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# Foraminiferal species responses to in situ experimentally induced anoxia in the Adriatic Sea

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#### Abstract

Anoxia was successfully induced in four benthic chambers installed at 24 m depth in the northern Adriatic Sea, for periods varying from 9 days to 10 months. During the 10 months period, species richness significantly decreased. Although no significant change in Shannon diversity and Evenness is observed, the composition of the

- <sup>5</sup> cant change in Shannon diversity and Evenness is observed, the composition of the foraminiferal assemblages changes with time. This change is due to interspecific differences in tolerance with respect to anoxia and free sulphides. *Leptohalysis scottii, Textularia agglutinans* and *Quinqueloculina* cf. *stelligera* all showed a significant decrease with time, strongly suggesting they are sensitive to the anoxia and sulphides. Con-
- versely, Eggerella scabra, Bulimina marginata, Lagenammina atlantica, Hopkinsina pacifica and Bolivina pseudoplicata appear to be resistant to the experimental conditions. Quinqueloculina seminula also appears to be sensitive to anoxia but shows a clear standing stock increase during the first month of the experiment, which we interpret as an opportunistic response to increasing organic meters availability due to the
- degradation of the dead macrofaunal organisms. It appeared that none of the anoxia sensitive species is capable to accumulate intracellular nitrates. Such a capacity could be shown for some tested specimens of the dominant anoxia tolerant species *E. scabra* and *B. marginata*. However, tests on the denitrification capacity of these taxa yielded negative results, suggesting that their resistance to long-term anoxia is not due to a
- 20 capacity to denitrify.

#### 1 Introduction

Over the last decades, bottom water hypoxia has occurred increasing number of coastal areas as a result of human-induced eutrophication. For semi-enclosed basins, hypoxia may appear seasonally as a response to combined pelagic productivity and water column stratification. In the benthic environment increased organic matter con-

<sup>25</sup> water column stratification. In the benthic environment, increased organic matter consumption leads to decreased dissolved oxygen concentration when seasonal water Discussion Paper

column stratification  $\implies$  its oxygen renewal. Seasonal oxygen depletion has been observed in the Adriatic Sea (e.g. Stachowitsch, 1984; Jorissen et al., 1992) with different intensity, varying from hypoxia (defined as < 63 µmol L<sup>-1</sup>; Helly and Levin, 2004) to anoxia (defined as 0 µmol L<sup>-1</sup>; Bernhard and Sen Gupta, 1999; Middelburg and Levin, 2009). The frequency of these low oxygen events increased from the 1970s to the

1990s, to decrease again after the 1990s as a result of reduced continental nutrient inputs (Giani et al., 2012).

Oxygen depletion events can drastically affect benthic faunas. Macrofaunal organisms (> 1 mm) are apparently more sensitive than meiofaunal organisms (< 1 mm) to

- hypoxic and anoxic conditions (Moodley et al., 1997; Diaz and Rosenberg, 2008). Among meiofauna, copepods are most sensitive, whereas several nematode genera can survive up to 60 days of anoxia (Wieser and Kanwisher, 1961). Benthic foraminifera appear to be most tolerant (Moodley et al., 1997) and can survive up to 10 months of anoxia (Langlet et al., 2013) rnhard and Reimers, 1991). This surprisingly long pe-
- riod may be due to a shift to anaerobic metabolism: the eukaryotic benthic foraminifera are capable of accumulating large quantities of nitrates in their cells and of denitrifying during anoxia (Risgaard-Petersen et al., 2006; Høgslund et al., 2008; Piña-Ochoa et al., 2010; Bernhard et al., 2012). Nonetheless, despite their high to prove to anoxia, foraminiferal density and diversity tend to decrease in low oxygenation environments
- 20 (Blackwelder et al., 1996; Schumacher et al., 2007; Bouchet et al., 2012). Furthermore, not all foraminiferal taxa respond similarly. For instance, in the Adriatic Sea some taxa (*Nouria polymorphoides, Leptohalysis scottii* and *Textularia* spp.) appear to be sensitive to hypoxia/anoxia, whereas others (*Nonionella turgida, Bolivina* spp., *Eggerella* spp., *Bulimina* spp., *Hopkinsina pacifica* and *Stainforthia fusiformis*) are more toler-
- ant (Jorissen et al., 1992; Moodley et al., 1998; Duijnstee et al., 2004; Ernst et al., 2005; Pucci et al., 2009). Note, however, that these valuable earlier results cannot be taken at face value ause they are based on inventories of Rose Bengal stained faunas. Rose Bengal is a bulk stain which adheres to proteins in the protoplasm (Walton, 1952; Bernhard, 2000). Since foraminiferal protoplasm may remain present after

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death of the organism, Rose Bengal can stain a non-negligible number of dead organisms (Boltovskoy and Lena, 1970; Bernhard, 1988; Hannah and Rogerson, 1997; Murray and Bowser, 2000). The amount of false positives could even be more important in anoxic and hypoxic conditions due to the slow degradation of organic matter (Bur-

- <sup>5</sup> dige, 2006; Glud, 2008). To avoid such artefacts we used CellTracker Green<sup>TM</sup> (CTG) to recognize living individuals. CTG is a non-fluorescent probe that passes through the cellular membrane into the cytoplasm, where hydrolysis with nonspecific esterase produces a fluoroscent compound (Bernhard et al., 2006). Consequently, fluorescent specimens were enzymatically active when sampled. This approach enabled us to ac-
- curately determine live individuals in our experimentally induced anoxia, and to distinguish between species more tolerant or more sensitive to anoxia.

The present study focuses on the northeastern Adriatic Sea, specifically the Gulf of Trieste. To control the environmental conditions as best as possible, we adopted an in situ approach and experimentally generated anoxia by placing benthic chambers on the

- <sup>15</sup> sea floor. The foraminiferal analyses reported in this study and in Langlet et al. (2013) are complemented by geochemical analyses, demonstrating the changes of redox conditions during the experiment (Koron et al., 2013; Metzger et al., 2013). Studies of macrofaunal behaviour (Blasnig et al., 2013; Riedel et al., 2008, 2012, 2013) and of copepod and nematode assemblages (De Troch et al., 2013; Grego et al., 2013) show
- the impact of experimentally induced anoxia on other biota. In a first paper Langlet et al. (2017), we determined the effect of anoxia on the total foraminiferal standing stocks. Benthic foraminifera were alive at all sampling times, proving that foraminifera can survive up to 10 months of anoxia with co-occurring hydrogen sulphides. In this second study we focus on the effects of long-term anoxia on individual species, and on
- the resulting biodiversity. We determine how the density of the major species change in response to anoxia, and distinguish between more or less resistant taxa. Finally, we investigate whether survival of tol species can be explained by a shift to an anaerobic metabolism (denitrification).

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#### 2 Material and methods

### 2.1 Study area

Two sites were sampled for the present study: a site in the Gulf of Trieste where the incubation experiment was conducted and a second site in the NW Adriatic Sea, where

