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Discussions

Foraminiferal survival after long term experimentally induced anoxia

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Abstract

Anoxia has been successfully induced in four benthic chambers installed on the Northern Adriatic seafloor from 1 week to 10 months. To accurately determine whether benthic foraminifera can survive experimentally induced prolonged anoxia, the CellTrackerGreen method has been applied. Numerous individuals have been found living at all sampling times and at all sampling depths, showing that benthic foraminifera can survive up to 10 months of anoxia with co-occurring hydrogen sulphides. However, foraminiferal standing stocks decrease with sampling time in an irregular way. A large difference in standing stock between two cores samples in initial conditions indicates the presence of a large spatial heterogeneity of the foraminiferal faunas. An unexpected increase in standing stocks after 1 month is tentatively interpreted as a reaction to increased food availability due to the massive mortality of infaunal macrofaunal organisms. After this, standing stocks decrease again in a core sampled after 2 months of anoxia, to attain a minimum in the cores sampled after 10 months. We speculate that the trend of overall decrease of standing stocks is not due to the adverse effects of anoxia and hydrogen sulphides, but rather due to a continuous diminution of labile organic matter.

1 Introduction

Over the last decade numerous marine environments have been affected by hypoxia (Diaz and Rosenberg, 2008). The increase in the frequency of occurrence of hypoxia and the spatial distribution of such oxygen-depleted areas are assumed to be linked to eutrophication and climate change (Stramma et al., 2008; Rabalais et al., 2010). Hypoxia can dramatically affect pelagic and benthic biodiversity (Levin, 2003; Gooday et al., 2009; Stramma et al., 2010). The mechanisms leading to low-oxygen conditions differ between the open ocean and coastal enclosed basins. In many oceanic upwelling areas, hypoxia is permanent and the dissolved oxygen concentration remains below

0.5 mL L^{-1} (20 µmol L^{-1}, Helly and Levin, 2004; Middelburg and Levin, 2009). Hypoxia is caused by a combination of high pelagic productivity and slow renewal of oxygen in the water column. In most semi-enclosed coastal areas, hypoxia is related to continental inputs (of nutrients and organic matter) and seasonal water column stratification.

5 Pelagic production is a response to natural and/or anthropogenic nutrient input, and hypoxia is mainly caused by benthic oxygen consumption. In such coastal environments hypoxia appears seasonally.

Generally, bottom water oxygen concentrations are strongly linked to several other environmental parameters. Increased organic matter supplies stimulate benthic pro-

10 duction, leading to an increased sediment oxygen demand and, finally, to lowered bottom water oxygenation. In extreme cases, when the seafloor becomes anoxic, organic matter degradation continues via anaerobic metabolic pathways, eventually leading to sulphate reduction near the sediment-water interface (Middelburg and Levin, 2009). In such conditions toxic hydrogen sulphide may diffuse into the bottom water.

15 Various terminologies have been proposed to describe the intensity of hypoxia, mainly based on the effect of oxygen concentration on marine biota (reviewed in Hofmann et al., 2011; Altenbach et al., 2012). For the purpose of the present study we will use only three terms defined as follows (following Tyson and Pearson, 1991; Bernhard and Sen Gupta, 1999, Middelburg and Levin, 2009): (1) oxic – the measured

20 oxygen concentration above 1.5 mL L^{-1} (> 63 µmol L^{-1}); (2) hypoxic – concentration below 1.5 mL L^{-1} (< 63 µmol L^{-1}), which is the threshold at which an impact has been recorded on coastal biota, and (3) anoxic – no detectable dissolved oxygen present.

In the past, several field and laboratory studies have been conducted to better understand the response of benthic faunas to hypoxia (i.e. to low oxygen concentration

25 and its co-occurring processes such as high organic matter availability and increased hydrogen sulphide concentration) in coastal environments. These studies demonstrate that for macrofauna (invertebrates larger than 1 mm) the first responses to low oxygenation are usually behavioral or physiological changes (e.g. body extension, shallowing of burial depth, emergence from the sediment; see Levin et al. (2009) for a review),

and the organisms only exhibit a high mortality after 2 to 7 days of anoxia (Riedel et al., 2008; Stachowitsch et al., 2012). Among meiofauna (invertebrates measuring 45 µm to 1 mm), copepods are among the taxa most sensitive to anoxia (Moodley et al., 1997; Diaz and Rosenberg, 2008), whereas several nematode species can survive up to 60 days of anoxia (Wieser and Kanwisher, 1961). Benthic foraminifera appear to be more tolerant to anoxia than most meiofaunal metazoans (Josefson and Widbom, 1988; Moodley et al., 1997; Levin et al., 2009).

In field studies, the foraminiferal density tend to decrease during anoxic and/or hypoxic events (e.g., Jorissen et al., 1992; Duijnstee et al., 2004) and infaunal taxa migrate upward in hypoxic conditions (Alve and Bernhard, 1995; Duijnstee et al., 2003). Laboratory studies show that multispecific foraminiferal faunas can survive up to 78 days of anoxia (Alve and Bernhard, 1995; Moodley et al., 1997). Conversely, benthic foraminiferal densities respond positively to increased input of fresh organic matter if it is not accompanied by oxygen depletion (Heinz et al., 2001; Ernst and van der Zwaan, 2004; Ernst et al., 2005; Nomaki et al., 2005). Some evidence suggests that an exposure to hydrogen sulphide for up to 21 days does not significantly affect foraminiferal density, and that after 66 days of exposure some individuals were still found alive (Moodley et al., 1998).

Most previous studies were based on the analysis of foraminiferal assemblages stained by Rose Bengal. Rose Bengal is a bulk-stain which adheres to the proteins present in the protoplasm (Walton, 1952; Bernhard, 2000). However, protoplasm may be preserved in the foraminiferal shell several days to weeks after the death of the organism in well-oxygenated conditions (Boltovskoy and Lena, 1970; Bernhard, 1988; Murray and Bowser, 2000) and up to three months in anoxia (Hannah and Rogerson, 1997), leading to the staining of dead organisms. The expectation is that protoplasm degradation is especially slow in anoxic conditions (Burdige, 2006; Glud, 2008). Consequently, using Rose Bengal can lead to substantial overestimation of the living foraminiferal standing stocks, and probably does not reliably reflect the living faunas in low-oxygen settings. Although Rose Bengal staining may yield a rapid overview of

living faunas in distributional studies, it is unsatisfactory for studies with a high temporal resolution, where the time between successive samplings may be shorter than the time needed for degradation of the foraminiferal protoplasm. Several techniques have recently been developed to more accurately identify the vitality of benthic foraminifera (reviewed in Bernhard, 2000). As noted above, proteins can still be present after the organism has died and all energetic and enzymatic activities have stopped. These activities are the basis for more accurate foraminiferal vitality discrimination techniques. For instance, based on an Adenosine TriPhosphate (ATP) concentration, an absence of living individuals was determined in the Santa Barbara basins after a 10 months anoxic period (Bernhard and Reimers, 1991). A laboratory experiment using the ATP concentration method determined, in four species from a shallow site (45 m) in Drammensfjord (Norway), that all these species survived up to three weeks of anoxia. Three species, however, showed a lower ATP concentration in anoxic conditions (Bernhard and Alve, 1996). A recent laboratory experiment (Piña-Ochoa et al., 2010b) using the enzymatic activity sensor Fluorescein DiAcetate (FDA; Bernhard et al., 1995) showed that the deep infaunal species *Globobulimina turgida* can survive anoxia (with presence of nitrate) for up to 84 days (with a survival rate of 46 %). A subsequent study, using the same method, demonstrated that the individuals do not migrate vertically during anoxia (Koho et al., 2011). CellTrackerGreen[TM] (CTG; Bernhard et al., 2006), another enzymatic activity probe, has been used to determine that Adriatic Sea foraminiferal assemblages can survive up to 69 days of hypoxia and that their vertical migrational behavior is largely linked to the labile organic matter availability (Pucci et al., 2009). Finally, it has been shown recently that the life activity of foraminifera can be confirmed by sequencing a taxonomic marker extracted from the sediment metatranscriptome (Lejzerowicz et al., 2013).

