Biogeosciences Discuss., 11, 251–280, 2014
www.biogeosciences-discuss.net/11/251/2014/
doi:10.5194/bgd-11-251-2014
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Biogeosciences Discussions Open Access

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Please refer to the corresponding final paper in BG if available.

Uptake of algal carbon and the synthesis of an "essential" fatty acid by *Uvigerina* ex. gr. *semiornata* (Foraminifera) within the Pakistan margin oxygen minimum zone: evidence from fatty acid biomarker and ^{13}C tracer experiments

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Received: 5 September 2013 – Accepted: 29 September 2013 – Published: 6 January 2014

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Published by Copernicus Publications on behalf of the European Geosciences Union.

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11, 251–280, 2014

Abstract

Foraminifera are an important component of benthic communities in oxygen depleted settings, where they potentially play a significant role in the processing of organic matter. We tracked the uptake of a ^{13}C-labeled algal food source into individual fatty acids in the benthic foraminiferal species, *Uvigerina* ex. gr. *semiornata*, from the Arabian Sea oxygen minimum zone (OMZ). The tracer experiments were conducted on the Pakistan Margin during the late/post monsoon period (August–October 2003). A monoculture of the diatom *Thalassiosira weisflogii* was ^{13}C-labeled and used to simulate a pulse of phytoplankton in two complementary experiments. A lander system was used for in situ incubations at 140 m and for 2.5 days duration, whilst a laboratory incubation used an oxystat system to maintain ambient dissolved oxygen concentrations. These shipboard experiments were terminated after 5 days. Uptake of diatoms was rapid, with high incorporation of diatom fatty acids into foraminifera after ~2 days in both experiments. Ingestion of the diatom food source was indicated by the increase over time in the quantity of diatom biomarker fatty acids in the foraminifera and by the high percentage of ^{13}C in many of the fatty acids present at the endpoint of both in situ and laboratory-based experiments. These results indicate that *U*. ex. gr. *semiornata* rapidly ingested the diatom food source and that this foraminifera will play an important role in the short-term cycling of organic matter within this OMZ environment. The experiments also suggested that *U*. ex. gr. *semiornata* consumed non-labeled bacterial food items, particularly bacteria, and synthesised the polyunsaturated fatty acid 20 : 4(*n*-6) de novo. 20 : 4(*n*-6) is often abundant in benthic fauna yet its origins and function have remained unclear. This study demonstrates that *U*. ex. gr. *semiornata* is capable of de novo synthesis of this "essential fatty acid" and is potentially a major source of this dietary nutrient in benthic food webs.

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Uptake of algal carbon by foraminifera

K. E. Larkin et al.

1 Introduction

Benthic foraminifera are a highly successful and diverse group of heterotrophic protists in all marine environments. Some species are carnivorous (Suhr et al., 2008; Dupey et al., 2010), but many typically feed at a low trophic level on algae, bacteria, sedimentary organic matter, and phytodetritus (e.g., Gooday et al., 2008 and references therein). In the deep ocean, phytodetritus derived from primary productivity in the euphotic zone provides an important source of fresh organic matter for benthic organisms. Deep-sea foraminifera exhibit rapid physiological and feeding responses when presented with labile food (Linke, 1992; Altenbach et al., 1992; Linke et al., 1995). Field and laboratory studies suggest that some species undergo reproduction and population growth following the deposition of phytodetritus in both bathyal and abyssal settings (e.g. Gooday and Rathburn, 1999; Koho et al., 2008; Gooday et al., 2010 and references therein). Based on these observations, it has been suggested that foraminifera are important in the processing of organic carbon on the ocean floor (Gooday et al., 2008).

Pulse-chase experiments using the stable isotope ^{13}C as a tracer of faunal OM uptake provide a powerful approach to understanding trophic pathways in benthic marine systems (Blair et al., 1996; Middelburg et al., 2000; Moodley et al., 2005); many of these studies have included benthic foraminifera (Levin et al., 1999; Moodley et al., 2000, 2002; Witte et al., 2003a; Enge et al., 2011). In situ experiments at a mildly oxygen-depleted bathyal site in Sagami Bay, Japan (1450 m depth) suggest that broad differences exist in the trophic preferences of different deep-sea foraminiferal species, as well as the degree to which they respond to pulses of labile food. Thus, some species (including *Uvigerina akitaensis*) in Sagami Bay consume only fresh algae and are particularly active in the short-term processing of algal-derived organic matter, while others will ingest sedimentary organic matter as well as more labile material (Nomaki et al., 2005a, 2006, 2011). Subsequent experiments in which labeled carbon was tracked into specific fatty acids within foraminiferal cells suggested that some species (again

including *U. akitinensis*) are able to degrade and/or synthesize some fatty acids (Nomaki et al., 2009).

Foraminifera are particularly abundant in severely hypoxic settings, notably those within bathyal oxygen minimum zones (OMZs) (Phleger and Soutar, 1973). On the Pakistan margin of the Arabian Sea, where an OMZ is strongly developed, Woulds et al. (2007) report that faunal uptake of labile organic matter was dominated by macrofaunal foraminifera in the OMZ core (300 m water depth; dissolved oxygen (DO) concentration: 4.2 μmol L^{-1} = 0.1 mL L^{-1}), but by metazoan macrofauna in the lower part of the OMZ (940 m; DO: 6.7 μmol L^{-1} = 0.15 mL L^{-1}). At the 140 m site that is the subject of the present study, metazoan macrofauna outcompeted foraminifera in ^{13}C uptake in the pre-monsoon season, when bottom waters were oxygenated, whereas foraminifera became dominant when oxygen concentrations decreased in response to the summer monsoon (Woulds et al., 2007). Further south, on the Indian margin of the Arabian Sea, tracer uptake by foraminifera was much less than uptake by metazoan macrofauna (although exceeding that by metazoan meiofauna) in shipboard experiments performed under different oxygen concentrations using samples from an OMZ site (756 m) (Moodley et al., 2011). Pozzato (2012) conducted similar experiments, incubating samples obtained at 885 m (DO: 2 μmol L^{-1} = 0.05 mL L^{-1}) and 1791 m (DO: 45 μmol L^{-1} = 1.0 mL L^{-1}) on the Murray Ridge (northern Arabian Sea) with ^{13}C-labeled phytodetritus under oxic and hypoxic conditions. In both treatments she found that "shelled" meiofaunal foraminifera took up more tracer at the shallower hypoxic site than at the deeper, more oxygenated site. However, uptake by the polychaete *Linopherus* greatly exceeded that of the foraminifera when the sample from the shallower site was incubated with tracer under more oxygenated conditions, consistent with the previous experimental results of Woulds et al. (2007). Jeffreys et al. (2012) suggested that this polychaete may prey on foraminifera, as well as out-competing them for food, thereby keeping their densities low. Overall, these studies suggest that foraminifera are important in organic matter cycling at very low oxygen concentrations, but outcompeted by