- we collected living foraminifera for our laboratory experiments. The field experiment was conducted in the Gulf of Trieste (Northern Adriatic Sea) near the oceanographic buoy of the Piran Marine Biology Station (45° 32.90' N, 13° 33.00' E) at 24 m depth, on a poorly sorted silty sandy bottom experimental set-up has been adapted from an earlier experiment (Stachoriticch et al., 2007; Riedel et al., 2008) and is explained in detail in Langlet et al. (2015, in the field, we used the Experimental Anoxia Generating
- detail in Langlet et al. (201) the field, we used the Experimental Anoxia Generating Unit (EAGU), which is a 0.125 m<sup>3</sup> fully equipped benthic chamber which enables experimentally inducing and documenting small-scale anoxia (Stachowitsch et al., 2007). In the current study, we used four different chambers to produce anoxia for different periods of time (Table 1). The four chambers were installed on the sea floor, several
- meters apart, on substrates which were visually poor in macrofauna. Two reference 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 successive provide the terminology for all articles of the present Biogeosciences Special Issue, the successive provide the terminology for all articles of the present Biogeosciences Special Issue, the successive provide the terminology for all articles of the present Biogeosciences Special Issue, the successive provide the terminology for all articles of the present Biogeosciences Special Issue, the successive provide the terminology for all articles of the present Biogeosciences Special Issue, the successive provide the terminology for all articles of the present Biogeosciences Special Issue, the successive provide the terminology for all articles of the present Biogeosciences Special Issue, the successive provide the terminology for all articles of the present Biogeosciences Special Issue, the successive provide the terminology for all articles of the present Biogeosciences Special Issue, the successive provide the terminology for all articles of the present Biogeosciences Special Issue, the successive provide the terminology for all articles of the present Biogeosciences Special Issue, the successive provide the terminology for all articles of the present Biogeosciences (terminology for all articles of the present Biogeosciences Special Issue, the successive provide the terminology for all articles of the present Biogeosciences (terminology for all articles of terminology for all articles of terminology for all articles of terminology for all articles (terminology for all articles of terminology for all articles of
- in Table 1. The first chamber, fully equipped with analytical devices, documented the onset of anoxia. The other three chambers, without oxygen sensors, were used to study the development of 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 inpact diameter (16 c cm<sup>2</sup> gurface corea). The replicate cores were taken by scuba divers using a plexiglas core with a same cores.
- 4.6 cm inner diameter (16.6 cm<sup>2</sup> surface area). Two replicate cores were taken in each of the benthic chambers at each sampling time.

Reliably measuring intracellular nitrate and denitrification requires a large number of fresh and living individuals. Therefore, in September 2012, sediment samples were

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collected with a Van Veen grab at 12 m depth at Portonovo Bay station D10 (43° 35.5′; 13° 34.9′), close to the site studied by Sabbatini et al. (2012). Samples were kept in plastic bottles in well oxygenated sea water (the lid of the bottles partly open) at in situ salinity and temperature (S = 35 and T = 22°C) until further analysis.

# 5 2.2 Faunal analysis

The methodology of the faunal analyses is described in detail in Langlet et al. (2013). Sediment cores of 4.6 cm inner diameter were sliced in 7 depth intervals, every 0.5 cm between 0 and 2 cm and every cm from 2 down to 5 cm. Each sediment slice was mixed with sea water, to which a CTG-DMSO (CellTracker<sup>TM</sup> Green CMFDA (5-

- <sup>10</sup> Chloromethylfluorescein Diacetate); Molecular Probes, Invitrogen Detection Technologies and Dimethyl sulfoxide) solution was added, with a final CTG concentration of 1 μmolL<sup>-1</sup> (Bernhard et al., 2006; Pucci et al., 2009). Next, the samples were kept in the dark at in situ temperature for at least 10 h, during which the originally non-fluorescent CTG molecule is hydrolysed by the living individuals and transformed
- into a molecule which fluoresces when excited at a specific wave-length. In order to perform the faunal analysis at a later stage, the CTG-reaction was fixed with Borax-buffered formalin (4%).

A specific aspect of this project is that soft-shelled (nematodes, copepods) and hardshelled meiofaunal organisms (foraminifera) have been studied in the same samples

- (De Troch et al., 2013; Grego et al., 2013; Langlet et al., 2013). In order to separate these two groups of organisms, a density separation was performed by adding a Levasil solution to the sample and centrifuging it at 3000 rpm for 10 min (McIntyre and Warwick, 1984; Burgess, 2001).
- sizes (63, 125, 150, 315 and 500 μm). Thus, soft-shelled foraminifera which are likely to be found in the supernatant after centrifugation are not considered in the present study. Samples were kept in a borax-buffered formalin solution until further analysis.

Foraminifera from the 63–125  $\mu$ m size fraction were separated from the sediment by the tetrachlorcarbon density separation method (e.g., Hohenegger et al., 1989; Murray, 2006) as described by Langlet et al. (2013). No density separation was performed for the size fractions larger than 125  $\mu$ m. Samples from all fractions were analyzed under an epifluorescence stereomicroscope (Olympus SZX12 with a light fluorescent source

Olympus URFL-T or Nikon SMZ 1500 with a PRIOR Lumen 200), and only clearly fluorescent individuals were considered as living.

#### 2.3 Nitrate content and denitrification measurements

The samples for living foraminifera, used in the denitrification experiments, have been selected from well oxygenated substrates (not from anoxic benthic chambers) from both study areas (Piran and station D10; see Sect. 2.1). These samples were kept under oxygenated conditions at all times Foraminifera were put on a thin layer of sediment (<  $38 \mu$ m), and living individuals, which moved on the sediment film, were picked and washed at least 3 times with a brush in nitrate-free artificial sea water, before being put

- <sup>15</sup> in a 1 mL centrifugation tube. Samples were kept at -20 °C until further analysis. The intracellular nitrate content of single specimens was measured using the VCl<sub>3</sub> reduction method and detected by chemiluminescence as described previously (Risgaard-Petersen et al., 2006; Høgslund et al., 2008). For the present study a total number of 33 individuals were measured for 7 different species.
- For denitrification measurements a maximum number of 7 living foraminifera was placed in an anoxic tube of 0.5 mm inner diameter containing nitrate-free sea water and acetylene (Risgaard-Petersen et al., 2006; Høgslund et al., 2008). Acetylene inhibits nitrous oxide reductase and therefore makes nitrous oxide the end product of denitrification. Vertical measurements of the nitrous oxide concentration were performed
- with a nitrous oxide microsensor with a 20  $\mu m$  tip diameter (Andersen et al., 2001), positioned  $\sim 100\,\mu m$  above the foraminifera. Vertical profiling enables identifying the production of N<sub>2</sub>O due to denitrification. By modelling the nitrous oxide production gradient, a denitrification rate can be determined according to Fick's first law of diffusion.

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a total of five experiments were performed. Note that some of these experiments are pseudoreplicates because some individuals from the first set of measurements were re-used in the second experiment, after adding some more individuals in the measurement chamber.

#### 5 2.4 Statistical analysis

After identifying all living individuals, they were sorted into different taxa, counted and expressed as standing stock (number of living individuals in the 0–5 cm depth interval normalized for a 10 cm<sup>2</sup> surface area), density (number of living individuals per depth interval normalized for a 10 cm<sup>3</sup> sediment volume) and relative frequency (percentage of a encoder of the total formal). Then, Special Biotheses the Shappen

of a species with respect to the total fauna). Then, Species Richness, the Shannon index and Evenness was calculated (e.g. Hayek and Buzas, 1997).

ters different procedures were used to estimate the effect of the different parameters the foraminiferal standing stocks and diversity indices systematically used Linear Models (Chambers and Hastie, 1992) in which the dependent (response) vari-

- <sup>15</sup> able must follow a normal distribution. The statistical analysis were performed with R version 2.14 (R Development Core Team, 2011). Therefore, data transformation was sometimes necessary. In these cases, we applied a log transformation for standing stocks and density, and an arcsin transformation for relative frequencies. Each of our four groups of models, which consider one to four dependent variables, is applied on
- <sup>20</sup> different data sets (see column "data selection" in Table 2). In Models 1, 2 and 4 the number of independent variables is reduced by backward selection (i.e. variables that do not have a significant effect on the dependent variable are progressively removed) to retain only the variables with a significant effect on the dependent variable.
- The first group of models tests the effect of Time and Time<sup>2</sup> on the Standing stock, Species richness, Shannon index and Evennes bur different samples categories (the > 125  $\mu$ m or > 63  $\mu$ m size fractions in the whole 0–5 cm core, or in the 0–0.5 cm interval).

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The second group of models is designed to identify whether Time, Time, Depth and their interactions significantly affect the standing stocks and relative frequencies of the major species (> 4 % in at least one core). The third group of models considers the vertical distribution of all major species. Note that in these models the dependent

variable is the standing stock at each sampling time, whereas the Time effect has not been considered.

Finally, the fourth group of models tests the effect of Time and Depth and their interaction on the total standing stocks and the cumulative relative frequencies of three groups of species, which have been selected in function of the results of the second

<sup>10</sup> group of models. Tables 3, 4, and 8 show only those dependent variables with a significant effect, whereas Table 5 and Supplement 4, Supplement 6 show how each variable affects the dependent variables.