Summarizing, previous laboratory and field studies suggest that foraminifera can survive strong hypoxia and anoxia for prolonged periods, in most cases without a clear decrease in population density. Many of these studies, however, are based on the problematic Rose Bengal staining method, which may substantially overestimate the

number of living individuals or even indicate "living" faunas where none are present, especially in anoxic conditions. This calls for testing the resistance of shallow-water foraminifera to anoxia with a more reliable vitality determination technique. Additionally, until now the foraminiferal response to strong hypoxia and/or anoxia has mainly been assessed by laboratory experiments and by ecological field studies lasting up to 3 months, but never (to our knowledge) by an in situ experiment during 10 months. The current paper applies such a long-term in situ experimental approach to document the foraminiferal response to anoxia, using the CTG technique. Our experimental setup was designed to reduce methodological biases as much as possible:

1. In order to ensure long-term anoxia (up to ∼ 10 months), several benthic chambers were positioned on the sea floor for different periods of time. After closure of the chambers, the respiration of the benthic organisms rapidly consumed all available oxygen, leading to anoxia. In order to quantify these processes, the chemical characteristics of the sediment and the overlying waters were monitored (Koron et al., 2013; Metzger et al., 2013).

2. An in situ experimental approach has the advantage of considerably reducing "laboratory effects" due to differences between experimental and natural conditions, and of reducing the natural temporal variability (in temperature, salinity, water transparency, food availability . . .) that can affect faunas in field studies.

3. Our experimental set-up allows us to apply the same method on different faunal elements. It has been successfully used to study the effect of anoxia on macrofauna (Blasnig et al., 2013; Riedel et al., 2013, 2008, 2012; Stachowitsch et al., 2012), on different meiofaunal groups such as copepods, nematodes (Grego et al., 2013; De Troch et al., 2013) and foraminifera (this study and Langlet et al., 2013).

Our study was conducted in the Northern Adriatic Sea, which is a typical coastal area impacted by seasonal hypoxia/anoxia. It combines many aspects commonly associated with low oxygenation events: a semi-enclosed, shallow (< 50 m) basin with a fine-grained substrate, a high riverine input, high productivity (leading to "marine snow"

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events), and long water residence times in summer/autumn (Ott, 1992). Several studies have reported the occurrence of bottom water hypoxia and anoxia here (e.g., Giani et al., 2012), with an increasing frequency from the 1970s to the mid-1990s. It appears that the frequency of marked hypoxia has decreased since the mid-1990s (Giani et al., 2012), likely due to a reduction of anthropogenic nutrient supplies and a reduced riverine nutrient input into the Northern Adriatic Sea.

In this context, we investigate the responses of the foraminiferal faunas to experimentally induced anoxia in benthic chambers. The present paper focuses on the foraminiferal survival and density response to long-term anoxia. Our main question was whether benthic foraminifera can survive 10 months of anoxia. In case of a positive answer, our goal was to determine how the foraminiferal density varies in function of the duration of anoxia. Finally, we studied the variation of density in the upper 5 cm of the sediment in order to identify potential vertical migration of the foraminiferal faunas in response to experimental conditions. The response of individual species to anoxia is treated in a second paper (Langlet et al., 2013).

2 Material and methods

2.1 Experimental set-up and sampling

The experiment was conducted in the Gulf of Trieste (Northern Adriatic Sea) near the oceanographic buoy of the Marine Biology Station Piran (45°32.90′ N, 13°33.00′ E) at 24 m depth, on a poorly sorted silty sandy bottom. The experimental set-up has been adapted from an earlier experiment conducted by Stachowitsch et al. (2007) and Riedel et al. (2008). Both experiments used the Experimental Anoxia Generating Unit (EAGU), which is a $0.125\,\text{m}^3$ fully equipped benthic chamber which enables experimentally inducing and documenting local-scale anoxia (see Stachowitsch et al., 2007).

In the current study, we used four different chambers to produce anoxia for four different durations (Table 1). The four chambers were installed on the sea floor, several

meters apart, on substrates which were visually poor in macrofauna. For comparison, two cores (termed "Normoxia") were sampled at the start of the experiment. In order to standardize the terminology for all articles of the present Biogeosciences Special Issue, the different sampling times (also named in the present paper "Sample ID" for "Sample Identification") have been termed "7 days", "1 month", "2 months" and "10 months". The exact duration (in days) of each of the experiments is given in Table 1. The first chamber, fully equipped with the EAGU analytical devices, documented the onset of anoxia during a 7 day period. The other three chambers were used to study the development of the meiofauna after ∼ 1 month, ∼ 2 months and about 10 months of anoxia, respectively. Foraminifera, copepods and nematodes were analyzed for the same cores. Sediment cores were taken by scuba divers using a Plexiglas corer with a 4.6 cm inner diameter (16.6 cm^2 surface area). Two replicate cores were taken in each of the benthic chambers at each sampling time.

2.2 Meiofaunal analysis

To identify whether the collected meiofaunal organisms were alive, we used CTG, or CellTracker Green 5-chloromethylfluorescein diacetate (CellTracker™ Green CMFDA; Molecular Probes, Invitrogen Detection Technologies). When living cells are incubated in CTG, this non fluorescent probe passes through the cellular membrane and reaches the cytoplasm, where hydrolysis with nonspecific esterase produces a fluorogenic compound. This fluorogenic compound does not leak out of the cell after fixation and can thus enable identifying the individuals exhibiting enzymatic activity when sampled (Bernhard et al., 2006).

For meiofaunal analyses, entire sediment cores were sliced every half centimeter from 0 to 2 cm and every centimeter between 2 and 5 cm. Within one hour after retrieval, sediments were stored in 100 cm^3 bottles, which were filled with sea water and CTG-DMSO (Dimethyl sulfoxide) at a final CTG concentration of 1 µmol L^{-1} (Bernhard et al., 2006; Pucci et al., 2009). Samples were gently shaken and immediately placed in a cold room at in situ temperature, where they remained for at least 10 h, in order to

obtain the hydrolysis of the CTG probe. After this reaction period, samples were fixed in 4 % formaldehyde buffered with Sodium Borax. The samples were further stored at room temperature.

Samples were then centrifuged to separate the soft meiofauna (i.e. copepods, nematodes; results presented in Grego et al., 2013; De Troch et al., 2013) from the sediment and the "hard-shelled" meiofauna (i.e. foraminifera; the present article and Langlet et al., 2013). Centrifugation has two main advantages: first it permits working on the same sample for both copepods/nematodes and foraminifera. These two groups are easily separated because of their different densities; foraminifera are heavier because of their agglutinated or calcareous test. Secondly, it simplifies the foraminiferal analysis because all the soft organisms, which show a stronger fluorescence than foraminifera, have been removed.

