Algal pigments in Southern Ocean abyssal foraminiferans indicate pelagobenthic coupling

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1. Introduction

The benthic life on the abyssal floor depends primarily on an input of mass and energy from the upper ocean. The quality and quantity of this input, the pelago-benthic coupling, will determine the structure and function of the communities (Dayton et al., 1994; Epping, 2013; Fabiano et al., 1997; Gooday, 2002; Graf, 1989; Graf et al., 1995; Grebmeier et al., 1988; Grebmeier and Barry, 1991; Hughes et al., 2007; Schnack-Schiel and Isla, 2005; Smith et al., 2006, 2013). This input occurs as sinking of particles produced at the surface (Asper et al., 1992; Fabiano et al., 1997). Faecal pellets and phytoplankton are the major source of such particles and they can sink individually or form aggregates (Asper et al., 1992; Isla et al., 2009). Aggregates, also called marine snow, sink faster than individual particles. The presence of ballast materials such as diatom frustules (opal) can increase the sinking speed (Iversen et al., 2010; Iversen and Ploug, 2010, 2013).

In recent years, attention is drawn upon an overlooked mechanism that can speed up the transportation of primary production to great depths. Active grazing by phytoplankton by large plankton organisms such as salps followed by their fast vertical migration to great depths where they are utilized by benthic organisms are alternative mechanisms that can by-pass the sedimentation of particles and aggregates. The combination of filtering of phytoplankton and vertical migration by salps forms a shortcut of the food chain. It reduces the loss of energy and makes the exchange between the surface layer and the bottom more efficient (Gili et al., 2006; Pfannkuche and Lochte, 1993; Schnack-Schiel and Isla, 2005). It has been estimated that salp carcasses deposit 16 t km⁻² yr⁻¹ of carbon in the Tasman Sea (Henschke et al., 2013).

Many factors affect the sinking particles and the largest part of the phytoplankton production is re-mineralized on its way down to the abyssal depths (Dayton et al., 1994; Grebmeier et al., 1988). It is estimated that only 0.01–1% remain after passage through the water column (Gooday, 2003).

The pelago-benthic coupling in the deep sea is demonstrated for various macro- and megafauna organisms. For example abyssal sponges (Kahn et al., 2012), cnidarians (Elías-Piera et al., 2013), holothurians (Hudson et al., 2004) and sea urchins (Campos-Creasy et al., 1994) contain large amounts of phytoplankton pigments that form the basis for their biological functions. Some of these organisms adapt their reproduction according to variation...
in the input of sedimenting algae, i.e. seasonal variation in primary production (Campos-Creasy et al., 1994).

Pelagic-benthic coupling has been demonstrated for shelf foraminifers (Cedhagen, 1988; Rathburn et al., 2001). But it has also been shown that many deep-sea foraminifers are associated with phytodetrital aggregates (Cornelius and Gooday, 2004) and can give a fast response to pulses of phytodetritus (Gooday, 1988, 2002). The response of foraminifers to phytodetritus exposure has been extensively documented both in situ and experimentally (Enge et al., 2011; Heinz et al., 2001; Nomaki et al., 2005, 2009; Witte et al., 2003). Indirectly, the distribution of benthic foraminiferal faunas, e.g., dominated by Epistominella exigua (Brady, 1884) in various Southern Ocean environments is a reflection of the importance of phytodetritus pulses (Mackensen et al., 1993, 1995).

The Antarctic is characterized by strong seasonality and vertical mixing due to oceanographic processes (Dayton et al., 1994; Schnack-Schiel and Isla, 2005; Smith et al., 2006). The food fluxes and their relation to oceanography are still not understood, but an extensive model in order to understand the structure and function of the food webs at multiple scales were developed by Murphy et al. (2012). The objective of the project SYSTCO II (SYSten COupling in the deep Southern Ocean II) was to investigate aspects of pelagic-benthic coupling between 50°S and 60°S. Upwelling deep-water masses interact here with the atmosphere and are ultimately subducted at the Sub-Antarctic Front. Preliminary results from this expedition are gathered by Wolf-Gladrow (2013).