#### 3 Results

#### 3.1 Effect of anoxia on standing stocks and diversity

- <sup>15</sup> In a first paper (Langlet et al., 2013), we showed that foraminifera are alive at all sampling times in fairly large numbers (Fig. 1). In the > 63  $\mu$ m fraction, total standing stocks in the whole (0–5 cm) cores varied ween ~ 1980 individuals 10 cm<sup>-2</sup> at the beginning of the experiment and ~ 530 inor 10 cm<sup>-2</sup> at the end. Both in the whole cores and in the 0–0.5 cm level, we observed a significant decrease with Time for the > 63  $\mu$ m
- fraction (Table 3 and Supplement 6). According to the data presented in this paper, also species richness tended to decrease with Time (Fig. 1). While 73 species were found in total, not all of them were found in all the cores. A maximum of 63 species was observed in the >  $63 \,\mu$ m fraction of one of the "Normoxia" cores (0–5 cm). Minimal values (49 species) were found in the "10 Months" cores (Fig. 1). Species richness
- $_{25}$  significantly decreased with Time in three out of the four treatments (0–5 cm,  $>63\,\mu m$  and  $>125\,\mu m$ ; 0–0.5 cm,  $>125\,\mu m$  (Table 3 and Supplement 6).

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Both other diversity indicators (Shannon Index and Evenness) showed important differences between the 4 treatments (Fig. 1). In the > 63  $\mu$ m fraction of the whole cores, the Shannon Index attained a maximum of 2.8 in one of the "Normoxia" cores. The Shannon Index showed a significant decrease with Time in the > 125  $\mu$ m fraction,

<sup>5</sup> but not in the > 63 µm fraction (Table 3 and Supplement 6). Evenness showed maximum values (from 0.64 to 0.85) in the > 125 µm fraction of 0–0.5 cm level. Only in the > 125 µm of the whole core 5 cm) a significant decrease with Time was observed (Table 3 and Supplement 6) $\nu$ 

#### 3.2 Species response to the experimental conditions

Here, we considered the nine major species (≥ 4% in one of the cores). The effect of Time, Time<sup>2</sup> and Depth on their standing stocks and relative frequencies was tested by backward variable selection in order to retain only the independent variables that have a significant effect on the dependent variable results of the analysis of variance are presented in Table 4, the estimation of the coefficients associated to the independent variables in Table 5.

Supplement 1 showed their absolute densities and relative frequencies in the whole cores (0–5 cm). Leptohalysis scottii, Quinqueloculina seminula, Hopkinsina pacifica, Bolivina pseudoplicata and Quinqueloculina cf. stelligera were largely restricted to the  $63-125\,\mu$ m fraction, whereas Bulimina marginata was mainly found in size fractions

<sup>20</sup> below 150 μm. Eggerella scabra and Textularia agglutinans occurred in all size fractions below 315 μm, whereas Lagenammina atlantica was present almost exclusively in the > 125 μm fraction.

The standing stock of *L. scottii* varied from ~ 780 ind. 10 cm<sup>-2</sup> in one of the two "Normoxic" cores to ~ 10 ind. 10 cm<sup>2</sup> in the "1 Year" cores (Fig. 2). The standing stocks significantly decreased with Time (Fig. 3 and Table 4), although a slight increase was observed in one of the "1 Month" cores. Also for *E. scabra* the highest standing stocks were recorded in one of the "Normoxic" cores (~ 330 ind. 10 cm<sup>-2</sup>), with the lowest standing stocks in the "9 Days" and "1 Month" samples (110 to 170 ind. 10 cm<sup>-2</sup>). The

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standing stock increased slightly after 1 month (Fig. 2), but this trend was not significant (Table 4).

Very similarly, also for *B. marginata* the highest standing stock was observed in one of the "Normoxic" cores ( $\sim 150$  ind.  $10 \text{ cm}^{-2}$ ), the lowest values in the "9 Days" sam-

- <sup>5</sup> ples (~ 50 ind.  $10 \text{ cm}^{-2}$ ; Fig. 2). Also for this species, the standing stock seemed to decrease in the first week, to remain stable thereafter until the end of the experiment. There was, however, no significant change with Time (Fig. 3 and Table 4). *Textularia agglutinans* showed a significant decrease of standing stock with Time, from 130 to 90 ind.  $10 \text{ cm}^{-2}$  in the "Normoxia" cores to ~ 15 ind.  $10 \text{ cm}^{-2}$  in the "10 Months" samples
- <sup>10</sup> (Fig. 2 and Fig. 3). *Quinqueloculina seminula* showed a very different pattern. Standing stocks were low at the beginning and end of the experiment ( $\sim 10$  ind.  $10 \text{ cm}^{-2}$ ). There was a statistically significant increase (Table 4) towards maximum values of  $\sim 50$  ind.  $10 \text{ cm}^{-2}$  in the "1 Month" samples (Fig. 3) followed by a decrease down to  $\sim 10$  ind.  $10 \text{ cm}^{-2}$  in the "10 Months" samples.
- <sup>15</sup> Lagenammina atlantica, H. pacifica and B. pseudoplicata all varied between 15 and 40 ind. 10 cm<sup>-2</sup> (Fig. 2). Lagenammina atlantica showed no clear changes with Time, and the variability between replicate cores was high. The other two species showed a maximum in one of the "Normoxia" cores. For none of these species the change with Time was statistically significant (Table 4).
- <sup>20</sup> Finally, the standing stock of *Quinqueloculina* cf. *stelligera* was 80 and 30 ind. 10 cm<sup>-2</sup> in the 2 "Normoxic" cores, but was less than 5 ind. 10 cm<sup>-2</sup> in all anoxic cores, from "9 Days" to "10 Months" (Fig. 2). Its standing stock decreased significantly with Time and Time<sup>2</sup> (Fig. 3 and Table 4).

#### 3.3 Three groups of species with a different response to long-term anoxia

<sup>25</sup> Based on the statistical tests presented in Tables 3 and 4, we determined whether Time and/or Time<sup>2</sup> had a significant relationship with the standing stocks of the dominant species. These test resulted in three groups of species (Table 5). The taxa of Group A, which consist of *L. scottii, T. agglutinans* and *Q.* cf. *stelligera*, all exhibited a 12075

significant decrease in standing stocks with Time. Group B is composed of *E. scabra*, *B. marginata*, *L. atlantica*, *H. pacifica* and *B. pseudoplicata*, which did not exhibit a significant effect of Time. Finally, group C contained only *Q. seminula*, which showed a significant effect of both Time (positive) and Time<sup>2</sup> (negative); its standing stock significantly increased in the first month of the experiment, and decreased thereafter until

the end of the experiment.

#### 3.4 Species microhabitats

For most of the species, density decreased gradually with sediment depth (Fig. 4 and Supplement 5). Nevertheless, some species exhibited fairly high densities at the sed-

- iment water interface as well as deeper in the sediment (between 1.5 and 4 cm). This was especially the case for group B species. *Eggerella scabra* and *B. marginata*, and to a minor degree for *H. pacifica*, which all showed maximum values both at the sediment water interface and in a deeper sediment layer. *Bolivina pseudoplicata* showed a maximum in the 0.5–2 depth interval, whereas *L. atlantica* and *Q. cf. stelligera* showed field here the sediment here.
- fairly high densities from the sediment water interface down to 2 cm depth (Fig. 4). For all these species, no major change of the vertical distribution was recorded between the different sampling times (Fig. 4). A complementary statistical model indicated that only *E. scabra*, *T. agglutinans*, *Q. seminula* and the *Q.* cf. *stelligera* group had significantly different vertical distributions at the different sampling times. These
- <sup>20</sup> differences, however, were largely due to density changes in the 0–0.5 cm layer and did not appear to reflect a potential vertical migration of the foraminiferal faunas.