Samples were first sieved over a 38 µm mesh to remove small sediment particles and formaldehyde. We then added a Levasil density medium and distilled water to achieve a density of about $1.17\,\mathrm{g\,cm^{-3}}$, and finally added about 10 g of kaolin powder to promote the sedimentation of the heavier particles (McIntyre and Warwick, 1984; Burgess, 2001). Next, we centrifuged for 10 min at 3000 rpm at ambient room temperature. After this, the supernatant (composed of soft meiofauna) and the deposit (composed of the hard-shelled meiofauna and sediment) were separately sieved over a 38 µm mesh to remove the kaolin and the Levasil medium. The two separated samples were stored in a Borax-buffered formaldehyde solution until further analysis.

The present study analyzes only the samples containing the hard-shelled meiofauna (soft-walled foraminiferal taxa are excluded). These samples were subsequently sieved over 315, 150, 125 and 63 µm meshes.

A major asset of the present study is that we also analyzed the $> 63\,\mu\mathrm{m}$ size fraction of the benthic foraminifera, and we systematically studied the complete sample, without splitting. Many studies fail to analyze the smaller size fractions (63–125 or 63–150 µm) because it is extremely time-consuming. The smaller size fraction, however,

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may contain abundant living individuals and can provide important information about small opportunistic species and about potential reproduction during the experiment.

For the 63–125 µm fractions is was necessary to use an accumulation method for the foraminifera to prevent splitting the sediment (leading to statistical problems) and/or unrealistic working times. Thus, all foraminiferal samples of the fractions 63–125 µm were washed to remove the borax-buffered formaldehyde solution and dried in a compartment dryer (Fa. Memmert) at 30–40 °C. The foraminiferal tests were then separated by treating the sediment with tetrachlorcarbon under a laboratory fume (Hohenegger et al., 1989; Murray, 2006). The tetrachlorcarbon was carefully decanted and the floating foraminifera were collected on paper filters, quickly dried, and stored in small glass vials.

Foraminiferal counts were performed in all fractions > 63 µm using an epifluorescence stereomicroscope (Olympus SZX12 with a light fluorescent source Olympus URFL-T or Nikon SMZ 1500 with a PRIOR Lumen 200). Samples were observed at the accurate excitation (492 nm) and emission (517 nm) wavelengths. The samples from the 63–125 fractions were dried and picked dry, while the samples from the > 125 µm fractions were not dried and picked wet. Dried specimens still show clearly the fluorescence produced by incubation with CTG. Only specimens showing clear green fluorescence were picked and counted as living.

2.3 Metatranscriptomic analysis

Total RNA was extracted from ca. 0.5 g of sediment from the top centimeter of a core collected in the "10 months" anoxia chamber, was retro-transcribed to cDNA and used as template to amplify, clone and sequence the foraminifera-specific hypervariable region 37f of the small subunit of the ribosomal RNA gene (SSU rDNA). The resulting sequences were filtered for chimeras using UChime and each sequence assigned to a foraminiferal species or genus based on global alignments as in Lejzerowicz et al. (2013). Pairwise global alignments were also used to cluster the assigned sequences into Operational Taxonomic Units (OTUs). A threshold of 4 % sequence

dissimilarity was chosen as no further sequence clustered until a distance of 16 %. The resulting OTUs were assigned according to the consensus of the individually assigned sequences.

2.4 Data analysis

After the discrimination of the living individuals, they were counted and expressed as standing stock (number of living individuals per core in the 0–5 cm depth interval normalized for a 10 cm^2 surface area) and as density (number of living individuals per depth interval normalized for a 10 cm^3 sediment volume).

Three statistical procedures were used to identify the effect of several parameters on the foraminiferal standing stocks or density. All the procedures are Linear Models (Chambers and Hastie, 1992) in which the dependent variable (the response variable) is the log-transformed foraminiferal density or standing stock. The log-transformation permits to respect the conditions for the application of parametrical statistics (i.e. the dependent variable must show a Gaussian distribution).

To be able to determine significant differences in the foraminiferal standing stocks between sampling times, two models test the effect of the Sample ID (i.e. a qualitative expression of the sampling time) on foraminiferal standing stocks in two depth intervals. The first model tests the Sample ID effect in the whole core (0–5 cm; Table 2; Model 1), the second only for the shallowest depth interval (0–0.5 cm; Table 2; Model 2).

To quantify the effect of anoxia on standing stocks in these two depth intervals (0–0.5 cm and 0–5 cm) we designed an analysis of covariance linear model. This model (Table 2; Model 3) tests the effect of both the Sampling Time (a quantitative expression of the duration of the experiment), the Depth Interval (a qualitative expression of the selected depth intervals: "0–0.5 cm" and "0–5 cm") and their Interaction. This model was designed to identify whether the variation of the standing stock in function of sampling time is similar for the two depth intervals or not. Note that the sampling Time has been log-transformed in order to avoid giving too much weight to the "10 months" chamber.

Finally, to quantify the effect of the anoxia and of sediment depth on the foraminiferal density, we designed an analysis of covariance linear model. The first computed model (Table 2; Model 4) tests the effect of both the Sample ID, the sediment depth (i.e. here a quantitative expression of the depth interval; the value used being the midpoint of the depth intervals) and their first-order interaction with foraminiferal density. Note that to increase the quality of the fit, sediment depth has been log-transformed and has been introduced with a polynomial component (the log transformed depth has been squared). The Depth times squared Depth interaction and the squared Depth times Sample ID interaction have also been added to the model.

Table 4 shows only the tested variables which have a significant effect. Table S2 shows how each of the variables affects the dependent variable.

3   Results

3.1   Foraminiferal survival under various experimental conditions

Living benthic foraminifera (i.e. positively labeled with CTG) were present from the beginning of the experiment to the end, after about 10 months of anoxia. Relatively large standing stocks were recorded from the surface down to 5 cm depth (Fig. 1; Table 3). In the 10 studied cores, the total number of individuals in the top 5 cm varied from ~ 1980 individuals per $10\,cm^2$ in "Normoxia" core 1, to ~ 530 individuals per $10\,cm^2$ in the "10 months" core 1. The living assemblages were dominated by *Leptohalysis scottii*, *Eggerella scabra* and *Bulimina aculeata*, whereas *Textularia agglutinans*, *Quinqueloculina seminula*, *Lagenammina atlantica*, *Hopkinsinella glabra* and *Bolivina pseudoplicata* were other conspicuous faunal elements. Census data are presented in Table S1, whereas a more complete description and analysis of the faunal composition will be presented in Langlet et al. (2013).

3.2 Density variation with time

Figure 1 shows that living benthic foraminifera (i.e. CTG-stained) were present at every sampling time. The standing stocks in the whole cores (0–5 cm depth interval, Fig. 1a) varied only slightly with incubation time. Although a clear maximum was found in "Normoxia" core 1, no significant differences existed between the pairs of replicate cores of the 5 sampling times (Table 4; Model 1). When only the 0–0.5 cm depth interval is considered, the highest standing stocks occurred in "Normoxic" core 1, very similar values were found in both "1 month" cores, whereas the values were lowest ($\sim$ 30 individuals per 10 cm^3) in the two "10 months" cores.