Benthic foraminifers were collected for other purposes during the expedition (Cedhagen et al., 2013a). Some foraminifers contained cytoplasm with a distinct brown or brown-green colour. We hypothesize that deep-sea foraminifers contain pigments from algae that settled from the euphotic zone and were ingested fresh or degraded. We analysed the algal pigment contents of four foraminifera species collected at two deep-sea benthic stations characterized by high net primary productivities and high bottom chlorophyll a concentrations (Lins et al., 2014).

2. Material and methods

Material was sampled during the PS79 expedition with the research vessel Polarstern to the Southern Ocean in 2012 (ANT-XXVIII/3). Sampling with Agassiz trawl (AGT) equipped with a net of 2.0 µm mesh size at two stations resulted in numerous very large foraminifers (Cedhagen et al., 2013a). Stations 141-8 and 175-3 were sampled at comparable water depths (~4100 m) during the austral summer on the 18th of February and the 3rd of March, respectively (Table 1). The first station is located under a very high sea-surface primary production being exported while the second station is located north of South Georgia, in an area of constantly high production during the austral summer (Jones et al., 2012; Lins et al., 2014). Extensive data about all stations are available in Janussen et al. (2013). The samples were already washed to a great extent when they reached the deck because the mesh size of the Agassiz trawl is primarily designed for large macro- and megafauna organisms. The remaining foraminifers were sieved in cold seawater in a +0 °C Lab container and all further handling was done on ice. They were sorted under a stereomicroscope (Wild M5) and photographed with a Canon EOS 50D camera with a double flash (Canon Macro Twin Lite MT-24EX) and an ocular adaptor from LM-Scope. The samples were then immediately shock-frozen in epen- dorf tubes in liquid nitrogen and stored at −80 °C until further pigment analysis using high performance liquid chromatography (HPLC) technique at Alfred-Wegener-Institute in Bremerhaven, Germany. Pigment contents were also analysed in sediment samples collected with a multicorer at the same stations as the Agassiz trawl samples. Collection date and coordinates of the multicorer samples are available in Cedhagen et al. (2013c). A summary of the pigment data from these replicates is given in Table 2.

Prior to HPLC analysis, foraminiferan samples were first weighted with a special accuracy balance. Afterwards samples were cleaned with MilliQ water on a Petri slide. Following the method of Knight and Mantoura (1985), the organisms were crushed using a glass rod and then centrifuged (730 g for 3 min). The supernatant was removed into a syringe previously wetted with 90% acetone, and its volume was recorded. Further 50 µl of 90% acetone was added and crushing, mixing and centrifugation repeated. Some samples with highly dense pigments were diluted with 90% acetone prior to HPLC analysis. All samples were analysed based on the HPLC method of Barlow et al. (1997), as detailed in Hoffmann et al. (2006). This method was adapted to our instrument (Waters 600, Waters, USA) and quality controlled as described in detail in Taylor et al. (2011).

3. Results

Various benthic foraminifers collected at all the abyssal stations during the expedition were observed to contain a cytoplas- tim that was more or less green or brown. The pigments in the cytoplasm became obvious when very large foraminifers were collected with a sampling gear designed for macro- and mega- fauna organisms. Four species were isolated for pigment analyses (Fig. 1). They were Bathysiphon aff. filiformis M. Sars, 1872 (resembles Jones, 1994, pl. 26, Fig. 15; Wiesner, 1931, pl. III, Fig. 28); Botellina aff. labyrinthica Brady, 1881 (resembles Jones, 1994, pl. 29, Fig. 8; Wiesner, 1931, pl. XIII, Fig. 158 and pl. XIV, Fig. 159); Nodosium gaussia (Rhumbler, 1913) (resembles Jones, 1994, pl. 31, Fig. 1–2, 5; Wiesner, 1931, pl. IX, Fig. 108), and Miliolinella aff. subrotunda (Montagu, 1803) (resembles Jones, 1994, pl. 4, Fig. 3; Wiesner, 1931, pl. XI, Fig. 178).