#### 3.5 Nitrate content and denitrification

Concerning the presence of nitrate in the foraminiferal protoplasm, only five individuals of the 33 tested individuals from seven different species exhibited cellular nitrate con-

<sup>25</sup> centrations above the detection limit (Table 6). Considering the tested major species, the two *L. scottii* individuals did not show measurable nitrate in their cells. Also the two

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tested *E. scabra* individuals from "Normoxic" conditions in Piran gave a negative result. Conversely, three of the nine tested individuals from station D10 from the western Adriatic Sea showed a nitrate content varying between 37 and 119 pmol. One of the nine tested *B. marginata* specimens from Piran contained 8 pmol of nitrate, whereas the base of the state of t

the only tested individual from station D10 yielded 433 pmol (Table 6). None of the three tested *T. agglutinans* and the seven tested individuals of three non-major species (*Fissurina* sp., *Nonion* spp., *Ammonia beccarii*) contained any nitrate.

Several individuals of *E. scabra* and *B. marginata* were tested for their capacity to denitrify. None of the tests revealed denitrification (no detectible  $N_2O$  production; Table 7).

#### 4 Discussion

# 4.1 Sediment geochemistry and faunal response to experimentally induced anoxia

In this experiment, not only benthic foraminifera, but also sediment geochemistry (Koron et al., 2013; Metzger et al., 2013), macrofaunal behaviour (Blasnig et al., 2013; Riedel et al., 2013) and soft-shelled meiofauna (copepods and nematodes; De Troch et al., 2013; Grego et al., 2013) have been analyzed. Sediment geochemistry analyses enabled following the development of the anoxia at the beginning of the experiment. The oxygen concentration starts to decrease as soon as the chamber is closed, and

- anoxia was reached after five days, two days before the "9 Days" benthic chamber was sampled. The available geochemical information (Metzger et al., 2013) indicates that the overlaying water remained anoxic with reducing conditions until the end of the experiment. The data also show that nitrates were available in the pore water at all times (Koron et al., 2013).
- <sup>25</sup> At the beginning of the experiment, 2 to 3 brittle stars were placed in each chamber. The brittle stars died after 7 to 15 days of deployment, and shortly after their death the

sediment surface turned black. From ~ 5 to 10 days several infaunal organisms such as polychaetes and bivalves emerged from the sediment, to finally die on the sediment surface some days later. Our interpretation is that the death of the macrofaunal organisms provided a large amount of labile organic matter that was degraded by aerobic

(in the first days), and later almost exclusively by anaerobic pathways. anaerobic degradation of this newly available organic matter released considerable amounts of hydrogen sulphides at the sediment water interface and in the overlaying , which has been clearly observed in the "1 Month" samples (Metzger et al., 2013).

# 4.2 Variation of foraminiferal standing stocks and diversity

- <sup>10</sup> The temporal variability of the total foraminiferal standing stock has been described in detail by Langlet et al. (2013). The data conclusively show that benthic foraminifera were alive at all the sampling times. Consequently, foraminifera can survive "10 Months" of anoxia. Nevertheless, the total foraminiferal standing stock significantly decreased with Time, interrupted by slightly higher values in the "1 Month" cores. Langlet
- <sup>15</sup> et al. (2013) tentatively interpreted this increase as a response to organic matter release due to macrofaunal mortality.

A total of 73 taxa were observed. Since the eight out of the nine major species were found alive in all samples, even after "10 Months" of anoxia (Fig. 1), the changes in species richness are exclusively caused by minor species and by *Quinqueloculina* cf.

- $_{20}$  stelligera, which does not show any living individuals in one of the replicates cores of the "1 Month" and "10 Months" chambers. Species richness of the whole cores significantly decreased with Time, but for the 0–0.5 cm interval, this trend was not significant in the > 63 µm fraction. This decrease is apparently explained by the disappearance of some of the less common species, which may be less resistant to the experimental conditions than the dominant species.
  - The two other diversity indexes (Shannon index and Evenness) show a significant decreasing trend only in the > 125  $\mu$ m fraction (Table 3). The lack of significant trends in the > 63  $\mu$ m fraction can be explained by the fact that the faunas of the small size

fraction (63–125  $\mu m$ ) are dominated (up to > 40 %) by *Leptohalysis scottii* (Fig. 2). The absolute as well as relative frequency of this species decrease with Time (Supplement 1 and 2), leading to a higher Evenness and Shannon Index for the fine fraction; this may compensate the significant decreasing tendency of these indices in the > 125  $\mu m$  fraction.

In general, the diversity of foraminiferal assemblages is low in permanently hypoxic areas, such as Oxygen Minimum Zones (reviewed in Koho and Piña-Ochoa, 2012) as well as in seasonally hypoxic areas, such as the Gulf of Movies (Blackwelder et al., 1996) or the shallow Skagerrak coast (Bouch and All 2012), this context, it is surprising that the decrease of foraminiferal density with Time is not clearer in our data.

# 4.3 Ecies responses to experimentally induced anoxia

Based on the temporal variability of the standing stocks of individual taxa, three groups with a different behaviour have been recognised: the taxa of group a show a significant decrease with Time, for the species of group B there is no significant trend, whereas

- Q. seminula shows a significant maximum in the "1 Month" cores. The next chapter considers all species of these three groups in more detail, in the hope that our observations may contribute to a better understanding of their ecology. For 64 taxa the relative frequency is always lower than 4%. These taxa have been arbitrarily separated into 7 groups with different responses to the experimental conditions. Four groups
- show a significant decrease of their cumulative standing stocks with Time: (1) Ammonia/Aubignina, (2) Elphidium/Nonion/Haynesina, (3) non fossilising agglutinated taxa and (4) miliolids. Conversely, bolivinids, buliminids and the group of epiphytic taxa do not show a significant decrease of their cumulative standing stocks.

# 4.3.1 Group A

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<sup>25</sup> Group a is composed of *L. scottii*, *T. agglutinans* and *Q.* cf. *stelligera*. The first two species, which are the most dominant in the assemblages are both agglutinan

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whereas *Quinqueloculina* cf. *stelligera* has a porcelaneous shell. All three species show a significant decrease of their standing stock with Time (Fig. 3 and Table 4). They appear therefore to be sensitive to the experimental conditions of long-lasting anoxia with a presence of hydrogen sulphides.

#### 5 Leptohalysis scottii

*Leptohalysis scottii* is present mainly in the 63–125 µm size fraction, and is only mentioned in papers which include the study of smaller size fractions. This species appears to be sensitive to the experimental conditions (Fig. 3 and Table 4). It has been described under different names (*Reophax scottii, Reophax nana* or *Acostata mariae*)

- and is common in coastal environments such as the Vigo estuary (Diz and Francés, 2008), the Rhone prodelta (Mojtahid et al., 2009; Goineau et al., 2012), the Adriatic Sea (Barmawidjaja et al., 1992; Sabbatini et al., 2010, 2012) and, more specifically, in the Gulf of Trieste (Hohenegger et al., 1993).
- In the literature, *L. scottii* has been described as an early colonizer of recently deposited sediment (Goineau et al., 2012). Moreover, its strong response to episodic pulses of organic matter shows an opportunistic capacity (Diz and Francés, 2008; Sabbatini et al., 2012). *Leptohalysis scottii* has therefore been proposed as a bioindicator of a high organic flux to the sediment surface (Scott et al., 2005). The slightly increased standing stocks observed in our "1 Month" cores (Fig. 2) could reflect an opportunis-
- <sup>20</sup> tic response to an increased availability of labile organic matter due to macrofaunal mortality in the first weeks of the experiment.