Model 2 (Table 4) was used to test whether the foraminiferal densities in the 0–0.5 cm depth interval (Fig. 1b), are significantly different at different sampling times. The test shows that the standing stocks of the "Normoxia" and the "1 month" cores are not significantly different, but both are significantly higher than the 0–0.5 cm densities of the "7 days" and "2 months" cores. The latter cores are not significantly different from each other (Table 4; Model 2 and Table S2). Finally, the standing stocks of the 0–0.5 cm layer of the "10 months" cores are significantly lower than those observed in all other cores (Table 4).

The analysis of covariance shows that the total standing stocks showed a significant exponential decrease both for the total cores (0–5 cm) and for the 0–0.5 cm level, in function of log-transformed experimental Time (Fig. 2, Table 4, Model 3). The foraminiferal density was significantly lower in the 0–0.5 cm depth interval than in the whole core (Table 4; Model 3), indicating that a significant part of the living fauna was present in deeper sediment levels.

The comparison of the slopes of the regression curves between the 0–5 cm and 0–0.5 cm intervals shows no significant difference (Table 4, Model 3). This means that the density decrease over Time was not significantly different between the whole core (0–5 cm) and the topmost sediment (0–0.5 cm).

3.3 Density variation with sediment depth

Figure 3 shows that foraminiferal density decreased strongly with sediment depth in the five pairs of replicate cores. In fact, all cores exhibited a significant exponential decrease of density in function of log-transformed sediment depth (Table 4, Model 4). Also the Sample ID (i.e. a qualitative expression of Time) effect was significant (Table 4, Model 4). When the various depth levels are considered individually, the densities in the "10 months" cores were significantly lower than those of the other 4 pairs of replicate cores (Table 4, Model 4 and Table S2). Additionally, the slopes of the density decrease with depth were not significantly different except for the one observed for the "1 month" cores, which is significantly steeper, mainly due to the very high density in the topmost level and the very strong decrease in the 0.5–1 cm level.

3.4 Metatranscriptomic analysis

A total of 61 filtered foraminiferal SSU rDNA sequences were obtained from the meta-transcriptomic sediment extract of the anoxic chamber. After clustering, 32 OTUs were obtained and included 15 unassigned OTUs (Fig. 4a). The remaining OTU were assigned to the calcareous order Rotaliida (9 OTUs), the agglutinated Textulariida (2 OTUs) and to the soft-walled monothalamiids (6 OTUs). One OTU represented by one sequence was perfectly assigned to the reference sequence of a specimen of *Eggerella scabra* collected in the same area (Fig. 4b).

4 Discussion

4.1 Variation of pore water geochemistry and macrofaunal behavior during the experiment

Parallel to the meiofaunal and macrofaunal analyses, sediment geochemical analyses were conducted to control whether the experimental conditions indeed led to anoxia

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in both the overlying water and in the pore water. Metzger et al. (2013) show that the oxygen concentration in the waters overlying the sediment (measured by two oxygen microsensors placed a few millimeters above the sediment-water interface in the "7 days" chamber) started to decrease as soon as the chamber was closed. The consumption rate was 950 nL cm^{-2} h^{-1} (i.e. 0.0115 nmol cm^{-2} s^{-1} or 9.93 mmol m^{-2} day^{-1}). Microsensor data show that hypoxia was reached after 2 to 6 days and anoxia after 7 days. The overlying water remained anoxic until the end of the deployment after 9 days. To estimate the contribution of the foraminiferal assemblages to the oxic respiration in our benthic chambers, we compared the oxygen consumption rate with the total foraminiferal respiration estimated using the power relation between foraminiferal biovolume and respiration rate (Geslin et al., 2011). The application of this relation, taking into account the foraminiferal densities in the oxygenated sediment layer (the 0–0.5 cm interval of the Normoxia cores) in 4 distinct size classes (63–125, 125–150, 150–315 and > 315 µm), yields a total foraminiferal respiration rate of 4.57 nL cm^{-2} h^{-1}. In comparison, the value is 950 nL cm^{-2} h^{-1} for total benthic respiration. Accordingly, foraminifera are responsible for about 0.5 % of the total benthic respiration in the chamber. These values are similar to those estimated for assemblages inhabiting the Rhone prodelta (0.6 and 1.2 % at 37 and 60 m depth, respectively; Geslin et al., 2011; Goineau et al., 2011).

At the beginning of each experiment, 2 to 3 brittle stars (*Ophiothrix quinquemaculata*) were placed into each chamber, which otherwise contained no visible macro-epifauna. *Ophiothrix* shows typical behavioral reactions to decreasing oxygen concentrations, such as arm-tipping at the transition from normoxia to hypoxia, probably to raise the position of the respiratory organs in the water column (see Riedel et al., 2008, 2012). This macrofaunal behavior (including emerging infauna species) was used as an additional in situ indicator for the onset of hypoxia in each chamber. The brittle stars and the other macrofaunal organisms died after 7 to 15 days of deployment. Shortly after the brittle stars died, the normally greyish-brown sediment turned black a few centimeters

around the dead organisms. About 2 days later (i.e. after 9 to 17 days of deployment), the whole surface of the sediment in the chambers was completely black.

After this 9 day period, direct oxygen measurements were no longer technically possible (due to the limited battery capacity of the data logging system), but other chemical indicators indicate that reductive conditions occurred and remained present until the opening of the "10 months" chamber. Upward shifts of the manganese, iron and sulfate reduction zones were observed during the experiment, confirming a shift from oxic to anoxic conditions at the sediment-water interface as well as in the overlying water and in the sediment column (Metzger et al., 2013). Whereas this change seems to be progressive in the sediment column, the chemical composition of the overlying waters shows a more complex pattern. The "1 month" probes showed a considerable increase of free sulphide hydrogen above the sediment water interface. No such intensive sulphide production was observed in the "2 months" and "10 months" samples. This particular observation caused an inversed sulphide gradient, and consequently, a downward sulphide flux into the sediment. In the "1 month" sample, sulphide was still detected 2 cm below the interface. These observations have been interpreted to reflect the degradation of the dead macrofaunal remains concentrated at the sediment surface. Previous studies at the same site, using the same experimental protocol, have shown that the macrofaunal organisms suffer massive mortality during the first week of anoxia (Riedel et al., 2008, 2012). The geochemical results (Metzger et al., 2013) suggest that the abundant labile organic matter resulting from macrofaunal mortality is consumed in the first month(s) of the experiment. Finally, the geochemical analyses also indicate that nitrates were present both in the overlying water and in the pore waters at all times, without any major changes in vertical distribution (Koron et al., 2013).

To summarize, the pore water chemistry indicates that the oxygen concentration decreased during the first days of the experiment. Anoxia was reached after 7 days, and was maintained until the end of the experiment after 315 days. Sediment geochemistry also suggests that a non-negligible amount of fresh organic matter was added to

the system due to the mortality of the macrofauna. This newly available labile organic matter was consumed in the first month(s) of the experiment.

4.2 Methodological strategy of the study

The present study is original for combining an in situ experiment with the use of the very accurate CTG labeling method (instead of the traditional Rose Bengal staining method) and for the very long duration of the anoxia.

