)]. *Fragilariopsis curta* was the most abundant species from 9 January onwards, with progressive increase of *Thalassiosira* spp. On 19 December high values of *Porannulus contentus* were found [Fig. 2(f)].

4 DISCUSSION

The first evidence from our results is that the occurrence of diatoms in the sponge tissues is a seasonal phenomenon. The absence of chlorophaeopigments and frustules at the beginning of November clearly indicates that the diatoms are not maintaining themselves during the winter season inside the sponges. This seasonal trend is also suggested by the decreasing

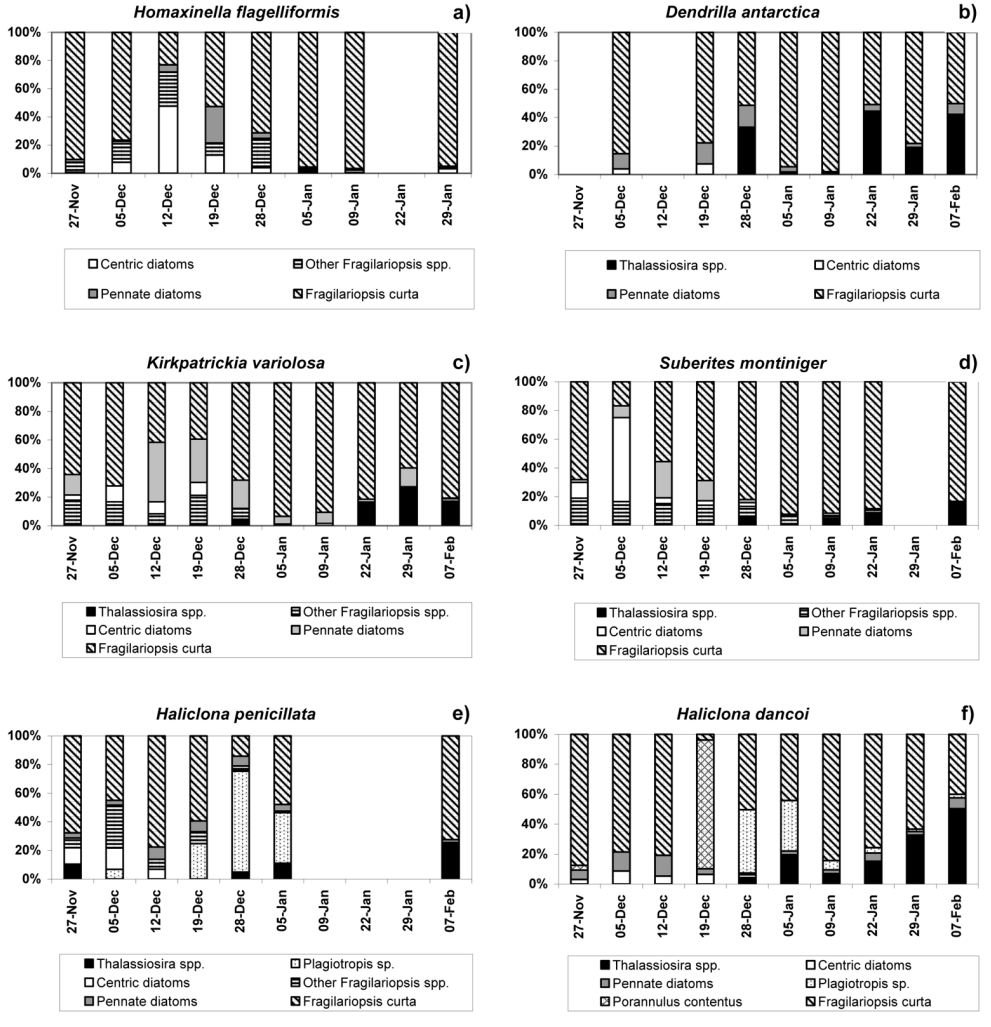

FIGURE 2 Diatom percent composition in sponge tissues from November 2001 to February 2002. Main *taxa* are represented and less abundant species were grouped into major categories.

values of diatom abundance and chlororophaeopigments observed in February for some of the investigated species (*Kirkpatrickia variolosa*, *Haliclona dancoi* and *Suberites montiniger*). The presence of diatoms inside the sponge tissues therefore appears to be a summer phenomenon related to the algal bloom in the water column.