metazoan macrofauna (notably polychaetes) where these are abundant outside the OMZ core.

The papers of Woulds et al. (2007) and Jeffreys et al. (2012) arose from a comprehensive study of carbon cycling by benthic communities during different seasons (spring intermonsoon and late pre-monsoon, 2003) and at different water depths (140–1850 m) across the Pakistan margin OMZ (Cowie and Levin, 2009). Within the framework of this broader investigation, we focussed on the calcareous foraminifera *Uvigerina* ex. gr. *semiornata*, the dominant macrofaunal (> 300 µm) species at 140 m depth (Larkin and Gooday, 2009). This species is also a dominant component of the macrofauna at 300 m in the OMZ core off Pakistan. At both sites it displayed an opportunistic response to the natural phytodetrital flux to the seafloor following the SW monsoon, with significant increases in both standing stock and percentage abundance among "live" foraminifera (> 300 µm) (Larkin and Gooday, 2009). Analyses of the natural fatty acid content of *U*. ex. gr. *semiornata* showed it to be omnivorous, consuming many types of food, ranging from bacteria and sediment to phytodetritus (Larkin, 2006). However, this species appears to prefer a herbivorous diet and selectively ingests phytodetritus when it is available (Larkin, 2006). Macrofaunal foraminifera in general are responsible for much of the cycling of freshly-deposited phytodetritus through the benthic ecosystem under the oxygen depleted conditions found at our study site, and *U*. ex. gr. *semiornata* is clearly the main agent of foraminiferal C uptake (Woulds et al., 2007). A combination of trophic flexibility, an ability to respond opportunistically to a natural flux event by preferentially ingesting algal-derived organic matter, an adaptation to the hydrostatic pressure at upper bathyal depths, and a tolerance of severe hypoxia, may explain its dominance in the upper OMZ off Pakistan.

The overall aim of this study was to elucidate the trophic ecology of calcareous foraminiferal species *Uvigerina* ex. gr. *semiornata* within a seasonally hypoxic environment. Shipboard and in situ feeding experiments were conducted on sediments from the 140 m site on the Pakistan margin, with ^{13}C-labeled diatoms as a food source. Fatty acid biomarkers and their ^{13}C label were used to track how rapidly the foraminifera re-

sponded to this pulse and how individual fatty acids were metabolised. We addressed the following specific questions. (1) How quickly does *U*. ex. gr. *semiornata* respond to an algal food pulse? (2) Does this species utilise other food sources? (3) Can ^{13}C be tracked into individual fatty acids within the foraminiferal cell? (4) Are differences apparent between the results of the shipboard and in situ incubations?

2 Methods

2.1 Study area

The northern Arabian Sea is characterised by an intense, mid-water oxygen minimum zone (OMZ) extending from the bottom of the euphotic zone (100–150 m) to depths greater than 1 km (Fig. 1). This is one of the most extensive and pronounced low-oxygen layers in the modern ocean (Wyrtki, 1971, 1973; Deuser et al., 1978; Olson, 1993; You and Tomczak, 1993; Helly and Levin, 2004) with oxygen concentrations falling well below 6.25 µmol L^{-1} (= 0.14 mL L^{-1}) in the OMZ core. Details of environmental parameters and site conditions across the Pakistan margin during the 2003 sampling campaign are summarised by Woulds et al. (2007; Table 1 therein) and Cowie and Levin (2009; Table 1 therein). Here we focus on the 140 m site, which experiences seasonal fluctuations from hypoxic to oxic conditions, linked to the influence of the monsoon. Our experiments were conducted during the post-monsoon season, when the site was within the OMZ and therefore strongly hypoxic. At the time of sampling the seafloor temperature at 140 m was 18.2 °C and the dissolved oxygen concentration was ~ 3.44 µmol L^{-1} (= 0.08 mL L^{-1}).

2.2 Experimental methods

Two complementary time-series experiments, one conducted in situ and the other in a shipboard laboratory, were carried out at the 140 m site during RRS *Charles Darwin* Cruise 151, following the SW monsoon (October 2003). A monoculture of ^{13}C-labeled

diatoms served as a food source and the ^{13}C-label was used to track its consumption. Full experimental methods, summarised below, are described in Woulds et al. (2007).

2.2.1 Diatom culture and incubation procedure

The diatoms (*Thalassiosira weisflogii*) were cultured in an autoclaved flask in ^{13}C-labeled seawater until the cells were ~90 % labelled with ^{13}C. The diatom detritus was then combined with kaolinite powder to act as a ballast. The slurry was freeze-dried and kept at −20 °C until required for the incubation experiments. Before being added to the experiments, the slurry was defrosted and re-suspended in Milli Q water. The diatom slurry was then added to the surface of the sediment in both shipboard and in situ lander feeding experiments using a carbon dose equivalent to 0.8 ± 0.3 % of the organic matter (OM) naturally present in the top 1 cm of sediment (Woulds et al., 2007).