A first advantage of the in situ approach is that it circumvents undesirable laboratory effects, such as stress from transport and/or culture maintenance, reproduction after incubation (e.g. Geslin et al., 2013; Ernst et al., 2006; Murray, 2006), and slightly differing environmental conditions (temperature, light, salinity, etc.). In comparison to non-experimental field studies, our experimental set-up allowed us also to prolong the anoxic period and to avoid the effect of seasonal variation in food supplies. Conversely, our closed set-up blocked food supply from the outside, and toxic components such as sulphides are not dispersed in the water column. Moreover, deployment by scuba divers in 24 m depth limits the available manpower and manipulation time, precluding sampling every month and obtaining a higher temporal resolution. Finally, our cores were taken a few decimeters from each other, potentially resulting in important variation related to spatial patchiness, especially in the distribution of macrofaunal organisms and burrows, and the related biogeochemical processes. Laboratory studies can avoid the effects of patchiness by sieving and homogenizing the sediment, which could in turn affect the results pertaining to more sensitive species (Langezaal et al., 2004). Nonetheless, we think that the advantages of our in situ approach outweigh the disadvantages.

A second advantage of our approach is avoiding the use of Rose Bengal, a bulk-stain which adheres to the proteins in the foraminiferal protoplasm. Since the protoplasm can be preserved weeks to months after death (Boltovskoy and Lena, 1970; Bernhard, 1988; Corliss and Emerson, 1990; Murray and Bowser, 2000), faunal inventories based on Rose Bengal can substantially overestimate foraminiferal densities,

especially in anoxic conditions, where organic matter degradation will be slow (Hannah and Rogerson, 1997; Glud, 2008). It can also yield false positives in experiments in anoxic conditions, calling for caution in interpreting the results of many earlier studies (e.g. Moodley et al., 1997; Heinz et al., 2001; Duijnstee et al., 2003). Our goal of follow-ing the faunal variation on a weekly to monthly scale required using a more accurate vitality discrimination technique. CTG is a fluorescent probe that is hydrolyzed within the foraminiferal cytoplasm by foraminiferal metabolism. Since such enzymatic activity should stop after the death of the organism, the CTG method should very accurately determine living specimens (Bernhard et al., 2006). This assumption has been con-firmed during a comparative study on copepods and nematodes (Grego et al., 2013) using the same experimental design as our study. These authors found that the two methods yielded similar estimations of copepod density but that Rose Bengal yielded higher densities of nematodes in well-oxygenated conditions. After 7 days of anoxia, the Rose Bengal method overestimated (compared to CTG) the density of living ne-matodes and copepods by about 30 % and 50 %, respectively (Grego et al., 2013). Ac-cordingly, especially in anoxic conditions, the new method produces considerably more reliable results. Nevertheless, recognizing pink tests using Rose Bengal and fluores-cent tests using CTG both have a subjective component and call for observer expertise. Several parameters can affect the observer's judgment: the various test types (hyaline, porcelaneous, agglutinated) influence the way the fluorescence of the cytoplasm is seen through the test, the presence of other fluorescent organic material close to the object (e.g. nematodes and copepods appear more fluorescent than foraminifera) can "hide" the foraminiferal fluorescence (we removed these organisms from our samples by centrifugation to avoid such a negative effect), the natural fluorescence of the sedi-ment, and the interspecific and inter-individual variability in the fluorescence intensity. Finally, in some cases the fluorescence intensity appeared low (patchy and "milky"). As with the Rose Bengal the identification of the living individuals can be affected by bacteria colonizing the tests post-mortem. Since living bacteria also react positively to CTG, we assume that the presence of bacterial films could be the cause of such

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a patchy/milky fluorescence. Fortunately, this type of fluorescence can be easily distinguished from actual foraminiferal fluorescence. In the present study the selection of fluorescent individuals was extremely strict; only individuals showing a clear fluorescence were selected and counted as alive. A third advantage of the experiment is the long term duration of the chamber deployment. While previous anoxia incubation experiments on foraminifera lasted up to three months, we extended this period to more than 10 months. Although it cannot be excluded that deeper-burrowing macrofauna may eventually find their way into the chambers, our geochemical analyses indicate that the conditions were strongly reductive and anoxic at the end of the evaluated deployment.

4.3 Foraminiferal survival under the experimental conditions

4.3.1 Foraminiferal survival under anoxia in the presence of hydrogen sulphides

Abundant CTG-labeled specimens were found in all cores. Thus, many foraminifera survived from 1 week to more than 10 months of anoxia, in all sediment layers down to 5 cm depth. To confirm that foraminifera were indeed alive after almost one year of anoxia, a complementary foraminiferal metatranscriptomic analysis was carried out. Ribosomal RNA marker sequences were successfully recovered from total sediment RNA extractions and assigned to diverse foraminifera as in Lejzerowicz et al. (2013). This showed that foraminifera were not only alive but active after 315 days of anoxia. Several groups of foraminifera have been identified by the molecular analysis (Rotaliida, Textulariida and monothalamiids). This molecular study further supports the accuracy of the CTG method.

Notwithstanding the caveats of the Rose Bengal approach, available data strongly suggest that foraminifera are tolerant to anoxia (Bernhard and Alve, 1996; Moodley et al., 1997; Piña-Ochoa et al., 2010b). It is nonetheless surprising that benthic foraminifera are still alive in large numbers after almost one year of anoxia (~ 530 and

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~ 680 individuals per 10 cm^2 down to 5 cm in the two replicate cores). To our knowledge this is the first time that such a long-term survival in anoxic conditions has conclusively been shown.

With the development of anoxia development, hydrogen sulphides were produced. The concentration has been roughly estimated at > 100 µmol L^{-1} in the overlying water of the "1 month" chamber (Metzger et al., 2013). Despite the potential toxicity (Giere, 1993; Fenchel and Finlay, 1995), the foraminiferal faunas survived this strong exposure.

4.3.2 Implications of the experiment for our understanding of the foraminiferal metabolism

We clearly show that foraminifera remain alive in anoxic conditions, in the presence of sulphides, for at least 315 days. Prior to this study, foraminifera were known to survive relatively short periods in anoxic conditions (Alve and Bernhard, 1995; Piña-Ochoa et al., 2010b). Several authors showed that certain foraminiferal taxa can respire nitrates in anoxic conditions (Risgaard-Petersen et al., 2006; Høgslund et al., 2008; Piña-Ochoa et al., 2010a). A gene for nitrate reduction has recently been found in *Bolivina argentea* (Bernhard et al., 2012), suggesting that, in this species, denitrification could at least partially be performed by the foraminifera themselves, and not by symbiotic bacteria, such has been shown for allogromiid foraminifera (Bernhard et al., 2011). Based on denitrification budget calculations, Risgaard-Petersen et al. (2006) estimated that the most efficient species could survive anoxia for 2 to 3 months by using their intracellular nitrate stock. In our experiment, however, all species, including some that do not store nitrate in large quantities (*Bulimina aculeata*, see Piña-Ochoa et al., 2010a, or *Eggerella scabra*, see Langlet et al., 2013), survived almost one year of anoxia. Two explanations can be proposed to explain this discrepancy: some species may indeed denitrify and continuously renew their nitrate stocks (nitrates are always available in pore and bottom waters; Koron et al., 2013). Others may shift to other

survival strategies, such as drastically decreasing their metabolic rates, or use as yet undescribed metabolic pathways.

4.4 Variations of the foraminiferal densities

Overall foraminiferal densities significantly decreased with time (Table 4, Model 2), but the values after one month are somewhat higher. This difference is much clearer in the values from the topmost 0.5 cm than from the whole core. In fact, the temporal density variation does not follow a gradual decrease. In the 0–0.5 cm depth interval the standing stocks in the "Normoxia" and the "1 month" chambers are both significantly higher than those in the "7 days" and "2 months" chambers. The cores of the latter two (which are not significantly different) both have significantly higher standing stocks in the 0–0.5 cm interval than the "10 months" cores (Table 4, Model 3). We hypothesize that these density variations are explained either by spatial variability, the effect of the anoxia, or by labile organic matter availability. These three hypotheses are discussed below.