Among the diatom species, *F. curta* was the most abundant in all the examined sponges, strongly dominating the diatom peak, while a progressive increase of *Thalassiosira* spp. (*T.* cf. *gracilis* and *T. perpusilla*) was always observed from the end of January onwards. *Plagiotropis* sp. increased from 19 December to 5 January in *Haliclona penicillata* and *Haliclona dancoi.* Although the phytoplankton assemblages in the water column were unfortunately not characterised during this study, diatom species observed into the sponge tissues resembled the composition of diatom assemblages previously described at Terra Nova Bay during the austral summer (Innamorati *et al.*, 1994; Marino and Cabrini, 1997; Nuccio *et al.*, 2000). This similarity indicates that the main source of diatoms for the sponges is

the phytoplankton population in the water column, penetrating through the inhalant aquiferous system or the exopinacoderm of the sponge surface. The capability of the sponge to incorporate siliceous particles has already been documented in several species (Bavestrello *et al.*, 1996; Cerrano *et al.*, 2000).

Notwithstanding the importance of phytoplanktonic diatoms, the high densities of *Porannulus contentus* (a species never recorded in the water column) observed in some samples of *Haliclona dancoi* suggest that other relationships, such as endosymbiosis (Hamilton *et al.*, 1997), may coexist between diatoms and sponges.

A second point, highlighted for the first time in this work, is the temporal shift between changes of chlorophaeopigment concentration and of the amount of frustules. In all the examined species the chlorophaepigments showed the highest values in the second half of December, in accordance with the occurrence of the phytoplankton peak as typically reported in the Ross Sea (Innamorati *et al.*, 2000; Nuccio *et al.*, 2000). The chlorophaeopigment maximum corresponded to a relatively low amount of diatom frustules, which increased until the second half of January when the chlorophaeopigment concentration progressively decreased. These data suggest that diatoms are incorporated alive in agreement with previously reported evidences (Gaino *et al.*, 1994; Bavestrello *et al.*, 2000) but they die inside the sponge tissues where the continuous incorporation of new diatoms caused a progressive accumulation of frustules. This evidence suggests that sponges use diatoms as trophic sources only during the summer season and not, as suggested by Gaino *et al.* (1994) as food reserve for the oligotrophic austral winter. Recent ultrastructural observations (Gaino and Rebora, 2003) indicate that freshwater sponges are able to incorporate diatoms into vacuole where they are digested.

The maximum amount of frustules strongly varied inside the tissues of different sponge species, ranging from 80 ± 36 to 1217 ± 178 cells $*10^6\,g^{-1}$ dw, respectively in *Dendrilla antarctica* and in *Haliclona dancoi.* However, chlorophaeopigment concentration showed less marked variations. This fact may be related to differences among sponge species in terms of different rates of filtration and/or turnover of diatoms inside the sponge tissues. *D. antarctica* showed high chlorophaeopigment concentration coupled with low frustule levels, indicating that the sponge has a slow rate of incorporation but diatoms remain alive for a long period of time inside the tissues. On the other hand, *H. dancoi* had lower chlorophyll concentration when the amount of frustules increased, suggesting that this species has a high rate of filtration and an efficient digestion of incorporated cells.

The lack of diatoms at the beginning of summer indicates that the accumulated frustules are eliminated during winter. Mechanisms involved in the turnover of diatom frustules may include the elimination through the excurrent canals or by the exopinacoderm or their dissolution inside the sponge cells.

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