2.2.2 Shipboard feeding experiments

Shipboard laboratory feeding experiments were conducted using six replicate megacores (78.5 cm^2 surface area) collected at the 140 m site and incubated in a constant temperature laboratory at the ambient seafloor temperature (18 °C) (Woulds et al., 2007). A core cap fitted with an O-ring, oxygen sensor, magnetic stirrer and sampling ports was used to isolate sediments and overlying seawater. The oxygen concentration in overlying waters was maintained at 3.44 $\mu mol\,L^{-1}$ (= 0.08 $mL\,L^{-1}$) by circulating core-top water through an "oxystat" system (Cowie and Levin, 2009). Between 100 and 150 mg of algal detritus was added to each megacore, equivalent to 685 ± 118 $mg\,C\,m^{-2}$ (Woulds et al., 2007). This ensured that a relatively constant dose of 5–8 mg of carbon was added to each megacore (Table 1). After a settling period of 30–60 min, a green layer of algal cells was visible on the surface of the cores. Gentle water column stirring was then initiated using a built-in magnetic stirrer in order to homogenise overlying water without resuspending sediment or algae. Megacores were covered in black sheeting during the experiment in order to exclude light. Pairs

of megacores were incubated for two or five days. Two megacores were used as time-zero ($t = 0$) controls. Immediately after the labeled algal slurry had been added, these control core were sliced into horizontal layers and the living fauna extracted, as described below. The $t = 0$ foraminifera were used to compare the fatty acid composition of natural *Uvigerina* ex. gr. *semiornata* with that of specimens exposed to the diatom food source after two and five days.

2.2.3 In situ feeding experiments

In situ feeding experiments were conducted at the 140 m site using a benthic lander (Black et al., 2001). Once on the seafloor, the lid was closed on the benthic chamber isolating an area of sediment and the overlying water. A known amount (~ 350 mg, equivalent to a carbon delivery of 25–35 mg of C or 361 ± 63 mg C m^{-2}) of ^{13}C-labeled algal slurry was added to the sediment chamber (900 cm^2 surface area) using an automated syringe. Gentle stirring of overlying water in the chamber was initiated after a period of 30–60 min to allow time for the algal detritus to settle. To maintain ambient dissolved oxygen concentration, chamber water was pumped through an oxystat gill in contact with bottom water for the duration of the experiment (Cowie and Levin, 2009). After an incubation period of ~ 2.5 days, the benthic lander was recovered and the sediment retained in the benthic chamber was sub-sampled for faunal analysis using two replicate megacores (78.5 cm^2 surface area). No time-zero controls were possible in the case of the in situ experiments. Therefore, the fatty acid composition of natural foraminifera was used to compare the fatty acid composition of *Uvigerina* ex. gr. *semiornata* before and after the 2.5 day exposure to the diatom food source.

2.2.4 Extraction of live foraminifera

At the end of each timed feeding experiment (both shipboard and in situ), the megacore samples were sliced at intervals of 0.5 cm to 2 cm depth, then at 1 cm intervals to 5 cm. Half of each layer was reserved for porewater extraction. The other half was wet sieved

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on a 300 µm screen to extract living fauna. The sieved residues from all layers were kept chilled (< 5 °C) in a refrigerator or over ice to prevent decomposition of the fatty acids and foraminifera were sorted as quickly as possible under a low-power binocular microscope. Individuals sampled were all from a modal size group (∼ 300–400 µm length) to ensure consistency throughout the experiment. Picked specimens were kept in a small glass petri dish containing chilled, filtered seawater placed in a larger dish containing ice. An organic stain such as rose Bengal was not used to distinguish "live" foraminifera as this could alter their fatty acid composition. Instead, specimens were judged to be "live" (and therefore feeding) at the time of sampling based on the presence of obvious test contents in most or all constituent chambers. Foraminifera were sorted into individual species and cleaned in filtered (2 µm screen) seawater; this removed attached organic particles including diatoms, although we cannot eliminate the possibility that some bacteria remained on the test surface. For fatty acid analysis, only foraminifera from the upper 1 cm of the sediment were used.

Two replicate cores were sampled for each time point in both shipboard and in situ experiments. However, only half a megacore section was available from each core for faunal extraction, so live specimens from both megacores sub-samples were pooled in order to produce enough specimens for replicate analyses. Four replicate samples (30 individuals each) of *Uvigerina* ex. gr. *semiornata* were extracted from sediment residues representing each time point, placed into 1.1 mL borosilicate glass vials with Teflon-lined screw-caps to avoid contamination, and frozen at −20 °C prior to fatty acid extraction. Lipid analyses of metazoan megafauna were conducted separately (Jeffreys et al., 2009).

2.3 Analytical methods

2.3.1 Lipid analysis

The foraminiferal fatty acids were derivatised to pentafluorobenzyl esters (PFB esters) and analysed using a gas chromatograph coupled to an electron capture detector (GC-

ECD). The method is highly sensitive and invaluable where quantities of material available for analysis are small. Full details of the method are given in Pond and Ward (2011). In brief, an internal standard (23 : 0 free fatty acid) was added to each sample (30 individuals of *Uvigerina* ex. gr. *semiornata*) to enable quantification of fatty acids. Lipids were then extracted by adding 500 µL Chloroform:Methanol (2 : 1 v/v) solution and stored at −20 °C for 24 h to ensure full extraction of lipids.

The sample was phase separated using 0.88 % (w/v) KCl and total lipid extracted following Folch et al. (1957). Total lipid was then saponified using 1 M KOH in ethanol (5 : 95 v/v) and acidified using 0.6 M HCl to produce free fatty acids (FFAs). After extraction of FFAs in diethylether and drying under a stream of nitrogen, free fatty acids were converted to PFB esters by reacting with 140 mL of acetonitrile: diisopropylamine/PFB bromide (1000 : 10 : 1 $v/v/v$) at 60 °C for 30 min. PFB esters of the fatty acids were purified using thin layer chromatography (Pond et al., 2011) and analysed using a Trace 2000 GC (Thermo) equipped with a Restek Stabilwax column (30 m × 0.32 mm) and an ECD. Hydrogen was used as the carrier gas and nitrogen as the ionizing gas for the ECD (Pond and Ward, 2011).