4.4.1 Spatial heterogeneity versus temporal variability

Since the living fauna has been analyzed in two replicate cores, we can to some extent assess the spatial variability of the meiofauna at a decimetric spatial scale. The two replicate cores that differ most are the "Normoxia" cores sampled before the beginning of the experiment, with total (0–5 cm) standing stocks of ~ 1980 and ~ 820 individuals per 10 cm^2, respectively. This difference is not due to a different vertical distribution, which is fairly similar (Fig. 3): the density differs at all depths. Considerable patchiness at this site is the logical explanation. The substrate here is a poorly sorted silty sand, which is colonized by very patchy macro-epibenthic assemblages, defined as multi-species clumps or bioherms (Fedra et al., 1976; Stachowitsch, 1984, 1991). Also the sediment geochemistry shows a non-negligible variability in the intensity and depth of the major diagenetic reactions (Metzger et al., 2013). We expect that the areas devoid

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of visible macro-epifauna (such as those selected for this experiment) also show important spatial patchiness. Burrowing activities of infaunal macrofauna, for example, may have affected the homogeneity of the sediment column. The presence of burrows generally leads to a deeper oxygen penetration depth (Aller, 1988), which can explain the higher infaunal foraminiferal standing stocks (e.g. Jorissen, 2003; Loubere et al., 2011; Phipps et al., 2012).

For comparison, at a 14.5 m-deep site located at the northern Gulf of Trieste, Hohenegger et al. (1993) compared the faunal composition (Rose Bengal method) of 16 sediment cores (4.8 cm inner diameter, as in our study), sampled in a single 1 m^2 surface. Foraminiferal standing stocks varied from $\sim$ 2450 to $\sim$ 6300 individuals per 100 cm^2, with an average of $\sim$ 3500 individuals per 100 cm^2 (Hohenegger et al., 1993). The authors explained this by the influence of burrows and specific food requirements. The ratio of about 2.6 between the richest and poorest cores is very similar to the maximum ratio of 2.4 found for our replicate cores at the beginning of the experiment. Such a difference may be typical for shallow Gulf of Trieste sites.

The spatial heterogeneity at our study site precludes definitively interpreting the differences in foraminiferal densities between the different chambers (and different sampling times) as being entirely the consequence of the experimental conditions. Nevertheless, all statistical tests indicate that the intra-chamber effect is less important than the other tested variables (Time, Sample ID or depth). Accordingly, experimental conditions appear to have a stronger impact on density differences between pairs of replicate cores than spatial patchiness.

4.4.2 Effect of anoxia and hydrogen sulphides on the foraminiferal faunas

The foraminiferal standing stocks exhibit a significant exponential decrease with the log transformed experiment time (Fig. 2). In detail, the densities do not decrease gradually with time, the highest densities occur in the initial conditions and after 1 month of anoxia, the densities are lower in the "7 days" and "2 months" cores and the lowest densities are observed in the "1 year" anoxia samples. If the observed density changes in

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the 0–0.5 cm interval are not entirely due to spatial patchiness, standing stocks could be negatively affected by the development of anoxia in the first week (Fig. 1). However, in an earlier experiment using the same protocol (in situ incubation, CTG probe), performed in 2009 at the same site, no significant difference in foraminiferal density was observed between a reference core (normoxic conditions) and a core sampled after 7 days of anoxia (Geslin, unpublished data). Also a previous experiment based on Rose Bengal-stained organisms using Adriatic Sea faunas did not show a significant impact of up to two months of anoxia on the hard-shelled foraminiferal density (Moodley et al., 1997). Conversely, in a study on the temporal variability of foraminiferal densities in the Northern Adriatic Sea (close to the Po delta), total foraminiferal standing stocks tended to decrease by roughly 20 % during periods of bottom water hypoxia in late summer (Duijnstee et al., 2004). A previous laboratory study conducted in the Northern Adriatic Sea (also using RB staining) found lower standing stocks (roughly 30 % fewer individuals) in cores incubated for 1.5 months in anoxic conditions (Ernst et al., 2005). Furthermore, in view of the survival of many foraminifera (of all common species) to more than 10 months of anoxia, the decrease in density in the first week can hardly reflect decreasing oxygen concentration, especially since the sediment surface was oxic most of the time. This observation and the literature suggest that short-term anoxia (0 to 1 month) cannot explain the decrease of foraminiferal densities; the observed differences might be explained by spatial patchiness. Nevertheless, the overall decrease of foraminiferal densities suggests that long-term anoxia (> 2 months) could negatively impact density.

In the present study, the vertical density profiles (Fig. 3) are very similar for all experimental cores, and are not significantly different from the "Normoxia" cores. In some other studies (Alve and Bernhard, 1995; Duijnstee et al., 2003; Ernst et al., 2005; Pucci et al., 2009), densities between 2 and 4 cm decreased rapidly after the onset of hypoxia/anoxia. This was ascribed to upward migration. In our study, apparently no major vertical migration took place. The standing stocks in the whole cores (0–5 cm) do not differ significantly over time, unlike the standing stock in the first depth interval

(0–0.5 cm). This difference may reflect biogeochemical changes (Metzger et al., 2013): they were much more important in the uppermost sediment interval (which became anoxic, where macrofaunal mortality and degradation resulted in hydrogen sulphides release) than in deeper sediment intervals, which were already anoxic at the start of the experiment (oxygen penetration about 0.5 cm).

Benthic foraminifera were still alive in large numbers in the "1 month" cores, for which the estimated hydrogen sulphides concentration in the water overlying the sediment exceeded 100 µmol L^{-1} (Metzger et al., 2013). The toxic nature of hydrogen sulphides could help explain the density decrease between the "1 month" and "2 months" cores (average values of $\sim$ 930 and $\sim$ 380 individuals per 10 cm^3 in the topmost 0–0.5 cm depth interval, respectively). In an earlier laboratory experiment using Northern Adriatic Sea sediments, Moodley et al. (1998) found that a 6 to 12 µmol L^{-1} concentration of dissolved sulphides led to a significant decrease of the Rose Bengal-stained foraminiferal densities, from about 700 to 200 individuals per 10 cm^3 after 30 days of incubation, corresponding to about 4 % per day. In our study the observed average decrease between 29 and 58 days from $\sim$ 940 to $\sim$ 760 individuals per 10 cm^3 in the 0–5 cm interval, or from 460 to 200 individuals per 10 cm^3 in the 0–0.5 cm layer, corresponds to a loss of 0.75 % and 1.3 % per day, respectively. These values are in the same order of magnitude as those obtained by Moodley et al. (1998). Thus, despite of the presence of high, toxic hydrogen sulphides concentrations (Giere, 1993; Fenchel and Finlay, 1995), a substantial part of the foraminifera survived up to 315 days of anoxic and sulphidic experimental conditions. The higher mortalities in other studies (Moodley et al., 1988) may be due to the presence of additional stress factors in their experimental set-ups.