Interestingly, Ernst et al. (2002) have reported that *L. scottii* maintained or even increased its population size in an experiment in which the upper 3 cm (with its living foraminiferal fauna) were homogenised, in spite of the fact that hypoxic conditions were

rapidly established below 1 cm depth. It has also been suggested, based on an assay of Rose Bengal stained assemblages, that *L. scottii* is sensitive to both anoxia and hydrogen sulphide (Moodley et al., 1997, 1998). Diz and Francés (2008) described *L. scottii* as predominantly inhabiting the uppermost oxygenated centimetre of the sediment; Paper

Duijnstee et al. (2003) also reported this species as a typical surface dweller. Despite its apparent sensitivity to anoxia, *L. scottii* is still alive in substantial numbers after "10 Months" of anoxia. We can therefore confirm that although it prefers welloxygenated settings, *L. scottii* is able to survive anoxia with co-occurring hydrogen sulphides. Nonetheless, the significant decrease of its relative frequencies (Supplement 2 Discussion Paper

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and 3) suggests that it is less resistant than most other dominant species

#### Textularia agglutinans

*Textularia agglutinans* was found alive in all size fractions, with larger numbers in the  $63-125 \,\mu$ m fraction (Fig. 2). Its standing stock significantly diminishes with Time, and

- in the sediment column it quickly disappears in deeper levels (Figs. 3, 4 and Table 4). Both observations suggest a limited tolerance of anoxia. *Textularia agglutinans* has been widely described in the Mediterranean, for instance in the Rhone prodelta (Mojtahid et al., 2009; Goineau et al., 2011), the Marmara Sea (Armynot du Châtelet et al., 2013) and also in the Adriatic Sea (Jorissen et al., 1992; Barmawidjaja et al., 1995;
- <sup>15</sup> Duijnstee et al., 2004; Sabbatini et al., 2010). Its ecological preferences are not well established. Duijnstee et al. (2003) described it as a deep-dwelling species which did not show any vertical migration or standing stock changes in experimentally induced anoxia. Conversely, *T. agglutinans* is a common species in the distal zone of the Rhone prodelta, where the surface sediment is well oxygenated and where the oxygen pen-
- etration depth is relatively high (Goineau et al., 2011). It is also abundant in less eutrophic and better oxygenated areas off the Po delta (Donnici and Serandrei Barbero, 2002). Most of these observations corroborate our conclusion that *T. agglutinans* is sensitive to bottom water anoxia and co-occurring hydrogen sulphide.

#### Quinqueloculina cf. stelligera

Quinqueloculina cf. stelligera is found alive only in the finest size fraction (63–125 μm;
 Fig. 2). Its standing stock show a strong and significant decrease with Time, as it is

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almost exclusively found alive in the "Normoxic" conditions (Fig. 3 and Table 4). In these well-oxygenated conditions it lives in the two uppermost centimetres of the sediment (Fig. 4). *Quinqueloculina. stelligera* has been described from infralittoral areas in the Gulf of Cadiz (Mendes et al., 2012) and in low quantities in sediment colonized

- <sup>5</sup> by *Posidonia oceanica* in the Mediterranean Sea (Mateu-Vicens et al., 2010). In the Tyrrhenian Sea the distribution of *Q. stelligera* seems to be controlled by the sediment type, since it is almost exclusively found in fine sands (Celia Magno et al., 2012). However, *Q. stelligera* is also described in stations polluted with Fe, Pb, Zn and PAHs and was consequently considered as pollution-tolerant (Romano et al., 2009). Although it
- appears to be resistant to heavy-metal pollution it has not been described before in lowoxygen environments. Consequently, in a paleontological record of the antique harbour of Claudius, the presence of *Q. stelligera* was interpreted as indicative of a well oxygenated environment (Di Bella et al., 2011). In the present study, the instantaneous (and significant) collapse of its standing stocks early in the experiment suggests that it is highly sensitive to anoxia.

# 4.3.2 Group B

Group B is composed of two agglutinated species (*E. scabra, L. atlantica*) and three calcareous species (*B. marginata, H. pacifica, B. pseudoplicata*). The standing stocks of these taxa do not decrease significantly with Time (Fig. 3 and Table 4), which suggests that these taxa are strongly resistant to the experimental conditions.

#### Eggerella scabra

In the present study, *Eggerella scabra* (= *Eggerelloides scabra*; Hohenegger et al., 1993; Diz and Francés, 2008) is present in all size fractions from 63 to  $315 \,\mu$ m (Fig. 2). Its standing stock does not change significantly with Time (Fig. 3 and Table 4). It penters relatively does not change significantly with Time (Fig. 3 and Table 4).

etrates relatively deep into the sediment, showing substantial numbers down to 5 cm depth (Fig. 4). *Eggerella scabra* is a very common species in subtidal, coastal and shelf

environments (Murray, 2006; Diz and Francés, 2008), and is a common species in the Adriatic Sea (Jorissen et al., 1992; Hohenegger et al., 1993; Stigter et al., 1998; Sabbatini et al., 2010, 2012). It has been reported in sediments enriched in organic matter (Stigter et al., 1998; Donnici and Serandrei Barbero, 2002), more specifically in sedi-

- ments with low-quality organic matter (Diz and Francés, 2008; Duchemin et al., 2008; Goineau et al., 2011). Eggerella scabra has also been considered as tolerant to hypoxic and/or anoxic conditions (Ernst et al., 2002; Diz and Francés, 2008). In a laboratory experiment, Pucci et al. (2009) observed an increase of the standing stocks of E. scabra after a 15 day incubation in strongly hypoxic conditions. Eggerella scabra is generally
- described as an infaunal species (Barmawidjaja et al., 1992; Jorissen et al., 1992; Ernst et al., 2002; Duijnstee et al., 2003). As in our cores, it is often found throughout the first 5 cm of the sediment column without a clear maximum at a specific depth (Ernst et al., 2006; Pucci et al., 2009). Our data clearly confirm the capacity of Eggerella scabra to survive anoxia with co-occurring hydrogen sulphides. This species can clearly be
- considered as highly resistant to such stressed conditions.

#### Bulimina marginata sensu lato

Bulimina marginata was alive in all size fractions from 63 to 315 µm and showed no significant change in its standing stock with Time (Figs. 2, 3 and Table 4). Bulimina marginata is a cosmopolitan taxon, well known in the Atlantic Ocean (e.g. Jorissen

- et al., 1998) and Mediterranean Sea (e.g. Jorissen, 1987; De Rijk et al., 2000; Schmiedl 20 et al., 2000; Donnici and Serandrei Barbero, 2002; Ernst et al., 2005; Di Leonardo et al., 2007; Mojtahid et al., 2009). It occurs in continental shelf and slope environments, and abundant faunas have been described from the Adriatic Sea (e.g. Jorissen et al., 1992) and especially from the Gulf of Trieste (Hohenegger et al., 1993). Jorissen (1988) noted
- that three main morphotypes occur in the Adriatic Sea. Typical morphotypes, with well-25 developed undercuts, occur in outer shelf and upper slope settings. Bulimina aculeata morphotypes, with a fusiform, slightly twisted initial part, are found mainly on the continental shelf, whereas B. denudata morphotypes, with weakly developed undercuts, 12083

are dominant at eutrophic inner shelf sites (Jorissen, 1988). Based on molecular phylogenetic analyses, Tsuchiya et al. (2008) concluded that these morphotypes are genetically different, and should be considered as biological species. In our material, denudata and aculeata types are dominant.

- Bulimina marginata sensu lato (s.l.) is generally considered to be an eutrophic taxon typical for areas with high organic carbon contents, where it may show an opportunistic behaviour (Zwaan and Jorissen, 1991; Schmiedl et al., 2000; Donnici and Serandrei Barbero, 2002; Langezaal et al., 2006; Mojtahid et al., 2006). Because this taxon can survive episodes of severe hypoxia/anoxia (Barmawidjaja et al., 1992; Donnici and
- Serandrei Barbero, 2002; Pucci et al., 2009), it is generally considered stress-tolerant, 10 although some authors suggested that it may prefer well-oxygenated conditions (Alve and Bernhard, 1995; Donnici and Serandrei Barbero, 2002; Ernst et al., 2005; Mojtahid et al., 2006). Our observations, with no significant changes of its standing stocks after "10 Months" of anoxia, fully confirm its resistance to anoxia with a presence of hydrogen sulphides.

The microhabitat of Bulimina marginata s.l. is not well defined. In the literature, it has been considered as a shallow, intermediate or even deep infaunal taxon (see review in Jorissen, 1999). Bulimina marginata s.l. may find suitable environmental conditions at the sediment surface as well as deeper in the sediment. In our study, abundant occur-

- rences in deeper sediment intervals confirm its potentially infaunal character (Fig. 4). 20 Several authors have suggested that Bulimina marginata s.l. can migrate rapidly through the sediment, which could explain their often chaotic vertical distribution (Barmawidjaja et al., 1992; Mackensen et al., 2000; Schmiedl et al., 2000; Ernst et al., 2005). In a laboratory experiment with strongly hypoxic conditions, however, it did not
- show vertical migration (Pucci et al., 2009). Alve and Bernhard (1995) considered Bu*limina marginata* s.l. as one of the few taxa potentially able to reproduce in strongly hypoxic conditions. In the present study, it is clearly strongly resistant to anoxia and co-occurring hydrogen sulphides, but its rather invariable standing stocks provide no indications that reproduction occurred during the 10 months of our experiment.