2.3.2 Gas chromatography mass spectrometry

In addition to the quantitative analysis of fatty acids using gas chromatography, the levels of ^{13}C-enrichment of twelve individual fatty acids extracted from *Uvigerina* ex. gr. *semiornata* and derviatised as PFB esters were analysed by selective ion monitoring (SIM) scan using a gas chromatograph mass spectrometer (GC-MS) equipped with a Chrompack CP wax fused 52CB column (30 m × 0.32 mm id, 0.25 micron film thickness). The GC-MS was operated in negative chemical ionization (NCI) mode with methane as the reagent gas (Bell et al., 2007). NCI of PFB esters of fatty acids generates 100 % yield of molecular ions.

Fatty acids were analysed from foraminifera sampled at each time point over the duration of both the shipboard and in situ feeding experiments. For each foraminiferal fatty acid, the relative proportions of 8 dominant mass ions (accounting for > 95 %

of all mass ions present in each fatty acid) were analysed. Two naturally occuring mass ions (100 % ^{12}C and +1^{13}C) were analysed to account for any natural fatty acid that was in the foraminifera before the experiment or any unlabeled fatty acid deriving from food other than the ^{13}C-labeled diatoms that could have been ingested during the experiments. In addition, the dominant six mass ions representing different degrees of ^{13}C-enrichment of the carbon chain in the fatty acid molecule (100 % ^{13}C, −1^{13}C, −2^{13}C, −3^{13}C, −4^{13}C, −5^{13}C) were quantified to determine the fatty acids in the foraminifera that originated from ingestion of the ^{13}C-labeled diatoms. Data are expressed as amount of ^{13}C as a percentage of total fatty acid carbon.

2.4 Statistical analysis

Data were analysed using the programme PRIMER (Clarke and Gorley, 2001). All statistical analysis was conducted on average quantity (ng per 30 foraminifera) data. A two sample *t* test assuming unequal variance was also conducted to test for significance in differences between univariate data, such as the average quantity (ng) of total fatty acid and individual fatty acids in *Uvigerina* ex. gr. *semiornata* at different time points during the experiments.

3 Results

3.1 Light microscope observations

In both the shipboard and in situ experiments, a positive uptake of the diatom monoculture was indicated by a bright green colouration of the cytoplasm, presumed to reflect the presence of chlorophyll. In the shipboard experiment, *Uvigerina* ex. gr. *semiornata* exhibited bright green cytoplasm after only two days. Specimens of *Uvigerina* also gathered diatom material around their apertures, again indicating that they were actively feeding.

3.2 Diatom fatty acid composition

Dominant fatty acids in the diatom food were the saturated fatty acids 14 : 0 and 16 : 0, the monounsaturates 16 : 1(*n*-7) and the polyunsaturates 16 : 2(*n*-4), 16 : 3(*n*-4) and 20 : 5(*n*-3) (Fig. 2).

5 ### 3.3 Tracking the uptake of ^{13}C-labeled diatoms by *Uvigerina* ex. gr. *semiornata*

3.3.1 Total quantity of fatty acids

The average quantity of total fatty acids in *U*. ex. gr. *semiornata* increased significantly ($P < 0.05$, 2-sample *t* test) over the duration of the 5 day shipboard experiment. Compared to the natural foraminifera ($t = 0$), there was a ~ 1.5-fold increase in the average
10 total quantity of fatty acids after 2 days and a ~ 3.5-fold increase after 5 days of exposure to the food source (Fig. 3). The average quantity of total fatty acids in this species also increased significantly ($P < 0.05$, 2-sample *t* test) over the 2.5 day period of the in situ feeding experiment, showing a ~ 1.2-fold increase in average total quantity compared to natural specimens (Fig. 3).

15 #### 3.3.2 Quantity of individual fatty acids

A total of thirty fatty acids were identified in the *U*. ex. gr. *semiornata* samples (Figs. 4 and 5). In most cases, average quantities of fatty acids increased over the course of the shipboard and in situ experiments. The amounts of three polyunsaturated fatty acids (PUFAs), 16 : 2(*n*-4), 16 : 3(*n*-4) and 20 : 5(*n*-3), and the monounsaturated fatty acid
20 (MUFA) 16 : 1(*n*-7) (all diatom fatty acid biomarkers), increased significantly ($P < 0.05$, 2-sample *t* test) between natural ($t = 0$) and experimental specimens exposed to the diatom food source in both the shipboard ($t = 2$ day and $t = 5$ day) and in situ ($t =$ 2.5 day) experiments. All four of these fatty acids were highly abundant in the diatom monoculture (Fig. 2) but were only present in small amounts in $t = 0$ samples (Figs. 4
25 and 5), indicating that diatoms were being consumed during the experiments. The

monounsaturated fatty acids 14 : 0 and 16 : 0 (dominant in the diatom food source) also increased significantly ($P < 0.05$, 2-sample t test) in foraminiferal samples from the start to the endpoints of both experiments (Figs. 4 and 5). Despite the PUFA 20 : 4(n-6) only being present in low amounts in the diatom food, levels of this fatty acid increased
5 dramatically in the foraminifera during both the in situ and shipboard experiments. For the shipboard experiments, where most data are available, the rate of increase of 20 : 4(n-6) was comparable with that of 16 : 0, the most abundant fatty acid in the diatoms (Fig. 6).