The following pigments were analysed but gave negative results: chlorophyll b (divinyl chlorophyll a and divinyl chlorophyll b, but not expected to appear in the Southern Ocean), peridinin, 19–butanoyloxyfucanoxanthin, neoaxanthin, violaxanthin, astaxanthin, dihydroxanthin, lutein, gonyxanthin diesters, α-carotene, and chlorophyllide a.

The analysis was, however, positive for the pigments listed in Table 2 and plotted in Fig. 2. The ratio between chlorophyll a and degraded chlorophyll a pigments (Pheo a, i.e. sum of pheophorbide a, ppyropeophorbide a, and pheophytin a) was 0.19 µg/g in

<table>
<thead>
<tr>
<th>Station number</th>
<th>Date</th>
<th>Lat.</th>
<th>Long.</th>
<th>Depth (m)</th>
<th>chlorophyll a</th>
<th>chlorophyll c1 + c2</th>
<th>Fucoxanthin</th>
<th>Diadinoxanthin</th>
<th>Diatoxanthin</th>
<th>Zeaxanthin</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS 79/141-8</td>
<td>2012-02-18</td>
<td>51°16.0'S</td>
<td>12°37.5°W</td>
<td>4110</td>
<td>0.700440883</td>
<td>0</td>
<td>0.622332781</td>
<td>0.159665768</td>
<td>0.132801238</td>
<td>0.136178391</td>
</tr>
<tr>
<td>PS 79/175-3</td>
<td>2012-03-03</td>
<td>51°49.9'S</td>
<td>39°24.0°W</td>
<td>4150</td>
<td>0.700440883</td>
<td>0</td>
<td>0.622332781</td>
<td>0.159665768</td>
<td>0.132801238</td>
<td>0.136178391</td>
</tr>
</tbody>
</table>
Botellina 1; 0.058 in Botellina 3; 0.13 μg/g in Nodosinum 2; 0.23 in Miliolinella -1; 0.84 in Miliolinella -2. Some foraminiferans such as Miliolinella 3 contained no chlorophyll a but only Pheo-a pigments.

The pigments in foraminiferan cytoplasm in Table 3 primarily originate from diatoms and secondarily from haptophytes. Indicative for both groups are fucoxanthin, chlorophyll c1, chlorophyll c2, chlorophyll c3, diadinoxanthin and diatoxanthin (Roy et al., 2011). Only 19-hexanoyloxyfucoxanthin (19-hex) is present in haptophytes but not in diatoms. Except for Nodosinum 2, the concentration of 19-hex is either much lower than fucoxanthin or not present in all samples which shows that diatoms were the primary pigment source. This is supported by the global relationship between fucoxanthin and chlorophyll a of 1.41 for living diatoms and between 19-hex and chlorophyll a of 1.27 for haptophytes (see Uitz et al., 2006). After chlorophyll a and pheophorbide a, fucoxanthin and pheophytin a were the most abundant. Except for Miliolinella 2, fucoxanthin was always detected in higher concentrations than chlorophyll a, and was even present in those cases when chlorophyll a was absent. The former pigment degrades much slower than the later. Low concentrations of alloxanthin, indicative of cryptophytes, and even lower concentrations of zeaxanthin, indicative for prokaryotes (cyanobacteria), are found in the Botellina 1 to 3; the later pigment is also found in Nodosinum 2 samples. In one sample (Miliolinella 2), β-carotene, a photoprotective pigment common in many algal groups such as haptophytes and diatoms, was observed. There are no pigments from chrysophytes, prasinophytes or dinoflagellates.