4.4.3 Potential response to labile organic matter availability

In the present experimental set-up, the availability of labile organic matter is another factor that could limit foraminiferal survival: the sealed benthic chamber blocks the sediment organic matter input by the pelagic system. This should considerably reduce labile organic matter availability and potentially starve the benthic faunas. Unfortunately

no complete (and conclusive) Organic Carbon dataset is available to confirm such a trend. Nevertheless, indirect indications from the geochemical analyses do show that organic matter availability varied during the experiment. A large input of hydrogen sulphides in the overlying water took place after one month of anoxia. Usually sulphides are produced deep in the sediment and diffuse upwards. Conversely, after 1 month of anoxia, the source of the sulphide pool was positioned at the sediment water interface (Metzger et al., 2013). This shallow production of sulphides has been interpreted as due to the death of macrofaunal organisms in the early stages of the experiment, and their subsequent anaerobic degradation.

Unexpectedly, the geochemical data suggest that the organic matter content at the sediment-water interface did not decrease with time (due to the sealing of the chamber), but rather increased, peaking after 1 month, due to macrofaunal decay. The coincidence between the periods with maximum foraminiferal standing stocks and maximum estimated organic matter availability (at "Normoxia" and "1 month") suggests a causal relationship between the two parameters. Numerous previous studies show a clear foraminiferal response to organic matter input (e.g., Heinz et al., 2001; Ernst and van der Zwaan, 2004; Duijnstee et al., 2005; Ernst et al., 2005; Nomaki et al., 2005; Pucci et al., 2009). These results suggest that increased labile organic matter (macrofaunal remains) could promote foraminiferal densities in the topmost sediment layer, despite the anoxic conditions. Consequently, organic matter availability would have a stronger effect on foraminiferal density than both anoxia and hydrogen sulphides.

5 Conclusions

We performed an in situ experiment at a 24 m-deep site in the Gulf of Trieste. Benthic chambers containing abundant benthic foraminiferal faunas were installed on the sediment surface and rapidly turned anoxic. Cores sampled in chambers opened after 1 week, 1 month, 2 months and 315 days all contained abundant foraminiferal assemblages down to 5 cm depth in the sediment. Benthic foraminifera in the Gulf of Trieste

are therefore capable of surviving anoxia with co-occurring sulphides for at least 315 days. Large differences between some of the replicate cores point to considerable spatial patchiness, which may be related to the irregular distribution of macro-infaunal burrowing activity, somewhat hampering the interpretation of the temporal trends in density. Nonetheless, our data show an exponential decrease in densities over time. Closer examination suggests slightly increased densities in the topmost 0.5 cm after one month, which we tentatively interpret as a response to increased labile organic matter availability due to macrofaunal mortality at the beginning of the experiment.

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Table 1. Deployment and sampling dates as well as duration of the experimental period for the cores representing in situ conditions ("Normoxia") and the various periods of anoxia in the four different benthic chambers.

Sample ID	Chamber deployment day	Core sampling day	Incubation duration
Normoxia	–	3 Aug 2010	0 days
7 days	2 Aug 2010	11 Aug 2010	9 days
1 month	27 Jul 2010	25 Aug 2010	29 days
2 months	27 Jul 2010	23 Sep 2010	58 days
10 months	24 Sep 2010	5 Aug 2011	315 days

Table 2. Summary of the variables used in each of the three linear models, describing the name of the variable, its type (dependent or independent), its unit, nature (quantitative or qualitative) and the used transformation. For the quantitative variables the minimum and maximum values of the non transformed data are presented in brackets, whereas for the qualitative variables the different categories are presented in brackets.

Name	Variable Type	Unit	Variable nature (values)	Transformation
Model 1: Analysis of variance				
Standing Stock (0–5 cm)	Dependent	Ind./10 cm^2	Quantitative (min = 533; max = 1979)	log (1 + Standing Stock)
Sample ID	Independent	–	Qualitative ("Normoxia", "7 days", "1 month", "2 months" and "10 months")	–
Model 2: Analysis of variance				
Standing Stock (0–0.5 cm)	Dependent	Ind./10 cm^2	Quantitative (min = 115; max = 529)	log (1 + Standing Stock)
Sample ID	Independent	–	Qualitative ("Normoxia", "7 days", "1 month", "2 months" and "10 months")	–
Model 3: Analysis of covariance				
Standing Stock	Dependent	Ind./10 cm^2	Quantitative (min = 115; max = 1979)	log (1 + Standing Stock)
Depth Interval	Independent	–	Qualitative ("0–5 cm" and "0–0.5 cm")	–
Time	Independent	days	Quantitative (min = 0; max = 315)	log (5 + Time)
Depth Interval × Time	Independent	–	interaction	–
Model 4: Analysis of covariance				
Density	Dependent	Ind./10 cm^3	Quantitative (min = 46; max = 1059)	log (1 + Density)
Depth	Independent	cm	Quantitative (min = 0.25; max = 4.5)	log (1 + Depth)
Depth^2	Independent	cm	Quantitative (min = 0.25; max = 4.5)	(log (1 + Depth))^2
Sample ID	Independent	–	Qualitative ("Normoxia", "7 days", "1 month", "2 months" and "10 months")	–
Depth × Depth^2	Independent	–	interaction	–
Depth × Sample ID	Independent	–	interaction	–
Depth^2 × Sample ID	Independent	–	interaction	–

Table 3. Total counts and standing stocks at every sampling time for every replicate core.

Sample ID	Time (days)	Replicate core	Total count (individuals)	Standing stock (indiv/10 cm^2)
Normoxia	0	core1	3289	1979
Normoxia	0	core2	1368	823
7 days	9	core1	1422	856
7 days	9	core2	1241	747
1 month	29	core1	1834	1104
1 month	29	core2	1291	777
2 months	58	core1	1263	760
2 months	58	core2	1274	767
10 months	315	core1	886	533
10 months	315	core2	1131	681

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Table 4. Results of the Models 1, 2, 3 and 4. For each Model, the degrees of freedom (Df), the sum and mean of squares (SumSq. And MeanSq.), the F value (F) and its p value (p) are given for each variable. The p values indicate whether the variable has a significant effect ($p < 0.05$) or not ($p > 0.05$).