3.3.3 ^{13}C-enrichment of individual fatty acids

10 The percentage of ^{13}C in twelve dominant fatty acids present in *U*. ex gr. *semiornata* before exposure to the ^{13}C-labeled diatom food source was very low, reflecting natural abundance of ^{13}C in the environment ($\leq 1.5\,\%$ ^{13}C). The percentage of ^{13}C present in the thirteen fatty acids increased substantially from $t = 0$ to $t = 5$ days (shipboard) and from natural samples to $t = 2.5$ (in situ), reflecting ingestion of labeled algae by
15 the foraminifera (Fig. 7a and b). However, the percentage of ^{13}C varied considerably between each individual fatty acid analysed. Fatty acids displaying the highest degree of ^{13}C labelling at the endpoints of both experiments included the diatom fatty acid biomarkers 16 : 1(n-7), (16 : 2(n-4) and 16 : 3(n-4), all of which were common in the diatom food source (Fig. 7a and b). Contradictory results were evident for 20 : 5(n-3),
20 a classic biomarker for diatoms. Although this fatty acid was highly labeled ($\sim 75\,\%$) in the shipboard experiments, it was only moderately labeled in the in *situ* experiments. The percentage of ^{13}C in 20 : 4(n-6) was low at the endpoint of both in situ and shipboard experiments, in contrast to the substantial increases in the amounts of this fatty acid in the foraminifera.

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4 Discussion

Fatty acid biomarker and ^{13}C tracer techniques established that the dominant macrobenthic foraminiferal species on the Pakistan margin rapidly ingested and metabolised algal phytodetritus. The amounts of 16 : 1(*n*-7), 16 : 2(*n*-4), 16 : 3(*n*-4) and 20 : 5(*n*-3) within *Uvigerina* ex. gr. *semiornata* increased between the start and the end of both shipboard (5 days duration) and in situ (2.5 days duration) feeding experiments. Because each foraminifera was cleaned thoroughly with filtered seawater before extracting the fatty acids, it is likely that the biomarkers originated from the cellular contents and not from diatoms attached to the outer surface of the test. The percentage of ^{13}C in these fatty acids generally increased with the increase in the quantity of the fatty acids, confirming that ^{13}C-labeled diatoms were being consumed throughout the feeding experiment. This is consistent with an increase in bulk cytoplasmic δ^{13}C values for the total foraminifera (all species combined) over the course of both experiments (Woulds et al., 2007). One surprising exception was 20 : 5(*n*-3). While specimens of *U*. ex. gr. *semiornata* sampled after $t = 2$ days during the shipboard experiments exhibited a high (78.9 %) level of ^{13}C-enrichment in 20 : 5(*n*-3), those recovered at the end of the in situ experiment (2.5 days) yielded only a low (2.9 %) level of ^{13}C-enrichment in this fatty acid. This result is difficult to explain, particularly given the fact that the PUFA 18 : 4(n-3) was heavily labeled with ^{13}C at the termination of both experiments. Occasional puzzling discrepancies between shipboard and in situ results were noted in other experiments conducted during the 2003 campaign on the Pakistan margin, including at the 140 m site (Woulds et al., 2009). In other respects, our in situ and shipboard experiments yielded similar results.

Many studies provide evidence for the consumption of algae by foraminifera, and in particular by calcareous species, in both shallow- and deep-water habitats. Nomaki et al. (2005a, 2006, 2011) reported that the shallow-infaunal, calcareous species *Uvigerina akitaensis* rapidly consumed algae, particularly the marine diatom *Chaetoceros sociale*, during in situ ^{13}C-labeled feeding experiments carried out at their bathyal

site in Sagami Bay. Goldstein and Corliss (1994) analysed the ultrastructure of *Uvigerina peregrina* from 710 m water depth in the San Pedro Basin (California Borderland) and found a variety of food items in the food vacuoles of this species. These included numerous aggregates of sediment, organic detritus and diatom frustules. Heeger (1990) concluded that phytodetritus was important in the diet of some calcareous species from the deep Greenland–Norwegian Sea, based on the occurrence of pennate diatoms in their food vacuoles. Experiments conducted by Ernst and van der Zwaan (2004) showed that a pulse of diatoms and other algae can maintain or lead to increased populations of opportunistic species such as *Epistominella exigua* and *Adercotryma glomeratum*. Finally, a higher foraminiferal population density was recorded 21 days after the addition of an algal food source to deep-sea sediments (from 919 m water depth, western Mediterranean) in laboratory culture experiments (Heinz et al., 2002).

The changes in quantity and ^{13}C content of other fatty acids indicate that *Uvigerina* ex. gr. *semiornata* was also consuming food sources other than diatoms during the feeding experiments. The increase in the quantity of 18 : 1(*n*-7) over the course of both the in situ and shipboard experiments is surprising, since this monounsaturated fatty acid is not known to be produced in significant amounts by eukaryotes (Gurr and Harwood, 1991) and constituted a very low percentage (1.7 %) of the total fatty acids in the diatom food source. It is likely that the 18 : 1(*n*-7) in the foraminiferal cells was derived from heterotrophic bacteria, which contain high amounts of this fatty acid (Sargent et al., 1987), ingested from the sediment over the course of the experiments. This idea is supported by the low ^{13}C content of 18 : 1(*n*-7) (< 30 % ^{13}C) at the endpoint of the five-day shipboard experiment. However, there was a substantial increase in the percentage of ^{13}C in 18 : 1(*n*-7) at the endpoint of the in situ incubation (2.5 days). Possibly, the foraminifera were ingesting bacteria that had already assimilated dissolved organic carbon derived from the ^{13}C-labeled diatoms and had incorporated these atoms into other fatty acids that they synthesised *de novo*. Andersson et al. (2008) reported the enrichment of ^{13}C in three bacterial fatty acids across the Pakistan margin OMZ,

including the 140 m site, during the same experiments. Nomaki et al. (2009) found “microbial” (presumably bacterial) biomarkers to be enriched in ^{13}C derived from labelled algae during experiments conducted in Sagami Bay.