Different foraminiferal species contain different pigments. One sample of Nodosinum (Nodosinum 1) contained no pigments at all, whereas Bathysiphon 1 contained only low concentrations of fucoxanthin. Concentrations of photoprotective pigments (diadinoxanthin, diatoxanthin, alloxanthin and β-carotene) were generally much lower than the photosynthetic pigments (all chlorophylls, fucoxanthin, 19-hex), which may indicate that light-inhibition for algae had been low. Some species contain higher concentrations of photosynthetic pigments (e.g. Miliolinella 1 and 2) while most other species (e.g. all Botellina, Miliolinella 3, and Bathysiphon 2) contain higher concentrations of degradation products such as pheophytin a and pheophorbide a. It shows that some species fed on fresh algae whereas others fed on degraded phytodetritus.

4. Discussion

The following pigments were analysed but gave negative results: chlorophyll b, peridinin, 19-butanoyloxyfucoxanthin, neoxanthin, violaxanthin, astaxanthin, dinoxanthin, lutein, gyro-xanthin diesters, α-carotene, and chlorophyllide a. The absence of peridinin, neoxanthin, lutein, and chlorophyll b suggests that dinoflagellates and chlorophytes were absent (Roy et al., 2011). Algae corresponding to these pigments were not found in the plankton samples (Klaas et al., 2013).

Some foraminiferans contained large amounts of pigments which shows that they were feeding on phytoplankton transported to the abyssal depth before being degraded, or on degraded algae. The composition of algal pigments in the foraminiferans reflects the plankton community that is dominated by diatoms, primarily Fragilariopsis kerguelensis (O’Meara, 1877), Pseudonitzschia spp. and Thalassiothrix antarctica Schimper ex Karsten, 1905 (Klaas et al., 2013). Salps have been shown to act as a mechanism that can increase the transportation of primary production to great depths (see above). Dense populations of the salp species Salpa thompsoni...
Table 3

Algal pigments in abyssal foraminifers from the Southern Ocean: Bathysiphon aff. liformis; aff. labyrinthica; Miliolinella subrotunda. All values are in μg pigment per gram foraminiferan dry weight.

<table>
<thead>
<tr>
<th>Foraminifera species</th>
<th>Bathysiphon</th>
<th>Botellina</th>
<th>Miliolinella</th>
<th>Nodosinum</th>
<th>Miliolinella</th>
<th>Nodosinum</th>
<th>Nodosinum</th>
<th>Nodosinum</th>
<th>Nodosinum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Chlorophyll a</td>
<td>0.094424754</td>
<td>0.014420232</td>
<td>0.01391913</td>
<td>0.014492023</td>
<td>0.014492023</td>
<td>0.014492023</td>
<td>0.014492023</td>
<td>0.014492023</td>
<td>0.014492023</td>
</tr>
<tr>
<td>Chlorophyll b</td>
<td>0.455843617</td>
<td>0.595543756</td>
<td>0.595543756</td>
<td>0.595543756</td>
<td>0.595543756</td>
<td>0.595543756</td>
<td>0.595543756</td>
<td>0.595543756</td>
<td>0.595543756</td>
</tr>
<tr>
<td>Pheophytin</td>
<td>0.704752759</td>
<td>0.752639389</td>
<td>0.752639389</td>
<td>0.752639389</td>
<td>0.752639389</td>
<td>0.752639389</td>
<td>0.752639389</td>
<td>0.752639389</td>
<td>0.752639389</td>
</tr>
<tr>
<td>Sum of Pheo a Chl a</td>
<td>0.187348671</td>
<td>0.342164771</td>
<td>0.342164771</td>
<td>0.342164771</td>
<td>0.342164771</td>
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<td>0.342164771</td>
<td>0.342164771</td>
<td>0.342164771</td>
</tr>
</tbody>
</table>

Foxton, 1961 were found in the investigated area (Pakhomov and Hunt, 2013) and could probably have contributed to the downward transport of the fresh phytodetritus. Both Miliolinella 1 and 2 samples from station 141_8 contain very high concentrations (16.52 and 45.58 μg/g, respectively) of chlorophyll a and the ratio between chlorophyll a and Pheo a pigments is also high (Table 3). It shows that they have been feeding on fresh phytodetritus. Some miliolid species from continental slope and deep-sea environments have been found to sit elevated on long tubes high above the sediment – a structure facilitating suspension feeding (Altenbach et al., 1993; Cedhagen et al., 2013b). Murray (2006) classified Miliolinella as a passive suspension feeder. The importance of the phenomenon could be grossly evaluated by counting the proportion of foraminifers displaying greenish cytoplasm, as observed experimentally for a deep-sea benthic species (Barras et al., 2009).