|	Df	SumSq.	MeanSq.	F	p
Model 1					
Sample ID	4	0.6	0.2	1.6	0.31
Residuals	5	0.5	0.1		
Model 2					
Sample ID	4	2.4	0.6	16.2	0.005
Residuals	5	0.2	0.0		
Model 3					
log(5+Time)	1	1.8	1.8	16.3	0.001
Depth Interval	1	7.3	7.3	66.4	4.37E-07
log(5+Time) × Depth Interval	1	0.1	0.1	1.1	0.32
Residuals	16	1.7	0.1		
Model 4					
Sample ID	4	3.5	0.9	7.1	1.12E-04
log(1+Depth)	1	16.7	16.7	134.6	2.77E-16
log(1+Depth)^2	1	2.7	2.7	22.0	1.89E-05
Sample ID × log(1+Depth)	4	3.1	0.8	6.3	3.25E-04
log(1+Depth) × log(1+Depth)^2	1	0.5	0.5	4.1	0.048
Sample ID × log(1+Depth)^2	4	1.0	0.3	2.0	0.10
Residuals	54	6.7	0.1		
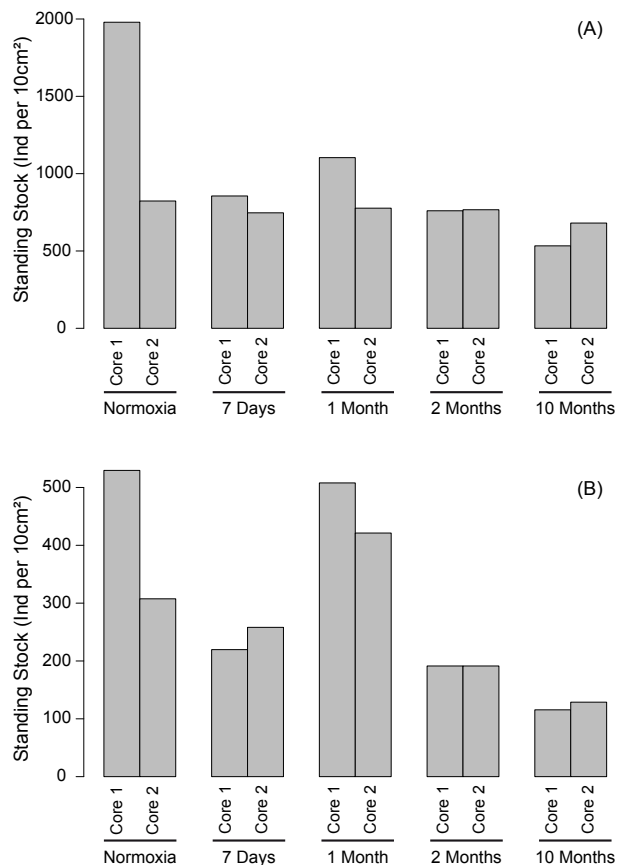
Fig. 1. Foraminiferal standing stocks in all sampled cores for the whole cores (0–5 cm; **A**) and for the 0–0.5 cm depth intervals **(B)**.
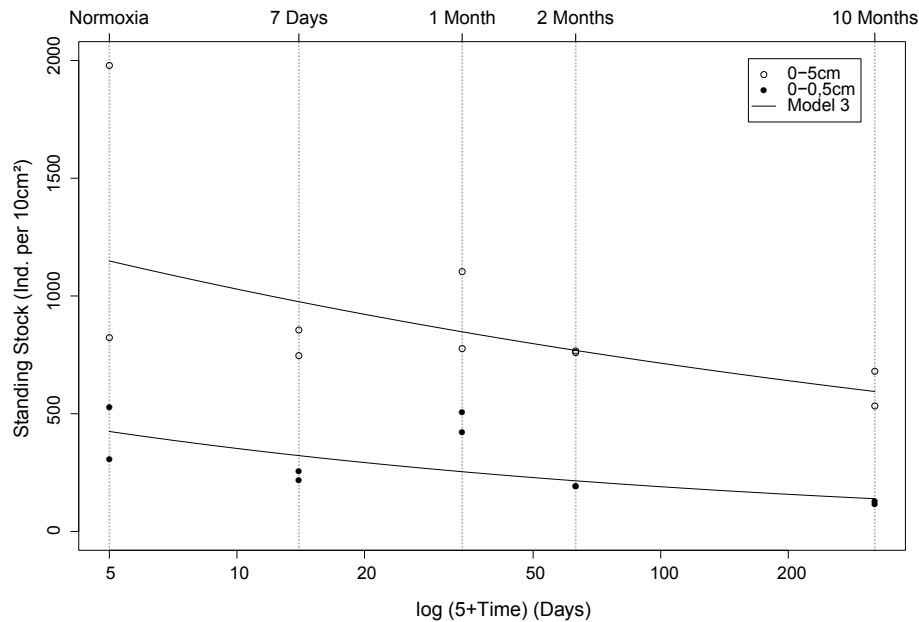
Fig. 2. Foraminiferal standing stocks plotted against deployment time. Open circles represent standing stock in the 0–5 cm depth interval, full black circles correspond to the standing stock in 0–0.5 cm depth. Full black lines represent the modeled standing stocks plotted against time, for the two depth intervals, after Model 3. Note that incubation time is represented on a transformed log scale (5 + time).
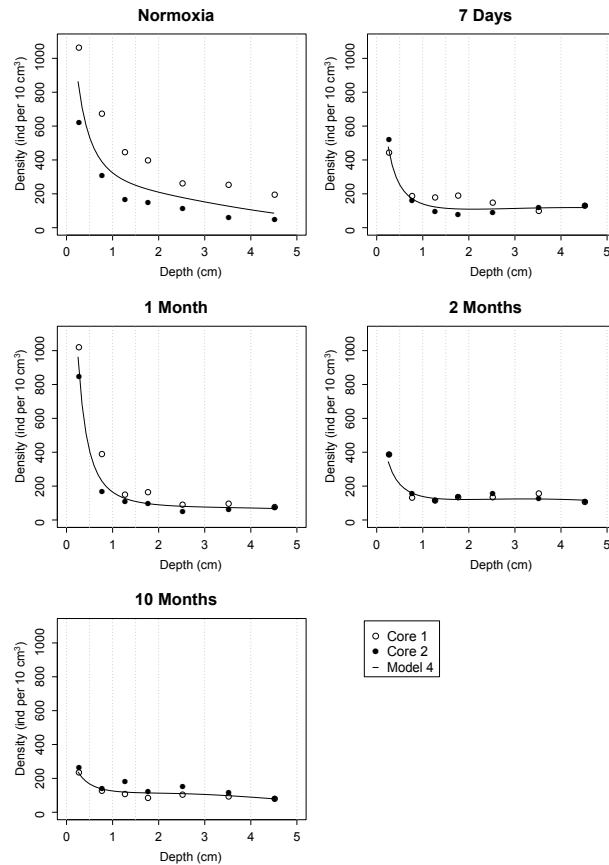
Fig. 3. Foraminiferal density plotted against sediment depth for all sampled cores. Open circles represent the density in replicate core 1, full black circles that in core 2. Full black line represents the modeled density according to Model 4.
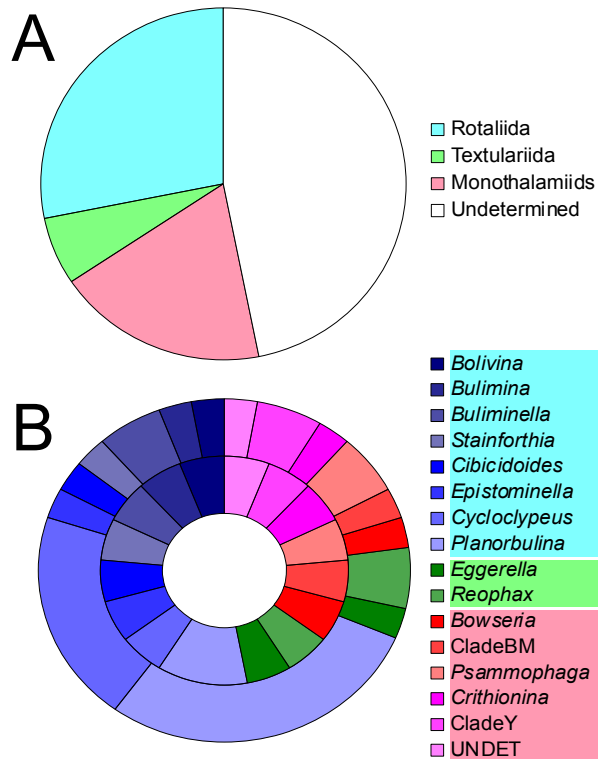
Fig. 4. Taxonomic composition of the foraminiferal metatranscriptomic data from the top centimeter of the sediment in the "10 months" chamber. **(A)** Relative proportions of the 32 OTUs assigned to the order level or unassigned. **(B)** Relative proportion of OTUs (inner circle) and sequences (outer circle) assigned to the genus level.