Nomaki et al. (2009) also presented evidence for the possible production of certain sterols by the modification of dietary molecules by the deep-sea foraminifera *Globobulimina affinis*. In our experiments there was a particularly dramatic increase in 20 : 4(*n*-6), another fatty acid normally found only in bacteria, during the course of the incubations. This PUFA was the second most abundant fatty acid in the in situ experiment after 2.5 days (Fig. 4) and in the shipboard experiment after 5 days (Figs. 5 and 6). In the latter case it accounted for 12.9 Mol% after 5 days compared to 4.5 Mol% in the natural foraminiferal cells and only 0.9 Mol% in the diatom food source (Fig. 2). It could have originated from bacteria ingested from the sediment or adhering to the test surface or it may have been synthesised *de novo* by the foraminifera themselves. Synthesis of fatty acids such as *n*-6 PUFAS by marine eukaryotes, including protists, has indeed been hypothesized by Bowles et al. (1999). Given the scale of the 10-fold increase in the amount of 20 : 4(*n*-6) observed in our shipboard experiments, we believe that this is the most likely explanation. The fact that this PUFA contains only a small proportion of ^{13}C (Fig. 7) suggests that *Uvigerina* ex. gr. *semiornata* synthesised it from existing C reserves within the cell. We expect that the ^{13}C content would increase during a longer experiment. Many benthic foraminifera contain high amounts of this “essential” fatty acid (Gooday et al., 2002; Suhr et al., 2008), which is also dominant in deep-sea echinoderms (Howell et al., 2003; Hudson et al., 2004). It is possible that particular biomechanical properties of 20 : 4(*n*-6) facilitate life at increased hydrostatic pressures. *U*. ex. gr. *semiornata* was sampled from 140 m off Pakistan but its range extends to ∼ 500 m depth on this margin (Schumacher et al., 2007). Further studies are required to determine the functional significance of this fatty acid in the marine environment and the potential metabolic pathways for its synthesis de novo by foraminifera.

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5 Conclusions

Uvigerina ex. gr. *semiornata*, the dominant macrofauna-sized foraminifera at a 140 m-deep study site on the Pakistan margin, displayed a fast uptake (within 2 days) of a ^{13}C-labeled diatom food source in both shipboard and in situ pulse-chase experiments. This species is likely to play a key role in short-term benthic organic matter cycling in this outer shelf environment, as well as on the adjacent upper slope where it is also abundant. The substantial increase in quantity and ^{13}C enrichment of diatom biomarker fatty acids in the foraminifera over the duration of the feeding experiments clearly demonstrates that *U*. ex. gr. *semiornata* readily consumes labile algal material as its main food source. Increases in the bacterial biomarker fatty acid 18 : 1(*n*-7) suggests that *U*. ex. gr. *semiornata* also consumed some bacteria from the surrounding sediment. Most importantly, fatty acid and ^{13}C data imply that *U*. ex. gr. *semiornata* actively synthesised 20 : 4(*n*-6) during the experiments. This "essential" polyunsaturated fatty acid is often extremely abundant in deep-sea organisms, particularly foraminifera (Gooday et al., 2000; Suhr et al., 2008), yet its origins and functions in this environment remain unclear. Evidence presented here suggests that foraminifera could be a major source of 20 : 4(*n*-6) in benthic ecosystems with important consequences for the supply of this "essential" dietary nutrient to higher trophic levels.

Acknowledgements. The authors are indebted to the Captain, officers, crew and RVS staff of the RRS Charles Darwin. We would also like to thank Oli Peppe and Willie Thompson who ran the benthic lander and to the many shipboard scientists who assisted with sampling, sample processing and sorting at sea, particularly A. A. S. Aranda da Silva, B. J. Bett, M. Danish, and T. Masood Ali Khan. This work was conducted aboard the RRS Charles Darwin, and was made possible by grants from the UK Natural Environmental Research Council (Grant No. NER/A/S/2000/01280).

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Table 1. Summary of the use of ^{13}C-labelled diatoms in all shipboard and in situ enrichment incubations at 140 m during the SW monsoon, cruise CD151 (October 2003). Abbreviations: SF13 = Shipboard experiments (2 replicate megacores of 78.5 cm surface area for each time point). EF13 = Elinor Lander in situ incubations (sediment chamber, 30 cm^2 surface area). Mass values are dry weight (dwt).

Incubation ID	Station number	Core number	Dates of incubation	Mass (mg dwt)	% Carbon	Carbon mass (mg dwt)
SF13-2 day (A)	56101#4	6	20–22 Sep 2003	50.2	14	7.03
SF13-2 day (B)	56101#10	6	20–22 Sep 2003	50.4	14	7.06
SF13-5 day (A)	56101#2	3	20–25 Sep 2003	49.7	14	6.96
SF13-5 day (B)	56101#2	2	20–25 Sep 2003	49.1	14	6.87
EF13-2.5 day (A)	56101#29	N/A	25–27 Sep 2003	349.1	14	48.87