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# Lagenammina atlantica

Lagenammina atlantica (also known as Saccammina atlantica) was mainly found alive in the 150–315  $\mu$ m fraction. Its standing stocks differ considerably between the pairs of replicate cores, and there is no significant overall change with Time (Figs. 2, 3 and

<sup>5</sup> Table 4). Most individuals were found in the first 1.5 cm layer (Fig. 4). Little information is available about the ecology of this taxon. Murray (2006) considers it to be a shelf species that also inhabits brackish waters. Our data strongly suggest that this species is very resistant to the combination of anoxia and hydrogen sulphide.

# Hopkinsina pacifica

- Hopkinsina pacifica (also named Hopkinsinella glabra (Haig, 1993) or Hopkinsina altantica, Diz and Francés, 2008) was found alive mainly in the 63–125 μm fraction. Its standing stocks show no significant trend with Time. Unlike the other species of group B, *H. pacifica* is mainly found at the sediment water interface, and is apparently a surface dweller.
- Hopkinsina pacifica is a typical species of river-influenced inner shelf ecosystems) (Barmawidjaja et al., 1992; Jorissen et al., 1992; Diz and Francés, 2008; Mojtahid et al., 2009) such as in the Northern Adriatic Sea (Hohenegger et al., 1993; Moodley et al., 1997; Ernst et al., 2002, 2005; Duijnstee et al., 2003, 2004).
- On the basis of a laboratory experiment, Ernst et al. (2005) concluded that this species is favoured by fresh food supplies. Other observations corroborate such an opportunistic behavior (Jorissen et al., 1992; Ernst et al., 2002; Duijnstee et al., 2004; Goineau et al., 2012), which is a common trait of several small-sized taxa (e.g., *Epistominella exigua, Leptohalysis scottii*). *Hopkinsina pacifica* typically occupies the superficial sediment layer (Jorissen et al., 1992; Ernst et al., 2002; Duijnstee et al., 2003).
- <sup>25</sup> Our observations confirm that this species is a surface dweller. In an experimental study of oil pollution exposition with anoxic conditions, Ernst et al. (2006) observed an upward migration of this taxon. In our experiment, living individuals were always

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restricted to the upper 1 cm, and no upward migration was observed (Fig. 4). Ernst et al. (2006) reported that only part of their *H. pacifica* population survived experimental anoxia. In our data, this species showed no significant decrease with Time, and it belongs to the most anoxia-resistant taxa.

# **5** Bolivina pseudoplicata

*Bolivina pseudoplicata* is mainly found in the  $63-125 \,\mu$ m fraction and, like the other representatives of group B, its standing stocks do not show a significant change with Time. *Bolivina pseudoplicata* occurs in fair numbers down to 5 cm depth, without a clear trend.

- Bolivina pseudoplicata has been reported from transitional environments, such as estuaries and prodeltas (Cearreta et al., 2000; Debenay et al., 2006; Diz and Francés, 2008). This species has also been observed in the Adriatic Sea (Hohenegger et al., 1993; Barmawidjaja et al., 1995). Its ecology is not well known and little information is available about its microhabitat. Our data, which show abundant living individuals after "10 Months" of anaxia, demonstrate the high telerance of this appaired.
- <sup>15</sup> "10 Months" of anoxia, demonstrate the high tolerance of this species.

#### 4.3.3 Quinqueloculina seminula

*Quinqueloculina seminula* is found mostly in the 63–125 µm size fraction. It only occurs in the topmost centimetre of the sediment, suggesting a very shallow microhabitat. *Quinqueloculina seminula* is only present in small quantities in the "Normoxia" cores

- samples at the beginning of the experiment, but shows a tenfold increase in standing stock after "1 Month", putatively in response to increased labile organic matter availability after the death of all macrofauna. Thereafter, standing stocks decrease, and after "10 Months" of anoxia, standing stocks are comparable to those observed in the "Normoxia" cores.
- <sup>25</sup> *Quinqueloculina seminula* has been described from a wide range of environments, among them Mediterranean lagoons, marshes and continental shelves (reviewed in

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Murray, 2006). Species of the genus *Quinqueloculina* are also common in the Adriatic Sea (Frontalini and Coccioni, 2011) and in the Gulf of Trieste (Hohenegger et al., 1993). Concerning its tolerance to low oxygen conditions, contradictory evidence has been reported. For example, Moodley and Hess (1992) observed that it was resistant to 24 h

- <sup>5</sup> of anoxia, whereas a later study described it as sensitive to anoxia with co-occurring hydrogen sulphides (Moodley et al., 1998). Rather surprisingly in view of the general perception of miliolids as sensitive taxa, typical of oligotrophic conditions (e.g. Barras et al., 2013), *Q. seminula* has been observed as an early colonizer after volcanic ash deposits (Hess and Kuhnt, 1996) and as a pioneer species after sediment disturbance
- <sup>10</sup> in submarine canyons (Duros et al., 2011). Our observations suggest an opportunistic response to labile organic matter supplies combined with a resistance to short-term anoxia. As explained above, we hypothesize that the increase in standing stocks of several taxa in the "1 Month" cores, which is particularly spectacular for *Q. seminula*, is a response to increased labile organic matter availability after macrofauna death in the
- <sup>15</sup> first two weeks of the experiment. In the subsequent weeks (i.e. after the sampling of the "1 Month" cores), the anaerobic degradation of this organic matter released a large quantity of hydrogen sulphides at the sediment water interface, making the environment hostile for *Q. seminula* and the taxa of group A.

#### 4.4 Foraminiferal community response to the experimental conditions

- <sup>20</sup> Considering the response of the whole foraminiferal community, the impact of 10 months of anoxia, with co-occurring hydrogen sulphides, is apparently very limited. This is not really surprising because most of the dominant taxa are known for their resistance to low oxygen conditions, which is largely confirmed by our results. Such a dominance by low-oxygen-tolerant taxa is logical in ecosystems that are periodically
- <sup>25</sup> impacted by seasonal hypoxia. Such seasonal hypoxias have increased in frequency over the last fifty years, reflecting strongly increased anthropogenic nutrient inputs, but are basically a natural phenomenon (Barmawidjaja et al., 1995; Diaz and Rosenberg, 2008). In spite of the overall tolerance of low oxygen conditions, which is evident for 10007

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all major species (which all survived 10 months of anoxia), three differential types of response have been distinguished. The species of group a (composed of *Leptohalysis scottii, Textularia agglutinans* and *Quinqueloculina* cf. *stelligera*), which is mainly composed of rather superficially living organisms, show a significant decrease of their

- standing stocks, although many living specimens remain present until the end of the experiment. The species of group B (*Eggerella scabra, Bulimina marginata, Lagenammina atlantica, Hopkinsina pacifica* and *Bolivina pseudoplicata*), which is mainly composed of taxa with a stronger infaunal tendency, show an even higher tolerance for the combination of prolonged anoxia and hydrogen sulphide, and show no significant
- decline in standing stocks with Time. This higher tolerance may be partly explained by their deeper microhabitats, which forces these taxa to live in anoxic conditions even when the sediment water interface is oxic. Conversely, their higher tolerance to anoxia also enables them to live in deeper sediment intervals; this is important in ecosystems where oxygen penetration is normally limited to the upper few millimetres (Metzger et al., 2013).
  - Finally, the shallow infaunal taxon *Q. seminula* combines the slightly lower tolerance of anoxic conditions of the group a taxa with a very strong opportunistic tendency. The result is a spectacular increase of its standing stocks after 1 month. Surprisingly, the presence of anoxia did not inhibit a reproductive response of this species to inputs of labels another.

<sup>20</sup> labile organic matter.