Niche separation between different surface-sediment feeding foraminifers is demonstrated by the composition of algal pigments in the cytoplasm, confirming previous experimental results (Heinz et al., 2001; Nomaki et al., 2005, 2009; Witte et al., 2003). The high ratios of chlorophyll a and Pheo a pigments in Miliolinella 1 and Miliolinella 2 shows that they were feeding on fresh phytodetritus. The lower chlorophyll a/Pheo a ratios, on the contrary, shows that other specimens were feeding on degraded phytodetritus. The availability of food might have been better sustained in station 175 as the benthos lied below extremely productive phytoplankton communities dominated by Chaetoceros spp., which contributed to a longer and more efficient export of carbon as compared to the communities of station 141, dominated by Fragilariapopsis (Assmy et al., 2013).

The concentration of algal pigment in the foraminiferal cytoplasm was very much higher than in the surrounding sediment, in which degraded chlorophylls were absent (Table 2) indicating an important mechanism. Pheo a, which could have resulted from zooplankton grazing and/or senescent cells (Jeffrey, 1974), have been applied recently as marker of detritus in the Southern Ocean (Wright et al., 2010). Diluted and degraded phytodetritus is a food resource of relatively low quality. Many foraminifers cover a fairly large sediment surface area with their pseudopodia and gather food particles that are transported to, and concentrate in their cytoplasm. Photographs of foraminifers with extended pseudopodial networks gathering food particles are published by Travis and Bowser (1991), Cedhagen (1988, 2010), Richardson and Cedhagen (2001), and Goldstein (1999). The granuloreticulopodial pseudopodia are able to collect even a sparse food resource. This phenomenon – covering a large area with numerous granuloreticulopodia without spending much energy on active searching for food – gives the foraminifers an advantage over other organisms and contributes to the explanation of their great quantitative success, particularly in the deep sea. The proportion of benthic foraminifers is generally increasing by depth in the deep sea where they gradually become the dominant organism group (Gage and Tyler, 1999). Moreover, one Agassiz trawl encompasses diverse foraminiferal communities resulting from the great patchiness of the deep-sea benthos. Indeed, subsamples taken within a single sediment core can greatly differ in terms of taxonomic composition, as shown using environmental DNA sequencing (Lejzerowicz et al., 2014). From our four species, only a relative of Bathysiphon could be sequenced while numerous species washed through the Agassiz trawl mesh such as those belonging to the small genus Epistominella may also contribute to the phytodetritus uptake (Enge et al., 2011; Gooday, 1988).

The Miliolinella subrotunda specimens were unusually large. We have no explanation for this particular phenomenon but gigantism has been described in other organisms from the deep Southern Ocean (Moran and Woods, 2012). Most foraminifers in Fig. 1 show large amounts of cytoplasm outside their tests. Similar reactions have been observed in sublittoral foraminifers as a
response to unfavourable conditions, particularly increased temperature (Cedhagen, 1993).

Foraminifers create food resources for benthic metazoans by concentrating phytodetritus and building up their own biomass. Some megabenthos such as holothurians are adapted to directly feed on the phytodetritus but some other groups in the deep sea do not easily utilize this food resource directly. Deep-sea foraminifers are known to be an important food source for isopods, a common organism group in the deep sea (Brøkeland et al., 2010 and further references therein). It shows that foraminifers are an important mechanism for using the residual of the settling primary production, also degraded algae, and transferring it back to the active food chains in the deep sea.

Acknowledgements


References