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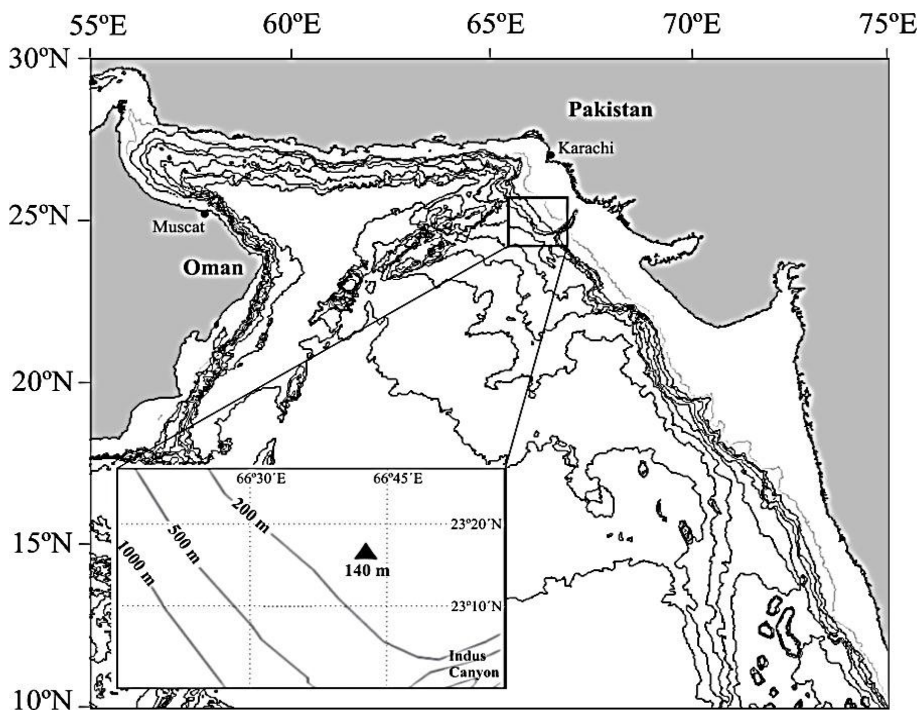

Fig. 1. Sampling location on the Pakistan margin of the Indian Ocean.

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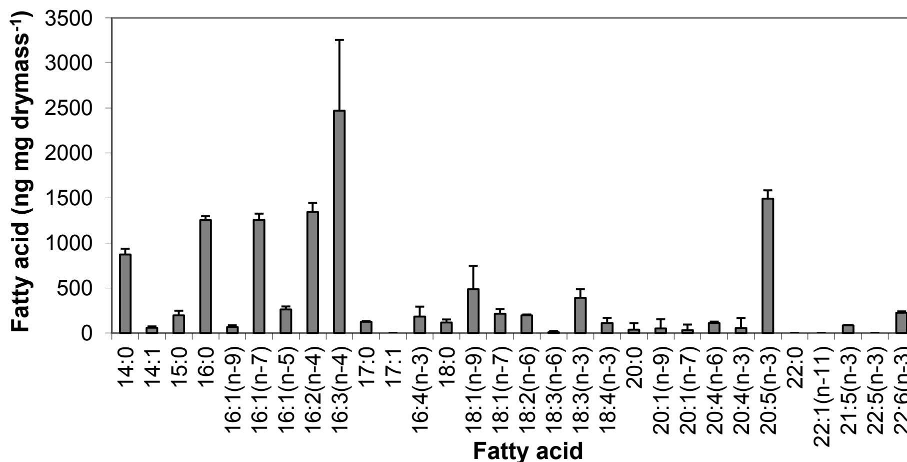

Fig. 2. Quantity (ng mg drymass^{-1}) of fatty acids in the ^{13}C-labelled diatom, *Thalassiosira weisflogii* ($n = 2$).

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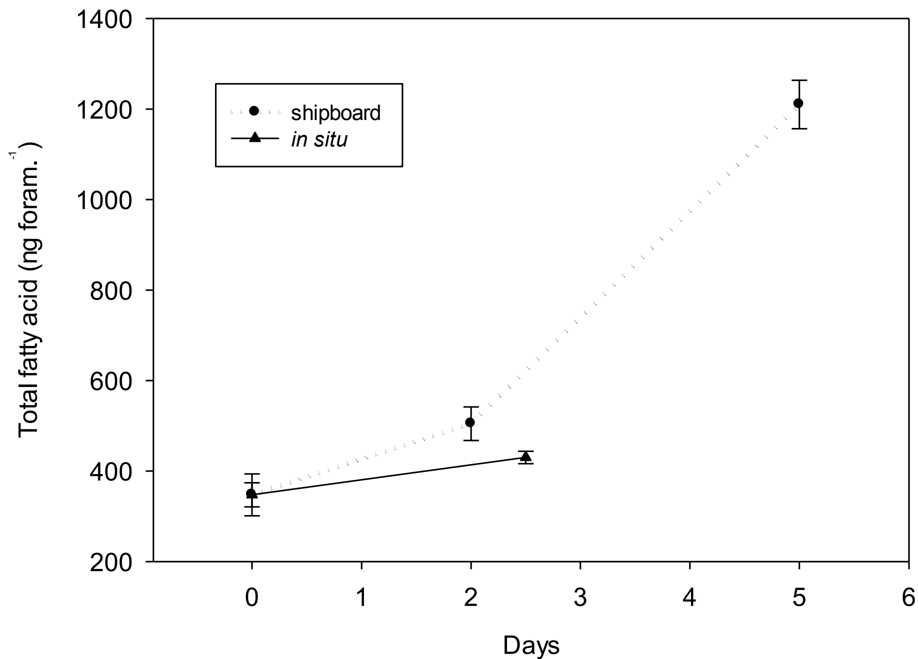

Fig. 3. Fatty acid composition (%) in the foraminiferan *Uvigerina* ex. gr. *semiornata* from shipboard and laboratory feeding experiments ($n = 4$).

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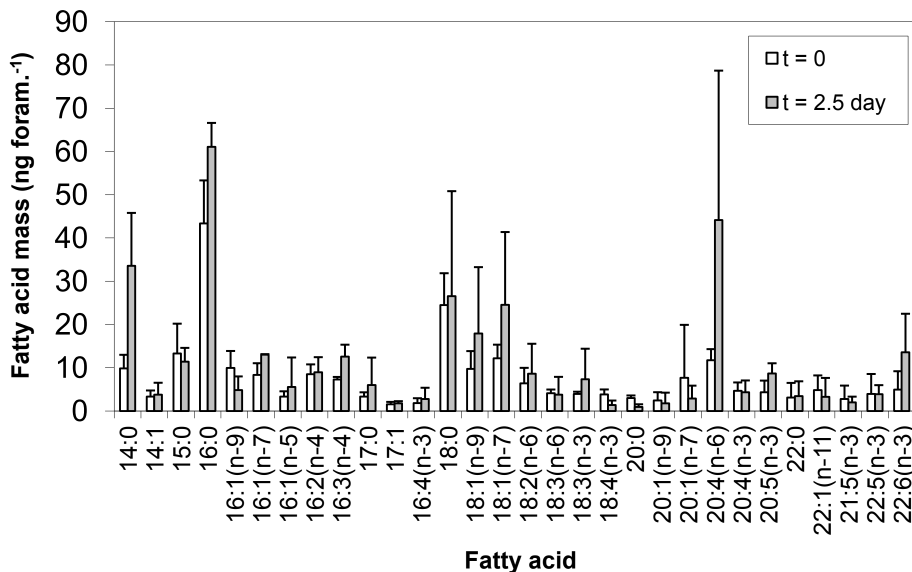