Examining the behaviour of these three species groups enables three successive stages to be distinguished. Figure 5 shows that during a first stage, until 2 to 3 weeks, the faunas tend to be dominated by species of group A. During a second stage, which lasts until about 2 months, *Q. seminula*, and perhaps also *Leptohalysis scottii* and

Lagenammina atlantica, show an opportunistic response (reproduction and growth) to putative labile organic input resulting from the death of enclosed macrofauna. The third and final stage shows an increasing dominance of the highly anoxia-resistant group B species (Fig. 5). This three-stage temporal succession is very similar to the spatial patterns along strong eutrophication gradients, such as those described around point Discussion Paper | Discussion Paper

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sources of organic pollution. Along such gradients, sensitive taxa progressively disappear, and slightly stress-tolerant taxa are little by little replaced by taxa with maximum tolerance for stressed conditions. When stress is not lethal, some species may show a strong opportunistic response, yielding very high standing stocks. Such patterns have

<sup>5</sup> been described repeatedly for macrofauna (e.g. Pearson and Rosenberg, 1976), but have recently also be shown for foraminifera (Mojtahid et al., 2006, 2008) Symmarising, the foraminiferal response to anoxia and co-occurring eutrophication be very similar to the macrofaunal response, with the essential difference that many foraminiferal taxa are much more resistant to anoxic conditions than macrofauna.

#### 10 4.5 Understanding foraminiferal survival during exposure to anoxia

The intracellular nitrate content and denitrification measurements could not be performed for all the major species of our study area, mainly because of the difficulty to select living foraminifera in the sandy sediments of our two sampling stations vertheless, intracellular nitrate measurements are available for species of each of the

- three identified groups. We performed measurements on species from groups A and B, whereas the genus *Quinqueloculina* has been tested by Piña-Ochoa et al. (2010). Our results show that none of the 12 tested individuals of 5 species belonging to group A were able to accumulate nitrate in their cells. Conversely, 5 of the tested 21 individuals of *E. scabra* and *B. marginata*, both belonging to group B, contained non-negligible
- amounts of intracellular nitrates, with values ranging from 8 to 433 pmol per cell. Unfortunately, no data are available for the three other species of group B (*L. atlantica*, *H. pacifica* and *B. pseudoplicata*). Piña-Ochoa et al. (2010) measured 16 specimens (from the Peru OMZ, Skagerrak, Bay of Biscay and Rhône Delta) of *Quinqueloculina* spp., without any positive results. Although the available information is still inconclu-
- sive, a picture emerges that group B species can accumulate nitrate in their cytoplasm, whereas group A species and *Q. seminula* can not. Although this picture remains to be confirmed or informed by additional measurements, the present data suggest that the higher tolerance to anoxia by the group B species could reflect their ability to 12080.

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accumulate nitrate and thus to shift to an anaerobic metabolism (denitrification) under anoxic conditions. Nonetheless, the 5 measurements of denitrification we performed for *E. scabra* and *B. marginata* gave only negative results. We see two possible explanations for the absence of denitrification in these experiments: (1) the measured

<sup>5</sup> individuals did not accumulate nitrate before being measured, perhaps because the well-oxygenated pre-measurement laboratory conditions did not incite them to do so, or (2) they are incapable of denitrifying. This calls for additional analyses to establish whether the foraminiferal taxa that survive 10 months of anoxia are capable of denitrifying or not. If not, another explanation must be sought for their highly surprising long-term survival.

#### 5 Conclusions

We performed an in situ experiment at a 24m-deep site in the Gulf of Trieste. Benthic chambers 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 con-

- tained abundant foraminiferal assemblages down to 5 cm depth in the sediment. During the experiment the foraminiferal species richness significantly decreased while the diversity indexes showed no changes, probably due to the anoxia sensitivity of one species which was dominant in the original conditions. Despite the minor changes in diversity, the species composition changed because some species are less resistant
- than others to anoxia. The present study identified *Leptohalysis scottii*, *Textularia ag-glutinans* and especially *Quinqueloculina* cf. *stelligera* as more sensitive to the experimental conditions. Conversely, *Eggerella scabra*, *Bulimina marginata*, *Lagenammina atlantica*, *Hopkinsina pacifica* and *Bolivina pseudoplicata* appear to be highly resistant to long-term anoxia and co-occurring hydrogen sulphide. The foraminiferal faunas
- not only responded by an altered species composition, but some taxa also apparently show an opportunistic behaviour to the organic matter release due to the degradation of the dead macrofauna after 1 month of experiment. *Quinqueloculina seminula*, and

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perhaps also Leptohalysis scottii and Lagenammina atlantica exhibit such a potentially opportunistic behaviour. Finally, the intracellular nitrate and denitrification measurements we performed provided no positive evidence that foraminiferal survival to anoxia is related to their denitrification capacity. This differential reaction to anoxia, and the

survival of set of species to long-term disturbance, has important implications for the status and recovery potential of coastal areas increasingly affected by large-scale oxygen depletion events.

### Supplementary material related to this article is available online at: http://www.biogeosciences-discuss.net/10/12065/2013/ bgd-10-12065-2013-supplement.zip.

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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
9 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.** Name and number of the linear models. For each model type the different dependent and independent variables are listed and the used transformation is presented in brackets. As the models are executed on different data, the categories of the selection are presented.

Models names	Dependent variable (Transformation)	Independent variable (Transformation)	Selection of the independent variable	Data selection
Models 1 (x 16)	Standing Stock (log)	Time (log)	Yes	Size (> 125 $\mu$ m and > 63 $\mu$ m)
	Species Richness Shannon Index Evenness	Time <sup>2</sup> (log)		Depth (0–0.5 cm and 0–5 cm)
Models 2 (× 18)	Standing Stock (log)	Time (log)	Yes	Species (L. scottii, E. scabra,
	Frequency (arcsin)	Time <sup>2</sup> (log) Depth (0–0.5 cm and 0–5 cm) Time*Depth Time <sup>2</sup> × Depth		B. marginata, T. agglutinans, Q. seminula, L. atlantica, H. pacifica and B. pseudoplicata and Q. cf. stelligera)
Models 3 (× 9)	Density (log)	Depth (log) Depth <sup>2</sup> (log) Depth × Depth <sup>2</sup> (log)	No	Species (L. scottii, E. scabra, B. marginata, T. agglutinans, Q. seminula, L. atlantica, H. pacifica and B. pseudoplicata and Q. cf. stelligera)
Models 4 (× 6)	Standing Stock (log) Frequency (arcsin)	Time (log) Time <sup>2</sup> (log)	Yes	Group (A, B, C)

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**Table 3.** Statistical parameters (Df: degrees of freedom, Sum Sq: Sum of Square, Mean Sq: Mean of Square, *F* value: value of the *F* test, Pr (> *F*): probability of the *F* test) for the Models 1 at all sampled Size fractions and Depth intervals for the 4 tested dependent variables. For the cases where no significant effect of either the Time or Time<sup>2</sup> were observed, only the "Residuals" parameters are estimated.

Dependent variable	Depth	Size	Independant variable	Df	Sum Sq	Mean Sq	F value	Pr (> <i>F</i> )
Standing Stock	0–5 cm	> 63 µm	log(5 + Time)	1	0.5	0.5	6.6	0.03
			Residuals	8	0.6	0.1	NA	NA
		> 125 µm	Residuals	9	0.7	0.1	NA	NA
	0–0.5 cm	> 63 µm	log(5 + Time)	1	1.4	1.4	9.8	0.01
			Residuals	8	1.1	0.1	NA	NA
		> 125 µm	Residuals	9	2.9	0.3	NA	NA
Species Richness	0–5 cm	> 63 µm	log(5 + Time)	1	92.6	92.6	10.0	0.01
			Residuals	8	74.3	9.3	NA	NA
		> 125 µm	log(5 + Time)	1	58.2	58.2	7.7	0.02
			Residuals	8	60.7	7.6	NA	NA
	0–0.5 cm	> 63 µm	Residuals	9	346.4	38.5	NA	NA
		> 125 µm	log(5 + Time)	1	66.6	66.6	7.2	0.03
			Residuals	8	73.8	9.2	NA	NA
Shannon Index	0–5 cm	> 63 µm	Residuals	9	0.2	0.0	NA	NA
		> 125 µm	log(5 + Time)	1	0.5	0.5	8.8	0.02
			Residuals	8	0.5	0.1	NA	NA
	0–0.5 cm	> 63 µm	Residuals	9	0.5	0.1	NA	NA
		> 125 µm	log(5 + Time)	1	0.2	0.2	5.6	0.05
			Residuals	8	0.3	0.0	NA	NA
Evenness	0–5 cm	> 63 µm	Residuals	9	0.0	0.0	NA	NA
		> 125 µm	log(5 + Time)	1	0.0	0.0	6.4	0.04
			Residuals	8	0.0	0.0	NA	NA
	0–0.5 cm	> 63 µm	Residuals	9	0.0	0.0	NA	NA
		> 125 µm	Residuals	9	0.0	0.0	NA	NA

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Sur obal	n Sq: Su bility of th	ım of S ne <i>F</i> tes	quare, I st) for th	Vean Sq: le Models
Sq	Mean Sq	F value	Pr (> F)	
29	4.29	21.0	0.00	
05	3.05	14.9	0.00	
47	0.20	NA	NA	
82	16.82	60.1	0.00	

Dependant Species	Independant variables	Df	Sum Sq	Mean Sq	F value	Pr (> F)
Leptohalysis scottii	log(5 + Time)	1	4.29	4.29	21.0	0.00
	Depth	1	3.05	3.05	14.9	0.00
	Residuals	17	3.47	0.20	NA	NA
Eggerella scabra	Depth	1	16.82	16.82	60.1	0.00
	Residuals	18	5.04	0.28	NA	NA
Bulimina marginata	Depth	1	12.49	12.49	76.1	0.00
	Residuals	18	2.96	0.16	NA	NA
Textularia agglutinans	log(5 + Time)	1	14.50	14.50	60.1	0.00
	Depth	1	11.43	11.43	47.4	0.00
	Residuals	17	4.10	0.24	NA	NA
Quinqueloculina seminula	log(5 + Time)	1	0.10	0.10	0.3	0.58
	Depth	1	3.27	3.27	10.1	0.01
	log(5 + Time <sup>2</sup> )	1	17.02	17.02	52.6	0.00
	Residuals	16	5.17	0.32	NA	NA
Lagenammina atlantica	Depth	1	6.63	6.63	15.1	0.00
	Residuals	18	7.92	0.44	NA	NA
Hopkinsina pacifica	Depth	1	2.70	2.70	17.4	0.00
	Residuals	18	2.80	0.16	NA	NA
Bolivina pseudoplicata	Depth	1	26.48	26.48	83.9	0.00
	Residuals	18	5.68	0.32	NA	NA
Quinqueloculina cf. stelligera	log(5 + Time)	1	18.03	18.03	55.9	0.00
	Depth	1	2.14	2.14	6.6	0.02
	log(5 + Time <sup>2</sup> )	1	12.66	12.66	39.2	0.00
	Residuals	16	5.161323	0.3225827	NA	NA

Table 5. Estimation of the coefficient associated to independent variables after Models 2 for the 9 major species. Only the independent variables having a significant effect on the Standing Stock are retained by the backward model selection. Depending on the presence or absence of a significant effect of Time on the density and the coefficient sign, three groups of species have been determined.

Species	Group	Time	Time <sup>2</sup>	Depth
Textularia agglutinans	А	-0.61		-1.51
Leptohalysis scottii	А	-0.33		-0.78
Quinqueloculina cf. stelligera	А	-3.73	0.41	-0.65
Eggerella scabra	В			-1.83
Bulimina marginata	В			-1.58
Lagenammina atlantica	В			-1.15
Hopkinsina pacifica	В			-0.74
Bolivina pseudoplicata	В			-2.30
Quinqueloculina seminula	С	3.49	-0.48	-0.81

			Nitrate content (pm)					
Species	Group	Site	n	$n \; (N \neq 0)$	mean	min	min ( $N \neq 0$ )	max
Leptohalysis scotii	А	Piran	2	0	0	0	_	0
Textularia agglutinans	Α	Piran	3	0	0	0	-	0
<i>Fissurina</i> sp.	Α	Piran	1	0	0	0	_	0
<i>Nonion</i> spp.	Α	Piran	2	0	0	0	-	0
Ammonia becarii	Α	D10a	4	0	0	0	_	0
Eggerella scabra	В	Piran	2	0	0	0	-	0
Eggerella scabra	В	D10a	9	3	22	0	37	119
Bulimina marginata	В	Piran	9	1	1	0	8	8
Bulimina marginata	В	D10a	1	1	433	433	433	433

Table 7. Denitrification rate of the two tested species from two different sites. The number of individuals added to the measurement chamber is indicated. In two cases some individuals have been reused in a second set of measurements, generating pseudoreplication; the number of individual used in a second set of measurement is indicated.

Species	Site	Nb Indiv	Pseudoreplication	Rate (pmol indiv <sup><math>-1</math></sup> d <sup><math>-1</math></sup> )
Eggerella scabra	Piran	4	4 indiv. (set 1)	0
Eggerella scabra	Piran	7	4 indiv. (set 2)	0
Eggerella scabra	D10a	7		0
Bulimina marginata	Piran	3	3 indiv. (set 1)	0
Bulimina marginata	Piran	6	3 indiv. (set 2)	0

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**Table 8.** Statistical parameters (Df: degrees of freedom, Sum Sq: Sum of Square, Mean Sq: Mean of Square, *F* value: value of the *F* test, Pr (> *F*): probability of the *F* test) for the Models 5 for all the 3 groups of species and for the two tested dependant variables (Standing Stock and Frequency).

Dependent variable	Group	Independent variable	Df	Sum Sq	Mean Sq	F value	Pr (> <i>F</i> )
Standing Stock	GroupA	log(5 + Time)	1	2.4	2.4	25.4	0.00
		Residuals	8	0.8	0.1		
	GroupB	log(5 + Time)	1	0.0	0.0	0.1	0.80
		Residuals	8	0.7	0.1		
	GroupC	log(5 + Time)	1	0.2	0.2	0.8	0.40
		log(5 + Time <sup>2</sup> )	1	5.8	5.8	18.8	0.00
		Residuals	7	2.2	0.3		
Frequency	GroupA	log(5 + Time)	1	0.1	0.1	44.6	0.00
		Residuals	8	0.0	0.0		
	GroupB	log(5 + Time)	1	0.2	0.2	23.6	0.00
		Residuals	8	0.1	0.0		
	GroupC	log(5 + Time)	1	0.0	0.0	0.4	0.56
		log(5 + Time <sup>2</sup> )	1	0.0	0.0	15.9	0.01
		Residuals	7	0.0	0.0		



**Fig. 1.** Standing Stock, Species Richness, Shannon Index and Evenness variations with Time at two depth intervals (open circles: 0–5 cm and full circles: 0–0.5 cm) and in two size fractions (in red: > 63  $\mu$ m and in black: > 125  $\mu$ m). The lines are estimated after the "Models 1" group, full line indicates that at the treated depth and size the relationship between the dependant variable and the independent variable is significant, while grey dashed lines indicate the lack of significance of the relationship.







Fig. 3. Standing Stock variation with Time and sampling depth (open circles: 0-5 cm and full circles 0-0.5 cm) for the 9 major species. The lines are estimated after the "Models 2" group, full lines indicating a significant effect of Time and dashed lines indicating a non-significant effect of Time. When two lines are drawn the depth effect is significant, while if only one line is drawn, no significant effect of the sampling depth.



Fig. 4. Relationship between the depth and the foraminiferal density for the 9 major species at all the sampling times. The line is estimated after the "Models 3" group.



Fig. 5. Standing Stock (upper panel) and relative density (lower panel) variation with time for the 3 groups of species. Note that for representation purposes the standing stock and relative density of the group C were multiplied by 10 (refer to axis on the right). The lines are estimated after the the "Models 4" group, full lines indicating a significant effect of Time and dashed lines indicating a non-significant effect of Time.