Fig. 4. Changes in the amounts of fatty acids (ng foram^{-1}) in *Uvigerina* ex. gr. *semiornata* during the in situ experiment ($n = 4$). The numerical nomenclature for the different lipids indicates the number of carbon atoms (14–22), followed by the number of double bonds (0–6) and the location of the first double bond in relation to the terminal methyl carbon (e.g. *n*-6).

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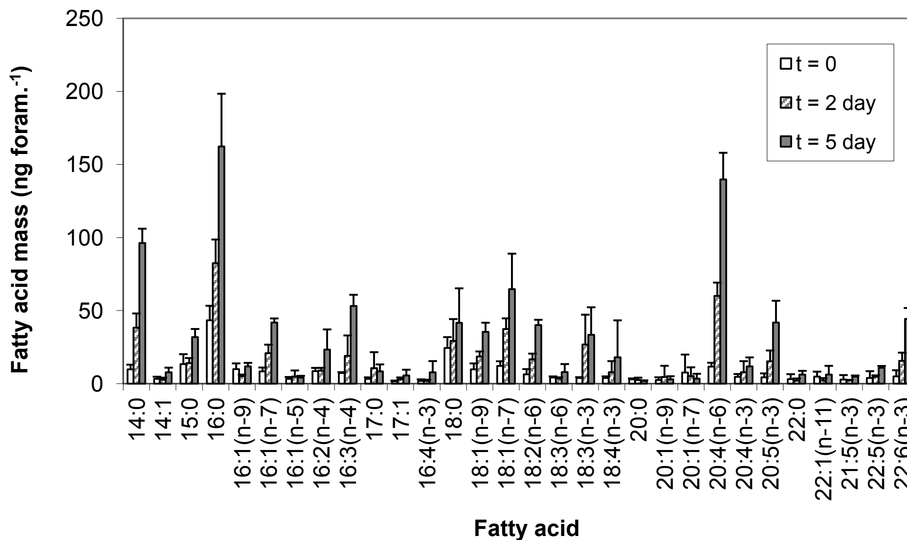

Fig. 5. Changes in the amounts of fatty acids (ng foram.$^{-1}$) in *Uvigerina* ex. gr. *semiornata* during the shipboard experiment ($n = 4$).

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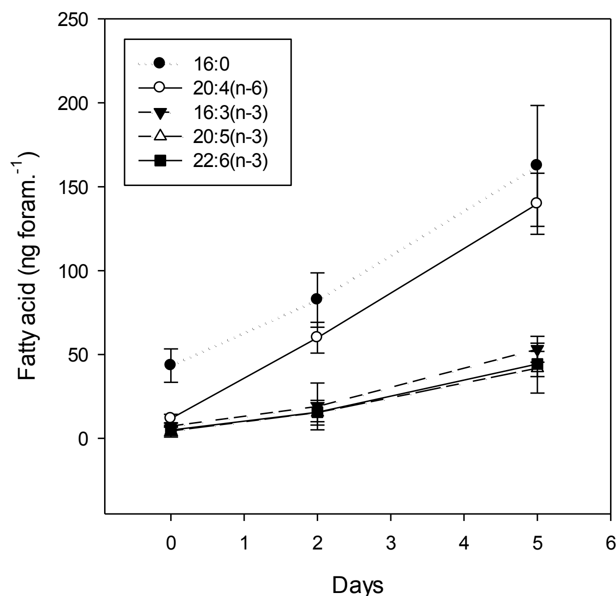

Fig. 6. Increase in amounts of 5 key fatty acids in *Uvigerina* ex. gr. *semiornata* during the shipboard experiment.

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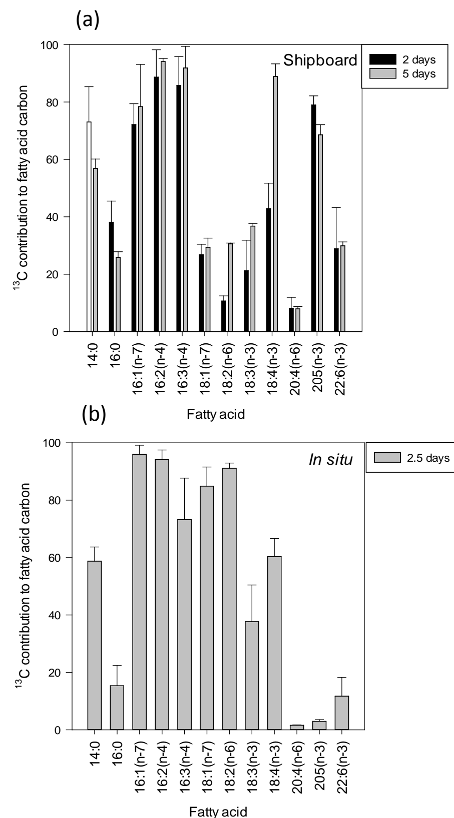

Fig. 7. Percentage ^{13}C labelling of twelve dominant fatty acids in **(a)** shipboard and **(b)** in situ incubations. Data are average values of 4 replicate samples (each of 30 Foraminifera). 95 % Confidence intervals are shown for ^{13}C values.

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